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Chemical contamination alters the interactions between bacteria and phytoplankton

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Abstract:	<p>Bacteria and phytoplankton are key players in aquatic ecosystem functioning. Their interactions mediate carbon transfer through the trophic web. Chemical contamination can alter the function and diversity of phytoplankton and bacterioplankton, with important consequences for ecosystem functioning. The aim of the present study was to assess the impact of chemical contamination on the interactions between both biological compartments. Two contrasting marine coastal ecosystems, offshore waters and lagoon waters, were exposed to chemical contamination (artificial or produced from resuspension of contaminated sediment) in microcosms in four seasons characterized by distinct phytoplankton communities. Offshore waters were characterized by a complex phytoplankton–bacterioplankton network with a predominance of positive interactions between both compartments, especially with Haptophyta, Cryptophyta, and dinoflagellates. In contrast, for lagoon waters, the phytoplankton–bacterioplankton network was simpler with a prevalence of negative interactions with Ochrophyta, Cryptophyta, and flagellates. Contamination with an artificial mix of pesticides and trace metal elements resulted in a decrease in the number of interactions between phytoplankton and bacterioplankton, especially for offshore waters. Resuspension of contaminated sediment also altered the interactions between both compartments. The release of nutrients stored in the sediment allowed the growth of nutrient limited phytoplankton species with marked consequences for the interactions with bacterioplankton, with a predominance of positive interactions, whereas in lagoon waters, negative interactions were mostly observed. Overall, this study showed that chemical contamination and sediment resuspension resulted in significant effects on phytoplankton–bacterioplankton interactions that can alter the functioning of anthropogenic coastal ecosystems.</p>
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Chemical contamination alters the interactions between bacteria and phytoplankton

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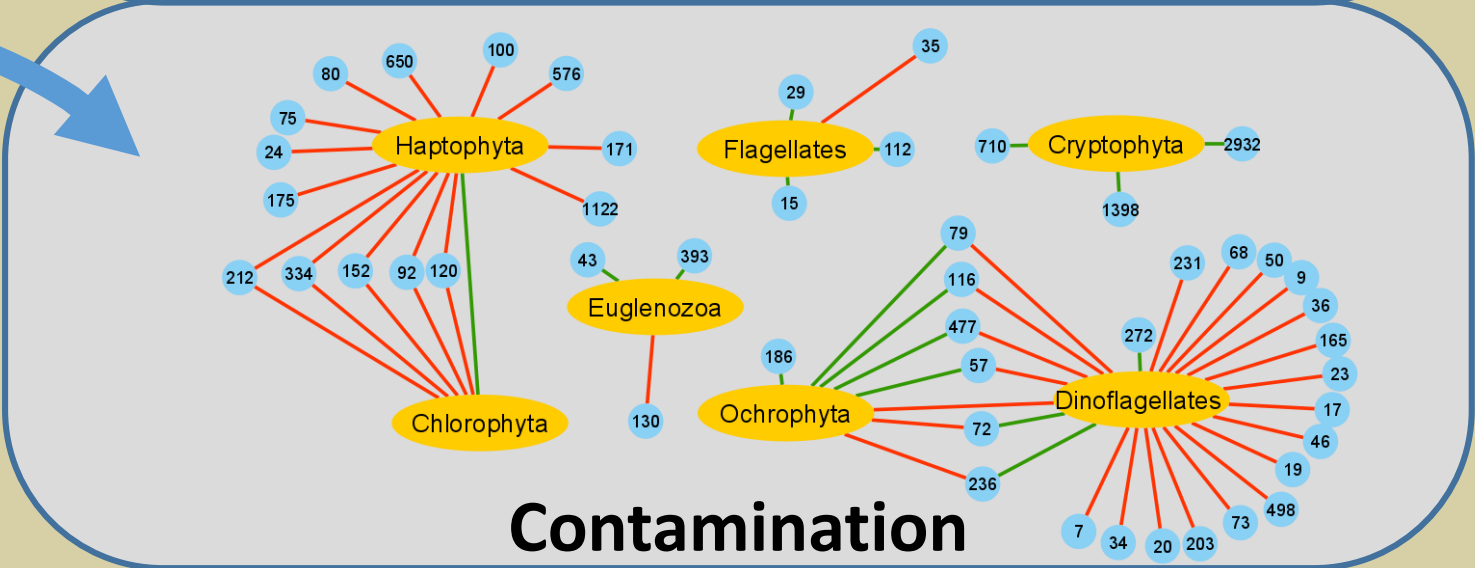
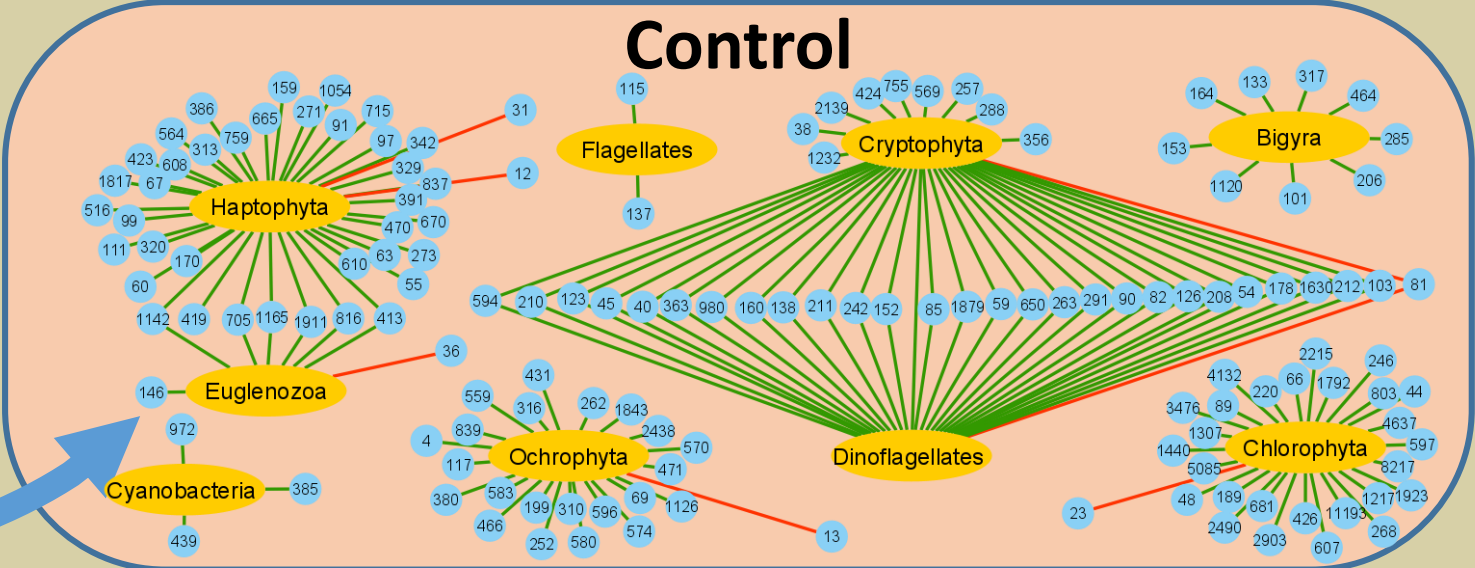
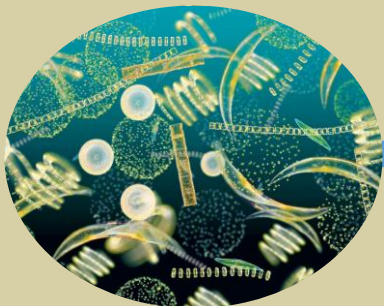
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Highlights

- Phytoplankton-bacterioplankton interactions were studied in lagoon and offshore waters
- The two ecosystems exhibited specific phytoplankton-bacterioplankton networks
- A more complex network was observed in offshore waters
- Positive interactions between phytoplankton and bacterioplankton dominated in offshore waters
- Contamination resulted in a simplification of the phytoplankton-bacterioplankton network



1 **Abstract**

2 Bacteria and phytoplankton are key players in aquatic ecosystem functioning. Their
3 interactions mediate carbon transfer through the trophic web. Chemical contamination
4 can alter the function and diversity of phytoplankton and bacterioplankton, with
5 important consequences for ecosystem functioning. The aim of the present study was
6 to assess the impact of chemical contamination on the interactions between both
7 biological compartments. Two contrasting marine coastal ecosystems, offshore waters
8 and lagoon waters, were exposed to chemical contamination (artificial or produced
9 from resuspension of contaminated sediment) in microcosms in four seasons
10 characterized by distinct phytoplankton communities. Offshore waters were
11 characterized by a complex phytoplankton–bacterioplankton network with a
12 predominance of positive interactions between both compartments, especially with
13 Haptophyta, Cryptophyta, and dinoflagellates. In contrast, for lagoon waters, the
14 phytoplankton–bacterioplankton network was simpler with a prevalence of negative
15 interactions with Ochrophyta, Cryptophyta, and flagellates. Contamination with an
16 artificial mix of pesticides and trace metal elements resulted in a decrease in the
17 number of interactions between phytoplankton and bacterioplankton, especially for
18 offshore waters. Resuspension of contaminated sediment also altered the interactions
19 between both compartments. The release of nutrients stored in the sediment allowed
20 the growth of nutrient limited phytoplankton species with marked consequences for
21 the interactions with bacterioplankton, with a predominance of positive interactions,
22 whereas in lagoon waters, negative interactions were mostly observed. Overall, this
23 study showed that chemical contamination and sediment resuspension resulted in
24 significant effects on phytoplankton–bacterioplankton interactions that can alter the
25 functioning of anthropogenic coastal ecosystems.

26

27 **Keywords**

28 Pesticides, trace metal elements, coastal ecosystems, phytoplankton–bacterioplankton
29 networks

30 1 Introduction

31 In aquatic pelagic systems, phytoplankton and bacteria represent the most important
32 producers of particulate organic material from inorganic and dissolved organic
33 sources (Berglund et al., 2007). The organic matter can be transferred to higher trophic
34 levels through different food webs, ranging from the microbial loop to the classic
35 herbivore pathways depending on resource availability and prey size (Azam et al.,
36 1983; Berglund et al., 2007). As a general rule in marine coastal and estuarine
37 ecosystems, bacteria mediate more carbon than is produced *in situ*, resulting in net
38 heterotrophy of the system (Morán et al., 2013). The ecosystem is thus depending on
39 the availability of other sources of carbon such as recycled autochthonous carbon
40 sources (Fouilland et al., 2014) or allochthonous terrestrial sources (Fouilland et al.,
41 2018a; Morán et al., 2002). Since bacterioplankton and phytoplankton represent the
42 first levels of pelagic marine food webs, any perturbation (natural or anthropogenic)
43 impacting these key players is likely to impact the higher trophic levels and the
44 ecosystem functioning depending on the strength and nature of the biological
45 interactions within the aquatic trophic web (Sakka Hlaili et al., 2014).

46 Phytoplankton–bacterioplankton interactions have received increasing attention in
47 recent decades. These interactions comprise four major modes as clearly described by
48 Grossart and Simon (2007) and Amin et al. (2012): (1) a symbiotic association between
49 phytoplankton bacteria and, in which bacteria benefit from phytoplankton exudates
50 and phytoplankton growth is in turn favored by bacterial products (re-mineralized
51 nutrients, vitamins, and other growth factors); (2) a parasitic association, where algae
52 can inhibit bacterial growth by releasing antibiotic compounds, while bacteria can also
53 act as parasites on phytoplankton leading to cell lysis and death of their hosts, (3) a
54 commensal association with no positive or negative effect between both compartment;
55 and (4) phytoplankton is only loosely associated with bacteria, thus allowing
56 competition for limiting nutrients. The ecological relationships between
57 phytoplankton and bacterioplankton represent the most important inter-organism

58 association in aquatic environments (Seymour et al., 2017). Phytoplankton–
59 bacterioplankton interactions strongly influence carbon and nutrient cycles, regulate
60 the productivity and stability of aquatic food webs (Seymour et al., 2017), and affect
61 ocean–atmosphere fluxes of climatically relevant chemicals such as CO₂ (Cole, 1982)
62 or dimethyl sulfide (DMS) (Yoch, 2002). The shared evolutionary history of these
63 organisms (Cirri and Pohnert, 2019) has played an important role in shaping global
64 biogeochemistry and aquatic ecosystem functioning (Seymour et al., 2017).

65 Anthropogenic perturbations like global warming (Hoppe et al., 2008), acidification
66 (Hornick et al., 2017) or nutrient loading (Prieto et al., 2015) can alter the interactions
67 between phytoplankton and bacterioplankton. For example, a nutrient increase can
68 modify the metabolism of phytoplankton-associated bacteria and consequently the
69 physiology of phytoplankton (Russo et al., 2016). Recent studies have shown that toxic
70 contaminants such as herbicides can indirectly affect bacterial communities through
71 their tight coupling with herbicide-sensitive phytoplankton (Goni-Urriza et al., 2018).
72 Natural or artificial exposure to contaminants can also promote increased tolerance to
73 toxic compounds within phytoplankton and bacterioplankton communities (Blanck,
74 2002). In addition, recent works have demonstrated that bacteria can increase toxic
75 tolerance of their associated phytoplankton species in culture (Fouilland et al., 2018;
76 Levy et al., 2009). Sensitivity of phytoplankton to contaminants is also influenced by
77 the seasons, since seasonal environmental conditions can increase or decrease the
78 success of tolerant species (Berard et al., 1999; Gregorio et al., 2012). Although the
79 phytoplankton–bacteria relationships through metabolite exchanges are well studied,
80 little is known regarding the impact of chemical contaminants on the interactions
81 between phytoplankton and associated bacteria.

82 The aim of the present study was to assess the consequences of chemical contamination
83 on the interactions between bacterioplankton and phytoplankton in two contrasting
84 ecosystems characterized by distinct phytoplankton species according to season. For
85 that purpose, microcosm incubations were performed during different seasons with
86 offshore and lagoon waters exposed to a sediment elutriate or an artificial mixture of

87 contaminants mimicking the main contaminants present in sediment elutriate
88 (pesticides and trace metal elements (TME)). Previous works have shown that
89 chemical contamination and sediment resuspension impacted the coastal marine
90 chemical environment differently (Bancon-Montigny et al., 2019) depending on the
91 water masses (open or semi enclosed) with important consequences for ecosystem
92 functioning (Pringault et al., 2020, 2016). The present study is based on the same
93 sampling and experimental approach extensively described in Bancon-Montigny et al.
94 (2019) and Pringault et al. (2020), dealing respectively with contaminant dynamics in
95 water and plankton, and with biogeochemical coupling between bacteria and
96 phytoplankton. Here we focus on the consequences of contamination for
97 phytoplankton and bacterioplankton diversity, and aim to decipher the
98 phytoplankton-bacterioplankton interactions and the resulting microbial networks.

99 2 Materials and Methods

100 2.1 Study sites and sampling

101 This study was performed in Southwestern Mediterranean ecosystems, the bay and
102 the lagoon of Bizerte (Fig. S1, Supplementary materials), during four seasons, spring
103 (April 2014), winter (February 2015), fall (November 2015), and summer (July 2016).
104 Like most of the Mediterranean coastal lagoons, the Bizerte lagoon (lagoon station, L),
105 to the North of Tunisia, is a polluted coastal ecosystem subject to intense
106 industrialization, urbanization, and agriculture pressures, as well as pressures from
107 commercial and naval shipping harbors (Pringault et al., 2020). Consequently,
108 sediments are strongly contaminated by a wide range of pollutants, including metals
109 and organic compounds (polycyclic aromatic hydrocarbons (PAHs) and pesticides)
110 (Barhoumi et al., 2014a, 2014b; Triki et al., 2017). The Bizerte bay (offshore station, O)
111 is less contaminated than the lagoon (Pringault et al., 2020), although polar pesticides
112 including herbicides have been detected in the lagoon watershed (Mhadhbi et al., 2019)
113 and local PAH contamination has been recorded in the effluent from an oil refinery
114 located on the shore of the bay (Zrafi-Nouira et al., 2009, 2008). Water sampling was

115 carried out from 2 m depth in a lagoon station (station L, 37° 12' 43.96'' N, 9° 50' 79.78''
116 E) and an offshore station (station O, 37° 16' 46.46'' N, 9° 53' 50.98'' E) (Fig. S1,
117 Supplementary materials) following the sampling procedure described in Pringault et
118 al. (2016). Water samples were collected from 2 m depth, corresponding to the
119 chlorophyll *a* maximum along the water column (Sakka Hlaili et al., 2007). Upon
120 sampling, water was filtered through 200 µm mesh to remove larger particles and the
121 metazooplankton thus minimizing grazing effects during incubation.

122 Sediment elutriate was obtained from polluted sediment resuspension following the
123 protocol described by Bonnet et al. (2000) and recommended by the Environmental
124 Protection Agency (US EPA). Briefly, polluted sediment was sampled using a Van
125 Veen grab at 4 m depth in front of a cement factory in the lagoon channel (station CH,
126 37° 15' 40.22'' N, 9° 51' 30.49'' E Fig. S1), one of the most polluted sites in the Bizerte
127 lagoon (Ben Said et al., 2010; Pringault et al., 2020; Triki et al., 2017). Overlying channel
128 water was also sampled at the same time following the sampling procedure used for
129 the offshore and lagoon waters. Just after sampling, the sediment was sieved (2 mm
130 mesh) to remove large particles and stored in a cool box until its return to the
131 laboratory. In the laboratory, sediment was mixed with channel water (1:4 w/v ratio),
132 and sediment was gently stirred for 8 h. After a 12 h settling period, the overlying
133 solution, thereafter called "elutriate", was smoothly siphoned out and stored in the
134 dark at 4 °C until spiking in microcosms few days later (Pringault et al., 2016). The
135 same protocol for sediment elutriate preparation was applied for the four studied
136 seasons. This protocol has been successfully used to characterize the contamination
137 level and toxicity of sediments using non-adapted species (Bonnet et al., 2000; Carr
138 and Chapman, 1995).

139 2.2 Incubation procedure

140 During the four studied seasons, seawater was incubated for four days in 9 L glass
141 microcosms (22.5 cm diameter and 23 cm height). The microcosms were covered with
142 a quartz lid to allow full penetration of natural sunlight, including UV radiation,

143 according to the protocol described in Pringault et al (2020, 2016) and briefly detailed
144 below. A series of triplicate microcosms was filled with 1.5 L of elutriate and
145 completed with 4.5 L of sample water (L and O stations) to achieve a final dilution of
146 25% (contaminated water microcosms (CW). Another series of triplicate microcosms
147 (artificial contaminated water microcosms (ACW)) was filled with 6 L (O and L
148 stations) and spiked with an artificial solution of TME (Ni, Cu, Zn, Cd, As, Pb), and
149 pesticides and some metabolites (acetochlore, alachlore, DCPU 1-(3,4-
150 dichlorophenyl)urea, DIA deisopropylatrazine, diuron, linuron, and simazine). The
151 final concentrations of metals and pesticides were adjusted to mimic the
152 concentrations observed in elutriate (Table S1, Supplementary materials) (see Bancon-
153 Montigny et al., 2019 for more details). This treatment was used to assess the impact
154 of the main chemical compounds observed in the elutriate without the possible
155 confounding effects of enhanced nutrients, DOC or benthic microorganisms that are
156 released during sediment resuspension and present in the elutriate in the CW
157 treatment. Lastly, a third series of triplicate microcosms (Control, C) was filled with 6
158 L of sample water (O and L stations). All microcosms were incubated during 96h
159 outdoors under natural sunlight in a 3 m³ pool with circulating seawater (open system)
160 to maintain *in situ* water temperature. Two light sensors (Skye, England), measuring
161 incident ultraviolet A and B radiation (315–380 nm, SKU 420 and 280–315 nm, SKU
162 430, respectively) and photosynthetically active radiation (PAR; 400–700 nm, quantum
163 SKP 215) were installed to monitor the light received at the surface of the microcosms.

164 2.3 Chemical analyses

165 Chemical analyses of dissolved organic carbon (DOC), Chlorophyll *a* (Chl*a*); the
166 nutrients (SiOH₄, NH₄, NO₃, NO₂), and TME and organic contaminants (pesticides and
167 PAHs) were performed using the analytical protocols described in Bancon-Montigny
168 et al. (2019) and Pringault et al. (2016).

169 2.4 Phytoplankton diversity

170 Water samples (245 mL) immediately fixed with buffered formaldehyde at 2% final
171 concentration were collected at the two stations O and L, and at the end of the 96h
172 incubations in each microcosm. Species identification and counts were performed with
173 a BX 60 Olympus microscope equipped with a Progress C3-cooled Janoptik camera
174 following the procedure of Pringault et al (2020). Each species was determined to the
175 lowest taxonomic level according to Tomas et al. (1997), Hoppenrath et al. (2009),
176 Kraberg et al. (2010), Viličić (2014), and the WoRMS database
177 (<http://www.marinespecies.org>). Counts were performed according to the Utermöhl's
178 concentration technique (Utermöhl, 1958) under an inverted microscope (Olympus
179 IX70). Each taxon was counted individually, except for Cyanophyceae spp, *Chaetoceros*
180 spp, *Pseudo-nitzschia* spp, *Prymnesiophyceae* spp and *Euglenoides* spp which were
181 grouped into global taxa due to the difficulties of species identification. Two groups of
182 flagellates were distinguished by size class (< 10 µm and >10-15 µm).

183 2.5 Bacterioplankton diversity

184 Bacterioplankton was sampled at the two stations O and L, and at the end of the 96h
185 incubations in each microcosm. Samples were processed as previously described
186 (Goni-Urriza et al., 2018). Briefly, after water filtration (1000 ml) using sterile filters (0.2
187 µm porosity), filters were immediately frozen in liquid nitrogen and stored at -80 until
188 DNA and RNA co-extraction. Nucleic acid extraction was performed coupling the Fast
189 RNA ProSoil direct kit (MpBIO) with the AllprepDNA/RNA mini kit (QIAGEN). DNA
190 contamination in RNA samples was eliminated with the RNase-Free DNase Set
191 (QIAGEN). The RNA reverse transcription was performed using the M-MLV RT
192 enzyme (Invitrogen) with 20-50 ng of RNA, RNase OUT (Invitrogen) and 50ng of
193 random primers, according to the manufacturer's instructions.

194 The composition of the bacterial community was assessed targeting the V3-V4 region
195 of the 16S rRNA gene as described elsewhere (Goni-Urriza et al., 2018). Briefly, PCR
196 amplifications of extracted DNA and synthesized cDNA were performed with the

197 AmpliTaq Gold 360 Master mix (Applied Biosystems) and 343F-784R primers' set
198 following the procedure described in Goni-Urriza et al. (2018). PCR amplicons were
199 sequenced using the MiSeq 250-paired technology (Illumina). Raw sequences are
200 available on the NCBI SRA database (National Center for Biotechnology Information
201 Sequence Read Archive) under the accession number PRJNA391504.

202 QIIME pipeline was used to analyze raw sequences. Briefly, sequences with a quality
203 score <20 were discarded before to remove chimeras (UCHIME, denovo).
204 USEARCH61 was used to generate OTUs and UCLUST with Silva database (v. 119) to
205 determine the affiliation of the OTUs' representative sequences. Prior to data
206 normalization (rarefyseq.py), reads affiliated with chloroplasts and singletons were
207 removed.

208 2.6 Statistical analysis

209 Phytoplankton and bacterioplankton species richness (S) was determined by the
210 number of taxa (phytoplankton) and OTUs (bacterioplankton). The Shannon diversity
211 (H) index was estimated using the following equation (Hill et al., 2003):

$$212 \quad H = \sum_{i=1}^S P_i \log P_i$$

213 where P_i represents the relative abundance of species or OTU i in each sample and S
214 the species richness. Analysis of variance (ANOVA) was performed to test the
215 significance of the differences observed between treatments, stations and seasons.
216 Prior to ANOVA, homogeneity of variance (Levene's test) and normality (Shapiro–
217 Wilk test) were determined. When these conditions were not met, differences between
218 treatments were tested using the non-parametric Kruskal–Wallis ANOVA test. A
219 posteriori paired multiple-comparisons were then performed using Tukey's HSD test.
220 All statistical tests were carried out with the level of significance set at $p < 0.05$. For
221 multivariate analysis of the phytoplankton and bacterioplankton diversity, the
222 triplicate data were averaged and the relative abundance was transformed with arcsin
223 ($x^{0.5}$) to obtain a normal data distribution (Legendre and Legendre, 1998) according

224 to the procedure described in Pringault et al (2016). Three canonical correspondence
225 analyses (CCA) were performed, following the recommendations Ramette (2007)
226 using the MVSP v3.12d software (Kovach Computing Service, Anglesey Wales). The
227 first two CCAs used either the 100 most abundant OTUs or the phytoplankton groups
228 as response variables and concentrations of chemicals, TMEs, nutrients and DOC as
229 explanatory variables. The explanatory variables with variance inflation factors (VIF)
230 > 20 were removed to eliminate collinearity of environmental variables. For
231 bacterioplankton diversity, only OTUs with at least ten reads were considered in order
232 to reduce the effects of rare OTUs (Goni-Urriza et al., 2018). Since similar
233 environmental variables were identified as structuring factors for bacterioplankton
234 and phytoplankton communities, a third CCA was performed with the 100 most
235 abundant bacterioplankton OTUs as response variables and the phytoplankton groups
236 as explanatory variables, in order to determine whether phytoplankton were
237 structuring the bacterioplankton communities. Spearman correlation was calculated
238 with XLSTAT software (Addinsoft, Bordeaux France) between the phytoplankton
239 groups and the 100 most abundant OTUs. Significant ($p < 0.05$) Spearman correlation
240 values $> |0.8|$ were then used to design phytoplankton–bacterioplankton networks
241 with the help of the CYTOSCAPE software. Phytoplankton species and bacterial OTU
242 are represented as nodes, and node-node interactions (edges) represented as links
243 (Shannon et al., 2003).

244 3 Results

245 3.1 *In situ* bacterioplankton and phytoplankton diversity

246 The two stations were characterized by distinct environmental conditions with clear
247 seasonal effects for T °C and Chl*a*. Chl*a* concentrations did not exceed 1 $\mu\text{g L}^{-1}$ in
248 offshore waters, with a minimum observed in winter and a maximum during spring.
249 On the contrary, maximum Chl*a* was observed during fall in lagoon waters with a
250 value close to 5 $\mu\text{g L}^{-1}$. Dissolved organic carbon was significantly ($p < 0.05$) higher in
251 lagoon waters, with concentrations up to 2.5 mg C L^{-1} in winter, whereas in offshore

252 waters, DOC was around 1 mg C L⁻¹. Similarly, nutrient concentrations were higher in
253 lagoon waters (Table S1, Supplementary materials). Pesticides were most abundant in
254 Lagoon waters especially DIA or Simazine, as well as some TME, Ni, Zn or Mn (Table
255 S1, Supplementary materials).

256 These distinct environmental conditions were concomitant to specific phytoplankton
257 communities in offshore and lagoon waters with clear seasonal effects for both
258 ecosystems (Fig. 1 upper panel). In spring and fall, dinoflagellates, with high
259 abundances of *Gonyaulax* sp., *Heterocapsa* sp., and *Prorocentrum* sp. were the dominant
260 phytoplankton group in lagoon waters representing up to 55% relative abundance
261 (spring), while in offshore waters flagellates and dinoflagellates (mostly *Scrippsiella*
262 sp.) accounted together for more than 70% of the relative abundance in both seasons.

263 Summer was characterized by a strong dominance of Ochrophyta (93%) in the lagoon
264 with a dense population of *Chaetoceros* sp. (68% of relative abundance), and to lesser
265 extent in offshore waters (56%). During winter, Haptophyta (*Coccolithophorids* sp.)
266 were the dominant (42%) phytoplankton taxa in offshore waters, while in the lagoon,
267 flagellates (38%) and Ochrophyta (27%) accounted for up to 65% of the relative
268 abundance. Diversity indices (Shannon index and species richness) exhibited distinct
269 seasonal patterns with significant ($F_{3,20} = 58.25$, $p < 0.05$, $F_{3,20} = 3.42$ $p < 0.05$, for Shannon
270 index and species richness, respectively) differences between lagoon and offshore
271 waters (Fig. S2, Supplementary materials). Species richness (S) and Shannon index (H)
272 were significantly higher ($F_{1,22} = 26.82$ $p < 0.05$, $F_{1,22} = 8.07$ $p < 0.05$, for Shannon index and
273 species richness, respectively) in offshore waters with maximum values observed for
274 fall ($H = 2.91 \pm 0.07$, $S = 27.7 \pm 5.5$), whereas the lowest values for the Shannon diversity
275 index were observed during summer in both ecosystems.

276 A total of 203 562 distinct OTUs were identified from the 16S rDNA library in the
277 whole dataset (*in situ* and microcosm incubation). As observed for phytoplankton, *in*
278 *situ* bacterioplankton diversity also showed variations according to season and
279 ecosystem (Fig. 1 lower panel). Gammaproteobacteria (dominated by *Alteromonas* sp.,
280 *Marinobacterium* sp., *Litoricola* sp., and SAR86 clade) represented the most dominant

281 groups irrespective of the season for both offshore and lagoon waters, with up to 42%
282 of the relative abundance in offshore waters during winter. Flavobacteria (21–34%)
283 and Alphaproteobacteria (12–26%) were the other dominant groups with a relative
284 abundance greater than 10%. Regarding the less dominant groups, Cytophagia (1.6–
285 4.9%) and Betaproteobacteria (2.2–3.8%) abundances were greater in lagoon waters,
286 whereas Deltaproteobacteria (1.4–5.0%) and *Deferribacter* sp. (0.6–3.7%) abundances
287 were higher in offshore waters. Diversity indices (Shannon index) exhibited distinct
288 significant ($F_{3,20} = 6.51$, $p < 0.05$) seasonal patterns (Fig. S2) in lagoon and offshore
289 waters, with minimum values during spring (4.30 ± 0.28 and 4.03 ± 0.05 for lagoon and
290 offshore waters, respectively) and winter (4.20 ± 0.06 and 4.47 ± 0.04 for lagoon and
291 offshore waters, respectively). Maximum values were observed during summer (6.75
292 ± 0.08 and 6.74 ± 0.12 for lagoon and offshore waters, respectively) and fall (6.74 ± 0.16
293 and 6.94 ± 0.12 for lagoon and offshore waters, respectively). No significant ($p > 0.05$)
294 differences were observed for the Shannon index and species richness between lagoon
295 and offshore waters.

296 3.2 Impact of contamination on bacterioplankton and phytoplankton communities

297 The impact of chemical contamination on the phytoplankton community was
298 ecosystem and season dependent. As a general rule, the most pronounced effects on
299 the relative abundance of the phytoplankton groups (compared to the control
300 treatment) were observed with elutriate exposure especially during fall, whereas
301 artificial contamination by a pesticide and TME mixture provoked minor changes in
302 phytoplankton community structure (Fig. 2, upper panel). The changes in relative
303 abundances were generally more marked in offshore waters relative to lagoon waters,
304 especially in fall where flagellates (60% relative abundance) became the dominant
305 group in the CW microcosm while they represented less than 10% of *in situ* relative
306 abundance. When all seasons were taken into account, exposure to elutriate provoked
307 a slight but significant ($F_{2,33} = 13.41$, $p < 0.05$) increase in species richness in lagoon
308 waters, whereas no significant effect was observed for the Shannon diversity index. In
309 contrast, in offshore waters, both treatments (ACW and CW) resulted in a significant

310 ($F_{2,33} = 16.58$, $p < 0.05$) reduction of the Shannon index (Fig. S3) and the specific richness
311 ($F_{2,33} = 7.83$, $p < 0.05$) (Fig. S4). CCA performed with the phytoplankton groups as
312 response variables, and the contaminants and nutrients as explanatory variables (Fig.
313 S5), showed that the impact of contaminants on phytoplankton structure was less
314 marked than that imposed by spatial and temporal effects. Seasons were discriminated
315 along axis 1, whereas stations were separated along axis 2. Nitrate, ammonium,
316 silicate, DOC, Ni, and Zn were identified as positive structuring factors for the
317 phytoplankton community in the lagoon ecosystem, especially during winter and
318 spring. In contrast, offshore phytoplankton communities were negatively impacted by
319 Zn, Ni, pesticides, and Cu, especially during spring and summer (Fig. S5). Among the
320 different phytoplankton groups, dinoflagellates and Haptophyta exhibited the lowest
321 abundance in ACW and CW treatments relative to the control (Fig. S6), whereas
322 Ochrophyta and Cryptophyta abundances were similar in ACW relative to the control
323 value, with an even higher abundance in CW for Cryptophyta (Fig. S6).

324 For the bacterioplankton community, the impacts of contamination followed the same
325 patterns as those observed for phytoplankton, with a more marked effect for the
326 sediment elutriate relative to the artificial mixture contamination (Fig. 2, lower panel).
327 The relative abundance of Gammaproteobacteria significantly increased ($F_{2,33} = 21.40$,
328 $p < 0.05$, $F_{2,33} = 56.63$, $p < 0.05$, for offshore waters and lagoon waters, respectively) in
329 the CW treatment especially in fall and winter, irrespective of the ecosystem, while
330 relative abundance of Alphaproteobacteria ($F_{2,33} = 14.07$, $p < 0.05$ for offshore waters)
331 and Flavobacteria ($F_{2,33} = 23.24$, $p < 0.05$, $F_{2,33} = 78.28$, $p < 0.05$, for offshore waters and
332 lagoon waters, respectively) significantly decreased. Nevertheless, no significant
333 changes were observed for the diversity indices (Figures S3 and S4, Supplementary
334 materials). As observed for the phytoplankton community, the consequences of
335 contamination were more evident in the offshore station although changes were
336 partially masked by the seasonal and spatial effects as indicated by the canonical
337 correspondence performed with the 100 most abundant OTUs as response variables,
338 and the contaminants and nutrients as explanatory variables (Fig. S5, Supplementary

339 materials). Interestingly, the structuring environmental chemical factors identified for
340 the bacterioplankton community were roughly similar to those observed for the
341 phytoplankton community. Lagoon communities were structured by nitrate, DOC, Ni,
342 Zn, Cd, and Cu, especially during spring and winter, whereas they negatively
343 impacted the offshore community in summer and fall.

344 3.3 Impact of contamination on bacterioplankton-phytoplankton interactions

345 Since similar environmental variables were identified as structuring factors for
346 bacterioplankton and phytoplankton communities, CCA was performed with the 100
347 most abundant OTUs as response variables and the phytoplankton groups as
348 explanatory variables (Fig. 3). The two first axes explained almost 30% of the variance
349 observed. Seasons were separated along axis 1 and ecosystem along axis 2. The impact
350 of phytoplankton groups on the bacterioplankton community was clearly season and
351 ecosystem dependent. Winter bacterial communities were mostly explained by
352 flagellates especially for offshore waters and, by Haptophyta and Euglenozoa for
353 lagoon waters. In both ecosystems, summer communities were structured by
354 Ochrophyta, whereas dinoflagellates were identified as a structuring factor during fall.
355 In order to estimate the possible interactions between bacterioplankton and
356 phytoplankton, Spearman correlation coefficients were calculated between the relative
357 abundance of the 100 most abundant bacterioplankton OTUs and the phytoplankton
358 groups observed at the end of the microcosm treatments for both ecosystems (Fig. 4).
359 Spearman correlation coefficients were also calculated within the 100 most abundant
360 bacterial OTUs, in order to assess interactions among the bacterioplankton (Fig. S7,
361 Supplementary materials). Highly significant Spearman correlations (> 0.8) were then
362 used to draw bacterioplankton–phytoplankton networks as a function of the
363 microcosm treatment for lagoon (Fig. 5) and offshore waters (Fig. 6). The nature
364 (positive or negative) and the number of significant interactions ($p < 0.05$) were
365 strongly dependent on the ecosystem and the microcosm treatments (Fig. 4). In the
366 control treatment, positive interactions dominated, especially in offshore waters (451
367 positive vs. 113 negative) and to a lesser extent in lagoon waters (384 positive vs. 227

368 negative). Differences were also observed depending on the phytoplankton groups:
369 Cyanobacteria, Bigyra, and Haptophyta showed mostly positive interactions with
370 bacterioplankton, whereas a predominance of negative interactions was observed for
371 flagellates and Ochrophyta, especially in lagoon waters. Contamination effects on the
372 bacterioplankton–phytoplankton networks depended on the impacted ecosystem. In
373 offshore waters, contamination resulted in an increase of negative interactions
374 concomitant with a reduction of positive interactions., irrespective of the microcosm
375 treatment (Fig. 6 and Fig. 4 lower panel). Interestingly, negative interactions became
376 dominant for dinoflagellates, Chlorophyta, and Haptophyta in the CW treatment,
377 whereas these groups exhibited a predominance of positive interactions with bacteria
378 in control conditions (Fig. 6). In contrast, for lagoon waters, no marked changes for
379 positive and negative interactions were observed between control and CW treatments
380 (Fig. 4), whereas exposure to artificial contamination resulted in a similar decrease for
381 both positive and negative interactions (Fig. 5 and Fig. 4 upper panel). Among the
382 Gammaproteobacteria, the dominant bacterial genus, *Marinobacterium* sp. exhibited
383 positive interactions with several phytoplankton taxa, irrespective of the treatments,
384 whereas *Litoricola* sp. appeared less associated with phytoplankton (Table S2,
385 Supplementary materials). Interactions among the most abundant OTUs were largely
386 dominated by positive interactions (Fig. S7, Supplementary materials) irrespective of
387 the treatments and the sampling site. Interestingly, for both ecosystems, chemical
388 contamination and sediment elutriate resulted in an increase of interactions between
389 OTUs; the large proportion of positive interactions (79–85%) observed in control
390 microcosms remained similar in ACW and CW treatments.

391 4 Discussion

392 The aim of the present study was to assess the consequences of chemical contamination
393 on bacterioplankton-phytoplankton interactions in two contrasting ecosystems, a semi
394 enclosed lagoon ecosystem versus an open coastal ecosystem. The four seasons were
395 studied to address the eventual modifications of the microbial community

396 composition driven by seasons. It is noteworthy, however, that the four seasons were
397 studied from April 2014 to July 2016, excluding so the possible seasonal succession and
398 the "ecological memory" of the microbial community between two consecutive seasons
399 (Padisák et al., 2010). The two Mediterranean ecosystems exhibited contrasting
400 environmental conditions during the four seasons studied with marked differences in
401 chemical contamination (Table S1, Supplementary materials); the lagoon waters were
402 significantly more contaminated than the offshore waters (Bancon-Montigny et al.,
403 2019; Pringault et al., 2020). According to the concept of pollution-induced community
404 tolerance that can be triggered by the chemical environment and the possible selection
405 of tolerant species (Blanck, 2002), these two contrasting environments allowed testing
406 the hypothesis that the microbial response to chemical contamination would be
407 different in the two sampling sites, with possible consequences for the interactions
408 between the two key players of the marine trophic web.

409 4.1 *In situ* phytoplankton and bacterioplankton diversity

410 The number of *in situ* phytoplankton species (129) identified by microscopy was in the
411 same order of magnitude as that observed in similar Mediterranean ecosystems
412 (Draredja et al., 2019; Percopo et al., 2011) or in other coastal regions (Srichandan et al.,
413 2019) using the same counting technique. Similarly, the number of OTUs (19 169 for
414 the *in situ* database) estimated by the 16S rRNA gene considered as a proxy of the
415 bacterioplankton richness, was comparable to bacterial specific richness estimates
416 determined with similar molecular tools in other coastal ecosystems (Goni-Urriza et
417 al., 2018; Mohapatra et al., 2020). As generally observed in Mediterranean coastal
418 ecosystems (Pulina et al., 2018; Quero et al., 2017), phytoplankton and
419 bacterioplankton diversity exhibited clear seasonal variations in lagoon and offshore
420 waters. Surprisingly, higher phytoplankton diversity indices were observed during
421 spring and fall, seasons where phytoplankton blooms are likely to occur in
422 Mediterranean coastal ecosystems (Ribera d'Alcalà et al., 2004); algal blooms often
423 being concomitant with low phytoplankton diversity (Lehtinen et al., 2017; Livingston,
424 2007). Phytoplankton diversity was significantly higher ($p < 0.05$) in offshore waters

425 relative to lagoon waters, contrasting that observed in other lagoons where
426 phytoplankton diversity in the enclosed system can be higher (Srichandan et al., 2019)
427 or equivalent (Minicante et al., 2019) to their adjacent waters. These contrasting results
428 show the particular trophic status played by semi enclosed ecosystems relative to their
429 counterpart adjacent marine waters (Newton et al., 2014), differences that might be
430 explained by the anthropogenic pressure exerted on enclosed systems that can result
431 in a reduction of diversity. Both ecosystems shared common bacterioplankton species
432 (1 739 OTUs) and phytoplankton species (51 species) such as OTUs 17 or 19 affiliated
433 to the SAR 11 clade, the dinoflagellate *Heterocapsa minima*, or the diatom *Cylindrotheca*
434 *closterium*, suggesting connectivity between lagoon and offshore waters (Minicante et
435 al., 2019). Nevertheless, the environmental conditions were sufficiently different to
436 allow the occurrence of distinct phytoplankton communities (48 and 30 species
437 uniquely observed in offshore and lagoon waters, respectively) and bacterioplankton
438 communities (9 033 and 8 397 OTUs specifically observed in offshore and lagoon
439 waters, respectively). In fact, despite a large open channel to the coastal sea, the
440 watershed influence and the very long water residence time (> 200 days) observed in
441 the Bizerte lagoon (Béjaoui et al., 2017) relative to the offshore adjacent waters (< 20
442 days) (Sayol et al., 2013) allows accumulation of nutrients, DOC, and chemical
443 contaminants within the lagoon (Bancon-Montigny et al., 2019; Pringault et al., 2020).
444 Moreover, salinity and temperature variations showed distinct patterns with higher
445 salinity and temperature during spring and winter in offshore waters compared to the
446 lagoon ecosystem, whereas the opposite trends was observed during summer where
447 temperature and salinity values were maximal in the semi enclosed system (Béjaoui et
448 al., 2016; Pringault et al., 2020). The dissimilarities observed for bacterioplankton and
449 phytoplankton communities between lagoon and offshore waters might result in
450 distinct responses to anthropogenic pressure such as chemical contamination.

451 4.2 Contamination impact on bacterioplankton and phytoplankton communities

452 The impact of chemical contamination on bacterioplankton and phytoplankton
453 communities was more marked in offshore waters than in lagoon waters with a clear

454 seasonal effect. A reduction of phytoplankton diversity concomitant to changes of
455 phytoplankton structure was observed during spring and fall, especially upon
456 sediment elutriate exposure (CW treatment). As a general rule, the structural changes
457 were less pronounced when the communities were exposed to the artificial mixture of
458 pesticides and TME (ACW treatment) than when exposed to sediment elutriate. The
459 artificial contamination was designed to mimic the concentrations of pesticides and
460 TME observed in sediment elutriate (Bancon-Montigny et al., 2019), but did not
461 include increased levels of other elements such as nutrients, DOC or benthic
462 microorganisms that were present in the sediment elutriate. This artificial cocktail
463 resulted in a significant reduction of metabolic activities involved in the carbon cycle
464 (Pringault et al., 2020, 2016), especially in offshore waters. Nevertheless, the toxic
465 effects observed on microbial metabolic activities were alleviated when the toxic
466 chemical compounds were accompanied by high concentrations of nutrients and DOC,
467 chemical conditions observed in sediment elutriate (Bancon-Montigny et al., 2019).
468 This exogenous supply of nutrients may promote the growth of nutrient limited
469 phytoplankton species, especially during summer and winter, the post bloom periods,
470 resulting in an increase of diversity and structural changes of the community.
471 Similarly, the exogenous DOC supply by sediment elutriate may promote the growth
472 of heterotrophic bacteria (Uchimiya et al., 2016), loosening their dependency on
473 phytoplankton exudates to sustain their carbon requirements.

474 Enclosed systems under anthropogenic pressure, such as lagoons, promote favorable
475 conditions for selective pressure of resistant species to chemical contamination
476 according to the pollution-induced community tolerance concept proposed by Blanck
477 et al. (1988) and illustrated with benthic phototrophic biofilms (Belando et al., 2017) or
478 meiofauna (Boufahja and Semprucci, 2015). Dense populations of the diatom
479 *Chaetoceros* sp. were observed in the lagoon ecosystem especially during summer,
480 representing up to 68% of the total relative abundance. The *Chaetoceros* genus is
481 considered one of the most abundant diatoms in marine phytoplankton (De Luca et
482 al., 2019) and this phytoplankton species has been proposed as a biological indicator

483 for detecting environmental stress, considering its ability to thrive in contaminated
484 marine environments (Verlecar et al., 2006). In this vein, the Gammaproteobacteria
485 *Marinobacterium* sp. or *Alteromonas* sp. were often observed *in situ* in the lagoon waters
486 with a relative abundance often close to 1%, and their abundance could reach up 10%
487 (*Alteromonas* sp.) in contaminated microcosms, either by sediment elutriate (CW) or by
488 the artificial mixture of pesticides and TME (ACW). The great abundance of
489 *Marinobacterium* sp. and *Alteromonas* sp. under chemical contamination is in agreement
490 with previous studies reporting their dominance in bacterial communities exposed to
491 various stressors, including chemical contamination by oil or hydrocarbons (Catania
492 et al., 2015; Dos Santos et al., 2011; Yakimov et al., 2005) or eutrophication (Wang et al.,
493 2020). In addition, the great abundance of *Alteromonas* sp. in the ACW treatment
494 confirms that this species could be used as a bioindicator of metal polluted
495 environments (Selvin et al., 2009). The difference in phytoplankton or bacterioplankton
496 communities observed in offshore and lagoon waters with the possible selection of
497 resistant species in the enclosed ecosystem might affect the interactions between
498 bacterioplankton and phytoplankton, considering that the microalgae–bacteria
499 association is species dependent (Seymour et al., 2017).

500 4.3 Contamination and bacterioplankton-phytoplankton interactions

501 Phytoplankton–bacterioplankton interactions and the resulting networks were
502 assessed with the Spearman correlation calculated with abundance data, thus allowing
503 detection of positive (mutualism or symbiose) or negative (resource competition,
504 parasitism, or predation) interactions among taxa (Goni-Urriza et al., 2018), bearing in
505 mind the limitations of using co-occurrence as a proxy for ecological interactions
506 (Blanchet et al., 2020; Freilich et al., 2018). This similarity based technique is often
507 employed to construct microbial networks in aquatic ecosystems (Mikhailov et al.,
508 2019; Zhu et al., 2018) although more complex modeling approaches allow
509 distinguishing unidirectional ecological interactions (such as amensalism) from
510 bidirectional ecological interactions (such as competition and mutualism) or

511 commensalistic association with no negative or positive effects between the considered
512 species (Faust and Raes, 2012).

513 The lagoon ecosystem and its adjacent open waters were characterized by distinct
514 phytoplankton–bacterioplankton interactions. In offshore waters, positive interactions
515 largely dominated over negative interactions (451 positive vs. 113 negative) resulting
516 in a complex bacterio–phytoplankton network. For the lagoon ecosystem, the
517 proportion of negative interactions was higher (227 negative vs. 384 positive) drawing
518 a simpler phytoplankton–bacterioplankton network, involving less bacterial species
519 relative to the offshore situation. A similar tendency was observed in the Venice
520 lagoon, with less interactions within the microbial communities in benthic and pelagic
521 lagoon biotopes relative to the adjacent open waters (Minicante et al., 2019; Quero et
522 al., 2017), suggesting that environmental heterogeneity in these adjacent and
523 connected ecosystems can be strong enough to allow for ecological segregation
524 (Minicante et al., 2019).

525 In both ecosystems, negative interactions were mainly observed in contaminated
526 treatments (CW and ACW) with dinoflagellates whereas positive interactions
527 dominated in control treatments. Negative correlations may indicate competition,
528 inhibition, or predation among taxa. Dinoflagellates are known to produce toxin
529 compounds resulting in allelopathic interactions with bacteria through cell lysis,
530 blistering, or growth inhibition (Legrand et al., 2003; Paul et al., 2007). Nevertheless,
531 dinoflagellates can also be specifically associated through positive interactions with
532 particular bacterial species, such as *Roseobacter* sp. or *Marinobacter* sp. (Jasti et al., 2005).

533 In contrast, bacterial interactions with Cryptophyta were dominated by positive
534 correlations, especially in offshore waters in control conditions, as also observed in
535 freshwater ecosystems where this phytoplankton group can form dense blooms
536 (Mikhailov et al., 2019) knowing that this mixotrophic species can also be considered
537 as bacterivores (Grujic et al., 2018). Interestingly, this mixotrophic regime might be
538 controlled by the presence of contaminants, since the negative interactions between
539 Cryptophyta and prokaryotes in ACW and CW treatments suggest an increase of

540 predation, as previously demonstrated by Losey & Denno (1999). Chemical
541 contamination as well as sediment elutriate provoked significant changes in bacteria–
542 phytoplankton interactions resulting in a simplification of the microbial pelagic
543 network. A similar simplification effect of oil contamination was also observed in soils
544 with a reduction of interactions and connectivity among bacteria (Liang et al., 2016).
545 Anthropogenic stressors are known to impact the functioning of the aquatic primary
546 producers and consequently, primary production is more sensitive to chemical
547 contamination than respiration (Johnston et al., 2015). Reduction of phytoplankton
548 biomass upon chemical contamination has been observed with a wide range of
549 contaminants including metal (Rochelle-Newall et al., 2008), pesticides (Pelletier et al.,
550 2006), plastic derivatives (M'Rabet et al., 2019), PAHs (Ben Othman et al., 2018; Hjorth
551 et al., 2008, 2007), or with a contaminant mixture (Pringault et al., 2020). These
552 inhibitory effects of chemical contaminants on the primary producers alter the
553 synchrony between the phytoplankton and bacterioplankton (Goni-Urriza et al., 2018).
554 Indeed, a reduction of phytoplankton biomass upon chemical stress, as observed in
555 the ACW treatment, will also reduce the interactions with bacterioplankton,
556 considering the interdependency observed between both compartments, especially in
557 open waters where phytoplankton and bacterioplankton can be strongly coupled
558 (Morán et al., 2002; Pringault et al., 2020).

559 In contrast to the artificial chemical contamination with TME and pesticides, exposure
560 to sediment elutriate provoked a strong stimulation of phytoplankton biomass
561 (Pringault et al., 2020) concomitant with a shift in the phytoplankton community
562 structure, especially in offshore waters. The important release of dissolved organic
563 matter (DOM) and nutrients from sediment resuspension (Bancon-Montigny et al.,
564 2019) alleviated the toxic effects of chemical contaminants present in the sediment
565 elutriate. Interestingly, the stimulation of phytoplankton biomass (Pringault et al.,
566 2020) was concomitant with a reduction of phytoplankton diversity, leading to a
567 simplification of the microbial network in lagoon and offshore waters. Phytoplankton
568 blooms are known to strongly impact the interactions with bacterioplankton (Teeling

569 et al., 2012), especially in the case of harmful algal bloom (Paerl and Otten, 2013). A
570 reduction of phytoplankton diversity during blooms has been already observed
571 (Livingston, 2007), with no negligible impacts for the diversity of the associated
572 bacterial community (Wemheuer et al., 2014), and for the interactions among
573 phytoplankton species depending on which taxa dominates the bloom (Escalas et al.,
574 2019). With phytoplankton and bacteria associations being species dependent
575 (Grossart et al., 2005; Seymour et al., 2017), a shift (natural or anthropogenic triggered)
576 in the phytoplankton community structure can impact the bacterial association, as
577 differences in the phytoplankton community structure can lead to changes in quality
578 and quantity of exuded organic matter that can be mineralized by bacteria for their
579 growth (Kent et al., 2007, 2004; Liu et al., 2014). In addition, sediment resuspension
580 provoked strong DOM release but also a significant increase in bacterial biomass, both
581 conditions resulting in stimulation of the heterotrophic metabolism (Pringault et al.,
582 2020). The simplified networks observed in CW treatments relative to the control
583 clearly showed that this external supply of exogenous bacteria and dissolved organic
584 matter decreases the interdependency between phytoplankton and bacterioplankton.
585 Bacteria (indigenous and exogenous) have access to an external carbon source, so
586 loosening their dependency on phytoplankton exudates to sustain their carbon
587 demand, while interactions among bacteria increased. Increasing bacterial cooperation
588 and association is important to allow biodegradation of recalcitrant biopolymers
589 (Ebrahimi et al., 2019) such as those found in sediment-derived DOM, a complex
590 mixture of organic compounds with different lability properties (Wu et al., 2018). Such
591 a “priming effect” mechanism was widely reported in soils but rarely in marine waters
592 (Bianchi, 2011; Blanchet et al., 2017).

593 5 Conclusion

594 The present study clearly showed that a semi-enclosed ecosystem and its adjacent
595 open waters exhibited distinct phytoplankton and bacterioplankton communities that
596 differ depending on season. These differences result in specific networks, suggesting

597 that environmental heterogeneity can be strong enough to allow for ecological
598 segregation despite sea-lagoon connectivity. Offshore waters were characterized by a
599 complex phytoplankton–bacterioplankton network dominated by positive
600 interactions between primary producers and bacteria, while the semi-enclosed
601 ecosystem harbored a simplified network where negative interactions were more
602 prevalent. Chemical contamination resulted in a decrease of phytoplankton biomass
603 especially during the bloom periods (spring and autumn) in offshore waters, leading
604 to a simplification of the phytoplankton–bacterioplankton network dominated by
605 negative interactions. Nutrients and DOM released by sediment resuspension
606 alleviated the toxic effects of chemical compounds (mainly pesticides and TME)
607 present in the elutriate, provoking stimulation of phytoplankton and bacterial biomass
608 concomitant with a significant shift in the phytoplankton community structure and
609 reduction of phytoplankton diversity. These structural changes favored the loosening
610 of the bacteria–phytoplankton coupling (Pringault et al., 2020) with strong
611 consequences for the trophic web and ecosystem functioning.

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Figure legends

Figure 1: Relative abundances of groups of phytoplankton and bacterioplankton observed *in situ* in the two sampling stations during the four seasons of the study.

Figure 2: Relative abundances of groups of phytoplankton and bacterioplankton observed at the end of the microcosm incubation as a function of treatments during the four seasons. C: Control, ACW: Artificial Contaminated Water and CW: Contaminated Water with sediment elutriate.

Figure 3: Correspondence analysis with the 100 most abundant OTUs and the phytoplankton groups as environmental variables measured at the end of the microcosm incubations. C: Control, ACW: Artificial Contaminated Water and CW: Contaminated Water with sediment elutriate.

Figure 4: Significant interactions ($p < 0.05$) between phytoplankton groups and the 100 most abundant OTUs estimated from Spearman correlations (Positive or Negative) as a function of the microcosm treatments. C: Control, ACW: Artificial Contaminated Water and CW: Contaminated Water with sediment elutriate.

Figure 5: Simplified network between phytoplankton groups and bacterial OTUs (indicated by numbers) in Lagoon station as a function of microcosm treatments. Green lines indicate significant ($p < 0.05$) positive correlations (Spearman) and red lines indicate significant ($p < 0.05$) negative correlations. Solid lines indicate bacterial interactions with other OTUs and with phytoplankton groups, dotted lines indicate interactions between phytoplankton groups. Only correlations $> |0.8|$ are indicated. C: Control, ACW: Artificial Contaminated Water and CW: Contaminated Water with sediment elutriate. See materials and methods for more details.

Figure 6: Simplified network between phytoplankton groups and bacterial OTUs (indicated by numbers) in Offshore station as a function of microcosm treatments. Green lines indicate significant ($p < 0.05$) positive correlations (Spearman) and red lines indicate significant ($p < 0.05$) negative correlations. Solid lines indicate bacterial interactions with other OTUs and with phytoplankton groups, dotted lines indicate interactions between phytoplankton groups. Only

1014 correlations $> |0.8|$ are indicated. C: Control, ACW: Artificial Contaminated Water and CW:
1015 Contaminated Water with sediment elutriate. See materials and methods for more details.
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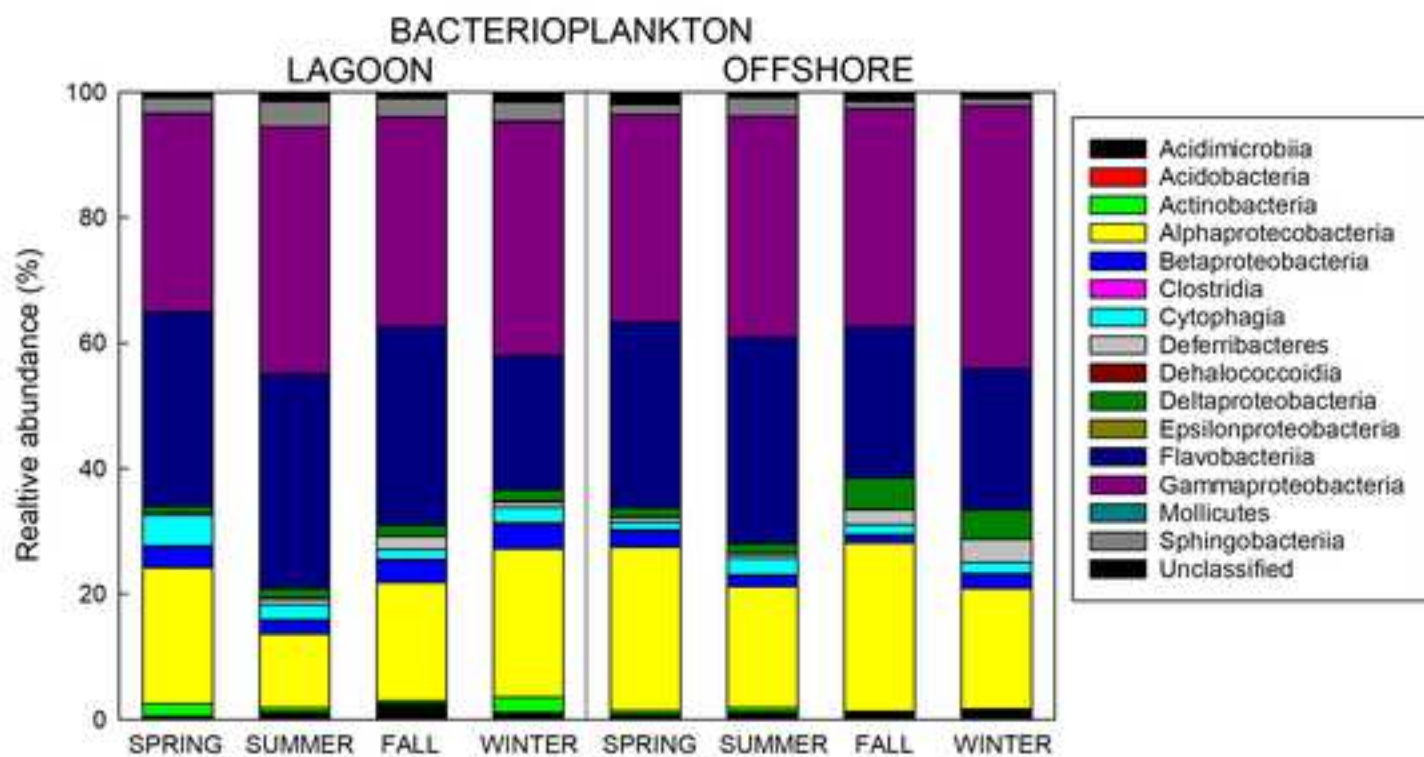
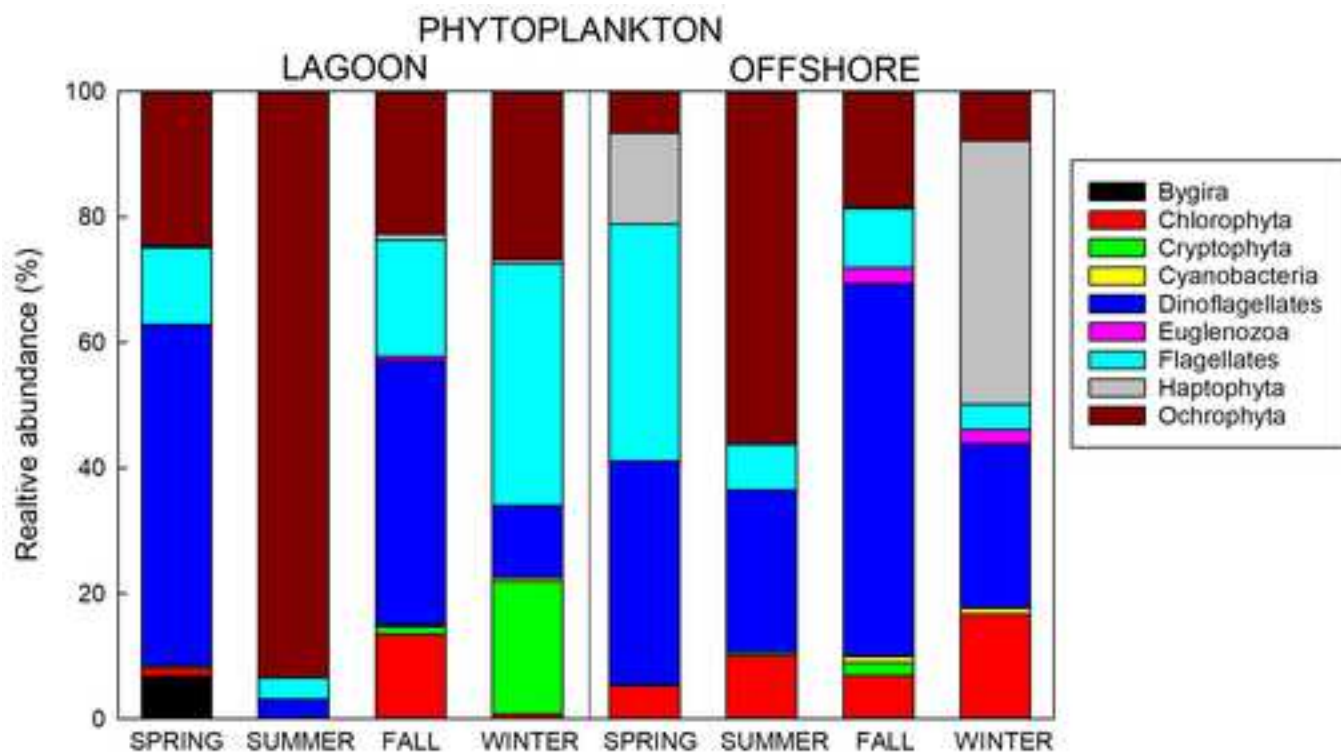
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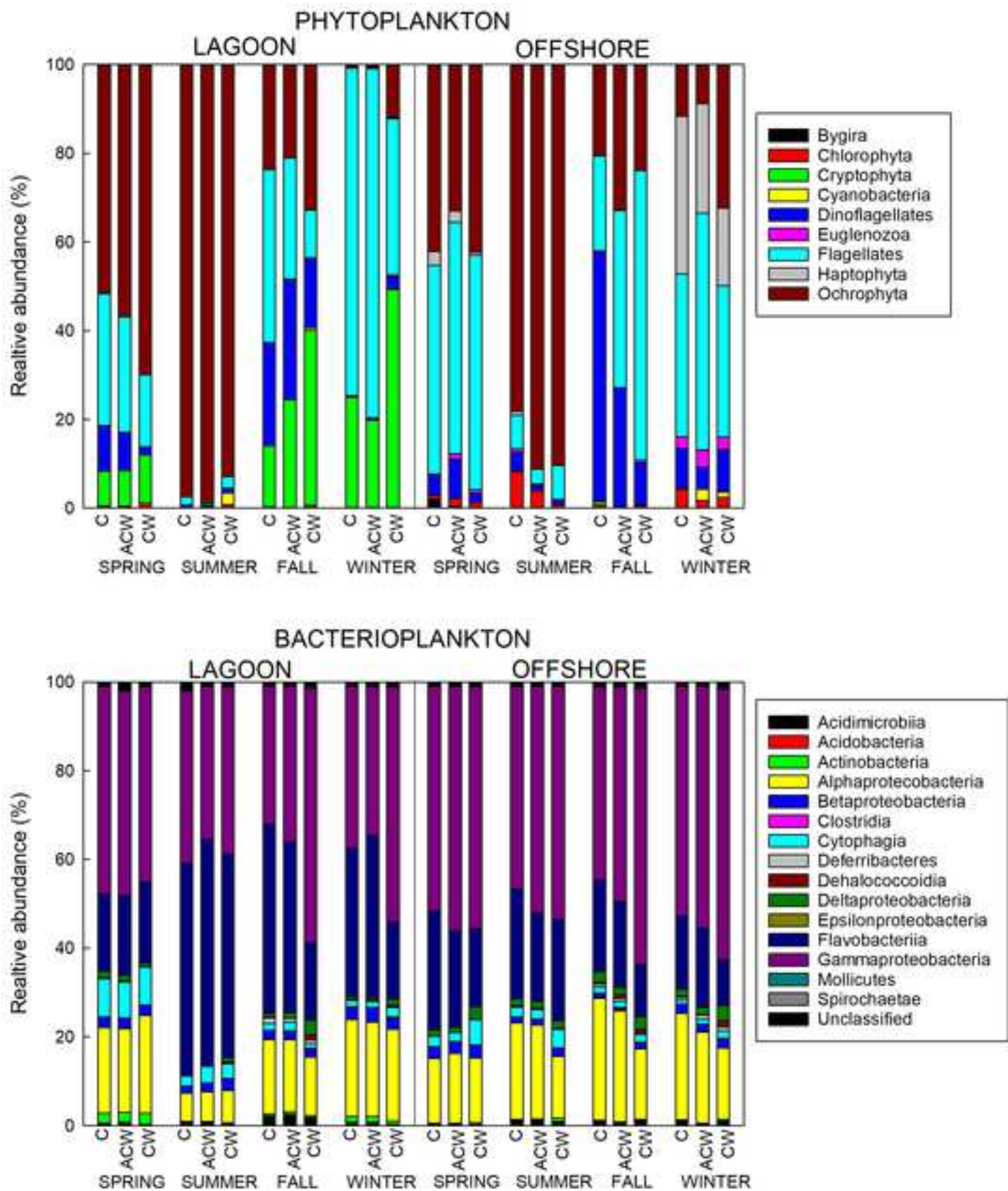
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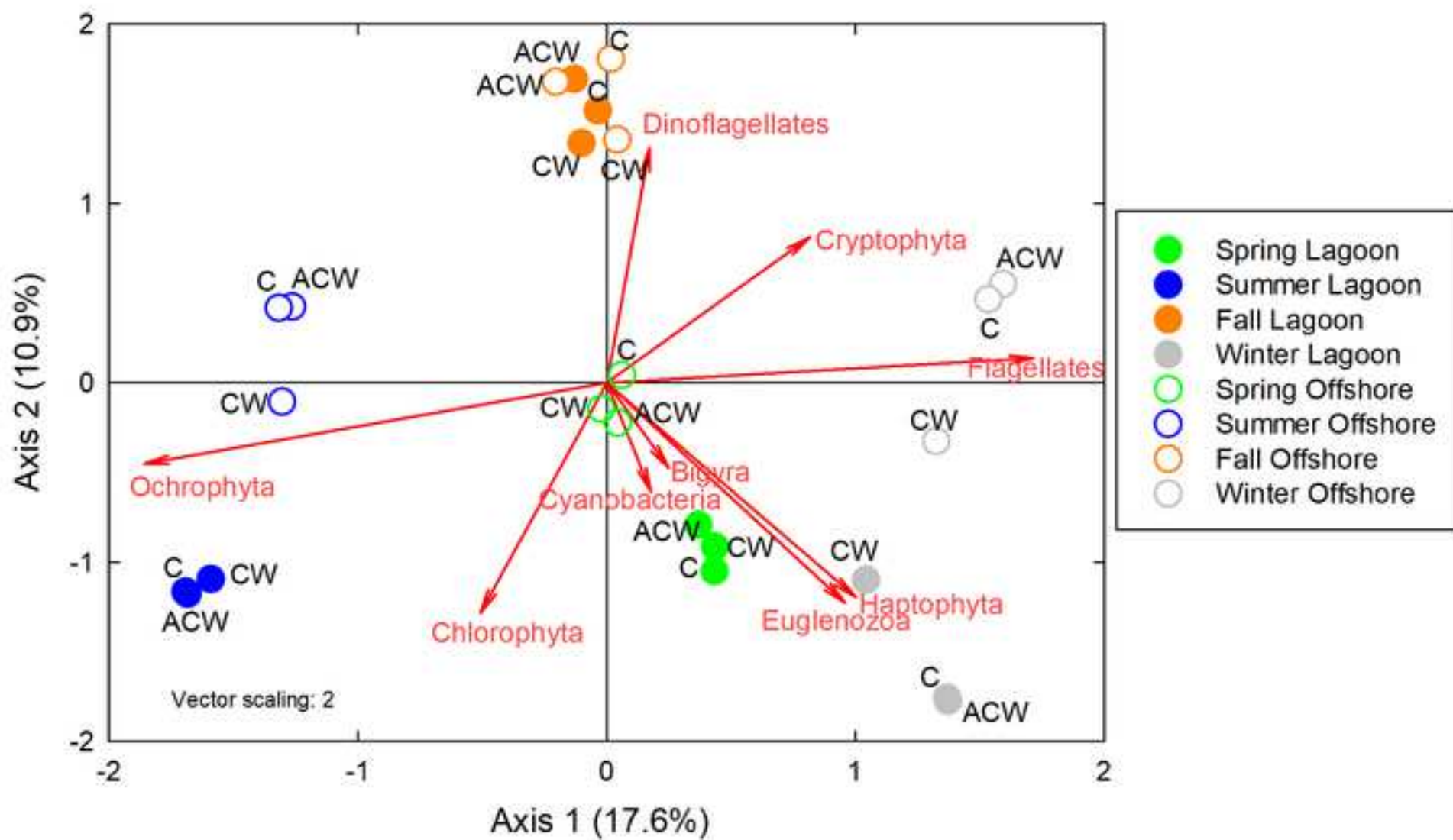
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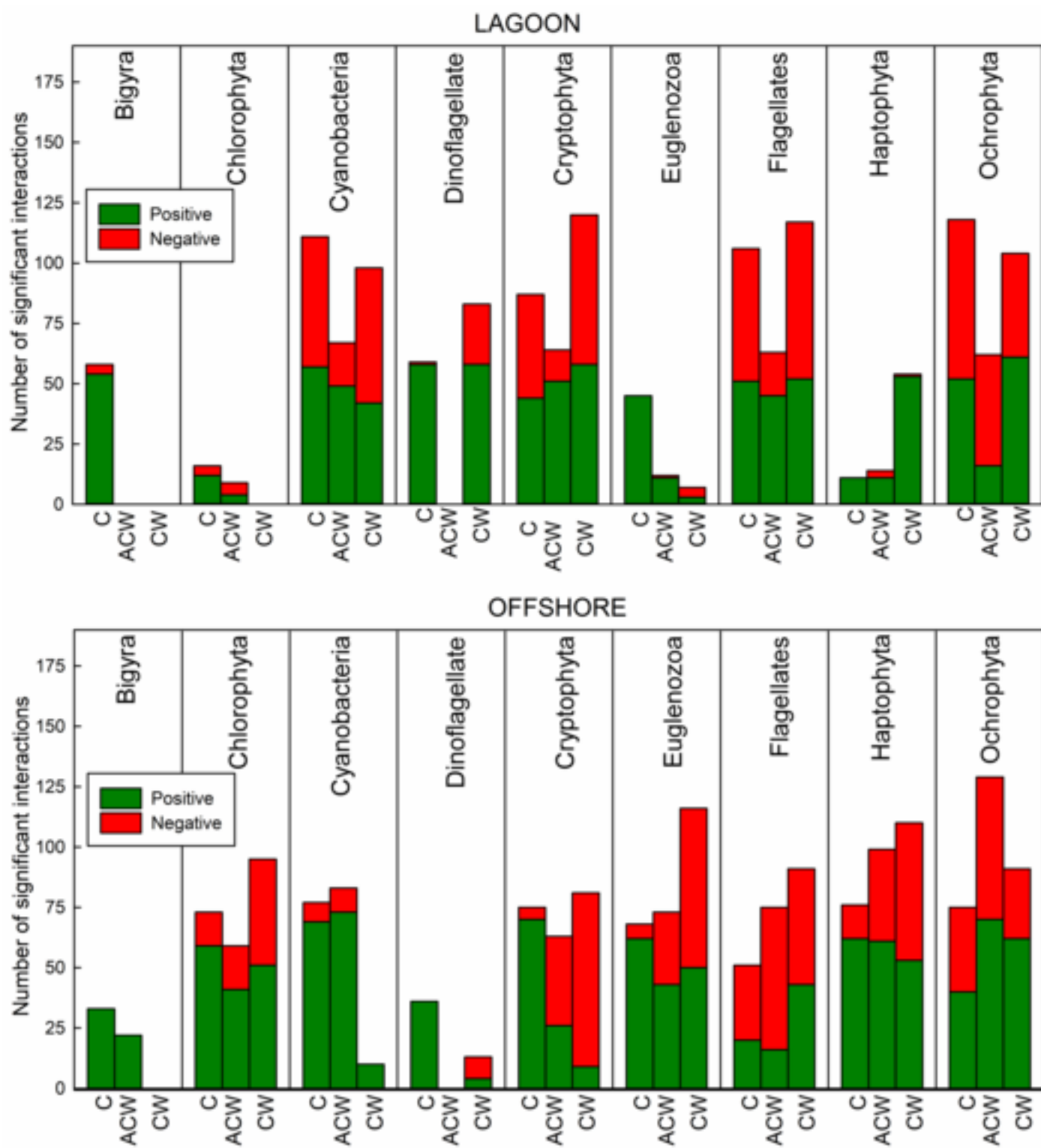
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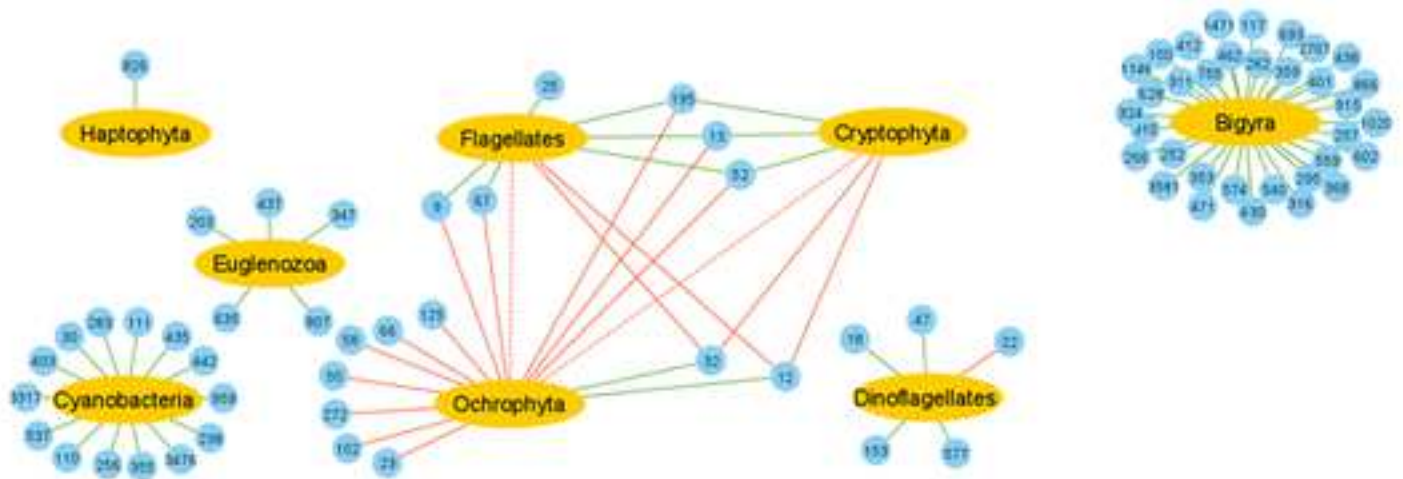




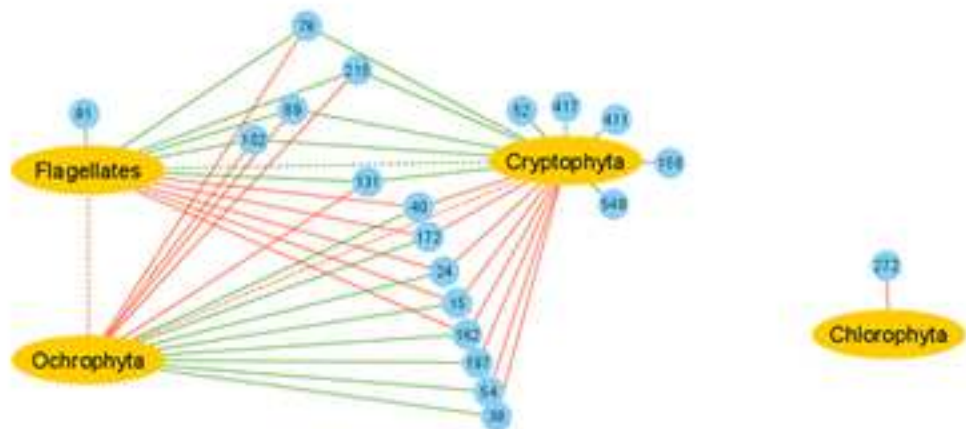


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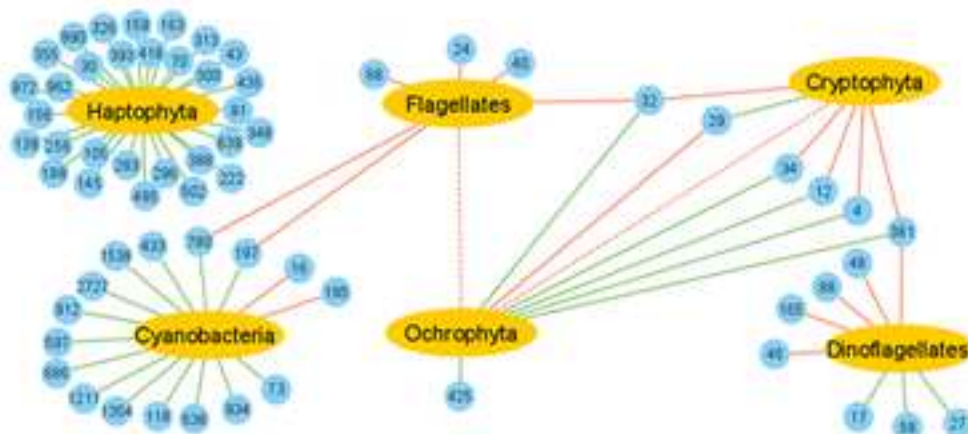
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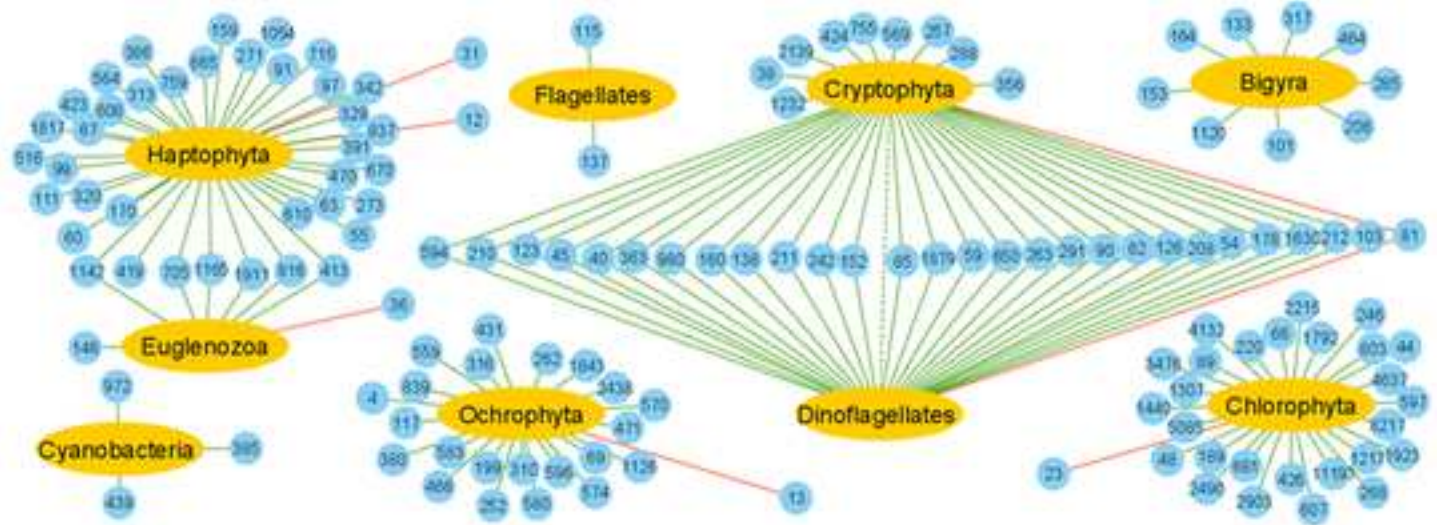


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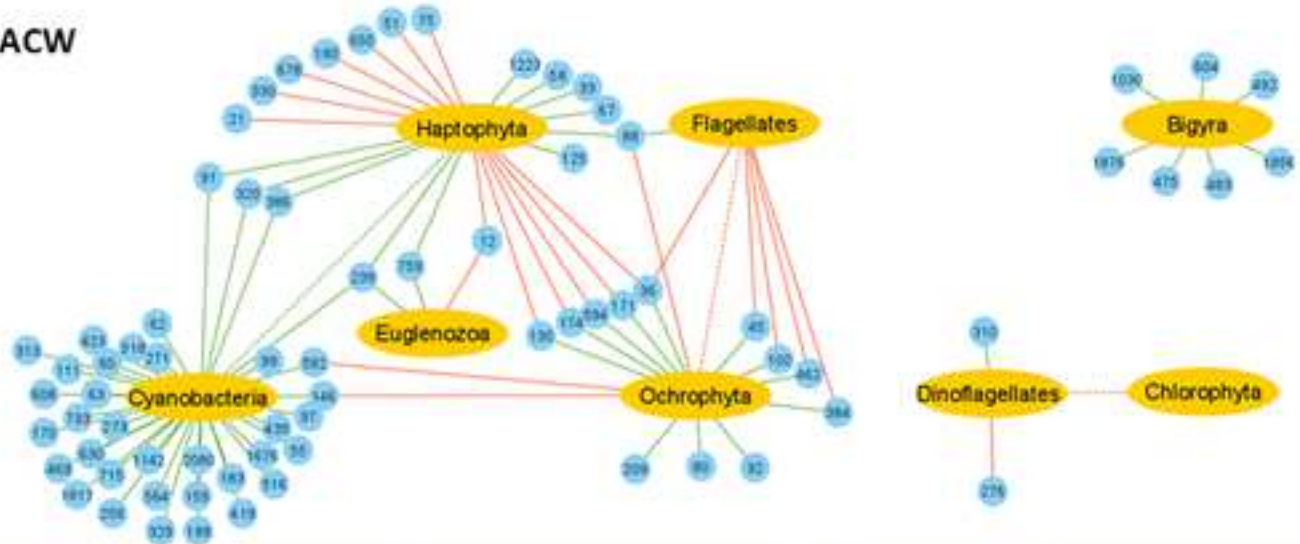


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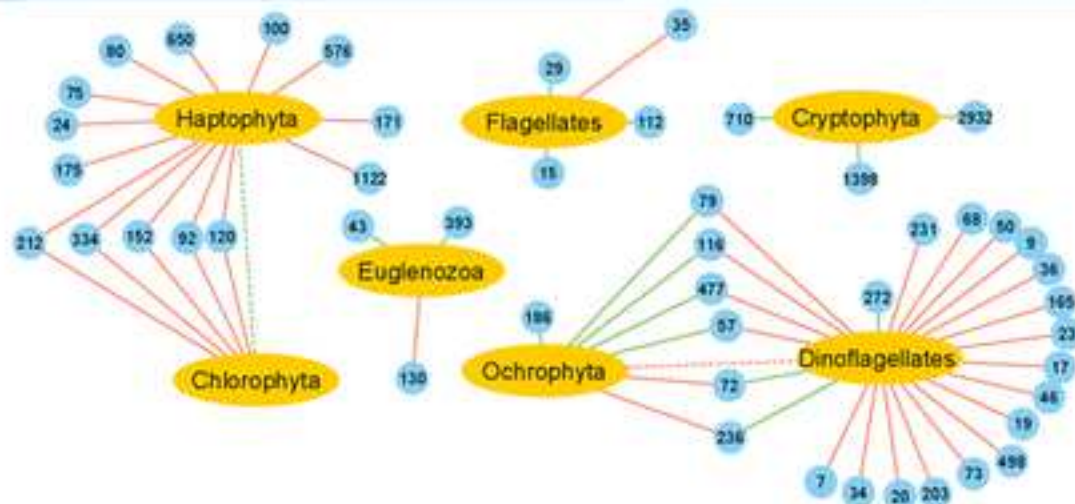
CONTROL



ACW



CW





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