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1 **Seasonal marine microorganisms change neighbors under contrasting**  
2 **environmental conditions**

3

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22 **Author Contributions:** FYB and PEG designed research; SL and JCL performed research;

23 FYB, PEG and SL analyzed data; and FYB, PEG, SL wrote the paper.

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25

26 **Originality-Significance Statement**

27 Planktonic microorganisms in the ocean play a key role for oxygen production, carbon  
28 sequestration and nutrient recycling. These microbial functions are directly associated with  
29 the composition of the communities, which changes regularly with seasons and shows strong  
30 and reproducible patterns year after year. Remarkable weather events can dramatically  
31 change the environment, but their effect on the stability of the microbial communities is not  
32 known. Here we show that environmental perturbations transform and weaken the networks  
33 of co-occurring eukaryotes, bacteria and archaea. In addition, individual taxa changed  
34 neighbors from year to year, which could suggest switches between ecologically equivalent  
35 microbes. Such unexpected unfaithful relationships between marine microorganisms may  
36 promote the long term stability of the ecosystem.

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51 **Summary**

52 Marine picoplankton contribute to global carbon sequestration and nutrient recycling. These  
53 processes are directly related to the composition of communities, which in turn depends on  
54 microbial interactions and environmental forcing. Under regular seasonal cycles, marine  
55 communities show strong predictable patterns of annual re-occurrences, but little is known  
56 about the effect of environmental perturbation on their organization. The aim of our study  
57 was to investigate the co-occurrence patterns of planktonic picoeukaryote, bacteria and  
58 archaea under contrasting environmental conditions. The study was designed to have high  
59 sampling frequency that could match both the biological rhythm of marine microbes and the  
60 short time scale of extreme weather events. Our results show that microbial networks  
61 changed from year to year depending on conditions. In addition, individual taxa became less  
62 interconnected and changed neighbors, which revealed an unfaithful relationship between  
63 marine microorganisms. This unexpected pattern suggests possible switches between  
64 organisms that have similar specific functions, or hints at the presence of organisms that  
65 share similar environmental niches without interacting. Despite the observed annual changes,  
66 the time series showed re-occurring communities that appear to recover from perturbations.  
67 Changing co-occurrence patterns between marine microorganisms may allow the long-term  
68 stability of ecosystems exposed to contrasting meteorological events.

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## 76 **Introduction**

77 The global carbon and nutrient cycle in the surface waters of the ocean is strongly driven by  
78 marine microbial communities (Falkowski et al., 2008). As microbial communities' function  
79 and productivity are directly linked to community diversity and composition (Bell et al.,  
80 2005), understanding patterns and processes of community assembly is crucial for the  
81 prediction of ecosystem functioning (Nemergut et al., 2013). Microbial community assembly  
82 is the outcome of biotic interactions such as competition, predation and mutualism, and  
83 abiotic selection by local environmental conditions (Vellend, 2010; Nemergut et al., 2013). In  
84 the ocean, there is a general consensus that microbial planktonic communities respond to the  
85 physical chemical features of sea water. In temperate and polar regions, these features are  
86 strongly associated with the seasonality of the ocean marked by changing temperature  
87 regimes (Fuhrman et al., 2015). The dynamics of the ocean may be hard to capture by  
88 discrete sampling schemes, and long-term oceanographic time-series studies have proven to  
89 be very useful to elucidate links between microbial communities and environmental  
90 conditions. Temperature, day length, photosynthetically available radiation, salinity and  
91 nutrient concentrations are the main abiotic factors that individually or together strongly  
92 impact the seasonal composition of microbial communities (e.g., (Treusch et al., 2009; Eiler  
93 et al., 2011; Gilbert et al., 2012; Chow et al., 2013; Cram et al., 2015b; Salter et al., 2015;  
94 Parada and Fuhrman, 2017; Auladell et al., 2019; Lambert et al., 2019)). Long term time-  
95 series of microbial communities have also shown the importance of biotic forcing (Chow et  
96 al., 2014; Ahlgren et al., 2019). Top-down controls, which include predation and viral lysis,  
97 and bacteria–bacteria interactions are important factors that shape community assembly.  
98 Microbial communities are complex systems of co-occurring species and the use of  
99 interaction networks has strongly increased our understanding on the possible connections

100 between microorganisms within a community (Beman et al., 2011; Steele et al., 2011; Gilbert  
101 et al., 2012; Chow et al., 2014; Cram et al., 2015a; Needham et al., 2017).

102 Time-series studies have demonstrated the strong predictability of microbial  
103 community composition season after season, and year after year (Fuhrman et al., 2006). This  
104 key feature has been seen repeatedly for bacteria and archaea (Hugoni et al., 2013; Chafee et  
105 al., 2018; Galand et al., 2018), but also eukaryotes (Giner et al., 2019), and appears to be  
106 maintained under irregular environmental perturbations (Lambert et al., 2019). This strong  
107 pattern of re-occurrence has been explained by the invisible hand metaphor, which refers to  
108 the cumulative effect of individuals working in their own self-interest that leads to well-  
109 regulated community function without any centralized control mechanism (Fuhrman et al.,  
110 2015). This long-term pattern has been observed at monthly sampling frequencies and the  
111 question remains as to whether such a motif could be seen at higher sampling frequency.  
112 Generation times of phytoplankton and marine bacteria can vary a lot, but are estimated to  
113 range from a few hours to a few days (Kirchman, 2016). Daily or twice a week sampling  
114 would thus better match the biological rhythm of the microbial sea (Fuhrman et al., 2015).

115 Higher frequency time-series studies have recently emerged and showed the rapid  
116 transitions between closely related strains (Needham and Fuhrman, 2016; Ward et al., 2017;  
117 Martin-Platero et al., 2018) and recurrent patterns of microdiversity in a coastal ecosystem  
118 (Chafee et al., 2018). They have followed the succession of species during blooms over a few  
119 months at a daily frequency (Needham and Fuhrman, 2016; Martin-Platero et al., 2018;  
120 Needham et al., 2018) or twice a week (Teeling et al., 2012). Only a few have monitored the  
121 dynamics of communities over longer time period: one year at a twice a week frequency  
122 (Lindh et al., 2015) or 3 years at weekly/twice a week frequency (Ward et al., 2017; Chafee  
123 et al., 2018). These studies have focused on prokaryotes and information on the long-term

124 high frequency dynamics of the 3 domains of life is lacking, and especially for coastal  
125 ecosystems.

126         Temperate coastal ecosystems are impacted by land and particularly in the  
127 Mediterranean Sea, where sudden and irregular weather events, such as strong precipitations  
128 causing flash flooding, storms triggering sediment resuspension, or wind inducing water  
129 column mixing, can have a strong impact (Charles et al., 2005; Thyssen et al., 2014; Tinta et  
130 al., 2015). These contrasting weather conditions are predicted to increase with a changing  
131 climate (Scoccimarro et al., 2016). The question remains as to whether community  
132 successions, and interactions between marine microorganisms, can remain stable under  
133 irregular climate events, or if the predicted increases in temperature and strong precipitations  
134 could disrupt individual interactions within communities (Berner et al., 2018).

135         The aim of our study was to investigate the patterns of planktonic picoeukaryote,  
136 bacteria and archaea co-occurrences under contrasting environmental conditions. The study  
137 was designed to have a sampling frequency that could match both the biological rhythm of  
138 marine microbes and the short time scale of extreme events such as flooding and storms.  
139 Furthermore, the multiyear sampling was intended to discriminate recurrent microbial  
140 community dynamics from stochastic events. We sampled the Banyuls Bay microbial  
141 observatory (SOLA), a coastal site of the NW Mediterranean Sea, twice a week during the  
142 most productive winter months (January-March) and weekly the rest of the time. Samples in  
143 this study included those collected during an “average” year, with physical and chemical  
144 parameters close to the long term mean, and two years marked by environmental winter  
145 perturbations in terms of seawater temperature and freshwater influx.

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## 147 **Results**

### 148 **Overall community composition, diversity and environmental conditions**

149 A canonical correspondence analysis (CCA) was carried out to compare the composition of  
150 communities during the 3 high frequency sampling winter periods for the prokaryote (Fig.  
151 1A) and eukaryote (Fig. 1B). Communities grouped generally by month of sampling but there  
152 were some exceptions for both eukaryotes and prokaryotes, in particular samples from  
153 December 2016, February 2017 and March 2017 were separated from the others for both  
154 domains of life.

155 In order to precisely identify dissimilarities in community composition, we compared  
156 systematically differences between successive samples (i.e.  $t$  sample vs  $t_{+1}$  sample) for the 3  
157 winter periods (Supplementary Fig. 1). The most important dissimilarities were identified as  
158 values above the annual average. They indicate abrupt changes in community composition  
159 within a short period of time. Their occurrence was indicated by arrows on top of the  
160 environmental conditions (Fig. 1C), and squares around CCA markers (Fig. 1A and B).  
161 Seven out of the ten highest dissimilarities in community composition occurred in 2017.

162 During the winter season, which is the most productive time of the year at the site  
163 {Galand, 2015 #2028}, the strong changes in community composition, marked by arrows,  
164 corresponded most of the time to changes in salinity, but not always (Fig. 1C). These changes  
165 were associated with increased nitrate concentrations and often to higher river discharge (Fig.  
166 1C). This was clearly visible in March 2015 (red arrow), December 2016 (blue arrow),  
167 February 2017 (blue arrow) and March 2017 (blue arrow).

168 The abrupt changes did not, however, impact the annual seasonality of the microbial  
169 communities, which was characterized by repeatable temporal patterns in the community  
170 composition, as shown by the higher similarity observed between communities sampled one  
171 year apart and lower similarity for communities taken 6 months apart for both eukaryotes and  
172 prokaryotes (Supplementary Fig. 2).



173           The Shannon diversity index pattern varied from year to year. Its range of variation  
174 was low in 2015, decreased from January to April in 2016, and it was highly variable in 2017  
175 (Supplementary Fig. 3).

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### 178 **Community composition at the class level**

179 Among all the eukaryotic classes found in the dataset, four main classes dominated:  
180 *Bacillariophyta*, *Dinophyceae*, *Mamiellophyceae* and *Syndiniales* (Supplementary Fig. 4).

181 Overall, for a given month, the average sequence abundance of these major groups varied  
182 from year to year (Fig. 2). *Bacillariophyta*, which showed relatively low levels of average  
183 sequence abundance throughout the sampling period, were more abundant in February and  
184 March 2017. *Bacillariophyta* were almost absent in March 2016. Average abundance of  
185 *Dinophyceae* remained relatively stable during the three years of sampling, with higher levels  
186 in January and February 2017. *Mamiellophyceae* had more variable patterns of abundance. In  
187 2015, they were more abundant in January and February, in 2016 they were more abundant in  
188 March, and in 2017 in January. *Mamiellophyceae* were mainly composed of the genera  
189 *Bathycoccus* and *Micromonas*. These two picoeukaryotes had different dynamics  
190 characterized by a *Micromonas* bloom in early winter followed by *Bathycoccus* (ASV5 vs  
191 ASV 4, Supplementary Fig. 5). *Syndiniales* abundance did not vary much in 2015, unlike in  
192 2016 and 2017 when they were more abundant in March, and January/March respectively.

193           For prokaryotes, abundance of the main classes also varied from year to year and  
194 especially in 2017 (Fig. 2). For *Alphaproteobacteria* decreases in abundance were seen in  
195 February and March 2017. *Cyanobacteria* average abundances were relatively low and stable  
196 during the three years of sampling, except for an increase in March 2017. Average abundance  
197 for *Flavobacteria* in 2015 and 2016 showed an increase in March for both years.

198 *Flavobacteria*, in contrast, had a higher than average relative abundance in February 2017.  
199 Finally, *Gammaproteobacteria* had similar levels of abundance between years from January  
200 to March, except for February 2017 when they were less abundant (Fig. 2).

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### 203 **Co-occurrence networks**

204 The co-occurrences of ASVs was investigated for each high frequency sampling winter  
205 seasons (January, February and March) by network analysis based on the Maximal  
206 Information Coefficient (MIC). MIC identifies correlations between variables and is best  
207 adapted to the non-linear temporal nature of the data (Reshef et al., 2011). The networks,  
208 based on significant MIC values ( $p > 0.05$ ) with a false discovery rate (FDR) of 5%, show that  
209 there were clear differences in the topology between years (Fig. 3). The 2015 network was  
210 narrower, more clustered and more centralized than the 2016 and 2017 networks  
211 (Supplementary Table 3).

212 The 2015 network was composed of 2 main modules, with a modularity of 12.4 and  
213 5.7, respectively, as well as 4 smaller modules (modularity < 1) (Supplementary Table 4). The  
214 ASVs that were more abundant in January and February grouped in one module (Fig. 3A). In  
215 January, the hub ASVs defined as the nodes with highest degree, belonged first to  
216 *Euryarchaeota* Marine Group II (Archaea), and then a number of Eukaryotes including  
217 *Syndiniales*, diatoms (*Chaetoceros brevis*), a flagellate (*Florenciella parvula*) and  
218 *Micromonas* (Mamiellophyceae) (Supplementary Table 5, Fig 3A). The hub ASVs of  
219 February belonged to *Acidimicrobiales* (OM1 clade), *Gammaproteobacteria* clades ZD0405  
220 and SAR86, and diatoms (*Chaetoceros curvisetus*). The second main module was composed  
221 of ASVs abundant in March and included SAR11 surface 1 clade (*Alphaproteobacteria*),  
222 *Flavobacteriales*, *Rhodobacteraceae* and *Syndiniales* (Eukaryota) as hubs. The 2015 network

223 did not show any discernible pattern with respect to temperature or salinity, but the highest  
224 values were associated with the January ASVs (Fig. 3B, 3C).

225 The 2016 network consisted of 3 modules with a modularity of 14.5, 6 and 3,  
226 respectively and followed a seasonal pattern, but with a distinct March module (Fig. 3A,  
227 Supplementary Table 4). In January, the hub ASVs belonged to the cyanobacteria  
228 *Prochlorococcus*, *Acidimicrobiales* OM1 clade, *Deltaproteobacteria* SAR324 clade, the  
229 SAR11 surface 2 clade (*Alphaproteobacteria*) and to the dinoflagellate *Gymnodiniales*. In the  
230 February group, the hubs were diatoms from the *Florenciellales* and Raphid-pennate  
231 families, *Flavobacteriales* of the NS9 marine group and *Syndiniales*. The second module  
232 contained ASVs that were more abundant in March. The hub ASVs were members of the  
233 *Flavobacteriaceae*, MAST 6 (Stramenopiles), and *Syndiniales* (Supplementary Table 5).  
234 *Micromonas* (*Mamiellophyceae*) was connected with both modules (Fig. 3). The 2016  
235 network was separated into a low temperature (11-12°C) and a high temperature (13-14°C)  
236 module (Fig. 3B), and the ASVs were mainly related to samples with higher salinity (38)  
237 (Fig. 3C).

238 The 2017 network formed 8 low modularity modules (modularity between 4.4 and  
239 0.5) (Fig. 3, Supplementary Table 4). A seasonal pattern within the ASVs was not as clear as  
240 in 2015 and 2016. In January, abundant hub ASVs belonged to *Micromonas*  
241 (*Mamiellophyceae*), *Thaumarchaeota* Marine group I (Archaea) and the flagellate algae  
242 *Pelagomonas* (Supplementary Table 5). The ASVs abundant in February were separated in 2  
243 groups. On the top of the network (Fig. 3A), diatoms (*Skeletonema*) were abundant, and the  
244 hubs belonged to *Flavobacteriales* and, among Eukaryotes, *Lauderia* diatoms and the  
245 phagotrophic flagellate *Ebria* (Cercozoa). In the second part of the network containing ASVs  
246 abundant in February, a *Syndiniales* was abundant. The ASVs that were abundant in March  
247 were in the middle of the network and the hubs were identified as *Synechococcus*

248 cyanobacteria, *Flavobacteriaceae* and the flagellate *Chrysochromulinaceae*. The 2017  
249 network showed a central module with higher temperature (13°C), surrounded by lower  
250 temperature modules (12°C) (Fig 3B). It was composed of modules of ASVs found under  
251 average salinity of 37, and one module of low salinity ASVs (35) (Fig. 3C).

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### 255 **ASV co-occurrences**

256 In the networks, a total of 42 ASVs were common to the three high frequency winter  
257 samplings (Supplementary Fig. 6). In order to assess if the first neighbor nodes of the 42  
258 common ASVs changed from year to year, we represented the positive co-occurrences in  
259 radar plots (Fig. 4). The radius of the spider net corresponds to the MIC score between the  
260 central ASV and its first neighbors.

261 Noteworthy, all of the 42 ASVs changed neighbors between years. The number of  
262 first neighbors also changed from year to year (Fig. 4, Supplementary Table 6). Overall, the  
263 total number of first neighbors decreases from 2015 to 2017 (542, 389 and 263 in 2015, 2016  
264 and 2017 respectively). Half of the ASV (21) had a larger number of first neighbors in 2015  
265 compared to the other years. A total of 16 ASVs had more first neighbors in 2016 and only 2  
266 had more neighbors in 2017. Three ASVs had years with similar number of neighbors (Fig.  
267 4). The total number of neighbors per year varied greatly between ASVs. The highest values  
268 were 51 for ASV38 (*Alphaproteobacteria*) and the lowest 6 for ASV10 (*Flavobacteria*) (Fig.  
269 4, Supplementary Table 6).

270 Among the common ASVs, 23 were earlier identified as rhythmic over 7 years  
271 (Lambert et al., 2019) (marked by a black frame, Fig. 4). They also had first neighbors that  
272 varied in number and identity between years (Fig. 4). Finally, bacterial ASVs were mostly

273 associated with bacteria (59% of the first neighbors) while eukaryotic ASVs were mostly  
274 associated with eukaryotes (67% of the first neighbors). However, the affiliation of the first  
275 neighbor to a given domain of life also changed from year to year. For a given ASV, some  
276 years showed more eukaryotic first neighbors while other displayed more prokaryotic  
277 neighbors.

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## 280 **Discussion**

281 Our high frequency sampling study, focusing on the productive winter months, showed that  
282 the patterns of the microbial networks linking members of the 3 domains of life changed from  
283 year to year. To the best of our knowledge, inter-annual variations of network topology have  
284 not been shown earlier. On the contrary, a recent high frequency sampling study from the  
285 North Sea reported that network topologies were similar for co-varying microbes between  
286 two different years for a same season (Chafee et al., 2018). Networks have, however, been  
287 shown to change between seasons in both freshwater (Kara et al., 2013; Hu et al., 2017) and  
288 marine environments (Chafee et al., 2018; Cui et al., 2019). Different temporal patterns in  
289 network topology have also been observed along shorter time series (Martin-Platero et al.,  
290 2018). These temporal changes reflect variations in community composition with changing  
291 environmental conditions along a year (Fuhrman et al., 2015). Nevertheless, these earlier  
292 studies did not test the year to year stability of the networks. Many correlation-based  
293 association networks have focused only on the overall trend of microbial interconnectivity  
294 without distinguishing seasons and years, the goal being to identify time-dependent  
295 associations among ecologically important taxa (Steele et al., 2011; Chow et al., 2013;  
296 Needham et al., 2013; Chow et al., 2014; Cram et al., 2015a; Milici et al., 2016; Needham et

297 al., 2017; Parada and Fuhrman, 2017). The fact that network topologies can change from year  
298 to year should be considered in future temporal studies.

299         The long-term stability of co-occurrences has not been tested earlier. Co-occurrences  
300 could have been considered to be robust and maintained over time since similar microbial  
301 communities have been shown to reoccur, at a same site, year after year (Fuhrman et al.,  
302 2006; Gilbert et al., 2012; Lambert et al., 2019). We also observed annual patterns of  
303 reoccurrence, but our data revealed that despite these reproducible patterns, the microbial  
304 networks are not necessarily robust as their topology can be broken when climatic conditions  
305 changes.

306         Interestingly, the abrupt or gradual changes (salinity and temperature) that we  
307 observed did not impact the overall rhythmicity of the microbial sea over the long term  
308 (Lambert et al., 2019) nor in this study. Regardless of the environmental perturbations, some  
309 ASVs reoccur every year. However, here we observed that these reoccurring ASVs rarely had  
310 the same first neighbors, which indicate very strong changes of co-occurrence patterns from  
311 year to year. The changing patterns probably correspond to the changing environmental  
312 conditions that included higher sea water temperature in 2015-2016 and numerous salinity  
313 drops in 2016-2017. Strong changes were also seen in the overall number of first neighbors  
314 within a network, which was halved from 2015 to 2017. Nodes with fewer neighbors may be  
315 an indication of a less robust network (Röttjers and Faust, 2018) and may thus illustrate a  
316 possible weakening of the network of co-occurrence by environmental perturbations.

317         The observed changes in co-occurrence patterns may have different explanations.  
318 First, if we hypothesize that co-occurrences represent interactions between microorganisms,  
319 such as cross feeding, predation or parasitism (Fuhrman et al., 2015), it could mean that some  
320 species might be replaced by others having a similar function. It could be the case for  
321 *Micromonas* (ASV5) that co-occurred with different potential ciliate predators between 2015

322 and 2017. The possibility for a partial functional redundancy at the individual level has been  
323 described earlier (Galand et al., 2018). Although there may not be functional redundancy at  
324 the community level (Galand et al., 2018), at the individual level, a switch between different  
325 organisms that have a same specific function is possible. In our study, a gene level functional  
326 redundancy may exist for the *Rhodobacterales* and the *Oceanospirillales*, or the  
327 *Thaumarchaeota*, which all could produce B12 (Doxey et al., 2015; Gómez-Consarnau et al.,  
328 2018), and that change co-occurrences with the *Mamiellophyceae* that are B12 auxotrophs  
329 (*Bathycoccus* ASV\_euk4, *Micromonas* ASV\_euk22). Our study is, however, focused on the  
330 small size fraction of the microbial communities and thus misses larger planktonic organisms.  
331 Phytoplankton is known to interact with prokaryotes, but these interactions, and their possible  
332 stability over time, cannot be accounted for in our analysis.

333         The other hypothesis for the change of neighbors is that there are only limited  
334 interactions between species, and that the co-occurrence patterns only represent species  
335 having preferences for similar environmental conditions, i.e they share similar niches. This  
336 would imply that the strong seasonal patterns, such as the ones observed for dominant ASVs  
337 like *Mamiellophyceae* or SAR11, would be driven by structuring environmental parameters  
338 like temperature and day length (Gilbert et al., 2012; Lambert et al., 2019). The reality  
339 probably reflects a mix of both hypotheses with the presence of microorganisms that are  
340 unfaithful in their interactions, and cohorts that respond to similar environmental conditions.

341         The abrupt perturbations that we observed at the Banyuls microbial observatory  
342 (SOLA) were characterized by lower sea water salinity. These drops corresponded to  
343 episodes of heavy rain leading to higher water level in the local river. Large rivers further up  
344 the coast have also been shown to impact the study site (Laghdass et al., 2010). Episodes of  
345 heavy rain are often concomitant with storms in the North Eastern Mediterranean (Charles et  
346 al., 2005; Guizien et al., 2007). Storms mix the sea floor sediments through the water column,

347 which increases nutrient concentrations. Nutrients are also brought from land by the river and  
348 can induce blooms measured as peak of chlorophyll, as seen earlier along the coast of the  
349 eastern Mediterranean (Charles et al., 2005; Nunes et al., 2018).

350 At the Banyuls site, the strongest low salinity events were observed during winter 2016-2017.  
351 The perturbations resulted in lower relative abundance of the predominant marine bacteria  
352 SAR11 (*Alphaproteobacteria*) and an increase of *Flavobacteria*. Among picoeukaryotes, the  
353 parasitic *Syndiniales* increased in relative abundance. The corresponding microbial network  
354 was characterized by a low salinity module that was composed of diatoms of the genus  
355 *Skeletonema* and *Lauderia*, and highly connected bacteria of the family *Flavobacteriaceae*  
356 and *Rhodobacteraceae*. Our results suggest that the freshwater input, and the associated  
357 nutrients, favored the bloom of specific diatoms species that were co-occurring with bacteria  
358 that are usually not abundant in the Bay of Banyuls at that time of the year. These bacteria  
359 have been seen associated with diatom blooms earlier (Teeling et al., 2016), and diatoms  
360 have been shown to bloom following episodic fertilization events associated with freshwater  
361 runoff (Tinta et al., 2015; Nunes et al., 2018). *Skeletonema* in particular was observed to  
362 bloom following heavy rains along the Catalan coast (Estrada, 1979). It should, however, be  
363 noted that microorganisms like diatoms are not expected to pass through our 3 µm filters. The  
364 fact that we detected sequences from larger cells suggests that DNA from damaged or lysed  
365 organisms passed through the pre-filter. The interpretation of the co-occurrence patterns is  
366 thus done within the frame of this technical limitation.

367 Winter 2015-2016 was characterized by higher sea water temperatures in January and  
368 February compared to average. These higher temperatures may have impacted the bloom of  
369 *Micromonas* that took place later that year compared to average (February instead of  
370 January). They may have been replaced by Cyanobacteria (*Prochlorococcus*) that increased  
371 in sequence abundance and became hub species in the network in January. *Prochlorococcus*



372 are overall less abundant than *Synechococcus* in the NW Mediterranean Sea (Schauer et al.,  
373 2003), but appear to bloom in autumn when solar radiations are weaker (Sommaruga et al.,  
374 2005). Our results suggest that when sea water temperature remains relatively high in early  
375 winter, the bloom of *Prochlorococcus* can persist at the expense of *Micromonas*  
376 development. Such a switch could have an impact on the functioning of the coastal NW  
377 Mediterranean. It has, for example, been shown that after a mild winter in the Baltic Sea the  
378 spring bloom was reduced, thus impacting the carbon cycle (Legrand et al., 2015). Such a  
379 scenario could lead to lower carbon export to the marine food web (Berner et al., 2018).

380 Winter 2014-2015 had environmental conditions that were the most similar to an  
381 average year. The corresponding microbial network was marked by a clear separation  
382 between seasonal modules. It corresponded to the temporal succession of species in the  
383 coastal NW Mediterranean, which is marked by a strong seasonality (Hugoni et al., 2013;  
384 Galand et al., 2018; Lambert et al., 2019). In January, the hub species were picoeukaryotes  
385 identified as *Micromonas* (class *Mamiellophyceae*), *Euryarchaeota* Marine Group II  
386 (*Archaea*), and diatoms (*Chaetoceros brevis*). A *Micromonas* bloom at the beginning of the  
387 year has been seen earlier at the Banyuls site (Lambert et al., 2019), but its co-occurrence  
388 with *Euryarchaeota* has not been reported. In February, the communities were characterized  
389 by diatoms of the genus *Chaetoceros* and bacteria belonging to the ZD0405 and SAR86  
390 clades of *Gammaproteobacteria*. These bacteria have been reported as associated with  
391 diatom blooms earlier (Lucas et al., 2015; Teeling et al., 2016). In March, the hub species  
392 corresponded to classical pelagic microbial communities composed of the SAR11 surface 1  
393 clade (*Alphaproteobacteria*) and *Bathycoccus* for the pico-eukaryotes, which is known to  
394 bloom in late winter at the site (Lambert et al., 2019). As *Bathycoccus* and SAR11 are both  
395 auxotroph to vitamin B1 precursors, their co-occurrence may reflect shared ecological niches

396 rather than interaction as proposed for co-occurring *Micromonas* and SAR11 (Lambert et al.,  
397 2019).

398

## 399 **Conclusions**

400 Our high frequency sampling of NW Mediterranean microbial communities revealed that co-  
401 occurrence networks of picoeukaryote, bacteria and archaea changed from year to year.  
402 Weather perturbations in temperature or salinity seem to alter the topology of the microbial  
403 networks. In addition, individual taxa changed neighbor from year to year suggesting an  
404 unfaithful relationship between marine microorganisms. However, the global seasonal  
405 patterns of rhythmic ASVs were maintained, which indicates that the communities recover  
406 from these perturbations. It implies a strong environmental control, but it also suggests that  
407 changing co-occurrences between marine microorganisms may allow the long-term stability  
408 of the ecosystem. The resilience of seasonal microbial ecosystems remains, however, to be  
409 assessed under the more extreme climatic events predicted for the future. Well-established  
410 time series are essential for the monitoring of anthropological impacts on the sea and to get a  
411 good understanding of the functioning of the microbial ocean.

412

## 413 **Experimental Procedures**

414

### 415 **Sampling**

416 Surface water (3m) was collected with Niskin bottles from January 2015 to March 2017 at  
417 the Banyuls Bay microbial observatory (SOLA) (42°31'N, 03°11'E) in the Bay of Banyuls-  
418 sur-Mer, North Western Mediterranean Sea, France. A total of 5 L of seawater was  
419 prefiltered through 3 µm pore-size polycarbonate filters (Merck-Millipore, Darmstadt,  
420 Germany), and the microbial biomass was collected on 0.22-µm pore-size GV Sterivex

421 cartridges (Merck-Millipore) and stored at -80 °C until nucleic acid extraction. Samples were  
422 collected twice a week during winters: January to March 2015, January to April 2016 and  
423 December 2016 to March 2017, and once a week otherwise (spring, summer and autumn).  
424 The physicochemical (temperature, salinity, day length, nitrate, nitrite, silicate and phosphate  
425 concentrations) and biological (chlorophyll a) were provided by the Service d'Observation en  
426 Milieu Littoral (SOMLIT). The levels of the river discharging in the bay of Banyuls, the  
427 Baillaury, were obtained from the “Service Central d'Hydrométéorologie et d'Appui à la  
428 Prévision des Inondations” (<http://www.hydro.eaufrance.fr/>).

429

### 430 **DNA extraction, amplification and sequencing**

431 The nucleic acid extraction followed protocols published earlier (Lambert et al., 2019). To  
432 summarize, the Sterivex filters were thawed on ice, followed by addition of lysis buffer  
433 (40nM EDTA, 50nM Tris, 0.75M sucrose) and 25 µL of lysozyme (20 mg mL<sup>-1</sup>). The filters  
434 were then incubated for 45 minutes at 37°C on a rotary mixer. Subsequently, 8µL of  
435 Proteinase K (20mg mL<sup>-1</sup>) and 26µL of sodium dodecyl sulfate (20% v/v) were added before  
436 incubating for 1 hour at 55°C. Total DNA was then extracted and purified with the Qiagen  
437 AllPrep kit (Qiagen, Hilden, Germany) following the kit's protocol.

438       Specific primers were used to target the eukaryotic V4 region (TAReuk\_F1 [5'-  
439 CCAGCASCYGC GGTAATTCC] and TAReuk\_R [5'-ACTTTCGTTCTTGATYRATGA],  
440 (Stoeck et al., 2010) and the prokaryotic V4-V5 region (515F-Y [5'-  
441 GTGYCAGCMGCCGCGGTAA] and 926R [5'-CCGYCAATTYMTTTRAGTTT] (Parada  
442 et al., 2016). Library preparation and sequencing were carried out by the Genotoul platform  
443 (Toulouse, France), with the Illumina Miseq 2x250 bp kits. All sequences were deposited in  
444 NCBI under accession number PRJNA579489.

445

## 446 **Sequence analysis and preprocessing**

447 The standard pipeline of the DADA2 package (v1.6) (Callahan et al., 2016) in “R” was used  
448 to do the analysis of the raw sequences. The parameters used for the eukaryote dataset were:  
449 trimLeft=c(20, 21) ,truncLen=c(250,250), maxN=0, maxEE=c(2,2), truncQ=2; and for the  
450 prokaryote dataset: trimLeft=c(19, 20), truncLen=c(240,200), maxN=0, maxEE=c(2,5),  
451 truncQ=2. We analyzed 141 and 142 samples for the eukaryote and prokaryote datasets  
452 respectively, and obtained a total of 3.8 and 3.4 million reads respectively after DADA2 data  
453 processing (Supplementary Table 1). The taxonomy assignment was done with the The  
454 Protist Ribosomal Reference database (PR<sup>2</sup>)v.4.10.0 (Guillou et al., 2012) database for the  
455 eukaryotes and with SILVA v.128 database (Quast et al., 2013) for the prokaryote. The  
456 “assignTaxonomy” function in DADA2 implements the RDP naive Bayesian classifier  
457 method (Wang et al., 2007).

458 Taxa belonging to the supergroup “Opisthokonta” were removed from the eukaryote  
459 dataset and taxa belonging to eukaryotes were removed from the prokaryote dataset. Samples  
460 containing less than 5000 reads and 9000 reads were removed from the eukaryote and  
461 prokaryote dataset respectively. A total of 139 and 137 samples remained for the eukaryotes  
462 and the prokaryotes respectively. These preprocessing steps were done with the “R” package  
463 “Phyloseq” (McMurdie and Holmes, 2013).

464 Microbial communities in this study and in our previous study (Lambert et al., 2019)  
465 were not amplified with the same primer set. Here we chose to use universal primers shown  
466 to have a good coverage of both Bacteria and Archaea (Parada et al., 2016) instead of the  
467 two sets of separate primers used earlier (Lambert et al., 2019). In order to identify in this  
468 study the ASVs that were detected as rhythmic in our previous study, we compared both  
469 datasets by BLAST (Altschul et al., 1997) (blastn, >99% identity). For the eukaryotes, reads  
470 from both studies overlapped (~230 bp), and we could thus do a direct identification

471 (Supplementary Table 2). For the prokaryotes, however, the reads did not have an  
472 overlapping region. We therefore did a first BLAST of the rhythmic prokaryotic ASVs  
473 (Lambert et al., 2019) against the SILVA v.128 database. The matching sequences were then  
474 extracted from SILVA to create a novel custom database. Finally, we did a BLAST of the  
475 prokaryotic ASVs from this study against the custom database (Supplementary Table 2). We  
476 could then identify the ASVs from Lambert et al 2019 and the ASVs from this study that  
477 exactly matched the same SILVA reference sequence.

478

### 479 **Statistics**

480 Sequence counts for both datasets were normalized with the median-ratio method  
481 implemented in the “DESeq2” package (Love et al., 2014), which is mathematically  
482 equivalent to the compositional log-ratio (clr) transformation that accounts for the  
483 compositional nature of the data (Quinn et al., 2018).

484 The Bray-Curtis dissimilarity index was calculated with the “vegdist” function of the  
485 “Vegan” package (Oksanen et al., 2019) in “R”. Distances between samples were calculated  
486 based on community composition with a canonical correspondence analyses (CCA) with the  
487 “Vegan” package in "R". Contribution of environmental factors were added as arrows.

488 Abrupt changes in community composition between two samples were identified as  
489 Bray-Curtis dissimilarity values peaking above average. For that purpose, the Bray-Curtis  
490 dissimilarity index was calculated between two successive samples ( $t$  vs  $t_{+1}$ ), within each  
491 dataset, to identify variations of community composition with time.

492 Networks were based on the Maximal Information Coefficient (MIC), a measure that  
493 effectively identifies relationships between variables and that is best adapted to the non-linear  
494 temporal nature of the data, and behave well with small datasets (Reshef et al., 2011). The  
495 MIC was computed for each year based on ASV tables containing the most abundant ASVs.

496 They were selected as the 20 most abundant ASVs per sample, in each dataset, over each  
497 winter period (January, February, March), and represented over all a total of 153 ASVs in  
498 2015, 182 in 2016 and 187 in 2017. The MIC was then used to build a network that was  
499 visualized in Cytoscape (Shannon et al., 2003). The full network was pruned to visualize  
500 ASV interactions that had a Pearson linear regression  $> 0$  (positive interactions) and a MIC  $>$   
501  $0.75$ . These MIC values corresponded to significant interactions ( $p < 0.05$ ) when accepting a  
502 false discovery rate (FDR) of 5% at most as computed with MICtools (Albanese et al., 2018).  
503 The layout chosen for the network was edge-weighted spring embedded, using the MIC  
504 parameter. Module analyses were done with the CytoCluster app (Li et al., 2017) for  
505 Cytoscape, using the HC-PIN clustering algorithm with the default parameters (Weak,  
506 Threshold: 2.0 and ComplexSize Threshold: 3). Network analysis was done using the  
507 NetworkAnalyzer tool included in Cytoscape. Hub ASVs, which are the ones associated with  
508 a high number of other ASVs, were defined for each network as the nodes with highest  
509 degree (Röttjers and Faust, 2018). The networks were treated as undirected.

510 Radar plots were made with the "fmsb" package ([https://cran.r-](https://cran.r-project.org/web/packages/fmsb)  
511 [project.org/web/packages/fmsb](https://cran.r-project.org/web/packages/fmsb)) in "R". The heatmap was made with the "Phyloseq"  
512 package. For this figure, the eukaryotic and prokaryotic datasets were merged. Only ASVs  
513 with more than 10 reads were represented.

514

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523

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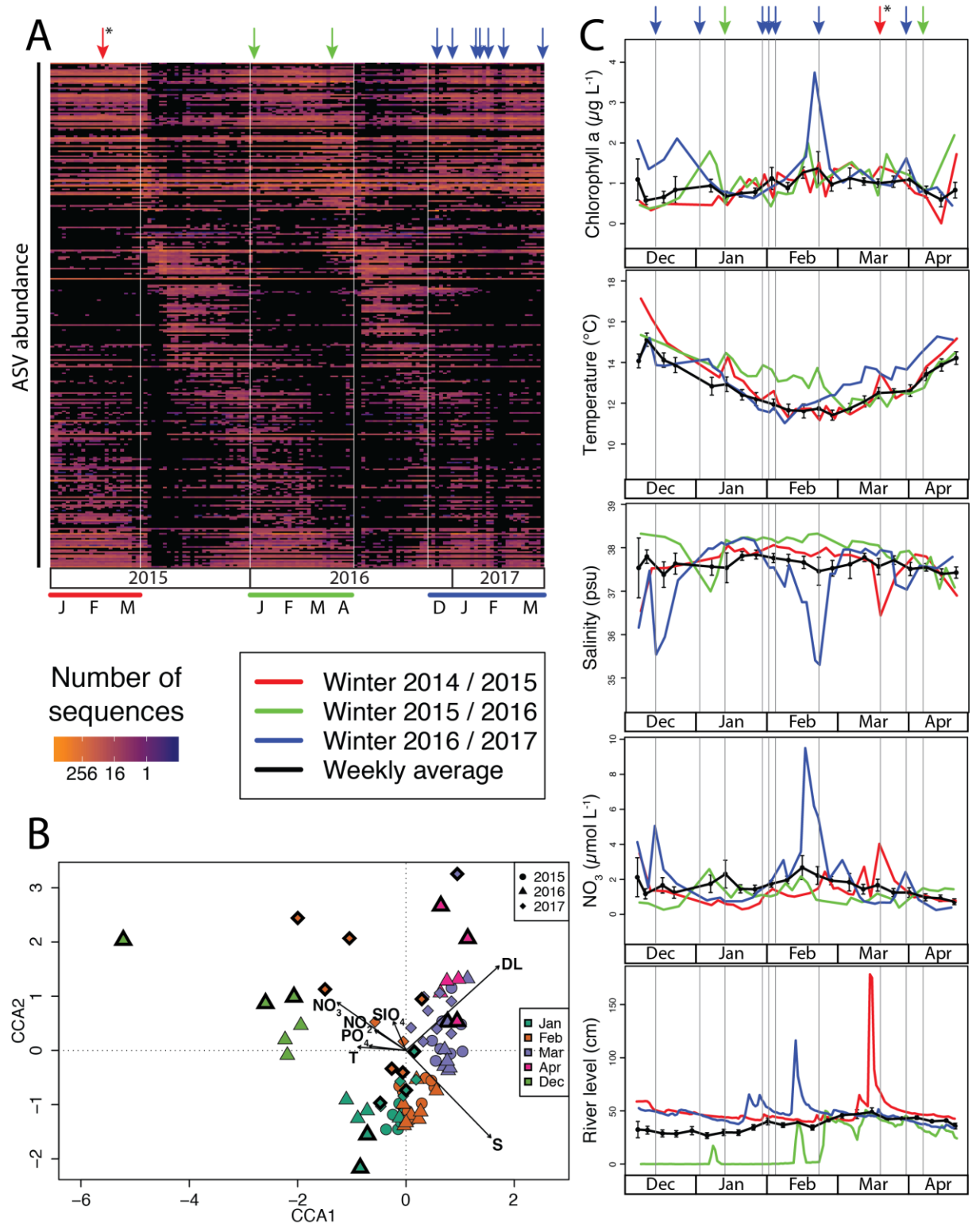
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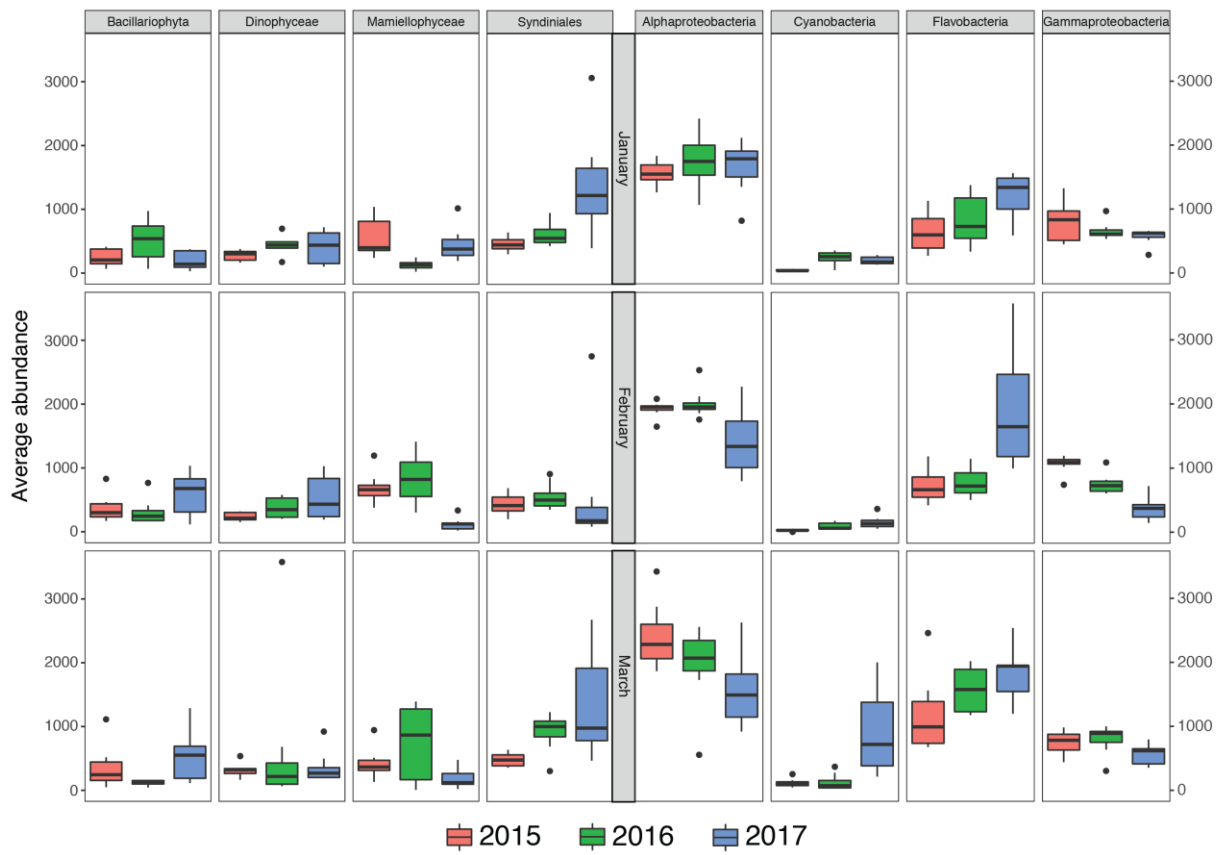
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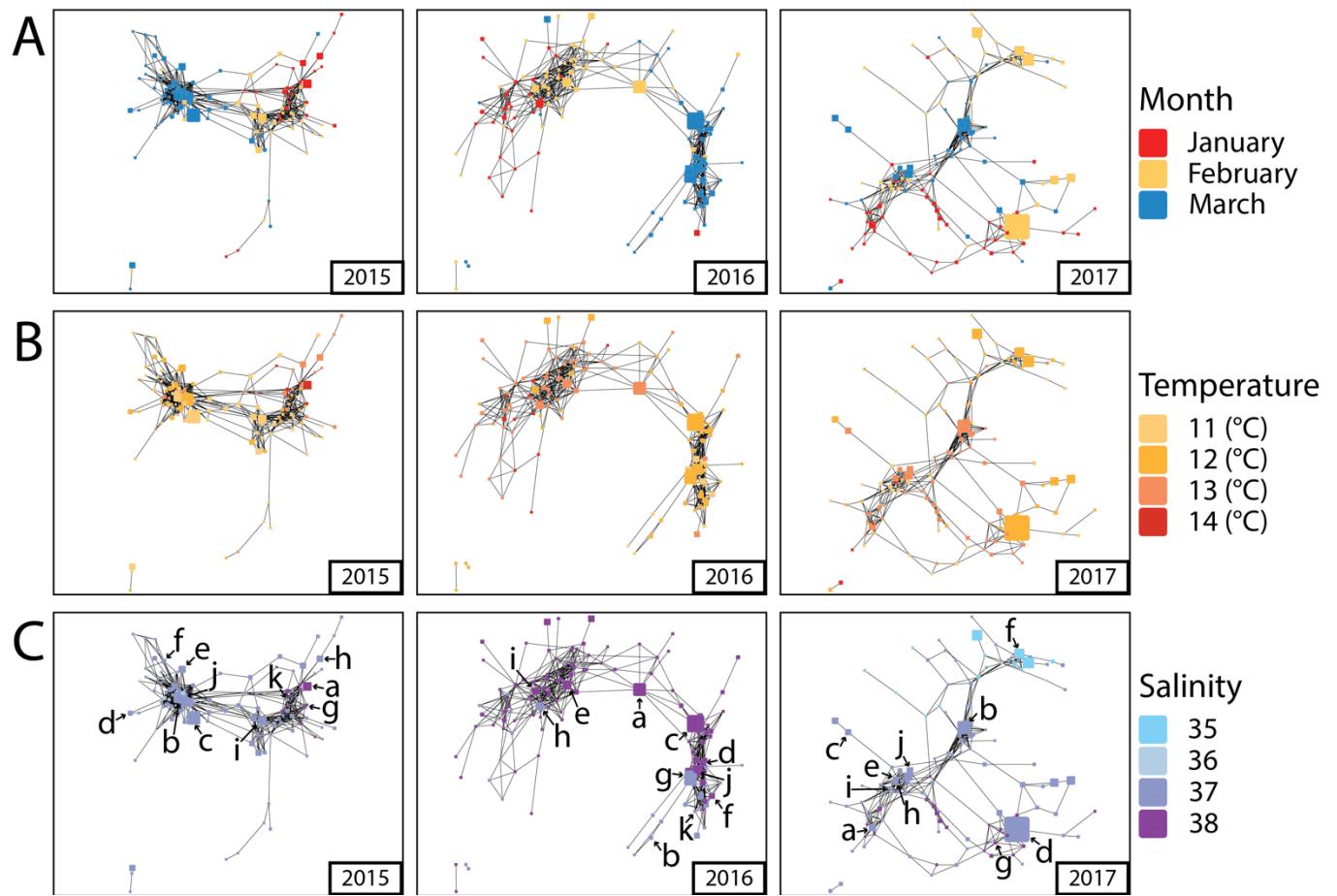
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Fig. 1

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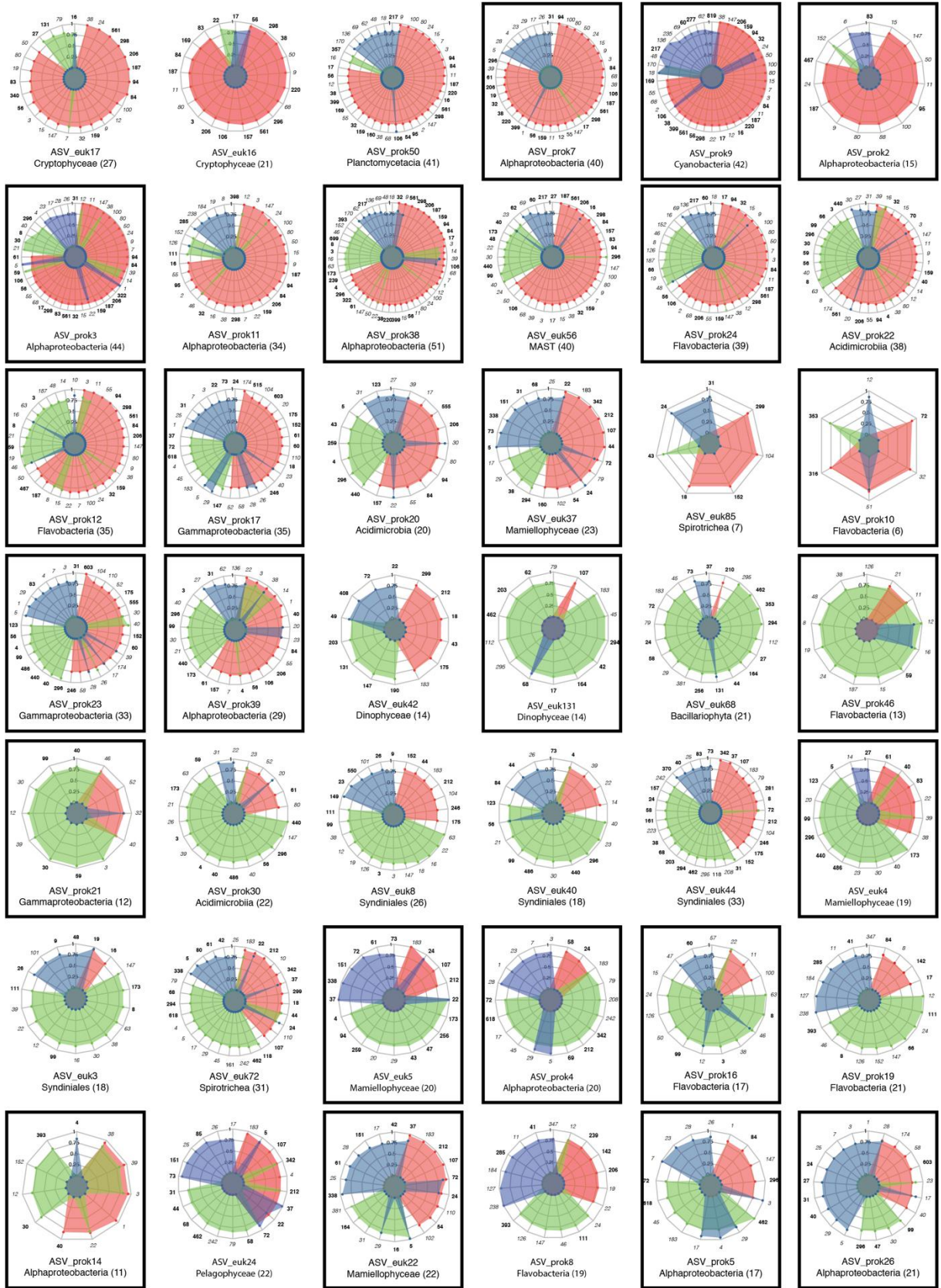


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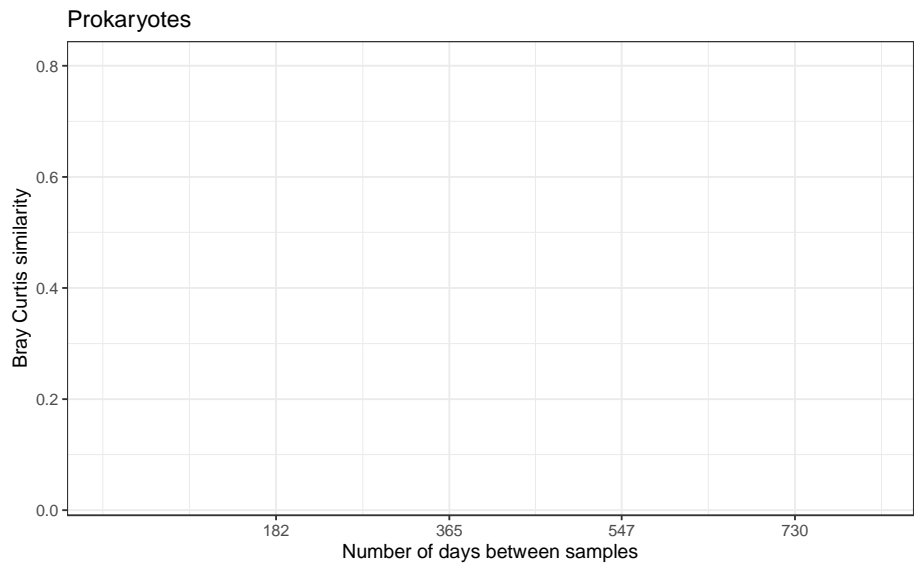
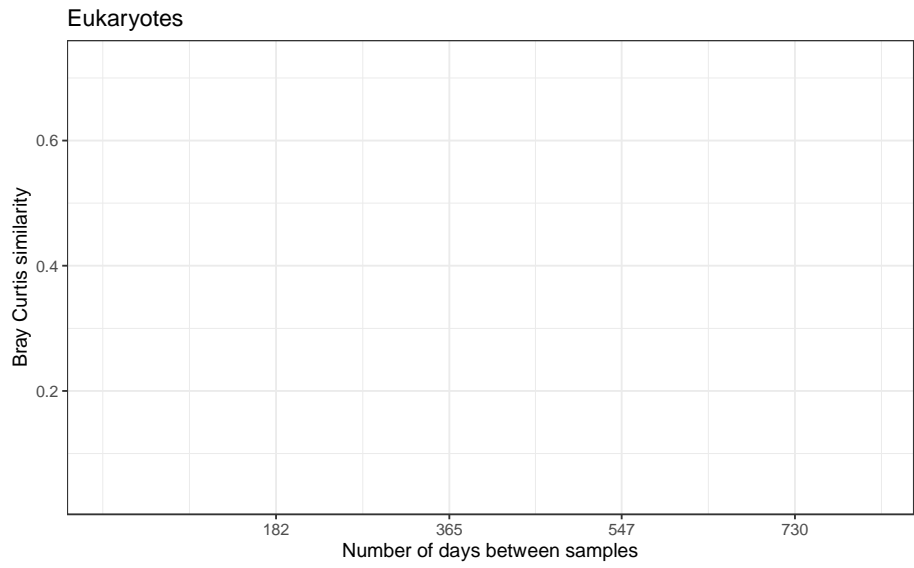
Fig 3



● 2015 ● 2016 ● 2017

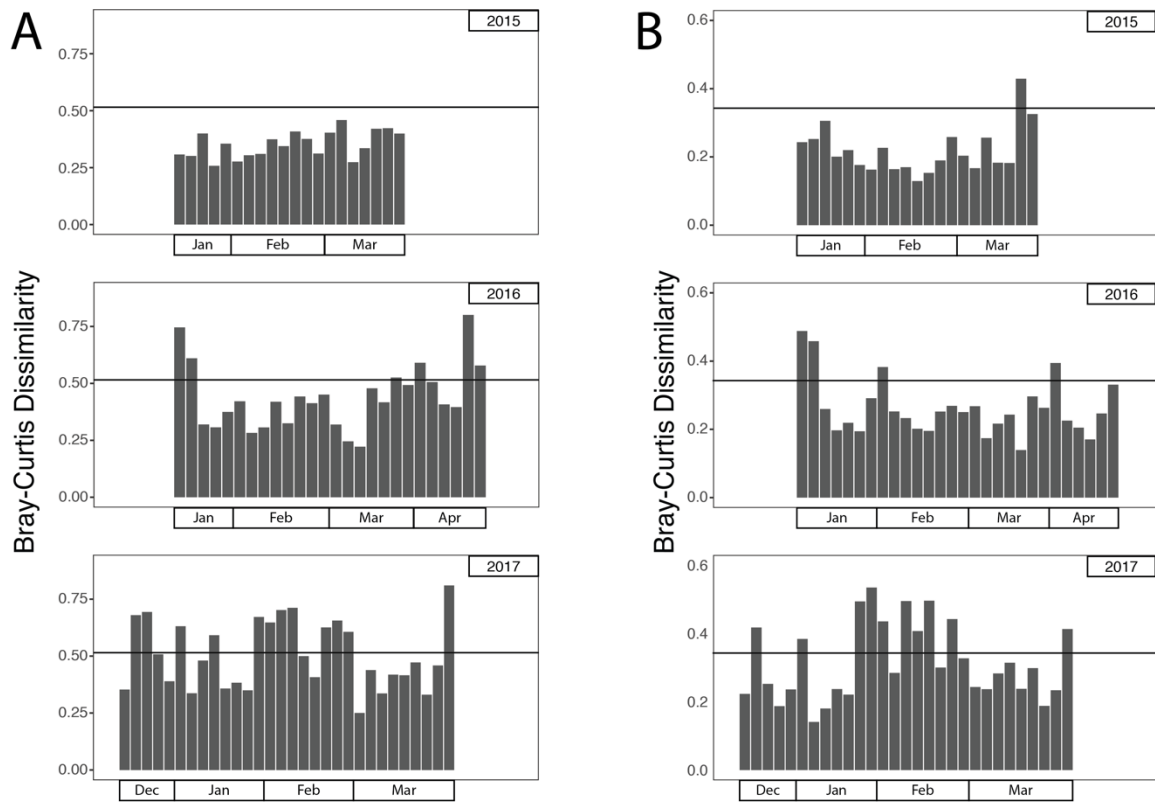
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Fig 4



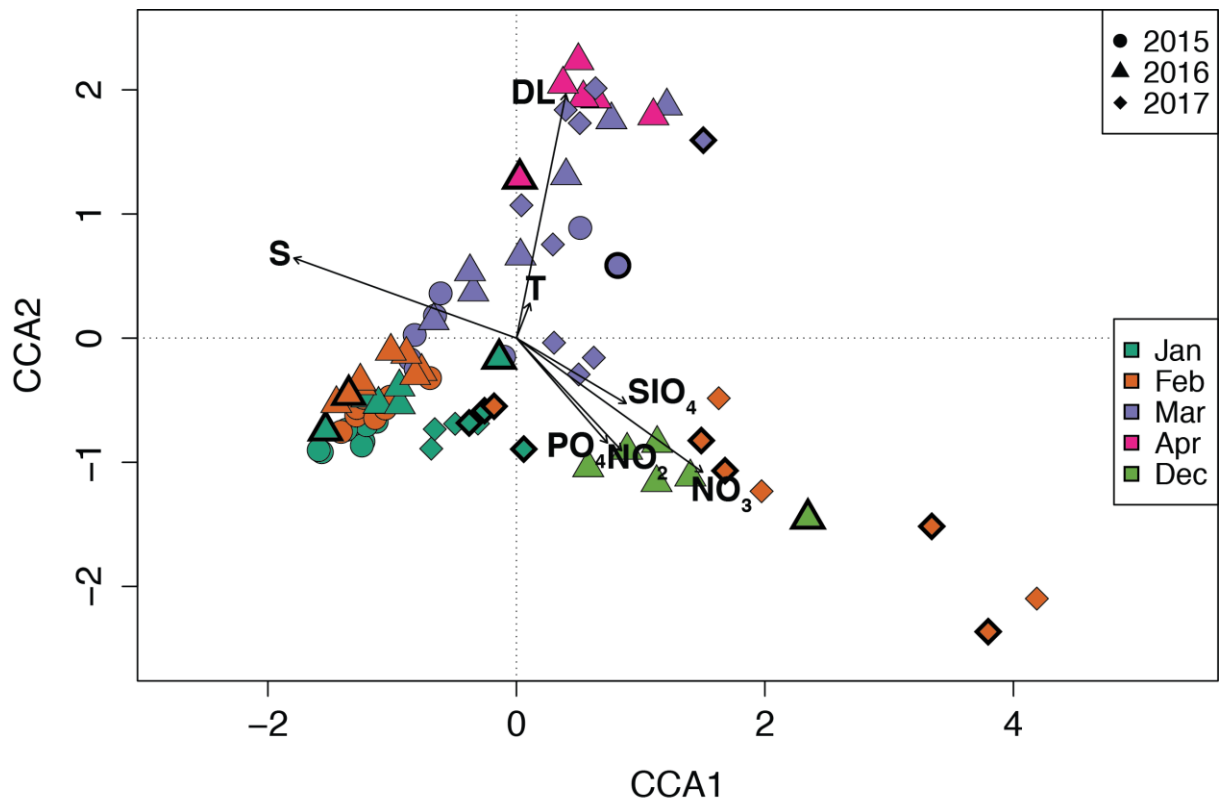
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757 Supplementary Fig. 1





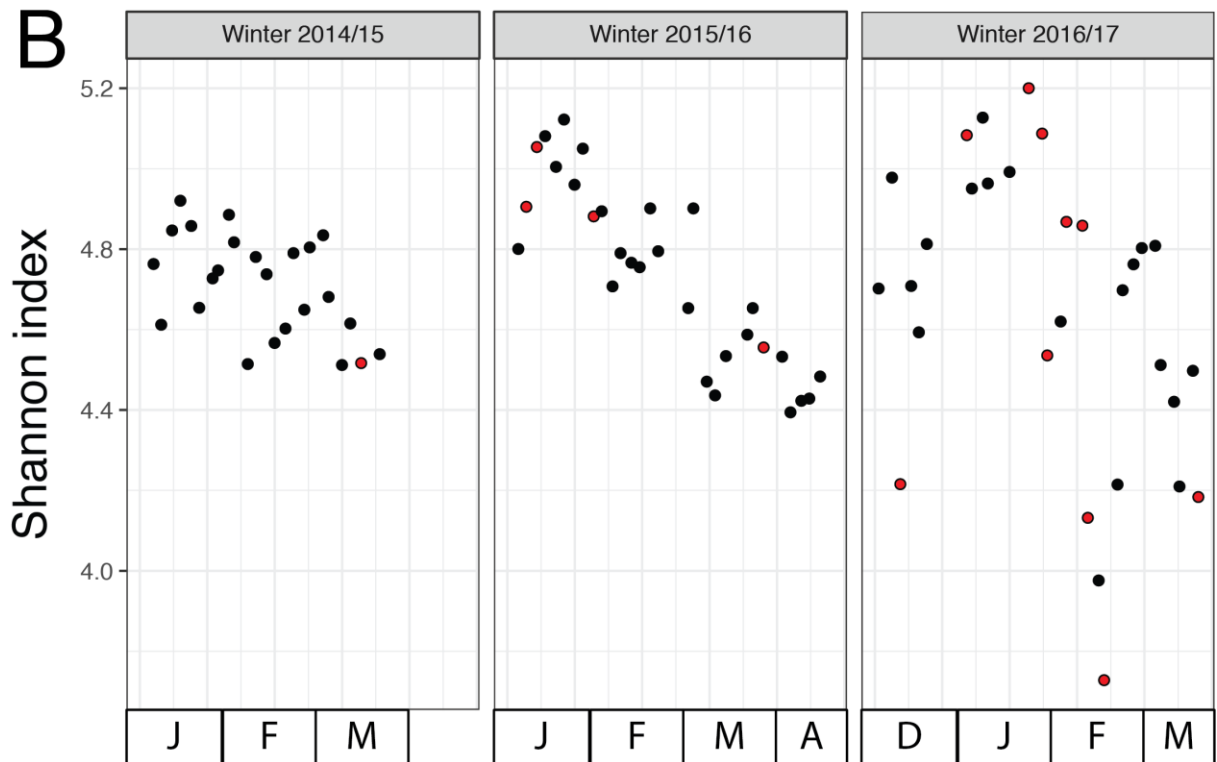
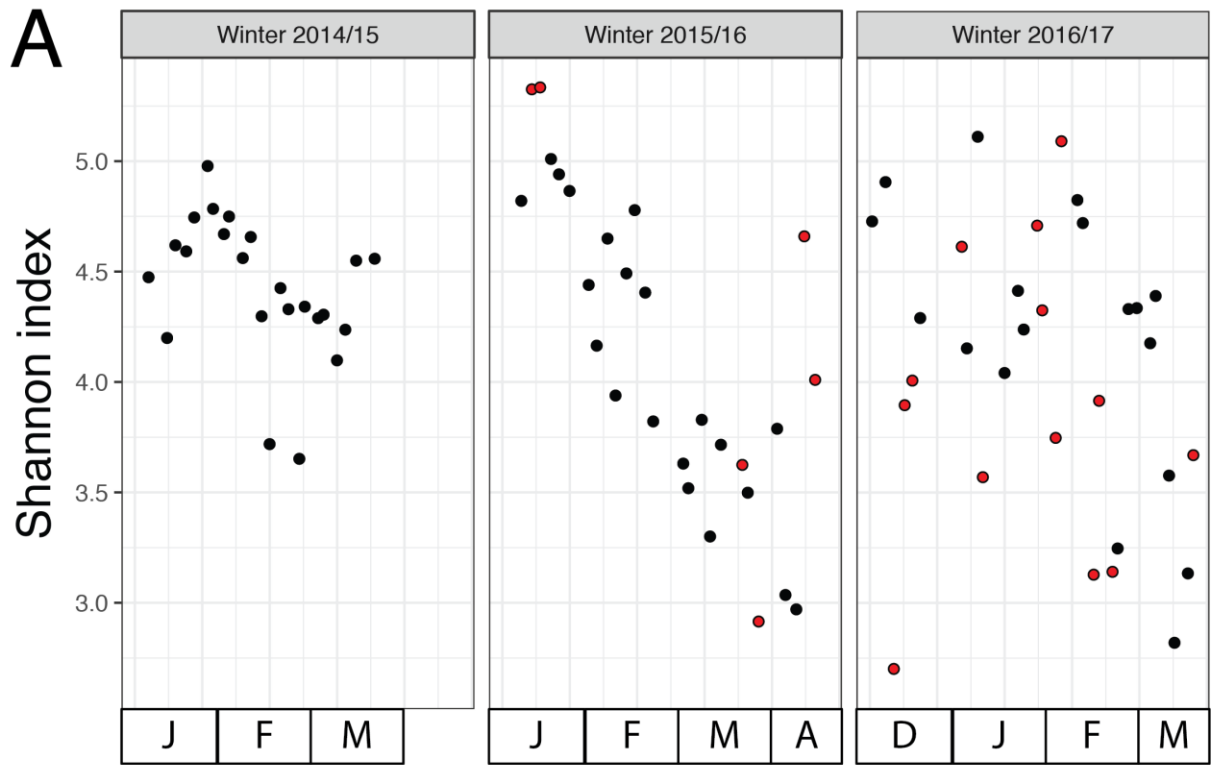
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Supplementary Fig. 2



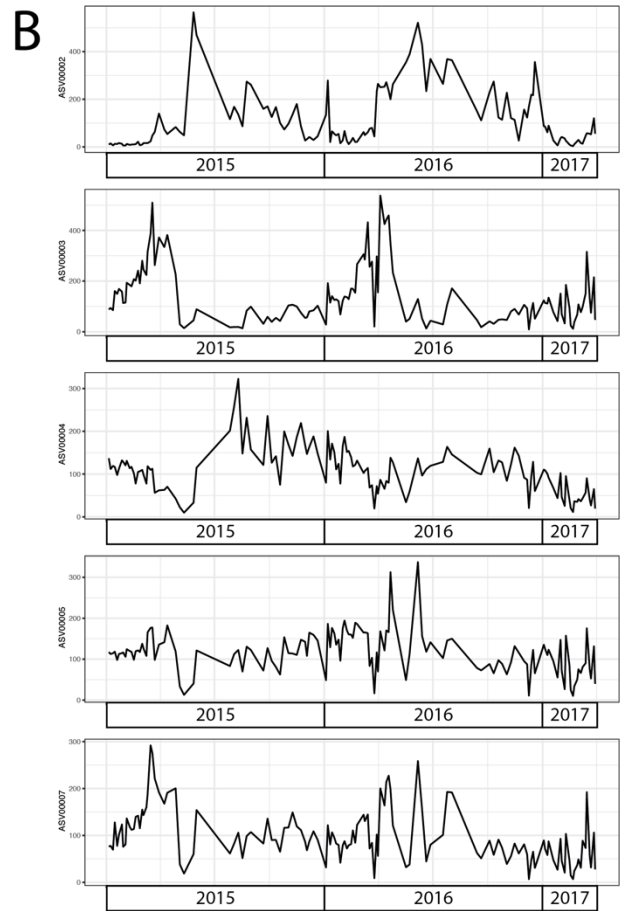
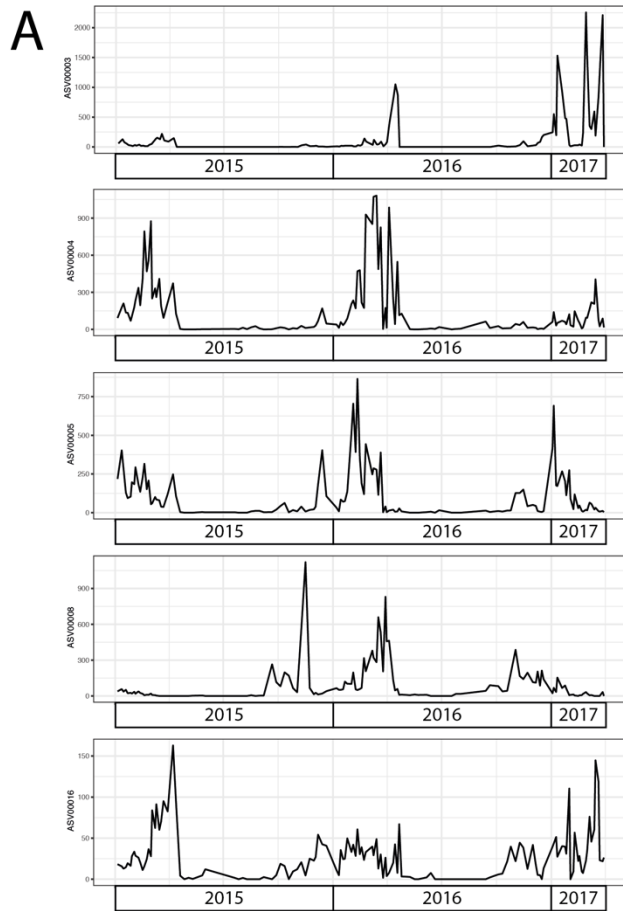
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Supplementary Fig. 3



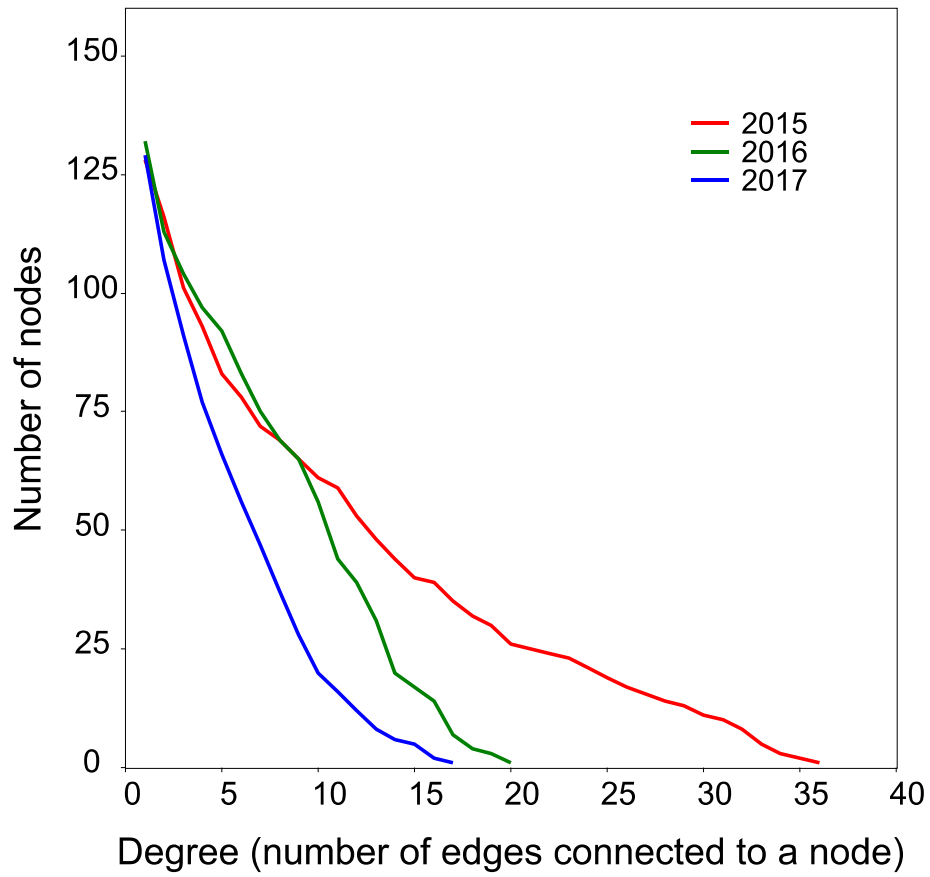
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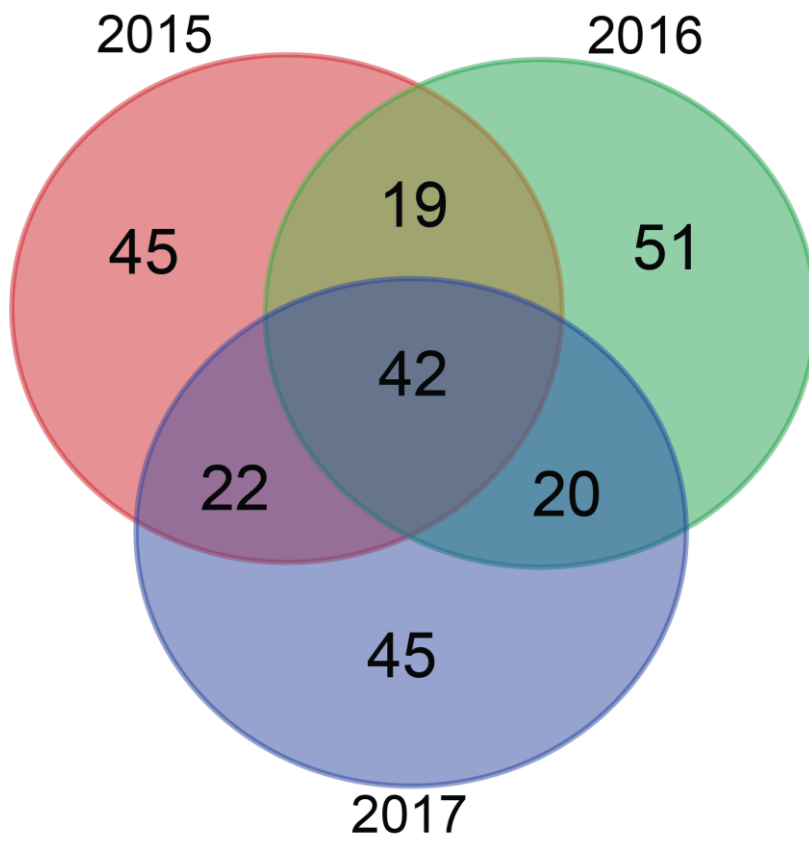


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Supplementary Fig. 5



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 774 Supplementary Fig. 6  
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Supplementary Fig. 7