

Disentangling temporal associations in marine microbial networks

Ina Maria Deutschmann, Anders Krabberød, Francisco Latorre, Erwan Delage, Cèlia Marrasé, Vanessa Balagué, Josep Gasol, Ramon Massana, Damien Eveillard, Samuel Chaffron, et al.

▶ To cite this version:

Ina Maria Deutschmann, Anders Krabberød, Francisco Latorre, Erwan Delage, Cèlia Marrasé, et al.. Disentangling temporal associations in marine microbial networks. 2022. hal-03781901v1

HAL Id: hal-03781901 https://hal.science/hal-03781901v1

Preprint submitted on 16 Nov 2022 (v1), last revised 7 Sep 2023 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Deutschmann et al.

1	Disentangling temporal associations in marine microbial
2	networks
3	
4	Ina Maria Deutschmann ^{1*} (ina.m.deutschmann@gmail.com), Anders K. Krabberød ²
5	(a.k.krabberod@ibv.uio.no), Francisco Latorre ¹ (latorre@icm.csic.es), Erwan Delage ^{3,4}
6 7	(erwan.delage@univ-nantes.fr), Célia Marrasé (celia@icm.csic.es), Vanessa Balagué ¹
8	(volague@icm.csic.es), Josep W. Gasor (pepgasor@icm.csic.es), Kanton Wassana (ramonm@icm.csic.es) Damien Eveillard ^{3,4} (damien eveillard@univ-nantes fr) Samuel Chaffron ^{3,4}
9	(samuel.chaffron@univ-nantes.fr), Ramiro Logares ^{1*} (ramiro.logares@icm.csic.es)
10	
11	
12 13	¹ Institute of Marine Sciences, CSIC, Passeig Marítim de la Barceloneta, 37-49, 08003, Barcelona, Spain.
14 15	² Department of Biosciences/Section for Genetics and Evolutionary Biology (EVOGENE), University of Oslo, p.b. 1066 Blindern, N-0316, Oslo, Norway.
16 17	³ Nantes Université, École Centrale Nantes, CNRS, LS2N, UMR 6004, F-44000 Nantes, France.
18 19	⁴ Research Federation for the Study of Global Ocean Systems Ecology and Evolution, FR2022/Tara Oceans GOSEE, F-75016 Paris, France.
20	
21	
22	*Corresponding authors
23	
24	
25 26	The authors declare that they have no competing interests.
20 27	
28	
29	
30	
31	
32	
33	
34	
35	

Deutschmann et al.

36 ABSTRACT

37 Background

38 Microbial interactions are fundamental for Earth's ecosystem functioning and 39 biogeochemical cycling. Nevertheless, they are challenging to identify and remain barely 40 known. Omics-based censuses are helpful in predicting microbial interactions through the 41 statistical inference of single (static) association networks. Yet, microbial interactions are 42 dynamic and we have limited knowledge of how they change over time. Here we investigate 43 the dynamics of microbial associations in a 10-year marine time series in the Mediterranean 44 Sea using an approach inferring a time-resolved (temporal) network from a single static 45 network.

46

47 *Results*

48 A single static network including microbial eukaryotes and bacteria was built using 49 metabarcoding data derived from 120 monthly samples. For the decade, we aimed to identify 50 persistent, seasonal, and temporary microbial associations by determining a temporal 51 network that captures the interactome of each individual sample. We found that the temporal 52 network appears to follow an annual cycle, collapsing and reassembling when transiting 53 between colder and warmer waters. We observed higher association repeatability in colder 54 than in warmer months. Only 16 associations could be validated using observations reported 55 in literature, underlining our knowledge gap in marine microbial ecological interactions.

56

57 *Conclusions*

Our results indicate that marine microbial associations follow recurrent temporal dynamicsin temperate zones, which need to be accounted for to better understand the functioning of

Deutschmann et al.

60	the ocean microbiome. The constructed marine temporal network may serve as a resource for						
61	testing season-specific microbial interaction hypotheses. The applied approach can be						
62	transferred to microbiome studies in other ecosystems.						
63							
64	Keywords: association network; temporal network; time series; microbial interactions;						
65	microorganisms; ocean; plankton						

66

67 **INTRODUCTION**

68 Microorganisms are the most abundant life forms on Earth, being fundamental for global 69 ecosystem functioning [1–3]. The total number of microorganisms on the planet is estimated 70 to be $\approx 10^{12}$ species [4] and $\approx 10^{30}$ cells [5, 6]. In particular, microorganisms dominate the 71 largest biome, the ocean, which harbors $\approx 10^{29}$ microbial cells [6] accounting for ~70% of

the total marine biomass [7, 8].

73 Microbial communities are highly dynamic and their composition is determined 74 through a combination of ecological processes: selection, dispersal, drift, and speciation [9]. 75 Selection is a prominent community structuring force exerted via multiple abiotic and biotic 76 factors [10, 11]. Several studies have addressed the role of *abiotic* factors in structuring 77 microbial communities. For example, temperature is one of the main factors exerting 78 selection in the ocean microbiome over spatiotemporal scales [12–15]. *Biotic* factors can also 79 strongly affect microbial communities [16]. However, a mechanistic understanding of how 80 they affect community structure is currently lacking, as the diversity of microbial interactions 81 is barely known [3, 17].

82

The vast microbial diversity and the fact that most microorganisms are still uncultured

Deutschmann et al.

[18, 19] make it impossible to experimentally test all potential interactions between pairs of
microbes. However, omics-technologies allow estimating microbial relative abundances over
spatiotemporal scales, which permits determining statistical associations between taxa. These
associations can be summarized as a network with nodes representing microorganisms and
edges representing potential interactions [20, 21].

88 As microorganisms are highly interconnected [21], association networks provide a 89 general overview of the entire microbial system and have been tremendously valuable for 90 generating novel hypotheses about putative interactions. In particular, time series have 91 allowed identifying potential ecological interactions among marine microorganisms [22–28]. 92 For example, previous work characterized ecological links between marine archaea, bacteria, 93 and eukaryotes [22], including links with viruses [24, 26], also investigating within- and 94 between ocean-depth relationships [25, 27]. These studies not only identified time-dependent 95 associations among ecologically important taxa, but also potential synergistic or antagonistic 96 relationships, as well as possible 'keystone' species and potential niches [22, 23]. Moreover, 97 several studies have reported more associations among microorganisms than between 98 microorganisms and environmental variables, suggesting the importance of biotic 99 relationships in structuring microbial community assemblages [22, 28].

Previous studies have used temporal microbial abundance data to infer static networks summarizing all potential associations in space and time. This static abstraction assumes that the network topology does not change (static) and edges represent persistent associations assumed as interactions [29]; that is, edges are present throughout time and space. This assumption cannot represent the reality of most microbial interactions. Thus, a single static network usually contains persistent, temporary, and recurring (including seasonal) associations that need to be disentangled.

Deutschmann et al.

107 Despite the contribution of static networks to our understanding of microbial 108 interactions in the ocean, it is necessary to incorporate the temporal dimension. Using a time-109 resolved, i.e., temporal network instead of a single static network would allow investigation 110 of the dynamic nature of microbial associations and how they change over time, whether the 111 change is deterministic or stochastic, and how environmental selection influences network 112 architecture. Addressing these questions is fundamental for a better understanding of the 113 dynamic interactions that underpin ecosystem function in the ocean. Here, we investigated 114 marine microbial associations through time by determining a temporal network from a single 115 static network.

116

117 MATERIALS AND METHODS

118 The Blanes Bay Microbial Observatory (BBMO)

119 The BBMO is a coastal oligotrophic site in the North-Western Mediterranean Sea (41°40'N 120 2°48'E) without major natural disturbances and little anthropogenic pressure, except for the 121 construction of a nearby harbor between 2010 and 2012 [30, 31]. The seasonal cycle is 122 typical for a temperate coastal system [30], and the main environmental factors influencing 123 seasonal microbial succession have been well studied and are known [12]. Shortly, the water 124 column is slightly stratified in summer before it destabilizes and mixes with water from 125 offshore in late fall, increasing the availability of inorganic nutrients with maximum 126 concentrations in winter, between November and March. The high amount of nutrients and 127 increasing light induce phytoplankton blooms, mostly in late winter-early spring. During 128 summer, inorganic nutrients become limiting, the primary production is minimal, and 129 dissolved organic carbon accumulates [30].

Deutschmann et al.

130

131 From sampling to microbial relative abundances

132	We sampled surface water (≈ 1 m depth) monthly from January 2004 to December 2013 to
133	determine microbial community composition and also measured ten environmental variables,
134	which were previously described [13, 30]: water temperature (°C) and salinity (obtained in
135	situ with a SAIV-AS-SD204 Conductivity-Temperature-Depth probe), day-length (hours of
136	light), turbidity (Secchi depth in meters), total chlorophyll-a concentration (μ g/l, fluorometry
137	of acetone extracts after 150 ml filtration on GF/F filters [30]), and five inorganic nutrients:
138	PO4 ³⁻ , NH4 ⁺ , NO2 ⁻ , NO3 ⁻ and SiO2 (μ M, determined with an Alliance Evolution II
139	autoanalyzer [32]).

140 Sampling of microbial communities, DNA extraction, rRNA-gene amplification, 141 sequencing, and bioinformatic analyses are explained in detail in [28]. In short, 6 L of water 142 were prefiltered through a 200 µm nylon mesh and subsequently filtered through another 20 143 μ m nylon mesh and separated into nanoplankton (3 – 20 μ m) and picoplankton (0.2 – 3 μ m) 144 using a 3 µm and 0.2 µm pore-size polycarbonate and Sterivex filters, respectively. Then, the 145 DNA was extracted from the filters using a phenol-chloroform protocol [33], which has been 146 modified for purification with Amicon units (Millipore). We amplified the 18S rRNA genes 147 (V4 region) with the primers TAReukFWD1 and TAReukREV3 [34], and the 16S rRNA 148 genes (V4 region) with Bakt 341F [35] and 806RB [36]. Amplicons were sequenced in a 149 MiSeq platform (2x250bp) at RTL Genomics (Lubbock, Texas). Read quality control, 150 trimming, and inference of Operational Taxonomic Units (OTUs) delineated as Amplicon 151 Sequence Variants (ASVs) were done with DADA2 [37], v1.10.1, with the maximum number 152 of expected errors set to 2 and 4 for the forward and reverse reads, respectively.

Deutschmann et al.

153 Microbial sequence abundance tables were obtained for each size fraction for both 154 microbial eukaryotes and prokaryotes. Before merging the tables, we subsampled each table 155 to the lowest sequencing depth of 4907 reads with the *rrarefy* function from the Vegan R-156 package [38], v2.4-2, (see details in [28]). We excluded 29 nanoplankton samples (March 157 2004, February 2005, May 2010 - July 2012) due to suboptimal amplicon sequencing. In 158 these samples, abundances were estimated using seasonally aware missing value imputation 159 by the weighted moving average for time series as implemented in the *imputeTS* R-package, 160 v2.8 [39]. These imputed values did not introduce biases in the analyses [28].

161 Sequence taxonomy was inferred using the naïve Bayesian classifier method [40] 162 together with the SILVA database [41], v.132, as implemented in DADA2 [37]. Additionally, 163 eukaryotic microorganisms were BLASTed [42] against the Protist Ribosomal Reference 164 (PR2) database [43], v4.10.0. The PR2 classification was used when the taxonomic 165 assignment from SILVA and PR2 disagreed. We removed ASVs that were identified as 166 Metazoa, Streptophyta, plastids, mitochondria, and Archaea since the 341F-primer is not 167 optimal for recovering this domain [44]. Besides, Haptophyta is known to be missed by the 168 primer TAReukREV3 [45].

169 The resulting table contained 2924 ASVs (Table 1A). Next, we removed rare ASVs 170 keeping ASVs with sequence abundance sums above 100 reads and prevalence above 15% 171 of the samples, i.e., we considered taxa present in at least 19 months. The resulting table 172 contained 1782 ASVs (Table 1B). An ASV can appear twice, in the nanoplankton and 173 picoplankton size fractions. However, an ASV may be detected in both size fractions due to 174 dislodging cells or particles and filter clogging, which can introduce biases in our analysis. 175 To reduce these biases, and as done previously [28], we divided the abundance sum of the

Deutschmann et al.

176 larger by the smaller size fraction for each ASV appearing in both size fractions and set the 177 picoplankton abundances to zero if the ratio exceeded 2. Likewise, we set the nanoplankton 178 abundances to zero if the ratio was below 0.5. This operation removed two eukaryotic ASVs 179 and 41 bacterial ASVs from the nanoplankton, and 30 bacterial ASVs from the picoplankton

180 (Table 1C). The resulting abundance table was used for network inference.

181

182 From sequence abundances to the single static network

183 First, we constructed a preliminary network using the tool eLSA [46, 47], as done in [28, 48], 184 including default normalization and z-score transformation, using median and median absolute 185 deviation. Although we are aware of time-delayed interactions, we considered our 1-month 186 sampling interval too large for inferring time-delayed associations with a solid ecological basis, and focused on contemporary interactions between co-occurring microorganisms. 187 188 Using 2000 iterations, we estimated p-values with a mixed approach that performs a random 189 permutation test of a co-occurrence if the comparison's theoretical *p*-values are below 0.05. 190 The Bonferroni false discovery rate (q) was calculated based on the p-values using the 191 *p.adjust* function from the stats R-package [49]. We used the 0.001 significance threshold for 192 the p and q values, as suggested in other studies [20]. We refrained from using an association 193 strength threshold since it may not be appropriate to differentiate between true interactions 194 and environmentally-driven associations [48]. Furthermore, changing thresholds have been 195 shown to lead to different network properties [50]. The preliminary network contained 754 196 nodes and 29820 edges (24458, 82% positive, and 5362, 18% negative).

197 Second, for environmentally-driven edge detection, we applied EnDED [48], 198 combining the methods Interaction Information (with a 0.05 significance threshold and 199 10000 iterations) and Data Processing Inequality. We inserted artificial edges connecting

Deutschmann et al.

each node to each environmental parameter. We identified and removed 3315 (11.12%)
edges that were environmentally driven; 26505 edges (23405, 88.3% positive, and 3100,
11.7% negative) remained (Supplementary Tables 3 and 4).

203 Third, we determined the Jaccard index, *I*, for each microorganisms pair associated 204 through an edge, in order to remove associations between microorganisms that have a low 205 co-occurrence. Let S_i be the set of samples in which both microorganisms are present (sequence abundance above zero), and S_{ν} be the set of samples in which one or both 206 207 microorganisms are present. Then, we can calculate the Jaccard index as the fraction of 208 samples in which both appear (intersection) from the number of samples in which at least one appears (union): $J = S_i/S_u$. We chose J > 0.5 as in previous work [48], which removed 209 210 9879 edges and kept 16626 edges (16481, 99.1% positive and 145, 0.9% negative). We 211 removed isolated nodes, i.e., nodes without an associated partner in the network. The number 212 and fraction of retained reads are listed in Table 1. The resulting network is our single static 213 network.

214

215 *From the single static network to the temporal network*

We determined the temporal network comprising 120 sample-specific (monthly) subnetworks through the three conditions indicated below and visualized in Figure 1. The subnetworks are derived from the single static network and contain a node subset and an edge subset of the static network. Let *e* be an association between microorganisms *A* and *B*, with association duration $d = (t_1, t_2)$, i.e., the association starts at time point t_1 and ends at t_2 . Then, considering month *m*, the association *e* is present in the monthly subnetwork N_m , if

1) *e* is an association in the single static network,

Deutschmann et al.

- 223 2) the microorganisms A and B are present within month m, and
- 224 3) *m* is within the duration of association, i.e., $t_1 \le m \le t_2$.

225 With the 2^{nd} condition, we assumed that an association was present in a month if both 226 microorganisms were present, i.e., the microbial abundances were non-zero for that month. 227 However, we cannot assume that microbial co-occurrence is a sufficient condition for a 228 microbial interaction because different mechanisms influence species and interactions, and 229 the environmental filtering of species and interactions can differ [51]. Using only the species 230 occurrence assumption would increase association prevalence. To lower this bias, we also 231 required that the association was present in the static network, 1st condition, and within the association duration, 3rd condition, both inferred by eLSA [46, 47]. Lastly, we removed 232 233 isolated nodes from each monthly subnetwork.

234

235 Network analysis

236 We computed global network metrics to characterize the single static network and each 237 monthly subnetwork using the igraph R-package [52]. Some metrics tend to be more 238 correlated than others implying redundancy between them, grouping them into four groups 239 [53]. Thus, we selected one metric from each group: edge density, average path length, 240 transitivity, and assortativity based on node degree. In addition, we also computed the 241 average strength of positive associations between microorganisms using the mean, and 242 assortativity based on the nominal classification of nodes into bacteria and eukaryotes. 243 Assortativity (bacteria vs. eukaryotes) is positive if bacteria tend to connect with bacteria and 244 eukaryotes tend to connect with eukaryotes. It is negative if bacteria tend to connect to 245 eukaryotes and vice versa. We also quantified associations by calculating their prevalence as

Deutschmann et al.

246 the fraction of monthly subnetworks in which the association was present for all ten years 247 (recurrence), and monthly. We visualized highly prevalent associations with the *circlize* R-248 package [54]. We tested our hypotheses of environmental factors influencing network 249 topology by calculating the Spearman correlations between global network metrics and 250 environmental data. We used Holm's multiple test correction to adjust p-values [55], with the 251 function corr.test in the psych R-package [56]. We used Gephi [57], v.0.9.2, and the 252 Fruchterman Reingold Layout [58] for network visualizations.

253

254 Test of network construction tool

255 We have used eLSA to estimate the duration of an association, which we used as the third 256 condition (*m* is within the duration of association, i.e., $t_1 \le m \le t_2$) to infer the sample-specific

257 subnetworks. Other methods may perform better on compositional data such as ours [59] 258 (although this is not necessarily the case; see [60]). Therefore we tested another network 259 construction approach (FlashWeave [61]) for comparative purposes. FlashWeave performed 260 better than eLSA in some benchmark tests run by other authors, while eLSA performed better 261 than FlashWeave in other tests [61]. FlashWeave can handle sparse datasets taking zeros into 262 account and avoiding spurious correlations between ASVs that share many zeros. However, 263 it neglects the temporal variation. To control data compositionality [59], we applied a 264 centered-log-ratio transformation separately to the bacterial and eukaryotic read abundance 265 tables before merging them. Then, we inferred a network using FlashWeave [61], selecting 266 the options "heterogeneous" and "sensitive". We have run analyses including the 267 environmental data (10 variables; see above). The resulting network had 932 nodes and 1440 268 edges. Next, we determined a temporal network using conditions 1) and 2) but not 3) since

Deutschmann et al.

the temporal duration is not estimated by FlashWeave. FlashWeave results are used hereafter to compare against eLSA, although eLSA is kept as the main network construction tool in our work, given that it allows determination of the duration of the associations and there is no evidence suggesting a poor performance of this tool. Thus, unless specified otherwise, we refer to the static and temporal network determined by eLSA.

274

275 Cyanobacteria

276 Our dataset contained 19 cyanobacterial ASVs, which all appeared in the nano-, and nine in 277 the picoplankton. We blasted the sequences against the Cyanorak database [62], v.2. against 278 the nucleotide database containing all Synechococcus and Prochlorococcus RNAs with the 279 option -evalue 1.0e-5. We found 2812 sequences comprising 95 different ecotypes 280 (considering name, clade and subclade), with 93.84-100% identity. A total of 11 BBMO 281 ASVs obtained 63 hits with 100% identity, and within these 63 reference sequences there 282 were 34 different ecotypes. Most matching sequences were found for *Synechococcus* ASV 1. 283 While Synechococcus ASV 5 had only two 100% hits, they did not 100% match ASV 1 284 (Supplementary Table 5). Finding Synechococcus in both size fractions was against 285 expectations, as this genus is part of the pico-plankton. Yet, they have been observed in 286 fractions above 3 µm at BBMO [63]. Recovering Synechococcus ASVs from the 287 nanoplankton may be due to cell aggregation, particle attachment, clogging of filters, or being 288 prey to larger microorganisms. Synechococcus could be also picked up in the 3 µm filters 289 during cell division.

290

291 Validated associations

Deutschmann et al.

292 As a general rule, the validation of associations tends to be limited as both true interactions 293 and microorganisms that do not interact with each other are poorly known. As done in [48], 294 we determined true genus-genus interactions as those known in the literature, which are 295 compiled within the Protist Interaction Database, PIDA [17]. On October 15th 2019, PIDA 296 contained 2448 interactions. Although our dataset contains protists and bacteria, we could 297 not evaluate interactions between them through PIDA. The ambiguity in taxonomic 298 classification and the large number of edges challenged the validation. We validated 299 associations between microbial eukaryotes via exact string matching as done previously [48]. 300

301 **RESULTS**

302 *Extracting a temporal network from a single static association network*

303 From ten years of monthly samples from the Blanes Bay Microbial Observatory (BBMO) in 304 the Mediterranean Sea [30], we computed sequence abundances for 488 bacteria and 1005 305 microbial eukaryotes from two organismal size-fractions: picoplankton $(0.2 - 3 \mu m)$ and 306 nanoplankton $(3 - 20 \,\mu\text{m})$. We removed Archaea since they are not very abundant in the 307 BBMO surface and primers were not optimal to quantify them. We inferred Amplicon 308 Sequence Variants (ASVs) using the 16S and 18S rRNA-gene. After filtering the initial ASV 309 table for sequence abundance and shared taxa among size fractions, we kept 285 and 417 310 bacterial and 526 and 481 eukaryotic ASVs in the pico- and nanoplankton size fractions, 311 respectively. We found 214 bacterial ASVs that appeared in both size fractions, but only two 312 eukaryotic ASVs: a Cryothecomonas (Cercozoa) and a dinoflagellate (Alveolate).

313 We used 1709 ASVs to infer a preliminary association network with the tool eLSA 314 [46, 47]. Next, we removed environmentally-driven edges with EnDED [48]. We only

Deutschmann et al.

315 considered edges involving partners that co-occurred more than half of the times together 316 than alone (see Methods and Figure 1A-B). Our filtering strategy removed a higher fraction 317 of negative than positive edges (see Methods and Supplementary Table 1). The resulting 318 network is our single static network connecting 709 nodes via 16626 edges (16481 edges, 319 99.1%, positive and 145, 0.9% negative).

320 Next, we developed an approach to determine a temporal network. Building upon the 321 single static network, we determined 120 sample-specific (monthly) subnetworks (see 322 Methods for details). These monthly subnetworks represent the 120 months of the time series 323 and together comprise the temporal network. Each monthly subnetwork contains a subset of 324 the nodes and a subset of the edges of the single static network. We used the ASV abundances 325 indicating the presence (ASV abundance > 0) or absence (ASV abundance = 0) as well as the 326 estimated start and duration of associations inferred with the network construction tool eLSA 327 [46, 47] for determining which nodes and edges are present each month (Figure 1, see 328 Methods).

329

330 The single static network metrics differed from most monthly subnetworks

Since each monthly subnetwork was derived from the single static network, they were smaller, containing between 141 (August 2005) and 571 (January 2012) nodes, median \approx 354 (Figure 2A), and between 560 (April 2006) to 15704 (January 2012) edges, median \approx 6052 (Figure 2B). For further characterization, we computed six global network metrics (Figure 2C and Methods). The results indicated that the single static network differed from most monthly subnetworks and it also differed from the average. In general, the single static network was less connected (edge density) and more clustered (transitivity) with higher

Deutschmann et al.

distances between nodes (average path length) and stronger associations (average positive
association score) than most monthly subnetworks (Figure 2C). In addition, the single static
network was usually more assortative according to the node degree but less assortative
according to the domain (bacteria vs. eukaryote) than most monthly subnetworks (Figure
2C). High assortativity indicates that nodes tend to connect to nodes of a similar degree and
domain.

344

345 Monthly subnetworks display seasonal behavior with yearly periodicity

346 Over the analyzed decade, the network became more connected and clustered in colder

347 months, with stronger associations and shorter distances between nodes (Figure 2C,

348 Supplementary Figures 1 and 2). Most global network metrics indicated seasonal behavior

349 with yearly periodicity (Figure 2C). For instance, edge density, average positive association

350 score, and transitivity were highest at the beginning and end of each year, while average

351 path length and assortativity (bacteria vs. eukaryotes) were highest in the middle of each

352 year. Assortativity (degree), in contrast to other metrics, usually had two peaks per year

353 corresponding to April-May, and November (Figure 2C). Some metrics (number of nodes

and edges, and average path length) presented similar seasonal behavior with yearly

355 periodicity in the temporal network determined from the single static FlashWeave network

356 (Supplementary Figure 3). However, edge density and transitivity displayed patterns

357 contrary to those observed in the temporal network determined from the stingle static eLSA

anetwork.

We found mainly temperature and day length, and to a lesser extent nutrient concentrations (mainly SiO₂, NO₃⁻ and NO₂⁻, less PO₄³⁻), and total chlorophyll-a

Deutschmann et al.

361 concentration to affect network topologies as indicated by correlation analyses 362 (Supplementary Figure 2). For example, edge density was highest and temperature lowest in 363 January-March. Then, edge density dropped as temperature increased. April-June displayed 364 edge densities slightly above or similar to those in the warmest months July-September, while 365 October-December had similar or slightly lower edge densities than the coldest months 366 January-March. Edge density vs. hours of light (day length) indicated a yearly recurrent 367 circular pattern for September-April (Supplementary Figure 1). Yet, May-August were not 368 part of the circular pattern. May-August had the highest day length and their corresponding 369 networks low edge density (Supplementary Figure 1).

370 Next, we quantified how many edges were preserved (kept), lost, and gained (new) 371 in consecutive months. We found the highest loss of edges in April, pointing to a network collapse. The overall number of edges (preserved and gained) was lowest during April-372 373 September and increased towards the end of each year (Figure 2B). The number of 374 associations changed over time in a yearly recurring pattern with few associations being 375 preserved when transitioning from colder to warmer waters. We observed a steep network 376 change when transiting from colder to warmer months, reflecting a large reorganization. In 377 turn, the network change from warmer to colder months was less abrupt. Thus, network 378 change between cold and warm waters was not symmetrical over the studied decade at 379 BBMO.

We defined summer and winter as in [28] and compared both seasons between consecutive years in terms of preserved, gained, and lost associations and ASVs. We observed higher repeatability in edges (Supplementary Figure 4) and ASVs (results not shown) in colder than in warmer months, indicating higher predictability during lowtemperature seasons.

Deutschmann et al.

385

386 *Potential core associations*

387 A single static network can comprise permanent, seasonal, and temporary associations. By 388 comparing monthly subnetworks, we identified edges that remain (preserved), appear 389 (gained), or disappear (lost) over time (Figure 2B). Intuitively, we would classify permanent 390 associations through 100% recurrence. However, no association fulfilled the 100% criteria. 391 Most associations had a low recurrence, with three-quarters of the associations present in no 392 more than 38% (total 46) of the monthly subnetworks. The average association prevalence 393 was similar across taxonomic ranks (Supplementary Figure 5). Considering the 100 most 394 prevalent associations, which appeared in 71.7-98.3% (total 86-118) of the monthly 395 subnetworks, 87 were associations among bacteria (Supplementary Table 2).

396 Although the temporal recurrence of associations over the ten years was low, we 397 found high recurrence in corresponding months from different years. We quantified the 398 fraction of subnetworks in which each association appeared (Supplementary Figure 6). We 399 observed the highest prevalence from December to March, and the lowest prevalence from 400 June to August (Supplementary Figure 6). For each month, we taxonomically characterized 401 prevalent associations appearing in at least nine out of the ten monthly subnetworks (e.g., 9 402 out of 10 Januarys; Figure 3). We found a larger number of prevalent associations in colder 403 waters compared to warmer waters, with Alphaproteobacteria dominating these associations, 404 especially in April and May (Figure 3). The Alphaproteobacteria ASVs featuring highly 405 prevalent associations belonged to Pelagibacter ubique (SAR11 Clades Ia & II), 406 Rhodobacteraceae, Amylibacter, Puniceispirillales (SAR116), Ascidiaceihabitans, Planktomarina, Parvibaculales (OCS116), and Kiloniella. Between April and May, we 407

Deutschmann et al.

408 noticed a large increase in the fraction of associations including Cyanobacteria or 409 Bacteroidetes as association partners. While Cyanobacteria associations were a small fraction 410 during November-April, they had a dominant role from May-October along with 411 Bacteroidetes and Alphaproteobacteria associations (Figure 3). Overall, this underlines the dynamic 412 nature of associations over the year, pointing to recurring annual associations that may be essential for 413 ecosystem function.

414

415 Dynamic associations within main taxonomic groups: the case of Cyanobacteria

416 Our results indicated that associations are dynamic within specific taxonomic groups. 417 Therefore, we investigated their behavior in Cyanobacteria given the importance of this 418 group as primary producers in the ocean. We found 661 associations for *Synechococcus*, Prochlorococcus, and Cyanobium ASVs (Figure 4 and Supplementary Figure 7). Most 419 420 associations between cyanobacterial ASVs were positive (63 of 65), and only a 421 Synechococcus (referred to as bn ASV 5) was negatively associated (association score -0.5) 422 with other Synechococcus (bn_ASV_1 and bn_ASV_25), which, in turn, were positively 423 associated (association score 0.8). While bn_ASV_5 appeared mainly in colder months, the 424 other two appeared mainly in warmer months (Supplementary Figure 7). All Cyanobacteria 425 had more associations with other bacteria (in total 433) than with eukaryotes (in total 163).

Within the temporal network, the fraction of Cyanobacteria associations was highest in April-October (Figure 4A), which are the months with the largest cyanobacterial abundances (Supplementary Figure 7), and the fewest edges in the entire temporal network (Figure 2B), for example, in the year 2011 (Figure 4B). We found that cyanobacterial ASVs, although being evolutionarily related, behaved differently in terms of the number of associations over time, and association partners (Supplementary Figure 7). For example,

Deutschmann et al.

432 Synechococcus bn ASV 5 had fewer partners than bn ASV 1 according to numbers of 433 associations, but more according to taxonomic variety (Supplementary Figure 7). Only a tiny 434 fraction of Prochlorococcus (e.g. bp ASV 18) association partners were other 435 Cyanobacteria, which contrasted with Synechococcus and Cyanobium (Supplementary 436 Figure 7). Moreover, we observed that Cyanobium (bn_ASV_20) connected to one 437 Deltaproteobacteria (SAR324) ASV during the first eight years, but the association 438 disappeared in the last two years. In particular, the inferred association duration was 101 439 months, starting in March 2004 and ending in July 2012. After summer 2012, the 440 Deltaproteobacteria ASV was not detected except a few reads in November and December 441 2012 and 2013. This Cyanobacteria example may also illustrate the dynamics of associations 442 within other main taxonomic groups.

443

444 Validating associations using known ecological interactions

445 We checked how many potential interactions could be validated using a database of observed 446 ecological interactions (PIDA; [17]). In total, 16 associations (out of 16626) in the temporal 447 network were validated by PIDA (Supplementary Table 6). These 16 associations describe 448 six unique interactions between seven taxa (at the genus-level). For instance, the reoccurring 449 association between a diatom from genus *Thalassiosira* and a Flavobacteriia starts mainly 450 around October and often ends around March (Supplementary Figure 8). In contrast, the 451 reoccurring association between a dinoflagellate from genus Gyrodinium and one from 452 Heterocapsa appears for a shorter time and during the summer months (Supplementary 453 Figure 8).

Deutschmann et al.

455 **DISCUSSION**

456 Previous work identified yearly recurrence of microbial community composition at the 457 BBMO [13, 28, 64], and similarly at the nearby Bay of Banyuls [14], both in the North-West 458 Mediterranean Sea and in other temperate sites around the world [12, 65]. We focused here 459 in the connectivity of microorganisms and how they organize themselves from a network 460 perspective. In general, the measured global network metrics (edge density, transitivity, and 461 average path length) are within the range reported in previous studies [22–25, 66–68] (Table 462 2). Contrary to early studies reporting biological networks generally being disassortative 463 (negative assortativity based on degree) [69], our single static network and the monthly 464 subnetworks were assortative. Microorganisms had more and stronger connections and a 465 tighter clustering in colder than in warmer waters. To some extent, this might reflect species 466 richness, which has been shown for the resident microorganisms to increase during the colder 467 months at BBMO using the same dataset [28]. However, the exact effect of richness on 468 ecological interactions among microorganisms needs further analysis. Seasonal bacterial 469 freshwater networks [67] also showed higher clustering in fall and winter than in spring and 470 summer, but, in contrast to our results, networks were most extensive in summer and smallest 471 in winter. In agreement with our results, Chaffron et al. [68] reported higher association 472 strength, edge density, and transitivity in cold polar regions compared to other warmer 473 regions of the global ocean. Colder waters in the Mediterranean Sea are milder than polar 474 waters. However, together, these results suggest that either microorganisms interact more in 475 colder environments, or that their recurrence is higher due to higher environmental selection 476 exerted by low temperatures. Additionally, limited resources (mainly nutrients) in summer 477 or in the tropical and subtropical open ocean may prevent the establishment of several 478 microbial interactions. In any case, temperature is likely not the only driver of network

Deutschmann et al.

479 architecture [68].

480 The effects of environmental variables on network metrics are unclear [70], yet, our 481 approach allowed us to identify potential environmental drivers of network architecture. 482 Correlation analyses pointed to variables that have been found to influence microbial 483 abundances in the ocean. For instance, our results indicated that temperature and day length, 484 key variables driving microbial assemblages in seasonal time series [12-14], and to a lesser 485 extent inorganic nutrients, were the main factors influencing global network metrics. It also 486 agrees with earlier works indicating that phosphorus and nitrogen are the primary limiting 487 nutrients in the Western Mediterranean Sea [71, 72]. Altogether, our correlation analysis is a 488 step forward towards elucidating the effects of environmental variables on network metrics. 489 However, we did not consider several other variables that could affect network architecture 490 (e.g. organic matter).

491 Our preliminary network (significant associations derived with eLSA) contained 18% 492 negative edges compared to 0.9% in the single static network (after filtering). Thus, our 493 filtering strategy removed proportionally more negative edges. Associations may represent 494 positive or negative interactions, but they can also indicate high niche overlap (positive 495 association) or divergent niches (negative association) between microorganisms [73]. We 496 hypothesize that most of the removed negative edges represented associations between 497 microorganisms from divergent niches, most likely corresponding to colder or warmer 498 months.

We found more highly prevalent associations within specific months than when considering all ten years of data. Furthermore, our results indicate a potentially low number of core interactions and a vast number of non-core ones. Usually, core microorganisms are defined based on sequence abundances, as those microorganisms (or taxonomical groups)

Deutschmann et al.

503 appearing in all samples or habitats being under investigation [74]. Shade & Handelsman 504 [74] suggested that other parameters, including connectivity, should create a more complex 505 portrait of the core microbiome and advance our understanding of the role of key 506 microorganisms and functions within and across ecosystems [74]. Using a temporal network 507 we identified core associations based on recurrence, which contributes to our understanding 508 of key interactions underpinning microbial ecosystem functions. Considering associations 509 within each month, we found more highly-prevalent associations in colder than in warmer 510 months. Our results indicate microbial connectivity is more repeatable (indicating higher 511 predictability) in colder than in warmer waters. On the one hand, the microbial community 512 in colder waters being more recurrent [13] may explain our observations indicating a more 513 robust connectivity during this period. Alternatively, it may be the stronger connectivity what 514 leads to more similar communities in colder waters at the BBMO. Last but not least, the 515 interplay of both species dynamics and interactions may determine community turnover in 516 the studied ecosystem. From a technical viewpoint, our monthly sampling strategy and/or the 517 overall single static network may have not been able to detect interactions appearing solely 518 in summer resulting in smaller monthly subnetworks. For instance, previous work on 519 freshwater lakes constructed season-specific networks and found more associations in 520 summer than in winter [67].

521 Several network-based analyses have been used to particularly study Cyanobacteria 522 associations. For example, in the southern Californian coast, Chow et al. [24] identified 44 523 potential relationships of 12 Cyanobacteria (*Prochlorococcus* and *Synechococcus*) with two 524 potential eukaryote grazers (a ciliate and a dinoflagellate), 39 to other bacteria, and three 525 between Cyanobacteria, which were all positive. Similarly, all cyanobacterial ASVs in our 526 study connected primarily to other bacterial ASVs, and featured mainly positive associations.

Deutschmann et al.

527 Furthermore, Cyanobacteria displayed primarily positive associations with other 528 microorganisms in a global ocean network [66]. This suggests that other sampling or 529 computational approaches are needed to detect negative associations involving marine 530 cyanobacteria.

531 Identifying different potential association partners for closely related Cyanobacteria 532 may indicate adaptations to different niches. A recent study found distinct seasonal patterns 533 for closely related bacterial taxa indicating niche partitioning at the BBMO, including 534 Synechococcus ASVs [64]. Our approach can complement and further characterize "sub-535 niches" by providing association partners for different ASVs. Moreover, in contrast to a 536 single static network, temporal networks allow identifying associated partners in time 537 (Supplementary Figure 7). An increase in the abundance of a microorganism may promote 538 the growth of associated partners and a decrease may hinder the growth of partners or cause 539 predators to prey on other microorganisms. Moreover, given the majority of association 540 partners being other bacteria, the growth of Cyanobacteria may affect other bacteria and their 541 growth, which is why it is necessary to identify potential interaction partners [67].

542 Our approach allowed us to disentangle in time the associations captured by a single 543 static network using monthly samples for ten years. Future studies should determine whether 544 higher sampling frequency (e.g., daily samples during a month) can capture other 545 associations not present in our networks. Thus, our results should be considered taking into 546 account the used (monthly) sampling frequency. In addition, certain network metrics may 547 depend on the tool used to infer the single static network, e.g., edge density, and, therefore, 548 should be interpreted with care. An additional consideration is that we disregarded local 549 network patterns by using global network metrics. Future work could use the local-550 topological metric based on graphlets [75]. Counting the number of graphlets a node is part

Deutschmann et al.

- 551 of quantifies their local connection patterns, which allows inferring seasonal microorganisms
- through recurring connection patterns in a temporal network.

553

554 CONCLUSION

Incorporating the temporal dimension in microbial association analysis unveiled multiple patterns that often remain hidden when using single static networks. Investigating a coastal marine microbial ecosystem over ten years revealed a one-year-periodicity in the network topology. The temporal network architecture was not stochastic, but displayed a modest amount of recurrence over time, especially in winter. Future efforts to understand the ocean microbiome should consider the dynamics of microbial interactions as these are likely fundamental for ecosystem functioning.

562

563 Ethics approval and consent to participate

564 Not applicable.

565

- 566 **Consent for publication**
- 567 Not applicable.
- 568

569 Availability of data and material

570 The BBMO microbial sequence abundances (ASV tables), taxonomic classifications, 571 environmental data including nutrients, networks and R-Markdowns for data analysis 572 including commands to run eLSA and EnDED (environmentally-driven-edge-detection and

573computingJaccardindex)arepubliclyavailable:574https://github.com/InaMariaDeutschmann/TemporalNetworkBBMO.

575

576 Competing interests

- 577 The authors declare that they have no competing interests.
- 578

579 Funding

580 This project and IMD received funding from the European Union's Horizon 2020 research 581 and innovation program under the Marie Skłodowska-Curie grant agreement no. 675752 582 (ESR2, http://www.singek.eu) to RL. RL was supported by a Ramón y Cajal fellowship 583 (RYC-2013-12554, MINECO, Spain). This work was also supported by the projects 584 INTERACTOMICS (CTM2015-69936-P, MINECO, Spain), MicroEcoSystems (240904, 585 RCN, Norway) and MINIME (PID2019-105775RB-I00, AEI, Spain) to RL. FL was 586 supported by the Spanish National Program FPI 2016 (BES-2016-076317, MICINN, Spain). 587 SC was supported by the CNRS MITI through the interdisciplinary program Modélisation 588 du Vivant (GOBITMAP grant). DE and SC were supported by the H2020 project AtlantECO 589 (award number 862923). A range of projects from the EU and the Spanish Ministry of 590 Science funded data collection and ancillary analyses at the BBMO. We acknowledge 591 funding of the Spanish government through the 'Severo Ochoa Centre of Excellence' 592 accreditation (CEX2019-000928-S).

593

594 Author's contributions

595 The overall project was conceived and designed by RL and AKK. VB, JMG, and RM were

Deutschmann et al.

responsible for the sampling and contextual data at the BBMO. RL processed the amplicon data from BBMO generating the ASV tables. AKK constructed the initial preliminary network which was the starting point of the present study. IMD developed the conceptual approach and DE, SC, and RL contributed to its finalization. IMD performed the data analysis. ED, DE, SC, FL, AKK, CM, JMG, and RL contributed with the biological interpretation of the results. IMD wrote the original draft. All authors contributed to manuscript revisions and approved the final version of the manuscript.

603

604 Acknowledgements

605 We thank all members of the Blanes Bay Microbial Observatory (http://bbmo.icm.csic.es)

team with the multiple projects funding this collaborative effort. Part of the analyses have

607 been performed at the Marbits bioinformatics core at ICM-CSIC

608 (https://marbits.icm.csic.es). We thank L. Felipe Benites for fruitful discussions regarding

- 609 microorganisms and their interactions.
- 610

611 **References**

- 612 1. Falkowski PG, Fenchel T, Delong EF. The Microbial Engines That Drive Earth's
- 613 Biogeochemical Cycles. Science. 2008;320:1034–9.
- 614 2. DeLong EF. The microbial ocean from genomes to biomes. Nature. 2009;459:200–6.
- 615 3. Krabberød AK, Bjorbækmo MFM, Shalchian-Tabrizi K, Logares R. Exploring the
- 616 oceanic microeukaryotic interactome with metaomics approaches. Aquatic Microbial
- 617 Ecology. 2017;79:1–12.
- 4. Locey KJ, Lennon JT. Scaling laws predict global microbial diversity. Proceedings of the
 National Academy of Sciences. 2016;113:5970–5.
- 5. Kallmeyer J, Pockalny R, Adhikari RR, Smith DC, D'Hondt S. Global distribution of
 microbial abundance and biomass in subseafloor sediment. Proceedings of the National
- 622 Academy of Sciences. 2012;109:16213–6.

Deutschmann et al.

- 623 6. Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: The unseen majority. Proceedings 624 of the National Academy of Sciences. 1998;95:6578–83.
- 7. Bar-On YM, Milo R. The Biomass Composition of the Oceans: A Blueprint of Our Blue
 Planet. Cell. 2019;179:1451–4.
- 8. Bar-On YM, Phillips R, Milo R. The biomass distribution on Earth. Proceedings of the
 National Academy of Sciences. 2018;115:6506–11.
- 9. Vellend M. The theory of ecological communities (MPB-57). Princeton UniversityPress; 2020.
- 631 10. Lindström ES, Langenheder S. Local and regional factors influencing bacterial
 632 community assembly. Environmental Microbiology Reports. 2012;4:1–9.
- 633 11. Mori AS, Isbell F, Seidl R. β-Diversity, Community Assembly, and Ecosystem
 634 Functioning. Trends in Ecology & Evolution. 2018;33:549–64.
- 635 12. Bunse C, Pinhassi J. Marine Bacterioplankton Seasonal Succession Dynamics. Trends
 636 in Microbiology. 2017;25:494–505.
- 637 13. Giner CR, Balagué V, Krabberød AK, Ferrera I, Reñé A, Garcés E, et al. Quantifying
 638 long-term recurrence in planktonic microbial eukaryotes. Molecular Ecology. 2019;28:923–
 639 35.
- 640 14. Lambert S, Tragin M, Lozano J-C, Ghiglione J-F, Vaulot D, Bouget F-Y, et al.
- 641 Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite irregular 642 environmental perturbations. The ISME Journal. 2019;13:388.
- 643 15. Logares R, Deutschmann IM, Junger PC, Giner CR, Krabberød AK, Schmidt TSB, et
- al. Disentangling the mechanisms shaping the surface ocean microbiota. Microbiome.2020;8:55.
- 646 16. Barraclough TG. How Do Species Interactions Affect Evolutionary Dynamics Across
 647 Whole Communities? Annu Rev Ecol Evol Syst. 2015;46:25–48.
- 17. Bjorbækmo MFM, Evenstad A, Røsæg LL, Krabberød AK, Logares R. The planktonic
- 649 protist interactome: where do we stand after a century of research? The ISME Journal.
- 650 2019. https://doi.org/10.1038/s41396-019-0542-5.
- 18. Baldauf SL. An overview of the phylogeny and diversity of eukaryotes. Journal ofSystematics and Evolution. 2008;46:263.
- 653 19. Lewis WH, Tahon G, Geesink P, Sousa DZ, Ettema TJG. Innovations to culturing the 654 uncultured microbial majority. Nature Reviews Microbiology. 2020.
- 655 https://doi.org/10.1038/s41579-020-00458-8.
- 656 20. Weiss S, Van Treuren W, Lozupone C, Faust K, Friedman J, Deng Y, et al. Correlation

Deutschmann et al.

- 657 detection strategies in microbial data sets vary widely in sensitivity and precision. The 658 ISME Journal. 2016;10:1669–81.
- 659 21. Layeghifard M, Hwang DM, Guttman DS. Disentangling Interactions in the
- 660 Microbiome: A Network Perspective. Trends in Microbiology. 2017;25:217–28.
- 22. Steele JA, Countway PD, Xia L, Vigil PD, Beman JM, Kim DY, et al. Marine bacterial,
 archaeal and protistan association networks reveal ecological linkages. The ISME Journal.
 2011;5:1414–25.
- 664 23. Chow C-ET, Sachdeva R, Cram JA, Steele JA, Needham DM, Patel A, et al. Temporal
- variability and coherence of euphotic zone bacterial communities over a decade in the
- 666 Southern California Bight. The ISME Journal. 2013;7:2259–73.
- 667 24. Chow C-ET, Kim DY, Sachdeva R, Caron DA, Fuhrman JA. Top-down controls on
- bacterial community structure: microbial network analysis of bacteria, T4-like viruses and
 protists. The ISME Journal. 2014;8:816–29.
- 670 25. Cram JA, Xia LC, Needham DM, Sachdeva R, Sun F, Fuhrman JA. Cross-depth

analysis of marine bacterial networks suggests downward propagation of temporal changes.
The ISME Journal. 2015;9:2573–86.

- 673 26. Needham DM, Sachdeva R, Fuhrman JA. Ecological dynamics and co-occurrence
 674 among marine phytoplankton, bacteria and myoviruses shows microdiversity matters. The
 675 ISME Journal. 2017;11:1614–29.
- 676 27. Parada AE, Fuhrman JA. Marine archaeal dynamics and interactions with the microbial
 677 community over 5 years from surface to seafloor. The ISME Journal. 2017;11:2510–25.
- 678 28. Krabberød AK, Deutschmann IM, Bjorbækmo MFM, Balagué V, Giner CR, Ferrera I,
- et al. Long-term patterns of an interconnected core marine microbiota. EnvironmentalMicrobiome. 2022;17:22.
- 681 29. Blonder B, Wey TW, Dornhaus A, James R, Sih A. Temporal dynamics and network
 682 analysis. Methods in Ecology and Evolution. 2012;3:958–72.
- 683 30. Gasol JM, Cardelús C, G Morán XA, Balagué V, Forn I, Marrasé C, et al. Seasonal
- 684 patterns in phytoplankton photosynthetic parameters and primary production at a coastal
- NW Mediterranean site. Scientia Marina. 2016;80:63–77.
- 686 31. Ferrera I, Reñé A, Funosas D, Camp J, Massana R, Gasol JM, et al. Assessment of
- microbial plankton diversity as an ecological indicator in the NW Mediterranean coast.
 Marine Pollution Bulletin. 2020;160:111691.
- 689 32. Grasshoff K, Kremling K, Ehrhardt M. Methods of seawater analysis. John Wiley &690 Sons; 2009.
- 691 33. Schauer M, Balagué V, Pedrós-Alió C, Massana R. Seasonal changes in the taxonomic

Deutschmann et al.

- 692 composition of bacterioplankton in a coastal oligotrophic system. Aquatic Microbial
 693 Ecology. 2003;31:163–74.
- 34. Stoeck T, Bass D, Nebel M, Christen R, Jones MDM, Breiner H-W, et al. Multiple
 marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic
 community in marine anoxic water. Molecular Ecology. 2010;19:21–31.
- 697 35. Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF.
- Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea.
 The ISME Journal. 2011;5:1571–9.
- 36. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA
 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic
- 702 Microbial Ecology. 2015;75:129–37.
- 37. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2:
- High-resolution sample inference from Illumina amplicon data. Nature Methods.
- 705 2016;13:581–3.
- 38. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan:Community Ecology Package. 2019.
- 39. Moritz S, Gatscha S. imputeTS: Time Series Missing Value Imputation. 2017.
- 40. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian Classifier for Rapid
- 710 Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and
- 711 Environmental Microbiology. 2007;73:5261–7.
- 41. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA
- ribosomal RNA gene database project: improved data processing and web-based tools.
- 714 Nucleic Acids Research. 2012;41:D590-6.
- 42. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search
 tool. Journal of Molecular Biology. 1990;215:403–10.
- 43. Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, et al. The Protist
- 718 Ribosomal Reference database (PR\$^2\$): a catalog of unicellular eukaryote Small Sub-
- 719 Unit rRNA sequences with curated taxonomy. Nucleic Acids Research. 2012;41:D597–
- 604.
- 44. McNichol J, Berube PM, Biller SJ, Fuhrman JA, Gilbert JA. Evaluating and Improving
- 722Small Subunit rRNA PCR Primer Coverage for Bacteria, Archaea, and Eukaryotes Using
- 723 Metagenomes from Global Ocean Surveys. mSystems. 2021;6:e00565-21.
- 45. Balzano S, Abs E, Leterme SC. Protist diversity along a salinity gradient in a coastal
 lagoon. Aquat Microb Ecol. 2015;74:263–77.
- 46. Xia LC, Steele JA, Cram JA, Cardon ZG, Simmons SL, Vallino JJ, et al. Extended local

Deutschmann et al.

- similarity analysis (eLSA) of microbial community and other time series data with
- replicates. BMC Systems Biology. 2011;5:S15.
- 47. Xia LC, Ai D, Cram J, Fuhrman JA, Sun F. Efficient statistical significance
- approximation for local similarity analysis of high-throughput time series data.
- 731 Bioinformatics. 2013;29:230–7.
- 48. Deutschmann IM, Lima-Mendez G, Krabberød AK, Raes J, Vallina SM, Faust K, et al.
- Disentangling environmental effects in microbial association networks. Microbiome.2021;9:232.
- 49. R Core Team. R: A Language and Environment for Statistical Computing. Vienna,
 Austria: R Foundation for Statistical Computing; 2019.
- 50. Connor N, Barberán A, Clauset A. Using null models to infer microbial co-occurrence
 networks. PLOS ONE. 2017;12:1–23.
- 51. Poisot T, Canard E, Mouillot D, Mouquet N, Gravel D. The dissimilarity of species
 interaction networks. Ecology Letters. 2012;15:1353–61.
- 52. Csardi G, Nepusz T. The igraph software package for complex network research.
 InterJournal. 2006;Complex Systems:1695.
- 53. Jamakovic A, Uhlig S. On the relationships between topological measures in real-world
 networks. Networks & Heterogeneous Media. 2008;3:345–59.
- 54. Gu Z, Gu L, Eils R, Schlesner M, Brors B. circlize implements and enhances circular
 visualization in R. Bioinformatics. 2014;30:2811–2.
- 55. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. ScandinavianJournal of Statistics. 1979;6:65–70.
- 56. Revelle W. psych: Procedures for Psychological, Psychometric, and PersonalityResearch. Evanston, Illinois: Northwestern University; 2020.
- 57. Bastian M, Heymann S, Jacomy M. Gephi: An Open Source Software for Exploringand Manipulating Networks. ICWSM. 2009;3.
- 58. Fruchterman TMJ, Reingold EM. Graph drawing by force-directed placement.
 Software: Practice and Experience. 1991;21:1129–64.
- 59. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome Datasets Are
 Compositional: And This Is Not Optional. Frontiers in Microbiology. 2017;8:2224.
- 60. Hirano H, Takemoto K. Difficulty in inferring microbial community structure based onco-occurrence network approaches. BMC Bioinformatics. 2019;20:329.
- 759 61. Tackmann J, Rodrigues JFM, von Mering C. Rapid Inference of Direct Interactions in
- 760 Large-Scale Ecological Networks from Heterogeneous Microbial Sequencing Data. Cell

Deutschmann et al.

761 Systems. 2019;9:286-296.e8.

62. Garczarek L, Guyet U, Doré H, Farrant GK, Hoebeke M, Brillet-Guéguen L, et al.
Cyanorak v2.1: a scalable information system dedicated to the visualization and expert
curation of marine and brackish picocyanobacteria genomes. Nucleic Acids Research.

765 2021;49:D667–76.

- 63. Mestre M, Höfer J, Sala MM, Gasol JM. Seasonal Variation of Bacterial Diversity
- Along the Marine Particulate Matter Continuum. Frontiers in Microbiology. 2020;11:1590.
- 64. Auladell A, Barberán A, Logares R, Garcés E, Gasol JM, Ferrera I. Seasonal niche
 differentiation among closely related marine bacteria. The ISME Journal. 2022;16:178–89.
- 65. Fuhrman JA, Cram JA, Needham DM. Marine microbial community dynamics and
 their ecological interpretation. Nature Reviews Microbiology. 2015;13:133–46.
- 66. Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, et al. Determinants
 of community structure in the global plankton interactome. Science. 2015;348:1262073.
- 67. Zhao D, Shen F, Zeng J, Huang R, Yu Z, Wu QL. Network analysis reveals seasonal
- variation of co-occurrence correlations between Cyanobacteria and other bacterioplankton.
 Science of The Total Environment. 2016;573:817–25.
- 68. Chaffron S, Delage E, Budinich M, Vintache D, Henry N, Nef C, et al. Environmental
 vulnerability of the global ocean epipelagic plankton community interactome. Sci Adv.
- 779 2021;7.
- 780 69. Newman MEJ. Assortative Mixing in Networks. Phys Rev Lett. 2002;89:208701.
- 781 70. Röttjers L, Faust K. From hairballs to hypotheses-biological insights from microbial
 782 networks. FEMS Microbiology Reviews. 2018;42:761–80.
- 783 71. Estrada M. Primary production in the northwestern Mediterranean. 1996.
- 784 72. Sala MM, Peters Francesc, Gasol JM, Pedrós-Alió C, Marrasé C, Vaqué D. Seasonal
- and spatial variations in the nutrient limitation of bacterioplankton growth in the
- northwestern Mediterranean. Aquatic Microbial Ecology. 2002;27:47–56.
- 787 73. Hernandez DJ, David AS, Menges ES, Searcy CA, Afkhami ME. Environmental stress
 788 destabilizes microbial networks. The ISME Journal. 2021. https://doi.org/10.1038/s41396789 020-00882-x.
- 74. Shade A, Handelsman J. Beyond the Venn diagram: the hunt for a core microbiome.
 791 Environmental Microbiology. 2012;14:4–12.
- 792 75. Pržulj N, Corneil DG, Jurisica I. Modeling interactome: scale-free or geometric?
 793 Bioinformatics. 2004;20:3508–15.

Deutschmann et al.

795 **FIGURE LEGENDS**

796 Figure 1: Estimating a temporal network from a single static network via subnetworks. A) 797 A complete network would contain all possible associations (edges) between microorganism 798 (nodes). B) The single static network inferred with the network construction tool eLSA and 799 the applied filtering strategy considering association significance, the removal of 800 environmentally-driven associations, and associations whose partners appeared in more 801 samples together than alone, i.e., Jaccard index being above 0.5. An association having to be 802 present in the single static network is the first out of the three conditions for an association 803 to be present in a monthly subnetwork. C) In order to determine monthly subnetworks, we 804 established two further conditions for each edge. First, both microorganisms need to be 805 present in the sample taken in the specific month. Second, the month lays within the time window of the association inferred through the network construction tool. Here, three months 806 807 are indicated as an example. D) Example of monthly subnetworks for the three months. The 808 colored nodes correspond to the abundances depicted in C).

809

810 Figure 2: Global (sub)network metrics. A) Number of ASVs (counting an ASV twice if it 811 appears in both size fractions) for each of the 120 months of the Blanes Bay Microbial 812 Observatory time series. There are 1709 ASVs, of which 709 ASVs are connected in the 813 static network. In black, we show the number of nodes connected in the temporal network, 814 and in red the number of nodes that are isolated in the temporal network, i.e., they are 815 connected in the static network and have a sequence abundance above zero for that month 816 ("non-zero"). In dark grey, we show the number of ASVs that are non-zero in a given month 817 but were not connected in the static and subsequently temporal network. In light grey, we

Deutschmann et al.

818	show the number of ASVs with zero-abundance in a given month. The sum of connected and
819	isolated nodes and non-zero ASVs represents each month's richness (i.e., number of ASVs).
820	B) By comparing the edges of two consecutive months, i.e., two consecutive monthly
821	subnetworks, we indicate the number of edges that have been lost (red), preserved (black),
822	and those that are gained (blue), compared to the previous month. C) Six selected global
823	network metrics for each sample-specific subnetwork of the temporal network. The colored

- 824 line indicates the corresponding metric for the static network.
- 825

Figure 3: Associations with a monthly prevalence of at least 90%. Bacteria and eukaryotes are separated and ordered alphabetically. We provide in parentheses the number of associations that appeared in at least nine out of ten monthly subnetworks.

829

Figure 4: *Cyanobacteria* associations. A) Fraction of edges in the temporal network containing at least one *Cyanobacteria* ASV. B) Location of *Cyanobacteria* associations in the temporal network and the single static network. Here we show, as an example, selected months of year 2011. The number and fraction of cyanobacterial edges and total number of edges is listed below each monthly subnetwork and the single static network.

Deutschmann et al.

836 **TABLES**

837 Table 1: Number and fraction of ASVs and reads (total, bacterial and eukaryotic) for the sequence abundance 838 tables (A, B, and C), the preliminary network with significant edges (D), and the single static network (E) obtained 839 after removing environmentally-driven edges and edges with association partners appearing more often alone than with the partner. If an ASV appeared in the nano- and pico-plankton size fractions, it was counted twice. 840

Count tables	ASVs	Reads	Eukaryote	Eukaryotic reads	Bacteria	Bacterial reads
А	2 924	2 273 548	1 365	1 121 855	1 559	1 151 693
В	1 782	2 155 318	1 009	1 057 599	773	1 097 719
С	1 709	2 062 866	1 007	1 057 263	702	1 005 603
D	754	1 657 885	306	730 025	448	927 860
Е	709	1 621 959	294	719 558	415	902 401

Fractions	ASV	Reads	Eukaryote	Eukaryotic reads	Bacteria	Bacterial reads
B/A*100	60.94	94.80	73.92	94.27	49.58	95.31
C/A*100	58.45	90.73	73.77	94.24	45.03	87.32
D/C*100	44.12	80.37	30.39	69.05	69.05	92.27
E/C*100	41.49	78.63	29.20	68.06	59.12	89.74

841 842 A – raw sequence abundance table; B – sequence abundance table without rare ASVs; C – sequence abundance table after size-fraction

filtering; D – preliminary network with significant edges; E – single static network

Deutschmann et al.

	844	Table 2: Global network r	netrics of previously	v described microbi	al association networks
--	-----	---------------------------	-----------------------	---------------------	-------------------------

Location & Depth	Edge density	Transitivity	Average path length	Sampling	Domains	Notes	Reference
SPOT (Off the southern California coast); Deep chlorophyll maximum.	0.04	0.26	3.05	Monthly. August 2000 - March 2004	Archaea, bacteria, and eukaryotes	Edge density for microbial network including environmental factors. Transitivity and average path length for microbial network.	[22]
SPOT; Surface ocean and deep chlorophyll maximum	0.14	0.33	1.94	Monthly. August 2000 - January 2011	Free-living bacteria and picoeukaryotes	Metrics from surface layer network.	[23]
SPOT; Surface	0.02	0.24		Monthly. March 2008 - January 2011	Free-living eukaryotes (0.7–20 μm), bacteria (0.22–1 μm) and viruses (30 kDa–0.22 μm)		[24]
SPOT; Five depths (5 m - Surface, the deep chlorophyll maximum layer, 150 m, 500 m and 890 m - just above the sea floor)	0.04	0.28	2.07	Monthly. August 2003 - January 2011	Free-living bacteria	Metrics for 5 m layer network.	[25]
52 samples from freshwater lakes in China; Surface	(0.023) W:0.033, Sp:0.032 , S:0.036, F:0.029	(0.472) W:0.518, Sp:0.480, S:0.475, F:0.573	(4.84) W:2.16, Sp:5.03 S:7.26, F:3.04	Spatial	Bacteria	Metrics for (whole network) and seasonal networks: W: winter, Sp: spring, S: summer, and F: fall	[67]
68 stations from the Tara Oceans expedition across eight oceanic provinces; Surface and Deep chlorophyll maximum	0.005, 0.003, 0.008	0.2, 0.0, 0.43	3.05, 3.02, 2.56	Spatial	Organisms from seven size fractions	Metrics from surface networks including eukaryotes only, eukaryotes and prokaryotes (0.5-5 µm), and prokaryotes only (0.2-1.6 µm)	[66]
115 stations from the Tara Oceans expedition covering all major oceanic provinces from pole to pole; Surface and deep chlorophyll maximum	0.002	0.036		Spatial	Bacteria, archaea, and eukaryotes from six size fractions.	Metrics represent the means of sample- specific subnetworks.	[68]





only keeping associations with Jaccard index > 0.5

D) temporal network constituted from monthly subnetworks



Single static network







