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2 **Spatial and temporal variability in the response of phytoplankton and**
3 **bacterioplankton to B-vitamin amendments in an upwelling system**

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13 **Abstract.** We evaluated the temporal (inter-day and inter-season) and spatial variability
14 in microbial plankton responses to vitamins B12 and B1 supply (also in combination with
15 inorganic nutrients) in coastal and oceanic waters of the northeast Atlantic ocean.
16 Phytoplankton and, to a lesser extent, bacteria were strongly limited by inorganic
17 nutrients. Inter-day variability in microbial plankton responses to B-vitamins was
18 unimportant, suggesting that B-vitamins availability was controlled by factors operating
19 at larger temporal scales. Phytoplankton and bacteria positively responded to B-vitamin
20 amendments in 13% and 21%, respectively, of the 216 cases (36 experiments x 6
21 treatments). Negative responses represented 21% for phytoplankton and 26% for bacteria.
22 Most positive responses were produced by treatments containing either B12 alone or B12
23 combined with B1 in oceanic waters, which was consistent with the significantly lower
24 average vitamin B12 ambient concentrations compared to that in the coastal station.
25 Growth stimulation by B1 addition was more frequent on bacteria than in phytoplankton,
26 which is coherent with their widespread dependence on exogenous sources for this growth
27 factor. Negative responses to B-vitamins were generalized in coastal waters in summer,
28 and were associated to a high contribution of Flavobacteriales to the prokaryote
29 community. This observation suggests that the external supply of B12 and/or B1 may
30 promote negative interactions between microbial components when B-vitamins
31 auxotrophs are abundant. The microbial response patterns to B12 and/or B1 amendments
32 were significantly correlated with changes in the prokaryotic community composition,
33 highlighting the pivotal role of prokaryotes in B-vitamins cycling in marine ecosystems.

34 **1 Introduction**

35 Phytoplankton accounts for almost half of the global net primary production (Field et al.,
36 1998) and may eventually cause toxic episodes entailing human health problems and large
37 economic losses (Hallegraeff, 1993; van Dolah et al., 2001). Recent emerging evidence

38 suggests the role of biologically active organic compounds, such as B-vitamins, on the
39 control of marine productivity in both coastal and oceanic waters (Bertrand et al., 2007;
40 Browning et al., 2017, 2018; Gobler et al., 2007; Koch et al., 2011; Panzeca et al., 2006).
41 B-vitamins act as cofactors for enzymatic reactions and are involved in many important
42 metabolic pathways (Madigan et al., 2005; Marsh, 1999; Monteverde et al., 2017).
43 Vitamin B12 (B12 herein), which is exclusively synthesized by some bacteria and archaea
44 (Roth et al, 1996; Martens et al., 2002; Warren et al., 2002), acts as a cofactor of three
45 enzymes in eukaryotes (methionine synthase, methylmalonyl-coA mutase and
46 ribonucleotide reductase type II) (Bertrand and Allen 2012, Helliwell et al., 2011). In
47 comparison, over 20 different **B12**-dependent enzymes are found in bacteria (Roth et al.,
48 1996), making B12 critically important also for these organisms. Vitamin B1 (B1 herein)
49 plays a pivotal role in intermediary carbon metabolism and is a cofactor for a number of
50 enzymes involved in primary carbohydrate and branched-chain amino acid metabolism
51 (Croft et al., 2006).

52 Most eukaryote phytoplankton species are auxotrophs for one or more B-vitamins,
53 consequently requiring an exogenous supply of these molecules (Carlucci and Bowes,
54 1970; Haines and Guillard, 1974; Croft et al., 2005; Tang et al., 2010; Helliwell et al.,
55 2011; Bertrand and Allen, 2012). Moreover, genomic data also indicate widespread B-
56 vitamins auxotrophy among many bacterial taxonomic groups (Sañudo-Wilhelmy et al.,
57 2014; Paerl et al., 2018), which implies that phytoplankton and bacteria may eventually
58 compete for the acquisition of these compounds (Koch et al., 2012). Auxotrophic
59 microorganisms may acquire the required vitamins from the environment or through
60 biotic interactions with prototrophic (biosynthetically competent) microorganisms
61 (Droop 2007; Kazamia et al., 2012, Grant et al., 2014). A well-known example is the

62 mutualistic interaction between B12-dependent phytoplankton and bacteria (Croft et al.,
63 2005; Amin et al., 2012; Cooper and Smith, 2015).

64 Even though B-vitamins appear to be important and potentially limiting factors for
65 microbial plankton, our understanding of B-vitamins cycling in the ocean is largely
66 limited by the complex and still evolving analytical methodology for its quantification in
67 natural waters (Okbamichael and Sañudo-Wilhelmy, 2004, 2005; Suffridge et al., 2017).
68 Sañudo-Wilhelmy et al. (2012) found extensive areas of coastal waters with close to
69 undetectable B12 concentrations, suggesting that microbes might be well adapted to drive
70 under limiting conditions for this growth factor.

71 The factors limiting phytoplankton and bacterial growth in marine ecosystems are known
72 to vary over different spatial and temporal scales (Cullen et al., 1992; Arrigo 2005;
73 Church 2008; Saito et al., 2008, Martínez-García et al., 2010a, 2010b, Moore et al., 2013),
74 in accordance with the dynamic nature of microbial communities (Pinhassi et al., 2003;
75 Pommier et al., 2007; Fuhrman et al., 2008; Carlson et al., 2009, Hernando-Morales et
76 al., 2018, Hernández-Ruiz et al., 2018). Compared to mineral nutrient and trace elements,
77 much less is known about B vitamin limitation and its spatial and temporal variability in
78 marine ecosystems.

79 Some studies have shown enhanced phytoplankton biomass associated to B12
80 amendments in both temperate coastal and polar waters (Bertrand et al., 2007; Gobler et
81 al., 2007; Koch et al., 2011; Koch et al., 2012). The simultaneous effect of vitamin B12
82 supply on both phytoplankton and bacteria has been barely explored (Koch et al., 2011,
83 Barber-Lluch et al., 2019). To our knowledge, the effect of B1 amendments on marine
84 natural microbial plankton community succession has been only assessed by Gobler et al.
85 (2007), who suggested that high concentration of B-vitamins, associated with high
86 bacterial abundance, caused an increase in auxotrophs, mostly dinoflagellates.

87 The Ría de Vigo (NW Spain) is a coastal embayment affected by intermittent upwelling
88 of subsurface cold and inorganic nutrient-rich water from March to September and the
89 downwelling of open ocean surface water from October to March (Fraga, 1981; Barton
90 et al., 2015). In addition to this seasonality, fluctuations of wind patterns in the area
91 generate upwelling and downwelling events occurring within each season (Alvarez-
92 Salgado et al., 1993; Figueiras et al., 2002). A recent study by Barber-Lluch et al. (2019)
93 at a shelf station off the Ría de Vigo (NW Spain) showed monthly variation in the
94 response of phytoplankton and bacteria to nutrient and/or B12 additions in surface waters,
95 likely related to variation in the ambient concentration of B12 and the taxonomic
96 community composition. Unfortunately, the role of these factors on the microbial
97 response to the amendments were not specifically assessed by these authors.

98 Within this context, the aim of our study was to explore spatial (horizontal and vertical)
99 and temporal (seasonal and short-term) variability patterns in B12 and B1 vitamin
100 limitation in relation to the prevailing initial abiotic (e.g., nutrient and B12
101 concentrations) and biotic (eukaryote and prokaryote community composition)
102 conditions in this productive ecosystem. We conducted a total of 36 microcosm bioassays
103 in February, April, and August 2016 to evaluate the response of heterotrophic bacteria
104 and phytoplankton to the addition of B12 and/or B1.

105 Considering that a large fraction of eukaryotic phytoplankton and bacterial taxa require
106 exogenous B-vitamins and considering the different requirements and capabilities to
107 synthesize B-vitamins by different microbial taxa, we hypothesize that microbial
108 community composition play a relevant role in explaining B-vitamins limitation patterns
109 in microbial plankton.

110 **2 Methods**

111 **2.1 Experimental design**

112 Thirty-six enrichment experiments were performed in the upwelling system near Ría de
113 Vigo on board “B/O Ramón Margalef” in three different oceanographic cruises
114 (ENVISION I, II & III) conducted in 2016. Two different locations of the East Atlantic
115 Ocean, one coastal station (st3) (42° N, 8.88° W) and one oceanic station (st6) (42° N,
116 9.06° W) (Fig. 1), were sampled during three different seasons aimed to cover a wide
117 range of initial hydrographic and ecological conditions. The 10-day cruises were
118 conducted in February (ENVISION I), coinciding with the spring bloom, and April
119 (ENVISION II) and August (ENVISION III) during the early and late summer upwelling,
120 respectively. During each cruise, 12 enrichment experiments were carried out on board,
121 3 experiments in each station (3a, 3b & 3c and 6a, 6b & 6c, respectively) with water from
122 two different depths. Water was collected using 20 l Niskin metal-free bottles. Surface
123 and sub-surface chlorophyll maximum (SCM) samples were taken at 5 m and at the
124 maximum fluorescence depth, between 10 m and 50 m according to the CTD data,
125 respectively (Fig. 2). We failed to sample the SCM on two occasions, due to large vertical
126 displacements between the downward and the upward casts. Vertical profiles of
127 temperature, salinity and chlorophyll fluorescence were obtained using a regular stainless
128 CTD-rosette down to 60 m in the coastal station and to 200 m in oceanic station. Samples
129 for phytoplankton and bacterial biomasses, dissolved nutrient concentration, including
130 vitamin B12, and microbial plankton community were collected at the beginning of each
131 experiment. Upwelling indexes (UI) were estimated by calculating the Ekman transport
132 from surface winds at fix-station (stn3) located at 42° N and 8.88° W. Daily UI values
133 were computed by the Instituto Español de Oceanografía (www.indicedeafloramiento.
134 ieo.es/) using data from atmospheric pressure at sea level, derived from the WXMAP
135 model (Gonzalez-Nuevo et al., 2014).

136 Seawater samples were gently pre-filtered through a 200 μm mesh to exclude large
137 zooplankton in order to ensure good replicability and collected into a 20 l acid-cleaned
138 polyethylene carboy. Following sample collection, 300 ml PAR and UVR transparent and
139 non-toxic (whirl-pak) bags were filled and nutrients were added establishing eight
140 different enrichment treatments as follows: (1) control treatment (C): no nutrients added;
141 (2) inorganic nutrient treatment (I): 5 μM nitrate (NO_3^-), 5 μM ammonium (NH_4^+), 5 μM
142 silicate (SiO_4^{2-}) and 1 μM phosphate (HPO_4^{2-}); (3) vitamin B12 (Sigma, V2876)
143 treatment: 100 pM; (4) vitamin B1 (Sigma, T4625) treatment: 600 pM); (5) Inorganic
144 nutrients and vitamin B12 (I+B12) treatment; (6) Inorganic nutrients and vitamin B1
145 (I+B1) treatment; (7) vitamins B12 and B1 (B12+B1) treatment and (8) Inorganic
146 nutrients with vitamins B12 and B1 (I+B12+B1) treatment. Inorganic nutrients were
147 added to avoid that inorganic nutrient limitation masked the responses to B vitamins. It is
148 important to note that incidental trace-metal contamination could have occurred during
149 water collection, as we could not strictly follow trace metal clean procedures onboard.
150 Each treatment had 3 replicates resulting in 24 whirl-pack bags per experiment. To assess
151 short-term effects of nutrient inputs, experimental bags were incubated on-deck during
152 72 h under natural light conditions. In-situ temperature and light were reproduced by
153 submerging the bags in tanks connected to the surface-water pump system, and covered
154 with screens simulating the light intensity at the sampling depth.

155 **2.2 Chlorophyll-*a***

156 Chlorophyll-*a* (Chl-*a*) concentration was measured at time-zero and after 72 h incubation
157 as a phytoplankton biomass proxy. 300 ml of water samples were filtered through 0.2 μm
158 polycarbonate filters and frozen at -20°C until further analysis. Chl-*a* was extracted with
159 90 % acetone and kept in darkness at 4°C overnight. Fluorescence was determined with a

160 TD-700 Turner Designs fluorometer calibrated with pure Chl-*a* (absorption coefficient at
161 665 nm = 12.6) standard solution.

162 **2.3 Flow cytometry**

163 Samples for heterotrophic bacteria abundance quantification (2 ml) were preserved with
164 1 % paraformaldehyde + 0.05 % glutaraldehyde (final concentrations). Samples were
165 incubated 20 min for the fixative to act on cells and frozen at -80°C after 15 min.
166 immersion in liquid nitrogen. Abundance of heterotrophic bacteria was determined using
167 a FACSCalibur flow cytometer equipped with a laser emitting at 488nm. Samples were
168 stained with SYBR Green DNA fluorochrome, and bacterial abundance was detected by
169 their signature of side scatter (SSC) and green fluorescence as described by Gasol and
170 Del Giorgio, 2000. The empirical calibration between light side scatter (SSC) and cell
171 diameter described by Calvo-Díaz and Morán (2006) were used to estimate the biovolume
172 (BV) of bacterioplankton cells. BV was converted into biomass by using the allometric
173 factor of Norland (1993: $\text{fg C cell}^{-1} = 120 \times \text{BV}^{0.72}$) for the coastal experiments and using
174 the open ocean conversion factor for the oceanic experiments ($\text{fg C cell}^{-1} = 350 \times \text{BV}$).

175 **2.4 Nutrients**

176 Aliquots for inorganic nutrient determinations (ammonium, nitrite, nitrate, phosphate,
177 and silicate) were collected in first place and directly from the Niskin bottle in order to
178 avoid contamination. Polyethylene bottles 50 ml precleaned with HCl 5 % were filled
179 with the sample employing free-contamination plastic gloves and immediately frozen at
180 -20°C until analysis by standard colorimetric methods with a Bran-Luebbe segmented
181 flow analyzer (Hansen and Grasshoff 1983). The detection limit was 0.1 $\mu\text{mol l}^{-1}$ for
182 nitrate, 0.02 $\mu\text{mol l}^{-1}$ for nitrite and phosphate and 0.05 $\mu\text{mol l}^{-1}$ for ammonium and

183 silicate. Dissolved inorganic nitrogen (DIN) concentration was calculated as the sum of
184 the ammonium, nitrite and nitrate concentrations.

185 **2.5 Vitamin B12**

186 Seawater samples for dissolved vitamin analysis were taken at surface and SCM depth in
187 the coastal and oceanic station on the first, third and fifth (or sixth) day of each cruise
188 (Table S1 in the Supplement). Samples were filtered through 0.2 μm sterivex filters and
189 frozen at -20°C until further analysis. Samples (1 l) were preconcentrated using a solid-
190 phase extraction with a C18 resin (Bondesil C18, Agilent) at pH 6.5 and rate of 1 ml/min.
191 Elution was performed with 12 ml of methanol (MeOH) LCMS grade that was removed
192 via evaporation with nitrogen in a Turbovap. Residual water behind (300-500 μl) was
193 frozen at -20°C until further analysis using liquid chromatography coupled to mass
194 spectrometry system.

195 Detection and quantification of dissolved vitamin B12 (cyanocobalamin and
196 hydroxocobalamin) was conducted using an Agilent 1290 Infinity LC system (Agilent
197 Technologies, Waghäusel-Wiesental, Germany), coupled to an Agilent G6460A triple
198 quadrupole mass spectrometer equipped with an Agilent Jet Stream ESI source. The LC
199 system used a C18 reversed-phase column (Agilent Zorbax SB-C18 Rapid Resolution HT
200 (2.1×50 mm, $1.8 \mu\text{m}$) with a 100 μl sample loop. Agilent Technologies software was
201 used for data acquisition and analysis. Chromatographic separation was performed using
202 MeOH and water LCMS grade, both buffered to pH 5 with 0.5 % acetic acid, as mobile
203 phases in a 15 minutes' gradient. Gradient starting at 7 % MeOH for 2 min, changing to
204 100 % MeOH by minute 11, continuing at 100 % MeOH until 13.5 min and returning to
205 initial conditions to complete 15 min. The average B12 recovery percentage after pre-
206 concentration and extraction of B-vitamin spiked samples was 93%. B-vitamin free

207 seawater was spiked with cyanocobalamin and hidroxocobalamin standards for recovery
208 percentage analysis.

209 **2.6 Microbial plankton community**

210 DNA samples were taken during the experimental period at surface and SCM depth in
211 the coastal and oceanic station. In particular, sampling of the microbial plankton
212 community was carried out on the first, second, fourth and sixth day of each cruise.
213 Community composition was assessed by sequencing the V4 and V5 regions from 16S
214 rRNA gene (16S rDNA) for prokaryotes and the V4 region from 18S rRNA gene (18S
215 rDNA) for eukaryotes. Two liters of water samples were sequentially filtered through 3
216 μm pore size polycarbonate filters and 0.2 μm pore size sterivex filter and immediately
217 frozen in liquid nitrogen and conserved at $-80\text{ }^{\circ}\text{C}$. DNA retained in the 3 μm and 0.2 μm
218 filters was extracted by using the PowerSoil DNA isolation kit (MoBio Laboratories
219 Inc., CA, USA) and the PowerWater DNA isolation kit (MoBio Laboratories Inc.,
220 CA, USA), respectively, according to the manufacturer's instructions. Prokaryotic DNA
221 from 0.2 μm filters was amplified using the universal primers "515F and 926R" and
222 eukaryotic DNA from both, 3 μm and 0.2 μm filters, using the primers
223 "TAReuk454FWD1" and "TAReukREV3". Amplified regions were sequenced in an
224 Illumina MiSeq platform and the sequences obtained were analyzed with software
225 package DADA2 (Callahan et al., 2016). SILVA reference database (Quast et al., 2012)
226 was used to taxonomic assignment of 16S amplicon sequence variants (ASVs) and PR2
227 (Guillou et al., 2012) and the marine protist database from the BioMarks project (Massana
228 et al., 2015) were used to taxonomic assignment of 18S ASVs. ASV table is an analogue
229 of the traditional OTU table which records the number of times each exact amplicon
230 sequence variant was observed in each sample (Callahan et al., 2016).

231 The raw ASV tables of prokaryotes and eukaryotes were subsampled to the number of
232 reads present in the sample with the lowest number of reads, which was 2080 and 1286,
233 for 16S rDNA and 18S rDNA, respectively. The abundance of ASVs was averaged for
234 coastal and oceanic samples, differentiating surface and SCM. A total of 1550 unique
235 ASVs of prokaryotes were identified. As many ASVs of eukaryotes were present in both
236 size fractions, we combined datasets derived from the 0.2 and the 3 μm filters for
237 eukaryotic community analyses. As explained in Hernández-Ruiz et al. (2018), we
238 normalized the reads from each filter size by the filter DNA yield, as recommended in
239 Dupont et al. (2015), obtaining 2293 unique ASVs. The sequence abundances of the
240 subsampled ASV tables were transformed using the centered log ratio (clr) (Fernandes et
241 al., 2014; Gloor et al., 2017). Zeros were replaced by the minimum value that is larger
242 than 0 divided by 2.

243 **2.7 Statistical analysis**

244 To compare the effect of different nutrient additions on the response variables,
245 phytoplankton and bacterial biomasses, we calculated response ratios (RR) by dividing
246 each observation (mean of triplicates) of each treatment by the respective control
247 treatment mean. A value equal to 1 implies no response, a value < 1 implies a negative
248 response and a value > 1 implies growth stimulation after nutrient addition. Secondary
249 limitation by B vitamins was calculated by dividing the mean biomass value in the
250 inorganic nutrients and B vitamin combined treatment by the mean biomass value in the
251 inorganic nutrient addition treatment. In the same way, a value < 1 implies a negative
252 effect of B vitamins and a value > 1 implies growth stimulation by B vitamin through
253 secondary limitation.

254 Normal distribution was tested by a Kolmogorov-Smirnov test and variables were log
255 transformed if necessary to attain normality. All statistical analysis were considered

256 significant at the 0.05 significance level and p-value was standardized as proposed by
257 Good (1982) in order to overcome the low number of replicates. Differences between
258 station and depth (spatial variability) and among sampling months (temporal variability)
259 in the responses to B vitamins were evaluated with factorial analysis of variance
260 (ANOVA). Bonferroni post hoc tests analyses were conducted to test which treatments
261 were significantly different from the control treatment in each experiment. Z-test was
262 used to evaluate the significance of the average B vitamins response ratios for each period,
263 sampling site and depth. In order to determine which factors better explain B-vitamin
264 response patterns, we calculated the correlation between the B vitamin response
265 resemblance matrix and the corresponding resemblance matrices of (a) abiotic variables,
266 (b) prokaryote community composition, and (c) eukaryotic community, using the
267 RELATE analysis implemented in PRIMER6 (Clarke and Warwick, 2001; Clarke and
268 Gorley, 2006). In order to highlight which specific taxonomic groups are associated to
269 changes of microbial plankton (bacterioplankton and phytoplankton) responses to vitamin
270 B1 and B12, we conducted a distance based redundancy analysis (dbRDA) combined
271 with a distance linear-based model (DistLM) using a step-wise procedure and adjusted r^2
272 as selection criteria) using the PRIMER6 software. Correlations among the prokaryotic
273 taxa best explaining the microbial plankton responses to B-vitamins and phytoplankton
274 and bacterial responses to different B vitamin treatments (including primary and
275 secondary responses) were calculated using Pearson's correlations.

276 **3 Results**

277 **3.1 Initial conditions**

278 Different hydrographic conditions were found during each cruise (Fig. 1, Fig. 2). In
279 February, heavy rainfall combined with relaxed winds (Fig. 1) caused a halocline at 10
280 meters depth (Fig. 2). High levels of Chl-*a* (as derived from the calibrated CTD

281 fluorescence sensor) were observed at the coastal station, being maximum ($4.97 \mu\text{g l}^{-1}$)
282 by the end of the cruise. At the oceanic station, Chl-*a* levels remained low (less than $3 \mu\text{g}$
283 l^{-1}) throughout the cruise, being slightly higher in the subsurface layer.

284 Strong precipitation during the April cruise (Fig. 1) caused a persistent surface halocline
285 at the coastal station (Fig. 2). Maximum Chl-*a* concentrations ranged from 0.99 to 2.73
286 $\mu\text{g l}^{-1}$, declining from day 5 onwards, coinciding with an increase in water temperature
287 associated to a downwelling situation. At the oceanic station, a persistent subsurface Chl-
288 *a* maximum (up to $1.61 \mu\text{g l}^{-1}$) was observed throughout the cruise.

289 In August, strong thermal stratification was observed at both stations (Fig. 2). At the
290 beginning of the cruise, high Chl-*a* concentration (close to $20 \mu\text{g l}^{-1}$) was observed in
291 subsurface water. These high Chl-*a* levels were maintained until day 4 and then
292 decreased, reaching minimum values by day 7, coinciding with upwelling relaxation (Fig.
293 1b, Fig. 2). Salinity minima during day 1 and 5 reflect precipitation events. Chl-*a* was
294 relatively low at the oceanic station, an increased by the end of the sampling period as a
295 consequence of an upwelling event, that brought cold and nutrient rich water to the
296 surface, at day 5 (Fig. 2).

297 Abiotic and biotic conditions at the beginning of each experiment are shown in Fig. 3 and
298 in the supplementary Table S2. Overall, the concentration of dissolved inorganic nitrogen
299 (DIN) was higher at the coastal than at the oceanic station, where very low levels were
300 measured in August (Fig. 3). At the coastal station, higher DIN concentrations were
301 observed in surface compared to subsurface waters. The DIN:DIP (dissolved inorganic
302 phosphorous) ratio was always lower in open ocean than in the coastal station and mostly
303 below of Redfield ratio. Phosphorous limitation ($\text{DIN:DIP} > 16$) was frequent in coastal
304 subsurface waters in February and April.

305 Phytoplankton biomass, estimated as Chl-*a* concentration greatly varied between stations
306 and seasons but was always higher at the coastal (st3) than at the oceanic (st6) station
307 (Fig. 3). Bacterial biomass (BB) increased from winter (February cruise) to summer
308 (August cruise) at the two stations. In February, Chl-*a* concentrations increased by the
309 end of the cruise at both coastal and oceanic stations, while bacterial biomass remained
310 very low throughout this sampling period. In April, both BB and Chl-*a* were similar in
311 the ocean and the coast, and showed reduced temporal variability, irrespective of the
312 observed nutrient variability (Fig. 3). In August, Chl-*a* concentration was much higher at
313 the coastal than at the oceanic station, and showed reduced temporal variability (except
314 at the SCM in the coast) (Fig. 3). At the beginning of the sampling period, BB was higher
315 in the ocean than in the coast, and tended to decline by the end of the cruise.

316 A MDS analysis revealed that microbial community composition showed a relatively
317 reduced within period variability, with samples clustering according to the sampling
318 period (ANOSIM, $p = 0.001$) (Fig. S3 in the Supplement). Consequently, we averaged
319 the microbial community composition for each period and sampling site. The sampling
320 period-averaged composition of the eukaryote community showed a clear variability
321 among sampling dates, while differences between sampling locations and depths were
322 less pronounced (Fig. 4a). At the coastal location, *Mamiellophyceae* were relatively
323 abundant in February and April, but their abundance sharply decreased in August. By
324 contrast, the relative abundance of *Dinophyceae* was highest in August at both sampling
325 locations. The contribution of diatoms (*Bacillariophyta*) was very low in summer at the
326 oceanic station and MALV were most representative in February at both locations.
327 Flavobacteriales and Rhodobacteriales were the dominant prokaryotes (Fig. 4b) in coastal
328 waters, particularly in August, when both represented more than 80 % of sequences, while
329 Cyanobacteria were mostly present in February and April. In oceanic waters,

330 Flavobacteriales and Cyanobacteria were the dominant prokaryotes. SAR11 clade and
331 Archaea were most abundant in February at both sampling locations.

332 B12 concentration was low, ranging from 0.06 to 0.55 pM (Table S1 in the Supplement)
333 Mean B12 concentration was significantly higher in the coast (0.30 ± 0.13 pM) than in the
334 ocean (0.15 ± 0.12 pM) (t-test, $p = 0.001$), and showed less variability at the coastal than
335 at the oceanic station (Fig. 4c).

336 **3.2 Short-term phytoplankton and bacteria responses to inorganic nutrients and** 337 **vitamin additions**

338 The magnitude of phytoplankton and bacteria responses (i.e., the response ratios) to the
339 different addition treatments differed between sampling stations (ANOVA, $p = 0.018$)
340 and among sampling periods (ANOVA, $p = 0.014$). The most prominent responses of
341 phytoplankton, compared to the control treatment, occurred after inorganic nutrient
342 amendments, especially in surface oceanic waters (Fig. S1 in the Supplement). The
343 magnitude of the phytoplankton response to inorganic nutrients was significantly higher
344 in oceanic than in coastal waters (ANOVA, $p = 0.028$). Bacteria responded comparatively
345 less than phytoplankton to inorganic nutrients and there were no significant differences
346 between coastal and oceanic waters (ANOVA, $p = 0.203$). The addition of inorganic
347 nutrients caused significant increases in phytoplankton biomass in 31 out of the 36
348 experiments, and in 19 out of 36 experiments in bacterial biomass (Fig. S1 in the
349 Supplement).

350 The addition of B12 stimulated phytoplankton growth in 5 out of 36 experiments while
351 bacteria responded positively to B12 in 6 experiments (Fig. 5 and Fig. 2c in the
352 Supplement). Phytoplankton biomass increased in 3, and bacterial biomass in 7 out of 36
353 experiments after adding B1. B vitamins also caused negative responses of phytoplankton
354 and bacterial biomass (Fig. 5 and Fig. S2 in the Supplement). The addition of vitamins

355 induced decreases of phytoplankton biomass in 6 experiments (4 after adding B12 and 2
356 after adding B1) and bacterial biomass in 14 experiments (6 after adding B12 and 8 after
357 adding B1). Additions of inorganic nutrients combined with B-vitamins caused a similar
358 increase in phytoplankton or bacterial biomass than the inorganic addition alone in most
359 of the experiments. Secondary limitation by B1 and/or B12 was occasionally observed
360 when inorganic nutrients were limiting, leading to a higher biomass increase in the
361 treatments including both inorganic nutrients and vitamins as compared to the inorganic
362 nutrient addition alone (Fig. 5 and Fig. S2 in the Supplement). In the case of
363 phytoplankton, secondary limitation by B-vitamins was found in the 3b-surface, 6a-SCM
364 and 6b-SCM experiments in February, in the 3b-surface and 3b-SCM experiments in
365 April, and in the 3b-SCM, 6b-SCM and 6c-surface experiments in August.

366 In order to quantify the relevance of inter-day variability, we calculated the mean
367 coefficient of variation (CV) of the responses to B vitamins (i.e., excluding the responses
368 to inorganic nutrients, and normalizing the responses of the nutrient and vitamin
369 combined treatments to the corresponding response to inorganic nutrients alone) within
370 sampling periods for each sampling point (4 sites during 3 periods). The CV ranged from
371 9%, in subsurface oceanic waters in April, to 34% in surface coastal waters in April,
372 averaging 16 ± 6 (SD) % (data not shown). Considering that short-term (within sampling
373 period) variability was overall very low, and for simplicity, we averaged the responses to
374 B vitamins in the 3 experiments conducted at each of the 12 sampling points to further
375 describe spatial and temporal patterns in the response to B vitamin amendments (Fig. 6).

376

377 **3.3 B-vitamin response patterns in relation to abiotic and biotic factors**

378 When averaging the responses within each sampling point (Fig. 6), some general patterns
379 emerge. Both phytoplankton and bacteria showed more negative than positive responses

380 to B1 and/or B12 amendments. Most positive responses occurred at the oceanic station,
381 while negative responses dominated in the coast. Phytoplankton significant positive
382 responses mostly occurred in February, showing an average increase of up to 1.2-fold in
383 coastal subsurface waters after B12+B1 amendment (Fig. 6). The largest significant
384 increase in phytoplankton biomass (ca. 1.4-fold) occurred in April after the combined
385 addition of B12 and B1 in coastal surface waters. Significant positive bacterial responses
386 mainly occurred in August, when the largest increase (ca. 1.3-fold) occurred in coastal
387 subsurface waters after B1 amendment (Fig. 6). Most positive responses were associated
388 with treatments containing B12 either alone or combined with B1 (Fig. 6). Phytoplankton
389 primary B1 limitation was only found at the oceanic SCM in February (Fig. 6), while
390 bacterial primary B1 limitation only occurred at the coastal SCM in August. In addition,
391 bacterial secondary B1 limitation occurred in oceanic surface waters in February and
392 August.

393 In order to explore the controlling factors of the observed B-vitamin response patterns,
394 the correlation between the B-vitamin response resemblance matrix and the
395 corresponding resemblance matrices obtained from the abiotic factors, the prokaryotic
396 community composition, or the eukaryotic community composition was calculated. Only
397 the prokaryotic community composition significantly correlated with the B-vitamin
398 responses (Spearman Rho = 0.31, $p = 0.041$). We then used distance-based linear
399 modelling (DistLM) to identify the prokaryotic taxa which best explained the microbial
400 plankton responses to B-vitamins (Fig. 7). The resulting model explained 78 % of the
401 variation and included seven prokaryotic groups. The sequential test identified
402 *Planktomarina* as the taxon explaining the largest fraction of variation (ca. 24 %) (Fig.
403 7). The total variation explained by the db-RDA1 and db-RDA2 was 59.4 %. The db-
404 RDA1 axis tended to separate coastal, where negative responses to B vitamins dominated,

405 from oceanic samples, where most positive responses were found (Fig. 6 and Fig. 7). The
406 db-RDA plot showed that Cellvibrionales and *Planktomarina* highly and positively
407 correlated with axis 1, while SAR11 and *Synechococcus* showed negative correlation with
408 axis 1. Flavobacteriales and Actinobacteria mostly correlated with the db-RDA2 axis.

409 Statistically significant correlations were found between several prokaryotic taxa and
410 microbial plankton responses to B vitamins. A statistically significant negative
411 correlation was found between *Planktomarina* abundance and the phytoplankton
412 response to B12 ($r = -0.69$, $p = 0.014$) and the phytoplankton response to B1 ($r = -0.58$, p
413 $= 0.048$). Flavobacteriales abundance showed a strong significant negative correlation
414 with the secondary response of bacteria to B1 addition (i.e. response to I+B1 compared
415 to I) ($r = -0.9$, $p < 0.001$) and the phytoplankton response to B1 ($r = -0.59$, $p = 0.045$). A
416 significantly positive correlation was found between Actinobacteria and the response of
417 bacteria to B12 ($r = 0.61$, $p = 0.036$) and the secondary response of bacteria to B1 with
418 ($r^2 = 0.50$, $p = 0.01$). *Synechococcus* and SAR11 also showed a significant positive
419 correlation with secondary responses of bacteria to B vitamins (Table 1).

420

421 **4 Discussion**

422 Although the dependence of phytoplankton on B vitamin has been previously observed
423 in cultures (Droop, 2007) and in natural phytoplankton assemblages in coastal areas
424 (Sañudo-Wilhelmy et al., 2006; C. J. Gobler et al., 2007; Koch et al., 2012, Barber-Lluch
425 et al., 2019), this is, to the best of our knowledge, the most complete study about responses
426 of phytoplankton and bacteria to vitamin B12 and/or B1 addition. The 36 experiments
427 developed in this study have allowed to explore the role of vitamins B12 and B1 at
428 different scales. On the one hand, spatial and seasonal differences were evaluated with

429 experiments in the coastal and oceanic stations during the spring bloom in February, April
430 and the upwelling in August. On the other hand, the role of B-vitamins on a very short
431 scale (inter-day) has been studied.

432 Contrary to our expectations, the frequency of the experiments (every 2-3 days)
433 conducted at different locations during contrasting hydrographic conditions revealed a
434 reduced short-term variability of microbial plankton community composition. The
435 reduced short-term variability in the responses to B vitamins additions suggested that B
436 vitamin availability was controlled by factors operating at larger temporal scales, such as
437 the succession of microbial communities associated to seasonal environmental variation
438 (Hernández-Ruiz et al., 2018; Hernando-Morales et al., 2018). Considering this, and for
439 further discussion, we averaged the responses from the three experiments conducted
440 during each sampling period, resulting in a total of 12 experimental situations (2 stations
441 \times 2 depths \times 3 periods). Overall, phytoplankton and/or bacterial growth enhancement
442 upon B vitamin supply was frequent but relatively moderate in this productive ecosystem,
443 showing 1.1 to 2.4-fold increases in 75% of the experimental situations for phytoplankton
444 and in 50% for bacteria, while negative responses to at least one B vitamin treatment
445 occurred in all but one of the experimental situations (Fig. 6). The low and constant B12
446 ambient concentration and the observed microbial response patterns suggest a close
447 balance between production and consumption of this growth factor. Different patterns of
448 response to B-vitamin amendments were observed in phytoplankton and bacteria, which
449 appear to be mostly explained by the prokaryotic community composition, suggesting
450 that B vitamin bioavailability might be largely controlled by the community assemblage.

451 **4.1 Positive responses to vitamin B1 and B12 amendments**

452 The experimental design allowed the detection of two categories of B vitamin dependency
453 of the microbial plankton community. A primary limitation by B vitamins occurs when

454 microorganisms respond to additions of B vitamins alone, while a secondary limitation
455 by B vitamins arises when the response to the combined addition of B vitamins and
456 inorganic nutrients is significantly higher than that to inorganic nutrients alone, as a result
457 of the ambient B-vitamin depletion associated to the plankton growth after inorganic
458 nutrient enrichment. Most positive (72% for phytoplankton and 60 % for bacteria)
459 responses occurred after single B-vitamins additions, suggesting that inorganic nutrient
460 availability enhance B-vitamin production by the prototrophic microbes. Under nutrient-
461 limiting conditions, the external supply of vitamins could reduce the energy costs
462 associated to its synthesis (Jaehme and Slotboom, 2015), stimulating the growth not only
463 of auxotrophs but also of prototrophs.

464 The significant positive effects of B12 and/or B1 addition, suggest that these compounds
465 may be eventually limiting microbial growth in this area, as previously observed by other
466 authors (Panzeca et al., 2006; Sañudo-Wilhelmy et al., 2006; Bertrand et al., 2007; Gobler
467 et al., 2007; Cruz-López and Maske, 2016). Most positive responses to B vitamin
468 amendments were observed in oceanic waters, where B12 concentration was significantly
469 lower than in coastal waters (Fig. 4c). Unfortunately we lack B1 measurements in this
470 study, but, according to previous field studies in other oceanographic regions, a similar
471 pattern to that observed for B12 can be expected (Cohen et al., 2017; Sañudo-Wilhelmy
472 et al., 2012; Suffridge et al., 2018). The overall low and stable concentration of B12 at
473 both sampling locations is consistent with the expected high turnover time of this
474 compound in productive, well-lit waters (Bertrand et al., 2015), due to both biological
475 uptake (Koch et al., 2012; Taylor and Sullivan, 2008) and photochemical degradation
476 (Carlucci et al., 1969; Juzeniene and Nizauskaite, 2013; Juzeniene et al., 2015). The
477 measured B12 concentrations were in the lower range reported for coastal sites, and

478 similar to that found in the upwelling system off the California coast in the San Pedro
479 Basin during winter, spring and summer (Panzeca et al., 2009).

480 The increase of phytoplankton biomass was mostly associated to B12 amendments, which
481 is consistent with the known incapability of eukaryotes to synthesize this vitamin (Croft
482 et al., 2005; Tang et al., 2010; Sañudo-Wilhelmy et al., 2014). Considering the very low
483 concentration of B12 in the sampling area, the limited phytoplankton response to B
484 vitamins is consistent with the presence of species that may have adapted to overcome
485 B12 limitation in the environment by using alternative enzymes. For example, changes in
486 external B12 availability may cause shifts from vitamin B12-dependence to vitamin B12-
487 independence in taxa possessing the vitamin B12-independent methionine synthase
488 (MetE) gene (Bertrand et al., 2013; Helliwell et al., 2014). Other strategies used by
489 phytoplankton to cope with low cobalamin concentration include, increased cobalamin
490 acquisition machinery, decreased cobalamin demand, and management of reduced
491 methionine synthase activity through changes in folate and S-adenosyl methionine
492 metabolism (Bertrand et al., 2012). The available data on B12 half-saturation constants
493 for phytoplankton (0.1-10 pM) (Droop, 1968, 2007; Taylor and Sullivan, 2008; Tang et
494 al., 2010; Koch et al., 2011) are similar or higher than the B12 concentrations measured
495 here (0.3 pM in the coastal and 0.15 pM in the oceanic waters, on average), reinforcing
496 the hypothesis of a phytoplankton community adapted to B12 limiting concentrations in
497 this upwelling system.

498 The positive responses of phytoplankton in surface oceanic waters in February were
499 associated with high abundance of *Synechococcus* and SAR11 (Fig. 4, 7). *Synechococcus*
500 produce a B12 analog known as pseudocobalamin, where the lower ligand base adenine
501 replaces 5,6-dimethylbenzimidazole (DMB) (Helliwell et al., 2016). In natural
502 conditions, pseudocobalamin is considerably less bioavailable to eukaryotic algae than

503 other cobalamin forms (Heal et al., 2017; Helliwell et al., 2016). SAR11 do not require
504 B12 and do not have pathways for its synthesis, suggesting that phytoplankton responds
505 to B12 when its synthesis is likely reduced, due to the low abundance of B12 producers.
506 The higher abundance of *Synechococcus* in oceanic compared to coastal waters may
507 explain the low concentration of B12 (Fig. 4).

508 There were positive effects of B1 addition on phytoplankton and bacteria in subsurface
509 oceanic waters in winter, also associated to high abundance of *Synechococcus* and, to
510 some extent, of Actinobacteria (Fig. 6 and Fig. 7). While *Synechococcus* is capable of B1
511 synthesis (Carini et al., 2014; Sañudo-Wilhelmy et al., 2014; Gómez-Consarnau et al.,
512 2018), Actinobacteria has a strong dependence on this vitamin (Gómez-Consarnau et al.,
513 2018) and both prokaryotic groups showed a strong positive correlation with secondary
514 responses of bacteria to B1 amendments (Table 1). Among the sequenced eukaryote
515 genomes, only Stramenopiles contain genes codifying for the synthesis of thiamine
516 monophosphate (Cohen et al., 2017; Sañudo-Wilhelmy et al., 2014). The ubiquitous
517 presence of Stramenopiles in the sampling area, dominated by Bacillariophyta, could
518 explain the relatively restricted response of phytoplankton to B1. The simultaneous
519 stimulation of phytoplankton and bacteria by B1 addition in subsurface oceanic waters in
520 winter suggest a strong demand for this compound under these particular conditions,
521 however what triggers the observed responses remain unclear.

522

523 Even though B1 caused a significant effect on phytoplankton only in subsurface waters
524 in winter, half of the positive responses of bacteria were associated to B1 supply (Fig. 6).
525 This pattern is consistent with the recently described widespread dependence of
526 bacterioplankton on external B1 supply (Paerl et al., 2018). B1 stimulated bacterial
527 growth in subsurface coastal waters and surface oceanic waters in summer, associated to

528 high abundance of *Planktomarina* and Actinobacteria (Fig. 6 and Fig. 7), which are
529 expected to strongly depend on external B1 sources (Giebel et al., 2013; Gómez-
530 Consarnau et al., 2018). The generalized significant and positive bacterial responses to
531 vitamin treatments in surface oceanic waters in summer, when the bacterial biomass was
532 high and dissolved inorganic nitrogen concentration was very low (Fig. 3) suggest that
533 bacteria may have an advantage in the uptake and assimilation of B vitamins under
534 nitrogen limiting conditions.

535

536 **4.2 Negative responses to vitamin B1 and B12 amendments**

537 Similar experiments conducted in this area also reported negative responses of microbial
538 plankton to vitamin B12 additions (Barber-Lluch et al., 2019). The generalized bacterial
539 negative responses after vitamin amendments during summer (Fig. 5, Fig. 6, and Fig. S2
540 in the Supplement), when nutrient concentrations were low (Fig. 3), suggest either a
541 strong competition between phytoplankton and bacteria or a stimulation of grazing and/or
542 bacterivory. Dinoflagellates were particularly abundant in summer at both sampling sites
543 and depths. Many dinoflagellate species are auxotrophs for B1 and/or B12 (Tang et al.,
544 2010), and also many of them are phagotrophs (Sarjeant and Taylor, 2006; Smayda, 1997;
545 Stoecker et al., 2017; Stoecker and Capuzzo, 1990), thus the external supply of B vitamins
546 may have promoted their growth, ultimately leading to net decreases in microbial biomass
547 at the end of the experiments. Several studies demonstrated that vitamin B12 is implicated
548 in the occurrence of dinoflagellate blooms around the world (Aldrich, 1962; Carlucci and
549 Bowes, 1970; Takahashi and Fukazawa, 1982; Yu and Rong-cheng, 2000). It has been
550 suggested that the B12-dependent enzyme methylmalonyl-CoA mutase in dinoflagellate,
551 euglenoid, and heterokont algae allows them to grow heterotrophically when B12 is

552 available (Croft et al., 2006). Therefore, the B12 enrichment could trigger such nutritional
553 strategy, particularly in summer, when mineral nutrients are less available.

554 Strikingly, phytoplankton and bacteria biomass systematically decreased upon B vitamins
555 supply in surface coastal water during summer (Fig. 6), associated to high abundance of
556 Flavobacteriales (Fig. 7). All isolates of Bacteroidetes sequenced so far are predicted to
557 be B12 auxotrophs (Gómez-Consarnau et al., 2018; Sañudo-Wilhelmy et al., 2014) and
558 recent metatranscriptomic analyses reveal that B1 synthesis gene transcripts are relatively
559 low in Flavobacteria as a group (Gómez-Consarnau et al., 2018). Therefore, the
560 systematically negative response of bacteria to B vitamins in surface coastal water in
561 summer is most likely associated to increased predation rather than to competition with
562 phytoplankton. By contrast, the negative responses observed in subsurface coastal waters
563 in summer were mostly associated to high abundances of *Planktomarina* and
564 Cellvibrionales (Fig. 7). Both bacterial groups showed a significantly negative correlation
565 with the phytoplankton response to B1 and/or B12 (Table 1) enrichments, which suggests
566 competition between phytoplankton and bacteria. This hypothesis is reinforced by the
567 opposite patterns of response of these two microbial components, while phytoplankton
568 responded negatively only to single B vitamin additions, bacteria responded negatively
569 only when both inorganic nutrients and B vitamins were added (Fig. 6). It is conceivable
570 that phytoplankton had an advantage over bacteria when mineral nutrients were added.

571 In conclusion, our findings indicate that the heterogeneous responses of microbial
572 plankton to B1 and B12 vitamins supply in this coastal upwelling system is mainly driven
573 by the composition of the prokaryote community, which is consistent with their major
574 role as B12 producers and B1 consumers. The overall moderate responses in terms of
575 biomass together with the low ambient B12 concentration, suggest that the microbial

576 plankton in this area is well adapted to cope with B vitamin shortage and that a close
577 balance exists between production and consumption of these important growth factors.

578

579 *Author contribution.*

580 Eva Teira designed the experiments and Vanessa Joglar carried them out with
581 contributions from all co-authors. Vanessa Joglar analyzed the data, Vanessa and Eva
582 Teira interpreted the results and Vanessa Joglar prepared the manuscript under Eva Teira
583 supervision.

584 *Competing interests.* The authors declare that they have no conflict of interest.

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593

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839 **6 Tables and Figures**

840 **Table 1:** Pearson correlation coefficient of phytoplankton and bacterial responses to
 841 different B vitamin treatments (including primary and secondary responses) with the
 842 seven prokaryotic taxa which best explained the microbial plankton responses to B-
 843 vitamins. Asterisks mean statistically significant Pearson correlation.

844

	Actinobacteria	Flavobacteriales	Synechococcus	SAR 11	<i>Planktomarina</i>	Cellvibrionales	Euryarchaeota	
Bacteria	B12	0.609*	-0.402	0.407	0.33	-0.202	-0.147	-0.141
	B1	0.003	0.264	-0.112	-0.365	0.097	0.182	-0.211
	B12B1	0.545	-0.158	0.398	0.038	-0.207	0.103	-0.272
	IB12/I	0.566	-0.571	0.576	0.459	-0.239	-0.252	0.087
	IB1/I	0.709*	-0.900*	0.757*	0.818*	-0.487	-0.442	0.297
	IB12B1/I	0.441	-0.568	0.401	0.635*	-0.464	-0.292	0.419
Phytoplankton	B12	0.451	-0.43	0.527	0.536	-0.686*	-0.552	0.499
	B1	0.474	-0.587*	0.368	0.566	-0.580*	-0.600*	0.459
	B12B1	0.124	-0.078	0.26	0.233	-0.53	-0.314	0.412
	IB12/I	0.496	-0.302	0.519	0.359	-0.184	-0.287	0.058
	IB1/I	0.029	-0.027	-0.0149	-0.024	0.148	-0.0311	0.109
	IB12B1/I	0.598*	-0.422	0.381	0.347	-0.318	-0.497	0.138

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846

847 **Figure 1:** (a) The NW Iberian margin (rectangle) and locations of the stations that were
848 sampled in the Ría de Vigo and on the shelf (diamonds), (b) distribution of daily coastal
849 upwelling index (I_w) and (c) registered precipitations during each sampling period
850 showing the initial time of each experiment (3a, 3b, 3c and 6a, 6b, 6c). ns: no sampling
851 day.

852 **Figure 2:** Vertical distribution in the coastal station of (a) fluorescence ($\mu\text{g l}^{-1}$), (b)
853 temperature ($^{\circ}\text{C}$) and (c) salinity (PSU) over time for February, April and August and
854 vertical distribution in the oceanic station of (d) fluorescence ($\mu\text{g l}^{-1}$), (e) temperature ($^{\circ}\text{C}$)
855 and (f) salinity (PSU) over time for February, April and August.

856 **Figure 3:** Initial biological conditions and abiotic factors at the coastal (st3) and oceanic
857 (st6) sampling stations. Each bar corresponds to one of the 3 experiments performed in
858 each depth and station during February, April and August. (a), Chl-*a*, total Chl-*a* ($\mu\text{g l}^{-1}$);
859 (b) BB, bacterial biomass ($\mu\text{g C l}^{-1}$); (c) DIN, dissolved inorganic nitrogen ($\mu\text{mol N l}^{-1}$)
860 and (d) DIN:DIP, ratio nitrogen:phosphate.

861 **Figure 4:** (a) Averaged relative contribution of reads to the major taxonomic groups of
862 eukaryotes and prokaryotes at surface and SCM in the coastal and oceanic station in
863 February, April and August. (b) Averaged B12 concentration (pM) at surface and SCM
864 in the coastal and oceanic station in February, April and August.

865 **Figure 5:** (a) Phytoplankton biomass (estimated as Chl-*a* concentration) ($\mu\text{g l}^{-1}$) and (b)
866 bacterial biomass in the time-zero of each experiment (striped bars) and in the final-time
867 of each treatment (colored bars) applied to surface and SCM experiments conducted in
868 the coastal and oceanic station in February, April and August.

869 **Figure 6:** Monthly averaged response ratio (RR) of (a) total phytoplankton community
870 and of (b) bacterial community at surface and SCM in the coastal and oceanic station.
871 Horizontal line represents a response equal to 1, that means no change relative to control

872 in the pink bars (treatments with vitamins alone) and no change relative to inorganic (I)
873 treatment in the green bars (vitamins combined with I treatments). Asterisks indicate
874 phytoplankton or bacterial significant response relative to control or I (Z-test; * $p < 0.05$)
875 and a indicate response with a level of significance between 0.05 and 0.1 (Z-test; ^a $p =$
876 0.05-0.06).

877 **Figure 7:** Distance based redundancy analysis (dbRDA) of B vitamin responses by
878 microbial plankton based on Bray-Curtis similarity. Filled and open symbols represent
879 samples from coastal and oceanic station, respectively, numbers correspond to the
880 sampling station, triangles and circles represent samples from surface and SCM,
881 respectively, and colours correspond to the months: (green) February, (blue) April and
882 (pink) August. Only prokaryotic taxa that explained variability in the B vitamin responses
883 structure selected in the DistLM model (step-wise procedure with adjusted R^2 criterion)
884 were fitted to the ordination.

885

Supplement information

- 1 **Table S1:** concentration of hydroxocobalamin (OHB12) and cyanocobalamin (CNB12)
- 2 in seawater samples corresponding to the initial time of the experiments. Abbreviations:
- 3 Not detected (nd) and lower concentration of the quantification limit (<LOQ).

Sample ID	Station	Depth	Month	OHB12 pM	CNB12 ⁴ pM ⁵
1602_st3_d1_p1	coast	surface	February	0.21	nd
1602_st3_d3_p1	coast	surface	February	0.20	nd ⁶
1602_st3_d5_p1	coast	surface	February	0.26	nd ⁷
1604_st3_d1_p1	coast	surface	April	0.47	nd
1604_st3_d3_p1	coast	surface	April	0.66	nd
1604_st3_d5_p1	coast	surface	April	0.23	nd
1608_st3_d1_p1	coast	surface	August	0.30	nd
1608_st3_d3_p1	coast	surface	August	0.38	nd
1608_st3_d5_p1	coast	surface	August	0.19	nd
1602_st3_d1_p2	coast	SCM	February	0.36	nd
1602_st3_d3_p2	coast	SCM	February	0.10	nd
1602_st3_d5_p2	coast	SCM	February	0.41	nd
1604_st3_d1_p2	coast	SCM	April	0.32	nd
1604_st3_d3_p2	coast	SCM	April	0.27	nd
1604_st3_d5_p3	coast	SCM	April	0.15	nd
1608_st3_d1_p2	coast	SCM	August	0.46	nd
1608_st3_d3_p2	coast	SCM	August	0.21	nd
1608_st3_d5_p2	coast	SCM	August	0.39	nd
1602_st6_d1_p1	ocean	surface	February	0.31	nd
1602_st6_d3_p1	ocean	surface	February	0.09	nd
1602_st6_d5_p1	ocean	surface	February	0.06	nd
1604_st6_d1_p1	ocean	surface	April	0.13	nd
1604_st6_d3_p1	ocean	surface	April	0.09	nd
1604_st6_d6_p1	ocean	surface	April	0.04	nd
1608_st6_d1_p1	ocean	surface	August	0.20	nd
1608_st6_d3_p1	ocean	surface	August	0.09	nd
1608_st6_d6_p1	ocean	surface	August	0.14	nd
1602_st6_d1_p2	ocean	SCM	February	0.21	0.77
1602_st6_d3_p2	ocean	SCM	February	0.08	nd
1604_st6_d1_p2	ocean	SCM	April	nd	nd
1604_st6_d3_p2	ocean	SCM	April	0.07	nd
1604_st6_d6_p2	ocean	SCM	April	0.05	nd
1608_st6_d1_p2	ocean	SCM	August	0.19	nd
1608_st6_d3_p2	ocean	SCM	August	0.09	nd
1608_st6_d6_p2	ocean	SCM	August	0.16	nd

8 **Table S2:** Summary of initial conditions for each experiment (expt). Sampling months
 9 were February (Feb), April (Apr) and August (Aug).

Station	Depth	Month	Expt	Temp °C	Sal	NO ₃ ⁻ μM	NO ₂ ⁻ μM	NH ₄ ⁺ μM	HPO ₄ ²⁻ μM	DIN:P	SiO ₄ ²⁻ μM	Chl-a μg l ⁻¹	BB μgC l ⁻¹		
Coast	surface	Feb	3a	13.75	35.02	2.86	0.19	0.35	0.17	19.65	3.62	1.39	1.84		
			3b	13.22	34.27	4.89	0.36	0.51	0.33	17.25	6.77	0.73	1.91		
			3c	13.43	34.21	4.63	0.19	0.09	0.18	27.68	8.57	4.86	3.45		
		Apr	3a	12.96	34.58	2.21	0.24	0.32	0.19	14.55	5.24	2.73	7.88		
			3b	13.31	34.25	12.46	0.36	0.54	0.41	32.73	12.57	1.40	9.17		
			3c	14.04	31.83	4.18	0.16	0.55	0.19	25.90	10.52	2.18	4.30		
		Aug	3a	14.14	35.60	0.50	0.10	0.84	0.12	11.77	1.11	5.73	14.64		
			3b	14.36	35.61	0.81	0.08	1.08	0.20	9.95	0.28	5.52	6.39		
			3c	13.66	35.16	3.93	0.17	0.12	0.33	12.78	3.86	5.64	10.61		
SCM		Feb	3a	13.73	35.71	3.58	0.14	0.04	0.31	12.13	5.25	0.21	1.30		
			3b	13.91	35.27	4.16	0.15	0.07	0.37	11.91	4.63	0.99	1.83		
			3c	13.45	34.66	2.94	0.09	0.10	0.17	18.37	6.13	4.98	2.36		
		Apr	3a	12.80	35.34	3.22	0.34	0.46	0.28	14.34	4.39	0.99	5.90		
			3b	13.22	35.28	0.24	0.07	0.12	0.04	10.19	2.83	2.15	9.47		
			3c	13.92	34.95	0.21	0.07	0.10	0.06	6.52	3.41	2.18	9.51		
		Aug	3a	13.58	35.62	0.91	0.13	0.23	0.15	8.32	1.68	20.75	12.71		
			3b	13.82	35.61	1.40	0.16	0.14	0.23	7.49	1.40	20.07	1.73		
			3c	13.38	35.63	5.29	0.13	0.14	0.41	13.47	3.93	4.63	9.21		
Ocean	surface	Feb	6a	13.98	30.20	1.32	0.18	0.11	0.16	10.07	3.23	0.82	2.38		
			6b	14.16	35.86	0.90	0.11	0.04	0.12	9.15	2.29	1.20	2.98		
			6c	14.10	35.40	1.03	0.15	0.13	0.16	8.43	2.97	2.08	2.92		
		Apr	6a	13.44	35.68	0.95	0.11	0.06	0.12	9.63	2.31	1.51	6.58		
			6b	13.59	35.66	0.47	0.11	0.06	0.08	8.33	2.71	1.29	7.37		
			6c	13.93	35.57	0.12	0.03	0.06	0.04	4.90	2.08	0.75	11.76		
		Aug	6a	15.97	35.61	0.05	0.01	0.06	0.02	4.88	1.46	0.65	39.38		
			6b	16.04	35.59	0.26	0.01	0.09	0.05	7.46	3.21	0.99	11.46		
			6c	15.34	35.53	0.45	0.04	0.05	0.07	7.38	1.37	1.30	5.63		
		SCM		Feb	6a	14.08	35.75	1.73	0.20	0.04	0.18	11.18	3.47	0.88	2.28
					6b	14.10	35.76	1.60	0.19	0.02	0.15	11.75	2.86	1.22	3.18
					6c	14.13	35.82	1.13	0.18	0.12	0.16	9.17	2.92	2.39	3.49
				Apr	6a	13.28	35.69	1.63	0.31	0.10	0.18	11.51	3.16	1.61	5.38
					6b	13.28	35.68	1.45	0.33	0.12	0.16	11.88	2.42	1.50	6.96
					6c	13.72	35.60	0.03	0.06	0.07	0.05	3.01	1.89	1.45	11.74
Aug	6a			14.90	35.60	0.00	0.04	0.10	0.03	4.20	1.44	0.84	26.55		
	6b			15.95	35.60	0.27	0.00	0.07	0.05	6.45	2.79	1.11	6.04		
	6c			15.41	35.62	0.35	0.06	0.06	0.07	6.51	1.66	1.41	5.45		

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12 **Figure S1:** Response ratio (RR) to inorganic nutrient addition (averaged biomass at the
13 end of the experiments by the averaged value in the control) of total phytoplankton
14 community (smooth bars) and of bacterial biomass (striped bars) at (a) coastal and (b)
15 oceanic station. Each bar corresponds to one of the 3 experiments (a, b or c) performed
16 in each depth and station during February, April and August. Colours represent samples
17 from (light grey) surface and (dark grey) SCM. Horizontal line represents a response
18 equal to 1, that means no change relative to control. Asterisks indicate phytoplankton
19 significant response relative to control (t-test; * $p < 0.05$) and circle indicate bacterial
20 significant response relative to the control (t-test; ⁰ $p < 0.05$). Note that different scales
21 were used.

22 **Figure S2:** Response ratio (RR) of total phytoplankton community (smooth bars) and of
23 bacterial biomass (striped bars) at (a) surface and (b) SCM in the coastal station and at
24 (c) surface and (d) SCM in the oceanic waters. Treatments represented are: B12; B1;
25 B12+B1 in pink tones and I+B12/I; I+B1/I; I+B12+B1/I in green tones. Pink bars
26 represent primary responses to B vitamins and green bars represent secondary responses
27 to B vitamins. Horizontal line represents a response equal to 1, that means no change
28 relative to control in the primary responses, and no change relative to inorganic treatment
29 in the secondary responses. Asterisks indicate phytoplankton significant response (t-test;
30 * $p < 0.05$) and circle indicate bacterial significant response (t-test; ⁰ $p < 0.05$). Note that
31 different scales were used.

32 **Figure S3:** A multidimensional scaling (MDS) showing the distance according to
33 similarity in the microbial plankton composition at the beginning of each experiment
34 (each symbol). Filled and open symbols represent samples from coastal and oceanic
35 station, respectively, numbers correspond to the sampling station, triangles and circles

36 represent samples from surface and SCM, respectively, and colours correspond to the
37 months: (green) February, (blue) April and (pink) August.

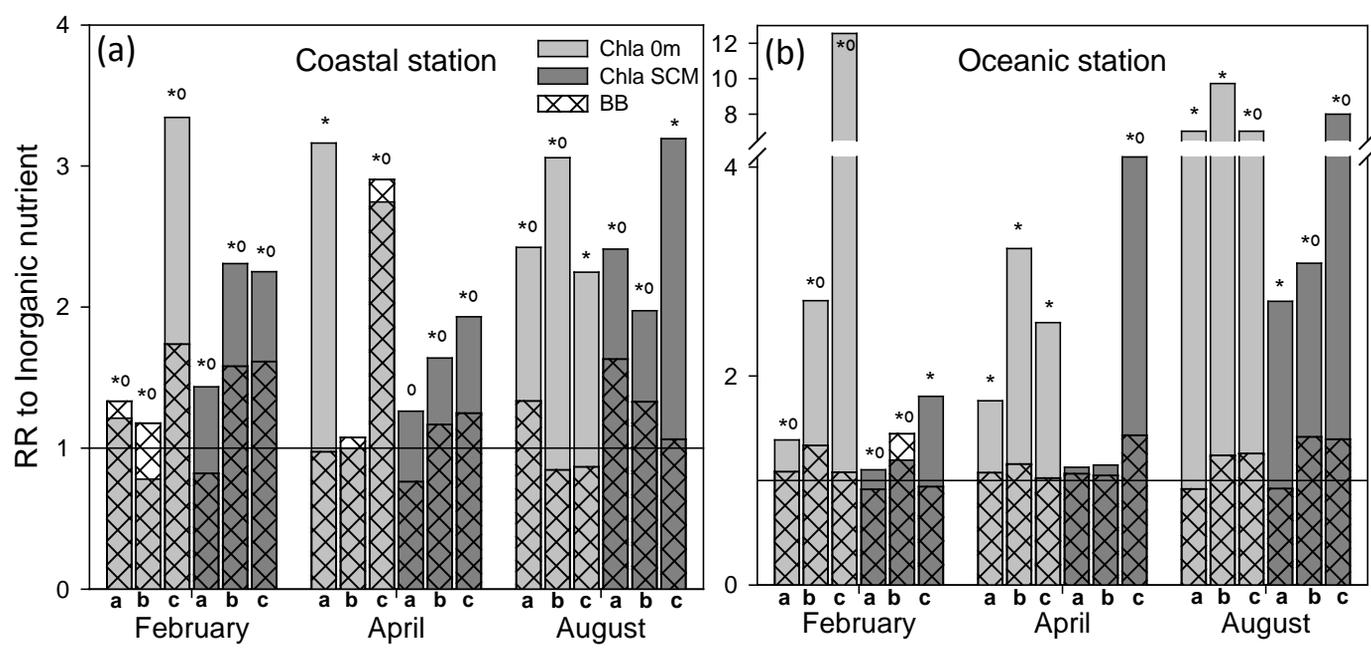


Figure S1

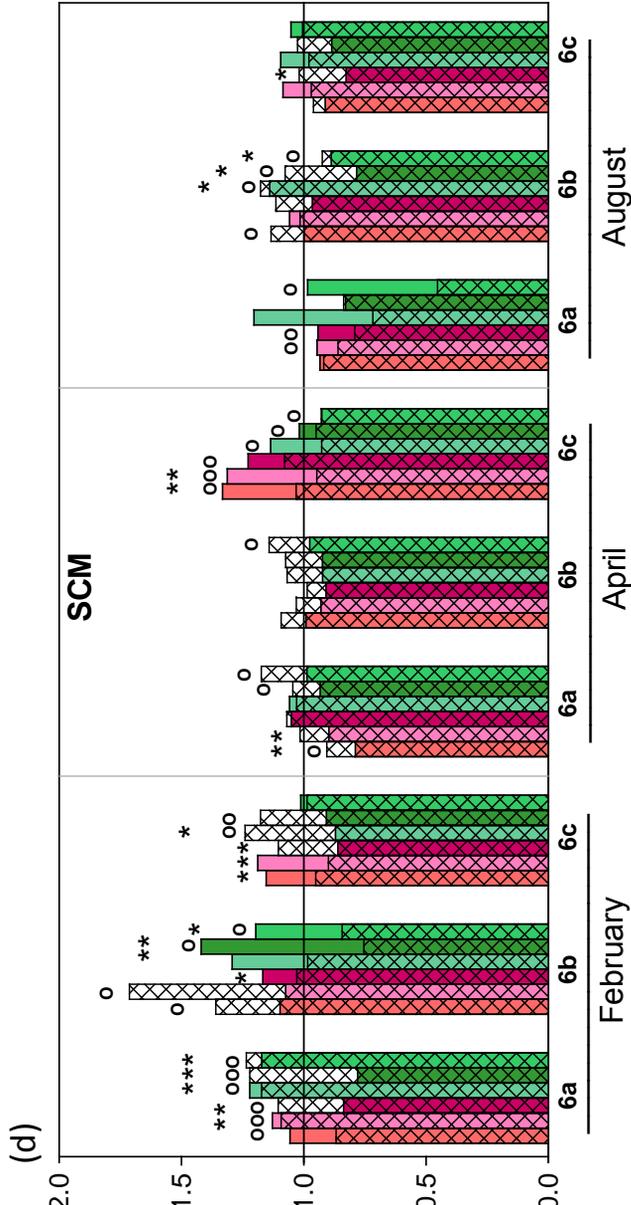
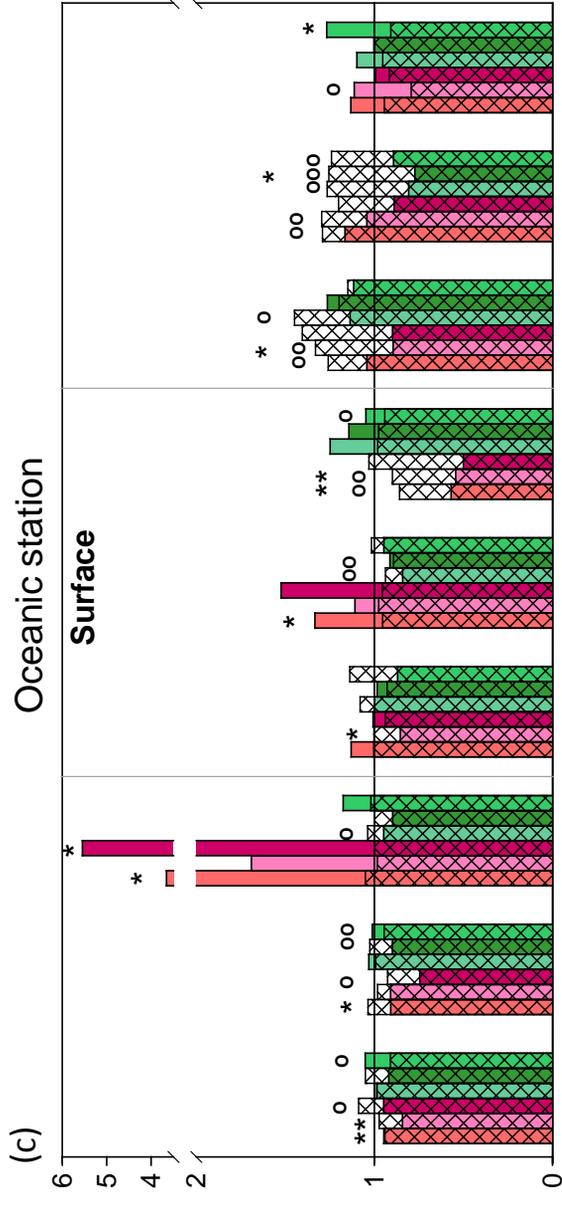
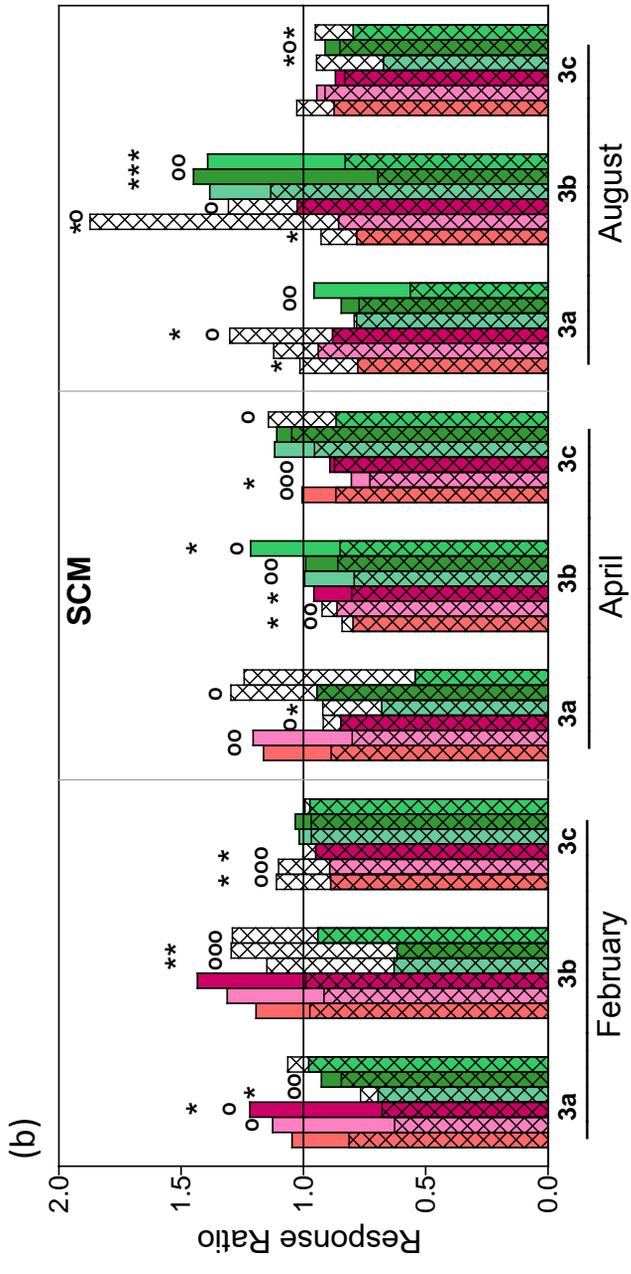
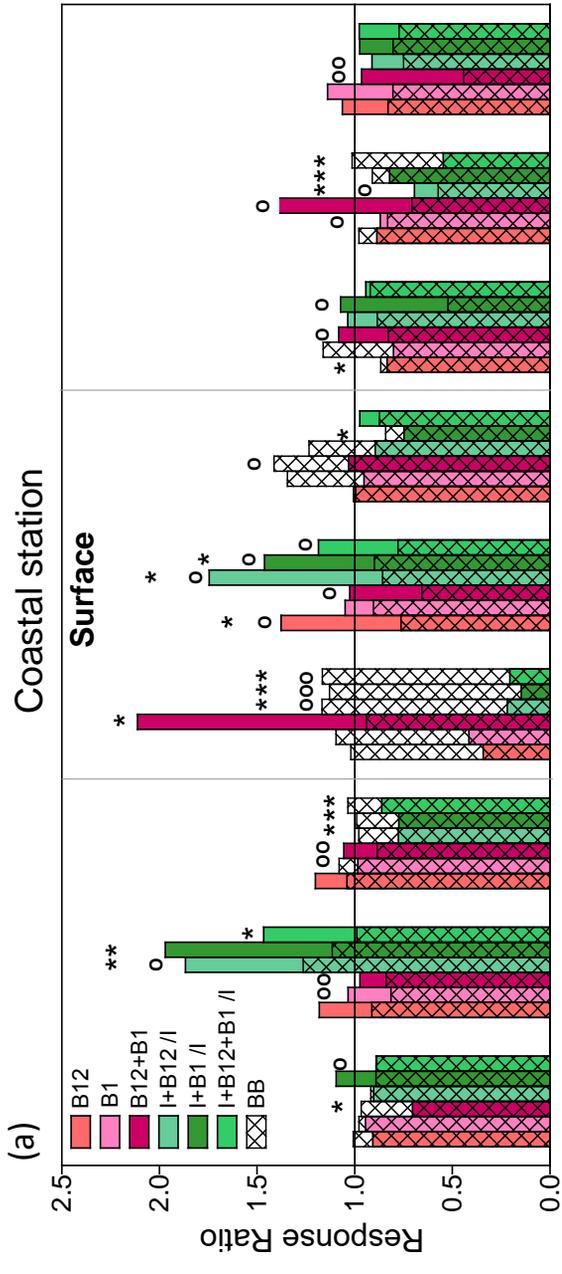


Figure S2

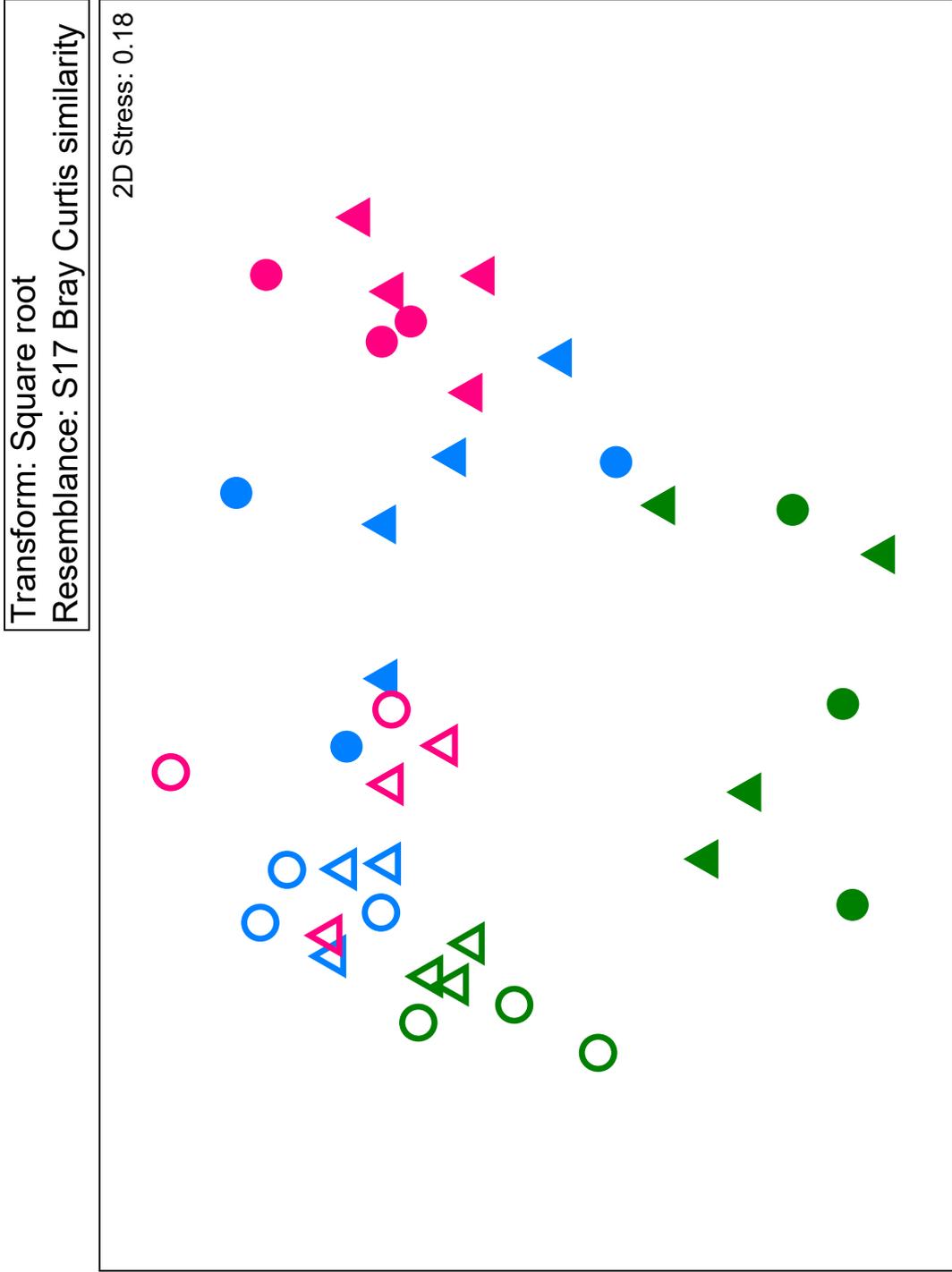


Figure S3