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1 **Seasonality of amino acid enantiomers and microbial community at**
2 **MOLA time series in the NW Mediterranean**

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4
5 **Highlights**

- 6
7
- 8 • Ship-based time series can be combined with remote sensing to close
9 sampling gaps.
 - 10 • Phytoplankton spring bloom occurs between February and May in
11 NW Mediterranean Sea.
 - 12 • Phytoplankton and river discharge affect the seasonality of L-DHAA
13 concentrations.
 - 14 • Accumulation of d-DHAA suggests bacterial processing of labile DOC
15 in summer.
 - 16 • DOM-microbe interactions are most apparent in lineages associated
17 with blooms
- 18

**Seasonality of amino acid enantiomers and microbial community at
MOLA time series in the NW Mediterranean**

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1 **Abstract**

2 Seasonality in light, temperature, and nutrient availability are well-known to
3 regulate phytoplankton blooms and the bacterioplankton community. During the
4 spring bloom, phytoplankton release biomolecules as part of the dissolved organic
5 matter (DOM) pool exploited by the bacterioplankton. Here, we investigated the
6 seasonal variability of phytoplankton biomass, enantiomers of dissolved
7 hydrolyzable amino acids (DHAA), bacterioplankton abundances and community
8 composition at the Microbial Observatory Laboratory Arago (MOLA) in the NW
9 Mediterranean Sea from 2019 to 2021. Phytoplankton biomass estimated from
10 pigment biomarkers suggests a spring bloom succession from cryptophytes,
11 haptophytes, and prasinophytes in March to diatoms in April. The spring bloom
12 coincided with a 50% increase in L-enantiomers of DHAA and an increase in
13 bacterial abundance. After the spring bloom, elevated concentrations of D-
14 enantiomers of DHAA and gamma-aminobutyric acid suggest bacterial processing
15 of labile biomolecules contributed to the seasonal accumulation of dissolved
16 organic carbon (DOC). Linking organic molecules with the free-living
17 bacterioplankton community showed a seasonal succession of niches and substrate
18 regimes. The parallel analysis of DOM and bacterioplankton community provides
19 an important baseline for bacteria-substrate relationships over the seasonal cycle
20 in the northwestern Mediterranean Sea.

21

22 1 Introduction

23 Marine dissolved organic matter (DOM) is a diverse mixture of compounds that
24 account for 662 Pg of organic carbon and play a critical role in global
25 biogeochemical cycles (Hansell, 2013). A single water sample can contain up to
26 20,000 molecular formulas, most of which remain uncharacterized to date (Riedel
27 and Dittmar, 2014; Wagner et al., 2020). The characterizable fraction of DOM is
28 frequently described based on its physical properties, i.e., size, and/or
29 characterizing major biochemical groups, such as amino acids, to determine the
30 bioavailability, diversity, and origin. Specifically, amino acids can be used to
31 estimate the labile, semi-labile, and refractory DOC reservoirs (Davis and Benner,
32 2007).

33 DOM sources and sinks can be estimated using L- and D-enantiomers of
34 amino acids. The main sources of marine DOM are phytoplankton biomass that
35 produce L-enantiomers of amino acids, which are essential building blocks for all
36 living organisms (Jørgensen et al., 1999). DOM is remineralized by heterotrophic
37 bacterioplankton (i.e., archaea and bacteria), which are enriched in D-enantiomers
38 as key constituents of the peptidoglycan, capsules, lipopolysaccharides,
39 siderophores, and antimicrobial peptides (Park and Strominger, 1957; Hancock,
40 1960; Lam et al., 2009). Additionally, most bacterioplankton preferentially use L-
41 over D-enantiomers of amino acids (Azúa et al., 2014; Zhang and Sun, 2014). Some
42 cultural representatives do use D-amino acids, with a preference for D-Alanine, D-
43 Glx, D-Asx, and D-Serine over D-Methionine, D-Valine, and D-Leucine, which are
44 assumed to be less bioavailable (Zhang et al., 2016). Therefore, tracing the
45 evolution of both pools of enantiomers in dissolved hydrolyzable amino acids

46 (DHAA) can provide information on whether the main DOM sources are
47 phytoplanktonic versus bacterial origin.

48 DOM bioavailability varies seasonally and controls the bacterioplankton
49 community. Organic carbon is predominantly derived from phytoplankton blooms
50 that provide pulses of labile and semi-labile compounds, which sustain bacterial
51 carbon and energy requirements during a given year (Piontek et al., 2011; Liu et
52 al., 2020, 2022; von Jackowski et al., 2022). Upon the decay of the spring
53 phytoplankton bloom, members of the *Roseobacter* clade (class:
54 Alphaproteobacteria) and Flavobacteria (class: Bacteroidetes) typically dominate
55 the bacterioplankton community in early successional stages (Pinhassi and
56 Hagström, 2000; Teeling et al., 2016; Bunse and Pinhassi, 2017). In summer,
57 SAR86 (class: Gammaproteobacteria) and SAR11 (class: Alphaproteobacteria)
58 become dominant clades in the bacterioplankton community (Alderkamp et al.,
59 2006; Alonso-Saez et al., 2007; Salter et al., 2015). Simultaneously, semi-labile
60 DOM compounds tend to accumulate since they resist rapid bacterial turnover and
61 possibly remineralized during fall or exported to the deep ocean during wintertime
62 convective mixing (Carlson et al., 1994; von Jackowski et al., 2020).

63 In the NW Mediterranean Sea, abiotic factors control the seasonal niche
64 preferences of the autotrophic and heterotrophic community (Salter et al., 2015;
65 Galand et al., 2018; Lambert et al., 2019). Seasonal changes in the DOM pool are
66 driven by the interplay between bacterioplankton during the spring bloom and low
67 salinity water (LSW, PSU < 37.9) events in summer (Laghdass et al., 2010;
68 Gonzalez et al., 2019). Additionally, the release of phytoplankton-derived
69 transparent exopolymer particles (TEP) have been linked to a peak in bacterial

70 extracellular enzyme activities resulting in an accumulation of recalcitrant
71 dissolved organic carbon (DOC) in the euphotic zone (0-200 m) during the summer
72 stratification period (Copin-Montégut and Avril, 1993; Romera-Castillo et al.,
73 2010; Vila-Reixach et al., 2012; Jones et al., 2013; Ortega-Retuerta et al., 2018). It
74 is possible that the seasonal accumulation of recalcitrant DOC causes a
75 malfunctioning of the microbial loop in summer to late fall (Thingstad et al., 1997;
76 Sánchez-Pérez et al., 2020). To understand seasonal DOC accumulation and its
77 factors in regulating bacterioplankton community patterns, we conducted a 3-year
78 survey at the Microbial Observatory Laboratory Arago (MOLA) in the Gulf of Lion,
79 northwestern Mediterranean Sea (NWMED). We hypothesized that the
80 pronounced seasonal variability of DHAA regulates the bioavailability, which has
81 consequences for microbial loop dynamics. Assessing relationships between
82 microbes (i.e., phytoplankton and bacterioplankton) and the DOM pool over the
83 seasonal cycle is important to understanding carbon cycling and substrate regimes
84 in the NW Mediterranean Sea.

85

86 **2 Material and Methods**

87 **2.1 *Sampling Site***

88 The “Microbial Observatory Laboratory Arago” (MOLA) time series station is
89 located at the edge of the continental shelf in the NW Mediterranean Sea
90 (42°27'205"N - 3°32'565"E) (Fig. 1). MOLA core parameters are sampled almost
91 every month since 2004 and the time series was incorporated into the
92 Mediterranean Ocean Observing System for the Environment (MOOSE) research
93 infrastructure in 2010. At MOLA, the conductivity, temperature, and depth sensor

94 (CTD) is equipped with two temperature probes, two conductivity probes, and a
95 Digiquartz pressure sensor. Seawater is collected using 12-L Niskin bottles to
96 sample nine discrete depths: 5 m, 20 m, 40 m, 80 m, 120 m, 150 m, 200 m, 300 m,
97 and 500 m.

98 MOLA was sampled 20 times for MOOSE core parameters and additional
99 microbial parameters between 2019 and 2021 (Supplementary Table 1). In brief,
100 MOOSE core parameters include the concentration of oxygen determined with
101 titration (Winkler, 1888), inorganic nutrients determined on a continuous flow
102 nutrient analyzer (AA3HR AutoAnalyzer, Bran-Luebbe, Ireland), chlorophyll-a
103 (chl-a) extracted from GF/F filters (Whatman, USA) with acetone and determined
104 fluorometrically (Evans et al., 1987), particulate organic carbon and nitrogen
105 determined on a CHNS-O Elemental Analyzer (Sharp, 1974), and bacterial cell
106 counts determined using flow cytometry (Cytoflex, Beckman Coulter, USA) and
107 converted into abundances using the software FlowJo (v7.6, USA). The protocols
108 for the core parameters and data management are available on the MOOSE
109 website (<https://www.moose-network.fr/>). Additional microbial parameters
110 included pigment-based chemotaxonomy, dissolved organic carbon (DOC), total
111 dissolved nitrogen (TDN), total dissolved phosphorus (TDP), dissolved
112 hydrolyzable amino acids (DHAA), and bacterioplankton community composition
113 using the 16S rRNA marker gene.

114

115 **2.2 Characterization of *DOM and Amino Acid Enantiomers***

116 Seawater for DOM was filtered through a double-layer of combusted GF/F filters
117 (450°C for 8 hours, Whatman, USA), equivalent to 0.2 µm pore-size, and collected

118 at all MOLA depths between 5-500 m. Samples for DOC were fixed with 10 μ L
119 phosphoric acid (pH < 2), and measured using high-temperature catalytic
120 oxidation (Sugimura and Suzuki, 1988; Qian and Mopper, 1996). Samples for TDN
121 and TDP were submitted to persulfate wet-oxidation (Pujo-Pay and Raimbault,
122 1994) and using inorganic nutrients were then used to derive dissolved organic
123 nitrogen and phosphorus (DON and DOP) concentration.

124 Samples for DHAA were collected at 5 and 500 m. Seawater was filtered
125 through a double-layer of combusted GF/F filters (Whatman, USA) and collected
126 in combusted glass vials and frozen (-20°C). Samples and blanks using ultrapure
127 water were analyzed following the protocol by Escoubeyrou and Tremblay (2014).
128 In brief, hydrolysis (110°C for 20 h) with hydrochloric acid (30% Suprapur, Merck
129 Millipore, USA) under vacuum and o-phthaldialdehyde derivatization with either
130 N-Isobutyryl-L-Cysteine (Sigma-Aldrich, USA) for the L-DHAA reagent run or N-
131 Isobutyryl-D-Cysteine for D-DHAA reagent run separated enantiomeric amino
132 acids and achiral amino acids by reversed-phase high-performance liquid
133 chromatography (HPLC). The HPLC (UltiMate 3000, Thermo Fisher Scientific,
134 USA) was equipped with a Gemini C18 column (Phenomenex, USA), an
135 autosampler, and a fluorescence detector (excitation at 335 nm, emission at 450
136 nm). A gradient of the organic phase (methanol, acetonitrile) and phosphate buffer
137 was used with a constant flow rate of 0.8 mL per minute (Escoubeyrou and
138 Tremblay, 2014). DHAA identification and quantification in unknown samples
139 were achieved by comparing the retention times and peak areas with those of
140 standard amino acid solutions and procedural blanks were subtracted from final
141 concentrations. Asparagine and Glutamine were deaminated during the

142 hydrolysis and were quantified as Aspartic acid (Asx) and Glutamic acid (Glx).
143 Overall, 13 L-DHAA were detected: Alanine, Arginine, Asx, Glx, Histidine,
144 Isoleucine, Leucine, Lysine, Phenylalanine, Serine, Threonine, Tyrosine, and
145 Valine. A total of 6 D-DHAA were detected: Alanine, Asx, Glx, Leucine, Serine,
146 and Valine. Additionally, we detected Beta-Alanine, Glycine, and Gamma-
147 aminobutyric acid (GABA).

148

149 ***2.3 Phytoplankton Pigment Analysis***

150 Seawater (1-2 L) for pigment-based chemotaxonomy was collected at 5 m, filtered
151 onto combusted GF/F filters (Whatman, USA), and stored frozen (-20°C). The
152 filters were extracted using 3 mL methanol (100%) for two hours, disrupted by
153 sonication, and clarified by vacuum filtration through GF/F filters. The extracts
154 were quantified using reversed-phase HPLC (Agilent Technologies, USA), after
155 Ras et al. (2008). Phytoplankton taxonomy was assessed from pigment
156 concentrations (Table 1) and converted into class abundances and pigment-to-chl-
157 a ratios (Supplementary Table 2) using r-package “phytclass” (v.1.0.0) (Hayward
158 et al., 2023) that is based on CHEMTAX program (Mackey et al., 1996).

159 Table 1. List of the pigments used in this study and their taxonomic significance
160 (Roy et al., 2011).

Chlorophylls	Taxonomic or biochemical significance
Alloxanthin	Cryptophytes
Bacteriochlorophyll-a	Photoheterotrophic bacteria
Chlorophyll-b	Chlorophytes, Prasinophytes
19-Butanoyloxyfucoxanthin	Haptophytes, Pelagophytes

Fucoxanthin	Diatoms, Haptophytes, some Dinoflagellates, Pelagophytes
19-Hexanoyloxyfucoxanthin	Haptophytes
Lutein	Chlorophytes, Prasinophytes,
Neoxanthin	Prasinophytes, Chlorophytes
Peridinin	Dinoflagellates
Prasinoxanthin	Prasinophytes
Violaxanthin	Chlorophytes, Prasinophytes
Zeaxanthin	Cyanobacteria, Chlorophytes, Prasinophytes, Flavobacteria

161

162 **2.4 Bacterioplankton Community Analysis**

163 Samples for genomic DNA were collected at 5 and 500 m. Around 5 L of seawater
 164 was filtered through 3.0- μm and then 0.22- μm filters (Nucleopore, USA) using a
 165 peristaltic pump and stored frozen (-80°C). The genomic DNA was lysed with
 166 lysozyme (20 mg mL⁻¹, 45 min at 37°C, Sigma-Aldrich, USA), proteinase K (20 mg
 167 mL⁻¹, 60 min at 55°C, Sigma-Aldrich, USA), and extracted using Zymobionics
 168 DNATM Miniprep Kit (Cat. No.: D6005, Zymo Research, USA). PCR-amplification
 169 using the universal 16S rRNA 515F and 926R primer pair covering the v4-v5
 170 hypervariable region (Parada et al., 2016) and sequenced on a MiSeq platform
 171 (Illumina, USA) at LGC Genomics GmbH (Berlin, Germany).

172 Amplicon reads were processed in RStudio (v2023.09.1+). FastQ files were
 173 processed into amplicon sequence variants (ASVs) using the R-package ‘dada2’
 174 (v1.16.1) (Callahan et al., 2016). Filtering settings were truncLen=c(190,200),
 175 maxN=0, minQ=2, maxEE=c(3,3) and truncQ=0, followed by merging using

176 minOverlap=10 and chimera removal. After singleton removal, we obtained an
177 average of 53,387 reads per sample. ASVs were taxonomically classified using the
178 SILVA SSU Reference dataset (release v138.1) (McLaren and Callahan, 2021).
179 ASVs with less than three counts in less than 3% of samples were excluded. ASVs
180 were further analyzed using R-packages ‘phyloseq’ (v1.42.0) (McMurdie and
181 Holmes, 2013), ‘ampvis2’ (v2.8.3) (Andersen et al., 2018), and ‘microeco’ (v1.4.0)
182 (Liu et al., 2021).

183

184 ***2.5 Statistical Analyses***

185 Data processing and statistical analyses were conducted in RStudio (v2023.09.1+).
186 Statistical analyses focused on changes within a Julian year, since the sampling
187 gaps over the course of the three-year time series made it inapt to determine
188 monthly averages or inter-annual variation within the discrete variables.

189 For all environmental parameters, arithmetic means and standard
190 deviations were calculated using the ‘base’ package (v4.2.1) and Spearman
191 correlations were corrected using a false discovery rate (FDR) adjusted p-value
192 (also called q-value) of 0.05. Degradation indices of DHAA were calculated based
193 on Kaiser and Benner (2009) by summarizing L- and D-enantiomer
194 concentrations. Integrations of the upper 500 m were performed using trapezoidal
195 integration.

196 For the amplicon data, alpha-diversity was analyzed with ‘iNEXT’ (v3.0.0)
197 (Hsieh et al., 2016) and permutational multivariate analysis of variance
198 (PERMANOVA) were calculated using vegan (‘adonis2’ and ‘vegdist’ functions,
199 v2.6.4) (Oksanen et al., 2022). Log₂fold-change (log₂FC) had an adjusted p-value

200 of 0.05 using the ‘DESeq2’ (v1.38.3) (Love et al., 2014) to investigate the differences
201 in bacterioplankton community between the free-living (FL) and particle-attached
202 (PA) size fractions. Distance-based redundancy analysis (dbRDA) was fit with
203 significant environmental vectors using vegan (‘envfit’ function) to investigate
204 seasonal environmental drivers of FL bacterioplankton community at 5 m
205 (Buttigieg and Ramette, 2014). Similarly, Pearson correlations with an FDR-
206 adjusted p-value was performed between ASVs and the environmental data using
207 ‘microeco’ (function ‘cal_cor’, v1.4.0) to investigate drivers of taxa (Liu et al., 2021).

208 Visualizations were performed in MATLAB with M_Map (Pawlowicz, 2020)
209 and using R-package ggplot2 (v3.4.3) (Wickham, 2016). All scripts are publicly
210 available via <https://github.com/anabelvonjackowski/>.

211

212 ***2.6 Remote Sensing and Simulation Approaches***

213 Remote sensing data products were used to fill in the gaps between discrete
214 sampling events of temperature, salinity, and chl-*a* at MOLA. For sea surface
215 temperature (SST), L4 satellite data was downloaded from the Copernicus Marine
216 Environment Monitoring Service (CMEMS), resulting in a high-resolution dataset
217 (VHR, 1/40°, ~1.4 km) of daily SST averages (Buongiorno Nardelli et al., 2013).

218 For salinity, we used two remote sensing data products to fill in the gaps
219 between discrete sampling events. Sea surface salinity (SSS) lacks a high-
220 resolution satellite-derived data for the Mediterranean (SSS, > 30 km with the
221 SMOS satellite) (Olmedo et al., 2018) and artifacts (>40) in global ocean datasets.
222 Consequently, we chose to apply two simulations with assimilations of
223 observations: a reanalysis for the January 2019 to June 2021 period and an

224 operational model from August to December 2021 to finalize the time series. The
225 high resolution ($1/24^\circ$, ~ 4 km) of the daily reanalysis (Escudier et al., 2021), the
226 daily CMEMS analysis, and the forecast model (Clementi et al., 2019) allowed the
227 assimilation of *in situ* temperature and salinity vertical profiles and satellite sea
228 level anomaly along the track data through an OceanVar assimilation scheme.

229 For sea surface chl- α , mass concentration data was downloaded from the L4
230 CMEMS satellite-derived product at 1km resolution (Berthon and Zibordi, 2004;
231 Volpe et al., 2018, 2019). The data was reconstructed using satellite-merged
232 (SeaWiFS, MODI, MERIS, VIIRS-SNPP & JPSS1) bio-optical multi-sensors that
233 use empirical and semi-analytical algorithms to calculate the remotely sensed chl-
234 α . Daily outputs were reprocessed with climatological data for the smoothing
235 procedure of the sensor merging, in addition with the DINEOF (Data
236 INterpolating Empirical Orthogonal Functions) algorithm to fill in missing data
237 due to cloud cover. Since oligotrophic waters of the Mediterranean are greener and
238 less blue than other ocean basins, the algorithm parameterized at a regional level
239 (an updated version of MedOC4) provides a more accurate estimate compared to
240 the overestimated chlorophyll by the global algorithm. Furthermore, algorithm
241 assessment and validation concluded that the product is in good agreement with
242 historical *in situ* chlorophyll data.

243

244 **3 Results**

245 Discrete sampling and remote-sensing approaches showed well-defined seasonal
246 patterns in temperature and salinity. The *in situ* surface (5 m) temperatures
247 ranged from 13 to 24°C ($n=20$), while satellite-derived sea surface temperatures

248 (SST) ranged between 13 to 25°C ($n=1096$) with rapid increases from ~15°C in
249 May to >18°C in June 2019 and 2021 compared to ~15°C in April to >18°C in June
250 in 2020. The *in situ* surface salinity ranged from 37.7 to 38.2 PSU ($n=20$), while
251 satellite-derived sea surface salinity (SSS) ranged between 37.5 and 38.4 PSU
252 ($n=1057$), both suggesting the occurrence of LSW (PSU < 37.9, >3 days) events but
253 with inter-annual variability between 2019 to 2021. LSWs likely ranged from 17
254 June to 29 July 2019, 09 June to 21 July 2020, and 28 May to 28 June 2021 (Fig.
255 1c). At 500 m, temperatures remained ~14°C and salinity ~39 PSU ($n=5$) year-
256 round, but data are only available for 2021.

257 The remote-sensing approach confirmed that spring bloom in the NWMED
258 occurred between February and May. Satellite-derived sea surface chl-*a* ranged
259 from 0.05 to 0.60 µg L⁻¹ ($n=1096$), which was slightly lower than the *in situ* surface
260 chl-*a* that ranged from 0.07 to 1.0 µg L⁻¹ ($n=21$; Fig. 1c). Both satellite-derived and
261 *in situ* chl-*a* show inter-annual variability in bloom dynamics between 2019 to
262 2021. Discrete measurements of chl-*a* increased by 85% from February to March
263 before declining 37% from March to April until the beginning of the new productive
264 season in November (Fig. 1c). Phytoplankton taxonomy estimated from pigment
265 biomarkers suggests that the spring bloom was dominated by cryptophytes,
266 haptophytes, and prasinophytes at the beginning of March and diatoms in early
267 February as well as the end of April (Fig. 2a). Accordingly, chl-*a* showed significant
268 positive correlations with cryptophytes ($r=0.83$, $p<0.001$), haptophytes ($r=0.84$,
269 $p<0.001$), prasinophytes ($r=0.88$, $p<0.001$), dinoflagellates ($r=0.86$, $p<0.001$), and
270 diatoms ($r=0.63$, $p=0.01$, Supplementary Fig. 1). Chlorophytes were only observed

271 in spring 2021, while *Synechococcus* appeared to grow in spring, summer, and fall
272 (Fig. 2a and 3).

273 Maximal inorganic nutrient concentrations were observed in winter, which
274 gradually decreased during the productive season until late fall. At 5 m, nitrate
275 (NO_3^-) ranged from below the detection limit to $2.3 \mu\text{mol L}^{-1}$ ($n=21$), nitrite (NO_2^-)
276 ranged from 0.010 to $0.25 \mu\text{mol L}^{-1}$ ($n=21$), and silicate (SiO_4) ranged from 0.25 to
277 $2.0 \mu\text{mol L}^{-1}$ ($n=21$). Ammonium (NH_4^+) ranged from below the detection limit to
278 $0.068 \mu\text{mol L}^{-1}$ ($n=21$), and phosphate (PO_4^{3-}) ranged from 0.010 to $0.80 \mu\text{mol L}^{-1}$
279 ($n=21$). At 500 m, NO_3^- ranged from 7.7 to $9.1 \mu\text{mol L}^{-1}$ ($n=18$), NO_2^- ranged from
280 0.010 to $0.31 \mu\text{mol L}^{-1}$ ($n=18$), PO_4^{3-} ranged from 0.31 to $0.41 \mu\text{mol L}^{-1}$ ($n=18$), and
281 SiO_4 ranged from 5.1 to $7.6 \mu\text{mol L}^{-1}$ ($n=18$).

282 Maximal POC concentrations and minimum DON concentrations were
283 observed in winter, while DOC and DOP concentrations decreased to a minimum
284 spring and accumulated during the productive season. At 5 m, concentrations of
285 POC ranged from 4.1 to $19 \mu\text{mol L}^{-1}$ ($n=21$) and generally decreased from winter
286 and throughout the productive season until fall (Supplementary Fig. 2). DOC
287 ranged from 58 to $102 \mu\text{mol L}^{-1}$ ($n=21$), DON concentrations ranged from 4.5 to 7.6
288 $\mu\text{mol L}^{-1}$ ($n=19$), and DOP concentrations ranged from 0.038 to $0.13 \mu\text{mol L}^{-1}$ ($n=19$;
289 Supplementary Fig. 2). DOC:DON:DOP ratios increased from 13:92:1 in spring,
290 14:96:1 in summer, 15:68:1 in fall, and decreased to 15:77:1 in winter. DOC
291 significantly negatively correlated with chl- α ($r=-0.59$, $p<0.001$). At 500 m, POC
292 concentrations ranged from 1.8 to $16 \mu\text{mol L}^{-1}$ ($n=20$) or 1529 to 4733 mmol m^{-2}
293 and peaked in winter and late summer ($n=20$; Supplementary Fig. 3). DOC
294 concentrations ranged from 45 to $94 \mu\text{mol L}^{-1}$ ($n=18$), DON ranged from 2.8 to 5.9

295 $\mu\text{mol L}^{-1}$ ($n=18$), and DOP ranged from below the detection limit to $0.13 \mu\text{mol L}^{-1}$
296 ($n=18$; Supplementary Fig. 3).

297 DHAA concentrations showed seasonal variability with an accumulation of
298 L-DHAA in March and July compared to a gradual accumulation of D-DHAA until
299 late summer. At 5 m, DHAA ranged from 0.65 to $1.17 \mu\text{mol L}^{-1}$ ($n=13$) that
300 negatively correlated with salinity ($r=-0.62$, $p<0.05$) and diatoms ($r=-0.82$, $p<0.01$).
301 Calculating degradation indices (DI) showed positive DIs (less degraded) in spring
302 and during the LSW events in June compared to negative DIs (more degraded) in
303 winter (Fig. 2b). Further investigating the enantiomer composition showed that
304 L-DHAA ranged from 0.38 to $0.67 \mu\text{mol L}^{-1}$ ($n=13$; Fig. 2c) and significantly
305 negatively correlated to diatoms ($r=-0.77$, $p<0.01$; Supplementary Fig. 1). In
306 contrast, D-DHAA ranged from 40 to 68 nmol L^{-1} ($n=15$; Fig. 2c) and significantly
307 increased with the Julian Day (JD) ($r=0.72$, $p=0.0025$), while significantly
308 negatively correlating with chl- a ($r=-0.69$, $p<0.01$) and phytoplankton groups that
309 bloomed in March (Supplementary Fig. 1). Specifically, individual monomers
310 showed that all L-DHAA increased more than two-fold from February to March
311 (Fig. 2d), while several L-DHAA (Histidine, Lysine, Serine, Tyrosine, and Valine)
312 decreased more than 1.3-fold in April (Fig. 2e). Similar to their L-DHAA
313 counterparts, B-Alanine, D-Alanine, and D-Glx also increased in April, whereas
314 D-Serine and D-Valine decreased in April (Fig. 2f). Generally, B-Alanine and D-
315 DHAA increased throughout the year (Fig. 2f). At 500, DHAA ranged from 0.42 to
316 $4.6 \mu\text{mol L}^{-1}$ with L-DHAA ranging from 0.17 to $1.7 \mu\text{mol L}^{-1}$ ($n=15$) peaking in
317 spring and D-DHAA ranging from 27 to 142 nmol L^{-1} ($n=15$) increasing towards
318 fall (Supplementary Fig. 3).

319 Seasonal changes were observed in the bacterioplankton abundance and
320 community composition at 5 m. Bacterioplankton abundances ranged from 3.8 to
321 13×10^5 cells mL⁻¹ ($n=20$) with a notable shift in high (HNA) and low nucleic acid
322 (LNA) subgroups. HNA cells ranged from 1.8 to 5.2×10^5 cells mL⁻¹ ($n=20$) and
323 peaked in late February to May and July to September. LNA cells ranged from 1.6
324 to 8.9×10^5 cells mL⁻¹ ($n=20$) and peaked in late February to early March and July
325 to August (Supplementary Fig. 2). Assessing the amplicon data showed that the
326 sequencing depth sufficiently covered the bacterioplankton diversity free-living
327 (FL) fraction and particle-attached (PA) ~99.7% (Supplementary Fig. 4). At 5 m,
328 the FL community significantly changed with the JD (PERMANOVA, $p<0.01$)
329 along with the PA community that also significantly changed with the JD
330 (PERMANOVA, $p<0.05$; Supplementary Table 3). Furthermore, alpha-diversity
331 indices of the FL showed a significant increase in richness ($r=0.69$, $p=0.0041$) and
332 Shannon diversity ($r=0.52$, $p=0.045$) with the JD (Supplementary Fig. 5, outlier
333 JD 27). At the class level, FL and PA were represented by Alphaproteobacteria,
334 Gammaproteobacteria, Bacteroidetes, and Cyanobacteria up to 80%. At the family
335 level, FL community was dominated by SAR11 clade I ~18.8%, SAR11 clade II
336 ~6.2%, and Flavobacteriaceae ~12.8% year-round, while Cyanobiaceae showed
337 monthly fluctuations. The PA community was dominated by Cyanobiaceae ~21.7%
338 and families in Bacteroidetes ~19.8% but also reoccurring Pirellulaceae ~2.8%,
339 Puniceococcaceae 2.6%, and Vibrionaceae ~3.5% (Fig. 3). Testing the seasonal
340 enrichment showed significant changes in SAR11 clades, SAR86 clade, and
341 AEGEAN-169 marine group during spring, summer, or winter in FL (\log_2FC

342 $q < 0.001$) compared to Planctomycetes in winter in PA (\log_2FC $q < 0.001$,
343 Supplementary Fig. 6).

344 Seasonal changes were less pronounced in the bacterioplankton abundance
345 and community composition at 500 m. Bacterial abundances ranged from 0.97 to
346 9.6×10^5 cells mL^{-1} ($n=19$) with HNA ranging from 0.48 to 5.1×10^5 cells mL^{-1} ($n=19$)
347 and LNA ranged from 0.47 to 4.5×10^5 cells mL^{-1} ($n=19$). Assessing the amplicon
348 data showed that the sequencing depth sufficiently covered the bacterioplankton
349 diversity FL fraction and PA $\sim 99.3\%$ (Supplementary Fig. 4). The alpha-diversity
350 indices gradually decreased by JD in the FL, while alpha-diversity peaked in
351 summer within the PA (Supplementary Fig. 5). At the class level, the FL was
352 dominated by Nitrososphaeria, whereas the PA community had representatives of
353 Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes up to 50% (Fig. 3). At
354 family level, FL community was dominated by Nitrosopumilaceae $\sim 32.3\%$ along
355 with SAR11 clades I $\sim 8.5\%$ and clade II $\sim 4.7\%$. PA community was almost year-
356 round, represented by Nitrosopumilaceae, Pirellulaceae, Crocinitomicaceae, and
357 Phycisphaeraceae $\sim 4\%$.

358 Combining environmental parameters with the FL bacterioplankton
359 community suggests DOM-microbe interactions. A distance-based redundancy
360 analysis (dbRDA) was used to assess the influence of environmental explanatory
361 variables (arrows) on the ASV abundance data. At 5m, the dbRDA showed that
362 salinity, dinoflagellates, and pelagophytes significantly constrained the ordination
363 space of the response variables (points) corresponding to the winter and spring
364 samples ($p < 0.05$, Fig 4a). In contrast, temperature, D-Asx, and DON significantly
365 constrained the ordination space corresponding to summer and fall ($p < 0.05$, Fig.

366 4a). Attempting direct links between environmental parameters and the FL
367 bacterioplankton community showed that *Candidatus* Nitrosopumilus and
368 common bloom-associated genera within the Roseobacter lineage and
369 Flavobacteriiaeae positively correlated with phytoplankton groups, while
370 negatively correlating with temperature ($p\text{-adj}<0.05$; Fig. 4b). In contrast, genera
371 such as *Candidatus* Aquiluna, *DS001*, and other Roseobacter group members
372 positively correlated to several L-DHAA ($p\text{-adj}<0.05$, Fig. 4b). At 500 m, data were
373 insufficient to derive the significant environmental variables, but L-DHAA
374 constrained the ordination space corresponding to winter samples, while DON,
375 DOP, and D-DHAA constrained the ordination space of summer and fall samples
376 (Supplementary Fig. 7). Direct links were limited in the mesopelagic depth with
377 few genera showing significant links to L-DHAA, B-Alanine, and D-Serine
378 (Supplementary Fig. 7).

379

380 **4 Discussion**

381 *4.1 The spring bloom in the NW MED*

382 The haptophyte- and diatom-dominated blooms caused L-DHAAs and the
383 degradation index to peak at 5 m during spring (Fig. 2). The phytoplankton spring
384 bloom composition at MOLA was similar to nearby coastal time series *Service*
385 *Observation Laboratory Arago* (SOLA), *Blanes Bay* near Barcelona, Spain, and
386 *DYFAMED* near Villefranche-sur-Mer, France (Marty et al., 2002; Latasa et al.,
387 2010; Gutiérrez-Rodríguez et al., 2011; Nunes et al., 2018). However, relative
388 abundances of *Synechococcus* were higher at MOLA compared to the Adriatic Sea
389 (Trano et al., 2022). The increase of phytoplankton-derived L-DHAA could be

390 similar across the NWMED with a release of hydroxyl-amino acids (L-Serine and
391 L-Threonine) and glycine from diatom cell walls post-bloom in March (Hecky et
392 al., 1973; Dauwe and Middelburg, 1998). In June, the peak of aromatic-DHAA (L-
393 Phenylalanine and L-Tyrosine) and L-Asx might have been released by cell
394 exudation or lysis of diatoms (Hecky et al., 1973). Furthermore, the different
395 pattern of L-Glx, which was low during the first diatom bloom but increased to 135
396 nmol L⁻¹ during the second diatom bloom in April, might suggest a succession of
397 diatom populations. *Chaetoceros* sp. and *Rhizosolenia* sp. tend to bloom in early
398 spring, while *Pseudo-nitzschia* sp. has been observed to bloom at SOLA between
399 March and May (Charles et al., 2005; Quiroga, 2006). Overall, chl-*a* and L-DHAA
400 concentrations were over 2-fold higher in surface waters at MOLA compared to
401 more oligotrophic sites during spring and summer, such as Bermuda Ocean Time
402 Series Station (BATS) and Hawaii Ocean Time Series (HOT) (Lomas et al., 2013;
403 Wirtz and Smith, 2020; Ianiri et al., 2022). Studying phytoplankton bloom
404 dynamics is essential to understand biogeochemical and microbial interactions.
405 We strongly encourage time series to analyze larger phytoplankton via microscopy
406 or automated imaging systems, e.g., imaging flow cytobot (IFCB) (Olson and Sosik,
407 2007; Sosik and Olson, 2007), and additional sampling efforts for smaller
408 phytoplankton, e.g., flow cytometry, to better understand the mechanisms of
409 bloom formation and links to biochemistry at offshore time series.

410 DOM bioavailability positively correlated with bacterial abundances and
411 coincided with copiotroph ecological strategies. Heterotrophs can be generally
412 distinguished as either HNA or LNA, where HNA are considered to be a more
413 substrate-driven and metabolically active subpopulation than LNA (Robertson

414 and Button, 1989; Sherr et al., 2006; Bouvier et al., 2007). Furthermore,
415 heterotrophs can be distinguished as either copiotrophs that compete under high
416 DOM concentrations or oligotrophs adapted to low concentrations (Koch, 2001;
417 Giovannoni et al., 2014). In terms of composition, Bacterioidetes (Flavobacteriaceae)
418 and members of the *Roseobacter* group are non-seasonal and known copiotroph
419 bacteria since they are more competitive under bioavailable DOM levels in the
420 NWMED (Lambert et al., 2019; Auladell et al., 2022). The positive correlations
421 with phytoplankton groups are indicative of labile DOM and diatom-derived DOM
422 consumption (Sarmiento et al., 2013; Landa et al., 2018). Alongside the
423 copiotrophs, we find oligotrophs such as SAR11 (Fig. 3). Within SAR11, the
424 ecotype-like clades exhibit pronounced seasonal cycles and confirm previous
425 observations that SAR11 clade *Ib* is associated with replete nutrient
426 concentrations and phytoplankton biomass, while clade *Ia* are adapted to thrive
427 under basal DOM levels (Salter et al., 2015). SAR11 ecotypes appear to be
428 differentially involved in the labile and humic substrate processing during the
429 spring bloom and can not be classified into these over-simplified ecological
430 strategies (Malmstrom et al., 2004; Alonso-Sáez and Gasol, 2007; Laghdass et al.,
431 2012). Still, a bacterioplankton community with fairly balanced proportions of
432 copiotrophs and oligotrophs can simultaneously process the phytoplankton-
433 derived protein-like substances that contribute to the labile DOC pool and humic-
434 like fluorescent organic matter that contribute to the recalcitrant DOC pool
435 released during the spring bloom (Yamashita and Tanoue, 2003; Romera-Castillo
436 et al., 2010; Sánchez-Pérez et al., 2020). Furthermore, experiments have shown
437 that picocyanobacteria and bacterioplankton produce humic-like fluorescent

438 organic matter under phosphorus-replete conditions during the spring bloom
439 (Romano et al., 2014; Zhao et al., 2017; Bouchachi et al., 2023). The humic-like
440 fluorescent organic matter would be less bioavailable than phytoplankton-derived
441 compounds and possibly serve oligotrophs. Overall, the copiotrophs appear to be
442 processing labile DOM, like *Roseobacter*, while oligotrophs might be processing
443 relatively refractory DOM, like SAR11 clade *Ia*, and supports the notion that
444 trophic strategies follow a gradient from copiotrophy to oligotrophy at the end of
445 spring (Lemonnier et al., 2020).

446

447 *4.2 Microbial loop dynamics from summer until winter*

448 LSWs are diluted mesoscale structures that detach from the Rhône River plume
449 to the open Mediterranean Sea and impact biogeochemical processes around June
450 to July each year (Diaz et al., 2008). Our study confirms that LSWs are low in chl-
451 *a* and dissolved inorganic nitrogen (DIN) but contain bioavailable DOM that
452 sustains bacterial activity (Laghdass et al., 2010; Gonzalez et al., 2019). In 2020,
453 we observed a strong increase in several L-DHAA and DI, therefore
454 bioavailability, during the LSW (JD 175) compared to after the LSW (JD 203; Fig.
455 2). Enrichments of phenylalanine and valine could have been released from
456 *Chaetoceros* sp. (Poulet and Martin-Jézéquel, 1983; Sarmiento et al., 2013).
457 Isoleucine and leucine are known to contribute to the highly labile fraction of DOM
458 (Carlson and Ducklow, 1995; Cherrier et al., 1996), promoting bacterioplankton
459 growth in summer.

460 Bacterioplankton were increasingly exposed to D-DHAA towards fall (Fig.
461 2). DHAA accumulated on the surface waters during summer, which appeared as

462 relatively short-term fluctuations in individual L-DHAA but a steady increase of
463 D-DHAA concentrations until fall (Fig. 2). Since D-DHAA amino acids are proxies
464 of bacterial-derived DOM, their presence indicates more degraded DOM at the end
465 of the accumulation period. To further understand the accumulation in the D-
466 DHAA pool, “canonical” DHAA (D-Alanine, D-Asx, D-Glx) showed relatively
467 higher concentrations and greater seasonal variability than “non-canonical”
468 DHAA (D-Leucine and D-Valine; Fig. 2), which is similar to previous observations
469 (Broek et al., 2019; Ianiri et al., 2022). Canonical DHAA are more abundant in the
470 HMW material and non-canonical DHAA are more abundant in the LMW material
471 (Ianiri et al., 2022), which could suggest a preference of bacterioplankton for
472 canonical D-DHAA. For example, D-Alanine is a key component of autotrophic and
473 heterotrophic bacterial peptidoglycan (Schleifer and Kandler, 1972; Kaiser and
474 Benner, 2008; Cava et al., 2011), which would be relatively rapidly remineralized
475 compared to recalcitrant DHAA that accumulates as part of the LMW DON
476 towards fall (Ianiri et al., 2022). Nonetheless, D-DHAA can be used as a nitrogen
477 source for bacterial growth, to modulate cell wall biogenesis or act as a chemotactic
478 warning signal, but many of the related functions and metabolic pathways remain
479 unknown (Zhang et al., 2016; Yu et al., 2020; Irazoki et al., 2023). The seasonal
480 changes in the DHAA pool amid few correlations to the bacterioplankton
481 community (Fig. 4) highlight the complex interactions among the abiotic and biotic
482 factors in the microbial loop. More research with longer sampling frequencies of
483 time series-based studies and higher resolution of ‘omic approaches (e.g., meta-
484 and transcriptomics) are needed to better unravel the interactions between the

485 composition of the amino acid pool and microbial communities in the NW
486 Mediterranean.

487

488 ***4.3 Mesopelagic DOM-Bacterioplankton interactions***

489 Organic matter seasonal variations in euphotic and mesopelagic zones will affect
490 the deep ocean bacterioplankton community and their metabolism. In MOLA,
491 mesopelagic POC and L-DHAA concentrations peaked during the spring and
492 summer, while D-DHAA concentrations remained fairly constant at depth
493 (Supplementary Fig. 3). In comparison, individual and total L-DHAA
494 concentrations were over 2-fold higher at MOLA than previously reported from
495 more oligotrophic sites during spring and summer, such as BATS and HOT (Kaiser
496 and Benner, 2008; Ianiri et al., 2022). The enrichment of L-DHAA at MOLA could
497 be due to a reduced DHAA degradation since bacterial production, which is around
498 2-3 times lower at the bottom of the euphotic zone relative to BATS (Gonzalez et
499 al., 2019; Liu et al., 2022). Alternatively, the elevated chl-*a* at the surface relative
500 to previous observations (section 4.1) could have enhanced organic matter export
501 at MOLA through various pathways (phytoplankton, aggregates zooplankton
502 migration or mixing processes (Siegel et al., 2016; Le Moigne, 2019). Surface
503 productivity combined with strong continental (westerly/northerly) winds that
504 cause dense shelf water cascading events favor POC export in the southwestern
505 Gulf of Lyon, near MOLA (Puig et al., 2008; Sanchez-Vidal et al., 2008; Many et
506 al., 2021). Furthermore, assuming that sinking or ascending particles contributed
507 to the POC pool at MOLA, these sinking particles release amino acids into the
508 surrounding water (Smith et al., 1992; Grossart and Ploug, 2000; Baumas et al.,

509 2023). Finally, our 500m samples are taken near the seafloor, where sediment
510 resuspensions into the nepheloid layer could have also been a source of L-DHAA.
511 The diverse pool of available organic substrates shapes the diverse community
512 across the different fractions in mesopelagic and relative to the euphotic zone
513 (Ghiglione et al., 2012; Wilson et al., 2017; Baumas et al., 2021; Sebastián et al.,
514 2021). We encourage future studies and time series that measure microbial
515 variables to observe organic matter degradation, aggregation, and sinking in the
516 meso- and bathypelagic zones since anthropogenic climate change affects the
517 entire water column (Buttigieg et al., 2018; González-Dávila and Santana-
518 Casiano, 2023; Baumas and Bizic, 2024).

519

520 **5 Conclusion**

521 Our assessment of the DOM pool and links to the phyto- and bacterioplankton
522 communities revealed a seasonality in the surface waters and mesopelagic zone.
523 In the surface, springtime phytoplankton blooms released L-DHAA, which
524 increased HNA bacteria abundances and lowered alpha-diversity, thereby
525 promoting bacterioplankton with copiotroph ecological strategies. A notable input
526 of bioavailable DOC occurred during LSW events during summer. During
527 summer, the accumulation of DOC and D-DHAA, the presence of LNA bacteria,
528 and declining richness suggest that a broader community of less active
529 bacterioplankton target more refractory substrates into fall. Our study highlights
530 the seasonal variation in L- and D- enantiomers of DHAA and possible
531 associations with the microbial community. We strongly encourage microbial time
532 series observatories to incorporate or expand the range of discrete parameters to

533 incorporate biochemical measurements, such as DHAA, as tracers of bacterial
534 molecules in the DON pool.

535
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538 review & editing. Nawal Bouchachi; Conceptualization, Investigation, and Data
539 Curation. Quentin-Boris Barral; Methodology, Software, and Visualization. Paul
540 Labatut; Investigation. Barbara Marie; Investigation and Formal analysis. Olivier
541 Crispi; Investigation and Formal analysis. Karine Escoubeyrou; Investigation and
542 Formal analysis. Charles-Hubert Paulin; Investigation and Formal analysis.
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567 **Data Availability, Products, and Datasets.** Environmental data for DOM,
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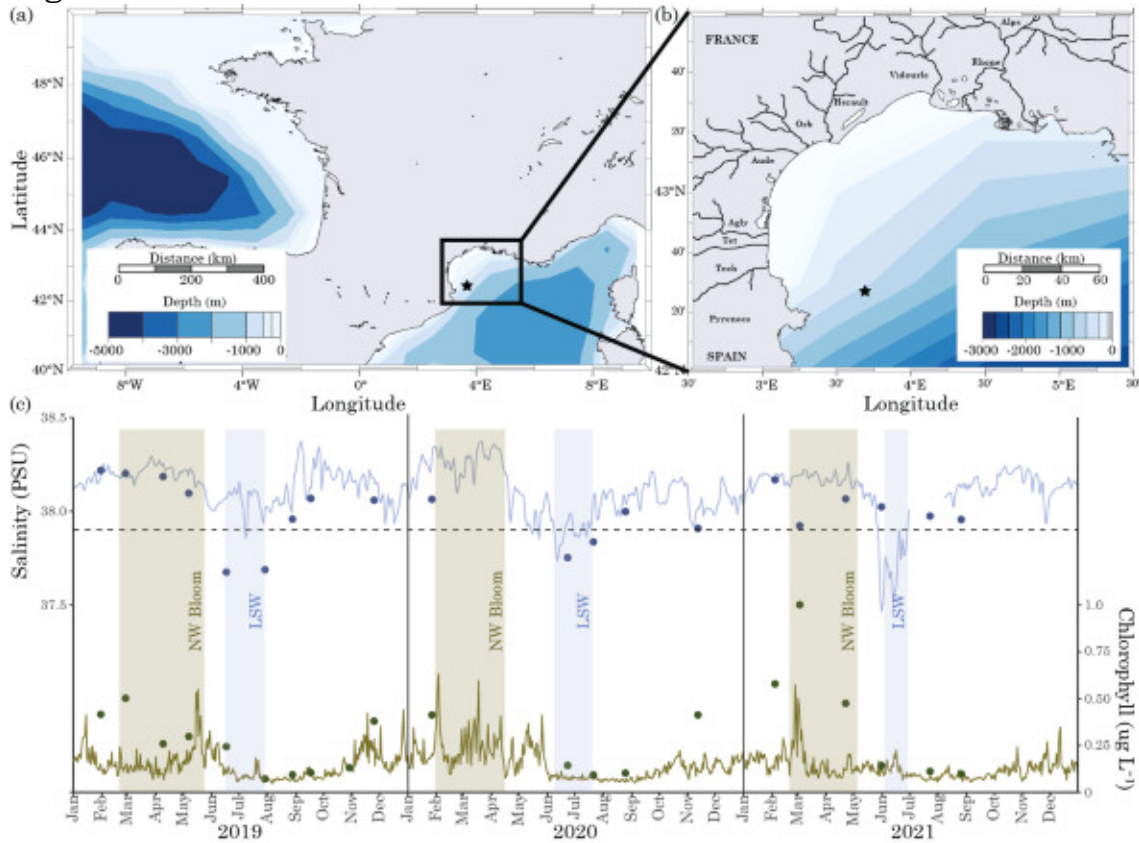
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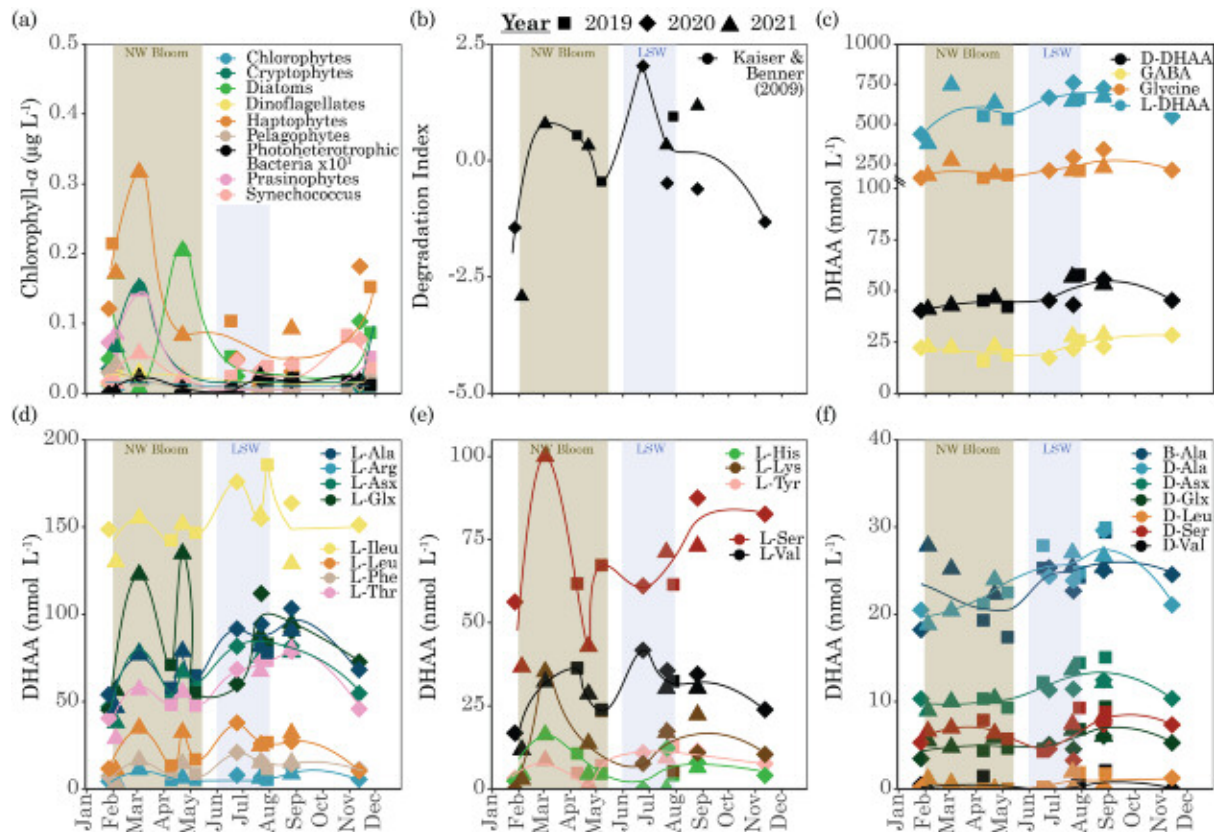
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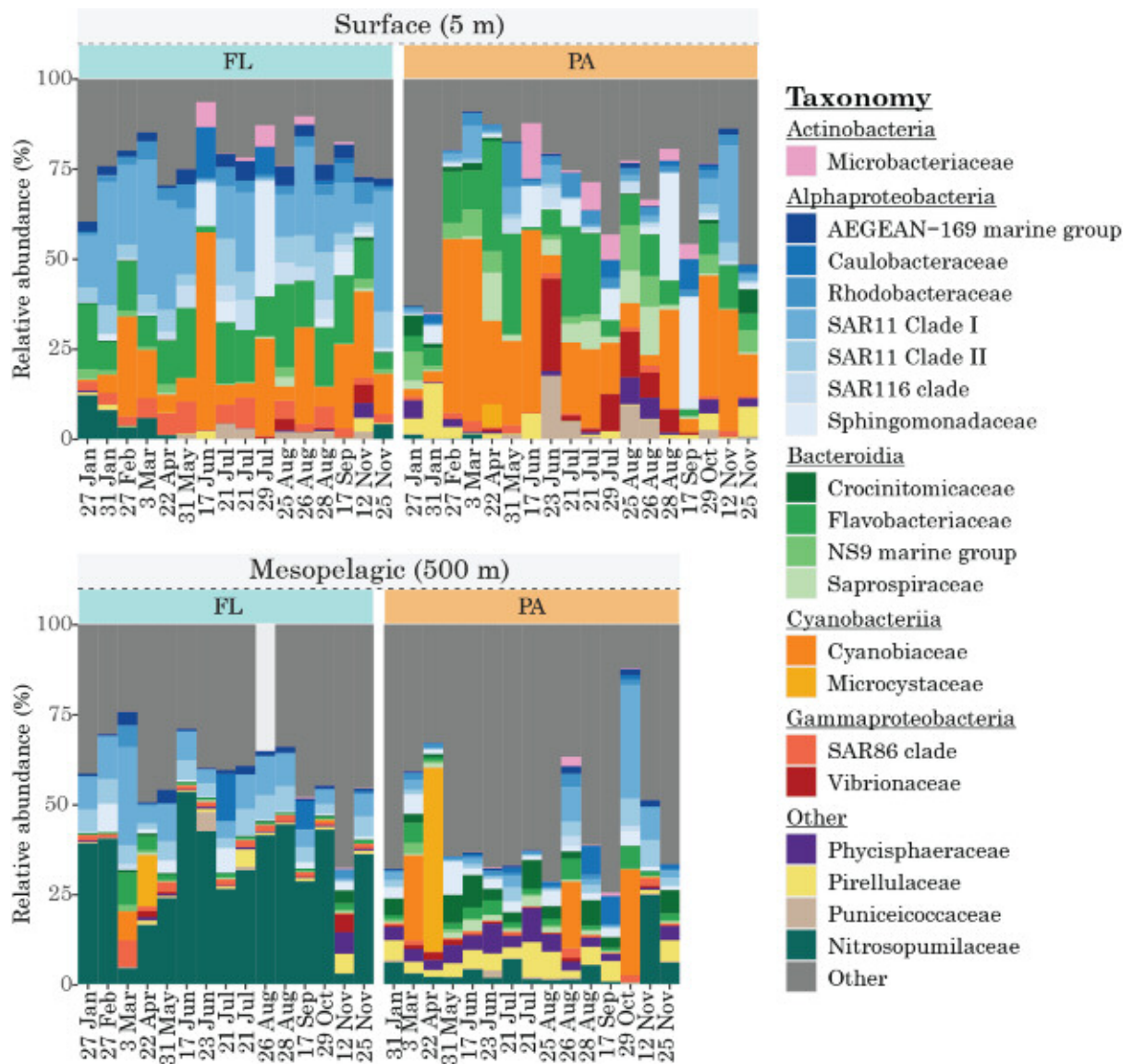
983 **Figures**



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 985 **Figure 1. Bathymetry and temporal conditions at Microbial Observatory**
 986 **Laboratory Arago (MOLA) in the northwestern Mediterranean Sea**
 987 **between 2019 and 2021.** The MOLA time series station (star) is located off (a)
 988 northern Catalonia in the (b) drainage basin of the Agly, Aude, Hérault, Orb,
 989 Rhône, Tech, Têt, and Vidourle into the Gulf of Lyon. The (c) satellite-derived (line)
 990 and subsurface (5 m, circles) of salinity (blue) and chlorophyll-a (green) at MOLA.
 991 Remote sensing allowed for a differentiation between coastal and offshore
 992 northwestern blooms. Low salinity water (LSW) events are below 37.9 PSU
 993 (dashed line) for more than three consecutive days.
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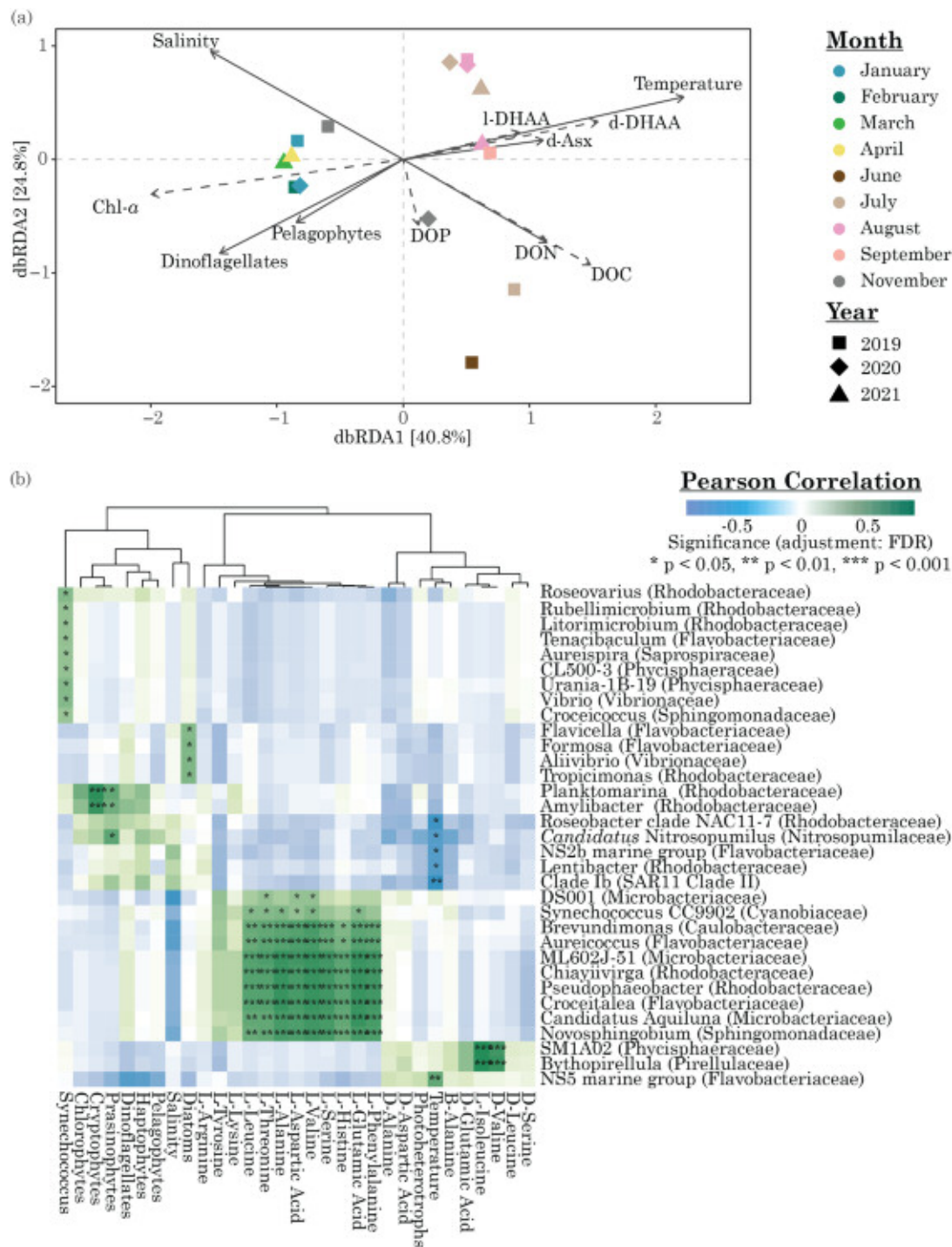


995
 996 **Figure 2. Seasonal dynamics of phytoplankton biomass and dissolved**
 997 **hydrolyzable amino acids (DHAA) at 5 meters of the Microbial**
 998 **Observatory Laboratory Arago.** The (a) phytoplankton biomass estimated
 999 from pigment biomarkers and bacteriochlorophyll-a for photoheterotrophic
 1000 bacteria, (b) DHAA degradation index, (c) total concentrations of L- and D-DHAA
 1001 and non-isomers gamma-aminobutyric acid (GABA) and glycine, (d) L-DHAA that
 1002 peak twice during the NWMED bloom, (e) L-DHAA that only peak once during the
 1003 NWMED bloom, (f) B- and D-DHAA. The shape corresponds to the year and
 1004 abbreviations refer to: Ala, Alanine; Asx, Aspartic acid and Asparagine; Arg,
 1005 Arginine; Glx, Glutamic acid and Glutamine; His, Histidine; Ileu, Isoleucine; Leu,
 1006 Leucine; Lys, Lysine; Phe, Phenylalanine; Ser, Serine; Thr, Threonine; Tyr,
 1007 Tyrosine; Val, Valine.
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Figure 3. Seasonal dynamics of the top 20 bacterioplankton families at the Microbial Observatory Laboratory Arago. Dominant orders in the free-living (FL) and particle-attached (PA) community at 5 m and 500 m depth.



1014
 1015 **Figure 4. Links between DOM and the free-living (FL) bacterioplankton**
 1016 **community at 5 meters of the Microbial Observatory Laboratory Arago.**
 1017 The redundancy analysis shows the significant environmental parameters and
 1018 ordination of the samples. A (b) pearson correlation between environmental
 1019 parameters and the bacterioplankton community at genus level within the top 20
 1020 families and false discovery rate (FDR) adjusted p-values (* p<0.05, ** p<0.01, ***
 1021 p<0.001). Abbreviations refer to: Asx, Aspartic acid and Asparagine; Chl-a,
 1022 Chlorophyll-a; dissolved hydrolyzable amino acid, DHAA; dissolved organic
 1023 carbon, DOC; dissolved organic nitrogen, DON; dissolved organic phosphorus,
 1024 DOP.