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Marine bacterial enrichment in the sea surface microlayer, and surface taxa aerosolization potential in the Western Mediterranean Sea

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1 **Abstract**

2
3 The sea surface microlayer (SSML) is critical to air-sea exchanges of gases and primary
4 aerosols. However, despite the extent of this boundary layer, little is known about its specific
5 bacterial community (bacterioneuston) and how it may affect ocean-atmosphere exchanges.
6 Here, we studied the bacterial community composition in the surface waters of three different
7 basins of the Western Mediterranean Sea and assessed the selective air-sea transfer of marine
8 bacteria through experimental nascent sea spray aerosol production in a 10 L tank with plunging
9 jets. In situ, the bacterioneuston harbored basin-specific enriched taxa and followed a similar
10 spatial pattern as the underlying bacterioplankton community. Aerosolization potential showed
11 that sea spray taxa might be recruited from both the underlying water and the SSML, and that
12 taxa enriched in the bacterioneuston were not always aerosolized. Our results suggest that the
13 Mediterranean nutrient gradient, as well as pulse events such as dust deposition, affect the
14 distribution of the bacterial community at the ocean-atmosphere interface, which may impact
15 biogeochemical processes, climate regulation and bacterial dispersal through aerosolization.

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30 **Introduction**

31
32 The sea surface microlayer (SSML) is a thin (1-1000 μm) gel-like matrix at the surface of the
33 ocean (Engel *et al.*, 2017 and reference therein). This ocean skin harbors a specific ecology and
34 chemistry in comparison to the underlying water (ULW). The biogeochemical and physical
35 processes there, are critical to air-sea exchanges of energy, gases and primary aerosols which
36 affect climate (Engel *et al.*, 2017, Barthelmeß *et al.*, 2021, Hendrickson *et al.*, 2021).
37 Atmospheric deposition can also accumulate at the SSML, influencing biogeochemical processes
38 (Astrahan *et al.*, 2016) before being exported to deeper waters. However, despite the geographic
39 extent of the SSML, the biogeographic distribution of the bacterial community living there (the
40 bacterioneuston) and its potential role in ocean-atmosphere connectivity is still poorly
41 understood.

42 The bacterioneuston is a key player in the SSML biogeochemical cycles and in air-sea
43 processes. Its activities affect organic matter composition and concentration, gas fluxes, physical
44 properties of the SSML through gel formation, and thus, aerosol composition and numbers (e.g.
45 Upstill-Goddard *et al.*, 2003, Reinthaler *et al.*, 2008, Kurata *et al.*, 2016, Engel *et al.*, 2018,
46 Freney *et al.*, 2020, Sun *et al.*, 2020, Sellegri *et al.*, 2021). The bacterioneuston composition
47 appears to be tightly linked to the ULW bacterial community. Nevertheless, while some studies
48 found that the bacterioneuston was enriched in specific taxa compared to the ULW (Franklin *et*
49 *al.*, 2005, Zäncker *et al.*, 2018), others report no significant differences between the two layers
50 (Agogue *et al.*, 2005, Obernosterer *et al.*, 2008). These discrepancies might be the results of
51 spatiotemporal variations, winds, and SSML chemical properties but also be the results of the
52 SSML collection method (Stolle *et al.*, 2010, Cunliffe *et al.*, 2011, Rahlff *et al.*, 2017, Wong *et*
53 *al.*, 2021).

54 Sea spray aerosols (SSA) represents one of the most abundant sources of natural aerosol
55 particles in the atmosphere and contribute significantly to global aerosol fluxes (McNeill, 2017).
56 SSA are produced mainly by rising bubbles bursting at the air-sea interface. As bubbles rise
57 through the water column they scavenge inorganic and biological material, which play a
58 significant role in the SSML formation (Engel *et al.*, 2017). Thus, bacterial accumulation at the
59 SSML might be a precursor to their aerosolization; and SSML properties might affect the
60 selective transfer of bacteria to SSA (Aller *et al.*, 2005, Michaud *et al.*, 2018). Indeed, nascent
61 SSA have been shown to contain a large diversity of bacteria which appear to be a subset of the
62 community present in the surface water (Fahlgren *et al.*, 2015, Rastelli *et al.*, 2017, Michaud *et*
63 *al.*, 2018, Freitas *et al.*, 2022). These studies suggest that bacterial aerosolization is selective,
64 which might be influenced by cellular properties and environmental conditions (Rastelli *et al.*,
65 2017, Michaud *et al.*, 2018). Some of these airborne bacteria can directly influence atmospheric
66 processes, cloud formation and climate through their activities and icenucleating capacities
67 (Amato *et al.*, 2017, Failor *et al.*, 2017, Beall *et al.*, 2021, Trueblood *et al.*, 2021, Šantl-Temkiv
68 *et al.*, 2022). Moreover, as SSA travel long distance (Bondy *et al.*, 2017, Mayol *et al.*, 2017,
69 Šantl-Temkiv *et al.*, 2018, Johansson *et al.*, 2019), they are a potential vector of microbial
70 dispersal, influencing bacterial biogeography.

71 Here, we studied the bacterial community composition in the surface waters of three different
72 basins of the oligotrophic Western Mediterranean Sea. We compared the spatial distribution of
73 the bacterioneuston with the bacterioplankton in the ULW, and evaluated the taxa enriched in the
74 SSML between the studied basins. We further assessed the aerosolization potential of surface
75 taxa through onboard experimental nascent SSA production.

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78 **Material and Methods**

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Sea water sampling

81 Seawater was collected during the ‘ProcEss studies at the Air-sEa Interface after dust
82 deposition in the MEditerranean sea’ cruise (PEACETIME), onboard the R/V “Pourquoi Pas ?”
83 in May/June 2017 (Guieu *et al.*, 2020). Stations were sampled in the Ionian Sea (ION),
84 Tyrrhenian Sea (TYRR), and in the Algerian basin (WEST) from a zodiac (Fig. S1). Water
85 collection is described in detail in Zäncker *et al.* (2021). Briefly, at each station sea surface
86 microlayer (SSML) water was sampled using the glass plate method, and uUnderlying water
87 (ULW) was sampled 20 cm below the SSML with two acid-cleaned bottles that were opened ca.
88 20 cm below the surface. At station FAST (the western most station), we sampled surface water
89 before a wet dust deposition event, and then 2h, 24h and 48h after the rain (SSML was not
90 sampled before the rain).

91 Nascent sea spray aerosols

92 Nascent sea spray aerosols (nSSA) were produced onboard, as described in Trueblood *et al.*
93 (2021). Briefly, nSSA were generated in a 10 L glass tank with a plunging jet system linked to a
94 continuous flow of surface water (5 m) from the ship underway system. nSSA filters were
95 collected for bacterial community composition at two stations in the Ionian Sea, and three
96 stations in the Algerian basin (Fig. S1),. and at station FAST 2h and 24h after the wet dust
97 deposition event. nSSA were filtered onto glass fiber filters over 24h at 16.5 LPM and
98 immediately stored at -20°C. Samples for bacterial abundance in nSSA were collected from 28
99 stations using a PILS sampler. 0.5 mL samples were fixed with glutaraldehyde (0.5% final), flash
100 frozen and stored at -80°C.

101 Bacterial abundance

102 2mL samples were fixed with glutaraldehyde (1% final concentration) for bacterial abundance in
103 the SSML and the ULW. Samples were stored immediately at -20°C. Abundance was measured
104 by flow-cytometry as described in Zäncker *et al.* (2021).

105 For bacterial abundance in the nSSA, the samples were stained with Sybr Green and measured
106 by flow cytometry (BD-FACS Calibur). True count beads and 2µm beads were used as
107 reference. Potential autofluorescent particles were subtracted from the counts.

108 DNA sampling, sequencing and sequence analysis

109 400 mL water for SSML and ULW were filtered through a 0.2µm filter (Durapore) after a pre-
110 filtration through a 100 µm mesh, and immediately stored at -80°C. DNA was extracted as
111 described in Zäncker *et al.* (2021). DNA extracts were quantified and normalized and used as
112 templates for PCR amplification of the V4-V5 region of the 16S rRNA gene (Parada *et al.*,
113 2016), as described in Dinasquet *et al.* (2022). The nSSA filters were extracted with a Zymo
114 quick Fungal/Bacterial DNA miniprep as described in Dadaglio *et al.* (2018). DNA extracts were
115 quantified and normalized and used as templates for PCR amplification of the V4 region of the
116 16S rRNA gene (Apprill *et al.*, 2015).

117 All reads were processed using the Quantitative Insight Into Microbial Ecology 2 pipeline
118 (QIIME2 v2020.2, Bolyen *et al.*, 2019). Reads were truncated to 250bp to cover the V4 region,
119 based on sequencing quality, denoised, merged and chimera-checked using DADA2 (Callahan *et al.*
120 *al.*, 2016). A total of 953 amplicon sequence variants (ASVs) were obtained. Taxonomy
121 assignments were made against the database SILVA 132 (Quast *et al.*, 2013). All sequences
122 associated with this study have been deposited under the BioProject ID: PRJNA693966.

123 Statistics

124 Alpha and beta-diversity indices for community composition were estimated after
125 randomized subsampling to 7888 reads for the analysis with nSSA samples and to 24728 for the
126 analysis with only the surface water samples 16S rDNA. Analyses were run in QIIME 2 and in
127 Primer v.6 software package (Clarke & Warwick, 2001). Differences between the samples
128 richness and diversity were assessed using Kruskal-Wallis pairwise test. Beta diversities were
129 run on Bray Curtis dissimilarity. Differences between samples' beta diversity were tested using
130 PERMANOVA (Permutational Multivariate Analysis of Variance) with pairwise test and 999
131 permutations. The sequences contributing most to the dissimilarity between layers were
132 identified using the ANalysis of Composition of Microbiomes (ANCOM)(Mandal *et al.*, 2015).

133

134

135 **Results and discussion**

136 The Mediterranean Sea is the largest semi-enclosed ocean basin. It is also a large Low-
137 Nutrient-Low-Chlorophyll (LNLC) region, characterized by a long summer stratification period
138 and a west-to-east gradient of increasing oligotrophy, where microbial growth is generally
139 limited by phosphorus availability (Durrieu de Madron *et al.*, 2011). The Mediterranean bacterial
140 biogeography was shown to reflect the oligotrophy gradient and physical barriers from surface to
141 deep waters (Mapelli *et al.*, 2013, Sebastián *et al.*, 2021). Here, we further describe, for the first
142 time to our knowledge, the spatial distribution of the Mediterranean bacterial community at the
143 air-sea interface and the aerosolization potential of surface taxa.

144 *Spatial distribution of bacteria community composition in the SSML and ULW*

145 The biogeochemical context of the SSML sampled during this cruise is described in Tovar-
146 Sanchez *et al.* (2020) and Zäncker *et al.* (2021). Briefly, these studies show that trace metal and
147 TEP were consistently enriched in the SSML. DOC was also enriched in the SSML (as shown
148 here, Fig.S2). Picophytoplankton were generally more abundant in the SSML, but no significant
149 difference was observed in the eukaryotic community composition and diversity (Zäncker *et al.*,
150 2021). The bacterial abundance decreased from west to east in both the SSML and ULW but
151 showed no significant enrichment in the SSML (Table 1, Tovar-Sánchez *et al.*, 2020, Zäncker *et*
152 *al.*, 2021). The bacterioplankton community, however, showed significant differences between
153 layers. Evenness was significantly lower in the SSML compared to the ULW (Fig.S3, Kruskal
154 Wallis $p=0.006$).

155 The bacterioneuston has been shown to be tightly linked to the bacterial community in the
156 ULW just below (Cunliffe *et al.*, 2013). Overall, reflecting previous studies from surface mixed

157 layer communities, the spatial distribution of the bacterial community in the first micrometers of
158 the SSML followed the Mediterranean Sea oligotrophic gradient and physical barriers. The
159 overall surface bacterioplankton (combined SSML and ULW) was significantly different
160 between basins (Fig.1, Permanova $p < 0.01$). Similar spatial distribution of the main taxa reported
161 for surface mixed layers (Mapelli *et al.*, 2013, Sebastián *et al.*, 2021) were also observed here.
162 Indeed, the relative abundance of the Gamma-Proteobacteria, Oceanospirillales,
163 Alteromonadales and Pseudoalteromonadales increased from West-to-East in both the SSML and
164 ULW, while the relative abundance of cyanobacteria (*Synechococcus*, but especially
165 *Prochlorococcus*) decreased (Fig.2, S4).

166 Nevertheless, due to the weak cloud coverage, the Mediterranean bacterioneuston is subject
167 to strong solar radiation that may influence its composition compared to lower surface layers.
168 The bacterioneuston has only been studied at a few coastal stations of the North-west
169 Mediterranean Sea (Agogue *et al.*, 2005, Joux *et al.*, 2006), and despite strong potential stressors
170 no significant differences were observed between the ULW and the SSML. Here, closely
171 comparing the ULW and SSML communities showed that the bacterioneuston in the Ionian and
172 Tyrrhenian Seas were significantly different between the two layers (Fig.1, $p < 0.05$). Generalist
173 Gamma-Proteobacteria (Oceanospirillales, Alteromonadales and Pseudoalteromonadales) were
174 particularly enriched in the SSML compared to the ULW (Fig.2, S4), which may be due to
175 different physico-chemical properties of the SSML.

176 No significant differences were observed between SSML and ULW communities in the
177 Western basin. However, at station FAST (Fig.S1) which was influenced by a wet dust
178 deposition event (Tovar-Sánchez *et al.*, 2020, Van Wambeke *et al.*, 2020, Desboeufs *et al.*,
179 2022), the SSML and ULW clustered separately with specific taxa changing in response to dust

180 compared to other Western stations (Fig.1, S4). This might be due to the Mediterranean
181 bacteria's capacity to rapidly respond to dust input (Tsiola *et al.*, 2017, Dinasquet *et al.*, 2022),
182 especially in the SSML (Astrahan *et al.*, 2016) or to the direct deposition of dust attached
183 bacteria (Rahav *et al.*, 2016, Mescioglu *et al.*, 2019, Aalismail *et al.*, 2020) to the SSML.

184

185 *Aerosolization potential of surface taxa to sea spray aerosols*

186 It is challenging to disentangle the role of marine bacteria on the atmospheric microbiome
187 due to the large influence of bacteria attached to anthropogenic, terrestrial and mineral aerosols
188 (e.g.Lang-Yona *et al.*, 2022), in particular in the semi-enclosed and dust impacted Mediterranean
189 Sea (Rahav *et al.*, 2016, Gat *et al.*, 2017, Mescioglu *et al.*, 2019). To isolate the aerosolization
190 potential of marine bacteria, complex experimental procedures with nSSA production are
191 necessary. But only few studies have investigated the selective transfer of marine taxa from
192 surface waters into nSSA (Fahlgren *et al.*, 2015, Rastelli *et al.*, 2017, Michaud *et al.*, 2018,
193 Freitas *et al.*, 2022).

194 Sea spray aerosols are formed by bubbles rising through the water column and potentially
195 scavenging material from the surface and SSML. Hence, bacterial accumulation at the SSML
196 might be a precursor to their aerosolization (Aller *et al.*, 2005).In order to better understand the
197 aerosolization potential of bacteria in the surface Mediterranean Sea, we compared the sequential
198 enrichment of bacterial taxa from the ULW to the SSML, to onboard experimentally produced
199 nSSA with surface water from the same stations. Concentration of bacteria in the nSSA likely
200 followed the same west-to-east gradient of decreasing abundance as the ULW and SSML (Table
201 1) and were in line with previous observations in marine aerosols in situ, in the Mediterranean

202 Sea (from 7 to 25x10³ cells m⁻³)(Mescioglu *et al.*, 2019) and in the North Atlantic (~8.02x10³
203 cells m⁻³)(Mayol *et al.*, 2014). The nSSA bacterial community showed significantly lower
204 diversity but higher evenness than the surface communities (Fig. S3, Kruskal-Wallis p <0.001).
205 The community structure also appeared to be basin specific with the Ionian and Western nSSA
206 communities clustered separately from ULW and SSML water communities (Fig. S5),
207 suggesting selective transfers of bacterial taxa, as observed in previous studies (Fahlgren *et al.*,
208 2015, Rastelli *et al.*, 2017, Michaud *et al.*, 2018, Freitas *et al.*, 2022). Interestingly the
209 transferred cyanobacteria followed a similar spatial gradient as in surface layers, with
210 *Prochlorococcus* better aerosolized in the Western basin where it is more abundant and
211 *Synechococcus* in the Ionian Sea. Sequential enrichment of some taxa (e.g. Rhizobiales,
212 *Sphingobium*, *Pseudomonas*, *Vibrio* and *Staphylococcus*) was observed from ULW, SSML to
213 nSSA but generally aerosolized taxa appeared to be present but not abundant throughout the
214 surface waters. Some taxa that were enriched in the SSML had a low aerosolization potential,
215 which suggests that not all bacterioneuston taxa are scavenged into the aerosols, and this might
216 be specific to cellular properties or environmental factors especially the physical properties of
217 the SSML.

218 Most of the aerosolized taxa observed here (Table 2, Fig 2, 3, S4) are commonly observed in
219 marine aerosols in other oceanic regions (e.g. Cho & Hwang, 2011, Fahlgren *et al.*, 2015,
220 Michaud *et al.*, 2018, Mescioglu *et al.*, 2019, Lang-Yona *et al.*, 2022), suggesting that cellular
221 properties consistent within these groups independent from environmental factors, influence their
222 sea-to-air transfer. A few of these taxa (Table 2, Fig 2, 3, S4) are related to known opportunistic
223 pathogens (e.g. *Vibrio*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*) for marine organisms.
224 Others are associated to known phototrophs or have specific sulfur requirements (e.g. SAR11,

225 SAR86, *Ectothiorhodobacterales*, *Coralimargarita*), which may be an advantage to survive
226 harsh atmospheric conditions. Some of the taxa with high aerosolization potentials have also
227 been shown to increase in response to higher temperature or to dust deposition (e.g. Westrich *et*
228 *al.*, 2016, Sato-Takabe *et al.*, 2019, Dinasquet *et al.*, 2022), which suggests that their dispersal
229 may have a greater impact on marine biogeochemical cycles and ecosystem health in the future
230 Mediterranean Sea.

231

232 Conclusion

233 The biogeographic distribution of the bacterial community in the very first centimeters of the
234 water column – the sea-air interface, is influenced by similar horizontal and vertical gradients, as
235 previously described for lower depths. This surface community appears to follow the
236 oligotrophic gradient but may also be influenced by pulse events like dust deposition that
237 stimulate the growth of specific opportunistic taxa. The bacterioneuston plays a crucial role in
238 the ocean and the atmosphere connectivity, where they are critical to air-sea exchange affecting
239 climate. Here, we show through onboard experiments that surface Mediterranean taxa have a
240 range of aerosolization potential. As described in other oceanic regions, these taxa can be
241 sequentially enriched through the surface layers and selectively transferred into the aerosols.
242 Sequential enrichment in the SSML and aerosolization of bacteria may be affected by future
243 climate conditions and aerosol deposition in the Mediterranean Sea. This could have a large
244 impact on climate, microbial biogeography and ecosystem services.

245

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248

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260 **Conflict of interest statement**

261 The authors declare that the research was conducted in the absence of any conflict of interest.

262

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Tables and figures

Table 1: Bacterial abundance in the three sampled basins, in the nascent sea spray aerosols (cells m^{-3}) and in the surface water (SSML and ULW, cells mL^{-1}).

	WEST	TYRR	ION	Total
SSML*	$6.62 \times 10^5 \pm 2.04 \times 10^5$ (n=8)	$4.96 \times 10^5 \pm 5.37 \times 10^4$ (n=4)	$2.92 \times 10^5 \pm 8.08 \times 10^4$ (n= 5)	$5.14 \times 10^5 \pm 2.17 \times 10^5$ (n=17)
ULW*	$5.87 \times 10^5 \pm 1.24 \times 10^5$ (n=8)	$4.83 \times 10^5 \pm 2.62 \times 10^4$ (n=4)	$2.85 \times 10^5 \pm 6.39 \times 10^4$ (n=5)	$4.74 \times 10^5 \pm 1.59 \times 10^5$ (n=17)
nSSA	$5.68 \times 10^3 \pm 3.49 \times 10^3$ (n=4)	$4.03 \times 10^3 \pm 3.62 \times 10^2$ (n=2)	1.92×10^3 (n=1)	$4.67 \times 10^3 \pm 2.86 \times 10^3$ (n=7)

*Bacterial abundance data in the SSML and ULW are also presented between Basin in Zäncker *et al.*, 2021 and details per station are presented in in Tovar-Sanchez *et al.*, 2020.

Table 2: Enrichment in the SSML and aerosolization potential in the nSSA and of the most abundant genus responsible for the difference between the communities in the ULW, SSML and nSSA based on Analysis of composition of microbiome (ANCOM).

Class	Order	Genus	SSML enrichment	Aerosolization potential	
			From ULW	from SSML	from ULW
Alpha-proteobacteria	Pelagibacterales	SAR11 clade	52%	189%	98%
Alpha-proteobacteria	Puniceispirillales	SAR116 clade	108%	31%	33%
Alpha-proteobacteria	Rhizobiales	<i>Bradyrhizobium</i>	111%	53300%	59012%
Alpha-proteobacteria	Rhizobiales	<i>Methylobacterium</i>	260%	1662%	4320%
Alpha-proteobacteria	Rhodobacterales	<i>Ascidiaceihabitans</i>	61%	47%	29%
Alpha-proteobacteria	Rhodospirillales	AEGEAN-169	60%	182%	110%
Alpha-proteobacteria	Sphingomonadales	<i>Sphingobium</i>	720%	639%	4598%
Beta-proteobacteria	Burkholderiales	<i>Delftia</i>	63%	1924%	1216%
Delta-proteobacteria	Bdellovibrionales	OM27 clade	448%	91%	407%
Gamma-proteobacteria	Alteromonadales	<i>Pseudoalteromonas</i>	542%	12%	67%
Gamma-proteobacteria	Alteromonadales	<i>Alteromonas</i>	246%	17%	41%
Gamma-proteobacteria	Cellvibrionales	OM60 clade	88%	18%	16%
Gamma-proteobacteria	Oceanospirillales	<i>Halomonas</i>	363%	1%	3%
Gamma-proteobacteria	Oceanospirillales	<i>Litoricola</i>	79%	4%	3%
Gamma-proteobacteria	Oceanospirillales	<i>Marinomonas</i>	81%	25029%	20154%
Gamma-proteobacteria	Pseudomonadales	<i>Acinetobacter</i>	152%	9%	13%
Gamma-proteobacteria	Pseudomonadales	<i>Pseudomonas</i>	197%	181%	356%
Gamma-proteobacteria	Vibrionales	<i>Vibrio</i>	173%	2739%	4732%
Gamma-proteobacteria	SAR86 clade		72%	491%	352%
Gamma-proteobacteria	Xanthomonadales	<i>Stenotrophomonas</i>	233%	0%	0%
Bacilli	Bacillales	<i>Staphylococcus</i>	146%	8975%	13140%
Bacteroidetes	Balneolales	<i>Balneola</i>	76%	74%	56%
Bacteroidetes	Flavobacteriales	NS4	64%	23%	14%
Bacteroidetes	Flavobacteriales	NS9	92%	91%	84%
Bacteroidetes	Flavobacteriales	NS5	85%	51%	43%
Cyanophyceae	Synechococcales	<i>Prochlorococcus</i>	71%	202%	142%
Cyanophyceae	Synechococcales	<i>Synechococcus</i>	77%	19%	14%
Verrucomicrobia	Opitutales	<i>Coraliomargarita</i>	69%	354%	252%
Verrucomicrobia	Opitutales	<i>Lentimonas</i>	79%	181%	147%

Dinasquet et al. Surface Microbiome in the Mediterranean Sea

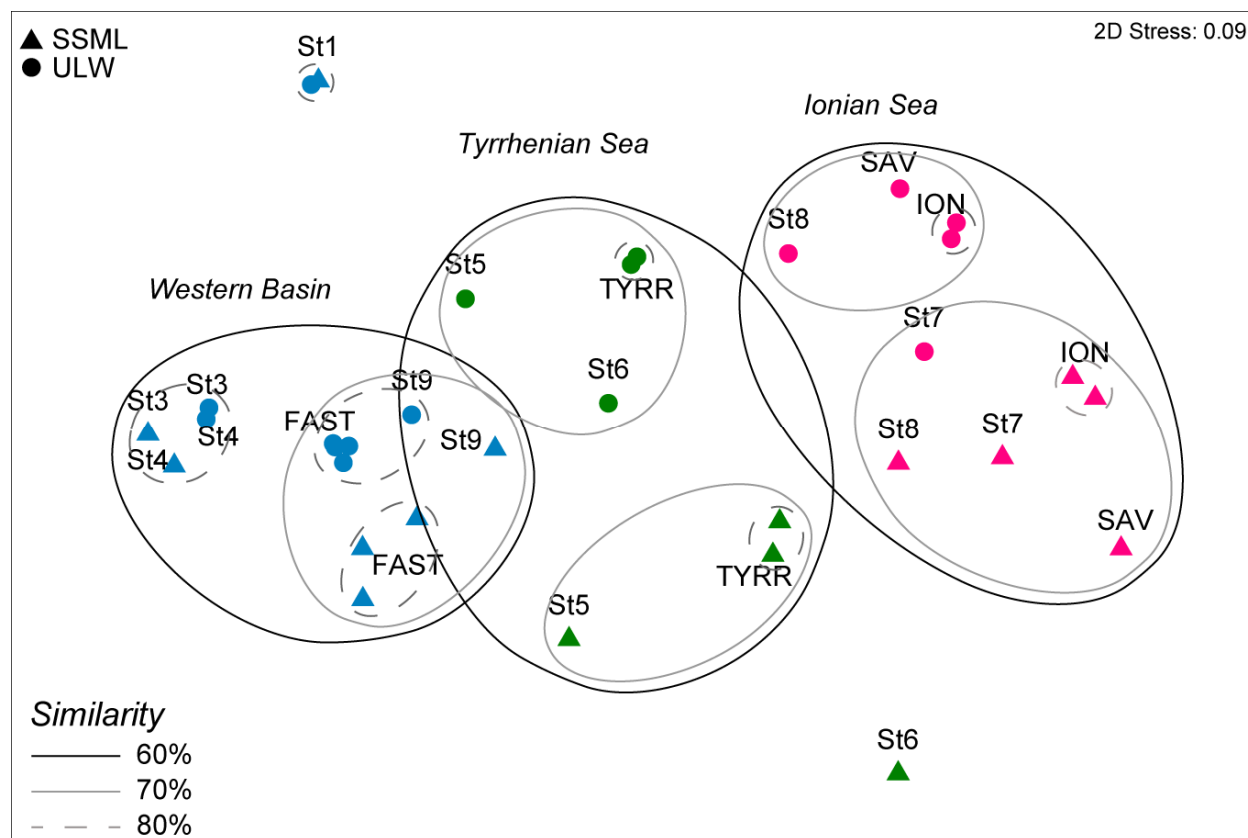


Figure 1: nMDS plot of bacterial community composition in the SSML and ULW at each station based on Bray-Curtis dissimilarities of the 16S rDNA sequences. Samples clustering at different level of similarity are circled together, basin groups are significantly different from each other ($p < 0.01$) based on a PERMANOVA test.

Dinasquet et al. Surface Microbiome in the Mediterranean Sea

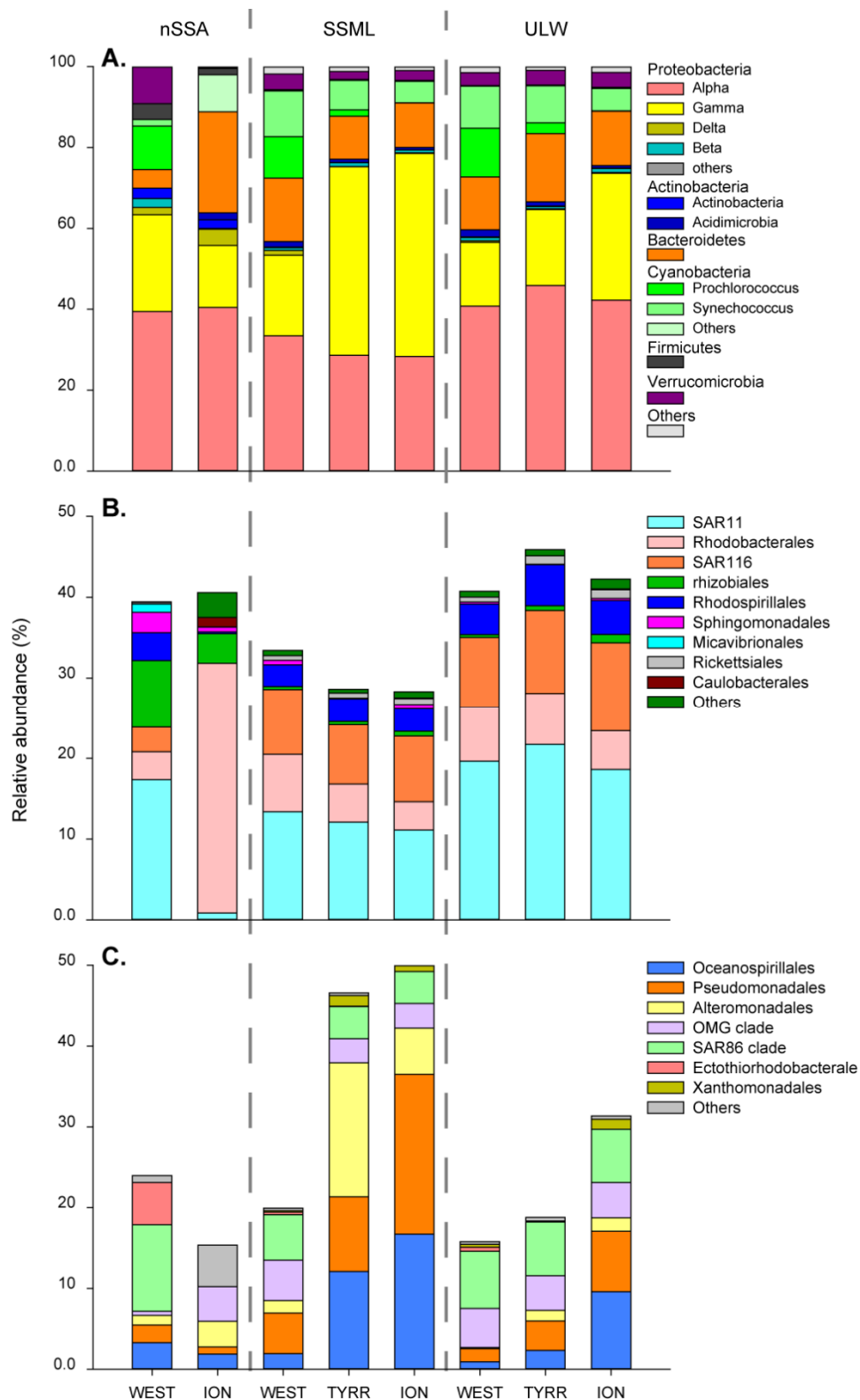


Figure 2: Average community composition at the phylum level (A), Alpha-proteobacteria family level (B) and Gamma-proteobacteria family level (C) distribution in the different basins in the nSSA, SML and ULW.

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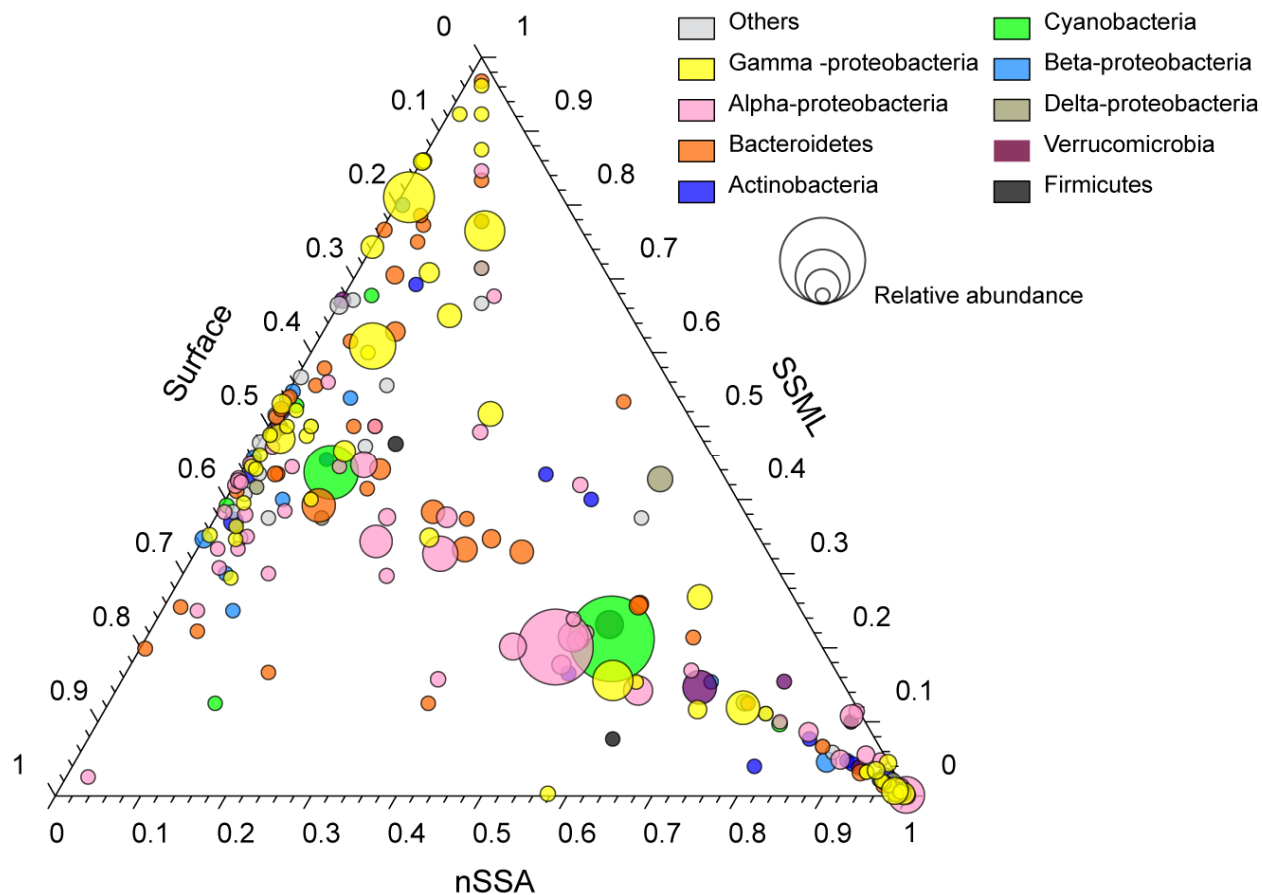


Figure 3: Ternary plot bacterial taxa enriched across the three fractions: surface water, sea surface microlayer (SSML) and nascent sea spray aerosol (nSSA). Each bubble represents one major bacterial group (collapsed at the genus level colored per phylum), the size of the bubble represents the overall relative abundance of the group in the three fractions. “Others” is an average of low abundance phyla.