Sea foams are ephemeral hotspots for distinctive bacterial communities contrasting sea-surface microlayer and underlying surface water

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Key words

Air-sea interface, surfactants, particles, 16S rRNA amplicon sequencing, neuston

One-sentence summary

Floating foams at the oceans' surface have a unique bacterial community signature in contrast to sea-surface microlayer and underlying water but receive and select for bacterial inhabitants from surface habitats.

Abstract

The occurrence of foams at oceans' surfaces is patchy and generally short-lived but a detailed understanding of bacterial communities inhabiting sea foams is lacking. Here we investigated how marine foams differ from the sea-surface microlayer (SML), a <1 mm thick layer at the air-sea interface and underlying water from 1 m depth. Samples of sea foams, SML and underlying water collected from the North Sea and Timor Sea indicated that foams were often characterized by a high abundance of small eukaryotic phototrophic and prokaryotic cells as well as a high concentration of surface-active substances (SAS). Amplicon sequencing of 16S rRNA (gene) revealed a distinctive foam bacterial community compared to SML and underlying water, with high abundance of *Gammaproteobacteria.* Especially *Pseudoalteromonas* and *Vibrio*, typical SML dwellers, were highly abundant, active foam inhabitants and thus might enhance foam formation and stability by producing SAS. Despite a clear difference in the overall bacterial community composition between foam and SML, the presence of SML bacteria in foams supports previous assumptions that foam is strongly influenced by the SML. We conclude that active and abundant bacteria from interfacial habitats potentially contribute to foam formation and stability, carbon cycling and air-sea exchange processes in the ocean.

Introduction

Foams are patches floating on the water surface and may appear in any aquatic habitat. Foam is loosely defined as a dispersion of gas in liquid in the presence of surface-active substances (SAS) (Schilling and Zessner 2011). Convergence at zones of downwelling water and fronts, currents, and breaking waves compress SAS and lead to foam formation at the sea surface and occasionally cause massive foam aggregates at beaches and in coastal zones (Bärlocher, *et al.* 1988; Eisenreich, *et al.* 1978; Jenkinson, *et al.* 2018; Kesaulya, *et al.* 2008; Thornton 1999). Furthermore, rising bubbles that do not burst immediately but accumulate at the surface can cause foam formation (Schilling and Zessner 2011). The nature, distribution and occurrence of foam in the marine environment is elusive, since its lifespan is limited to hours or days (Pugh 1996; Velimirov 1980), and the mean coverage of the ocean's surface by foams (white caps) is 1 - 6% based on satellite observations (Anguelova and Webster 2006).

One major prerequisite for foam formation are SAS, representing a complex mixture of mainly organic compounds. Due to their amphipathic nature, SAS accumulate at the sea surface (Wurl, *et al.* 2009) and influence CO_2 air-sea gas exchange (Pereira, *et al.* 2018; Ribas-Ribas, *et al.* 2018). In foams, SAS can originate from a variety of sources such as marine bacteria (Satpute, *et al.* 2010), kelp mucilage (Velimirov 1980), exudates of alive or broken phytoplankton cells (Frew, *et al.* 1990; Velimirov 1980; Velimirov 1982; Wegner and Hamburger 2002), or other organic detritus (Velimirov 1980). In addition, organic materials such as biogenic lipids and amino acids that accumulate at the sea surface during phytoplankton blooms, are important substrates for the formation of foam (Eberlein, *et al.* 1985; Hunter, *et al.* 2008; Riebesell 1993). Even if foam is generally short-lived, its high concentration of organic matter (Eisenreich, *et al.* 1978; Johnson, *et al.* 1989), especially of proteins and carbohydrates (Stefani, *et al.* 2016), allows these nutrient-rich islands to function as microbial habitats. Despite being ephemeral feeding grounds, foams are remarkably rich and diverse in microorganisms (Tsyban 1971), including bacteria (Gobalakrishnan, *et al.* 2014), protists, and algae (Harold and Schlichting 1971; Maynard 1968).

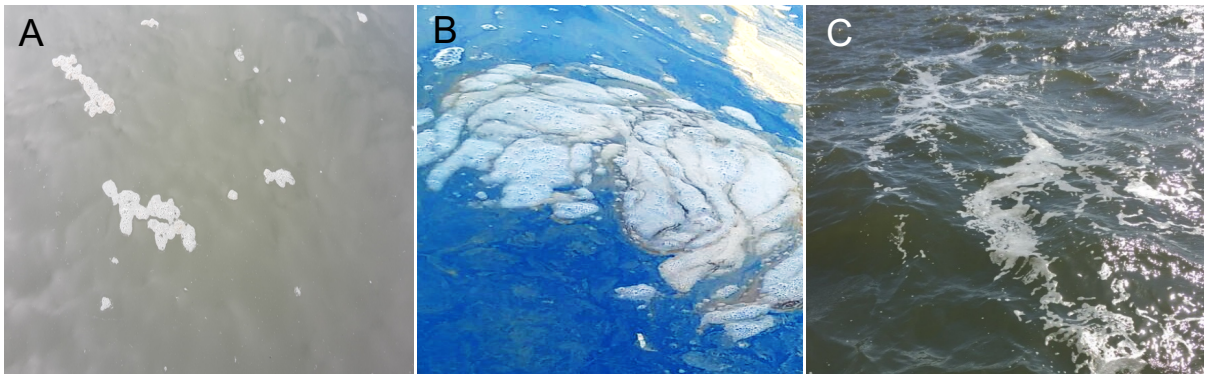
In addition, foams were shown to enclose copepods, polychaete, and tunicate larvae (Armonies 1989; Castilla, *et al.* 2007) and to trap microalgae (Roveillo, *et al.* 2020), thus forming potentially important food sources for the higher trophic levels of the food web (Bärlocher, *et al.* 1988; Craig, *et al.* 1989; Scully 2009). In addition, a pharmaceutical potential of sea foam inhabiting microbes was recently suggested (Oppong-Danquah, *et al.* 2020).

The sea-surface microlayer (SML) is a <1 mm thick, biofilm-like layer (Wurl and Holmes 2008; Wurl, *et al.* 2016), located at the air-sea boundary of all aquatic ecosystems (Supplementary Figure 1). It is characterized by remarkably different physicochemical and biological properties that allow its differentiation from the underlying water (ULW) (Cunliffe, *et al.* 2013; Hardy 1982). Research throughout the last decades revealed that the accumulation of inorganic and organic substances and particles (including microorganisms) at the sea surface is a widespread phenomenon with important implications for carbon cycling processes (Engel, *et al.* 2017; Rahlff 2019; Wurl, *et al.* 2017). The interfacial position of the SML represents a challenging environment for inhabiting organisms also known as neuston (Naumann 1917). Differences in bacterial community composition between SML and ULW have been related to meteorological conditions (Agogué, *et al.* 2005b; Rahlff, *et al.* 2017a; Stolle, *et al.* 2011), however the specific adaptation of bacteria to the SML habitat remains an open question (Agogué, *et al.* 2005a).

Neuston and plankton have a demonstrated role in gas exchange across air-water interfaces (Calleja, *et al.* 2005; Upstill-Goddard, *et al.* 2003). Moreover, the impact of the neuston on carbon cycling can be high due to its higher abundance of bacteria compared to the ULW (Hardy 1982). This includes enhanced heterotrophic activity (Obernosterer, *et al.* 2005), turnover of organic matter (Reinthaler, *et al.* 2008), colonization of buoyant and sinking particle aggregates (Bigg, *et al.* 2004; Leck and Bigg 2017), as well as viral lysis of prokaryotic cells (Ram, *et al.* 2018). First approaches to quantify the contributions of neuston activity to underlying water oxygen concentrations revealed maximum neuston contributions of ≤7.1% (Rahlff, *et al.* 2019).

100 Napolitano and Cicerone (1999) suggested that 1 L of foam water represents 2 m^2 of SML of 50
101 µm thickness, i.e. foams are essentially compressed SML. Supporting this idea, enrichment of
102 bacteria in foams compared to SML and/or ULW has been reported (Kuznetsova and Lee 2002;
103 Rahlff, *et al.* 2017a; Tsyban 1971). In addition, during blooms of *Trichodesmium erythraeum*, high
104 concentrations of this species were also found in the corresponding foam (Maynard 1968).
105 However, a thorough characterization of foam microbial community composition compared to the
106 SML and ULW has never been performed. Using a microscopic approach, Druzhkov, *et al.* (1997)
107 found a highly identical taxonomic composition of heterotrophs (nanoflagellates <5 µm and
108 bacteria), nano- and microphytoplankton in foam and the SML. The authors further described
109 higher abundances (one order of magnitude) of autotrophs, but not of heterotrophs in foams
110 compared to the SML.
111 In this study, we investigated the bacterial community composition of marine foams in direct
112 comparison to SML and ULW. Based on the theory that foam is an extreme condensed form of the
113 SML (Napolitano and Cicerone 1999), we hypothesized that the bacterial community composition
114 of foam and SML are more similar than between foam and ULW. Since the SML is also considered
115 an extreme habitat (Maki 1993) likely comprising many dead or dormant cells, we differentiated
116 between active and total bacteria as inferred from a complementary DNA (cDNA) and DNA-based
117 16S rRNA amplicon sequencing, respectively. Overall, we provide a detailed understanding of the
118 bacterial community composition associated with marine foams in comparison to SML and ULW
119 with likely implications for foam formation and stability, air-sea exchange processes and carbon
120 cycling.
121
122 **Materials & Methods**
123 **Field sample collection**
124 Field sampling was conducted from the bow of a small boat in the Jade Bay, North Sea offshore
125 Wilhelmshaven, Germany (Supplementary Table 1) in spring and summer 2016. Foams originated

from different sources such as phytoplankton exudates and convergence of surface water (Figure 1A, Figure 1C, Supplementary Table 1). Additional samples were collected during a *Trichodesmium* sp. bloom encountered in the Timor Sea at stations 4, 5b and 8 (Figure 1B, Supplementary Table 1) in October 2016 during *R/V* Falkor cruise FK161010 as described by Wurl, *et al.* (2018). Foam, and corresponding SML and ULW samples (here referred to as 'set') were collected from each location. From the Jade Bay, six sets were sampled in total, i.e., two from each month in April, May and July 2016. From the Timor Sea, one set was collected from each of three stations, but sequencing was only done for station 8, i.e., one sampling set. Foam and SML were collected using the glass plate technique (Harvey and Burzell 1972) with a withdrawal rate of 5-6 cm s^{-1} as suggested by Carlson (1982). The glass plate was cleaned with 70% ethanol and rinsed with sample water before use. Material adhering on the glass plate was removed by wiping its surface with a squeegee into a sample-rinsed brown bottle. The procedure was repeated until the required volume of approximately 100 mL was collected (~20 dips). SML samples were collected between the foam patches and any dips contaminated with foam were rejected, and the glass plate was cleaned with ethanol again. Collected foams were not generated by the small boat whose engine was turned off. Samples from the ULW were taken at a depth of 1 m around the foams by using a syringe connected to a hose. All samples were kept on ice and immediately processed after sampling, since Velimirov (1980) showed that bacterial density in old foam was significantly higher than in fresh foam.



147 **Figure 1.** Marine foam originating from A) presumptive phytoplankton exudates (Jade Bay), B)
148 *Trichodesmium* bloom (Timor Sea) and C) whitecaps produced by convergence of surface water
149 (Jade Bay, North Sea).
150

151 **Concentration of surface-active substances**

152 The concentration of surface-active substances (SAS) was measured by the voltammetry VA Stand
153 747 (Methrom, Herisau, Switzerland) with a hanging drop mercury electrode as previously
154 described (Ćosović and Vojvodić 1998; Wurl*, et al.* 2011). The quantification is based on SAS
155 adsorption on the Hg electrode measured by the change of capacity current (ΔIc) at an applied
156 potential (E) of -0.6 V (Ćosović and Vojvodić 1998). Before measurement, concentrated samples
157 such as foam samples were diluted with artificial seawater (0.55 M of NaCl solution) to achieve
158 measurements within the linear calibration range. A standard addition technique was used with
159 non-ionic surfactant Triton X-100 (Sigma Aldrich, Taufkirchen, Germany) as a standard. SAS
160 concentration in the samples was measured using two to three analytical replicates, resulting in
161 relative standard deviations below 6% (Rickard*, et al.* 2019). Concentration of SAS is expressed
162 as the equivalent concentration of the additional Triton X-100 (µg Teq L^{-1}).
163

164 **Determination of microbial abundance**

165 For determination of prokaryotic and small (< 50 µm) eukaryotic phototrophic cell numbers in all
166 Jade Bay samples and for three stations from the Timor Sea, foam and water samples were fixed
167 with glutaraldehyde (1% final concentration), incubated at room temperature for 1 hour, and stored
168 at -80°C until further analysis. Prior staining and counting by flow cytometry, the particle-enriched
169 foam samples were pre-filtered by gravity onto CellTrics® 50 µm filter (Sysmex Partec, Münster,
170 Germany) to avoid clogging of the instrument by particulate matter. Autofluorescence analysis was
171 used to count small eukaryotic phototrophic cells (Marie*, et al.* 2000), and prokaryotic cells were
172 stained with SYBR® Green I Nucleic Acid Gel Stain (9x in final concentration, Thermo Fisher

173 Scientific, Darmstadt, Germany) following a protocol of Giebel*, et al.* (2019). We did not measure
174 biological replicates of samples because the coefficient of variation for SML and ULW flow
175 cytometry samples is <5% (Rahlff*, et al.* 2017a).
176
177 **Calculation of Enrichment Factors (EF)**
178 Enrichment factors (EF) of SAS and cell counts were calculated for the pairings foam/SML (F/S),
179 foam/ULW (F/U) and SML/ULW (S/U) (Table 1). Since the concentration of SAS or the
180 abundance of cells in a foam or SML sample was divided by its SML or ULW counterpart, an
181 EF>1 implies enrichment of the parameter, whereas an EF<1 indicates depletion.
182

Table 1: Absolute and relative abundances given as enrichment factor (EF) of prokaryotes, small phototrophic eukaryotes and surface-active substances (SAS) in foam (F), sea-surface microlayer, SML (S) and underlying water, ULW (U), NS=North Sea, NA=not available, Teq=Triton X-100 equivalents, TS=Timor Sea

	Foam	SML	ULW	EF (F/S)	EF (F/U)	EF (S/U)
Prokaryotes (cells mL^{-1})	**Absolute values (10^6 cells mL^{-1})**			**Relative values**		
NS_St1_210416	4.89	NA	2.56	NA	1.9	NA
NS_St2_210416	2.63	2.62	2.48	1.0	1.1	1.1
NS_St1_190516	13.70	3.34	3.23	4.1	4.2	1.0
NS_St3_190516	46.20	4.57	3.13	10.1	14.8	1.5
NS_St1_190716	6.61	3.90	3.71	1.7	1.8	1.1
NS_St2_190716	4.99	3.39	3.48	1.5	1.4	1.0
TS_St4_151016	9.97	1.77	1.07	5.6	9.3	1.7
TS_St5b_171016	33.90	NA	1.01	NA	33.6	NA
TS_St8_191016	5.83	0.98	1.18	5.9	4.9	0.8
Small phototrophic eukaryotes (cells mL^{-1})	**Absolute values (10^4 cells mL^{-1})**			**Relative values**		
NS_St1_210416	1.85	1.03	1.61	1.8	1.1	0.6
NS_St2_210416	1.38	1.41	2.24	1.0	0.6	0.6
NS_St1_190516	29.60	0.85	2.30	34.8	12.9	0.4
NS_St3_190516	57.10	1.02	1.88	56.0	30.4	0.5
NS_St1_190716	9.10	3.97	4.17	2.3	2.2	1.0
NS_St2_190716	4.23	2.49	2.82	1.7	1.5	0.9
TS_St4_151016	2.14	0.37	0.11	5.8	20.2	3.5
TS_St5b_171016	10.50	NA	0.13	NA	81.4	NA

TS_St8_191016	2.73	0.12	0.26	23.7	10.4	0.4

SAS (µg Teq L^{-1})	**Absolute values**			**Relative values**		
NS_St1_210416	NA	NA	NA	NA	NA	NA
NS_St2_210416	NA	NA	NA	NA	NA	NA
NS_St1_190516	77496	576	213	134.4	364.5	2.7
NS_St3_190516	148233	1753	223	84.6	665.0	7.9
NS_St1_190716	900	716	180	1.3	5.0	4.0
NS_St2_190716	1397	270	133	5.2	10.5	2.0
TS_St4_151016	69370	240	268	288.9	258.5	0.9
TS_St5b_171016	67546	66	109	1020.5	618.7	0.6
TS_St8_191016	28797	255	171	113.1	168.5	1.5

184

185 **Nucleic acid extraction and PCR**

186 A two-step filtration of foam, SML and ULW samples was conducted as performed in previous
187 studies (Garneau*, et al.* 2009; Stolle*, et al.* 2011). Sample water was filtered through 3 µm pore
188 size (particle-associated cells) polycarbonate filters, after which the filtrate was filtered onto 0.2
189 µm pore size (free-living cells) polycarbonate filters (Merck Millipore, Darmstadt, Germany).
190 Foam from the Timor Sea (Station 8) collected during a bloom of *Trichodesmium* sp. was
191 additionally pre-filtered on a 100 µm mesh before subsequent filtration on the 3 µm pore size filter.
192 All filters were initially stored at -80°C prior analysis. Extraction of DNA and RNA from the filters
193 was performed by using the DNA + RNA + Protein Extraction Kit (Roboklon, Berlin, Germany)
194 with a modified protocol (Rahlff*, et al.* 2017a). Remaining DNA in RNA samples was digested on-
195 column using 3 U of DNase and subsequently checked for contaminations with genomic DNA by
196 PCR (Supplementary Figure 2). A quantity of 10 ng RNA was reversely transcribed to cDNA using
197 the NG dART Kit (Roboklon, Berlin, Germany) including negative controls either without reverse
198 transcriptase or without RNA (Supplementary Figure 2). The reaction was incubated using the
199 primer 1492R (5'-GGTTACCTTGTTACGACTT-3', adapted from (Lane 1991)) for 60 minutes at
200 50°C followed by 5 minutes at 85°C. All DNAs and cDNAs were quantified using the Quant-iT™
201 PicoGreen™ dsDNA assay (Thermo Fisher Scientific, Darmstadt, Germany).

202

203 **16S rRNA library preparation, sequencing run and data analysis**

204 The bacterial 16S rRNA gene was amplified using Bakt_341F (5'-CCTACGGGNGGCWGCAG-
205 3') and Bakt_805R (5'-GACTACHVGGGTATCTAATCC-3') (Herlemann*, et al.* 2011) with the
206 following modifications. Genomic DNA was amplified with 35 cycles prior Index-PCR. The
207 cDNA samples were amplified with 25 cycles prior Index-PCR. Amplicon PCR, Index PCR,
208 quantity and quality control and sequencing of the individual libraries as pool in one Illumina

209 MiSeq run was performed by an external provider (Eurofins Genomics, Ebersberg, Germany). Raw
210 sequencing data were deposited at the European Nucleotide Archive (ENA) under accession
211 number PRJEB34343. For data analysis, the resulting sequences were assembled using
212 QIIME 1.9.1 (Caporaso, *et al.* 2010) "joins paired-end Illumina reads" function with default
213 settings to merge forward and reverse sequence with an overlap of at least 30 bp. Sequences without
214 overlap were removed. After converting fastq to fasta using the "convert_fastaqual_fastq" function
215 the resulting sequences were evaluated using the SILVA NGS pipeline. The SILVA next -
216 generation sequencing (NGS) pipeline (Glöckner, *et al.* 2017) performs additional quality checks
217 according to the SINA-based alignments (Pruesse, *et al.* 2012) with a curated seed database in
218 which PCR artifacts or non-SSU reads are excluded (based on SILVA release version 128 (Pruesse,
219 *et al.* 2007). The longest read serves as a reference for the taxonomic classification in a BLAST
220 (version 2.2.28+) search against the SILVA SSU Ref dataset. The classification of the reference
221 sequence of a cluster (98% sequence identity) is mapped to all members of the respective cluster
222 and to their replicates. Best BLAST hits were only accepted if they had a (sequence identity +
223 alignment coverage)/2 ≥ 93% or otherwise defined as unclassified. SILVA NGS classified a total
224 of 9182084 reads (2% were rejected by the quality control). Sequences assigned to chloroplasts,
225 mitochondria, eukaryotes and *Archaea* were removed since the primer set employed in the analysis
226 has only a very limited coverage of these groups.

227

228 **Statistical analyses**

229 Operational taxonomic unit (OTU) counts based on genus level were rarefied to 43,500 reads per
230 sample using the single_rarefraction.py script implemented in QIIME. Venn diagrams were
231 calculated using the ugent webtool (http://bioinformatics.psb.ugent.be/webtools/Venn/). We
232 visualized the differences in the bacterial community composition through non-metric

233 multidimensional scaling (NMDS) plots using Bray–Curtis dissimilarity indices based on a genus
234 rank classification. A linear discriminant analysis effect size (LEfSe) analysis was performed to
235 determine bacterial groups which are significantly different between the samples using the 'one
236 against all' strategy for multi-class analysis (Segata*, et al.* 2011). The program LEfSe uses a non-
237 parametric test that combines standard tests for statistical significance with additional tests
238 encoding biological consistency and effect relevance. $P<0.05$ was regarded as statistical
239 significance.

240 Differences in the number of 16S rRNA and 16S rRNA gene-derived OTUs between habitats
241 (foam, SML, ULW) and attachment status were statistically analyzed using a Kruskal-Wallis test,
242 and *post hoc* multiple pairwise comparisons were conducted based on Dunn's z statistic
243 approximations to the actual rank statistics within the package "dunn.test" (Dinno and Dinno 2017)
244 in R version 4.0.3 (Team 2017). The null hypothesis was rejected if $p>0.05$. For the same samples,
245 comparisons on phylum and OTU-based differences were statistically investigated using a one-
246 way Analysis of Similarities (ANOSIM) based on Bray Curtis dissimilarity and 9999 permutations,
247 Bonferroni correction and a significance level of 95%.

248

249 **Results**

250 **Foams are enriched with surface-active substances and microorganisms**

251 Overall, foams from both sampling areas (North Sea and Timor Sea), were enriched with
252 prokaryotic microorganisms, small eukaryotic phototrophic cells, and SAS (Table 1). Cell counts
253 of prokaryotic microorganisms ranged between 2.63 - 46.2 x 10^6, 0.98 – 4.57 x 10^6, and 1.01 -
254 3.71 x 10^6 cells mL^{-1} in foam, SML and ULW, respectively (Figure 2A). Thus, prokaryotic
255 microorganisms in foams were enriched with a maximum EF (enrichment factor) of 10.1 and 5.9
256 over SML, and with a maximum EF of 14.8 and 33.6 over ULW in North Sea and Timor Sea,
257 respectively (Table 1). However, these numbers likely represent an underestimation of cell counts

because pre-filtration of foam samples onto a 50 µm sieve before the flow cytometry measurement likely excluded some bigger aggregates of cells and colloid material. Prokaryotic cells in the SML were enriched with a maximum EF of 1.5 and 1.7 over ULW in North Sea and Timor Sea, respectively. Likewise, the total number of small eukaryotic phototrophic cells, was always higher in foam (range=1.38 - 57.1 x 10^4 cells mL^{-1}) compared to SML (range=1.15 - 39.7 x 10^3 cells mL^{-1}) and ULW (range=1.06 - 41.7 x 10^3 cells mL^{-1}, Figure 2B). Thus, the maximum EF was 3.5 and 81.2 for SML over ULW and foam over ULW, respectively. The absolute number of small eukaryotic phototrophic cells was two orders of magnitude lower compared to the prokaryotic cell counts (Figure 2 A&B). Interestingly, small eukaryotic phototrophic cells were often depleted in the SML compared to the ULW (S/U minimum EF=0.4), while they were enriched in foams over ULW at the same time (F/U EF=12.9 (Table 1)).

Foams contained the highest SAS concentrations compared to the other two habitats (Figure 2C). SAS concentrations in foams varied between 900 to 148,233 µg Teq L^{-1} in North Sea and Timor Sea, whereas SML SAS concentrations were in a range of 66 to 1,753 µg Teq L^{-1}, and ULW SAS concentrations in a range of 109 to 223 µg Teq L^{-1} (Table 1). While SAS concentrations in the SML were enriched with a maximum EF of 7.9 compared to ULW, their concentration in foams compared to ULW was typically enriched by two orders of magnitude (EF ranging from 5 to 665).

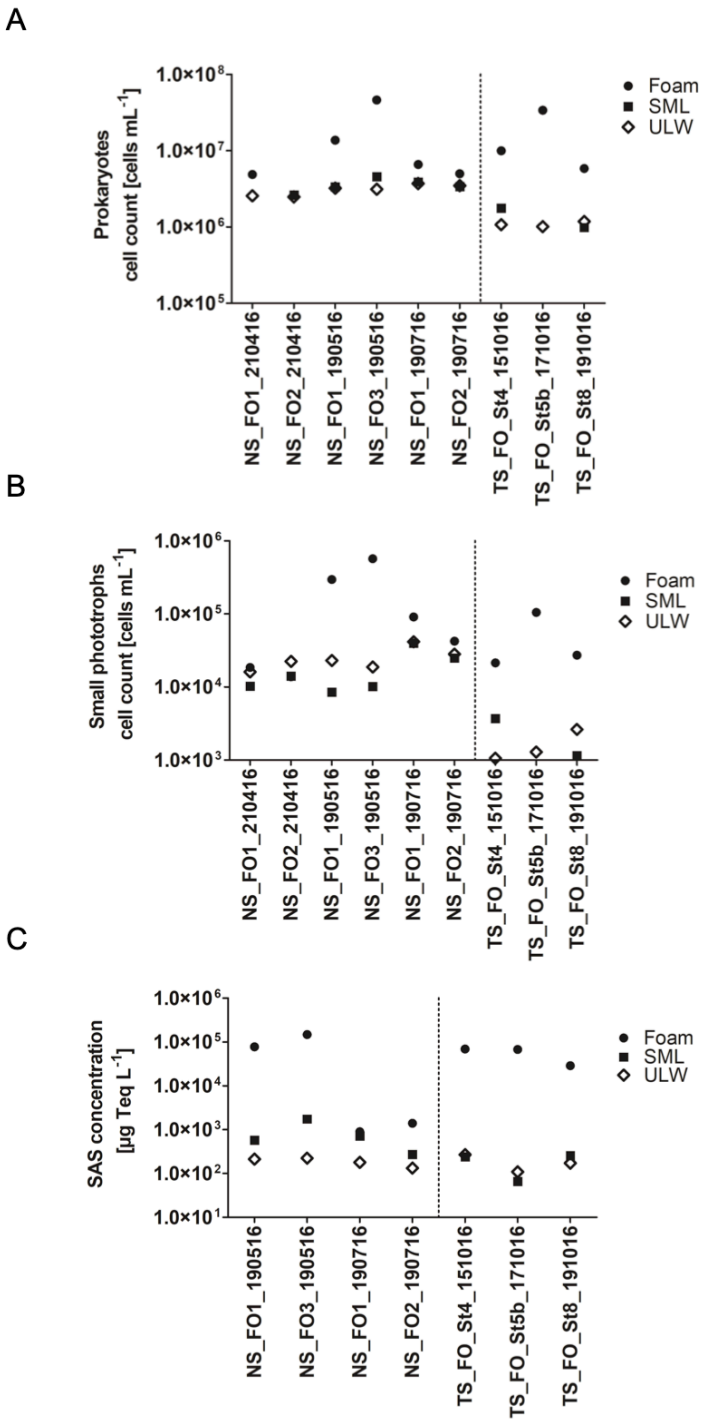


Figure 2: Absolute cell counts mL^{-1} for A) prokaryotes and B) small eukaryotic phototrophic cells and C) concentration of surface-active substances (SAS) in µg Teq L^{-1} for foam, sea-surface microlayer (SML) and underlying water (ULW). Foam samples from the North Sea NS_FO1_210416, and NS_FO2_210416 were produced by waves, whereas NS_FO1_190516, NS_FO2_190516 and NS_FO1_190716 are foams likely derived from phytoplankton exudates. Foams from the Timor Sea (TS) originated from a *Trichodesmium* sp. bloom.

282

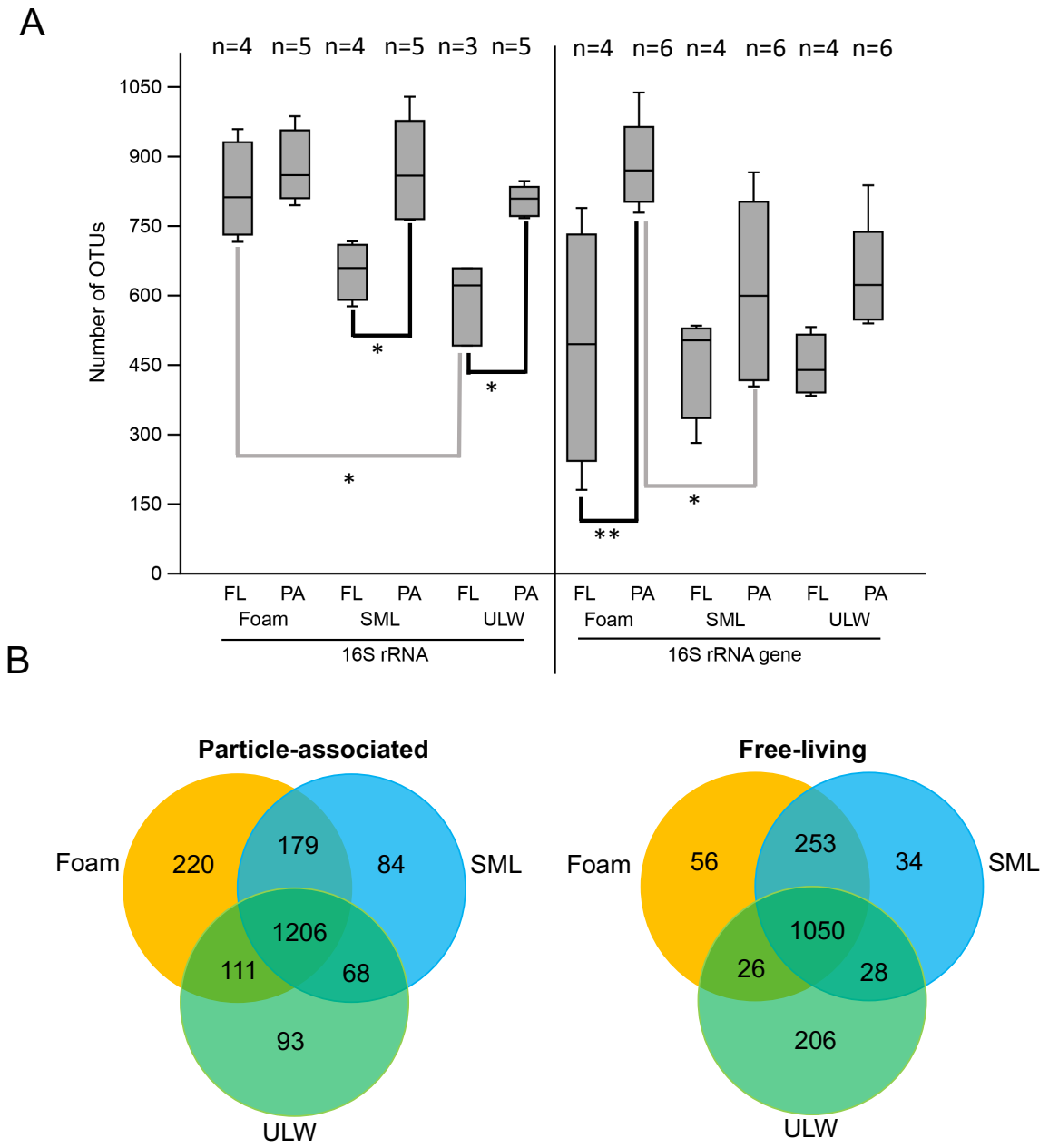
283 **Changes in the number of OTUs among foam, sea-surface microlayer and underlying water**

284 We analyzed the bacterial community composition of all North Sea samples and could not detect
285 any obvious difference between sampling dates (Supplementary Figure 3). In the following, we did
286 not differentiate between sampling dates, but by habitat (foam, SML, ULW) attachment status
287 (particle-associated, free-living), and nucleic acid type for 16S rRNA analysis (16S rRNA gene,
288 16S rRNA) (Figure 3A). Analyses revealed overall higher alpha diversity (numbers of OTUs) in
289 active communities (median=786.5) compared to total communities (median=571).

290 In 16S rRNA gene-based samples, the mean number of foam OTUs was significantly increased for
291 particle-associated over free-living communities (Dunn's test, p=0.0090), and also significantly
292 higher compared to the SML particle-associated fraction with (p=0.035, Figure 3A). OTU numbers
293 derived from 16S rRNA were significantly different between the particle-associated and the free-
294 living fractions of SML (Dunn's test, p=0.015) and ULW (p=0.040) as well as between the free-
295 living communities of foam and ULW (p=0.032, Figure 3A). Further significant differences
296 existing between different biomes and different attachment states are shown in Supplementary
297 Table 2. The Shannon-Wiener index (Supplementary Figure 4), which accounts for both abundance
298 and evenness of OTUs, confirmed the above-mentioned trends reflected by the total number of
299 OTUs.

300 When comparing the 16S rRNA gene-based and 16S rRNA-based as well as the particle-attached
301 and free-living communities, foam, SML and ULW generally shared a high number of similar
302 OTUs (953-1206). Interestingly, foams and SML always had more OTUs in common compared to
303 foam-ULW and ULW-SML (Figure 3B, Supplementary Figure 5). In the particle-attached fraction,
304 foam had the highest fraction of specific OTUs (220) and also shared many with the SML (179).
305 In addition, SML and foam shared many OTUs in the free-living fraction (253) with less specific
306 OTUs in foam (56) and the SML (34) compared to the particle-associated fraction (Figure 3B).

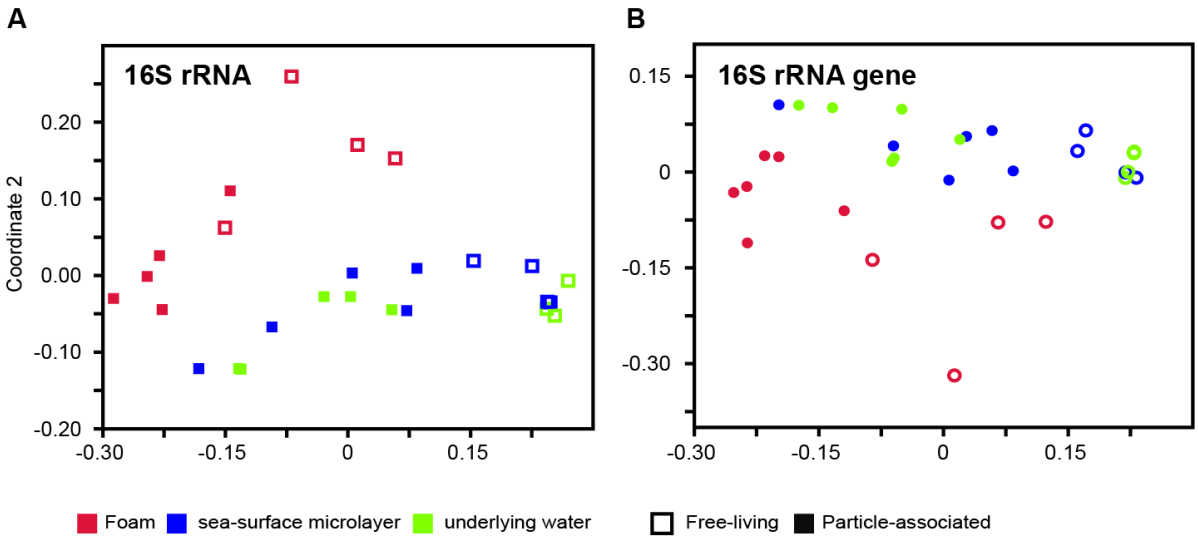
307



308

309 **Figure 3:** A) 16S rRNA and 16S rRNA gene-derived numbers of operational taxonomic units
310 (OTUs) for foam, sea-surface microlayer (SML) and underlying water (ULW) habitat of pooled
311 North Sea stations. The total number of OTUs of the three habitats is further distinguished between
312 free-living (FL) and particle-associated (PA) bacterial communities. Grey and black lines indicate
313 inter- and intra-habitat comparisons, respectively. The boxplot shows the 25–75 percent quartiles;
314 the median is indicated by the horizontal line inside the box. Error bars show minimal and maximal
315 values. Asterisks indicate the level of significant differences: * $p \leq 0.05$, ** $p \leq 0.01$; For reasons of
316 the different number of observations (n) see Supplementary Table 1. B) 3-Venn diagram showing

317 overlapping and unique OTUs for foam, SML and ULW separated by free-living and particle-
318 associated OTUs.
319
320 **Bacterial community composition of sea foam from the Jade Bay, North Sea**
321 Non-metric multidimensional scaling plots comparing the bacterial community composition based
322 on the abundance of OTUs, revealed that the foam bacterial community composition was distinct
323 from SML and ULW communities, irrespective of differentiating 16S rRNA and its gene, or free-
324 living and particle-associated bacterial communities (Figure 4, Supplementary Figure 6). This was
325 supported by the pairwise ANOSIM test, which revealed that the 16S rRNA and 16S rRNA gene-
326 based community differed significantly between foam and SML (both p=0.01) as well as between
327 foam and ULW (p=0.01 and 0.04, respectively). In contrast, SML and ULW bacterial community
328 composition were more similar to each other as shown by the clustering (Figure 4, Supplementary
329 Figure 6).
330



331
332 **Figure 4:** Non-metric multidimensional scaling plot shows distinct clustering of foam (red), sea-
333 surface microlayer (blue) and underlying water (green) bacterial communities. Further separation
334 of communities into A) 16S rRNA-based with free-living (open symbols) and particle-associated

335 (filled symbols) stress=0.14; B) 16S rRNA gene-based with free-living (open symbols) and
336 particle-associated (filled symbols) stress=0.11;
337
338 On a phylum-level, 16S rRNA gene-based community analyses showed that for all three habitats
339 *Gammaproteobacteria*, *Verrucomicrobia* and *Cyanobacteria* formed a higher portion of particle-
340 associated than free-living communities with the exception of *Cyanobacteria* in foam (Figure 5).
341 In contrast, *Alphaproteobacteria* and *Actinobacteria* were more abundant in the free-living fraction
342 (Figure 5, Supplementary Table 3).
343 Most phyla were similarly distributed in 16S rRNA and 16S rRNA gene-derived bacterial
344 communities, suggesting that many of the total phyla were also active. *Gammaproteobacteria*, as
345 one exception, tended to have higher relative abundance in the 16S rRNA-based community
346 composition in foam (37.4% and 35.0% of free-living and particle-associated OTUs, respectively)
347 compared to the 16S rRNA gene-based community composition (22.7% and 26.0% of free-living
348 and particle-associated OTUs, respectively). In contrast, *Verrucomicrobia* had a higher portion in
349 all habitats and fractions based on 16S rRNA gene compared to 16S rRNA. This was particularly
350 striking for particle-associated communities in foams, where *Verrucomicrobia* were quite abundant
351 among the total community (24.9%) but little active (3.1%). The 16S rRNA-based communities of
352 foam contained less *Alphaproteobacteria* but more *Gammaproteobacteria* compared to SML and
353 ULW communities (Figure 5).
354

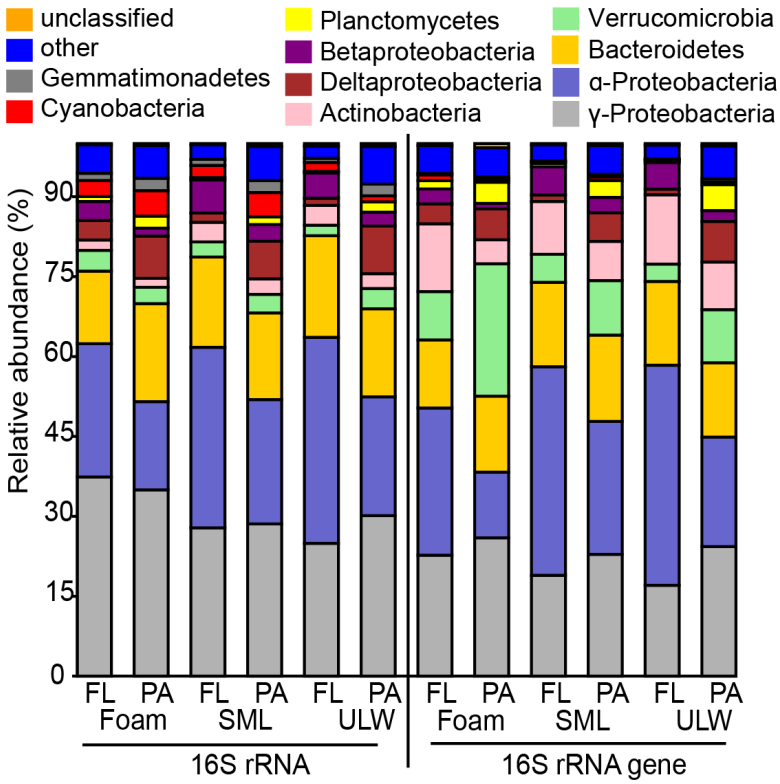


Figure 5: Composition of phylum-level of foam, sea-surface microlayer (SML) and underlying water (ULW) samples of 16S rRNA and 16S rRNA gene-based relative abundance of operational taxonomic units (OTUs) of pooled North Sea stations. Each habitat is further separated into free-living (FL) and particle-associated (PA) bacterial communities.

MB11C04 marine group (*Verrucomicrobia*), SAR11 clade (*Alphaproteobacteria*) and *Oceanospirillales* (*Gammaproteobacteria*) had a higher relative abundance in ULW and SML compared to foam based on 16S rRNA gene and 16S rRNA and when comparing the respective attachment status, i.e., free-living and particle-associated fractions. (Supplementary Figures 7, 8, 9). A higher relative abundance of active OTUs (based on 16S rRNA) in foam compared to SML and ULW was found among the *Puniceicoccales* (*Verrucomicrobia*), *Sphingomonadales* (*Alphaproteobacteria*), *Alteromonadales* and *Vibrionales* (both *Gammaproteobacteria*) (Supplementary Figure 7, 8, 9). Active, free-living OTUs belonging to the order *Flavobacteriales* and *Oceanospirillales* were more, whereas free-living *Sphingobacteriales* were less numerous than their particle-associated counterparts in all three habitats (Supplementary Figures 7 & 10).

371 Except from the order *Rhodobacterales* (Supplementary Figure 9), foam generally had less
372 alphaproteobacterial 16S rRNA gene-based OTUs compared to SML and ULW. However, foam
373 contained a higher 16S rRNA gene-based relative abundance of *Verrucomicrobia* and
374 *Gammaproteobacteria* compared to SML and ULW (Figure 5). Among the *Gammaproteobacteria*,
375 especially more OTUs of the orders *Cellvibrionales, Vibrionales, Legionellales, Alteromonadales*
376 were increasingly detected in foam compared to the SML and ULW, whereas the order
377 *Oceanospirilliales* was more depleted in foam (Supplementary Figure 7).
378
379 ***Gammaproteobacteria* are typical sea foam colonizers**
380 Using the linear discriminant analysis effect size (LefSe) method we could identify OTUs that were
381 significantly more abundant in foam compared to SML and ULW (Figure 6). The analysis does not
382 refer to the most abundant OTUs in terms of absolute numbers but points out the largest differences
383 between foam and the other two habitats. Members of the *Gammaproteobacteria* were typically
384 abundant active and total foam colonizers (Figure 6). Taxa including *Winogradskyella*, *Vibrio*,
385 *Halioglobus* and *Pseudoaltermonas* were particularly abundant in both 16S rRNA and 16S rRNA
386 gene derived foam samples as well as when compared to SML and ULW habitats. *Persicirhabdus*
387 and other *Verrucomicrobiaceae* were typical foam-dwellers with 11% and 7% relative abundance
388 according to their presence in 16S rRNA gene-based samples, but with low activity according to
389 16S rRNA samples.
390 Typical SML populating bacteria belonged to taxa, which were phylogenetically related to
391 *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteria*. Strikingly, abundance of total and
392 active foam specific OTUs often indicated a decreasing gradient from foam to SML to ULW
393 (Supplementary Figure 1). High relative abundances (>5%) of *Planktomarina*, SAR116 and
394 SAR86 were found for 16S rRNA and 16S rRNA gene-based SML samples. SAR11 and
395 Candidatus *Actinomarina* typically occurred in high abundances in the ULW.
396

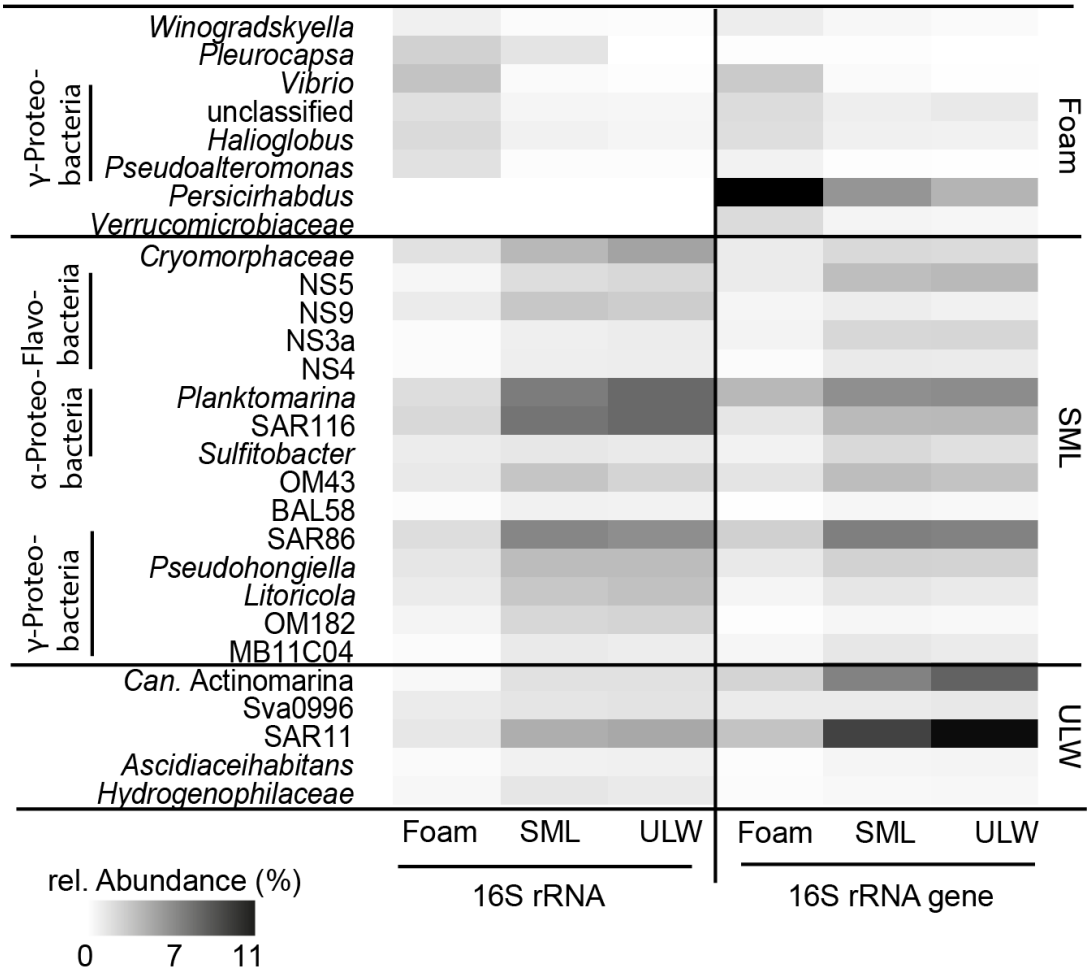


Figure 6: Heat-map showing operational taxonomic units (OTUs) with significant different relative abundance in foam, sea-surface microlayer (SML) and underlying water (ULW) based on LEfSe analysis from pooled North Sea samples; OTUs are derived from sequencing amplicons derived from 16S rRNA and its gene. The vertical axis indicates the key species for these three biomes, respectively, while the horizontal axis shows how abundant these key OTUs were in the other two habitats.

***Trichodesmium* sp.-produced foam – a case study**

Due to technical restrictions we could only obtain a single sample from the Timor Sea (Station 8) but found it valuable to analyze because the immediate source (*Trichodesmium* sp.-produced foam) was clear. Among the 16S rRNA gene-based community in foam we found most particle-associated OTUs assigned to *Trichodesmium* (relative abundance=33.4%), *Alteromonas* (26.4%) and

410 *Rhodobium* (5.4%), whereas free-living OTUs were mostly assigned to *Alteromonas* (18.0%) and
411 *Rhodobium* (10.2%) (Supplementary Table 4). Particle-associated OTUs were mainly assigned to
412 *Trichodesmium* (68%) and *Rhodobium* (10.9%) in the SML, and to *Trichodesmium* (23.8%) and
413 *Oscillatoria* (26.7%) in the ULW. Bacteria of the genus *Saprospira* were also detected in foam and
414 SML, mainly in the particle-associated fractions. Most free-living OTUs from SML and ULW were
415 assigned to *Synechococcus* with 15.7% and 21.6% relative abundance, respectively. In all 16S
416 rRNA samples, *Trichodesmium* was also the most abundant among active OTUs in foam and SML,
417 only in the ULW *Oscillatoria* (48.2%) had higher relative abundance compared to *Trichodesmium*
418 (29.1%). The relative abundance of 16S rRNA-based OTUs assigned to *Alteromonas* in foams
419 (particle-associated: 17.8%, free-living: 12.6%) was comparatively enhanced to the SML (particle-
420 associated: 0.2%, free-living: 1.2%) (Supplementary Table 4).
421
422 **Discussion**
423
424 **Foams form ecological niches for selected water bacterial communities and opportunistic**
425 ***Gammaproteobacteria***
426 Foams are peculiar but understudied microbial habitats at air-water interfaces, and as soon as they
427 subside, their material becomes part of the SML (Kuznetsova and Lee 2002). As foam has been
428 suggested to represent compressed SML (reviewed by Schilling and Zessner (2011)), we
429 hypothesized that foams contain typical SML bacteria. Our analysis showed that a considerable
430 number of OTUs (>1000) overlap between all three habitats (Figure 3B), supporting that bacteria
431 are regularly dispersed between the biomes by wind-induced mixing processes, small-scale
432 turbulences (Wu*, et al.* 2019) and bubble transport (Robinson*, et al.* 2019). However, also the
433 sampling method, being identical for foam and SML, causes some cross-contamination between
434 habitats. As expected for a compressed form of SML, we found high concentrations of SAS (max.
435 148,233 µg Teq L^{-1}) in foams compared to reported values of 4,989 µg Teq L^{-1} in nearshore SML

436 (Wurl, *et al.* 2011). In line with previous reports, we also found a strong enrichment of cells in
437 foam (Kuznetsova and Lee 2002; Rahlff, *et al.* 2017a; Robinson, *et al.* 2019). Decreasing
438 abundance of small eukaryotic phototrophic cells in the SML during simultaneous increase of those
439 in the respective foam sample supports passive transport from SML to foam, and that SML
440 compression is a mechanism for foam formation (Schilling and Zessner 2011). In our study, typical
441 abundant and free-living groups from the ULW including *Planktomarina*, SAR116 and SAR86
442 were found in foams at lower relative abundance (Figure 6, Supplementary Figure 11) suggesting
443 that these groups were passively transferred to the sticky SML and further into the foam.
444
445 The foam environment is an ecological niche with unique properties, that harbors a distinctive
446 bacterial community when compared to the SML and ULW (Figure 4), which contradicts our initial
447 hypothesis that SML and foam communities would be more similar to each other than foam and
448 ULW. The occurrence of specific foam bacterial communities most likely resembles the
449 particularities of the foam habitat, e.g., its dynamics in formation and disruption, its ephemerality,
450 and high organic matter accumulation. Foam consists of 90% air (Napolitano and Cicerone 1999)
451 and contains channels, referred to as Plateau borders (Schramm and Wassmuth 1994) tending to
452 trap living, motile algae, but draining dead ones (Roveillo, *et al.* 2020). If this mechanism also
453 applies to bacteria, it may explain why most OTUs found in foams were also active (Figure 3A).
454 In addition, foam is heavily enriched in nutrients but also in pollutants (Eisenreich, *et al.* 1978;
455 Pojasek and Zajicek 1978) probably affecting its bacterial community structure. The type of foam
456 under investigation also seems to be important: Foams originating from phytoplankton exudates
457 (Supplementary Table 1), contained high loads of SAS (Figure 2C) and were linked to higher
458 abundances of microbes (Figure 2A&B) compared to foams formed by convergence of surface
459 water (Figure 1C, Supplementary Table 1). However, the role of the foam source for shaping its
460 microbiome requires further research.
461

462 The overall difference in the bacterial community composition of foams compared to other habitats
463 (Figure 4) could be due to bacteria originating from SML and ULW that were selectively enriched
464 in foam, including *Vibrio*, *Pseudoalteromonas,* and *Halioglobus* (Figure 6). Foams associated with
465 phytoplankton biomass likely contain substantial amounts of labile organic matter and nutrients.
466 Labile organic matter particularly selects for fast-growing, opportunistic, and active bacteria in
467 foams such as *Gammaproteobacteria* (Landa*, et al.* 2013; Teeling*, et al.* 2012), which are known
468 to be dominant in the SML (Sun*, et al.* 2020) and on transparent exopolymer particles (Zäncker*, et*
469 *al.* 2019). For instance, *Alteromonas* sp., of which we found an OTU among the free-living bacteria
470 present in *Trichodesmium* foam (Supplementary Table 4), were previously shown to be highly
471 abundant and active degraders of alginate, a cell wall component from marine macroalgae (Mitulla*,*
472 *et al.* 2016), and of labile dissolved organic carbon (Pedler*, et al.* 2014).
473
474 **The role of particles for foam-populating bacteria and biogenic foam formation**
475 Foams in aquatic habitats contain large numbers of benthic or symbiotic rather than free-living
476 bacteria (Maynard 1968). Since particulate organic matter is frequently enriched in both the SML
477 (Aller*, et al.* 2005) and foams (Johnson*, et al.* 1989) compared to the ULW, foams are ideal
478 substrates for particle-attached colonization. Particle-associated and free-living bacteria form
479 separate communities in many aquatic environments (Crespo*, et al.* 2013; Crump*, et al.* 1999)
480 including all habitats from this study, especially foams (Figure 4). SML bacteria are rather attached
481 to substrates than occurring in the free-living state (Cunliffe*, et al.* 2009), and particle-associated
482 bacteria of the SML are generally more prone to changes in community composition than free-
483 living ones (Stolle*, et al.* 2010). In agreement with that and former studies (Parveen*, et al.* 2013;
484 Rieck*, et al.* 2015), we found higher OTU numbers present on particles independent of the habitat
485 under investigation (Figure 3A).
486 Although attachment to particles might have some drawbacks for bacteria regarding grazing
487 (Albright*, et al.* 1987), disadvantages might be easily outweighed by benefits of particles providing

488 organic material and shelter from extreme levels of ultraviolet and solar radiation experienced at
489 the air-sea boundary. The LefSe analysis revealed that *Winogradskyella* (*Flavobacteriaceae*), often
490 being associated with brown algae or sponges (Park and Yoon 2013; Schellenberg*, et al.* 2017;
491 Yoon and Lee 2012), was abundant among the metabolically active OTUs in foams (Figure 6). As
492 broken algal cells and detritus are major parts of foams, high relative abundance of
493 *Winogradskyella* in the foam particle-associated fraction (Supplementary Figure 11) might be due
494 to its attachment to algal-derived substrates. *Persicirhabdus,* known for particle adherence in
495 sediments (Freitas*, et al.* 2012) or on plastic debris (Oberbeckmann*, et al.* 2016), was abundant in
496 particle-attached total communities in foam but not very active (Figure 6). Both *Persicirhabdus*
497 and *Winogradskyella* are well-known for their polysaccharide-degrading capacities (Cardman*, et*
498 *al.* 2014; Yoon and Lee 2012) and, hence, might prefer to stick to organic materials feeding or
499 sheltering them. Microbes with a free-living lifestyle, such as *Planktomarina*, for example,
500 represent a very prominent and active group of the *Rhodobacteraceae* in marine temperate and
501 (sub-)polar areas of the oceans and coastal waters (Giebel*, et al.* 2009; Giebel*, et al.* 2011; Selje*,*
502 *et al.* 2004; Voget*, et al.* 2015). *Planktomarina* is not a typical biofilm colonizer, but due to its high
503 abundances in surface waters, we assume it was "glued" in the SML and foam fraction and can
504 benefit from enriched dissolved organic material, from aerobic anoxygenic photosynthesis, and
505 oxidation of carbon monoxide as complementary energy sources (Giebel*, et al.* 2019).
506
507 Foam events were observed in association with blooms of the haptophyte *Phaeocystis globosa* or
508 *P. pouchettii, Cyanobacteria,* or linked to certain plants (Eberlein*, et al.* 1985; Seuront*, et al.* 2006;
509 Velimirov 1980; Wegner and Hamburger 2002; Wu*, et al.* 2019). Experiments by Velimirov (1980)
510 demonstrated foam formation in the presence of *Ecklonia maxima* while most bacterial growth was
511 antibiotically inhibited. The author showed that an algal component was crucial for the production
512 of stable foam. Nevertheless, foam bacteria might contribute to the foam formation process (Heard*,*
513 *et al.* 2008), because, like phytoplankton, they produce SAS and exopolysaccharides (Satpute*, et*

514 *al.* 2010). Foam samples from our study contained bacterial OTUs likely capable of producing
515 SAS, as previously demonstrated for *Vibrio* and *Pseudoalteromonas* (Dang*, et al.* 2016; Hu*, et al.*
516 2015). Moreover, *Saprospira* found in *Trichodesmium*-associated foam can produce sticky
517 substances such as acidic polysaccharides, which enhance aggregate formation (Furusawa*, et al.*
518 2015). We further speculate that in the Jade Bay, foam formation could be additionally supported
519 by blooming *Phaeocystis* sp. (OSPAR_Assessments_HASEC17/D503 2017). Surface scum
520 formation by cyanobacteria using intracellular gas vacuole formation as described for
521 *Trichodesmium* sp. (Walsby 1992) could be an ecological strategy to reach atmospheric carbon
522 dioxide supplies at the air-sea interface (Paerl and Ustach 1982). *Trichodesmium* sp. was active in
523 our Timor Sea samples (Supplementary Table 4). During research in the Timor Sea, we incubated
524 surface water with 1 mL *Trichodesmium* foam and found complete oxygen depletion after <14
525 hours. Since samples without foam showed incomplete O_2 consumption (Rahlff*, et al.* 2017b), we
526 assume that complete O_2 depletion was attributable to highly active, heterotrophic bacteria that
527 accompanied *Trichodesmium* and their associated foam.
528
529 Overall, our cell count and SAS data support the previous assumption that foams are strongly
530 influenced by the SML and, as an ephemeral and discrete habitat, select for specific bacteria
531 including the typical SML inhabitants *Vibrio* and *Pseudoalteromonas*. Moreover,
532 ultramicrobacteria in foams can probably be easily be aerosolized to the atmosphere and dispersed
533 to land (Rahlff*, et al.* 2020). Future studies should elucidate how wind-stirred bulk water and
534 bubbles transfer colonies to SML and foams, and whether bacteria use these habitats as nutrient-
535 rich "rest stop" before being transferred to sea-spray aerosols, dispersed to beaches or returned to
536 bulk water. The SML spans 71% of the Earth's surface and much remains to be learned about
537 patchy surface phenomena such as foams and their ecological implications for the functioning of
538 the marine food web and carbon turnover.
539

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Conflict of interests:

The authors declare no conflict of interests.

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