Title: Marine foams represent compressed sea-surface microlayer with distinctive bacterial communities

Running title: Foams are ephemeral hotspots for microbial life

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Key words

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Originality-Significance Statement

Marine foams are highly underexplored microbial habitats at the air-sea interface. Here, we provide the first comparison of the bacterial community composition of foams, sea-surface microlayer (SML, the uppermost layer on top of aquatic ecosystems) and seawater collected from 1 m depth. Our work shows that the foam environment selects for bacterial taxa common to the SML but overall harbors a distinctive bacterial community compared to the other two habitats, allowing the conclusion that foams are a highly compressed version of the SML.

Summary

The occurrence of foams at the oceans' surface is patchy and generally short-lived. Here we investigated if marine foams as important sea surface phenomena represent a compressed form of the sea-surface microlayer (SML), a <1 mm thick film at the air-sea interface. The comparison of marine foams, SML and underlying water (ULW) collected from the North Sea and Timor Sea revealed that foams were often characterized by high abundance of small phototrophic and prokaryotic cells as well as high concentrations of surface-active substances (SAS) in contrast to SML and ULW. Amplicon sequencing also revealed distinctive bacterial communities in foams including species of *Persicirhabdus* and *Winogradskyella* that were part of the particle-attached, bacterial communities. Comparison of rRNA and DNA based sequenced data suggests that *Pseudoalteromonas* sp. are highly active and thus might enhance foam formation and stability by producing SAS. Presence of motile *Vibrio* might indicate an active migration of *Vibrio* towards

ephemeral and nutrient-rich foams. Our study supports that foam is a compressed version of the SML due to increased cell numbers and SAS concentration, and bacterial taxa found in foam were also present in the SML entailing major implications for air-sea exchange processes, biogeochemical cycling and food web functioning.

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 (Eisenreich et al., 1978; Bärlocher et al., 1988; Thornton, 1999; Kesaulya et al., 2008; Jenkinson et al., 2018). Furthermore, bubbles that do not burst immediately but rise to and 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 (Velimirov, 1980; Pugh, 1996), 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, which represent 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 (Velimirov, 1980; Velimirov, 1982; Frew et al., 1990; Wegner and Hamburger, 2002), or other organic detritus (Velimirov, 1980). In addition, during phytoplankton blooms organic material accumulates at the sea surface, and mainly biogenic lipids and amino acids are important substrates for the formation of foam (Eberlein et al., 1985; Riebesell, 1993; Hunter et al., 2008). 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 functioning as microbial habitats. By comprising ephemeral feeding grounds, foams are "remarkably rich and diverse" in microorganisms (Tsyban, 1971), including bacteria (Maynard, 1968; Gobalakrishnan et al., 2014), protists and algae (Maynard, 1968; Harold and Schlichting, 1971). In addition, foams were shown to enclose Metazoans including copepods, polychaete and tunicate larvae (Armonies, 1989; Castilla et al., 2007) thus forming vital food sources for the higher trophic levels of the food web (Bärlocher et al., 1988; Craig et al., 1989; Scully, 2009).

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. It is characterized by remarkably different physicochemical and biological properties that allow its differentiation from the underlying water (ULW) (Hardy, 1982; Cunliffe et al., 2013). Increasing interest in the SML 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 biogeochemical cycles (Engel et al., 2017; Wurl et al., 2017; Rahlff, 2019). The interfacial position of the SML makes it a challenging environment for its inhabiting organisms termed as neuston (Maki, 1993). Differences in bacterial community composition between SML and ULW have been related to meteorological conditions (Agogué et al., 2005b; Stolle et al., 2011; Rahlff et al., 2017b), however the specific adaptation of bacteria to the SML habitat remains an open question (Agogué et al., 2005a).

Napolitano and Cicerone (1999) suggested that 1 L of foam water would represent 2 m^2 of SML, i.e. foams are essentially compressed SML. Supporting this idea, enrichment of bacteria in foams compared to SML and/or ULW has been reported (Tsyban, 1971; Kuznetsova and Lee, 2002; Rahlff et al., 2017b). However, a thorough characterization of foam microbial community

composition compared to the SML and the ULW is missing. Using a microscopic approach, Druzhkov et al. (1997) found a highly identical taxonomic composition of heterotrophs (nanoflagellates <5 µm and bacteria), nano- and microphytoplankton in foam and the SML. The authors further described higher abundances (one order of magnitude) of autotrophs but not of heterotrophs in foams compared to the SML. If microorganisms are rather passively transported to foams by, e.g., bubbles or SML compression, or whether they actively seek the presence of foams remains to be elucidated.

In this study, we investigated the bacterial community composition of marine foams in direct comparison to non-foamy SML and ULW. Collected foam samples were associated with different events such as surface slicks, cyanobacterial blooms, presumptive phytoplankton exudates and surface compression by wave action (Figure 1, Table S1; supporting information). Based on the theory that foam is an extreme condensed form of the SML (Napolitano and Cicerone, 1999), we hypothesized that the bacterial community composition of foam and SML are more similar than between foam and ULW. Since the SML is considered as an extreme habitat (Maki, 1993) likely comprising many dead or dormant cells, we also considered the community composition among active and abundant bacteria as inferred from a cDNA and DNA-based 16S rRNA amplicon sequencing approach, respectively. We expect that nutrient-rich foams harbour a distinct bacterial community and favour fast-growing heterotrophic bacteria. Overall, we provide a detailed understanding of the bacterial community composition associated with marine foams with implications for the uppermost sea surface in air-sea exchange processes and biogeochemical cycling.

Results

Foams are enriched with surface-active substances and microorganisms

Overall, foams from both sites, North Sea (NS) and Timor Sea (TS), were enriched with prokaryotic microorganisms, small phototrophs and SAS (Table 1). Cell counts of prokaryotic microorganisms, which mainly consisted of heterotrophs, ranged between 2.63 x 10^6 and 4.62 x 10^7, 9.83 x 10^5 and 4.57 x 10^6, and 1.01 x 10^6 and 3.71 x 10^6 cells mL^{-1} in foam, SML and ULW, respectively (Figure 2A). Thus, prokaryotic microorganisms in foams were enriched with a maximum EF (enrichment factor) of 10.1 and 5.9 over SML, and with a maximum EF of 14.8 and 33.6 over ULW in NS and TS, respectively (Table 1). Prokaryotic cells in the SML were enriched with a maximum EF of 1.5 and 1.7 over ULW in NS and TS, respectively. Likewise, the total number of small phototrophs, which also contained some cyanobacteria, was always higher in foam (range=1.38 x 10^4 to 5.71 x 10^5 cells mL^{-1}) compared to SML (range=1.15 x 10^3 to 3.97 x 10^4 cells mL^{-1}) and ULW (range=1.06 x 10^3 to 4.17 x 10^4 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 phototrophs was two orders of magnitude lower compared to the prokaryotic cell counts (Figure 2 A&B). Interestingly, small phototrophs 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 also contained the highest SAS concentrations compared to the other two habitats (Figure 2C). SAS concentrations in foams varied between 900 to 148233 µg Teq L^{-1} in NS and TS whereas SML SAS concentrations were in a range of 66 to 1753 µg Teq L^{-1}, and ULW SAS

141 concentrations in a range of 109 to 223 µg Teq L^{-1} (Table 1). While SAS concentrations in the
142 SML were enriched and depleted compared to ULW, their concentration in foams compared to
143 ULW was typically enriched by three orders of magnitude (EF ranging from 5 to 665).

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

146 We analyzed the bacterial community composition of all NS samples to compare the diversity
147 between the different habitats (foam, SML, ULW). We furthermore differentiated the attachment
148 status of bacteria between particle-attached (PA) and free-living (FL) as well as the community
149 composition between abundant (based on DNA) and active (based on cDNA) operational
150 taxonomic units (OTUs, Figure 3). Analyses revealed overall higher numbers of OTUs, i.e.
151 higher diversity, in cDNA-based communities (reflecting active taxa, median=786.5) compared
152 to DNA-based communities (reflecting abundant taxa, median=571). In DNA-derived samples,
153 the number of foam OTUs was significantly increased for PA over FL communities (Dunn's test,
154 p=0.0031), and also significantly higher compared to the SML and ULW PA fraction (Figure 3)
155 with p=0.0103 and 0.0146, respectively. OTUs derived from cDNA were significantly more
156 diverse among the PA samples of foam (p=0.037) and SML (p=0.042) compared to the respective
157 FL samples (Figure 3). We found indications for a higher diversity of FL OTUs in foams (cDNA-
158 based evaluation) compared to their SML and ULW counterparts, although these trends lacked
159 significance (Figure 3).

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Bacterial community composition of North Sea foams

On a phylum-level, the bacterial community composition of foam, SML and ULW was comparable (Figure 4). The most dominant group was assigned to *Gammaproteobacteria* (maximum=37.4%). DNA-based community analyses showed that for all three habitats *Gammaproteobacteria*, *Verrucomicrobia* and *Cyanobacteria* formed a higher portion of PA than FL communities. In contrast, *Alphaproteobacteria* and *Actinobacteria* were more abundant in the FL form (Figure 4, Table S2; supporting information). Differences between cDNA and DNA-derived bacteria were only minor, indicating that abundant phyla were also active. *Gammaproteobacteria*, as a single exception, showed high relative abundance in the cDNA-based community composition (37.4% and 35.0% of FL and PA OTUs, respectively) compared to the DNA-based community composition (22.7% and 26.0% of FL and PA OTUs, respectively). The cDNA-based communities of foam contained less *Alphaproteobacteria* but more *Gammaproteobacteria* compared to SML and ULW communities (Figure 4). Non-metric multidimensional scaling plots revealed that foam bacterial communities were clearly distinct from SML and ULW communities, irrespective of differentiating cDNA and DNA or FL and PA (Figure 5). In contrast to this, SML and ULW bacterial community composition were more similar to each other as shown by the clustering (Figure 5). On the order-level, the difference between cDNA- and DNA-based communities became more obvious: A depletion of the relative abundance of active OTUs in foam in contrast to the other two surface habitats was most apparent in the MB11C04 marine group (*Verrucomicrobia*), SAR11 clade (*Alphaproteobacteria*) and *Oceanospirillales* (*Gammaproteobacteria*) (Figure S1, S2, S3; supporting information). A higher relative abundance of active OTUs in foam compared to SML and ULW was found among the *Puniceicoccales* (*Verrucomicrobia*), *Sphingomonadales* (*Alphaproteobacteria*),

186 *Alteromonadales* and *Vibrionales* (both *Gammaproteobacteria*) (Figure S1, S2, S3; supporting
187 information). Active FL OTUs of the order *Flavobacteriales* and *Oceanospirilliales* were more –
188 whereas free-living *Sphingobacteriales* were less numerous than their PA counterparts in all three
189 habitats (Figure S1, S4; supporting information).

190 Apart from the order *Rhodobacterales* (Figure S3; supporting information), foam generally had
191 less alphaproteobacterial DNA-based OTUs compared to SML and ULW. However, foam
192 contained a higher DNA-based relative abundance of *Verrucomicrobia* and
193 *Gammaproteobacteria* (Figure 4). Among the *Gammaproteobacteria*, especially more OTUs of
194 the orders *Cellvibrionales, Vibrionales, Legionellales, Alteromonadales* were increasingly
195 detected in foam compared SML and ULW, whereas the order *Oceanospirilliales* was more
196 depleted in foam (Figure S1; supporting information).

197

198 **Foam-specific bacteria**

199 Using the linear discriminant analysis (LDA) effect size (LefSe) method we could identify OTUs
200 that were enriched in foam compared to SML and ULW (Figure 6). The analysis does not refer to
201 the most abundant OTUs in terms of absolute numbers but points out the largest differences
202 between foam and the other two habitats. Members of the *Gammaproteobacteria* were typical
203 active and abundant foam colonizers (Figure 6). Taxa including *Winogradskyella*, *Vibrio*,
204 *Halioglobus* and *Pseudoaltermonas* were particularly abundant in both cDNA and DNA-derived
205 foam samples as well as when compared to SML and ULW habitats. *Persicirhabdus* and other
206 *Verrucomicrobiaceae* were typical foam-dwellers with 11% and 7% relative abundance
207 according their presence in DNA samples but seemed not very active according to cDNA

208 samples. Typical SML populating bacteria belonged to taxa which were phylogenetically related
209 to *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteria*. Strikingly, abundance and
210 activity profiles for SML microbes were hardly different from the ULW but more different from
211 foam samples. High relative abundances (>5%) of *Planktomarina*, SAR116 and SAR86 could be
212 revealed for SML samples for cDNA and DNA. Microbial taxa of SAR11 and Candidatus
213 *Actinomarina* typically occurred in high abundances in the ULW.

214

215 **_Trichodesmium_ sp.-produced foam – a case study**

216 Due to technical restrictions we could only obtain a single DNA/cDNA sample from the Timor
217 Sea (Station 8). Among the DNA-based community in foam we found most PA OTUs assigned
218 to *Trichodesmium* (relative abundance=33.4%), *Alteromonas* (26.4%) and *Rhodobium* (5.4%),
219 whereas FL OTUs were mostly assigned to *Alteromonas* (18.0%) and *Rhodobium* (10.2%) (Table
220 S3; supporting information). PA OTUs were mainly assigned to *Trichodesmium* (68%) and
221 *Rhodobium* (10.9%) in the SML, and to *Trichodesmium* (23.8%) and *Oscillatoria* (26.7%) in the
222 ULW. Most FL OTUs from SML and ULW were assigned to *Synechococcus* with 15.7% and
223 21.6% relative abundance, respectively. In all cDNA samples, *Trichodesmium* was also the most
224 abundant among active OTUs in foam and SML, only in the ULW *Oscillatoria* (48.2%) had
225 higher relative abundance compared to *Trichodesmium* (29.1%). The relative abundance of
226 cDNA-based OTUs assigned to *Alteromonas* in foams (PA: 17.8%, FL: 12.6%) was
227 comparatively enhanced to the SML (PA: 0.2%, FL: 1.2%). The ten most abundant OTUs found
228 in the three habitats are given in Table S3 (supporting information).

229

Discussion

Foams comprise an extreme form of SML

Foams are peculiar but understudied microbial habitats at the air-sea interface. They stem from the SML and bursting bubbles, and as soon as foams subside, their material becomes part of the SML again (Kuznetsova and Lee, 2002). The idea that foams are essentially compressed SML is supported by our results. The SML is usually enriched in bacterial cells compared to the ULW (Hardy, 1982). As expected for an extreme form of the SML, we found high concentrations of SAS in foams as well as an enrichment of prokaryotic microorganisms (determined by flow cytometry and DNA-based amplicon sequencing) and small phototrophic cells (flow cytometry only), matching previous observations (Kuznetsova and Lee, 2002; Rahlff et al., 2017b; Robinson et al., 2019). Foams likely originating from cyanobacterial cells and presumptive phytoplankton exudates (Figure 1 A&B) that principally contain high loads of SAS (□utić et al., 1981) were also linked to higher amounts of microbes compared to foams formed by convergence of surface water (Figure 1C, Table S1; supporting information). This indicates that foams originating from photoautotrophic biomass contain substantial amounts of labile organic matter, stimulating enhanced growth of heterotrophic bacteria. The presumptive phytoplankton-associated foam was mostly found in slicks, which are visible sea surface features that result from SAS causing dampening of capillary waves, and which are known to comprise distinct microbial communities (Wurl et al., 2016). Interestingly, abundance of small phototrophic cells declined in the SML while it was enhanced in the respective foam sample. This observation might argue for passive transport of microbes from SML to foam, e.g. by SML compression, and some transferred, fast-growing, opportunistic bacteria took the advantage and thrived. An OTU assigned to *Alteromonas* was the most abundant FL bacterium in the *Trichodesmium*-associated

253 foam (Table S3; supporting information), and a high relative abundance among the active OTUs
254 was found in foam compared to SML in both FL and PA fractions. The increased relative
255 abundance of active OTUs being restricted to the foam environment allows speculations on active
256 migration of certain taxa towards the foam, e.g. *Vibrio* may use flagella to actively move there
257 (Atsumi et al., 1992).

258

259 One striking finding of our study is that we mostly observed a higher diversity within the cDNA-
260 based compared to the DNA-based bacterial community, suggesting that bacteria being enriched
261 in foams were generally alive and most likely active. Sequencing of cDNA-derived amplicons of
262 small subunit RNA gives a rough estimate for assessing activity of bacterial taxa because cellular
263 rRNA concentration is linked to cell growth and activity (Schaechter et al., 1958; Poulsen et al.,
264 1993; Lanzén et al., 2011). Detecting both amplicon types allows to find rare and active
265 communities (Campbell et al., 2011) and to detect two distinct communities, for instance if small
266 cells having a general lower RNA content are highly active. However, the taxonomic resolution
267 of 16S rRNA (gene) amplicon sequencing cannot discriminate between closely related species
268 (Fox et al., 1992), and especially between cyanobacteria.

269 Long residence time of microbes at the air-sea interface and thus prolonged exposure to the cell-
270 inhibitory effects of high solar and ultraviolet (UV) radiation (Santos et al., 2013) and wind-wave
271 dynamics (Stolle et al., 2011) are neither expected for foam being highly ephemeral (Pugh, 1996)
272 nor the SML being prone to sudden changes in physical and chemical properties (Zhang et al.,
273 2003). Some bacteria, such as *Trichodesmium* sp. show more tolerance towards photoinhibition at
274 the air-sea interface (Sieburth et al., 1976), tend to accumulate in slicks (Sieburth and Conover,

1965), and can remain active as our results for the Timor Sea revealed (Table S3; supporting information).

The role of particles for foam-populating bacteria

According to Figure 5, PA and FL bacteria form distinctive communities in all of the studied habitats. Particulate organic matter is frequently enriched within the SML (Aller et al., 2005) as well as in foams (Johnson et al., 1989) compared to the ULW. In addition, SML bacteria are more attached to substrates than occurring in the free-living state (Cunliffe et al., 2009), and PA bacteria are generally more prone to changes in community composition (Stolle et al., 2010). In agreement with that and former studies (Parveen et al., 2013; Rieck et al., 2015), we found higher OTU numbers being linked to the PA lifestyle independent of the habitat under investigation. For instance, the LefSe analysis revealed that *Winogradskyella* was particularly abundant in the cDNA fraction, reflecting active OTUs. Previous work has shown that *Winogradskyella* spp. is often extracted from other species such as brown algae or sponges (Yoon and Lee, 2012; Park and Yoon, 2013; Schellenberg et al., 2017). As broken algal cells and detritus are major parts of foams, high relative abundance of *Winogradskyella* in the foam PA fraction (Figure S5; supporting information) might be due to its attachment to algal-derived particles. Especially *Verrucomicrobia* were attached to particles rather than occurring in the FL form, which has been previously suggested (Freitas et al., 2012), and one of its members, *Persicirhabdus* was particularly abundant in foam-derived cDNA samples. *Persicirhabdus* might have a preference for particle adherence since it also occurs in higher abundance in sediments compared to free water column (Freitas et al., 2012) or colonizes plastic debris (Oberbeckmann et al., 2016). In addition, *Persicirhabdus* and *Winogradskyella* are well-known for their polysaccharide-degrading

298 capacities (Yoon and Lee, 2012; Cardman et al., 2014) and, hence, might prefer to stick to
299 organic materials feeding them. Certain bacteria that were particularly active in foams, e.g.
300 *Alteromonas* sp., were previously shown to be highly abundant and active degraders of alginate, a
301 cell wall components from marine macroalgae (Mitulla et al., 2016), and of labile dissolved
302 organic carbon (Pedler et al., 2014). By using 16S rRNA gene sequencing of individual
303 transparent exopolymer particles (TEP) from the SML, Zäncker et al. (2019) found that
304 *Alteromonadaceae* on TEP was significantly increased compared to subsurface water. Though
305 being attached to particles might have some drawbacks for bacteria when it comes to grazing
306 (Albright et al., 1987), this might not be necessarily true for the SML, or this disadvantage is
307 easily outweighed by the benefits of particles providing food and shelter for extreme levels of UV
308 and solar radiation. In this regard, foam bacteria might be important key players in
309 biogeochemical cycling, jump starting the microbial loop from the air-sea interface.

310

311 **Ecological implications of sea foam bacteria**

312 Sea foams are often perceived as aesthetically unpleasant or are associated with pollutants
313 (Schilling and Zessner, 2011). In this study we detected bacteria of the orders *Cellvibrionales*,
314 *Vibrionales* and *Legionellales*, all of which include potentially pathogenic bacteria. Likewise, sea
315 foam bacteria near a sandy beach on Havelock Island, India were shown to contain high portions
316 of *Vibrio* and *Salmonella*, as well as of fecal coliforms such as *E.coli* (Gobalakrishnan et al.,
317 2014). A general awareness for the presence of pathogens in sea foams can benefit public health
318 and the recreational value of coastal areas and beaches where foams frequently occur.

Recent work has shown that massive foam events in association with blooms of *Phaeocystis globosa* and an unknown plankton species can have devastating effects for local residents (Jenkinson et al., 2018). Likewise, *Phaeocystis pouchetii*, the kelp species *Ecklonia maxima* and the river water-crowfoot *Ranunculus fluitans* contributed to foam formation in their respective habitats (Velimirov, 1980; Eberlein et al., 1985; Wegner and Hamburger, 2002). Heterotrophic bacteria in foams might be an important but overlooked component in the foam formation process, because also bacteria can produce SAS and exopolysaccharides (Satpute et al., 2010), which may contribute to foam production and stabilization (Jenkinson et al., 2018). Experiments by Velimirov (1980) revealed that the metabolic products of growing bacteria in kelp bed foams would not remarkably contribute to foam stability and formation time. The author demonstrated foam formation in the presence of *E.maxima* while bacterial growth was antibiotically inhibited. Our foam samples contained bacterial OTUs which are likely capable of producing SAS, as previously demonstrated for the genus *Vibrio* and *Pseudoalteromonas* (Hu et al., 2015; Dang et al., 2016). However, if these bacteria enhance foam formation and stability in the absence of a major SAS-producing algae however requires further experiments.

The SML is an important component for the regulation of gas-exchange (Frew, 1997) but foams covering up to 6% of the ocean's surface (Anguelova and Webster, 2006) are rarely part of this concept. While performing research in the Timor Sea, we used a free-floating Surface In Situ Incubator (Rahlff et al., 2017a) to incubate water from 5 m depth supplemented with 1 mL *Trichodesmium* foam and found complete oxygen (O_2) depletion after less than 14 hours (data not shown), while samples without foam showed incomplete O_2 consumption (Rahlff et al., 2017a). We assume that complete O_2 depletion was attributable to highly active bacteria associated with the foam. Since a recent study found that even pronounced biological activity within the SML

had no major contribution to O_2 air-water gas exchange (Rahlff et al., 2019), if extensive surface foams and therein enhanced bacterial activity may play a role in air-sea gas exchange consequently merits further investigation.

This study presents first detailed insights into the distinctive bacterial communities associated with marine foams in contrast to SML and ULW. Our study identified particularly well-adapted bacteria including *Vibrio*, *Winogradskyella* and *Pseudoalteromonas* for foam and foam particle colonization. Although foams contain distinctive bacterial communities and a higher diversity compared to the other two habitats, a selection towards typical SML taxa, e.g. *Vibrio* or *Pseudoalteromonas* cannot be dismissed. It follows that foam represents an ephemeral and compressed version of the SML and studying its microbes aids our understanding of air-sea exchange and bacterial transport processes. While sticking on rising bubbles, bacteria might benefit from SML and foams as a nutrient-rich "rest stop" before being transferred to sea-spray aerosols and clouds or return to bulk water. Air-sea interfaces span 70% of the Earth's surface and much remains to be learned about patchy surface phenomena such as foams and their ecological implications for the functioning of the marine food web, biogeochemical cycles and human health.

Experimental Procedures:

Field sample collection

Field sampling was conducted from the bow of a small boat in the Jade Bay, North Sea (NS) offshore Wilhelmshaven, Germany (Table S1; supporting information) in spring and summer 2016. Foams originated from different sources such as from presumptive phytoplankton exudates

and convergence of surface water (Figure 1A, Figure 1C, Table S1; supporting information). Additional samples were collected during a *Trichodesmium* sp. bloom encountered in the Timor Sea (TS) (Figure 1B, Table S1; supporting information) in October 2016 during *R/V* Falkor cruise FK161010 as recently described (Wurl et al., 2018). A set of foam, SML and ULW samples was collected from each location. Foams and SML were sampled with the glass plate technique (Harvey and Burzell, 1972) using 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 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 not running. 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.

Concentration of surface-active substances

The concentration of SAS was measured by automated VA Stand 747 (Methrom, Herisau, Switzerland) with a hanging drop mercury electrode as previously described (Ćosović and Vojvodić, 1998; Wurl et al., 2011). The quantification is based on SAS adsorption on the Hg electrode measured by the change of capacity current (ΔIc) at an applied potential (E) of -0.6 V (Ćosović and Vojvodić, 1998). Before measurement, thick samples such as foam samples were diluted with artificial seawater (0.55 M of NaCl solution) to achieve measurement within the

387 linear range. Standard addition technique was utilized where non-ionic surfactant Triton X-100
388 (Sigma Aldrich, Taufkirchen, Germany) was used as a standard. SAS concentration in the
389 samples was measured using two to three technical replicates, resulting in relative standard
390 deviations below 6% (Rickard et al., 2019). Concentration of SAS is expressed as the equivalent
391 concentration of the additional Triton X-100 (µg Teq L^{-1}).

392

393 **Determination of microbial abundance**

394 For determination of prokaryotic and small (< 50 µm) phototrophic cell numbers, foam and water
395 samples were fixed with glutardialdehyde (1% final concentration), incubated at room
396 temperature for 1 hour, and stored at -80°C until further analysis. Prior staining and counting by
397 flow cytometry, the particle-enriched foam samples were pre-filtered by gravity onto CellTrics®
398 50 µm filter (Sysmex Partec, Muenster, Germany) to avoid clogging of the instrument by
399 particulate matter. Autofluorescence analysis was used to count small phototrophic cells (Marie
400 et al., 2000), and prokaryotic cells were stained with SYBR® Green I Nucleic Acid Gel Stain (9x
401 in final concentration, Thermo Fisher Scientific, Darmstadt, Germany) following a protocol after
402 Giebel et al. (2019). Enrichment factors (EF) were calculated for the pairings foam/SML (F/S),
403 foam/ULW (F/U) and SML/ULW (S/U) (Table 1). This means that the relative abundance of
404 cells in a foam or SML sample was divided by its SML or ULW counterpart. Therefore, an EF>1
405 implies an enrichment of cells, whereas an EF<1 indicates a depletion.

406

407 **Nucleic acid extraction and PCR**

A two-step filtration of foam, SML and ULW samples was conducted. Sample water was filtered through 3 µm pore size polycarbonate (PC) filters, after which the filtrate was filtered onto 0.2 µm pore size PC filters (Merck Millipore, Darmstadt, Germany). These two size fractions were defined to contain the particle-associated (PA, on 3 µm filter) and free-living (FL, on 0.2 µm filter) bacterial community, respectively. Foam from the Timor Sea (Station 8) collected during a bloom of *Trichodesmium* sp. was additionally pre-filtered on a 100 µm mesh before subsequent filtration on the 3 µm pore size filter. All filters were initially stored at -80°C prior analysis. Extraction of DNA and RNA from the filters was performed by using the DNA + RNA + Protein Extraction Kit (Roboklon, Berlin, Germany) with a modified protocol (Rahlff et al., 2017b). RNA was digested on-column using 3 U of DNase and subsequently checked for contaminations with genomic DNA by PCR. A quantity of 10 ng RNA was converted to cDNA using the NG dART Kit (Roboklon, Berlin, Germany) including negative controls either without reverse transcriptase or without RNA. The reaction was incubated for 60 minutes at 50°C followed by 5 minutes at 85°C. All DNAs and cDNAs were quantified using the Quant-iT™ PicoGreen™ dsDNA assay (Thermo Fisher Scientific, Darmstadt, Germany).

16S rRNA library preparation, sequencing run and data analysis

The bacterial 16S rRNA gene was amplified according to Herlemann et al. (2011) with the following modifications. Genomic DNA was amplified with 35 cycles prior Index-PCR. The cDNA samples were amplified with 25 cycles prior Index-PCR. Amplicon PCR, Index PCR, quantity and quality control and sequencing of the individual libraries as pool in one Illumina MiSeq run was performed by an external provider (Eurofins Genomics, Ebersberg, Germany). Raw sequencing data were deposited at the European Nucleotide Archive (ENA) under accession

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

Statistical analyses

Operational taxonomic unit (OTU) counts based on genus level were rarefied to 43500 reads per sample using the single_rarefraction.py script implemented in QIIME. We visualized the differences in the bacterial community composition through non□metric multidimensional

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

460 Differences in alpha diversity between habitats, nucleic acid types and attachment status were
461 statistically analyzed using a Kruskal-Wallis test and Dunn's multiple pairwise comparisons
462 within the R package "dunn.test" (R version 3.4.3, Team (2014)). The null hypothesis was
463 rejected if $p \leq 0.05$. Comparisons were made between FL and PA status within a habitat (foam,
464 SML, ULW) and between habitats for each DNA and cDNA, respectively.

465

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Competing interests:

The authors declare no conflict of interests.

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Figures

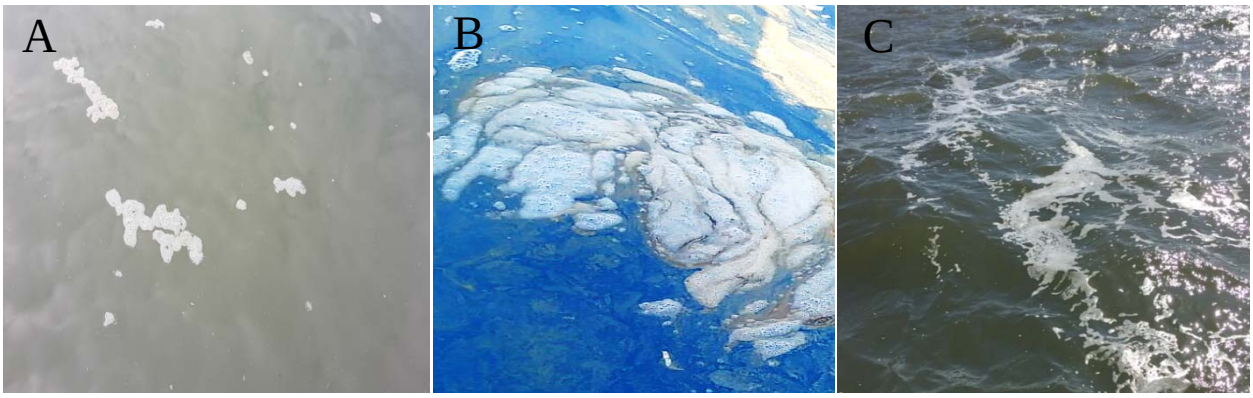

Figure 1: Marine foam originating from A) presumptive phytoplankton exudates (Jade Bay), B) a *Trichodesmium* bloom (Timor Sea) and C) whitecaps produced by convergence of surface water (Jade Bay, North Sea).

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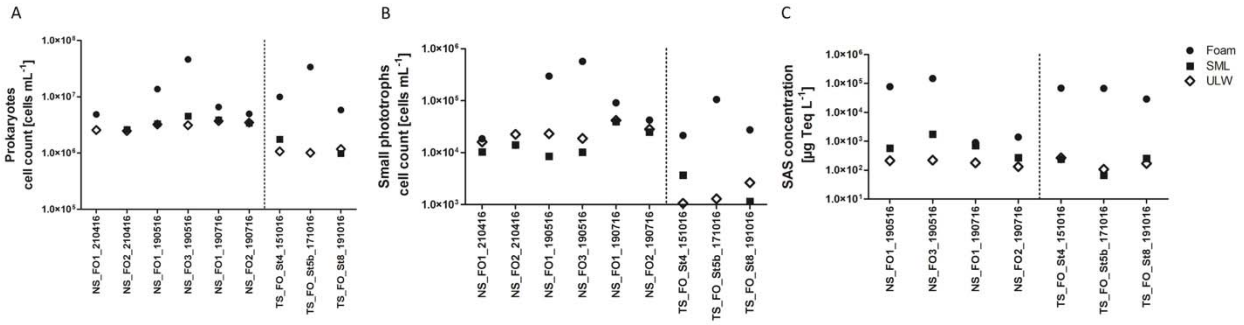

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723 **Figure 2:** Absolute cell counts mL^{-1} for A) prokaryotes and B) small phototrophic cells and C)

724 concentration of surface-active substances (SAS) in µg Teq L^{-1} for foam, sea-surface microlayer

725 (SML) and underlying water (ULW).

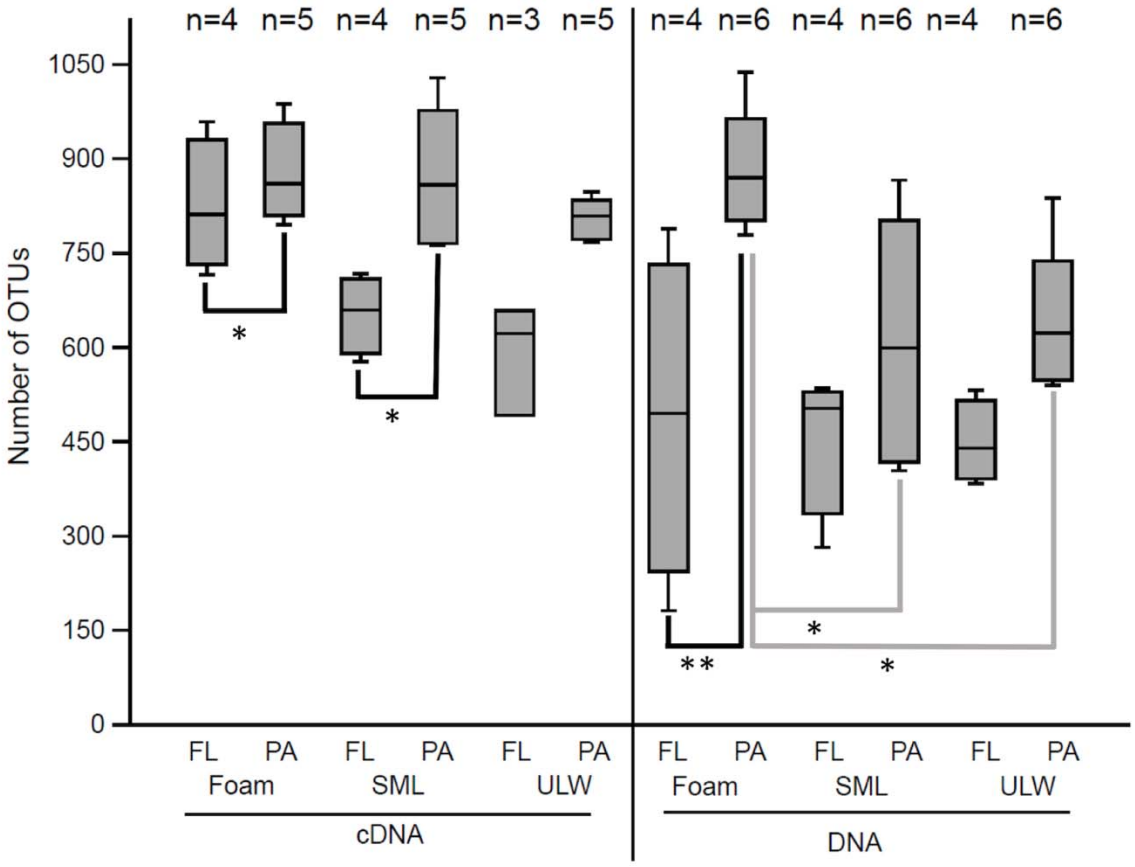
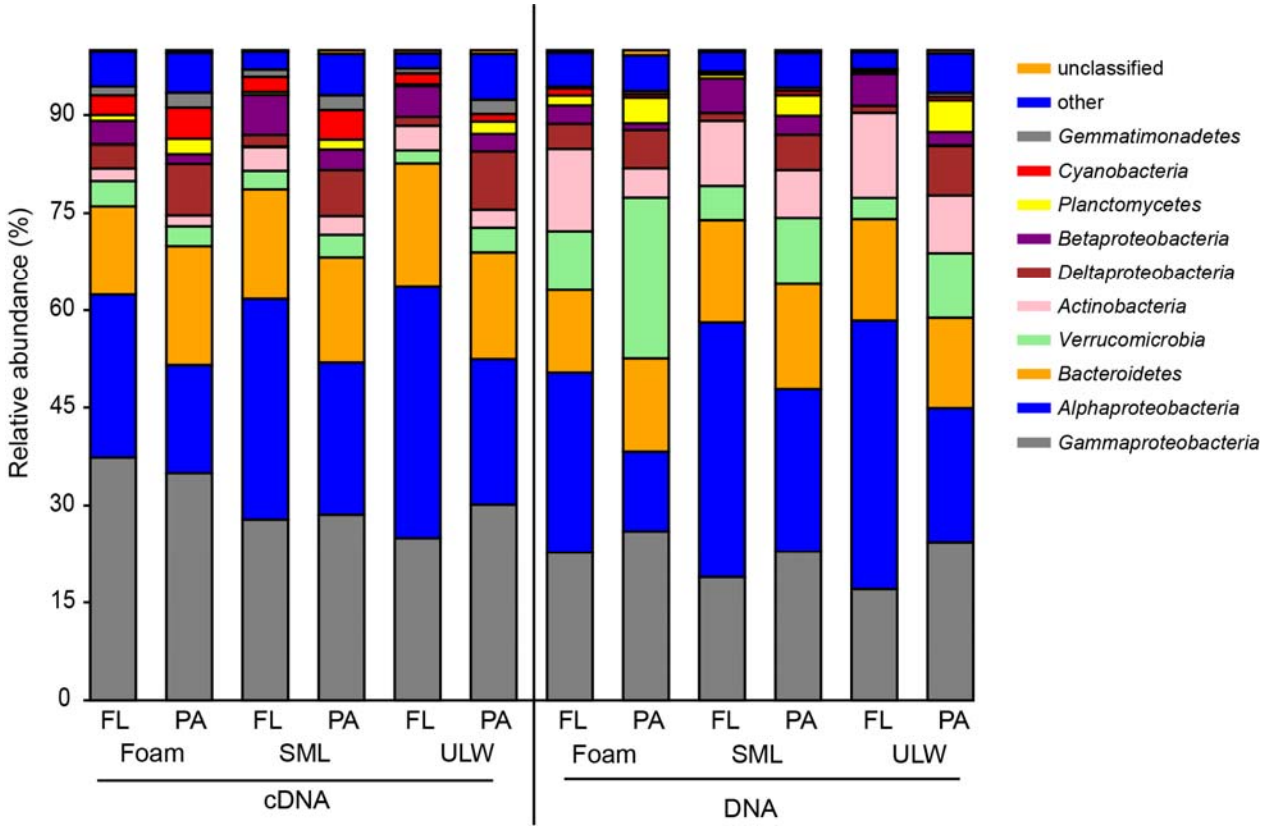


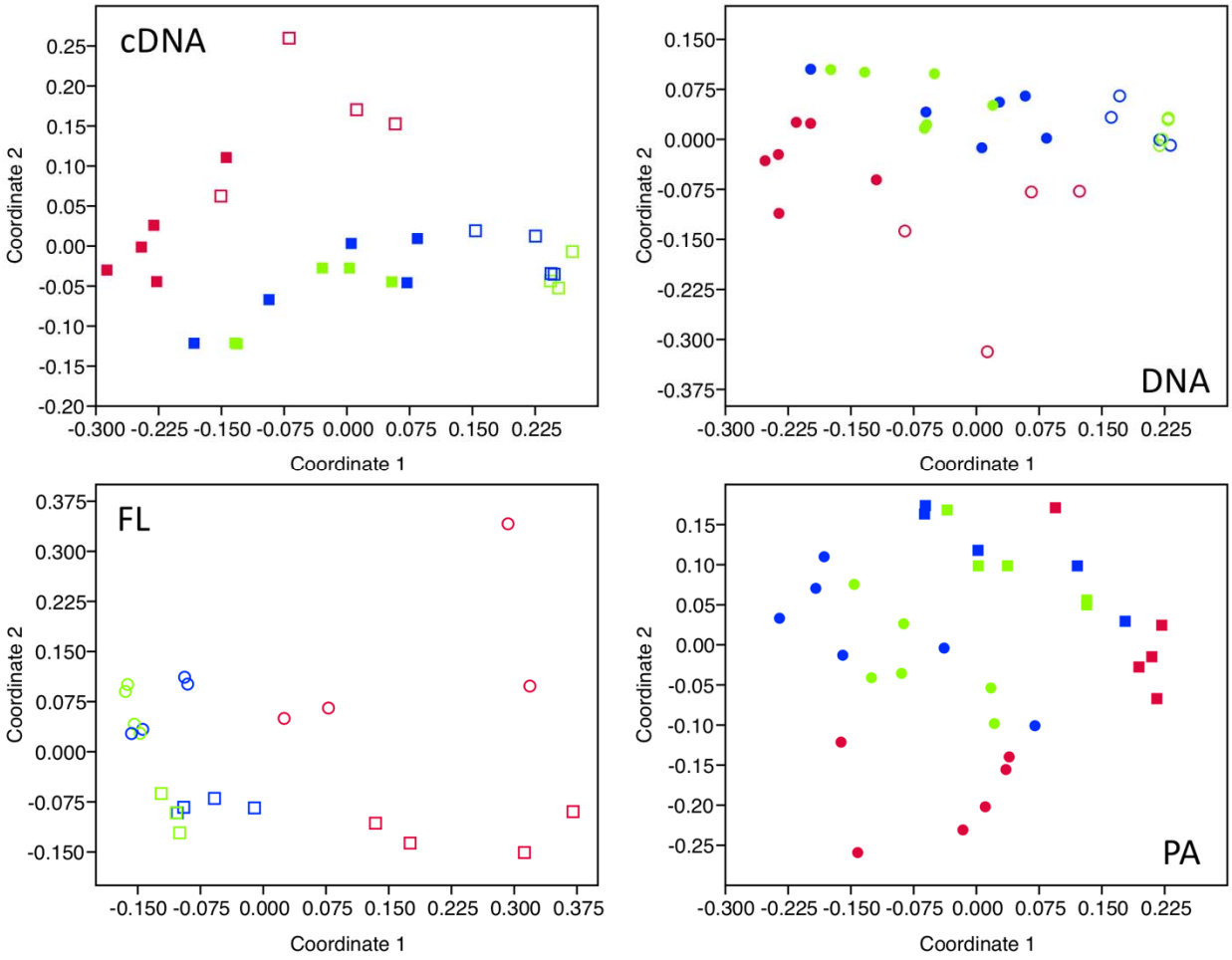
Figure 3: cDNA and DNA-derived numbers of operational taxonomic units (OTUs) for foam, SML and ULW habitat of pooled North Sea stations. Alpha diversity of the three habitats is further distinguished between free-living (FL) and particle-associated (PA) bacterial communities. Grey and black lines indicate inter- and intra-habitat comparisons, respectively. The boxplot shows the 25–75 percent quartiles; the median is indicated by the horizontal line inside the box. Error bars show minimal and maximal values. Asterisks indicate the level of significant differences:

* $p \leq 0.05$, ** $p \leq 0.01$



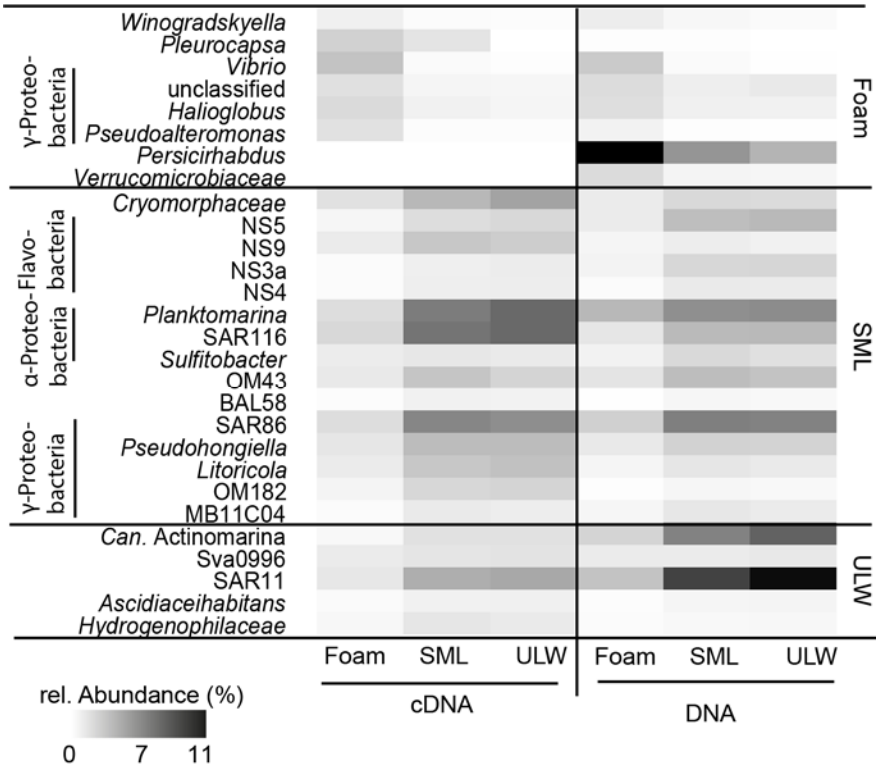
735

736 **Figure 4:** Beta diversity at the phylum-level of foam, SML and ULW samples of cDNA and
737 DNA-based relative abundance of operational taxonomic units (OTUs) of pooled North Sea
738 stations. Each habitat is further separated into free-living (FL) and particle-associated (PA)
739 bacterial communities.



740

741 **Figure 5:** Non-metric multidimensional scaling plot shows distinct clustering of foam (red), SML
742 (blue) and ULW (green) bacterial communities. Further separation according to nucleic acid
743 source (cDNA=squares and DNA=circles) as well as free-living (open symbols) and particle-
744 associated (filled symbols) attachment style has been conducted.



745

746 **Figure 6:** Heat-map showing the relative abundance of most different foam OTUs compared to

747 SML and ULW according to the linear discriminant analysis (LDA) effect size (LEfSe) method.

Table 1: Absolute and relative abundances of prokaryotes, small phototrophs and surface-active substances (SAS) in foam (F), SML (S) and ULW (U), NA=not available, Teq=Triton X-100 equivalents

	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 cells (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

748

Supporting Information

Marine foams represent compressed sea-surface microlayer with distinctive bacterial communities

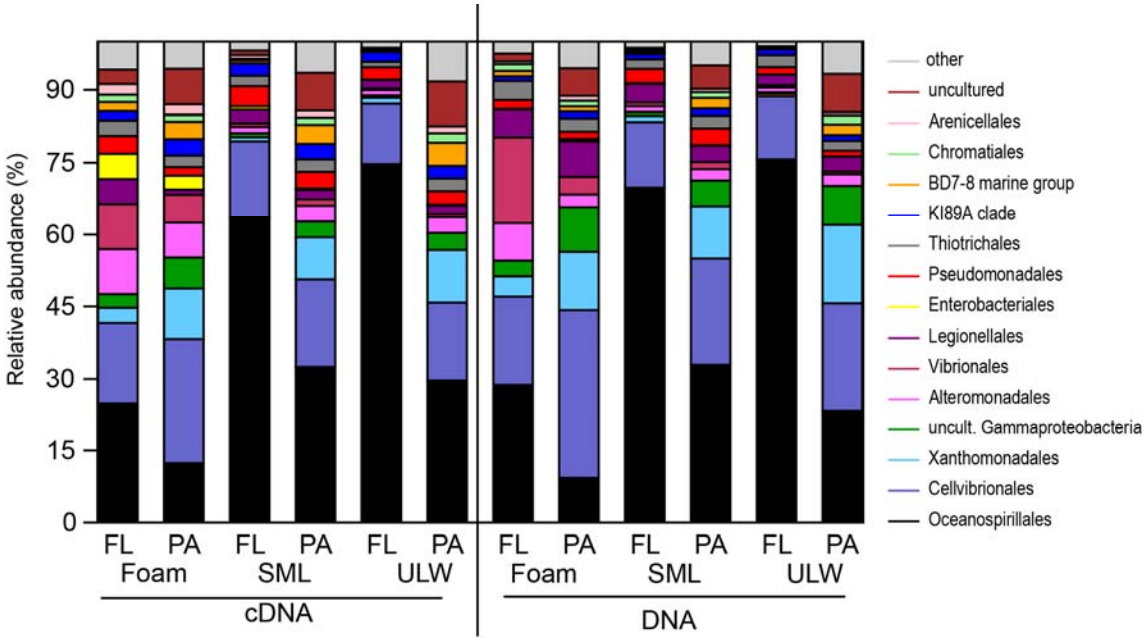


Figure S1: Beta diversity among *Gammaproteobacteria* in foam, sea-surface microlayer (SML) and underlying water (ULW) samples of cDNA and DNA-based operational taxonomic units (OTUs). Each habitat contains further information on free-living (FL) and particle-associated (PA) bacterial community composition.

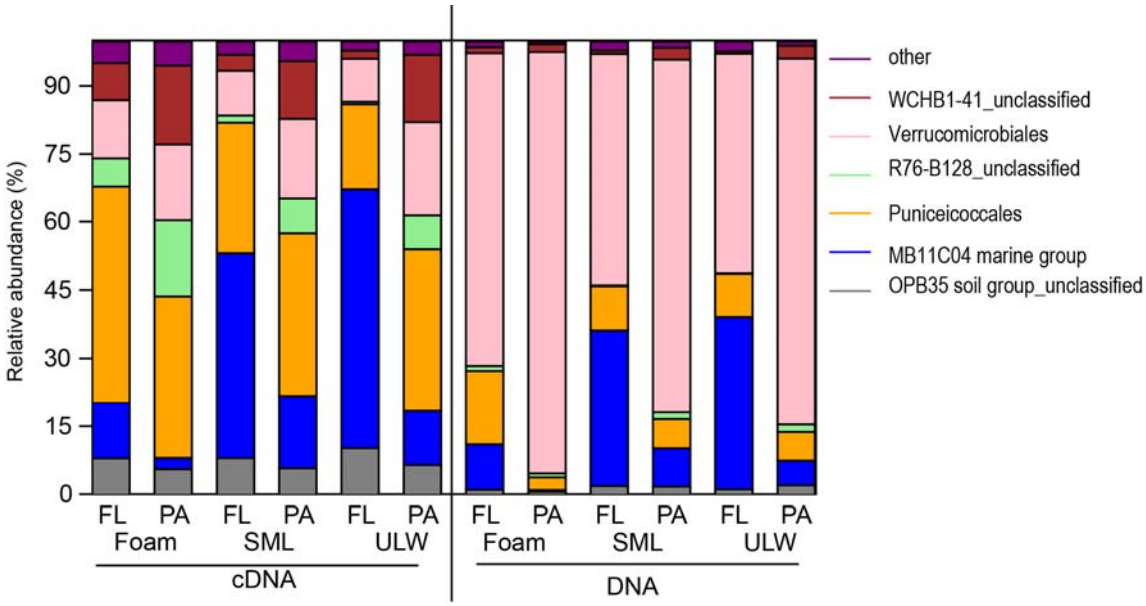


Figure S2: Beta diversity among *Verrucomicrobia* in foam, SML and ULW samples of cDNA and DNA-based operational taxonomic units (OTUs). Each habitat contains further information on free-living (FL) and particle-associated (PA) bacterial community composition.

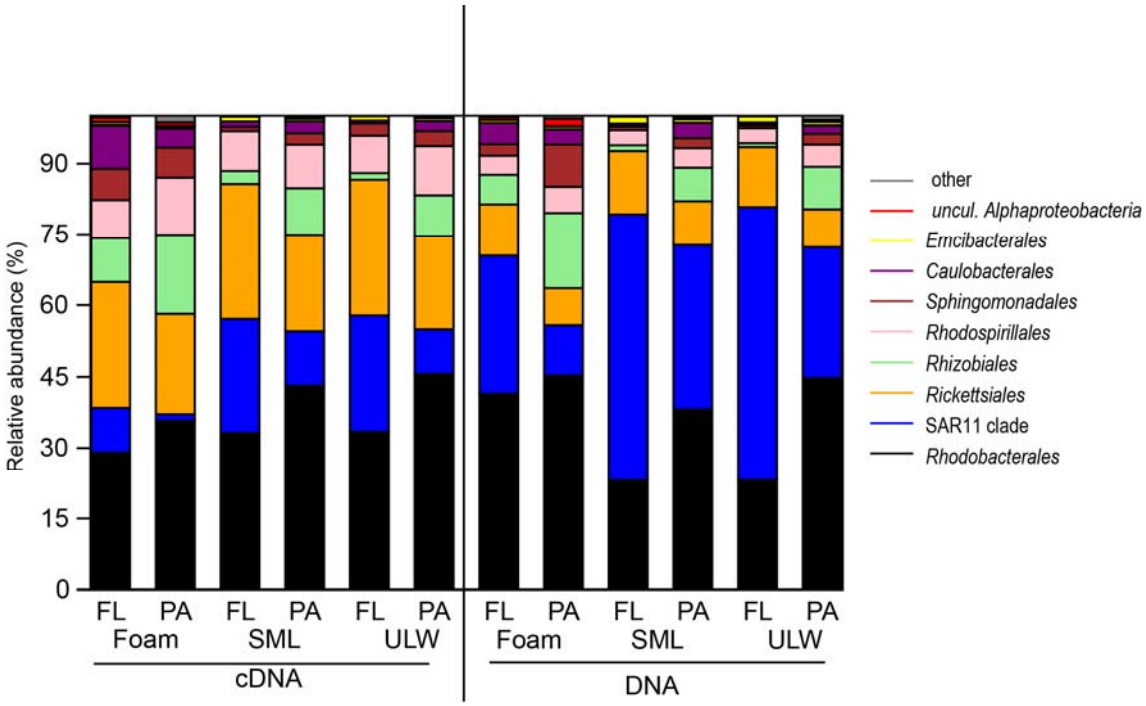


Figure S3: Beta diversity among *Alphaproteobacteria* in foam, SML and ULW samples of cDNA and DNA-based operational taxonomic units (OTUs). Each habitat contains further information on free-living (FL) and particle-associated (PA) bacterial community composition.

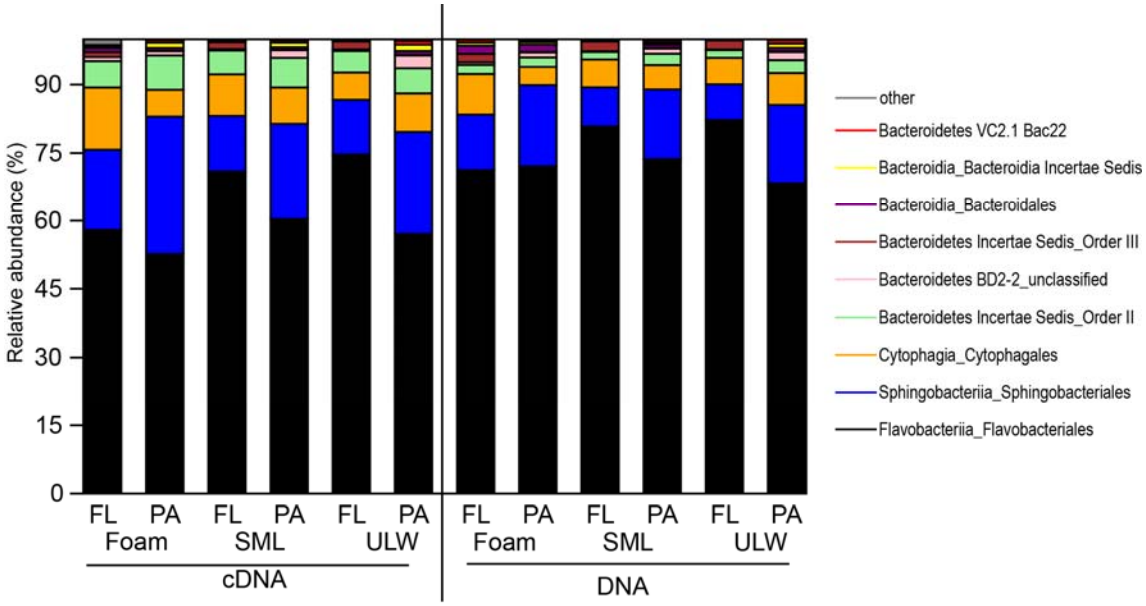


Figure S4: Beta diversity among *Bacteroidetes* in foam, SML and ULW samples of cDNA and DNA-based operational taxonomic units (OTUs). Each habitat contains further information on free-living (FL) and particle-associated (PA) bacterial community composition.

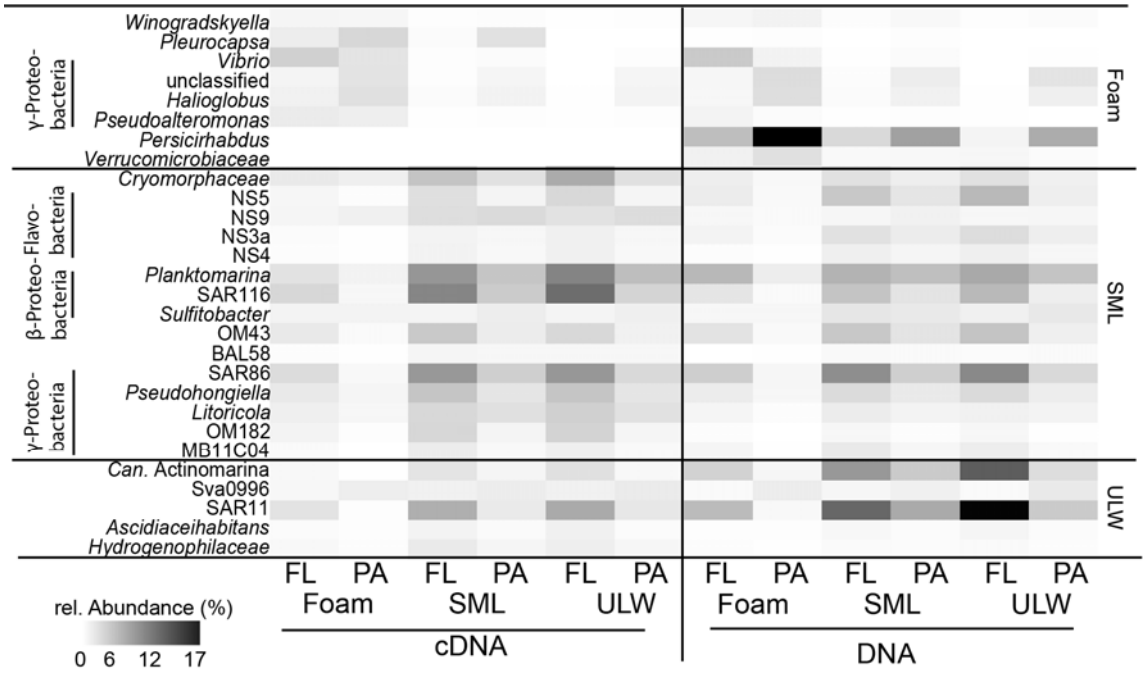


Figure S5: Heat-map showing the relative abundance of most different foam OTUs compared to SML and ULW among free-living (FL) and particle-attached (PA) fractions according to the linear discriminant analysis (LDA) effect size (LEfSe) method.

Table S1: Sampling notes

Sample	Position	Remarks
NS_FO1_210416	53°30.374'N, 08°08.963'E	Foam produced by waves
NS_FO2_210416	53°31.089'N, 08°09.998'E	Foam produced by waves
NS_FO1_190516	53°29.916'N, 08°07.9380'E	Slick-associated, probably phytoplankton exudates
NS_FO3_190516	NA (similar to FO1 190516)	Slick-associated, probably phytoplankton exudates
NS_FO1_190716	53°30.627'N, 08°08.031'E	Close to beach, probably phytoplankton exudates
NS_FO2_190716	53°30.327'N, 08°07.854'E	-
TS_FO_St4_151016	-12°15.49'S, 126°22.36'E	Slick-associated, *Trichodesmium* bloom, little true foam
TS_FO_St5b_171016	-12°15.46'S, 125°58.60'E	Slick-associated foam, *Trichodesmium* bloom, SML and ULW no slick area
TS_FO_St8_191016	-13°41.51'S, 127°31.27'E	*Trichodesmium* bloom, little true foam, sudden rain and squalls during SML and ULW sampling

Table S2: Relative abundance (%) of operational taxonomic units as shown in Figure 4. SML=sea-surface microlayer, ULW=underlying water, PA=particle-attached, FL=free-living

DNA	Foam_PA	Foam_FL	SML_PA	SML_FL	ULW_PA	ULW_FL
>*Gammaproteobacteria*	25.98	22.70	22.85	18.91	24.33	17.05
>*Alphaproteobacteria*	12.31	27.65	24.98	39.18	20.56	41.33
Bacteroidetes	14.26	12.77	16.21	15.85	13.93	15.71
Verrucomicrobia	24.86	9.09	10.23	5.26	9.99	3.28
Actinobacteria	4.51	12.70	7.36	9.92	8.92	13.01
>*Deltaproteobacteria*	5.80	3.76	5.36	1.22	7.63	1.06
>*Betaproteobacteria*	1.04	2.82	2.90	5.30	2.03	4.93
Planctomycetes	3.96	1.54	3.16	0.64	4.87	0.42
Cyanobacteria	0.52	1.10	0.67	0.33	0.55	0.25
Gemmatimonadetes	0.48	0.27	0.47	0.11	0.60	0.08
other	5.50	5.22	5.40	3.00	6.11	2.57
unclassified	0.80	0.39	0.40	0.29	0.48	0.32

cDNA	Foam_PA	Foam_FL	SML_PA	SML_FL	ULW_PA	ULW_FL
>Gammaproteobacteria	34.98	37.40	28.59	27.83	30.14	24.94
>Alphaproteobacteria	16.56	25.00	23.33	33.91	22.31	38.66
Bacteroidetes	18.38	13.64	16.28	16.93	16.52	19.08
Verrucomicrobia	3.08	3.91	3.48	2.85	3.81	1.98
Actinobacteria	1.66	1.94	2.88	3.67	2.77	3.70
>Deltaproteobacteria	7.95	3.64	7.08	1.76	8.95	1.36
>Betaproteobacteria	1.48	3.58	3.14	6.18	2.61	4.75
Planctomycetes	2.27	0.93	1.44	0.45	1.93	0.28
Cyanobacteria	4.79	3.02	4.55	2.31	1.13	1.67
Gemmatimonadetes	2.31	1.35	2.28	1.13	2.21	0.78
other	6.13	5.36	6.38	2.74	7.05	2.32
unclassified	0.41	0.23	0.56	0.24	0.57	0.49

Table S3: Relative abundance (%) of most abundant operational taxonomic units in foam, SML and ULW among free-living (FL) and particle-attached (PA) fractions from Station 8, Timor Sea.

DNA	**Foam_PA**	**Foam_FL**	**SML_PA**	**SML_FL**	**ULW_PA**	**ULW_FL**
Cyanobacteria;Cyanobacteria;SubsectionIII;FamilyI;Trichodesmium;	33.39	2.11	67.96	6.17	23.78	0.02
Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;Synechococcus;	3.36	8.71	0.99	15.69	4.19	21.63
Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;	26.40	18.02	1.83	2.30	3.65	2.06
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium;	5.43	10.23	10.97	2.45	8.93	0.80
Cyanobacteria;Cyanobacteria;SubsectionIII;FamilyI;Oscillatoria;	0.47	0.04	0.46	0.06	26.57	0.00
Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 1;	0.26	1.65	0.03	7.57	0.29	8.58
Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;Prochlorococcus;	0.30	0.93	0.06	8.10	0.48	7.50
Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Saprospira;	4.52	3.36	5.90	0.02	0.99	0.01
Proteobacteria;Gammaproteobacteria;Oceanospirillales;SAR86 clade;	0.29	1.27	0.03	6.05	0.15	6.05
Proteobacteria;Alphaproteobacteria;Rickettsiales;SAR116 clade;	0.22	1.19	0.06	5.11	0.64	6.59

cDNA	**Foam_PA**	**Foam_FL**	**SML_PA**	**SML_FL**	**ULW_PA**
Cyanobacteria;Cyanobacteria;SubsectionIII;FamilyI;Trichodesmium;	47.44	21.71	85.44	38.84	29.08
Cyanobacteria;Cyanobacteria;SubsectionIII;FamilyI;Oscillatoria;	0.65	0.49	0.06	0.36	48.15
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium;	7.96	14.44	9.17	7.20	5.77
Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;Synechococcus;	8.33	6.44	1.26	16.05	1.92
Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;	17.66	12.63	0.16	1.22	0.57

Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Saprospira;	2.88	6.63	2.00	1.37	0.90
Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;Prochlorococcus;	0.14	0.85	0.00	8.45	0.05
Proteobacteria;Alphaproteobacteria;Rickettsiales;SM2D12;	0.67	1.98	0.03	0.93	1.70
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured;	0.22	0.40	0.02	3.08	0.42
Bacteroidetes;Bacteroidetes Incertae Sedis;Order III;Unknown Family;Balneola;	0.56	2.04	0.35	0.50	0.42