**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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**One-sentence summary**

While foams at the oceans' surface have a unique bacterial community signature, they represent a compressed version of the sea-surface microlayer with typical bacterial inhabitants.

**Abstract**

The occurrence of foams at oceans' surfaces is patchy and generally short-lived. However, a detailed understanding of bacterial communities inhabiting foams is lacking. In this study, we investigate if marine foams differ from the sea-surface microlayer (SML), a <1 mm thick layer at the air-sea interface. The comparison of marine foams, SML and underlying water collected from the North Sea and Timor Sea showed that foams were often characterized by highest abundance of small phototrophic and prokaryotic cells as well as highest concentrations of surface-active substances (SAS). Amplicon sequencing based on 16S rRNA revealed a comparable bacterial community in SML and foam. DNA and rRNA based sequence data suggest that *Pseudoalteromonas*, a typical member of the SML, was highly active and thus might enhance foam formation and stability by producing SAS. Although foams contained some specific taxa, our study supports the hypothesis that foam represents a compressed version of the SML. Foam is characterized by increased cell numbers, high SAS concentration, and a significant fraction of overlapping bacteria with SML. Due to the compressed nature of foam and SML compared to the underlying water, bacteria thriving in both surface phenomena have likely implications for biogeochemical cycling and air-sea exchange processes.

## 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, 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 (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, 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 (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 in copious amounts 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) 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 (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 biogeochemical cycles (Engel, *et al.* 2017; Rahlff 2019; Wurl, *et al.* 2017). 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.* 2005a; 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.* 2005b).

Bacteria have a demonstrated role in gas exchange across air-water interfaces. Neustonic methanogens enhanced trace gas flux by active metabolic control in a tank experiment (Upstill-Goddard, *et al.* 2003), and near-interface plankton governed air-sea carbon dioxide anomalies in the subtropical Northeast Atlantic (Calleja, *et al.* 2005). Moreover, the impact of the neuston on biogeochemical cycling can be high due to their 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).

Napolitano and Cicerone (1999) suggested that 1 L of foam water represents 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 (Kuznetsova and Lee 2002; Rahlff, *et al.* 2017a; Tsyban 1971). In addition, during blooms of *Trichodesmium erythraeum*, high concentrations of this species were also found in the corresponding foam (Maynard 1968). However, a thorough characterization of foam microbial community composition compared to the SML and the ULW has never been performed. 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. In this study, we investigated the bacterial community composition of marine foams in direct comparison to SML and ULW. 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 an extreme habitat (Maki 1993) likely comprising many dead or dormant cells, we differentiated between active and abundant bacteria as inferred from a cDNA and DNA-based 16S rRNA amplicon sequencing approach, respectively. Overall, we provide a detailed understanding of the bacterial community composition associated with marine foams in comparison to SML and ULW with likely implications for air-sea exchange processes and biogeochemical cycling.

## Materials & Methods

### Field sample collection

Field sampling was conducted from the bow of a small boat in the Jade Bay, North Sea offshore Wilhelmshaven, Germany (Supplementary Table 1) 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, Supplementary Table 1). Additional samples were collected during a *Trichodesmium* sp. bloom encountered in the Timor Sea (Figure 1B, Supplementary Table 1) in October 2016 during *R/V* Falkor cruise FK161010 as described by Wurl*, et al.* (2018). Foam, SML and ULW samples were collected from each location 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 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 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.

**Concentration of surface-active substances**

The concentration of surface-active substances (SAS) was measured by the voltammetry 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, concentrated samples such as foam samples were diluted with artificial seawater (0.55 M of NaCl solution) to achieve measurements within the linear calibration range. A standard addition technique was used with non-ionic surfactant Triton X-100 (Sigma Aldrich, Taufkirchen, Germany) as a standard. SAS concentration in the samples was measured using

two to three analytical replicates, resulting in relative standard deviations below 6% (Rickard, *et al.* 2019). Concentration of SAS is expressed as the equivalent concentration of the additional Triton X-100 (µg Teq $L^{-1}$).

**Determination of microbial abundance**

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

**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 (particle-associated cells) polycarbonate filters, after which the filtrate was filtered onto 0.2 µm pore size (free-living cells) polycarbonate filters (Merck Millipore, Darmstadt, Germany). 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.* 2017a). Remaining DNA in RNA samples 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 using the primer 1492R (5'-GGTTACCTTGTTACGACTT-3', adapted from Lane (1991)) 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 using Bakt_341F (5'-CCTACGGGNGGCWGCAG-3') and Bakt_805R (5'-GACTACHVGGGTATCTAATCC-3') (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 43,500 reads per sample using the single_rarefraction.py script implemented in QIIME. 3-Venn diagrams were calculated using the webtool http://bioinformatics.psb.ugent.be/webtools/Venn/. We visualized the differences in the bacterial community composition through non-metric multidimensional scaling (NMDS) plots using Bray–Curtis dissimilarity indices based on a genus rank classification. A linear discriminant analysis effect size (LEfSe) analysis was performed to determine bacterial groups which are significantly different between the samples using the 'one against all' strategy for multi-class analysis (Segata, *et al.* 2011). The program LEfSe uses a non-parametric test that combines standard tests for statistical significance with

additional tests encoding biological consistency and effect relevance. $P<0.05$ was regarded as statistical significance.

Differences in the total number of OTUs between habitats, nucleic acid types and attachment status were statistically analyzed using a Kruskal-Wallis test and *post hoc* multiple pairwise comparisons based on Dunn's z statistic approximations to the actual rank statistics within the package "dunn.test" (Dinno and Dinno 2017) in R version 3.4.3 (Team 2017). The null hypothesis was rejected if $p \leq 0.05$. Comparisons were made between free-living and particle-associated status and between nucleic acids templates within a habitat (foam, SML, ULW) and between habitats for each DNA and cDNA, respectively. For the same samples, comparisons on phylum-based differences were statistically investigated using a one-way Analysis of Similarities (ANOSIM) based on Bray Curtis dissimilarity and 9999 permutations, Bonferroni correction and a significance level of 95%.

## Results

### Foams are enriched with surface-active substances and microorganisms

Overall, foams from both sampling areas (North Sea and Timor Sea), were enriched with prokaryotic microorganisms, small phototrophs, and SAS (Table 1). Cell counts of prokaryotic microorganisms ranged between 2.63 - 46.2 x $10^6$, 9.83 - 45.7 x $10^5$, and 1.01 - 3.71 x $10^6$ cells $mL^{-1}$ 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 North Sea and Timor Sea, respectively (Table 1). 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 phototrophs, 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 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 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 and depleted compared to ULW, their concentration in foams compared to ULW was typically enriched by three orders of magnitude (EF ranging from 5 to 665).

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

We analyzed the bacterial community composition of all North Sea samples to compare the diversity between foam, SML, and ULW. We furthermore differentiated the attachment status of bacteria between particle-associated and free-living as well as the community composition between present (based on DNA) and active (based on cDNA) OTUs (Figure 3A).

Analyses revealed overall higher diversity (numbers of OTUs) in cDNA-based communities (reflecting active taxa, median=786.5) compared to DNA-based communities (reflecting present taxa, median=571). Differences between cDNA and DNA were significant for the foam free-living bacterial fraction (Dunn's test, $p$=0.037) and for the SML particle-associated fraction ($p$=0.044).

In DNA-derived samples, the mean number of foam OTUs was significantly increased for particle-associated over free-living communities (Dunn's test, $p$=0.0031), and also

significantly higher compared to the SML and ULW particle-associated fraction (Figure 3A) with $p$=0.010 and 0.015, respectively. OTU numbers derived from cDNA were weakly significantly higher in particle-associated samples of foam ($p$=0.037) and SML ($p$=0.042) compared to the respective free-living samples (Figure 3A). The Shannon-Wiener index (Supplementary Figure 2), which accounts for both abundance and evenness of OTUs, confirmed the abovementioned trends reflected by the total number of OTUs.

When considering the presence of OTUs from pooled North Sea samples, foam, SML and ULW shared a high number of OTUs (953-1206; Figure 3B, Supplementary Figure 3), while foams and SML always had more OTUs in common compared to foam-ULW and ULW-SML (Figure 3, Supplementary Figure 3). In the particle-attached fraction, foam had the highest fraction of specific OTUs (220) and also shared many with the SML (179). In addition, SML and foam shared many OTUs in the free-living fraction (253) with less specific OTUs in the foam (56) and the SML (34) compared to the particle-associated fraction.

**Bacterial community composition of North Sea foams**

Non-metric multidimensional scaling plots comparing the bacterial community composition based on the abundance of OTUs, revealed that the foam bacterial community composition was distinct from SML and ULW communities, irrespective of differentiating cDNA and DNA, or free-living and particle-associated bacterial communities (Figure 4). In contrast to this, SML and ULW bacterial community composition were more similar to each other as shown by the clustering (Figure 4).

On a phylum-level, DNA-based community analyses showed that for all three habitats *Gammaproteobacteria*, *Verrucomicrobia* and *Cyanobacteria* formed a higher portion of particle-associated than free-living communities (Figure 5). In contrast, *Alphaproteobacteria* and *Actinobacteria* were more abundant in the free-living fraction (Figure 5, Supplementary Table 2). ANOSIM revealed significant differences between foam and SML ($p$=0.012), and

between foam and ULW ($p$=0.030). Differences between cDNA and DNA-derived bacteria were only minor, suggesting that the abundant phyla were also active. *Gammaproteobacteria*, as a single exception, showed high relative abundance in the cDNA-based community composition in foam (37.4% and 35.0% of free-living and particle-associated OTUs, respectively) compared to the DNA-based community composition (22.7% and 26.0% of free-living and particle-associated OTUs, respectively). The cDNA-based communities of foam contained less *Alphaproteobacteria* but more *Gammaproteobacteria* compared to SML and ULW communities (Figure 5).

On OTU level, differences based on cDNA-derived were significant between foam and SML (Pairwise ANOSIM, $p$=0.001) as well as on foam and ULW ($p$=0.008). MB11C04 marine group (*Verrucomicrobia*), SAR11 clade (*Alphaproteobacteria*) and *Oceanospirillales* (*Gammaproteobacteria*) were more abundant in ULW and SML compared to foam. (Supplementary Figures 4, 5, 6). A higher relative abundance of active OTUs in foam compared to SML and ULW was found among the *Puniceicoccales* (*Verrucomicrobia*), *Sphingomonadales* (*Alphaproteobacteria*), *Alteromonadales* and *Vibrionales* (both *Gammaproteobacteria*) (Supplementary Figure 4, 5, 6). Active free-living OTUs belonging to the order *Flavobacteriales* and *Oceanospirilliales* were more – whereas free-living *Sphingobacteriales* were less numerous than their particle-associated counterparts in all three habitats (Supplementary Figures 4 & 7).

Apart from the order *Rhodobacterales* (Supplementary Figure 6), foam generally had less alphaproteobacterial DNA-based OTUs compared to SML and ULW. However, foam contained a higher DNA-based relative abundance of *Verrucomicrobia* and *Gammaproteobacteria* (Figure 5). Among the *Gammaproteobacteria*, especially more OTUs of the orders *Cellvibrionales, Vibrionales, Legionellales, Alteromonadales* were increasingly detected in foam compared to the SML and ULW, whereas the order *Oceanospirilliales* was more depleted in foam (Supplementary Figure 4).

**Foam-enriched bacteria**

Using the linear discriminant analysis effect size (LefSe) method we could identify OTUs that were enriched in foam compared to SML and ULW (Figure 6). The analysis does not refer to the most abundant OTUs in terms of absolute numbers but points out the largest differences between foam and the other two habitats. Members of the *Gammaproteobacteria* were typical active and abundant foam colonizers (Figure 6). Taxa including *Winogradskyella*, *Vibrio*, *Halioglobus* and *Pseudoaltermonas* were particularly abundant in both cDNA and DNA-derived foam samples as well as when compared to SML and ULW habitats. *Persicirhabdus* and other *Verrucomicrobiaceae* were typical foam-dwellers with 11% and 7% relative abundance according to their presence in DNA samples but with low activity according to cDNA samples. Typical SML populating bacteria belonged to taxa, which were phylogenetically related to *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteria*. Strikingly, abundance and activity profiles of foam specific OTUs often indicated a decreasing gradient from foam to SML to ULW (Supplementary Figure 1). High relative abundances (>5%) of *Planktomarina*, SAR116 and SAR86 were found for cDNA and DNA SML samples. Microbial taxa of SAR11 and Candidatus *Actinomarina* typically occurred in high abundances in the ULW.

***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 DNA-based community in foam we found most particle-associated OTUs assigned to *Trichodesmium* (relative abundance=33.4%), *Alteromonas* (26.4%) and *Rhodobium* (5.4%), whereas free-living OTUs were mostly assigned to *Alteromonas* (18.0%) and *Rhodobium* (10.2%) (Supplementary Table 3). Particle-

associated OTUs were mainly assigned to *Trichodesmium* (68%) and *Rhodobium* (10.9%) in the SML, and to *Trichodesmium* (23.8%) and *Oscillatoria* (26.7%) in the ULW. Bacteria of the genus *Saprospira* were also detected in foam and SML, mainly in the particle-associated fractions. Most free-living OTUs from SML and ULW were assigned to *Synechococcus* with 15.7% and 21.6% relative abundance, respectively. In all cDNA samples, *Trichodesmium* was also the most abundant among active OTUs in foam and SML, only in the ULW *Oscillatoria* (48.2%) had higher relative abundance compared to *Trichodesmium* (29.1%). The relative abundance of cDNA-based OTUs assigned to *Alteromonas* in foams (particle-associated: 17.8%, free-living: 12.6%) was comparatively enhanced to the SML (particle-associated: 0.2%, free-living: 1.2%). The ten most abundant OTUs found in the three habitats are shown in Supplementary Table 3.

## Discussion

### Foams comprise an extreme form of SML

Foams are peculiar but understudied microbial habitats at the air-sea interface. Foam is derived from the SML and bursting bubbles of the ULW, and as soon as foams subside, their material becomes part of the SML (Kuznetsova and Lee 2002). As expected for an extreme form of the SML, we found high concentrations of SAS in foams, and an enrichment of phototrophic and prokaryotic cells, matching previous observations (Kuznetsova and Lee 2002; Rahlff*, et al.* 2017a; Robinson*, et al.* 2019). Foams from presumptive phytoplankton exudates (Supplementary Table 1), contained high loads of SAS (Figure 2C) and were also linked to higher amounts of microbes (Figure 2A&B) compared to foams formed by convergence of surface water (Figure 1C, Supplementary Table 1). This indicates that foams originating from photoautotrophic biomass contain substantial amounts of labile organic matter. 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).

The community composition analyses showed that *Gammaproteobacteria* were highly abundant in foams. Nevertheless, they were also present to a lesser extent in the SML and in the ULW (Figure 6, upper panel). Foams and SML often had more OTUs in common compared to foam-ULW and ULW-SML (Figure 3B). Considerable overlap between SML and foam bacterial community was found among the particle-attached bacteria and free-living fraction. The clear overlap between SML and foam OTUs supports our conclusion that foams represent a compressed version of the SML. However, this is based on a subsample of reads, and by increasing the sequencing depth the similarity between the habitats could become more similar. Despite this similarity, foams contained also specific bacteria according to the LefSe analysis (Figure 6, upper panel) that are significantly less abundant in the other two habitats, including *Vibrio*, *Pseudoalteromonas,* and *Halioglobus.* The significant differences in abundance caused also that foam and SML are different in the NMDS analysis (Figure 4). Interestingly, the 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. As a consequence of the high organic load in foams, some passively transferred, fast-growing, opportunistic bacteria, like many *Gammaproteobacteria* (Figure 6, upper panel), could benefit and thrive within foam islands as they usually do on algal-derived organic matter (Teeling*, et al.* 2012; von Scheibner*, et al.* 2018). An OTU assigned to *Alteromonas* was the most abundant free-living bacterium in the *Trichodesmium*-associated foam (Supplementary Table 3), and a high relative abundance among the active OTUs was found in foam compared to SML in both free-living and particle-associated fractions. Commonly found OTUs of oceanic pelagic clusters of *Planktomarina*, SAR116 and SAR86 were found in ULW, SML and foam with decreasing relative abundance (Figure 6, Supplementary Figure 8), leading to the assumption that cells of

these abundant and free-living groups were passively transferred to the sticky SML and foam. Members of these clusters carrying streamlined genomes specialized on the uptake of monomers, are metabolically active and are able the use light energy as additional energy sources (Dupont, *et al.* 2012; Giebel, *et al.* 2011; Lee, *et al.* 2019; Voget, *et al.* 2015). Certain bacteria that were particularly active in foams, e.g., *Alteromonas* sp., were previously shown to be highly abundant and active degraders of alginate, a cell wall component from marine macroalgae (Mitulla, *et al.* 2016), and of labile dissolved organic carbon (Pedler, *et al.* 2014). By using 16S rRNA gene sequencing of individual transparent exopolymer particles from the SML, Zäncker, *et al.* (2019) found that *Alteromonadaceae* on those particles was significantly increased compared to subsurface water. Though being attached to particles might have some drawbacks for bacteria when it comes to grazing (Albright, *et al.* 1987), this might not be necessarily true for the SML, or this disadvantage is easily outweighed by the benefits of particles providing organic material and shelter for extreme levels of UV and solar radiation. In this regard, foam bacteria could be important key players in biogeochemical cycling, jump-starting the microbial loop from the air-sea interface.

**The role of particles for foam-populating bacteria**

Early work showed that foams from various aquatic habitats contained large numbers of benthic or symbiotic bacteria of diatoms rather than free living bacteria (Maynard 1968). The authors concluded that foams form ideal substrates for attached colonization. Our study showed that SML and foams shared most OTUs although in different abundances (Figure 3B). This provides evidence for our hypothesis that foams represent compressed SML, since particulate organic matter is frequently enriched in both the SML (Aller, *et al.* 2005) and foams (Johnson, *et al.* 1989) compared to the ULW. Particle-associated and free-living bacteria are well known to form separate communities in many aquatic environments (Crespo, *et al.* 2013; Crump, *et al.* 1999), and they also formed distinctive communities in all of the

here studied habitats (Figure 4). SML bacteria are more attached to substrates than occurring in the free-living state (Cunliffe*, et al.* 2009), and particle-associated bacteria of the SML are generally more prone to changes in community composition than free-living ones (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 particle-associated lifestyle independent of the habitat under investigation.

The LefSe analysis revealed that *Winogradskyella* was particularly abundant in the cDNA fraction, reflecting active OTUs. Previous work has shown that *Winogradskyella* is often associated with other species such as brown algae or sponges (Park and Yoon 2013; Schellenberg*, et al.* 2017; Yoon and Lee 2012). As broken algal cells and detritus are major parts of foams, high relative abundance of *Winogradskyella* in the foam particle-associated fraction (Supplementary Figure 8) might be due to its attachment to algal-derived particles. Freitas*, et al.* (2012) suggested that *Verrucomicrobia* show particle attachment rather than occurring in the free-living form, and we found that one of its members, *Persicirhabdus,* was particularly abundant in foam-derived DNA samples (Figure 6). *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 capacities (Cardman*, et al.* 2014; Yoon and Lee 2012) and, hence, might prefer to stick to organic materials feeding them. We also found *Saprospira* sp. on *Trichodesmium*-associated foam and the particle fraction of the SML. Bacteria of the *Saprospiraceae* might be of ecological relevance for interfacial habitats as they are well-known producers of sticky substances such as acidic polysaccharides enhancing aggregate formation (Furusawa*, et al.* 2015).

**Biogenic foam formation and bacterial activity**

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 the local community when reaching beaches (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 (Eberlein*, et al.* 1985; Velimirov 1980; Wegner and Hamburger 2002). 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 *Ecklonia maxima* while bacterial growth was antibiotically inhibited and claimed that at least one algal component should be present for the production of stable foam. 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). Our foam samples contained bacterial OTUs, which are likely capable of producing SAS, as previously demonstrated for the genus *Vibrio* and *Pseudoalteromonas* (Dang*, et al.* 2016; Hu*, et al.* 2015). Foam formation by bacterial SAS production is theoretically possible (Heard*, et al.* 2008). However, if the foam-associated bacteria from our study have the potential to enhance foam formation and stability in the absence of a major SAS-producing algae, requires further experiments.

We observed a higher alpha diversity within the cDNA-based compared to the respective DNA-based bacterial community (significant for foam free-living and SML particle-associated bacteria), suggesting that a higher diversity of bacteria being enriched in foams were most likely active. Sequencing of cDNA-derived amplicons of small subunit RNA gives a rough estimate for assessing activity of bacterial taxa because cellular rRNA concentration is linked to cell growth and activity (Lanzén*, et al.* 2011; Poulsen*, et al.* 1993; Schaechter*, et al.* 1958).

Neustonic inhabitants often have to endure prolonged exposure to the cell-inhibitory effects of high solar and UV radiation (Santos*, et al.* 2013) and wind-wave dynamics (Stolle*, et al.* 2011). However, long interfacial residence times of microbes are neither expected for those attached to foams being highly ephemeral (Pugh 1996) nor for those in the SML, where bacterial enrichment declines at wind speeds around 4-5 m $s^{-1}$ (Rahlff*, et al.* 2017a). Some bacteria, such as *Trichodesmium* sp. show high tolerance towards photoinhibition at 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 (Supplementary Table 3). Paerl and Ustach (1982) suggested that surface scum formation by cyanobacteria using intracellular gas vacuole formation could be an ecological strategy to reach atmospheric carbon dioxide supplies at the air-sea interface. This mechanism may be also applied to $CO_2$-depleted *Trichodesmium* sp.*,* that also uses gas vesicles for floating (Walsby 1992).
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.* 2017b) to incubate water from 5 m depth supplemented with 1 mL *Trichodesmium* foam and found complete oxygen depletion after less than 14 hours, while samples without foam showed incomplete $O_2$ consumption (Rahlff*, et al.* 2017b). We assume that complete $O_2$ depletion was attributable to highly active bacteria associated with the foam, but future work is definitively required to demonstrate the activity profile of foam bacteria.

Overall, our study supports the hypothesis that foams are essentially compressed SML. In addition to high loads of microbial cells and SAS in foams compared to the SML, we also found a large overlap between foam and SML OTUs. Among those we identified specific foam bacteria including *Vibrio*, *Winogradskyella* and *Pseudoalteromonas* with significant

higher abundances in foam. While attached to rising bubbles, bacteria might benefit from SML and foams as a nutrient-rich “rest stop” before being transferred to sea-spray aerosols and clouds, dispersed to land or returned to bulk water. The SML spans 71% 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 and biogeochemical cycles.

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**Conflict of interests:**

The authors declare no conflict of interests.

## References


Agogué H, Casamayor EO, Bourrain M *et al.* A survey on bacteria inhabiting the sea surface microlayer of coastal ecosystems. *FEMS Microbiol Ecol* 2005a;**54**: 269-80.

Agogué H, Joux F, Obernosterer I *et al.* Resistance of marine bacterioneuston to solar radiation. *Appl Environ Microbiol* 2005b;**71**: 5282-9.

Albright LJ, Sherr EB, Sherr BF *et al.* Grazing of ciliated protozoa on free and particle-attached bacteria. *Mar Ecol Prog Ser* 1987;**38**: 125-9.

Aller JY, Kuznetsova MR, Jahns CJ *et al.* The sea surface microlayer as a source of viral and bacterial enrichment in marine aerosols. *J Aerosol Sci* 2005;**36**: 801-12.

Anguelova MD, Webster F. Whitecap coverage from satellite measurements: A first step toward modeling the variability of oceanic whitecaps. *J Geophys Res-Oceans* 2006;**111**.

Armonies W. Occurrence of meiofauna in *Phaeocystis* seafoam. *Mar Ecol Prog Ser* 1989;**53**: 305-9.

Bärlocher F, Gordon J, Ireland RJ. Organic composition of seafoam and its digestion by *Corophium volutator* (Pallas). *J Exp Mar Biol Ecol* 1988;**115**: 179-86.

Bigg EK, Leck C, Tranvik L. Particulates of the surface microlayer of open water in the central Arctic Ocean in summer. *Mar Chem* 2004;**91**: 131-41.

Calleja ML, Duarte CM, Navarro N *et al.* Control of air-sea $CO_2$ disequilibria in the subtropical NE Atlantic by planktonic metabolism under the ocean skin. *Geophys Res Lett* 2005;**32**.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;**7**: 335-6.

Cardman Z, Arnosti C, Durbin A *et al. Verrucomicrobia* are candidates for polysaccharide-degrading bacterioplankton in an arctic fjord of Svalbard. *Appl Environ Microbiol* 2014;**80**: 3749-56.

Carlson DJ. A field evaluation of plate and screen microlayer sampling techniques. *Mar Chem* 1982;**11**: 189-208.

Castilla JC, Manriquez PH, Delgado AP *et al.* Bio-foam enhances larval retention in a free-spawning marine tunicate. *P Natl Acad Sci USA* 2007;**104**: 18120-2.

Ćosović B, Vojvodić V. Voltammetric analysis of surface active substances in natural seawater. *Electroanalysis* 1998;**10**: 429-34.

Craig D, Ireland RJ, Bärlocher F. Seasonal variation in the organic composition of seafoam. *J Exp Mar Biol Ecol* 1989;**130**: 71-80.

Crespo BG, Pommier T, Fernandez-Gomez B *et al.* Taxonomic composition of the particle-attached and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA. *Microbiologyopen* 2013;**2**: 541-52.

Crump BC, Armbrust EV, Baross JA. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. *Appl Environ Microbiol* 1999;**65**: 3192-204.

Cunliffe M, Engel A, Frka S *et al.* Sea surface microlayers: A unified physicochemical and biological perspective of the air–ocean interface. *Progr Oceanogr* 2013;**109**: 104-16.

Cunliffe M, Whiteley AS, Newbold L *et al.* Comparison of bacterioneuston and bacterioplankton dynamics during a phytoplankton bloom in a fjord mesocosm. *Appl Environ Microbiol* 2009;**75**: 7173-81.

Dang NP, Landfald B, Willassen NP. Biological surface-active compounds from marine bacteria. *Environ Technol* 2016;**37**: 1151-8.

Dinno A, Dinno MA. Package ‘dunn. test’, 2017.

Druzhkov NV, Makarevich PR, Bardan SI. Sea foam as an object of sea-surface film studies. *Polar Res* 1997;**16**: 117-21.

Dupont CL, Rusch DB, Yooseph S *et al.* Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. *ISME J* 2012;**6**: 1186-99.

Eberlein K, Leal MT, Hammer KD *et al.* Dissolved organic-substances during a *Phaeocystis pouchetii* bloom in the German Bight (North-Sea). *Mar Biol* 1985;**89**: 311-6.

Eisenreich SJ, Elzerman AW, Armstrong DE. Enrichment of micronutrients, heavy metals, and chlorinated hydrocarbons in wind-generated lake foam. *Environ Sci Technol* 1978;**12**: 413-7.

Engel A, Bange HW, Cunliffe M *et al.* The ocean's vital skin: Toward an integrated understanding of the sea surface microlayer. *Front Mar Sci* 2017;**4**.

Freitas S, Hatosy S, Fuhrman JA *et al.* Global distribution and diversity of marine *Verrucomicrobia*. *ISME J* 2012;**6**: 1499-505.

Frew N. The role of organic films in air-sea gas exchange. In: Liss PSD, R.A. (ed.) *The sea surface and global change*. Cambridge, UK: Cambridge University Press, 1997.

Frew NM, Goldman JC, Dennett MR *et al.* Impact of phytoplankton-generated surfactants on air-sea gas exchange. *J Geophys Res-Oceans* 1990;**95**: 3337-52.

Furusawa G, Hartzell PL, Navaratnam V. Calcium is required for ixotrophy of *Aureispira* sp. CCB-QB1. *Microbiology* 2015;**161**: 1933-41.

Giebel HA, Kalhoefer D, Lemke A *et al.* Distribution of *Roseobacter* RCA and SAR11 lineages in the North Sea and characteristics of an abundant RCA isolate. *ISME J* 2011;**5**: 8-19.

Giebel HA, Wolterink M, Brinkhoff T *et al.* Complementary energy acquisition via aerobic anoxygenic photosynthesis and carbon monoxide oxidation by *Planktomarina temperata* of the *Roseobacter* group. *FEMS Microbiol Ecol* 2019;**95**: fiz050.

Glöckner FO, Yilmaz P, Quast C *et al.* 25 years of serving the community with ribosomal RNA gene reference databases and tools. *J Biotechnol* 2017;**261**: 169-76.

Gobalakrishnan R, Sivakumar K, Sethubathi GV *et al.* Seafoam bacteria from the Havelock island of the Andamans, India. *Indian J Geo-Mar Sci* 2014;**43**: 1562-5.

Hardy JT. The sea surface microlayer: Biology, chemistry and anthropogenic enrichment. *Progr Oceanogr* 1982;**11**: 307-28.

Harold E, Schlichting JR. A preliminary study of the algae and protozoa in seafoam. *Bot Mar* 1971;**14**: 24-8.

Harvey GW, Burzell LA. A simple microlayer method for small samples *Limnol Oceanogr* 1972;**17**: 156-7.

Heard J, Harvey E, Johnson BB *et al.* The effect of filamentous bacteria on foam production and stability. *Colloids Surf B Biointerfaces* 2008;**63**: 21-6.

Herlemann DPR, Labrenz M, Jurgens K *et al.* Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* 2011;**5**: 1571-9.

Hu X, Wang C, Wang P. Optimization and characterization of biosurfactant production from marine *Vibrio* sp. strain 3B-2. *Front Microbiol* 2015;**6**: 976.

Hunter TN, Pugh RJ, Franks GV *et al.* The role of particles in stabilising foams and emulsions. *Adv Colloid Interface Sci* 2008;**137**: 57-81.

Jenkinson IR, Laurent S, Ding H *et al.* Biological modification of mechanical properties of the sea surface microlayer, influencing waves, ripples, foam and air-sea fluxes. *Elem Sci Anth* 2018;**6**.

Johnson BD, Zhou X, Parrish CC *et al.* Fractionation of particulate matter, the trace metals Cu, Cd, and Zn, and lipids in foam and water below Niagara Falls. *J Great Lakes Res* 1989;**15**: 189-96.

Kesaulya I, Leterme SC, Mitchell JG *et al.* The impact of turbulence and phytoplankton dynamics on foam formation, seawater viscosity and chlorophyll concentration in the eastern English Channel. *Oceanologia* 2008;**50**: 167-82.

Kuznetsova M, Lee C. Dissolved free and combined amino acids in nearshore seawater, sea surface microlayers and foams: Influence of extracellular hydrolysis. *Aquat Sci* 2002;**64**: 252-68.

Lane D. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds.) *Nucleic Acid Techniques in Bacterial Systematics*. Chichester, UK: John Wiley & Sons, 1991, 115-75.

Lanzén A, Jørgensen SL, Bengtsson MM *et al.* Exploring the composition and diversity of microbial communities at the Jan Mayen hydrothermal vent field using RNA and DNA. *FEMS Microbiol Ecol* 2011;**77**: 577-89.

Leck C, Bigg EK. Biogenic particles in the surface microlayer and overlaying atmosphere in the central Arctic Ocean during summer. *Tellus B Chem Phys Meteorol* 2017;**57**: 305-16.

Lee J, Kwon KK, Lim SI *et al.* Isolation, cultivation, and genome analysis of proteorhodopsin-containing SAR116-clade strain Candidatus *Puniceispirillum marinum* IMCC1322. *J Microbiol* 2019;**57**: 676-87.

Maki J. The air-water interface as an extreme environment. In: Ford T (ed.) *Aquatic Microbiology: An Ecological Approach*. Boston, MA: Blackwell Scientific Publication, 1993, 409-39.

Marie D, Simon N, Guillou L *et al.* Flow Cytometry Analysis of Marine Picoplankton. In: Diamond RA, Demaggio S (eds.) *In Living Color Springer Lab Manuals*, DOI 10.1007/978-3-642-57049-0_34. Berlin, Heidelberg: Springer, 2000, 421-54.

Maynard NG. Aquatic foams as an ecological habitat. *Zeitschrift für allgemeine Mikrobiologie* 1968;**8**: 119-26.

Mitulla M, Dinasquet J, Guillemette R *et al.* Response of bacterial communities from California coastal waters to alginate particles and an alginolytic *Alteromonas macleodii* strain. *Environ Microbiol* 2016;**18**: 4369-77.

Napolitano GE, Cicerone DS. Lipids in water-surface microlayers and foams*Lipids in Freshwater Ecosystems*, DOI 10.1007/978-1-4612-0547-0: Springer, 1999, 235-62.

Oberbeckmann S, Osborn AM, Duhaime MB. Microbes on a bottle: Substrate, season and geography influence community composition of microbes colonizing marine plastic debris. *Plos One* 2016;**11**: e0159289.

Obernosterer I, Catala P, Reinthaler T *et al.* Enhanced heterotrophic activity in the surface microlayer of the Mediterranean Sea. *Aquat Microb Ecol* 2005;**39**: 293-302.

Paerl HW, Ustach JF. Blue-green algal scums: An explanation for their occurrence during freshwater blooms. *Limnol Oceanogr* 1982;**27**: 212-7.

Park S, Yoon JH. *Winogradskyella undariae* sp. nov., a member of the family *Flavobacteriaceae* isolated from a brown algae reservoir. *Antonie Van Leeuwenhoek* 2013;**104**: 619-26.

Parveen B, Mary I, Vellet A *et al.* Temporal dynamics and phylogenetic diversity of free-living and particle-associated Verrucomicrobia communities in relation to environmental variables in a mesotrophic lake. *FEMS Microbiol Ecol* 2013;**83**: 189-201.

Pedler BE, Aluwihare LI, Azam F. Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. *Proc Natl Acad Sci U S A* 2014;**111**: 7202-7.

Pereira R, Ashton I, Sabbaghzadeh B *et al.* Reduced air–sea $CO_2$ exchange in the Atlantic Ocean due to biological surfactants. *Nat Geosci* 2018;**11**: 492-6.

Poulsen LK, Ballard G, Stahl DA. Use of rRNA fluorescence in situ hybridization for measuring the activity of single cells in young and established biofilms. *Appl Environ Microbiol* 1993;**59**: 1354-60.

Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;**28**: 1823-9.

Pruesse E, Quast C, Knittel K *et al.* SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* 2007;**35**: 7188-96.

Pugh RJ. Foaming, foam films, antifoaming and defoaming. *Adv Colloid Interface Sci* 1996;**64**: 67-142.

Rahlff J. The virioneuston: A review on viral-bacterial associations at air-water interfaces. *Viruses-Basel* 2019;**11**: 191.

Rahlff J, Stolle C, Giebel HA *et al.* High wind speeds prevent formation of a distinct bacterioneuston community in the sea-surface microlayer. *FEMS Microbiol Ecol* 2017a;**93**.

Rahlff J, Stolle C, Wurl O. SISI: A new device for in situ incubations at the ocean surface. *J Mar Sci Eng* 2017b;**5**: 46.

Ram ASP, Mari X, Brune J *et al.* Bacterial-viral interactions in the sea surface microlayer of a black carbon-dominated tropical coastal ecosystem (Halong Bay, Vietnam). *Elementa-Sci Anthrop* 2018;**6**: 13.

Reinthaler T, Sintes E, Herndl GJ. Dissolved organic matter and bacterial production and respiration in the sea-surface microlayer of the open Atlantic and the western Mediterranean Sea. *Limnol Oceanogr* 2008;**53**: 122-36.

Ribas-Ribas M, Helleis F, Rahlff J *et al.* Air-sea $CO_2$-exchange in a large annular wind-wave tank and the effects of surfactants. *Front Mar Sci* 2018;**5**: 457.
Rickard PC, Uher G, Upstill-Goddard RC *et al.* Reconsideration of seawater surfactant activity analysis based on an inter-laboratory comparison study. *Mar Chem* 2019;**208**: 103-11.
Riebesell U. Aggregation of *Phaeocystis* during phytoplankton spring blooms in the southern North-Sea. *Mar Ecol Prog Ser* 1993;**96**: 281-9.
Rieck A, Herlemann DP, Jürgens K *et al.* Particle-associated differ from free-living bacteria in surface waters of the Baltic Sea. *Front Microbiol* 2015;**6**: 1297.
Robinson TB, Wurl O, Bahlmann E *et al.* Rising bubbles enhance the gelatinous nature of the air–sea interface. *Limnol Oceanogr* 2019;**64**: 2358-72.
Santos L, Santos AL, Coelho FJ *et al.* Heterotrophic activities of neustonic and planktonic bacterial communities in an estuarine environment (Ria de Aveiro). *J Plankton Res* 2013;**36**: 230-42.
Satpute SK, Banat IM, Dhakephalkar PK *et al.* Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. *Biotechnol Adv* 2010;**28**: 436-50.
Schaechter M, Maaløe O, Kjeldgaard NO. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. *Microbiology* 1958;**19**: 592-606.
Schellenberg J, Busse HJ, Hardt M *et al. Winogradskyella haliclonae* sp. nov., isolated from a marine sponge of the genus *Haliclona*. *Int J Syst Evol Microbiol* 2017;**67**: 4902-10.
Schilling K, Zessner M. Foam in the aquatic environment. *Water Res* 2011;**45**: 4355-66.
Scully EP. Utilization of surface foam as a food source by the Hermit crab, *Pagurus longicarpus* say, 1817. *Mar Behav Physiol* 2009;**5**: 159-62.
Segata N, Izard J, Waldron L *et al.* Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;**12**: R60.
Sieburth JM, Conover JT. Slicks associated with *Trichodesmium* blooms in the Sargasso Sea. *Nature* 1965;**205**: 830-1.
Sieburth JM, Willis PJ, Johnson KM *et al.* Dissolved organic matter and heterotrophic microneuston in the surface microlayers of the north atlantic. *Science* 1976;**194**: 1415-8.
Stefani F, Salerno F, Copetti D *et al.* Endogenous origin of foams in lakes: a long-term analysis for Lake Maggiore (northern Italy). *Hydrobiologia* 2016;**767**: 249-65.
Stolle C, Labrenz M, Meeske C *et al.* Bacterioneuston community structure in the southern Baltic sea and its dependence on meteorological conditions. *Appl Environ Microbiol* 2011;**77**: 3726-33.
Stolle C, Nagel K, Labrenz M *et al.* Succession of the sea-surface microlayer in the coastal Baltic Sea under natural and experimentally induced low-wind conditions. *Biogeosciences* 2010;**7**: 2975-88.
Team RC. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria* 2017.
Teeling H, Fuchs BM, Becher D *et al.* Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 2012;**336**: 608-11.
Thornton DCO. Phytoplankton mucilage production in coastal waters: a dispersal mechanism in a front dominated system? *Ethol Ecol Evol* 1999;**11**: 179-85.
Tsyban A. Marine bacterioneuston. *Journal of the Oceanographical Society of Japan* 1971;**27**: 56-66.
Upstill-Goddard RC, Frost T, Henry GR *et al.* Bacterioneuston control of air-water methane exchange determined with a laboratory gas exchange tank. *Global Biogeochem Cy* 2003;**17**.
Velimirov B. Formation and potential trophic significance of marine foam near kelp beds in the Benguela upwelling system. *Mar Biol* 1980;**58**: 311-8.
Velimirov B. Sugar and lipid components in sea foam near kelp beds. *Mar Ecol* 1982;**3**: 97-107.
Voget S, Wemheuer B, Brinkhoff T *et al.* Adaptation of an abundant Roseobacter RCA organism to pelagic systems revealed by genomic and transcriptomic analyses. *ISME J* 2015;**9**: 371-84.
von Scheibner M, Herlemann DPR, Lewandowska AM *et al.* Phyto- and Bacterioplankton During Early Spring Conditions in the Baltic Sea and Response to Short-Term Experimental Warming. *Front Mar Sci* 2018;**5**: 231.

Walsby AE. The Gas Vesicles and Buoyancy of *Trichodesmium, Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs*, DOI 10.1007/978-94-015-7977-3_9: Springer, 1992, 141-61.

Wegner C, Hamburger M. Occurrence of stable foam in the upper Rhine River caused by plant-derived surfactants. *Environ Sci Technol* 2002;**36**: 3250-6.

Wurl O, Bird K, Cunliffe M *et al.* Warming and inhibition of salinization at the ocean's surface by cyanobacteria. *Geophys Res Lett* 2018;**45**: 4230-7.

Wurl O, Ekau W, Landing WM *et al.* Sea surface microlayer in a changing ocean – A perspective. *Elem Sci Anth* 2017;**5**: 31.

Wurl O, Holmes M. The gelatinous nature of the sea-surface microlayer. *Mar Chem* 2008;**110**: 89-97.

Wurl O, Miller L, Ruttgers R *et al.* The distribution and fate of surface-active substances in the sea-surface microlayer and water column. *Mar Chem* 2009;**115**: 1-9.

Wurl O, Stolle C, Van Thuoc C *et al.* Biofilm-like properties of the sea surface and predicted effects on air–sea $CO_2$ exchange. *Progr Oceanogr* 2016;**144**: 15-24.

Wurl O, Wurl E, Miller L *et al.* Formation and global distribution of sea-surface microlayers. *Biogeosciences* 2011;**8**: 121-35.

Yoon JH, Lee SY. *Winogradskyella multivorans* sp. nov., a polysaccharide-degrading bacterium isolated from seawater of an oyster farm. *Antonie Van Leeuwenhoek* 2012;**102**: 231-8.

Zäncker B, Engel A, Cunliffe M. Bacterial communities associated with individual transparent exopolymer particles (TEP). *J Plankton Res* 2019;**41**: 561-5.

**Figures**

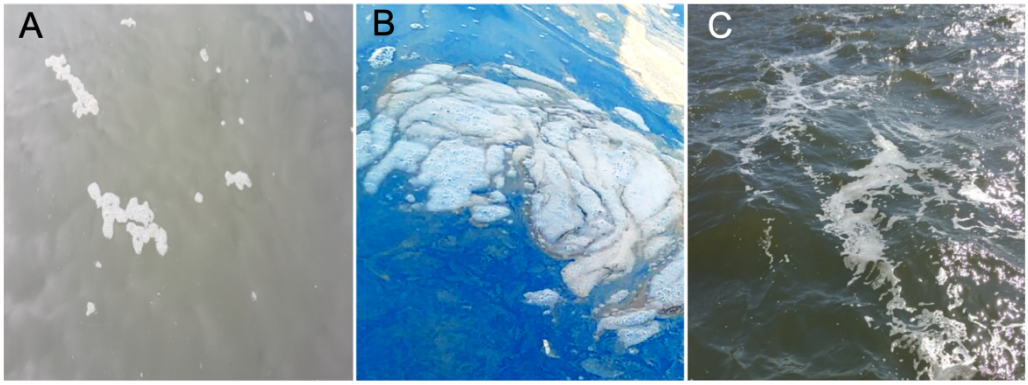


**Figure 1.** Marine foam originating from A) presumptive phytoplankton exudates (Jade Bay), B) a *Trichodesmium* bloom (Timor Sea) and C) whitecaps produced by convergence of surface water (Jade Bay, North Sea).

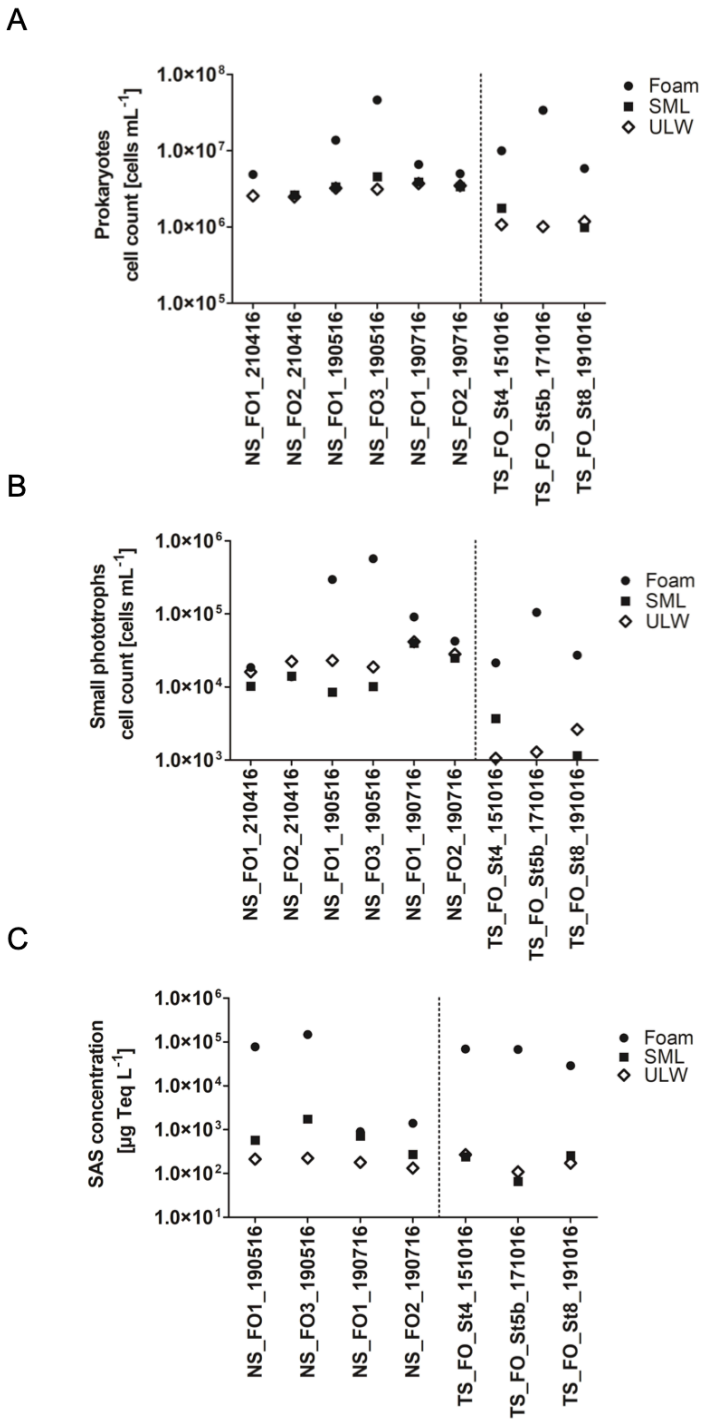


**Figure 2:** Absolute cell counts $mL^{-1}$ for A) prokaryotes and B) small phototrophic cells and C) concentration of surface-active substances (SAS) in µg Teq $L^{-1}$ for foam, sea-surface microlayer (SML) and underlying water (ULW).

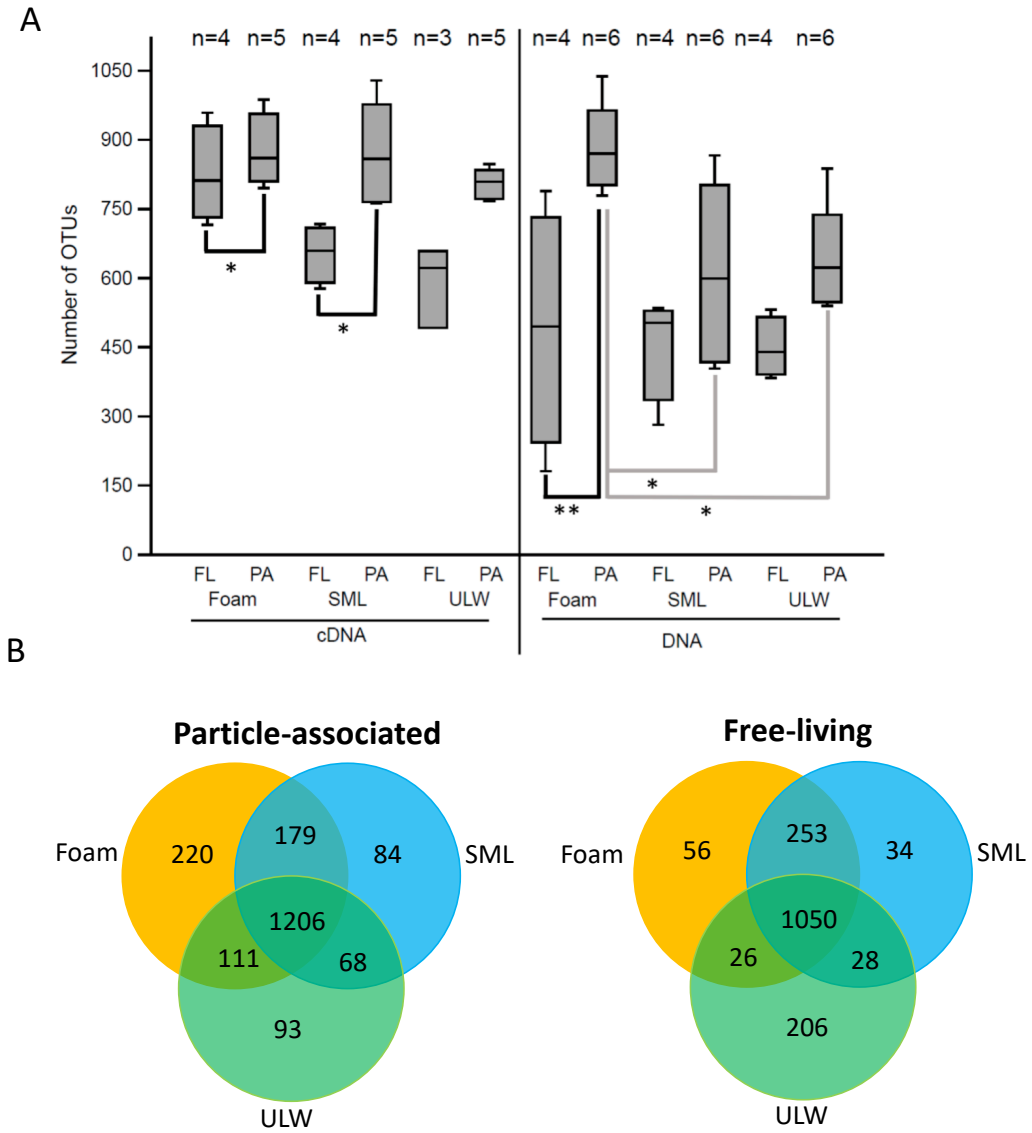


**Figure 3: A)** cDNA and DNA-derived numbers of operational taxonomic units (OTUs) for foam, SML and ULW habitat of pooled North Sea stations. The total number of OTUs of the three habitats is further distinguished between free-living (FL) and particle-associated (PA) bacterial communities. Grey and 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: * $p \leq 0.05$, ** $p \leq 0.01$ B) 3-Venn diagram showing overlapping and unique OTUs for foam, sea-surface microlayer (SML) and underlying water (ULW) separated by free-living and particle-associated OTUs.

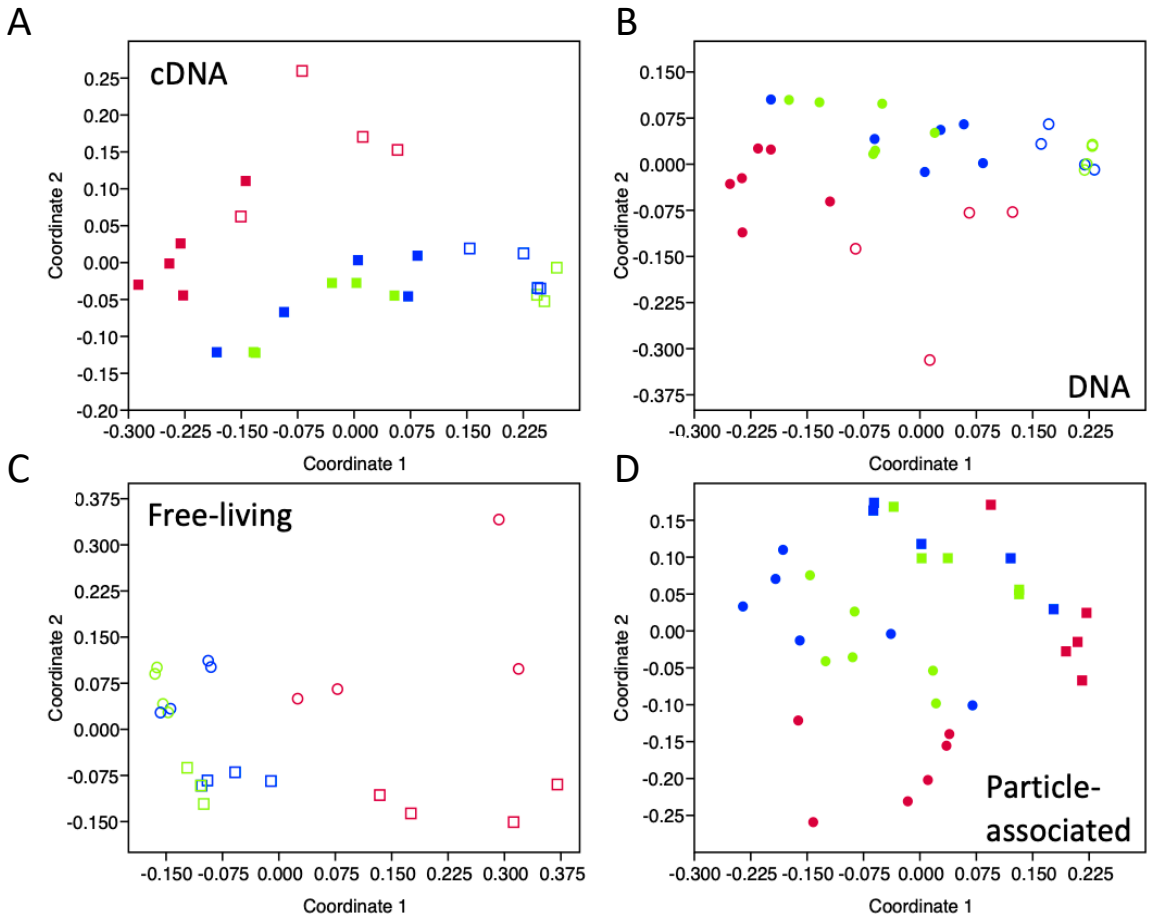


**Figure 4:** Non-metric multidimensional scaling plot shows distinct clustering of foam (red), SML (blue) and ULW (green) bacterial communities. Further separation of communities into A) cDNA-based with free-living (open symbols) and particle-associated (filled symbols) (stress=0.14); B) DNA-based with free-living (open symbols) and particle-associated (filled symbols) (stress=0.11); C) free-living with different nucleic acid source (cDNA=squares and DNA=circles) (stress=0.11); and D) particle-associated with different nucleic acid source (cDNA=squares and DNA=circles) (stress=0.15).

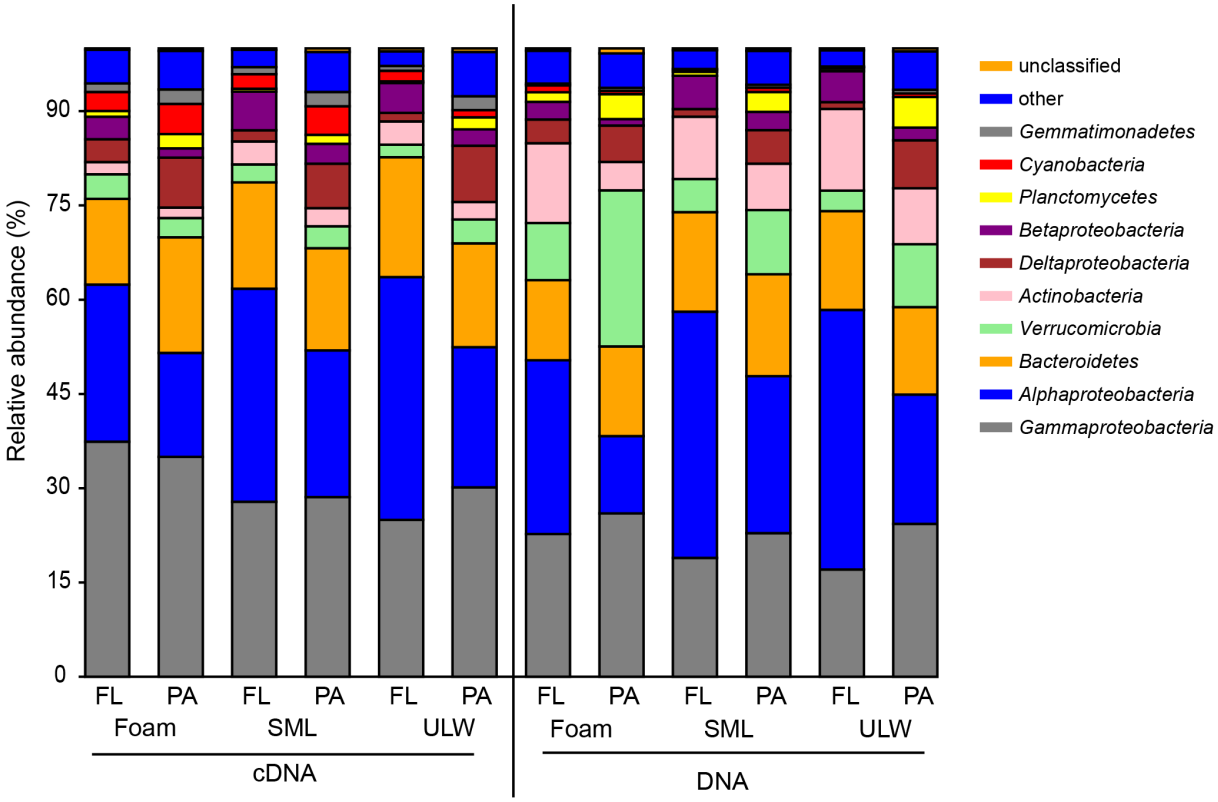


**Figure 5:** Composition of phylum-level of foam, SML and ULW samples of cDNA and DNA-based relative abundance of operational taxonomic units (OTUs) of pooled North Sea stations. Each habitat is further separated into free-living (FL) and particle-associated (PA) bacterial communities.

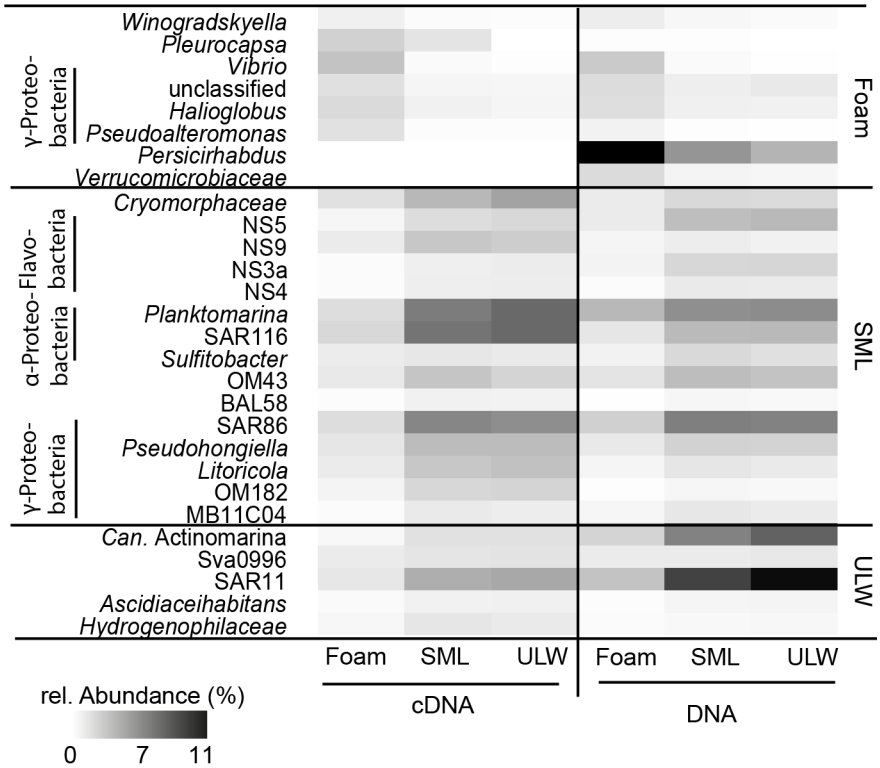


**Figure 6:** Heat-map showing OTUs with significant different abundance in foam, SML and ULW based on relative abundances of pooled samples.

**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 |

**Supporting Information**

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

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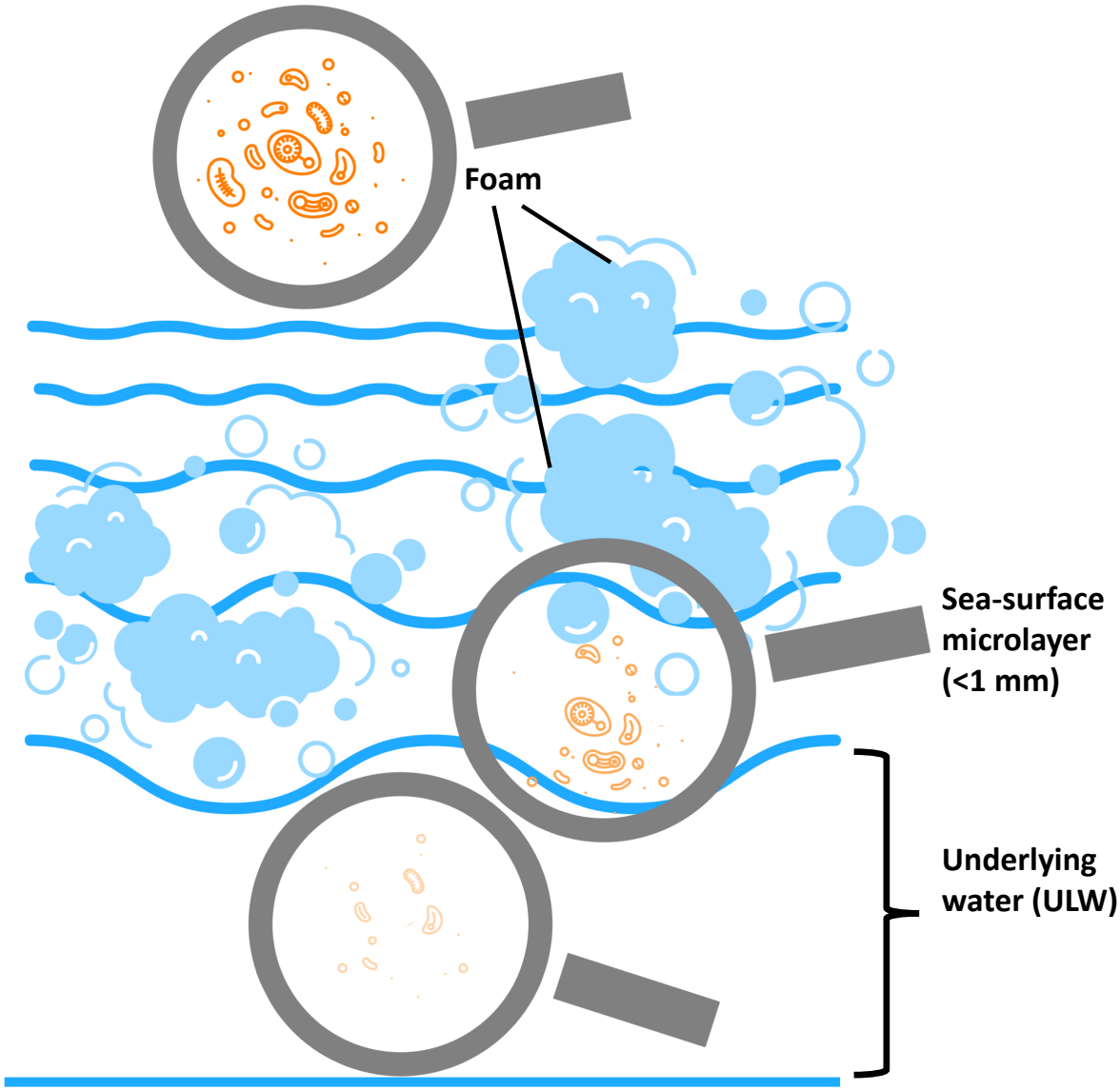
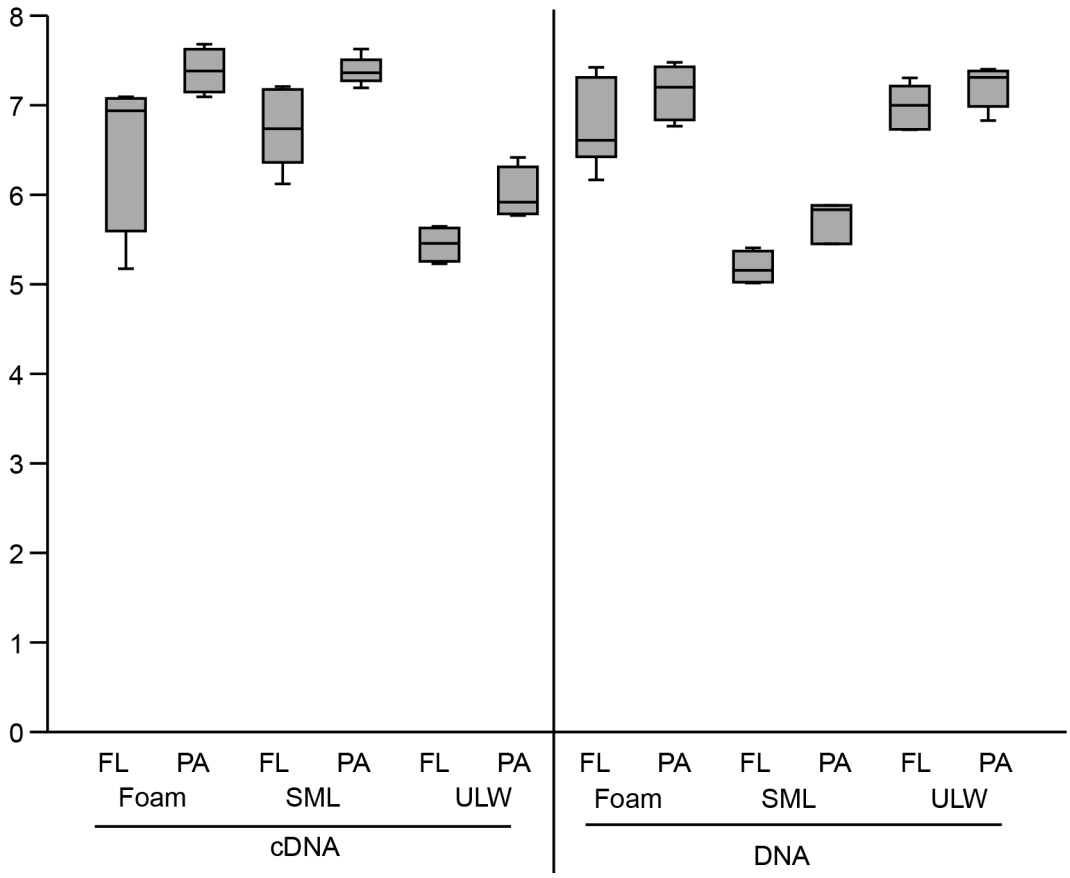
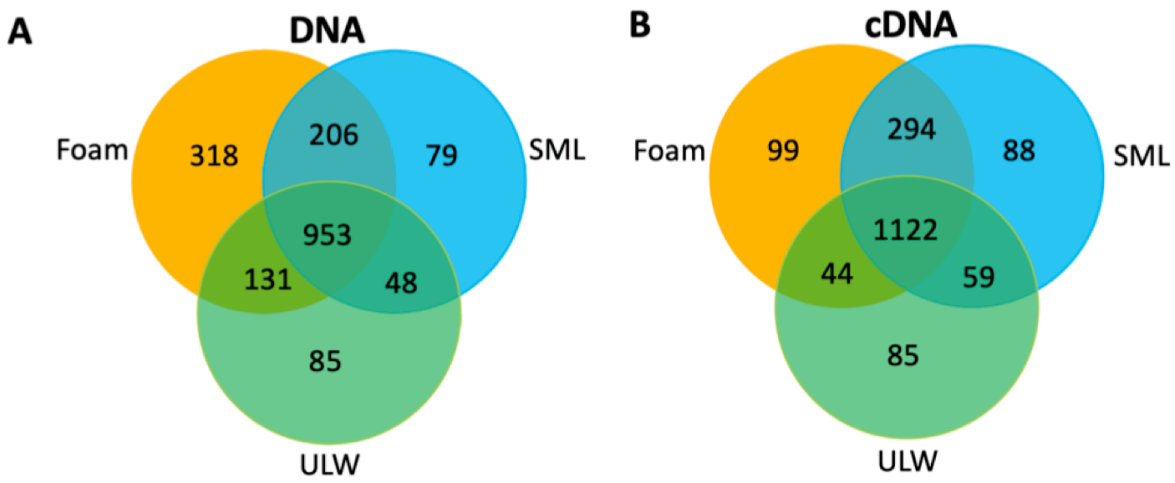


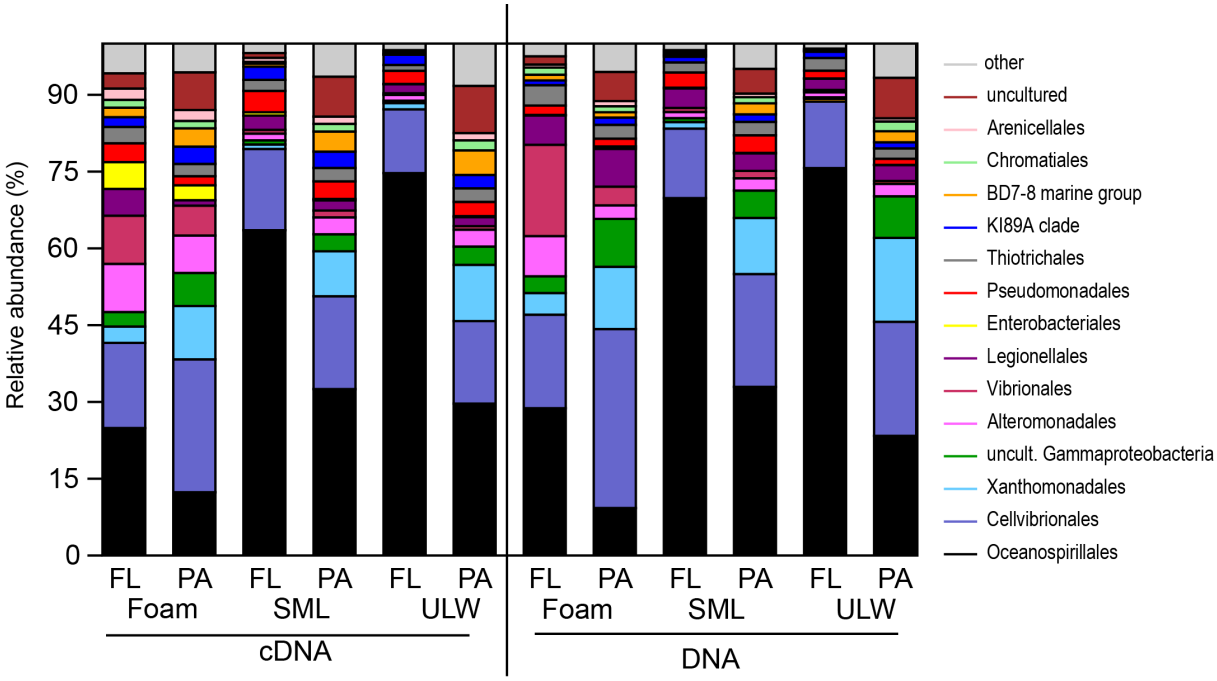

**Supplementary Figure 1:** Concept of the sea-surface microlayer (SML) and floating foams.



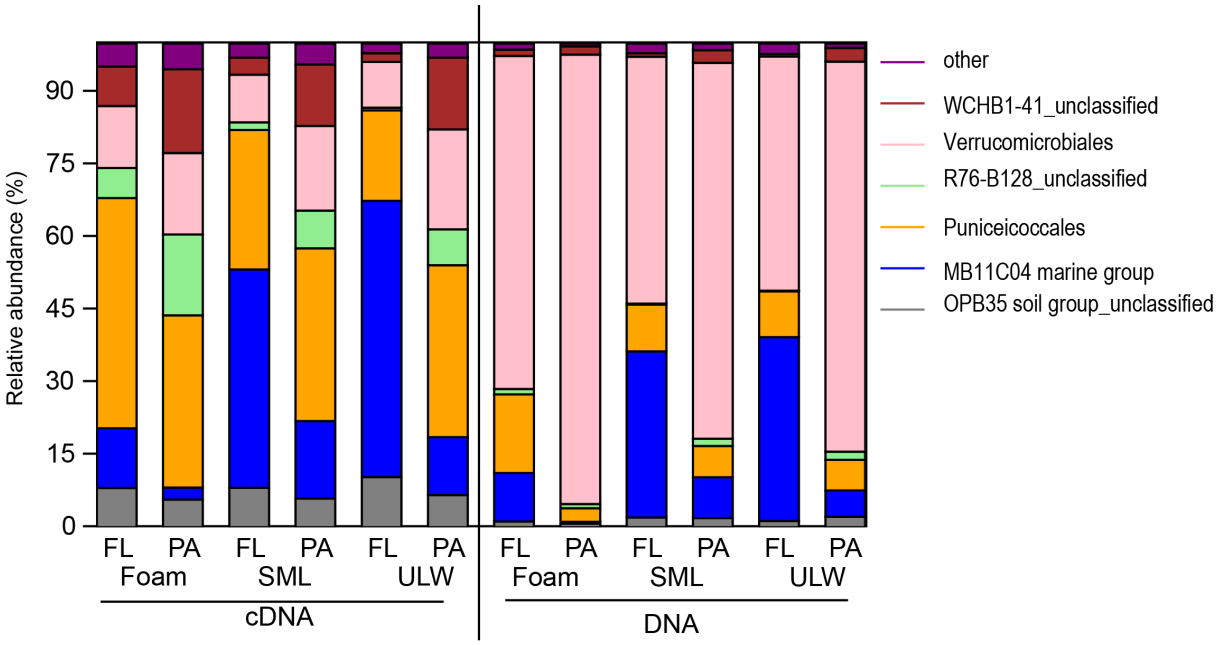

**Supplementary Figure 2:** Shannon index reflecting abundance and evenness of North Sea-derived OTUs separated by habitat (foam, sea-surface microlayer (SML) and underlying water (ULW), free-living (FL) or particle-attached (PA) status and nucleic acid template used for sequencing.



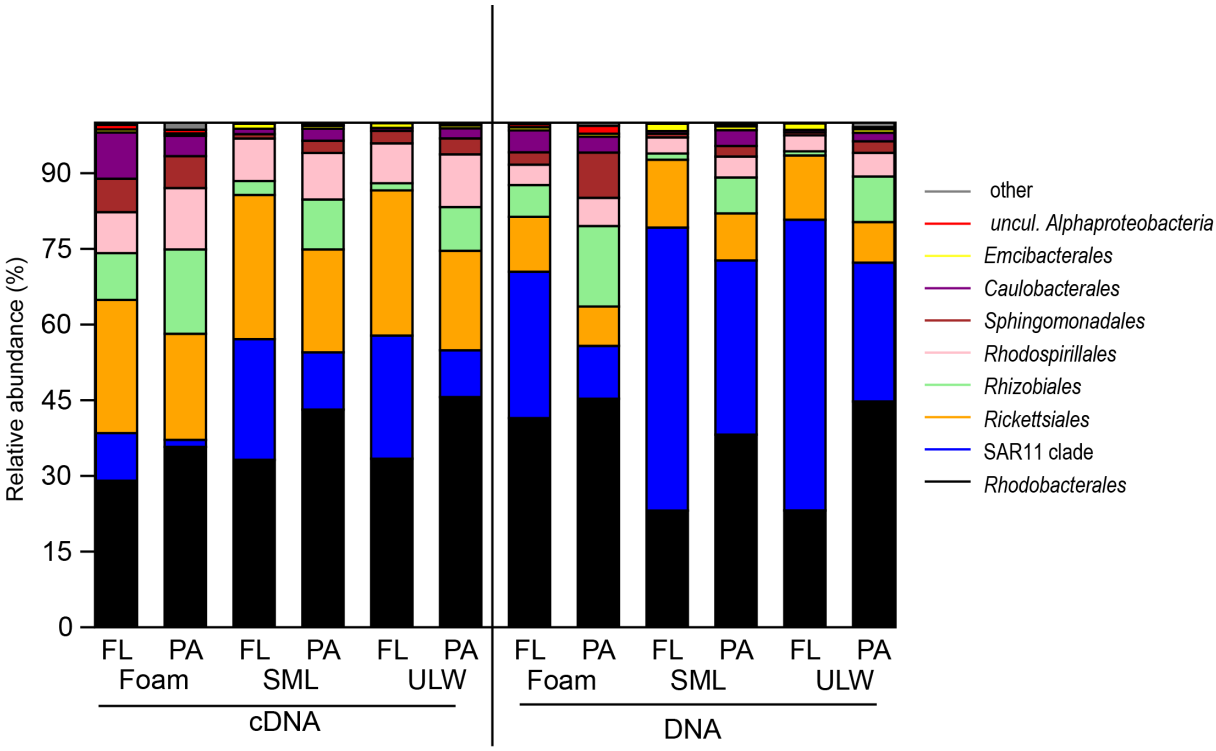

**Supplementary Figure 3:** 3-Venn diagram showing overlapping and unique A) DNA-based and B) cDNA-based OTUs for foam, sea-surface microlayer (SML) and underlying water (ULW).



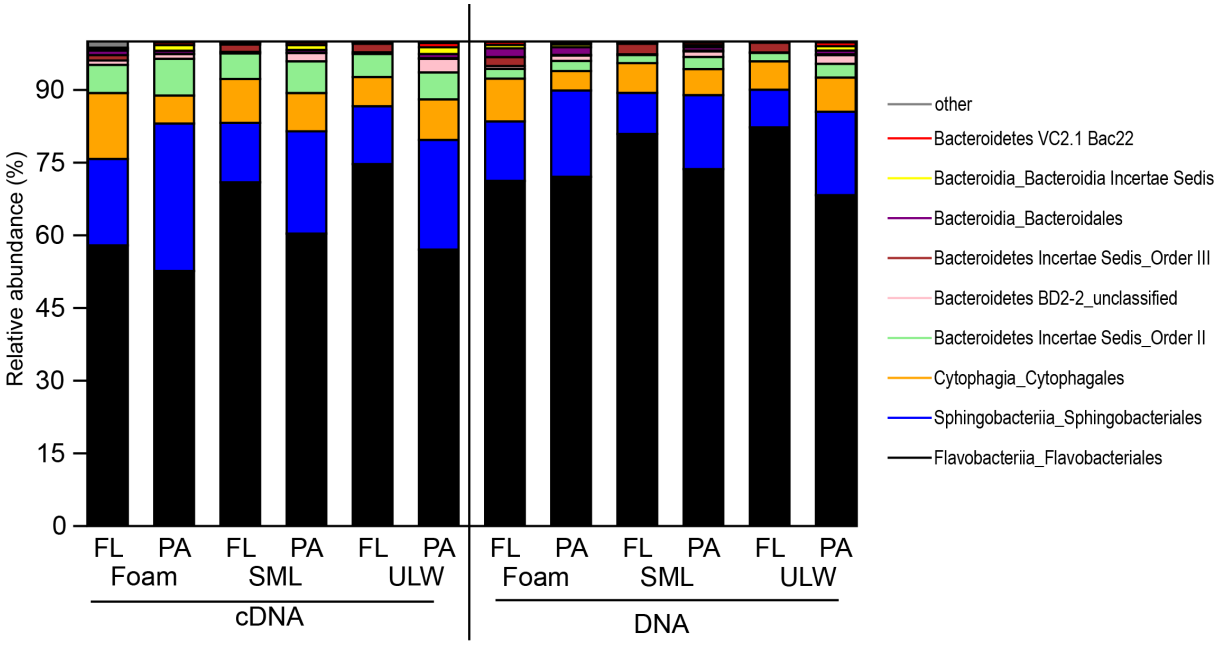

**Supplementary Figure 4:** Beta diversity among *Gammaproteobacteria* in foam, sea-surface microlayer (SML) and underlying water (ULW) samples of cDNA and DNA-based operational taxonomic units (OTUs). Each habitat contains further information on free-living (FL) and particle-associated (PA) bacterial community composition.



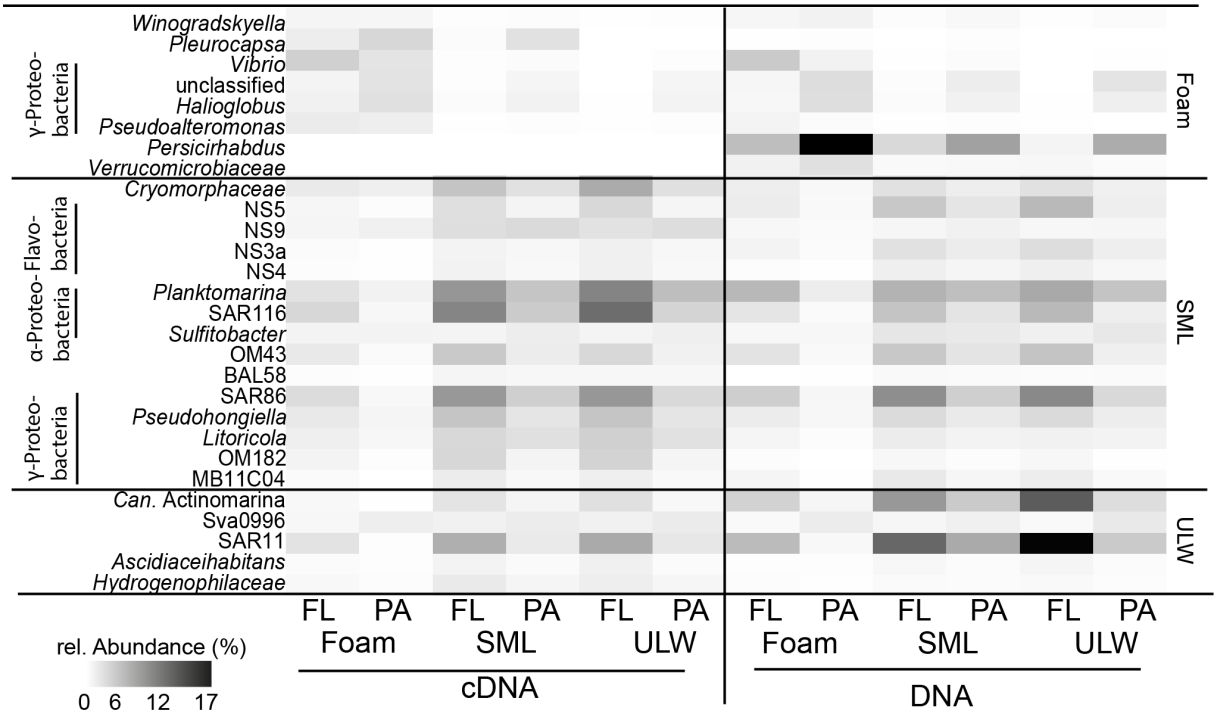

**Supplementary Figure 5:** Beta diversity among *Verrucomicrobia* in foam, SML and ULW samples of cDNA and DNA-based operational taxonomic units (OTUs). Each habitat contains further information on free-living (FL) and particle-associated (PA) bacterial community composition.




**Supplementary Figure 6:** 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.




**Supplementary Figure 7:** 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.




**Supplementary Figure 8:** 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 effect size (LEfSe) method.

**Supplementary Table 1:** 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 |

**Supplementary Table 2:** Relative abundance (%) of operational taxonomic units as shown in Figure 5. 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 |

**Supplementary Table 3:** 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. The ULW_FL sample based on cDNA is missing.

| **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 |