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ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Chemical and biological dispersants differently affect the bacterial communities of uncontaminated and oil-contaminated marine water

Camila Rattes de Almeida Couto[1] · Deborah Catharine de Assis Leite[1] · Diogo Jurelevicius[1] · Jan Dirk van Elsas[2] · Lucy Seldin[1,3]

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Abstract

The use of dispersants in marine environments is a common practice worldwide for oil spill remediation. While the effects of chemical dispersants have been extensively studied, those of biosurfactants, mainly surfactin that is considered one of the most effective surfactants produced by bacteria, have been less considered. We constructed microcosms containing marine water collected from Grumari beach (W_GB, Brazil) and from Schiermonnikoog beach (W_SI, The Netherlands) with the addition of oil (WO), Ultrasperse II plus oil (WOS), surfactin plus oil (WOB), and both dispersants (WS or WB) individually. In these treatments, the composition of bacterial communities and their predictive biodegradation potential were determined over time. High-throughput sequencing of the *rrs* gene encoding bacterial 16S rRNA revealed that Bacteroidetes (Flavobacteria class) and Proteobacteria (mainly Gammaproteobacteria and Alphaproteobacteria classes) were the most abundant phyla found among the W_GB and W_SI microbiomes, and the relative abundance of the bacterial types in the different microcosms varied based on the treatment applied. Non-metrical multidimensional scaling (NMDS) revealed a clear clustering based on the addition of oil and on the dispersant type added to the GB or SI microcosms, i.e., WB and WOB were separated from WS and WOS in both marine ecosystems studied. The potential presence of diverse enzymes involved in oil degradation was indicated by predictive bacterial metagenome reconstruction. The abundance of predicted genes for degradation of petroleum hydrocarbons increased more in surfactin-treated microcosms than those treated with Ultrasperse II, mainly in the marine water samples from Grumari beach.

Keywords Dispersants · Biosurfactant · Bacterial community · Oil · Marine water

Introduction

Oil spills occur recurrently in marine environments worldwide. Dispersants, i.e., compounds that are able to split oil slicks into micron-sized droplets, are applied as a strategy to enhance the bioavailability of petroleum hydrocarbons to indigenous microorganisms and, consequently, to improve environmental remediation [1, 2]. However, different studies show contradictory results regarding the efficiency of chemical dispersants that have been applied, such as Ultrasperse II (a mixture of alcohol, alcohol sulfate, and the fatty ester ethoxylate diluted in a solvent containing non-aromatic hydrocarbons) and Corexit 9500 (a mixture of hydrocarbons, glycols, and dioctylsulfosuccinate—DOSS) [3, 4].

Hamdan and Fulmer [5] showed that high concentrations of Corexit 9500 reduced the viability of different oil-degrading bacteria isolated from the beach sand of Elmer's Island (LA, USA) contaminated with oil from the Deep Water Horizon spill. By contrast, Kleindienst et al. [3]

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✉ Lucy Seldin
lseldin@micro.ufrj.br

[1] Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

[2] Microbial Ecology Department, University of Groningen, Groningen, The Netherlands

[3] Laboratório de Genética Microbiana, Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Bloco I, Ilha do Fundão, Rio de Janeiro, RJ CEP 21941.902, Brazil

demonstrated that the addition of only Corexit 9500 altered the microbial community composition, with the increase of the relative abundance of *Colwellia* in a study using microcosms containing seawater sampled from an active natural hydrocarbon seep in the northern Gulf of Mexico. Techtmann et al. [6] also observed that the addition of Corexit 9500 resulted in a decrease in the relative abundance of *Marinobacter* in microcosms constructed using samples collected near the site of the Deepwater Horizon oil spill and incubated at 25 °C. However, other OTUs were stimulated by the addition of the dispersant, many of which were identified as known hydrocarbon-degrading bacteria.

The effect of Ultrasperse II has been less studied than that of Corexit 9500 in marine oil-contaminated environments, even though it has been used in a few countries including Brazil [7]. A recent study of our group showed that the addition of Ultrasperse II to oil-contaminated marine water did not increase the abundance of the total bacterial community and of the alkane-degrading bacteria [7]. However, these results did not elucidate the effect of this substance on the composition and diversity of the oil-contaminated and uncontaminated marine water bacterial communities.

Although widely used, chemical surfactants are generally derived from petroleum and/or have hydrocarbons in their composition, thus being toxic to different life forms such as plants, animals, and microorganisms, including hydrocarbon-degrading bacteria [8–10]. On the other hand, biosurfactants produced by several microorganisms are considered to be environmentally friendly. They represent an alternative to chemical dispersants, as they are active under conditions of extreme temperature, pH, and salinity and are less toxic than synthetic surfactants to some invertebrate species [8, 11, 12]. However, they are still understudied for marine bioremediation applications. Among the well-known biosurfactants, surfactin—a polypeptide produced by *Bacillus subtilis* and related species—is considered to be one of the most effective [13]. With respect to its effects, Couto et al. [7] demonstrated that the abundance of genes potentially involved in alkane degradation was higher in surfactin-treated than in Ultrasperse II-treated oil-contaminated marine waters. However, the effects of either surfactin or Ultrasperse II on the structural and functional composition of bacterial communities of oil-contaminated and uncontaminated marine water are still not well known.

The aim of this study was to compare the effects of two dispersants—Ultrasperse II and surfactin—on the relative abundance and diversity of the bacterial communities in oil-contaminated and uncontaminated marine water samples collected from Grumari beach (Rio de Janeiro, Brazil) and from Schiermonnikoog beach, Island of Schiermonnikoog (Groningen, The Netherlands)—two countries with contrasting climatic conditions (tropical and temperate weather). We used high-throughput sequencing of the *rrs* gene encoding 16S rRNA to achieve our goals, thereby contributing to the understanding of the possible effects on marine bacterial communities.

Materials and methods

Dispersant sources

The biosurfactant produced by *Bacillus velezensis* H_2O-1, a strain originally isolated from connate water from an oil reservoir in Brazil and previously described by Korenblum et al. [14] and Guimarães et al. [15], was produced and purified as previously described by Guimarães et al. [15]. In brief, it was produced in a mineral salt medium with the following composition (% *w*/*v*): glucose 1.0, NaCl 1.0, Na_2HPO_4 0.5, KH_2PO_4 0.2, $MgSO_4$ 0.02, and $(NH_4)_2SO_4$ 0.2. The medium was inoculated with 20 mg cells L^{-1} of a pre-inoculum of strain H_2O-1 grown in the same culture medium for 14 h at 30 °C and 170 rpm. The biosurfactant was characterized as surfactin by Korenblum et al. [16]. Ultrasperse II was obtained from Oxiteno, São Paulo, Brazil.

Sample sites and construction of the microcosms

Marine water samples (5 L) were collected from Grumari beach (GB) in Rio de Janeiro, Brazil (23° 2′ 59″ S 43° 31′ 35″ W), and from Schiermonnikoog Island (SI), located in the Netherlands (53° 29′ 14″ N 6° 14′ 3″ E) in December 2014 and April 2015, respectively. The oil samples were supplied by Petrobras in Brazil and by Royal Dutch Shell in the Netherlands. Both were considered medium oils. Microcosms were constructed in triplicate using 25 mL of water, and they were submitted to different treatments as previously described in detail in Couto et al. [7]. They were as follows: (a) control—microcosms containing only water—W; (b) microcosms with water and the addition of biosurfactant—WB (surfactin, 40 μg mL^{-1}); (c) microcosms with water and the addition of chemical surfactant—WS (Ultrasperse II, 1 μL mL^{-1}); (d) microcosms with water contaminated with crude oil—WO (1% *v*/*v*); (e) microcosms with water contaminated with crude oil and the addition of biosurfactant—WOB; and (f) microcosms with water contaminated with crude oil and the addition of chemical surfactant—WOS. The microcosms were incubated at 20 °C, under shaking conditions (75 rpm) and in the dark, during 30 days.

DNA extraction

After 30 days of incubation, the content of each microcosm (samples in triplicate) was filtered through a Millipore membrane (0.45 μm), and the community DNA was then extracted

using FastDNA® Spin Kit for Soil (BIO 101 Systems, OH, USA) and then stored at 4 °C prior to PCR amplification.

High-throughput sequencing and data analyses

DNA obtained from the different microcosms was PCR-amplified using primers 515F/806R [17], which target the V4 region of the 16S rRNA encoding gene. Amplification, pooling, and purification were performed by Macrogen (South Korea). All samples were sequenced using a MiSeq Platform and the MiSeq Reagent kit v3 (Illumina, USA). All subsequent analyses were carried out with the software package QIIME (Quantitative Insights into Microbial Ecology toolkit; [18]). The sequences were trimmed using parameters such as quality score (> 30) and sequence length (> 100), maximum homopolymer length of 6, and 0 mismatched bases in the primers and barcodes. Barcodes and adapters were also removed. The forward and reverse sequences were merged using the fastq-join tool [19], demultiplexed and quality-filtered as described by Bokulich et al. [20]. The sequence analysis tool USEARCH was used to cluster sequences in operational taxonomic units (OTUs) at 97% sequence identity and to remove chimeras from the sequences. Then, representative sequences from each OTU were aligned with the Greengenes Core Set [21] using PyNAST [22]. Taxonomy was assigned to sequences using the BLAST tool [23]. Before analysis, singletons, chloroplast plastid, mitochondrion, and archaeal sequences were manually removed from the dataset. All sequences were deposited in the sequence read archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number SRP151584.

Statistical analyses

The OTU-generated matrices were exported into PAST software [24] for non-metrical multidimensional scaling (NMDS—Bray-Curtis distance) analyses. ANOVA was used to check whether the sampling sites (GB and SI) and the applied treatments significantly influenced the bacterial communities, and Tukey's test was used to determine the significance of differences ($p < 0.05$).

Bacterial community functional predictions

Predicted genes potentially related to metabolic pathways involved in petroleum hydrocarbon degradation were analyzed in all microcosms containing only oil and in those with oil added with either surfactin or Ultrasperse II using the software Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) [25]. Metagenomic functional predictions were compared using Tukey's pairwise comparisons ($p < 0.05$).

Results

Diversity and composition of the bacterial communities in the different microcosms

The diversity and composition of the bacterial communities from the different microcosms (36 in total) were evaluated using high-throughput sequencing of the 16S rRNA gene after 30 days of incubation. A total of 1,885,728 sequences were obtained. After filtering out of low-quality sequences, OTU tables were rarified to analyze 12,000 sequences for each sample (based on the lowest number of sequences obtained per sample).

OTU numbers and the diversity measures obtained from the GB and SI samples are shown in Tables 1 and 2, respectively. Rarefaction curve expressing the estimated OTUs obtained from Grumari Beach and Schiermonnikoog Island samples and a graphic representation expressing the values of Chao and Shannon alpha diversity are shown in Figs. S1 and S2, respectively. Significant differences within the diversity values were determined using Tukey's pairwise comparison tests ($p < 0.05$). Among the GB samples, the bacterial richness values (Chao1) were highest in WOS_GB (347.24 ± 40.56), whereas the lowest richness values were found in the W_GB (255.39 ± 32.63) and WO_GB (249.01 ± 26.02) samples (Table 1). However, W_GB and WO_GB were statistically similar to WB_GB, WS_GB, and WOB_GB. Conversely, bacterial diversity values (Shannon-Weaver index) were higher in the W_GB (6.10 ± 0.39) and WO_GB (5.96 ± 0.61) systems, as compared to WS_GB (4.30 ± 0.35), WOB_GB (4.29 ± 0.15), and WOS_GB (4.29 ± 0.39). In the SI systems, bacterial richness values (Chao1) were lower in WO_SI (207.40 ± 26.08) than in all other systems (Table 2). Moreover, whereas bacterial diversity (Shannon-Weaver index) was high in W_SI (5.60 ± 0.40), samples WS_SI (4.35 ± 0.23) and WOS_SI (4.58 ± 0.37) showed the lowest diversity indices (Table 2). Nonetheless, WS_SI and WOS_SI were statistically similar to the other samples.

Multivariate analyses of 16S rRNA gene–based OTUs

NMDS ordination based on the OTU-generated matrices was used to compare the GB or SI samples, in separate and together. The analysis revealed clear clusterings based on the parameters "addition of oil" and "dispersant type" added to the GB or SI microcosms (Fig. 1A, B). WB and WOB were separated from W, WO, WS, and WOS in both marine waters studied, indicating the effect of the biosurfactant used. In general, oil-contaminated samples were separated from uncontaminated samples in GB (Fig. 1A). WB and WOB showed to be closer to each other (Fig. 1A). The separation of WS and WB in SI is clearly shown in Fig. 1B.

Table 1 Estimated OTUs, richness, and diversity indices based on the OTU-generated matrices of samples from Grumari Beach

Samples	Observed OTUs	Chao (richness)	Shannon (diversity)	Phylogenetic distance
W_GB	222.53 ± 37.17[a*]	255.39 ± 32.63[a]	6.10 ± 0.39[a]	24.87 ± 1.2[a]
WB_GB	248.3 ± 18.45[a]	311.85 ± 28.63[ab]	5.03 ± 0.60[ab]	23.74 ± 1.04[a]
WS_GB	243.73 ± 8.97[a]	331.01 ± 4.32[ab]	4.30 ± 0.35[b]	23.5 ± 1.51[a]
WO_GB	207.8 ± 28.19[a]	249.01 ± 26.02[a]	5.96 ± 0.61[a]	24.8 ± 1.25[a]
WOB_GB	210.1 ± 25.17[a]	270.35 ± 37.10[ab]	4.29 ± 0.15[b]	22.13 ± 2.15[a]
WOS_GB	259.63 ± 26.62[a]	347.24 ± 40.56[b]	4.29 ± 0.39[b]	23.82 ± 2.14[a]

GB, Grumari Beach; *W*, water; *WB*, water and surfactin; *WS*, water and Ultrasperse II; *WO*, water and oil; *WOB*, water, oil, and surfactin; *WOS*, water, oil and Ultrasperse II

*Different letters indicate statistically significant differences based on Tukey's test ($p < 0.05$)

Analysis of all samples (GB and SI) using NMDS revealed a separation of the samples with the addition of oil and/or dispersants—WS, WB, WOS, and WOB—from those only contaminated with oil—W and WO (when coordinates 1 and 2 were analyzed; Fig. 1C), suggesting an influence of the dispersant addition. The influence of the type of dispersant used was observed when the samples contaminated with Ultrasperse II (WS_GB, WOS_GB, WS_SI, and WOS_SI) were separated from those samples treated with surfactin (WB_GB, WOB_GB, WB_SI, and WOB_SI) when coordinates 2 and 3 were analyzed (Fig. 1D). Furthermore, a clear distinction between the GB and SI samples was observed (Fig. 1D).

Relative abundance of bacterial types in the different microcosms

In a separate analysis of each replicate with respect to the OTU types, we found similar bacterial groups at similar percentages of the total among the replicates of the same treatment. Hence, in further analyses, we considered the averages of the relative abundances of the different taxa found in the different microcosms incubated for 30 days. Only the taxonomic groups with more than 5% relative abundance were considered for further analyses, and the remainder (less than 5% of the relative abundance) being denoted as "others" (Fig. 2).

Identical phyla were found among the W_GB and W_SI microbial communities, albeit at different relative abundances. Thus, Bacteroidetes (Flavobacteria class) and Proteobacteria (mainly Gammaproteobacteria and Alphaproteobacteria classes) were the most abundant phyla found. The presence of oil and dispersants in the GB and SI samples incited different responses in the respective bacterial communities (Fig. 2).

In the GB samples, slight increases of Alphaproteobacteria and Gammaproteobacteria and decreases of Bacteroidetes were observed with the addition of surfactin (W_GB versus WB_GB). On the other hand, an increase of Bacteroidetes and a decrease of Gammaproteobacteria were observed with the addition of Ultrasperse II (WS_GB). The presence of oil (WO_GB) resulted in an increase of Alphaproteobacteria and a decrease of Gammaproteobacteria. The most prominent alterations were observed with the combined addition of oil and dispersants. When surfactin was added to the oil-contaminated microcosms (WOB_GB), the relative abundance of Planctomycetes increased up to more than 30% when compared with W_GB. In addition, a decrease of Gammaproteobacteria and an increase of Alphaproteobacteria were also detected. The main effect was observed with the combination of oil and Ultrasperse II (WOS_GB). While a considerable increase of the relative abundance of OTUs related to Bacteroidetes was observed, increases of those related to Planctomycetes were not found in WOS_GB (as observed in WOB_GB).

The results obtained in the GB and SI systems were different (Fig. 2). With the addition of surfactin but not of Ultrasperse II in SI, an increase of the relative abundance of OTUs related to Bacteroidetes was observed. Conversely, an increase of Gammaproteobacteria was observed in WS_SI compared with W_SI. With oil addition (WO_SI), we observed the appearance of OTUs related to Actinobacteria which were not found in the surfactin- or Ultrasperse II-amended water systems. Furthermore, an increase of Alphaproteobacteria was also observed in WO_SI. OTUs related to the Gammaproteobacteria dominated in the systems treated with oil and Ultrasperse II (WOS_SI).

Using the genus level, different bacterial communities were observed in GB versus SI (Fig. 3A and B, respectively). Briefly, OTUs related to *Alcanivorax*, Sinobacteriaceae and *Parvibaculum* were enriched by the presence of surfactin in the GB samples. Members of the Flavobacteriaceae were enriched with the addition of Ultrasperse II with or without the presence of oil (WS_GB or WOS_GB). While OTUs related to *Planctomyces* increased with the addition of surfactin and oil (in WOB_GB), they were not detected with Ultrasperse II as the dispersant used (WOS_GB) (Fig. 3A).

The relative abundance of OTUs related to Rhodobacteriaceae, Microbacteriaceae, and *Loktanella* increased in oil-containing SI samples (WO_SI). *Marinobacter*,

Table 2 Estimated OTUs, richness, and diversity indices based on the OTU-generated matrices of samples from Schiermonnikoog Island

Samples	Observed OTUs	Chao (richness)	Shannon (diversity)	Phylogenetic distance
W_SI	247.65 ± 8.41[a*]	293.44 ± 24.72[a]	5.60 ± 0.40[a]	24.36 ± 2.37[a]
WB_SI	224.4 ± 18.61[a]	292.69 ± 17.02[a]	5.07 ± 0.11[ab]	22.84 ± 1.50[ac]
WS_SI	227.96 ± 8.39[a]	295.80 ± 12.30[a]	4.35 ± 0.23[b]	21.91 ± 1.25[ac]
WO_SI	165.7 ± 16.60[b]	207.40 ± 26.08[b]	4.93 ± 0.71[ab]	19.46 ± 1.35[bc]
WOB_SI	218.73 ± 23.60[a]	271.63 ± 25.71[a]	5.23 ± 0.48[ab]	20.63 ± 1.45[bc]
WOS_SI	225.13 ± 21.47[a]	275.78 ± 26.60[a]	4.58 ± 0.37[b]	20.48 ± 1.83[bc]

SI, Schiermonikoog Island; *W*, water; *WB*, water and surfactin; *WS*, water and Ultrasperse II; *WO*, water and oil; *WOB*, water, oil, and surfactin; *WOS*, water, oil, and Ultrasperse II

*Different letters indicate statistically significant differences based on Tukey's test ($p < 0.05$)

Kordia, and *Winogradskyella* were enriched with the addition of surfactin (WB_SI) but decreased with the presence of oil (WOB_SI). OTUs related to *Pseudoalteromonas* were mainly enriched in oil- and Ultrasperse II-containing samples (representing 24% in WOS_SI) (Fig. 3B).

Predicting functions involved in oil degradation

Using PICRUSt, fifteen predicted genes potentially related to metabolic pathways involved in petroleum hydrocarbon degradation were analyzed in all microcosms containing only oil and in those with oil added with either surfactin or Ultrasperse II (Fig. 4). The abundances of genes encoding enzymes potentially involved in aminobenzoate, benzoate, chloroalkane, chloroalkene, chlorocyclohexane, chlorobenzene, fluorobenzene, naphthalene, styrene, toluene and xylene degradation, and butanoate metabolism were significantly increased in surfactin-treated GB samples (Fig. 4A). Conversely, 9 and 14 of the 15 predicted genes potentially related to hydrocarbon degradation significantly decreased in Ultrasperse II-treated GB and SI microcosms, respectively, when compared with oil-contaminated microcosms (Fig. 4A, B).

Discussion

The search for an efficient and environmentally friendly technology to remediate spills of crude oil and derived products is still a challenge. The rate of oil biodegradation is related to the presence of populations of microorganisms that will transform oil components into degradable compounds; this process is governed by pollutant bioavailability [26, 27]. Therefore, the use of dispersants is a common practice in terrestrial and marine environments to improve the availability of oil to microbial degraders.

In a previous study, Couto et al. [7] used quantitative PCR (*alkB* genes) and genetic fingerprint analyses (PCR-DGGE) to demonstrate that the addition of either surfactin or Ultrasperse II influenced the structure and abundance of total and oil-degrading bacterial communities of the oil-contaminated and uncontaminated marine waters collected from two regions with contrasting climatic conditions also used in this study. The enhancement of the oil-degrading bacteria with the addition of surfactin was greater than that observed with Ultrasperse II [7]. Here, the compositions of the bacterial communities of these two marine waters are compared using sequence analyses to better understand the effects of oil, surfactin, and Ultrasperse II on their dynamics and diversity.

Our finding that the diversity indices (Shannon) decreased with the addition of either Ultrasperse II or surfactin (with or without oil) in both GB and SI was in line with that of Liu et al. [28], who showed that the application of oil and dispersant typically led to the lowest alpha diversity of microbial communities from on-ship microcosms set up immediately after water collection in Eastern Mediterranean Deep Sea. Possibly, both agents incited selective (toxicity or growth related) pressures resulting in such lowered diversities.

Evaluating bacterial community structures in the oil collected from the sea surface and sediment in the northern Gulf of Mexico after the Deepwater Horizon oil spill, Liu and Liu [29] showed that Proteobacteria was the most dominant phylum in both the oil spill and the control site. Lee and Eom [30] also demonstrated that the phylum Proteobacteria accounted for 68.44% of the bacterial community in the seawater collected from Mallipo, South Korea. Similarly, we observed more than 50% and about 40% Proteobacteria (Gammaproteobacteria and Alphaproteobacteria)-related OTUs in uncontaminated GB and in SI samples, respectively. Indeed, both marine waters contained largely the same phyla but at different proportions (Fig. 2). However, with the introduction of the different dispersants, the bacterial communities responded differently. With the addition of Ultrasperse II in GB, an increase of the OTU numbers related to Bacteroidetes was observed. This effect was accentuated with the introduction of oil when Bacteroidetes corresponded to more than 60% of the OTUs. On the other hand, the major effect of Ultrasperse II in SI was the increase of

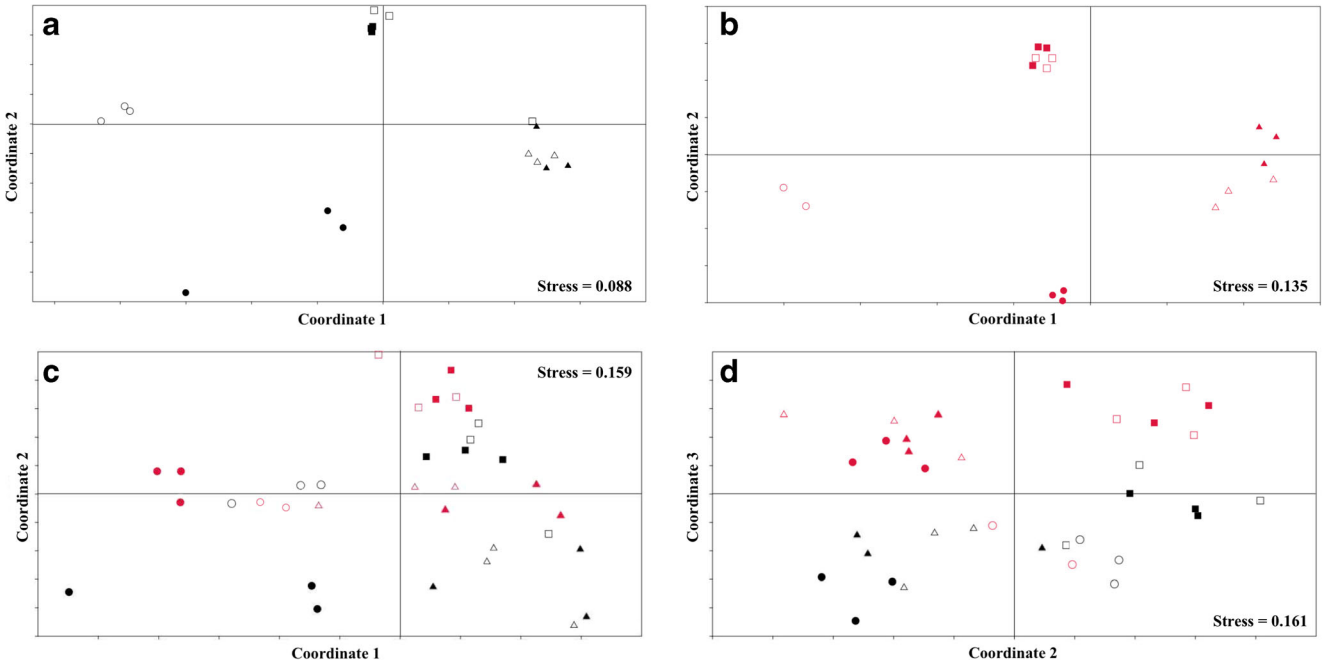

Fig. 1 Non-metric multidimensional scaling (NMDS) ordination diagram based on the OTU-generated matrices obtained after sequencing using primers for the 16S rRNA-coding gene. A—samples from Grumari beach (GB); B—samples from Schiermonikoog Island (SI), and C and D—all samples analyzed together. ○ water; ● water and oil; Δ water and surfactin; ▲ water, oil, and surfactin; □ water and ultrasperse II; ■ water, oil, and ultrasperse II. Black and red colors represent GB and SI, respectively

Gammaproteobacteria (constituting almost 70% of the OTUs). Considering the use of the biosurfactant, OTUs related to Planctomycetes were clearly stimulated with the addition of surfactin in GB, reaching over 30% abundance with the addition of oil. Oppositely, Yu et al. [31] suggested strong negative impacts of the oil contamination and/or biostimulation treatments on Planctomycetes and other phyla in the saline soil of Yellow River Delta, China. In that environment, *Alcanivorax*-related Gammaproteobacteria rapidly dominated and contributed to the biodegradation of easily degradable portion of the heavy crude oil [31].

Various drivers of observed differences included OTUs affiliated with bacterial groups that are considered (or at least suspected) to be hydrocarbon degraders [32]. In treatments

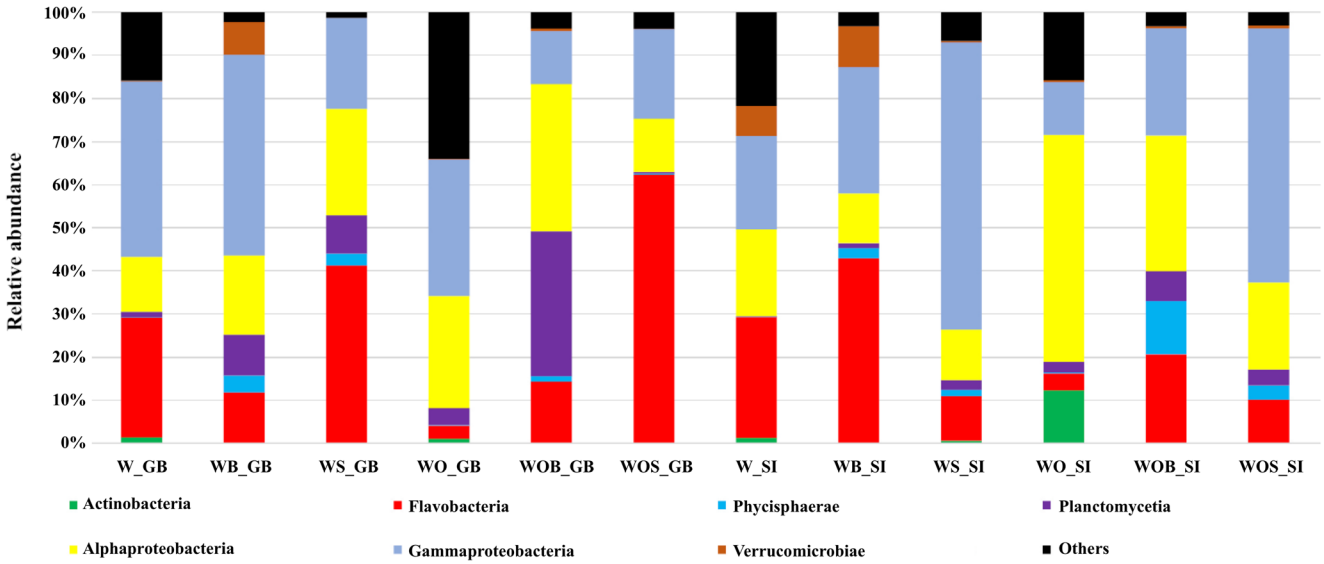

Fig. 2 Relative abundances of the most abundant classes found in the marine water samples from Grumari Beach (GB) and Schiermonnikoog Island (SI) as determined by high-throughput sequencing. W, microcosms containing only water; WB, microcosms with water and surfactin; WS, microcosms with water and Ultrasperse II; WO, microcosms with water contaminated with crude oil (1% *v*/*v*); WOB, microcosms with oil-contaminated water plus surfactin; WOS, microcosms with oil-contaminated water plus Ultrasperse II

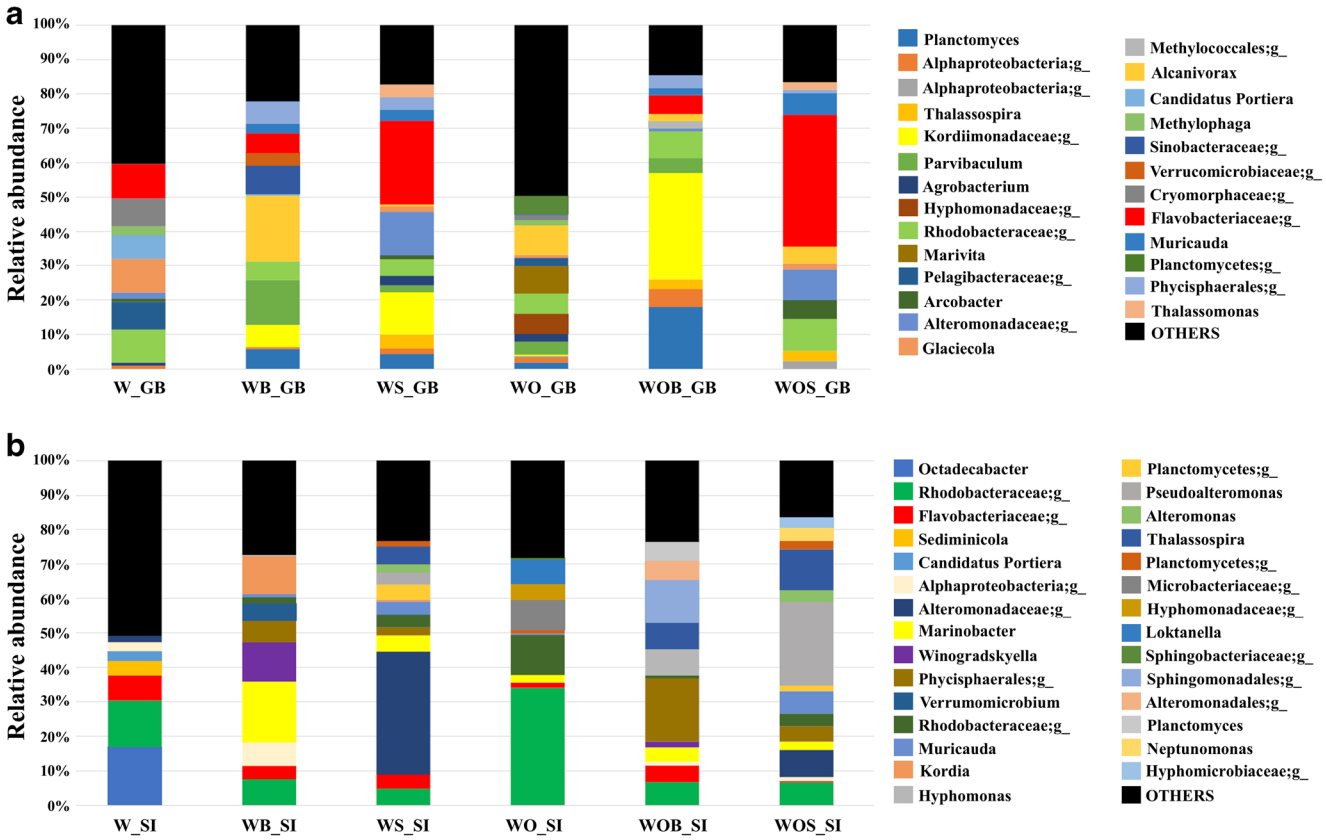

Fig. 3 Relative abundances of the most abundant bacterial genera found in the marine water samples from (A) Grumari Beach—GB and (B) Schiermonnikoog Island—SI as determined by high-throughput sequencing. W, microcosms containing only water; WB, microcosms with water and surfactin; WS, microcosms with water and Ultrasperse II; WO, microcosms with water contaminated with crude oil (1% *v*/*v*); WOB, microcosms with oil-contaminated water plus surfactin; WOS, microcosms with oil-contaminated water plus Ultrasperse II

containing surfactin in GB, we observed the increase of OTUs related to *Alcanivorax*, *Planctomyces*, and Kordiimonadaceae. These two latter groups were stimulated with the addition of oil. *Alcanivorax* has been previously related to petroleum hydrocarbon degradation [31–33]. Flavobacteriaceae- and Kordiimonadaceae-related OTUs were present in Ultrasperse II–treated GB microcosms. While the former is stimulated with oil addition, the other was not found among the most abundant OTUs in WOS_GB. In treatments containing surfactin in SI, significant increases in the abundance of OTUs related to *Winogradskyella* and *Marinobacter* were observed, also suggesting an ability of these microorganisms to take advantage of the presence of biosurfactant and outcompete other microorganisms. Members of *Winogradskyella* have been already involved with the degradation of naphthalene and fluorene [34] and the genus *Marinobacter* has also been reported as a suspected taxa known to contain oil-degraders [5, 6, 32, 35]; however, in our study, OTUs related to this genus decreased in abundance with oil addition. On the other hand, other hydrocarbon degraders, such as *Hyphomonas* and others, were stimulated by the presence of oil. A similar situation was observed with the addition of Ultrasperse II in SI microcosms. OTUs related to *Pseudoalteromonas* reached to 25% of the total in WOS_SI. This latter genus was found in oil-polluted water column of the North Sea as a potential hydrocarbon degrader [36].

Finally, PICRUSt was used in an attempt to assign a function to phylogeny. It is very useful to build hypotheses, based on data concerning the enrichment of bacterial groups related to the hydrocarbon degradation in oil and/or dispersant-marine water samples. For example, after the DWH oil spill, many genes related to aromatic and aliphatic biodegradation were found to be enriched in the bacterial communities from both surface and deep water and strong biodegradation of those compounds was detected [34, 37, 38]. In our study, PICRUSt revealed a potential enrichment of several genes that were potentially involved in oil degradation, mainly after the surfactin addition in GB (Fig. 4). In contrast, a decrease of the abundance of several predicted biodegradative genes was observed after the use of Ultrasperse II in either oil-contaminated GB or SI. Couto et al. [7] have previously shown that significantly higher alkane-monooxygenase AlkB (*alkB*) copy numbers were observed in surfactin and oil-containing microcosms from GB and SI, suggesting that the addition of surfactin stimulated oil-degrading bacteria

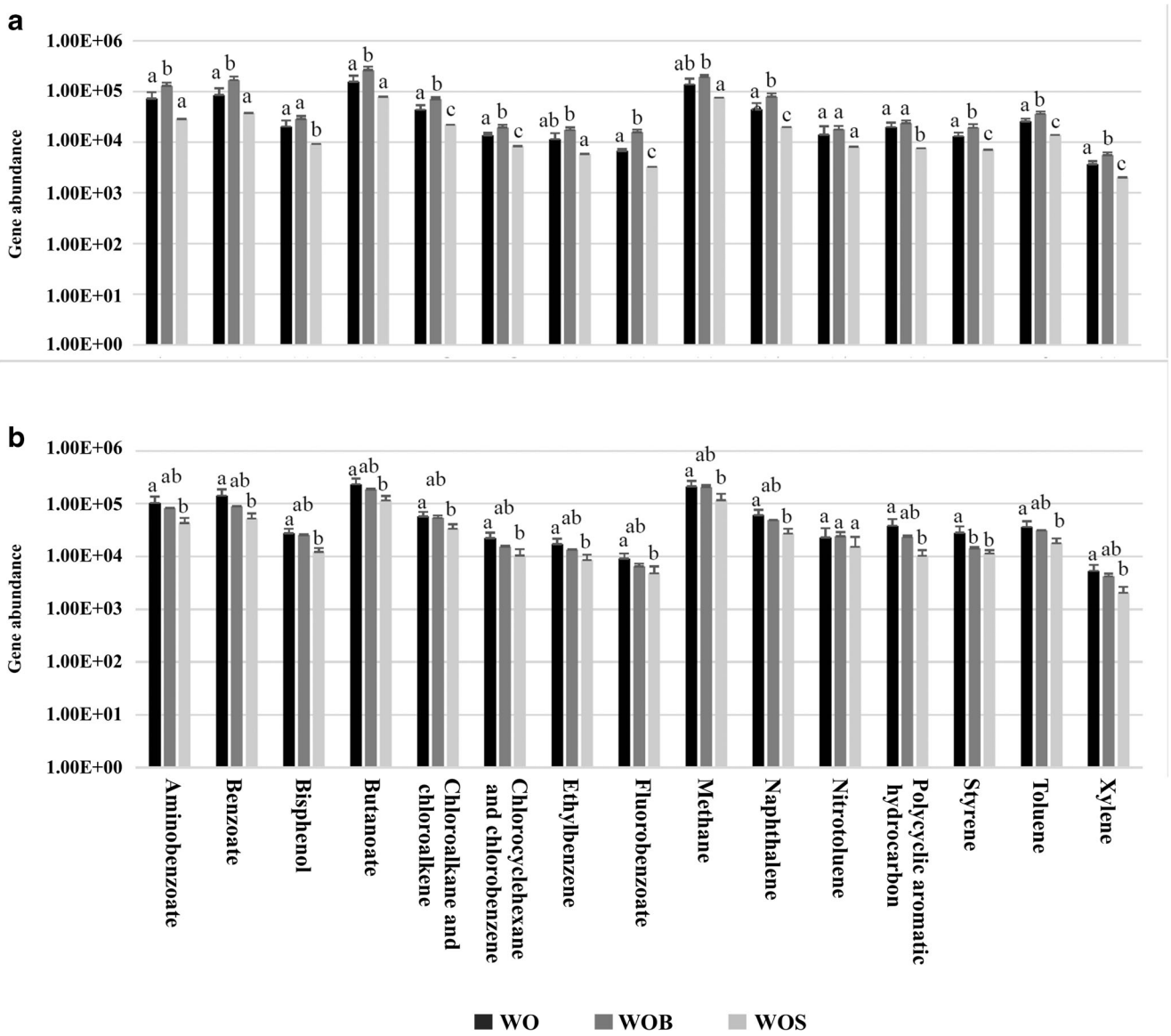

Fig. 4 Abundance of predicted genes coding for metabolic pathways related to hydrocarbon degradation in samples (using PICRUSt) from (A) Grumari beach—GB and (B) Schiermonikoog Island—SI containing oil (WO), oil with surfactin (WOB), and oil with Ultrasperse II (WOS). Different letters indicate statistically significant differences ($p < 0.05$)

more than the chemical surfactant used here. Therefore, data obtained in the present study not only corroborate surfactin as a promising alternative to the recovery of oil-contaminated environments but also demonstrate its effects on the dynamics and diversity of the bacterial communities in two marine waters.

Conclusions

The effects of chemical surfactant (Ultrasperse II) and biosurfactant (surfactin) on the bacterial communities differed in both oil-contaminated and uncontaminated marine water from both regions (GB and SI). The dispersants used influenced more the original bacterial communities than the addition of oil. Different bacteria related to hydrocarbon degradation were stimulated by the addition of both surfactants. The use of surfactin resulted in an increase of gene abundance related to hydrocarbon degradation mainly in the tropical region (GB), while the use of Ultrasperse II led to the decrease of abundance of these genes in both regions studied (GB and SI). Therefore, surfactin is a promising alternative to the petroleum industry, with the potential to contribute to the restoration of oil-contaminated environments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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