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# Spatio-temporal variation of the microbial community of the coast of Lebanon in response to petroleum hydrocarbon pollution

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ABSTRACT

In this study, the coast of Lebanon was analyzed for the dynamic changes in sediment microbial communities in response to a major petroleum oil spill and tar contamination that occurred in the summer of 2021. Spatio-temporal variations in the microbial structure along the shores of Lebanon were assessed in comparison to baseline microbial structure determined in 2017. Microbial community structure and diversity were determined using Illumina MiSeq technology and DADA2 pipeline. The results show a significant diversity of microbial populations along the Lebanese shore, and a significant change in the sediment microbial structure within four years. Namely, *Woeseia*, *Blastopirellula*, and *Muriicola* were identified in sediment samples collected in year 2017, while a higher microbial diversity was observed in 2021 with *Woeseia*, *Halogranum*, *Bacillus*, and *Vibrio* prevailing in beach sediments. In addition, the results demonstrate a significant correlation between certain hydrocarbon degraders, such as *Marinobacter* and *Vibrio*, and measured hydrocarbon concentrations.

## 1. Introduction

The increased recent interest in oil and gas exploration and extraction along the Eastern shore of the Mediterranean basin has raised concerns over the potential occurrence of spills and their associated long-lasting damage to marine ecosystems. One example of such a devastating event is the Jiyeh oil spill in Lebanon which occurred in 2006 and resulted in around 15,000 tons of heavy fuel oil spreading across hundreds of kilometers over the shorelines of Lebanon and Syria, and threatening the shoreline of Turkey (Little et al., 2021). Another recent oil spill event in this area occurred in 2021 along the Syrian coasts, where 15,000 tons of fuel oil were released into the Mediterranean Sea, spreading across >800 $km^2$ and threatening nearby countries including Cyprus and Turkey (Kundu, 2021). In addition, a notable and unusual petroleum-based contamination along the Eastern Mediterranean basin happened recently during the summer of 2021 along the southern coast of Lebanon, from an unknown source, during which large amounts of tar residues washed out on the shores, heavily contaminating the areas with tar balls clinging to sediments (Zodiatis et al., 2021). Furthermore, the heavy maritime oil-tanker traffic within the Mediterranean basin, which reaches about 20 % of the global traffic, increases risks of oil spills and continuously threaten the highly diverse ecosystems across this marine basin (Cucco and Daniel, 2016; Daffonchio et al., 2013; Kostianoy and Carpenter, 2018; Miller and Spoolman, 2019).

It is well known that one of the most highly diverse ecosystems occur along shorelines across the world. The Mediterranean basin is especially notable for being a biodiversity hotspot, especially along its shores, with many endemic and native species belonging to its unique ecosystems. It is estimated that >17,000 species thrive in the Mediterranean Sea and many others are discovered every year (Coll et al., 2010). Such an ecological significance of the Mediterranean basin stresses the importance of prompt and effective oil spill response to minimize damage and protect natural resources.

Several methods exist to contain and clean up oil spills in aquatic environments. Physical, chemical, and thermal methods are widely used for this aim but are associated with undesirable environmental impacts and the disturbance of the ecological balance at contaminated sites (Ndimele et al., 2018). Bioremediation constitutes an alternative oil cleanup technique that is safer for the environment (Fragkou et al., 2021; Hassanshahian et al., 2010), relying on the ability of naturally occurring microbial populations to degrade petroleum hydrocarbon pollutants (Perelo, 2010). Those hydrocarbon degraders form a highly

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dynamic and diverse sediment microbial ecosystem that can, in many cases, efficiently eliminate the pollution (Adams et al., 2015; McGenity et al., 2012).

Many microbial species are usually involved in the degradation of petroleum-based pollution in marine environments, each capable of degrading specific groups of hydrocarbons. This entails a microbial community succession during the oil biodegradation process and dynamic changes in the composition of the microbial consortium in response to the changing composition of the pollution (Alegbeleye et al., 2017; Hassanshahian et al., 2010; Viñas et al., 2005). This highlights the impact of the pollution characteristics in shaping the microbial community structure and further stresses the importance of understanding the dynamics of the microbial ecosystem in affected sites (Ceccaldi et al., 2011; Hassanshahian et al., 2010; Militon et al., 2016). In addition, it is noteworthy to mention that the microbial community structure and function is highly dependent on the physical and chemical characteristics of the impacted site (Hamdan et al., 2019). Hence, marine microbial ecosystems can spatially and temporally change, which would highly affect the capability of the polluted area to degrade petroleum hydrocarbons.

Understanding the structure and dynamics of indigenous marine microbial communities at contaminated sites is essential for successful execution of bioremediation strategies. In a previous study (Hamdan et al., 2019), we characterized the sediment microbial communities along the shores of Lebanon, establishing a baseline microbiome composition of the Eastern side of the Mediterranean basin, which was still ill-characterized. In this study, we evaluate the spatial and temporal microbial dynamics and structural changes along the shores of Lebanon in response to petroleum hydrocarbon pollution. Namely, we aim at evaluating changes in the sediment microbial structure in response to a major petroleum oil spill that occurred in summer 2021 along the Southern shores of Lebanon, and which resulted in a heavy contamination with tar residues. The baseline for comparison is the microbial community characterized in a previous study conducted in 2017 along the Lebanese shores. In addition, we aim at evaluating the spatial variation in the microbial community along the coast of Lebanon, as affected by the spill and other potential sources of hydrocarbon pollution. Spatio-temporal variations of microbial communities across the coast of Lebanon were not assessed before and are significant when considering the high risks of oil spills on the Eastern shores of the Mediterranean basin, requiring effective bioremediation response based on informed decisions.

Sediment samples were collected along the shores of Lebanon from eleven different sites at two distinct points in time: first in year 2017 and then in year 2021 following a major oil spill in summer 2021 polluting the Lebanese shores with tar balls. The sediment microbial community structure was determined and analyzed for all collected samples. DADA2 pipeline, an open-source high-resolution pipeline for analysis of Illumina amplicon sequencing data, was used to process the sequencing files and establish the microbial composition of the sediment samples. Sediment microbial communities were correlated to measured levels of Total Petroleum Hydrocarbons (TPHs), and were analyzed to better understand the long-term dynamics of microbial sediments in response to continuous and emerging sources of contamination affecting pollution type and levels. The results from this study would permit to determine the potential of the Lebanese shores to assimilate petroleum-based pollution through the evolution of its microbial communities towards the dominance of hydrocarbon degraders. They also constitute valuable addition to the state of knowledge of the coastal microbial communities across the Eastern Mediterranean basin, which are still poorly characterized.

## 2. Materials and methods

### 2.1. Sediment collection

The Lebanese shore, which is mostly sandy and extends over 225 km along the Eastern Mediterranean, was sampled for sediments from eleven different sampling sites that span the length of the country. The sites were chosen to be equidistant from one another, in major coastal cities, and far away from any major source of point pollution, to ensure a better representation of the overall sediment contamination profile. The sampling sites are presented in Fig. 1, along with the name of the cities. These sampling locations were assigned a number from one to eleven which represents the southern-most location to the northern-most location. This labeling was based on the northerly prevailing currents along the Lebanese shore, which usually spreads pollution from the south to the north of the country's coast.

The sediments were collected in triplicates at each sampling location. Sampling was repeated twice, first in year 2017 and then four years later following a major tar spill on the Southern coast of Lebanon in 2021. Both sampling events were performed during the dry season. Namely, the 2017 sampling event was carried out during the month of July, and the 2021 sampling event was performed during the month of June. This eliminated potential changes in microbial community structure due to seasonal variations. The sediments were collected from a depth of around 50 cm below the water surface, sealed in bags, placed on ice, and transported promptly for analysis. The sediment samples were then processed for determination of TPHs and microbial structure.

### 2.2. Extraction and measurement of TPHs

A DIONEX Accelerated Solvent Extractor (ASE 350) was used to extract petroleum hydrocarbons based on a method described by Richter (2000). Each of the three replicate samples collected at a specific location on the shoreline was extracted and analyzed separately.

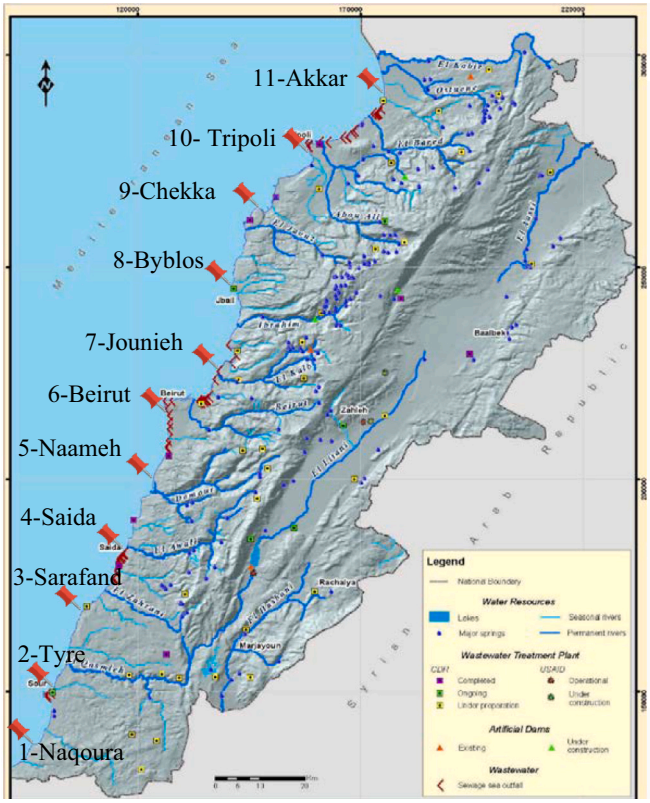


**Fig. 1.** Location of the sediment and seawater sampling sites.

Accordingly, a total 3 TPH extracts were generated per sampling location and were then analyzed separately for TPHs. Reported TPH values in the sediments at each location are thus average concentrations of triplicate samples. For the extraction of TPHs, sediment samples were first sieved through a 2 mm sieve, and then aliquots of around 40 g of wet sediments were mixed with diatomaceous earth (ThermoFisher SCIENTIFIC) as a drying agent, and the samples were then loaded into the ASE cells for TPHs extraction. Acetone and dichloromethane were used as the extraction solvents (acetone:dichloromethane = 1:1) at a temperature of 175 °C. The extraction parameters were set as follows: heat-up time: 8 min, static time: 5 min, flush: 70 %, and nitrogen purge: 60 s. The utilized method does allow for some water to be extracted along; hence, the extracts were passed through 5 g of sodium sulfate for further drying. To concentrate the dried extracts, a rotary evaporator (Buchi R-205 Rotavapor) was used, and the extract was ultimately reconstituted to a final volume of 10 mL using dichloromethane.

The extracts were then analyzed for a complete TPH profile, namely alkanes and PAHs, using an Agilent 7890A gas chromatography (GC) connected to an Agilent 5975C mass spectrometer (MS). Normal alkanes including hydrocarbon chains ranging from nC10 to nC35, and branched alkanes including pristine and phytane were analyzed. PAHs analysis included 2–3-, and 4-rings aromatic compounds along with their alkylated derivatives (C0–4-naphthalenes, C0–3-fluorenes, C0–3-dibenzothiophenes, C0–4-phenanthrenes, anthracene, fluoranthene, C0–3-naphthobenzothiophenes, C0–2-pyrenes, and C0–3-chrysenes). The analysis was performed using an internal standard method described by Campo et al. (2013). Labelled alkanes (D22 nC10, ND34 nC16, D24 nC20 and D62 nC30), and PAHs (D8 naphthalene, D10 anthracene, D12 chrysene) were used as internal standards. Sample injection was performed in splitless mode using 1 μL sample volume. The inlet temperature was 200 °C. A high-resolution gas chromatography column was used (Agilent 122-5532UIE, DB-5ms Ultra Inert GC Column, 30 m, 0.25 mm, 0.25 μm, 5-inch cage), and the flow through the column was set at 1 mL/min. The oven program was set at an initial temperature of 35 °C with a hold time of 2 min, followed by a ramp of 10 °C/min and a final hold time of 10.5 min at 310 °C.

### 2.3. Extraction of sediment microbial DNA

Each of the triplicate sediment samples collected at each sampling location along the Lebanese shore was extracted three times for DNA. The microbial DNA was extracted from the sediment using DNeasy PowerSoil Kit from QIAGEN according to the manufacturer's instructions. This resulted in a total of 9 DNA extracts for each of the sampling sites and for a given sampling year. The nine DNA extracts were then pooled together into a single composite sample representative of the average sediment microbial community for a given sampling site and year. This resulted in a total of 22 final DNA extracts, 11 corresponding to the 2017 sampling event, and another 11 corresponding to sediment DNA extracts of the same locations from the 2021 sampling event. The DNA extracts were then packaged and sent to MR DNA (Shallowater, TX, USA) for characterization of microbial communities by sequencing of the 16S V4 variable region of the rRNA gene on a MiSeq (Illumina, USA).

### 2.4. DNA amplification and sequencing

The V4 region of the 16S rRNA gene was amplified using the 515F (GTGYCAGCMGCCGCGGTAA) and the 806R (GGACTACHVGGGTWTCTAAT) PCR primers. A total of 30 amplification cycles were performed using QIAGEN HotStarTaq Plus Master Mix Kit. The following conditions were applied for the PCR: 95 °C for 5 min, followed by 30 cycles at 95 °C for 30 s (each or 1 s per cycle), 53 °C for 40 s and 72 °C for 1 min. A final elongation step was performed for 10 min at 72 °C. Samples were multiplexed using unique dual indices, and then pooled together in equal proportions based on their molecular weight and concentration of the DNA. Pooled samples were then purified using calibrated Ampure XP beads, and were then used to prepare an Illumina DNA library. Finally, sequencing was performed on a MiSeq following the manufacturer's guidelines.

The raw data generated from the sequencing process was further processed in Rstudio using DADA2, which offers an easy, simple, and high-resolution pipeline for processing of sequencing data (Callahan et al., 2016). The DADA2 pipeline allows for the generation of an Amplicon Sequence Variant (ASV) table. The ASV table provides a more accurate representation of the microbial community as compared to the usually produced Operational Taxonomic Unit (OTU) tables because it can resolve sequence differences as short as a single nucleotide (Callahan et al., 2016). The sequencing data is shared through NCBI as a Sequence Read Archive (SRA) submission with a citation accession of PRJNA966809.

### 2.5. Processing of the sequencing data and bioinformatics analysis

DADA2 package was utilized as the pipeline in Rstudio to process the generated demultiplexed DNA sequencing fastq files. Sequencing files were loaded into Rstudio. The sequences were first filtered and trimmed utilizing a standard truncation length of 240 and 160 for the forward and the reverse reads, respectively, based on the quality profiles of the reads. The filtered sequences were then dereplicated to combine all identical sequencing reads into unique sequences. Then, the core sample inference algorithm was performed for the forward and the reverse reads and then both reads were merged to obtain the full denoised sequences. Merging was performed by aligning the denoised forward and the complementary reverse reads. After the merging step, the ASV table was constructed. Finally, chimeras were removed to increase the accuracy of the results.

Finally, an important step of this analysis was to assign taxonomy to the identified sequence variants. For this aim, Silva SSU taxonomic training data V.138 (updated on March 10, 2021) that includes taxonomy down to the species level, was used as a reference training set. This allowed for finalizing the processing of the sequencing data by generating and exporting the final ASV table for further downstream processing.

The ASV table was then used for further bioinformatics analysis using the Rstudio ampvis2 package. This package was used to generate heatmaps, Principal Component Analysis (PCA), and diversity indices. Finally, STAMP software (Statistical Analysis of Taxonomic and Functional Profiles) was used to perform the statistical analysis of the samples in this study.

## 3. Results and discussion

### 3.1. Petroleum hydrocarbons

Concentrations of TPH across all sites are presented in Fig. 2, showing both PAHs and alkanes fractions. For the 2017 sampling event, TPH ranged around 0.64 ± 0.19 mg/kg of sediment, with PAHs and alkanes contributing to an average of 14.97 % and 85.03 % of the TPH, respectively. Collected samples during the 2021 sampling event exhibited considerably higher TPH concentrations. Excluding Tripoli (site 10), which showed a huge increase compared to all other locations, TPH levels exhibited an average of 2.79 ± 1.07 mg/kg of sediment, with PAHs and alkanes contributing to an average of 9.61 % and 90.39 % of the TPH, respectively. This indicates a significant rise in the TPH pollution levels across the shoreline over four years across the entire shoreline. It is noteworthy to mention that this rise was observed across all sampling sites and was statistically significant ($p < 0.05$; *t*-test) between the 2017 and the 2021 sampling events, except for a single site, Byblos. In Byblos, the TPH pollution levels did not significantly change in four years when compared together ($p = 0.5$). Excluding the substantial TPH pollution measured in Tripoli (site10), reported increase in

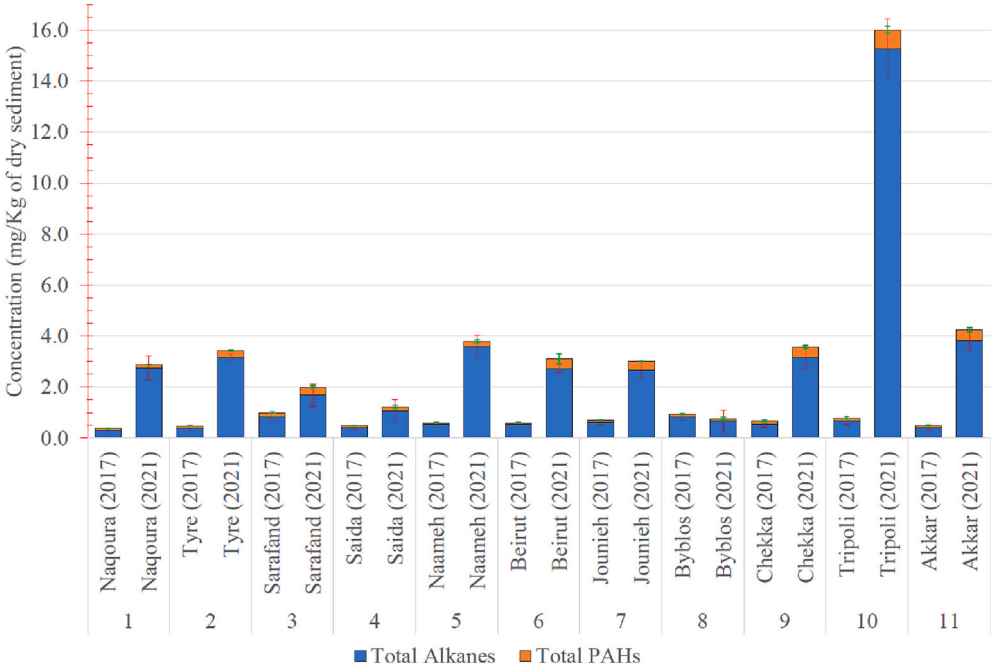


**Fig. 2.** TPH concentrations along the Lebanese shoreline.

TPH levels along the shore of Lebanon ranged from 98 % in Sarafand, up to 681 % in Naqoura. Several recent studies reported hydrocarbon pollution levels across the Mediterranean basin. The concentration of petroleum hydrocarbons along the shorelines of Turkey, located in the Northeast of the Mediterranean, was recently reported to range between 6.5 and 11.2 mg/kg of sediment (Ünlü and Özgür, 2022). Higher pollution levels with PAHs were reported along the Egyptian shorelines, located in the Southeastern Mediterranean, and ranged between 13.1 and 34.8 mg/kg of sediment (El-Maradny et al., 2023). Furthermore, the concentration of PAHs along the Italian shores (Northern Mediterranean) ranged from 0.4 to 0.8 mg/kg of sediment (Montuori et al., 2021). In addition, it was reported that alkanes pollution across the Mediterranean basin varied significantly, and varied between 0.8 and 6.63 mg/kg of sediment at the Eastern shores of the Basin (Gadelha et al., 2019). This highlights the significant pollution levels across the Mediterranean shorelines, and further stresses the importance of understanding the dynamics of its microbial populations in response to the encountered pollution type and extent.

The significant increase in the TPH levels is most probably associated to the recent oil spill which occurred in summer 2021 on the Southern shores of Lebanon, where large amounts of sticky tar balls contaminated beaches stretching from the town of Naqoura to the city of Tyre, and spread along the northern shores (Skynews Arabia, 2023). In addition, small amounts of tar balls and fragments were still reportedly washing up on the shoreline following the 2021 spill, but those were likely coming from other sources elsewhere in the Mediterranean carried by sea current. Pollution with petroleum tar is more resistant to natural attenuation as compared to petroleum oil and may become persistent (Al-Hawash et al., 2018; Shinde et al., 2020). Other sources of petroleum hydrocarbon contamination existing along the Lebanese shores may also have contributed to the increased levels of TPH. Those include ports, recreational sites, industrial effluents, and dumpsites, and contribute to hydrocarbon pollution ranging from moderate to high levels of contamination (Dai et al., 2022; Hamdan et al., 2019). The exceptionally higher increase in TPH levels in Tripoli may be attributed to increased anthropogenic activities at Tripoli port facilities (such as shipping and transport operations) especially after the explosion of the port of Beirut in 2020 and the redirection of the majority of the Lebanese imports through the port of Tripoli, the second largest port in the country. In addition, sitting just 30 Km from the Syrian border, Tripoli has witnessed increased urbanization over the last years due to the influx of Syrian refugees. This, in addition to the uncontrolled coastal dumping of solid wastes and the extensive commercial fishing in the area may have also contributed to the high measured levels of petroleum hydrocarbons. It is expected that this temporal increase in the levels of hydrocarbon pollution would have entailed a shift in sediment microbial population along the Lebanese shoreline, favoring hydrocarbons degraders.

### 3.2. Microbial community analysis

#### 3.2.1. Microbial diversity

All sediment samples exhibited highly diverse microbial ecosystems. The Shannon diversity index was calculated based on 10,000 reads per sample using the defaults of the function 'amp_alphadiv' within the package ampvis2 in Rstudio. The Shannon diversity indices of the

**Table 1**
Diversity indices of the sediment microbial communities.

| Location | Year | Shannon diversity index |
|---|---|---|
| Akkar | 2017 | 6.96 |
| | 2021 | 7.93 |
| Beirut | 2017 | 6.73 |
| | 2021 | 6.86 |
| Chekka | 2017 | 7.05 |
| | 2021 | 7.62 |
| Jbeil | 2017 | 6.47 |
| | 2021 | 7.75 |
| Jounieh | 2017 | 6.68 |
| | 2021 | 6.98 |
| Naameh | 2017 | 6.75 |
| | 2021 | 7.33 |
| Naqura | 2017 | 6.49 |
| | 2021 | 7.87 |
| Tyre | 2017 | 6.70 |
| | 2021 | 6.72 |
| Saida | 2017 | 7.05 |
| | 2021 | 7.70 |
| Sarafand | 2017 | 6.58 |
| | 2021 | 7.60 |
| Tripoli | 2017 | 6.87 |
| | 2021 | 7.65 |

samples ranged from 6.47 to 7.92 (Table 1). The number of observed ASVs ranged from 796 to 3974, with taxonomy assignment being relatively high reaching 71 % at the family level, 48.3 % at the genus level, and 9.52 % at the species level, out of the different identified sequence reads.

Principal coordinate analysis (PCoA) was conducted to determine the clustering of the microbial communities and assess their relatedness to each other. Fig. 3 shows the PCA plot of identified microbial populations in each of the 2017 and 2020 sampling years.

The PCA plot shows two distinct clusters of points corresponding to the microbial populations identified at each of the sampling years, indicating a shift in the microbial composition over the study period at all sampled locations. In addition, the points in the 2021 cluster are more spread out as compared to the points in the 2017 cluster, indicating further divergence of the microbial community along the Lebanese shoreline in year 2021. This can be explained by the higher hydrocarbon pollution levels reported in this sampling year and their higher variability among sampled sites entailing further selectivity across the shoreline of Lebanon. Such a high diversity within the microbial community of the Lebanese shore sediment, within the same sampling event and across the years, indicates a high potential for a dynamic change of the microbial community in response to the ever-changing site conditions. In general, the microbial community of sediments is highly diverse, especially near the shoreline. Mahmoudi et al. (2020) reported that the diversity in the sediment microbial ecosystems decreases with increased depth below the water (Mahmoudi et al., 2020). Furthermore, Lalzar et al. (2023) recently demonstrated that the diversity and composition of the sediment microbial communities in Mediterranean sediments were influenced directly by many environmental factors and anthropogenic stressors, such as petroleum hydrocarbons pollution (Lalzar et al., 2023).

### 3.2.2. *Microbial structure*

Fig. 4 shows the heatmaps of the 2017 and 2021 overall microbial communities determined along the shore of Lebanon. Statistical differences among the taxa were calculated using STAMP, utilizing Welch's two-sided t-test with a p of 0.05.

A significant presence of the phylum proteobacteria was observed in both of the 2017 and 2021 microbial communities, with an average relative abundance of 39.7 ± 6.1 % and 41.5 ± 11.6 %, respectively, with no significant difference ($p = 0.64$). Similarity was also observed for the third most abundant Phylum, which was actinobacteriota, at an average relative abundance of 8.2 % ± 2.3 % and 9.4 % ± 4.6 %, respectively ($p = 0.47 > 0.05$). Bacteroidota, the second most abundant phylum in both sampling years, was significantly different between 2017 and 2021 sediments ($p < 0.05$), composing 18.7 % ± 5.4 % and 12.2 % ± 3.7 %, respectively. Planctomycetota showed a major presence in the 2017 sediment microbial community (10.8 % ± 3.5 %) compared to much lower average abundance in the 2021 sediment community (3.5 % ± 2.7 %), while firmicutes and halobacteria were mainly present in the 2021 microbial community (8.5 % ± 14.4 % and 8.1 % ± 16.6 %, respectively), and were negligible in the 2017 microbial communities. It is noteworthy to highlight such differences as it gives an insight into the overall variability in the sediment microbial structure over the years, though it does not indicate very specific functional differences at such a high taxonomic level.

To further investigate functional differences in the sediment microbial communities across the years, analysis of more closely grouped taxonomic levels was performed. Comparison at the class level showed a significant average relative abundance of gammaproteobacteria, at 27.2 % ± 5.3 % and 32 % ± 10.6 % for the 2017 and 2021 microbial communities, respectively, which was not significantly different ($p = 0.25$). The class gammaproteobacteria is well known to encompass many important generalist hydrocarbon-degrading genera as well as obligate hydrocarbon degraders, especially in marine environments (Gutierrez, 2017; Hamdan and Salam, 2020; Romanenko et al., 2010). Hassanshahian et al. (2010) reported that hydrocarbon contamination in marine sediment could significantly stimulate the activity of genera within the gammaproteobacterial class, such as Alcanivorax, which can easily outcompete with other microbial taxa by utilizing alkanes as a sole source of carbon. Bacteroidia was significantly more abundant ($p < 0.05$) in the 2017 community (17.4 % ± 5.6 %) compared to the 2021 community (10.6 % ± 3.9 %), being the second most abundant class in both years. Class bacteroidia was reported to be present in many marine

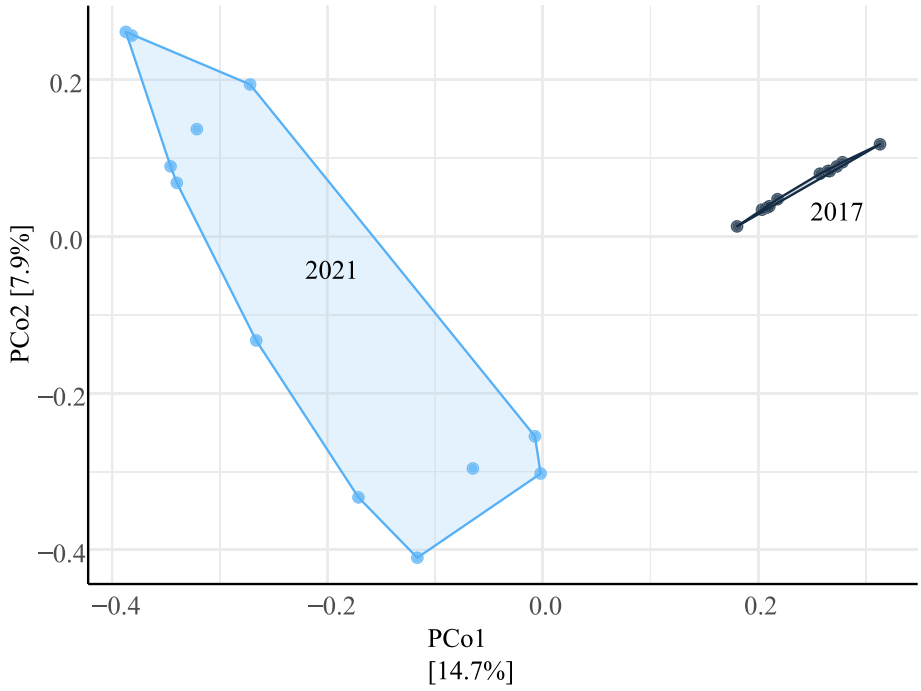


**Fig. 3.** PCoA plot based on Bray-Curtis distance and Hellinger transformation showing the relatedness of the bacterial community composition between the different samples.

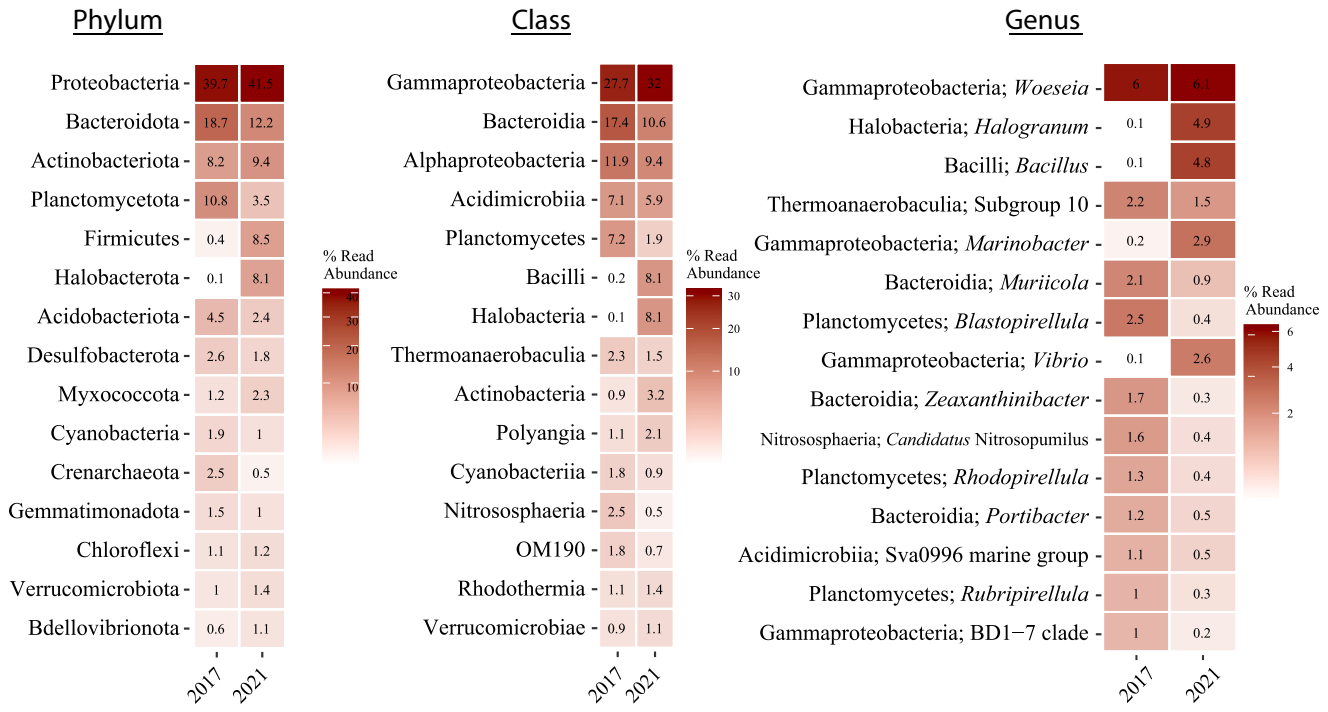


**Fig. 4.** Heatmaps of the overall shoreline microbial community during 2017 and 2021.

sediment samples and cultures, and was especially noted in hydrocarbon-polluted sediments (Dell'Anno et al., 2021). It was also reported for its involvement in the degradation of complex hydrocarbons, and its potential capacity to degrade plastics (Näkki et al., 2021). Alphaproteobacteria and acidimicrobiia were statistically similar in both sampling years ($p = 0.19$ and 0.37, respectively), ranging in average relative abundance between 5.9 % and 11.9 %. Alphaproteobacteria is well known to dominate sediments of marine environments following exposure to oil pollution, but in lower average abundance compared to gammaproteobacteria (Kostka et al., 2011), while acidimicrobiia was reported for its potential bioremediation capabilities in highly contaminated marine sediments (Dell'Anno et al., 2021). The observed similarity in microbial communities determined in 2017 and 2021 as indicated by the general profile of the most abundant classes indicates that the continuous exposure to hydrocarbon pollution over four years has shaped the associated microbial communities involved in the pollution degradation.

On the other hand, significant differences in the average relative abundance were observed in other classes, including planctomyces, bacilli, and halobacteria. Planctomyces was a major component of the 2017 microbial community, comprising 7.2 % ± 3.0 % of the total microbial community compared to only 1.9 % ± 1.3 % in the 2021 community. Bacilli and halobacteria were negligible in the 2017 sediment microbial community but composed 8.1 % ± 13.1 % and 8.1 ± 16.6 %, respectively, in the 2021 microbial community. Class planctomyces is reported to be widely distributed in marine environments, especially in the sediments, and is known to be responsible for some unique processes such as anaerobic oxidation of methane and ammonium (Hamdan et al., 2019; Jasmin et al., 2017). This class was also reported to be able to tolerate at a wide range of oxygen concentrations, permitting its existence in both oxic and suboxic zones of marine sediments (Fuchsman et al., 2012). Finally, class bacilli is comprised of orders bacillales and lactobacillales, both known to contain hydrocarbon-degrading genera (Kim and Kwon, 2010). Such observations may indicate that lower-level pollution detected during year 2017 favored certain classes such as bacteroidia, acidimicrobiia, and planctomyces, and that a higher-level pollution with heavy hydrocarbons such as tar residues might have favored other groups such as Bacilli and halobacteria. This also suggests a general involvement of the aforementioned groups with degradation of the corresponding lower- or higher-level petroleum pollution.

To better understand the dynamic variability of the microbial groups in response to the existing levels of hydrocarbon pollution, further investigation was performed at the genus level to identify the most active microbial taxa during each of the sampling years. Among the identified genera, during both sampling events, Woeseia was the highest in average relative abundance at 6 % ± 1.3 % and 6.1 % ± 5.3 % during 2017 and 2021, respectively, with no statistical difference between the two years. Woeseia was recently reported for being a chemoheterotrophic genus with the ability to degrade hydrocarbons (Bacosa et al., 2018). Its presence in high average relative abundance at almost same levels across both sampling years, irrespective of the hydrocarbon pollution levels, suggests that it has a role as a general hydrocarbon degrader.

Nonetheless, at the genus level, significant differences were observed in multiple groups, being abundant during one of the sampling years and negligible in the other. For example, in the 2017 samples, Muriicola, Blastopirellula, Zeaxanthinibacter and Candidatus Nitrosopumilus where significant at 2.1 % ± 1.1 %, 2.5 % ± 1.2 %, 1.7 % ± 0.8 % and 1.6 % ± 0.8 %, respectively, while being negligible in the sediment samples collected in 2021. Muriicola was previously reported to be present in marine sediments and even in some soils, but its role in hydrocarbon degradation is yet to be reported (Hamdan et al., 2019; Hu et al., 2015; Kahng et al., 2010). Blastopirellula was repeatedly reported in marine environments (Kallscheuer et al., 2020; Rensink et al., 2020; Schlesner et al., 2004), but no clear information is available currently about its involvement in hydrocarbon degradation. Although this Genus was reported in multiple aquatic environments, such as marine and estuarine sediments and water (Asker et al., 2007; Lee et al., 2016), no clear correlation with petroleum pollution degradation or other functions were reported. Finally, Candidatus Nitrosopumilus belongs to the group of autotrophic and aerobic Archaea, with some species of this Genus reported to utilize urea as a source of energy for growth. This Genus is commonly found in brackish and marine environments, and is reported to have an obligate requirement for salt (Qin et al., 2016). It was not reported though for its involvement in hydrocarbon degradation.

On the other hand, Genera Halugranum, Bacillus, Marinobacter, and Vibrio, presented a relatively higher average abundance in the 2021 microbial community. These genera comprised 4.9 % ± 10.5 %, 4.8 % ± 9.9 %, 2.9 % ± 3.0 %, and 2.6 % ± 5.6 % of the total 2021 microbial community, respectively. Halogranum was previously identified in marine salterns (Cui et al., 2010), indicating its correlation with salty environments; however, it was not reported to be involved in degradation of petroleum hydrocarbons. Nonetheless, Bacillus, Marinobacter, and Vibrio were all previously reported for their ability to degrade petroleum-based pollution as well as for their involvement in various marine sediment bioremediation processes of petroleum hydrocarbons. For example, Bacillus was reported for its degradation of petroleum hydrocarbons in marine environments, including heavy PAHs such as pyrene and benzo[a]pyrene (Wang et al., 2019), Marinobacter is a widely-spread and common marine bacterium known for its ability to efficiently degrade aliphatic and aromatic hydrocarbons (Duran, 2010; Hamdan and Salam, 2020, 2021; Handley and Lloyd, 2013), and Vibrio is known to be a marine PAH-degrading bacterium (Gezginci and Uysal, 2015; Hamdan and Salam, 2021; Hedlund and Staley, 2001). The emergence of hydrocarbon-degrading genera in year 2021, namely genera known for their ability to degrade PAHs, could be correlated to the tar spill which happened in summer of 2021 and might have encouraged the thriving of hydrocarbon degraders that are specialized with the removal of heavy PAHs and long chain alkanes such as those present in tar.

### 3.2.3. Site-specific differences

Spatial variations in the microbial structure were observed in both sampling events as evidenced by the weak clustering of the points on the PCoA plots. Higher variation was reported in the case of samples collected in 2021 as demonstrated by the larger distances between data points corresponding to this year (Fig. 3). The higher microbial variation observed across the shoreline in 2021 compared to 2017 could be due the higher measured TPH levels during this year in beach sediments, and their relatively higher variability among sampled sites (Fig. 5). Notable examples are apparent at multiple sites and for various genera, such as *Halogranum* in Tyre and Chekka, *Bacillus* in Tyre, Naameh and Beirut, and *Vibrio* in Beirut and Tripoli.

*Halogranum* is especially apparent in site 2 (Tyre) and site 9 (Chekka) during the 2021 sampling event, and practically non-existent in most other sites across both years. *Halogranum* reached a relative abundance of 35.7 % in Tyre during 2021, accounting for more than third of the total sediment microbial community at that location. While *Halogranum* is yet to be described in relation to its involvement in the degradation of petroleum hydrocarbons, its presence at a relatively high abundance at Tyre, the location most impacted by the tar spill in 2021, suggests its correlation with the emerging heavy pollution with the sticky tar balls. The fact that the relative abundance of *Halogranum* was also high in Chekka far northern the spill location, could be attributed to other sources of petroleum hydrocarbons pollution in the area, which is home of some of the biggest industrial facilities in northern Lebanon.

*Bacillus*, as described earlier, can degrade recalcitrant petroleum hydrocarbons such as high molecular weight PAHs, which are major constituents of petroleum tar residues such as tar balls following a contamination event (Shinde et al., 2020). Composing 34.6 % of the total sediment microbial community in Beirut in 2021 (site 6; located in the middle of the shoreline of Lebanon), *Bacillus* occurrence could be mostly correlated with lingering contamination with TPH at this location. Indeed, the capital of Lebanon, Beirut, hosts the main commercial and recreational ports in the country, which are source of petroleum and PAHs contamination, namely through loading and unloading operations, shipping, transportation, fishery activities, and industrial and urban discharges. The absence of *Bacillus* genus in year 2017 at Beirut shores could be due to significantly lower hydrocarbon contamination levels reported in that year, along with the occurrence of other hydrocarbon degrading genera such as *Muriicola* and *Marinobacter*. Same observation was noted also in Beirut for the Genus *Vibrio*, another hydrocarbon and PAH degrader, composing 18.4 % of the total sediment microbial community during 2021, while being absent in sediment samples collected in 2017. Hence, a total of 53 % of the total sediment microbial community occurring in 2021 at Beirut shores was composed of two major hydrocarbon degraders, *Bacillus* and *Vibrio*. The prevalence of hydrocarbon and PAH degraders could be confidently attributed to the increased TPH levels measured in Beirut over the four years sampling period, and the continuous exposure of the beach sediments to hydrocarbons and PAHs pollution at this location, which had significantly shaped the microbial consortia towards specialized taxa in response to the persistent pollution.

Furthermore, the microbial community in Tripoli (site 10) was dominated by hydrocarbon degraders *Marinobacter* (4.7 %) and *Vibrio* (9.3 %) during 2021. Those genera were negligible four years earlier in 2017, when the sediment microbial community was dominated by *Woeseia* (4.7 %) and an unclassified genus (subgroup 10, belonging to class thermoanaerobaculia) (4.4 %), which are not reported for being

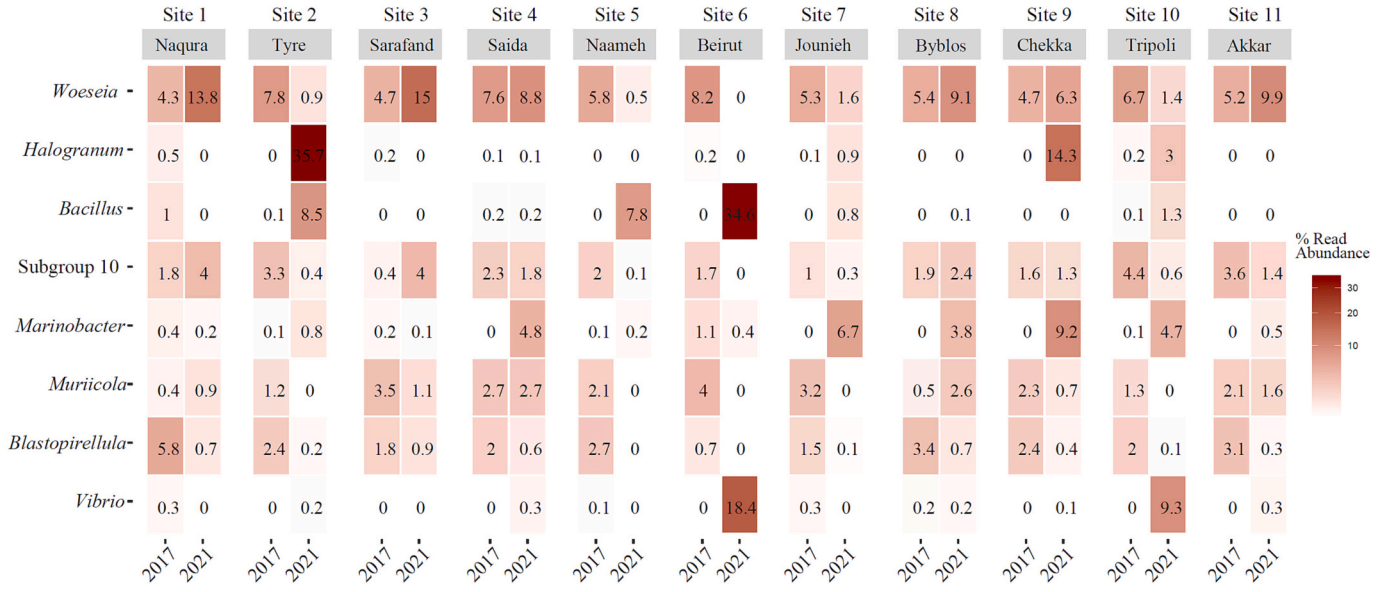


**Fig. 5.** Site-specific genera heatmaps.

hydrocarbon degraders. Considering the major increase in TPH concentration in Tripoli from 0.785 mg/kg of sediment in year 2017 to 16.002 mg/kg of sediment in 2021, it is expected that the sediment microbial community would observe a significant change, further highlighted by the significant difference in the average relative abundance of all of the most abundant genera at that site, with a higher contribution from hydrocarbon degraders. Besides activities undertaken at the port of Tripoli, the second major port in Lebanon, coastal solid waste dumping and discharge of raw industrial effluents are main contributors to hydrocarbons and PAHs pollution in Tripoli. In addition, the large increase in TPH levels in Tripoli beach sediments in 2021, exceeding by far the increase observed across all other locations along the shoreline of Lebanon, could be mostly due to the fact that Tripoli port, usually operating at only 40 % of its capacity, had to accommodate all Lebanese imports following the 2020 Beirut explosion. This definitely contributed to major increase in hydrocarbons and PAHs contamination along Tripoli's shoreline. All of these observations further highlight the potential close relationship between the levels of petroleum hydrocarbon pollution and the dynamic change of the sediment microbial communities across the Lebanese shore. This also stresses the high potential of the Lebanese shore to withstand any significant source of petroleum pollution. Yet, it is also important to note that the variation in the sediment microbial structure, although heavily influenced by high levels of petroleum hydrocarbons, is also affected by other sediment and seawater parameters and sources of pollution, such as nitrogen and phosphorus levels, temperature, salinity, etc. To better understand the potential correlation of highly abundant genera with the TPH pollution levels, further analysis was performed to identify any potential trend.

### 3.2.4. *Abundant taxa in relation to pollution levels*

Analysis of the sediment microbial communities determined in 2017 and 2021 was performed in relation to the TPH pollution levels observed at the different studied locations. The TPH levels ranged from 0.368 mg/kg of sediment, observed in Naqoura (site 1) in 2017, up to 16.002 mg/kg of sediment, observed in Tripoli (site 10) in 2021. The pollution levels that were measured in this study were assigned to five different categories: Trace (0–0.5 mg/kg of sediment), Low (0.5–1 mg/kg of sediment), Moderate (1–2 mg/kg of sediment), High (2–4 mg/kg of sediment), and Heavy (>4 mg/kg of sediment). Genera heatmaps were then generated based on the assigned pollution levels (Fig. 6).

*Woeseia* exhibited a high average relative abundance under different pollution levels across the Lebanese shoreline. This genus also showed no significant difference in average relative abundance across the two sampling events (Fig. 5). This could imply that *Woeseia* is not involved in hydrocarbon degradation or its involvement is limited to being a generalist degrader. Under heavy contamination, there was notable presence of *Vibrio* at 4.8 % ± 4.5 %, which was significantly different in this group compared to all other contamination levels. *Vibrio* was also apparent under high contamination at 2.7 % ± 6.4 %, and its average relative abundance decreased with decreasing contamination levels. This suggests a strong correlation between the occurrence of *Vibrio* and increased levels of TPH contamination across the Lebanese shoreline.

Furthermore, under high levels of contamination, there was major occurrence of *Halogranum* and *Bacillus*, at 7.3 % ± 12.6 %and 7.4 % ± 11.6 % of the total sediment microbial community. Those genera were negligible under other pollution levels, including heavy pollution. This suggests that these two genera might be highly active within the high range of petroleum hydrocarbon pollution. This also suggests that *Halogranum*, which is still not reported as hydrocarbon degrader, might be involved in the degradation of petroleum hydrocarbons.

For the remaining lower levels of contamination (moderate, low and trace), there was no major abundance of a single genus in the sediment microbial community, but rather a more even distribution of multiple genera such as *Muriicola*, *Blastopirellula*, *Zeaxanthinibacter*, *Candidatus* Nitrosopumilus, as well as others, ranging from1.4 % ± 0.3 % to 3.6 % ± 1.6 %. This suggests that, at lower pollution levels, microbial communities include more generalist microbes, while special degraders emerge at high concentrations of persistent pollutants which determine the evolution of the microbial community.

Pearson's correlation coefficients were also calculated using the GGally package in Rstudio by correlating the concentration of TPH to the occurrence of the ten most abundant Classes and the ten most abundant Genera (Fig. 7). This analysis complements the correlation between TPH levels and relative abundance of the most abundant taxa

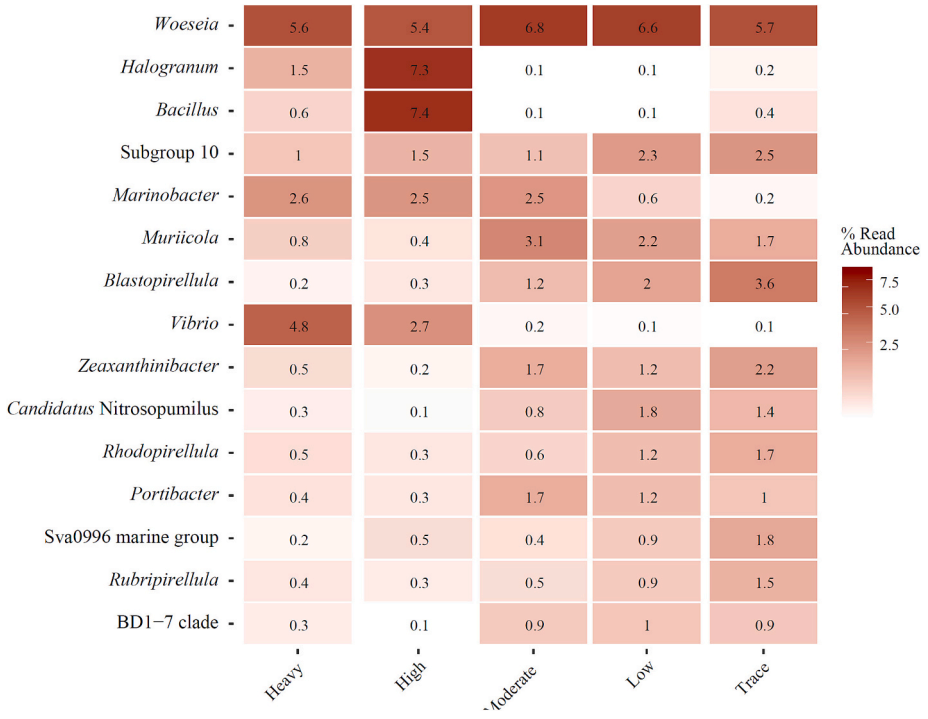


**Fig. 6.** Heatmap of genera based on pollution level.

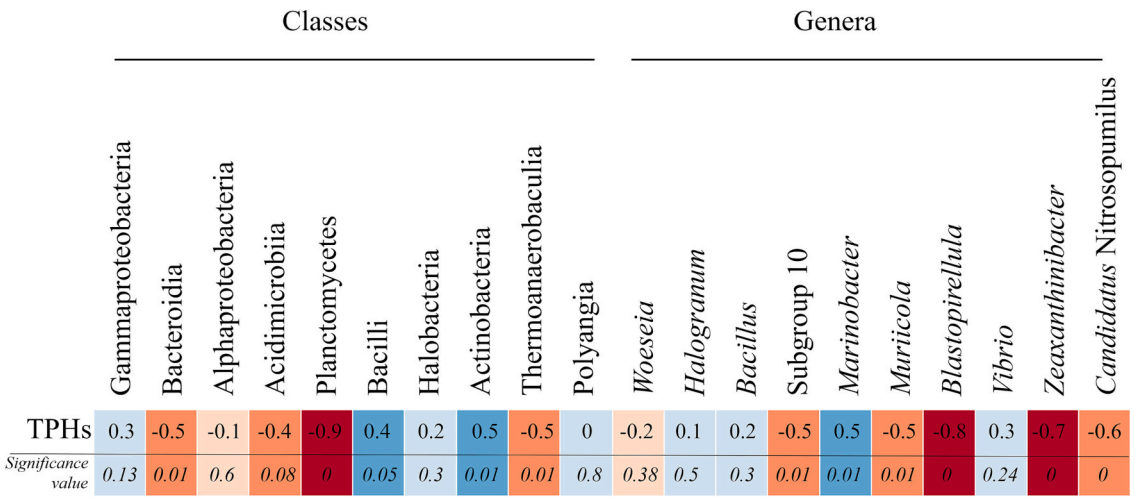


**Fig. 7.** Pearson's correlation coefficients of most abundant genera and TPH levels.

(Fig. 6), and permits determining the potential involvement of certain sediment microbial taxa in degrading petroleum hydrocarbons.

The strongest negative correlations with TPH concentration were observed with the class planctomycetes (−0.4–0.9), and with the genera *Blastopirellula* (−0.8), *Zeaxanthinibacter* (−0.7), and *Candidatus* Nitrosopumilus (−0.6), which are yet to be described in terms of their hydrocarbon degradation capabilities. These Taxa were present in moderate average relative abundances compared to other groups as shown in the heatmaps (Figs. 4 and 6).

On the other hand, no strong positive correlations were observed between TPHs and the most abundant genera. Only moderate positive correlations were observed in the case of the class actinobacteria (0.5) and the genus *Marinobacter* (0.5) with *Marinobacter* being highly abundant under heavy and high TPH pollution levels (Fig. 6). *Marinobacter* already appeared in multiple locations where the pollution was significant (Fig. 5). This further suggests the involvement of this genus in the degradation of petroleum hydrocarbons across the Lebanese shoreline.

While the results demonstrate that higher abundances of hydrocarbon degraders are well associated with higher pollution levels, several genera which were suggested to be involved in hydrocarbon degradation, such as *Halogranum*, did not show neither a strong positive nor a strong negative correlation with increased TPH concentrations. This, however, does not eliminate their possible involvement as potential hydrocarbon degraders, especially that they were highly abundant in heavily polluted sites.

## 4. Conclusion

This study provides a thorough investigation of the spatio-temporal changes in sediment microbial communities along the shoreline of Lebanon, and provides insights of the dynamic nature of the sediment microbial consortia on the Eastern side of the Mediterranean coastline, highly prone to petroleum pollution events. Fully understanding the capabilities and dynamics of the marine ecosystems is extremely essential to plan remediation strategies. This becomes even more significant when considering the continuous exposure of the sediment to sources of pollution including petroleum hydrocarbons. The results from this study clearly highlight the extremely changing nature of the sediment along the coastline of Lebanon in response to new and continuous hydrocarbons contamination, and its potential to rapidly evolve and adapt to the changing environmental conditions. This reflects the potential of the Lebanese shoreline to assimilate hydrocarbons pollution and permits, accordingly, the planning of bioremediation strategies in response to potential petroleum oil spills.

## CRediT authorship contribution statement

**H.Z.H.:** Investigation, Methodology, Analysis, Data curation, Writing, Visualization; **F.A.A.:** Investigation, Analysis, Data curation; **R. M.Z.:** Investigation; **D.A.S.:** Conceptualization, Methodology, Validation, Analysis, Resources, Data curation, Review & Editing, Supervision, Project administration, Funding acquisition; All authors read and approved the final manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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