

A STUDY OF MICROBIAL CRUDE OIL DEGRADATION IN WATER AND SEDIMENT FROM THE GREENLAND SEA

Addendum to the DCE report no.347

Scientific briefing from DCE – Danish Centre for Environment and Energy Date: 11 August 2022 | **48**



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Front page photo: Greenland Sea, September 1, 2017. (Photo: Anders R. Johnsen)

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Background

This is an addendum to the main report “A study of microbial crude oil degradation in water and sediment from the Greenland Sea” (Johansen et al. 2019, <http://dce2.au.dk/pub/SR347.pdf>) submitted as a part of the Northeast Greenland Environmental Study Program.

To provide a more detailed insight into the microbial community data created by DNA sequencing, as presented in the main report, additional in-depth analysis is presented here.

The sequencing data were divided in abundant (figures 1, 4 and 6) and rare (figures 2, 5 and 7) taxa (operational taxonomic units; OTUs). Even though there is not a universal definition of abundant and rare taxa, various studies have used different threshold values for rare taxa, ranging from 0.01% to 5 % of the relative abundance. This is based on the resolution, depth, and richness of the dataset as well as the sampled environment. In the present briefing, 0.1% is used as the threshold based on the richness and previous optimizations (Malard et al., 2019). The division of data into dominant and rare taxa allows understanding the domination of major taxa, without the potential skewness caused by the large number of taxa that have a significantly lower abundance in such studies where a certain taxon can be heavily dominating. The division in abundant and rare taxa shows that around 7.1 (± 3.7) % of all OTUs have an abundance of at least 0.1% in all three datasets. The datasets were: Prokaryotic community composition (i.e. bacteria and archaea) of taxa present in the raw sediment samples of ten sampling stations, microcosm experiments with sediment microbiota, and filtride microcosms, respectively. Illustration of the compositional differences (Beta-diversity) between the microbiomes of the ten sampling stations is given in figure 3.

Archaeal Insights

In addition to the archaeal analysis in the main report, we here observed that the pool of abundant taxa in both sediment and seawater filtride was heavily dominated by the archaean class *Nitrososphaeria*, especially the family *Nitrosopumilaceae* which dominates with at least 75% of the abundant taxa (see figures 1, 4 and 6). This taxon is also seen in a few other studies, such as in Campeão et al. (2017) where the same taxon family (*Nitrosopumilaceae*) was reported to have an abundance of 21% to 35% in the archaeal community in the deep sea from the Amazonian Basin associated with oil degradation. *Nitrosopumilaceae* was also reported to be abundant in another study (Mason et al., 2012), where the authors investigated the microbial response to the Deepwater Horizon oil spill in the Gulf of Mexico using metagenomics, metatranscriptomics and single-cell sequencing approaches.

The pool of rare taxa in both sediments and seawater filtride suggests relative high abundance of general archaean classes, such as *Woesearchaeia*, Marine Benthic Group A, *Bathyarchaeia* and *Halobacteria* (Figure 2, 5 and 7) which are also found in different studies on sediments and deep seawater (Baricz et al., 2019; Lauer et al., 2016; Yu et al., 2017). This could indicate that the rare taxa are not affected by the oil contamination as they are also present at other similar habitats. There is no indication that the rare taxa influence the degradation of the oil compounds.

Bacterial Insights

In addition to bacterial community data presented in the main report, the outcome of additional data analysis is described here. Another dominant taxon, found in abundant bacteria (>0.1%), is *Alteromonadales*, order of *Gammaproteobacteria*, which is observed in both sediment samples and seawater filtrate taxa (figure 1, 4 and 6). Representatives of this taxonomic group have been observed as oil bio-degrading bacteria in Amazonian basin (Campeão et al., 2017). However, other bacterial families, such as *Pseudomonodaceae* and *Oceanospirillaceae*, were found to be oil degraders in similar studies. *Pseudomonodaceae* is present among the abundant OTUs, but only in microcosm/enrichment conditions which may indicate that they are associated with oil degradation.

In the pool of rare bacterial OTUs, sub-groups of *Acidobacteria*, *Chloroflexi*, *Firmicutes* and *Proteobacteria* seem to dominate (Figure 2). These groups are generally observed in deep-sea water and sediments as also pointed out by Zheng et al. (2019) in their study on microbial communities in sediments of the Bohai Sea, Yellow Sea and South China Sea (Zhang et al., 2019). Like the Archaea, the bacterial community at the present sampling sites in Greenland indicates a microbiota similar to other likewise habitats.

Conclusions

Although the data from the sampling stations and microcosm experiments with sediment microbiota cannot be compared directly, they show a remarkable resemblance with regards to both common and rare taxa.

Finally, the enrichments revealed insights into prokaryotic taxa, which are potentially involved in the oil degradation. The rare members of the communities appeared to be more frequently associated with natural deep-sea habitats.

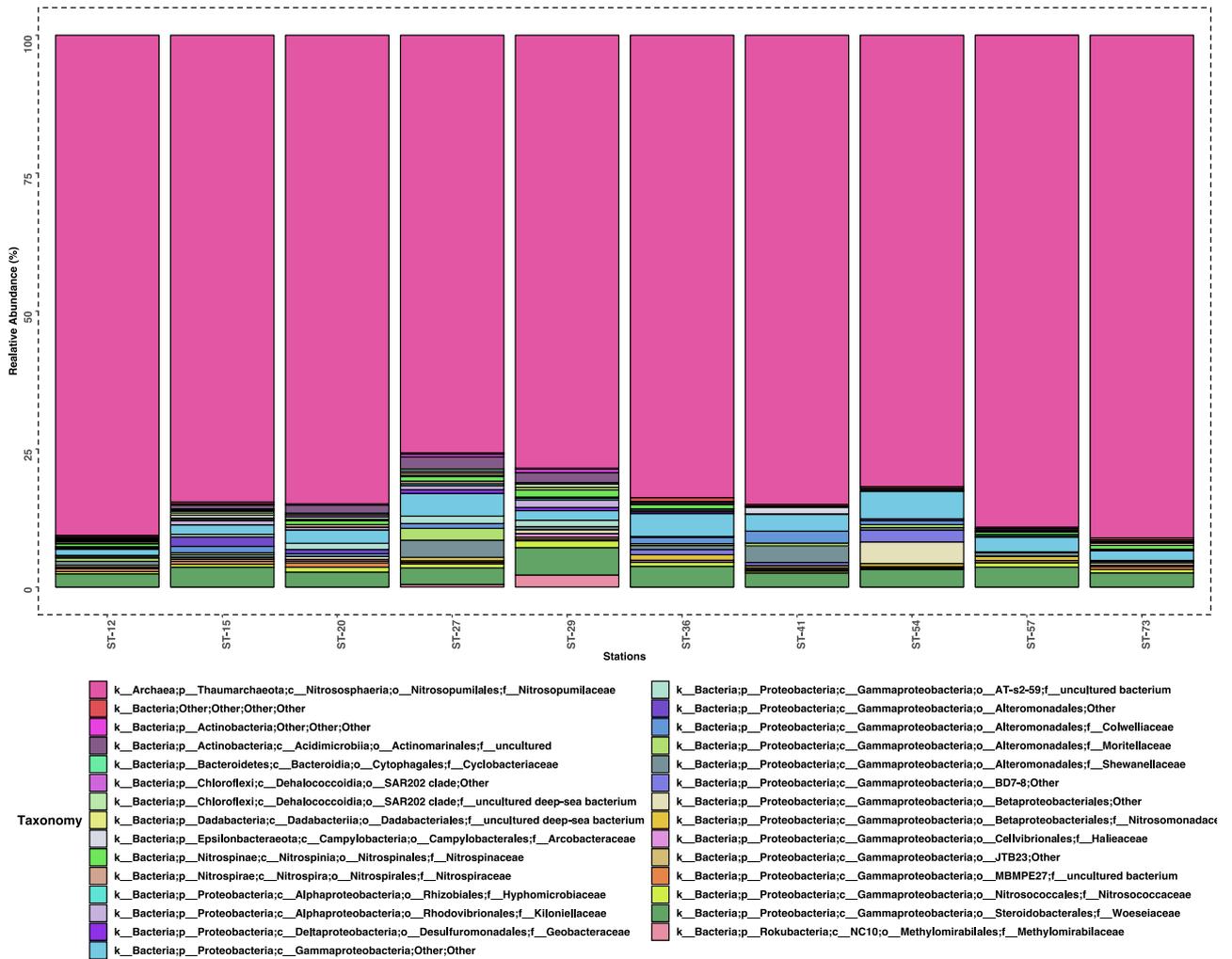


Figure 1. Prokaryotic community composition (i.e. bacteria and archaea) of abundantly taxa present in the raw sediment samples of the 10 sampling stations. The taxonomy of the groups is determined to family level where possible.

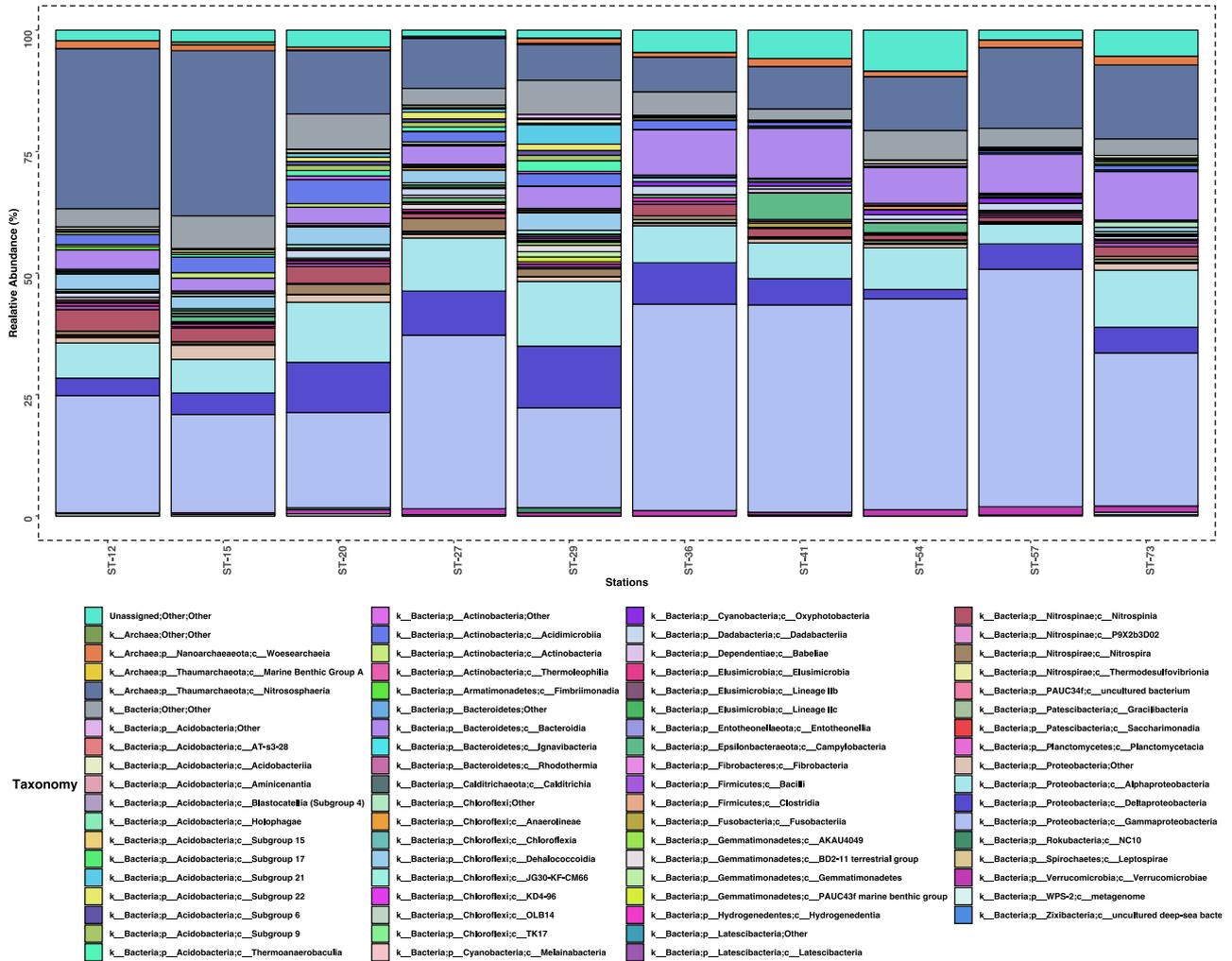


Figure 2. Prokaryotic community composition (i.e. bacteria and archaea) of rare taxa in the raw sediment samples of the 10 sampling stations.

Figure 3A. NMDS plot to highlight the compositional differences (Beta-diversity) of the microbiomes between sampling stations. Beta-diversity was calculated based on Bray-Curtis matrix of dissimilarity. The plot illustrates that the microbiomes, at the three sampling sites (see Figure 3B), are taxonomically very different. The distribution of stations along axis-1 represents the geographical location of the stations. Microbial communities from the shelf break stations (St12, St15, St20 and St73) are more similar and well separated from the other samples along axis-1. Microbial communities from the coastal shelf stations (St36, St41, St49 and St57) are likewise similar. The communities from the shelf stations close to the shelf break (St27 and St29), on the other hand, are found in-between the two groups.

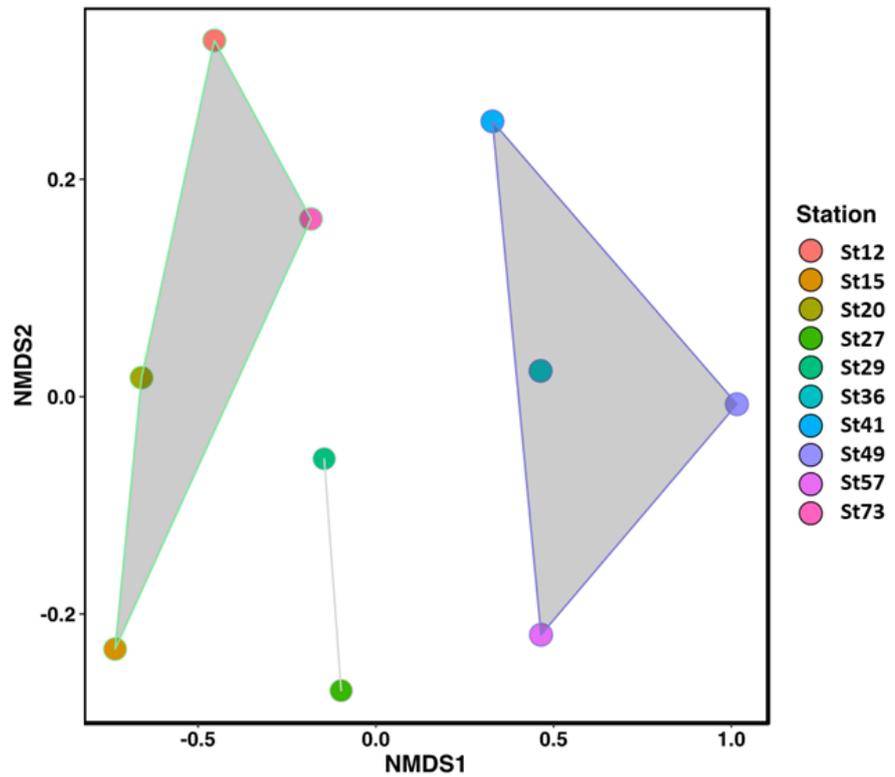
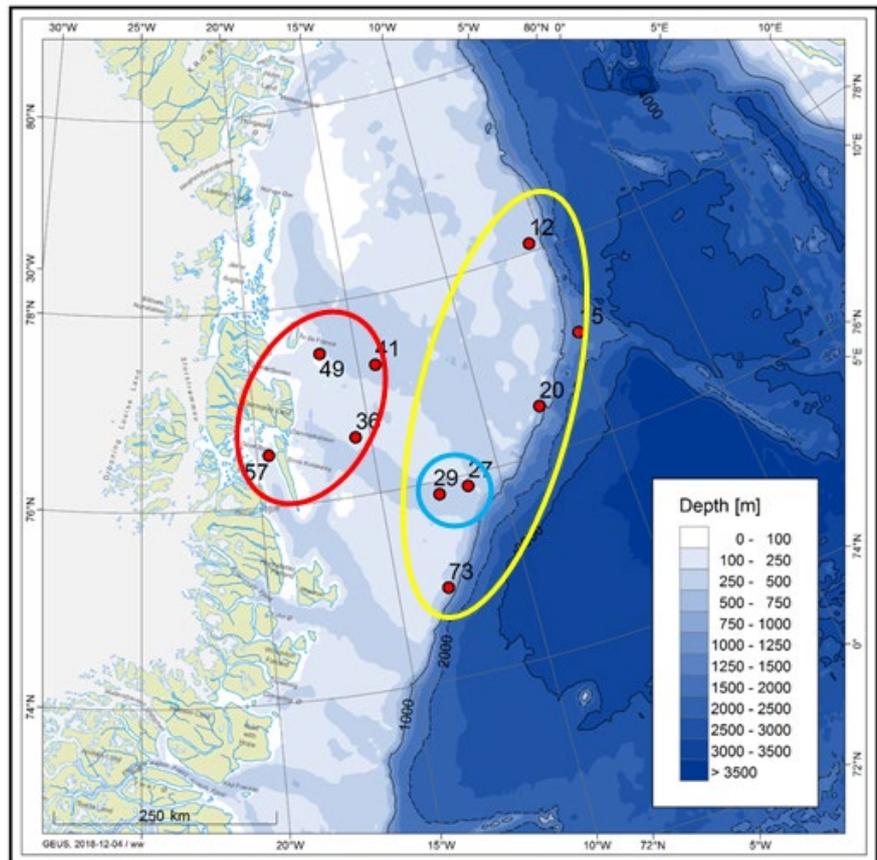
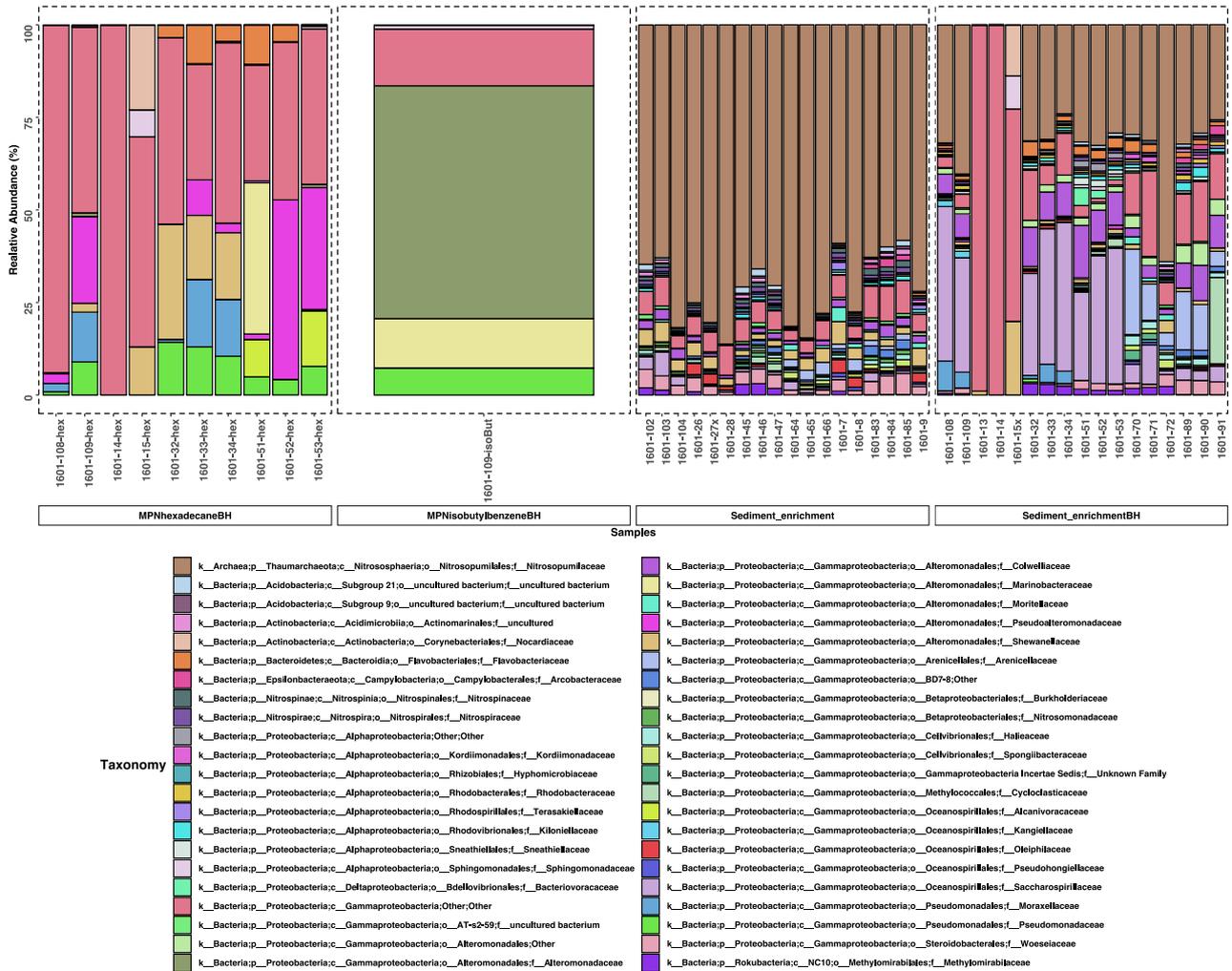


Figure 3B. Map of the Greenland Sea showing the stations sampled for sediment microbiota characterization. The sampling sites were distributed on the continental shelf ranging from close to the coast (stations 36, 41, 49 and 57), over stations close to the shelf break (stations 27 and 29), to stations at the shelf break (stations 12, 15, 20 and 73). The depth ranged from relatively shallow at Station 49 (117 m) to much deeper at the shelf break (1441 m, Station 15). The stations closest to the coast (stations 36, 49 and 57) were oxidized only in the top layer, whereas sediment from the stations further away from the coast were oxidized at least down to 25 cm depth.





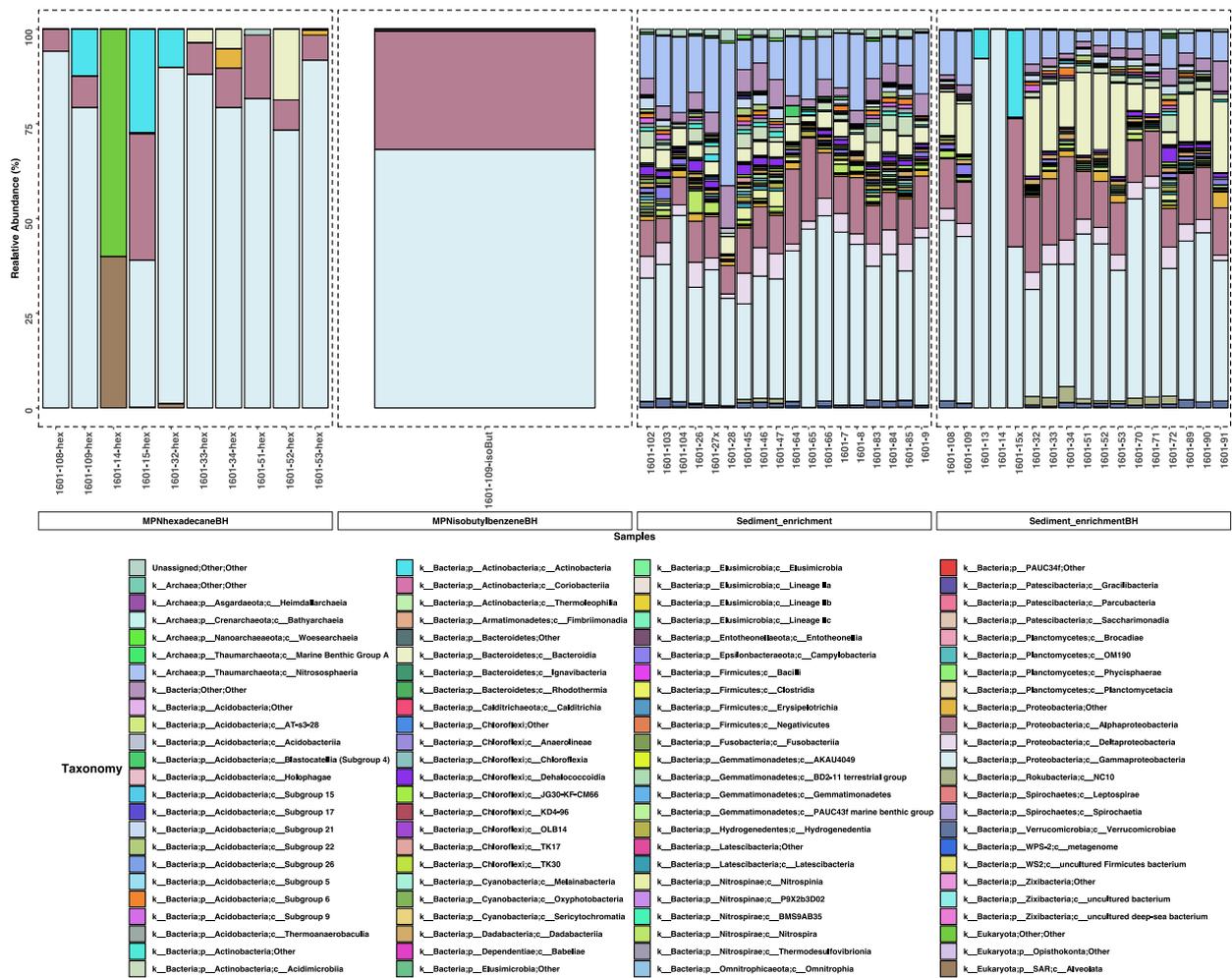


Table 1. Origins of the sediments for the respective sample IDs in figures 4 and 5.

Sample ID	Sampling station	Sample ID	Sampling station	Sample ID	Sampling station
1601-13-hex	St27	1601-7	St27	1601-13	St27
1601-14-hex	St27	1601-8	St27	1601-14	St27
1601-15-hex	St27	1601-9	St27	1601-15x	St27
1601-32-hex	St29	1601-26	St29	1601-32	St29
1601-33-hex	St29	1601-27x	St29	1601-33	St29
1601-34-hex	St29	1601-28	St29	1601-34	St29
1601-51-hex	St36	1601-45	St36	1601-51	St36
1601-52-hex	St36	1601-46	St36	1601-52	St36
1601-53-hex	St36	1601-47	St36	1601-53	St36
1601-108-hex	St57	1601-64	St41	1601-70	St41
1601-109-hex	St57	1601-65	St41	1601-71	St41
1601-109-isoBut	St57	1601-66	St41	1601-72	St41
		1601-83	St49	1601-89	St49
		1601-84	St49	1601-90	St49
		1601-85	St49	1601-91	St49
		1601-102	St57	1601-108	St57
		1601-103	St57	1601-109	St57
		1601-104	St57		

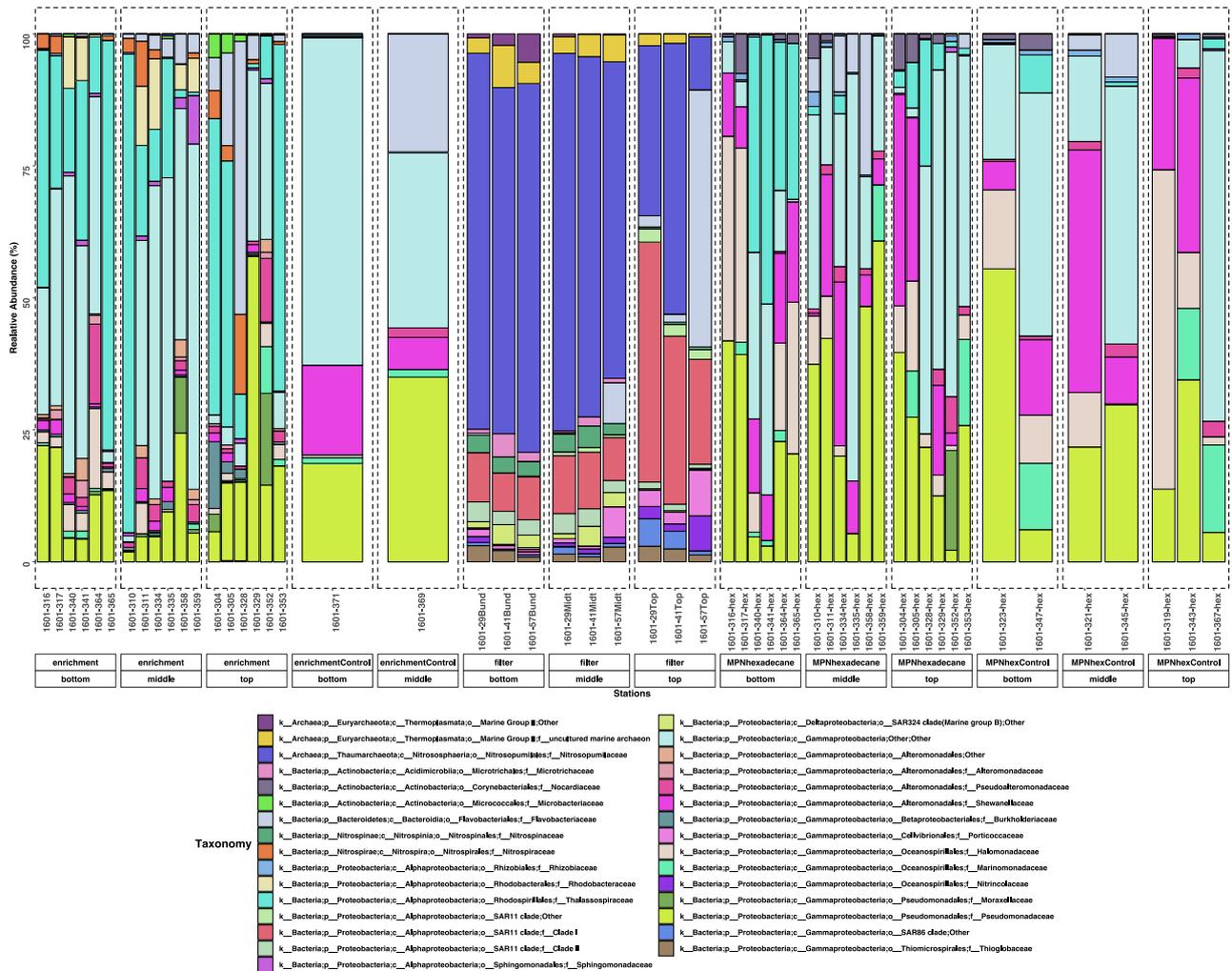


Figure 6. Abundant prokaryotic community composition (i.e. bacteria and archaea) of filtride microcosms added either crude oil and BH mineral nutrients (enrichment) or BH mineral nutrients only (control). The communities were sampled after 84 days incubation. The composition of growth-positive MPN wells (cultivable hexadecane degraders) is shown for comparison. The taxonomy (16S rRNA gene) of the groups in this figure is filtered as abundant taxa (from data shown as Figure 33 in main report). The taxonomy of the groups is determined to family level where possible. Origins of the filtride for the respective sample IDs can be seen in table 2.

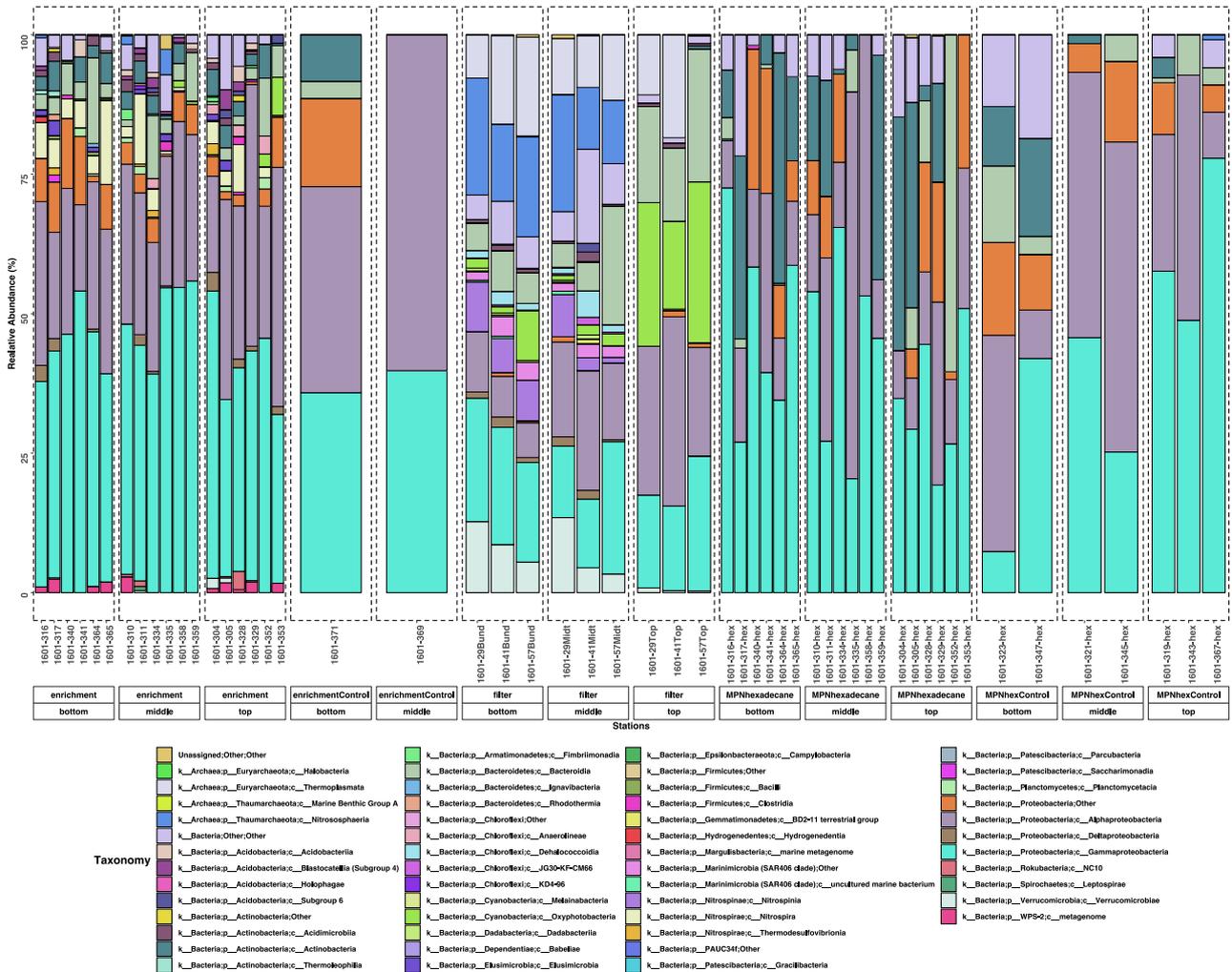


Figure 7. Rare Prokaryotic community composition (i.e. bacteria and archaea) of filtride microcosms added either crude oil and BH mineral nutrients (enrichment) or BH mineral nutrients only (control). The communities were sampled after 84 days incubation. The composition of growth-positive MPN wells (cultivable hexadecane degraders) is shown for comparison. The taxonomy (16S rRNA gene) of the groups in this figure is filtered as rare taxa (from data shown as Figure 33 in main report). Origins of the filtride for the respective sample IDs can be seen in table 2.

Table 2. Origins of the sediments for the respective sample IDs in figures 6 and 7.

Sample ID	Sampling station	Sample ID	Sampling station	Sample ID	Sampling station
1601-304	St29	1601-29Top	St29	1601-304-hex	St29
1601-305	St29	1601-29Midt	St29	1601-305-hex	St29
1601-310	St29	1601-29Bund	St29	1601-310-hex	St29
1601-311	St29	1601-41Top	St41	1601-311-hex	St29
1601-316	St29	1601-41Midt	St41	1601-316-hex	St29
1601-317	St29	1601-41Bund	St41	1601-317-hex	St29
1601-328	St41	1601-57Top	St57	1601-319-hex	St29
1601-329	St41	1601-57Midt	St57	1601-321-hex	St29
1601-334	St41	1601-57Bund	St57	1601-304-hex	St29
1601-335	St41			1601-328-hex	St41
1601-340	St41			1601-329-hex	St41
1601-341	St41			1601-334-hex	St41
1601-352	St57			1601-335-hex	St41
1601-353	St57			1601-340-hex	St41
1601-358	St57			1601-341-hex	St41
1601-359	St57			1601-343-hex	St41
1601-364	St57			1601-345-hex	St41
1601-365	St57			1601-347-hex	St41
1601-369	St57			1601-352-hex	St57
1601-371	St57			1601-353-hex	St57
				1601-358-hex	St57
				1601-359-hex	St57
				1601-364-hex	St57
				1601-365-hex	St57
				1601-367-hex	St57

References

- Baricz, A., Cecilia, C., Paul-Adrian, B., Adorján, C., Zsolt G, K., Tudor, T., et al. (Jun2019). Patterns of prokaryotic diversity in sediments of brackish to hypersaline salt lakes in Romania. *STUDIA UNIVERSITATIS BABEȘ-BOLYAI BIOLOGI* 64.
- Campeão, M. E., Reis, L., Leomil, L., de Oliveira, L., Otsuki, K., Gardinali, P., et al. (2017). The Deep-Sea Microbial Community from the Amazonian Basin Associated with Oil Degradation. *Front Microbiol* 8. doi:10.3
- Lauer, A., Sørensen, K. B., and Teske, A. (2016). Phylogenetic Characterization of Marine Benthic Archaea in Organic-Poor Sediments of the Eastern Equatorial Pacific Ocean (ODP Site 1225). *Microorganisms* 4, 32. doi:10.3390/microorganisms4030032.
- Malard, L. A., Anwar, M. Z., Jacobsen, C. S., and Pearce, D. A. (2019). Biogeographical patterns in soil bacterial communities across the Arctic region. *FEMS Microbiol Ecol* 95. doi:10.1093/femsec/fiz128.
- Mason, O. U., Hazen, T. C., Borglin, S., Chain, P. S. G., Dubinsky, E. A., Fortney, J. L., et al. (2012). Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. *ISME J* 6, 1715–1727. doi:10.1038/ismej.2012.59.
- Yu, T., Liang, Q., Niu, M., and Wang, F. (2017). High occurrence of Bathyarchaeota (MCG) in the deep-sea sediments of South China Sea quantified using newly designed PCR primers. *Environmental Microbiology Reports* 9, 374–382. doi:10.1111/1758-2229.12539.
- Zhang, J., Chen, M., Huang, J., Guo, X., Zhang, Y., Liu, D., et al. (2019). Diversity of the microbial community and cultivable protease-producing bacteria in the sediments of the Bohai Sea, Yellow Sea and South China Sea. *PLoS One* 14. doi:10.1371/journal.pone.0215328.