1 # Comparison of microbial community dynamics induced by distinct crude oil
2 dispersions reveals compositional differences

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Abstract

To understand the impact of oil contamination on marine microbial communities, numerous studies have been conducted following microbial dynamics after oil spills and concerning the effects of different environmental parameters on oil biodegradation potential. Nevertheless, there is a lack of understanding of how distinct oil crude types might influence the dynamics of microbial communities of identical origin. Here we show that different crude oils affect the community composition by shaping it distinctly over the course of incubation. We have used chemical dispersion of three crudes with different properties (paraffinic, paraffinic-asphaltenic and naphthenic). *Oceanospirillaceae*, *Colwelliaceae*, *Porticocacceae*, *Flavobacteriaceae* and *Piscirikettsiaceae* were highly abundant in all three oil dispersions. However, comparing group distances of the communities at each time point, as well as pairwise fold comparison of OTUs, has revealed significant differences in microbial composition between the oils ($p < 0.05$), but also between the major families related to biodegradation ($p < 0.01$). Furthermore, the PAH degradation rates proved to be significantly higher in naphthenic oil ($p < 0.05$), while the n-alkane degradation was slower, however not significant ($p > 0.05$). We conclude that different crude oils can shape microbial community distinctively over exposure time, therefore altering community biotransformation potential and causing different degradation rates of targeted oil compounds.

1 Introduction

Marine oil spills may cause major environmental impacts to the biota in the seawater, but also after stranding of the oil. Rapid removal of oil from the environment by response actions is therefore important. Efficient oil spill responses depend on a variety of factors, including environmental conditions and oil weathering properties. Oil biodegradation is a natural attenuation process, which may be significantly improved by the use of dispersants [1-7]. Oil biodegradation in the ocean is caused by

32 microbial organisms and is affected by a number of factors, including seawater temperature, nutrient
33 and oxygen availabilities, microbial community compositions, and physical properties of the oil [8, 9].
34 While the effects of environmental conditions on biodegradation have been studied extensively [2, 10-
35 14], the effects of different crude oil types on biodegradation dynamics have been mostly neglected.

36 Varying abiotic parameters have significant impact on microbial community structures and their
37 biodegradation potential. Changes in temperature, nutrient and oxygen concentration, salinity and pH
38 have been observed to affect microbiome compositions and functional potential of communities [15,
39 16]. Following the Deepwater Horizon (DWH) oil spill, deep sea microbial communities exhibited multiple
40 shifts in composition over the period of contamination [17]. Surface water communities were
41 substantially different compared to subsurface communities for the reason of temperature difference
42 between these layers (30 °C and 4 °C, respectively) [18]. Changes within each of the communities along
43 the exposure timeline in the deep sea plume were significant and were not related to temperature effect
44 [19]. Experimental studies with Norwegian and Alaskan seawater when amended with crude oil are in
45 good accordance with taxonomic alterations observed within surface and subsurface communities
46 during the DWH spill [11, 20]. These shifts are a product of sequential degradation of different
47 hydrocarbon compounds and their susceptibility to biodegradation. Short-chain saturates are quickly
48 utilized, while more complex ones (PAHs) require additional time to be consumed [10]. Different
49 components demand distinct mechanisms for biotransformation, therefore different types of
50 microorganisms may be expected to be involved in these processes [21, 22]. As a consequence, microbial
51 communities can be controlled by substrate availability, and compositional changes during
52 biodegradation may occur, although some bacteria may be ubiquitous [23] . This becomes obvious when
53 different sources of individual hydrocarbons are introduced to microbial consortia and compositional
54 and functional differences arise [24].

55 In contrast to single hydrocarbons, crude oils are complex mixtures of hundreds of thousands of
56 inorganic and organic chemical compounds [25]. Based on the resolvable compound content, crude oils
57 can be differentiated as paraffinic (containing larger fraction of saturated compounds- paraffins-
58 alkanes), naphthenic (predominant in cycloalkanes) or asphaltenic (contain high fraction of asphaltenes,
59 resins and aromatics). However, crude oils are actually dominated by an unresolved complex mixture
60 (UCM), which can account for more than 95% of the oil [26].

61 It is logical to expect that oils with different compound ratio may have the ability to induce distinct
62 microbial responses. Nevertheless, there is a lack of understanding how different and complex crude oils
63 may influence microbial community structures if spilled into marine environment. Therefore, in this
64 study we aim to assess the hypothesis that different crude oils may cause distinct microbial community
65 response and affect oil compound biodegradation kinetics. For that purpose, we have chosen three oils
66 with different physical properties, which were dispersed by a commercial chemical dispersant in natural
67 non-amended seawater. A biodegradation study was performed at temperate conditions, corresponding
68 to "summer" temperatures in the North Sea, and microbial community successions was compared during
69 biodegradation of the three oils.

70 # 2 Methods

71 ## 2.1 Experimental setup

72 Seawater of salinity 34 PSU supplied via a pipeline system to our laboratories was collected from a depth
73 of 80 m (below thermocline) in a Norwegian fjord (Trondheimsfjord; 63°26'N, 10°23'E). The seawater
74 was incubated at 13°C overnight before start of the experiments.

75 Dispersions with nominal median diameter of 10 µm droplets were prepared from premixed fresh
76 paraffinic (Statfjord crude), naphthenic (Troll crude) and a paraffinic-asphaltenic mixture (Balder crude)

77 oils, pre-mixed with the dispersant Slickgone NS (Dasic International Ltd., Romsey, Hampshire, UK) at

78 dispersant to oil ratio (DOR) 1:100, as previously described [10, 27]. Stock oil dispersions (200 mg/L)

79 were diluted with seawater to a final concentration of 3 mg/L in 2-L pre-sterilized (autoclaved 120°C, 15

80 min) flasks (SCHOTT), based on Coulter Counter measurements. Natural seawater with oil dispersions

81 (NSOD) were generated in unfiltered non-amended seawater, while sterilized seawater with oil

82 dispersions (hereinafter referred to as "chemical control") were prepared in seawater filtered through 1

83 µm Nalgene™ Rapid-Flow™ filters (ThermoFisher Scientific, MA USA), autoclaved (120°C, 15 min) and

84 preserved with 100 mg/L (final concentration) $HgCl_2$. In addition, flasks of natural seawater without oil

85 were included as biological controls (hereinafter referred to as "biological control"). The flasks were

86 mounted on a carousel system with continuous slow rotation (0.75 r.p.m.) and incubated at 13°C for up

87 to 64 days. Flasks with dispersions (NSOD and chemical controls) and biological controls were sacrificed

88 for analyses after 0, 3, 7, 14, 21, 30 and 64 days. At each sampling date flasks with NSOD (duplicate),

89 chemical control (duplicate) and biological control (one replicate) were sampled. Each sample was

90 analyzed for semi-volatile and volatile oil compounds, while microbiological analyses (community

91 characterization by 16S rRNA amplicon sequencing) were performed on NSOD and biological control

92 treatment of all samples.

93 2.2 Microbiological analysis (16S rRNA gene)

94 Seawater samples without oil and oil dispersions (approximately 500 ml) were filtered through 0.22 µm

95 filters (Millipore), and DNA was extracted from filters by employing FastDNA Spin kit for soil (MP

96 biomedicals) according to the manufacturer's instructions. DNA yields were quantified using Qubit 3.0

97 (ThermoFisher Scientific, MA, USA) with dsDNA High Sensitivity kit (ThermoFisher Scientific, MA, USA).

98 16S rDNA amplicons were generated according to Illuminas "16S Metagenomic Sequencing Library

99 Preparation" protocol using S-D-bact-0341-b-S-17 and S-bact-0785-a-A-21 primer set [28]. Amplicons

100 generated by PCR were isolated using magnetic beads (Agencourt Amoure XP Beads). Libraries have
101 been quantified using Quant iT Picogreen Dye and the Fragment Analyzer (Advanced Analytical) as well
102 on Agilent's Bioanalyzer. All amplicons were pooled equimolar and then sequenced paired-end on the
103 Illumina MiSeq platform, 2x300nt following manufacturer instructions.

104 Raw pair-end reads were assembled with fastq-join in QIIME 1.9.1 [29]. Assembled sequences were
105 demultiplexed and quality filtered to remove low quality reads (Phred score < 20; -q 19). UCHIME was
106 employed for chimera detection on assembled quality filtered reads [30]. Operational Taxonomic Units
107 (OTUs) were determined by clustering assembled sequences on 97% nucleotide identity using UCLUST
108 [31] with open reference clustering option. Representative sequences were aligned with PyNAST [32]
109 and taxonomy assignment was performed with RDP classifier [33] based on SILVA-123 database [28]. In
110 order to evaluate differences in microbial community composition within and between oil types alpha
111 and beta diversity were calculated using QIIME's core_diversity_analysis.py script. Samples were rarefied
112 to the equal number of reads based on the sample containing the least number of reads (5045). For
113 statistical analysis t-test was applied on alpha diversity output (PD-whole_tree matrix) and PERMANOVA
114 on beta diversity output (weighted-UniFrack matrix), using QIIME's scripts compare_alpa_diversity.py
115 and compare_categories.py, respectively. Non-parametric two-sample t-test using Monte Carlo
116 permutations was employed to calculate the p-values for statistical comparison of alpha diversity
117 between oil types since the OTU data are not exactly normally distributed [34]. For statistical analysis of
118 weighted-UniFrack distance matrix and for the hypothesis testing PERMANOVA test was chosen.
119 PERMANOVA tests whether two or more groups of samples are significantly different based on a
120 provisional categorical variable, in this case oil type. Furthermore, to evaluate for potential differences in
121 dynamics of microbial communities between different oil types at separate time points, subset of
122 weighted-UniFrack distance matrix data from each time point was used as input for PERMANOVA as
123 described above. To visualize taxonomical composition, relative abundances of OTUs on each sampling

124 point were calculated from the raw reads and plotted with ggplot2 package v.2.2.1 in R-studio v.3.2.2.
125 For the purpose of statistical analysis of differentially abundant OTUs between oil types, the R package
126 DESeq2 [35] was used to standardize the counts between samples rather than rarefying to the number
127 of reads present in the sample with least number of reads [36]. Statistical analysis was performed within
128 the Phyloseq package v.1.12.2 [37] in R-studio.

129 Nucleotide sequence data for 16S rRNA amplicon sequences were deposited to the European Nucleotide
130 archive (ENA) with the title "PETROMAKS_E8" from sample ID "ERS1814682" to sample ID
131 "ERS1814729". Sample group can be found under study accession number PRJEB14899 entitled "Oil spill
132 dispersant strategies and bioremediation efficiency".

133 2.3 Chemical analysis

134 The chemical analyses included GC-FID for determination of total extractable organic carbon (TEOC) and
135 GC-MS analyses of targeted oil compounds and groups.

136 Samples of dispersions and seawater were solvent-solvent extracted with dichloromethane (DCM) for
137 measurements of semi-volatile organic compounds (SVOC) by gas chromatographic methods. The flask
138 glass walls were also rinsed with DCM after removal of dispersions to extract material attached to the
139 glass walls.

140 2.3.1 GC-FID

141 A gas chromatograph coupled to a flame ionization detector (GC-FID; Agilent 6890N with 30 mDB1
142 column; Agilent Technologies) was used for quantification of semi-volatile C_{10}-C_{36} saturates extracted by
143 DCM, total extractable organic carbon (TEOC). *o*-Terphenyl (10 µg/mL) was used as surrogate internal
144 standard (SIS) and 5α-androstane (10 µg/mL) as recovery internal standard (RIS). Based on a signal-to-
145 ratio of 10, a lower detection limit (LOD) of 0.1 µg/L was used in the analyses. Experimental blanks

146 (deionized water) and a QA oil spike were used (standard fresh paraffinic oil) were included in all oil

147 batches [38].

148 ### 2.3.2 GC-MS analyses

149 More than 80 individual targeted compounds or compound groups (C_{10}-C_{36} n-alkanes, decalines, phenols,

150 2- to 5-ring poly-aromatic hydrocarbons (PAH) and 17α(H),21β(H)-Hopane (30ab Hopane) were analyzed

151 in a gas chromatograph coupled to a mass spectrometer (GC-MS; Agilent 6890 plus GC coupled with an

152 Agilent 5973 MSD detector, operated in Selected Ion Monitoring [SIM] modus; Agilent Technologies), as

153 recently described [10]. Deuterated SIS-PAH (naphthalene, phenanthrene, chrysene, perylene; 50-250

154 µg/ml) and RIS-PAH (acenaphthene, fluorene; 100 mg/ml) were included for analyses. The LOD of these

155 analyses were 0.01 µg/L for the individual target compounds (signal-to-noise ratio of 10). In addition to

156 experimental blanks and a QA oil spike, a QA PAH spike was included in all GC-MS test batches [38].

157 The SVOC target compound concentrations were normalized against 30ab Hopane [39] and percentage

158 depletion measured as % compounds of concentrations in corresponding sterilized controls:

159 *% of original concentration caused by biodegradation:* $100\left(\frac{(t_{c/Hop_c})nSW}{(t_{c/Hop_c})sterSW}\right)$, where

160 t_c – target compound concentration; Hop_c – Hopane concentration; nSW – normal seawater; sterSW –

161 sterilized seawater.

162 The VOC compounds were not normalized against any internal standard, and % depletion of target

163 compound concentrations in the normal seawater was measured as % of concentrations in sterilized

164 seawater.

165 Statistical analysis was conducted using percentages of concentration values of n-alkanes and PAHs

166 between oil types and incubation days by applying post-hoc TukeyHSD after two-way ANOVA in Stats

167 package in R v.3.2.2.

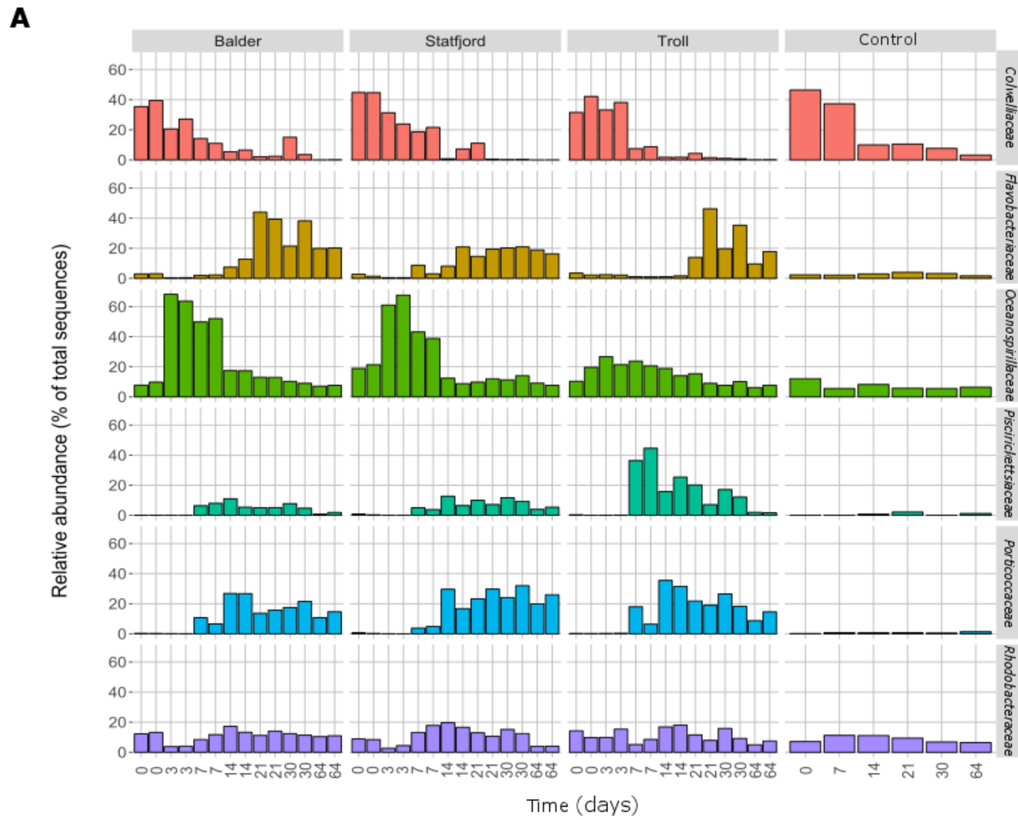
168 # 3 Results and discussion

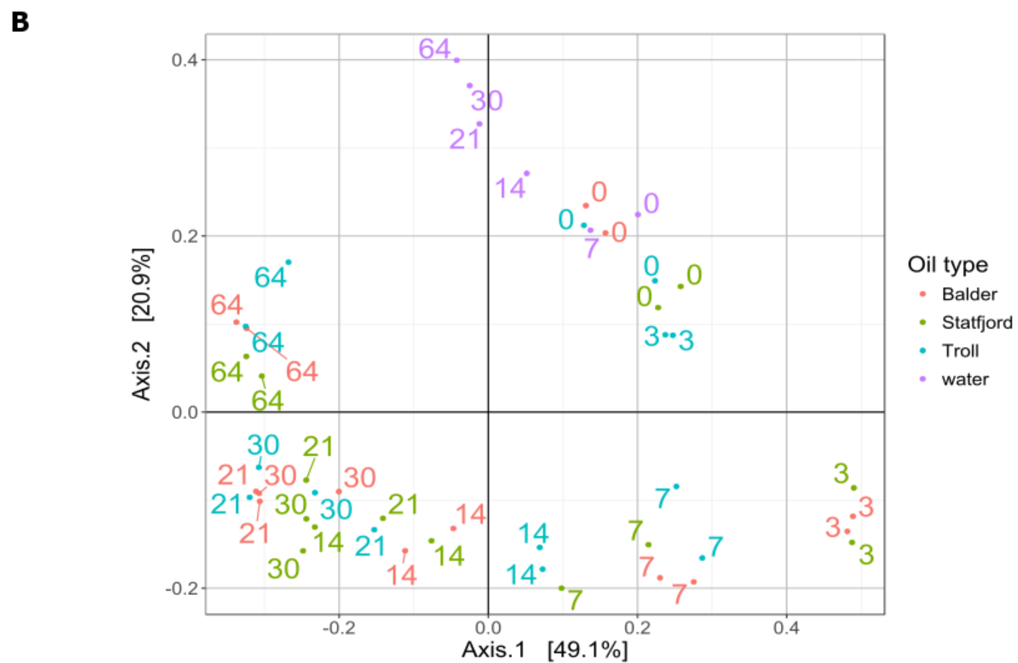
169 Microbial community dynamics can vary depending on environmental factors changing with seasonality
170 and location such as oxygen and nutrient concentration or temperature, salinity and pH [40, 41]. With
171 respect to oil degradation, hydrocarbonoclastic bacteria are also susceptible to those environmental
172 factors [15, 16]. An example of different community dynamics to oil contamination, in the same
173 geographical location but in completely different environment, was the Deepwater Horizon (DWH) oil
174 spill. DWH spill triggered different community dynamics in the deep-sea compared to community
175 response to oil residue reaching surface [18, 19, 42], primarily owing the temperature contrast between
176 subsurface (4°C) and surface (30°C) waters. While previously mentioned factors influencing community
177 dynamics have been well studied, the effects of oil composition as a driver for distinct community
178 dynamics in ocean have not been much investigated as compared to studying the effects on soil
179 microbial communities [43]. In order to test whether different crude oils can influence distinct
180 community dynamics, we performed a 16S rRNA gene amplicon study of microcosms (2L flasks)
181 containing natural seawater spiked with three different oil dispersions (final conc. 3 mg/L), incubated at
182 13°C and run over a period of 64 days.

183 We originally planned to test three different crudes; a paraffinic (Statfjord), a naphthenic (Troll) and an
184 asphaltenic (Balder) oil. The Statfjord and Balder blends showed n-alkane patterns, demonstrating
185 paraffinic properties of these oils, while the Troll oil showed high content of unresolved complex mixture
186 (UCM). Although the Balder oil was reported to be an asphaltenic oil, a low asphaltene content (see
187 Table S2) showed that this oil was not a true asphaltenic oil, and later examination showed this oil to be
188 a blend of a wax-rich paraffinic (Ringhorne, 60%) and an asphaltenic (Balder 40%) oil. These
189 characteristics were further shown by comparison of targeted versus unresolved groups in
190 dichloromethane (DCM) extracts of fresh oils by comparison of GC-FID and GC-MS analyses (Fig. S3). Oils
191 were not treated prior to dispersion (i.e. no sterilization). During oil spills crudes are not sterile and do

192 carry autochthonous microbial community. However, we believe that the small concentrations of
193 autochthonous oil microbes inoculated to our microcosoms (3 mg/L final conc. made from 200 ppm oil
194 dispersion) do not possess the potential to alter the overall microbial community or in any other way
195 influence the dynamics of the native seawater community, since oil microbes are notably outnumbered
196 by seawater microbes (ca. $1x10^6$ cell/mL in seawater compared to ca. 3 cell/mL from the oil assuming ca.
197 $1x10^6$ cell/mL in oil before the dilution [44]). Additionally, reservoir communities are adapted to high
198 pressure, temperature (> 60° C) and they are usually anaerobic bacteria and archaea [44, 45]. Microcosm
199 conditions (surface pressure, low temperature of 13° C and oxygen presence) are therefore considered
200 as extreme environments for reservoir oil microbes. This should reduce their potential influence on the
201 microcosms, which is supported by the fact that abundant reservoir microbes (often
202 thermophilic/thermotolerant methanogenes and sulfate reducing bacteria) are not detected in
203 biodegradation studies with local seawater [11, 46].

204 Microbial community composition was mainly dominated by *Oceanospirillaceae*, *Colwelliaceae*,
205 *Porticocacceae*, *Flavobacteriaceae* and *Piscirikettsiaceae* (Fig. 1a). All of the families are known to
206 contain hydrocarbon degraders and have been found in many oil degradation experiments and real oil
207 spill studies [1, 2, 11, 17, 46-48].





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209 **Fig 1. A) Microbial community structure of most abundant families that are present in > 1% of relative sequence abundance in**
210 **at least two samples. It is possible to observe duplicates for different incubations at each sampling point on the x-axis. B)**
211 **PCoA plot recapturing differences of all samples based on total microbial community composition. Different incubations are**
212 **color coded and the numbers represent incubation days. Axis title numbers show fraction of variance explained.**

213 *Colwelliaceae* exhibited the highest values in all of the oils, as well as in control samples, at day 0 (> 35%
214 of relative sequence abundance), decreasing afterwards. Local seawater is often highly abundant in
215 *Colwelliaceae* during winter-spring season (which is the season when the current experiment was
216 conducted), whereas during summer-autumn season *Colwelliaceae* are low in abundance (< 2%), which
217 usually increases to > 50% during incubation period (days 3-16) [11]. Here, by day 7 *Colwelliaceae*
218 decreased in abundance to < 10% in paraffinic-asphaltenic and naphthenic incubation and to about 20%
219 in paraffinic incubation. It is interesting that *Colwelliaceae* showed the same trend in control samples as
220 in oil incubations. *Oceanospirillaceae* exhibited highest abundance in all three oil incubations at day 3,
221 reaching 68% (paraffinic-asphaltenic), 67% (paraffinic) and 26% (naphthenic) of relative sequence
222 abundance (Fig. 1a). *Oceanospirillaceae* are usually associated with degradation of n-alkane compounds
223 and increase in abundance very soon after the input of hydrocarbons [47]. On the other hand,
224 *Flavobacteriaceae* tended to increase in abundance later during incubation and are usually associated
225 with degradation of aromatics and their co-products [47, 49]. Here, the increase was from < 5% of
226 relative sequence abundance at the start of experiment to 46% (naphthenic incubation), 44% (paraffinic-
227 asphaltenic incubation) and 20% (paraffinic incubation) on day 21. Similar trends can be observed for
228 another specialized aromatics degrader, *Piscririkettsiaceae* (mainly genus *Cycloclasticus*) which exhibited
229 rapid increase in particular in naphthenic oil (Troll) incubation, from < 1% of relative sequence
230 abundance at the start of experiment to 44% at day 7. Response was delayed to day 14 with 12% and
231 11% in relative sequence abundance in paraffinic and paraffinic-asphaltenic incubation, respectively.
232 *Porticoccaceae*, a genus associated with aromatics degradation [48] showed a similar trend as
233 *Flavobacteriaceae* and *Piscirikettsiaceae*. With less than 1% of sequences at the start of experiment, the
234 highest values could be observed at day 14 in naphthenic (35%) and paraffinic-asphaltenic incubation
235 (27%) and at day 30 in paraffinic incubation (31%), but with high values already observed from day 14 (>
236 29%). Another highly abundant family observed in our experiment was *Rhodobacteraceae*. However, the

237 abundance of this family was mainly stable along the experimental timeline and was varying from 5% to

238 20%. We found that the beta diversity exhibited significant differences when comparing oil incubations

239 pairwise on a day-to-day basis. As seen in Table 1 and Fig. 1b, there are substantial differences between

240 oil types in early development of the communities (day 3-14).

241 **Table 1 Statistical analysis of microbial community group distances between oil types using PERMANOVA test. Asterisk**
242 **symbol indicates statistically significant p values ($p < 0.05$).**

Days	*pseudo*-F	p-value	Sample size	Number of groups
all days	0.746	0.570	42	3
d0	1.616	0.227	6	3
d3	21.821	0.046*	6	3
d7	11.213	0.066	6	3
d14	2.493	0.140	6	3
d21	2.590	0.260	6	3
d30	1.729	0.298	6	3
d64	2.168	0.057	6	3

243

244 Furthermore, pairwise fold change between families revealed that the major player associated with

245 degradation of aromatics, the *Piscirikettsiaceae* family, was significantly enriched in the naphthenic oil

246 incubations (day 7) compared to others (Table S1). Also, the same could be observed for the

247 *Oleiphilaceae* family (day 7 and 14), an n-alkane degrader [50]. However, *Oleiphilaceae* were rather low

248 in abundance (< 2%). On the other hand, *Colwelliaceae*, *Oceanospirillaceae* and *Flavobacteriaceae*, all

249 major families abundance-wise, were found to be significantly enriched either in paraffinic, paraffinic-

250 asphaltenic or both incubations compared to naphthenic incubation. Paraffinic and paraffinic-asphaltenic

251 incubations exhibited differences, but not as substantial as compared to naphthenic incubation.

252 Biotransformation of n-alkanes in our study seemed to be slightly (although not significantly) slower in

253 naphthenic oil incubation compared to paraffinic and paraffinic-asphaltenic oil (Fig. 2).

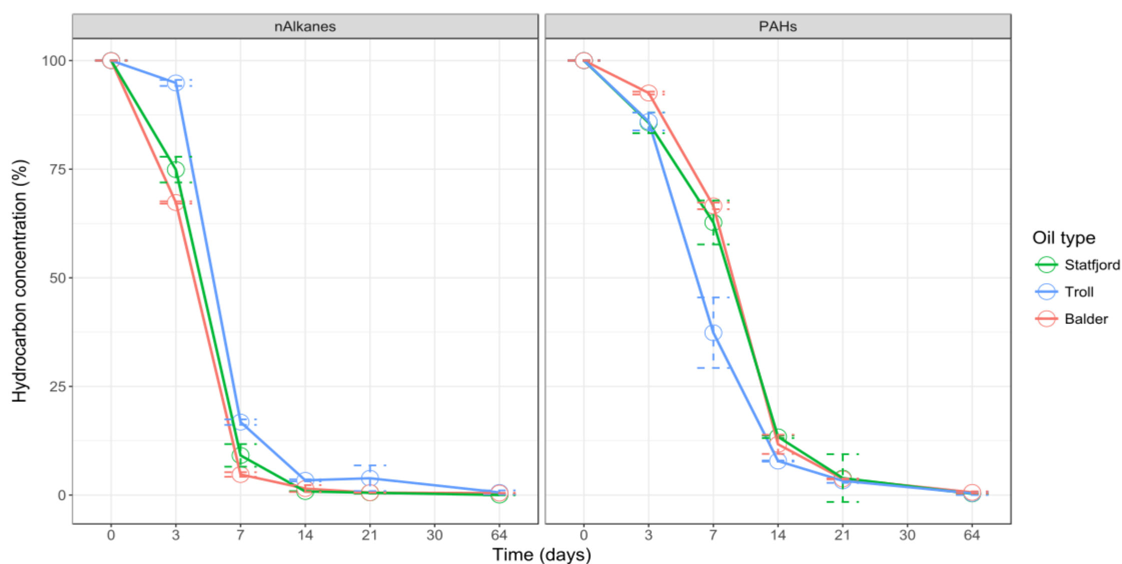

Fig. 2. Concentration of total n-Alkanes and PAHs during incubation period of 64 days calculated as percentages after standardization using 30ab Hopane. Different colors represent different oil incubations. Dashed lines represent standard deviation.

PAH biotransformation, on the other hand, was significantly different between naphthenic and the other two oil types (Table 2; two-way ANOVA, p=0.045). Target-specific biotransformation results are shown in Fig. S1 for n-alkanes and in Fig. S2 for PAHs.

Table 2 ANOVA analysis of n-Alkane and PAH concentration between oil types. Significance codes: ** p < 0.01, * p < 0.05. A *post-hoc* Tukey test was additionally applied to PAHs degradation data in order to see exactly which oil types showed significant difference revealed previously by ANOVA analysis.

	ANOVA test	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PAHs	Oil Type	2	3533	1766	3.102	0.0458*
	Sampling Day	5	657087	131417	230.819	<2e-16**
	Oil Type:Sampling Day	10	6253	625	1.098	0.3615
	Residuals	486	276705	569		
	Tukey-HD *post-hoc* test	**diff**	**lwr**	**upr**	**p-adj**	
	Statfjord-Balder	-3.905	-10.026	2.2145	0.2916	
	Troll-Balder	-6.436	-12.556	-0.315	0.0366*	
	Troll-Statfjord	-2.530	-8.6507	3.5903	0.5950	
n-Alkanes	**ANOVA test**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**

Oil Type	2	937	468	1.08	0.341
Sampling Day	2	284329	142165	327.83	<2e-16***
Oil Type:Sampling Day	4	1059	265	0.611	0.655
Residuals	240	104076	434		

In 1975, Atlas [15] performed biodegradability study of seven different oils using *Pseudomonas sp*. inoculums. A more recent study, employing a bacterial consortium of six known biodegraders, tested biodegradability of eight different crudes in order to differentiate between biological and physical weathering of oils [51]. In both studies different degradation rates were determined between tested oil types and concluded that differences in chemistry of oils is responsible for different degree of susceptibility to biodegradation. A study by Sugiura et al. [52] has obtained similar results after testing physicochemical properties of four different oils during biodegradation by two defined bacterial consortia. Degradation of saturates was shown to be faster in light paraffinic oils compared to heavier aromatic oils, while the degradation of aromatic compounds in respective oils was opposite compared to n-alkanes. They hypothesized that bioavailability of targeted compounds and distinct community development in different crudes could be the key to distinct biodegradation rates. In the current study we have observed slower degradation of n-alkanes in naphthenic incubation compared to other two. This is related to the fact that n-alkane fraction accounts for less than 5% in naphthenic Troll oil compared to 20 % and 45 % in paraffinic Statfjord and paraffinic-asphaltenic Balder, respectively (Fig. S3). Moreover, microbial community structure showed significant difference between these oils at day 3 (PERMANOVA, *pseudo*-F = 21.8, p = 0.045) and noteworthy at day 7 (PERMANOVA, *pseudo*-F =11.2, p = 0.066) (Table 1). The main n-alkane degraders were observed to be less abundant in naphthenic incubations, as *Oceanospirillaceae* peaked to only 26% of sequences compared to > 65% in paraffinic and paraffinic-asphaltenic incubation. Initial selective mechanism probably includes so called bottom up control, where the substrate stipulates and controls the abundance of consumer. In this case

285 *Oceanospirillaceae* in paraffinic and paraffinic-asphaltenic incubations was sustained by the higher
286 content and bioavailability of n-alkane fraction (Fig. S3). Naphthenic oil incubation, having lower content
287 of n-alkanes (Fig. S3), was not able to provide enough substrate for *Oceanospirillaceae* to proliferate to
288 the same extent as in other incubations. However, after only 14 days nearly all n-alkanes were
289 transformed in all three incubations (Fig. S2). A smaller fraction of *Oceanospirillaceae* may therefore
290 have been enough to degrade the low n–alkane substrate in the naphthenic oil, compared to the oils
291 with high n-alkane content. Accordingly, oils which are rich in aromatics will influence microbial
292 community by selecting for aromatics degraders sooner than paraffinic oil because of substrate
293 abundance and bioavailability which can support higher biomass of particular degraders.
294 *Piscirikettsiaceae* as a canonical aromatics degrader showed therefore unusual high abundance in
295 naphthenic oil (predominant in cycloalkane derivatives). However, naphthenic oil exhibited larger
296 fraction of UCM compared to other two oils (Fig. S3). It has been observed that UCM can contain up to
297 250,000 different compounds, of which heavily resolved aromatic compounds like branched
298 alkylbenzenes, aromatic sulfoxides or triaromatic steroids can be highly abundant [53, 54].Hence, we
299 speculate that the abundance of aromatics, within UCM, was able to trigger and sustain
300 *Piscirikettsiaceae* bloom, but also *Porticoccaceae* and *Flavobacteriaceae* increase in naphthenic
301 incubation. However, it is not excluded that the bloom may have been triggered solely by naphthenic
302 acid compounds present in oil. Additionally, while still substantially abundant, previously mentioned
303 families contributed to significantly higher PAHs transformation rates in the naphthenic than in the other
304 two oil types (Fig. 2). After 30 days more than 90 % of targeted PAHs were biotransformed in all
305 dispersions (Fig. 2). Due to low PAHs concentration in both paraffinic incubations, less represented
306 *Piscirikettsiaceae* with the help of more abundant *Porticoccaceae* and *Flavobacteriaceae* was still
307 sufficient for effective degradation.

The current study explained some essential driving mechanisms towards distinct biodegradation dynamics of different crude oils based on detailed microbiological and chemical analysis. We further showed that differences in oil types resulted in differences in dynamics of microbial communities of identical origin. This has implications on metabolic biodegradation potential of the local seawater community, since transformation rates can vary depending on the crude present at the time of the contamination.

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6 Author contributions

D.R. has performed incubation experiment, analyzed sequence data and has written the manuscript. R.N. has designed and performed incubation experiment and contributed to manuscript writing. A.W has performed sequencing and contributed to manuscript writing. O.G.B has designed incubation experiment and contributed to manuscript writing.

327 # 7 References

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