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Seafloor deposition of water-based drill cuttings generates distinctive and lengthy sediment bacterial community changes

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ABSTRACT

The spatial extent and persistence of bacterial change caused by deposition of water-based drill cuttings on the seafloor were explored by a community-wide approach. Ten centimeter sediment cores were sampled along transects extending from ≤15 m to 250 m from three nearby drilling sites in the southern Barents Sea. Eight months, 8 years and 15 years, respectively, had passed since the completion of the drillings. At locations heavily affected by drill cuttings, the two most recent sites showed distinct, corresponding deviances from native Barents Sea bacterial community profiles. Otherwise marginal groups, including Mollicutes and Clostridia, showed significant increases in relative abundance. Beyond 100 m from the boreholes the microbiotas appeared undisturbed, as they did at any distance from the 15-years old borehole. The extent of the biological distortion, as indicated by the present microbial study, agreed with previously published macrofaunal surveys at the same drilling sites.

1. Introduction

Offshore drilling operations for oil and natural gas cause perturbation of the seafloor surrounding the borehole through deposition of rock cuttings and residual drilling mud, collectively named drill cuttings (DC) in the following text. The extent and severity of the consequences of such depositions are related to the quantity and composition of the DC and the susceptibility of the local environment. The latter point includes the character of the native biota, the strength of ocean currents and the physical nature of the seafloor. Until the 1990s the liquid component of the drilling muds used in e.g. the Norwegian offshore industry was predominantly petroleum-based. Due to anaerobicity and accompanying slow biodegradation of the hydrocarbons, these oil-based DC deposits have shown stability for decades in e.g. parts of the North Sea (Daan et al., 2006; Breuer et al., 2008). On this background, stricter regulations which comply with the OSPAR Decision 2000/3 (OSPAR, 2000) are implemented on the Norwegian continental shelf area. A principal requirement is that the oil component of DC released to the environment should not exceed 1% (w/w dry material). Consequently, the common practice today is to send oil-infested DC to onshore treatment facilities where base-oil is recovered and recycled by thermal treatment. Releases at the drilling sites may be permitted by the Norwegian Environmental Agency, and are usually limited to purely water-based drilling fluid varieties. The severity of local environmental impacts of drilling waste depositions has consequently been reduced. However, the bulky parts of the drilling waste, i.e. the rock cuttings and the mineral components of the drilling muds, still lead to evident deposits on the seafloor in the vicinity of drilling sites. Furthermore, non-hydrocarbon organic constituents, e.g. formate and various polymers, are frequently added to improve the functional properties of the muds. Therefore, some degree of local influence on the benthic biota is observed even with water-based fluids (Breuer et al., 2004; Gates and Jones, 2012; Bakke et al., 2013; Cochrane et al., 2019).

Historically, the deposition of oil-based DC on the seafloor has caused profound and lasting distortions of the microbiota, characterized by the emergence of bacteria associated with aerobic and anaerobic hydrocarbon degradation (Artz et al., 2002; Potts et al., 2019). Less pronounced consequences should be expected by employing water-based drilling fluids with lower quantities of organic constituents. Even such material may, however, affect the native microbial communities. The structure and activity of the microbiota are susceptible to changes in the oxygenation level of the sediment. Penetration of O_2 can

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be reduced directly by the smothering effect of fines from DC or indirectly by inactivating the bioturbating sediment fauna. As well, organic and inorganic constituents of the drill fluids may affect the community structure by stimulating or inhibiting the growth of specific bacterial groups.

The prokaryotic communities in upper sediment layers of productive shelf oceans, including the Barents Sea, harbor diversities comparable to rich soils, i.e. in the order of 10^9 bacteria, representing thousands of different operational taxonomic units (OTUs), in each gram of sediment (Evans et al., 2017; Qiao et al., 2018). At the higher taxonomic ranks of phylum and class, much the same gross distribution between bacterial groups is found in seafloor sediments on a global scale and, to some extent, even independently of differing environmental conditions. Characteristic universal features are dominance by groups of Gamma- and Deltaproteobacteria, which together frequently constitute more than half of all 16S rRNA genes in such communities, and lower percentages of Alphaproteobacteria than commonly found in e.g. the pelagic microbiota. Furthermore, the upper sediment layers harbor substantial fractions of otherwise rare bacterial groups, like the Planctomycetes, Chloroflexi, Gemmatimonadetes, Nitrospirae and Verrucomicrobiae. Some high-abundance taxa in the bacterioplankton, e.g. the cyanobacteria and the SAR 11 group, are virtually absent from the sediment microbiomes (Kouridaki et al., 2010; Zinger et al., 2011; Oni et al., 2015). This extensive knowledge of native seafloor bacterial community structure provides a robust baseline for evaluating the consequences of anthropogenic impacts like drilling operations.

In the present study, we explore the temporal and spatial extent of change in the southern Barents Sea benthic bacterial community structure brought about by deposition of water-based DC. In a previous, comparable study at a Barents Sea continental slope location, marked perturbation of the sediment microbiota was observed at up to 50 m distance from a recently drilled well (Nguyen et al., 2018). The present microbial survey was done in concert with accompanying analyses of DC perturbations at the same locations. These studies have included sediment macrofauna and foraminifera profiling, and geochemical analyses (Aagaard-Sørensen et al., 2018; Cochrane et al., 2019).

Two questions are specifically addressed in the present study. Firstly, to what degree do changes in the microbiota reflect the spatial and temporal extent of seafloor perturbation, as manifested by established surveying approaches, like visual inspection, macrofaunal diversity studies and geochemical analyses? Secondly, can specific bacterial taxa be unequivocally associated with the community perturbations imposed by water-based DC deposition? If so, there is a potential for utilizing these bacteria as bioindicators in rapid, DNA-based screenings for this kind of anthropogenic impact.

2. Materials and methods

2.1. Sampling

Push corer (50 × 8 cm i.d. PVC tubes; Planet Ocean Ltd., UK) sediment samples were obtained by a remotely operated vehicle (Oceaneering Magnum Plus) from the offshore supply ship M/V Njord Viking (Viking Supply Ships, Gothenburg) at three abandoned exploration drilling sites at the Goliat oil field in the southern Barents Sea (Fig. 1). The Goliat field is operated by Vår Energi AS, a Norwegian independent oil and gas company. Water depths in the area were in the range 360–420 m. At the surveyed sites only the top hole DC material had been deposited at the seafloor near the drillholes, while the material from the deeper well sections were collected at the drilling rig and shipped to shore for treatment and recycling. A transect obtained next to a drilling operation terminated in December 2006 (Y2006) was sampled on 29–30 November 2014, while transects from drillings terminated in October 2000 (Y2000) and January 2015 (Y2015) were collected on a cruise in the period 3–12 September 2015. Duplicate cores, separated by ≤1 m, were collected along linear transects in southeastern direction, stretching from the closest achievable distance from the well, varying in the range 5–15 m, via 30 m, 60 m, 125 m to the most remote sampling distance at 250 m (hereafter denoted D<5–15>, D30, D60, D125, D250). At two sampling sites, the Y2000/D30 and Y2015/D60, just a single sediment core was successfully collected.

Oxygen profiles were recorded on board in triplicate from each core by pushing a 1.1 mm diameter needle sensor (Unisense A/S, Aarhus, Denmark) stepwise downwards, with readings at 0.5, 1.5, 3.5 and 7.5 cm. Aerated seawater and 100 mM ascorbate in 100 mM NaOH were used as saturation and anoxic calibration points, in accordance with the manufacturer's recommendation.

The upper 10 cm of the sediment cores were divided into 4 sections as follows: 0–1 cm, 1–2 cm, 2–5 cm and 5–10 cm. The sampled sections were packed aseptically into plastic bags and frozen at −25 °C on board. In the laboratory, the samples were stored at −72 °C until analyses.

2.2. Geochemical analyses

Geochemical analyses were achieved in cooperation with the Department of Geosciences, UiT The Arctic University of Norway and for the Y2000 and Y2006 transects, more extensive data are published elsewhere (Aagaard-Sørensen et al., 2018; Dijkstra et al., 2020). The

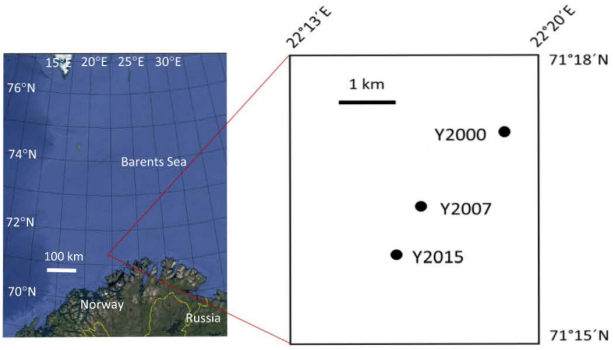

Fig. 1. Location of the surveyed area in the southern Barents Sea and positions of the 3 drilling sites within that area. Image: Google Earth.

Y2015 data were provided as unpublished by Juho Junttila. The material for the geochemical analyses was obtained from separate cores collected simultaneously and within 1 m distance from the cores for microbiota analyses. The original geochemical data were reorganized in accordance with the above-stated core sectioning pattern of the present study (Supplementary Fig. S1). In short, sediment grain size distribution was determined by a Beckman Coulter LS 13 320 Laser diffraction particle size analyzer. The particles were separated into two size classes; clay/silt (<63 μm) and sand/ gravel (>63 μm), and fraction of clay/silt was used as grain size indicator. Organic carbon content was estimated by weight loss on ignition methodology, with approximately 3 g starting material, heating at 550 °C for 12 h and 0.58 as conversion factor (Wang et al., 2011). Heavy metals concentrations were determined by Inductively Coupled Plasma Spectroscopy according to Environmental Protection Agency (US) methods 200.7 and 200.8 (http://www3.epa.gov) (Junttila et al., 2018).

2.3. Generation of 16S rRNA gene sequence-based community data

Extraction of DNA from sediment samples, 16S rRNA gene amplification and sequencing were done in accordance with a previous study (Nguyen et al., 2018). In short, duplicate 0.5 g samples of sediment were DNA-extracted (PowerSoil™ DNA Isolation kit; Mo Bio Labs, Carlsbad, CA, USA). The 16S rRNA genes were amplified by primers covering the V3-V4 region and 2 × 300 bp paired-end sequenced on an Illumina MiSeq instrument (Illumina Inc., San Diego, USA) according to the manufacturer's protocol (Barents Biocentre Lab, Tromsø, Norway). Joining of forward and reverse sequence reads, quality filtering, operational taxonomic unit (OTU) clustering and taxonomic annotation of OTU sequence tags (Greengenes database, v. 13.8; http://greengenes.lbl.gov) were all done within the framework of the Quantitative Insights Into Microbial Ecology (QIIME v.1.8) pipeline (Caporaso et al., 2010). The sequence data have been submitted to the EMBL database under the accession numbers PRJEB39105 (Y2000 data), PRJEB39106 (Y2006 data) and PRJEB 39126 (Y2015 data).

2.4. Statistical analyses

The bacterial community alpha-diversities of individual samples were quantified by the richness estimators abundance-based coverage estimator (ACE) (Chao and Lee, 1992) and Chao1 index (Chao, 1983), by the Simpson diversity index (Simpson, 1949) and by evenness, as defined by the ratio between the actual, OTU-based Shannon diversity index (H′) (Shannon, 1948) and the maximum possible Shannon index if all OTUs were equally represented ($H'_{max} = \ln S$, where S is the number of OTUs in the sample). All indices were estimated on the basis of average OTU distributions after 100 resamplings of 2000 sequence reads, as generated by the QIIME rarefaction tool. Comparisons of community composition (beta-diversities) were based on the taxonomic groupings that resulted from the OTU annotation. Non-proteobacterial groups were included at the level of class and the Proteobacteria at order level. Multivariate and statistical analyses were carried out by use of the R software (https://cran.r-project.org). Bray-Curtis distance after square root transformation of sequence read frequencies was used as beta-diversity measure. Multivariate ordination based on these distances was principally obtained by non-metric multidimensional scaling (NMDS), as implemented in the package *vegan* (Oksanen et al., 2019). Permutational analysis of variance on the NMDS data was performed by *adonis*. Environmental variables and experimental factors (drilling year, distance from drilling site, sediment depth) were fitted onto the NMDS plot by the function *envfit* and the goodness of fit tested by 999 permutations. Taxonomic groups that contributed significantly to explained community alterations at heavily DC affected sampling sites were sorted out by univariate permutation testing of generalized linear models, established by the package *mvabund* (Wang et al., 2012), with barium concentration as dependent variable.

3. Results

3.1. Bacterial community changes

Bacteria-specific partial 16S rRNA gene libraries were generated by identical procedures for all sediment samples (3 years × 5 distances × 4 sediment depths). The number of qualified reads per sample varied from $2.3 * 10^3$ to $5.9 * 10^5$, with average $6.4 * 10^4$. At most sampling locations two cores, separated by less than 1 m, were recovered, resulting in replicate libraries. However, at two sampling sites, Y2000/D30 and Y2015/D60, just a single core was successfully obtained. Additionally, PCR amplification failed for individual sediment samples and in one case, the 5–10 cm layer at Y2000/D<5–15>, the PCR reaction did not result in products from either of the two cores. In total, 107 separate 16S rRNA gene libraries were generated. After removal of consistently low abundance OTUs, i.e. the ones constituting <0.1% in all samples, the remaining 34,734 OTUs were binned into 146 groups at class level. Further subdivision of the Proteobacteria, which constituted an average of 47% of total bacterial reads, into the rank of order extended the list to 187 taxonomic groups.

Unconstrained multivariate ordination based on this grouping is shown in Fig. 2. All samples collected beyond 100 m from the drillhole clustered in the enclosed core section of the NMDS plot and they showed a random distribution according to drilling year and 125 m versus 250 m distance from the wells, as substantiated statistically by permutation test of variance. Three samples collected in year 2009 from the upper 4 cm of seafloor at remote and undisturbed locations in the southern Barents Sea (sampling stations 1–3 in Nguyen and Landfald (2015)) were reanalyzed with the 16S rRNA gene primers and sequencing technology of the present study. Their community profiles showed agreement with those of corresponding sediment depths in the present D125 and D250 cores (Fig. 2). A consistent community stratification pattern was manifested along axis 2 in the NMDS plot. There were significant differences between the two deepest segments, 2–5 cm and 5–10 cm and between each of them and the communities of the upper 2 cm ($P < 0.01$), while the 0–1 cm and 1–2 cm segments were not confidently distinguishable. In summary, the overall independence of time since drilling and distance from the drillhole, combined with the stable stratification pattern and the similarity with remote Barents Sea sediment communities, indicated that the microbiotas beyond 100 m were largely unaffected by the DC depositions.

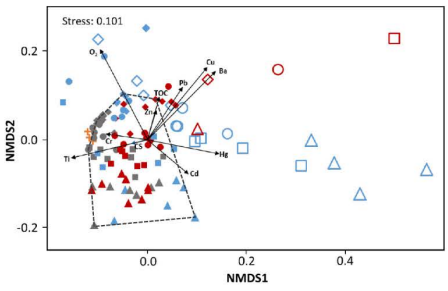

Fig. 2. Non-metric multidimensional scaling ordination of sediment samples based on 16S rRNA gene distribution data, and vector fitting of environmental variables. Colours: gray, Y2000 transect; blue, Y2006 transect; red, Y2015 transect; orange crosses, Barents Sea reference samples. Sediment depth division by markers: diamonds, 0-1 cm; circles, 1-2 cm; squares, 2-5 cm; triangles, 5-10 cm. Enlarged open markers represent the Y2006/D<5-15> - D30 (blue) and Y2015/D60 (red) cores. All samples collected at 125 m and 250 m distance are within the dashed line.

The Proteobacteria were dominant at all sampling sites, and the Delta and Gamma classes constituted, by far, the most prevalent groups both in the upper and lower parts of the 10 cm cores (Fig. 3). Univariate permutation tests based on generalized linear modeling pointed to reduced downward abundances of Bacteroidetes, Verrucomicrobia and orders of Gammaproteobacteria, including Alteromonadales, and increases in anaerobic bacteria like Desulfarculales and Dehalococcoidetes, as the strongest contributors to explained vertical community variation in the essentially undisturbed sediments beyond 100 m.

The bacterial communities of the Y2000 transect did not deviate significantly from the general D125 and D250 community profiles at any distance from the drilling site. This contrasted the conditions at the Y2006 and Y2015 sites where marked perturbations of the native microbiotas were observed at up to 30 m distance (Y2006) and at 60 m (Y2015). The NMDS plot showed the communities below 2 cm in the Y2006/D<5–15> and D30 cores and in the Y2015/D60 core as distinct "outliers" relative to the remaining material, principally manifested as large positive values along NMDS1.

All four alpha diversity indices, i.e. community evenness, as estimated by the H'/H'_{max} ratio, the Simpson diversity index, and the two phylotype richness estimators Chao1 and ACE, consolidated the pattern of localized community perturbations (Table 1). Stable high diversities, with evenness values in the range 0.85–0.91 and richness estimates from approximately 3000 to 4600 (based on resampling of 2000 OTUs) were observed in the anticipated little DC affected communities beyond 100 m and through the complete Y2000 transect. While these samples showed just a slight lowering of bacterial diversity in the bottom 5–10 cm segments of the cores, this trend was much stronger in the Y2006/D<5–15> and D30 cores, where the average value of the 5–10 cm segments dropped below 0.7 for evenness and 1200 for Chao1 and ACE richness. The Y2015/D60 core also showed a markedly deviating diversity pattern, with diversity minima in the 1–2 cm and 2–5 cm sections.

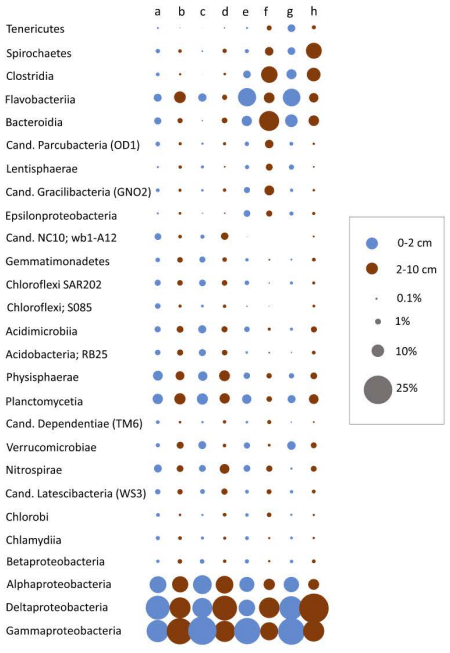

Fig. 3. Relative distribution of bacterial taxa in various sample categories, as grouped by drilling year, distance from drillhole and sediment depth (0-2 cm and 2-10 cm). Circle areas correspond to percentage 16S rRNA gene sequence tags for each phylum (class for Protebacteria). Only taxa that constituted an overall average of ≥1%, or ≥2% at one or more individual sampling sites, are included. Columns: **a** and **b**, all years, 125 m - 250 m; **c** and **d**, Y2000, ≤60 m; **e** and **f**, Y2006, ≤30 m, **g** and **h**, Y2015, 60 m.

Table 1
Bacterial community diversity estimates for sediment selections with different levels of drill cuttings deposition.

Sample group	Core depth range (cm)	H'/H'_{max} [a]	Simpson (%) [b]	Chao1 [c]	ACE [b]
All years; ≥125 m	0–1	0,90 ± 0,01	0.1 ± 0.0	4116 ± 300	3969 ± 562
	1–2	0,90 ± 0,01	0.1 ± 0.0	4178 ± 285	4143 ± 425
	2–5	0,89 ± 0,01	0.2 ± 0.1	4123 ± 285	3676 ± 549
	5–10	0,85 ± 0,03	0.4 ± 0.2	3317 ± 290	2936 ± 736
Y2000; ≤60 m	0–1	0,91 ± 0,01	0.1 ± 0.0	4610 ± 280	4033 ± 291
	1–2	0,89 ± 0,01	0.2 ± 0.0	4188 ± 304	3842 ± 483
	2–5	0,90 ± 0,02	0.2 ± 0.1	4093 ± 268	4430 ± 706
	5–10	0,87 ± 0,02	0.2 ± 0.1	3781 ± 250	4027 ± 459
Y2006; ≤30 m	0–1	0,88 ± 0,02	0.3 ± 0.1	3490 ± 245	3430 ± 716
	1–2	0,86 ± 0,02	0.3 ± 0.1	3455 ± 244	3183 ± 660
	2–5	0,83 ± 0,07	0.5 ± 0.6	2895 ± 234	2157 ± 781
	5–10	0,67 ± 0,08	3.8 ± 4.6	1178 ± 213	1152 ± 871
Y2015; 60 m	0–1	0,87	0.2	3499 ± 330	2595
	1–2	0,67	3.4	1558 ± 133	1161
	2–5	0,50	5.4	1222 ± 220	1014
	5–10	0,83	0.2	3420 ± 272	3293

All estimates are based on distribution of 2000 OTUs after 100 resamplings of original OTU data.
[a] H'_{max}: ln2000 = 7.601.
[b] Uncertainty range given as standard deviation between individual samples of each category.
[c] Uncertainty range generated by permutation in QIIME software.

3.2. Environmental variations

Accumulation of barium (Ba) and additional heavy metals is the most established geochemical tracer of DC residuals in marine sediments (Kennicutt et al., 1983; Hartley, 1996; Neff, 2005) and was employed as the principal quantitative indicator of remaining DC also in the present study. Increased levels of Ba were evident in the vicinities of all three drilling sites. The Ba concentrations were heightened relative to the baseline level of 31–81 mg kg^{-1}, reported in a pre-drilling survey (Veritas, 2014), throughout the 250 m transects, as the present post-drilling levels largely exceeded 100 mg kg^{-1} in the top 1 cm layer (Supplementary Fig. S1). However, the heavily DC deposited areas, as manifested by 1–2 orders of magnitude increases in Ba concentrations, were found within 30 m and 60 m, respectively, from the Y2000 and Y2006 drilling sites. At the Y2015 site, the Ba level peaked around 60 m, but showed high levels in the top layer throughout the sampling range.

Vector fitting of standardized geochemical variables to the NMDS ordination indicated association between the above-mentioned, most diverging bacterial communities and increased levels of the four co-varying heavy metals barium, copper, lead and mercury (Fig. 2). On the other hand, no causal effect on the microbiota of high residual heavy metals was substantiated, as increased metal concentrations were just as conspicuous near the Y2000 drilling site where no community distortions was observed. Hence, the metals indicated high levels of DC remains in the sediment, but did not appear to play a vital role as drivers of lasting bacterial community perturbation.

The direction of the oxygen vector reflected the sediment-depth stratification pattern of the bacterial communities by pointing in the direction of the top 2 cm communities. Although the spread between replicate recordings of O_2 profiles in the same cores was high, the measurements indicated that DC deposition led to a narrowing of the oxic layer in the sediment (Fig. 4). In the Y2006 transect, the O_2 concentrations in the upper 2 cm of sediment were significantly lower up to D60 than in the presumed marginally affected sediments beyond 100 m ($P < 0.01$). The uppermost 1-cm layer in the Y2015 transect showed O_2 concentrations of 75 μM or less up to D125 and only the D250 cores approached the levels observed along the Y2000 and Y2006 transects. Contrary to the conditions at the more recent Y2006 and Y2015 drilling sites, the Y2000 transect showed no relationship between high barium or other heavy metals and steep drops in oxygen concentration.

The two recorded non-metallic environmental variables, i.e. total organic carbon and clay + silt fraction, showed no significant correlation with bacterial community variation.

3.3. Emerging taxonomic groups in DC affected sediment

The bacterial community data were subjected to univariate significance tests in a generalized linear model, with barium concentration as the response variable, to disentangle the taxonomic groups with strong correlations with sediment DC loads. The complete Y2006 and Y2015 community data, but not those of the apparently unaltered Y2000 transect, were included. The GLM showed negative relationships between Ba concentration and a number of taxonomic groups with ubiquitous presence at less affected locations. These reductions were balanced by significant increases in just 5 groups, the Mollicutes, Clostridia, Spirochaetes, Desulfuromonadales and Bacteroidia, which all showed statistically robust positive relationships with Ba level ($P < 0.001$). The prevalence of these groups at the most DC affected locations was also evident as peak abundances of 16S rRNA gene reads in the untransformed, original data (Fig. 5). The pattern of DC-induced bacterial community change was similar in the Y2006 and Y2015 transects, but increased levels of the Bacteroidia and Clostridia groups was more pronounced in the Y2006 material than at the recently drilled Y2015 well. The Spirochaetes and Desulfuromonadales showed the opposite pattern. Contrary to the Desulfuromonadales and Bacteroidia, the presence of the Mollicutes, Clostridia and Spirochaetes was very marginal outside the most DC-affected locations. The percentage for the Mollicutes peaked at 2.0% of sequence tags in the Y2015/D60 core, while the figures never exceeded 0.1% the in the assumed unperturbed communities. The corresponding figures for the Clostridia were 14.2% (Y2006/D<5–15>) versus 0.7%, and for the Spirochaetes 13.7% (Y2006/D<5–15>) versus 1.1%. Although the Bacteroidia and Desulfuromonadales showed more ubiquitous presences throughout the transects, their fractions of total 16S rRNA gene tags showed distinct peaks in the Y2006/D<5–15> m cores for the Bacteroidia and the Y2015/D60 core for the Desulfuromonadales.

Taxonomic annotation to lowest reliable rank affiliated the majority of the Clostridia sequences to the families Christensenellaceae and Lachnospiraceae and there was no clear pattern of predominance by one or the other family related to drilling years. While no identity beyond class level was disclosed for the Mollicutes, the Spirochaetes were exclusively affiliated with the genus *Spirochaeta*. All Bacteroidia sequences were identified as affiliated to the order Bacteroidales and the Desulfuromonadales to the family Desulfuromonadaceae.

4. Discussion

4.1. The native microbiota

The sediment bacterial community profiles at the locations that were assumed insignificantly affected by DC, i.e. the samples collected beyond 100 m from the drilling sites, consolidated a pattern of spatial and temporal stability in the southern Barents Sea region. Upper sediment samples collected more than 5 years prior to the present survey, at locations more than 200 km north of the Goliat field, showed close match with the outcome of the present study (Nguyen and Landfald, 2015). Undisturbed sediment material collected at a deep-water continental slope site some 200 km west of the Goliat field also showed a high degree of community identity (Nguyen et al., 2018). The spatial stability was just as evident on the local scale, as there were insignificant differences both between the communities in the D125 and D250 cores at each drilling site and between these cores from the three drilling sites separated by up to 3 km. Hence, the microbiota of the undisturbed seafloor appeared less sensitive to random environmental variations than the macrofauna. In a related study, marked differences were observed with respect to polychaete populations at D125 and D250 in both the Y2000 and Y2006 transects (Cochrane et al., 2019).

4.2. Effects of water-based drilling muds on the bacterial community

The present Barents Sea survey and the preceding one addressing the deeper continental slope site (Nguyen et al., 2018) are, to the best of our knowledge, the first studies to address the in situ microbiotal effects of water-based DC. Both studies confirm marked, localized bacterial community changes, as evidenced by reduction in diversity and altered taxonomic profile. Moreover, the locations with the most strongly affected microbiotas, i.e. the Y2006/D<5–15> and D30 and the Y2015/D60 sites of the present study, coincided with the most affected locations as revealed by optical and macrofaunal surveys (Cochrane et al., 2019). There was also agreement between the different surveying approaches

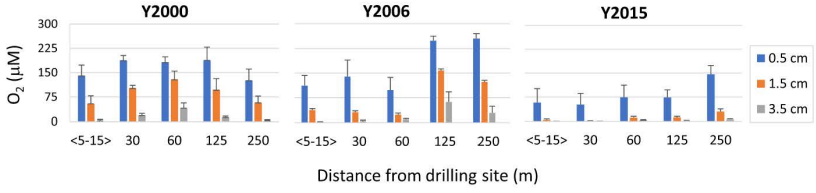

Fig. 4. Profiles of pore water oxygen concentrations down the sediment, separated by distance from the drilling site. At 7.5 cm sediment depth all measurements showed <1 mM and are not included in the graphs.

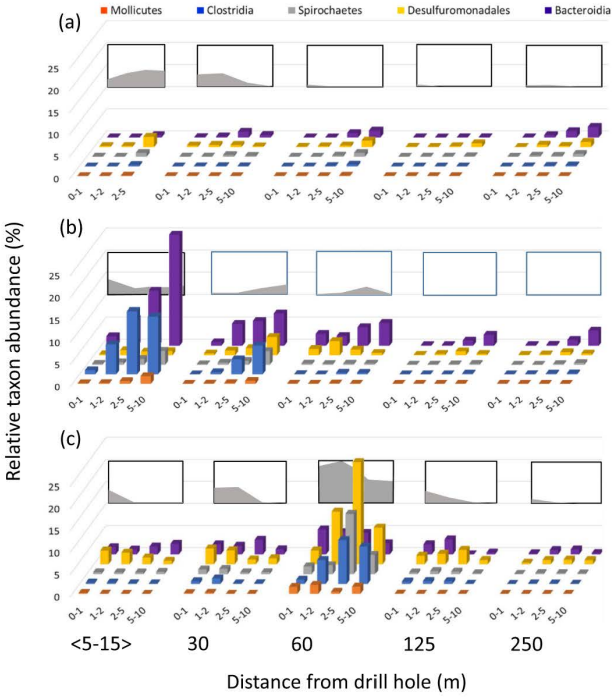

Fig. 5. Relative abundances of 16S rRNA sequence tags of the five bacterial classes that showed significant positive correlation with barium concentration in the Y2006 and Y2015 transects. Drilling years: (a) Y2000, (b) Y2006 and (c) Y2015. The values for each taxon are partitioned by sediment depth and distance from the drilling site. Gray area charts: Depth profiles of barium concentrations at each sampling site, with same scale in all graphs (0-15 mg g^{-1}).

as they all indicated marginal disruption of the seafloor's natural biota beyond 100 m from the drilling sites.

The bacterial profiles presented an ambiguous picture with respect to the resilience of the microbiota at DC-affected locations. The deeper parts of the cores collected close to the Y2006 drilling site maintained marked deviations from native sediment for 8 years, while no distortion of community structure was observed next to the Y2000 drilling site. We find it unlikely that the difference is purely attributable to the longer time span since the Y2000 drilling. More plausibly, the time effect added to a less radical distortion of the Y2000 microbiota in the first place, related to differences in DC composition or quantity.

Reduction of the oxic layer thickness in sediments due to water-based DC deposition has been documented in mesocosm and in situ studies. Stimulated heterotrophic activity by organic constituents in the drilling mud is pointed out as a contributing factor and the glycol component proposed as a main carbon source (Schaanning et al., 2008; Trannum et al., 2011). Reduced pore water O_2 concentrations at DC-affected locations were also observed in our in situ surveys. There was, however, no strong spatial association between impaired oxygen penetration and the altered bacterial community structures, implying that additional factors to general O_2 stress contributed to the observed community change. One or more organic drilling fluid constituents, common to the Y2006, Y2015 and continental slope (Nguyen et al., 2018) drilling operations, appear as likely candidates. However, no drilling mud ingredients except seawater and mineral components (bentonite, ilmenite, barite) have been specified for the top hole drilling operations at the surveyed drilling sites. Hence, the identity of putatively influential chemical factors remains unresolved.

4.3. *The bacterial markers of water-based DC impact*

Three out of five bacterial groups that showed significantly increased presence at the heavily DC impacted sampling sites in the present study, i.e. the Mollicutes, Clostridia and Desulfuromonadales, were coincident with the groups that showed the same response in the preceding continental slope survey (Nguyen et al., 2018), while the Spirochaetes and Bacteroidia groups remained unaffected by DC in the previous study. The five taxa belong to different phyla and vary widely with respect to morphology and physiology. Some are conventionally associated with microbial ecosystems that diverge widely from marine sediments. However, high-performance ribosomal RNA gene sequencing, as employed in this study, tend to reveal low relative abundances of much broader selections of taxonomic groups in natural environments than previously appreciated. The Mollicutes showed the statistically strongest affiliation to the DC-affected locations This class of bacteria, commonly known as mycoplasmas, are without exception categorized as commensal or parasitic organisms in plants, invertebrates and vertebrates (Brown, 2010). Demonstrated presence in marine sediments is

limited to a study by Koo et al. (2015) who observed an increase from undetectable level to 4.4% in relative abundance when adding crude oil to a salt marsh sediment microcosm system. The host organisms of the Mollicutes in the present marine sediments remain unclarified, but it appears unlikely they are macroscopic. The small quantity of sediment sample (0.5 g) and the procedure used for DNA extraction should reveal organisms that are visible by eye. We therefore anticipate that the population of Mollicutes was hosted by protists or microscopic invertebrates and that unidentified components in the DC induced their proliferation. This increase may be due to higher numbers of host organisms or higher bacterial densities within a stable population of hosts. Well-documented association between protists and mycoplasmas is, however, limited to the human pathogen *Trichomonas vaginalis*, which harbor *Mycoplasma hominis* in a symbiotic relationship (Dessi et al., 2019).

The Clostrida – as endospores or vegetative cells – are ubiquitous in oceanic marine sediments, but are largely found in low relative abundances. Higher fractions of Clostridia are commonly found in coastal areas with increased primary production, accompanied by stronger flux of organic material onto the seafloor and more anoxic conditions in the uppermost sediment layer (Schauer et al., 2010; Zinger et al., 2011). Sediments affected by anthropogenic organic pollution from e.g. aquaculture or oil production are, likewise, characterized by substantial fractions of Clostridia (Hasegawa et al., 2014; Koo et al., 2015; Ape et al., 2019). The two emerging Clostridia groups in the present sediment material, Christiansenellaceae and Lachnospiraceae, are both primarily characterized in the context of human gut microbiota (Morotomi et al., 2012; McLellan and Eren, 2014), but are also identified as degraders of freshwater algal material (Morrison et al., 2017) and as inhabitants of polluted marine sediment systems (Espinola et al., 2018; Ape et al., 2019; Cupit et al., 2019). Like the Clostridia, the genus *Spirochaeta* is mostly categorized as obligate anaerobic bacteria with a fermentative metabolism. They have been demonstrated as a minor, but rather common component of marine sediments, and significant presence is observed at locations with increased amounts of organic material through a natural phenomenon like whale falls (Goffredi and Orphan, 2010), or pollution (Nogales et al., 2011; Shivani et al., 2015; Dong et al., 2018).

In general, the anoxic layers of native marine sediments have a high fraction of Deltaproteobacteria with capacity for various types of anaerobic respiration. The family Desulfuromonadaceae was a ubiquitous, although minor group of the Deltaproteobacteria in the assumed native sediments in the present study. This group includes genera with capacity for degradation of organic molecules coupled to reduction of sulfur compounds, nitrate and various metal ions (Greene, 2014). Increased abundances of representatives of the Desulfuromonadaceae have been correlated with anthropogenic pollution by polyaromatic and aliphatic hydrocarbons in Polar sediments (Espinola et al., 2018). However, the present study did not give grounds for concluding which specific factors led to the strong emergence of the Desulfuromonadaceae at the DC-affected locations.

The Bacteroidales (class Bacteroidia) constituted close to one-quarter of total 16S rRNA gene tags generated from the Y2006/D<5–15> cores. These bacteria were, however, also found in significant proportions in the deep sediment sections of the unaffected sampling sites. The Bacteroidales are dominated by anaerobic, fermentative bacteria (Krieg et al., 2010). There is very limited documentation of Bacteroidia as inhabitants of marine sediments, but they have recently been shown to make up substantial fractions of Polar sediment microbiotas both in the Northern and Southern hemisphere (Espinola et al., 2018). With some reservation for the Mollicutes, we conclude that the bacterial taxa that showed marked increase at the heavily DC-affected sampling sites corroborated a selective role of one or more organic components as essential drivers of community change.

5. Concluding remarks

The present study has firmly established that deposition of water-based DC causes perturbation of the bacterial communities in the receiving sediment. The spatial extent of significant microbiota change appears not to extend beyond what is manifested by established environmental survey methods like visual inspection and benthic macrofaunal community perturbation. The study confirmed that DC-induced community shifts can last for at least 8 years, but it was inconclusive regarding the long-term resilience of the microbiota after this type of environmental insult.

A few taxonomic groups of bacteria show distinct increases in relative abundance at DC-affected locations, despite geographical and temporal variations between the drilling sites. Provided that this observation represents a more universal bacterial response to deposition of water-based DC, one or more of these bacteria are interesting candidates as indicator organisms of the state of the sediment by an environmental DNA based approach (Cordier et al., 2020). The overall agreement between the spatial extent of detectable bacterial change and severe seafloor change at the macroscopic level makes a rapid, DNA-based method for identifying informative bacterial taxa an interesting supplement to established surveying technologies. Such an approach would most likely be based on the development of taxon-specific oligonucleotides to be used as primers in ordinary or real-time PCR gene amplification.

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CRediT authorship contribution statement

Tan T. Nguyen: Formal analysis, Investigation, Writing – review & editing. **John E. Paulsen:** Resources, Writing – review & editing. **Bjarne Landfald:** Conceptualization, Writing – original draft, Supervision.

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.

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