Potential microbial contamination from drilling lubricants into subseafloor rock cores

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Abstract. International Ocean Discovery Program (IODP) Expedition 357: “Serpentinization and Life” drilled shallow cores into the Atlantis Massif near the Mid-Atlantic Ridge in October 2015 using seabed drills. Serpentinization and other geochemical processes occurring within the Atlantis Massif release hydrogen, methane, and other chemicals that can potentially fuel microorganisms through chemosynthesis. The subseafloor rock cores collected during IODP Exp. 357 are the first of their kind, meaning the analysis and interpretation of these samples required new methodologies, including a specialized approach for distinguishing endemic subsurface inhabitants from potential contaminants from various sources. Background samples of various potential contamination sources were collected during sampling: 109 samples of seawater collected before, during, and after drilling; 20 samples of greases and oils associated with the drilling equipment; and samples of the laboratory’s ambient air. Despite the widespread usage of drilling lubricants and the importance of controlling contamination in drill-core samples for microbiological analyses, no studies to date have looked at DNA in drilling greases and oils. In this study, drilling lubricants were analyzed as possible sources of microbial contamination of subseafloor rock core samples by environmental sequencing of 16S rRNA genes. We find that microbial signatures from drilling lubricants are only found in low abundance in seafloor samples (at most a few percent of total sequence counts), with laboratory contaminants being a greater source of contamination.

1 Introduction

Due to the low biomass of many subsurface habitats (Kallmeyer, 2017; Smith et al., 2000b), there is a need for improved quality control metrics in order to distinguish between endemic microbial communities and those introduced through contamination (Friese et al., 2017; Smith et al., 2000b; Wilkins et al., 2014; Kallmeyer 2017; Yanagawa et al., 2013; Santelli et al., 2010; Smith et al., 2000b). Contamination of drill core samples can occur from multiple sources before, during, and after drilling. Tracers introduced during drilling (e.g., Friese et al., 2017; Kallmeyer, 2017; Kallmeyer et al., 2006; Lever et al., 2006; Smith et al., 2000a; Yanagawa et al., 2013) are an essential tool for tracking environmental contamination that occurs during drilling, but such tracers cannot identify all possible sources of contamination. Methods for tracking and monitoring the level of contamination introduced during drilling can be generally grouped into three categories: (1) particle tracers (e.g., microspheres), (2) chemical or dissolved tracers (e.g., perfluorocarbon compounds such as perfluoromethylcyclohexane – PFC), and (3) microbiological analyses (e.g., 16S rRNA, fatty acids) (Kallmeyer et al., 2006). Depending on the choice of tracer, various techniques can be used to determine the level of contamination of the drill core samples.

The Lost City is an iconic hydrothermal vent system located on the Atlantis Massif, near the Mid-Atlantic Ridge and Atlantis Fracture Zone (Kelley et al., 2005). The Atlantis Massif is a site of active seafloor serpentinization, and hydrothermal fluids venting through the Lost City chimneys contain products of serpentinization reactions, including hydrogen and methane gas, that can fuel chemosynthetic microorganisms (Kelley et al., 2005; Lang and Brazelton, 2020). During International Ocean Discovery Program (IODP) Expedition 357: “Serpentinization and Life”,

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shallow rock cores were drilled in several locations along the Atlantis Massif to recover serpentinite cores where serpen
tinization is actively occurring, using seabed drills (Früh-
Green et al., 2018). Seabed drills differ from traditional plat-
form drilling as they are lowered directly to the drilling site
and use bottom seawater as the drilling fluid instead of
fluid from a non-indigenous source (Freudenthal and We-
fer, 2007). Two seabed drills were used on the Royal Re-
search Ship *James Cook* for this expedition: RD2 (British
Geological Survey) and MARUM-MeBo70 (Center for Ma-
rine Environmental Sciences at the University of Bremen)
(Früh-Green et al., 2017a; Freudenthal and Wefer, 2007).
IODP Exp. 357 employed the use of the synthetic tracer PFC
(Smith et al., 2000b) mixed into flushing seawater in an ef-
tort to assess the level of contamination introduced into the
cores (Orcutt et al., 2017).

The subseafloor rock cores collected during IODP
Exp. 357 are the first oceanic crust samples to be collected
with seabed drills and suitable for microbiology, so the de-
velopment of new methodologies, including a specialized ap-
proach for distinguishing true subsurface microbial inhab-
itiants from surface contaminants (Motamedi et al., 2020).
In addition to investigating seawater and lab air as poten-
tial sources of contamination of the core samples, the industrial
oils and greases used during the drilling process and which
potentially came into direct contact with the core samples
were previously examined as potential sources of organic
molecule contaminants (Hickok et al., 2018). Here, we re-
port an investigation of potential microbial contamination in
these same greases and oils, as measured by DNA sequenc-
ing.

2 Methods

2.1 Sample collection and processing

Drill core and seawater samples were collected during
IODP Exp. 357 and are described in detail in Motamedi et
al. (2020). Briefly, core subsamples identified for microbi-
ological analyses were immediately retrieved from the drill
upon its return to the ship deck, wrapped in acid-washed and
autoclaved teflon, and stored at −80°C. Core samples were
then shipped to the Kochi Core Center (Japan) for further
processing and subsampling under sterile conditions (Früh-
Green et al., 2017b; Orcutt et al., 2017). Please see Früh-
Green et al. (2017a, 2018) for more details on lithology and
other details of the recovered cores.

Prior to the deployment of the drill at each site, the ship’s
conductivity, temperature, and depth (CTD) Niskin bottle
rosette was cast to collect a water-column profile, with six
10 L bottles being triggered approximately 2–3 m above
the seafloor and three 10 L bottles collected at even intervals
throughout the water column (Früh-Green et al., 2017b).
Additionally, a 4 L bucket was used to collect surface seawater
surrounding the ship. Ten-liter Niskin bottles were also at-
tached to each drill and were used to collect bottom seawater
during and immediately after drilling. A total of 109 sea-
water samples were collected during IODP Exp. 357. All sea-
water samples were filtered with a peristaltic pump through
0.22 µm Sterivex™ filters onboard the ship and stored at
−80°C until DNA extraction (Motamedi et al., 2020).

The drill grease and oil samples were collected in sterile
15 mL tubes directly from their original packaging. Additionally,
one methanol-soaked filter used to wipe down a stain-
less-steel core liner, plastic shards shaved from a plastic
core liner, and samples of spray paint that were used on the
drill were collected. These samples were all stored at −80°C
until extraction. Two sets of these samples were collected,
one for testing of organic chemical contamination (Hickok
et al., 2018) and the other for microbial contamination test-
ing, described here. All grease/oil extractions took place in
a HEPA-filtered room using an aseptic technique. The lab
bench was wiped down with 80 % ethanol prior to each set
of extractions. Lab air samples were collected by filtering
air through 0.1 µm Puradisc 25 mm PTFE syringe filters (GE
Healthcare Whatman, Pittsburgh, Pennsylvania, USA) by a
dual-head Air Cadet Model 420-2901-007K (Thermo Fisher
Scientific, Waltham, Massachusetts, USA). The lab air was
vacuumed through a total of three different filters for 9 h each
and was combined during DNA extraction. DNA extraction
and purification were performed using the same protocols
and reagents as outlined in Motamedi et al. (2020).

2.2 DNA extraction and purification

Test DNA extractions were performed to determine the best
protocol for use on the industrial grease and oil samples. For
the test samples, LMX “Red” Grease (Plews & Edelmann,
IL, USA), WD-40 spray (CA, USA), and mineral oil were se-
lected for their accessibility and similarity to industrial grade
oils and greases used during IODP Exp. 357. The quality and
quantity of extracted DNA from six different protocols were
evaluated (Table 1). Two sets of extractions were performed:
the first set were unaltered test samples and the second set
were spiked with 10 µL of a turbid suspension of *E. coli*
cells to assess DNA recovery. Once the samples were ex-
tracted, both sample sets underwent DNA purification via 2x
SPRI beads (Rohland et al., 2012). After each step in the pu-
"
Table 1. Comparison of DNA-extraction methods with three test samples. Six DNA-extraction methods were compared on three test samples (WD-40, mineral oil, and LMX “Red” grease), chosen for their similar properties to industrial grade lubricants. The test samples were spiked with E. coli cells. All DNA yields from un-spiked test samples were below the detection limit (BDL). Each column shows the total amount of DNA extracted (ng).

| Extraction method                  | WD-40 Total ng | Mineral oil Total ng | LMX “Red” Grease Total ng |
|-----------------------------------|----------------|----------------------|---------------------------|
| MP FastDNA® SPIN Kit              | 7.1            | 5.8                  | 6.2                       |
| MoBio PowerLyzer                  | BDL            | BDL                  | BDL                       |
| MoBio PowerSoil                   | BDL            | 10.5                 | BDL                       |
| Wizard® Magnetic DNA Purification system for food | 6.5            | 5.5                  | BDL                       |
| Phenol: chloroform (Brazelton et al., 2017) | 8.6            | 15.5                 | BDL                       |
| ZR Fecal DNA MiniPrep             | BDL            | BDL                  | BDL                       |

instrument. The quantity of starting material for the extraction ranged from 0.25 to 1.0 g (Table 2). Extracted DNA was purified via 2x SPRI beads (Rohland et al., 2012). If replicate extractions of the same sample were possible, the extracted DNA from those replicates was pooled together during the DNA purification step. Additionally, six blank samples of Invitrogen UltraPure™ distilled water were extracted alongside the grease and oil samples as an additional precaution to test for kit contamination, potential contamination introduced during the extraction process, and sequencing contamination (Salter et al., 2014). The extraction of DNA from rock core, seawater, and laboratory air samples was previously described in detail in Motamedi et al. (2020).

2.3 Sequencing and analysis of 16S rRNA genes

Purified DNA preparations from IODP Exp. 357 rock cores, seawater, laboratory air, and greases and oils were sent to the Michigan State University Research and Technology Support Facility Genomics Core for sequencing of the V4 region of the 16S rRNA gene using the duel-indexed Illumina fusion primers 515F-806R (Kozich et al., 2013). All grease and oil samples were submitted for sequencing twice (i.e., sequencing replicates), and the results from both replicates are included in our analysis, with the exception of a single sample, GREd003, one replicate of which was determined to have been compromised during sequencing. Sequences from seawater and rock core samples were previously reported in Motamedi et al. (2020). Analysis of the 16S rRNA gene amplicon sequences from the greases and oils, rock cores, seawater, laboratory air, and extraction blanks was conducted with the mothur (v.1.39.5) software platform (Schloss et al., 2009). Sequences with > 8 homopolymers and > 0 ambiguous bases were removed from downstream analyses, and the sequences were then pre-clustered with the mothur command pre.cluster (diffs = 1) to remove rare sequences most likely created by sequencing errors (Schloss et al., 2011). Operational taxonomic units (OTUs) were formed with a 97% similarity threshold using the VSEARCH DGC clustering algorithm (Rognes et al., 2016) in mothur. Of the 31406783 paired sequences, 75189 OTUs were identified among the greases and oils, seawater, rock cores, laboratory air, and extraction blanks. Taxonomic classification of all OTUs was performed with mothur using the SILVA reference alignment (SSURefv132) and taxonomy outline (Pruesse et al., 2012). The proportion of contamination from seawater, laboratory air, or industrial grease and oil into each rock core sample was estimated using SourceTracker2 v.2.0.1 (Knights et al., 2011). All sequence data are available via NCBI SRA Bio-Project PRJNA575221.

3 Results and discussion

3.1 DNA-extraction tests for industrial greases and oils

To our knowledge, the extraction of DNA from industrial greases and oils has not been previously published, either in the context of scientific drilling projects or in other uses of industrial lubricants. DNA-extraction protocols have been published for testing the integrity of food oils such as olive oil (Busconi et al., 2003; Consolandi et al., 2008; Testolin and Lain, 2005) and soybean oil (Pauli et al., 1998), but not industrial oils. Multiple DNA-extraction protocols were evaluated for this study using three test samples (LMX “Red” Grease, WD-40 spray, and mineral oil) that were spiked with E. coli cells before DNA extraction. The MPBio FastDNA® SPIN Kit (Qbiogene, Inc., CA) was the only method able to extract detectable DNA from all three of the test samples (Table 1). Extractions from these test samples highlighted viscosity as a key challenge for adapting extraction protocols for greases and oils. In general, less viscous oils were easier to extract than the more viscous grease samples due to difficulties in implementing the physical lysing of thick greases. 16S rRNA gene amplicon sequencing of the spiked test samples confirmed that the recovered DNA was dominated by E. coli (data not shown).

3.2 Microbial composition of grease and oil samples

To assess the potential of drilling equipment to introduce contamination into drill core samples, we collected 20 samples of greases, oils, plastic shards, spray paint, and a cotton filter that wiped a stainless-steel core liner, all of which were used during IODP Exp. 357 (Table 2). All materials that were sampled had some interaction with the drills and introduced a potential for contamination into the rock cores. Six samples of Invitrogen UltraPure™ distilled water were extracted
Table 2. Description and DNA concentration of drill-associated samples. Twenty samples were taken during the duration of the cruise, including grease, oil, plastic shards, spray paint, and a cotton filter liner. Total DNA (ng) values represent measurements post DNA purification.

| Sample name | Sample type           | Drill | Purpose/location on drill                                      | Amount extracted (g) | DNA extracted (ng) |
|-------------|-----------------------|-------|----------------------------------------------------------------|--------------------|-------------------|
| 0GREd001    | Atlantis 22 hydraulic oil | RD2   | Hydraulic oil used in both MeBo and RD2 drills                  | 1.0 g               | BDL               |
| 0GREd002    | MeBo transformer fluid  | MeBo  | Used on MeBo drill                                              | 1.0 g               | BDL               |
| 0GREd003    | Loclite 638            | RD2   | Used on the threads of the drill rods                           | 1.0 g               | BDL               |
| 0GREd004    | K Nate BGS drill       | RD2   | On bolts and drill rods                                         | 0.50 g              | BDL               |
| 0GREd005    | B30 transformer oil    | RD2   | Used on RD2 drill                                               | 1.0 g               | BDL               |
| 0GREd006    | Contact grease         | RD2   | Located on electrical connections                                | 1.0 g               | BDL               |
| 0GREd007    | MeBo Anti-Seize        | MeBo  | Greases threads at the top of the core barrel                   | 1.0 g               | 6.25              |
| 0GREd008    | MeBo Aqua Shield       | MeBo  | Greases threads at the top of the core barrel                   | 0.50 g              | 5.25              |
| 0GREd009    | RD2 grease             | RD2   | Used on RD2 drill                                               | 0.25 g              | BDL               |
| 0GREd010    | Fincox GC Mei Belpask  | MeBo  | Greased threads of the rods                                     | 1.0 g               | 7.55              |
| 0GREd011    | Tuflube                | RD2   | Launch and recovery system on drill                             | 0.75 g              | 5.35              |
| 0GREd012    | Sapphire Aqua 2        | RD2   | Launch and recovery system on drill                             | 0.75 g              | 3.18              |
| 0GREd013    | MeBo seawater grease   | MeBo  | Used on core lifter case and core breaker                       | 0.75 g              | 2.56              |
| 0GREd014    | Brit Lube              | RD2   | Drill rods, packers                                             | 0.75 g              | 1.72              |
| 0GREd015    | Umbilical cord grease  | RD2   | Used to lubricate umbilical cord for RD2 drill                  | 0.50 g              | 40.4              |
| 0GREd016    | Atlantis 22 hydraulic oil | MeBo | Hydraulic oil used in both drills                               | 1.0 g               | 5.4               |
| 0GREd017    | Panolin hydraulic oil  | MeBo  | Used on MeBo drill                                              | 1.0 g               | 6.3               |
| 0GREd018    | Plastic shard liner    | MeBo  | Shards from the plastic core liners                             | 3 thin strips       | BDL               |
| 0GREd019    | Split cotton liner     | MeBo  | Wiped down core liner with methanol-soaked filter               | One liner           | BDL               |
| 0GREd020    | Spray paint            | RD2   | Dried spray paint located on drill and flaked off               | 0.25 g              | BDL               |

Alongside the grease and oil samples to account for any possible contamination introduced from the DNA-extraction kit or during the extraction and sequencing process.

From these 26 samples, we obtained a total of 4,339,588 paired sequences of 16S rRNA gene amplicons, which were clustered into 5629 OTUs at a 97% sequence similarity threshold. Any OTUs detected in the DNA-extraction blank samples were removed from the dataset, leaving 4,694 OTUs (Table S1 in the Supplement). Gammaproteobacteria constituted the highest percentage of taxa in the oil and grease samples (32% of total sequences; Fig. 1), and the most abundant Gammaproteobacteria OTUs could not be classified below the class level (Table S1). Alphaproteobacteria (17% of total sequences) were primarily represented by Sphingomonadaceae, Rhodobacteraceae, and Acetobacteraceae. Bacteroidia (10% of total sequences) were primarily represented by Flavobacteriaceae, Spirosomaceae, and Hymenobacteraceae. Betaproteobacteriales (4% of total sequences, but note that Betaproteobacteriales are classified as an order within class Gammaproteobacteria in the SILVA taxonomy) were dominated by various genera of the Burkholderiaceae.

DNA sequences in the oils and greases had high similarity to sequences from a wide range of environments, including soil (e.g., NCBI accessions HM104622, MG716681, AM940870, KP786168, NR_163645), glaciers (HQ333317, MN880348), lake sediments (MT067094), a geothermal plant (KY077452), a shallow marine hydrothermal vent (GU369930), and seawater (JN233022, KX177824). Sequences associated with petroleum-contaminated environments were also identified (EU328045, KY190357). Many of the matching sequences were associated with drilling projects, such as an IODP borehole (KR072759), marine
seds (CP004387, MF977474), a continental borehole (KP901594), and groundwater wells (KC606558). A few OTUs obtained from a swipe of a core liner barrel matched those from continental subsurface studies (MT067098, HM185963, HM641526). Notably, several OTUs that were abundant in the grease that is used on RD2’s umbilical cord were nearly identical to clones from a deep-sea drilling and coring contamination study (Yanagawa et al., 2013), notably including those recovered from the drilling fluid in that study (e.g., AB824901). A summary of best sequence matches is provided in the Supplement (Table S2).

3.3 Potential oil and grease contamination of seawater samples

All of the most abundant grease/oil OTUs were also identified in samples of seawater collected during the expedition. Because the greases and oils were sampled directly from their commercial product packages (except for the core liner swab and paint chips), contamination from seawater into the grease/oil samples seems unlikely. The grease/oil OTUs that are most abundant in seawater samples were almost exclusively derived from GRED008 (Aquashield, lubrication for MeBo core barrel threads) and GRED015 (RD2 umbilical cord grease). Furthermore, seawater samples collected with Niskin bottles mounted on MeBo and RD2 were especially likely to contain OTUs from the Aquashield grease (Table S1). However, overall, these potential grease/oil contaminants represent a small fraction of the total sequence dataset from seawater (<1 % of all sequence counts).

3.4 Minimal oil and grease contamination of rock cores

The rock core samples collected during IODP Exp. 357 were exposed to potential contamination sources before, during, and after drilling (Fig. 2). The extent of DNA contamination from seawater into the rock cores was investigated by Motamedi et al. (2020), and here, we extend that analysis to include grease and oil samples as additional potential sources of contamination.

Of the 4694 OTUs identified in all grease and oil samples, 565 OTUs were also identified in at least one rock core sample from IODP Exp. 357 (Table S3). However, 86 of these OTUs were also identified in the ambient lab air, suggesting that some of these sequences represent general contamination from dust particles during laboratory handling. In addition, the taxonomic classifications of many of these OTUs suggest that they are derived from commercial reagents (e.g., Burkholderia) or the human microbiota (e.g., Enterobacteriaceae), based on previous studies (Sheik et al., 2018; Salter et al., 2014), even though they were not detected in the extraction blanks or ambient lab air during our study. OTUs that are suspected to be contaminants on the basis of their taxonomic classification are highlighted in (but not removed from) the tables in the Supplement.

The remaining 479 OTUs that represent potential grease/oil contaminants of the rocks (Table S3) comprise 16 % of the total OTUs and 24 % of the total sequence counts in the rock core samples. However, most (90 %) of these sequence counts in rocks are contributed by OTUs that were found in low abundance in our samples of greases and oils (<100 total counts across all GRE samples), casting doubt that the greases and oils were the source of most of these contaminants into the rock cores. Abundant OTUs from greases and oils were generally very rare in the rock cores.

We estimated the proportion of DNA sequences from each rock core sample that could be attributed to each potential source of contamination (i.e., seawater, laboratory air, or drill grease and oil) using SourceTracker2 (Fig. 3). OTUs with “unknown” sources could not be assigned to a single contamination source and may represent true inhabitants of the rock cores. Lab air was the largest source of contamination into the rock cores, and contamination from other sources was minimal. Greases and oils were estimated to contribute at most a few percent of the sequences in each rock core sample, and their contribution was nearly zero in many of the samples. Nevertheless, the detectable levels of contamination from grease/oil and seawater are notable, considering the extensive precautions employed during handling and processing of the rock core samples (Früh-Green et al., 2017a, 2018; Hickok et al., 2018; Motamedi et al., 2020). These precautions (including the use of bottom seawater as the drilling fluid, immediate freezing of core samples, and shaving of
| Phylum               | Class                      | Order                          | Family                          | Genus/Species             | Most Abundant in Sample of          |
|---------------------|----------------------------|--------------------------------|--------------------------------|---------------------------|-------------------------------------|
| Acidobacteria       | Blastocatellia_(Subgroup_4)| Blastocatellales                | Blastocatellaceae              | Blastocatellaceae_unclassified | Umbilical cord grease              |
| Actinobacteria      | Actinobacteria Corynebacteriales | Corynebacteriaceae                | Turicella                      | Panolin                    | Hydraulic oil                      |
| Chloroflexi         | Chloroflexia                | Thermomicrobiales                | JG30-KF-CM45                   | JG30-KF-CM45_ge            | Umbilical cord grease              |
| Cyanobacteria       | Oxyphotobacteria Nostocales | Nostocales_unclassified          | Nostocales_unclassified         | Nostocales_unclassified    | B30 transformer oil                |
| Cyanobacteria       | Oxyphotobacteria_Incertae_Sedis | Unknown_Family                  | Calothrix_KVSF5                | MeBo                      | Seawater grease                   |
| Firmicutes          | Bacilli                     | Bacillales                       | Planococcaceae                 | Planococcaceae_unclassified | Loclite 638                        |
| Firmicutes          | Clostridia                  | Clostridiales                    | Clostridiaceae_1               | Clostridium_sensu_stricto_1 | Atlantis 22 hydraulic oil           |
| Firmicutes          | Clostridia                  | Family_XI                        | Lachnospiraceae_unclassified   | Lachnospiraceae_unclassified | Atlantis 22 hydraulic oil           |
| Firmicutes          | Clostridia                  | Ruminococcaceae                 | Ruminococcaceae_unclassified   | Ruminococcaceae_unclassified | MeBo                                |
| Firmicutes          | Clostridia                  | Peptostreptococcaceae            | Romboutsia                     | Romboutsia                | Atlantis 22 hydraulic oil           |
| Firmicutes          | Clostridia                  | Alphaproteobacteria              | Xanthobacteraceae              | Xanthobacteraceae_unclassified | MeBo                                |
| Firmicutes          | Betaproteobacteriales       | Nitrosomonadaceae                | DSSD61 Spray paint             | DSSD61 Spray paint         |                                                   |
| Firmicutes          | Gammaproteobacteriales      | Nitrosococcaceae                | wb1-P19                        | wb1-P19                   | Atlantis 22 hydraulic oil           |
| Firmicutes          | Gammaproteobacteriales      | Oceanospirillales                | Alcanivoracaceae               | Alcanivorax                | MeBo Aqua Shield                   |
| Firmicutes          | Gammaproteobacteriales      | Pseudomonadales                 | Moraxellaceae                  | Alkanindiges               | K Nate BGS drill                   |
| Proteobacteria      | Gammaproteobacteriales      | Xylophagococcales               | Thiobacillales                 | Thiobacillales             | Plastic shard liner                |
| Proteobacteria      | Gammaproteobacteriales      | Chondromonadaceae               | Inoculum Family                | Inoculum Family            |                                                   |
| Proteobacteria      | Gammaproteobacteriales      | Cyanobacteriales                 | Synechococcus                  | Synechococcus              |                                                   |
| Proteobacteria      | Gammaproteobacteriales      | Oxysphaerella_Sedula            | Nostocaceae                    | Nostocaceae                |                                                   |
| Proteobacteria      | Gammaproteobacteriales      | Oxysphaerella_Incertae_Sedis    | Nostocaceae                    | Nostocaceae                |                                                   |
| Proteobacteria      | Gammaproteobacteriales      | Achromatiales                    | Thiobacillales                 | Thiobacillales             |                                                   |
| Proteobacteria      | Gammaproteobacteriales      | Achromatiales                    | Achromatiales                  | Achromatiales              |                                                   |
| Proteobacteria      | Gammaproteobacteriales      | Alphaproteobacteriales           | Achromatiales                  | Achromatiales              |                                                   |

Table 3. Condensed phylogenetic table of 27 most likely contaminant OTUs from the grease and oil samples.
core exteriors with a sterile rock saw in a dedicated facility) are not practical for many drilling projects, suggesting that these contamination levels may be higher in other studies.

We assembled a final list of 27 likely contaminant OTUs from greases and oils (Table S4 and summarized in Table 3) based on their absence in extraction blanks and lab air and their much higher abundance in greases and oils compared to seawater (i.e., > 5 × greater abundance and > 500 total counts in greases and oils; see Table S3 for numbers). These likely contaminants were mostly derived from GREd001 (hydraulic oil used on both RD2 and MeBo) and GREd015 (RD2 umbilical cord grease) and were also moderately abundant in GREd004 (K Nate grease), GREd006 (electrical contact grease), and GREd008 (AquaShield grease). They represent 8 phyla and 10 classes, with Clostridia the most frequently appearing. As noted in Sect. 3.2, the most abundant of these sequences had high similarity to database sequences reported from a wide range of environments.

4 Conclusions

The possibility of contaminant DNA introduced by greases and oils associated with drilling equipment had not been previously explored. We have demonstrated that DNA can be detected in industrial greases and oils and that these same DNA sequences can also be found at low levels in low-biomass rock cores and in seawater samples. Nevertheless, our results indicate that, for our study, contamination from greases and oils was much less prevalent compared to contamination during laboratory handling, as measured by DNA extracted from dust particles in ambient lab air. Even though we do not expect greases and oils to be the most important source of contamination in most studies, levels of contamination from different sources will vary according to the particular circumstances of each project. Therefore, we recommend that future studies should monitor potential contamination from greases and oils associated with drilling and sampling equipment.

Data availability. All sequence data are available via NCBI SRA BioProject PRJNA575221. The data are accessible with the provided accession ID at the following link for the SRA database: https://www.ncbi.nlm.nih.gov/sra/ (last access: 18 March 2021).

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