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Perturbation of seafloor bacterial community structure by drilling waste discharge

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ABSTRACT

Offshore drilling operations result in the generation of drill cuttings and localized smothering of the benthic habitats. This study explores bacterial community changes in the in the upper layers of the seafloor resulting from an exploratory drilling operation at 1400 m water depth on the Barents Sea continental slope. Significant restructurings of the sediment microbiota were restricted to the sampling sites notably affected by the drilling waste discharge, i.e. at 30 m and 50 m distances from the drilling location, and to the upper 2 cm of the seafloor. Three bacterial groups, the orders *Clostridiales* and *Desulfuromonadales* and the class *Mollicutes*, were almost exclusively confined to the upper two centimeters at 30 m distance, thereby corroborating an observed increase in anaerobicity inflicted by the drilling waste deposition. The potential of these phylogenetic groups as microbial bioindicators of the spatial extent and persistence of drilling waste discharge should be further explored.

1. Introduction

Drilling for oil and gas generates quantities of waste that originate from the release of drilling muds and rock cuttings, collectively referred to as drill cuttings, and often also excess cement or other materials used. The amount of waste depends on the drilling depth, and the environmental impact of this discharge depends on its quantity and composition, local oceanographic conditions and the discharge strategy adopted. Since the early 1990s, the Norwegian environmental regulatory authorities have prohibited the discharge of drill cuttings using oil-based drilling muds, such that either water based muds or closed systems are used. The oil content of released drill cuttings with accompanying drilling mud residuals should not exceed 1%. Materials exceeding this threshold level are slurrified and reinjected or transported onshore for cleanup. Hence, the drilling waste currently deposited on the seafloor in the vicinity of the drilling sites largely comprises rock debris, mineral weight material (e.g. barite, ilmenite) and smaller amounts of residual, water-soluble chemical components. As baseline levels of barium (Ba) in sediments are generally low, the barite-derived Ba is commonly used as a sensitive tracer of dispersal and persistence of discharges from drilling operations (Kennicutt et al., 1983; Phillips et al., 1998; Ellis et al., 2012).

Although the environmental impacts of the discharges of water-based drill cuttings are considerably less than those of the previously-used oil-based varieties (Bakke et al., 2013), mesocosm and field experiments have demonstrated effects on the benthic macrofauna (Schaanning et al., 2008; Trannum et al., 2010). As might be expected, the intensity of these effects is dependent on the thickness of the deposited layer. However, contrary to previous assumptions, oxygen depletion, anticipated to be induced by microbial catabolism of organic components in the drilling fluids, seems to have a stronger negative impact on the benthos than the plain burial effect caused by drill cuttings sedimentation (Trannum et al., 2010). The radius of seriously affected benthic communities coincides rather closely with the visually evident spread of drilling waste and is rarely reported to extend beyond 100–200 m. The affected area is furthermore shown to diminish gradually over time after termination of the drilling operations (Daan et al., 2006; Gates and Jones, 2012; Jones et al., 2012).

Only few studies have been published on the microbiological effects of drilling muds and cuttings deposition, and their focus has largely been on the consequences of using oil-based muds. In a North Sea field study, Sanders and Tibbetts (1987) demonstrated increased hydrocarbon-degrading and sulfate-reducing activity as far out as 500 m from the center of a drill cuttings pile if aromatics-rich diesel based drilling muds were employed, whereas less toxic aliphatics based muds led to less far-reaching effects. Anaerobic degradation of hydrocarbon components in drill cuttings was demonstrated by Artz et al. (2002). A mesocosm study by Dow et al. (1990) showed less distinct and long-lasting, but still significant changes in microbial activity after covering sediment with water based drill cuttings.

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A number of factors, both biotic and abiotic, could be expected to influence the microbial community structure in the upper seafloor sediment. These factors include climate, water depth, intensity and character of benthic-pelagic coupling, geo-chemical characteristics of the sediment and level of macrofaunal bioturbation. However, some universal features still characterize upper sediment microbiotas and distinguish them from the overlaying pelagic realm on the one hand and the deep sediment biosphere on the other. The phylogenetic richness is very high both at species and higher taxonomic levels and compares with that of e.g. unperturbed soil communities (Torsvik et al., 1998; Roesch et al., 2007). *Gammaproteobacteria* and *Deltaproteobacteria* commonly constitute major taxonomic groups and otherwise rather uncommon taxa like *Planctomycetes* and *Chloroflexi* are also amply represented (Bertics and Ziebis, 2009; Orcutt et al., 2011; Zinger et al., 2011; Bienhold et al., 2012; Nguyen and Landfald, 2015).

A well-documented effect of drilling waste discharge is an increased anoxicity in the upper sediment strata. The suggested primary cause of this effect is increased oxygen demand by components in the muds, but reduced O_2 diffusion due to fines that settle onto the seabed, as well as reduced bioturbation by burrowing animals, may also contribute to oxygen depletion in the affected sediment (Trannum et al., 2011; Ellis et al., 2012). Furthermore, chemical components in the muds may have selective effects by promoting or inhibiting the growth of specific groups of microorganisms, thereby distorting the pristine phylogetic structure of the sediment communities.

In the present study we explore the character and spatial extent of changes in bacterial community structure following exposure to discharged drilling waste at a recently abandoned drilling location on the south-western continental slope of the Barents Sea. By combining a comprehensive partial 16S rRNA gene based community analysis with analyses of relevant environmental variables, we aimed at elucidating the closeness between community change and variations in direct and indirect influences of drilling waste discharge. Furthermore, using community analyses, we aimed to determine if affected sediments were characterized by specific phylogenetic groups of bacteria which, if so, would have a potential as bioindicator of the spatial and temporal extent of this type of seafloor perturbation.

2. Material and methods

2.1. Sampling

Sediment push corer samples (8 cm i.d., 50 cm length PVC tubes; Planet Ocean Ltd., UK) were collected by means of a working class remotely operated vehicle (ROV; Magnum Plus, Oceaneering AS) on 1–2 December 2013. The ROV was operated from the sub-sea anchor-handling vessel M/V Njord Viking (Viking Supply Ships, Gothenburg). The sampling was done in the vicinity of an drilling location at close to 1400 m depth at the Bønna location, situated on the continental slope of the Barents Sea towards the Norwegian Sea (Fig. 1) with Eni Norge as main operator. The site was drilled during the period 14th of July-8th of November 2013, implying that the drilling of the top hole (567 m) which led to discharge of drilling waste on the seafloor, took place in the first month of this period (Paulsen et al., 2014). The estimated quantity of drilling mud discharged to the seafloor from the top hole of the drilling site was about 1260 m^3. Eight corer samples were collected at distances of 28, 33, 43, 55, 100, 155, 210 and 210 m from the drilling location (Fig. 1). In the following data presentations, the two first-mentioned cores are treated as 30 m duplicates, the next two as 50 m duplicates and the last two as 210 m duplicates.

On board, oxygen concentrations were recorded at 0.5, 1.5 and 3.5 cm from the core surface, corresponding to the mid-depths of the three uppermost core sections (see below), by use of a ϕ 1.1 mm needle sensor coupled to an amplifier unit (Unisense AS, Aarhus, Denmark). Aerated seawater and 100 mM ascorbate in 100 mM NaOH were used as saturation and anoxic calibration points, respectively, according to the manufacturer's recommendation. Each core was subsequently sliced into four sections, 0–1, 1–2, 2–5 and 5–10 cm from the surface, mixed to homogeny in sterile plastic bags and frozen immediately at − 25 °C. On arrival to the onshore laboratory facilities, the samples were transferred to a – 72 °C freezer for storage. The individual samples were subjected to geochemical and 16S rRNA gene based bacterial community analyses. However, in one of the 30 m cores, the amount of sediment recovered from the uppermost 1 cm layer was sufficient only for bacterial community analysis, not for geochemical analyses.

2.2. Sediment characteristics

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), in accordance with the Wentworth Scale (Wentworth, 1922). 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). The pore water nitrate concentration was determined by azo dye formation after reduction of nitrate to nitrite by cadmium, according to the following protocol: sediment samples (0.5 ml) were spun at 10000 rpm for 10 min in polyethersulfone centrifugal filters with 30 kD cutoff (VWR prod.no. 516-0231). The filtrate volume was precisely determined and diluted to 0.5 ml with 3% NaCl, then added 0.5 ml Mixed Acid Reagent (LaMotte Company; prod.no. V-6278). After 2 min, 20 mg of Nitrate Reducing Agent (LaMotte Company; prod.no. V-6279) was added followed by tube inversion each second for 1 min. After 10 min, absorbance at 540 nm was recorded spectrophotometrically (Spekol 2000, Analytik, Jena, Germany) in a 1 cm light path cuvette. Standard solutions (0–50 μM) were made by diluting KNO_3 in 3% NaCl. Barium, lead, iron (III) oxide, and manganese dioxide were determined by Inductively Coupled Plasma Spectroscopy according to Environmental Protection Agency (US) methods 200.7 and 200.8 (http://www3.epa.gov).

2.3. DNA extraction

Total DNA was extracted from duplicate 0.5 g samples using the PowerSoil™ DNA Isolation kit (Mo Bio Labs, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and quality of extracted DNA were determined by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.4. Amplification and sequencing of partial 16S rRNA genes

Bacterial 16S rRNA genes were amplified and prepared for sequencing with the Illumina MiSeq system (Illumina Inc., San Diego, USA), according to the manufacturer's protocol. Primers 341F and 785R, with added overhang adapters, were used for the primary amplification with the KAPA HiFi™ Hotstart ReadyMix (KAPA Biosystems Inc., Wilmington, MA, USA). To minimize potential random PCR biases, each DNA preparation was amplified in triplicate. After indexing PCR, the amplicons were purified with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA), normalized, and pooled. The pooled sample was sequenced at the Barents Biocentre Lab, Tromsø, Norway on an Illumina MiSeq platform, using a 2 × 300 bp paired end protocol.

The raw sequence data have been submitted to the EMBL database under the accession number ERP023770.

2.5. Sequence analysis

Sequence analyses were carried out using the Quantitative Insights Into Microbial Ecology (QIIME v.1.8) pipeline (Caporaso et al., 2010). The forward and reverse sequence reads were joined using the *join_paired_end* script (Erik Aronesty 2011; http://qiime.org/scripts/join_