Scientific Drilling

Wood

Reports on Deep Earth Sampling and Monitoring
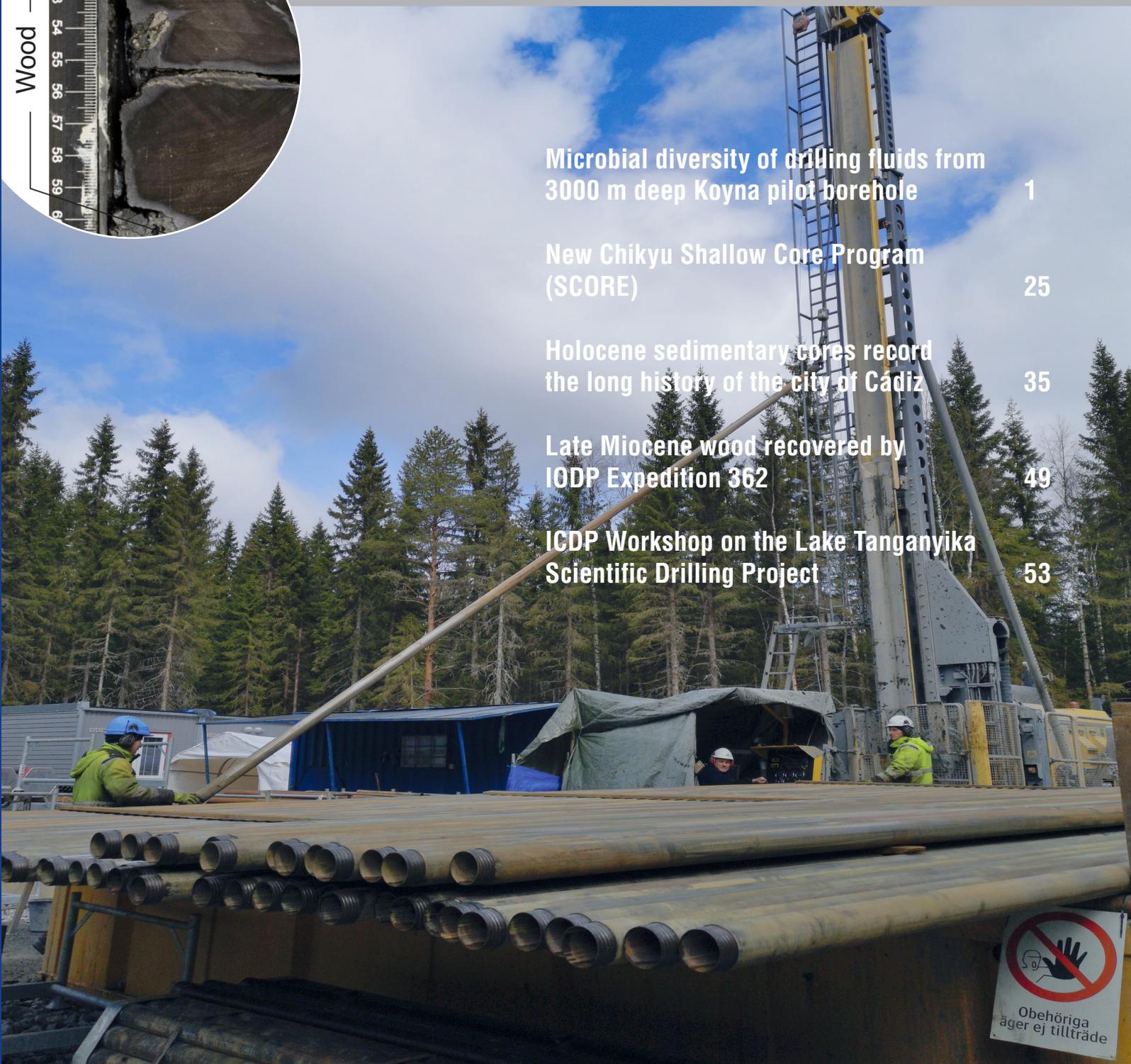
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Editorial preface

Dear Reader,

This volume of SCIENTIFIC DRILLING (SD 27) comprises five publications on completed or upcoming scientific drilling projects in different geological settings. Common to all publications in this volume is the important role of investigations on organic material. Scientific boreholes can sample organic material of different sizes from macroscopic to microscopic scale, and serve a variety of scientific purposes. For the studies presented in this SD volume, organic material is used for radiocarbon dating and to reveal important information e.g. on mass deposit processes from submarine fluvial fans or tsunamis, to study the deep biosphere and the evolution of endemic species, and, little surprising, for paleoclimate research.
IODP Expedition 362 in the eastern Indian Ocean encountered probably the largest fragment of well- preserved wood ever recovered in scientific ocean drilling, as reported in SD-2019-17 (p. 49). The wood is Late Miocene in age, is buried beneath 800m of the Bengal–Nicobar Fan sediments and is either transported as part of the submarine fan sedimentation or as a megathrust tsunami deposit. The preservation state of the wood fragment may provide qualitative information on seafloor conditions (e.g., oxygen and burial rate) that can be used to augment expedition sedimentary analyses.
SCORE, the new coring programme of the research vessel Chikyu, provides the ocean drilling community with the opportunity to conduct short-term drilling expeditions for the drilling of shallow holes of up to 100 meter below sea floor (mbsf). SD-2019-15 (p. 25) summarizes the first SCORE expedition that drilled three holes down to a maximum depth of 100 mbsf off Cape Erimo, northern Japan, to study submarine mass transport units and the local subseafloor sedimentary biosphere.
SD-2019-11 (p. 1) reports on the geomicrobiology observed in drilling fluids during ICDP drilling of the deep subsurface of the Deccan Traps and underlying Precambrian basement at Koyna, India. Drilling fluids, often regarded as a source of contamination during investigations of deep subsurface microbiology, served here as a vector for sampling of geological fluids and signatures of microbial life from terrestrial, granite-rock-hosted habitats. The drilling fluids samples from Koyna show enrichment of anaerobic, thermophilic sulphate-reducing and deep subsurface relevant microbial populations.
Lake Tanganyika in Africa probably preserves the longest and most continuous continental climate record from the mid-Miocene to the present anywhere in the tropics. It also harbours incredibly diverse endemic biota and an entirely unexplored deep microbial biosphere. SD-2019-19, p. 53 outlines the results of an ICDP workshop, attended by more than 70 scientists representing 12 countries and a variety of scientific disciplines who recognized Lake Tanganyika as a top-priority site for a major international scientific drilling project.
In the framework of an international, joint archaeological and geoarchaeological project, three cores recovered a thick Holocene sedimentary sequence from a marine palaeochannel that ran through the ancient city of Cádiz. This sedimentary archive will allow reconstructing the palaeogeographical evolution of this specific coastal area, to trace the intensity of activities of the city of Cádiz and to identify and date high-energy event deposits such as storms and tsunamis and is discussed in SD-2019-20, p. 35.

We wish the readers of this volume a positive outlook on future scientific objectives in difficult virus-impacted times.

Your Editors
Ulrich Harms, Thomas Wiersberg, Jan Behrmann,
Tomoaki Morishita, and Will Sager

Cover figure: Drilling of the Collisional Orogeny in the Scandinavian Caledonides in Sweden is in full swing (Photo credits: Henning Lorenz, Uppsala University).
Insert 1: Pore water sampling on board the drilling vessel Chikyu (Kubo et al., 2020, this volume).
Insert 2: Late Miocene wood recovered in Bengal–Nicobar submarine fan sediments by IODP Expedition 362 (McNeill et al., 2020, this volume).

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Science Reports

Microbial diversity of drilling fluids from 3000 m deep Koyna pilot borehole provides insights into the deep biosphere of continental earth crust

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Abstract. Scientific deep drilling of the Koyna pilot borehole into the continental crust up to a depth of 3000 m below the surface at the Deccan Traps, India, provided a unique opportunity to explore microbial life within the deep granitic bedrock of the Archaean Eon. Microbial communities of the returned drilling fluid (fluid returned to the mud tank from the underground during the drilling operation; designated here as DF) sampled during the drilling operation of the Koyna pilot borehole at a depth range of 1681–2908 metres below the surface (m b.s.) were explored to gain a glimpse of the deep biosphere underneath the continental crust. Change of pH to alkalinity, reduced abundance of Si and Al, but enrichment of Fe, Ca and SO_4^{2-} in the samples from deeper horizons suggested a gradual infusion of elements or ions from the crystalline bedrock, leading to an observed geochemical shift in the DF. Microbial communities of the DFs from deeper horizons showed progressively increased abundance of *Firmicutes, Gammaproteobacteria* and *Actinobacteria* as bacterial taxa and members of *Euryarchaeota* as the major archaeal taxa. Microbial families, well known to strive in strictly anaerobic and extremophilic environments, (e.g. *Thermoanaerobacteraceae*, *Clostridiaceae*, *Bacillaceae*, *Carnobacteriaceae*, *Ruminococcaceae*), increased in the samples obtained at a depth range of 2000 to 2908 m b.s. Phylogenetic analysis of common and unique operational taxonomic units (OTUs) of DF samples indicated signatures of extremophilic and deep subsurface relevant bacterial genera (*Mongoliitalea*, *Hydrogenophaga*, *Marinilactibacillus*, *Anoxybacillus*, *Symbiobacterium*, *Geosporobacter*, *Thermoanaerobacter*). Thermophilic, obligatory anaerobic sulfate-reducing bacterial taxa known to inhabit the deep subsurface were enriched from DF samples using sulfate as a terminal electron acceptor. This report on the geomicrobiology of the DF obtained during drilling of the deep subsurface of the Deccan Traps showed new opportunities to investigate deep life from terrestrial, granite-rock-hosted habitats.

1   Introduction

The earth's deep continental crust was found to be of geologically varied morphology with extreme conditions (temperature, pressure, pH etc.) which made it almost impossible for life to survive (Fredrickson and Balkwill, 2006; Colwell and D'Hondt, 2013). Nevertheless, the "deep subsurface" of the earth has been known to host diverse arrays of ecosystems which harbour numerous populations of extremophilic microbial life, comprising a significant (about 19 %) but mostly unexplored parts of earth's biosphere (Whitman et al., 1998; McMahon and Parnell, 2014, 2018; Kieft, 2016; Magnabosco et al., 2018; Soares et al., 2019). In fact, the deep subsurface is among the five main "big habitats" of bacteria and archaea containing about 3×10^{29} cells (Flemming and Wuertz, 2019). Exploration of deep life within the continental crust mostly aims to provide answers to some of the fundamental questions related to the limits of life on our planet and its evolution, energy mechanisms and metabolism as well as its involvement in cycling of the essential elements of life (Hoehler and Jørgensen, 2013; Breuker et al., 2011; Purkamo et al., 2018; Borgonie et al., 2019).

Deep drilling has been an important method for obtaining subsurface samples for geomicrobiological investigations (Escudero et al., 2018). Generally, for acquiring the sample of more than 300 m depth (especially from crystalline – granite and basalt – rock) a rotary drilling technique has been used which requires the drilling fluid to facilitate the drilling process (Keift et al., 2007; Onstott et al., 1998). Drilling fluid prepared by mixing multiple prepackaged powdered components, including bentonite and a variety of organic constituents with water to lubricate and cool the drill bit, maintain the hydrostatic pressure during the drilling operations so that intact cores can be retrieved and ultimately carry the rock cuttings back to the surface (Zhang et al., 2006; Keift, 2010). Bentonite (sodium bentonite: aluminium phyllosilicate clay consisting mostly of montmorillonite) has been widely used as drilling fluid in several continental and oceanic drilling operations (Masui et al., 2008; Zhang et al., 2006; Struchtemeyer et al., 2011). Specific advantages of bentonite-based fluids included protection of the drilling tools from corrosion, reduced fluid and gas penetration (Gandhi and Sarkar, 2003), and owing to its characteristic "thixotrophic reaction" it hinders the rock material from dropping back into the drilled shaft (İşçi and Turutoğlu, 2011).

These fluids are essentially considered as potential contaminants to rock cores not only from chemical but also from a biological perspective. Owing to the presence of a variety of complex organic compounds (e.g. xanthan gum, guar gum, glycol, carboxymethylcellulose, polyanionic cellulose (PAC) or starch), diverse heterotrophic microbial populations (bacteria, archaea, fungi) could grow in drilling fluid (Keift et al., 2007; Beeman and Suflita, 1989; Rabia, 1985). A circulatory system in which the fluid remained in continuous use throughout the drilling operation and the scope of the invasion of microorganisms from the upper stratum to the strata below may also pose a matter of further concern (Pedersen et al., 1997; Yanagawa et al., 2013; Smith et al., 2000; Miteva et al., 2014). In spite of the shortcomings, the fluid that returned back to the mud tank has been a subject of geomicrobiological investigation since it can act as a window enabling sampling of the deep subsurface environment and may give us an opportunity to analyse the microbial communities from the geological fluids from faults and fractures (Zhang et al., 2006).

Drilling fluids' microbial diversity as a marker for deep life was studied during a number of drilling operations. Drilling fluid obtained from deep drilling at the Cretaceous-age Piceance basin in Colorado, Triassic-age Taylorsville basin in Virginia, Chinese continental scientific drilling operations (CCSD) at a 240 Ma old ultra-high pressure metamorphic (UHPM) belt located in Jiangsu Province in China and during the first riser drilling operation on the deep-earth research vessel Chikyu was used for microbiological studies (Liu et al., 1997; Roh et al., 2002; Zhang et al., 2005, 2006; Masui et al., 2008). The presence of microorganisms able to survive the extreme oligotrophic conditions of the deep subsurface was detected in these studies. Several strains of an extremely thermophilic, anaerobic chemoorganotroph bacterium *Thermoanaerobacter ethanolicus* have been previously isolated from the fluids obtained during drilling in the Piceance basin in Colorado and Taylorsville basin in Virginia at different depths of the geological formation (Liu et al., 1997; Roh et al., 2002). This particular organism was also retrieved from the DF samples of CCSD. Extremophilic deep subsurface microorganisms (*Caldicellulosiruptor lactoaceticus*, *Anaerobranca gottschalkii* and *Sulfolobus solfataricus*) were reported from the fluids obtained from the CCSD project (Zhang et al., 2005, 2006). Deep subsurface archaeal populations (Marine Crenarchaeota Group I and II, South African Gold Mine Euryarchaeotic Group (SAGMEG), Soil Group Archaea) were detected in the fluid samples at the time of the first riser drilling operation on Chikyu (Masui et al., 2008). Overall, the microbial ecology of circulating fluids collected from various deep drilling operations (continental and oceanic) showed strong signatures of subsurface microbial populations. Investigating these communities remained scientifically intriguing. Additionally, the drill cuttings along with the fluids also leave behind tons of residue without adequate treatment, generating a large environmental liability. Knowledge about the microbial community of such drilling residue may be useful for developing techniques for drilling mud bioremediation (Guerra et al., 2018).

The present research was undertaken to study the microbial communities present in the returned drilling fluids (designated here as DF) as a proxy for deep granitic subsurface microbial diversity in the Koyna Intra-plate Seismic zone (Deccan Traps, India; https://www.icdp-online.org/projects/

world/asia/koyna-india/, last access: 27 January 2020). This unique opportunity to study microbial life within deep terrestrial igneous rocks came to us through scientific deep drilling in this region by the Inter Continental Drilling Programme (ICDP) and Ministry of Earth Sciences (MoES), Government of India, to study the earthquakes. The Deccan Traps, representative of a continental flood basalt province, covers an area of over 0.5 million km^2 of lava flows (~ 65 Ma) with a total thickness of over 2000 m near the eruptive centre in western India. This thick pile of Deccan basalts has been resting on a ~ 2.5 Ga Archaean crystalline basement. With respect to varying temperature, pressure regimes (15 °C increase in temperature and 26.7 MPa increase in lithostatic pressure per 1000 m b.s. in granite) and geochemical conditions, this study provided an excellent opportunity to study life in deep igneous terrestrial subsurface (Roy and Rao, 2000). The specific goal of this work was to systematically investigate the microbial diversity of DFs in the depth range of 1681–2908 m b.s. by a culture-independent approach and explore the presence of deep life relevant organisms within these systems. Our results indicated that there was a change in the microbial community structure of DF samples on increasing borehole depth. The phylogenetic positions of unique and shared operational taxonomic units (OTUs) of the DF samples suggest the presence of deep subsurface and extremophilic microbial populations in drilling fluid samples.

2 Materials and methods

2.1 Sampling site and drilling

A 3 km deep pilot borehole (KFD-1) was drilled in the Koyna region (17°24′6″ N, 73°45′8″ E) of the Deccan Traps, Maharashtra during March–June 2017 under the pilot phase of the Koyna scientific drilling project to comprehend the genesis of earthquakes triggered by artificial water reservoirs in the Koyna intraplate seismic zone. Several modifications to the standard drilling procedure were made in order to collect the core and drilling fluid samples in such a way that they could be later used reliably for geochemical and geomicrobiological analysis. All instruments in contact with the core samples were cleaned properly before use. The drilling fluid (bentonite) was kept in a separate tank and sodium fluorescein (500 mg m^{-3}) was added to the tank before the coring process to evaluate the penetration of drill mud into the core. Drilling fluid samples that returned after circulating to the depths ranging from 1681 to 2908 m b.s. were collected following aseptic techniques along with the rock cores (core 1–core 7) at the time coring at the depths ranging from 1681 to 2908 m b.s. These returned DF samples were designated as DF1 to DF7. Along with that the in-flow fluid (before its introduction in the borehole) was also collected. A list of samples, depth and other physiochemical parameters has been presented in Table S1 in the Supplement. Samples were

stored in sterile containers at 4 °C for shipment. In laboratory, the samples were stored at -80 °C.

2.2 Geochemical analysis

The DF samples (DF1–DF7) were dried overnight in a hot air oven (80 °C) to remove the water content. The dried cakes were ground to powder using a mortar and pestle. The powdered DFs were again kept overnight at 80 °C to remove any leftover moisture and stored in a vacuum desiccator. The powdered samples were analysed for their elemental composition by energy-dispersive X-ray spectroscopy (EDX) using a field emission scanning electron microscope (Zeiss Merlin) attached to an EDX detector. For pH and conductivity measurements, DF samples were incubated in distilled water overnight at a $1 : 10$ ratio (w/v). All measurements were done using highly sensitive probes fitted with a multiparameter (Orion) (Thermo electron corporation, Beverly, MA) (Islam et al., 2014). Temperature for each depth (at which rock cores and DF samples were collected) was measured on-site during drilling.

2.3 DNA extraction

Microbial diversity and community composition of DFs were studied using an approach based on next-generation sequencing (NGS) and using the extracted DNA from the respective samples. Environmental DNA from DFs (250 mg of DF was taken for each extraction) was extracted in triplicates using the DNeasy® PowerSoil® kit (Qiagen 12888-50) according to the manufacturer's protocol. A blank DNA extraction was performed (no sample) at the same time as a negative control and was used subsequently in the PCR and sequencing steps to check chances of any possible contamination. The quality of the extracted environmental DNA and its concentration was measured using a NanoDrop 2000 spectrophotometer, followed by fluorometric quantitation using Qubit (Thermo-Fisher Scientific).

2.4 Quantitative real time PCR (qPCR)

Quantification of the bacterial and archaeal populations in DFs was performed by estimating the copy number of bacteria- and archaea-specific 16S rRNA genes. Details of the qPCR primers used are provided in Table S2 in the Supplement. A total of 2 µL of the environmental DNA was added to the PCR mastermix with a total volume of 10 µL. All the reactions were set in triplicates. Quant Studio 5 was used to perform qPCR with a Power SYBR green PCR Master Mix (Invitrogen), primer concentration of 5 pM and the following amplification conditions: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 30 s. A melting curve analysis was run after each assay to check PCR specificity. Genes encoding archaeal and bacterial 16S rRNA genes were PCR amplified from the metagenome and cloned

in a TA cloning vector and plasmid DNA for each, with copy numbers 10^2 to 10^{10} used as standards for quantification purposes.

2.5   16S rRNA gene amplification and sequencing

Environmental DNA was subjected to amplification of the V4 region of the 16S rRNA gene using a Veriti 96 well thermal cycler (Applied Biosystems, Foster City, CA). The V4 region of the 16S rRNA gene was amplified using a 515F′–806R′ primer (Table S2) (Bates et al., 2010) and an amplicon library was prepared and sequenced on IonS5 NGS platform (Thermo Scientific) as per the manufacturer's protocol. The sequence reads obtained were submitted to the sequence read archive (SRA) under SRA accession: SRP155468.

2.6   Bioinformatic and statistical analysis

The reads obtained were quality filtered using the Quantitative Insights Into Microbial Ecology (QIIME 1.9.1) (Caporaso et al., 2010) bioinformatics pipeline, according to which sequences with lengths outside the bounds of 210 and 310, a mean quality score below a minimum of 25 and a maximum homopolymer run exceeding a limit of 6 were filtered out. De novo-based clustering of reads to form OTU was performed using UCLUST under QIIME workflow. Sequences with greater than 97 % similarity were assigned to the same OTU. Representative reads from each OTU were assigned taxonomy using the UCLUST trained SILVA 128 database (Quast et al., 2012). OTUs which were present in the reagent control were removed from the OTU pool of the samples. Alpha diversity parameters were calculated using alpha_diversity.py under the QIIME workflow. The OTU overlap among the DF samples and OTU pool of different DFs were elucidated using InteractiVenn (Heberle et al., 2015). Core (OTUs present in all samples) and unique OTU sequences (sequences detected in only one of the samples) were analysed using the National Center for Biotechnology Information (NCBI) BLASTn nucleotide database. A phylogenetic tree was constructed by MEGA7 software using a neighbour-joining method with 1000 bootstraps (Kumar et al., 2016).

2.7   Enrichment of sulfate-reducing bacteria (SRB) from DF samples

Two DF samples (DF1 and DF3, 10 mL in a 100 mL medium) were incubated in a Postgate medium (Postgate, 1963) containing sulfate as the sole terminal electron acceptor for 6 months under anaerobic conditions at 50 °C for the enrichment of sulfate-reducing bacteria. Subsequently, samples were sub-cultured thrice in the same medium and the final culture obtained was used for total DNA extraction (in triplicates) using the DNeasy® PowerSoil® kit (Qiagen 12888-50). Total DNA was extracted from 10 mL of enrichment

cultures. The extracted DNA from the enrichment was processed further for 16S rRNA gene amplicon sequencing and subsequent analysis as described above.

3   Results

3.1   Geochemical properties of DF samples

DF samples were analysed for their major geochemical properties (Table S1). Silicon and oxygen were the major elements present in the DF (on an average 19 % and 29 % respectively) along with Ca, S, N, Al, C and Mn. These elements were found to be abundant in bentonite-based DF (Schlumberger Oilfield Glossary, 2010). All the samples (except DF1 – pH 7.3) were found to be alkaline in nature, with the maximum for DF7 (9.17). The concentration of sulfide (S^{2-}) was found to be in the range of 0.3–0.8 mg kg^{-1}. High levels of barium were detected in all the DFs (except DF1) with the maximum in DF4 (46 %). With increasing sample depth, the change of pH to alkalinity reduced relative abundance of Si and Al, but enrichment of Fe, Ca and SO_4^{2-} was noted (Fig. 1). A shift in geochemical parameters suggested the gradual infusion of elements from the crystalline bedrock during the course of drilling.

3.2   Bacterial and archaeal abundance in DF

The abundance of bacterial and archaeal populations was determined by estimating the copy number of bacterial- and archaeal-specific 16S rRNA gene using a real-time PCR-based quantitative approach. With respect to the sampling depth, DF used for drilling rocks of a deeper horizon showed marginal change in bacterial (2.02×10^{10}–3.43×10^9 g^{-1} of DF) and archaeal (2.54×10^4 to 4.42×10^3 g^{-1} of DF) gene copy numbers (Fig. 2). Assuming an average of 4.7 and 1.7 16S rRNA gene copies per genome of bacteria and archaea (Stoddard et al., 2015), we could estimate bacterial cell abundance ranging between 1.38×10^{10} and 7.29×10^8 g^{-1} of DF and archaeal cell abundance ranging between 1.22×10^4 and 2.55×10^3 g^{-1} of DF.

3.3   Sequencing data

Sequencing of a hypervariable V4 region of 16S rRNA gene generated 20 83 060 reads from 6 samples (Table 1). On average 613 669 reads were found usable and these obtained reads were grouped into 49 570 operational taxonomic units (OTUs). A total of 46 950 OTUs belonged to bacteria and 56 OTUs belonged to archaea. A small fraction of OTUs (0.03 %–2.12 %) remained unassigned across all the samples. A considerable portion of OTUs (17 % to 73 %) were found to be unique and these unique OTUs represented 6 %–26 % of the total microbial community across all the samples. A total of 148 OTUs (0.29 %) were found to be common among all samples, which comprised about 25 % of the total micro-
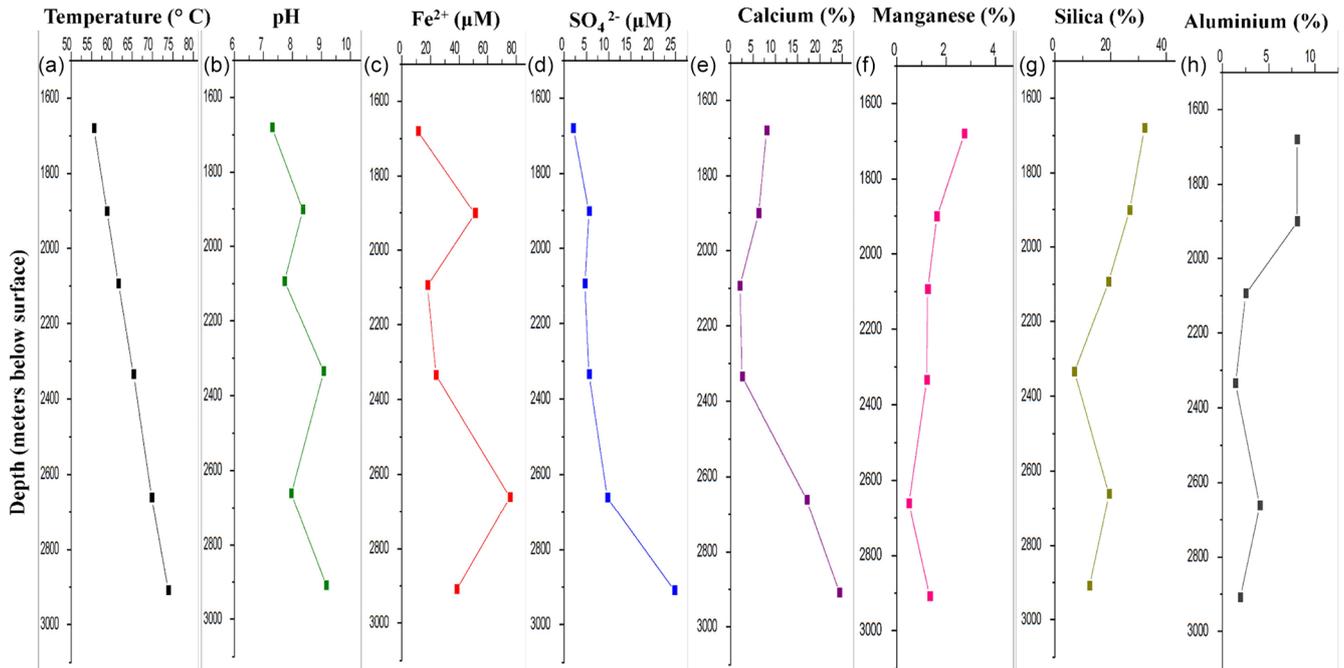
Figure 1. Major geochemical parameters of DF samples. There was a distinct variation in the geochemistry of DF samples obtained at different depths. Increase in temperature **(a)**, alkalinity **(b)**, iron **(c)**, sulfate **(d)** and calcium **(e)** was observed whereas the concentration of manganese **(f)**, silica **(g)** and aluminium **(h)** showed a decreasing trend.
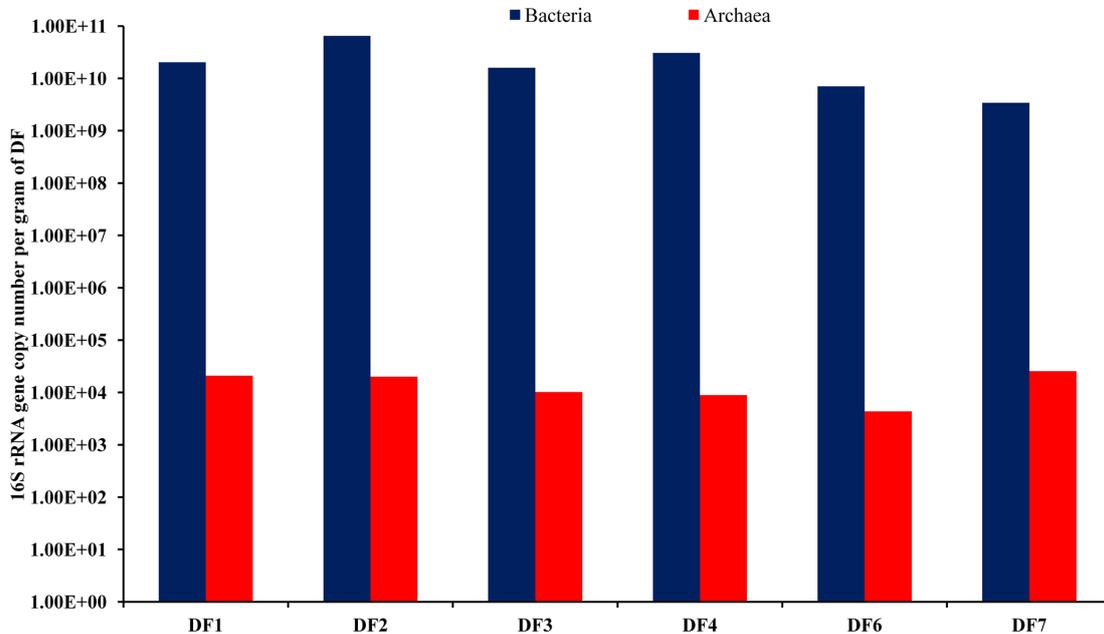
Figure 2. Archaeal and bacterial 16S rRNA gene copy number detected by qPCR.

bial abundance. Alpha diversity analysis was performed for the sample set. Chao 1 estimator analysis suggested that samples from the deepest location (DF7) contained the highest number of OTUs. The Shannon index was in the range of 3.84–7.3 across all samples. The Simpson index ranged be-

tween 0.78 and 0.95. It could be noted that the lowest values of the Shannon and Simpson indices were obtained for DF1 and in general both the values showed an increasing trend from DF1 to DF7 (Fig. 3a).

Table 1. Read details and alpha diversity.

	DF1	DF2	DF3	DF4	DF6	DF7
No. of raw reads	311 959	27 804	447 627	243 598	223 603	828 469
No. of quality controlled reads	238 061	19 648	311 609	184 537	165 243	671 354
No. of reads after control subtraction	33 319	4055	127 385	74 140	98 994	275 776
Percentage of bacterial reads	99.118	99.459	99.440	96.69	99.433	98.578
Percentage of archaeal reads	0.003	0.024	0.005	0.033	0.055	0.417
Percentage of unassigned reads	0.879	0.517	0.555	3.277	0.512	1.005
No. of observed OTUs	3408	1148	7229	8002	9035	20 748
No. of bacterial OTUs	3291	1131	6883	7303	8692	19 660
No. of archaeal OTUs	1	1	3	6	12	33
No. of unassigned OTUs	116	16	341	693	331	1055
Percentage of unique OTUs	46.24	17.42	54.61	58.93	43.54	73.05
Percentage abundance of unique OTUs	12.29	6.51	7.82	20.94	6.89	25.98
Percentage of core OTUs	3.84	11.41	1.90	1.63	1.44	0.63
Percentage abundance of core OTUs	31.94	30.87	31.13	12.45	42.93	29
Estimated total OTUs (chao1)	6442.04	2805.21	13 162.85	14 339.015	17 092.29	38 931.388
Shannon evenness index	3.84	5.46	5.48	6.57	7.04	7.30
Simpson index	0.78	0.93	0.84	0.95	0.954	0.944
Goods coverage	0.99	0.96	0.99	0.98	0.97	0.983
Equitability	0.326027	0.531948	0.424991	0.504811	0.534176	0.504093
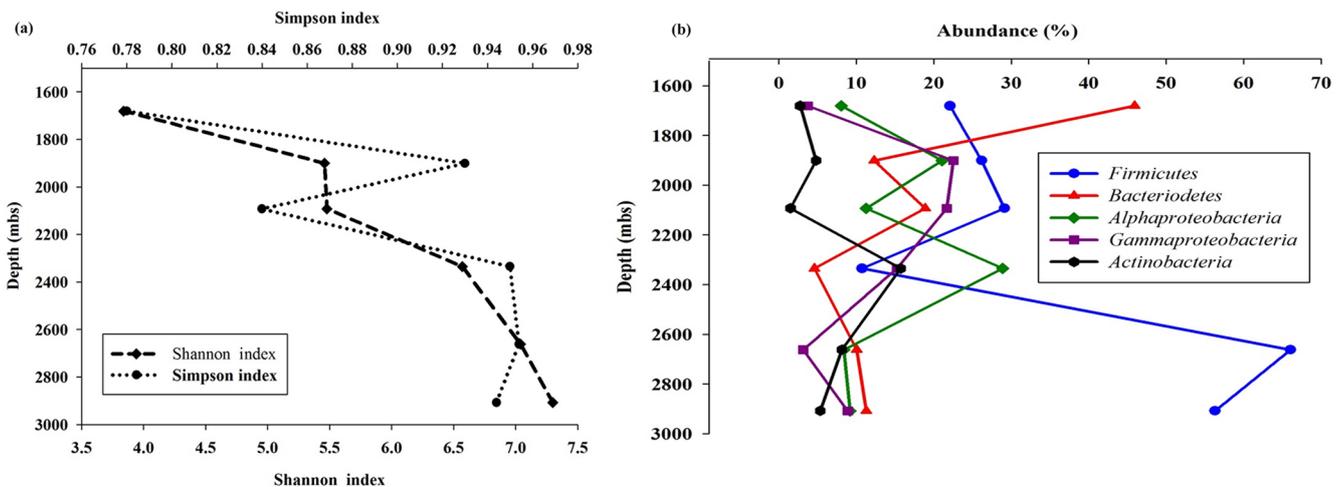
Figure 3. (a) Variation in Shannon and Simpson diversity indices across depths and **(b)** abundance of the top five bacterial groups across depths.

3.4 Microbiological assessment of DF

The number of sequences assigned to bacteria covered 35 phyla, 87 classes, 174 orders, 350 families and 848 genera. Sequences assigned to archaea covered 2 phyla, 5 classes, 6 orders, 6 families and 7 genera (Table 2). Based on average abundance at phylum level (phylum *Proteobacteria* has been further divided into its different classes), *Firmicutes* (10 %–66 %), *Bacteriodetes* (4 %–45 %), *Alphaproteobacteria*, (10 %) *Gammaproteobacteria*, (3 %–21 %) and *Actinobacteria* (2 %–15 %) constituted more than 90 % of the communities in the DF (Fig. 4a). *Firmicutes* was the most abundant phylum across all the samples except DF4. Figure 3b revealed changes in the abundance of these phyla depending on the depth. With respect to depth, the abundance of *Firmicutes* (20 % to 60 %), *Gammaproteobacteria* (3 % to 21 %) and *Actinobacteria* (2 % to 15 %) increased, whereas *Bacteriodetes* (45 % to 10 %) showed a decreasing trend. The abundance of *Alphaproteobacteria* remained nearly consistent across the depths (10 %–30 %). *Verrucomicrobia*, *Planctomycetes*, *Spirochaetae*, *Tenericutes*, *Chloroflexi* and *Deinococcus-Thermus* (0.07 %–3 %) were some of the other phyla observed in the DF samples. Only two archaeal phyla, namely (Fig. 4b) *Euryarchaeota* and *Thaumar-*

chaeota, were detected in DF samples, with relatively low abundance (0.01 %–0.4 %).

Microbial diversity of the in-flow drilling fluid was assessed and compared with that of returning DFs (DF1–DF7). *Bacteriodetes* (45 %) was the most abundant phyla in the in-flow DF, followed by *Firmicutes* (23 %), *Alphaproteobacteria* and *Deltaproteobacteria* (Fig. 4a). These four phyla constituted more than 90 % of the total community in the in-flow fluid sample. A distinct shift in microbial community composition was observed in the returning DFs (DF1–DF7). *Bacteriodetes* was identified as one of the major phyla present in DF1 (44 %), and its diversity decreased in DF samples retrieved from increasing depths. Similarly, abundance of *Firmicutes*, *Alphaproteobacteria* and *Gammaproteobacteria* increased in the DF samples collected at lower depths. Interestingly, no archaeal phylum was detected in the in-flow sample but they were detected in the DF samples collected from the lower depths.

Clostridia, *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Cytophagia Actinobacteria*, *Bacteroidia* and *Betaproteobacteria* (> 80 %) were the major bacterial classes in DFs. Noticeably, there was an increase in the abundance of *Clostridia* and *Bacilli* in the DF samples obtained from lower depths. DF1 was majorly dominated by *Cytophagia* (38 %) followed by *Betaproteobacteria* (15 %), *Clostridia* (14 %), *Alphaproteobacteria* (8 %) and *Bacteroidia* (6 %). DF2 was comprised of *Gammaproteobacteria* (22 %) and *Alphaproteobacteria* (21 %), with *Clostridia* (11 %) being the third most abundant class. *Clostridia* (26 %) was the most abundant class in DF3 followed by *Gamma-* (21 %), *Alphaproteobacteria* (11 %) and *Deltaproteobacteria* (9 %). DF4 was majorly dominated by *Alphaproteobacteria* (28 %) and *Gammaproteobacteria* (15 %). DF4 also had a higher abundance of *Actinobacteria* (15 %) than other DFs. DF6 had a high abundance of *Clostridia* (31.5 %) and *Bacilli* (26.5 %) with *Alphaproteobacteria* (8.5 %), *Actinobacteria* (6 %), *Bacteroidia* (6 %) and *Gammaproteobacteria* (8 %) being the other prominent classes. DF7 had a significant abundance of *Clostridia* (36 %) and *Bacilli* (18 %) followed by *Alphaproteobacteria* (9 %) and *Actinobacteria* (5 %). Among archaeal classes *Methanobacteria* (DF6 and DF7) and *Methanomicrobia* (DF3–DF7) showed the highest abundance followed by *Thermoplasmata* (DF3 and DF7) and the Soil Crenarchaeotic Group (SCG) (DF6 and DF7).

3.5 Abundance of major microbial families and genera in DF

Figure 5 displays the abundance of different microbial families in DFs. DF1 was dominated by *Cyclobacteriaceae* (*Mongoliitalea*, *Cecembia*) (39 %), *Comamonadaceae* (*Hydrogenophaga*) (14 %) and *Clostridiales* (unclassified *Clostridium*) (9 %). *Pseudomonadaceae* (*Pseudomaonas*) (11 %), *Rhodobacteraceae* (*Rhodobacter*, *Pannonibacter*, *Paracoccus*) (10 %), *Erysipelotrichaceae* (*Erysipelothrix*)

(8 %), *Cyclobacteriaceae* (*Mongoliitalea*, *Cecembia*) (6 %) and *Alteromonadaceae* (*Alishewanella*) (5 %) were the top five families in DF2. *Shewanellaceae* (*Shewanella*) (16 %), *Clostridiaceae* 1 (unclassified *Clostridiaceae*) (15 %), *Bacteroidetes Incertae Sedis* (8 %) and *Desulfovibrionaceae* (*Desulfovibrio*) (7 %) were the major families in DF3. The prominent microbial families in DF4 were *Hyphomonadaceae* (Hyphomonas) (12 %), *Bacteria* (10 %), *Opituraceae* (*Opitutus*) (9 %), *Cellulomonadaceae* (*Actinotalea*) (8 %) and *Caulobacteraceae* (*Brevundimonas*) (6 %). *Carnobacteriaceae* (*Marinilactibacillus*, *Alkalibacterium*) (32 %) was found to be the most abundant microbial family in DF6 followed by *Clostridiaceae 1* (*Clostridium sensu stricto 1*) (13 %) and *Lachnospiraceae* (unclassified *Lachnospiraceae*) (7 %). *Bacillaceae* (*Geobacillus*, *Anoxybacillus*, *Anaerobacillus*, *Aeribacillus*) (9 %), *Clostridiaceae 1* (*Clostridium sensu stricto 1*) (7 %), *Rhodobacteraceae* (*Pannonibacter*) (7 %) and *Thermoanaerobacteraceae* (*Thermoanaerobacter*, *Gelria*) (6 %) were the major microbial families present in DF7.
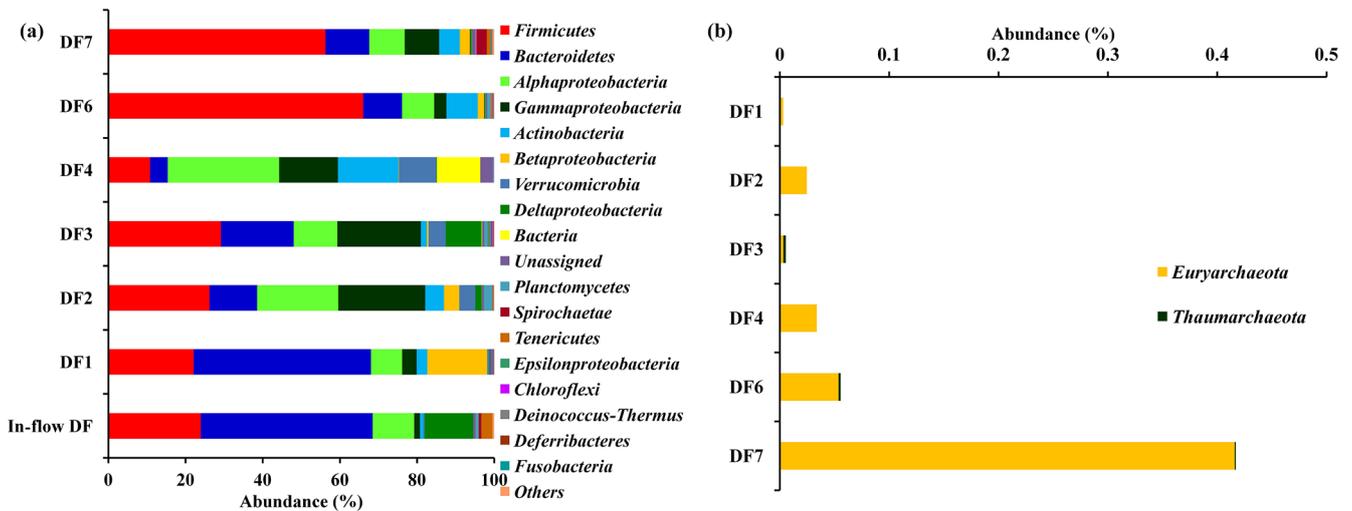
Sample-wise community profiling at lower taxonomic levels highlighted the distinct microbial assemblages with change in depth. Overall community profile highlighted that with changes in depth, the DF microbial communities underwent a pronounced shift. However, members of a few bacterial taxa remained ubiquitously abundant across all the samples. *Clostridiaceae*, *Marinilibiaceae*, *Rhodobactereaceae* and *Erysipelotrichaceae* were ubiquitous in all the samples. Samples from deeper horizon (2662–2908 m b.s.) showed a distinct abundance of *Clostridiaceae 4*, *Desulfovibrionaceae*, *Veillonellaceae*, *Aeromonadaceae* and *Xanthomonadaceae*. On the other hand, samples recovered from shallow levels (1681–1801 m b.s.) (like DF1, DF2) were more populated with *Peptococcaceae* and *Opitutaceae*. Members of *Shewanellaceae*, *Hyphomonadaceae* and *Microbacteriaceae* were relatively more abundant in samples recovered from DF3 and DF4, which represent a medium depth range (2093–2335 m b.s.).

3.6 Core OTU analysis

OTUs common (core OTUs) across all six DF samples were analysed. Out of the total 49 570 OTUs present across these six samples only 148 OTUs were identified as core OTUs (Fig. 6a). All of these OTUs together could cover 30 %–45 % of the total communities (Fig. 6b), with the exception of DF4 (12 %) and DF7(18 %). The relative abundance of different taxonomic groups (Fig. 7a) representing the core OTUs indicated that the abundance of *Firmicutes* remained higher in samples from deeper horizons (highest in DF6; 71 %). OTUs affiliated with *Alphaproteobacteria* represented 7 % to 31 % from DF1 to DF7. Members of *Bacteriodetes* present in the core OTUs represented 50 % of total communities of DF1 and 11 % to 15 % in DF2, DF3 and DF6 but reduced substantially to 2 % and 6 % in DF4

Table 2. Analysis of the unique operational taxonomic units (OTUs).

Sample name	Total no. of unique OTUs	No. of reads	No. of extremophilic OTUs among top 10 unique OTUs	Total no. of reads of extremophilic communities among top 10 unique OTUs	Percent abundance of top 10 unique OTUs
DF1	1577	4093	9	335	8.21
DF2	200	264	5	26	9.84
DF3	3947	9966	7	377	4.3
DF4	4716	15 534	6	1884	11.22
DF6	3934	6819	7	176	2.58
DF7	15 159	71 647	6	11 047	15.148

Figure 4. (a) Bacterial community composition of DF samples (original and returned DF samples) at phylum level (phylum *Proteobacteria* has been further divided into its different classes). Phyla which had minimum 1 % abundance in the DF samples were selected and the remaining ones were grouped into "Others". **(b)** Abundance of archaeal communities in DFs (phylum level).

and DF7 respectively. Actinobacterial core OTUs in general constituted 2 % to 9 % of the communities, except for DF4 where these OTUs represented 53 % of total OTUs. Core OTUs affiliated with *Deltaproteobacteria* represented a relatively lower proportion of most of the communities except DF2 (4 %) and DF3 (19 %). Members of *Verrucomicrobia*, present as core OTUs, constituted 16 % of DF1 and 3 % of DF2 communities, while in the rest of the communities their abundance was very low. *Planctomycetes* members were generally less abundant except in DF2 (3 %). *Betaproteobacterial* core OTUs were no very abundant (0.004 % to 0.03 %) except in DF1 (0.13 %) and DF2 (0.29 %). Archaeal OTUs were not detected in the core communities of DF. *Clostridia* (24 %) and *Alphaproteobacteria* (18 %) were the major classes detected in the core OTUs of DF followed by *Bacilli* (14 %), *Actinobacteria* (12 %) and *Cytophagia* (9 %) (Fig. 7b). However, the absence of *Gammaproteobacteria* and lower abundance of *Betaproteobacteria* was noteworthy in the core OTUs of DF. The unique presence of *Negativicutes* and *Coriobacteria* (with very low abundance) was also noted within the DF core communities

(Fig. 7b). *Erysipelotrichia*, *Clostridia*, *Alphaproteobacteria* and *Deltaproteobacteria* (Fig. 7b) were the other major microbial classes. *Carnobacteriaceae*, *Rhodobacteraceae*, *Clostridiaceae 1*, *Cellulomonadaceae*, *Erysipelotrichaceae*, *Cyclobacteriaceae*, *Opitutaceae*, *Bradyrhizobiaceae* and *Desulfovibrionaceae* were the major families present as the core community members of DF (Fig. 8). These microbial families constituting the core OTUs of DF obtained from 1681 to 2908 m b.s. were quite different from the microbial families detected in the core OTUs of granitic bedrock at higher depths (up to 1500 m b.s.). *Idiomarinaceae*, *Moraxellaceae*, *Methylophilaceae*, *Nocardioidaceae*, *Nitrosomonadaceae*, *Enterobacteriaceae*, *Comamonadaceae* and *Chitinophagaceae* were the major microbial families detected in the core OTUs of granitic bedrock up to 1500 m b.s. (Dutta et al., 2018). *Marinilactibacillus*, *Youngilibacter*, *Clostridium sensu stricto 8*, *Geosporobacter* and *Pannonibacter* were the highly abundant bacterial genera present in the core communities of DF.
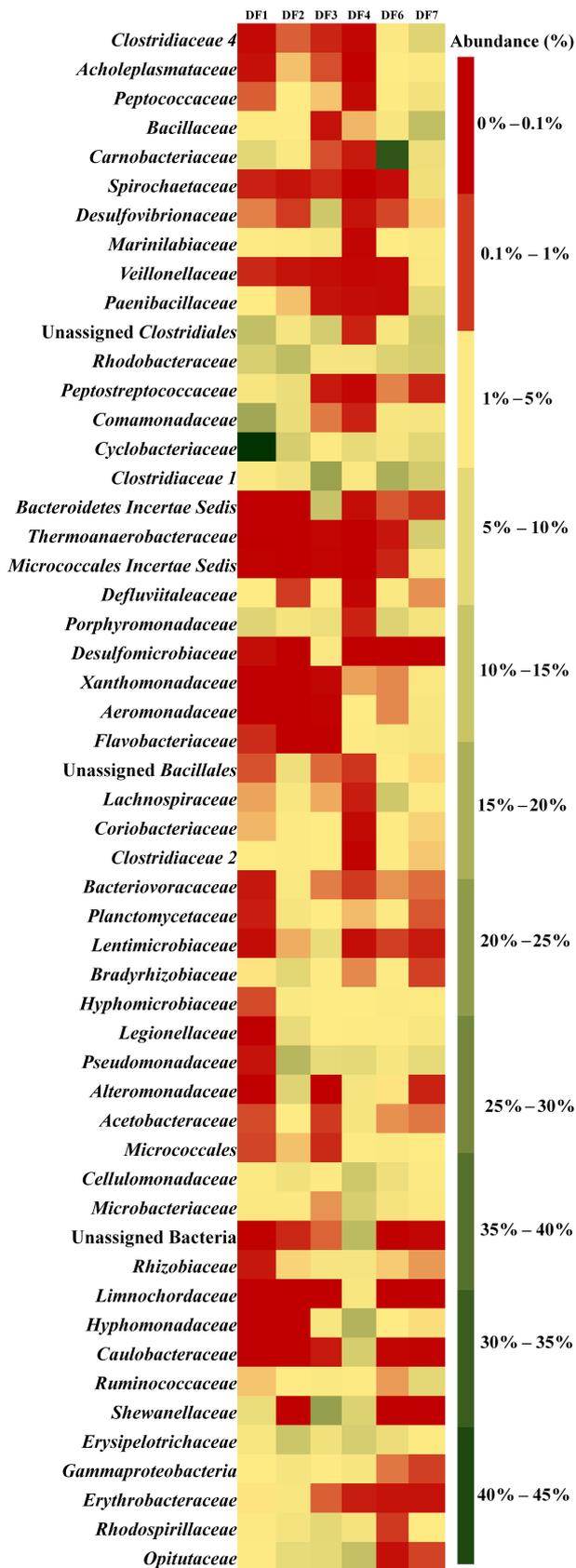
Figure 5. Microbial community composition and their abundance (> 1 %) in DF samples at family level.

3.7 Phylogeny of the core OTUs of DF samples

The phylogeny of the top 20 core OTUs (relative abundance 82 %) were analysed using the NCBI BLASTn nucleotide database, and a phylogenetic tree (Fig. 9) was constructed using MEGA7 software. Phylogenetic analysis revealed that the most of the core OTUs of all six DF samples displayed a significant match with known (cultured and uncultured) anaerobic, thermo-alkaliphilic microorganisms obtained from extremophilic habitats and the deep subsurface. *Amphibacillus, Marinilactibacillus, Pannonibacter phragmitetus, Thermotalea metalivorans, Crassaminicella profunda, Geosporobacter subterraneus, Anaerobranca horikoshii, Anaerobranca californiensis, Crassaminicella profunda* and *Mongoliitalea lutea* were some of the major bacteria which displayed close lineage with the OTUs of DF samples. Some of the OTUs also showed a match with uncultured thermophilic, anaerobic iron and sulfate and nitrate reducers (*Clostridia, Geosporobacter, Desulfovibrio* etc.).

3.8 Phylogeny of the unique OTUs of DF samples

The top 10 OTUs (on the basis of relative abundance), unique to each specific DF sample, were subjected to phylogenetic analysis to investigate their identity with extremophilic lineages or microorganisms reported from the deep subsurface (Fig. 10). The relative abundance of the unique OTUs (Table 2), which showed matches with extremophiles or microorganisms reported from deep subsurface microbiome, pertaining to a specific sample, was highest for DF7 (15.42 %) and lowest for DF6 (2.58 %). Unique OTUs corresponding to DF1 showed close NCBI BLASTn matches with *Hydrogenophaga aquatica, Tepidicella* and other uncultured bacteria from extreme environments (hypersaline lakes) and deep environments. The top 10 unique OTUs corresponding to DF2 showed matches with *Peptostreptococcaceae* and *Sphingobacteriales*. Here also some of the OTUs showed matches with *Pseudomonas* strains derived from surface environments. Apart from a few OTUs pertaining to DF3 (which showed a match with bacteria from waste water and sewage sludge) most of them displayed close similarity to *Desulfomicrobium* and some uncultured bacterial population from deep subsurface environments. Most of the top 10 OTUs of DF4 showed correspondence to *Aishwanella* and other haloalkaliphilic bacteria, whereas few OTUs were related to bacteria derived from hydrocarbon-contaminated soil and anaerobic sludge. The unique OTUs (top 10) of sample DF6 exhibited close lineage with *Marinilactibacillus piezotollerans* and other representatives of uncultured bacteria from deep subsurface rocks and geologically derived fluids. Subsequently most of the top 10 unique OTUs of DF7 were similar to *Thermincola, Thermosinus*, extremotolerant *Paenibacillus* and other representatives from geothermal deep aquifers.
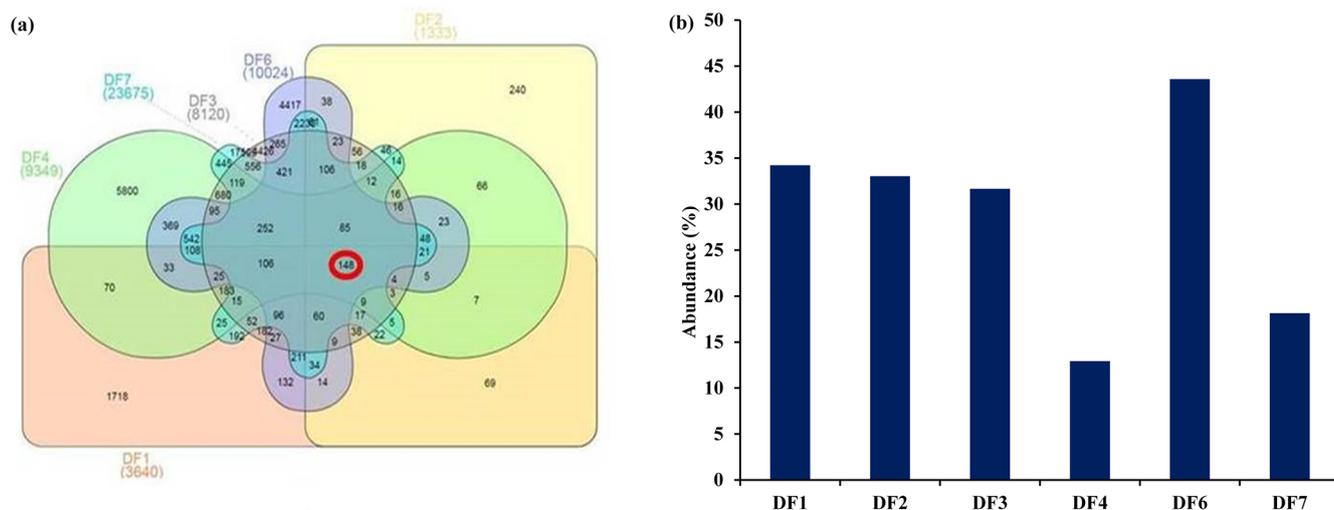
Figure 6. (a) Venn diagram displaying the number of common OTUs among the DF samples; 148 OTUs were found common in the DF samples (1–7). **(b)** Percentage abundance of common OTUs across the DF samples.

3.9 Enrichment of SRB from DF samples

16S rRNA gene amplicon sequencing and subsequent analysis revealed an assessable shift in microbial community composition of DF samples (DF1 and DF3) following anaerobic incubation under sulfate-reducing conditions. A comparison with the microbial community composition of DFs before and after enrichment is given in Fig. S1 in the Supplement. Following incubation under sulfate-reducing conditions a distinct shift in community composition with great enhancement of *Firmicutes* (60 %) was observed. *Bacteriodetes*, *Alphaproteobacteria* and *Betaproteobacteria*, which dominated the DF1 sample, decreased significantly in the enrichments. Members of the phyla *Gammaproteobacteria* (12 %) and *Deltaproteobacteria* (4 %) increased in DF1 SRB enrichments. Similarly, in the DF3 sample *Firmicutes* (29 %), Bacteriodetes (18 %), *Gammaproteobacteria* (21 %) and *Alphaproteobacteria* (11 %) were the major phyla. Following enrichment under sulfate-reducing conditions, a sharp increase in the abundance of *Firmicutes* (98 %) was noted (Fig. S1a). A 3- to 4-fold enhancement of *Firmicutes* was observed in the SRB enrichments for both the DF samples. Analysis of genus-level data (Fig. S1b) showed that known anaerobic sulfate reducers such as *Anaerobranca*, *Anoxybacillus*, *Bacillus* and *Clostridium sensu stricto* 8 were most prevalent in the SRB enrichments for both the DFs.

4 Discussion

Rotary drilling has been one of the most important techniques to get the crystalline rock samples and geological fluids from deeper depths (Keift et al., 2007). The returned drilling fluid obtained on site, during the drilling of Koyna pilot borehole (at the Deccan Traps), India, contained chemical signatures of bentonite-based drilling mud (Table S1). These drilling fluids were mostly silica-rich, with Na, Al, N and C being the other major elements, and also contain a variety of organic constituents (Schlumberger Oilfield Glossary, 2010; Keift et al., 2007; Rabia, 1985). The presence of compounds such as calcium (Ca), manganese (Mn^{2+}), sulfur (S), iron (Fe^{2+}), sulfate (SO_4^{2-}) and minor amounts of sulfide (S^{2-}) in DF samples was quite noteworthy, and their concentrations increased with increased sampling depth. Normally, those compounds should not exist in the bentonite-based DF (Montmorillonite – Handbook of Mineralogy, 2000; http://rruff.geo.arizona.edu/doclib/hom/montmorillonite.pdf, last access: 27 January 2020). With increasing sample depth, a change of pH to alkalinity and a reduced relative abundance of Si and Al was noted in the samples (Fig. 1). The granitic subsurface of the Koyna–Warna region was reported to be sulfur- and iron-rich (Misra et al., 2017). The availability of sulfate in deeper horizons (> 1000 m) was widely reported through geochemical analysis of deep granitic aquifers around the globe (Ino et al., 2017, and references within). Compared to other metallic elements present in mineral form, SO_4^{2-} and Fe^{2+} present in rock were able to dissolve in the DF during drilling; hence it got enriched with sulfate with an increase in depth. The shift in geochemical parameters suggested gradual infusion of elements from the crystalline bedrock during the course of drilling. More evidence of this infusion came from the alkaline nature of the DF samples obtained from lower depths. These granites have been reported to contain minerals responsible for creating alkaline conditions in those environments (Misra et al., 2017). The mixing of the granitic bedrock with the drilling fluid had provided alkalinity to the samples. There was a constant increase in temperature with increasing depth (55.6 to 73.9 °C) from 1681 to 2908 m b.s. In the subsurface of the
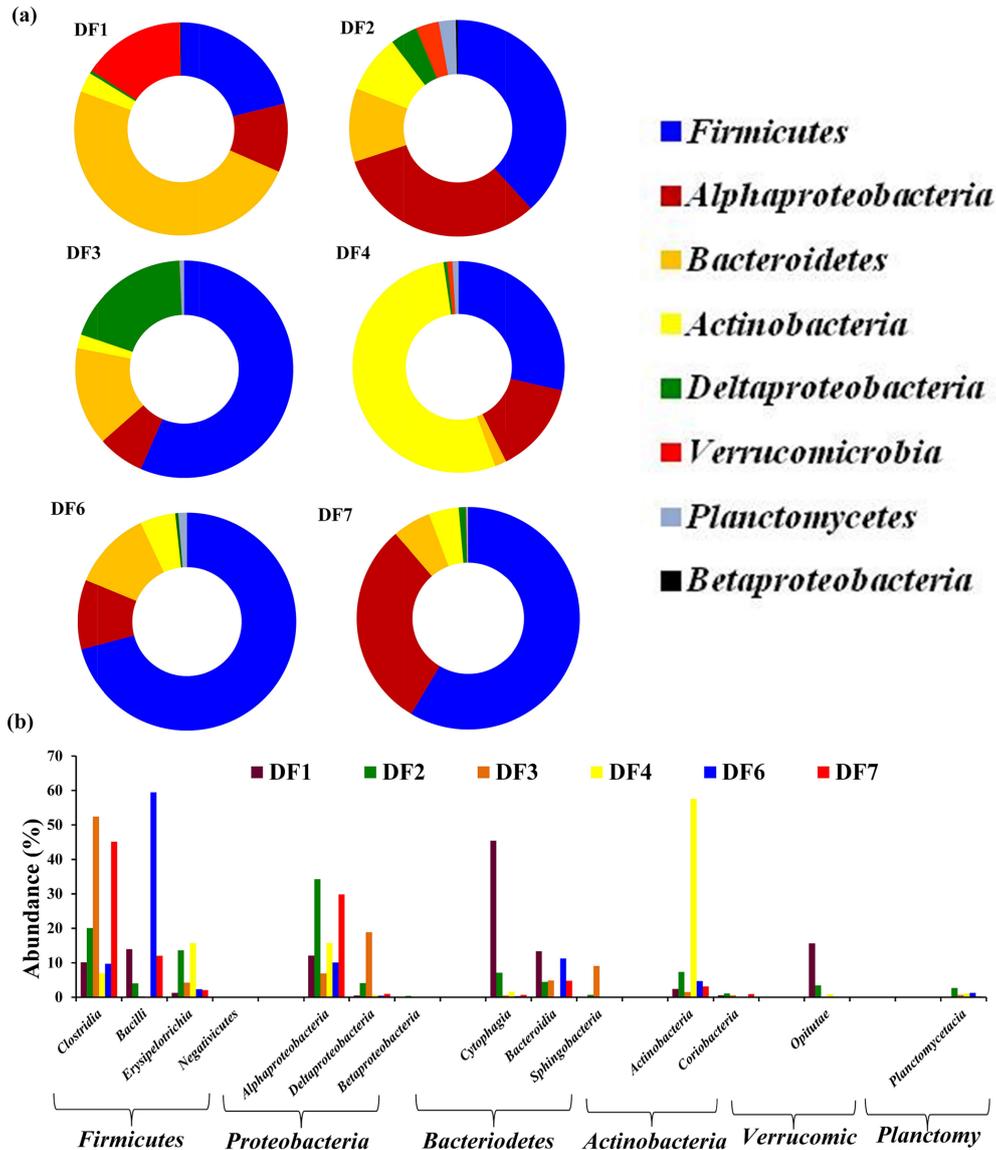
Figure 7. Microbial abundance of core OTUs at phylum **(a)** and class **(b)** level.

Deccan Traps temperature increased with depth at a rate of $\sim 15\,^{\circ}\mathrm{C}\,\mathrm{km}^{-1}$ in granitic bedrock and lithostatic pressure increased by 26.7 MPa km^{-1} (Roy and Rao, 1999). The initial fluid used might have contained surface-derived microbial populations but the deep extreme subsurface environments, through which it had circulated, could have tended to favour indigenous, subsurface-adapted microorganisms (Zhang et al., 2006; Masui et al., 2008). It could also contain the microbial diversity of rock chips and geological fluids from the deep subsurface carried along with the DFs to the surface during drilling. The bacterial and archaeal cell abundance were detected using qPCR. No significant change in bacterial and archaeal abundance was observed with respect to sampling depth (Fig. 2). Microbial load in the DF was observed to be persistent across depths. Hence, it could be assumed that the change in biomass and its decay have been at equilibrium across depths in DF samples. Dynamic conditions encountered by the DF (due to drilling and coring process) might also have favoured the growth of some of the microbial populations, whereas others were not able to sustain themselves, thereby keeping the overall microbial load consistent throughout.

The microbial diversity of DFs were studied by amplicon sequencing of the V4 region of the 16S rRNA gene. This primer set (515F–806R) to amplify this region was developed by Bates et al., 2011, and was designed to be universal for nearly all bacterial and archaeal taxa. They even demonstrated in silico that this primer set could amplify 16S rRNA genes from a broad range of archaeal and bacterial groups with few biases or excluded taxa. In spite of the short-read
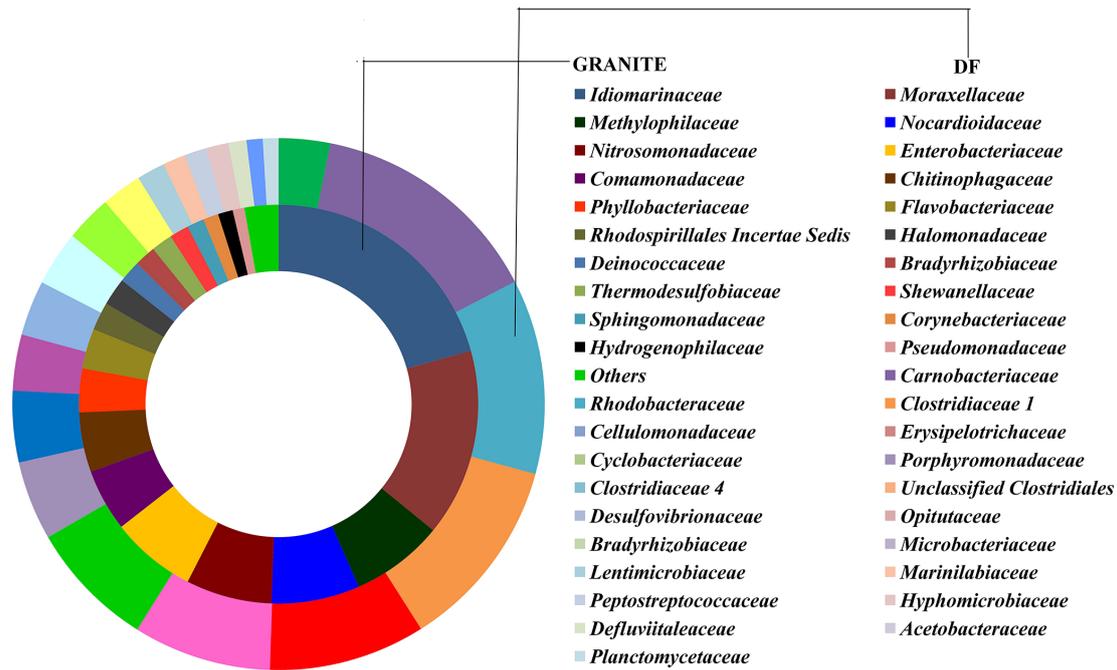
GRANITE
- *Idiomarinaceae*
- *Methylophilaceae*
- *Nitrosomonadaceae*
- *Comamonadaceae*
- *Phyllobacteriaceae*
- *Rhodospirillales Incertae Sedis*
- *Deinococcaceae*
- *Thermodesulfobiaceae*
- *Sphingomonadaceae*
- *Hydrogenophilaceae*
- *Others*
- *Rhodobacteraceae*
- *Cellulomonadaceae*
- *Cyclobacteriaceae*
- *Clostridiaceae 4*
- *Desulfovibrionaceae*
- *Bradyrhizobiaceae*
- *Lentimicrobiaceae*
- *Peptostreptococcaceae*
- *Defluviitaleaceae*
- *Planctomycetaceae*

DF
- *Moraxellaceae*
- *Nocardioidaceae*
- *Enterobacteriaceae*
- *Chitinophagaceae*
- *Flavobacteriaceae*
- *Halomonadaceae*
- *Bradyrhizobiaceae*
- *Shewanellaceae*
- *Corynebacteriaceae*
- *Pseudomonadaceae*
- *Carnobacteriaceae*
- *Clostridiaceae 1*
- *Erysipelotrichaceae*
- *Porphyromonadaceae*
- *Unclassified Clostridiales*
- *Opitutaceae*
- *Microbacteriaceae*
- *Marinilabiaceae*
- *Hyphomicrobiaceae*
- *Acetobacteraceae*

Figure 8. Microbial abundance comparison at family level of core OTUs of granitic rock cores (up to 1500 m b.s.) (Dutta et al., 2018) and DF samples (DF; up to 2908 m b.s.).

lengths (∼ 250 bp), this region provided sufficient resolution for the accurate taxonomic classification of microbial sequences. Since then there have been many studies which have used the V4 region of the 16S rRNA gene to study the microbial diversity of different environments, including the deep subsurface (Dutta et al., 2018; Gupta et al., 2018; Purkamo et al., 2017). Considerably higher diversity and bacterial abundance was observed in DF with an increase in depth (Table 1). There was a marginal increase in archaeal abundance in DF with an increase in depth. Increases in Shannon and Simpson index values (Fig. 3a) confirmed the increase in the microbial diversity of DF as we went further down (Simpson, 1949; Pielou, 1966). Continuous exposure to the deep subsurface environment and its constant mixing with the granitic subsurface rocks might have enriched the microbial diversity of DF with an increase in depth (Struchtemeyer et al., 2011; Zhang et al., 2006). The phylum-level composition of microbial communities of the DF samples was similar to some of the earlier studies on the microbial diversity of DFs (Fig. 4a and b). Struchtemeyer et al., 2011, reported the dominance of *Firmicutes* (average 55 %) in all the DF samples from thermogenic natural gas wells of the Barnett Shale. *Firmicutes* were found to be the major microbial community in six DF samples collected from 2290 to 3350 m b.s. in the Chinese continental scientific drilling (CCSD) project (Zhang et al., 2006). The original in-flow DF which was collected from the tank before its introduction in the borehole had different microbial community composition than the returned DF samples (DF1–DF7) from each depth (Fig. 4a). *Bacteriodetes*,

which dominated the in-flow DF, decreased considerably in the returned DF samples (DF1 to DF7). The phylum *Bacteriodetes* is widely distributed in the environment, including in soil, sediments and sea water, as well as in the guts and on the skin of animals; hence it might be indigenous to drilling fluid (Gibino et al., 2018; Rainey and Oren, 2011). Similarly, *Firmicutes*, *Alphaproteobacteria*, *Actinobacteria* and *Gammaproteobacteria* were increased in the returned DF samples. Hence, it can be seen that indigenous microbial community composition of in-flow DF was altered considerably during its interaction with the granitic rock cores during drilling. It was evident that microbial communities of the returned DF samples also varied with increases in depth due its extended exposure to deep subsurface conditions and interaction with granitic rocks. The relative abundance of some bacterial phyla such as *Bacteriodetes*, *Betaproteobacteria* and *Gammaproteobacteria* decreased and the relative abundance of some bacterial phyla such as *Firmicutes*, *Alphaproteobacteria* and *Actinobacteria* (relevant to deep subsurface) increased (Fig. 3b). There are several reports that confirmed the dominance of these phyla in the deep subsurface (Sahl et al., 2008; Leandro et al., 2018; Ino et al., 2017; Purkamo et al., 2017; Leblanc et al., 2019). The concentration of some archaeal phyla (*Euryarchaeota* and *Thaumarchaeota*) also increased in the lower depths. Interestingly, archaea were not detected in the in-flow DF. These archaeal phyla have been predominantly reported in deep igneous rocks (Nyyssönen et al., 2014; Breuker et al., 2011; Labonté et al., 2017). A dynamic shift in microbial populations was particularly note-
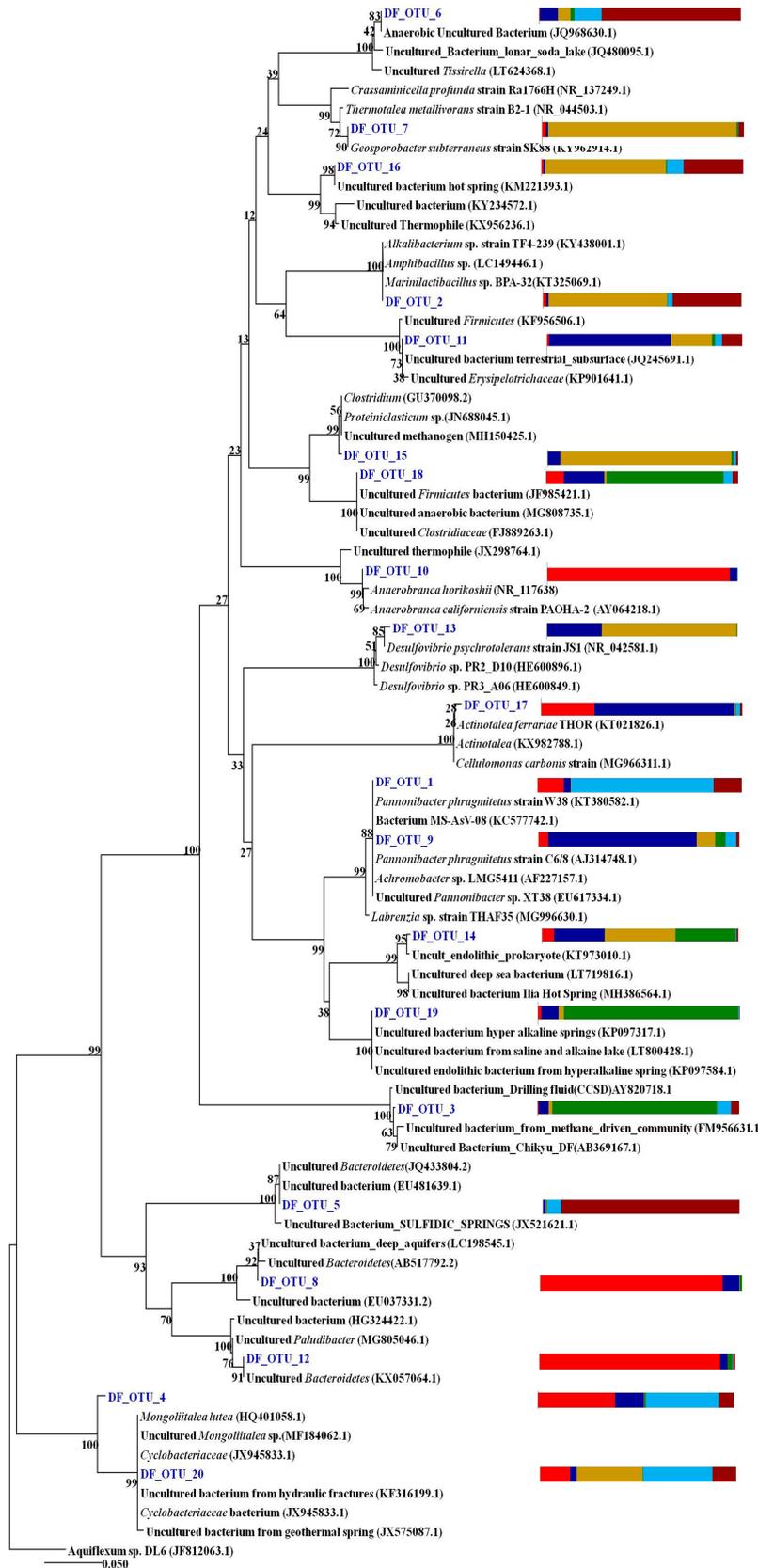
Figure 9. The phylogeny of the top 20 core OTUs (relative abundance > 82 %) constructed in MEGA7. Coloured stack bar represent their relative abundance in all the DF samples. Each sample is represented by different colours as follows: DF1: red, DF2: blue, DF3: yellow, DF4: green, DF6: light blue, DF7: dark red.
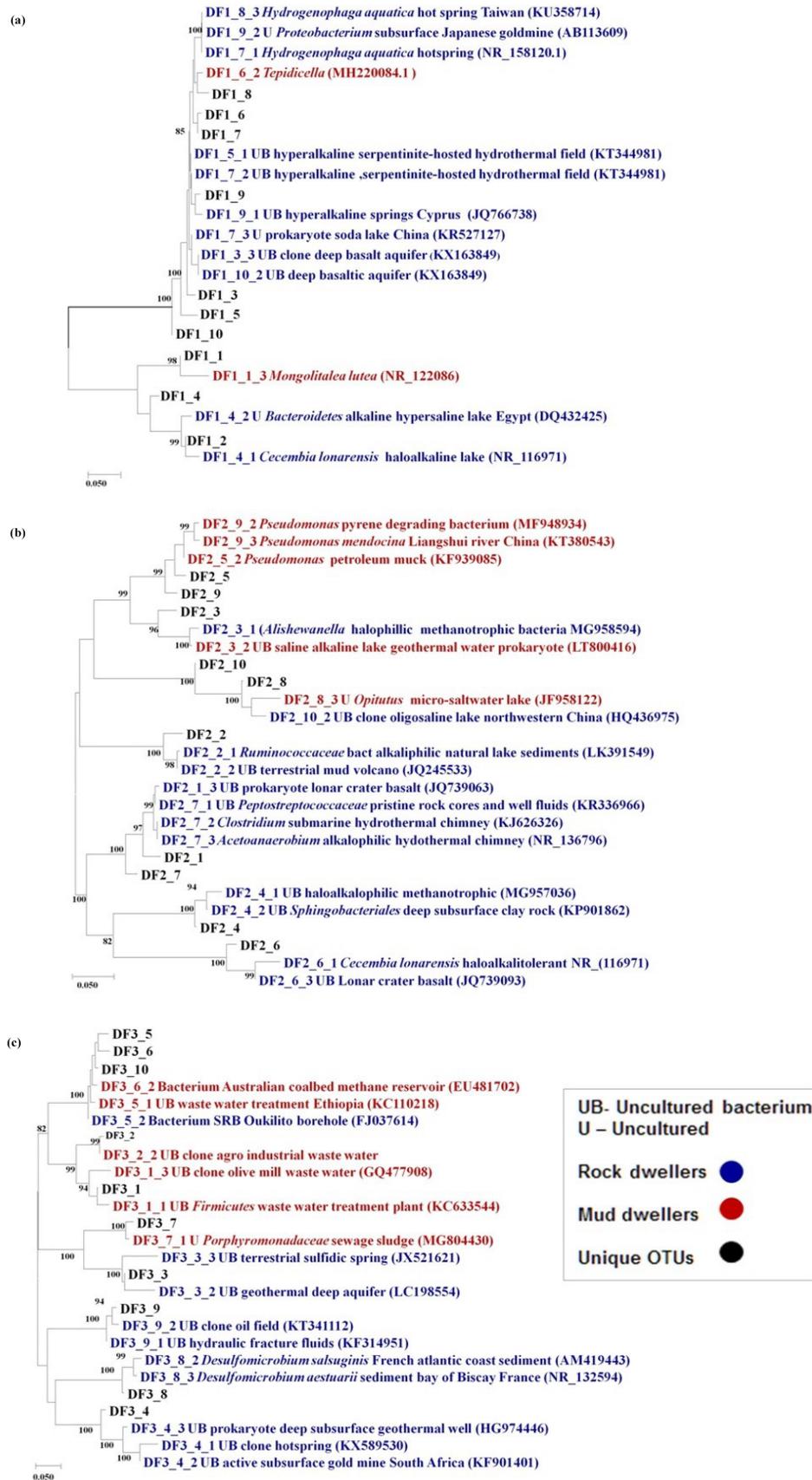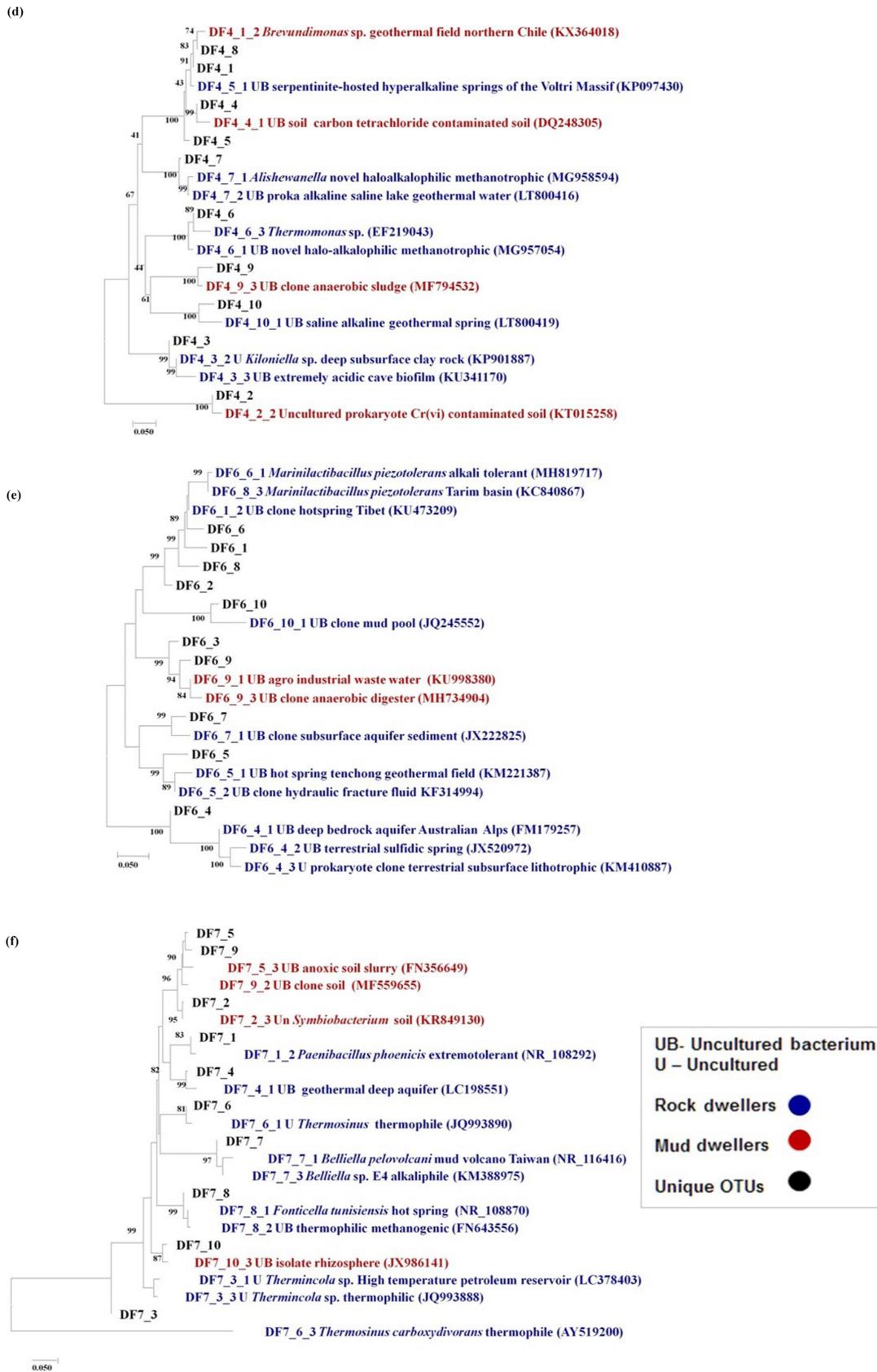
Figure 10.

Figure 10. The phylogeny of the top 10 unique OTUs of DF1 (**a**), DF2 (**b**), DF3 (**c**), DF4 (**d**), DF6 (**e**) and DF7 (**f**). A phylogenetic tree was constructed using MEGA 7 software.

worthy in the DF samples from the deep granitic subsurface of the Koyna–Warna region. These microbial communities must have intruded from the deep granitic rocks into the DFs during the drilling process.

The family- and genus-level data gave us better insight into the changing microbial dynamics of returning DF samples by depth (Fig. 5). *Comamonadaceae* and *Pseudomonadaceae* were observed to be dominant in DF1 (1500 m b.s.) and DF2 (1901 m b.s.) and their abundance decreased in the lower depths. Several microorganisms belonging to *Commamonadaceae* and *Pseudomonadaceae* families were mesophilic in nature, mostly found in soil and water (Palleroni, 1981; Moore et al., 2006; Willems, 2014), and they might have come either from DF or the surface. *Cyclobacteriaceae* and *Rhodobacteriaceaeae*, though dominant in DF1 and DF2, were also present in all the DF samples. These two microbial families, found in diverse and extreme habitats (freshwater bodies, algal mats, marine waters, alkaline soda lakes, hot springs, mud volcanoes etc.), manifested the ability to degrade a number of polysaccharides and have been deeply involved in sulfur and carbon biogeochemical cycling (Pujalte et al., 2014; Pinnaka and Tanuku, 2014). Major populations of these two families in DF1 and DF2 could be indigenous to DFs and surface soil or water. There is a reasonable chance that the extremophilic microbial population belonging to *Cyclobacteriaceae* and *Rhodobacteriaceaeae* might have become enriched in the DFs sampled at lower depths due to the extreme conditions (Zhang et al., 2006; Kumar et al., 2012). Alternatively, it could also be concluded that they might be part of the deep subsurface microbiome (Russell et al., 2016; Hubalek et al., 2016; Leboulanger et al., 2017). Subsequently the relative abundance of some of the microbial families, known to strive in strictly anaerobic and extremophilic environments (e.g. *Thermoanaerobacteraceae*, *Clostridiaceae*, *Bacillaceae*, *Carnobacteriaceae*, *Ruminococcaceae*) (Tomás et al., 2013; Deep et al., 2013; Glaring et al., 2015; Toffin et al., 2005; Ishikawa et al., 2009; Song and Dong, 2009; Horino et al., 2014), increased in the DFs sampled from 2000 to 3000 m b.s. Some of these microbial families such as *Thermoanaerobacteraceae* and *Bacillaceae* have been reported from deep subsurface environments as well as DFs from other deep subsurface studies (Chakraborty et al., 2018; Gaboyer et al., 2015; Zhang et al., 2006; Slobodkina et al., 2012; Fang et al., 2017) These groups might have intruded the DFs from the rocks at lower depths during drilling operations (Struchtemeyer et al., 2011). High concentrations of sulfate and iron in the DFs from lower depths are correlated with the presence of these microbial families which have been reported previously to utilize sulfate and iron as terminal electron acceptors (Sylvan et al., 2015; Reyes et al., 2017; Kjeldsen et al., 2007; Kanso et al., 2002; Emmerich et al., 2012; Ogg et al., 2010). The presence of thermophilic, alkaliphilic, halophilic and anaerobic fermentative bacterial genera relevant to the deep subsurface (*Opitutus*, *Mongoliitalea*, *Hydrogenophaga*, *Marinilactibacillus*, *Anoxybacillus*, *Sym-*

biobacterium, *Geosporobacter*, *Thermoanaerobacter* etc.) in the DFs (Lee et al., 2012; Willems et al., 1989; Toffin et al., 2005; Ueda et al., 2004; Klouche et al., 2007; Hong et al., 2015) and their dominating presence in the samples from lower depths (2500 m b.s.) confirmed the hypothesis of a possible interaction between the granitic bedrock and DF and was indicative of the possible alteration in the DF microbial community due to its exposure to the highly extreme conditions at these depths.

Core OTU analysis revealed similar observations to those stated above (Figs. 6 and 7). Some of the microbial phyla (*Cyanobacteria*, *Chloroflexi*, *Chlamydiae* and *Euryarchaeota*) indigenous to deep subsurface environments (Puente-Sánchez et al., 2014, 2018; Fullerton and Moyer, 2016; Rempfert et al., 2017) were only observed in the core OTUs of lower depths (DF4 onwards). The microbial families (*Carnobacteriaceae*, *Rhodobacteraceae*, *Clostridiaceae 1*, *Cellulomonadaceae*, *Erysipelotrichaceae*, *Cyclobacteriaceae*, *Opitutaceae*, *Bradyrhizobiaceae* and *Desulfovibrionaceae*) detected in the core OTUs of DF were different from the microbial families (*Idiomarinaceae*, *Moraxellaceae*, *Methylophilaceae*, *Nocardioidaceae*, *Nitrosomonadaceae*, *Enterobacteriaceae*, *Comamonadaceae*, *Chitinophagaceae*) detected in granitic core OTUs at higher depths (1500 m b.s.) (Dutta et al., 2018) (Fig. 8). These microbial families might have either come from the intrusions of subsurface deep granitic bedrock at lower depths (1600 m b.s. onwards) or might have got enriched due to the extreme environmental conditions at the deep granitic subsurface. Phylogenetic analysis of the core OTUs revealed that most of the sequences belonged to anaerobic, deep subsurface microbial communities (Fig. 9). Core OTUs displayed a good match with extremophilic microorganisms (cultured and uncultured), namely *Amphibacillus* (anaerobic, alkaline), *Marinilactibacillus* (halo-alkaliphilic, peizotolerant), *Pannonibacter phragmitetus* (alkalitolerant), *Thermotalea metalivorans* (anaerobic, thermophilic), *Crassaminicella profunda* (anaerobic, chemo-organotrophic bacterium), *Geosporobacter subterraneus* (obligate anaerobe), *Anaerobranca horikoshii* (alkalitolerant thermophile), *Anaerobranca californiensis* (obligately anaerobic, alkalithermophilic, chemo-organotroph), *Crassaminicella profunda* (anaerobic, chemo-organotrophic) and *Mongoliitalea lutea* (alkaliphilic, halotolerant). These organisms have been reported from deep subsurface environments and extreme habitats such as alkaline soda lakes, hot and alkaline springs, sulfur-containing springs, methane bedrock, deep sea sediments, and aquifers (Engle et al., 1995; Borsodi et al., 2003; Gorlenko et al., 2004; Toffin et al., 2005; Klouche et al., 2007; Wu et al., 2010; Yang et al., 2012; Lakhal et al., 2015; Ogg and Patel, 2009). Very few OTUs showed a match with both types of habitats, namely moderate as well as extreme. Phylogenetic analysis of unique OTUs pertaining to each DF sample revealed similar results (Fig. 10). Some of the OTUs unique to each sample (3 %–15 % relative abun-

dance per sample) showed relatedness to extremophilic as well as deep subsurface microbial groups while few OTUs showed a match with mesophilic bacteria reported from surface environments. Unique OTUs corresponding to the DF1 sample showed close NCBI BLASTn matches with *Hydrogenophaga aquatica*, which is isolated from the hot spring (Lin et al., 2017) and *Tepidicella* (alkaliphilic bacteria isolated from hot spring runoff) (França et al., 2006) and other uncultured bacteria from hyperalkaline saline lakes and deep basaltic aquifers. The top 10 unique OTUs corresponding to DF2 showed matches with *Peptostreptococcaceae* (a moderately thermophilic anaerobic bacterial family which consists of bacteria reported from pristine rock cores, deepsea hydrothermal vents and other extreme environments) (Slobodkin, 2014) and *Sphingobacteriales* (reported from deep rocks) (Dutta et al., 2018). They are similar to various *Pseudomonas* strains reported from river and petroleum muck environment and other uncultured bacteria from mud microbiomes as well. Uncultured bacteria from the Oukilito borehole, geothermal deep aquifer, Atlantic coast sediment (*Desulfomicrobium*) (Dias et al., 2008) and hydraulic fracture fluids showed close lineage with most of the top 10 unique OTUs pertaining to sample DF3, whereas a few uncultured bacteria from waste water and sewage sludge (*Porphyromonadaceae*) (Sakamoto, 2014) also showed relatedness to some of the unique OTUs of this sample. Some unique OTUs among the top 10 of the DF4 sample showed correlation with *Alishawanella* (Salah et al., 2016) and other haloalkalophilic bacteria and were representative of hypersaline lake and deep subsurface clay rocks, and some unique OTUs were related to hydrocarbon-contaminated soil and anaerobic sludge. Unique OTUs (top 10) of sample DF6 exhibited close lineage with *Marinilactibacillus piezotollerans*, which is a known peizotolerant bacterium, and other representatives from hydraulic fracture fluids, deep bedrock aquifer and subsurface aquifer sediments (Toffin et al., 2005). Subsequently most of the top 10 unique OTUs of DF7 were similar to *Thermincola* (anaerobic, thermophilic, chemolithotrophic organism), *Thermosinus* (anaerobic, thermophilic and carbon monoxide oxidizing bacterium), extremotolerant *Paenibacillus* and other representatives from geothermal deep aquifers (Zavarina et al., 2007; Sokolova et al., 2004). Based on these observations we hypothesized that due to the prolonged interaction of DF with the subsurface granitic crust these organisms might have become infused in the DF. The interaction between DF and crust has altered the geochemistry of DF, which is evident from the altered geochemical properties of the DF. This interaction (infusion of crustal particle carrying cells) led to the enrichment of DF with extremophilic microbial populations pertaining to the deep granitic subsurface (represented by the core OTUs). On the contrary in the OTUs unique to each DF, a mixed bacterial population (both extremophilic as well as mesophilic) was observed. The presence of deep subsurface and extremophilic bacterial populations in unique as well as core OTUs confirms our hypothesis

of the presence of deep subsurface microbial populations in DF samples collected at different depths below the surface. Apart from that, the presence of diverse heterotrophic bacterial populations in DF samples is quite interesting, and during core OTU analysis we observed that many OTUs showed lineages with heterotrophic bacteria. As already discussed above, DF consists of a variety of organic carbon compounds (Keift et al., 2007; Rabia, 1985). These organic carbon compounds could be efficiently utilized by heterotrophic bacteria as a carbon source and hence played an active role in shaping up the functional diversity of the DF microbial communities. Incubation of DF samples (DF1 and DF3) under sulfate-reducing conditions was quite successful and it led to increase in the abundance of *Firmicutes* members (*Anaerobranca*, *Clostridium sensu stricto* 8, *Anoxybacillus*, *Bacillus*) capable of anaerobic sulfate reduction (Fig. S1). All these members of the phylum *Firmicutes* were well known for their facultative to strict anaerobic metabolism and have been reported from several deep subsurface studies and extremophilic environments (Sahl et al., 2008; Purkamo et al., 2017; Leblanc et al., 2019; Gupta et al., 2018). Purkamo et al. (2017) detected *Anaerobranca* in their enrichment samples, which were set up to understand the response of acetate (carbon source) and sulfate (electron acceptor) in the fracture water collected at a depth of 2516 m b.s. at the Outokumpu drill site. As already shown these genera were also detected in DF samples (DF1 to DF7) and showed phylogenetic lineages with the bacteria isolated from extremophilic environments or the deep subsurface. A dynamic shift in the microbial community structure of DF with increases in depth was observed due to its exposure to deep subsurface environmental conditions.

5 Conclusion

Drilling fluid, often regarded as a source of contamination during investigations of deep subsurface microbiology, could be a vector for the sampling of geological fluids and signatures of microbial life from such environments. The geochemical investigation of DF that circulated through the deep borehole (down to 3000 m b.s.) from the granitic subsurface confirmed a possible interaction of DFs and igneous rocks. These interactions had conferred the DF with unique conditions (reducing, hot, alkaline and saline environments) that partly reflected the deep subsurface. Our investigations of the DFs suggested that although such circulating fluids could be responsible for contaminating the deep subsurface rock samples by introducing extraneous microbes, enrichment of these fluids with subsurface organisms was inevitable due to prolonged interaction with subsurface rocks. Successful identification and enrichment of anaerobic, thermophilic sulfate-reducing and deep subsurface relevant microbial populations from DF samples confirmed that DF could be used as a proxy to study the deep subsurface. Hence, the study of the mi-

crobial ecology of the DF that circulated through the deep borehole (down to 3000 m) from granitic subsurface of the Koyna–Warna region of the Deccan Traps provided a window through which to look into the broader spectrum of deep life residing within the deep crystalline bedrock of the Deccan Traps. The DF microbiome could also be used as a proxy for bedrock to isolate and characterize deep biosphere related microorganisms.

Data availability. The sequence reads obtained were submitted to the sequence read archive (SRA) under SRA accession: SRP155468. It can be accessed from the following link: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA482760/ (Sar, 2018).

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Author contributions. HB performed the DNA extraction, geo-microbiological analysis, statistical analysis, data organization and analysis and prepared the manuscript. AD and AG performed the amplicon sequencing of the samples and helped in statistical analysis and bioinformatics pipeline optimization. JS performed the qPCR analysis. HB, AD, AG, AR and BM did the periodic sampling. SM assisted in phylogenetic study and data interpretation. SR organized sample collection and gave inputs about the geophysical and geochemical characterization of drilling fluid, Deccan lava flow and the Koyna–Warna seismic zone. PS and SKK conceived the study, designed experiments, compiled and analysed the data, wrote the manuscript and did overall mentoring.

Competing interests. The authors declare that they have no conflict of interest.

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Scientific Drilling
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New *Chikyu* Shallow Core Program (SCORE): exploring mass transport deposits and the subseafloor biosphere off Cape Erimo, northern Japan

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Abstract. The *Chikyu* Shallow Core Program (SCORE) has been started to provide more opportunities for the scientific ocean drilling of shallow boreholes (up to 100 m) during a short-term expedition. The proposal flow is a simplified version of that of the International Ocean Discovery Program (IODP). Although there are several limitations for a SCORE project, the opportunity to retrieve 100 m of continuous core samples will be of great interest for the scientific ocean drilling community in multiple disciplines. The first expedition of the SCORE program was implemented off Cape Erimo, Hokkaido, northern Japan. The target of the drilling was to investigate the impact of submarine mass transport on the subseafloor sedimentary biosphere. In the preliminary observation of the core samples, including X-ray computed tomography (CT) scan image analysis, chaotic and inclined beds were found and interpreted as mass transport deposit (MTD) units.

1 Introduction

The drilling vessel *Chikyu* has implemented more than 20 scientific ocean drilling expeditions for the IODP (Integrated Ocean Drilling Program and International Ocean Discovery Program) since 2007. Because the *Chikyu* is the only riser drilling platform for scientific ocean drilling, previous IODP expeditions have mainly focused on very deep subseafloor targets such as seismogenic zones in accretionary prisms and the deep biosphere within coal and shale beds. However, opportunities for utilizing the *Chikyu* might have been limited for the exploration of relatively shallow horizons, because the riser drilling expeditions often tend to be long, expensive, and complicated, taking years from the initial proposal submission to the implementation. To maximize the use of the *Chikyu* for the broader science and educational community, a new framework for scientific ocean drilling, the *Chikyu* Shallow Core Program (SCORE), has been initiated under a collaboration between the Japan Drilling Earth Science Consortium (J-DESC) and Japan Agency for Marine-Earth Science and Technology (JAMSTEC). SCORE is designed to implement short-term expeditions in a relatively simple process using hydraulic piston coring. It would add extra value to the *Chikyu* if more opportunities were made available for the ocean drilling community to take part in *Chikyu* expeditions without the time, effort, and cost necessary for major long-term expeditions. In this article, the concept of SCORE and preliminary results from its first expedition (910) off Cape Erimo in September 2017 are introduced.

1.1 SCORE

SCORE is a new programme of J-DESC to utilize the *Chikyu* for drilling shallow boreholes during a short-term expedition. Any scientist from a member institution of J-DESC can submit a drilling proposal with a format similar in length to an IODP pre-proposal. Although the lead proponent must be associated with a member institution of J-DESC, any scientist may be a co-proponent and is eligible to participate in an expedition. The proposal flow of SCORE is a simplified version of that of the IODP (the format is available at the J-DESC website https://www.j-desc.org/score/, last access: 13 February 2020). The IODP section in J-DESC reviews the proposal and forwards it to the Institute for Marine-Earth Exploration and Engineering (MarE3) in JAMSTEC with recommendations, if the proposal is scientifically mature enough for implementation in the future. MarE3 determines whether the proposal fits within operational constraints (budget, schedule, logistics, etc.).

Due to the short timeframe of the drilling, there are several limitations for a SCORE project. First, drilling operations are limited to hydraulic piston coring of sediment down to ~ 100 m below seafloor (m b.s.f.). Second, proposed drilling sites located on, or nearby, the planned ship track of the *Chikyu* (see the J-DESC website) are preferred. Third, ship-

board flow of analyses is limited as compared to the standard measurement procedures on an IODP expedition due to the short duration of the expedition and a reduced number of shipboard technical staff. Typically, non-destructive analysis of whole-round cores and image scanning after core splitting are conducted onboard. In addition to the limitations in such operational and analytical conditions, proponents should be aware that J-DESC provides no financial support for expedition participants (as of today). While MarE3 implements drilling operations and provides support for core curation and minimal data collection, proponents and team members should prepare necessary costs for travel and accommodations, sample shipping, onboard and post-expedition research activities, etc. Nevertheless, in addition to supplementing full IODP expeditions, the opportunity to retrieve 100 m of continuous core samples will be of great interest for the scientific ocean drilling community in multiple disciplines.

1.2 Expedition 910: drilling west off Cape Erimo

The first SCORE expedition with the *Chikyu* was conducted from 19 to 23 September 2017, based on the proposal "Deep Learning of Deep Life: Exploring impact of submarine landslides on the deep biosphere-evolution off Cape Erimo" proposed by the lead proponent Fumio Inagaki of JAMSTEC. Since the Ocean Drilling Program (ODP) Leg 201, numerous microbiological and biogeochemical studies have demonstrated that Earth's biosphere extends far below its surface, at least down to ~ 2.5 km beneath the ocean floor on the continental margin (Inagaki et al., 2015). Based on the accumulated evidence of microbial biomass in subseafloor sediments in various oceanographic and geological settings, a recent numerical model study estimated that a total of 10^{29} microbial cells are present in the global subseafloor biosphere, accounting for 4 Pg of biomass carbon on Earth (Kallmeyer et al., 2012). Previous scientific ocean drilling also demonstrated that activity of aerobic and anaerobic subseafloor microbial communities is generally extraordinarily low; i.e. mean organic-fuelled respiration rates range from 2.8×10^{-18} moles e- per cell per year in anaerobic sediments in the eastern equatorial Pacific to 1.1×10^{-14} moles e- per cell per year in aerobic SPG sediments (D'Hondt et al., 2004, 2015; Hoehler and Jørgensen, 2013). In addition, these sedimentary communities in both aerobic and anaerobic subseafloor habitats consist mainly of species evolutionarily very distinct from known microbes in Earth's surface biosphere (e.g. Inagaki et al., 2003, 2006). Previous metagenomic and metatranscriptomic studies have found that those physiology and metabolic functions are also very distinct and adapted to the sedimentary biosphere where the energy flux is extremely low (Biddle et al., 2006; Orsi et al., 2013; Tully and Heidelberg, 2016; Valentine, 2007). To date, however, little is known about how such deeply buried "slow life" responds to changes in the surrounding environment.

In SCORE Expedition 910 off Cape Erimo, we targeted the community response and succession of the sedimentary biosphere associated with earthquake- and/or climate change-triggered mass transport deposits (MTDs) in that area. During the past decade, our knowledge of submarine mass movements including submarine landslides has significantly expanded worldwide, with realization of their destructive impact and tsunami-generation potential (e.g. Yamada et al., 2012). However, it still remains unknown how environmental changes caused by submarine landslides have impacted the modern subseafloor biosphere, how the deeply buried microbial communities have responded to the physical and chemical changes in their stable habitat, and what the succession and resilience of the microbial ecosystem and potential roles are in biogeochemical element cycling. Establishing high-resolution depth–age profiles of various environmental factors in this site is also useful for considering the occurrence mechanism of the MTDs. These scientific objectives are highly relevant to Biosphere Frontiers: Challenge 7 "How sensitive are ecosystems and biodiversity to environmental change?" and Earth in Motion: Challenge 12 "What mechanisms control the occurrence of destructive earthquakes, landslides, and tsunami?" in IODP Science Plan 2013–2023.

2 Geological background

The drilling site is located in the Hidaka Trough, which corresponds to an arc–arc junction between the north-eastern Japan arc and Kuril arc in the southern part of Hokkaido and extends southward to offshore of the Shimokita Peninsula (Fig. 1). The Hidaka Trough originated from a fore-arc basin that developed along the Pacific Plate subduction zone ($\sim 8\,\mathrm{cm\,yr^{-1}}$, west–northwest plate motion vector; Seno et al., 1996) and later converted to a foreland basin adjacent to the Hidaka Block collision zone due to the westward migration of the Kuril forearc sliver (Kimura and Kusunoki, 1997; Takano 2017). A large-scale fore-deep subsidence and a tremendous amount of clastic inflow that derived from the uplifted Hidaka Mountains resulted in the thick sedimentary piles of the Neogene foreland basin succession in the Hidaka Trough (Itoh and Tsuru, 2005; Noda et al., 2013). The 5000 m thick foreland basin-filling succession comprises submarine-fan turbidites, MTDs, siliceous shale and hemipelagic mudstones, depending on the background tectonic conditions at the depositional time and the inside-basin locations (JOGMEC, 2013).

Multiple seismic surveys have been conducted in the Hidaka Trough (JOGMEC, 2013, 2014), providing seismic stratigraphic and structural information on both the deeper and shallower parts of the basin-filling succession. Some seismic sections indicate that the shallower part below the seafloor is dominated by interbedded MTDs and hemipelagic shales. The 3-D topographic maps of the seafloor clearly in-
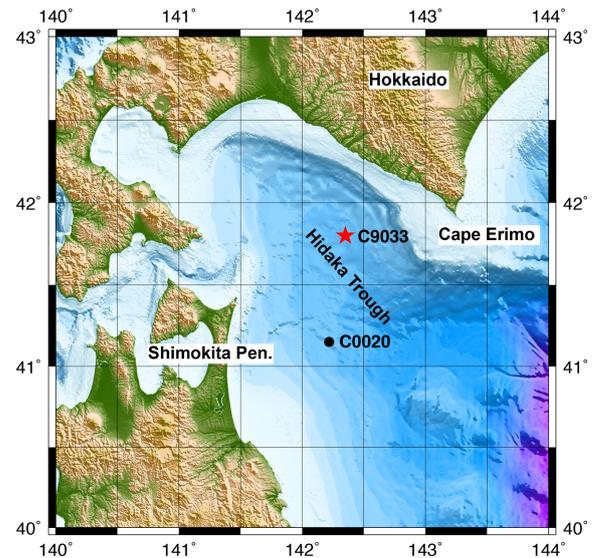

Figure 1. Site C9033 is located west off Cape Erimo, Hokkaido, northern Japan. The site is located 70 km north of IODP Site C0020.

dicate that a large-scale slump scar structure (escarpment) developed along the shelf break westward offshore from Cape Erimo and debris flow lobes and fragmented slump blocks widely distributed in the downstream part of the shelf break slump scar (Fig. 2).

The shallowest sedimentary sequence ($\sim 100\,\mathrm{m\,b.s.f.}$) at the drilling site primarily consists of a Quaternary sedimentary sequence including two MTDs (Upper and Middle MTDs in Fig. 3), overlying Pliocene units with a thicker MTD (Lower MTD in Fig. 3) (Takano, 2017; von Huene et al., 1980).

3 Coring operation and analytical flow

In the 4 d SCORE expedition, the *Chikyu* successfully drilled three holes using the hydraulic piston coring system (Table 1). Coring of the first hole, C9033A, reached 100 m b.s.f. and formation temperature measurements using the APCT-3 tool were attempted five times. Core samples from C9033A were used for shipboard sampling and analysis. Plug-sediment samples for cell count and headspace gas analysis were collected from the interiors of freshly cut section ends. After whole sections were scanned with X-CT and logged with MSCL-W, whole round core samples for geochemistry and microbiology were retrieved, and the remaining sections were split for shipboard observation and sampling. The other cored sections were used for physical property measurements such as moisture and density, thermal conductivity and penetration strength. Basic geochemistry of both interstitial water and gas components was obtained by the shipboard scientific party. For microbiology, whole round core samples were collected immediately after
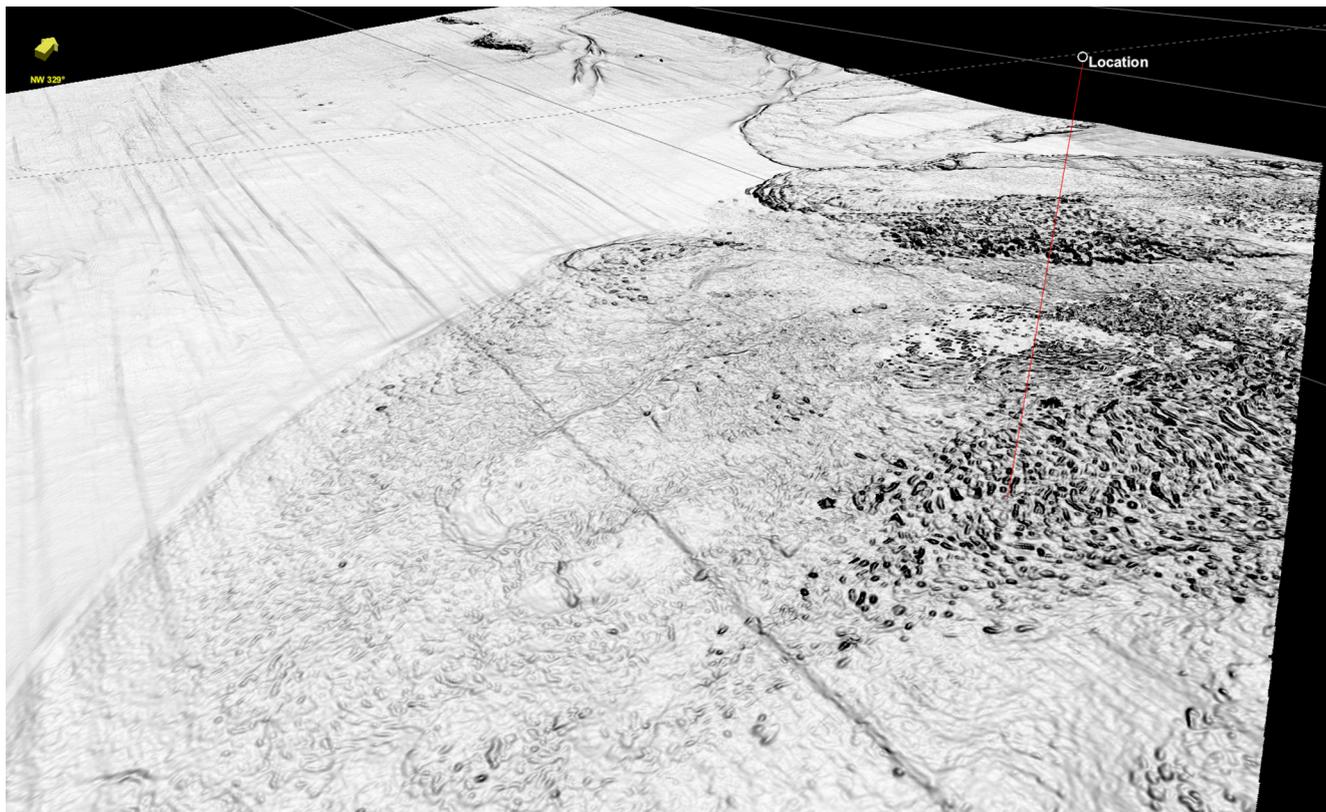
Figure 2. Three-dimensional view of topography around the drill site C9033. Rugged features indicate occurrences of mass transport. Seafloor topography detection from the seismic survey data (JOGMEC, 2013, 2014).
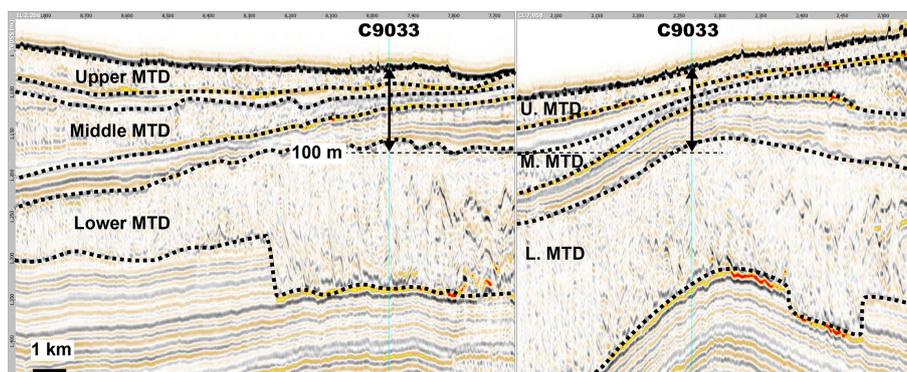

Figure 3. Seismic profiles at the drill site C9033 show transparent and/or chaotic intervals interpreted as mass transport deposits. There are three such intervals in the top 100 m. Seismic profiles are from JOGMEC (2013, 2014).

X-CT scan and processed for the appropriate storage prior to the post-cruise analysis (Morono and Inagaki, 2016).

Because of time constraints of the cruise, only one core from the seafloor was recovered from the second hole C9033B, and the entire core was used for high-frequency sampling of interstitial water after X-CT observation (Fig. 4). The sections were then packed under the anaerobic condition (filled with N_2 and vacuumed with oxygen-impermeable bags) for post-cruise use. From the third hole C9033C, 11 cores to 99.5 m b.s.f. were taken mainly for geological observations and post-cruise studies. The cores were split onboard after the non-destructive analysis and the split surface of archive halves was scanned for image archive. All sections were packed and stored at $+4\,°C$ for shipping to Kochi Core Center, where all split sections were observed and described for lithostratigraphic study.

Table 1. Hole and coring summary.

Hole	Latitude	Longitude	Water depth (m b.s.l.)	Cores (*N*)	Cored (m)	Recovered (m)	Recovery (%)
910-C0033A	41°48′08.33″ N	142°21′27.88″ E	1068.5	12	100.0	109.64	109.6
910-C0033B	41°48′08.33″ N	142°21′27.88″ E	1069.0	1	7.0	7.25	103.6
910-C0033C	41°48′08.33″ N	142°21′27.88″ E	1069.0	11	99.5	107.47	108.0
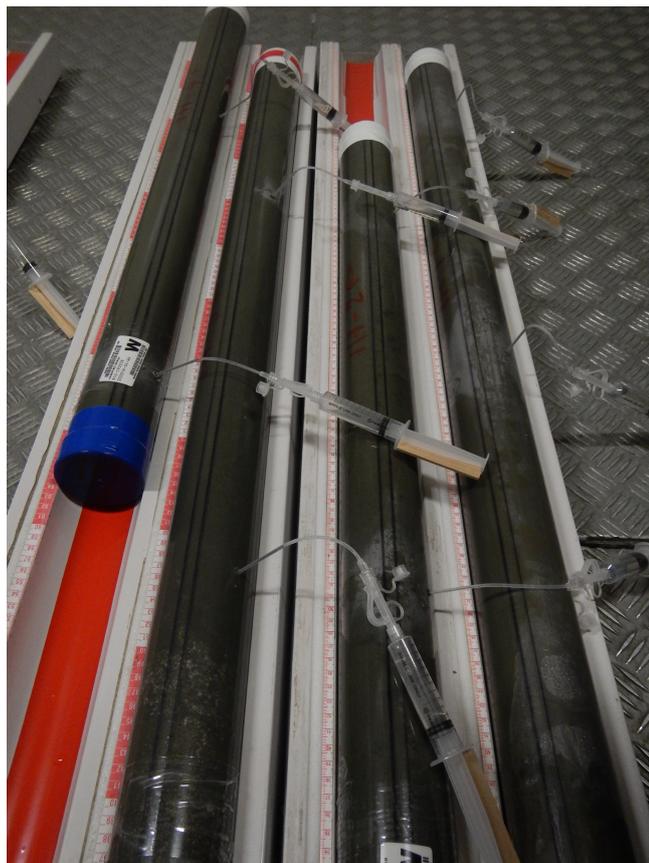
Figure 4. High-frequency pore water samples were taken from C9033B core sections using Rhizon samplers in a refrigerator room on the *Chikyu*.

4   Preliminary results and discussion

The main lithology of the cores was dark olive to olive grey silty clay with quartz, feldspar, volcanic glass, and microfossils of calcareous nannofossils, diatoms, and sponge spicules (Fig. 5). These massive sediments were unconsolidated, moderately bioturbated, and intercalated with sandy layers of various thicknesses commonly showing sharp bases and normal grading. There were several layers of volcaniclastic sediments, consisting of pumice, scoria, and volcanic glass (Fig. 5). The volcaniclastic sediments in the top 10 m b.s.f. contained coarse pumice, whereas tuffaceous sands were dominant in deeper intervals. Many of the volcaniclastic lay-

ers showed inverse grading, and some thick layers were comprised of multiple units, each of which showed inverse or normal grading.

Four chaotic deposits characterized by scattered mud clasts and lack of stratification were observed in both Holes A and C (Fig. 5). Neither normal nor inverse grading was observed in the chaotic deposits. Mud clasts were matrix supported without preferred orientation (Fig. 6). The mud clasts were predominantly silty clay, usually darker olive grey in colour than that of the surrounding matrix, with sub-rounded to sub-angular forms. The size of the clasts varied from centimetres to tens of centimetres in length.

The most distinct chaotic deposits were observed in 60.3–64.4 m b.s.f. in C9033A. A folded mud clast was found at C9033A-8H-10, 63 cm (Fig. 6). An overturned sand bed, which apparently showed a sharp top and inversed grading in appearance, was observed at C9033A-9H-4, 19 cm (Fig. 6). Another good example of chaotic bedding was found in 45.0–49.4 m b.s.f. in C9033C, which included a sandy turbidite at the top.

The chaotic deposits were accompanied by intervals showing inclined bedding. The bedding angle was mostly 5–15°, but was up to 40° in some cases. Except for the inclination, the lithologies of the inclined bedding were similar to those of other hemipelagic sediments with horizontal laminae.

The combinations of inclined bedding and chaotic deposits are often reported from MTDs in other present deepwater basins (e.g. Algar et al., 2011; Alves, 2015) and exposed ancient MTDs (Ogata et al., 2012; Sola et al., 2018). Assuming the combination of the two features indicates MTD, there were three MTD units in both Holes A and C (Fig. 5). The first unit (46.0–56.6 m b.s.f. in 9033A and 45.0–56.1 m b.s.f. in 9033C) is about 10 m thick and contains two chaotic deposits, which sandwich an interval of inclined bedding. The second unit (60.3–69.0 m b.s.f. in 9033A and 59.5–69.0 m b.s.f. in 9033C) consists of a chaotic deposit underlain by silty clay with inclined laminae. The third unit consists of the bottom 8 m, continuing deeper, in 9033A and has thin chaotic deposits and silty clay with inclined bedding. The upper boundary of this unit at Hole 9033C is unclear due to flow-in material during coring. In addition to these three MTD units, inclined bedding was observed without chaotic deposits at the top of all three holes.

The observed MTD units in the core can be clearly correlated with those observed in the seismic profile (Fig. 3). The
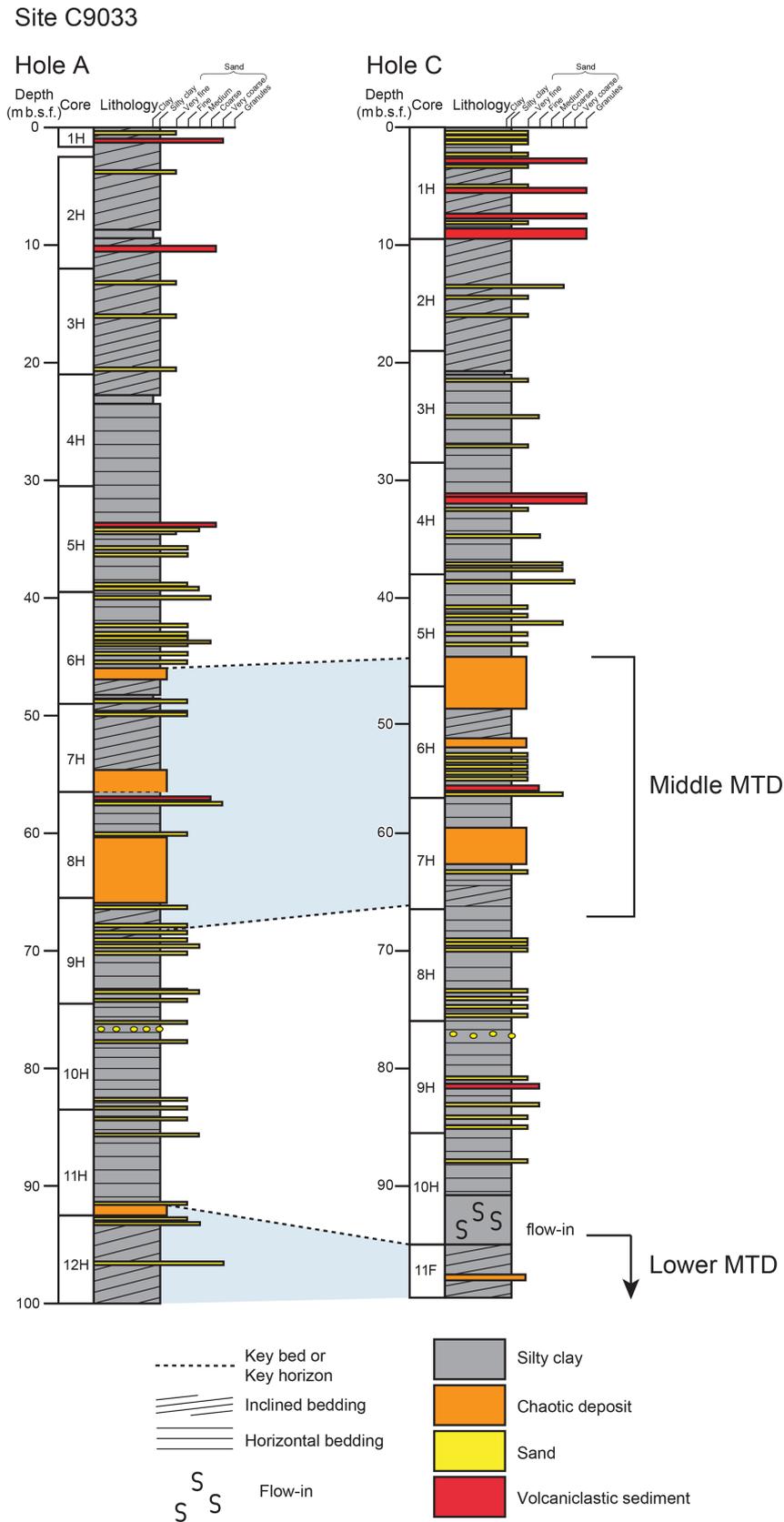
Figure 5. Lithologic column figure of Holes A and C at Site C9033. The combination of chaotic deposits and inclined beds is interpreted as mass transport deposits (MTDs). Two MTD units observed in the middle part of the core comprise "Middle MTD" in the seismic profile, whereas the MTD unit in the bottom corresponds to the top of "Lower MTD". Inclined bedding in the top 20 m may be another MTD layer.
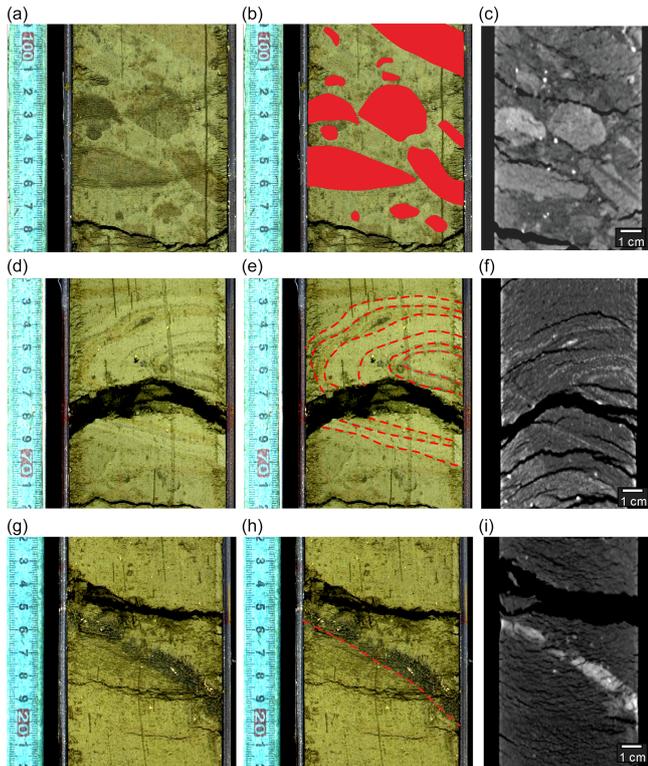
Figure 6. Three examples of deformation structures in the core samples: scattered mud clasts in C9033A-8H-6 **(a, b, c)**, folded block in C9033A-8H-10 **(d, e, f)** and overturned bed with a sharp top and inverse grading in C9033A-9H-4 **(g, h, i)**.

first and second MTD units comprise the Middle MTD in the seismic profile, whereas the third MTD corresponds to the top of the Lower MTD. Similarly, the inclined bedding at the top of all three holes may correspond to the Upper MTD of the seismic profile.

5 Summary

Chikyu's new coring programme, SCORE, provides the ocean drilling community with the opportunity to conduct short-term drilling expeditions for the drilling of shallow holes of up to 100 m b.s.f. With the simplified proposal review process, SCORE will open up possibilities for new research in a timely manner. The first SCORE expedition drilled three holes down to a maximum depth of 100 m b.s.f. off Cape Erimo, northern Japan. The sediment cores showed features such as chaotic deposits and inclined bedding, indicating repeated occurrences of mass transport deposits in the foreland basin. Post-cruise shore-based analyses will investigate the impact of such geological events on the local subseafloor microbial biosphere.

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Scientific Drilling
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High-resolution late Holocene sedimentary cores record the long history of the city of Cádiz (south-western Spain)

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Abstract. Today, coastal cities worldwide are facing major changes resulting from climate change and anthropogenic forcing, which requires adaptation and mitigation strategies to be established. In this context, sedimentological archives in many Mediterranean cities record a multi-millennial history of environmental dynamics and human adaptation, revealing a long-lasting resilience. Founded by the Phoenicians around 3000 years ago, Cádiz (south-western Spain) is a key example of a coastal resilient city. This urban centre is considered to be one of the

first cities of western Europe and has experienced major natural hazards during its long history, such as coastal erosion, storms, and also tsunamis (like the one in 1755 CE following the destructive Lisbon earthquake).

In the framework of an international, joint archaeological and geoarchaeological project, three cores have been drilled in a marine palaeochannel that ran through the ancient city of Cádiz. These cores reveal a $\geq 50\,\mathrm{m}$ thick Holocene sedimentary sequence. Importantly, most of the deposits date from the 1st millennium BCE to the 1st millennium CE. This exceptional sedimentary archive will allow our scientific team to achieve its research goals, which are (1) to reconstruct the palaeogeographical evolution of this specific coastal area; (2) to trace the intensity of activities of the city of Cádiz based on archaeological data, as well as geochemical and palaeoecological indicators; and (3) to identify and date high-energy event deposits such as storms and tsunamis.

1 Introduction

Focusing on the city of Cádiz (Figs. 1, 2, 3), this starting international, geoarchaeological project offers outstanding research perspectives for two main reasons. To begin with, Cádiz is considered to be one of the first cities of western Europe and has remained a maritime crossroads since its creation (Botto, 2014). Given its prominent position, directly northward of the Strait of Gibraltar, Cádiz has been a first-order port since its founding (Bernal Casasola, 2012). When Phoenicians originally settled, Cádiz was on the tin road between the Mediterranean and Atlantic coasts (Bendala Galán, 1988; Domínguez Monedero, 2012). Afterwards, its history was closely linked to the main Mediterranean societies developing along its shores, experiencing successive Punic (Ramírez Delgado, 1982) and Roman domination (Bernal Casasola, 2008; Lara Medina, 2016; Bernal Casasola et al., 2019a). During late antiquity, it was conquered by the Visigoths, and later by Moors in the 8th century (Fresnadillo García et al., 2008). Finally, Christians took over the city in the 13th century during the Reconquista. Whilst the city port of Cádiz was of secondary importance during the Medieval period, it regained a major role during the modern period, coinciding with the discovery of the Americas and the development of transatlantic trade (O'Flanagan, 2016).

Secondly, Cádiz was founded in a specific geographic situation off the mainland of Spain (Carayon, 2011) and provides Late Holocene high-resolution sedimentary archives located in the heart of the city. Based on topographical observations and descriptions by ancient authors, an E–W-oriented palaeochannel running through Cádiz was first identified by Ponce Cordones (1985). The topography of Cádiz reveals lowlands across the city (Fig. 2), and ancient texts mention a channel in the city (Strabo, 1923, 3, 5, 3; Pliny, 1942, 4, 119–120) (Corzo Sánchez, 1980). Two decades later, complementary geoarchaeological studies gathered geotechnical reports, and new sedimentary cores reaching a maximum depth of 9 m were drilled (Arteaga et al., 2001). The bedrock contact was identified at several places along the lateral borders of this palaeochannel, but this information remains unknown in its central part (Arteaga et al., 2001). More recently, it was demonstrated that this east–west palaeochannel was void

of any antique archaeological remains (Lara Medina, 2018). Other hypotheses of palaeochannels running through the spit of Cádiz were also proposed, but no clear evidence is available yet (Márquez Carmona and Alonso Villalobos, 2016).

In 2018, the University of Cádiz started to rehabilitate the Valcárcel building located above the Bahía-Caleta palaeochannel. In this context, an interdisciplinary project was initiated: archaeological excavations were conducted alongside drilling of three sedimentary cores from the same area. The excavations demonstrate that La Caleta beach was a built area only from the 18th century CE (Bernal Casasola et al., 2018) and confirm the absence of structures from antiquity within the limits of the palaeochannel defined by Lara Medina (2018) (Fig. 3). The cores reveal a significant depth of at least 50 m along the northern side of the marine palaeochannel (Val-18/Core 3) (Fig. 4). The three cores yielded a high density of artefacts, dated from the beginning of the 1st millennium BCE to the Roman period, at depths between 15 and 40 m below sea level (b.s.l.) (Bernal-Casasola et al., 2020b). These archaeological findings associated with silty-clay and sand deposits highlight the existence of a deep anchorage (Bernal-Casasola et al., 2020a).

From a geological and geomorphological perspective, the palaeochannel filled by these thick sedimentary sequences points to the existence of a deep gorge in the past, probably of Pleistocene age. From a geoarchaeological perspective, the marine deposits filling this palaeochannel represent high-resolution sedimentary archives to study the evolution of the local palaeogeography and trace the urban activities of the city. Initial geochronological results via radiocarbon and thermoluminescence (TL) dating attest that at least the upper 40 m b.s.l. of the sequence date from the 1st millennium BCE and later. Whilst these sedimentary sequences represent a unique opportunity to undertake high-resolution palaeoenvironmental analyses, the associated high sedimentation rates simultaneously challenge future geomorphological reconstructions. Finally, given its location off the mainland of Spain and within a tectonically active area, storms and tsunamis could have struck the city, like in 1755 CE right after the destructive Lisbon earthquake. Sedimentological and palaeoenvironmental evidence of high-energy events could be documented in this palaeochannel.
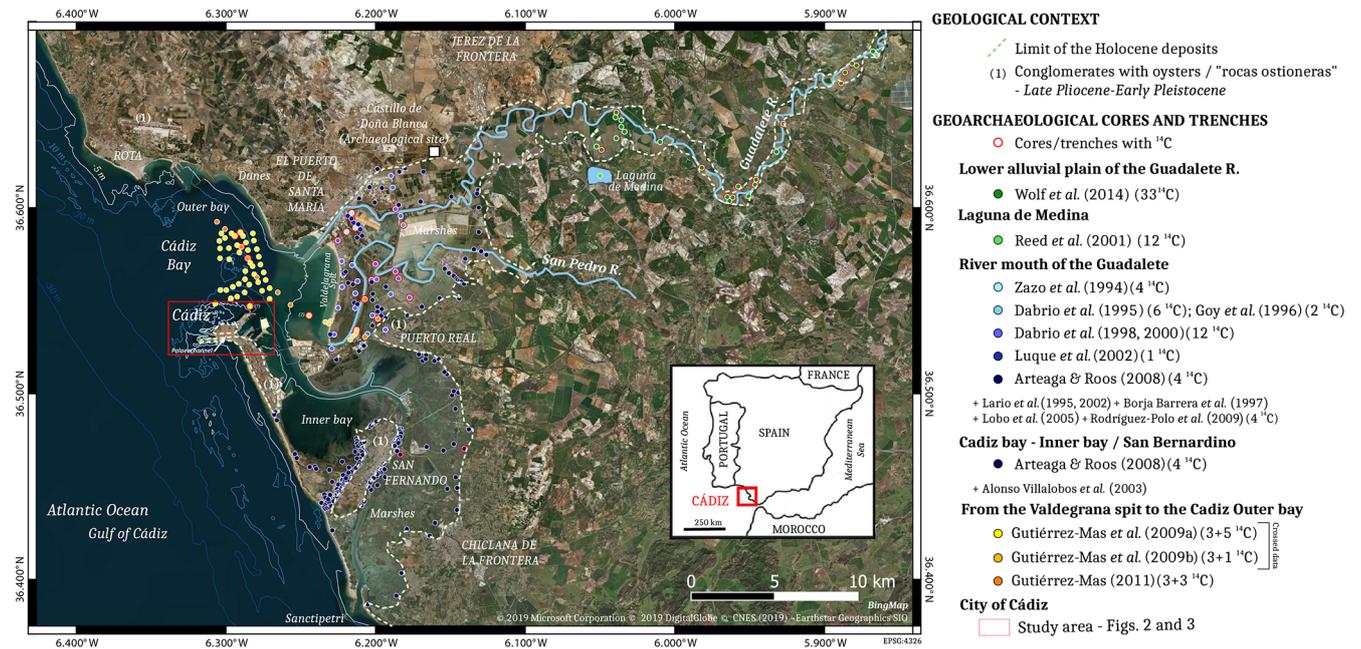
Figure 1. Location of the study area and overview of the geological, geomorphological, and geoarchaeological researches focusing on the lower part of the Guadalete River to the Bay of Cádiz. It includes core and trench locations and radiocarbon dates available.
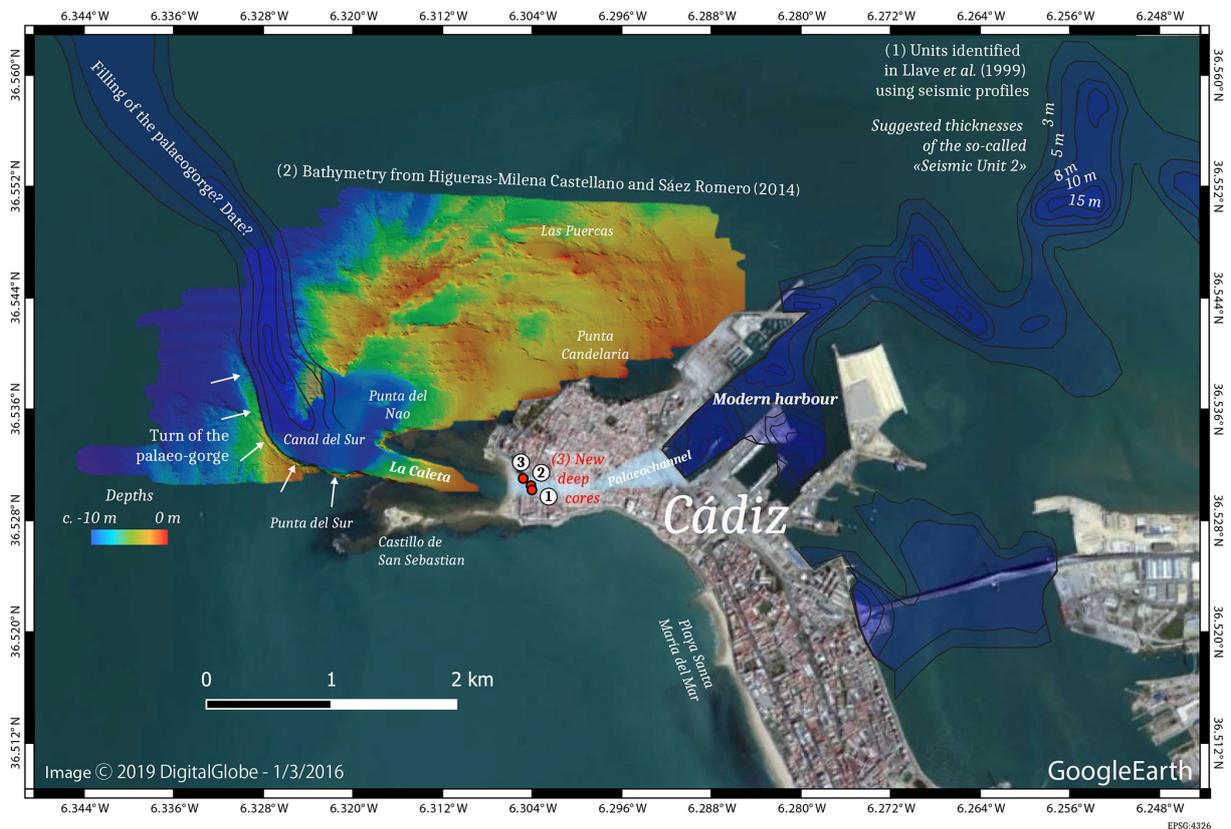

Figure 2. Palaeogorge of Cádiz – evidence from (1) seismic profiles possibly recording the filling of the palaeogorge of Cádiz (Llave et al., 1999); (2) bathymetric data showing a turn of the palaeogorge west of Cádiz (Higueras-Milena Castellano and Sáez Romero, 2014); and (3) new deep cores in Cádiz (this paper).

Figure 3. Locations of the three new deep cores in the context of the studied area. Bathymetric data (http://www.gpsnauticalcharts.com, last access: 11 February 2020), topographical data (collected in 1911; Ramírez Delgado, 1982), and previous cores (Arteaga et al., 2001) are reported in this figure.

This project aims to understand how this city faced coastal hazards and adapted to environmental change across its history, especially during the 1st millennium BCE to the 1st millennium CE. This includes how the landscape of Cádiz appeared at the time of its foundation and how it evolved through time.

2   Geological and geomorphological context of the Bay of Cádiz

2.1   Geodynamic background

The Bay of Cádiz and its neighbouring coastal areas are affected by active Quaternary tectonics and seismicity. This is materialised by an intricate network of SW–NE and SSE–NNW-striking normal faults, sometimes prolonged by strike–slip faults (e.g. underneath the present extension of the city; Gracia et al., 2008). The low to moderate historical seismicity in this area, i.e. magnitudes between 2.5 and 4 (Gracia et al., 2008), is apparently related to the Azores–Gibraltar transform fault zone and, more specifically, to N–S compressive motion in the Gibraltar area (Buforn et al.,

1988; Luque et al., 2002). In the latter, a mean uplift rate of 0.1 to 0.15 mm yr^{-1} is suggested over the Late Pleistocene–Holocene (Zazo et al., 1999). Given this geodynamic context, the whole Bay of Cádiz and coastal areas were regularly impacted by high-energy events. Offshore sediments in the bay (Gutiérrez-Mas et al., 2009a), the Valdelagrana spit bar (Luque et al., 2002), and the marshlands of the Guadalete River bay (Gutiérrez Mas, 2011) bear witness to historical tsunamis (Fig. 1).

2.2   Coastal areas around the Bay of Cádiz

According to the 1 : 50 000 geological map (Baena Pérez et al., 1984), two main types of rocks or deposits constitute the coastal areas of the Bay of Cádiz. The first one, a rock formation locally known as "*roca ostionera*", is composed of well-compacted conglomerates and calcareous sandstones enriched in oyster shells assigned to the Late Pliocene–Pleistocene (Llave et al., 1999; Vázquez et al., 2000) (Fig. 1). These rocks exhibit a folded structure (MacPherson, 1873; Gutiérrez Mas et al., 1991), referred to as the Cádiz anticline,
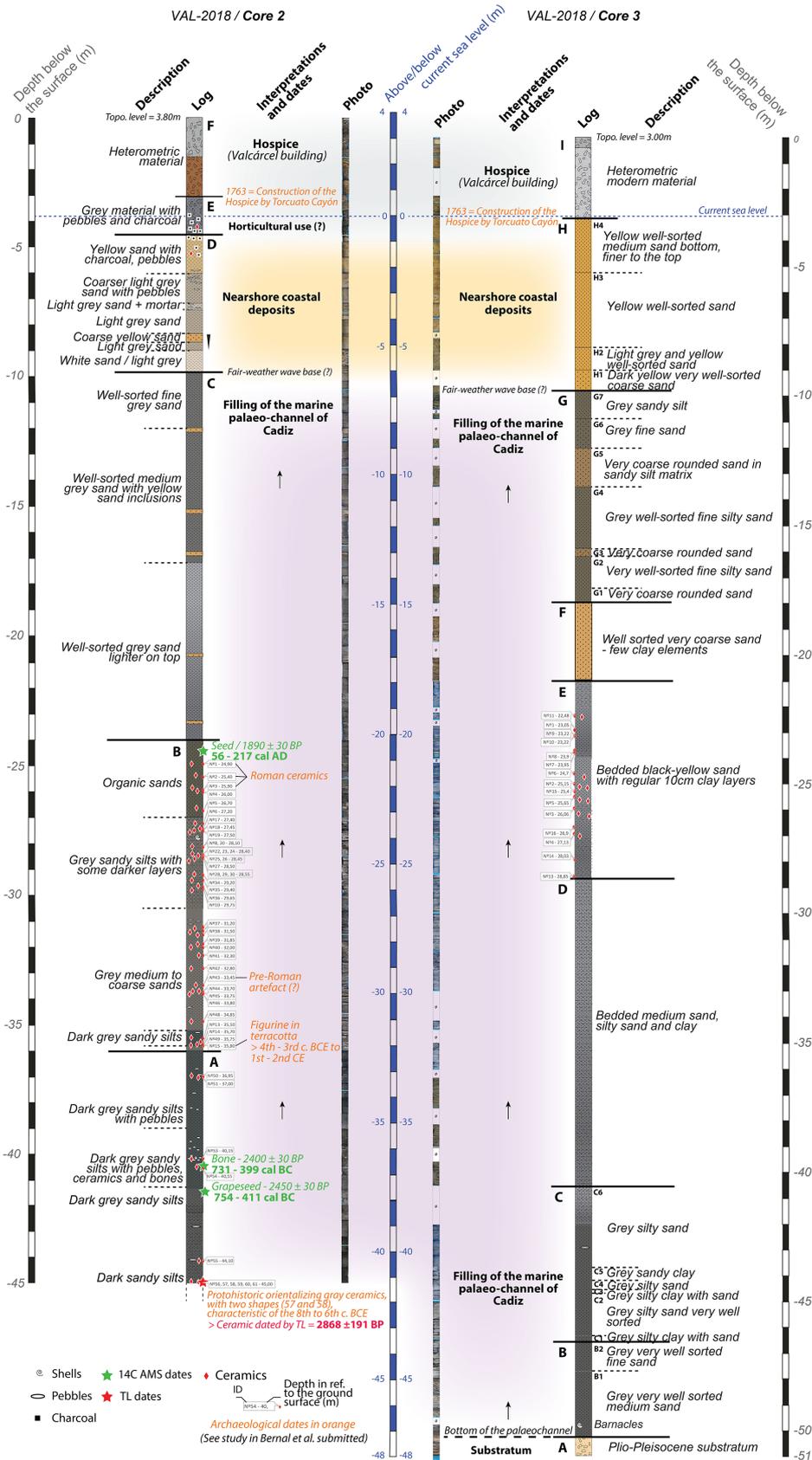
Figure 4. Stratigraphic sequences of VAL-18/Cores 1, 2, and 3 with preliminary dates (radiocarbon dates, thermoluminescence dates, and archaeological dates available from the cores under investigation – see also Bernal-Casasola et al., 2020b).

and form the bulk of the Cádiz peninsula upon which the city lies at the northernmost tip (Domínguez-Bella, 2008).

The second types of deposits encompass Holocene sediments (Luque et al., 2002; Moral Cardona, 1994; Moral Cardona et al., 1996; Gutiérrez Mas et al., 1996) (Fig. 1). To the south, estuarine, intertidal silts and clays (marshlands or *slikke*) occur around the city of San Fernando (Arteaga and Roos, 2008). They are limited to the west by a sandy coastal spit. To the north, the mesotidal, N–S-oriented Valdelagrana spit-barrier system exhibits sandy beaches and beach ridges, this structure having been extensively studied over the last 3 decades (Zazo et al., 1994; Dabrio et al., 1995, 1998, 2000; Lario et al., 1995; Goy et al., 1996; Borja Barrera et al., 1997; Luque et al., 2002; Lobo et al., 2005; Arteaga and Roos, 2008; Rodríguez-Polo et al., 2009; Gutiérrez-Mas et al., 2009a, b; Gutierrez-Mas, 2011). It encloses Holocene intertidal clays and silts to the east. The lower part of the San Pedro River most probably corresponds to a palaeocourse of the Guadalete River (Dabrio et al., 1998). Finally, fringing the Cádiz peninsula, Holocene bioclastic sandy sediments delimit the "Bahía-Caleta" palaeochannel to the north and the south (Fig. 3).

2.3 Geoarchaeological and geomorphological studies of the Guadalete River

The paragraphs below synthesise the research conducted on the Guadalete River and towards the Bay of Cádiz since the 1990s.

Fluvial dynamics in the lower reach of the Guadalete River

Recent research mostly focused on the reconstruction of the floodplain evolution in this lower reach (Wolf et al., 2014; Wolf and Faust, 2015). In relation to the post-LGM sea-level rise, terrace formation is reported during the Late Pleistocene, with contrasting morphologies along the valley reach. The prominent sub-continuous landform upstream is buried below the Holocene floodplain deposits downstream. Holocene floodplain aggradation is recorded for the last 10 000 years and enhanced phases of fluvial sedimentation are identified around 6050, 4150, 2650, and 250 BCE and 1050 CE (Wolf et al., 2014). The strongest sedimentation episode occurred around 1550 CE during the Little Ice Age. Stability phases are suggested during the Bølling–Allerød interstadials, prior to 6050, 4150, and 3150 BCE and after 2350 BCE and 50 CE (Wolf et al., 2014). High-resolution analysis of lake sediments from the neighbouring Laguna de Medina reveals an aridification during the Late Holocene (Reed et al., 2001). Whilst local palaeoenvironmental studies suggest important wine production already existing in the 8th–7th centuries BCE at Castillo de Doña Blanca (Chamorro, 1994; Buxó, 2008; Iriarte-Chiapusso et

al., 2017), a generalised human impact seems more difficult to infer along this river reach (Wolf et al., 2014).

Evolution of the Guadalete River mouth

Concomitant to a decelerated sea-level rise, progradation at the mouth of the Guadalete River began in the middle of the 5th millennium BCE. Goy et al. (1996) identified two periods of progradation: between 4500 and 1050 BCE with a gap around 2050 BCE and from 800 BCE to the present with a gap between 750 and 900 CE. The formation of the Valdelagrana spit likely started in the 2nd millennium BCE (1850–1650 BCE) based on archaeological dates (in Gómez Ponce et al., 1997) and radiocarbon dates (in Dabrio et al., 2000), accelerated around 450 BCE (radiocarbon dates in Zazo et al., 1996), and reduced during 50–150 CE (based on archaeological and radiocarbon dates in Gómez Ponce et al., 1997). High sedimentation recorded during the Little Ice Age (Dabrio et al., 2000; Lario et al., 1995) was related to strong human impact as well as land-use changes (Dabrio et al., 2000). The formation of the Valdelagrana spit is likely to have affected the sedimentation of the delta front and to have contributed to the sedimentation in the palaeochannel of Cádiz (Gracia and Benavente, 2000).

3 Methodology

Three new sedimentary sequences were extracted from the area of the Valcárcel building in Cádiz: Val-18/Core 1 (36°31′48.73″ N,   6°18′14.45″ W;   3.78 m a.s.l.),   Val-18/Core 2 (36°31′49.85″ N, 6°18′14.75″ W; 3.80 m a.s.l.), and   Val-18/Core 3   (36°31′51.70″ N,   6°18′17.27″ W; 3.00 m a.s.l.) (Figs. 2, 3, and 4).

These cores were retrieved by the Concadiz company using a rotary drilling device between August and October 2018. The cores have a diameter of 10 cm. Val-18/Core 1 was drilled down to 31.50 m, Val-18/Core 2 to 45.00 m, and Val-18/Core 3 to 50 m. Only Val-18/Core 3 reached the substratum. Val-18/Core 1 is not described in this paper since the stratigraphy is shorter and similar to Val-18/Core 2. These drillings are due to a great opportunity. It is generally very difficult to drill in a dense urban area. However, due to the renovation of the Valcárcel building by the University of Cádiz, an archaeological assessment was undertaken (Lara Medina et al., 2020). The sedimentary cores were drilled on that occasion.

Visual recognition of sedimentary units and core description was completed quickly after extraction and followed by sampling. The first samples extracted were reserved for optically stimulated luminescence (OSL) datings. OSL samples were chosen from the best preserved sections within the stratigraphic sequence. Archaeological material was extracted during the sampling and analysed by specialists (see Bernal-Casasola et al., 2020a, b). The analysis of the archaeological artefacts presented a preliminary chronologi-

cal framework. The absolute datings were implemented on Val-18/Core 2. Radiocarbon dates were performed by Beta Analytics on seeds and bones, and a ceramic was dated by the thermoluminescence technique by the Dating and Radiochemistry Laboratory of the Universidad Autónoma of Madrid (Table 1).

4 New sedimentary sequences from the city centre of Cádiz

Figure 4 displays the combined stratigraphy of VAL-18/Core 2 and VAL-18/Core 3, respectively drilled in the central and northern parts of the palaeochannel. Because VAL-18/Core 2 exhibits the highest concentration of artefacts, it was first targeted for numerical dating (radiocarbon and TL). VAL-18/Core 3 reached the Plio-Pleistocene substratum. Detailed analysis of the artefacts is available in Bernal-Casasola et al. (2020a, b).

4.1 Val-18/Core-2

This core reaches a depth of 41.20 m b.s.l. The bottom Unit A is at least 9 m thick (41.20 to 32.20 m b.s.l.) since the bedrock was not attained. It is composed of dark grey sandy silts with some pebbles. A fragment of ceramic found at the bottom of the unit is dated by TL at 850 ± 191 BCE (Table 1). Additionally, radiocarbon dates of a grape seed (41.57 m b.s.l.) and a bone fragment (40.85 m b.s.l.) respectively yield similar ages of 755–410 BCE (2450 ± 30 BP) and 730–400 BCE (2400 ± 30 BP) (Table 1). The consistent dating results indicate that Unit A was deposited in the first part of the 1st millennium BCE.

Unit B (32.20 to 20.20 m b.s.l.) is composed of grey sandy silts and coarse sands. Additionally, several silty-clay layers are intercalated at depths between 23.00 and 20.20 m b.s.l. This unit is characterised by a high content of artefacts and ecofacts. At the bottom of the unit (32 m b.s.l.), a figurine in terracotta was dated to the 4th–3rd centuries BCE (artefact 15 in Bernal-Casasola et al., 2020a). Between 20.20 and 23.00 m, several ceramics are identified and date to the Roman period (artefacts 2, 3, and 5 in Bernal-Casasola et al., 2020a). This chrono-typological date is confirmed by a radiocarbon-dated seed at 55–215 CE (1890 ± 30 BP) in the upper part of the unit (20.48 m b.s.l.; Table 1). At 24.00 and 25.15 m b.s.l., a murex *Bolinus brandaris* and a murex *Hexaplex trunculus* are respectively found.

Unit C (20.20 to 6.05 m b.s.l.) is characterised by a noticeable sedimentation change, i.e. mostly very well-sorted lighter grey sands, together with the absence of artefacts. The first yellow sand layers are observed in this unit too. The sedimentation turns into beige–yellow sands in the upper sequence of VAL-18/Core 2 in Unit D (6.05 to 0.70 m b.s.l.). Past hydrodynamic conditions seem to have quickly changed within this unit made of medium- to coarse-sand deposits containing some pebbles. Ceramic fragments are found again

Table 1. Radiocarbon and thermoluminescence dates.

Core	Sample for radiocarbon date	Depth below surface (m)	Lab. code	Depth below sea level (m)	Dated material	^{14}C yr BP	±	Age calibrated BCE–CE (Reimer et al., 2013) – 2σ	Age calibrated cal. BP (Reimer et al., 2013) – 2σ
VAL-18/Core 2 +3.80 m	VAL-18/Core 2/24.28 m	24.28	Beta – 509383	−20.48	Seed	1890	30	55 to 215 CE	1895 to 1735 cal. BP
VAL-18/Core 2 (+3.80 m)	VAL-18/Core 2/40.44 m	40.44	Beta – 509385	−36.64	Bone	2400	30	730 to 400 BCE	2680 to 2350 cal. BP
VAL-18/Core 2 (+3.80 m)	VAL-18/Core 2/41.57 m	41.87	Beta – 509384 VAL-18	−38.07	Grape seeds	2450	30	755 to 410 BCE	2360 to 2705 cal. BP

Core	Sample for thermoluminescence date	Depth below surface (m)	Lab. code	Depth below sea level (m)	Equiv. dose	Plateau (°C)	Factor K	Archaeological dose (Gy)	Annual dose (mGy yr^{-1})	Age
VAL-18/Core 2 (+3.80 m)	TL1 (no. de inventario 57)	45.00	MADN6650 bb.binx	−41.20	4.13 ± 0.25	270–350	0.07	4.13	1.44	2868 ± 191 BP 850 ± 191 BCE

in the upper part of the unit (not identifiable) along with charcoals.

Unit E (0.70 m b.s.l. to 0.80 m a.s.l), mostly composed of sands embedding pebbles and charcoals, corresponds to a transitional environment between coastal deposits (Unit D) and the uppermost archaeological layers (Unit F). Unit F is composed of heterometric material of anthropic origin. These deposits were excavated and studied using archaeological trenches (Lara Medina et al., 2020).

4.2 Val-18/Core-3

This core reaches a depth of 48.00 m b.s.l. No dates are available for this core yet. The bottom Unit A (48.00 to 47.30 m b.s.l.) corresponds to the Plio-Pleistocene conglomerates, upon which the city of Cádiz presently lies.

The first unconsolidated deposit appears in Unit B (47.30 to 43.50 m b.s.l.). It is composed of very well-sorted, dark grey medium to fine sands (marine environment). Very well-preserved barnacles are observed at 46.40 m b.s.l. Barnacles are suspension feeders and mostly inhabit the intertidal zone fixed on the substrate.

Unit C (43.50 to 37.50 m b.s.l.) is subdivided into six sub-units (C1 to C6). Sub-unit C1 suggests a hydrodynamic change with grey silty-clay deposits. Other sub-units express regular alternations from very well-sorted grey sands (C2, C4, and C6) to grey silty clay (C3 and C5). Some small pebbles are observed in C6 too.

Unit D (37.50 to 26.00 m b.s.l.) is composed of bedded silty clay, sands, and silty sands. Although technical difficulties experienced during the drilling impede a clear identification of the bedding limits, ongoing analyses will hopefully provide a better picture of the shift in the energy controlling the deposition.

Unit E (26.00 to 18.00 m b.s.l.) is composed of bedded black–grey sands with regularly intercalated 10 cm thick clay layers. This sedimentation change is coeval with the presence of ceramics, which are not observed in Units B to D.

A sharp limit is observed between Units E and F at 18.00 m b.s.l. Unit F (18.00 to 15.00 m b.s.l.) is composed of yellow very well-sorted coarse sands (> 1 mm) and small gravels (> 2 mm). Few clayey elements are scarcely distributed and no ceramics are observed in this unit. This coarse deposit can be related to a quick change in the hydrodynamic conditions.

Unit G (15.00 and 6.95 m b.s.l.) is subdivided into seven sub-units (G1 to G7), which are mostly characterised by alternations between grey sandy silts and silty sands. The coarsest, mostly yellow deposits of G3 and G5 are very well rounded and are similar to the sedimentary facies in Unit F. Unit H (6.95 to 0.10 m b.s.l.) is subdivided into four sub-units (H1 to H4) and is composed of well-sorted yellow sands. H1 is characterised by the coarsest sediments at the bottom, while other subunits show medium to fine sands at the top.

Unit I (0.10 m b.s.l. and 3.00 m a.s.l.) is composed of heterometric material belonging to the archaeological layers of Cádiz. Excavations on modern archaeological materials located in the Valcárcel building were conducted by Bernal Casasola et al. (2018).

5 The Bahía-Caleta palaeochannel in Cádiz: a deep palaeogorge?

Based on the new cores, a maximum depth of the Bahía-Caleta palaeochannel around 47 m below the current sea level was recorded (i.e. the depth at which the contact with the Pliocene–Pleistocene deposits was reached in Val-18/Core-3; see Sect. 4.2). Unfortunately, these data cannot be extrapolated to the whole palaeochannel. A cross section in the palaeochannel of Cádiz would be necessary to infer the maximum bathymetry above the Plio-Pleistocene conglomerate. However, a sharp height difference in the underwater topography of the conglomerate is observed. The substratum is located close to the subaerial surface below the shallow archaeological deposits northwards of Val-18/Core-3, and it drops down to 47 m b.s.l. in the palaeochannel where Val-18/Core-3 was drilled. Also, based on the ca. 1 km long offshore palaeochannel visible to the west of the coring (Fig. 2), its width is estimated between 150 and 250 m. Considering these morphological features (i.e. large height difference combined with the channel width), we suggest that the palaeochannel of Cádiz may represent a palaeogorge. Interestingly, very well-sorted marine sands are deposited directly over the Plio-Pleistocene substratum in VAL-18/Core3 (Unit B). No fluvial deposits are observed. A marine origin of these sediments is confirmed by the occurrence of well-preserved barnacles (currently processed for a ^{14}C dating) at 46.40 m b.s.l. If the fluvial origin of the palaeogorge can hardly be questioned, the presence of fluvial deposits in other areas of the palaeogorge may be expected.

This palaeogorge can be tracked to the west using high-resolution bathymetry proposed by Higueras-Milena Castellano and Sáez Romero (2014) and reported in Fig. 2. This topographical survey clearly shows that the palaeochannel was flowing northwards after passing through the area of Cádiz. This is confirmed by a geophysical survey, where geopulse seismic data exhibit an identical offshore bathymetry westwards of our corings (northward orientation of the palaeogorge, Fig. 2; Llave et al., 1999). As for its origin, two main hypotheses were suggested. First, the currently underwater gorge might be related to a palaeocourse of the Guadalete River (Dabrio et al., 2000; Wolf et al., 2014) or secondly to the Guadalquivir River (Chic García, 1979; Gracia and Benavente, 2000; Arteaga Matute and Roos, 2002). As for its age, it has been suggested that given the different palaeogeography of the Bay of Cádiz during the marine lowstand associated with the LGM, generalised fluvial incision might have occurred at that time in this area (Hernández

Molina et al., 1996). Future results obtained in the framework of this project will provide new insights into these unanswered questions.

6 Late Holocene coastal dynamics and preliminary results

6.1 A fast coastal sedimentation rate inferred from VAL-18/Cores 2 and 3

The dated VAL-18/Core 2 reveals an important sedimentation rate over more than 20 m. Over the entirety of Units A and B (41.2 to 20.2 m b.s.l.), the sedimentation rate is estimated between 1.6 and 2.8 cm yr^{-1}. The time range of this sedimentation starts at least from the beginning of the 1st millennium BCE and ends after the beginning of the 1st millennium CE. This is concomitant with the Phoenician origins of the city and includes the development of Cádiz during the Roman period, when Cádiz was one of the major ports of the western Mediterranean.

The interpretation of the high sedimentation rate, possibly lasting over 1 millennium, will be a key question to future research. It probably results from a combination of local factors, related to the waste of the ancient city of Cádiz, and regional factors, related to the progradation of the river mouth of the Guadalete, or else to sediment trapped during tsunami/storm events. Additionally, these local and regional considerations will take into account possible anthropic impacts within the Guadalete River watershed as well as global palaeoclimatic drivers.

Some authors have already argued that waste from the city could have contributed to the filling of the palaeochannel (Ramírez Delgado, 1982). The high content of archaeological material (e.g. Unit B of VAL-18/Core 2) points to this hypothesis, but this must be completed by complementary and systematic analyses. The > 45 m thick sedimentary archives of the 3 millennium long history of Cádiz are also to be compared with the 7 m thick sequence related to the history of Cádiz studied in the "Testaccio haliéutico de Gades" (Bernal Casasola et al., 2019a). This garbage mount was recently excavated close to the cores drilled in the Bahía-Caleta palaeochannel (Bernal Casasola et al., 2019a). Debris could have been transported into the palaeochannel either naturally or anthropogenically.

6.2 High-energy events from the sea: palaeostorms and palaeotsunamis

The palaeochannel of Cádiz corresponds to an offshore sedimentation trap for high-energy events such as palaeostorms and palaeotsunamis. Yellow coarse sand layers observed in the upper sequences of Cores 2 and 3 will be thoroughly investigated and dated. The difference between yellow–beige deposits in the upper 10 m (Unit D in VAL-18/Core 2 and Unit H in VAL-18/Core 3) and the dark grey deposits below

this limit is most probably due to local factors. This limit at ca. 10 m b.s.l. is probably the fair-weather wave base. However, the coarse-grained yellow–beige layers observed below 10 m in VAL-18/Core 2/Unit C and VAL-18/Core 3/Unit G and F are most probably related to regional events, possibly storms or tsunamis.

These high-energy events will contribute to the reconstruction of the history of the coastal hazards of this littoral area (Lario et al., 2010, 2011; Ruiz et al., 2013). Whilst the spit of Cádiz and the palaeo-islands are exposed to storms (Benavente et al., 2006; Del Río et al., 2012), palaeotsunami occurrences are evidenced in various depositional settings in and around the Bay of Cádiz. This includes lithofacies recording high-energy events in offshore sediments (Gutiérrez-Mas et al., 2009a), washover fans on the Valdelagrana spit (Luque et al., 2002), and coarse-grained shelly layers intercalated in the marshlands of the Guadalete/San Pedro River (Dabrio et al., 1997; Luque et al., 2002; de Duque, 2008; Gutiérrez-Mas, 1992, 2011; Gutiérrez-Mas et al., 2009b). The Lisbon tsunami of 1755 CE was the focus of much research in this context (Dabrio et al., 1997; Cuven et al., 2013; Font et al., 2013).

7 Future research perspectives

The palaeogeographical considerations listed above will be considered in regard to new analyses in process (e.g. sedimentological data, bioindicator identification, and absolute dating). Three main research topics will be explored.

7.1 Palaeogeography

Palaeogeographical and palaeotopographical reconstructions will be proposed for the infill of the palaeogorge in relation to regional palaeoclimatic data, sea-level evolution, and sediment input from the watershed. Ultimately, we will discuss the interconnection of the Cádiz archipelago during the Phoenician–Punic and Roman periods. A more robust chronology of the stratigraphic sequences will be proposed with additional OSL and radiocarbon dates, along with palaeoenvironmental analysis (e.g. grain size, mineralogy, organic and inorganic geochemistry, ostracod, foraminifera, and pollen analysis).

7.2 Urban geoarchaeology

The impact of the city through time will also be explored based on the analysis of the high-resolution sedimentary sequences. Based on new dates, geochemical analyses (e.g. ICP-MS and GC-MS analyses, especially looking at lead pollution), pollen analyses, plant macroremains, and charcoals, our research group will characterise the origins of the settlement, the resilience of the city against coastal hazards, the evolution of the urban impacts, the possible productions

in the city, the evolution of the vegetal landscape, and the palaeopollution records.

7.3　Palaeoeconomy

Finally, new information related to the palaeoeconomy of the city of Cádiz will be proposed (e.g. plant macroremains, charcoals, pollens, malacofauna, organic and inorganic geochemistry). Notably, we will look at the introduction of some cultivated plants, the development of vineyards and olive trees, or *Murex* exploitation during the Phoenician–Punic and Roman periods. A first date obtained from radiocarbon dating of a grape seed in VAL-18/Core3 (755–410 BCE) may confirm observations made in Spain (Buxó, 2008; Pérez-Jordà et al., 2017) and locally in the Bay of Cádiz at Castillo de Doña Blanca (Chamorro, 1994). Important wine production is confirmed in the 8th–7th centuries BCE in this archaeological site (Chamorro, 1994). Complementary and systematic analyses will shed new light on commercial activity in Cádiz.

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Late Miocene wood recovered in Bengal–Nicobar submarine fan sediments by IODP Expedition 362

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Abstract. Drilling and coring during IODP Expedition 362 in the eastern Indian Ocean encountered probably the largest wood fragment ever recovered in scientific ocean drilling. The wood is Late Miocene in age and buried beneath ~ 800 m of siliciclastic mud and sand of the Bengal–Nicobar Fan. The wood is well preserved. Possible origins include the hinterland to the north, with sediment transported as part of the submarine fan sedimentary processes, or the Sunda subduction zone to the east, potentially as a megathrust tsunami deposit.

IODP Expedition 362 drilled offshore at two sites (U1480 and U1481) in August–October 2016 on the Indian oceanic plate subducting at the Sumatra seismogenic zone (Fig. 1). The primary objective of the expedition was to characterize the input sediments that control the properties of the plate boundary fault zone responsible for the December 2004 magnitude 9.1 Sumatra–Andaman earthquake and resulting tsunami. The drill sites are situated on the Nicobar Fan – part of the wider Bengal–Nicobar fan system composed of deep-water siliciclastic sediments derived from the uplifting Himalaya–Tibetan Plateau collisional system (McNeill et al., 2017a). At 3° N, the latitude of sites U1480 and U1481, 1.5 km of sediment overlies the oceanic basement with water depths of 4.5 km. In situ temperature measurements document a local geothermal gradient of $44.4\,°\text{C km}^{-1}$ with a seafloor temperature of approximately $1.5\,°\text{C}$ (McNeill et al., 2017b).

During coring at Site U1480, two adjacent cores of interbedded sediment gravity flow and hemipelagic siliciclas-

tic sediment containing large pieces of fossilized wood were recovered at $\sim 840–860$ m below the seafloor. The sediments at this depth are Late Miocene in age (~ 9 Ma), based on biostratigraphic analysis (Backman et al., 2019). The largest wood fragment (in Core 362-U1480G-11R) is 14 cm in length along the core axis (Fig. 2). The second fragment is 6 cm in length within the next underlying core (U1480G-12R). Both fragments have a black, lignitic appearance. Binocular microscope and SEM images of the large fragment from Core 11R (Fig. 3) reveal cellular and tubular structures that are slightly compressed in the plane of bedding by sediment compaction (Fig. 3c). This preliminary examination indicates that these are examples of angiosperm wood, based on pitted ray cells and fibers as well as the stacked layers of horizontal ray cells. Although the definitive vessels of angiosperm wood are not observed, these other characteristics support an angiosperm interpretation; moreover, we do not find any convincing evidence for the wood being conifer. The wood appears to be mummified rather than charcoalified
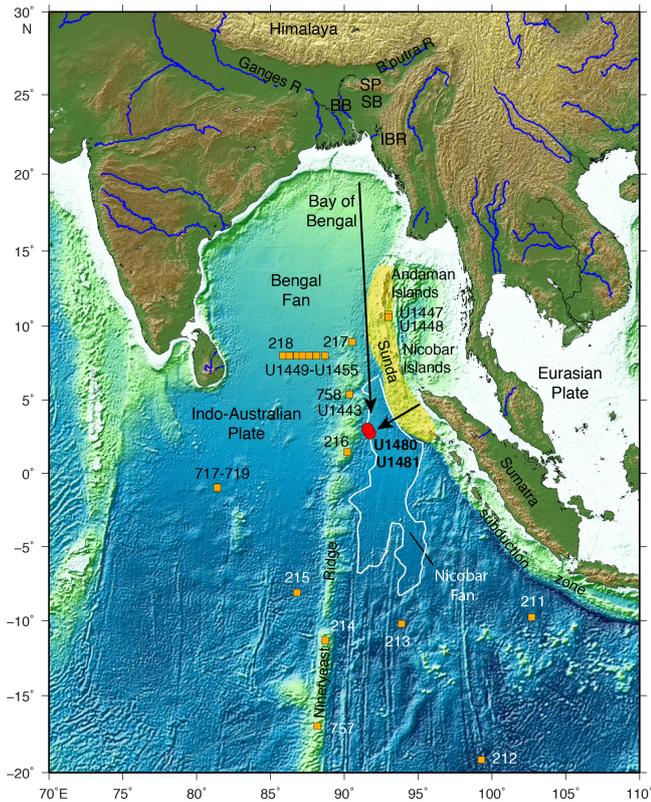
Figure 1. Eastern Indian Ocean map showing the IODP Expedition 362 drill sites (red dots) and the extent of the Nicobar Fan (white line). The yellow shaded area indicates the rupture area of the 2004 Sumatra–Andaman subduction earthquake. The wood could have originated from the north as part of the Bengal–Nicobar Fan transport or from the islands of the Sunda subduction zone to the northeast and east; this includes a possible tsunami transport origin triggered by a northern Sunda subduction zone earthquake, similar to the 2004 Sumatra–Andaman event (black arrows indicate possible pathways). Map uses GEBCO bathymetric data from the GEBCO_08 Grid data set obtained from the National Centers for Environmental Information (NCEI; previously NGDC). After McNeill et al. (2017a).

Figure 2. Section of Core 362-U1480G-11R, including the largest of the wood fragments. The image is lightened to highlight these fragments.

or coalified (Mustoe, 2018). We rule out charcoal because this would display crushing rather than compressional bending and plastic deformation in compaction, whereas coalified wood would require greater burial and thermal maturity (Mustoe, 2018). Mummification of the wood indicates that it was buried in a manner that protected it from oxidation and microbial degradation (Mustoe, 2018).

This find is extremely unusual, in terms of the size of the wood pieces, the age of the sediments within which they were deposited and preserved, and the depth of burial. Based on a search through previous DSDP, ODP, and IODP reports, the fragment from Core 362-U1480G-11R is believed to be the largest wood fragment cored in scientific ocean drilling history. Investigations are ongoing to try to identify the wood/tree type as well as its taxonomy and geographic
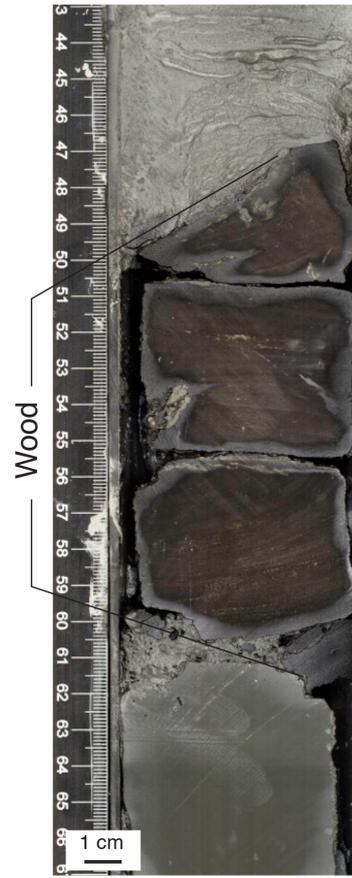
origin. The preservation state of these wood fragments may also provide qualitative information on seafloor conditions (e.g., oxygen and burial rate) that augment expedition sedimentary analyses. It is likely that the along-core length represents the width of the tree trunk and that the wood sample's long axis is laying horizontal, meaning that it is perpendicular to the core axis as a consequence of deposition on the seafloor. Therefore, we infer that the coring process sampled what would be a much larger section of woody material. Inspection of the wood fragment surfaces revealed no borings or epibionts.

Possible origins and transport processes of this wood material include (1) large-scale flooding and long-distance transport of material from the north within the Bengal–Nicobar submarine fan system, (2) more regional transport from the islands of the Sunda subduction zone to the northeast and east (Sumatra, the Nicobar islands, and the Andaman islands), or (3) subduction zone tsunami debris similar to that created by the 2004 Sumatra–Andaman earthquake and tsunami that removed and washed away thousands of trees, whose settling locations are not known. Figure 1 shows
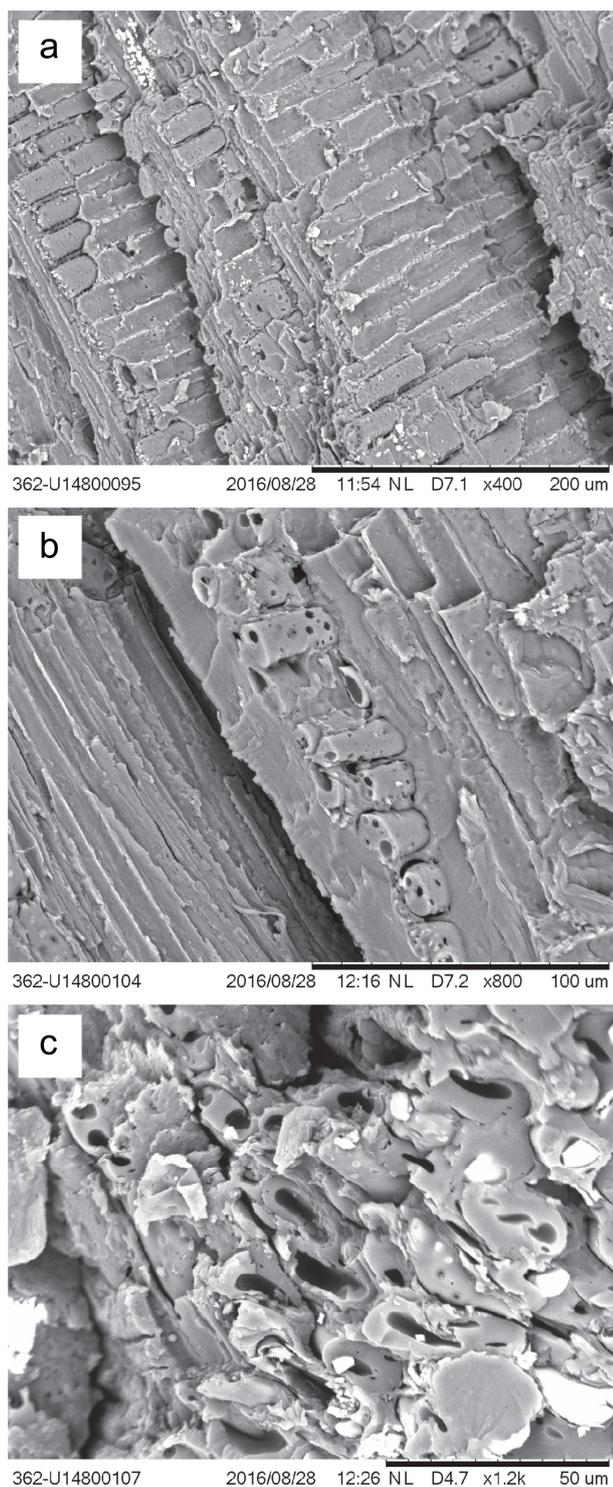
these geographic regions relative to the drill sites and potential transport pathways.

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Author contributions. All authors contributed to writing the paper. LM, BD, KP, KM, and the Expedition 362 scientists all participated in Expedition 362 and the collecting of cores and logs as well as their preliminary analysis. KM generated and performed the initial analysis of the thin sections and the SEM images. JF was responsible for the technical wood analysis.

Competing interests. The authors declare that they have no conflict of interest.

Figure 3. Shipboard SEM images of angiosperm wood fragments from IODP Site U1480 cores. **(a)** Radial view of ray cells showing rays cells (horizontal orientation in the original tree) with tiny pits on ray cell walls, and several ray cell lumen with infills. **(b)** Oblique view showing the tangential cross section of a ray composed of numerous cells (some with infilling). Vertical fibers are visible on the left. **(c)** Oblique transverse view showing the transverse section of fiber walls, which have been slightly compressed.

that improved the paper, in particular the technical descriptions of the wood.

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Scientific Drilling
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ICDP workshop on the Lake Tanganyika Scientific Drilling Project: a late Miocene–present record of climate, rifting, and ecosystem evolution from the world's oldest tropical lake

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Abstract. The Neogene and Quaternary are characterized by enormous changes in global climate and environments, including global cooling and the establishment of northern high-latitude glaciers. These changes reshaped global ecosystems, including the emergence of tropical dry forests and savannahs that are found in Africa today, which in turn may have influenced the evolution of humans and their ancestors. However, despite decades of research we lack long, continuous, well-resolved records of tropical climate, ecosystem changes, and surface processes necessary to understand their interactions and influences on evolutionary processes. Lake Tanganyika, Africa, contains the most continuous, long continental climate record from the mid-Miocene (~ 10 Ma) to the present anywhere in the tropics and has long been recognized as a top-priority site for scientific drilling. The lake is surrounded by the Miombo woodlands, part of the largest dry tropical biome on Earth. Lake Tanganyika also harbors incredibly diverse endemic biota and an entirely unexplored deep microbial biosphere, and it provides textbook examples of rift segmentation, fault behavior, and associated surface processes. To evaluate the interdisciplinary scientific opportunities that an ICDP drilling program at Lake Tanganyika could offer, more than 70 scientists representing 12 countries and a variety of scientific disciplines met in Dar es Salaam, Tanzania, in June 2019. The team developed key research objectives in basin evolution, source-to-sink sedimentology, organismal evolution, geomicrobiology, paleoclimatology, paleolimnology, terrestrial paleoecology, paleoanthropology, and

geochronology to be addressed through scientific drilling on Lake Tanganyika. They also identified drilling targets and strategies, logistical challenges, and education and capacity building programs to be carried out through the project. Participants concluded that a drilling program at Lake Tanganyika would produce the first continuous Miocene–present record from the tropics, transforming our understanding of global environmental change, the environmental context of human origins in Africa, and providing a detailed window into the dynamics, tempo and mode of biological diversification and adaptive radiations.

1　Introduction

The Earth has experienced enormous environmental changes during the last 10 million years, including global cooling that culminated in permanent ice in the northern high latitudes, the expansion of C_4 grasslands and other dry tropical biomes, and the development of globally synchronized glacial–interglacial cycles (Cerling et al., 1993; Zachos et al., 2001). Our knowledge of these transitions and their global impacts is primarily based on deep-sea drill cores, yet many of these transitions unfolded on land, where they reshaped the continents and influenced organismal evolution and dispersal, including that of our own species. Scientific drilling under the auspices of the International Continental Drilling Program (ICDP) has begun to elucidate the terrestrial environmental changes that accompanied these events (Soreghan and Cohen, 2013), yet we still lack long, continuous, independently dated sedimentary records to document the rates, amplitudes, and dynamics of continental environmental change from the Miocene to the present. This is particularly the case in the tropics, despite their critical role in maintaining Earth's climate and biodiversity.

Lake Tanganyika (LT), East Africa (Fig. 1) is one of the oldest, largest, and deepest lakes on Earth and is a truly unrivaled site for scientific drilling. Its stratigraphy, which spans the Miocene–present, is the most continuous continental record for this time interval known in the tropics (Colman, 1996). Scientific drilling in LT could thus provide a unique, high-resolution record of tropical continental climate in the late Cenozoic. LT is also one of the most biodiverse lakes on Earth (Salzburger et al., 2014, and fills one of the most deeply subsided parts of the East African rift (Ebinger, 1989). Drilling LT offers outstanding opportunities to investigate evolutionary transitions in aquatic and terrestrial organisms and ecosystems, and the geological evolution of a large continental rift system. In the last 6 years we have convened a series of disciplinary workshops that developed a strong consensus that drilling LT will transform our understanding of climatic, evolutionary, and rift processes (Cohen and Salzburger, 2017; McGlue and Scholz, 2016; Russell et al., 2012). To expand on these objectives, and to develop a fully integrated, interdisciplinary scientific drilling program on Lake Tanganyika, we held an ICDP workshop in Dar es Salaam, Tanzania, from 17 to 20 June 2019, attended by more than 70 scientists. Workshop attendees defined the scientific
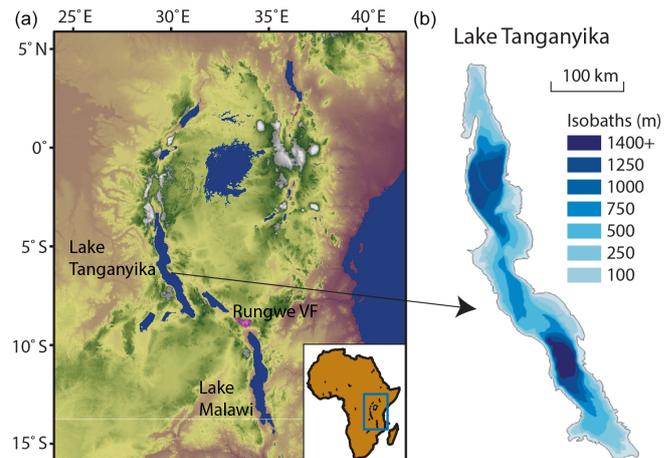

Figure 1. Topographic map of East Africa from SRTM data showing the location of Lake Tanganyika; figure modified from Russell et al. (2012). The lower left inset shows the location of the region within Africa. In panel **(b)**, a bathymetric map of Tanganyika illustrates the major depositional sub-basins within the lake.

rationale, drilling targets and logistics, and other plans for the Lake Tanganyika Scientific Drilling Project (TSDP).

2　Lake Tanganyika: a world-class site for scientific drilling

Lake Tanganyika ($32\,600\,\mathrm{km}^2$, $1470\,\mathrm{m}$ deep, 4–9° S, 29–31° E) is near the center of the western branch of the East African Rift on the border between Tanzania, the Democratic Republic of Congo (DRC), Burundi, and Zambia (Fig. 1). Precipitation is strongly seasonal, with a pronounced dry season from June to August when the tropical rain belt shifts northward. During this season, strong southerly winds associated with the East African and Indian monsoons flow over the basin and cause lake upwelling that drives primary production by algae, especially diatoms, forming the basis for a fishery that has yielded up to $\sim 200\,000\,\mathrm{t}$ of fish annually (Descy et al., 2005), one of the largest inland fisheries in the world. All of these components of LT vary in response to climate, as documented in intricate detail by geochemical and fossil records in shallow sediment cores (Cohen and Salzburger, 2017; Tierney and Russell, 2007).
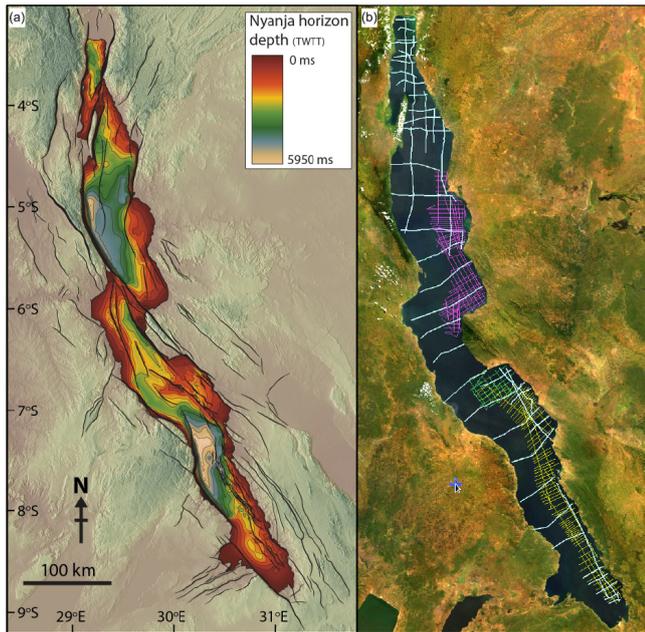
Figure 2. In panel **(a)**, a contour map shows the two-way travel time from lake surface to acoustic basement (i.e., the Nyanja Event) deduced from seismic reflection data shown within a regional digital elevation model based on 90 m Surface Radar Topography Mission data. Major interpreted faults are shown. In panel **(b)**, a map of seismic lines collected from Lake Tanganyika overlain on a satellite image (© Esri imagery – source: Esri, i-cubed, USDA, USGS, AEX, GeoEye, Getmapping, Aerogrid, IGN, IGP, UPR-EGP, and the GIS). Light blue lines are 24-fold multichannel seismic lines collected by Project PROBE 1993–1984. Green lines are single-channel sparker profiles collected during the Nyanza Project (McGlue et al., 2008). Yellow profiles are 60-fold data acquired in 2012 as part of a commercial exploration program. Magenta lines are single-channel high-resolution air-gun profiles collected in 1997.

Lake Tanganyika is part of East Africa's western rift (Rosendahl, 1987; Ebinger, 1989). Extension is accommodated by steeply dipping border faults that commonly form the coastlines of the lake (Fig. 2). Nine border faults link together to form the Lake Tanganyika rift, producing several sub-basins with water depths of 1000 m or more separated by deep-water horsts (Scholz and Rosendahl, 1988). The Rungwe Volcanic Province (RVP) lies ∼ 250 km south of LT. RVP volcanism has been ongoing since at least 9 Ma and is dominated by effusive and explosive eruptions, with the oldest pyroclastic units dated to ∼ 8.6 Ma (Fontijn et al., 2012). Several volcanic ash beds derived from the RVP have been found in shallow cores from southern LT (e.g., Livingstone, 1965), suggesting great potential for tephra-based age control in our project.

Several thousand kilometers of reflection seismic data have been acquired on Lake Tanganyika (McGlue et al., 2008; Muirhead et al., 2019; Rosendahl, 1988; Scholz et

al., 2003), including a commercial survey of the southeastern part of the Tanzanian side of the lake completed in 2012 (Fig. 2). LT's seismic stratigraphic section is comprised of a set of four major depositional sequences (Fig. 3) that overlie a set of reflections referred to as the "Nyanja Event", which is interpreted to mark the onset of the current phase of rifting and the initiation of the present-day LT (Rosendahl, 1988). Its Cenozoic sedimentary succession has been divided into six sequences (Muirhead et al., 2019) that vary widely in thickness across the basin. These sequences include the following:

- *Sequence S1, characterized by low-amplitude, discontinuous reflections.* The relatively uniform thickness and character of this sequence implies low-relief, shallow lacustrine and fluvial environments formed during the earliest phase of the formation of LT.

- *Sequence S2, which consists of high-frequency reflectors and thickens westward, indicating subsidence of the western border fault.* Zones of high continuity extend over tens of kilometers, implying that a deep rift lake was in place by this time.

- *Sequences S3–S5 are characterized by alternating high- and low-amplitude reflectors, often with incised channels and paleodeltaic deposits.* This suggests varying lake levels, probably caused by Plio-Pleistocene climatic changes, with water-level changes of up to 600 m.

- *Sequence S6, characterized by low-amplitude but high-frequency, high-continuity sediments.* The character, lateral extent, and external draping form are all similar to fine-grained hemipelagic muds drilled in nearby Lake Malawi (Scholz et al., 2011).

Most core-based research at LT has focused on the last glacial cycle to the present, as only short piston cores (∼ 10 m or less from within S6) are available. Sedimentological data documented a lake lowstand of ∼ 200 m during the Last Glacial Maximum (McGlue et al., 2008), when temperatures ∼ 3 °C cooler than present allowed Afromontane forests to expand around the lake (Ivory and Russell, 2016). Following these cold, dry conditions, climate rebounded during the Pleistocene–Holocene transition, marked by a very warm, wet early-Holocene interval known as the "African Humid Period" (Tierney et al., 2008). These events occurred throughout much of equatorial and northern Africa (Otto-Bliesner et al., 2014), highlighting LT's potential as a "master record" of African environmental history. Organic geochemical analyses of short cores have shown that the lake has warmed by 1–2 °C in the last century in response to anthropogenic greenhouse gas forcing, resulting in significant reductions in nutrient upwelling, primary productivity, mollusks, and fish (Cohen et al., 2016). These results highlight the potential of an LT drill core to provide quantitative estimates of tropical climate, to record climate variations, and to
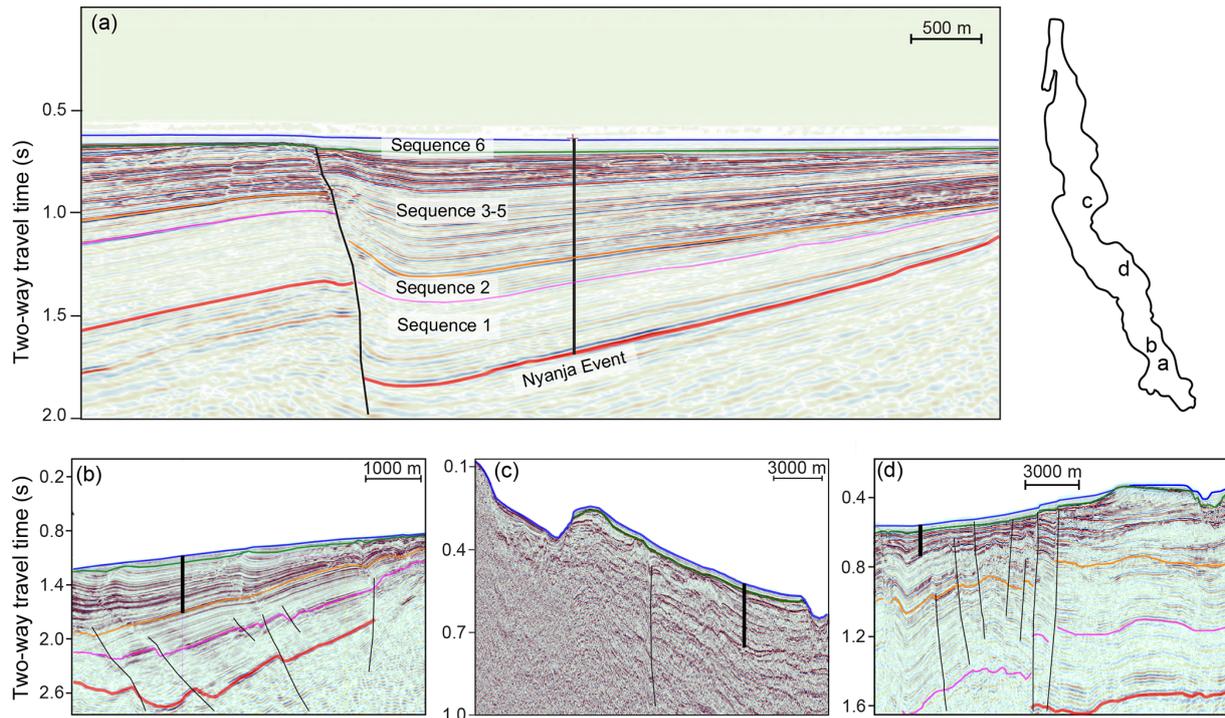
Figure 3. Seismic reflection profiles showing potential drilling sites at Lake Tanganyika. Panel **(a)** illustrates a potential drilling site that captures the major seismic sequences (S1–S6) present in the lake. Sequences S3–S5 are condensed and likely truncated by erosion at this site, but these sequences can be drilled at a nearby site in deeper water **(b)**. Panels **(c)** and **(d)** illustrate sections in shallower water where fossiliferous sediments can be drilled for evolutionary biological studies. In all panels, black lines indicate potential drill holes, blue lines trace the lake floor; red lines indicate the Nyanja event (the interpreted base of the modern rift); and pink, orange, and green lines trace the boundaries between sequences 1 and 2, 2 and 3, and 5 and 6, respectively.

record the response of equatorial climate to global forcings and processes.

3   Workshop structure and findings

We convened a workshop to define scientific priorities and analyses, logistics, drilling targets, and education and out-reach plans for a deep scientific drilling project in Lake Tanganyika. Presentations on the first day focused on the limnology of Lake Tanganyika and the evolutionary history of its biota, the structural geology of the East African and Tanganyikan rifts, the sedimentary architecture of LT, and the environmental history of East Africa. Participants then spent the next three days in breakout groups to de-velop scientific hypotheses and strategies in the broad ar-eas of paleoclimatology, basin evolution, source-to-sink sed-imentology, organismal evolution, paleolimnology, terres-trial paleoecology, paleoanthropology, geomicrobiology, and geochronology. These scientific breakout discussions led to prioritized research goals within each group linked to drilling targets, from which we developed a coordinated, parsimo-nious drilling plan.

Sub-Saharan Africa is highly socioeconomically vulner-able to future climate change. However, considerable uncer-

tainty remains in climate predictions for the continent (Niang et al., 2014), demanding that we test climate model simula-tions against reconstructions of climate under higher green-house gas concentrations than the present. The Miocene–Pliocene presents the best analog for future climate, as conti-nental configurations were similar to the present yet green-house gas concentrations were higher than present (Hay-wood et al., 2016; Zhang et al., 2013). Shallow cores from LT have provided outstanding records of late Quaternary to recent changes in climate (Cohen et al., 2016; Tierney et al., 2008) and benchmark targets for late-Pleistocene pale-oclimate modeling (Otto-Bliesner et al., 2014), highlighting the lake's potential to establish a tropical paleoclimate ref-erence section for the late Neogene. Thus, LT offers an un-matched opportunity to evaluate the response of tropical rain-fall and temperature to changes in high-latitude glaciation, greenhouse gas concentrations, insolation forcing, and other changes in global climate boundary conditions during the last ~ 10 million years. The occurrence of severe hydroclimate fluctuations and lake level draw-downs over the past 200 kyr in the Malawi Rift are well-documented (Scholz et al., 2011), and other extant lakes in Africa, including LT (Burnett et al., 2011), show evidence of similar variability. Drilling in LT is critical for determining the phasing of this high-amplitude

variability across the African tropics. Moreover, TSDP will represent the culmination of several decades of scientific drilling and coring in East African lakes and paleolakes. ICDP records from Lake Malawi, the Hominin Sites and Paleolakes Drilling Project (HSPDP), and Lake Challa have provided considerable insight into East African climate but continuously span only the last ~ 1.2 Myr at Lake Malawi (Ivory et al., 2016), with more discontinuous HSPDP records back to 3.3 Ma (Campisano et al., 2017). A long, continuous record from LT will therefore provide a master stratigraphy from the region to contextualize the Lake Malawi, HSPDP, and Lake Challa records within late Miocene–present environmental change.

The western branch of the East African rift is the global archetype of an active, amagmatic early-stage rift, and its thick sediments preserve a multimillion-year record of extensional tectonics and landscape evolution. The western branch of the rift experiences the largest magnitude earthquakes of the African continent, and presents textbook examples of rift segmentation and fault behavior (Lavayssière et al., 2019). This deep, anoxic, freshwater body is also commonly cited as a classic example of a continental basin accumulating lacustrine petroleum source rocks (Katz, 1996), yet stratigraphic prediction in ancient low-latitude rift basins has been a major challenge. TSDP will provide opportunities to investigate (1) how along-strike basin segmentation and fault growth impact sedimentation and source-to-sink processes; (2) the dynamics of fault slip, propagation and linkage, and whether they conform to rifting models; (3) the history of magmatic activity and geothermal gradients and how they relate to basin evolution in a "cold rift"; and (4) how these processes influence Tanganyika's limnological and biological evolution, stratigraphy, and resources.

Understanding how ecosystems are assembled and altered through time, and how speciation, dispersal, and extinction shape species assemblages and communities has been a fundamental problem in ecology and evolutionary biology ever since Darwin. LT harbors spectacular endemic faunas, with hundreds of unique species of fish, in particular cichlids, as well as mollusks, and crustaceans that have evolved over the lake's long history (Salzburger et al., 2014). These endemic species form unique communities in benthic and pelagic habitats, and many of these organisms have left fossil records in LT's sediments (e.g., Cohen et al., 2016). Together with ancient DNA (aDNA) analyses these fossils will provide records of the evolution, radiation, and extinction of endemic taxa and coevolved ecosystems. The combination of LT's antiquity and size probably accounts for its extraordinary diversity, but the role of limnological, climatic, and tectonic changes in shaping LT's flora and fauna remain largely hypothetical. The availability of a continuous paleolimnological record of the lake together with fossil and, in the younger intervals, aDNA records of the lake's endemic organisms would allow us for the first time to understand the

rates, dynamics, and drivers of adaptive radiation – a truly transformative advance for evolutionary biology.

Lake Tanganyika is surrounded by Miombo woodlands, part of the largest dry forest tropical biome on Earth. It is generally assumed that these ecosystems arose at the expense of the Guineo-Congolian rainforests to the west, but there is little evidence to support this hypothesis. Palynological records from LT have highlighted the sensitivity of Miombo and other surrounding ecosystems to climate and environmental changes, including human impacts (Ivory and Russell, 2016). Our understanding of the processes that generated the present-day structure of these ecosystems would be greatly enhanced by records spanning the larger range of climate variations occurring from the Miocene to the present. Moreover, Africa has a long history of hominin–environmental interactions. A Miocene–present record from LT will provide a benchmark record of the environmental context in which our ancestors lived and evolved.

While Lake Tanganyika is renowned for its aquatic biodiversity, its microbial diversity in the water column and sediments remain largely unknown. Drill cores will allow us to explore LT's deep biosphere, the role it plays in the lake's carbon, nitrogen, sulfur, and other elemental cycles, and how the water column and sedimentary microbial communities vary in relation to climatic and tectonic changes. Changes in the microbial community, acting in concert with limnological processes and climatically and tectonically driven changes in the lake's physical structure, should govern key aspects of the lake ecosystem, including primary productivity, and ultimately its biodiversity.

Investigations of Lake Tanganyika's depositional history will require a robust geochronology. Participants reviewed state-of-the-art geochronological techniques and their applicability to LT sediments. In addition to the Rungwe Volcanic Province (RVP) in southern Tanzania, significant Neogene–Quaternary volcanic events in the Central Kenyan Rift, northern Tanzania and the Virunga Volcanic Province could have transported ash to the LT basin. RVP-derived tephra are relatively rich in potassium (Fontijn et al., 2012), and several are known to be present in Holocene-age sediments from southern LT (e.g., Livingstone, 1965). Ar–Ar ages from RVP tephra in the lake core, or from coarser correlative proximal outcrops, will provide multiple anchor points for our core chronology, and this opportunity guides us to concentrate our drilling efforts in LT's southern basin. Ar–Ar, together with ^{14}C, luminescence dating, paleomagnetic, and other dating techniques, will provide vital independent age estimates for the LT core chronology, as well as contributing to future regional geochronologic and earth system studies. This effort will provide a new eastern African palaeomagnetic reference curve back to the Miocene and a stratified multi-million-year record of explosive volcanism from the southern East African rift volcanic provinces.

Ultimately our goal is to integrate information from these different fields to understand the coupled climatic, geologic,

and biological processes that control the evolution of Africa's largest rift lake. Participants discussed possible drilling targets and strategies to address these issues, and the need for continuity, resolution, and lithologies through the different sedimentary units (sequences S1–S6) to address the goals outlined above. Participants emphasized the importance of determining LT's age and early conditions, and the transformative nature of a Miocene–present paleoclimate record from the tropics. Subsidence and sedimentation rate estimates suggest the Nyanja Event occurred between 9 and 12 Ma (Cohen et al., 1993). Accordingly, sequences S1 and S2 could date to the Miocene and Pliocene, and S3–S6 to the Pleistocene and Holocene. Thus, the team agreed on the importance of obtaining a complete representative section from the lake – i.e., a record extending to the Nyanja Event. Participants also highlighted the importance of obtaining fine-grained, continuous sediments for state-of-the-art paleoenvironmental analyses and for essential geochronologic control. It is prohibitively expensive to drill 2–3 km holes from LT's deep basins, where sedimentation rates are rapid ($0.5 \, \mathrm{m \, kyr^{-1}}$ or more). However, our team has identified hemipelagic sections that include all of the major sedimentary units in LT, in water depths below the maximum depth of lake lowstands and with combined water and sediment depths of ~ 1500 m (Fig. 3), achievable with intermediate-scale drilling technology.

Based on these needs, we anticipate an offset drilling program at two sites in southern Lake Tanganyika, proximal to the Rungwe volcanoes and where we have excellent seismic stratigraphic constraints. Two offset holes drilled to ~ 1500 m depth (combined water and sediment) will allow recovery of a relatively complete Miocene–present record (Fig. 3a and b). A shallow (~ 100 m) hole at one of these sites will provide sediment for geomicrobiological investigations across the strong biogeochemical gradients that should exist in the uppermost sediment column. We anticipate also recovering a set of shallow holes in central LT to evaluate evolutionary and paleolimnological gradients during lake level lowstands that might bifurcate LT into multiple basins (Fig. 3c and d). We are now pursuing pre-drilling logistical and scientific activities including the assembly of a Tanganyika database to improve access to information about the lake, drilling platform design, safety evaluations and project permitting, methodological tests using existing sediment cores, and educational and outreach activities within the riparian countries.

Data availability. No data sets were used in this article.

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Message from ECORD/IODP by Gilbert Camoin, Director ECORD Management Agency

Science knowledge over the last 50 years of ocean drilling has greatly enhanced our understanding of the Earth system. Since its creation in 2003, ECORD has played a leading role in the successive ocean drilling programmes. During 2019, the scientific ocean drilling community took a unique multi-decadal approach to formulating the future of this international program in the new 2050 Science Framework: Exploring Earth by Scientific Ocean Drilling. The unprecedented health crisis related to the COVID-19 disease outbreak is severely affecting the activities of our programme, but the scientific ocean drilling community remains mobilized for a brighter future. In these different times, I do hope that you and your loved ones will stay safe and healthy.

Message from the ICDP Executive Director Marco Bohnhoff

COVID-19 is having a huge impact on society as a whole and the personal life of most of us has been turned upside down. However, ICDP is also active in times of COVID-19. A new ICDP Science Plan for the time after 2021 is currently being prepared and will be published in the second half of 2020. For those who submitted drilling or workshop proposals to ICDP this year: the ICDP Panels met online between May 11–15 and decided about your proposals. Good news is also that the second phase of drilling into the

Collisional Orogeny in the Scandinavian Caledonides (COSC-2) in Sweden runs very successful, passing a depth of 1000 m on May 16. Whether the ICDP training course can take place in October as planned is currently still open. Please check the ICDP website or our social media channels regularly for updates.

EGU 2020 General Assembly

Due to the COVID-19 situation, the EGU 2020 General Assembly was held online May 3–8. The joint IODP-ICDP session "Achievements and perspectives in scientific ocean and continental drilling" was performed via chat and joined by 177 interested participants on Tuesday afternoon. 18 contributions were presented in the chat and lively discussed. Even if the chat has been received very positively, ICDP and IODP are looking forward to meet the community in Vienna in 2021.

GeoUtrecht 2020

The upcoming GeoUtrecht 2020 conference will take place August 24–26 as online conference (https://www.geoutrecht2020.org/) free of charge. The chairs of the scientific drilling session 'Latest Achievements in Scientific Ocean and Continental Drilling', Martin Ziegler, Timme Donders, Jan Behrmann, and Lucas Lourens, kindly invite to submit abstracts. Abstract submission is possible until June 26.

Schedules

IODP – Expedition schedule http://www.iodp.org/expeditions/

USIO operations	Platform	Dates	Port of origin
1 Exp 395: Reykjanes Mantle Convection and Climate	JOIDES Resolution	26 Jun–26 Aug 2020	Reykjavik
2 Exp 390: South Atlantic Transect #1	JOIDES Resolution	5 Oct–5 Dec 2020	Rio de Janeiro

ICDP – Project schedule http://www.icdp-online.org/projects/

ICDP project	Drilling dates	Location
1 GRIND	Jun 2019–summer 2021	Namibia, Brazil, China
2 COSC-2	Apr–Jul 2020	Jämtland, Sweden
3 JET	after July 2020	Wales, UK
4 Trans-Amazon	after August 2020	Brazil (multiple locations)

Locations
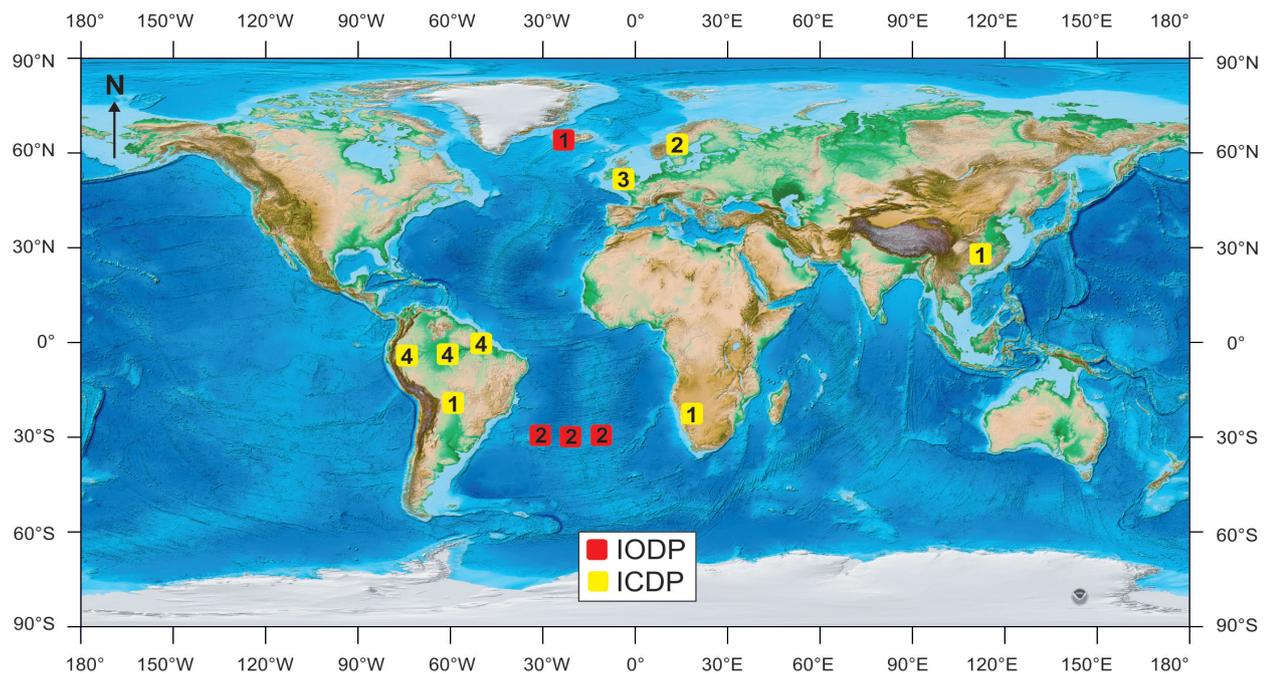
Topographic/Bathymetric world map with courtesy from NOAA (Amante, C. and B.W. Eakins, 2009. ETOPO1 1 Arc-Minute Global Relief Model: Procedures, Data Sources and Analysis. NOAA Technical Memorandum NESDIS NGDC-24. National Geophysical Data Center, NOAA. doi:10.7289/V5C8276M).