



Workshop to develop deep-life continental scientific drilling projects

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Abstract. The International Continental Scientific Drilling Program (ICDP) has long espoused studies of deep subsurface life, and has targeted fundamental questions regarding subsurface life, including the following: "(1) What is the extent and diversity of deep microbial life and what are the factors limiting it? (2) What are the types of metabolism/carbon/energy sources and the rates of subsurface activity? (3) How is deep microbial life adapted to subsurface conditions? (4) How do subsurface microbial communities affect energy resources? And (5) how does the deep biosphere interact with the geosphere and atmosphere?" (Horsfield et al., 2014) Many ICDP-sponsored drilling projects have included a deep-life component; however, to date, not one project has been driven by deep-life goals, in part because geomicrobiologists have been slow to initiate deep biosphere-driven ICDP projects. Therefore, the Deep Carbon Observatory (DCO) recently partnered with the ICDP to sponsor a workshop with the specific aim of gathering potential proponents for deep-life-driven ICDP projects and ideas for candidate drilling sites. Twenty-two participants from nine countries proposed projects and sites that included compressional and extensional tectonic environments, evaporites, hydrocarbon-rich shales, flood basalts, Precambrian shield rocks, subglacial and subpermafrost environments, active volcano–tectonic systems, megafan deltas, and serpentinizing ultramafic environments. The criteria and requirements for successful ICDP applications were presented. Deep-life-specific technical requirements were discussed and it was concluded that, while these procedures require adequate planning, they are entirely compatible with the sampling needs of other disciplines. As a result of this workshop, one drilling workshop proposal on the Basin and Range Physiographic Province (BRPP) has been submitted to the ICDP, and several other drilling project proponents plan to submit proposals for ICDP-sponsored drilling workshops in 2016.

1 Background: current state of sampling opportunities for deep continental biosphere studies

It has been recognized for decades that deep continental environments contain active, diverse communities of microorganisms functioning in subsurface ecosystems that collectively contain half or more of the Earth's microbial biomass (Whitman et al., 1998). However, despite the global significance of subterranean life, opportunities to study it remain limited. The coring of continental settings for microbiology began in the 1950s, with Russians examining petroleumbearing sediments. Their goal had been to determine whether the microorganisms discovered by Ginsburg-Karagitscheva (1926), Bastin et al. (1926) and Zobell (1945) in the fluids removed from petroleum reservoirs were indigenous to the formations in which they were found or contaminants from the oil exploration process. Subsequently, drilling for microbes really took off in the mid-1980s with support from the U.S. Department of Energy, the U.S. Environmental Protection Agency, and the U.S. Geological Survey as concerns mounted over the contamination of groundwater by a wide spectrum of pollutants created by the petroleum industry and by the fabrication of nuclear weapons. The success of these drilling programs in identifying indigenous subsurface microbial communities resulted from the pivotal development of tracers for quantifying drilling contamination (Phelps et al., 1989; Colwell et al., 1992; Russell et al., 1992). These pioneering subsurface programs extended the known depth limit of the biosphere, quantified the sizes and activities of subsurface microbial communities, and documented the direct consequences of microbial metabolism on the geochemistry of subsurface environments. Modern molecular biology tools have greatly extended the capabilities for characterizing the phylogeny and metabolic activities of deep subsurface communities. The Deep Sea Drilling Program (now the International Ocean Discovery Program, IODP), which began exploring the subseafloor biosphere in 1990 off the coast of Peru, adopted these tracer technologies for deeplife-driven drilling expeditions in 1999 on Leg 185 (Smith et al., 2000a, b). The first IODP expedition that was designed and carried out with deep life as the primary driver was the Leg 201 drilling of the Peru Margin, the success of which paved the way for subsequent biologically motivated expeditions. The IODP has made exceptional progress over the past 25 years in quantifying marine subsurface microbial abundance, characterizing their diversity, and relating microbial activities to geochemical conditions.

The International Continental Scientific Drilling Program (ICDP) is the continental counterpart to the IODP. Although deep life is a major ICDP theme (Zoback and Emmermann, 1994; Horsfield et al., 2007; Kallmeyer and Kieft, 2014), deep-life studies have so far only piggybacked onto ICDP drilling projects planned for other purposes. To date, no ICDP projects have been conceived and executed with deep life as the primary objective, in part because the deeplife community has been slow to initiate a bio-driven ICDP project. The objective of this Deep Carbon Observatory (DCO)-sponsored workshop, therefore, was to develop one or more deep-life continental drilling projects, which would essentially become the continental equivalent of Leg 201. The workshop was held at the GFZ German Research Center for Geosciences in Potsdam, Germany, on 3 and 4 November in 2014 with support from the DCO and local support from the ICDP. There were 22 participants from nine countries; nineteen of the participants were on site (Fig. 1) and three participated remotely.

2 The deep biosphere

The majority of deep continental subsurface microbes are prokaryotes (bacteria and archaea) living in darkness without



Figure 1. Group photograph of on-site participants of the workshop. From left to right, Heath Mills (USA), Lasse Ahonen (Finland), Bert Engelen (Germany), Phil Long (USA), P.-L. Wang (Taiwan), L.-H. Lin (Taiwan), Jens Kallmeyer (Germany), Eric Gaidos (USA), Sergiu Fendrihan (front, Romania), Dirk Schultz-Makuch (USA/Germany), Duane Moser (USA), Mike Wilkins (USA), Brandi Kiel Reese (USA), Tom Kieft (USA), Uli Harms (Germany), Vanni Aloisis (France), Pinaki Sar (India), T. C. Onstott (USA), Dirk Wagner (Germany) (photo courtesy of Helga Stan-Lotter, Austria).

exposure to photosynthetically generated O_2 . In the deepest sites, microorganisms function without access to photosynthetically generated organic carbon, as well; instead, being fueled by hydrogen (H₂) generated from rock–water reactions (Fig. 2) (Stevens and McKinley, 1995; Chapelle et al., 2002; Sleep et al., 2004; Lin et al., 2006; Chivian et al., 2008). A wide diversity of bacteria and archaea has been detected in the continental subsurface, with the majority of these appearing to be indigenous and adapted to subterranean life (Heim, 2011; Colwell and D'Hondt, 2013; Lau et al., 2014). These microbes are active, albeit at slow metabolic rates, and thus are important in the biogeochemical cycling of carbon (Head et al., 2003), nitrogen (Lau et al., 2014), and other biologically relevant, redox-sensitive elements (Pedersen et al., 2008).

Although bacteria and archaea have been the major focus of continental deep-life studies to date, participants with expertise in subseafloor drilling highlighted other biological groups, as well. For example, recent investigations suggest that viruses, including bacteriophages and archaeophages, play an important role in the deep biosphere (Kyle et al., 2008; Eydal et al., 2009). High virus-to-cell ratios found in oligotrophic deep marine sediments indicate ongoing viral production (Engelhardt et al., 2014). Viral lysis might not only control prokaryotic biomass but also release N- and Prich organic compounds. The integration of this viral shunt into biogeochemical models could modify estimated rates of carbon cycling in the subsurface. Fungi have long been studied in freshwater lakes, soils, surface sediments and, more recently, marine deep subsurface sediments (Nagano et al., 2010; Edgcomb et al., 2011), but are currently not known to be important players in the continental subsurface. In the marine deep subsurface, fungi appear to be reducing nitrate and degrading lignin (Cathrine and Raghukumar, 2009; Gubernatorova and Dolgonosov, 2010), and have been reported in biological samples collected from the deep continental subsurface (Sinclair and Ghiorse, 1989; Reitner et al., 2005). Other eukaryotic components found in the deep continental biosphere include yeasts (Ekendahl et al., 2003), protists (Sinclair and Ghiorse, 1989), and nematodes (Borgonie et al., 2011). Exploration for these ecologically important, but numerically less abundant, members of subsurface ecosystems will require the capability of accessing high volume subsurface material (fluids and/or solids) from any proposed ICDP site.

Most current microbiology-based research efforts that aim to describe subsurface microbial communities utilize so-called next generation sequencing approaches (e.g., Wrighton et al., 2012; Baker and Dick, 2013) that can detect and identify microorganisms present in deep systems even when they comprise < 1 % of the total community. Because this technology is sensitive to trace constituents in DNA extracts, maintaining sample quality and conducting proper controls is essential for reducing the likelihood of sequencing contaminants infesting the community database. Methodological and reagent blanks should be included to account for DNA contamination that might occur during sampling and in the laboratory or that might be present in reagents, enzymes, or buffers. By consulting databases that catalog classical contaminant sequences, such as those present in DNA extraction kits (cf. Salter et al., 2014), indigenous minor or rare biosphere microorganisms can be identified with higher confidence. In some cases, oligotyping may differentiate closely related but distinct taxa (McLellan et al., 2013) as well as the respective origin of these taxa (i.e., from the subsurface vs. introduced at some point during the analysis) (cf. Magnabosco et al., 2014).

3 Drilling technology for deep-life projects

Concerns are often expressed by non-biologists that deeplife studies impose onerous methodological costs and constraints. Drilling for microbial investigations does require additional effort to implement quality control and quality assurance (QA/QC) procedures; however, established protocols exist (Kieft et al., 2007; Kieft, 2010, 2015b; Wilkins et al., 2014), and they are compatible with and, in many cases, facilitate the needs of other disciplines, such as biomarker analyses and pore water chemistry. Biological QA/QC involves use of tracers in drilling fluids, subsampling from the center of cores, and quantifying tracers and thus drilling fluid infiltration into the subcores (Fig. 3). Good QA/QC practice



Figure 2. Overview of subsurface continental environments and parameters that typically vary with depth (modified from EarthLab, National Science Foundation, 2003; from Kieft, 2015a).



Figure 3. Use of tracers and subcoring for geomicrobiological sampling in a granitic subsurface environment (Sahl et al., 2008). (a) Fluorescent microbead tracers deployed in the core shoe, (b) subcoring using a hammer and chisel in a laminar flow hood, (c) subcore samples in Whirl-Pak[®] bags, and (d) fluorescent microbead tracers in drilling mud, viewed by epifluorescence microscopy.

also entails eschewing biodegradable drilling fluid additives, steam cleaning of core barrels, using disinfected plastic inner core liners, and rapidly processing samples on site. New technologies include foam drilling fluids and freezing the core while it is still underground (Kieft, 2015b). Once drilled, the borehole can be completed with packers sealing off discrete layers or fractures and instrumented to measure environmental parameters and biomass at depth. The addition of fluidsampling and solid sample immersion and extraction would provide for long-term monitoring of and in situ experimenta-



Figure 4. Worldwide locations of drilling sites proposed during the workshop from west to east. (1) Cenozoic Basin and Range Physiographic Province (BRPP), Nevada, USA. (2) Paleozoic Marcellus and Utica shales, Ohio, USA. (3) Mesozoic sediment and salt of Axel Heiberg Island, Nunavut, Canada. (4) Cenozoic Amazon River delta megafan, Brazil. (5) Vatnajökull sub-glacial lakes, Iceland. (6) Cenozoic Eger Continental Rift, Germany–Czech Republic. (7) Cenozoic and Paleozoic salt deposits, circum-Mediterranean. (8) Proterozoic Outokumpu borehole, Finland. (9) Cretaceous–Tertiary Deccan flood basalts, India. (10) Tertiary mud volcano province, Taiwan.

tion on the subsurface microbial communities, thereby transforming the borehole into a deep-life observatory.

4 Criteria for drilling projects

The ICDP, International Ocean Discovery Program (IOPD), and DCO have each listed compelling deep-life research questions and, not surprisingly, these lists share many of the same questions. The ICDP asks the following: "(1) What is the extent and diversity of deep microbial life and what are the factors limiting it? (2) What are the types of metabolism/carbon/energy sources and the rates of subsurface activity? (3) How is deep microbial life adapted to subsurface conditions? (4) How do subsurface microbial communities affect energy resources? And (5) how does the deep biosphere interact with the geosphere and atmosphere?" (Kallmeyer and Kieft, 2014). Additionally, the IODP asks how environmental change affects subsurface diversity and ecosystem function (http://www.iodp. org/Science-Plan-for-2013-2023/), and the DCO questions mechanisms of evolution and dispersal and also focuses on microbial transformations of carbon (https://deepcarbon. net).

The continental subsurface is more varied than the marine subsurface in terms of physical and chemical properties, and thus its microbiology is likely correspondingly more varied, as well. Workshop participants discussed developing a systematic approach to the global subsurface biosphere and its biomes, defining them by their physical (T, P), geological (sedimentary vs. igneous), geohydrological (high vs. low connectivity), and geochemical (salinity, low organic C, organic-rich shale, abiotic H₂, etc.) parameters. This discussion of the categorization of globally significant subsurface habitats or biomes led to consideration of which subsurface biomes may have been neglected by previous deep-life investigations. Participants also agreed that the strongest possible deep-life drilling proposals should meet the following list of criteria:

- The proposal should address compelling research questions, as outlined above.
- The proposal should also meet the ICDP selection criteria (http://www.icdp-online.org).
- The 3-D geological structure and the geological history of the proposed site should be understood well enough to formulate ecological hypotheses that can be tested by targeting specific depths or horizons.
- The site should encompass a high diversity of physical, geochemical, and potentially biological attributes.
- The site should have a high probability of possessing active microbial communities.
- The site should have the potential to intersect the depth and temperature limit for life.
- The site should be readily accessible, and should permit long-term access to the completed borehole(s).

5 Proposed projects and sites

Eleven different locations distributed around the world (see Table 1 and Fig. 4) were discussed. Seven of the sites were situated in predominantly sedimentary rock strata, and four sites were located in predominantly igneous-metamorphic rock strata. The geological age of the formations varied from Holocene to Precambrian. Many of the sites either have marine sub-seafloor analogs or provide an opportunity for exploring the continental-marine transition. The sites span the complete range of continental tectonic and hydrogeological settings, some of which have never been explored for subsurface microbial activity. Some of the sites are tectonically active, representing a very dynamic subsurface environment; conversely, other sites represent ancient settings that are tectonically and hydrologically quiescent except for humaninduced activity.

5.1 Active continental rift environments

The Eger Rift in Germany hosts a diverse lithology of surficial sediments overlying crystalline rocks, active CO₂ fluxing from the deep crust, and mineral-rich hot springs. An ICDP-supported drilling project called the Probe Intra-continental magmatic activity by drilling the Eger Rift (PIER drilling project) is being planned (Dahm et al., 2013). The main objective of a deep-life component to this drilling project would be to determine how microbial communities respond to variable lithology and fluid fluxes, including CO₂, from deeper strata. Several geological and geophysical studies have already been completed in this area, providing critical background information.

5.2 Active extensional crustal environments

The Basin and Range Physiographic Province (BRPP) covers much of the western United States and represents the largest continental extensional zone in the world. Its hydrogeological characteristics are similar to those of smaller systems, e.g., Rio Grande, East African, Baikal, and Rhine continental rift systems. Extensional systems are characterized by large-scale listric faults, which facilitate the flow of groundwater to great depths and sometimes over tens to hundreds of kilometers laterally. Because the BRPP is located in an arid region, meteoric water recharge in the mountain ranges drives fluid flow towards internally drained or endorheic basins. The surface water ultimately terminates in evaporative sinks, such as the Death Valley Salt Pan in California, where a chronosequence of non-marine salt deposits exist stretching back several hundred thousand years. High geothermal gradients, corresponding with thin crust (e.g., 17-25 km under Death Valley) (Collier, 1990), should enable drilling to the 120 °C isotherm (e.g., the approximate upper temperature limit for known life) at relatively shallow depths. The regional hydrology of this system is well characterized due to U.S. Government studies of groundwater resources and potential contaminant transport from nearby underground nuclear tests. Specific sites for deep-life study in and around Death Valley were proposed based on prior microbiological observations of groundwater transmitted along

Table	1.	Proposed	drilling	projects	and	sites.
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Proposed drift site	Tectonic environment	Geological leatures	themes.
Eger Continental Rift, Germany	Paleogene–Miocene conti- nental rift with active seis- micity	Sediments and volcanics	Elevated CO ₂ concentrations, seismically induced microbial activity.
Basin and Range Physiographic Province (BRPP), USA	Miocene to currently active crustal extension	Paleozoic limestones in endorheic basin with Pleistocene evaporates and a high geothermal gradient	Temperature limits of life, sub- surface microbial transport in carbonate system, microbial sur- vival in young salt deposits.
Ancient evaporites, Austria and Romania	Paleozoic–Miocene marine evaporite sediments	Gypsum to halite and siliclastic interbeds	Salinity limits of life. Sulfate- reducing microbial activity and longevity of microorgan- isms trapped in mineral fluid inclusions.
Amazon Delta, Brazil	Miocene to currently ac- tive continental to marine megafan-delta complex	Rapidly buried, organic-rich, sediments in fresh to saline water	Rates of microbial carbon cycling under rapid burial as a function of salinity. Continental to marine transition.
Mud volcano province, southern Taiwan	Pliocene to active continen- tal oceanic crustal collision	Tertiary sediments actively deformed by thrust faults with fluidized mud and groundwater flow	Microbial communities adapta- tion to tectonic displacement and pulse heat sterilization generated by fault shearing. Continental to marine transition.
Axel Heiberg Island, Canada	Eocene continental fold and thrust belt	Subpermafrost envi- ronment in Paleozoic evaporites	Active sub-zero saline ground- water migration as it relates to subsurface microbial activity, and lower temperature limit for life.
Western Appalachian Basin, USA	Paleozoic organic-rich shale	Hydrocarbon-rich shales/sandstone interfaces	Relationship of organic-rich gra- dients to microbial activity in a hydrocarbon reservoir.
Deccan Traps, India	Cretaceous–Tertiary flood basalts plus induced active seismicity	Basalts, interbedded sediments overlying Precambrian granite	Multiple mechanisms for abiotic and biotic H_2 -fueled microbial ecosystems, seismically induced microbial activity.
Vatnajökull Glacier, Iceland	Tertiary to currently active subaerial oceanic rift zone	Subglacial hydrother- mal environment in fractured basalt	Temperature extremes of life. Abiotic H_2 -fueled microbial ecosystems in comparison to deep sea vents.
Oman ophiolite, Al Hajar Mountains, Oman	Late Cretaceous oceanic crust obducted onto Pre- cambrian continental crust	Marine ocean crust exposed to meteoric groundwater flow along fractures	Serpentinization leading to abi- otic H_2 -fueled microbial ecosys- tems, comparison to subseafloor ocean crust, microbial ecosys- tems, carbon sequestration.
Fennoscandian shield, Finland	Precambrian metamorphic	Deep saline fracture water in metasedi- ments/metavolcanics/ granite	Abiotic H ₂ -fueled microbial ecosystems.



Figure 5. Possible drilling site near Death Valley, California, on the eastern flank of the Funeral Mountains. A consortium of interests, including the U.S. Department of Energy, U.S. Geological Survey, Inyo County, California, and the Hydrodynamics Group, LLC, drilled a well (BLM-1) to 883 m depth through a range of sedimentary and volcanic lithologies and conducted two hydrologic pump tests. The underlying carbonate aquifer contains anoxic thermal water (58 °C) and abundant sulfate-reducing bacteria including *Candidatus Desulforudis* sp. and *Desulfotomaculum* spp. (Thomas, 2013).

fault zones with known seismic activity (Thomas et al., 2013; Fig. 5). The complex geology creates the potential to examine deep life across conditions ranging from saturated aerobic to anoxic conditions and from mesophilic to hyperthermal temperatures in substrates ranging from ancient evaporites and sedimentary carbonates to young volcanics, sometimes within the same borehole. Characterization of the deep biosphere of this endorheic, continental extensional zone should provide an interesting contrast to that of the oceanic spreading centers.

5.3 Ancient evaporitic basins

Microbiologists have explored the preservation potential of ancient marine salt crystals for trapped microorganisms for decades, but ancient marine evaporitic sequences have never been explored for their deep biosphere potential despite their widespread distribution in space and through geological time. Because of the variety of chemical environments they produce, evaporite deposits have the potential to harbor correspondingly diverse microorganisms (Stan-Lotter and Frendihan, 2011). Gypsum and anhydrite provide a source of oxidants (sulfate and CO₂). A range of interacting extremes (temperature, pressure, salinity) and pore fluid compositions may have selected for phylogenetically diverse deep biosphere communities. Fluid inclusions in halite and gypsum provide refugia where microbes may survive for tens of thousands to millions of years (Mormile et al., 2003). Salt deposits ranging in age from the late Miocene (Mediterranean Salt Giant) to Permian (e.g., Alpine deposits in Austria and Romania) were reviewed as potential candidates for ICDP drilling. The IODP Deep Sea Record of Mediterranean Messinian Events or (DREAM) drilling proposal for the Miocene age evaporites of the Mediterranean provides an opportunity to reveal the secrets of the deep biosphere of subseafloor marine evaporite ecosystems. The primary objective of ICDP drilling into continental evaporite deposits would be to test the long-term survivability of microbes within fluid inclusions.

5.4 Active megafans

Deltaic fans transport continental detrital sediments and terrigenous biota into a marine environment and represent the most rapidly deposited, organic-rich end member of seafloor sediments. Megafans provide opportunities to explore the transition from continental to marine subsurface biomes and to study how organic matter, salinity, and porosity affect microbial composition and function. One megafan discussed was the Amazon delta. Tectonic uplift of the Andes led to a sediment megafan deposited during the last \sim 5–7 million years. Goals of a combined ICDP–IODP transect would be to document Amazon megafan evolution and to characterize this subsurface biome under continental and marine hydrogeological settings.

5.5 Active oceanic–continental crustal collision environment

In Taiwan, the ongoing arc-continent collision associated with the convergence between the Philippine Sea and Eurasian plates uplifts and exposes Mesozoic metamorphic complexes and Oligocene–Quaternary marine and continental sediments sequentially through a series of thrusts and folds. Such imbricate fault systems influence the compartmentalization of strata, hydrological circulation through discrete units, and channeling of deeply sourced carbon to shallow depths via fluid flow and mud volcanism. The geological context provides unique opportunities to address how microbial communities are shaped by and/or adapted to tectonic displacement of strata, pulse heat sterilization generated by fault shearing, and substrate availability and flux associated with lithological transitions and active faulting. Previous analyses have revealed diverse and active microbial communities present at 1.5 km depth (Wang et al., 2007). At the workshop, the mud volcanoes in southwestern Taiwan were proposed as a potential ICDP–IODP drilling target as they extend along the same fault zone beneath the South China Sea. This tectonic feature provides an ideal setting for studying deep life in a terrestrial–marine transition.

5.6 Subpermafrost environments in ancient fold and thrust belt

Permafrost covers 24 % of the Northern Hemisphere. Contiguous permafrost effectively sequesters the subsurface biosphere from the overlying photosphere and meteorically driven fluid flow. Difficulty in drilling permafrost makes it the least explored of subsurface biomes. The ~ 650 m thick permafrost on Axel Heiberg Island has chemotrophic bacteria in saline mineral springs sustained by snowmelt recharge through salt diapirs, Mesozoic shale, and sandstone that were structurally deformed in the Eocene (Andersen et al., 2008). This setting provides a unique opportunity to study the effect of fluid flow on the subpermafrost biosphere, while also providing a terrestrial analog for the exploration of life on Mars.

5.7 Black shale interfaces in an ancient foreland basin

Phanerozoic black shale formations are ubiquitous in continental basins and represent important targets for future deeplife studies. The western Appalachian Basin preserves one of the best records of marine black shales, the Ordovician age Utica Shale and Devonian age Marcellus Shale, that were deposited in a foreland basin during the formation of the Appalachian Mountains by arc-continent collisions. The depths of these shale units range from hundreds to thousands of meters. Previous studies of Cretaceous black shales at shallower depths suggest that shale interfaces represent hotspots for microbial heterotrophic activity due to high concentrations of organic substrates that diffuse from the shale into more porous sandstone (Krumholz et al., 1997) and limestone. The heterogeneous nature of the carbon substrate may support diverse microbial metabolisms. Unconventional gas and oil extraction (fracking) adds further interest to this subsurface biome. A major goal will be to compare subsurface microbial diversities and processes between pristine and hydraulically fractured shale interface regions to elucidate the effects of natural gas extraction.

5.8 Ancient continental flood basalts with active seismicity

Continental flood basalts represent another subsurface biome that has only been partially explored in the 15 My old Columbia River Basalt Province (Stevens and McKinley, 1995; Lavalleur and Colwell, 2013). Examining the microbial communities within flood basalts of the 65 Ma Deccan traps was proposed with the goals of gaining a better understanding of (i) H_2 -supported ecosystems and (ii) the role of lithotrophic microbes in biogeochemical processes. The seismically active zone of deep basalt, sedimentary interlayers, and underlying Precambrian granite of the Deccan (Koyna-Warna region, India) offers an excellent opportunity to explore three different modes of H_2 generation (i.e., anaerobic oxidation of reduced iron in the basalt, radiolytic production in the granite, and cataclastic production) in one location. An ICDP drilling project to investigate reservoir-triggered earthquakes is already underway and providing some samples for initial microbial characterization (Gupta et al., 2014).

5.9 Active subglacial hydrothermal environments

Iceland's subaerial exposure of the Mid-Atlantic Ridge and its Arctic proximity combine to produce unusual hydrogeology and geochemistry. Volcanic melting of glacial ice maintains lakes beneath the 300 m thick Vatnajökull ice cap and recharges an underlying aquifer in the permeable basaltic crust. The lakes host thriving chemoautotrophic bacteria exploiting volcanic and geothermal sources of sulfur species, CO_2 , and H_2 (Gaidos et al., 2009). Goals for ICDP exploration of the subglacial aquifer would be to characterize the microbial diversity, relate microbial metabolism to geochemical energy sources, and probe the lower depth limit of the active biosphere. The site also has direct applications to the search for life in icy worlds, the origin and early evolution of life on Earth, and the potential for carbon sequestration in the mafic crust.

5.10 Ancient ultramafic environments

Ophiolites represent oceanic crust tectonically removed from its marine environment and deposited onto a continental crustal environment where they are exposed to meteoric groundwater. As such, ophiolites comprise an important continental subsurface biome, where serpentinization generates abiotic H₂ and associated CH₄ and high pH, that can be compared to the sub-seafloor biome of oceanic crust. Anaerobic processes include fermentation and sulfur reduction. Ultimately, fluids and near-surface microbes interact with O2 and CO₂ to consume H₂ and precipitate carbonate. The late Cretaceous Samail Ophiolite site in Oman is ideal for testing hypotheses regarding H₂-fueled chemoautotrophic ecosystems and also carbon sequestration scenarios. The ICDP will soon drill the Samail Ophiolite (Kelemen et al., 2013). However, dedicated geomicrobiology drilling is needed to establish the relationships between the microbial communities, mineral, structure and formation water geochemistry as a function of depth.

5.11 Ancient Precambrian shield environments

The subsurface microbiology of ancient fractured crystalline rocks has been studied for decades and the fractures have been shown to host diverse microbial communities (Pedersen, 1997). The Outokumpu deep drill hole in Finland, which provides access to 2.5 km of Proterozoic mica schist, granite, and serpentinized ophiolite, represents an unusually diverse lithological example of Precambrian shield environments. CH_4 , H_2 , and N_2 serve as the essential nutrients for life in the deep saline fracture waters (Nyyssönen et al., 2012). Hydrogeological, geochemical and microbiological studies since 2006 indicate high potential for future studies to further test the importance of abiotic H_2 as the energetic driver of subsurface microbial ecosystems.

6 Outcomes

ICDP Executive Secretary Uli Harms advised project proponents on the proposal preparation process, which begins with an ICDP-sponsored drilling workshop proposal. As a result of this workshop, one drilling workshop proposal has been submitted for the BRPP and several other drilling project proponents plan to submit proposals for ICDP-sponsored drilling workshops in 2016. A full drilling proposal was submitted in January 2015 for drilling in the Deccan Traps, India, and this proposal now has a deep-life component that resulted from Pinaki Sar's workshop participation. When funded, these drilling workshops will provide opportunities for continental subsurface deep-life investigators to reach out to the earth science community and to build momentum for deep-life-driven drilling.

Workshop participants

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References

- Andersen, D. T., Pollard, W. H., and McKay, C. P.: The perennial springs of Axel Heiberg Island as an analogue for groundwater discharge on Mars, in: Ninth international conference on permafrost, edited by: Kane, D. L. and Hinkel, K. M., Fairbanks, Alaska, Institute of Northern Engineering, University of Alaska Fairbanks, 43–48, 2008.
- Baker, B. J. and Dick, G. J.: Omic approaches in microbial ecology: charting the unknown, Microbe, 8, 353–360, 2013.
- Bastin, E. S., Greer, F. E., Merritt, C. A., and Moulton, G.: The presence of sulphate reducing bacteria in oil field waters, Science, 63, 21–24, 1926.
- Borgonie, G., García-Moyano, A., Litthauer, D., Bert, W., Bester, A., van Heerden, E., and Onstott, T. C.: Nematoda from the terrestrial deep subsurface of South Africa, Nature, 474, 79–82, doi:10.1038/nature09974, 2011.
- Cathrine, S. J. and Raghukumar, C.: Anaerobic denitrification in fungi from the coastal marine sediments off Goa, India, Mycol. Res., 113, 100–109, doi:10.1016/j.mycres.2008.08.009, 2009.
- Chapelle, F. H., O'Neill, K., Bradley, P. M., Methe, B. A., Ciufo, S. A., Knobel, L. L., and Lovley, D. R.: A hydrogen-based subsurface microbial community dominated by methanogens, Nature, 415, 312–315, doi:10.1038/415312a, 2002.
- Chivian, D., Brodie, E. L., Alm, E. J., Culley, D. E., Dehal, P. S., DeSantis, T. Z., Gihring, T. M., Lapidus, A., Lin, L.-H., Lowry, S. R., Moser, D. P., Richardson, P. M., Southam, G., Wanger, G., Pratt, L. M, Andersen, G. L., Hazen, T. C., Brockman, F. J., Arkin, A. P., and Onstott, T. C.: Environmental genomics reveals a single species ecosystem deep within the Earth, Science, 322, 275–278, doi:10.1126/science.1155495, 2008.
- Collier, M.: An Introduction to the Geology of Death Valley, Death Valley, California, Death Valley Natural History Association, LCN 90-081612, 1990.
- Colwell, F. S. and D'Hondt, S.: Nature and Extene of the Deep Biosphere, in: Carbon in Earth. Mineralogical Society of America and Geochemical Society, Reviews in Mineralogy and Geochemistry, edited by: Hazen, R. H., Jones, A. P., and Baross, J. A., 75, 547–566, doi:10.2138/rmg.2013.75.17, 2013.
- Colwell, F. S., Stormberg, G. J., Phelps, T. J., Birnbaum, S. A., McKinley, J. P., Rawson, S. A., Veverka, C., Goodwin, S., Long, P. E., Russell, B. F., Garland, T., Thompson, D., Skinner, P., and Grover, S.: Innovative techniques for collection of saturated and unsaturated subsurface basalts and sediments for microbiological characterization, J. Microbiol. Meth., 15, 279–292, doi:10.1016/0167-7012(92)90047-8, 1992.
- Dahm, T., Hrubcová, P., Fischer, T., Horálek, J., Korn, M., Buske, S., and Wagner, D.: Eger Rift ICDP: an observatory for study of non-volcanic, mid-crustal earthquake swarms and accompanying phenomena, Sci. Dril., 16, 93–99, doi:10.5194/sd-16-93-2013, 2013.
- Deep Carbon Observatory: https://deepcarbon.net//, last access: 14 April 2015.
- Edgecomb, V. P., Beaudoin, D., Gast, R., Biddle, J. F., and Teske, A.: Marine subsurface eukaryotes: the fungal majority, Environ. Microbiol., 13, 172–183, doi:10.1111/j.1462-2920.2010.02318.x, 2011.
- Ekendahl, S., O'Neill, A. H., Thomson, E., and Pedersen, K.: Characterisation of yeasts isolated from deep igneous rock

aquifers of the Fennoscandian shield, Microb. Ecol., 46, 416–428, doi:10.1007/s00248-003-2008-5, 2003.

- Engelhardt, T., Kallmeyer, J., Cypionka, H., and Engelen, B.: High virus-to-cell ratios indicate on-going production of viruses in deep subsurface sediments, ISME J., 8, 1503–1509, doi:10.1038/ismej.2013.245, 2014.
- Eydal, H. S. C., Jagevall, S., Hermansson, M., and Pedersen, K.: Bacteriophage lytic to Desulfovibrio aespoeensis isolated from deep groundwater, ISME J., 3, 1139–1147, doi:10.1038/ismej.2009.66, 2009.
- Gaidos, E., Marteinsson, V., Thorsteinsson, T., Jóhannesson, T., Rúnarsson, A. R., Stefansson, A., Glazer, B., Lanoil, B., Skidmore, M., Han, S., Miller, M., Rusch, A., and Foo, W.: An oligarchic microbial assemblage in the anoxic bottom waters of a volcanic subglacial lake, ISME J., 3, 486–497, doi:10.1038/ismej.2008.124, 2009.
- GFZ German Research Centre for Geosciences: http://www. gfz-potsdam.de/en/home/, last access: 14 April 2015.
- Ginsburg-Karagitscheva, T. L.: Microbiological research in the sulphurous and salty waters of Apsheron, Azerbajdzanskoe Neftjanoe Khozjajstvo, Nos. 6–7, 1926.
- Gubernatorova, T. N. and Dolgonosov, B. M.: Modeling the biodegradation of multicomponent organic matter in an aquatic environment: 3. Analysis of lignin degradation mechanisms, Water Resources, 37, 332–346, doi:10.1134/S0097807810030085, 2010.
- Gupta, H., Nayak, S., Ellsworth, W., Rao, Y. J. B., Rajan, S., Bansal, B. K., Purnachandra Rao, N., Roy, S., Arora, K., Mohan, R., Tiwari, V. M., Satyanarayana, H. V. S., Patro, P. K., Shashidhar, D., and Mallika, K.: Probing reservoir-triggered earthquakes in Koyna, India, through scientific deep drilling, Sci. Dril., 18, 5–9, doi:10.5194/sd-18-5-2014, 2014.
- Head, I. M., Jones, D. M., and Larter, S. R.: Biological activity in the deep subsurface and the origin of heavy oil, Nature, 426, 344–352, doi:10.1038/nature02134, 2003.
- Heim, C.: Terrestrial deep biosphere, in: Encyclopedia of geobiology, edited by: Reitner, J., Thiel, V., and Dordrecht, The Netherlands, Springer Science+Business Media B.V., 871–876, 2011.
- Horsfield, B., Kieft, T. L., Amann, H., Franks, S. G., Kallmeyer, J., Mangelsdorf, K., Parkes, R. J., Wagner, D., Wilkes, H., and Zink, K.-G.: The GeoBiosphere, in: Continental scientific drilling: A decade of progress and challenges for the future, edited by: Harms, U., Koeberl, C., and Zoback, M. D., Berlin, Heidelberg, New York, Springer, 163–211, 2007.
- Horsfield, B., Knebel, C., Ludden, J., and Hyndman, R. (Eds.): Unraveling the complexities of planet earth: science plan for 2014–2019, Potsdam, Germany, International Continental Scientific Drilling Program, 2014.
- International Continental Scientific Drilling Program: http://www. icdp-online.org/, last access: 14 April 2015.
- International Ocean Discovery Program: http://www.iodp.org/, last access: 14 April 2015.
- Kallmeyer, J. and Kieft, T. L.: The ubiquitous hidden biosphere, in: unraveling the complexities of planet earth: science plan for 2014–2019, edited by: Horsfield, B., Knebel, C., Ludden, J., and Hyndman, R., Potsdam, Germany, International Continental Scientific Drilling Program, 56–65, 2014.
- Kelemen, P., Al Rajhi, A., Godard, M., Ildefonse, B., Köpke, J., MacLeod, C., Manning, C., Michibayashi, K., Nasir, S., Shock,

E., Takazawa, E., and Teagle, D.: Scientific drilling and related research in the Samail ophiolite, Sultanate of Oman. Sci. Dril., 15, 64–71, doi:10.2204/iodp.sd.15.10.2013, 2013.

- Kieft, T. L.: Sampling the deep sub-surface using drilling and coring techniques, in: Microbiology of hydrocarbons and lipids, edited by: Timmis, K. N., Berlin, Springer Verlag, 3427–3441, 2010.
- Kieft, T. L.: Microbiology of the deep continental biosphere. Ch. 6, in: Advances in environmental microbiology, Volume 1, Their world: a diversity of environments, edited by: Hurst, C. J., New York, Springer, in press, 2015a.
- Kieft, T. L.: Sampling the subsurface, in: Hydrocarbons and lipid microbiology, edited by: McGenity, C. J., Timmis, K. M., and Nogales, B., Heidelberg, Springer Protocols Handbooks, Springer-Verlag, in press, 2015b.
- Kieft, T. L., Phelps, T. J., and Fredrickson, J. K.: Drilling, coring, and sampling subsurface environments, in: Manual of environmental microbiology, Third Edition, edited by: Hurst, C. J., Washington, D. C., ASM Press, 799–817, 2007.
- Krumholz, L. R., McKinley, J. P., Ulrich, G. A., and Suflita, J. M.: Confined subsurface microbial communities in Cretaceous rock, Nature, 386, 64–66, doi:10.1038/386064a0, 1997.
- Kyle, J. E., Eydal, H. S. C., Ferris, F. G., and Pedersen, K.: Viruses in granitic groundwater from 69 to 450 m depth of the Äspö hard rock laboratory, Sweden, ISME J., 2, 571–574, doi:10.1038/ismej.2008.18, 2008.
- Lau, M. C. Y., Cameron, C., Magnabosco, C., Brown, C. T., Schilkey, F., Grim, S., Hendrickson, S., Pullin, M., Sherwood Lollar, B., van Heerden, E., Kieft, T. L., and Onstott, T. C.: Phylogeny and phylogeography of functional genes shared among seven terrestrial subsurface metagenomes reveal N-cycling and microbial evolutionary relationships, Front. Microbiol., 5, 531, doi:10.3389/fmicb.2014.00531, 2014.
- Lavalleur, H. J. and Colwell, F. S.: Microbial characterization of basalt formation waters targeted for geological carbon sequestration, FEMS Microbiol. Ecol., 85, 62–73, doi:10.1111/1574-6941.12098, 2013.
- Lin, L. H., Wang, P.-L., Rumble, D., Lippmann-Pipke, J., Boice, E., Pratt, L. M., Sherwood Lollar, B., Brodie, E., Hazen, T., Andersen, G., DeSantis, T., Moser, D. P., Kershaw, D., and Onstott, T. C.: Long term biosustainability in a high energy, low diversity crustal biome, Science, 314, 479–482, doi:10.1126/science.1127376, 2006.
- Magnabosco, C., Tekere, M., Lau, M. C. Y., Linage, B., Kuloyo, O., Erasmus, M., Cason, E., van Heerden, E., Borgonie, G., Kieft, T. L., and Onstott, T. L.: Comparisons of the composition and biogeographic distribution of the bacterial communities occupying South African thermal springs with those inhabiting deep subsurface fracture water, Front. Microbiol., 5, 1–17, doi:10.3389/fmicb.2014.00679, 2014.
- McLellan, S. L., Newton, R. J., Vandewalle, J. L., Shanks, O. C., Huse, S. M., Murat Eren, A., and Sogin, M. L.: Sewage reflects the distribution of human faecal Lachnospiraceae, Environ. Microbiol., 15, 2213–2227, doi:10.1111/1462-2920.12092, 2013.
- Mormile, M. R., Biesen, M. A., Gutierrez, M. C., Ventosa, A., Pavolich, J. B., Onstott, T. C., and Fredrickson, J. K.: Isolation of *Halobacterium salinarum* retrieved directly from halite brine inclusions, Environ. Microbiol., 5, 1094–1102, doi:10.1046/j.1462-2920.2003.00509.x, 2003.

- Nagano, Y., Nagahama, T., Hatada, Y., and Nunoura, T.: Fungal diversity in deep-sea sediments – the presence of novel fungal groups, Fungal Ecol., 3, 316–325, doi:10.1016/j.funeco.2010.01.002, 2010.
- National Science Foundation: EarthLab, NSF-sponsored report of underground opportunities in GeoSciences and GeoEngineering, Washington, DC, National Science Foundation, 2003.
- Nyyssönen, M., Bomberg, M., Kapanen, A., Nousiainen, A., Pitkänen, P., and Itävaara, M.: Methanogenic and sulphate-reducing microbial communities in deep groundwater of crystalline rock fractures in Olkiluoto, Finland, Geomicrobiol. J., 29, 863–878, doi:10.1080/01490451.2011.635759, 2012.
- Pedersen, K.: Microbial life in deep granitic rock, FEMS Microbiol. Rev., 20, 399–414, 1997.
- Pedersen, K., Aringer, J., Eriksson, S., Hallbeck, A., Hallbeck, L., and Johansson, J.: Numbers, biomass and cultivable diversity of microbial populations relate to depth and borehole-specific conditions in groundwater from depths of 4–450 m in Olkiluoto, Finland, ISME J., 2, 760–775, doi:10.1038/ismej.2008.43, 2008.
- Phelps, T. J., Fliermans, C. B., Garland, T. R., Pfiffner, S. M., and White, D. C.: Methods for recovery of deep subsurface sediments for microbiological studies, J. Microbiol. Meth., 9, 267– 280, doi:10.1016/0167-7012(89)90069-9, 1989.
- Reitner, J., Schumann, G. A., and Pedersen, K.: Fungi in subterranean environments, in: Fungi in Biogenchemical Cycles, edited by: Gadd, G. J., Cambridge, Cambridge University Press, 788– 1002, 2005.
- Russell, B. F., Phelps, T. J., Griffin, W. T., and Sargent, K. A.: Procedures for sampling deep subsurface microbial communities in unconsolidated sediments, Ground Water Monitor Rev., 12, 96– 104, doi:10.1111/j.1745-6592.1992.tb00414.x, 1992.
- Sahl, J. W., Schmidt, R., Swanner, E. D., Mandernack, K. W., Templeton, A. S., Kieft, T. L., Smith, R. L., Sanford, W. E., Callaghan, R. L., Mitton, J. B., and Spear, J. R.: Subsurface microbial diversity in deep-granitic fracture water in Colorado, Appl. Environ. Microbiol., 74, 143–152, doi:10.1128/AEM.01133-07, 2008.
- Salter, S., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., Turner, P., Parkhill, J., Loman, N., and Walker, A. W.: Reagent contamination can critically impact sequence-based microbiome analyses, BioRxiv, 87, 1–12, doi:10.1101/007187, 2014.
- Sinclair, J. L. and Ghiorse, W. C.: Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments, Geomicrobiol. J., 7, 15–31, 1989.
- Sleep, N. H., Meibom, A., Fridriksson, T., Coleman, R. G., and Bird, D. K.: H₂-rich fluids from serpentinization: geochemical and biotic implications, P. Natl. Acad. Sci. USA, 101, 12818– 12823, doi:10.1073/pnas.0405289101, 2004.

- Smith, D. C., Spivack, A. J., Fisk, M. R., Haveman, S. A., Staugudigel, H., and Ocean Drilling Program Leg 185 Shipboard Scientific Party: Tracer-based estimates of drilling-induced microbial contamination of deep sea crust, Geomicrobiol. J., 17, 207–219, doi:10.1080/01490450050121170, 2000a.
- Smith, D. C., Spivack, A. J., Fisk, M. R., Haveman, S. A., Staugudigel, H., and Ocean Drilling Program Leg 185 Shipboard Scientific Party: Methods for quantifying potential microbial contamination during deep ocean coring, ODP Technical Note 28, 2000b.
- Stan-Lotter, H. and Frendihan, S.: Deep biosphere of salt deposits, in: Encyclopedia of geobiology, edited by: Reitner, J. and Thiel, V., Dordrecht, The Netherlands, Springer Science+Business Media B.V., doi:10.1007/978-1-4020-9212-1, 2011.
- Stevens, T. O. and McKinley, J. P.: Lithoautotrophic microbial ecosystems in deep basalt aquifers, Science, 270, 450–454, 1995.
- Thomas, J. M., Moser, D. P., Fisher, J. C., Reihle, J., Wheatley, A., Hershey, R. L., Baldino, C., and Weissenfluh, D.: Using water chemistry, isotopes and microbiology to evaluate groundwater sources, flow paths and geochemical reactions in the Death Valley flow system, USA, Procedia Earth and Planetary Science, 7, 842–845, doi:10.1016/j.proeps.2013.03.033, 2013.
- Wang, P.-L., Lin, L.-H., Yu, H.-T., Cheng, T.-W., Song, S.-R., Kuo, L.-W., Yeh, E.-C., Lin, W., and Wang, C.-Y.: Cultivation-based characterization of microbial communities associated with deep sedimentary rocks from Taiwan Chelungpu Drilling Project cores, Terr. Atmos. Ocean. Sci., 18, 395–412, doi:10.3319/TAO.2007.18.2.395(TCDP), 2007.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J.: Prokaryotes: the unseen majority, P. Natl. Acad. Sci. USA, 95, 6578–6583, 1998.
- Wilkins, M. J., Daly, R., Mouser, P. J., Trexler, R., Wrighton, K. C., Sharma, S., Cole, D. R., Biddle, J. F., Denis, E., Fredrickson, J. K., Kieft, T. L., Onstott, T. C., Petersen, L., Pfiffner, S. M., Phelps, T. J., and Schrenk, M. O.: Trends and Future Challenges in Sampling the Deep Terrestrial Biosphere, Frontiers Microbiol., 5, 481, doi:10.3389/fmicb.2014.00481, 2014.
- Wrighton, K. C., Thomas, B. C., Sharon, I., Miller, C. S., Castelle, C. J., VerBerkmoes, N. C., Wilkins, M. J., Hettich, R. L., Lipton, M. S., Williams, K. H., Long, P. E., and Banfield, J. F.: Fermentation, hydrogen and sulfur metabolism in multiple uncultivated bacterial phyla, Science, 337, 1661–1665, doi:10.1126/science.1224041, 2012.
- Zoback, M. D. and Emmermann, R. (Eds.): Scientific rationale for the establishment of an international program of continental scientific drilling, Potsdam, International Lithosphere program, Coordinated Committee Continental Drilling (CC4), 150 pp., 1994.
- Zobell, C. E.: The role of bacteria in the formation and transformation of petroleum hydrocarbons, Science, 102, 364–369, 1945.