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IODP ADVANCES IN THE UNDERSTANDING OF SUBSEAFLOOR LIFE

By Steven D'Hondt, Fumio Inagaki, Beth N. Orcutt, and Kai-Uwe Hinrichs



CORK borehole observatory deployment on *JOIDES Resolution* during IODP Expedition 336 Mid-Atlantic Microbiology. Photo credit: William Crawford, IODP-USIO

ABSTRACT. The most recent decadal phase of scientific ocean drilling through the International Ocean Discovery Program (IODP) has resulted in paradigm-shifting understanding of life below the seafloor. Enabled by new drilling and coring approaches, cutting-edge methodologies, and novel observatory science, IODP expeditions have significantly advanced understanding of the amount and diversity of subseafloor life, the metabolic strategies that this life uses to survive under extreme energy limitation, and consequences of this life for the Earth system. Here, we summarize highlights from recent IODP expeditions focused on life beneath the seafloor and emphasize remaining major science challenges in investigating the form and function of life in this environment.

INTRODUCTION

Study of subseafloor life is a central objective of the International Ocean Discovery Program (IODP) and its immediate predecessor, the Integrated Ocean Drilling Program. Exploration of “Biosphere Frontiers,” one of four guiding themes of the IODP Science Plan 2013–2023 (IODP, 2011), includes three challenges: What are the origin, composition, and global significance of subseafloor communities? What are the limits of life in the subseafloor? How sensitive are ecosystems and biodiversity to environmental change?

The first two challenges focus solely on subseafloor life. They guide the biological objectives of individual drilling expeditions. They also guide IODP decisions about shipboard facilities, sampling procedures, and shipboard measurements. Within the context of the current IODP Science Plan, the ultimate objective is to use scientific ocean drilling to advance understanding of:

- The composition and diversity of subseafloor communities, the processes by which they are established, and the ease by which they disperse and find new resources
- The physical and chemical limits to life in the subseafloor, including mechanisms that microbes use to generate energy and fix carbon far from the influence of Earth’s surface (photosynthetic) environments (IODP, 2011).

A wide range of projects has used Deep Sea Drilling Project (DSDP), Ocean Drilling Program (ODP), and IODP samples and data to address these challenges. Most have been scientific ocean drilling

expeditions dedicated to study of subseafloor life and the deep biosphere. A few have been ancillary programs on expeditions with other central foci (such as paleoceanography). Other projects have used archived data and/or samples from DSDP, ODP, and IODP to identify global patterns of subseafloor life and habitability. Here, we describe some of the major discoveries that have resulted from these scientific ocean drilling projects, beginning with dedicated expeditions and ending with studies that mined samples or data.

PRE-IODP EXPLORATION OF SUBSEAFLOOR LIFE

Deliberate exploration of subseafloor life began during DSDP. A serious program of DSDP porewater chemical measurements began with DSDP Leg 15 (Caribbean Sea) in 1970 (Broecker, 1973). Within a few years, concentration profiles and stable isotopic signatures of methane (CH_4) and sulfate (SO_4^{2-}) provided the first evidence that microbial activity occurs deep in marine sediment, even at burial depths as great as 800 m and in sediment that has been buried for tens of millions of years (Claypool and Kaplan, 1974). Subsequently, direct measurements of CH_4 concentrations in incubations of DSDP Leg 64 (Gulf of California) sediment showed that methane is produced in sediment recovered from depths as great as 12 meters below seafloor (mbsf) (Oremland et al., 1982). This demonstration was bolstered by the discovery of organic molecules typical of methanogens in the sediment of a Leg 64 site (Thomson et al., 1982). On

the last two DSDP expeditions (Legs 95 and 96), radiotracer incubations were introduced to the shipboard scientific arsenal to identify sulfate-reducing and methanogenic activity in sediment recovered from depths as great as 167 mbsf in the Gulf of Mexico (Whelan et al., 1986) and the New Jersey Margin (Tarafa and Whelan, 1987). A deliberate objective of these DSDP radiotracer studies was to develop methodology for microbiological experiments on future ODP expeditions (Whelan et al., 1986).

Early in ODP, direct counts on core samples from multiple expeditions showed microbial cells to be ubiquitous in subseafloor sediment throughout much of the ocean (Parkes et al., 1994). ODP radiotracer studies showed that SO_4^{2-} reduction, CH_4 production, and CH_4 oxidation occur in continental margin sediment throughout the world ocean (Parkes et al., 2000). Research with contamination tracers during Leg 185 (Izu-Mariana Margin) showed the influence of drilling contamination to be so low that the vast majority of cells in ODP piston cores are indigenous to the sediment (D.C. Smith et al., 2000).

Other studies of ODP core samples suggested that microbial life and activity might be ubiquitous in the igneous crust that underlies marine sediment. Fisk and colleagues (1998) showed that altered surfaces of subseafloor basalt from DSDP and ODP core samples from the Atlantic, Pacific, and Indian Oceans display textures suggestive of microbial colonization. Torsvik and coauthors (1998) found evidence of DNA in the altered surfaces of subseafloor lava from ODP Hole 896A (Costa Rica Rift). Cowen and colleagues (2003) sampled the hydrologic observatory at ODP Hole 1026B (Juan de Fuca Ridge flank) to discover that bacteria and archaea are abundant in warm water flowing through subseafloor basalt. Other studies of the same site documented connectivity between microbial communities in the sediment and communities in the underlying basement (Engelen et al.,

2008) and identified the predominant origin of dissolved organic carbon in the basaltic aquifer from microbial communities that subsist on inorganic chemicals (McCarthy et al., 2011).

ODP Leg 201, the first ocean drilling expedition dedicated solely to study of seafloor life, occurred late in ODP (2002). It laid the foundation for IODP studies of seafloor life by bringing large numbers of microbiologists and biogeochemists into the field and advancing understanding on multiple fronts. Leg 201 explored the seafloor sedimentary ecosystems of the Peru margin and the equatorial Pacific. The expedition showed that seafloor sedimentary communities are characterized by diverse metabolic activities (D'Hondt et al., 2004), including previously unknown activities (microbial production of ethane and propane; Hinrichs et al., 2006), and by diverse bacterial and archaeal taxa (D'Hondt et al., 2004; Inagaki et al., 2006). Most of the sedimentary cells were found to have been active recently enough that they contained detectable RNA concentrations (Schippers et al., 2005). Study of the bulk RNA pool in sediment samples from Peru margin Site 1227 indicated that the active community in the seafloor sulfate-reducing methane-oxidizing zone is very different than the active communities in the sediment above and below that zone (Sørensen and Teske, 2006). Both Bacteria (Schippers et al., 2005; Schippers and Neretin, 2006) and Archaea (Biddle et al., 2006, 2008; Lipp et al., 2008) are abundant in Leg 201 sediment. Although cell abundance and community metabolic activity generally decrease exponentially with sediment depth, cell concentrations and rates of potential activities can be much higher at seafloor sulfate-methane transitions than in the overlying or underlying sediment (Parkes et al., 2005).

For more than a decade, study of archived samples and data from Leg 201 has continued to advance understanding of seafloor life. For example, organic geochemical study of Leg 201 samples stored at -80°C showed that endospores

(compositionally distinct dormant bacterial cells) are as abundant as vegetative (metabolically active) cells in Peru margin sediment, and amino-acid racemization (molecular handedness) data indicate that microbial biomass turnover times range from centuries to millennia (Lomstein et al., 2012). Study of community RNA signatures in archived Leg 201 samples indicated that seafloor sedimentary microbes engage in a wide range of metabolic activities, repair DNA, undertake some degree of cell division, and span all three domains of life (Bacteria, Archaea, Eukarya) (Orsi et al., 2013).

A few studies used data or samples from many DSDP and ODP expeditions to advance understanding of seafloor life. D'Hondt and colleagues (2002) integrated chemical and microbiological data from DSDP and ODP expeditions through ODP Leg 182 to show that most seafloor catabolic activity occurs in a relatively narrow zone of sulfate-reducing methane oxidation along continental margins, that methane is produced in anoxic sediment throughout the world ocean (whether dissolved sulfate is present or absent), and that per-cell respiration rates are orders of magnitude lower in seafloor sediment than in the surface world. Bach and Edwards (2003) analyzed data from multiple DSDP and ODP sites to infer that most oxidation of seafloor igneous crust occurs within 10–20 million years following crust formation at mid-ocean-ridge spreading centers. Their quantification of basaltic-crust oxidation rates indicates that water-rock reactions may support a significant quantity of microbial life within mid-ocean-ridge flank systems. Inagaki and colleagues (2006) used genomic techniques to identify bacterial and archaeal taxa in seafloor sediment samples from three different regions of the ocean—the eastern equatorial Pacific (ODP Leg 201), the Peru margin (Leg 201) and the Cascadia margin (ODP Leg 204). Their results significantly advanced understanding of seafloor biogeography by demonstrating that

(1) similar seafloor sedimentary environments in distant parts of the ocean contain similar microbial communities, and that (2) the communities of different seafloor environments (hydrate-bearing sediment vs. hydrate-free sediment) differ significantly.

MAJOR IODP SUBSURFACE LIFE DISCOVERIES

Life in Seafloor Sediment

There are many good reasons to study microbial life in seafloor sediment. Although the community structure, metabolic interactions, and origin of seafloor sedimentary communities are poorly known, sediment chemistry studies show that they strongly influence Earth's near-surface biogeochemical cycles (Bowles et al., 2014). In affecting these cycles, seafloor sedimentary communities directly affect Earth's climate and surface oxidation state. These communities provide an accessible model for subsurface sedimentary life on other worlds. The environmental variation in seafloor sediment provides exciting opportunities to test limits to life on Earth (Morita and Zobell, 1955; LaRowe et al., 2017).

During IODP, several expeditions dedicated to the study of seafloor life, some expeditions of opportunity, and several post-expedition syntheses of IODP data and samples have focused on (1) documenting the composition, global significance, and origin of microbial life in seafloor sediment; and (2) testing the limits to life in seafloor sediment. We briefly describe some of these scientific ocean drilling expeditions and their primary discoveries below.

The Origin and Composition of Seafloor Sedimentary Communities (Terrestrial Life Deep Beneath the Seafloor)

IODP Expedition 337 in 2012 discovered the most deeply buried seafloor microbial communities to date (Inagaki et al., 2015; Figure 1). Using the riser drilling technology of *D/V Chikyu*,

Expedition 337 cored more deeply than any previous scientific ocean drilling expedition—2,466 mbsf—to study microbial life and biogeochemical processes in terrigenous coal and shale underlying marine sediment off Shimokita, Japan (Inagaki et al., 2015). In doing so, the expedition yielded fundamental insight about the origin and persistence of deep subseafloor communities.

The microorganisms that inhabit the ~20 million-year-old subseafloor coalbeds are most closely related to lineages found in peat or modern tropical forest soil (Inagaki et al., 2015). Furthermore, diverse fungal species (Ascomycota and Basidiomycota) related to terrestrial wood-rotting fungi were isolated from a thin layer of soft brown coal at 2,457 mbsf, interbedded with sand (Liu et al., 2016). These coal-associated microbial communities are highly distinct from commonly observed subseafloor sedimentary microbes, which are typically of marine origin. This discovery suggests that the in

situ communities are relicts of the original depositional communities that inhabited continental soils or swamps ~20 million years ago, rather than microbial migrants into the coal from the ocean or elsewhere in the subseafloor.

Geochemical and microbiological data indicate that this coal-associated deep subseafloor ecosystem contains heterotrophs that may be active in situ (Inagaki et al., 2015; Glombitza et al., 2016). Post-expedition cultivation experiments show that microbes from the 2 km deep sediment are capable of feeding on simple organic compounds, but are characterized by biomass generation times exceeding hundreds of years, even under laboratory incubation conditions (Trembath-Reichert et al., 2017; Figure 2). Position-specific analysis of stable carbon isotopes of lignite-derived methoxy groups (methyl groups bound with oxygen) suggests their transformation into methane by methanogenic communities over geologic time (Lloyd, 2018).

The Limit to Life in Subseafloor Sediment

IODP Expedition 329 (2010) showed that there is no limit to sedimentary life in the largest ocean desert (D’Hondt et al., 2015). More than 60 years ago, Morita and ZoBell (1955) studied shallow gravity cores to report “the lower limits of the biosphere” at 3.9–7.5 mbsf in abyssal clay of the oligotrophic North Pacific Gyre. Expedition 329 set out to test their claim in subseafloor clay of the most oligotrophic region in the world ocean—the South Pacific Gyre. Expedition 329 found that low concentrations of microbial cells and low rates of microbial respiration occur throughout the sediment column in this region, even in sediment deposited more than 100 million years ago (D’Hondt et al., 2015). The sediment is so thin and respiration rates are so low that dissolved oxygen penetrates the entire sediment column, from seafloor to the sediment/basement interface (Figure 3). As in anoxic sediment

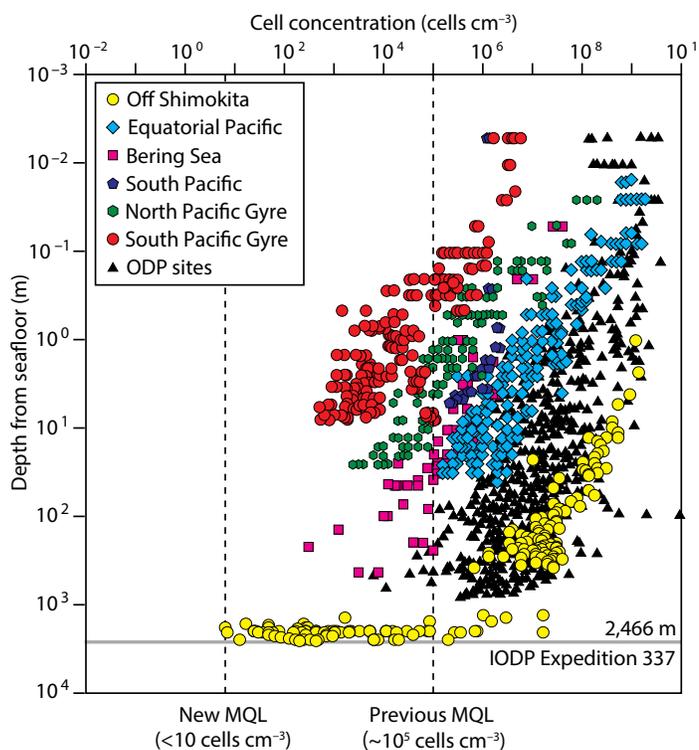


FIGURE 1. Cell concentrations in marine sediment. The yellow dots clustered around 2,466 meters below seafloor line identify cell concentrations in the deepest subseafloor sediment samples examined for life to date (IODP Expedition 337 Site C0020; Inagaki et al., 2015). MQL = minimum quantification limit for sedimentary microbial cells.

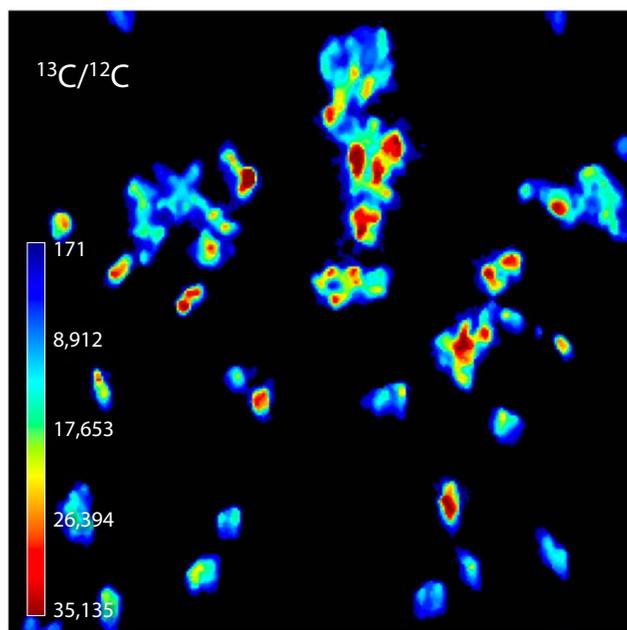


FIGURE 2. A NanoSIMS image of subseafloor sedimentary cells that incorporated ^{13}C from a mixture of stable isotope (^{13}C)-labeled amino acids, showing that microbial life in ~2 km deep coal formation is metabolically active (see Table T13 in Inagaki et al., 2013; Trembath-Reichert et al., 2017). The color gradient represents ^{13}C abundance expressed as $^{13}\text{C}/^{12}\text{C}$. Figure courtesy of Yuki Morono and Motoo Ito, JAMSTEC

and in the ocean, abundances of virus-like particles exceed microbial cell abundances by one to two orders of magnitude (Engelhardt et al., 2014).

As described above, Expedition 337 demonstrated that microbial life extends more than 2 km beneath the ocean

floor in thick nearshore sediment (off Shimokita, Japan). To quantify microbial cells at extremely low concentrations so deep beneath the seafloor, novel techniques were established to improve cell enumeration by four to five orders of magnitude relative to the conventional

manual counting techniques (Morono et al., 2013; Morono and Inagaki, 2016). The resulting concentrations were $\sim 10^2$ to 10^3 cells cm^{-3} , with local peaks in the brown coal horizons. These low concentrations suggest that this deeply buried sediment, with moderately elevated temperatures of 40°C to 60°C, may be situated close to the limit of habitability. Based on thermal data (Tanikawa et al., 2016), temperature-related increases in energetic costs of biomolecule repair may place an important constraint on the viability and size of subseafloor communities (Inagaki et al., 2015). It is also conceivable that physical factors such as low porosity, low permeability, and low free-water availability influence key fluxes of fluids, nutrients, and waste products in this semi-closed sedimentary basin (Tanikawa et al., 2018).

Temperature is commonly hypothesized to estimate the lower boundary of the deep biosphere (LaRowe et al., 2017). The currently known high-temperature limit of life for microorganisms from energy-rich hydrothermal vents is 122°C (Takai et al., 2008). However, little is known about the temperature limit to subseafloor life. Two IODP expeditions have focused on this issue. IODP Expedition 331 (2010) tackled this challenge by drilling in the Iheya North hydrothermal field, which lies within the Okinawa Trough, an actively spreading back-arc basin. At Expedition 331 sites, evidence of subseafloor microbial communities was limited to the first 10–30 mbsf, which were characterized by relatively cool temperatures (less than a few tens of degrees Celsius). The absence of identifiable life at greater depths was interpreted as possibly due to recent sterilization by intense subseafloor hydrothermal activity (Brandt and House, 2016; Yanagawa et al., 2016).

To extensively study the influence of temperature in a more stable subseafloor environment, IODP Expedition 370 (2016) cored a 1.2 km thick sediment sequence where the thermal gradient is much less steep, but temperature reaches

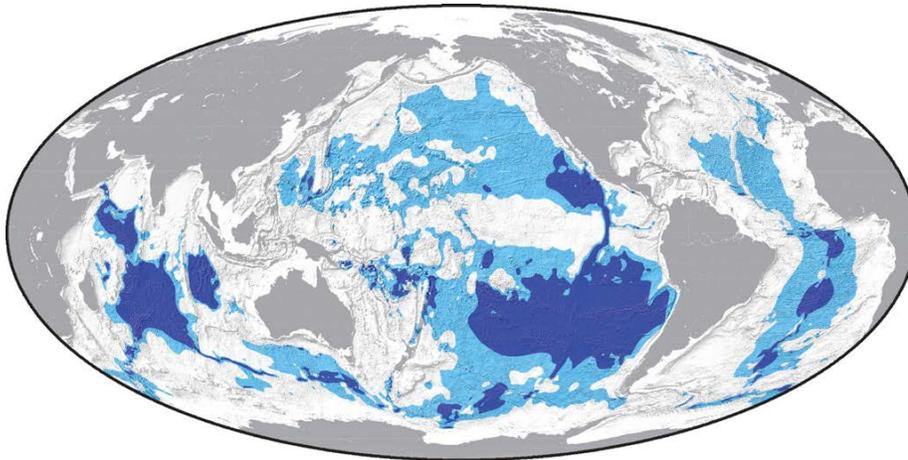


FIGURE 3. Geographic distributions of predominant net electron-accepting activities in subseafloor sediment. Sulfate reduction is the predominant net electron-accepting activity in the white regions. Oxygen reduction is the dominant net electron-accepting activity from seafloor to the sediment/basement interface in the dark blue regions and perhaps the light blue regions. *Figure modified from D'Hondt et al. (2015)*

How hot is too hot for Earth-style life?

International research mission seeks temperature limit to life deep beneath the seafloor

D/V Chikyu: World's largest scientific research vessel, capable of sampling to 7,000 meters below the seafloor.

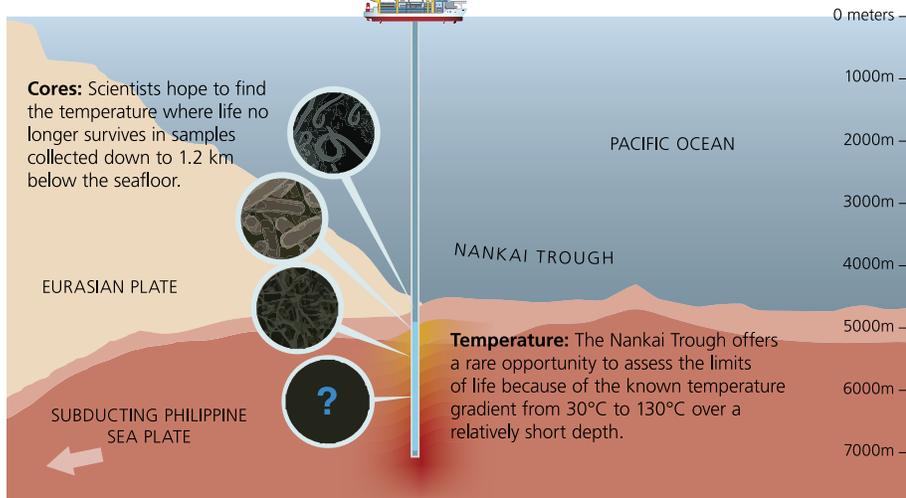


FIGURE 4. How deep is Earth's habitable zone? What are the factors that limit life's maximum depths? IODP Expedition 370 "Temperature-Limit of the Deep Biosphere off Muroto (T-Limit)" tackled these fundamental questions by drilling through the sediments at Site C0023 in the central Nankai Trough off Cape Muroto, Japan (Hinrichs et al., 2016; Heuer et al., 2017). Anomalously high heat flow regimes in this area result in temperature of 120°C at the sediment-basement interface and make the site an ideal target for in-depth examination of subseafloor microbial life close its upper temperature limit. *Figure courtesy of Deep Carbon Observatory and JAMSTEC*

~120°C at the sediment-basement interface (Hinrichs et al., 2016; Heuer et al., 2017; Figure 4). This sequence is in a well-characterized geological setting—along the Muroto Transect in the Nankai Trough off Japan. The coring site, IODP Site C0023, is located at the seaward end of the Nankai Trough accretionary prism, where the Shikoku Basin subducts beneath Japan. The 1.2 km of recovered seafloor sediment thus spanned the range of suitable conditions for psychrophilic (optimal growth temperature range <20°C), mesophilic (20°C to 43°C), thermophilic (43°C to 80°C), and hyperthermophilic (>80°C) microorganisms. In order to effectively study very low-biomass communities at the limit of the biosphere, Expedition 370 researchers made an unprecedented effort to trace and minimize contamination of core samples, including in the processes of X-ray CT scans of cores before sampling and super-clean shore-based subsampling technologies (Heuer et al., 2017; Morono et al., 2018). Selected samples were transported daily by helicopter to the Kochi Core Center for microbiological analyses. In 2018, scientists used JAMSTEC's R/V *Kairei* and remotely operated vehicle (ROV) *Kaiko* to recover a 1.5 year record of in situ temperature data from an observatory installed in the drill hole. The data and samples from Expedition 370 and the post-drilling ROV expedition are now being analyzed to pinpoint the thermal limit to seafloor sedimentary life.

The Composition and Global Significance of Life in Subseafloor Sediment

Several studies have used IODP data or samples from expeditions of opportunity to advance understanding of seafloor sedimentary communities. For example, participants in IODP Expedition 317 (2010) found microbial communities present to nearly 2 km below seafloor in the Canterbury Basin (New Zealand) that contained members from all three domains of life (Ciobanu et al., 2014).

Bering Sea IODP Expedition 323 (2009) scientists showed that taxonomic richness of marine sedimentary communities declines exponentially with sediment age and organic oxidation rate (Walsh et al., 2016). Other studies have used IODP data or samples from multiple expeditions to quantify global distributions of several key properties of seafloor ecosystems, including microbial abundance and biomass in sediment (Kallmeyer et al., 2012), sedimentary microbial activities (Bowles et al., 2014; D'Hondt et al., 2015), potential thermal limits to sedimentary life (LaRowe et al., 2017), and relative abundances and diversities of Bacteria and Archaea (Hoshino and Inagaki, 2018). The last study found archaea to comprise 37% of the prokaryotic (bacterial and archaeal) cells in seafloor sediment (similar to their relative abundance in the deep ocean).

Life in Subseafloor Crust

A rocky crust consisting mainly of basalt, but also including gabbro, peridotite, and serpentinite, exists beneath the sediment that blankets most of the seafloor. This rocky crust is a potentially expansive habitat for life (Edwards et al., 2011, 2012a; Orcutt et al., 2011b). However, before the current phase of the scientific ocean drilling program, there were few studies of microbial life and its consequences in this environment. Uncovering whether and how life exists in oceanic crust is important because (1) chemical reactions in the crust impact broader ocean systems, (2) it is a great analog for extraterrestrial life on other ocean worlds, and (3) it will greatly advance understanding of microbial mechanisms for survival between a “rock and a hard place” (IODP, 2011).

In the current phase of IODP, several major expeditions and observatory programs have focused on documenting the nature, extent, and function of microbial life in oceanic crust, augmented by additional studies on expeditions of opportunity. We briefly describe these scientific ocean drilling expeditions and their primary discoveries below.

The Composition and Origin of Subseafloor Crustal Communities

IODP expeditions have targeted the origin and composition of subseafloor life in diverse crustal environments, including warm anoxic aquifers (IODP Expeditions 301, 327) and cool oxic aquifers (IODP Expeditions 329, 336), basalt of the upper crust (Expeditions 327, 329, 330, 336) and gabbro of the lower crust (Expedition 360), young crust (less than 10 million years old; Expeditions 301, 327, 336) and old crust (up to 100 million years old; Expeditions 329, 330), normal seafloor (Expeditions 327, 329, 336, 360), ultramafic crust (Expedition 357), and seamounts (Expedition 330).

Three of these expeditions, 301 (2004), 327 (2010), and 336 (2011) set up observatories for long-term studies of microbial communities in crustal aquifers (Figures 5 and 6). Expeditions 301 (2004) and 327 (2010) built on the long history of research on the Juan de Fuca Ridge (following the earlier work of ODP Leg 168) to examine fluid-flow properties and the nature of life in oceanic crust. Expeditions 301 and 327 drilled multiple holes into basement and installed four



FIGURE 5. CORK borehole observatories, like this one installed by IODP Expedition 336 at the North Pond site on the Mid-Atlantic Ridge, allow access to the deep biosphere in oceanic crust. Photo courtesy of AT39-01 cruise chief scientist C. Geoff Wheat, University of Alaska Fairbanks, US National Science Foundation, ROV Jason, 2017, © Woods Hole Oceanographic Institution

state-of-the-art borehole observatories to enable long-term microbiological studies. In addition, Expedition 327 cored several holes at a location where fluid was suspected to flow into the basement in order to study transitions of microbial communities from seawater, overlying sediment, and within basement (Fisher et al., 2011). Expedition 336 (2011) built on a decades-long history of ocean drilling and observatory science at Hole 395A (DSDP Leg 45) on the western flank of the Mid-Atlantic Ridge (Figure 6). This expedition focused on documenting the nature of life in cool basaltic ocean crust, which constitutes much of the global subseafloor (Edwards et al., 2014). It cored the entire sediment column and upper basaltic basement and installed three borehole observatories.

IODP Expeditions 301, 327, and 336 enabled unprecedented discoveries regarding the composition and structure of subseafloor crustal communities. Direct study of rock recovered by Expedition 301 provided evidence for microbial methane and sulfur cycling within the crust of the Juan de Fuca Ridge flank (Lever et al., 2013). Rock colonization experiments in the 301 and 327 observatories revealed the dynamic nature of microbial biofilms that form on rock surfaces in this warm anoxic habitat (Figure 7). Microbes readily colonize rock

substrates incubated in the subseafloor crustal boreholes (Orcutt et al., 2011a; A. Smith et al., 2011). Temperature plays an important role in structuring the biofilm communities that colonize the incubated substrates (Baquiran et al., 2016). Comparison of the observatory results to sedimentary communities indicates that proximity to basement and/or seawater recharge locations does not impact sedimentary community structure (LaBonté et al., 2017), even though fluid diffuses from the permeable oceanic crust into sediment and stimulates microbial activity (Engelen et al., 2008). The first viral study of subseafloor crust found that, as in marine sediment and seawater, novel archaeon-infecting viruses are an order of magnitude more abundant than the archaeal cells that might possibly host them (Nigro et al., 2017). By measuring nanoscale changes in heat capacity during microbial growth, nanocalorimetry experiments with crustal fluid samples show that these microbial communities have potential for high metabolic rates (Robador et al., 2016).

Expedition 336 studies demonstrated that the subseafloor crustal habitat of the mid-Atlantic North Pond is fundamentally different than the warm anoxic habitat explored by Expeditions 301 and 327 on the Juan de Fuca Ridge flank. The North Pond system is relatively cool and

oxic (Orcutt et al., 2013). Diffusion of oxygen from the basaltic crust into the overlying sediment stimulates a unique nitrogen cycling microbial community within the sediment (Reese et al., 2018). Unlike the Juan de Fuca ecosystem, the North Pond system exhibits significant overlap in microbial community structure between basal sediment and the basaltic rocks below, with both showing dominance of Proteobacteria groups that are also observed on exposed seafloor basalts (Jørgensen and Zhao, 2016). Fluid sampling from the North Pond observatories shows that the ecological structure of the planktonic microbial community in the aquifer is relatively stable, despite large shifts in dominant taxonomic groups over time and space (Tully et al., 2017). Although chemosynthetic carbon fixation could be linked in this ecosystem to oxidation of sulfide, elemental sulfur, thiosulfate, and hydrogen and ferrous iron (Meyer et al., 2016; Tully et al., 2017), biological imprints on the organic-carbon signature of fluid venting into the overlying ocean indicates that organic-fueled respiration exceeds organic production within the ecosystem (Shah Walter et al., 2018). Observatory studies continue at these sites, extending the legacy of deep-biosphere observatory research into a new era. Expedition 336 observatories were revisited in 2017, and

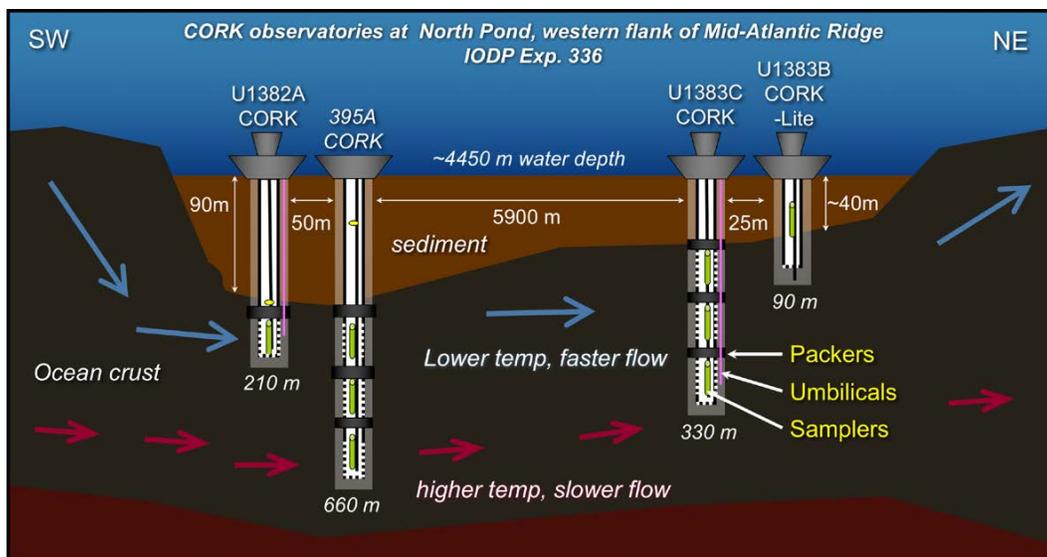


FIGURE 6. Schematic of borehole observatories installed by IODP Expedition 336 to study the deep biosphere in cool oxic oceanic crust. The CORK and CORK-Lite observatories enable microbial colonization experiments at depth within oceanic crust, as well as collection of pristine crustal fluids from depth. Modified with permission of Edwards et al. (2012b)

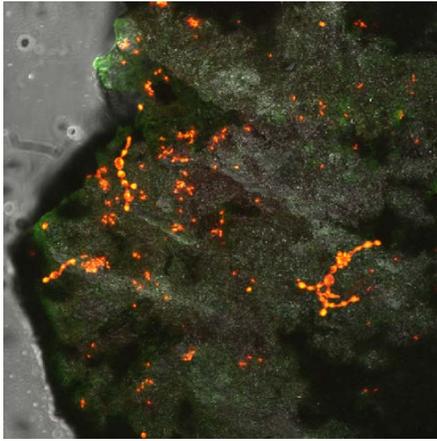


FIGURE 7. Confocal laser scanning micrograph of microbial colonization of mineral incubation experiments in subseafloor oceanic crust on the Juan de Fuca Ridge flank (Orcutt et al. 2011a). The orange dots are individual microbial cells. Image dimensions 175 μm x 175 μm .

Expedition 327 observatories will be visited again in 2019.

Several recent IODP expeditions have explored subseafloor life in crust environments other than the relatively young basaltic crust of Expeditions 301, 327, and 336. Expedition 329 scientists are testing the extent to which microbial communities persist in cool, oxic subseafloor crust of widely different ages, ranging from 13.5 to 104 million years old. Expedition 330 sampled older basaltic crust from submarine extinct volcanoes in the Louisville Seamounts from which a new species of a manganese oxidizing bacillus was isolated (Sylvan et al., 2015). Expedition 357 (2015) collected samples of ultramafic crust from an ocean core complex on the Mid-Atlantic Ridge to examine microbial life associated with serpentinization (Früh-Green et al., 2017). So far, results from this expedition have documented very low biomass in these shallow ultramafic samples from an actively serpentinizing system, in contrast to the elevated density of life in highly altered samples from the nearby Lost City hydrothermal vent field (Früh-Green et al., 2018). Expedition 360 (2016) collected gabbro from the lower oceanic crust at the Southwest Indian Ridge, where initial measurements indicated microbial life

in many of the samples (MacLeod et al., 2017). Continued analyses of these samples will further expand our understanding of the nature, extent, and activity of life in the rocky subseafloor.

FUTURE RESEARCH

As described above, scientific ocean drilling is greatly advancing understanding of the quantity, diversity, and global significance of life in subseafloor sediment. It is also significantly enhancing knowledge of the composition and origin of communities in subseafloor igneous crust. These are major advances in the fundamental understanding of life on Earth as they extend the known biosphere to sediment depths as great as ~2.5 km below seafloor (Inagaki et al., 2015) and to sediment ages older than 100 million years (D'Hondt et al., 2015). These advances also radically challenge understanding of the low-energy limit to life (Hoehler and Jørgensen, 2013), because communities in subseafloor sediment appear to survive at per-cell metabolic rates that are orders of magnitude lower than rates in the surface world (D'Hondt et al., 2002, 2015). Knowledge of the microbial features and strategies that enable survival at these extraordinarily low metabolic rates will ultimately inform understanding of the limits to intraterrestrial life on Earth, the potential for life on other worlds, and the nature of persister cells in bacterial infections.

More complete understanding of subseafloor life and its effects on the world we live in requires a new generation of drilling expeditions and projects. For example, the limits to life remain unknown in both subseafloor sediment and igneous crust, because active microbial communities have been recovered from almost all subseafloor environments cored to date. More complete understanding will also require much closer focus on the diverse ways that organisms interact with each other in subseafloor communities. The global significance of subseafloor crustal communities remains unknown, because the extent to which those communities

drive chemical alteration in their habitats is not yet clear. The processes by which subseafloor communities are established, and the ease by which they disperse and find new resources, are not yet well understood either. Finally, we do not yet fully understand the diverse mechanisms that they use to generate energy and fix carbon far from the influence of Earth's surface (photosynthetic) environments. 

REFERENCES

- Bach, W., and K.J. Edwards. 2003. Iron and sulfide oxidation within the basaltic ocean crust: Implications for chemolithoautotrophic microbial biomass production. *Geochimica et Cosmochimica Acta* 67:3,871–3,887, [https://doi.org/10.1016/S0016-7037\(03\)00304-1](https://doi.org/10.1016/S0016-7037(03)00304-1).
- Baquiran, J.-P., G.A. Ramírez, A.G. Haddad, B.M. Toner, S. Hulme, C.G. Wheat, K.J. Edwards, and B.N. Orcutt. 2016. Temperature and redox effect on mineral colonization in Juan de Fuca Ridge Flank subsurface crustal fluids. *Frontiers in Microbiology* 7:396, <https://doi.org/10.3389/fmicb.2016.00396>.
- Biddle, J.F., J.S. Lipp, M.A. Lever, K.G. Lloyd, K.B. Sorenson, R. Anderson, H.F. Fredricks, M. Elvert, T.J. Kelly, D.P. Schrag, and others. 2006. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proceedings of the National Academy of Sciences of the United States of America* 103:3,846–3,851, <https://doi.org/10.1073/pnas.0600035103>.
- Biddle, J.F., S. Fitz-Gibbon, S.C. Schuster, J.E. Brenchley, and C.H. House. 2008. Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proceedings of the National Academy of Sciences of the United States of America* 105:10,583–10,588, <https://doi.org/10.1073/pnas.0709942105>.
- Bowles, M.W., J.M. Mogollón, S. Kasten, M. Zabel, and K.-U. Hinrichs. 2014. Global rates of marine sulfate reduction rates and implications for subseafloor metabolic activities. *Science* 344(6186): 889–891, <https://doi.org/10.1126/science.1249213>.
- Brandt, L.D., and C.H. House. 2016. Marine subsurface microbial community shifts across a hydrothermal gradient in Okinawa Trough sediments. *Archaea* 2016:2690329, <https://doi.org/10.1155/2016/2690329>.
- Broecker, W.S. 1973. Interstitial water studies, Leg 15, Introduction and Summary. Pp. 1,069–1,073 in *Initial Reports of the DSDP 15*. N.T. Edgr, J.B. Saunders, et al., eds, US Government Printing Office, Washington, DC, <https://doi.org/10.2973/dsdp.proc.15.139.1973>.
- Ciobanu, M.C., G. Burgaud, A. Dufresne, A. Breuker, V. Rédou, S.B. Maamar, F. Gaboyer, O. Vandenabeele-Trambouze, J.S. Lipp, A. Schippers, and P. Vandenkoornhuyse. 2014. Microorganisms persist at record depths in the subseafloor of the Canterbury Basin. *The ISME Journal* 8(7):1,370–1,380, <https://doi.org/10.1038/ismej.2013.250>.
- Claypool, G.E., and I.R. Kaplan. 1974. The origin and distribution of methane in marine sediments. Pp. 99–139 in *Natural Gases in Marine Sediments*. I.R. Kaplan, ed., Plenum Press, New York.

- Cowen, J., S.J. Giovannoni, F. Kenig, H.P. Johnson, D.A. Butterfield, M.S. Rappé, M. Hutnak, and P. Lam. 2003. Fluids from aging ocean crust that support microbial life. *Science* 299 (5603):120–123, <https://doi.org/10.1126/science.1075653>.
- D'Hondt, S., S. Rutherford, and A. Spivack. 2002. Metabolic activity of subsurface life in deep-sea sediments. *Science* 295:2,067–2,070, <https://doi.org/10.1126/science.1064878>.
- D'Hondt, S., B.B. Jørgensen, D.J. Miller, A. Batzke, R. Blake, B.A. Cragg, H. Cypionka, G.R. Dickens, T. Ferdelman, K.-U. Hinrichs, and others. 2004. Distributions of microbial activities in deep seafloor sediments. *Science* 306:2,216–2,221, <https://doi.org/10.1126/science.1101155>.
- D'Hondt, S., F. Inagaki, C. Alvarez Zarikian, L.J. Abrams, N. Dubois, T. Engelhardt, H. Evans, T. Ferdelman, B. Gribsholt, R.N. Harris, and others. 2015. Presence of oxygen and aerobic communities from seafloor to basement in deep-sea sediment. *Nature Geoscience* 8:299–304, <https://doi.org/10.1038/NNGEO2387>.
- Edwards, K.J., C.J. What, and J.B. Sylvan. 2011. Under the sea: Microbial life in volcanic oceanic crust. *Nature Reviews Microbiology* 9:703–712, <https://doi.org/10.1038/nrmicro2647>.
- Edwards, K.J., A.T. Fisher, and C.G. Wheat. 2012a. The deep subsurface biosphere in igneous ocean crust: Frontier habitats for microbiological exploration. *Frontiers in Microbiology* 3, Article 8, <https://doi.org/10.3389/fmicb.2012.00008>.
- Edwards, K.J., C.G. Wheat, B.N. Orcutt, S. Hulme, K. Becker, H. Jannasch, A. Haddad, T. Pettigrew, W. Rhinehart, K. Grigar, and others. 2012b. Design and deployment of borehole observatories and experiments during IODP Expedition 336, Mid-Atlantic Ridge flank at North Pond. In *Proceedings of the Integrated Ocean Drilling Program, Volume 336*. Edited by K.J. Edwards, W. Bach, A. Klaus, and the Expedition 336 Scientists, Integrated Ocean Drilling Program Management International, Inc., Tokyo, <https://doi.org/10.2204/iodp.proc.336.109.2012>.
- Edwards, K.J., W. Bach, A. Klaus, and IODP Expedition 336 Scientific Party. 2014. IODP Expedition 336: Initiation of long-term coupled microbiological, geochemical, and hydrological experimentation within the seafloor at North Pond, western flank of the Mid-Atlantic Ridge. *Scientific Drilling* 17:13–18, <https://doi.org/10.5194/sd-17-13-2014>.
- Engelen, B., K. Ziegelmueller, L. Wolf, B. Kopke, A. Gittel, H. Cypionka, T. Treude, S. Nakagawa, F. Inagaki, M.A. Lever, and B.O. Steinbu. 2008. Fluids from the oceanic crust support microbial activities within the deep biosphere. *Geomicrobiology Journal* 25(1):56–66, <https://doi.org/10.1080/01490450701829006>.
- Engelhardt, T., J. Kallmeyer, H. Cypionka, and B. Engelen. 2014. High virus-to-cell ratios indicate ongoing production of viruses in deep subsurface sediments. *The ISME Journal* 8:1,503–1,509, <https://doi.org/10.1038/ismej.2013.245>.
- Fisher, A.T., T. Tsuji, K. Petronotis, C.G. Wheat, K. Becker, J.F. Clark, J. Cowen, K. Edwards, H. Jannasch, the IODP Expedition 327, and Atlantis Expedition AT18-07 Shipboard Parties. 2011. IODP Expedition 327 and Atlantis Expedition AT18-07: Observatories and experiments on the eastern flank of the Juan de Fuca Ridge. *Scientific Drilling* 13:4–11, <https://doi.org/10.2204/iodp.sd.13.01.2011>.
- Fisk, M.R., S.J. Giovannoni, and I.H. Thorseth. 1998. Alteration of oceanic volcanic glass: Textural evidence of microbial activity. *Science* 281:978–980, <https://doi.org/10.1126/science.281.5379.978>.
- Früh-Green, G., B.N. Orcutt, S.L. Green, C. Cotterill, S. Morgan, N. Akizawa, G. Bayraktci, J.-H. Behrmann, C. Boschi, W.J. Brazelton, and others. 2017. Expedition 357 summary. In *Proceedings of the International Ocean Discovery Program, Volume 357*. G.L. Früh-Green, B.N. Orcutt, S.L. Green, C. Cotterill, and the Expedition 357 Scientists, College Station, TX, <https://doi.org/10.14379/iodp.proc.357.101.2017>.
- Früh-Green, G., B.N. Orcutt, S. Rouméjon, M.D. Lilley, Y. Morono, C. Cotterill, S. Green, J. Escartin, B.E. John, A.M. McCaig, and others. 2018. Magmatism, serpentinization and life: Insights through drilling the Atlantis Massif (IODP Expedition 357). *Lithos* 323:137–155, <https://doi.org/10.1016/j.lithos.2018.09.012>.
- Glombitza, C., R.R. Adhikari, N. Riedinger, W.P. Gilhooly III, K.-U. Hinrichs, and F. Inagaki. 2016. Microbial sulfate reduction potential in coal-bearing sediments down to ~2.5 km below the seafloor off Shimokita Peninsula, Japan. *Frontiers in Microbiology* 7:1576, <https://doi.org/10.3389/fmicb.2016.01576>.
- Heuer, V.B., F. Inagaki, Y. Morono, Y. Kubo, L. Maeda, and the Expedition 370 Scientists. 2017. Expedition 370 Preliminary Report: Temperature Limit of the Deep Biosphere off Muroto. International Ocean Discovery Program, <https://doi.org/10.14379/iodp.pr.370.2017>.
- Hinrichs, K.-U., J.M. Hayes, W. Bach, A. Spivack, L.R. Hemel, N. Holm, C.G. Johnson, and S.P. Sylva. 2006. Biological formation of ethane and propane in the deep marine subsurface. *Proceedings of the National Academy of Sciences of the United States of America* 103:14,684–14,689, <https://doi.org/10.1073/pnas.0606535103>.
- Hinrichs, K.-U., F. Inagaki, V.B. Heuer, M. Kinoshita, Y. Morono, and Y. Kubo. 2016. Expedition 370 Scientific Prospectus: T-Limit of the Deep Biosphere off Muroto (T-Limit). International Ocean Discovery Program.
- Hoehler, T.M., and B.B. Jørgensen. 2013. Microbial life under extreme energy limitation. *Nature Reviews Microbiology* 11:83–94, <https://doi.org/10.1038/nrmicro2939>.
- Hoshino, T., and F. Inagaki. 2018. Abundance and distribution of Archaea in the seafloor sedimentary biosphere. *The ISME Journal* 13:227–231, <https://doi.org/10.1038/s41396-018-0253-3>.
- Inagaki, F., T. Nunoura, S. Nakagawa, A. Teske, M. Lever, A. Lauer, M. Suzuki, K. Takai, M. Delwiche, F.S. Colwell, K.H. Nealson, K. Horikoshi, S. D'Hondt, and B.B. Jørgensen. 2006. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proceedings of the National Academy of Sciences of the United States of America* 103(8):2,815–2,820, <https://doi.org/10.1073/pnas.0511033103>.
- Inagaki, F., K.-U. Hinrichs, Y. Kubo, and the Expedition 337 Scientists. 2013. Proceedings of the Integrated Ocean Drilling Program, Volume 337. Integrated Ocean Drilling Program Management International Inc., Tokyo, <https://doi.org/10.2204/iodp.proc.337.2013>.
- Inagaki, F., K.-U. Hinrichs, Y. Kubo, M.W. Bowles, V.B. Heuer, W.-L. Long, T. Hoshino, A. Ijiri, H. Imachi, M. Ito, and others. 2015. Exploring deep microbial life in coal-bearing sediment down to ~2.5 km below the ocean floor. *Science* 349:420–424, <https://doi.org/10.1126/science.aaa6882>.
- IODP (International Ocean Discovery Program). 2011. *Illuminating Earth's Past, Present, and Future: Science Plan for 2013–2023*. Integrated Ocean Drilling Program Management International, Washington, DC, <https://www.iodp.org/about-iodp/iodp-science-plan-2013-2023>.
- Jørgensen, S.L., and R. Zhao. 2016. Microbial inventory of deeply buried oceanic crust from a young ridge flank. *Frontiers in Microbiology*, <https://doi.org/10.3389/fmicb.2016.00820>.
- Kallmeyer, J., R. Pockalny, R.R. Adhikari, D.C. Smith, and S. D'Hondt. 2012. Global distribution of microbial abundance and biomass in subsurface sediment. *Proceedings of the National Academy of Sciences of the United States of America* 109:16,213–16,216, <https://doi.org/10.1073/pnas.1203849109>.
- LaBonté, J.M., M.A. Lever, K.J. Edwards, and B.N. Orcutt. 2017. Influence of igneous basement on deep sediment microbial diversity on the eastern Juan de Fuca Ridge flank. *Frontiers in Microbiology* 8:1434, <https://doi.org/10.3389/fmicb.2017.01434>.
- LaRowe, D.E., E. Burwiicz, S. Arndt, A.W. Dale, and J.P. Amend. 2017. Temperature and volume of global marine sediments. *Geology* 45:275–278, <https://doi.org/10.1130/G386011>.
- Lever, M.A., O.J. Rouxel, J.C. Alt, N. Shimizu, S. Ono, R.M. Coggon, W.C. Shanks III, L. Lapham, M. Elvert, X. Prieto-Mollar, and others. 2013. Evidence for microbial carbon and sulfur cycling in deeply buried ridge flank basalt. *Science* 339(6125):1,305–1,308, <https://doi.org/10.1126/science.1229240>.
- Lipp, J.S., Y. Morono, F. Inagaki, and K.-U. Hinrichs. 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature* 454:991–994, <https://doi.org/10.1038/nature07174>.
- Liu, C.-H., X. Huang, T.-N. Xie, N. Duan, Y.-R. Xue, T.-X. Zhao, M.A. Lever, K.-U. Hinrichs, and F. Inagaki. 2016. Exploration of cultivable fungal communities in deep coal-bearing sediments from ~1.3 to 2.5 km below the ocean floor. *Environmental Microbiology* 19:803–818, <https://doi.org/10.1111/1462-2920.13653>.
- Lloyd, M.K. 2018. *Clumped and Intramolecular Isotopic Perspectives on the Behavior of Organic and Inorganic Carbon in the Shallow Crust and Deep Biosphere*. PhD dissertation, California Institute of Technology, <https://doi.org/10.7907/Z96T0JV5>.
- Lomstein, B.A., A.T. Langerhuus, S. D'Hondt, B.B. Jørgensen and A.J. Spivack. 2012. Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment. *Nature* 484:101–104, <https://doi.org/10.1038/nature10905>.
- MacLeod, C.J., H.J.B. Dick, P. Blum, N. Abe, D.K. Blackman, J.A. Bowles, M.J. Cheadle, K. Cho, J. Cizžela, J.R. Deans, and others. 2017. Site U1473. In *Southwest Indian Ridge Lower Crust and Moho*. Proceedings of the International Ocean Discovery Program, vol. 360. Edited by C.J. MacLeod, H.J.B. Dick, P. Blum, and the Expedition 360 Scientists, College Station, TX, <https://doi.org/10.14379/iodp.proc.360.103.2017>.
- McCarthy, M.D., S.R. Beaupré, B.D. Walker, I. Voparil, T.P. Guilderson, and E.R.M. Druffel. 2011. Chemosynthetic origin of ¹⁴C-depleted dissolved organic matter in a ridge-flank hydrothermal system. *Nature Geoscience* 4(1):32–36, <https://doi.org/10.1038/ngeo1015>.
- Meyer, J.L., U. Jaekel, B.J. Tully, B.T. Glazer, C.G. Wheat, H.-T. Lin, C.-C. Hsieh, J.P. Cowen, S.M. Hulme, P.R. Girguis, and J.A. Huber. 2016. A distinct and active bacterial community in cold oxygenated fluids circulating beneath the western flank of the Mid-Atlantic Ridge. *Scientific Reports* 6:22541, <https://doi.org/10.1038/srep22541>.
- Morita, R.Y., and C.E. ZoBell. 1955. Occurrence of bacteria in pelagic sediments collected during the Mid-Pacific Expedition. *Deep Sea Research* 3:66–73, [https://doi.org/10.1016/0146-6313\(55\)90036-8](https://doi.org/10.1016/0146-6313(55)90036-8).
- Morono, Y., T. Terada, J. Kallmeyer, and F. Inagaki. 2013. An improved cell separation technique for marine subsurface sediments: Applications for high-throughput analysis using flow cytometry and cell sorting. *Environmental Microbiology* 15:2,841–2,849, <https://doi.org/10.1111/1462-2920.12153>.
- Morono, Y., and F. Inagaki. 2016. Analysis of low-biomass microbial communities in the deep biosphere. *Advances in Applied Microbiology* 95:149–178, <https://doi.org/10.1016/bs.aambs.2016.04.001>.

- Morono, Y., T. Hoshino, T. Terada, T. Suzuki, T. Sato, H. Yuasa, Y. Kubota, and F. Inagaki. 2018. Assessment for capacity to capture DNA aerosols by clean filters for molecular biology experiments. *Microbes and Environment* 33:222–226, <https://doi.org/10.1264/jsme2.ME18012>.
- Nigro, O.D., S.P. Jungbluth, H.-T. Lin, C.-C. Hsieh, J.A. Miranda, C.R. Schvarcz, M.S. Rappé, and G.F. Steward. 2017. Viruses in the oceanic basement. *mBio*, <https://doi.org/10.1128/mBio.02129-16>.
- Orcutt, B.N., W. Bach, K. Becker, A.T. Fisher, M. Hentscher, and B.M. Toner, C.G. Wheat, and K.J. Edwards. 2011a. Colonization of subsurface microbial observatories deployed in young ocean crust. *The ISME Journal* 5:692–703, <https://doi.org/10.1038/ismej.2010.157>.
- Orcutt, B.N., J.B. Sylvan, N.J. Knab, and K.J. Edwards. 2011b. Microbial ecology of the dark ocean above, at, and below the seafloor. *Microbiology and Molecular Biology Reviews* 75(2):361–422, <https://doi.org/10.1128/MMBR.00039-10>.
- Orcutt, B.N., C.G. Wheat, O.J. Rouxel, S. Hulme, K.J. Edwards, and W. Bach. 2013. Oxygen consumption rates in subseafloor basaltic crust derived from a reaction transport model. *Nature Communications* 4:2539, <https://doi.org/10.1038/ncomms3539>.
- Oremland, R.S., C. Culbertson, and B.R.T. Simoneit. 1982. Methanogenic activity in sediment from Leg 64, Gulf of California. Pp. 759–762 in *Initial Reports of the Deep Sea Drilling Project, Volume 64*. US Government Printing Office, Washington, DC, <https://doi.org/10.2973/dsdp.proc.64.122.1982>.
- Orsi, W., V. Edgcomb, G. Christman, and J. Biddle. 2013. Gene expression in the deep biosphere. *Nature* 499:205–208, <https://doi.org/10.1038/nature12230>.
- Parkes, R.J., B.A. Cragg, S.J. Bale, J.M. Getliff, K. Goodman, P.A. Rochelle, J.C. Fry, A.J. Weightman, and S.M. Harvey. 1994. Deep bacterial biosphere in Pacific Ocean sediments. *Nature* 371:410–413, <https://doi.org/10.1038/371410a0>.
- Parkes, R.J., B.A. Cragg, and P. Wellsbury. 2000. Recent studies on bacterial populations and processes in subseafloor sediments: A review. *Hydrogeology Journal* 8:11–28, <https://doi.org/10.1007/PL00010971>.
- Parkes, R.J., G. Webster, B.A. Cragg, A.J. Weightman, C.J. Newberry, T.G. Ferdelman, J. Kallmeyer, B.B. Jorgensen, I.W. Aiello, and J.C. Fry. 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* 436:390–394, <https://www.nature.com/articles/nature03796>.
- Reese, B.K., L.A. Zinke, M.S. Sobol, D.E. LaRowe, B.N. Orcutt, X. Zhang, U. Jaekel, F. Wang, T. Dittmar, D. Defforey, and others. 2018. Nitrogen cycling potential of active bacteria within oligotrophic sediment of the Mid-Atlantic Ridge flank. *Geomicrobiology Journal* 35:468–483, <https://doi.org/10.1080/01490451.2017.1392649>.
- Robador, A., D.E. LaRowe, S.P. Jungbluth, H.-T. Lin, M.S. Rappé, K.H. Nealson, and J.P. Amend. 2016. Nanocalorimetric characterization of microbial activity in deep subsurface oceanic crustal fluids. *Frontiers in Microbiology* 7:454, <https://doi.org/10.3389/fmicb.2016.00454>.
- Schippers, A., L.N. Neretin, J. Kallmeyer, T.G. Ferdelman, B.A. Cragg, R.J. Parkes, and B.B. Jorgensen. 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. *Nature* 433:861–864, <https://doi.org/10.1038/nature03302>.
- Schippers, A., and L.N. Neretin. 2006. Quantification of microbial communities in near-surface and deeply buried marine sediments on the Peru continental margin using real-time PCR. *Environmental Microbiology* 8:1,251–1,260, <https://doi.org/10.1111/j.1462-2920.2006.01019.x>.
- Shah Walter, S.R., U. Jaekel, H. Osterholz, A.T. Fisher, J.A. Huber, A. Pearson, T. Dittmar, and P.R. Girguis. 2018. Microbial decomposition of marine dissolved organic matter in cool oceanic crust. *Nature Geoscience* 11:334–339, <https://doi.org/10.1038/s41561-018-0109-5>.
- Smith, A., R. Popa, M.R. Fisk, M. Nielsen, C.G. Wheat, H.W. Jannasch, A.T. Fisher, K. Becker, S.M. Sievert, and G. Flores. 2011. In situ enrichment of ocean crust microbes on igneous minerals and glasses using an osmotic flow-through device. *Geochemistry, Geophysics, Geosystems* 12(6), Q06007, <https://doi.org/10.1029/2010GC003424>.
- Smith, D.C., A.J. Spivack, M.R. Fisk, S.A. Haveman, H. Staudigel, and ODP Leg 185 Shipboard Science Party. 2000. Tracer-based estimates of drilling-induced microbial contamination of deep sea crust. *Geomicrobiology Journal* 17:207–219, <https://doi.org/10.1080/01490450050121170>.
- Sørensen, K.B., and A. Teske. 2006. Stratified communities of active archaea in deep marine subsurface sediments. *Applied and Environmental Microbiology* 72:4,596–4,603, <https://doi.org/10.1128/AEM.00562-06>.
- Sylvan, J.B., C.L. Hoffman, L.M. Momper, B.M. Toner, J.P. Amend, and K.J. Edwards. 2015. *Bacillus rigiliprofundii* sp. nov., an endospore-forming Mn-oxidizing, moderately halophilic bacterium isolated from deep subseafloor basaltic crust. *International Journal of Systematic and Evolutionary Microbiology* 65:1,992–1,998, <https://doi.org/10.1099/ijs.0.000211>.
- Takai, K., K. Nakamura, T. Toki, U. Tsunogai, M. Miyazaki, J. Miyazaki, H. Hirayama, S. Nakagawa, T. Nunoura, and K. Horikoshi. 2008. Cell proliferation at 122°C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. *Proceedings of the National Academy of Sciences of the United States of America* 105:10,949–10,954, <https://doi.org/10.1073/pnas.0712334105>.
- Tanikawa, W., O. Tadai, S. Morita, W. Lin, Y. Yamada, Y. Sanada, K. Moe, Y. Kubo, and F. Inagaki. 2016. Thermal properties and thermal structure in the deep-water coalbed basin off the Shimokita Peninsula, Japan. *Marine and Petroleum Geology* 73:445–461, <https://doi.org/10.1016/j.marpetgeo.2016.03.006>.
- Tanikawa, W., O. Tadai, Y. Morono, K.-U. Hinrichs, and F. Inagaki. 2018. Geophysical constraints on microbial biomass in subseafloor sediments and coal seams down to 2.5 km off Shimokita Peninsula, Japan. *Progress in Earth and Planetary Science* 5:58, <https://doi.org/10.1186/s40645-018-0217-2>.
- Tarafa, M.E., and J.K. Whelan. 1987. Evidence of microbiological activity in Leg 95 (New Jersey Transect) sediments. Pp. 635–640 in *Initial Reports of the Deep Sea Drilling Project, Volume 95*. US Government Printing Office, Washington, DC, <https://doi.org/10.2973/dsdp.proc.95.125.1987>.
- Thomson, I.D., S.C. Brassell, G. Eglinton, and J.R. Maxwell. 1982. Preliminary lipid analysis of section 481-2-2. Pp. 913–919 in *Initial Reports of the Deep Sea Drilling Project, Volume 64*. US Government Printing Office, Washington, DC.
- Torsvik, T., H. Furnes, K. Muehlenbachs, I.H. Thorseth, and O. Tumyr. 1998. Evidence for microbial activity at the glass-alteration interface in oceanic basalts. *Earth and Planetary Science Letters* 162:165–176, [https://doi.org/10.1016/S0012-821X\(98\)00164-2](https://doi.org/10.1016/S0012-821X(98)00164-2).
- Trembath-Reichert, E., Y. Morono, A. Ijiri, T. Hoshino, K.S. Dawson, F. Inagaki, and V.J. Orphan. 2017. Methyl-compound use and slow growth characterize microbial life in 2-km-deep subseafloor coal and shale beds. *Proceedings of the National Academy of Sciences of the United States of America* 114:E9206–E9215, <https://doi.org/10.1073/pnas.1707525114>.
- Tully, B.J., C.G. Wheat, B.T. Glazer, and J.A. Huber. 2017. A dynamic microbial community with high functional redundancy inhabits the cold, oxic subseafloor aquifer. *The ISME Journal* 12:1–16, <https://doi.org/10.1038/ismej.2017.187>.
- Walsh, E.A., J.B. Kirkpatrick, R. Pockalny, J. Sauvage, A.J. Spivack, R.W. Murray, M.L. Sogin, and S. D'Hondt. 2016. Relationship of bacterial richness to organic degradation rate and sediment age in subseafloor sediment. *Applied and Environmental Microbiology* 82(16):4,994–4,999, <https://doi.org/10.1128/AEM.00809-16>.
- Whelan, J.K., R. Oremland, M. Tarafa, R. Smith, R. Howarth, and C. Lee. 1986. Evidence for sulfate-reducing and methane-producing microorganisms in sediments from Sites 618, 619, and 222. Pp. 767–775 in *Initial Reports of the Deep Sea Drilling Project Volume 96*. US Government Printing Office, Washington, DC, <https://doi.org/10.2973/dsdp.proc.96.147.1986>.
- Yanagawa, K., A. Ijiri, A. Breuker, S. Sakai, Y. Miyoshi, S. Kawagucci, T. Noguchi, M. Hirai, A. Schippers, J.-I. Ishibashi, and others. 2016. Defining boundaries for the distribution of microbial communities beneath the sediment-buried, hydrothermally active seafloor. *The ISME Journal* 11:529–542, <https://doi.org/10.1038/ismej.2016.119>.

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