Microbial Life in Oceanic Crust

Beth N. Orcutt^{1,2}, Timothy D'Angelo¹, Sean P. Jungbluth³, Julie A. Huber³, Jason B. Sylvans

¹ Bigelow Laboratory for Ocean Sciences, 60 Bigelow Drive, East Boothbay, ME, 04544, USA;
borcutt@bigelow.org

² Hanse Institute for Advanced Study, Delmenhorst, Germany

³ Department of Energy, Joint Genome Institute, Walnut Creek, CA, USA

⁴ Woods Hole Oceanographic Institution, Woods Hole, MA, USA

⁵ Texas A&M University, College Station, TX, USA

Abstract. Oceanic crust comprises a vast but virtually unexplored habitat for life on Earth, characterized by massive global flows of water, heat, and dissolved chemicals. Uncovering where and how life exists in oceanic crust is important because chemical reactions occurring in this environment impact broader ocean systems and also because it is an earth analog for considering the possibility of extraterrestrial life on other ocean worlds. Over the past decade, several major oceanographic expeditions focused on characterizing the ocean crust microbiome, enabled by advances in seafloor drilling and observatory technologies. Here we review what is known about the crustal ocean microbial biosphere, focusing on a new synthesis of recent studies on the diversity of microbial life in oceanic crust to reveal common and unique taxa in this environment.

Keywords: microbial, oceanic crust, crustal fluids, basalts, subsurface, Juan de Fuca, North Pond

1. Introduction

Oceanic crust – the upper part of the oceanic lithosphere – underlies the entire seafloor (Figs. 1, 2). Thus, the 70% of Earth's surface covered with ocean is underlain by oceanic crust, making it the most abundant solid crust on Earth. Much of the oceanic crust is blanketed in sediment that has deposited out of the water column, although there are many areas of the seafloor where rocks are exposed at the seafloor. Where such rocks are exposed, seawater moves in and out of oceanic crust, ventilating heat from the Earth's interior [1-3]. As the seawater moves through the cracks and fissures within oceanic crust, it undergoes fluid-rock reactions with silicates and other minerals in the rock, changing the chemical content of the fluid over time. Spectacular hydrothermal vents are charismatic end-members of these fluid-rock reactions, with highly altered high-temperature fluids exiting the crust into the water column, in turn supporting a rich diversity of novel animal and microbial life [Vetriani et al., Chapter 10 this book]. However, the majority of fluid flow and chemical exchange in the crust occurs at much cooler temperatures and in areas of more diffuse, lower-temperature venting where the chemical differences are subtler [1,4-16]. The movement of fluids through oceanic crust is very heterogenous over space and time [17-20], as new veins, cracks and fissures form and older ones fill in with alteration products. This dynamic environment is estimated to be on the order of 10^{18} – 10^{19} m³ in total volume (solid rock + pore/void space), slightly larger than the estimated volume of the entire ocean and ten times the volume of marine sediment [21,22]. Roughly 2% of the ocean volume exists within oceanic crust at any given time, and the volume equivalent of the ocean is recirculated through the oceanic crustal aquifer on the order of tens to hundreds of thousands of years [9,11,20], making the fluid-rock reactions that occur as water moves through oceanic crust relevant on geological timescales. More recently, studies have suggested that some fluid-rock reactions in oceanic crust can be accelerated to be relevant on human time scales, such as possible carbon capture and sequestration [23,24].

Despite the immensity and importance of the oceanic crust environment on Earth, the first investigations into microbial life in this habitat began just two decades ago, many decades behind the study of microbial life in the ocean water column, marine sediment, and even hydrothermal vents. This is not surprising considering the considerable technological investment to recover seafloor oceanic crust and crustal fluids through seafloor drilling, and the additional methodological challenges of adapting microbiological techniques to work on hard rock and low biomass samples [25]. The first decade of investigation into life “on the rocks” generated a dozen or so studies, with just a handful describing microbial community structure. Improvements in DNA sequencing and sampling technologies, coupled with an invigorated scientific community interested in the study of the deep biosphere in marine sediment and oceanic crust, has ushered in an abundance of studies of life in oceanic crust in the past few years [26,27]. This review summarizes these recent findings and highlights remaining gaps in our understanding of the form and function of microbial life in the oceanic crust habitat. Considering that other planetary bodies in our solar system may have salty ocean and silicate crust interactions [28,29], resolving how life exists on planet Earth in oceanic crust also has relevant implications for the astrobiological search for life elsewhere in Earth’s Solar System [30]. Finally, emergent human activities in the deep-sea, such as the potential for deep-sea mining and carbon capture and sequestration in seafloor oceanic crust (Fig. 2), underscore the need to understand the form, function, and resilience of the crustal ocean biosphere to natural and anthropogenic perturbations [31].

2. Oceanic Crust Habitat Types

There is a spectrum of habitat types within oceanic crust, varying in age, rock composition, depth, and alteration state, as reviewed elsewhere [32,33,25,22]. The majority of new ocean crust seafloor is formed along mid-ocean ridges from magmatic injection and extrusion as plates spread apart from plate tectonics (Fig. 1a). This crust is typically mafic – basaltic or gabbroic, a solid solution silicate relatively enriched in magnesium and iron. At ocean plate margins where plates are pulling apart more slowly, tectonic and deformation processes can allow for deeper sections of ocean mantle to be exhumed. This mantle-derived crust material is considered ultramafic and is even more enriched in magnesium and iron than basalts and gabbros. The ultramafic blocks are often referred to as “core complexes”.

As new crust moves away from the ridge axis into the ridge flank with plate tectonic motion, the crust subsides and starts to become buried in sediment particles depositing from the water column. Topographic highs with steeper slopes, such as outcrops and seamounts, can remain sediment free. Volcanic seamounts can also form over mid-plate “hot spots”, such as along the Hawaiian Island seamount chain. Seamounts and outcrops are critical conduits for fluid flow in and out of the ocean crust, ventilating heat from the Earth’s interior [20,1,2,14]. Large fluid flow discharge rates from outcrops may be driven by relatively small differences in pressure and temperature [34], and this flow may be concentrated within just a subset of the total porosity of the crust [35]. Satellite altimetric mapping techniques predict that there are 10^5 – 10^7 seamounts and outcrops dotted across the ocean floor [36], although improvements in mapping sensitivity may reveal even more in the future. Due to the exposure of hard substrate and often higher water currents, there is an accumulation of animal life at seamounts and outcrops (Fig. 1b, Fig. 2) [37,38]. In areas where two oceanic plates collide, one plate bends and slides beneath the other, forming a deep depression called a trench (Fig. 1a). Frictional heat and compression from the plate collision and subduction leads to the formation of a series of mud and arc volcanoes, as well as hydrothermal vent systems, on the over-riding plate. Marine transform faults and fracture zones are also places where fluid circulation can occur [39].

Where mafic oceanic crust is exposed at the seafloor, the basalts oxidize over time due to reaction with seawater (see [40-43] for reviews of these processes). Centimeter thick rinds of

manganese and iron oxides can form on the exteriors of the basalts (Fig. 1b-c, Fig. 2), which also form nucleation centers for the precipitation of rare and critical metals that may be attractive for future deep-sea mining [44,45,31]. Due to its charge, phosphate is scavenged by the iron oxides, which is an important consideration for microbial life that requires this essential nutrient. The iron-rich minerals in mafic and ultramafic rocks, like peridotite, can also react with seawater in a reaction process called serpentinization to release substrates such as hydrogen that can be used by microbial life [46,41,47]. Alteration also occurs below the seafloor where conduits and fracture networks allow for fluid movement, as described in more detail in the next section. Some geochemical evidence suggests that oceanic crust is reactive with circulating fluids for up to about 10 million years [48]; however, there are indications that reactive conditions can still exist beyond this age cutoff.

Our knowledge of processes occurring in subseafloor rocks comes from a variety of sources: from drilling into oceanic crust and recovering cores, from examining the nature of fluids exiting the seafloor at hydrothermal vents and seamounts, and through the placement of subseafloor observatories into oceanic crust after drilling (Fig. 1d-e, Fig. 2). Like groundwater wells on land, these borehole observatories – referred to as “CORKs” and described in detail elsewhere [49-52] – allow the subseafloor crustal environment to return to pre-drilling conditions after fluid circulation flushes out drilling disturbance [6,53-56]. CORKs allow access points to the crustal subsurface, either through sampling of deep crustal fluids that vent or are pumped from depth, or through the deployment of experiments within the borehole at depth, as reviewed elsewhere [25]. Microbiologists use this infrastructure to examine the microbial ecology of the subseafloor [57,58,56,59], as reviewed elsewhere [25] and highlighted later in this chapter.

3. Evidence of Life in Oceanic Crust

The concept of microbial activity within oceanic crust originated soon after the discovery of deep-sea hydrothermal vents [60]. Based on high cell counts in discharging vent fluids, it was suggested that there must be a large microbial population living at depth in the basaltic rocks [60]. Karl et al. [61] used ATP measurements as a proxy for microbial biomass to estimate primary production in vent fluids from the Galapagos, and these rates exceeded those in deep-sea water by more than an order of magnitude. Soon after, the first thermophilic and hyperthermophilic microbes were isolated from high temperature vent fluids and chimneys, and the presence of a thermophilic subseafloor population of microbes proposed [62,63]. Tuttle et al. [64] suggested that the bulk of bacterial biomass found in venting fluids must have originated at elevated temperatures on rock surfaces and in cracks of pillow basalts “at an undetermined depth below the sea floor.” A demarcation between a cooler shallow oxic zone and a warmer deep anoxic zone of the subseafloor soon became evident [65]. These early studies led to a new generation of subseafloor investigations using venting fluids as an indicator of processes occurring beneath the seafloor, reviewed elsewhere in this book [Vetriani et al., Chapter 10 this book].

The earliest visual suggestions of microbial life in oceanic crust came in the 1990s from textural features in seafloor and subseafloor rock sample thin sections that were attributed to microbial activity [66,21,67-73]. Recent studies suggest that the “tunnel” textural features may be formed by proton exchange at the basalt glass-seawater interface [74]. Scanning electron microscopy later documented various cellular structures on seafloor rock samples [75,76], confirming that microorganisms did indeed colonize and attach to these surfaces. Microbial morphologies on rock surfaces include coccoids, bacillus rod-shapes, and various stalk forms (filaments, branching, and twisted), often with webbed networks of fossilized cellular material observed [75-77]. For example, Fig. 3 provides examples of microbial cell morphologies and alteration structures formed during colonization experiments with basalts incubated below the

seafloor at two ocean drilling sites that tap into subseafloor oceanic crust with CORK borehole observatories (experimental descriptions provided elsewhere [78,51,56,79]). The cellular features are often enriched or encrusted in iron and/or manganese [76,75,56], leading to the suggestion that the microbes that form them are involved in iron and/or manganese cycling. Although microscopic Bacteria are often assumed to be the source of these fossilized networks, some of these features have recently been attributed to microscopic eukaryotic fungi living within basalts [80-82]. There is no known role of Archaea in producing mineralized cellular structures within seafloor rocks.

Additional early evidence for microbial life in oceanic crust came from sulfur stable isotope surveys of ocean drilling core samples from peridotite-rich ultramafic seafloor [73]. Sulfide enrichments and highly depleted sulfur isotopes in sulfides precipitated in the core samples were argued to be sourced from the microbial reduction of seawater sulfate fueled by reductants generated by fluid-rock interactions in the subsurface, with this process representing a sink of seawater sulfate. Deeper analysis of multiple sulfur isotopes provided more evidence that microbial sulfate reduction occurs in subseafloor ocean crust, perhaps under low metabolic rates [83]. More recently, combining sulfur stable isotope analysis with DNA sequencing revealed that subseafloor microbial communities with sulfate reduction capability are associated with these stable isotope depletions, although modern sulfate reduction rates were not measurable [84]. Subsequent examination of pristine crustal fluids collected from CORKs from the same environment as the DNA study finally documented the modern potential for microbial sulfate reduction [85] and further confirmed that microbial communities in these fluids were operating under low energy conditions [86].

Although these early studies on microbial activity in oceanic crust focused on warm and anoxic settings, oxic conditions within some portions of subseafloor oceanic crust were assumed based on secondary mineral assemblages formed during fluid-rock reactions [48]. The confirmation of oxygen within large portions of the crustal environment only happened within the past few years [87-89,13,55]. Spatial patterns in oxygen concentrations in oceanic crust suggest oxygen consumption within crust [87,13], and aerobic microbial processes in oxic crustal fluids and basalts were recently measured [90,55]. Both of these studies examined the potential for carbon cycling under aerobic conditions and confirmed the potential for chemolithoautotrophy, although oceanic crust is net heterotrophic based on organic carbon loss [91-93]. Moreover, empirical rates of carbon fixation potential in basalts matched earlier predictions based on modeling of secondary alteration features [90,48]. Finally, even net oxic systems may have anaerobic niche that form as fluids stagnate and continue to react with the rock substrate. The formation of reduced substrates within these areas, for example from the radiolytic production of hydrogen [94,43], can contribute to oxygen removal.

4. Microbial Community Structure in Oceanic Crust

Pioneering investigations of the microbial community structure on seafloor basalts started at the beginning of the 21st century. These early studies used Sanger sequencing of clone libraries of the 16S ribosomal RNA (16S rRNA) gene, with results indicating that various groups of Proteobacteria seemed to dominate this habitat type, with Actinobacteria, Bacteroidetes, and Firmicutes bacteria also being common groups [75,95-99]. Thaumarchaeota were also identified in these early studies as the dominant archaeal species on seafloor basalts [97,75,99,98,100,101], although the relative abundance of Archaea is much lower than of Bacteria [101]. Advances in both DNA sequencing technology and sample collection methodology have enabled deeper synthesis of microbial community structure on seafloor and subseafloor rocks than was possible even a decade ago [22], with a relative explosion of datasets from these environments in the past few years enabled by focused drilling and observatory efforts of the international scientific ocean drilling programs [78,51,102].

1 To summarize crustal bacterial and archaeal taxa for this review (Fig. 4), we synthesized
2 publicly-available sequence datasets of the 16S rRNA gene in environmental DNA extracted
3 from seafloor and subseafloor basalts generated using 454, Illumina and Ion Torrent amplicon
4 sequencing platforms. These include seafloor basalts from the Dorado Outcrop [103] and the
5 Lō'ihi Seamount [104] in the Pacific Ocean and subseafloor basalts from North Pond on the
6 western flank of the Mid-Atlantic Ridge [105] and the Juan de Fuca Ridge flank in the
7 northeastern Pacific Ocean [106]. Datasets from rock colonization experiments conducted in the
8 subseafloor at the Juan de Fuca Ridge flank site [107,79] were also included, as well as
9 microbial community surveys of the subseafloor crustal fluids from the anoxic Juan de Fuca site
10 [108] and the oxic North Pond site [109,55]. For comparison, we included select reference
11 datasets from oxic [110,111] and anoxic sediment [106] and the overlying bottom seawater
12 [103] from these same study sites. A reference map of these locations can be found in an earlier
13 review [25].

14 For individuals seeking a deeper dive into phylogeny and nearly full-length 16S rRNA gene
15 sequence data, we recommend these earlier clone library based studies for comparison: from
16 Juan de Fuca crustal fluids [112,113], from rock colonization experiments in the Juan de Fuca
17 subsurface [56,59] and at the seafloor [114-116], from seafloor basalts from a variety of
18 locations [117,118,75,101,119,97,103,120,121,99,122,96]. There are also 16S rRNA genes
19 constructed in metagenome samples from the oxic and cool crustal fluids from the North Pond
20 site [109] and the anoxic crustal fluids from the Juan de Fuca site [123,108]. These earliest
21 clone library datasets from crustal samples were not included in this analysis due to small
22 sample sizes and vast differences in diversity compared to the non-clone library based
23 sequencing approaches, but they are valuable references for considering the history of
24 examining microbial diversity in oceanic crust.

25 A detailed description of the dataset origins, sequence quality analysis steps, and taxonomic
26 assignments for this review are available elsewhere [[https://github.com/orcuttlab/ocean-crust-](https://github.com/orcuttlab/ocean-crust-micro)
27 [micro](https://github.com/orcuttlab/ocean-crust-micro), and at the Biological and Chemical Oceanography Data Management Office (BCO-
28 DMO) dataset 789136 [124]]. It is important to keep in mind that different DNA extraction
29 methodologies, primer sets and sequencing platforms were used to generate the various datasets,
30 which may introduce bias, although others have demonstrated that these issues can be overcome
31 to document community differences in complex environmental samples [125]. It is also
32 important to keep in mind that many of these datasets were generated from low biomass
33 samples, which are known to have issues with sequencing contamination [126,127,106,128,79].
34 Because of the different dataset types, a universal removal of possible sequence contaminants
35 was not possible in this study, so some datasets presented here may include sequence
36 contaminants. Also, our analysis pipeline generated a few mismatches to the taxonomic
37 presentations in earlier studies due to the use of newer reference databases: namely, (1)
38 taxonomic assignment using the latest release of the SILVA sequence reference database [129-
39 131] now classifies Betaproteobacteria as an order within the Gammaproteobacteria, which
40 were previously identified in many of these studies as a separate class of the Proteobacteria; (2)
41 several new “microbial dark matter” groups now have taxonomic names; and (3) several
42 samples have an abundance of unclassified sequences.

43 At a broad level, this synthesis reveals similar major taxa in seafloor-exposed basalts and
44 subseafloor basalts and the crustal fluids within cool and oxic aquifers, despite variations in age,
45 composition, depth, and geography (Fig. 4). Consistent with prior studies [101,103], there is a
46 high diversity of taxa in the crustal samples, and bacterial taxa are more prevalent than archaeal
47 taxa. Gammaproteobacteria are the dominant group in most of these sample types (as shown in
48 more detail in Supplemental Figure S1 at BCO-DMO dataset 789136 [124]).
49 Alphaproteobacteria and Deltaproteobacteria are also abundant in many of these samples,
50 particularly in the older and deeper basalts. By contrast, Epsilonbacteraeota abundance
51 fluctuates, with notable abundances in deep basalts from the North Pond site and some of the
52 crustal fluid samples from this location as well as in seafloor rock colonization experiments.
53 While many of the basalt samples indicate a high relative abundance of Actinobacteria, this

1 should be viewed cautiously as many of the Actinobacteria taxa have overlap with common
2 sequencing contaminants and may not be truly from the subsurface, as discussed in detail
3 elsewhere [106,126].

4 Various groups of Firmicutes appear in subseafloor basalt and fluid samples, and this group is
5 especially dominant in several anoxic subseafloor rock colonization experiments (Fig. 4).
6 Firmicutes are known for spore production, and it is speculated that spores may travel long
7 distances through the surface and subsurface until conditions are right for revival [132,133].
8 Supporting this, a recent metagenomic study identified similarities between subseafloor and
9 continental subsurface Firmicute lineages [134,135], suggesting that a common genetic
10 repertoire exists between marine and continental subsurface microbes. Spores are gaining
11 increasing recognition as an important component of the oceanic crust deep biosphere, and
12 further studies in more diverse crustal habitat types like the cool and oxic North Pond system
13 are needed. It is also worth noting that Firmicutes may be more prone to differential DNA
14 extraction efficiencies due to their relatively rigid cell membranes, which may impact their
15 presence in environmental sequence libraries.

16 Other common but lower abundance taxonomic groups in oxic basalts include the
17 Acidobacteria, Bacteroidetes, and Planctomycetes, and occasionally members of the Candidate
18 Phyla Radiation (CPR) like Schekmanbacteria (Fig. 4). Within anoxic crustal fluids, the
19 Nitrospirae group has variable abundance; this group is also present in many older, highly
20 altered basalt samples at low abundance. Within the archaeal domain, Thaumarchaeota
21 (previously referred to as Marine Group I Crenarchaeota in earlier studies of basalt microbial
22 ecology) are the dominant archaeal group in basalt rocks and biofilms, although warm and
23 anoxic crustal fluids have a broader diversity of archaeal groups including Crenarchaeota and
24 Euryarchaeota. Intriguingly, Thaumarchaeota appear to be enriched in older seafloor basalts and
25 deep oxic subseafloor basalts. These patterns in Thaumarchaeota, Nitrospirae, and
26 Planctomycetes – all groups known to be involved in nitrogen cycling – suggest that nitrogen
27 cycling may be more pronounced in older or more altered crustal environments. Other CPR and
28 Archaeal taxa are members of the rare biosphere in crustal samples (Supplemental Figure S2,
29 BCO-DMO dataset 789136 [124]).

30 Due to the different primer sets and sequencing platforms, a species-level comparison of the
31 microbial communities in these samples is not robust. Non-metric dimensional scaling of Bray-
32 Curtis distances between the sequence datasets at the family level of sequence similarity (Fig.
33 5), however, reveals some patterns in microbial community biogeography in crustal
34 environments (see Supplemental Material at BCO-DMO dataset 789136 [124] for more details
35 about the analysis process). For example, the community structures in the cool and oxic North
36 Pond subsurface crustal fluids and basalts are highly overlapping (despite different sequencing
37 platforms and primer sets) yet distinct from the overlying sediment microbial communities.
38 These cool and oxic subsurface basalts microbial communities from North Pond are also distinct
39 from cool and oxic seafloor basalts from the Dorado Outcrop and the Lōʻihi Seamount. The
40 more altered crustal fluids from the Juan de Fuca show a range of microbial community
41 structure, which may be a reflection of the increasing purity of the crustal fluid sampling
42 overtime, as noted elsewhere [108]. There is little overlap between the microbial community
43 structures of the Juan de Fuca crustal fluids and the microbial biofilms that formed on rocks in
44 subsurface colonization incubations (i.e., FLOCS) at this site (Fig. 5). A recent study suggested
45 that the biofilm-forming microbial community members at this site only represent a subset of
46 the fluid community, and that these two communities have functional differences [79],
47 emphasizing the need to examine both rock-attached and fluid microbial communities to have a
48 full understanding of this biosphere.

49 The microbial community structure of seafloor and subseafloor gabbros and ultramafic rocks
50 is poorly understood at present, although this will hopefully change in the near future after
51 analysis of gabbro and serpentinite samples collected during the recent ocean drilling
52 expeditions in 2015-16 to the Atlantis Bank and the Atlantis Massif, respectively [136-139]. An
53 early study from subseafloor gabbros suggests some overlap in Proteobacteria when compared

1 to basalts, although low sequence recovery from these samples limits deeper comparison [121].
2 Highly altered crustal fluids escaping from serpentinizing ultramafic systems are known to
3 contain unique archaeal groups and sulfate reducing bacteria [140-143], but it is currently
4 unknown if these same groups are within the actively serpentinizing subsurface ecosystem.

5 The ecology of other microscopic denizens of the crustal ecosystem – microscopic
6 Eukaryotes and viruses – has developed significantly in the past few years, although much still
7 remains to be explored. As mentioned above, there are reports of hyphae-like structures in
8 basalts that are suggested to be formed by fungi [80,82,81], and yeast of the genus *Rhodoturula*
9 has been isolated from crustal habitats [59], but there are no descriptions of fungal community
10 structure from this environment. Considering that appreciation of the existence of microscopic
11 eukaryotes in the marine subsurface is in its infancy [144,145], it is not surprising that
12 description of this community in oceanic crust has lagged behind. So far, there is only one
13 published study on the existence of viruses in oceanic crust [146], although they have been
14 observed in the hydrothermal fluids exiting the oceanic crust [147-150]. In this study of viruses
15 in the warm, anoxic, and highly altered fluids from the Juan de Fuca Ridge flank, viruses
16 outnumber Bacteria and Archaea by roughly a factor of 10, which is consistent with other
17 habitats. Many unique viral types (e.g. lemon-shaped viruses) were observed in this ecosystem,
18 likely reflecting the bacterial and archaeal community found in this environment. Whether or
19 not viruses in other oceanic crust settings are similarly unique, or if they are more similar to
20 other ecosystems, remains to be seen with expanded sampling to diverse habitat types.

21 **5. Microbial functions in oceanic crust from metagenomic analysis**

22 The broad biogeographic survey described above is only now possible because of the
23 increasing amount of taxonomic marker (i.e., 16S rRNA gene) sequence data that has become
24 available from diverse ocean crust habitats over the past decade. While these taxonomic profiles
25 might be tempting to use for predicting possible metabolic functions based on functions of close
26 cultured representatives, as is done for human microbiome studies, this approach has its
27 limitations (see supplemental materials at BCO-DMO dataset 789136 [124] for comparison).
28 Excitingly, the next horizon for ocean crust microbiome research is beginning with the recent
29 release of metagenomic datasets from the crustal environment [109,123,151,152]. For example,
30 a time series of metagenome samples from the cool and oxic crustal fluids from the North Pond
31 site [109] reveal temporal variation in microbial community members (based on presence of
32 metagenome assembled genome bins in different samples over time) although there is functional
33 redundancy in these communities over time. Similar patterns were also recently observed in
34 hydrothermal fluids from the Mariana back-arc [153] and the Mid-Cayman Rise [154]. The
35 metagenomic analysis of North Pond fluids indicates both aerobic and anaerobic metabolic
36 functions occur within the crustal habitat over time, including the oxidation of sulfide, sulfur,
37 thiosulfate, hydrogen, and ferrous iron and the respiration of oxygen, nitrate, and sulfate. The
38 metagenomic analysis also supported prior evidence for autotrophy in this environment, with
39 the identification of known carbon fixation genes for the Calvin Benson-Bassham cycle as well
40 as the reverse citric acid cycle. Likewise, metagenomic analysis of the warm and anoxic crustal
41 fluids from the Juan de Fuca site revealed new metabolic functions of *Archaeoglobi* Archaea
42 [155] and *Desulfuridis*-related Bacteria [134], and suggest that the Wood-Ljungdahl pathway is
43 an important mechanism for carbon fixation in this system [152,156]. With continued
44 exploration of these metagenomes as well as generation of new metagenomic,
45 metatranscriptomic, and single cell genomic analyses, combined with stable isotope incubation
46 approaches, much more will be learned about metabolic function in the crustal ecosystem. For
47 example, stable isotope incubation studies coupled with genomics in hydrothermal fluids reveal
48 autotrophic potential in diverse microbial groups [153,157,158,55,159].

6. Conclusions and Future Directions

While our knowledge about the form and function of microbial life in oceanic crust has increased considerably in the past decade, there are still gaps in our understanding about life in this vast habitat. So far, only a relatively small range of crustal habitats have been investigated in detail, focused on relatively young basaltic crust environments. On-going efforts to examine life in older crust, such as within the Louisville Seamount chain [160] and underneath the South Pacific Gyre [88], and in ultramafic crust as described above, will test the hypotheses that there are similar microbial communities living throughout oceanic crust. More drilling and observatory work at diverse sites will be needed to expand this horizon [102], such as in young and old oceanic crust, sites at ultraslow-spreading ridges, and along transform faults. These expanded datasets are also critical to calibrate models of the amount of microbial biomass contained within oceanic crust, as estimates vary widely [161,162]. Recent advances in standardized and low-detection-limit cell density measurement methods from crustal subsurface samples [138] make this goal possible. Although genomic approaches are revealing information about possible microbial functions in these habitats, further work is needed to validate functional inferences and measure rates of process. Efforts to isolate cultivars from the subsurface are also necessary to evaluate adaptations to subsurface conditions.

The desire for better understanding of the crustal ocean microbial biosphere is driven by both basic and applied science demands. In the applied spectrum, the increasing interest in human activities in the deep-sea, such as deep-sea mining and carbon capture and sequestration in oceanic crust (Fig. 2), makes it essential to understand the form, function and resilience of the crustal ocean biosphere to natural and anthropogenic activities. A recent study suggests a wide range of possible impacts of deep-sea mining to the ecosystem services provided by microbial life in crustal habitats [31], but there is a severe lack of studies on the resilience of these services to perturbations that should be addressed with new field work. New results from North Pond documenting the ability for cross-hole tests on the order of a few days [163] set this site up for possible perturbation experimentation. On the basic science spectrum, interest in the possibility for life on ice-covered ocean worlds such as Enceladus and Europa, where salty liquid oceans are thought to be in contact with mafic-type crust, underscores the utility of understanding how life survives and thrives in Earth's crustal ocean [30]. The primary mechanism for accessing the crustal ocean microbial biosphere remains through the international scientific ocean drilling program – currently called the International Ocean Discovery Program (IODP) – which is evaluating the decadal science questions, and the assets needed, to drive scientific ocean drilling moving forward [164]. Continuing research into the microbiome of oceanic crust will certainly require a strong ocean drilling program moving forward.

Acknowledgments. We thank all of the generators of the publicly available sequence datasets used in this review, and the Census of Deep Life of the Deep Carbon Observatory (funded by the Alfred P. Sloan Foundation) for supporting many of those sequencing projects. We also thank Josh Wood for the graphic design of Figure 2, and to the anonymous reviewer of this chapter for their constructive suggestions. Support for this work came from the U.S. National Science Foundation (NSF; awards 1536539 and 1737017 to BNO, 1745589 to JAH, and 1658118 to JBS) and the NSF-funded Center for Dark Energy Biosphere Investigations Science and Technology Center (C-DEBI, NSF award 0939564). BNO also thanks the Hanse Institute for Advanced Study, Delmenhorst, Germany, for a fellowship that partially supported this study. This is C-DEBI contribution number 522.

1

2

Fig. 1. Schematic of oceanic crust habitats (a) and example images of crustal environments on the seafloor (b-e). Panel (a), modified from Schrenk et al. 2009, shows the location of cool (blue) and warm (red) hydrothermal vent systems along the mid-ocean ridge, on ridge flanks, and along arcs; Panels (b) and (c) of altered seafloor basalts from the Dorado Outcrop (licensed from cruise AT26-09 chief scientist C. Geoff Wheat, University of Alaska Fairbanks, NSF, ROV Jason-II Dive J2-572, 2013 © Woods Hole Oceanographic Institution); Panels (d) and (e) of crustal fluid sampling from CORK subseafloor borehole observatories on the Juan de Fuca Ridge flank (licensed from cruise AT26-18 chief scientist Andrew Fisher, University of California, Santa Cruz, NSF, HOV Alvin Dives 4760 and 4759 respectively, 2014, © Woods Hole Oceanographic Institution).

11

The Crustal Ocean Biosphere and Emerging Human Activities in the Deep Sea

Fig. 2. Schematic of the crustal ocean biosphere, emphasizing emergent human activities in these habitats, such as deep-sea mining and carbon capture and sequestration. Figure created by Josh Wood.

Fig. 3. Examples of subseafloor rock colonization experiments incubated at the Juan de Fuca Ridge flank (*a-b*) and at North Pond (*c-d*) and the microbial biofilm morphologies and alteration products that form on basalt surfaces in these experiments as imaged by scanning electron microscopy. Panels (*e-f*) from Juan de Fuca incubations after four years and (*g-h*) from North Pond incubations after six years. These morphologies resemble those observed in some of the earliest studies of cells on crustal materials [76,75]. Photographs in (*a-d*) by B.N. Orcutt, micrographs in (*e-h*) by T. D'Angelo.

Fig. 4. Relative abundance of common (taxa >5% abundance in at least one sample) bacterial and archaeal taxonomic groups in publicly-available 454, Illumina, and Ion Torrent 16S rRNA gene sequence datasets from crustal rocks, biofilms, and fluids in comparison to sediment and seawater samples (SW). Blue shaded boxes indicate samples from North Pond (NP), green from Dorado Outcrop (DO), yellow from Lō'ihi Seamount, and red from Juan de Fuca (JdF). Column labels indicate sample names, with further details available at BCO-DMO dataset 789136 [124].

Fig. 5. Non-metric dimensional scaling of microbial community composition from 16S rRNA gene sequence data compared at the family level of taxonomic-relatedness for crustal samples compared to sediment and seawater, with symbol shape representing sample type, color representing study dataset, and shading representing seafloor or subsurface origin, as shown in the legend. Subsurface rock samples from Juan de Fuca (JdF) and North Pond (NP) that are suspected to have high sequence contamination were removed from this analysis; for further detail on this subject see [126,106,105]. Further details about sample origins available at BCO-DMO dataset 789136 [124].

7. References

1. Fisher AT, Davis EE, Hutnak M, Spleess V, Zühlsdorff L, Cherkaoul A, Christiansen L, Edwards K, Macdonald R, Villinger HW, Mottl MJ, Wheat CG, Becker K (2003) Hydrothermal recharge and discharge across 50 km guided by seamounts on a young ridge flank. *Nature* 421:618-621. doi:10.1038/nature01352
2. Fisher AT, Wheat CG (2010) Seamounts as conduits for massive fluid, heat, and solute fluxes on ridge flanks. *Oceanography* 23:74-87. doi:10.5670/oceanog.2010.63
3. Hutnak M, Fisher AT, Harris R, Stein C, Wang K, Spinelli G, Schindler M, Villinger HW, Silver E (2008) Large heat and fluid fluxes driven through mid-plate outcrops on ocean crust. *Nature Geoscience* 1:611-614. doi:10.1038/ngeo264
4. Mottl MJ, Wheat CG (1994) Hydrothermal circulation through mid-ocean ridge flanks: Fluxes of heat and magnesium. *Geochimica et Cosmochimica Acta* 58 (10):2225-2237. doi:10.1016/0016-7037(94)90007-8
5. Mottl MJ, Wheat CG, Baker E, Becker K, Davis EE, Feely RA, Grehan A, Kadko DC, Lilley MD, Massoth GJ, Moyer C, Sansone FJ (1998) Warm springs discovered on 3.5 Ma oceanic crust, eastern flank of the Juan de Fuca Ridge. *Geology* 26 (1):51-54. doi:10.1130/0091-7613(1998)026<0051:WSDOMO>2.3.CO;2
6. Wheat CG, Elderfield H, Mottl MJ, Monnin C (2000) Chemical composition of basement fluids within an oceanic ridge flank: Implications for along-strike and across-strike hydrothermal circulation. *Journal of Geophysical Research* 105 (B6):13437-13447. doi:10.1029/2000JB900070
7. Wheat CG, Fisher AT (2008) Massive, low-temperature hydrothermal flow from a basaltic outcrop on 23 Ma seafloor of the Cocos Plate: Chemical constraints and implications. *Geochemistry Geophysics Geosystems* 9:Q12O14. doi:10.1029/2008GC002136
8. Wheat CG, Jannasch HW, Kastner M, Plant JN, DeCarlo EH (2003) Seawater transport and reaction in upper oceanic basaltic basement: chemical data from continuous monitoring of sealed boreholes in a ridge flank environment. *Earth and Planetary Science Letters* 216:549-564. doi:10.1016/S0012-821X(03)00549-1
9. Wheat CG, McManus J, Mottl MJ, Giambalvo E (2003) Oceanic phosphorus imbalance: Magnitude of the mid-ocean ridge flank hydrothermal sink. *Geophysical Research Letters* 30 (17):1895. doi:10.1029/2003GL017318
10. Wheat CG, Mottl MJ (2000) Composition of pore and spring waters from Baby Bare: Global implications of geochemical fluxes from a ridge flank hydrothermal system. *Geochimica et Cosmochimica Acta* 64 (4):629-642. doi:10.1016/S0016-7037(99)00347-6
11. Elderfield H, Schultz A (1996) Mid-ocean ridge hydrothermal fluxes and the chemical composition of the ocean. *Annual Reviews of Earth and Planetary Sciences* 24:191-224. doi:10.1146/annurev.earth.24.1.191
12. Elderfield H, Wheat CG, Mottl MJ, Monnin C, Spiro B (1999) Fluid and geochemical transport through oceanic crust: a transect across the eastern flank of the Juan de Fuca Ridge. *Earth and Planetary Science Letters* 172 (1-2):151-165. doi:10.1016/S0012-821X(99)00191-0

13. Wheat CG, Fisher AT, McManus J, Hulme SM, Orcutt BN (2017) Cool seafloor hydrothermal springs reveal global geochemical fluxes. *Earth and Planetary Science Letters* 476:179-188. doi:10.1016/j.epsl.2017.07.049
14. Fisher AT (2005) Marine hydrogeology: recent accomplishments and future opportunities. *Hydrogeology Journal* 13 (1):69-97. doi:doi.org/10.1007/s10040-004-0400-y
15. Villinger HW, Müller P, Bach W, Becker K, Orcutt BN, Kaul N, Wheat CG (2019) Evidence for low-temperature diffuse venting at North Pond, western flank of the Mid-Atlantic Ridge. *Geochemistry Geophysics Geosystems* 20:2018GC008113. doi:10.1029/2018GC008113
16. Wheat CG, Hartwell AM, McManus J, Fisher AT, Orcutt BN, Schlicht LEM, Niedenzu S, Bach W (2019) Geology and fluid discharge at Dorado Outcrop, a low temperature ridge-flank hydrothermal system. *Geochemistry Geophysics Geosystems* 20. doi:10.1029/2018GC007933
17. Becker K, Fisher AT (2008) Borehole packer tests at multiple depths resolve distinct hydrologic intervals in 3.5 Ma upper oceanic crust, eastern flank of Juan de Fuca Ridge. *Journal of Geophysical Research* 113:B07105. doi:10.1029/2007JB005447
18. Becker K, Fisher AT (2000) Permeability of upper oceanic basement on the eastern flank of the Endeavor Ridge determined with drill-string packer experiments. *Journal of Geophysical Research* 105 (B1):897-912. doi:10.1029/1999JB900250
19. Fisher AT (1998) Permeability within basaltic oceanic crust. *Review of Geophysics* 36 (2):143-182. doi:10.1029/97RG02916
20. Fisher AT, Becker K (2000) Channelized fluid flow in oceanic crust reconciles heat-flow and permeability data. *Nature* 403:71-74. doi:10.1038/47463
21. Fisk MR, Giovannoni SJ, Thorseth IH (1998) Alteration of oceanic volcanic glass: Textural evidence of microbial activity. *Science* 281:978-980. doi:10.1126/science.281.5379.978
22. Orcutt BN, Sylvan JB, Knab NJ, Edwards KJ (2011) Microbial ecology of the dark ocean above, at, and below the seafloor. *Microbiology and Molecular Biology Reviews* 75 (2):361-422. doi:10.1128/MMBR.00039-10
23. Matter JM, Stute M, Snæbjörnsdóttir SÓ, Oelkers EH, Gislason SR, Aradóttir ES, Sigfusson B, Gunnarsson I, Sigurdardóttir H, Gunnlaugsson E, Axelsson G, Alfredsson HA, Wolff-Boenisch D, Mesfin K, Fernandez de la Reguera Taya D, Hall J, Dideriksen K, Broecker WS (2016) Rapid carbon mineralization for permanent disposal of anthropogenic carbon dioxide emissions. *Science* 352 (6291):1312-1314. doi:10.1126/science.aad8132
24. Goldberg DS, Takahashi T, Slagle A (2008) Carbon sequestration in deep-sea basalt. *Proceedings of the National Academy of Science USA* 105 (29):9920-9925. doi:10.1073/pnas.0804397105
25. Orcutt BN, Edwards KJ (2014) Life in the Ocean Crust: Lessons from Subseafloor Laboratories. In: Stein R, Blackman DK, Inagaki F, Larsen H-C (eds) *Earth and Life Processes Discovered from Subseafloor Environment - A decade of science achieved by the Integrated Ocean Drilling Program (IODP)*, vol *Developments in Marine Geology* 7. Elsevier, Amsterdam/New York, pp 175-196. doi:10.1016/B978-0-444-62617-2.00007-4

26. IODP (2001) Initial Science Plan, 2003-2013: Earth, oceans, and Life - Scientific investigations of the Earth system using multiple drilling platforms and new technologies. International Working Group Support Office, Washington DC
27. IODP (2011) Science plan for 2013-2023: Illuminating Earth's past, present and future. Integrated Ocean Drilling Program Management International, Washington DC
28. Vance S, Harnmeijer J, Kimura J, Hussman H, DeMartin B, Brown JM (2007) Hydrothermal systems in small ocean planets. *Astrobiology* 7:987-1005
29. Russell MJ, Barge LM, Bhartia R, Bocanegra D, Bracher PJ, Branscomb E, Kidd R, McGlynn SE, Meier DH, Nitschke W, Shibuya T, Vance S, White L, Kanik I (2014) The drive to life on wet and icy worlds. *Astrobiology* 14 (4):308-343. doi:10.1089/ast.2013.1110
30. Jones RM, Goordial JM, Orcutt BN (2018) Low energy deep subsurface environments as extraterrestrial analogs. *Frontiers in Microbiology* 9:1605. doi:10.3389/fmicb.2018.01605
31. Orcutt BN, Bradley J, Brazelton WJ, Estes E, Goordial JM, Huber JA, Jones RM, Mahmoudi N, Marlow JJ, Murdock SA, Tivey MK (2020) Impacts of Deep-Sea Mining on Microbial Ecosystem Services. *Limnology and Oceanography* In press. doi:10.1002/lno.11403
32. Edwards KJ, Bach W, McCollom TM (2005) Geomicrobiology in oceanography: microbe-mineral interactions at and below the seafloor. *TRENDS in Microbiology* 13 (9):449-456. doi:10.1016/j.tim.2005.07.005
33. Schrenk MO, Huber JA, Edwards KJ (2009) Microbial provinces in the subseafloor. *Annual Reviews of Marine Sciences* 2:85-110. doi:10.1146/annurev-marine-120308-081000
34. Winslow DM, Fisher AT (2015) Sustainability and dynamics of outcrop-to-outcrop hydrothermal circulation. *Nature Communications* 6:Article Number 7567. doi:10.1038/ncomms8567
35. Neira NM, Clark JF, Fisher AT, Wheat CG, Haymon RM, Becker K (2016) Cross-hole tracer experiment reveals rapid fluid flow and low effective porosity in the upper oceanic crust. *Earth and Planetary Science Letters* 450:335-365. doi:10.1016/j.epsl.2016.06.048
36. Wessel P, Sandwell DT, Kim S-S (2010) The Global Seamount Census. *Oceanography* 23 (1):24-33. doi:10.5670/oceanog.2010.60
37. Etnoyer PJ, Wood J, Shirley TC (2010) How Large is the Seamount Biome. *Oceanography* 23 (1):206-209. doi:10.5670/oceanog.2010.96
38. Shank TM (2010) Seamounts: Deep-ocean Laboratories of Faunal Connectivity, Evolution, and Endemism. *Oceanography* 23 (1):108-122. doi:10.5670/oceanog.2010.65
39. Hensen C, Duarte JC, Vannucchi P, Mazzini A, Lever MA, Terrinha P, Géli L, Henry P, Villinger H, Morgan JP (2019) Marine transform faults and fracture zones: a new perspective integrating seismicity, fluid flow and life. *Frontiers in Earth Science* 7:39. doi:10.3389/feart.2019.00039
40. McCollom TM, Shock EL (1997) Geochemical constraints on chemolithoautotrophic metabolism by microorganisms in seafloor hydrothermal systems. *Geochimica et Cosmochimica Acta* 61 (20):4375-4391. doi:10.1016/S0016-7037(97)00241-X

41. Bach W (2016) Some compositional and kinetic controls on the bioenergetic landscapes in oceanic basement. *Frontiers in Microbiology* 7:107. doi:10.3389/fmicb.2016.00107
42. Bach W, Humphris S, Fisher AT (2004) Fluid flow and fluid-rock interaction within ocean crust: Reconciling geochemical, geological, and geophysical observations, vol 144. *Geophysical monograph*. American Geophysical Union. doi:10.1029/144GM07
43. Türke A, Nakamura K, Bach W (2015) Palagonitization of basalt glass in the flanks of Mid-Ocean Ridges: Implications for the bioenergetics of oceanic intracrustal ecosystems. *Astrobiology* 15 (10):793-803. doi:10.1089/ast.2014.1255
44. Hein JR, Conrad TA, Staudigel H (2010) Seamount mineral deposits: A source of rare metals for high-technology industries. *Oceanography* 23 (1):184-189. doi:10.5670/oceanog.2010.70
45. Hein JR, Mizell K, Koschinsky A, Conrad TA (2013) Deep-ocean mineral deposits as a source of critical metals for high- and green-technology applications: Comparison with land-based resources. *Ore Geology Reviews* 51:1-14. doi:10.1016/j.oregeorev.2012.12.001
46. McCollom TM (2007) Geochemical constraints on sources of metabolic energy for chemolithoautotrophy in ultramafic-hosted deep-sea hydrothermal systems. *Astrobiology* 7 (6):933-950. doi:10.1089/ast.2006.0119
47. Schrenk MO, Brazelton WJ, Lang SQ (2013) Serpentinization, carbon, and deep life. *Reviews in Mineralogy and Geochemistry* 75 (1):575-606. doi:10.2138/rmg.2013.75.18
48. Bach W, Edwards KJ (2003) Iron and sulfide oxidation within the basaltic ocean crust: Implications for chemolithoautotrophic microbial biomass production. *Geochimica Et Cosmochimica Acta* 67 (20):3871-3887. doi:10.1016/s0016-7037(00)00304-1
49. Becker K, Davis EE (2005) A review of CORK designs and operations during the Ocean Drilling Program. *Proceedings of the Integrated Ocean Drilling Program*, 301. College Station, TX. doi:doi: 10.2204/iodp.proc.301.104.2005
50. Davis EE, Becker K, Pettigrew TL, Carson B, Macdonald R (1992) CORK: a hydrologic seal and downhole observatory for deep-ocean boreholes. In: Davis EE, Mottl MJ, Fisher AT (eds) *Proceedings of the Ocean Drilling Program, Initial Reports, Vol. 139*. Ocean Drilling Program, College Station, TX, pp 43-53
51. Fisher AT, Wheat CG, Becker K, Cowen J, Orcutt B, Hulme S, Inderbitzen K, Haddad A, Pettigrew TL, Davis EE, Jannasch H, Grigar K, Aduddell R, Meldrum R, Macdonald R, Edwards KJ (2011) Design, deployment, and status of borehole observatory systems used for single-hole and cross-hole experiments, IODP Expedition 327, eastern flank of Juan de Fuca Ridge. In: Fisher AT, Tsuji T, Petronotis K, Expedition327Scientists (eds) *Proc. IODP, 327*. Integrated Ocean Drilling Program Management International, Inc., Tokyo. doi:10.2204/iodp.proc.327.107.2011
52. Fisher AT, Wheat CG, Becker K, Davis EE, Jannasch HW, Schroeder D, Dixon R, Pettigrew TL, Meldrum R, Macdonald R, Nielsen M, Fisk MR, Cowen J, Bach W, Edwards KJ (2005) Scientific and technical design and deployment of long-term, subseafloor observatories for hydrogeologic and related experiments, IODP Expedition 301, Eastern Flank of Juan de Fuca Ridge. In: Fisher AT, Urabe T, Klaus A (eds) *Proceedings IODP, Expedition 301*. Integrated Ocean Drilling Program, College Station, TX. doi:10.2204/iodp.proc.301.103.2005

53. Wheat CG, Jannasch HW, Fisher AT, Becker K, Sharkey J, Hulme S (2010) Subseafloor seawater-basalt-microbe reactions: Continuous sampling of borehole fluids in a ridge flank environment. *Geochemistry Geophysics Geosystems* 11:Q07011. doi:10.1029/2010GC00305
54. Wheat CG, Mottl MJ (2004) Geochemical fluxes through mid-ocean ridge flanks. In: Davis EE, Elderfield H (eds) *Hydrogeology of the Oceanic Lithosphere*. Cambridge University Press, Cambridge, UK, pp 627-658
55. Meyer JL, Jaekel U, Tully BJ, Glazer BT, Wheat CG, Lin H-T, Hsieh C-C, Cowen JP, Hulme S, Girguis PR, Huber JA (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. doi:10.1038/srep22541
56. Orcutt BN, Bach W, Becker K, Fisher AT, Hentscher M, Toner BM, Wheat CG, Edwards KJ (2011) Colonization of subsurface microbial observatories deployed in young ocean crust. *The ISME Journal* 5:692-703. doi:10.1038/ismej.2010.157
57. Cowen J, Giovannoni SJ, Kenig F, Johnson HP, Butterfield DA, Rappé MS, Hutnak M, Lam P (2003) Fluids from aging ocean crust that support microbial life. *Science* 299. doi:10.1126/science.1075653
58. Cowen JP, Copson DA, Jolly J, Hsieh C-C, Lin H-T, Glazer BT, Wheat CG (2012) Advanced instrument system for real-time and time-series microbial geochemical sampling of the deep (basaltic) crustal biosphere. *Deep-Sea Research I* 61:43-56. doi:10.1016/j.dsr.2011.11.004
59. Smith A, Popa R, Fisk MR, Nielsen M, Wheat CG, Jannasch HW, Fisher AT, Becker K, Sievert SM, Flores G (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. doi:10.1029/2010GC003424
60. Corliss J, Dymond J, Gordon LI, Edmond J, von Herzen R, Ballard R, Green K, Williams D, Bainbridge A, Crane K, van Andel T (1979) Submarine thermal springs on the Galapagos Rift. *Science* 203:1073-1083. doi:10.1126/science.203.4385.1073
61. Karl DM, Wirsén CO, Jannasch HW (1980) Deep-Sea Primary Production at the Galapagos Hydrothermal Vents. *Science* 207 (4437):1345-1347. doi:10.1126/science.207.4437.1345
62. Baross JA, Lilley MD, Gordon LI (1982) Is the CH₄, H₂ and CO venting from submarine hydrothermal systems produced by thermophilic bacteria? *Nature* 298:366-368. doi:10.1038/298366a0
63. Baross JA, Deming JW (1983) Growth of 'black smoker' bacteria at temperatures of at least 250 C. *Nature* 303 (5916):423. doi:10.1038/303423a0
64. Tuttle J, Wirsén C, Jannasch H (1983) Microbial activities in the emitted hydrothermal waters of the Galapagos Rift vents. *Marine Biology* 73 (3):293-299. doi:10.1007/BF00392255
65. Jannasch HW, Mottl MJ (1985) Geomicrobiology of deep-sea hydrothermal vents. *Science* 229:717-725. doi:10.1126/science.229.4715.717

66. Furnes H, Staudigel H, Thorseth IH, Torsvik T, Muehlenbachs K, Tumyr O (2001) Bioalteration of basaltic glass in the oceanic crust. *Geochemistry, Geophysics, Geosystems* 2:2000GC000150
67. Furnes H, Staudigel H (1999) Biological mediation in ocean crust alteration: how deep is the deep biosphere? *Earth and Planetary Science Letters* 166 (3-4):97-103. doi:10.1016/S0012-821X(99)00005-9
68. Giovannoni SJ, Fisk MR, Mullins T, Furnes H (1996) Genetic evidence for endolithic microbial life colonizing basaltic glass/seawater interfaces. *Proceedings of the Ocean Drilling Program, Scientific results, Vol. 148. Ocean Drilling Program, College Station, TX*
69. Torsvik T, Furnes H, Muehlenbachs K, Thorseth IH, Tumyr O (1998) Evidence for microbial activity at the glass-alteration interface in oceanic basalts. *Earth and Planetary Science Letters* 162 (1-4):165-176. doi:10.1016/S0012-821X(98)00164-2
70. Thorseth IH, Torsvik T, Furnes H, Muehlenbachs K (1995) Microbes play an important role in the alteration of oceanic crust. *Chemical Geology* 126 (2):137-146. doi:10.1016/0009-2541(95)00114-8
71. Furnes H, Thorseth IH, Tumyr O, Torsvik T, Fisk MR (1996) Microbial activity in the alteration of glass from pillow lavas from Hole 896A. In: Alt JC, Kinoshita H, Stokking LB, Michael PJ (eds) *Proceedings of the Ocean Drilling Program, Scientific Results, Vol. 148. Ocean Drilling Program, pp 191-206*
72. Thorseth IH, Furnes H, Heldal M (1992) The importance of microbiological activity in the alteration of natural basaltic glass. *Geochimica et Cosmochimica Acta* 56:845-850
73. Alt JC, Shanks III WC (1998) Sulfur in serpentinized oceanic peridotites: Serpentinization processes and microbial sulfate reduction. *Journal of Geophysical Research* 103 (B5):9917-9929. doi:10.1029/98JB00576
74. Fisk MR, Popa R, Wacey D (2019) Tunnel formation in basalt glass. *Astrobiology* 19 (1):132-144. doi:10.1089/ast.2017.1791
75. Thorseth IH, Torsvik T, Torsvik V, Daae FL, Pedersen RB, Keldysh-98 Scientific Party (2001) Diversity of life in the ocean floor basalt. *Earth and Planetary Science Letters* 194:31-37. doi:10.1016/S0012-821X(01)00537-4
76. Thorseth IH, Pedersen RB, Christie DM (2003) Microbial alteration of 0-30-Ma seafloor and sub-seafloor basaltic glasses from the Australian Antarctic Discordance. *Earth and Planetary Science Letters* 215:237-247. doi:10.1016/S0012-821X(03)00427-8
77. Fisk MR, McLoughlin N (2013) Atlas of alteration textures in volcanic glass from the ocean basins. *Geosphere* 9 (2):317-341. doi:10.1130/GES00827.1
78. Edwards KJ, Wheat CG, Orcutt BN, Hulme S, Becker K, Jannasch H, Haddad A, Pettigrew TL, Rhinehart W, Grigar K, Bach W, Kirkwood W, Klaus A (2012) Design and deployment of borehole observatories and experiments during IODP Expedition 336 Mid-Atlantic Ridge flank at North Pond. *Proc. IODP, 336. Integrated Ocean Drilling Program Management International, Inc., Tokyo. doi:10.2204/iodp.proc.336.109.2012*

79. Ramírez GA, Garber AI, Lecoivre A, D'Angelo T, Wheat CG, Orcutt BN (2019) Ecology of subseafloor crustal biofilms. *Frontiers in Microbiology* 10:1983. doi:10.3389/fmicb.2019.01983
80. Bengtson S, Ivarsson M, Astolfo A, Belivanova V, Broman C, Marone F, Stampanoni M (2014) Deep-biosphere consortium of fungi and prokaryotes in Eocene subseafloor basalts. *Geobiology* 12:489-496. doi:10.1111/gbi.12100
81. Ivarsson M, Schnürer A, Bengtson S, Neubeck A (2016) Anaerobic fungi: A potential source of biological H₂ in the oceanic crust. *Frontiers in Microbiology* 7:674. doi:10.3389/fmicb.2016.00674
82. Ivarsson M, Bengtson S, Belivanova V, Stampanoni M, Marone F, Tehler A (2012) Fossilized fungi in subseafloor Eocene basalts. *Geology* 40 (2):163-166. doi:10.1130/G32590.1
83. Rouxel OJ, Ono S, Alt J, Rumble D, Ludden J (2008) Sulfur isotope evidence for microbial sulfate reduction in altered oceanic basalts at ODP Site 801. *Earth and Planetary Science Letters* 268:110-123. doi:10.1016/j.epsl.2008.01.010
84. Lever MA (2013) Functional gene surveys from ocean drilling expeditions - A review and perspective. *FEMS Microbiology Ecology* 84:1-23. doi:10.1111/1574-6941.12051
85. Robador A, Jungbluth SP, LaRowe DE, Bowers RM, Rappé MS, Amend JP, Cowen JP (2015) Activity and phylogenetic diversity of sulfate-reducing microorganisms in low-temperature subsurface fluids with the upper oceanic crust. *Frontiers in Microbiology* 5:748. doi:10.3389/fmicb.2014.00748
86. Robador A, LaRowe DE, Jungbluth SP, Lin H-T, Rappé MS, Nealson KH, Amend JP (2016) Nanocalorimetric characterization of microbial activity in deep subsurface oceanic crustal fluids. *Frontiers in Microbiology* 7:454. doi:10.3389/fmicb.2016.00454
87. Orcutt BN, Wheat CG, Rouxel OJ, Hulme S, Edwards KJ, Bach W (2013) Oxygen consumption rates in subseafloor basaltic crust derived from a reaction transport model. *Nature Communications* 4:2539. doi:10.1038/ncomms3539
88. D'Hondt S, Inagaki F, Zarikian CA, Abrams LJ, Dubois N, Engelhardt T, Evans H, Ferdelman T, Gribsholt B, Harris RN, Hoppie BW, Hyun J-H, Kallmeyer J, Kim J, Lynch JE, McKinley CC, Mitsunobu S, Morono Y, Murray RW, Pockalny R, Sauvage J, Shimono T, Shiraishi F, Smith DC, Smith-Duque CE, Spivack AJ, Steinsbu BO, Suzuki Y, Szpak M, Toffin L, Uramoto G, Yamaguchi YT, Zhang G-l, Zhang X-H, Ziebis W (2015) Presence of oxygen and aerobic communities from sea floor to basement in deep-sea sediments. *Nature Geoscience* 8:299-304. doi:10.1038/ngeo2387
89. Ziebis W, McManus J, Ferdelman T, Schmidt-Schierhorn F, Bach W, Muratli J, Edwards KJ, Villinger H (2012) Interstitial fluid chemistry of sediments underlying the North Atlantic Gyre and the influence of subsurface fluid flow. *Earth and Planetary Science Letters* 323-324:79-91. doi:10.1016/j.epsl.2012.01.018
90. Orcutt BN, Sylvan JB, Rogers DR, Delaney J, Lee RW, Girguis PR (2015) Carbon fixation by basalt-hosted microbial communities. *Frontiers in Microbiology* 6:904. doi:10.3389/fmicb.2015.00904

91. Lang SQ, Butterfield DA, Lilley MD, Johnson HP, Hedges JI (2006) Dissolved organic carbon in ridge-axis and ridge-flank hydrothermal systems. *Geochim Cosmochim Acta* 70:3830-3842. doi:10.1016/j.gca.2006.04.031
92. Lin H-T, Cowen JP, Olson EJ, Amend JP, Lilley MD (2012) Inorganic chemistry, gas compositions and dissolved organic carbon in fluids from sedimented young basaltic crust on the Juan de Fuca Ridge flanks. *Geochimica et Cosmochimica Acta* 85:213-227. doi:10.1016/j.gca.2012.02.017
93. Shah Walker SR, Jaekel U, Osterholz H, Fisher AT, Huber JA, Pearson A, Dittmar T, Girguis PR (2018) Microbial decomposition of marine dissolved organic matter in cool oceanic crust. *Nature Geoscience* 11:334-339. doi:10.1038/s41561-018-0109-5
94. Dzaugis ME, Spivack AJ, Dunlea AG, Murray RW, D'Hondt S (2016) Radiolytic hydrogen production in the subseafloor basaltic aquifer. *Frontiers in Microbiology*. doi:10.3389/fmicb.2016.00076
95. Edwards KJ, McCollom TM, Konishi H, Buseck PR (2003) Seafloor bioalteration of sulfide minerals: Results from in-situ incubation studies. *Geochimica et Cosmochimica Acta* 67:2843-2856. doi:10.1016/S0016-7037(03)00089-9
96. Rogers DR, Santelli CM, Edwards KJ (2003) Geomicrobiology of deep-sea deposits: Estimating community diversity from low-temperature seafloor rocks and minerals. *Geobiology* 11:109-117. doi:10.1046/j.1472-4669.2003.00009.x
97. Lysnes K, Thorseth IH, Steinsbu BO, Øvreås L, Torsvik T, Pedersen RB (2004) Microbial community diversity in seafloor basalt from the Arctic spreading ridges. *FEMS Microbiology Ecology* 50:213-230. doi:10.1016/j.femsec.2004.06.014
98. Mason OU, Di Meo-Savoie CA, Van Nostrand JD, Zhou J, Fisk MR, Giovannoni SJ (2009) Prokaryotic diversity, distribution, and insights into their role in biogeochemical cycling in marine basalts. *The ISME Journal* 3:231-242. doi:10.1038/ismej.2008.92
99. Mason OU, Stingl U, Wilhelm LJ, Moeseneder MM, Di Meo-Savoie CA, Fisk MR, Giovannoni SJ (2007) The phylogeny of endolithic microbes associated with marine basalts. *Environmental Microbiology* 9 (10):2539-2550. doi:10.1111/j.1462-2920.2007.01372.x
100. Santelli CM, Edgcomb VP, Bach W, Edwards KJ (2009) The diversity and abundance of bacteria inhabiting seafloor lavas positively correlate with rock alteration. *Environmental Microbiology* 11 (1):86-98. doi:10.1111/j.1462-2920.2008.01743.x
101. Santelli CM, Orcutt BN, Banning E, Bach W, Moyer CL, Sogin ML, Staudigel H, Edwards KJ (2008) Abundance and diversity of microbial life in ocean crust. *Nature* 453:653-656. doi:10.1038/nature06899
102. Edwards KJ, Fisher AT, Wheat CG (2012) The deep subsurface biosphere in igneous ocean crust: frontier habitats for microbiological exploration. *Frontiers in Microbiology* 3:Article 8. doi:10.3389/fmicb.2012.00008
103. Lee MD, Walworth NG, Sylvan JB, Edwards KJ, Orcutt BN (2015) Microbial communities on seafloor basalts at Dorado Outcrop reflect level of alteration and highlight global lithic clades. *Frontiers in Microbiology* 6:1470. doi:10.3389/fmicb.2015.01470

104. Jacobsen Meyers ME, Sylvan JB, Edwards KJ (2014) Extracellular enzyme activity and microbial diversity measured on seafloor exposed basalts from Loihi Seamount indicate importance of basalts to global biogeochemical cycling. *Applied and Environmental Microbiology*. doi:10.1128/AEM.01038-14
105. Jørgensen SL, Zhao R (2016) Microbial inventory of deeply buried oceanic crust from a young ridge flank. *Frontiers in Microbiology* 7:820. doi:10.3389/fmicb.2016.00820
106. LaBonté JM, Lever MA, Edwards KJ, Orcutt BN (2017) Influence of igneous basement on deep sediment microbial diversity on the eastern Juan de Fuca Ridge flank. *Frontiers in Microbiology* 8:1434. doi:10.3389/fmicb.2017.01434
107. Smith AR, Fisk MR, Thurber AR, Flores G, Mason OU, Popa R, Colwell FS (2016) Deep crustal communities on the Juan de Fuca Ridge are governed by minearology. *Geomicrobiology Journal*. doi:10.1080/01490451.2016.1155001
108. Jungbluth SP, Bowers RM, Lin H-T, Cowen JP, Rappé MS (2016) Novel microbial assemblages inhabiting crustal fluids within mid-ocean ridge flank subsurface basalt. *The ISME Journal* 10:2033-2047. doi:10.1038/ismej.2015.248
109. Tully BJ, Wheat CG, Glazer BT, Huber JA (2017) A dynamic microbial community with high functional redundancy inhabits the cold, oxic subseafloor aquifer. *The ISME Journal* 12:1-16. doi:10.1038/ismej.2017.187
110. Reese BK, Zinke LA, Sobol MS, LaRowe DE, Zhang X-X, Jaekel U, Wang F, Orcutt BN, Dittmar T, Defforey D, Tully BJ, Paytan A, Sylvan JB, Amend JP, Edwards KJ, Girguis PR (2018) Nitrogen cycling potential of active bacteria within oligotrophic sediment of the Mid-Atlantic Ridge Flank. *Geomicrobiology Journal* 35:468-483. doi:10.1080/01490451.2017.1392649
111. Zinke LA, Kiel Reese B, McManus J, Wheat CG, Orcutt BN, Amend JP (2018) Sediment microbial communities influenced by cool hydrothermal fluid migration. *Frontiers in Microbiology* 9:1249. doi:10.3389/fmicb.2018.01249
112. Huber JA, Johnson HP, Butterfield DA, Baross JA (2006) Microbial life in ridge flank crustal fluids. *Environmental Microbiology* 8 (1):88-99. doi:10.1073/pnas.0605127103
113. Jungbluth SP, Lin H-T, Cowen JP, Glazer BT, Rappé MS (2014) Phylogenetic diversity of microorganisms in subseafloor crustal fluids from Holes 1025C and 1026B along the Juan de Fuca Ridge flank. *Frontiers in Microbiology* 5:119. doi:10.3389/fmicb.2014.00119
114. Orcutt B, Wheat CG, Edwards KJ (2010) Subseafloor ocean crust microbial observatories: Development of FLOCS (FLOw-through Osmo Colonization System) and the evaluation of borehole construction materials. *Geomicrobiology Journal* 27 (2):143-157. doi:10.1080/01490450903456772
115. Barco RA, Hoffman CL, Ramírez GA, Toner BM, Edwards KJ, Sylvan JB (2017) *In situ* incubation of iron-sulfur mineral reveals a diverse chemolithoautotrophic community and a new biogeochemical role for *Thiomicrospira*. *Environmental Microbiology* 19:1322-1337. doi:10.1111/1462-2920.13666

116. Baquiran J-P, Ramírez GA, Haddad AG, Toner BM, Hulme S, Wheat CG, Edwards KJ, Orcutt BN (2016) Temperature and redox effect on mineral colonization in Juan de Fuca Ridge Flank subsurface crustal fluids. *Frontiers in Microbiology* 7:396. doi:10.3389/fmicb.2016.00396
117. Sudek LA, Templeton AS, Tebo BM, Staudigel H (2009) Microbial ecology of Fe(hydr)oxide mats and basalts rock from Vailulu'u Seamount, American Samoa. *Geomicrobiology Journal* 26 (8):581-596. doi:10.1080/01490450903263400
118. Sylvan JB, Sia TY, Haddad AG, Briscoe LJ, Toner BM, Girguis PR, Edwards KJ (2013) Low temperature geomicrobiology follows host rock composition along a geochemical gradient in Lau Basin. *Frontiers in Microbiology* 4:61. doi:10.3389/fmicb.2013.00061
119. Fisk MR, Storrie-Lombardi MC, Douglas S, Popa R, McDonald G, Di Meo-Savoie C (2003) Evidence of biological activity in Hawaiian subsurface basalts. *Geochemistry, Geophysics, Geosystems* 4 (12):1103. doi:10.1029/2002GC000387
120. Nitahara S, Kato S, Urabe T, Usui A, Yamagishi A (2011) Molecular characterization of the microbial community in hydrogenetic ferromanganese crusts of the Takuyo-Daigo Seamount, northwest Pacific. *FEMS Microbiology Letters* 321 (2):121-129. doi:10.1111/j.1574-6968.2011.02323.x
121. Mason OU, Nakagawa T, Rosner M, Van Nostrand JD, Zhou J, Maruyama A, Fisk MR, Giovannoni SJ (2010) First investigation of the microbiology of the deepest layer of ocean crust. *PLoS ONE* 5 (11):e15399. doi:10.1371/journal.pone.0015399
122. Ehrhardt CJ, MHaymon RM, Lamontagne MG, Holden PA (2007) Evidence for hydrothermal Archaea within the basaltic flanks of the East Pacific Rise. *Environmental Microbiology* 9 (4):900-912. doi:10.1111/j.1462-2920.2006.01211.x
123. Jungbluth SP, Amend JP, Rappé MS (2017) Metagenome sequencing and 98 microbial genomes from Juan de Fuca Ridge flank subsurface fluids. *Scientific Data* 4:170037. doi:10.1038/sdata.2017.37
124. Orcutt BN, D'Angelo T (2020) Synthesis of publicly-available sequence datasets of the 16S rRNA gene in environmental DNA extracted from seafloor and subseafloor samples from the Dorado Outcrop, Lō'ihi Seamount, North Pond, and Juan de Fuca Ridge flank. . Dataset version 2020-02-04 edn., Biological and Chemical Oceanography Data Management Office (BCO-DMO). doi:10.1575/1912/bco-dmo.789136.1
125. Ramirez KS, Knight CG, De Hollander M, Brearley FQ, Constantinides B, Cotton A, Creer S, Crowther TW, Davison J, Delgado-Baquerizo M (2018) Detecting macroecological patterns in bacterial communities across independent studies of global soils. *Nature Microbiology* 3 (2):189-196. doi:10.1038/s41564-017-0062-x
126. Sheik CS, Reese BK, Twing KI, Sylvan JB, Grim SL, Schrenk MO, Sogin ML, Colwell FS (2018) Identification and removal of contaminant sequences from ribosomal gene databases: Lessons form the Census of Deep Life. *Frontiers in Microbiology* 9:840. doi:10.3389/fmicb.2018.00840
127. Santelli CM, Banerjee N, Bach W, Edwards KJ (2010) Tapping the subsurface ocean crust biosphere: Low biomass and drilling-related contamination calls for improved quality controls. *Geomicrobiology Journal* 27 (2):158-169. doi:10.1080/01490450903456780

- 1 128. Lever MA, Rouxel OJ, Alt JC, Shimizu N, Ono S, Coggon RM, Shanks III WC, Lapham
2 L, Elvert M, Prieto Mollar X, Hinrichs KU, Inagaki F, Teske A (2013) Evidence for microbial
3 carbon and sulfur cycling in deeply buried ridge flank basalt. *Science* 339 (6125):1305-1308.
4 doi:10.1126/science.1229240
- 5 129. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO
6 (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-
7 based tools. *Nucleic Acids Research* 41 (D1):D590-D596. doi:10.1093/nar/gks1219
- 8 130. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J,
9 Ludwig W, Glöckner FO (2014) The SILVA and "all-species Living Tree Project (LTP)"
10 taxonomic frameworks. *Nucleic Acids Research* 42:643-648. doi:10.1093/nar/gkt1209
- 11 131. Glöckner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, Bruns G, Yarza P,
12 Peplies J, Westram R, Ludwig W (2017) 25 years of serving the community with ribosomal
13 RNA gene reference databases and tools. *Journal of Biotechnology* 261:169-176.
14 doi:10.1016/j.jbiotec.2017.06.1198
- 15 132. Hubert C, Loy A, Nickel M, Arnosti C, Baranyi C, Brüchert V, Ferdelman T, Finster K,
16 Christensen FM, de Rezende JR, Vandieken V, Jørgensen BB (2009) A constant flux of diverse
17 thermophilic bacteria into the cold arctic seabed. *Science* 325:1541-1544.
18 doi:10.1126/science.1174012
- 19 133. Müller AL, Rosa de Reszende J, Hubert C, Kjeldsen KU, Lagkouvardos I, Berry D,
20 Jørgensen BB, Loy A (2014) Endospores of thermophilic bacteria as tracers of microbial
21 dispersal by ocean currents. *The ISME Journal* 8:1153-1165. doi:10.1038/ismej.2013.225
- 22 134. Jungbluth SP, Glavina del Rio T, Tringe SG, Stepanauskas R, Rappé MS (2017) Genomic
23 comparisons of a bacteriophage lineage that inhabits both marine and terrestrial deep subsurface
24 systems. *PeerJ* 5:e3134. doi:10.7717/peerj.3134
- 25 135. LaBonté JM, Field EK, Lau M, Chivian D, van Heerden E, Wommack KE, Kieft TL,
26 Onstott TC, Stepanauskas R (2015) Single cell genomics indicates horizontal gene transfer and
27 viral infections in a deep subsurface Firmicutes populations. *Frontiers in Microbiology* 6:349.
28 doi:10.3389/fmicb.2015.00349
- 29 136. Früh-Green GL, Orcutt BN, Green S, Cotterill C, Expedition 357 Scientists (2016)
30 Expedition 357 Preliminary Report: Atlantis Massif Serpentinization and Life. *International*
31 *Ocean Discovery Program*. doi:10.14379/iodp.pr.357.2016
- 32 137. Dick HJB, MacLeod CJ, Blum P, The Expedition 360 Scientists (2016) Expedition 360
33 Preliminary Report: Southwest Indian Ridge lower crust and Moho. *International Ocean*
34 *Discovery Program*. doi:10.14379/iodp.pr.360.2016
- 35 138. Früh-Green G, Orcutt BN, Rouméjon S, Lilley MD, Morono Y, Cotterill C, Green SL,
36 Escartín J, John BE, McCaig AM, Cannat M, Ménéz B, Schwarzenbach EM, Williams M,
37 Morgan S, Lang SQ, Schrenk MO, Brazelton WJ, Akizawa N, Boschi C, Dunkel KG,
38 Quéméneur M, Whattham SA, Mayhew LE, Harris M, Bayrakci G, Behrmann J-H, Herrero-
39 Bervera E, Hesse K, Liu HQ, Sandaruwan Ratnayake A, Twing K, Weis D, Zhao R, Bilenker L
40 (2018) Magmatism, serpentinization and life: Insights through drilling the Atlantis Massif
41 (IODP Expedition 357). *Lithos*. doi:10.1016/j.lithos.2018.09.012

139. Dick HJB, MacLeod CJ, Blum P, Abe N, Blackman DK, Bowles JA, Cheadle MJ, Cho K, Ciazela J, Deans JR, Edgcomb VP, Ferrando C, France L, Ghosh B, Ildefonse B, John BE, Kendrick MA, Koepke J, Leong JAM, Liu C, Ma Q, Morishita T, Morris A, Natland JH, Nozaka T, Pluempert O, Sanfilippo A, Sylvan JB, Tivey MA, Tribuzio R, Viegas LGF (2019) Dynamic accretion beneath a slow-spreading ridge segment : IODP Holes 1473A and the Atlantis Bank Oceanic Core Complex. *Journal of Geophysical Research-Solid Earth* 124:12631-12659. doi:10.1029/2018JB016858
140. Brazelton WJ, Ludwig KA, Sogin ML, Andreishcheva EN, Kelley DS, Shen C-C, Edwards RL, Baross JA (2010) Archaea and bacteria with surprising microdiversity show shifts in dominance over 1000-year time scales in hydrothermal chimneys. *Proceedings of the National Academy of Science USA*. doi:10.1073/pnas.0905369107
141. Brazelton WJ, Morrill PL, Szponar N, Schrenk MO (2013) Bacterial communities associated with subsurface geochemical processes in continental serpentinite springs. *Applied and Environmental Microbiology* 79:3906-3916. doi:10.1128/AEM.00330-13
142. Brazelton WJ, Schrenk MO, Kelley DS, Baross JA (2006) Methane- and sulfur-metabolizing microbial communities dominate the Lost City Hydrothermal Field ecosystem. *Applied and Environmental Microbiology* 72 (9):6257-6270. doi:10.1128/AEM.00574-06
143. Schrenk MO, Kelley DS, Bolton SA, Baross JA (2004) Low archaeal diversity linked to subsurface geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge. *Environmental Microbiology* 6 (10):1086-1095. doi:10.1111/j.1462-2920.2004.00650.x
144. Edgcomb VP, Beaudoin D, Gast RJ, Biddle JF, Teske A (2011) Marine subsurface eukaryotes: the fungal majority. *Environmental Microbiology* 13 (1):172-183. doi:10.1111/j.1462-2920.2010.02318.x
145. Orsi W, Biddle JF, Edgcomb VP (2013) Deep sequencing of subsurface eukaryotic rRNA reveals active Fungi across marine subsurface provinces. *PLoS ONE* 8 (2):e56335. doi:10.1371/journal.pone.0056335
146. Nigro OD, Jungbluth SP, Lin H-T, Hsieh C-C, Miranda JA, Schvarcz CR, Rappé MS, Steward GF (2017) Viruses in the oceanic basement. *mBio* 8 (2):e02129-02116. doi:10.1128/mBio.02129-16
147. Anderson R, Sogin ML, Baross JA (2014) Evolutionary strategies of Viruses, Bacteria, and Archaea in hydrothermal vent ecosystems revealed through metagenomics. *PLoS ONE* 9 (10):e109696. doi:10.1371/journal.pone.0109696
148. Anderson RE, Brazelton WJ, Baross JA (2011) Is the genetic landscape of the deep subsurface biosphere affected by viruses? *Frontiers in Microbiology* 2:219. doi:10.3389/fmicb.2011.00219
149. Anderson RE, Brazelton WJ, Baross JA (2013) The Deep Viriosphere: Assessing the viral impact on microbial community dynamics in the deep subsurface. In: Hazen RM, Jones AP, Baross JA (eds) *Reviews in Mineralogy and Geochemistry*, vol 75. The Mineralogical Society of America, Chantilly, Virginia, USA, pp 649-675. doi:10.2138/rmg.2013.75.20
150. Williamson SJ, Cary SC, Williamson KE, Helton RR, Bench SR, Winget D, Wommack KE (2008) Lysogenic virus-host interactions predominate at deep-sea diffuse-flow hydrothermal vents. *The ISME Journal* 2 (11):1112-1121. doi:10.1038/ismej.2008.73

151. Zhang X, Feng X, Wang F (2016) Diversity and metabolic potential of subsurface crustal microorganisms from the western flank of the Mid-Atlantic Ridge. *Frontiers in Microbiology* 7:363. doi:10.3389/fmicb.2016.00363
152. Smith AR, Kieft B, Mueller RS, Fisk MR, Mason OU, Popa R, Colwell FS (2019) Carbon fixation and energy metabolisms of a subseafloor olivine biofilm. *The ISME Journal* 13 (7):1737-1740. doi:10.1038/s41396-019-0385-0
153. Trembath-Reichert E, Butterfield DA, Huber JA (2019) Active subseafloor microbial communities from Mariana back-arc venting fluids share metabolic strategies across different thermal niches and taxa. *The ISME Journal*. doi:10.1038/s41396-019-0431-y
154. Galambos D, Anderson RE, Reveillaud J, Huber JA (2019) Genome-resolved metagenomics and metatranscriptomics reveal niche differentiation in functionally redundant microbial communities at deep-sea hydrothermal vents. *Environmental Microbiology* 21:4395-4410. doi:10.1111.1462-2920.14806
155. Boyd J, Jungbluth S, Leu A, Evans P, Woodcroft B, Chadwick G, Orphan V, Amend J, Rappe M, Tyson G (2018) Divergent methyl-coenzyme M reductase genes in a deep-subseafloor *Archaeoglobi*. *bioRxiv*. doi:10.1101/390617
156. Carr SA, Jungbluth SP, Elie-Fadrosch EA, Stepanauskas R, Woyke T, Rappé MS, Orcutt BN (2019) Carboxydolithotrophy potential of uncultivated Hydrothermarchaeota from the subseafloor crustal biosphere. *The ISME Journal* 13 (9):1457-1468. doi:10.1038/s41396-019-0352-9
157. Fortunato CS, Huber JA (2016) Coupled RNA-SIP and metatranscriptomics of active chemolithoautotrophic communities at a deep-sea hydrothermal vent. *The ISME journal* 10 (8):1925. doi:10.1038/ismej.2015.258
158. Fortunato CS, Larson B, Butterfield DA, Huber JA (2018) Spatially distinct, temporally stable microbial populations mediate biogeochemical cycling at and below the seafloor in hydrothermal vent fluids. *Environmental microbiology* 20 (2):769-784. doi:10.1111/1462-2920.14011
159. McNichol J, Stryhanyuk H, Sylva SP, Thomas F, Musat N, Seewald JS, Sievert SM (2018) Primary productivity below the seafloor at deep-sea hot springs. *Proceedings of the National Academy of Sciences*:201804351. doi:10.1073/pnas.1804351115
160. Expedition 330 Scientists (2011) Louisville Seamount Trail: Implications for geodynamic mantle flow models and the geochemical evolution of primary hotspots. *IODP Preliminary Report, Vol 330*. doi:10.2204/iodp.pr.330.2011
161. Heberling C, Lowell R, Liu L, Fisk MR (2010) Extent of the microbial biosphere in the oceanic crust. *Geochemistry Geophysics Geosystems* 11 (8):Q08003. doi:10.1029/2009GC002968
162. Bar-On YM, Phillips R, Milo R (2018) The biomass distribution on Earth. *Proceedings of the National Academy of Science USA*. doi:10.1073/pnas.1711842115
163. Wheat CG, Becker K, Villinger HW, Orcutt BN, Fournier T, Hartwell AM, Paul C (2020) Subseafloor cross-hole tracer experiment reveals hydrologic properties, heterogeneities, and

- 1 reactions in slow-spreading oceanic crust. *Geochemistry Geophysics Geosystems* 21 (1).
2 doi:10.1029/2019GC008804
- 3 164. Koppers AAP, Escutia C, Inagaki F, Pälke H, Saffer D, Thomas DN (2019) Quo vadis:
4 Look into the future. *Oceanography* 32 (1):218-219. doi:10.5670/oceanog.2019.150
5