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An interdisciplinary approach to sampling hard rock cores of the oceanic crust for microbiological and biogeochemical research

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Abstract. Studying life in the deep, rocky subseafloor involves many layers of technological and methodological challenges, and each drilling project or expedition must make a series of choices to address these challenges. We report on the workflow for sampling hard rock cores for microbiological and biogeochemical research that was developed and optimized during International Ocean Discovery Program (IODP) Expedition 399. All steps of the workflow, including selection of the sample and its shipboard homogenization and subsampling for specific

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analyses, were designed to maximize the potential for interdisciplinary collaborations and syntheses of results. Furthermore, multiple strategies to minimize and detect potential microbial and organic chemical contamination of the core samples were employed, including the shipboard detection of a fluorescent chemical tracer pumped into the drill fluid. Contamination tracer levels were detectable on the exterior surfaces of the core but absent in the homogenized interiors of most core samples. Based on the experiences and preliminary results of this expedition, recommendations for processing samples on future expeditions are presented.

1 Introduction

Our biological knowledge of the oceanic crust remains severely limited, in large part due to the methodological challenges of accessing and sampling the deep, rocky subseafloor. Most studies that utilize drilling to explore the subseafloor biosphere have relied on samples of marine sediments or borehole fluids (Becker et al., 2019; Cowen et al., 2003; D'Hondt et al., 2019; Edwards et al., 2011; Heuer et al., 2020; Inagaki et al., 2015; Jungbluth et al., 2016; Kallmeyer et al., 2012; Orcutt et al., 2021; Wheat et al., 2010), but a complete understanding of the possibilities and limits of subsurface life also requires effective sampling of the hard, rocky crust (Edwards et al., 2011; Orcutt et al., 2011; Sheik et al., 2026; Templeton and Caro, 2023).

Access to the oceanic crust has been enabled primarily by seafloor drilling expeditions of the International Ocean Discovery Program (IODP). The first investigations of microbial communities deep within oceanic crust were conducted with rock cores recovered during Expeditions 301 (basalt from the Juan de Fuca Ridge: Lever et al., 2013) and 304/305 (gabbro from the Atlantis Massif: Mason et al., 2010). Since then, a few IODP expeditions have investigated the density and diversity of microbial communities within the rocky crust, including Expeditions 330, 336, 357, 360, 390/393, 395, and 399 (Coggon et al., 2024b; Edwards et al., 2012; Früh-Green et al., 2018; Li et al., 2020; Lissenberg et al., 2024; McCaig et al., 2025; Motamedi et al., 2020; Parnell-Turner et al., 2025; Sylvan et al., 2024; Wee et al., 2021; Zhang et al., 2016). Recent continental scientific drilling projects have also contributed to the optimization of sampling protocols and analytical procedures for exploring the microbiology of rocky subsurface environments (Dai et al., 2021; Magnabosco et al., 2018; Purkamo et al., 2020; Templeton et al., 2021; Twing et al., 2025).

IODP Expedition 399 (April–June 2023) pursued several interdisciplinary scientific goals related to the geology, geochemistry, and microbiology of the Atlantis Massif (McCaig et al., 2025). The massif is an oceanic core complex where uplifted mantle and lower crustal rocks are exposed to seawater, triggering water–rock reactions and creating a hydrothermal system that produces the Lost City hydrothermal field (Blackman et al., 2002; Kelley et al., 2005).

Drilling of a new borehole (Hole U1601C, located 800 m north of Lost City) during this expedition was much more successful than expected (Lissenberg et al., 2024). While previous seafloor drilling of mantle rocks had only reached $\sim$ 200 m b.s.f., Hole U1601C reached 1268 m b.s.f. The surprising depth of Hole U1601C provided the unexpected opportunity to document the distribution of life through $>$ 1.2 km of the Atlantis Massif's subseafloor, possibly spanning environmental conditions from highly favorable to too extreme for life. Furthermore, the borehole may have reached zones of the subseafloor where water–rock reactions create a "chemical kitchen" where organic compounds can be synthesized abiotically (Lang et al., 2025a). Therefore, detection of low-molecular-weight organic compounds was also a priority science objective for this expedition.

Here we describe the strategy, workflow, and methods for sampling hard rock cores employed during Expedition 399. Because of the unexpected depth of Hole U1601C, $>$ 200 microbiological samples (whole round cores) were collected, providing the opportunity to intensively test and optimize sampling methods. With the recent retirement of the *JOIDES Resolution* in mind, we provide this report as a record of the microbiological methods that were developed for that platform over multiple decades, and we conclude with recommendations for future expeditions that wish to explore the microbiology of the rocky subseafloor.

2 MBIO rock sample workflow

2.1 Overview

The primary goal of our strategy for selecting and processing hard rock core samples during Expedition 399 was to maximize the potential for collaborative interdisciplinary research. This goal informed each step of the sample-handling workflow from initial sample selection to subsampling of core material for specific measurements and analyses. Additional goals included the preservation of geological context for each chosen sample, the ability to conduct time-sensitive experiments of biological and chemical activities, distribution of representative subsamples of the same core material among many different collaborating research groups, the detection of drilling-associated contamination, and handling all samples with sterile and organic carbon-free techniques in a controlled environment with minimal air particles and chemical contaminants.

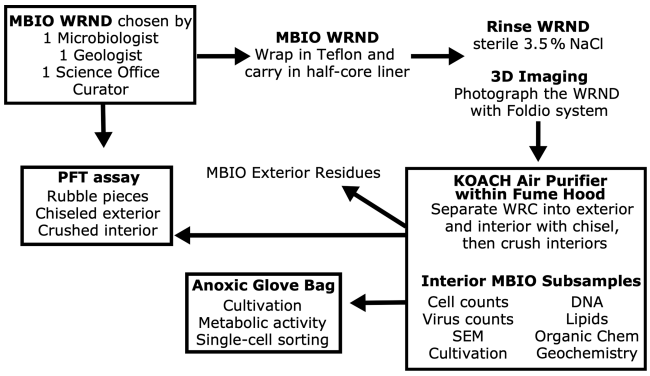

Figure 1. Overall workflow for shipboard handling of rock core samples intended for microbiological and organic chemistry analyses.

The overall sampling workflow was designed to reveal pristine, uncontaminated interior zones of the core material for microbiological and organic chemical analyses, while enabling direct links with later geological and geochemical analyses (Fig. 1). Early development of the workflow during pre-expedition planning was informed by the recommendations of Sylvan et al. (2021) and prior experiences with Expedition 357 (Hickok et al., 2018; Motamedi et al., 2020; Orcutt et al., 2017; Pendleton et al., 2021). The final protocols described here closely resemble those of IODP Expeditions 390/393 (Coggon et al., 2024a), with additional modifications to accommodate simultaneous trace-level organic geochemical analyses, as the investigation of abiotic synthesis of organic compounds was a major scientific goal of Expedition 399.

2.2 Sample selection

Within a few minutes of recovery, cores were brought from the catwalk into the core splitting laboratory and shaken out into split core liners that had been cleaned and sterilized with isopropanol. To minimize contamination during sample selection, all personnel examining the core wore masks and nitrile gloves. Gloves and tools were rinsed with methanol prior to handling the core material, and all personnel handling and describing the cores removed jewelry from their hands and wrists to avoid contamination of the core with platinum group elements and gold.

A whole-round sample of the core to be used for microbiological and biogeochemical research (hereafter referred to as *the MBIO sample*) was selected by a sample selection team, consisting of one microbiologist, one geologist, one co-chief scientist, and the core curator. Approximately one MBIO sample (~ 10–15 cm in length) was collected from each 5 m core, although two MBIO samples were collected from a few cores, and occasionally zero MBIO samples were collected from a core. After removing the MBIO sample, a styrofoam insert was placed in the core as a placeholder and marker of the sample's location within the core.

A fundamental challenge of selecting MBIO samples is that they were removed from the core as quickly as possible, to minimize impact on time-sensitive microbiological sampling, creating a gap in the core record before the rest of the scientific party were able to examine the core. Therefore, the primary responsibility of the MBIO sample selection team was to choose a sample that is biologically interesting while minimizing the permanent loss of geological information. Therefore, zones of the core that appeared to be unique or of particular geological interest were avoided.

In addition to minimizing loss of geological information, selection of MBIO samples during Exp. 399 also attempted to balance the targeted selection of features exhibiting visible signs of biological potential (e.g., indicators of extensive fluid flow and porosity) with a more regular selection of samples that are representative of the lithological unit. MBIO samples included "typical" serpentinites and gabbros as well as contacts between different rock types and particularly interesting and unusual features that might have significant biological interest. Discussions between the geologist and microbiologist serving on the MBIO sample selection team were vitally important for assessing the expected geological and biological value of each potential sample. Such a deliberative approach to sample selection is not always practical. During IODP Expedition 357, for example, a smaller shipboard science party required a more standardized approach to selecting MBIO samples, which were collected from the end of each core. Targeted selection of particularly interesting zones or attempting to avoid the loss of unique geological features was not possible due to operational constraints during that expedition. For example, shipboard examination of cores could only be conducted through the transparent core liner during that expedition.

The selected MBIO sample was photographed in situ before being removed from the core (Fig. 2), wrapped in a sheet of polytetrafluoroethylene (PTFE) that had been washed with 10 % hydrochloric acid, and carried to the ship's microbiology laboratory for processing. Previous tests indicated that PTFE sheeting has minimal lipid and amino acid contributions, making it well-suited for both microbiological and organic geochemical analyses (Hickok et al., 2018).

2.3 Shipboard processing of MBIO rock samples

In the ship's microbiology laboratory, each MBIO sample was rinsed with a 3.5 % sodium chloride solution (combusted NaCl dissolved in filter-sterilized Milli Q water), labeled with its sample ID and core orientation, placed on a sheet of acid-washed PTFE on top of a rotating turntable, and photographed with a Foldio 360 system (Orangemonkey). The Foldio system captured a still image after each 15° rotation of the turntable, as described for Expeditions 390/393 (Coggon et al., 2024a). These photographs document sample ap-

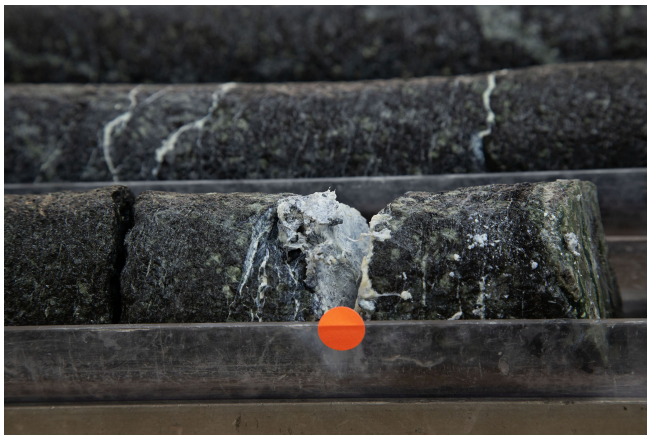

Figure 2. The MBIO sample (orange circle) is photographed before it is removed from the core. This example shows a fractured zone that was targeted for microbiological investigation. Photo credit: Erick Bravo, IODP JRSO.

pearance prior to homogenization and are intended to assist later reconstructions of its relationship to the remainder of the core.

After the MBIO sample was rinsed and photographed, it was placed in a clean and methanol-wiped stainless steel rock box and carried to a chemical fume hood containing a KOACH clean air system (Model T 500-F, Koken Ltd.) and an ELIMINOSTAT (Shishido Electrostatic Ltd.) air static eliminator (Fig. 3). A very similar setup was employed during Expeditions 390/393 (Coggon et al., 2024a). The use of the chemical fume hood as the work area during Exp. 399 was determined by administrative concerns regarding the presence of chrysotile fibers in the serpentinite cores (otherwise, shipboard processing of MBIO samples was unaffected by other shipboard procedural variations introduced because of concerns regarding chrysotile). Although the negative pressure environment of the fume hood was not ideal for contamination reduction, the ability of the KOACH clean air system to eliminate dust particles within the working area was confirmed with a portable dust particle detector. In the future, a positive-pressure work area would be preferable to further reduce potential contamination of the core samples.

All chiseling, crushing, and subsampling tools were washed with soap and water, rinsed three times with ultrapure water, and wiped with methanol before use. Tools were not autoclaved due to concerns with potential chemical contamination from the autoclave. Autoclaved tools may be desirable for crushing of samples intended for microbiological analyses, but samples crushed with autoclaved tools are likely to be unsuitable for organic chemistry research. Similarly, methanol was used in place of ethanol as the primary sanitizing agent, because its greater volatility makes it easier to remove from surfaces and therefore less likely to interfere with downstream organic chemical analyses. Furthermore, the tools were not flamed because combustion of organic matter generates a complex smear of smaller carbon compounds that are difficult to track as chemical markers of contamination. The rock core itself was also not sprayed with ethanol nor flamed, as has been done in some previous expeditions (Früh-Green et al., 2018; MacLeod et al., 2017; Motamedi et al., 2020; Wee et al., 2021). Consequently, the tools could be considered non-sterile and not entirely DNA-free. As with any similar sample set, regardless of the cleaning and sterilization procedures, future studies should evaluate the potential for background contamination originating from shipboard handling of the samples via the analysis of extensive control samples, as described below.

All chiseling, crushing, and subsampling of the MBIO sample was conducted within the KOACH clean air system. The exterior of each core sample was removed with a hammer and chisel, including the top, bottom, and sides of the whole-round sample's cylindrical shape. These exterior portions, sometimes > 1 cm thick, were placed into sterile bags and returned to the section half core liner from where the MBIO sample was taken for potential description and sampling by the rest of the scientific party. Many of these MBIO "residues" were used for shipboard physical properties measurements and geochemical analyses.

An alternative technique for removing exterior material from the MBIO sample using an electric Dremel tool was explored with a few samples, but this technique was considered unfavorable due to the production of dust, the likely contribution of hydrocarbon contamination from the tool, and the lack of significant "residues" available to be returned to the general core workflow for further descriptions and sampling.

After the exterior material was chiseled away, the interior rock material was transferred to a different (clean) rock box. This two-box strategy was intended to limit the transfer of potential contaminants from the exterior material into the interior portion. The interior zones were crushed with a hammer within the rock box along grain boundaries into roughly millimeter-sized pieces and subsampled for various microbiological and biogeochemical analyses. Thus, replicate subsamples, representative of the whole MBIO sample, were made available for each downstream analysis. One exception to this procedure was that intact pieces of each MBIO interior, prior to homogenization, were collected for future imaging analyses, such as microscopy and spectroscopy. This strategy of homogenizing all samples on the ship during the expedition was chosen to avoid the scenario where different research groups work on slightly different portions of the original sample, which can create challenges for synthesizing results at the publication stage.

A potential disadvantage of this homogenization strategy is that it can increase the processing time for time-sensitive analyses such as microbiological and biochemical activity experiments. A streamlined version of the workflow described here could prioritize time-sensitive experiments by subsetting one portion of the rock core for immediate transfer to a controlled environment such as an anoxic chamber

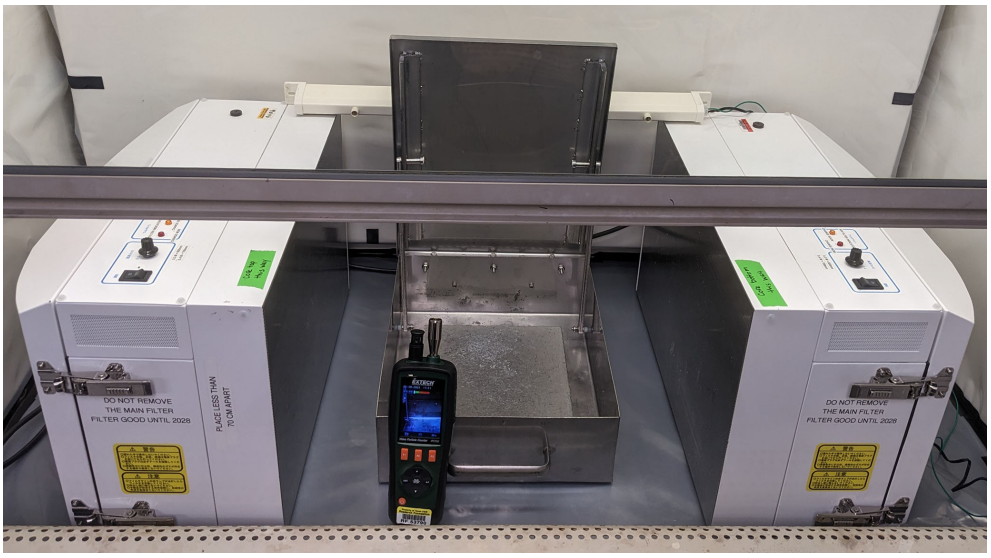

Figure 3. Rock core samples are chiseled, homogenized, and subsampled in a stainless-steel rock box placed within a KOACH clean air system and below an air static eliminator.

or cold room. This approach is typical of microbiological experiments with sediment cores and has also been used with hard rock cores (e.g., Templeton et al., 2021). Sectioning the MBIO sample in this way comes at the cost of these experiments being conducted with material that is adjacent to, but not true replicates of, the homogenized material that is frozen and distributed for future laboratory-based research such as DNA sequencing and organic chemistry analyses. Furthermore, a separate shipboard procedure for time-sensitive experiments could require additional personnel resources. The evaluation of this cost–benefit analysis depends on the expected environmental gradients of the system being studied as well as operational constraints.

Manual chiseling and crushing of the MBIO samples consumed most of the shipboard working hours by the four microbiologists on Expedition 399. A planetary ball mill was not used for crushing of MBIO samples because no practical method for cleaning the ball mill equipment between samples could be conceived. Therefore, the risk of cross-contamination between samples was judged to be too great. In contrast, the simple rock box, hammer, and chisel could be easily cleaned by hand between each sample. An impact mortar was also used to facilitate crushing particularly hard rock cores, but with practice, the hammer alone proved to be the most efficient tool for crushing multiple samples per day. For some shore-based laboratory analyses, additional powdering of the core homogenate will be necessary, but the shipboard crushing achieved sufficient homogenization to produce replicate subsamples.

These procedures were optimized during Expedition 399 in response to the unexpectedly high number of MBIO samples that were collected (typically 3–4 samples per 12 h shift for a total of 191 MBIO samples collected from 172 cores in Hole U1601C), thanks to unusually high rates of drill penetration for hard rocks (Lang et al., 2025b). The complete MBIO workflow described above was initiated immediately upon recovery of each core, and subsamples of homogenized core interiors were typically collected and stored in their final destinations (freezer, refrigerator, preservative, anoxic chamber, etc.) approximately 2 h after core recovery.

Shipboard processing of so many samples immediately after recovery was only possible because four microbiologists were selected for Expedition 399, who could work in two-person teams in two separate 12 h shifts, supported by the ship's technical staff. Expeditions with fewer personnel available for the processing of MBIO samples will need to eliminate shipboard crushing and homogenization or else process fewer samples. Reasonable workloads for expeditions employing one or two microbiologists compared to three or more microbiologists has been discussed by Sylvan et al. (2021).

2.4 Tracing of drilling-associated contamination

An important component of the MBIO sampling workflow during Expedition 399 was the shipboard tracing and detection of drilling-associated contamination. The overall approach was based on methods developed during many previous IODP expeditions (Lever et al., 2006; Orcutt et al., 2017; Smith et al., 2000). Sylvan et al. (2021) recommended against the use of fluorescent microspheres because of the difficulty in delivering them to the borehole in a consistent and quantitative manner (House et al., 2003) and due to their high rate of false negative detections, especially in environments where temperatures exceed ∼ 95 °C (Yanagawa et al., 2013). Accordingly, recent IODP expeditions (including 301,

360, 366, 376, 385, 390/393, 399, 400, 402, and 403) have utilized a commercial perfluorocarbon tracer (PFT), which has very high sensitivity and can be quantitatively measured if the appropriate equipment is available at the time of sampling (reviewed by Kallmeyer, 2017). During Expedition 399, a pure perfluorodecalin fluid ($C_{10}F_{18}$; mol wt 462; Oakwood Products P/N 003283; a less volatile alternative to perfluoromethylcyclohexane) was injected into the drill fluid without dilution using a high-pressure liquid chromatography pump at a variable rate during drilling operations. The injection rate was automatically tuned to the mud pump rate to achieve a target concentration of 1 mg PFT L^{-1} of drilling fluid with software developed by *JOIDES Resolution* technical staff. Occasional software malfunctions resulted in sudden spikes of PFT injection into the drilling fluid, and a PFT injection rate of 0.3–0.5 mL min^{-1} was manually set during software troubleshooting. In general, an injection rate of 0.3 mL min^{-1} was sufficient for detection of the tracer on the exterior of cores, and slightly elevated rates of 0.4–0.5 mL min^{-1} provided increased sensitivity for detection of potential contamination in rinsed and chiseled MBIO sample interiors.

PFT measurements were conducted according to procedures first described for IODP Expedition 360 (MacLeod et al., 2017). Samples for PFT measurements were sealed in 20 mL glass headspace vials and heated to 70 °C in a shaking oven. An aliquot of the headspace was injected into an HP 6890 gas chromatograph equipped with a microelectron capture detector (GC-μECD) and a megabore separation column (Rt-Alumina BOND/KCl, 50 m, 0.53 mm ID, 10 μm Agilent column). Headspace concentrations of the PFT were determined by calibration with a seven-point standard curve.

Successful delivery of PFT into the borehole during drilling was confirmed by collecting small pieces of rubble from each recovered core. PFT levels in these loose rubble samples were in the range of 1–25 ppb (of headspace) in most samples. A few extreme outliers up to 257 ppb were the result of accidental increases in the rate of PFT delivery during drilling. The fairly consistent and high levels of PFT in loose rubble samples confirmed the successful delivery of PFT during drilling.

Contamination of core samples was tested by collecting approximately 4 cm^3 of the chiseled exterior shavings and homogenized interiors of MBIO samples during shipboard sample homogenization. PFT was absent or present at only trace levels in nearly all samples of crushed interiors from Hole U1601C (Fig. 4). PFT levels that were below the level of quantification were divided into two categories: trace (a visible peak was manually identified but could not be quantified) and zero (no peak could be detected, even by manual inspection). Exterior shavings of MBIO samples frequently contained levels of PFT ranging from trace to 5 ppb, whereas most samples of the revealed interiors contained zero PFT. Only two interior samples contained quantifiable PFT levels, and these were very low (0.007 and 0.028 ppb). These results

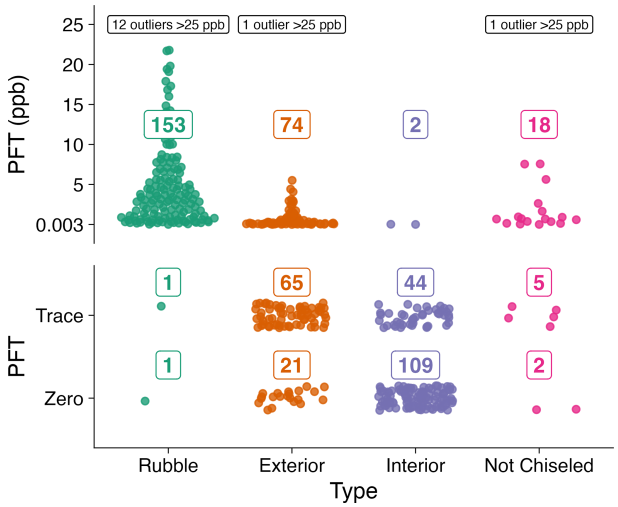

Figure 4. Detection of perfluorocarbon tracer (PFT) of drilling-induced contamination in rock cores from Hole U1601C. Samples for PFT assay were collected from (1) loose rubble during core shakeout, (2) chiseled whole-round exteriors, (3) whole-round interiors remaining after exterior chiseling, and (4) samples that could not be chiseled because of their unconsolidated, mushy compositions. Trace = detectable peak but not quantifiable. Zero = no detectable peak. Jittering of data points along *x* axis (and also along *y* axis for Trace and Zero) is for aesthetic purposes only. The number of data points in each category is reported in boxes.

indicate that drilling-associated contamination, as measured by PFT, was largely removed from the homogenized interiors of the MBIO samples.

An estimate of the expected contamination level of drilling fluid into the rock core samples can be obtained by converting the PFT headspace concentrations to a corresponding quantity of drilling fluid. The detection limit of PFT-bearing drilling fluid was not measured during Expedition 399, but a limit of 0.004 μL of drilling fluid per gram of rock was obtained during Expedition 301 using similar equipment and the same concentration of 1 mg PFT L^{-1} of drilling fluid (Lever et al., 2006). Lever et al. (2006) used perfluoromethylcyclohexane instead of perfluorodecalin, but a similar detection limit can be assumed, at least to a rough approximation. If 0.004 μL of drilling fluid corresponds to our minimum quantifiable detection of headspace PFT (0.003 ppb), then we would expect samples with minimal PFT to be contaminated with a number of cells corresponding to 0.004 μL of drilling fluid. Assuming 10^6 cells per milliliter of drilling fluid, minimally contaminated samples should have no more than 4 cells derived from drilling fluid per gram of rock. Accordingly, samples with high levels of headspace PFT (e.g., 30 ppb) can be expected to have $\sim 10^4$ cells from drilling fluid per gram of rock.

A subset of MBIO samples from Hole U1601C could not be chiseled to reveal interior zones because of their unconsolidated, often crumbly nature (Fig. 4). Attempting to sepa-

rate exterior surfaces from these mushy samples proved to be impractical. These samples appeared to have high biological potential, however, precisely because of their highly porous, apparently altered compositions. Therefore, instead of being discarded, these non-cohesive samples were rinsed with the sterile 3.5 % NaCl solution and then directly homogenized and subsampled without sectioning of exterior and interior zones. As expected, PFT levels in these non-cohesive unchiseled MBIO samples were similar to those of exterior shavings and higher than those of isolated interiors. The detection of PFT in unchiseled samples demonstrates the success of the PFT detection procedure and that volatilization of PFT during sample handling, for example, does not prevent its detection. Furthermore, the higher level of PFT in unchiseled samples compared to chiseled samples is evidence that decontamination of the core is necessary and that manually chiseling exterior surfaces is an effective means for removing drilling-associated contamination. Downstream microbiological and geochemical results from these unchiseled samples with high PFT levels should be interpreted with caution. Nevertheless, these mushy, highly altered rocks were among the most biologically interesting samples collected during the expedition and should not be ignored. Future research could potentially distinguish native taxa from likely contaminants using DNA sequencing analyses, for example.

The lack of drilling-associated contamination in the MBIO interiors does not indicate the lack of any other kinds of contamination (Labonté et al., 2025). All types of contamination that occur after drilling, including any potential contamination that might occur during the shipboard processing of MBIO samples described here, are not measured by the PFT analysis. To that end, samples of ambient air dust were collected from the ship's microbiology laboratory throughout the expedition, as well as many samples of the ship's tap water, drilling mud, surface seawater, and bottom seawater near the borehole. These samples will serve as potential contamination sources to be evaluated by future laboratory research, including DNA sequencing (e.g., Motamedi et al., 2020).

This comprehensive monitoring of drilling-associated contamination was implemented and largely executed by the shipboard technical staff, especially the two chemistry lab specialists. Because these procedures require specialized equipment and technical familiarity with drilling operations, it would not be practical for project scientists to carry them out without training and assistance. Therefore, we recommend that budgeting personnel resources required to carry out daily monitoring of drilling-associated contamination should be a major component of pre-expedition planning.

3 Considerations for future research

3.1 Sampling for interdisciplinary collaborations

The MBIO sample processing workflow was designed to maximize the potential for collaborative, interdisciplinary research via two specific mechanisms. The first is that the procedures described above produced plenty of homogenized material that could be subsampled and distributed to as many analyses as desired. For example, a subsample of every MBIO sample homogenate was collected for geochemistry analyses to complement the full suite of microbiological analyses including cultivation, metabolic activity experiments, cell counts, DNA sequencing, lipid molecular characterization, and organic chemistry. A portion of these MBIO subsamples intended for geochemistry were used for shipboard total carbon/inorganic carbon calorimetry assays, and the remaining material was shared among multiple members of the scientific party for future research.

Widespread distribution of the MBIO homogenates in this way provided additional samples for geochemical research to complement those sampled from the core in the standard way. The additional geochemical data also benefit the microbiology team because they are generated from the same homogenized material used for microbiological analyses. This can be especially valuable in subseafloor environments with small-scale gradients or highly heterogenous lithologies, where measurements conducted on nearby samples located only centimeters apart in the same core section can yield significantly different results. Furthermore, the criteria used to select core samples intended for microbiological or geochemical research can be quite different; microbiologists are attracted to unusual and altered rocks while geochemists may be more likely to seek samples that are un-weathered and representative of the core.

The second mechanism for facilitating collaborative research was the return of chiseled shavings of MBIO sample exteriors to the core for further description and sampling by the rest of the scientific party. When the potential value of these "residues" or "off-cuts" became evident, efforts were made to chisel the exteriors in a manner that produced larger, more intact pieces, sometimes even preserving their orientation in the core. By making these pieces available to the scientific party, essential shipboard data could be obtained from a part of the core that had been removed, partially recovering what would otherwise have been a permanent gap in the core record.

Because these MBIO off-cuts were not collected in the standard way for shipboard measurements (e.g., not cut as standard cubes), validation tests were conducted to assess their suitability. For example, shipboard measurements of the density and porosity of irregularly shaped MBIO samples were found to be within 5 % of shore-based measurements of cube samples (Dickerson et al., in review, 2026).

3.2 Recommendations

We recommend this MBIO sample processing workflow to future seafloor drilling expeditions that wish to facilitate microbiological research. Specifically, the most important principles are

- the tracing of drilling-induced contamination with the ability to evaluate the efficacy of the removal of this contamination from processed samples during the expedition so that shipboard procedures can be adjusted where necessary;
- the distribution of homogenized MBIO samples among the scientific party, not only microbiologists, to maximize the number of analyses that can be conducted on the same sampled material;
- the inclusion of multiple microbiologists (at least two and preferably four or more) in the shipboard scientific party to enable rapid processing of MBIO samples as well as time-sensitive experiments;
- the use of simple tools that are easy to clean and sanitize between samples, not only to remove microbial contaminants but also problematic organic molecules such as hydrocarbons.

One improvement that was lacking during Expedition 399, but that we recommend for future expeditions, is the creation of a clean room for MBIO sample handling. Although the KOACH air purifier system appeared to perform well, it represents only a small area within a large and busy laboratory. Samples and supplies must be frequently moved into and out of the area covered by the KOACH system, creating abundant opportunities for the transfer of dust and other potential contaminants into the sample processing area. Indeed, dust particles from ambient air in the laboratory has been shown to be the dominant source of contamination into low-biomass subseafloor rocks collected during IODP Expedition 357 (Motamedi et al., 2020; Pendleton et al., 2021). An enclosed, controlled environment with air purifiers and sufficient space for multiple workers would greatly reduce the probability that samples are exposed to significant contamination during shipboard processing. The "super-clean room" of the Kochi Core Center is ideal (Heuer et al., 2017), although a shipboard implementation would require modifications. A further potential improvement would be to chisel and homogenize samples within an oxygen-free chamber, although this is unlikely to be practical for immediate shipboard processing of large numbers of hard rock cores.

4 Conclusions

The MBIO sample workflow employed during IODP Expedition 399 succeeded in producing hundreds of homogenized subsamples from > 200 hard rock core samples, while tracking and mostly eliminating drilling-associated contamination. Portions of almost every MBIO sample were made available to the entire scientific party, facilitating future interdisciplinary collaborations and syntheses of results. These procedures and results are presented in the hope that they benefit future studies of the deep subseafloor biosphere.

Data availability. All data reported in this text are publicly available via the IODP LIMS database (https://web.iodp.tamu.edu/LORE/, last access: 6 August 2026).

Author contributions. All authors contributed to the design of the workflow and participated in shipboard sample processing. WJB, OC, JAR, GS, JS, FW, and SQL performed the contamination tracing procedures. WJB wrote the original manuscript draft with editing from all co-authors.

Competing interests. The contact author has declared that none of the authors has any competing interests.

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References

Becker, K., Austin Jr., J. A., Exon, N., Humphris, S., Kastner, M., McKenzie, J. A., Miller, K. G., Suyehiro, K., and Taira, A.: 50 Years of Scientific Ocean Drilling, Oceanography, 32, 17, https://doi.org/10.5670/oceanog.2019.110, 2019.

Blackman, D. K., Karson, J. A., Kelley, D. S., Cann, J. R., Früh-Green, G. L., Gee, J. S., Hurst, S. D., John, B. E., Morgan, J., and Nooner, S. L.: Geology of the Atlantis Massif (Mid-Atlantic Ridge, 30 N): Implications for the evolution of an ultramafic oceanic core complex, Mar. Geophys. Res., 23, 443–469, 2002.

Coggon, R. M., Teagle, D. A. H., Sylvan, J. B., Reece, J., Estes, E. R., Williams, T. J., Christeson, G. L., and Expedition 390/393 Scientists: Expedition 390/393 methods, in: Proceedings of the International Ocean Discovery Program, 390/393, International Ocean Discovery Program, College Station, TX, https://doi.org/10.14379/iodp.proc.390393.102.2024, 2024a.

Coggon, R. M., Teagle, D. A. H., Sylvan, J. B., Reece, J., Estes, E. R., Williams, T. J., Christeson, G. L., and Expedition 390/393 Scientists: Expedition 390/393 summary, in: Proceedings of the International Ocean Discovery Program, 3909/393, International Ocean Discovery Program, College Station, TX, https://doi.org/10.14379/iodp.proc.390393.101.2024, 2024b.

Cowen, J. P., Giovannoni, S. J., Kenig, F., Johnson, H. P., Butterfield, D., Rappé, M. S., Hutnak, M., and Lam, P.: Fluids from Aging Ocean Crust That Support Microbial Life, Science, 299, 120–123, https://doi.org/10.1126/science.1075653, 2003.

Dai, X., Wang, Y., Luo, L., Pfiffner, S. M., Li, G., Dong, Z., Xu, Z., Dong, H., and Huang, L.: Detection of the deep biosphere in metamorphic rocks from the Chinese continental scientific drilling, Geobiology, 19, 278–291, https://doi.org/10.1111/gbi.12430, 2021.

D'Hondt, S., Pockalny, R., Fulfer, V. M., and Spivack, A. J.: Subseafloor life and its biogeochemical impacts, Nat. Commun., 10, 3519, https://doi.org/10.1038/s41467-019-11450-z, 2019.

Edwards, K. J., Wheat, C. G., and Sylvan, J. B.: Under the sea: microbial life in volcanic oceanic crust, Nat. Rev. Microbiol., 9, 703–712, https://doi.org/10.1038/nrmicro2647, 2011.

Edwards, K. J., Bach, W., Klaus, A., and Expedition 336 Scientists: Mid-Atlantic Ridge Microbiology: initiation of long-term coupled microbiological, geochemical, and hydrological experimentation within the seafloor at North Pond, western flank of the Mid-Atlantic Ridge, in: Proceedings of the International Ocean Drilling Program, 336, Integrated Ocean Drilling Program, College Station, TX, https://doi.org/10.2204/iodp.proc.336.2012, 2012.

Früh-Green, G. L., Orcutt, B. N., Rouméjon, S., Lilley, M. D., Morono, Y., Cotterill, C., Green, S., Escartin, J., John, B. E., McCaig, A. M., Cannat, M., Ménez, B., Schwarzenbach, E. M., Williams, M. J., Morgan, S., Lang, S. Q., Schrenk, M. O., Brazelton, W. J., Akizawa, N., Boschi, C., Dunkel, K. G., Quéméneur, M., Whattam, S. A., Mayhew, L., Harris, M., Bayrakci, G., Behrmann, J.-H., Herrero-Bervera, E., Hesse, K., Liu, H.-Q., Ratnayake, A. S., Twing, K., Weis, D., Zhao, R., and Bilenker, L.: 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, 2018.

Heuer, V. B., Inagaki, F., Morono, Y., Kubo, Y., Maeda, L., and Expedition 370 Scientists: Temperature Limit of the Deep Biosphere off Muroto, in: Proceedings of the International Ocean Discovery Program, 370, International Ocean Discovery Program, College Station, TX, https://doi.org/10.14379/iodp.proc.370.2017, 2017.

Heuer, V. B., Inagaki, F., Morono, Y., Kubo, Y., Spivack, A. J., Viehweger, B., Treude, T., Beulig, F., Schubotz, F., Tonai, S., Bowden, S. A., Cramm, M., Henkel, S., Hirose, T., Homola, K., Hoshino, T., Ijiri, A., Imachi, H., Kamiya, N., Kaneko, M., Lagostina, L., Manners, H., McClelland, H.-L., Metcalfe, K., Okutsu, N., Pan, D., Raudsepp, M. J., Sauvage, J., Tsang, M.-Y., Wang, D. T., Whitaker, E., Yamamoto, Y., Yang, K., Maeda, L., Adhikari, R. R., Glombitza, C., Hamada, Y., Kallmeyer, J., Wendt, J., Wörmer, L., Yamada, Y., Kinoshita, M., and Hinrichs, K.-U.: Temperature limits to deep subseafloor life in the Nankai Trough subduction zone, Science, 370, 1230–1234, https://doi.org/10.1126/science.abd7934, 2020.

Hickok, K. A., Nguyen, T. B., and Lang, S. Q.: Assessment of apolar lipids in subseafloor rocks and potential contaminants from the Atlantis Massif (IODP Expedition 357), Org. Geochem., 122, 68–77, 2018.

House, C. H., Cragg, B. A., Teske, A., and Leg 201 Scientific Party: Drilling Contamination Tests during ODP Leg 201 Using Chemical and Particulate Tracers, in: Proceedings of the Ocean Drilling Program, 201 Initial Reports, vol. 201, Ocean Drilling Program, https://doi.org/10.2973/odp.proc.ir.201.2003, 2003.

Inagaki, F., Hinrichs, K.-U., Kubo, Y., Bowles, M. W., Heuer, V. B., Hong, W.-L., Hoshino, T., Ijiri, A., Imachi, H., Ito, M., Kaneko, M., Lever, M. A., Lin, Y.-S., Methé, B. A., Morita, S., Morono, Y., Tanikawa, W., Bihan, M., Bowden, S. A., Elvert, M., Glombitza, C., Gross, D., Harrington, G. J., Hori, T., Li, K., Limmer, D., Liu, C.-H., Murayama, M., Ohkouchi, N., Ono, S., Park, Y.-S., Phillips, S. C., Prieto-Mollar, X., Purkey, M., Riedinger, N., Sanada, Y., Sauvage, J., Snyder, G., Susilawati, R., Takano, Y., Tasumi, E., Terada, T., Tomaru, H., Trembath-Reichert, E., Wang, D. T., and Yamada, Y.: 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, 2015.

Jungbluth, S. P., Bowers, R. M., Lin, H.-T., Cowen, J. P., and Rappé, M. S.: Novel microbial assemblages inhabiting crustal fluids within mid-ocean ridge flank subsurface basalt, ISME J., 10, 2033–2047, https://doi.org/10.1038/ismej.2015.248, 2016.

Kallmeyer, J.: Contamination Control for Scientific Drilling Operations, Adv. Appl. Microbiol., 98, 61–91, https://doi.org/10.1016/bs.aambs.2016.09.003, 2017.

Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C., and D'Hondt, S.: Global distribution of microbial abundance and biomass in subseafloor sediment, P. Natl. Acad. Sci. USA, 109, 16213–16216, 2012.

Kelley, D. S., Karson, J. A., Früh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A. S., Brazelton, W. J., Roe, K., Elend, M. J., Delacour, A., Bernasconi, S. M., Lilley, M. D., Baross, J. A., Summons, R. E., and Sylva, S. P.: A Serpentinite-Hosted Ecosystem: The Lost City Hydrothermal Field, Science, 307, 1428–1434, https://doi.org/10.1126/science.1102556, 2005.

Dickerson, K. L., Fisher, A. T., Deans, J. R., Abe, N., Lang, S. Q., and McCaig, A.: Integration of Core, Log, and Machine Learning Analyses from a Deep Borehole in the Atlantis Massif: Implications for the Structure and Hydrogeology of an Ultramafic- and Mafic-Dominated Oceanic Core Complex, J. Geophys. Res.-Sol. Ea., in review, 2026.

Labonté, J. M., Campbell, K. L., Walker, J. R., and Rodriguez-Pilco, M. A.: Ubiquitous microbial contaminants associated with scientific ocean drilling, Access Microbiology, 7, 000865.v3, https://doi.org/10.1099/acmi.0.000865.v3, 2025.

Lang, S. Q., Wheat, C. G., Dickerson, K. L., Reagan, M. K., Savov, I. P., Robare, J. A., Brazelton, W. J., Suhonen, J., Cavazos, O., McCaig, A., Blum, P., Abe, N., Coltat, R., Deans, J. R., Godard, M., John, B. E., Klein, F., Kuehn, R., Lin, K.-Y., Lissenberg, C. J., Liu, H., Lopes, E. L., Nozaka, T., Osborne, W., Parsons, A. J., Pathak, V., Rodgers, J., Seewald, J. S., Sims, K. W., Southam, G., Sylva, S., and Wang, F.: Hydrogen, single-carbon compounds, and thermal regime in the oceanic ultramafic-dominated lithosphere: insights from a deep borehole on the Atlantis Massif, Geochim. Cosmochim. Ac., 408, 84–104, https://doi.org/10.1016/j.gca.2025.09.024, 2025a.

Lang, S. Q., McCaig, A. M., Blum, P., and Expedition 399 Scientists: Site U1601, in: Proceedings of the International Ocean Discovery Program, 399, International Ocean Discovery Program, College Station, TX, https://doi.org/10.14379/iodp.proc.399.104.2025, 2025b.

Lever, M. A., Alperin, M., Engelen, B., Inagaki, F., Nakagawa, S., Steinsbu, B. O., and Teske, A.: Trends in Basalt and Sediment Core Contamination During IODP Expedition 301, Geomicrobiol. J., 23, 517–530, https://doi.org/10.1080/01490450600897245, 2006.

Lever, M. A., Rouxel, O., Alt, J. C., Shimizu, N., Ono, S., Coggon, R. M., Shanks, W. C., Lapham, L., Elvert, M., Prieto-Mollar, X., Hinrichs, K.-U., Inagaki, F., and Teske, A.: Evidence for Microbial Carbon and Sulfur Cycling in Deeply Buried Ridge Flank Basalt, Science, 339, 1305–1308, https://doi.org/10.1126/science.1229240, 2013.

Li, J., Mara, P., Schubotz, F., Sylvan, J. B., Burgaud, G., Klein, F., Beaudoin, D., Wee, S. Y., Dick, H. J. B., Lott, S., Cox, R., Meyer, L. A. E., Quémener, M., Blackman, D. K., and Edgcomb, V. P.: Recycling and metabolic flexibility dictate life in the lower oceanic crust, Nature, 579, 250–255, https://doi.org/10.1038/s41586-020-2075-5, 2020.

Lissenberg, C. J., McCaig, A. M., Lang, S. Q., Blum, P., Abe, N., Brazelton, W. J., Coltat, R., Deans, J. R., Dickerson, K. L., Godard, M., John, B. E., Klein, F., Kuehn, R., Lin, K.-Y., Liu, H., Lopes, E. L., Nozaka, T., Parsons, A. J., Pathak, V., Reagan, M. K., Robare, J. A., Savov, I. P., Schwarzenbach, E. M., Sissmann, O. J., Southam, G., Wang, F., Wheat, C. G., Anderson, L., and Treadwell, S.: A long section of serpentinized depleted mantle peridotite, Science, 385, 623–629, https://doi.org/10.1126/science.adp1058, 2024.

MacLeod, C. J., Dick, H. J., Blum, P., and Expedition 360 Scientists: Southwest Indian Ridge Lower Crust and Moho, in: Proceedings of the International Ocean Discovery Program, 360, International Ocean Discovery Program, College Station, TX, https://doi.org/10.14379/iodp.proc.360.2017, 2017.

Magnabosco, C., Lin, L.-H., Dong, H., Bomberg, M., Ghiorse, W., Stan-Lotter, H., Pedersen, K., Kieft, T. L., van Heerden, E., and Onstott, T. C.: The biomass and biodiversity of the continental subsurface, Nat. Geosci, 11, 707–717, https://doi.org/10.1038/s41561-018-0221-6, 2018.

Mason, O. U., Nakagawa, T., Rosner, M., Nostrand, J. D. V., Zhou, J., Maruyama, A., Fisk, M. R., and Giovannoni, S. J.: First Investigation of the Microbiology of the Deepest Layer of Ocean Crust, PLOS ONE, 5, e15399, https://doi.org/10.1371/journal.pone.0015399, 2010.

McCaig, A. M., Lang, S. Q., Blum, P., and Expedition 399 Scientists: Building Blocks of Life, Atlantis Massif, in: Proceedings of the International Ocean Discovery Program, 399, International Ocean Discovery Program, College Station, TX, https://doi.org/10.14379/iodp.proc.399.2025, 2025.

Motamedi, S., Orcutt, B. N., Früh-Green, G. L., Twing, K. I., Pendleton, H. L., and Brazelton, W. J.: Microbial Residents of the Atlantis Massif's Shallow Serpentinite Subsurface, Appl. Environ. Microb., 86, e00356-20, https://doi.org/10.1128/AEM.00356-20, 2020.

Orcutt, B. N., Sylvan, J. B., Knab, N. J., and Edwards, K. J.: Microbial Ecology of the Dark Ocean above, at, and below the Seafloor, Microbiol. Mol. Biol. R., 75, 361–422, https://doi.org/10.1128/mmbr.00039-10, 2011.

Orcutt, B. N., Bergenthal, M., Freudenthal, T., Smith, D., Lilley, M. D., Schnieders, L., Green, S., and Früh-Green, G. L.: Contamination tracer testing with seabed drills: IODP Expedition 357, Sci. Dril., 23, 39–46, https://doi.org/10.5194/sd-23-39-2017, 2017.

Orcutt, B. N., D'Angelo, T., Wheat, C. G., and Trembath-Reichert, E.: Microbe-mineral biogeography from multi-year incubations in oceanic crust at North Pond, Mid-Atlantic Ridge, Environ. Microbiol., 23, 3923–3936, https://doi.org/10.1111/1462-2920.15366, 2021.

Parnell-Turner, R. E., Briais, A., LeVay, L. J., and Expedition 395 Scientists: Reykjanes Mantle Convection and Climate, in: Proceedings of the International Ocean Discovery Program, vol. 395, International Ocean Discovery Program, College Station, TX, https://doi.org/10.14379/iodp.proc.395.2025, 2025.

Pendleton, H. L., Twing, K. I., Motamedi, S., and Brazelton, W. J.: Potential microbial contamination from drilling lubricants into subseafloor rock cores, Sci. Dril., 29, 49–57, https://doi.org/10.5194/sd-29-49-2021, 2021.

Purkamo, L., Kietäväinen, R., Nuppunen-Puputti, M., Bomberg, M., and Cousins, C.: Ultradeep Microbial Communities at 4.4 km within Crystalline Bedrock: Implications for Habitability in a Planetary Context, Life, 10, 2, https://doi.org/10.3390/life10010002, 2020.

Sheik, C. S., Steen, A. D., Reese, B. K., Wang, D. T., Osburn, M. R., Cantner, K. A., Kieft, T. L., Colwell, F. S., Spear, J. R., and Toner, B. M.: Subsurface microbiology and the pressing societal need to support future exploration, FEMS Microbiol. Ecol., 102, fiaf121, https://doi.org/10.1093/femsec/fiaf121, 2026.

Smith, D. C., Spivack, A. J., Fisk, M. R., Haveman, S. A., and Staudigel, H.: Methods for Quantifying Potential Microbial Contamination during Deep Ocean Coring, ODP Tech. Note, 28, https://doi.org/10.2973/odp.tn.28.2000, 2000.

Sylvan, J. B., Estes, E. R., Bogus, K., Colwell, F. S., Orcutt, B. N., and Smith, D. C.: Technical Note 4: Recommendations for microbiological sampling and contamination tracer use aboard the JOIDES Resolution following 20 years of IODP

deep biosphere research, International Ocean Discovery Program, https://doi.org/10.14379/iodp.tn.4.2021, 2021.

Sylvan, J. B., Tully, B. J., Morono, Y., Alt, J. C., Grim, S. L., Inagaki, F., Koppers, A. A. P., and Edwards, K. J.: Bacterial abundance and diversity in 64–74 Ma subseafloor igneous basement from the Louisville Seamount Chain, mLife, 3, 578–583, https://doi.org/10.1002/mlf2.12148, 2024.

Templeton, A. S. and Caro, T. A.: The Rock-Hosted Biosphere, Annu. Rev. Earth Pl. Sc., 51, 493–519, https://doi.org/10.1146/annurev-earth-031920-081957, 2023.

Templeton, A. S., Ellison, E. T., Glombitza, C., Morono, Y., Rempfert, K. R., Hoehler, T. M., Zeigler, S. D., Kraus, E. A., Spear, J. R., Nothaft, D. B., Fones, E. M., Boyd, E. S., Munro-Ehrlich, M., Mayhew, L. E., Cardace, D., Matter, J. M., Kelemen, P. B., and the Oman Drilling Project Science Party: Accessing the Subsurface Biosphere Within Rocks Undergoing Active Low-Temperature Serpentinization in the Samail Ophiolite (Oman Drilling Project), J. Geophys. Res.-Biogeo., 126, e2021JG006315, https://doi.org/10.1029/2021JG006315, 2021.

Twing, K. I., Brazelton, W. J., McCollom, T. M., Schubotz, F., Pendleton, H. L., Harris, R. L., Brown, A. R., Richins, S. M., Kubo, M. D. Y., Hoehler, T. M., Cardace, D., and Schrenk, M. O.: Heterogeneity of rock-hosted microbial communities in a serpentinizing aquifer of the Coast Range Ophiolite, Front. Microbiol., 16, https://doi.org/10.3389/fmicb.2025.1504241, 2025.

Wee, S. Y., Edgcomb, V. P., Beaudoin, D., Yvon-Lewis, S., and Sylvan, J. B.: Microbial Abundance and Diversity in Subsurface Lower Oceanic Crust at Atlantis Bank, Southwest Indian Ridge, Appl. Environ. Microb., 87, e01519-21, https://doi.org/10.1128/AEM.01519-21, 2021.

Wheat, C. G., Jannasch, H. W., Fisher, A. T., Becker, K., Sharkey, J., and Hulme, S.: Subseafloor seawater-basalt-microbe reactions: Continuous sampling of borehole fluids in a ridge flank environment, Geochem. Geophy. Geosy., 11, https://doi.org/10.1029/2010GC003057, 2010.

Yanagawa, K., Nunoura, T., McAllister, S. M., Hirai, M., Breuker, A., Brandt, L., House, C. H., Moyer, C. L., Birrien, J. L., Aoike, K., Sunamura, M., Urabe, T., Mottl, M. J., and Takai, K.: The first microbiological contamination assessment by deep-sea drilling and coring by the D/V chikyu at the iheya north hydrothermal field in the mid-okinawa trough (IODP Expedition 331), Front. Microbiol., 4, https://doi.org/10.3389/fmicb.2013.00327, 2013.

Zhang, X., Feng, X., and Wang, F.: Diversity and Metabolic Potentials of Subsurface Crustal Microorganisms from the Western Flank of the Mid-Atlantic Ridge, Front. Microbiol., 7, https://doi.org/10.3389/fmicb.2016.00363, 2016.