

Subsurface microbiology and the pressing societal need to support future exploration

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Editor: Marcus Horn

Abstract

Subsurface microbiology is at a crossroads, evolving from asking ‘who’s home’ to seeking clarity on microbes’ functionality and the key processes that constrain subsurface life. Importantly, the processes subsurface microorganisms mediate are central to societal needs to mitigate climate change and address waste storage, as proposed solutions to both involve subsurface habitats. However, subsurface sampling opportunities and funding remain limited and, in some cases, have diminished. This perspective article is aimed at scientists who have or might develop an interest in the geomicrobiology of the subsurface, for funding agencies worldwide, and for scientists and engineers engaged in the extractive and waste disposal industries. It briefly reviews subsurface science’s history and current status and proposes some actions for moving forward. In particular, we see the continued need for engaging early-career microbiologists in drilling projects, increasing access through industry partnerships, microbiology-led drilling projects, and creating interdisciplinary drilling projects by including microbiologists during the drilling project planning.

Keywords: deep life; subsurface microbiology; continental subsurface

The deep biosphere is the habitable zone within the Earth’s entire lithosphere (continental and marine, which each have historically and wrongly been siloed) where some well-adapted microbial life (from all three domains, Bacteria, Archaea, and Eukarya) thrives, albeit at low biomass and limited cell activities per gram of material, despite challenging conditions (Fig. 1) (Gold 1992, 1999, Coleman et al. 2017). The previous century of microbiology research has revealed a vast, active, and geochemically adapted biosphere in Earth’s subsurface, especially at < 80°C (Head et al. 2003, Colwell and D’Hondt 2013, Heuer et al. 2020) and in hotter zones (80–120°C) where energy flux is sufficient to fuel metabolism (Beulig et al. 2022). These subsurface ecosystems are dynamic and depend on the interplay of the *in situ* physico-chemical conditions, geological history, and biogeochemical feedbacks from microbial activity (Osburn et al. 2014, Lau et al. 2016, Momper et al. 2017, Casar et al. 2020, Sheik et al. 2021, Schuler et al. 2024). Within the habit-

able zone, microbial metabolisms drive ecosystem function, composition, and complexity. Within shallow subsurface systems, microorganisms shape the chemistry of soils and sediments and provide critical habitat/symbiosis for plant root zones (shallow and deep). In deeper subsurface environments, microbes metabolize carbon and other elements over geologic time, even influencing the formation of ore deposits (e.g. roll-front uranium or iron ore).

From our scant studies, we see that subsurface microbial life is fueled through oxidation-reduction (redox) couplings that drive chemosynthetic and heterotrophic metabolisms (Osburn et al. 2014, Dowd et al. 2022). In some geological formations, this includes recycling ancient carbon (Inagaki et al. 2015, Schmidt et al. 2021), abiotically produced volatiles (Dowd et al. 2022), microbial biomass (Bird et al. 2019, Patsis et al. 2025), and newly fixed carbon via chemosynthesis (Fullerton et al. 2021). These processes, happening at various timescales, can alter the geochemistry of deep

Received 16 July 2025; revised 3 December 2025; accepted 3 December 2025

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Figure 1. Happy microbes feasting and living their best lives in the subsurface. We understand that while the subsurface is an extreme environment, microorganisms have adapted over time to thrive in this environment and provide necessary ecosystem functions. We have only begun to understand the extent of life in the subsurface, its ecological roles, and, importantly, the feedbacks with the rest of Earth's biosphere. Figure credit: Zirun Luo.

fluids and solids, and likely also influence aboveground geochemical cycling (Ijiri et al. 2018, Snyder et al. 2024). Our current view of subsurface life's diversity (Ruff et al. 2024), both terrestrial and marine, is derived from a relatively small number of core samples (Voelker et al. 2024), boreholes sampled via mines (Pedersen 1997, Sahl et al. 2008, Lau et al. 2016, Purkamo et al. 2016, Momper et al. 2017, Sheik et al. 2021), caves (Macalady et al. 2006, Spear et al. 2007, Zhu et al. 2022, Osburn et al. 2023), and produced fluid samples (Daly et al. 2016, Mouser et al. 2016, 2019, Hernandez-Becerra et al. 2023). As such, despite its importance, the subsurface remains one of the most poorly characterized microbiomes on Earth (Whitman et al. 1998, Magnabosco et al. 2018). Despite the lack

of understanding, we continue to permanently alter subsurface environments with deep-well injection of various surface-derived compounds, some of which are hazardous, and now the injection of supercritical CO₂ to mitigate climate change via carbon sequestration could inadvertently stimulate other microorganisms (Tyne et al. 2023). Unfortunately, as we have seen before (Carson 1962), engineering a system without understanding how critical biomes will be affected or respond could result in ecological catastrophe. The rapidly expanding demands on the subsurface for resources and as a waste repository underscore the urgent need to intensify rather than scale back subsurface research.

The foundations of modern deep life exploration

Much like the organisms that inhabit the subsurface (Fig. 1), the study of continental and marine subsurface biospheres has a long history. The isolation of methane from sediments by Volta (1777) and the discovery of chemosynthesis by Winogradsky (Dworkin and Gutnick 2012) helped lay the foundations of modern subsurface microbiology. Subsequent breakthroughs and research investments would spur the exploration of the subsurface for life. Oilfield development led to the early recognition of sulfate-reducing microorganisms in petroleum reservoir waters and showed the potential for life in deep subsurface environments (Bastin et al. 1926). Later, ZoBell greatly expanded on the breadth of subsurface life and its geologic role in diagenesis (Ehrlich 1999, and references within). With the space race in full swing, NASA's early investments in exobiology research fueled work by Carl Woese that redefined how microbiologists assay life in these extreme environments and revealed the vast microbial diversity that exists outside a test tube (Stahl et al. 1985). Similarly, culture-independent analysis by D.C. White ushered in a new era of quantifying microbial biomass and diversity using the lipids they manufacture (Morris et al. 2008). Then, nearly ~80 years after the idea of chemosynthesis was formed, the discovery of hydrothermal vents showed how these reactions drive deep ocean biospheres (Corliss et al. 1979). Similarly, H₂-fueled subsurface lithoautotrophic microbial ecosystems were discovered in the continental subsurface (Stevens and McKinley 1995).

A confluence of microbiology research in the 1970s (e.g. Colwell et al. 1977, Thauer et al. 1977, Woese and Fox 1977, Balch et al. 1979, Jannasch and Wirsén 1979, White et al. 1979), 1980s (e.g. McInerney and Bryant 1981, Suflita et al. 1982, Pace et al. 1986, Lovley and Goodwin 1988, 1989a, Phelps et al. 1989b), and 1990s (e.g. Evans et al. 1991, Pedersen 1993, Stevens and McKinley 1995, 1997, Murphy and Schramke 1998, Chapelle et al. 2002), coupled with the increasing need for remediating contaminated soils, sediments, and aquifers, helped drive the exploration of terrestrial subsurface environments. Equally, exploration of life in deep, isolated sediments progressed quickly in the marine realm, and mounting evidence pointed to an active deep biosphere. Early work funded through the US Environmental Protection Agency (Wilson et al. 1983, Harvey and George 1987), US Geological Survey (Oremland et al. 1982), and US Department of Energy (DOE) (Colwell et al. 1992, Lehman et al. 1995, Stevens and McKinley 1995, McKinley and Colwell 1996) accelerated our knowledge of the extent subsurface life, how these organisms thrive in these environments, and the metabolic capabilities of these organisms. Specifically, Dr. Frank Wobber's vision as Program Manager for the DOE's Subsurface Science Program (SSP) brought many disparate scientists together to explore the deep continental biosphere. In a short time, 1986–1995 (Onstott 2017), a series of technological advances allowed the use of drilling platforms to access the microbes living deep within the Earth and how to effectively retrieve these organisms while minimizing and quantifying contamination from the surface (Colwell et al. 1992, Phelps et al. 1989a). Many of the scientists responsible for those advances would continue work in the deep subsurface and spur a new generation of deep science explorers. While work in the UNITED STATES was happening rapidly, it was not in a vacuum, and groundbreaking work on deep biosphere microorganisms was underway in deep mines in the Fennoscandian shield (Pedersen 1993) and later in South Africa (Takai et al. 2001). Additionally, drilling deep boreholes in Germany provided a basis for the next generation of con-

tinental drilling (Emmertmann and Lauterjung 1997). This work helped establish a temperature limit for life in the subsurface and showed that molecular hydrogen is a base economy for many subsurface microbes (Gold 1992, Stevens and McKinley 1995, Spear et al. 2005, Colman et al. 2017).

Investigations spawned from the early research networks, like the SSP, served as templates for developing cross-disciplinary collaborations. Continued investment in the (International) Ocean Drilling Program (ODP/IODP) and International Continental Scientific Drilling Program (ICDP) helped microbiologists gain access to the deep biosphere. Microbe-focused ODP projects, starting with Leg 201 in 2002, highlighted how international and cross-disciplinary collaborations can drive science and serve as a touchstone for the next generation of subsurface exploration. The establishment of science communities, e.g. those fostered by Deep Carbon Observatory (Alfred P. Sloan Foundation), the Center for Dark Energy Biosphere Investigations (NSF), Life Underground (NASA), Rock-Powered Life (NASA), National Lacustrine Core Facility (LacCore, NSF), and Continental Science Drilling (CSD, NSF), brought interdisciplinary teams, including microbiologists, together for over a decade and facilitated new collaborations.

The future of drilling and experimentation with deep life

We pose (Table 1) questions that extend beyond the marine and continental realms, which have historically been seen as divided, but rather represent a functional continuum (Ruff et al. 2024). However, our ability to address these questions is threatened as access to the subsurface has become increasingly difficult due to global divestment from key programs and infrastructure. The universality of life, finding ways to thrive in deep ecosystems, and mediating ecosystem services should act as a driving force for future research. We believe that with the increasing number of techniques used to assess microbial life, we, as a field, have moved well beyond the 'stamp collecting' phase. That being said, we do realize the great value in understanding the taxonomic identities and genetic capabilities of these organisms that have evolved in these isolated environments. Novel gene discovery remains essential, and will be facilitated especially by the symbioses of the subsurface 'micro'-microorganisms, members of the candidate phyla radiation, viruses (Daly et al. 2019, Cai et al. 2023), and eukaryotes (Borgonie et al. 2011, Ivarsson et al. 2018), and further subsurface exploration will provide new avenues for technology innovation.

We further argue that subsurface microbial ecology should act as the 'tip of the wedge' for driving subsurface geoscience questions rather than piggybacking on existing drilling projects. The unique geochemical conditions in the subsurface—particularly the low energy available for metabolism—drive unique microbial communities. Microbial life in these systems can be long-lived, both in the sense of long cell doubling time and from an evolutionary perspective, especially in formations that remain isolated for hundreds to millions of years (Holland et al. 2013). These ultra-slow-growing organisms have been termed 'aeonophiles' (Lloyd and Steen 2025). This presence on geologic timescales provides ample opportunity for life to impact the geochemistry of the waters and the rocks surrounding them, on which humanity continually depends. Given the diversity of lithologies across the globe and the dearth of samples from most, what are we missing, and what novel discoveries lie beneath our feet? Specifically, subsurface microbes continually live against all odds, as

Table 1. Major questions and broader impacts for future subsurface microbiology to address as we contemplate new drilling and monitoring.

Major Questions	Broader impacts
What is the role of tectonics (e.g. fault movement), magmatism, planetary collisions, and other large-scale geological or planetary processes in releasing substrates for microbial growth and what role does this play in the evolution of life?	Earth's crust is dynamic but operates on time scales that are counter to much of life on Earth's surface. However, these long-scale processes impact subsurface microorganisms' life cycles by altering their immediate environment. These long scale processes have direct impact on the storage of long-lived contaminants and other proposed subsurface engineering climate solutions. These interactions have direct impact on the movement of microbes and flux of solutes in fracture networks. How microbes disperse in the subsurface is a driving question on the evolution of subsurface microorganisms. Furthermore, the ability of microbes to open or close fractures by mediating mineral dissolution, precipitation, or biofilm formation has great interest for hydrocarbon retrieval via fracking or the ability to stop the flow of contaminants.
What biotic-abiotic interactions create or destroy habitat suitability (e.g. fracture formation or closure)? How do these interactions affect the hydrology, lithology, and ultimately subsurface life's distribution, dispersal, and metabolism?	These potentially ancient lineages may harbour novel genes that could be used for bioengineering new microorganisms that are adapted to subsurface life and could be used for bioremediation or other process.
What diversity (viral, bacterial, archaeal, or eukaryotic) remains to be discovered and how do we define evolution when population sizes are vanishingly small and cell growth and metabolic rates are extremely slow? Are there ancient lineages remaining to be discovered in the subsurface?	How life thrives in the subsurface is one of the lingering questions that has broad appeal, not just for life on Earth but on other celestial bodies.
What are the energy requirements and microbial strategies for long-term survival and maintenance of subsurface life?	If we do find life in the subsurface, how do we know its age? How do we distinguish between contamination or true subsurface life? These are broader questions directed at exploration of life but have implications for bioremediation and other technologies.
How do we observe, date, and understand the relative antiquity of cells and their lineages within the deep biosphere in their hydrogeological context?	

most are exposed to multiple stressors simultaneously, like energy and/or nutrient-limited conditions, and maintenance under high pressures and elevated temperatures. We feel microbiology can provide information that will help geologists and geochemists holistically understand the geobiology of the habitable zones of Earth and the imprint of biology on subsurface conditions.

Subsurface microbiology is deeply rooted in hydrocarbon exploration and bioremediation and has been driven by partnerships with government agencies and industry around the world. In the coming decades, exploration for minerals and energy will intensify to satisfy human demand. Thus, continued investment, both monetarily and through industry partnerships, in subsurface microbiology exploration will continue to reap both intellectual and practical benefits. Our view of the deep biosphere continually evolves, but the realization that subsurface and surface processes are intertwined is fundamental. This is especially apparent when human activities can short-circuit and accelerate subsurface microbiological processes (Amundson et al. 2025) through mineral and hydrocarbon extraction (Jenneman et al. 1984, Daly et al. 2016), contaminant spills (Harvey and George 1987), energy storage (Dopffel et al. 2024), or contaminant storage (Amundson et al. 2025). These activities raise the question of whether we are unknowingly contaminating and/or poisoning subsurface ecosystems to our detriment. Furthermore, bio-corrosion and biofouling (Beech 2004) (i.e. microbially influenced corrosion (MIC)) in subsurface infrastructure is a long-recognized problem: D.C. White termed it ‘the venereal disease of industry—it is painful, incapacitating, and expensive, but usually unmen-

tioned’ (White et al. 1990, Morris et al. 2008). By studying and experimenting with the subsurface biosphere continuum, we can inform and improve strategies for waste storage (e.g. near-surface sanitary landfills, deeper injection wells, and safer nuclear waste storage), better building foundations, energy generation and exploitation, mineral extraction, development of a hydrogen economy, search for natural products, and storage of carbon dioxide.

We are at a crossroads for subsurface exploration. Funding drilling projects has always been and will continue to be a rate-limiting step to subsurface exploration, as drilling is costly and time-consuming. For instance, the length of an ICDP project from pre-proposal to drilling can exceed four years, depending on funding post-ICDP acceptance. Often, these projects outlive graduate students, postdoctoral researchers, and late-career investigators. Furthermore, no matter where a research program is housed, funding cycles and grant longevity are typically limited to 2–5 years and may not fully align with the drilling project. Because of this funding discontinuity, institutional knowledge of basic subsurface techniques, e.g. drilling, coring, tracer deployment, etc., may be lost. A clear example of this is the recent US divestment from IODP and the decommissioning of the R/V JOIDES Resolution, which means that a whole generation of early-career subsurface scientists from around the world will have only limited access, through other international drilling platforms, to subsurface marine life. While programs like the United States-based CSD and ICDP help investigators plan and execute drilling projects, there is an imminent threat of brain drain from subsurface science.

Table 2. Suggestions for future subsurface drilling.

Recommendations	Benefits
Establish and fund research communities that mentor and support diverse, early career subsurface scientists and provide mentorship for studying subsurface life	Numerous studies have shown that bringing together scientists from diverse backgrounds enhances scientific discovery. Moreover, mentoring early career scientists in subsurface projects will insure longevity of subsurface research by facilitating the passing of knowledge gained from previous drilling expeditions. These networks will foster new ideas and drive drilling future expeditions.
Link continental and marine subsurface science, both at the level of funding agencies, and in individual projects, e.g. transects across subduction zones.	Leveraging both systems will give us insight into the universal rules for how microorganisms overcome energetic hurdles of slow growth. Combining marine and continental organizations and infrastructure will also leverage limited resources
Strategically select new drilling sites that focus on the biology of understudied geological provinces	The subsurface, especially beneath the continents, is geologically diverse, but understudied. Expanding to new geological provinces will lead to new discoveries. What makes for an interesting geological investigation may not yield interesting microbiology! Microbes live in mundane to extreme geologic settings. Let's focus on the microbes! If microbial life in these deep systems are long lived, then why should we sample once and be done? Cores can be sampled only once, but subsurface fluids can be monitored, sampled, and experimented with in situ for many years. Longterm investment in resampling and monitoring should be a priority and will give insight to microbial community dynamics many years after drilling has commenced and boreholes have equilibrated.
Post drilling, create long-term ecological monitoring stations, by installing multi-level samplers and downhole instrumentation or CORK-like, autonomous, robotic devices ⁷² .	Industry is the primary driver of subsurface exploration. Direct pathways to partner with, fund, and publish research with industry has benefits for both academia and industry.
Create funding routes for subsurface microbial exploration with industry partners.	Engagement of local stakeholders is essential for gaining local knowledge, recruiting a diversity of scientists who bring added skillsets to subsurface science, and avoiding 'scientific piracy'.
Seek local collaborators and stakeholders during the pre-drilling process and work with them throughout the drilling process (pre, during, and post).	

At the same time, this is a moment of promise for subsurface microbiology. Multidisciplinary continental drilling projects, like now defunct NASA Astrobiology Institute's partial funding of the Oman Drilling Project (Fones et al. 2019, Templeton et al. 2021), are successful models for future subsurface microbial exploration. Additionally, partnerships with industry have also proved worthwhile and have facilitated groundbreaking work in the subsurface (Colwell et al. 2011, Daly et al. 2016, McIntosh et al. 2023). Continued advances in methodologies to study microorganisms, such as high-throughput cultivation, BONCAT labelling, nano-SIMS, fluorescence-activated cell sorting, bioinformatic tools, super-resolution microscopy, isotopologue analyses, and sampling methodologies, will continue to transform our view of life in the subsurface and across Earth's biomes. The field of subsurface microbial ecology has taken the forefront and will continue to yield a broad, holistic understanding of how microorganisms interact with this vast environment.

Based on this legacy of subsurface study and after extensive discussions, we put forward several recommendations for future subsurface drilling (Table 2). Careful consideration should be given to preserving future boreholes, e.g. by casing and installing multilevel samplers or CORK-like devices (Becker and Davis 2000) to create long-term microbial observatories. The long-term study of sediment and fractured rock fluids can provide valuable access to deep microbiomes and facilitate the study of community

dynamics and evolutionary processes in a geochemical context that may occur on long timescales. The consequences of subsurface microbe-fluid-rock interactions are increasingly important, as they are and have been critical for the evolution of life across time on this planet. Designing subsurface drilling and sampling projects to prioritize geomicrobiology is therefore a pressing requirement. Furthermore, the ability to study these microbes and geochemistry *in situ* will aid laboratory studies that try to recreate these subsurface microbial habitats and give insight to how these low-biomass and activity systems operate. As such, by understanding how these microbes operate in these vast ecosystems, we may be better positioned to develop technologies that leverage the physiology and behaviour of subsurface microorganisms to enhance subsurface storage of waste or carbon dioxide. These types of solutions have broad implications for life on Earth's surface and, importantly, for the subsurface water sources we rely on in some regions. As we move into the next phase of subsurface study, the incorporation of early-career researchers at every facet of the drilling process, from project inception to core processing, will be critical for sustaining our field. Finally, the creation and long-term investment in international working groups will be key to linking researchers together and facilitating the open exchange of ideas, outside of the normal modes of publishing and conference presentations. Together, we hope this perspective spurs inspiration to study subsurface microbes and to seek new pathways for gaining access to the subsurface.

Acknowledgements

We thank the Continental Scientific Drilling Program and the US National Science Foundation (NSF) for facilitating discussions and bringing us together to build this paper. In the early conceptions of the two-page community outlook, many researchers brought ideas, joined the conversation, and edited. We thank them for their hard work and dedication to the field.

Conflict of Interest: None declared.

Funding

NSF OCE-2145434 funded A.D.S., NSF EAR-1813526 funded C.S.S. and B.M.T., and NSF EAR-2026858 funded T.L.K. NASA PICASSO23-0026 funds J.R.S.

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