

# Marine Cold Seeps As A Gateway Of Deep Subsurface Extremophiles To The Seafloor

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## Abstract

In the Earth's deep subsurface life is comprised exclusively of microorganisms, and estimates indicate 12-45% of the global prokaryotic biomass, on the order of  $10^{29}$  microbes, is found in subseafloor sediments. Investigating how this enormous microbial biomass is maintained in the extreme habitats below seafloor is critical for understanding the rules of life in the deep biosphere. Furthermore, Earth's subseafloor habitats often present analog environments detected in other planets such as the recently discovered "ocean worlds", i.e., planetary bodies in our solar system which consist of large subsurface oceans including Saturn's moons Titan and Enceladus and Jupiter's moon Europa. Therefore, investigating life in and beneath Earth's oceans remains at the forefront of the current astrobiological research endeavors.

Despite the inhospitable nature of the subseafloor sedimentary realm, active microbial populations including bacteria capable of transforming into dormant endospores have been demonstrated to inhabit deeply buried anoxic sediments and oil reservoirs, permeable ocean crust, and around hydrothermal vents. These extreme habitats often remain physically connected to the seafloor by unique geological features such as marine cold seeps that transmit hydrocarbon-rich fluids originating in deep sediment layers. It remains unclear how fluid migration in cold seeps influence the composition of the seabed microbiome and if they transport deep subsurface life up to the surface. In this study, we addressed this knowledge gap by analyzing over 180 marine surficial sediments from the Gulf of Mexico and the Monterey Bay to assess whether hydrocarbon fluid migration serves as a mechanism for the dispersal of subsurface extremophiles and their introduction into the seabed via cold seeps.

Seafloor samples were collected either by piston coring or ROV-operated push coring and were stored at  $-20^{\circ}\text{C}$  upon collection. Presence of hydrocarbons in the piston core sediments was characterized by gas chromatography mass spectrometry and fluorescence spectroscopy whereas gas seepage was determined in the ROV push cores by visual confirmation of gas bubbles emanating from the seafloor. Sediment microbiome composition was determined by high throughput 16S rRNA gene amplicon

sequencing. Metabolic diversity was assessed via a genome-centric metagenomics approach aided by shotgun metagenomic sequencing of selected samples. Additionally, viable bacterial endospore communities were investigated from a subset of over 120 of the above samples by allowing endospore germination using a high-temperature incubation assay followed by amplicon sequencing.

While 132 of the piston core sediments contained migrated liquid hydrocarbons, evidence of continuous advective transport of thermogenic alkane gases was observed in 11 sediments. Gas seeps harbored distinct microbial communities featuring bacteria and archaea that are well known inhabitants of deep biosphere sediments. Specifically, 25 distinct sequence variants within the bacterial lineages *Atribacterota* and *Aminicenantia* and the archaeal lineage *Thermoprofundales* occurred in significantly greater relative sequence abundance along with well-known seep-colonizing members of the bacterial genus *Sulfurovum*, in the gas-positive sediments. Metabolic predictions guided by metagenome-assembled genomes suggested these organisms are anaerobic heterotrophs capable of non-respiratory breakdown of organic matter, likely enabling them to inhabit energy-limited deep seafloor ecosystems. In addition, eight different lineages of anaerobic bacterial endospores activated by sediment incubation assays were strongly associated with hydrocarbon-bearing sediments. These lineages were most closely related to *Clostridiales* previously detected in oil reservoirs from around the world.

These results cumulatively point to petroleum geofluids as a vector for the advection-assisted upward dispersal of deep biosphere microbes from subsurface to surface environments, shaping the microbiome of cold seep sediments and providing a general mechanism for the maintenance of microbial diversity in the deep sea.

## Keywords

Deep biosphere, seafloor microbial ecology, microbial dispersal, anaerobic thermophiles, bacterial endospores, hydrocarbon seep

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## Conflicts of interest

The authors have declared that no competing interests exist.