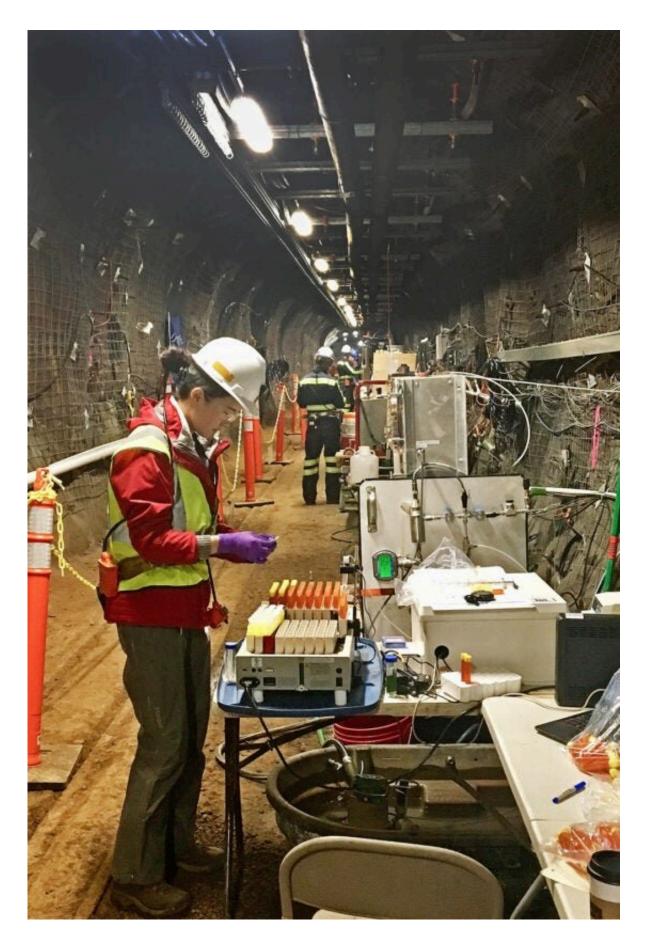


Geological activity can rapidly change deep microbial communities

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Lead study author Yuran Zhang working deep underground at the field site in South Dakota. Zhang and her colleagues used samples collected from the facility to show that microbial community changes can be driven by geological activity. Credit: Yuran Zhang

In the deep subsurface that plunges into the Earth for miles, microscopic organisms inhabit vast bedrock pores and veins. Belowground microorganisms, or microbes, comprise up to half of all living material on the planet and support the existence of all life forms up the food chain. They are essential for realizing an environmentally sustainable future and can change the chemical makeup of minerals, break down pollutants, and alter the composition of groundwater.

While the significance of bacteria and archaea is undeniable, the only evidence of their existence in the deep <u>subsurface</u> comes from traces of biological material that seep through mine walls, cave streams, and drill holes that tap into aquifers.

Many scientists have assumed that the composition of microbial communities in the deep subsurface is primarily shaped by local environmental pressures on microbial survival such as temperature, acidity, and oxygen concentration. This process, environmental selection, can take years to millennia to cause significant community-level changes in slow-growing communities like the subsurface.

Now, with data collected nearly 5,000 feet below ground, Stanford University researchers have shown that deep subsurface microbial communities can change in a matter of days, and the shifts can be driven by geological activity—not only by environmental pressures. The



findings were published last month in *Proceedings of the National Academy of Sciences (PNAS)*.

"In the deep subsurface, we can no longer understand environmental selection to be the dominant driver in community dynamics—it could be just a changing flow rate or movement of the groundwater through the crevices and cracks in the subsurface that's driving what we observe," said lead study author Yuran Zhang, who conducted the research as a Ph.D. student in energy resources engineering.

Filling in gaps

Like reading a random page of someone's 1000-word biography, previous studies on deep subsurface microbes have only offered glimpses into the chronicles of their existence. By collecting water samples from multiple geothermal wells weekly over 10 months, the Stanford researchers showed how these populations can change over space and time, demonstrating the first evidence of geological activity as a driver for microbial community change—and therefore evolution.

"There is previous research on the composition of microbial communities in the deep subsurface, but it's almost always using samples from a single time point," said geomicrobiologist Anne Dekas, a senior study author and assistant professor of Earth system science. "To have a time series over 10 months—especially at a weekly resolution—is a really different perspective that allowed us to ask different questions about how and why these communities change with time."

Dekas said that while microbial ecologists might have guessed that geological activity was at play, she was surprised by the extent of the community shifts that occurred after a change in the flow network.



Boreholes and test tubes

The technique used in the study involved processing samples from a flow test conducted at the Sanford Underground Research Facility (SURF), formerly the Homestake Gold Mine, in South Dakota. Zhang said the experience of moving from a borehole sample setting to a test-tube-filled lab with a PCR machine on campus was "like connecting two totally different worlds," referring to how this work unites the distinct fields of microbial ecology and geothermal engineering.

In analyzing the properties of the water samples, the researchers identified microbial DNA fingerprints. Each of the 132 water samples supplied tens of thousands of unique sequencing IDs. Those data were used to show that when geological activity occurred, it could quickly mix disparate biological communities—and from locations that weren't previously known to be connected.

"One of the additional pieces of information from this microbiology study is that we've seen populations of microbes that have moved not just directly from place to place, but as a consequence of the network in between," said senior study author Roland Horne, the Thomas Davies Barrow Professor of Earth Sciences. "That's so important from the reservoir point of view because it reveals something that isn't revealed by normal geothermal analytical methods."

Geology meets biology

The level of data collected by current geothermal techniques is like only having access to highways that are cut off from the side roads that will take you all the way home. Investigation of microorganism populations opens the potential for mapping the complex intricacies of the deep subsurface in more detail, Horne said.



Being able to use biology as a tool may also bring insights into the deep subsurface as a frontier for geological storage, such as nuclear waste and carbon sequestration. But combining biology and geology requires fundamental knowledge of both subjects.

"On the geothermal underground project, I realized that reservoir engineers or geologists or geophysicists usually aren't that familiar with microbiology," said Zhang, who was co-advised by Horne and Dekas. "There is common knowledge about geochemistry, but not so much in geomicrobiology."

This work could even be meaningful beyond Earth-based disciplines: If some of the oldest life forms in the deep subsurface of Earth can change and diversify because of geological activity, maybe we can have similar expectations for the origin and diversification of life on other tectonic planetary bodies.

"What we observe could potentially connect to the early story of life's evolution," Zhang said. "If geological activity is a driver for early life formation or diversification, then maybe we should look for extraterrestrial life on planets that are geologically active."

More information: Yuran Zhang et al, Geological activity shapes the microbiome in deep-subsurface aquifers by advection, *Proceedings of the National Academy of Sciences* (2022). DOI: 10.1073/pnas.2113985119

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