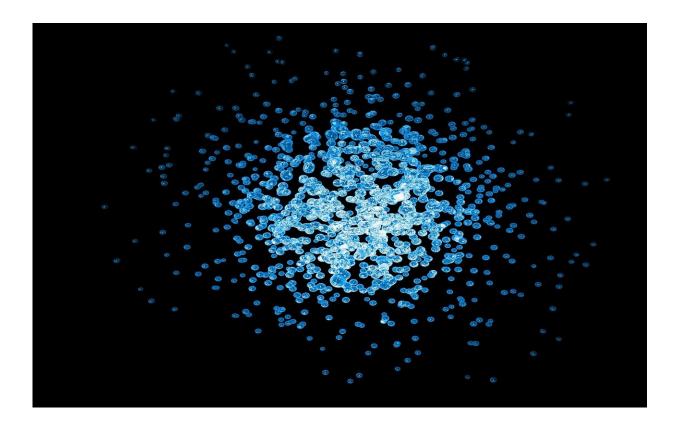


Microbes buried at the bottom of the sea start flourishing after 80,000 years

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In otherwise energetic deserts at the bottom of the sea, researchers have found oases where microbes can harvest energy. Remarkably, the microbes first have to be buried under starving conditions for 80,000 years. An international group of researchers, amongst them José



Mogollón from the Insitute of Environmental Sciences (CML) at Leiden University, has published this finding in *PNAS*.

The researchers studied microbes from the genus Scalindua in the Greenland/Norwegian Sea. Microbes of this species were able to reactivate and increase their population size by more than 4 orders of magnitude long after burial.

Metabolic activity to an absolute minimum

Millions of <u>microbial cells</u> populate every inch of the more than 360,000,000 km² of Earth's seafloor. Over time, they become buried deep into the sediments due to the continuous rain of particles from above to become part of what is known as the deep sedimentary biosphere. Once the <u>food supply</u> from the surface world is cut off, the energy becomes increasingly limited with depth and as a consequence, the population slowly succumbs to the inhospitable conditions and gradually diminishes over time. Here, metabolic activity is slowed down to an absolute minimum, providing barely enough energy for basic cell maintenance. Survival thus becomes a matter of persistence and perseverance rather than growth. In the new study published in *PNAS*, however, the research team describes how a small group of organisms that obtain their energy by oxidizing ammonium under anaerobic conditions, known as anammox, are an exception from this general rule and manage to proliferate after extensive time under unfavorable conditions.

80,000 years going through hell

"It is really quite astonishing what these cells are able to endure," says senior author Steffen L. Jørgensen from Centre for Deep Sea research at the University of Bergen (Norway). He continues to explain: "From the



moment the Scalindua cells are deposited on the ocean floor they find themselves in a hostile environment where conditions are far from optimal. In fact, the presence of oxygen in the surface could be downright lethal to them as their metabolism is inhibited by free oxygen and, if that wasn't enough, then their food source (ammonium and nitrite) is very scarce. After deposition on the ocean floor they must survive an 80,000 year-long journey facilitated by slow burial through a place that is toxic and basically devoid of any energy sources they can use. Not until then are they deep enough into the sediments to reach the energy rich zone where fluxes of ammonium and nitrate meet, the ammonium-nitrate transition zone (NATZ). Here the few cells that make it to this depth starts to grow and the <u>population size</u> increases. Their residence time in the NATZ is however limited as the zone itself stays at a fixed depth relative to the surface over time, whereas the microbes will be buried even further down as a consequence of the relentless burial process. In principle, these cells have spent 80,000 years going through hell to finally reach a place where they can get served a proper meal only to find themselves kicked back out after the appetizer."

Outlier result

The researcher's discovery is an example of how results that could have easily been rejected as outliers can lead to new insights when pursued. Lead author Rui Zhao describes how he had observed increases in cell abundances at depths that could not be explained by textbook knowledge. "We have collected and analyzed many sediment cores over the years and I often saw an unexpected elevation in cell numbers right below the oxic zone that puzzled me," Rui tells. After contemplating at the geochemical context data with collaborator and co-author José Mogollón from Leiden University, the team found that the elevated abundances of Scalindua coincided with increased energy availability present at the depth where reduced and oxidized species of nitrogen meets. "These geochemical transitions zones are the opportune locations



where interdisciplinary scientific approaches that bring together laboratory and computational techniques can drive our understanding of microbial life dynamics," says Mogollón. Rui Zhao: "This is exactly the place you would expect that microbes able to utilize ammonium and nitrite thrive and so it was a great feeling to see that our geochemical and microbial data supports each other."

Genetic explanation

One of the obvious questions the researchers were left with was what traits that enable these microbes to survive for such a long time under highly unfavorable conditions. In order to investigate this further, they sequenced the entire genome from these organisms and compared it to the genome content of their surface world relatives. They found that the organisms possess specific genes that allow them to utilize different sources of nitrogen containing compounds in order to obtain the ammonium that they need, says Sophie Abby, a researcher then at the University of Vienna (Austria), who helped investigate the genome content along with professor Christa Schleper. "In addition, it is not unlikely that they can use exploit other reactions than anammox in order to obtain energy, and that this metabolic versatility might be what help them survive until they reach the NATZ," Abby says. "However, looking at the low number of microbes that actually survives that long, it seems to be by the skin of their teeth."

Population dynamics are not so simple after all

When asked about the ramifications of their discovery Rui Zhao mentions that: "This has important implications for how we perceive population dynamics in the deep biosphere, which largely has been viewed as a function of different microbial groups' ability to stay alive for variable timespans. While this might still be the case, the study shows



that this view might not be so simple after all. In addition, and very importantly, these microbes play important roles in regulating fluxes across the seafloor of both critical nutrients in the form of nitrogen species and of the potent greenhouse gas CO_2 ." He follows up by reminding us that the high energy NATZ is not unique to the specific study site, but seem to be widespread in the subsurface and hence the environmental significance relevant on a global scale.

Exactly how important these microbes are for the environment on a global scale is something that Jørgensen is eager to learn more about and he considers this current study the first step on the way to provide such information. "There are many more questions that needs to be addressed," he says. "For example, how are other microbes benefitting from this increase in energy and biomass? Or how fast do the <u>microbes</u> react to changes in the energy landscape and what are their growth rates? In addition, we would like to investigate other high energy zones further down into the sediments where <u>energy</u> is also available if you have the right metabolic machinery to harvest it and this too might affect fluxes between the surface and the subsurface worlds. These are all aspects we are trying to investigate."

More information: Rui Zhao et al. Geochemical transition zone powering microbial growth in subsurface sediments, *Proceedings of the National Academy of Sciences* (2020). DOI: 10.1073/pnas.2005917117

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