MOIRA HOUGH

A boardwalk for traversing Sweden's Stordalen Mire sinks in the thawing landscape.

THE BURIED CARBON BOMB

Scientists are scrambling to understand how the microbes in thawing permafrost might alter climate-change predictions. **By Monique Brouillette**

In May, as the temperatures in northern Sweden begin to creep to several degrees above freezing, scientists will once again descend on the squelchy peat of Stordalen Mire. They'll tread across sagging wooden boardwalks, past clusters of clear plexiglass boxes placed among the cotton grass.

Once every three hours during the mire's short growing season, the lids on the boxes will close, allowing them to fill with methane — a powerful greenhouse gas — seeping up from the soil beneath. After 15 minutes, the gas will get sucked through a labyrinth of tubes into a nearby trailer for analysis.

Meanwhile, the scientists have a messier job. They will push metal cores into the squishy mud and pull out samples to take back to the laboratory. There, they will study the microorganisms producing the methane by sequencing their genes. Although there are other efforts to study the microbes that dwell in permafrost, this project, known as IsoGenie, is one of the largest and longest-running field studies of its kind. "We put together measurements in geochemistry and microbial ecology, two things that are in completely different areas, to find out something new," says Scott Saleska, an ecologist at the University of Arizona in Tucson and a co-founder of the project.

Several decades ago, Stordalen Mire was covered in permafrost. But today, thanks to rising global temperatures, most of it has degraded into a patchwork of bogs and grassy wetlands, leaving behind raised mounds known as palsas, in which permafrost remains partially insulated by dry peat. As the palsas continue to thaw, scientists are eager to document changes to the microbial communities within.

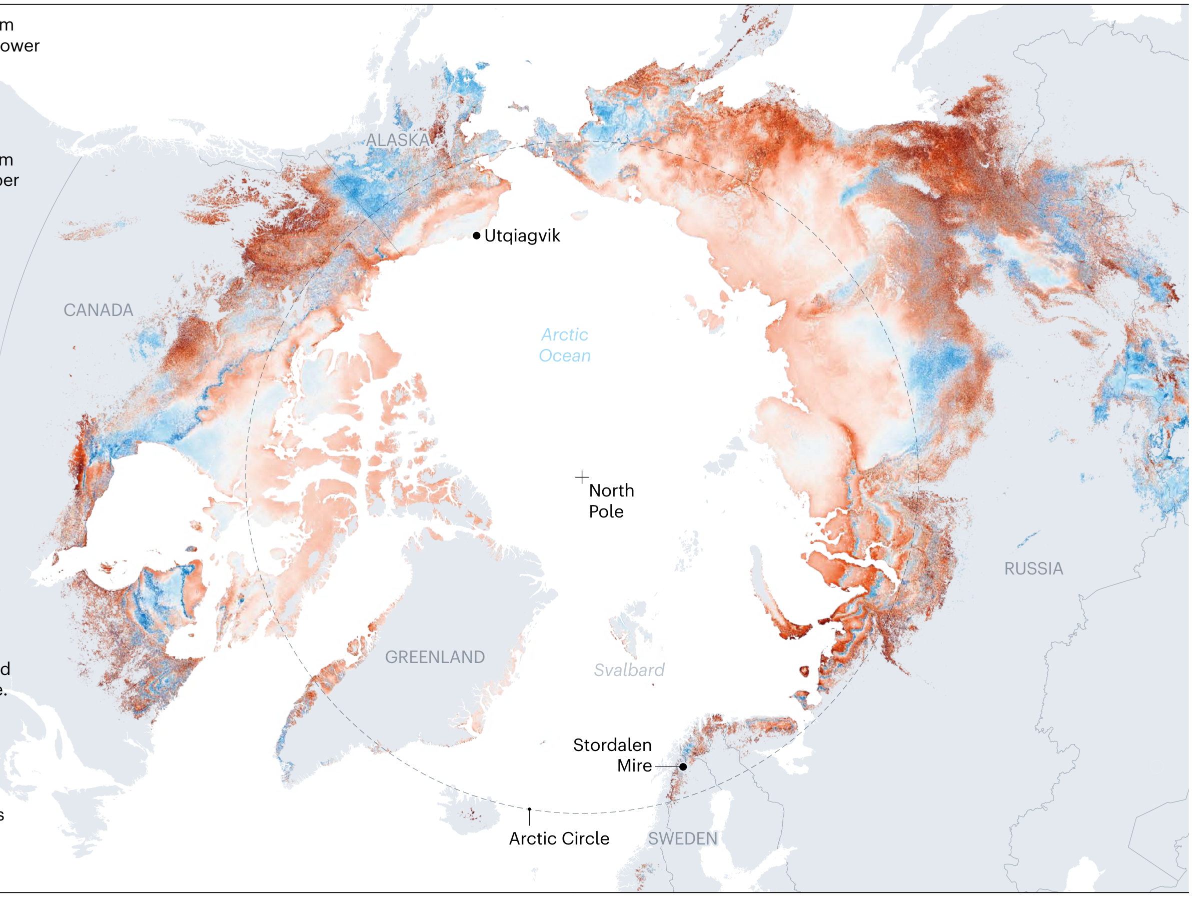
For most of human history, permafrost has been Earth's largest terrestrial carbon sink, trapping plant and animal material in its frozen layers for centuries. It currently stores about 1,600 billion tonnes of carbon — more than twice the amount in the atmosphere today. But thanks to rising temperatures, permafrost is fracturing and disappearing, leaving behind dramatic changes in the landscape (see 'The big thaw').

Scientists are becoming increasingly worried that the thaw will lead to an epic feast for bacteria and archaea that produce carbon dioxide and methane. And although climate models have long accounted for the carbon-emitting capacity of Arctic permafrost and Arctic lakes, the microbial activity within has largely been treated as a black box, changing in sync with the physical properties of the ecosystem, including temperature and moisture. That's a problem, says Carmody McCalley, a biogeochemist at the Rochester Institute of Technology in New York. "If your model doesn't get the mechanism right, it's probably not going to do a great job of making predictions," she says.

As scientists look more closely at the organisms living in these environments, the findings are beginning to bubble up. The identity of the dominant microbes in transitional permafrost settings can make a difference to the types of greenhouse gas emitted, for example[1]. The depths of Arctic lakes could be more sensitive to climate change than expected, owing to the types of microbes they host[2]. And the availability of iron and other nutrients in the soil could accelerate greenhouse-gas production in some locations.

Although there are still unknowns about how the landscape will change in response to warming — and questions such as the role of viruses in the soil remain largely unanswered

THE BIG THAW



Scientists can track the loss of permafrost using satellite data. The active layer, the soil that thaws and refreezes seasonally, deepened by an average of 2.5 cm across the Northern Hemisphere during 2007–16 compared with the previous decade. For about 5% of the area, the active layer has deepened by more than 30 cm. The deepening active layer destabilizes the landscape and makes more carbon available to microbes in the soil.

SOURCES: DATA FROM PERMAFROST CCI; J. OBU *ET AL.* DATA SET AT CEDA ARCHIVE HTTPS://DOI.ORG/GHJKB2 (2020)

– gathering data on the microbes is leading to a more holistic view of what's going on. "It let us see under the hood," says Virginia Rich, a microbiologist at the Ohio State University in Columbus and the other co-founder of IsoGenie. "In the permafrost system, this is an acutely pressing need, because these systems are thawing before our very eyes."

A long history

Several research projects are investigating the microbes in thawing permafrost. Some, such as the Alaska Peatland Experiment funded by the US National Science Foundation (NSF), study microbial communities in environments that are similar to Stordalen's carbon-rich soil. Another big project is the Next-Generation Ecosystem Experiment – Arctic, funded by the US Department of Energy. It is investigating the mineral-rich terrain of Alaska's North Slope, near Utqiagvik (formerly Barrow). The US Army conducts research on how microbial communities shift and change in its Permafrost Tunnel, a 110-metre chamber carved into a frozen hillside near Fairbanks.

Other large-scale efforts include the Center for Permafrost at the University of Copenhagen, which conducts metagenomics analysis on soil from various sites in Greenland, Russia, Sweden and Svalbard. And a joint effort from Russian and US scientists in northeastern Siberia is comparing microbial communities in permafrost samples of different ages, from a few thousand to a few million years old. The researchers have found intact permafrost with cyanobacteria and microalgae that can become active after thawing[3].

Stordalen Mire is one of the most heavily studied sites in the Arctic, with more than a century of detailed information collected about its temperatures, soil content and plant communities. Bo Svensson, a microbiologist at Linköping University in Sweden, was one of the first researchers to start taking measurements of methane emitted from the soil, in the 1970s. He used buckets and coffee cans to capture the gas, often spending hours in the mire fending off mosquitoes and black flies with thick tar-oil repellent purchased from a local Sami community. Back then, there were no facilities or electricity, and Svensson would often have to hike 10 kilometres or more to and from Sweden's Abisko Scientific Research Station with gas-filled syringes and other equipment tucked securely in his pack.

Today, one of Svensson's rusted coffee cans sits among the updated equipment in the mire – a physical reminder of how much the science has progressed. "Stordalen Mire has become an international hub," he says. Its physical position on the leading edge of thaw in the region has made it an attractive research site for scientists interested in climate change. The addition of electricity and an access road built in the 1980s hasn't hurt.

In 2010, the launch of the IsoGenie project brought a new suite of molecular-biology tools to the site. Funded by the US Department of Energy, the project was spearheaded by Rich, who developed environmental DNA-sampling techniques for studying ocean microbes, and Saleska, who created laser-based systems for measuring trace-gas concentrations. IsoGenie brought together scientists from a range of disciplines and has amassed a tremendous collection of data over the past decade.

Not long ago, scientists would have to culture microbes in the lab to learn much about them, but they have increasingly been sampling and sequencing DNA from environmental samples and using metagenomics to piece together the communities in soils, oceans, lakes and more. Not only can they identify the species that are present, they can also see which genes are active – providing a powerful picture of the metabolic strategies at work and the relationships between microbes.

Rich estimates that her team has assembled 13,000 genomes from microbes living in the site's soils. The community is vast, spanning the entire microbial tree of life. It includes a newly discovered order of methane-emitting archaea and 15,000 soil viruses that are thought to infect the microbes living there. It is a trove that has provided fresh insights into methane production.

Methane makers

The first big finding came in 2014, when the team showed that the various landscape features in the mire have distinct microbial communities that churn out methane at different rates[1]. In partially thawed muddy bogs, for example, most of the microbes present produce methane through a process called hydrogenotrophic methanogenesis, in which they consume carbon dioxide and hydrogen. But in fully thawed fens, the microbial community becomes more complex, and microbes move in that produce methane through a process called acetoclastic methanogenesis, in which acetate and carbon dioxide are used to produce methane. Rich says this is important because the two processes respond differently to environmental conditions such as temperature and pH.

The finding was a wake-up call for the scientists, because it means that areas of the mire in later stages of thaw could be producing more or less methane depending on environmental conditions, which is important to incorporate into models when extrapolating into the future. "What we showed in our paper is that the kind of methane produced varied a lot from one place to another depending on the amount of thaw and who was there," says Saleska.

"That was a really huge step," says Patrick Crill, a biogeochemist at Stockholm University and an IsoGenie collaborator. "Now, we could see a link between the landscape and the biogeochemical signal that was coming out, and that's because of the 'omics."

"The fact that they were able to put the pieces together from microbes to climate models was really cool," says Ted Shuur, an ecosystem ecologist at Northern Arizona University in Flagstaff.

Into the depths

Next, the team turned its attention to Arctic lakes. According to Ruth Varner, a biogeochemist at the University of New Hampshire in Durham and an IsoGenie collaborator, current efforts to forecast climate change pay little attention to how the various regions in a lake might emit methane differently. It has long been assumed that shallow waters, which heat up faster during the warm months, produce more methane than do the depths. But this had never been tested.

Using metagenomics and measurements of gas emissions from two lakes in Stordalen Mire, Varner and her colleagues have found that this long-held assumption might need to be revised. In work that has yet to be peer reviewed[2], they show that microbial communities in the deeper parts of the lakes contain more methane-producing microbes than do those in the shallow regions. They are also more sensitive to increasing temperatures. This means a slight rise in temperature could result in a disproportionate release of methane from the middle of the lake. Varner warns that if global temperatures continue to rise "we think there'll be more methane coming out than we would expect".

Last September, Varner and Rich announced their next undertaking — a project called EMERGE, which stands for 'emergent ecosystem response to change'. The venture, backed by US$12.5 million from the NSF, gathers 33 researchers across 15 disciplines to continue the metagenomics work that IsoGenie began. They aim to improve understanding of the evolution of microbes in response to climate change, and even the role of viruses.

One aspect of the coming work will look to correlate different microbial communities with landscape features that can be monitored remotely, such as plants. Making these links should allow the researchers to use satellite technology to map methane-producing microbes across the Arctic.

Relating the observations at Stordalen Mire and a few other research sites around the Arctic to permafrost carbon stores elsewhere will not be straightforward. The size, variety and remoteness of these landscapes pose a challenge for scientists. In fact, it is estimated that almost one-third of all Arctic research has been conducted within 50 kilometres of just two sites — Abisko and Toolik Lake in the North Slope. Mark Waldrop, a microbial ecologist at the US Geological Survey in Menlo Park, California, has spent more than a decade studying the Alaskan permafrost, and thinks there is a lot of value in learning how the microbiology there works at local and regional scales, but he points out that there are still many unknowns about what will happen to different permafrost habitats as they thaw across the Arctic. To combat this sampling bias, he is working with NASA to amass the largest pan-Arctic database of permafrost microbe samples. Waldrop is excited about using this database to study under-sampled regions of the Arctic.

"The fact that they were able to put the pieces together from microbes to climate models was really cool."

Modelling matters

Another challenge will be understanding how terrestrial environments change when they thaw. Whether a particular location drains and becomes dry and rocky, or gets swamped with water, will have major impacts on microbial communities and their resulting emissions, according to Waldrop. Janet Jansson, a microbial ecologist at the Pacific Northwest National Laboratory in Richland, Washington, echoes these sentiments, and underscores the importance of identifying the unique signatures of the microbial life that inhabits these diverse ecosystems. She thinks knowledge about microorganisms will aid the modelling of future carbon emissions. "They are the little factories that are producing these greenhouse gases. And so, of course, we have to understand how that is happening. We can't just be ignorant and say, 'Oh, these gases are, are somehow being produced.'"

Jansson has been leading a team studying microbial communities on a low-lying, lake-studded region in the North Slope. As the permafrost there freezes and thaws, it cracks and buckles to form geometric formations called ice-wedge polygons that are a combination of ice, bog and lake. This heterogeneous landscape covers about 20% of this region in Alaska, and over the past decade or so, Jansson has been incorporating metagenomic and gas analysis into her work to understand how emissions differ in the diverse habitats.

In 2015, her metagenomics work led to a new understanding of how microbes can survive for long periods in the nutrient-poor and freezing permafrost conditions[4]. She and her team found genes that encoded proteins involved in iron metabolism, indicating that the microbes used the mineral as an energy source to survive in harsh conditions. The discovery shed light on a mechanism that later proved to be a predominant survival strategy for microbes in permafrost[4]. And last December, researchers at the Abisko research station showed that, as microbes thaw and awaken, the presence of iron in the soil could actually hasten the release of carbon dioxide[5].

Going forward, Jansson is interested in studying the viruses that infect many of these soil microbes and unpicking their role in carbon processing. Some viruses will kill off their hosts, altering the balance of microbes in the community. Others contain auxiliary metabolic genes that encode proteins that will release carbon locked up in plant matter. "It's not a normal thing that you would expect a virus to do well, and we have a lot of unpublished data showing that they can do a lot more than that, potentially," she says.

As temperatures rise in the Northern Hemisphere, scientists are preparing to return to the Arctic research sites. At Stordalen Mire, snow still covers the ground and temperatures are stuck well below freezing. But the thaw is coming, and Rich and Varner are looking forward to continuing to chip away at the mysteries of the microbes within.

Monique Brouillette is a science journalist in Cambridge, Massachusetts.

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