

CASE STUDY

# Metagenomic analysis of microbial communities in permafrost thaw

Taking advantage of the long sequencing reads generated by nanopore technology, Devin Drown and his team at the University of Alaska Fairbanks, USA, have been researching how the thawing of permafrost may affect soil microbial communities<sup>1</sup>. Permafrost is a permanently frozen layer of soil, gravel, and sand, on or under the Earth's surface.

To be considered permafrost, the area must have been continuously frozen for at least two consecutive years but, in most cases, it has been frozen for hundreds or thousands of years. Permafrost covers large regions of the Earth, in areas with cold climates at high latitudes or altitudes. Sitting on top of the permafrost is a thin layer of soil, known as the active layer. Devin and his team wanted to understand how permafrost thawing, caused by climate change, affects the microbial communities in the active layer and how, in turn, this affects the crops which grow within the soil. Interactions between microbes and plants are critical for the acquisition and cycling of nutrients, and even small changes in soil microbial communities can disrupt plant-microbial interactions<sup>2</sup>.

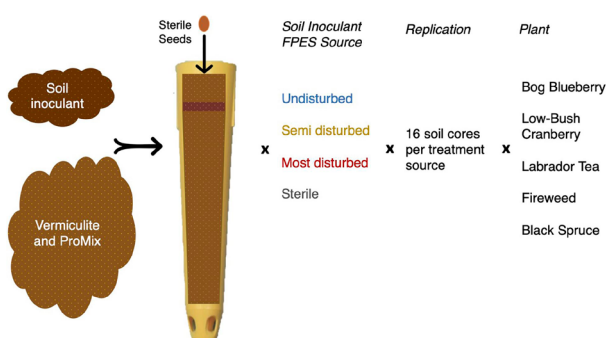
Based in Alaska, the team applied nanopore metagenomic sequencing to the study of microbial communities in soils associated with differing permafrost conditions that can be found in boreal forests, also known as snow forests. These forests are home to many plants that are integral to the diets of native Alaskan communities. Using metagenomics

“soil microbes have the capability to alter plant growth in response to climate change”

means microorganisms do not need to be cultured. Devin and his team chose nanopore technology for a rapid, and thorough characterisation of the complex microbial communities in the active layers. Long, PCR-free nanopore sequencing reads enable access to regions that are difficult to sequence with traditional short-read sequencing technologies, facilitating the assembly of accurate microbial genomes from complex communities. Native DNA can be sequenced in real time, enabling streamlined library preparation and rapid turnaround times.

Using plots of artificially induced permafrost thaw, created over 60 years ago at the Fairbanks Permafrost Experiment Station in Alaska, the team conducted plant growth experiments with five plant species found in boreal forests: *Vaccinium vitis-idaea* (low-bush cranberry), *Vaccinium uliginosum* (bog blueberry), *Picea mariana* (black spruce), *Ledum groenlandicum* (Labrador tea), and *Chamerion angustifolium* (fireweed). The plants were grown in soils with differing degrees of permafrost thaw, termed ‘undisturbed’, ‘semi-disturbed’, and ‘most disturbed’ (Figure 1).

Most of the plants inoculated with microbial communities from the active layer above thawing permafrost showed decreased productivity compared to plants inoculated with microbes from the undisturbed active layer. To analyse the microbial communities within the differing active layers, the team used the Ligation Sequencing Kit to sequence 48 metagenomes across four MinION™ Flow Cells. The combined datasets showed a mean read length of



**Figure 1**  
Experimental design of the plant growth experiment. Image from Seitz *et al.*<sup>1</sup> and available under Creative Commons license ([creativecommons.org/licenses/by/4.0](https://creativecommons.org/licenses/by/4.0)).

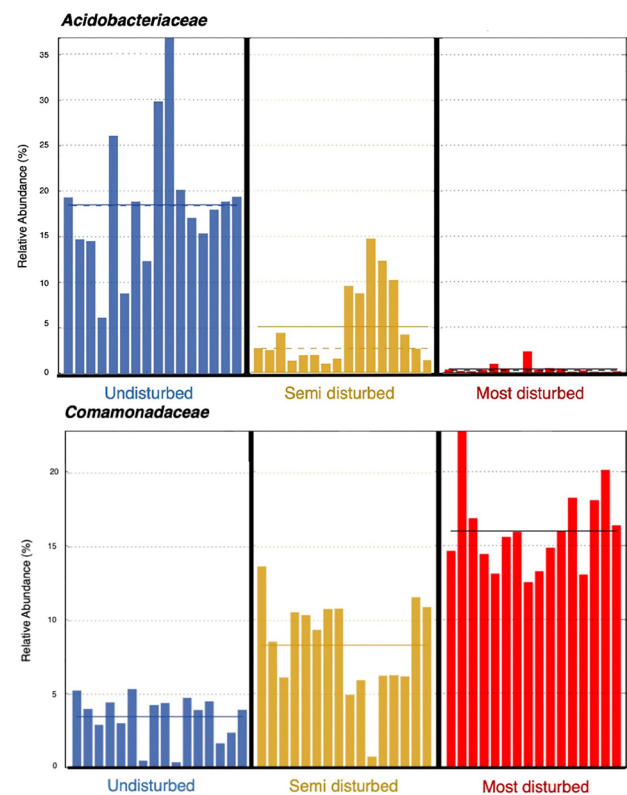
2,594 bp, and a read length N50 of 5,531 bp. The reads were then processed using Kraken<sup>3</sup> and Bracken<sup>4</sup> to detect taxa and estimate abundance, respectively. The team identified 24 bacterial phyla within the microbial communities. Visualisation of the data showed that the microbial community populations differed between the different soil types, with the largest difference observed between the undisturbed and most disturbed active soil layers.

Beneficial soil microbes are known to enhance nutrient availability to plants, enabling increased plant growth and productivity<sup>2</sup>. The team analysed biomarkers associated with healthy plant growth and found microbes that have been positively correlated with plant productivity, such as *Acidobacteriaceae* and *Bacillales*, were highly represented in undisturbed soil samples compared with most disturbed soil samples. In contrast, members of the *Comamonadaceae* family, which are known to exhibit pathogenic effects on a variety of plants, were more abundant in the most disturbed soils compared with undisturbed soils (Figure 2). The team hypothesised that the known plant pathogens found in the most disturbed soil led to reduced plant growth, due to a disruption in nutrient cycling and direct alterations to the plant rhizosphere, the area around a plant root.

#### Products used

<b>Kits</b>	Ligation Sequencing Kit Native Barcoding Expansion
<b>Device</b>	MinION
<b>Tools</b>	Kraken 2   Bracken
<b>Find out more:</b>	<a href="https://nanoporetech.com/products">nanoporetech.com/products</a>

This environmental metagenomics study formalises the links between climate change, thawing permafrost, changing microbial communities, plant health, and broader community health<sup>2</sup>.



**Figure 2**

Relative abundances of bacterial families in the different soil types. Solid lines represent mean relative abundance. Image from Seitz *et al.*<sup>1</sup> and available under Creative Commons license ([creativecommons.org/licenses/by/4.0](https://creativecommons.org/licenses/by/4.0)).

## NANOPORE SEQUENCING

- enabled streamlined and accurate identification of microorganisms from metagenomic soil samples
- delivered fast access to results through rapid workflows

“Our results suggest that a decrease in plant growth can be linked to changes in the taxonomic makeup of microbial communities<sup>1</sup>”

Find out more about microbial sequencing using nanopore technology:  
[nanoporetech.com/applications/microbiology](https://nanoporetech.com/applications/microbiology)

#### References

1. Seitz, T.J. *et al.* *Frontiers in Microbiology* 12:619711 (2022).
2. Devin Drown. <https://nanoporetech.com/resource-centre/video/lc21/evaluating-the-effects-of-the-changing-permafrost-ecosystem-through-the-lens-of-genomics> [Accessed 24 August 2022]
3. Wood, D.E., Lu, J. & Langmead, B. *Genome Biol* 20:257 (2019).
4. Lu, J. *et al.* *PeerJ Comp. Sci.* 3:e104 (2017).