

## Genome-resolved Insights Into Permafrost Thaw Processes

Gene Tyson<sup>1</sup>, Ben Woodcroft<sup>1</sup>, Caitlin Singleton<sup>1</sup>, Miguel Martinez<sup>2</sup>, Dylan Cronin<sup>2</sup>, The IsoGenie Project Team<sup>3</sup>, Eoin Brodie<sup>4</sup>, Ruth Varner<sup>5</sup>, Jeff Chanton<sup>6</sup>, Patrick Crill<sup>7</sup>, Matt Sullivan<sup>2</sup>, **Scott Saleska<sup>8</sup>, and Virginia Rich<sup>\*2</sup>** (rich.270@osu.edu)

<sup>1</sup>Australian Centre for Ecogenomics, University of Queensland, Brisbane; <sup>2</sup>The Ohio State University, Columbus; <sup>3</sup>10 IsoGenie Project Institutions; <sup>4</sup>Berkeley Labs, Berkeley, California; <sup>5</sup>University of New Hampshire, Durham; <sup>6</sup>Florida State University, Tallahassee; <sup>7</sup>Stockholm University, Stockholm; <sup>8</sup>University of Arizona, Tucson.

<http://isogenie.osu.edu>

**Project Goals:** The objective of the IsoGenie3 Project is to discover how microbial communities mediate the fate of carbon in thawing permafrost landscapes under climate change. We are engaged in a systems approach integrating (a) molecular microbial and viral ecology, (b) molecular organic chemistry and stable and radiocarbon isotopes, and (c) state-of-the-art modeling, across an interconnected system of thawing permafrost and post-glacial lakes in Arctic Sweden.

Our IsoGenie Project examines microbial carbon cycling and climate feedbacks along a natural permafrost thaw gradient in Arctic Sweden, where permafrost-underlain palsas sink into partially-thawed bogs and fully-thawed fens, within a mosaic landscape of interconnected post-glacial lakes. Using an ecosystems approach with nearly a decade of high-resolution characterization of microbes, organic matter and carbon gas isofluxes, our team has mapped how thaw's simultaneous impacts on hydrology and vegetation lead to different microbial communities and processes.

In the seasonally-thawed active layer, microbial diversity and cell numbers increase overall from palsa to bog to fen, with different 'type' communities present at each stage of thaw. Metagenomic recovery of genomes for ~60% of the mire's microbial genera has illuminated phylogenetic and functional novelty, bringing genomic representation to many phyla and clades previously lacking any (including the globally relevant methanotroph lineages USC $\alpha$ ), connecting new functionality to known lineages (such as the encoded potential for methanotrophy in the Hyphomicrobiaceae), and expanding the phylogenetic breadth of biogeochemically relevant enzymes (such as the presence of an active 'fungal' pathway for xylose degradation in bacterial MAGs). Beyond novelty, the MAGs also revealed thaw-associated shifts in the web of carbon degradation that fuels microbial carbon gas emissions. The dominant microbial lifestyle encoded by the mire's MAGs was the breakdown of polysaccharides, which are the primary source of both carbon and energy, as inorganic terminal electron acceptors are rare. While the dissolved organic matter itself becomes more labile and less diverse in the fully-thawed habitat (see companion geochemistry poster, Wilson et al.), the microbiota encoding its breakdown becomes more diverse, shifting from dominance in the MAGs of genes for complex polysaccharide degradation towards that of simpler polysaccharides, along with fermentation, acetogenesis, and methanogenesis. Concomitantly, the lineages encoding these processes become more diverse.

Methane emissions provide a potent positive feedback to climate warming from these sites, and are proximately controlled by methane cyclers, whose presence, composition, and activity are dramatically impacted by thaw. Methanogens are absent from the dry palsas, and become more abundant, diverse, and active from the bog to the fen, in parallel with increasing methane emissions. High-affinity methanotrophs in the palsa drive slight atmospheric methane uptake, then peak in abundance in the bog and mitigate emissions (as inferred by isotopes), before becoming more diverse and physiologically active in the fen, but playing a lesser role in mitigating emissions. Demonstrating the importance of more explicit microbial representation in models, a single novel genus of methanogens is the best predictor - better than typical environmental drivers or various guild-level aggregations of the methanogens - of the bog methane's isotopic signature (a characteristic used to partition sources of atmospheric CH<sub>4</sub>) over multiple years and methods.

Below the seasonally-thawed active layer at the depth of the permafrost itself, thaw also increases microbial diversity, and drives development of a community distinct from those of both the initial permafrost and the overlying seasonally-thawed 'active layer'. Of particular note is a deeply-branching lineage related to the phylum Caldiserica (novel at the level of phylum or class by 16S or genome-based taxonomies, respectively), which rises to ~60% of the community (by metagenome recruitment) in the permafrost itself, has been seen at similarly

high abundances at another Mire nearby, and is present at other global high-carbon sites. Via inferences from 7 metagenome-assembled genomes, this lineage is a fermenter of carbohydrates and possibly amino acids, that can use labile plant compounds and peptides, and encodes adaptations to low temperature. Meta- transcriptome and - proteome analyses indicate that this lineage is active after thaw, but its role as the dominant member of the permafrost microbiome is not yet known.

As microbes shift with thaw, so do viruses (see companion virus poster, Sun et al.), which infect a range of carbon-cycling hosts including methanogens and methanotrophs, based of *in silico* virus-host linkages. Viral diversity loosely tracks microbial diversity across the thaw stages, peaking in the fen. The mire's viruses also carry at least one active 'host' gene responsible for carbon substrate transformation.

In the lakes, which are responsible for half the system's methane emissions, and are the main portal for 'old carbon' loss (whereas mire's methane is from recently fixed 'new' carbon), differences in resident methanogens' temperature sensitivity highlights the importance of understanding acclimation to accurately predict future fluxes.

IsoGenie's MAGs and extensive multi-disciplinary data are housed within a neo4j graph database with a map-based user interface (at <http://isogenie.osu.edu>; see companion database poster, Hodgkins et al.). By connecting these datasets, and the arising ecosystem meta-omics insights, to a hierarchy of models (including explicit representation of MAG-derived information), IsoGenie is endeavoring to improve predictions of climate feedbacks from these rapidly thawing systems.

*This study was funded by the Genomic Science Program of the United States Department of Energy Office of Biological and Environmental Research, grants DE-SC0010580 and DE-SC0016440.*