

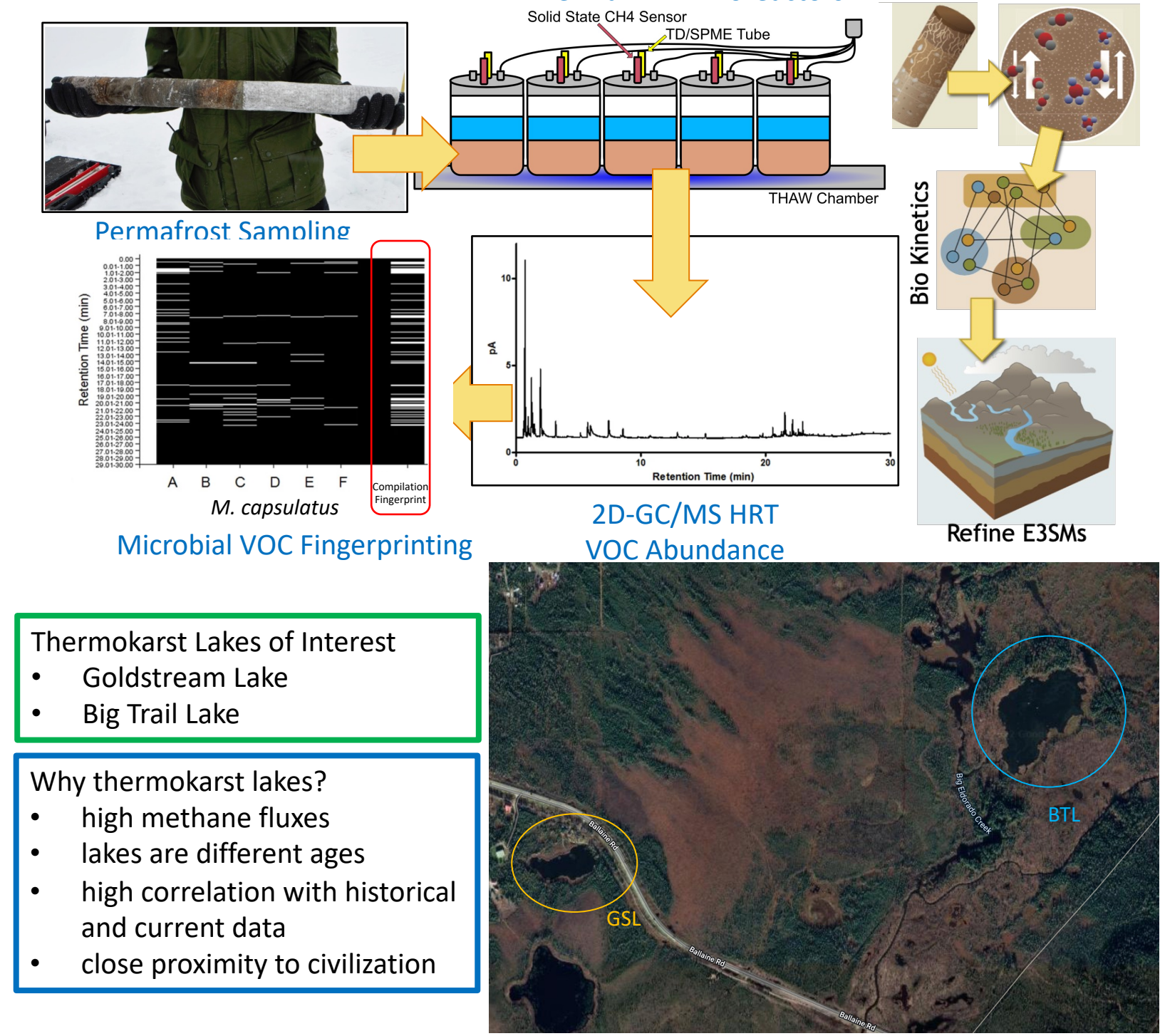


## Abstract

The enhanced rate of Arctic warming will accelerate permafrost degradation contributing to a large magnitude of uncertainty for greenhouse gas emissions. Accurate measurements of biogeochemical changes are needed to address climate feedbacks through high resolution modeling and develop potential mitigation strategies. Our work seeks to increase biological fidelity of greenhouse gas emissions to improve understanding of the role of biology on permafrost degradation, resilience, and subsequent greenhouse gas release. This work will ultimately allow us to define the climate feedbacks and improve existing earth systems models in permafrost ecosystems.

Here, we utilized PermaTHAW Pod bioreactors to evolve thermokarst soil samples and measure high-resolution chemical profiles to identify mVCs microbial fingerprints. Our goals are to explore and understand ecological patterns arising from long dormant permafrost-bound organisms and their impact on the biogeochemical climate feedback cycles occurring in ecological hotspots such as thermokarst lakes. Our proposed approach will develop innovative real-time measurement of mVCs over time to monitor microbial metabolic dynamics in a simulated thermokarst permafrost microcosms.

### Project Summary



Thermokarst Lakes of Interest

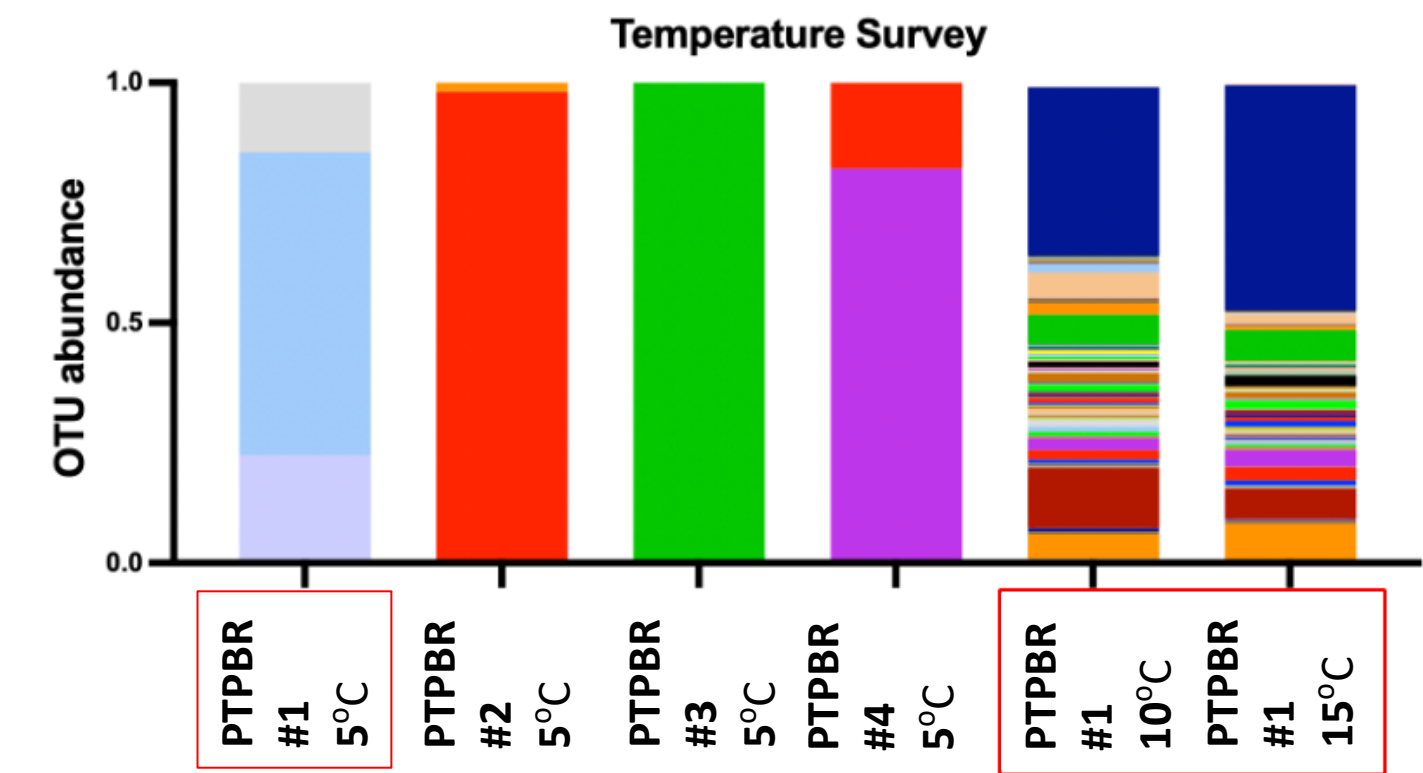
- Goldstream Lake
- Big Trail Lake

Why thermokarst lakes?

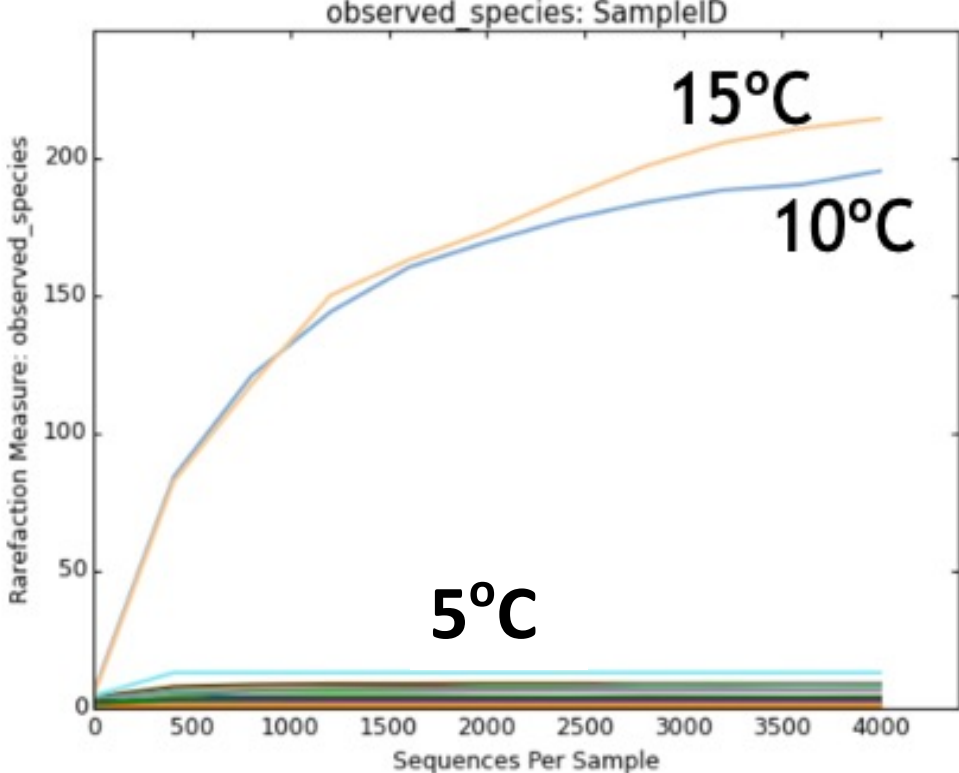
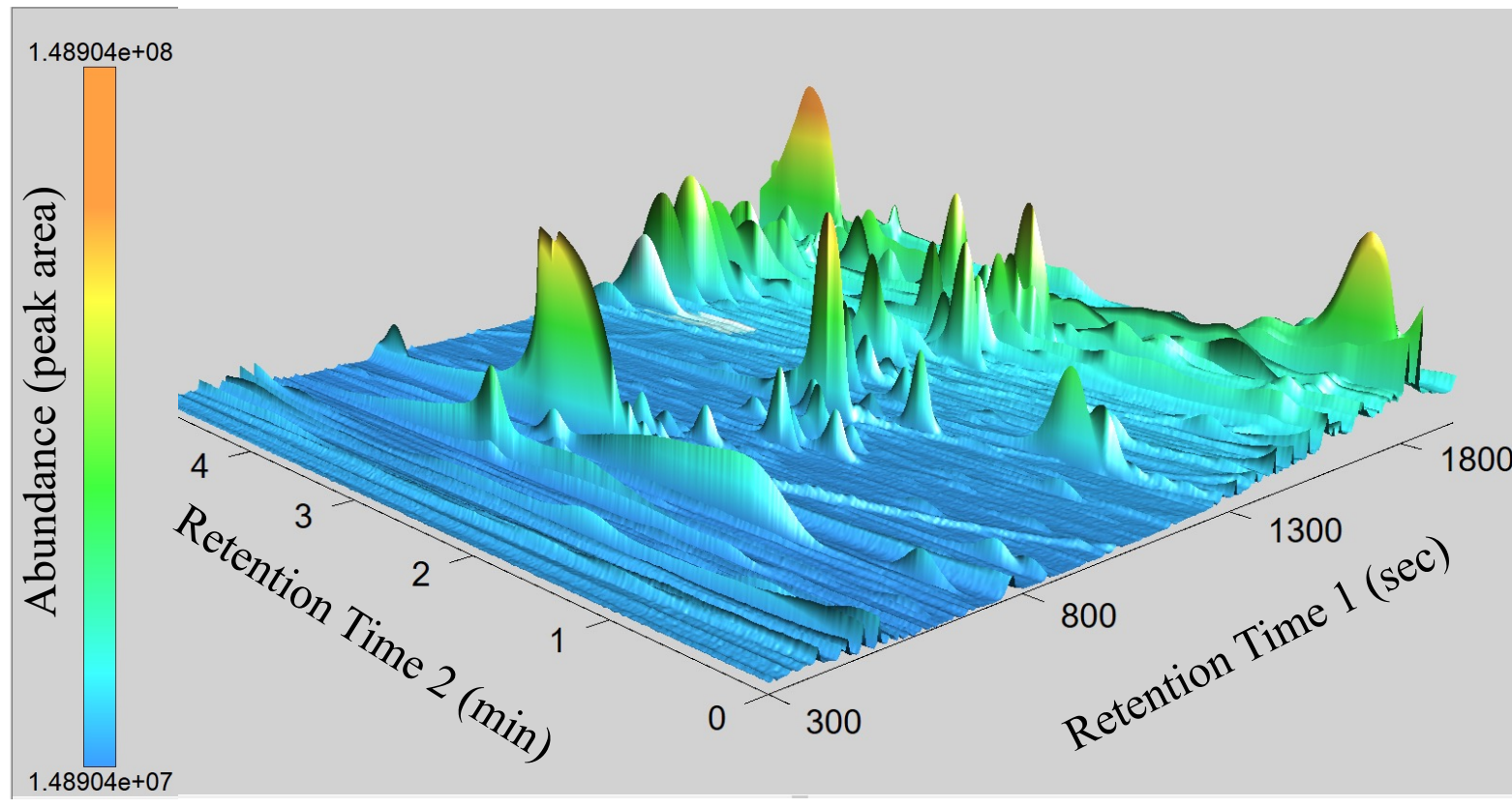
- high methane fluxes
- lakes are different ages
- high correlation with historical and current data
- close proximity to civilization

## 16S rRNA Sequencing & mVCs Analysis

### Permafrost 16S rRNA Gene Sequence Evolution Results



### Evolution Experiment Gas Sampling at 15°C



- Diversity of microbes grows logarithmically as temperature rises from 5°C
- Thousands of mVCs detected were not identified by HRMS libraries
- Over 50% of identified mVCs were biologically relevant
- Majority of biologically relevant compounds have not been linked to specific bacterial strains in literature

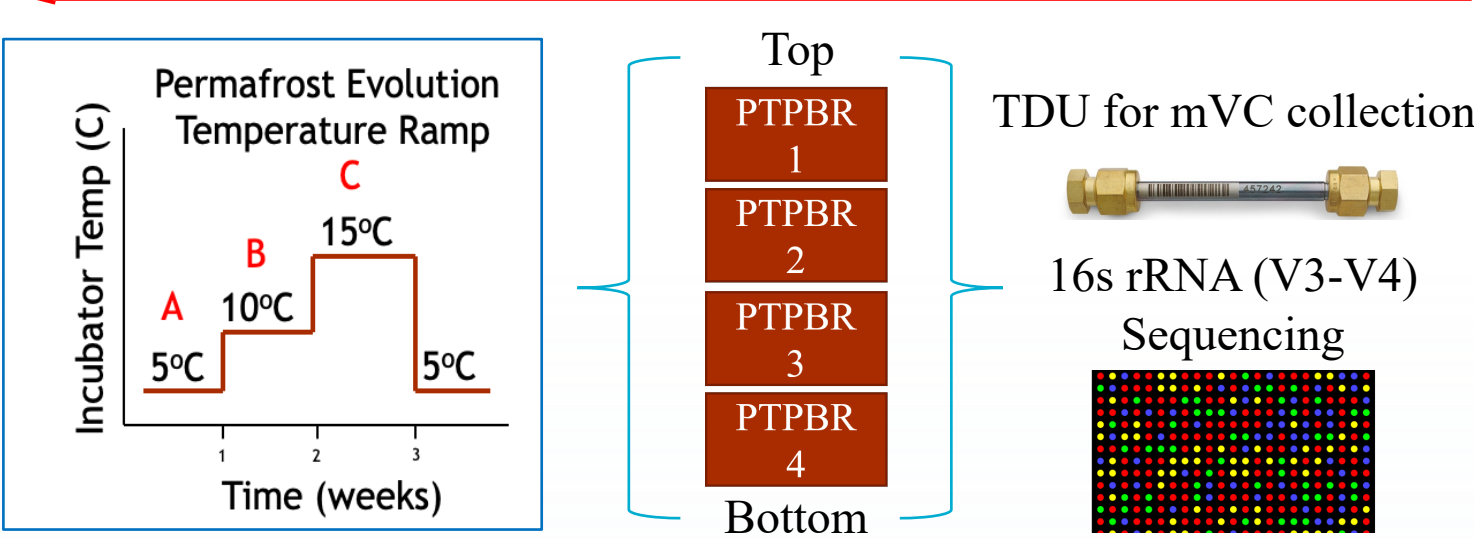
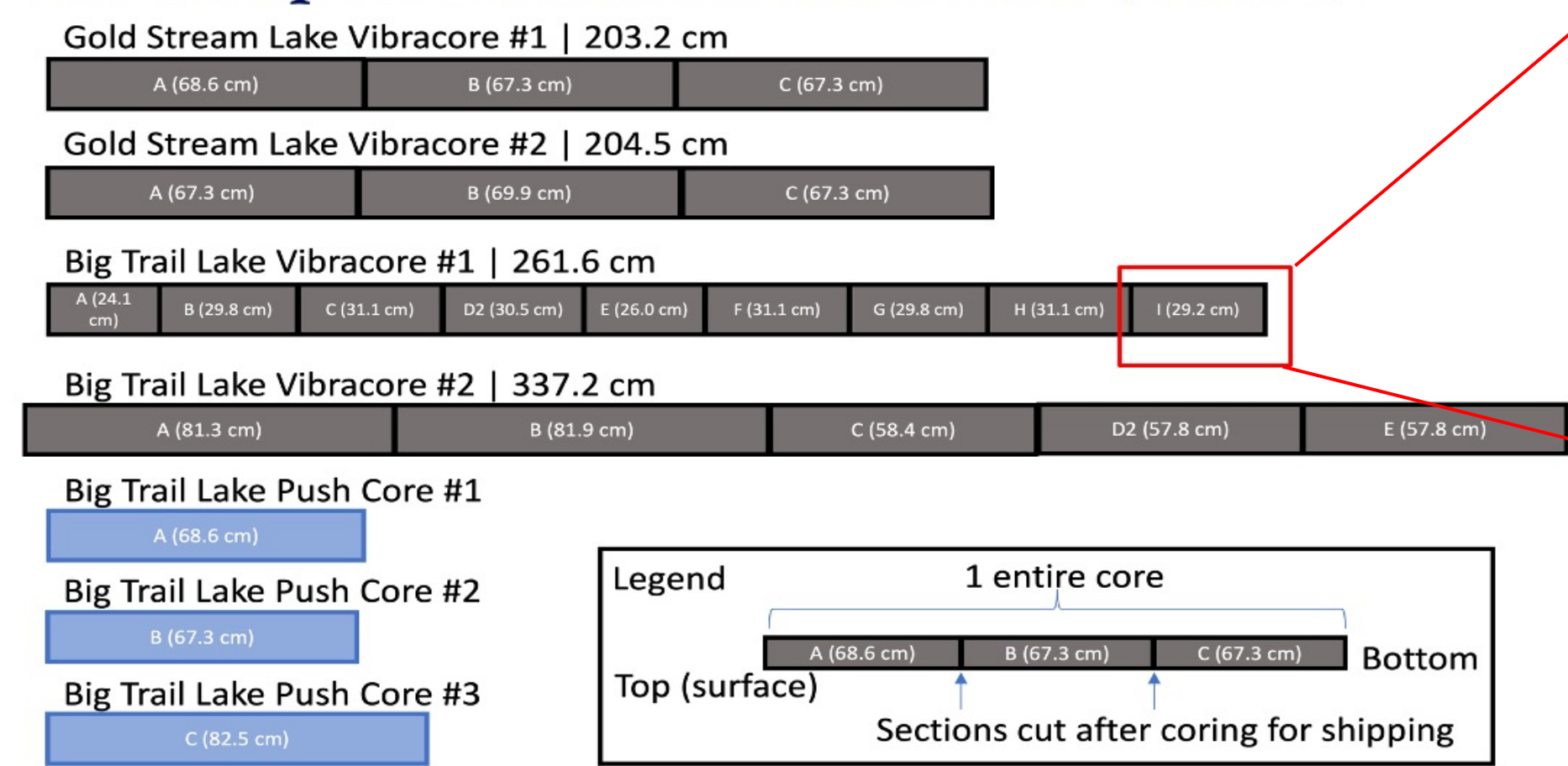
### Detected Microbial Evolution mVCs

Compound	Potential Role	Temp	Microbial Source
Etoxidrine	Metabolite	5°C	E.coli
2,4,6-trimethyldecane	Metabolite	10°C	Bacillus
L- Glutamic Acid	Metabolite	10°C	E.coli
Indole	Secondary metabolite	10°C/15°C	Shikimate pathway
2,3-indolinedione	Antifungal Metabolite	10°C/15°C	Pseudomonas
L-Pyroglutamic acid	Metabolite	15°C	Cyano/proteobacteria
Hexadecanenitrile	Lipid, VOC	All	Pseudomonas
Benzoic acid	Secondary metabolite	All	E.coli

Highlighted cells correspond to bacteria identified in 16s rRNA sequencing results

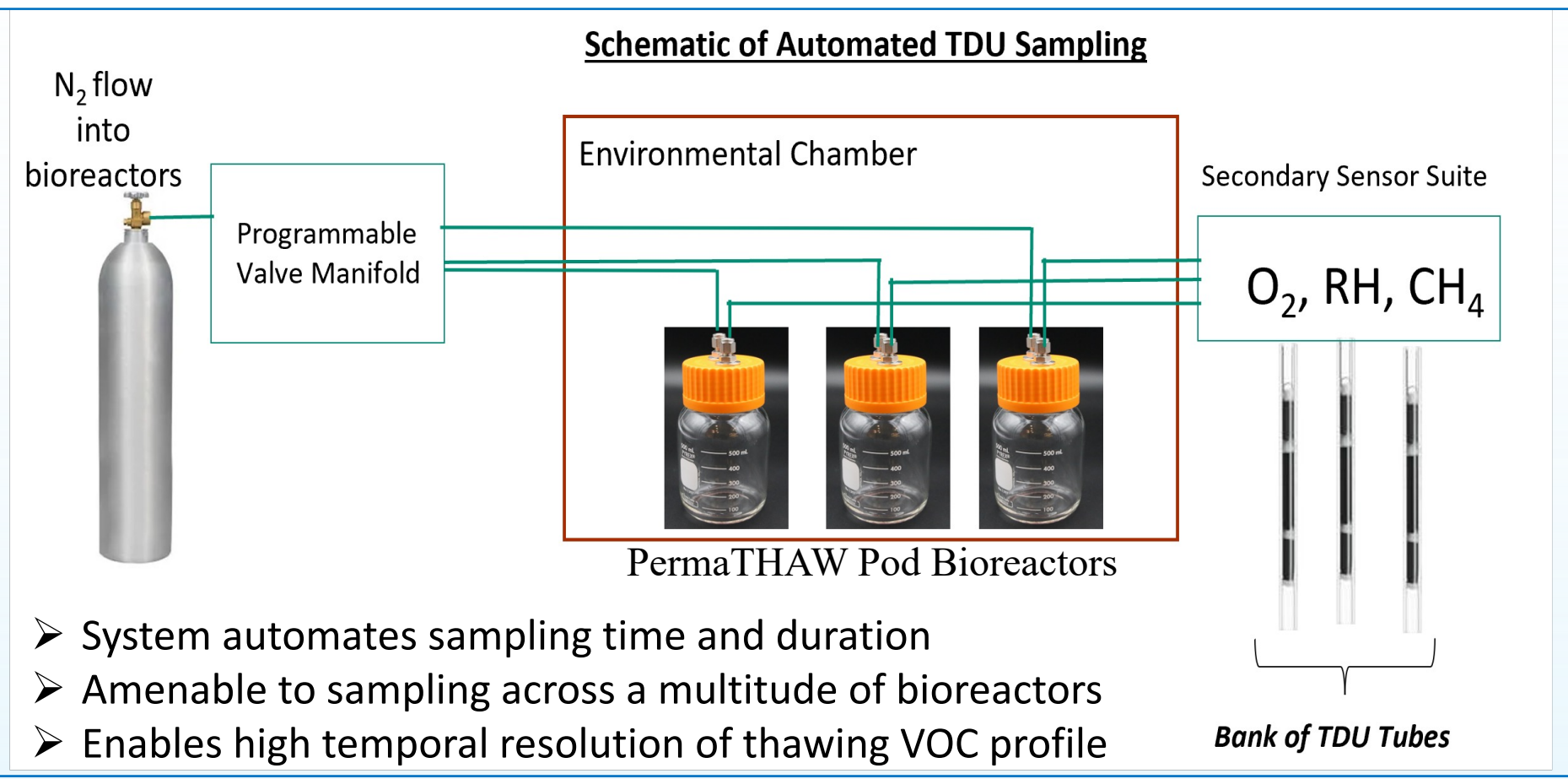
## mVCs & Metagenomics Pilot Evolution Experiments

### Core Samples Collected Before Thaw (March 2022)



- Samples collected from thermokarst lakes stored at 4°C
- Sectioned cores further divided into four equal parts and put into sealed PermaTHAW Pod Bioreactors (PTPBR)
- DNA extraction and 16s rRNA (V3-V4) sequencing of all sections
- PTPBRs equilibrated at 5°C for 1 week, then temperature ramped to 10°C and 15°C for 1 week respectively
- During each temperature, thermal desorption units (TDUs) packed with sorptive material collected volatile organic compounds (mVCs)
- TDUs analyzed on GCxGC -HRMS

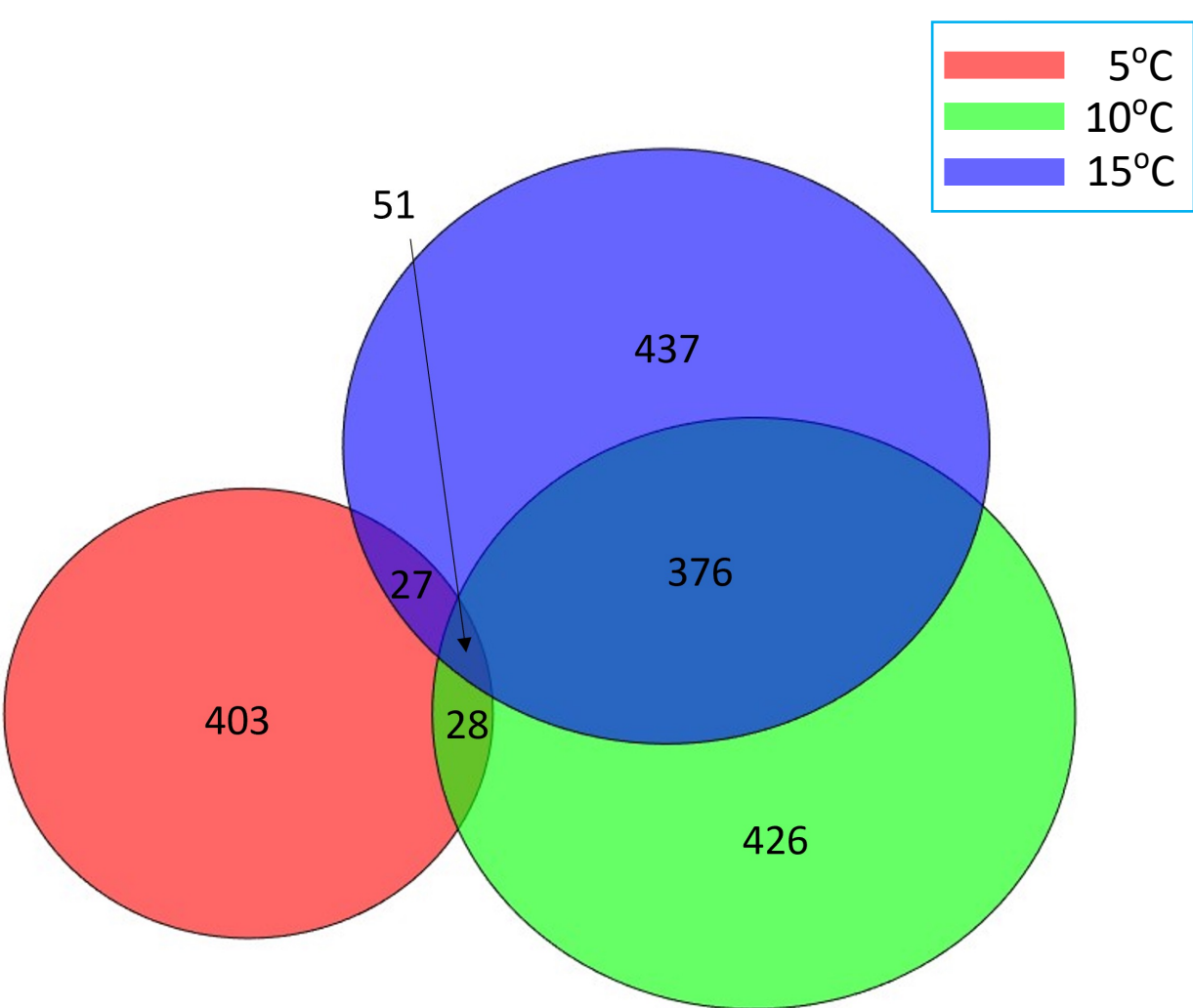
### VOC Pilot Evolution Experiment:



Please contact Haley Bennett for literature references  
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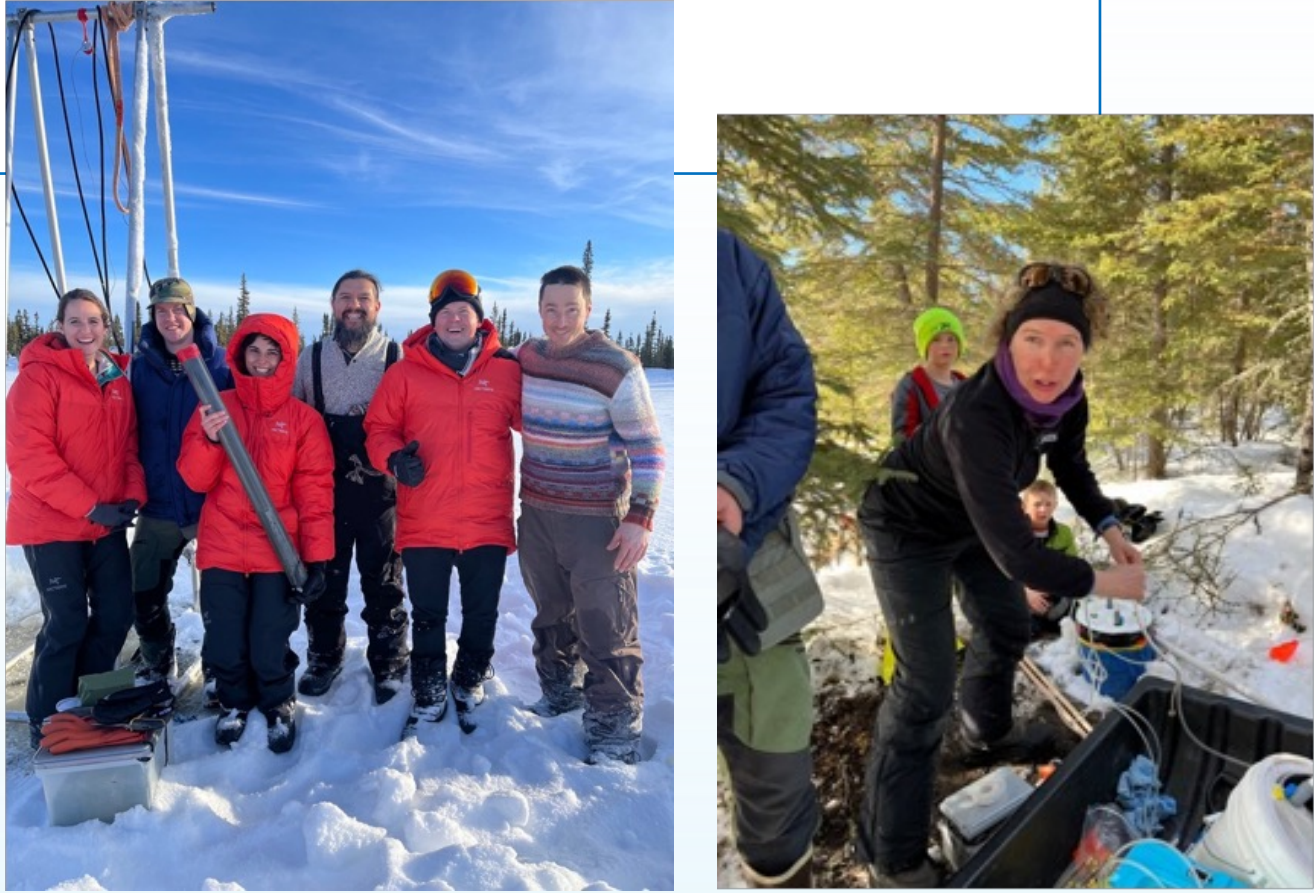
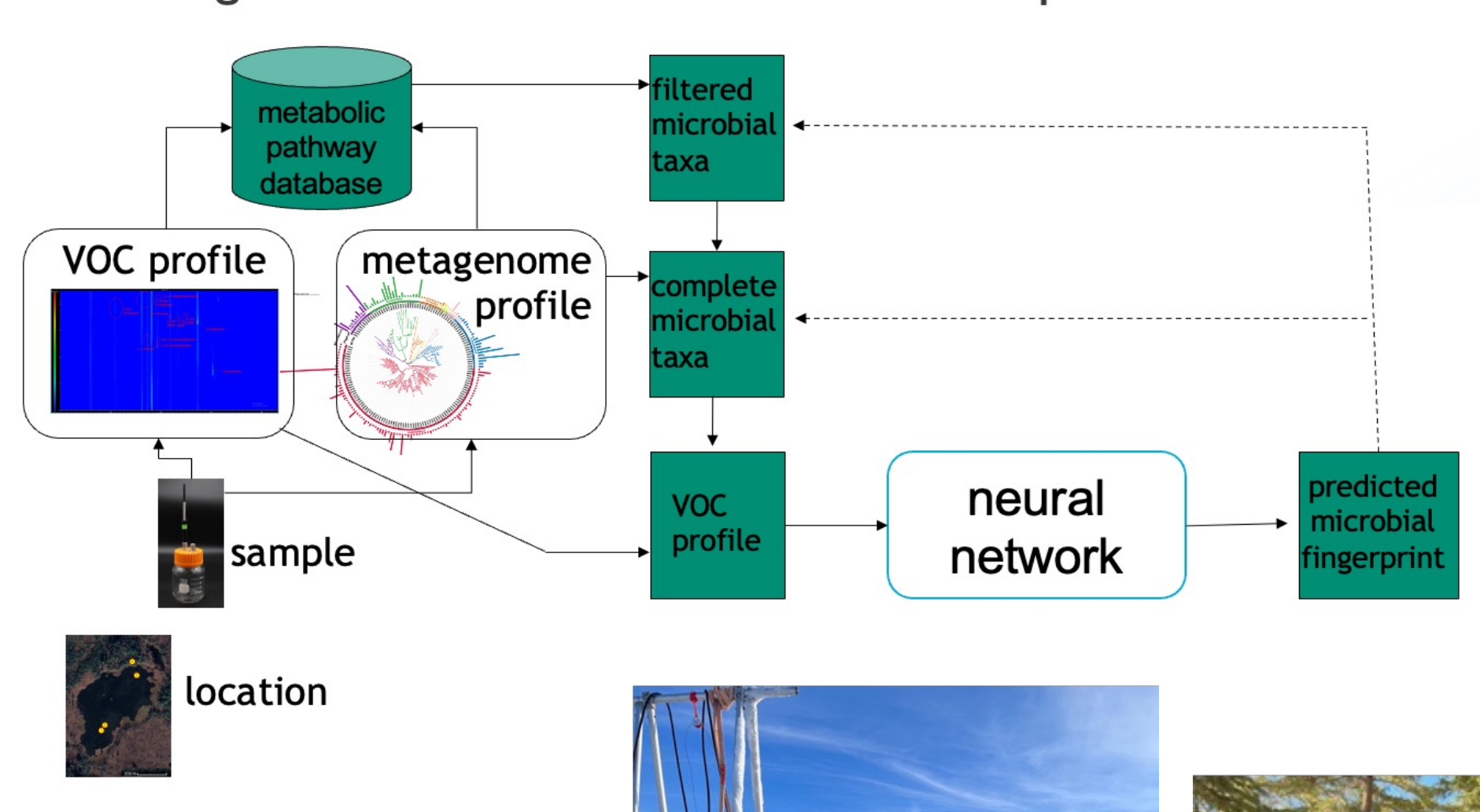
## Permafrost Volatolomics Library

### Comparison of Identified Evolution VOCs



- Successful elicitation of microbial activity at higher temperatures compared to the lower base temperature. Complete 16s rRNA sequencing (V1-V9) on Oxford Nanopore PromethION should reveal species specific microbes in permafrost
- HRMS libraries used for identification can be biased against biochemicals. Investigation could reveal previously unknown mVCs
- Employing machine learning tactics on the megavariate data collection to automate the linking of the mVCs to microbial sources. Builds volatolomic library
- Culturing/isolation of higher temperature microbes from PTPBR and study individual mVCs fingerprint to deconvolute the megavariate data and identify species-unique mVCs

### Predicting microbial communities from VOC profiles



A collaborative project with:



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