

Microorganisms as bio-filters to mitigate greenhouse gas emissions from high-altitude permafrost revealed by Nanopore-based metagenomics

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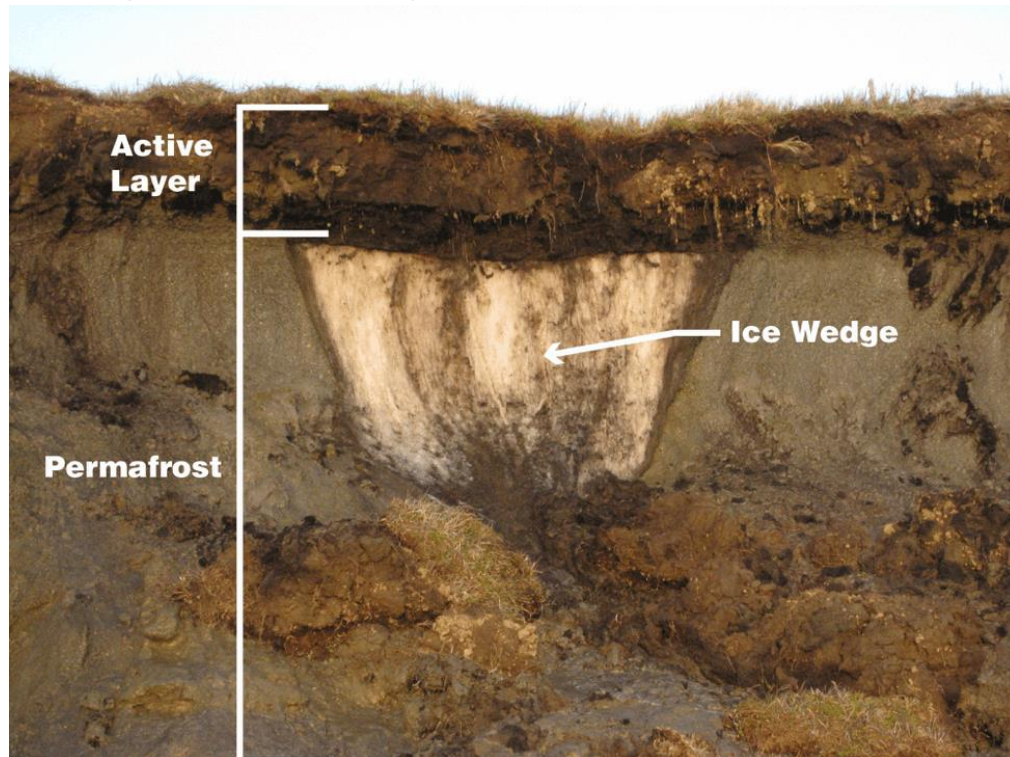


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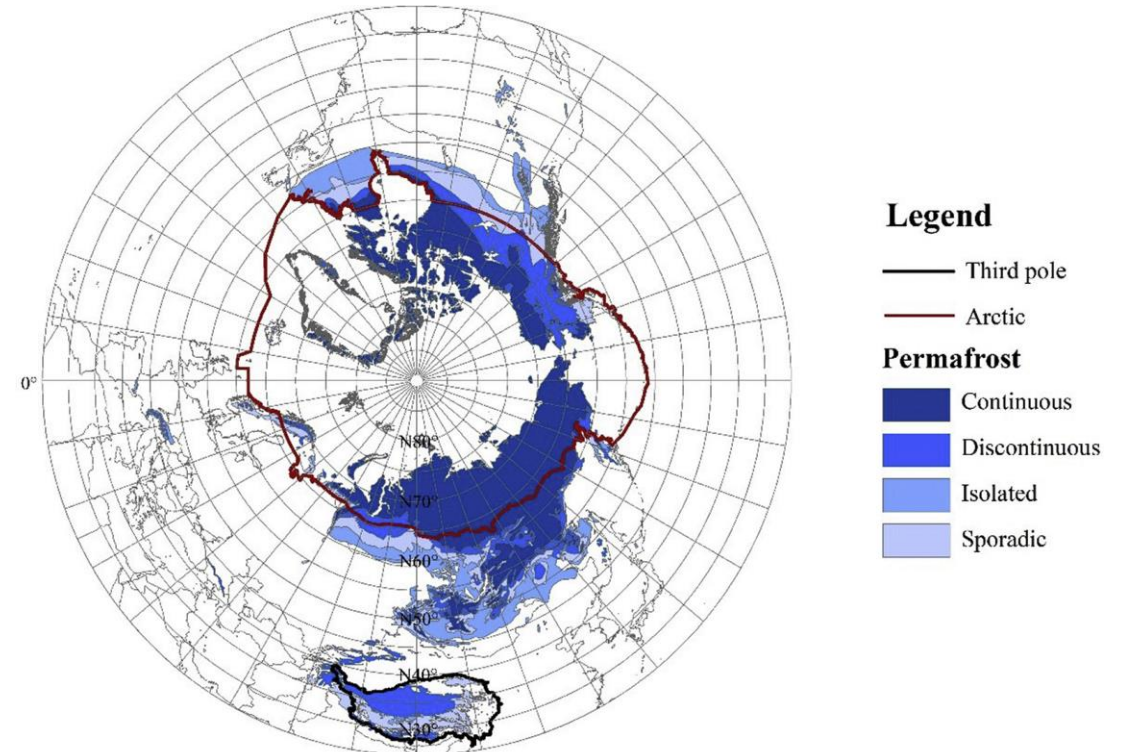
Introduction

- About 25% of the Earth's terrestrial surface is covered by permafrost. With thawing, it can be predicted that an estimated 174 Pg carbon will be accessible for microbial degradation by 2100.

- China has the third largest permafrost area in the world, just behind Russia and Canada. Specially, most permafrost in China is controlled by elevation and is called high-altitude permafrost.



<https://climatekids.nasa.gov/permafrost/>



Earth-Science Reviews: 2020, 211, 103433.

Goals

- However, the microbial responses associated with greenhouse gas production in thawing permafrost remains obscured.
- Here, we investigated the microbiome in the active layer of permafrost and compared microbial metabolic activities between thawed (sampling in August) and frozen (sampling in November) permafrost soils at the Qilian Mountain in the Qinghai-Tibet Plateau, using the Oxford Nanopore Technologies (ONT)-based metagenomic and Illumina-based RNA-seq:
 - (1) provide a new bioinformatic framework to analyze the functionality of the large unassembled proportion of soil metagenome with the aid of nanopore long read sequencing;
 - (2) reveal the shift of microbial activities involved in greenhouse gas emission between thawed and frozen state of high-altitude permafrost soil.

Field photos

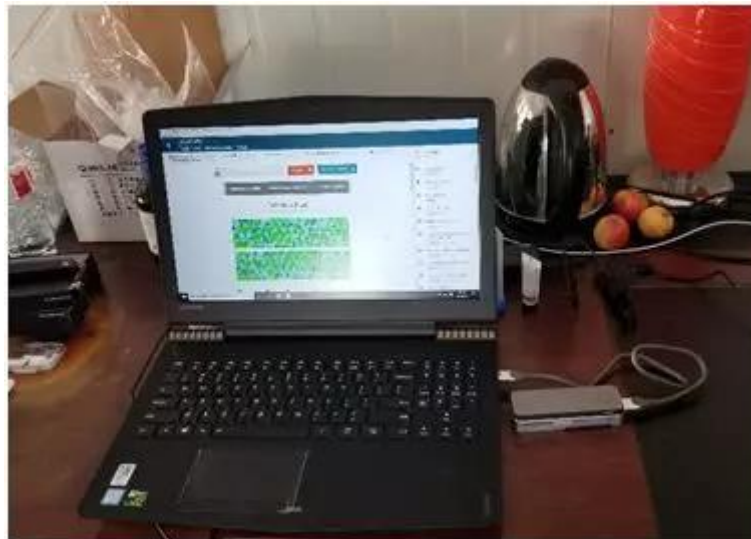
DNA extraction: DNeasy PowerSoil Kit

Purification: AMPure XP beads

Library: SQK-LSK108 1D ligation genomic DNA kit

Sequencing: R9.4 flow cells (FLO-MIN 106) on MinION

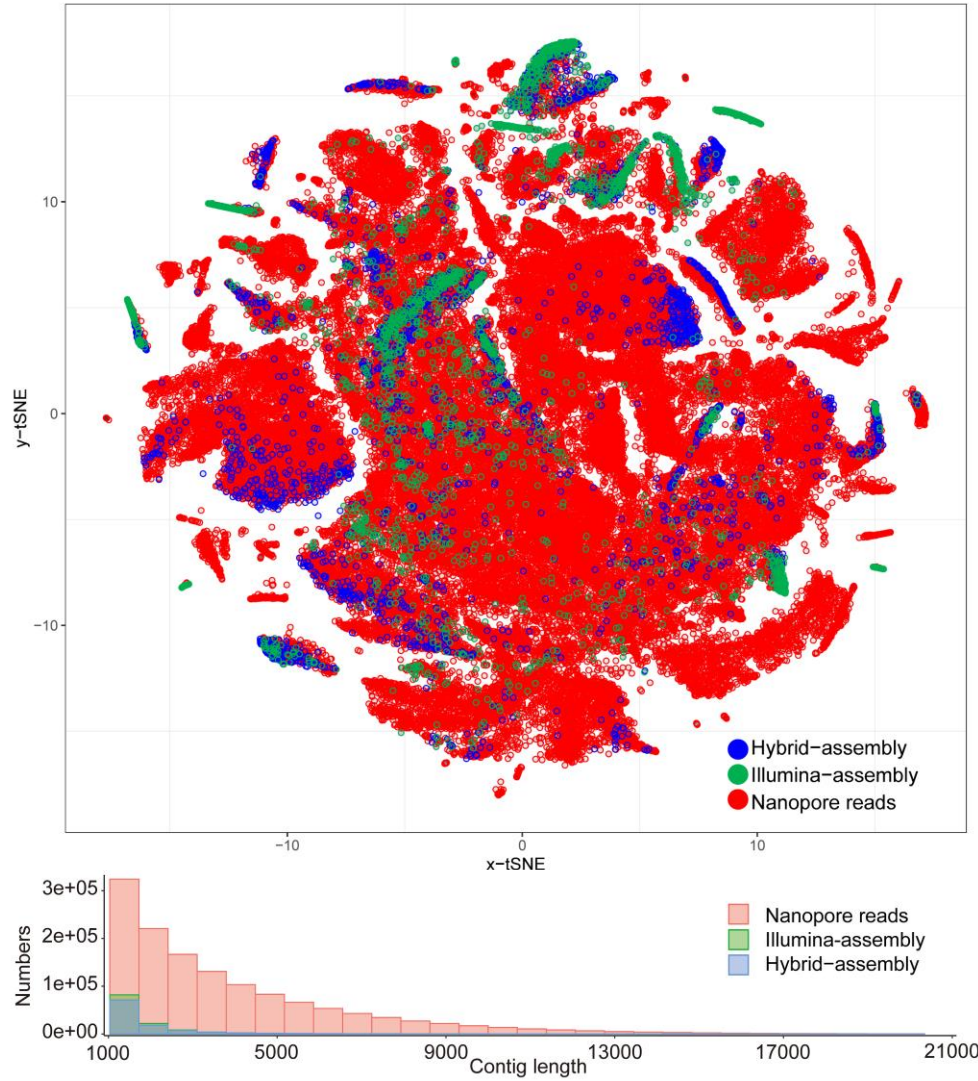
Data analysis: MinKNOW + Guppy basecaller + Porechop



DNA extraction, sequencing in field



Comparison of three methods

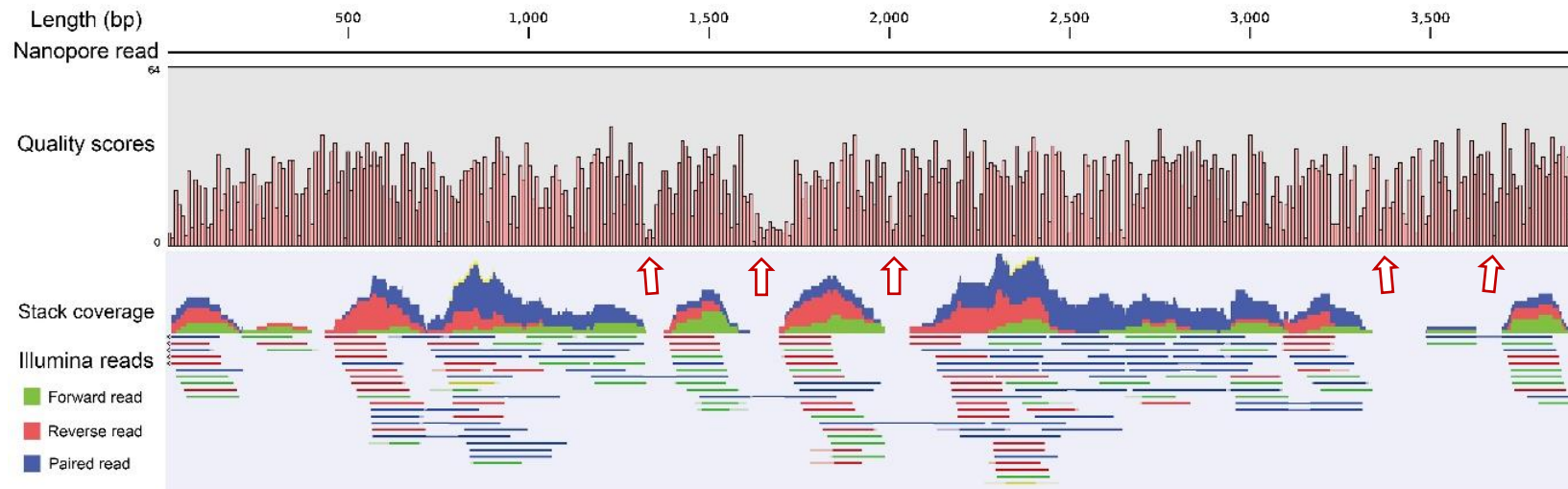


- Compared to Illumina-assembly, the hybrid-assembly of 5.2 Gbp nanopore dataset (3.8 kb) and 42 Gbp (150 bp) Illumina reads using popular hybrid assemblers of metaSPAdes and Opera-MS barely improved the contig continuity.
- 67.4 % of the nanopore dataset were unused in the hybrid assembly.

Nanopore reads correction

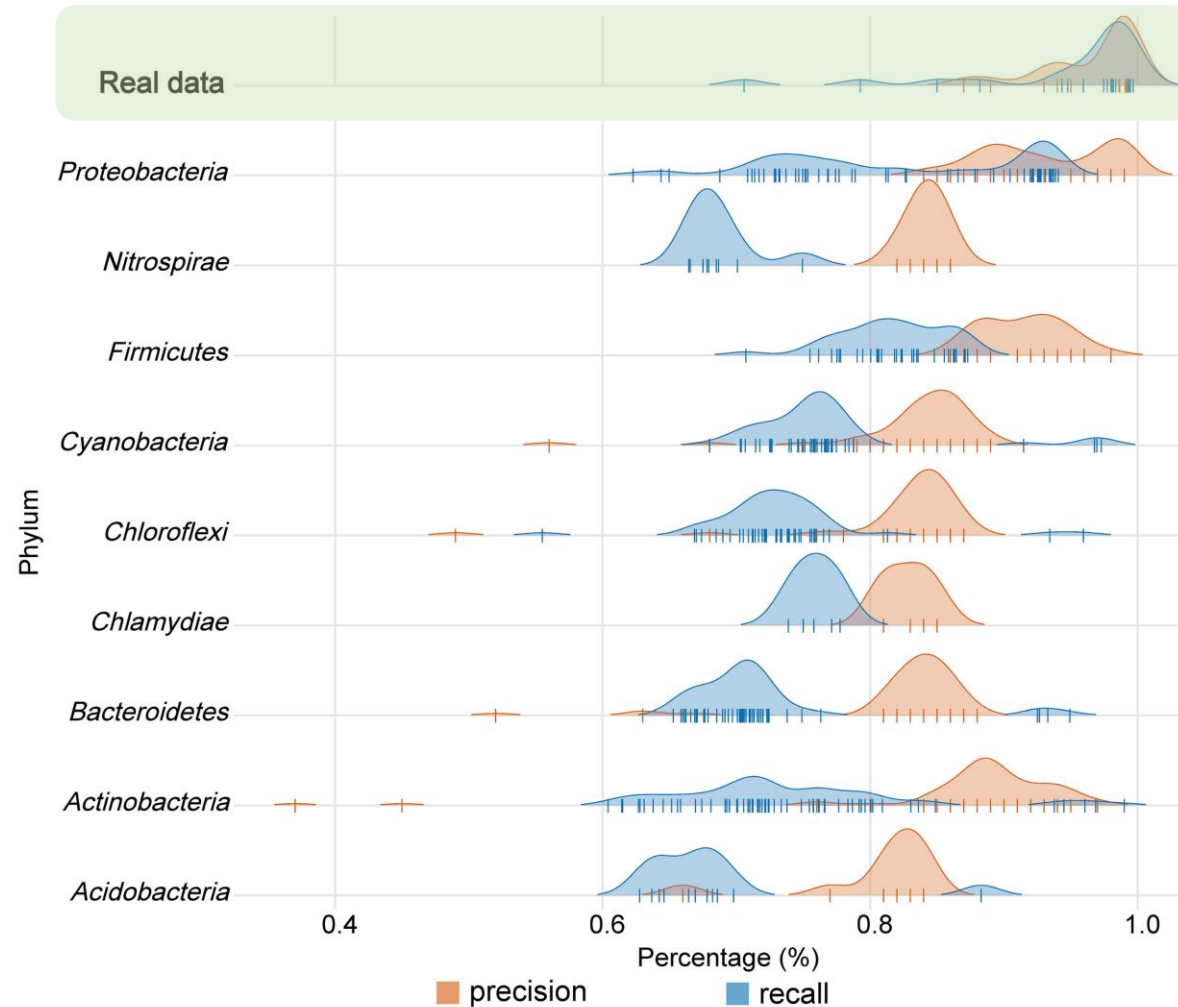
Table S5. The assembly information using different assembly strategies

	Input fasta data	Assembly data	Total reads number	N50	N90	Min read length	Max read length	Average read length
Raw nanopore reads	5.2	—	1375351	5146	1824	871	510592	3823.48
Polished nanopore reads by Pilon	5.1	—	1358631	5179	1851	871	510619	3860.4
CLC assembly (Illumina reads only)	42	306	148227	2043	1116	1000	482534	2064.93
Opera assembly (Illumina + raw nanopore reads)	42+5.2	317	134333	2566	1136	1000	588631	2362.81
Opera assembly (Illumina + polished nanopore reads)	42+5.1	316	135944	2494	1134	1000	588631	2327.25
CLC assembly (Illumina reads only)	10.5	127	163368	706	526	500	85864	—
metaSPAdes assembly (Illumina+polished nanopore reads)	10.5+5.1	152	173392	809	533	500	90061	—
Miniasm assembly (raw nanopore reads only)	5.2	5.3	308	20999	10354	273	79134	17942.31
Miniasm assembly (polished nanopore reads only)	5.1	3.3	196	20522	9940	839	84067	17389.42
Wtdbg2 assembly (raw nanopore reads only)	5.2	28	2533	12738	5642	4075	254519	11188.08
Wtdbg2 assembly (polished nanopore reads only)	5.1	20	1910	12392	5360	3417	101159	10729.9



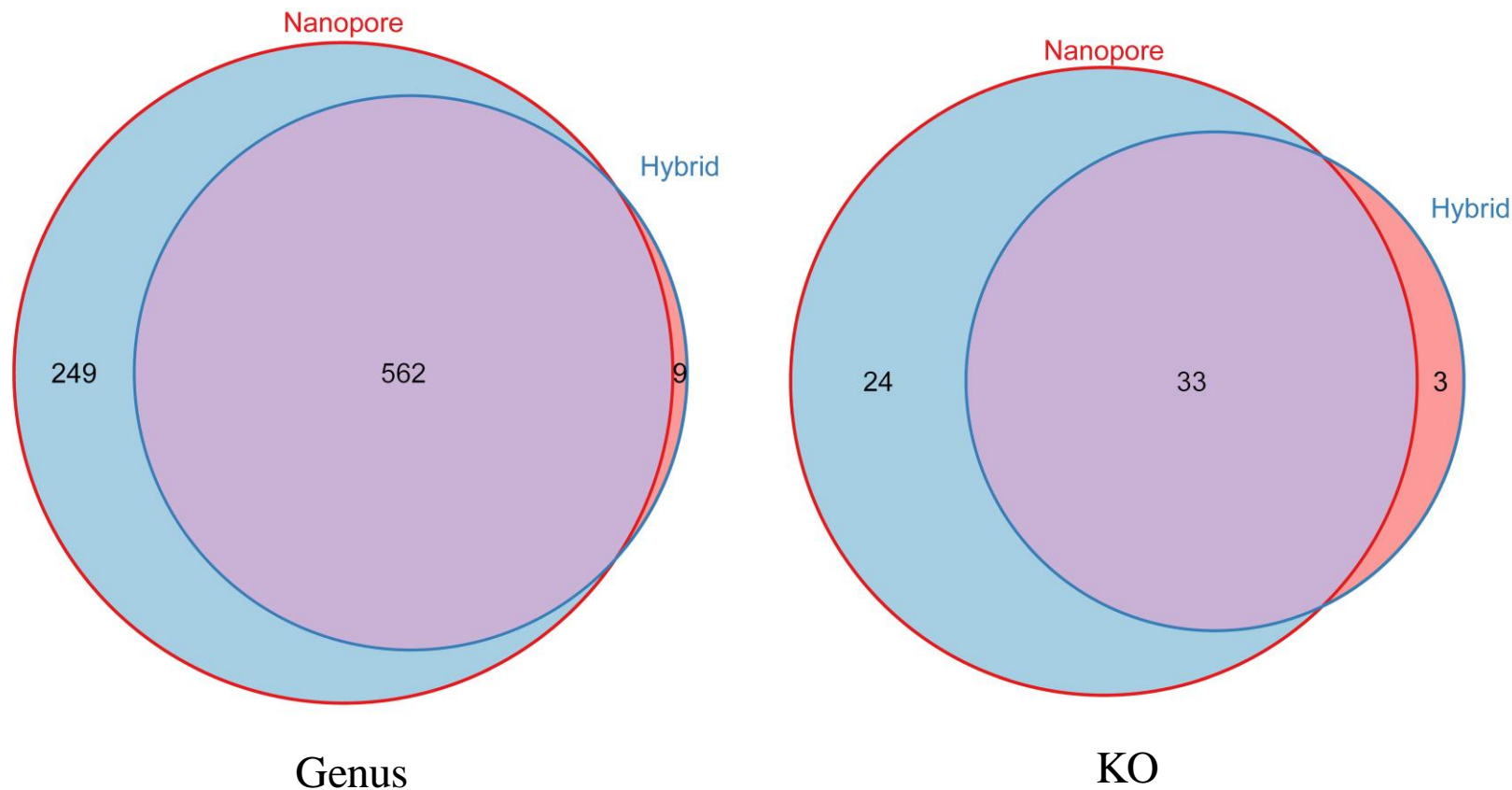
- These regions could not be mapped by any of the shotgun reads, leaving an assembly identically fragmented as that of assembling short-read datasets alone.

Self-made frame-shift correction evaluation



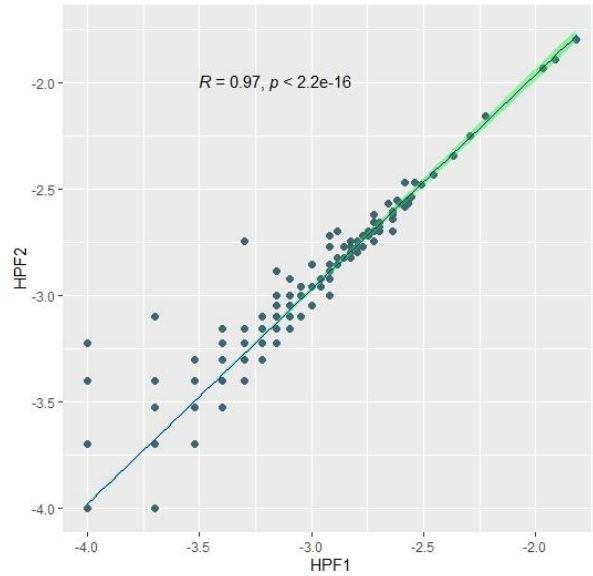
- The precision and recall of KO perform good in real sequencing data.
- The mock data show the performance difference in the different phylum.

FUNpore Advantage

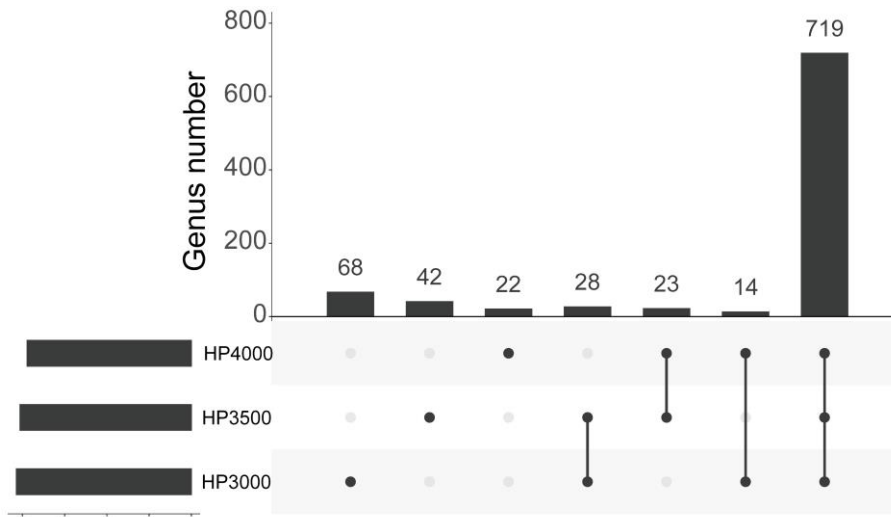


- Compared to hybrid-assembly, 42% more of the permafrost microbiome could be annotated at genus level. Also, the KO categories involved in nitrogen and methane metabolism (the main research object in this study) were enlarged by 58% with our strategy.

Microbial community in thawed permafrost



biological repetition results at genus level

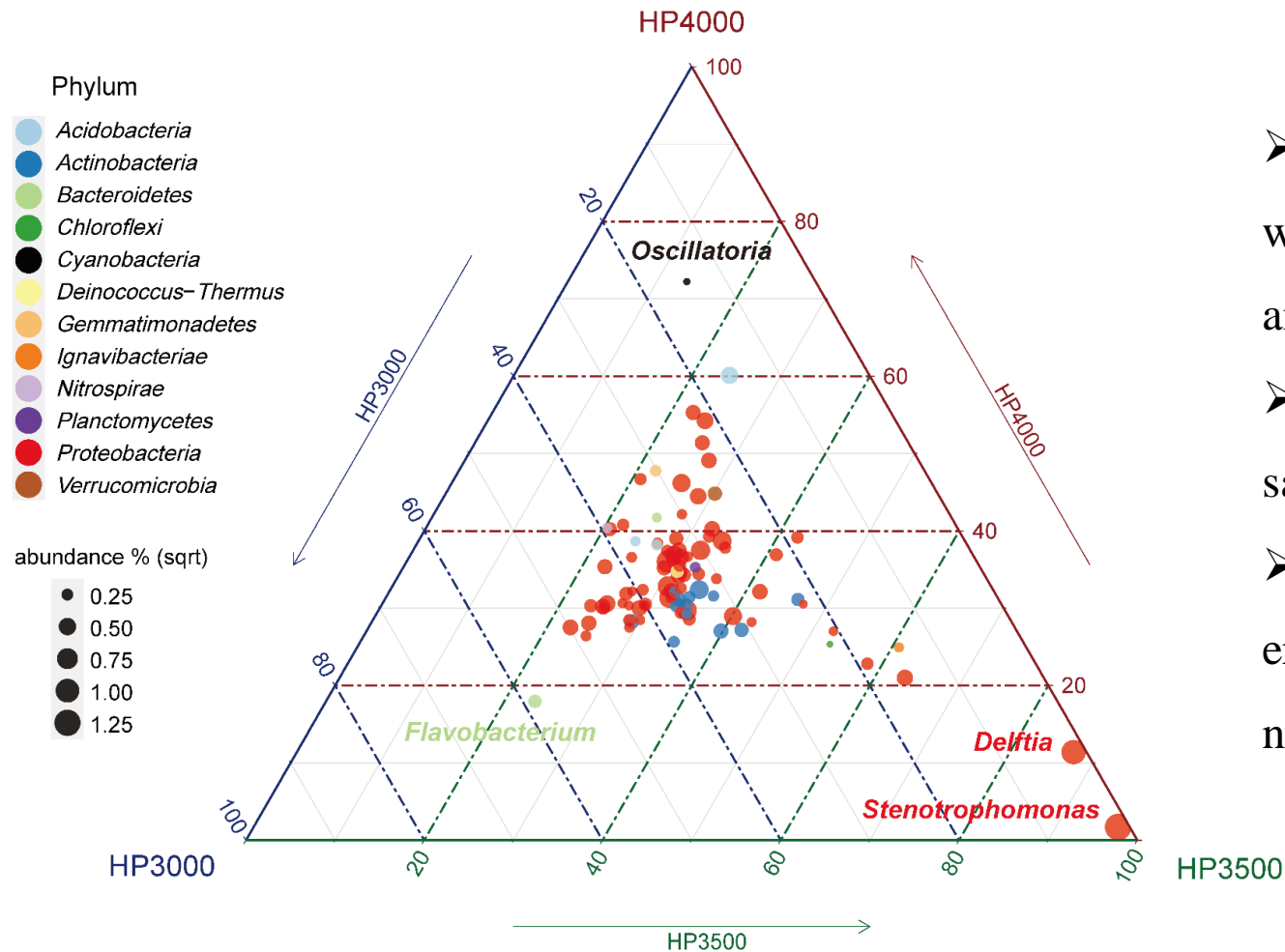


➤ Community composition of two biological reproducibility samples was compared at genus level.

➤ Results were reliable!

➤ 78.4% (719 out of 915) of the genera observed were shared in all permafrost samples at different altitudes, the abundance of shared genera accounted for more than 99% of the total annotated genera.

Microbial community in thawed permafrost



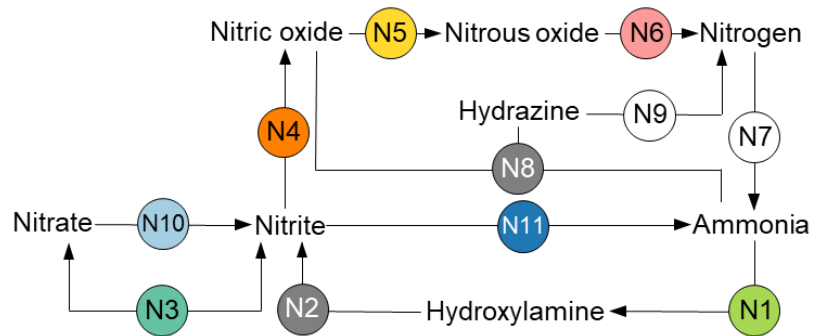
➤ 93.8% of these dominant genera were affiliated to Proteobacteria and Actinobacteria

➤ *Oscillatoria* enriched in HP4000 sample (strong solar radiation)

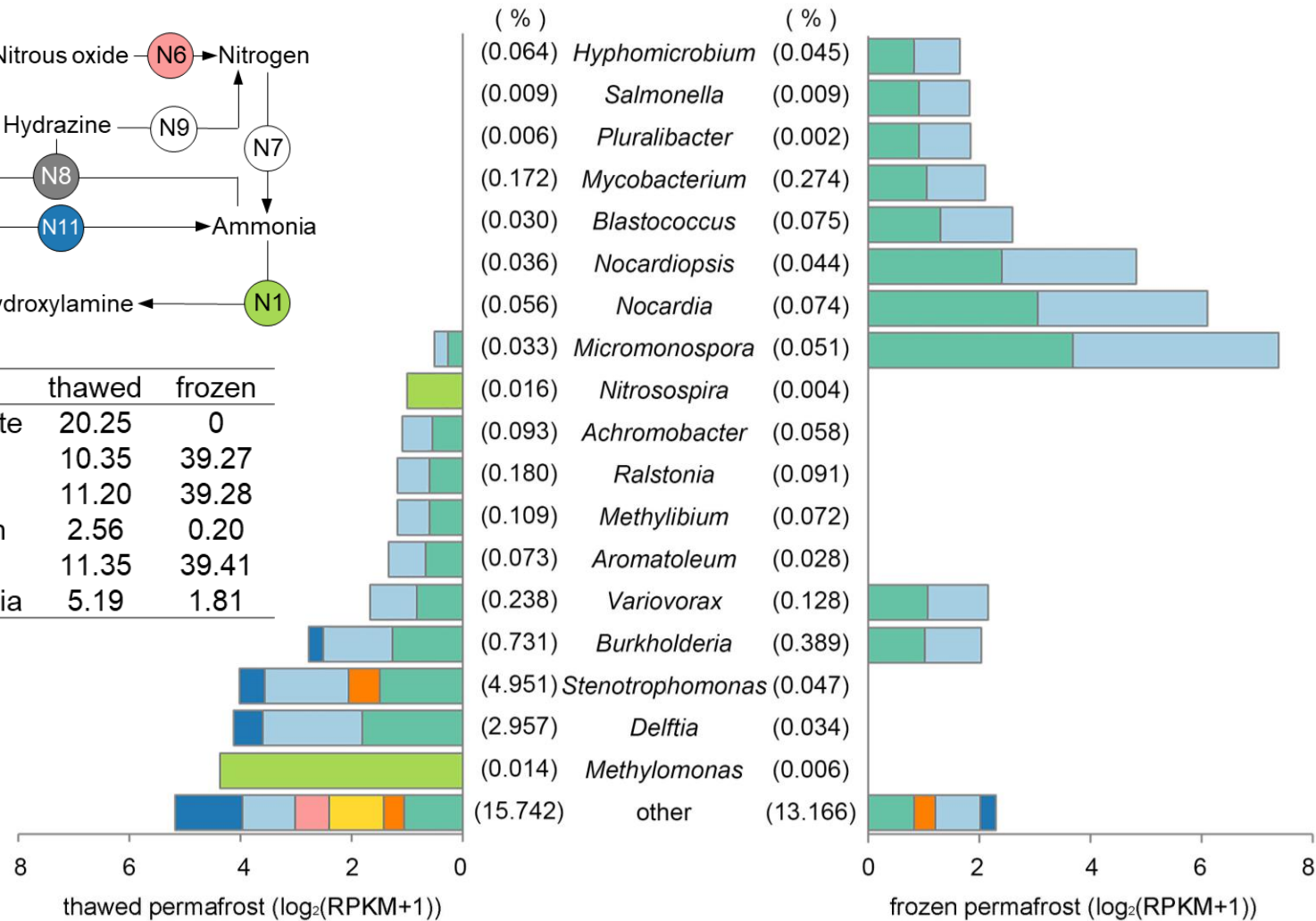
➤ *Stenotrophomonas* and *Delftia* enriched in HP3500 sample (high nitrate concentration)

➤ Although the well adapted core microbial community in permafrost were resistant to environmental changes, the strong light at the top and the high nitrate nitrogen content at the middle could lead to permafrost community differentiation.

Nitrogen metabolism



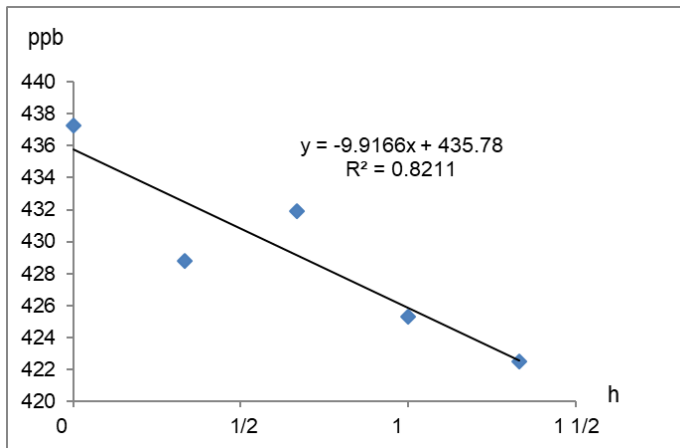
Pathways		thawed	frozen
Nitrification	ammonia > nitrite	20.25	0
	nitrite > nitrate	10.35	39.27
Denitrification	nitrate > nitrite	11.20	39.28
	nitrite > nitrogen	2.56	0.20
Nitrate reduction	nitrate > nitrite	11.35	39.41
	nitrite > ammonia	5.19	1.81



- The active microbial community involved in nitrogen metabolism evidently shifted from copiotrophic/heterotrophic microbes from *Proteobacteria* in summer, to oligotrophic/autotrophic microbes from *Actinobacteria* in winter.

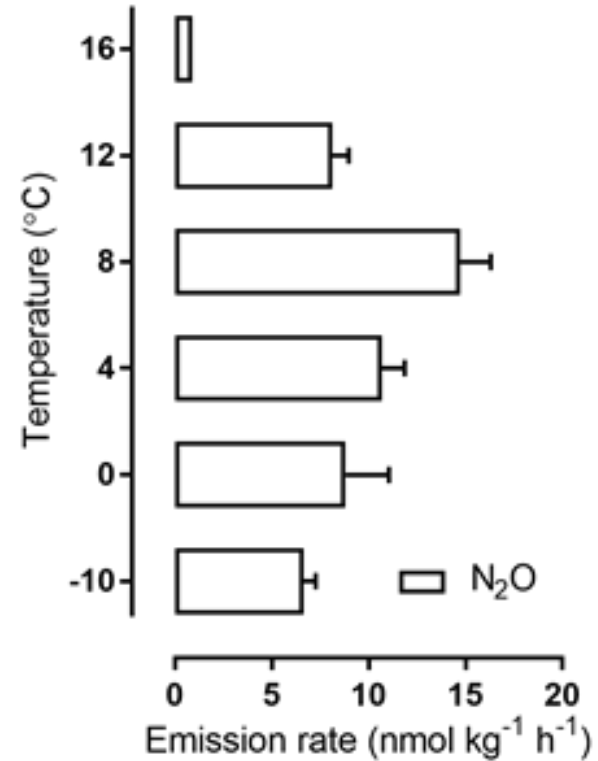
Nitrogen metabolism

Emission rate in the field



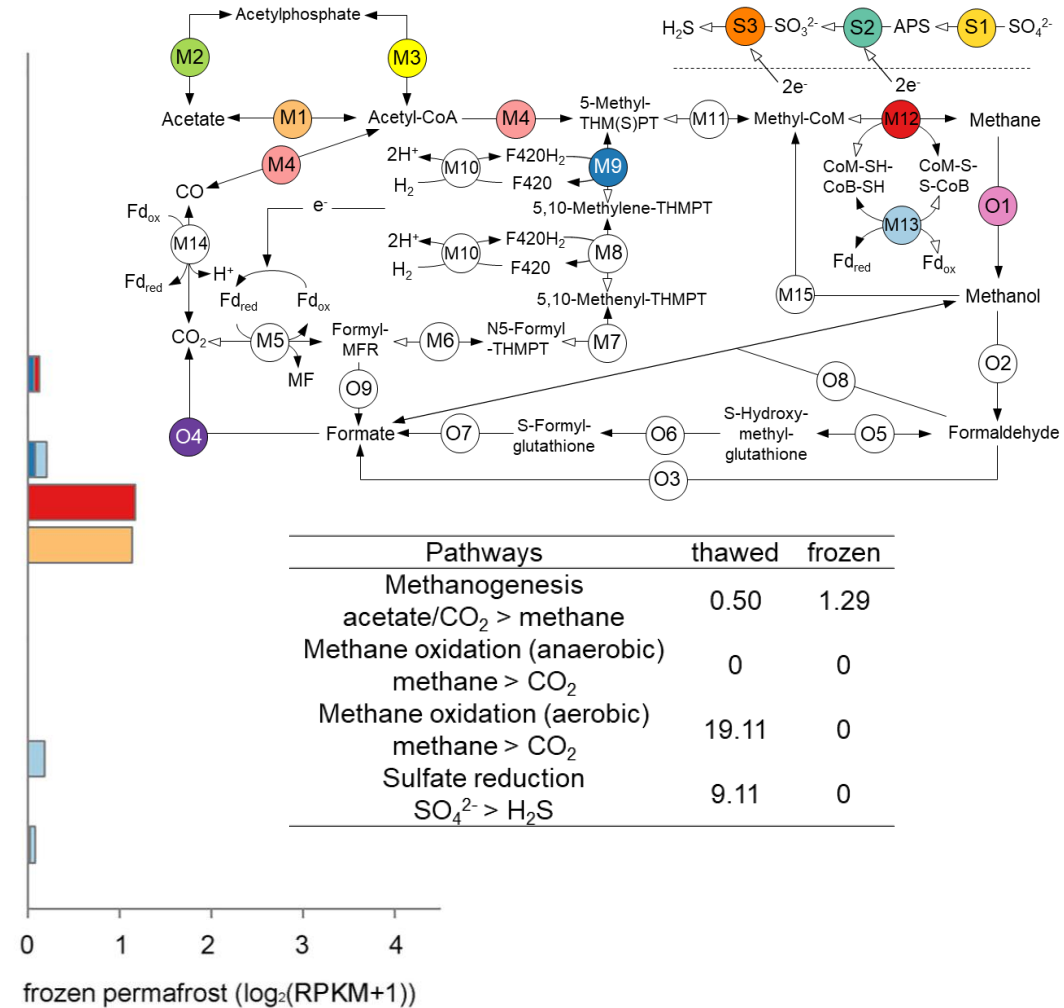
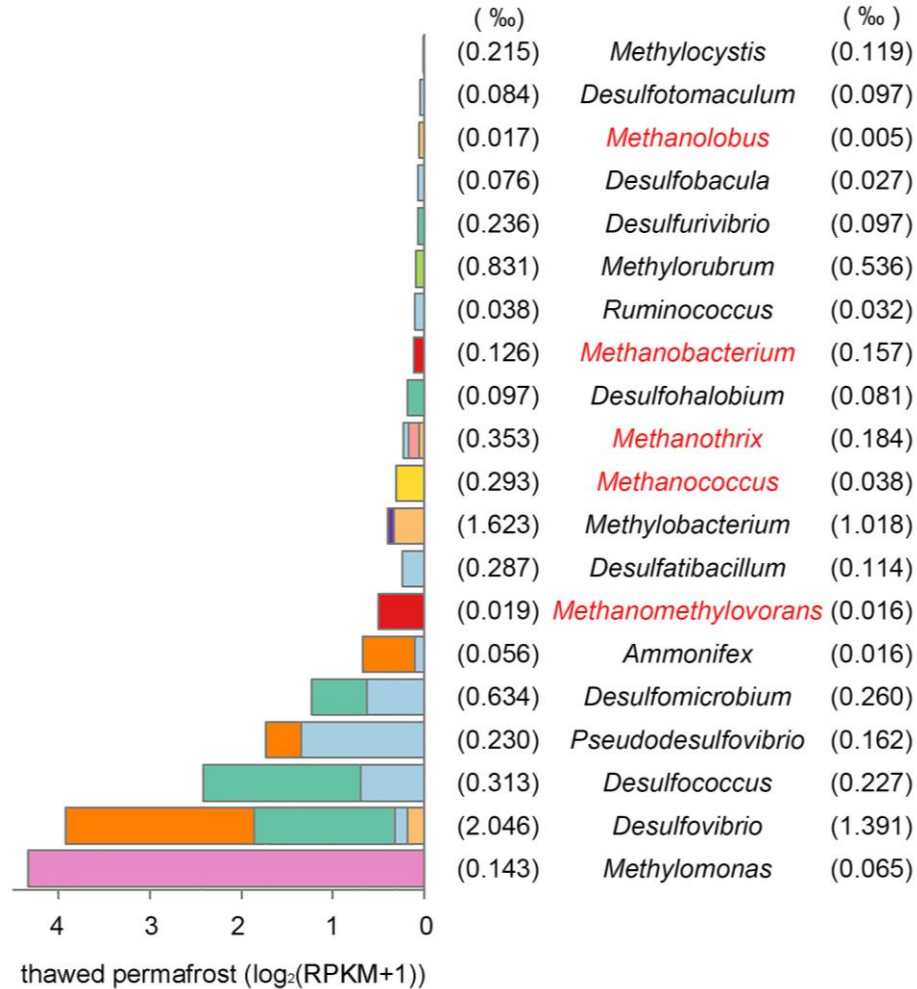
The gas phase measurement of the thawed permafrost in the field (about 15 °C) showed a slow consumption rate (18.8 nmol m⁻² h⁻¹) of N₂O.

Emission rate in the incubation experiment



Our soil incubation experiment further proved that the N₂O emission increased with thawing of permafrost soil from -10 °C to 8 °C, and N₂O emission decreased as the temperature continued to rise to above 10 °C (8 °C to 16 °C)

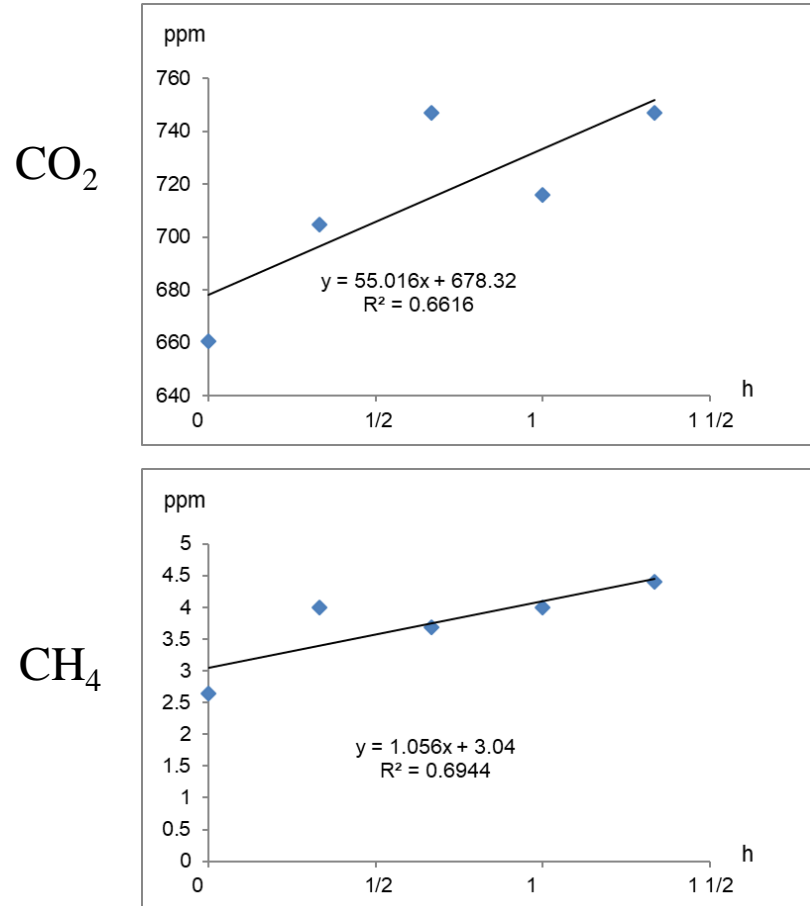
Active methane oxidation in thawed permafrost



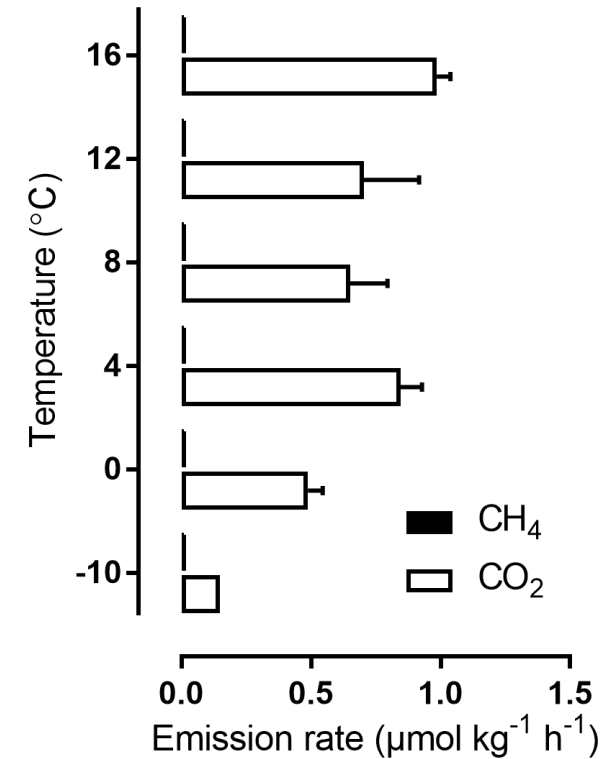
- The active methanogenesis switched from aceticlastic methanogens in summer to hydrogenotrophic methanogens in winter. Aerobic methane oxidation was the major methanotrophy process.

Active methane oxidation in thawed permafrost

Emission rate in the field



Emission rate in the incubation experiment



- This speculation was consistent with the low CH₄ emission rate of 2.0 μmol m⁻² h⁻¹ observed in the gas phase on-site, while the CO₂ emission rate was 50 times higher (104.5 μmol m⁻² h⁻¹).

Summary

- FUNpore can take advantage of ONT long-read data.
- The active nitrogen metabolism in permafrost trend to be a closed cycle.
Increased denitrification activities were observed in thawed permafrost.
- With thawing, the methanogenesis switched from H₂- to acetate-dependent.
- The vigorous aerobic methane oxidation by *Methylomonas* could greatly mitigate CH₄ emissions in thawed permafrost.
- The increasing thawed state will change the nitrogen and methane metabolism, and microorganisms could serve as bio-filters to relieve greenhouse gas emissions (N₂O → N₂, CH₄ → CO₂).



“iMeta” is an open-access Wiley partner journal and launched by scientists of the Chinese Academy of Sciences. iMeta aims to promote metagenomics, microbiome and bioinformatics development by publishing original researches, methods or protocols, and reviews. The goal is to publish highly quality papers (Top 10%, IF > 15) targeting broad audience. Unique features including video submission, reproducible analysis, figure polishing, APC waiver, and promotion by social media with 500,000 followers. The first issue will be released in March 2022.

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