Temperature- and time- dependent microbial community dynamics modulates methane cycle in permafrost-affected soils

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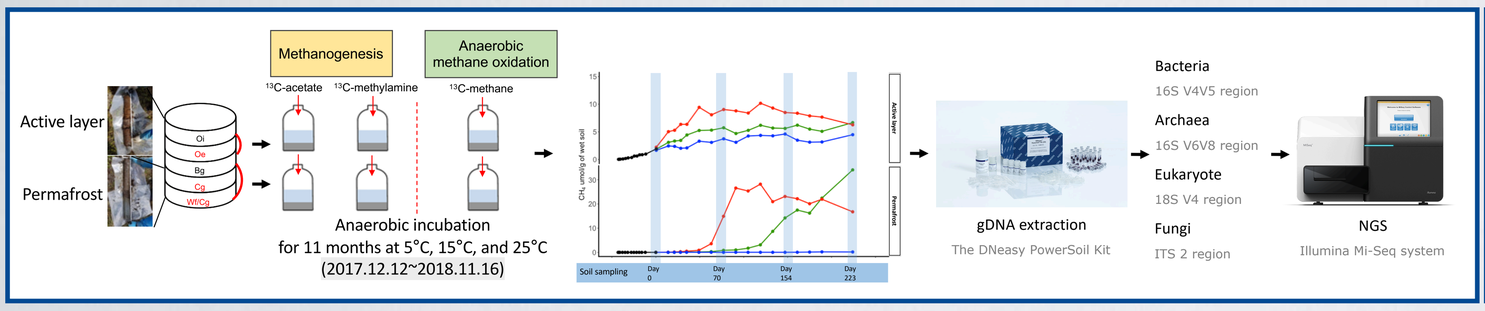
Introduction

Arctic permafrost soils store large amounts of SOC (soil organic carbon). Thawing permafrost promotes microbial degradation of SOC leading to the biogenic production of greenhouse gases (GHGs) as carbon dioxide and methane. However, the dynamics of microbial community, including bacteria, archaea, protists, and fungi, in methane production and oxidation during thawing remains poorly understood. This study aims to understand the temperature- and time dependent modulation of microbial production and oxidation of methane under anaerobic condition. To demonstrate the potential for methanogenesis and anaerobic oxidation of methane at permafrost, we anaerobically incubated Alaska Council soil cores with ^{13}C-labeled substrates. Furthermore, amplicon sequence data was analyzed by amplicon sequence variants (ASVs). ASVs improves the precision, comprehensiveness and reproducibility of marker-gene data analysis than read clustering based operational taxonomic units (OTUs) analysis.



Methods

Alaska tundra soil were anaerobically incubated at 5°C, 15°C and 25°C for 11 months with ^{13}C labeled substrates. Soil DNA was extracted and sequenced targeting bacterial, archaeal 16S, protistan 18S and fungal ITS2 region using MiSeq platform. 18S ASVs belonging to Metazoa, Streptophyta, and Plantae were removed for protistan and fungal community analysis. Gas concentration of CO_2 and CH_4 in headspace was measured to confirm microbial respiration and methanogenesis by gas chromatography systems.

Results

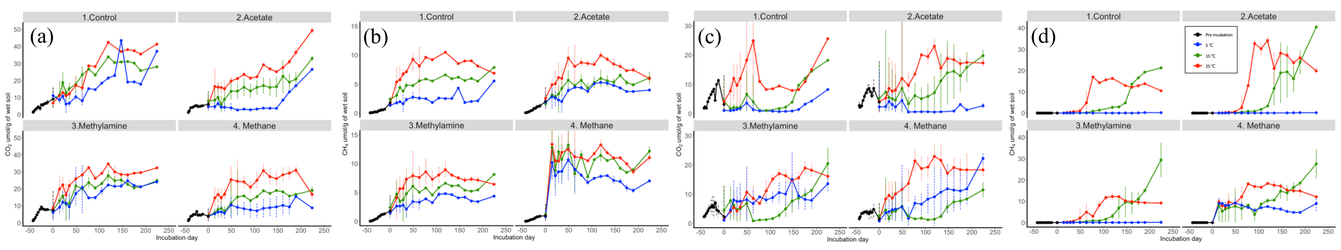


Fig 1. GHGs concentration of head space gas.
(a) CO_2 and (b) CH_4 concentration at active layer. (c) CO_2 and (b) CH_4 concentration at permafrost.

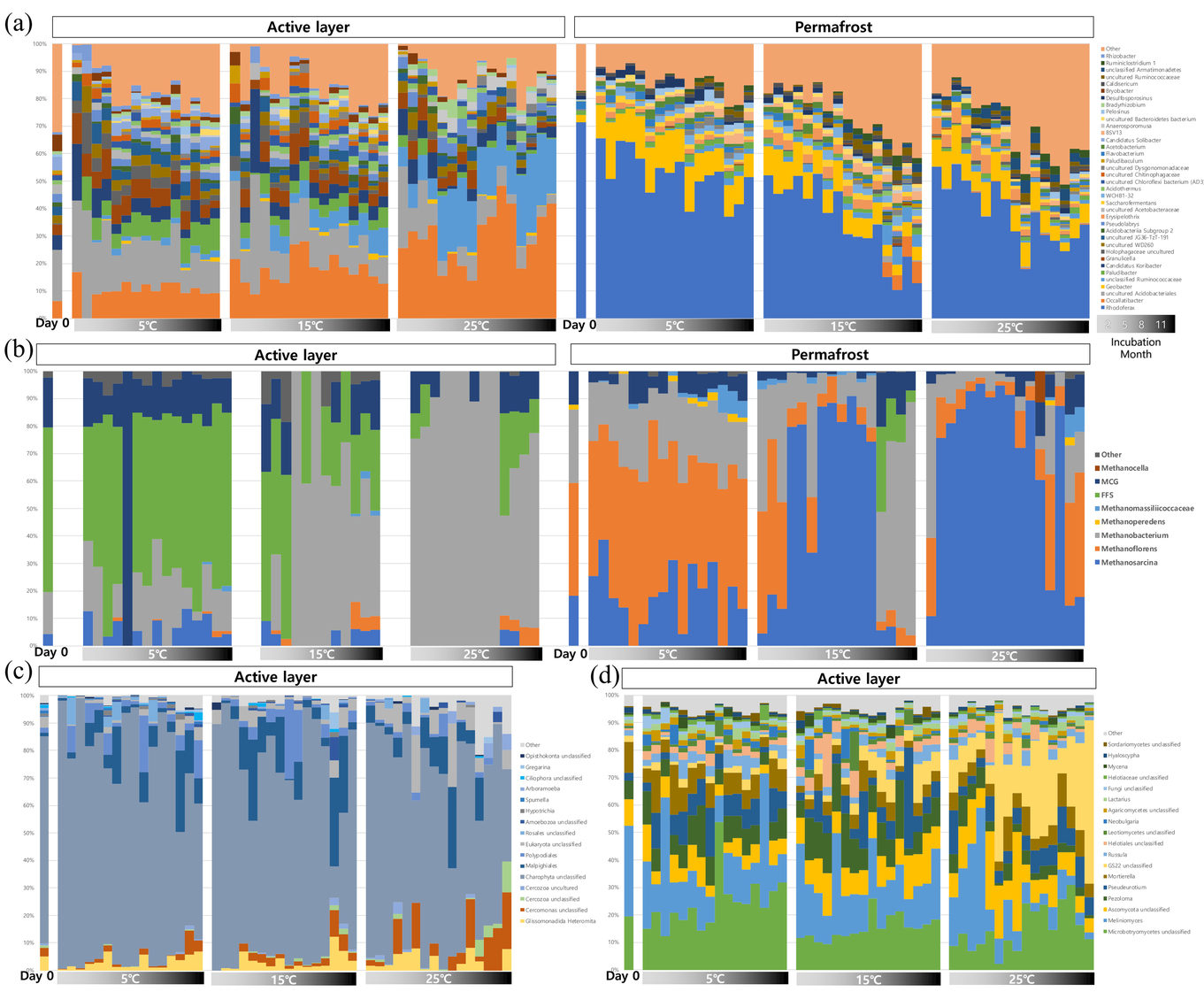


Fig 2. Relative abundance of bacteria, archaea, protists and fungi at genus level
(a) Bacteria: 16S V4-V5, (b) Archaea: 16S V6-V8, (c) protists: 18S V4, (d) fungi: ITS2 region. Amplicon sequencing for protists and fungi were successful on active layer solely.

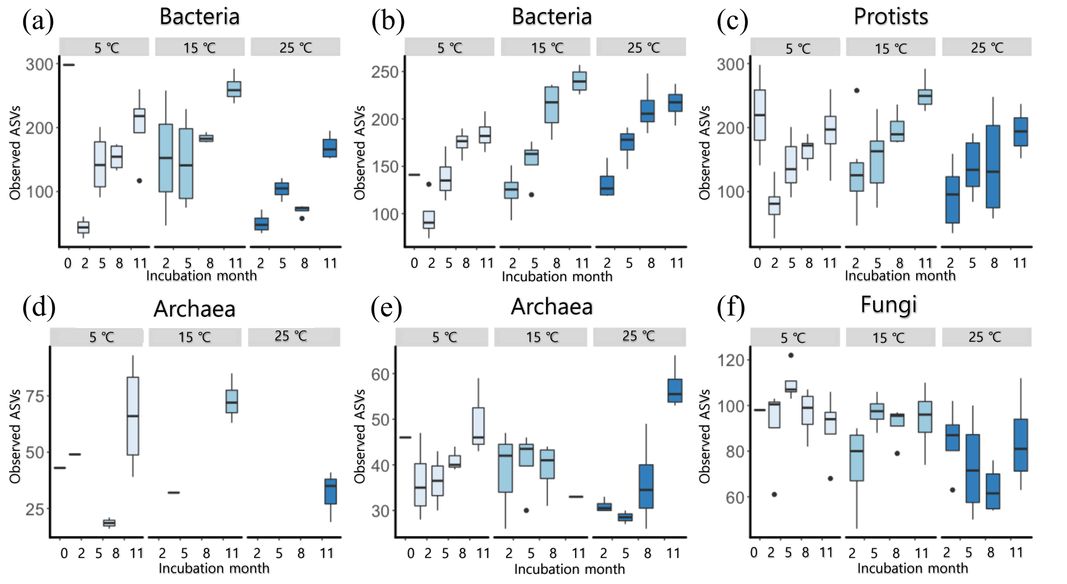


Fig 3. Richness of microbial communities depending on incubation temperature and time.
Differences in mean ASV richness across the time and temperature. Richness was calculated as the number of ASVs out of 1000 reads per sample. (a)-(c) and (f) were active layer richness. (d) and (e) were permafrost richness.

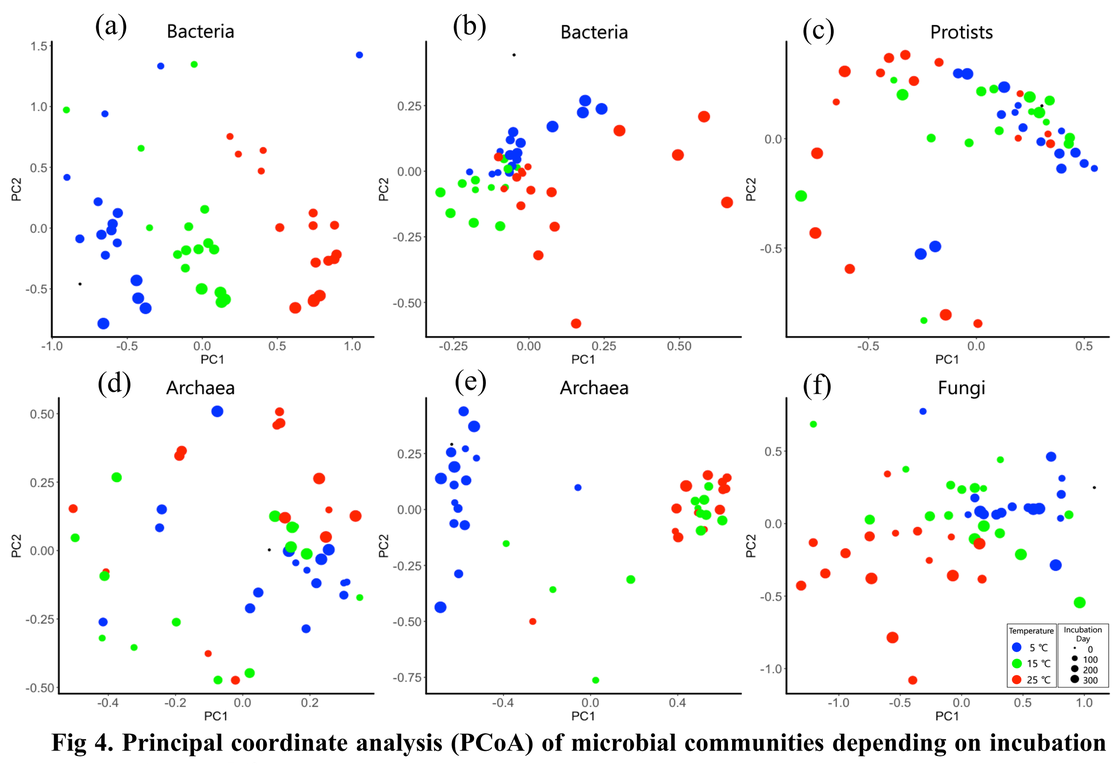


Fig 4. Principal coordinate analysis (PCoA) of microbial communities depending on incubation temperature and time
PCoA plot with Bray-Curtis dissimilarity calculated by ASVs. Colors and point sizes mean incubation temperature and time, respectively. (a)-(c) and (f) were active layer. (d) and (e) were permafrost.

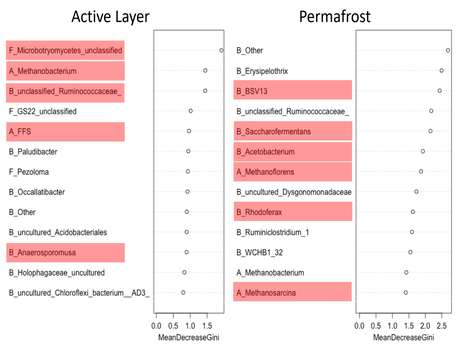
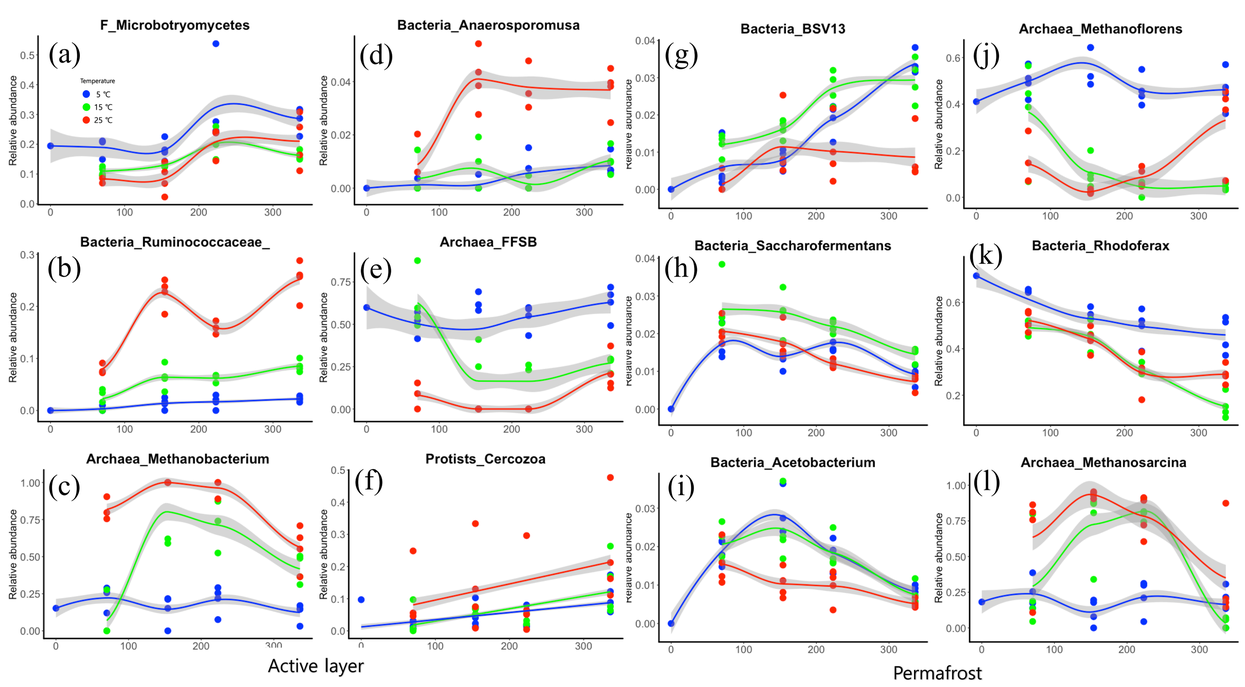


Fig 5. Microbial abundance sorted by MeanDecreaseGini top13

MeanDecreaseGini was calculated by the relative abundance of bacteria, archaea, protists and fungi together using RandomForest R packages. And active layer and permafrost were separately analyzed. (a) - (f) are active layer, (g) - (l) are permafrost.



Summary

- GHGs concentration

CO_2 and CH_4 concentration increased with time at all temperatures. CO_2 concentration patterns are similar at both active and permafrost layer. However, in permafrost CH_4 production increased abruptly at later stage of incubation. Finally, permafrost CH_4 concentration reached two times than that of active layer at all temperatures.

- Bacterial community

In active layer, there were clear shifts in major bacterial groups depending on temperature and incubation time. Anaerobic bacteria Ruminococcaceae and *Anaerosporomusa* were increased by temperature after incubation 150 days.

- Archaeal community

The relative abundance of the *Methanobacterium* increased with temperature at active layer. However, at permafrost, the relative abundance of the *'Ca* Methanoflorens*'* dominant at 5℃ and *Methanosarcina* dominant at over 15℃. Also *Methanoperedens* known as methane oxidation archaea were identified in permafrost samples incubated at 5℃.

- Protistan community

The relative abundance of Cercozoa increased at higher incubation temperatures of 15 ℃ and 25 ℃ at active layer.

Conclusion

Our study provides a first covered microbial community changes by long term anaerobic incubation on both active layer and permafrost soils from Alaska permafrost cores. Patterns of GHG concentration and microbial community differed by soil layer, temperature, and incubation time. There are distinct patterns in bacterial and archaeal community composition between active layer and permafrost. The relative abundance of '*Ca* Methanoflorens' maintained high at 5℃ but less abundant or almost absent at 15℃ and 25 ℃, suggesting that they have long adapted to the cold niches of the Arctic tundra environment. Further metagenome study using ^{13}C-labeled by DNA and RNA are ongoing to demonstrate temperature dependent micro-organisms have methane modules and activation. These results help to understand the direct effect of different temperature and substrates on metabolic interactions among anaerobic microbes in an Arctic tundra.

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