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TITLE: *Metagenomic Analysis of Alaskan Permafrost Microbial Communities*

Little information is known about the microbial communities and interactions present in permafrost (perennially frozen ground) soils. These microbial communities survive for centuries in extreme conditions such as sub-zero temperatures, drought, lack of oxygen and/or sunlight. In addition, global temperature rises are expected to significantly alter the community dynamics of permafrost soils. These soils contain vast amounts of the greenhouse gasses carbon and methane, so increased metabolic rates of microbial communities at higher temperatures could increase the atmospheric release of these gasses, thereby further accelerating global temperature rises. Detailed prediction of microbial community responses to anthropogenic change requires an in-depth understanding of community composition and interactions.

To this end, we extracted (mini-)metagenome-assembled genomes (MAGs) from a dataset of contiguous DNA sequences (contigs) assembled from a permafrost soil microbial community from West Dock, Prudhoe Bay oil field, Alaska (depth: 30cm, T=0.17ºC). Phylogenetically, the MAGs group into 8 bacterial phyla and 1 archaeal phylum. We then performed metabolic reconstruction on the resulting 116 MAGs, focussing on energy metabolism in general, and methane and carbon metabolism in particular.

We discovered 34 MAGs that contained modules related to methane metabolism, 13 to nitrogen metabolism, and 74 to carbon fixation. The key methanogenesis gene cluster methyl-coenzyme M reductase was observed in 1 of 116 MAGs - a Euryarchaeotum - indicating that methanogenesis is restricted to this phylum. The key carbon fixation gene cluster CO dehydrogenase/acetyl-CoA synthase was observed in 24 of 116 MAGs, indicating that this is a more common process in the permafrost sample. Most of these genes fall within the Bacteroidetes and Proteobacteria MAGs. Further in-depth study is required to gain full understanding of the West Dock microbial community interactions and metabolisms. This will allow us to make quantitative predictions concerning the community carbon and methane metabolism.

Metagenomic Analysis of Alaskan Permafrost Communities

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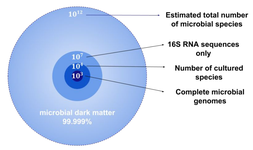
Background

Little information is currently known about the microbial communities and interactions present in **permafrost** (perennially frozen ground) soils. These microbial communities survive for centuries in extreme conditions such as:

- **sub-zero temperatures**
- **drought**
- **lack of oxygen and/or sunlight**



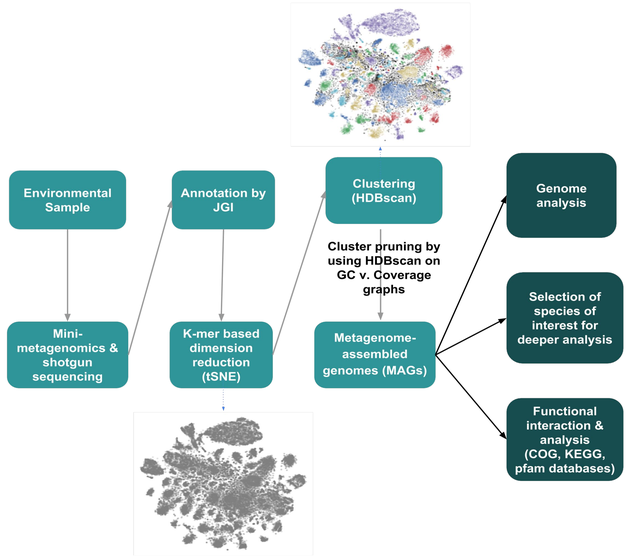
In addition, the rise of global temperatures is expected to significantly alter the community dynamics of permafrost soils. As these soils are known to contain vast amounts of the greenhouse gasses carbon and methane, increased metabolic rates of microbial communities at higher temperatures could increase the atmospheric release of these gasses, thereby further accelerating global temperature rises. **Detailed prediction of microbial community responses to anthropogenic change requires an in-depth understanding of community composition and interactions.**

Objectives

The primary **goals** of this study were to explore the types and functions of the microbes present in the permafrost from West Dock, Prudhoe Bay oil field, Alaska as a vehicle to:

1. **Investigate the boundaries of life**
2. **Discover new species**
3. **Find useful compounds**
4. **Understand how the carbon and methanogen pathways might be affected by rising temperatures**

Methods & Materials



Results

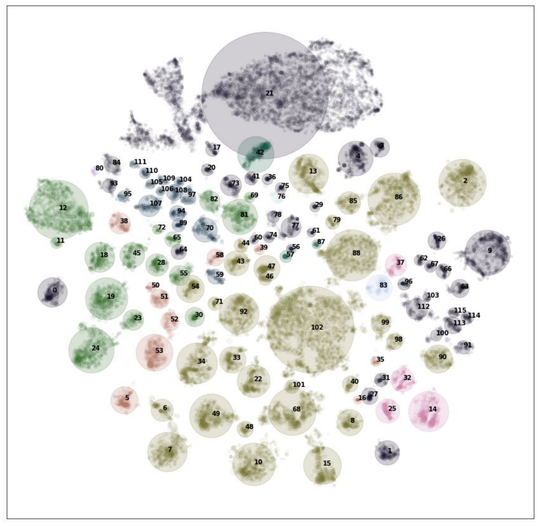


Figure 1. tSNE plot colored by majority phylum and numbered by genome; individual genomes enclosed in circle with radius proportional to the respective genome size (minimum genome size = 0.2mb, maximum = 60mb)

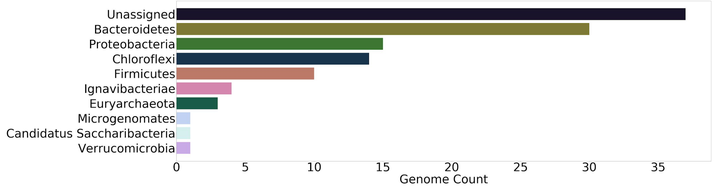


Figure 2 . (left) Microbial phylum arranged in order from most to least number of MAGs

Results (Cont.)

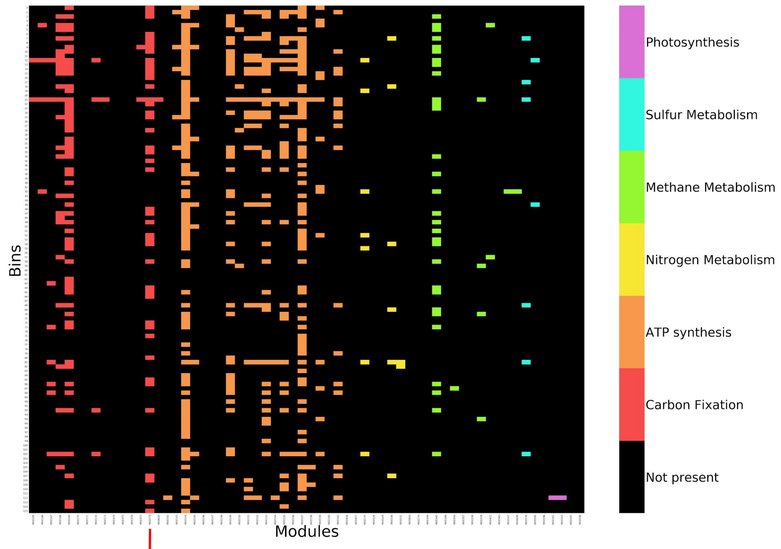


Figure 3. Presence map of KEGG modules involved in energy metabolism (grouped by modules used for photosynthesis, sulfur metabolism, methane metabolism, nitrogen metabolism, ATP synthesis, and carbon fixation) in genomes

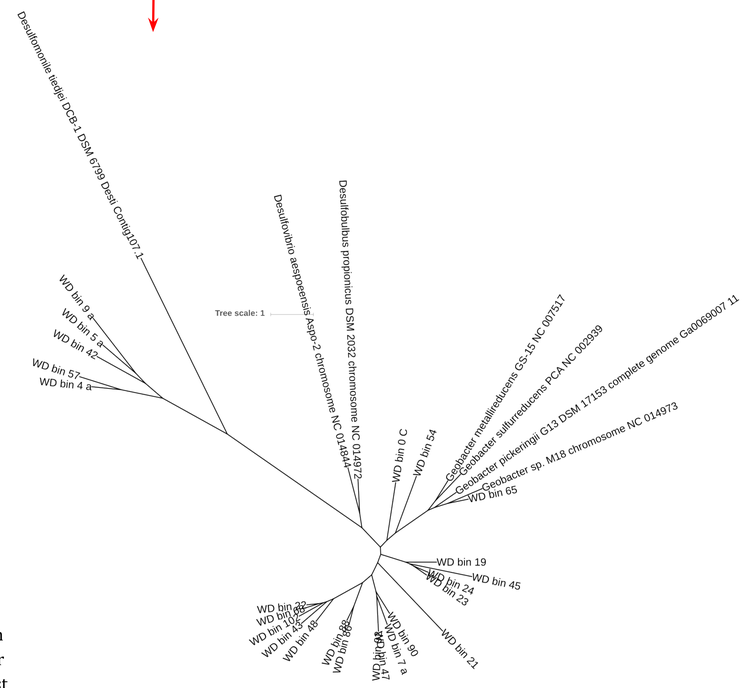


Figure 4. Phylogenetic tree of the CO dehydrogenase/acetyl-CoA synthase gamma subunit

Conclusion

At this point in the metagenomic data analysis, we observed:

1. **116 unique MAGs belonging to 8 bacterial and 1 archaeal phylum**
2. **Bacteroidetes was the most common genome phylum (37 bins)**
3. **Key methanogenesis gene cluster Methyl Coenzyme M Reductase in 1 of 116 MAGs**
4. **Key autotrophic carbon fixation gene cluster CO dehydrogenase/acetyl-CoA synthase in 24 of 116 MAGs**

To improve the analysis pipeline, we employed:

1. **A novel method of using HDB scan on GC vs coverage graphs to further prune the clusters in an unsupervised way**

Future Directions

Further steps with this data will include:

1. **Improving unsupervised cleaning of the MAGs (increasing completeness and decreasing contamination and strain heterogeneity)**
2. **Placing cleaned MAGs in a reference genome tree to determine exact phylogeny (noting metabolic comparison to neighboring genomes)**
3. **Building a conceptual model of Carbon, Nitrogen, and Methane cycling**

References

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2. Johnston, E. R. *et al.* Metagenomics reveals pervasive bacterial populations and reduced community diversity across the Alaska tundra ecosystem. *Frontiers in Microbiology* 7, (2016).

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Acknowledgments

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