58. Metaproteomics reveals key aspects of microbial community mediated carbon cycling in thawing Arctic Permafrost

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Project Goals:

1. Perform ~2 year thawing experiments on well-characterized, intact cores of the Arctic active- layer and permafrost from a proposed reference ecosystem site where CO_2 and CH_4 fluxes, temperatures, humidity, soil moisture, nutrients, microbial diversity and activities and isotopic analyses are currently being measured.
2. Characterize the vertical flux of volatile organic acids, O_2, H_2, CO_2 and CH_4 the isotopic systematics of CO_2 and CH_4 and changes in the transcriptomics, proteomics, and C cycle networks in these cores as the permafrost thaws under water saturated and water unsaturated conditions.
3. Compare the fluxes measured for the cores with those measured in the field.
4. Based upon these thawing experiments, construct a 1D biogeochemical reaction/transport model that predicts the CO_2 and CH_4 release into the atmosphere as permafrost thaws and compare these predictions with observations at the reference ecosystem site.

http://www.princeton.edu/southafrica/permafrost-project/

Microbial activity plays an important role in the fate of carbon compounds sequestered in permafrost ecosystems. Nonetheless, how the microorganisms influence carbon cycling is not clear due to the paucity of data on cellular activity of indigenous microbial communities in Arctic permafrost. Our work focuses on studying microbial activity in cryosol, (mineral cryosols above the permafrost table) obtained from Axel Heiberg Island (AHI), Canada. We sampled soil cores from different sites within AHI to explore the range of microbial activities in the soil. The microbes and their activities in various soil cores were examined in (1) unaltered state (control), (2) after thawing (4^{o}C and 10^{o}C and (3) after a combination of nutritional amended microcosms (glucose, acetate and lactate) incubated at temperatures above freezing. Microbial proteins were extracted using established protocols and identified with liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The resulting MS datasets were matched against a compiled protein database assembled from genome sequences of closely related bacteria identified in AHI samples using a 16S rRNA 454 pyrosequencing approach. Metaproteomics of 5g of unaltered cryosols yielded limited protein information, suggesting dormant cryosol microflora and/or low biomass. In contrast, 5g of thawed (4°C or 10^{o}C, 1- 3 months) cryosol layers and/or nutritionally amended microcosms yielded increased protein identifications (~350 proteins), including DNA polymerase, energy metabolism proteins (phosophoribulokinase, malate dehydrogenase etc.), GroEl, DnaK, F0F1 ATP synthase, acetoacetyl CoA reductase, acetoacetyl CoA transferase. In particular, proteome data was obtained for species of *Bradyrhizobium* and known methanotrophs *Methylosinus* and *Methylocystis*, the latter of which are known to express high affinity methane monooxygenase capable of oxidizing atmospheric methane.

The cryosols were characterized with respect to bulk soil organic carbon, as analyzed by C K-edge x-ray absorption near edge structure, and extractable soil organic carbon (OC), as analyzed by ^{1}H nuclear magnetic spectroscopy and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Results indicated that both the unaltered and thawed soils were highly aliphatic in composition. Although the soils are composed of 1-5% total OC, the amount of extractable soil OC was low (< 10% of the total organic carbon) and the composition did not significantly vary after a year of thawing. The limited variability and low amounts of extractable OC in AHI cryosols suggests limited bioavailability of this OC pool.

To ascertain if increasing amounts of readily bioavailable carbon can promote higher microbial growth in cryosol, another round of experiments was set up (1 gm soil in 10 ml culture media) and incubated at $10^{o}C$ for 3 months with different carbon sources [Glucose, Tryptic Soy Broth (TSB), Potato dextrose broth (PDB) and diluted R2A]. Increases in microbial biomass, as evidenced by increases in the amount of total extracted protein, was noted in all cultures except the relatively low nutrient 1/10 R2A supplemented cultures. MS analysis of the enrichment cultures yielded identification of ~ 1500 proteins spanning across multiple functional categories, indicating the presence of robust, actively dividing cells with varied metabolic pathways.

Metagenomic sequencing using a 2 x 100 bp paired-end Illumina protocol was performed on all enrichment cultures to measure shifts in the microbial community profile due to differences in carbon substrate types. The metagenomic and metaproteomics analyses indicated that *Bacilli* (phylum Firmicutes) was highly enriched in the PDB, glucose and TSB enrichment cultures, *Arthrobacter* (phylum Actinobacteria) was highly enriched in PDB, 1/10 R2A and glucose enrichment cultures, and *Clostridium* (phylum Firmicutes) was highly enriched in PDB, glucose and TSB enrichment cultures. Other genera enriched only in one type of media were *Pseudomonas* in PDB and *Bacteroidetes* class in 1/10 R2A. All media enrichments showed a relative decline in Alpha-Proteobacteria, including the order rhizobiales which is more dominant in the native upper active layer of the cryosol. The deeper cryosol layers revealed an abundance of spore formers belonging to order *Clostridiales*

In conclusion, AHI cryosols represent a unique permafrost system, which is low in utilizable organic carbon leading to a predominantly dormant microbial consortium in cryosol layers. However, upper active layers have a high abundance of Alpha-Proteobacteria (rhizobiales), some of which are capable of utilizing CO_2 and methane. With the gradual warming of cryosols coupled with an increase in greenhouse gases, the upper layer microbial consortia will likely cycle C1 carbon and thus provide utilizable carbon source to the deeper cryosol layers, reviving the dormant microbial community.

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