BIOCHEMICAL CHARACTIZATIONS OF ARCTIC PERMAFROST ALONG AN AGE GRADIENT. S. A. Giatpaiboon[1], R. Mackelprang[2], and R. Mogul[1], [1]California Polytechnic University, Pomona, 3801 W. Temple Ave, Pomona, CA 91768 (rmogul@cpp.edu), [2]California State University, Northridge, 18111 Nordoff St, Northridge, CA 91330.

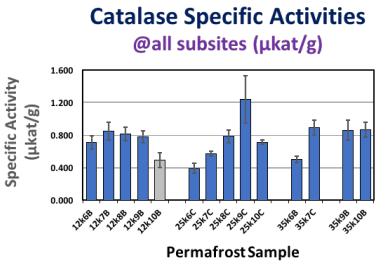
Introduction: Studies on the microbiology of Arctic permafrost clearly show that microbial life thrives at anaerobic subzero temperatures. As such, permafrost environments serve as excellent terrestrial analogs for the Mars subsurface, which contains a globally distributed permafrost, and experiences prolonged subzero temperatures. In this poster presentation, we will present biochemical analyses of Alaskan permafrost collected along a geochronosequence gradient of 12-35 kyr. Given the significane of omics-based analyses, our ultimate aim is to provide biochemical contexts (*i.e.*, enzyme specific activities, metabolites abundances, and respiration rates) to the growing understanding of permafrost metagenomics.

Methods: Catalase specific activities were measured using a custom-built volume displacement apparatus using 2 g permafrost samples in 50 mM HEPES (pH 7.5) and 300 mM H_2O_2. Total and free ATP abundances were measured by luminescence using an ATP Water Test device and SystemSURE Plus by Hygeina. Colorimetric cellular respiration assays were performed using absorbance spectroscopy (465 nm) by following the reduction of 5 mM XTT in 50 mM HEPES (pH 7.5) at 4˚C using 0.02 g sample.

Results:
Catalase specific activities, as measured at ~22˚C and calculated per gram of permafrost (n=6), were quite similar across the transect, with relative values of 0.82 ± 0.03, 0.69 ± 0.11, and 0.87 ± 0.02 µkat/g at 12, 25, and 35 kyr, respectively. In contrast, ATP abundances, as measured by luminescence (n=2), decreased as age of the permafrost increased, where both the total and intracellular ATP were highest in the 12 kyr samples and significantly lower in the 25 and 35 kyr samples (12 kyr > 25 kyr = 35 kyr). Preliminary cell respiration analysis of 25k samples show reproducible reduction of XTT and support the presence of metabolically active permafrost (at 4˚C).

Conclusions:
While preliminary, our catalase results show similar activities across the transect; while metagenomic data on identical samples also show no discernable changes in catalase gene abundances across the transect. The role of temperature in these assays along with comparison to other genes involved in oxidative stress will be dicussed. Our measurements of decreased ATP abundances across the transect were suggestive of lower microbial abundances; these results were directly supported supported by observed decreases in operational taxonomic units across the gradient. Future experiments will include community metabolomics analyses and the kinetic measurement of additional enzymes identified through molecular genetics as being invovlved in permafrost metabolism over time.

(A)



(B)

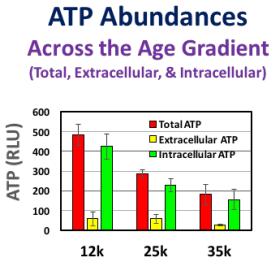


Figure 1. (A) Catalase specific activities (µkat/g permafrost) and (B) total, extracellular, and intracellular ATP abundances (relative light units) across the age gradient.

References: [1] Mackelprang, R., *et al*. (2011) *Nature, 480,* 368-371.