Identifying Changes in the Active, Dead, and Dormant Microbial Community Structures across a Chronosequence of Ancient Alaskan Permafrost. A. Burkert[1], T. Mahendrarajah[2], R. Mackelprang[2], [1] CSU Northridge 18111 Nordhoff St. Northridge, CA 91330, burkertalex@gmail.com, [2]CSU Northridge

Microbial communities within permafrost (perennially frozen ground) survive and reproduce for millennia despite extreme conditions such as water stress, subzero temperatures, high salinity, and low nutrient availability. However, we do not fully understand how these organisms are able to survive. Previous studies have used metagenomic and 16S rRNA gene sequencing to characterize community structure and functional potential in attempts to understand how these organisms adapt to the challenges associated with long-term survival in a permanently frozen environment. However, freezing temperatures may preserve DNA from dead organisms for extended periods of time. Because metagenomic and 16S rRNA gene sequencing do not distinguish between live, dead, and dormant cells, it is difficult to determine which organisms are viable. This study focuses on developing strategies to differentiate the live, dead, and dormant populations of low biomass permafrost microbial communities. Fluorescene microscopy coupled with Live/Dead staining revealed that live cells exist in ancient permafrost and allowed us to observe how the number of cells and the live:dead ratio changes in increasingly ancient permafrost. Preliminary data suggests that live and dead cells can be separated via Live/Dead staining coupled with fluorescence activated cell sorting. Further, we were able to enrich for endospores using treatments that lyse vegetative cells and we were able to inhibit PCR amplification of DNA from dead cells. These findings suggest that the combined protocols can be used to isolate the live, dead, and dormant microbial communities from low biomass environmental samples. Ongoing work focuses on applying these methods to a chronosequence (19 kyr, 27 kyr, and 33 kyr) of Pleistocene permafrost located near Fairbanks, Alaska and sequencing the 16S rRNA gene of the live, dead, and dormant cell populations to observe changes over geologic time.