Summer Undergraduate Research Fellowship (SURF) USA

Permafrost Microbial Community Characterization along Depth and Salinity Gradients

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Research Location: James Hall Rooms 123, 178, and G40E – UNH Durham

***The following example SURF proposal has been adapted from a longer original proposal written by Torin Scalora/Environmental Science – Ecosystems (Faculty Mentor: Jessica Ernakovich). The example has been modified and edited to fit the current, more concise SURF proposal guidelines.**

Proposal prompts in blue are included for instructional purposes only, based on the SURF proposal guidelines. Applicants should not include these prompts when submitting a final application.

Project Summary (one page maximum, single-spaced)

1. Abstract (200 words maximum): concisely summarize your project and its goals

Permafrost, which is soil that is perennially frozen year-round, is an essential component of the global carbon cycle as it contains a carbon stock equal to the size of the atmospheric carbon stock. Microorganisms in permafrost are also active players in the carbon cycle, as they are capable of both carbon fixation and respiration, making them an integral part of the carbon cycle. Microbial communities inhabiting permafrost are still being investigated, and with the Arctic warming up to four times faster than the rest of the planet, it is crucial to study the microbial diversity currently found within permafrost to understand microbial carbon dynamics better. Two environmental factors worth considering are salinity and depth, as both dictate the presence and abundance of microorganisms in soils; however, there have only been a few studies investigating these parameters in permafrost. I will study the impact of permafrost depth and salinity on microbial communities by analyzing three depths and salinity levels in three permafrost cores from Alaska's North Slope. I will extract DNA from permafrost samples to prepare them for PCR amplification and genomic sequencing. By conducting this research, we will gain insight into the ecology of an ecosystem that is rapidly transforming.

2. Outcomes (list 2-5, one bullet point each):

- Analysis of three depths and salinity levels in three permafrost cores from Alaska to contribute to our understanding of global warming
- Data reports for DNA from permafrost samples, PCR amplification, and genomic sequencing that will build a strong foundation for my plans to pursue a graduate degree in this field
- Paper and poster for the Undergraduate Research Conference (URC) and, with additional funding, national conferences such as the American Geophysical Union (AGU)

3. Timetable (one line or row per week maximum):

Activity	Week									
	1	2	3	4	5	6	7	8	9	10
	Date									
	5/20	5/27	6/3	6/10	6/17	6/24	7/1	7/8	7/15	7/22
Physicochemical Analysis										
DNA Extraction/PCR										
Submission for Sequencing										
Bioinformatics Pipeline & R Practice										
Bioinformatic Processing, Data Analysis, Statistics										
Troubleshooting/Flex Time										

Project Background **(two pages maximum, single-spaced)**

1. **Project History and Significance** **(one paragraph maximum, 1-2 sentences each):**
   - general problem, theme, or issue to be addressed
   - most relevant previous research, scholarship, or artistry on this topic
   - project's specific question, hypothesis, or objective
   - wider implications of your project to the problem and field

Carbon sequestration is an urgent topic in climate change, where in removing atmospheric carbon (C), we may mitigate the impacts of a warming Earth. The Arctic is one of Earth's largest C sinks, containing a third of the world's soil C, in part because of the low activity of microorganisms in cold arctic soils (Schuur et al., 2022). However, as the planet warms and microbial life respires more of the ancient C found in permafrost, we risk higher concentrations of atmospheric C (Conrad R, 1996). By characterizing the microbes that have the potential to release C from this C sink, we will better understand how the planet cycles C and be able to make more accurate climate predictions for a rapidly changing and globally important Arctic. While studies have looked at the microbial communities in the active layer, the seasonally thawed layer of ground overlaying permafrost (Harris et al., 1988), and near-surface permafrost, they often fail to characterize microbial communities in permafrost deeper than a few meters (Tripathi et al., 2018; Song et al., 2023). There is also minimal research examining saline permafrost and the differences between communities occupying saline versus non-saline permafrost. This research aims to better understand how depth and salinity structure microbial communities and their C-cycling functions. This study asks 1) How do salinity and depth impact microbial community abundance and diversity? and 2) How do salinity and depth impact microbial interaction with the permafrost C stock? I hypothesize that microbial diversity and abundance will decrease in response to increased depth and diversity will increase in response to increased salinity. I also hypothesize that there will be a negative relationship in microbe interaction with permafrost C as depth and salinity increase.

2. **Approach and Methodology** **(two paragraphs maximum):**
   - methods, theories, procedures, or lines of thinking and/or creating you will use to address the topic
   - material and sources needed to pursue your project, and overcome any foreseeable challenges
   - how you will analyze, interpret, and/or evaluate your findings

Our lab has three permafrost cores 100 meters in depth from the North Slope of Alaska, courtesy of PND Engineers. Sample preparation will involve scraping the outermost layer from the cores under sterile conditions to ensure sample integrity and prevent microbial contamination from the coring process (Doherty et al., 2020). From three depths in each core, I will take three samples for DNA extraction, three for total C, and three for pH; for a total of 81 samples, 27 for each analysis. I will conduct three analyses: (1) to understand the role of salinity, (2) to characterize the microbial community, and (3) to quantify richness and diversity.

(1) To understand the role of salinity and gain insight into microbial interactions with permafrost C stocks, I will measure each core's pH/electroconductivity and total organic carbon (TOC) of each core. Soil pH and electroconductivity will be measured to determine soil salinity (Rhoades,

1993). TOC will be measured by grinding a sample in a ball grinder and then analyzing samples via combustion in a Costech elemental analyzer. Electroconductivity and pH will be measured using conductivity and pH electrodes. (2) To characterize the microbial community, we will use DNA amplicon sequencing of the 16S rRNA gene – a gene that can be used to identify and count prokaryotic species in environmental samples. I will extract DNA from the samples using a standard soil DNA extraction kit (Qiagen, Carlsbad CA). Next, I will use Polymerase Chain Reactions (PCR) to amplify (make small copies of) a small section of the 16S rRNA gene (V4-V5 region) with the 515F and 926R "universal" microbial sequencing primers. After PCR, the samples will be sequenced at the Hubbard Center for Genome Studies, and I will identify species with the DADA2 Bioinformatics pipeline (Callahan et al., 2016). (3) To quantify alpha (richness) and beta (composition) diversity I will use the Vegan package on R (Dixon, 2003). To understand the strength of depth and salinity in structuring the community, I will use permutational multivariate analysis of variance (PERMANOVA) (Anderson & Walsh, 2013). To quantify physicochemical differences (e.g., salinity, pH, and C content) I will use a two-way analysis of variance (ANOVA) of depth and salinity factors (freshwater, brackish, and saline) (Ståhle & Wold, 1989).

3. My Role/Preparation/Experience (one paragraph maximum, 1-2 sentences each):

- your preparation and qualifications to undertake the project (e.g., previous coursework, jobs, extracurricular experiences, other research or training)
- your plans for further preparing yourself before undertaking the project, prior to the start date
- your unique role in the project as compared to the role of your faulty mentor and others (graduate students, technicians, collaborators), including your plan for regular communication with your mentor
- if a group project, the unique role of each student on the project (use an additional paragraph if necessary for this question)

In my first summer at UNH, I participated in the Emergent Ecosystems Response to Change Research Experience for Undergraduates, where I learned how to effectively sample, extract DNA, conduct PCR, and interpret data. As a laboratory technician in my mentor's lab, I have helped graduate students with numerous lab techniques, including DNA extraction, PCR, gel electrophoresis, soil enzyme assays, and microbial biomass quantification. I thus have a strong understanding of the lab techniques I propose to use. My UNH coursework has also given me vital background knowledge. In Studio Soils, I learned foundational concepts in soil studies, soil microbiology, and the intersection of soil and the C cycle. In Applied Biostatistics I learned how to conduct and interpret statistical analyses of data using R and applied those skills to data from my previous research in the final course project. Throughout this research, I will have the support of my mentor, Professor [A], as well as Dr. [B] and the rest of the lab. Attending lab group meetings and working closely with them gives me a group of experienced researchers to go to with any research questions or problems that arise during this project. During weekly lab meetings, I plan to present my research progress to the group and get feedback, which will prove invaluable.

Appendices

References

Ahmad, I., Ahmad, F., & Pichtel, J. (2011). *Microbes and Microbial Technology: Agricultural and Environmental Applications*. Springer Science & Business Media.

Anderson, M. J., & Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, *83*(4), 557–574. https://doi.org/10.1890/12-2010.1

Bhattacharyya, S. S., Ros, G. H., Furtak, K., Iqbal, H. M. N., & Parra-Saldívar, R. (2022). Soil carbon sequestration – An interplay between soil microbial community and soil organic matter dynamics. *Science of The Total Environment*, *815*, 152928. https://doi.org/10.1016/j.scitotenv.2022.152928

Bilal, M., & Iqbal, H. M. N. (2020). Microbial bioremediation as a robust process to mitigate pollutants of environmental concern. *Case Studies in Chemical and Environmental Engineering*, *2*, 100011. https://doi.org/10.1016/j.cscee.2020.100011

Biskaborn, B. K., Smith, S. L., Noetzli, J., Matthes, H., Vieira, G., Streletskiy, D. A., Schoeneich, P., Romanovsky, V. E., Lewkowicz, A. G., Abramov, A., Allard, M., Boike, J., Cable, W. L., Christiansen, H. H., Delaloye, R., Diekmann, B., Drozdov, D., Etzelmüller, B., Grosse, G., … Lantuit, H. (2019). Permafrost is warming at a global scale. *Nature Communications*, *10*(1), 264. https://doi.org/10.1038/s41467-018-08240-4

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Conrad R. (1996). Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). *Microbiological Reviews*, *60*(4), 609–640. https://doi.org/10.1128/mr.60.4.609-640.199610

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Doherty, S. J., Barbato, R. A., Grandy, A. S., Thomas, W. K., Monteux, S., Dorrepaal, E., & Ernakovich, J. G. (2020). The Transition From Stochastic to Deterministic Bacterial Community Assembly During Permafrost Thaw Succession. *Frontiers in Microbiology*, *11*, 596589. https://doi.org/10.3389/fmicb.2020.596589

George, S. F., Fierer, N., Levy, J. S., & Adams, B. (2021). Antarctic Water Tracks: Microbial Community Responses to Variation in Soil Moisture, pH, and Salinity. *Frontiers in Microbiology*, *12*. https://www.frontiersin.org/articles/10.3389/fmicb.2021.616730

Gurung, N., Ray, S., Bose, S., & Rai, V. (2013). A Broader View: Microbial Enzymes and Their Relevance in Industries, Medicine, and Beyond. *BioMed Research International*, *2013*, e329121. https://doi.org/10.1155/2013/329121

Harris, S. A., & Permafrost Subcommittee, Associate Committee on Geotechnical Research, National Research Council of Canada (Eds.). (1988). *Glossary of permafrost and related ground-ice terms*.

Li, X., Cui, Y., Ma, D., Song, D., & Liu, L. (2022). Vertical distribution of bacterial community diversity in the Greater Khingan Mountain permafrost region. *Ecology and Evolution*, *12*(7), e9106. https://doi.org/10.1002/ece3.9106

Rampelotto, P. H. (2010). Resistance of Microorganisms to Extreme Environmental Conditions and Its Contribution to Astrobiology. *Sustainability*, *2*(6), Article 6. https://doi.org/10.3390/su2061602

Rantanen, M., Karpechko, A. Yu., Lipponen, A., Nordling, K., Hyvärinen, O., Ruosteenoja, K., Vihma, T., & Laaksonen, A. (2022). The Arctic has warmed nearly four times faster than the11 globe since 1979. *Communications Earth & Environment*, *3*(1), 168. https://doi.org/10.1038/s43247-022-00498-3

Rhoades, J. D. (1993). Electrical Conductivity Methods for Measuring and Mapping Soil Salinity. In D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 49, pp. 201–251). Academic Press. https://doi.org/10.1016/S0065-2113(08)60795-6

Schuur, E. A. G., Abbott, B. W., Commane, R., Ernakovich, J., Euskirchen, E., Hugelius, G., Grosse, G., Jones, M., Koven, C., Leshyk, V., Lawrence, D., Loranty, M. M., Mauritz, M., Olefeldt, D., Natali, S., Rodenhizer, H., Salmon, V., Schädel, C., Strauss, J., … Turetsky, M. (2022). Permafrost and Climate Change: Carbon Cycle Feedbacks From the Warming Arctic. Annual Review of Environment and Resources, 47(1), 343–371. https://doi.org/10.1146/annurev-environ-012220-011847

Sipes, K., Almatari, A., Eddie, A., Williams, D., Spirina, E., Rivkina, E., Liang, R., Onstott, T. C., Vishnivetskaya, T. A., & Lloyd, K. G. (2021). Eight Metagenome-Assembled Genomes Provide Evidence for Microbial Adaptation in 20,000- to 1,000,000-Year-Old Siberian Permafrost. Applied and Environmental Microbiology, 87(19), e00972-21. https://doi.org/10.1128/AEM.00972-21

Song, Y., Sun, L., Song, C., Li, M., Liu, Z., Zhu, M., Chen, S., Yuan, J., Gao, J., Wang, X., & Wang, W. (2023). Responses of soil microbes and enzymes to long-term warming incubation in different depths of permafrost peatland soil. *Science of The Total Environment*, *900*, 165733. https://doi.org/10.1016/j.scitotenv.2023.165733

Ståhle, L., & Wold, S. (1989). Analysis of variance (ANOVA). *Chemometrics and Intelligent Laboratory Systems*, *6*(4), 259–272. https://doi.org/10.1016/0169-7439(89)80095-412

Stimmler, P., Priemé, A., Elberling, B., Goeckede, M., & Schaller, J. (2022). Arctic soil respiration and microbial community structure driven by silicon and calcium. *Science of The Total Environment*, *838*, 156152. https://doi.org/10.1016/j.scitotenv.2022.156152

Tripathi, B. M., Kim, M., Kim, Y., Byun, E., Yang, J.-W., Ahn, J., & Lee, Y. K. (2018). Variations in bacterial and archaeal communities along depth profiles of Alaskan soil cores. *Scientific Reports*, *8*(1), 504. https://doi.org/10.1038/s41598-017-18777-x

Trumbore, S. (2006). Carbon respired by terrestrial ecosystems – recent progress and challenges. *Global Change Biology*, *12*(2), 141–153. https://doi.org/10.1111/j.1365-2486.2006.01067.x

Wu, X., Almatari, A. L., Cyr, W. A., Williams, D. E., Pfiffner, S. M., Rivkina, E. M., Lloyd, K. G., & Vishnivetskaya, T. A. (2023). Microbial life in 25-m-deep boreholes in ancient permafrost illuminated by metagenomics. *Environmental Microbiome*, *18*(1), 33. https://doi.org/10.1186/s40793-023-00487-9

Zimov, S. A., Davydov, S. P., Zimova, G. M., Davydova, A. I., Schuur, E. a. G., Dutta, K., & Chapin III, F. S. (2006). Permafrost carbon: Stock and decomposability of a globally significant carbon pool. *Geophysical Research Letters*, *33*(20). https://doi.org/10.1029/2006GL027484