

Summer Undergraduate Research Fellowship (SURF) USA

Permafrost Microbial Community Characterization along Depth and Salinity Gradients

Torin Scalora, Environmental Science: Ecosystems

Mentor: Jessica Ernakovich, Natural Resources and the Environment

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.**

[illegible]

Project Background (two pages maximum, single-spaced)

1. Project History, Definition, and Significance

- general problem, theme, or issue to be addressed
- historical context: i.e., most relevant previous research, scholarship, or artistry on this topic by other researchers, scholars, or artists (cite sources)
- project's specific question, hypothesis, or objective
- contribution of your project to the problem and your field
- broader significance or importance of your research (e.g., social, cultural, intellectual, creative, practical, theoretical)

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

- methods, theories, procedures, or lines of thinking and/or creating you will use to address your research topic and answer the question(s) you pose
- if you plan to conduct interviews or surveys: what assumption/hypothesis/general principle you will test; the number of participants you will seek and how you will recruit/select them; sample interview/survey questions (to be included in Appendices)
- materials and sources needed to pursue your project
- how you will analyze, interpret, and/or evaluate your findings – including how/why this mode of analysis will enable you to accomplish your project objectives and/or answer your research question(s)
- foreseeable challenges, obstacles, or difficulties – and how you are prepared to address them

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) (Stähle & Wold, 1989).

3. My Role/Preparation/Experience

- 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 faculty 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-Saldivar, 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 the 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](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., Lorant, 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](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>