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):** tangible products of your project (e.g. report, database, results of quantitative/qualitative/textual analysis, paper, thesis, presentation, production, exhibition, film, article submitted for conference or publication, etc.); also include at least one outcome stating how this project contributes to your personal, educational, and/or professional goals
   - 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):** using bullet points or a table, list your planned research activities and specific goals for each week of the project

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

- 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) (Ståhle & 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 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

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