

SOAR Proposal for Summer Research 2018

Project Title: Microbiome Analysis of Soil from Palmerton Zinc Pile Superfund Site
Faculty Mentor: Dr. Kara Mosovsky, Assistant Professor, Department of Biological Sciences
Student: Alayna Koch, Interdepartmental Major in Biology and Management, class of 2019
Project Duration: Standard SOAR schedule, starting June 4th and lasting 10 weeks

Project Description:

General Background:

From 1898 to 1980, zinc smelter plants operated on land adjacent to the Kittatinny Ridge (Blue Mountain) in eastern Pennsylvania. Emissions from these plants contained, among other things, heavy metals such as zinc, lead, cadmium and arsenic, which contaminated 3,000 acres of the surrounding area. As a result of the pollution, acid rain, and topsoil erosion off the slopes, the vegetation was killed off and the mountainside became a denuded, barren landscape. This once valuable forested habitat could no longer support plant life, nor microscopic organisms.¹¹

This contaminated landscape posed risks to wildlife and humans and was therefore placed on the National Priorities List and designated as the Palmerton Zinc Pile Superfund Site. In 2002, about 750 acres of this land was purchased by the Lehigh Gap Nature Center (LGNC). Along with the Environmental Protection Agency (EPA), responsible party, and other stakeholders, the LGNC identified restoration goals that included re-vegetation with native plants and rebuilding and returning organic matter to the soil.

Remediation efforts have included limestone and compost application and prescribed burns at the permanent test plots that have been established on the LGNC property. Warm seasonal grasses have successfully been grown on some of these test plots but not others. The end goal of these restoration treatments is the growth of native grasses on the contaminated plots. These grasses have the potential to replenish the topsoil by building other organic matter, which will eventually support re-vegetation processes with other plant species.¹

Specific Project Background and Proposed Project:

Previous microbiology research at the site has determined which bacteria are present in the soils and have tracked relative numbers of bacteria in the soil over time, but these studies relied exclusively on identifying bacterial numbers and species based on what grew in a laboratory environment (ex. in petri plates).¹ Since most microbes are not capable of growing in the laboratory (outside of their specific natural environment), it is likely that there has been a severe underestimation of the bacterial abundances, diversity, and recovery in the test plots over time. Instead of counting and characterizing only the bacteria which can grow on a laboratory petri plate, we will isolate and analyze all bacterial DNA from the soil as a much more advanced and complete way to survey which microbes are present in the soil sample. Portions of bacterial DNA are so unchanging that we can identify bacteria based only on a particular sequence of DNA. We can also group bacteria together based on similar sequences of DNA. Such studies that use genetic sequencing to group related microbes together and analyze community structures of microbes are called microbiome studies.

¹ Latham, R. E., D. B. Steckel, H. M. Harper, C. Steckel and M. Boatright. 2007. Lehigh Gap Wildlife Refuge Ecological Assessment. For the Lehigh Gap Nature Center, Slatington, Pennsylvania, by Natural Lands Trust, Media, Pennsylvania; Continental Conservation, Rose Valley, Pennsylvania; and Botanical Inventory, Allentown, Pennsylvania. 62 pp. + 8 maps, 5 appendices.

To this end, the aim of our summer research project is to conduct a microbiome study to determine if there are differences in the communities of bacteria present between sites that have been successfully re-vegetated with native grasses versus those that have not. Soil microbes play a big role in what plant species are capable of growing in the soil, so the goal of studying differences in bacterial communities is to be able to identify if a certain community of bacteria supports the re-growth of native grasses. If we find differences between sites this summer, in the future we will be able to perform microbiome studies that look at soil microbes before and after a particular treatment, to help determine direct treatment effects to the microbial communities. Eventually we hope to steer treatments in a way that would generate healthy communities of bacteria in the soils.

To start this project, we will become familiar with the site and various test plots, and meet with other researchers to learn about previous research. We have already been introduced to some other key researchers at the site and they are excited to collaborate with us on this project. Then we would collect core samples of soil from various treated test plots (some that have been successfully re-vegetated with native grasses, and others which have not). We will extract the bacteria and isolate their DNA in the laboratory. Then, we'd sequence a particular part of the DNA that can be used to determine relatedness of bacteria to each other (the gene for 16s rRNA) and we would analyze the differences between bacterial communities in the soil samples. In particular we want to determine total and relative numbers of different groups of bacteria, and we want to determine the diversity of bacteria present in the soils. We plan to order about 20,000 DNA sequences from each soil sample, so the organization and analysis of data will be the most extensive part of the project (elaborated on below: organization and analysis of data). In the end, we hope that understanding the soil microbiome will help with the restoration efforts and recovery of the soil ecosystems at the Superfund site.

Organization and Analysis of Data

As microbiome analysis is new to us both, we will spend a great deal of the summer understanding how to use two software programs needed to analyze microbial sequencing data: Quantitative Insights into Microbial Ecology (QIIME) and R for statistical analyses. Both of these programs are free and available online. Further, we have already established a contact at the City University of New York (CUNY) who is willing and able to help us with these programs and analysis of our data should we find the need. We will have some sample data (from an unrelated microbiome project this semester) to analyze while our Superfund soil samples are being sequenced. It is our hope that we will be ready to analyze our sequence data using QIIME and R when our data is returned to us during the second half of the summer.

In addition to analyzing the raw sequencing data, we will also receive some analysis of the data from the sequencing company. We hope to be able to compare our analysis of the data to the version given to us by the sequencing company, so that we can verify that our methods are accurate. While learning to use QIIME and R programs would be a valuable tool for microbiome research in general, we realize that it may not be within our abilities to fully comprehend both programs in one summer. In this case, the data analysis from the sequencing company will provide us with a fail-safe for answering our research questions. In using their analysis, we would cut out a few steps of analysis for ourselves, but would still need to sort through the summaries, organize the analysis, and draw conclusions about our samples. In this second scenario, we would probably be able to ask and answer a few additional questions about the microbial communities on the different test plots, as we'd have a bit more time for our project. We feel as though there will be great benefit to both student and faculty mentor to first try to conduct the analysis and organization of DNA sequence data ourselves, but we are happy to have a fallback plan should our technology abilities fail to meet the

demands of the task. Either way, we are confident that we can answer our basic research questions about the differences and similarities between types of bacteria present in the soils at the Superfund site, and either way of reaching these answers will be a valuable learning opportunity for Alana.

Roles and responsibilities of student vs. faculty mentor: Alayna will experience all aspects of her research project working side-by-side with myself. This is as much a new project for me as for her, and I am thrilled to have someone so enthusiastic as a partner in this project for every step of the way. We have already started by reading about the history of the site and communicating with collaborators both at the LGNC as well as CUNY. We will both visit the Lehigh Gap Nature Center (LGNC) when the vegetation returns, to learn more about the site and permanent test plots. In summer, we will select our survey sites and collect soil samples together in the field. We will process the samples back in our laboratory and then a large chunk of the summer will involve learning to analyze sample microbiome sequencing data using QIIME and R, as well as organization and analysis of the data when it returns from sequencing. She and I will be equal partners in learning to use the software programs to analyze our data.

Student Engagement in Discipline-Appropriate Scholarly Research: All aspects of this project serve as relevant hands-on application and reinforcement of her classroom learning about biology and biochemistry. Since this is a new project for my research lab, she will also get a very rich experience with the culture of science because she is experiencing the project from its very beginnings, with all its inevitable ups and downs. She will gain experience with fieldwork, molecular laboratory techniques, as well as computer analysis of extremely large amounts of data. The ability to organize and analyze such “big data” is an increasingly valuable skill in biology, but one that few of our biology students master. Alayna will present her work at Moravian College’s Scholar’s Day in 2019, as well as submit an abstract for acceptance to the National Council for Undergraduate Research student conference and the Pennsylvania Academy of Sciences conference. We also expect that she will have the opportunity to share our results with collaborators working at the LGNC contaminated lands. Pending the results of her work, we hope that she will be able to publish her study in an undergraduate journal.

Expected Timeline of Project:

Week 1-2: Read primary and secondary literature about the microbiology of soils contaminated by heavy metals, as well as bioremediation strategies for such soils. Visit Superfund site; meet important researchers and key players in remediation efforts

Week 3-4: Identify most suitable test plots to conduct our study, and take multiple samples of soil from these plot areas. Isolate bacterial DNA from the soil samples in the laboratory and send samples out to be sequenced.

Week 4-6: Learn how to use QIIME and R software to analyze microbiome sequencing data

Week 6-8: Practice QIIME and R with sample sequencing data; learn to organize results into professional figures and graphs typically used for microbiome analysis; collaborate with LGNC to design future studies at the Superfund site

Weeks 8-9: Analyze *our* sequencing data; organize results into professional graphs and tables

Week 10: Wrap-up project, share results with other researchers at LGNC, prepare a poster for Scholar’s Day

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Alayna Koch
Interdepartmental Major in Biology and Management
Spring 2019
Mentor: Dr. Kara Mosovsky
Request On-Campus Housing

Rationale to Participate in Project

Following my education at Moravian College, I plan on attending medical school to pursue a career in pediatrics or neonatology. At a young age, I immediately recognized a passion and desire within myself to contribute to the care and healthy development of children. The development and well being of children and adolescents is a complex process; physical, mental, and emotional health all intertwined. There are several ways in which this summer research project, at the Palmerton Zinc Pile Superfund Site, would enhance my career objectives.

Everything has a microbiome, including people, and thus, experience with soil microbiomes will translate towards a future research direction involving human subjects. Identifying what microbes are present in an environment, and understanding how they interact with each other is important, whether these microbes come from soil, water, or humans. Most microbial ecosystems are fragile and disruptions to the community structure can lead to direct and indirect changes in an environment. For example, disruption of the human gut microbiota (the microbes that live in our intestines) has been associated with the progression of human diseases including, but not limited to, infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, and autoimmune diseases.²

Since I aspire to pursue a career in pediatrics or neonatology, it is important that I understand the impact that microbiome communities, present in the body, can have on a baby's health. We already know that the first few months of life are the most crucial time period for a baby to develop a healthy microbiota. Furthermore, young children consume the majority of antibiotics, with an average of 17 courses of antibiotics administered before the age of 20.³ The normal community structure of the human microbiota is disrupted with antibiotic use, and many "good bacteria are killed off in the process, so it would be unsurprising if there were health impacts of such rampant antibiotic use in babies."³

It is apparent that microbes and health care are more closely related than one would predict. The human microbiota impacts bodily processes, as well as diseases and illnesses. As a result, microbiota research could impact one's health and outcomes and drive a shift towards a more individualized health care system based on a person's microbiota. Even if specialized treatments cannot be developed, researchers could still aim to understand the ecology of someone's microbes and how various treatments will affect them. My summer research project will help me strive to reach my fullest potential as a physician. My medical judgment would incorporate the idea that any treatments, medications, or other healthcare would undoubtedly affect a patient's microbes. This experience with microbiome analyses will make me more consciously aware of the inadvertent affects that treatments, antibiotics or medications could have on one's microbes, which then, impacts one's health. Rather than simply treating the

² Wang B, Yao M, LV L, Ling Z, Li L. The Human Microbiota in Health and Disease. *Engineering*, 3 (1), 71-82.

³ Blaser, Martin J. *Missing Microbes: How Killing Bacteria Creates Modern Plagues*. Oneworld, 2015.

³ Knott, Dr. Laurence. "Microbes, Germs and Antibiotics. What Are Microbes?" . What Are Microbes? | Patient, Patient.info, 12 Dec. 2014, patient.info/health/antibiotics-leaflet/microbes-germs-and-antibiotics.

symptoms, I can utilize my background and knowledge of microbiome differences to provide well-rounded, quality care and treatment to patients.

Expected Outcomes for Project

Moravian College has provided me the opportunity to participate in several course based research projects. Throughout my time at Moravian College I have gained a deeper appreciation for the laboratory experiences that the science courses have to offer. The Palmerton Zinc Pile Superfund Site is an exceptional research opportunity that I take great interest in, and one that will help me broaden my horizons and grow as a student.

Restoration efforts have been a primary focus for ongoing research at this site. I am eager to explore the superfund site and conduct further analysis of the microbiome communities present at the various test plots. The over-arching goal of this project would be for Dr. Mosovsky and I to use two software programs, Quantitative Insights into Microbial Ecology (QIIME) and R, to successfully analyze a trial run microbiome project at the superfund site. Part of this goal will involve us learning how to use the software programs QIIME and R, which are programs that can analyze the ecology of microbes. For future direction, this research could lead to understanding the affects that the treatments have on the composition of the microbiome communities, and vice versa. Through this summer research project, I would have the opportunity to acquire a wide array of new skills and techniques. I would gain experience in designing an experiment to solve a problem, collecting and analyzing data, drawing conclusions, as well as learning new computer analysis skills and modern microbiology lab techniques. These critical analysis and analytic skills could then be applied to future research projects.

Any research experience will connect what I have learned in lecture to a more hands-on application. It will enable me to gain a deeper understanding of the topics covered in class lectures. This summer project would incorporate a wide variety of disciplines in order to solve this problem. Through this research, I would expect for Dr. Mosovsky and I to both understand and apply these two software programs in order to analyze microbiomes from soil samples, and compare relative abundances of certain species of bacteria present in these samples. We will construct a graph to examine the bacterial populations, and to compare the diversity and differences between soil samples. If given the opportunity to participate in SOAR, I would be honored to contribute to a successful and meaningful summer research project. Following SOAR, I would hope to conduct an honors project relating to microbiome studies. Thus, this summer research project would help pave the way for a future honors project.

Expense Proposal

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Expected Expenses:

- 1) Sequencing of bacterial DNA from 12-15 soil samples
(allowing for triplicate samples from any single site)
@ ~\$80/sample for Mr. DNA (sequencing company) \$960-1200

This is absolutely essential to our project and we do not have the means to sequence microbial DNA on Moravian's campus, therefore we will send our samples out to another company for sequencing. This is typical for such microbiome studies.

- 2) Travel expenses (gas and tolls) for several trips \$30
to the Superfund site to meet with researchers, to be trained in safety, to scope out test plots, and to collect soil samples

Total = ~\$1000-1200

Funds requested from SOAR = \$500

**The Department of Biological Sciences and/or Dr. Mosovsky's start-up funds will cover the remaining expected costs as well as any unforeseen costs throughout the length of the project.