Enrichment of Subsurface Microbes with Differing Carbon Substrates

[1]Mariam Alsaid, [2]Kristine Cabugao, [2]Mon Oo Yee, [2]Romy Chakraborty
[1]Sacramento City College; [2]Department of Ecology, Lawrence Berkeley National Laboratory

2021 Transfer-to-Excellence Research Experiences for Undergraduates Program (TTE REU Program)

EARTH & ENVIRONMENTAL SCIENCES

Berkeley UNIVERSITY OF CALIFORNIA

BERKELEY LAB Lawrence Berkeley National Laboratory

ABSTRACT

Carbon sequestration is a necessary mechanism that mitigates the threats of climate change by preventing the accumulation of greenhouse gases (like CO2) that trap heat in the atmosphere. Soil functions as a massive carbon sink, constituting a negative feedback loop to climate change. A majority of research on global carbon cycling focuses on surface soils and related plant-microbial interactions. However, subsurface carbon-mineral associations and microbial communities can significantly impact soil organic matter dynamics. To investigate this, we enriched and isolated microbial community members from various soil depths in different enrichment cultures. We found that microbial community compositions differ depending on the initial soil core depth, as well as the carbon source of each enrichment. Refining our enrichment and isolation methods to capture new microbial isolates for more detailed studies will improve our understanding of microbial communities that impact subsurface carbon cycling.

BACKGROUND

WHAT?

- Microbes are critical to biogeochemical processes that impact carbon cycling, however, a majority of soil microbial ecology focuses on rhizosphere and surficial soil environments, neglecting subsurface sediments where over 50% of soil carbon resides.

WHY?

- Standard and commercial practices with rich media favor fast growing microbes, impeding our ability to understand other, potentially more important, slow growing microbes.
- There is a need to develop new types of media with customized carbon sources to capture microbial community members that would otherwise be excluded

HOW?

- Isolating microbes from these subsurface environments is a critical first step in understanding the metabolic activities that impact carbon cycling and sequestration.

1. **Soil Core Collection**
Auger was used to collect 3 soil cores at different depths at Oak Ridge Field Research Center

2. **Sample Enrichments**
Sediments were placed in customized media associated with nitrogen-reducing and methanogenic enrichment conditions

3. **Sequencing and Analyzing**
Samples were sequenced using 16S amplicon sequencing and were then analyzed using QIIME2

OBJECTIVES

- Enrich and isolate microbial community members from different depths from a field site at Oak Ridge Field Research Center (OR FRC) using different carbon sources.
- We hypothesize that each carbon source will lead to different relative abundances of microbial community members even at the same depth since some microbes will utilize each carbon source better than another.

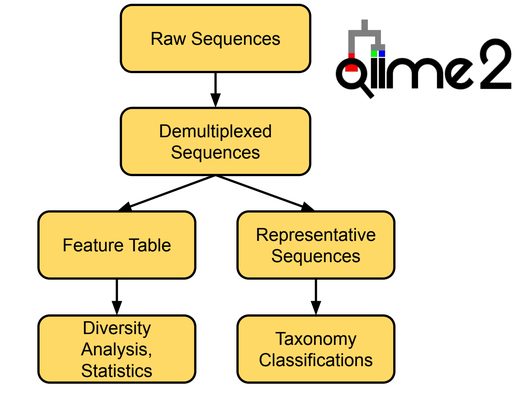
MATERIALS & METHODS

Soil Samples Extracted From Three Different Soil Cores and their Associated Enrichment Conditions and Substrates

Sample name	Enrichment Condition	Inoculum source	Substrates fed
F153	Iron-oxidizing/Nitrate reducing	EB271(05-03)	Acetate 2mM, FeCl2 2mM, NO3 5mM
F221	Iron-oxidizing/Nitrate reducing	EB271(02-01)	Acetate 2mM, FeCl2 2mM, NO3 5mM
H253B	Methanogenic	EB271(05-03)	2 bar, N2:H2 (80:20)
H223B	Methanogenic	EB271(02-03)	1 bar, N2:H2 (80:20)
MH223B	Methanogenic	EB271(02-03)	Methanol 50mM
MH253B	Methanogenic	EB271(05-03)	Methanol 50mM

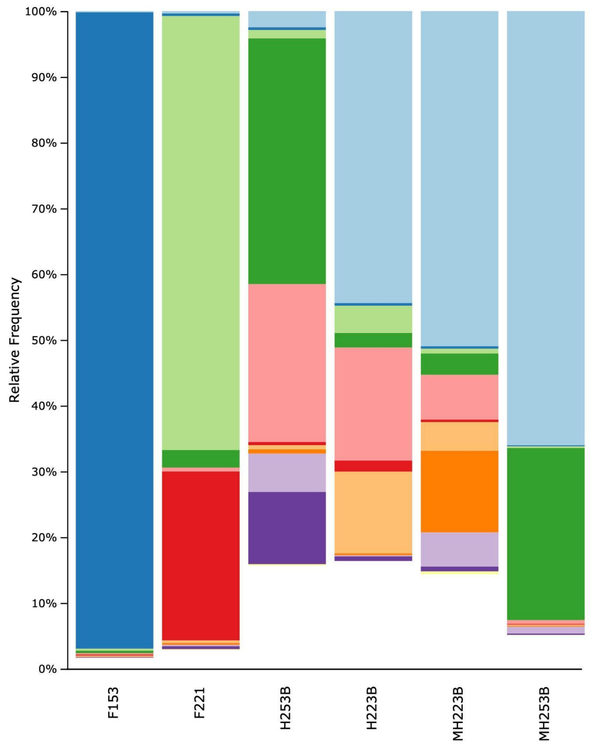
Samples were fed one of three different substrates to create either Iron-oxidizing/nitrate-reducing or methanogenic enrichment conditions. Inoculum source refers to the soil core ID associated with each sample

Pathway for DNA Analysis of 16S Amplicon Sequencing Using Quantitative Insights into Microbial Ecology (QIIME2) Package



RESULTS

Ten Most Abundant Microbes Found in Soil Samples Enriched with Different Carbon Sources (Genus level)



KEY: Family, Genus (if detected)

- *Methanobacteriaceae, Methanobacterium*
- *Rhodospirillaceae*
- *Order Bacillales**
- *Clostridiaceae, Clostridium*
- *Veillonellaceae, Sporomusa*
- *Rhizobiaceae*
- *Gracilibacteraceae, Gracilibacter*
- *Lachnospiraceae, Clostridium*
- *Veillonellaceae*
- *Ruminococcaceae, Ruminococcus*

DISCUSSION

Inoculum Source | **Characteristics of Each Sample**

EB271(02-01)

F221
- Oxygen still present at this depth
- Microbes present are predominantly aerobic (*Order Bacillales and Family Rhizobiaceae*)

EB271(02-03)

H223B and MH223B
- No oxygen present at this depth
- Roughly similar microbial populations but at different compositions
- Discrepancies possibly due to different concentrations of N2:H2

EB271(05-03)

F153
- Nitrate-reducing enrichment condition
- Large population of Family *Rhodospirillaceae*, a group of nitrogen-fixing bacteria
- Lack of biodiversity possibly because substrate was too harsh

H253B and MH253B
- Similar trend as above soil core but differs as a result of different initial microbial community composition

The main factors impacting final microbial community composition are:
- Soil depth
- Chemistry of enrichment substrate
- Initial microbial composition

This field of research will enhance our understanding of the carbon cycle and help us achieve more accurate quantitative estimates of global carbon emission versus sequestration

ACKNOWLEDGMENTS

This work was funded by the National Science Foundation REU site grant #1757690. The Transfer-to-Excellence Program is hosted and supported by the Center for Energy Efficient Electronics Science. I would like to thank my mentor Kristine Cabugao, our principal investigator, Romy Chakraborty, and everyone at the Lawrence Berkeley Lab for all their insight and words of encouragement. I would also like to give a special thank you to everyone involved with the TTE REU, namely Nicole, Tony, Sam, as well as all of the interns who made this summer of research a memorable one.