



Summer experience analyzing microbial communities from Tims Branch watershed exposed to heavy metals

solution driven

Juan Morales, DOE Fellow

Environmental Health Sciences PhD Student



FLORIDA INTERNATIONAL UNIVERSITY



Proposed Agenda



- 1. Background (Savannah River Site Tims Branch watershed)
- 2. Aim of the study
- 3. Materials and methods
 - Sample strategy and experimental design
 - DNA Extractions
 - NGS Laboratory procedures
 - Bioinformatics
- 4. Preliminary results
- 5. Conclusion (experience gained)



Savannah River Site – SRS



Metals Contamination at Steed Pond and Tims Branch. Gaines and Seaman (2010) Savannah River Site – Former nuclear production facility covering 780 km².

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- Resulted in the release of various contaminants into the SRS environment.
- Sits in the Sand Hills ecoregion Upper coastal plain of South Carolina
- Many of the industrial areas that received and may still receive contaminants through surface transport mechanisms may not be fully evaluated or controlled.



Tims Branch Watershed

- Second order stream
- Flow is strongly influenced by groundwater pumping
- Historical discharges (Radionuclides, heavy metals and spent fuel)
- 2007, <u>remediation technologies</u> (SnCl₂) reduces Hg(II) →Hg(0)
 - Aqueous mercury concentrations decreased 90 %
- Metal concentrations are greater in Steed Pond sediments



Aerial image of Tims Branch stream and historical discharge and outfall locations. *Applied Research Center 2016*



Aim of the Study

Investigate the microbial community response to heavy metal pollution from different soil types in Tims Branch watershed.

Objectives while at ANL:

- 1. Extract DNA from TBW sediment samples
- 2. Prepare PCR amplicon libraries (targeting 16rRNA gene)
- 3. Amplify region specific primer (V4) of the 16s rRNA gene
- 4. Evaluate microbial diversity from raw sequencing data
 - Using downstream analysis (QIIME)



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Sampling strategy and locations



1. Rank sample locations according to metal concentration.

Control **	Beaver Pond 1**	
Low	Rip Rap / Met Lab channel	
Mid	Upstream Steed's Pond	
High	Downstream Steed's Pond	

- 1. Numerate polythene bags with sample and replicate IDs.
 - 1. (RR_1), (Ctrl_1), (Upst_1)and (Down_1)
 - 2. Collect 8 replicate samples from each location and label
- 2. Use individual sterile syringe corer and collect 30 g of sediment from the uppermost layer (0-6 cm) of the stream bed.
- 3. Store samples in a secure location to preclude conditions which could alter the properties of the samples.
- 4. Ship and label collected samples to Argonne National Lab Sequencing Center upon completion.





Next generation sequencing

- **NGS** is a method for sequencing genomes at a high speed and at low cost.
- It provides a relatively easy approach to analyze microbiome and its composition.

Main workflow:

- 1. Library preparation
- 2. Cluster generation
- 3. Sequencing
- 4. Data Analysis



Cluster 1 > Read 1: GAGT ...

Cluster 4 > Read 4: ATAC ... Text File

Cluster 2 > Read 2: TTGA.. Cluster 3 > Read 3: CTAG..

Digital Image





Data is exported to an output file

DNA Extraction Materials and Methods

Equipment and Reagents

- Savannah River Site Sediments
- Reagents QIAGEN DNEasy PowerSoil Kit ®
- Equipment Microcentrifuge (10, 000 x g)
- **Pipettor** (50-1000 μl)
- Vortex and Adapter for 24





Library design, PCR, and clustering



<u>Library preparation</u>: DNA is broken down and specialized adapters are (glued) to the ends of the fragmented DNA.

<u>Adapter-ligated</u> fragments are amplified via PCR and gel purified.

<u>Pooling</u>: Once PCR is completed, DNA libraries are placed into a flow cell with complimentary oligonucleotides.

Quantification: Run pool through a qubit assay.



Amplification 16rRNA gene





1.

2.

3.

4.

5.

6.

enables researchers to:

and visualization





Preliminary results



Basic statistics of OTU table.

This lists the taxonomy with greatest depth allowed by confidence threshold (80 percent)

- Figure represents a summary OTU table.
- If the representative sequence file is counted. The same number of sequences should be displayed.

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JM-UPS-8: 43665.0 denovo41478 k_Bacteria; p_OP3; c_koll11; o_; f_; g_; s_ 1.00 3	JM-RR-3: 23213.0	denovo41475 k_Bacteria; p_Ver	ucomicrobia; c[Pedosphaerae]; o[Pedosphaerales]; fEllin515; g; s 1.00 3
	JM-UPS-8: 43665.0	denovo41478 k_Bacteria; p_OP3	c_koll11; o_; f_; g_; s_ 1.00 3
JM-UPS-4: 50258.0 denovo414/9 K_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_; s_ 1.00 3	JM-UPS-4: 50258.0	denovo41479 k_Bacteria; p_Firm	icutes; cClostridia; oClostridiales; fLachnospiraceae; g; s1.00 3
JM-CTRL-5: 61369.0 denovo15140 k_Bacteria; p_Acidobacteria; c_Solibacteres; o_Solibacterales; f_Solibacteraceae; g_Candidatus Solibacter; s_ 1.00 3	JM-CTRL-5: 61369.0	denovo15140 k_Bacteria; p_Acio	obacteria; cSolibacteres; oSolibacterales; fSolibacteraceae; gCandidatus Solibacter; s1.00 3



Summarize communities by taxonomic composition



- The degree of sharing microbial taxa in 34 samples from 4 different location points.
- The summary microbial composition by sample appears to have Acidobacteria-5 and 6 in greater amounts.





Investigating Alpha Diversity



Identifies how diversity is each sample species according to location and soil type.

- 1. Identifies how many species are in each sample
- 2. Chao1 diversity metric predicts the OTUs richness at high depth sequencing
- 3. Rarefaction curve identifies how many sequences are necessary to capture most of the microbial diversities





Beta diversity metrics of microbial communities in TBW



- Identifies how closely samples are related to another sample.
- Loamy sand (blue) the distance between each data point is proportionally to the weighed UniFrac distance.
- Clusters are represented to have similar microbial communities with very close phylogenetic diversities.





Conclusion



- Short term analyses of microbial communities in Tims Branch watershed resulted in:
- ➢For each sample, we quantified the microbial communities found in each sample by soil types.
- >Alpha and Beta diversity metrics were calculated for individual samples.
- Samples contained similar classes of bacterial with highest quantity of Acidobacteria (bright red) present in all soil types.
- >As a preliminary exploration, only a small set of the pipeline was utilized.
- Visualization and tools will need to be revaluated in order to determine further assessment.

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