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


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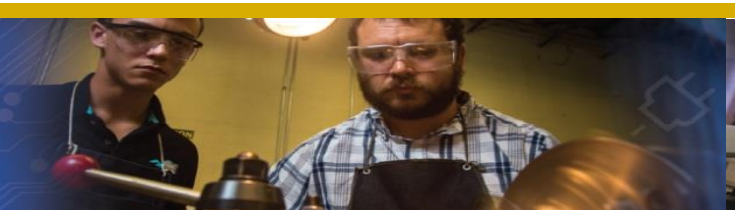
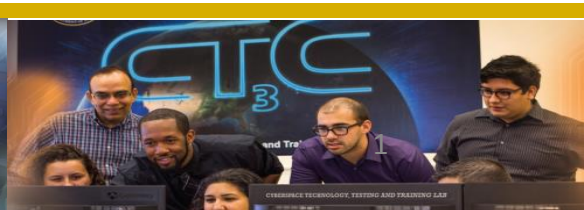
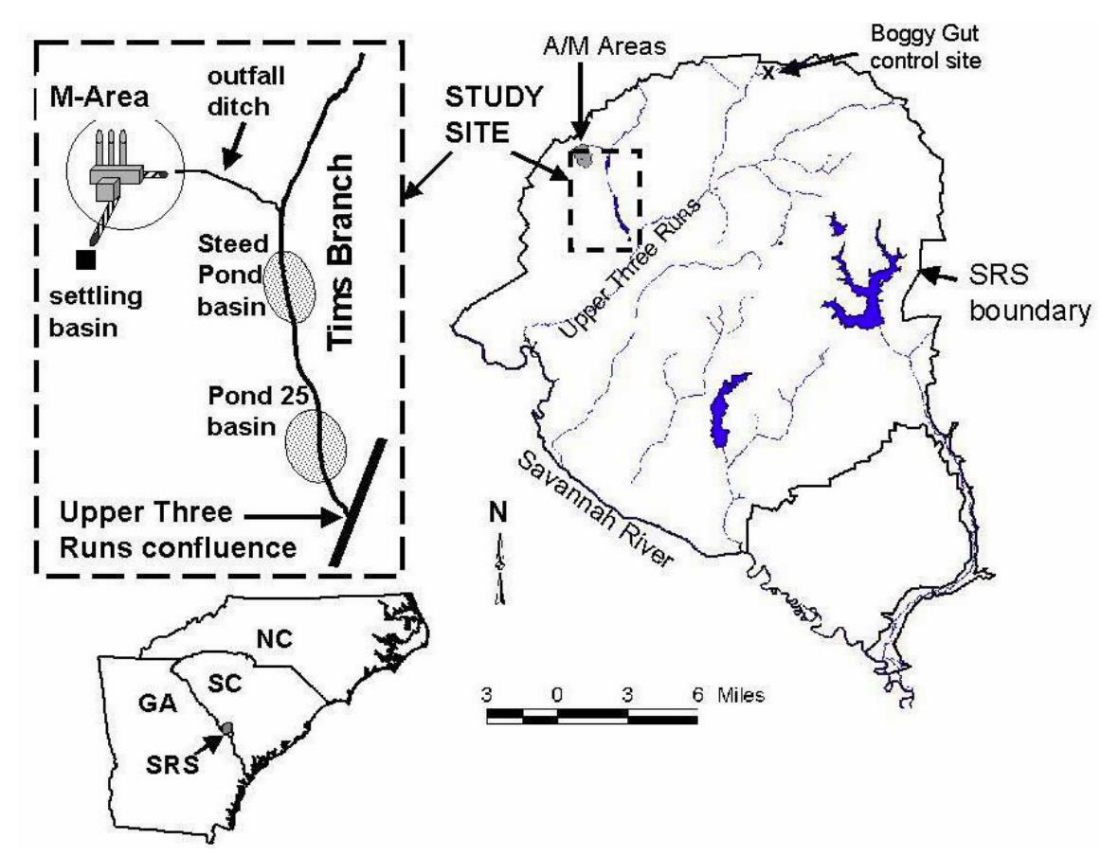


# Proposed Agenda

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



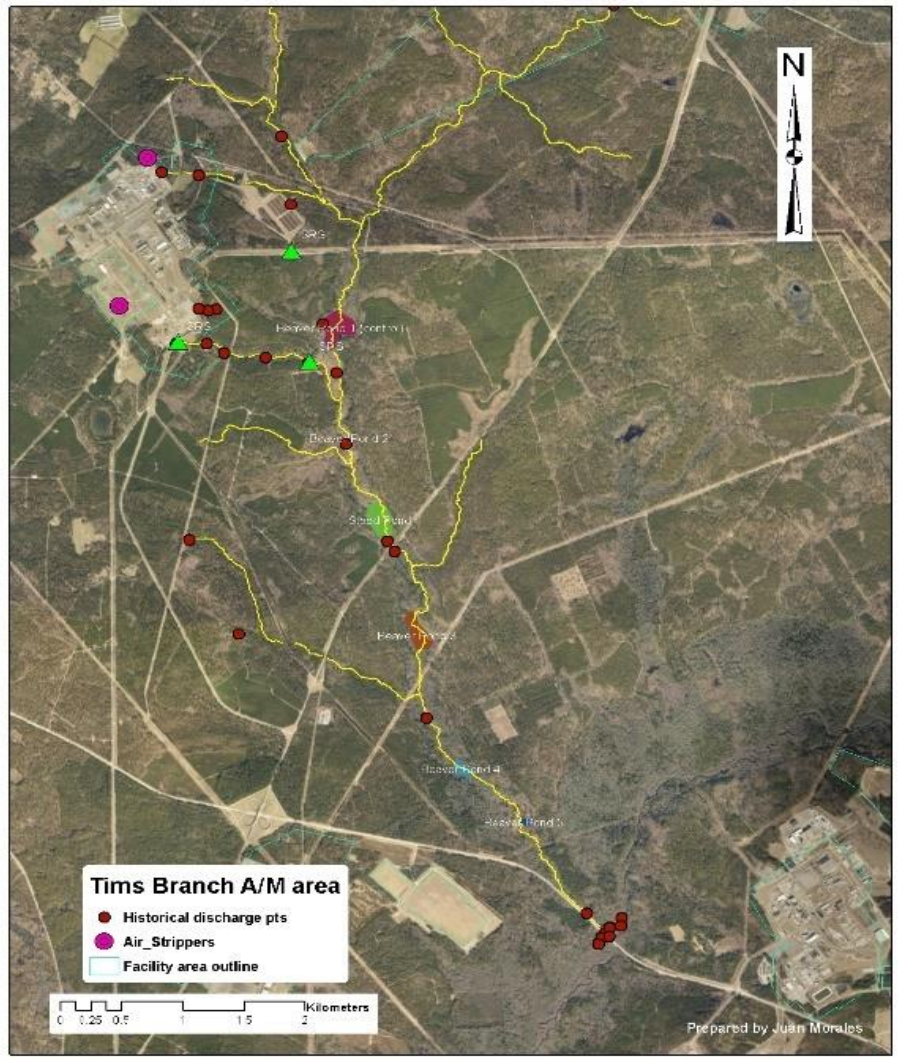

Metals Contamination at Steed Pond and Tims Branch.
*Gaines and Seaman (2010)*

- **Savannah River Site** – Former nuclear production facility covering 780 km$^2$.
- 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, remediation technologies ($SnCl_2$) reduces Hg(II) →Hg(0)
  - Aqueous mercury concentrations decreased 90 %
- Metal concentrations are greater in Steed Pond sediments



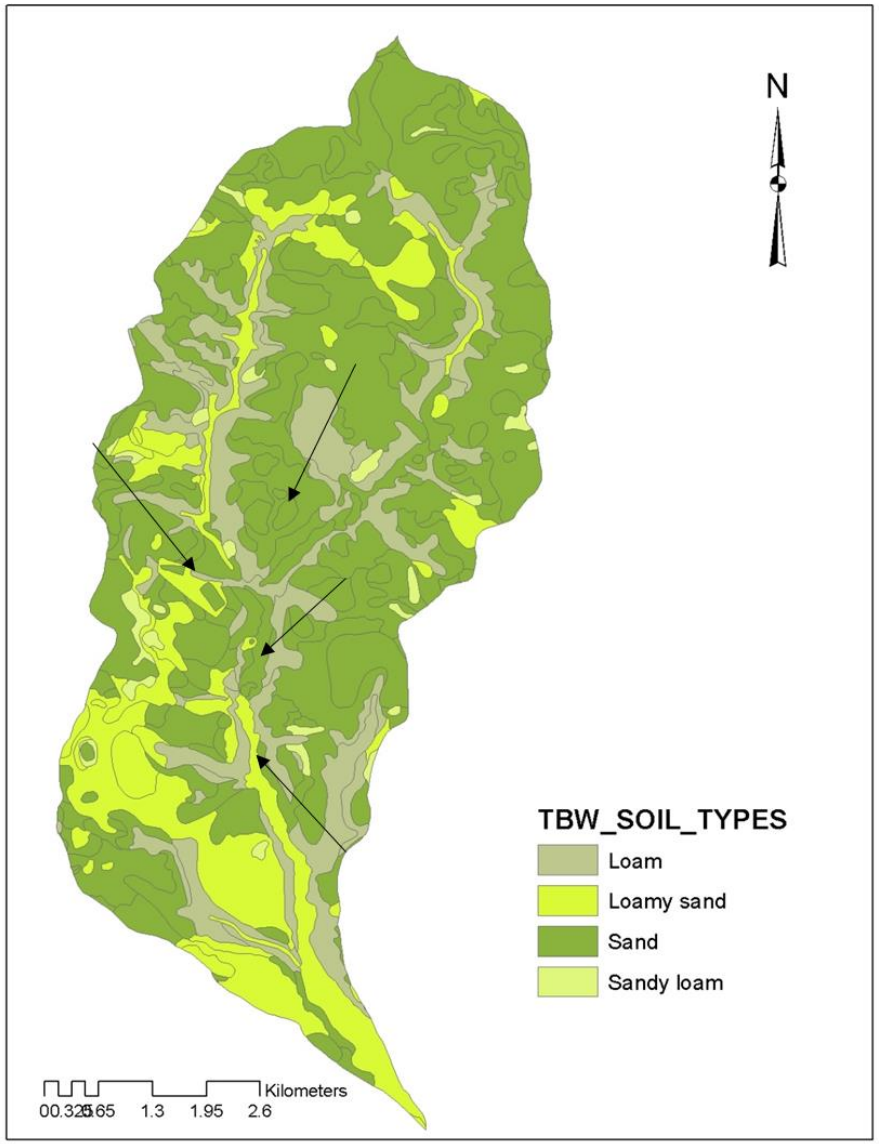

Aerial image of Tims Branch stream and historical discharge and outfall locations. *Applied Research Center 2016*

# 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)

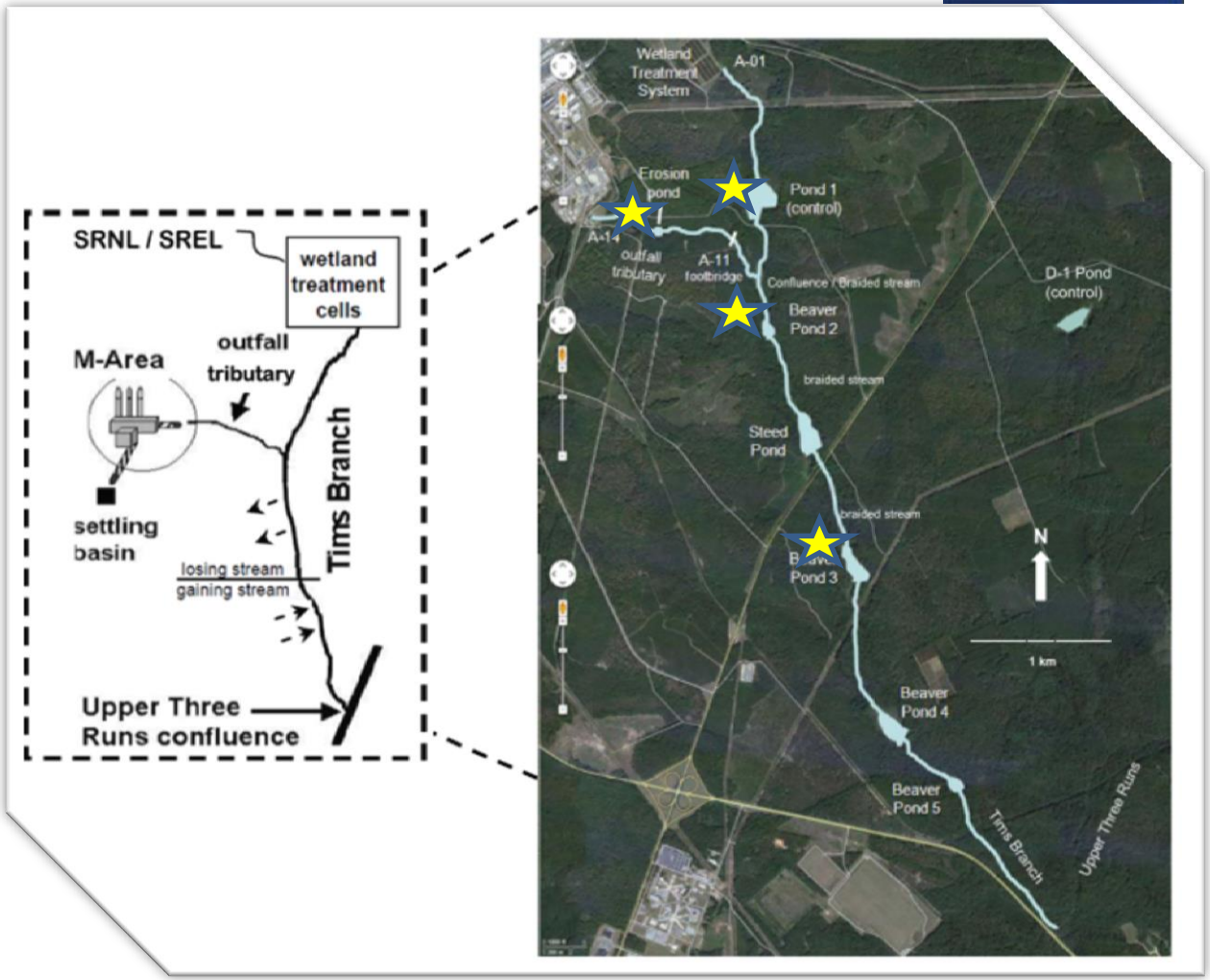


# Sampling strategy and locations

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.**

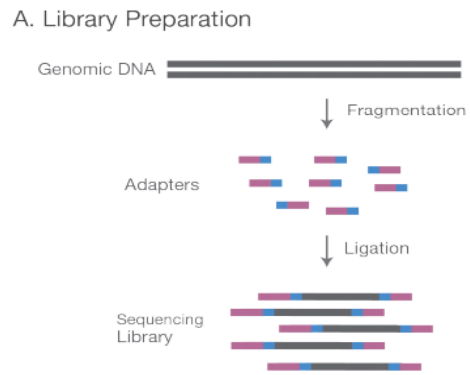


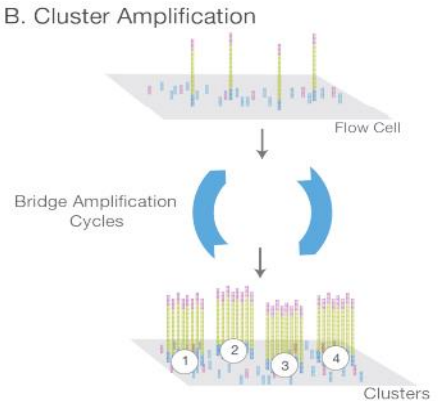
# Next generation sequencing

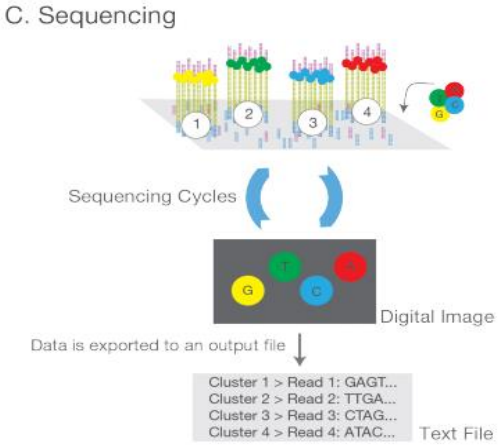
- **NGS** is a method for sequencing genomes at a high speed and at low cost.
- It provides a relatively easy approach to analyze microbiome and its composition.

**Main workflow:**

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



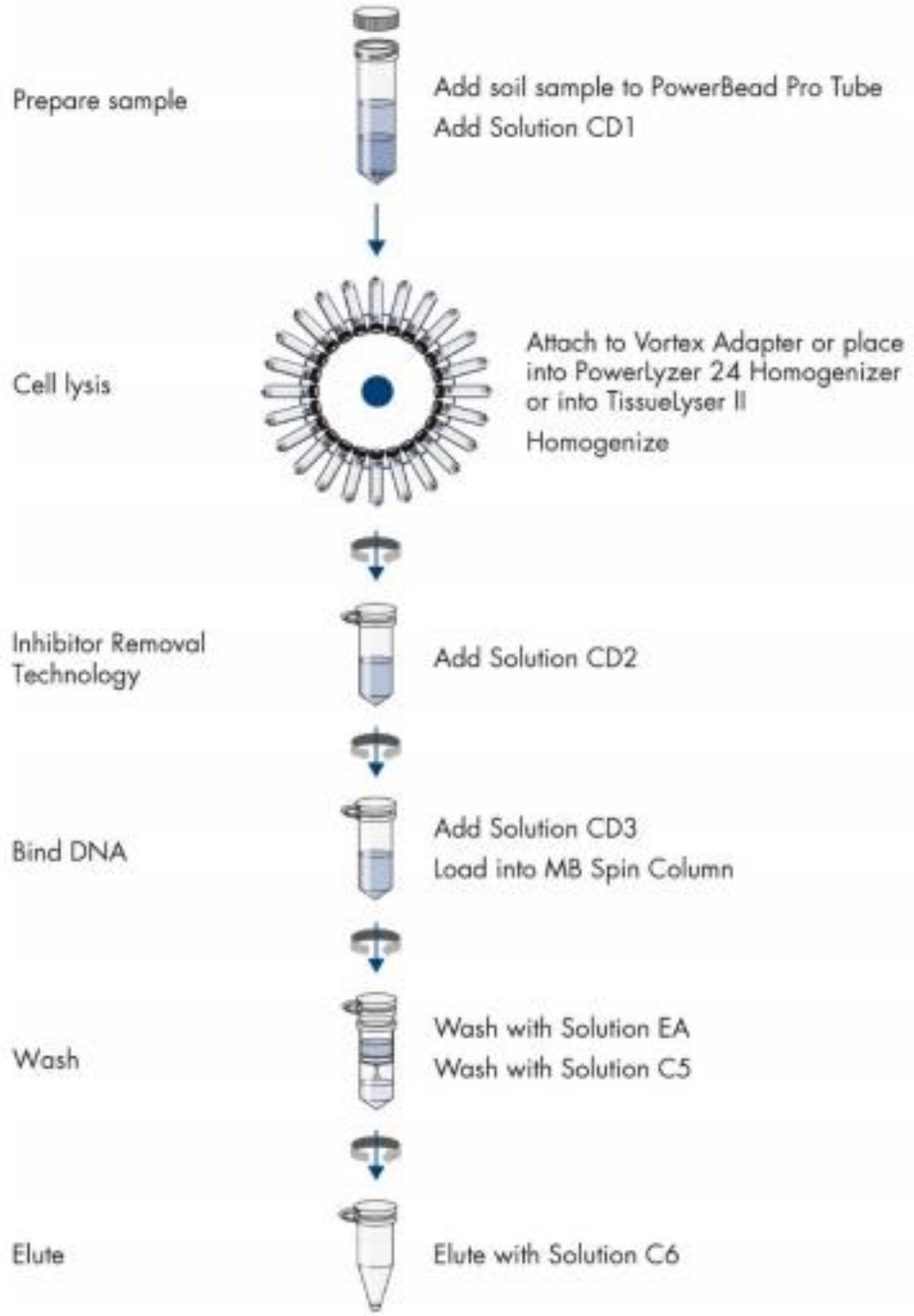




# DNA Extraction Materials and Methods

**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

FIU Applied Research Center

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

Adapter-ligated fragments are amplified via PCR and gel purified.

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

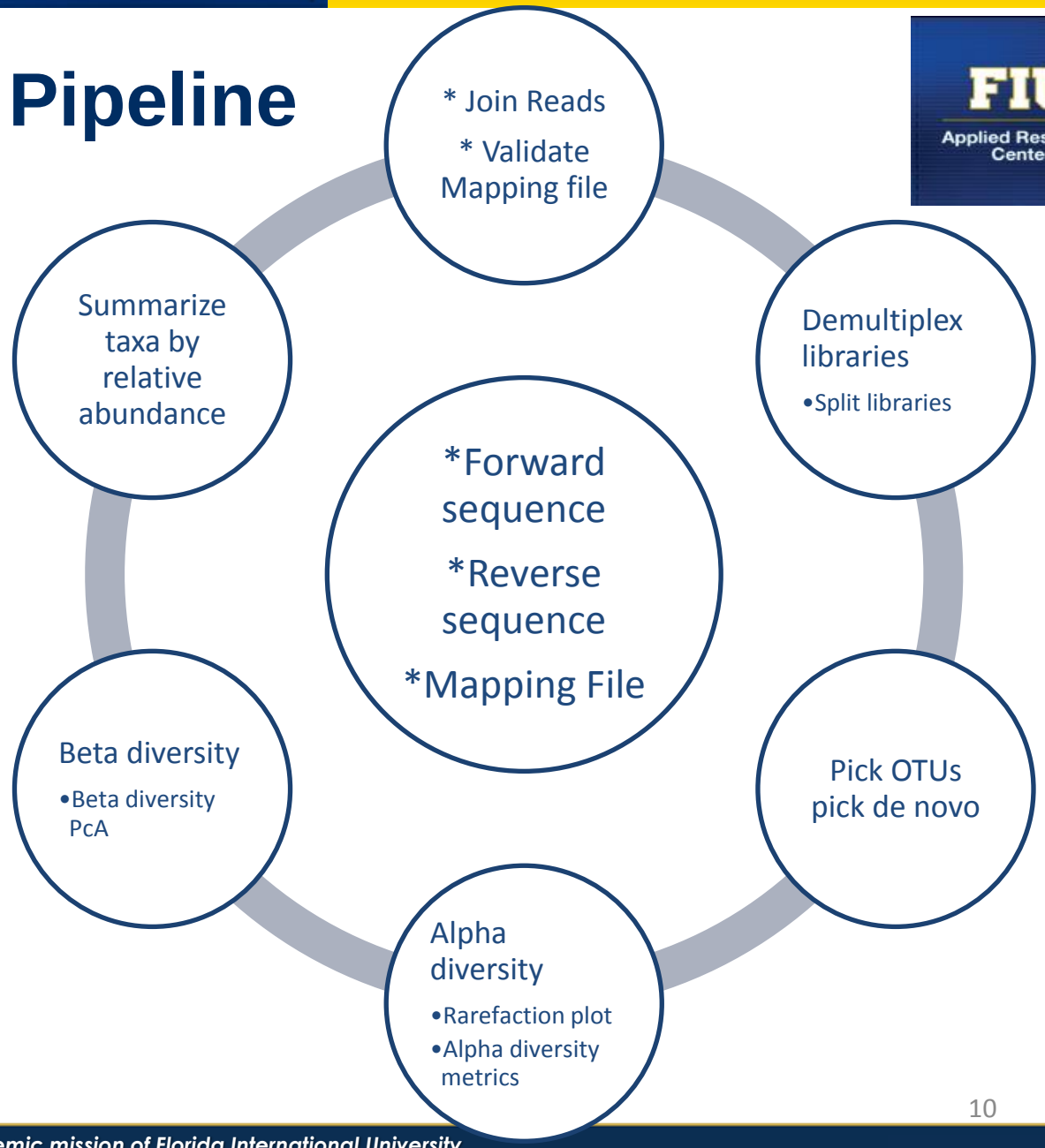
Quantification: Run pool through a qubit assay.

Amplification 16rRNA gene

# QIIME Pipeline

Opensource bioinformatic pipeline that enables researchers to:

1. Process raw DNA sequenced data
2. Joining the sequences
3. Validate the mapping file
4. Demultiplexing reads
5. Identification of OTUs
6. Phylogenetic diversity analysis and visualization



# Preliminary results

Basic statistics of OTU table.

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

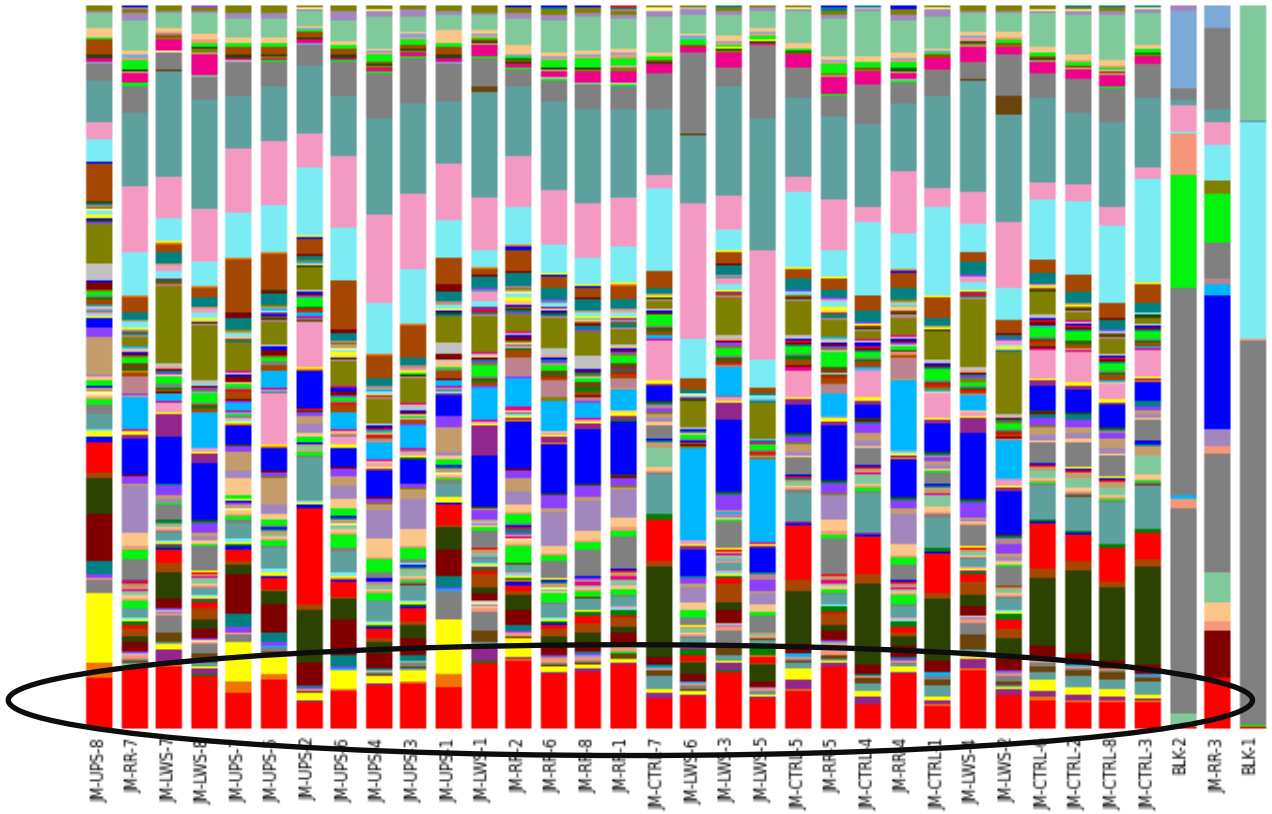
- Figure represents a summary OTU table.
- If the representative sequence file is counted. The same number of sequences should be displayed.

```
Num samples: 34
Num observations: 212448
Total count: 2690999
Table density (fraction of non-zero values): 0.062

Counts/sample summary:
 Min: 720.0
 Max: 123632.0
 Median: 84389.500
 Mean: 79147.029
 Std. dev.: 26585.576
 Sample Metadata Categories: None provided
 Observation Metadata Categories: taxonomy

Counts/sample detail:
 BLK-1: 720.0
 BLK-2: 13531.0
 JM-RR-3: 23213.0
 JM-UPS-8: 43665.0
 JM-UPS-4: 50258.0
 JM-CTRL-5: 61369.0
```

```
denovo15142     k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Gemmatales; f__Gemmataceae; g__; s__      1.00    3
denovo123377    Unassigned      1.00    1
denovo123376    k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__A31; f__; g__; s__     1.00    3
denovo123375    k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__BPC076; f__; g__; s__        1.00    3
denovo123374    Unassigned      1.00    1
denovo123373    k__Bacteria; p__Acidobacteria; c__[Chloracidobacteria]; o__11-24; f__; g__; s__ 1.00    3
denovo123372    k__Bacteria; p__Acidobacteria; c__Acidobacteriia; o__Acidobacteriales; f__Koribacteraceae; g__Candidatus Koribacter; s__        1.00    3
denovo123371    k__Bacteria; p__Acidobacteria; c__Solibacteres; o__Solibacterales; f__; g__; s__        0.67    3
denovo123370    k__Bacteria; p__Acidobacteria; c__DA052; o__Ellin6513; f__; g__; s__    1.00    3
denovo123379    k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__; f__; g__; s__  1.00    3
denovo123378    k__Bacteria; p__NC10; c__12-24; o__JH-WHS47; f__; g__; s__      0.67    3
denovo41472     k__Bacteria; p__Chloroflexi; c__Dehalococcoidetes; o__Dehalococcoidales; f__Dehalococcoidaceae; g__; s__        1.00    3
denovo41473     k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Alteromonadales; f__211ds20; g__; s__        0.67    3
denovo41470     k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Planctomycetales; f__Planctomycetaceae; g__Planctomyces; s__      1.00    3
denovo41471     k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__Gaiellales; f__Gaiellaceae; g__; s__     1.00    3
denovo41476     Unassigned      1.00    1
denovo41477     Unassigned      1.00    1
denovo41474     k__Bacteria; p__Acidobacteria; c__Acidobacteriia; o__Acidobacteriales; f__Koribacteraceae; g__; s__     0.67    3
denovo41475     k__Bacteria; p__Verrucomicrobia; c__[Pedosphaerae]; o__[Pedosphaerales]; f__Ellin515; g__; s__  1.00    3
denovo41478     k__Bacteria; p__OP3; c__koll11; o__; f__; g__; s__      1.00    3
denovo41479     k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__       1.00    3
denovo15140     k__Bacteria; p__Acidobacteria; c__Solibacteres; o__Solibacterales; f__Solibacteraceae; g__Candidatus Solibacter; s__    1.00    3
```

# Summarize communities by taxonomic composition

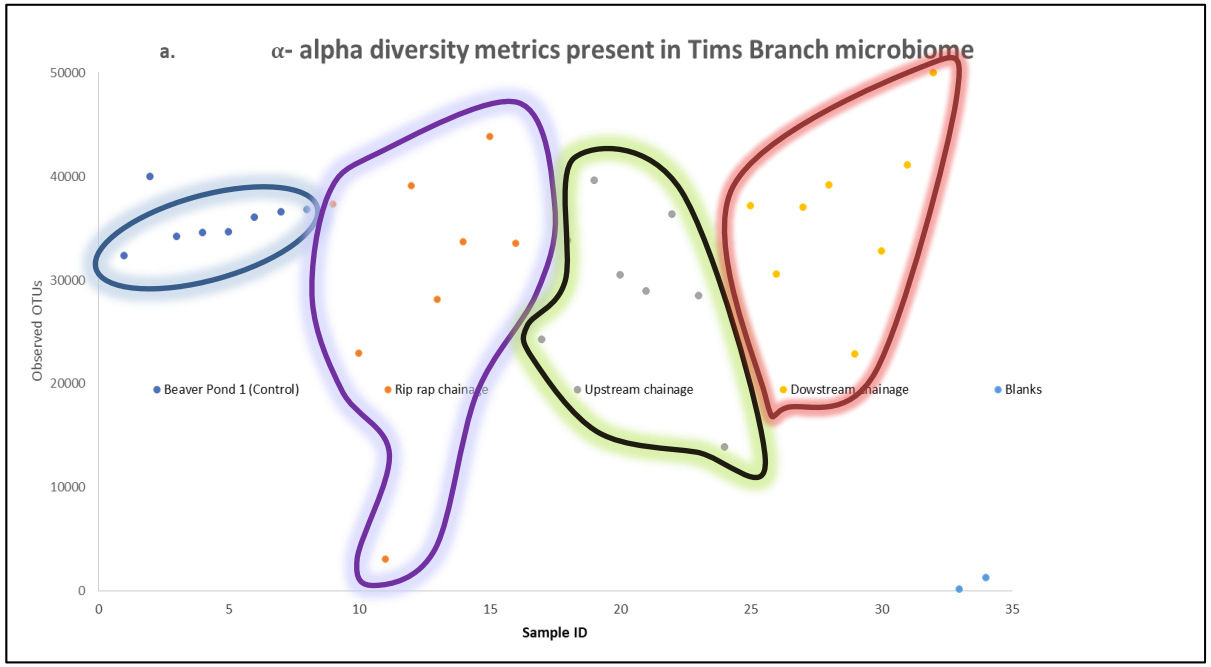
- The degree of sharing microbial taxa in 34 samples from 4 different location points.
- The summary microbial composition by sample appears to have Acidobacteria-5 and 6 in greater amounts.

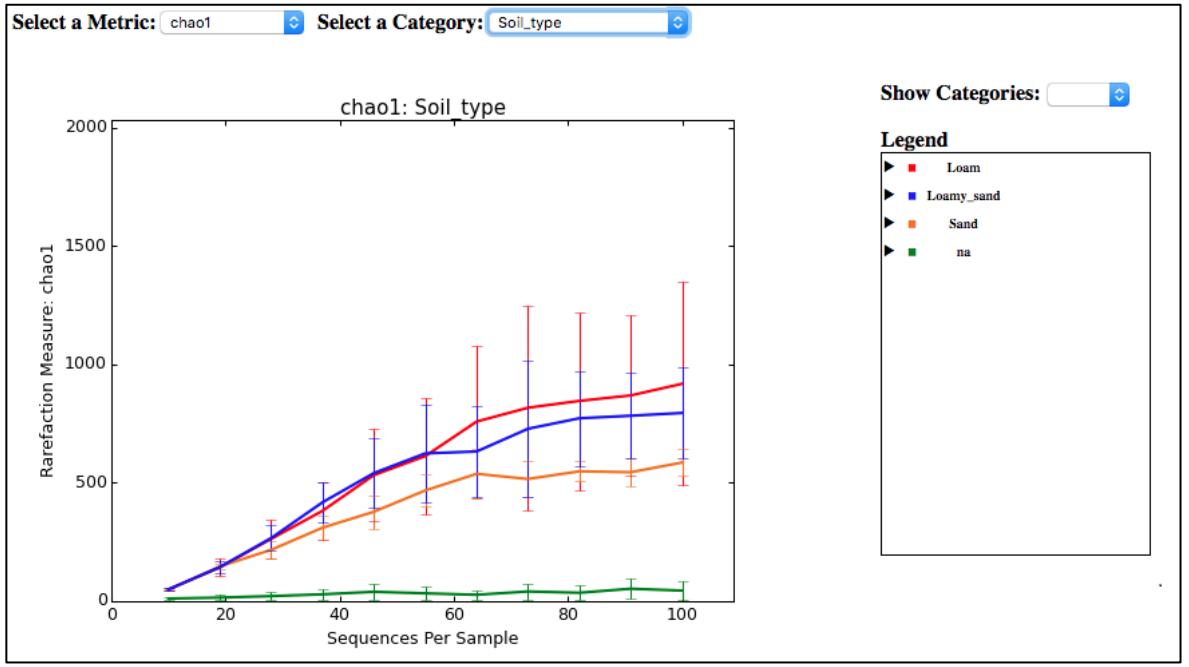


# Investigating Alpha Diversity

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

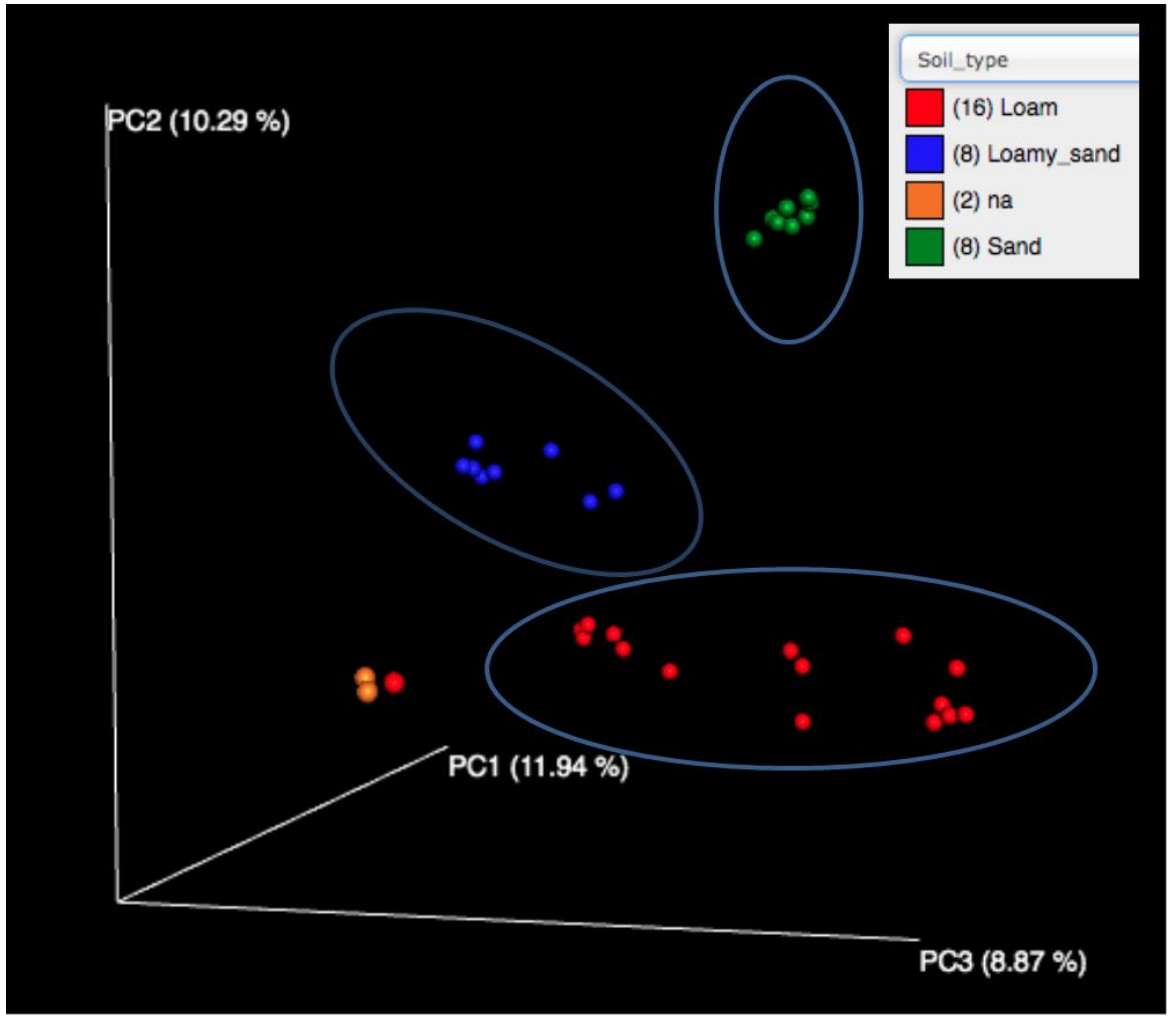
1. Identifies how many species are in each sample
2. Chao1 diversity metric predicts the OTUs richness at high depth sequencing
3. Rarefaction curve identifies how many sequences are necessary to capture most of the microbial diversities



# 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.

# Acknowledgements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