How Does Pipe Material Affect the Diversity of Microbial Communities in Drinking Water Distribution Systems?

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Biofilms in Drinking Water Distribution Systems

Biofilms in DWDS

- Ubiquitous and persistent, these communities form on virtually any surface in contact with water
- Affect the turbidity, taste, odor, and color of the water, and promote decay of residual disinfectants
- Secrete acid metabolites that corrode cast iron and cement pipes
- Pose a major health threat by harboring pathogens
    - Contaminated water is responsible for over 40,000 hospitalizations per year in the U.S. alone (Lui et. al, 2016)

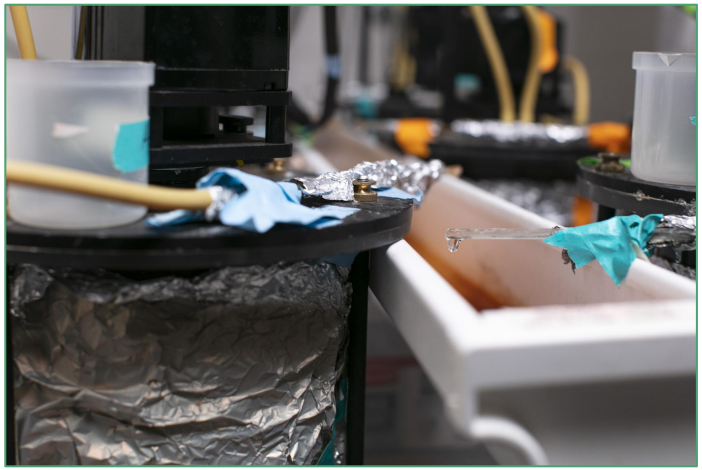
The goal: Characterize the community composition of biofilms in DWDS and investigate the factors that influence their growth and development

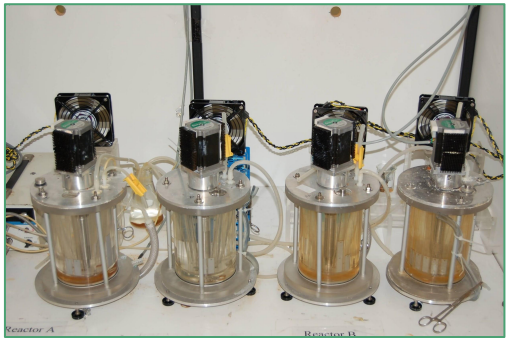
The Experiment

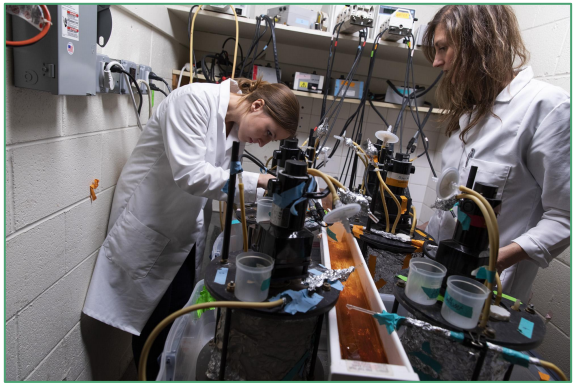
The Experiment

- Two sets of three annular reactors (ARs) were connected to a city water main
- Each AR contained 20 coupons made of pipe material upon which biofilm could accumulate
  - 3 ARs with **Cast Iron** Coupons
  - 3 ARs with **Cement** Coupons
- A steady state biofilm was allowed to develop
- After steady state biofilm formation, a coupon from each set was aseptically sampled weekly for 40 weeks
- High-throughput sequencing of the 16s rRNA V3-V4 region was used to characterize microbial communities (MiSeq 2x300 bp; ≅460 bp amplicon)

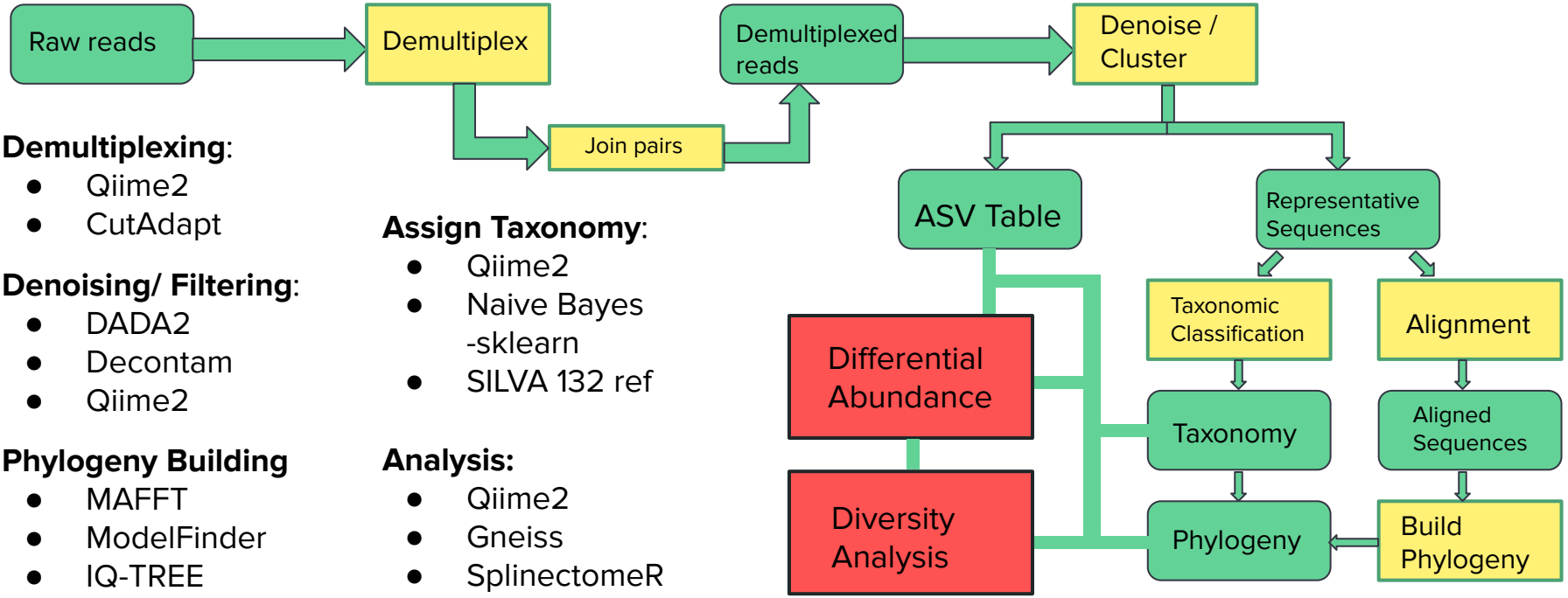
Experimental Setup







Amplicon Workflow



Demultiplexing:
- Qiime2
- CutAdapt

Denoising/ Filtering:
- DADA2
- Decontam
- Qiime2

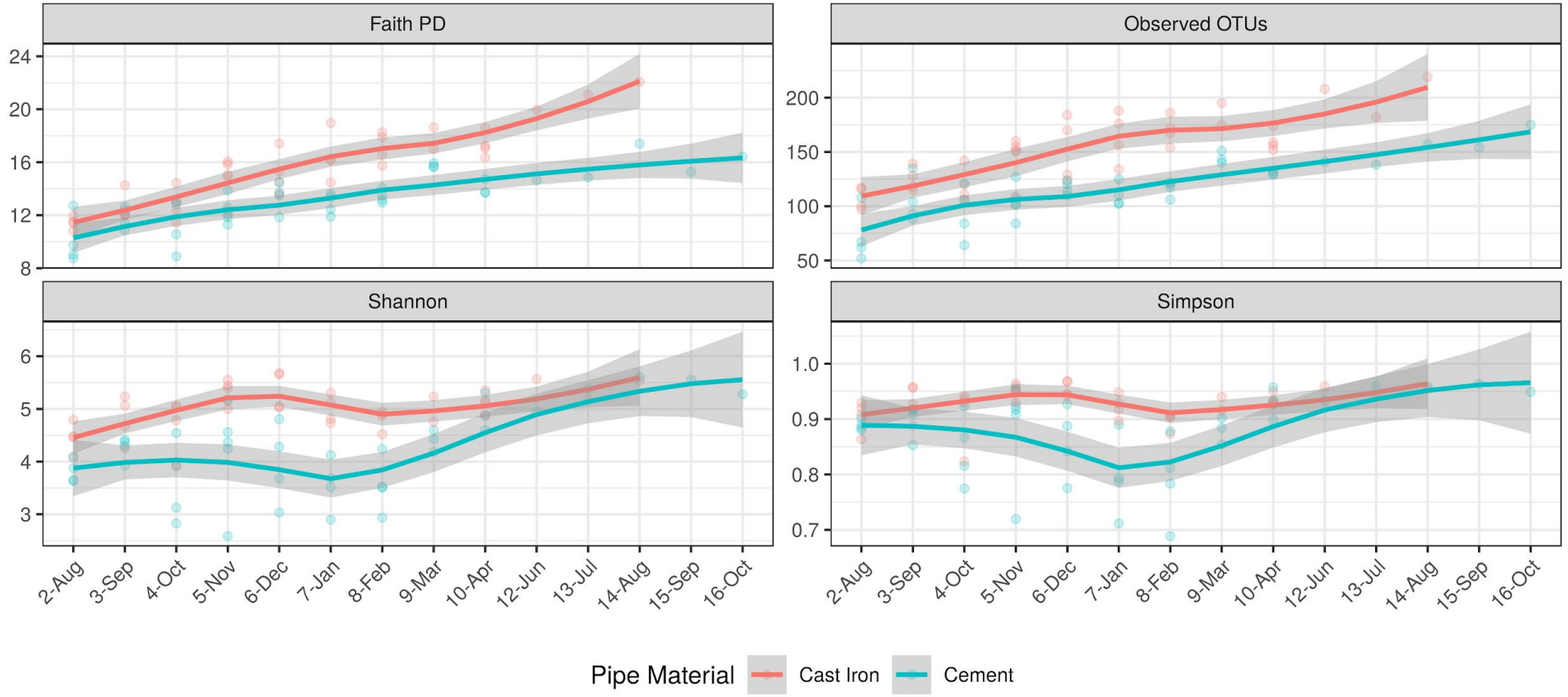
Phylogeny Building
- MAFFT
- ModelFinder
- IQ-TREE

Assign Taxonomy:
- Qiime2
- Naive Bayes -sklearn
- SILVA 132 ref

Analysis:
- Qiime2
- Gneiss
- SplinectomeR

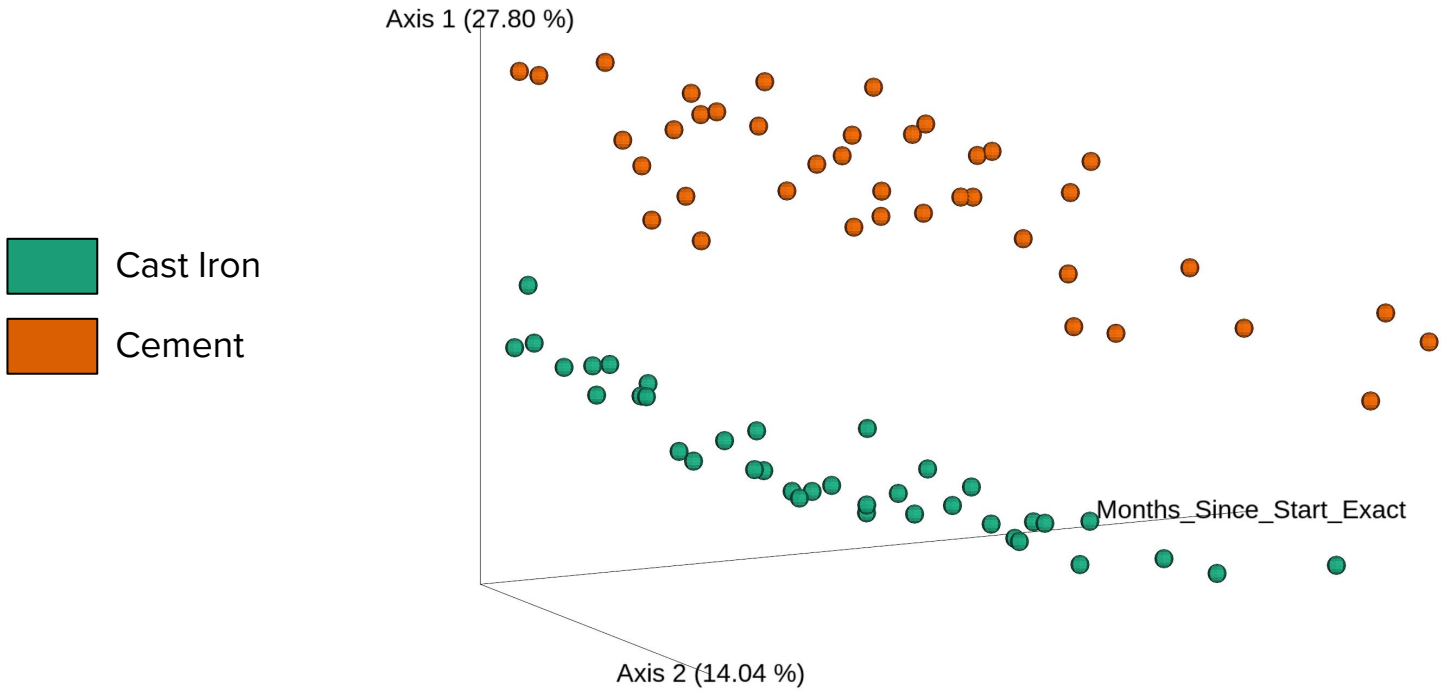
Results - Alpha Diversity

α-Diversity Increases Over Time in Both Pipe Materials

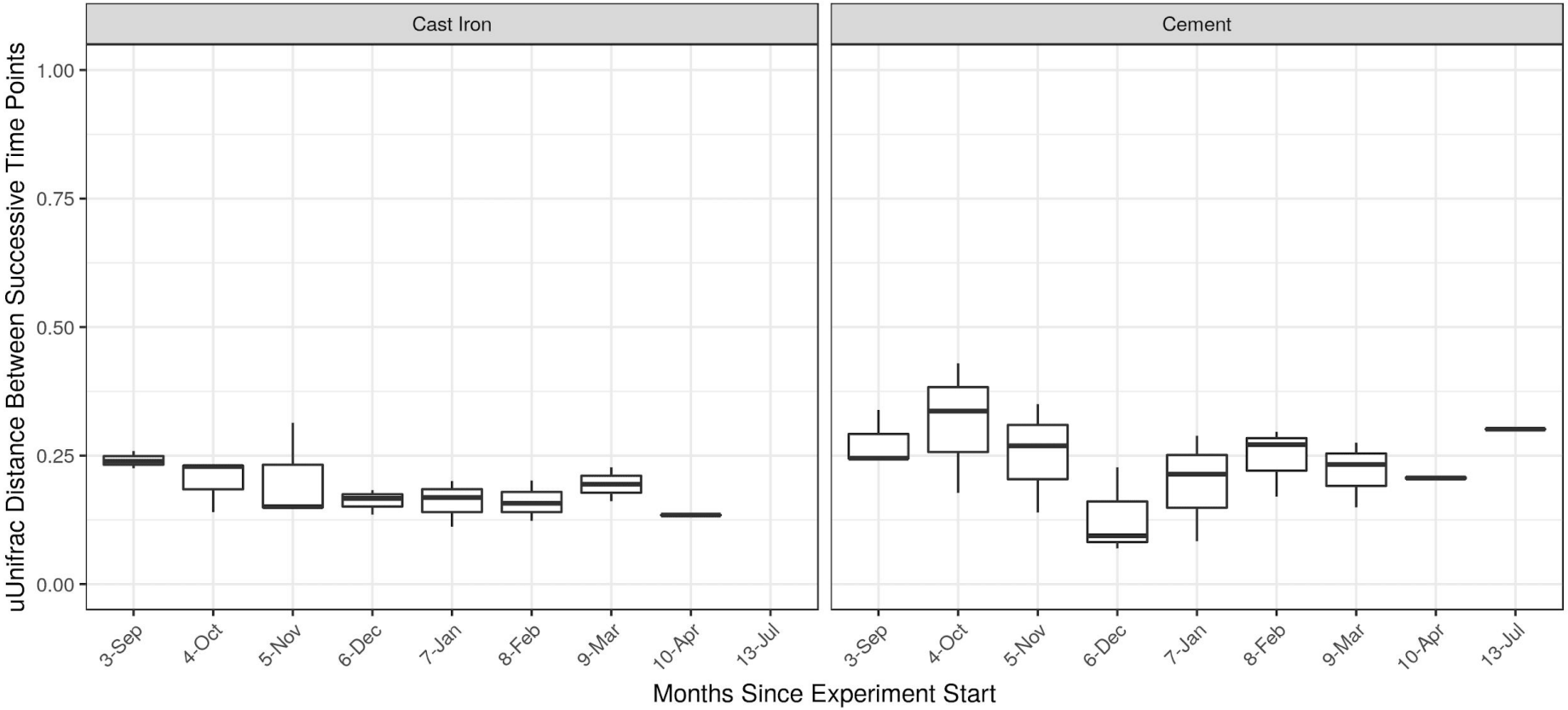


Results - Beta Diversity

Cast Iron and Cement Communities are Compositionally Different



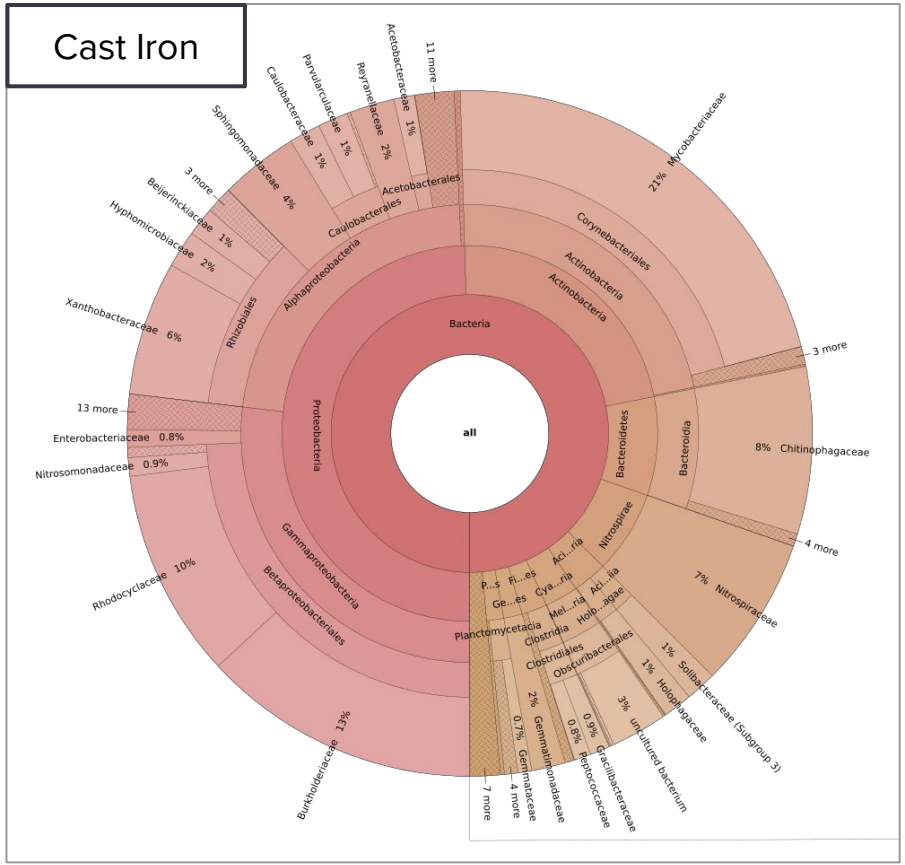
Rate of Change in Each Community is Constant - Distances Between Successive Time Points



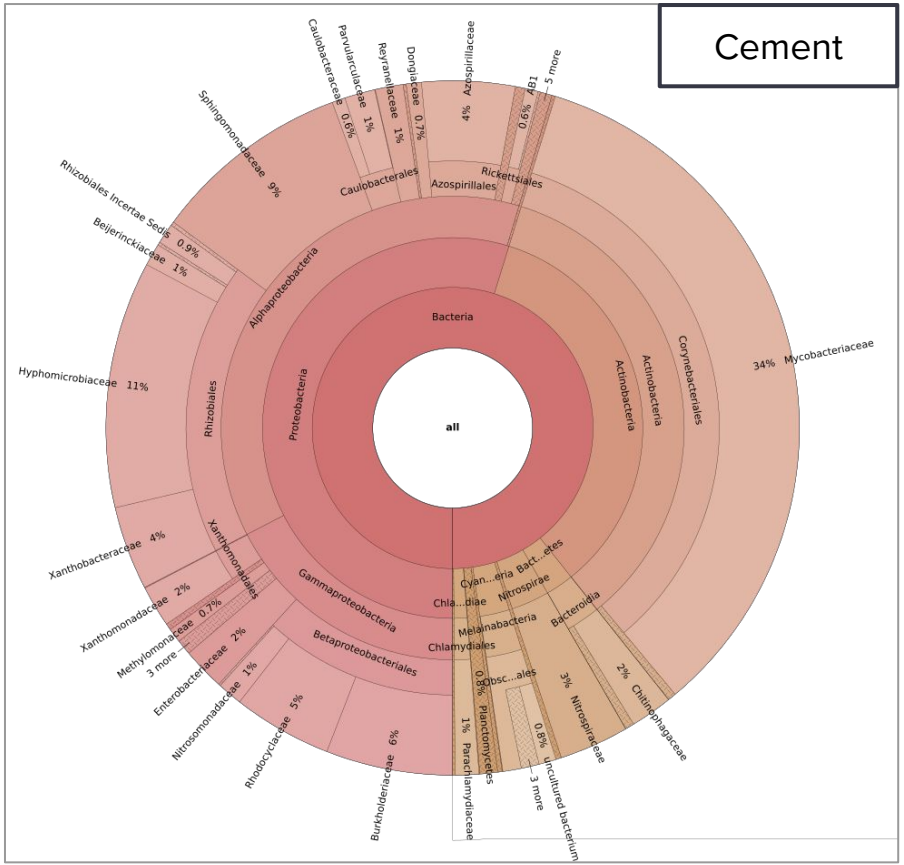
Results - Differential Abundance

Krona Plots Show Overall Structure

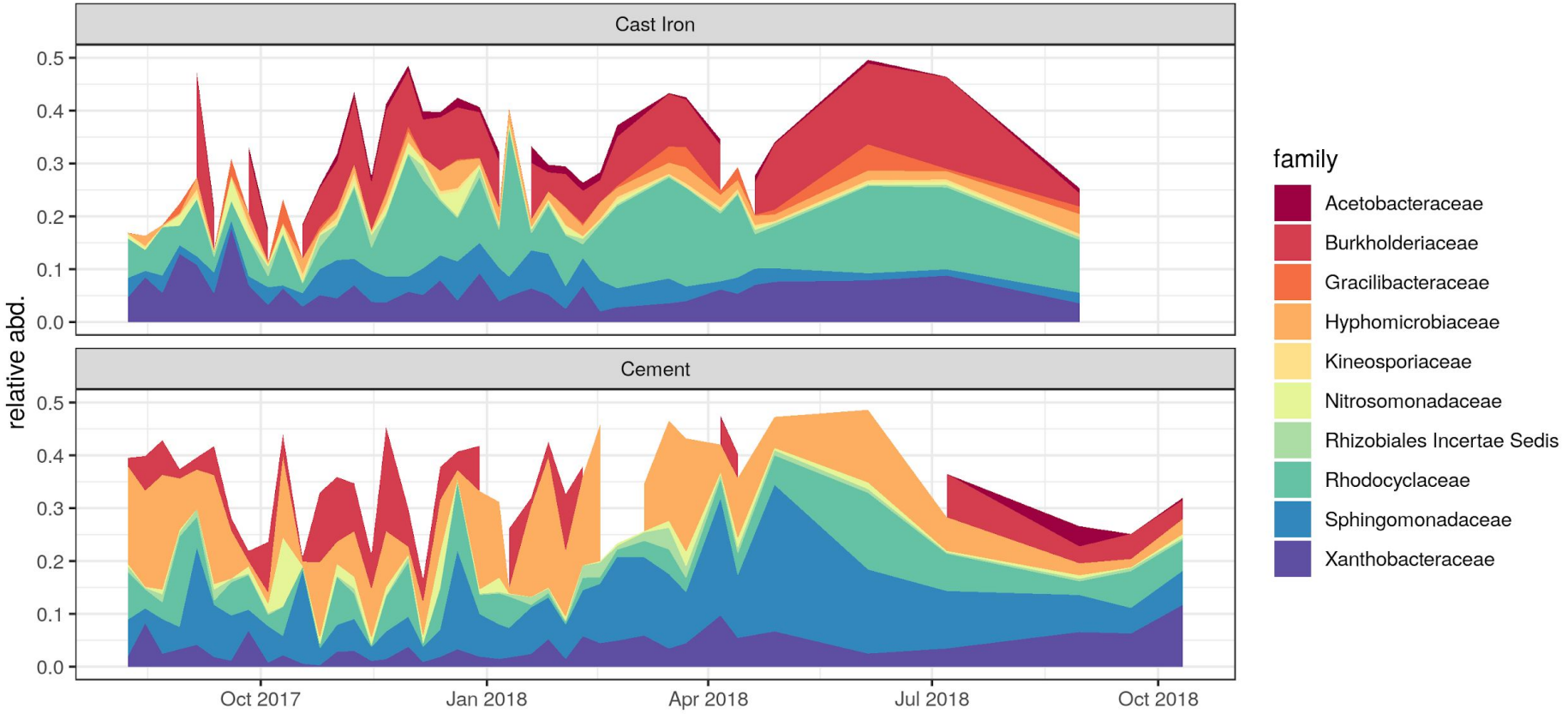
Cast Iron



Cement



Relative Abundance of Differentially Abundant Taxa Over Time

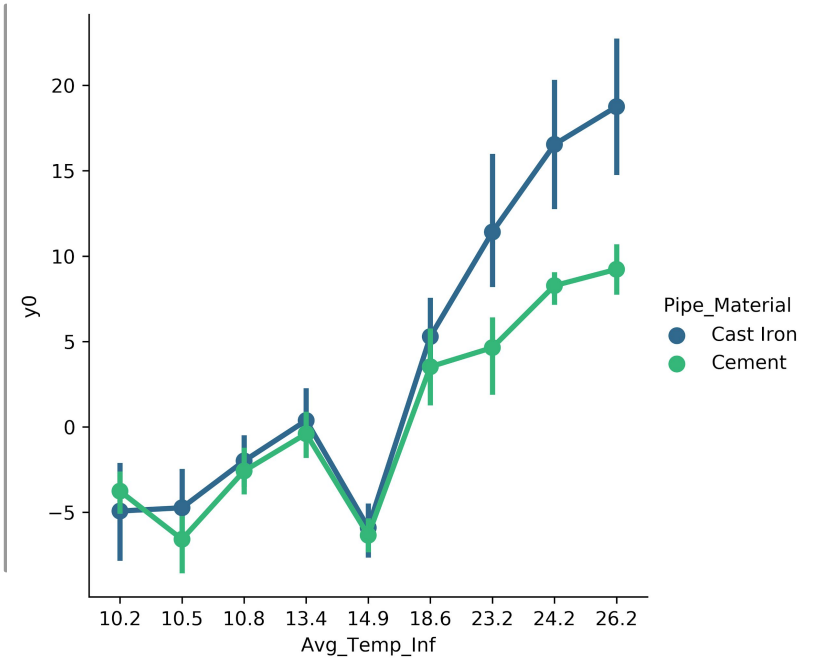


Water Quality & Microbial Niche Differentiation

Balance Trees Reveal Ecological Niche Differentiation

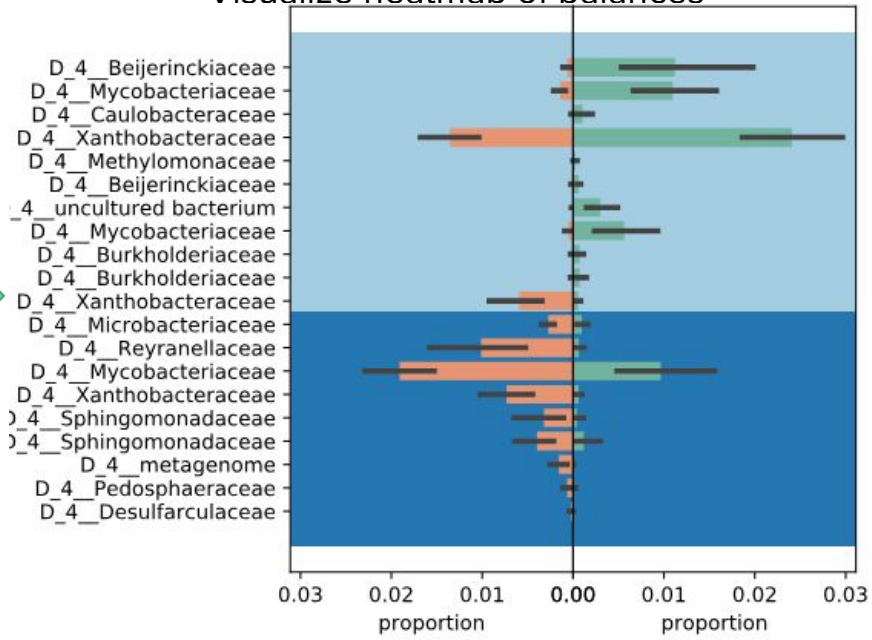
Create Balance Tree

Visualize y0 Balance over temperature gradient



Identify numerator and denominator taxa

Visualize heatmap of balances



< 17.5 C

> 17.5 C

Conclusions

Putting it all together...

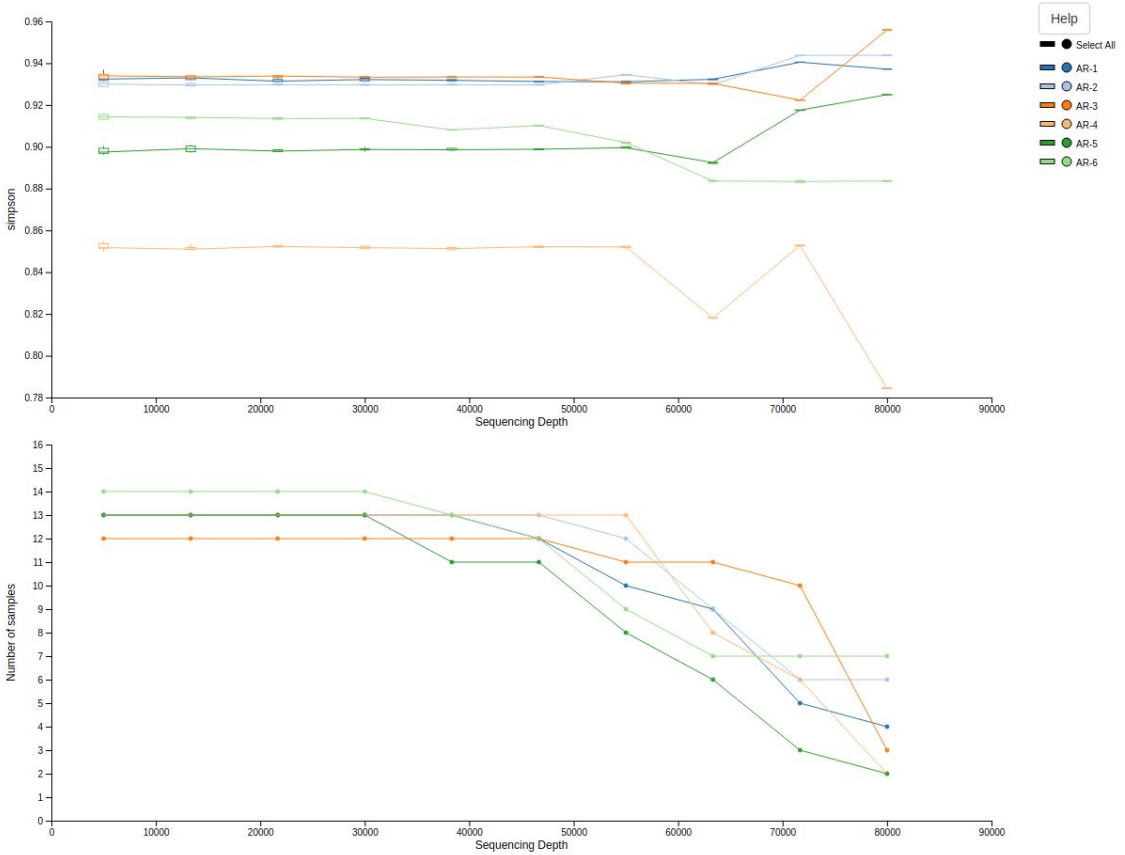
- Cast Iron and Cement pipe materials develop different communities
- These communities change at a fairly constant rate and increase in diversity over time
- Biofilm communities share many of the same taxa but differ in significant ways
  - β- and γ-Proteobacteria dominate Cast Iron but not Cement
    - β-Proteobacteria may serve as prey for host amoebae of *L. pneumophila*
  - α-Proteobacteria dominate Cement but not Cast Iron
    - α-Proteobacteria are slower growing and more amoebae resistant
  - *Legionella* were observed in both pipe materials but at higher relative abundances in cast iron
- Water quality may affect biofilm composition
  - Higher temperatures increase the concentration of easily assimilable organic compounds promoting biofilm growth
  - The result is that nitrogen-fixing bacteria begin to proliferate (especially in cast iron) at higher temperatures

Thank You!

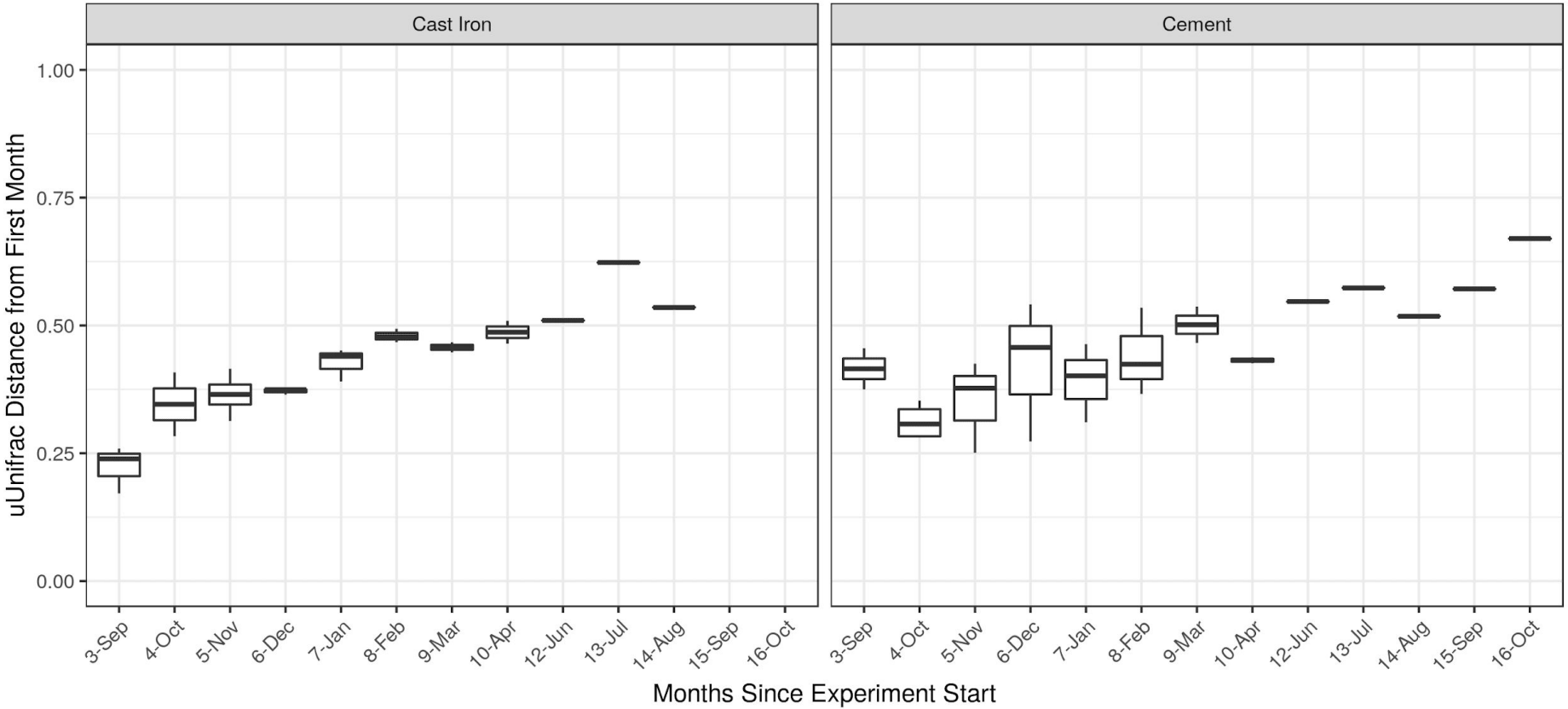
- Dr. Murphy & the Murphy Lab
- Dr. Debbie Lee
- Dr. Rebekah Henry- Monash University, Melbourne, AU
- PSM Steering Committee

Supplementary Slides

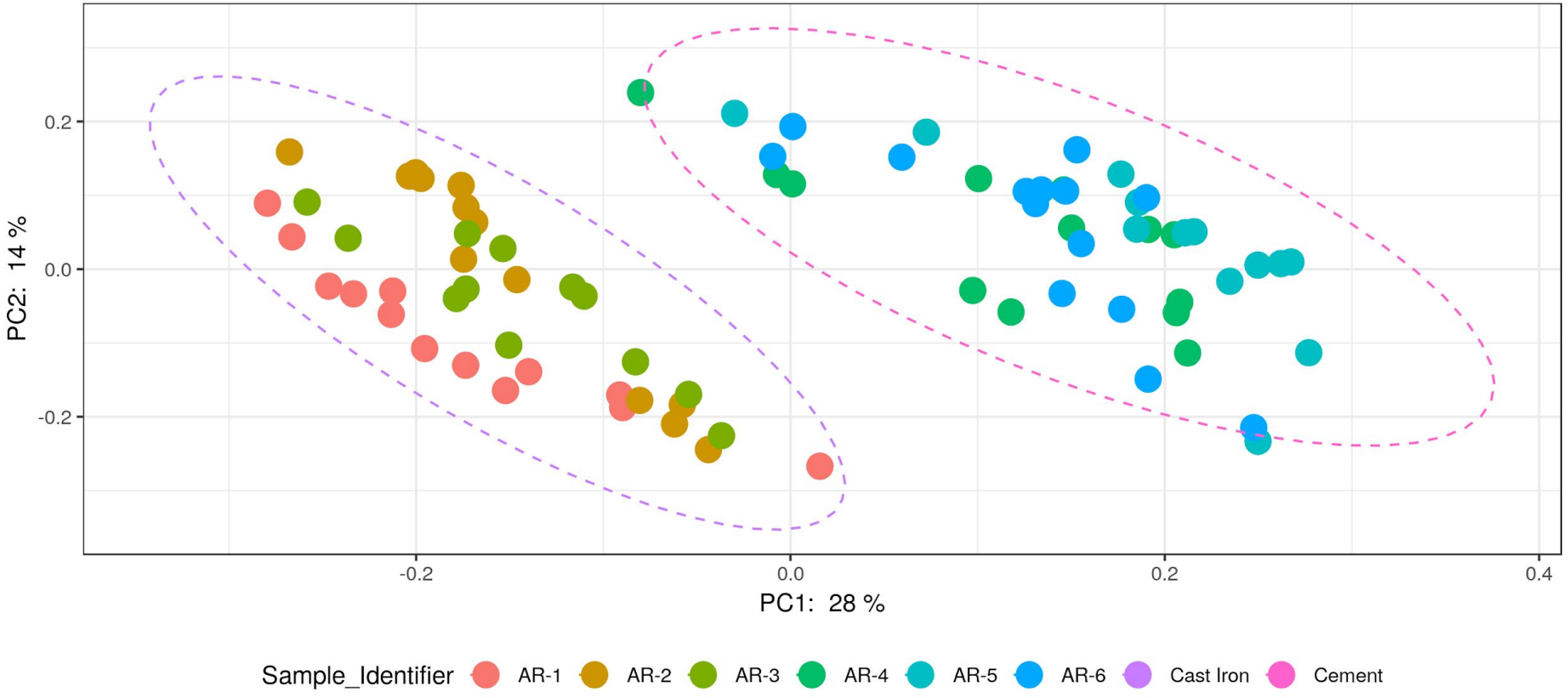
Alpha Rarefaction - Did we capture as much diversity as possible?



Rate of Change in Each Community is Constant - Distances from First Month



PCoA Plot of uUnifrac Distances



PCoA Plot of Bray-Curtis Dissimilarity