Phylogenetic Analysis of the Metabolically Active Fraction of FRC Sediment Microbial Communities

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Overall project objectives

- Quantify microbial activity using geochemical analysis of groundwater/ sediments (push-pull activity tests)
  - Jack Istok's lab
- To determine structure/ function relationships of metal-reducing bacteria and competing heterotrophs during *in situ* bioremediation in acidic subsurface environments
  - Lainie Petrie's research (Joel Kostka's lab)
- In parallel, quantify the change in the abundance/ diversity of sedimentary microbial communities using cultivation-independent methods
  - Focus on site/sample specific differences between the metabolically active fractions of the microbial communities

Sediment sample characteristics

Area 1, FRC

Sample	pH	Nitrate	Fe- oxalate extract	Fe -HCl extract
61-01-00	**6.7**	**0.6**	**31.5**	**27.6**
61-01-24	**6.1**	**0.1**	**17**	**1.8**
61-02-00	3.9	25.4	30.4	1.6
61-02-24	3.9	15.9	21.6	3.6
61-03-00	3.9	17.8	17.3	3
61-03-24	3.7	40.1	18.6	5.5
61-04-00	3.7	29.8	9.6	12.3
61-04-22	3.5	73.1	196	4.2
61-05-00	3.7	36	25.3	5.2
61-05-22	**4.1**	**35.2**	**12.1**	**22.8**
61-05-22(bottom)	4.4	23.3	7.4	18.2

All measurements besides pH are μmoles/gram

Lainie Petrie's Area 1 slurries

- Biostimulation clearly demonstrated
- Activity and geochemistry varies even within core
- Microbial groups display similar heterogeneity?

Conclusions from slurry experiments

- Metabolism inhibited under acidic conditions (pH 4)
- Electron donor/ acceptor appear to be secondary controls to pH
- Electron donors vary in their biostimulation potential:

  Glucose > Ethanol > Lactate > Hydrogen
- Nitrate and Fe(III) reduction are dominant TEAPs
- Acidic conditions shift nitrate-reducers toward incomplete reduction pathways, away from complete denitrification

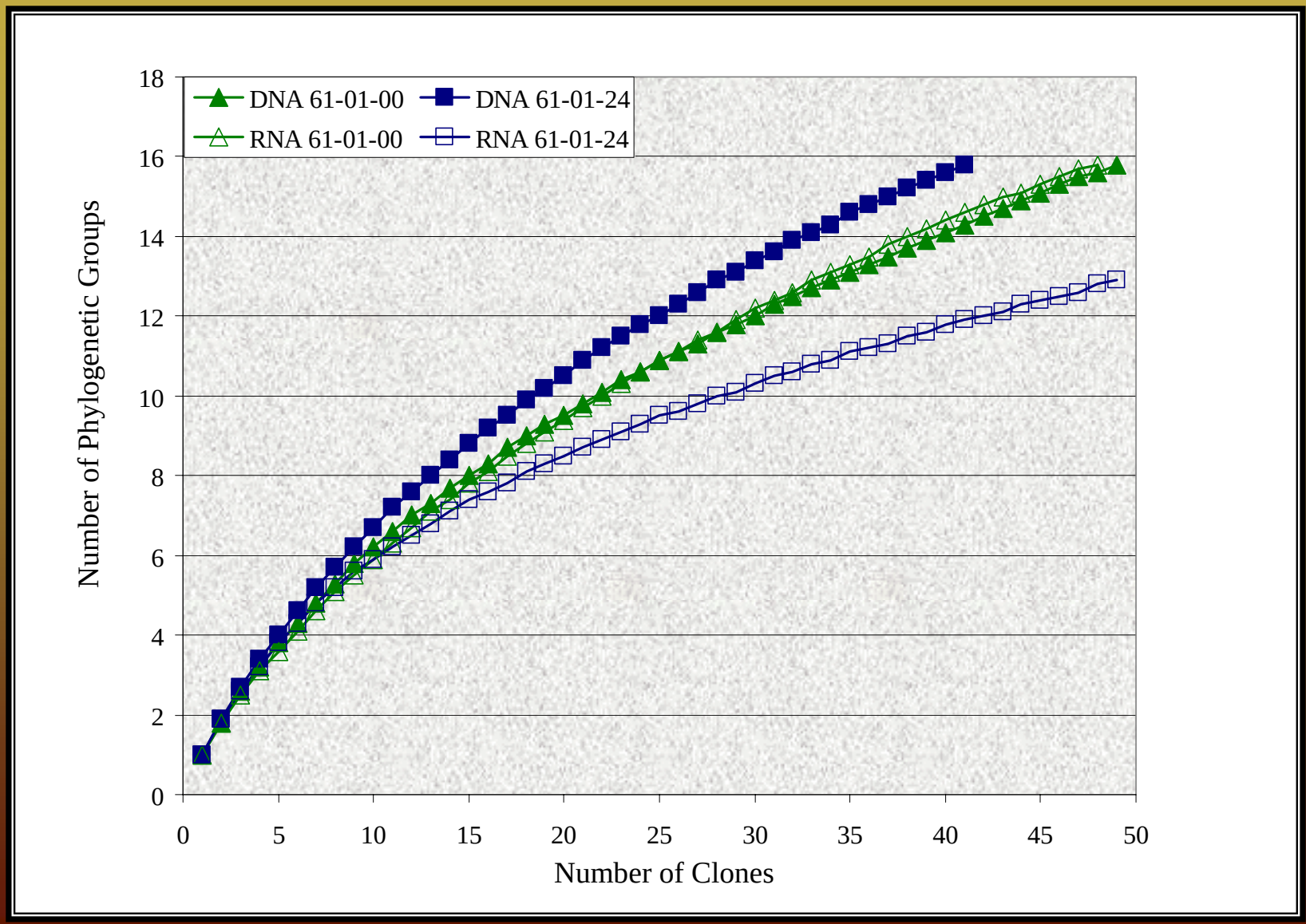
Sample collection and processing

- Sediment samples were collected using the geoprobe at a depth of:
  - 61-01-00 – 8-10 ft
  - 61-01-24 – 10-12 ft
- Total DNA and RNA was extracted using the method described in Hurt et al. 2001. AEM 67:4495-4503.
- DNA and RNA was separated and purified using Qiagen RNA/DNA system tip 500

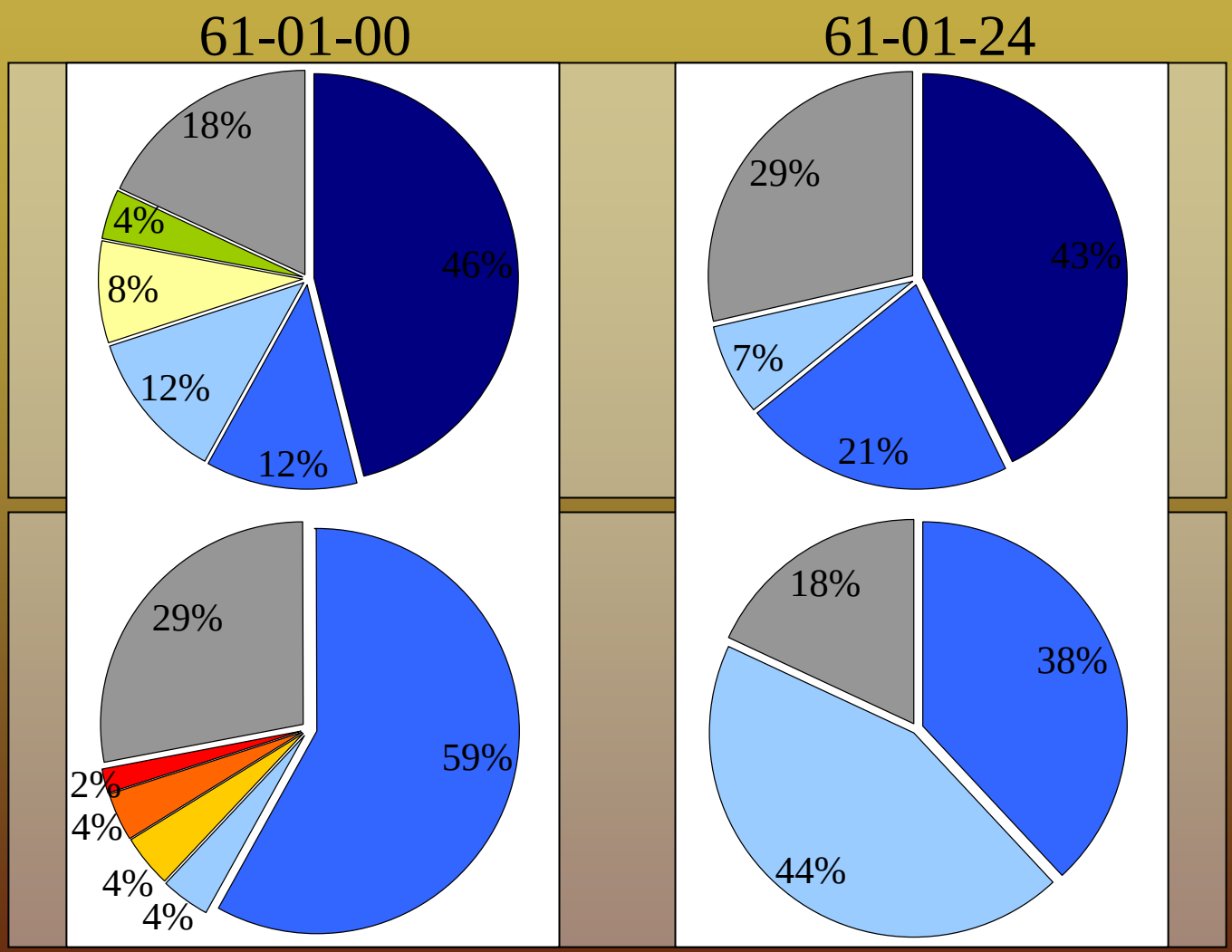
Clone library statistics

	Sample	No. of clones analyzed	No. of phylotypes	Percent coverage
DNA-derived	61-01-00	50	16	82.0%
	61-01-24	42	16	81.0%
RNA-derived	61-01-00	49	16	83.7%
	61-01-24	50	12	88.0%

Rarefaction analysis



Clone library analysis



Lineages linked to nitrogen cycling

- Denitrification (24% - RNA; 25% - DNA)
  - *Alphaproteobacteria*
    - *Sphingomonadaceae* (5% - DNA)
  - *Betaproteobacteria*
    - *Alcaligenaceae* (24% -RNA)
    - *Comamonadaceae* (7% - DNA)
  - *Gammaproteobacteria*
    - *Moraxellaceae* (13% - DNA)
- Nitrogen fixation (42% - DNA)
  - *Alphaproteobacteria*
    - *Methylobacteriaceae* (35% - DNA)
  - *Betaproteobacteria*
    - *Oxalobacteraceae* (7% - DNA)

Direction of future work

- Additional nucleic acid extractions from sediments with varying pH and nitrate concentrations
- Characterize the active fraction of the microbial communities present at the end of the slurry incubations
- Target specific functional genes for nitrification (amoA) and denitrification (nosZ)
- Propose a model for bioremediation potential of FRC sediments based on *in situ* and phylogenetic data