Analysis of Microbial Community Composition and Activity in Sediments from Area 1 and Area 2

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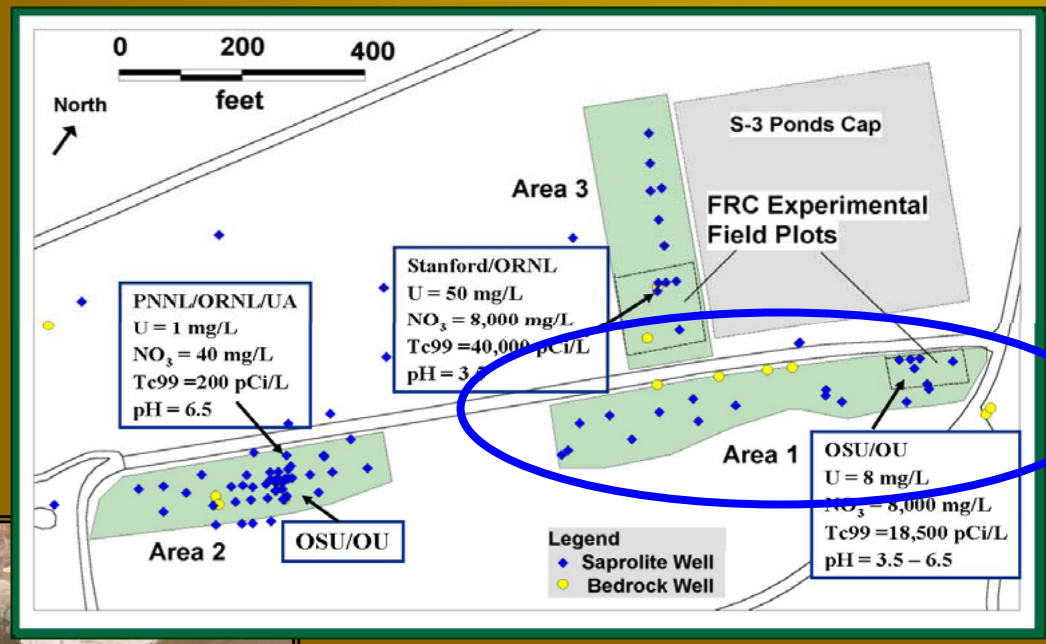
Project 1: Microbial Community Analysis of Sediments from Area 1 Based on SSU rRNA Clone Libraries

Project 2: Microbial Activity and Community Composition in Microcosms of FRC Area 2 Uranium Contaminated Subsurface Sediment and Groundwater

Project 1:
Community Analysis In Unamended Area 1 Sediments



Sediment characteristics of Area 1 borehole FW61

Depth Interval	Depth (m)	pH	Nitrate[1]	Fe-oxalate extract[1]	Nitrate reduction rates[2]	Fe(II) production rates[2]
61-01-00	2.4-3.1	6.7	0.6	31.5	0.70 - 2.84	0.00 - 1.44
61-01-24	3.1-3.7	6.1	0.1	17.0	0.70 - 1.30	0.01 - 0.25
61-03-00	4.9-5.5	3.9	17.8	17.3	0.70 - 1.30	ND[3]
61-03-25	5.5-6.1	3.7	40.1	18.6	0.01 - 2.84	0.00 - 0.82

[1]Units in µmol g^{-1}. [2]Units in µmol g^{-1} d^{-1}, data reported in Petrie et al. in review. [3]Not determined.

From these samples, SSU rRNA (RNA-derived) and SSU rRNA gene (DNA-derived) clone libraries were constructed.

Statistical Indices

Target	Primer Set	Samples	No. of Clones	OTUs[1]	Species Richness	Shannon Weiner[2]	1/D[3]	Percent Coverage[4]	$\theta(\pi)$[5]	Nucleotide Diversity[6]	Gene Diversity[7]
DNA	27F/1392R	61-01-00	90	20	29 (22, 56)[8]	2.09	4.22	90.0	172.8 ± 82.7[9]	0.15 ± 0.07	0.76 ± 0.04
		61-01-24	77	20	27 (22, 49)	2.19	4.72	88.3	167.4 ± 80.3	0.14 ± 0.07	0.79 ± 0.05
		61-03-00	62	11	21 (13, 63)	1.86	5.25	91.9	172.3 ± 82.9	0.15 ± 0.07	0.81 ± 0.03
		61-03-25	109	13	14 (13, 21)	1.98	5.78	97.3	204.3 ± 97.6	0.18 ± 0.08	0.83 ± 0.02
RNA	1055F/1392R	RR61-01-00	36	7	7 (7, 7)	1.39	2.93	97.2	22.78 ± 11.32	0.06 ± 0.03	0.73 ± 0.04
		RR61-01-24	43	8	11 (8, 33)	1.72	5.13	93.0	21.69 ± 10.83	0.06 ± 0.03	0.81 ± 0.03
	27F/518R	R61-01-00	14	6	8 (6, 21)	1.57	5.35	78.6	68.32 ± 35.22	0.16 ± 0.08	0.81 ± 0.07
		R61-03-00	23	16	30 (20, 63)	2.67	28.11	52.2	88.36 ± 43.99	0.19 ± 0.09	0.96 ± 0.02
		R61-03-25	43	6	7 (6, 16)	0.7	1.43	93.0	32.12 ± 15.87	32.1 ± 14.3	0.30 ± 0.09

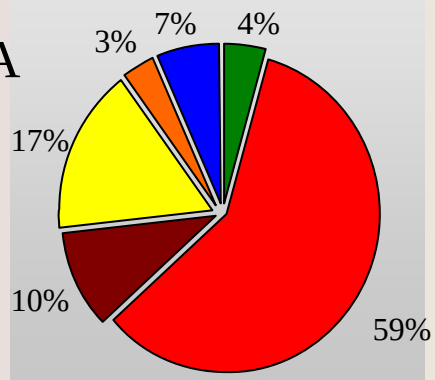
- **Rarefaction, species richness, and percent coverage estimators suggest an adequately sampled clone library for each sample**
- **DNA-derived clone libraries were more diverse in terms of species detected however, both RNA- and DNA-derived nucleotide and gene diversity estimators were similar**

Neutral pH Sediments

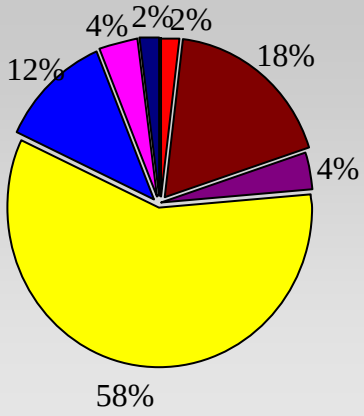
Acidic pH Sediments

61-01-00

DNA

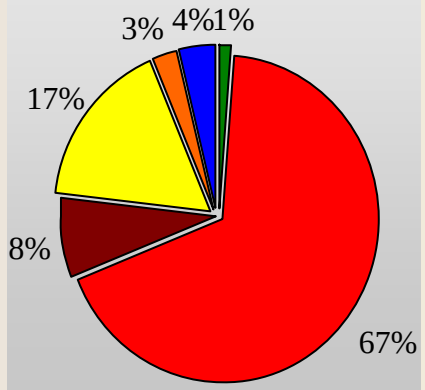


RNA

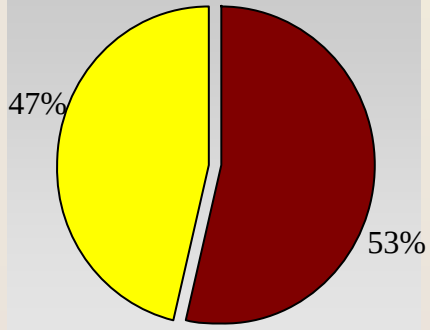


61-01-24

DNA

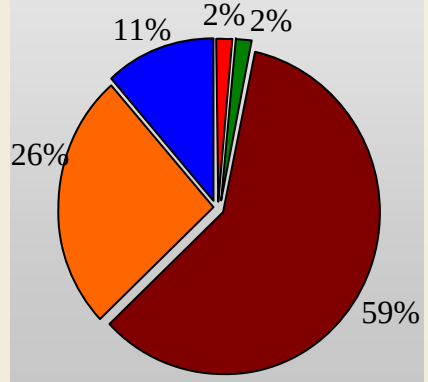


RNA

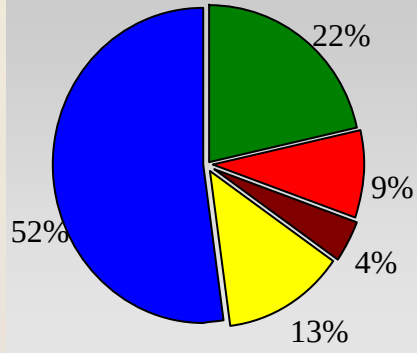


61-03-00

DNA

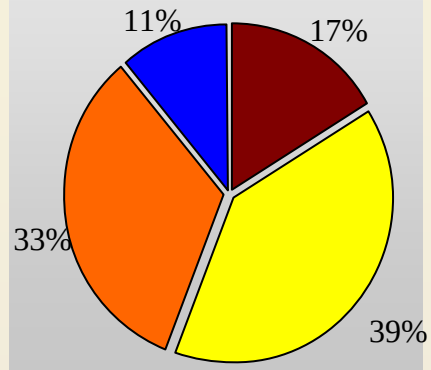


RNA

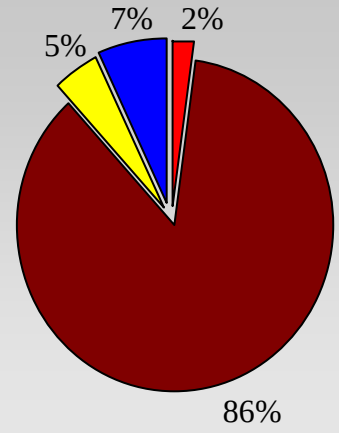


61-03-25

DNA



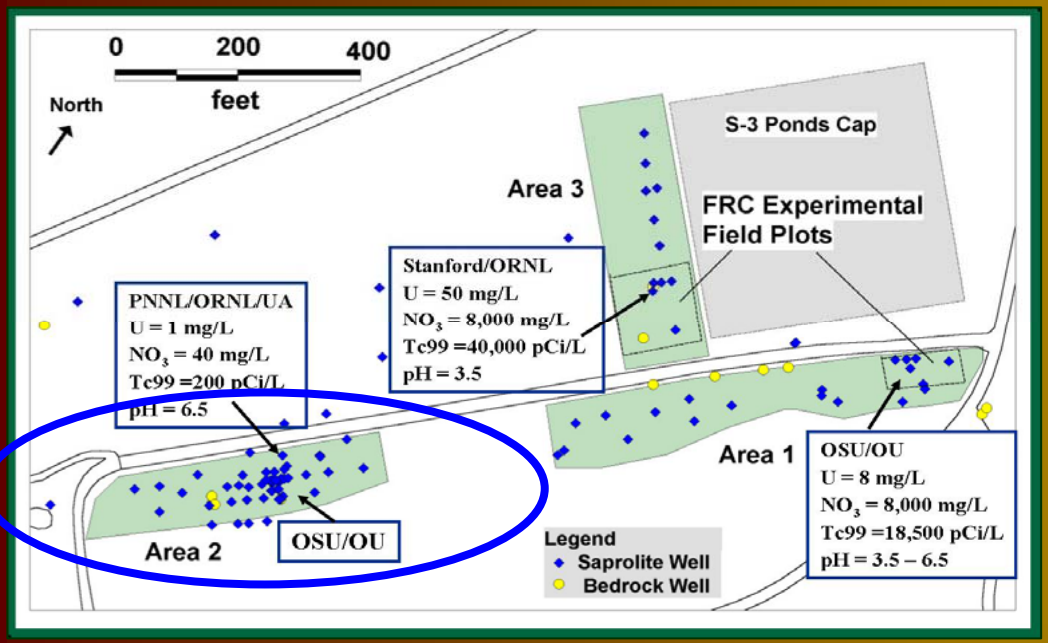
RNA



- *Actinobacteria*
- *Alphaproteobacteria*
- *Betaproteobacteria*
- *Deltaproteobacteria*
- *Gammaproteobacteria*
- *Bacteroides*
- *Firmicutes*
- *Planctomycetes*
- Unclassified

Observed Trends

- Numerous phylotypes had high sequence similarity to cultured organisms capable of nitrate reduction and clones from other FRC studies of groundwater and sediments microbial communities
  - Should these be the taxa that are tracked during biostimulation experiments?
- Library similarity at the phyla level not always detected at the species level
  - At what taxonomic level does diversity become important?
  - How can differences in physiology be assessed with no closely related cultured isolate?
- RNA-derived libraries were predominantly subsets of the DNA-derived library from the same sample
  - In low cell mass environments, is DNA-based analyses sufficient?

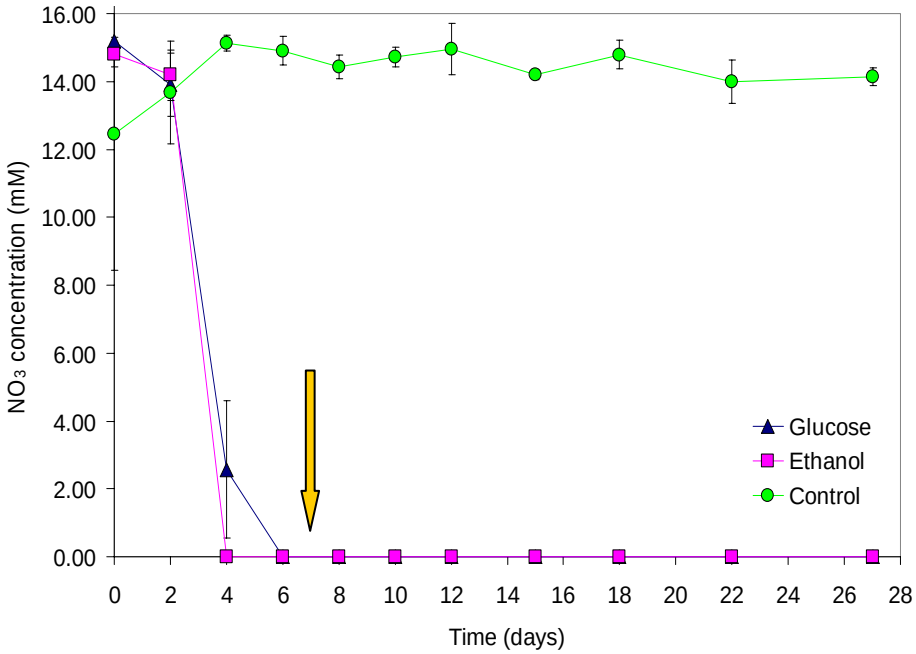


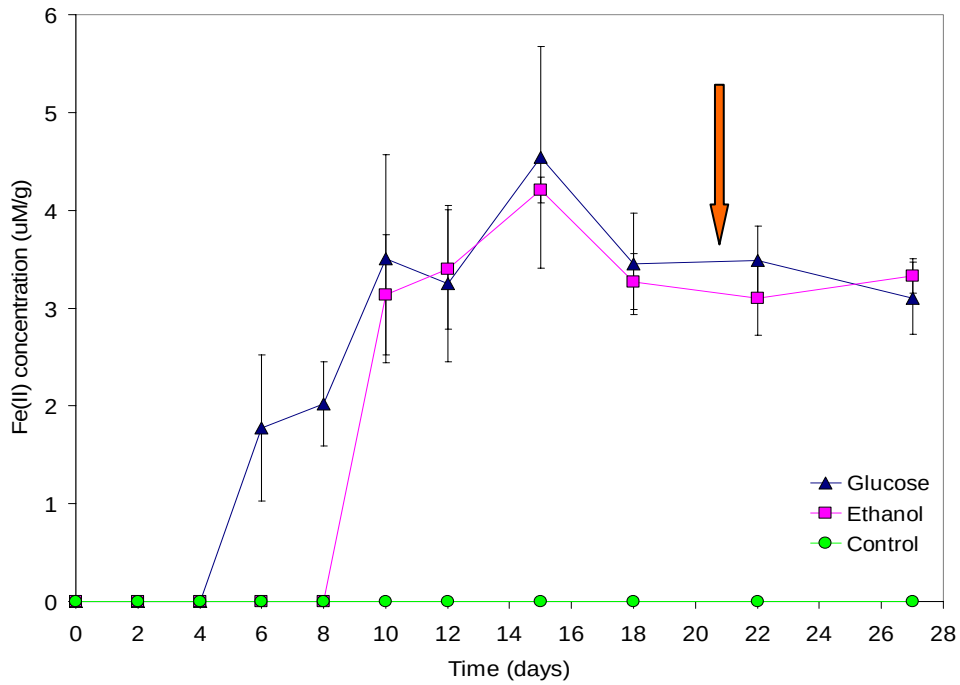
Project 2: Community analysis of microcosm sediments from Area 2

Microcosm Design

- Area 2 sediment was pH neutralized and then flushed with N_2 to create anoxic conditions.
- Treatments (3 replicates each):
  - 20 mM Ethanol
  - 10 mM Glucose
  - No carbon control
- Incubated at 30˚ C and sampled for geochemical analysis every 1-5 days.
  - High performance liquid chromatography (HPLC) for electron donor usage (*in progress*)
  - Fe-mineral analysis (*in progress*)

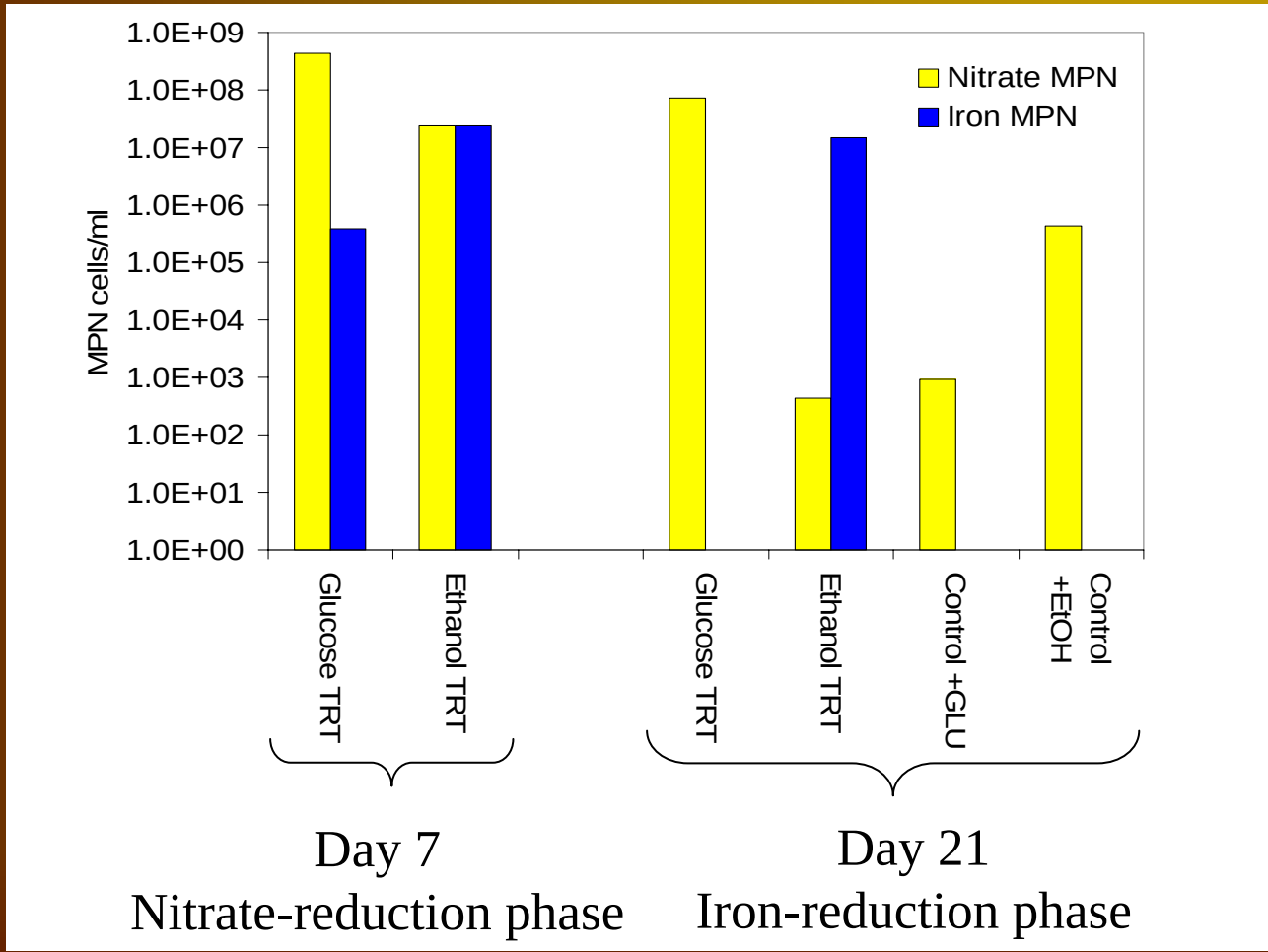
Microcosm Analysis





Preliminary electron acceptor utilization data

Microcosm Analysis



Preliminary MPN data

Preliminary Results and Future Direction

- **The shift between nitrate- and iron-reduction phases in the microcosms occurred at different times in the two electron donor treatments.**

- **Further analysis using both cultivation dependent and independent techniques will be used to determine the differences in community composition during nitrate- and iron-reducing conditions.**

- **Future Direction:**
  - Analyze MPN data for nitrate- and iron-reducing bacteria.
  - Perform molecular-based analysis on homogenized sediment, microcosm material, and MPN dilution tubes.