Oak Ridge *IFRC*

Microbial Community Response Parallels Uranium Immobilization and Remobilization during *In Situ* Field Manipulation

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Project Team

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  - Kenneth Lowe
  - Jack Carley
  - Susan Carroll

Project Objectives

Task C. Enhanced Contaminant Stability Strategies for Source Control

- **Goal = reduce the soluble contaminant concentration at the source**

- **Objectives**:
  - Explore novel strategies for enhancing the subsurface stability of immobilized metals and radionuclides
  - Understand the long-term impacts of geochemical and hydrologic heterogeniety on the remobilization of immobilized radionuclides
  - Improve ability to predict the long-term effectiveness of remedial activities

Project Objectives

Task C. Enhanced Contaminant Stability Strategies for Source Control

- **Hypothesis** = **Enhanced subsurface stability of U and Tc** can be achieved through remedial strategies that maintain a favorable geochemistry and microbial ecology, minimize biogeochemical heterogeniety, and counteract or inhibit mechanisms of reoxidation and remobilization

Overall Objectives - Microbiology Team

- Heterogeniety of subsurface + complexity of microbial communities has obscured our view of the coupling of contaminant transformation mechanisms to microbial groups that mediate them
- Objectives are to:
  - Conduct high throughput, quantitative, statistically defensible interrogation of microbial communities for the field-scale testing of hypotheses derived from lab and environmental genomics studies
  - Elucidate the active microbial groups impacting the fate and transport of U for models of *in situ* bioremediation and natural attenuation

Uranium reduction mechanisms

- U mobility controlled largely by complexation and redox reactions
- Extensive data on enzymatic U(VI) reduction
- Catalyzed by metabolically and phylogenetically diverse group of indigenous microorganisms
- In situ experiments show that O_2 and nitrate must be depleted first

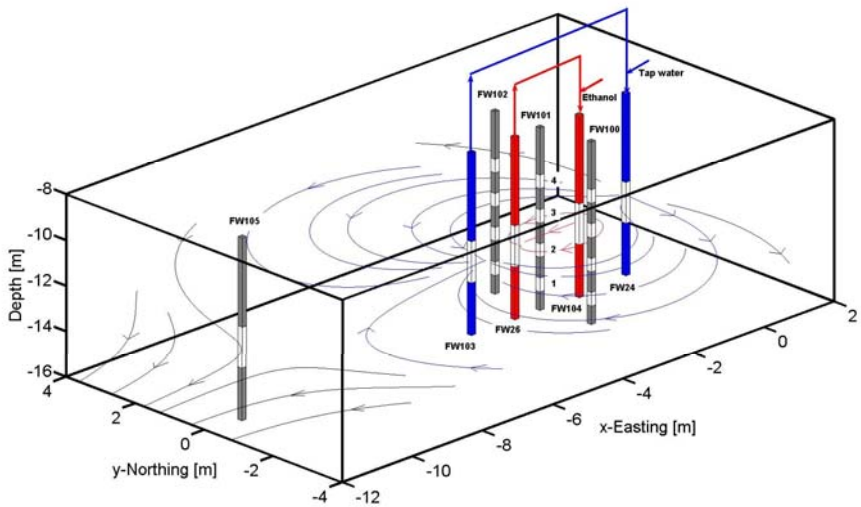
Uranium oxidation mechanisms

- Less information available
- Enzymatic oxidation coupled to nitrate reduction, mostly tested in lab
- Abiotic oxidation by poorly crystalline metal oxides and denitrification intermediates

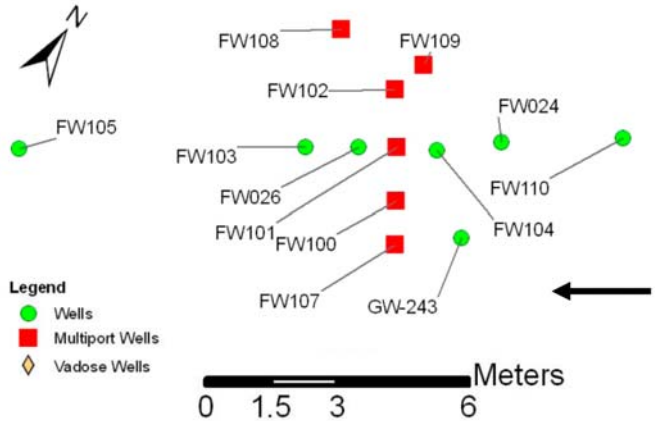
Background

Pilot-scale test system

- Developed for the field testing of U bioremediation strategies (Criddle or Stanford site)
- This system has been tested in a number of previous studies- Wu et al. (2006a; 2006b; 2007)
- Bioreduction and immobilization of U to below U.S. EPA MCL achieved through intermittent ethanol injection
- Current study focused on influence of nitrate on the mobility of U in the presence and absence of ethanol



Pilot-scale test system for in situ field manipulation

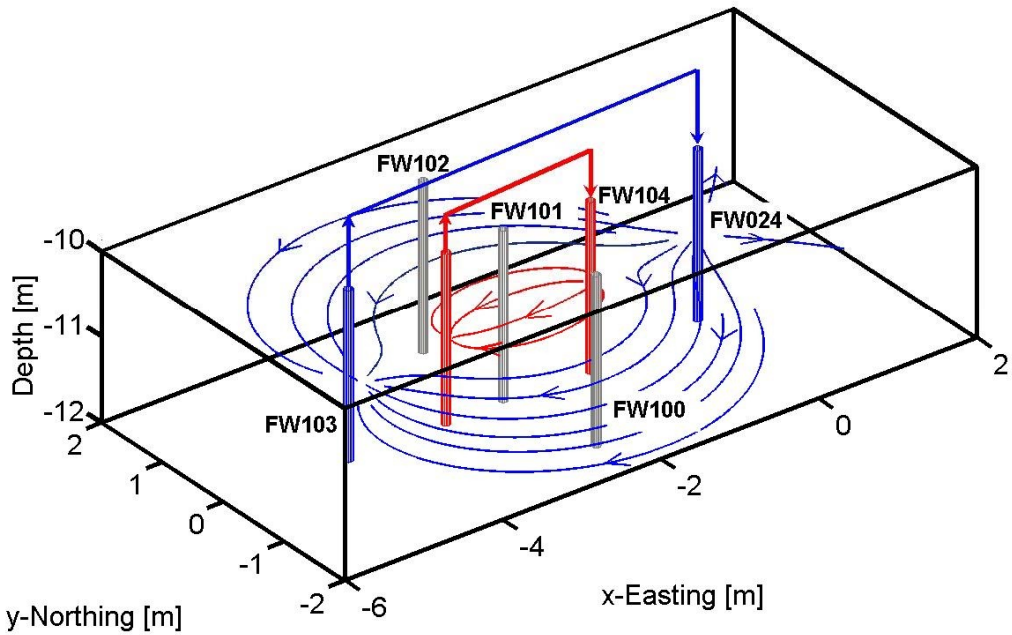


Outer recirculation loop (blue)

Inner loop for field manipulation (red)

MLS wells for monitoring

Injection at 13.8 m depth in high flow zone



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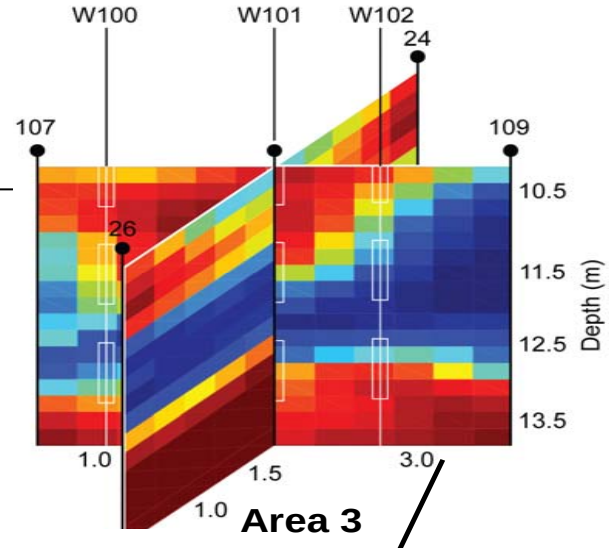
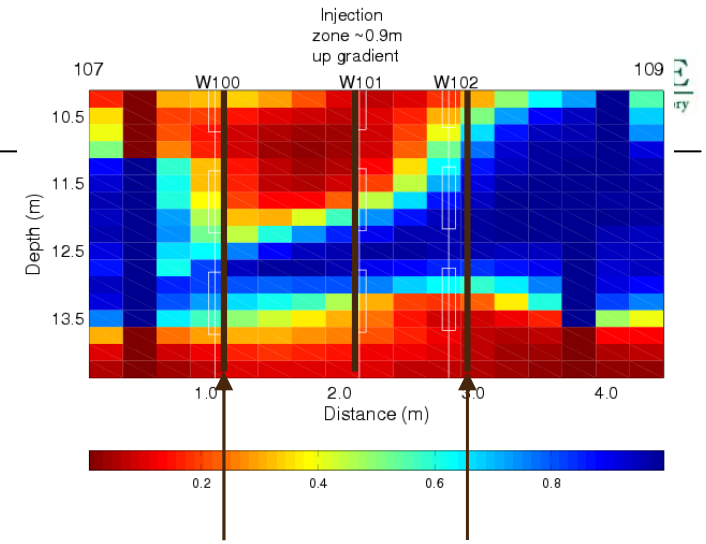
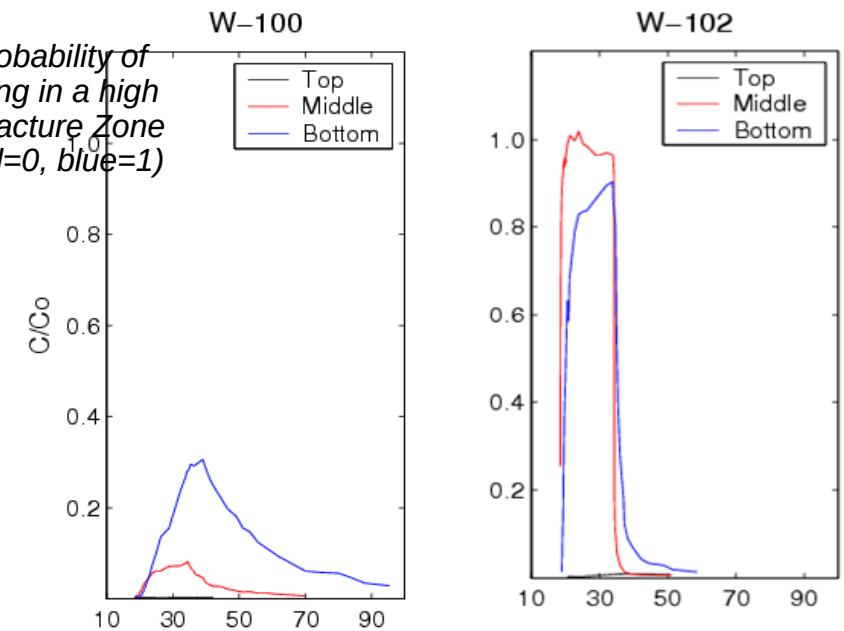
Fracture transition (high hydraulic conductivity) zonation at Criddle site





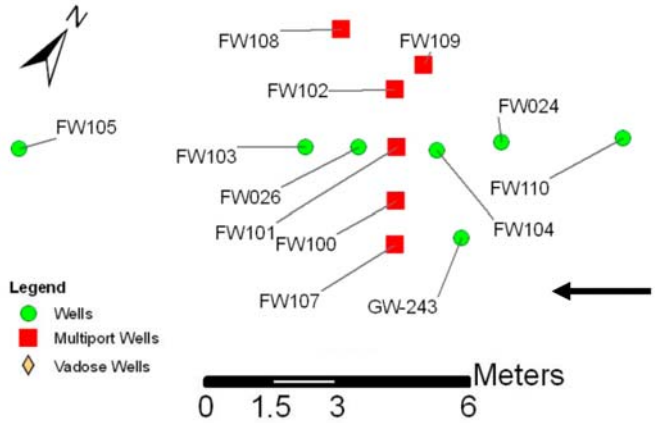
Probability of being in a high K fracture Zone (red=0, blue=1)



- **Low seismic tomographic velocity** indicative of high hydraulic conductivity fracture zones.
- Method developed to jointly invert wellbore flowmeter and seismic tomographic travel times in the **estimate of fracture zonation** (Chen et al., WRR, 2006)

Seismic P-Wave Velocity

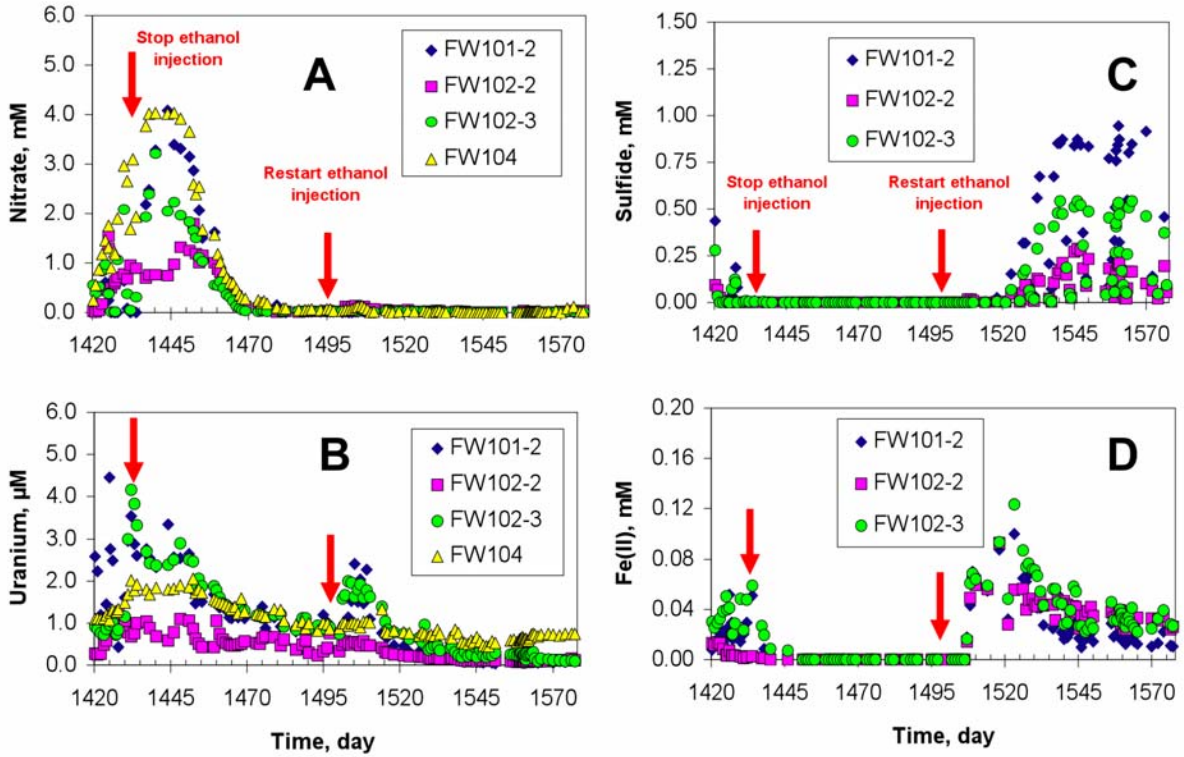
Pilot-scale test system for in situ field manipulation



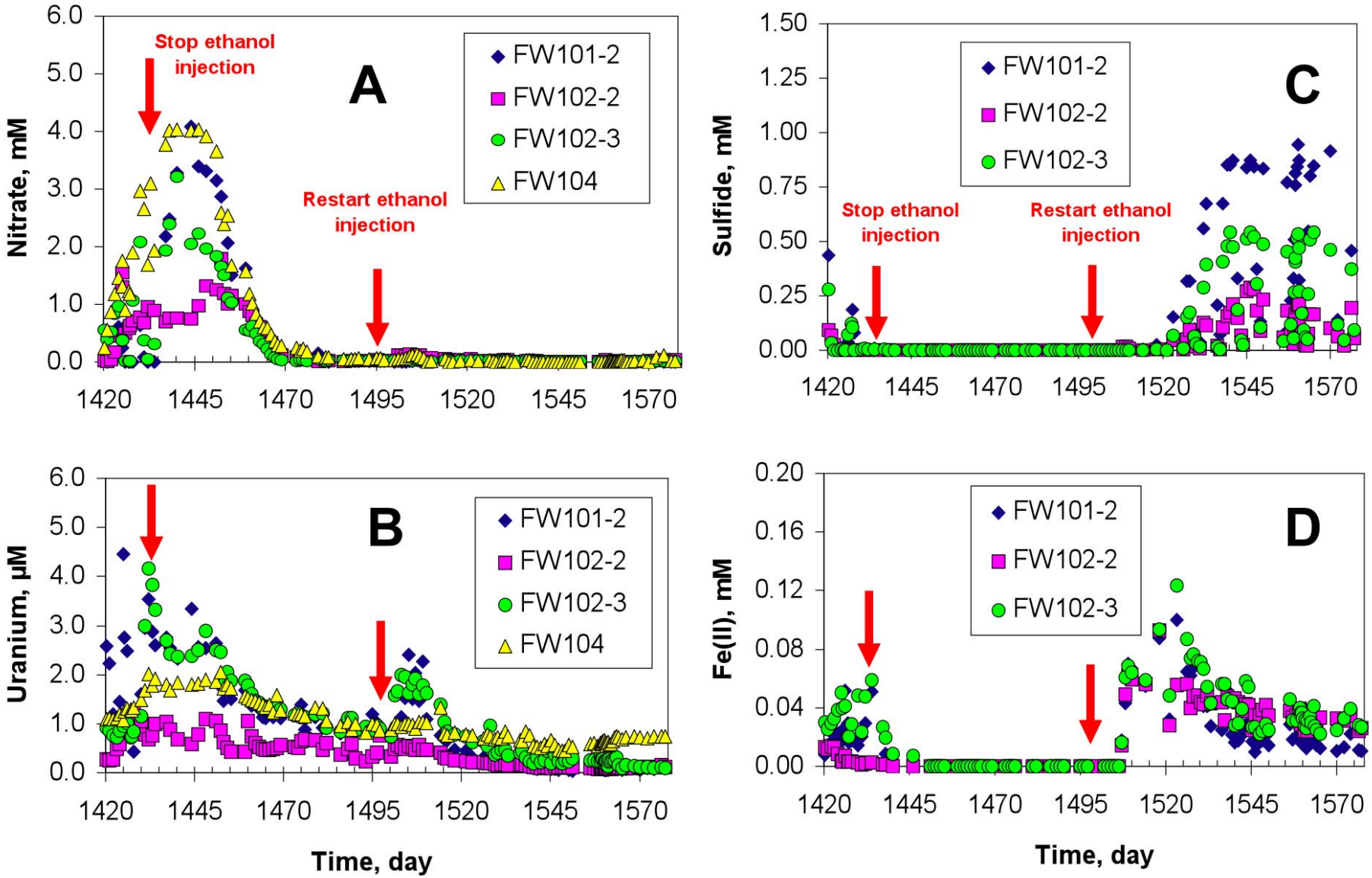
- Injection of nitrate/Br/ethanol- day 1398-1404
- Exposure to nitrate w/o ethanol- day 1420-1496
- Resumption of ethanol injection- day 1496-1578
- Hydraulic connectivity:
  - 101-2 > 102-3 > 102-2 > 101-3
- 101-3 lost connection
- Thus, study focused on first 3 samplers

Nitrate exposure

- 1) Injection of nitrate + Br
- 2) Injection of nitrate + ethanol
- 3) Ethanol alone
- 4) No injection



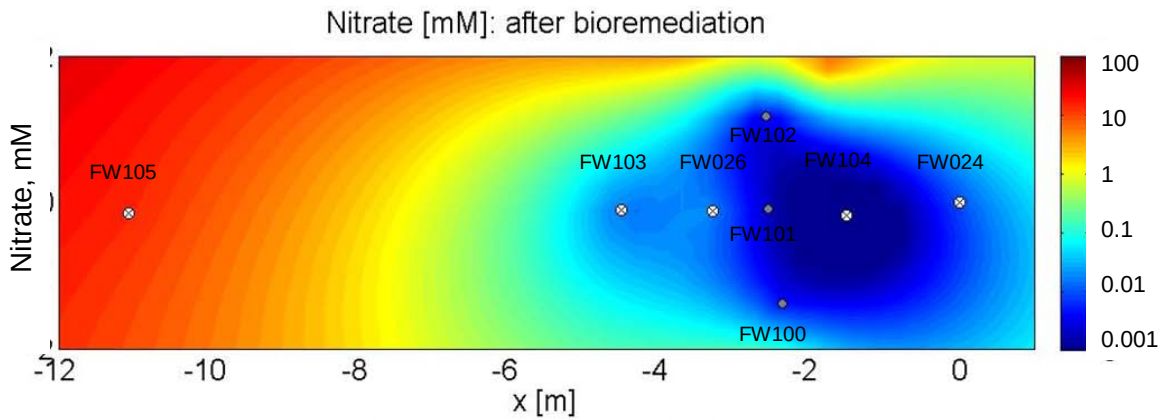
Nitrate exposure



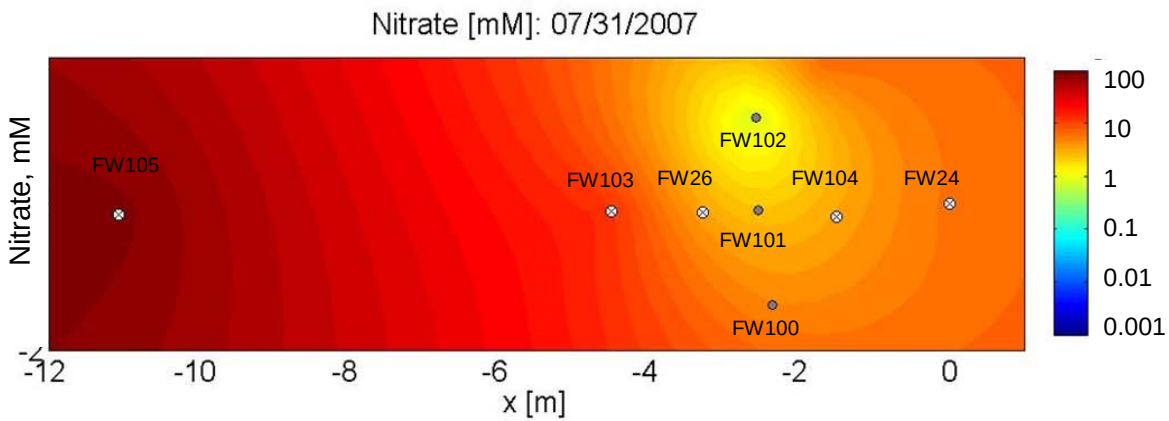
Nitrate concentration before and after reoxidation

Nitrate distribution before and after nitrate-reoxidation test

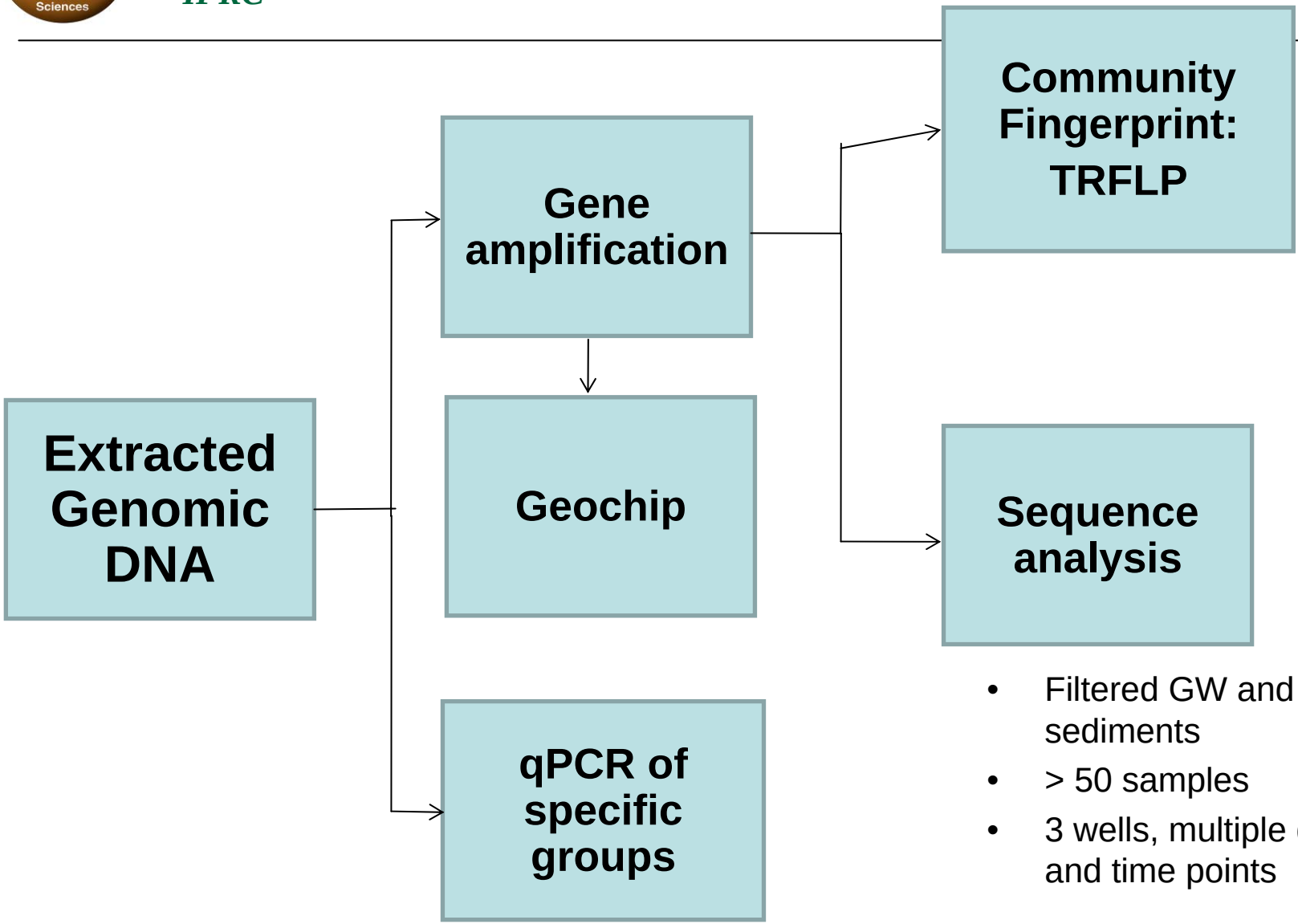
12 / 2007



7 / 2007

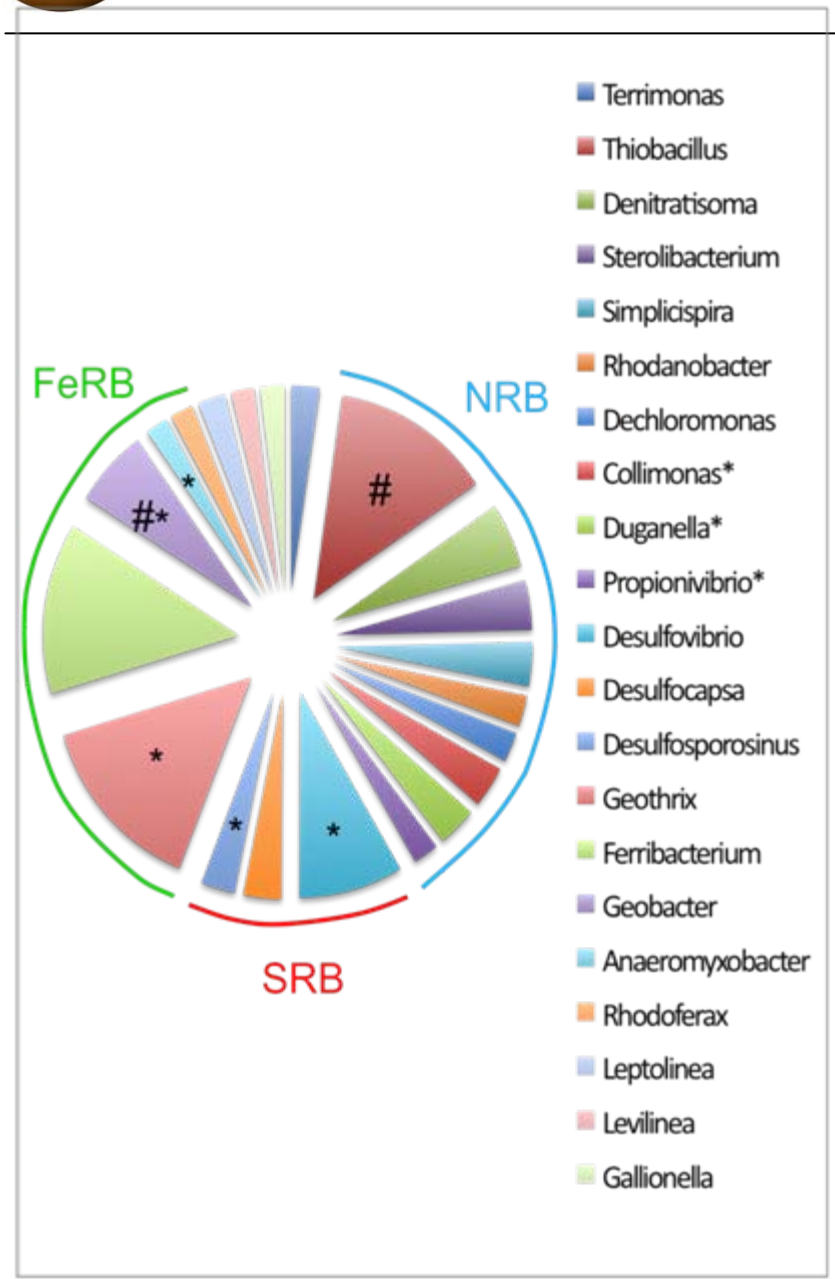


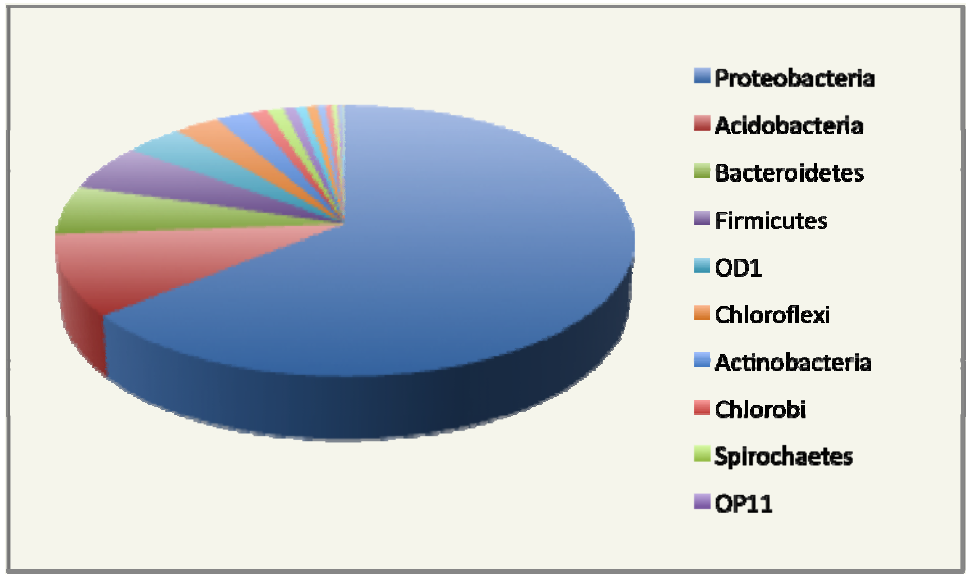
Microbiological Approach

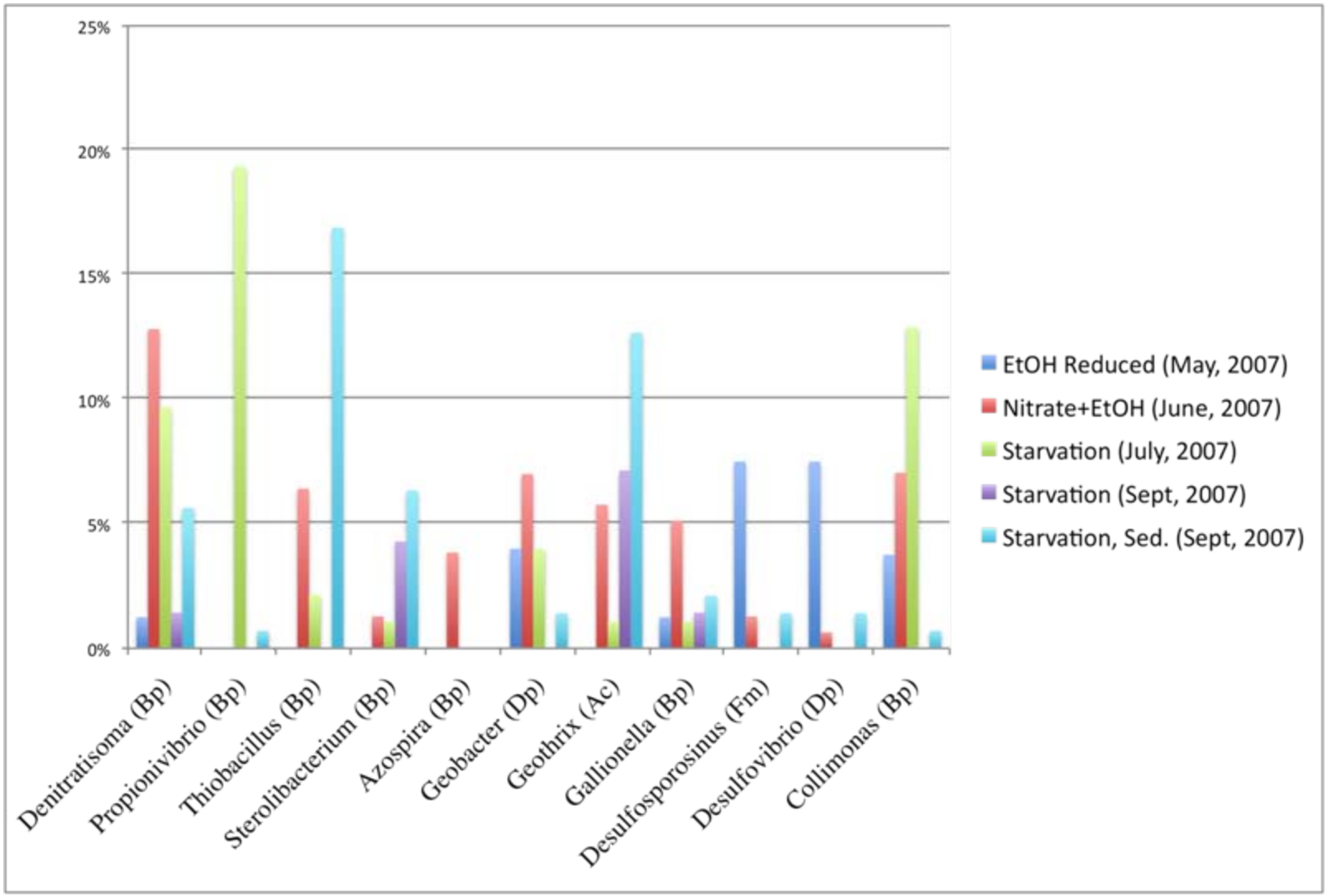


- Filtered GW and “surge” sediments
- > 50 samples
- 3 wells, multiple depths and time points

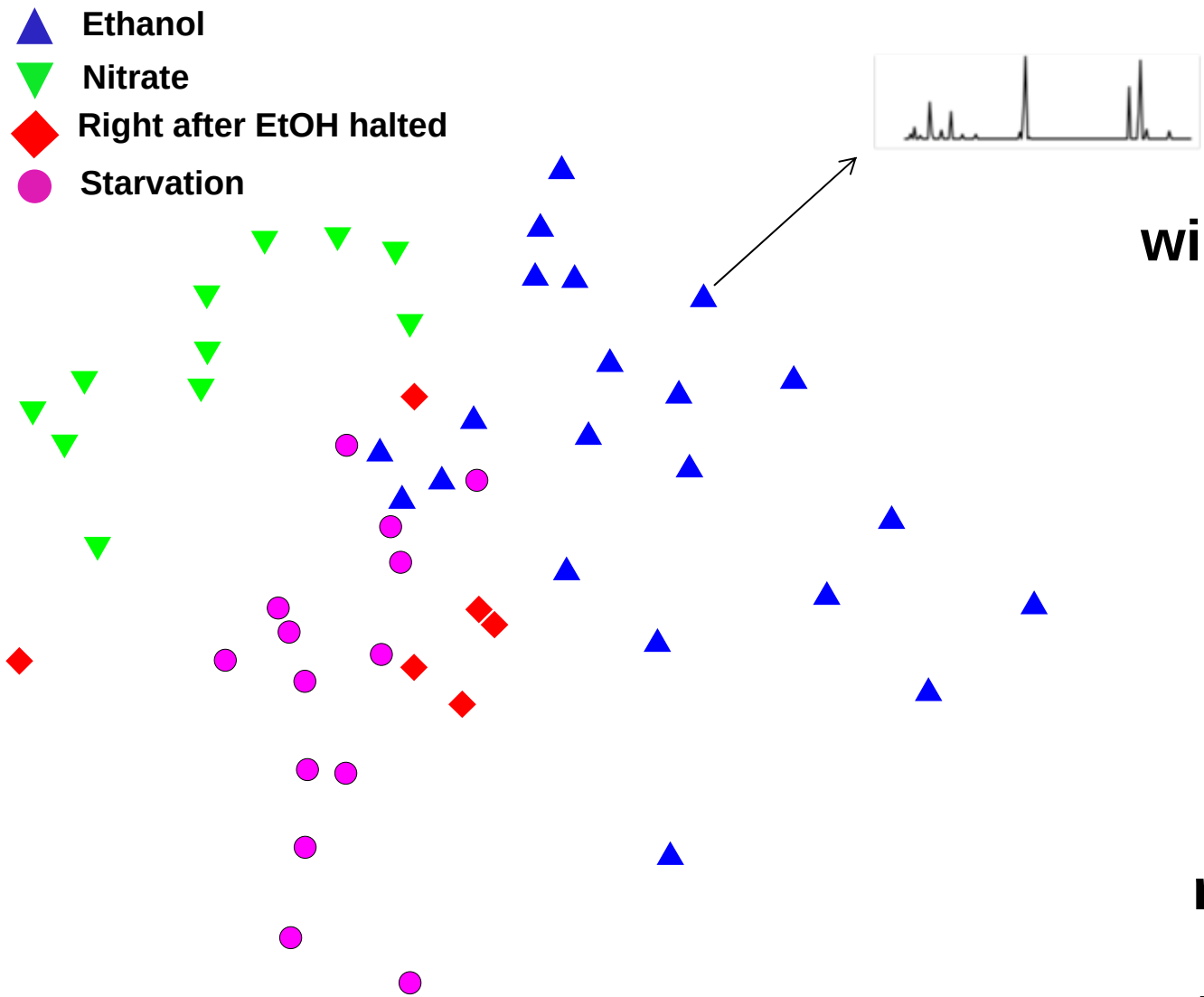
Community Structure – 16S rRNA






25%
20%
15%
10%
5%
0%
Denitratisoma (Bp)
Propionivibrio (Bp)
Thiobacillus (Bp)
Sterolibacterium (Bp)
Azospira (Bp)
Geobacter (Dp)
Geothrix (Ac)
Gallionella (Bp)
Desulfosporosinus (Fm)
Desulfovibrio (Dp)
Collimonas (Bp)
EtOH Reduced (May, 2007)
Nitrate+EtOH (June, 2007)
Starvation (July, 2007)
Starvation (Sept, 2007)
Starvation, Sed. (Sept, 2007)

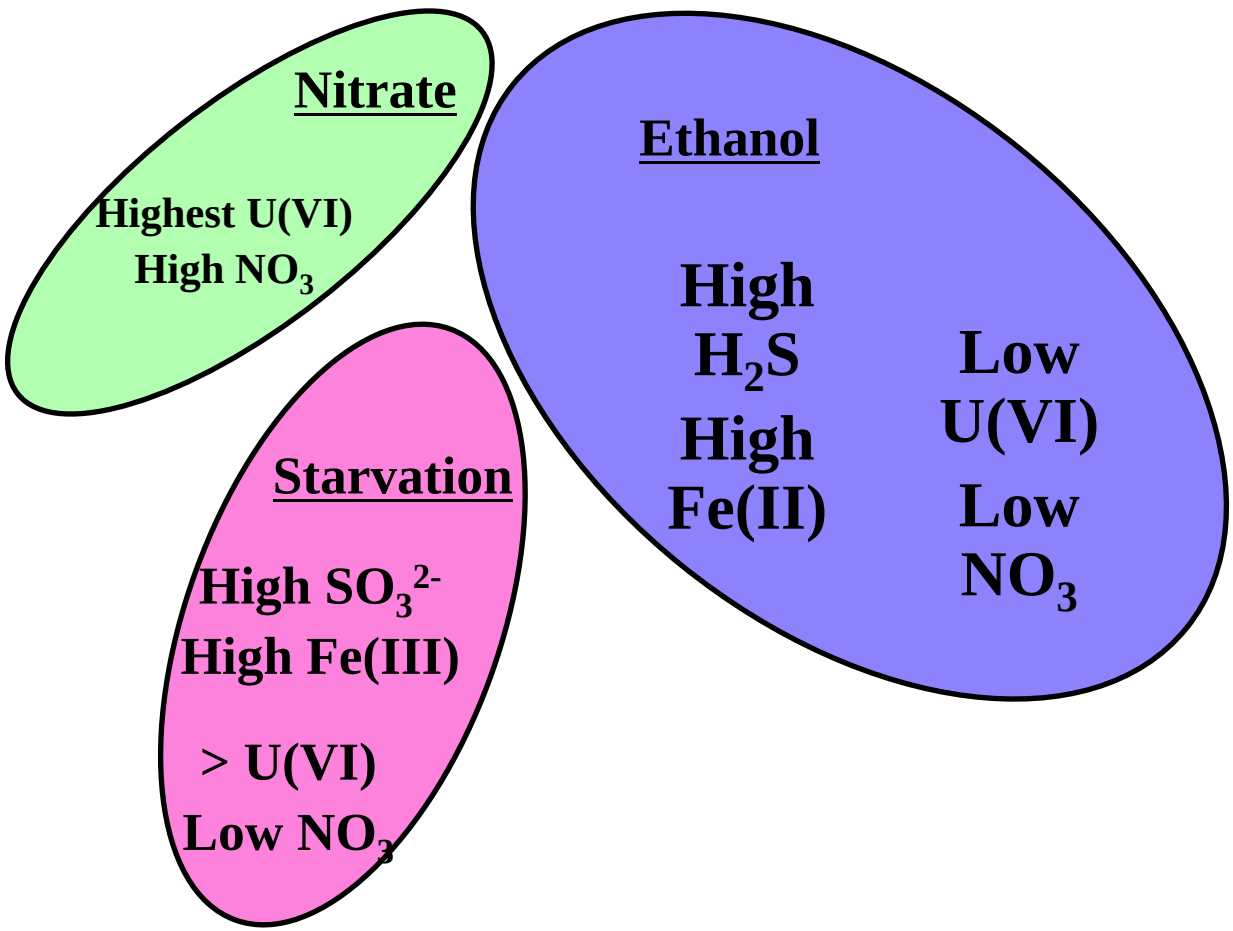
Microbial Community Shifts



Bray-Curtis Similarity
Stress – 0.2

- Microbial community structure varies widely after ethanol amendment
- Ethanol and nitrate together dramatically alter community structure
- Communities under starvation conditions are more similar than those under ethanol conditions

Geochemical Correlations



- Ethanol does not select for a single microbial community, but ethanol treated samples are *distinct* from other treatments
- Geochemical measurements for ethanol treated and starvation samples are much more uniform than microbial community structure analyses
- Geochemical conditions in ethanol & nitrate samples are highly sensitive to hydrological conditions, but generally yield increase U(IV)

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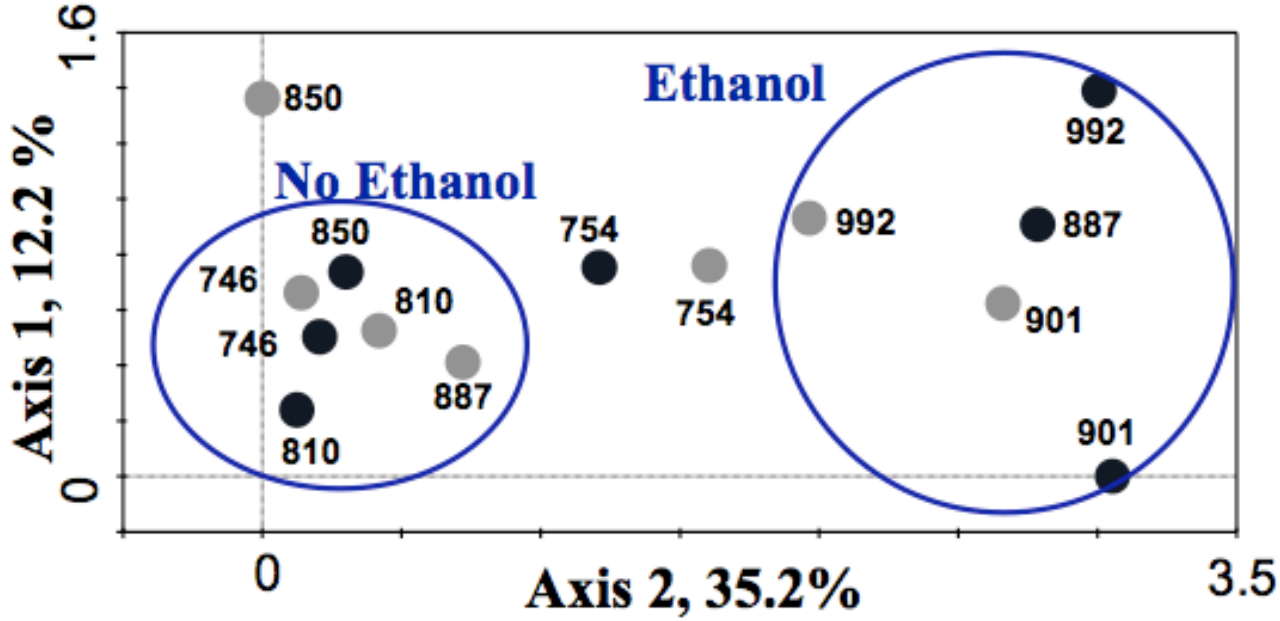
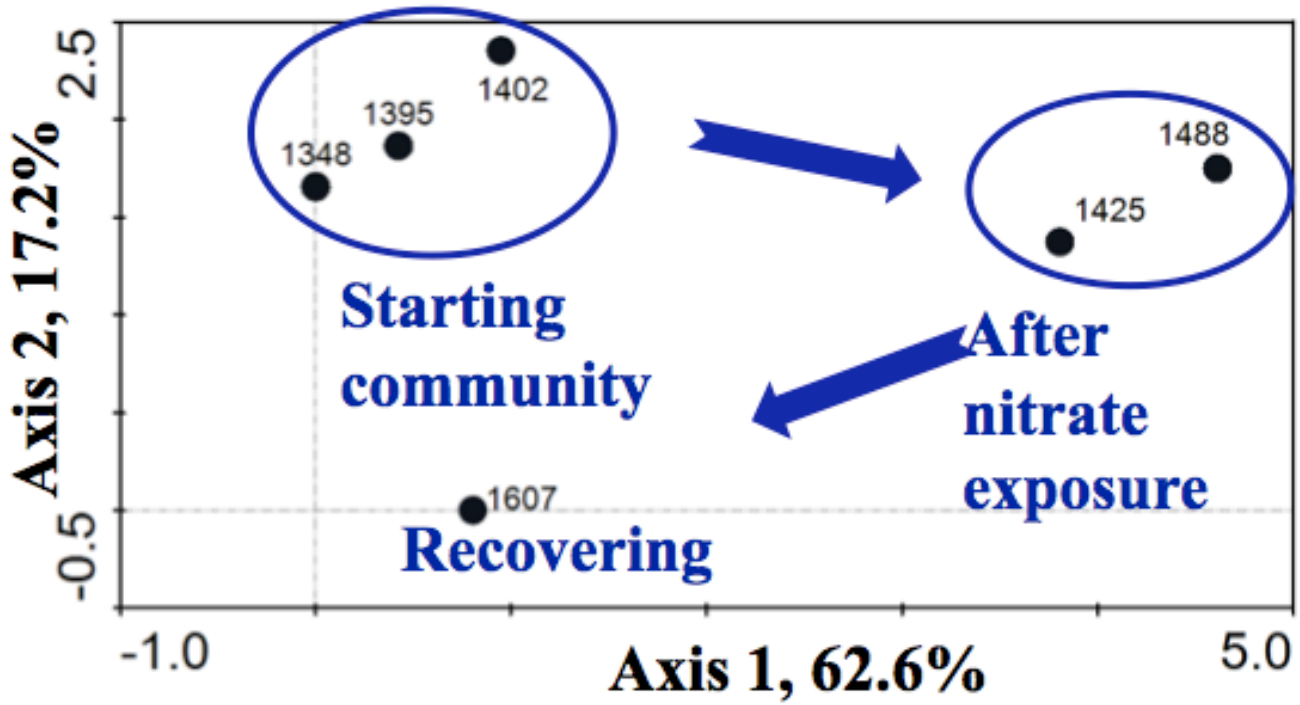
Geochip Analysis

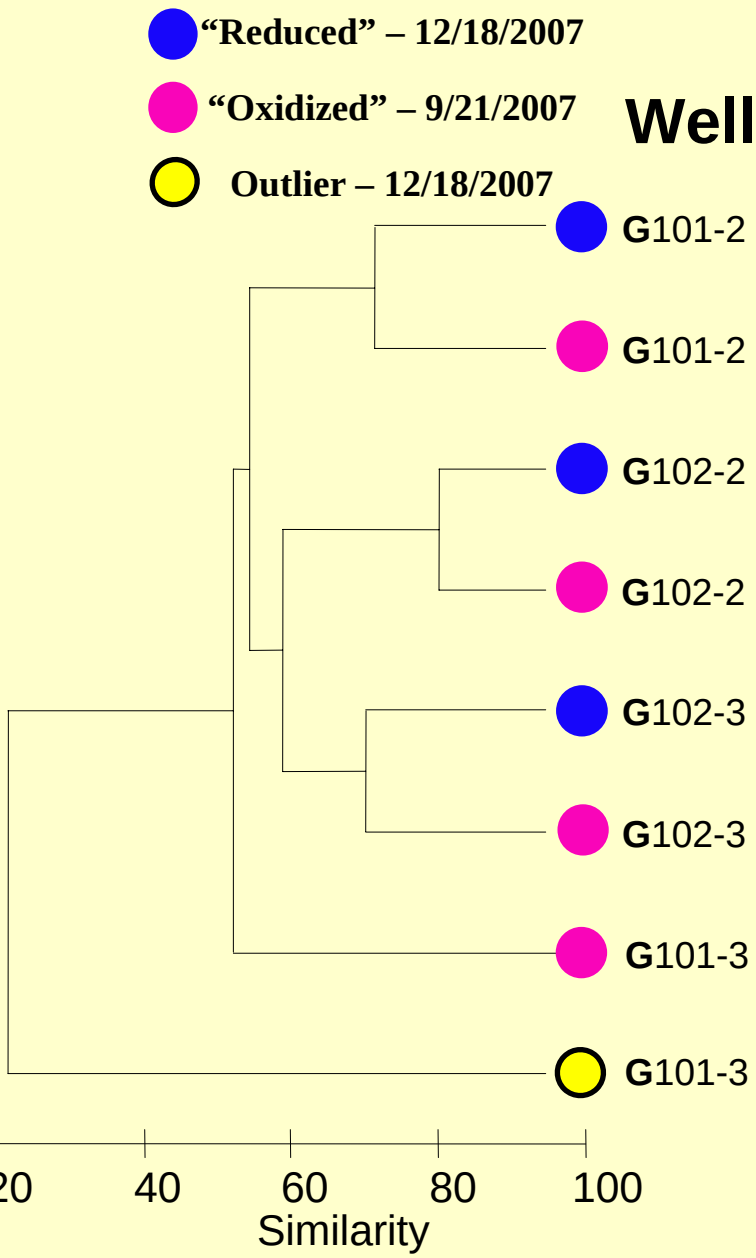
- Top panel = Reoxidation by DO
- Bottom panel = Nitrate exposure



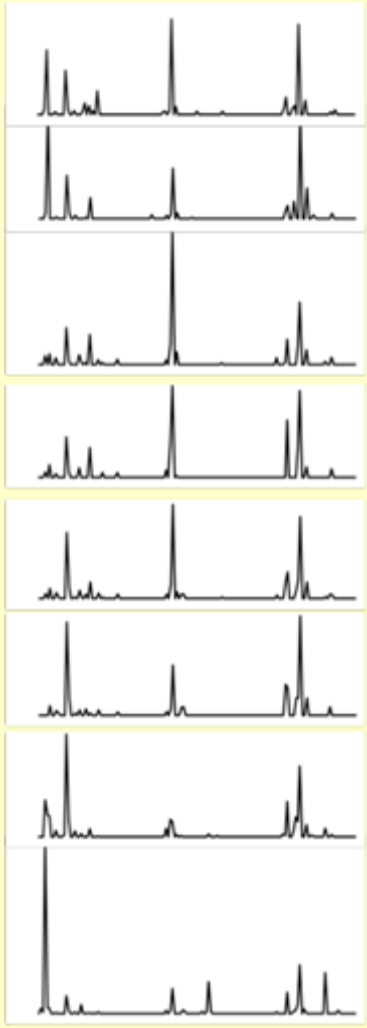


Tracking Hydraulic Connectivity and Sulfate-reducing Bacteria

Linkage of Communities to Site Hydrology



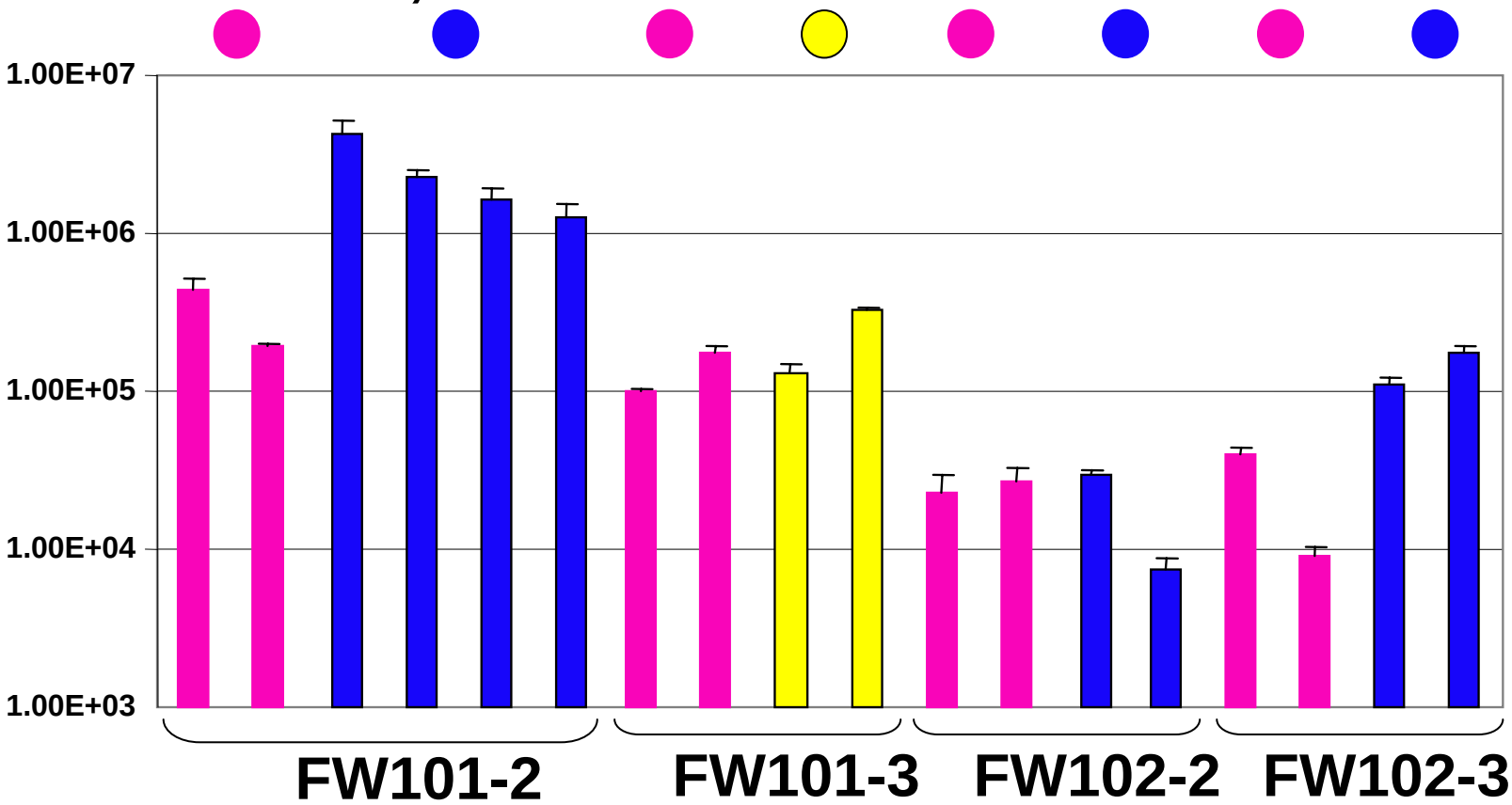
TRF Profile



- Ethanol induces community shift
- This shift is small relative to well to well differences
- Hydrology greatly effects stimulated community
- Functionally important groups: FeRP, SRP, NRP
- Ratio of dsrA/16s reflects hydraulic connectivity

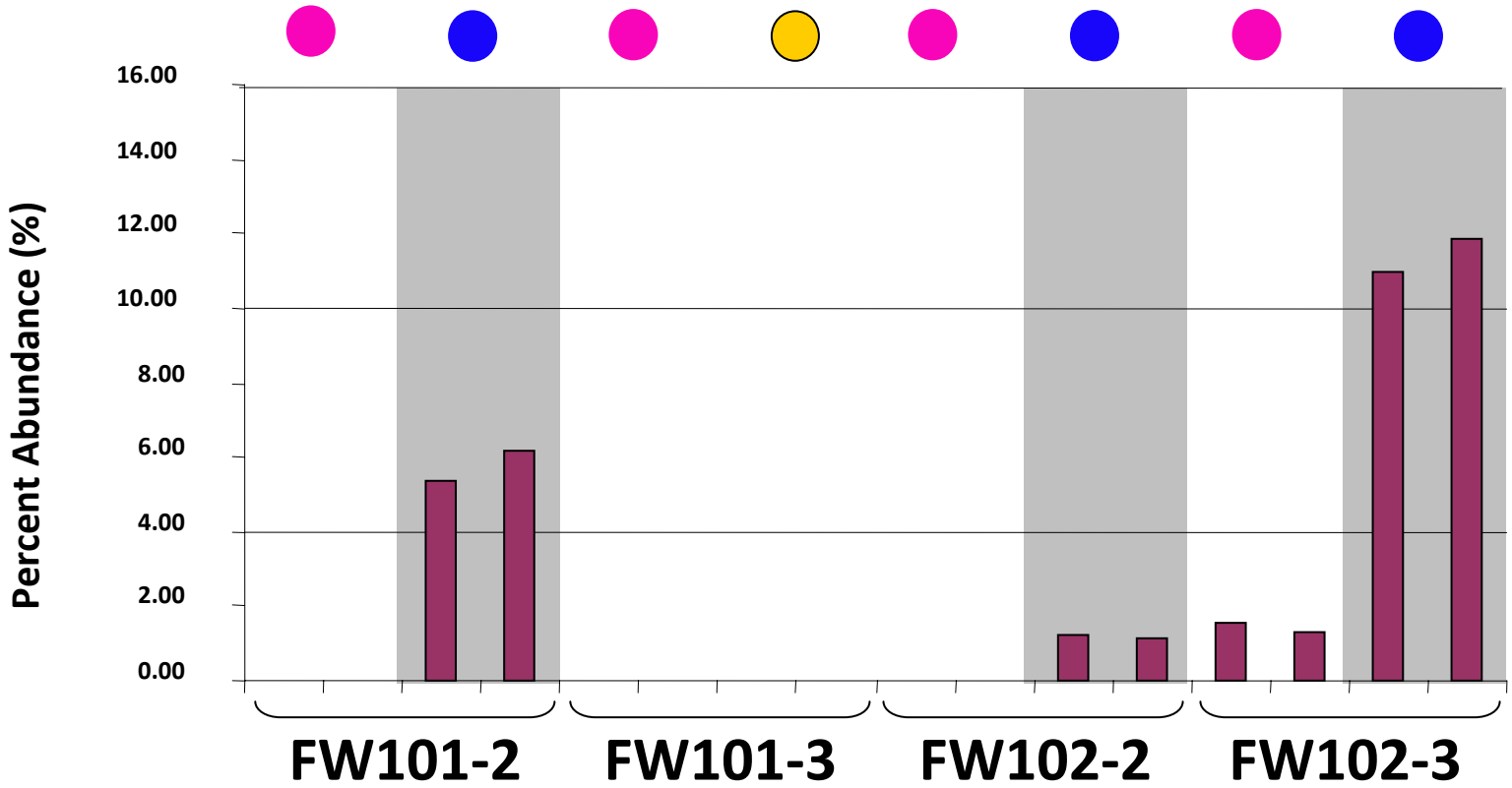
Quantitative Analysis of SRP

- Dec 2007; Ethanol bio-reduced
- Sept 2007; Oxidized
- Dec 2007; FW101-3



- Hydraulic connectivity: 101-2 > 102-3 > 102-2 > 101-3

Tracking *Desulfosporosinus*



- Excellent agreement with qPCR of dsr as well as shifts in overall community
- Hydraulic connectivity: 101-2 > 102-3 > 102-2 > 101-3

Summary / Conclusions

- High throughput screening of microbial community structure
- PCA shows community structure closely correlated with accumulation of reductants (FeII, sulfide) during ethanol treatment and oxidants (nitrate, UVI) during reoxidation (please see OR-IFRC poster by Green et al.)
- Clear impact of hydraulic connectivity

Summary / Conclusions

- ***Thiobacillus*** **and other nitrate-reducing** ***Betaproteobacteria*** **(*Denitratisoma*, *Sterolibacterium*) predominate during nitrate exposure**
  - **Chemolithotrophic metabolism may allow *Thiobacillus* to thrive**
- ***Geothrix*** **(*Acidobacteria*) and *Desulfosporosinus* (*Firmicutes*) predominate during bioreduced conditions over other metal-reducing taxa; diminish during nitrate exposure**
  - ***Geothrix*** **also reduces nitrate and is capable of fermentation, oxidation of reduced metals?**
  - ***Desulfosporosinus*** **tolerates high salt, uses nitrate?**

Conclusions - Geochip

- Ethanol addition resulted in increase in the overall number and diversity of functional genes
- Nitrate treatment also resulted in a substantial shift in community structure
- DO did not have such a pronounced effect (although *dsr* decreased)
- Under starvation conditions, diversity and richness were relatively stable

Outline

- Project objectives and hypotheses
- Background
- Experimental Approach
- Results
  - Geochemistry and Contaminant Chemistry
  - Microbiology
- Summary/ Conclusions

Research Needs

- Need **tools for monitoring** microbial processes during NA and targeted immobilization (Tasks B and C)
- Need to refine and verify microbial **inputs to conceptual and quantitative models**: abundance/ biomass, community structure, activity/ rates (Task D)
- Need to understand and **predict major controls** (pH, redox, recharge) of microbial growth and metabolism at watershed scale for monitoring of natural attenuation (Task B)
- Need to determine **impact of biomanipulations** (electron donors, organophosphate) on microbial dynamics for optimization of targeted immobilization and longterm stability of contaminants from secondary sources (Task C)

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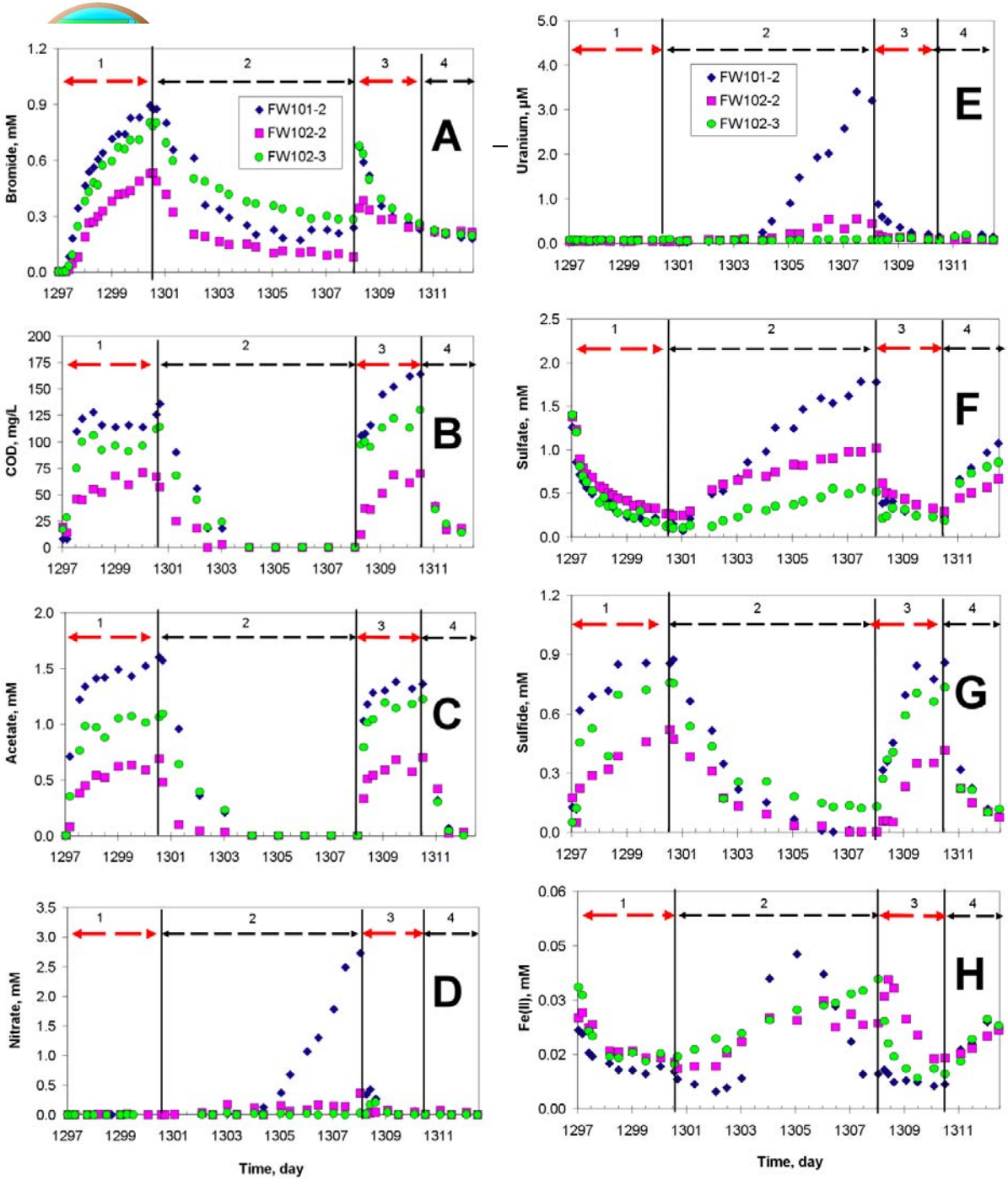
ion of flow control



Flow control suspension

- 1) Injection of nitrate+Br
- 2) Flow suspended
- 3) Flow resumed, ethanol injected
- 4) Ethanol injection stopped

Similarities between Treatments

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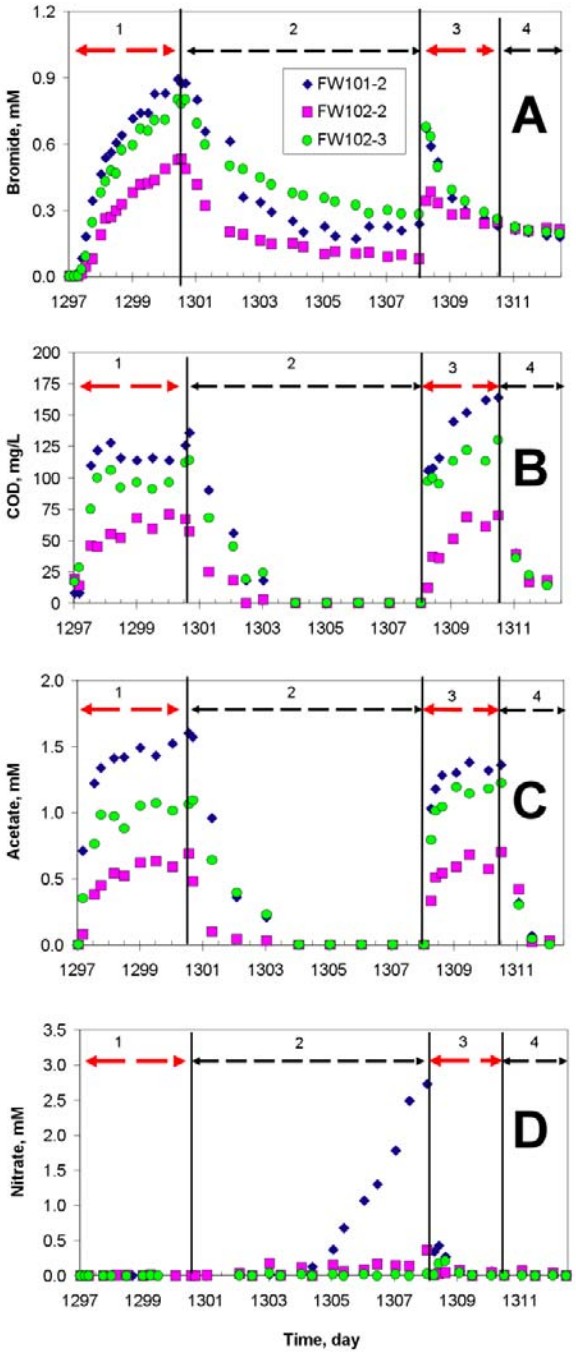
Group Name/ Comparisons	Average Percent Similarity	Average Similarity between Groups	Key TRFs Contributing to Similarities	Percent TRFs contribute
0-2 EtOH	37.57	-	388, 206, 109	41.99
10 -21 NO3	-	25.09	206, 386, 388	31.42
52-54 EtOH	-	29.68	92, 388, 206	23.08
10 EtOH	-	33.36	382, 388, 206	30.03
NO3	-	34.83	206, 385, 388	38.22
10-21 NO3	46.52	-	206, 386, 388	66.03
52-54 EtOH	-	30.19	206, 92, 386	36.49
10 EtOH	-	33.16	382, 206, 386	40.57
NO3	-	40.71	386, 206, 385	39.65
52-54 EtOH	49.88	-	92, 206, 388	53.25
10 EtOH	-	39.52	382, 92, 388	35.31
NO3	-	31.7	206, 92, 388	39.53
10 EtOH	55.36	-	382, 206, 388	52.32
NO3	-	30.58	382, 206, 388	46.63
NO3	65.48		206, 388, 385	87.47

TRFs	Possible Identites
388	Thiobacillus, Simplicispira, Gallionella, Ferribacterium
206	Rhodoferax, Simplicispira, Denitratisoma,
109	Desulfovibrio, Geobacter, Alkaliflexus
386	Simplicispira, Ferribacterium, Geothrix
92	Geothrix
382	Thermacetogenium, Gemmatimonas, Dokdonia,
385	Simplicispira, Dokdonia, Geothrix,

- **Amendments select for communities that are more highly similar to each other than they are to other treatments**
- **Often β-proteobacteria, likely denitrifiers, are determining TRFs in the sample set**



Geochemical changes during suspension of flow control

Flow control suspension

- 1) Injection of nitrate+Br
- 2) Flow suspended
- 3) Flow resumed, ethanol injected
- 4) Ethanol injection stopped

Geochemical Conditions

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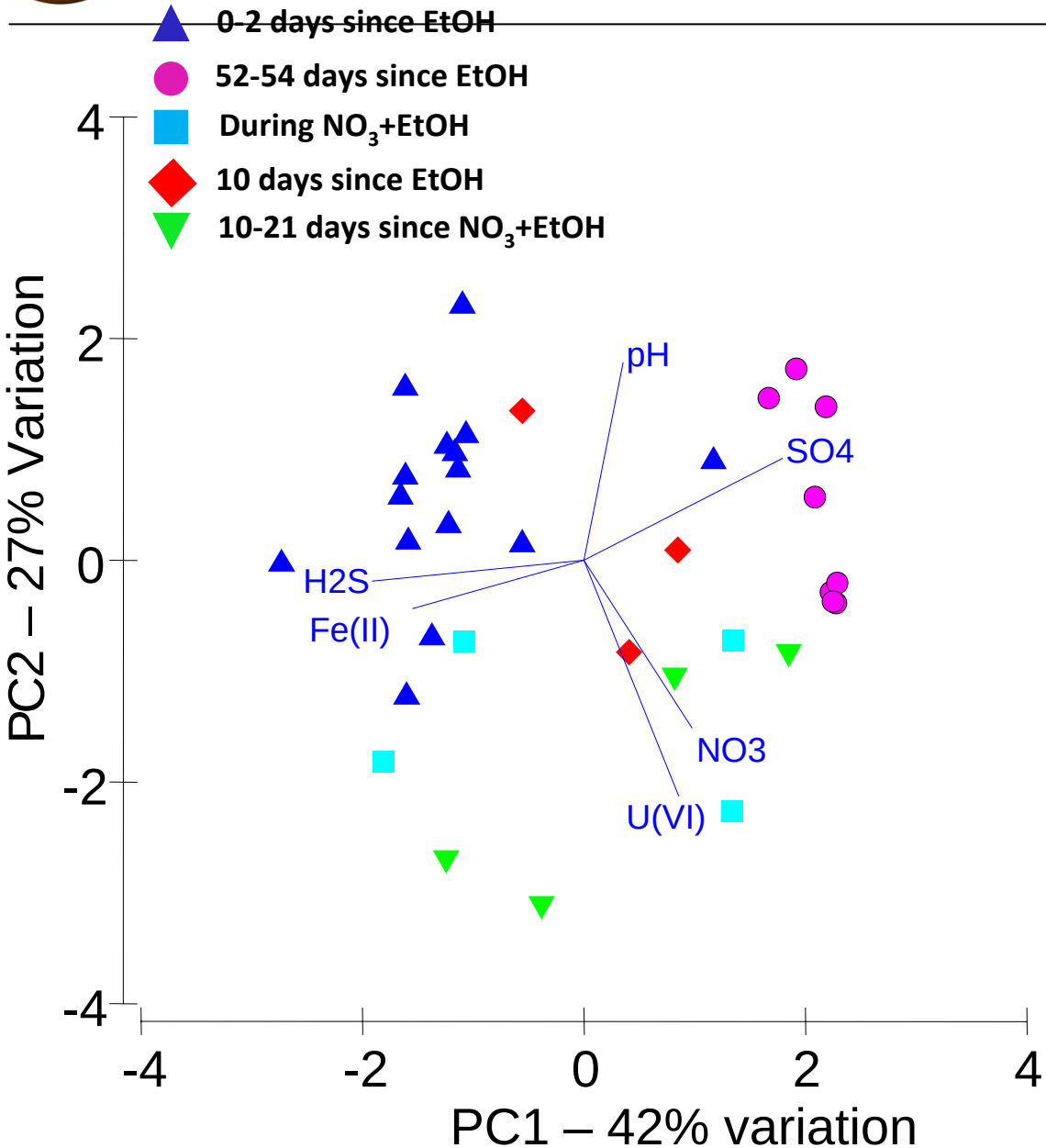

- Samples experiencing ethanol characterized by lower nitrate and U(VI) levels and elevated H2S and Fe^{2+}
- Samples from starvation characterized by high sulfate, low nitrate, relatively low U(VI)
- Samples during nitrate stimulation widely vary. All have high uranium and nitrate levels

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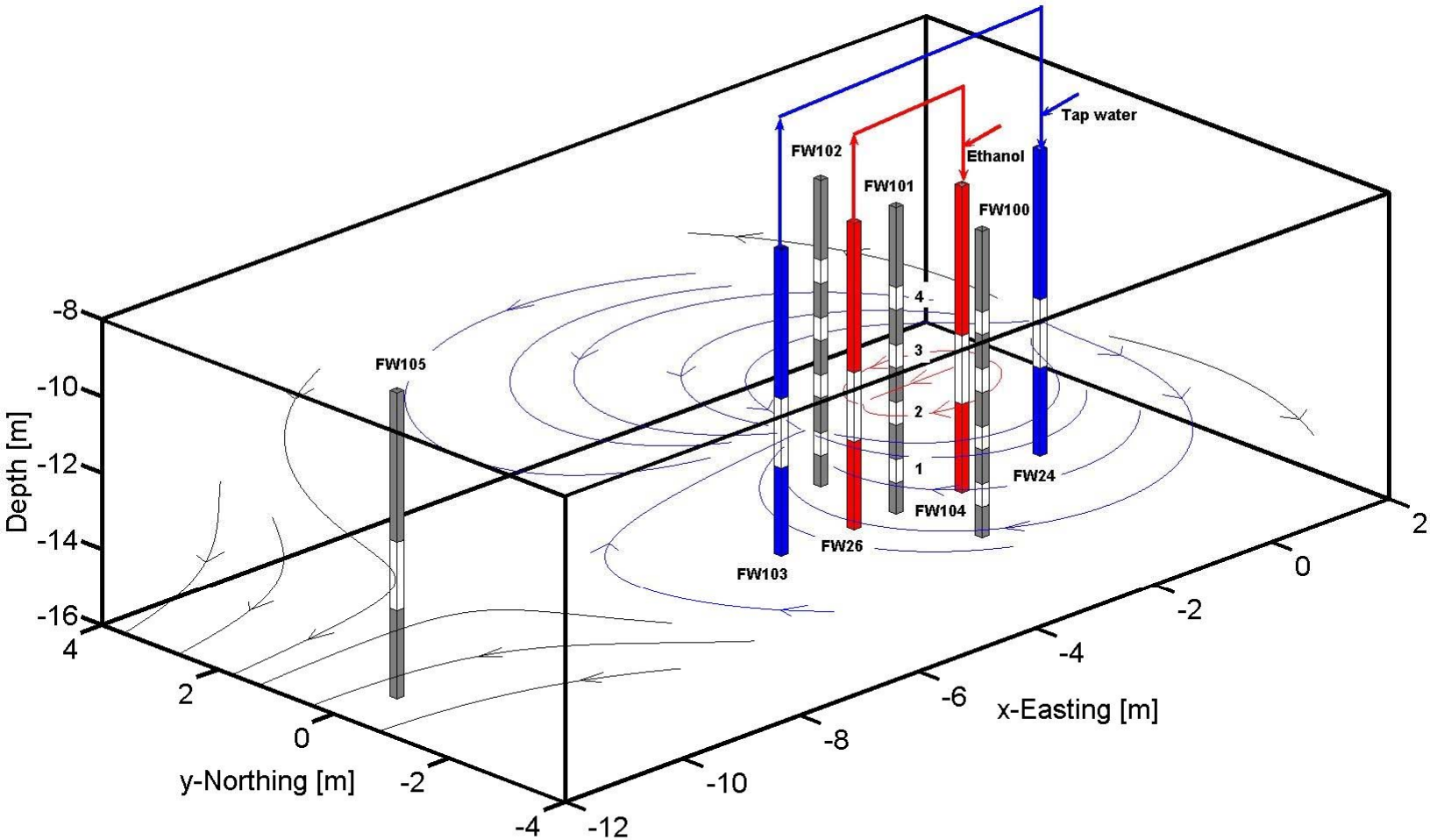
Microbial community response To Uranium Transformation



HYPOTHESES - MICROBIOLOGY

- **Microbial denitrification** is the only mechanism for permanently decreasing nitrate flux, where the denitrification rates are governed by pH and electron donor concentration
- **Groundwater recharge** dictates temporal and spatial variability of microbiological processes in the saturated zone
- **Transition zones**, characterized by steep gradients in subsurface properties, are the most active zones with respect to microbially-mediated contaminant transformation
- **Enhanced subsurface stability of U and Tc** can be achieved through remedial strategies that maintain a favorable microbial ecology, minimize biogeochemical heterogeneity, and counteract or inhibit mechanisms of reoxidation and remobilization.