



U.S. Department of Energy

Office of Science

Oak Ridge Field Research Center

Environmental Remediation and Stewardship Research

## Integrated Field-Scale Challenge at ERKP686

**Multiscale investigations of subsurface microbial communities that mediate targeted immobilization and natural attenuation (NA) of co-contaminants**



**Joel E. Kostka**

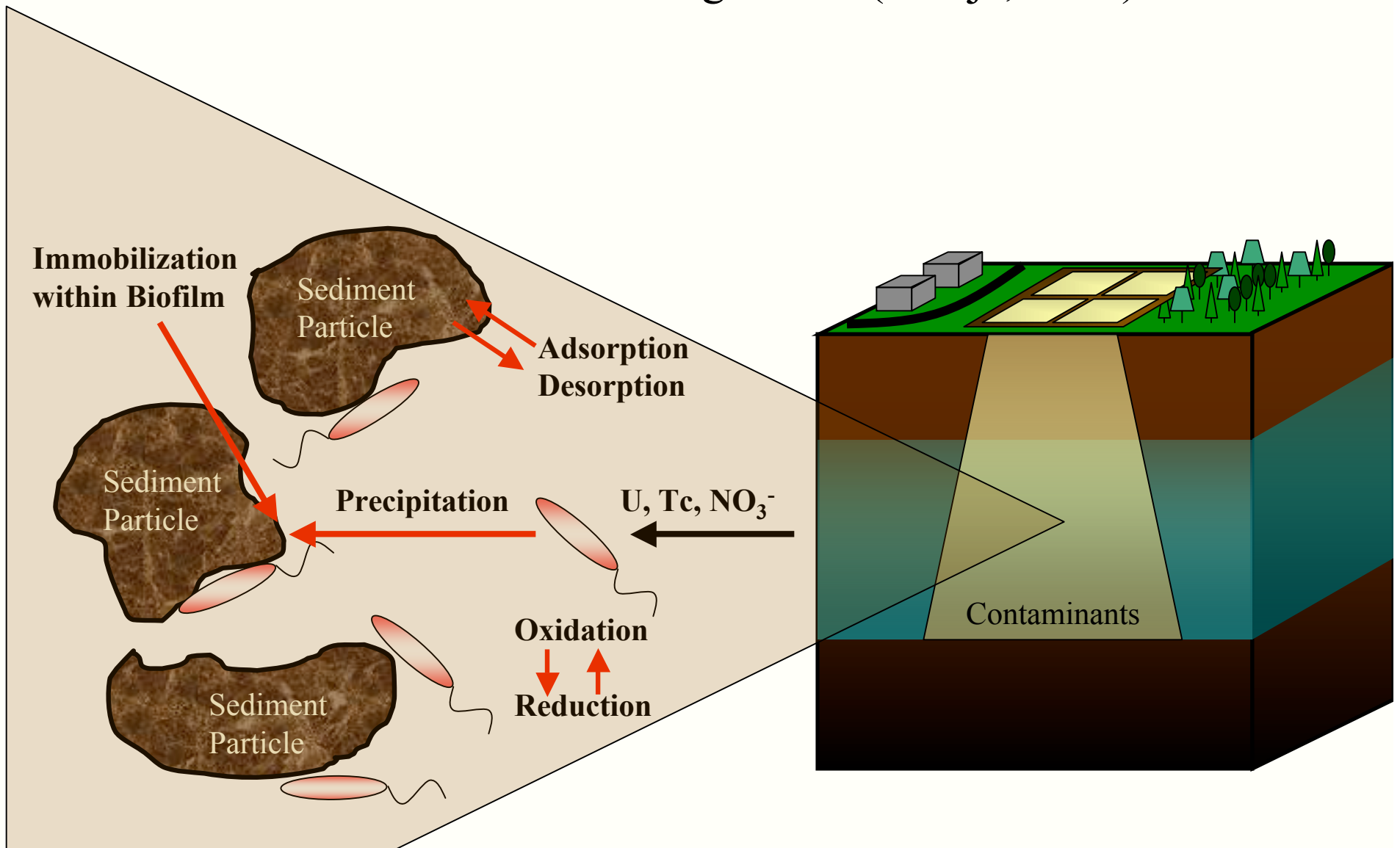
**Florida State University**

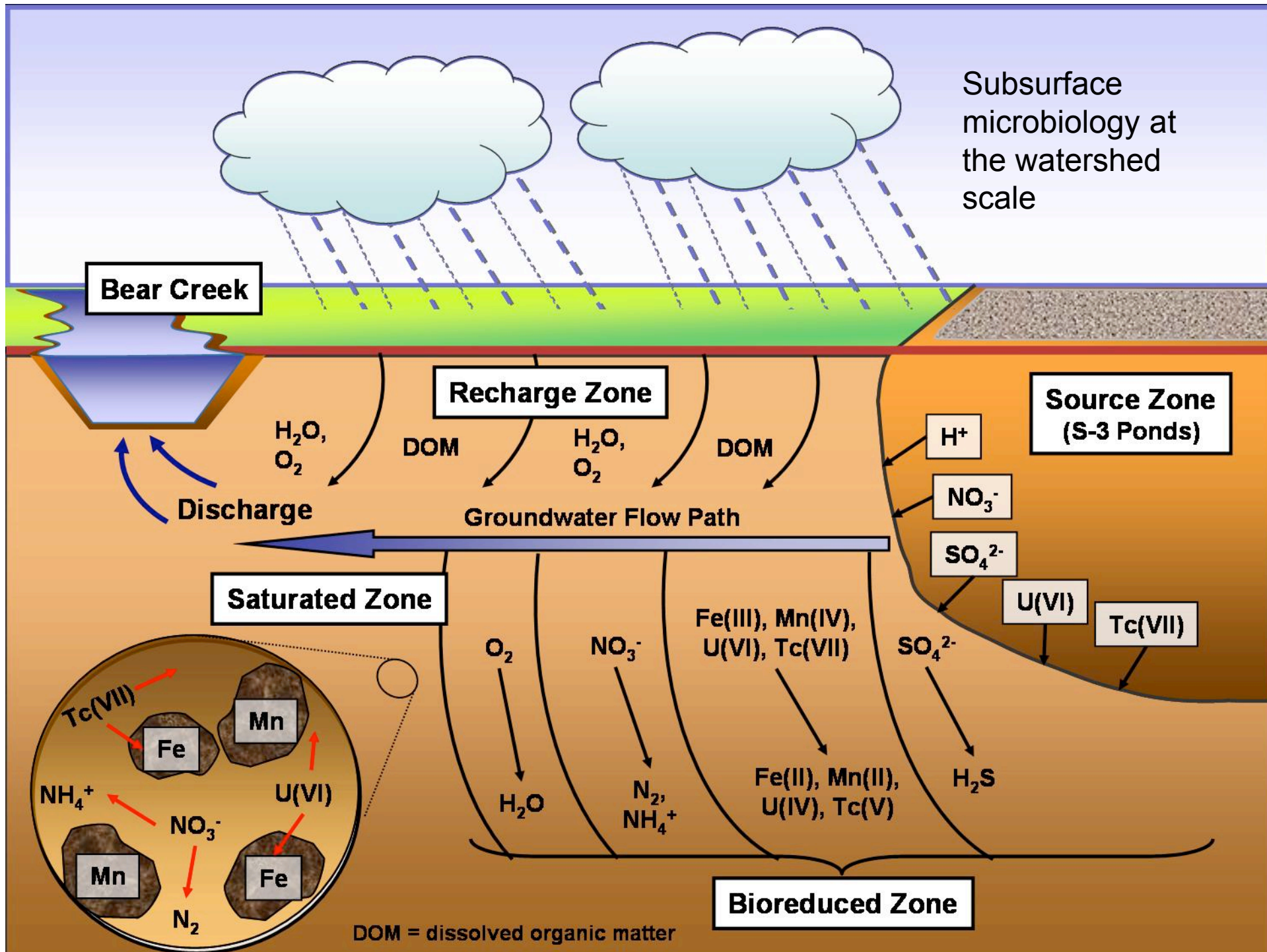
ERSP Annual PI Meeting, Lansdowne, VA

April 16-19, 2007



- **PROBLEM STATEMENT:** Bioremediation potential dictated by the physiological requirements for the growth and metabolism of microorganisms (Tiedje, 1993)





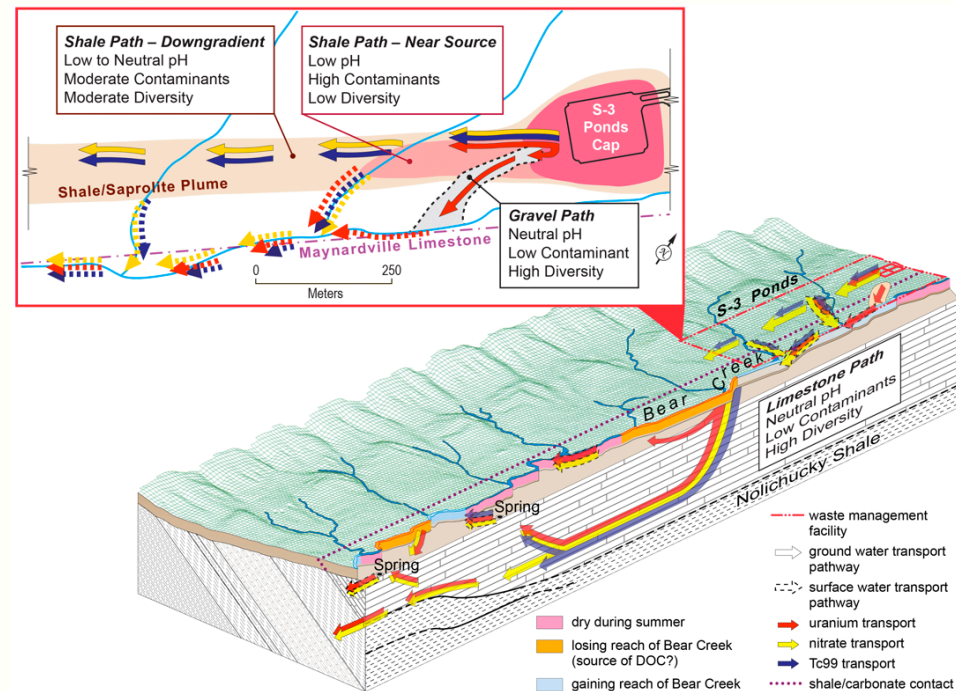


## RESEARCH NEEDS

- 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)



- ORFRC contains a unique infrastructure and well-defined subsurface contaminant plumes with contrasting parent rock lithologies, pH, contaminant load, and redox conditions
- Provides an ideal natural laboratory to elucidate subsurface microbial communities under conditions representative of radionuclide-contaminated DOE sites exposed to mixed wastes



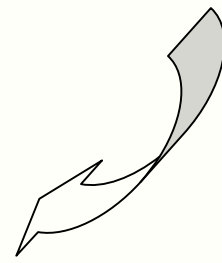
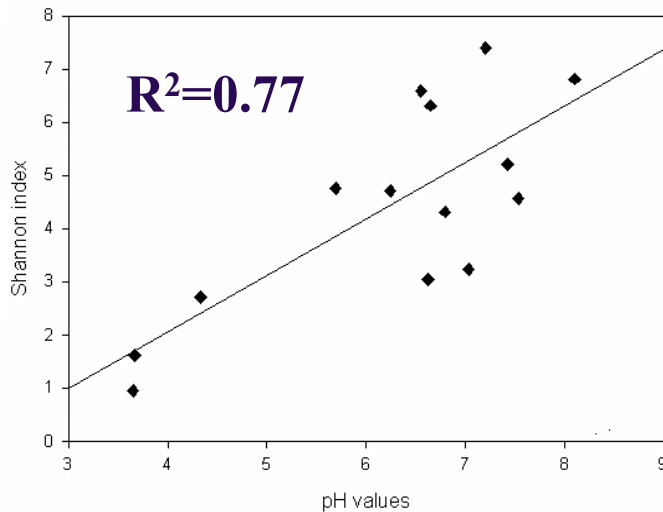
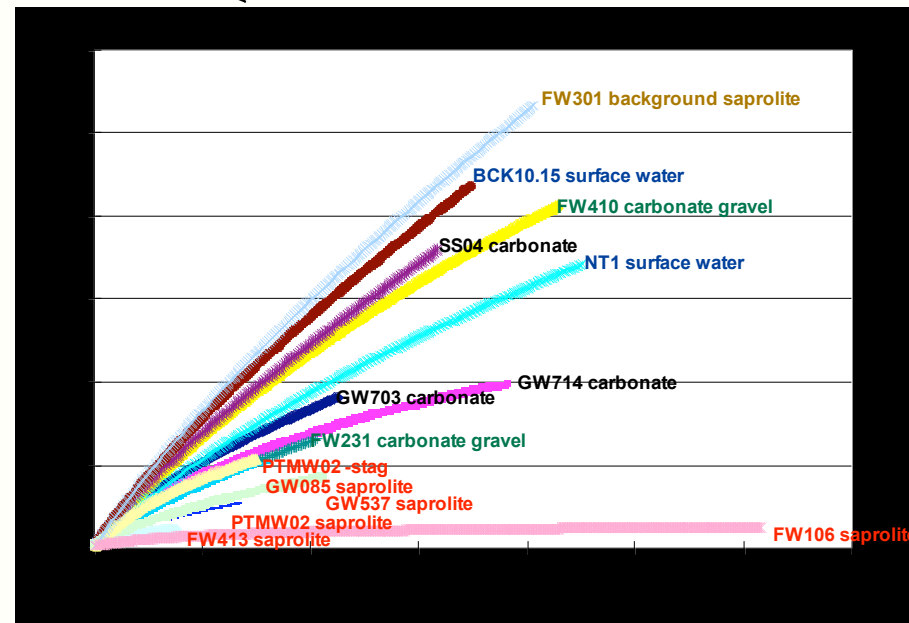
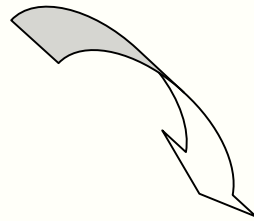
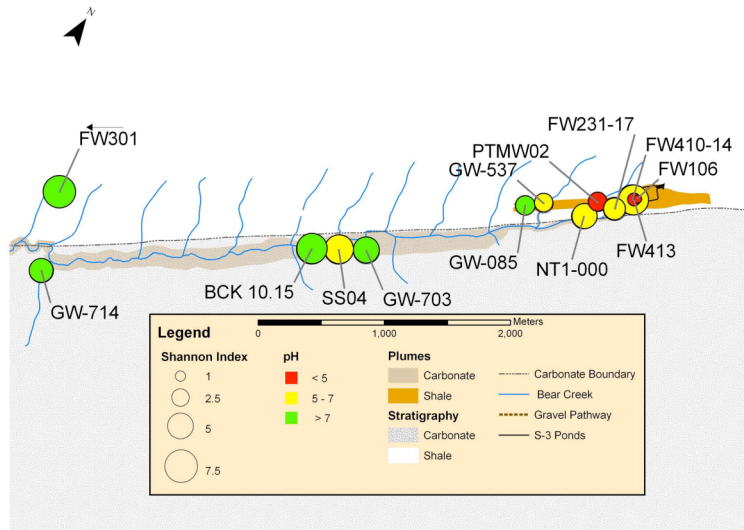


## PREVIOUS RESEARCH

- Extensive field experiments indicate that indigenous microorganisms reduce U, Tc, and nitrate in the subsurface via biomanipulation
- Subsurface microorganisms have been characterized in unprecedented detail at the ORFRC
- Several thousand genetic sequences from groundwaters and sediments have been retrieved by numerous investigators, compiled, and annotated by our group into the only sequence database of its kind for DOE sites.
- Field studies have shown that in concert with prevailing physicochemical conditions, contaminant concentrations directly impact the diversity and activity of subsurface microbial communities.
- Construction of the most comprehensive functional gene arrays (FGAs) has allowed the characterization of groundwater microbial communities at high resolution.



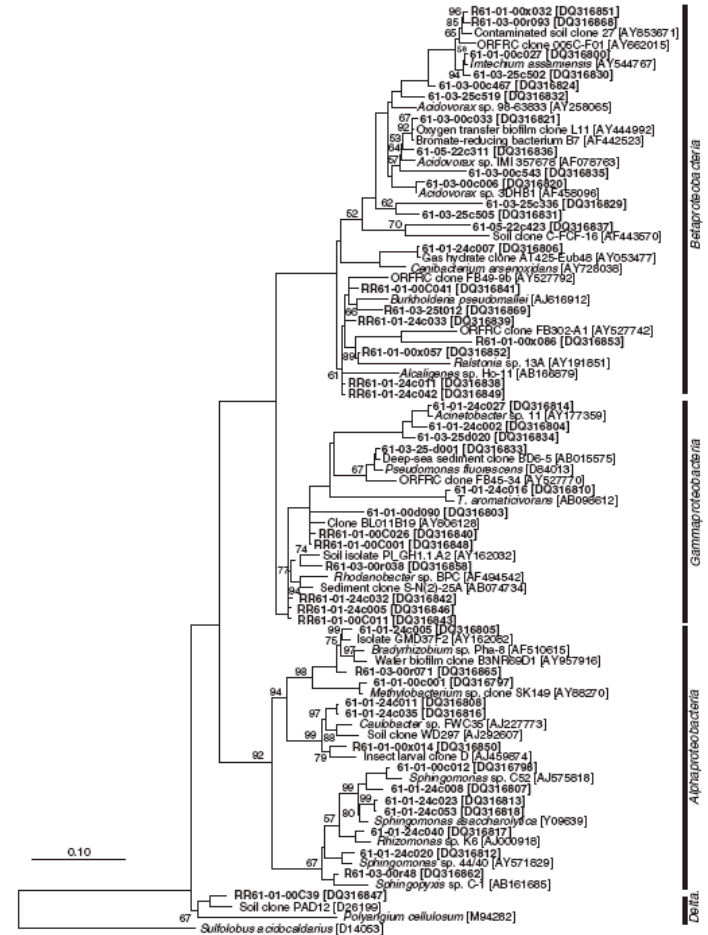
# Shannon Index vs pH across Watershed



Preliminary watershed scale surveys indicate strong influence of pH



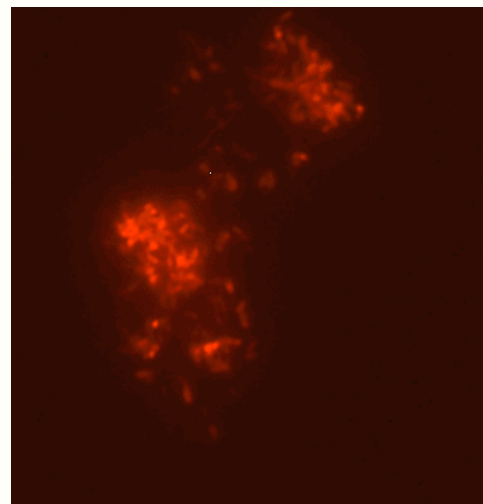
- Large amount of sequence information on who's there, but we don't know how many or whether they are active in catalyzing bioremediation reactions
- “Ecstasy and agony of environmental genomics”
- Little information on metabolic function *in situ*
- No consensus on:
  - Whether there are predominant, “metabolically-active” groups or many such populations that catalyze the attenuation and immobilization of contaminants *in situ*
  - Their distribution across changes in the relevant environmental parameters likely to control bioremediation potential







- **In situ quantification and characterization** of microbial groups under low nutrient conditions of subsurface
  - Techniques remain tedious
  - Most studies semiquantitative
  - ORFRC subsurface analogous to “deep biosphere”
- **Scale:** no systematic studies exist that could be used to predict community metabolism at the *watershed* scale
- **Heterogeneity:** variation along fracture zones, physicochemical gradients
- **Free-living + attached microorganisms:** groundwater collection is the only practical means at present for monitoring over large scales, yet the bulk of contaminants and active microbes reside in subsurface sediments
  - Use wells, sediments, or both for monitoring of microbial reactions during NA and targeted immobilization? If sediments, at what scale and frequency must they be sampled?





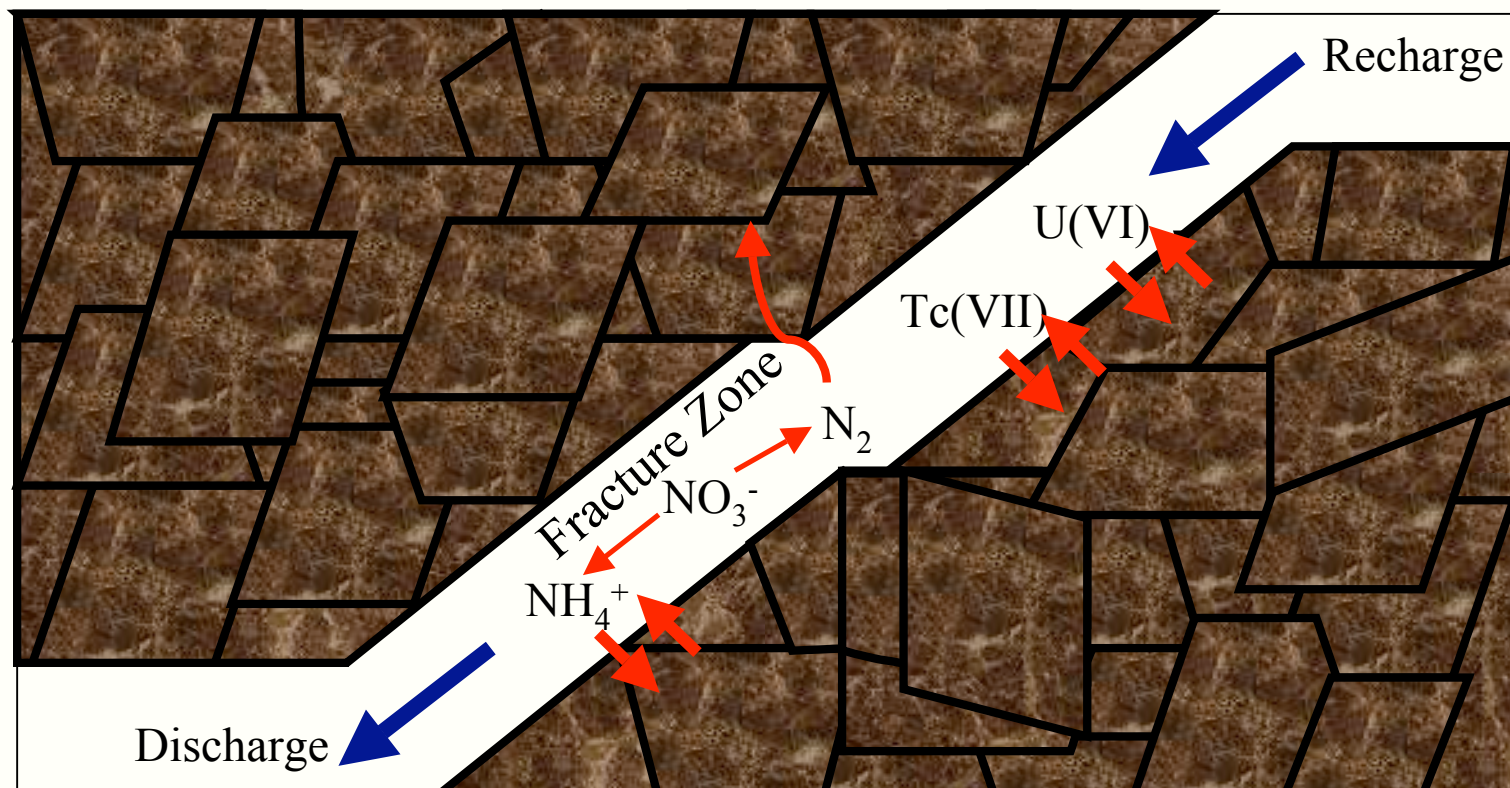
## TECHNICAL APPROACH - NATURAL ATTENUATION

- Task B1. Microbially-mediated mechanisms (rates and pathways) of contaminant transformation during longterm natural attenuation
  - Develop high throughput, cultivation-independent methods that allow quantification and characterization under oligotrophic conditions of subsurface
  - Address heterogeneity and scaling issues for microbial assessment
  - Quantify microbial mediation of U, Tc, and nitrate transformation along shale and carbonate plume pathways
    - Cross correlate dynamics of groundwater and solid phase geochemistry with dynamics of metal- and nitrate-reducing prokaryote groups
      - Rates of N species transformation and metal reduction will be correlated to message RNA analysis of key gene targets
  - Quantify metabolically-active microbial groups on a site-wide scale along temporal and spatial gradients in pH and redox conditions



## TECHNICAL APPROACH - NATURAL ATTENUATION

- Task B3. Impacts of recharge on microbial community dynamics
  - Impacts will be determined by correlating microbial assessments with geophysical imaging, water levels, and geochemical parameters in a time series
  - Drainage ditches and wells





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## TECHNICAL APPROACH - TARGETED IMMOBILIZATION

- Task C. Microbiological contribution to targeted immobilization strategies and longterm contaminant stability for secondary source control
  - Population dynamics of metabolically active microbial communities will be monitored in field and laboratory experiments where pH and nutrient levels are amended
  - Impacts of mixed electron donors on functional diversity
  - Change in microbial community dynamics upon increasing pH
    - For example, how do Fe(III) vs. sulfate-reducers respond to increasing pH in the biostimulated zone?
    - Which denitrifiers become active after raising pH?
  - Identify microbial functional groups predominating in response to organophosphate and oleate amendments



## APPROACH - METHODS

- Determine abundance/ biomass, distribution, community composition, activity
- Elucidate distribution of functional groups (metal and nitrate-reducers)
- Functional microbial diversity will be investigated over the site-wide scale using high throughput methods including community fingerprinting (TRFLP) and functional gene arrays (FGAs)
- High throughput methods will be verified through clonal analysis.
- Microbial groups will be quantified using state-of-the-art methods including realtime RT-PCR of rRNA and mRNA targets and catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH)



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## APPROACH - METHODS

- **Community structure**
- Quantification of metabolically active groups
- Rate measurements



## New features for GeoChip 3.0

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- **GeoChip 3.0 is more comprehensive and more representative.** It covers **>37,700** gene sequences of **290** gene families.
- Automatically retrieved sequences by key words is verified by HUMMER and then **unrelated sequences are removed.**
- **A software package** for sequence retrieval, probe and array design, probe verification, array construction, array data analysis, and information storage.
- **Automatic update** greatly facilitates the management of such a complicated functional gene array.



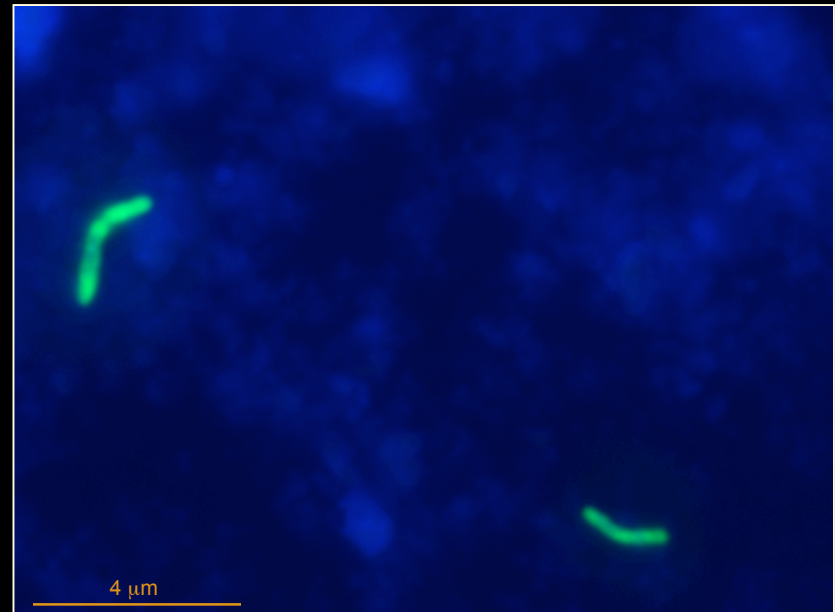
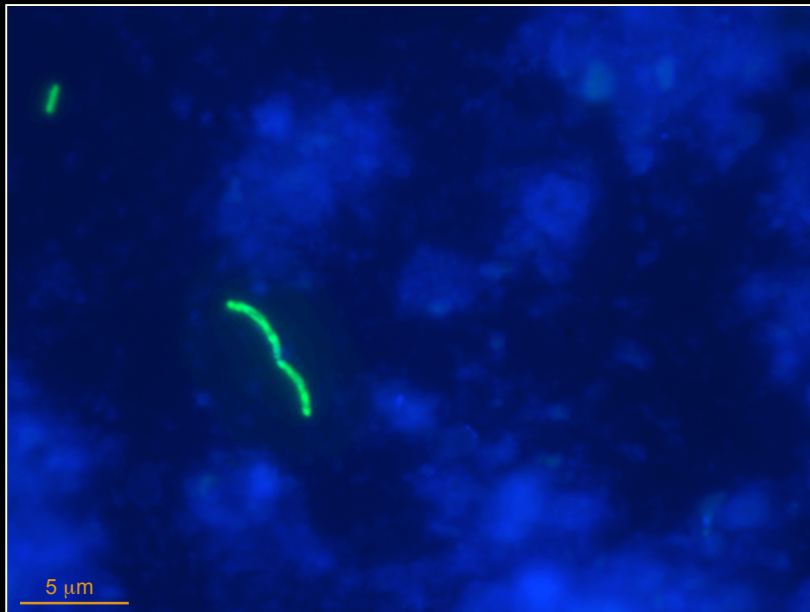
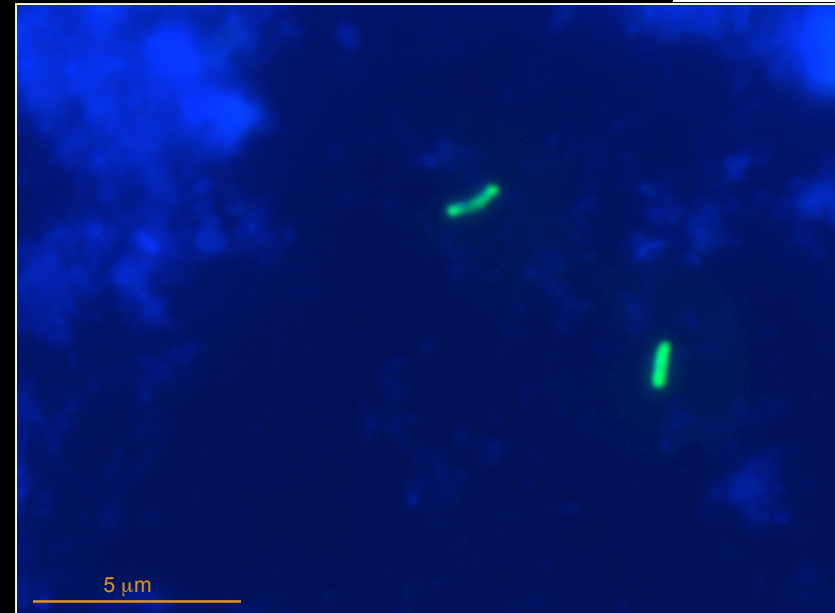
## APPROACH - METHODS

- Community structure
- **Quantification of metabolically active groups**
- Rate measurements





- CARD-FISH, EUBI-III + DAPI
- Collaboration with Dr. Rudi Amann, Max Planck Inst. for Marine Microbiology-Bremen
- FW106: unstimulated, low pH, high contaminant load well





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## APPROACH - METHODS

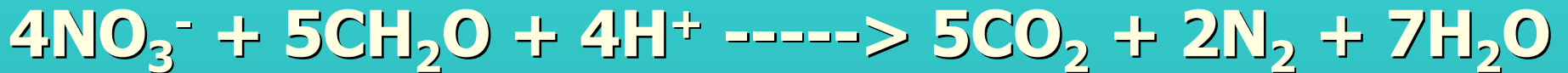
- Community structure
- Quantification of metal- and nitrate-reducing prokaryote groups
- **Rate measurements**



# Nitrate Reduction

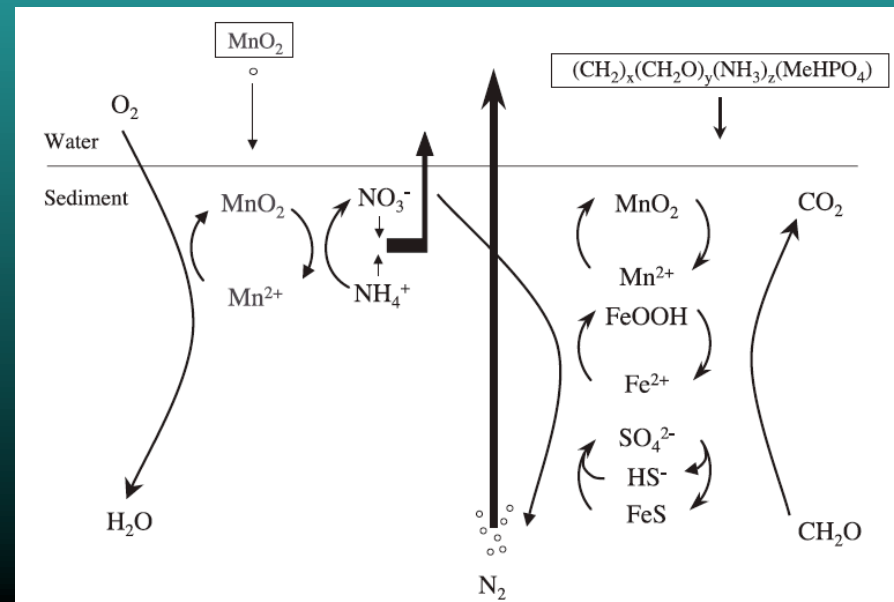


- Denitrification



- Alternative pathways

- Anaerobic ammonium oxidation (anammox)

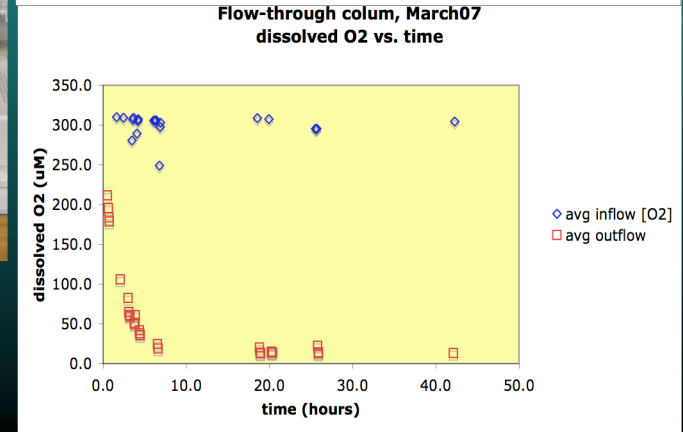
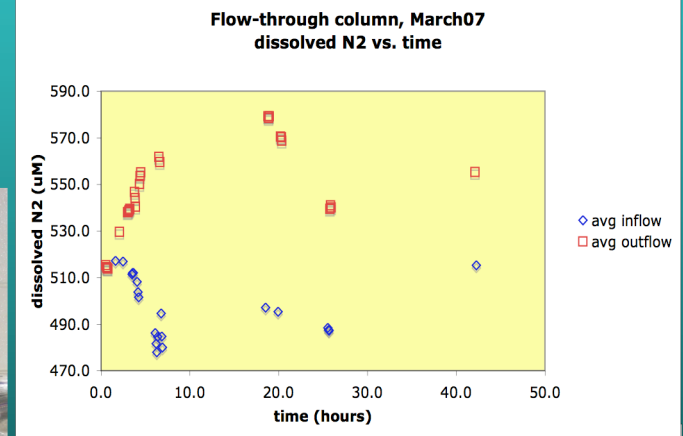




# Dissolved Gas Analysis ( $O_2$ , $N_2$ , Ar)

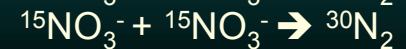
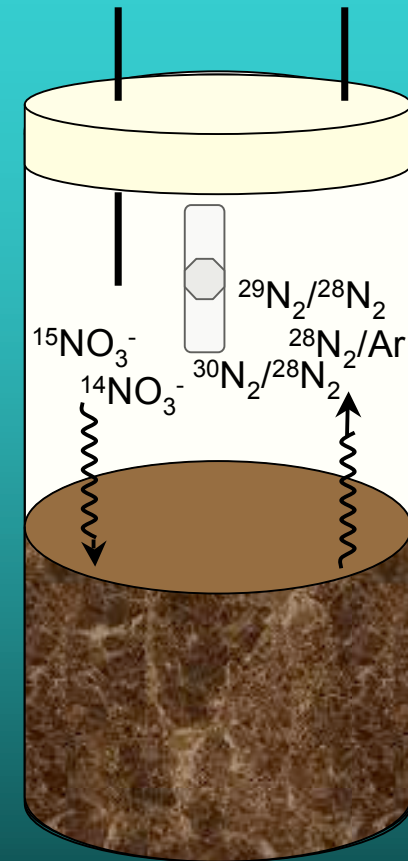
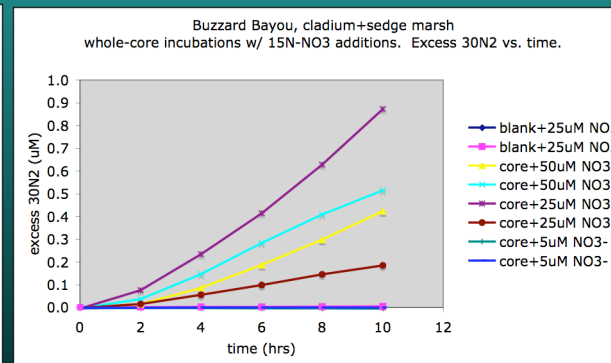
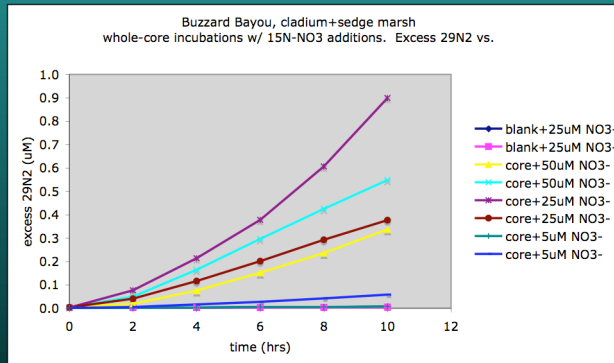
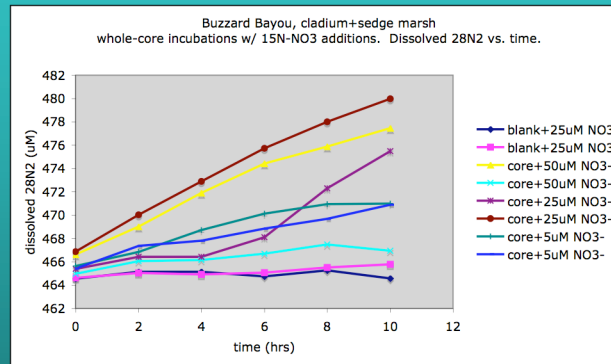
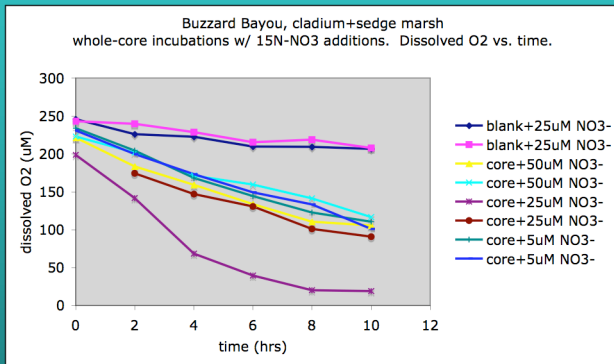


- Membrane Inlet Mass Spectrometry (MIMS)
- High precision gas analysis in water samples
- Silicone capillary membrane extracts gases in vacuum line
- Cryotrap to remove water,  $CO_2$
- Quadrupole mass spectrometer





- Addition of  $^{15}\text{N}$ -labeled  $\text{NO}_3^-$  and other N species
- Quantify various mass abundances of  $\text{N}_2$  to track pathways





- **High throughput molecular tools** for monitoring microbial populations with a high remediation potential at low biomass (Tasks A to D)
- **Assessment of microbial communities** and their heterogeneity over a range of scales up to the entire watershed (Task A)
- Better understanding of **impacts of pH, redox, and recharge** on in situ microbial community dynamics during natural attenuation (Task B)
- Better understanding of **biomanipulations** ( $e^-$  donor, organophosphate, pH) on microbial dynamics for optimization of immobilization and stability of contaminants from secondary sources (Task C)
- **Rates of microbial processes** determined using geochemical methods will provide inputs/verification of kinetic parameters and molecular monitoring tools in modeling (Task D)
- Cultivation-independent **quantification of the abundance/ biomass of active microbial groups** as well as “unwanted” microbial groups will also provide important inputs for modeling efforts (Task D)



- FSU: Heath Mills, Denise Akob, Tom Gihring
- ERSP Student Travel Fellowships
- Transatlantic Biotechnology Exchange Fellowship Program, EU-US Task Force on Biotechnology Research
  - Judy Wall, Joe Suflita, Anna Palmisano





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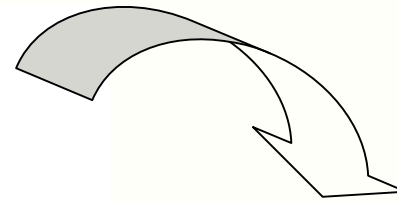
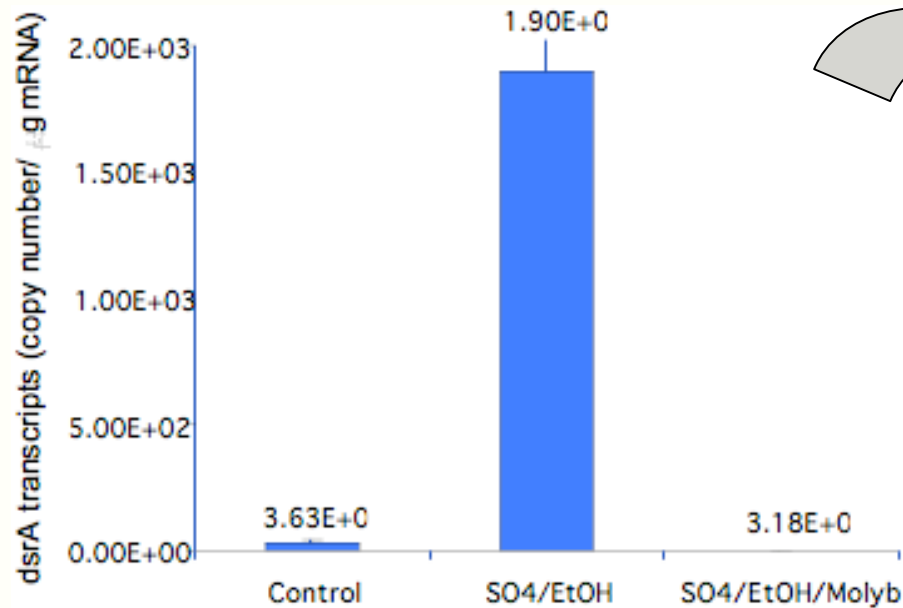
## **Presentations to follow: *Detailed Research Tasks and Descriptions***

- **(Task B) Natural Attenuation**: Rates and Mechanisms along pathways and within source zones (speaker: Watson) 20 min
- **(Task C) Targeted Manipulations**: Enhanced contaminant stability of source zones (speaker: Criddle) 20 min
- **(Tasks A-C) Geophysics**: Characterization and monitoring (speaker: Hubbard) 20 min
- **(Tasks B & C) Microbiology**: Characterization and monitoring as a function of scale (speaker: Kostka) 20 min
- **(Task D) Numerical Modeling**: Multiscale flow and transport modeling, upscaling, and advanced pattern recognition (speaker: Parker) 15 min
- **Research Outcomes, Site Contributions, and Opportunities**: (speaker: Jardine) 5 min

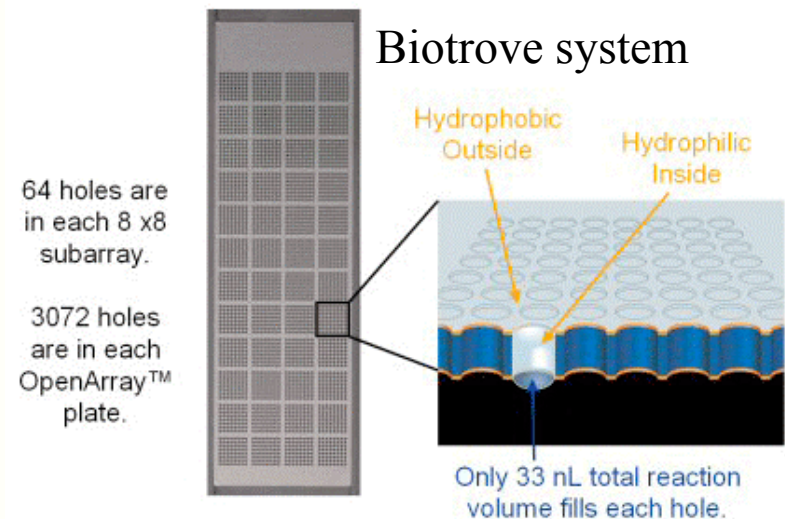


## Quantification of gene expression in ORFRC subsurface sediments

- *dsrAB* gene expression



May need a scalable platform  
for application to watershed  
scale analysis and detailed  
experimental studies



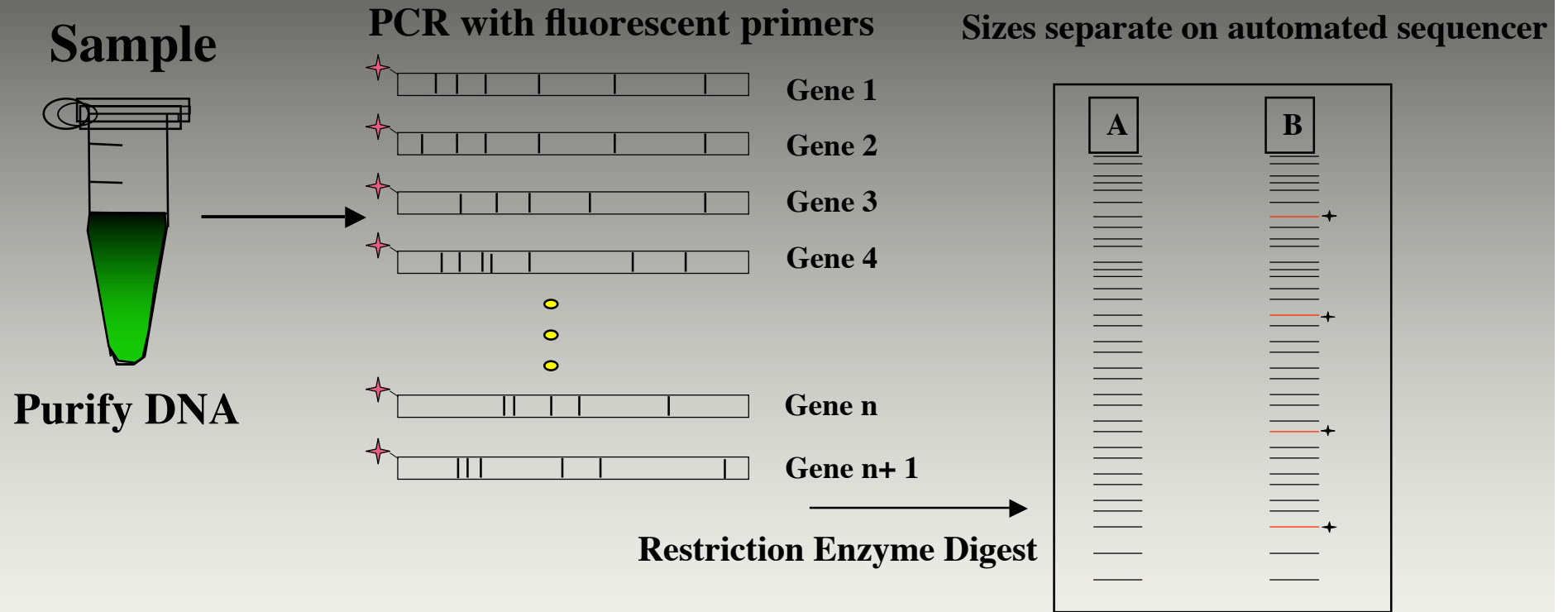
Collaboration with Dr. Kuki Chin,  
Georgia State University



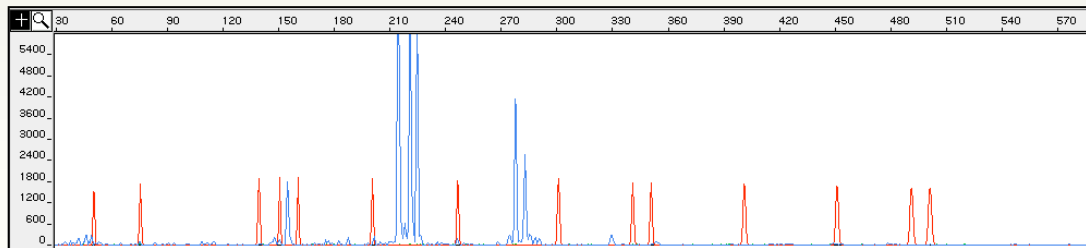
## HYPOTHESES - MICROBIOLOGY

- **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.

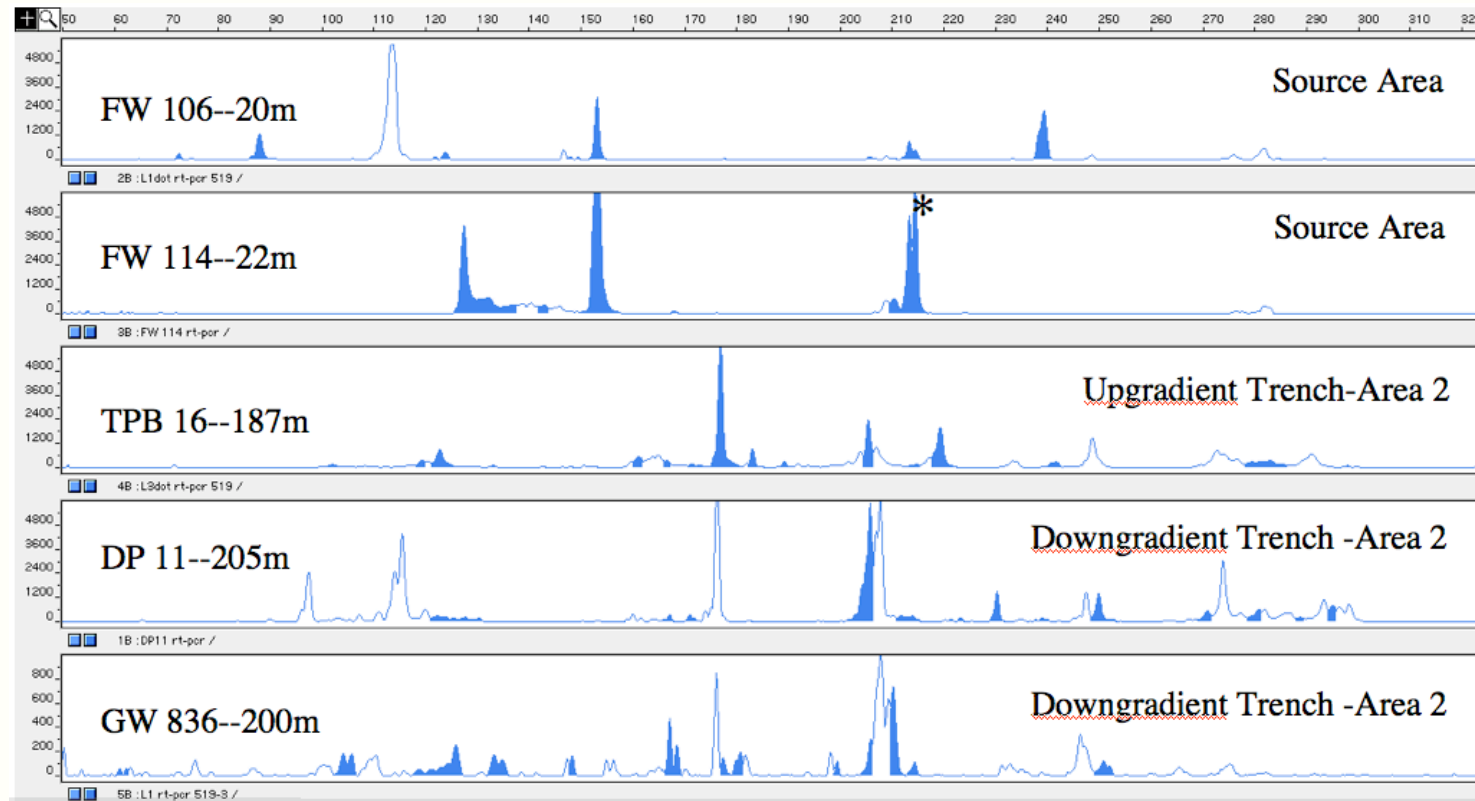
# How does Terminal Restriction Fragment Length Polymorphism work?



**Only labeled fragments appear as peaks**



**ABI Software Analysis**

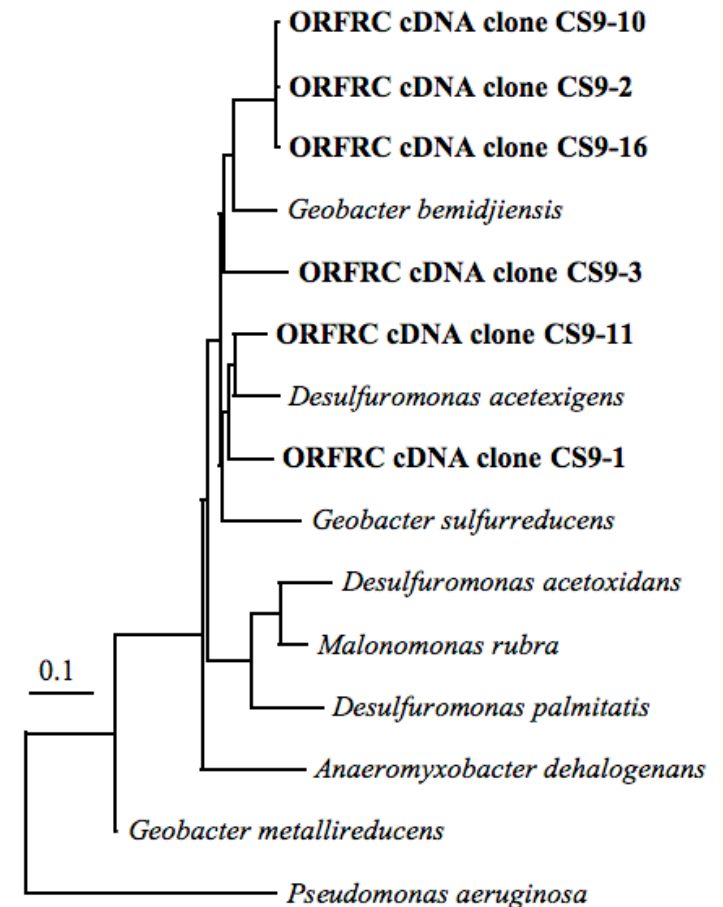
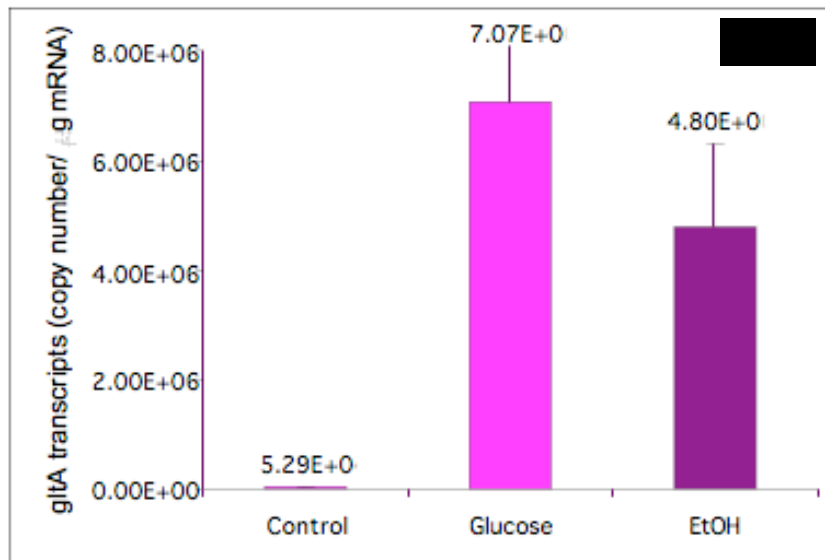


- T-RFLP profiles of RT-PCR reactions of 16S rRNA extracted along the contaminant plume
- Metal-reducing *Deltaproteobacteria* (*Anaeromyxobacter* and *Geobacter*) detected often at DNA level



## Quantification of gene expression in ORFRC subsurface sediments

- mRNA extracts
- Quantification by reverse transcription realtime PCR during metal reduction phase
- *Geobacteraceae* citrate synthase (*gltA*) gene
- Phylogenetic analysis



Collaboration with Dr. Kuki Chin, Georgia State University



# TEAM MEMBERS

- **Joel Kostka - Florida State University**
- **Anthony Palumbo, Christopher Schadt - Oak Ridge National Laboratory**
- **Jizhong Zhou - University of Oklahoma**