Linking biodiversity to the assessment of bioremediation potential in the subsurface of DOE sites

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Welcome to Florida
We are here

Acknowledgements

- FSU research team: Heath Mills, Denise Akob, Tom Gihring, Lainie Petrie (graduated, 12/04)
- Collaborators: Joe Stucki, Bernard Goodman, Lee Kerkhof, Jack Istok, Lee Krumholz, Tony Palumbo, Susan Pfiffner
- FRC staff, especially Dave Watson, Barry Kinsall
- EU-US Task Force on Biotech.; Transatlantic Environ. Biotech. Fellowship (Judy Wall, Joe Suflita, Anna Palmisano)
- The NABIR program, Biological and Environmental Research (BER), U.S. DOE (grant DE-FG02-DOER62986)

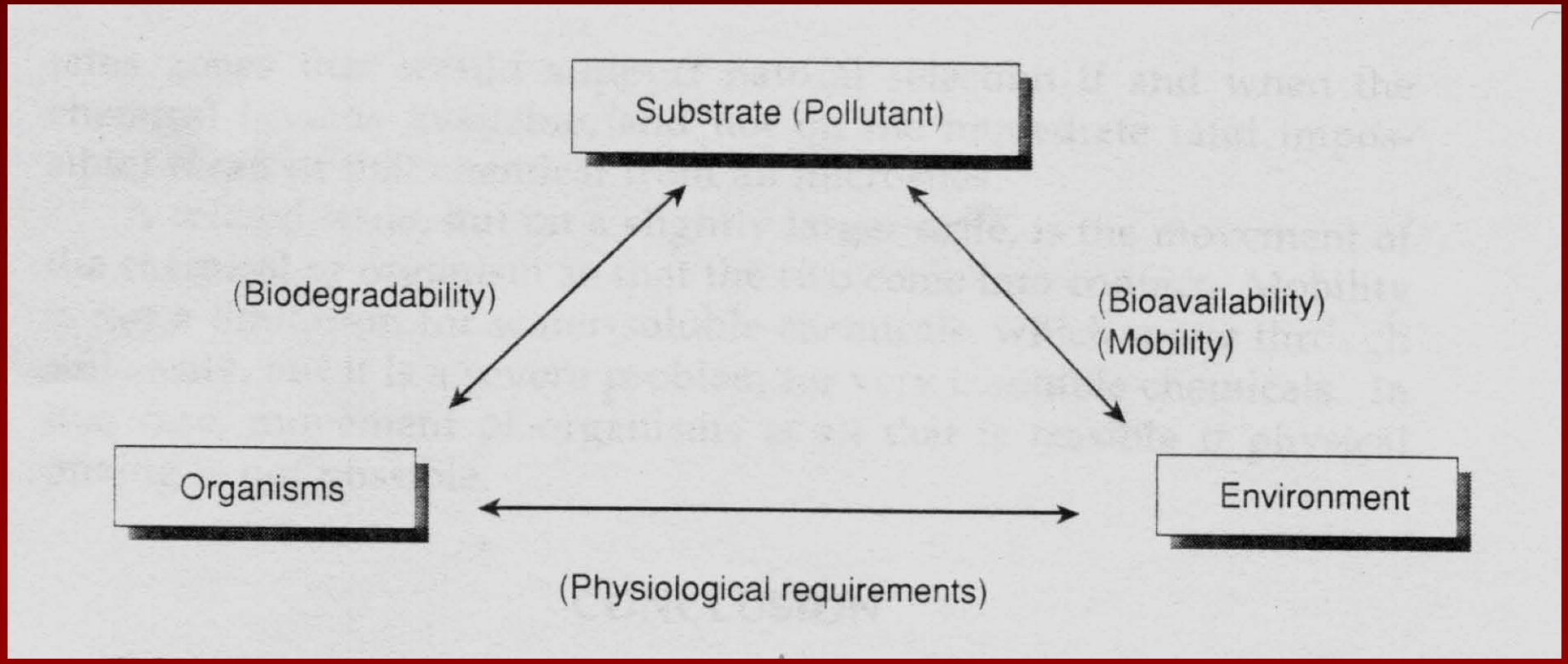
Outline

- Focus on recent work in shallow subsurface aquifers, mostly saturated, at DOE sites prior to manipulation; metal- and nitrate-reducing bacteria
- Introduction
- Assessment of microbial diversity using cultivation-independent approaches
- Cultivation of organisms with high remediation potential
- Parallels between approaches
- Conclusions

Critical Issues

- To identify, isolate, characterize microorganisms or microbial groups with a high metabolic potential for bioremediation
- To quantify distribution and biomass of remediating organisms
- To determine the mechanisms controlling the metabolism of remediating organisms, groups

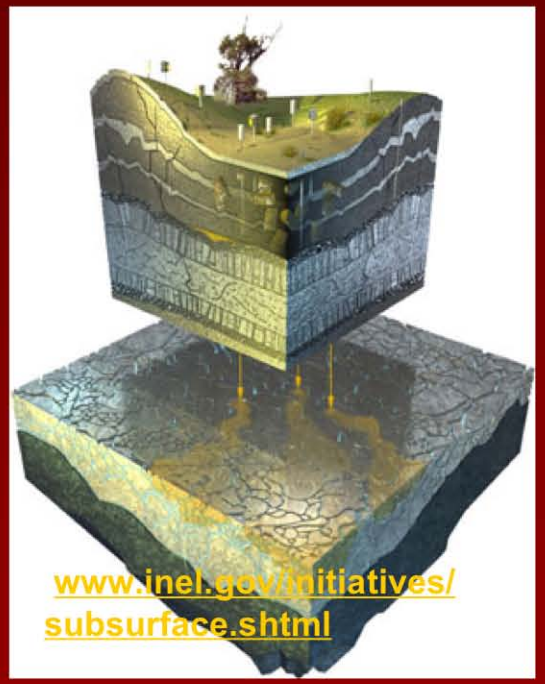
Ecological approach - bioremediation potential (Tiedje, 1993)



- ✓ Remediation potential dictated by physiological requirements for growth and metabolism
- ✓ Microbial ecology of contaminated ecosystems, interaction between remediating organisms and their surrounding environment

Microbial diversity = key to functioning of contaminated ecosystems

- Enzymes of key microbial groups catalyze processes that limit fate and transport of contaminants
- Enzyme production controlled by environmental effects and ecological interactions
- Environmental parameters (pore structure, water content, minerals, substrates, toxicity) determine which organisms thrive and whether enzymes are turned on



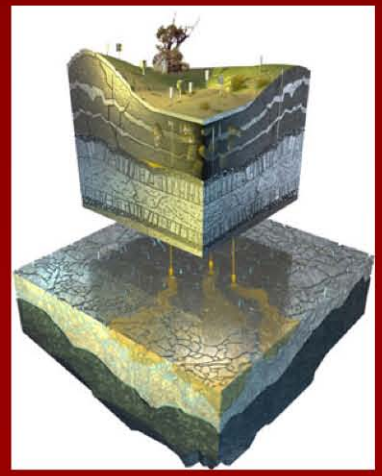
Why Diversity?

- If only necessary to identify important functional groups, then perhaps diversity not as critical
- However, complete understanding of competitive interactions, selective forces controlling distribution/ activity of microbial groups impossible without knowledge of phylogenetic composition
- Functional diversity allows prediction of status of community, response to perturbations/ stresses, interactions with surrounding environment

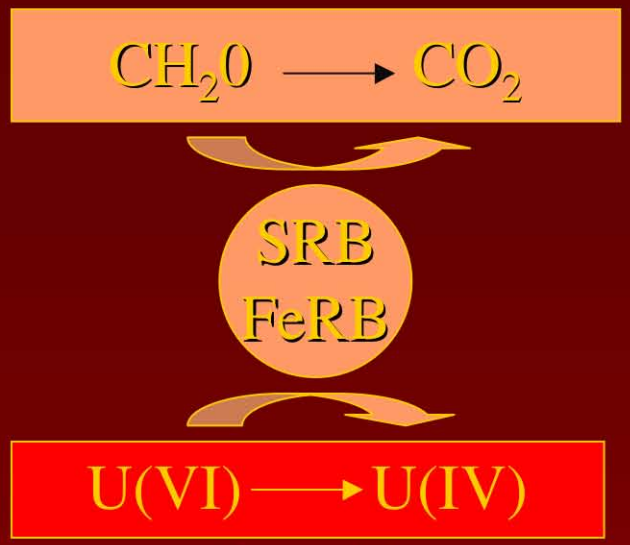
www.inel.gov/initiatives/subsurface.shtml

Diversity in subsurface

- Microbial communities remain largely unexplored
- Now have tools and conceptual framework to begin comprehensive characterization, thanks to many DOE PIs
  - Tiedje, White, Marsh, Balkwill, Hazen, Brockman, Chandler, Kuske, Palumbo, Zhou
- What is diversity?
  - Composed of 2 elements
  - Richness = number of unique taxa
  - Evenness = relative abundance of each taxon



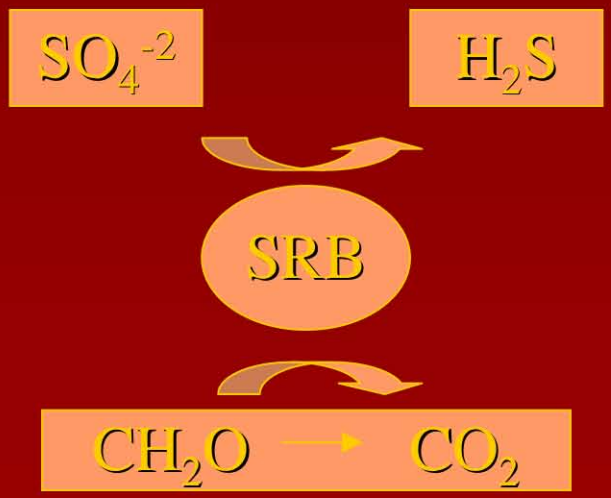
FeRB and SRB catalyze the direct (enzymatic) and indirect (abiotic) reduction of U(VI)

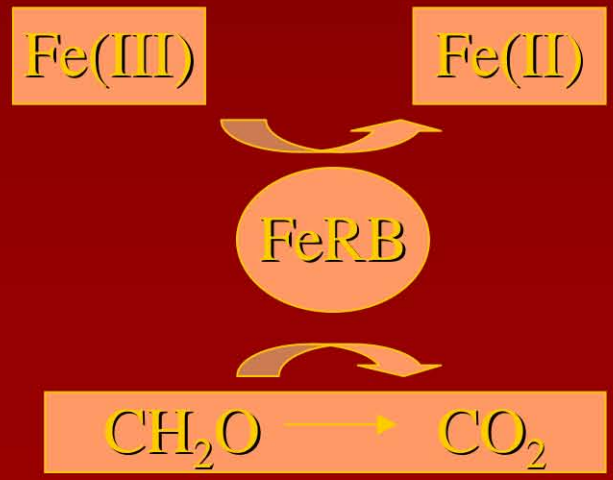


Populations capable of reducing metals, nitrate, halogenated compounds largely overlap

Abiotic reaction

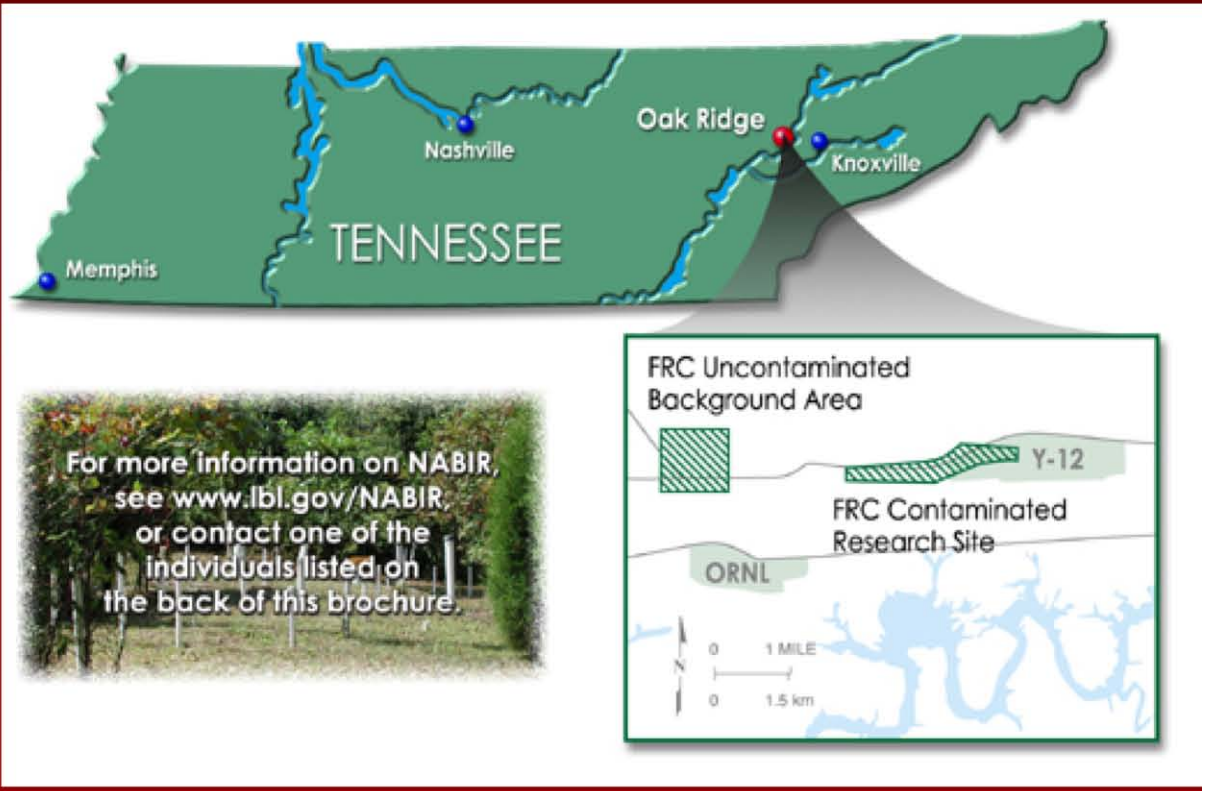
Abiotic reaction





U.S. DOE NABIR Field Research Center

- Located at Y-12 on Oak Ridge Reservation
- Site constructed as part of the secret WWII Manhattan Project to produce nuclear weapons
- FRC (Field Research Center) is centered on groundwater plumes that originate from former waste disposal ponds



Contaminated Area - Waste Ponds During Operation

Background Area

✓Pristine site with a similar parent rock mineralogy and sediment characteristics to contaminated areas of the FRC

FRC - site characterization

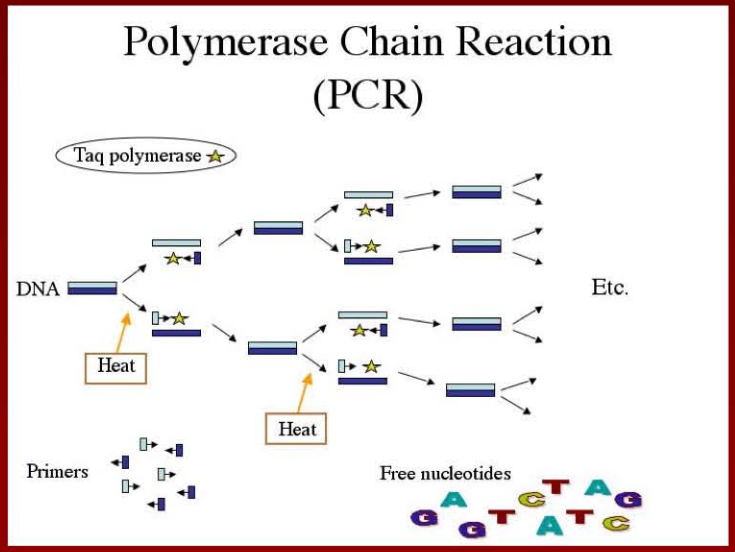
- Contaminants present: uranium, nitrate, technetium, chlorinated compounds (TCE, PCE), fuel hydrocarbons (toluene, benzene)
- Uranium and nitrate are primary contaminants driving remediation; therefore focus has been on metal- and nitrate-reducers
- Harsh subsurface environment for microorganisms; pHs 3-4, [nitrate] mM to M
- Microbial metabolism believed to be limited by: labile carbon, acidic pH, and high nitrate, toxic metals (Al, Ni)
- Upon addition of electron donor and pH neutralization, extensive nitrate and metal reduction have been observed
- Thus, “Biostimulation” or substrate addition is a promising strategy for U(VI) immobilization by indigenous microorganisms

Microbial Community Composition or Diversity

- Mostly assessments of richness or number of types
- Groundwater, biofilms, sediments
- Range of sampling strategies
- DNA, PLFA, RNA as molecular markers
- Mostly studies of 16S rRNA genes

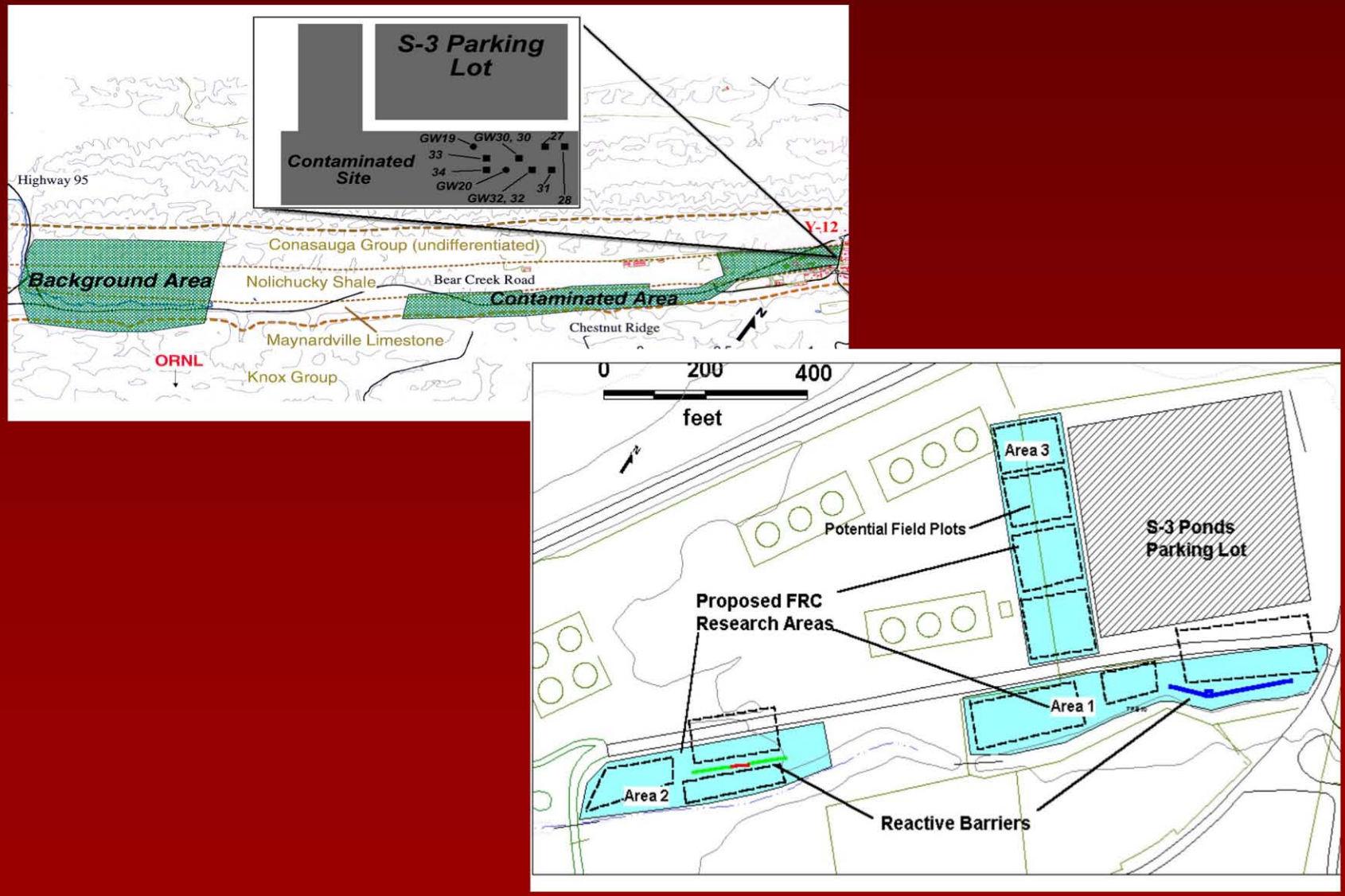
Cultivation-independent methods

- Community composition or distribution- in situ
  - PCR/cloning/ sequencing- DNA, RNA targets
  - Fingerprinting- DGGE, TRFLP
  - Quantitative PCR
  - High density oligonucleotide arrays of 16S rRNA targets
  - Functional gene arrays
  - Environmental genomics
- Methods that link phylogeny with function (generally involve substrate addition)
  - Stable isotope probing (SIP)
  - Micro-FISH



Groundwater

Sampling sites- Field Research Center



16SrRNA - FRC, Areas 1-3, bkgd

- Contaminated wells in close proximity to S-3 source ponds compared to pristine bkgd site
- Wells represent range in concentration of contaminant drivers (pH, nitrate, U)
- Most extensive spatial comparison of areas within FRC published to date
- Metagenomic sequencing of FW-010 community to follow

Table 1
The pH, nitrate, uranium, aluminum and nickel levels in the groundwater samples from four characterized FRC groundwater samples

Well	pH	Nitrate (mM)	Uranium (μM)	Nickel (μM)	Aluminum[a] (mM)
FW-300	5.8 ± 0.4	0.04 ± 0.05	ND	0.45 ± 0.5	0.01 ± 0.01
FW-005	4.0 ± 0.2	4.6 ± 2.5	30 ± 4.8	84 ± 1.0	1.74
FW-010	3.5 ± 0.1	694 ± 28	0.8 ± 0.1	310 ± 17	41.5
FW-015	3.5 ± 0.2	154 ± 27	31 ± 2.7	148 ± 2.5	22.9

FW-300 represents the background area. ND, not detected.
[a] Some samples measured once.

✓ Fields et al., 2005, FEMS Microbio. Ecol., in press

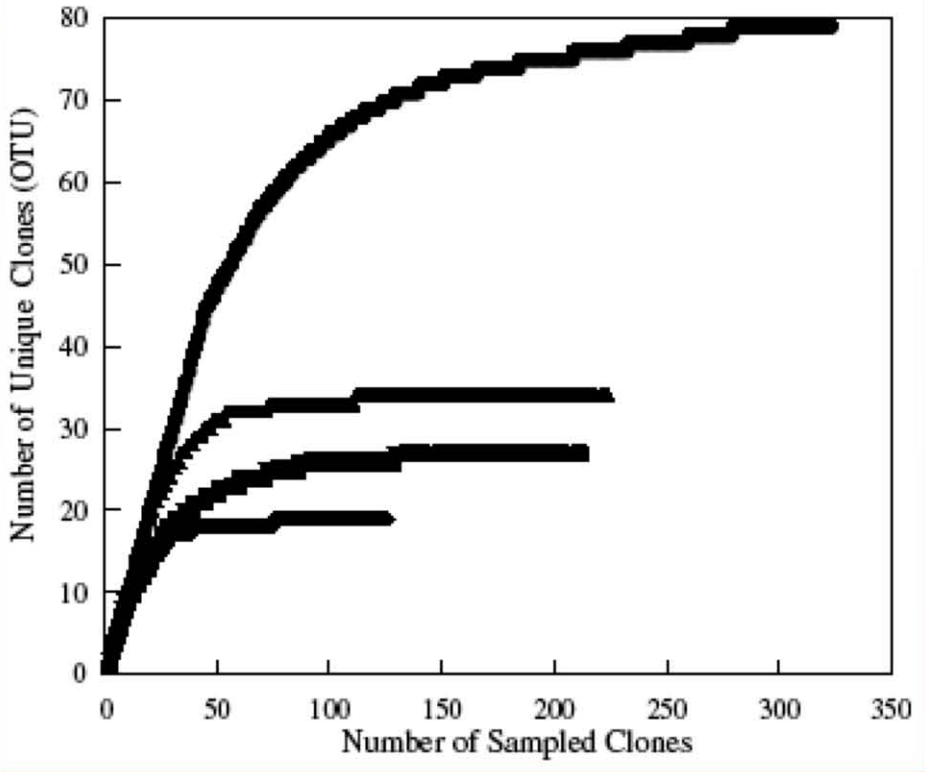
16S rRNA - FRC, Areas 1-3, bkgd

- Bkgd library showed highest diversity and evenness
- Majority of OTUs from bkgd differed from acidic sites
- Libraries of acidic sites dominated by *Betaproteobacteria*
- Nitrate-reducing bacteria (*Azoarcus*, *Ralstonia*) but not metal- or sulfate-reducers detected
- No significant correlation between diversity and geochemical parameters

Table 3
Characteristics and diversity estimates for SSU rRNA gene clones from four FRC groundwater samples

Well	Number of clones[a]	OTU[b]	H′[c]	1/*D*[d]	Evenness[e]
FW-300	321	79	5.17	22.7	0.97
FW-005	211	27	3.18	4.91	0.82
FW-010	113	19	2.62	3.81	0.77
FW-015	231	34	2.84	3.43	0.73

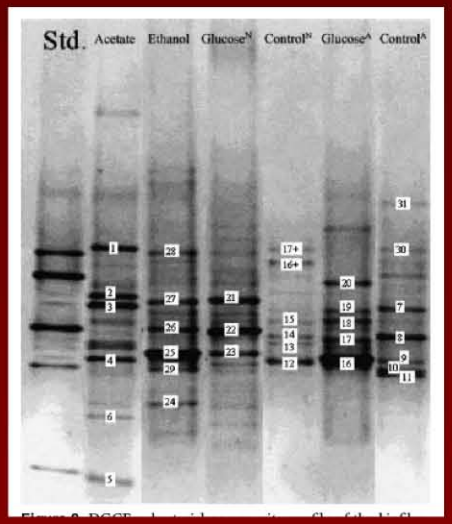
FW-300 represents the background area.
[a] Number of clones analyzed from each library.

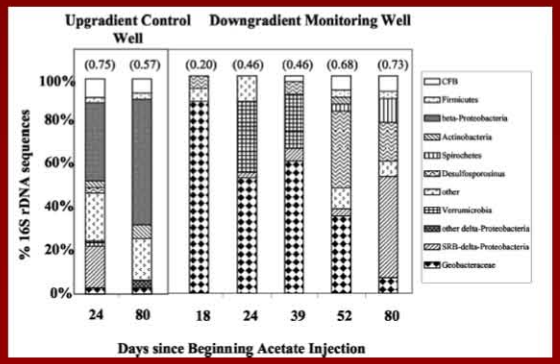


✓ Fields et al., 2005, FEMS Microbiol. Ecol., in press

Other studies from groundwater

- FRC, Old Rifle site, Colo.
- Clone libraries, DGGE profiling of 16S genes
- *Betaproteobacteria* dominated in control wells
- Nitrate- (*Alcaligenes*, *Ralstonia*) but not metal-or sulfate-reducers detected in controls



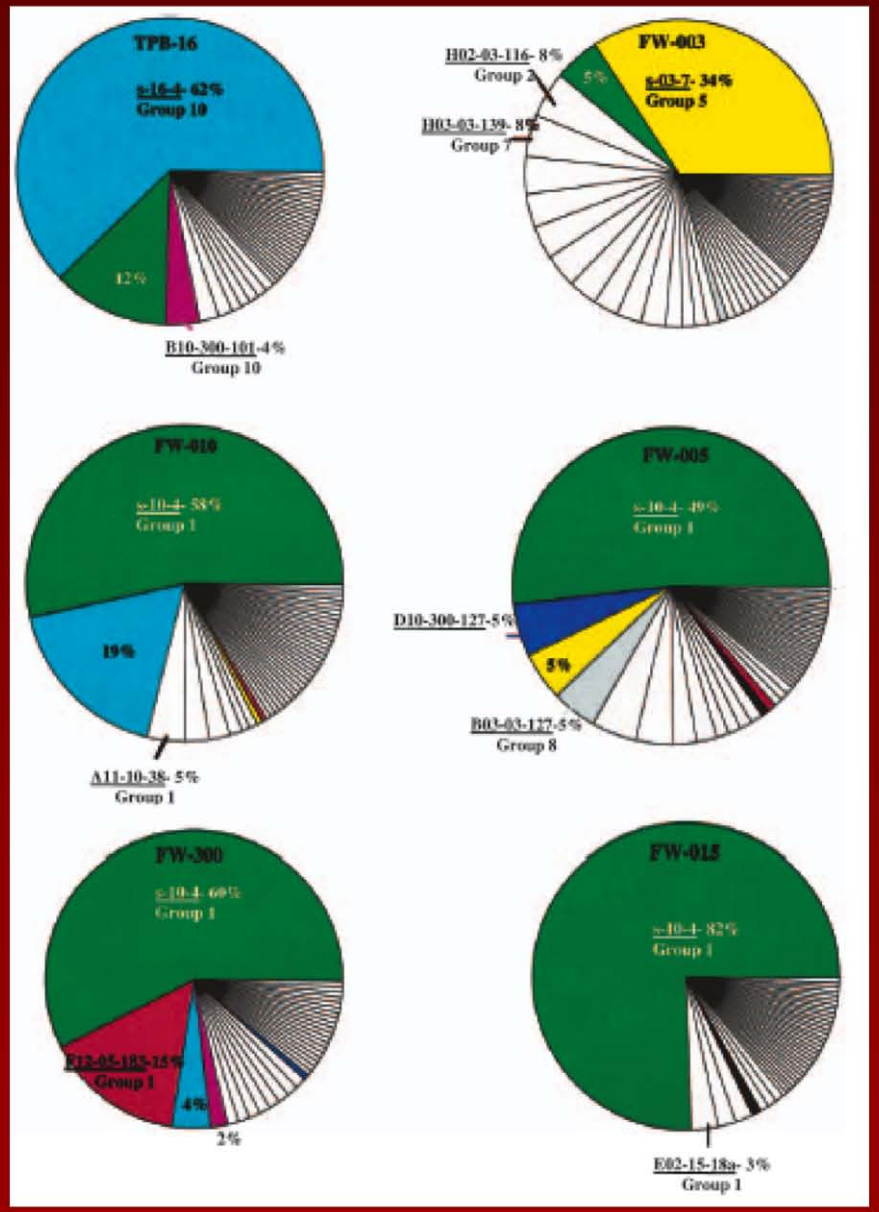


✓ Peacock et al., 2004, Microbial Ecology 47: 284

✓ Anderson et al., 2003, AEM 69: 5884

Denitrifiers - FRC, Areas 1-3, bkgd

- Functional genes for denitrification; nitrite reductases
- Expanded series of wells
- 958 nirK and 1162 nirS clones screened
- Dominant phylotype for nirS 95% similar to *Alcaligenes*
- Diversity of nirS larger and different from nirK
- Diversity not strongly related to any geochemical characteristic

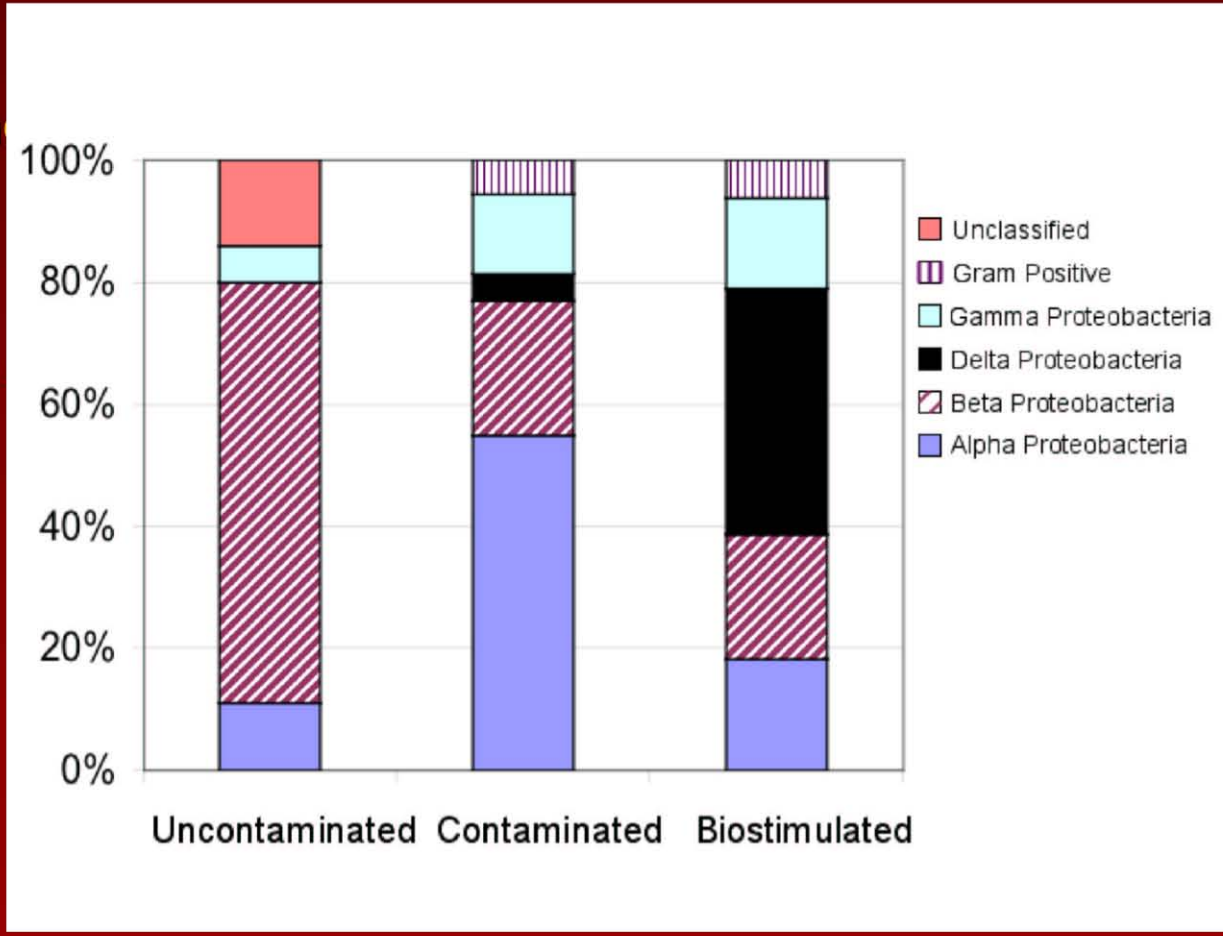


✓Yan et al., 2003, Environ. Microbiol. 5: 13

Sediments

Sediments - FRC, bkgd and Area 1

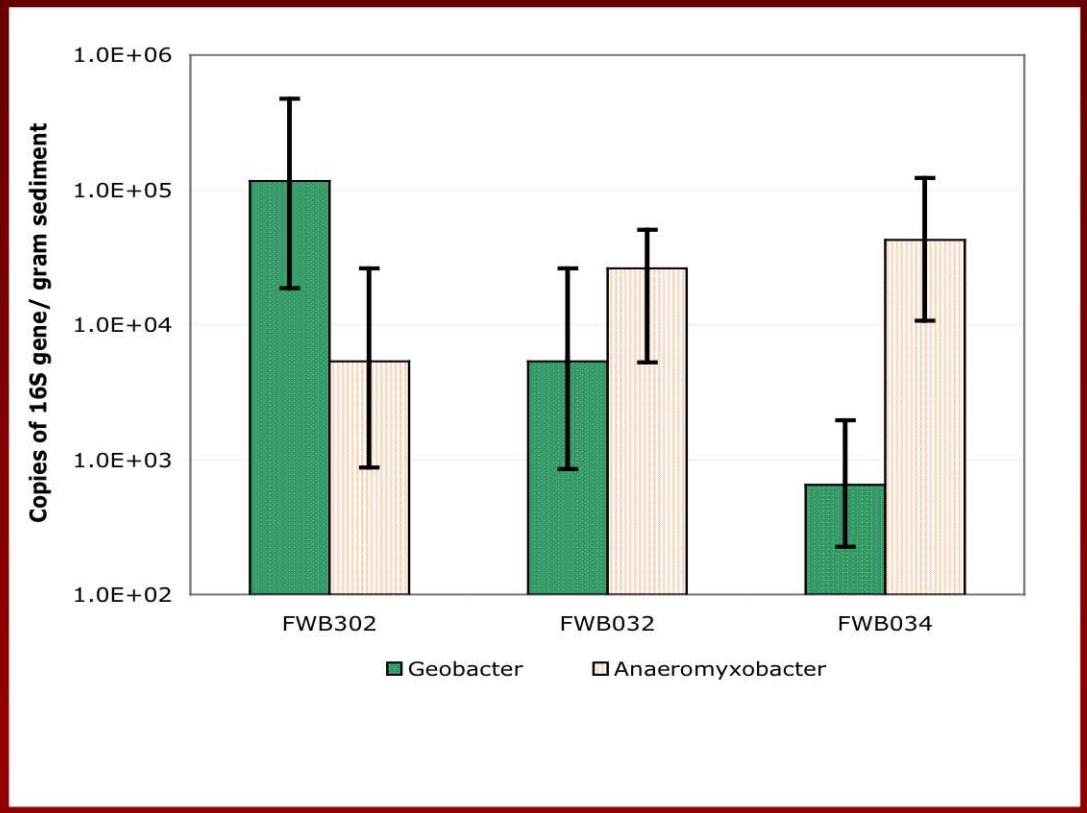
- Diversity relatively high; lower in bkgd relative to contaminated
- Libraries contained mostly *Alpha-*, *Beta-*, an *Gammaproteobacteria*
- Nitrate-reducers (*Ralstonia*, *Pseudomonas*) and one metal-reducer (*Anaeromyxobacter*) detected



✓North et al., 2004, AEM 70: 4911

Sediments - FRC, bkgd and Area 1

- MPN-PCR of 16S targets
- *Geobacteraceae* sequences were one to two orders of magnitude less abundant in contaminated (FWB032, FWB034) as compared to background (FWB302) sediment
- *Anaeromyxobacter* sequences were more abundant in contaminated sediments



✓Petrie et al., 2003, AEM 69: 7467

Comparison downcore

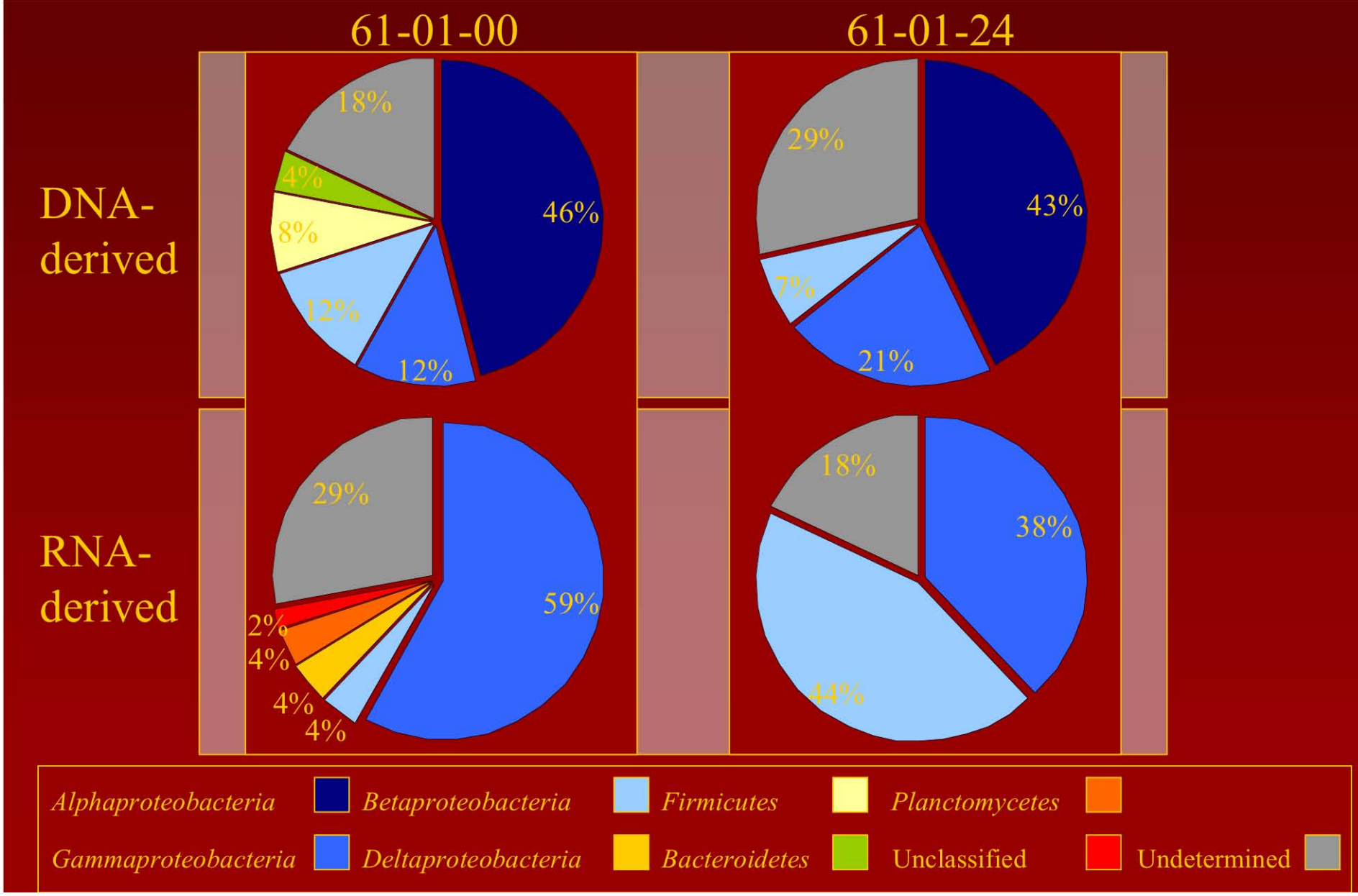
- Core depths range from 3 to 6 m below surface
- Lower OTU diversity and greater dominance by fewer OTUs
- Like other high carbon sites, uniform diversity pattern observed with depth
- No clear relationship with contamination
- No significant difference in diversity indices observed between DNA libraries
- Some differences in the frequency of specific taxa were observed

Table 1: Sediment characteristics of Area 1 borehole FB61.

Depth Interval	pH	Nitrate (umol/g)	Fe-oxalate extract (umol/g)	Fe-HCl extract (umol/g)
61-01-00	6.7	0.6	31.5	27.6
61-01-24	6.1	0.1	17	1.8
61-03-00	3.9	17.8	17.3	3
61-03-25	3.7	40.1	18.6	5.5
61-05-22	4.1	35.2	12.1	22.8

✓Mills, Akob, Kostka, in prep.

Clone library analysis



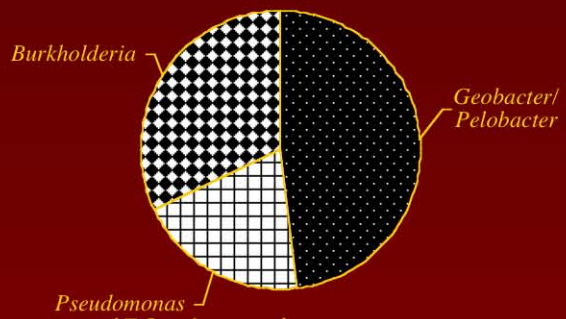
Cultivation

- Only a fraction of in situ community can be cultured
- Required to gain a comprehensive understanding of physiology of potential remediating organisms
- For example, application of genomics and testing of *in silico* models

Background:

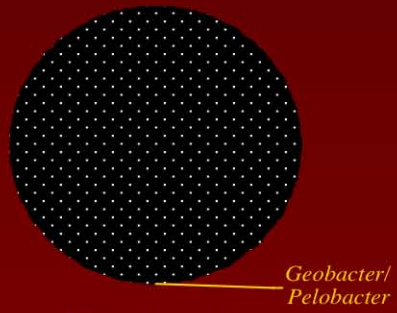
Cultured at pH 7

Acetate



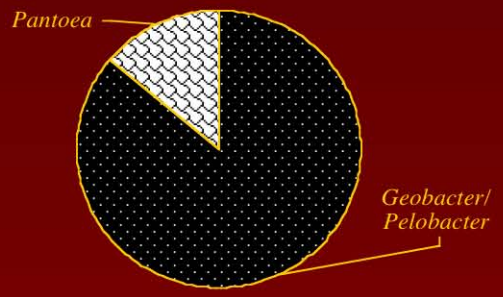
(50 clones)

Lactate



(39 clones)

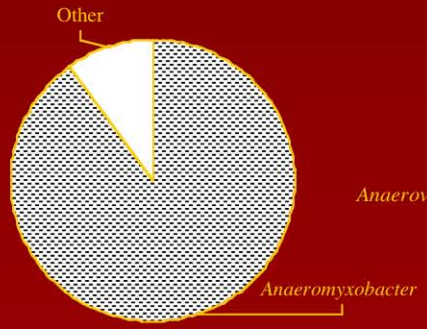
Glycerol



(49 clones)

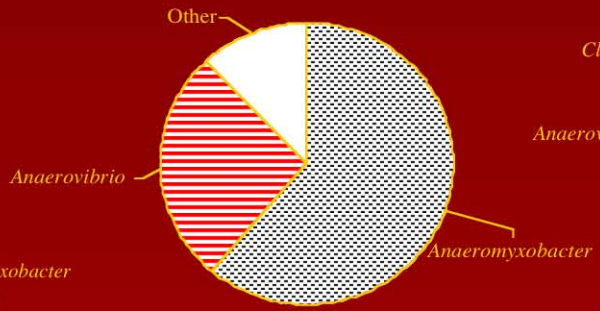
Contaminated:

Acetate



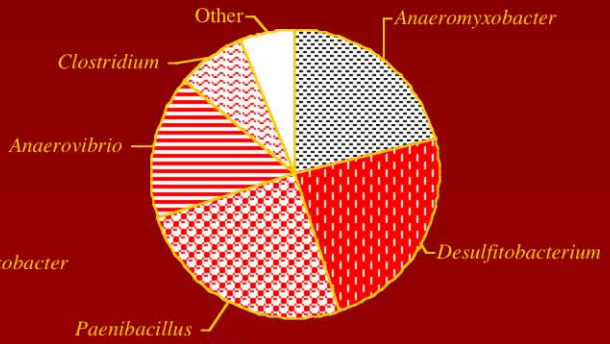
(152 clones)

Lactate



(41 clones)

Glycerol



(238 clones)

Conclusions: cultivation-approach

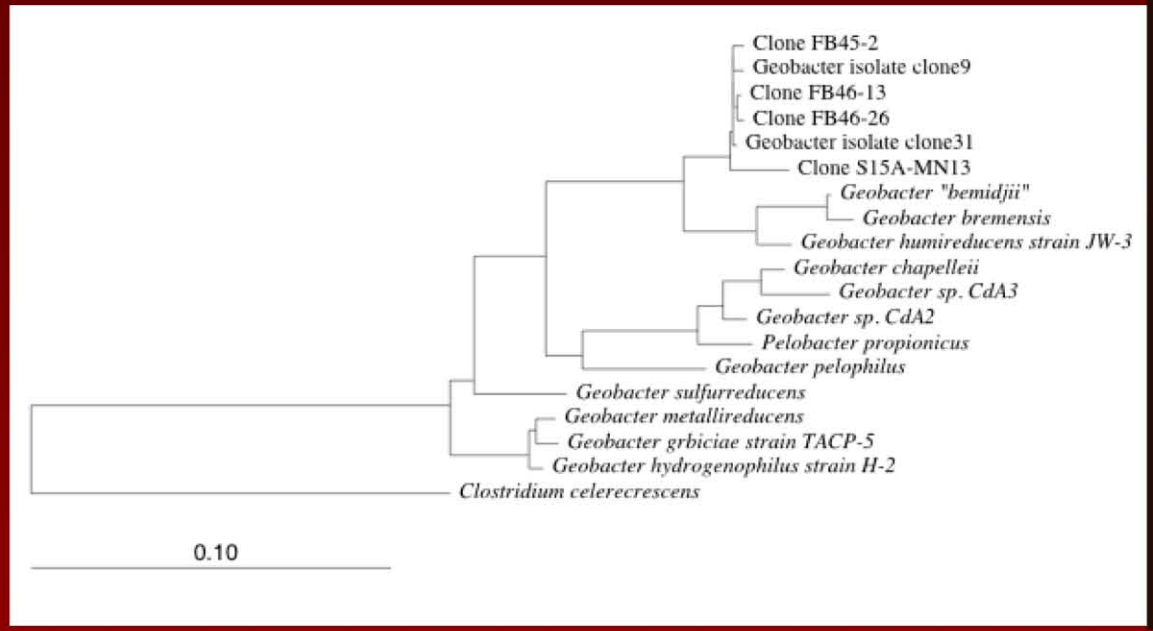
- The abundance and community composition of culturable FeRB is dependent upon geochemical parameters (pH, nitrate)
- Members of the *Geobacteraceae* dominated cultures from pristine subsurface
- Microorganisms capable of producing spores or spore-like bodies were representative of acidic U(VI)-contaminated sediments; parallels with metal-reducers isolated from deep terrestrial subsurface (*Desulfotomaculum*, *Bacillus*)
- Neutrophilic organisms cultured from contaminated acidic sediment likely to be important since pH neutralization used for bioremediation

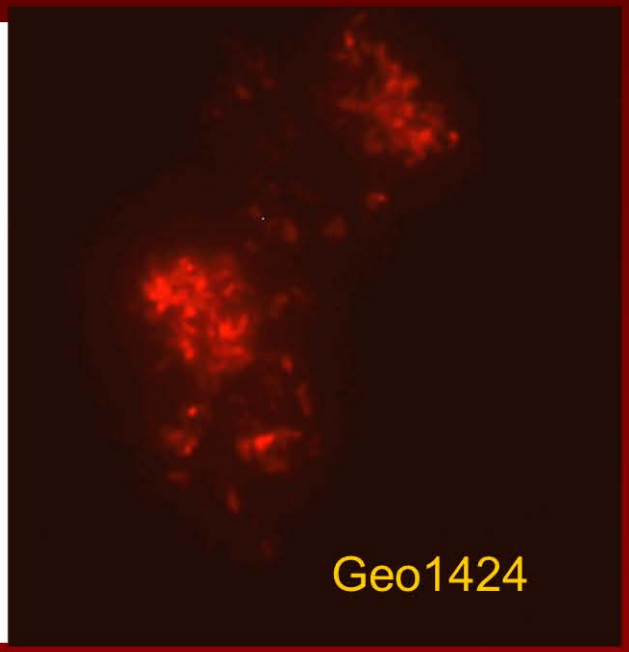
Petrie et al., 2003, AEM 69: 7467

Isolation of pure cultures from acidic subsurface

- Enrichment by successive transfer in liquid culture with FeOOH + carbon substrate (acetate, lactate, ethanol, glucose)
- Isolation in repeated Gelrite dilutions with AQDS + carbon substrate
- Screening with 16S rRNA gene cloning/ sequencing
- Strains: *Geobacter sp.* FRC32 (acet), *Clostridium* sp. CITR8 (acet), *Desulfotomaculum sp.* (lact)
- Please see poster for further information

Geobacter strain FRC 32





- ✓Isolate shares high sequence identity with phylotypes from acidic FRC subsurface (North et al., AEM, 2004)
- ✓Growth with FeOOH as sole electron acceptor
- ✓Limited carbon substrate utilization: acetate, ethanol, formate
- ✓Currently at JGI for draft genome sequencing

Target Organisms- Metal-reducers

- Dissimilatory metal-reducers
  - *Deltaproteobacteria*: *Geobacter* (I), *Anaeromyxobacter*
  - *Betaproteobacteria*: *Rhodoferax*
  - *Gammaproteobacteria*: *Salmonella* (I)
  - Gram positives: *Desulfitobacterium*, *Desulfosporosinus*, *Desulfotomaculum* (I)
  - *Acidobacteria*: *Geothrix*
- Fermentative metal-reducers
  - Gram positives: *Clostridium* (I), *Anaerovibrio*, *Bacillus*, *Paenibacillus*
  - *Gammaproteobacteria*: *Pseudomonas*, *Serratia*

✓I = Isolated

✓Published evidence: Petrie et al., 2003; Istok et al., 2003; Peacock et al., 2003; Shelobolina et al., 2003; North et al., 2004

Target Organisms- Nitrate-reducers

- Dissimilatory reduction of nitrate to ammonium
  - *Deltaproteobacteria*: *Geobacter* (I), *Anaeromyxobacter*
  - Gram positives: *Desulfitobacterium*
- Denitrification
  - *Betaproteobacteria*: *Alcaligenes* (I), *Ralstonia*, *Azospirillum*, *Acidovorax* (I), *Dechloromonas*
  - *Gammaproteobacteria*: *Pseudomonas*(I), *Klebsiella* (I)
  - *Alphaproteobacteria*: *Hyphomicrobium*, *Bradyrhizobium*, *Rhizobium*, *Blastobacter*, *Agrobacterium* (I)

✓Published evidence: Yan et al., 2003; Fields et al., 2005

Conclusions

- Nearly all published cultivation-independent studies of community composition from shallow subsurface based on PCR to date
- Mostly qualitative to semi-quantitative
- Groundwater and biofilm communities:
  - *Betaproteobacteria* dominated at FRC and Rifle sites
  - Functional markers retrieved for denitrifiers, sulfate-reducers
- Sediment communities: less data, more mixed assemblages dominated by various proteobacteria
  - Low template concentration likely limits reduction of PCR artifacts
  - Heterogeniety and scaling an issue for molecular techniques

Conclusions

- Too early to generalize about variation in distribution/ diversity across physicochemical gradients
  - Due to sampling/ methodological problems
  - Small sample sizes, inadequate extraction, tediousness of methods
  - Large range in biomass has not been adequately addressed

Conclusions

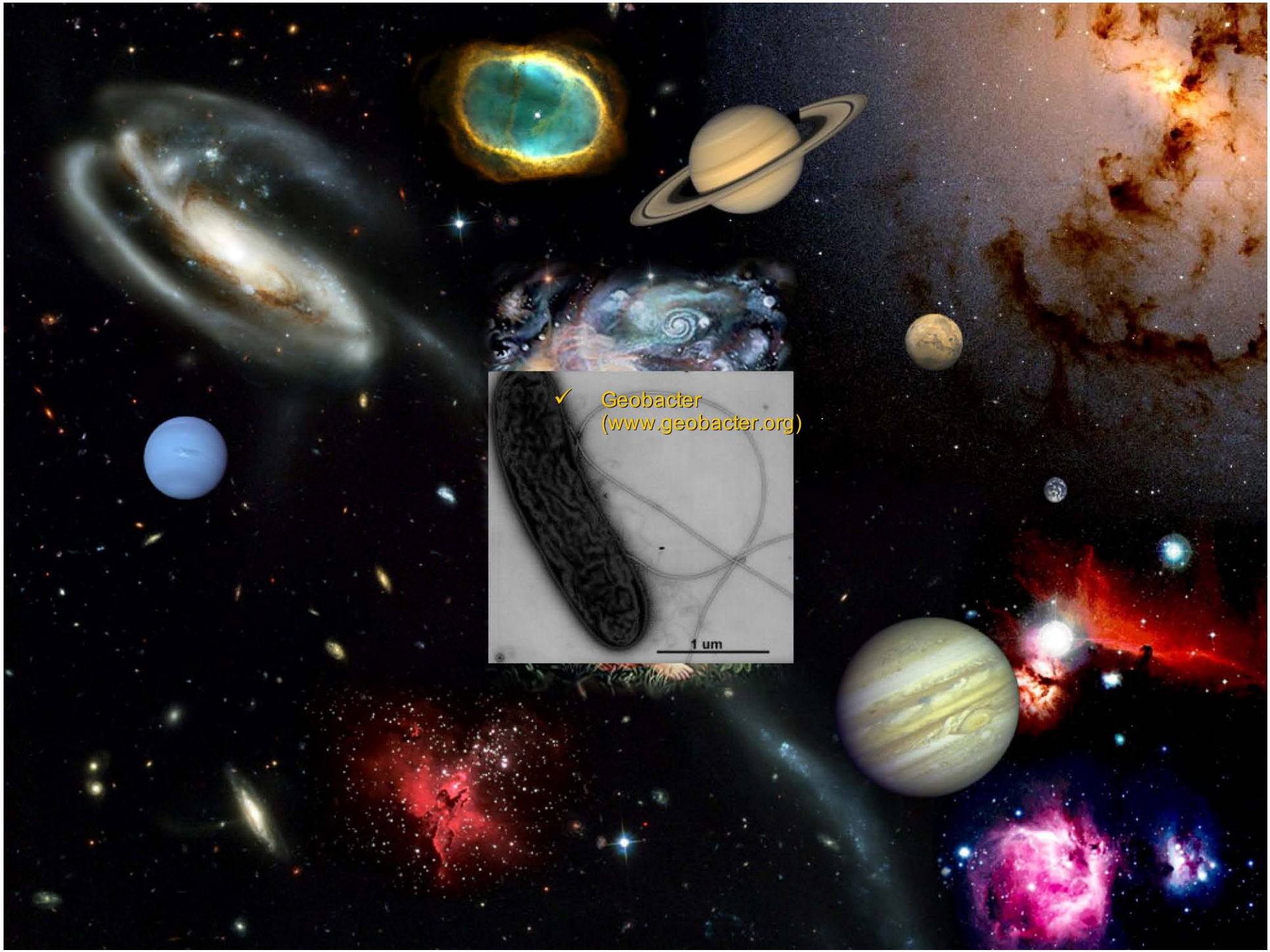
- Some interesting parallels have emerged between molecular approaches and cultivation
  - Studies indicate lower diversity and different metabolism in contaminated environments (low pH, high nitrate) at FRC
  - However,results equivocal, effect may not be that large, even at high contaminant levels
  - 16S and functional targets point to organisms with high remediation potential, some of which have been cultivated (*Alcaligenes*, *Geobacter*)
- Robust comparisons employ larger sequence datasets and statistical methods for testing coverage, Chao1 or LIBSHUFF

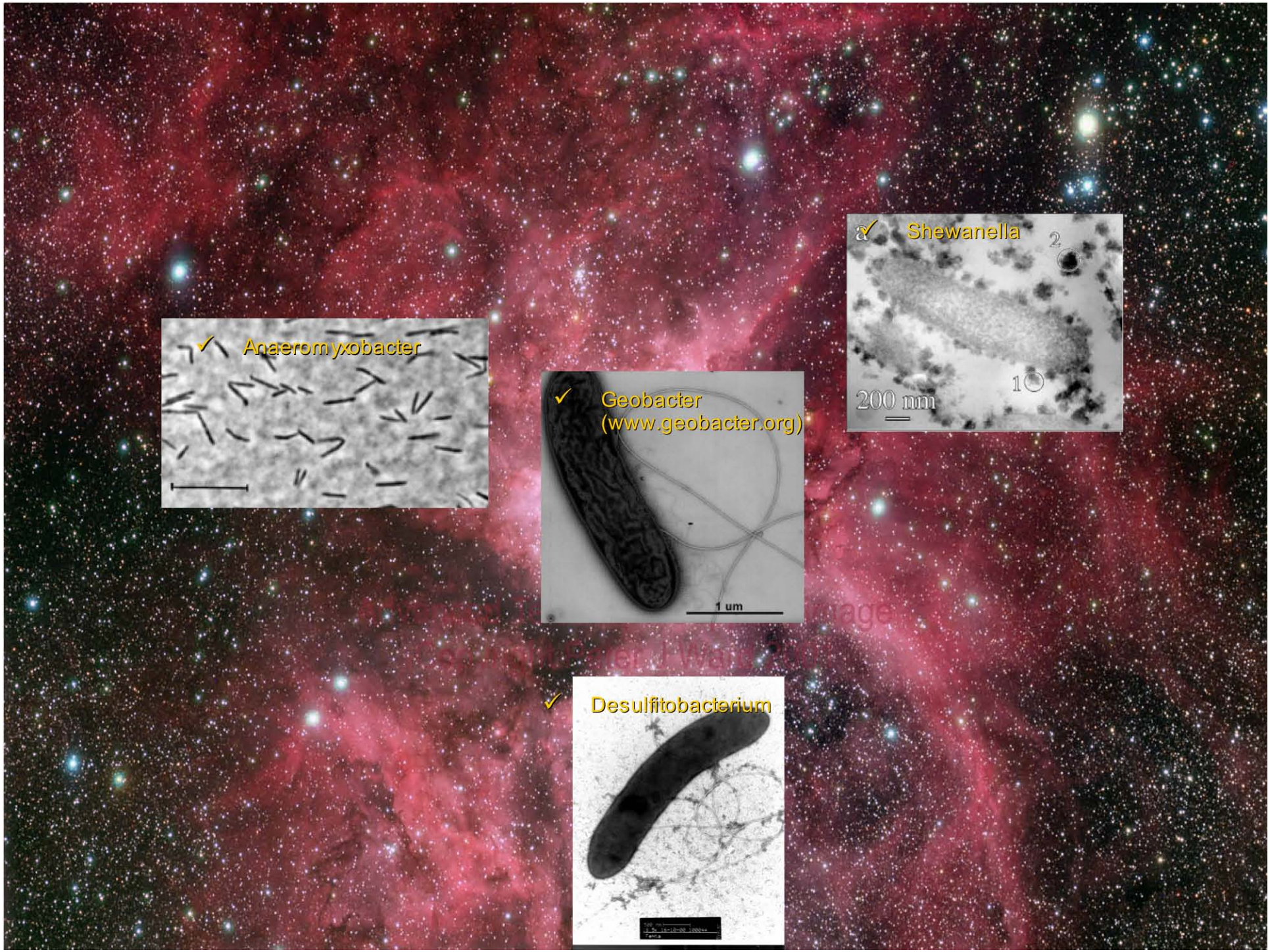
Future Work

- Use existing methods as foundation and build with new technologies that acquire information on in situ physiology, genomics
- Reach consensus
  - Sampling, analysis, data synthesis protocols for robust comparisons
- Refine existing molecular approaches
  - Hone for high throughput, scale up for site-wide comparisons
  - Larger molecular marker databases, faster collection techniques
  - Where possible, use quantitative approaches
  - Standardize, intercalibrate
  - Robust statistical comparisons
  - Increased replication
  - Deal with issues of heterogeniety, scaling

Future Work

- Polyphasic plan of action
  - Clone libraries, fingerprinting to survey for important groups
  - PLFA for biomass, verification of broader scale phylogenetic relationships
  - FISH, SIP, microarrays for determination of in situ physiology
  - Cultivation for model organisms, to confirm/ test hypotheses on physiology
  - Direct methods that do not rely on PCR?
  - New quantitative tools that address expression?

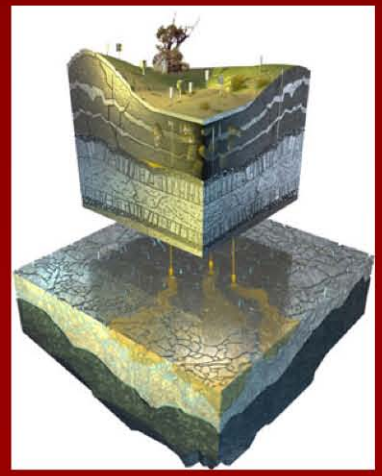

✓ Geobacter
(www.geobacter.org)
1 um


✓ Anaeromyxobacter
✓ Geobacter
(www.geobacter.org)
1 um
✓ Desulfitobacterium
✓ Shewanella
200 nm


NCC-1701-A
200 nm
The Light Works

Diversity in Soils

- Processes mediated by a less diverse microbial group more likely to be influenced by changes
- Diversity has a buffering effect on critical processes
- More diverse phylogenetic groups are more resistant, resilient to environmental perturbations
- Example: community composition has a major effect on N transformations in soil
  - Diazotrophs
    - Highly diverse, functionally redundant
    - Despite environmental fluctuations, N_2 fixation remains constant
  - Denitrifiers
    - Community composition influences total amount of denitrification as well as various products/ intermediates formed

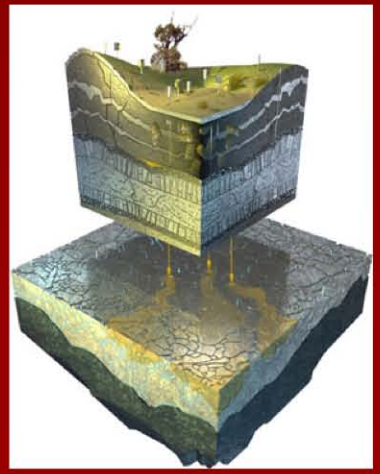


Evidence from Field Studies in Subsurface

- Majority of environments acidic, carbon-poor, aerobic, cocontaminated with NO_3^-, SO_4^{2-}
- Upon addition of an electron donor, U(VI) reduced concurrently w/ Fe(III) after NO_3^- depleted (Finneran et al., 2002; Senko et al., 2002; Anderson et al., 2003; Istok et al., 2004)
- Members of Geobacter family often detected in abundance in metal-reducing sediments (Holmes et al., 2002; Anderson et al., 2003; North et al., 2004)
- Most studies have been carried out under neutrophilic conditions, much less is known about acidic subsurface

Diversity in Soils

- Often 2 or more genetically distinct organisms have the capacity to mediate a single process; organisms are said to be “functionally redundant”
- Concept led to hypothesis that biodiversity leads to stability of ecosystem function
- Redundant organisms may exist in soil matrix due to spatial isolation or physiological differences
- Processes mediated by a less diverse microbial group more likely to be influenced by changes
- Diversity has a buffering effect on critical processes
- More diverse phylogenetic groups are more resistant, resilient to environmental perturbations



Conclusions

- Temporal as well as spatial variability need to be further addressed with more robust approaches
- Tighter coupling when sampling for microbial and environmental parameters
- Need an integrated approach, beginning with existing methods applied at higher throughput, while developing newer methods that detect in situ activity
- Don't give up on "tried and true" methods- Existing methods for community characterization suitable for surveying composition and scaling up
  - Cloning/ sequencing, fingerprinting
- New "tricks" that quantify "active" members of community still tedious but rapidly improving
- Polyphasic approach warranted that includes: cloning/ sequencing of nucleic acids, lipid biomarkers, cultivation, methods that link phylogeny with physiology, environmental genomics

Future Work

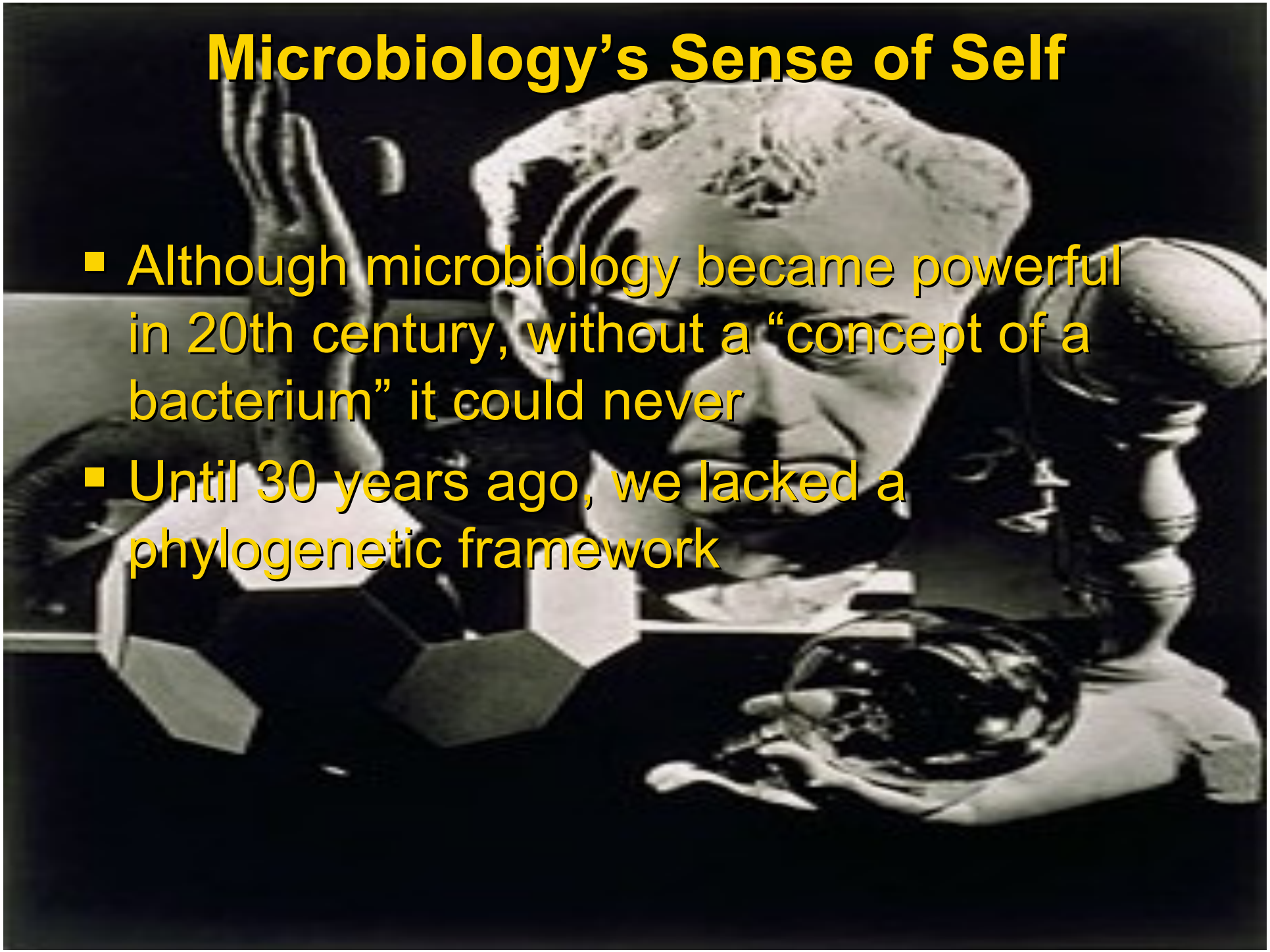
- Couple microbiology with extensive engineering,physical and geochemical characterization at similar scales and locations
- Quantitatively address groundwater as a surrogate for sediment communities
- Compare groundwater, biofilm, and sediment communities in same area
  - For example, in multilevel sampler adjacent to coring site
  - Sample sediments in “saturated” zone likely to exchange with surrounding groundwater
- Use modeling to address scaling and sampling issues

Ecological approach- what do we need to know?

- Determination of biomass and distribution of organisms driving desired metabolism
- Variation in microbial parameters across physical-chemical gradients
- Quantification of “active” metabolic groups and/ or determination of physiological potential
- Significance of diversity
  - Functional diversity, redundancy- competition or overlap between populations
- Use the above basic science information to refine remediation strategies
- Use shallow subsurface as a model for deep subsurface biosphere

Biofilms

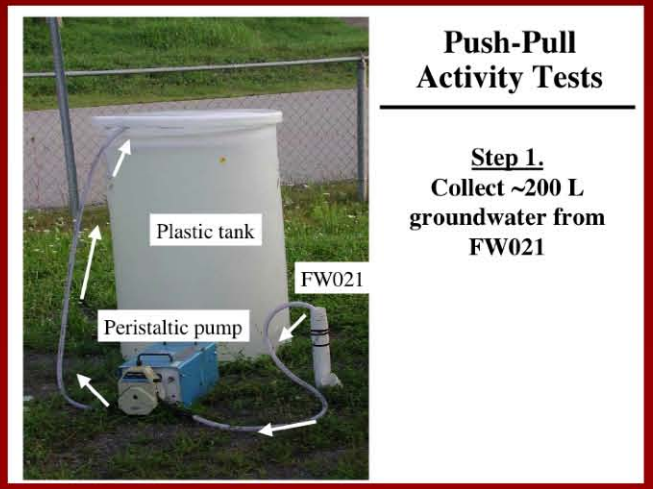
Microbiology's Sense of Self



- Although microbiology became powerful in 20th century, without a "concept of a bacterium" it could never
- Until 30 years ago, we lacked a phylogenetic framework

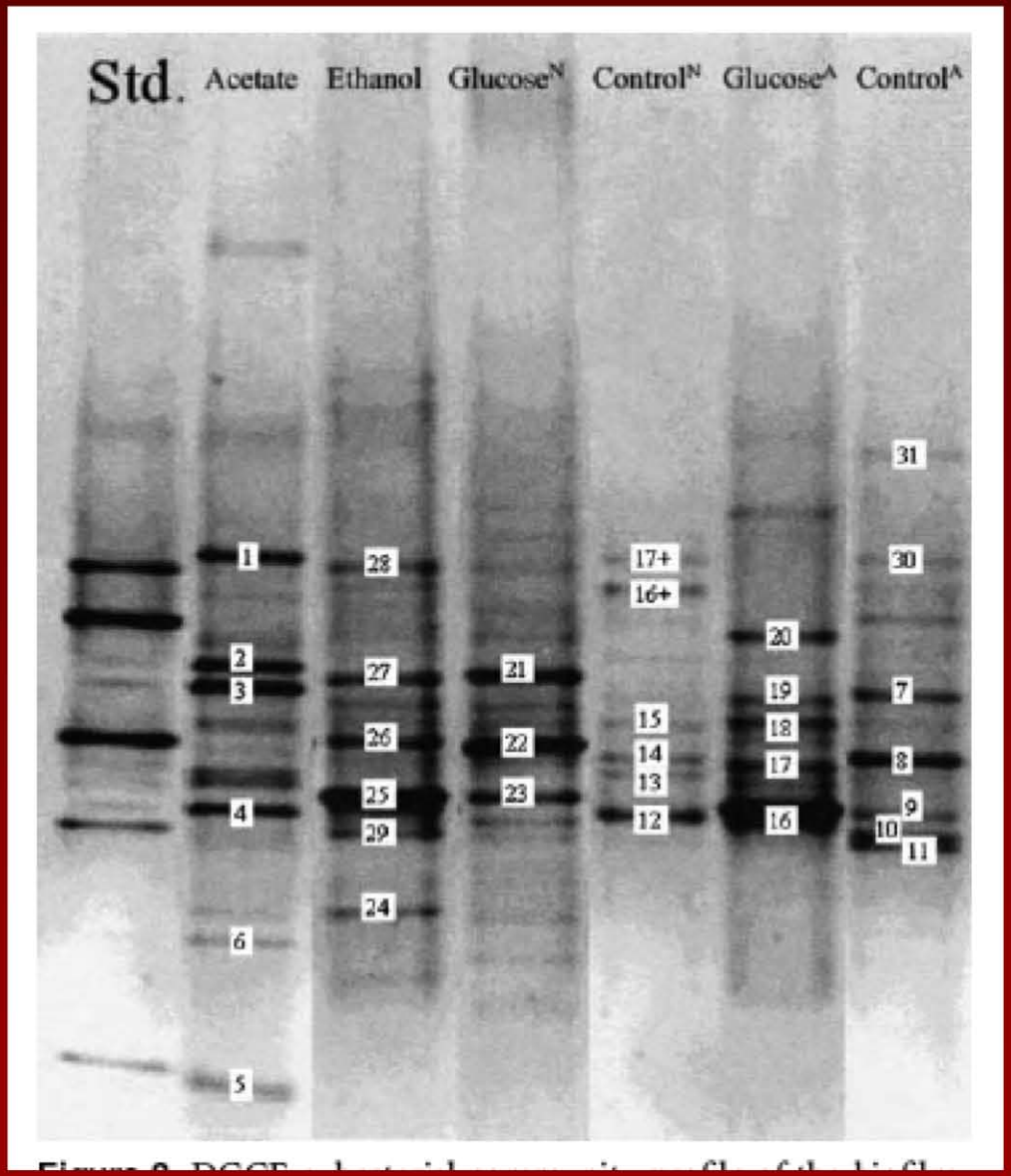
Bio-Sep bead samplers - FRC, Area 1 groundwaters

- PLFA biomass higher at neutral relative to acidic pH
- DGGE of 16S targets
- Impact of pH on diversity?
- *Betaproteobacteria* dominated
- Nitrate- (*Alcaligenes*, *Ralstonia*) but not metal- or sulfate-reducers detected in controls



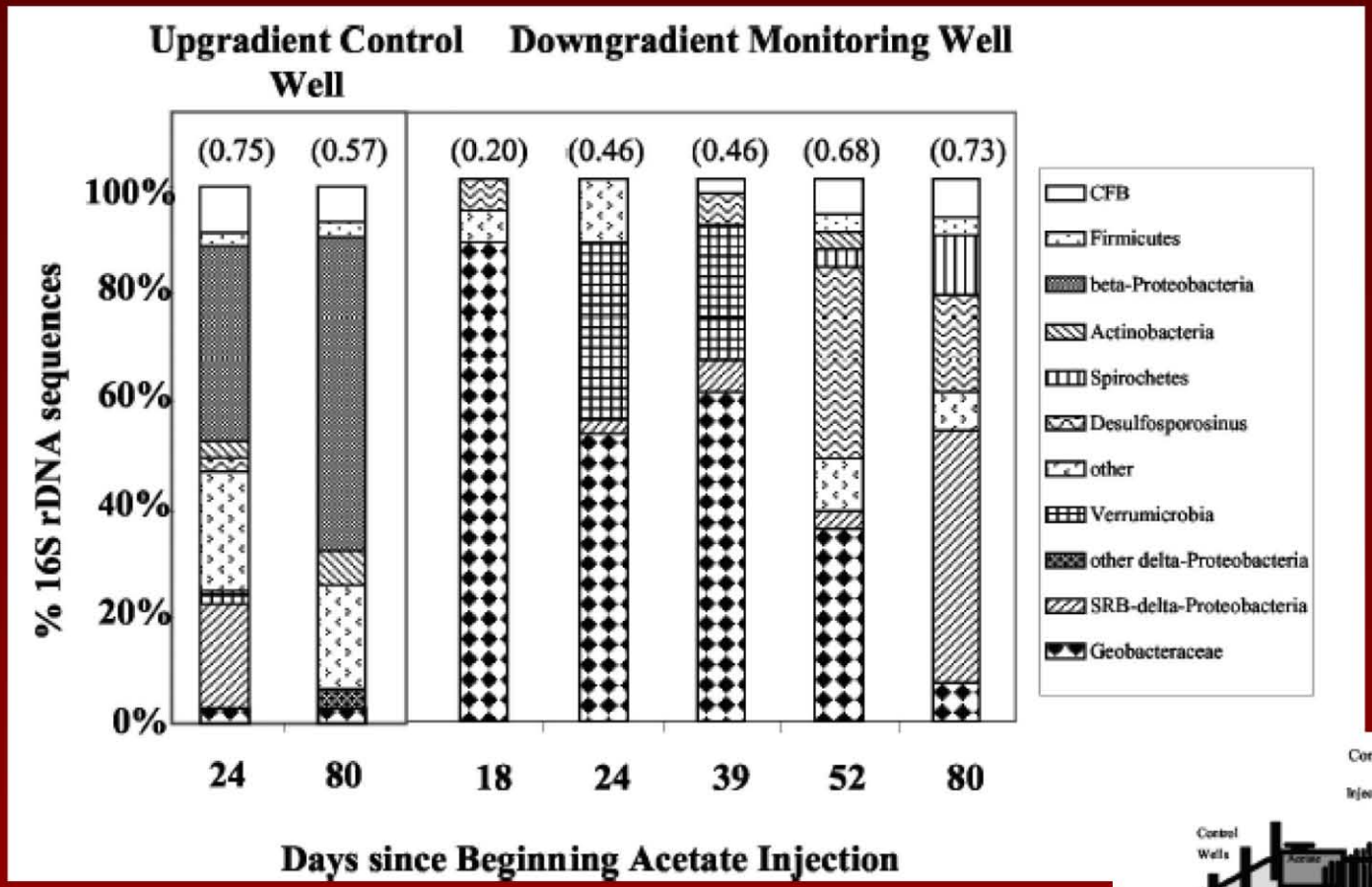
Push-Pull Activity Tests

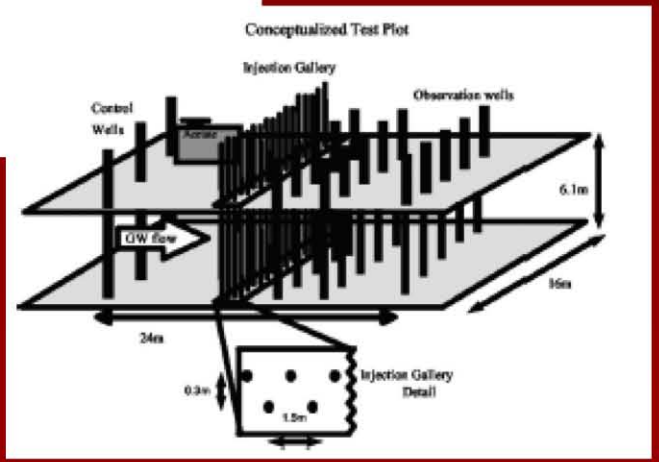
Step 1.
Collect ~200 L groundwater from FW021



✓Peacock et al., 2004, Microbial Ecology 47: 284

16S rRNA - UMTRA site, Rifle, Colorado





- Permeable aquifer, neutrophilic pH
- *Betaproteobacteria* dominated clone libraries in upgradient control well

✓ Anderson et al., 2003, AEM 69: 5884

Hematite coupon samplers - FRC, bkgd and Area 3

TABLE 3. Bacterial 16S rDNA clones from communities formed on hematite in FRC Area 3 well FW026

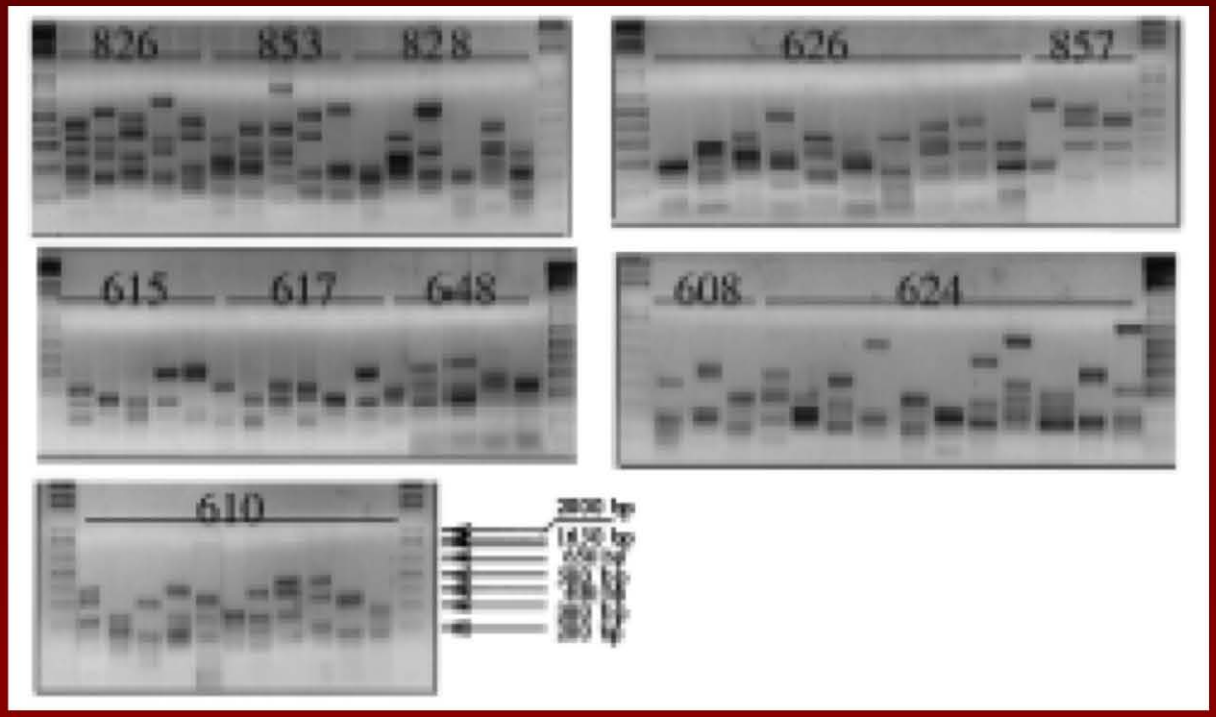
Clone ID	GenBank no.	Frequency[a]	Affiliation[b] (% similarity)	Putative division
C-CG17	AY622228	59	*Alcaligenes* sp. strain L6 (95)	β-*Proteobacteria*
C-CS3	AY622233	24	*Frateuria* sp. NO-16 (96) (AF376025)	γ-*Proteobacteria*
C-CF16	AY622227	4	*Methylobacterium radiotolerans* (99)	α-*Proteobacteria*
C-CU62	AY622234	3	*Pseudomonas straminea* (99)	γ-*Proteobacteria*
C-CJ32	AY622229	2	*Beutenbergia cavernosa* (96)	*Actinobacteria*
C-CY80	AY622237	1	*Herbaspirillum seropedicae* (96)	β-*Proteobacteria*
C-DA88	AY622239	1	*Burkholderia* sp. A6.2 (98)	β-*Proteobacteria*
C-CZ82	AY622238	1	*Duganella zoogloeoides* (98)	β-*Proteobacteria*
C-CL42	AY622230	1	*Pseudomonas syringae* (89)	γ-*Proteobacteria*
C-CX74	AY622236	1	*Sphingobacterium antarcticum* (99)	γ-*Proteobacteria*
C-CO51	AY622232	1	*Microbacterium* sp. VKM Ac-2050 (99)	*Actinobacteria*
C-CV63	AY622235	1	*Nocardioides* sp. MWH-CaK6 (98)	*Actinobacteria*
C-CM46	AY622231	1	Clone MTAC17 (92)	Unknown

[a] Frequency of a given RFLP-type out of 100 total clones.
[b] Closest identified match in the GenBank database followed by percent similarity. Clone C-CM46 did not match any identified sequences in the database.

- Diversity lower in acidic, contaminated (13 OTUs) relative to bkgd (34 OTUs) groundwater
- Groundwater and sediment communities distinct
- Coupon communities more similar to those of groundwater
- Libraries dominated by beta and gammaproteobacteria
- Nitrate-reducers (*Alcaligenes*, *Pseudomonas*) and one metal-reducer (*Rhodoferax*) detected

✓ Reardon et al., 2004, AEM 70: 6037

Sulfate-reducers - UMTRA site, Shiprock New Mexico

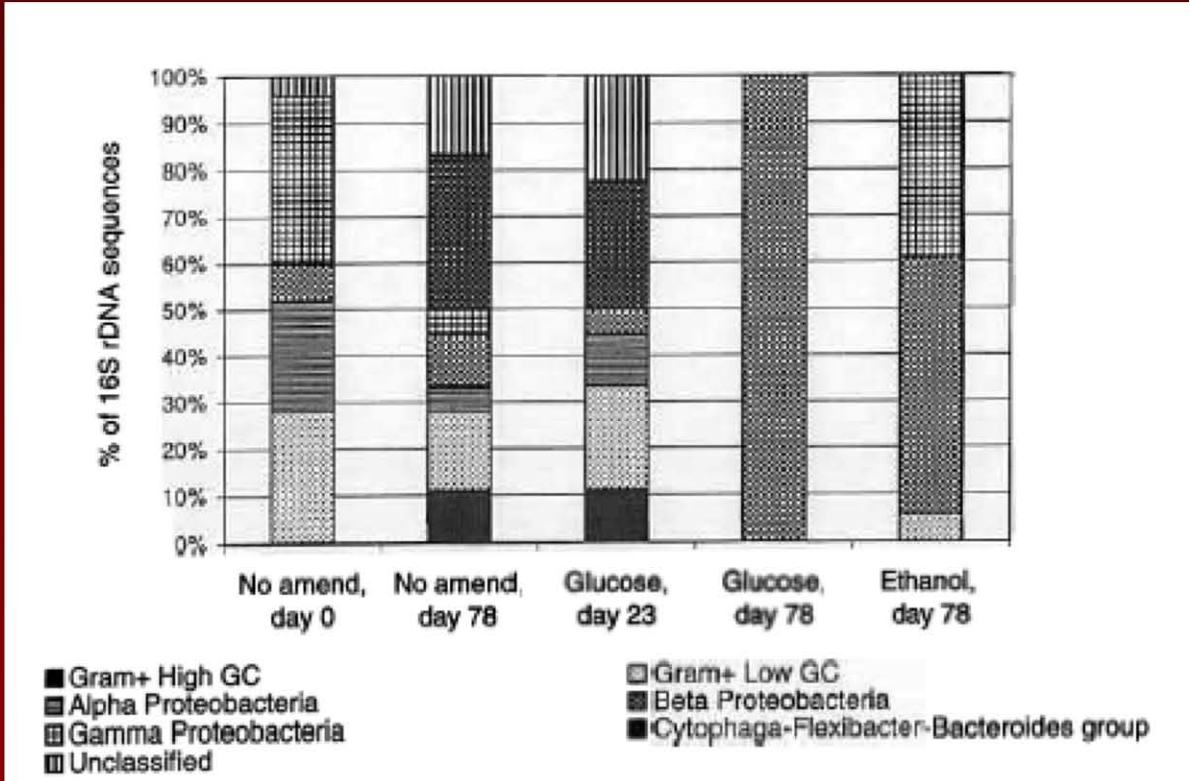


- Permeable aquifer, neutrophilic pH
- Functional gene for sulfate reduction, dsr
- High diversity of gram positives, *Deltaproteobacteria*, novel sequences observed
- PLFA biomarkers for SRB provided quantitative confirmation of presence of viable organisms
- *Desulfotomaculum* sequences dominant and positively correlated with uranium concentrations

✓Chang et al., 2001, AEM 67: 3149

Sediments - FRC, Area 3

- Homogenized sediment + groundwater (FW-021) from highly contaminated area
- Relatively high diversity observed
- Library contained *Alpha-*, *Beta-*, *Gammaproteobacteria* , low G+C gram positives
- Metal- and sulfate-reducers not detected
- Nitrate-reducers not discussed



✓ Shelobolina et al., 2003, Soil Sed. Contamin. 12: 865

Characteristics and diversity estimates for SSU rRNA gene clones from three FRC acidic sediment samples

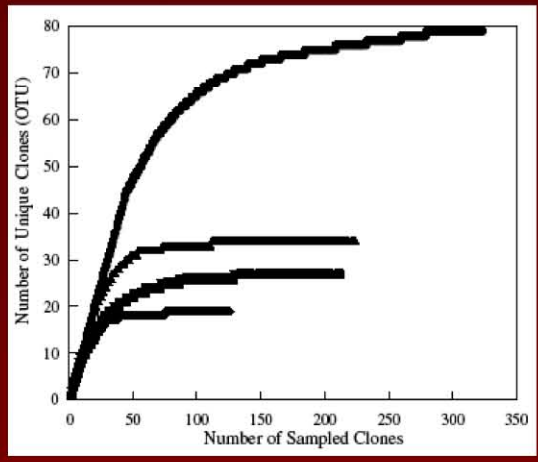
Sample	No. of Clones	OTU[1]	Species Richness[2]	Shannon-Weiner[3]	1/D[4]	Evenness[5]
61-01-00	88	21	38 (25, 96)	2.35	6.92	0.65
61-01-24	81	22	27 (23, 43)	2.56	9.64	0.78
61-03-00	30	10	18 (11, 75)	2.05	8.06	0.71
61-03-25	31	10	13 (10, 29)	1.9	5.47	0.74
61-05-22	44	20	35 (24, 82)	2.71	15.51	0.76

[1] Operational taxonomic units based on RFLP analysis
[2] Species Richness determined by Choa1, parenthesis indicate 95% confidence intervals
[3] Shannon-Weiner Index, higher number represents higher diversity
[4] Simpson's Reciprocal Index, higher number represents higher diversity
[5] Evenness, as value approaches 1, the population is more evenly distributed

- **No significant difference in diversity indices observed between DNA libraries**
- **Some differences in the frequency of specific taxa were observed**
- ***Betaproteobacteria* and *Flavobacteria* more frequently detected in libraries from acidic sediments**
- **Together with culture studies, indicates that *Betaproteobacteria* are important nitrate-reducers in highly contaminated subsurface at FRC**

16S rRNA - FRC, Areas 1-3, bkgd

- Increased diversity of bkgd sample contained: *Alpha-*, *Beta-*, *Gamma-*, *Deltaproteobacteria* , *Acidobacteria*, high G+C, *Verrucomicrobia*
- *Azoarcus* phylotypes showed high sequence identity to cultured denitrifiers capable of organic contaminant degradation
- No significant correlation between diversity and geochemical parameters

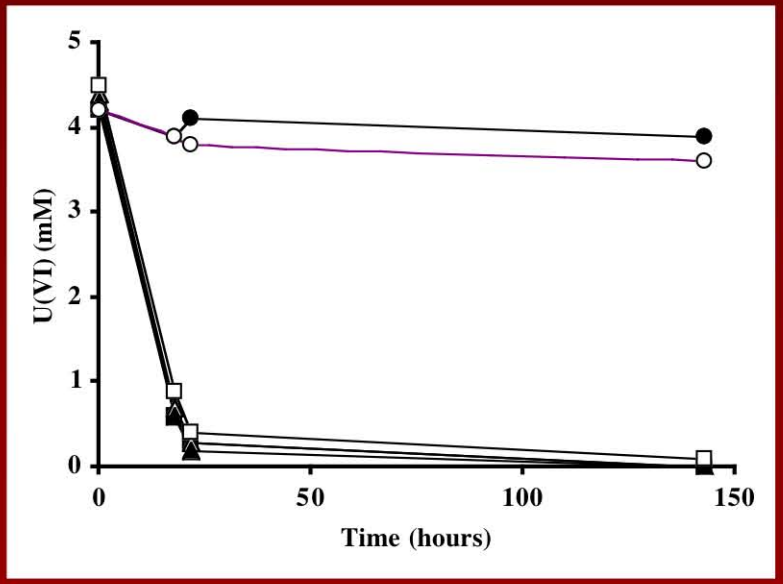


✓ Fields et al., 2005, FEMS Microbiol. Ecol., in press

Functional markers

- Community characterization based on 16S alone can underestimate species richness
- Protein-encoding genes generally change more rapidly and tend to reveal higher levels of diversity
- Allow for elucidation of broad phylogenetic groups, such as denitrifiers, that carry out a specific process

Fe(III)-reducing consortia enriched from FRC sediment rapidly reduce U(VI) and grow with smectite clay minerals as the sole electron acceptor

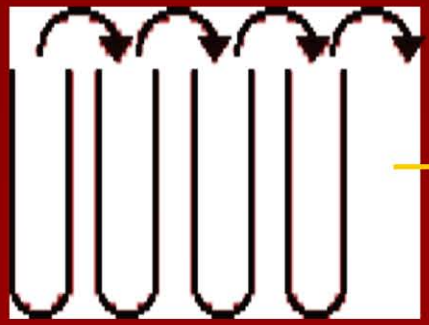


Kostka et al., 2002, AEM 68: 6256

MPN Serial Dilution Assay

- Minimal media
- Variety of EDs and buffers used
- >2000 enrichments
- Enrichments conducted from contaminated and background sediments at pH 7 and pH 5
- In some treatments, sediments washed

Sediment Core

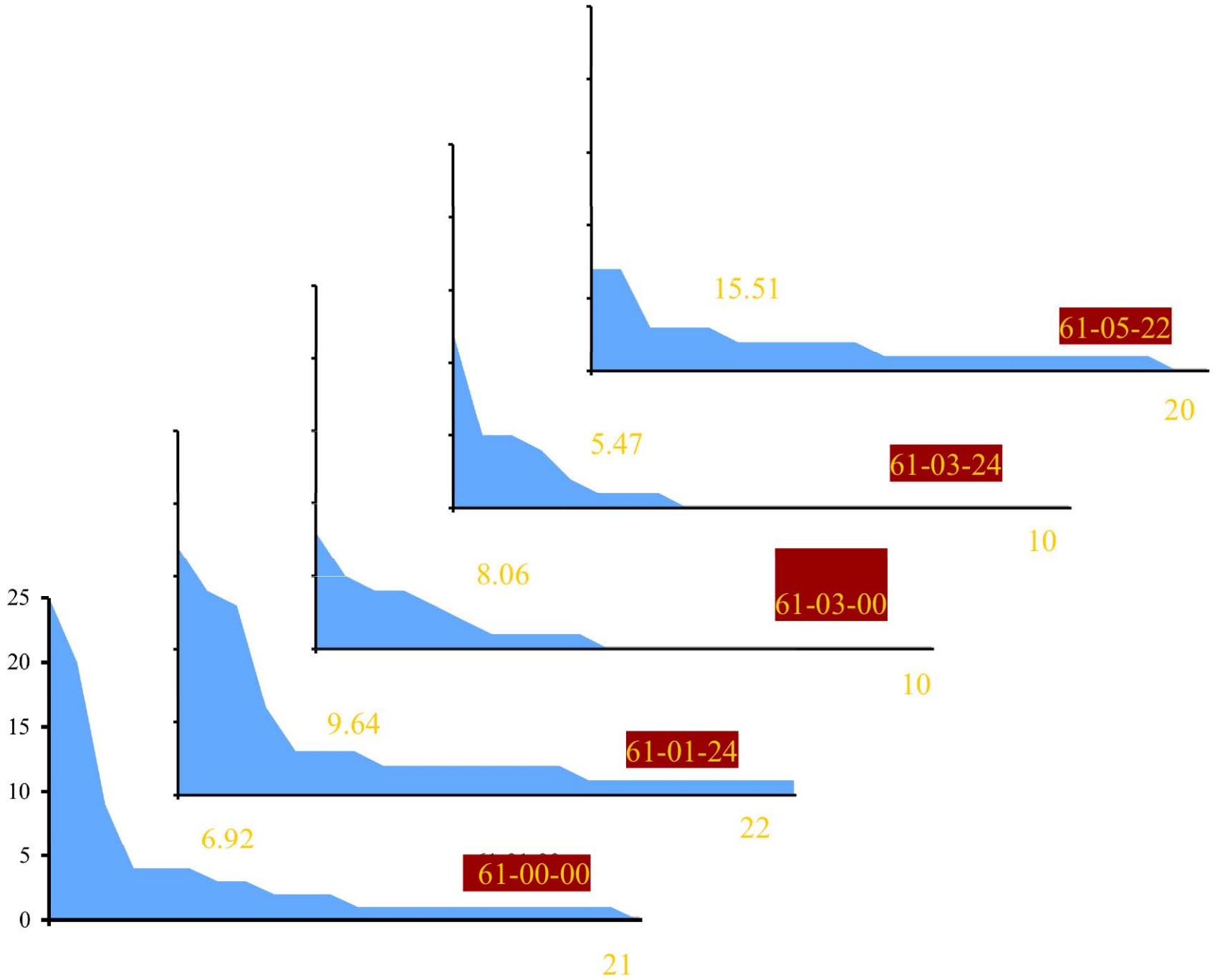


Serial Dilutions

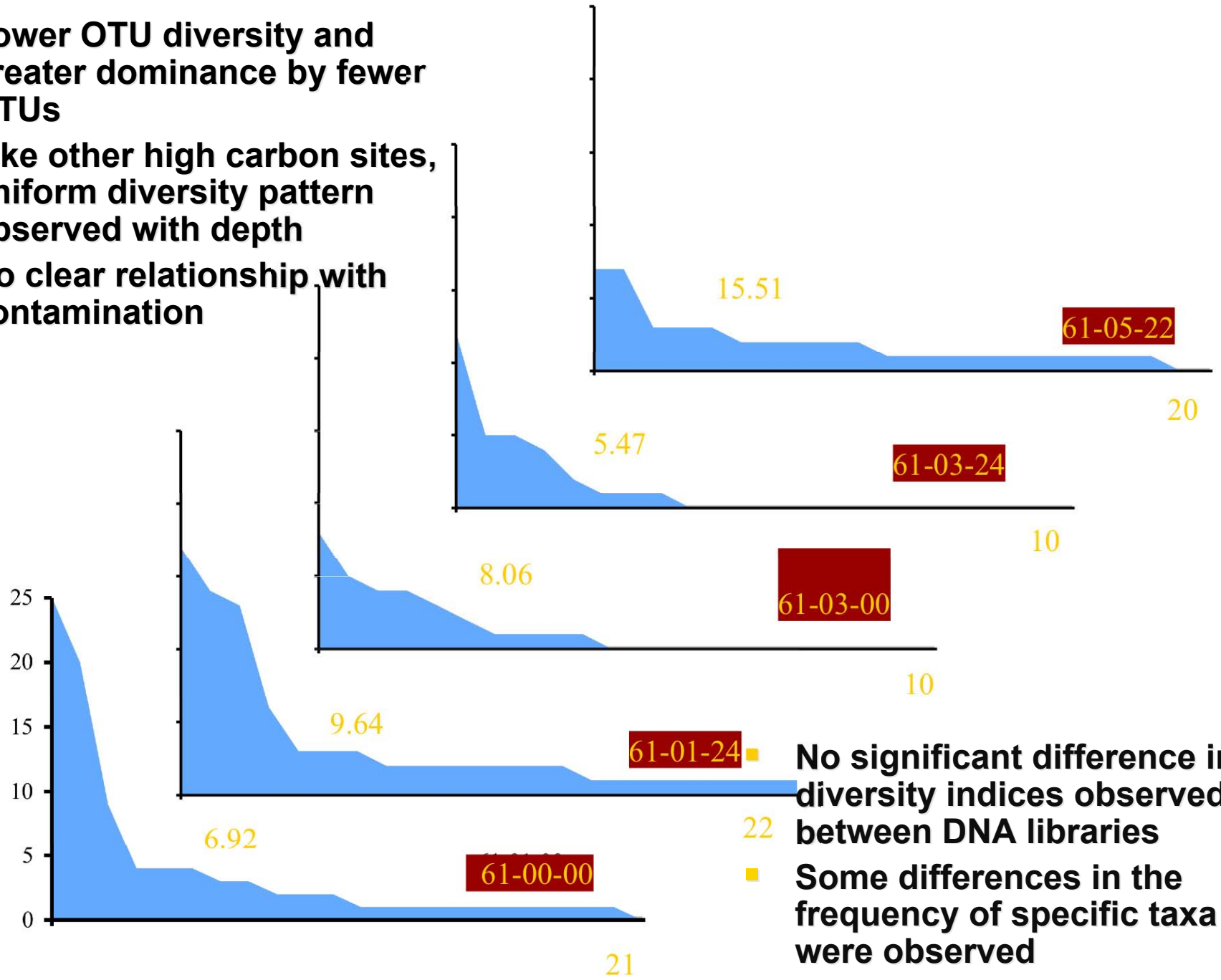
Score Tubes to Extinction

Fe(III)-reducing Enrichments

- ✓ Up to 50% of 4-6 mM U(VI) was reduced in <48 hours


25
20
15
10
5
0
6.92
61-00-00
21
9.64
61-01-24
22
8.06
61-03-00
10
5.47
61-03-24
10
15.51
61-05-22
20

- Lower OTU diversity and greater dominance by fewer OTUs
- Like other high carbon sites, uniform diversity pattern observed with depth
- No clear relationship with contamination



- No significant difference in diversity indices observed between DNA libraries
- Some differences in the frequency of specific taxa were observed

Enumeration summary MPN serial dilution assay

SRB:

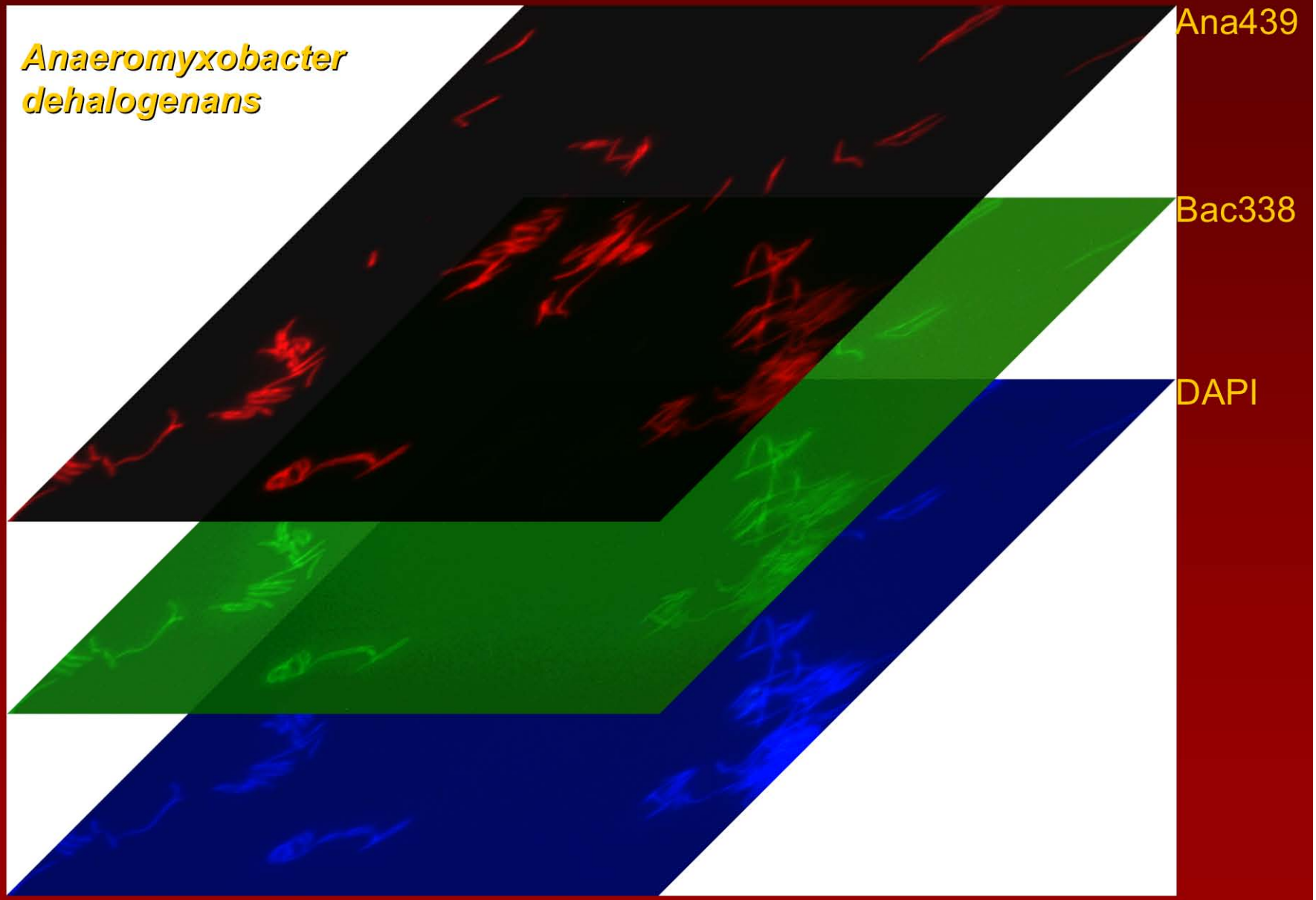
- none detected
- Corroborated by low sulfate reduction rates

FeRB:

- Growth detected in 87% of bkgd and 37% of contaminated sediment samples enriched at pH 7
- Higher counts in neutral enrichments than acidic enrichments
- Growth detected at low pH after 4 months
- Little growth detected in unwashed contaminated sediments, whereas washing had no effect on counts in background sediments

Petrie et al., 2003, AEM 69: 7467

FISHing

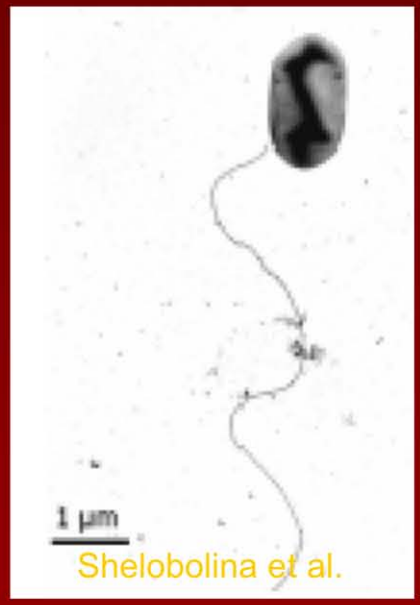


Summary of culture work

- Nitrate-reducers
  - Fields et al. (2005):
    - most isolates members of the *Betaproteobacteria*
    - *Pseudomonas* isolate similar to predominant groundwater phylotypes
    - Isolates grew down to pH 4 and tolerated high [Ni]
  - Krumholz et al.:
    - *Alpha-, Beta-, Gammaproteobacteria* isolated
    - *Alcaligenes* isolate similar to phylotypes retrieved from groundwater and sediments at site

Summary of culture work

- Metal- and Sulfate-reducers
  - Shelobolina et al. (2003):
    - Many known Fe(III)- and sulfate-reducers detected in U(VI)-reducing enrichments including *Geobacter*, *Desulfitobacterium* , *Geothrix*, *Desulfosporosinus*
    - Not detected in clone library from same sediment
  - Shelobolina et al. (2004):
    - Isolation and description of U(VI)-reducer, *Salmonella subterranea* strain FRC-1
    - Acid-resistant, grows to pH 4
    - Capable raising pH of medium
    - Facultative anaerobe, capable of using nitrate, thiosulfate, fumarate, malate as electron acceptors
    - Nitrate reduced to nitrite
    - Hydrogen, acetate, lactate, citrate, butyrate, succinate, methanol, ethanol, glycerol, butanol used as electron donors
  - *Geobacter uraniumreducens* - isolated from Rifle site



New techniques for determination of activity or function of uncultured microorganisms

- SIP
- Micro-FISH
- BrDU

Future Work

- Reach consensus on sampling and analysis protocols for robust comparisons
- Refine existing approaches to scale up for site-wide comparisons (high throughput, increased replication, pooling of PCR products)
- Cultivation-independent methods should generate large enough sequence database and be amenable to robust statistical analysis
- Where possible, employ quantitative approaches and those that determine “active” members of microbial communities
- Use extensive biomass characterization to focus and refine sampling for community analysis
- Address limitations of PCR at low biomass, low template suggested by Chandler, Brockman, Zhou, Tiedje, Marsh, Palumbo
- Develop methods for direct detection without amplification, PCR

Radioactive Metal Contamination

- Global problem- especially prevalent in U.S. and eastern Europe
  - Volume of contaminated groundwater > total volume of all lakes in U.S.!
- Contamination from various aspects of nuclear bomb production (mining, enriching, packaging, cleaning of machinery)
  - Mainly a subsurface problem
  - Site cleanup managed by Dept. of Energy
- Most prevalent contaminant in U.S. = uranium
  - Long half life
  - Chemically toxic, causing cancers
  - Usually present in oxidized state as U(VI), or reduced state, as U(IV)
  - In oxidized U(VI) state, uranium is mobile and can leach into drinking water reservoirs

Microbial Reduction of Uranium-Evidence from Pure Cultures

- Bacteria capable of uranium reduction include iron reducing bacteria (FeRB) and sulfate reducing bacteria (SRB)
- Model organisms studied in pure culture
  - FeRB = members of the *Geobacter* and *Shewanella* families
  - SRB = *Desulfovibrio* and *Desulfotomaculum*
  - Subset of these groups can conserve energy for growth by reducing U(VI)
  - Reduce U(VI) concurrently with primary EA