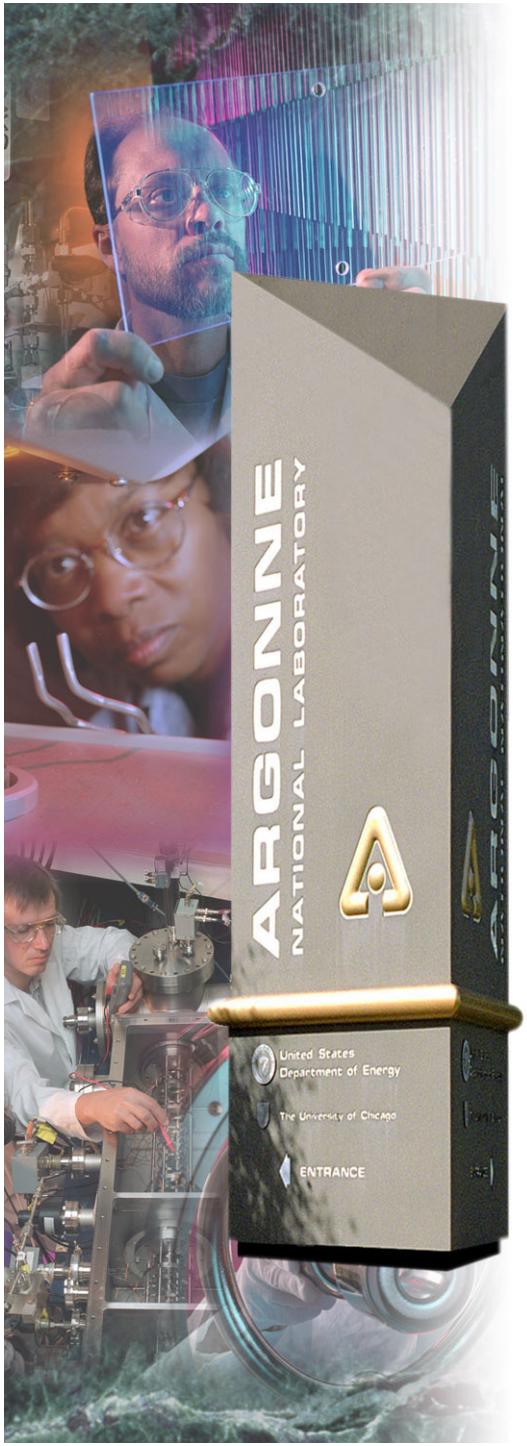

Taking Arrays to the Field

Making good on the Assessment promise

Darrell P. Chandler
Section and Technical Group Leader
Biodetection Technologies Section, Biochip Technology Center

Argonne National Laboratory

Office of Science
U.S. Department of Energy

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Operated by The University of Chicago

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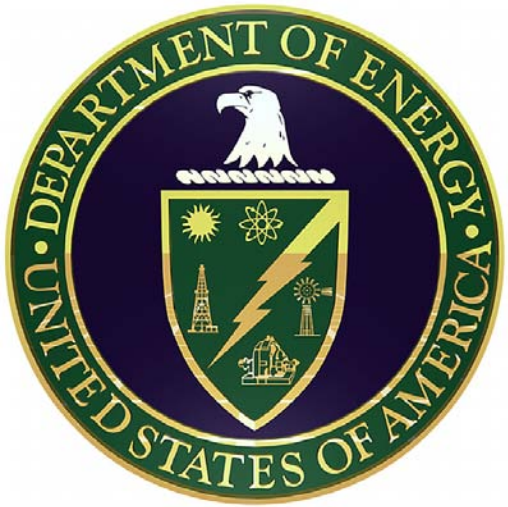
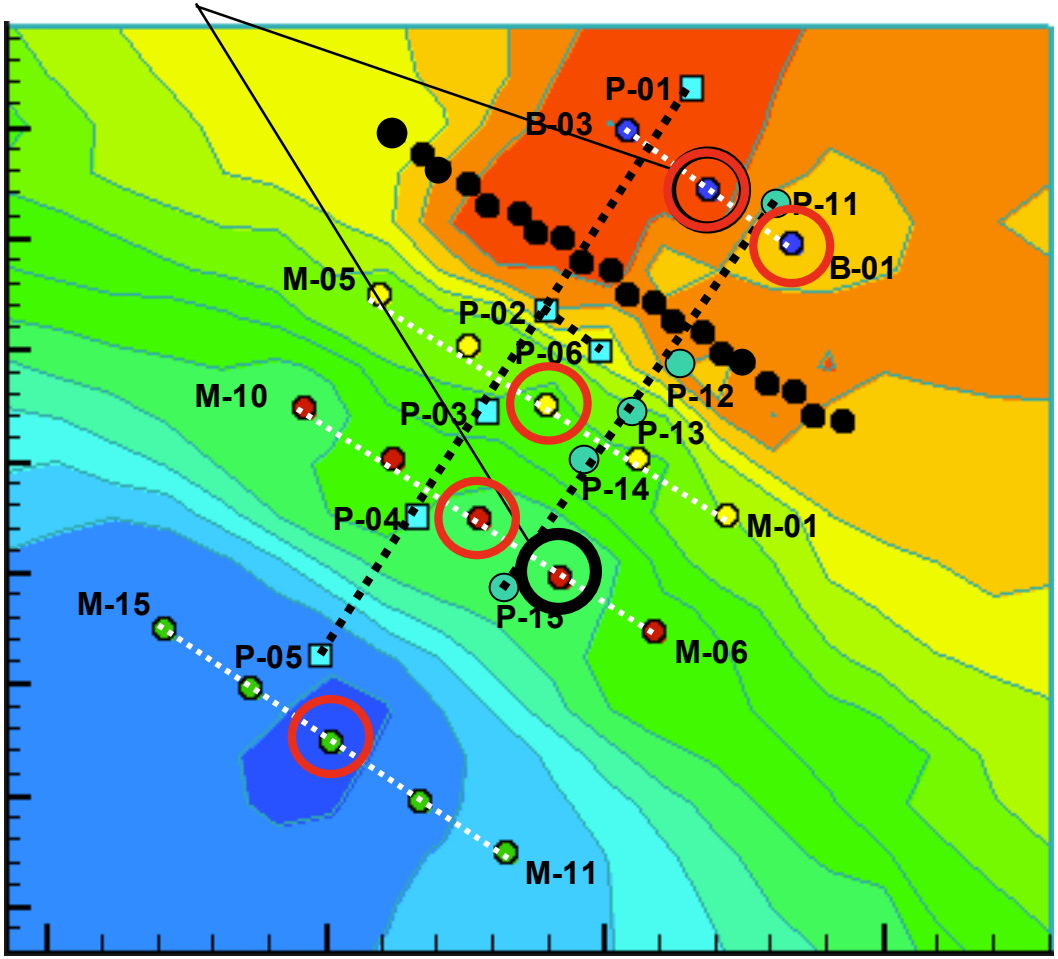


Old Rifle UMTRA Site, Rifle, CO

Map of NABIR Biostimulation Well Field and Water Table Contours (6/18/03)

Anderson et al. clone libraries. 2003. Appl. Environ. Microbiol. 69(10): 5884-5891



Published Groundwater Study

Anderson et al. 2003. Appl. Environ. Microbiol. 69(10): 5884-5891

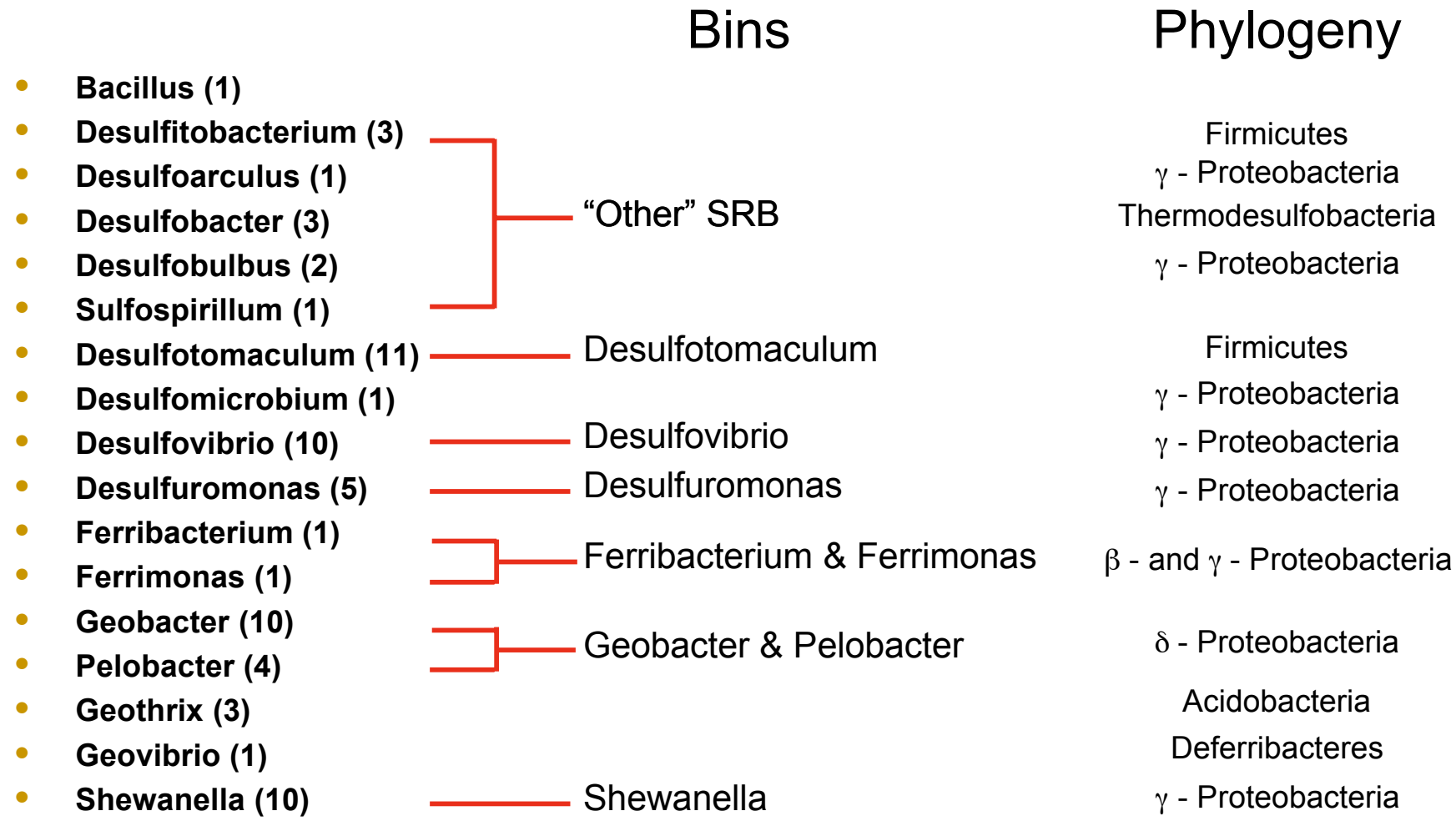
- **Continuous 1-3 mM acetate injection for 3 months in 2002**
- **Monitor U(VI), Fe(II), SO_4^{2-}, and acetate concentrations in B and M wells**
- **Clone libraries from groundwater samples, B-02 (background) and M-07 (monitoring well); PLFA from bead coupons**

- **Acetate injection resulted in substantial enrichment of "*Geobacteraceae*", to 89% of total groundwater microbial community (clones) 18 days after injection**
- **Other well-known U(VI) reducers were not detected in groundwater over the course of 39 days (i.e. *Shewanella, Desulfovibrio*)**
- **Beyond day 39, groundwater community (clones) shifted to sulfate-reducing genera**
- **Only 1 clone related to *Desulfotomaculum***

Bead Array Sediment Sample Set

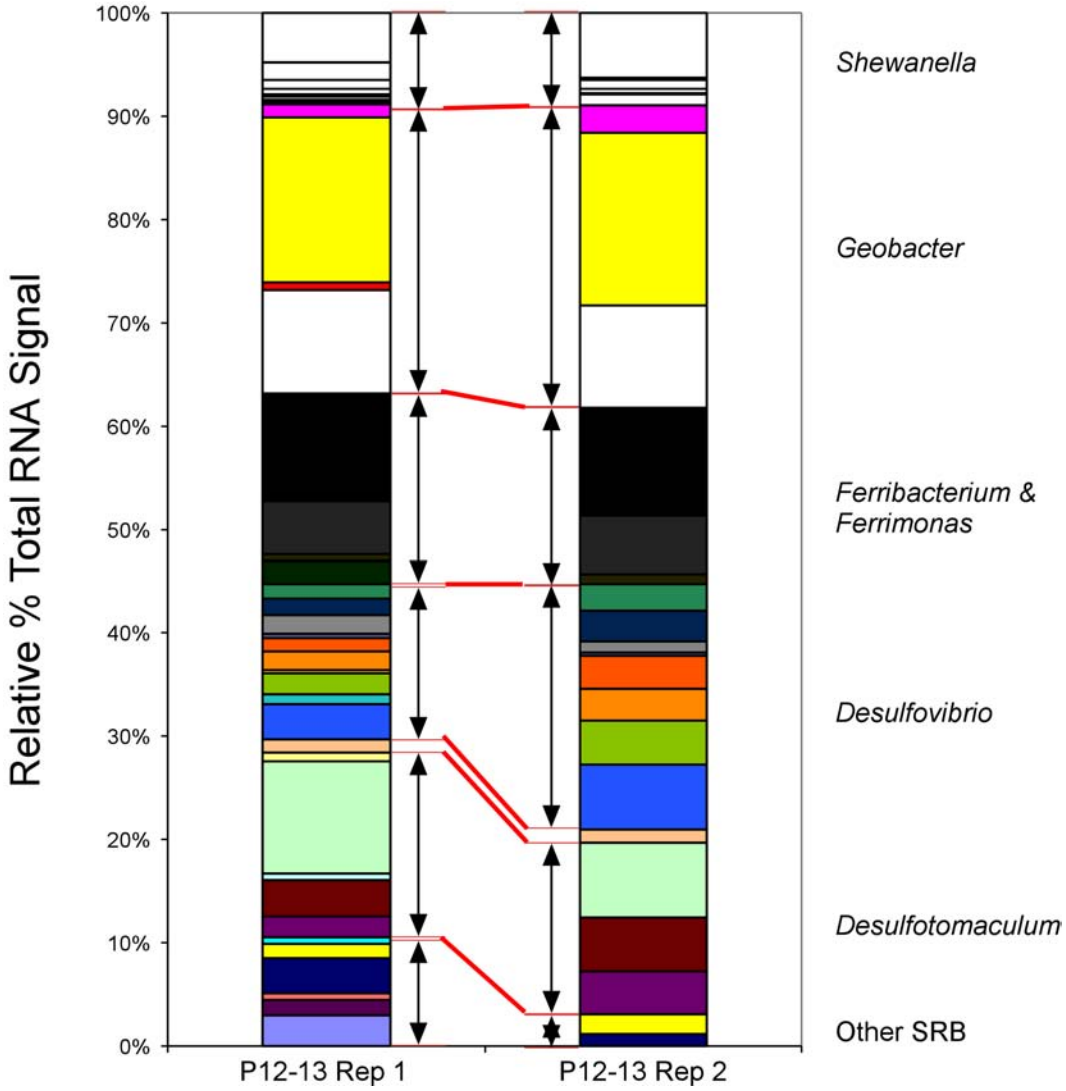
- **Backgrounds = B01 and B02; M03, M08 and M13 monitoring wells**
- **2002 Samples**
  - *P01-P06, 3 depths*
  - *All samples collected at the end of the experiment in 2002 and after 80 days of acetate injection @ 1-3 mM*
- **2003 Samples**
  - *P11-P15, 3 depths*
  - *All sediments collected at the end of the experiment after ~100 days of acetate injection @ 9-10 mM*
- **5 g sediment per sample, extract total RNA, hybridize to bead arrays for 2 hr in pH 5 tunable surface buffer (no PCR!)**
- **Bead arrays contained 67 species-specific probes for 16 genera of FeRB and SRB**

The Bead Array



Method-Level Reproducibility

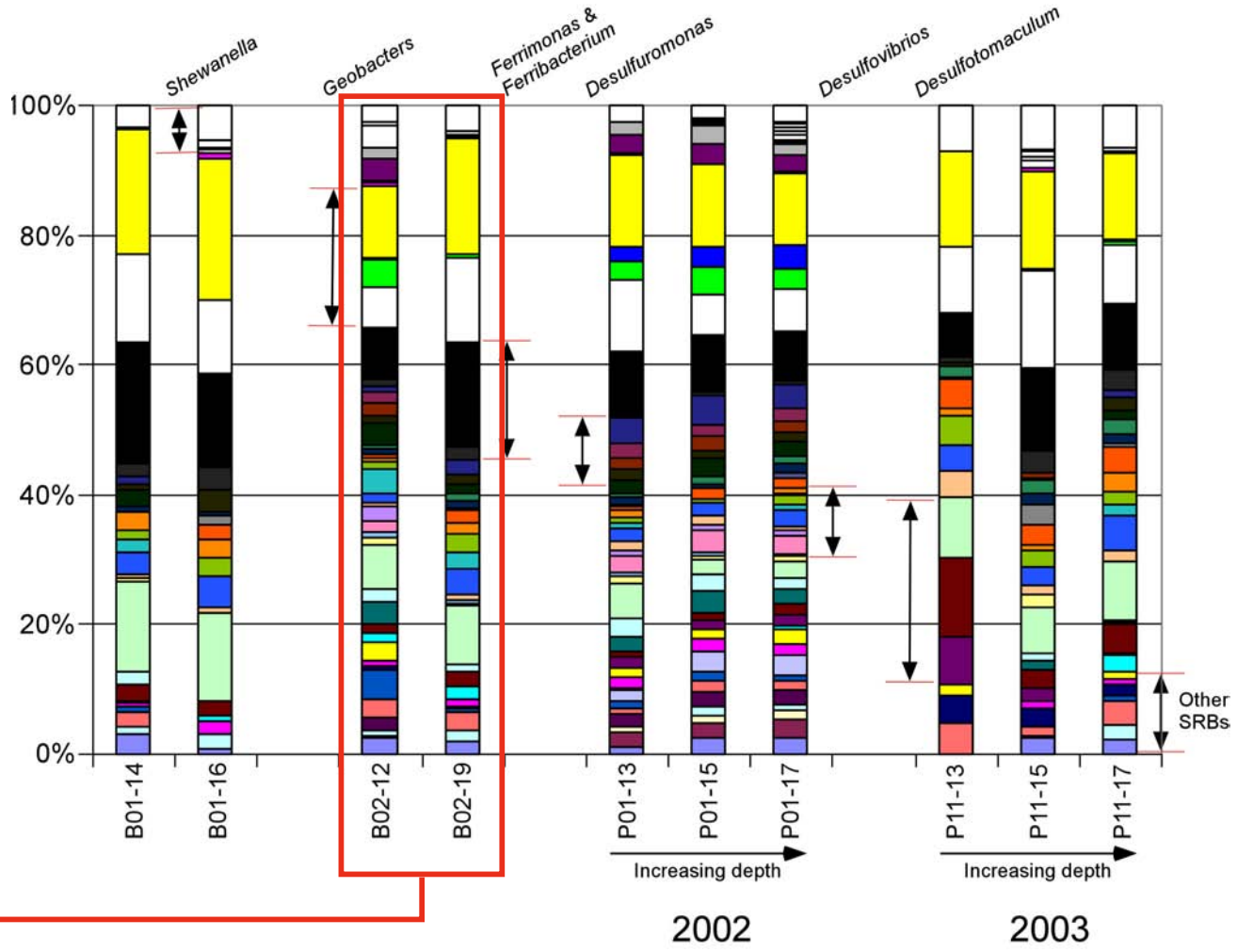
- **2 x 5 g sediment samples processed separately from P12-13** *(2003 treatment regime, 13 ft dept, closest to injection gallery)*
- **Total MFI = 1560 and 700, respectively**
- **Community profile as a % of total FeRB/SRB signal is similar between reps** *(how's that for a descriptive, non-quantitative conclusion?)*
  - ***Spatial heterogeneity in situ?***
  - ***Measurement error?***
  - ***Does it matter?***
- **Warm fuzzy to proceed**



The Background

B samples, P-01 and P-11 sediments, up-gradient from injection gallery; as % of Total Array Signal

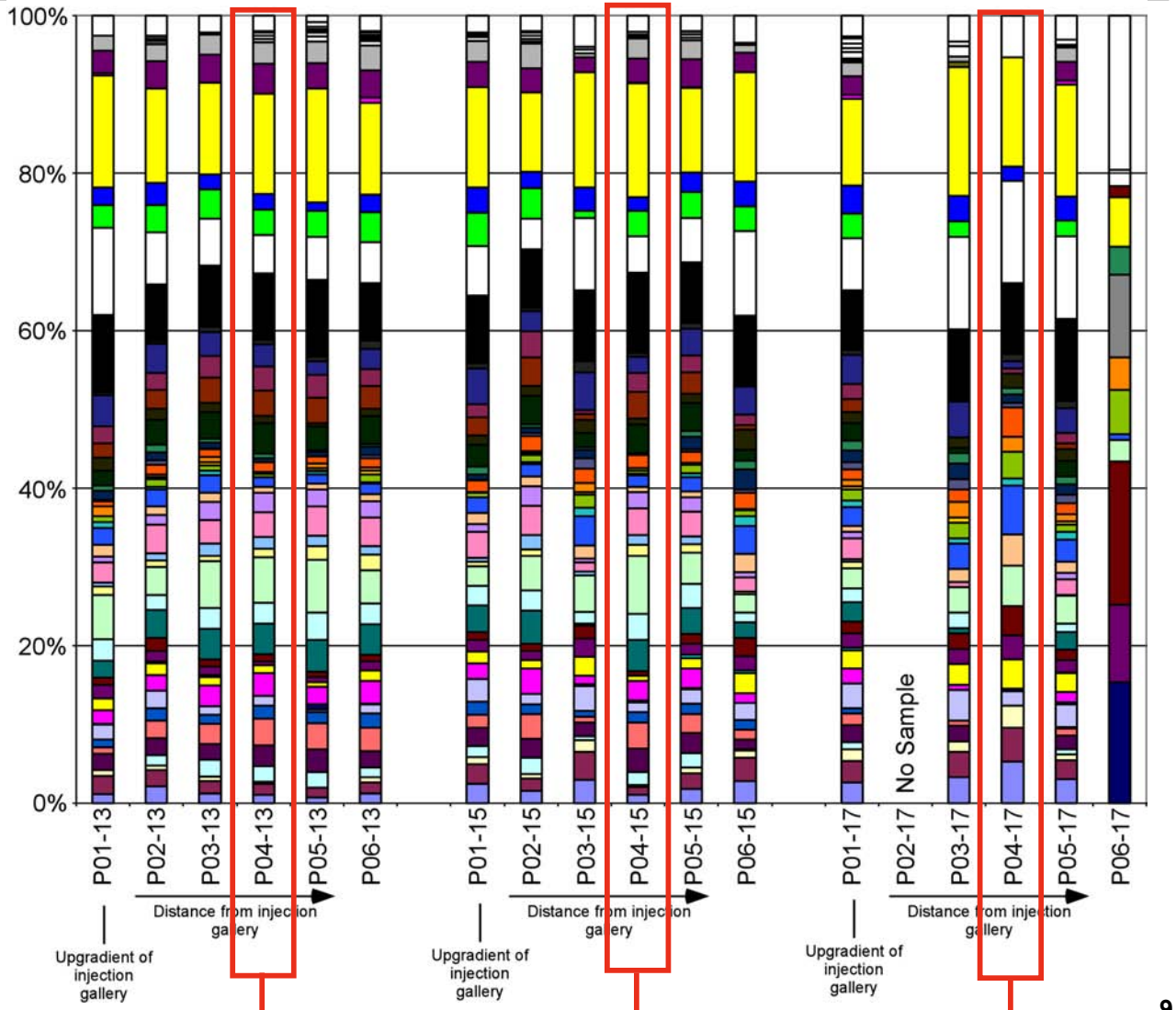
- ***5-7% Shewanella***
- ***23-32% Geobacter & Pelobacter***
- ***9-18% Ferrimonas & Ferribacterium***
- ***5-9% Desulfuromonas***
- ***9-15% Desulfovibrios***
- ***13-20% Desulfotomaculum***
- ***10-16% other SRBs***



2002 Community Response

In the sediments; as % of Total Array Signal

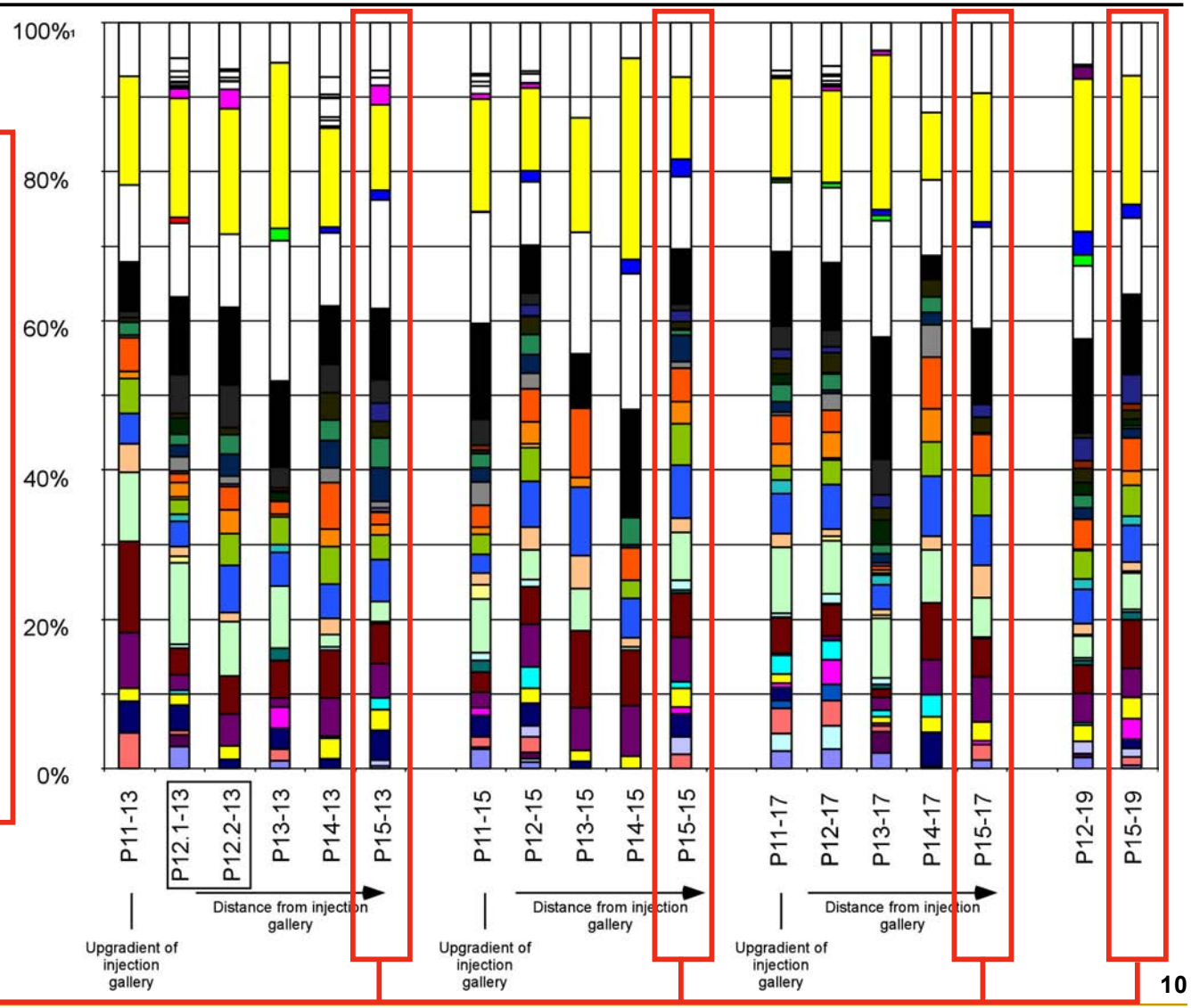
- ***3-5% Shewanella***
- ***23-29% Geobacter & Pelobacter***
- ***9-11% Ferrimonas & Ferribacterium***
- ***3-14% Desulfuromonas***
- ***4-19% Desulfovibrios***
- ***12-23% Desulfotomaculum***
- ***13-16% other SRBs***



2003 Community Response

In Sediments; as % of Total Array Signal

- ***7-9% Shewanella***
- ***23-32% Geobacter & Pelobacter***
- ***8-13% Ferrimonas & Ferribacterium***
- ***3-5% Desulfuromonas***
- ***18-25% Desulfovibrios***
- ***13-20% Desulfotomaculum***
- ***9-14% other SRBs***



Where's the Action?

Says something about size/activity of total community

	2002 P01/P04	2003 P11/P15
Total RNA (ug) from 5 g sediment	1.4-2.3 / **0.6-4.9**	0.3-2.0 / **0.7-14.7**
Estimated cell number (x 10^6)	21.0-34.5 / **9.0-73.5**	4.5-30.0 / **10.5-220.5**
Total FeRB/SRB Signal (MFI)	768-1386 / **323-2076**	493-1028/ **391-470**

Says something about size/activity of FeRB/SRB community

Does this metric say anything about the community?

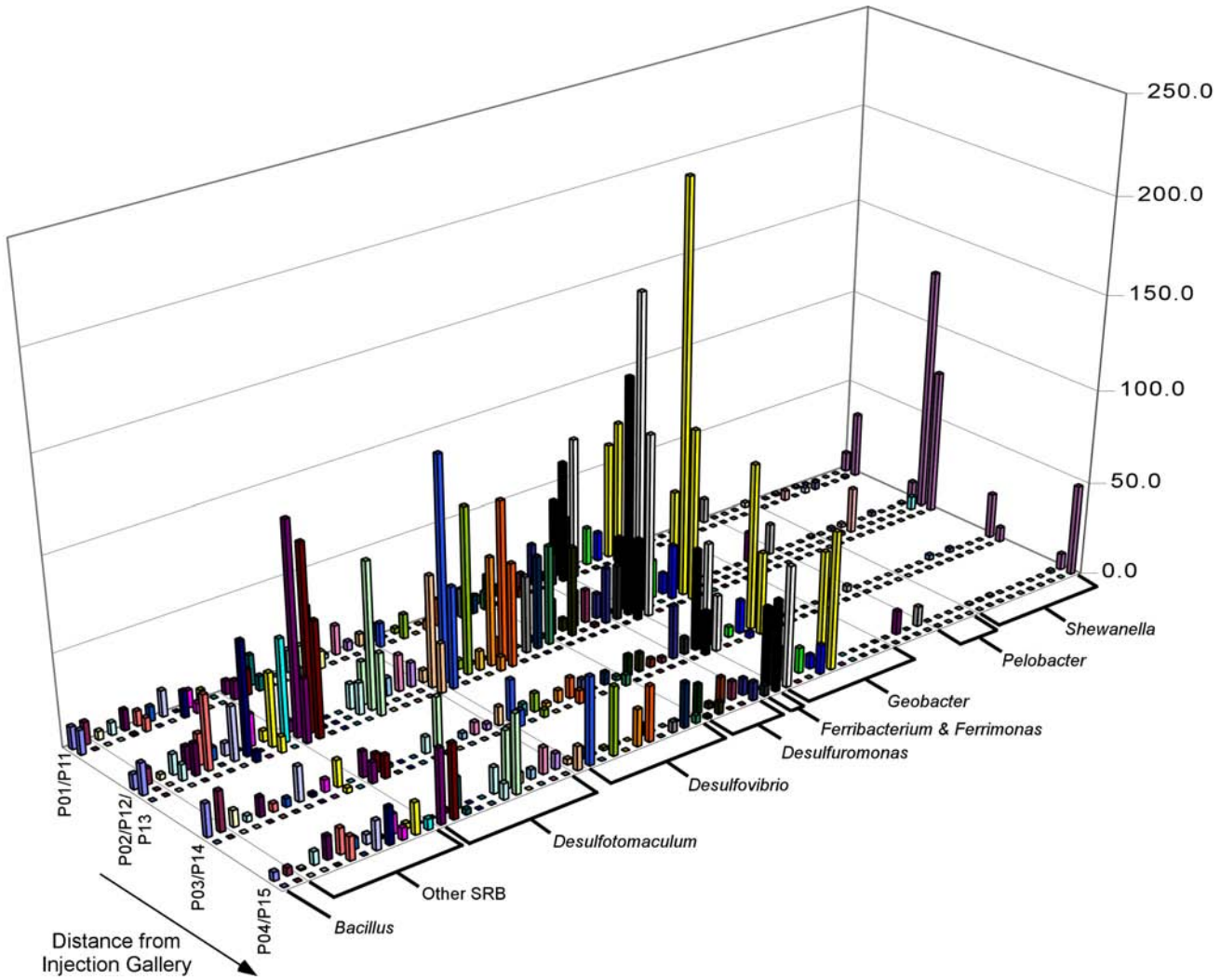
% of Total FeRB/SRB signal		
Shewanella	3-6% / **3-5%**	7-10% / **7-9%**
Geobacter & Pelobacter	25-31% / **23-39%**	23-31% / **23-32%**
Ferrimonas & Ferribacterium	8-10% / **9-11%**	7-16% / **8-13%**
Desulfuromonas	11-12% / **3-14%**	1-5% / **3-5%**
Desulfovibrio	6-11% / **4-19%**	16-20% / **18-25%**
Desulfotomaculum	15-18% / **12-23%**	14-29% / **13-20%**
Other SRBs	11-16% / **13-16%**	7-15% / **9-14%**

Do genus-level "bins" provide meaningful information?

A More Meaningful (?) View

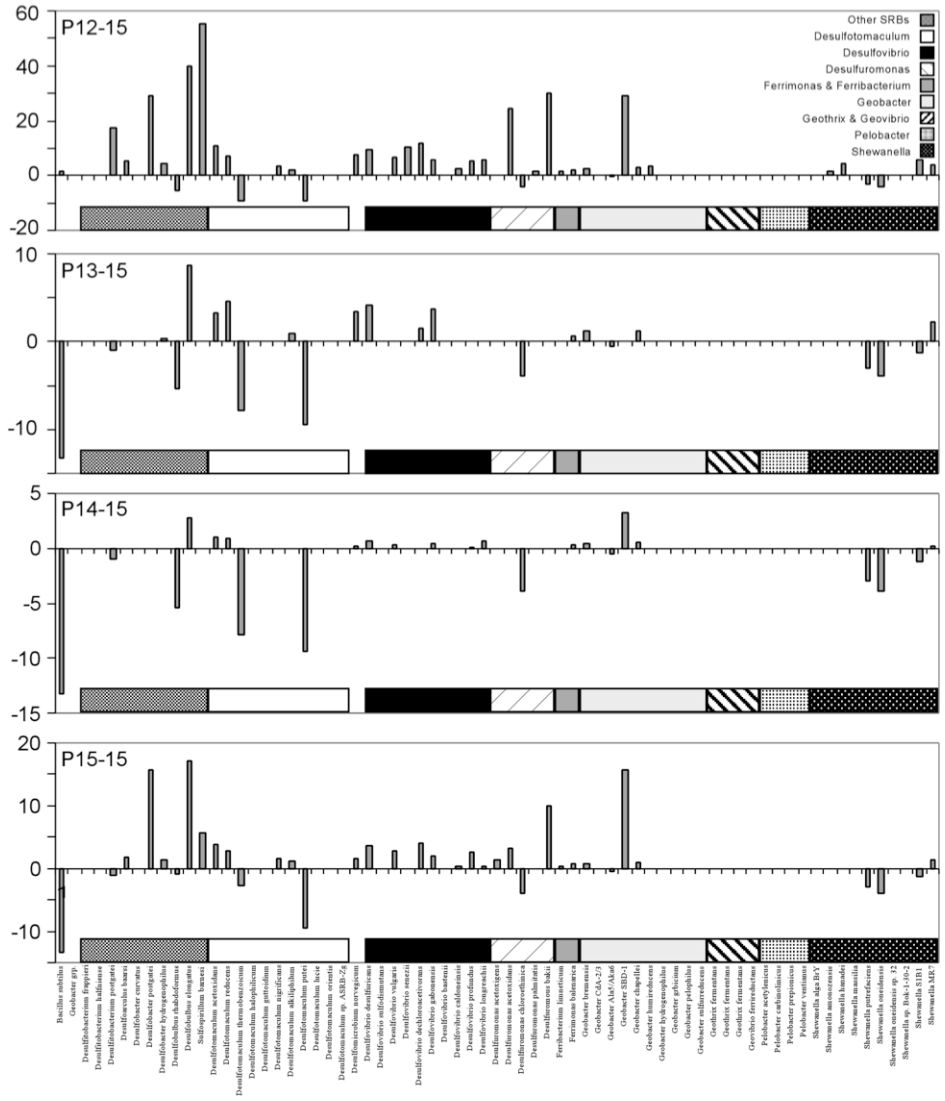
Bead array signal normalized per microgram total RNA; 15 ft depth contour

- **General decrease in specific signals with distance from injection gallery**
- ***Ferribacterium* signal almost as strong as best *Geobacter* probe response** *Is it biologically meaningful?*
- **Relatively strong 2003 *Dfm* response** *especially near injection gallery and compared to background*
- **How 'bout that *Shewanella* signal?**



2003 Change in rRNA Signal Relative to P11-15 Background

- **Strongest response closest to injection gallery, especially in "other" SRB probes** *(as expected)*
- **Changes in community response more visible in fine scale architecture** *(e.g. only one of the Geobacter probes seemed "responsive", even though several of the probes generated a strong signal as % of Total MFI and on a per microgram RNA basis; Ferribacterium did not seem to "respond")*
- **Do these data reflect a change in microbial activity?** *We are measuring rRNA directly, after all.*
- **Is this view of the data ecologically meaningful?**



Is It Believable?

- **Capture probes all target same rRNA region**
  - *Minimize or avoid differential hybridization due to 2° or 3° structure*
- **Bead array specificity validated as per "normal" array studies**
  - *Total RNA from 24 SMCC isolates of known FeRB and SRB*
- **Direct hybridization and detection of rRNA (no PCR)**
- **Ecologically relevant cell densities and detection limits** *(10^6-10^8 cell equivalents of total RNA applied to array)*
- **Community structure and response consistent with site chemistry, changes in 2002/2003 remediation procedures, and corollary molecular and microbial studies at the site**

- **If we accept clone libraries, PCR-DGGE and T-RFLP profiles as truth, then the bead array data and conclusions should be acceptable without hesitation**
- **BE CAREFUL, and QUESTION MICROARRAY ASSUMPTIONS –** ***2005 BioTechniques 38(4):591-600***

What's Next?

For the next performance period (Chandler/Roden)

- **Expand array for more thorough coverage**
- **Exercise technology on more samples = Assessment**
- **Methods for mRNA analysis = function**
- **Keep an eye on the ball – methods consistent with same day, in field, autonomous analysis and reporting**

Status of "The Box"

New fluidic design and system for developing fully autonomous DNA, rRNA and/or mRNA purification, fragmentation, labeling, hybridization, washing and bead array analysis methods

Thank You!