

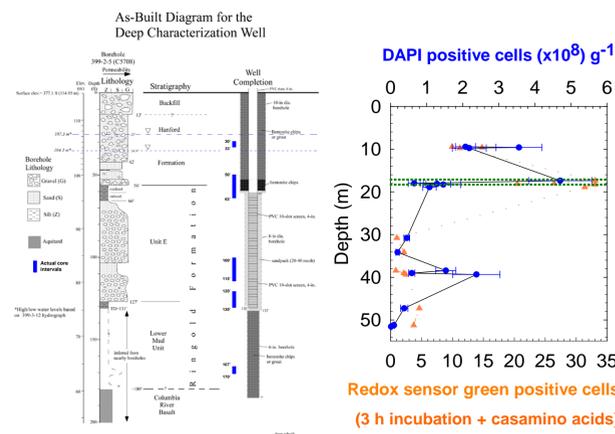
Microbial Ecology in Subsurface Sediments from Hanford 300A Area

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Total and metabolically active biomass

- Highest cell counts were found in the highly transmissive Hanford sediments
- From 0 to 19 m depth, 8-30% of the cells were metabolically responsive, with the highest proportion present within 2 m of the Hanford-Ringold interface
- Reactive cells declined to < 3% at 30 - 52 m



One goal of the PNNL Scientific Focus Area is to determine how the subsurface microbial community in Hanford's unconfined aquifer impacts contaminant fate and transport. The first essential element is a characterization of microbial biomass, phylogenetic diversity, and biogeochemically-relevant activities.

- Hanford IFRC project provided 12 m of core over 52 m Deep Characterization Borehole (well# C6209)
- Intensive analyses for 17 samples across Hanford and Ringold formations and transition zones

Cultivation-independent analyses

- Biomass**
 - Direct microscopic counts (DAPI staining)
 - Phospholipid fatty acids (by Aaron Peacock)
 - % Respiring cells (BaLight™ Redox Sensor dye)
- Phylogenetic and functional diversity and relative abundance**
 - Census of Bacterial/Archaeal 16S rRNA gene sequences
 - JGI CSP project for Sanger sequencing (~500 seq/sample)
 - Pyrosequencing by U Colorado collaborators (ongoing)
 - Real time PCR for specific phylogenetic and functional groups
- Assessment of potential for TEA reduction (see Fredrickson et al. poster)**
 - Amend samples with electron donor
 - Add "natural" TEA: Mn⁴⁺, Fe³⁺, NO₃⁻, SO₄²⁻
 - Add U or Tc.

Cultivation-dependent analyses

- Enrichment cultures with various TEA's
- Analysis of metabolic versatility in cultivars

Provide Hanford-relevant microbes for molecular- to pore-scale projects (see Believ et al poster)

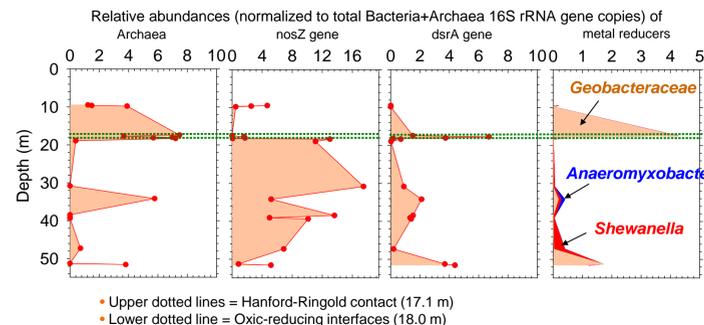
Multivariate statistical analysis of microbial census + geochemical / mineralogical data to generate hypotheses for field-scale studies

Profile of phospholipid fatty acids (PLFA) in two sediment samples

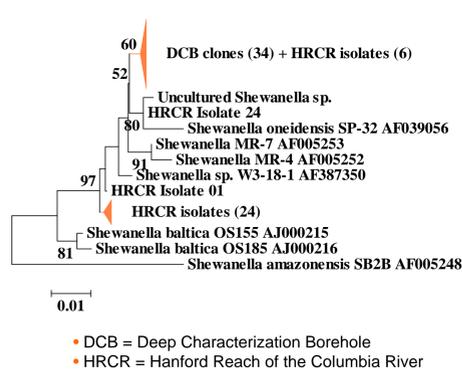
Sample depth (m)		9.6	51.2
Biomass	PLFA (pmols) / g soil	252	99
	cy17:0/16:1w7c	0.51	1.34
	cy19:0/18:1w7c	0.68	0.68
	16:1w7/16:1w7c	0.22	0.00
Metabolic Stress (P/S ratio)	Firmicutes (TerBrSats)	21.5	14.5
	Proteobacteria (Monos)	44.1	44.3
	Anaerobic metal reducers (BrMonos)	2.4	0.7
	Actinomycetes (MidBrSats)	7.4	0.0
Community Structure (% of total PLFA)	General (Nsats)	24.6	40.5
	Eukaryotes (polyenoics)	0.0	0.0

Quantitative real time PCR

- Microbial cells were dominated by Bacteria; Archaea comprised 0-8% of total 16S rRNA gene copies. The *nosZ* gene, associated with denitrification, was quite prevalent
- Sulfate-reducing bacteria (*dsrA* gene) ranged from below detection to 7% of total 16S rRNA genes in Ringold sediments, and were near the detection limit in Hanford sediments
- Metal-reducing Geobacteraceae, *Shewanella*, *Anaeromyxobacter* were sporadic and their abundance was relatively low

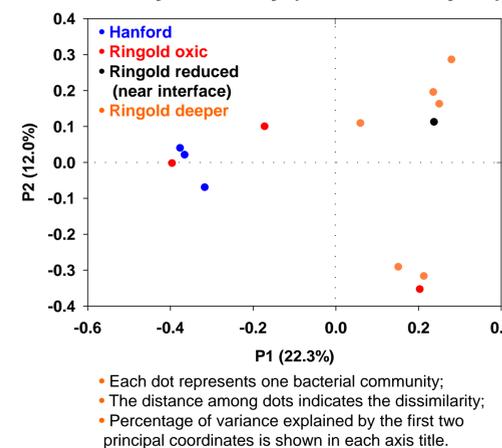


Shewanella DCB amplicons vs. Columbia River isolates (HRCR - Matt Marshall)

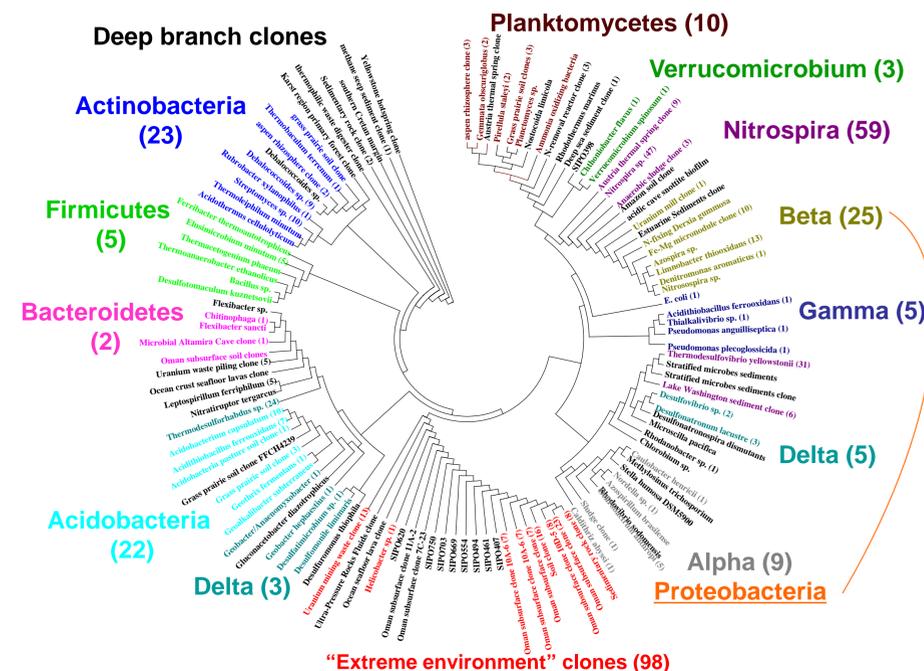


- DCB = Deep Characterization Borehole
- HRCR = Hanford Reach of the Columbia River

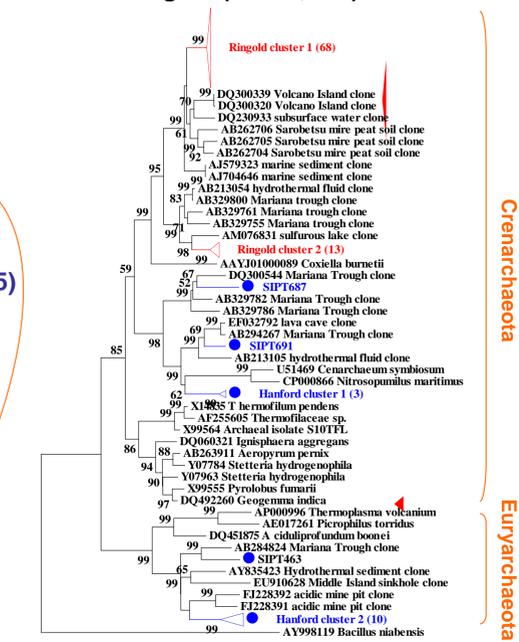
Principal coordinate display of bacterial community similarity (UniFrac analysis)



Bacterial 16S rRNA gene tree (total 346 sequences from well C6218, 10.7m)



Archaeal lineages in Hanford (9.8m, blue) and Ringold (18.3m, red) sediment



- Sequences were aligned with NAST algorithm using GreenGenes webtool and trees were constructed using maximum likelihood algorithm;
- For Bacteria tree (topology only), branching pattern with closest lineages are collapsed for clarity. Number of DCB sequences shown in parenthesis;
- For Archaea tree, bootstrap values are shown in each branch. Wedges are proportional to sequence abundances with number of sequences shown.

Preliminary Conclusions

- Microbial cells were most abundant in the highly transmissive Hanford sediment. As many as 30% of total cells were metabolically active in a short-term assay.
- Bacteria were predominant – Archaea accounted for less than 8% of total 16S rRNA gene copies.
- Microbial communities were dominated by *Proteobacteria* (31-59%), followed by *Nitrospira* (17%), *Actinobacteria* (7%), *Acidobacteria* (6%), *Planktomycetes* (4%), *Bacteroides* (3%), *Firmicutes* (1%), and *Verrucomicrobium* (1%). A group of unclassified "extremophiles" accounted for about 28% of total sequences in the library.
- Potential metal reducers were minor components of DCB microbial communities, while denitrifiers were quite prevalent throughout DCB.
- Tree-topology-based community comparison and statistical analyses indicated that distinct Bacterial and Archaeal lineages residing in Hanford, Ringold oxic, and Ringold reduced sediment.

Future Research

- Deeper phylogenetic analysis of JGI clone libraries / analysis of spatial heterogeneity by pyrosequencing
- In-situ experiments to quantify the activity and rates of target microbial populations and processes
- Test effective protocols to enhance protein recovery for proteomics analysis of microbial community



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