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Microbial and Isotopic Evidence of Concurrent Aerobic and Anaerobic Degradation of Chlorinated Benzenes in Wetland Sediments and a Bioaugmented-Activated Carbon Reactive Barrier

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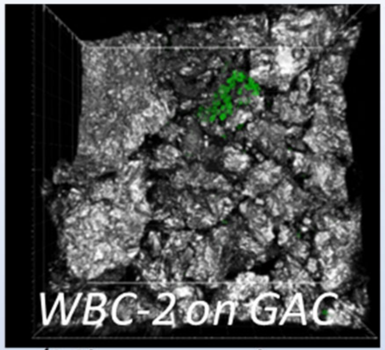

(Microscopy image from Staci Capozzi, Geosyntec

USGS

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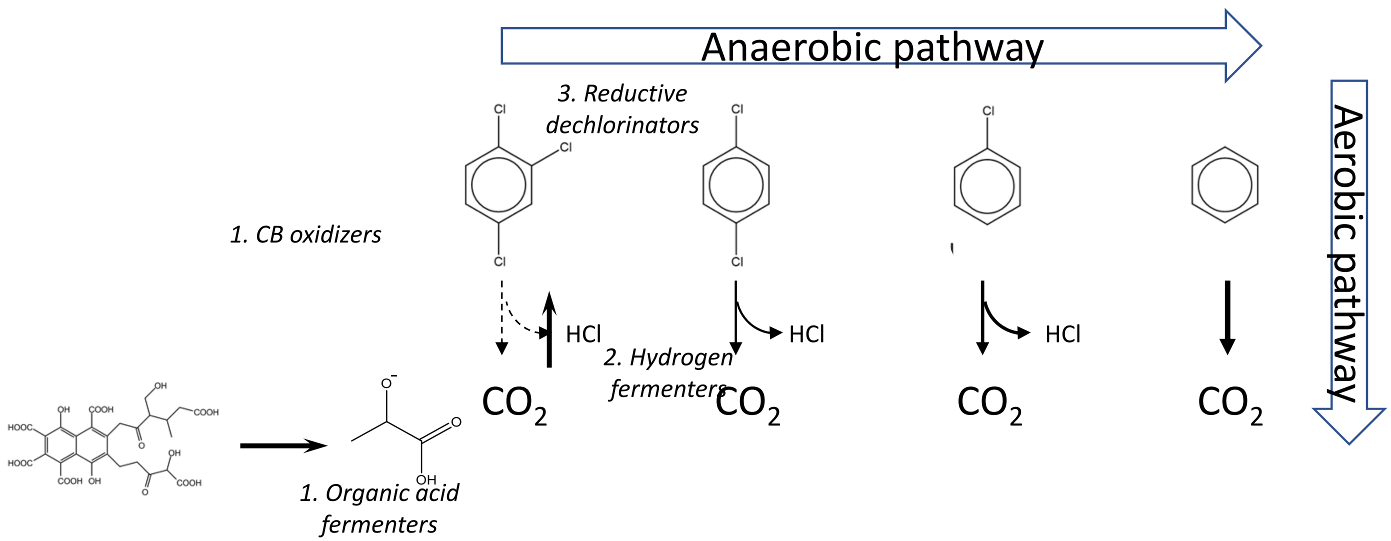
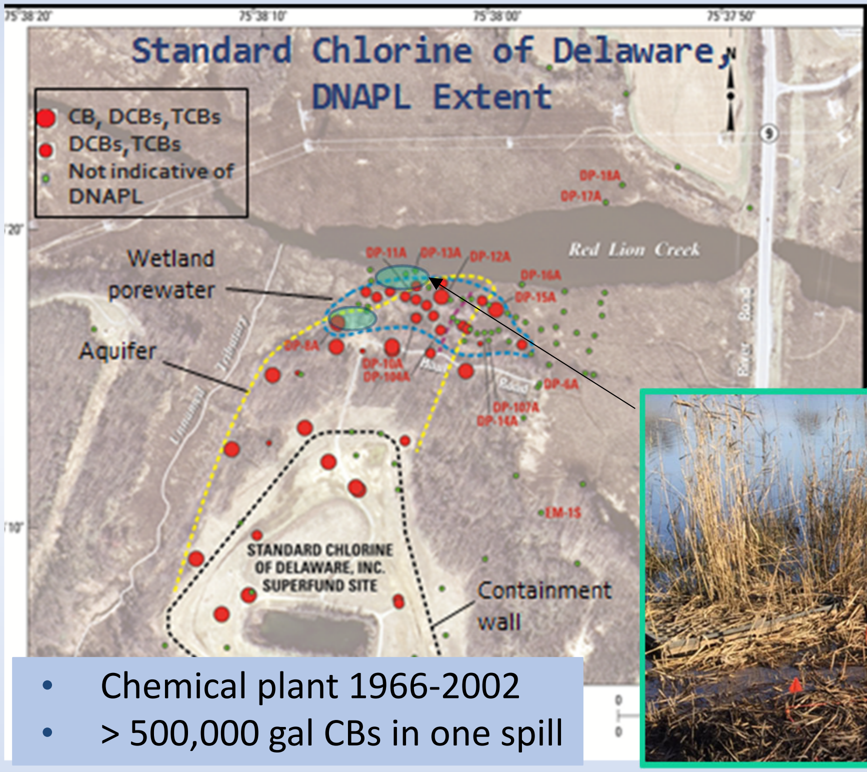
Coupled Anaerobic – Aerobic Biodegradation

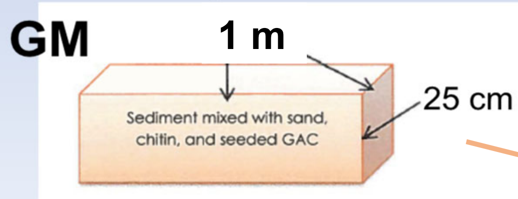
1. **Anaerobic:** reduce highly-chlorinated compounds to less-chlorinated products
   - Highly specialized degraders (*Dehalo…* organisms)
   - External substrate + CB e^- acceptor
   - Daughter compounds remain
2. **Aerobic:** oxidize less-chlorinated CBs to innocuous products
   - Generalist degraders (one of a variety of potential substrates)
   - CB substrate + O_2 e^- acceptor
   - Complete mineralization



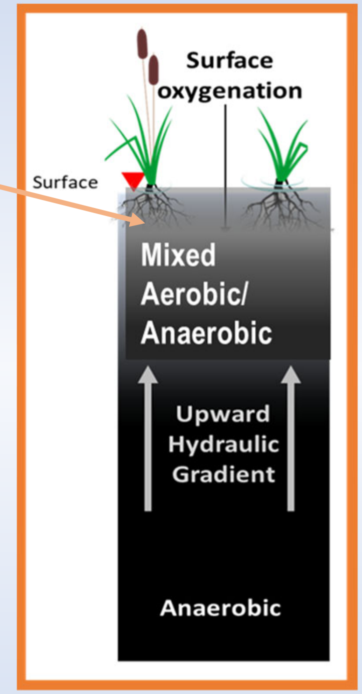
Demonstrated with CBs[1], PCBs[2], chloroethenes and chloroethanes[3], azo dyes[4], and others



- Chemical plant 1966-2002
- > 500,000 gal CBs in one spill



Vol %	GAC	Chiten	Sand
GM	5	3	12



Reactive barriers at 2 sites to pilot test goal of enhancing sorption and biodegradation

- intercept discharging groundwater contaminants (TCBs, DCBs, CB or MCB, benzene)
- decrease contaminant mass in near-surface wetland sediments

Bioaugmented GAC with an anaerobic dechlorinating culture (WBC-2) and a site-derived aerobic culture (15B).

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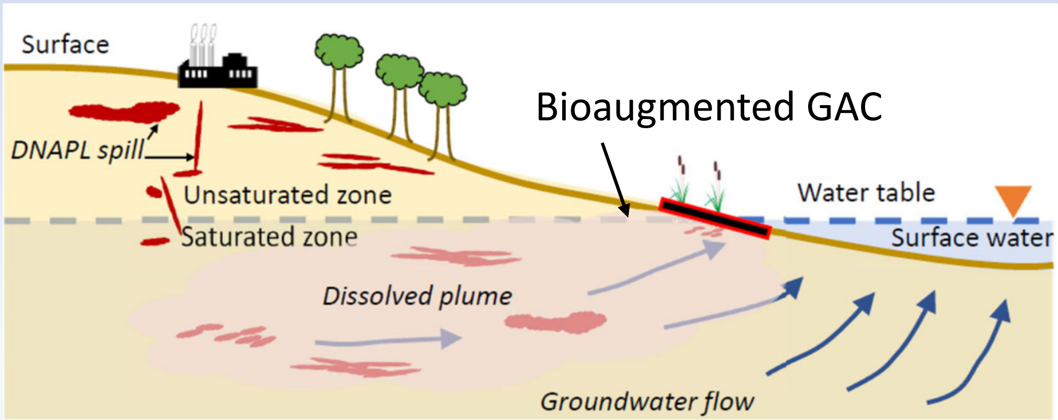
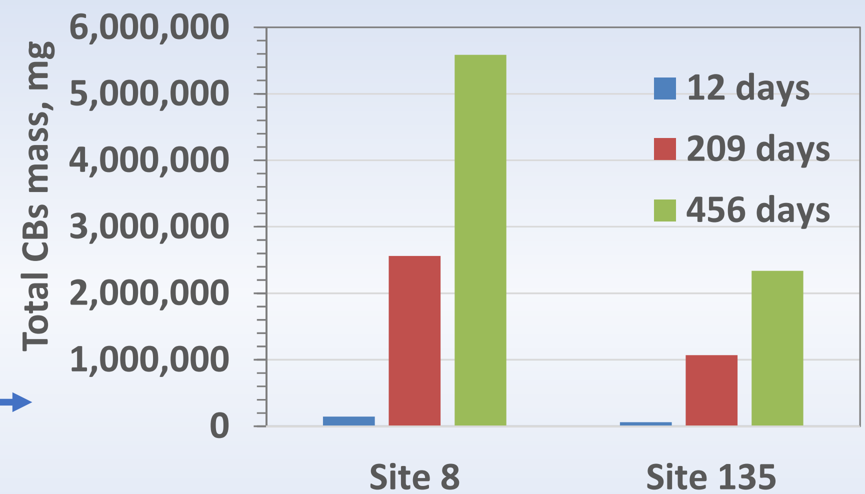
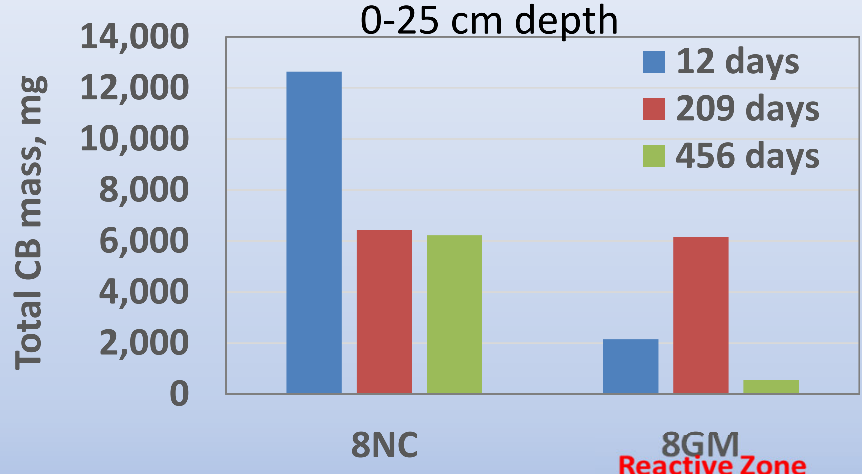


Cumulative CB mass, groundwater influx

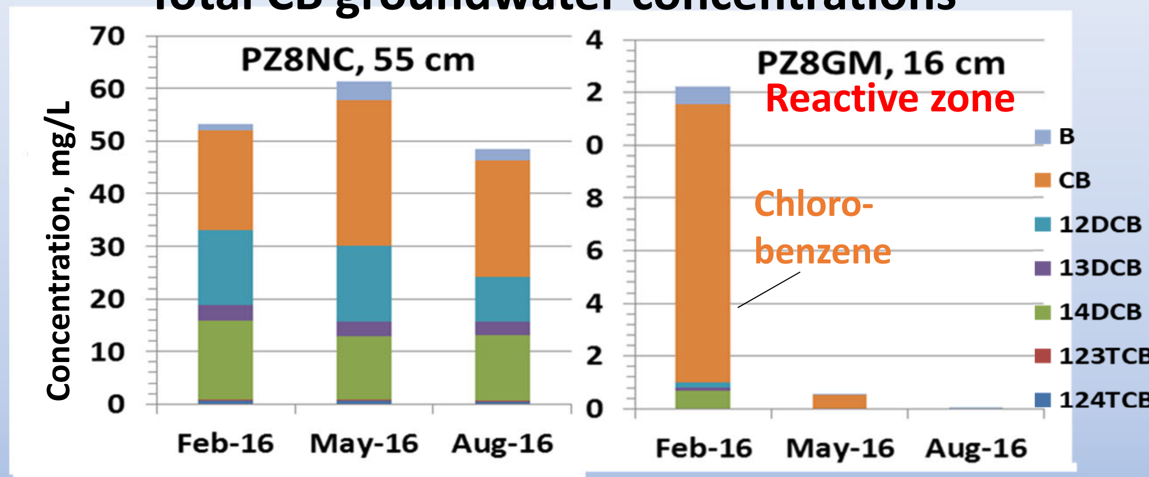


Specific discharge, q = 0.25 m/day

Site 8, Mass CBs in Sediment, 0-25 cm depth



Total CB groundwater concentrations

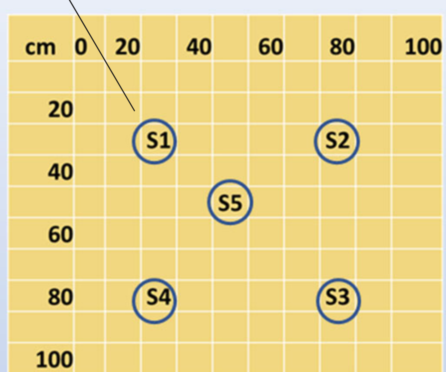


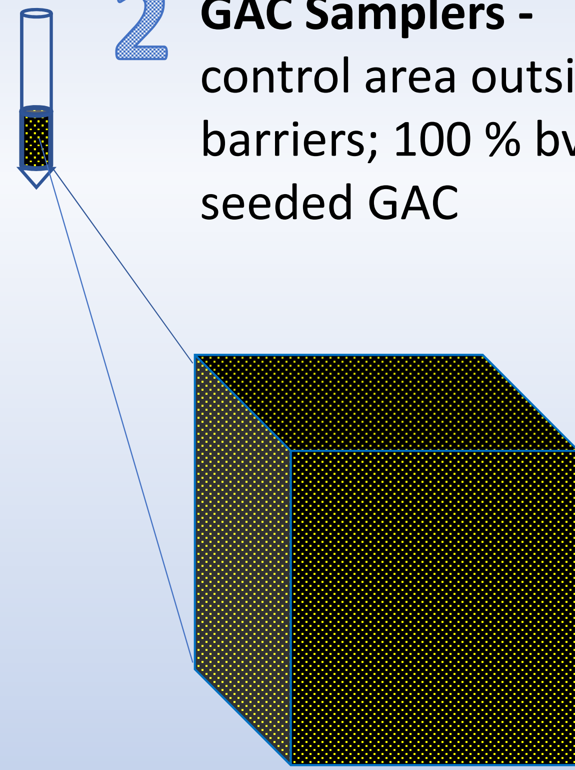
Microbial Samples

1 **Sediment samples-** cores from reactive barrier and control samples below and outside reactive zone

Reactive Barrier- 5% bv seeded GAC

2 **GAC Samplers -** control area outside barriers; 100 % bv seeded GAC





0.5 ml seeded GAC dispersed in 10 mL sediment sample

Sediment core sites in test plot.
5 to 10 cm depth intervals.
Dec. 2015, Aug. 2016, July 2017.

10 ml seeded GAC in 10 mL sample; depth integration from 7-15 cm bls.

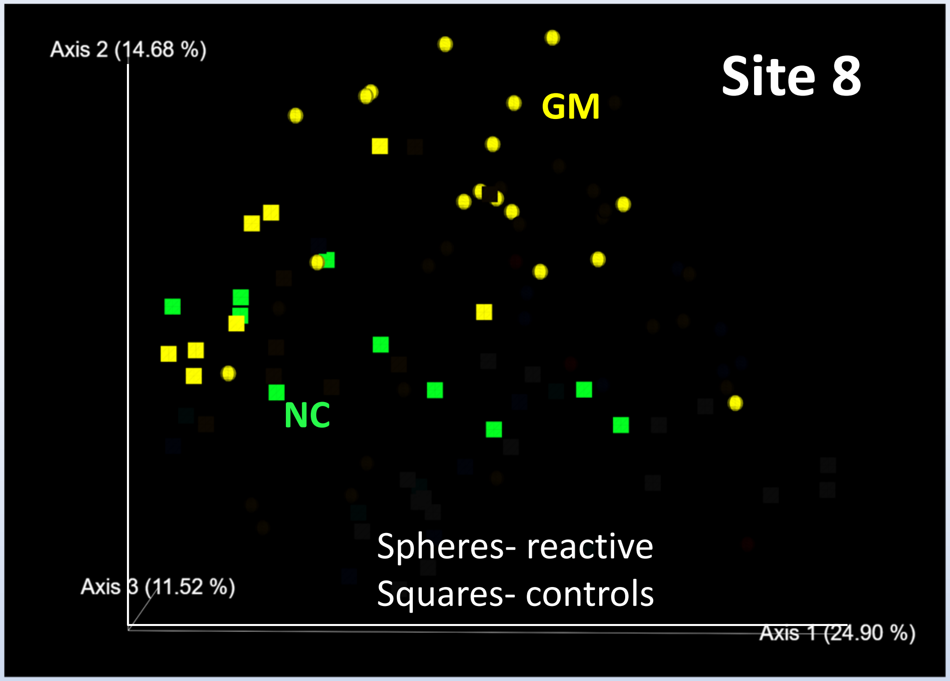
Questions:

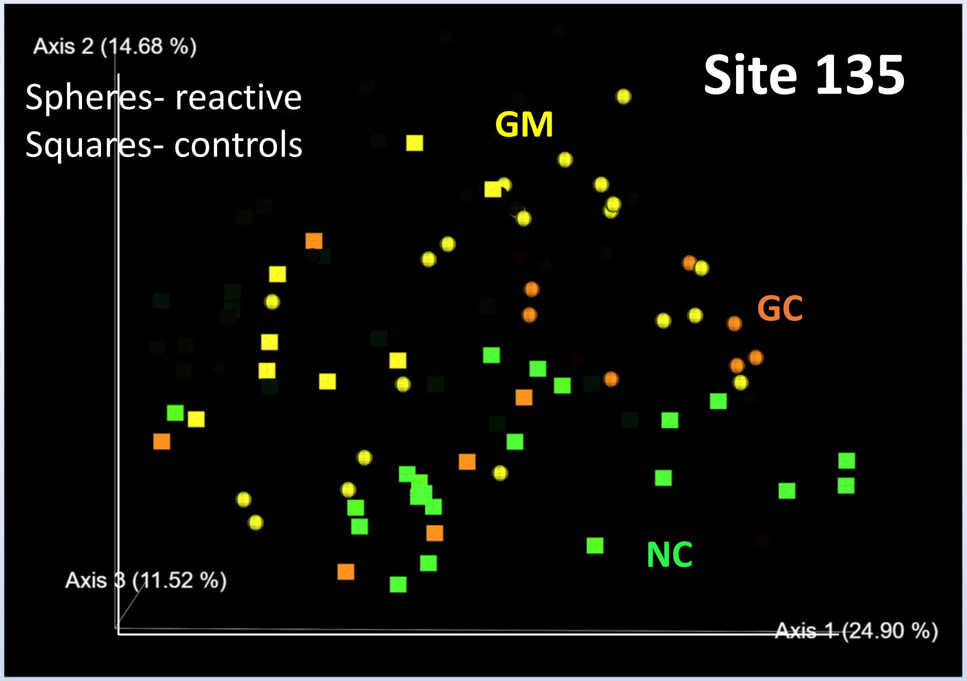
1. Is the microbial community in the wetland sediment changed in the reactive barrier?
   - presence of anaerobic and aerobic chlorobenzene degraders from the respective cultures in the test plots and control areas
   - similarities between the 2 constructed reactive barriers
   - community composition with depth and co-occurrence of the cultures

2. Can we relate microbial community composition changes in the reactive barriers to the bioaugmented GAC?
   - changes in the added microbial community with incubation in the wetland sediment

Principal Coordinate Analysis – Reactive barrier vs controls

(weighted UniFrac, phylogenetic β-diversity)

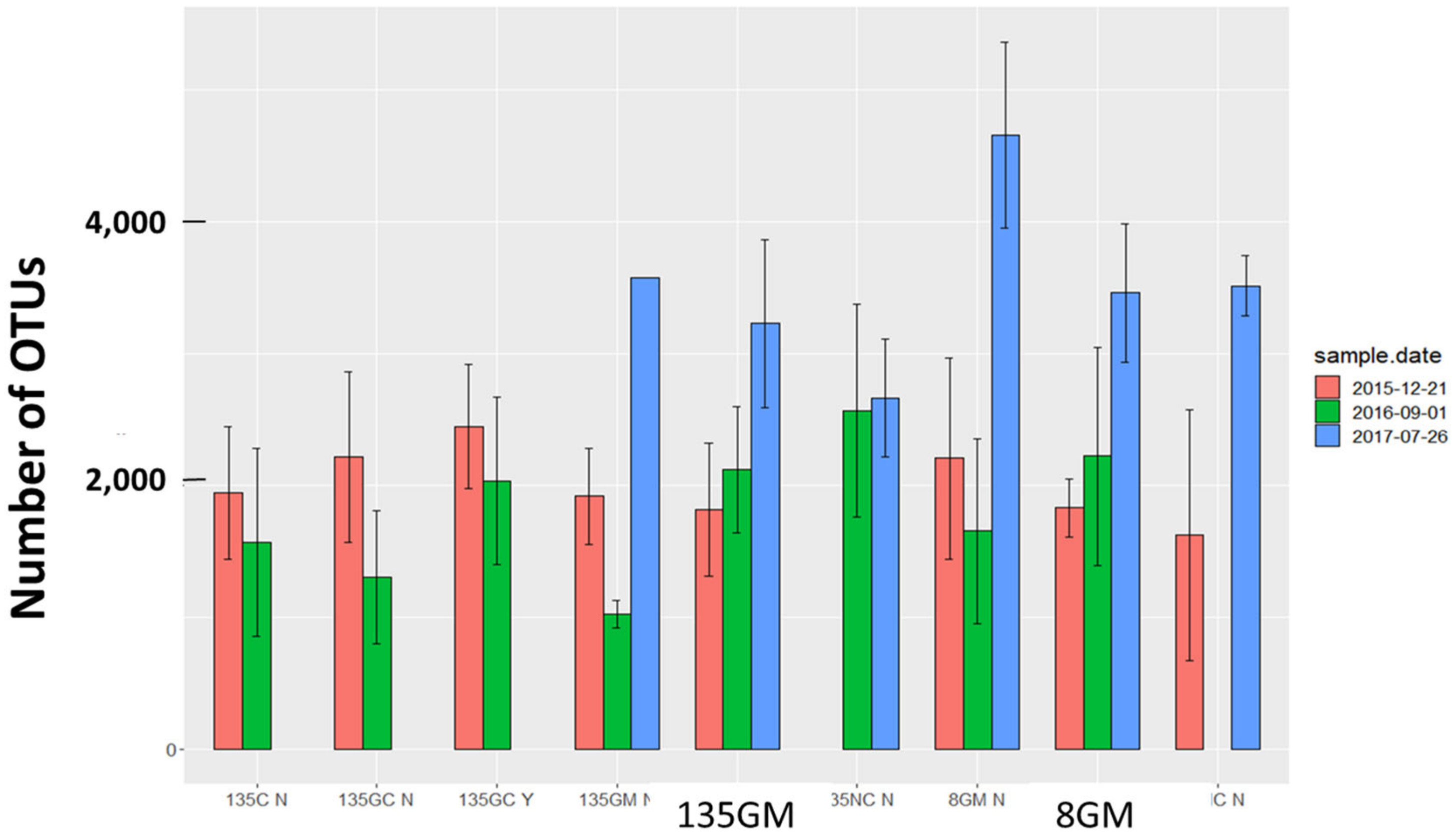




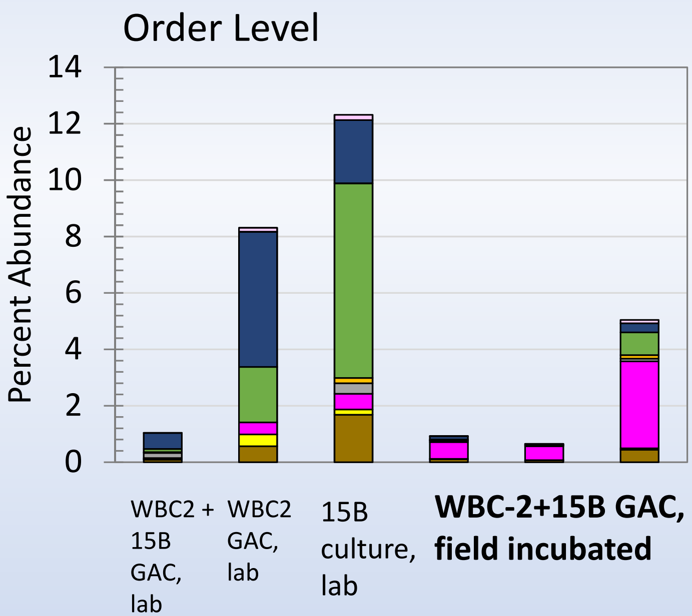
- Reactive (spheres) and control samples (squares) are distinct from each other (p-value of 0.001).
- Reactive 8GM and 135GM samples appear distinct from each other.
- Communities in controls beneath the reactive zones (yellow, orange squares) appear to be distinct from controls outside the barrier plots (green squares).

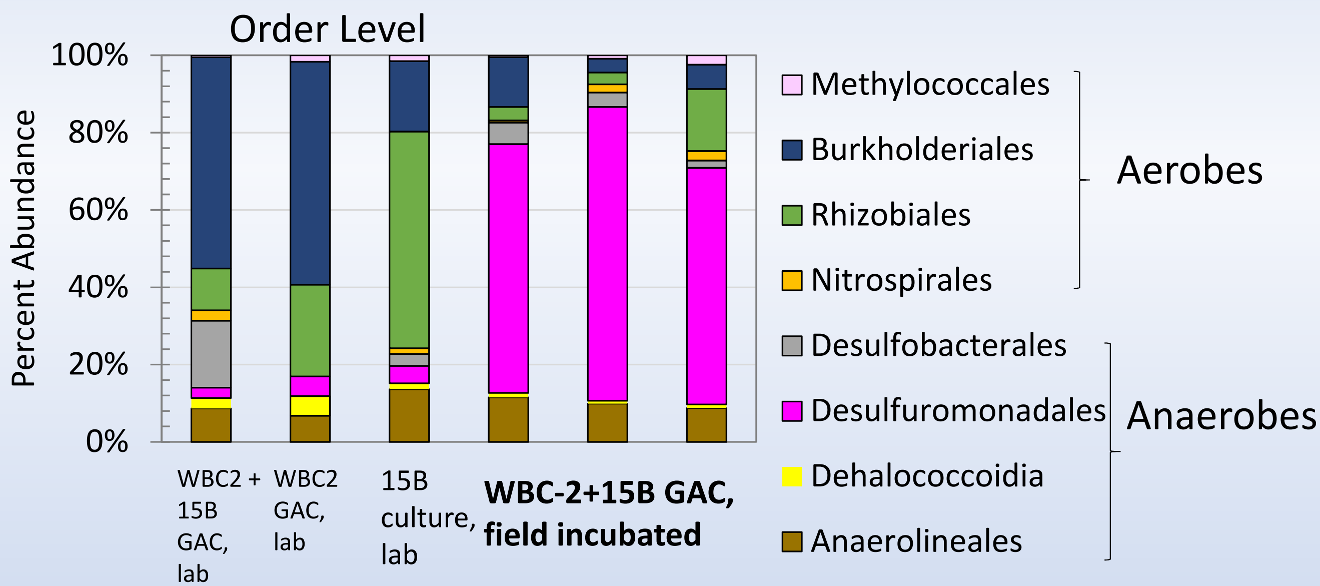
All data in this presentation are provisional.

Number of OTUs (operational taxonomic units)



GAC Samplers- Microbial Community





- Burkholderiales- a dominant order with aerobic degraders in 15B culture and in GAC in lab and field.
- Desulfuromonadales- dominant anaerobes in WBC2 culture and show a large increase in abundance on field incubated GAC compared to the lab.
- Dehalococcoidia- specialized anaerobes with increase on GAC in lab and consistently present in low percent abundance in field incubated GAC.

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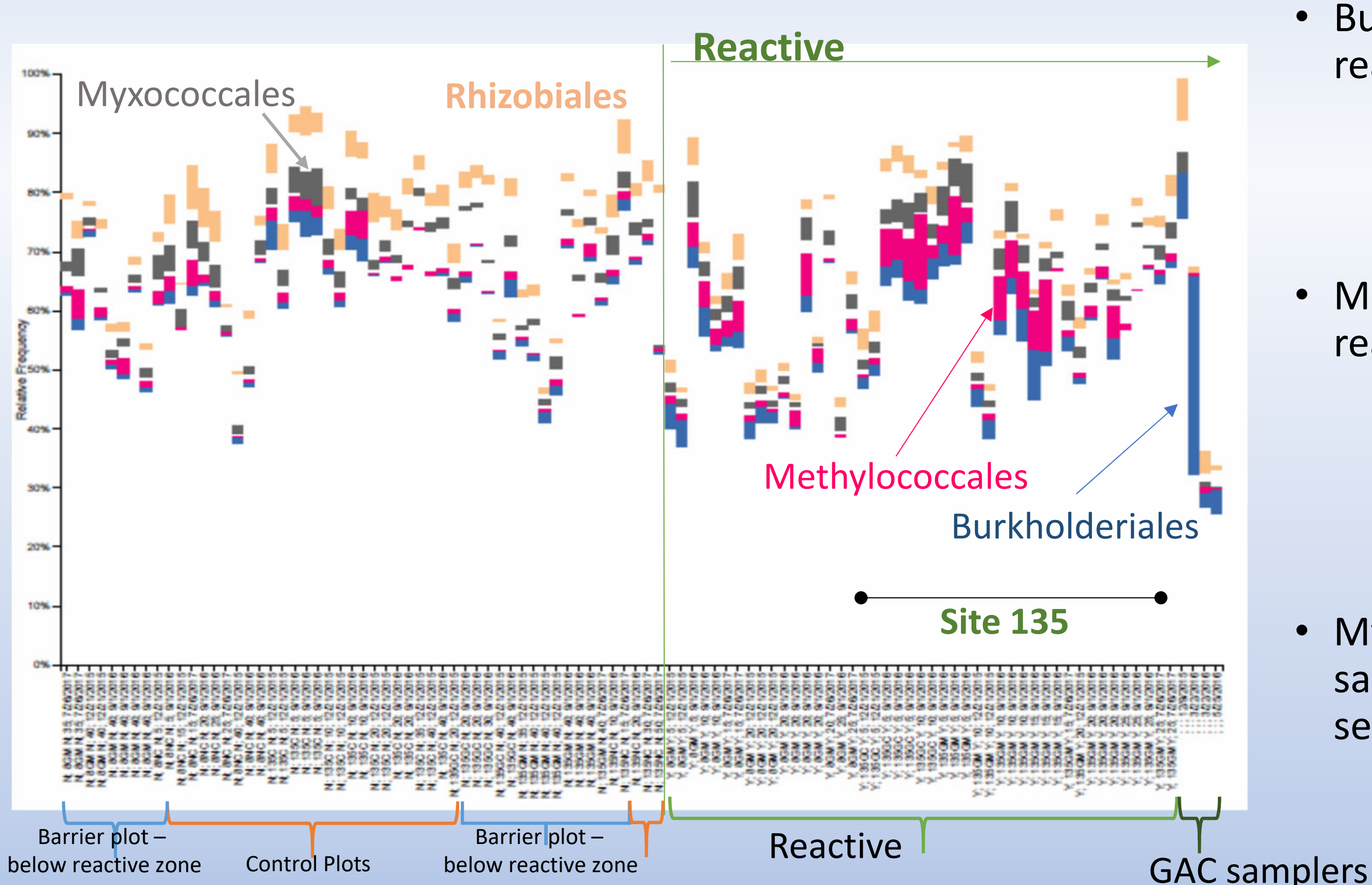
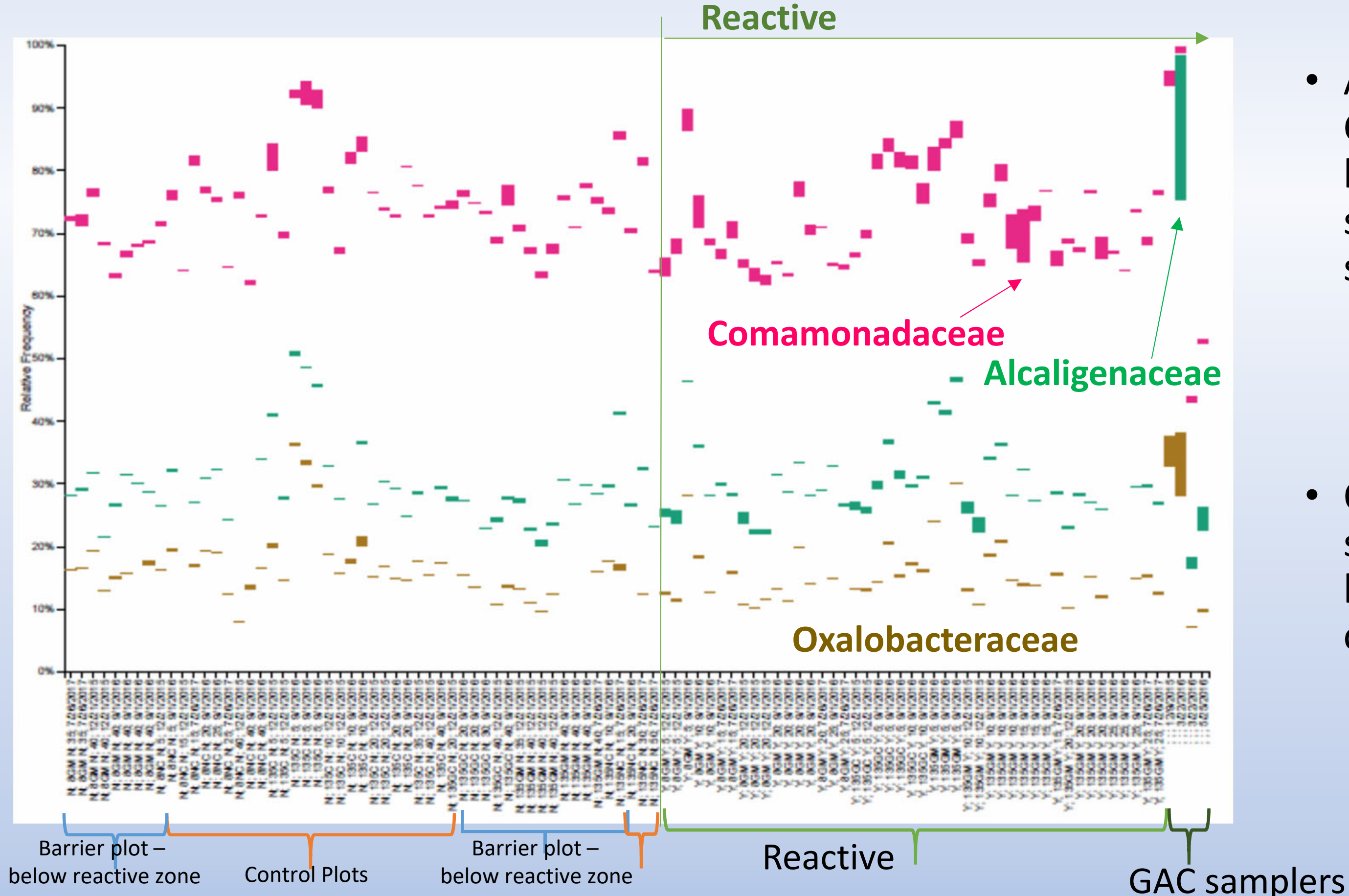
Sediment Samples Taxonomy plots – Aerobes, Order Level
(sorted by reactive vs unreactive and barrier plot)
Percent Abundance



- Burkholderiales higher abundance in reactive zone sediment than controls
  - High in 15B culture and GAC samplers
  - Highest at site 135
- Methylococcales higher abundance in reactive zone sediment than controls
  - Relatively low in 15B culture and GAC samplers
  - Highest at site 135
  - Methanotrophs
- Myxococcales and Rhizobiales about same or lower in reactive barrier sediment compared to controls

All data in this presentation are provisional.

Sediment Samples Taxonomy plots – Family Level, under Burkholderiales
(sorted by reactive vs unreactive and barrier plot)
Percent Abundance



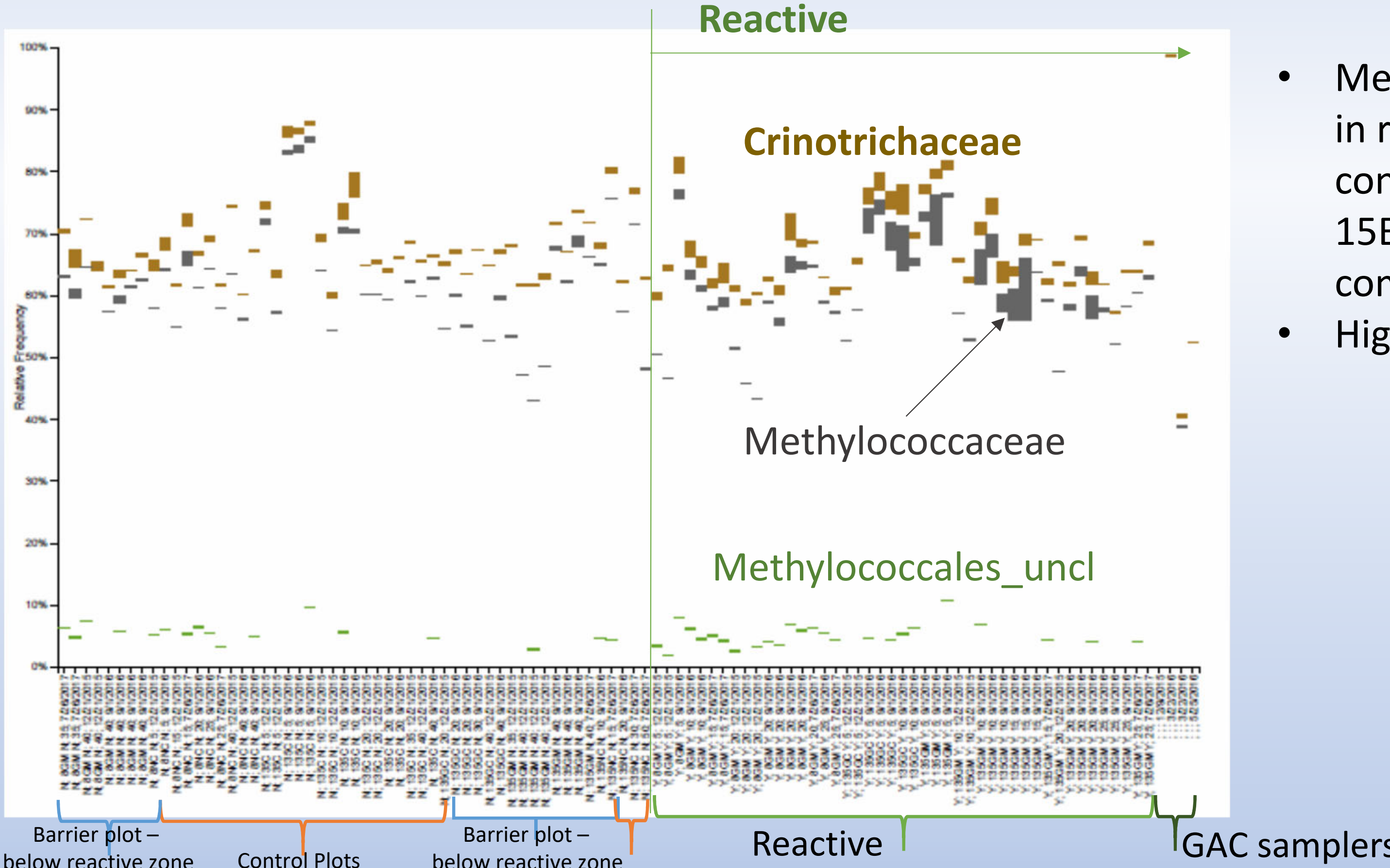
- Alcaligenaceae and Comamonadaceae showed highest abundance in GAC samplers and reactive zone sediment
  - Also high in the 15B culture
  - Known to include chlorobenzene degraders
- Oxalobacteraceae was high on some GAC samplers but was not higher in reactive zone sediment compared to controls
  - Direct vs. indirect effects?

All data in this presentation are provisional.

Sediment Samples Taxonomy – Family Level, under Methylococcales

(sorted by reactive vs unreactive and barrier plot)

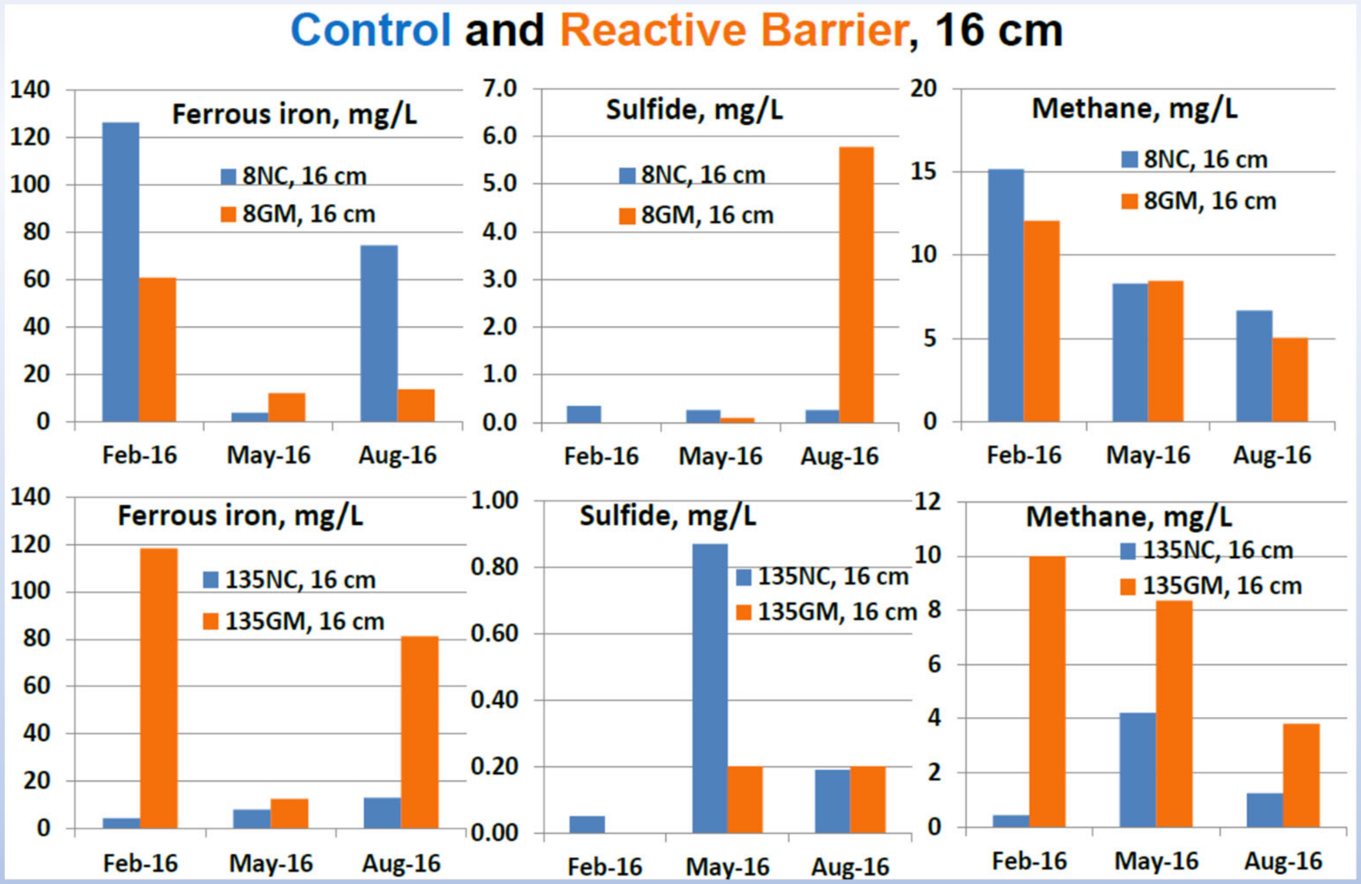
Percent Abundance



- Methylococcales higher abundance in reactive zone sediment than controls but were relatively low in 15B culture and GAC samplers compared to Burkholderiales
- Highest at site 135

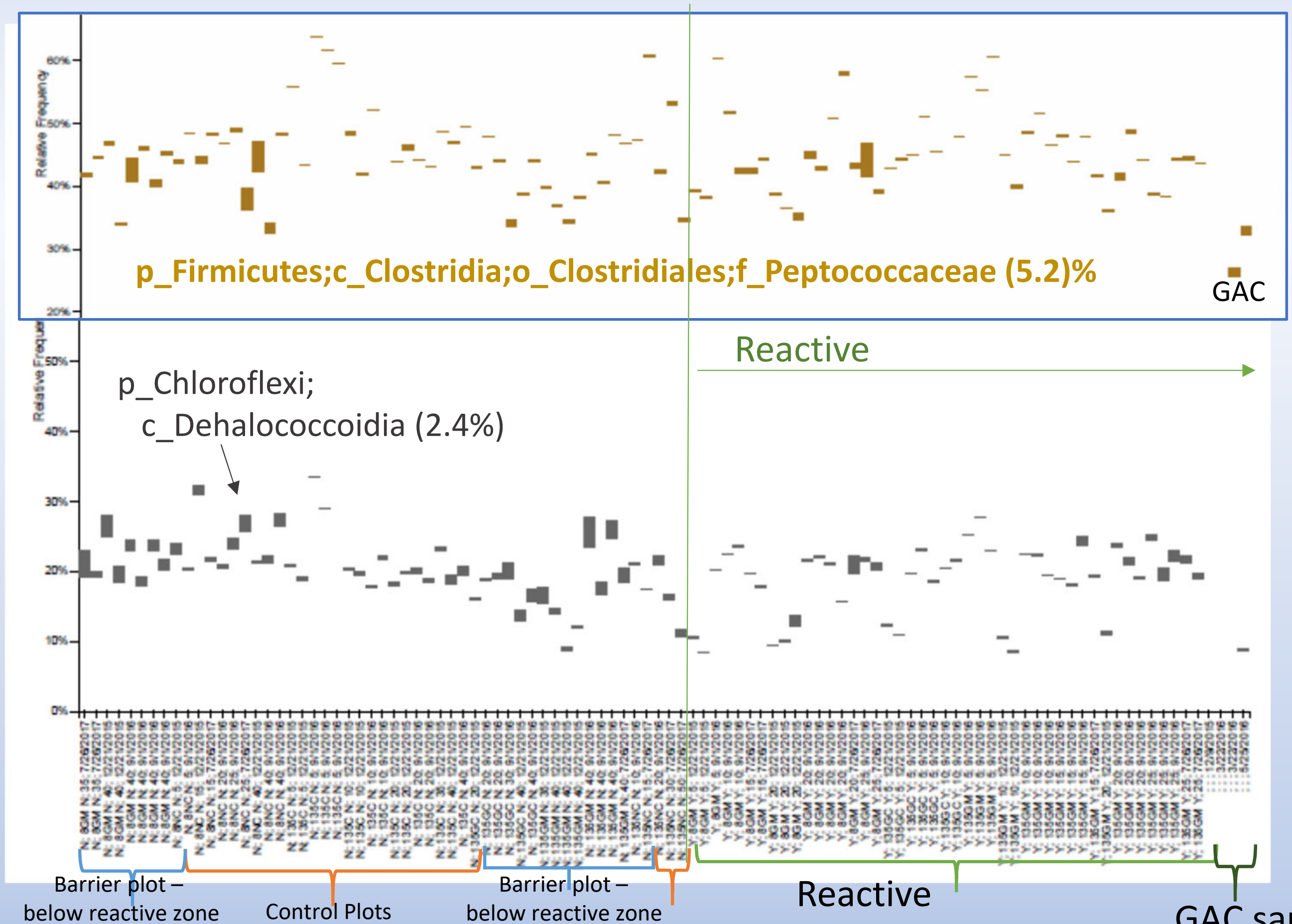
All data in this presentation are provisional.

Groundwater –Redox Constituents



- Groundwater chemistry shows anaerobic conditions with high methane.
- Higher methane production in site 135 reactive barrier – supports high methanotrophs

Sediment Samples Taxonomy – Anaerobes, Reductive Dechlorination (sorted by reactive vs unreactive and barrier plot) Percent abundance

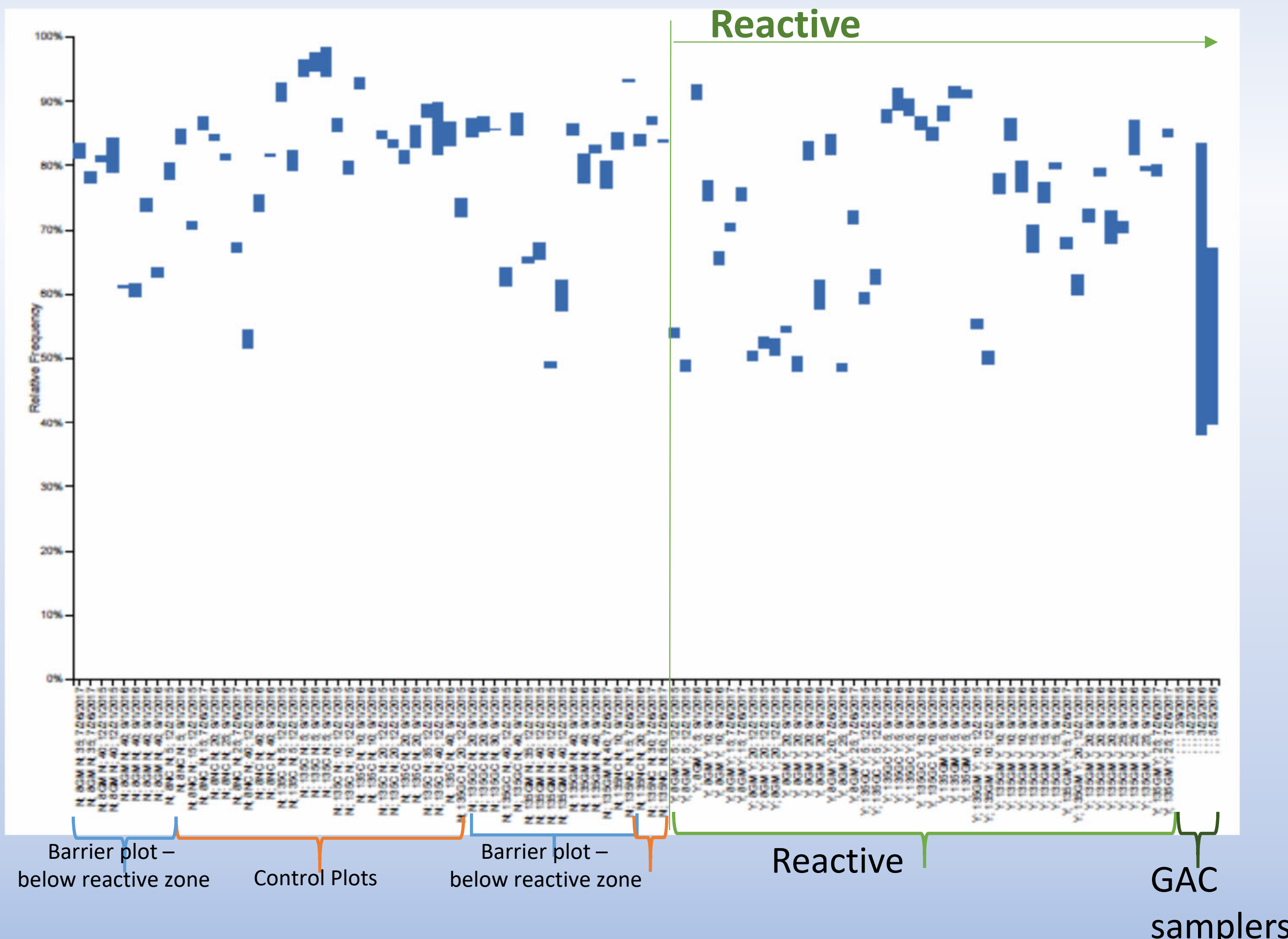


- Relatively low abundance and generally not higher in reactive sediment samples compared to controls.
- *Dehalobacter spp.* and Dehalococcoidia members known to reductively dechlorinate CBs
- Known syntrophic associations between Peptococcaceae members and anaerobic benzene degradation

Peptococcaceae:	**Dehalococcoidia:**
Dehalobacter	Dehalococcoidia (other)
Syntrophobotulus	Unclassified Dehalococcoidia
Desulfosporosinus spp.	Dehalococcoidales
Pelotomaculum spp.	FS117-23B-02
	GIF9

All data in this presentation are provisional.

Sediment Samples Taxonomy– Anaerobes, Desulfuromonadales (sorted by reactive vs unreactive and barrier plot) Percent abundance

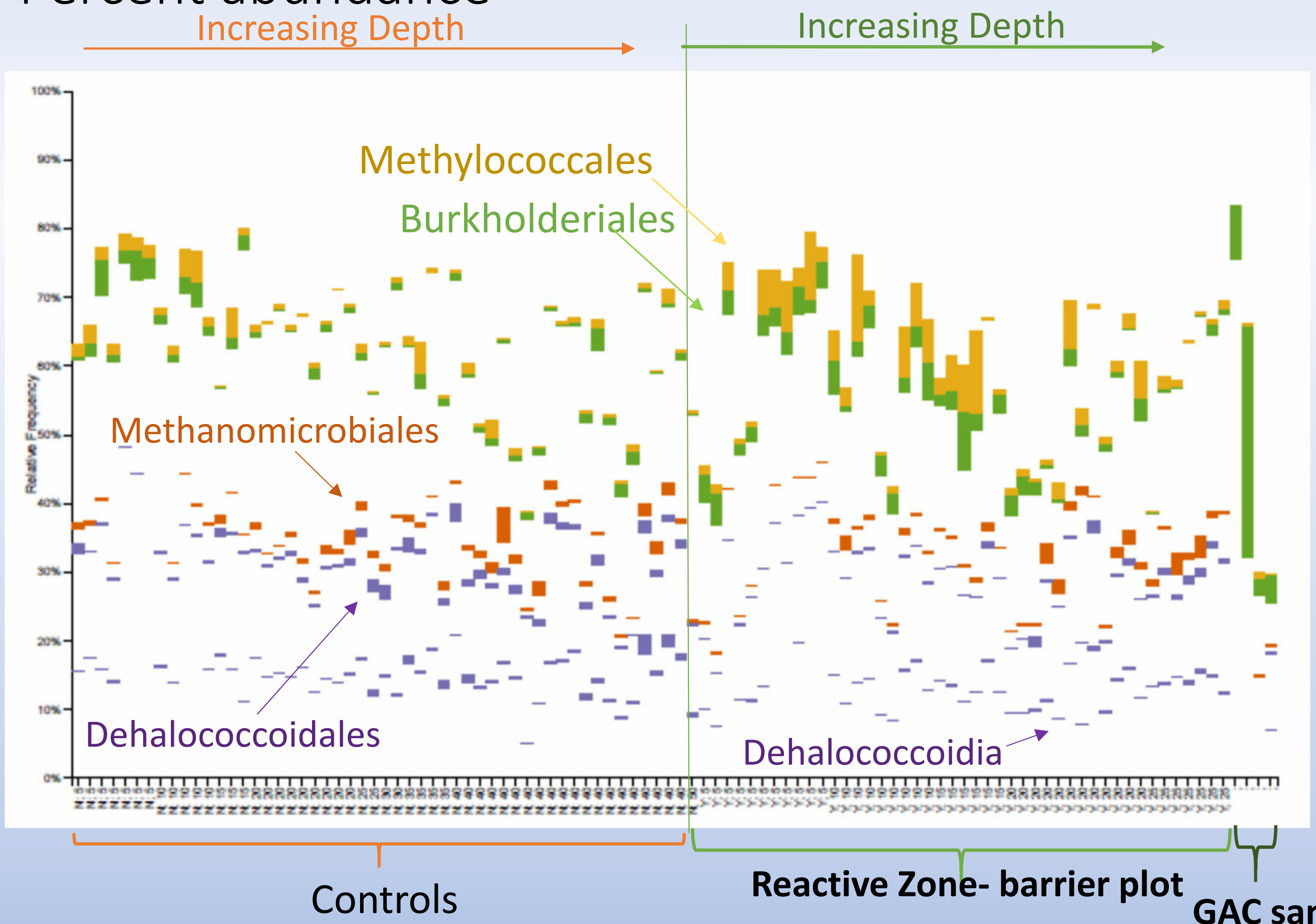


- Generally not higher in reactive sediment samples compared to controls, although high in GAC.
- *Geobacter spp.* known reductive dechlorinators.
- Known syntrophic associations between iron- and sulfate-reducers and anaerobic benzene degradation

<u>Desulfuromonadales:</u>
unclassified Desulfuromonadales
Geobacter spp.
Pelobacteraceae
Desulfuromonadaceae

All data in this presentation are provisional.

Sediment Samples Taxonomy – by Depth for Select Orders (sorted by reactive vs unreactive plot) Percent abundance



- Aerobes decrease in abundance in control plots.
- Aerobes remain relatively abundant at depth in the reactive barriers.
- Methanogens and anaerobic dechlorinators show an increase in abundance with depth in control and reactive barrier plots.

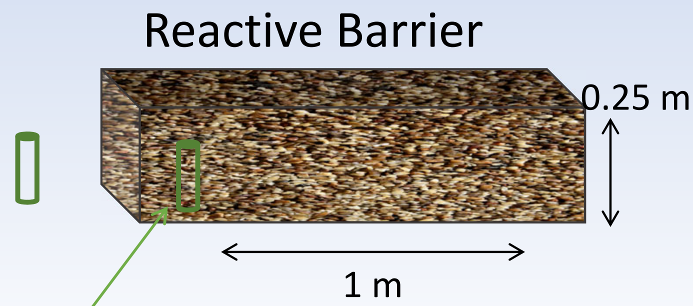
Although some depth stratification is evident, anaerobes and aerobes overlap even in the reactive plots where aerobic groups were greatly increased.

All data in this presentation are provisional.

3 In Situ Microcosms

Bio-Traps (Microbial Insights) used to conduct in situ microcosms, with and without Biosep beads that pre-loaded with ^{13}C-labeled monochlorobenzene.

- Concurrent microbial and isotopic data to verify biodegradation activity.
- Measure incorporation of ^{13}C in CO2 and PLFA.
- Analysis of functional genes to relate microbial presence to degradation ability.

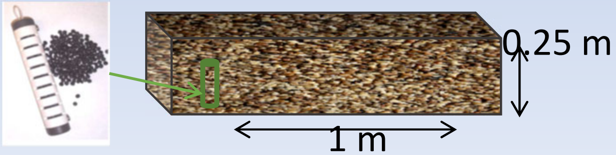


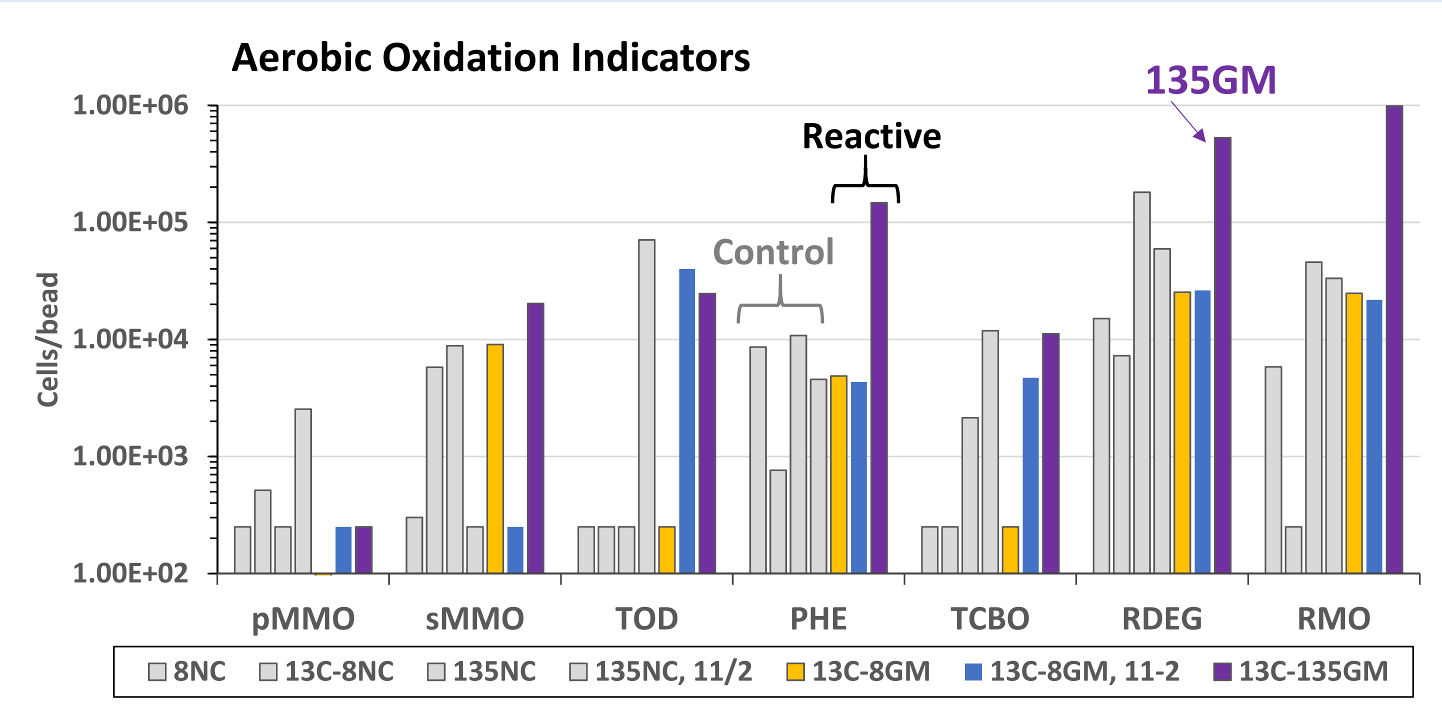
Installed at 10-20 cm bls

- inside and outside reactive barrier plots
- similar depth-integrated microbial sample as the GAC samplers
- time-integrated over 50-day incubation.

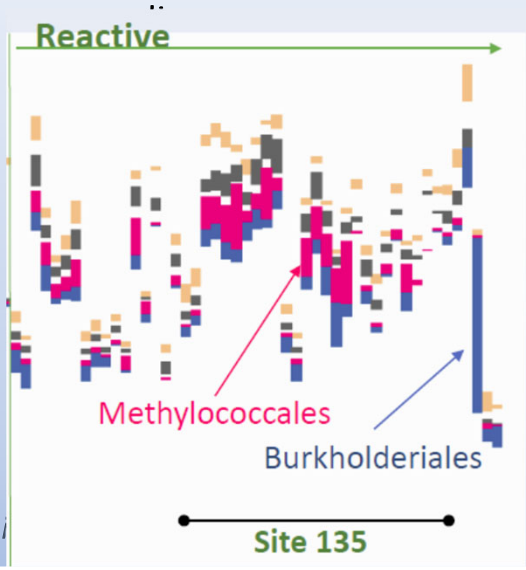
3. Is biodegradation in the reactive barriers enhanced compared to the control sediment areas, and does aerobic and anaerobic biodegradation co-occur?

In Situ Microcosms (QuantArray Analysis)





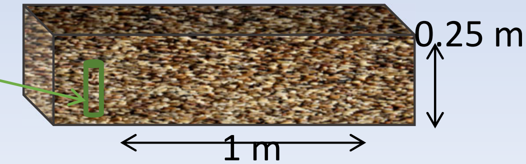
pMMO	particulate methane monooxygenase
sMMO	soluble methane monooxygenase
TOD	toluene dioxygenase
PHE	phenol hydroxylase; benzene monooxygenase
TCBO	trichlorobenzene

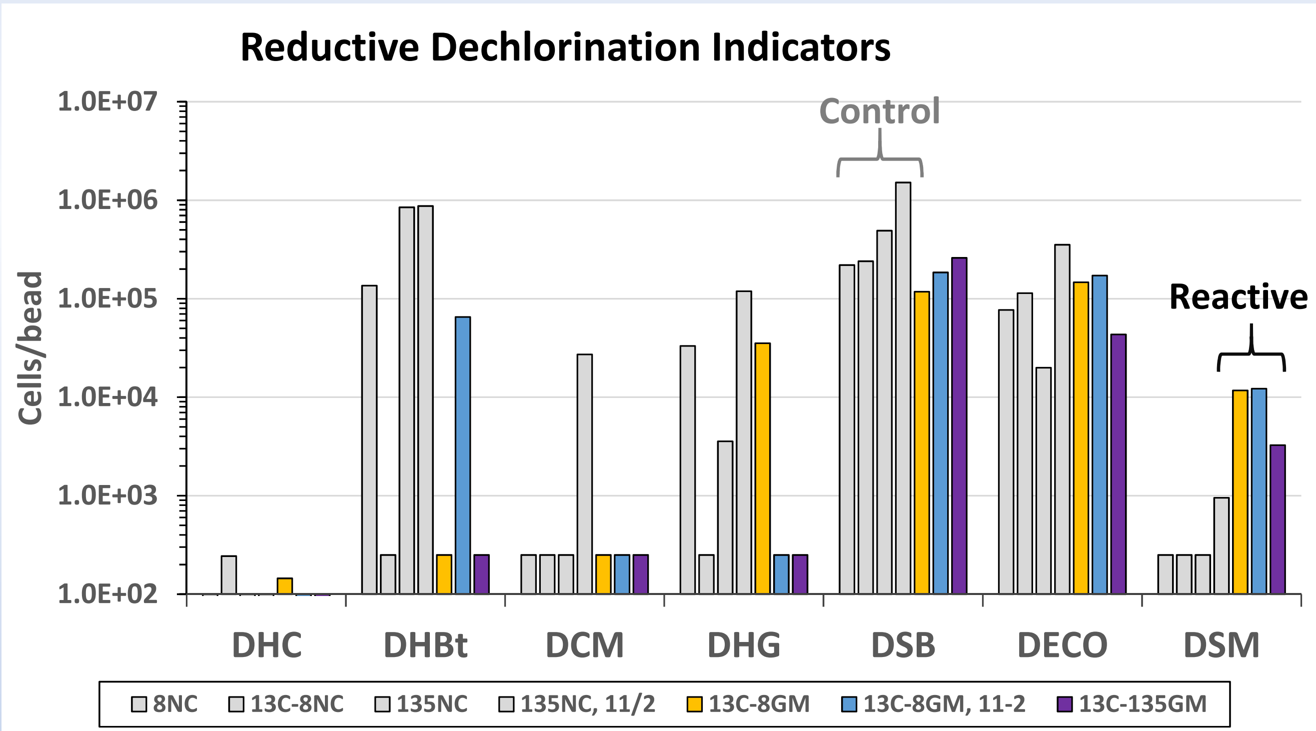


- Aerobic oxidation indicators higher in reactive barrier at site 135 compared to site 8 reactive barrier or controls.
- However, indicators of both aerobic oxidation activity and anaerobic reduction present in all ISMs.

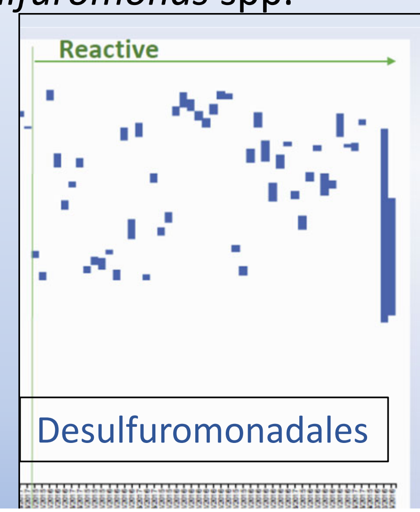
All data i

In Situ Microcosms (QuantArray Analysis)





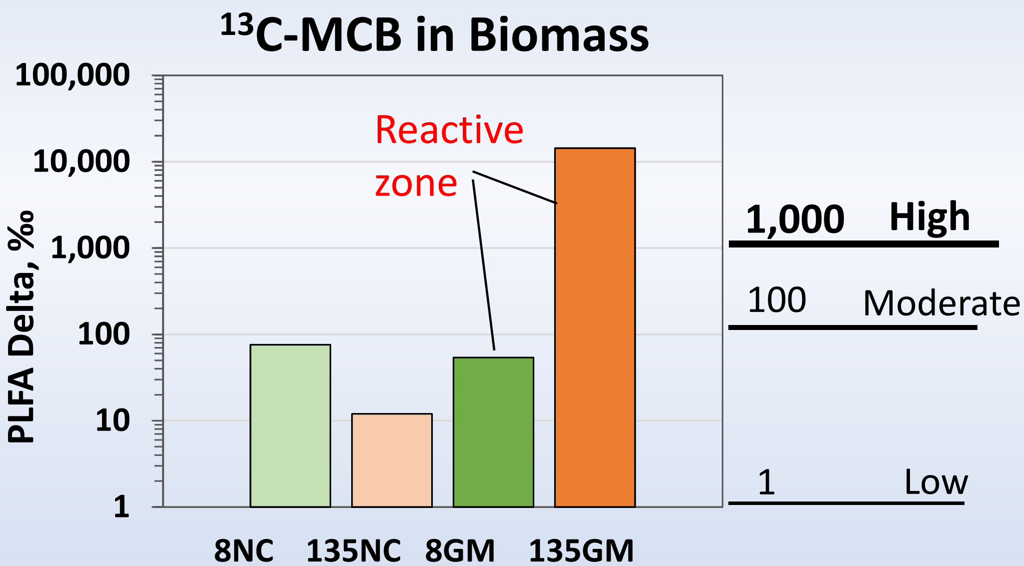
DHC ***Dehalococcoides***
DHBt ***Dehalobacter* spp.**
DCM *Dehalobacter* DCM
DHG *Dehalogenimonas* spp.
DSB *Desulfitobacterium* spp.
DECO ***Dehalobium chlorocoercia***
DSM *Desulfuromonas* spp.

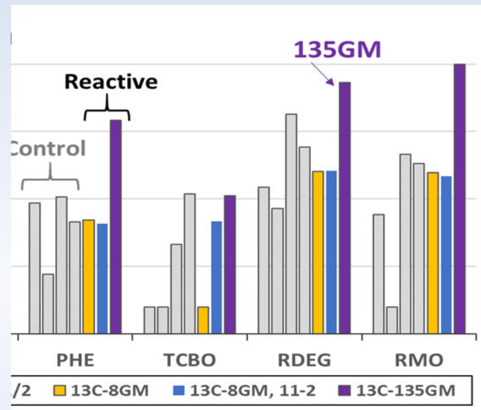


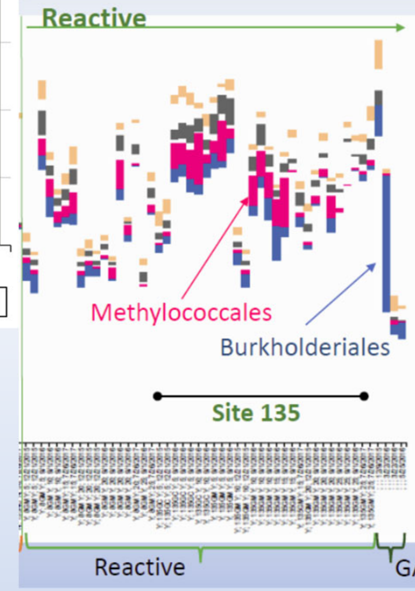
DSM was the only reductive dechlorination indicator that was consistently higher in the reactive barrier plots than controls.

All data in this presentation are provisional.

^{13}C-Monochlorobenzene in Biomass in Bio-Traps







- **High ^{13}C uptake in biomass** (PLFA) in the reactive barrier at site 135 indicates high aerobic oxidation of MCB.
- Agrees with the observed higher abundance of aerobic oxidizers and functional genes at site 135 compared to site 8.

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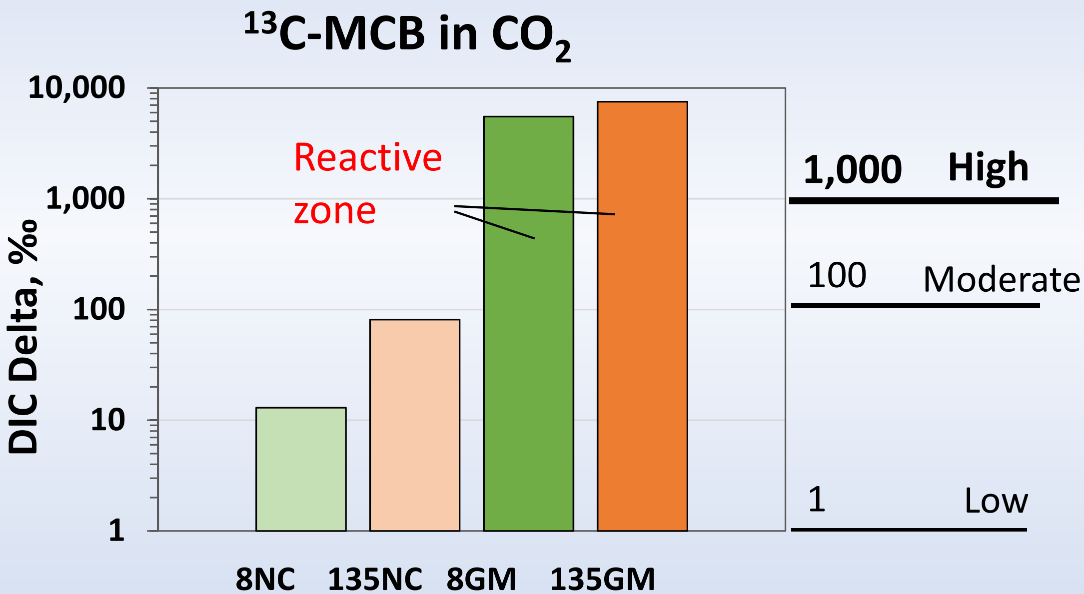
^{13}C-Monochlorobenzene in Bio-Traps



- Incorporation of ^{13}C in CO_2 was **high in both reactive barriers** and low in the controls, verifying complete enhanced biodegradation in the reactive barriers.
- Complete degradation to CO_2 is ~ equal in the two reactive barriers, despite the lower use of MCB as growth substrate at site 8. Indicates a combination of anaerobic (^{13}C for energy) and aerobic biodegradation processes in the reactive barrier.

All data in this presentation are provisional.

Conclusions

- Even with the large difference in sample type, indicator microbial groups of aerobic and anaerobic biodegradation could be seen in the reactive barrier sediment samples that corresponded to the GAC samples (field and lab).
- Differences in the microbial communities and in situ microcosm results show that biodegradation was enhanced in the reactive barrier plots.
- Overlap of anaerobes and aerobes and the ^{13}C results from in situ microcosms indicate concurrent aerobic and anaerobic biodegradation of chlorobenzenes.

Questions?