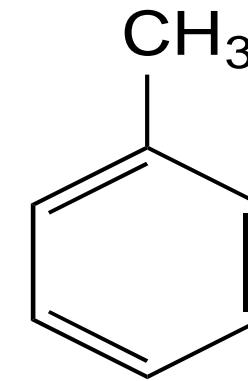
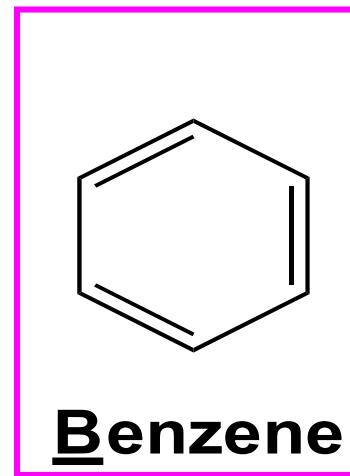


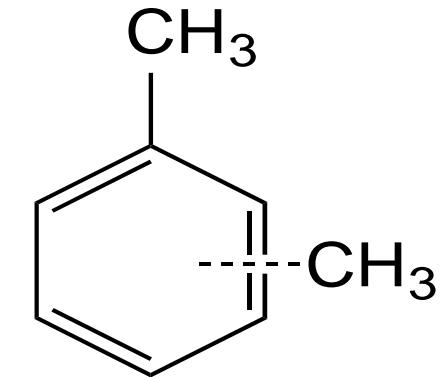
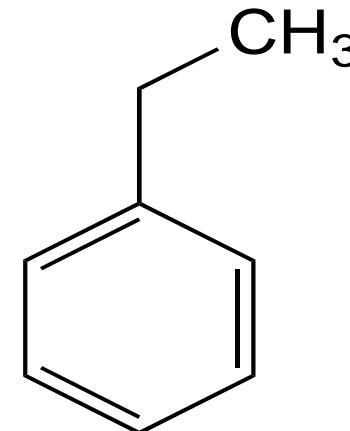
# **Monitoring and Enhancing Anaerobic Benzene Biodegradation in Groundwater Systems**

Fei Luo, Shen Guo, Nancy Bawa, Jennifer Webb, Sandra Dworatzek,  
Trevor Carlson, Kris Bradshaw, and Elizabeth Edwards

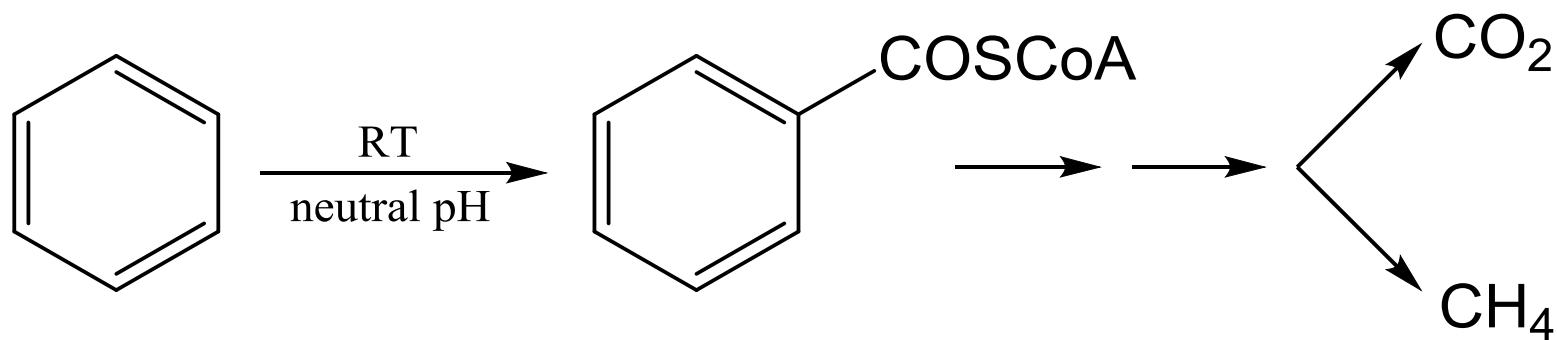
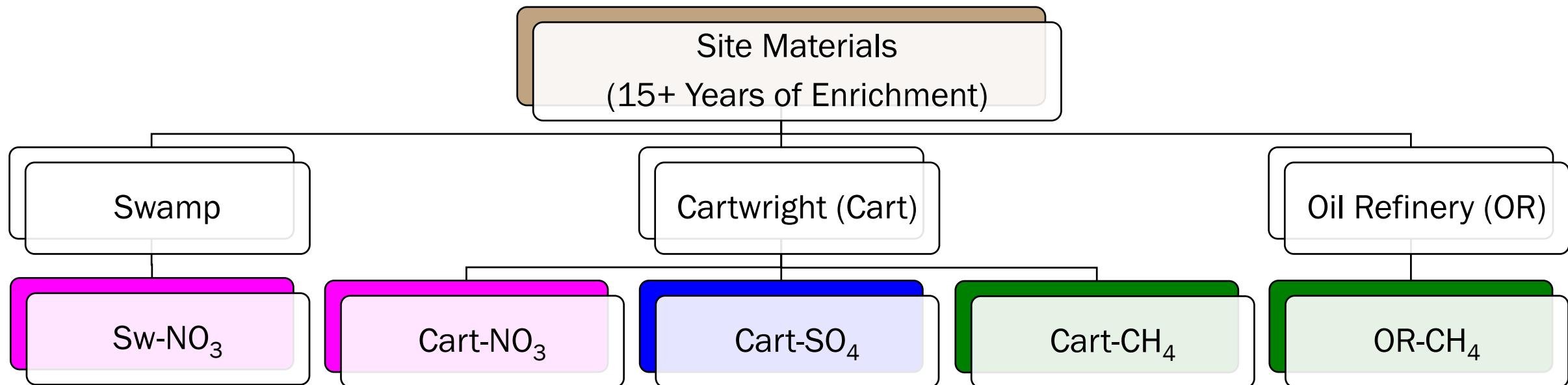
# Petroleum-Derived Aromatic Hydrocarbons



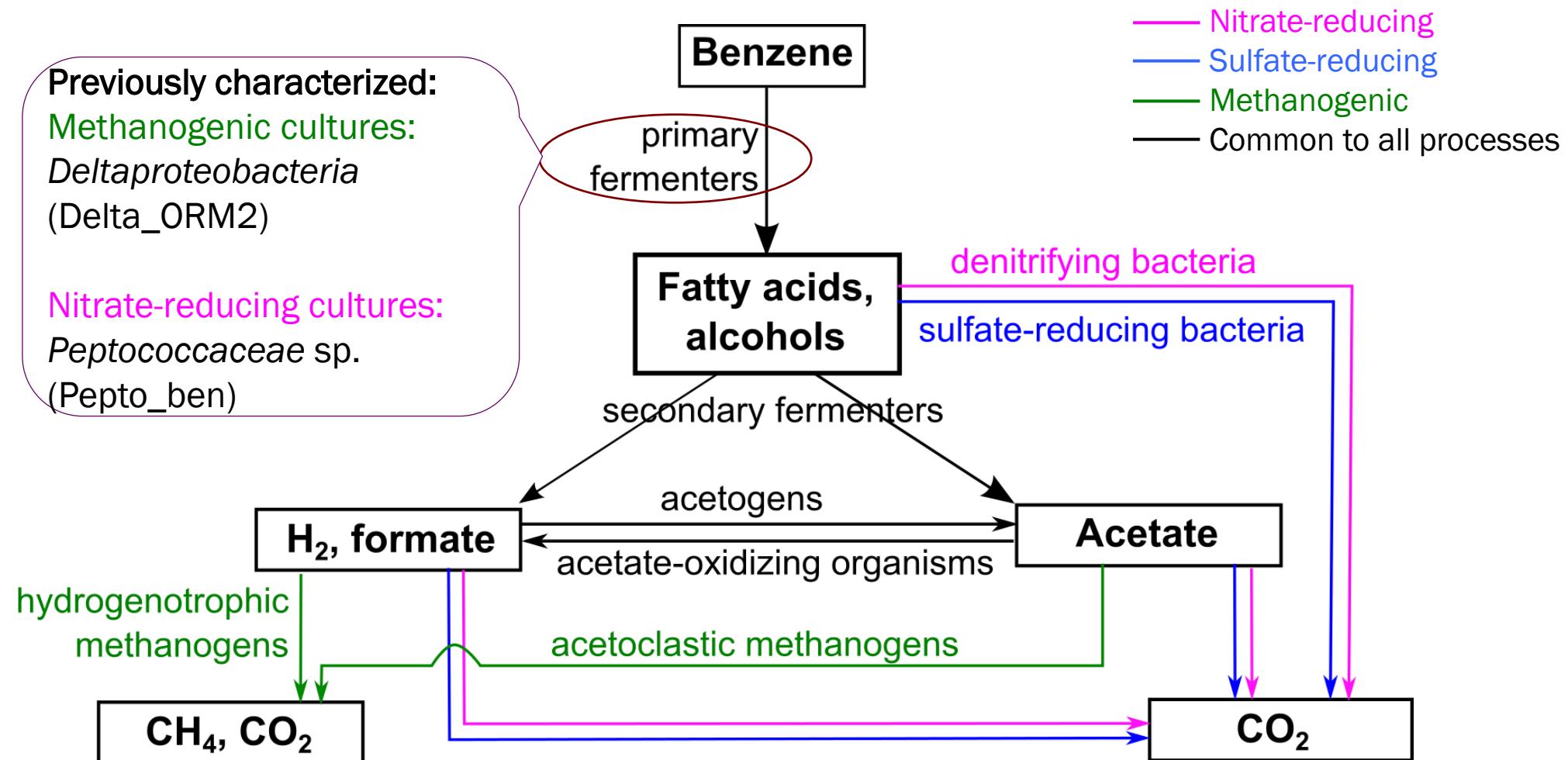
- Prevalent
- Hazardous to human health
- Relatively soluble
- Carcinogenic
- Persistent anaerobically due to the unsubstituted aromatic structure



# Edwards Lab Enrichment Cultures



# The Key Organisms and Their Syntrophic Partners

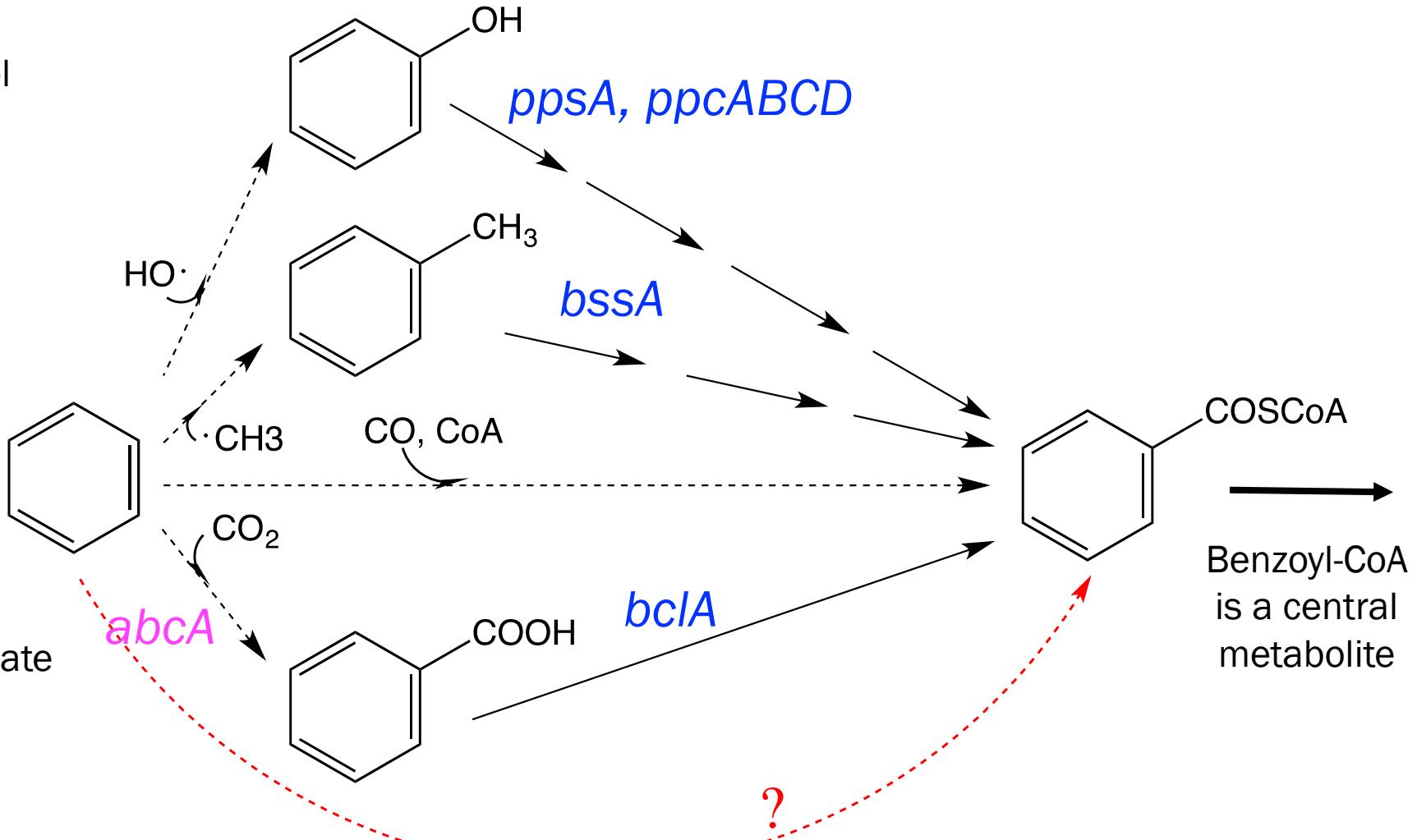


# Are ORM2 and *Pepto\_Ben* Found in Other Habitats?

Species	Habitat	Identity	Gene accession
<i>Delta_ORM2</i>	Methanogenic benzene-degrading culture, Japan	99%	AB291810
	Tar oil contaminant plume, toluene degrader	99%	EU266873
	Benzene-contaminated groundwater	98%	AY214193
	Terrestrial mud volcano	97%	JQ245639
	Sediments of lakes Cadagno and Rotsee	96%	FR729621
	Honghu Lake sediments	94%	HM243896
<i>Pepto_ben</i>	Iron-reducing benzene-degrading enrichment culture, Germany	97%	EU016442
	Nitrate-reducing benzene-degrading chemostat	97%	JN366441
	Hydrocarbon contaminated sites	97%	KJ955656
	Naphthalene-degrading culture	96%	JF820825
	Hydrocarbon contaminated aquifer	97%	JQ087045
	Wetland soil	97%	HE804426
	Rice paddy soil	97%	AB486907
	*Hydrocarbon-related sites		

# In Search of Elusive Anaerobic Benzene Activation Mechanisms

Hydroxylation to phenol



Methylation to toluene

Carbonylation to benzaldehyde

Carboxylation to benzoate

Others????

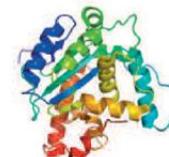
# GAPP Project: Scaling Up Anaerobic Benzene-Degrading Cultures and *in situ* Bioaugmentation



Culture  
Enrichment &  
Characterization



Contaminated Sites



**Biomarkers**  
3 qPCR assays developed



Field  
Injection



Treatability Testing



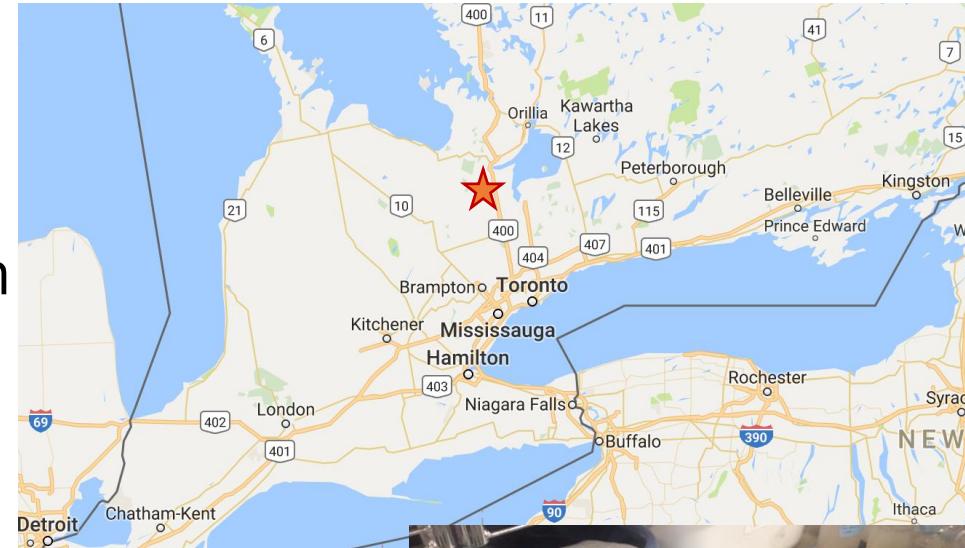
Culture Scale Up



Prof. Dunja Grbic-Galic

# Proof of Concept: The First Treatability Study

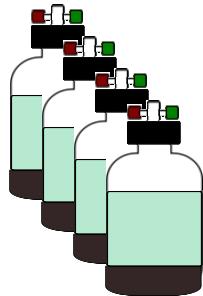
- Site:
  - Previous military facility
  - Sandy aquifer
  - Previously artificially contaminated with petroleum
  - Biostimulation trial with sulfate was successful
  - Gene *abcA* was transiently observed.



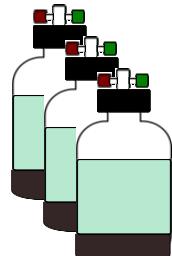
# Setup of Treatability Study

## Group 1: benzene only

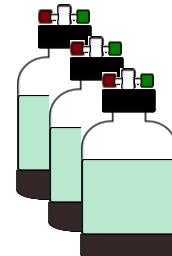
Sterile control



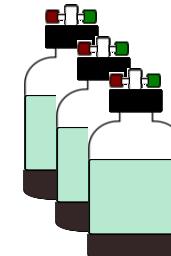
Active control



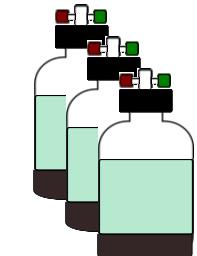
$\text{SO}_4^{2-}$ -amended



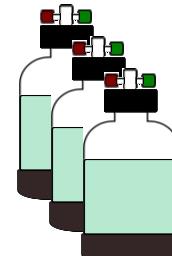
$\text{NO}_3^-$ -amended



Bioaugmented



Bioaugmented +  $\text{SO}_4^{2-}$



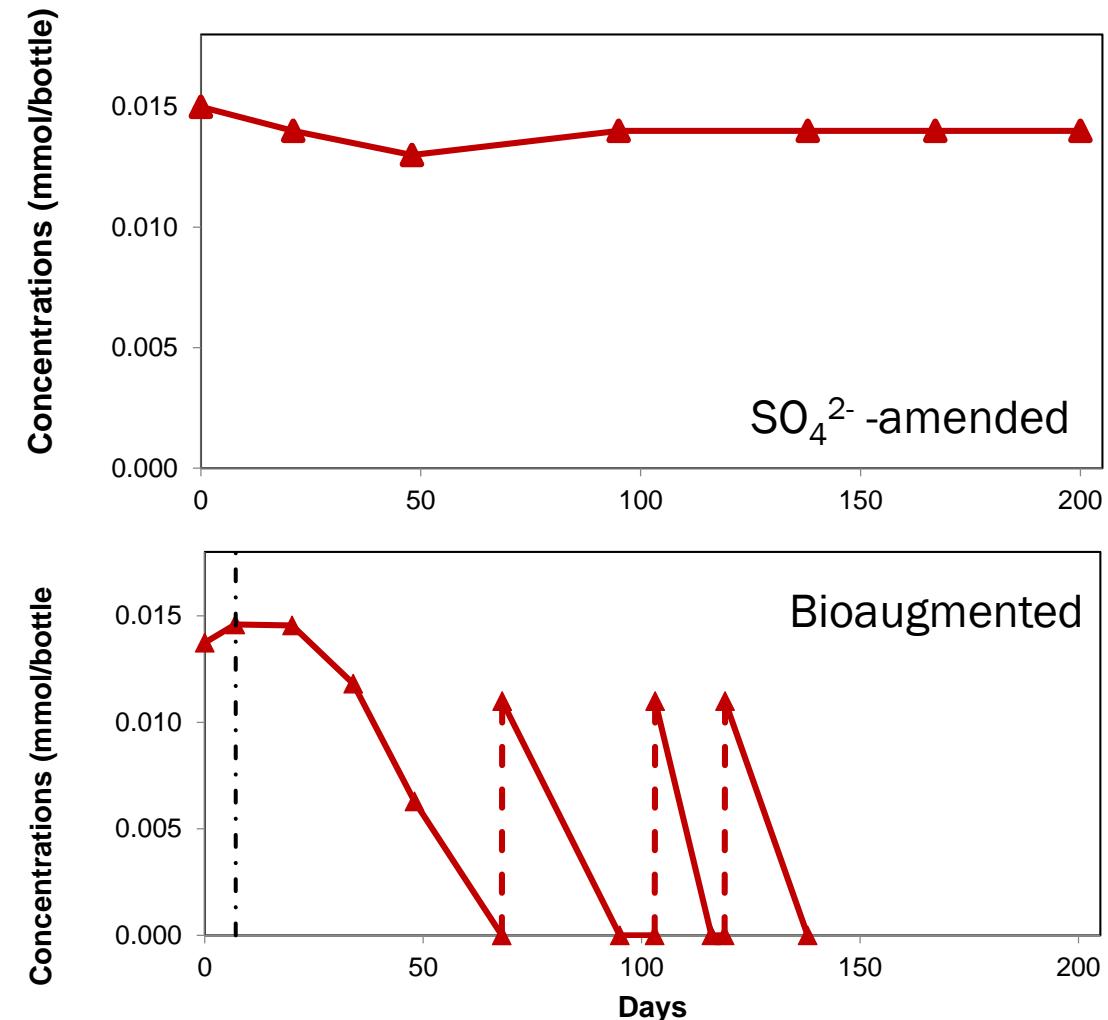
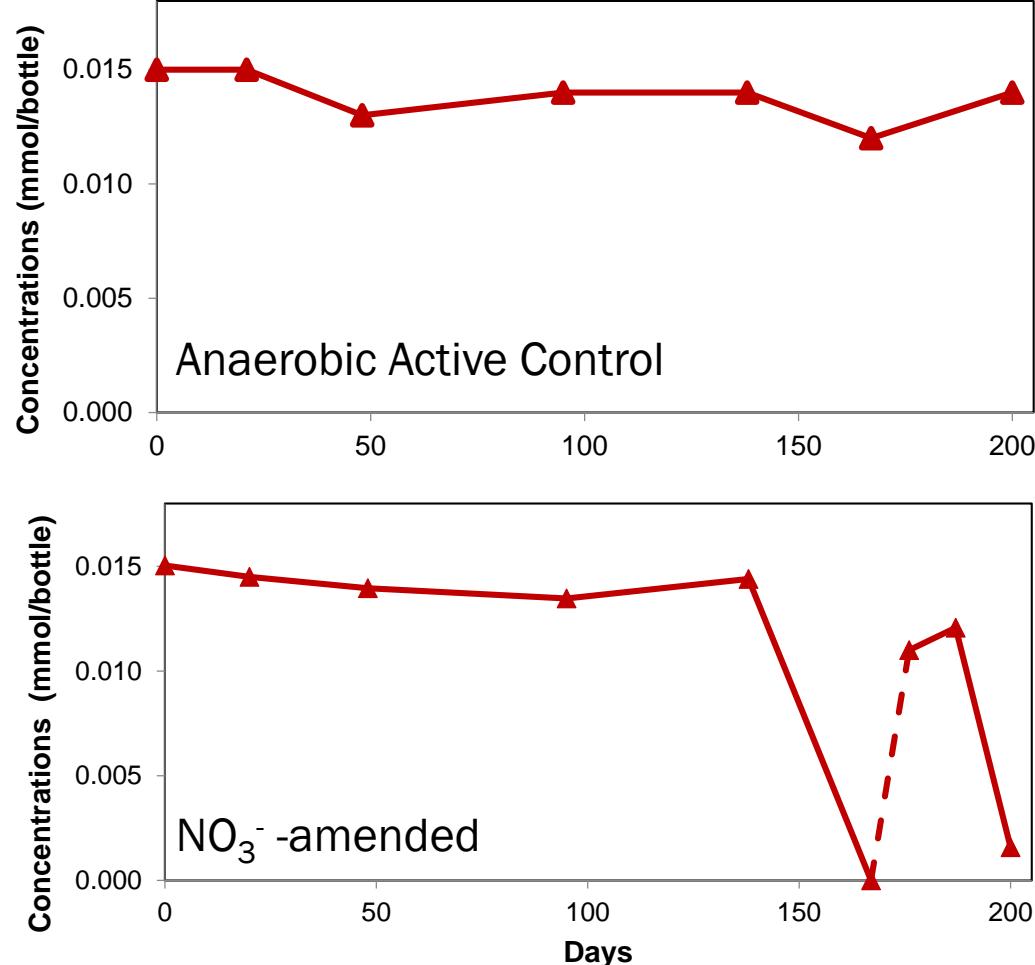
GC-FID: BTEX and  
methane

IC: inorganic ions

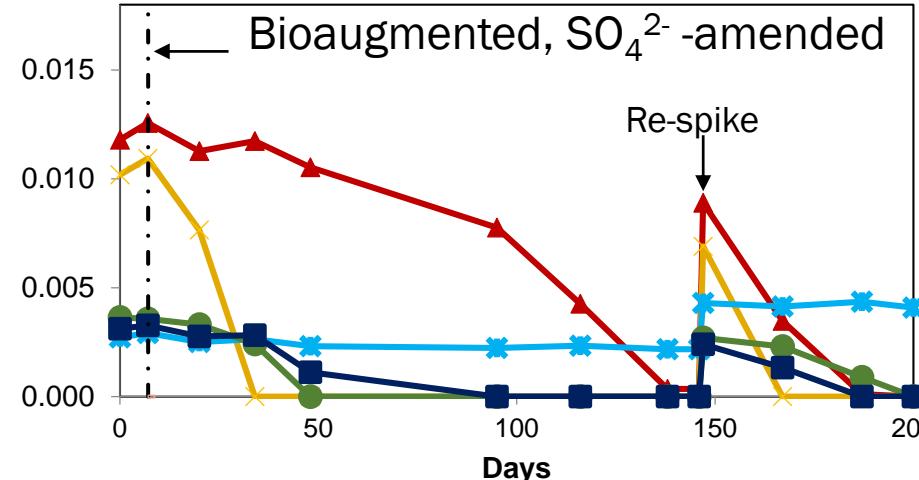
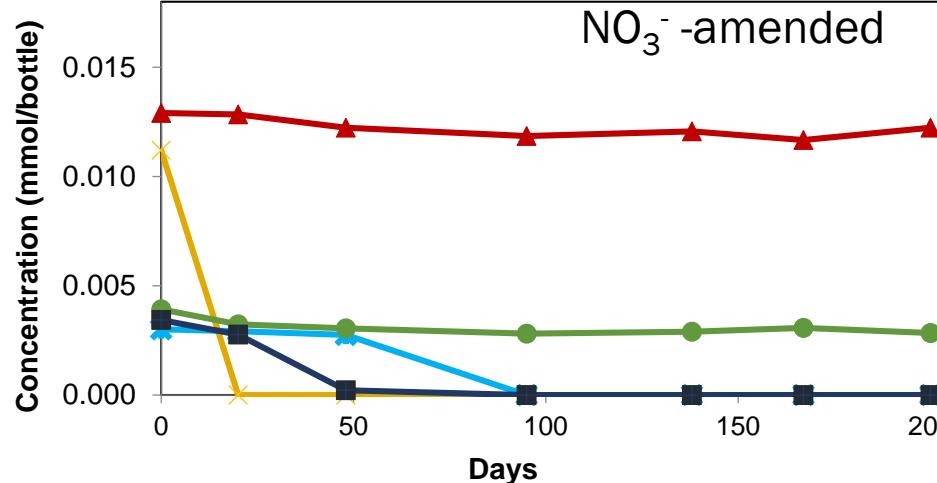
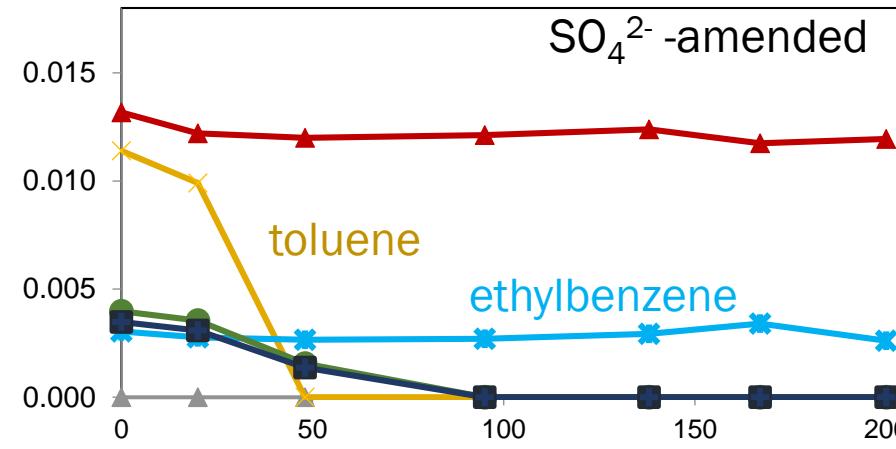
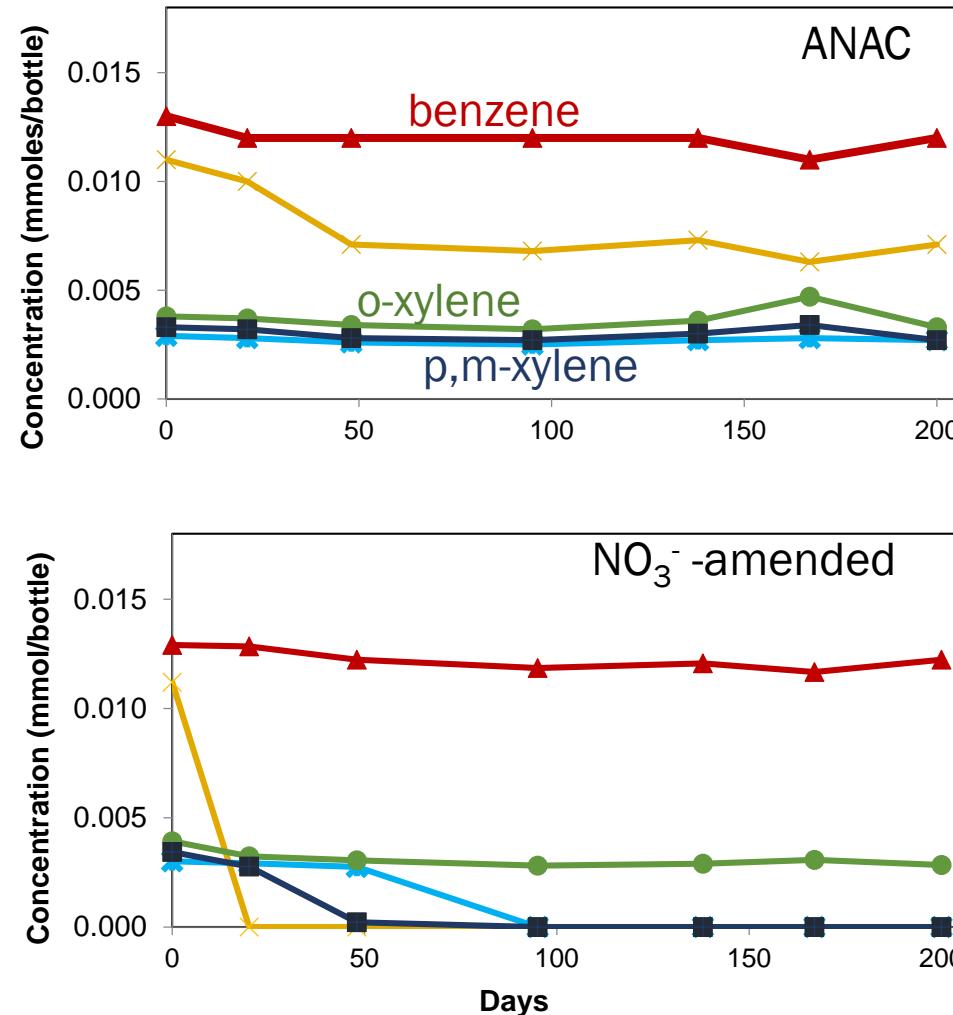
HPLC: naphthalene

qPCR: microbial  
populations

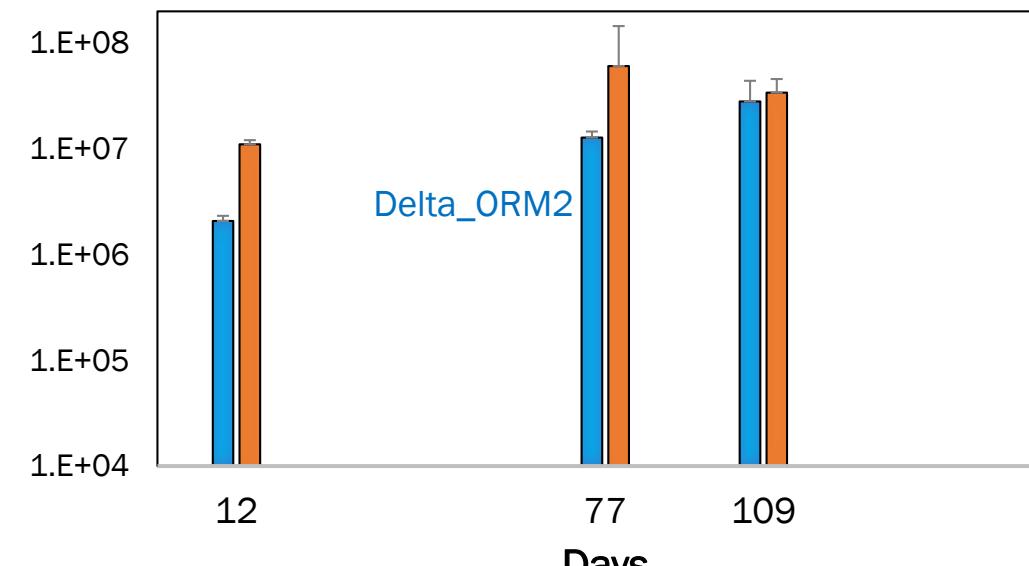
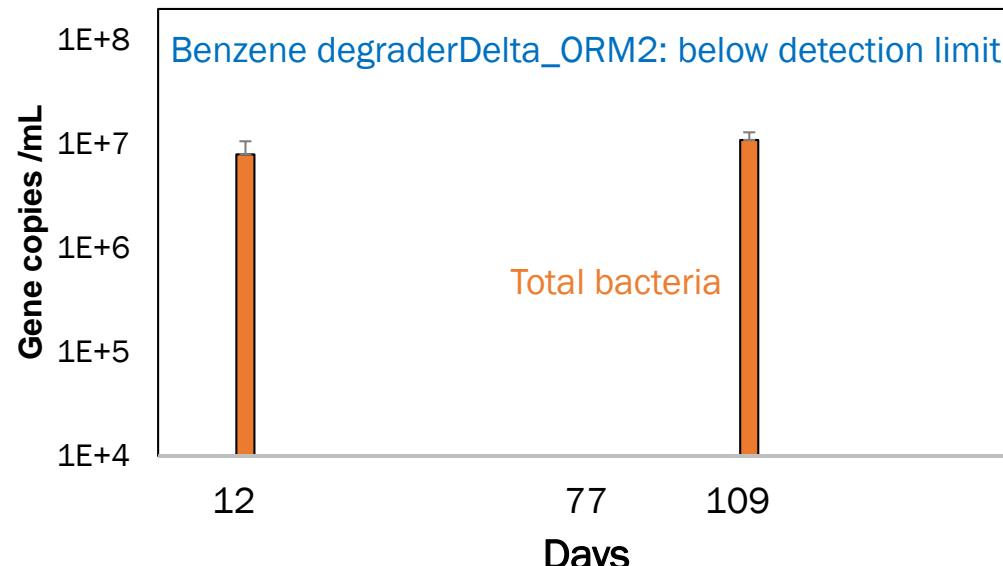
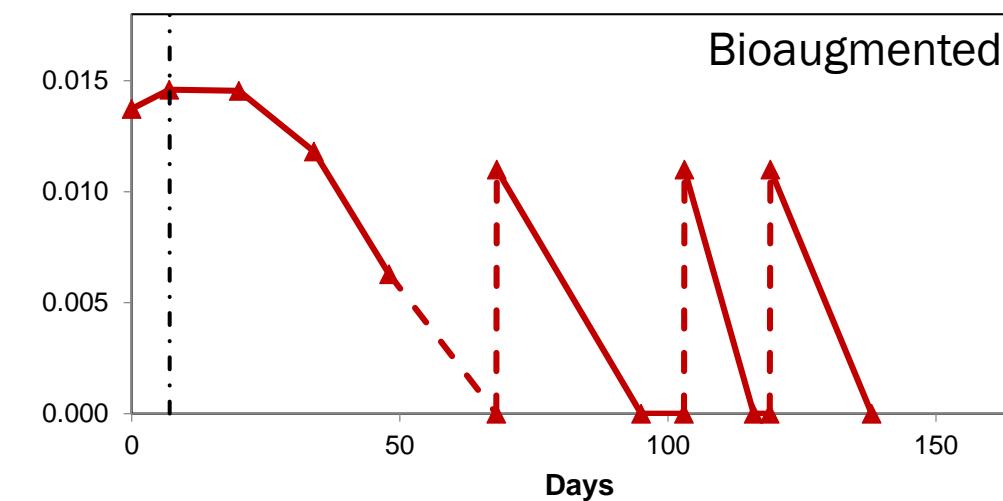
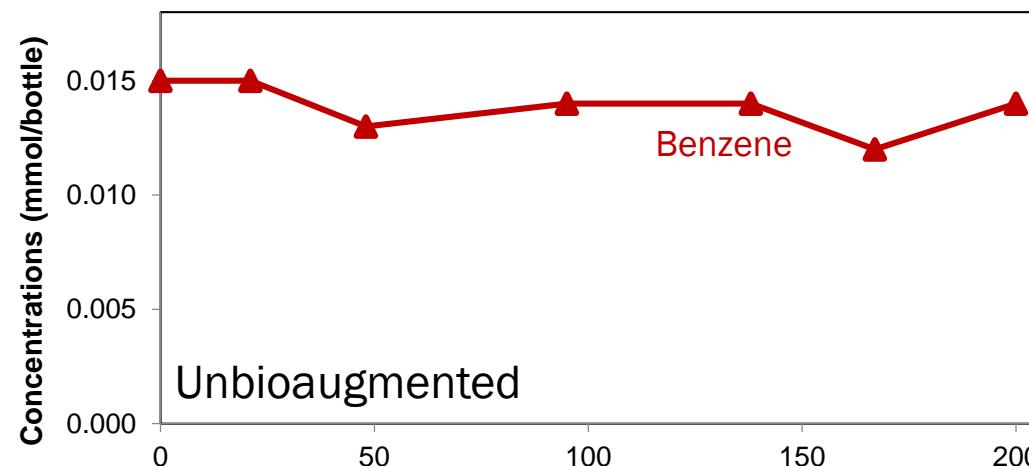
# Anaerobic Benzene Degradation: Benzene As the Only Contaminant



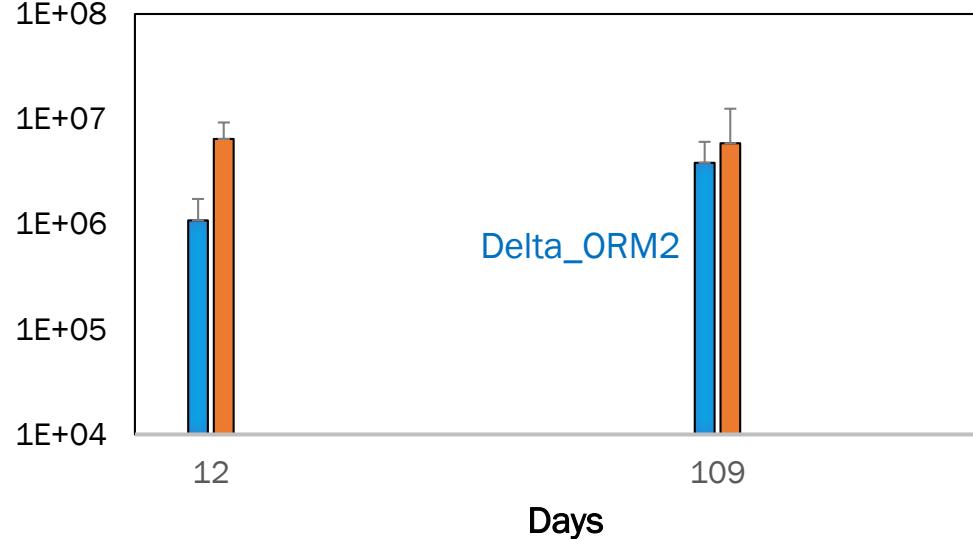
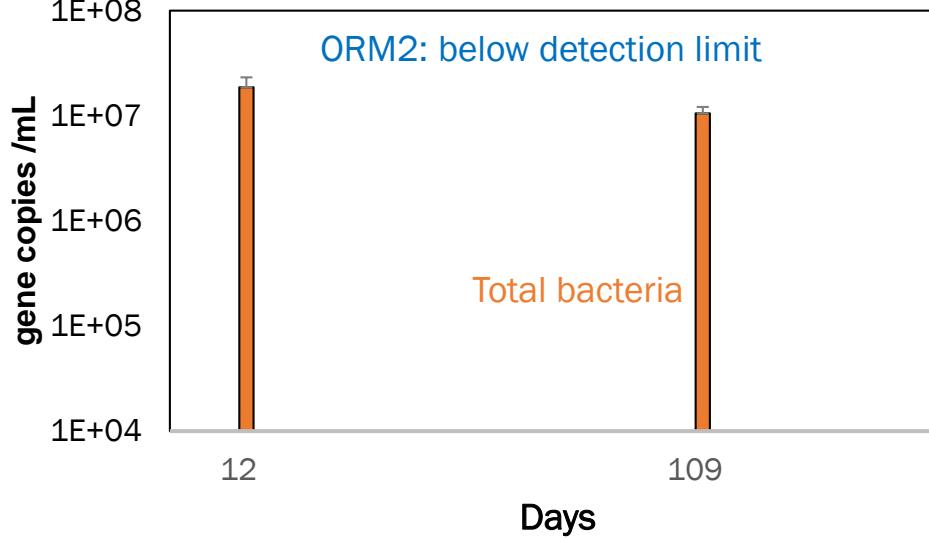
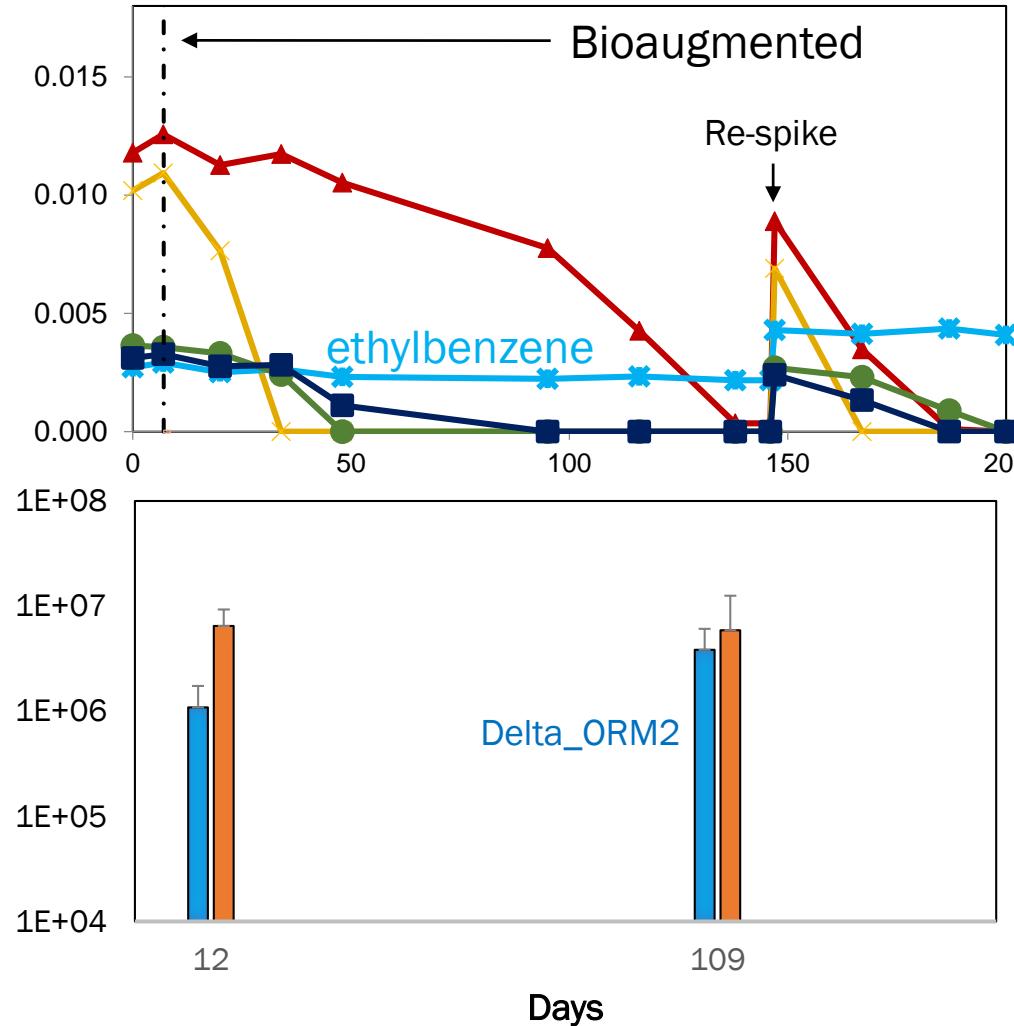
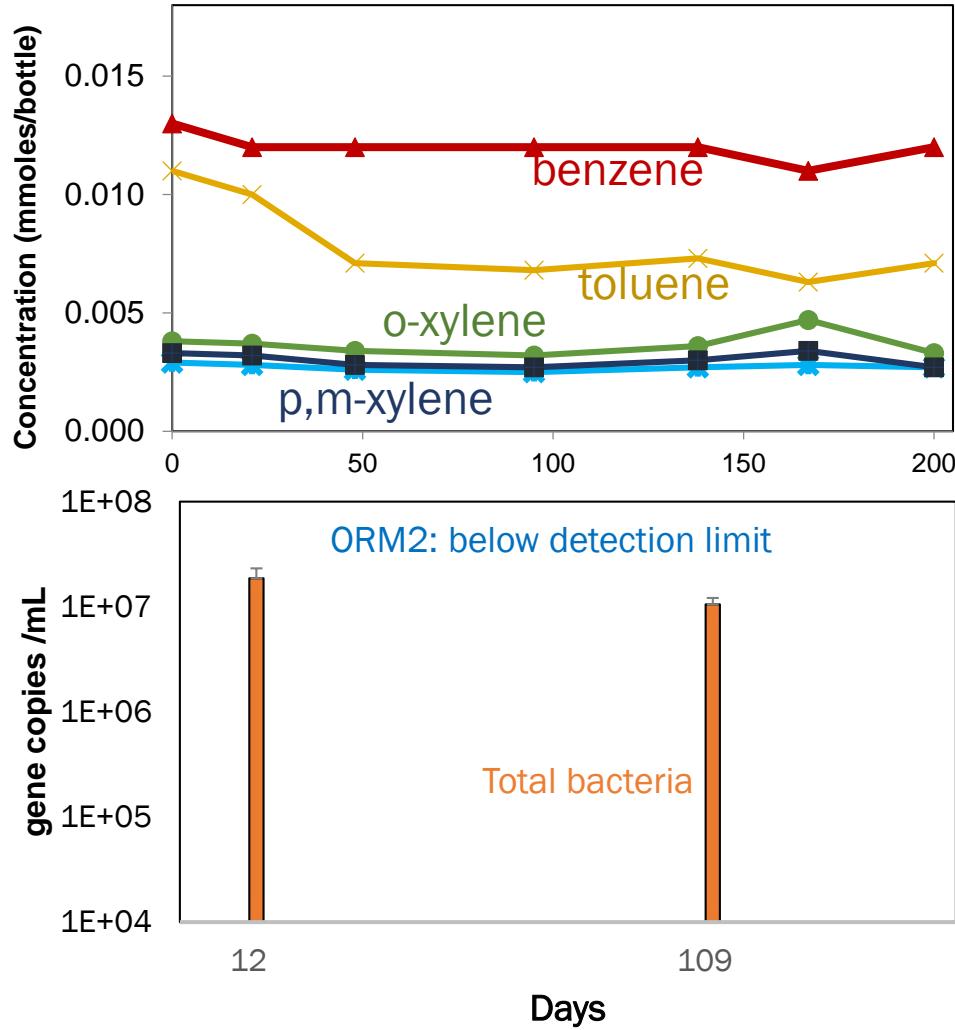
# Anaerobic Benzene Degradation: BTEX+Naphthalene



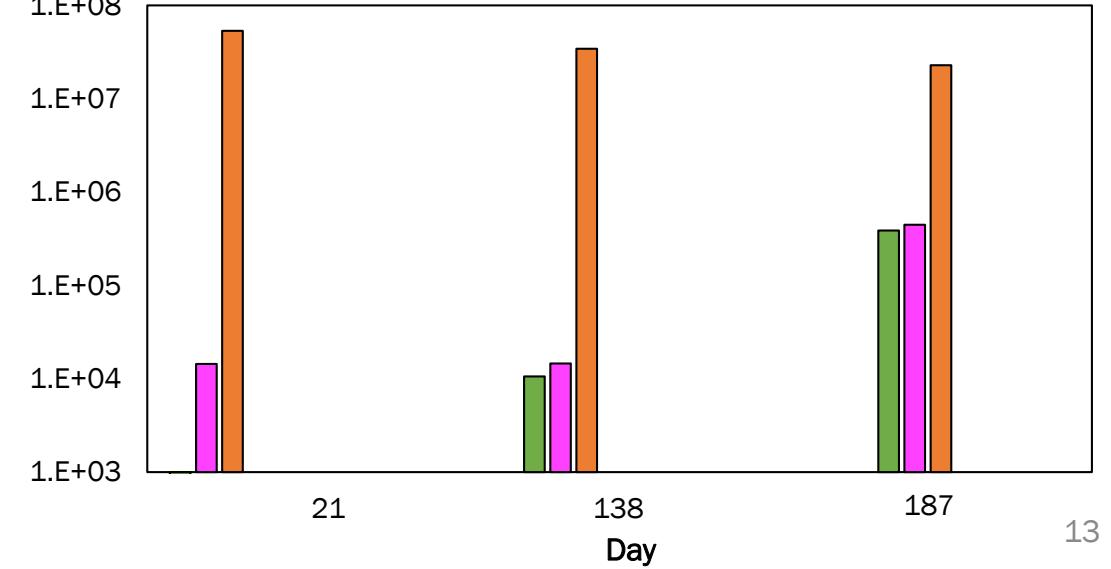
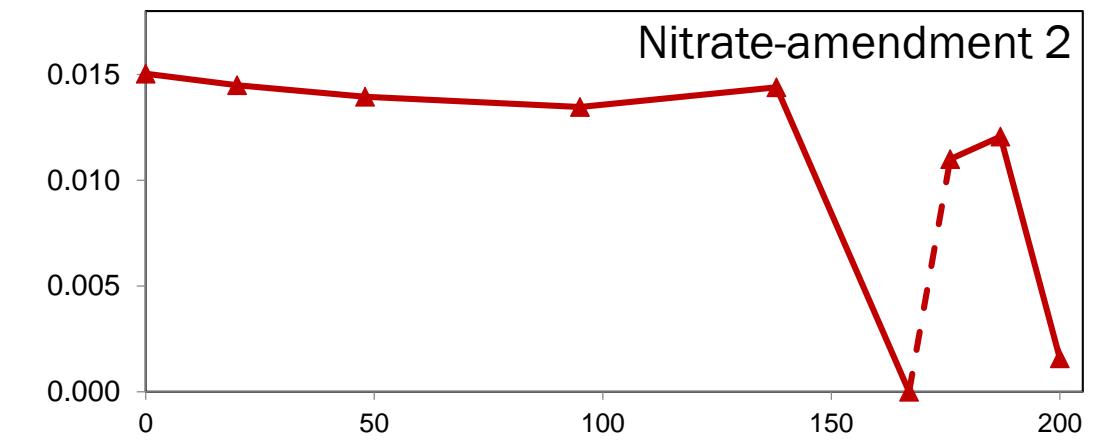
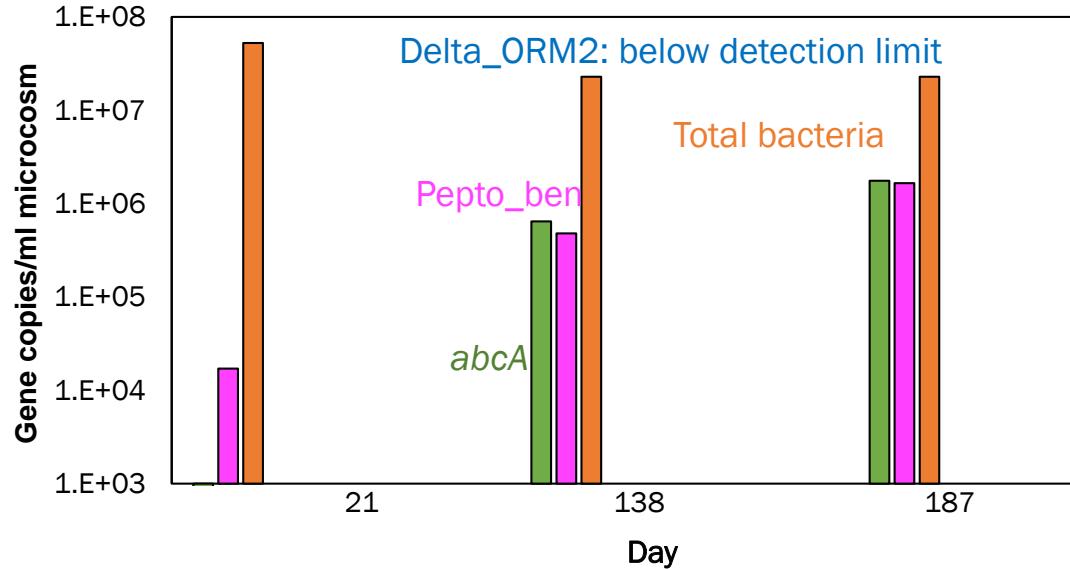
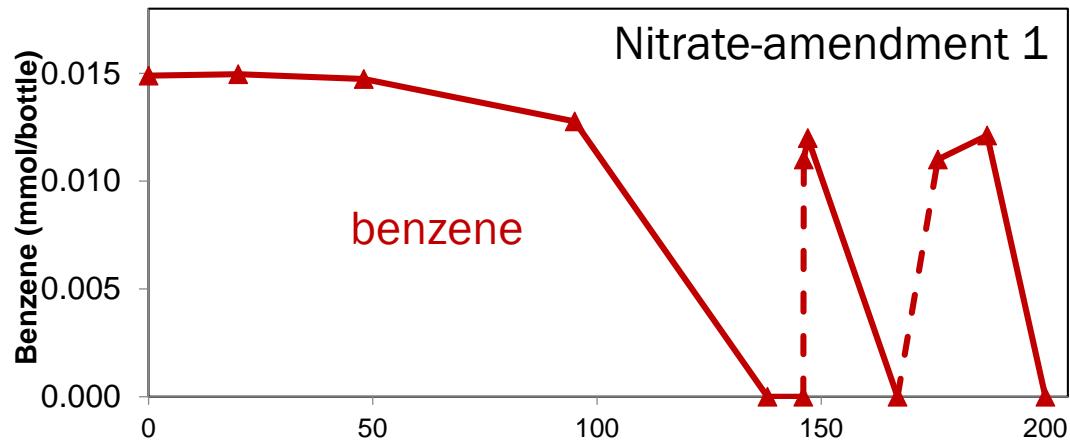
# Tracking the Growth of Benzene Degraders with qPCR Reactions: Benzene Only



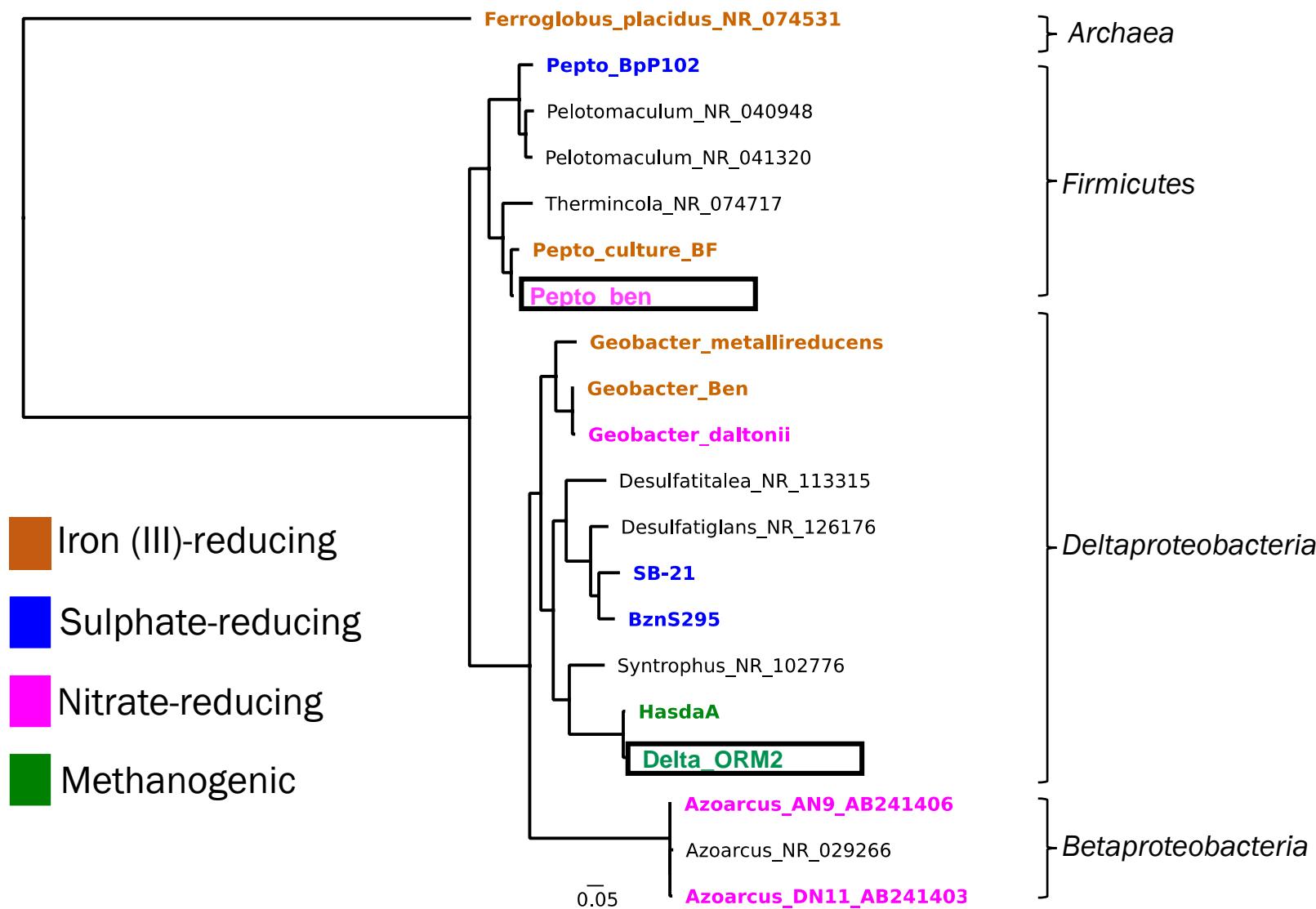
# Tracking the Growth of Benzene Degraders with qPCR Reactions: BTEX+Naphthalene



# Biostimulation: Tracking the Emergence of Indigenous Benzene Degrader



# The Indigenous Anaerobic Benzene Degraders



# qPCR VS DNA Amplicon Sequencing



qPCR



16S rRNA gene amplicon sequencing

## Pro:

- Accurate absolute quantification
- Potential low detection limit from groundwater

## Cons:

- Low multiplexing capacity.
- Time and reagent consuming when there are many targets.

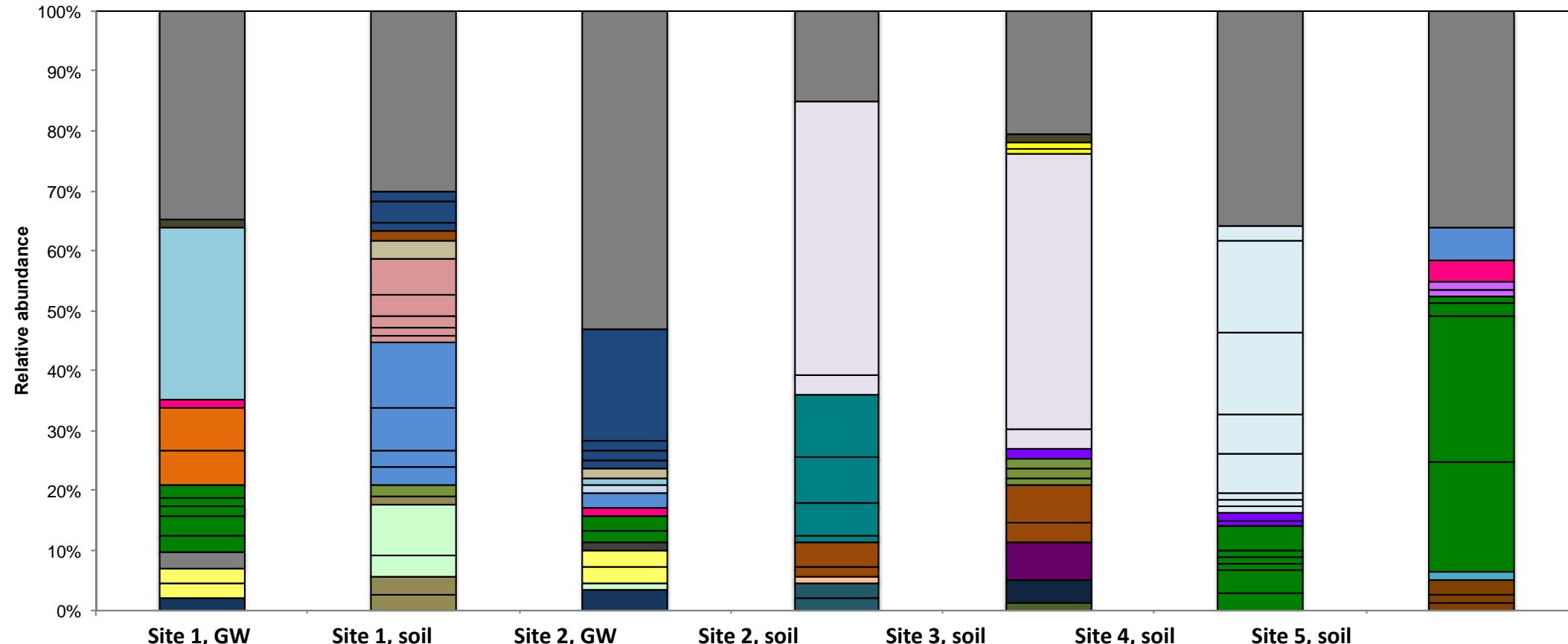
## Pro:

- Processing many samples at the same time (~180 samples per run)
- Obtain information about the entire microbial community

## Cons:

- Not very sensitive if the targeted organisms have low relative abundance;
- No detection for functional genes;

# Great Microbial Diversity in Environmental Samples



Methanoregula	Propionibacterium	OPB41	OPB41	Gaiellaceae	Paludibacter	Anaerolinaceae	Anaerolinaceae	Vagococcus
Vagococcus	Sedimentibacter	Pelotomaculum	Pelotomaculum	OPB92	WCHB1-07	Caulobacteraceae	Caulobacteraceae	Burkholderiales
Comamonadaceae	Comamonadaceae	Comamonadaceae	Comamonadaceae	Comamonadaceae	Comamonadaceae	Comamonadaceae	Comamonadaceae	Caulobacteraceae
Polaromonas	Polaromonas	Roseateles	Roseateles	Oxalobacteraceae	Oxalobacteraceae	Oxalobacteraceae	Oxalobacteraceae	Deltia
Ralstonia	Ralstonia	Thiobacillus	Thiobacillus	Rhodocyclaceae	Rhodocyclaceae	Rhodocyclaceae	Rhodocyclaceae	Ralstonia
Desulfobulbaceae	Desulfobulbaceae	Geobacter	Geobacter	Azoarcus	Desulfobulbaceae	Desulfobulbaceae	Desulfobulbaceae	Desulfobulbaceae
Enterobacteriaceae	Enterobacteriaceae	Pseudomonas	Pseudomonas	Desulfobulbaceae	Syntrophaceae	Sulfuricurvum	Sulfurimonas	Desulfobulbaceae
Spirochaetes	Treponema	Treponema	TM7	Pseudomonas	Pseudomonas	Pseudomonas	Pseudomonas	Pseudomonas
Enterobacteriophage	Enterobacteriophage	Betaproteobacteria	Minor	WS6	WS6	WS6	WS6	WS6
								WS6

# Example of Low Abundance OTUs from the Environmental Samples

---

	Pepto_ben		Delta_ORM2	
	# Reads	%	# Reads	%
Site1_groundwater	0	0.000%	1	0.003%
Site1_soil	1	0.002%	1	0.002%
Site2_groundwater	2	0.005%	3	0.007%
Site2_soil	0	0.000%	0	0.000%
Site3_soil	0	0.000%	1	0.002%
Site4_soil	0	0.000%	1	0.003%
Site5_soil	4	0.008%	1	0.002%

# Take Home Messages

- Anaerobic benzene-degrading organisms appear to be limited to specialized groups
- Bioaugmentation may therefore be a feasible approach to speed up in situ remediation of benzene
- qPCR method can be used to track the growth of microorganisms during bioaugmentation and possibly also during MNA (monitored natural attenuation)
- DNA amplicon sequencing and qPCR are complementary methods for detection of hydrocarbon degraders from environmental samples - amplicon sequencing does not provide absolute abundances that correlate more closely with rate.

# Future Work

- More qPCR analysis to establish the correspondence between the onset of benzene biodegradation and microbial abundance
- Analyzing DNA amplicon sequencing results more closely to identify if low number of reads can be used as evidence of hydrocarbon degraders
- Developing new diagnostic tools for more accurate detection of molecular biomarkers indicative of anaerobic hydrocarbon biodegradation

# Acknowledgements

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