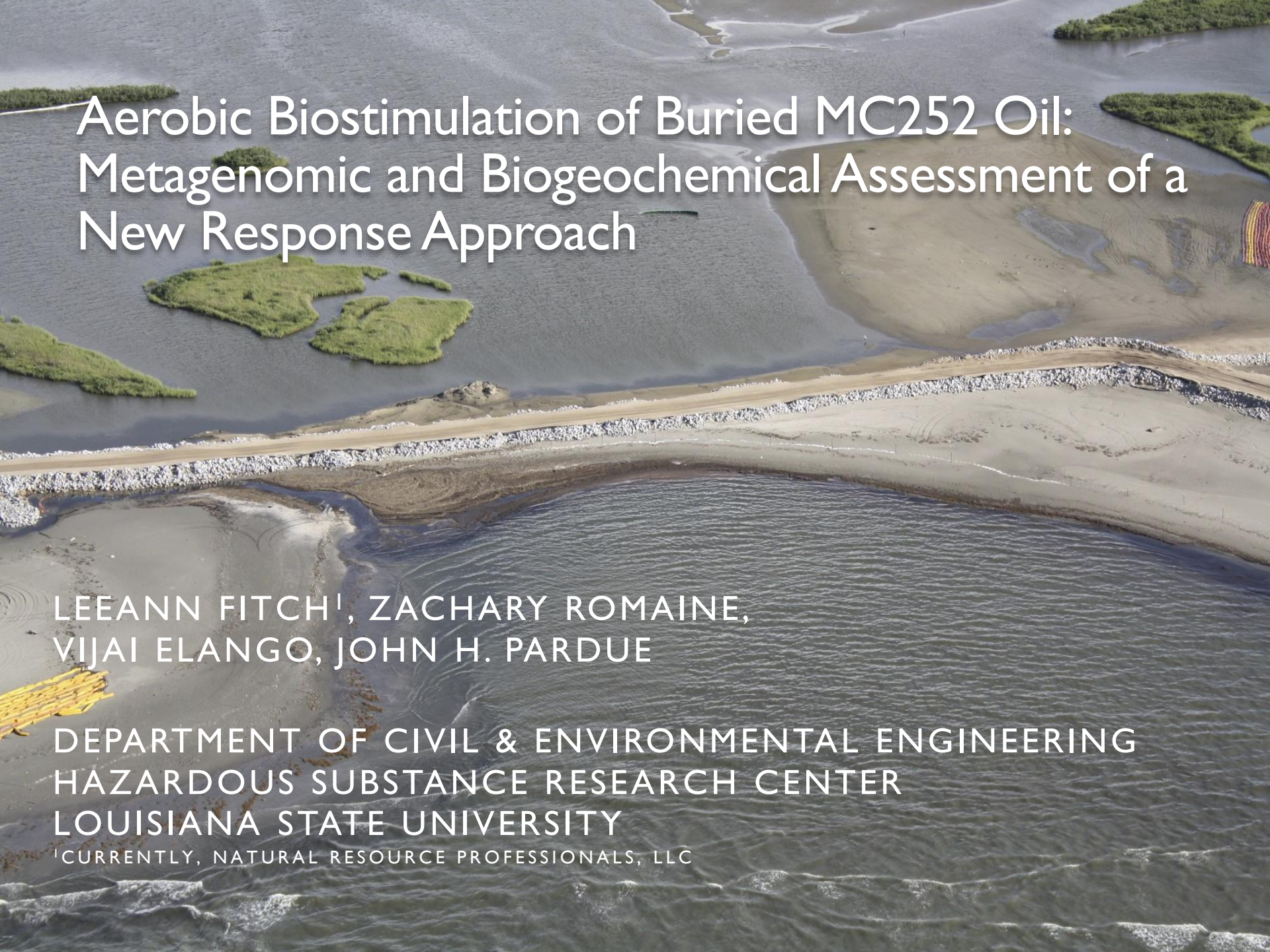

# Aerobic Biostimulation of Buried MC252 Oil: Metagenomic and Biogeochemical Assessment of a New Response Approach


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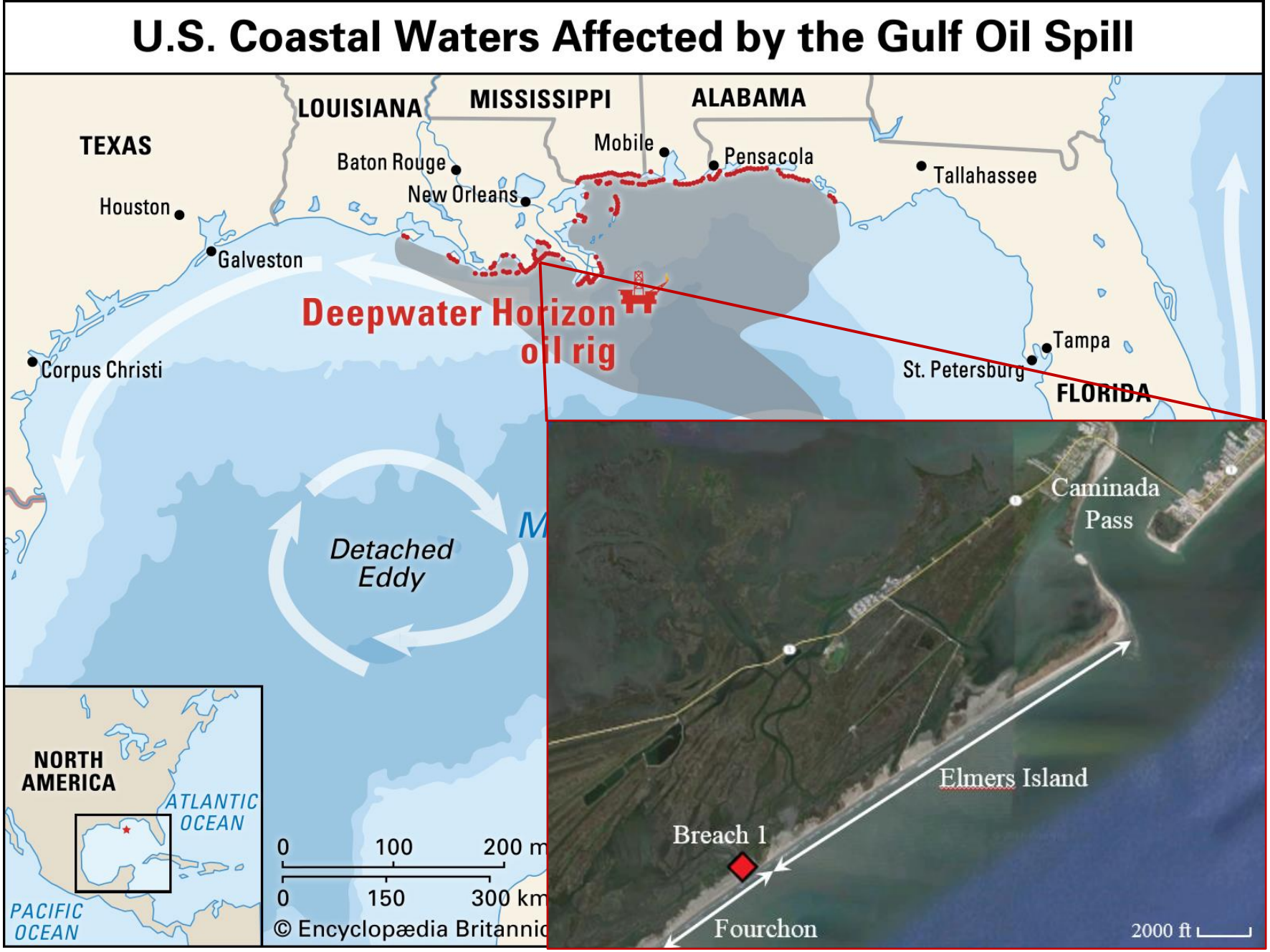

U.S. Coastal Waters Affected by the Gulf Oil Spill
TEXAS
LOUISIANA
MISSISSIPPI
ALABAMA
Houston
Galveston
Baton Rouge
New Orleans
Mobile
Pensacola
Tallahassee
Tampa
St. Petersburg
FLORIDA
Corpus Christi
Deepwater Horizon oil rig
Detached Eddy
NORTH AMERICA
ATLANTIC OCEAN
PACIFIC OCEAN
0 100 200 m
0 150 300 km
© Encyclopædia Britannic
Caminada Pass
Elmers Island
Breach 1
Fourchon
2000 ft


Breach 1: July 2010


Breach 1: November 2012
N
500 ft
Google earth


Google earth
N
500 ft

# Study Goal and Objectives

**Goal:** Enhance biodegradation of buried MC252 oil by *in situ* aerobic biostimulation in a hypersaline, anoxic beach environment.

**Objective 1:** Investigate efficacy of oxygen delivery methods

**Objective 2:** Relate biogeochemical indicators of crude oil mineralization with presence of oxygen

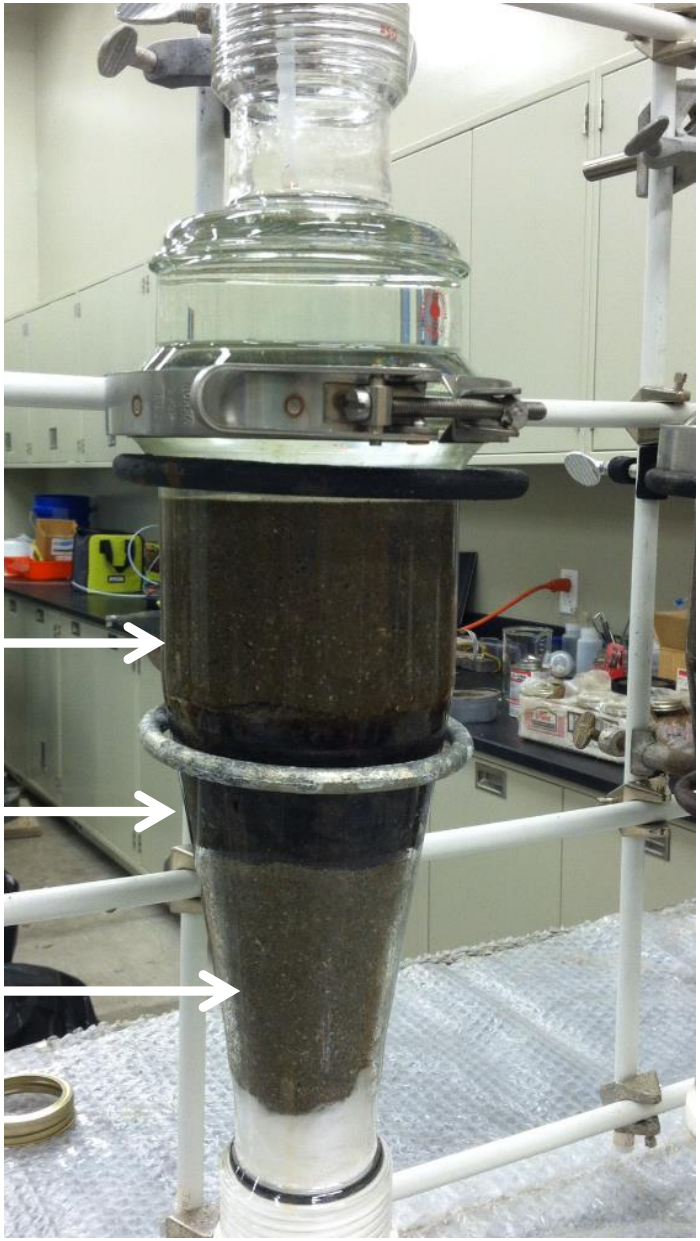
**Objective 3**: Relate changes in crude oil chemistry with the presence of oxygen

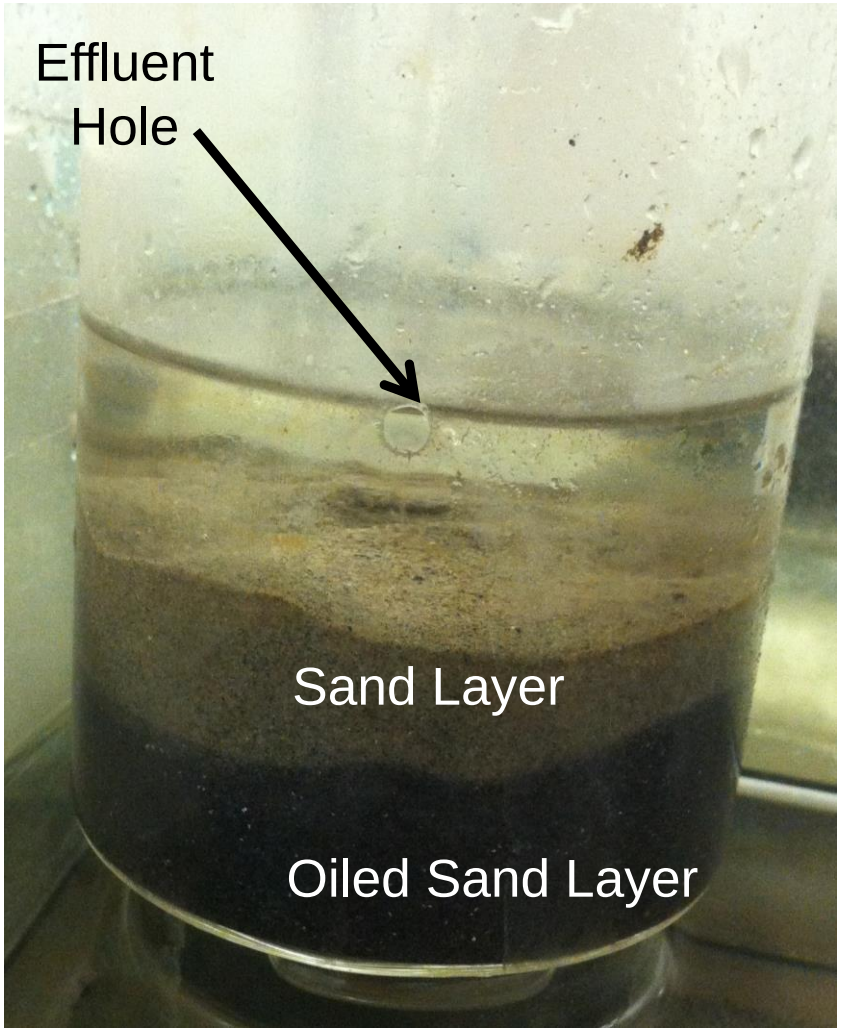
**Objective 4:** Relate changes in microbial community structure with the presence of oxygen

Upper Sand Layer

Oiled Sand Layer

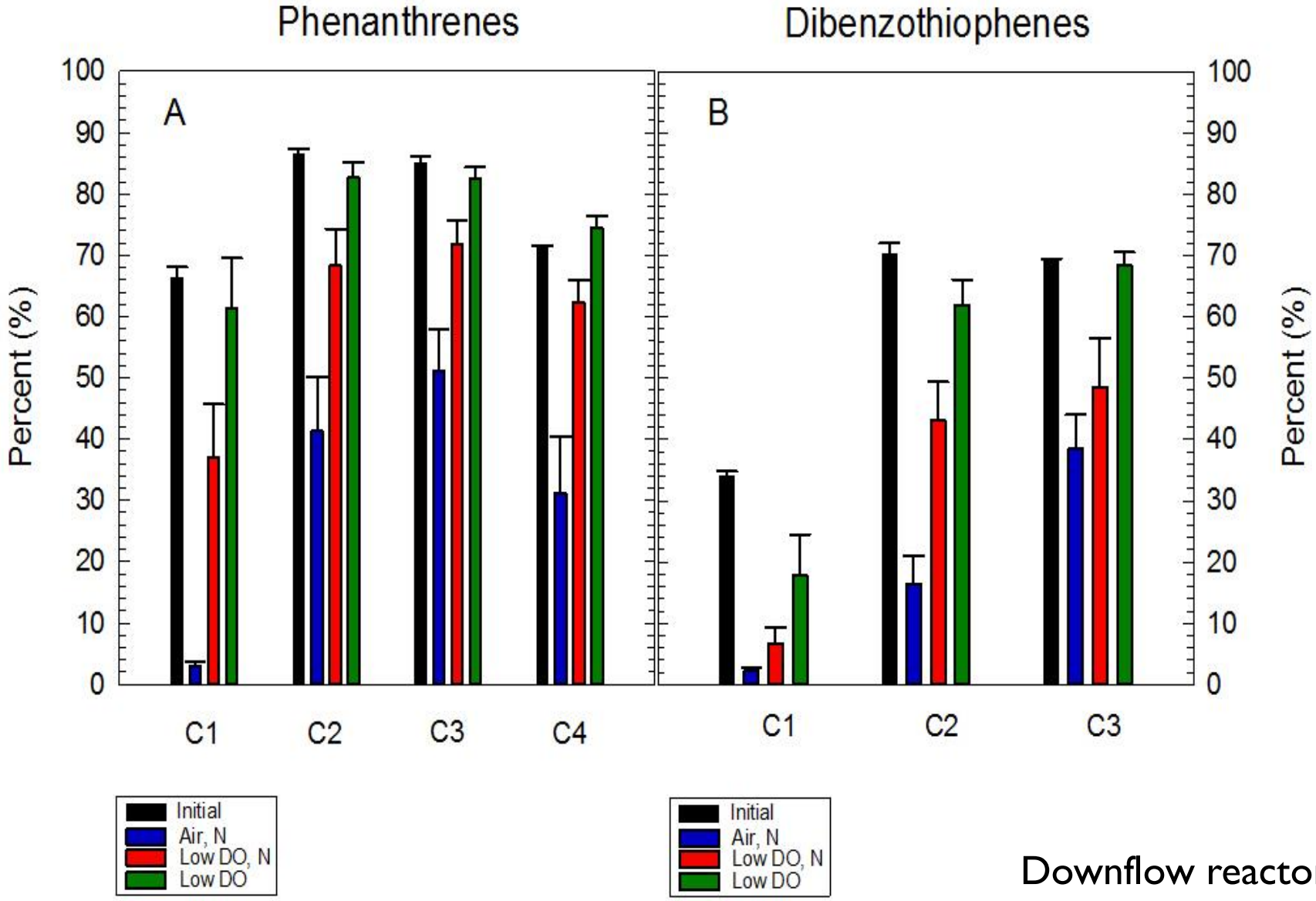
Lower Sand Layer






Downflow reactors

Cross-flow reactors




Downflow reactors

# Four phases

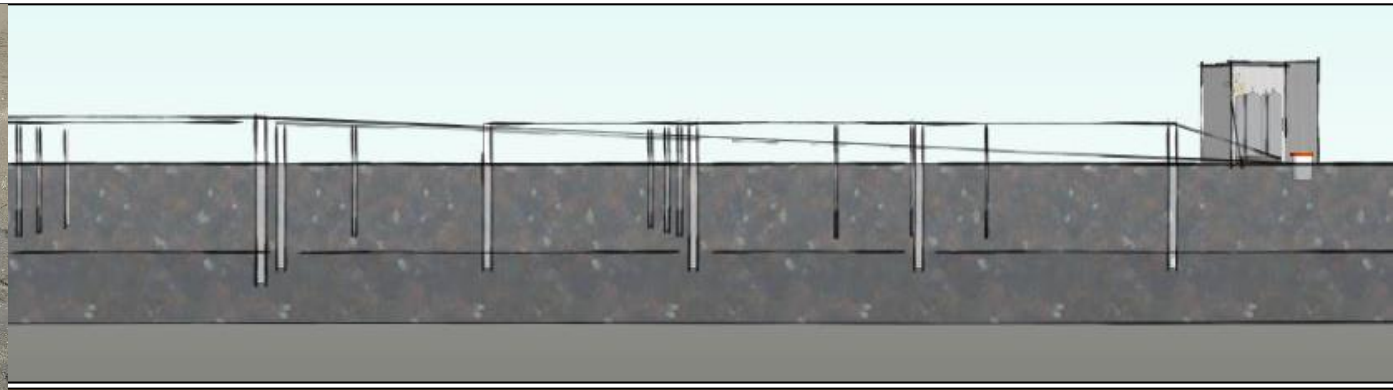
- **Breathing air addition (12 emitters)** July 2015-September 2015
- **Oxygen addition (12 emitters)** October 2015- May 2016
- Weekly circulation of oxygenated groundwater through an aboveground tank (May 2016 -February 2017)
- Advanced oxidation (persulfate) followed by solid oxidant (calcium peroxide) (February 2017-present)

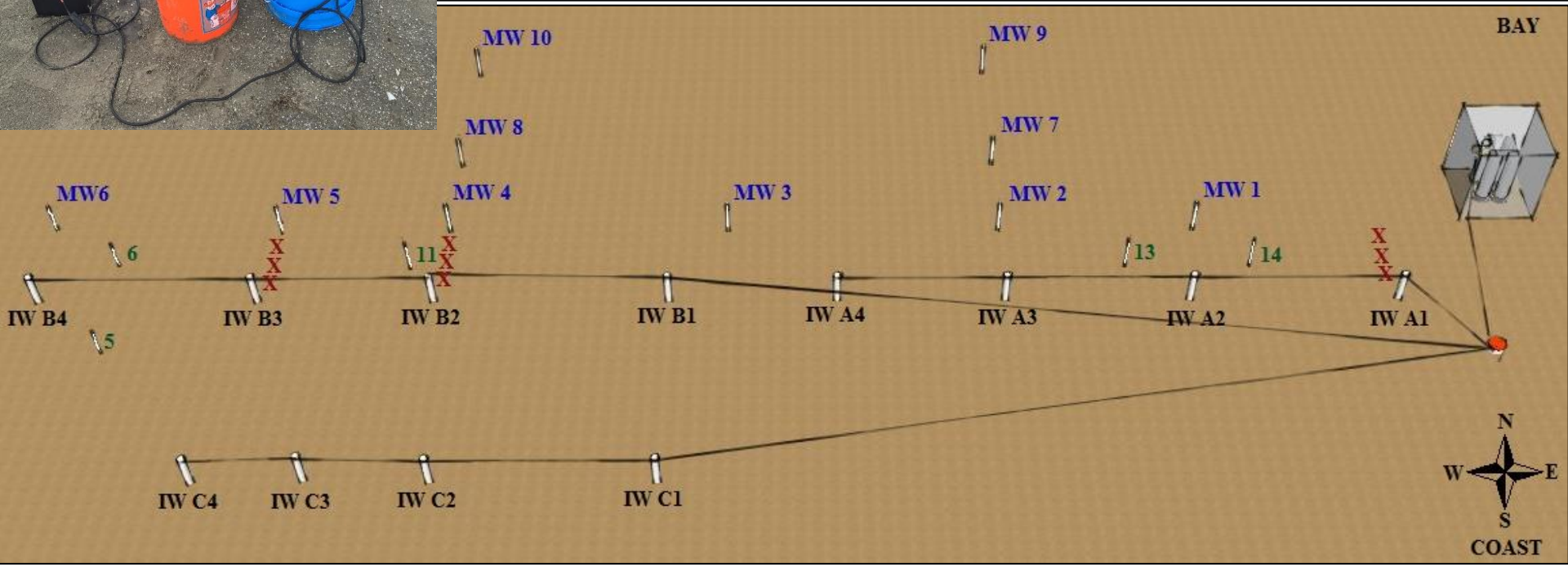
# Field Pilot Installation

Solinst Waterloo Emitters™

Twelve 8-foot screened wells

3.5 L/day per emitter @ 20 psi




MW 10
MW 9
BAY
MW 8
MW 7
MW6
MW 5
MW 4
MW 3
MW 2
MW 1
6
11
13
14
IW B4
IW B3
IW B2
IW B1
IW A4
IW A3
IW A2
IW A1
5
IW C4
IW C3
IW C2
IW C1
N
W
E
S
COAST

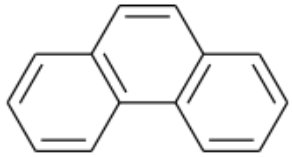
# Polycyclic aromatic hydrocarbons (PAHs)

| Naphthalene | Phenanthrene | Dibenzothiophene | Chrysene | Fluorene | Other |
|---|---|---|---|---|---|
| C1-naphthalene | C1-phenanthrene | C1-dibenzothiophene | C1-chrysene | C1-fluorene | acenaphthylene |
| C2-naphthalene | C2-phenanthrene | C2-dibenzothiophene | C2-chrysene | C2-fluorene | acenaphthene |
| C3-naphthalene | C3-phenanthrene | C3-dibenzothiophene | C3-chrysene | C3-fluorene | anthracene |
| C4-naphthalene | C4-phenanthrene | | | | Fluoranthene |
| | | | | | pyrene |
| | | | | | C1-pyrene/fluoranthene |
| | | | | | hopanes |

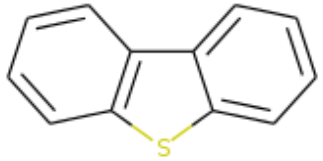
## Weathering Ratios

$$\frac{\Sigma\ (C1{-}3\ Dibenz)}{\Sigma\ (C1{-}3\ Dibenz + C1{-}3\ Chry)}$$

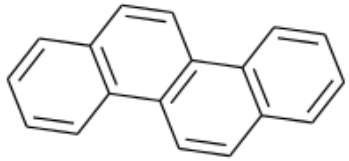
$$\frac{\Sigma\ (C1{-}4\ Phen)}{\Sigma\ (C1{-}3\ Phen + C1{-}3\ Chry)}$$



PHENANTHRENE




DIBENZOTHIOPHENE



CHRYSENE

# Carbon Mineralization

## $^{13}C$

- DIC in 100 mL groundwater
- 2 mL NaOH in test tube
- + BaCl → $BaCO_3$ (s)
- Analyzed for $\delta^{13}C$ using a natural abundance mass spectrometer
- Reported relative to v-PDB

## $^{14}C$

- DIC in 500 mL groundwater
- Acidify-gas strip
- Analyzed for percent modern carbon (PMC) using AMS


Trapping $CO_2$ in NaOH from beach groundwater.

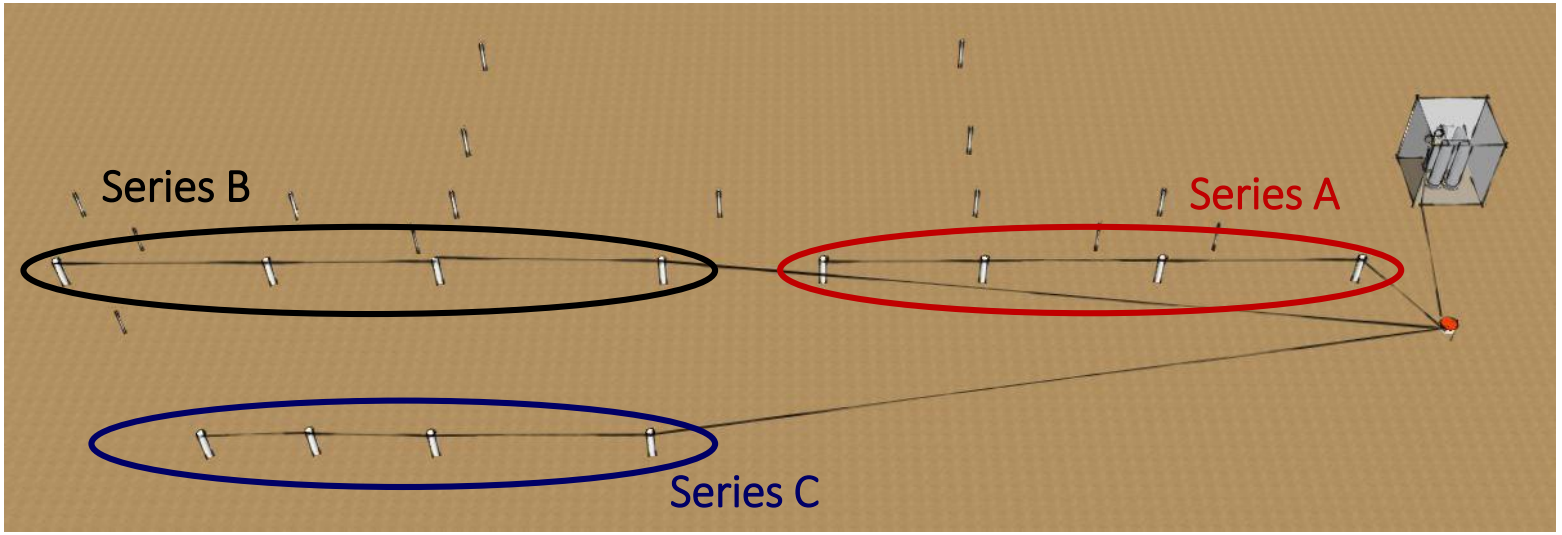
# Bacterial Extraction and Analysis

- Genomic DNA from soil were extracted and 16S rRNA from V4 region was sequenced by Illumina MiSeq platform
- Microbial diversity was characterized at genus level with reference to rdp classifier and sequences were aligned based on the Silva reference database using the mothur program

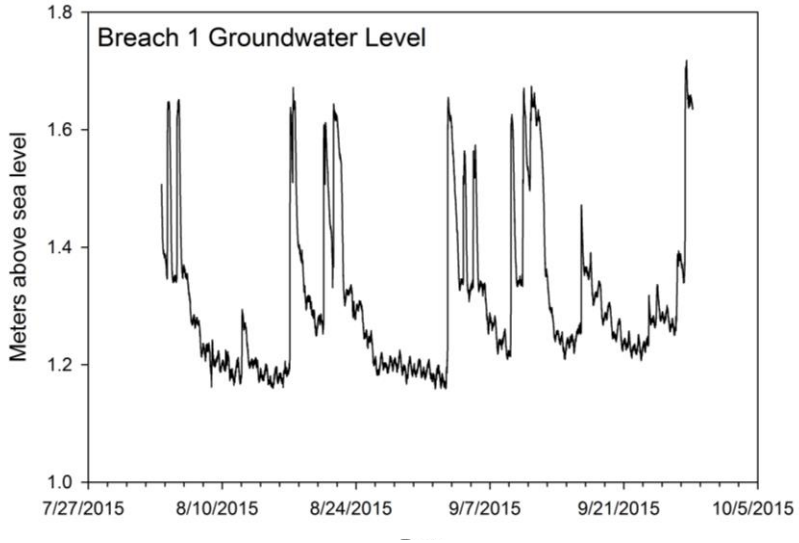
# Oxygen (mg/L)

| IW Series | Pretreatment | Air Phase[a] | $O_2$ Phase[b] |
|---|---|---|---|
| A | <<0.02 | 4.8 ± 2.1 | 16.9 ± 3.8 |
| B | <<0.02 | 2.1 ± 0.7 | 17.3 ± 4.4 |
| C | <<0.02 | 3.6 ± 1.5 | 15.3 ± 2.6 |

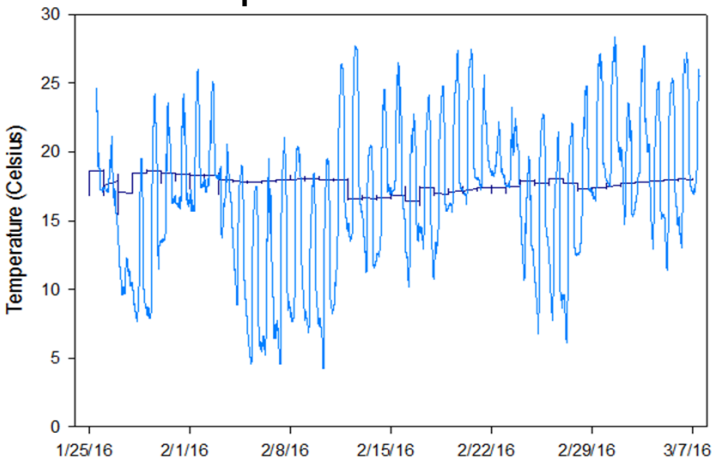
[a]7/22/15 through 9/30/15, [b]10/1/15 through 4/27/15



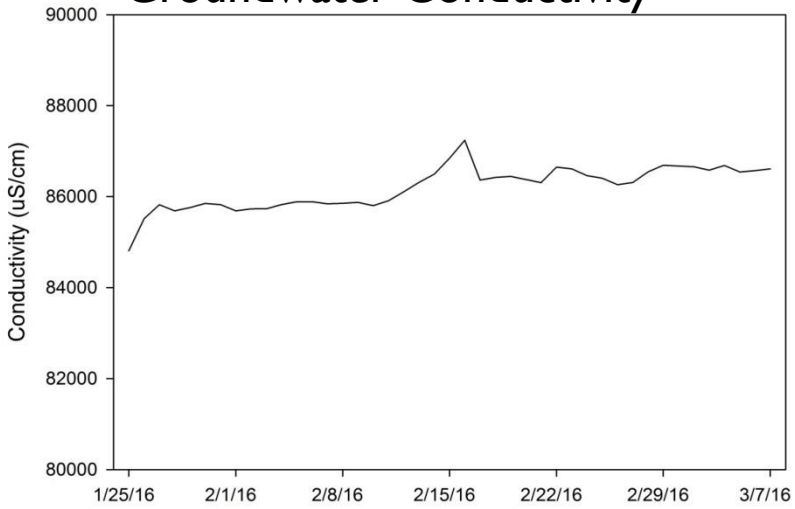
## Groundwater level



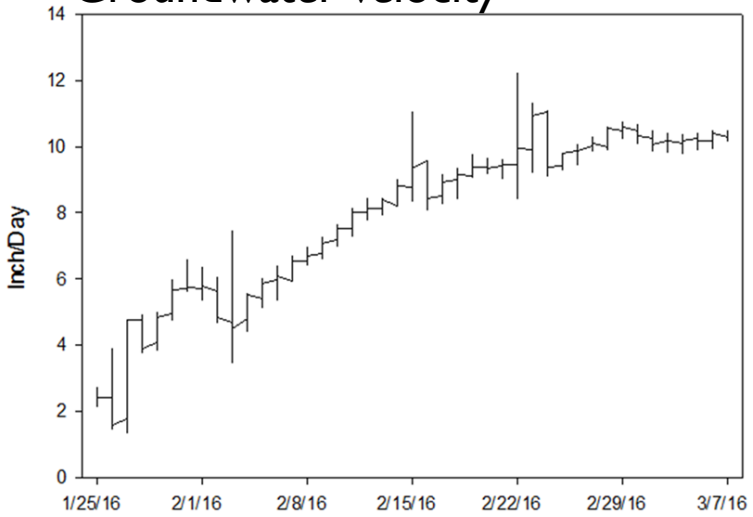

## Air Temperature

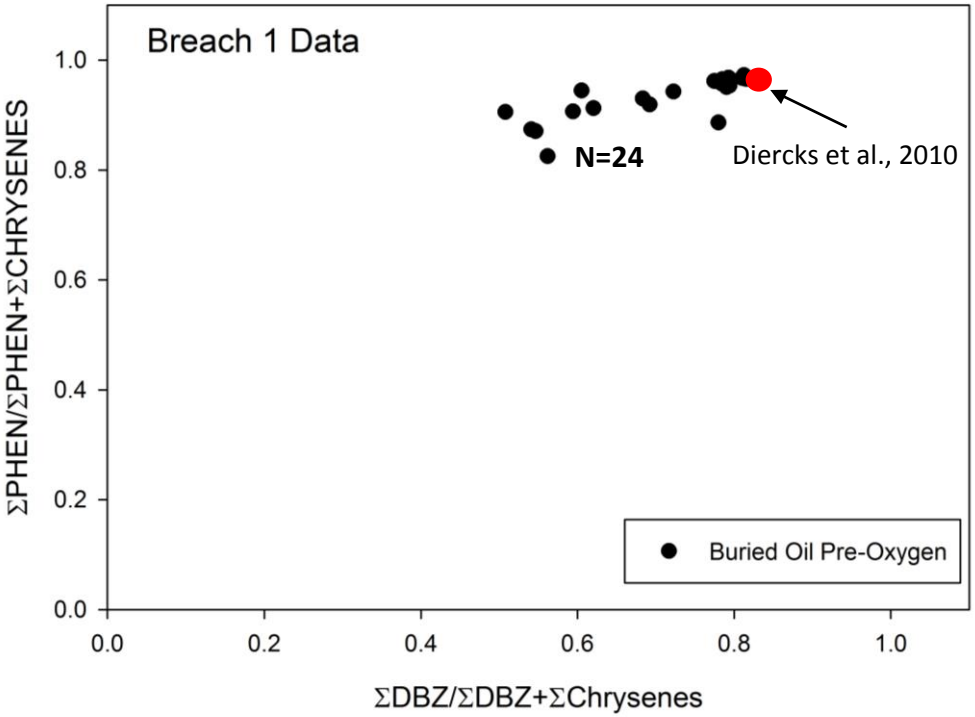


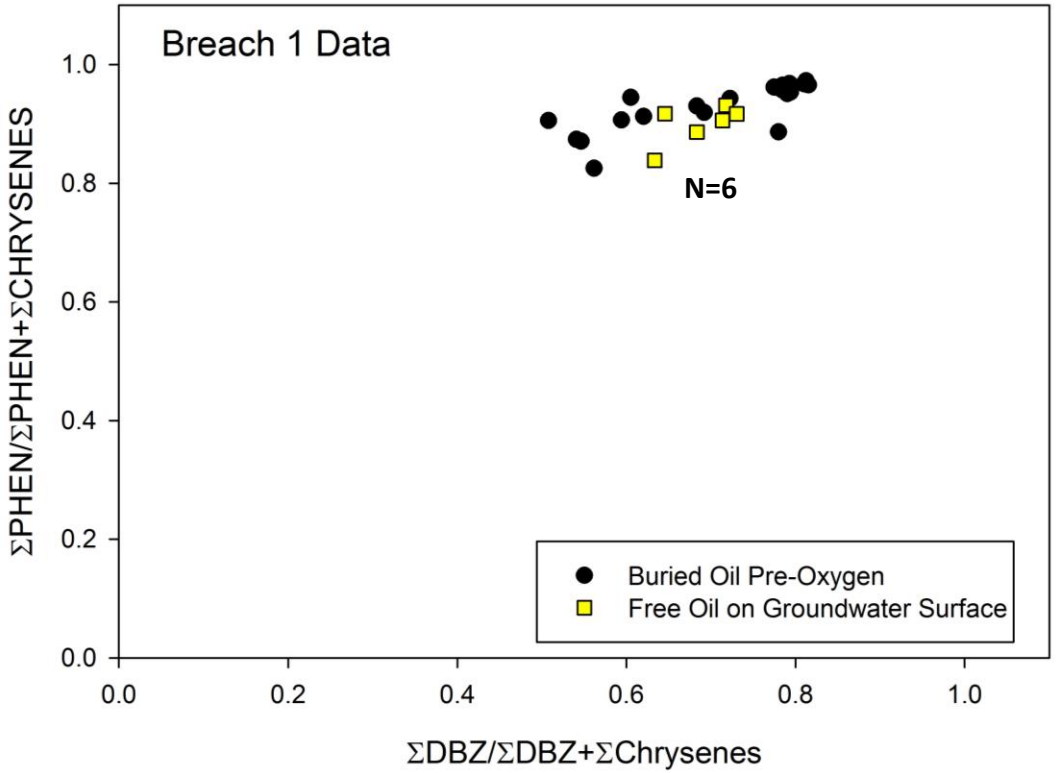

## Groundwater Conductivity

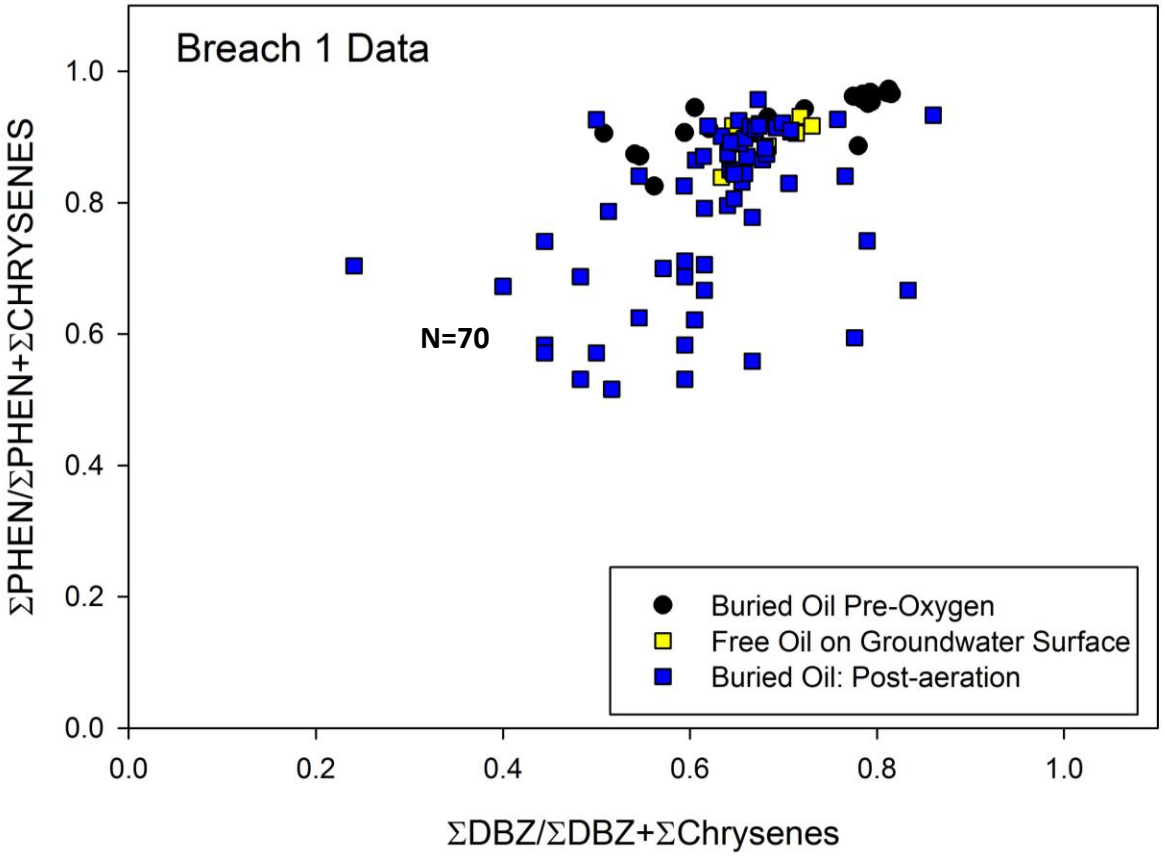


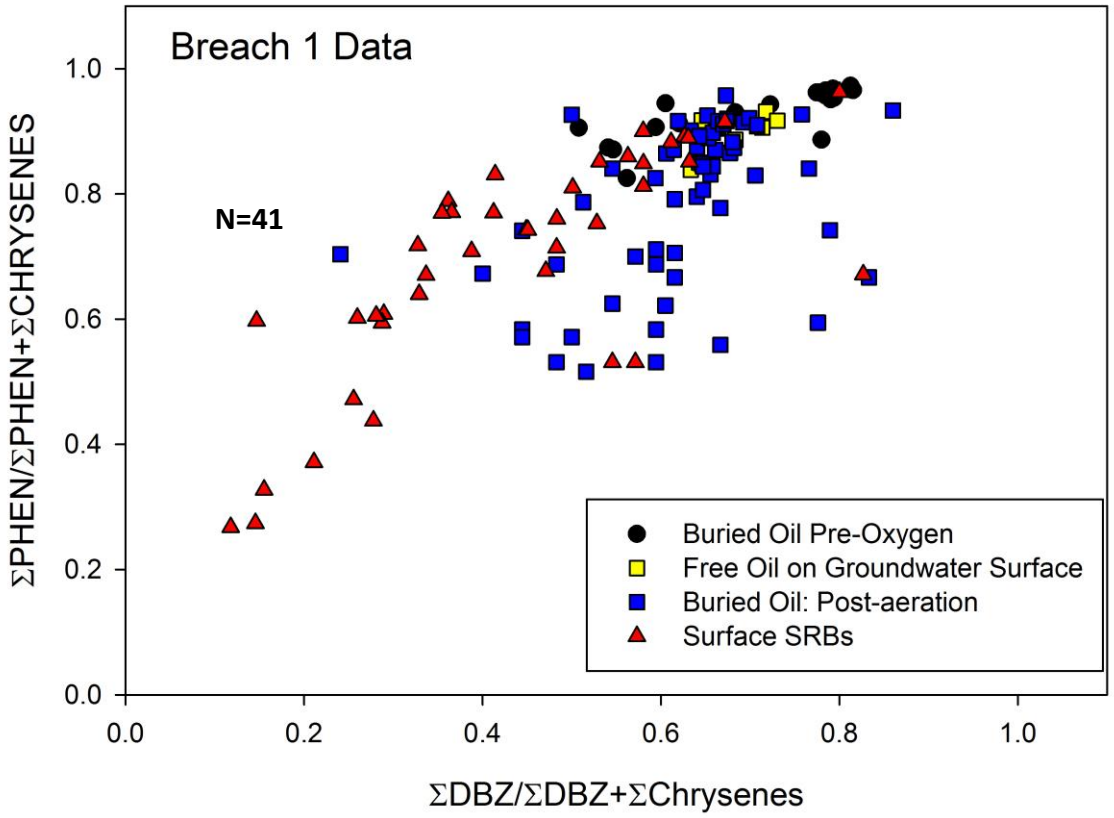

## Groundwater velocity

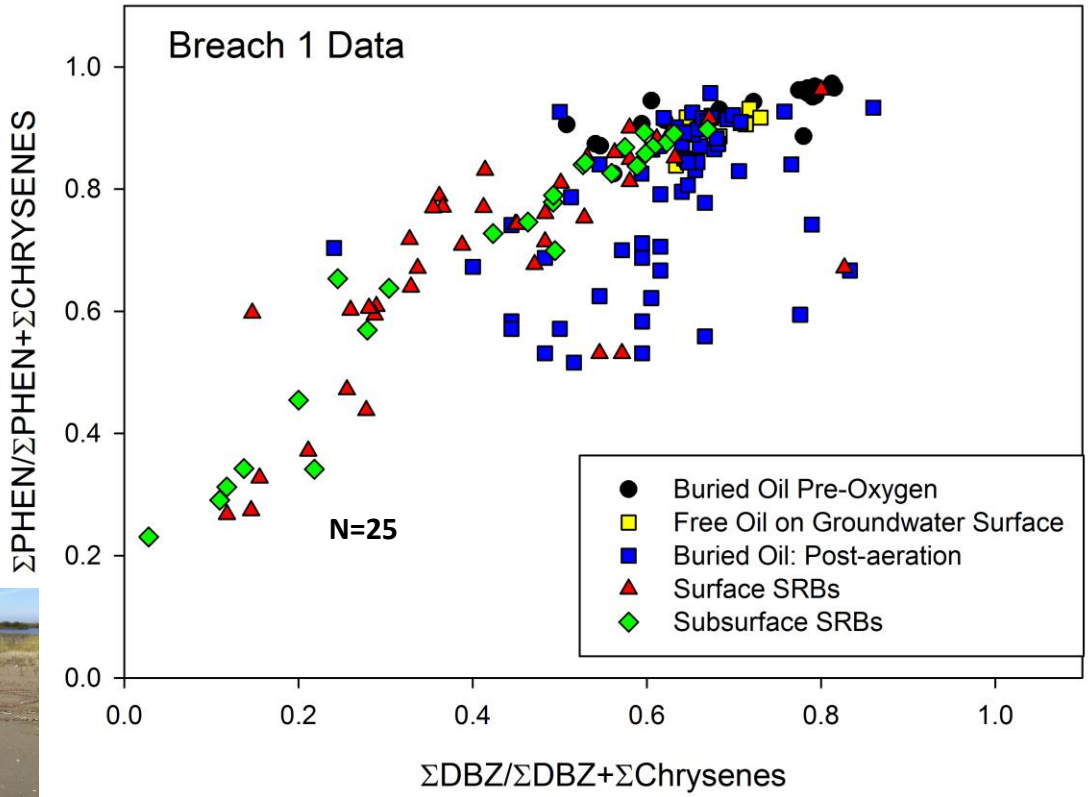



Breach 1 Data
N=24
Diercks et al., 2010
Buried Oil Pre-Oxygen
ΣPHEN/ΣPHEN+ΣCHRYSENES
ΣDBZ/ΣDBZ+ΣChrysenes
0.0
0.2
0.4
0.6
0.8
1.0


Breach 1 Data
N=6
ΣPHEN/ΣPHEN+ΣCHRYSENES
ΣDBZ/ΣDBZ+ΣChrysenes
0.0
0.2
0.4
0.6
0.8
1.0
Buried Oil Pre-Oxygen
Free Oil on Groundwater Surface

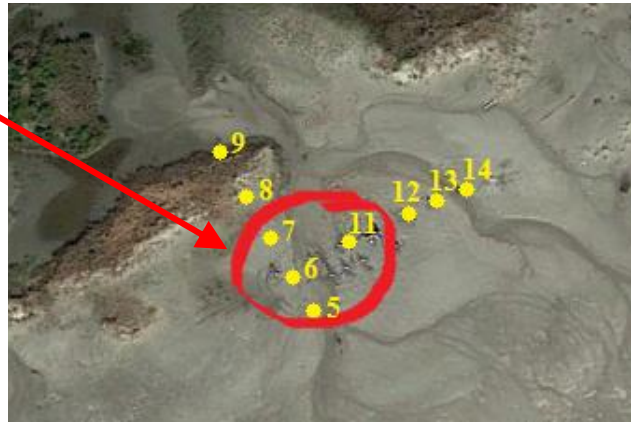

Breach 1 Data
N=70
ΣPHEN/ΣPHEN+ΣCHRYSENES
ΣDBZ/ΣDBZ+ΣChrysenes
0.0
0.2
0.4
0.6
0.8
1.0
Buried Oil Pre-Oxygen
Free Oil on Groundwater Surface
Buried Oil: Post-aeration


Breach 1 Data
N=41
ΣPHEN/ΣPHEN+ΣCHRYSENES
ΣDBZ/ΣDBZ+ΣChrysenes
0.0
0.2
0.4
0.6
0.8
1.0
Buried Oil Pre-Oxygen
Free Oil on Groundwater Surface
Buried Oil: Post-aeration
Surface SRBs


Breach 1 Data
ΣPHEN/ΣPHEN+ΣCHRYSENES
ΣDBZ/ΣDBZ+ΣChrysenes
0.0
0.2
0.4
0.6
0.8
1.0
N=25
Buried Oil Pre-Oxygen
Free Oil on Groundwater Surface
Buried Oil: Post-aeration
Surface SRBs
Subsurface SRBs

# Carbon Mineralization: Pre-aeration

| Piezometer | $d^{13}C$ (‰) | $CO_2$ Production from oil (%) | $CO_2$ from background carbon (%) |
|---|---|---|---|
| P-5 | $-13.1^{a} \pm 1.3$ | 1 | 99 |
| P-6 | $-15.8^{a,b} \pm 0.6$ | 8 | 92 |
| P-7 | $-15.8^{a,b} \pm 1.6$ | 10 | 90 |
| P-8 | | 21 | 79 |
| P-9 | | 30 | 70 |
| P-11 | | 14 | 86 |
| P-12 | | 91 | 9 |
| P-13 | | 92 | 8 |
| P-14 | | 68 | 32 |



# Carbon Mineralization: Post-aeration

| Location | PMC (%) | $d^{13}C$ (‰) |
|---|---|---|
| Reference | 82.7 | -16.2 |
| MW-1 | 72.3 | -17.2 |
| MW-4 | 63.9 | -15.8 |

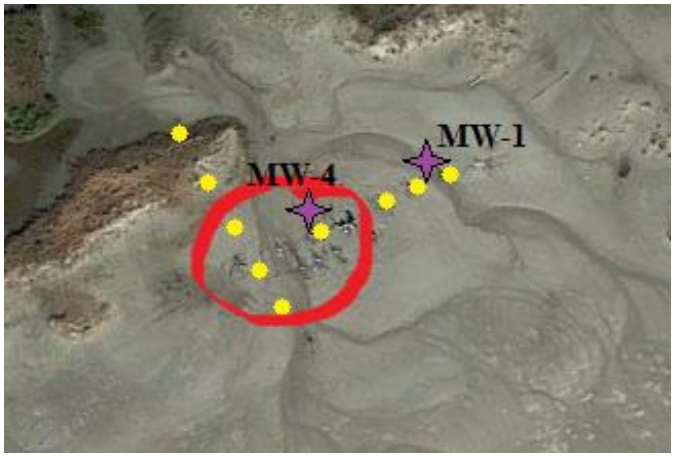
Reference (clean area)

MW-1 (moderately oiled area)

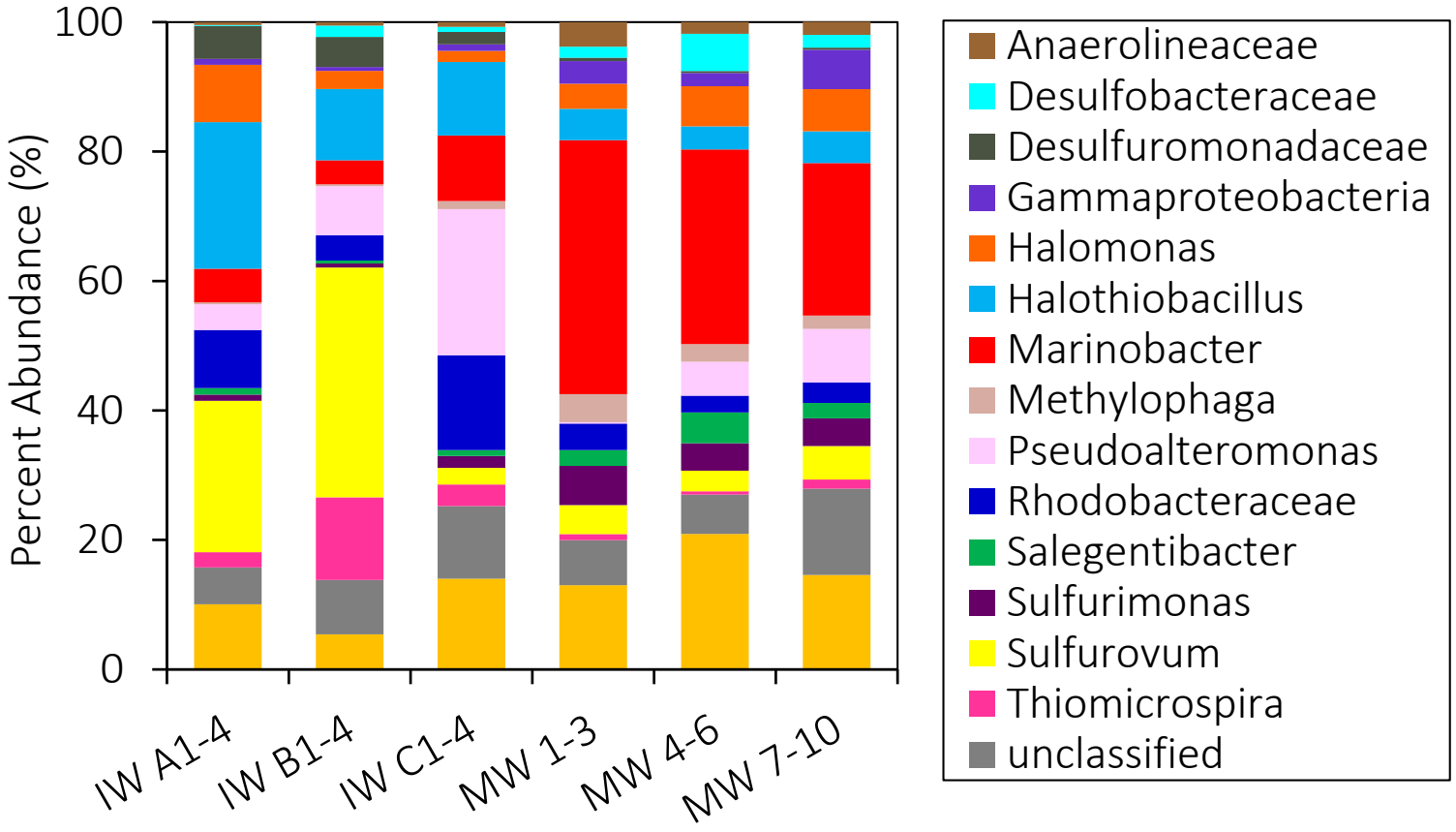
MW-4 (heavily oiled area)

Local Post-aeration Weathering Ratios

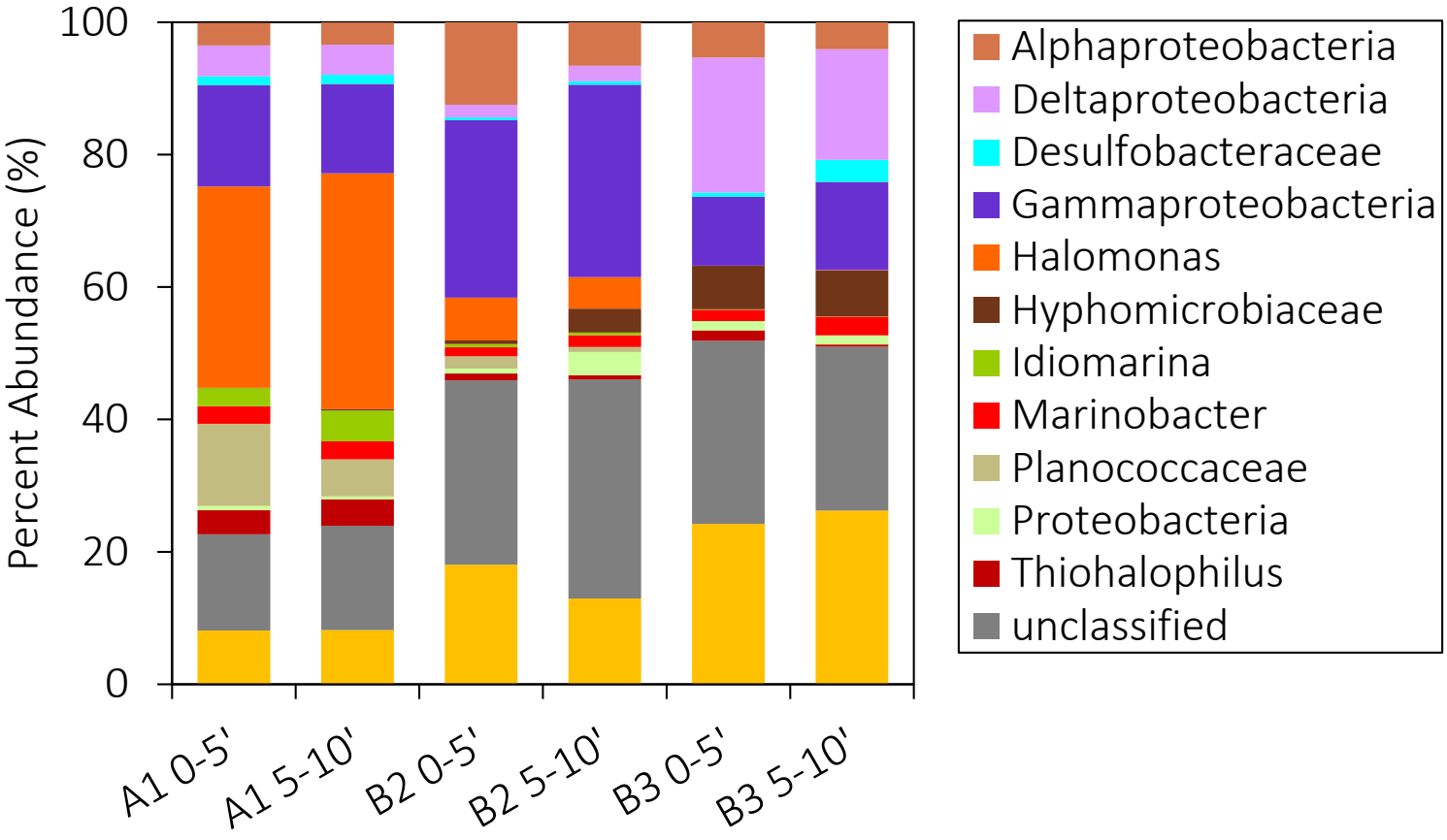
| Location | Average P Ratio | Average D Ratio |
|---|---|---|
| A1 | 0.77 ± 0.03 | 0.61 ± 0.02 |
| B2 | 0.82 ± 0.03 | 0.67 ± 0.03 |



# Bacterial Characterization: Pre-aeration 0-5’



# Bacterial Characterization: Post-aeration



# Bacterial Characterization

→ Increase in diversity

| | Shannon Index |
|---|---|
| Pre-aeration | $4.9 \pm 0.31$ |
| Post-aeration | $5.6 \pm 0.17$ |
| | |

→ Switch from *Marinobacter* to *Halomonas*

| | Number of Samples | | Percent Abundance (%) | |
|---|---|---|---|---|
| | *Marinobacter* | *Halomonas* | *Marinobacter* | *Halomonas* |
| Pre-aeration | 40 (40) | 40 (40) | 1.4 - 45 | 0.3 - 16 |
| Post-aeration | 26 (30) | 19 (30) | 0.1 - 6 | 0.1 - 42 |

# Questions?

GULF OF MEXICO RESEARCH INITIATIVE

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