

Transport of Crude Oil Aggregates and Associated Microbial Populations: Impact on Biodegradation Potential

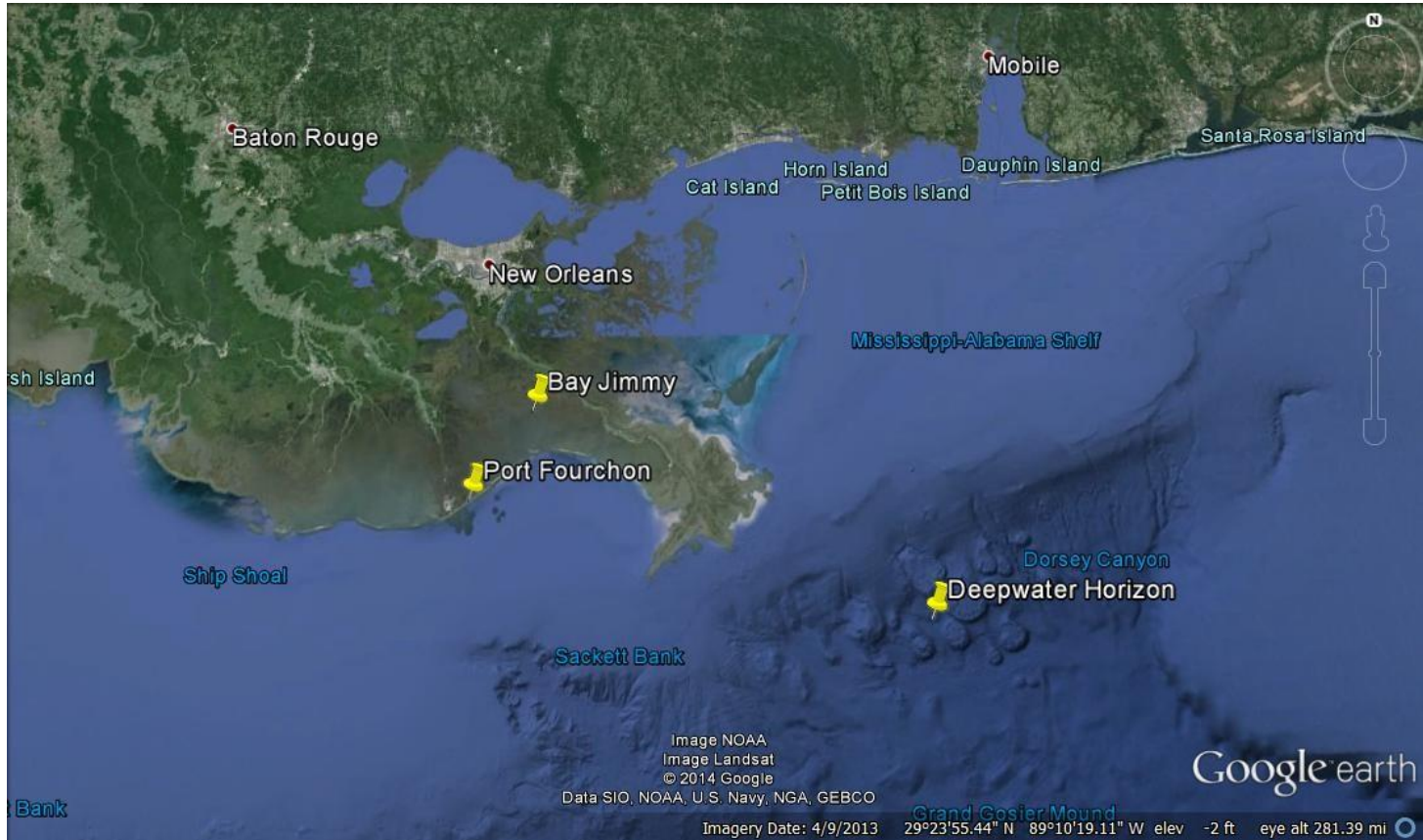
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Objectives

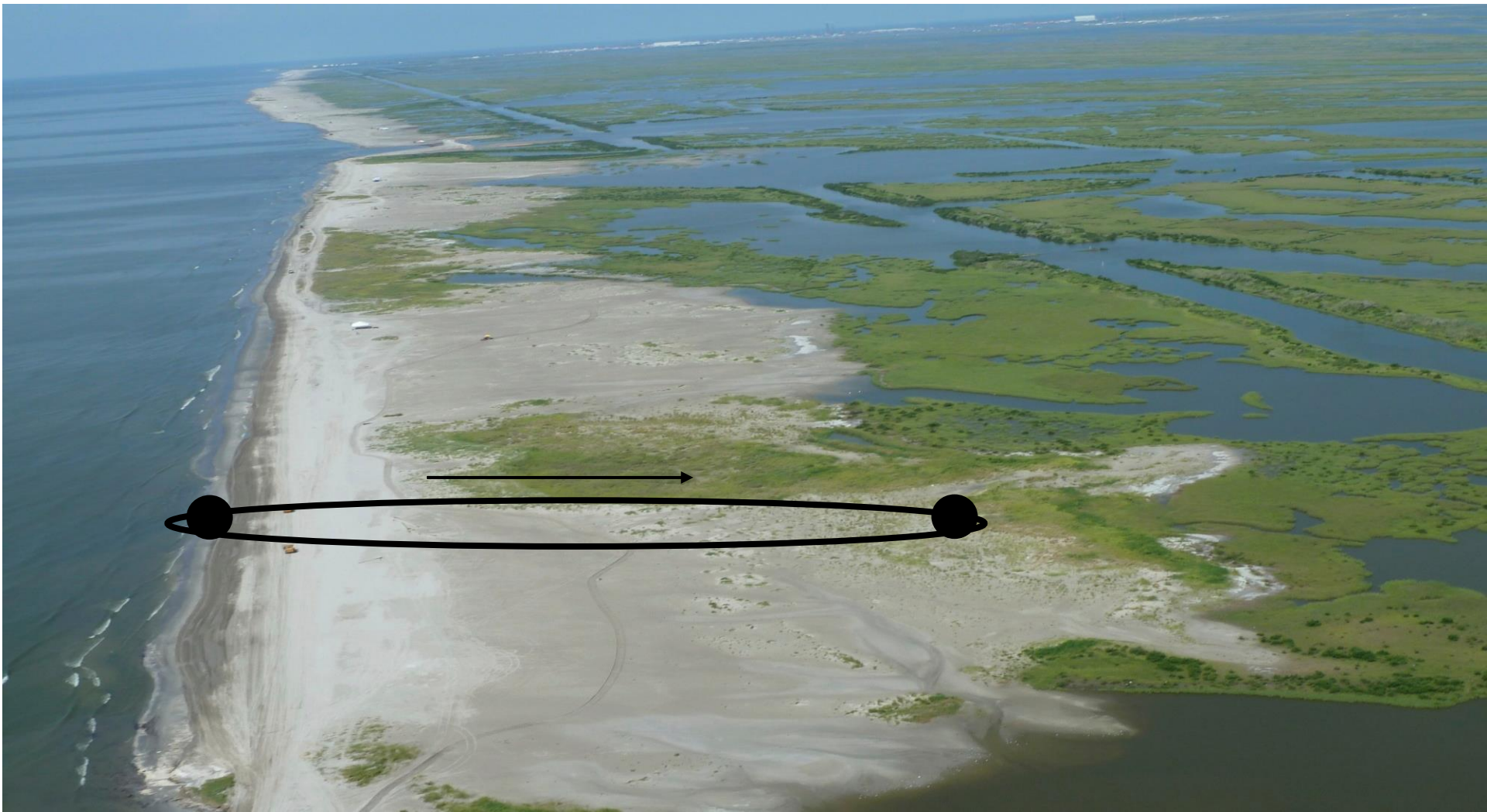
- **Extent of weathering in different oil forms; Submerged Oil Mats (SOMs) and Surface Residue Balls (SRBs)**
- **Characterization of Microbial Communities**

Site Description



SRB/SOM Transport, Port Fourchon, LA

- Storm-driven conveyor for sand and oil:sand:shell aggregates and associated microbial populations
- Erosion rates > 50 ft/year



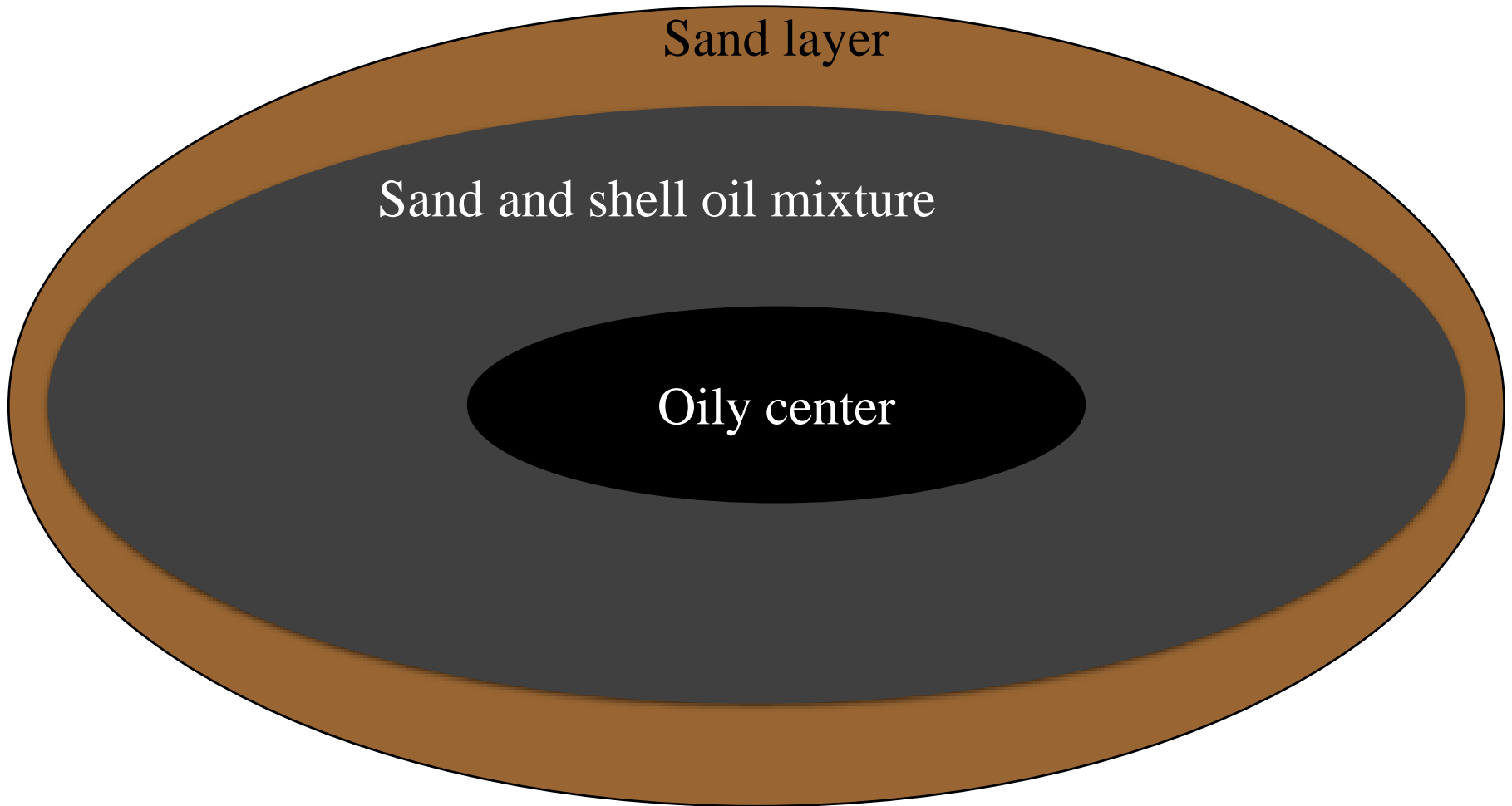
Surface Residue Balls (SRBs)



Submerged Oil Mat (SOM)



SOM/SRB Composition

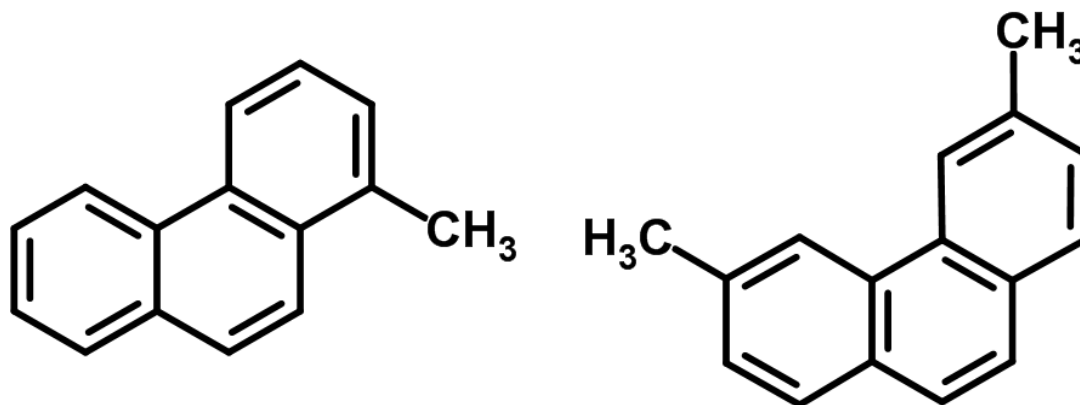


Density (g/cm^3) = 1.32 ± 0.11 , Moisture (%) = 0.16 ± 0.09

PAHs of Interest

Naphthalenes	Phenanthrenes	Dibenzothiophenes	Chrysenes
C ₀ -Naphthalene	C ₀ -Phenanthrenes	C ₀ -Dibenzothiophenes	C ₀ -Chrysenes
C ₁ -Naphthalenes	C ₁ -Phenanthrenes	C ₁ -Dibenzothiophenes	C ₁ -Chrysenes
C ₂ -Naphthalenes	C ₂ -Phenanthrenes	C ₂ -Dibenzothiophenes	C ₂ -Chrysenes
C ₃ -Naphthalenes	C ₃ -Phenanthrenes	C ₃ -Dibenzothiophenes	C ₃ -Chrysenes
C ₄ -Naphthalenes	C ₄ -Phenanthrenes		

Fluorenes	Others
C ₀ -Fluorenes	Hopanes
C ₁ -Fluorenes	Fluoranthene
C ₂ -Fluorenes	Pyrene
C ₃ -Fluorenes	Acenaphthylene
	Acenaphthene



PAHs: Data Analysis

Weathering Ratio:

- Weathering Ratio Phenanthrenes

$$\frac{(100 * \Sigma PHEN)}{(\Sigma PHEN + \Sigma CHRY)}$$

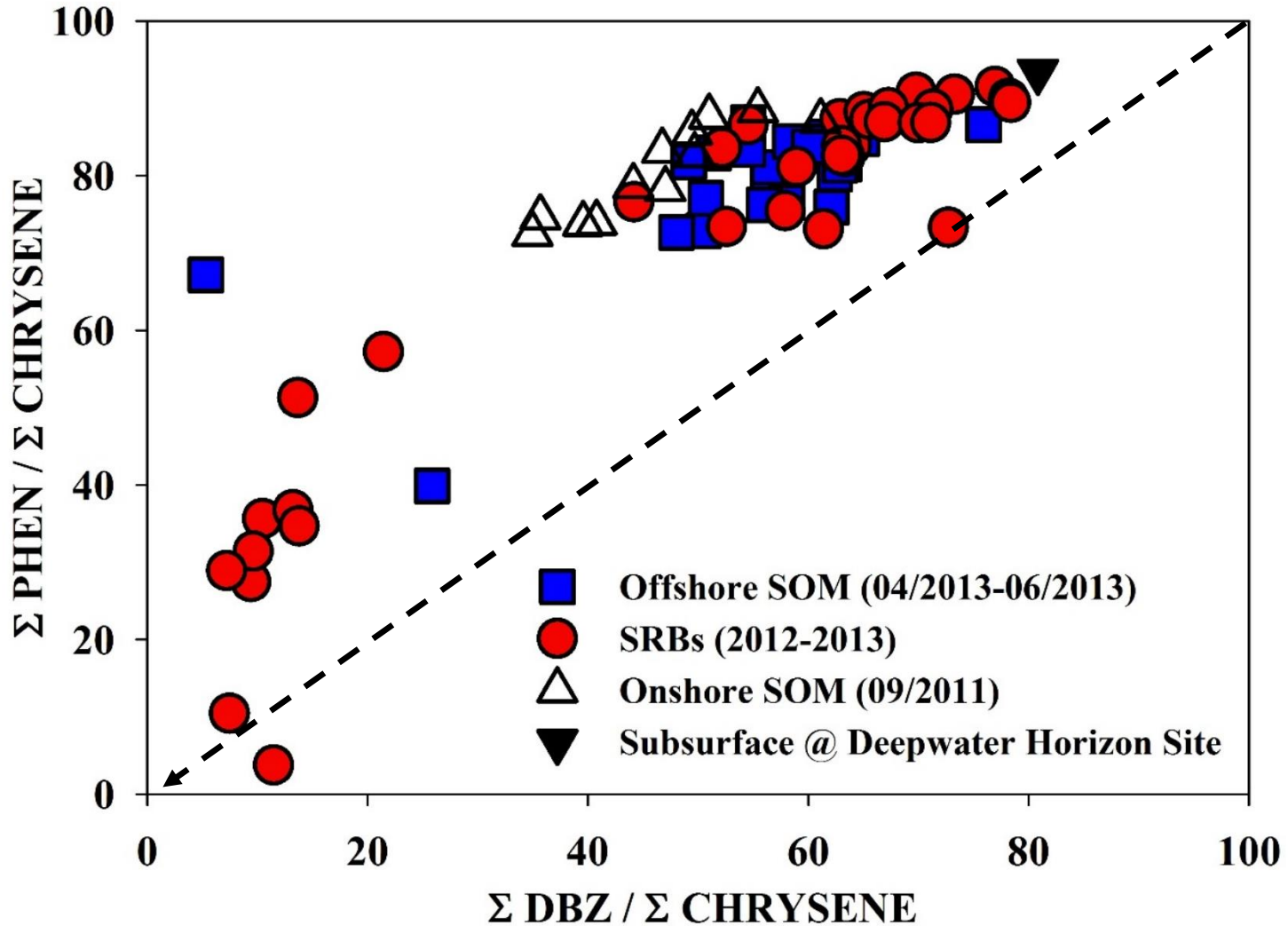
- Weathering Ratio Dibenzothiophenes

$$\frac{(100 * \Sigma DBZ)}{(\Sigma DBZ + \Sigma CHRY)}$$

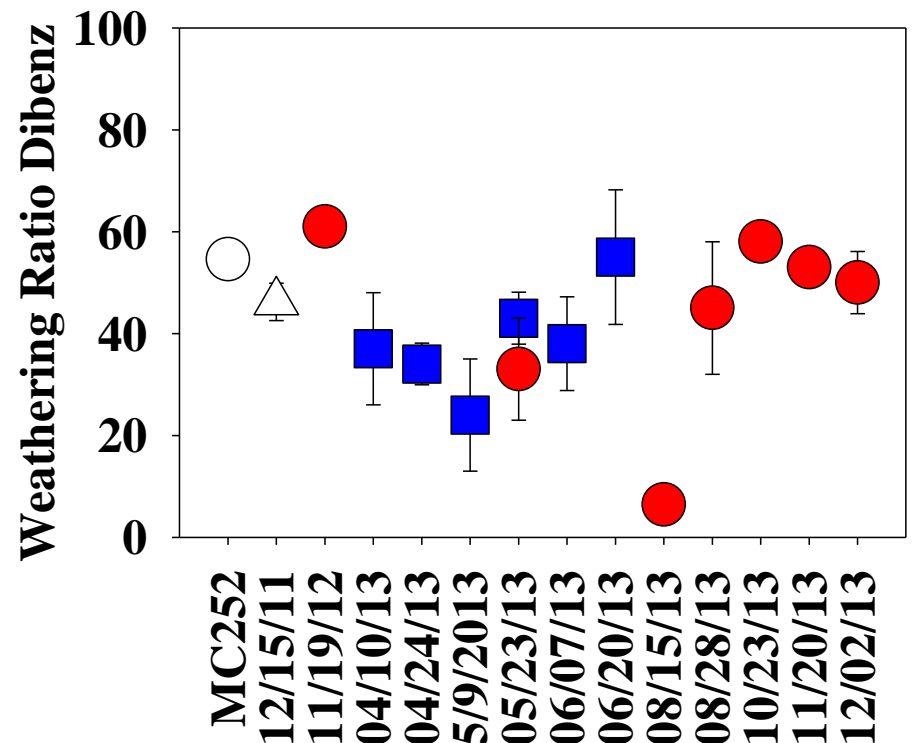
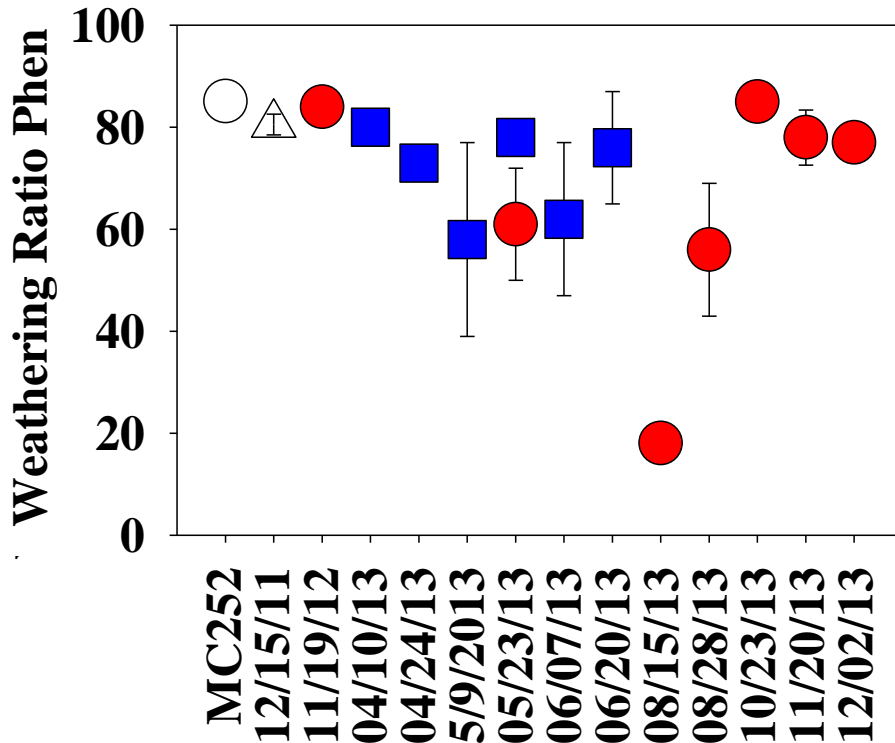
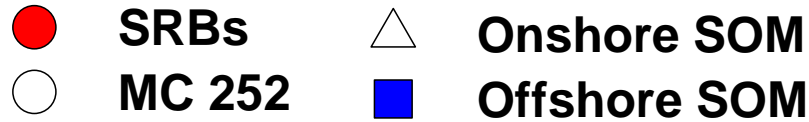
Microbial Community Analysis

- **Genomic DNA from soil were extracted and 16S rRNA from V4 region was sequenced by Illumina MiSeq platform**
- **Bioinformatics analysis by Mothur program**

Results: SOM and SRBs Weathering



Results: Impacts of Washover Events

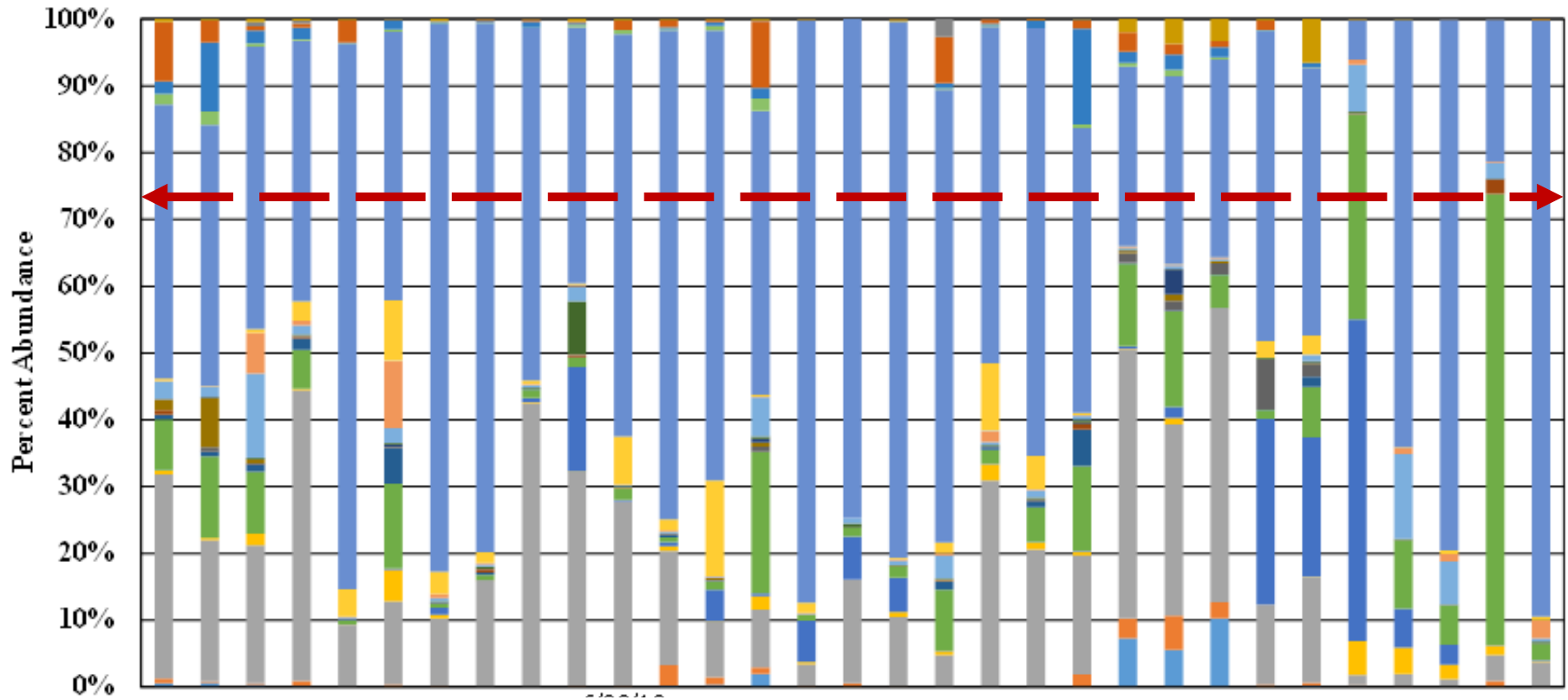


- 12/15/11, Lee
- 11/19/12, Isaac
- 10/23/13, Karen

Results: Impacts of Washover Events- Distribution of PAHs

Compounds	Percentage of Total PAH	
	MC 252	Port Fourchon
Naphthalene	8	<1
C ₁ -Naphthalenes	25	<1
C ₂ -Naphthalenes	12	<1
C ₃ -Naphthalenes	12	<1
C ₄ -Naphthalenes	6	<1
C ₁ -Phenanthrenes	1	3 to 35
C ₂ -Phenanthrenes	1	3 to 35
C ₃ -Phenanthrenes	1	3 to 35
C ₄ -Phenanthrenes	1	0.3 to 2
C ₁ -Dibenzothiophenes	1	≤1
C ₂ -Dibenzothiophenes	1	1 to 13
C ₃ -Dibenzothiophenes	1	1 to 13
C ₁ -Chrysenes	1	4 to 25
C ₂ -Chrysenes	1	4 to 25
C ₃ -Chrysenes	1	1 to 4

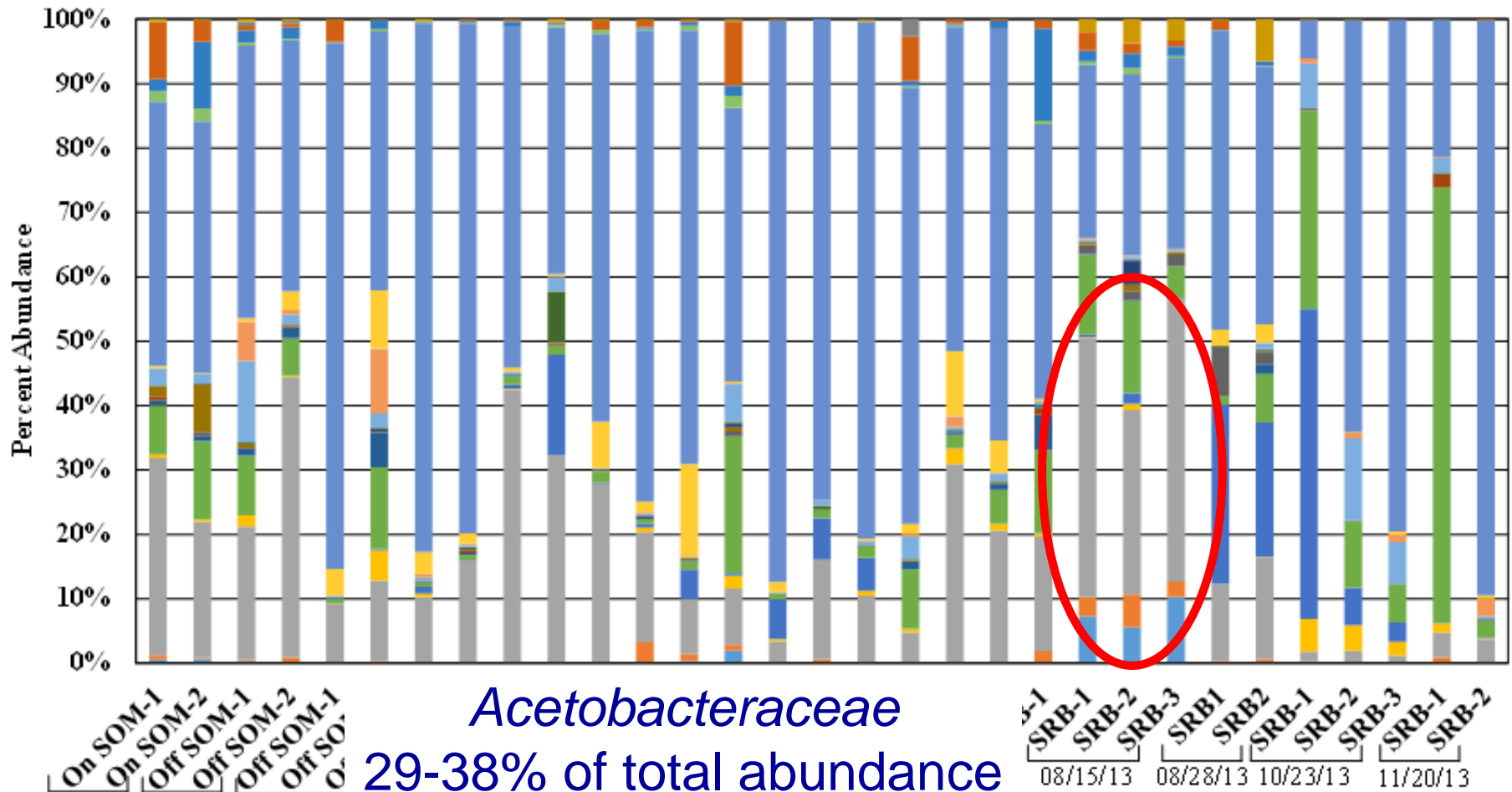
Results: Microbial Community



Alcanivorax, Altermonas, Idiomarina, Halothiobacillus, Halomonas, Marinobacter, Oceanospirillaceae, Porticoccus, Pseudoalteromonas, Singularimonas, Thiomicrospira, and Vibrio



Results: Microbial Community



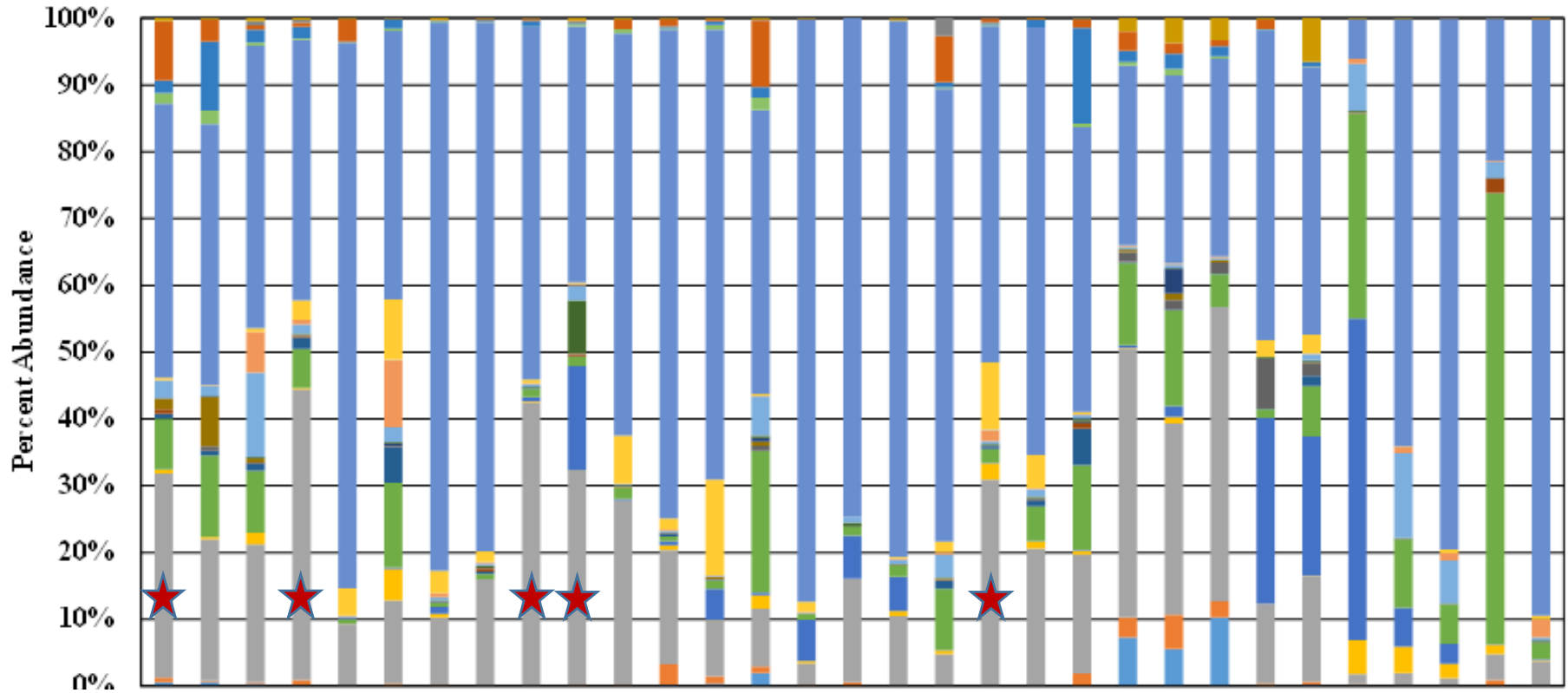
- Acidobacteria
- Bacilli
- Betaproteobacteria
- Deltaproteobacteria
- Gammaproteobacteria
- Spirochaetes

- Actinobacteria
- Bacteria
- Chlamydiae
- Epsilonproteobacteria
- Planctomycetacia
- Verrucomicrobia

- Alphaproteobacteria
- Bacteroidetes
- Chloroflexi
- Firmicutes
- Proteobacteria

- Anaerolineae
- Bacteroidia
- Clostridia
- Flavobacteria
- Sphingobacteria

Results: Microbial Community



Acetobacteraceae, not identified

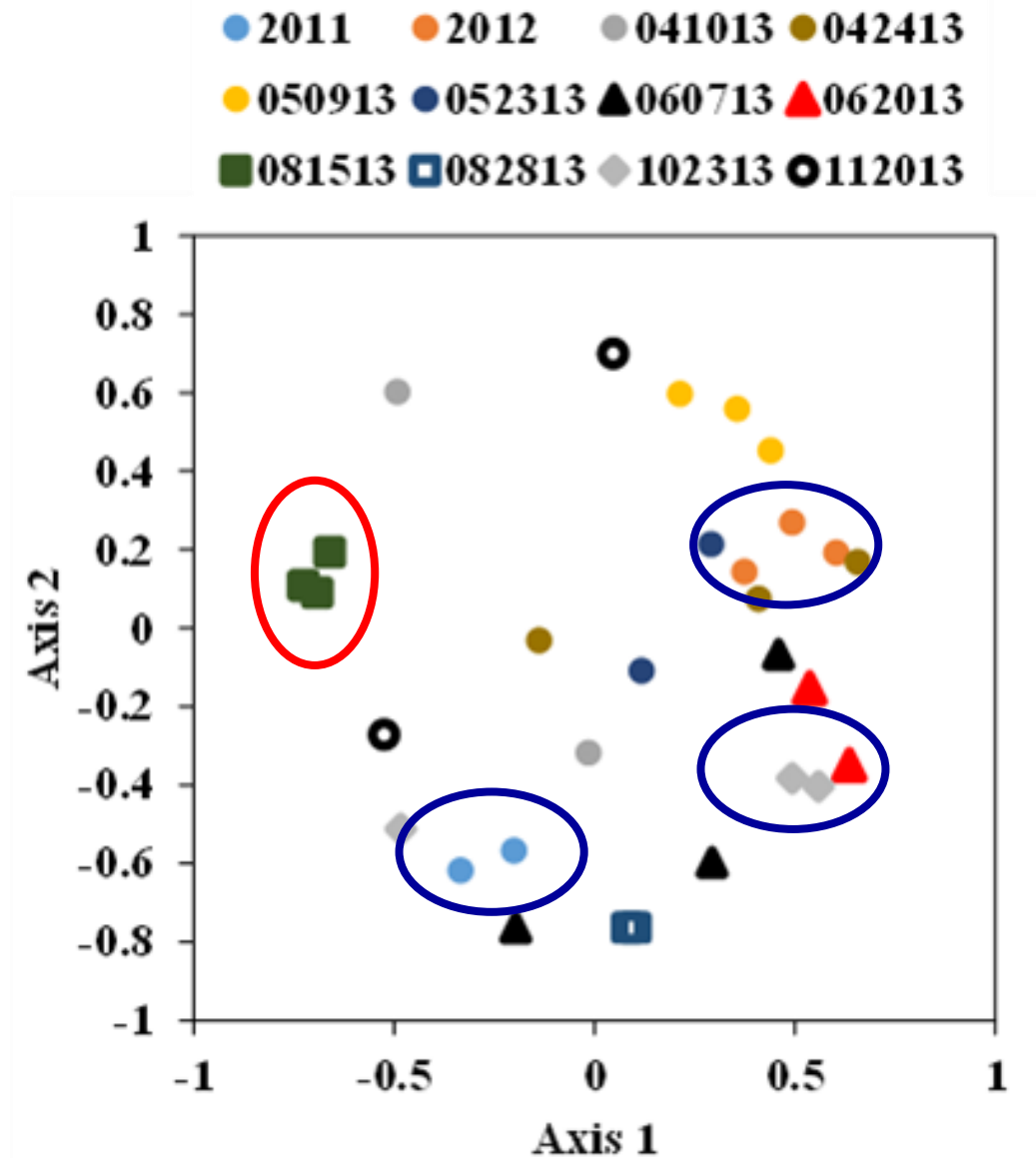
Hyphomonas, *Parvibaculum* and *Porphyrobacter*

L

12/15/11 04/10/13 04/24/13 05/07/13 06/07/13 06/20/13

- | | | | |
|-----------------------|-------------------------|-----------------------|-------------------|
| ■ Acidobacteria | ■ Actinobacteria | ■ Alphaproteobacteria | ■ Anaerolineae |
| ■ Bacilli | ■ Bacteria | ■ Bacteroidetes | ■ Bacteroidia |
| ■ Betaproteobacteria | ■ Chlamydiae | ■ Chloroflexi | ■ Clostridia |
| ■ Deltaproteobacteria | ■ Epsilonproteobacteria | ■ Firmicutes | ■ Flavobacteria |
| ■ Gammaproteobacteria | ■ Planctomycetacia | ■ Proteobacteria | ■ Sphingobacteria |
| ■ Spirochaetes | ■ Verrucomicrobia | | |

Results: Non-metric multidimensional Scaling



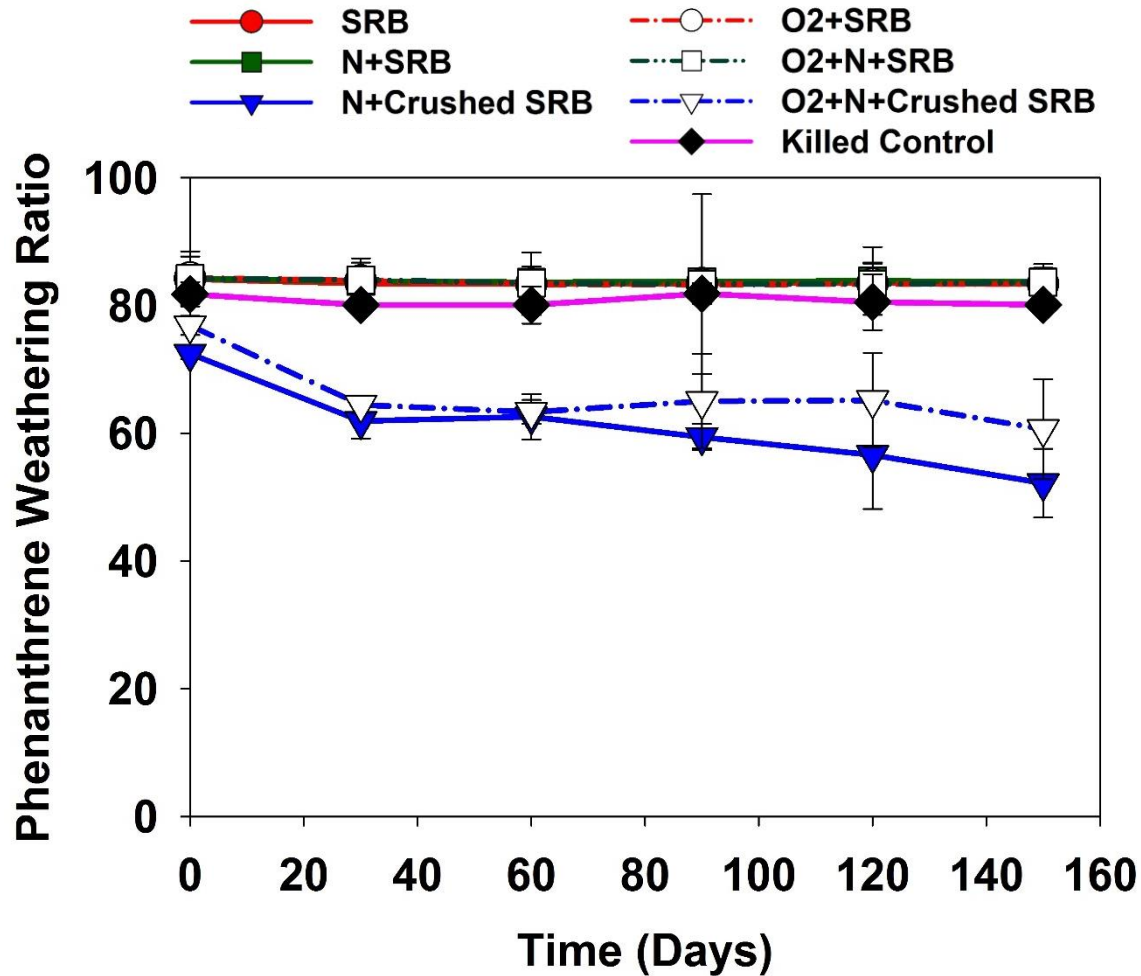
Microcosm Design

Anaerobic	SRB
	SRB + Nitrogen
	Crushed SRB + Nitrogen
Aerobic	SRB + Oxygen
	SRB + Nitrogen + Oxygen
	Crushed SRB + Nitrogen + Oxygen
Killed Control	

Duration: 150 days

Triplicate bottles sacrificed every 30 days

Results: Microcosm



Results: Microcosm Biodegradation Rates

Compound	First order rate (yr⁻¹)	
	Crushed Anaerobic	Crushed Aerobic
C1-Phenanthrenes	7.3 ± 4.2	3.2 ± 0.93
C2-Phenanthrenes	8.3 ± 4.8	8.5 ± 3.9
C3-Phenanthrenes	6.6 ± 2.0	7.3 ± 2.6
C4-Phenanthrenes	6.8 ± 2.2	9.4 ± 1.8

Summary

- Alkylated phenanthrenes and dibenzothiophenes poorly weathered in SOMs
- Crushed SRBs are more susceptible to weathering
- Alkylated dibenzothiophenes appears to be preferentially weathered relative to alkylated phenanthrenes
- *Acetobacteraceae* was the dominant phylotype in most weathered SRB
- *Gammaproteobacteria* was the most dominant phylotype

Questions?