

Little Things Rule the World: Microbial Responses to Oil

Asim Bej, PhD
Professor of Biology
University of Alabama at Birmingham



GoM (Gulf of Mexico)



Water

Sediment in the Gulf of Mexico. Credit: NASA

Bacteria are abundant in all marine habitats

BACTERIAL ACTIVITIES ARE VITAL FOR MAINTAINING A BALANCED ECOSYSTEM

BACTERIA DO GOOD THINGS !

- Decomposition of organic matters which maintain Earth's carbon, nitrogen and sulfur cycles
- Serve as food for some bottom-living organisms



OIL SPILL

BUT SOME BACTERIA ARE THREAT TO HUMAN and ANIMAL HEALTH !

- Some of the resident bacteria are pathogenic to humans (*e.g. Vibrio*)
 - Consumption of undercook or raw seafood
 - Wound infection – Septicemia
 - Diarrhea

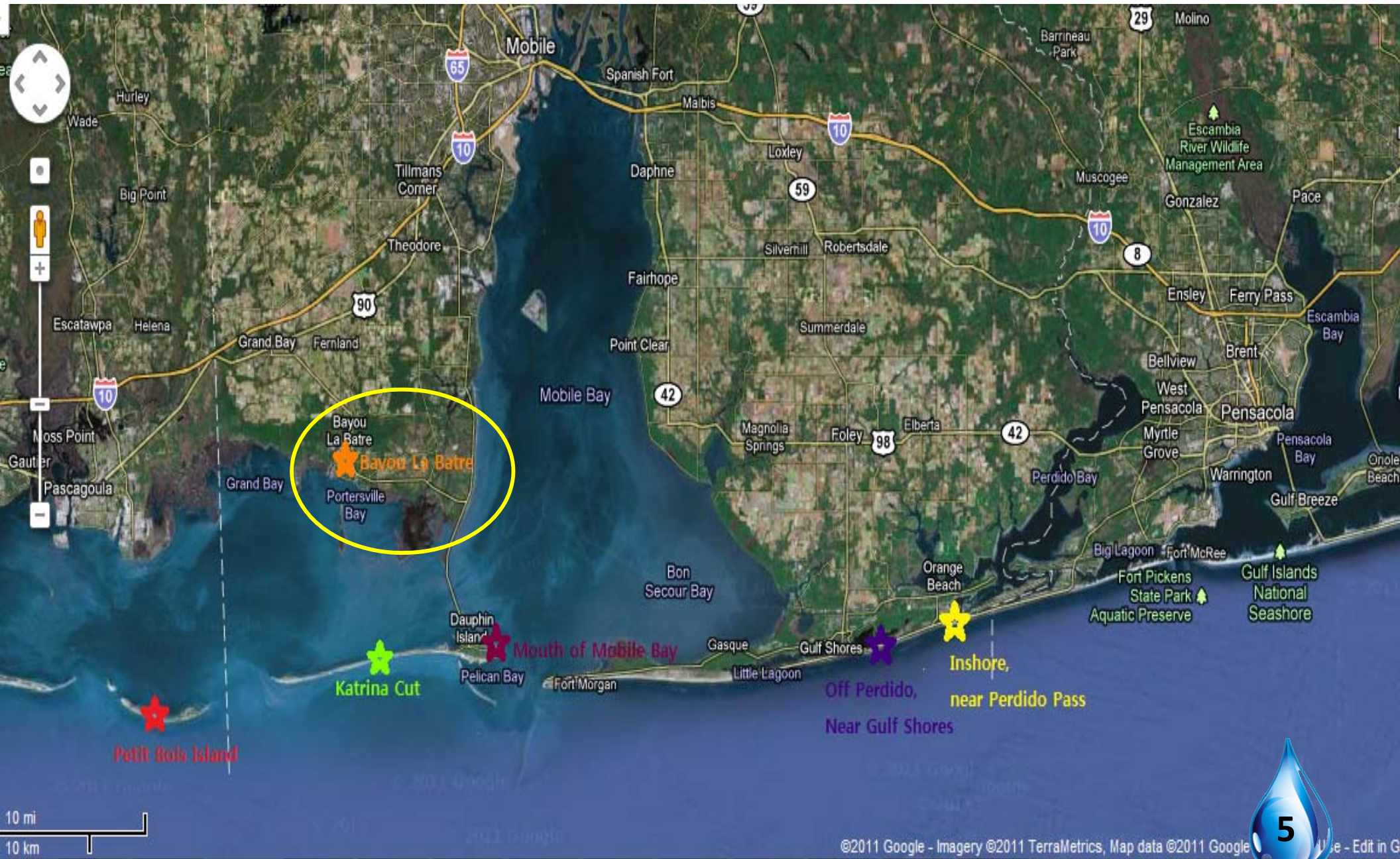


STUDY OBJECTIVES

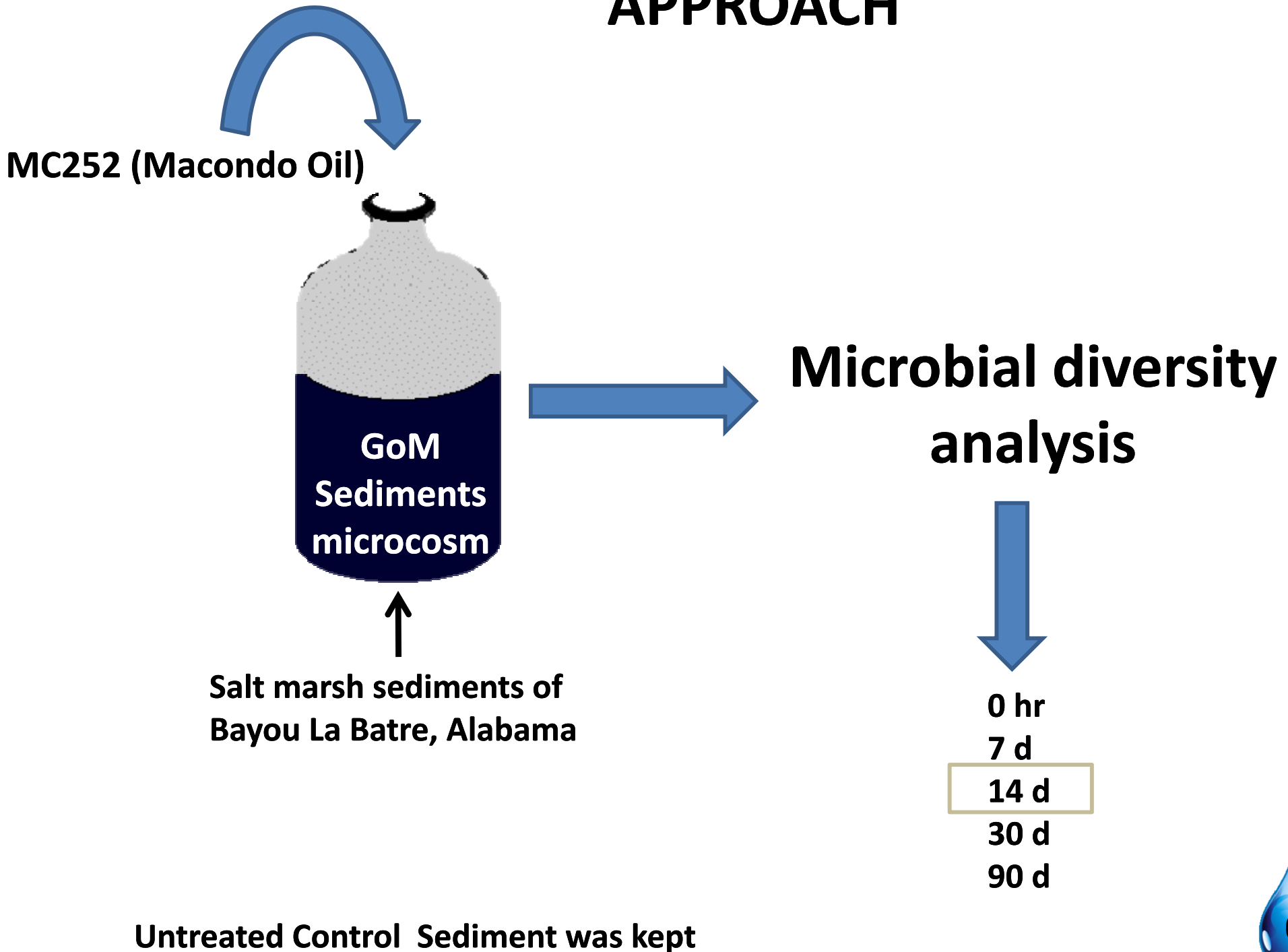
- Determine the effects of oil on the microbial population dynamics
- The oil degrading potential of the GoM sediments by looking at the “oil-eating” (biodegradative) bacteria



Study Sites

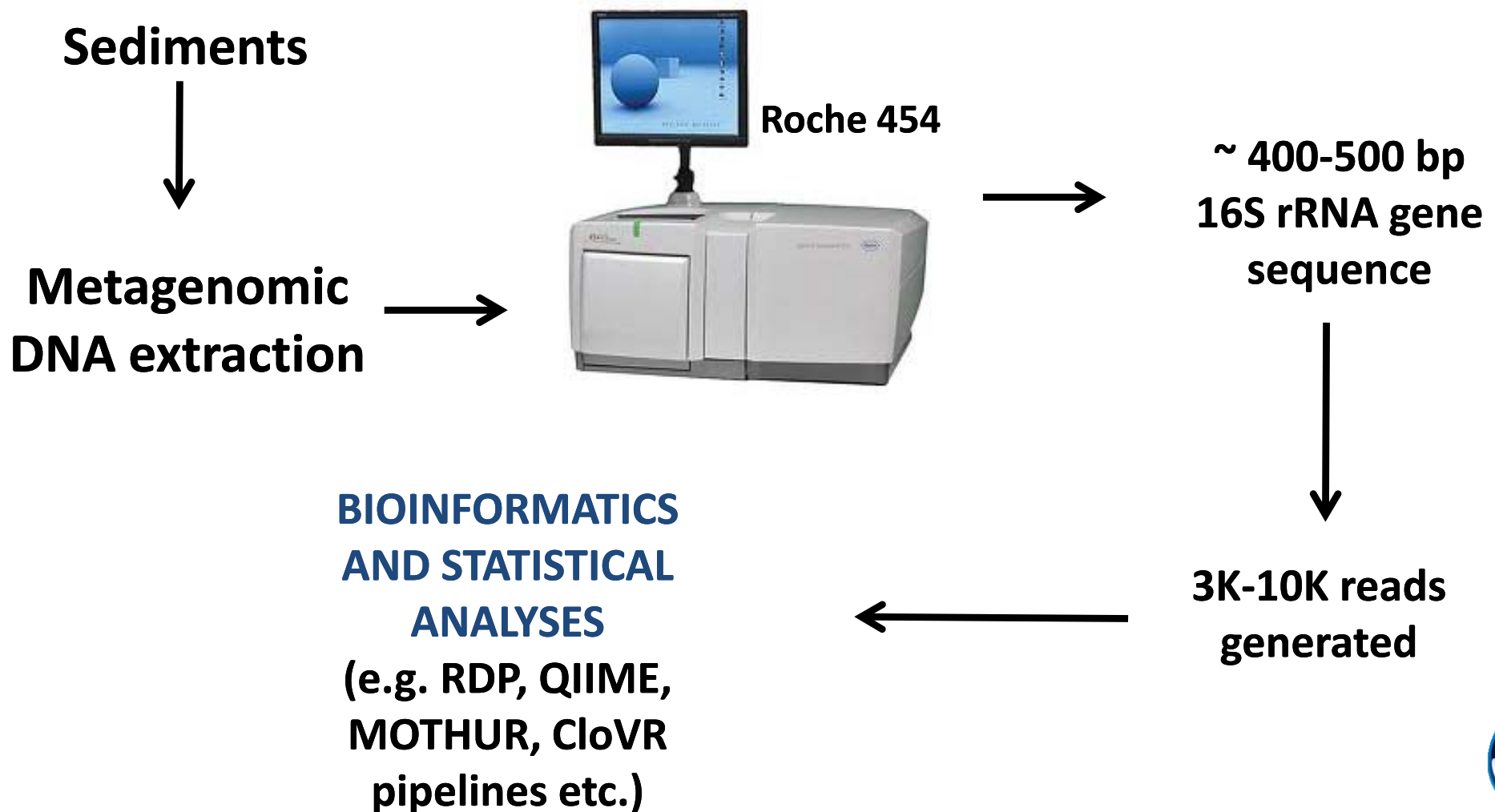


APPROACH



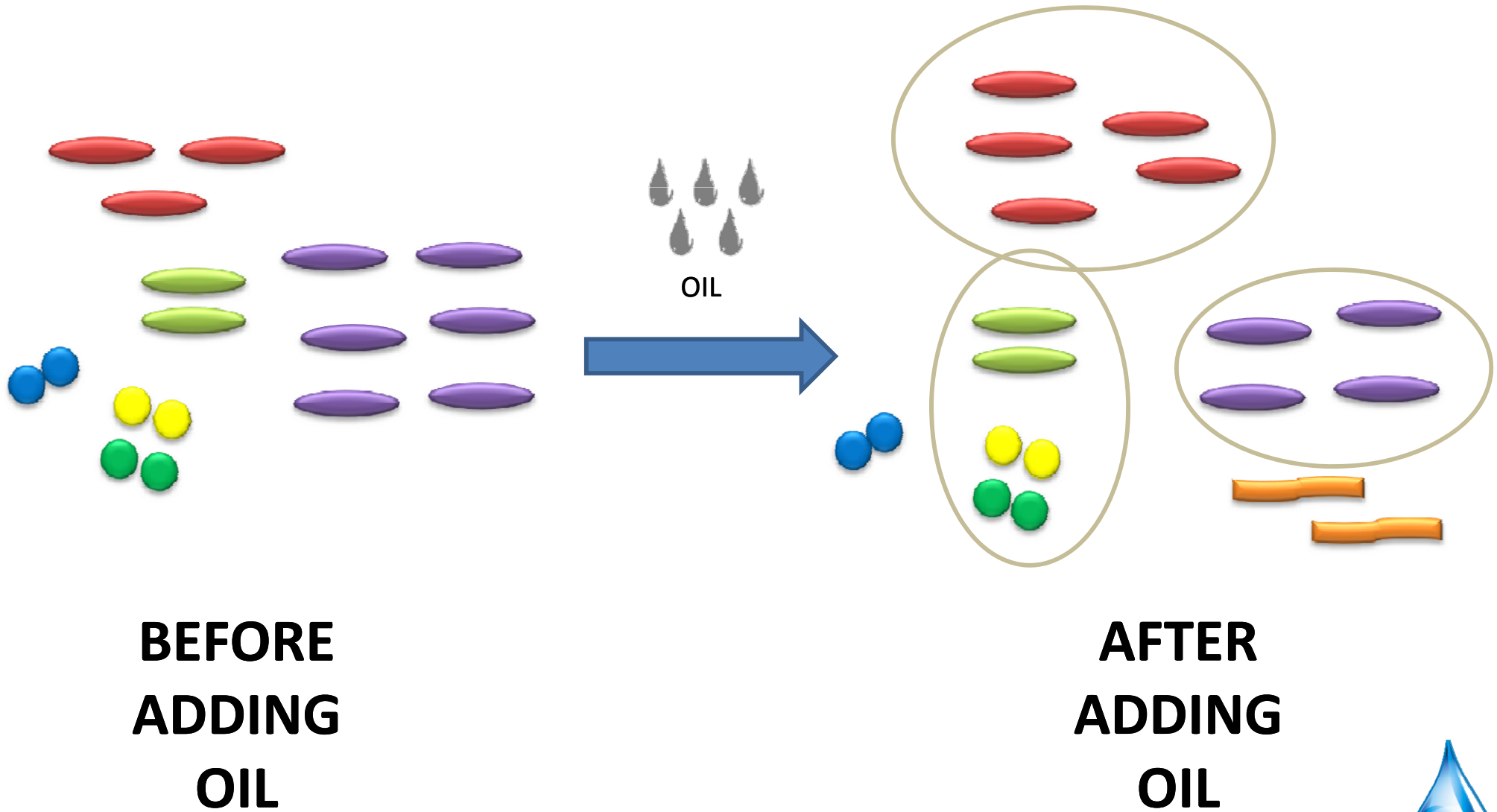
STATE OF THE ART GENOME TECHNOLOGY

bTEFAP for microbial diversity analysis

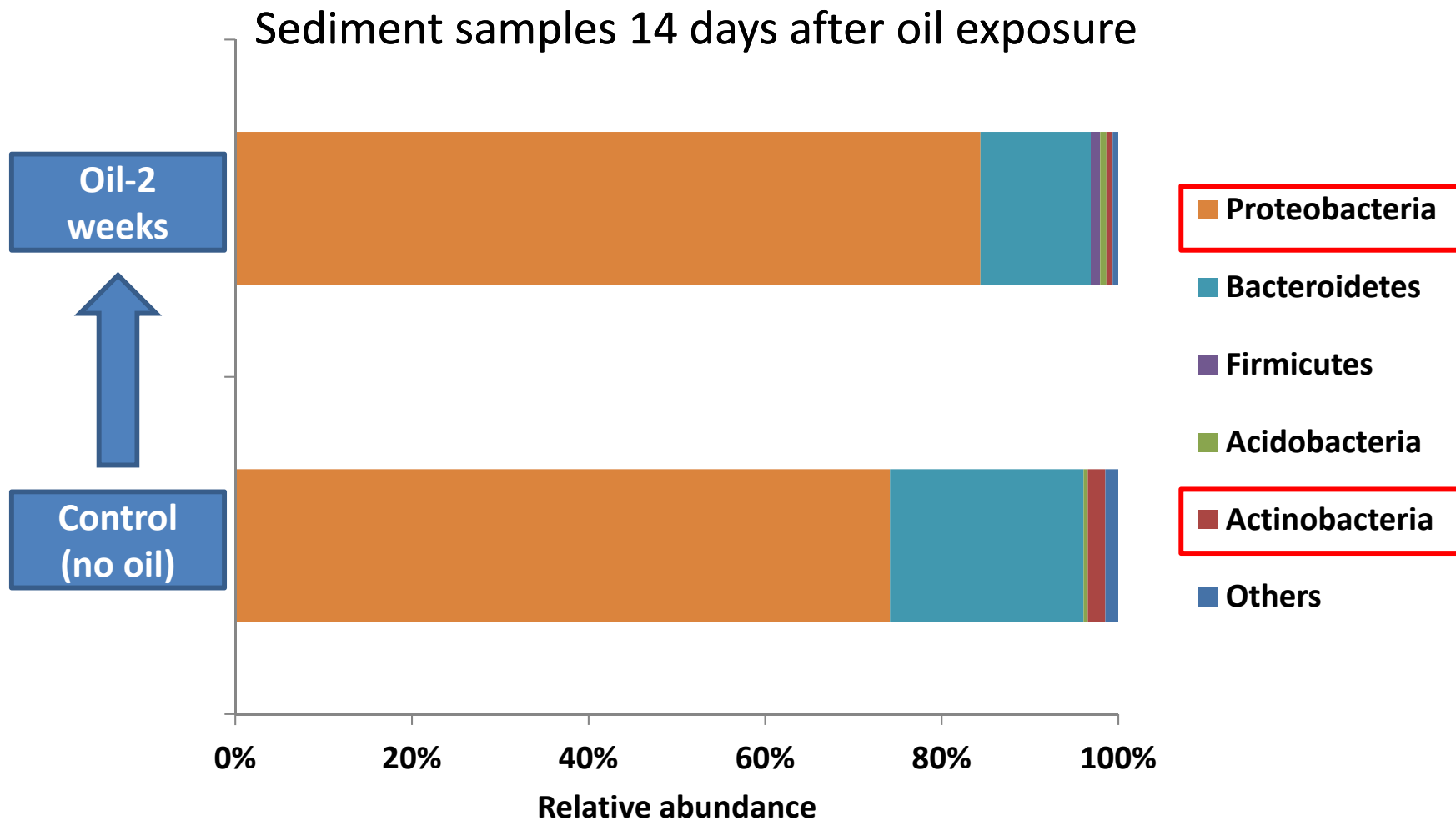


EXPECTED RESULTS

GoM sediments



Phylum level distribution



Bar charts depicting the phylum level distribution of the microbial population before (C, Control) and after two-week incubation with MC252 oil (O-2) of the salt marsh sediments from of Bayou La Batre, Alabama using RDP classifier.

Proteobacteria: Nitrogen fixation; pathogens; Bacteroidetes = distributed in the environment, including in soil, in sediments, sea water and in the guts and on the skin of animals;; Firmicutes: Gram positive; Acidobacteria = Important for sound ecosystem; found in soils and sediments; Actinobacteria = Soil and sediment bacteria, involved in carbon cycle;

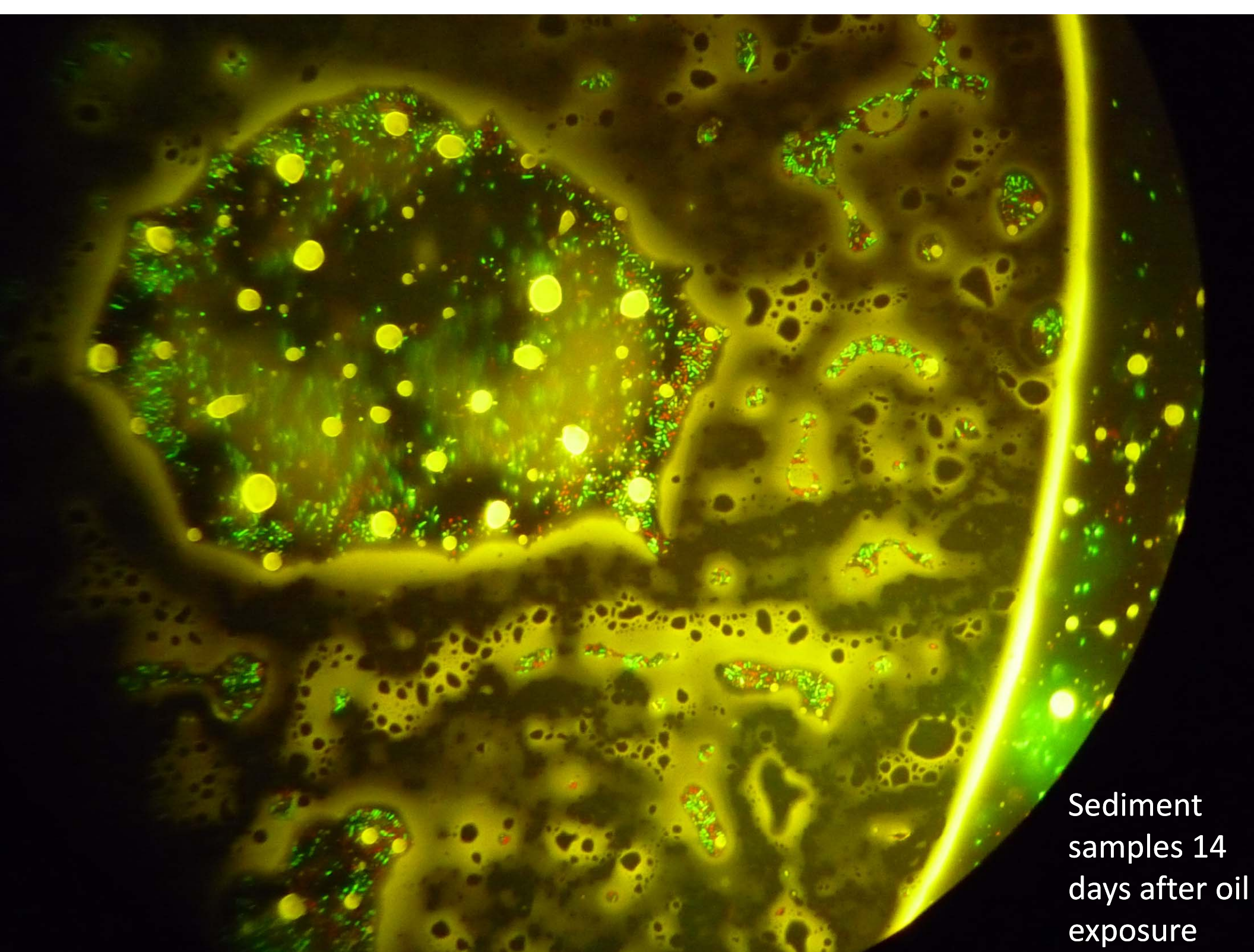


Class level distribution

Sediment samples 14 days after oil exposure

Control (no oil)		%	class	%	Oil-2 weeks	
		0.3	Epsilonproteobacteria *	3.7		
		0.3	Betaproteobacteria	0.1		
		15.1	Deltaproteobacteria *	7.2		
		5.6	Alphaproteobacteria *	8.7		
		37.5	Gammaproteobacteria *	46.3		
		3.0	unclassified "Proteobacteria"	3.2		

(* = significantly different at 0.01)



Sediment
samples 14
days after oil
exposure

OTU heatmap

Consensus Lineage	C	O2
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;Thiomicrospira;		19
Bacteria;Proteobacteria;Gammaproteobacteria;	16	19
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;		26
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Amphritea;		88
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Thalassolituus;		27
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;		86
Bacteria;Proteobacteria;Gammaproteobacteria;	13	18
Bacteria;Proteobacteria;Gammaproteobacteria;	3	19
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;		42
Bacteria;Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_incertae_sedis;Thiohalophilus;	6	11
Bacteria;Proteobacteria;Gammaproteobacteria;	17	30
Bacteria;Proteobacteria;Gammaproteobacteria;	7	11
Bacteria;Proteobacteria;Gammaproteobacteria;	8	19
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Desulfuromonadaceae;	6	10
Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Arcobacter;		34
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;		116

OTU heatmap showing the number of times each OTU was found in the sediment samples (C and O-2) including the taxonomic assignment. The OTU heatmap displays raw OTU counts per sample, where the counts are colored based on the contribution of each OTU to the total OTU count present in that sample (blue: contributes low percentage of OTUs to sample; red: contributes high percentage of OTUs). The lineages that increased or appeared after MC252 oil addition are highlighted with yellow color.



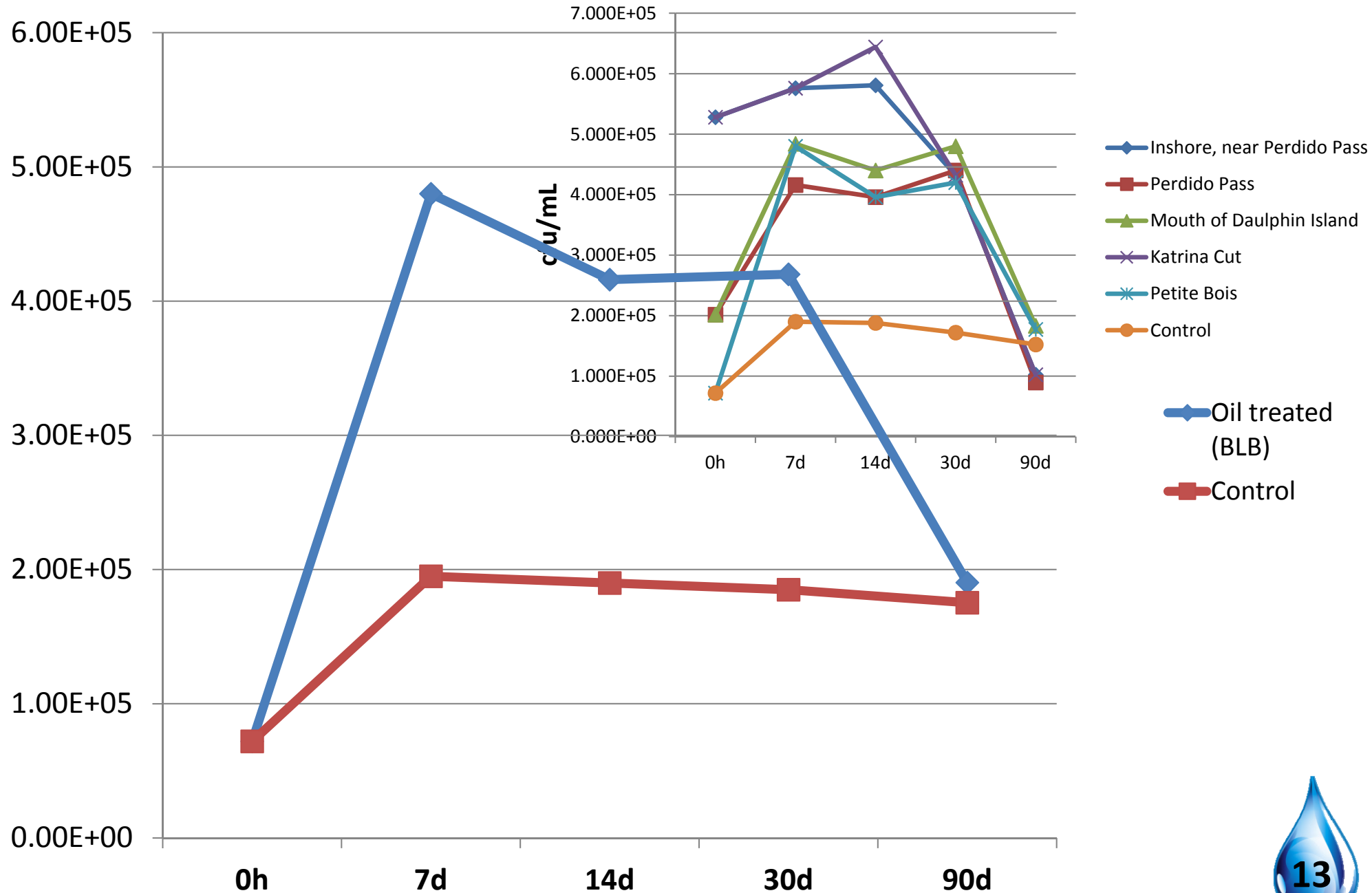
THE OIL DEGRADING POTENTIAL OF THE GoM SEDIMENTS

PCR amplification of the biodegradative genes

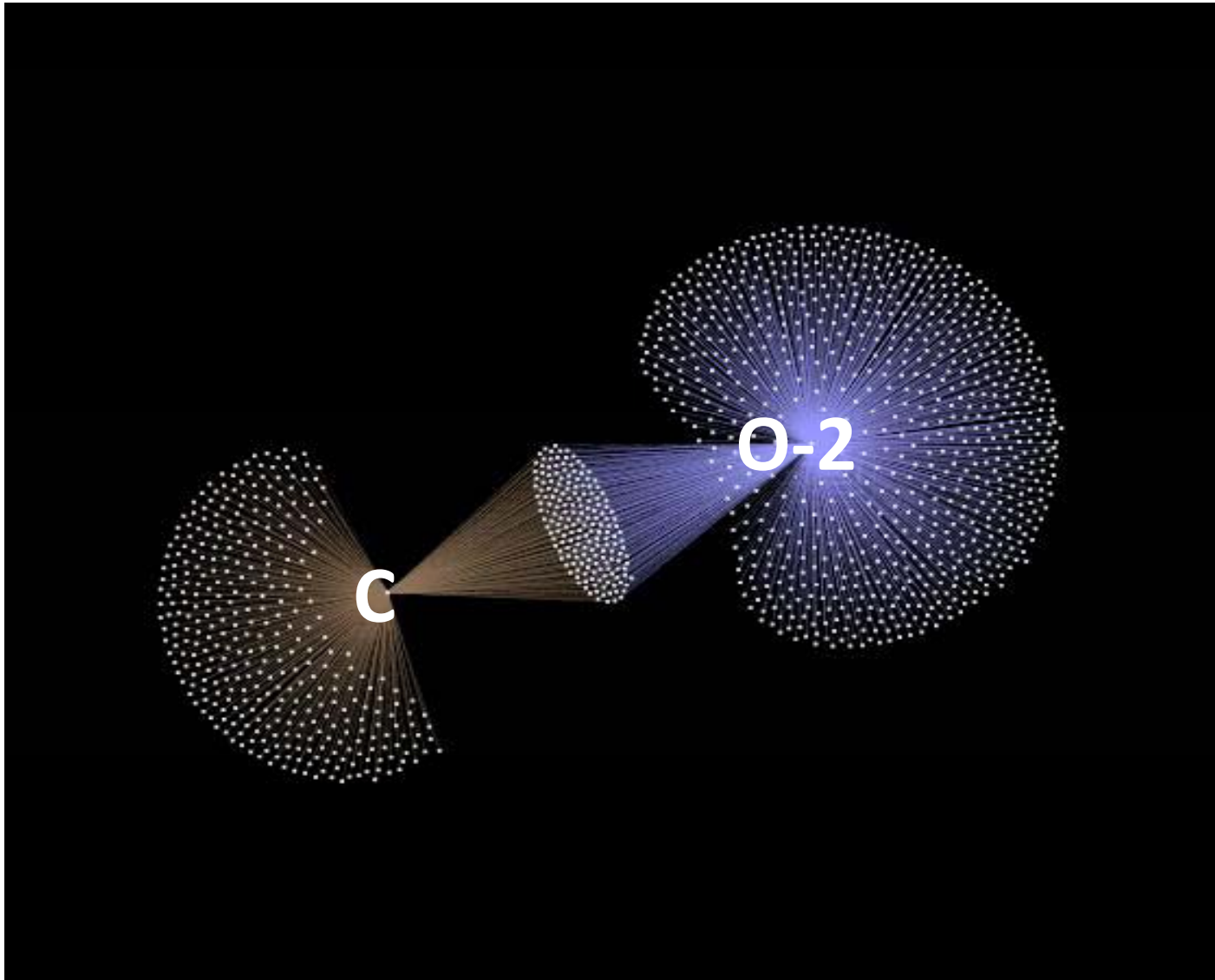
Table 1. Results for PCR amplification using different primer sets for biodegradative genes in community DNA extracted from control and oil enriched samples from Gulf coast.

Sample I.D.	Detail Description	alkB (546 bp)	Rh-alkB1 (629 bp)	Rh-alkB2 (552 bp)	Rh-alkB194 (194 bp)	alkB870G (870 bp)	TS2S/ deg1RE (550bp)	(Ac) alkM (496 bp)	ndoB (642 bp)	cndoB (501 bp)	C23DO (238 bp)	XylEb (834bp)	Cat2,31a (405-408 bp)	todC1 (560bp)	bphA1(830 bp)
		Alkane hydroxylase (<i>alk</i>)					Naphthalene dioxygenase (<i>ndoB</i>)			Catechol 2,3 dioxygenase (C23DO)			Toluene dioxygenase (<i>tod</i>)	Biphenyl dioxygenase (<i>bph</i>)	
C1	BLB-NSC-2 (no oil)	-	-	-	-	-	-	-	-	-	-	-	-	-	+
02	BLB-14D-2	-	-	-	-	+	-	-	-	-	+	-	+	-	+

Microbiology of the sediment samples collected from Gulf Coast



OTU (Operational Taxonomic Units) network map



OTU (Operational Taxonomic Units) network map generated by QIIME showing OTU interactions between the rarefied sediment samples without (C-Control) and after 2 weeks incubation with MC252 oil (O-2). The lines radiating from control samples C are colored brown and oil treated O-2 are colored blue and represent the OTUs present. Each white dot represents an OTU. OTU size is weighted with respect to sequence counts within the OTU.

Summary

- Overall, some changes in the microbial community occurred, but not drastic
- Certain oil eating (biodegradative) class of bacteria proliferated
- Pathogens such as *Vibrios* were not detected
- Long-term (90 days – 120 days) effects on the microbial community are being investigated

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