Confirming *in situ* Benzene Biodegradation Under Anaerobic Conditions Using Stable Isotope Probing

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Overview

Benzene Biodegradation

Stable Isotope Probing

Analysis of 300+ SIP Samples

Results by Treatment

Anaerobic Benzene Biodegradation

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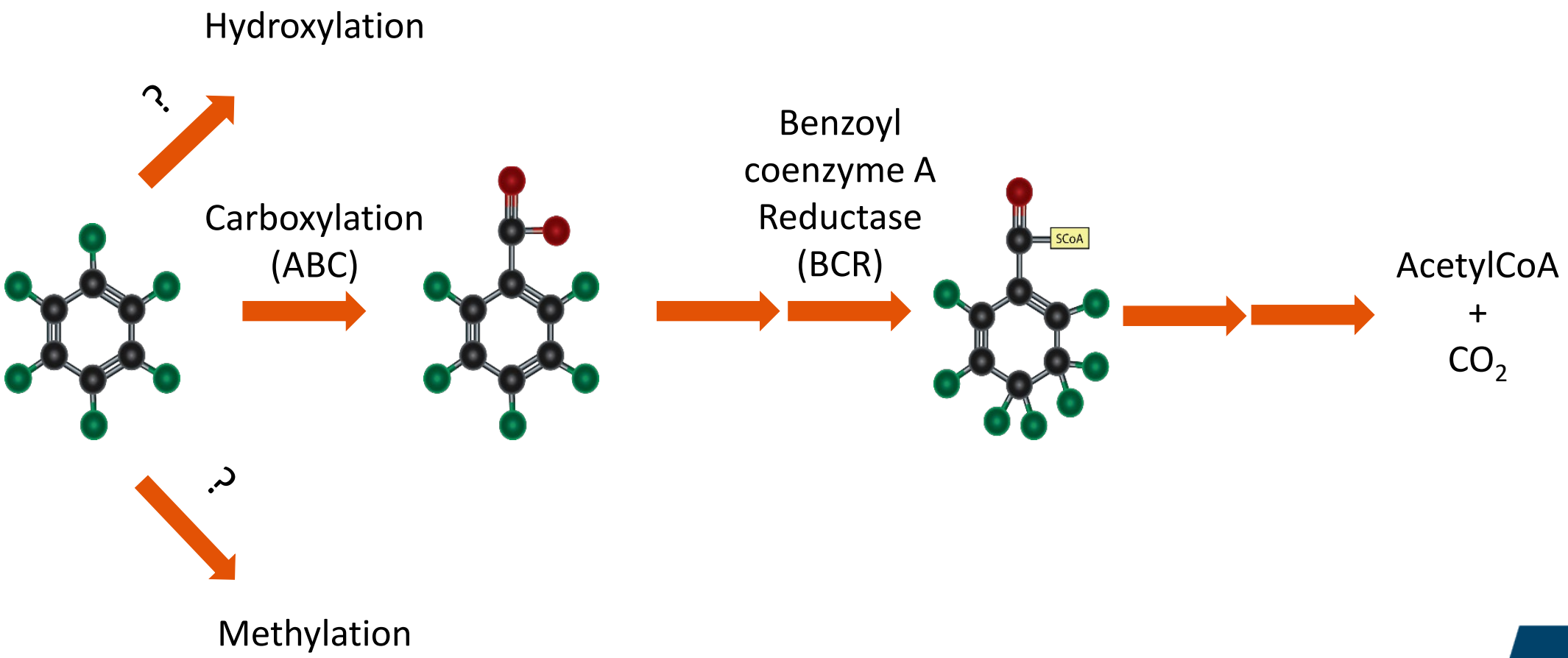
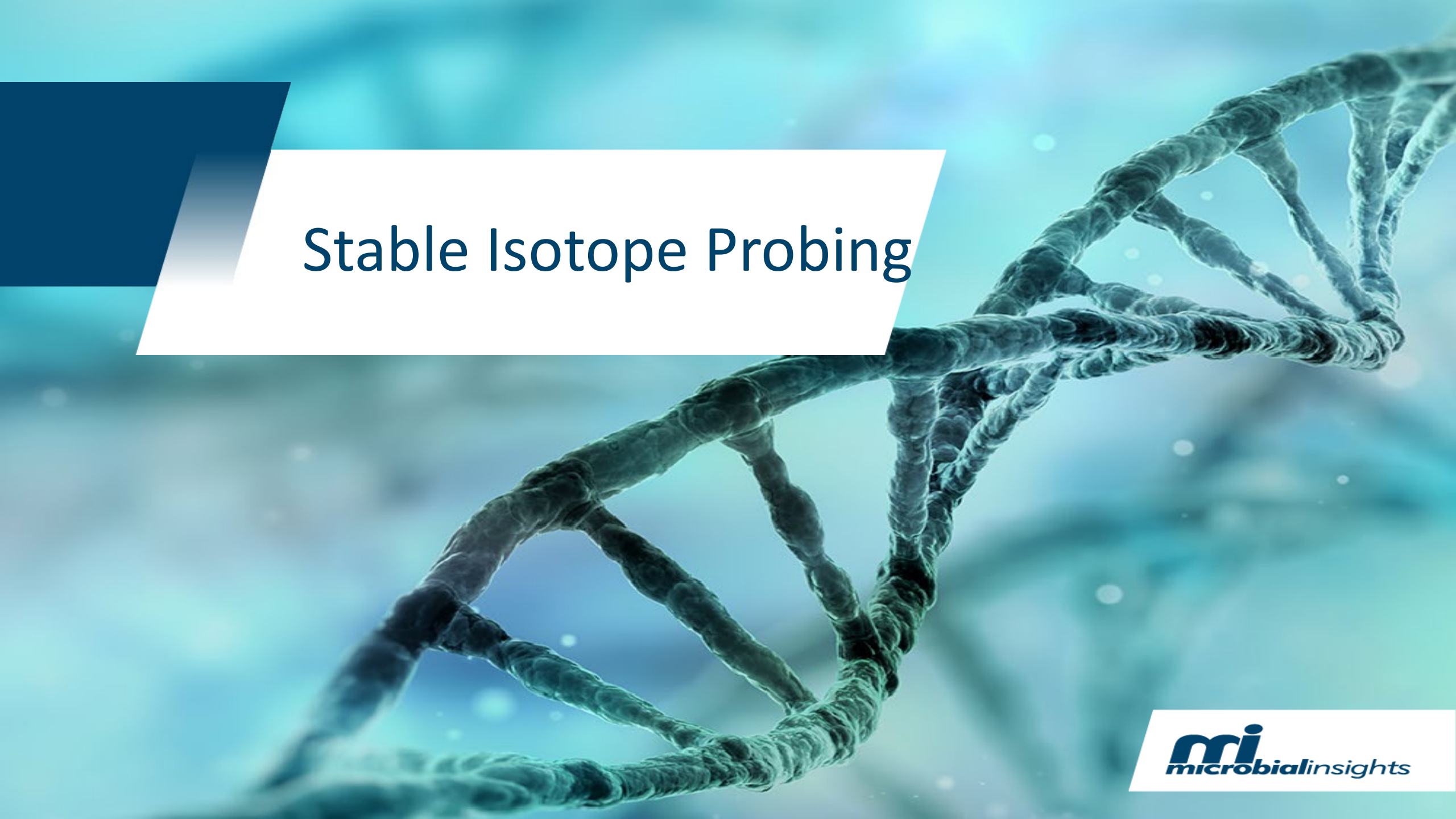
Anaerobic Benzene Degradation

- Anaerobic conditions common, particularly in source area
- Reported under all terminal electron accepting processes
- Degraders likely not ubiquitous
- May be slow with long lag times and/or inhibited by co-contaminants
- A determining factor in the success or failure of MNA

Limited qPCR Assays Available



Stable Isotope Probing



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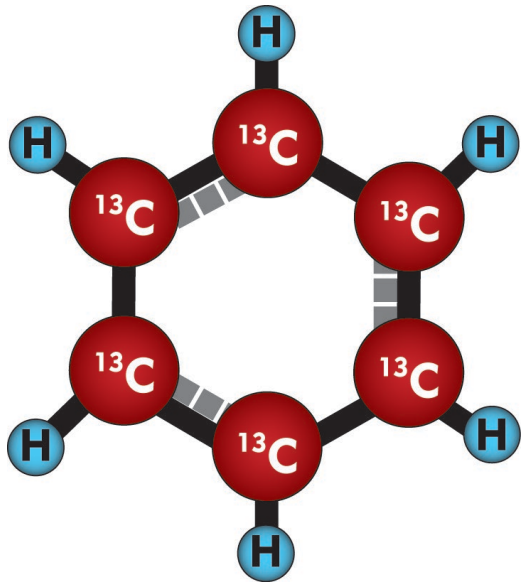
Stable Isotope Probing

- Specially produced “heavy” compounds which are composed of 99+% ^{13}C
  - Natural Compounds are 99% ^{12}C
  - Same characteristics as original compound
  - Behave similar to the natural compound

- Used as “tracers” to increase our understanding of contaminant fate
  - BTEX, fuel oxygenates, naphthalene, chlorobenzene, 1,4-dioxane

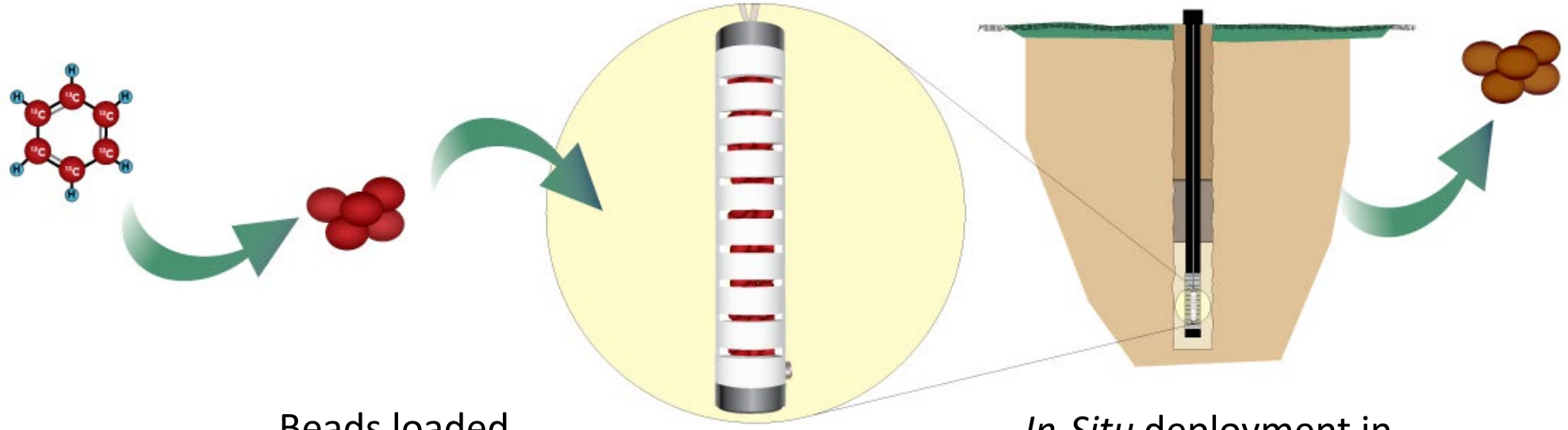


Stable Isotope Probing

^{13}C labeled Benzene

Bio-Trap® with ^{13}C-benzene loaded beads

Beads analyzed following deployment

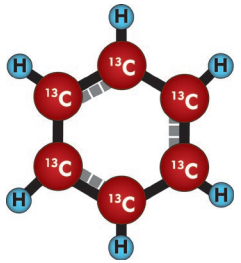


Beads loaded with ^{13}C compound

In-Situ deployment in monitoring well

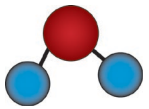
Bio-Trap SIP Analysis

Residual ^{13}C-Compound



Utilization

$^{13}C/^{12}C$ Dissolved Inorganic Carbon



Mineralization
(C for energy)

$^{13}C/^{12}C$ of Biomarkers

PLFA
DNA
RNA

Metabolism
(C for growth)

Unit of Measure

Amount of ^{13}C relative to ^{12}C is expressed by the $\delta^{13}C$ notation

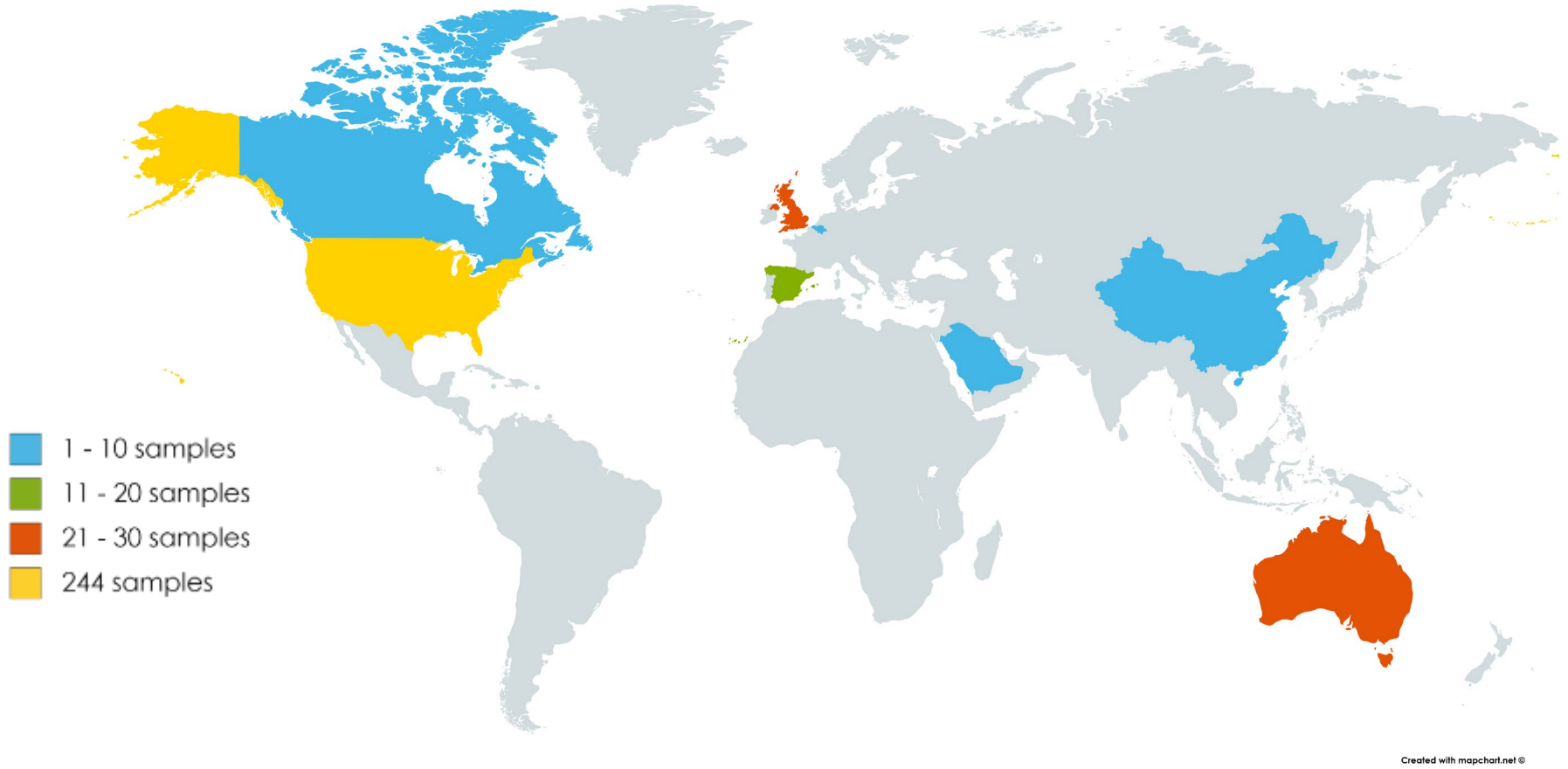
$$\delta^{13}C\,[‰] = \left(\frac{(^{13}C/^{12}C)_{\text{Sample}}}{(^{13}C/^{12}C)_{\text{Standard}}} - 1 \right) \cdot 1000$$

The standard is a specific carbon-containing mineral from a specific location: Pee Dee Belemnite (PDB)

Units of $\delta^{13}C$ are ‰ or “per mill”

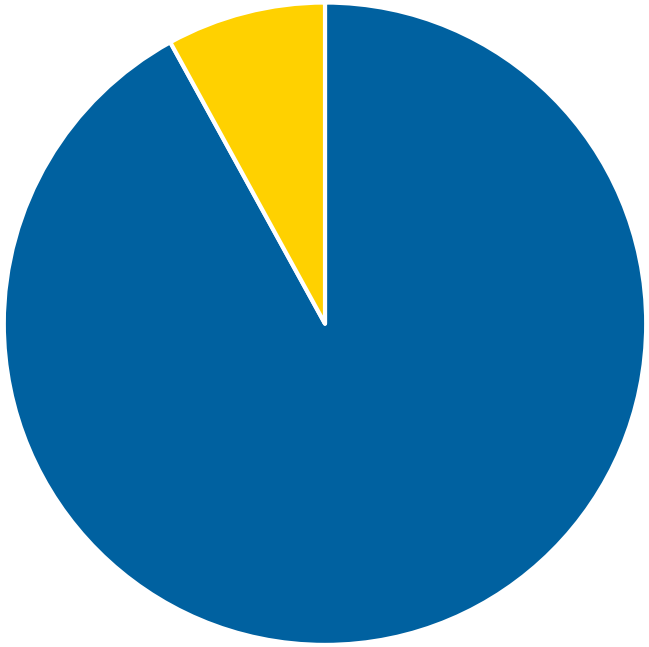
SIP Benzene Dataset

309 samples from 90 different sites



SIP Benzene Dataset

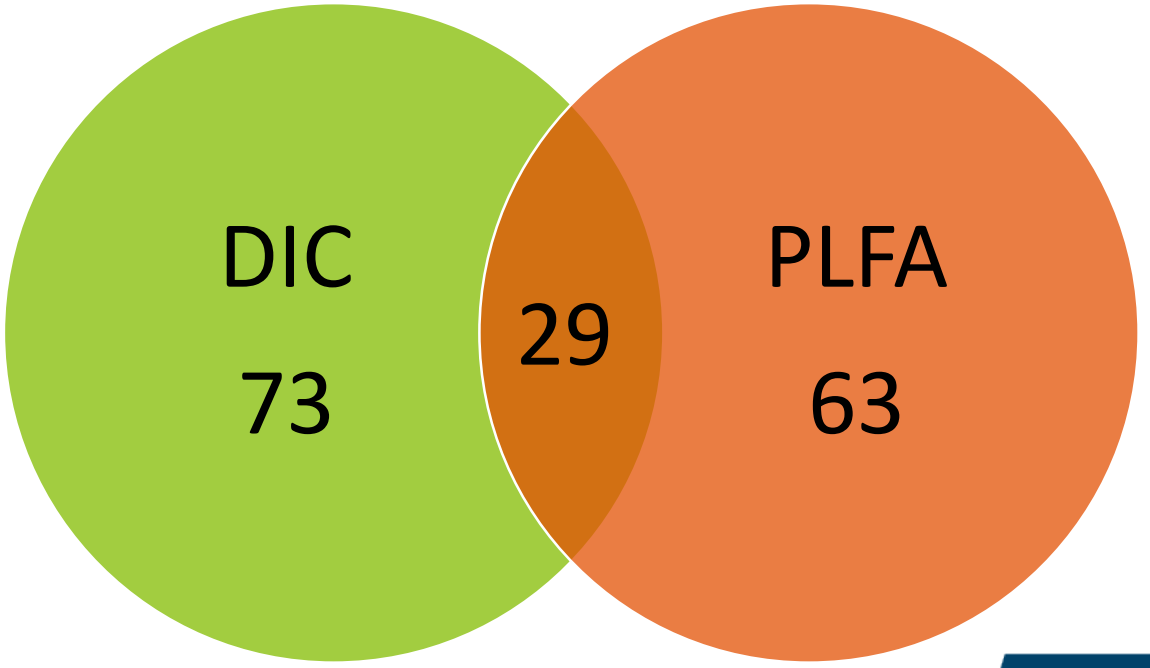
Benzene Biodegradation



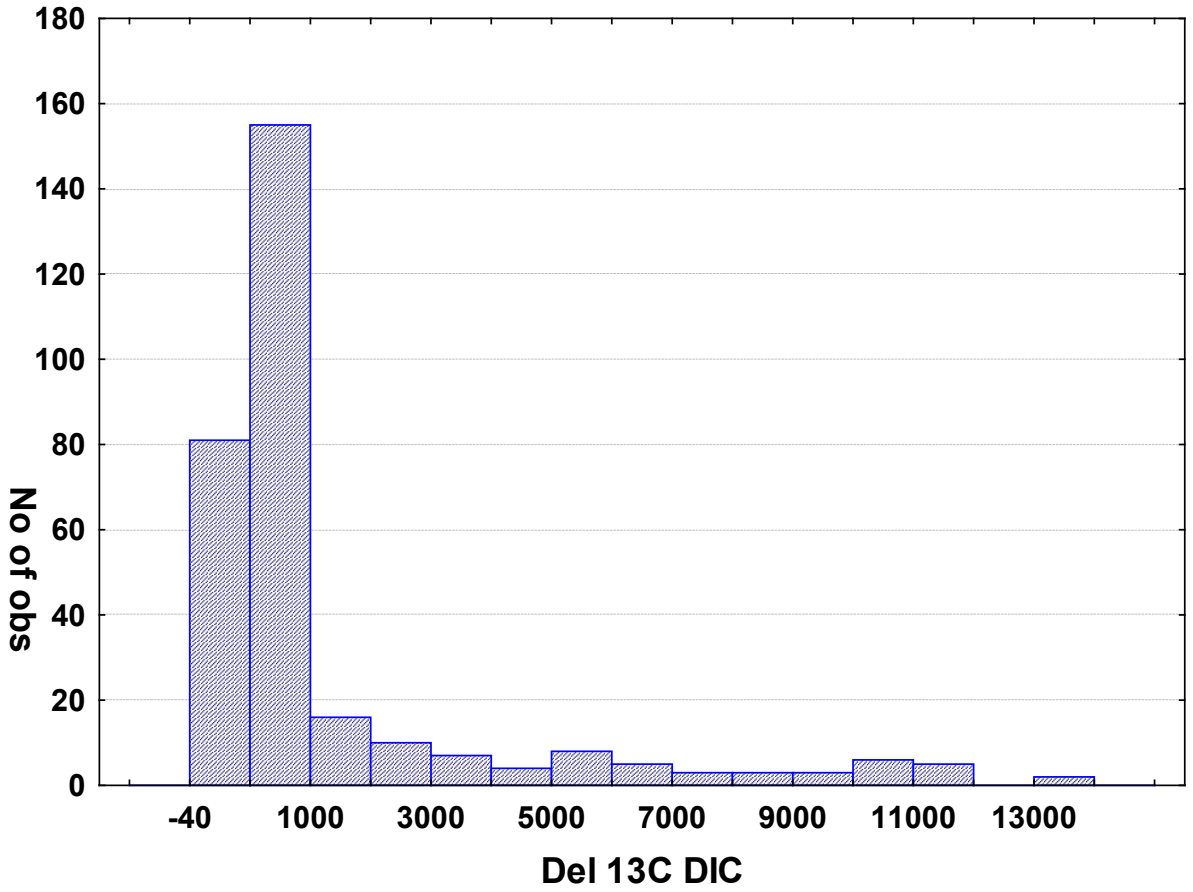
Yes
92%

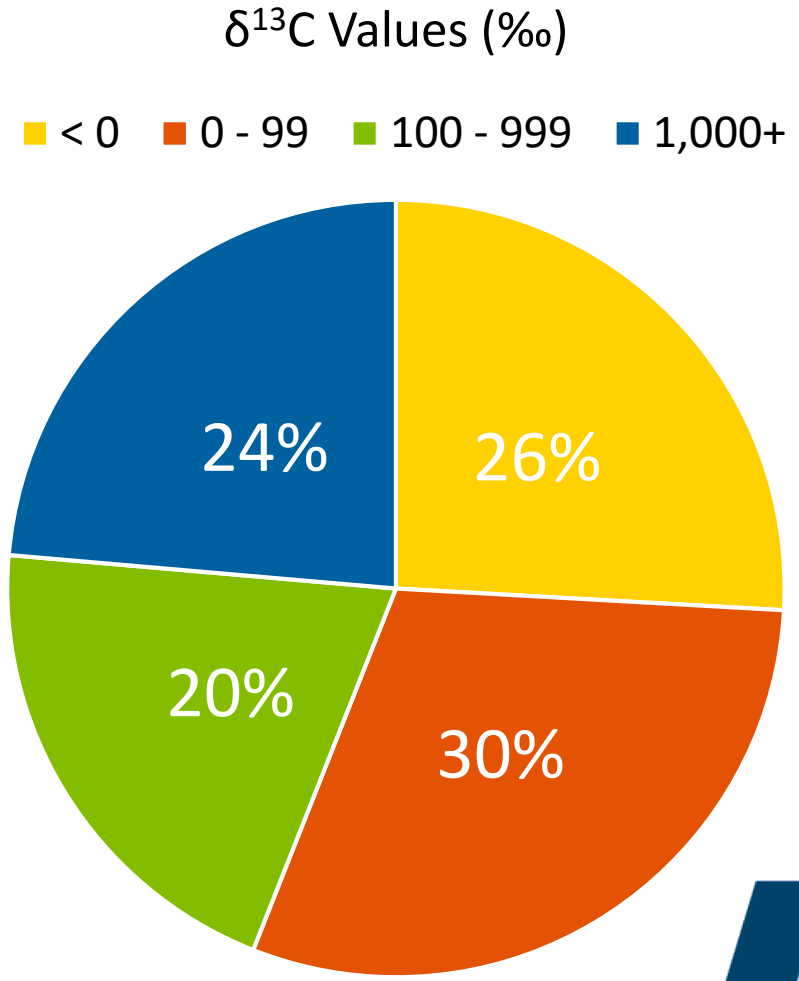
No
8%

Samples with high ^{13}C enrichment
($\delta^{13}C > 1{,}000‰$)

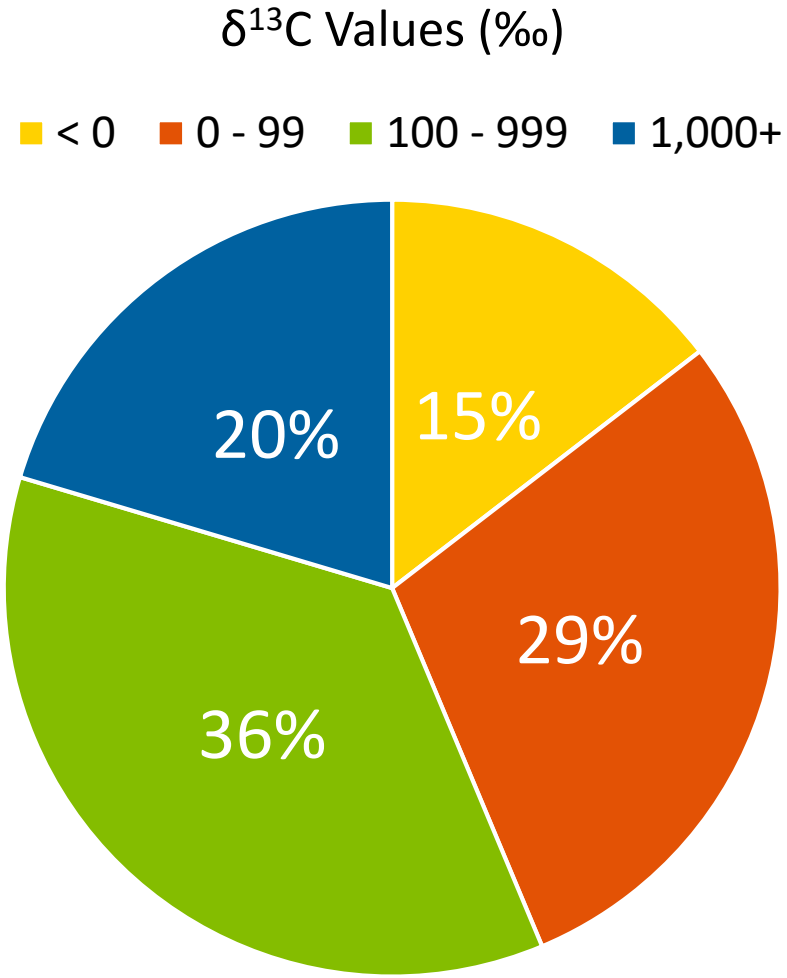


^{13}C-Enriched DIC





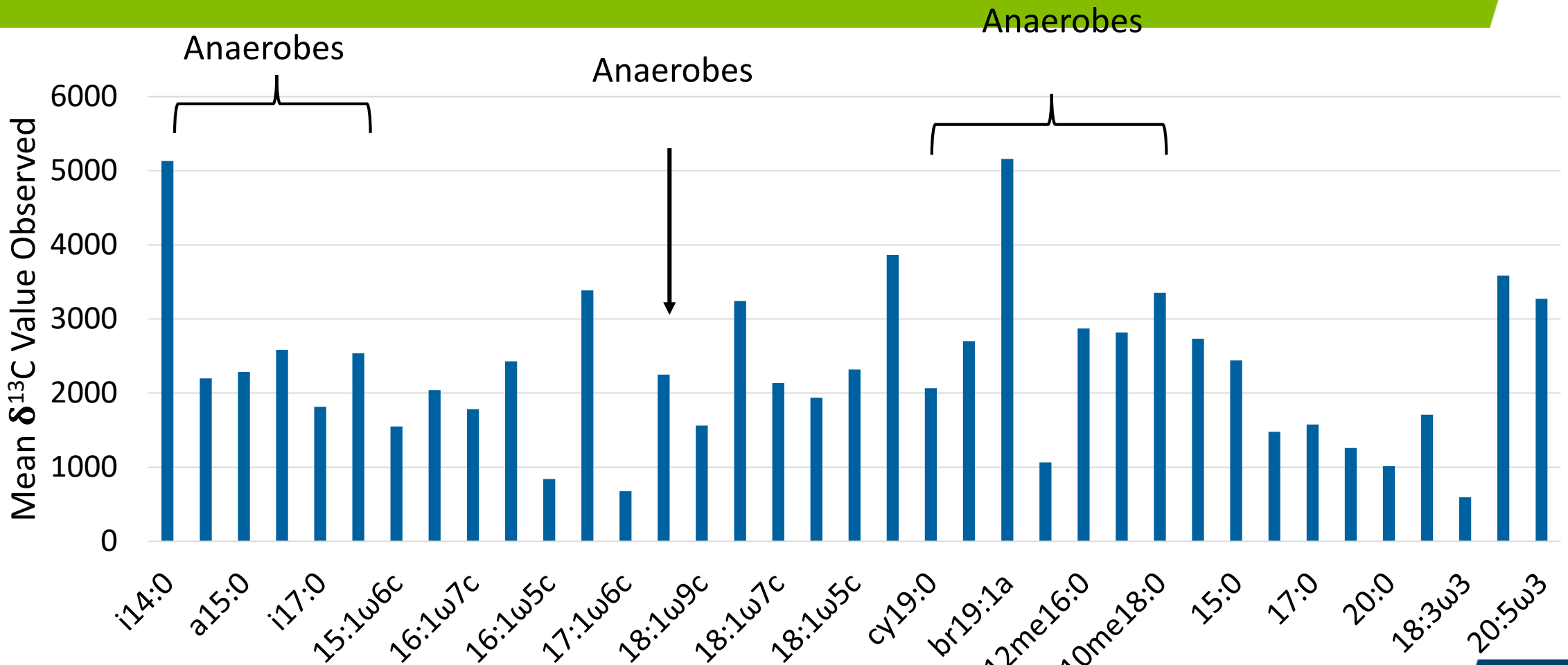
^{13}C-Enriched PLFA



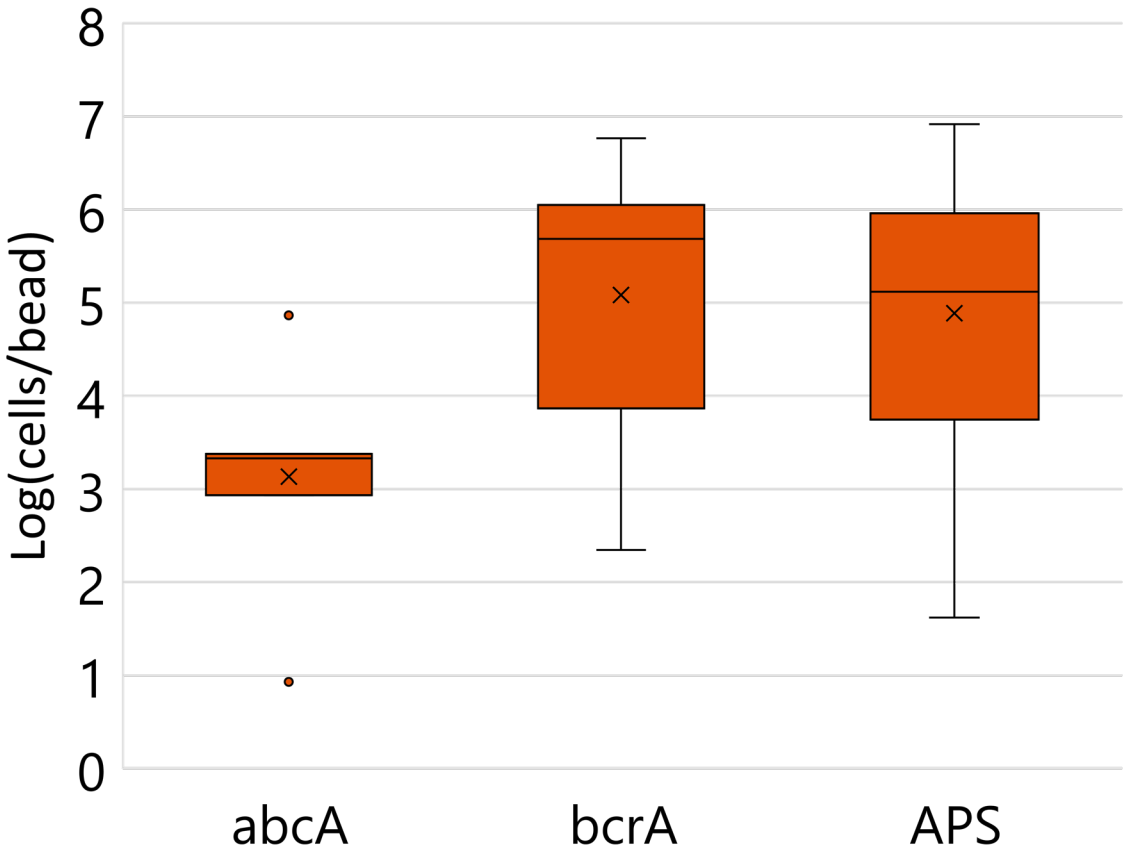
55% (169 of 309) had at least one ^{13}C-enriched anaerobic indicator

- Anaerobic Proteobacteria (cy17:0 and cy19:0)
- Firmicutes (Terminally Branched Saturated)
- Anerobic Metal Reducers (Branched Monoenoic)
- Sulfate Reducers/Actinomycetes (Mid-Chain Branched Saturated)

^{13}C-Enriched Fatty Acids



Subset with qPCR Analysis



Gene		Detection Frequency
Benzene carboxylase	abcA	11% (7 of 66)
Benzoyl coenzyme A reductase	bcrA	79% (34 of 43)
Sulfate reducers	APS	88% (64 of 73)

45 Samples from *in situ* Microcosm Studies

MNA Unit

BioStim Unit

BioStim Unit

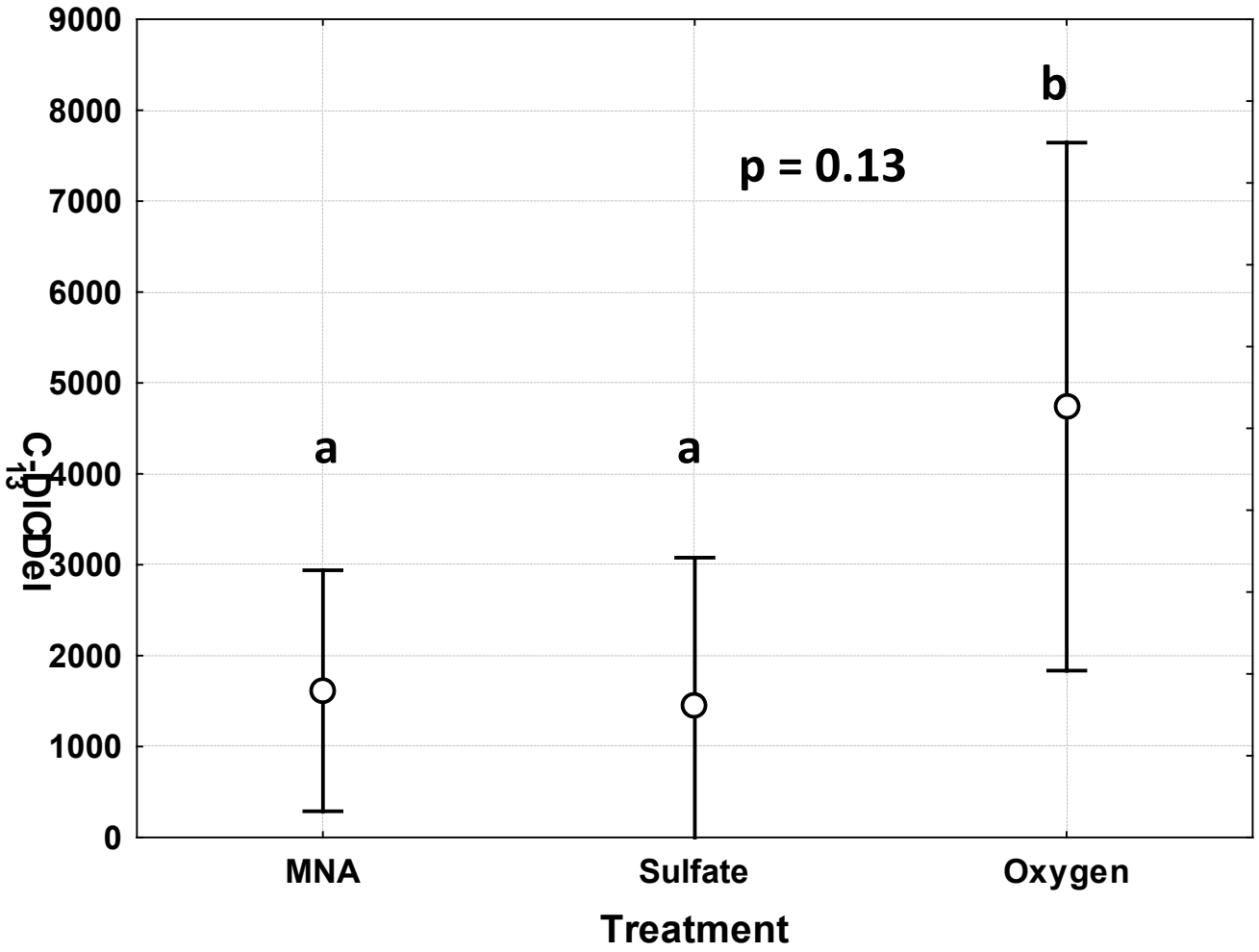
COC

^{13}C-Benzene Bio-Trap

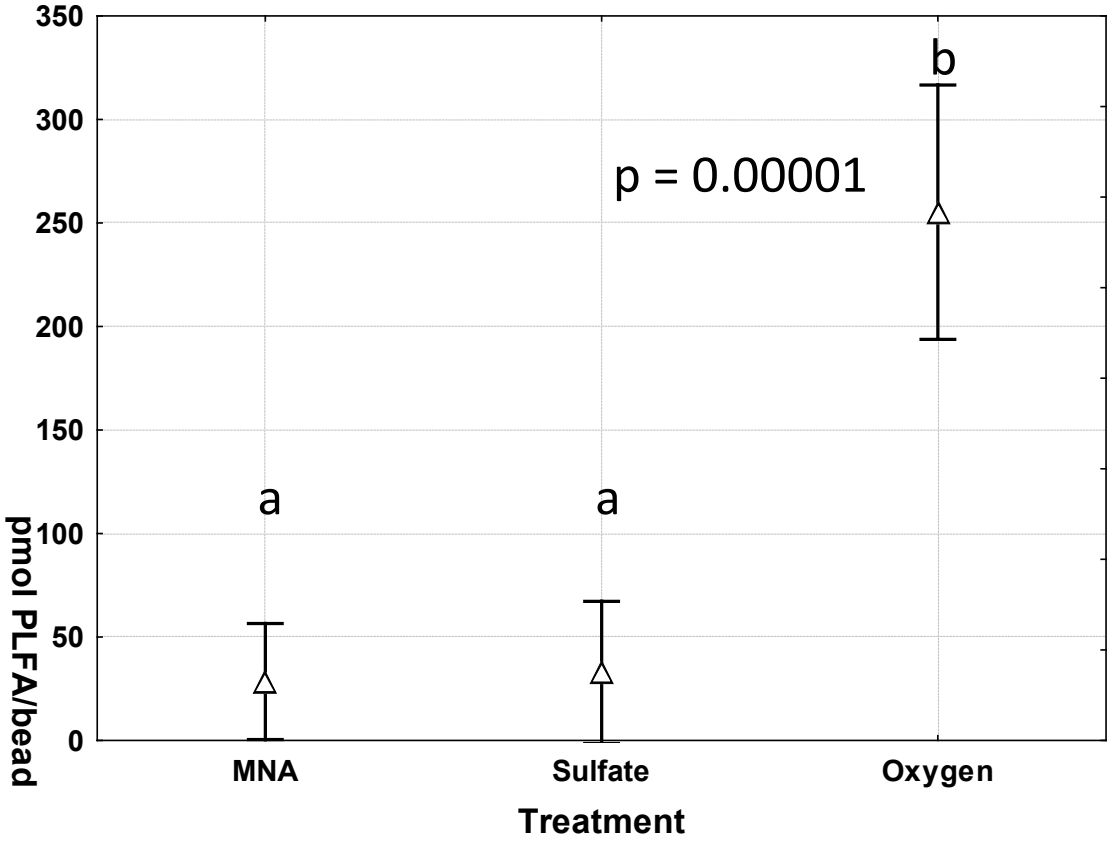
GEO

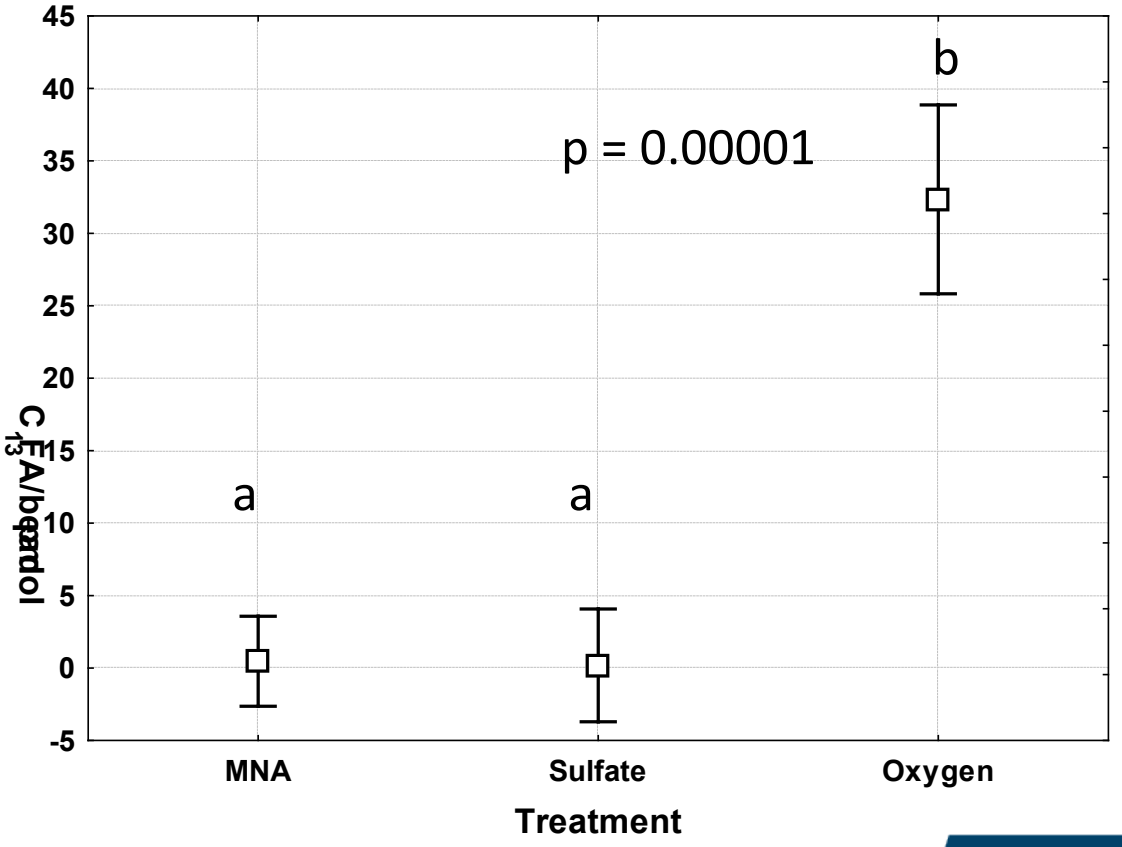
Oxygen or Sulfate

DIC δ^{13}C by Treatment

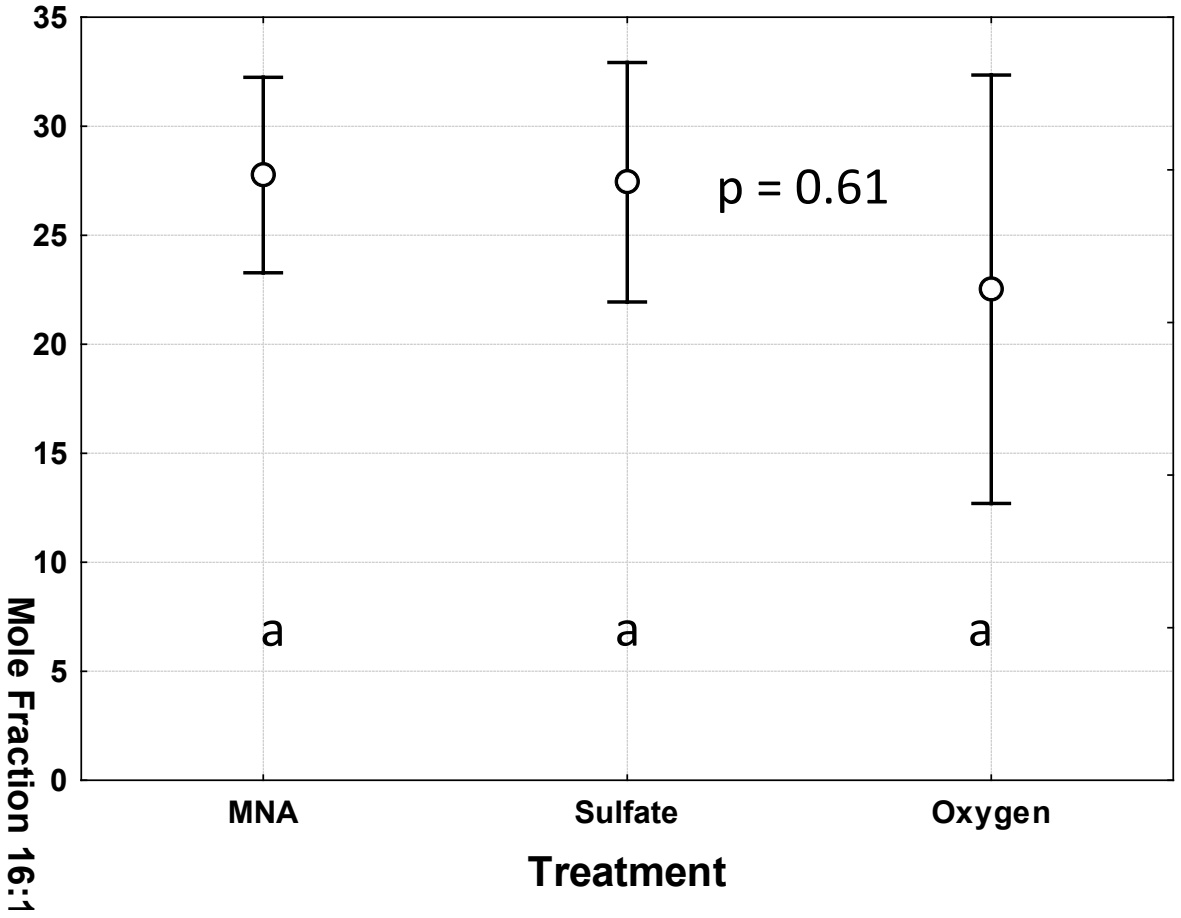


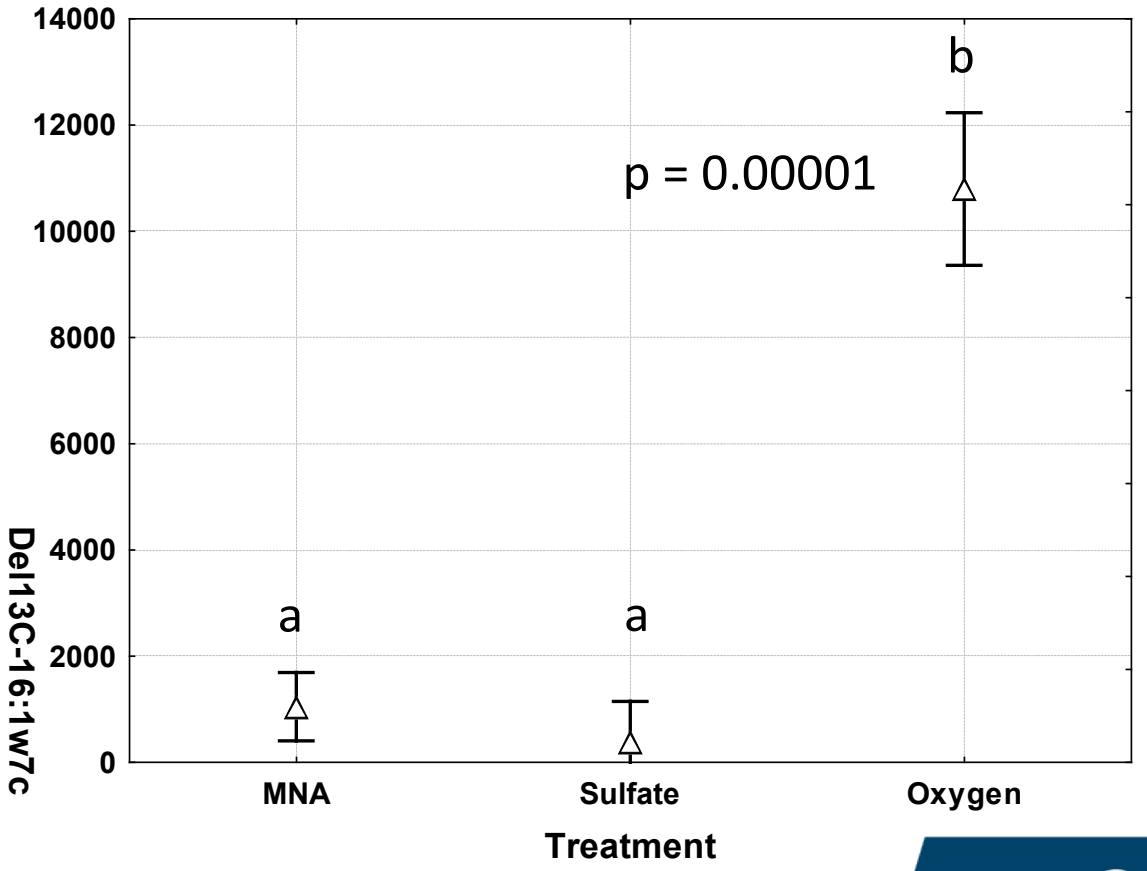
Total and ^{13}C-Enriched PLFA



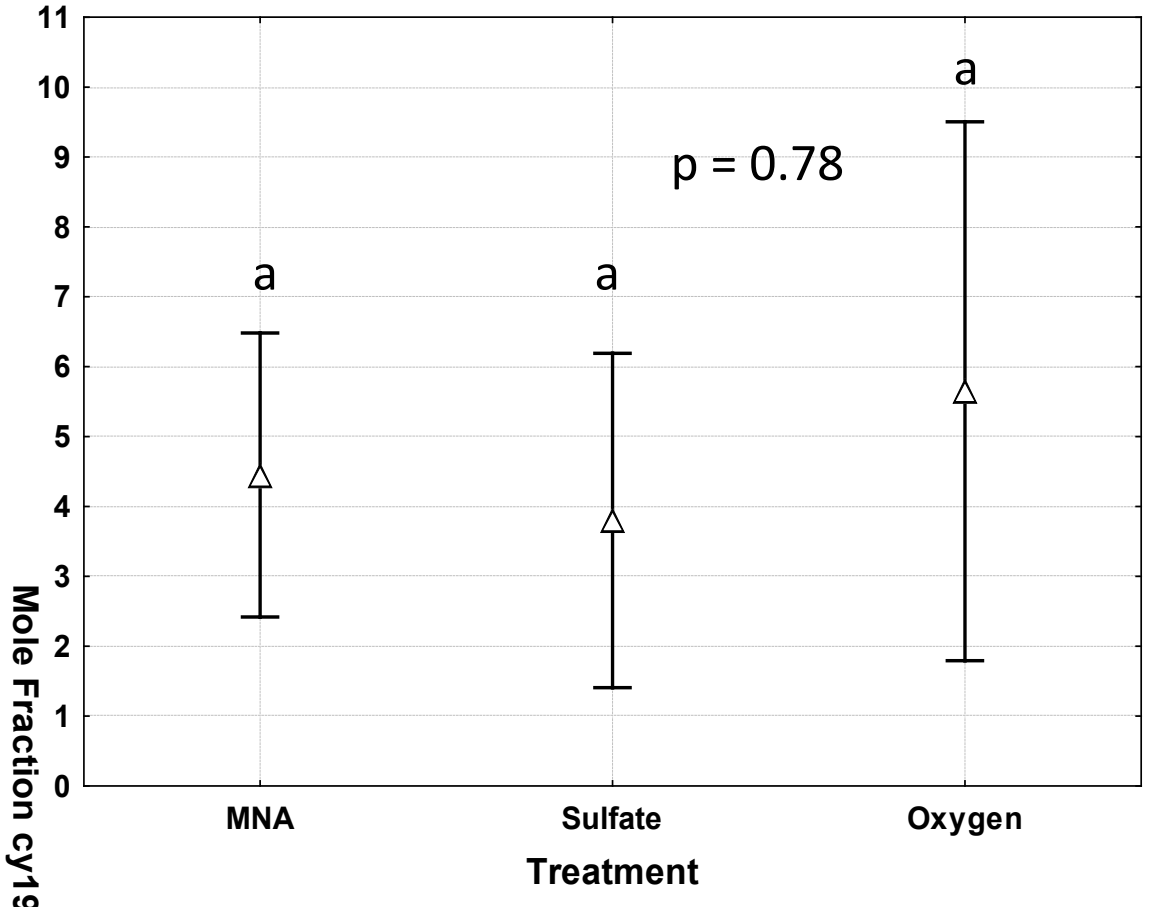


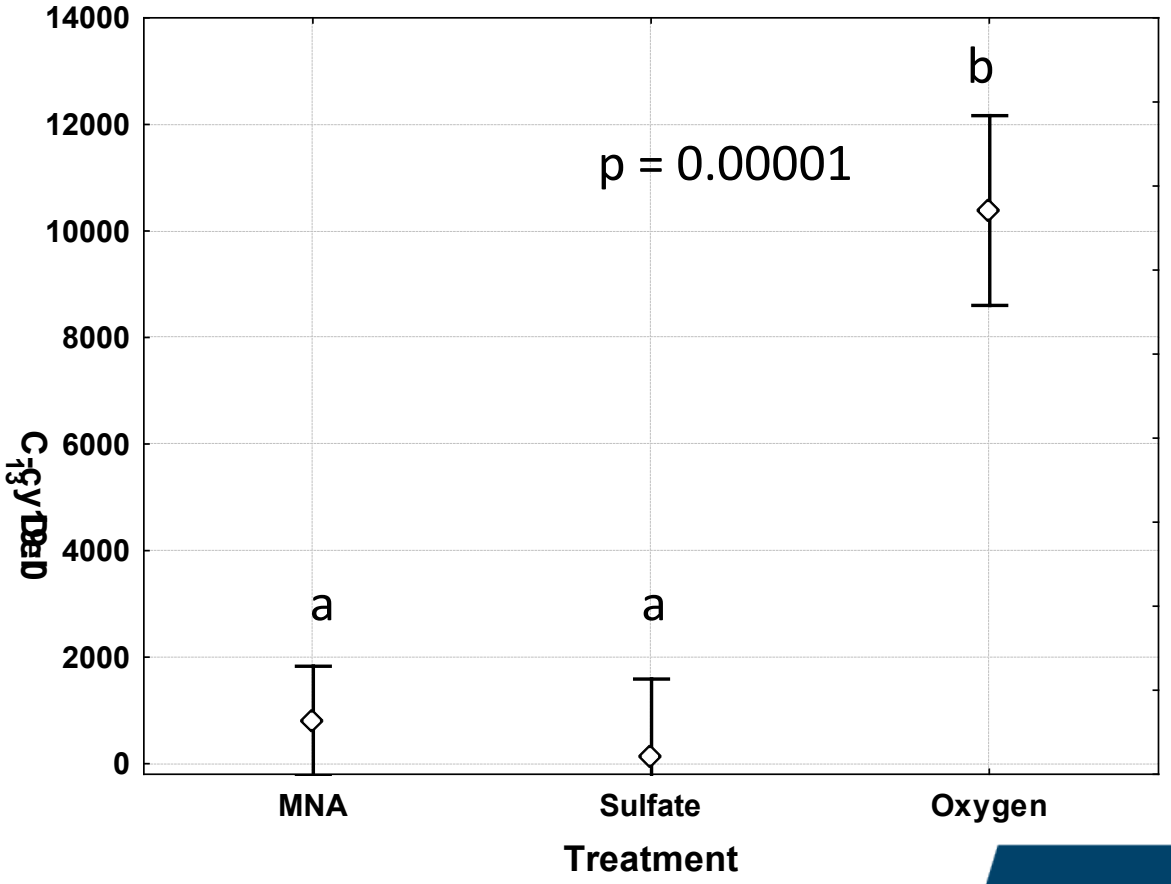
Aerobic Proteobacteria Marker - 16:1ω7c



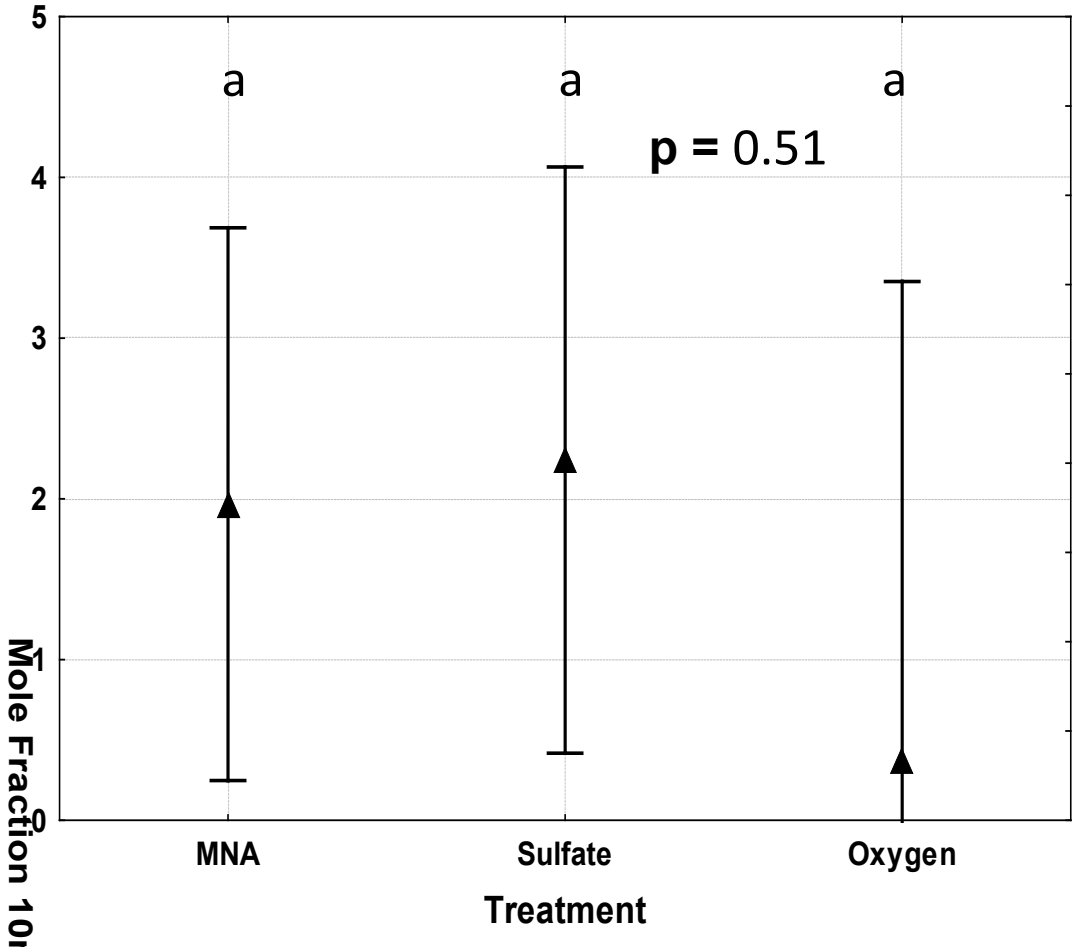


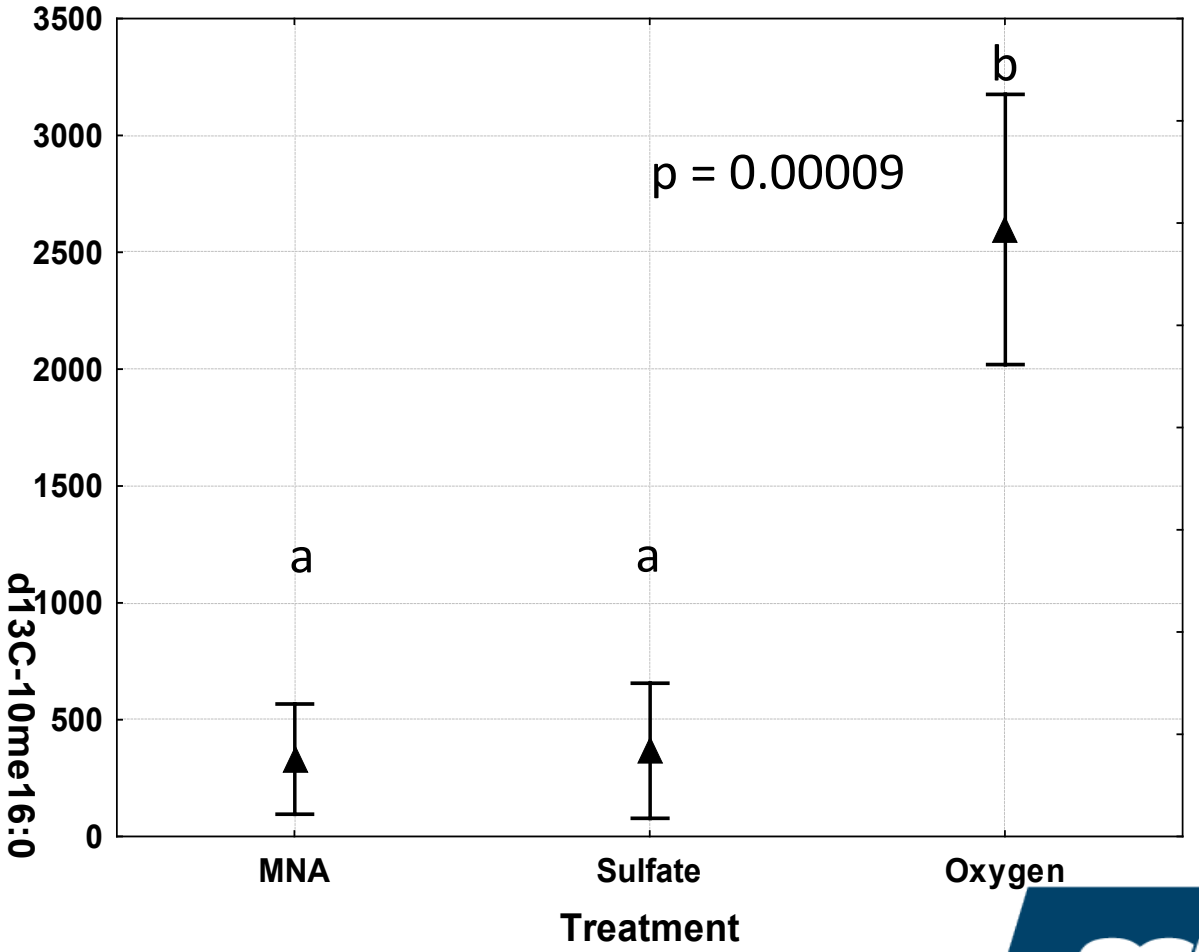
Anaerobic Proteobacteria Marker - cy19:0





Sulfate-Reducer Marker - 10me16:0





Conclusions

- Confirmed benzene degradation in 92% of samples
- ^{13}C-enriched fatty acids indicative of anaerobes in 55% of samples
- Benzene carboxylase gene less frequently detected
- Analysis of samples with known treatment suggested oxygen addition increased overall biomass and stimulated both aerobic and anaerobic degradation of benzene and its metabolites

Thank you for your time

Are there any questions?