Presented to
Fourth International Symposium on Bioremediation and Sustainable Environmental Technologies
Miami, Florida

# Characterization of MTBE Biodegradation Using Multiple Lines of Evidence: Equilibrium Partitioning, CSIA, and Microbial Analysis

May 24, 2017

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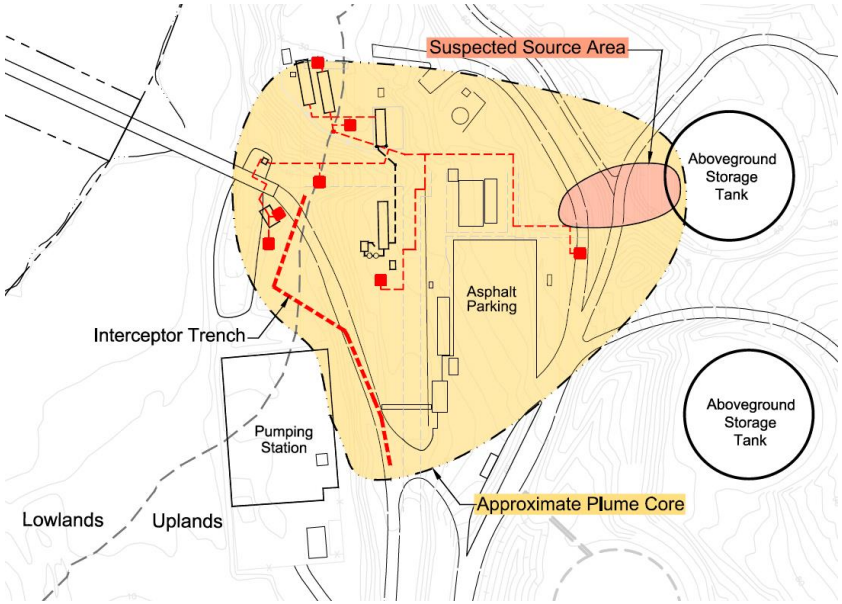
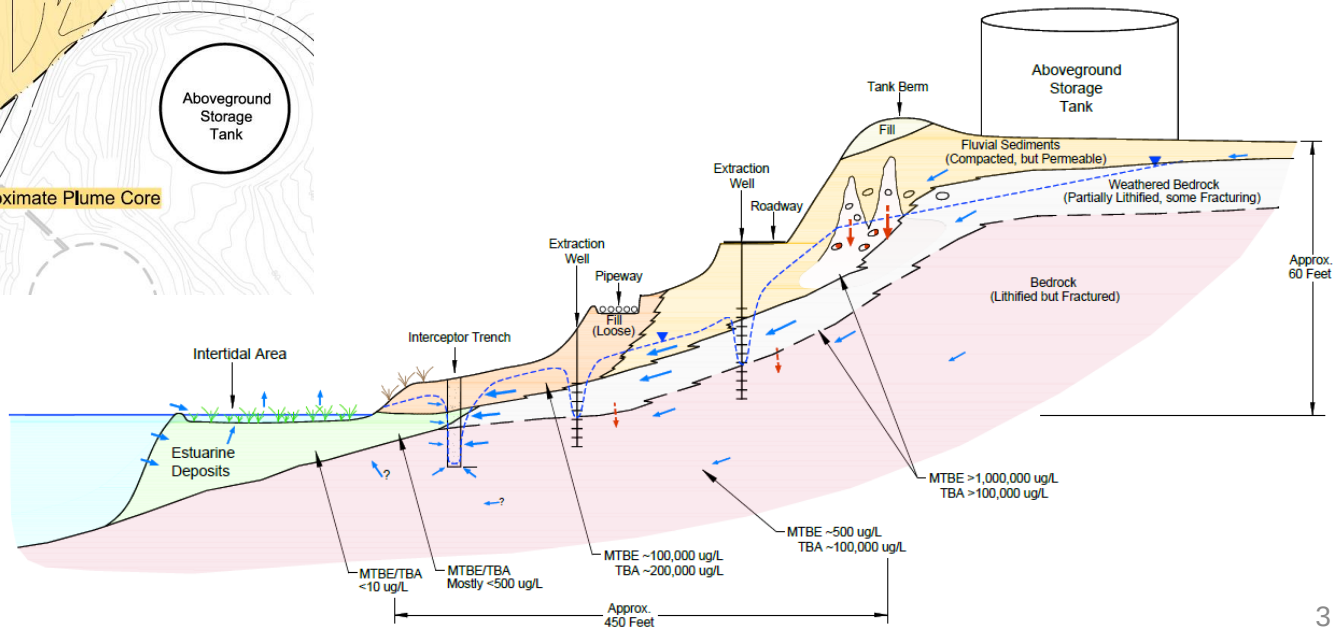
# MTBE Biodegradation Study Agenda

1. Project Background
2. Study Objectives
3. Field Work
4. Results
    - TBA/MTBE Ratios
    - Equilibrium Partitioning
    - TEAPs
    - CSIA
    - Microbial Analysis
5. Conclusions
6. Questions

# Project Background

- Release occurred over 20 years ago
- Gasoline-range petroleum hydrocarbons and pure phase MTBE
- Current remedies include downgradient hydraulic control and mid-plume pump and treat
- Enhanced bioremediation and MNA are critical remedial action components



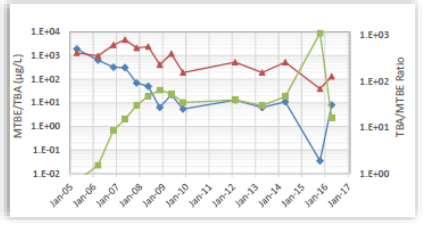



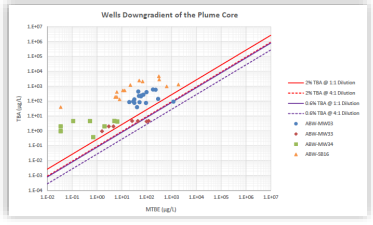
# Study Objectives

The following objectives were evaluated for the representative areas of the site:

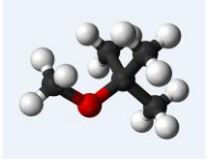
- Evaluate the presence of TBA as a product of dissolution or MTBE transformation
- Identify the terminal electron accepting processes and dominant degradation pathways
- Characterize the microbial community, including confirming the presence and quantifying the number of potential MTBE degraders
- Estimate the rate and extent of MTBE degradation and evaluate the effects of varying MTBE concentrations on degradation

Historical Data and Equilibrium Partitioning

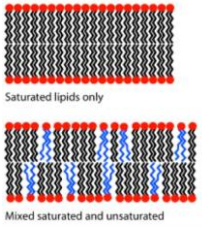



Microbial Analysis

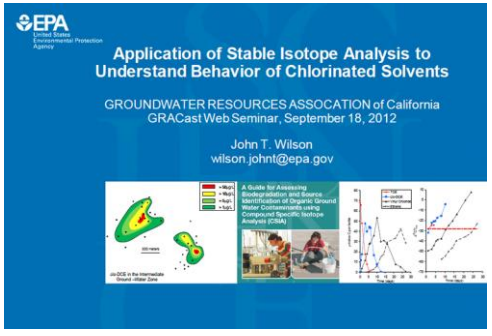


$^{13}$C-labelled MTBE loaded into Bio-Trap



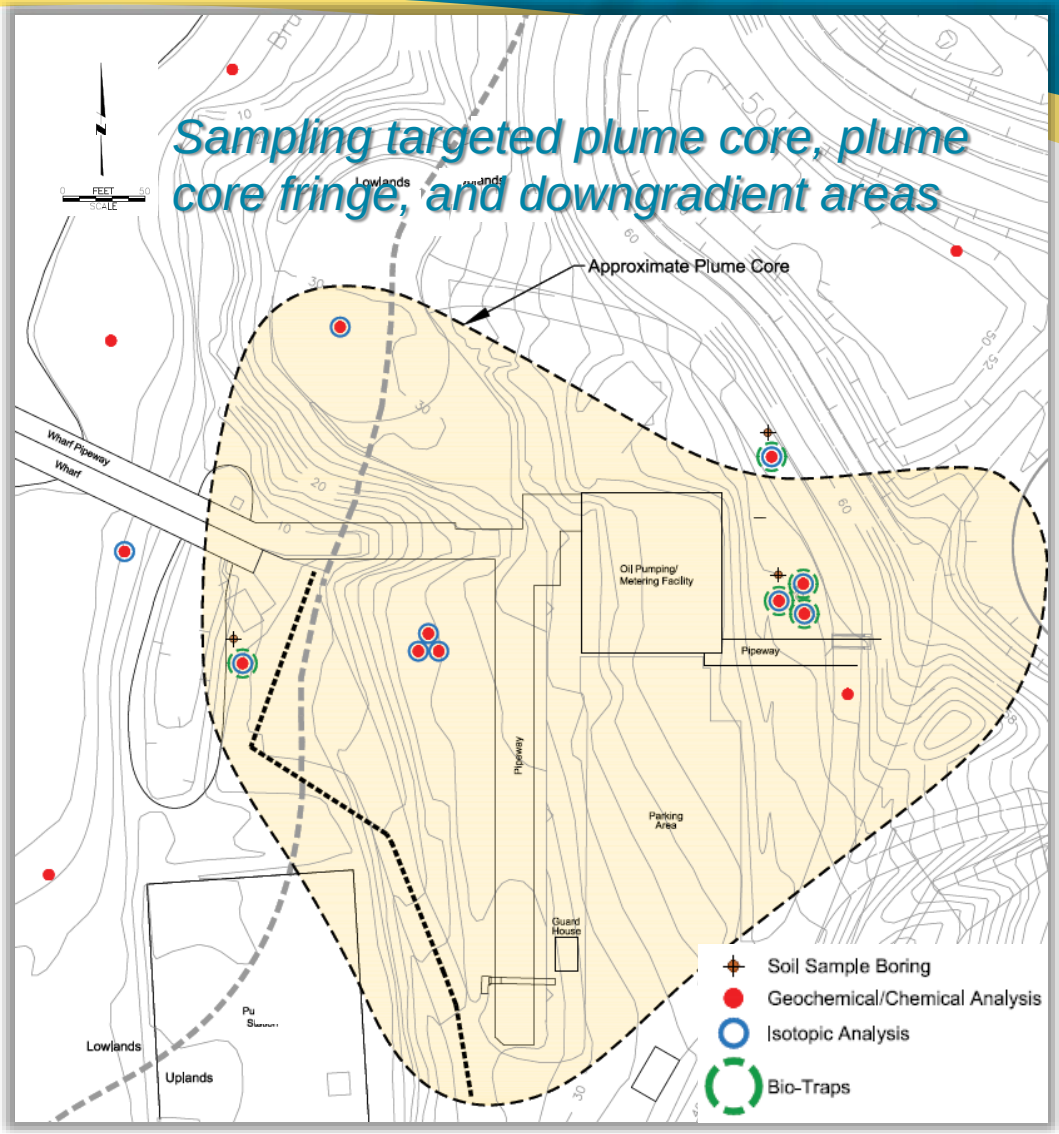

PLFA analysis

Compound Specific Isotope Analysis



# Field Work

- Soil sampling
  - 3 borings, 2 depths in each boring
  - Analyzed for:
    - VOCs
    - Microbial analysis (PLFAs, qPCR)
- Groundwater sampling
  - 15 wells sampled
  - Analyzed for:
    - VOCs
    - Geochemical parameters
    - CSIA
- Microbial Insights Bio-Traps®
  - Deployed in 5 wells
  - Analyzed for:
    - PLFAs
    - qPCR
    - SIP

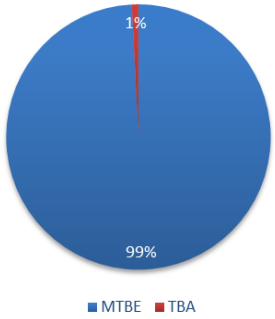


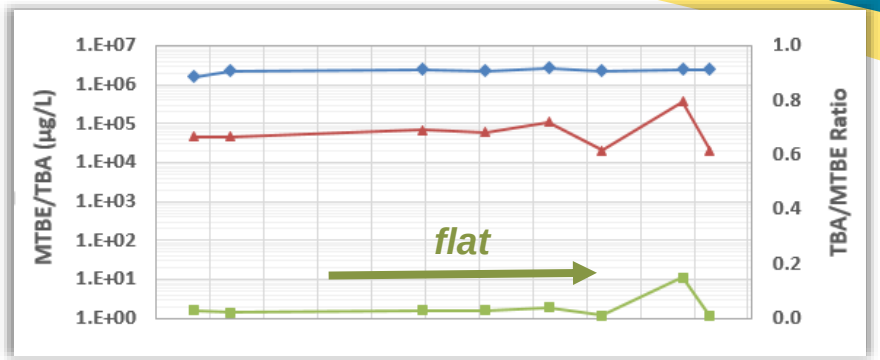
# Increasing TBA/MTBE ratios are an indicator of biodegradation

Spring 2016 results

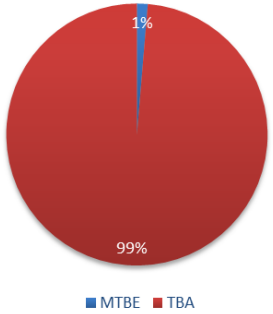
Historical results

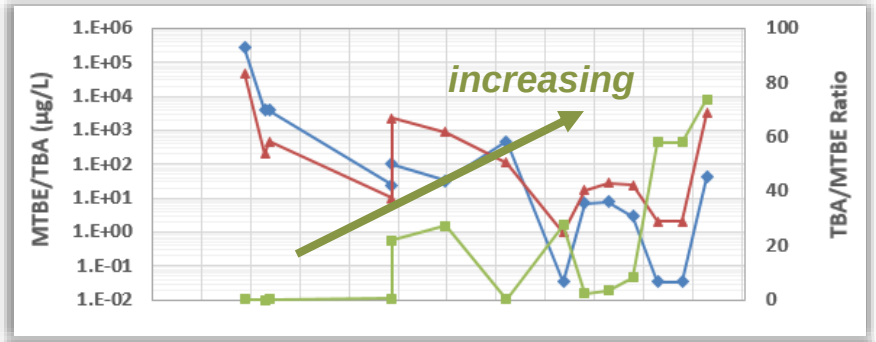
Plume core:



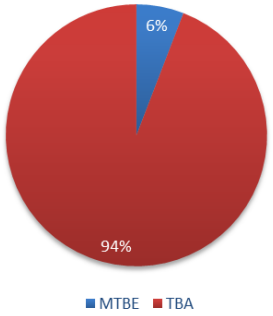



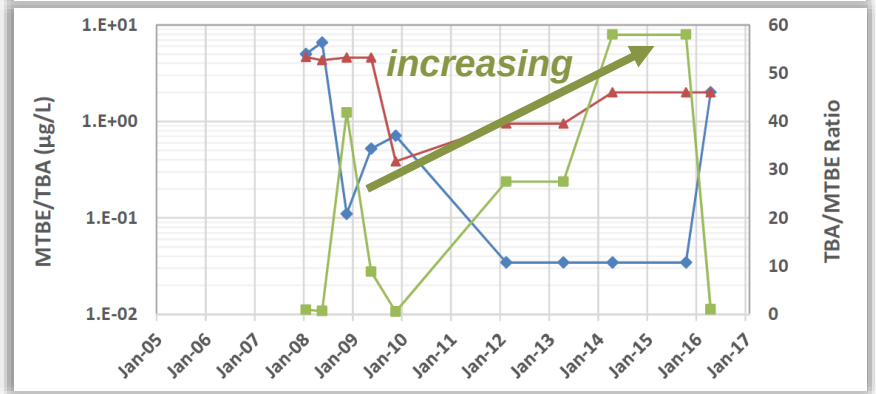

Plume core fringe:



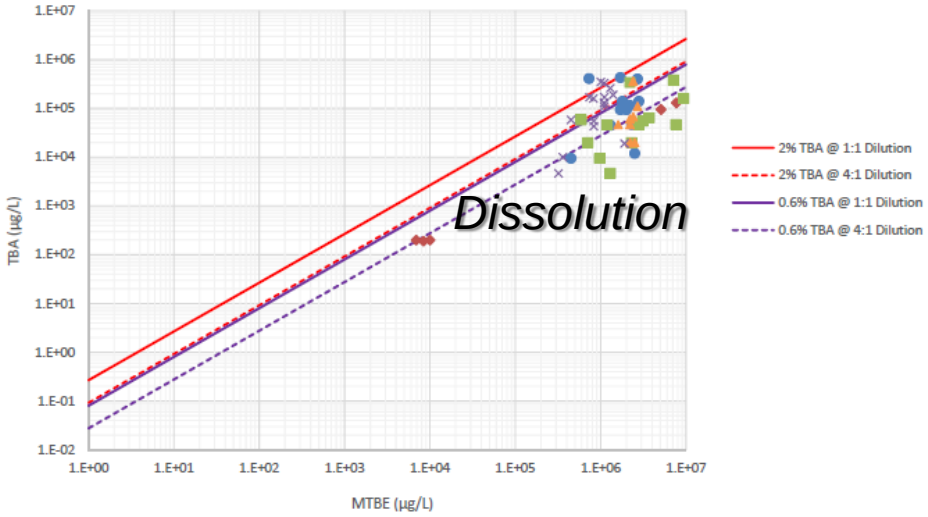

Downgradient:



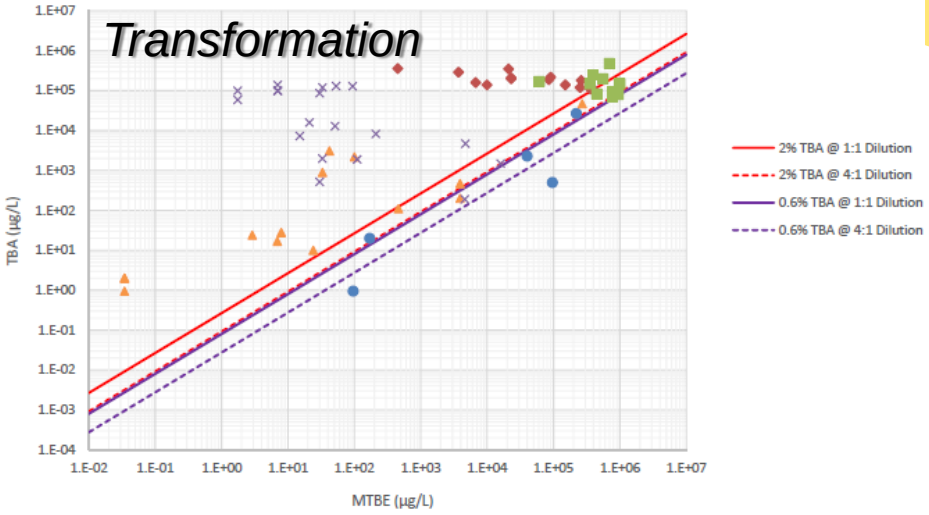



# Comparison of historical data against equilibrium partitioning curves

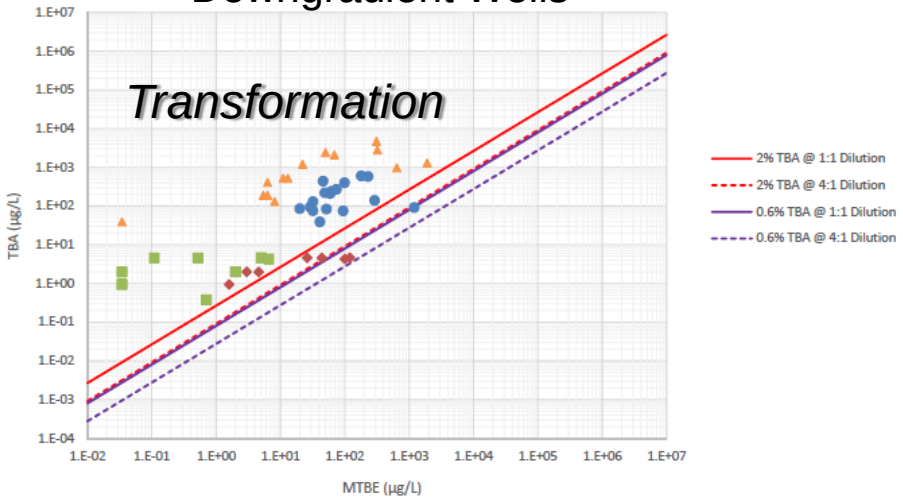
Plume Core Wells




Plume Core Fringe Wells




Downgradient Wells



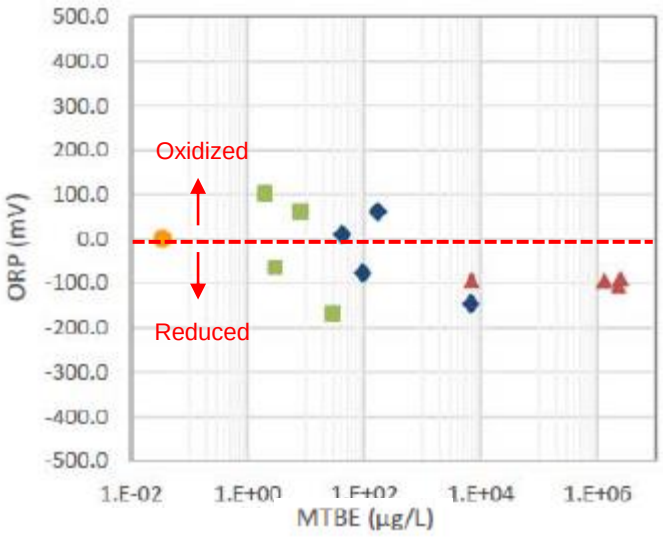

Equilibrium Partitioning Approach (Kolhatkar and Wilson, 2003):

$$C_{TBA}^{water} = C_{MTBE}^{water} * \left(\frac{TBA}{MTBE}\right)_{gasoline} * \left(\frac{K_{fw}^{MTBE} + \frac{V_{wat}}{V_{gas}}}{K_{fw}^{TBA} + \frac{V_{wat}}{V_{gas}}}\right)$$

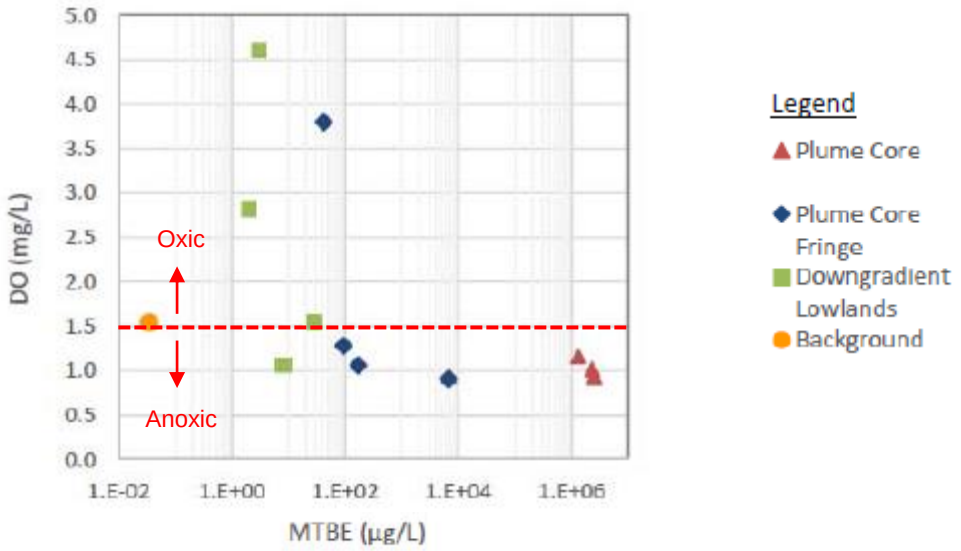
# Terminal electron accepting processes: ORP and DO

Energy available in TEAPs: $O_2$ > $NO_3^-$ > Mn(IV) oxide > Fe(III) oxide > $SO_4^-$ > $CH_4$

ORP




DO



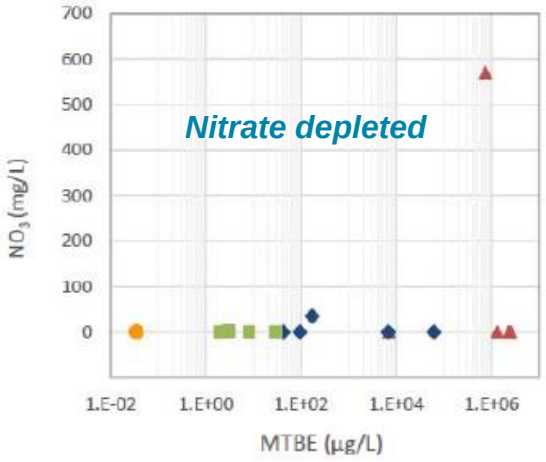

- ***Plume core reduced***
- ***Mildly oxidizing to reducing conditions in plume core fringe and downgradient areas***

- ***Anoxic conditions in plume core and fringe***
- ***Trending more oxic in downgradient areas***

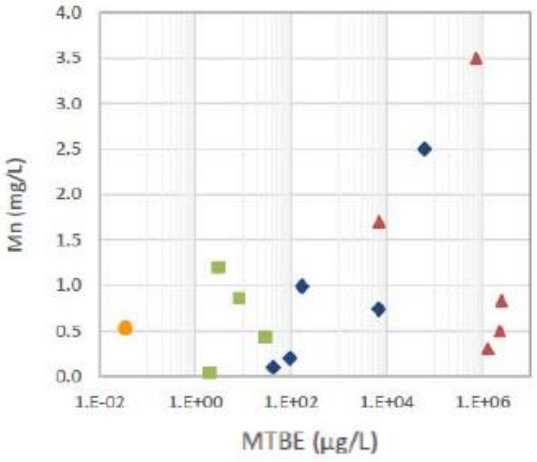
# TEAPs: conditions become more methanogenic as concentrations increase

***Mn and Fe indicate some reductive dissolution of metals***

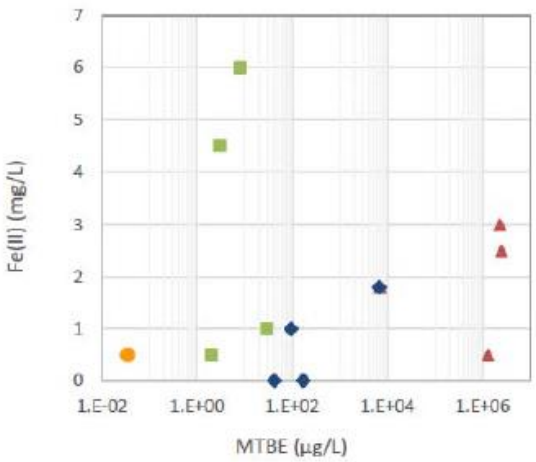
Nitrate




Manganese

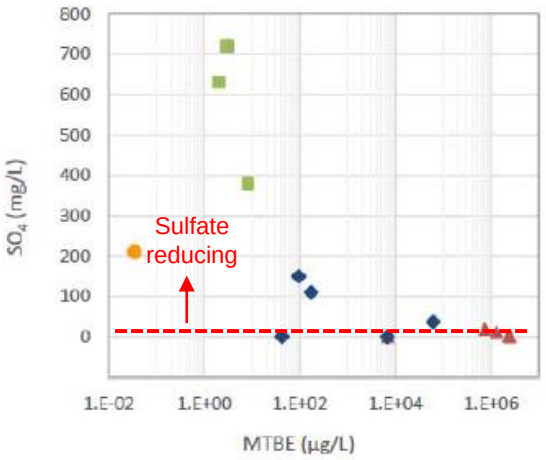



Iron (II)

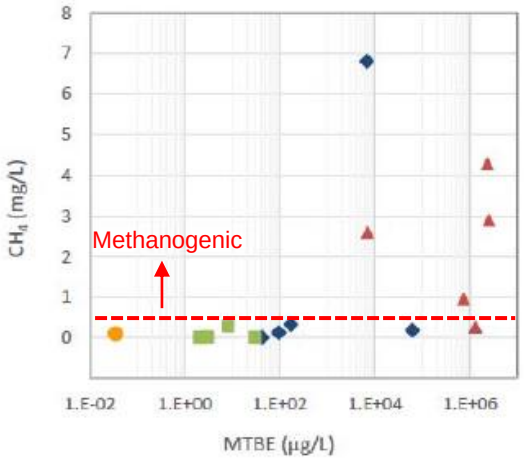



***Sulfate reducing conditions in plume core fringe and downgradient***

Sulfate




Methane

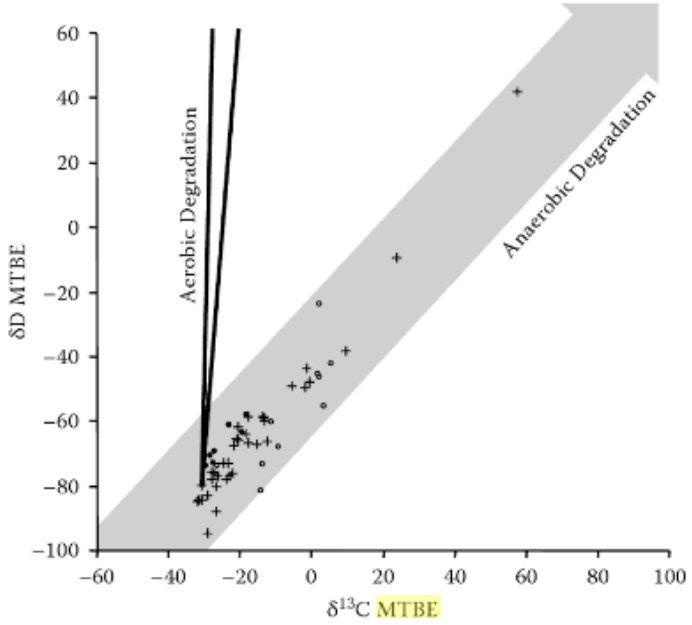



***Plume core is methanogenic, fringe is weakly methanogenic***

Legend

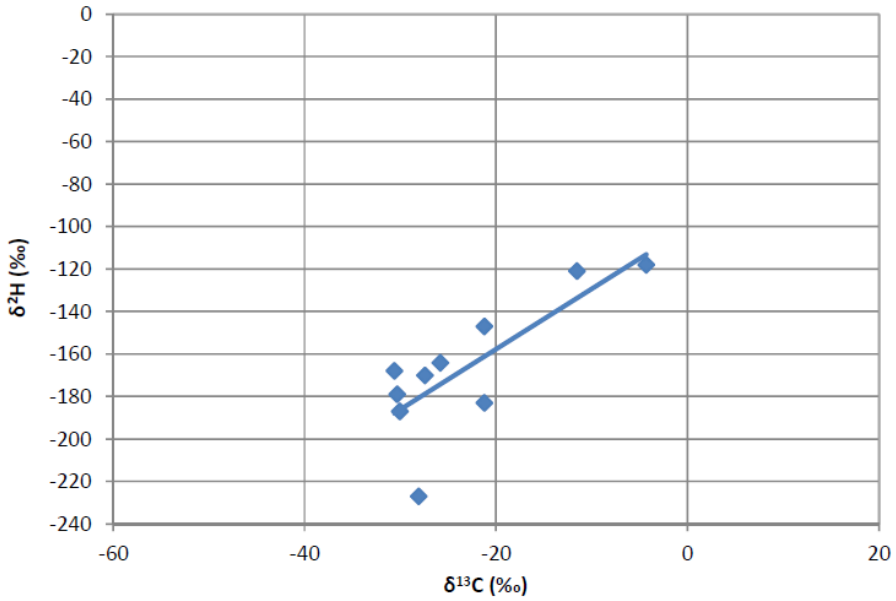
- Plume Core
- Plume Core Fringe
- Downgradient Lowlands
- Background

# CSIA indicates anaerobic degradation is dominant pathway




Source: Kuder, T. et al., Environmental Science and Technology, 39: 213-220, 2005

2-D CSIA Plot of $\delta^{13}C$ vs $\delta^{2}H$

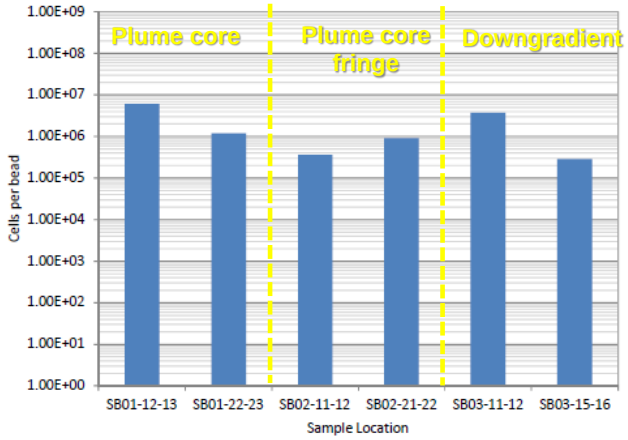


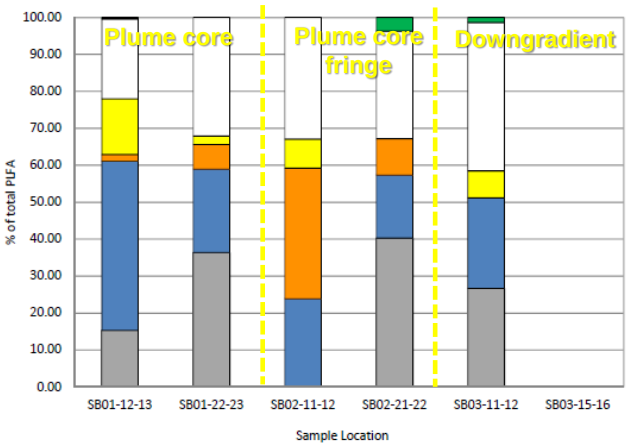
# Microbial Analysis: PLFA Analysis

## Total Biomass

## Microbial Community Structure

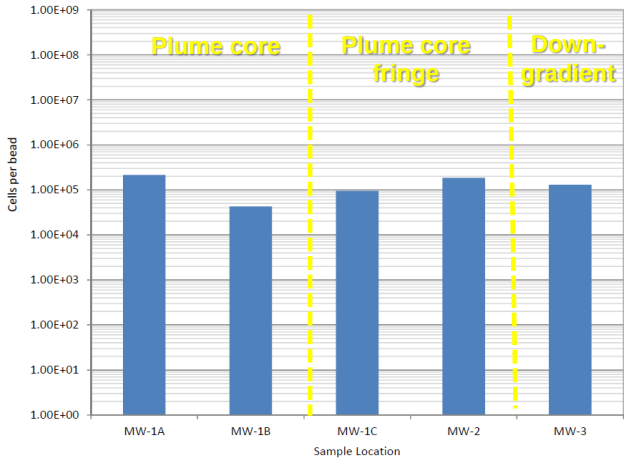
***Soil Sample Results:***

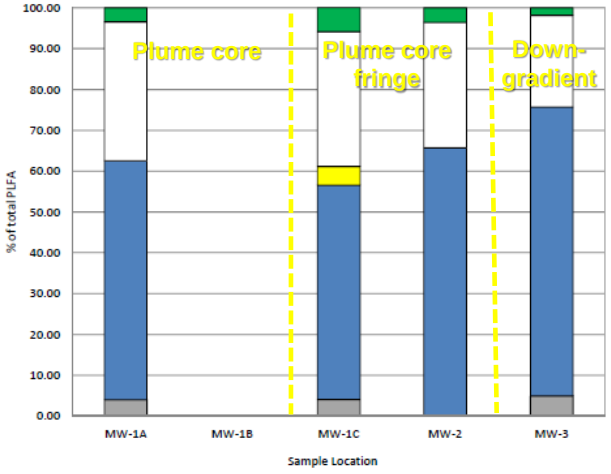



Legend
- Total Biomass
- Eukaryotes (polyenoics)
- General (Nsats)
- SRB/Actinomycetes (MidBrSats)
- Anaerobic Metal Reducers (BrMonos)
- Proteobacteria (Monos)
- Firmicutes (TerBrSats)

***Bio-Trap Sample Results:***

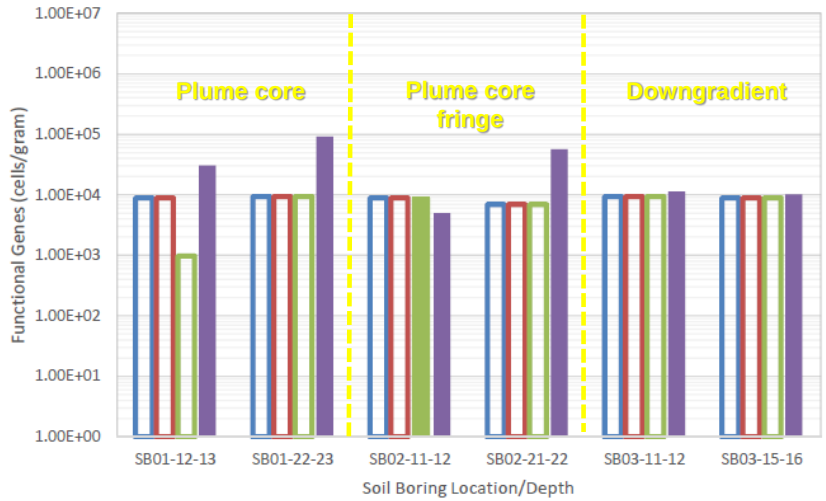


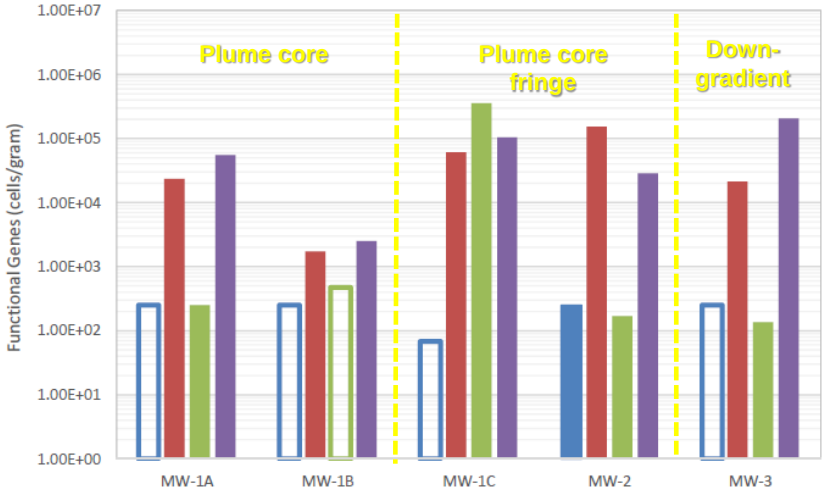

- ***Moderate levels of biomass***
- ***More diverse microbial community in soil***
- ***Presence of anaerobes and hydrocarbon degraders***

# Microbial Analysis: qPCR Analysis

Soil Sample Results




Bio-Trap Sample Results

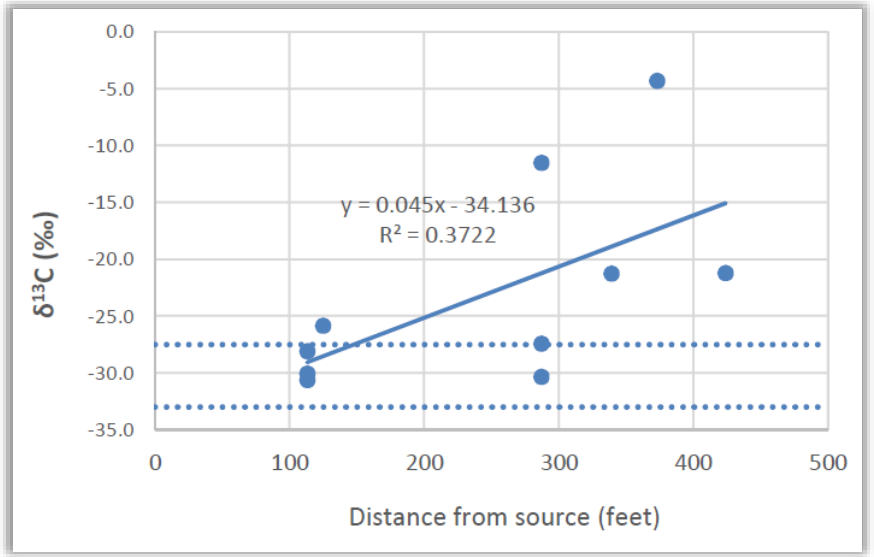


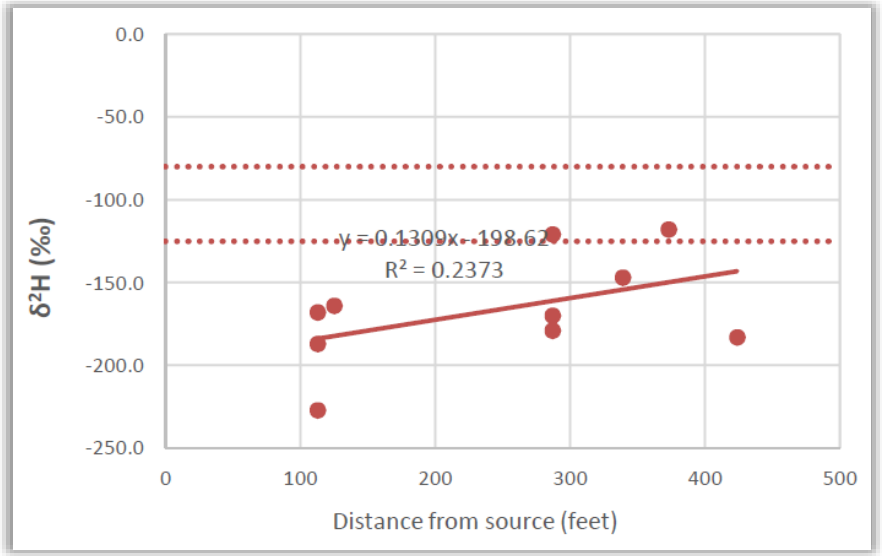

Legend
- ETHB
- PM1
- TBA
- HCMB

Acronyms
ETHB – ETBE monooxygenase
HCMB – HIBA Mutase
PM1 – Methylibium petroleiphilum
TBA – TBA monooxygenase

- ***Genetic material related to MTBE degradation detected***
- ***Higher levels on plume core fringe than plume core***

# CSIA: isotopic fractionation increases with distance and as MTBE concentrations decrease




Plume Core Wells

Plume Core Fringe Wells

Downgradient Lowlands Wells

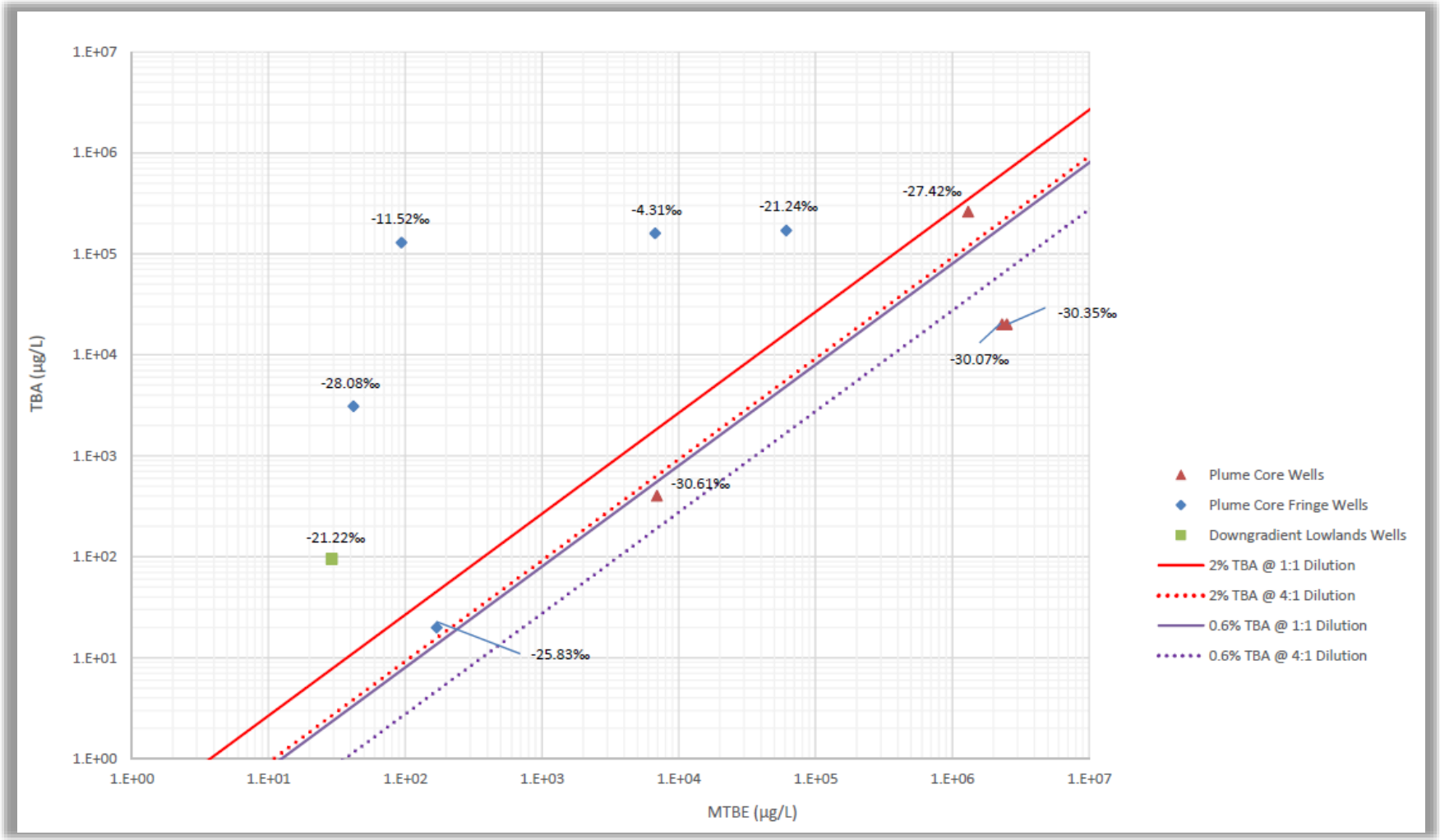
δ13C MTBE in gas - upper range (‰)

δ13C MTBE in gas - lower range (‰)

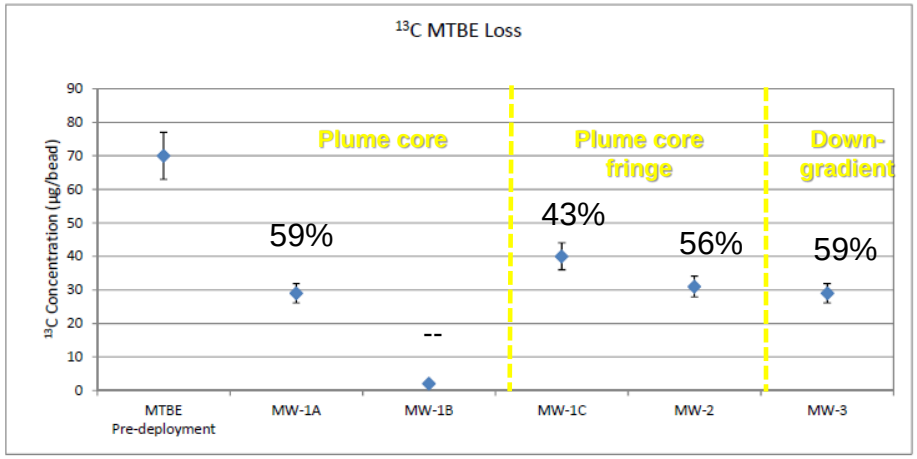
δ2H MTBE in gas - upper range (‰)

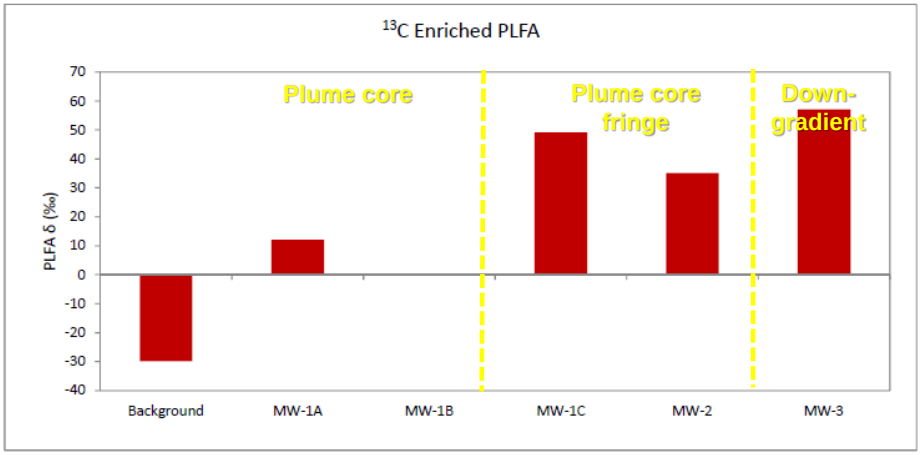
δ2H MTBE in gas - lower range (‰)

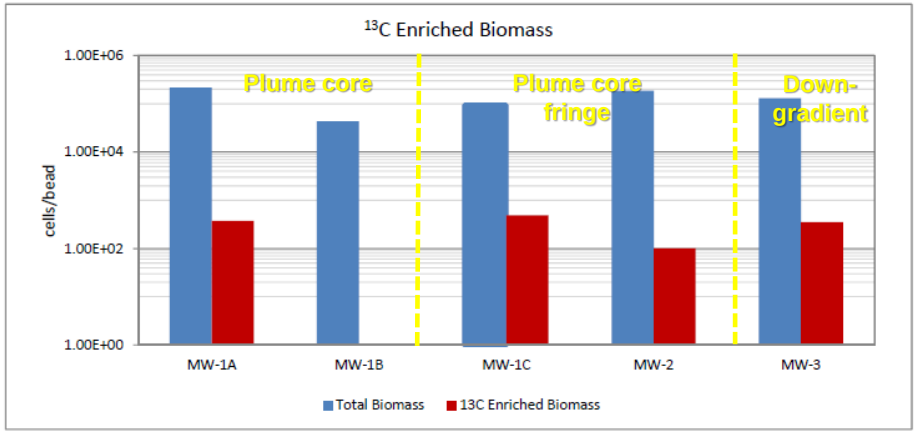
# Correlation of CSIA and Equilibrium Partitioning

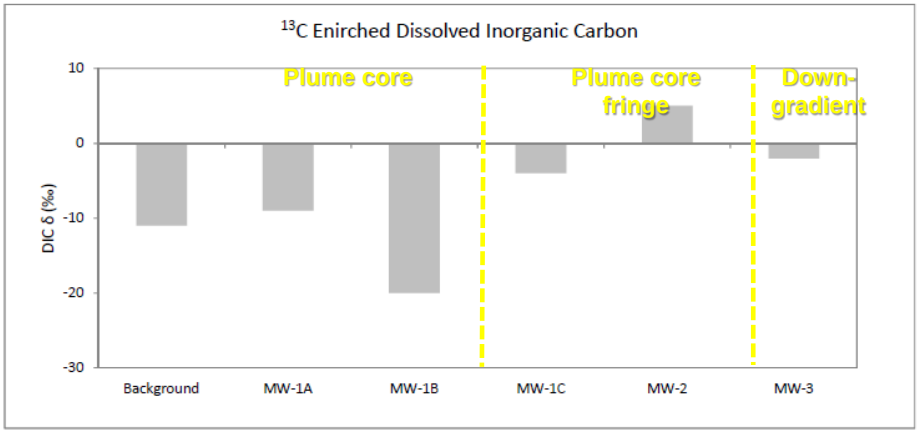


# Microbial Analysis: Stable Isotope Probing Analysis



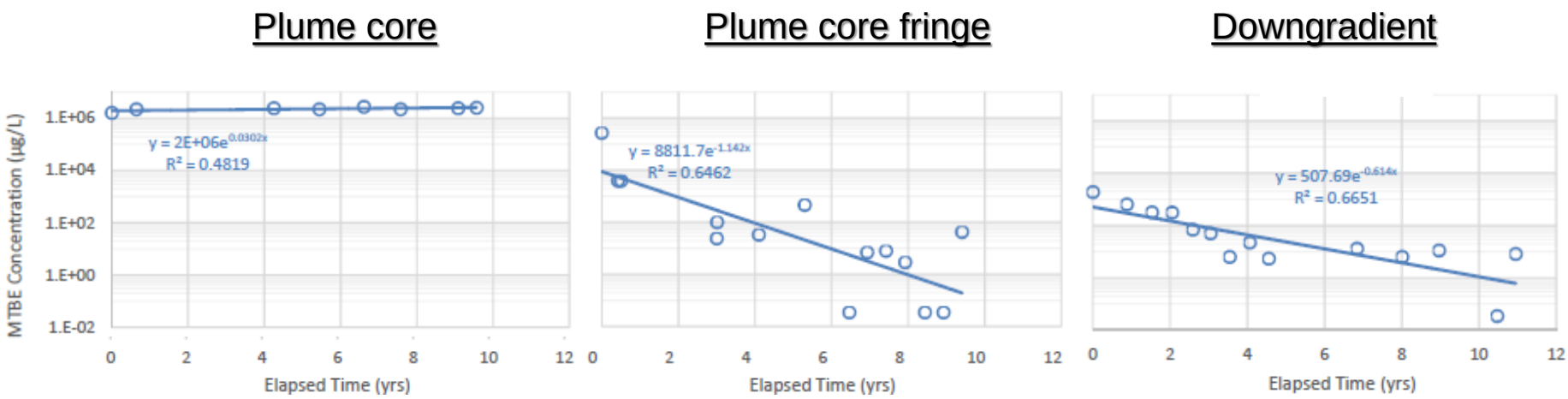




***4 of 5 wells showed:***

- ***Enriched MTBE mass loss***
- ***Enrichment in biomass (conversion of MTBE for growth) and DIC (conversion of MTBE for energy)***
- ***Highest levels of enrichment on plume core fringe***

# MTBE attenuation rates highest at plume core fringe and downgradient areas




- ***Rate constants in plume core fringe and downgradient wells ranged from 0.0001 to 0.0031 $d^{-1}$ equivalent to half-life's of 0.6 up to 21 years***
- ***Attenuation rates highest in plume core fringe and downgradient wells***

# MTBE Biodegradation Study Conclusions

- Results of the multiple lines of evidence correlated well
- TBA/MTBE ratios, eq partitioning, CSIA, microbial analysis, and MTBE attenuation rates indicated:
  - ***Biodegradation is occurring*** on the ***plume core fringe*** and in ***downgradient areas***
  - Little to ***no biodegradation is occurring*** in the ***plume core***
- CSIA and TEAPs indicated ***anaerobic degradation is the dominant pathway***
- Microbial analysis confirmed the presence and functionality of appropriate degraders across the site
- Suggests ***manipulation of the plume core*** to create favorable conditions should ***stimulate intrinsic microbial activity***
- ***Monitored natural attenuation is viable*** once plume core concentrations have been addressed


# Questions?