Investigating the *in situ* biodegradation of BTEX at an active refinery using bio-traps and molecular biological tools

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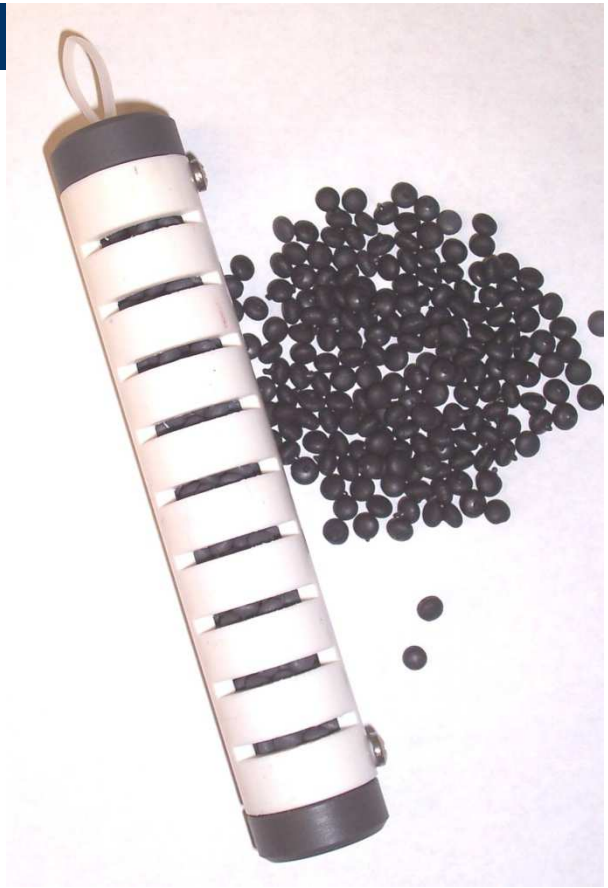
What are Bio-Trap® samplers?

- Passive sampling tool for microbes
- Collects **active** microbes
- Integrated sample vs. "snapshot"
- Analyzed using molecular biological tools, analytical chemistry, and stable isotope analysis



Bio-Sep® beads

- 3-4 mm in diameter
- 25% Nomex, 75% PAC
- Cleaned of fossil biomarkers by heating to 270 ^{o}C
- 74% porosity
- 600 m^2 of surface area/g
- Biofilms form rapidly on Bio-Sep® beads

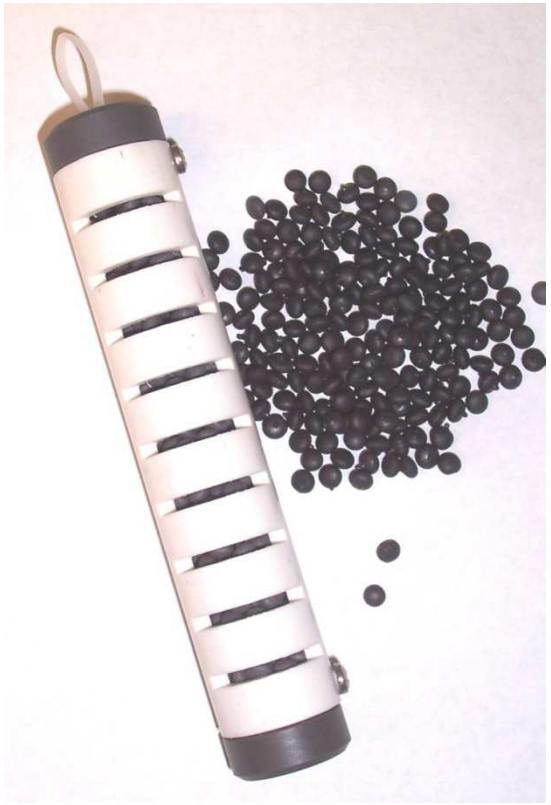
Molecular biological tools (MBTs)

- Culture independent technologies
- Use biomarkers to gain information about microbial populations and activity:
  - Phospholipid Fatty Acids (PLFA)
  - DNA (genetic potential)
  - RNA (gene expression)
- Stable Isotope Probing (SIP) tracks ^{13}C from a labeled contaminant as it is mineralized (CO_2 or methane) or metabolized (PLFA, DNA)

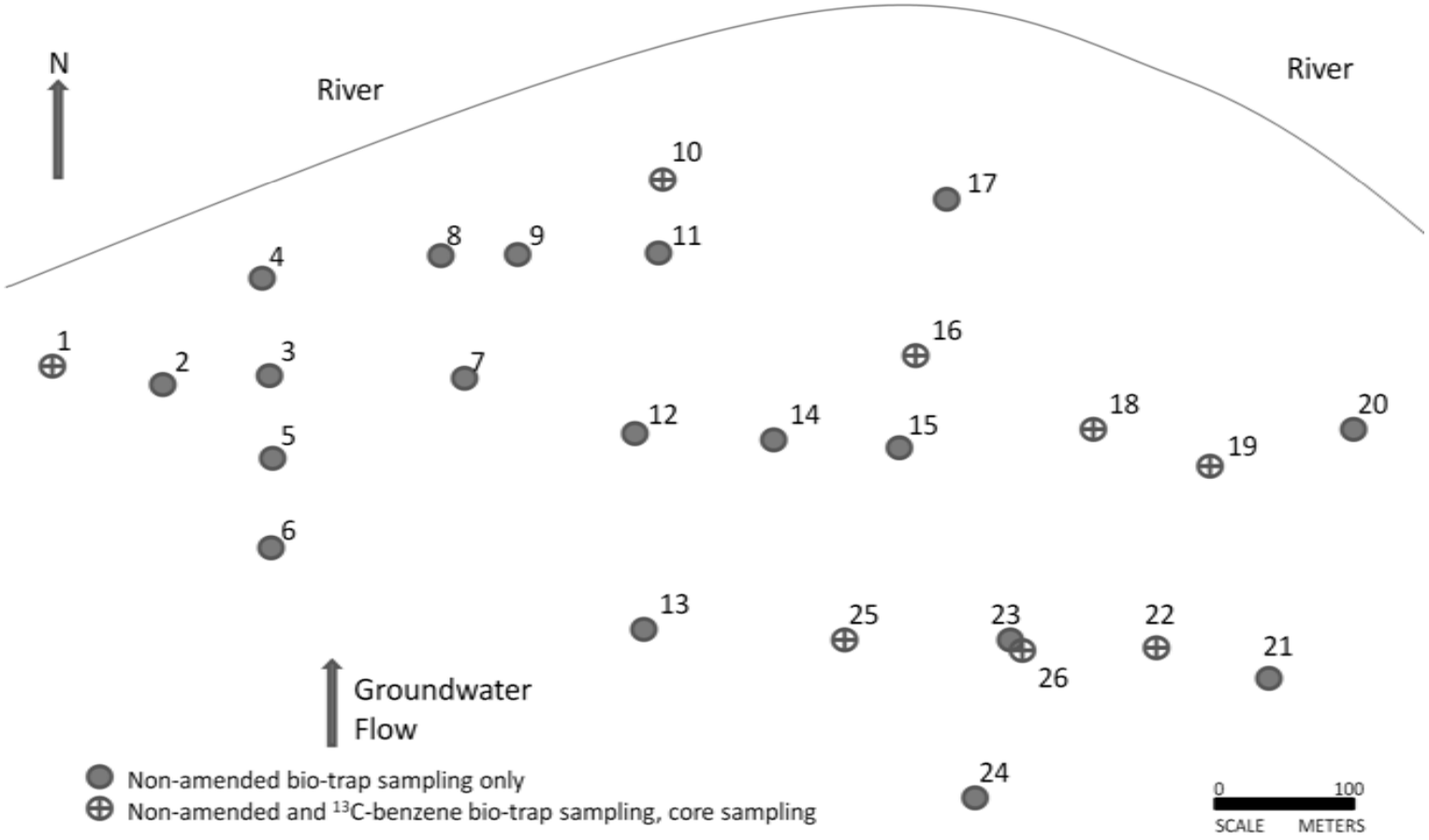
Assessing BTEX biodegradation potential at a refinery using molecular biological tools

Assessment of *in situ* BTEX biodegradation using MBTs

- Phase I: Groundwater survey using bio-traps and groundwater sampling
- Phase II: Microbial activity in the vadose zone studied in soil cores
- Phase III: SIP used to provide direct proof of benzene biodegradation in groundwater



Site map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Groundwater
Flow
24
Non-amended bio-trap sampling only
Non-amended and ^{13}C-benzene bio-trap sampling, core sampling
0
100
SCALE
METERS

Specific qPCR targets

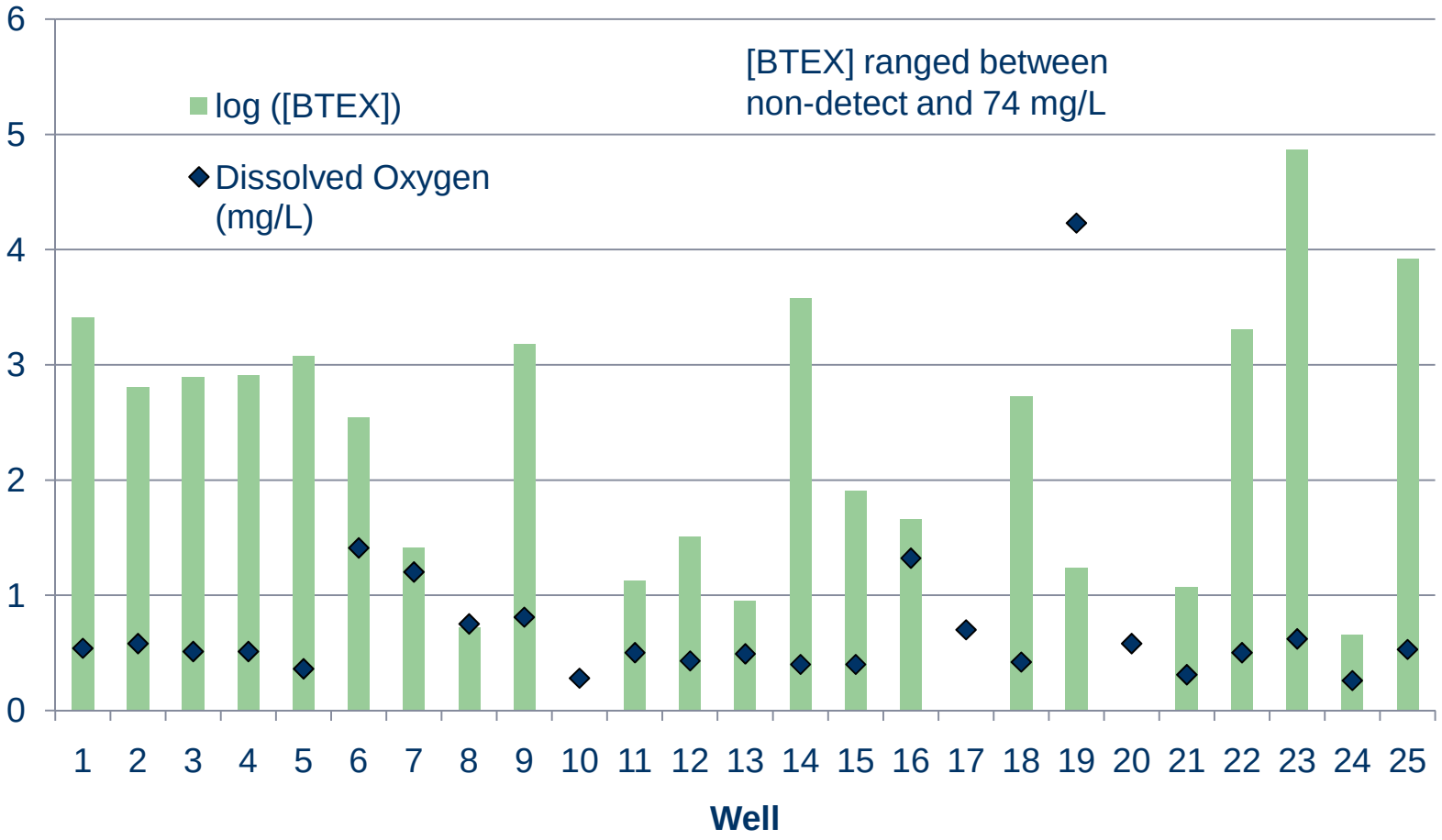
- 16S rRNA genes
  - EBAC: total eubacteria
  - PM1: aerobic MTBE degradation
- Functional genes—Taxonomic
  - *nirK* and *nirS*: denitrifiers
  - APS: sulfate-reducing bacteria
  - MGN: methanogens
- Functional genes:
  - NAH, PHE, TOD: aerobic hydrocarbon degradation

qPCR analysis of DNA extracted from bio-traps in groundwater

- All gene targets detected in all 25 wells
- Genetic potential for aerobic oxidation, sulfate reduction, denitrification, and methanogenesis present across the site

Correlations		r	p
log [EBAC]	log [nirS]	+0.61	0.001
log [EBAC]	log [APS]	+0.55	0.004
log [EBAC]	log [MGN]	+0.71	< 0.001
log [EBAC]	log [NAH]	+0.64	< 0.001
log [EBAC]	log [PM1]	+0.60	0.002
[Toluene]	log [PM1]	+0.51	0.011

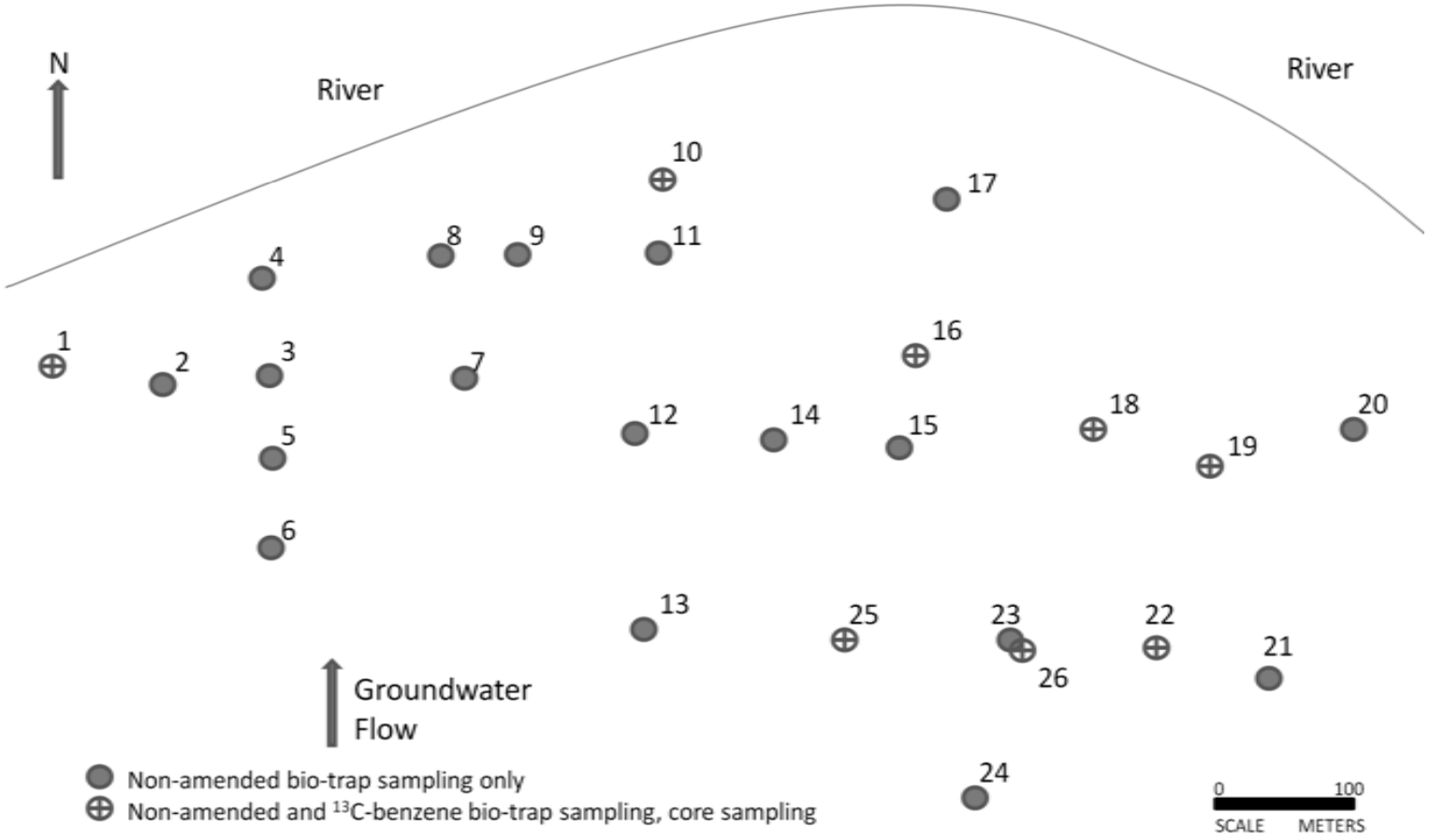
Groundwater BTEX concentrations



Geochemistry correlations

Correlations		r	p
log [BTEX]	[Fe^{+2}]	+0.54	0.009
log [BTEX]	[Methane]	+0.51	0.014
log [BTEX]	pH	-0.65	< 0.001
Alkalinity	ORP	+0.70	< 0.001
Alkalinity	[Ammonia-N]	+0.85	< 0.001

Site map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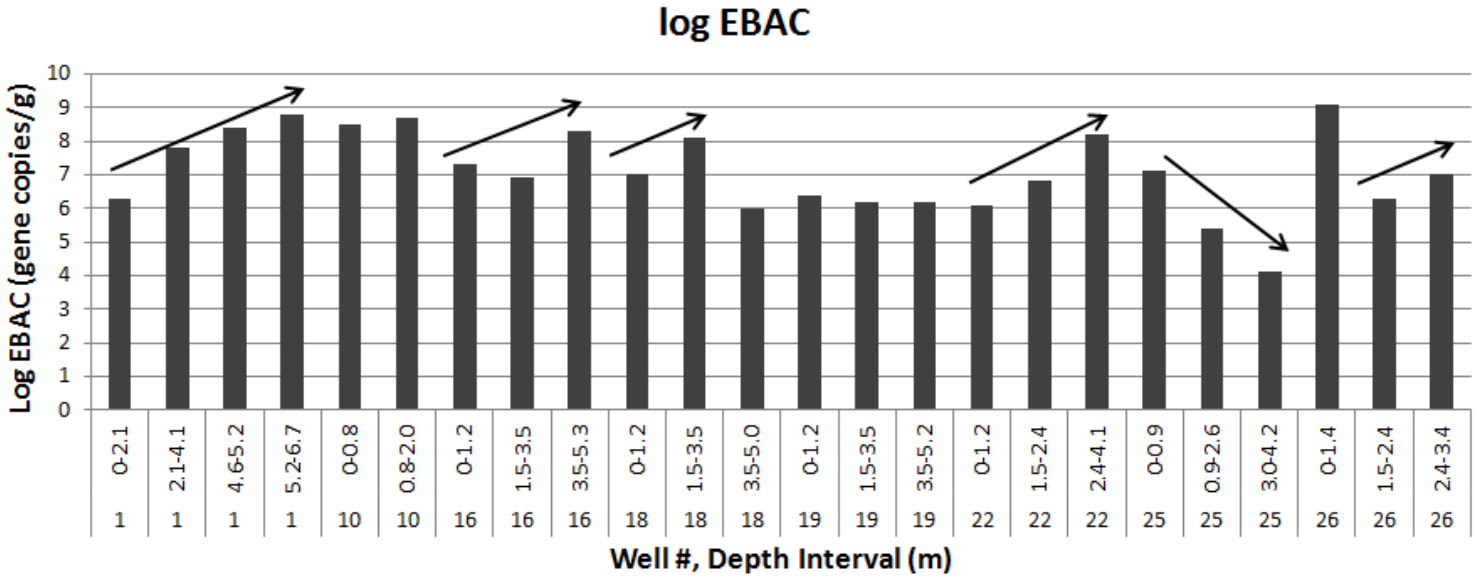
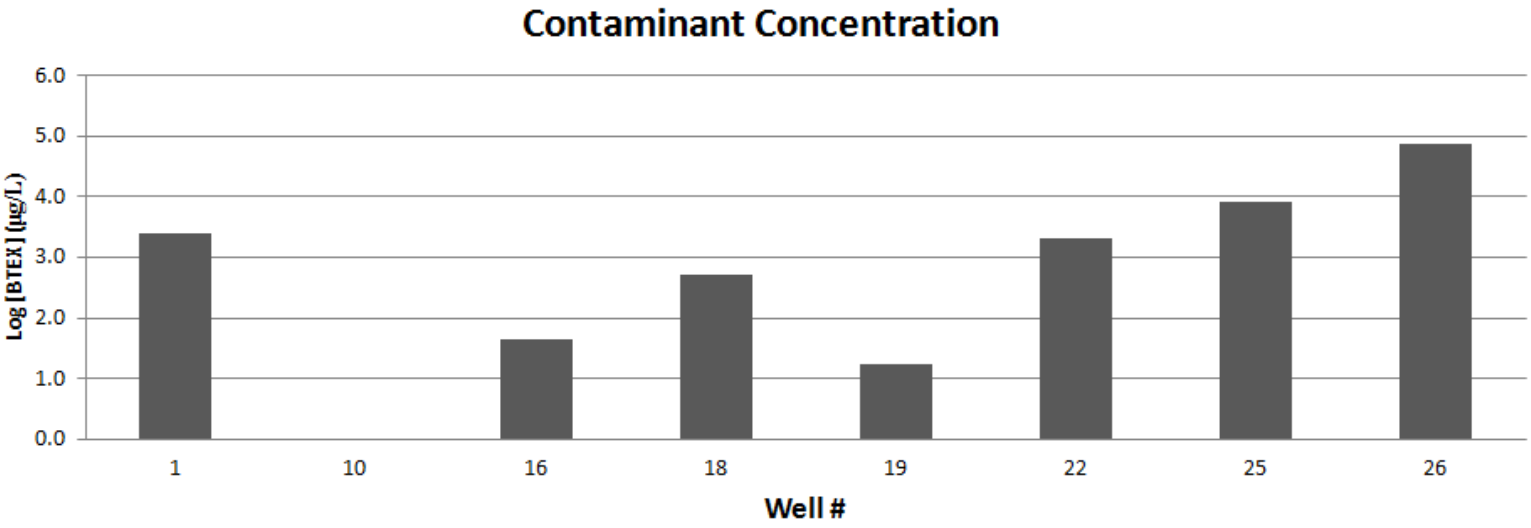
RT-qPCR analysis of RNA extracted from soil cores

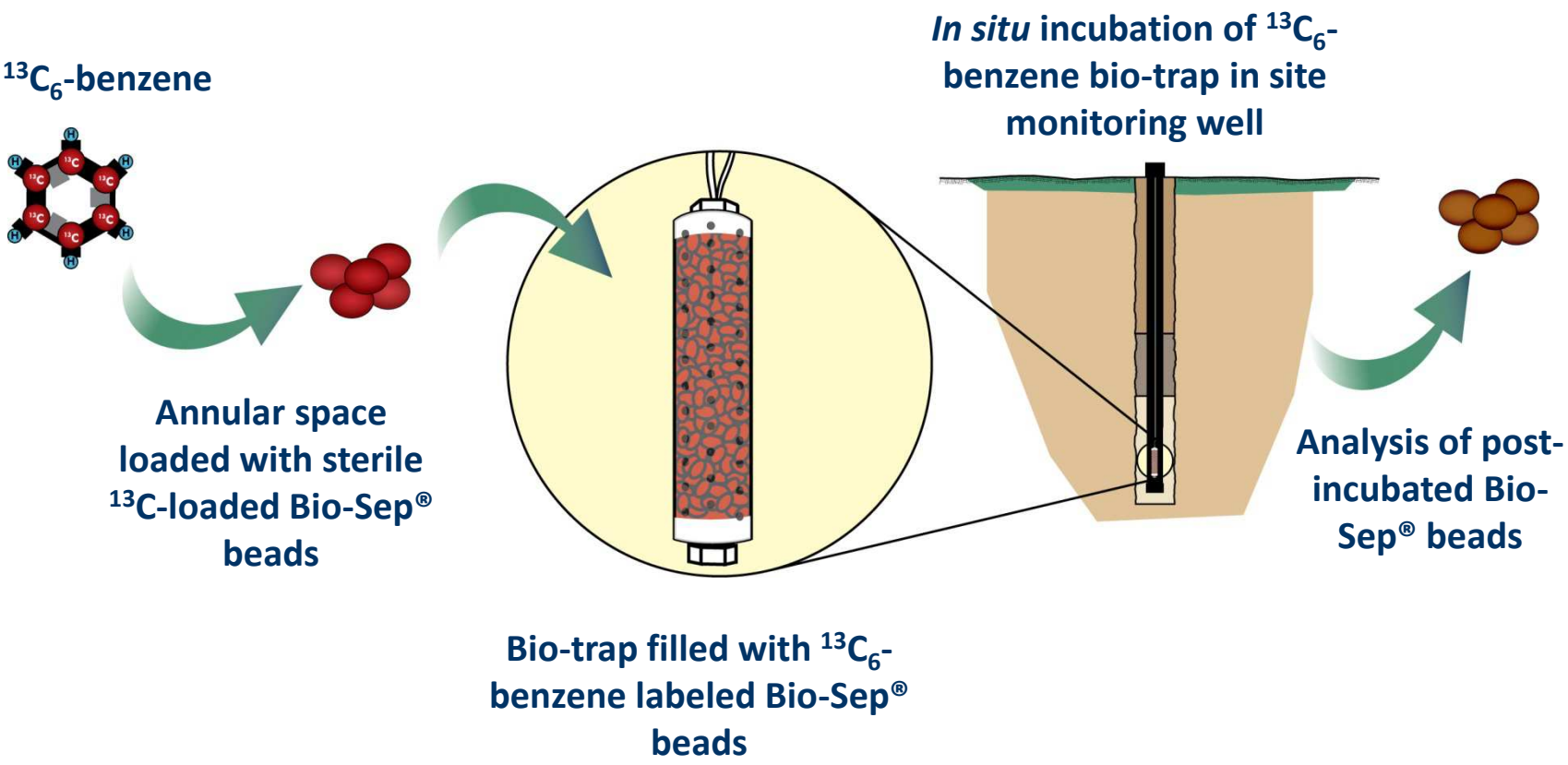
- Gene targets associated with denitrifiers and sulfate reducers present in all 8 locations
- Expression of aerobic hydrocarbon oxidation genes detected in 5 locations
- Methanogenic gene target detected in 3 locations

Eubacteria rRNA in the vadose zone and groundwater [BTEX]

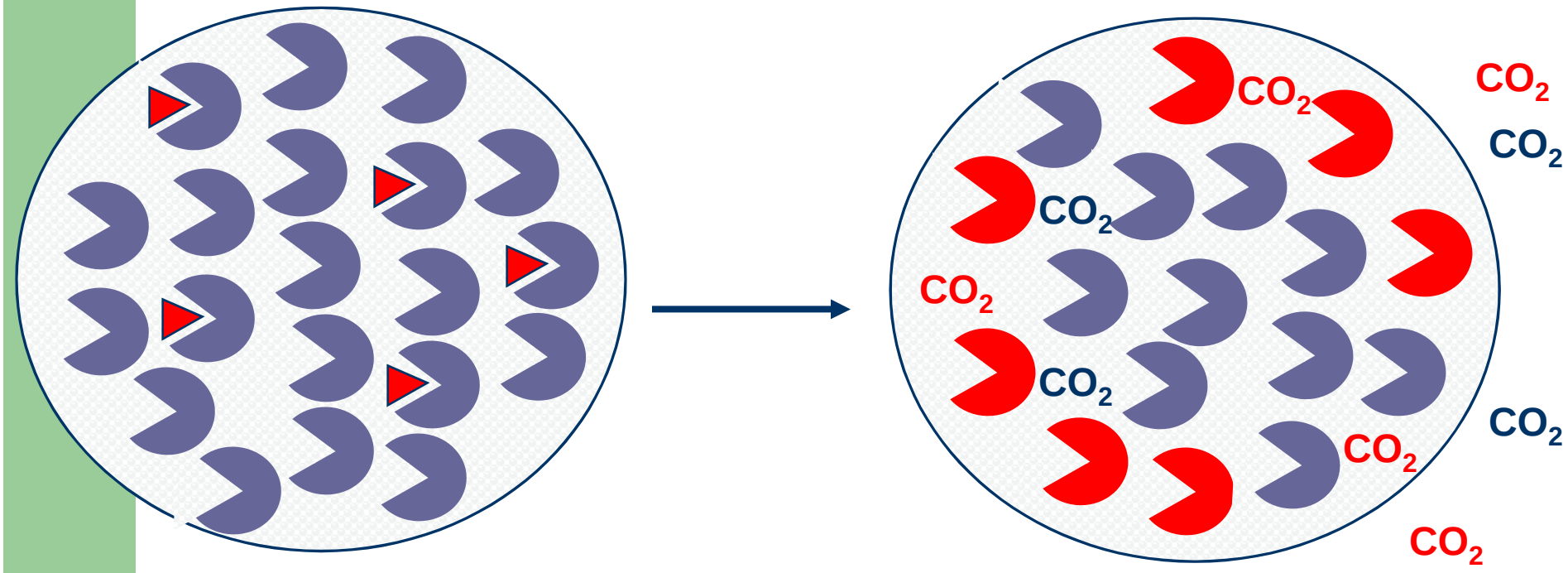




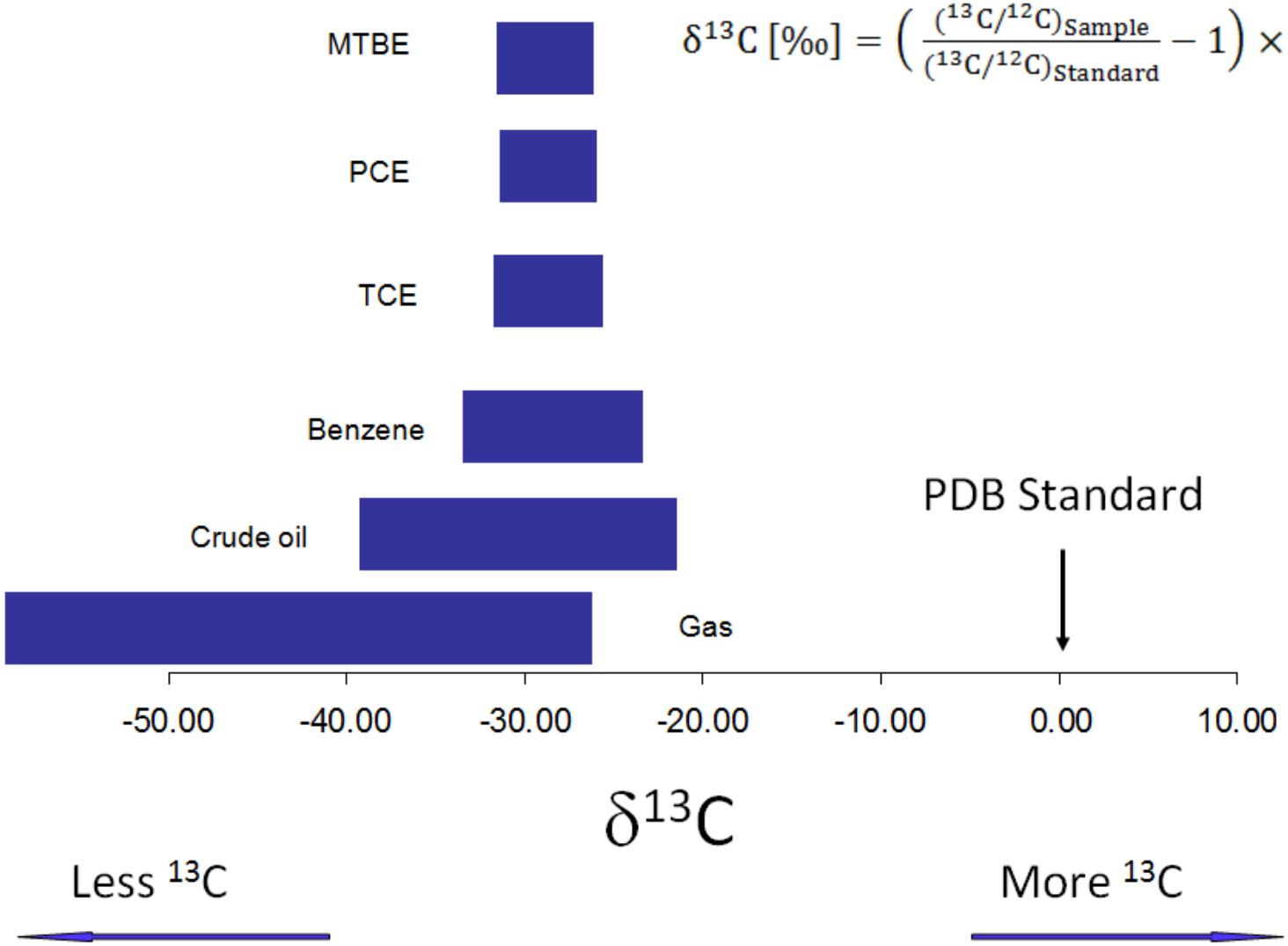
Overview of Bio-trap stable isotope probing (SIP) approach

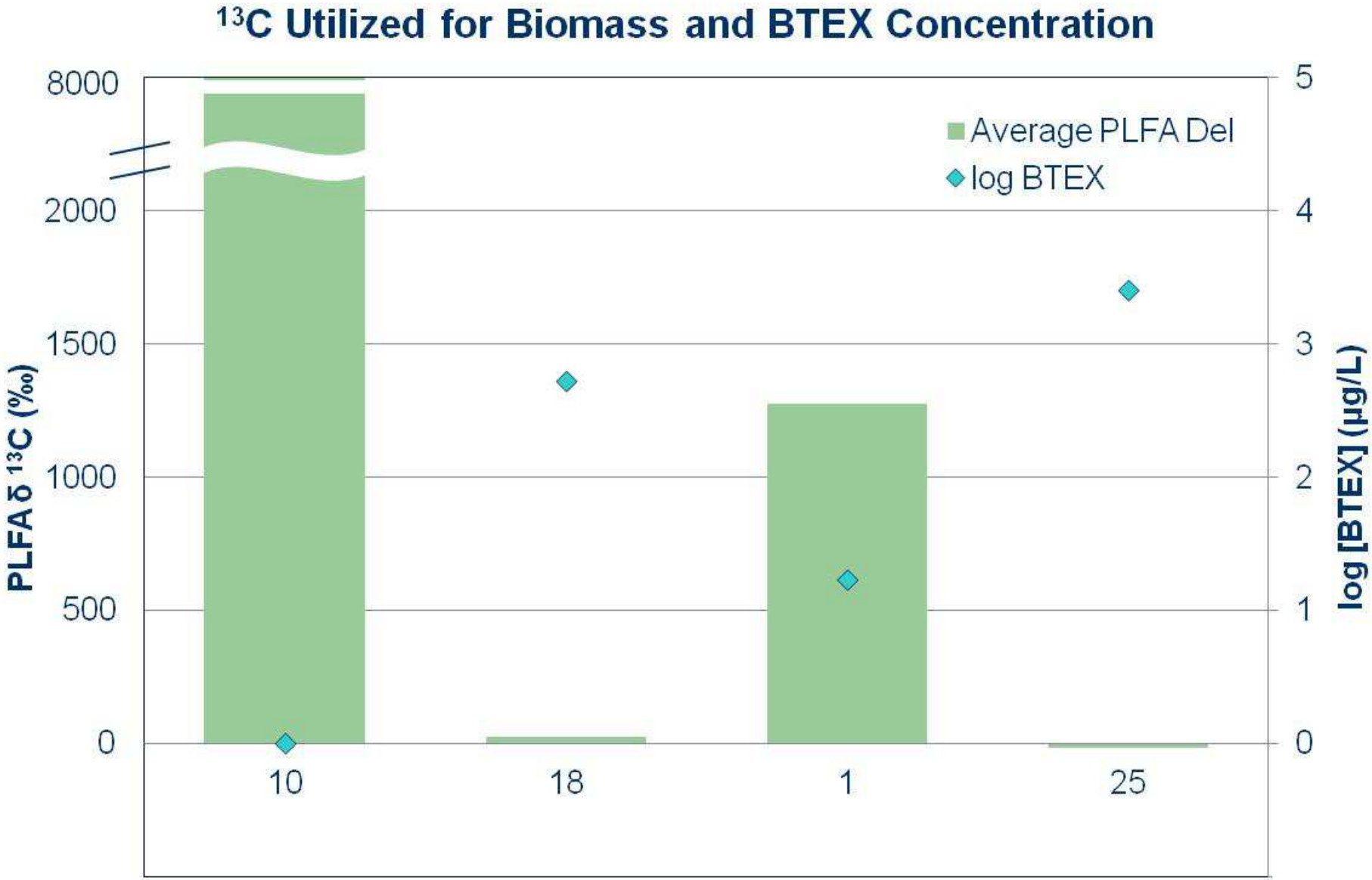


Stable isotope probing with Bio-Sep® beads

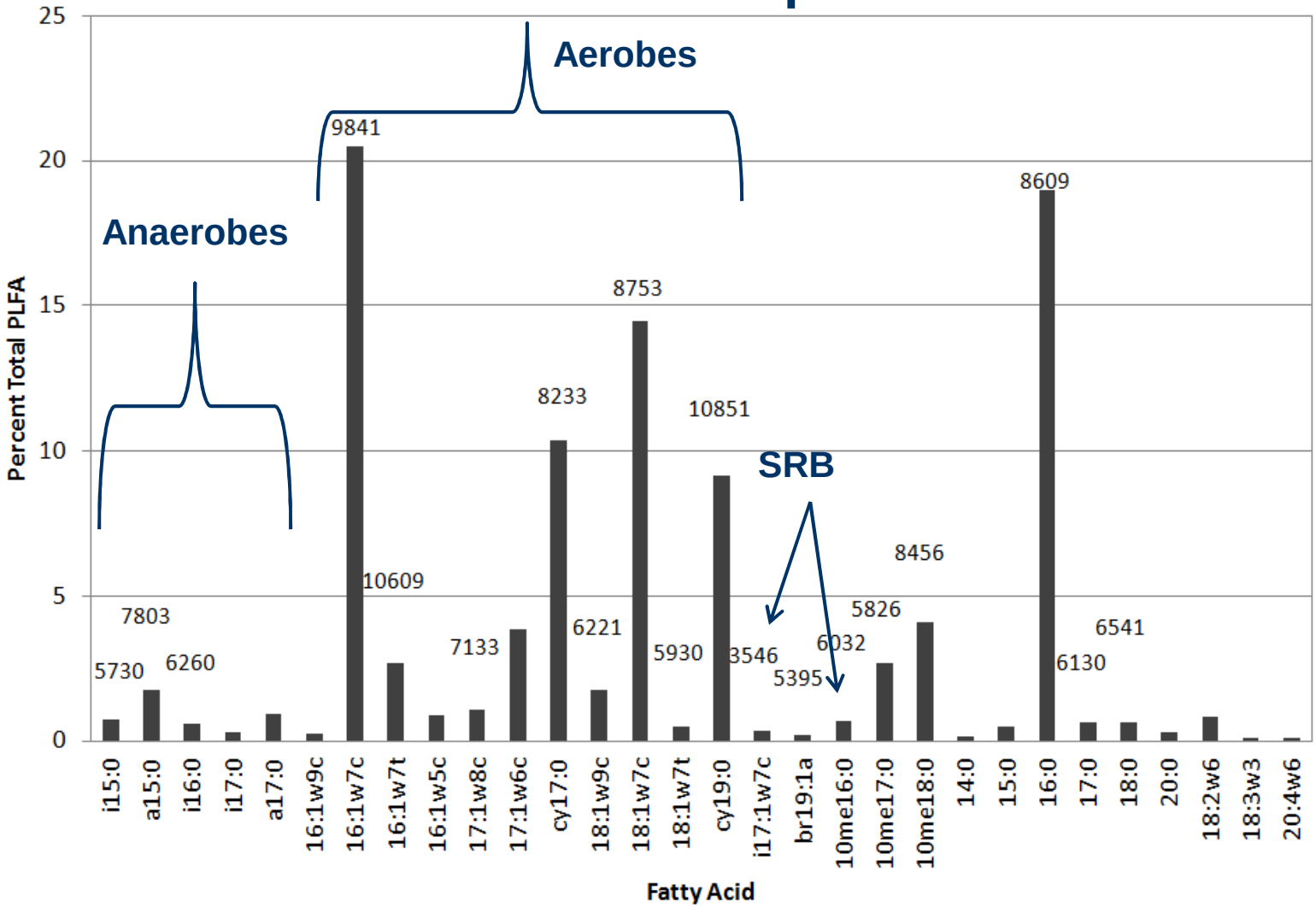


$$\delta^{13}C\ [‰] = \left(\frac{(^{13}C/^{12}C)_{Sample}}{(^{13}C/^{12}C)_{Standard}} - 1 \right) \times 1000$$

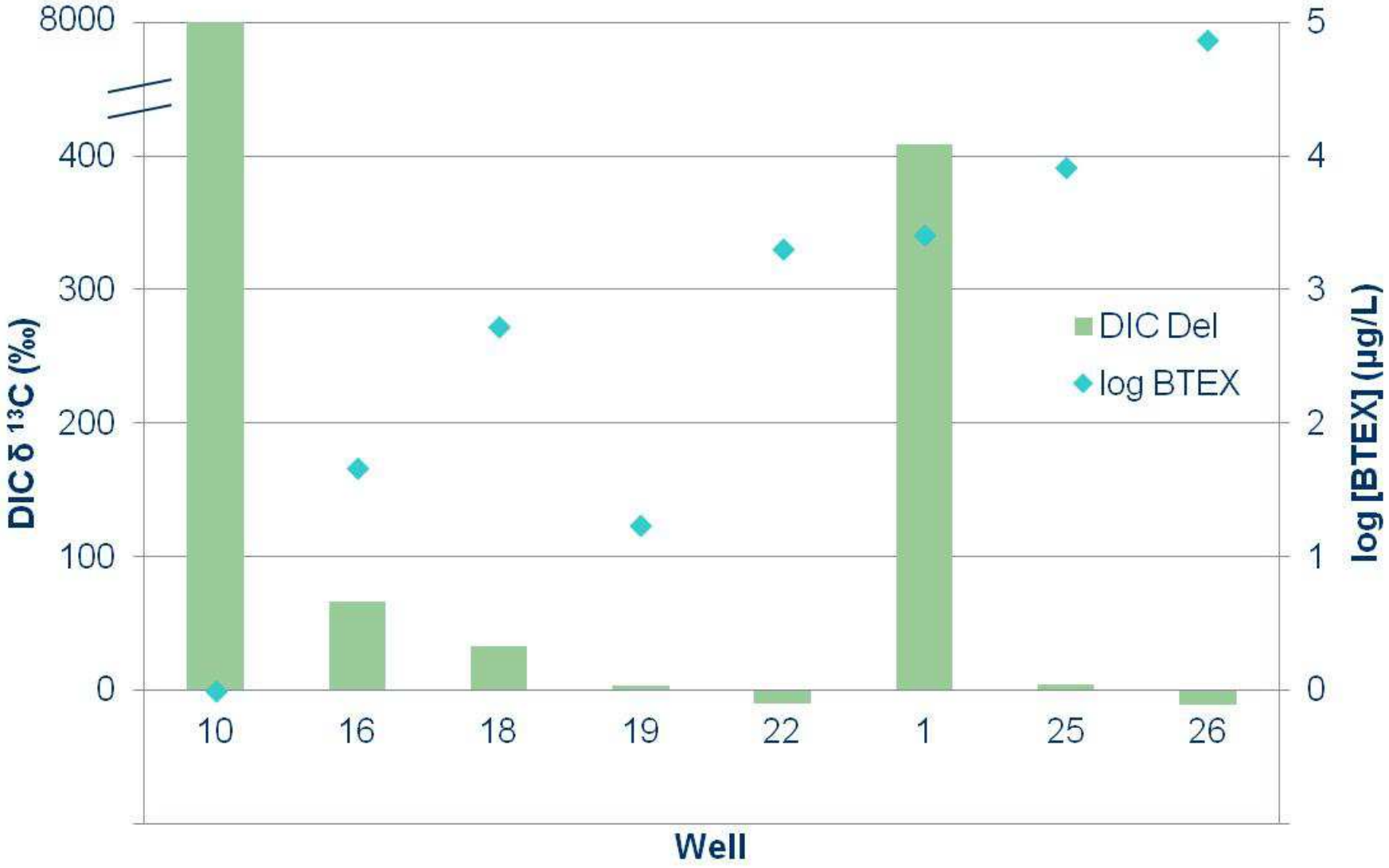



13C Utilized for Biomass and BTEX Concentration
8000
2000
1500
1000
500
0
PLFA δ 13C (‰)
5
4
3
2
1
0
log [BTEX] (µg/L)
Average PLFA Del
log BTEX
10
18
1
25

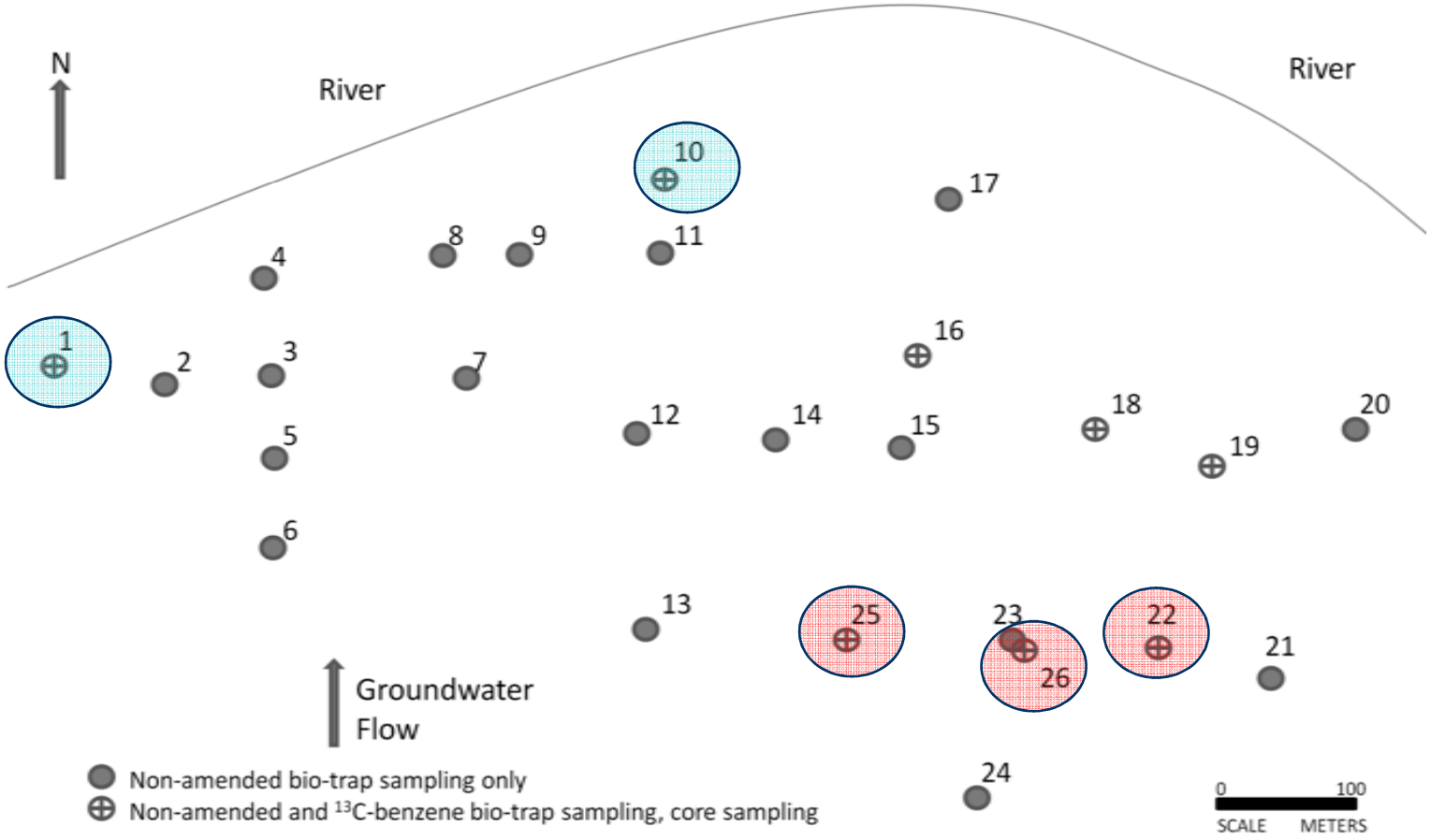
Relative proportion of fatty acids from the PLFA analysis of $^{13}C_6$-benzene-amended beads from the well MW10 bio-trap.



^{13}C Utilized for CO_2 and BTEX Concentration



Relationship between SIP results and proximity to the river



Geochemistry comparison between river and non-river associated sites

Parameter	River (n= 10)	Non-River (n=15)	p
ORP (mV)[1]	-58.1 ± 89.2	-96.4 ± 64.8	0.26
Dissolved Oxygen[1] (mg/L)	1.0 ± 1.2	0.57 ± 0.32	0.06
Methane (µg/L)[2]	4320 ± 2563	6934 ± 3212	0.04
Alkalinity (mg/L)[1]	522 ± 204	444 ± 89	0.11
Ferrous iron (mg/L)[2]	6.5 ± 5.8	9.1 ± 7.5	0.36

[1]Mann-Whitney U test
[2]Student's t test

Results of *in situ* BTEX biodegradation assessment

- A significant, diverse microbial community active in groundwater and vadose zone
- Likely BTEX biodegradation mechanisms: aerobic oxidation, denitrification, sulfate reduction, methanogenesis, and possibly Fe^{+3} reduction
- Microbial distribution in the vadose zone is more influenced by structural characteristics than groundwater hydrocarbon concentrations
- SIP provided direct evidence of benzene biodegradation in 6 of the 8 wells sampled

Questions?