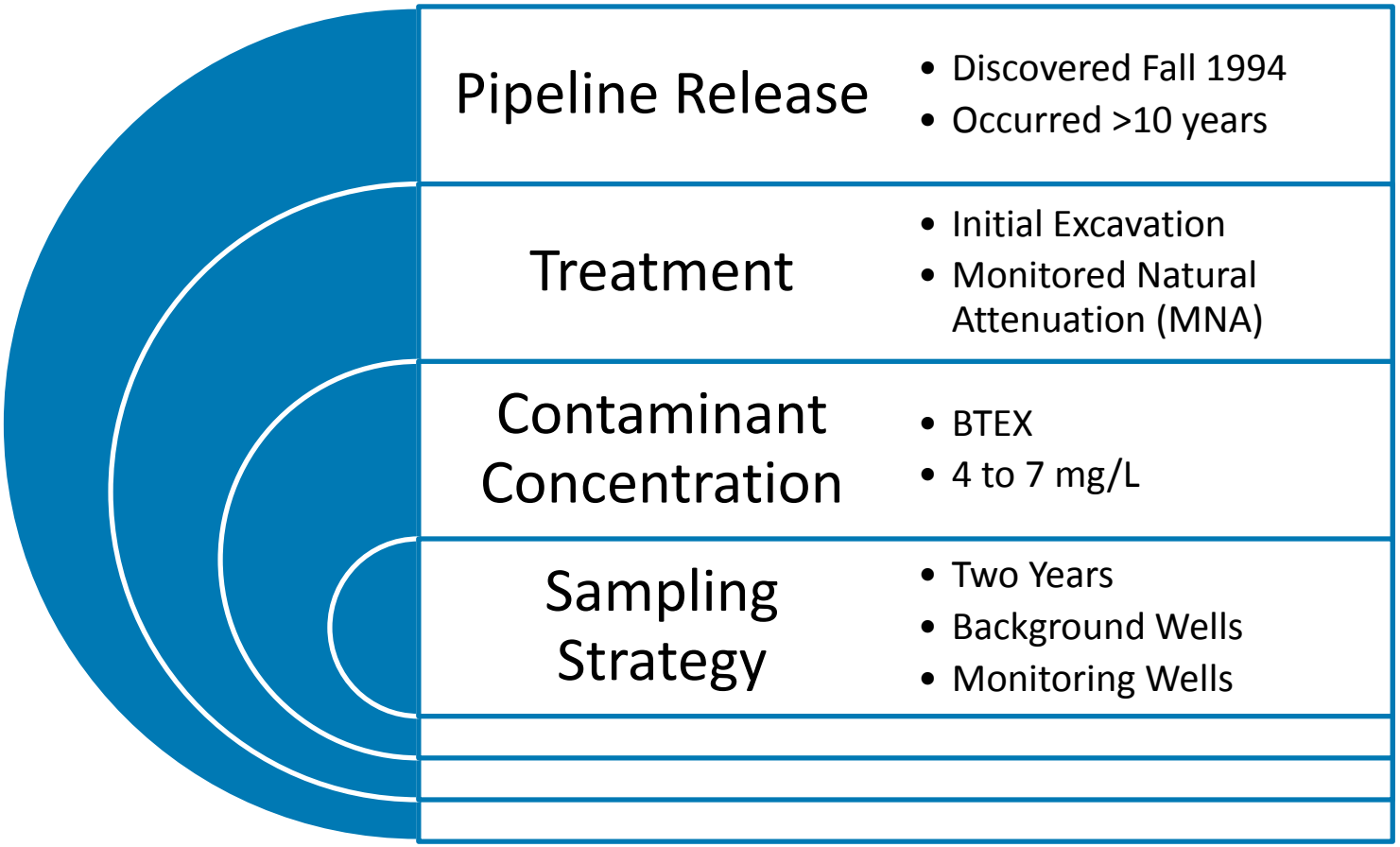
Next Generation Sequencing and qPCR as Complementary Approaches for Evaluating MNA at a Petroleum Hydrocarbon-Impacted Site

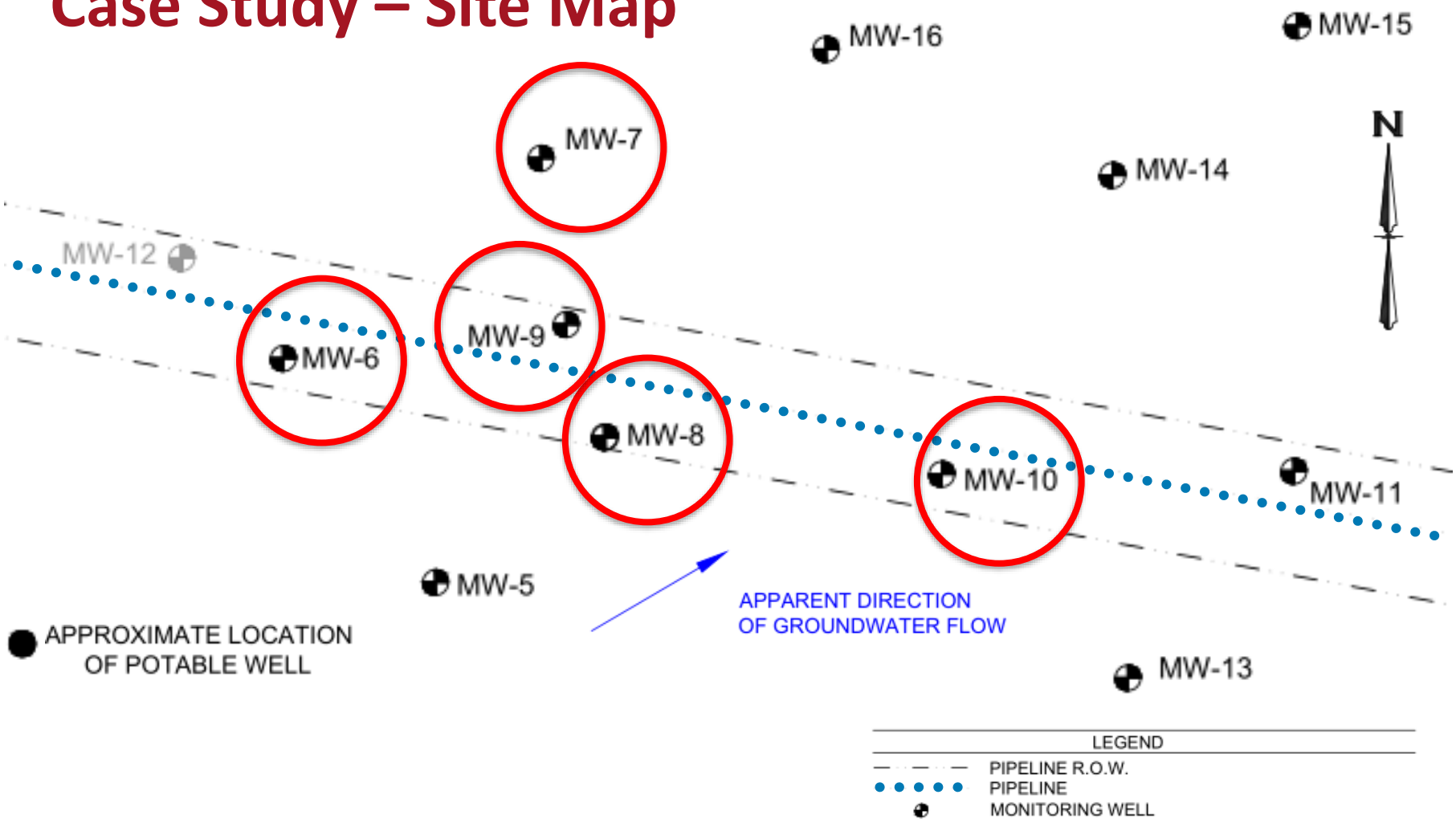
microbialinsights

Case Study – Site Background



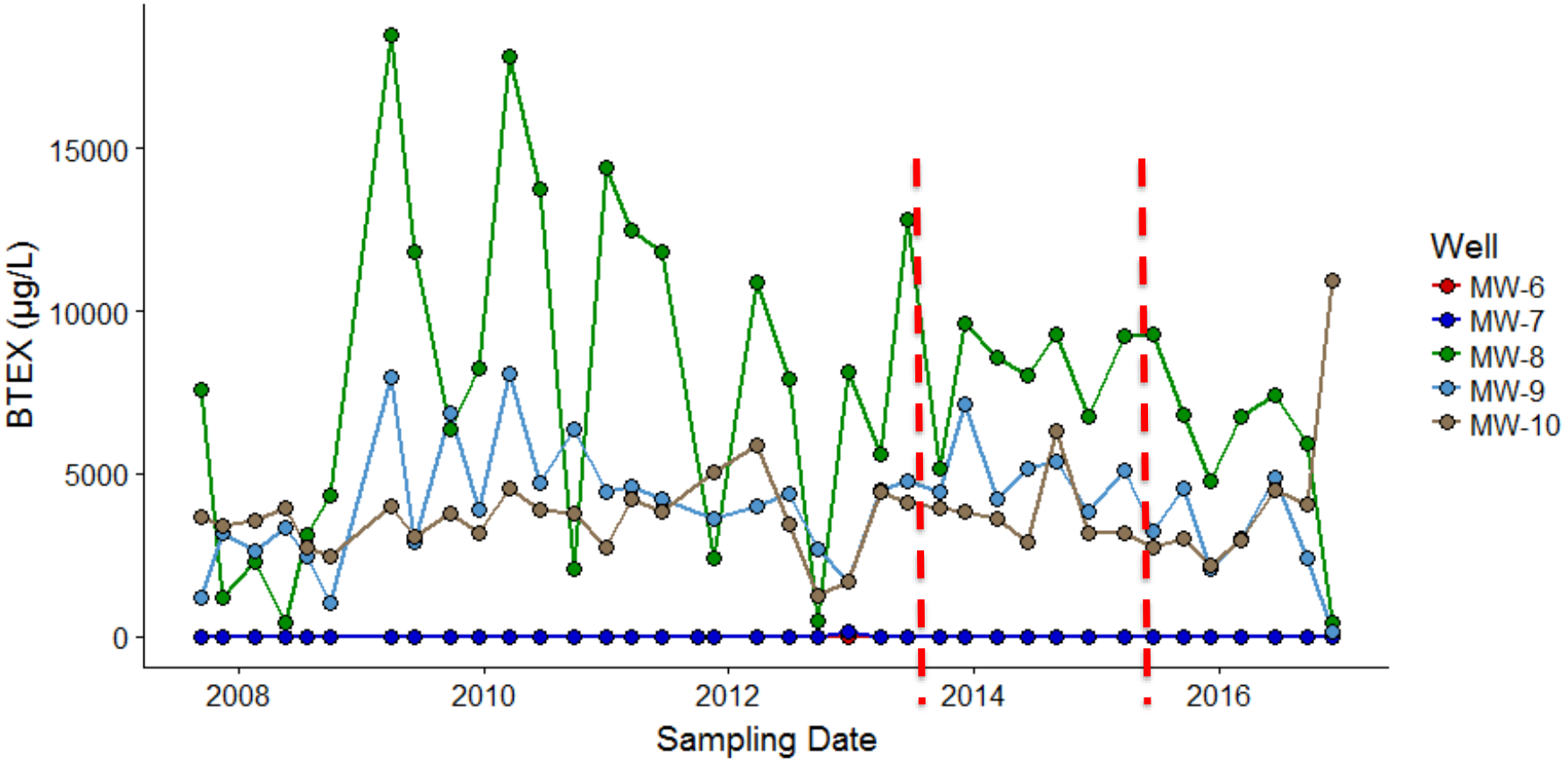
microbialinsights

Case Study – Site Map



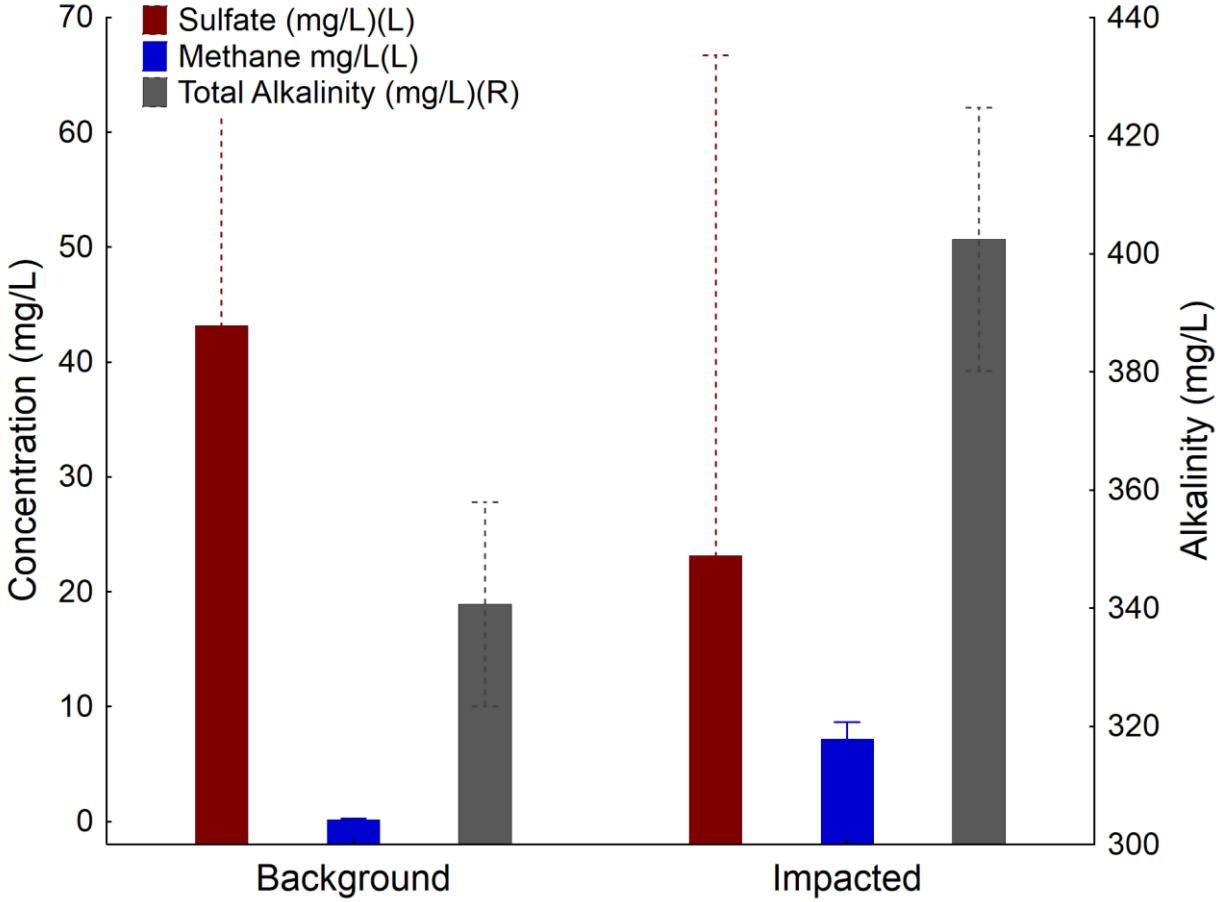
microbialinsights

BTEX Concentrations



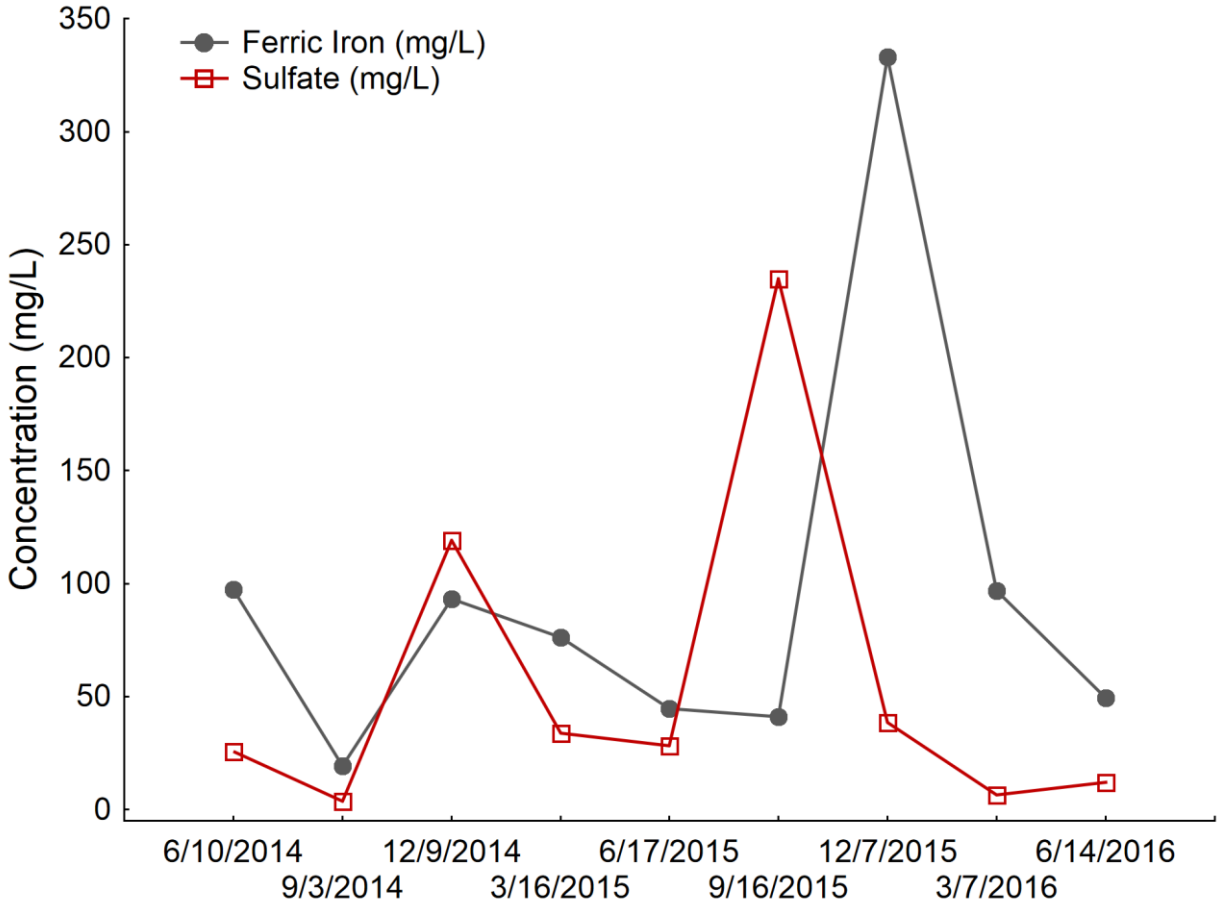
microbialinsights

Geochemistry



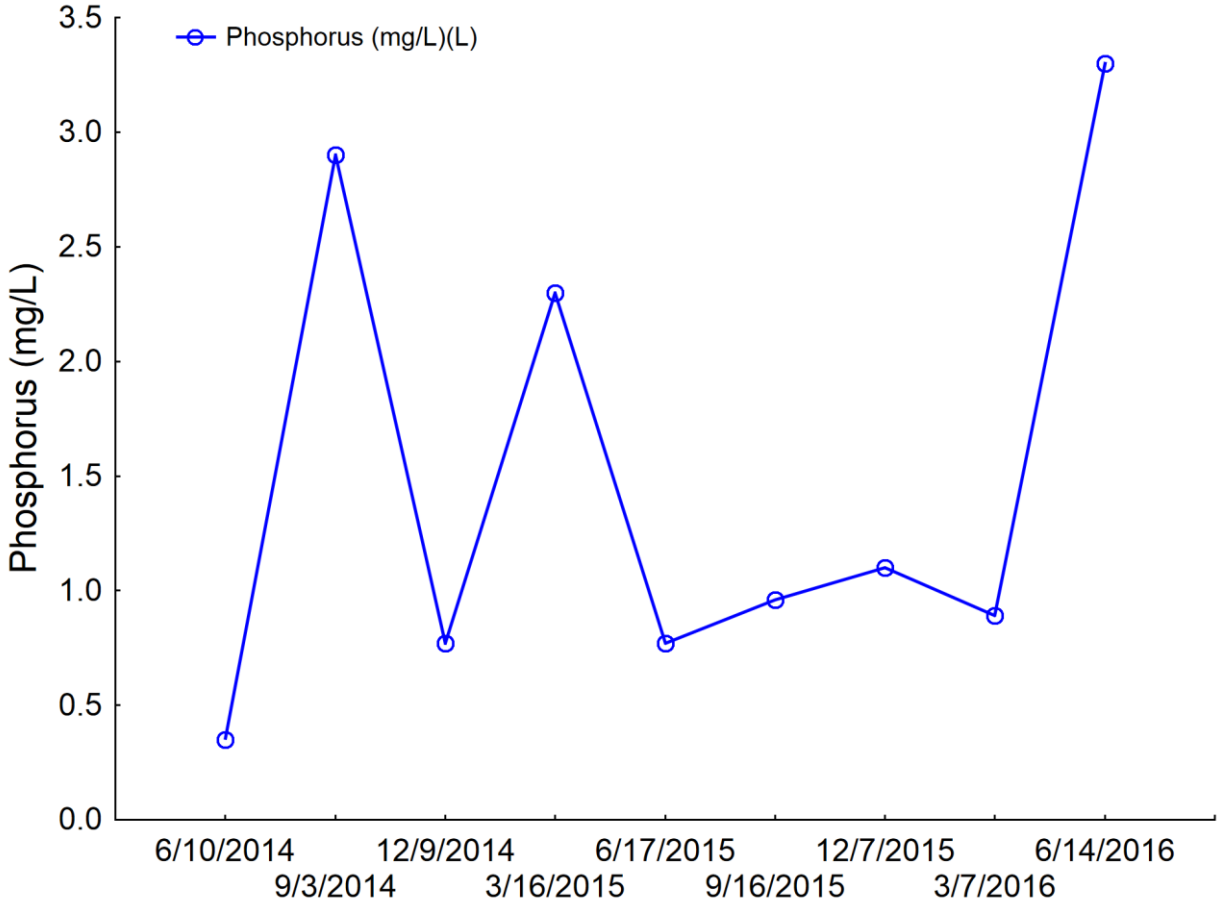
microbialinsights

Variability in Electron Acceptors



microbialinsights

Variability in Nutrients



microbialinsights

Evaluating MNA

- BTEX concentrations
  - stable/decreasing
- Geochemistry
  - Electron acceptor consumption
  - Variability in MNA parameters
  - Agricultural activity & wetland environment
- Microbiology
  - Quantify BTEX degraders (qPCR)
  - Assess changes in the microbial community (NGS)

Molecular Biological Tools

qPCR

What is the concentrations of BTEX degraders?

- ☐ Quantitative
- ☐ Absolute concentration (gene copes/mL)
- ☐ Target Specific
- ☐ Functional genes or taxonomic
- ☐ Small, specific dataset

NGS

Who is there?

- ☐ Not quantitative
- ☐ Relative abundance (% hits)
- ☐ Broad range
- ☐ Typically taxonomic only
- ☐ “Big Data”

microbialinsights

Questions Answered by qPCR and NGS

qPCR

What are the concentrations of BTEX degraders?

Higher concentrations of BTEX degraders within plume?

Specific biodegradation pathways…

What are the concentrations of anaerobic benzene degraders?

NGS

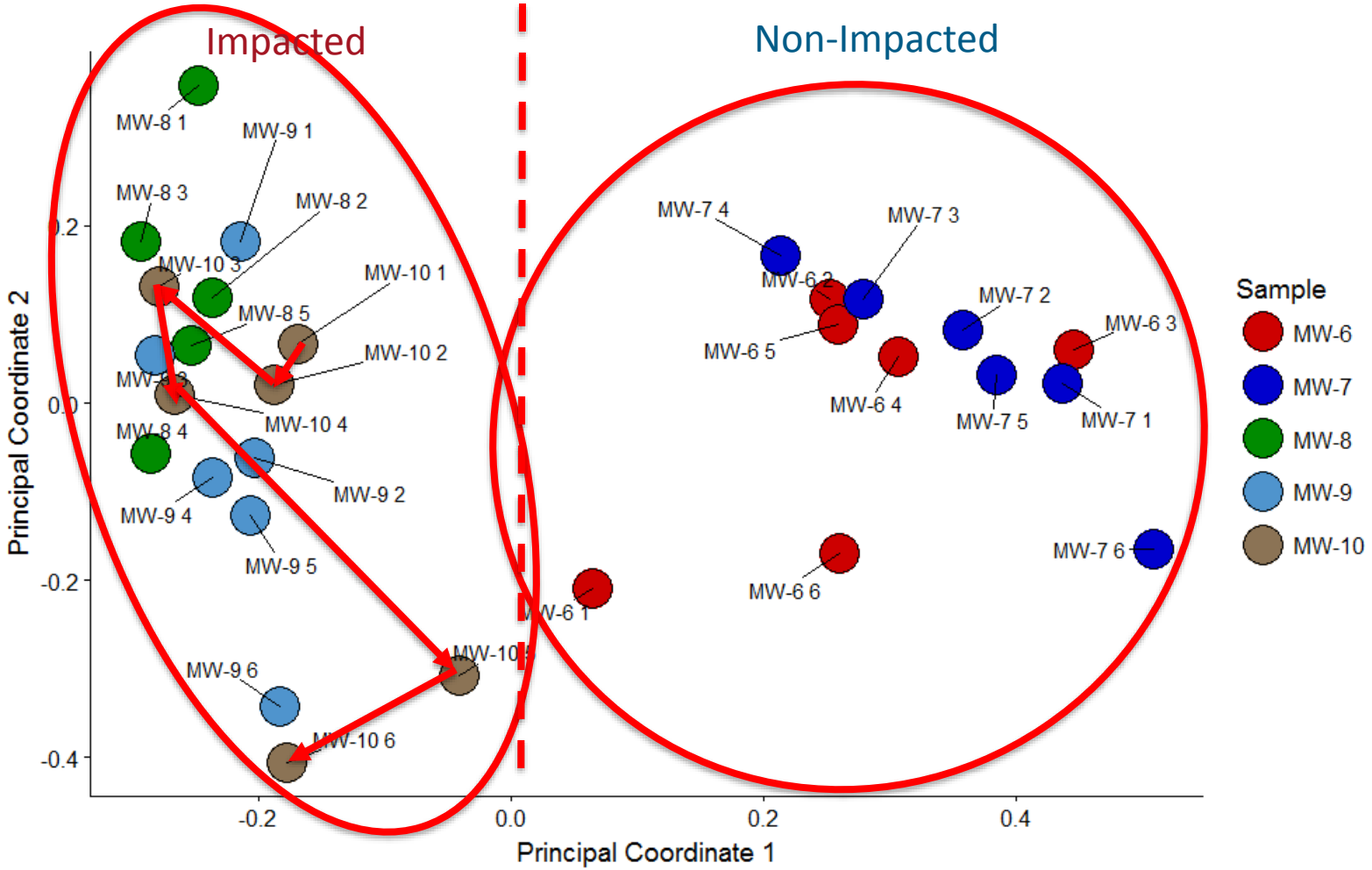
Who is there?

Differences in the microbial community in background vs plume?

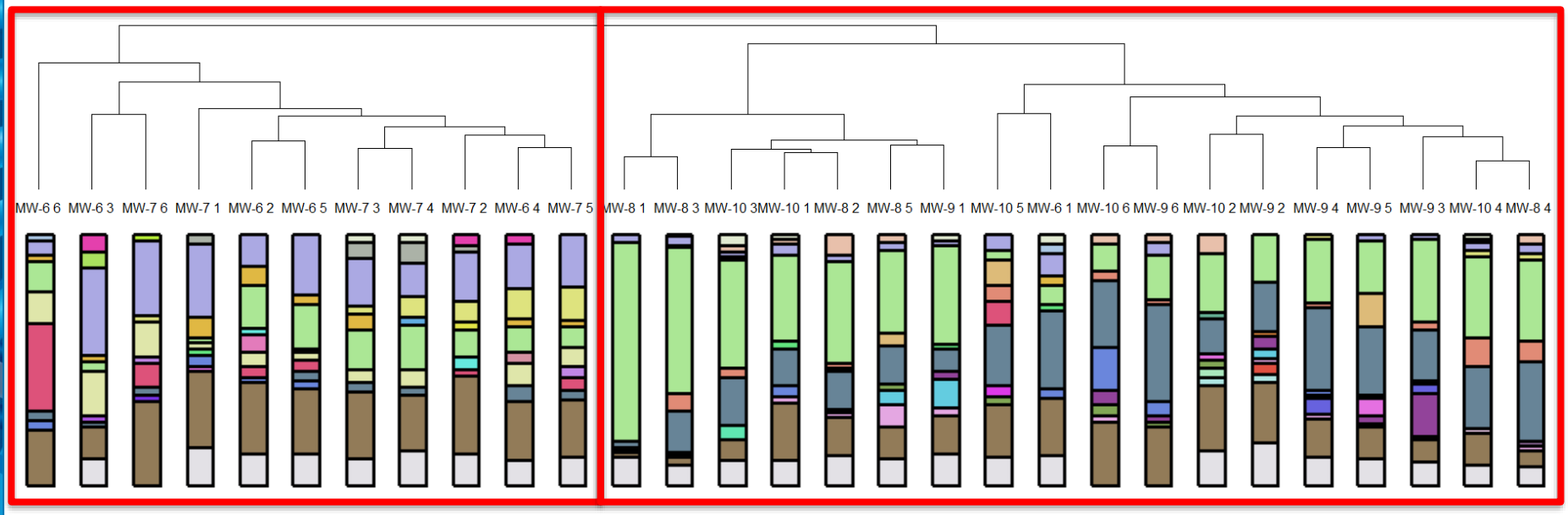
Changes in the microbial community over time?

Impacts on microbial diversity?

Microbial Communities

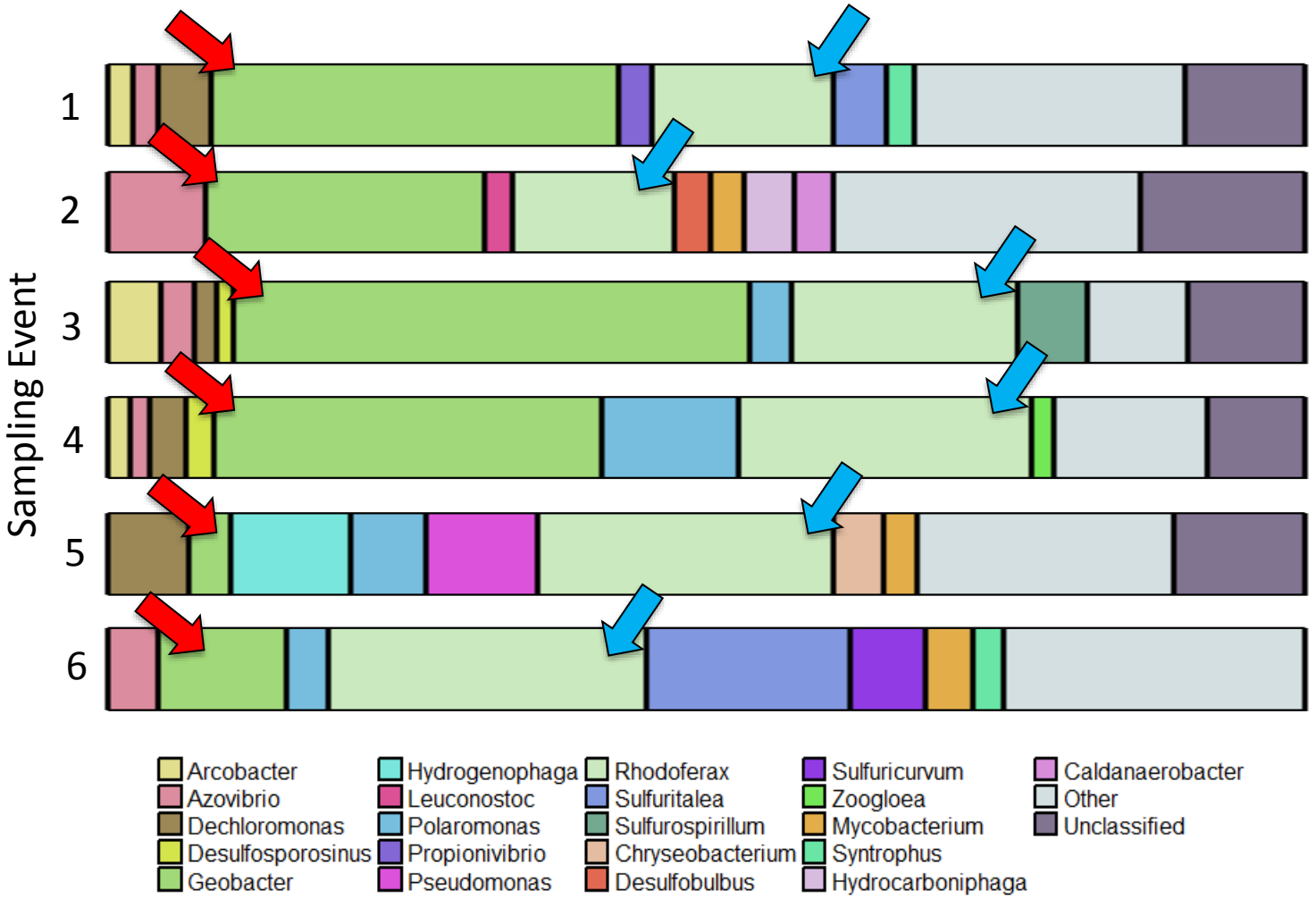


Shifts in Microbial Communities



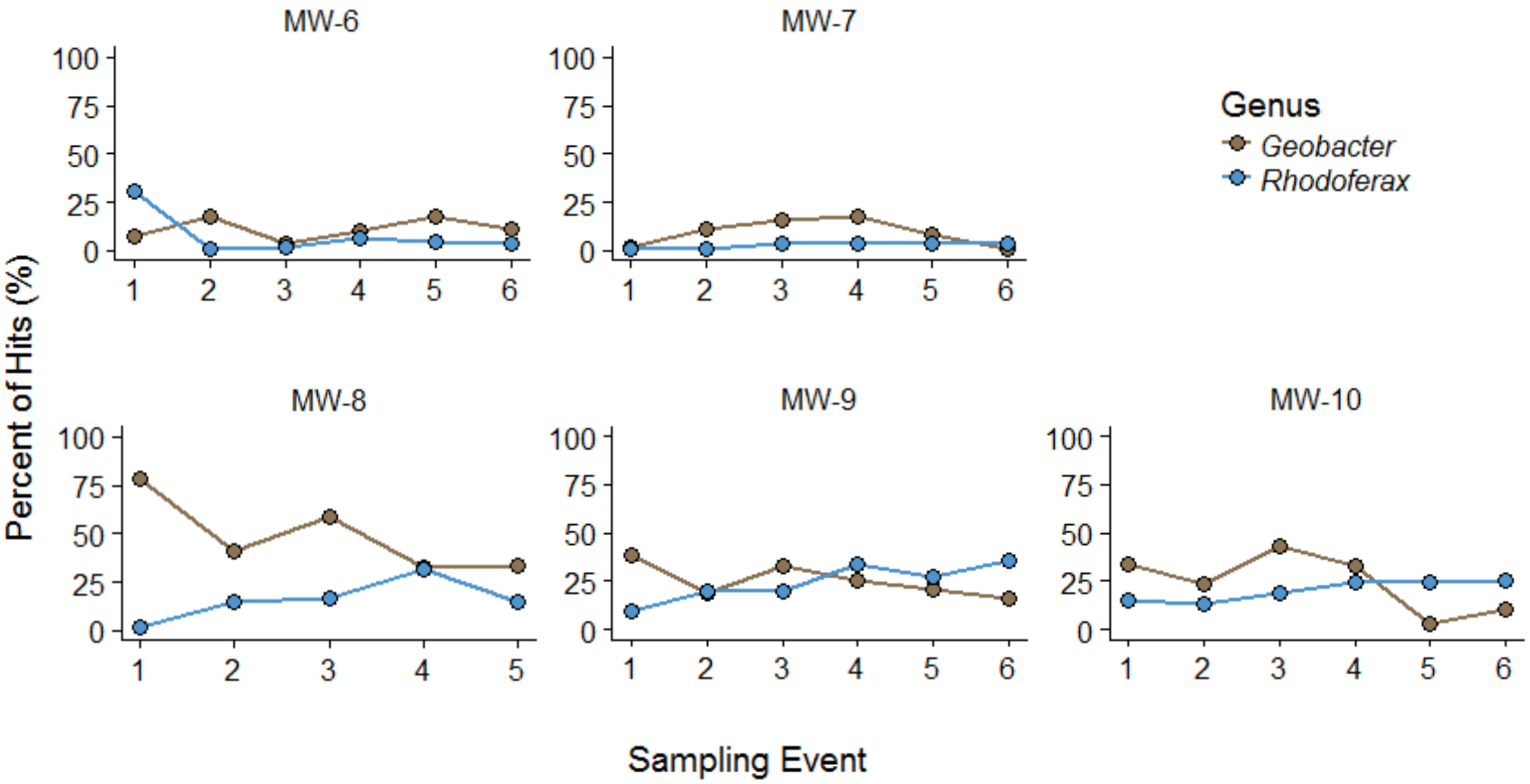
microbialinsights

Changes in MW-10 Over Time



microbialinsights

Rhodoferax and *Geobacter*



microbialinsights

Rhodoferax and *Geobacter*

- Agricultural activities contribute to:
  - Variability in Nitrogen Availability
  - Possible Influx of More Favorable Electron Acceptors

Rhodoferax (Albidoferax)	*Geobacter*
Iron-reducing Bacteria	Iron-reducing Bacteria
Utilize O_2 & NO_3^-	Tolerate O_2
Do Not Fix N_2	Fix N_2
Higher Biomass Yield	Lower Biomass Yield
Slower Growth Rate	Higher Growth Rate

microbialinsights

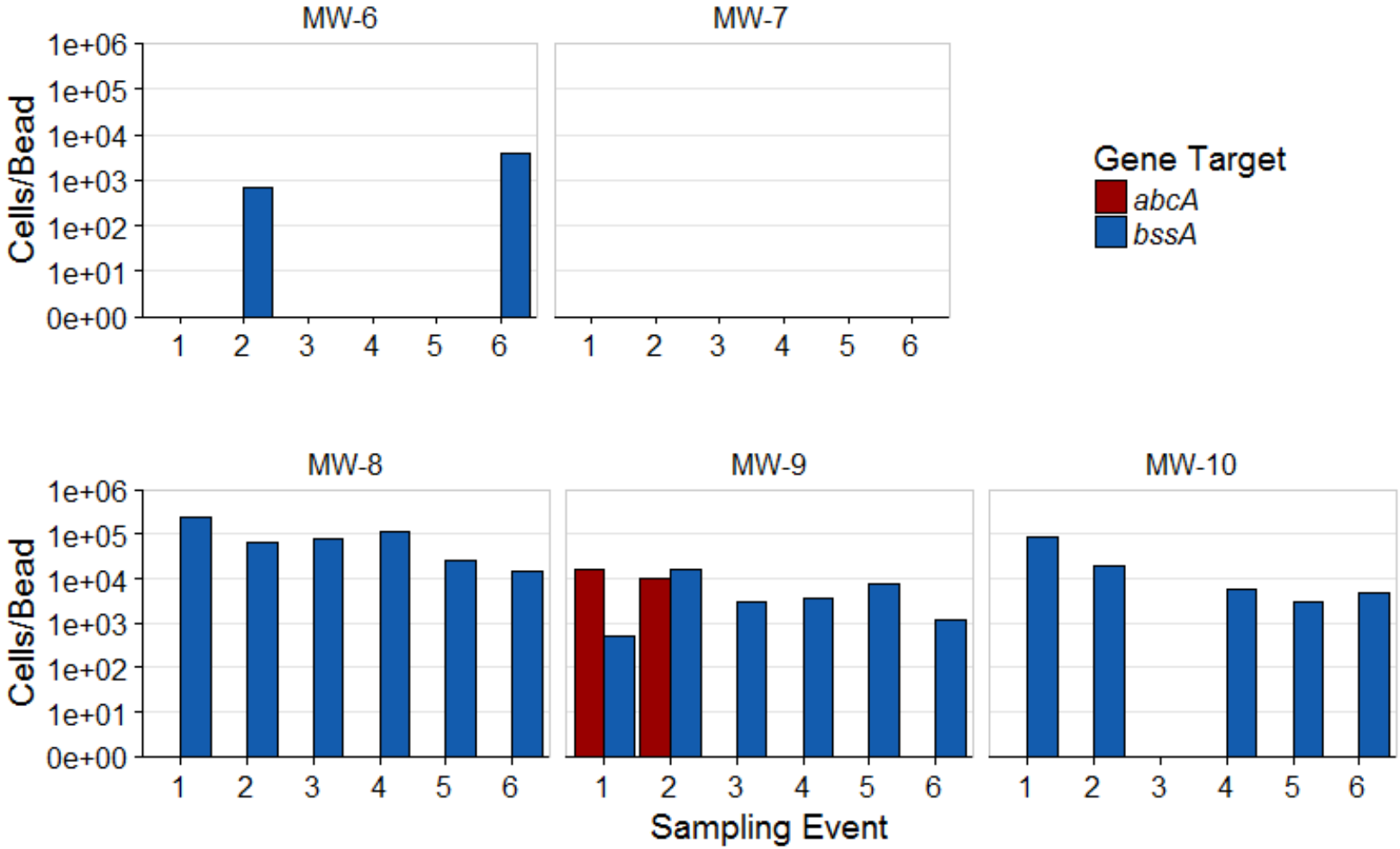
NGS Observations & Conclusions

- Background vs. Impacted
  - Substantial differences in microbial communities
  - Decrease in microbial diversity in impacted wells
  - Higher relative abundances of anaerobes
  - Consistent with TEAPs
- Populations shifts over time
  - *Rhodoferax* (*Albidoferax*) and *Geobacter* competition
  - Changes in electron acceptors or nutrients?

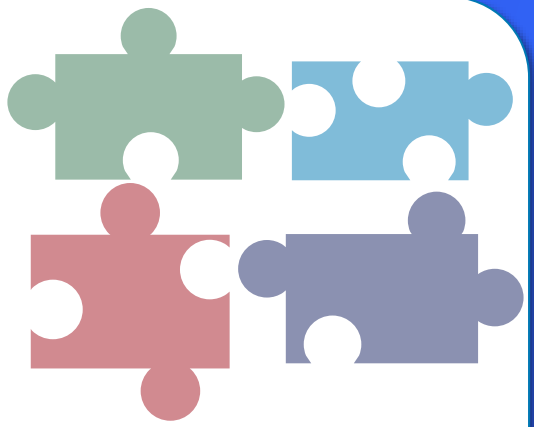
Questions answered by qPCR

- What are the concentrations of anaerobic TEX degraders?
  - Benzylsuccinate synthase (*bssA*)
  - First step in anaerobic biodegradation of TEX
- Concentrations of anaerobic benzene degraders?
  - Anaerobic benzene carboxylase (*abcA*)
  - Initial step in anaerobic benzene biodegradation
  - Other pathways are possible

Potential for Anaerobic BTEX Biodegradation



Putting It All Together



- NGS – Microbial community dynamics
  - Microbial diversity
  - Shift to anaerobic populations
  - Shifts and competition between microbial groups
- qPCR – Line of evidence for biodegradation
  - Quantification of functional genes responsible for anaerobic BTEX biodegradation
  - Supported MNA

Questions???

microbialinsights