In-Well Test to Determine Indigenous Naphthalene Biodegradation under Sulfate-Reducing and Methanogenic Conditions

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Former McCormick and Baxter Superfund Site

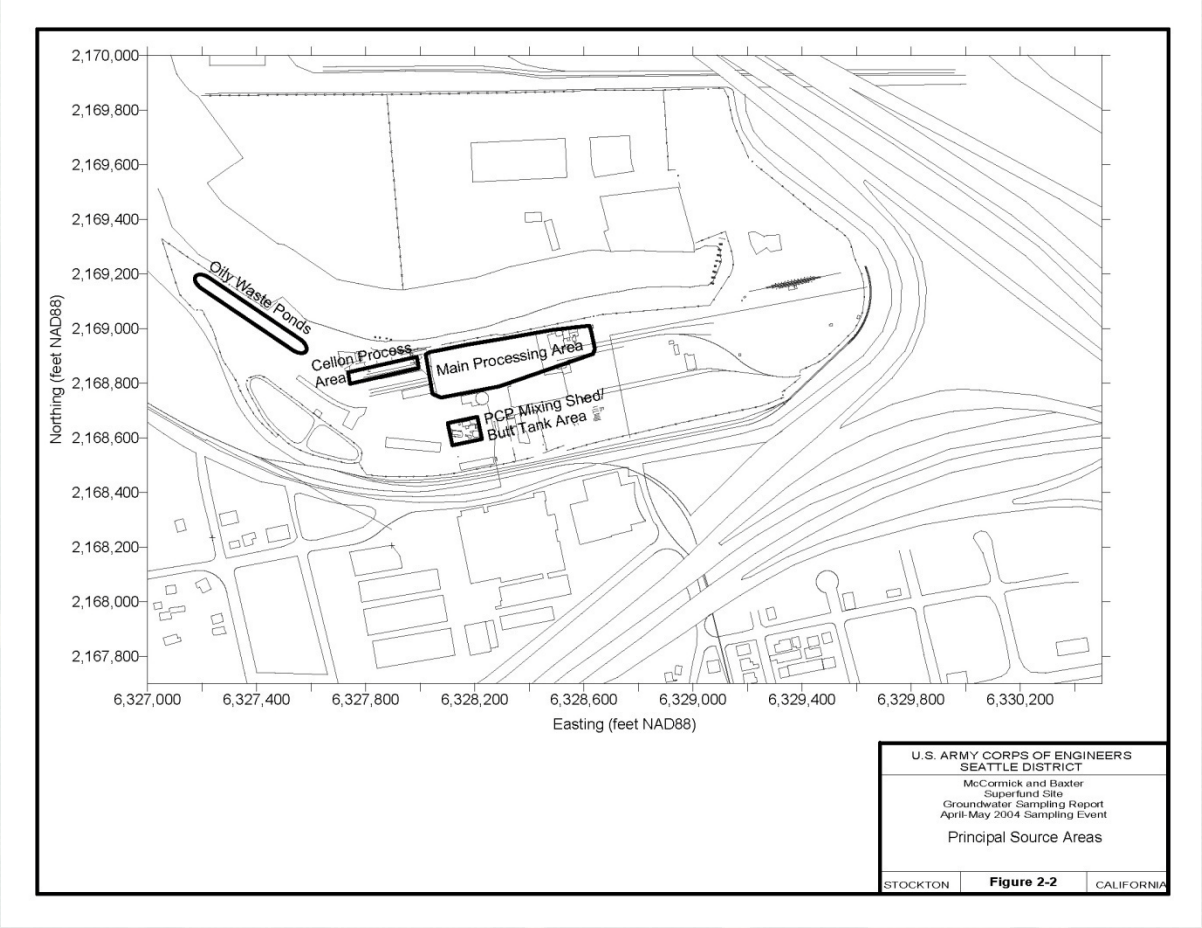
Former Tie and Treating Plant in Stockton, CA

EPA Fund-lead Superfund Site

USACE, Seattle District, with support from the USACE Environmental and Munitions Center of Expertise, providing technical assistance to EPA Region 9

Revision of Focused Feasibility Study to include rigorous evaluation of MNA, along with updates of in-situ thermal, pump and treat, and other technologies/alternatives

Former Creosote Manufacturing Plant



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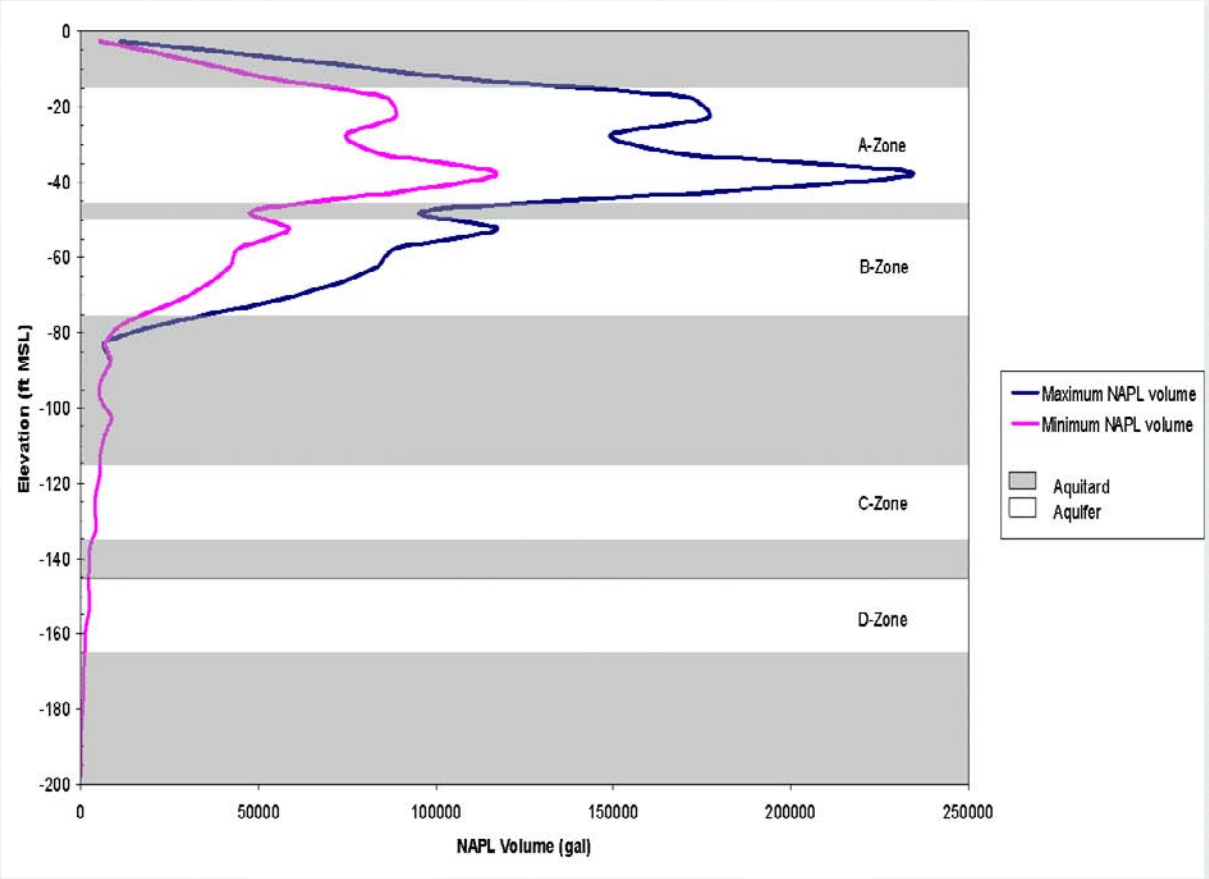
Significant Amounts of Creosote DNAPL (1-2 million gal in Four Aquifer Zones (Down to -200 ft)



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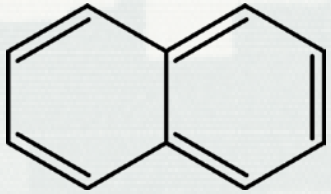
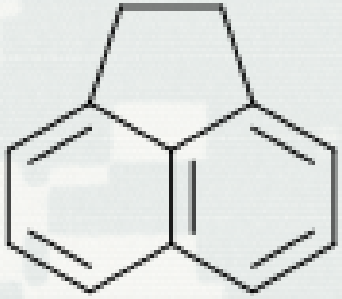
Polyaromatic Hydrocarbons (PAHs) of Interest

- Naphthalene – PAH with the highest source concentrations and potential risk driver



- Acenaphthene – PAH with furthest downgradient migration



Other EPA Superfund Sites with Similar Conditions

- Wyckoff, Brainbridge, WA
- McCormick and Baxter, Portland, OR
- Numerous Former Natural Gas Production Facilities

Why MNA Characterization

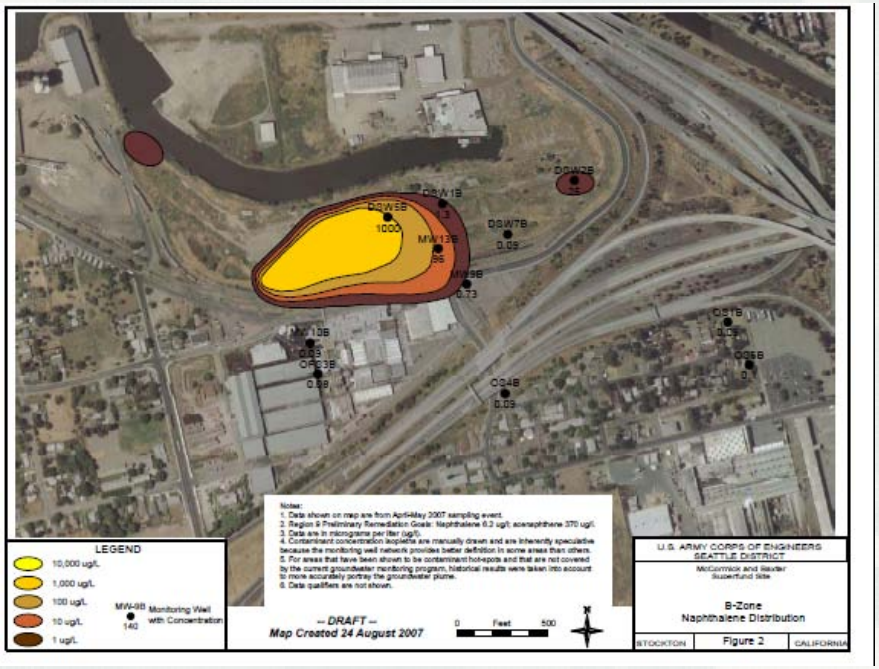
- 1-2 million gallons of creosote DNAPL in the groundwater – is MNA a viable technology?
- Questions to be answered in FFS
  - Can source removal technologies remove DNAPL to the extent it effectively reduces dissolved groundwater contaminant concentrations and the remediation time frame?
  - Can MNA alone control the dissolved phase plume?
  - What is the area that doesn't need to be actively treated, i.e. will naturally attenuate – optimization of the area of active source/dissolved phase treatment

Indications that Biodegradation of Naphthalene is Occurring

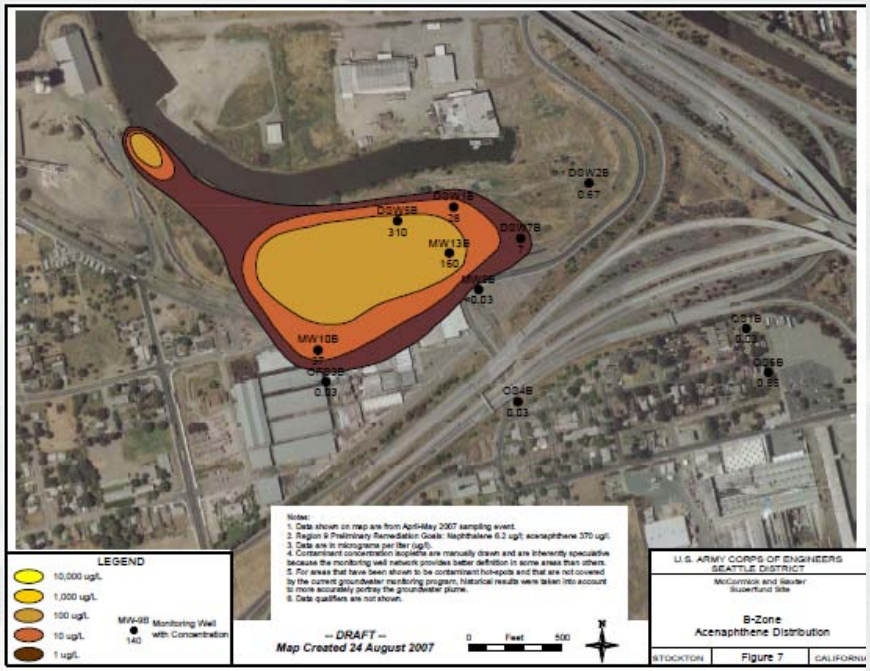
- Analyses by Cook College (Rutgers University) identified intermediate degradation products of naphthalene
- Naphthalene plume is relatively stable
- Comparison of Naphthalene and Acenaphthene Plumes
  - Naphthalene has highest source area concentrations
  - Naphthalene is lower molecular weight – expected to be more mobile compared to acenaphthene but naphthalene is less widespread

Comparison of Naphthalene and Acenaphthene Plumes

- B-Zone Naphthalene



- B-Zone Acenaphthene



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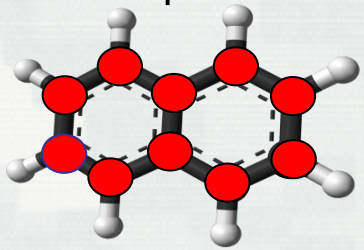
Questions To Be Answered for 3-D Modeling of Monitored Natural Attenuation

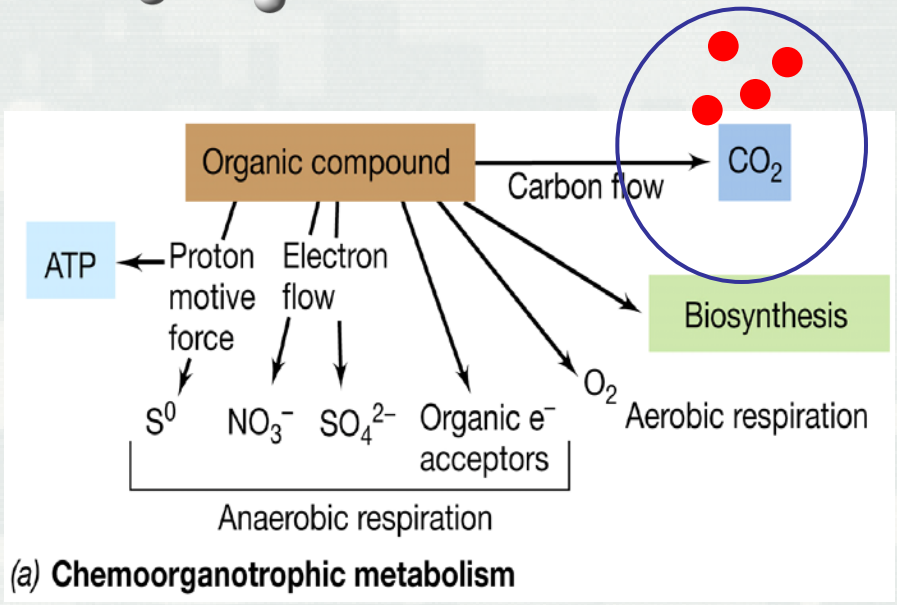
- Is naphthalene degrading under the predominant geochemical conditions in the aquifer (sulfate-reducing and methanogenic): In-Situ (Bio-trap) Study
- Rates of naphthalene degradation for modeling monitored natural attenuation: Lab Microcosm Study
- Spatial variability of naphthalene degradation: In-Situ (Bio-trap) Study
- Only naphthalene studied because of expense of 13C acenaphthene

What is Stable Isotope Probing (SIP)?

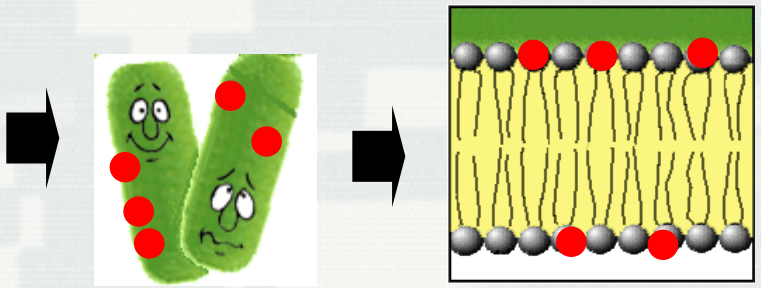
^{13}C Naphthalene



- **The coupling of molecular methods along with stable isotopic compounds provides a way to link biodegradation to the responsible organisms**



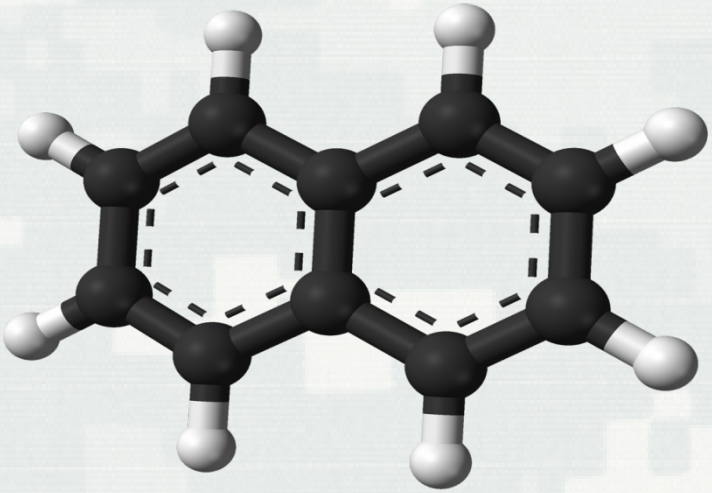
^{13}C Membrane PLFA



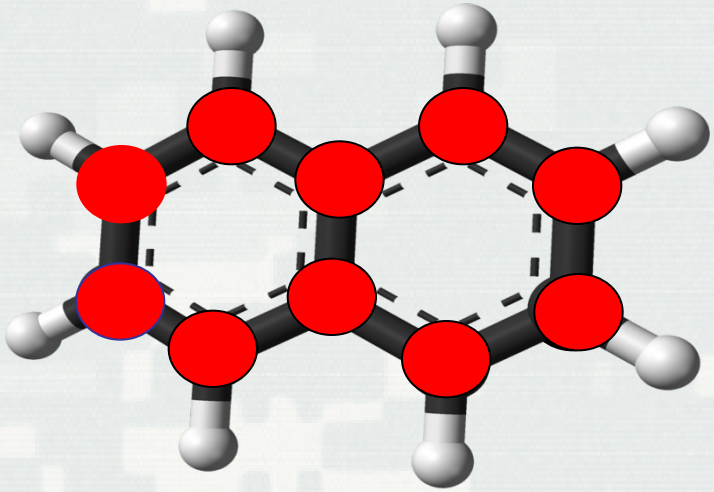
Answers the question is the COC being destroyed at this site?

Microcosms were loaded with ^{13}C Naphthalene

Regular ^{12}C Naphthalene



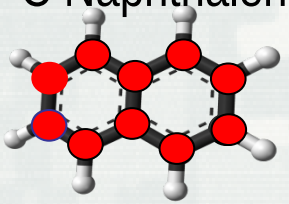
^{13}C Naphthalene



In Situ Bio-Trap Study Design

- Individual traps (18) over the five different aquifer zones and within sulfate-reducing and methanogenic conditions across each zone
- Two pairs, one amended with sulfate, in a well most likely to provide samples for microcosm study. One pair removed at intermediate times to proof analytical methods.

^{13}C Naphthalene



Bio-Sep Bead

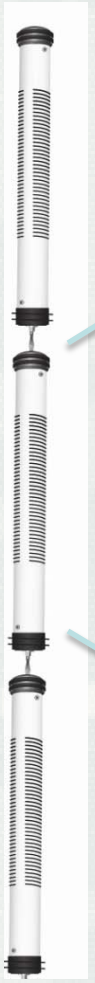
Assembly

Unit

Samplers

MNA

Sulfate amended



Geochemical

COC

MICRO Sampler w/^{13}C Naphthalene

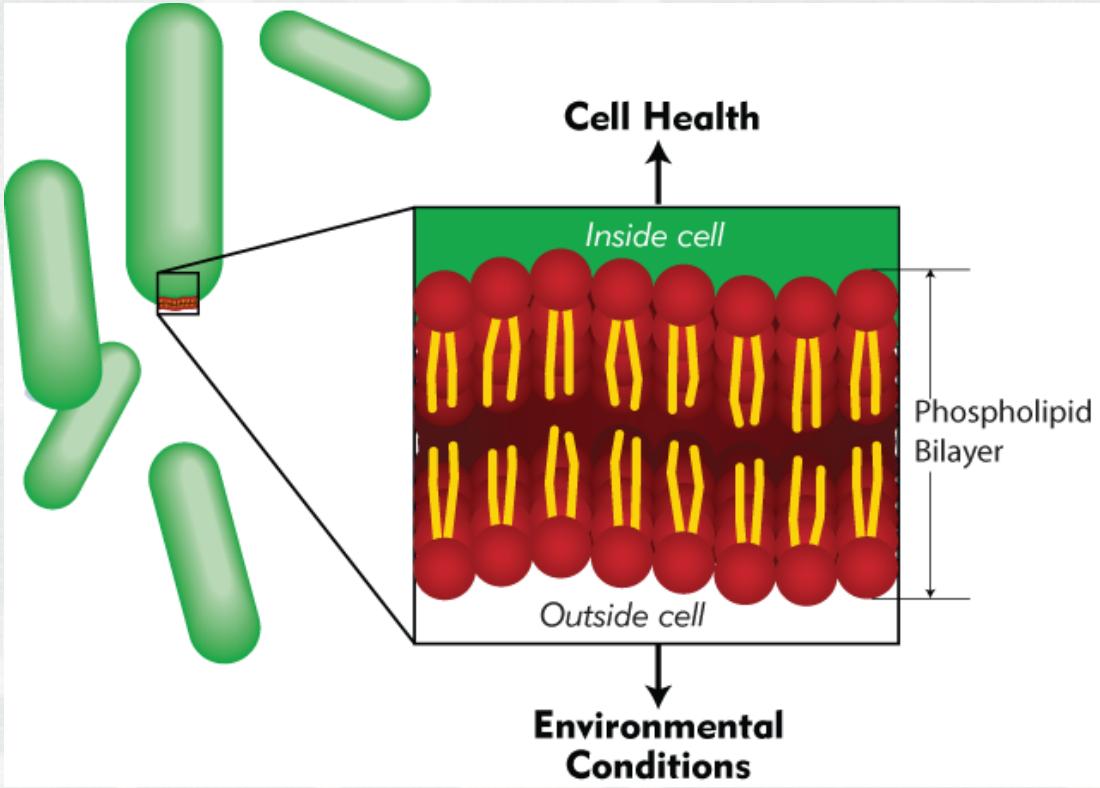
Sulfate Supplier

Demonstration of Naphthalene Biodegradation Decision Rules

- Detection of carbon-13 in **phospholipid fatty acids** (microbial biomass).
- Detection of carbon-13 in **carbon dioxide** (microbial metabolic activity).
- Detection of carbon-13 in **methane** (microbial metabolic activity).
- Decision criteria was based on establishing the background carbon-13 levels for each compound and then calculating the 99% upper tolerance limit. Carbon-13 levels above the 99% upper tolerance limit were accepted as carbon-13 enrichment.

Phospholipid Fatty Acid Analysis

- Essentially the “skin” of the microbe
- Monitor incorporation of ^{13}C Naphthalene
- Quantitative results



In Situ Bio-Trap Data

- Geochemical
  - ►sulfate, nitrate and dissolved methane
- Residual naphthalene concentrations on the Biosep beads.
- Carbon 13 in microbial biomass, CO_2 and methane.

^{13}C Incorporation Results – Microbial Biomass

Carbon-13 Delta Lipid Membrane Values from Field-Deployed Bio-Traps®

Well	Geochemical Condition	Estimated Total Microbial Biomass (Cells/mL)	Estimated Total ^{13}C Biomass[a] (Cells/mL)	Percent ^{13}C Incorporation[a]	Average $^{13}\delta$ ‰ Value[b]	Max $^{13}\delta$ ‰ Value	Confirmed Degradation
DSW7A	Sulfate Reducing	3.6E+05	2.6E+03	0.7%	58	260	Yes
MW1A	Methanogenic	5.8E+05	5.5E+02	0.1%	53	110	Yes
SW171A1	Nitrate Reducing	9.7E+05	5.8E+03	0.6%	126	447	Yes
DSW2B	Sulfate Reducing	1.2E+05	7.5E+02	0.6%	64	160	Yes
DSW5B	Methanogenic	3.5E+05	2.6E+03	0.8%	48	180	Yes
DSW7B	Iron to Sulfate Reducing	1.8E+05	1.0E+03	0.5%	120	320	Yes
MW15C	Methanogenic	8.7E+04	1.4E+02	0.2%	10	30	Yes
MW8C	Methanogenic	1.3E+05	9.0E+01	0.1%	37	37	Yes
OFS4C	Sulfate Reducing	2.7E+05	1.3E+02	0.1%	28	81	Yes
ONS1C	Methanogenic	2.0E+05	3.7E+02	0.2%	21	34	Yes
MW18D	Methanogenic	6.7E+04	4.2E+02	0.6%	550	1700	Yes
MW19D	Sulfate Reducing	1.6E+05	1.6E+01	0.0%	19	37	Yes
ONS1D	Methanogenic	3.8E+04	3.3E+02	0.9%	140	330	Yes
MW20E	Methanogenic	1.4E+05	1.7E+02	0.1%	27	120	Yes
MW21E	Methanogenic	1.9E+05	2.8E+02	0.2%	24	94	Yes
MW24E	Low Sulfate	4.4E+04	0.0E+00	0.0%	9.0	14	Yes
MW4E	Methanogenic	5.2E+04	1.3E+02	0.3%	30	45	Yes
MW6E	Methanogenic	1.7E+04	0.0E+00	0.0%	6.0	9.0	Yes

Carbon-13 Delta Carbon Dioxide (Dissolved Inorganic Carbon) Values from Field-Deployed Bio-Traps®

Carbon-13 Delta Carbon Dioxide (Dissolved Inorganic Carbon) Values from Field-Deployed Bio-Traps®

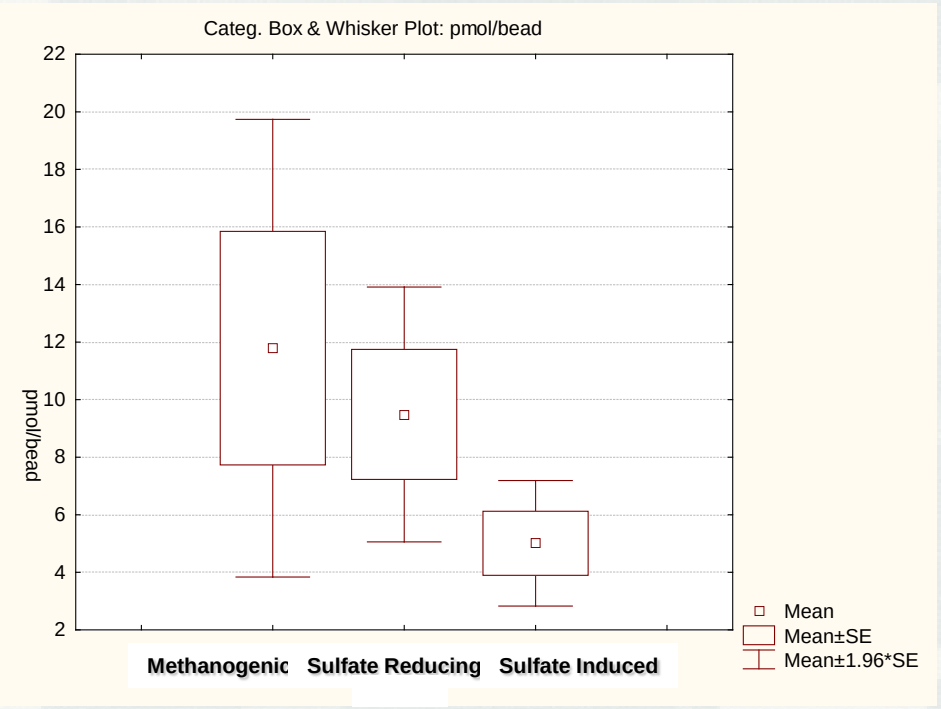
Well	Replicate 1 $^{13}\delta$ ‰	Replicate 2 $^{13}\delta$ ‰	Average DIC $^{13}\delta$ ‰	Percent ^{13}C	Confirmed Degradation
DSW7A	-30	-24	-27	1.1	No
MW1A	-14	-29	-21	1.1	No
SW171A1	-27	-28	-28	1.1	No
DSW2B	-14	-27	-20	1.1	No
DSW5B	-25	67	21	1.1	Yes
DSW7B	-32	142	55	1.2	Yes
MW15C	60	-17	22	1.1	Yes
MW8C	303	281	290	1.4	Yes
OFS4C	-17	-12	-15	1.1	No
ONS1C	-32	11	-10	1.1	No
MW18D	139	-28	55	1.2	Yes
MW19D	-27	-29	-28	1.1	No
ONS1D	-28	-32	-30	1.1	No
MW20E	-21	-32	-27	1.1	No
MW21E	122	112	120	1.2	Yes
MW24E	-12	42	15	1.1	Yes
MW4E	-23	-32	-28	1.1	No
MW6E	-28	-30	-29	1.1	No

^{13}C Incorporation Results

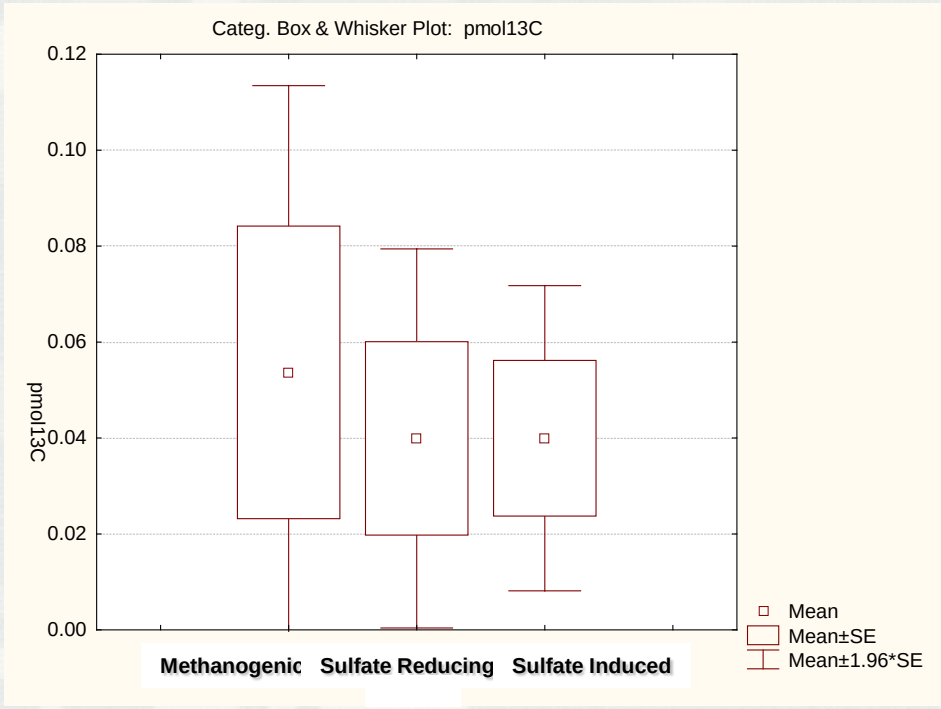
- ^{13}C incorporation from Naphthalene above the acceptance threshold in microbial biomass was recorded in all 17 locations.
- ^{13}C incorporation from Naphthalene above the acceptance threshold in CO_2 was recorded in 7 of the 17 locations.
- No ^{13}C incorporation was observed in methane.

Microbial Biomass (TEAP)

Microbial Biomass



^{13}C Microbial Biomass



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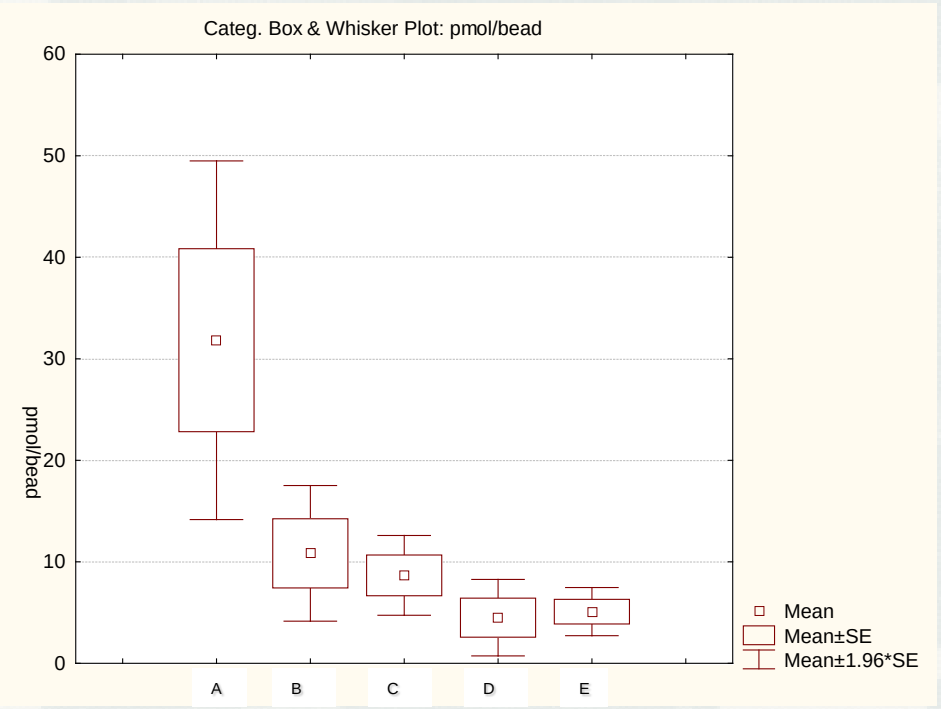
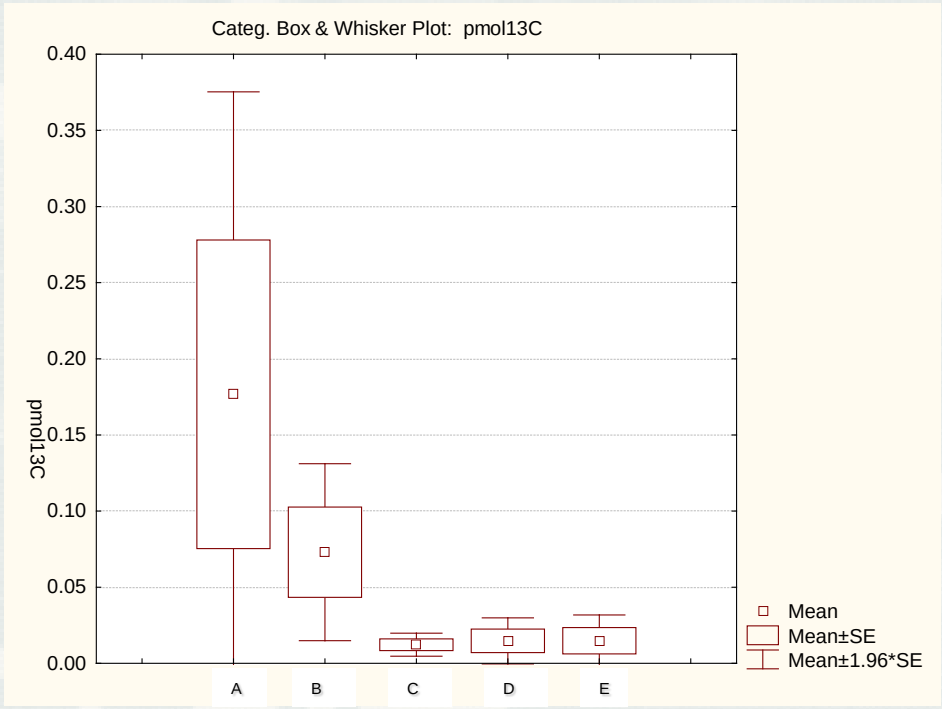
Microbial Biomass (Aquifer Zone)

Microbial Biomass



^{13}C Microbial Biomass



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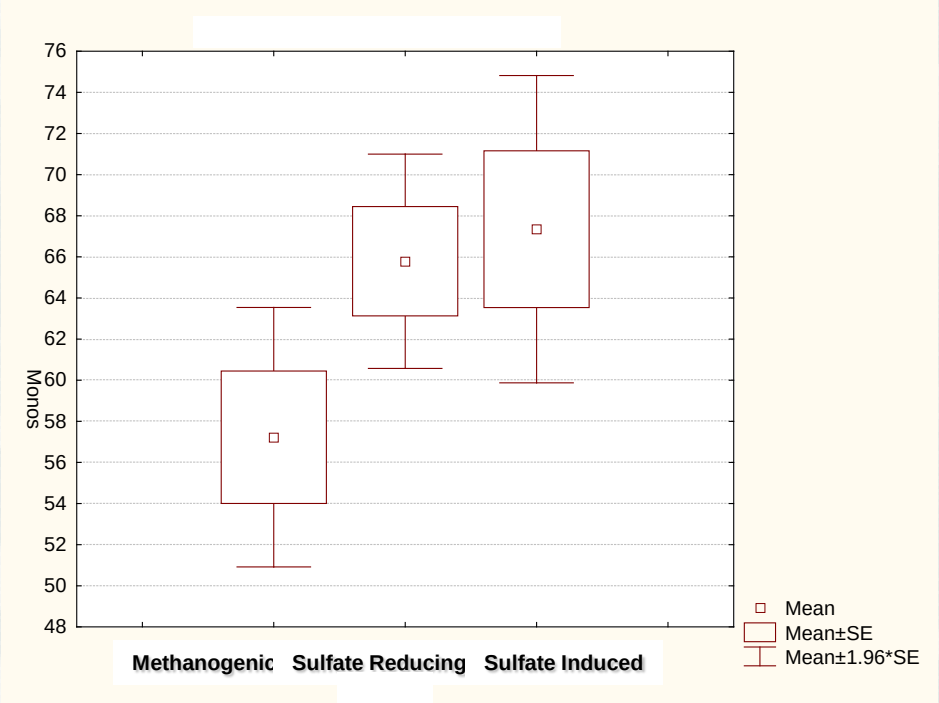
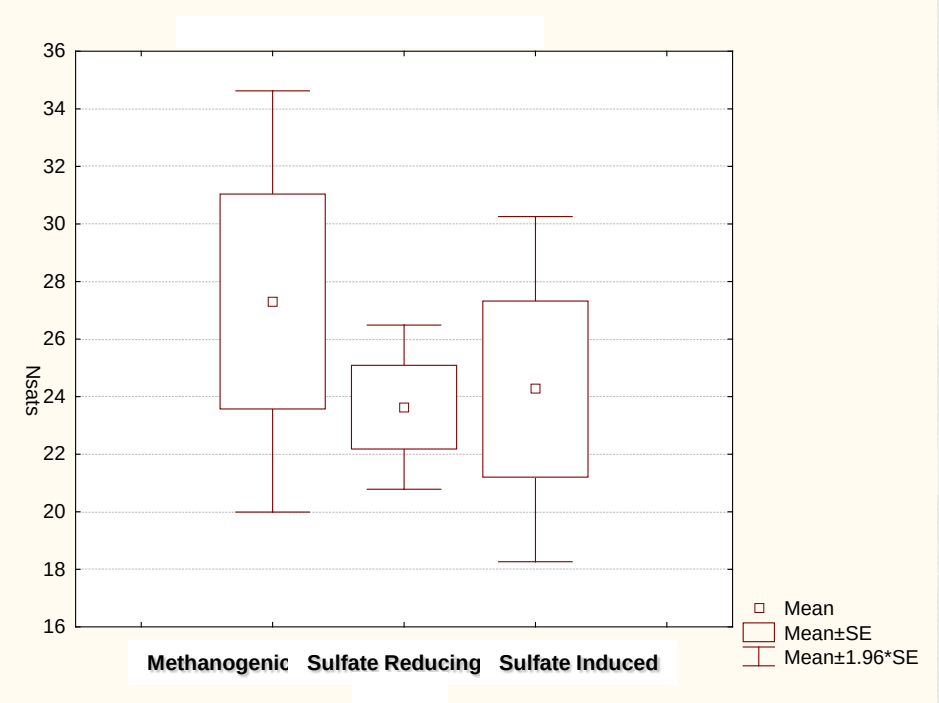
Community Composition (TEAP)

Monounsaturated PLFA



Normal Saturated PLFA



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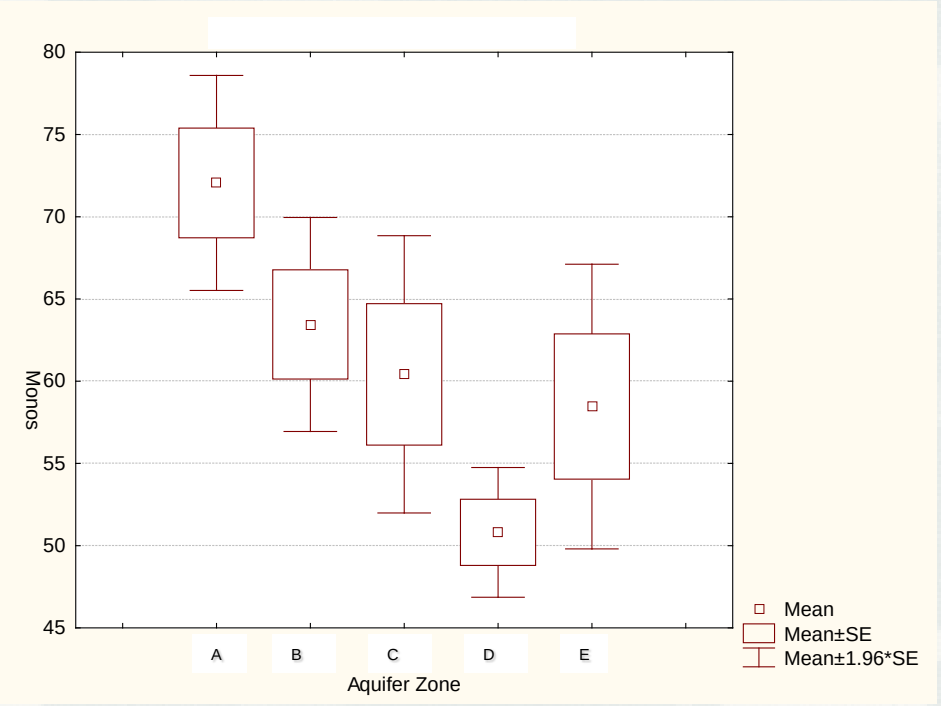
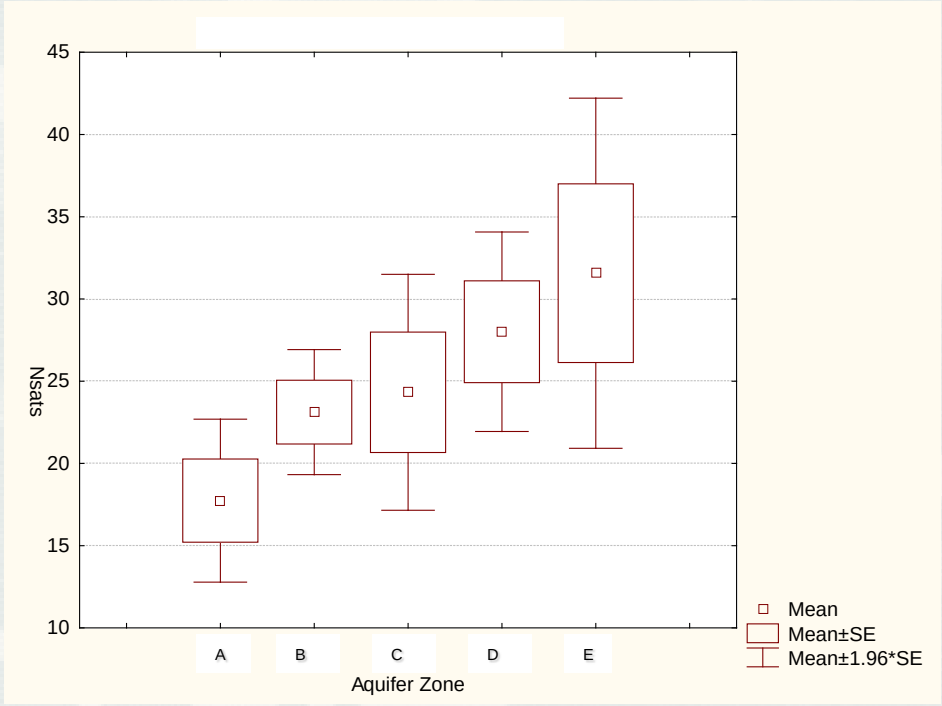
Community Composition (Aquifer Zone)

Monounsaturated PLFA



Normal Saturated PLFA



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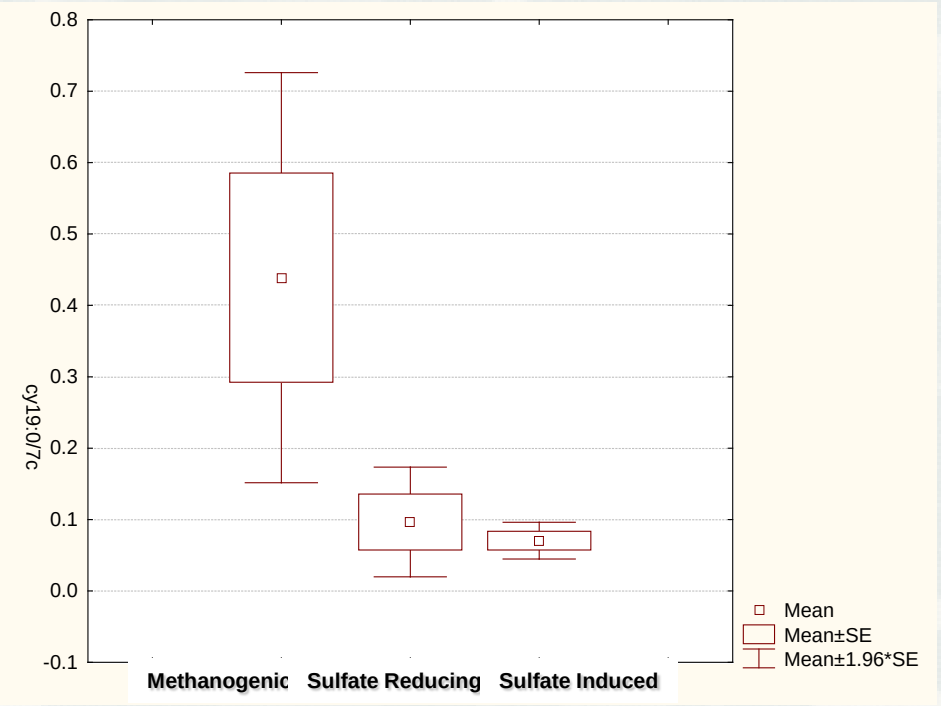
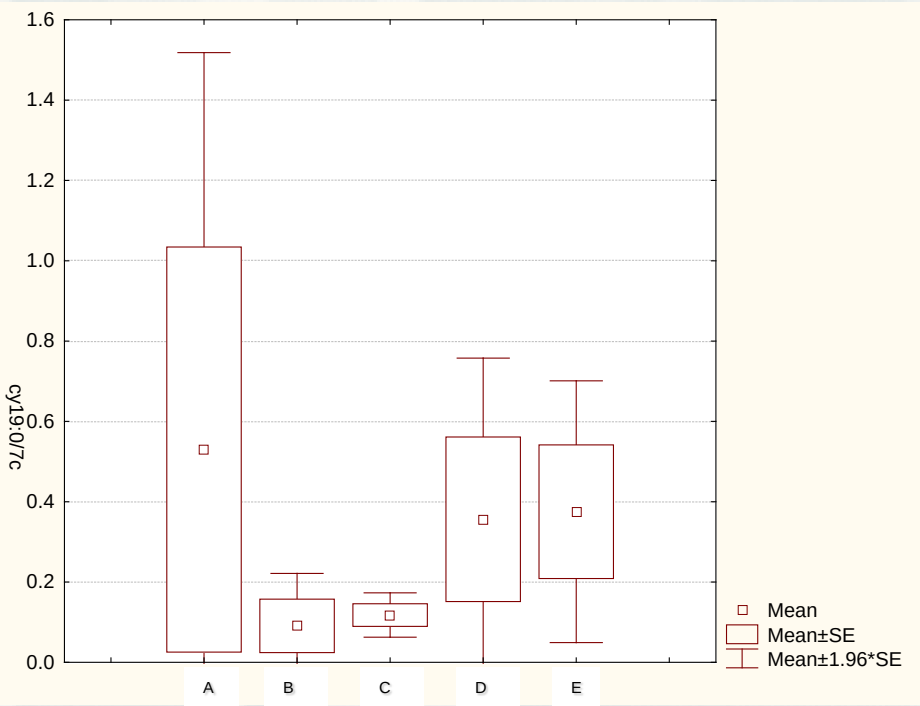
Microbial Activity Biomarkers

TEAP



Aquifer Zone



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Conclusions

- The ability of the microbial community to degrade naphthalene at the McCormick and Baxter Site appears to be (is) widespread.
- TEAPs and Aquifer Zone (Physical Location) both influenced the microbial community biomass, composition, physiological status and the resulting naphthalene degradation.

Questions?

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