

Use of MBTs for decision-making following thermal remedy

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Site conditions

- **Rutgers University- campus setting:** active maintenance area, nearby residential housing, student center, and other student activities
- **Contamination:** > 300 ppm PCE and up to 60 ppm carbon tetrachloride in bedrock groundwater, potential presence of DNAPL
- **Bedrock:** unconsolidated weathered shale deposits (Passaic Formation)
- **Static groundwater level:** 4 to 12 ft below ground surface (bgs)
- **Target treatment area:** 20,000 square feet from 6 - 30 ft bgs, approximately 17,000 cubic yards
- **Utilities/structures:** Occupied building with active utilities including fiber-optic communications, potable water, natural gas, and storm sewer

Background

- Site Remedial Strategy: Reduce source zone GW VOC concentrations to enable MNA closure with periodic groundwater monitoring
- Sodium permanganate and in situ chemical reduction (ISCR) previously implemented
- Electrical Resistance Heating (ERH) in situ thermal remediation selected for enhanced source removal (2016)



ERH system design elements

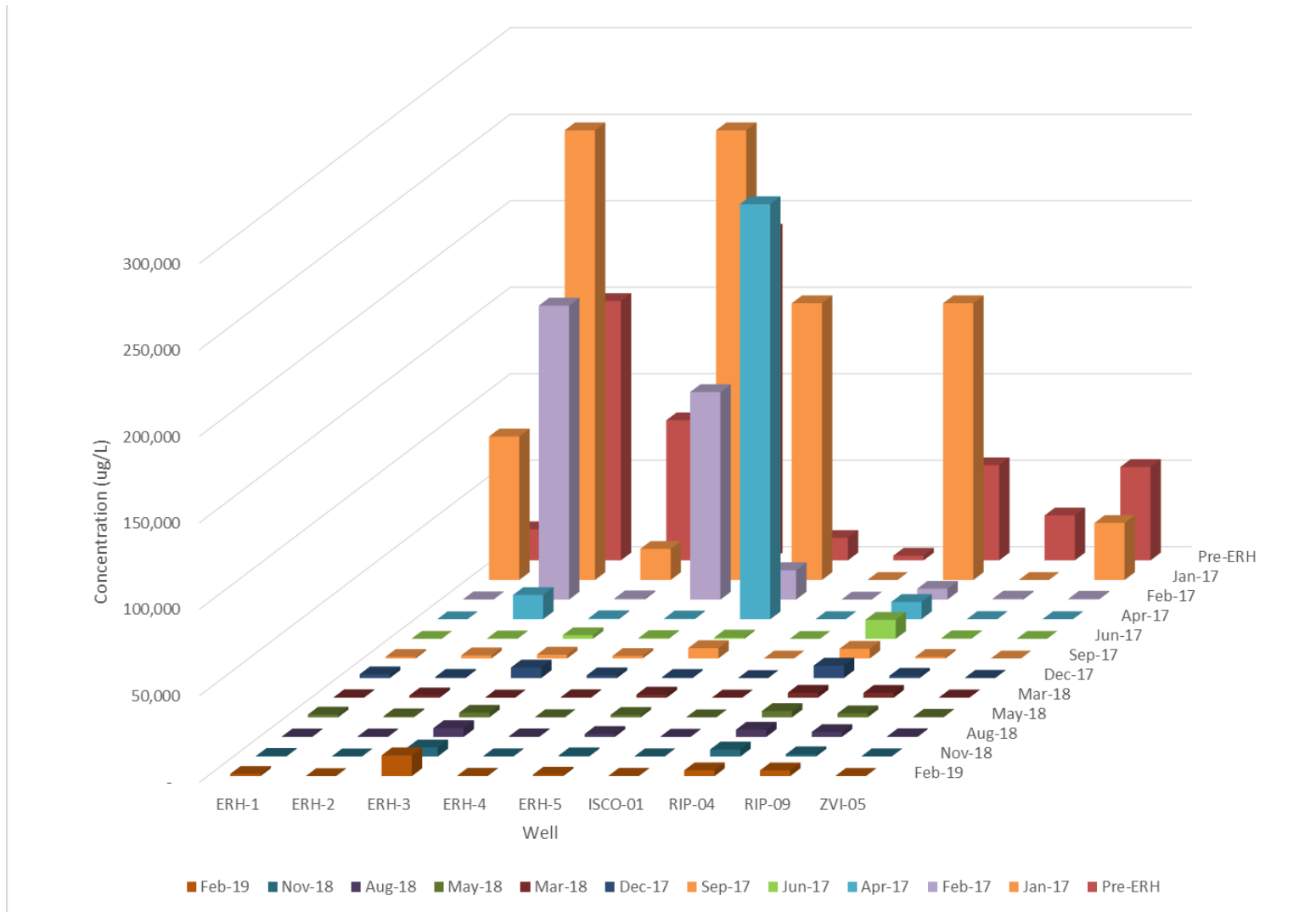
- 104 electrodes with co-located vapor recovery
- Delta pattern with 16-foot electrode spacing
- Treatment system
 - 40-hp vacuum blower
 - Water and vapor granulated activated carbon (GAC) vessels



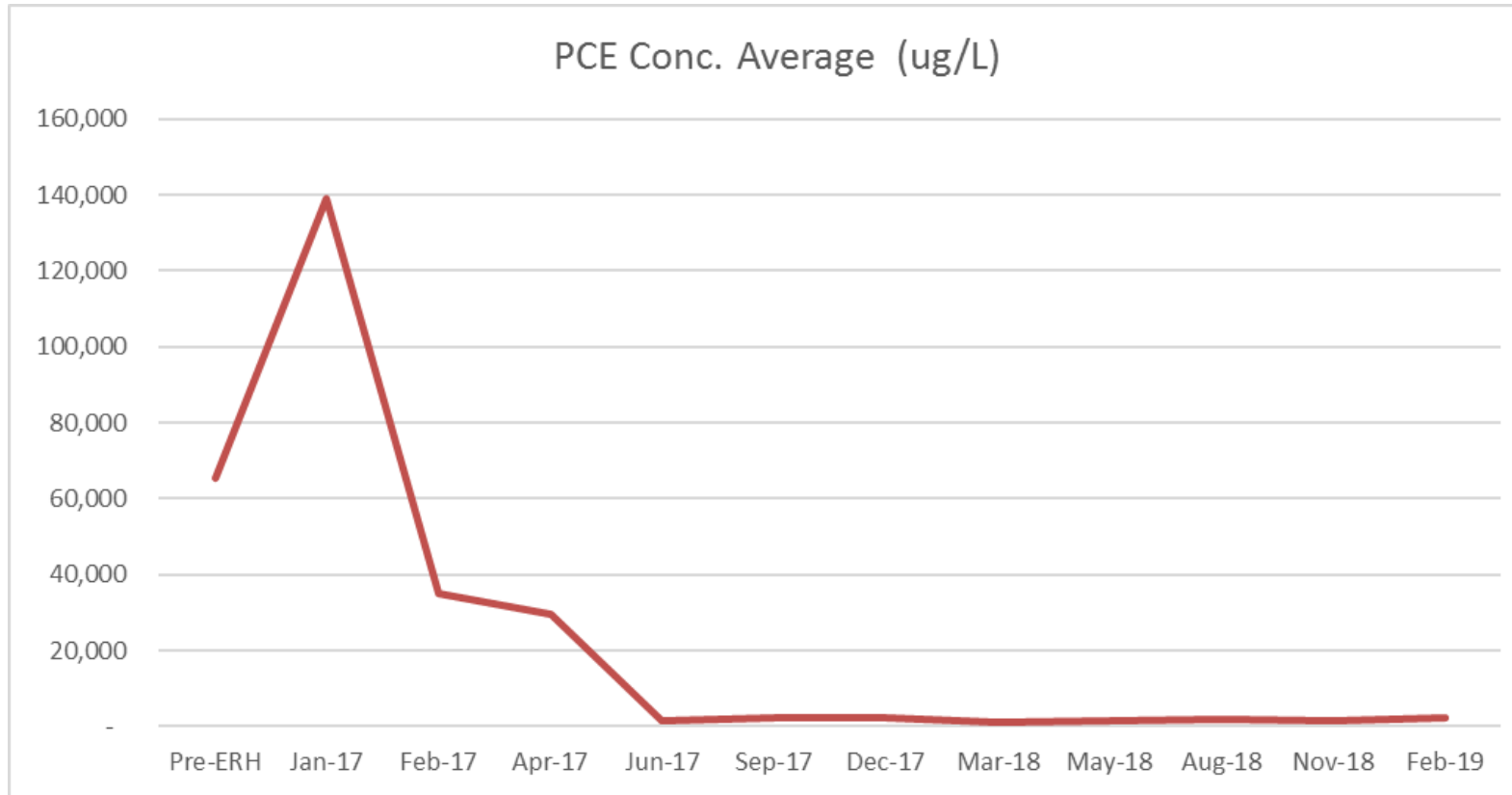
ERH system results

- ERH heating <8 months (Oct. 2016 - June 2017)
- 96% reduction in total VOC groundwater concentrations
- 100% removal of carbon tetrachloride
- 3,216 lbs. of VOCs removed from subsurface (40% more than design mass)
- Average in situ temperature > 100°C after 96 days

ERH performance (total VOCs)



ERH performance: Tetrachloroethene (PCE)



What's next?

Natural attenuation pathway or more remediation?

- While concentrations had decreased significantly (~96% reduction), concentrations of PCE >5,000 ppb in select monitoring points.
- Weathered bedrock stayed at 40°C for over a year following ERH shutdown
- Secondary lines of evidence not strong
- Tertiary lines?

Microbiological tool selection

- **16S gene sequencing:** snapshot of all the bacteria on the genus level and then provides the percent abundance as a function of population
- **Shotgun metagenomic sequencing:** analyzes all the DNA present down to the species level and then provides the percent abundance as a function of population
- **qPCR:** quantitative analysis for specific amount of bacterium

Evolving/easier sampling methodology, decreased costs, faster turnaround time

Examples of the MBT reporting

qPCR:

Exhibit 1. Gene copies per Liter (ground water)

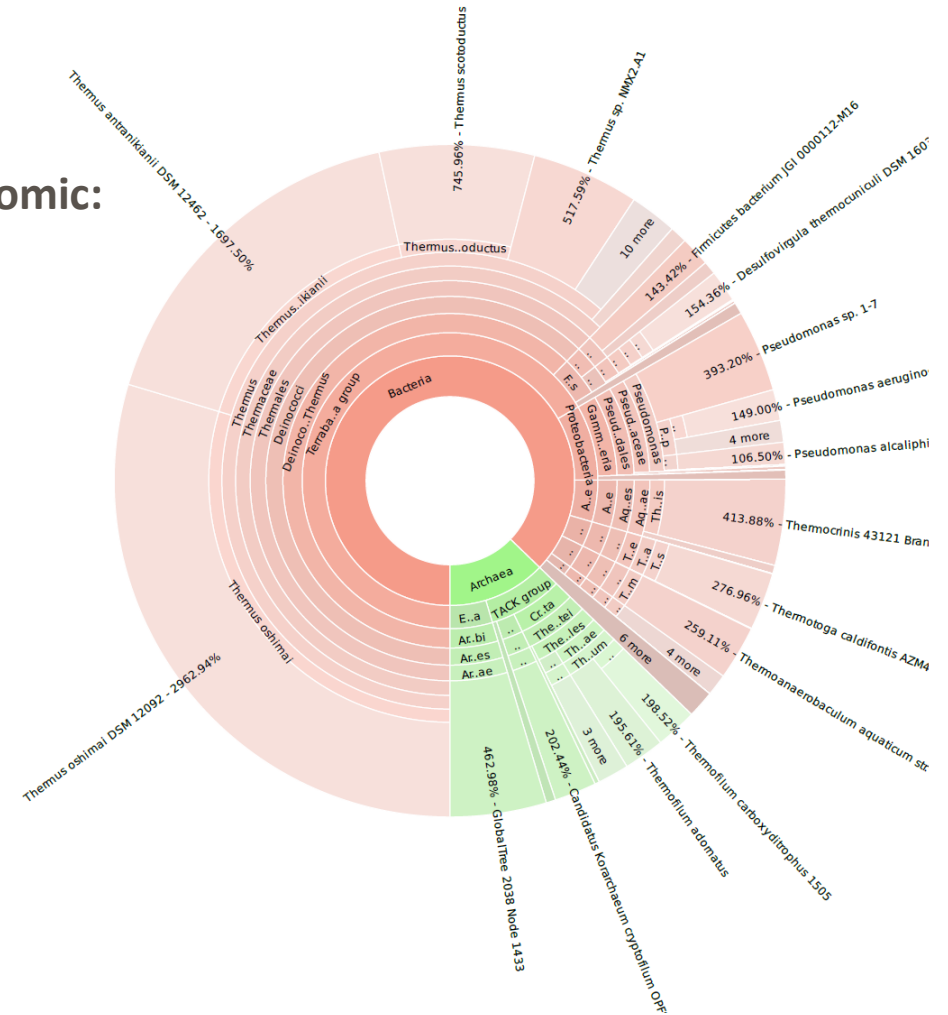
Lab ID	Client ID	Total Bacteria	Dehalobacter spp.	Dehalococcoides spp.
Z8E142-1	ERH-MW-5-27.5-052418	7.00E+06	<50	<50
Z8E142-2	RIP-04-27.5-052418	1.70E+10	<50	1.52E+06
Z8E142-3	HA-08-27.5-052418	2.70E+10	4.76E+02	9.08E+07
Z8E142-4	ERH-MW-3-27.5-052418	1.32E+07	1.66E+04	6.72E+03

16S:

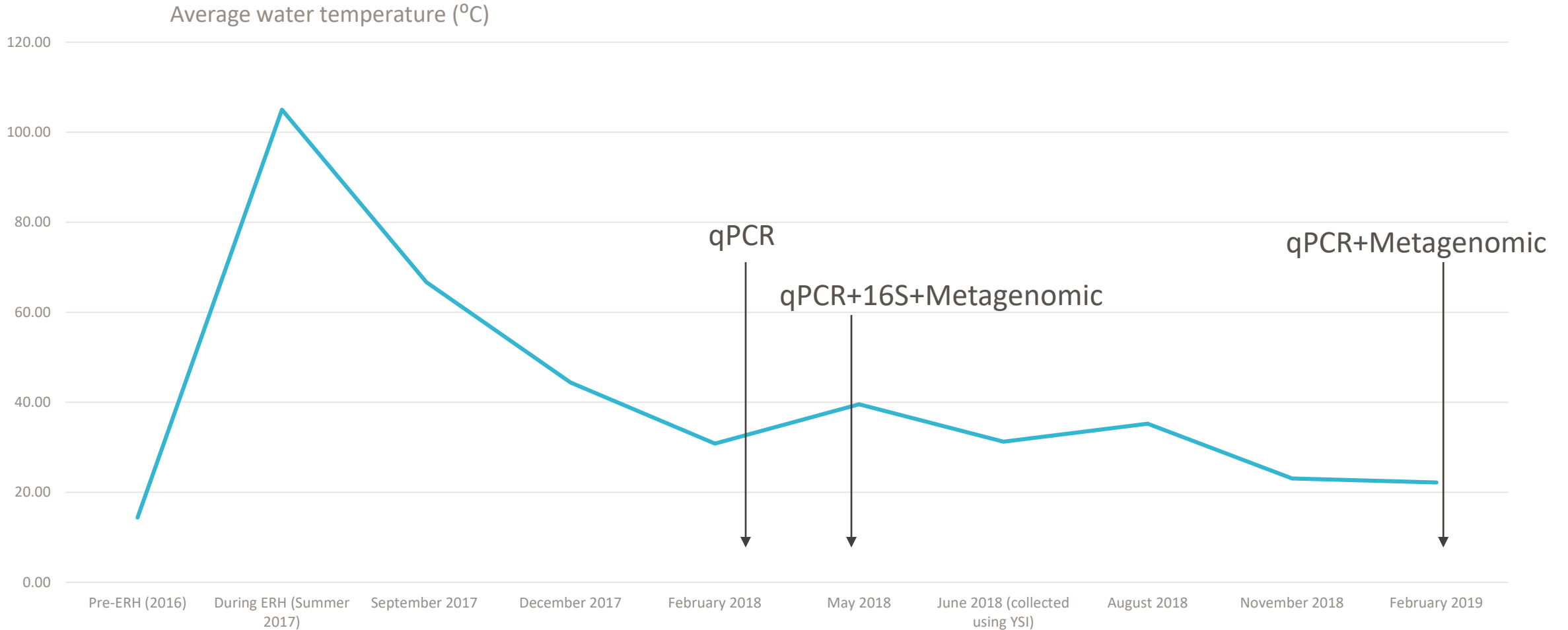
Exhibit 3. RIP-04-27.5

Identification	% of Profile	Metabolism	Gram
<i>Thermus oshimai</i> DSM 12092	29.6	Aerobic, NRB	Negative
<i>Thermus antranikianii</i> DSM 12462	17.0	Aerobic, NRB	Negative
<i>Thermus scotoductus</i> SA 01	7.5	Aerobic, NRB	Negative
<i>Thermus</i> sp NMX2 A1	5.2	Aerobic, NRB	Negative
GlobalTree 2038 Node 1433	4.6	--	--
<i>Thermocrinis</i> 43121 Branch	4.1	Aerobic	Negative
<i>Pseudomonas</i> sp 17	3.9	Aerobic	Negative
<i>Thermotoga caldifontis</i> AZM44c09	2.8	Anaerobic	Negative
<i>Thermoanaerobaculum aquaticum</i>	2.6	Anaerobic	Negative
<i>Candidatus Korarchaeum cryptofilum</i> OPF8	2.0	Anaerobic	--
<i>Thermofilum carboxyditrophus</i> 1505	2.0	Anaerobic	--
<i>Thermofilum</i> sp 1910b	2.0	Anaerobic	--
<i>Desulfoviregula thermocuniculi</i> DSM 16036	1.5	Anaerobic SRB	Positive
<i>Pseudomonas aeruginosa</i> str C 763	1.5	Aerobic	Negative
<i>Firmicutes bacterium</i> JGI 0000112 M16	1.4	--	--
<i>Pseudomonas alcaliphila</i> 34	1.1	Aerobic	Negative
<i>Pseudomonas mendocina</i> EGD AQ5	1.0	Aerobic	Negative

Shotgun metagenomic:



In situ groundwater temperatures



Microbiological testing

- 16S gene sequencing & Shotgun Metagenomics conducted May 2018 (avg temp 40°C) & February 2019 (avg temp 22°C)
- Shotgun vs 16S: Minimum of DNA for full metagenomic analysis; 16S or shallow shotgun metagenomic can be conducted instead
- Conducted testing on wells within ERH area & just outside treatment area

MBT sampling locations

ERH treatment
area MBT



Perimeter MBT

March 2018 (9 months post-ERH)

- Quantitative polymerase chain reaction (qPCR) testing

ERH-3 - qPCR Results (Mar-2018)

Dehalococcoides spp. 7.0E+05 / liter

TCE Reductase (tceA) 6.0E+05

VC Reductase (vcrA) 1.0E+05

ERH-5 - qPCR Results (Mar-2018)

Dehalococcoides spp. 3.0E+05 / liter

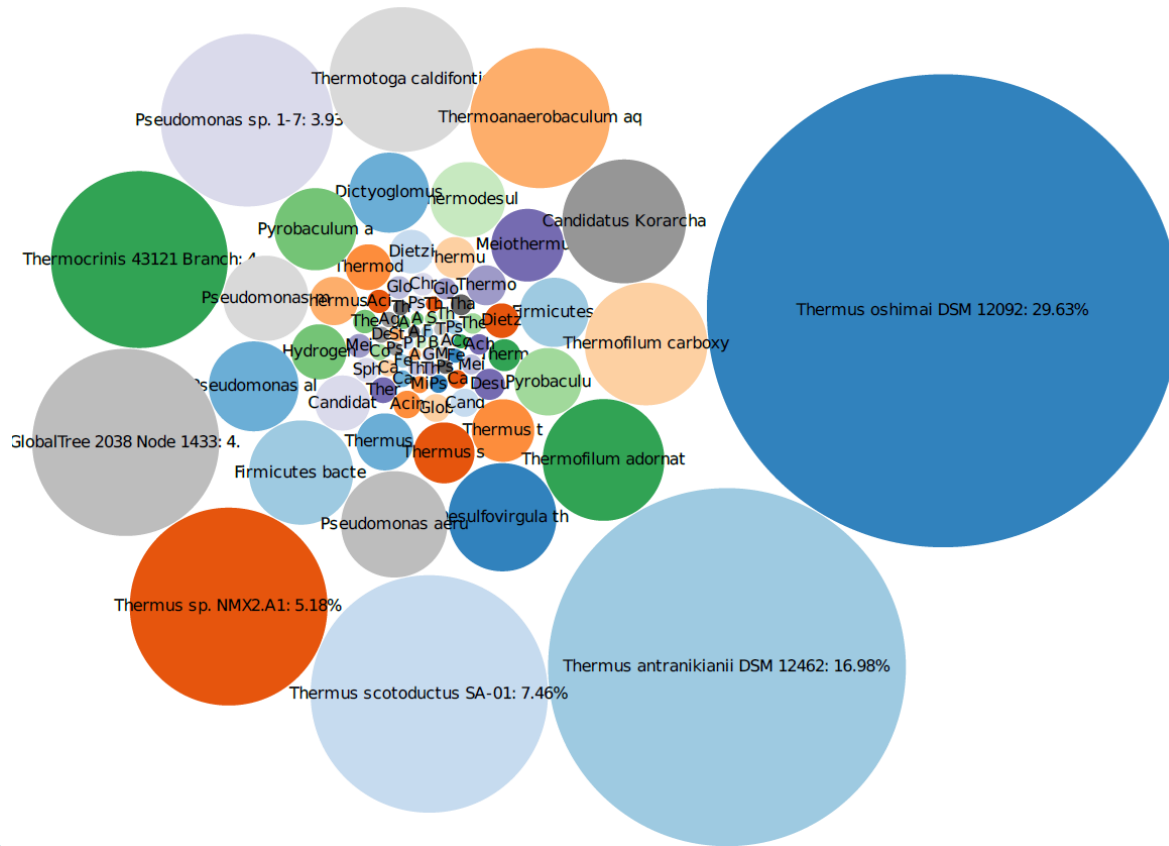
TCE Reductase (tceA) 2.0E+02

VC Reductase (vcrA) <50

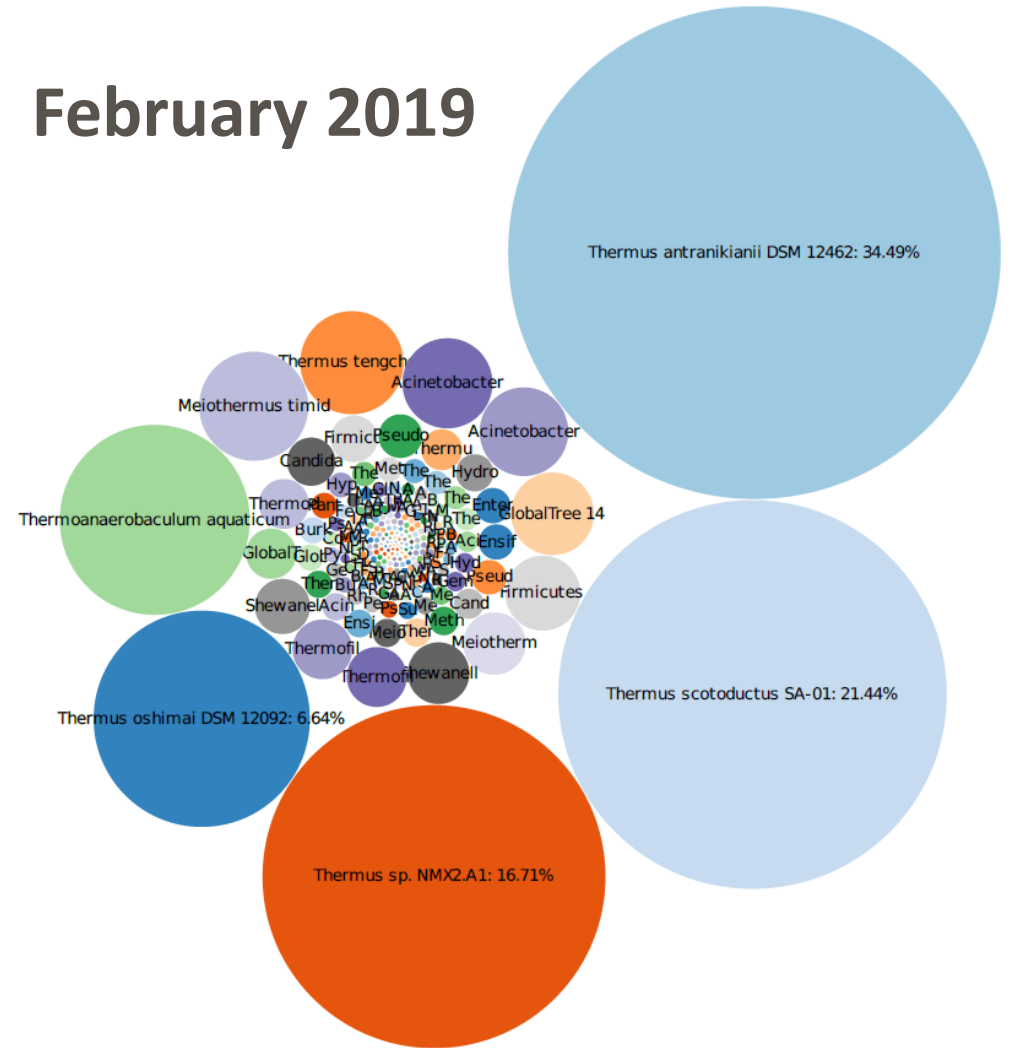
- DHC/DHB not present in high abundance due to elevated temperatures
- DHC concentrations looked ok, but.....

Treatment area well results - middle of ERH treatment area

May 2018



February 2019



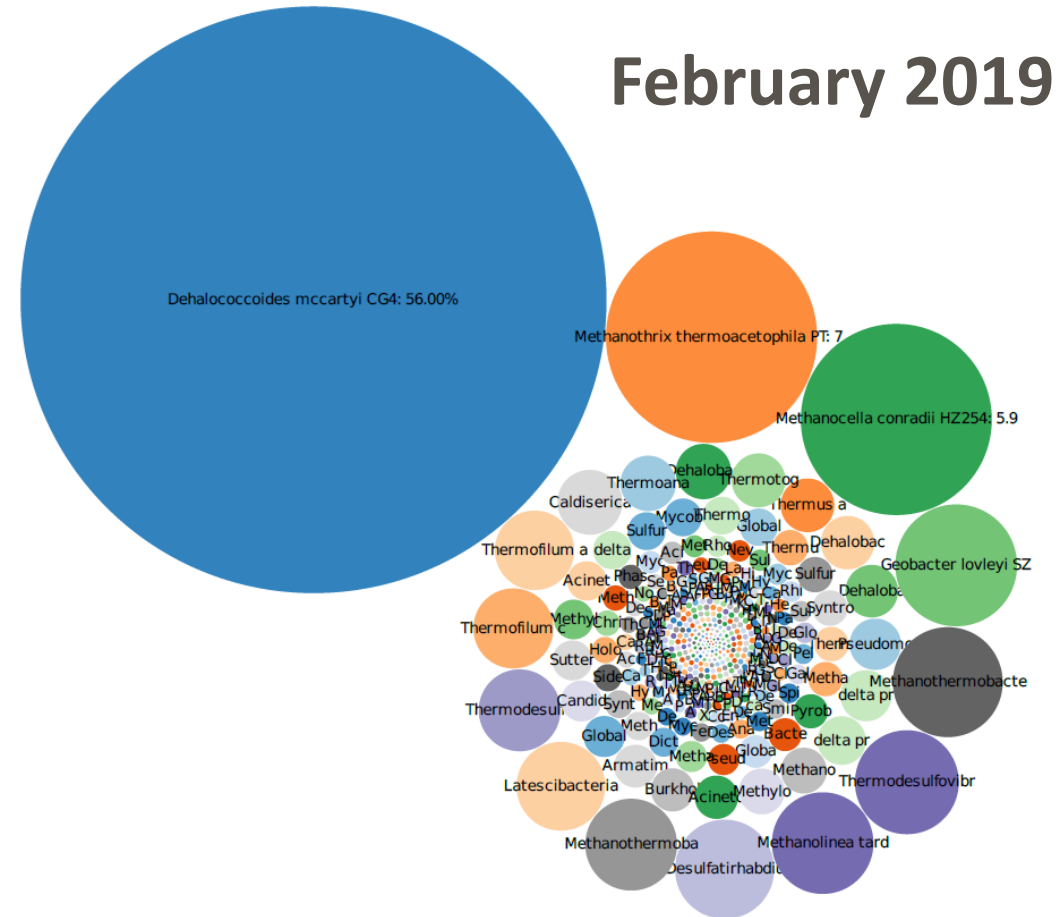
Treatment area well results - high concentration area

May 2018

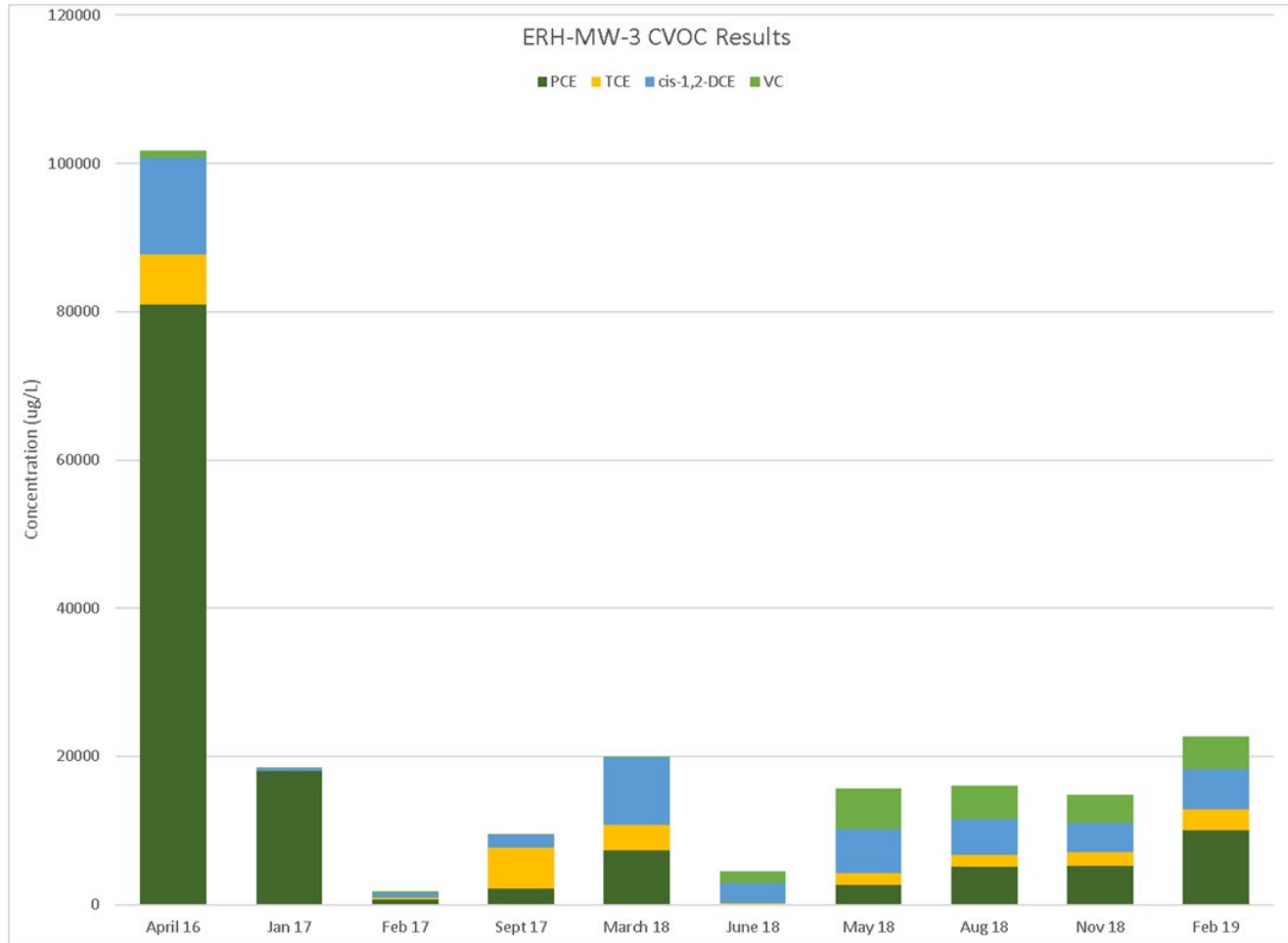
Exhibit 3. ERH-MW3-27.5

Genus (closest match)	% of Profile	Metabolism	Gram
geobacter	16.04	Anaerobic MRB	Negative
thermopallium	13.79	--	--
methanothermobacter	8.87	Anaerobic Methanogenic	Positive
burkholderia	7.43	Aerobic	Negative
synechococcus	5.42	Aerobic	Negative
eubacterium	4.96	Anaerobic	Variable
methylocystis	4.56	Aerobic MOB	Negative
roseibacillus	3.19	Aerobic	Negative
arthrobacter	2.74	Aerobic	Positive
thermacetogenium	2.51	Anaerobic	Positive
desulfosporosinus	2.16	Anaerobic SRB, Fermentive	Negative
dehalococcoides	1.74	Anaerobic	Positive
catenulispora	1.69	Aerobic	Positive
thiohalophilus	1.64	Anaerobic NRB	--
phormidium	1.41	--	--
prochlorococcus	1.03	Aerobic	Negative
desulfobacca	0.97	Anaerobic SRB	Negative
rickettsiella	0.93	intracellular	Negative

February 2019

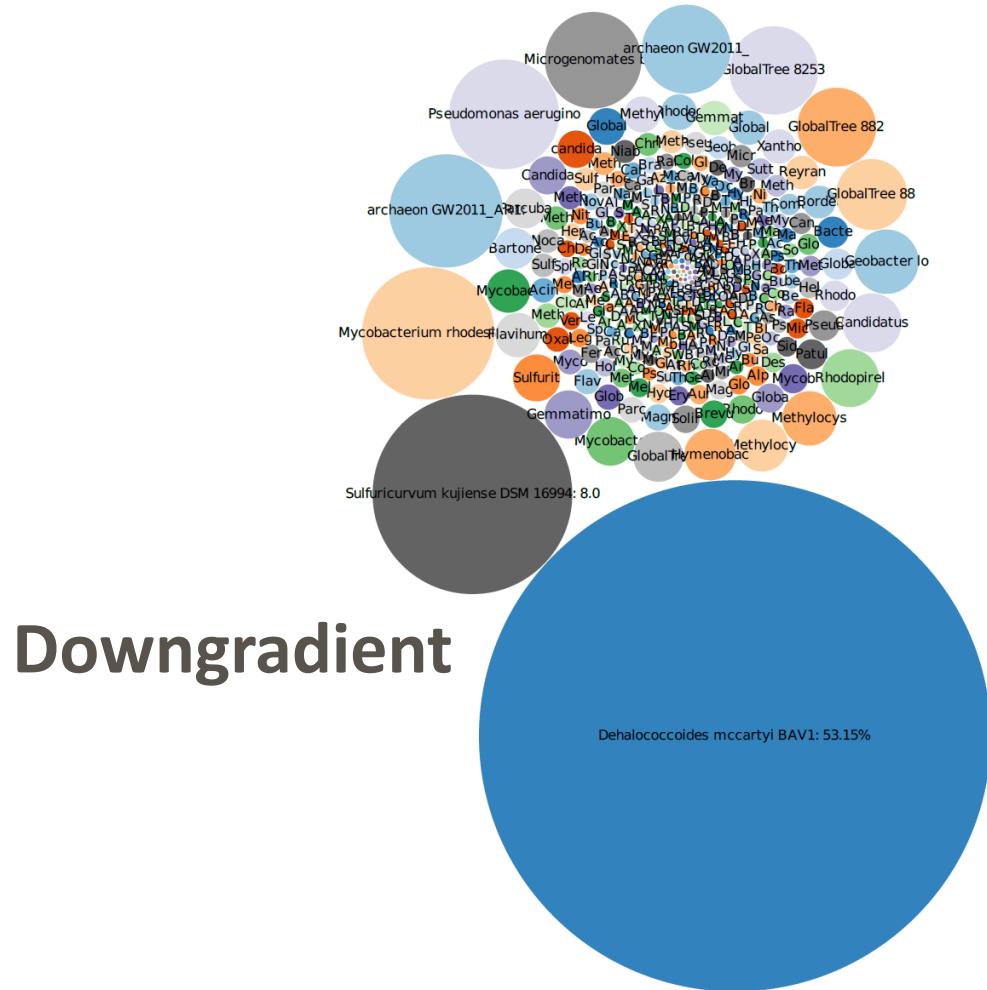


Treatment area well results - high concentration area

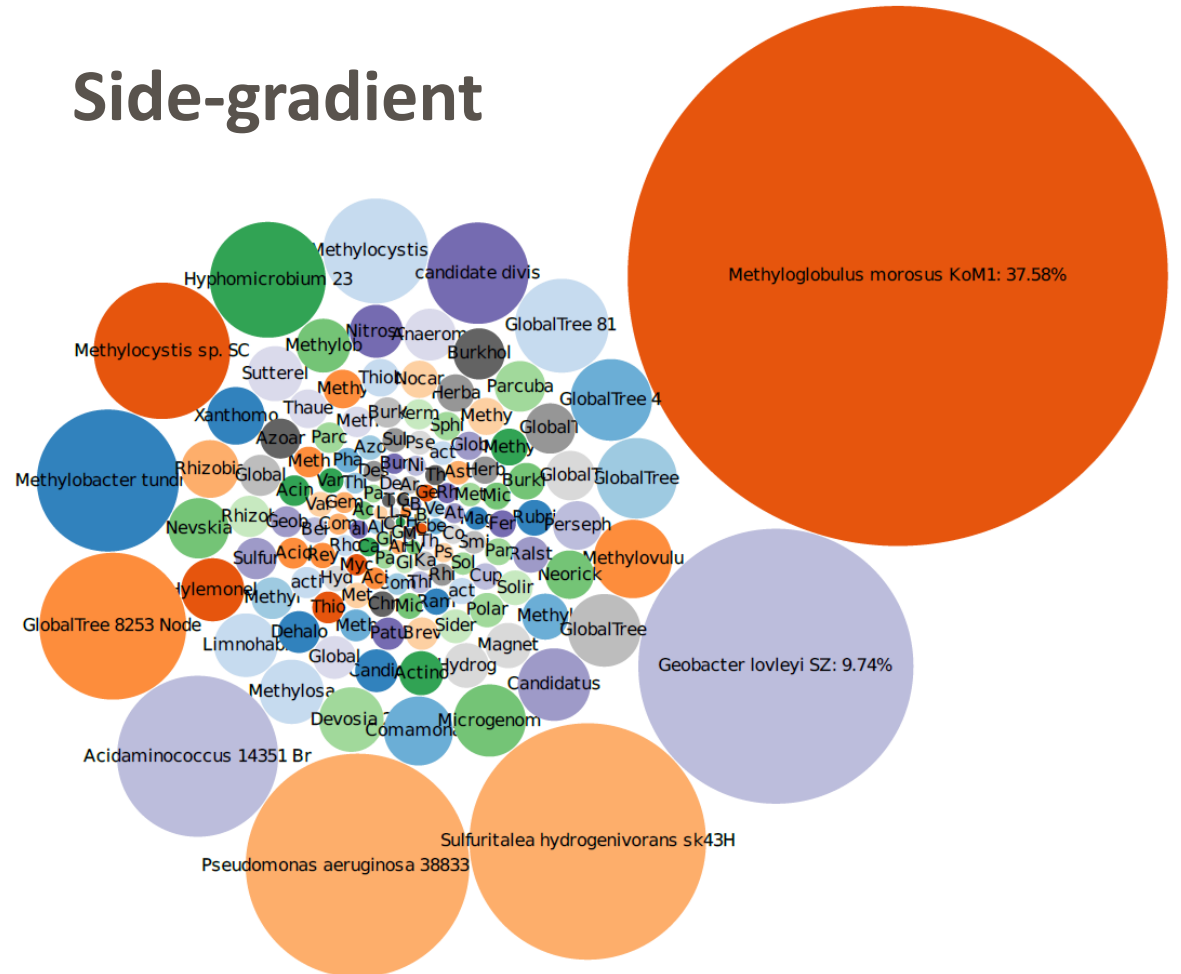


- MBT show positive bacterial populations
- Concentrations still elevated
- Bacterial concentrations only part of puzzle

Perimeter well results - outside ERH area



Side-gradient



Microbiological testing results for decision-making

- Use of advanced MBTs allowed for better understanding of subsurface
- **May 2018** - DHC & other dechlorinating bacteria present at low concentrations
 - Recommended allowing subsurface to cool and evaluating bacterial populations
- **February 2019**- DHC & other dechlorinating bacteria present at more elevated concentrations
 - Thermophilic bacteria still present at elevated concentrations
 - CVOC concentrations not decreasing at desired rate; intrinsic biodegradation appears to be stalled

Path forward

- Use of MBTs, has allowed us to identify predominant metabolic pathways occurring within ERH treatment area
- Additional polishing via enhanced in situ bioremediation (EISB) to increase desired bacterial populations within next 6-12 months
- Following EISB, additional MBT testing will be used to evaluate efficacy of natural attenuation for residual CVOCs

Conclusions

Conclusions

- ERH treatment does not inhibit intrinsic bacterial growth
- Cost-effective program of MBT analyses can be designed for evaluation of additional remediation options, as well as natural attenuation processes
- MBTs can be used to determine need & timing of remedial amendment injections to promote EISB following thermal treatment
- MBTs can provide a strong argument for MNA by identifying robust bacterial populations capable of direct and co-metabolic VOC degradation pathways

Thank you! Questions?



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