Advancing a Novel NGS Based Metric for Optimizing Bioremediation Performance: MCSI

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International Conference on Remediation of Chlorinated and Recalcitrant Compounds

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Why do we need an MCSI metric?

Microbial Community Structure Indexing (MCSI)

- Importance of the microbial community
- **Quarterback alone cannot win the game**
- **Need the whole team!**

Evaluating Bioremediation Performance

Evaluating Standard Molecular Tools:

- Typically focus only on contaminant degrading microorganisms
- For some contaminants (e.g., VOCs) levels of contaminant degraders can fail to predict bioremediation outcomes

16S rRNA next generation sequencing (NGS) data is now cost effective, but we lack **good metrics** for applying these data in the remediation industry!

Organohalide Respiring Bacteria (OHRB)

- **OHRB are the superstars of halogenated volatile organic compounds biodegradation**

- ***Dehalococcoides needs other species to:***
  - *Ferment organic matter into more readily useable electron donors*
  - *Produce corrinoids such as Vitamin B12*
  - *Conduct other syntrophic functions*
    - *e.g., Desulfovibrio, Clostridium, and Syntrophomonas*

Microbial Community Structure Indexing (MCSI)

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Hypotheses:

1. Next Generation Sequencing (NGS) data correlates with contaminant first-order degradation rates
2. Field data can be used to refine the list of key community members (MCSI)

NGS versus qPCR

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16S ribosomal ribonucleic acid (rRNA) NGS

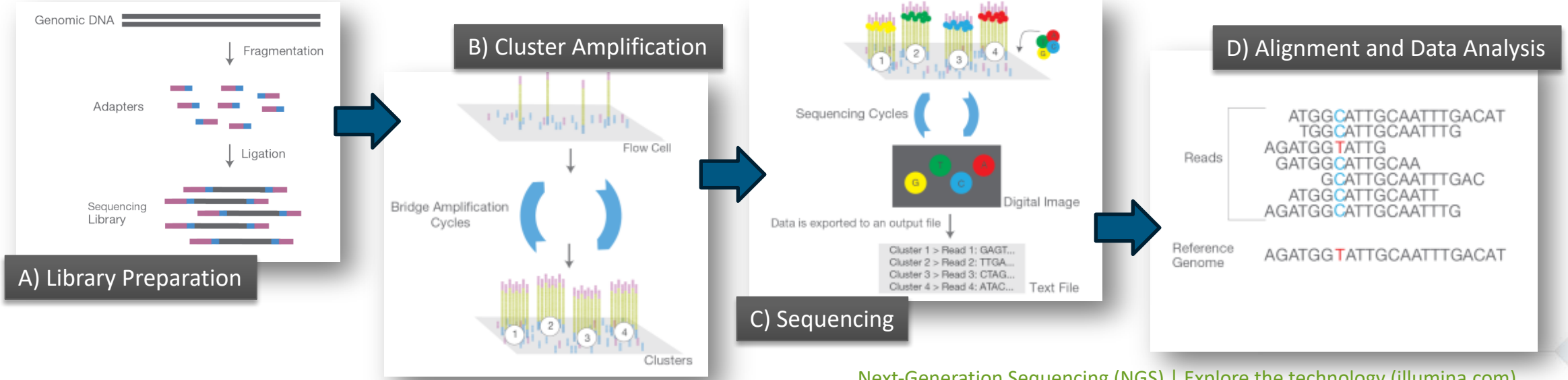
- Non-targeted
- Microbial Composition
- **Relative Abundance**

%

High-throughput quantitative PCR (qPCR)

- Targeted
- Requires Specific Primers
- **Bacteria Quantification**

cells/ mL



Next-Generation Sequencing (NGS) | Explore the technology (illumina.com)

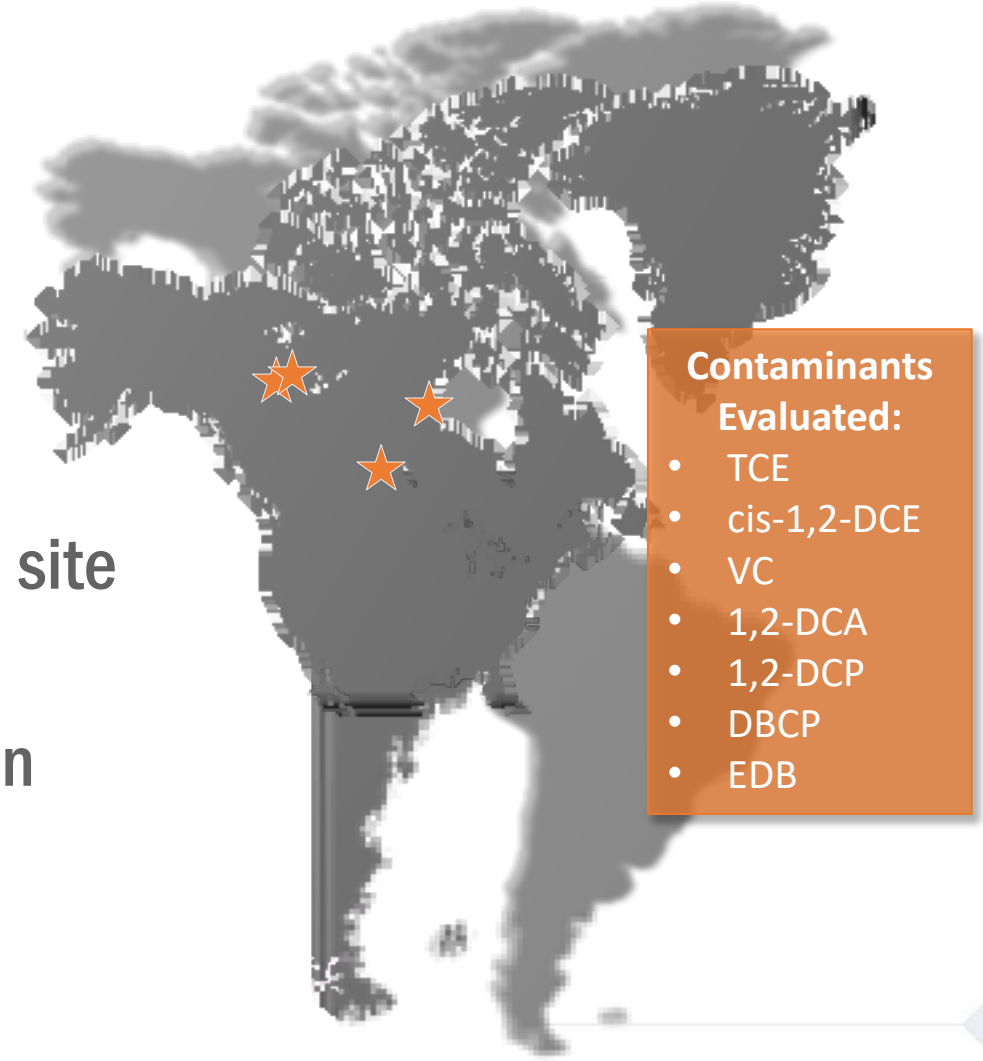
Testing the Hypotheses

- Sum the relative abundance (percentage) of bacteria in two main categories
  - **Organohalide-Respiring Bacteria (OHRB) list (primary degraders)**
  - **Microbial community members/cofactor generators (i.e., the "supporting cast")**
    - Community members/cofactor generators
    - Initially compiled from literature/laboratory studies

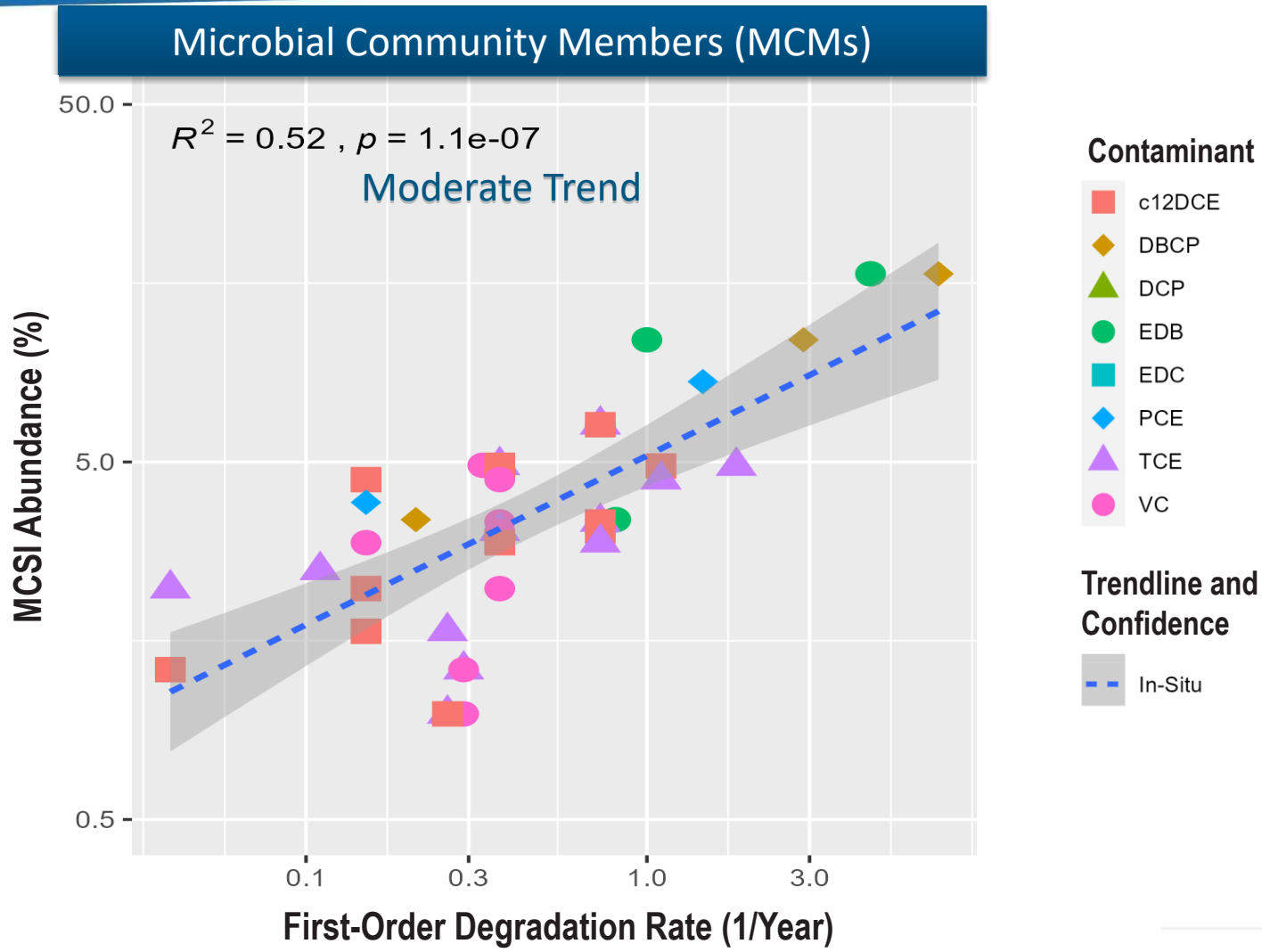
OHRB Relative Population %	1.21
Community Index Relative Population %	13.21

Interpretation of Preliminary Data Sets

- Comparison of NGS data distilled down to the MCSI parameters vs. first-order degradation rates
  - Groundwater data from 8 different plumes
  - Selected sites have well defined conceptual site models (CSMs)
  - Locations sampled through a bioremediation "process", either spatially, or over time



MCSI Metrics versus 1st Order Degradation Rate



Refine MSCI Metric: MCM List

- Moderate correlations observed with preliminary microbial community member (MCM) list compiled from literature
- Using Pearson's (r) and Spearman's rho (r_s) bivariate correlation coefficients:
  - Identified additional community members to refine MCSI metric
  - Genera abundance vs. first-order degradation rates

MCM List

Order	Genus
(1) *Alteromonadales*	(1.1) *Shewanella*
(2) *Clostridiales*	(2.1) *Acetobacterium*
	(2.2) *Eubacterium*
	(2.3) *Sporotalea*
	(2.4) *Syntrophomonas*
(3) *Desulfovibrionales*	(3.1) *Desulfovibrio*
(4) *Enterobacterales*	(4.1) *Citrobacter*
(5) *Eubacteriales*	(5.1) *Clostridium*
(6) *Geobacterales*	(6.1) *Geobacter*
(7) *Methanomicrobiales*	Multiple
(8) *Methanosarcinales*	(8.1) *Methanomethylovorans*
	(8.2) *Methanosarcina*
(9) *Selenomonadales*	(9.1) *Dendrosporobacter*
	(9.2) *Pelosinus*
	(9.3) *Sporomusa*
(10) *Spirochaetales*	(10.1) *Spirochetes*
(11) *Syntrophobacterales*	Multiple

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Order	Family	Genus
(1) *Acidobacteriales*	(1.1) *Acidobacteriaceae*	(1.1.1) *Granulicella**
(2) *Clostridiales*	(2.1) *Clostridiaceae*	(2.1.1) *Sporotalea*
	(2.2) *Desulfitobacteriaceae*	(2.2.1) *Desulfitobacterium**
		(2.2.2) *Desulfosporosinus**
	(2.3) *Eubacteriaceae*	(2.3.1) *Eubacterium*
		(2.3.2) *Acetobacterium*
	(2.4) *Syntrophomonadaceae*	(2.4.1) *Syntrophomonas*
(3) *Enterobacterales*	(3.1) *Enterobacteriaceae*	(3.1.1) *Citrobacter*
(4) *Eubacteriales*	(4.1) *Clostridiaceae*	(4.1.1) *Clostridium*
(5) *Geobacterales*	(5.1) *Geobacteraceae*	(5.1.1) *Geobacter*
(6) *Hyphomicrobiales*	(6.1) *Pleomorphomonadaceae*	(6.1.1) *Pleomorphomonas**
(7) *Methanomicrobiales*	(7.1) *Methanocalculaceae*	(7.1.1) *Methanocalculus*
	(7.2) *Methanoc[illegible]aceae*	(7.2.1) *Methanoc[illegible]pusculum*
	(7.3) *Methanomicrobiaceae*	(7.3.1) *Methanoculleus*
		(7.3.2) *Methanofollis*
		(7.3.3) *Methanogenium*
		(7.3.4) *Methanolacinia*
		(7.3.5) *Methanomicrobium*
		(7.3.6) *Methanoplanus*
	(7.4) *Methanoregulaceae*	(7.4.1) *Methanoregula*
		(7.4.2) *Methanosphaerula*
	(7.5) *Methanospirillaceae*	(7.5.1) *Methanospirillum*
(8) *Methanosarcinales*	(8.1) *Methanosarcinaceae*	(8.1.1) *Methanomethylovorans*
		(8.1.2) *Methanosarcina*
(9) *Opitutales*	(9.1) *Opitutaceae*	(9.1.1) *Opitutus**
(10) *Rhodocyclales*	(10.1) *Rhodocyclaceae*	(10.1.1) *Azospira**
(11) *Selenomonadales*	(11.1) *Sporomusaceae*	(11.1.1) *Dendrosporobacter*
		(11.1.2) *Pelosinus*
		(11.1.3) *Sporomusa*
(12) *Sphingobacteriales*	(12.1) *Sphingobacteriaceae*	(12.1.1) *Sphingobacterium**
(13) *Spirochaetales*	(13.1) *Spirochaetaceae*	(13.1.1) *Spirochaeta**
		(13.1.2) *Spirochetes*
(14) *Syntrophobacterales*	(14.1) *Syntrophaceae*	(14.1.1) *Desulfobacca*
		(14.1.2) *Smithella*

REVISED MICROBIAL COMMUNITY MEMBER (MCM) LIST BASED ON FIELD DATA CORRELATIONS

MCSI Refined Correlation

- 36 individual genera identified as MCMs in refined MCSI list
  - Includes 8 additional genera identified from field data
- Increased correlation between MCSI Abundance and first-order degradation rates using refined MCM list

Case Study: Applying MCSI

- EVO and pH buffering amendments injected at a VOC site

Time	Microbial Community Structure Index (MCSI)	Halogenated VOC Concentration (ug/L)
Baseline	Standard qPCR Targets $5x10^0$ Community Members 1.5%	1,200 No Trend
Emulsified vegetable oil (EVO) and pH buffering injections		
Month 3	Standard qPCR Targets ~$1x10^4$ Community Members 3%	200 Decrease ↓

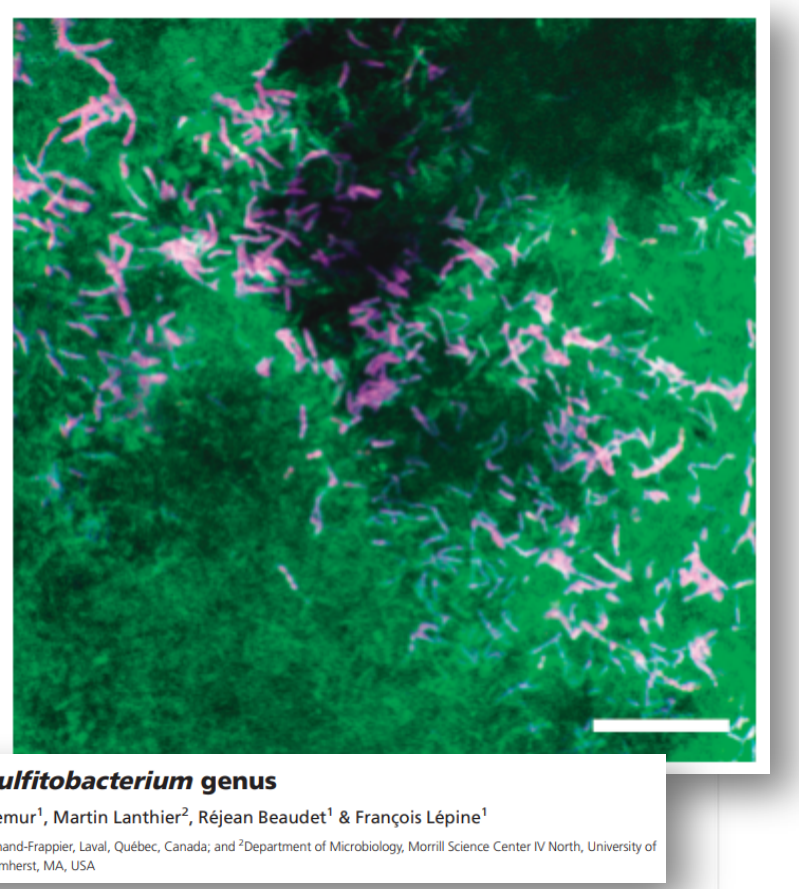
MCSI Case Study Explained

- Stall is seemingly explained by drop in community members
- **We need better performance monitoring metrics to confirm we are correctly diagnosing issues**

Time	Microbial Community Structure Index (MCSI)	Halogenated VOC Concentration (ug/L)
Baseline	Standard qPCR Targets $5x10^0$ Community Members 1.5%	1,200 No Trend
Emulsified vegetable oil (EVO) and pH buffering injections		
Month 3	Standard qPCR Targets ~$1x10^4$ Community Members 3%	200 Decrease ↓
Months 4-9	Standard qPCR Targets ~$1x10^{4-5}$ Community Members 3.9%	200-190 Stall ↔
pH buffering, no additional EVO		
Months 10.5+	Standard qPCR Targets ~$1x10^{4-5}$ Community Members 6%	45 Ultimately becoming consistent at 2.2 ug/L ↓

Conclusions

- Whole microbial community metrics may be better predictors of bioremediation performance
- Preliminary data suggest MSCI is a useful tool
  - **Case study example: MSCI helped identify a change in operation (pH buffering) that increased levels of supporting community members and improved biodegradation** (*with NO change in degrader levels*)
- Additional data may continue to refine MCSI list for HVOCs and other contaminants



The *Desulfitobacterium* genus

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Publication in review:
"Developing a Microbial Community Structure Index (MCSI) as an Approach to Evaluate and Optimize Bioremediation Performance"

Thank you!

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QUESTIONS?

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MCSI Metrics versus 1st Order Degradation Rate

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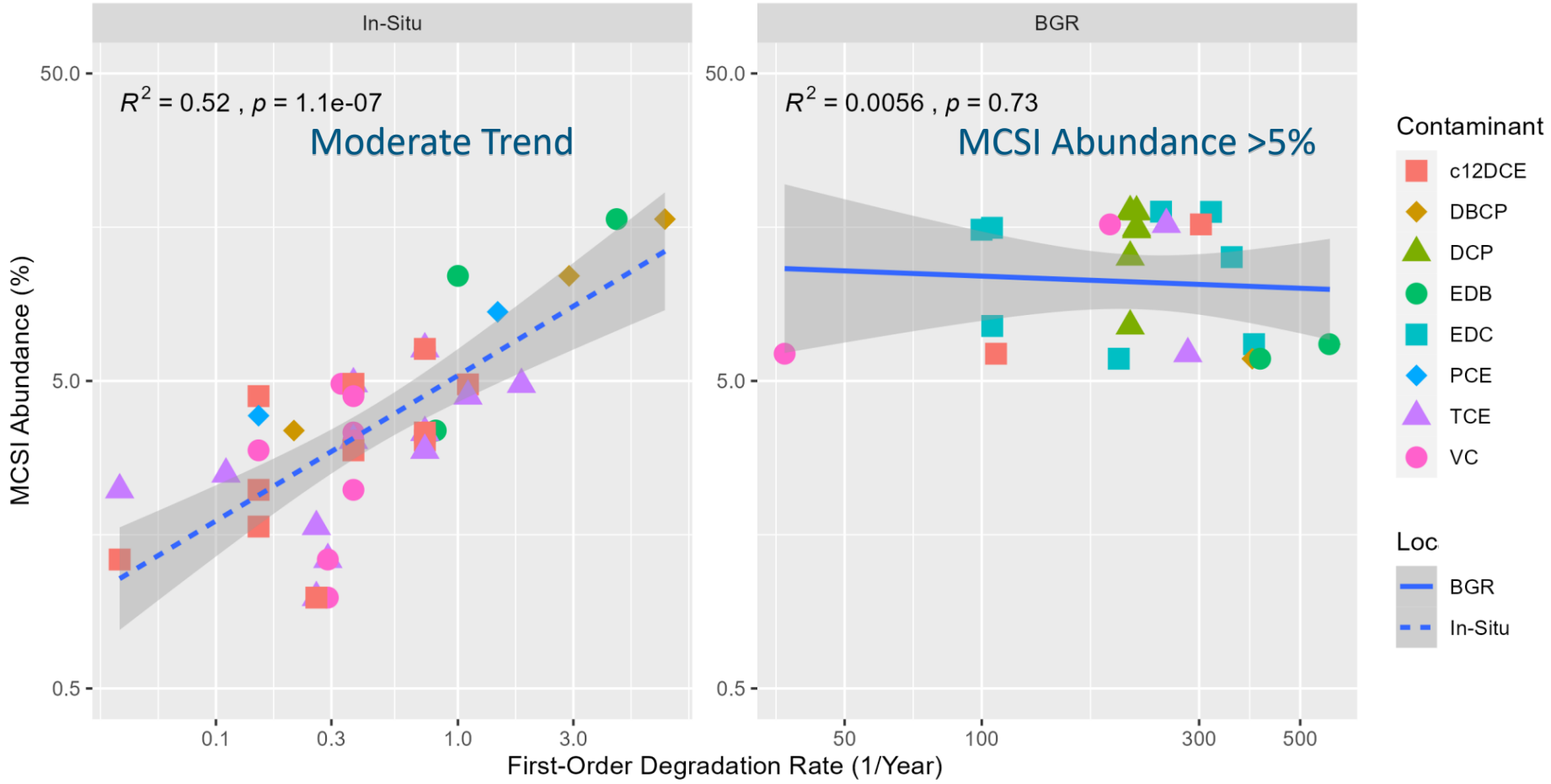
Microbial Community Members (MCSI)



MCSI Metrics versus 1st Order Degradation Rate

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Organohalide Respiring Bacteria (OHRB)