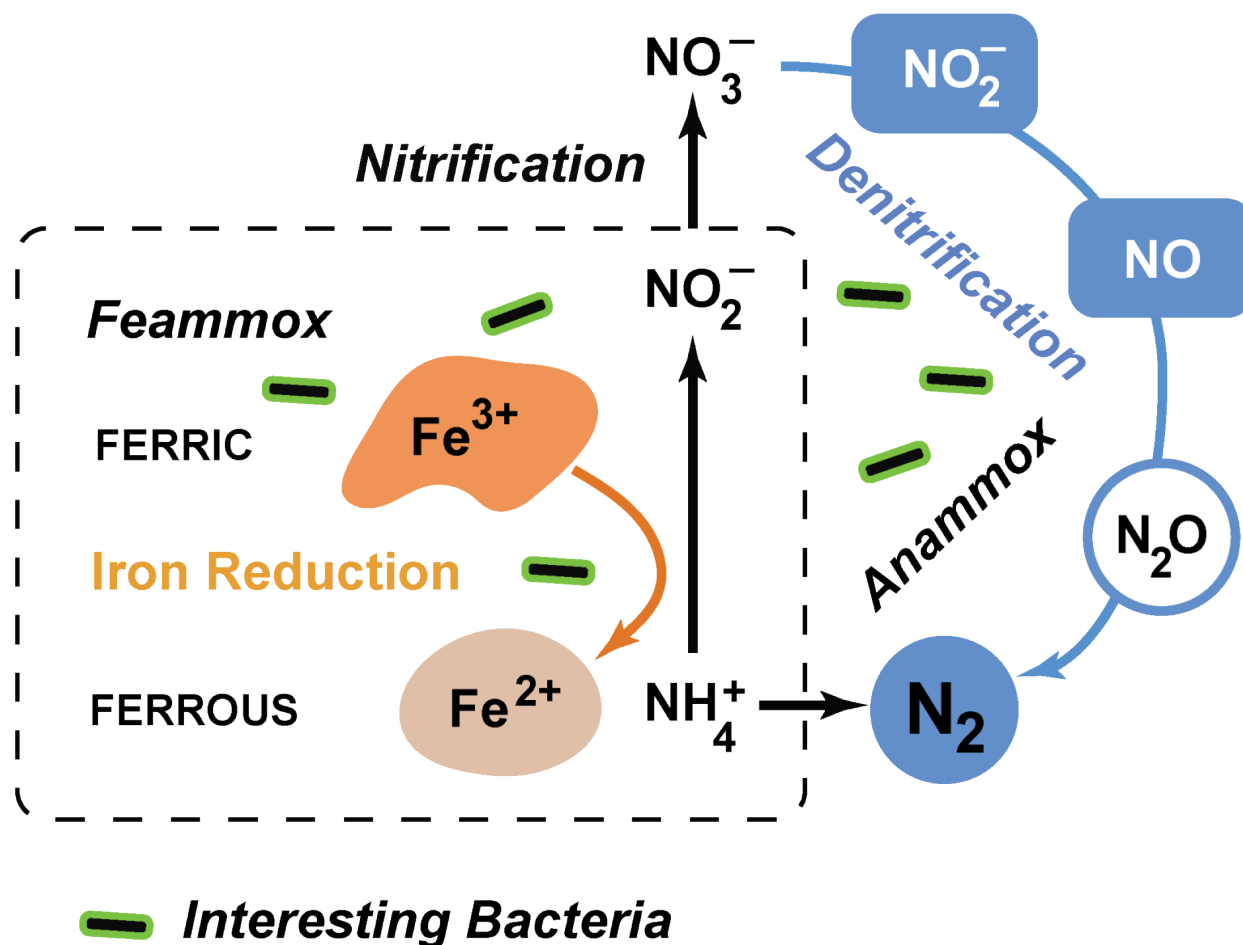


Natural Occurrence of Feammox Conditions and Anammox Microbiota within a PFAS Plume at the Ground Water-to-Surface Water Interface

**Barry J. Harding, Director of Nature-Based Solutions
AECOM Remediation West**

Feammox Conditions / Precedence

- Feammox = Oxidation of ammonia under iron reducing conditions
- Feammox also refers to bacteria associations
- Anammox = Anaerobic ammonium oxidation and associated bacteria
- *Acidimicrobium* sp. Strain A6, encoded for novel dehalogenases and studied for defluorination capabilities (Jaffé and others)



Feammox reaction modified from graphic derived from Jaffé, Huang, Park and Koel (Princeton U), April 15, 2022.

Purpose of Study

- Field studies documenting Feammox conditions are lacking
 - Studies to-date were in flooded rice paddies, manure composts, and bacteria harvesting in the New Jersey Pine Barrens
- Most Feammox studies are *in-vitro* (bench-scale reactor vessels and controlled experimentation)
- This study focused on documenting Feammox conditions in situ within a PFAS-containing groundwater-to-surface water discharge

Goal

- Document if Feammox conditions and related bacteria are present within a PFAS-laden groundwater seep to surface water and identified study area

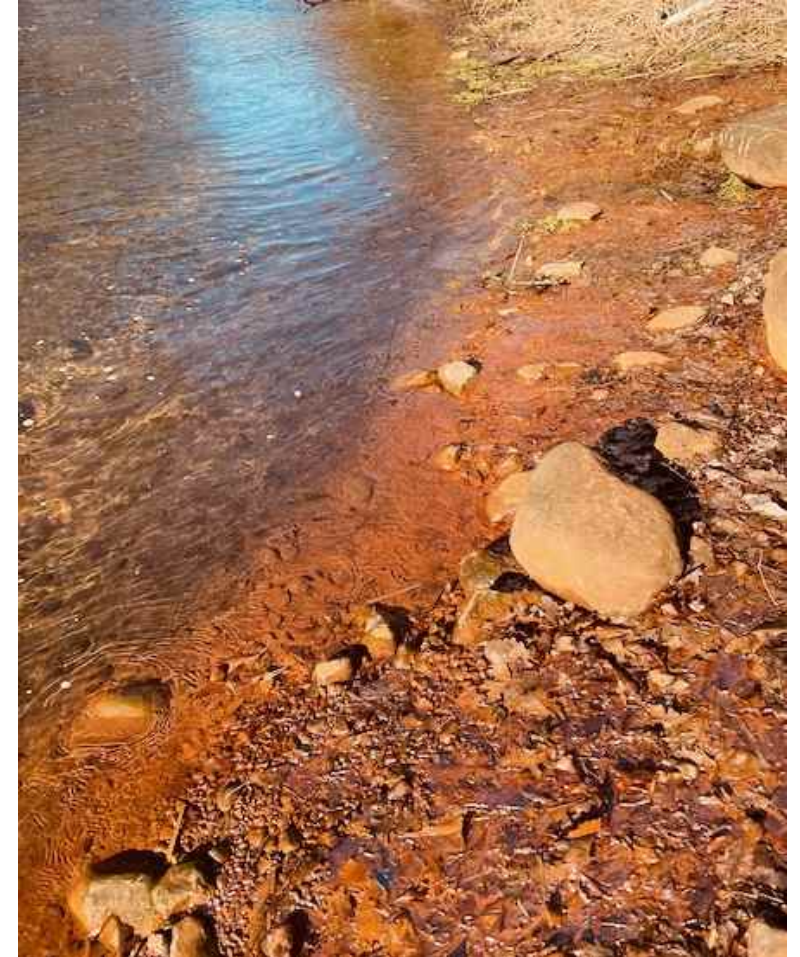
Field Study Design

- Pore-Water Sampling and Analysis
 - Three push-point samples
 - Low flow minimal drawdown and use of peristaltic pump
 - pH, ORP, Sp.Cond, DO
 - Feammox indicators: Ammonia, Iron, Ferrous Iron, Nitrate-N, Nitrate-N
 - PFAS-28; Two sampling events
- Sediment Sampling and Analysis
 - Three co-located hand-auger borings
 - PFAS-28
 - NGS, 16s rRNA Amplicon, preserved with DNA/RNA Shield® (Zymo Research)

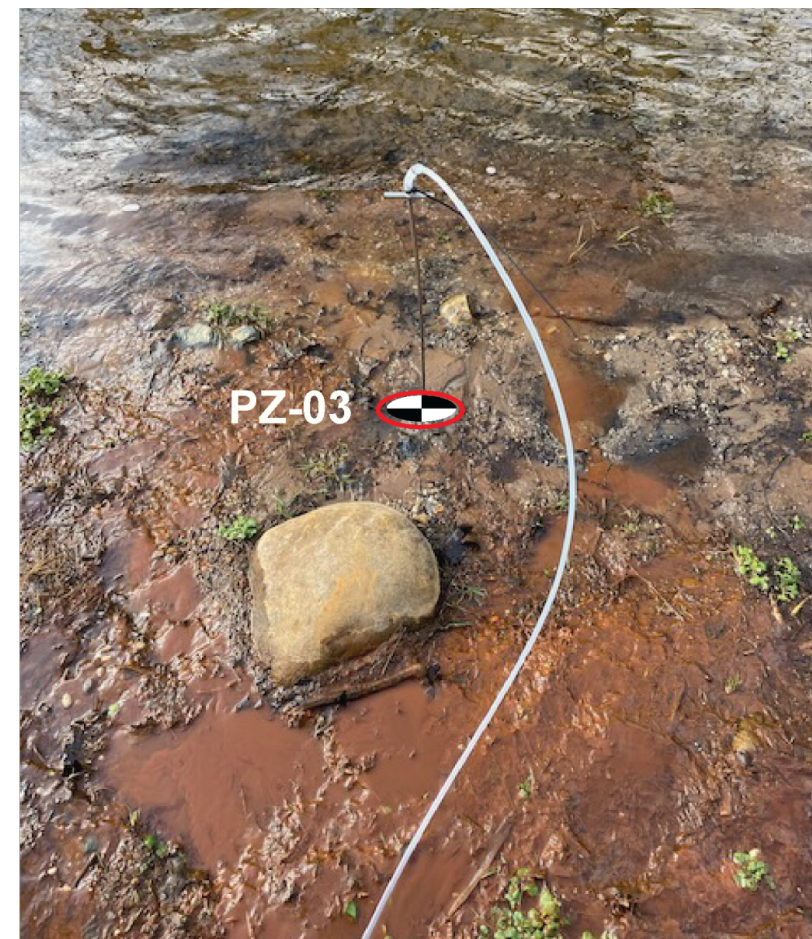
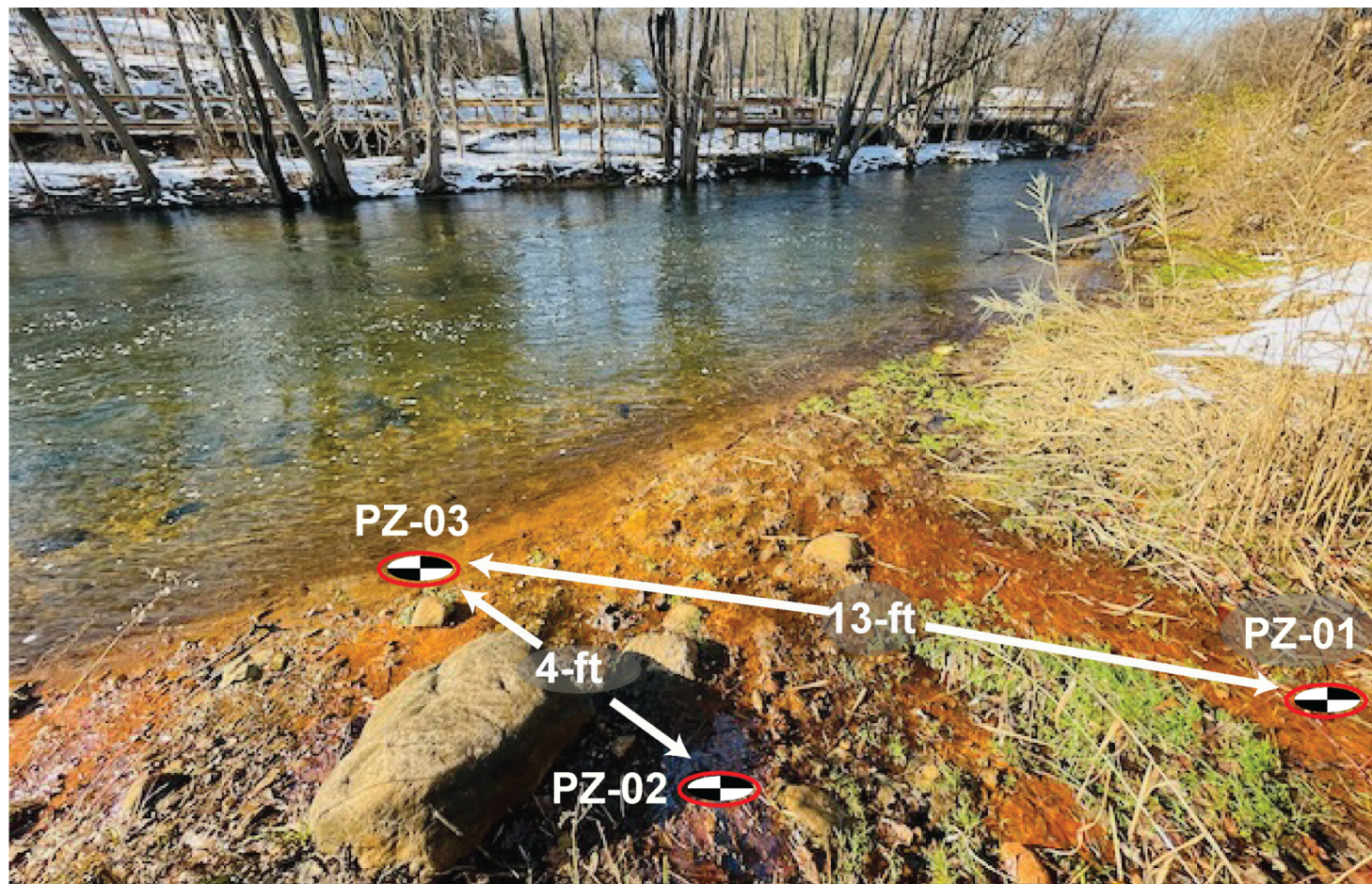


Study Area

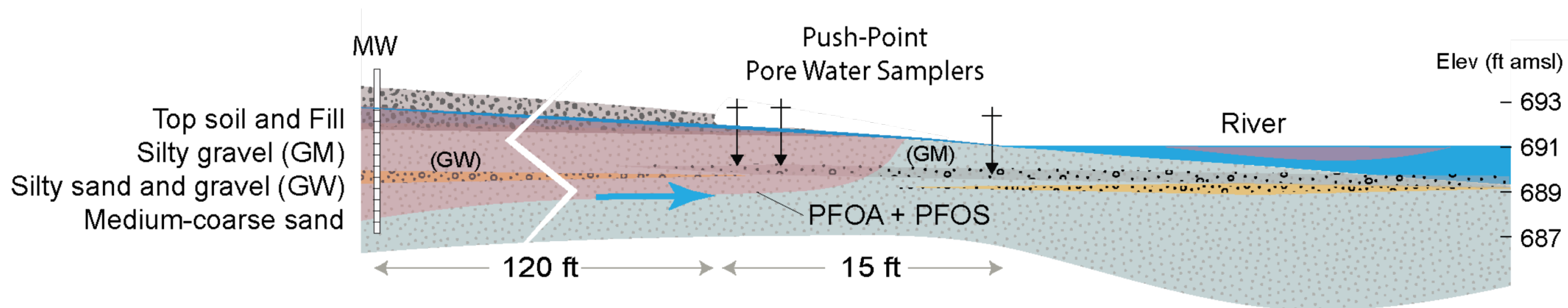
- Within an identified dissolved PFAS discharge adjacent to a Great Lakes tributary
- Stream was gaining during sampling activities in September 2022 and January 2023
- Iron-rich sediments including hematite, magnetite, goethite and various iron-hydroxides
- Study depth limited to < 3-feet below grade and consistent with known PFAS occurrences adjacent to tributary



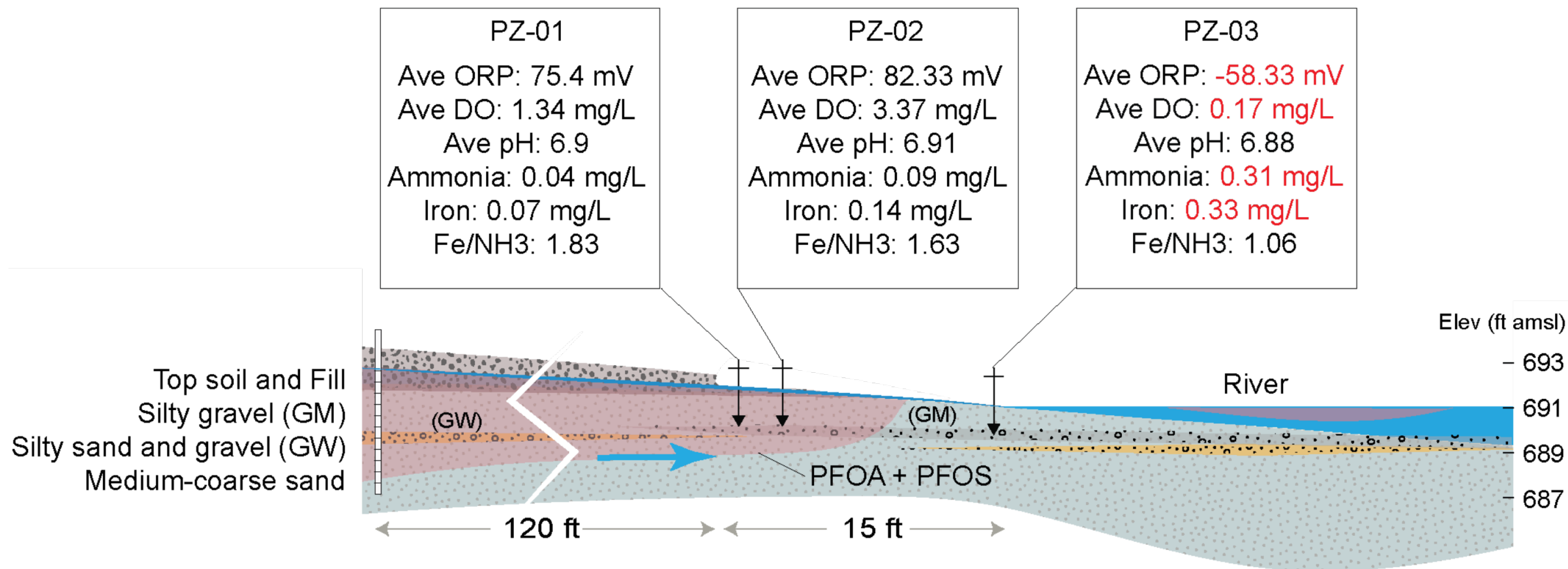
Pore-Water Sampling Locations



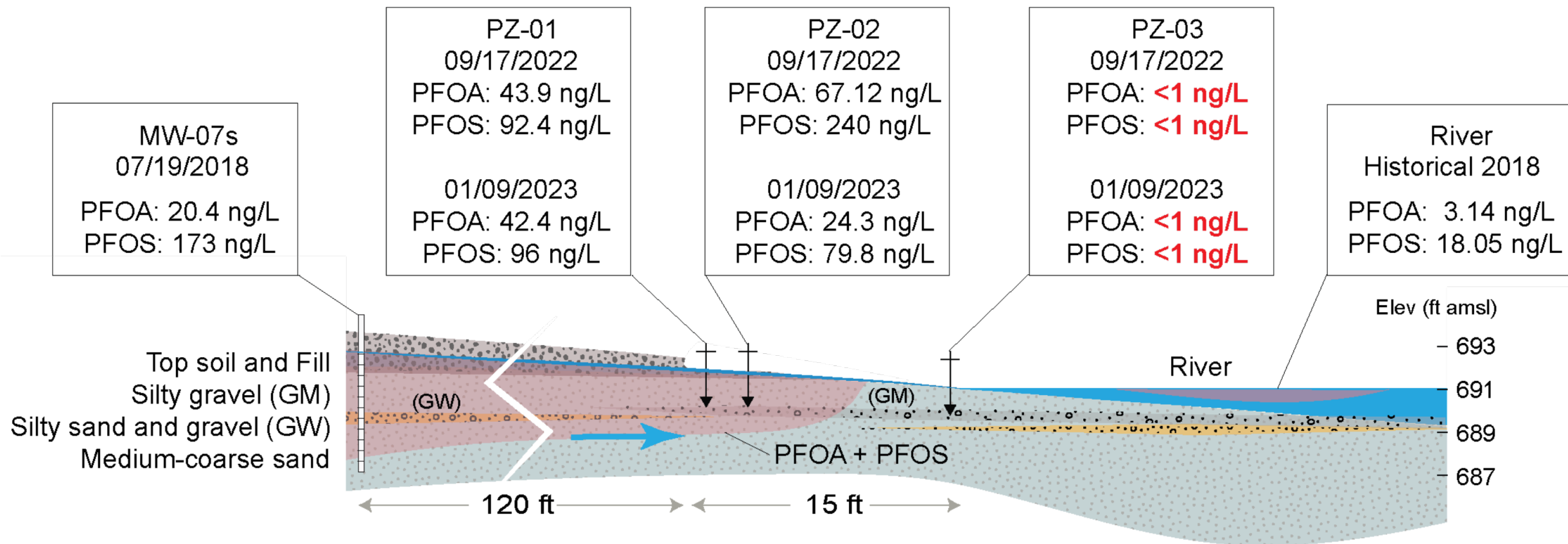
Study Area Cross-Sectional View



Water Quality & Feammox Indicators



PFAS Sampling Results



16S Amplicon Sequencing

- Nine sediment samples collected from each pore-water location with three discrete sampling depths (0.5, 1.0 and 1.5-foot intervals)
- Samples preserved in field with DNA/RNA® Shield
- Microbiomics Reporting, Zymo Research, Irvine California
 - ZymoBIOMICS® 96 MagBead DNA Kit
 - Quick-16S™ Primer Set V3-V4
 - Sequencing: Illumina® MiSeq™ v3 Reagent
 - Bioinformatics Analysis: Dada2 Pipeline

16S rRNA Amplicon Sequencing – ORDER LEVEL

PZ-01

PZ-02

PZ-03

	Taxon - Order	Gene %		Taxon - Order	Gene %		Taxon - Order	Gene %
1	<i>Rhizobiales</i>	11.54%	1	<i>Rhizobiales</i>	10.52%	1	<i>Anaerolineales</i>	7.16%
2	<i>Burkholderiales</i>	6.07%	2	<i>Nitrosomonadales</i>	5.54%	2	<i>Rhizobiales</i>	6.99%
3	<i>Acidimicrobiales</i>	5.33%	3	<i>Class Acidobacteria; Order NA</i>	5.48%	3	<i>Chloroflexi; Class and Order NA</i>	6.07%
4	<i>Chloroflexi; Class and Order NA</i>	5.30%	4	<i>Acidimicrobiales</i>	5.33%	4	<i>Acidobacteria; Order NA</i>	4.89%
5	<i>Rhodobacterales</i>	3.96%	5	<i>Chloroflexi; Class and Order NA</i>	4.49%	5	<i>Deltaproteobacteria; Order NA</i>	4.02%
6	<i>Xanthomonadales</i>	3.87%	6	<i>Nitrospirales</i>	4.29%	6	<i>Burkholderiales</i>	3.68%
7	<i>Acidobacteria; Order NA</i>	3.55%	7	<i>Burkholderiales</i>	4.06%	7	<i>Nitrospirales</i>	3.54%
8	<i>Nitrospirales</i>	3.15%	8	<i>Xanthomonadales</i>	3.66%	8	<i>Acidimicrobiales</i>	3.53%
9	<i>Anaerolineales</i>	3.00%	9	<i>Anaerolineales</i>	3.33%	9	<i>Gaiellales</i>	2.65%
10	<i>Nitrosomonadales</i>	2.56%	10	<i>Deltaproteobacteria; Order NA</i>	2.59%	10	<i>Xanthomonadales</i>	2.49%
		48.35%			49.28%			45.03%
	<i>Other Taxon</i>	51.65%		<i>Other Taxon</i>	50.72%		<i>Other Taxon</i>	54.97%

Rhizobiales:

Broad group of root-zone dwelling bacteria that fix atmospheric nitrogen and are involved in nitrogen cycling

Anaerolineales:

Filamentous, fermentative, strictly anaerobic chemo-organo-heterotrophs

16S rRNA Amplicon Sequencing – GENUS LEVEL

PZ-01

	Taxon - Genus	Gene %
1	Nitrospira	1.86%
2	Acidiferrobacter	1.26%
3	<i>Illumatobacter</i>	1.10%
4	Gaiella	0.99%
5	Rhodobacter	0.96%
6	Hyphomicrobium	0.96%
7	<i>Hydrogenophaga-Variovorax</i>	0.88%
8	<i>Tabrizicola</i>	0.87%
9	<i>Nocardioides</i>	0.83%
10	<i>Pedomicrobium</i>	0.82%
11	Leptothrix	0.72%
12	Methylomirabilis	0.68%
13	<i>Amaricoccus</i>	0.68%
14	Denitratisoma	0.65%
15	Luteolibacter	0.54%
16	<i>Alysiosphaera</i>	0.52%
17	<i>Haloferula</i>	0.50%
18	<i>Nordella</i>	0.50%
19	<i>Devosia</i>	0.46%
20	<i>Sphingomonas</i>	0.43%

PZ-02

	Taxon - Genus	Gene %
1	Nitrospira	2.45%
2	Hyphomicrobium	1.51%
3	<i>Illumatobacter</i>	0.94%
4	Leptothrix	0.93%
5	Methylomirabilis	0.86%
6	<i>Variibacter</i>	0.84%
7	<i>Nitrotoga</i>	0.68%
8	<i>Ornithinibacter</i>	0.66%
9	Nitrosomonas	0.66%
10	Denitratisoma	0.65%
11	<i>Rhodoferax</i>	0.65%
12	Gaiella	0.60%
13	Acidiferrobacter	0.59%
14	<i>Methylobacter</i>	0.57%
15	<i>Nordella</i>	0.56%
16	<i>Nocardioides</i>	0.52%
17	<i>Bryobacter</i>	0.51%
18	<i>Nakamurella</i>	0.49%
19	<i>Amaricoccus</i>	0.48%
20	<i>Pedomicrobium</i>	0.47%

PZ-03

	Taxon - Genus	Gene %
1	Hyphomicrobium	1.93%
2	Gaiella	1.29%
3	Leptothrix	1.14%
4	Nitrospira	0.65%
5	Brocadia	0.50%
6	<i>Bryobacter</i>	0.46%
7	<i>Nocardioides</i>	0.46%
8	<i>Pedomicrobium</i>	0.45%
9	<i>Clostridium</i>	0.44%
10	Acidiferrobacter	0.43%
11	<i>Desulfatiglans</i>	0.43%
12	Methylomirabilis	0.38%
13	<i>Desulfobacca</i>	0.37%
14	<i>Ignavibacterium</i>	0.37%
15	<i>Thiobacillus</i>	0.36%
16	<i>Rhodobacter</i>	0.36%
17	Denitratisoma	0.35%
18	<i>Haloferula</i>	0.34%
19	<i>Variibacter</i>	0.33%
20	Luteolibacter	0.32%

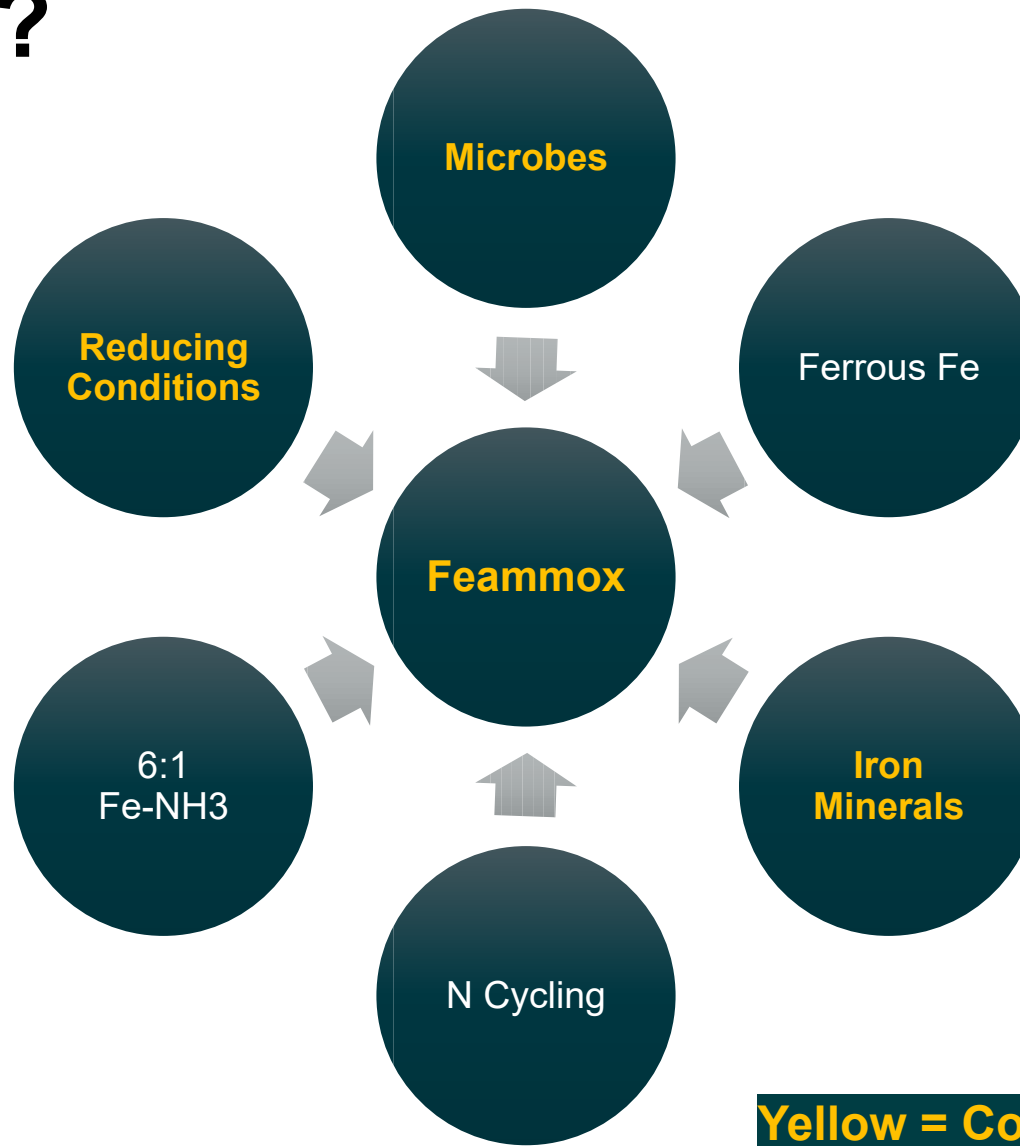
ANAMMOX BACTERIA

"IRON" BACTERIA

DENITRIFYING BACTERIA

DCM DEHALOGENASE

Feammox-Like?



Yellow = Condition Present at Study Area

Conclusions

- Feammox-like conditions were verified in the field through a weight of evidence including presence of iron mineralization, ammonia, reducing conditions (at the groundwater-to-surface water interface at PZ-03) and specific bacteria, including *Nitrospira*, *Brocadia*, and *Denitratisoma*.
- Optimal iron to ammonia ratio (6:1) for Feammox were not present in the study area
- One identified bacterium *Hyphomicrobium* sp. is included within a genus documented as being encoded with dehalogenase genes.
- PFAS were conspicuously absent during all sampling events at location PZ-03, which yielded the most representative Feammox conditions (redox and microbial footprint).

Next Steps

- Further temporal monitoring is warranted with additional sampling and analysis for Feammox indicators and PFAS
- Additional use of Molecular Biological Tools (MBTs) will focus on identifying functional genes associated with the promising bacteria
- Focused sampling and bench-scale microcosm culturing of study area sediments



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