The Role of Microbes on the Fate of PFAS in the Environment

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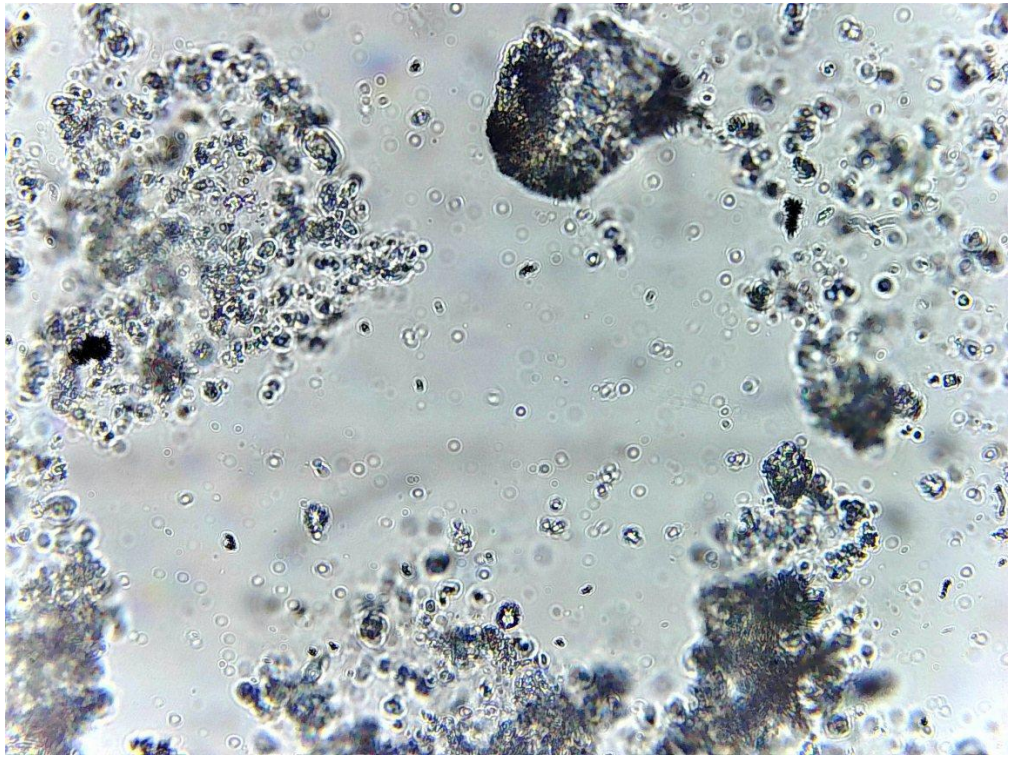
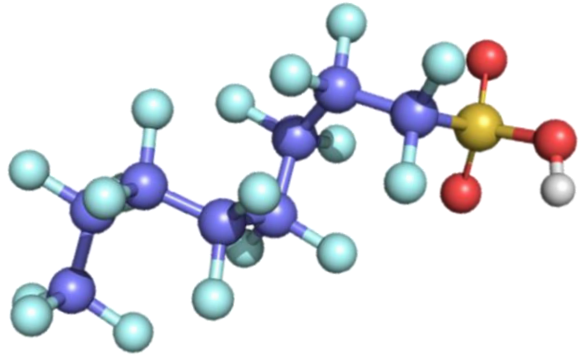
October 12, 2023

Outline

- Per and polyfluorinated alkyl substances (PFAS)
  - Background
  - Biotransformation
- Endemic Microbes - Alpena Hide and Leather (AHL)
  - Benchtop 1.0
  - Benchtop 2.0
  - Field Results





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- Fixed Earth - **Tim Repas**
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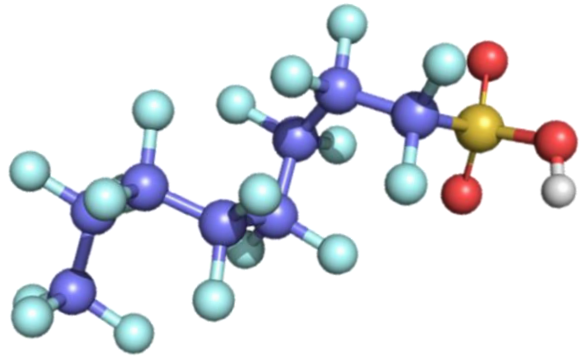
- Bureau Veritas Laboratory – Heather- Lynn Lord and Lori DeFour



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PFAS – General
Site Introduction
Biotransformation – The gift that keeps on giving

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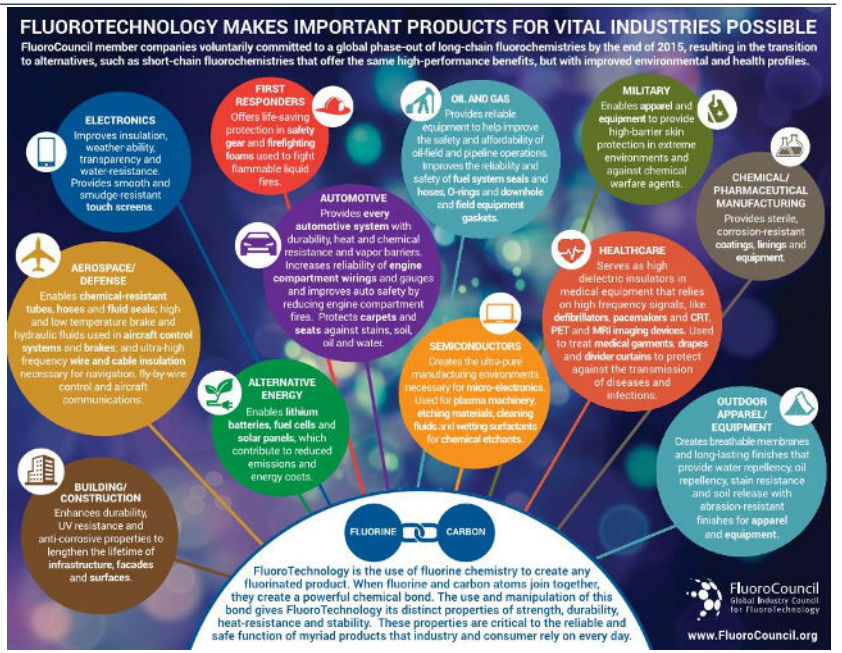
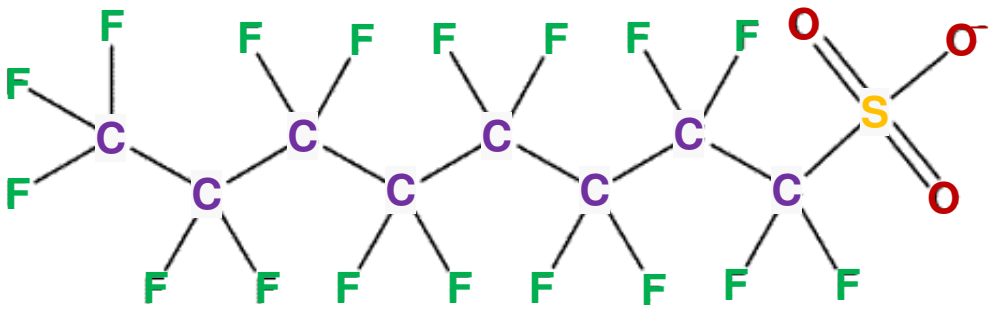
PFAS – Emerging Contaminants of Concern

- Family of widely used compounds
- ***Strong carbon-fluorine bond***
- Persistent in environment "Forever Chemicals"
- Surfactants with hydrophobic "tails" and hydrophilic "heads"
- Cationic (+), anionic (-), or zwitterionic (+ and -)
- Mechanisms to cleave the "head" debones the snake (Trang et al. 2022)



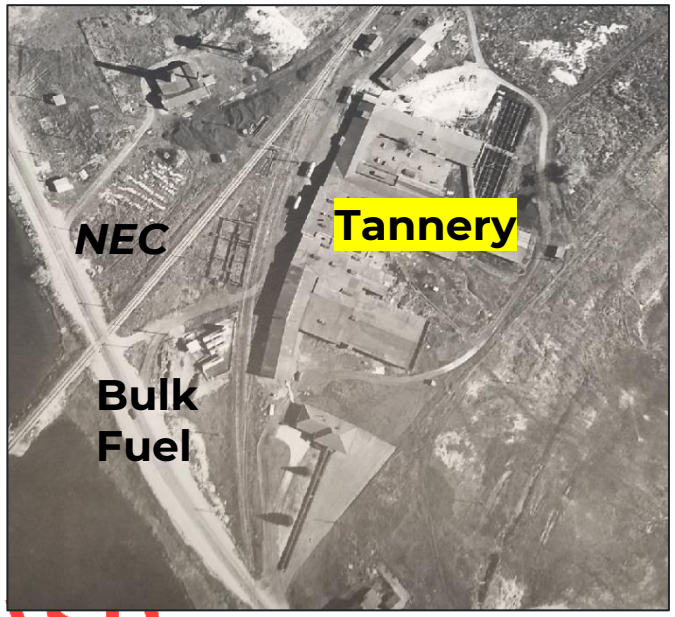
Hydrophobic Tail (Affinity: Air, NAPL, Carbon etc)

Hydrophilic Head (Affinity: Water)

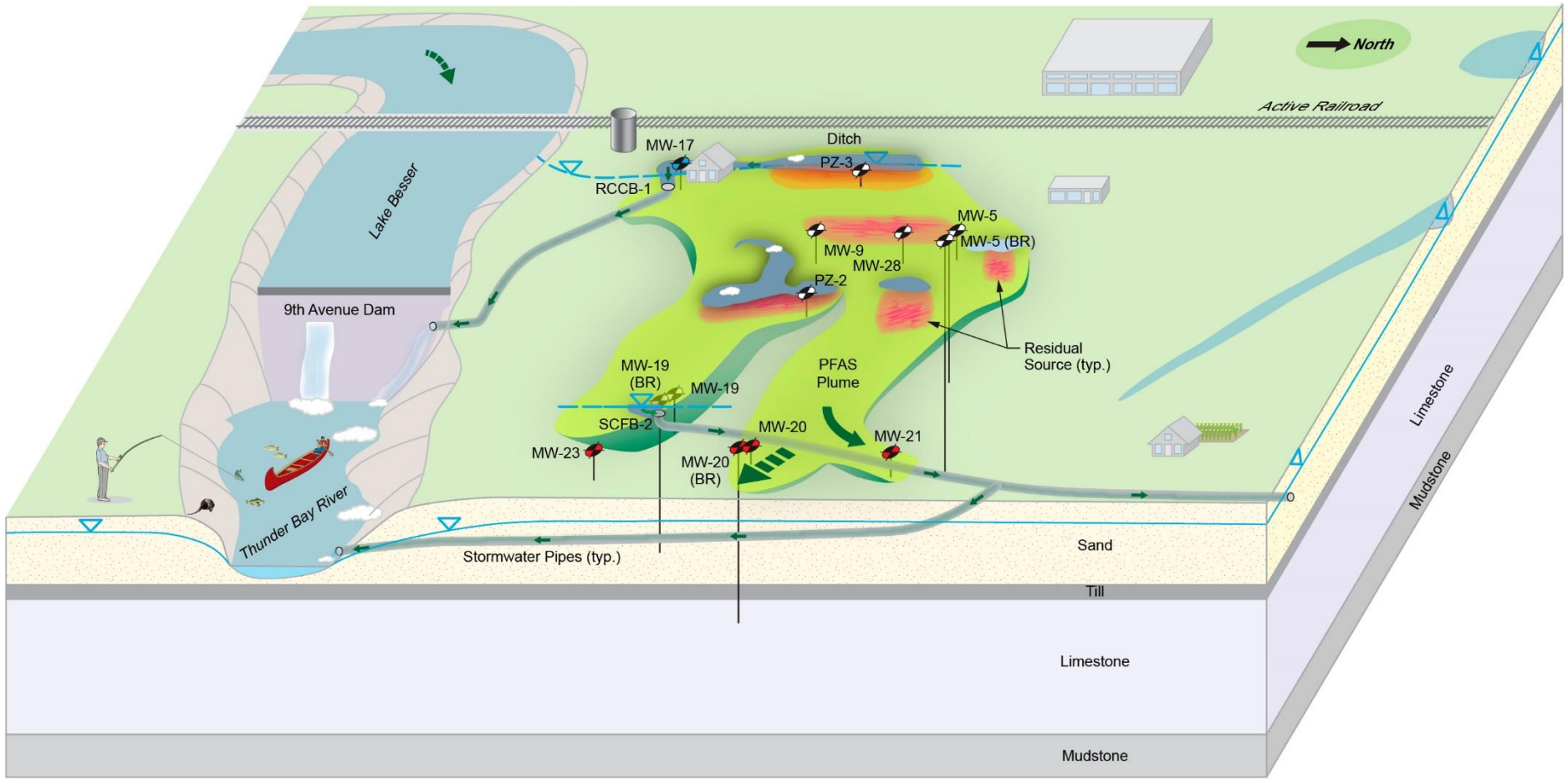


PFOS - perfluorooctanesulfonate

Alpena Hide and Leather – Site History



Alpena Hide and Leather – Conceptual Site Model

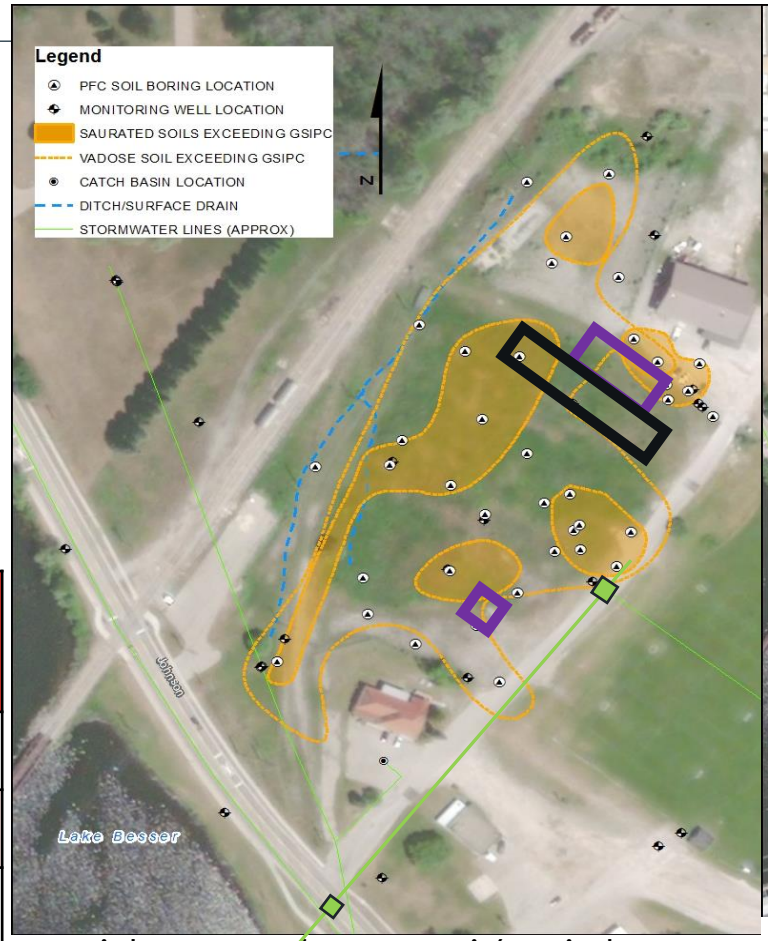


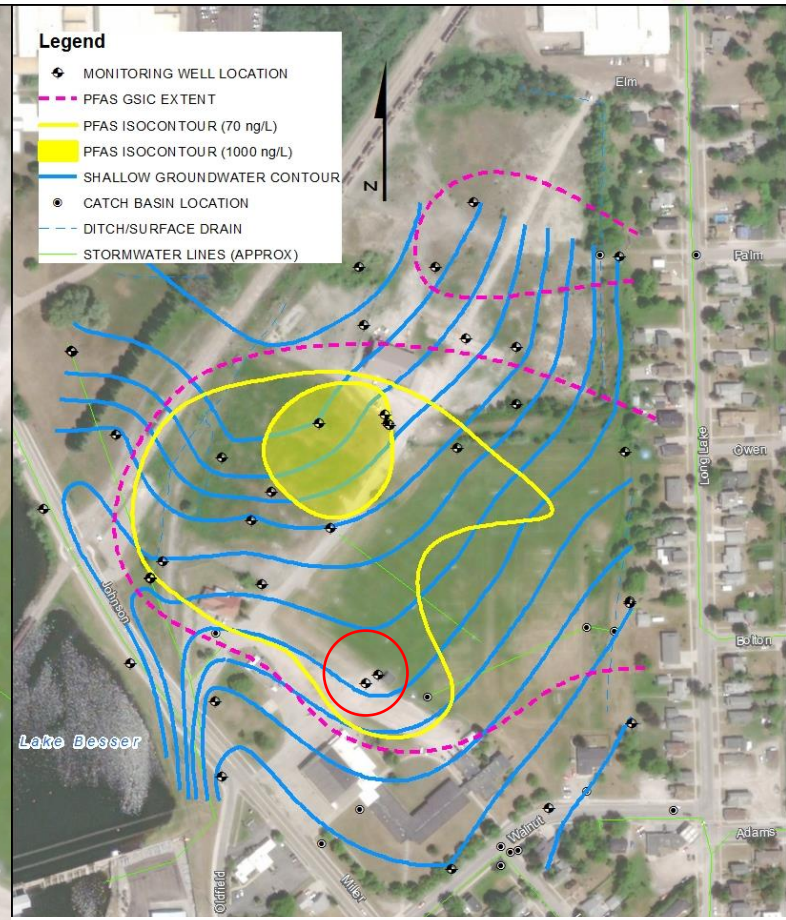
Not To Scale

PFAS in Soil & Groundwater

- Source: Surface to Capillary Fringe (low)
  - Migration to storm water?
- Soil to Groundwater: Detections "*not 1:1*"
  - "Flushing" fractionation
  - Precursor transformation
  - Limits of detection?

PFAS	Soil		Groundwater	
	ng/g (ppb)	*f* (%)	ng/L (ppt)	*f* (%)
PFBA	ND	0%	493	92%
PFBS	5.7	11%	*3,140*	85%
PFHxS	43	56%	*15,400*	79%
PFOS	264	63%	**8,270**	74%
PFOA	5.4	9%	**804**	79%
Total (537)	14 – 76% samples		20 – 92% samples	





—Widespread at 0-1.5' (~High Wate

—Hotspots at 4-5 feet (~Low Water)
- Former building footprint
- Topographic lows/infiltration areas

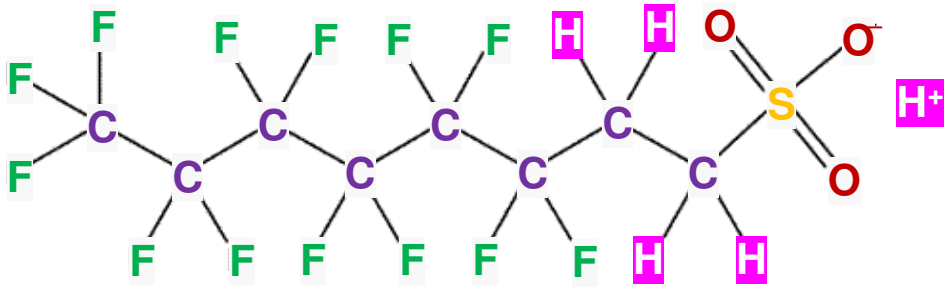
—Limited detections at base of aquifer

- PFOA Hot Spot - downgradient
- Expanding plume
- Offsite fractionation: PFBA+PFBS, followed by PFOA then PFOS

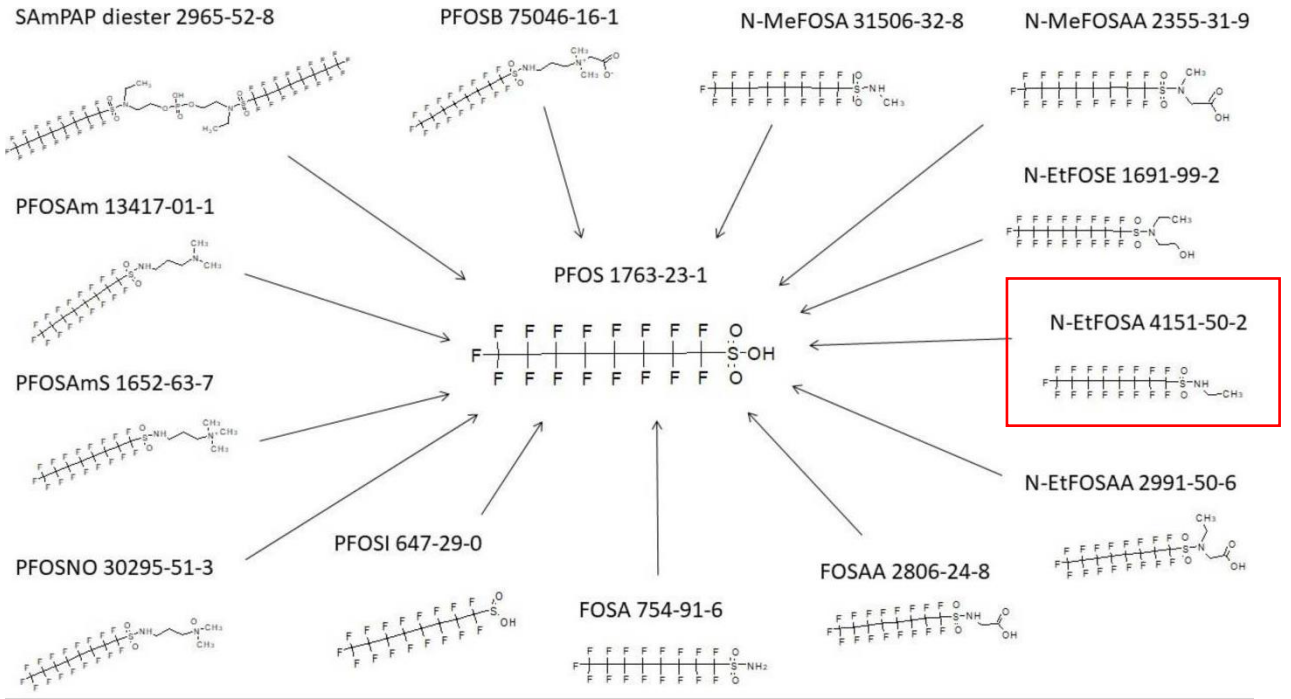
PFAS – Precursors

Polyfluorinated Alkyls

- Poly-fluoros: “precursors” that can transform to per-fluoros
- Bio-transformation of poly- to perfluoro alkyls has been demonstrated:
  - Anaerobic (e.g., Yi et. Al. 2014)
  - Aerobic (e.g., Zhang et al., 2020)
  - Fungal (e.g., Shah et al. 2023)
- Bio-transformation has been shown to be a “**team**” **sport**
- **Inorganic inhibitors** (e.g., sulfates) can shut down some transformation reactions (Yang et al, 2023)



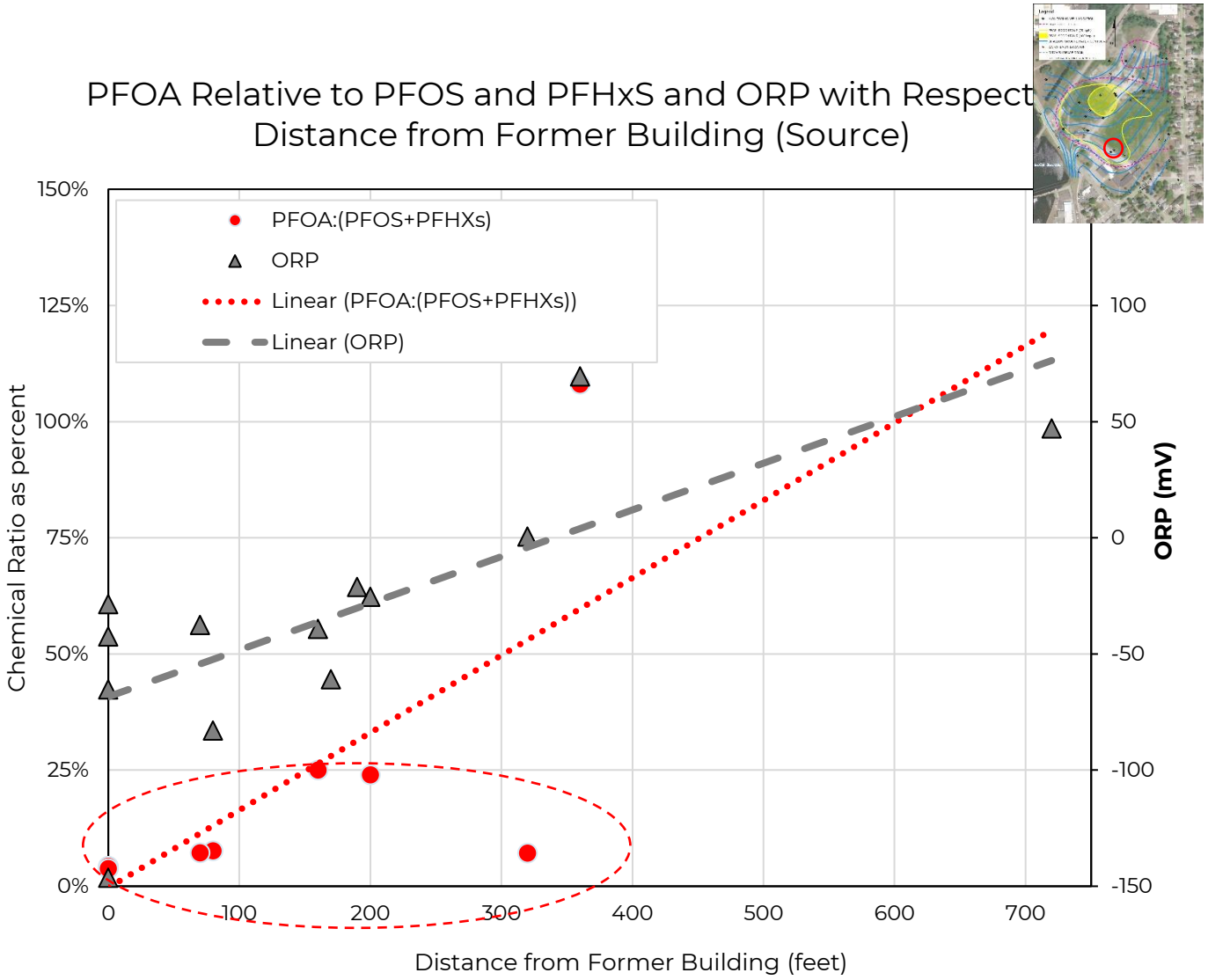
6:2FTS – fluorotelomer sulfonate



Kolanczyk RC, Saley MR, Serrano JA, Daley SM, Tapper MA. PFAS Biotransformation Pathways: A Species Comparison Study. Toxics. 2023 Jan 12;11(1):74. doi: 10.3390/toxics11010074. PMID: 36668800; PMCID: PMC9862377.

Precursor Compounds – PFOA

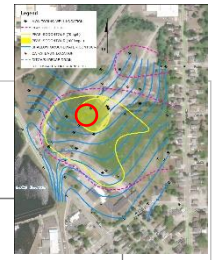
- PFOA increases in concentration ***off-site***
- PFOA increases relative to PFHxS and PFOS
- Formation becomes more aerobic off-site (increased ORP)
  - Separate source?
  - Mobility of PFOA > PFOS; example of fractionation?
  - Bio-oxidative transformation?



Precursor Compounds – PFOS

The gift that keeps on giving - precursor transformation…

- 8:2 & 6:2 Fluorotelomer Sulfonate (FTS) & Et-FOSA in soil, groundwater, storm water surface water and/or foam
- PFOS concentrations are increasing in groundwater in untreated source area. Potential bio-transformation?



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Biodesktop 1.0
Biodesktop 2.0

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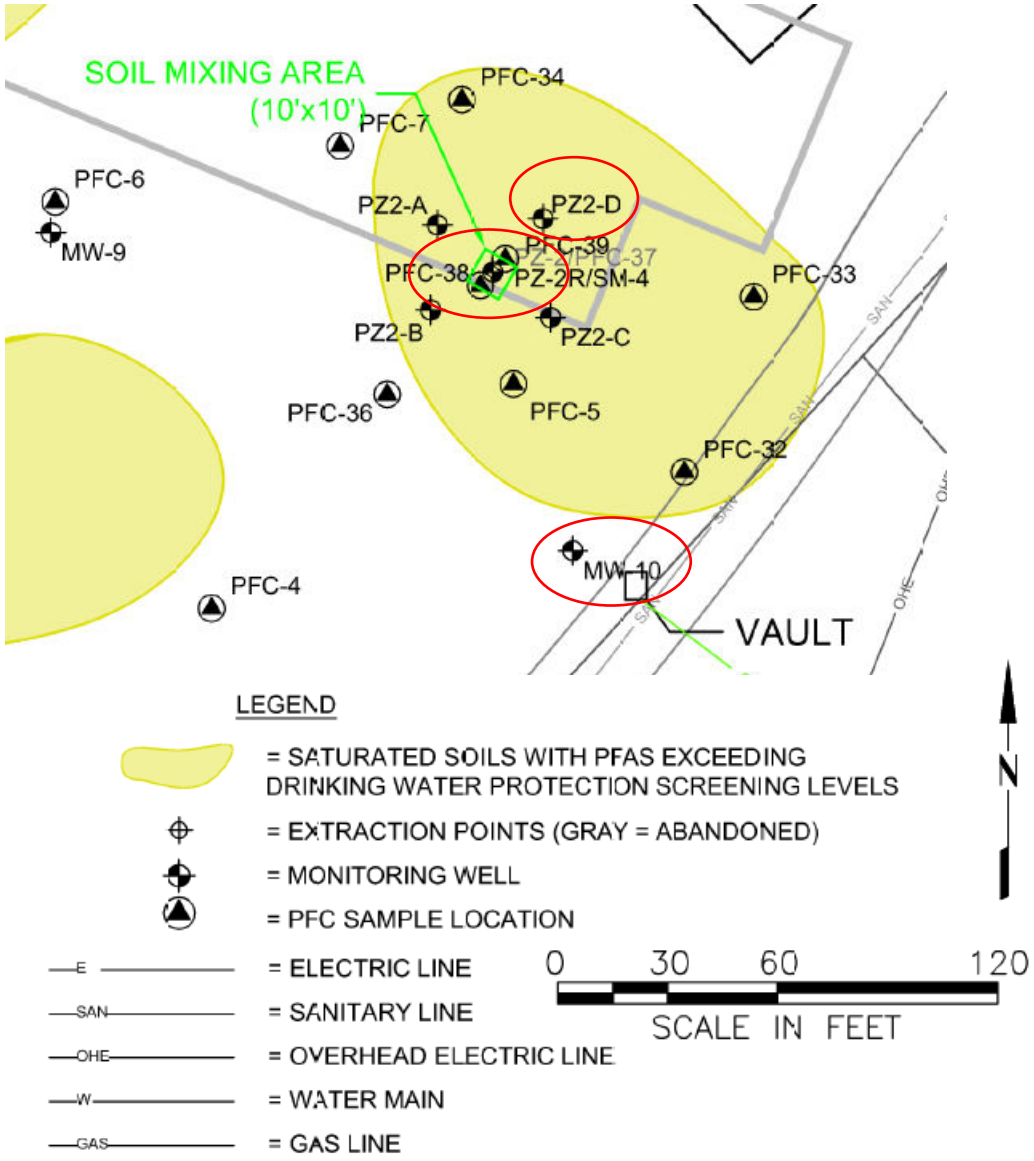
Microbial Benchtop Study – Seeking Endemic Opportunists

Why Microbes?

- An in-situ destructive mechanism is needed
- Microbes are resilient and adaptable
- The hunt for microbes is never easy (e.g., TCE, 1,4-Dioxane)
- Evidence building for bio-defluorination of PFOS and PFOA (e.g., Huang and Jaffe, 2019; Harding, 2023?)

2020 - Collected soil and groundwater to identify potential endemic, PFAS-degrading microbes (Fixed Earth LLC):

- Upgradient of soil mixing area (PZ-2D)
- Soil mixing area (PZ-2R)
- Aerobic transition zone where PFOA begins to increase (MW-10)



BioDesktop 1.0

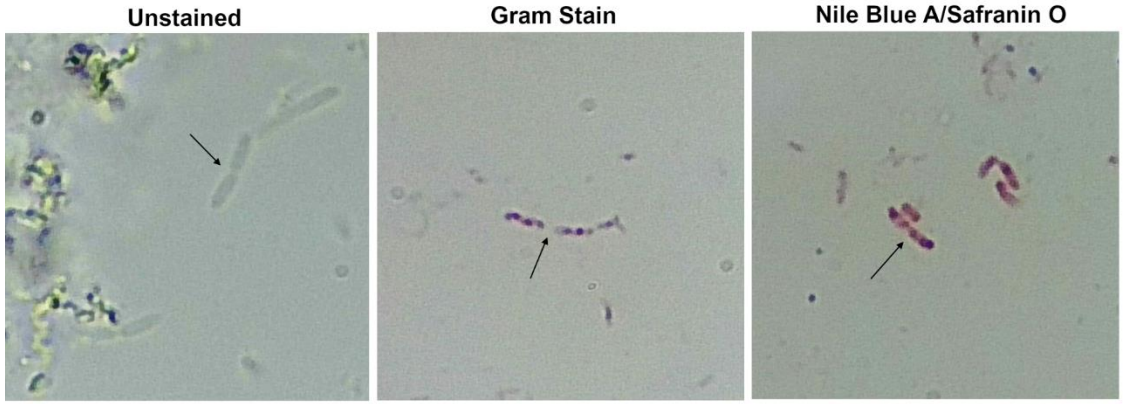
- Fixed Earth isolated 6-endemic microbial candidates from Site soil and groundwater
- Mixed microbial strains in Site groundwater:
  - Control (C);
  - Microbes (only; M);
  - Microbes + sugar + aeration (MFA)
- Mixed microbial strains in spiked PFOS/A tap water (1,000 ng/L ea.)
  - Control (C)
  - Microbes (only; M)
  - Microbes + sugar (MF)
  - Microbes + aeration (MA)
  - Microbes + sugar + aeration (MFA)

NAA-2D (Source)

NAA-2R (Soil Mix)

NAA-10 (~Aerobic)

The Zoo



Production of polyhydroxalkanoaes (PHA) suspected to give apparent positive gram g test

BioDesktop 1.0 – Tap Water Results

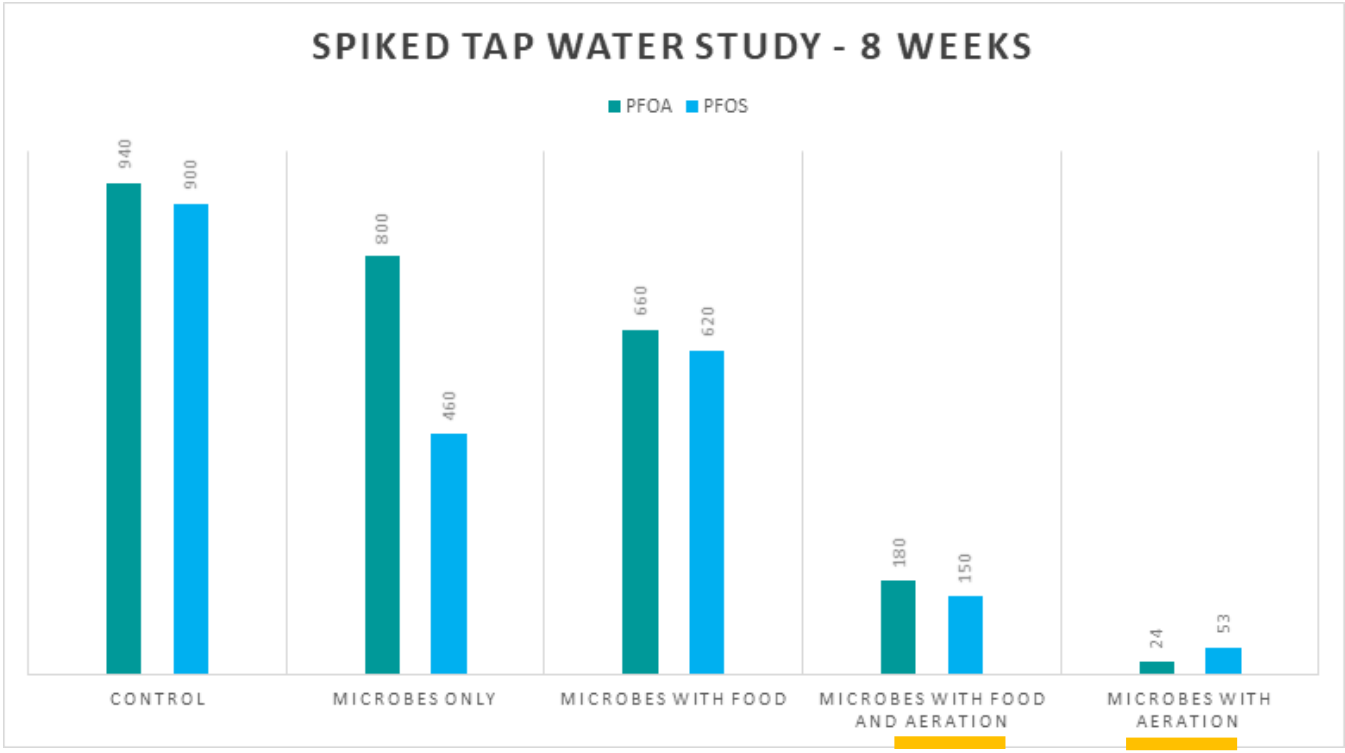
- PFOA/PFOS show significant reduction compared to control at 8 weeks:

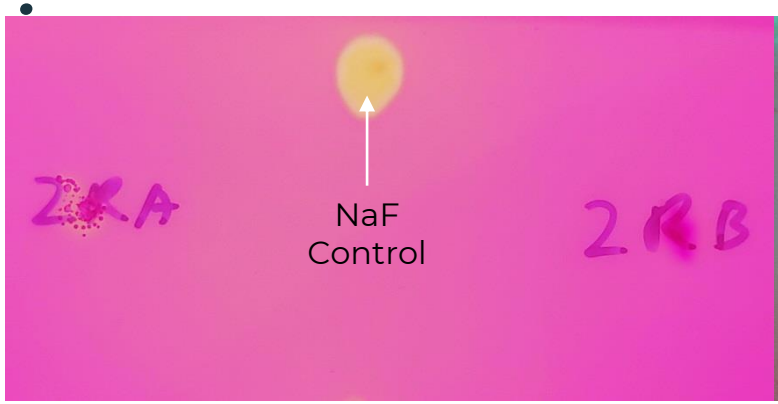
94% to 97% PFOS/A reduction

- No fluorinated VOCs detected (open scan)
- Positive inorganic fluorine response in PFOS/A enriched media in 2 of the 6 microbes isolates.

Note yellow glow in "biofilm"?

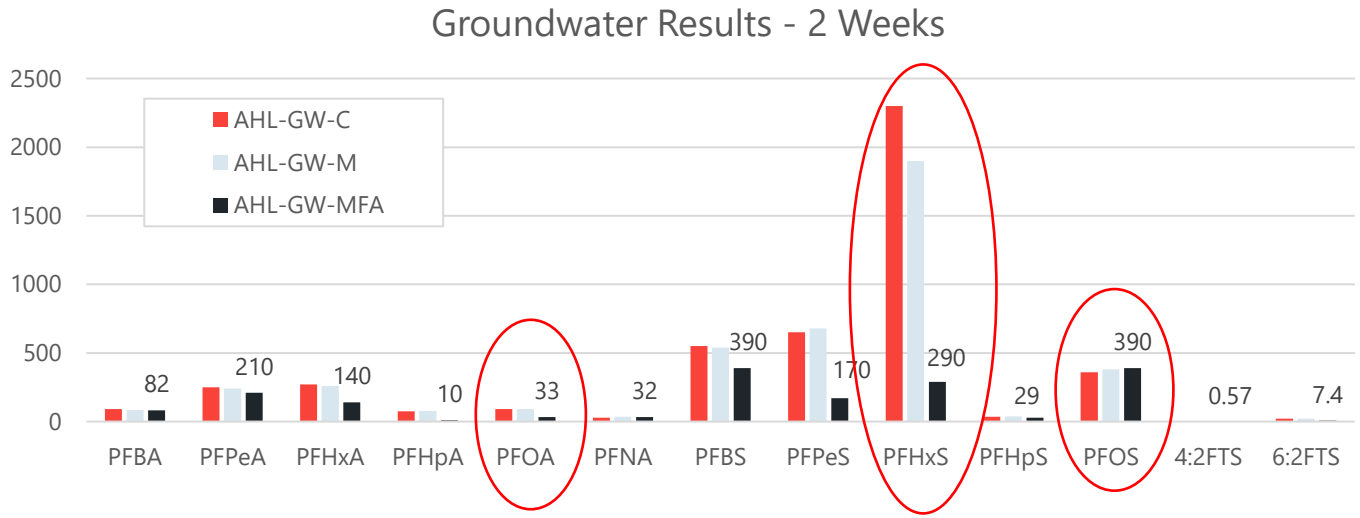
- No significant change in inorganic water chemistry

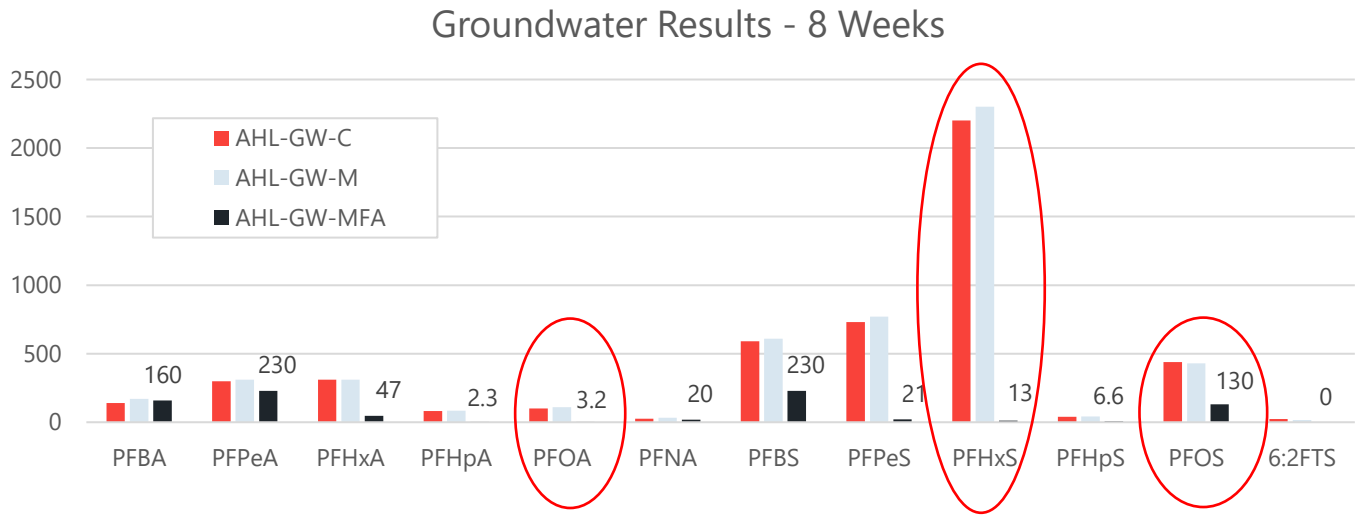




BioDesktop 1.0 – Groundwater Results

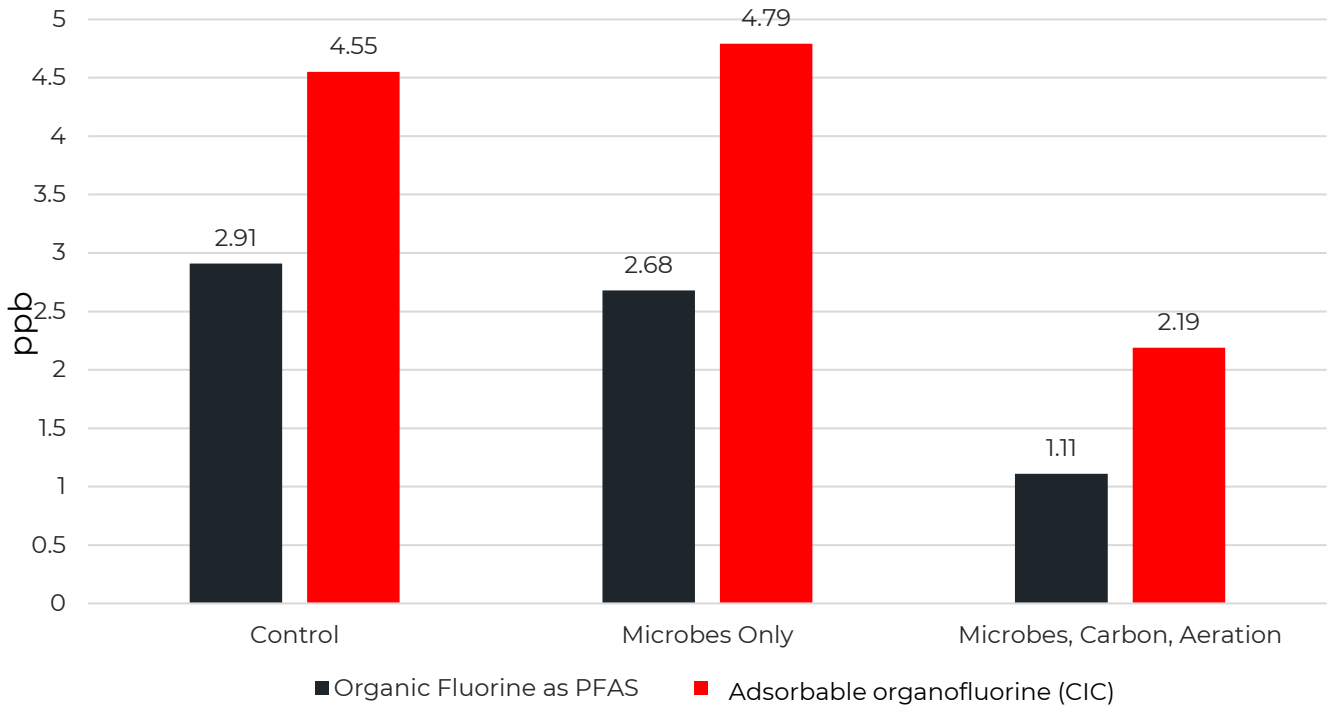
- Significant drop in PFHxS at 2- and 8-weeks with aeration
- At 2-weeks PFOS increased in MFA reactor
- At 8-weeks PFOS decreased in MFA
- Microbial augmentation alone showed modest changes – more reduction observed with aeration
- PFBA and PFPeA were only compounds to increase in MFA reactor at 8-weeks

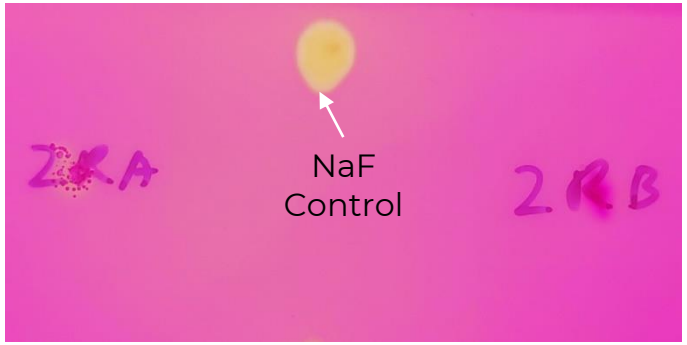




BioDesktop 1.0 – Groundwater “Precursor” Results

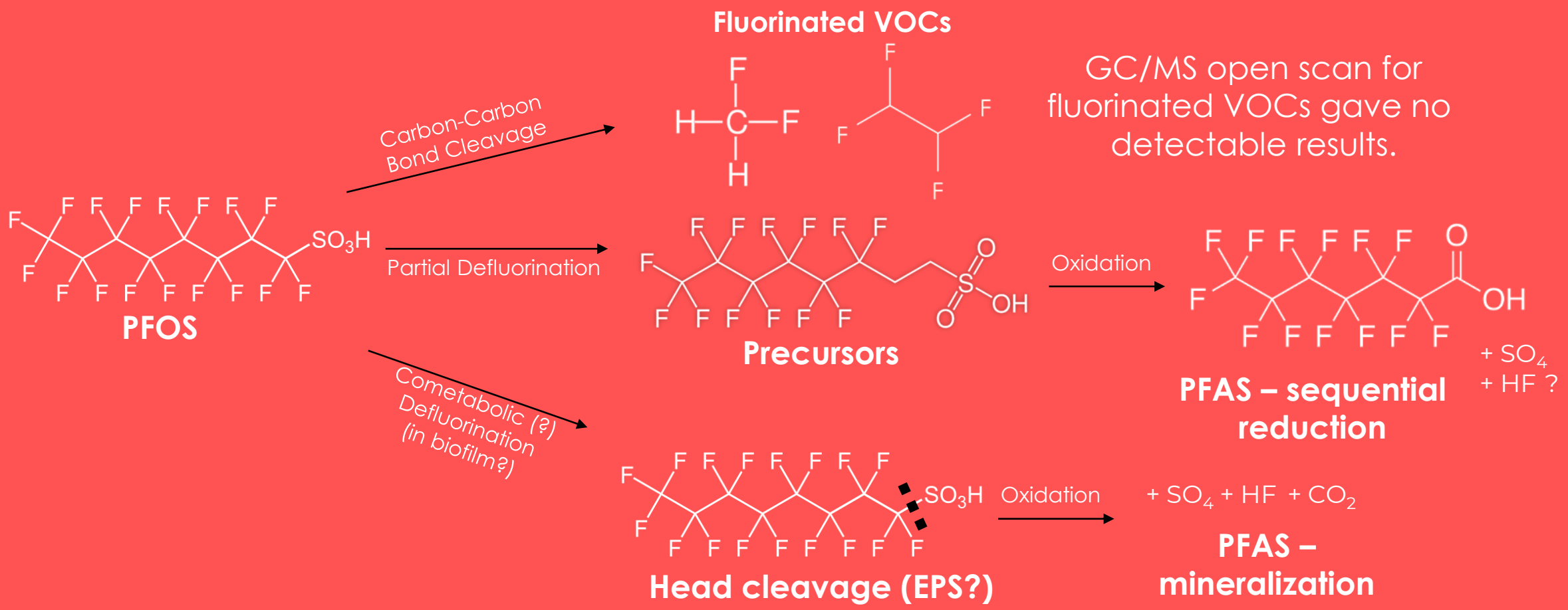
- PFAS scan (537) in control and microbes-only detected 64% and 56% of fluorine detected by AOF
- PFAS scan (537) in MFA detected 51% of fluorine detected by AOF
- 42% of the AOF reduced in the MFA sample when compared to control & microbes only
- AOF is “non-unique” but potential precursor transformation loss
- AOF does not reflect fluorine from short chains (<C4; sorption process)
- Precursor transformation may have contributed to PFOS increase at 2-weeks?





BioDesktop 1.0 – Roadmaps to Destruction?

– Mineralization model best fits observed results



PFOS/A Microbial Degradation Isn't Supposed to Happen

Conventional wisdom: Aerobic biodegradation is "unlikely" -what "went wrong"?

Q. Could aeration have caused loss via aerosol production or partitioning?

A. Potentially – we did not have a control on aeration only

Q. Used Method 537 (not whole bottle) extraction techniques – container loss?

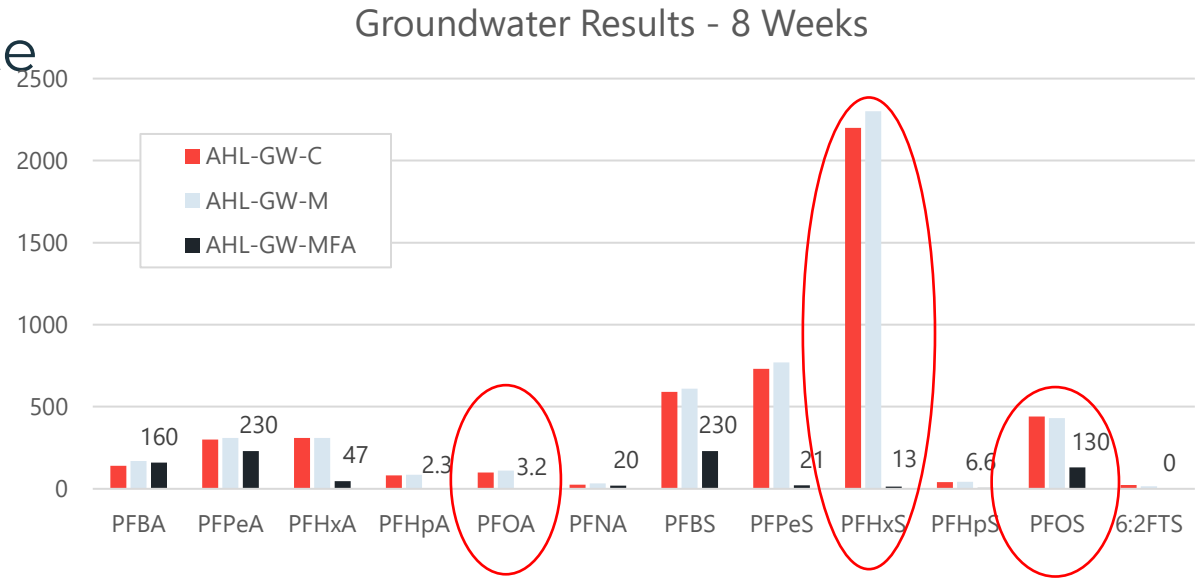
A. Controls which should also have been affected but were not and this does not explain the breadth of data.

Q. Work was not completed in triplicate

A. Fair point (but within budget ☺)

Q. No "killed" control to assess bioadsorption?

A. Differences in multiple microbe runs suggest it was not a factor



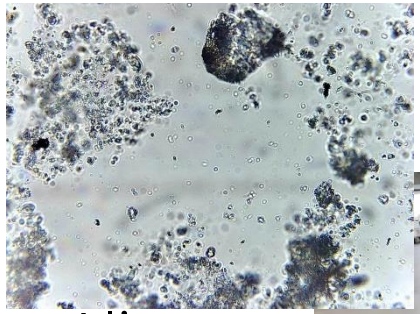
BioDesktop 2.0

WSP and Fixed Earth Sponsored

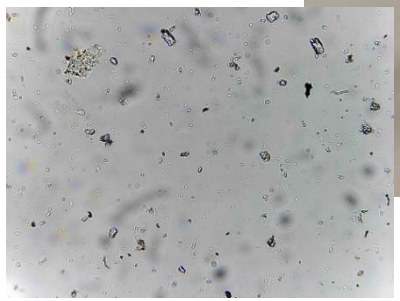
- Freezer stock of isolated microbial strains cultured and added to PFOS/A spiked tap water.
- Single (1-week) & duplicate (2-week) analyses
- 537 Method with whole bottle extraction
- Viability & qualitative inorganic fluorine testing
- Dose - 1.25 Million CFU/mL
- Additional controls added (including heat killed microbes [HKM] to assess bio-adsorption)
- Rock flour (RF) added to assess role of a substrate
- Use of a diffuse oxygen source, shaker table & peroxide (partitioning/ aerosol concerns)


Waking up


The Zoo?


^ Alive

v Not so much



BioDesktop 2.0/2.1 – Results at 2-weeks

- Microbes in aerated samples were viable
- Viable colonies then demonstrated positive inorganic fluorine response
- HKM (bio-adsorption: 0-12% Reduction)
- Partitioning (14-32 % diff between top & bottom)
- Live microbe reductions of 0-12%

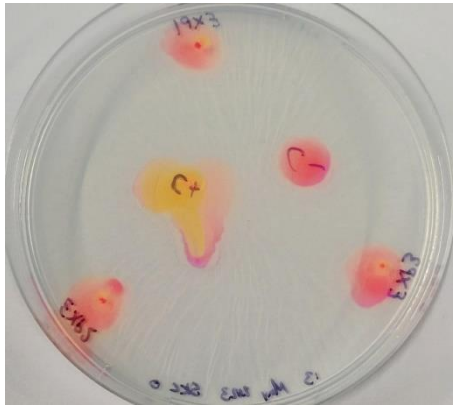
2.0: PFOS 1200 ng/L; PFOA 1400 ng/L

2.1: PFOS 3000 ng/L; PFOA 800 ng/L

2.0: 2-Week results; diffusive oxygen source

Condition	Average Duplicate Results at 2-wks (ng/L)			
	Control		Live Microbes	
	PFOS	PFOA	PFOS	PFOA
Stock Solution	1000	1300	--	--
Rock Flour (RF)	1100	1400	995	1300
Diffusive Oxygen (O2)	--	--	980	1300
RF+O2	1100	1400	1000	1300
RF+O2+HKM	1200	1400	--	--
RF+Peroxide	--	--	995	1300

Positive inorganic fluorine response in viable colonies



Viability test (growth); PFAS-enriched media, 6 E08 CFU/mL

2.1: 2-Week results; shaker oxygen source

Condition	Results at 2-wks (ng/L)			
	Control		Live Microbes	
	PFOS	PFOA	PFOS	PFOA
Stock (top)	3400	700	2200	660
Stock (bottom)	2300	600	--	--
RF+Shaker O2	3000	750	3400	830
Shaker O2	3000	810	2900	810
HKM (Sealed/shaken)	2500	770	--	--

PFOS/A Microbial Degradation Was Supposed to Happen

Bio-desktop 2.0 Wisdom: Aerobic biodegradation “worked”-what “went wrong”?

Q. Could aeration have caused loss via aerosol production/partitioning in 1.0?

A. Potentially – we did not have a control on aeration only during this experiment (**but will in 3.0**)

Q. Container loss?

A. 1.0 controls still should have been affected.

Q. “Killed” control to assess bio-adsorption?

A. Killed microbe sets suggest 0-12% adsorption (including standard 537 extraction to isolate biofilms from aqueous media). Biofilm consideration.

Q. What was different?

A. Refined microbial strain isolates used in 2.0; was something “lost” that caused enzyme expression? Fresh samples to be used for Bio-desktop 3.0

Dr. Chris Marshall (Marquette University, WI) performing third party, independent validation & genome mapping

The Role of Microbes on the Fate of PFAS in the Environment

This is driving me crazy – let's go outside
2021 & 2022 Pilot Testing

October 12, 2023

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Microbial Pilot Tests

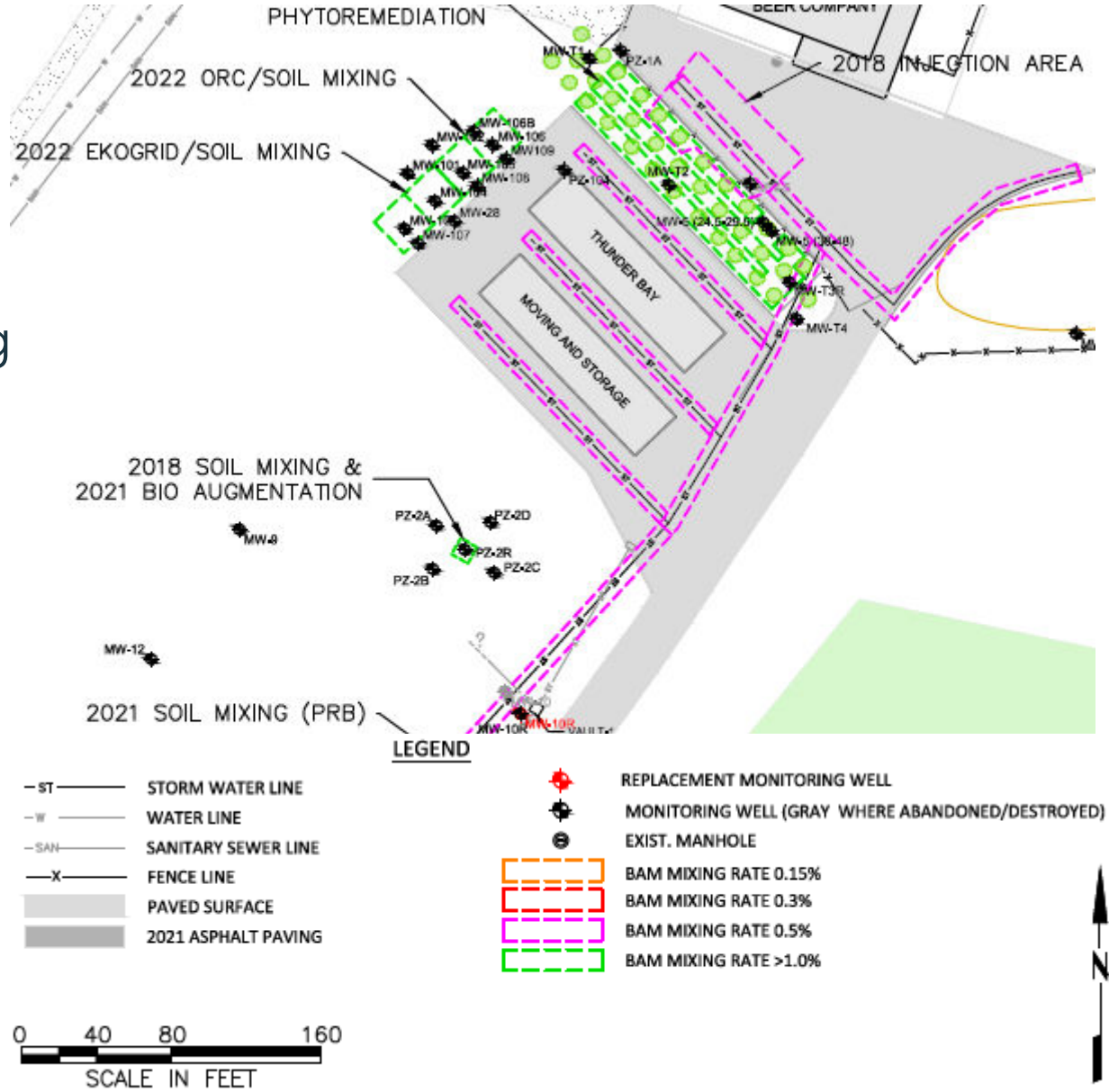
- BAM-Biochar used with bioaugmentation

Bioaugmentation Test 1 (2021):

- PZ-2R (biochar) – dissolved oxygen release compound (ORC) then air sparg (AS)
- PZ-2A – ORC-only (control)

Test 2-4 (2022):

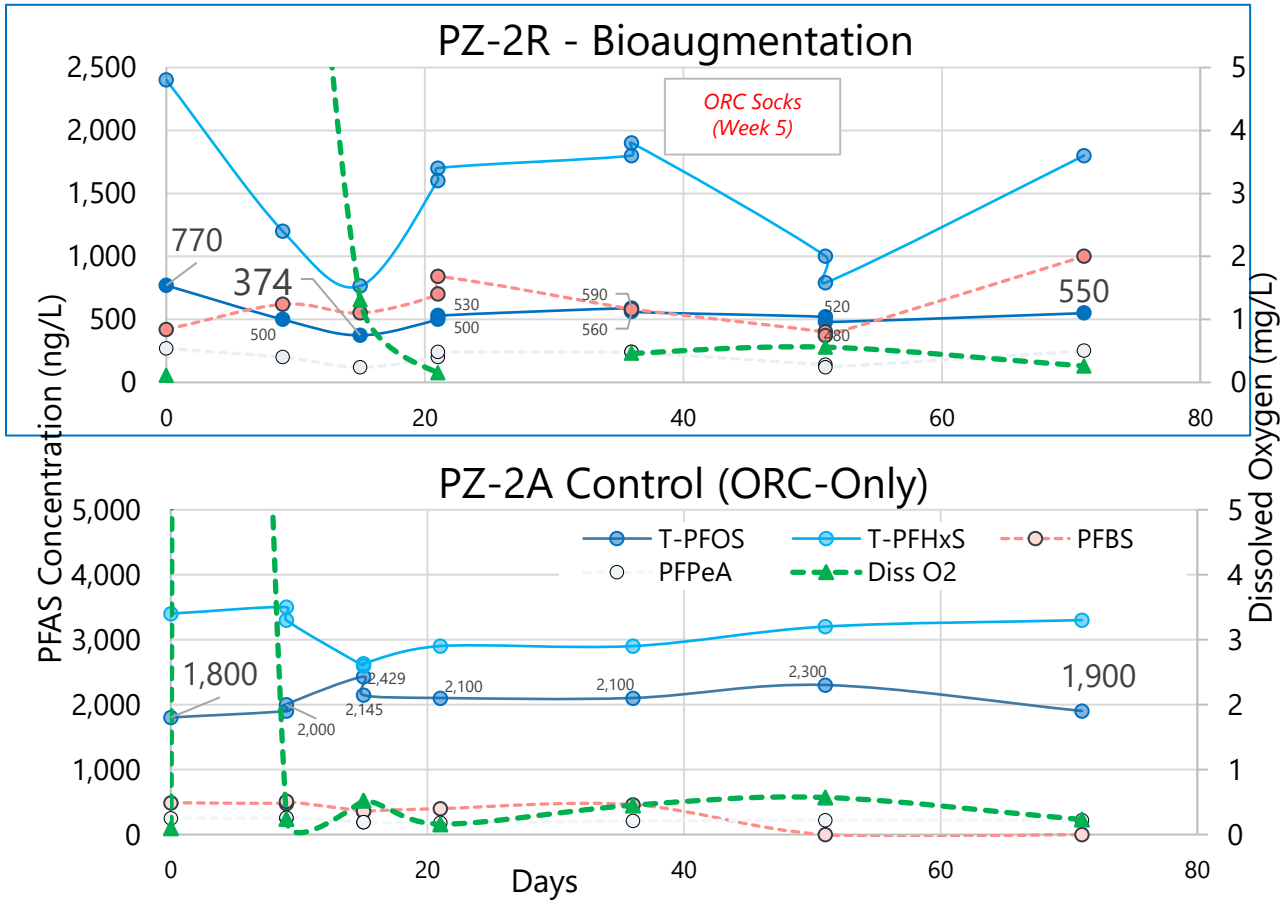
- Biochar+hydrolysis (EKOGrid)
- Biochar+ORC (Soil mixed)
- TreeWells + Biochar +/- ORC + AS



Microbial Pilot Test – 2021 Results

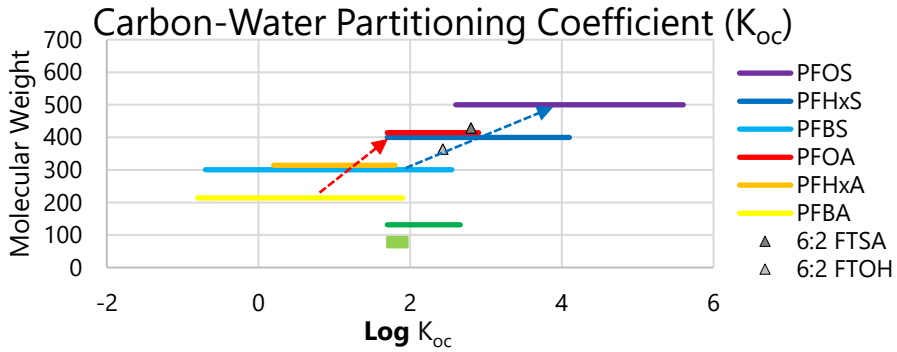
Delivery via three dedicated injection wells in treatment (PZ-2R) and control (PZ-2A) blocks

- Bioaugmentation: Significant declines in PFHxS & PFOS in first 2-weeks
- PFAS rebound when DO << 1 mg/L (AS added later)
- Short-term reduction inconclusive but declines > than ~10% bio-absorption observed in HKA (Bio-desktop 2.0)
- PFOS increased in ORC-only control Precursor “source” of PFOS masking breakdown?
- Displacement unlikely cause of observed reductions

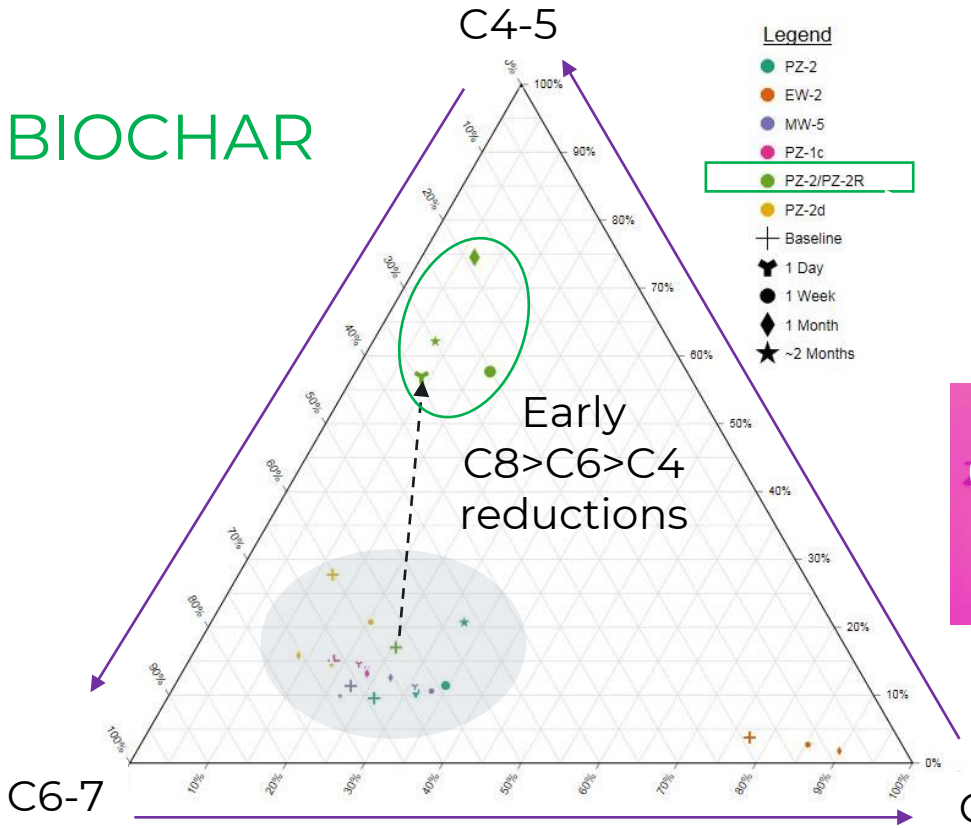


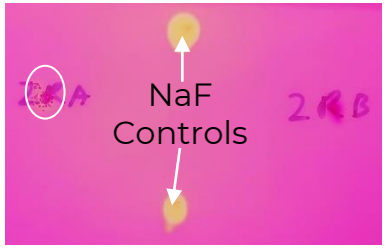
Is there a “Bio” signature?

- Looking for PFAS reductions beyond BAM-sorption
- Detection of free fluoride ions would be smoking gun
- Benchtop shows bio very efficient at reducing PFHxS (depleted faster); which is inconsistent with expected Koc. behavior

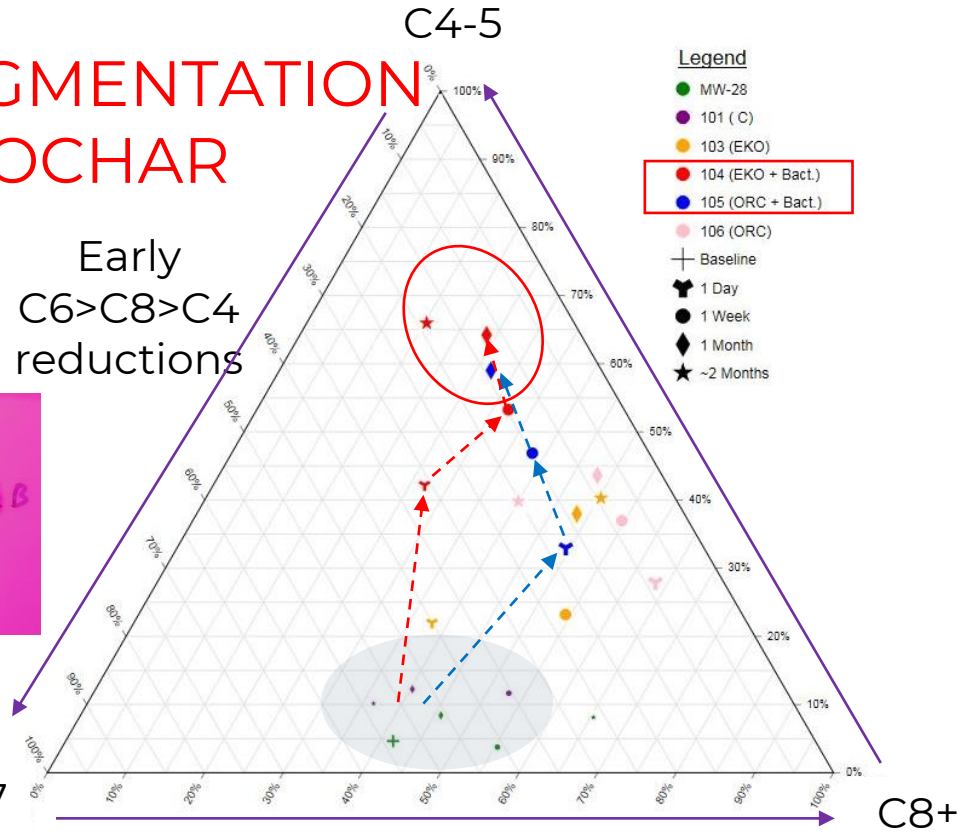


BIOCHAR



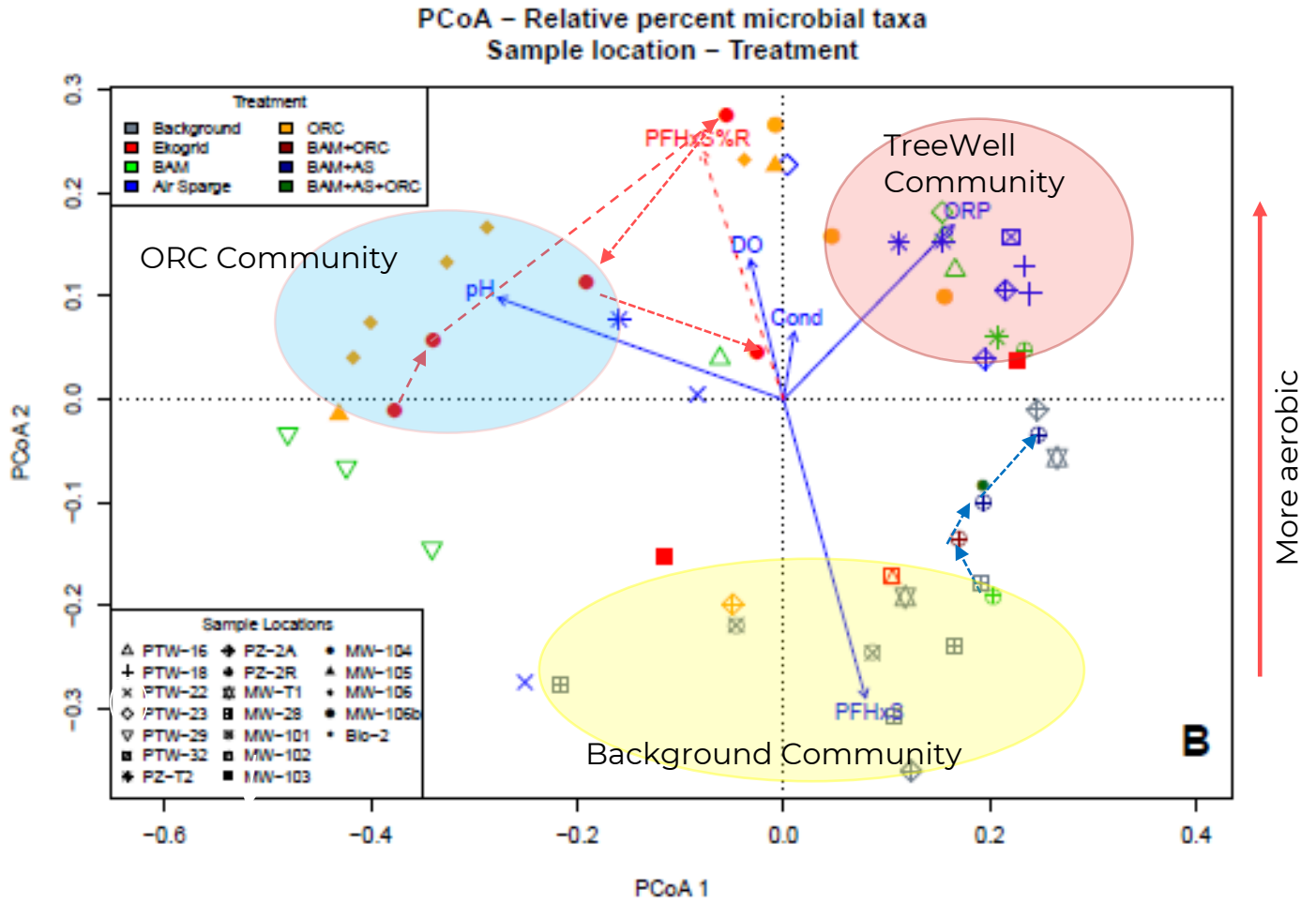


BIOAUGMENTATION with BIOCHAR



Is there a better way to assess aquifer buffering?

- Principal component analysis to statistically assess community similarities and differences
- Allows environmental parameters (Eigen vectors) to map across PCoA field to assess role of aquifer conditions on microbial communities.
- Can also be used to assess PFAS concentration (or percent reductions)
- Microbial communities change in response to treatment approaches



Summary – Biochar / Bio-desktop results

- Probably not a single microbe “silver bullet”
- Biodegradation results are inconclusive (1 for 2):
  - Microcosm Benchtop 1.0 provided compelling data suggesting degradation (PFAS mineralization and liberation of free fluoride) may occur under enhanced aerobic conditions.
  - Microcosm Benchtop 2.0 failed to show significant biodegradation or bio-adsorption processes.
    - Air-water partitioning NOT significant to explain lack of reduction
    - Processed strains lost key element for necessary gene expression?
    - Desktop 3.0 will use site groundwater and provide additional control on aeration mechanism
    - 3rd party validation & genome mapping underway
    - Microbes unable to be “separated” during genome mapping

Summary – Biochar / Bio-desktop results (cont'd)

- Mass balance continues to pose challenges and requires reliance on a multiple lines of evidence approach.
  - AOF and fluorinated VOC open scans suggest that large chain precursors were reduced and no cleavage of PFAS (C-C) evident (Desktop 1.0)
  - Better quantification of inorganic fluoride is one of the elements needed to improve mass balance control.
  - Qualitative results suggest microbial enhanced mineralization of PFAS occurred (Desktop 1.0 and in viable samples at 2-weeks in desktop 2.0/2.1).

Summary – Biochar / Bio Pilot

- 2021 Pilot mirrors Desktop 1.0: Early reduction in PFHxS linked to microbial degradation (microcosm and field results)? Partitioning onto carbon should favor PFOS over PFHxS.
- Precursor biotransformation to PFOA/PFOS may mask degradation of PFOS/PFOA (microcosm and PZ-2A field results)
- Bioaugmentation pilot results are inconclusive
  - Short term reductions in PFAS (Oxygen was limiting factor)
  - See decreased PFHxS relative to longer/shorter chains (ala benchtop)
  - Is there a critical loading rate? (e.g., Dhc 10^4)
  - Reductions exceeded benchtop bio-adsorption ranges
- Verifying biologic destruction of PFAS in the field remains extremely challenging.

Conclusion and Take Home Message

— Current state of the knowledge and technology is that microbial-related remediation is not there. But the NEED is there. These studies don’t disapprove or prove that, but it hopefully helps to get the ball rolling.

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Thank you!