Measuring *in situ* degradation by tracer stable isotope-labeled RDX groundwater release

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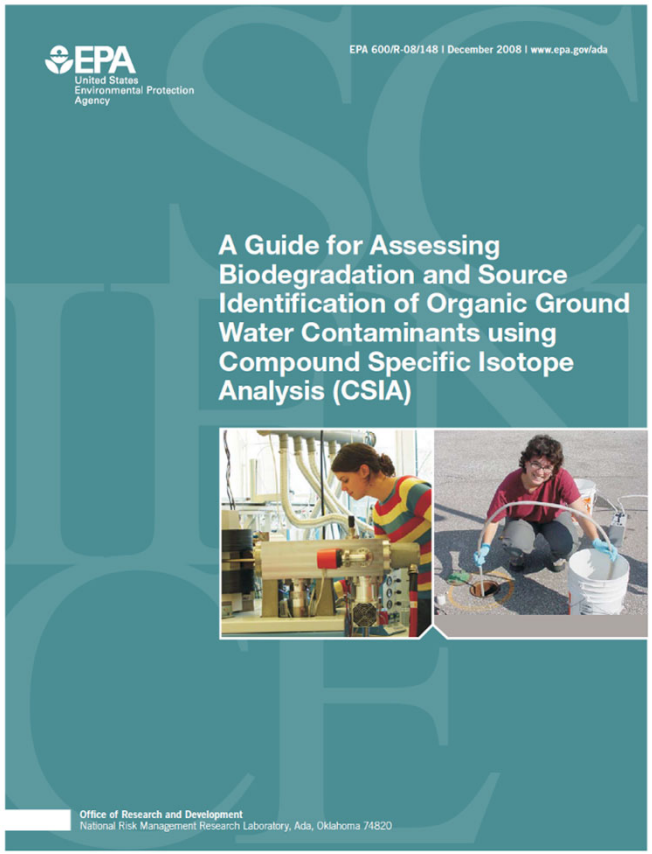
- "Traditional" use for compound-specific stable isotope analysis (~last 20 years) is to apply Rayleigh kinetics and lab- (or field-) derived fractionation factors to confirm and model *in situ* degradation
- Nothing "added" to system. Concentrations and isotope ratios determined from samples collected across a site (plume gradient)

Potential disadvantages

- Shifts may be minor
- Source(s) may be "mixed" over time
- Free product pools along gradient may obscure results
- Concentrations may not vary if CoC is relatively soluble



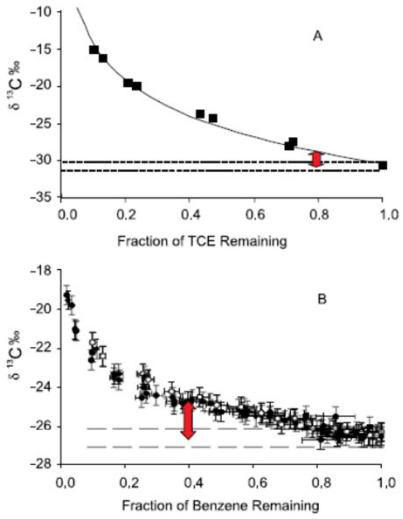


Figure 4.1. Degradation of (A) TCE and (B) benzene by enrichment cultures. The stable carbon isotope ratios in the substrate that remains after biodegradation are plotted against the fraction of the original concentration remaining. Data for TCE degradation are after Sherwood Lollar et al. (1999) and for benzene after Mancini et al. (2003). Dotted lines represent ± 0.5 ‰ around the $\delta^{13}C_0$ value of TCE and of benzene, respectively. The vertical solid red arrow represents the extent of fractionation necessary to recognize biodegradation in field data (2‰).

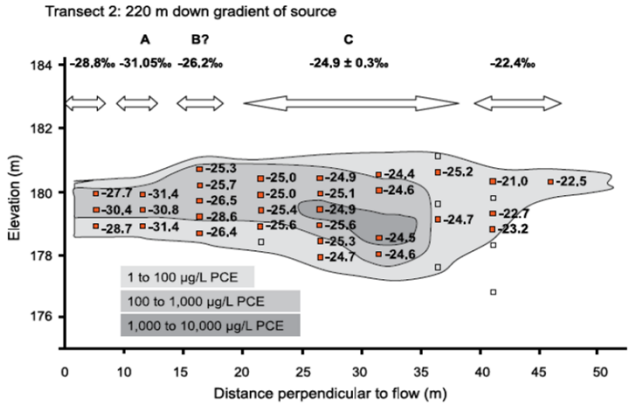


Figure 6.3. Concentrations and carbon isotope ratios of PCE in two transects downgradient of unidentified PCE sources. All values are given in ‰ relative to the V-PDB standard. Filled squares are depths sampled for determination of both concentration and $\delta^{13}C$. Open squares are depths sampled for concentration only. The figure is modified after Hunkeler et al. (2004).

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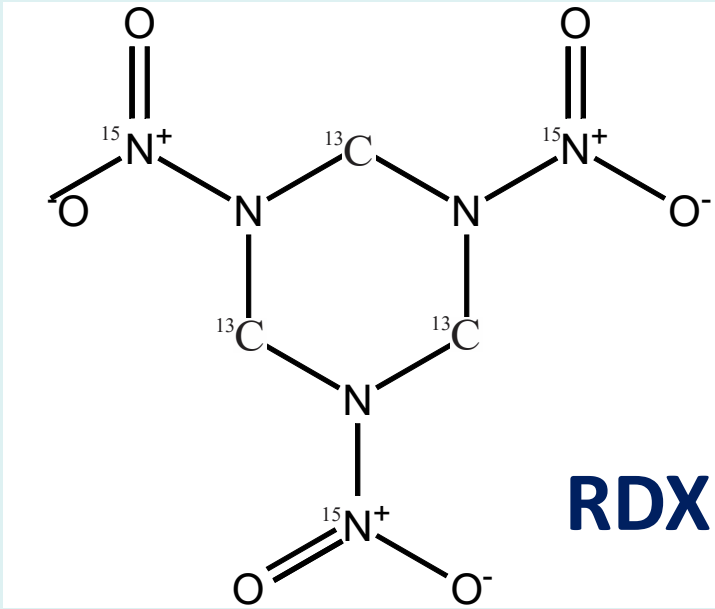
- Enriched compounds (^{13}C, ^{15}N) offer the ability to track conversion to degradation products (~100K and 400K higher signal respectively)
- Because the isotope is stable, no regulatory hurdles exist as would with radiolabeled substrates
- ^{13}C-, ^{15}N-Labeled compounds can be added at near tracer levels – easing regulatory issues
- Isotopic enrichment in mineralization products (CO_2, CH_4, NO_3^-, NO_2^-, NH_4^+, N_2O, N_2) provides concrete evidence for biological conversion. Isotopic enrichment in biomass proves the same

- 100% ^{13}C-, ^{15}N-RDX was synthesized at the Naval Air Weapons Station, China Lake
- The substrate was diluted to ~4 µM with groundwater taken from Site F
- The substrate was injected into 6 wells during push-pull tests (PPTs) in April 2018
- The substrate's fate was tracked by measuring mineralization compounds and biomass*



*separate experiment

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U.S. NAVAL RESEARCH LABORATORY

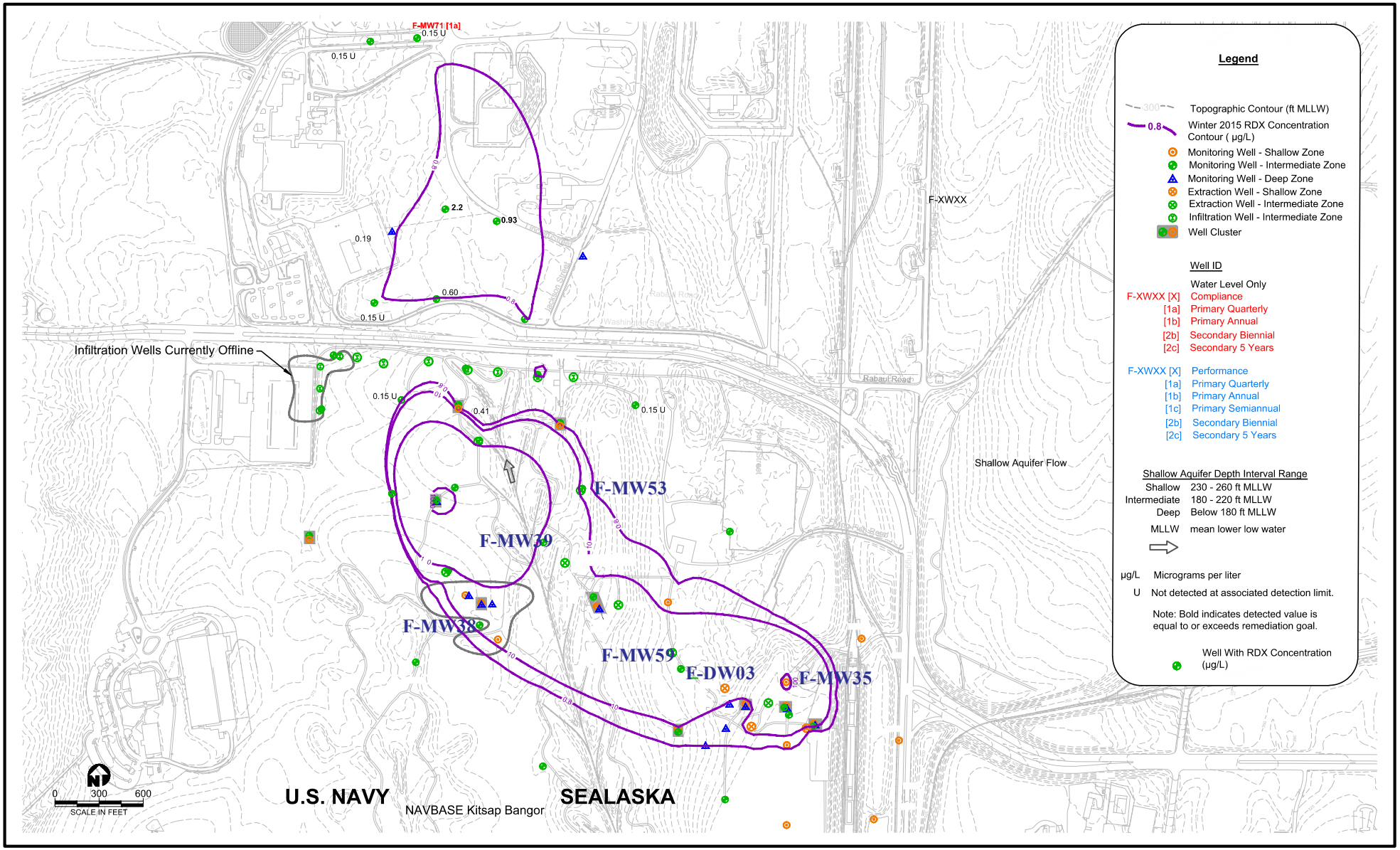
UCONN

NAVFAC Naval Facilities Engineering Command

NESDI

US Army Corps of Engineers

Naval Base Kitsap, Bangor Site F, Silverdale WA



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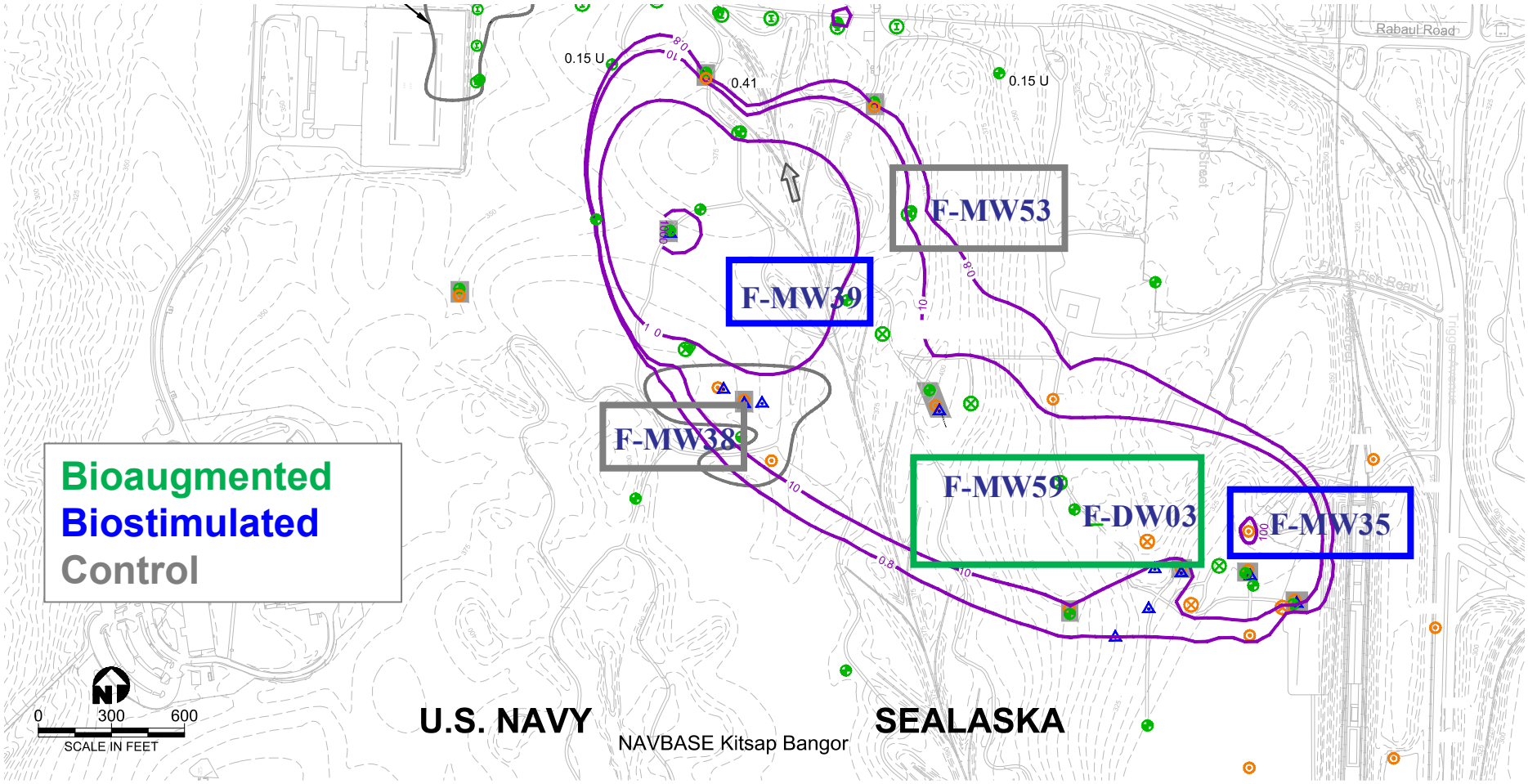
Site F, Bangor-Kitsap, WA, Treatment Conditions Tested



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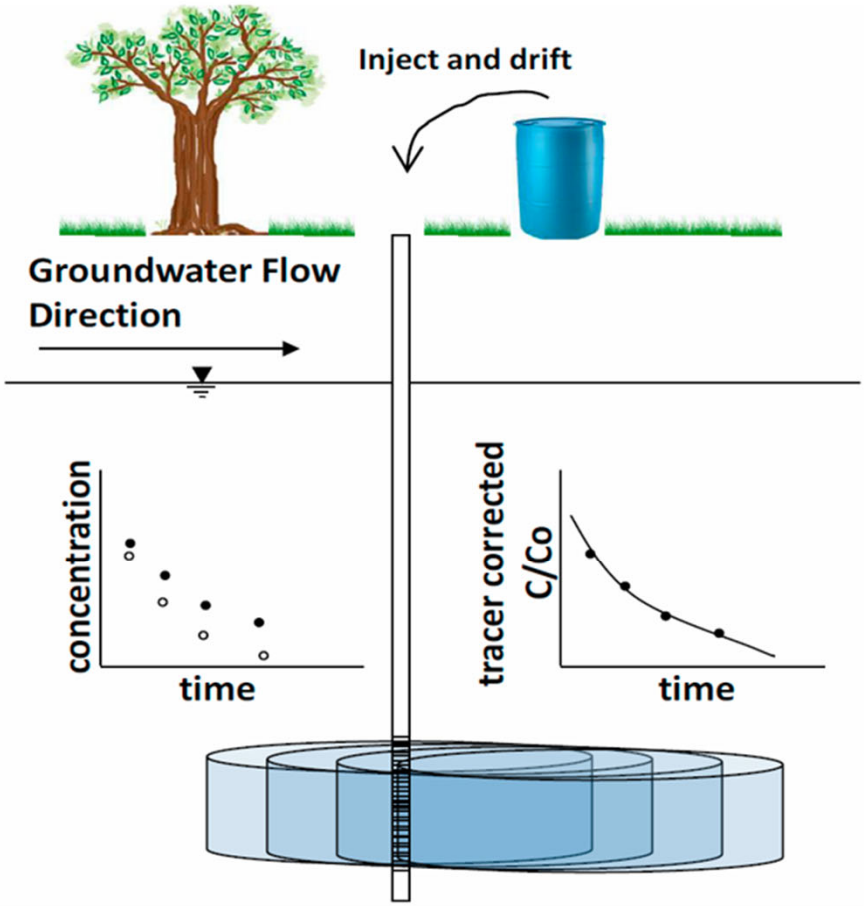
Push-Pull Tests April-May 2018

- *In situ* push-pull tests (PPTs) involve injecting a mixture of conservative (e.g. Br-) and reactive (e.g. RDX) tracers to quantify contaminant fate and transport characteristics and degradation rates

- Testing Sequence
  1) Borehole dilution tests
  2) Retardation factor tests
  3) Pre-biostimulation push-pull tests
  4) Carbon substrate "feedings"
  5) Post-biostimulation push-pull tests

***In situ* Push-Pull Test Schematic**



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Chloride concentrations were determined by ion chromatography according to EPA Method 300.0
RDX nitroso-RDX derivatives were determined by high performance liquid chromatography (HPLC) according to EPA Method 8330B.

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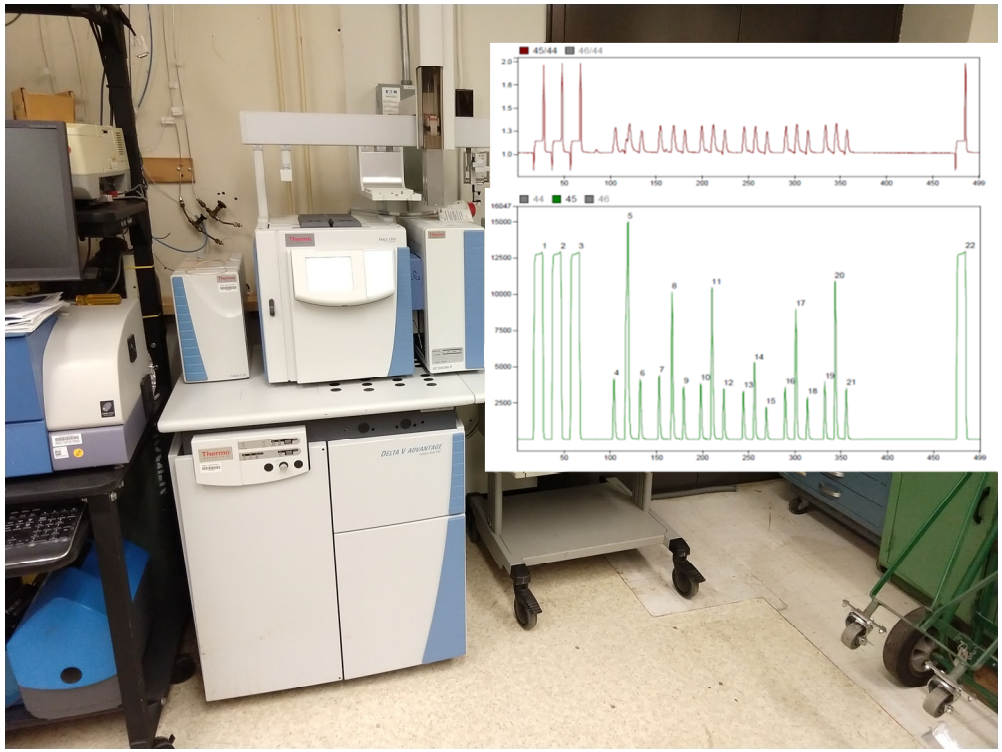
Carbon chemical analyses

- CH_4 concentration by GC-FID using packed PLOT column (Pohlman et al., Org. Geochem. 36, 703, 2005).

- CO_2 concentration by coulometry (Johnson et al., Mar.Chem 44:167, 1993)



- $\delta^{13}CH_4$ and $\delta^{13}CO_2$ by IRMS. GC-IRMS with PLOT column at NRL, gas bench used at UCD SIF. Scale anchors ranged from -66.5 to ~+2.4 ‰. (Zhong-Ping et al., Chinese Journal of Analytical Chemistry 35, 1455, 2007; Paul et al., Rapid Commun. Mass Spectrom. 21, 3006 2007)

- Isotope values converted to mole fraction in ^{13}C pool; then push value was subtracted to calculate "excess" ^{13}C in CO_2 and CH_4
- These values were converted to moles using concentration data
- Published fractionation factors were used for (minor) corrections, (Fuller et al., Appl. Environ. Microbiol. 82, 3297 2016; Zhang et al., Scientific Reports 6, 27065, 2016).
- Then – converted to µmoles RDX equivalent using 3 Cs per 1 RDX and dilution corrected (PPT)
- Growth efficiency was determined using short-term groundwater ^{14}C-RDX incubations (4 days, PPT concentration) and radioassaying cells and CO_2. Efficiency was determined as the percent incorporated into cellular biomass divided by both respiration and incorporation (Montgomery et al., Environ. Pollut. 174, 257, 2013)

$$\text{Mole fraction} = \frac{\frac{\delta^{13}C}{(1000+1)} X 0.0111796}{1+\frac{\delta^{13}C}{(1000+1)} X 0.0111796}$$

- The sum of mineralization products (CO_2, CH_4) was multiplied by the growth efficiency and summed to estimate complete RDX degradation

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Nitrogen chemical analyses

- Isotope values converted to mole fraction in ^{15}N pool; then push value was subtracted to calculate "excess" ^{15}N in NO_3^-, NO_2^-, NH_4^+, N_2O, N_2. These values were converted to moles using concentration data
- Then – converted to µmoles RDX equivalent using 3 NOs per 1 RDX and dilution corrected (PPT)
- Growth efficiency was determined using short-term groundwater ^{14}C-RDX incubations (4 days, PPT concentration) and radioassaying cells and CO_2. Efficiency was determined as the percent incorporated into cellular biomass divided by both respiration and incorporation (Montgomery et al., Environ. Pollut. 174, 257, 2013)
- The sum of mineralization products (NO_3^-, NO_2^-, NH_4^+, N_2O, N_2) was multiplied by the growth efficiency and summed to estimate complete RDX degradation

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RDX degradation kinetics varied within and between treatments

Treatment & Well		k, yr^{-1}
Bioaugmentation	F-DW03	66.0 ± 12.0
	F-MW59	3.2 ± 0.7
Biostimulation	F-MW35	57.0 ± 32.0
	F-MW39	78.0 ± 13.0
Control	F-MW53	7.6 ± 0.4
	F-MW38	0.4 ± 0.1

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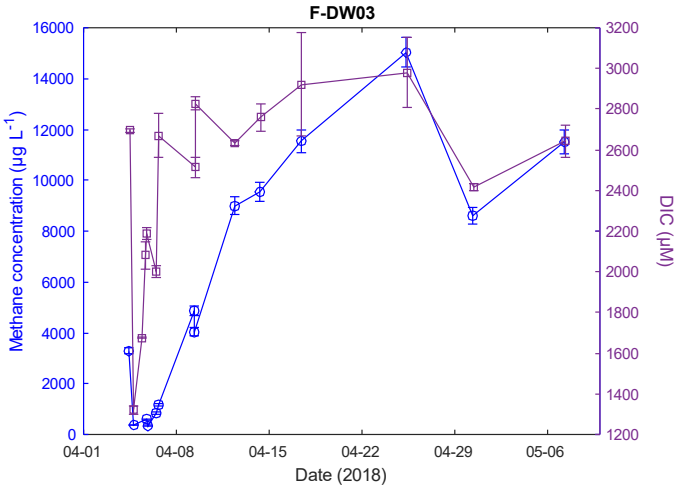
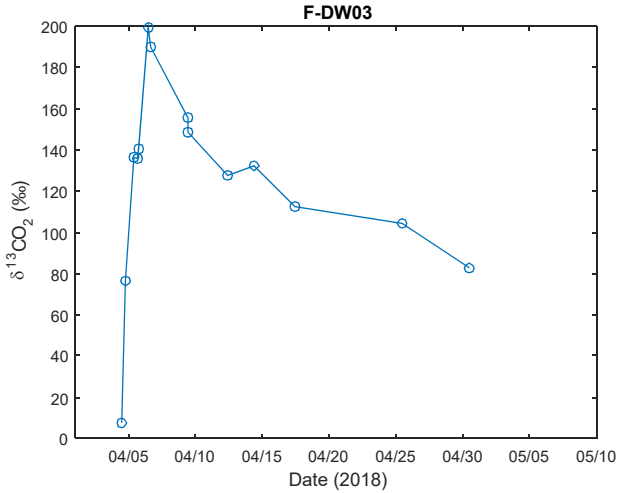
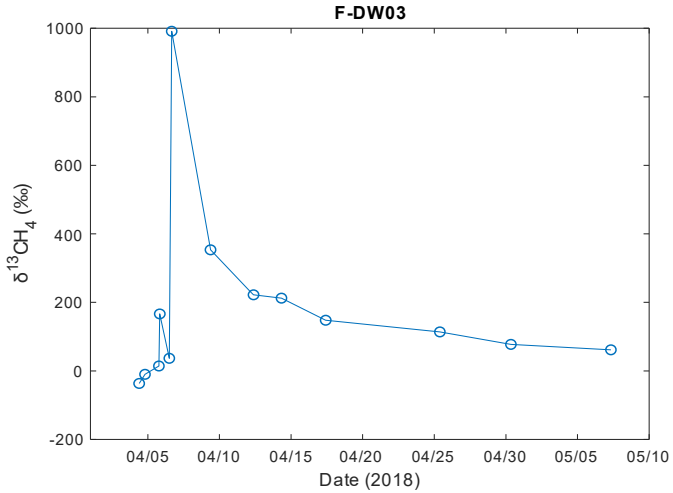
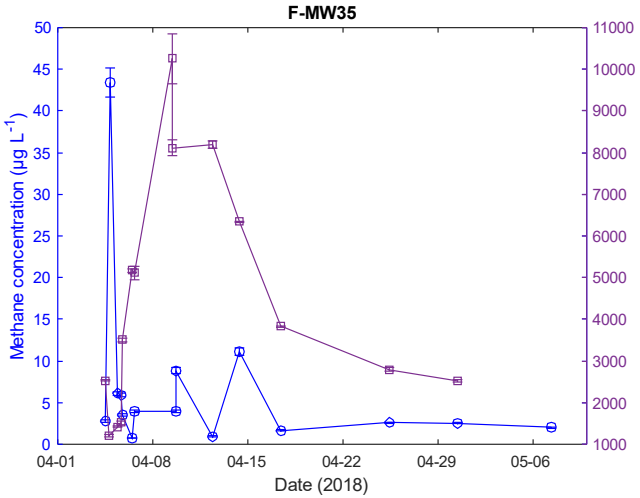
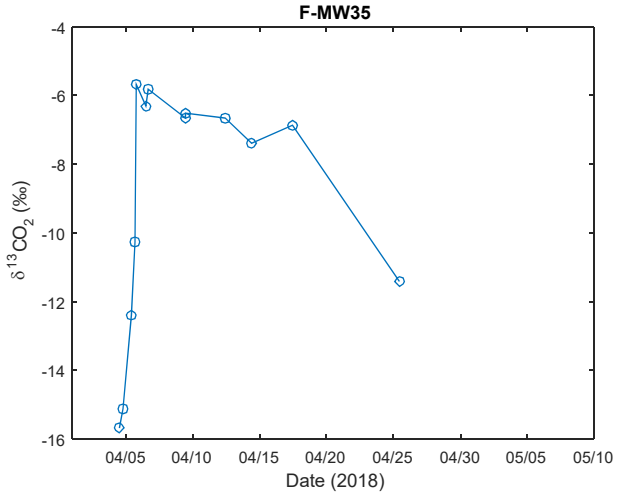
Carbon concentrations and isotopes

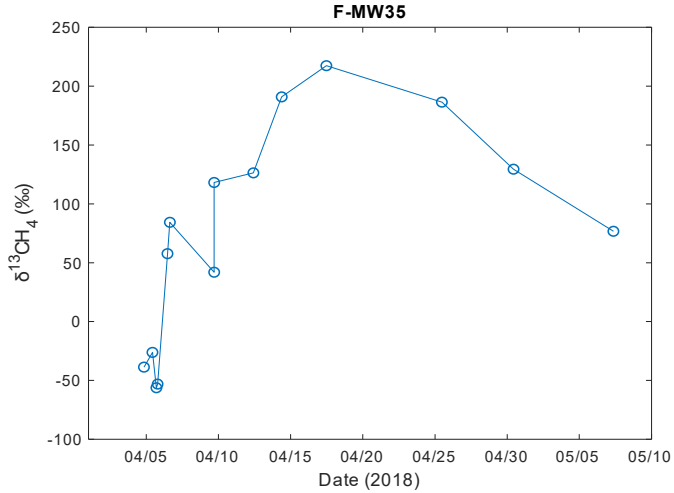




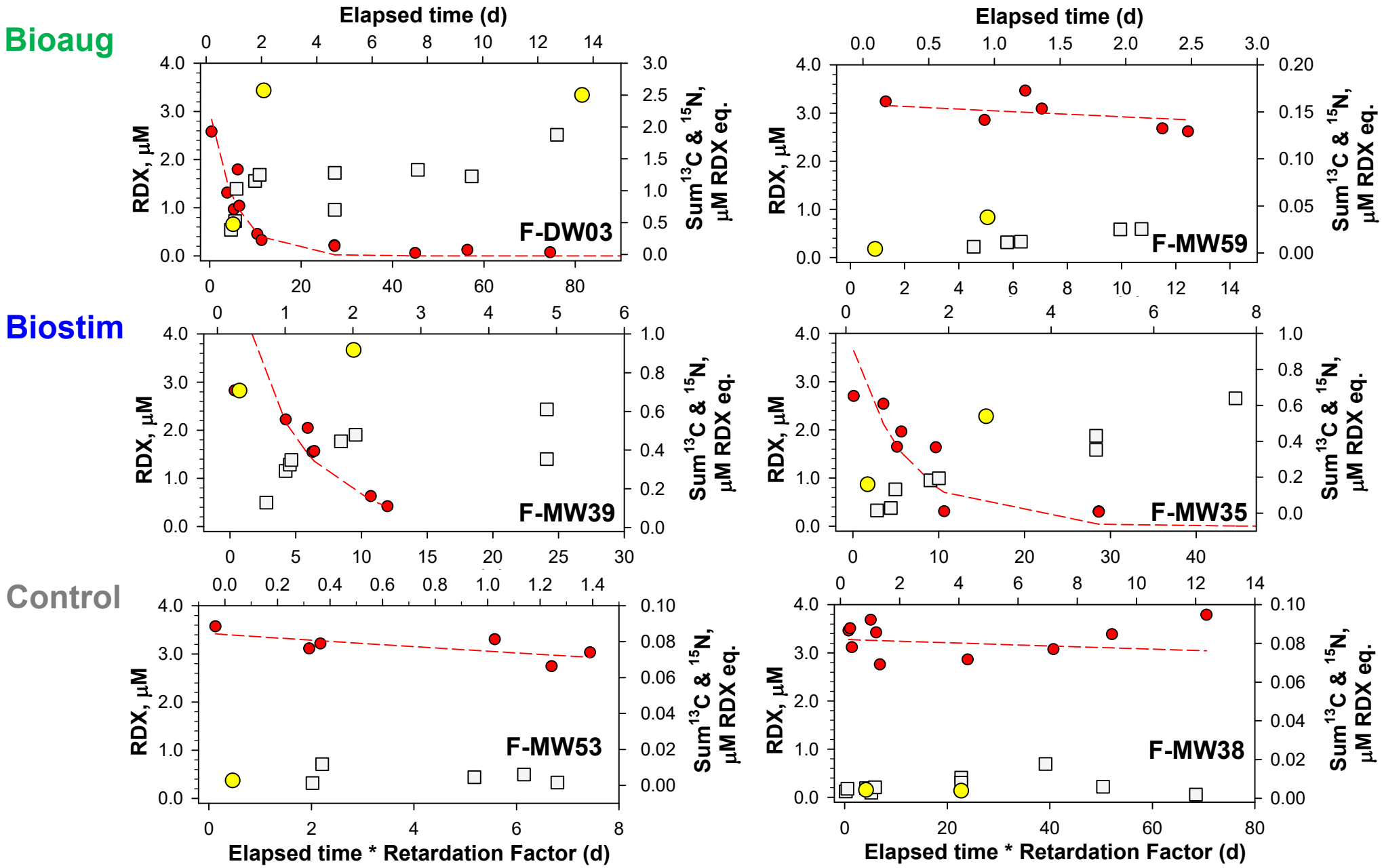






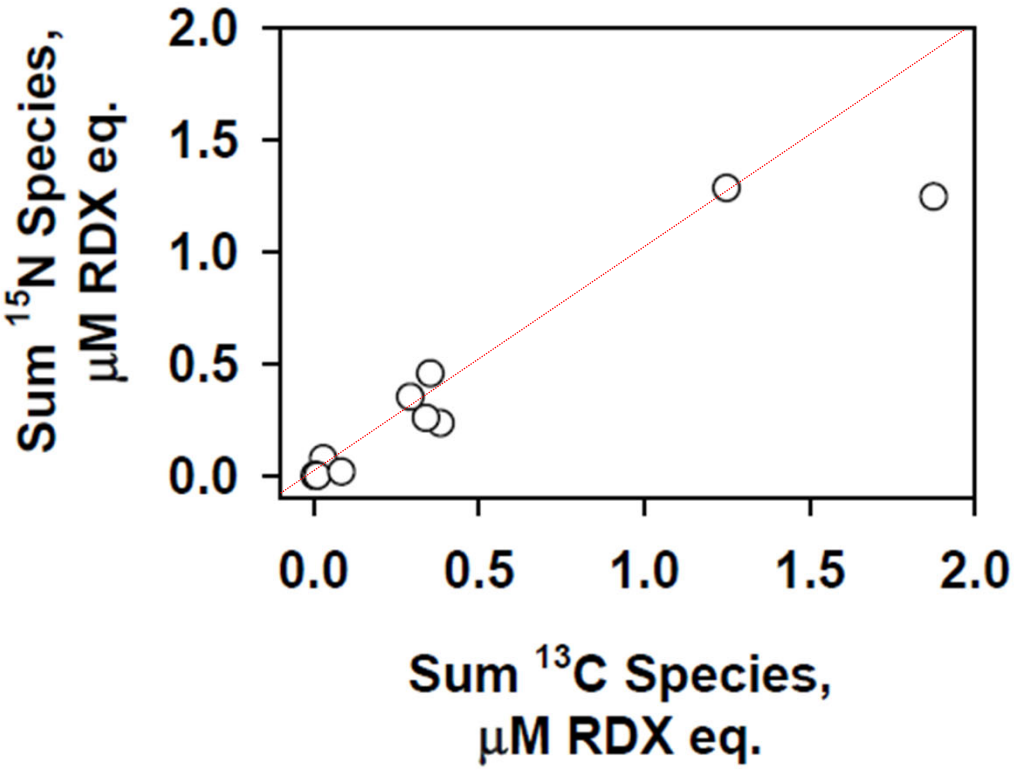


Push-Pull Test Results in Bioaugmented, Biostimulated and Control Wells



Observed and fitted RDX concentrations (red circles and dashed lines, respectively) vs. retarded time during the PPTs (lower x-axis); sum total of isotopically-labeled species measured during the test in RDX equivalent µM units ($^{13}CO_2$ and $^{13}CH_4$, open squares; $^{15}N_2O$, $^{15}NO_3$, $^{15}N_2$, and $^{15}NH_4$, yellow circles) vs. elapsed time during the test (upper x-axis).

Slope of excess ^{15}N vs. excess ^{13}C suggest tracers provided consistent information regarding release of labeled compounds during all tests.



Sum of the ^{15}N-labeled species measured during the test ($^{15}N_2O$, $^{15}NO_3$, $^{15}N_2$, and $^{15}NH_4$) vs. sum of the ^{13}C-labled species ($^{13}CO_2$ and $^{13}CH_4$)) both in RDX equivalent µM units

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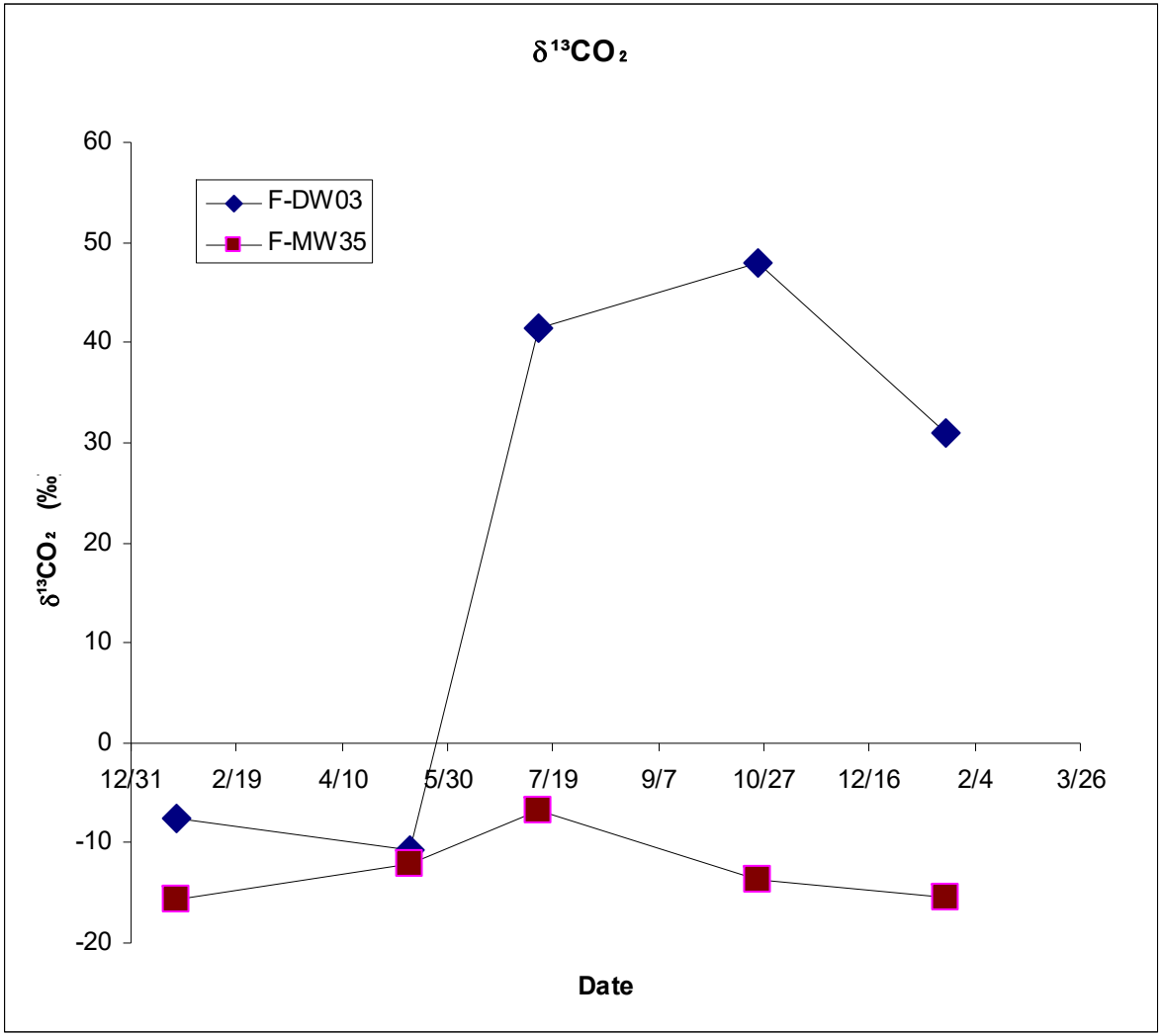
Sustained $^{13}CO_2$ in previously bioaugmented well compared to biostimulated well – cell turnover?



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Take Home Messages

- Study provided direct evidence of complete RDX mineralization under bioaugmented and biostimulated conditions in an RDX-contaminated aquifer
- Data analysis continues – manuscript in progress

Acknowledgements

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