Naval Research Laboratory
Washington, DC 20375-5320

NRL/6180/MR--2021/1

Stable Carbon Isotopes for Tracing in Situ RDX Remediation

THOMAS J BOYD

Naval Technology Center for Safety and Survivability Branch
Chemistry Division

RICHARD H. CUENCA
YUTAKA HAGIMOTO

HEI Inc.
Wooster, OH

MANDY M. MICHALSEN

USACE
Washington, DC

CRAIG TOBIAS

University of Connecticut
Storrs, CT

JOVAN POPOVIC

EXWC
Port Hueneme, CA

February 2, 2021

NAVFAC
Naval Facilities Engineering Command

Navy Environmental Sustainability Development to Integration Program

STABLE CARBON ISOTOPES FOR TRACING *IN SITU* RDX REMEDIATION

NESDI project number 537

PI: Thomas J Boyd, US NRL
Co-PIs: Richard H. Cuenca & Yutaka Hagimoto, HEI Inc

In collaboration with:

Mandy M. Michalsen, USACE
Craig Tobias, University of Connecticut
Jovan Popovic, EXWC

NESDI PROJECT FINAL REPORT

This page intentionally left blank.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
02-02-2021

2. REPORT TYPE
NRL Memorandum Report

3. DATES COVERED *(From - To)*
10/01/2017-09/30/2020

4. TITLE AND SUBTITLE
Stable Carbon Isotopes for Tracing in Situ RDX Remediation

5a. CONTRACT NUMBER
N0002519WR06115

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Thomas J Boyd, Richard H. Cuenca*, Yutaka Hagimoto*, Mandy M. Michalsen**,
Craig Tobias***, and Jovan Popovic+

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
5557

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Naval Research Laboratory
4555 Overlook Avenue, SW
Washington, DC 20375-5320

8. PERFORMING ORGANIZATION REPORT NUMBER

NRL/6180/MR--2021/1

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

NESDI
NFEC, Ste 1000
1322 Patterson Ave., SE
Washington, DC 20374-5065

10. SPONSOR / MONITOR'S ACRONYM(S)
NESDI

11. SPONSOR / MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
*HEI Inc., 443 W Liberty St, Wooster, OH 44691; **USACE, 441 G Street N.W., Washington, DC 20314-1000; ***University of Connecticut, Storrs, Connecticut 06269; +EXWC, 1100 23rd Ave, Port Hueneme, CA 93043

14. ABSTRACT

This report summarizes NESDI Project 537 activities to track in situ RDX biodegradation by adding small quantities of ^{13}C-labeled RDX to the environment using groundwater push-pull tests and analyzing groundwater respiration products (CO_2 and CH_4) over time. ^{13}C-enrichment in CO_2 and CH_4 was observed at two RDX-contaminated field sites at the Naval Base Kitsap-Bangor in Washington. The ^{13}C-enrichment was modeled and rate constants for RDX degradation were calculated. The technique was validated relative to RDX extinction models previously approved by federal and state regulators. A cost analysis for this technology relative to "standard" methods is included.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					Thomas Boyd
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified Unlimited	56	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified Unlimited	Unclassified Unlimited	Unclassified Unlimited			(202) 404-6424

This page intentionally left blank.

TABLE OF CONTENTS

FIGURES

TABLES

EXECUTIVE SUMMARY

Described is a validation demonstration for using ^{13}C-labeled substrates to determine *in situ* contaminant biodegradation rates. ^{13}C-labeled RDX was added instead of unlabeled RDX in groundwater well push-pull tests (PPTs) for evaluation. Two sites at the Naval Base Kitsap-Bangor were used for the demonstration. At each site, 6 PPTs were chosen based on previous bio-augmentation (with known RDX-degrading bacteria) and bio-stimulation (fructose addition). PPTs were conducted with ~500 gallons of natural groundwater containing a conservative tracer (Cl^- or Br^-) to calculate dilution, ^{13}C-RDX, and biostimulant (fructose) at selected wells. Post-push, subsamples were taken over ~1 month and analyzed for RDX, RDX biodegrdation daughter products (MNX, DNX, TNX), conservative tracers, and relevant C pools (CO_2, CH_4, $\delta^{13}CO_2$, $\delta^{13}CH_4$). Respiration (CO_2 and CH_4) and isotopic enrichment (calculated from $\delta^{13}CO_2$ and $\delta^{13}CH_4$) along with laboratory estimates of RDX growth efficiency were used to calculate *in situ* RDX degradation rate. Calculated rates were similar (but lower) than rates calculated by traditional PPT RDX extinction models. Differences were less than one order of magnitude and followed the general trend. Biomass recycling may be one reason as at several wells, ^{13}C enrichment in the CO_2 pool was observed for many months after the PPT conclusion. As demonstrated (using regulator-accepted PPTs), the technology offers some potential cost savings. $^{13}CO_2$ and $^{13}CH_4$ analyses are usually less than 10% of the cost for RDX and daughter products. ROI for the technology is ~5% for the Navy alone, but ~20% DoD-wide given modest adoption. Smaller scale deployments using less materials and long-term CO_2 in-well trapping offer very inexpensive options for using stable isotope technologies to definitively confirm biological contaminant degradation and estimate *in situ* degradation rates

ACKNOWLEDGEMENTS

This demonstration was made possible through the B-K RPM Malcolm Gander who provided matching funds, logistical support, and intellectual prowess. Collaboration with NESDI Project 544 (Segura/Popovic-EXWC, Tobias-UConn) was critical to successful field work, coordination, dual-isotope-labeling, and data interpretation. Shared field efforts with this also increased value to the Navy and DoD. Dr. Steve Fallis, Naval Air Warfare Center Weapons Division (NAWCWD) - China Lake, CA synthesized the ^{13}C-labeled RDX for the project. Dr. Mandy Michalsen (USACE) held the permitting for PPTs and was instrumental in performing this testbed component of the project. The field and scientific teams from Sealaska Inc (Poulsbo, WA) were instrumental in obtaining field samplings and site logistics. Federal and State regulators were helpful in refining the sampling and analyses plans. Finally, support from the NESDI Program was the prime financial source for this effort and made the demonstration possible.

This page intentionally left blank.

1. INTRODUCTION

1.1 BACKGROUND

Munitions explosives contamination plagues DoD facilities, costing considerable resources in time and money for assessment, cleanup, monitoring and site closure. Acceptable limits are low for these contaminants and their daughter products, making accurate degradation rate measurements critical for environmental site managers. If provided realistic degradation rates over proper spatial and temporal scales, site managers could confidently implement remediation strategies such as monitored natural attenuation (MNA) that would result in significant savings for the DoD. Most assessment methods for rate estimation are derived from indirect measures, therefore requiring an expensive multi-line, evidence approach with limited forecast capability. Methods that can discern the actual contaminant remediation, targeting the actual carbon backbone, for instance, would provide far more accurate and scientifically-defensible attenuation measures. As degradation rates may be low, it is critically important to regulators that the degradation pathway for explosive contaminants be tracked and validated in order to determine the effectiveness of a particular remediation method. More particularly, this means monitoring both the mineralization and incorporation of munitions by biomass at a contaminated site, as opposed to simply monitoring the loss of the parent molecule within a given plume.

1.2 REGULATORY DRIVERS

EPA has established groundwater concentration levels for RDX at 2 µg L^{-1} (lifetime). In soils, 6.1 mg kg^{-1} has been set as the screening level (*3*). For the purposes of this grant, an EPA National Priorities List (NPL) site was chosen - NAVBASE Kitsap, Bangor. Two sites (Site F and Site A) were chosen as test sites. Site F housed a wastewater lagoon servicing ordnance demilitarization. The lagoon was occasionally drained and residuals "burned off" or hauled to Site A (the other site) for incineration. During high flows, the lagoon could overflow spilling contaminated water into adjacent regions via drainage ditches. The resultant contamination leaves a groundwater plume exceeding 0.8 µg L^{-1}, the state regulatory level for RDX, extending ~700 X 250 meters. As stated, Site A also contained an ordnance demilitarization disposal lagoon where munitions burns were conducted in the 60's and 70's. Upon investigation, a groundwater plume exceeding state RDX compliance levels (0.8 µg L^{-1}) was found. The groundwater plume extends less than ~100 X 100 m and concentrations peak at as much as 140 µg L^{-1}. At both sites, treatment plants have been installed to remove RDX (and TNT) by sorption through a series of extraction wells (returning processed groundwater down-gradient). Costs for treatment plants approach $1M per year with very low recoveries. Innovative remediation solutions such as

---

Manuscript approved January 25, 2021.

bioaugmentation (adding known RDX-degrading microbial strains or consortia) or biostimulation (adding readily-degraded carbon to stimulate RDX degradation) are of interest because pump and treat has proven minimally effective over the long term (20+ years with concentrations still well above action levels). A consistent question posed by federal (EPA) and state (WA Ecology) regulators is what impact do innovative technologies have on the in situ RDX degradation rate. The regulators and RPM have agreed that definitive evidence (*e.g.* conversion of RDX to mineralization products) is needed to confirm remediation efficacy. Isotopes provide a definitive means to track contaminants to ultimate fate (respiration to harmless end products).

1.3 OBJECTIVE OF THE PROJECT

This project's objective was to validate using stable isotope-labeled contaminant tracers to track on-site degradation accurately and succinctly under challenging *in situ* conditions. ^{13}C-labeled RDX was used to assess degradation because one can straightforwardly track the label into various degradation pools (respiration products – CO_2 and CH_4) and optimally into cellular material (proteins) under both aerobic and anaerobic site conditions. Added RDX contains ~100K enrichment in ^{13}C relative to background RDX which allows accurate measurement of the label into these degradation pools. Measuring ^{13}C enrichment over space and time after injection (by subsampling a groundwater well network multiple times over the course of the project) and incorporating these data into a site-specific groundwater model allows an accurate estimate for site biodegradation. These estimates are more robust than using multiple indirect lines of evidence as they contain actual *in situ* RDX turnover information and provide RPMs and regulators a realistic picture of degradation kinetics. Removing considerable uncertainty from site conceptual models will allow the Navy and DoD to more carefully and reasonably implement cleanup and monitoring goals.

Because stable carbon isotope labeling is non-radioactive, obtaining permits for chemical release will be far less complicated and is currently permitted at the proposed site(s). Modern natural abundance isotope ratio mass spectrometers (IRMS) are designed to work at per mil (1 in 1,000) resolution, and, therefore, are sensitive enough to detect shifts in the RDX stable isotope ratios with relative ease. As RDX is moderately soluble in groundwater, the label will spread out over a defined region in a relatively short period of time. This approach allows push-pull or downgradient monitoring for the RDX signal in various degradation pools (respiration, $^{13}CO_2$ evolution, and incorporation into biomass) to accurately assess engineered and subsequent MNA.

The benefit of tracking the contaminant carbon backbone in impacted environments has been well documented in the literature in our laboratory with natural abundance ^{14}C (*4-9*). Compound-specific ^{13}C labeling would offer similar benefits because the tracer could be added to the existing contaminant pool (much like depleted ^{14}C defines industrial chemical contaminants made from petroleum feedstocks), in addition to allowing for very specific tracking of ^{13}C that can be linked directly to the injection point for ^{13}C-labeled RDX. CO_2 (the final respiration product from RDX degradation) is a very small component of ambient air (~0.04%) and if converted to CH_4 under anaerobic conditions, the ^{13}C label will be even more easily detected (CH_4 is a trace gas in ambient air). The carbon in RDX can also be incorporated into cellular biomass by growing bacteria. This process is relatively easy to assess by precipitating microbial proteins which can then be assayed by combustion-isotope ratio mass spectrometry. Using these tools, we can determine how much RDX is respired (CO_2), reprocessed (CH_4) and incorporated (protein), the sum of which can be used to determine the total degradation – under either aerobic or anaerobic conditions.

2. TECHNOLOGY DESCRIPTION

2.1 TECHNOLOGY OVERVIEW

This project relies on tracking contaminant fate and transport (into final degradation products or bacterial biomass) by stable isotope analysis. Carbon-based contaminants, like RDX, can be synthesized to contain ^{13}C within their carbon backbone. The abundance of ^{13}C naturally is ~1% in nature, so a labeled substrate is easily measured by instrumentation designed to measure natural abundance (thousands of time lower). ^{13}C-labeled RDX can thus be added at a very small amount to a field site and it's fate tracked over time or distance from the injection point. The main interest in this project is to assay RDX degradation products (CO_2, CH_4, and microbial biomass) either in association with a push-pull test or a point source release, with subsequent downgradient sampling. The prime advantage of this methodology relative to other measures is that it directly targets the carbon backbone in the contaminant itself. The measurement is direct - rather than a series of indirect lines of evidence measurement scheme. Guidance documents (*c.f. Natural Attenuation of Fuel Hydrocarbons Performance and Cost Results from Multiple Air Force Demonstration Sites* and *Natural Attenuation of Chlorinated Solvents Performance and Cost Results from Multiple Air Force Demonstration Sites*) offer ~70 different measurements which can be used as lines of evidence. However, none are capable of determining the contaminant fate by actually tracking its conversion to harmless end-products. This has a two-fold advantage. First, detecting isotopically-enriched degradation products confirms degradation is occurring on-site. Second, measuring these products over space and time allows a mass balance model to be created thus providing actual turnover rates which can be scaled to spatial or temporal attenuation estimates as suits the RPM.

Figure 1. Schematic representation with ^{13}C-RDX injected and labeled degradation products collected downgradient

The technology is applicable wherever RDX (or other carbon-based contamination) exists: ranges, weapons depots, etc. The method will allow RPMs to provide regulators with a clear picture of site-specific contaminant fate and transport to support decision making.

2.2 TECHNOLOGY DEVELOPMENT

Stable isotope techniques have been used for years to track elemental cycling in biological and geochemical systems (*c.f.* (*10, 11*). Isotope ratio mass spectrometry (IRMS) has advanced over the years to allow measuring very small variations in natural isotopes – usually around 1 in 1000 levels for ^{13}C (or ±1 ‰). However, because natural isotopic ratios are within a relatively narrow range band, it is difficult to reliably track contaminant carbon within ecosystems. For instance, RDX from multiple manufacturers was shown to have a carbon isotope range spanning 33 ‰ (*12*). The reported range (-20 to -40 $‰_{VPDB}$[1]) is roughly the same range in natural organic matter isotopic ratios, so trying to tease out a respiration product (*e.g.* CO_2) from RDX relative to natural background organic matter is highly problematic. By the same token, because ^{13}C is naturally found in only trace amounts (~1% of carbon on Earth), using ^{13}C-labeled substrates allows increasing analytical resolution for the labeled material ~100,000-fold. Tracking the label and its products in natural environments thus becomes analytically feasible.

Using isotopically-labeled substrates allows adding a contaminant of concern (CoC) to the environment at a concentration at or below that found on-site. This enables the analyst to minimally "spike" the system (*e.g.* adding contaminant far in excess of existing concentrations) so that the current degradation rate can be measured. Additionally, because the labeled substrate is so highly enriched in ^{13}C relative to ^{12}C, any ^{13}C found in mineralization products (CO_2, CH_4, biomass) can be directly ascribed to the added substrate. Measuring ^{13}C enrichment and product mass, one can calculate the original substrate degraded. Measuring these properties over time and spatial scales allows integrating contaminant degradation estimates over a particular site.

Delivering isotopically-labeled test substrates to the subsurface and determining their fate may be problematic. At "mature" sites with existing monitoring well networks, a rigorous means to track fate and transport is a push-pull test (PPT). While various field experiments using variations of this technique are found in the literature, Istok et al (1997) refined the technique for tracking contaminant utilization by co-injecting conservative tracers and biologically-active components along with physical modeling and breakthrough analysis (*13*). This development allows a researcher to inject a substrate of interest and track its degradation using existing wells. The substrates are "pushed" into the well by pumping well-mixed solutions to the screened interval. During the "pull" phase, subsamples are taken from the monitoring well and assayed for analytes of interest (including potential

---

[1] Vienna Pee Dee Belemnite. This is a standard by which isotope values are typically presented against (it is set at 0).

degradation products). Using these data, breakthrough curves for the conservative tracer, reactant materials and any degradation products can be calculated. A mass balance for these species can then be determined. By adding isotopically-labeled substrates, contaminant loss can be measured by assessing enrichment during pull-phase sampling.

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The main advantage of this demonstration is the ability to inexplicably tie any isotopically-enriched mineralization or degradation product to *in situ* contaminant degradation. No other current method, aside from limited proteomics use (which has only had minimal field validation) and perhaps identifying transient biological intermediates, can directly tie the contaminant carbon (or nitrogen) backbone to degraded product (CO_2, CH_4, N_x, biomass). Currently, a multiple lines of evidence approach (~70 available) is used to "infer" contaminant degradation in the field (*14-16*). While this increases confidence, in many instances, regulators and stakeholders require more definitive evidence. This is the case for the test sites used during this demonstration. As mentioned, transient increases in metabolic intermediates may be detected when contaminants are degraded *in situ*, however, these are, as stated, transient. Concentrations may be low, or below the limits of detection and if not sampled intensely, their transient generation and utilization may be missed analytically. An advantage to using isotopically-labeled substrates is the accumulation of final degradation products (CO_2, CH_4, N_x, biomass). These components are not transient and their concentration(s) accumulate in proportion to the starting contaminant concentration and degradation rate(s).

Because labeled substrates have analytical abundance(s) 100-400 thousand times higher than natural (unlabeled) substrates, they can be added at levels approaching background (depending on the site-specific contamination levels). In this manner, one may be able to assess the *in situ* degradation rate without artificially "spiking" the environment. For instance, if a given site had 100 $\mu g\ L^{-1}$ RDX, adding 1000 $\mu g\ L^{-1}$ RDX might stimulate the natural microbial community to degrade RDX at a higher rate than was actually occurring (with 1.0 $\mu g\ L^{-1}$ ambient RDX). Both ^{13}C- and ^{15}N-labeled RDX can be added at less than 100 $\mu g\ L^{-1}$ and mineralization products can easily be detected using isotope ratio mass spectrometry. Because the contaminant backbone is labeled, isotopic enrichment (e.g. more ^{13}C or ^{15}N) in mineralization products or biomass collected from the site after infusion with labeled substrate is definitive evidence that the contaminant has been degraded *in situ*.

Of course, no method is free from disadvantages. Effective isotope-labeled release and products recover and analysis may be limited by several factors. On prime enabler is regulator and stakeholder buy-in and approval. Because small contaminant (labeled) aliquots need to be released into the environment, regulatory approval may be difficult. For the sites included in this study, labeled RDX was added at or below the ambient concentration (see discussion above), so regulatory approval was not an issue. Another potential disadvantage for this technology could be rapid groundwater flow (*e.g.* dilution). As RDX (the substrate for this demonstration) is relatively soluble, high-flow systems might be responsible for diluting the initial label – or the mineralization products produced during degradation to levels below the limits of detection. Labeled substrate(s) may be prohibitively expensive depending on the deployment scale. For this demonstration, push-pull tests were used requiring large volumes – and thus large amounts of labeled RDX (10 grams). Cost was up to $28K for dual ^{13}C-^{15}N-labeled RDX (requiring a custom synthesis). At more modest scales (e.g. a single groundwater well or small group of wells), commercially available single- (^{13}C) and dual- (^{13}C, ^{15}N) labeled RDX is modestly priced (~$550 for 1 mg; https://shop.isotope.com/advancedsearchresults.aspx?id=0&keyword2=RDX&searchType=ALL%20Keywords&SearchSpecificField=0&SearchContent=0). Lastly, commercial laboratories providing stable isotope analyses may be difficult to find having state certification(s) if required by regulators. Many academic and commercial laboratories can measure stable isotope ratios and costs are low (for instance, UC Davis charges $11 for dissolved $^{13}CO_2$ analysis). While no certified laboratories may be available within a site's jurisdiction, state regulators are mandated to asses EPA acceptance for "newer" methodologies and EPA has long advocated using stable isotope analyses for contaminant assessment (*17*).

3. PERFORMANCE OBJECTIVES

Because this work is not a hardware development project or device, performance metrics are more difficult to solidly identify. Within the overall scope, performance will be assessed for each project phase, keeping in mind the need to procure, permit, release and monitor the labeled substrate. Performance metrics were also established for the modeling component (meta-analysis to scale both spatially and temporally). One difficulty is defining "established criteria" as there are no current methods which can directly track the contaminant carbon through degradation pathways to CO_2, CH_4 and cell protein. In this respect, performance achieved will represent the "new" metric. Our performance goal is a realistic estimate for RDX degradation on-site tailored to the needs of the RPMs, regulators, and stakeholders.

Table 1. Performance Measurement Basis

Performance Objective	**Data Requirements**	**Success Criteria**
QUANTITATIVE		
Measure carbon isotope ratios in DIC	CO_2 evolved from samples spatially and / or temporally before and after labeled RDX injection	• Significant difference between background $\delta^{13}CO_2$ and post-injection $\delta^{13}CO_2$ of > 2 ‰
Measure carbon isotope ratios in CH_4	CH_4 evolved from samples spatially and / or temporally before and after labeled RDX injection	• Significant difference between background $\delta^{13}CH_4$ and post-injection $\delta^{13}CH_4$ of > 2 ‰
Measure carbon isotope ratios in bacterial protein	Bacterial proteins evolved from samples spatially and / or temporally before and after labeled RDX injection	• Significant difference between background $\delta^{13}C$-protein and post-injection $\delta^{13}C$-protein of > 2 ‰
Groundwater model to interpolate between wells (spatially)	Basic hydrogeological parameters (already collected) and verification (planned under FFS)	• Simulation allowing between well calculation(s) for degradation rates
Overall degradation rate model to satisfy sponsor, RPM and stakeholder objectives	Data collected during the project (including related effort under NESDI funding)	• Refined estimates for *in situ* RDX degradation
QUALITATIVE		
Ease of preparation	Regulator acceptance, permitting, labeled-substrate procurement, time to inject (from project start)	• Regulator sign-off, permits completed if needed, substrate procured at

		reasonable cost, injection done in a timely fashion
Ease of deployment	Time, cost, effort associated with introducing the labeled tracer	• Cost, time and materials for injection and / or push-pull experiment
Ease of analysis	Instrumental requirements, analytical difficulty, commercial laboratory capabilities, ancillary data	• Availability of substrate(s) and commercial analysis. FS, IR or other on-site data collection for hydrogeologic data.
Ease of transition	Feedback from sponsor(s), RPM(s), regulators, stakeholders, etc.	• Successful demonstration at study site(s) and transition to other site(s)

4. FACILITY/SITE DESCRIPTION

4.1 FACILITY/SITE LOCATION AND OPERATIONS

Demonstrations were carried out at two separate sites at Naval Base Kitsap-Bangor in Washington State. NAVBASE Kitsap-Bangor covers 7,201 acres on the Kitsap Peninsula in Kitsap County, Washington near Hood Canal, approximately 10 miles north of Bremerton (Fig. 2). The Kitsap County 2012 Comprehensive Land Use Plan lists land immediately surrounding NAVBASE Kitsap, Bangor as rural residence (one dwelling unit per five acres). Land immediately surrounding NAVBASE Kitsap, Bangor is generally undeveloped or supports limited residential uses. Naval activities began at NAVBASE Kitsap, Bangor in June 1944, when the U.S. Naval Magazine, NAVBASE Kitsap, Bangor was established. From 1944 to the early 1970s, the U.S. Department of the Navy (Navy) facility at NAVBASE Kitsap, Bangor was primarily used as a trans-shipment and storage point for ordnance. Ordnance arrived by train and was shipped to support U.S. military efforts. In February 1977, NAVBASE Kitsap, Bangor was commissioned as the west coast homeport for the Trident Submarine Launched Ballistic Missile System.

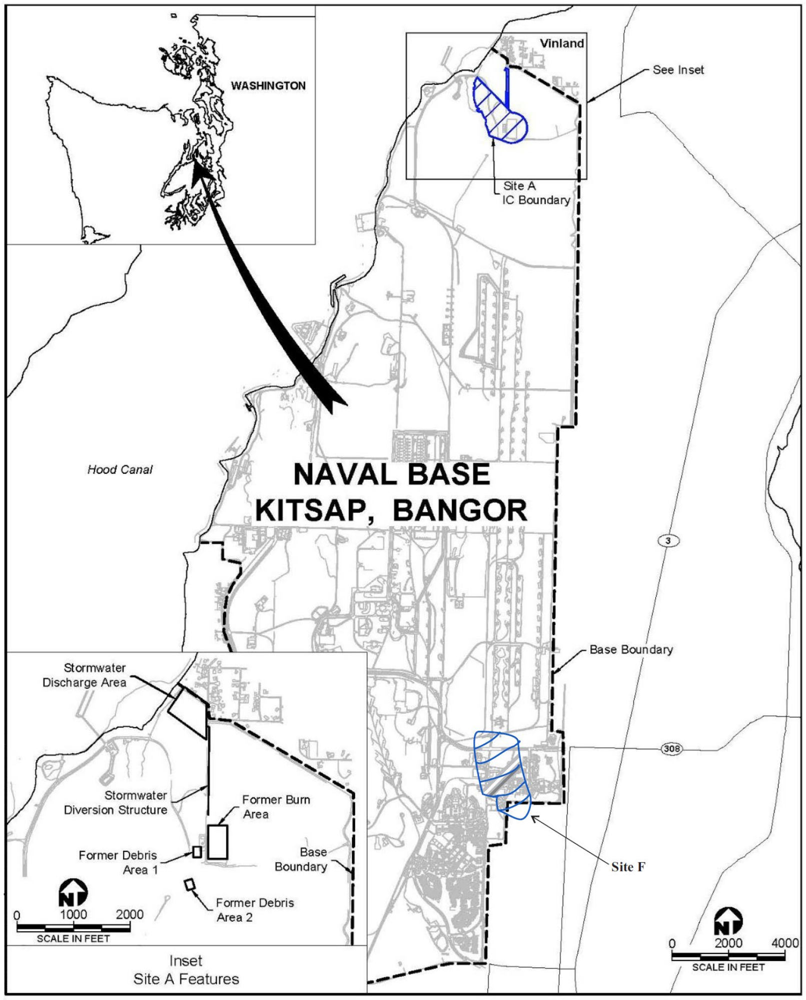


Figure 2. Naval Base Kitsap-Bangor

The first demonstration was conducted at Site F near the SE corner of the Base. Site F served as a wastewater lagoon and overflow ditch between approximately 1960 and 1970 for wastewater disposal. Site F wastewater was generated during ordnance demilitarization in an adjacent segregation facility building. Wastewater contained relatively high 2,4,6-trinitrotoluene (TNT) and RDX concentrations. Between approximately 1957 and 1978, the segregation facility's primary function was demilitarization of ordnance items using steam cleaning and/or steam melt-out procedures. Prior to 1972, wastewater from the demilitarization process was discharged into an unlined wastewater lagoon and infiltrated through the lagoon bottom. During periods of heavy discharge, wastewater overflowed the lagoon to a narrow ditch south of the lagoon. Periodically, the wastewater lagoon was allowed to drain, and waste materials at the surface of the lagoon were "burned off" in place or transported to Site A for burning and disposal. Beginning in about 1972, the lagoon was taken out of service, wastewater collected in barrels, and these barrels were disposed outside Site F's boundary. Multiple groundwater monitoring wells (64) have been installed as well as extraction (10) and infiltration (11) wells to support the site treatment system. Extraction, treatment and reinfiltration have been ongoing since 1994 with only modest energetics recovery. RDX remains above regulatory limits (0.8 µg L^{-1}) over much of the site (Fig. 3).

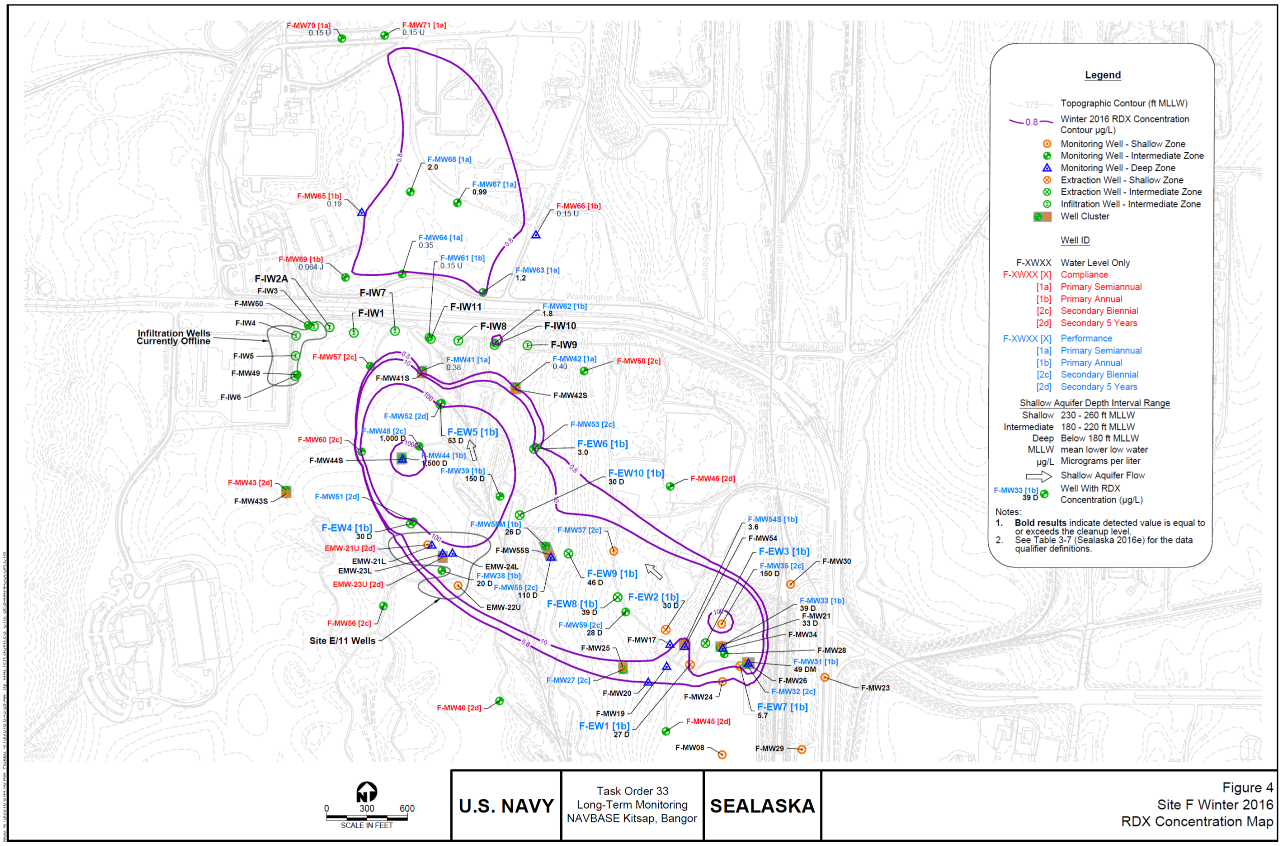


Figure 3. Site F well layout and RDX concentrations

Several remedial activities aside from the pump and treat facility have been implemented at Site F. Both biostimulation with fructose and bioaugmentation with KTR9 ande strain I-C (microbial consortia) were performed at select wells between 2015 and 2017 at Site F (*18, 19*). Wells previously biostimulated (F-MW35, F-MW39) and bioaugmented (F-DW03, F-MW59 – downgradient) along with control wells (F-MW38, F-MW53) were used to test the technology.

The second demonstration was conducted at Site A at the North end of the Base. Site A was used by the Navy as an explosives ordnance disposal facility. It operated from 1962 to 1975. In February 1972, 500 cubic feet of soil were excavated from the top several feet of the former lagoon at Site F and taken to Site A. Environmental investigations began at Site A in the mid-1970s. The Navy investigated the Site A ordnance disposal facility and bordering off-Base properties, which included portions of the community of Vinland to the north. Explosives contamination was found in soil and groundwater samples leading to installation of a groundwater Treatment Facility on the west side of Pintado Road in 1994. Leachate from a passive soil washing system was treated initially with extraction wells installed for present treatment. RDX is above compliance levels over the entire leach basin and in many monitoring wells down-gradient (Fig. 4). The treatment facility consists of two, 20,000-pound granular activated carbon (GAC) units that treat ordnance-contaminated groundwater recovered from the five extraction wells (A-EW4 through A EW8) and two other retrofitted monitoring wells (A-MW37 and A MW46). Three aquifers have been identified on-site with RDX contamination found primarily in the shallow aquifer (remains saturated during the year) and in a perched zone aquifer which may run

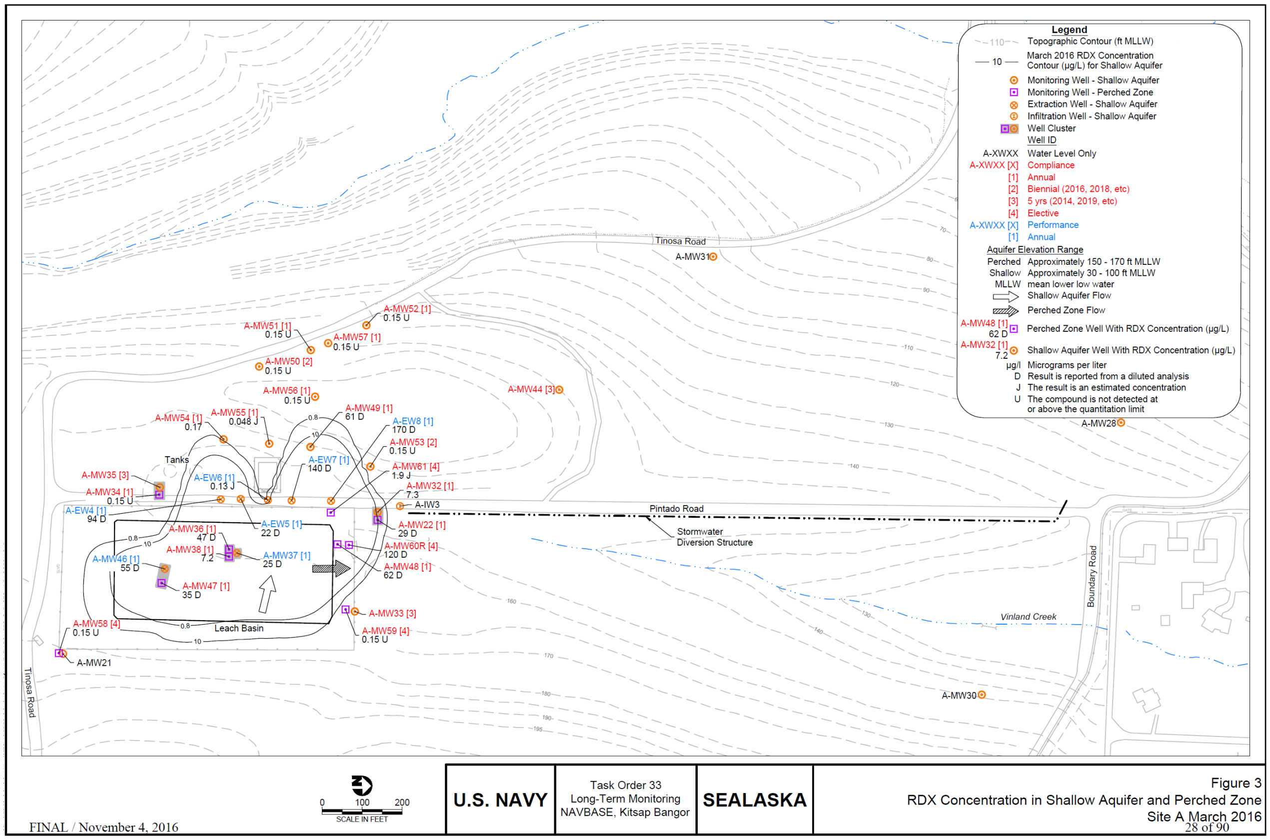


Figure 4. Site A well layout and RDX concentrations

dry seasonally. A deep aquifer is mostly devoid of RDX contamination. The aquifers are delineated by aquitards which restrict vertical groundwater flow. The P&T system extracts and treats water from the shallow aquifer.

As with Site F, Site A has had several remediation studies in attempts to biostimulate natural microbial communities to increase RDX degradation. Within the shallow aquifer, biostimulation was performed (fructose additions) at A-MW32 and A-MW62. Within the perched zone, A-MW22 and A-MW60R we also stimulated (fructose). Results from those initial studies are under review and publication. These wells were used for the demonstration along with two control wells (no previous stimulation), A-MW36 in the perched zone and A-MW37 in the shallow aquifer.

5. TEST DESIGN

5.1 CONCEPTUAL TEST DESIGN

Push pull tests (PPTs) and sampling events at each site went for around 1 month using six wells. Long-term in-well CO_2 traps were deployed 6 months before the PPTs at the test and adjacent wells to capture nature $\delta^{13}CO_2$ variation expected on-site. On site, 1,500 gallon tanks were filled to ~ 500 gal with pump and treat effluent from respective treatment plants (Site F for Site F PPTs and Site A for Site A PPTs). The tank was amended with ^{13}C-RDX (to ~600-700 µg L^{-1}) synthesized by Dr. Steve Fallis, Naval Air Warfare Center Weapons Division (NAWCWD) - China Lake, CA - and chloride (Site F) or bromide salt (Site A) to a ~100 mg L^{-1} final concentration and well mixed using recirculating pumps (Figs. 5-6). For biostimulated wells, fructose was added (different concentrations – see results) during the mixing. The amended well-mixed groundwater was then injected as rapidly as possible into PPT test wells. After injection, pull samples were taken over the course of ~5 weeks and divided into analytical subsamples: CO_2, microbial biomass[2], dissolved CH_4, and anion concentrations (Cl^{-1}, Br^{-1}) were measured by NRL. These fractions were also assayed at NRL for $\delta^{13}C$ ratios (or subcontract in the case of CH_4). Additionally, collaborators (Dr. Mandy Michalsen, USACE and Dr. Craig Tobias, UConn) performed additional measurements such as RDX concentration, daughter product concentrations (MNX, DNX, TNX), and $\delta^{15}N$ within mineralization products (N_2, N_2O, NH_4^+, NO_2^- and NO_3^-).

Figure 6. Adding ^{13}C-, ^{15}N-labeled RDX to PPT tank

Time-based data were used to calculate *in situ* dilution by groundwater. Cl^{-1} or Br^{-1} concentrations were used as conservative tracers and using retardation factors (*20*), RDX dilution was calculated. To determine labeled-RDX converted to mineralization products, stable isotope ratios were measured at t0 (before PPTs) and converted to mole fractions using standard conversions from delta notation reported by measurement software (*21*). Excess label was

Figure 5. Recirculation pump mixing PPT injectate

---

[2] Biomass was unable to be analyzed due to filtering difficulties. Biomass estimates are discussed in later sections.

then determined as the difference between the time-based measurements and the t_0 mole fraction for the heavy isotope (1):

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

where $\delta^{13}C$ is the value provided by instrument or commercial lab. 0.0111796 is the ^{13}C to ^{12}C ratio for Vienna Pee Dee Belemnite (VPDB - the de facto standard). Excess ^{13}C fraction is the mole fraction at t_x minus the mole fraction immediately after push. Excess fractions are multiplied by the ambient CO_2 or CH_4 to quantify the $^{13}CO_2$ and $^{13}CH_4$ in the groundwater sample(s). CO_2 and CH_4 is converted to RDX equivalents by multiplication (3 moles CO_2 or CH_4 per 1 mole RDX).

Conversion from excess label to RDX degradation equivalents was accomplished by multiplying the excess label and concentration for respective mineralization species, then converting to moles RDX using molecular formula (carbon or nitrogen number) and mass. For $^{13}CO_2$ calculations, known fractionation factors were used where appropriate: 1.0008 under aerobic conditions, and for F-DW03, which had extremely low dissolved oxygen levels, 1.0044 (*22*). For $^{13}CH_4$ calculations, an average literature fractionation factor of 1.05 was used (*23, 24*).

Filtering groundwater to obtain biomass for stable isotope analysis was not possible as filters clogged with <10 mL throughput. To account for RDX converted to biomass (in addition to CO_2, CH_4 and nitrogen mineralization products, a separate groundwater sampling was conducted after the Site F PPT (October 2018). Groundwater subsamples were shipped refrigerated back to the lab and incubations with ^{14}C-RDX (UL-^{14}C - 1.126 mCi mmol^{-1}) were conducted at similar RDX concentrations (4.0 µM) used during the PPTs in 10 mL test tubes with NaOH-soaked filter paper traps suspended in the headspace. A 5 mL groundwater sample was transferred to each tube pre-amended with ^{14}C-RDX. Groundwater from each well was represented with two additional incubations for F-DW03 and F-MW35 amended with 1 and 25 mM fructose (final concentration), respectively (to simulate PPT conditions). Triplicate samples and triplicate kills (1 mL 2 N H_2SO_4 added at t0) were created. After ~4 days incubation, live samples were killed with 1 mL 2 N H_2SO_4 and allowed to sit overnight for liberated CO_2 to be trapped on suspended filter papers (*25*). Filter papers were transferred to scintillation vials, amended with 5 mL scintillation cocktail, counted using a liquid scintillation counter and DPM converted to µM RDX mineralized d^{-1}. To determine ^{14}C-RDX incorporated into cellular biomass (proteins and

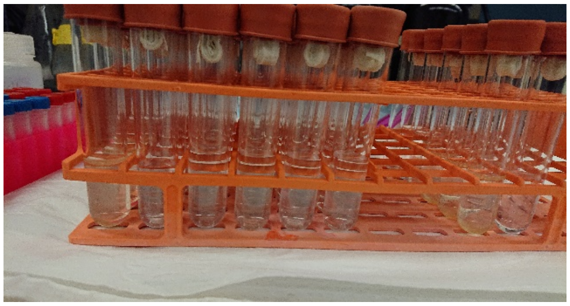


Figure 7. Short-term incubations with ^{14}C-RDX to determine growth efficiency

nucleic acids), 1 mL subsamples from the incubation tubes were transferred to 2 mL conical centrifuge tubes and processed similarly to microbial production samples (*26*). Briefly, each tube was amended with 50 µL 80% (w/v) trichloroacetic acid (TCA) and processed by centrifugation (10,000 X *g*, 10 minutes) to produce a cellular material pellet. The supernatant was aspirated and the pellets washed with 5% (w/v) TCA, re-centrifuged, aspirated, and washed with 80% (v/v) ethanol. After a final centrifugation, the supernatant was aspirated and each tube amended with 1 mL scintillation cocktail, counted and DPM converted to µM RDX incorporated d^{-1}. Growth efficiency (%) was calculated as the ^{14}C-RDX incorporated divided by the sum of the ^{14}C-RDX incorporated and mineralized X 100%.

All processed time-based data were compiled into master sheets for final modeling. The dilution factor (C/C_0 for conservative tracer Cl^{-1} or Br^{-1}) was calculated for each timepoint and used to determine isotopic dilution (dilution of ^{13}C-, ^{15}N-RDX with *in situ* RDX without label). This value was very small as added ^{13}C-, ^{15}N-RDX had a higher concentration than *in situ* “natural” RDX. When the conservative tracer dilution reached 70%, the PPT was considered to be complete. Assuming mineralization products (CO_2, CH_4, N_2, N_2O, NH_4^+, NO_2^- and NO_3^-) behaved conservatively, they were dilution corrected using conservative tracer C/C_0. Mineralization product conversions to RDX equivalents were summed and adjusted for efficiency (^{14}C-RDX incubations described above). These were summed over the course of the PPT to determine total RDX degradation occurring *in situ*. These measures were compared to 1st order degradation rate models using RDX retardation factors, dilution, and transient degradation intermediates concentrations calculated by USACE (*18, 20*).

5.2 DESIGN AND LAYOUT OF THE TECHNOLOGY COMPONENTS

As alluded to above in the previous section, no device was fabricated or tested. The most useful presentation for this section is a tablature view describing the demonstrations at both sites outlining dates, times, treatments, subsamples, and measurements collected. Referring to specific analyses described in Section 5.1 provides detailed context. Appendix B contains all data collected and further data presentation. Section 6 shows meta-analysis and comparison(s) to validate the technology.

The PPT test at Site F began with logistical preparation in early April 2018. 1,500 gallon tanks (*e.g.* Figs. 5-6) were transported and secured near wells of interest (Table 2). Ambient RDX and anion (conservative tracer), dissolved oxygen (DO), RDX degradation intermediate, and relevant stable isotope component concentrations were measured before injection. Subsamples were taken using Grundfos pumps at time intervals after the push injection (see Appendix B for spreadsheets containing exact times for each well). At least twelve subsamples were taken from each well, immediately chilled on ice and shipped to the appropriate laboratories for analyses (see list in Section 5.1).

Preparation for the Stie A PPT began in March 2019. Tanks were moved into place adjacent to test wells and logistical preparations for sample collection, short-term storage and shipping were set in place. Ambient concentrations for all analytes were taken (t0) before or while PPT tanks were mixing injectates. Injection conditions are compiled in Table 3. Subsamples were taken using peristaltic pumps from perched zone wells (A-MW22, A-MW36, A-MW60) and by bladder pumps from shallow aquifer wells (A-MW32, A-MW-60, A-MW37).

Table 3. Site F PPT parameters

Well ID	Ambient RDX (µM)	Added ^{15}N-^{13}C-RDX (µM)	Injection Date	Final Sampling Date	Biostim (mM fructose)
F-DW03	0.0045	3.26	4/4/2018	5/7/2018	1
F-MW35	0.64	2.25	4/4/2018	5/7/2018	25
F-MW38	0.058	3.17	4/5/2018	5/7/2018	-0-
F-MW39	0.0045	2.90	4/4/2018	5/7/2018	-0-
F-MW53	0.018	3.61	4/5/2018	5/7/2018	-0-
F-MW59	0.0043	3.11	4/4/2018	5/7/2018	1

Table 2. Site A PPT parameters

Well ID	Ambient RDX (µM)	Added ^{15}N-^{13}C-RDX (µM)	Injection Date	Final Sampling Date	Biostim (mM fructose)
A-MW22 (PZ)	0.045	2.84	4/4/2019	5/30/2019	25
A-MW32 (SA)	0.0017	2.86	4/5/2019	5/30/2019	25
A-MW36 (PZ)	0.051	2.80	4/3/2019	5/15/2019	-0-
A-MW37 (SA)	0.14	2.97	4/4/2019	5/30/2019	-0-
A-MW60 (SA	0.45	3.22	4/5/2019	5/15/2019	25
A-MW62 (PZ)	0.077	2.21	4/5/2019	5/15/2019	25

PZ – Perched zone; SA – Shallow aquifer

Initial processing for subsamples consisted of the following field and analytical treatment:

- Br^{-1} or Cl^{-1}: 0.7μm filtered and stored and shipped cold. Holding time - one year.
- RDX and degradation products: 0.7 μm filtered into amber glass bottles, shipped on ice in the dark. Holding time - 1 week.
- CO_2: Unfiltered and preserved with 0.01 (v/v) $CuSO_4$. Shipped on ice. Holding time - one year.
- CH_4: Transferred under water and sealed in serum bottle with suitable stopper. Holding time - one year.
- The DIN analytes NH_4^+, $^{15}NH_4^+$, NO_3^-, $^{15}NO_3^-$ samples 0.7um filtered, shipped frozen, with holding times of one year at -20 degrees C for all analytes.
- N_2O, $^{15}N_2O$, N_2 and $^{15}N_2$ samples unfiltered, pumped into He-flushed vials containing $KHSO_4$ as a preservative, shipped and stored at room temperature in the dark, with holding times of 4 months for all analytes.
- Microbial protein: Duplicate 25 mL samples amended with 1.5 mL 100% trichloroacetic acid (TCA), frozen and shipped on ice. Holding time is indefinite kept frozen.

Samples were shipped in coolers, double wrapped in plastic with ample blue ice, sample manifest and padding to the US Naval Research Lab, contract laboratories, or UConn where they will be stored until analysis. Shipping was done via FedEx next day.

Sample Documentation

The project team members used field notebook(s) to record field and sampling activities and significant events, observation, and measurements during field activities. The following general information was compiled in each daily log:

- Name, date, time entry
- Description of field activities and any problems encountered
- Field equipment calibration and maintenance
- Sample collection details including the number of samples, date and time, sampler and sample collection methods
- Field measurements and general observations

The sampling team made maximum use of preprinted forms and labels to track samples. Bottleware was pre-labeled when possible with analyte name, sample number and replicate. Maximum use of electronic records was made (spreadsheet, database). Samples were logged in the laboratory using electronic means. All instrument-produced data was be backed up within 24 hours of analysis. Residual sample will be archived until QA checks are complete. Forms and field books serve as permanent records and were completed with permanent ink. Electronic copies are kept in at least two separate locations, encrypted with access by two-factor authentication and backed up regularly.

5.3 OPERATIONAL TESTING

The PPTs were in effect an operational testing platform for stable isotope efficacy in determining *in situ* degradation rates for munitions contaminants. Observing labeled mineralization products during the PPTs was irrefutable evidence for biological RDX transformation in the field. PPTs were scheduled as part of ongoing USACE operations at Site F and Site A and offered a rather unique opportunity to evaluate stable isotope techniques within the context of alternative remediation assessment techniques. The PPT platform also offered a means to rapidly deliver labeled energetics to the groundwater with previously-described perfusion characteristics – obviating the need to determine that component de novo. A far smaller scale could have been implemented – requiring far less isotope injection (and far less initial cost – see Section 7). PPTs offered an independent means for estimating *in situ* RDX degradation rates so that cross validation could be performed and regulator acceptance could be documented for transition to the fleet and both site assessment and remediation communities.

6. PERFORMANCE ASSESSMENT

To gauge technology performance, evaluation was performed using PPTs which offer insight into RDX degradation on site using a 1st order degradation rate model (*18, 20*). PPTs rely on aquifer hydrogeologic parameters, a calculated retardation factor (for RDX in this case), and chemical concentration data collected at intervals post-push (RDX, daughter products, and conservative tracer). Several limitations are evident using standard PPTs. Because the hydrogeologic parameters are derived from soil samples and retardation factors are calculated using empirical tests with representative samples, these "single factor" model inputs are used to characterize a far more heterogenous environment than can be represented by a single value within the model(s). Additionally, because rate models predict loss due to biodegradation, given considerable site heterogeneity, it is not possible to definitively ascribe RDX loss to biodegradation relative to other processes - *e.g.* sorption to soil constituents poorly represented in retardation factor or other hydrogeologic estimates (sorption kinetics for example).

By monitoring the stable isotope ratios from labeled-RDX mineralization products, one can definitively confirm RDX has been degraded. Mineralization products are particularly useful because oxidized species will behave conservatively in oxic environments and are miscible with groundwater (*e.g.* no retardation). Therefore, chemicals such as CO_2, NO_3^-, N_2, and N_2O will reflect the isotopic ratio of their source, but will largely be excluded from biological processing. Direct monitoring with a dilution correction tracer (Cl^- or Br^-) allows reasonably direct conversion to parent RDX degraded. In the case where groundwaters become anaerobic, CH_4 may be produced but is readily analyzed and will reflect any isotopic enrichment. Ultimately, RDX incorporated into biomass is needed to determine added RDX fate *in situ*. Microorganisms in soils and groundwater will mineralize organic compounds (to mineralization products) but also incorporate some carbon and nitrogen into cellular material based on availability (*27*). Determining incorporation into biomass was not straightforward as filtering sufficient groundwater to analyze was not possible (filter clogging). The growth efficiencies for several test wells were determined using ^{14}C-RDX incubations. Because groundwaters may be both carbon and nutrient limited (*27*), we assumed relatively high efficiencies with the relatively abundant source of both carbon and nitrogen (RDX).

Tracking mineralization products from post-push samples was relatively straightforward as mentioned. After the push, both dissolved CO_2 (dissolved inorganic carbon – DIC) and dissolved CH_4 increased in wells that were stimulated and anaerobic, respectively. At Site F, DIC increased almost 10-fold at F-MW35 and F-MW39 (both stimulated with fructose) while following an initial decrease, DIC increased to previous levels in F-DW03 (which was anaerobic). Control wells (no stimulation) F-MW38 and F-DW59 showed little to no DIC increase after the push – although F-MW59 had very low DIC levels prior to the push

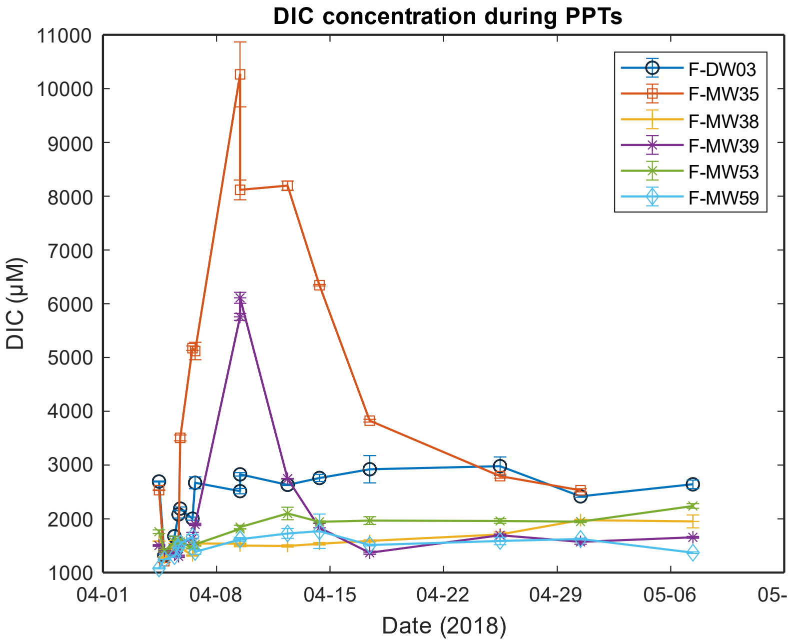


Figure 8. DIC during Site F PPTs

(and showed modest increase before trailing off to ~ pre-push levels). Recall that F-MW59 was downgradient of F-DW03 which was bioaugmented prior to these PPTs (Fig. 8).

At Site A, DIC increased at biostimulated wells (both in the perched zone and shallow aquifer). Within the perched zone, A-MW22 and A-MW60R and shallow aquifer, A-MW-32 and A-MW62 all showed several-fold increases in DIC after the push with A-MW22 and A-MW32 (very close geographically) showing peak DIC increase after about 10-15 days post-push. In the stimulated wells within the source area (A-MW60R (PZ) and A-MW62 (SA)), DIC increased during the first month after the PPTs and then began tailing off toward starting concentrations (Fig. 9). Control wells (A-MW36 (PZ) and MW37 (SA) showed very change in DIC post-push which could be expected because natural groundwater was used as the base for PPTs (Fig. 9). The relative increase in DIC for stimulated wells was assumed to be increased respiration (available carbon as fructose and, or carbon in added RDX). ^{13}C-stable isotope enrichment within the DIC was needed to determine the increased DIC's source.

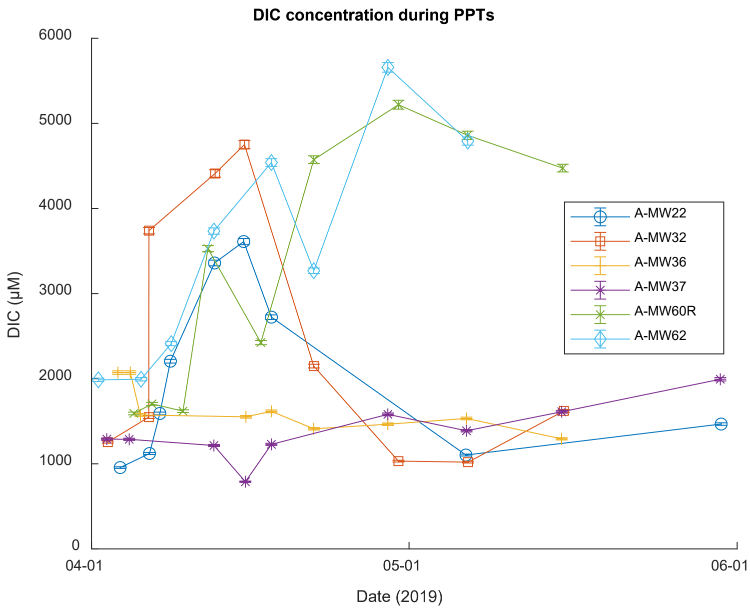


Figure 9. DIC during Site A PPTs

At biostimulated wells (and at F-DW59 downgradient from F-DW03 which was bioaugmented as well), significant ^{13}C enrichment occurred post-push (Fig. 10). F-DW03 (both bioaugmented and biostimulated) showed extreme ^{13}C enrichment (+200 ‰$_{\mathrm{VPDB}}$)

indicating considerable RDX degradation during the PPT (Fig. 10 – right axis). Biostimulated wells (F-MW35, F-MW39 – and by proximity to F-DW03) also showed enrichment in ^{13}C-DIC after the push. Control wells (F-MW38 and F-MW-53 showed little significant ^{13}C-DIC enrichment trend after the push. Minor transient enrichment can be seen immediately after the push, but most of that trend was evident only until about 7 days (Fig. 10). At site A, ^{13}C-DIC enrichment was also observed in stimulated wells after the push. Enrichment was not nearly as drastic as observed at Site F (with up to +200 ‰$_{\text{VPDB}}$ difference recorded). All stimulated wells showed ~+15 ‰$_{\text{VPDB}}$ enrichment post push with most wells retaining the signal a month after the push (Fig. 11). Control wells (A-MW36 (PZ) and A-MW37 (SA)) showed minimal to no initial enrichment. Some transient enrichment was noted after several weeks at A-MW37 (Fig. 11).

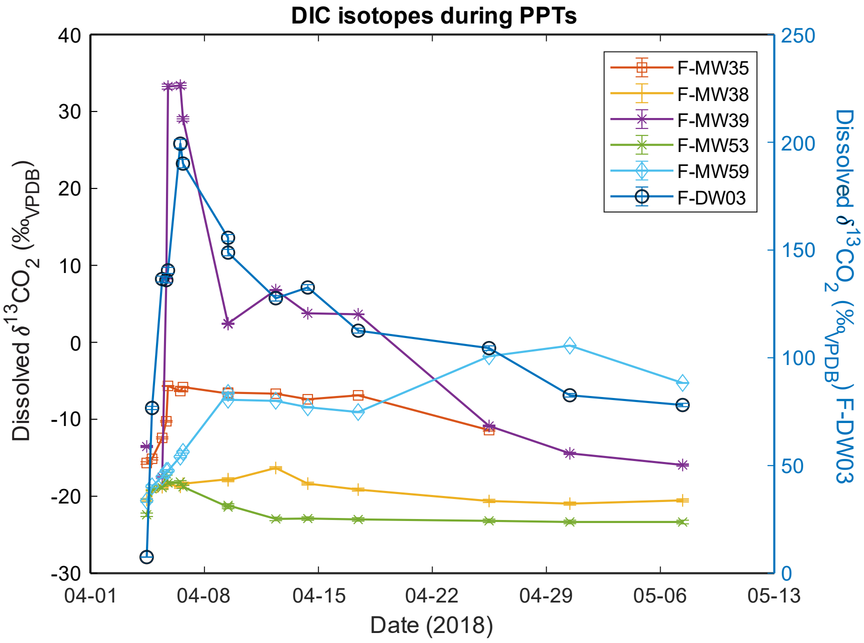


Figure 10. Site F DIC stable carbon isotopes during PPTs

Because a conservative tracer was added to each PPT to calculate dilution, a two end-member mixing model can be applied to the isotope values in order to estimate dilution impacts on measuring isotope ratios at a single point (*e.g.* the injection well) post-push. Given the δ^{13}C values for DIC at each time point (at each test well), and assuming the native groundwater DIC is constant over the PPT course (which may not be a valid assumption), one can estimate the δ^{13}C for CO_2 contributed by the labeled RDX degradation using the following formula (2):

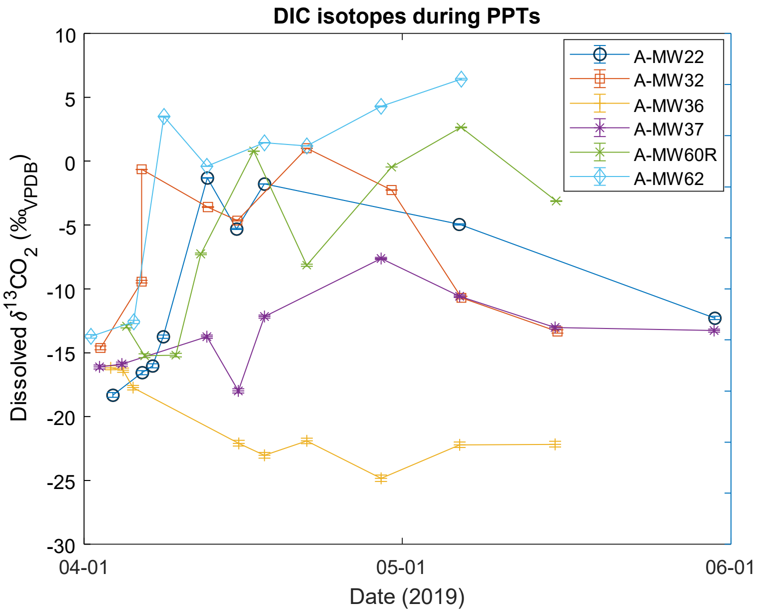


Figure 11. Site A DIC stable carbon isotopes during PPTs

$$(2)\ \delta^{13}C_{mix} = f_1\ \delta^{13}C_{original} + f_2\ \delta^{13}C_{labeled\ RDX}$$

Where f_1 and f_2 are the fractional contributions from CO_2 from each source (original – natural and from labeled RDX). As there are only 2 end-members, $f_1 + f_2 = 1$. The dilution factor (C/C_0) for Cl^- or Br^- allows assigning f_1. f_2 can be assigned as $1 - f_1$. Rearranging to solve for $\delta^{13}C$ from labeled RDX gives (3):

$$(3)\ \delta^{13}C_{labeled\ RDX} = (\delta^{13}C_{mix} - f_1\ \delta^{13}C_{original})\ /\ f_2$$

Using this model, dilution corrected isotopic values were calculated with a 90% dilution cutoff (keeping isotope ratio estimates reasonable). At Site F with relatively slow dilution kinetics, DIC isotopes are enriched similarly to what's observed in undiluted estimates – however, for several wells (F-DW03 and downgradient F-MW59), ^{13}C-DIC enrichement is observed to the end of the PPT without tailing off toward starting $\delta^{13}C$ values (Fig. 12). At Site A, dilution was much more rapid (one to two weeks to reach ~90%) so fewer time-based data points are available to display adjusted $\delta^{13}C$-DIC. Overall, the ^{13}C enrichment was far higher using dilution-adjusted values (up to 1000 ‰ ^{13}C- enrichment versus ~20 with uncorrected values). Similar to Site F, some wells appear to be increasing in enrichment to the end of the sampling (*e.g.* A-MW22, A-MW62). Control wells (no biostimulation) showed relatively flat response – and with A-MW36, a decrease in $\delta^{13}C$-DIC. This implies that no RDX was degraded (confirmed in other ways – see below). However, dilution at A-MW36 was so rapid, that the 10% dilution cutoff was reached one week after the push. Data shown from 12 May 2019 on are outside the model

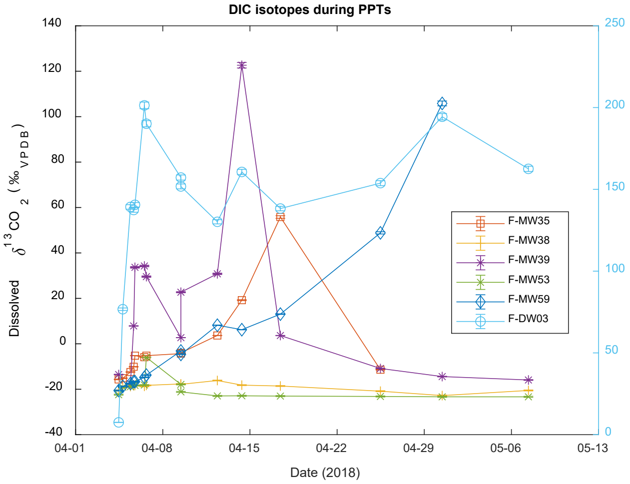


Figure 13. Site F dilution-adjusted DIC isotopes

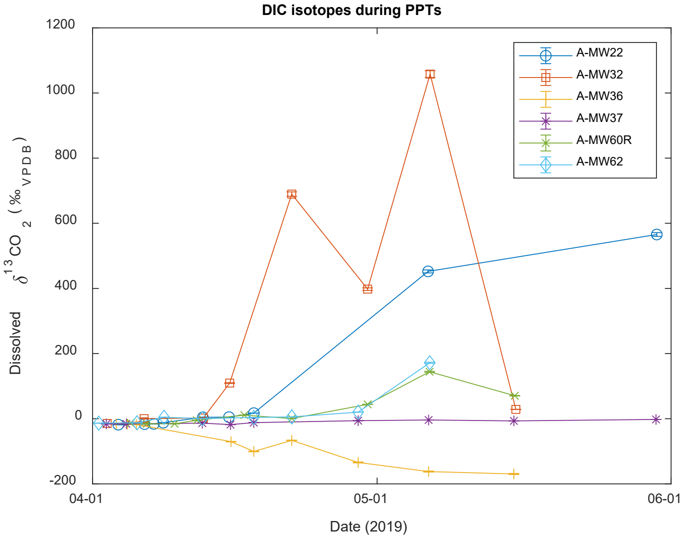


Figure 12. Site A dilution-adjusted DIC isotopes

confidence – but shown to visualize among the other lines (which cover completely if A-MW36 values are truncated (Fig. 13).

In addition to DIC, dissolved methane produced during RDX degradation should reflect the source isotopic signature (with appropriate fractionation factor). Enzymes for methanogenesis are highly discriminatory against $^{13}CO_2$. $^{13}CO_2$ derived from RDX degradation is less likely to be converted to methane than $^{12}CO_2$ (see fractionation factor discussion in Section 5). Additionally, appreciable methanogenesis is likely only at wells with anaerobic conditions. Because push waters were well mixed in aerobic conditions, methane concentrations are expected to drop immediately after the push – until anaerobic conditions reestablished. This can be observed at both sites. At Site F, two wells were anaerobic before the PPTs (F-DW03 and F-MW39). Several days after the push, methane concentrations increased in samples from both these wells up to several thousand ppm (Fig. 14).

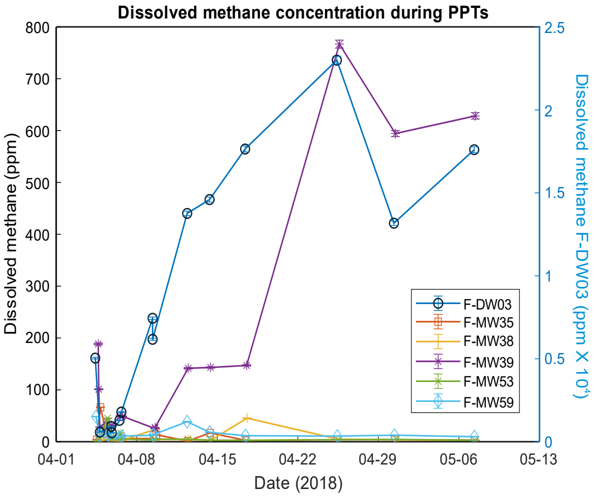


Figure 14. Site F methane concentrations during PPTs

At Site A, well A-MW62 had appreciable methane before the PPT began (~2500 ppm). Well A-MW32 had the highest initial methane concentrations (~7200 ppm) before the PPT began. After the push, A-MW32 did not "recover" with methane concentrations only around 1000 ppm at the end of the test. A-MW62 however, became anoxic (dissolved oxygen below 0.50) before two weeks post-push and had methane concentrations ~25,000 ppm after one-month post-push (Fig. 15). All other wells remained at least hypoxic post-push and appreciable methane concentrations were not measured (Fig. 15).

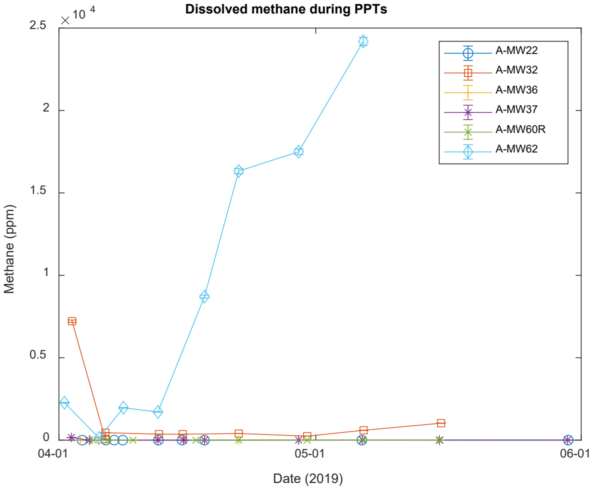


Figure 15. Site A methane concentrations during PPTs

Like DIC, methane isotopes were measured and recorded during PPTs. Although methane concentrations were very low at many wells, enough was available to obtain carbon isotope ratios for both Site F and Site A PPTs. Using conservative tracer dilution during the PPTs, methane isotopes were corrected (see above *Eq* (2) and *Eq* (3)) for

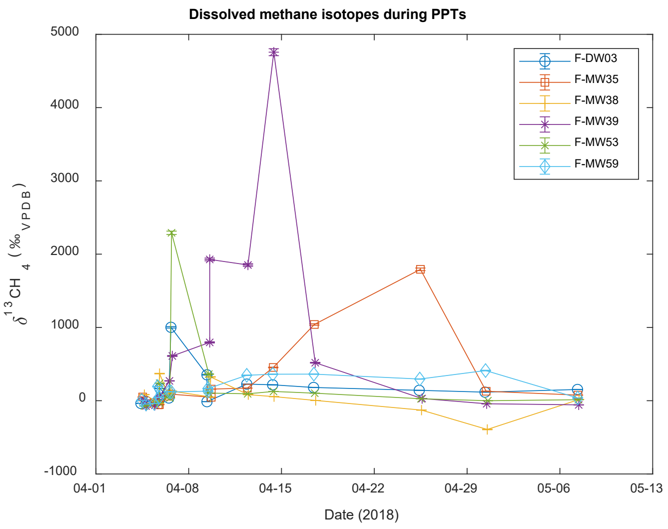


Figure 16. Site F dilution-corrected methane isotopes during PPTs

contribution from natural groundwater CH_4 (assuming a single source with the same isotope ratio as pre-PPT methane). At Site F, considerable ^{13}C enrichment was observed in methane for most wells (up to ~5,000 ‰$_{VPDB}$) although non-stimulated wells (F-MW38 in particular) showed a decrease down to starting levels by the end of the PPT. F-MW53, a control well, showed a considerable initial spike in ^{13}C ($\delta^{13}CH_4$ ~2,220 ‰$_{VPDB}$) but very quickly returned to levels near pre-PPT (Fig. 16).

At Site A, methane isotopes became enriched during PPTs – in fact, at well A-MW32, enrichment was well over +20,000 ‰$_{VPDB}$ when corrected for dilution (Fig. 17). Aside from A-MW32 (shallow aquifer), ^{13}C enrichment appeared to be transient post-push. At A-MW32, methane ^{13}C enrichment started after about 15 days post-push and continued until the end of the collection period (Fig. 17). Because flow rates (dilution) were relatively high at Site A (versus Site F), dilution was more rapid and the dilution correction led to extreme ^{13}C enrichments. It is obvious that the ^{13}C -labeled RDX signal is clearly seen in the methane generated on site during the PPTs.

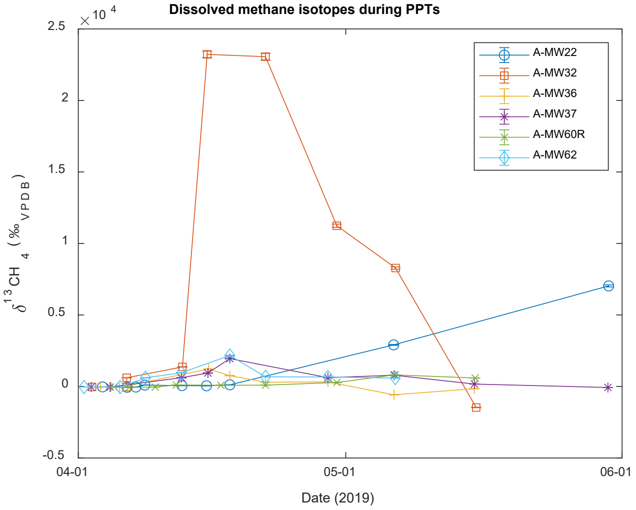


Figure 17. Site A dilution-corrected methane isotopes during PPTs

Laboratory experiments using ^{14}C-labeled RDX to determine growth efficiencies allowed us to estimate RDX conversion to biomass which was not able to be effectively measured (filter clogging). It is unknown what particulates in the groundwater at the test sites led to poor filtration efficacy. This was not only a problem for collecting biomass uptake samples, but impacted work by Dr. Craig Tobias for collecting nitrogen mineralization product samples (used many filters). This was an unforeseen issue but was adequately resolved by performing short-term laboratory studies to assess growth efficiencies. Because the relatively oligotrophic groundwaters on site were low in organic carbon and nitrogen, it seemed reasonable that growth efficiencies would be relatively high. They ranged between 45 and 90% (Table 4). Surprisingly, fructose addition (F-DW03) did not appear to decrease growth efficiency, so it's likely that microbial consortia on-site are nitrogen limited – thus adapted to incorporate organic nitrogen into cellular material when it is available.

Table 4. RDX growth efficiency data and calculations (Site F)

Well ID	RDX incorporation (nM h^{-1})	RDX mineralization (nM h^{-1})	RDX growth efficiency (%)	Microbial production (µg C L^{-1} h^{-1})
F-DW03	0.198	0.0987	68	0.72
F-MW35	0.223	N.D.	N/A	0.49
F-MW38	0.076	0.0672	53	0.58
F-MW39	0.120	0.0161	88	0.30
F-MW53	0.426	0.0462	90	N/A
F-MW59	0.054	0.0655	45	N/A
F-DW03 (fructose)	0.057	0.0348	62	0.83
F-MW35 (fructose)	0.136	0.0196	87	0.45

As discussed in Section 5, raw values for concentrations and isotopic enrichments were converted using dilution factors and signatures from initial (pre-PPT) samples to RDX degradation equivalents. As discussed in Sections 2 and 5, a major goal in deploying stable isotope labeling technology along-side on-going PPTs at Naval Base Kitsap-Bangor was the opportunity to place measurements in context with on-going estimates for RDX removal (using attenuation modeling during PPTs). This allowed Project 537 to not only demonstrate the technology in the field, but to validate the technology with

respect to regulator-approved tests ongoing at the sites. This technology also allowed an independent validation of attenuation modeling during PPTs – a technique which has industry acceptance, but cannot be irrefutably linked to RDX degradation (no direct evidence that CO_2 respiration products are derived from the on-site RDX). This was a golden opportunity to obtain concurrent validation data for more rapid transition to practitioners, managers and the fleet.

Mineralization products and their stable carbon isotope ratios (CO_2, CH_4), dilution corrections and estimates for efficiency were used to determine the RDX degradation equivalents at each time-point during the PPTs. Spreadsheets containing all data and formulas are contained in Appendix B. Here, we present the processed data for both measured and fitted RDX attenuation and confirmed degradation based on isotopic conservation in mineralization products. Although not presented in detail within this report, data will be shown for nitrogen-based mineralization products as for both field demonstrations, dual-labeled RDX was used (^{13}C-, ^{15}N-RDX).

As discussed, we chose existing PPTs at both sites as test-beds for this demonstration as the PPTs were approved by regulators and the data generated made a reasonable backdrop for presenting the isotopic results. After PPTs, standard 1st order degradation rate models were applied to RDX loss over the test timecourse. These rate models allow conversion to degradation time so are typically used (*20*). The rate models are developed using site-specific retardation factors (*e.g.* how likely the RDX is to partition to the soils versus remain in solution). The dilution factor is used to estimate loss of added RDX by dilution alone (versus degradation). The retardation-factored time after the push is used with the dilution-adjusted RDX concentration to create the final 1st order model which takes the general form:

$$(2)\ \text{Concentration} = a^{-b*t}$$

Where a represents a scaling factor for a given starting value and b represents the 1st order rate constant (often called k). Solving for t (time) gives the half-life or time to degrade for the modeled compound. PPT rate constants were calculated by fitting exponential decay models. At Site F, 1st order models were reasonably constrained for F-DW03, F-MW35, and F-MW-59. Models were poorly fitted ($r^2 < 0.7$) for F-MW38, F-MW39 and F-MW53 (Table 5). For well-constrained models, half-lives were on the order of days – around one week (Table 5). RDX concentrations decreased exponentially during these PPTs as visualized (Fig. 18). At F-MW39, decay appeared exponential, but the goodness of fit statistic was under the threshold value ($r^2 = 0.45$). From the graph, RDX attenuates to near zero within 30 days (retardation-adjusted) after injection (Fig. 18D). The half-life (~5 days) calculated by the 1st order model seems reasonable even though the fit is poor.

Table 5. RDX degradation rate models based on RDX loss at Site F

Parameter	F-DW03	F-MW35	F-MW38	F-MW39	F-MW53	F-MW59
a	0.61	0.31	0.00	0.22	0.00	0.07
b	-0.10	-0.37	0.01	0.15	0.01	-0.11
r^2	0.76	0.95	0.48	0.45	0.09	0.90
k	37.70	136.51	1.84	55.70	5.44	41.32
Half-life (*yr*)	0.02	0.01	0.38	0.01	0.13	0.02
Half-life (*days*)	6.71	1.85	137.20	4.54	46.52	6.12

For control wells (F-MW39 and F-MW53), almost no RDX loss was observed during the PPTs, explaining the poor exponential fit. A linear fit would perhaps be a better overall attenuation model, but this does not offer an attenuation coefficient that can be easily used to calculate an RDX half-life. At site F, biostimulated wells (F-DW03 and its downgradient neighbor F-MW59) showed appreciable RDX loss as did

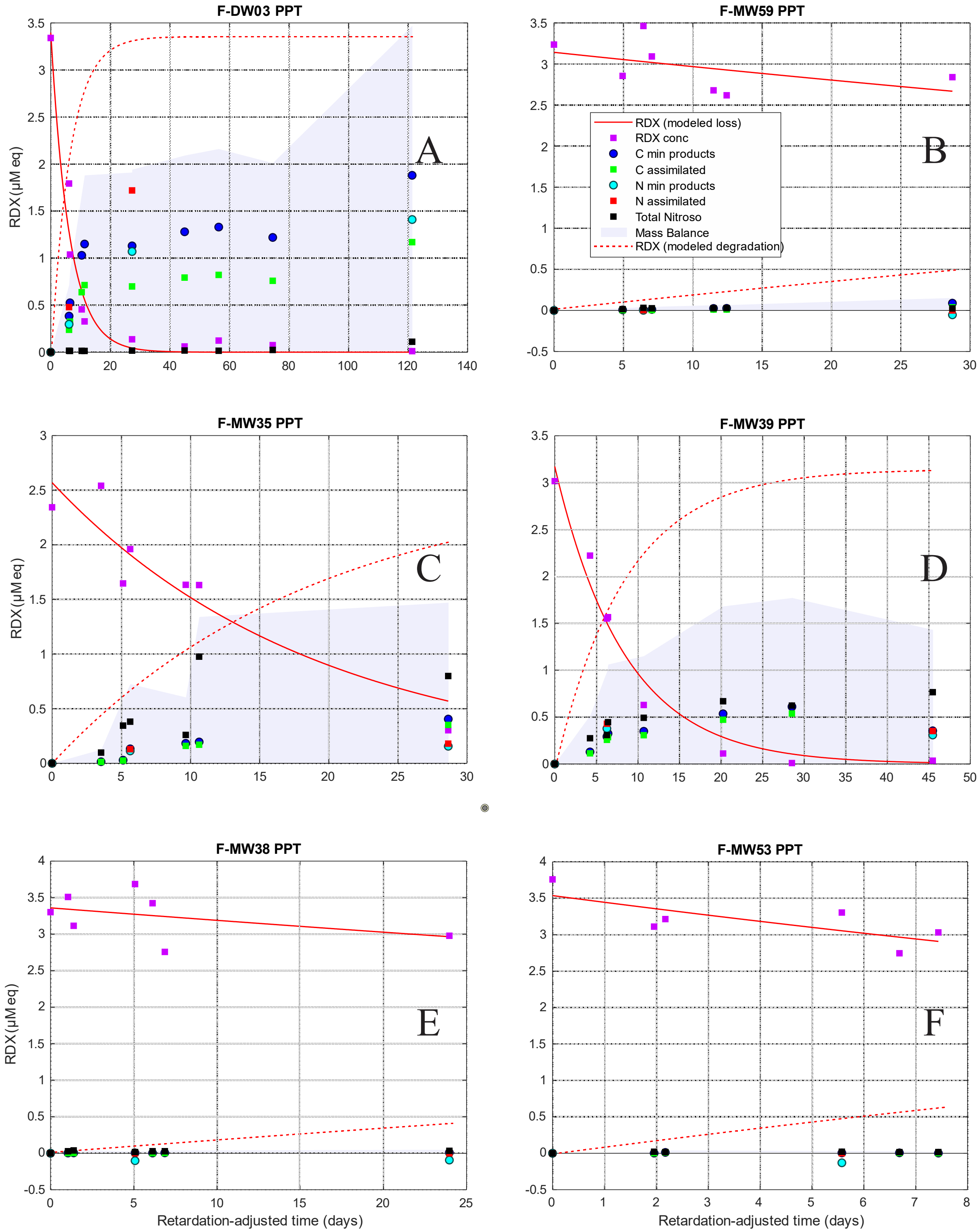


Figure 18. PPT results Site F

biostimulated wells (F-MW35 and F-MW39) (Figs 18A, 18B, 18C and 18D). Interestingly, the fastest degradation model at Site F was well F-MW35. It had the highest constrained model ($r^2 = 0.95$; Table 5). RDX attenuation modeling showed that biostimulated and bioaugmented groundwater wells demonstrated rapid RDX removal relative to background wells (non-augmented or stimulated) which showed little to no RDX removal.

In order to test isotopic rate measurements in context, 1st order rate models were applied to biodegradation rates tied isotopically to the added ^{13}C-RDX. ^{13}C-excess CO_2, CH_4 and biomass (estimated through efficiency studies) were summed and modeled in the time domain (see Fig. 18). Model fits varied as with RDX loss with background well F-MW53 failing to constrain ($r^2 = 0.1$; Table 6). At two stimulated wells (F-DW03 and F-MW39), fits were also poor ($r^2 < 0.7$; Table 6). At wells where good 1st order models were fit, half-lives calculated by isotopic mass balance were within one order of magnitude to those calculated by RDX loss (Tables 5 and 6). Rates are somewhat lower which should be expected as the isotopic method measures only account for actual conversion of the parent RDX (not just loss). Of note, the isotopic measurements show absolute evidence for RDX converted to mineralization products.

Table 6. RDX degradation rate models based on isotopic measurements (Site F)

Parameter	F-DW03	F-MW35	F-MW38	F-MW39	F-MW53	F-MW59
a	0.81	0.07	0.00	0.32	0.01	0.01
b	0.01	0.06	0.04	0.01	-0.10	0.09
r^2	0.56	0.77	0.78	0.15	0.10	0.98
k	2.26	20.23	13.67	3.32	36.54	32.12
Half-life (*yr*)	0.31	0.03	0.05	0.21	0.02	0.02
Half-life (*days*)	111.85	12.50	18.51	76.28	6.92	7.88

At Site A, the four biostimulated wells showed appreciable RDX removal during the PPT (A-MW22, A-MW32, A-MW60R, and A-MW62; Table 7). 1st Order models were well constrained for these wells and RDX half-lives were on the order of days to a little over a week for the Site. Wells with no biostimulation (A-MW36 and A-MW37) showed no appreciable RDX removal (aside from dilution) during the PPTs.

Table 7. RDX degradation rate models based on RDX loss at Site A

Parameter	A-MW22	A-MW32	A-MW36	A-MW37	A-MW60R	A-MW62
a	2.92	3.00	2.79	0.00	3.99	2.04
b	-0.11	-0.78	0.01	-0.04	-0.08	-0.16
r^2	0.95	1.00	NA	NA	0.81	0.97
k	40.84	286.27	3.31	NA	27.96	57.12
Half-life (*yr*)	0.02	0.00	0.21	NA	0.02	0.01
Half-life (*days*)	6.19	0.88	76.32	NA	9.05	4.43

Consequently, RDX loss was difficult to model in control wells. Degradation intermediates (MNX, DNX, TNX) became elevated in the two perched zone PPT wells

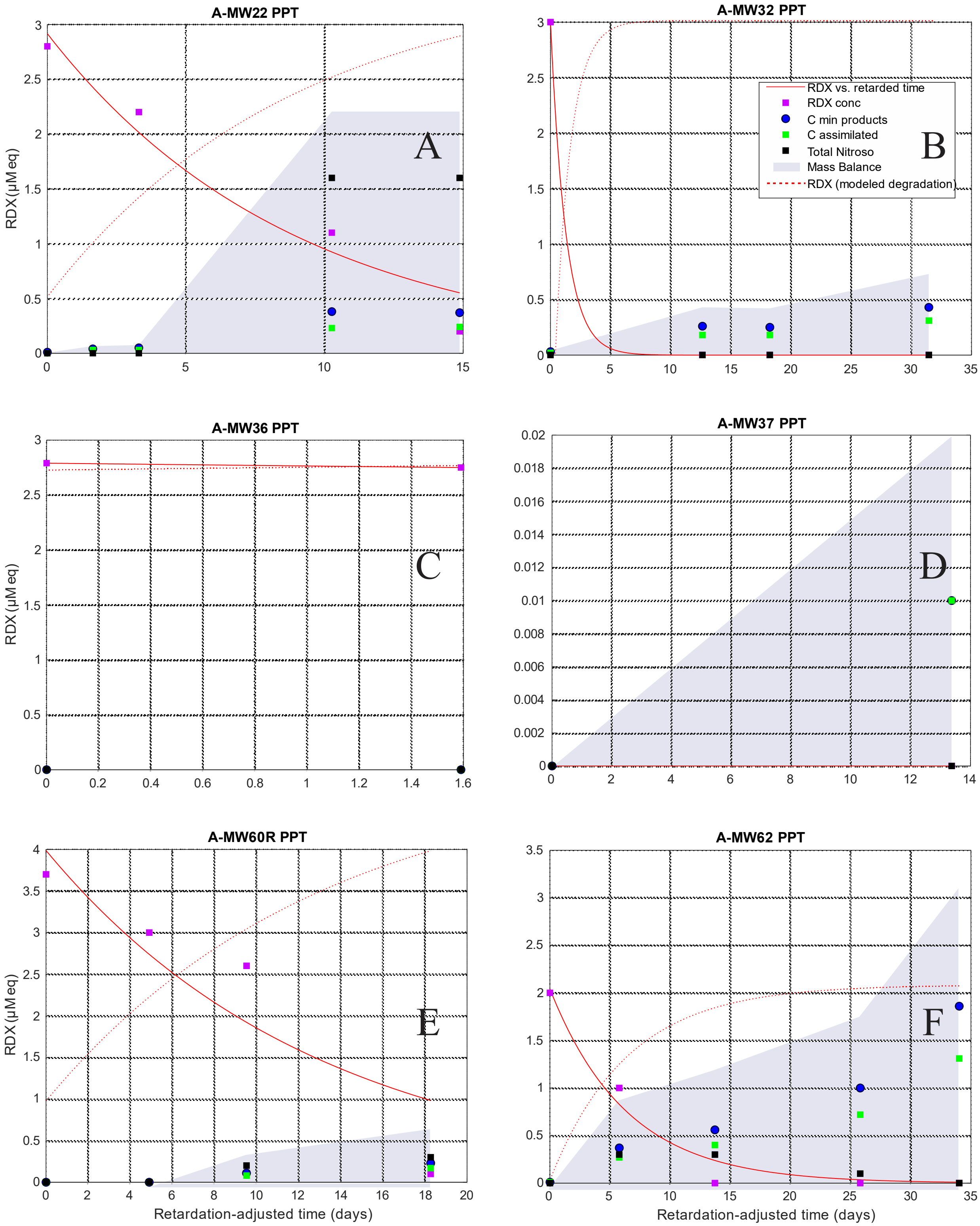


Figure 19. PPT results Site A

(A-MW22 and A-MW60R; Fig. 19A, 19E). At A-MW62 (shallow aquifer) there was also a transient increase in intermediates (Fig. 19F).

Calculating biodegradation based on isotopic balances, 1st order rate models could be produced for all but control wells with well-constrained fits ($r^2 > 0.7$) (Table 8). Similar to Site F, degradation rate estimates were within 1 order of magnate for RDX loss-based models (Table 7 & 8). For the poorest fitted model (A-MW22), the rate constant calculated by stable isotope analysis was slightly faster than that from RDX loss – but likely not a significant difference.

Table 8. RDX degradation rate models based on isotopic measurements (Site A)

Parameter	A-MW22	A-MW32	A-MW36	A-MW37	A-MW60R	A-MW62
a	0.11	0.18	0.00	0.00	0.03	0.32
b	0.13	0.05	0.00	0.29	0.14	0.07
r^2	0.80	0.87	NA	NA	0.91	0.97
K	46.21	16.57	0.00	106.58	50.77	24.46
Half-life (*yr*)	0.02	0.04	NA	0.01	0.01	0.03
Half-life (*days*)	5.48	15.26	NA	2.37	4.98	10.35

At both sites, isotopically-calculated degradation rate estimates were in-line with those derived from RDX loss. These results constitute a successful demonstration that adding stable-isotope labeled substrates provides an alternative method for obtaining contaminant degradation rate estimates *in situ*. While the PPTs have become a regulator-approved means for determining RDX degradation rate estimates, they have some drawbacks. Principally, PPTs are expensive and logistically difficult to perform. They require thousands of gallons of test water and considerable expense in post-push sampling and analysis. Additionally, field crews must sub-sample for a relatively large chemical suite to validate rate models (*e.g.* degradation intermediates confirm initial parent contaminant degradation). At both test sites, aerobic wells showed almost no daughter product production during the post-push sampling periods (Figs. 18 & 19). This makes absolute confirmation of biological RDX remediation problematic.

This demonstration made use of already approved PPTs to test stable isotope efficacy. The performance was consistent with the approved PPTs and was not considerably more cost-effective given the demonstrations' scale. However, the test concept was validated and the stable isotope method could be used at much smaller scale to significantly decrease costs. As discussed in the next section, smaller isotopically-labeled RDX preparations are inexpensive ($250) and stable isotope analysis for CO_2 is as well ($10 per sample). With well access needed post-push for this demonstration's tests, it was not possible to sample the well headspaces for $^{13}CO_2$ enrichment during the tests. However, after the tests, CO_2 traps were deployed in several well headspaces and CO_2 analyzed for concentration and stable isotope composition. At Site F stimulated wells (F-DW03 and F-MW35), traps installed after the tests showed $^{13}CO_2$ enrichment (Fig. 20). With F-DW03, $^{13}CO_2$ enrichment persisted for at least one year after the PPT ($\delta^{13}CO_2$ was +38 ‰ in July 2019). This may be the result of ^{13}C-RDX incorporated into biomass that is remineralized over time. RDX sequestration may also be a factor as any ^{13}C-RDX sorbed to soil material in excess of predicted retardation (limited number of measurements) may be more slowly degraded.

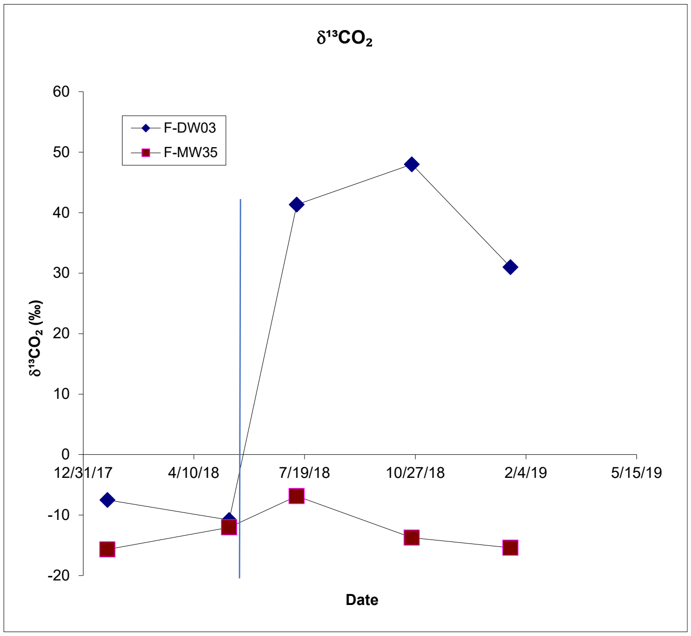


Figure 20. Well headspace CO_2 stable isotope ratio

At Site A, we did not have access to the test wells to establish long-term background CO_2 isotope characteristics. One well was sampled for previous to the PPTs and while perhaps not representative of all wells, we obtained a "baseline" $\delta^{13}CO_2$ in trap material of -32 ‰. After the PPTs, All wells but A-MW36 (control well) showed ^{13}C enrichment in the well headspace CO_2 (Fig. 21). Using ZOI modeling (*9*), the flux from RDX degradation can be estimated using enrichment factors. This has been completed and will be evaluated for the sites tested during this demonstration. As we were not able to seal the well headspace(s) because of PPTs, extreme care would be needed to interpret these results. Immediately evident is the enrichment picked up in a simple CO_2 trap ($5 to make) by deploying post-push of ^{13}C-RDX.

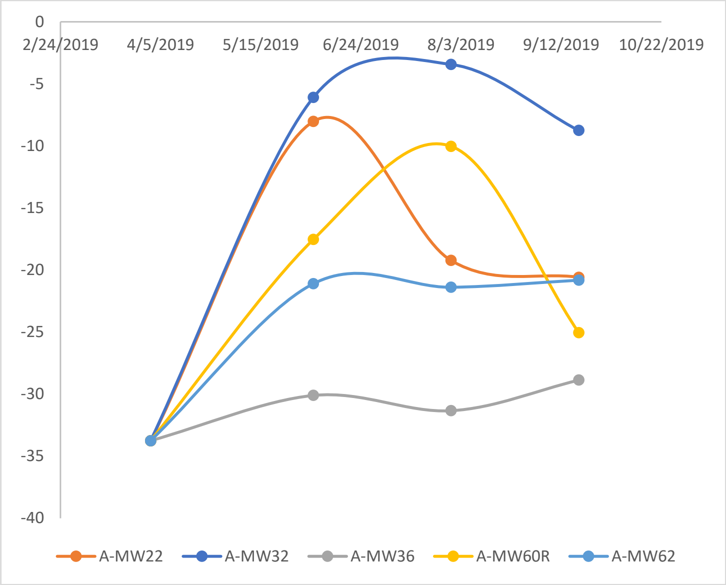


Figure 21. Well headspace CO_2 stable isotope ratio

While not covered in great detail in the next section, cost savings from smaller scale releases wherein degradation could be estimated from CO_2 respiration and its associated enrichment could be substantial as only several measurements would be needed and trap deployments would integrate respiration over longer time scales (obviating the need to sample intensely post-PPT. With inexpensive starting materials ($250 for ^{13}C-RDX, $10 for sampling equipment, and \$10 per $\delta^{13}CO_2$ analysis), and open-source models for calculating a zone of influence (*9*), a full sampling from multiple wells could be completed for less than \$5K.

7. COST ASSESSMENT

7.1 COST MODEL

This project was a technology demonstration as the fundamental application(s) of labeled isotopic tracers have been applied in many fields (medicine, biology, geology, *etc*.). Costs for the demonstration revolved around participant salaries, field supplies, isotope synthesis, analytical analyses and field work. Additionally, funds were used for developing Tier II SAPs for both sites – which was very time-consuming. Costs – and associated logistics with fielding this technology and collaborating with a complementary NESDI project (#544) and the USACE (for Push-Pull Tests) added to the labor and coordination for this demonstration – but were invaluable for validating the technology in the field and ultimately transition to the user community.

An initial cost model was created for the project itself to describe costs allocations by element types (Table 5). Total funding from NESDI was $653K over the three year project. Of that, $58K was distributed to Hydrologic Engineering Inc. to perform Zone of Influence modeling for test wells at Site F and Site A. Considerable costs were shared with other

Table 7. Cost structure for the project

Cost Element	Data Tracked During Project
1. Equipment capital costs	No capital equipment purchased for this project. Capital equipment was used.
2. Installation costs	No permanent installation(s) were needed during this project. Existing monitoring wells were used.
3. Consumables	Consumables included chemicals, tubing, rentals, field supplies. In the laboratory, gasses and chemicals were used. Special isotope synthesis (dual-labeled ^{13}C, ^{15}N-RDX) was ~$32K
4. Analytical costs	Analysis for CO_2 and CH_4 and anion concentrations were completed at NRL. Costs at comparable commercial laboratories range from $10-$50. Isotopic analyses were completed at NRL and UC Davis with costs ~$12 per sample. Analytical costs for RDX and daughter products (EPA 8330B ~$150 per sample) were covered by collaborative projects or the RPM.
5. Modeling costs	Modeling included determining zones of influence around wells for CO_2 traps (to obtain background isotopic information). Model(s) for RDX attenuation were 1st order and relatively easily calculated using spreadsheet(s)
6. Publication costs	Several manuscripts and this report will be published. Costs were obligated at $4K (for open access).
7. Labor and travel costs	Labor costs were the largest portion of the NESDI project as DoD Laboratory overhead is very high. Labor was ~75% of the total project costs. Travel amounted to ~10% with multiple field deployments and samplings.

projects on base led by the USACE (Dr. Mandy Michalsen) and NESDI #544 (Dr. Jovan Popovic and Dr. Craig Tobias). These were "value added" collaborations and as mentioned in Sections 5 and 6, the independent data allowed validating the technologies from NESDI #537 for transition.

A more relevant cost model for transitioning this demonstration to the user and regulatory community could be made – namely the transitioned technology. For this model, we can create a single-well cost estimate where the technology is deployed to confirm *in situ* RDX degradation is – or is not occurring. Under this cost model, a smaller push (release isotopic energetic, conservative tracer and stimulant if desired) followed by subsampling over several days or weeks could be accomplished. Commercially-available ^{13}C-labled RDX could be used for the release, and commercial or academic laboratories could be used for the δ^{13}C analyses. Using a recently funded proposal with a commercial environmental firm, per-well low-flow sampling and contaminant analysis is conservatively estimated at \$1,500 per well (labor, shipping, EPA 8330B energetics, low-flow sampling). Three such events would be needed (pre-push, post-push, end-point) at a minimum.

Measurements at each well would require well-specific calculations, however using a typical ~3 m screened interval and a 2" well casing, around 1/10 of a commercial ^{13}C-labled RDX ampule would give ~1.5 µM RDX final within a typical groundwater well. Regulatory limits of 0.8 µM are typical. If we assume 1-2 weeks for 50% dilution from on-site groundwater (*c.f.* Figs. 12-13, 16-17), ample signal should be available to detect ^{13}C-enriched CO_2 and CH_4 against background values should RDX mineralization occur. Using a commercial preparation costing \$525 per 1 mg ^{13}C-RDX (Cambridge Isotopes), 5-10 wells could be tested. Using 5 wells as a conservative estimate for one ^{13}C-RDX purchase, total costs can be calculated at approximately \$105 in isotopes and \$1,500 per well sampled and at least 3 samplings per well to equal \$3,605 per well times 5: \$18,025. This value is in line with "normal" monitoring costs for a suite of wells. In terms of cost structure (see Table 6), consumables, analytical and labor costs are the main elements for using the transitioned technology.

Cost analysis will be covered in section 7.3, but it bears pointing out here that the transitioned technology from this demonstration allows definitive information on the *in situ* RDX degradation rate. While extinction models can infer degradation, they require extensive PPTs to adequately monitor the RDX disappearance over time. They use limited and often single point hydrogeologic data (porosity, RDX retardation, conductivity, *etc.*) which are singularly used to represent heterogeneous soils. This demonstration was in response to a statement of need to be able to irrefutably tie mineralization products to the RDX carbon backbone to justify regulatory acceptance. Tracking the actual elements making up the RDX molecule offers proof of degradation should the labeled element (carbon or nitrogen) end up in final mineralization products (*e.g.* CO_2, CH_4, *etc.*).

Table 8. Cost structure for transitioned technology

Cost Element	Data Tracked During Project
1. Equipment capital costs	No capital equipment purchased for this project. Capital equipment was used.
2. Installation costs	No permanent installation(s) needed if existing monitoring wells are used.
3. Consumables	Consumables include chemicals (labeled RDX, stimulant(s), conservative tracers), tubing, rentals, field supplies.
4. Analytical costs	Costs for commercial laboratories range from \$10-\$50 for CO_2 and CH_4 concentrations. Isotopic analyses at UC Davis cost ~\$12 per sample. Analytical costs for RDX and daughter products (EPA 8330B) are ~\$150 per sample.
5. Modeling costs	Model(s) for RDX attenuation are fitted to 1st order and are relatively easily calculated using spreadsheet(s). Spreadsheet calculations for RDX equivalents from isotope values are a transition product from NESDI Project 537.
6. Publication costs	N/A
7. Labor and travel costs	Labor and transportation costs are encapsulated in the per-well cost of \$1,500 per sample.

7.2 COST DRIVERS

Cost drivers are covered referentially above in section 7.2. For this project, the main drivers were:

- salaries,
- custom isotope synthesis,
- field supplies and equipment (purchase and rental),
- analytical services (either in-house or academic / commercial).

Because this was a demonstration/validation project, analysis, collaboration, permitting and reporting were also significant cost drivers for this effort as labor rates are high for personnel. Appendix C contains Tier II Sampling and Analysis Plans (SAPs) for both Site F and Site A. These documents required significant labor hours to generate and get approved.

For the validated transition product, cost drivers would look different. The custom isotope synthesis would be unnecessary because small quantities are available commercially. Because example Tier II SAPs have been created and approved, a template is available which would greatly reduce labor hours for the transitioned product. Analytical services costs would likely be lower than analyses done in-house at NRL that carry a labor premium relative to commercial or subsidized academic laboratories. The modified cost drivers for the transitioned product are thus:

- salary / labor / overhead (consulting firm),
- field supplies and equipment,
- analytical services,
- data workup, synthesis and reporting.

These costs would be borne by an RPM or site manager with the per well (or event) costs outlined above (**7.1**).

7.3 COST ANALYSIS AND COMPARISON

As stated above (**7.1**, **7.2**), the project costs were substantially higher than the transitioned technology would be to implement. Because this project's focus was demonstration and validation, costs were not controlled as would be for limited implementation at DoD sites to provide definitive energetics degradation rate estimates. It is difficult to determine exactly how many DoD and Navy sites are currently under management for energetics pollution. As of 2012, an estimated 1,400 UXO sites were identified DoD-wide (*1*). The Navy's ERP Manual estimates ~400 munitions response sites within the DoN (*2*). Site F and Site A were chosen for project #537 demonstration because there was a need to convince regulators that on-site RDX was being degraded as predicted by PPTs and following the isotopically-labeled compound backbone into mineralization products is absolute evidence for *in situ* degradation. It is difficult to assess how many RPMs face regulator pressure to validate *in situ* degradation estimates. To calculate potential ROI for the project and for the transition product, a conservative 10% adoption rate is used. This means that for ~400 sites, there could be 40 within DoN that will use isotopic confirmation to verify on-site degradation estimates. For site costs, we will use the current annual site management costs for Site F and Site A (per RPM, ~$900K per year).

This project's intrinsic value could be estimated using a return on investment (ROI) analysis. While this analysis is more typical in the development and manufacturing industry, it is one of the most commonly applied. While Project #537 demonstrated, validated, and through this report and publication – transitioned stable isotope techniques for confirming *in situ* energetics degradation, RPM, stakeholder and regulator acceptance will take time. Transition(s) through peer-reviewed publications, OER2 webinar(s), TIPS and RITS seminar series and through other workgroups are planned. For these to be effective, word will have to get – and be – out. For this reason, ROI calculations have been made with several out-year horizons, assuming increasing acceptance. Additionally, some assumptions must be made for the isotope technology value relative to typical multiple lines of evidence strategies to convince regulators and stakeholders that site management objectives are being met. This is difficult to estimate because we don't have an accurate estimate for sites needing confirmational analysis. We do know that the two sites used in this project were prime candidates because state and federal regulators were not convinced that PPT biostimulation and bioaugmentation data alone were accurate and representative. As with other assessment technologies (or multiple lines of evidence approaches) uncertainty can always be suggested because measurements are indirect and unable to tie observation(s) to the contaminant's elemental backbone (carbon or nitrogen).

With those caveats in mind, various metrics for a ROI calculation can be estimated (Table 7). What becomes most difficult to estimate is the cost difference between this project's technology and "standard" multiple lines of evidence approaches which generally require chemical concentration analyses, nutrient analysis, redox and other physical analyses and may require multiple wells being analyzed to refine site conceptual model(s). In terms of annual monitoring costs, the sites used in this study average ~$120K per year (per RPM). This is for a large suite of analyses (multiple lines of evidence approach). If bi-annual sampling were pared to determine conversion via stable isotope-labeled contaminants, costs could be reduced to ~$19K per each round of sampling. At the most, $100K could be saved in annual monitoring costs. Again – this is if the regulators and stakeholders want only to know the fate of RDX or other energetic contaminant over time (presuming that is the only regulatory driver). There is no reliable means to estimate acceptance for new techniques and technologies. The PI's experience is that several RPMs who actively follow the RITS, ARTT, and other transition forums will gain interest in technologies which solve their regulatory issues. As an approximate acceptance rate, we estimate 10% of RPMs will adopt the technology to satisfy regulators and stakeholder concerns that energetics are being converted to CO_2 or other mineralization products on -site (Table 7).

Table 9. ROI calculation data for stable isotope technology

Metric	Value	Ref
DoD sites	1,400	(*1*)
MC contamination	10% (400)	(2)
Annual per-site cost	$115K	Site F, A RPM
Wells sampled to confirm degradation	5	This report
Estimated acceptance (RPMs, stakeholders, regulators	10%	This report
Typical per-well cost for low-flow sampling and analysis	$1,500	*This report
Cost for isotope tracer	$525	This report
Cost to develop and validate this technology (Project #537)	$653K	ςThis report

*Based on personal communication by 3 Navy RPMs and PI ESTCP Project
ςLow estimate because PPTs were supported also by USACE and RPM

With those estimates in hand, we can calculate an initial DoD-wide and Navy return on investment (ROI) for the development effort as:

$$(4)\ ROI_{DoD} = \frac{[1{,}400 \text{ sites} * 10\% \text{ site adoption} * (\$120K_{current} - \$19K_{isotope\ only})] - \$653K}{\$653K}$$

$$(5)\ ROI_{Navy} = \frac{[400 \text{ sites} * 10\% \text{ site adoption} * (\$120K_{current} - \$19K_{isotope\ only})] - \$653K}{\$653K}$$

The ROI_{DoD} (4) comes to 20.6% and the ROI_{Navy} (5) comes to 5.2 %. These ROI estimates are for "one" year site adoption for the technology, but we hope that increasing adoption over time might lead to increased ROI as more and more site managers adopt the technology. For instance, because the technology was fielded at two sites in a three-year effort, the initial year 1-3 ROI would be reflected in calculations (4) and (5). However, after the current effort, cost subtraction for investment would be zero (0), so substituting $0 for $653 in the numerator of equations (4) and (5) yields increasing ROI for every year post-study that the technology is used (*e.g.* ROI_{DoD} = 22% and ROI_{Navy} = 6.2). If savings were amortized each year, ROI would increase in both scenarios. We would expect site closures to decrease the number of potential sites over time, so those forcing factors could balance.

Unknowns in this analysis include actual numbers of sites, true RPM and regulator acceptance and true cost reductions using the transitioned technology. As with any dem/val project, we are providing the data to the RPM community so that informed decisions may be made to guide field efforts. The main advantage – as stated – is that this technology provides irrefutable evidence that the energetic's elemental backbone is being converted to a harmless end product *in situ*. No single or combination of indirect measurements can confirm this with the same certainty. From our work over the years with RPMs and regulators, this single piece of information appears to be critically valuable, so isotopic techniques appear to have a new, novel and extremely valuable place in the RPM and site manager's arsenal for developing and validating site conceptual models.

8. CONCLUSIONS, RECOMMENDATIONS, AND IMPLEMENTATION ISSUES

Stable isotopes were used to assess RDX mineralization from groundwater samples amended with ^{13}C-labled RDX. Mineralization products and their stable carbon isotope ratios (CO_2, CH_4), dilution corrections and estimates for efficiency were used to determine the RDX degradation equivalents at each time-point during push-pull tests designed to assess activity under various conditions (in this demonstration, biostimulation). Additionally, this demonstration was conducted in conjunction with NESDI Project #544 (PIs Popovic and Tobias). Limited data from this collaboration are provided in this report but joint studies will be published in the peer-reviewed literature when final analysis is complete. Multiple samplings over time after PPTs at multiple wells per site allowed us to evaluate the technology with different hydrogeologies and treatment conditions. The main strengths of this technology are:

- Able to estimate *in situ* degradation by directly measuring conversion to mineralization products (CO_2, CH_4),
- Confirming degradation by tracking isotopic signature within the contaminant backbone,
- Potentially replacing multiple lines of evidence measures with a single definitive analysis.

Vetting the technology by validation against traditional PPTs allowed placing results within the context of traditionally-applied methods. The regulatory drivers required confirming contaminant degradation which was not previously possible using indirect measures (concentration change, redox, transient intermediates). In this respect, the demonstration's goals were met and results have been tacitly accepted by regulators.

There were quite a few lessons learned in validating the technology. Initially, we planned to do a smaller release and track the isotope signature into mineralization pools. The unstimulated on-site biodegradation at both sites is very low. It would have been difficult to measure (using any techniques). PPTs allow one to add substrate (biostimulant) to impact the microbial communities. The PPTs allow stimulation over a zone at the well which increases in size with increasing water mass pushed in. This required larger initial isotopic releases (grams as opposed to milligrams). This increased the initial costs but allowed a more comprehensive validation. Having a dual-labeled preparation also increased costs. For ^{13}C-RDX alone, costs actually scale well as a 10g preparation is ~$8K while 1 mg costs $525. Depending on the scale necessary or desired, transition for this technology carries a reasonable cost-benefit ratio. On small scales, an initial tracer test or careful review of previous slug tests would be advantageous in determining sampling frequency post-release. Sites having very high dilution rates may necessitate frequent post-release sampling while relatively stagnant sites would warrant longer timescales between post-release samplings. The smaller the release, the more potential for isotope dilution – and more potential for "missing" any mineralization product enrichment.

Using in-well CO_2 traps may be a good means for mitigating these limitations. They are inexpensive and able to integrate CO_2 respiration over long time-scales (months if desired). CO_2 thus captured retains the isotopic signature from the respiration source. Deployed long after the PPT completion (several months), in-well CO_2 traps were able to pick up $^{13}CO_2$ enrichment – in one case over a year after the PPT (Figs. 20-21). The negligible cost for making and deploying these traps attractive. Interpretation would require some modeling, but all software tools are open-source and methods are carefully described (*9*).

Another lesson critical for employing the technology is estimating growth efficiency within the natural microbial community. We intended to measure this directly by assaying the cellular biomass for ^{13}C enrichment. Although the sites sampled have reasonably oligotrophic groundwaters, filtering was not possible due to almost immediate clogging. This necessitated performing a reasonably specialized laboratory study to determine growth efficiency. We used ^{14}C-RDX which we had on hand and a designated laboratory capable of supporting radioisotope use. These are not common in the contracting world, so we believe assumptions will need to be made when using the transitioned technology. While uncertainty can be promulgated into final utilization kinetics, it may bear fruit to attempt filtration for "new" sites as the technology is fielded. A small-scale filtration test could be done before the isotopic release to assess efficacy.

Ultimately, the technology has been validated and merely by the specificity (enriched ^{13}C-RDX has to be added and the actual atoms from the molecule can be definitively tracked), one can absolutely confirm biological RDX transformation occurring *in situ*. No combination of other analyses can absolutely confirm with this degree of certainty. While the ROI for this project may be seen as initially low, the minimal expense for deploying the validated methods in more limited fashion (small targeted releases vice PPTs) should allow future adoptions to be done far less expensively.

9. REFERENCES

References

1. J. Pichtel, Distribution and Fate of Military Explosives and Propellants in Soil: A Review. *Applied and Environmental Soil Science* **2012**, 617236 (2012).
2. *Department of the Navy Enrionmental Restoration Program (DON ERP) manual* (2018).
3. U. EPA, "Techical Fact Sheet - Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)," *US EPA* (US EPA, Washington, DC, 2017).
4. C. M. Aelion, B. C. Kirtland, P. A. Stone, Radiocarbon assessment of aerobic petroleum bioremediation in the vadose zone and groundwater at an AS/SVE site. *Environmental Science and Technology* **31**, 3363-3370 (1997).
5. B. C. Kirtland, C. M. Aelion, P. A. Stone, Assessing *in situ* mineralization of recalcitrant organic compounds in vadose zone sediments using $\delta^{13}C$ and $\Delta^{14}C$ measurements. *Journal of Contaminant Hydrology* **76**, 1-18 (2005).
6. R. B. Coffin *et al.*, Radiocarbon and Stable Carbon Isotope Analysis to Confirm Petroleum Natural Attenuation in the Vadose Zone. *Environmental Forensics* **9**, 75-84 (2008).
7. T. J. Boyd, M. J. Pound, D. Lohr, R. B. Coffin, Radiocarbon-depleted CO_2 evidence for fuel biodegradation at the Naval Air Station North Island (USA) fuel farm site. *Environmental Science: Processes & Impacts* **15**, 912-918 (2013).
8. T. J. Boyd, M. T. Montgomery, R. H. Cuenca, Y. Hagimoto, Combined radiocarbon and CO_2 flux measurements used to determine *in situ* chlorinated solvent mineralization rate. *Environmental Science: Processes & Impacts* **17**, 683 - 692 (2015).
9. T. J. Boyd, M. T. Montgomery, R. H. Cuenca, Y. Hagimoto, Measuring Carbon-based Contaminant Mineralization Using Combined CO_2 Flux and Radiocarbon Analyses. *Journal of Visualized Experiments*, e53233 (2016).
10. B. Fry, *Stable Isotope Ecology*. (Springer Science+Business Media, LLC, New York, NY, 2006), pp. 1-308.
11. Z. D. Sharp, *Principles of Stable Isotope Geochemistry*. (Pearson Eduction, Inc., Upper Saddle River, NJ, ed. 2nd, 2017).
12. J. D. Howa, M. J. Lott, L. A. Chesson, J. R. Ehleringer, Carbon and nitrogen isotope ratios of factory-produced RDX and HMX. *Forensic Science International* **240**, 80-87 (2014).
13. J. Istok, M. Humphrey, M. Schroth, M. Hyman, K. O'Reilly, Single-well,"push-pull" test for in situ determination of microbial activities. *Ground Water* **35**, 619-631 (1997).
14. P. Engineering, "Natural Attenuation of Fuel Hydrocarbons Performance and Cost Results from Multiple Air Force Demonstration Sites," (Parsons Engineering Science, Inc., Denver, CO, 1999).

15. P. Engineering, "Natural Attenuation of Chlorinated Solvents Performance and Cost Results from Multiple Air Force Demonstration Sites," (Parsons Engineering, Inc., Denver, CO, 1999).
16. P. Engineering, "Principles and practices of enhanced anaerobic bioremeidation of chlorinated solvents," (2004).
17. D. Hunkeler, R. U. Meckenstock, B. S. Lollar, T. C. Schmidt, J. T. Wilson, "A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA)," (EPA Office of Research and Development, Ada, OK, 2008).
18. M. M. Michalsen *et al.*, Evaluation of biostimulation and bioaugmentation to stimulate hexahydro-1,3,5-trinitro-1,3,5,-triazine degradation in an aerobic groundwater aquifer. *Environmental Science and Technology* **50**, 7625-7632 (2016).
19. M. M. Michalsen *et al.*, Spatially-distinct redox conditions and degradation rates following field-scale bioaugmentation for RDX-contaminated groundwater remediation. *Journal of Hazardous Materials* **387**, 121529 (2020).
20. M. M. Michalsen *et al.*, Push-pull tests for estimating rdx and tnt degradation rates in groundwater. *Groundwater Monitoring and Remediation* **33**, 61-68 (2013).
21. T. B. Coplen, Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry* **25**, 2538-2560 (2011).
22. M. E. Fuller *et al.*, Relating carbon and nitrogen isotope effects to reaction mechanisms during aerobic or anaerobic degradation of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) by pure bacterial cultures. *Applied and Environmental Microbiology* **82**, 3297-3309 (2016).
23. G. Zhang, H. Yu, X. Fan, J. Ma, H. Xu, Carbon isotope fractionation reveals distinct process of CH_4 emission from different compartments of paddy ecosystem. *Scientific Reports* **6**, 27065 (2016).
24. R. Botz, H.-D. Pokojski, M. Schmitt, M. Thomm, Carbon isotope fractionation during bacterial methanogenesis by CO2 reduction. *Organic Geochemistry* **25**, 255-262 (1996).
25. T. J. Boyd *et al.*, in *Microbial Ecology Research Trends,* T. van Dijk, Ed. (Nova Science Publishers, Inc., Hauppauge, NY, 2008), pp. 1-38.
26. D. C. Smith, F. Azam, A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3 H-leucine. *Mar.Microb.Food Webs* **6**, 107-114 (1992).
27. R. Hofmann, C. Griebler, DOM and bacterial growth efficiency in oligotrophic groundwater: absence of priming and co-limitation by organic carbon and phosphorus. *Aquatic Microbial Ecology* **81**, 55-71 (2018).

APPENDICES

Appendix A: Points of Contact

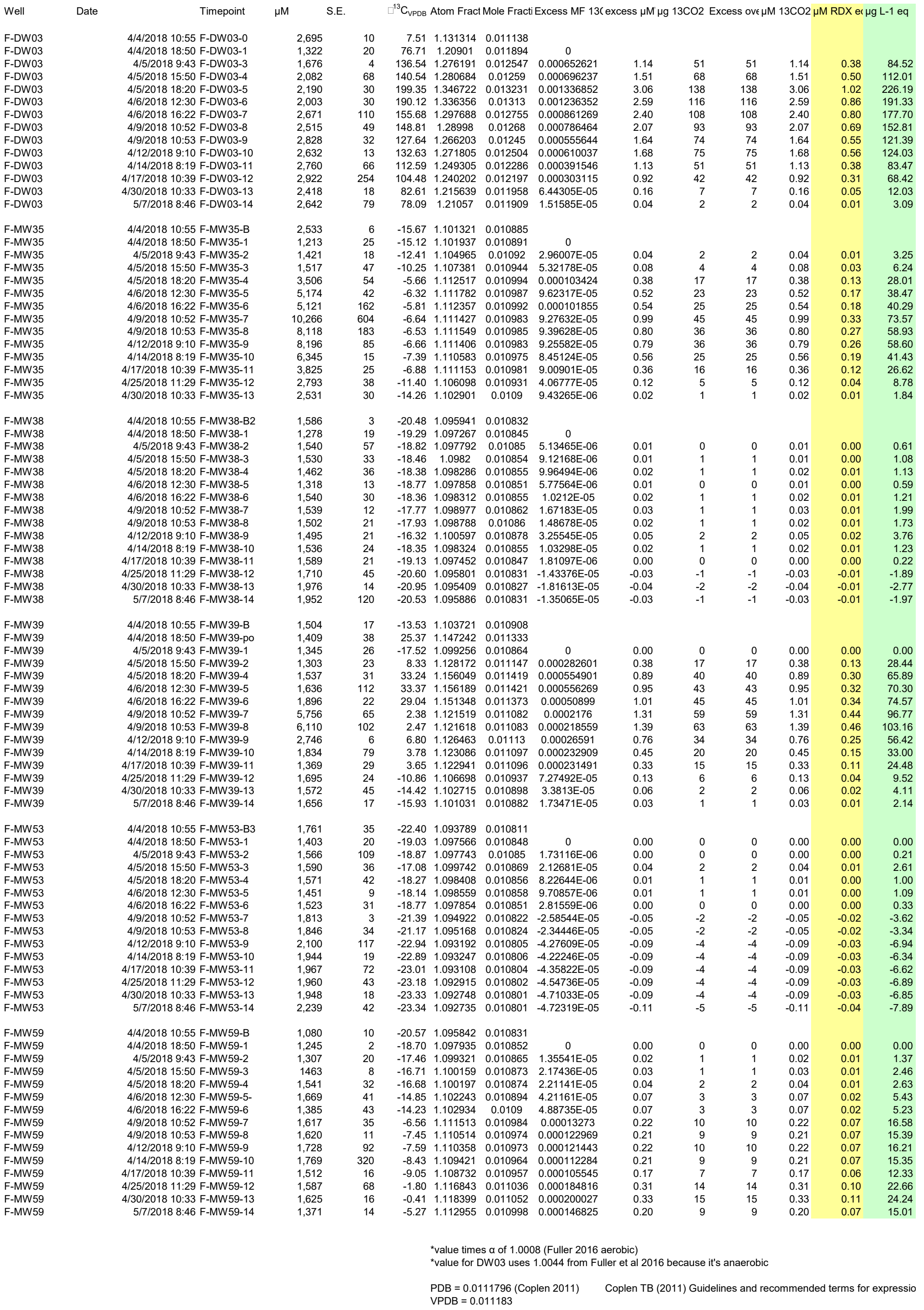
List all

Name	Organization	Phone	E-mail	Role in Project
Thomas J Boyd	US NRL	202-404-6424	thomas.boyd@nrl.navy.mil	PI
Richard H Cuenca	HEI/OR State	703-304-2692	cuenca@oregonstate.edu	Co-I
Yutaka Hagimoto	HEI	541-914-5373	hagimoty@gmail.com	Co-I
Mandy M. Michalsen	USACE	206-764-3324	Mandy.M.Michalsen@usace.army.mil	Collab
Craig Tobias	UConn	860-405-9140	craig.tobias@uconn.edu	Collab
Jovan Popovic	EXWC	815-212-3214	jovan.popovic@navy.mil	Collab
Tom Goodlin	Sealaska	206-842-4247	thomas.goodlin@sealaska.com	Collab
Malcolm J Gander	Navfac	360-315-2804	malcolm.gander@navy.mil	RPM

Appendix B. Spreadsheet data

Spreadsheets showing all field data and calculations are embedded here. Clicking will open excel for full viewing.

Site F DIC



Well	Date	Timepoint	µM	S.E.	□^{13}C$_{VPDB}$	Atom Fract	Mole Fracti	Excess MF 13(	excess µM	µg 13CO2	Excess ove	µM 13CO2	µM RDX ec	µg L-1 eq
F-DW03	4/4/2018 10:55	F-DW03-0	2,695	10	7.51	1.131314	0.011138							
F-DW03	4/4/2018 18:50	F-DW03-1	1,322	20	76.71	1.20901	0.011894	0						
F-DW03	4/5/2018 9:43	F-DW03-3	1,676	4	136.54	1.276191	0.012547	0.000652621	1.14	51	51	1.14	0.38	84.52
F-DW03	4/5/2018 15:50	F-DW03-4	2,082	68	140.54	1.280684	0.01259	0.000696237	1.51	68	68	1.51	0.50	112.01
F-DW03	4/5/2018 18:20	F-DW03-5	2,190	30	199.35	1.346722	0.013231	0.001336852	3.06	138	138	3.06	1.02	226.19
F-DW03	4/6/2018 12:30	F-DW03-6	2,003	30	190.12	1.336356	0.01313	0.001236352	2.59	116	116	2.59	0.86	191.33
F-DW03	4/6/2018 16:22	F-DW03-7	2,671	110	155.68	1.297688	0.012755	0.000861269	2.40	108	108	2.40	0.80	177.70
F-DW03	4/9/2018 10:52	F-DW03-8	2,515	49	148.81	1.28998	0.01268	0.000786464	2.07	93	93	2.07	0.69	152.81
F-DW03	4/9/2018 10:53	F-DW03-9	2,828	32	127.64	1.266203	0.01245	0.000555644	1.64	74	74	1.64	0.55	121.39
F-DW03	4/12/2018 9:10	F-DW03-10	2,632	13	132.63	1.271805	0.012504	0.000610037	1.68	75	75	1.68	0.56	124.03
F-DW03	4/14/2018 8:19	F-DW03-11	2,760	66	112.59	1.249305	0.012286	0.000391546	1.13	51	51	1.13	0.38	83.47
F-DW03	4/17/2018 10:39	F-DW03-12	2,922	254	104.48	1.240202	0.012197	0.000303115	0.92	42	42	0.92	0.31	68.42
F-DW03	4/30/2018 10:33	F-DW03-13	2,418	18	82.61	1.215639	0.011958	6.44305E-05	0.16	7	7	0.16	0.05	12.03
F-DW03	5/7/2018 8:46	F-DW03-14	2,642	79	78.09	1.21057	0.011909	1.51585E-05	0.04	2	2	0.04	0.01	3.09
F-MW35	4/4/2018 10:55	F-MW35-B	2,533	6	-15.67	1.101321	0.010885							
F-MW35	4/4/2018 18:50	F-MW35-1	1,213	25	-15.12	1.101937	0.010891	0						
F-MW35	4/5/2018 9:43	F-MW35-2	1,421	18	-12.41	1.104965	0.01092	2.96007E-05	0.04	2	2	0.04	0.01	3.25
F-MW35	4/5/2018 15:50	F-MW35-3	1,517	47	-10.25	1.107381	0.010944	5.32178E-05	0.08	4	4	0.08	0.03	6.24
F-MW35	4/5/2018 18:20	F-MW35-4	3,506	54	-5.66	1.112517	0.010994	0.000103424	0.38	17	17	0.38	0.13	28.01
F-MW35	4/6/2018 12:30	F-MW35-5	5,174	42	-6.32	1.111782	0.010987	9.62317E-05	0.52	23	23	0.52	0.17	38.47
F-MW35	4/6/2018 16:22	F-MW35-6	5,121	162	-5.81	1.112357	0.010992	0.000101855	0.54	25	25	0.54	0.18	40.29
F-MW35	4/9/2018 10:52	F-MW35-7	10,266	604	-6.64	1.111427	0.010983	9.27632E-05	0.99	45	45	0.99	0.33	73.57
F-MW35	4/9/2018 10:53	F-MW35-8	8,118	183	-6.53	1.111549	0.010985	9.39628E-05	0.80	36	36	0.80	0.27	58.93
F-MW35	4/12/2018 9:10	F-MW35-9	8,196	85	-6.66	1.111406	0.010983	9.25582E-05	0.79	36	36	0.79	0.26	58.60
F-MW35	4/14/2018 8:19	F-MW35-10	6,345	15	-7.39	1.110583	0.010975	8.45124E-05	0.56	25	25	0.56	0.19	41.43
F-MW35	4/17/2018 10:39	F-MW35-11	3,825	25	-6.88	1.111153	0.010981	9.00901E-05	0.36	16	16	0.36	0.12	26.62
F-MW35	4/25/2018 11:29	F-MW35-12	2,793	38	-11.40	1.106098	0.010931	4.06777E-05	0.12	5	5	0.12	0.04	8.78
F-MW35	4/30/2018 10:33	F-MW35-13	2,531	30	-14.26	1.102901	0.0109	9.43265E-06	0.02	1	1	0.02	0.01	1.84
F-MW38	4/4/2018 10:55	F-MW38-B2	1,586	3	-20.48	1.095941	0.010832							
F-MW38	4/4/2018 18:50	F-MW38-1	1,278	19	-19.29	1.097267	0.010845	0						
F-MW38	4/5/2018 9:43	F-MW38-2	1,540	57	-18.82	1.097792	0.01085	5.13465E-06	0.01	0	0	0.01	0.00	0.61
F-MW38	4/5/2018 15:50	F-MW38-3	1,530	33	-18.46	1.0982	0.010854	9.12168E-06	0.01	1	1	0.01	0.00	1.08
F-MW38	4/5/2018 18:20	F-MW38-4	1,462	36	-18.38	1.098286	0.010855	9.96494E-06	0.02	1	1	0.02	0.01	1.13
F-MW38	4/6/2018 12:30	F-MW38-5	1,318	13	-18.77	1.097858	0.010851	5.77564E-06	0.01	0	0	0.01	0.00	0.59
F-MW38	4/6/2018 16:22	F-MW38-6	1,540	30	-18.36	1.098312	0.010855	1.0212E-05	0.02	1	1	0.02	0.01	1.21
F-MW38	4/9/2018 10:52	F-MW38-7	1,539	12	-17.77	1.098977	0.010862	1.67183E-05	0.03	1	1	0.03	0.01	1.99
F-MW38	4/9/2018 10:53	F-MW38-8	1,502	21	-17.93	1.098788	0.01086	1.48678E-05	0.02	1	1	0.02	0.01	1.73
F-MW38	4/12/2018 9:10	F-MW38-9	1,495	21	-16.32	1.100597	0.010878	3.25545E-05	0.05	2	2	0.05	0.02	3.76
F-MW38	4/14/2018 8:19	F-MW38-10	1,536	24	-18.35	1.098324	0.010855	1.03298E-05	0.02	1	1	0.02	0.01	1.23
F-MW38	4/17/2018 10:39	F-MW38-11	1,589	21	-19.13	1.097452	0.010847	1.81097E-06	0.00	0	0	0.00	0.00	0.22
F-MW38	4/25/2018 11:29	F-MW38-12	1,710	45	-20.60	1.095801	0.010831	-1.43376E-05	-0.03	-1	-1	-0.03	-0.01	-1.89
F-MW38	4/30/2018 10:33	F-MW38-13	1,976	14	-20.95	1.095409	0.010827	-1.81613E-05	-0.04	-2	-2	-0.04	-0.01	-2.77
F-MW38	5/7/2018 8:46	F-MW38-14	1,952	120	-20.53	1.095886	0.010831	-1.35065E-05	-0.03	-1	-1	-0.03	-0.01	-1.97
F-MW39	4/4/2018 10:55	F-MW39-B	1,504	17	-13.53	1.103721	0.010908							
F-MW39	4/4/2018 18:50	F-MW39-po	1,409	38	25.37	1.147242	0.011333							
F-MW39	4/5/2018 9:43	F-MW39-1	1,345	26	-17.52	1.099256	0.010864	0	0.00	0	0	0.00	0.00	0.00
F-MW39	4/5/2018 15:50	F-MW39-2	1,303	23	8.33	1.128172	0.011147	0.000282601	0.38	17	17	0.38	0.13	28.44
F-MW39	4/5/2018 18:20	F-MW39-4	1,537	31	33.24	1.156049	0.011419	0.000554901	0.89	40	40	0.89	0.30	65.89
F-MW39	4/6/2018 12:30	F-MW39-5	1,636	112	33.37	1.156189	0.011421	0.000556269	0.95	43	43	0.95	0.32	70.30
F-MW39	4/6/2018 16:22	F-MW39-6	1,896	22	29.04	1.151348	0.011373	0.00050899	1.01	45	45	1.01	0.34	74.57
F-MW39	4/9/2018 10:52	F-MW39-7	5,756	65	2.38	1.121519	0.011082	0.0002176	1.31	59	59	1.31	0.44	96.77
F-MW39	4/9/2018 10:53	F-MW39-8	6,110	102	2.47	1.121618	0.011083	0.000218559	1.39	63	63	1.39	0.46	103.16
F-MW39	4/12/2018 9:10	F-MW39-9	2,746	6	6.80	1.126463	0.01113	0.00026591	0.76	34	34	0.76	0.25	56.42
F-MW39	4/14/2018 8:19	F-MW39-10	1,834	79	3.78	1.123086	0.011097	0.000232909	0.45	20	20	0.45	0.15	33.00
F-MW39	4/17/2018 10:39	F-MW39-11	1,369	29	3.65	1.122941	0.011096	0.000231491	0.33	15	15	0.33	0.11	24.48
F-MW39	4/25/2018 11:29	F-MW39-12	1,695	24	-10.86	1.106698	0.010937	7.27492E-05	0.13	6	6	0.13	0.04	9.52
F-MW39	4/30/2018 10:33	F-MW39-13	1,572	45	-14.42	1.102715	0.010898	3.3813E-05	0.06	2	2	0.06	0.02	4.11
F-MW39	5/7/2018 8:46	F-MW39-14	1,656	17	-15.93	1.101031	0.010882	1.73471E-05	0.03	1	1	0.03	0.01	2.14
F-MW53	4/4/2018 10:55	F-MW53-B3	1,761	35	-22.40	1.093789	0.010811							
F-MW53	4/4/2018 18:50	F-MW53-1	1,403	20	-19.03	1.097566	0.010848	0	0.00	0	0	0.00	0.00	0.00
F-MW53	4/5/2018 9:43	F-MW53-2	1,566	109	-18.87	1.097743	0.01085	1.73116E-06	0.00	0	0	0.00	0.00	0.21
F-MW53	4/5/2018 15:50	F-MW53-3	1,590	36	-17.08	1.099742	0.010869	2.12681E-05	0.04	2	2	0.04	0.01	2.61
F-MW53	4/5/2018 18:20	F-MW53-4	1,571	42	-18.27	1.098408	0.010856	8.22644E-06	0.01	1	1	0.01	0.00	1.00
F-MW53	4/6/2018 12:30	F-MW53-5	1,451	9	-18.14	1.098559	0.010858	9.70857E-06	0.01	1	1	0.01	0.00	1.09
F-MW53	4/6/2018 16:22	F-MW53-6	1,523	31	-18.77	1.097854	0.010851	2.81559E-06	0.00	0	0	0.00	0.00	0.33
F-MW53	4/9/2018 10:52	F-MW53-7	1,813	3	-21.39	1.094922	0.010822	-2.58544E-05	-0.05	-2	-2	-0.05	-0.02	-3.62
F-MW53	4/9/2018 10:53	F-MW53-8	1,846	34	-21.17	1.095168	0.010824	-2.34446E-05	-0.05	-2	-2	-0.05	-0.02	-3.34
F-MW53	4/12/2018 9:10	F-MW53-9	2,100	117	-22.94	1.093192	0.010805	-4.27609E-05	-0.09	-4	-4	-0.09	-0.03	-6.94
F-MW53	4/14/2018 8:19	F-MW53-10	1,944	19	-22.89	1.093247	0.010806	-4.22246E-05	-0.09	-4	-4	-0.09	-0.03	-6.34
F-MW53	4/17/2018 10:39	F-MW53-11	1,967	72	-23.01	1.093108	0.010804	-4.35822E-05	-0.09	-4	-4	-0.09	-0.03	-6.62
F-MW53	4/25/2018 11:29	F-MW53-12	1,960	43	-23.18	1.092915	0.010802	-4.54736E-05	-0.09	-4	-4	-0.09	-0.03	-6.89
F-MW53	4/30/2018 10:33	F-MW53-13	1,948	18	-23.33	1.092748	0.010801	-4.71033E-05	-0.09	-4	-4	-0.09	-0.03	-6.85
F-MW53	5/7/2018 8:46	F-MW53-14	2,239	42	-23.34	1.092735	0.010801	-4.72319E-05	-0.11	-5	-5	-0.11	-0.04	-7.89
F-MW59	4/4/2018 10:55	F-MW59-B	1,080	10	-20.57	1.095842	0.010831							
F-MW59	4/4/2018 18:50	F-MW59-1	1,245	2	-18.70	1.097935	0.010852	0	0.00	0	0	0.00	0.00	0.00
F-MW59	4/5/2018 9:43	F-MW59-2	1,307	20	-17.46	1.099321	0.010865	1.35541E-05	0.02	1	1	0.02	0.01	1.37
F-MW59	4/5/2018 15:50	F-MW59-3	1463	8	-16.71	1.100159	0.010873	2.17436E-05	0.03	1	1	0.03	0.01	2.46
F-MW59	4/5/2018 18:20	F-MW59-4	1,541	32	-16.68	1.100197	0.010874	2.21141E-05	0.04	2	2	0.04	0.01	2.63
F-MW59	4/6/2018 12:30	F-MW59-5-	1,669	41	-14.85	1.102243	0.010894	4.21161E-05	0.07	3	3	0.07	0.02	5.43
F-MW59	4/6/2018 16:22	F-MW59-6	1,385	43	-14.23	1.102934	0.0109	4.88735E-05	0.07	3	3	0.07	0.02	5.23
F-MW59	4/9/2018 10:52	F-MW59-7	1,617	35	-6.56	1.111513	0.010984	0.00013273	0.22	10	10	0.22	0.07	16.58
F-MW59	4/9/2018 10:53	F-MW59-8	1,620	11	-7.45	1.110514	0.010974	0.000122969	0.21	9	9	0.21	0.07	15.39
F-MW59	4/12/2018 9:10	F-MW59-9	1,728	92	-7.59	1.110358	0.010973	0.000121443	0.22	10	10	0.22	0.07	16.21
F-MW59	4/14/2018 8:19	F-MW59-10	1,769	320	-8.43	1.109421	0.010964	0.000112284	0.21	9	9	0.21	0.07	15.35
F-MW59	4/17/2018 10:39	F-MW59-11	1,512	16	-9.05	1.108732	0.010957	0.000105545	0.17	7	7	0.17	0.06	12.33
F-MW59	4/25/2018 11:29	F-MW59-12	1,587	68	-1.80	1.116843	0.011036	0.000184816	0.31	14	14	0.31	0.10	22.66
F-MW59	4/30/2018 10:33	F-MW59-13	1,625	16	-0.41	1.118399	0.011052	0.000200027	0.33	15	15	0.33	0.11	24.24
F-MW59	5/7/2018 8:46	F-MW59-14	1,371	14	-5.27	1.112955	0.010998	0.000146825	0.20	9	9	0.20	0.07	15.01

*value times α of 1.0008 (Fuller 2016 aerobic)
*value for DW03 uses 1.0044 from Fuller et al 2016 because it's anaerobic

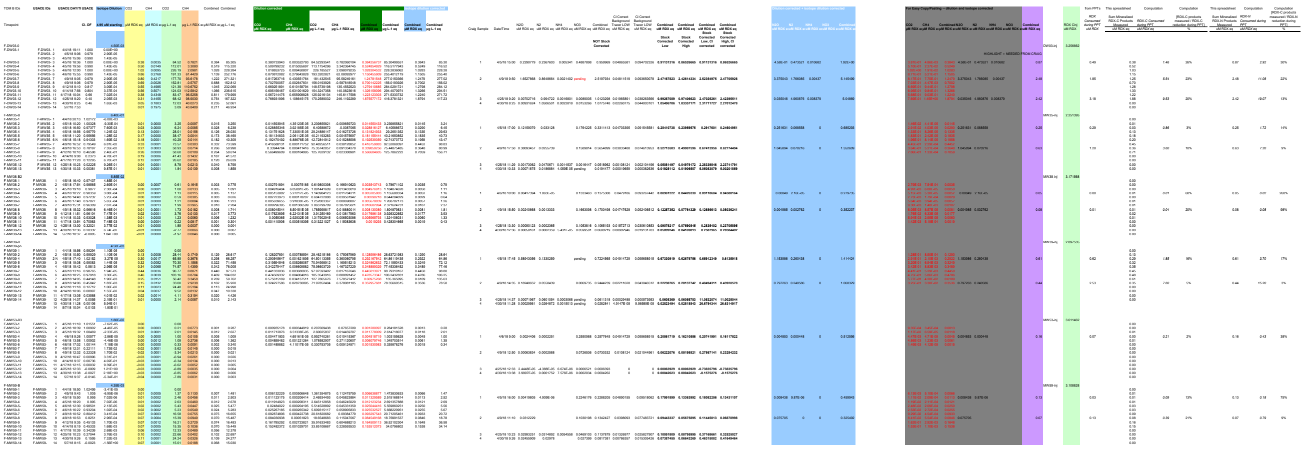
PDB = 0.0111796 (Coplen 2011)   Coplen TB (2011) Guidelines and recommended terms for expressio
VPDB = 0.011183

Site F methane

Site	Sample name	SIF sample	Sample	Sample date/Time	dilution	ppm	P	V	R	T	Moles	Moles/L	Moles / 30	Mol L-1 in	uM orig	ug L-1	$\delta^{13}C_{VPDB}$	Atom Frac	Mol Fracti	Excess MF	Excess MF	μM - 13CH	μM RDX ec	μg L-1 RDX
F-DW-03	F-DW03-B	NRL-M-076	F-DW-03B	4/4/2018 9:31		5039.21	0.005039	1.00E-03	0.08206	298.15	2.06E-07	2.06E-04	6.18E-06	2.06E-04	205.97	3,295.46	-36.74	1.13108	0.010654					
F-DW-03	F-DW03-1	NRL-M-077	F-DW-03 -1	4/4/2018 19:11		579.05	0.000579	1.00E-03	0.08206	298.15	2.37E-08	2.37E-05	7.10E-07	2.37E-05	23.67	378.68	-10.15	1.162293	0.010945	0	0			
F-DW-03	F-DW03-3	NRL-M-078	F-DW-03 -3	4/5/2018 18:36		920.36	0.00092	1.00E-03	0.08206	298.15	3.76E-08	3.76E-05	1.13E-06	3.76E-05	37.62	601.88	14.32	1.191029	0.011213	0.000268	0.000281	0.01	0.00	0.7821
F-DW-03	F-DW03-4	NRL-M-079	F-DW-03-4 4/05/18	4/5/2018 19:56		540.73	0.000541	1.00E-03	0.08206	298.15	2.21E-08	2.21E-05	6.63E-07	2.21E-05	22.10	353.62	166.33	1.369525	0.012871	0.001926	0.002023	0.04	0.01	3.3080
F-DW-03	F-DW03-5	NRL-M-080	F-DW-03-5 4/06/18	4/6/2018 12:00		1287.43	0.001287	1.00E-03	0.08206	298.15	5.26E-08	5.26E-05	1.58E-06	5.26E-05	52.62	841.93	36.79	1.217418	0.011458	0.000513	0.000539	0.03	0.01	2.0981
F-DW-03	F-DW03-6	NRL-M-081	F-DW-03-6 4/06/18	4/6/2018 15:55		1785.96	0.001786	1.00E-03	0.08206	298.15	7.3E-08	7.30E-05	2.19E-06	7.30E-05	73.00	1,167.96	991.37	2.338294	0.021778	0.010833	0.011375	0.83	0.28	61.4429
F-DW-03	F-DW03-7	NRL-M-082	F-DW-03-7 4/09/18	4/9/2018 9:05	744.01	7440.06	0.00744	1.00E-03	0.08206	298.15	3.04E-07	3.04E-04	9.12E-06	3.04E-04	304.10	4,865.53	353.60	1.589414	0.014907	0.003962	0.00416	1.27	0.42	93.6178
F-DW-03	F-DW03-8	NRL-M-083	F-DW-03-8 4/09/18	4/9/2018 9:06	617.18	6171.84	0.006172	1.00E-03	0.08206	298.15	2.52E-07	2.52E-04	7.57E-06	2.52E-04	252.26	4,036.16	-12.84	1.159139	0.010916	-2.9E-05	-3.1E-05	-0.01	0.00	-0.5757
F-DW-03	F-DW03-9	NRL-M-084	F-DW-03-9 4/12/18	4/12/2018 9:10	1376.01	13760.13	0.01376	1.00E-03	0.08206	298.15	5.62E-07	5.62E-04	1.69E-05	5.62E-04	562.41	8,998.63	222.02	1.434918	0.013478	0.002533	0.002659	1.50	0.50	110.6752
F-DW-03	F-DW03-10	NRL-M-085	F-DW-03-10 4/12/1	4/14/2018 7:56	1459.45	14594.46	0.014594	1.00E-03	0.08206	298.15	5.97E-07	5.97E-04	1.79E-05	5.97E-04	596.52	9,544.25	212.50	1.423739	0.013374	0.002429	0.00255	1.52	0.51	112.5842
F-DW-03	F-DW03-11	NRL-M-086	F-DW-03-11 4/17/1	4/17/2018 10:04	1763.97	17639.66	0.01764	1.00E-03	0.08206	298.15	7.21E-07	7.21E-04	2.16E-05	7.21E-04	720.98	11,535.70	147.68	1.347618	0.012668	0.001723	0.001809	1.30	0.43	96.5258
F-DW-03	F-DW03-12	NRL-M-087	F-DW03-12	4/25/2018 9:20		22996.82	0.022997	1.00E-03	0.08206	298.15	9.4E-07	9.40E-04	2.82E-05	9.40E-04	939.94	15,039.09	113.84	1.307893	0.012299	0.001354	0.001422	1.34	0.45	98.9030
F-DW-03	F-DW03-13	NRL-M-088	F-DW03-13	4/30/2018 8:25		13169.79	0.01317	1.00E-03	0.08206	298.15	5.38E-07	5.38E-04	1.61E-05	5.38E-04	538.29	8,612.57	77.44	1.265147	0.011902	0.000957	0.001005	0.54	0.18	40.0273
F-DW-03	F-DW03-14	NRL-M-089	F-DW03-14	5/7/2018 7:53		17598.96	0.017599	1.00E-03	0.08206	298.15	7.19E-07	7.19E-04	2.16E-05	7.19E-04	719.32	11,509.08	61.63	1.24658	0.011729	0.000784	0.000824	0.59	0.20	43.8409
F-MW-35	F-MW35-B	NRL-M-001	F-MW35B	4/4/2018 12:43		4.25	4.25E-06	1.00E-03	0.08206	298.15	1.74E-10	1.74E-07	5.21E-09	1.74E-07	0.17	2.78	-38.71	1.128759	0.010633					
F-MW-35	F-MW35-1	NRL-M-002	F-MW35-1	4/4/2018 20:13		66.41	6.64E-05	1.00E-03	0.08206	298.15	2.71E-09	2.71E-06	8.14E-08	2.71E-06	2.71	43.43	-26.13	1.143531	0.01077	0	0			
F-MW-35	F-MW35-2	NRL-M-003	F-MW35-2	4/5/2018 10:20		9.30	9.3E-06	1.00E-03	0.08206	298.15	3.8E-10	3.80E-07	1.14E-08	3.80E-07	0.38	6.08	-56.13	1.108309	0.010442	-0.00033	-0.00034	0.00	0.0000	-0.0097
F-MW-35	F-MW35-3	NRL-M-004	F-MW35-3	4/5/2018 16:50		9.04	9.04E-06	1.00E-03	0.08206	298.15	3.69E-10	3.69E-07	1.11E-08	3.69E-07	0.37	5.91	-53.12	1.111836	0.010475	-0.0003	-0.00031	0.00	0.0000	-0.0085
F-MW-35	F-MW35-4	NRL-M-005	F-MW35-4 4/05/18	4/5/2018 18:56		5.35	5.35E-06	1.00E-03	0.08206	298.15	2.19E-10	2.19E-07	6.56E-09	2.19E-07	0.22	3.50	57.75	1.242024	0.011687	0.000917	0.000963	0.00	0.0001	0.0156
F-MW-35	F-MW35-5	NRL-M-006	F-MW35-5 4/06/18	4/6/2018 11:20		1.14	1.14E-06	1.00E-03	0.08206	298.15	4.66E-11	4.66E-08	1.40E-09	4.66E-08	0.05	0.75	84.41	1.273327	0.011978	0.001208	0.001268	0.00	0.0000	0.0044
F-MW-35	F-MW35-6	NRL-M-007	F-MW35-6 4/06/18	4/6/2018 15:18		6.10	6.1E-06	1.00E-03	0.08206	298.15	2.49E-10	2.49E-07	7.47E-09	2.49E-07	0.25	3.99	42.00	1.223534	0.011515	0.000745	0.000782	0.00	0.0001	0.0144
F-MW-35	F-MW35-7	NRL-M-008	F-MW35-7 4/09/18	4/9/2018 16:52		6.06	6.06E-06	1.00E-03	0.08206	298.15	2.48E-10	2.48E-07	7.43E-09	2.48E-07	0.25	3.96	118.19	1.312997	0.012347	0.001576	0.001655	0.00	0.0001	0.0303
F-MW-35	F-MW35-8	NRL-M-009	F-MW35-8 4/09/18	4/9/2018 16:53		13.50	1.35E-05	1.00E-03	0.08206	298.15	5.52E-10	5.52E-07	1.65E-08	5.52E-07	0.55	8.83	126.44	1.322689	0.012437	0.001666	0.00175	0.00	0.0003	0.0714
F-MW-35	F-MW35-9	NRL-M-010	F-MW35-9 4/12/18	4/12/2018 10:10		1.44	1.44E-06	1.00E-03	0.08206	298.15	5.9E-11	5.90E-08	1.77E-09	5.90E-08	0.06	0.94	190.88	1.39835	0.013139	0.002368	0.002487	0.00	0.0000	0.0109
F-MW-35	F-MW35-10	NRL-M-011	F-MW-35-10 04/12/	4/14/2018 9:08		16.98	1.7E-05	1.00E-03	0.08206	298.15	6.94E-10	6.94E-07	2.08E-08	6.94E-07	0.69	11.10	217.41	1.429498	0.013427	0.002657	0.00279	0.00	0.0006	0.1432
F-MW-35	F-MW35-11	NRL-M-012	F-MW35-11 4/17/1	4/17/2018 11:28		2.51	2.51E-06	1.00E-03	0.08206	298.15	1.03E-10	1.03E-07	3.08E-09	1.03E-07	0.10	1.64	186.54	1.393256	0.013091	0.002321	0.002437	0.00	0.0001	0.0185
F-MW-35	F-MW35-12	NRL-M-013	F-MW35-12	4/25/2018 11:29		3.94	3.94E-06	1.00E-03	0.08206	298.15	1.61E-10	1.61E-07	4.84E-09	1.61E-07	0.16	2.58	129.45	1.326212	0.012469	0.001699	0.001784	0.00	0.0001	0.0213
F-MW-35	F-MW35-13	NRL-M-014	F-MW35-13	4/30/2018 10:33		3.894108	3.89E-06	1.00E-03	0.08206	298.15	1.59E-10	1.59E-07	4.77E-09	1.59E-07	0.16	2.55	76.68	1.264255	0.011894	0.001124	0.00118	0.00	0.0001	0.0139
F-MW-35	F-MW35-14	NRL-M-015	F-MW35-14	5/7/2018 8:46		3.067351	3.07E-06	1.00E-03	0.08206	298.15	1.25E-10	1.25E-07	3.76E-09	1.25E-07	0.13	2.01	83.73	1.27253	0.011971	0.0012	0.00126	0.00	0.0001	0.0117
F-MW-38	F-MW38-B	NRL-M-016	F-MW38B	4/4/2018 15:45		2.55	2.55E-06	1.00E-03	0.08206	298.15	1.04E-10	1.04E-07	3.13E-09	1.04E-07	0.10	1.67	-4.25	1.169225	0.01101					
F-MW-38	F-MW38-1	NRL-M-017	F-MW38-1	4/5/2018 13:12		4.81	4.81E-06	1.00E-03	0.08206	298.15	1.97E-10	1.97E-07	5.90E-09	1.97E-07	0.20	3.15	-35.65	1.132351	0.010666	0				
F-MW-38	F-MW38-2	NRL-M-018	F-MW-38-2 04/05/1	4/5/2018 17:54		11.80	1.18E-05	1.00E-03	0.08206	298.15	4.82E-10	4.82E-07	1.45E-08	4.82E-07	0.48	7.72	367.22	1.605413	0.015055	0.004389	0.004608	0.00	0.0007	0.1645
F-MW-38	F-MW38-3	NRL-M-019	F-MW38-3 4/05/18	4/5/2018 19:18		2.98	2.98E-06	1.00E-03	0.08206	298.15	1.22E-10	1.22E-07	3.65E-09	1.22E-07	0.12	1.95	92.67	1.283026	0.012068	0.001402	0.001472	0.00	0.0001	0.0133
F-MW-38	F-MW38-4	NRL-M-020	F-MW38-4 4/06/18	4/6/2018 10:22		2.37	2.37E-06	1.00E-03	0.08206	298.15	9.7E-11	9.70E-08	2.91E-09	9.70E-08	0.10	1.55	104.18	1.296539	0.012194	0.001528	0.001604	0.00	0.0001	0.0115
F-MW-38	F-MW38-5	NRL-M-021	F-MW38-5 4/06/18	4/6/2018 14:40		6.71	6.71E-06	1.00E-03	0.08206	298.15	2.74E-10	2.74E-07	8.23E-09	2.74E-07	0.27	4.39	129.66	1.326461	0.012472	0.001806	0.001896	0.00	0.0002	0.0385
F-MW-38	F-MW38-6	NRL-M-022	F-MW38-6 4/06/18	4/6/2018 17:40		2.75	2.75E-06	1.00E-03	0.08206	298.15	1.13E-10	1.13E-07	3.38E-09	1.13E-07	0.11	1.80	52.62	1.236004	0.011631	0.000965	0.001013	0.00	0.0000	0.0084
F-MW-38	F-MW38-7	NRL-M-023	F-MW-38-7 4/09/18	4/9/2018 15:31		24.27	2.43E-05	1.00E-03	0.08206	298.15	9.92E-10	9.92E-07	2.98E-08	9.92E-07	0.99	15.87	317.25	1.546734	0.014513	0.003847	0.004039	0.00	0.0013	0.2965
F-MW-38	F-MW38-8	NRL-M-024	F-MW-38-8 4/09/18	4/9/2018 15:32		4.42	4.42E-06	1.00E-03	0.08206	298.15	1.81E-10	1.81E-07	5.42E-09	1.81E-07	0.18	2.89	83.28	1.272009	0.011966	0.0013	0.001365	0.00	0.0001	0.0182
F-MW-38	F-MW38-9	NRL-M-025	F-MW-38-9 4/12/18	4/12/2018 11:51		4.09	4.09E-06	1.00E-03	0.08206	298.15	1.67E-10	1.67E-07	5.01E-09	1.67E-07	0.17	2.67	57.89	1.242186	0.011689	0.001022	0.001074	0.00	0.0001	0.0133
F-MW-38	F-MW38-10	NRL-M-026	F-MW-38-10 4/12/1	4/14/2018 10:33		2.77	2.77E-06	1.00E-03	0.08206	298.15	1.13E-10	1.13E-07	3.40E-09	1.13E-07	0.11	1.81	27.21	1.20617	0.011353	0.000687	0.000722	0.00	0.0000	0.0060
F-MW-38	F-MW38-11	NRL-M-027	F-MW38-11 4/17/1	4/17/2018 13:54		45.52	4.55E-05	1.00E-03	0.08206	298.15	1.86E-09	1.86E-06	5.58E-08	1.86E-06	1.86	29.77	16.02	1.19303	0.011231	0.000565	0.000593	0.00	0.0004	0.0817
F-MW-38	F-MW38-12	NRL-M-028	F-MW38-12	4/25/2018 13:30		4.69	4.69E-06	1.00E-03	0.08206	298.15	1.92E-10	1.92E-07	5.75E-09	1.92E-07	0.19	3.07	-12.97	1.158987	0.010914	0.000248	0.000261	0.00	0.0000	0.0037
F-MW-38	F-MW38-13	NRL-M-029	F-MW38-13	4/30/2018 12:36		3.633195	3.63E-06	1.00E-03	0.08206	298.15	1.48E-10	1.48E-07	4.45E-09	1.48E-07	0.15	2.38	16.87	1.19402	0.01124	0.000574	0.000603	0.00	0.0000	0.0066
F-MW-38	F-MW38-14	NRL-M-030	F-MW38-14	5/7/2018 10:37		2.939851	2.94E-06	1.00E-03	0.08206	298.15	1.2E-10	1.20E-07	3.60E-09	1.20E-07	0.12	1.92	9.46	1.185329	0.011159	0.000493	0.000518	0.00	0.0000	0.0046
F-MW-39	FMW39-B	NRL-M-061	FMW-39B	4/4/2018 15:45		188.55	0.000189	1.00E-03	0.08206	298.15	7.71E-09	7.71E-06	2.31E-07	7.71E-06	7.71	123.30	-33.62	1.134735	0.010688					
F-MW-39	F-MW39 post inj	NRL-M-062	F-MW39 post inj	4/4/2018 16:48		101.28	0.000101	1.00E-03	0.08206	298.15	4.14E-09	4.14E-06	1.24E-07	4.14E-06	4.14	66.23	-71.47	1.090289	0.010274	0				
F-MW-39	F-MW39-1	NRL-M-063	F-MW39-1	4/4/2018 18:56		28.43	2.84E-05	1.00E-03	0.08206	298.15	1.16E-09	1.16E-06	3.49E-08	1.16E-06	1.16	18.59	-63.56	1.099583	0.010361	8.67E-05	9.1E-05	0.00	0.0000	0.0078
F-MW-39	F-MW39-2	NRL-M-064	F-MW39-2	4/5/2018 10:50		37.77	3.78E-05	1.00E-03	0.08206	298.15	1.54E-09	1.54E-06	4.63E-08	1.54E-06	1.54	24.70	61.90	1.246893	0.011732	0.001458	0.001531	0.00	0.0008	0.1749
F-MW-39	F-MW39-4	NRL-M-065	F-MW39-4 4/05/18	4/5/2018 19:42		31.42	3.14E-05	1.00E-03	0.08206	298.15	1.28E-09	1.28E-06	3.85E-08	1.28E-06	1.28	20.55	266.34	1.486953	0.01396	0.003686	0.00387	0.00	0.0017	0.3678
F-MW-39	F-MW39-5	NRL-M-066	F-MW39-5 4/06/18	4/6/2018 13:16		49.69	4.97E-05	1.00E-03	0.08206	298.15	2.03E-09	2.03E-06	6.09E-08	2.03E-06	2.03	32.50	604.03	1.88348	0.017617	0.007343	0.00771	0.02	0.0052	1.1588
F-MW-39	F-MW39-6	NRL-M-067	F-MW39-6 4/06/18	4/6/2018 18:25		49.06	4.91E-05	1.00E-03	0.08206	298.15	2.01E-09	2.01E-06	6.02E-08	2.01E-06	2.01	32.08	780.42	2.090596	0.019516	0.009242	0.009704	0.02	0.0065	1.4399
F-MW-39	F-MW39-7	NRL-M-068	F-MW-39-7 4/09/18	4/9/2018 14:05		25.25	2.53E-05	1.00E-03	0.08206	298.15	1.03E-09	1.03E-06	3.10E-08	1.03E-06	1.03	16.52	856.87	2.180366	0.020337	0.010063	0.010566	0.01	0.0036	0.8071
F-MW-39	F-MW39-8	NRL-M-069	F-MW-39-8 4/09/18	4/9/2018 14:06		27.30	2.73E-05	1.00E-03	0.08206	298.15	1.12E-09	1.12E-06	3.35E-08	1.12E-06	1.12	17.85	854.67	2.177779	0.020313	0.010039	0.010541	0.01	0.0039	0.8704
F-MW-39	F-MW39-9	NRL-M-070	F-MW-39-9 4/12/18	4/12/2018 11:18		141.61	0.000142	1.00E-03	0.08206	298.15	5.79E-09	5.79E-06	1.74E-07	5.79E-06	5.79	92.61	613.01	1.894018	0.017713	0.007439	0.007811	0.05	0.0151	3.3458
F-MW-39	F-MW39-10	NRL-M-071	F-MW-39-10 4/12/1	4/14/2018 10:06		143.26	0.000143	1.00E-03	0.08206	298.15	5.86E-09	5.86E-06	1.76E-07	5.86E-06	5.86	93.69	519.19	1.783856	0.0167	0.006426	0.006748	0.04	0.0132	2.9238
F-MW-39	F-MW39-11	NRL-M-072	F-MW39-11 4/17/1	4/17/2018 13:05		147.12	0.000147	1.00E-03	0.08206	298.15	6.01E-09	6.01E-06	1.80E-07	6.01E-06	6.01	96.21	30.15	1.209614	0.011386	0.001112	0.001167	0.01	0.0023	0.5194
F-MW-39	F-MW39-12	NRL-M-073	F-MW39-12	4/25/2018 14:37		766.78	0.000767	1.00E-03	0.08206	298.15	3.13E-08	3.13E-05	9.40E-07	3.13E-05	31.34	501.45	-40.97	1.126109	0.010608	0.000334	0.000351	0.01	0.0037	0.8132
F-MW-39	F-MW39-13	NRL-M-074	F-MW39-13	4/30/2018 11:28		594.3412	0.000594	1.00E-03	0.08206	298.15	2.43E-08	2.43E-05	7.29E-07	2.43E-05	24.29	388.68	-56.02	1.108438	0.010443	0.000169	0.000178	0.00	0.0014	0.3194
F-MW-39	F-MW39-14	NRL-M-075	F-MW39-14	5/7/2018 10:04		628.3256	0.000628	1.00E-03	0.08206	298.15	2.57E-08	2.57E-05	7.70E-07	2.57E-05	25.68	410.90	-71.87	1.089819	0.01027	-4.4E-06	-4.6E-06	0.00	0.0000	-0.0087
F-MW-53	F-MW53-B	NRL-M-031	F-MW-53B	4/4/2018 15:57		1.08	1.08E-06	1.00E-03	0.08206	298.15	4.41E-11	4.41E-08	1.32E-09	4.41E-08	0.04	0.71	-31.36	1.137386	0.010713					
F-MW-53	F-MW53-1	NRL-M-032	F-MW53-1	4/5/2018 11:10		45.32	4.53E-05	1.00E-03	0.08206	298.15	1.85E-09	1.85E-06	5.56E-08	1.85E-06	1.85	29.64	-25.73	1.144007	0.010775	0				
F-MW-53	F-MW53-2	NRL-M-033	F-MW-53-2 04/05/1	4/5/2018 18:39		8.34	8.34E-06	1.00E-03	0.08206	298.15	3.41E-10	3.41E-07	1.02E-08	3.41E-07	0.34	5.45	241.83	1.458178	0.013693	0.002918	0.003064	0.00	0.0003	0.0773
F-MW-53	F-MW53-3	NRL-M-034	F-MW-53-3 04/05/1	4/5/2018 19:32		5.20	5.2E-06	1.00E-03	0.08206	298.15	2.13E-10	2.13E-07	6.38E-09	2.13E-07	0.21	3.40	54.68	1.238424	0.011654	0.000879	0.000923	0.00	0.0001	0.0145
F-MW-53	F-MW53-4	NRL-M-035	F-MW-53-4 04/06/1	4/6/2018 9:26		4.75	4.75E-06	1.00E-03	0.08206	298.15	1.94E-10	1.94E-07	5.82E-09	1.94E-07	0.19	3.10	37.84	1.218646	0.01147	0.000695	0.00073	0.00	0.0000	0.0105
F-MW-53	F-MW53-5	NRL-M-036	F-MW53-5 4/06/18	4/6/2018 13:58		16.61	1.66E-05	1.00E-03	0.08206	298.15	6.79E-10	6.79E-07	2.04E-08	6.79E-07	0.68	10.86	450.96	1.703736	0.015962	0.005188	0.005447	0.00	0.0012	0.2736
F-MW-53	F-MW53-6	NRL-M-037	F-MW53-6 4/06/18	4/6/2018 17:02		5.14	5.14E-06	1.00E-03	0.08206	298.15	2.1E-10	2.10E-07	6.30E-09	2.10E-07	0.21	3.36	25.51	1.204165	0.011335	0.00056	0.000588	0.00	0.0000	0.0091
F-MW-53	F-MW53-7	NRL-M-038	F-MW-53-7 4/09/18	4/9/2018 12:31		3.20	3.2E-06	1.00E-03	0.08206	298.15	1.31E-10	1.31E-07	3.93E-09	1.31E-07	0.13	2.10	105.01	1.297524	0.012203	0.001428	0.0015	0.00	0.0001	0.0145
F-MW-53	F-MW53-8	NRL-M-039	F-MW-53-8 4/09/18	4/9/2018 12:32		5.08	5.08E-06	1.00E-03	0.08206	298.15	2.08E-10	2.08E-07	6.23E-09	2.08E-07	0.21	3.32	95.41	1.286247	0.012098	0.001323	0.00139	0.00	0.0001	0.0213
F-MW-53	F-MW53-9	NRL-M-040	F-MW-53-9 04/12/1	4/12/2018 10:47		4.91	4.91E-06	1.00E-03	0.08206	298.15	2.01E-10	2.01E-07	6.03E-09	2.01E-07	0.20	3.21	127.40	1.323812	0.012447	0.001672	0.001756	0.00	0.0001	0.0261
F-MW-53	F-MW53-10	NRL-M-041	F-MW-53-10 04/12/	4/14/2018 9:37		2.98	2.98E-06	1.00E-03	0.08206	298.15	1.22E-10	1.22E-07	3.66E-09	1.22E-07	0.12	1.95	103.75	1.296039	0.012189	0.001414	0.001485	0.00	0.0001	0.0134
F-MW-53	F-MW53-11	NRL-M-042	F-MW53-11 4/17/1	4/17/2018 12:15		2.80	2.8E-06	1.00E-03	0.08206	298.15	1.14E-10	1.14E-07	3.43E-09	1.14E-07	0.11	1.83	27.65	1.206679	0.011358	0.000584	0.000613	0.00	0.0000	0.0052
F-MW-53	F-MW53-12	NRL-M-043	F-MW53-12	4/25/2018 12:33		3.82	3.82E-06	1.00E-03	0.08206	298.15	1.56E-10	1.56E-07	4.68E-09	1.56E-07	0.16	2.50	0.81	1.175165	0.011065	0.00029	0.000305	0.00	0.0000	0.0035
F-MW-53	F-MW53-13	NRL-M-044	F-MW53-13	4/30/2018 13:38		4.427082	4.43E-06	1.00E-03	0.08206	298.15	1.81E-10	1.81E-07	5.43E-09	1.81E-07	0.18	2.90	14.73	1.191507	0.011217	0.000442	0.000464	0.00	0.0000	0.0062
F-MW-53	F-MW53-14	NRL-M-045	F-MW53-14	5/7/2018 9:37		2.879343	2.88E-06	1.00E-03	0.08206	298.15	1.18E-10	1.18E-07	3.53E-09	1.18E-07	0.12	1.88	5.04	1.180137	0.011111	0.000337	0.000353	0.00	0.0000	0.0031
F-MW-59	F-MW59-B	NRL-M-046	F-MW59B	4/4/2018 10:55		48.50	4.85E-05	1.00E-03	0.08206	298.15	1.98E-09	1.98E-06	5.95E-08	1.98E-06	1.98	31.72	-45.09	1.121268	0.010563					
F-MW-59	F-MW59-1	NRL-M-047	F-MW59-1	4/4/2018 18:50		11.33	1.13E-05	1.00E-03	0.08206	298.15	4.63E-10	4.63E-07	1.39E-08	4.63E-07	0.46	7.41	-36.11	1.13181	0.010661	0				
F-MW-59	F-MW59-2	NRL-M-048	F-MW59-2	4/5/2018 9:43		14.12	1.41E-05	1.00E-03	0.08206	298.15	5.77E-10	5.77E-07	1.73E-08	5.77E-07	0.58	9.24	194.81	1.402967	0.013181	0.00252	0.002646	0.00	0.0005	0.1130
F-MW-59	F-MW59-3	NRL-M-049	F-MW59-3	4/5/2018 15:50		16.33	1.63E-05	1.00E-03	0.08206	298.15	6.67E-10	6.67E-07	2.00E-08	6.67E-07	0.67	10.68	44.30	1.226233	0.01154	0.000879	0.000923	0.00	0.0002	0.0456
F-MW-59	F-MW59-4	NRL-M-050	F-MW-59-4 04/05/1	4/5/2018 18:20		8.29	8.29E-06	1.00E-03	0.08206	298.15	3.39E-10	3.39E-07	1.02E-08	3.39E-07	0.34	5.42	123.94	1.319746	0.012409	0.001748	0.001836	0.00	0.0002	0.0460
F-MW-59	F-MW59-5	NRL-M-051	F-MW-59-5 04/06/1	4/6/2018 12:30		8.62	8.62E-06	1.00E-03	0.08206	298.15	3.52E-10	3.52E-07	1.06E-08	3.52E-07	0.35	5.64	113.21	1.307145	0.012292	0.001631	0.001713	0.00	0.0002	0.0447
F-MW-59	F-MW59-6	NRL-M-052	F-MW59-6 4/06/18	4/6/2018 16:22		11.06	1.11E-05	1.00E-03	0.08206	298.15	4.52E-10	4.52E-07	1.36E-08	4.52E-07	0.45	7.23	106.95	1.299792	0.012224	0.001563	0.001641	0.00	0.0002	0.0549
F-MW-59	F-MW59-7	NRL-M-053	F-MW-59-7 04/09/1	4/9/2018 10:52		12.48	1.25E-05	1.00E-03	0.08206	298.15	5.1E-10	5.10E-07	1.53E-08	5.10E-07	0.51	8.16	138.26	1.33656	0.012565	0.001904	0.002	0.00	0.0003	0.0755
F-MW-59	F-MW59-8	NRL-M-054	F-MW-59-8 04/09/1	4/9/2018 10:53		13.96	1.4E-05	1.00E-03	0.08206	298.15	5.71E-10	5.71E-07	1.71E-08	5.71E-07	0.57	9.13	159.99	1.362076	0.012802	0.002141	0.002248	0.00	0.0004	0.0949
F-MW-59	F-MW59-9	NRL-M-055	F-MW-59-9 04/12/1	4/12/2018 9:10		38.71	3.87E-05	1.00E-03	0.08206	298.15	1.58E-09	1.58E-06	4.75E-08	1.58E-06	1.58	25.31	167.24	1.370586	0.012881	0.00222	0.002331	0.00	0.0012	0.2729
F-MW-59	F-MW59-10	NRL-M-056	F-MW-59-10 04/12/	4/14/2018 8:19		18.20	1.82E-05	1.00E-03	0.08206	298.15	7.44E-10	7.44E-07	2.23E-08	7.44E-07	0.74	11.90	128.00	1.324519	0.012454	0.001793	0.001882	0.00	0.0005	0.1036
F-MW-59	F-MW59-11	NRL-M-057	F-MW59-11 4/17/1	4/17/2018 10:39		11.72	1.17E-05	1.00E-03	0.08206	298.15	4.79E-10	4.79E-07	1.44E-08	4.79E-07	0.48	7.67	84.02	1.272873	0.011974	0.001313	0.001378	0.00	0.0002	0.0489
F-MW-59	F-MW59-12	NRL-M-058	F-MW59-12	4/25/2018 10:23		10.89	1.09E-05	1.00E-03	0.08206	298.15	4.45E-10	4.45E-07	1.34E-08	4.45E-07	0.45	7.12	70.12	1.256548	0.011822	0.001161	0.001219	0.00	0.0002	0.0402
F-MW-59	F-MW59-13	NRL-M-059	F-MW59-13	4/30/2018 9:26		12.53815	1.25E-05	1.00E-03	0.08206	298.15	5.12E-10	5.12E-07	1.54E-08	5.12E-07	0.51	8.20	38.82	1.219803	0.01148	0.000819	0.00086	0.00	0.0001	0.0326
F-MW-59	F-MW59-14	NRL-M-060	F-MW59-14	5/7/2018 8:15		9.717238	9.72E-06	1.00E-03	0.08206	298.15	3.97E-10	3.97E-07	1.19E-08	3.97E-07	0.40	6.35	19.62	1.197258	0.011271	0.00061	0.00064	0.00	0.0001	0.0188

* Fractionation factor 1.067 Org.Geochem. 25:255 (1996)
* Soil 1.041-1.056 Zhang, Nature 2016, DOI: 10.1038/srep27065

Site F combined with ^{15}N studies and PPT calculations



Site A DIC

Well	Date	Sealaska nomenclature	Timepoint	µM	S.E.	□$^{13}C_{VPDB}$	Atom Fract	Mole Fracti	Excess MF 13C	excess µM	µg 13CO2	Excess ove	µM 13CO2	µM RDX e	µg L-1 eq
A-MW22	4/3/2019 17:00	A-19-219-1	A-MW22-B	955.52	28.70	-18.31	1.10232	0.010856							
A-MW22	4/4/2019 0:00	A-19-219-2	A-MW22-Inj1	2202.97	35.86	-18.91	1.101649	0.010849	-6.52915E-06						
A-MW22	4/5/2019 8:30	A-19-219-3	A-MW22-Inj2	2176.96	16.74	-18.91	1.101649	0.010849	-6.52915E-06						
A-MW22	4/5/2019 8:30	A-19-219-3a	A-MW22-Inj3			-18.91	1.101649	0.010849	-6.52915E-06						
A-MW22	4/6/2019 11:30	A-19-219-4	A-MW22-0	1120.71	48.41	-16.55	1.104291	0.010875	1.9199E-05	0.02	1	1	0.02	0.01	1.59
A-MW22	4/7/2019 11:00	A-19-219-5	A-MW22-1	1591.47	9.77	-16.03	1.104882	0.010881	2.49583E-05	0.04	2	2	0.04	0.01	2.94
A-MW22	4/8/2019 10:55	A-19-219-6	A-MW22-2	2205.44	19.31	-13.74	1.107454	0.010906	5.00141E-05	0.11	5	5	0.11	0.04	8.17
A-MW22	4/12/2019 15:00	A-19-219-7	A-MW22-3	3359.68	45.27	-1.32	1.121402	0.011042	0.000185854	0.62	28	28	0.62	0.21	46.24
A-MW22	4/15/2019 9:10	A-19-219-8	A-MW22-4	3611.25	22.61	-5.30	1.116927	0.010998	0.000142273	0.51	23	23	0.51	0.17	38.05
A-MW22	4/18/2019 0:00	A-19-219-9	A-MW22-5	2723.06	60.35	-1.79	1.120874	0.011036	0.00018071	0.49	22	22	0.49	0.16	36.44
A-MW22	5/6/2019 9:00	A-19-219-11	A-MW22-6	1102.76	32.67	-4.95	1.117325	0.011002	0.00014615	0.16	7	7	0.16	0.05	11.94
A-MW22	5/30/2019 11:45	A-19-219-12	A-MW22-7	1466.91	37.28	-12.28	1.10909	0.010922	6.59477E-05	0.10	4	4	0.10	0.03	7.16
A-MW32	4/2/2019 13:00	A-19-220-1	A-MW32-B	1254.95	27.93	-14.63	1.102489	0.010896							
A-MW32	4/5/2019 0:00	A-19-220-2	A-MW32-Inj1	1220.39	23.51	-15.29	1.101742	0.010889	-7.30269E-06						
A-MW32	4/6/2019 0:00	A-19-220-3	A-MW32-Inj2			-9.19	1.108577	0.010956	5.95125E-05						
A-MW32	4/6/2019 10:00	A-19-220-3a	A-MW32-Inj3	1218.42	13.36	-9.47	1.108259	0.010952	5.64053E-05						
A-MW32	4/6/2019 10:20	A-19-220-4	A-MW32-0	1548.52	13.12	-9.44	1.108296	0.010953	5.67676E-05	0.09	4	4	0.09	0.03	6.79
A-MW32	4/12/2019 16:20	A-19-220-5	A-MW32-1	3739.67	64.18	-0.64	1.11814	0.011049	0.000152976	0.60	27	27	0.60	0.20	44.20
A-MW32	4/15/2019 10:55	A-19-220-6	A-MW32-2	4411.30	190.01	-3.59	1.114835	0.011017	0.000120677	0.56	25	25	0.56	0.19	41.13
A-MW32	4/22/2019 0:00	A-19-220-7	A-MW32-3	4751.28	74.92	-4.64	1.113664	0.011005	0.000109236	0.54	24	24	0.54	0.18	40.10
A-MW32	4/30/2019 0:00	A-19-220-8	A-MW32-4	2147.95	51.32	1.02	1.11999	0.011067	0.000171056	0.38	17	17	0.38	0.13	28.39
A-MW32	5/6/2019 14:10	A-19-220-9	A-MW32-5	1031.35	18.96	-2.25	1.116333	0.011031	0.000135317	0.15	7	7	0.15	0.05	10.78
A-MW32	5/15/2019 15:10	A-19-220-10	A-MW32-6	1019.557	35.38505	-10.69	1.106898	0.010939	4.30998E-05	0.05	2	2	0.05	0.02	3.39
A-MW32	5/30/2019 11:50	A-19-220-11	A-MW32-7	1621.42	49.60	-13.33	1.10394	0.01091	1.41879E-05	0.02	1	1	0.02	0.01	1.78
A-MW36	4/3/2019 12:00	A-19-203-1	A-MW36-B	2072.06	11.17	-16.18	1.100749	0.010879							
A-MW36	4/3/2019 16:18	A-19-203-2	A-MW36-Inj1			-10.75	1.106827	0.010938	5.94178E-05						
A-MW36	4/4/2019 9:30	A-19-203-3	A-MW36-Inj2	1693.91	35.49	-22.77	1.093375	0.010807	-7.20856E-05						
A-MW36	4/4/2019 12:30	A-19-203-3a	A-MW36-Inj3	1939.77	14.80	-18.42	1.098241	0.010855	-2.45166E-05						
A-MW36	4/4/2019 16:00	A-19-203-4	A-MW36-0	2072.06	11.17	-16.37	1.100535	0.010877	-2.09076E-06	0.00	0	0	0.00	0.00	-0.32
A-MW36	4/5/2019 14:50	A-19-203-5	A-MW36-1	1573.44	2.69	-17.74	1.099011	0.010862	-1.69858E-05	-0.03	-1	-1	-0.03	-0.01	-1.98
A-MW36	4/15/2019 14:15	A-19-203-6	A-MW36-2	1552.23	23.66	-22.09	1.094142	0.010814	-6.45873E-05	-0.10	-5	-5	-0.10	-0.03	-7.42
A-MW36	4/18/2019 0:00	A-19-203-7	A-MW36-3	1616.31	46.14	-23.04	1.093078	0.010804	-7.49964E-05	-0.12	-5	-5	-0.12	-0.04	-8.98
A-MW36	4/22/2019 0:00	A-19-203-8	A-MW36-4	1411.24	25.61	-21.91	1.094342	0.010816	-6.26319E-05	-0.09	-4	-4	-0.09	-0.03	-6.55
A-MW36	4/29/2019 0:00	A-19-203-9	A-MW36-5	1465.17	11.88	-24.83	1.091075	0.010784	-9.4579E-05	-0.14	-6	-6	-0.14	-0.05	-10.26
A-MW36	5/6/2019 10:15	A-19-203-10	A-MW36-6	1532.76	8.18	-22.20	1.094014	0.010813	-6.58419E-05	-0.10	-5	-5	-0.10	-0.03	-7.47
A-MW36	5/15/2019 10:00	A-19-203-11	A-MW36-7	1293.35	35.82	-22.17	1.094049	0.010814	-6.54976E-05	-0.08	-4	-4	-0.08	-0.03	-6.27
A-MW37	4/2/2019 10:45	A-19-204-1	A-MW37-B	1290.17	8.16	-16.11	1.10083	0.01088							
A-MW37	4/3/2019 15:25	A-19-204-2	A-MW37-Inj1	1028.02	4.12	-17.48	1.099297	0.010865	-1.49886E-05						
A-MW37	4/4/2019 9:20	A-19-204-3	A-MW37-Inj2	1094.31	13.58	-15.22	1.101822	0.01089	9.6937E-06						
A-MW37	4/4/2019 12:10	A-19-204-3a	A-MW37-Inj3	1117.60	18.76	-15.43	1.101592	0.010887	7.44876E-06						
A-MW37	4/4/2019 13:30	A-19-204-4	A-MW37-0	1287.62	33.38	-15.89	1.101076	0.010882	2.39674E-06	0.00	0	0	0.00	0.00	0.23
A-MW37	4/12/2019 13:35	A-19-204-5	A-MW37-1	1214.80	3.19	-13.75	1.103475	0.010906	2.58559E-05	0.03	1	1	0.03	0.01	2.33
A-MW37	4/15/2019 13:00	A-19-204-6	A-MW37-2	789.19	9.79	-17.98	1.098734	0.010859	-2.04913E-05	-0.02	-1	-1	-0.02	-0.01	-1.20
A-MW37	4/18/2019 0:00	A-19-204-7	A-MW37-3	1228.50	16.97	-12.17	1.10524	0.010923	4.31048E-05	0.05	2	2	0.05	0.02	3.92
A-MW37	4/29/2019 0:00	A-19-204-8	A-MW37-4	1581.12	5.94	-7.61	1.110336	0.010973	9.29189E-05	0.15	7	7	0.15	0.05	10.88
A-MW37	5/6/2019 10:20	A-19-204-9	A-MW37-5	1388.21	45.92	-10.58	1.107019	0.01094	6.0496E-05	0.08	4	4	0.08	0.03	6.22
A-MW37	5/15/2019 10:15	A-19-204-10	A-MW37-6	1611.28	31.72	-13.02	1.104288	0.010914	3.38031E-05	0.05	2	2	0.05	0.02	4.03
A-MW37	5/30/2019 9:50	A-19-204-11	A-MW37-7	1993.46	6.48	-13.26	1.104018	0.010911	3.11629E-05	0.06	3	3	0.06	0.02	4.60
A--MW60R	4/5/2019 0:00	A-19-213-1	A-MW60R-B	1591.47	9.77	-12.93	1.104387	0.010915							
A--MW60R	4/5/2019 8:30	A-19-213-2	A-MW60R-Inj1	2213.09	4.75	-15.03	1.102036	0.010892	-2.29795E-05						
A--MW60R	4/5/2019 13:55	A-19-213-3	A-MW60R-Inj2			-13.11	1.104189	0.010913	-1.9374E-06						
A--MW60R	4/6/2019 12:30	A-19-213-3a	A-MW60R-Inj3	2315.88	79.69	-12.93	1.104387	0.010915	0						
A--MW60R	4/6/2019 17:00	A-19-213-4	A-MW60R-0	1703.87	9.18	-15.23	1.101816	0.010889	-2.51352E-05	-0.04	-2	-2	-0.04	-0.01	-3.17
A--MW60R	4/9/2019 15:25	A-19-213-5	A-MW60R-1	1618.93	16.92	-15.19	1.101863	0.01089	-2.46735E-05	-0.04	-2	-2	-0.04	-0.01	-2.96
A--MW60R	4/12/2019 0:00	A-19-213-6	A-MW60R-2	3528.80	18.39	-7.25	1.110739	0.010977	6.20836E-05	0.22	10	10	0.22	0.07	16.22
A--MW60R	4/17/2019 0:00	A-19-213-7	A-MW60R-3	2424.29	45.06	0.80	1.119749	0.011065	0.000150141	0.36	16	16	0.36	0.12	26.96
A--MW60R	4/22/2019 0:00	A-19-213-8	A-MW60R-4	4573.91	85.18	-8.15	1.10974	0.010967	5.23255E-05	0.24	11	11	0.24	0.08	17.72
A--MW60R	4/30/2019 0:00	A-19-213-9	A-MW60R-5	5219.50	156.18	-0.46	1.118339	0.011051	0.000136365	0.71	32	32	0.71	0.24	52.71
A--MW60R	5/6/2019 12:50	A-19-213-10	A-MW60R-6	4859.98	98.35	2.66	1.121835	0.011085	0.000170529	0.83	37	37	0.83	0.28	61.38
A--MW60R	5/15/2019 12:25	A-19-213-11	A-MW60R-7	4474.71	59.29	-3.12	1.115363	0.011022	0.000107282	0.48	22	22	0.48	0.16	35.55
A-MW62	4/1/2019 15:30	A-19-226-1	A-MW62-B	1986.62	19.79	-13.71	1.103513	0.010906							
A-MW62	4/4/2019 14:40	A-19-226-2	A-MW62-Inj1	2528.22	13.71	-13.28	1.103996	0.010911	4.71831E-06						
A-MW62	4/5/2019 8:45	A-19-226-3	A-MW62-Inj2	2539.81	61.35	-12.23	1.105169	0.010922	1.61852E-05						
A-MW62	4/5/2019 0:00	A-19-226-3a	A-MW62-Inj3			-12.23	1.105169	0.010922	1.61852E-05						
A-MW62	4/5/2019 16:25	A-19-226-4	A-MW62-0	1993.457	6.478334	-12.58	1.104777	0.010918	1.23594E-05	0.03	1	1	0.03	0.01	1.90
A-MW62	4/8/2019 12:50	A-19-226-5	A-MW62-1	2412.03	29.95	3.49	1.122764	0.011094	0.000188144	0.47	21	21	0.47	0.16	35.06
A-MW62	4/12/2019 13:35	A-19-226-6	A-MW62-2	3734.53	39.47	-0.40	1.118403	0.011052	0.000145535	0.57	26	26	0.57	0.19	41.99
A-MW62	4/18/2019 0:00	A-19-226-7	A-MW62-3	4540.28	58.64	1.44	1.120462	0.011072	0.000165656	0.79	35	35	0.79	0.26	58.11
A-MW62	4/22/2019 0:00	A-19-226-8	A-MW62-4	3268.55	25.63	1.20	1.120195	0.011069	0.000163043	0.56	25	25	0.56	0.19	41.17
A-MW62	4/29/2019 0:00	A-19-226-9	A-MW62-5	5658.26	58.12	4.30	1.123662	0.011103	0.000196923	1.16	52	52	1.16	0.39	86.08
A-MW62	5/6/2019 13:15	A-19-226-10	A-MW62-6	3873.81	42.39	6.42	1.126037	0.011126	0.000220132	0.89	40	40	0.89	0.30	65.88
A-MW62	5/15/2019 13:00	A-19-226-11	A-MW62-7	4792.56	28.97	7.02	1.126705	0.011133	0.000226652	1.13	51	51	1.13	0.38	83.92

*value times α of 1.0008 (Fuller 2016 aerobic)
*value for DW03 uses 1.0044 from Fuller et al 2016 because it's anaerobic

PDB = 0.0111796 (Coplen 2011)   Coplen TB (2011) Guidelines and recommended terms for expressio
VPDB = 0.011183

Site A methane

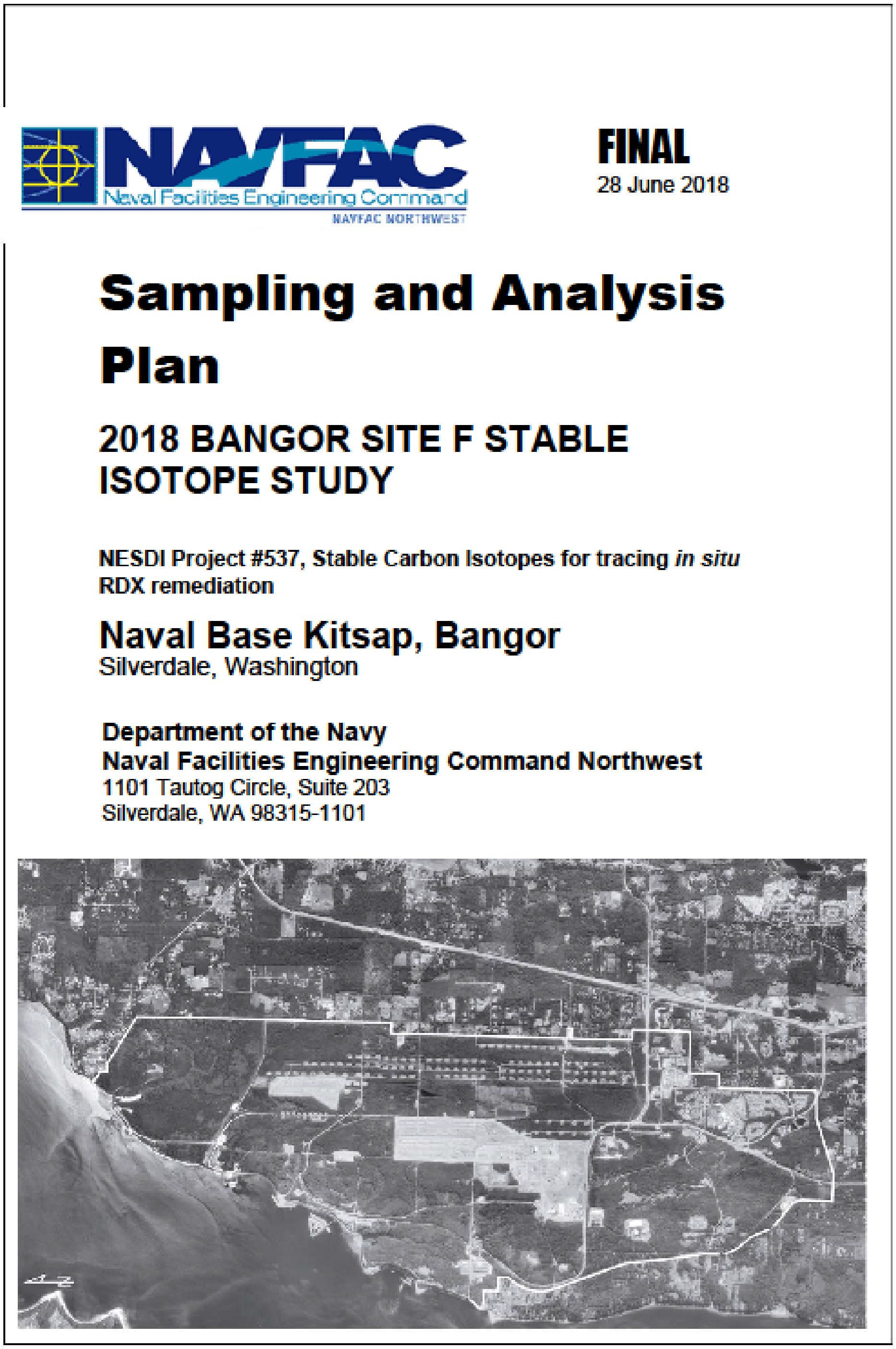
Site	Sealaska Sample	ppm	P	V	R	T	Moles	Moles/L	Moles / 30	Mol L-1 in	uM orig	ug L-1	$\delta^{13}C_{VPDB}$	Atom Frac	Mol Fracti	Excess MF	Excess MF	μM - 13CH	μM RDX e	μg L-1 RDX
A-MW22	A-19-219-1	0.047651	4.77E-08	1.00E-03	0.08206	298.15	1.95E-12	1.95E-09	5.84E-11	1.95E-09	0.00	0.03	-30.0827	1.138891	0.010727					
A-MW22	A-19-219-4	4.846081	4.85E-06	1.00E-03	0.08206	298.15	1.98E-10	1.98E-07	5.94E-09	1.98E-07	0.20	3.17	-58.43	1.105606	0.010417	0	0			
A-MW22	A-19-219-5	6.330484	6.33E-06	1.00E-03	0.08206	298.15	2.59E-10	2.59E-07	7.76E-09	2.59E-07	0.26	4.14	-42.53	1.124276	0.010591	0.000174	0.000183	0.0000	0.0000	0.0035
A-MW22	A-19-219-6	1.815512	1.82E-06	1.00E-03	0.08206	298.15	7.42E-11	7.42E-08	2.23E-09	7.42E-08	0.07	1.19	64.32	1.249741	0.011759	0.001342	0.001409	0.0001	0.0000	0.0077
A-MW22	A-19-219-7	1.048486	1.05E-06	1.00E-03	0.08206	298.15	4.29E-11	4.29E-08	1.29E-09	4.29E-08	0.04	0.69	35.55	1.215958	0.011445	0.001028	0.001079	0.0000	0.0000	0.0034
A-MW22	A-19-219-8	0.29558	2.96E-07	1.00E-03	0.08206	298.15	1.21E-11	1.21E-08	3.62E-10	1.21E-08	0.01	0.19	13.12	1.189621	0.011199	0.000783	0.000822	0.0000	0.0000	0.0007
A-MW22	A-19-219-9	0.273572	2.74E-07	1.00E-03	0.08206	298.15	1.12E-11	1.12E-08	3.35E-10	1.12E-08	0.01	0.18	37.64	1.218412	0.011467	0.001051	0.001103	0.0000	0.0000	0.0009
A-MW22	A-19-219-10	1.597443	1.6E-06	1.00E-03	0.08206	298.15	6.53E-11	6.53E-08	1.96E-09	6.53E-08	0.07	1.04	53.35	1.236859	0.011639	0.001222	0.001283	0.0001	0.0000	0.0062
A-MW22	A-19-219-11	1.255128	1.26E-06	1.00E-03	0.08206	298.15	5.13E-11	5.13E-08	1.54E-09	5.13E-08	0.05	0.82	42.83	1.224507	0.011524	0.001107	0.001163	0.0001	0.0000	0.0044
A-MW32	A-19-220-1	7217.774	0.007218	1.00E-03	0.08206	298.15	2.95E-07	2.95E-04	8.85E-06	2.95E-04	295.01	4,720.16	-36.7698	1.131039	0.010654					
A-MW32	A-19-220-4	64.18	6.42E-05	1.00E-03	0.08206	298.15	2.62E-09	2.62E-06	7.87E-08	2.62E-06	2.62	41.97	-42.83	1.123923	0.010587	0.000171	0.000179			
A-MW32	A-19-220-5	453.40	0.000453	1.00E-03	0.08206	298.15	1.85E-08	1.85E-05	5.56E-07	1.85E-05	18.53	296.51	572.37	1.8463	0.017275	0.006858	0.007201	0.1334	0.0445	9.8751
A-MW32	A-19-220-6	362.92	0.000363	1.00E-03	0.08206	298.15	1.48E-08	1.48E-05	4.45E-07	1.48E-05	14.83	237.34	903.3	2.234883	0.020835	0.010418	0.010939	0.1623	0.0541	12.0075
A-MW32	A-19-220-7	360.40	0.00036	1.00E-03	0.08206	298.15	1.47E-08	1.47E-05	4.42E-07	1.47E-05	14.73	235.69	1869.48	3.369386	0.031083	0.020666	0.021699	0.3196	0.1065	23.6536
A-MW32	A-19-220-8	404.38	0.000404	1.00E-03	0.08206	298.15	1.65E-08	1.65E-05	4.96E-07	1.65E-05	16.53	264.45	512.2	1.775648	0.016625	0.006208	0.006518	0.1077	0.0359	7.9726
A-MW32	A-19-220-9	242.64	0.000243	1.00E-03	0.08206	298.15	9.92E-09	9.92E-06	2.98E-07	9.92E-06	9.92	158.68	337.17	1.570125	0.014729	0.004312	0.004528	0.0449	0.0150	3.3229
A-MW32	A-19-220-10	598.90	0.000599	1.00E-03	0.08206	298.15	2.45E-08	2.45E-05	7.34E-07	2.45E-05	24.48	391.66	30.43	1.209946	0.011389	0.000972	0.00102	0.0250	0.0083	1.8485
A-MW32	A-19-220-11	1029.20	0.001029	1.00E-03	0.08206	298.15	4.21E-08	4.21E-05	1.26E-06	4.21E-05	42.07	673.06	-44.12	1.122409	0.010573	0.000157	0.000164	0.0069	0.0023	0.5120
A-MW36	A-19-203-1	0.134203	1.34E-07	1.00E-03	0.08206	298.15	5.49E-12	5.49E-09	1.65E-10	5.49E-09	0.01	0.09	-31.04	1.137767	0.010716					
A-MW36	A-19-203-4	17	0.000017	1.00E-03	0.08206	298.15	6.95E-10	6.95E-07	2.08E-08	6.95E-07	0.69	11.12	-29.71	1.139329	0.010731	0.000314	0.00033			
A-MW36	A-19-203-5	5.2	5.2E-06	1.00E-03	0.08206	298.15	2.13E-10	2.13E-07	6.38E-09	2.13E-07	0.21	3.40	-64.62	1.098337	0.010349	-6.8E-05	-7.1E-05	0.0000	0.0000	-0.0011
A-MW36	A-19-203-6	0.336303	3.36E-07	1.00E-03	0.08206	298.15	1.37E-11	1.37E-08	4.12E-10	1.37E-08	0.01	0.22	105.54	1.298142	0.012209	0.001792	0.001881	0.0000	0.0000	0.0019
A-MW36	A-19-203-7	16.99885	1.7E-05	1.00E-03	0.08206	298.15	6.95E-10	6.95E-07	2.08E-08	6.95E-07	0.69	11.12	32.71	1.212624	0.011414	0.000997	0.001047	0.0007	0.0002	0.0538
A-MW36	A-19-203-8	0.521114	5.21E-07	1.00E-03	0.08206	298.15	2.13E-11	2.13E-08	6.39E-10	2.13E-08	0.02	0.34	6.42	1.181753	0.011126	0.000709	0.000745	0.0000	0.0000	0.0012
A-MW36	A-19-203-9	9.481584	9.48E-06	1.00E-03	0.08206	298.15	3.88E-10	3.88E-07	1.16E-08	3.88E-07	0.39	6.20	-5.42	1.167851	0.010997	0.00058	0.000609	0.0002	0.0001	0.0175
A-MW36	A-19-203-10	3.839929	3.84E-06	1.00E-03	0.08206	298.15	1.57E-10	1.57E-07	4.71E-09	1.57E-07	0.16	2.51	-53.8	1.111042	0.010467	5.07E-05	5.32E-05	0.0000	0.0000	0.0006
A-MW36	A-19-203-11	0.742809	7.43E-07	1.00E-03	0.08206	298.15	3.04E-11	3.04E-08	9.11E-10	3.04E-08	0.03	0.49	-35.87	1.132096	0.010664	0.000247	0.000259	0.0000	0.0000	0.0006
A-MW37	A-19-204-1	162.2059	0.000162	1.00E-03	0.08206	298.15	6.63E-09	6.63E-06	1.99E-07	6.63E-06	6.63	106.08	-36.7698	1.131039	0.010654					
A-MW37	A-19-204-4	0.35	3.51E-07	1.00E-03	0.08206	298.15	1.44E-11	1.44E-08	4.31E-10	1.44E-08	0.01	0.23	-42.83	1.123923	0.010587	0.000171	0.000179			
A-MW37	A-19-204-5	0.00	0	1.00E-03	0.08206	298.15	0	0.00E+00	0.00E+00	0.00E+00	0.00	0.00	572.37	1.8463	0.017275	0.006858	0.007201	0.0000	0.0000	0.0000
A-MW37	A-19-204-6	0.00	0	1.00E-03	0.08206	298.15	0	0.00E+00	0.00E+00	0.00E+00	0.00	0.00	903.3	2.234883	0.020835	0.010418	0.010939	0.0000	0.0000	0.0000
A-MW37	A-19-204-7	0.68	6.76E-07	1.00E-03	0.08206	298.15	2.76E-11	2.76E-08	8.29E-10	2.76E-08	0.03	0.44	1869.48	3.369386	0.031083	0.020666	0.021699	0.0006	0.0002	0.0444
A-MW37	A-19-204-8	3.98	3.98E-06	1.00E-03	0.08206	298.15	1.63E-10	1.63E-07	4.88E-09	1.63E-07	0.16	2.60	512.2	1.775648	0.016625	0.006208	0.006518	0.0011	0.0004	0.0785
A-MW37	A-19-204-9	12.98	1.3E-05	1.00E-03	0.08206	298.15	5.3E-10	5.30E-07	1.59E-08	5.30E-07	0.53	8.49	337.17	1.570125	0.014729	0.004312	0.004528	0.0024	0.0008	0.1777
A-MW37	A-19-204-10	6.39	6.39E-06	1.00E-03	0.08206	298.15	2.61E-10	2.61E-07	7.83E-09	2.61E-07	0.26	4.18	30.43	1.209946	0.011389	0.000972	0.00102	0.0003	0.0001	0.0197
A-MW37	A-19-204-11	9.38	9.38E-06	1.00E-03	0.08206	298.15	3.83E-10	3.83E-07	1.15E-08	3.83E-07	0.38	6.13	-44.12	1.122409	0.010573	0.000157	0.000164	0.0001	0.0000	0.0047
A-MW60R	A-19-213-1	0.048989	4.9E-08	1.00E-03	0.08206	298.15	2E-12	2.00E-09	6.01E-11	2.00E-09	0.00	0.03	-30.0827	1.138891	0.010727					
A-MW60R	A-19-213-4	0.301284	3.01E-07	1.00E-03	0.08206	298.15	1.23E-11	1.23E-08	3.69E-10	1.23E-08	0.01	0.20	-58.43	1.105606	0.010417	0	0			
A-MW60R	A-19-213-5	0.680413	6.8E-07	1.00E-03	0.08206	298.15	2.78E-11	2.78E-08	8.34E-10	2.78E-08	0.03	0.44	-42.53	1.124276	0.010591	0.000174	0.000183	0.0000	0.0000	0.0004
A-MW60R	A-19-213-6	0	0	1.00E-03	0.08206	298.15	0	0.00E+00	0.00E+00	0.00E+00	0.00	0.00	64.32	1.249741	0.011759	0.001342	0.001409	0.0000	0.0000	0.0000
A-MW60R	A-19-213-7	0	0	1.00E-03	0.08206	298.15	0	0.00E+00	0.00E+00	0.00E+00	0.00	0.00	35.55	1.215958	0.011445	0.001028	0.001079	0.0000	0.0000	0.0000
A-MW60R	A-19-213-8	0.966618	9.67E-07	1.00E-03	0.08206	298.15	3.95E-11	3.95E-08	1.19E-09	3.95E-08	0.04	0.63	13.12	1.189621	0.011199	0.000783	0.000822	0.0000	0.0000	0.0024
A-MW60R	A-19-213-9	1.053627	1.05E-06	1.00E-03	0.08206	298.15	4.31E-11	4.31E-08	1.29E-09	4.31E-08	0.04	0.69	37.64	1.218412	0.011467	0.001051	0.001103	0.0000	0.0000	0.0035
A-MW60R	A-19-213-10	4.12	4.12E-06	1.00E-03	0.08206	298.15	1.68E-10	1.68E-07	5.05E-09	1.68E-07	0.17	2.69	53.35	1.236859	0.011639	0.001222	0.001283	0.0002	0.0001	0.0160
A-MW60R	A-19-213-11	1.231483	1.23E-06	1.00E-03	0.08206	298.15	5.03E-11	5.03E-08	1.51E-09	5.03E-08	0.05	0.81	42.83	1.224507	0.011524	0.001107	0.001163	0.0001	0.0000	0.0043
A-MW62	A-19-226-1	2272.542	0.002273	1.00E-03	0.08206	298.15	9.29E-08	9.29E-05	2.79E-06	9.29E-05	92.88	1,486.16	-36.7698	1.131039	0.010654					
A-MW62	A-19-226-4	133.18	0.000133	1.00E-03	0.08206	298.15	5.44E-09	5.44E-06	1.63E-07	5.44E-06	5.44	87.10	-42.83	1.123923	0.010587	0.000171	0.000179			
A-MW62	A-19-226-5	1962.67	0.001963	1.00E-03	0.08206	298.15	8.02E-08	8.02E-05	2.41E-06	8.02E-05	80.22	1,283.52	572.37	1.8463	0.017275	0.006858	0.007201	0.5777	0.1926	42.7469
A-MW62	A-19-226-6	1962.67	0.001963	1.00E-03	0.08206	298.15	8.02E-08	8.02E-05	2.41E-06	8.02E-05	80.22	1,283.52	903.3	2.234883	0.020835	0.010418	0.010939	0.8775	0.2925	64.9367
A-MW62	A-19-226-7	1711.18	0.001711	1.00E-03	0.08206	298.15	6.99E-08	6.99E-05	2.10E-06	6.99E-05	69.94	1,119.05	1869.48	3.369386	0.031083	0.020666	0.021699	1.5177	0.5059	112.3061
A-MW62	A-19-226-8	8707.85	0.008708	1.00E-03	0.08206	298.15	3.56E-07	3.56E-04	1.07E-05	3.56E-04	355.91	5,694.62	512.2	1.775648	0.016625	0.006208	0.006518	2.3200	0.7733	171.6794
A-MW62	A-19-226-9	16325.58	0.016326	1.00E-03	0.08206	298.15	6.67E-07	6.67E-04	2.00E-05	6.67E-04	667.27	10,676.34	337.17	1.570125	0.014729	0.004312	0.004528	3.0212	1.0071	223.5703
A-MW62	A-19-226-10	17497.79	0.017498	1.00E-03	0.08206	298.15	7.15E-07	7.15E-04	2.15E-05	7.15E-04	715.18	11,442.92	30.43	1.209946	0.011389	0.000972	0.00102	0.7298	0.2433	54.0068
A-MW62	A-19-226-11	24195.26	0.024195	1.00E-03	0.08206	298.15	9.89E-07	9.89E-04	2.97E-05	9.89E-04	988.93	15,822.82	-44.12	1.122409	0.010573	0.000157	0.000164	0.1627	0.0542	12.0361

Site A final calculations with PPTs

(placeholder)

Appendix C. Tier II SAPs (Site F and Site A)

Site F SAP – double click opens the document.



FINAL
28 June 2018

Sampling and Analysis Plan

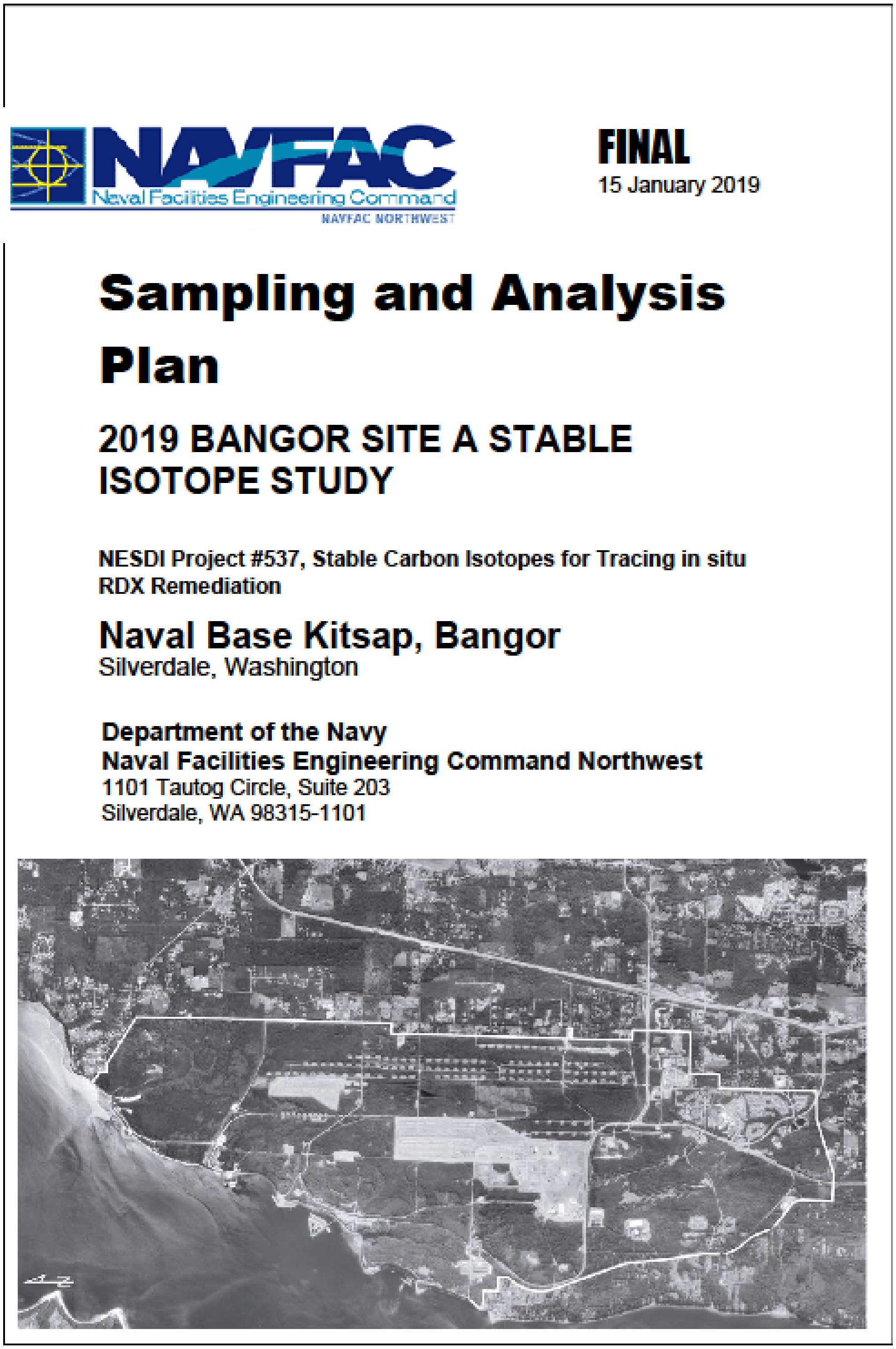
2018 BANGOR SITE F STABLE ISOTOPE STUDY

NESDI Project #537, Stable Carbon Isotopes for tracing *in situ* RDX remediation

Naval Base Kitsap, Bangor
Silverdale, Washington

Department of the Navy
Naval Facilities Engineering Command Northwest
1101 Tautog Circle, Suite 203
Silverdale, WA 98315-1101

Site A SAP. Double clicking opens the document.



NAVFAC
Naval Facilities Engineering Command
NAVFAC NORTHWEST

FINAL
15 January 2019

Sampling and Analysis Plan

2019 BANGOR SITE A STABLE ISOTOPE STUDY

NESDI Project #537, Stable Carbon Isotopes for Tracing in situ RDX Remediation

Naval Base Kitsap, Bangor
Silverdale, Washington

Department of the Navy
Naval Facilities Engineering Command Northwest
1101 Tautog Circle, Suite 203
Silverdale, WA 98315-1101