Heat-Enhanced In Situ Degradation for Treatment of Energetic Compounds Impacting Groundwater

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TRS Group Accelerating Value

WRIGHTLABS

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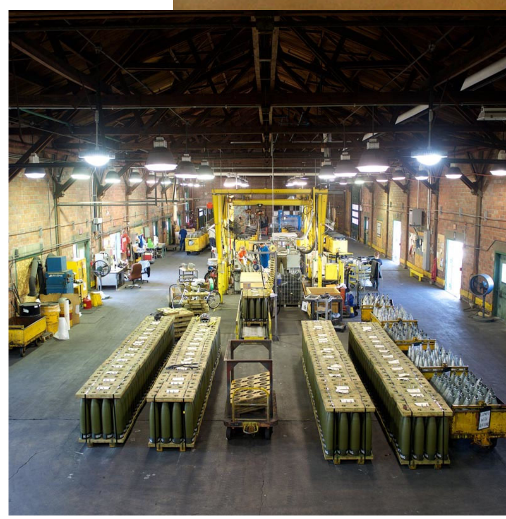
Problem Statement

- DoD extensively used energetic compounds
  - Explosives
    - 2,4,6-trinitrotoluene (TNT)
    - 1,3,5-hexahydro-1,3,5-trinitrotriazine (RDX),
    - oxtahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)
    - nitroguanidine (NQ)
    - 2,4-dinitroanisole (DNAN)
  - Propellants
    - 2,4-dinitrotoluene (DNT) with 2,5-DNT
    - nitroglycerin
    - perchlorate



Problem Statement

- Residual energetics in soil/ sediment can act as long-term sources of contamination to groundwater
  - operating ranges
  - former munitions manufacturing
  - storage, transportation, and staging areas
  - hand grenade range
  - open burn/open detonation
  - blow-in-place facilities
  - formerly used defense sites

Chemical	Sorption	Solubility		
	Log K_{ow}	Water (mg/L)	Acetone (mg/L)	Ethanol (mg/L)
TNT	1.86	0.13	1090	12.3
RDX	0.86	0.04	83	1.5
HMX	0.061	0.002	28	ND

Spanggord 1982, Yinon and Zitrin 1993, McGrath 1995

Chemical	Residential Soil	Drinking Water
	mg/kg	µg/L
TNT	19	2
RDX	5.5	2
HMX	8,300	400

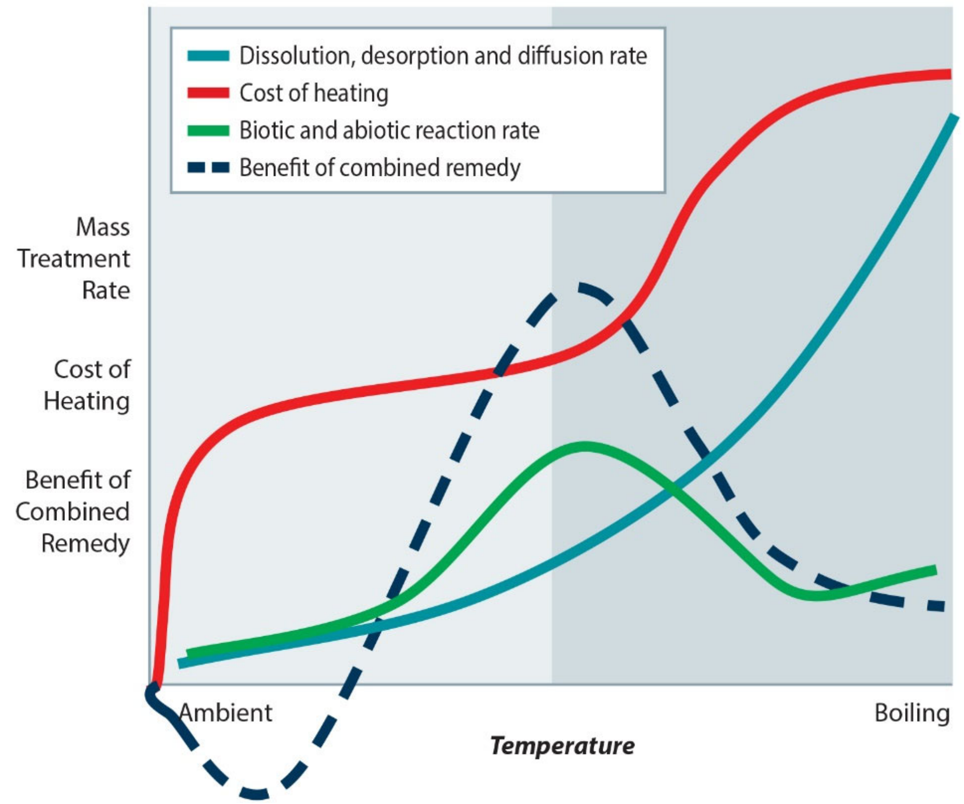
Limitations to Mass Treatment Rate

- Degradation kinetics often result in the rapid degradation of aqueous phase contaminants.
- The dissolution and desorption of the residual source material to the aqueous phase is often the rate-limiting factor for cleanup.
- ***Process optimization:*** *maximize the mass transfer of source material while stimulating effective in situ destruction of liberated chemicals*

Pink water in crater formed by the dissolution of TNT from a 500-lb. bomb. The concentration of TNT in the water was determined to be 19 mg/L.
https://clu-in.org/characterization/technologies/exp.cfm

HETEC Technology

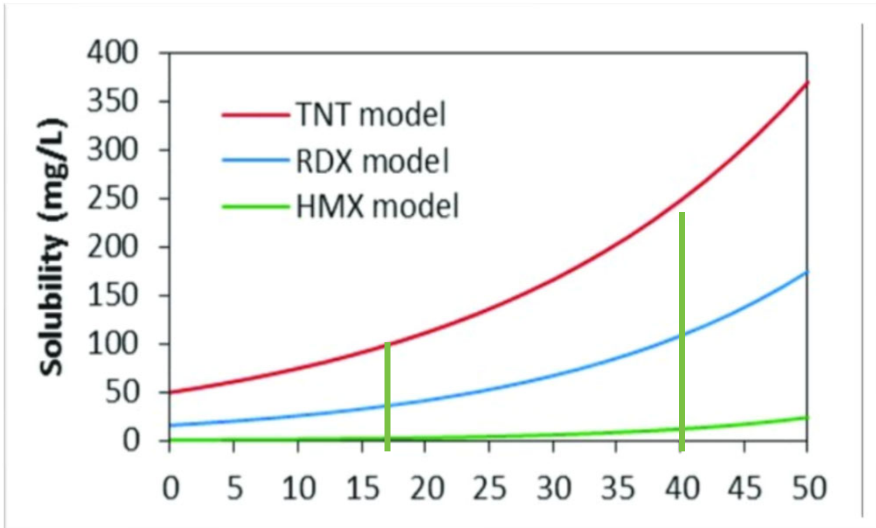
- The Heat-Enhanced Treatment for Energetic Compounds (HETEC patent-pending) relies on heating to accelerate mass transfer and degradation processes.
- Once the target site temperature is achieved, renewable energy sources (e.g., solar, wind) may provide sufficient energy for temperature maintenance.



Technology Description: Enhanced Mass Transfer

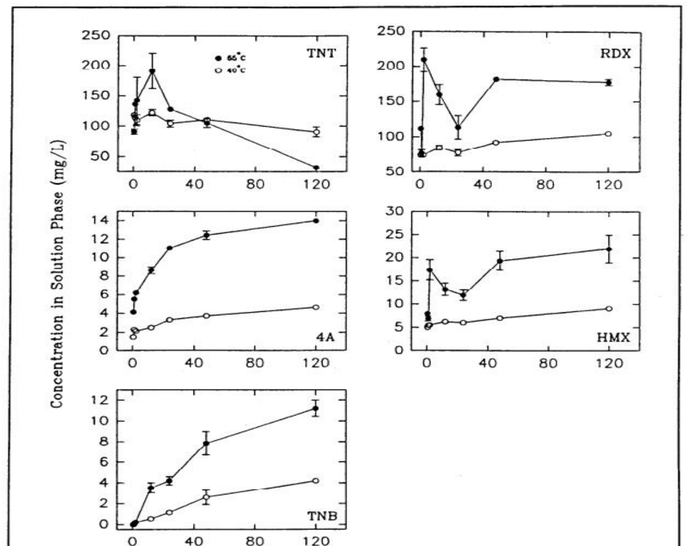
- Sources of Energetics
  - crystallized solid
  - sorbed mass onto soil solids, including organic matter and minerals
  - diffused mass in low-permeability soil/sediment strata
  - dissolved in porewater or groundwater
- Enhanced Mass Transfer- impact of increased temperatures
  - increase aqueous solubility of energetic solids (e.g. TNT, HMX, RDX)
  - increase desorption rates
  - increase diffusion rates into transmissive porewater

Heat-Enhanced Mass Transfer



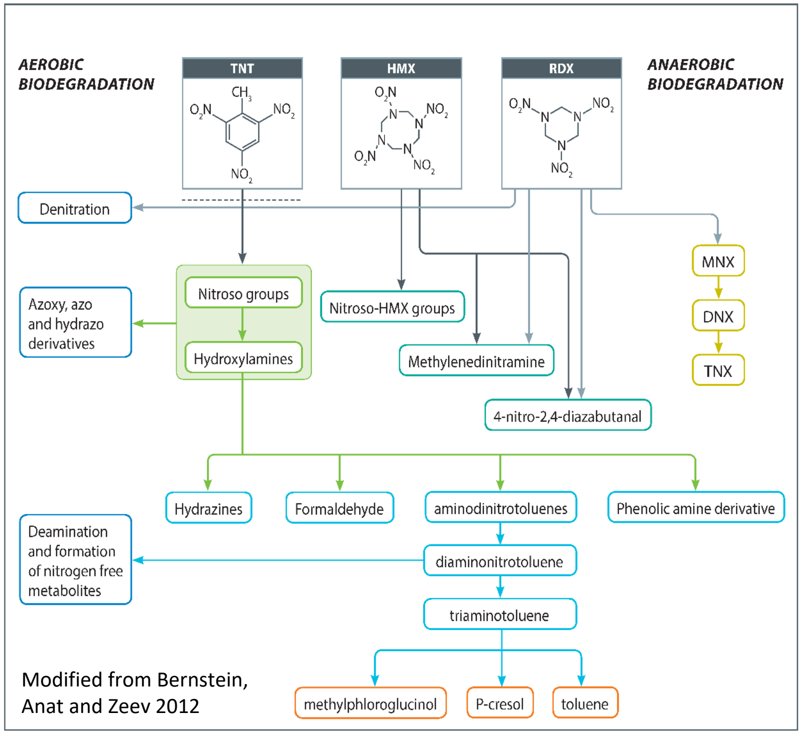
Letters identify data sources: a, Taylor and Rinkenbach (1923); b, Hale et al. (1979); c, Spanggord et al. (1983); d, Leggett (1985); e, Rosenblatt et al. (1989); f, Ro et al. (1996); g, Lynch et al. (2001); h, Phelan and Barnett (2001); i, (Composition B, tap water) (Phelan et al., 2002); j, (freshwater) (Luning Prak and O'Sullivan, 2006); k, (seawater) (Luning Prak and O'Sullivan, 2006); l, Banerjee (1980); m, Sikka et al. (1980); n, Bier et al. (1999); o, (pure RDX, deionized water) (Phelan et al., 2002); p, (pure RDX, tap water) (Phelan et al., 2002); q, Monteil-Rivera et al. (2004); r, Glover and Hoffsommer (1973); s, Spanggord et al. (1982); t, McLellan et al. (1988).

- Desorption/dissolution kinetics studies for TNT, RDX, HMX, 4A and TNB at 25, 40 and 55C.
  - 70% for RDX (170 mg/L vs. 100 mg/L)
  - Factor of 2.5 for HMX (21.9 mg/L vs. 8.8 mg/L)
  - TNT, 4A, and TNB in the effluent of the contaminated sediment columns increased from non-detect at ambient temperature to approximately 32, 11, and 8 mg/L.



Pennington, 1995

Energetic Constituents Biotic Degradation

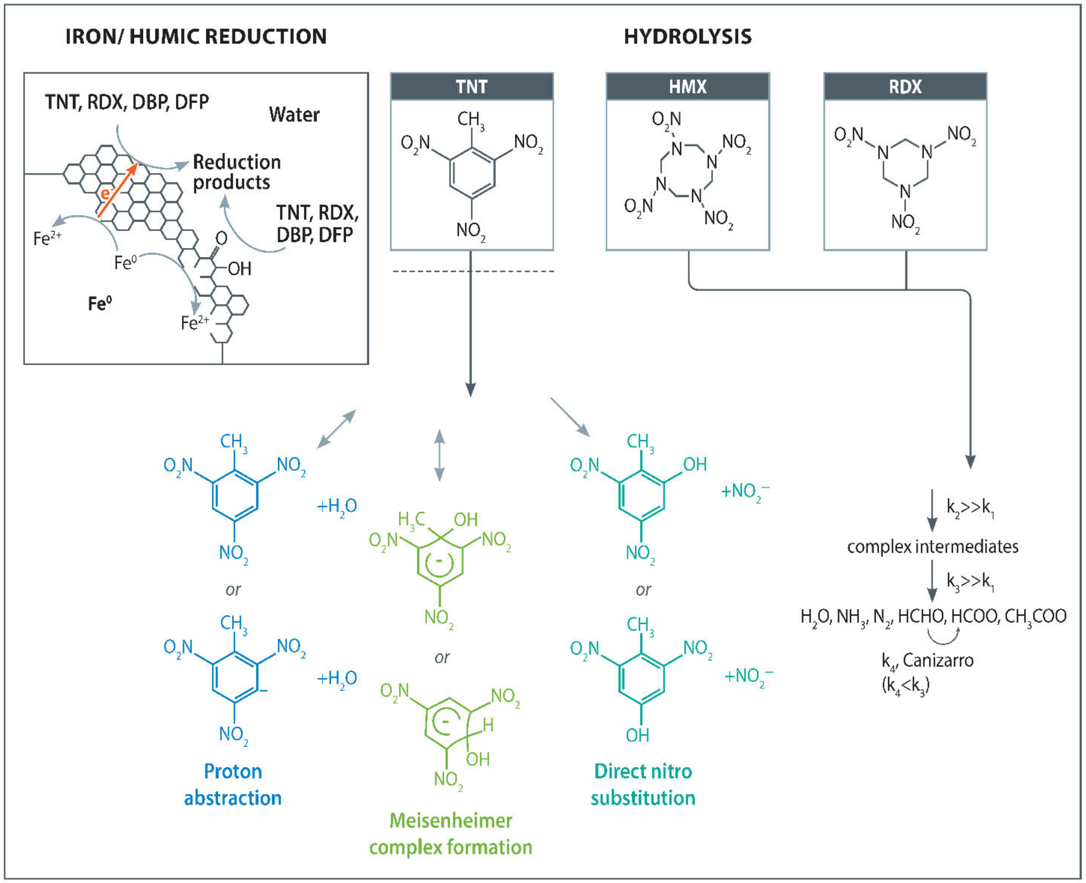


Modified from Bernstein, Anat and Zeev 2012

RDX, TNT and HMX bacterial degradation occurs under aerobic and anaerobic conditions and perchlorate transforms under anaerobic conditions.

- Bacterial Degradation
  - Aerobic denitration
  - Anaerobic denitration
  - Anaerobic reduction
- Fungal Degradation
  - Aerobic reduction (goes through the MEDINA and MNX pathway) - Phanaerocheate chrysosporium

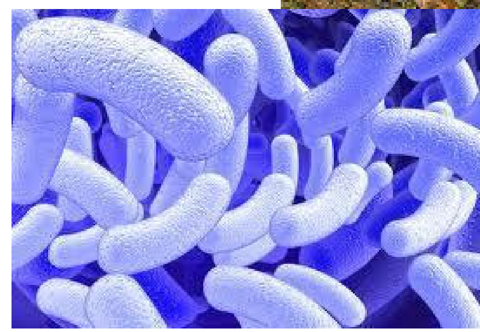
Energetic Constituents Abiotic Degradation

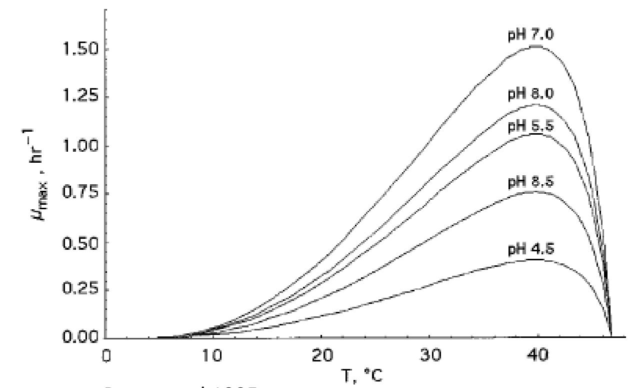


Modified from Oh 2017, Salter-Blac 2013, and Heilmann 1996

Temperature and Biotic Degradation Kinetics

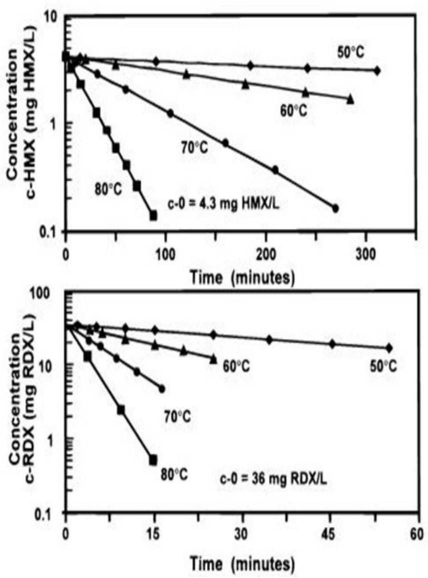
- Biological/Chemical Reaction Kinetics f(T)
  - Arrhenius - k_d doubles with every 10°C increase
- Fungal: optimum temperature is 40°C

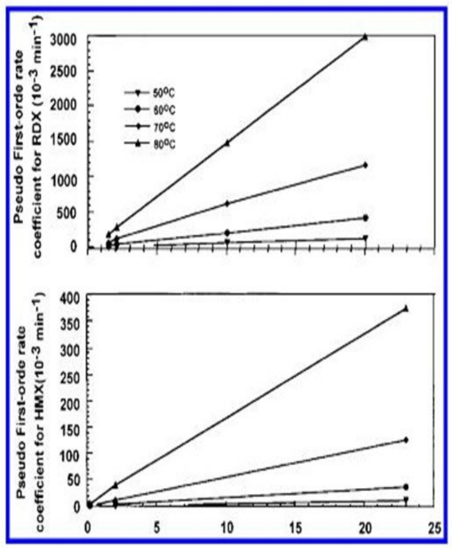


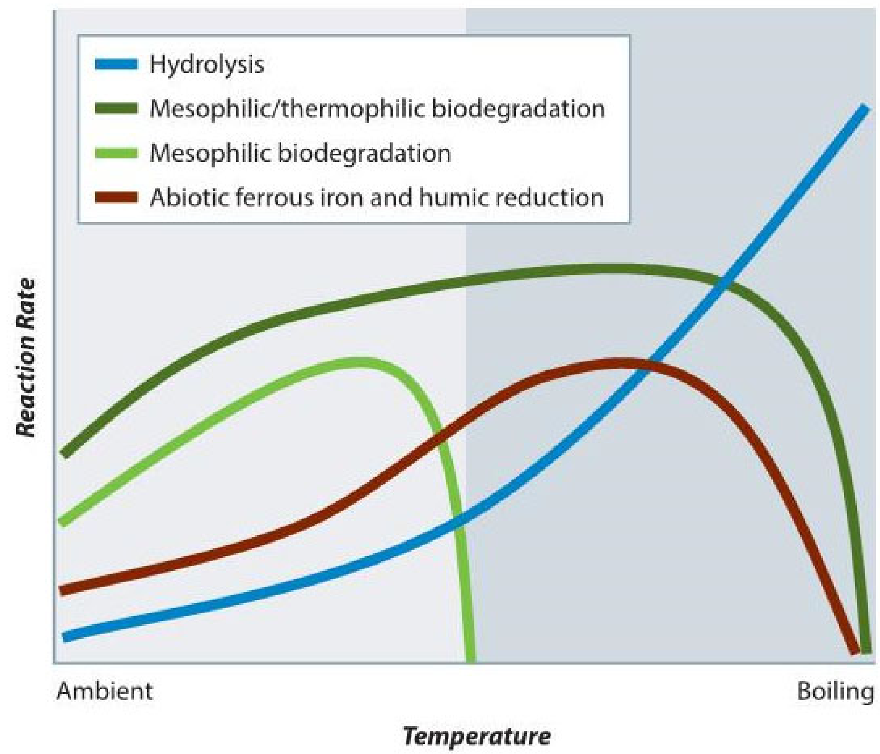


Rosso et al 1995

Temperature and Abiotic Degradation Reactions

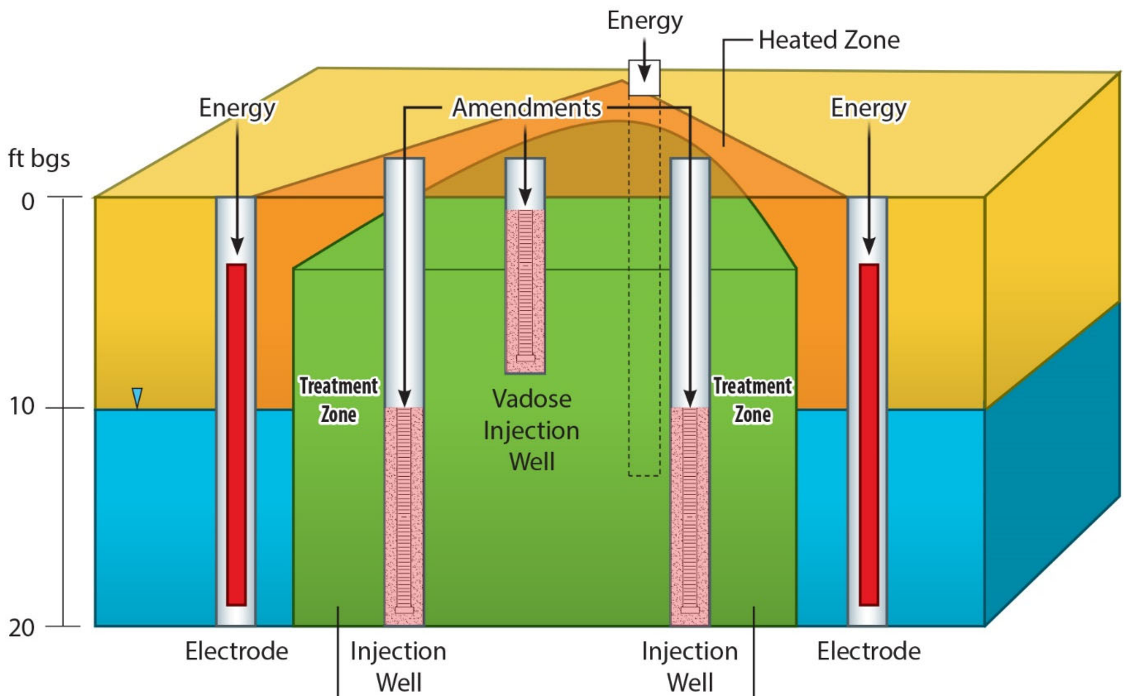






- Heilman et al, 1996: Increase in hydrolysis reactions increased substantially from 50-80C.

Treatment Conceptual Design



Enhanced Energetic Mass Transfer:

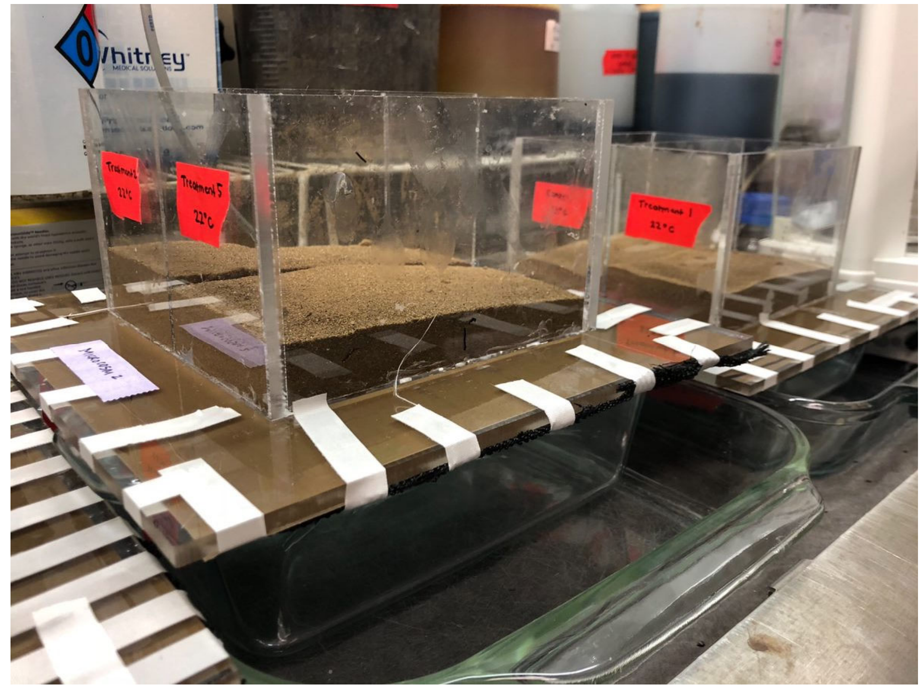
- Dissolution
- Desorption
- Diffusion

Enhanced Energetic Degradation:

- Alkaline Hydrolysis
- Heat-Enhanced Hydrolysis
- Biotic Degradation
- Abiotic Iron and Humic Degradation

Technology Maturity- Proof of Concept

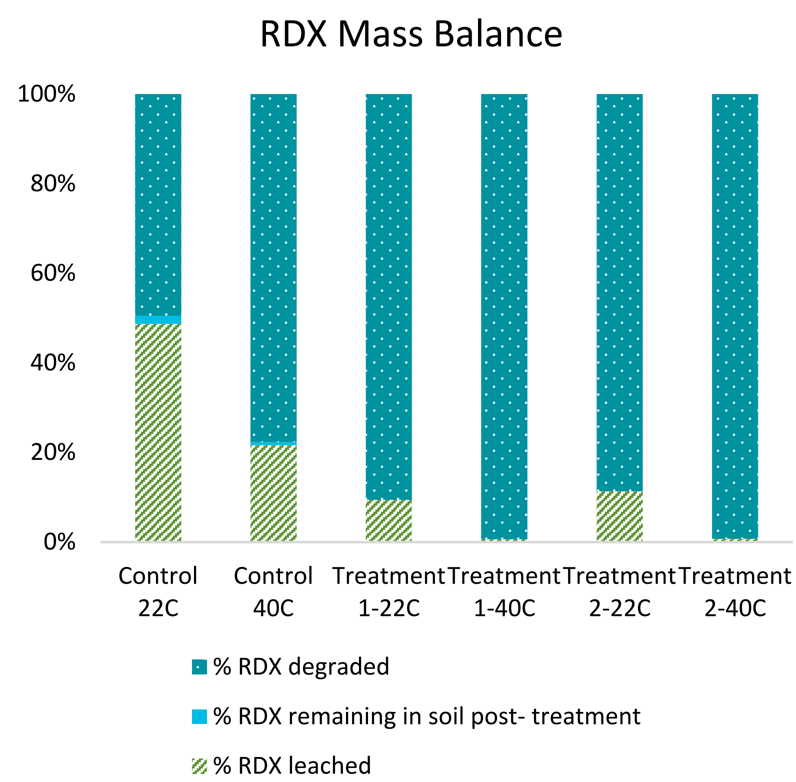
- Control Soil Microcosms (22°C and 40°C)
  - Four Leaching Events (Day 1, 4, 14 and 21)with simulated Rainwater
- Biostimulated Soil Microcosms (20°C and 40°C)
  - Amendment and 3 leaching events at Days, 4, 14, 21
  - Amendment – acetate, corn steep liquor, unsulfured molasses and urea (Treatment #1) or ammonium sulfate (#2)
- Leachate collected and monitored for:
  - pH, TOC, Alkalinity
  - HMX and RDX concentrations
  - Nitrate, Nitrite, Sulfate, Chloride (EPA 300/EPA 353.2)
  - Post-treatment samples sent for HMX/RDX analysis and molecular tools



Soil spiked with 50 mg/kg RDX and HMX was treated with one biostimulation application and four maintenance solution applications.

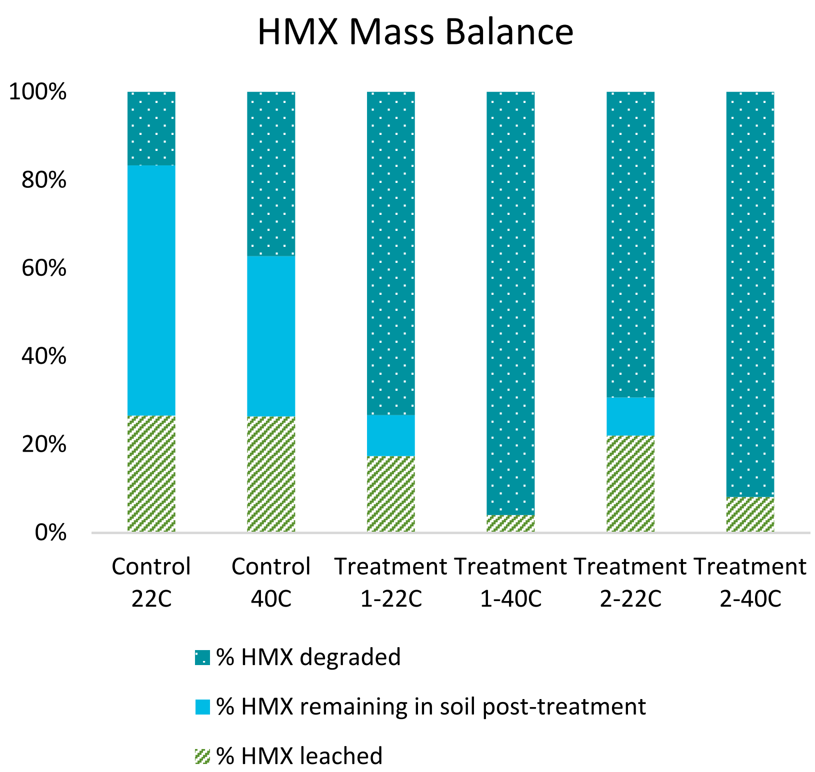
Energetic Compound Mass Balance

Final Mass Balance			
	Original mass of RDX in Microcosms (µg)	% RDX degraded	Half Life (Days)
Day	0	28	
Control 22C	20511	49%	27
Control 40C	20508	78%	13
Treatment 1-22C	20480	91%	8
Treatment 1-40C	20495	99%	4
Treatment 2-22C	20517	89%	9
Treatment 2-40C	20533	99%	4



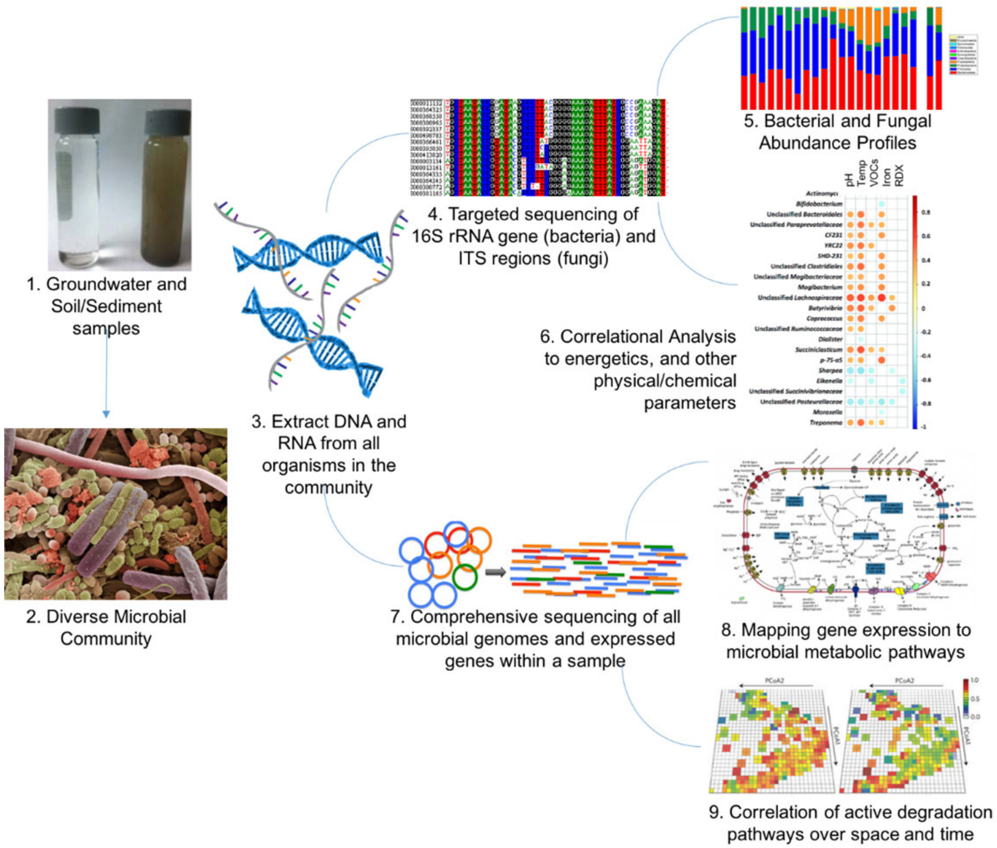
Energetic Compound Mass Balance

Final Mass Balance			
	Original mass of HMX in Microcosms (µg)	% HMX degraded	Half Life (Days)
Days	0	28	
Control 22C	22012	17%	106
Control 40C	22008	37%	41
Treatment 1-22C	21978	73%	14
Treatment 1-40C	21994	96%	6
Treatment 2-22C	22018	69%	16
Treatment 2-40C	22035	92%	8



Molecular Tools: Metagenomics

- Metagenomics used to evaluate bacterial and fungal microbial communities
- Samples analyzed by Wright Labs, LLC for the following analyses:
  - 16S rRNA sequencing and analysis
  - ITS sequencing and analysis
  - Shotgun metagenomic sequencing and analysis



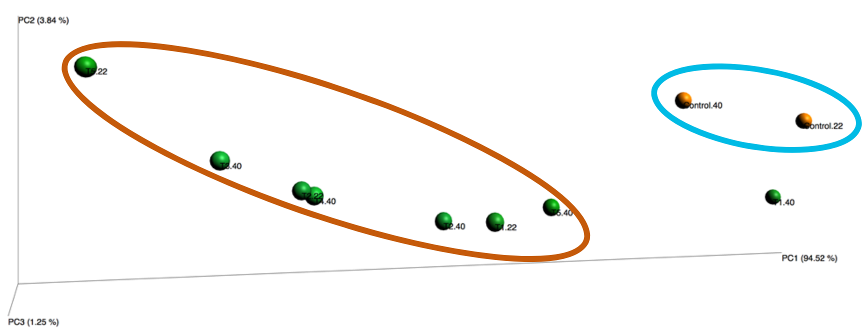
16S rRNA Sequencing Results

- All samples yielded high quality 16S rRNA data post de-noising and decontamination.
- All samples were able to be included in downstream bioinformatic analyses (> 1000 sequences per sample).

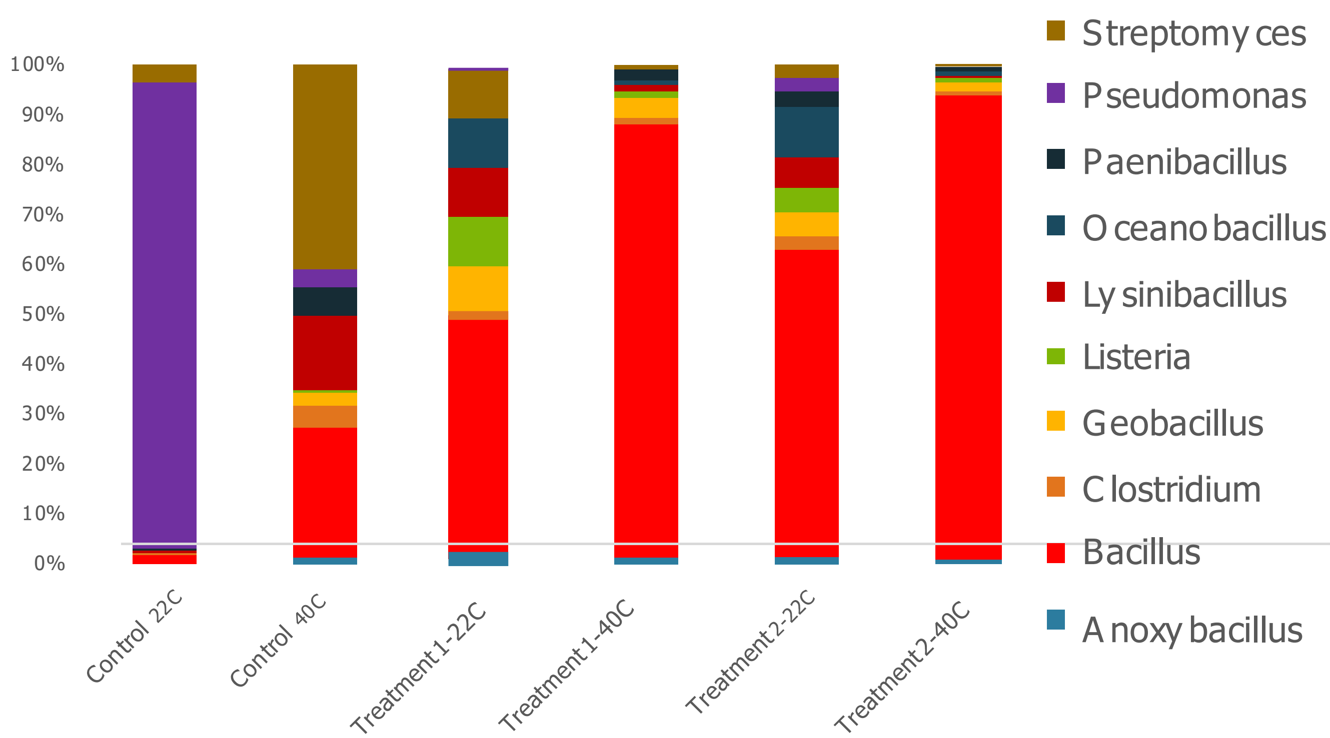
SampleID	ITS Filtered Sequences	16S rRNA Filtered Sequences	Metagenome Sequences
Control 22°C	56,526	30,643	8,334,739
Control 40°C	72,072	43,811	4,317,294
Treatment 1 22°C	1,432	46,813	12,676,832
Treatment 1 40°C	14,030	27,323	16,789,783
Treatment 2 22°C	32,626	45,602	13,122,613
Treatment 2 40°C	12,150	32,373	17,032,493

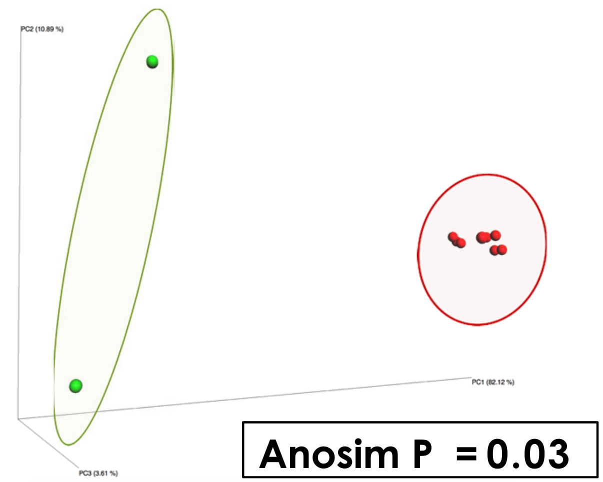
A Fungal Community is Observed within Each Sample

- A preliminary screening for a fungal signal within shotgun metagenome data revealed a fungal consortia.
- The preliminary data- more abundant fungal sequences within the majority of Treated samples in comparison to the Control samples.
- Clustering of fungal communities between Treated and Control samples was found to be statistically significant (Adonis p = 0.03)



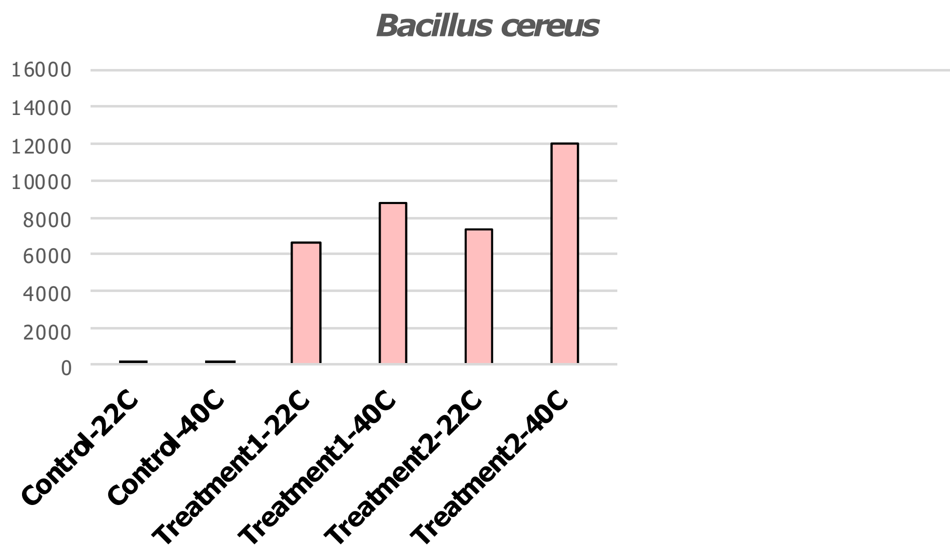
Relative Distribution of Prominent Genera (Metagenome)





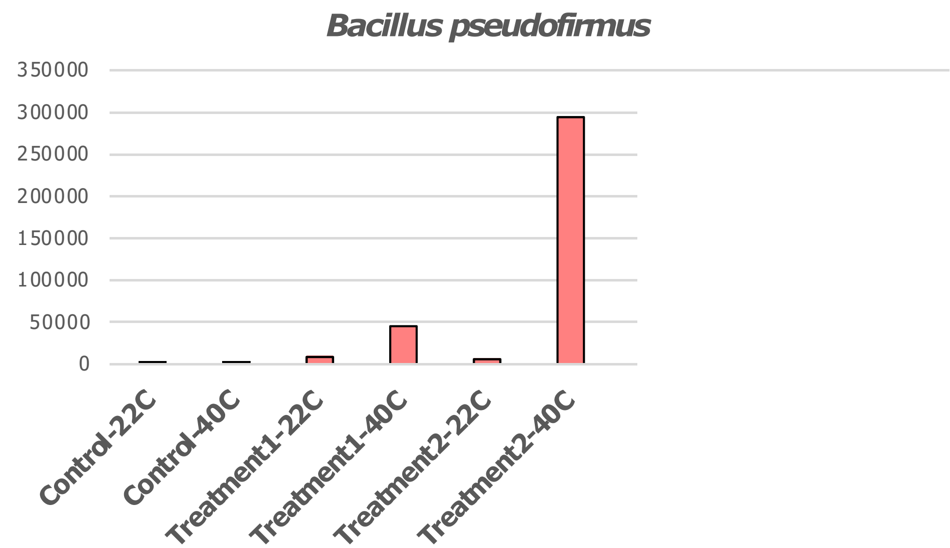
Significantly differential clustering between control samples (GREEN) and *Treatment* samples (RED).

Bacillus Species



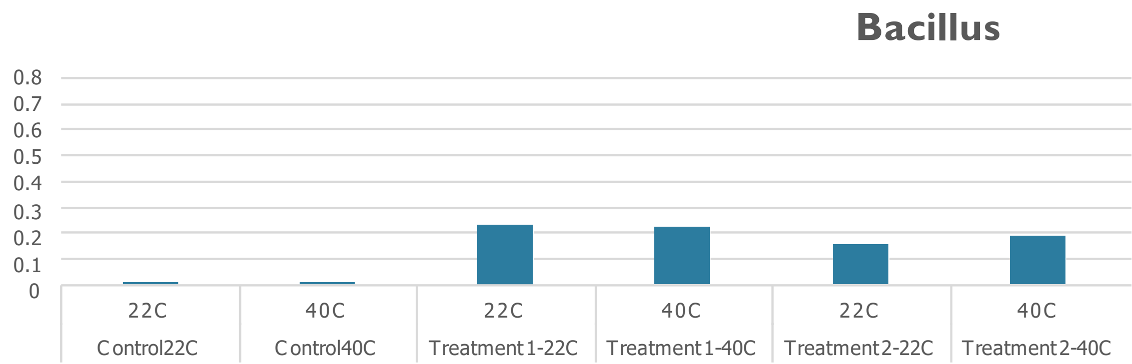
- Mercimek et. al found isolated B. cereus to be capable of TNT degradation in liquid medium (2013).
- At 75 mg L(-1), 77% degradation of TNT was observed within 96 hours.

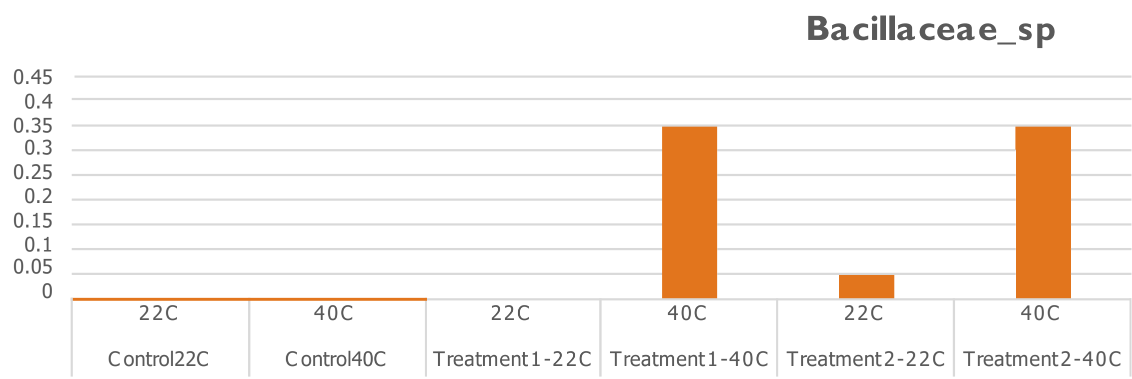
https://www.ncbi.nlm.nih.gov/pubmed/23715804



- In addition to B. cereus, the genome of B. pseudofirmus is cited to contain TNT degradation genes within the Nitrotoluene degradataion KEGG pathway:
  - Nitroreductase – nfnB
  - N-ethylmaleimide reductase - nemA

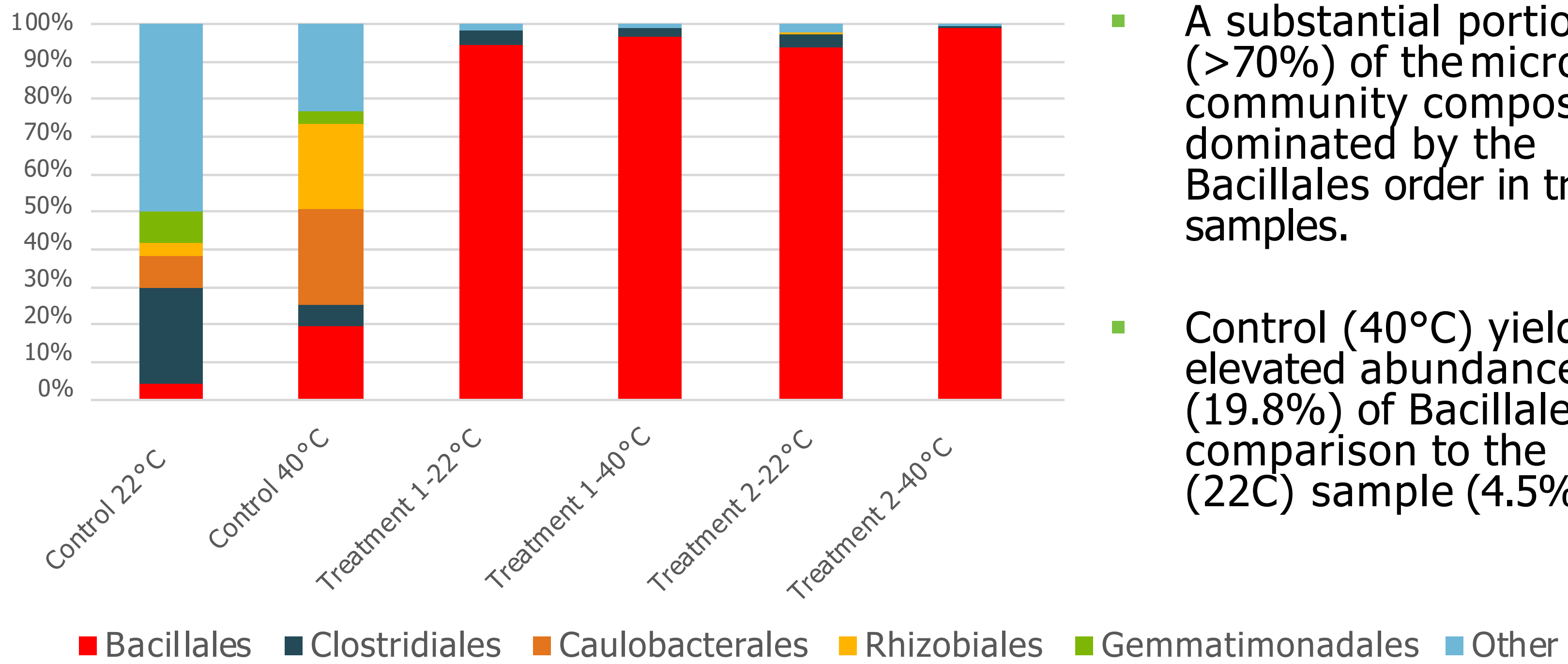
Genus Level Summaries





- A Bacillaceae not classified beyond the family rank was also found to be abundant within the treatment samples. It is also elevated within the 40C treatments for conditions 1 and 2.
- Trace amounts (<1%) of *Bacillus* sp. were identified within the control samples. They comprise >10% of community composition in the experimental treatments.

Order Level Summaries



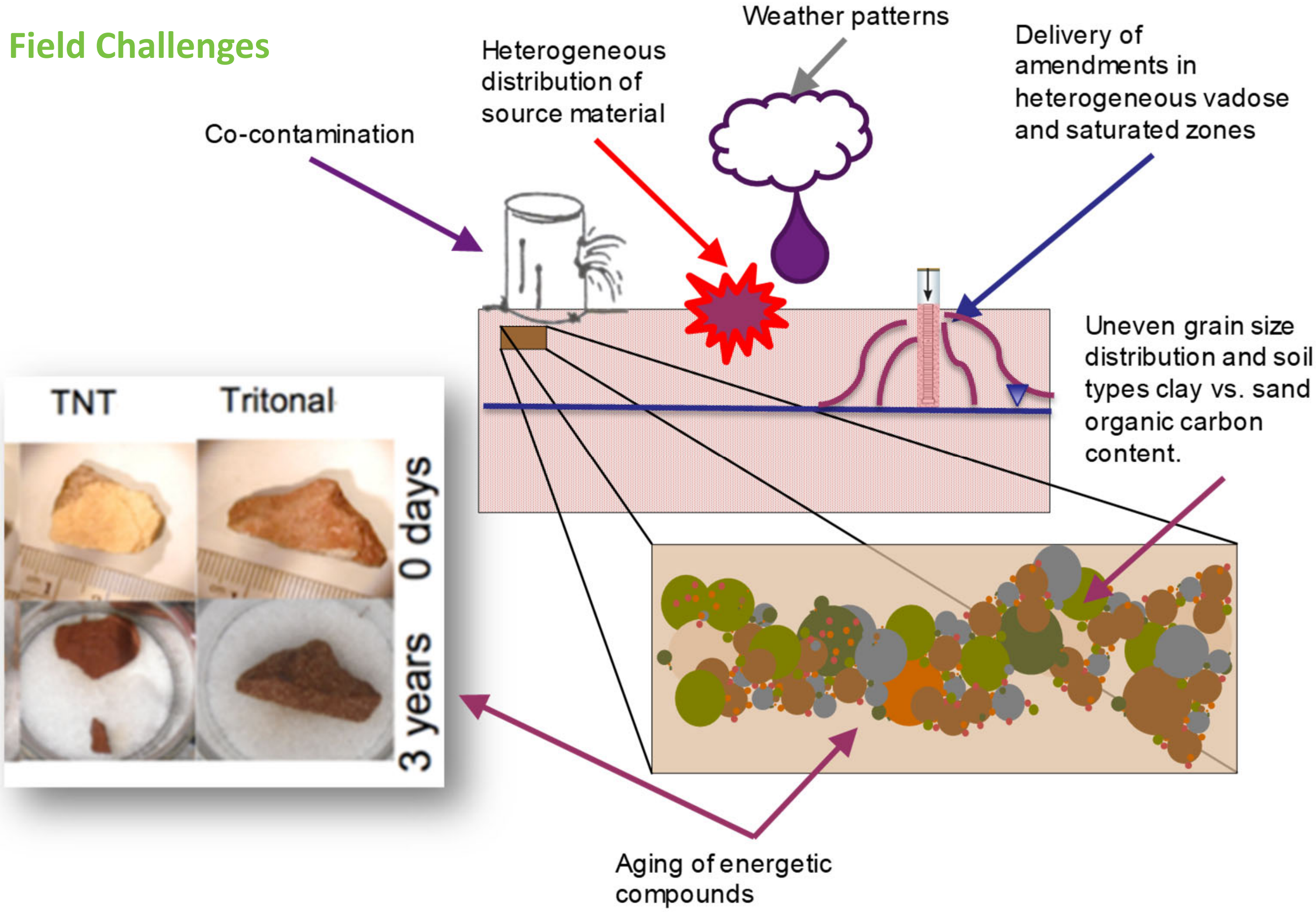
- A substantial portion (>70%) of the microbial community composition is dominated by the Bacillales order in treated samples.
- Control (40°C) yields an elevated abundance (19.8%) of Bacillales in comparison to the Control (22C) sample (4.5%).

Conclusions

- Enhanced desorption and degradation observed when heating occurred to moderate temperatures, factor of 3-6 increase in treatment rates at elevated temperature
- Munitions degrading community combination of fungal and bacterial populations
- Suite of populations different heated vs. non-heated
- Bacillus sp. predominate both the biostimulated heated and non-heated community

Need for Field Demonstration

Field Challenges



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