



# ***Phytoremediation- Humification Strategies for RDX in Surface Soil***

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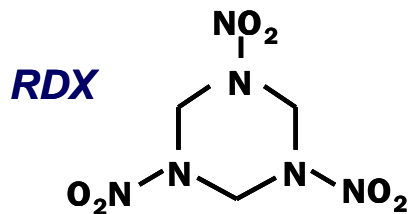


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# Problem

- RDX from low-order detonations
- Deposition onto surface soils
- Heterogeneous and widely dispersed
- Potential for range restrictions/closures



Hexahydro-1,3,5-trinitro-1,3,5-triazine



Relatively high solubility

Weak soil binding

- Potential human health effects
- Seizures
- Possible carcinogen effects

Remediation strategies?

- Cost effective, easily implemented, applicable to surface soils

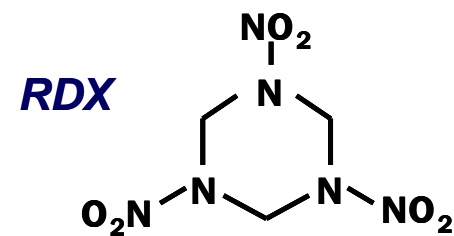




# RDX Degradation Background

## *RDX Biodegradation*

- Favored in saturated soils rather than surface soils
- Plant uptake of RDX is significant, but degradation in plants is limited
- RDX conjugated in plant tissue can be re-deposited onto soils as plants die
- Surface soils are not constant with regard to temperature, soil water potential, and carbon
- Can we identify, predict, or enhance processes that reduce the potential for RDX movement?



Hexahydro-1,3,5-trinitro-1,3,5-triazine







# Relationship to Other Phytoremediation

Previous research – rhizosphere enhanced remediation for petroleum

## Similarities

Surface soil                      Limited site access  
Root Accessible              Few alternatives

## Different mechanisms



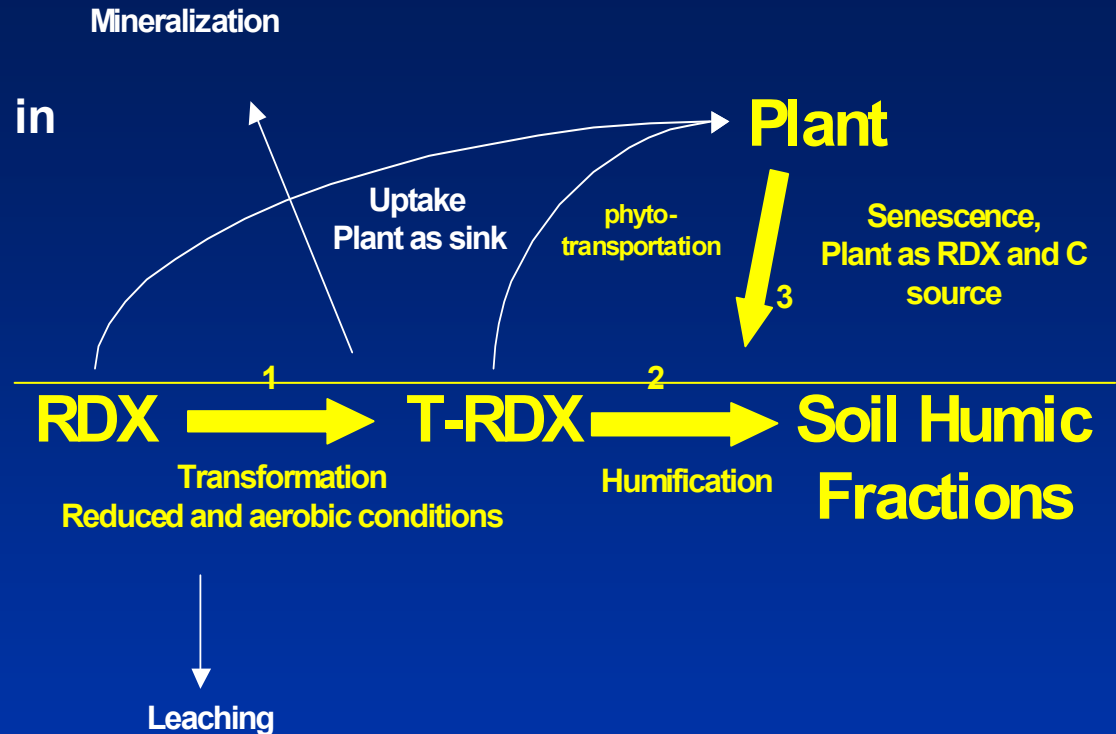
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# Objective/Description

## Objective

Sequester RDX-derived C in soil humic fractions



## Hypotheses

- Soil RDX concentrations can be decreased by microbially driven transformations and plant uptake
- Humification can serve as an RDX sink
- Bioavailable carbon drives the microbiology
- Mineralization-Immobilization Turnover (MIT) drives humification
- Plant-conjugated RDX gives a humification advantage
- There is characteristic microbiology associated with humification



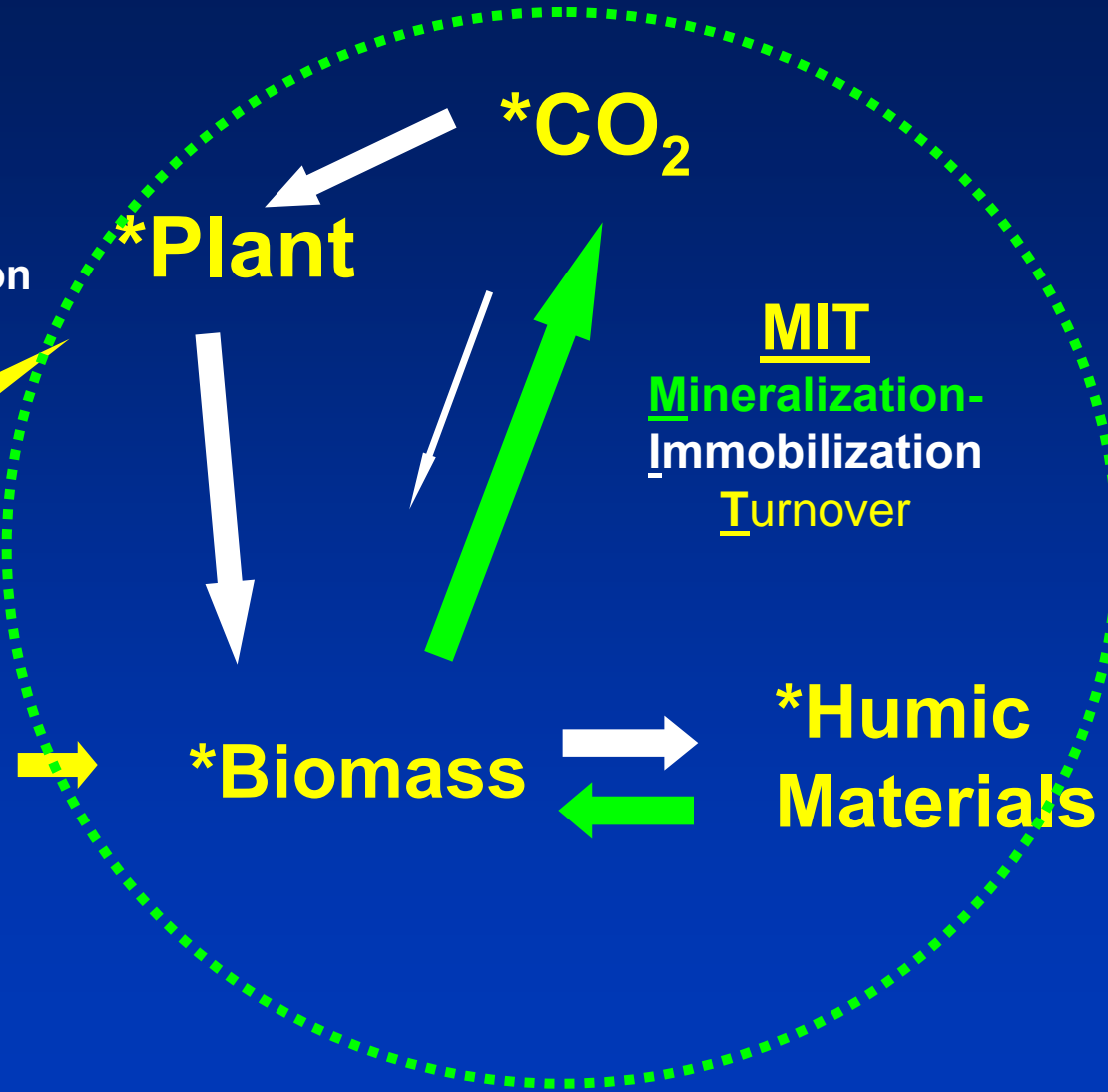


# Approach- Theory & Hypothesis

"2" cycles  
Native carbon  
RDX carbon

Native carbon >>>> RDX carbon  
MIT drives soil processes

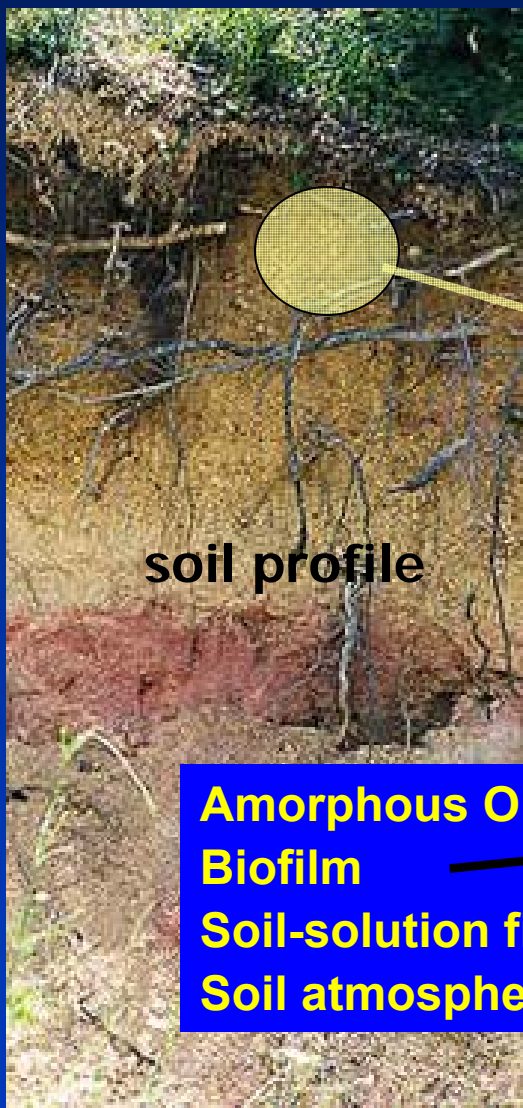
\*RDX  $\xrightarrow{\text{biotic}}$  \*T-RDX



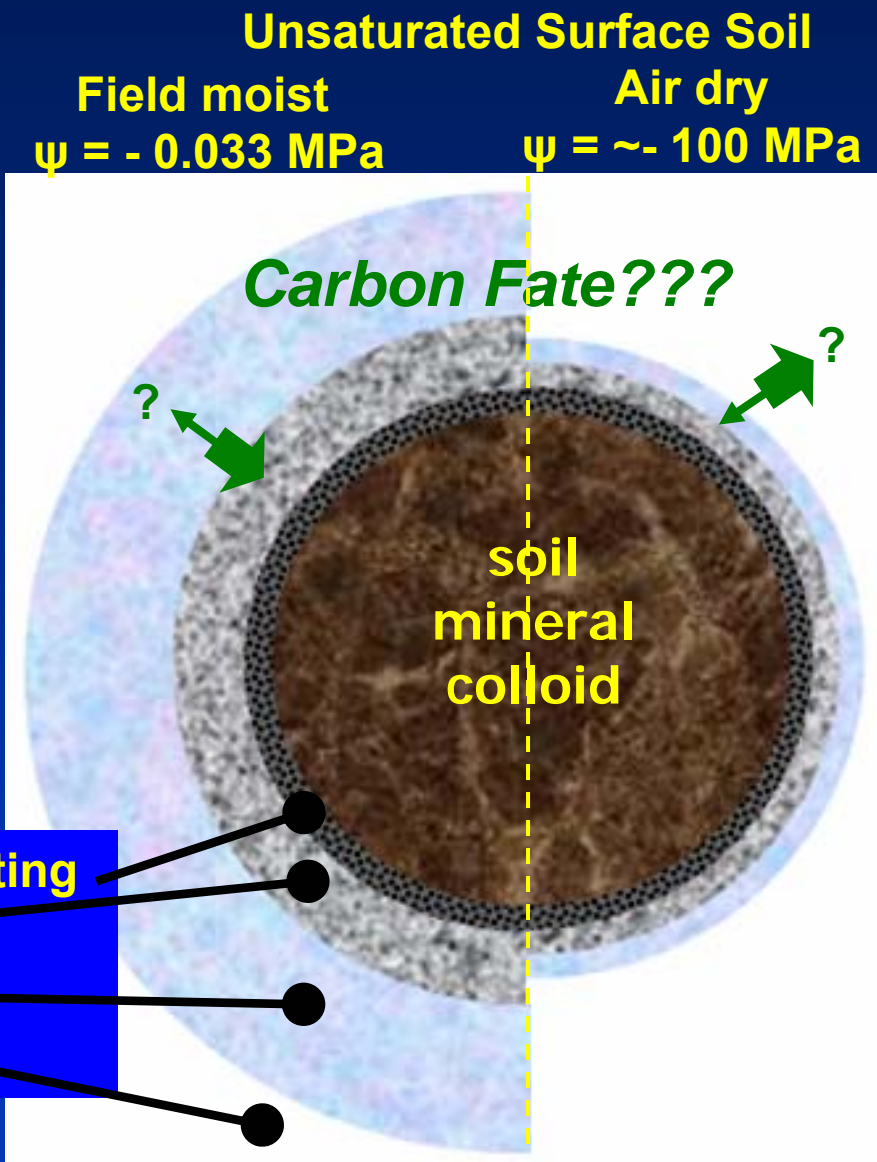




# Soil Water → Biofilm → A-OM → Particle



soil profile





# *Approach*

## **RDX humification in surface soils**

- Humification studies using both  $^{14}\text{C}$  and non-labeled RDX
- Add  $^{14}\text{C}$ -RDX directly to soil
- Use 2 soils with different OM levels
- Defined soil moisture and temperature conditions

## **Plant-associated RDX (underway)**

- Grow plants and load with  $^{14}\text{C}$ -RDX
- Add plant tissue with RDX-derived  $^{14}\text{C}$  to soils
- Use same soil moisture and temperature

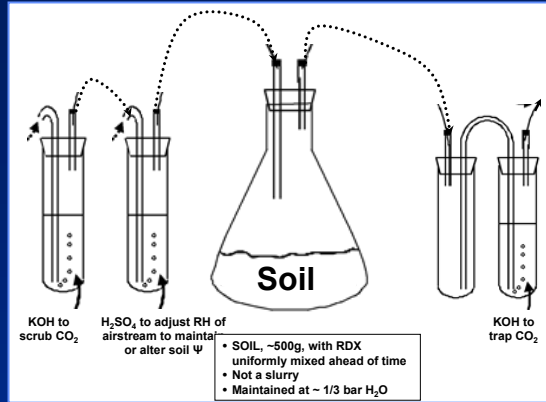
## **RDX photo-degradation using variegated plants (underway)**







# Approach- Methods



~ 1500g  
RT  
~1/3 bar

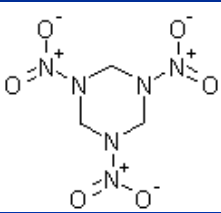
KOH trap  
BaCO<sub>4</sub>  
LSC

\*Plant

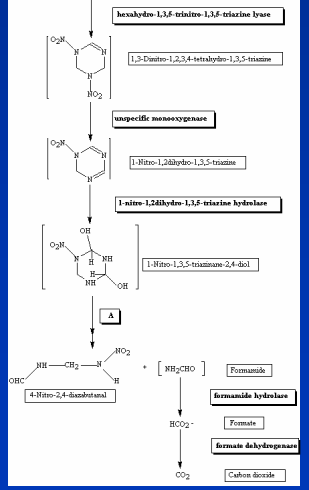
CO<sub>2</sub>

Mineralization-  
Immobilization  
Turnover

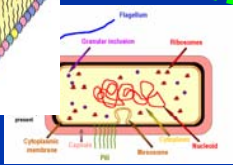
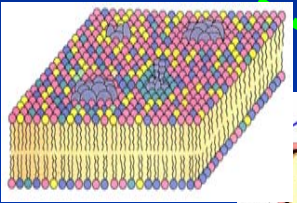
\*RDX  $\xrightarrow{\text{biotic}}$  \*T-RDX



RDX<sub>ACN</sub>  
HPLC  
LSC

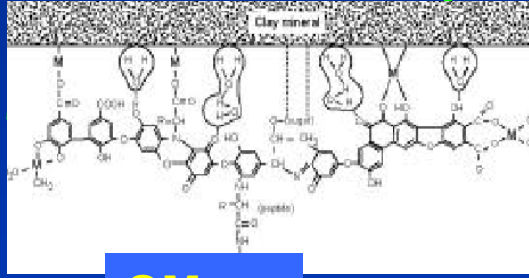


\*Biomass



Lipid<sub>BD</sub> & GCMS  
LSC  
T-RFLP

\*Humic  
Materials



OM<sub>MIBK</sub>  
LSC



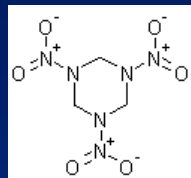


**4 reps**  
**2 soils (hi and lo OM)**  
**Controls (no RDX)**  
**Dark**  
**Mini-core sampling**





# Results – Partial Summary



**\*RDX**

biotic

**\*T-RDX**



**\*Plant**



**\*Biomass**

**CO<sub>2</sub>**



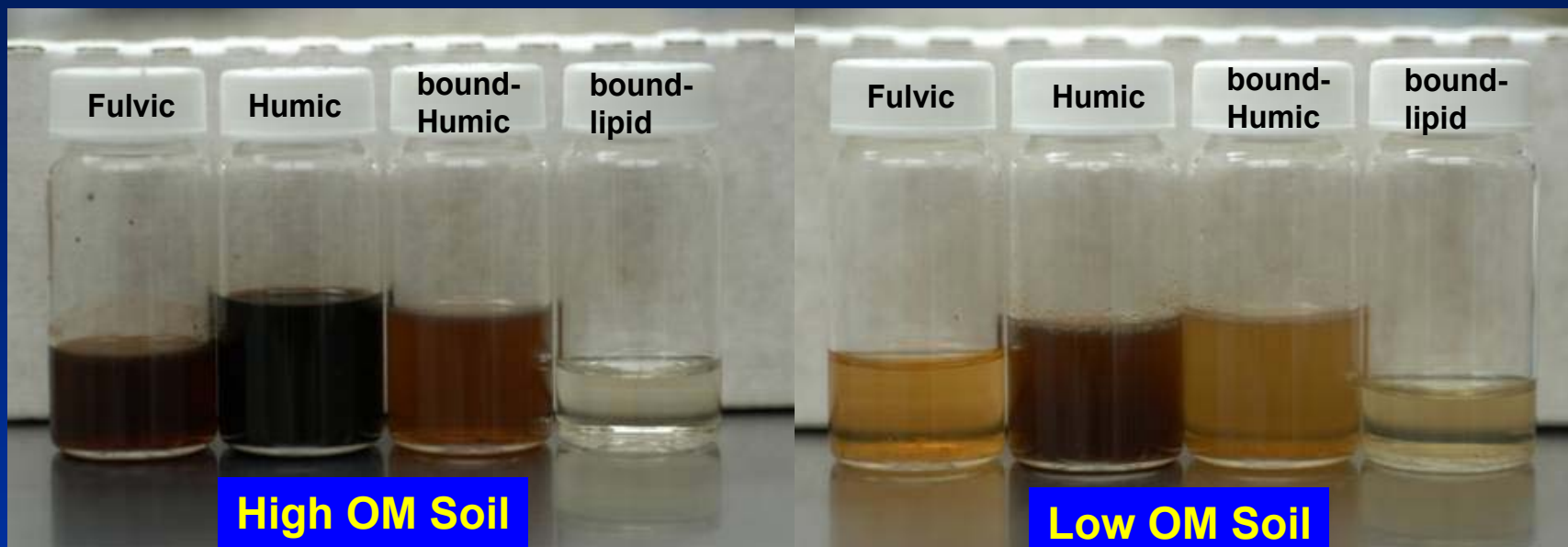
**\*Humics**

- RDX loss slow but consistent
- T-RDX transient
- \*C in microbial biomass low and consistent
- RDX-specific microbial community changes ...??
- Mass balance decreases with time...??
  - Cumulative error...??
  - Missing a pool...??





# Results – RDX directly to Soil



(OM fractionation – MIBK method)

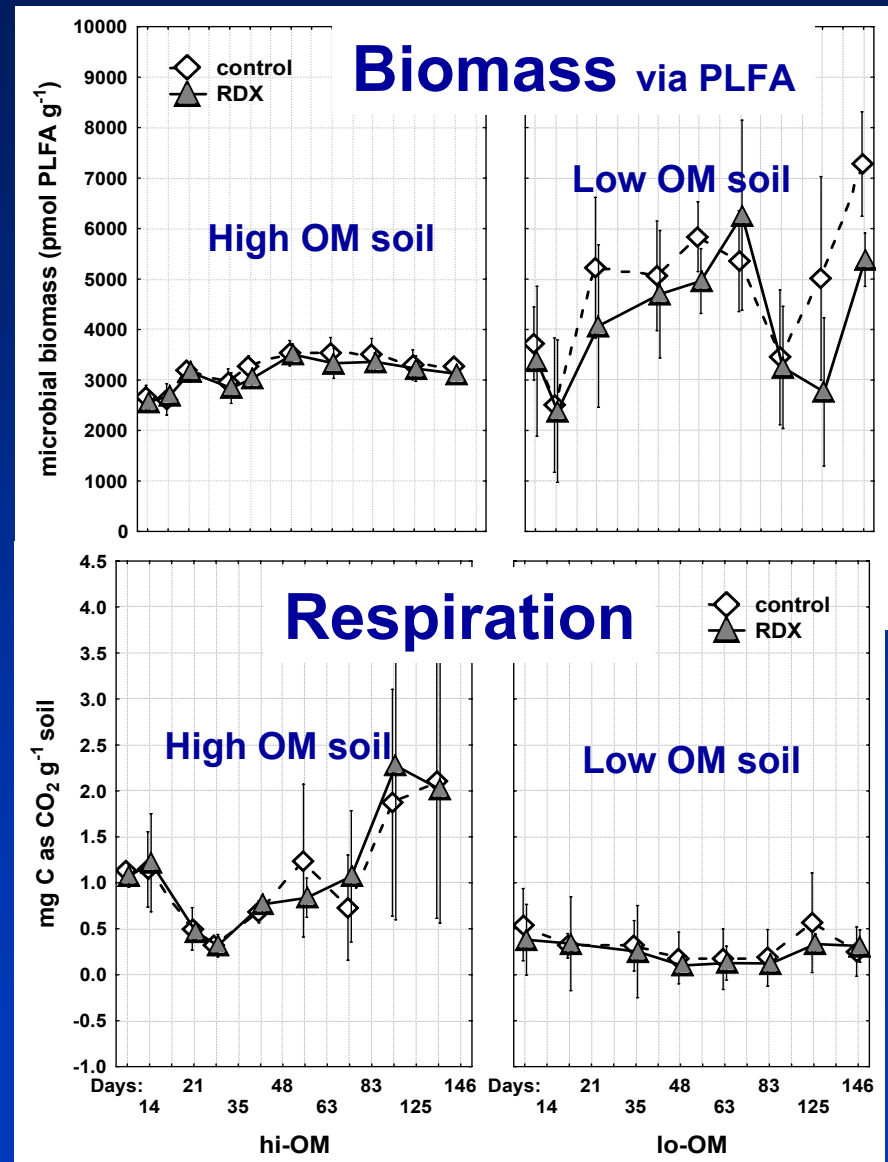




# Results – RDX directly to Soil

Soil biomass, normalized to soil dry weight showed:

- No sig. RDX effect on biomass
- More consistency in high OM soil (biomass responds to soil C rather than RDX-C)
- Yet respiration was greater in the high OM soil, and increased with time.
- Biomass and respiration sometimes viewed as “equivalent” but they diverge for both soils...
- These data suggest:
  - greater “activity” or “throughput” or MIT for the high OM soil,
  - greater cellular storage for the low OM soil







# Results – RDX to Soil

**Fate of  $^{14}\text{C}$  derived from RDX ?**

**Is there a difference between soils?**

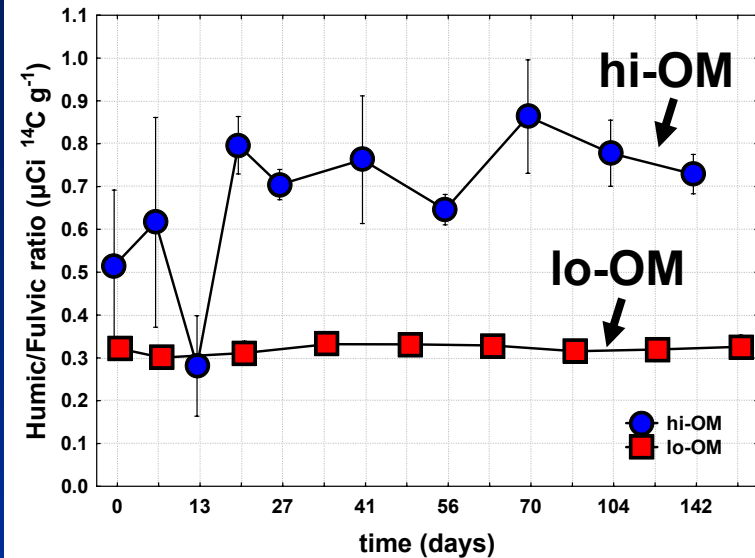
- In high OM soil, a significantly greater amount of RDX derived C moves into the bound humic fraction --“humification”

**And**

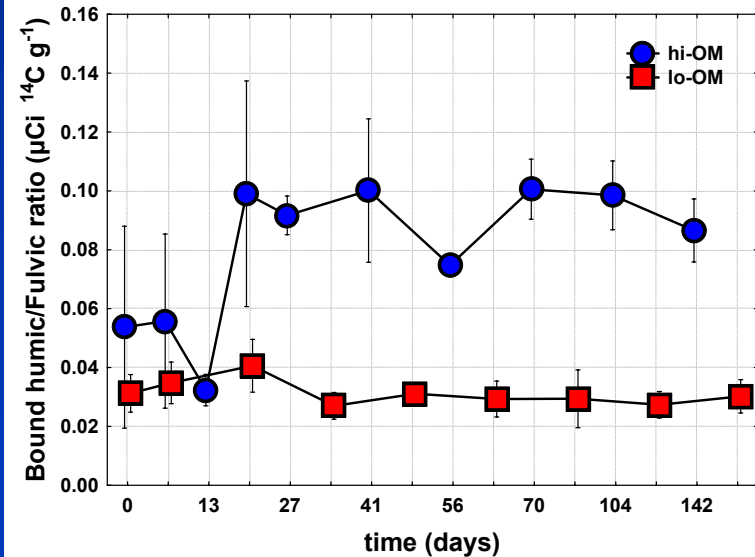
- This appears related to MIT

**$^{14}\text{C}$  location**

**Humic**



**Bound Humic**





# Results – Plant Tissue RDX to soil

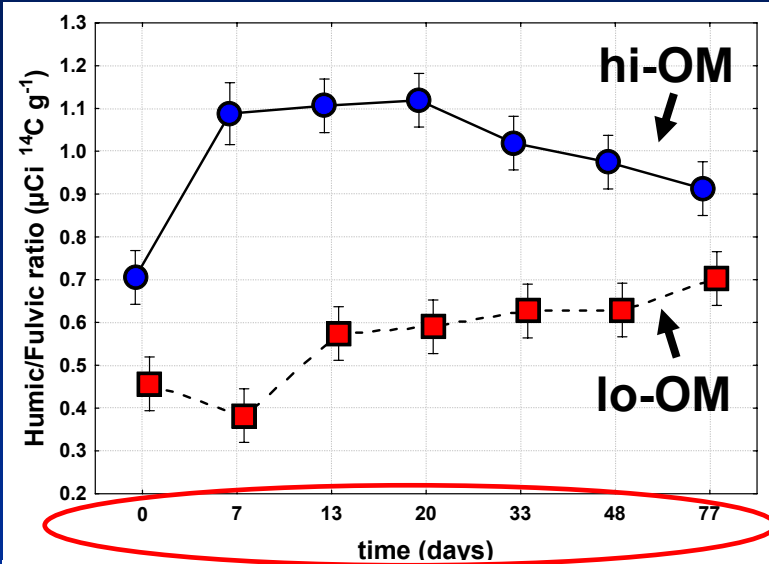
*Fate of  $^{14}\text{C}$  derived from Plant RDX ?*

*Is there a difference between soils?*

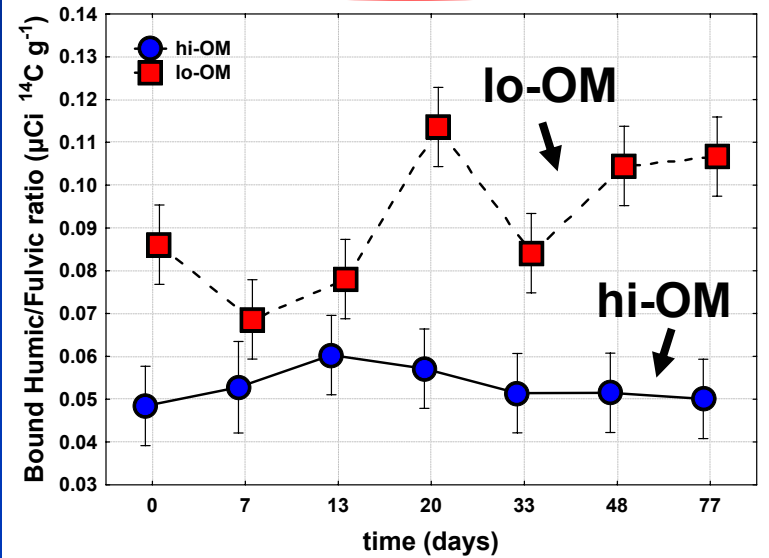
- Yes, but a different pattern than seen for RDX added directly to soil is emerging

14C location

Humic



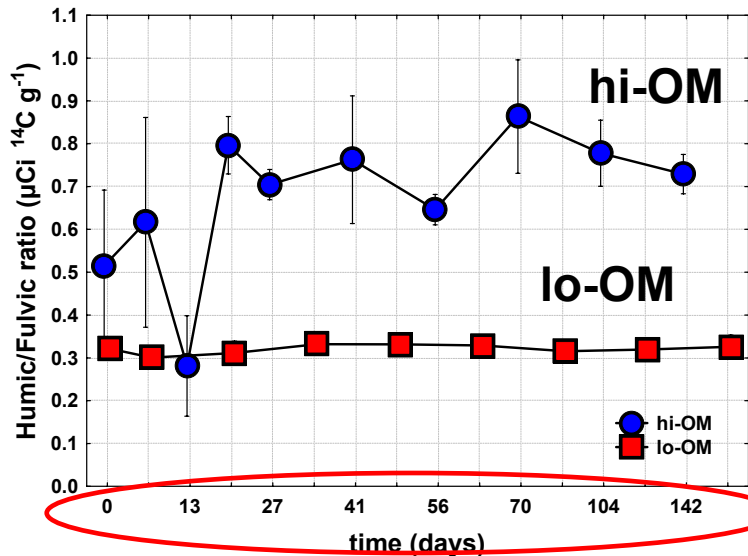
Bound Humic





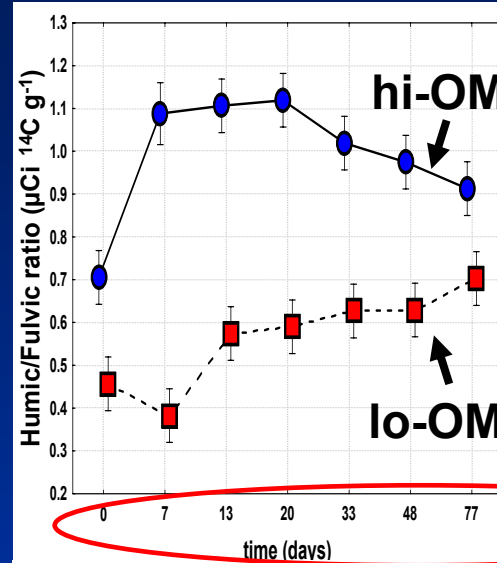
# Plant Tissue RDX to soil

RDX to soil

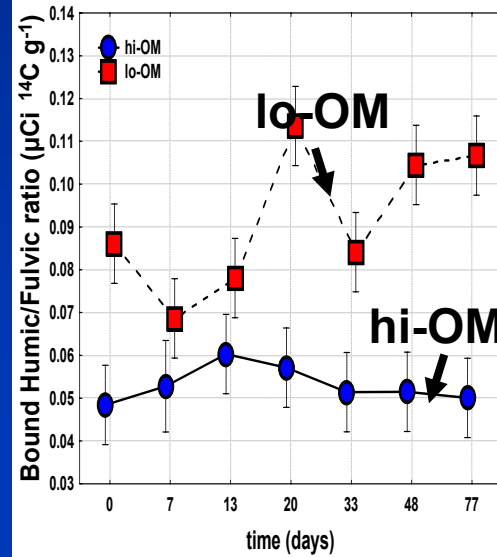
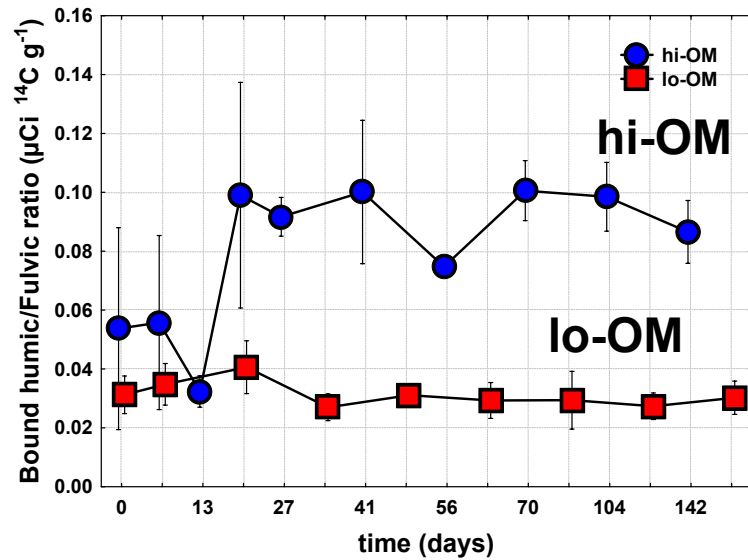


Plant Tissue RDX to soil

Humic



Bound Humic



14C location





# Results

Using Humic/Fulvic Ratio

Direct soil RDX vs Plant  
Tissue RDX

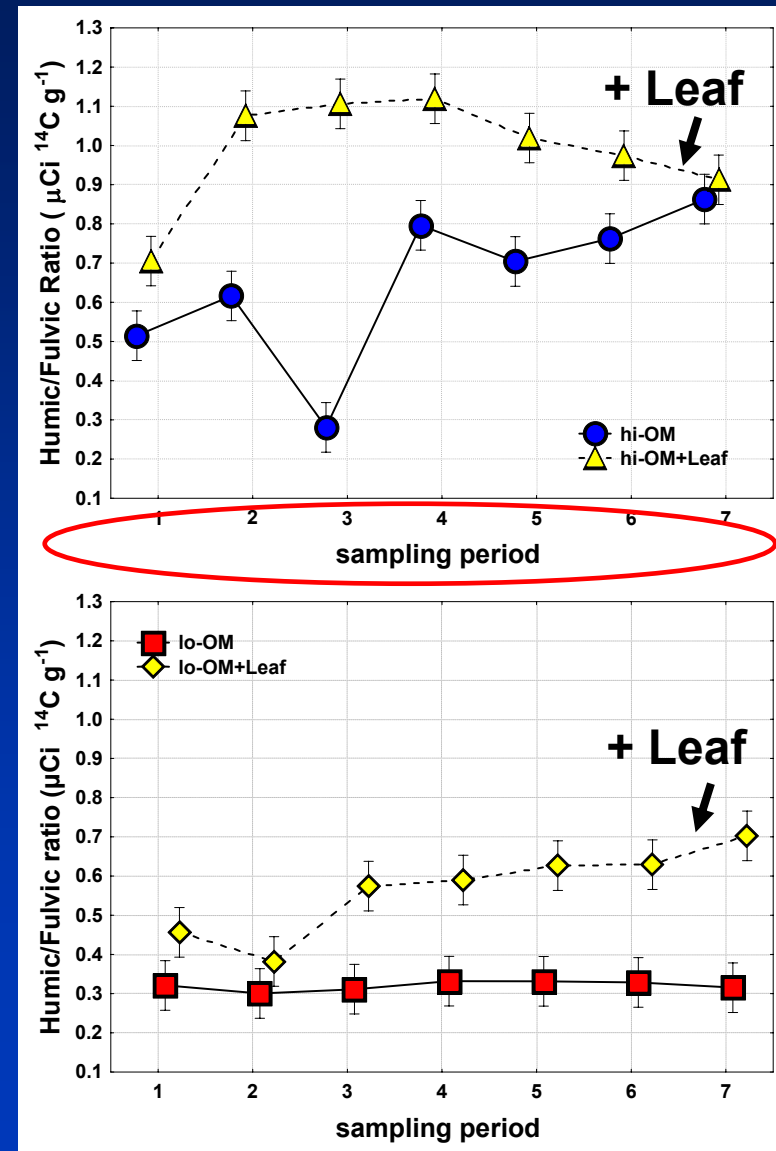
High OM soil – convergence

Low OM soil - divergence

14C location

High OM soil

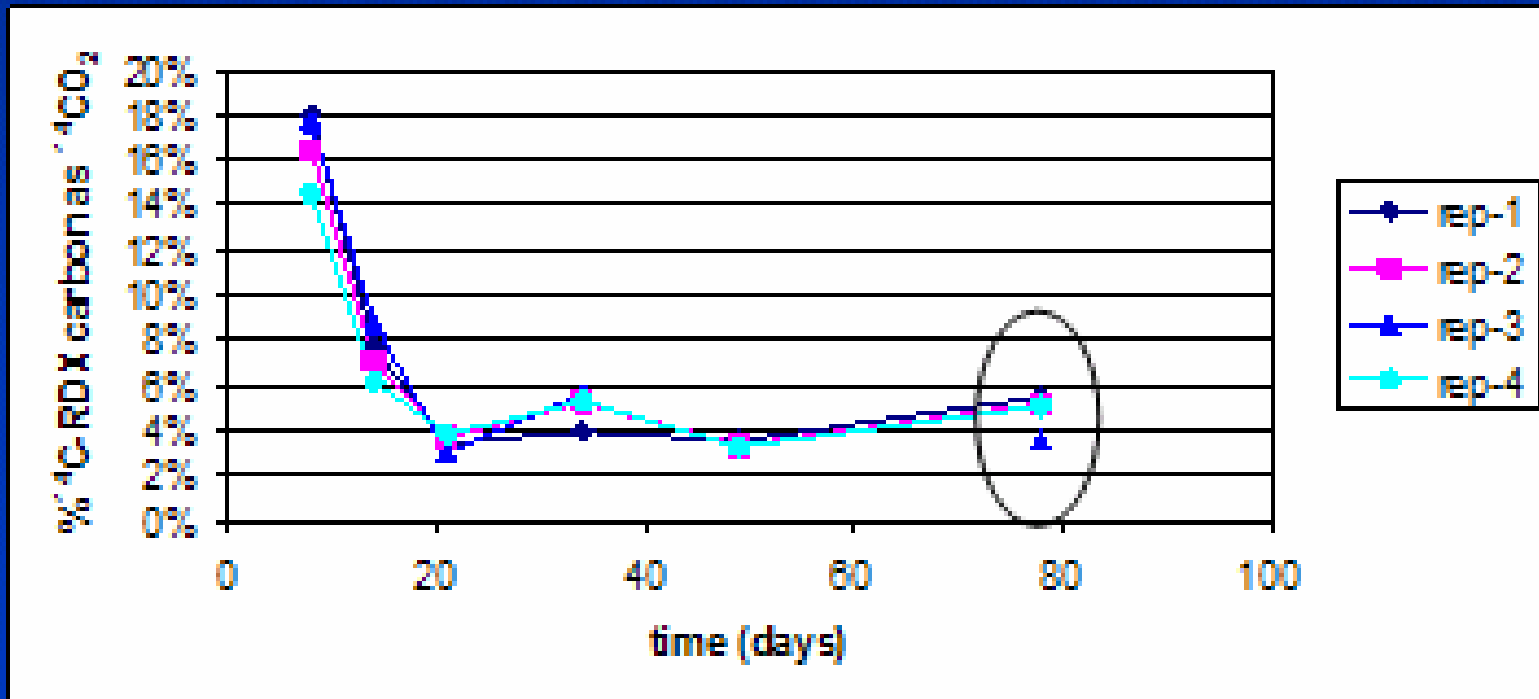
Low OM soil





# Evolving Soil Communities

## Sudden variability in $^{14}\text{C}$ evolution







# Results – Odd 14 C Rep for Plant Tissue RDX to soil

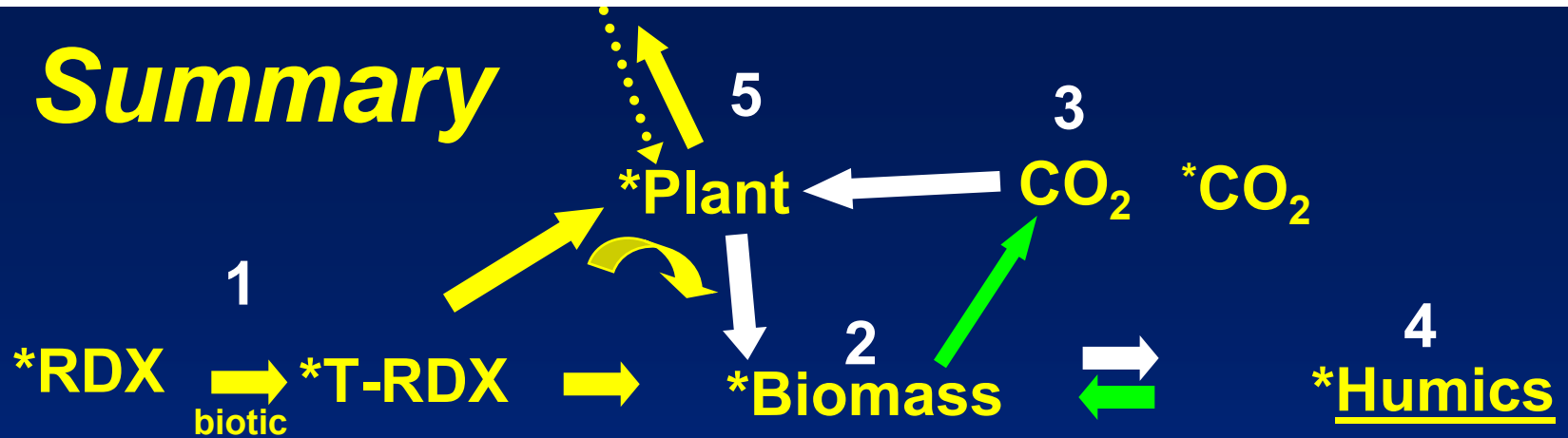


*Phanerochaete ?*





# Summary



1. Biotic transformation of RDX in surface soils is slow but does occur.
2. \*C in biomass low but constant – suggesting steady state role in flow of RDX into other pools.
3. \*CO<sub>2</sub>↑ low but constant. General CO<sub>2</sub>↑ may be important as an indicator of MIT
4. RDX (soil) - Greater amount of \*C associated with bound-humic fraction in the high OM, high respiration soil relative to the lower OM soil.  
RDX (plant) – changes in \*C in humic fractions for both soils, more so for low OM soil.
5. Photo-degradation in plant tissue, variegated plants (underway)

Data suggest possible plant-based, agronomic site management practices that encourage binding of RDX residues to soil

