



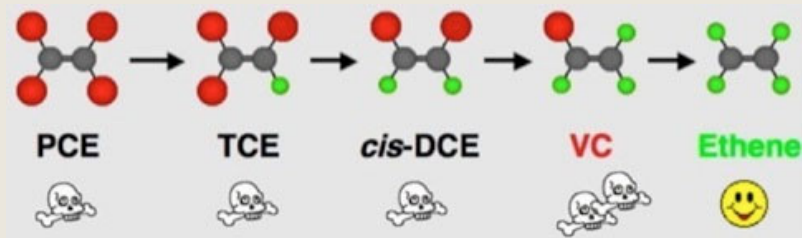
*BIOSTIMULATION OF TRICHLOROETHENE
DECHLORINATION BY ORGANOHALIDE-
RESPIRING BACTERIA*

Presented by Xuewen Wang



Environmental Problem

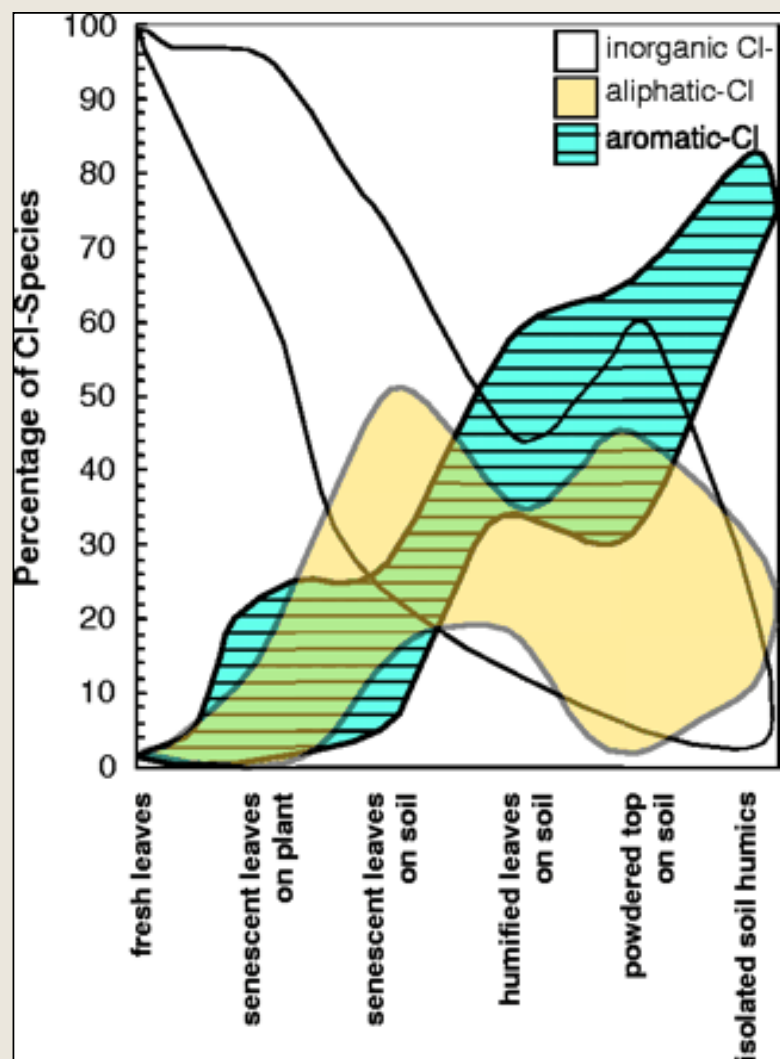
- Soils and groundwater contaminated with chlorinated ethenes is extensive
- PCE and TCE used in dry-cleaning fluids and degreasing



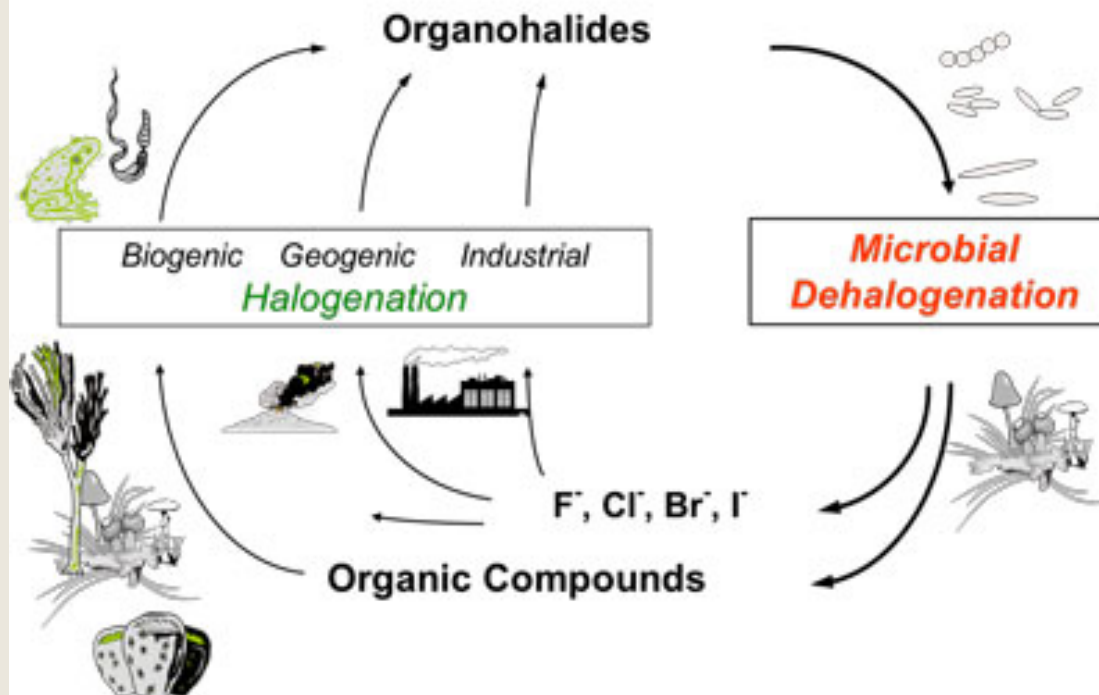
- Many anaerobic bacteria can dechlorinate chlorinated ethenes

Halogenation of 'Natural' chlorinated compounds

- Cl as inorganic form in the fresh leaves turn into aliphatic Cl and aromatic Cl as the leaves decay.



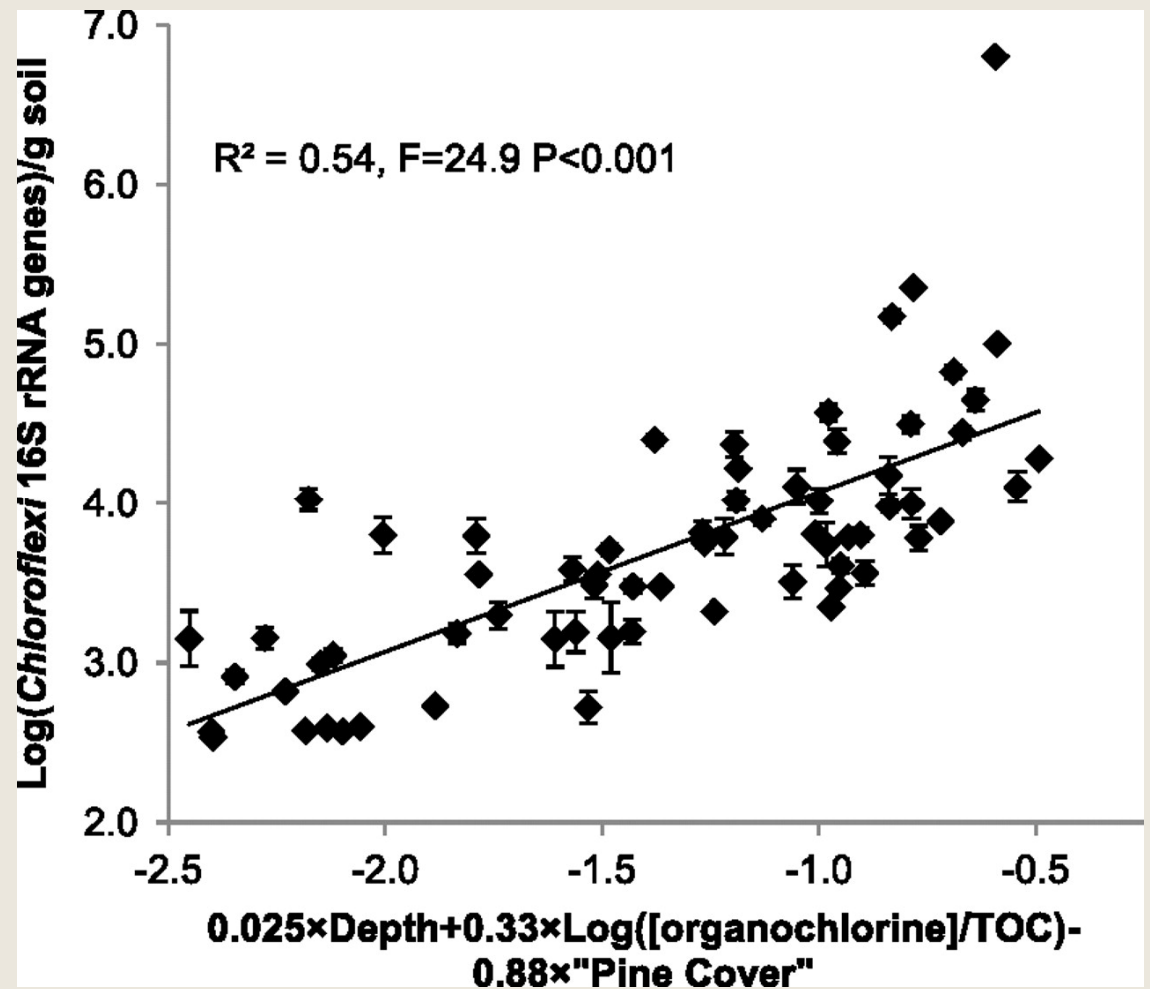
A Natural Organohalogen Cycle



Why Organohalide Respiring Bacteria?

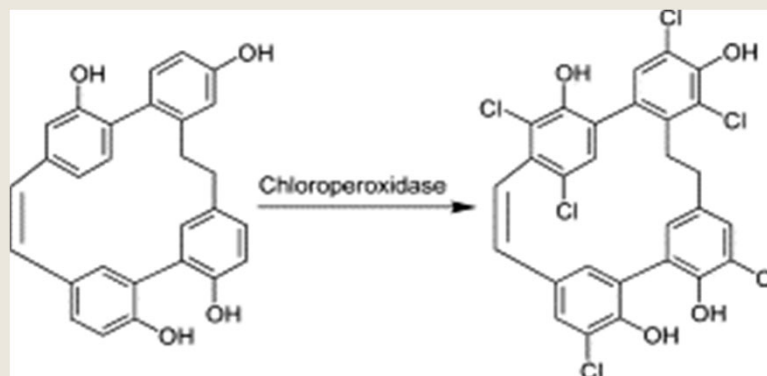
- OHRB are anaerobes and can reside in anoxic habitats
- Organohalogenes are excellent electron acceptors
- Some members of the class Dehalococcoidia within the phylum Chloroflexi are exclusively dependent on OHR
- Many OHRB are capable of reducing highly halogenated organohalogenes that usually resist aerobic degradation.

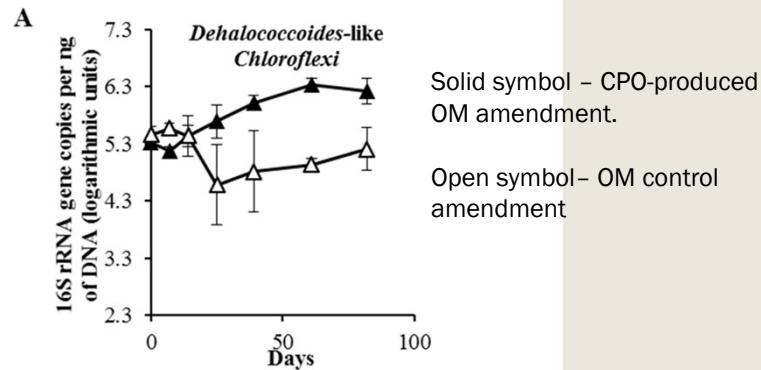
The "*Dehalococcoides*-like *Chloroflexi*" is correlated to the amount of organochlorine in natural soil, which gives the idea that chloroflexi would prefer the environment which has natural organochlorines.



Biosynthesis of organochlorine compounds

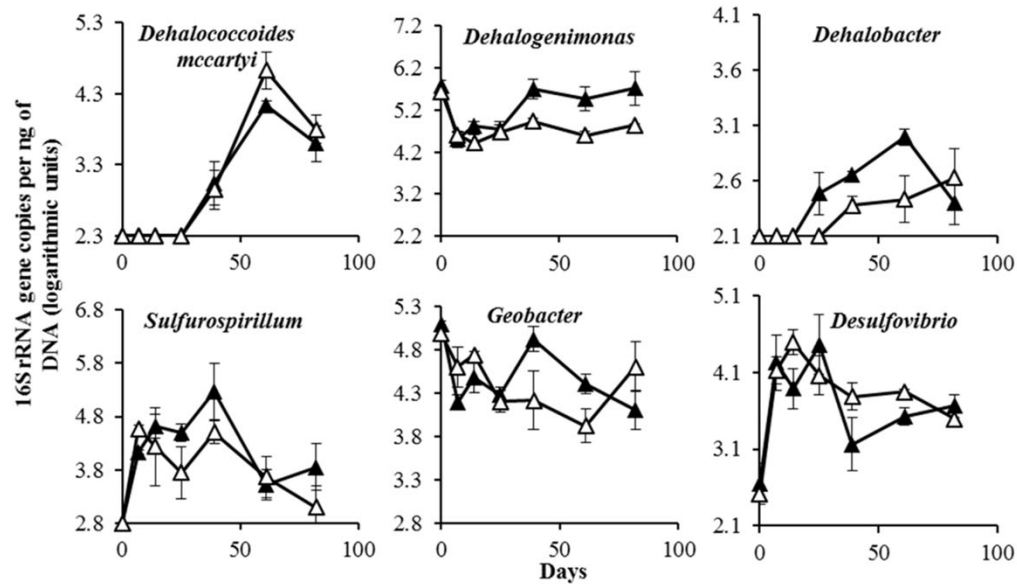
Chloroperoxidase (CPO) reactions





The “*Dehalococcoides-like Chloroflexi*” were enriched as a whole – however no individual strains contributed more than a few reads in the Illumina sequencing.

Illumina and qPCR found 15 groups of uncultured novel bacteria that were enriched.

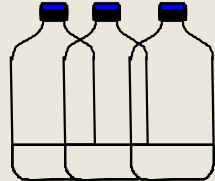


Directly targeting known organochloride-respirers with qPCR, found that *Dehalogenimonas* and *Dehalobacter* were enriched, though others were not.

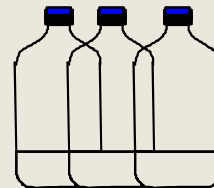
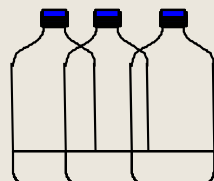
First question is...

Can CPO-produced organochlorides stimulate trichloroethene biodegradation?

CPO Treated OM



Organic Matter Control



Using **water-based** soil extraction
(for CPO reaction and OM control).

Measure TCE dechlorination
with GC-ECD

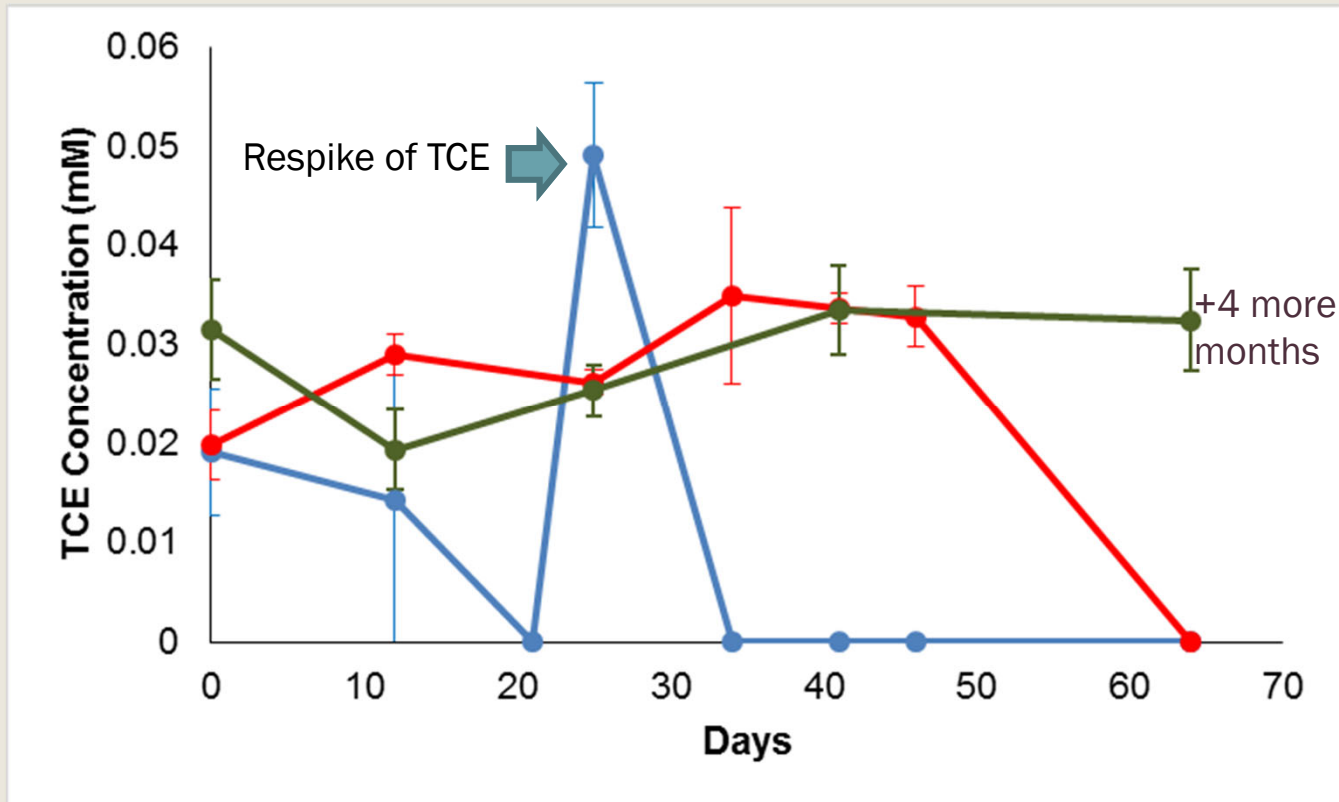
Anaerobic conditions

Methanol for electron
donor



+ Trichloroethene

TCE concentrations in Batch Reactors with **CPO-treated OM**, **OM-control extract**, and **no-coamendment**

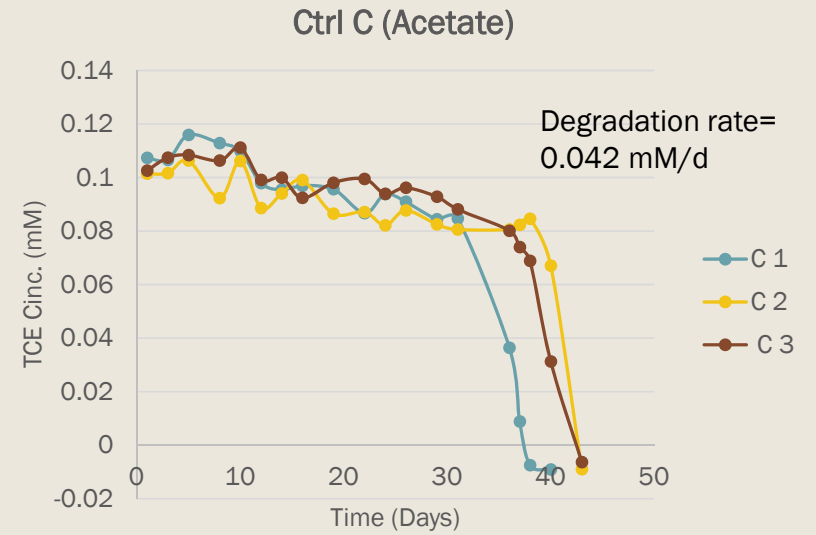
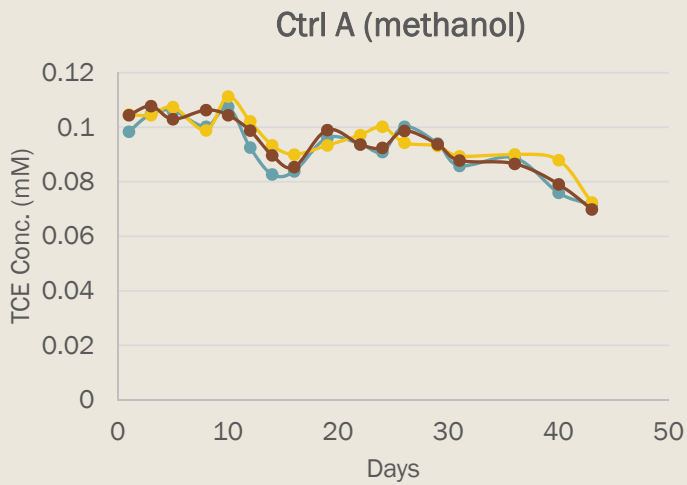
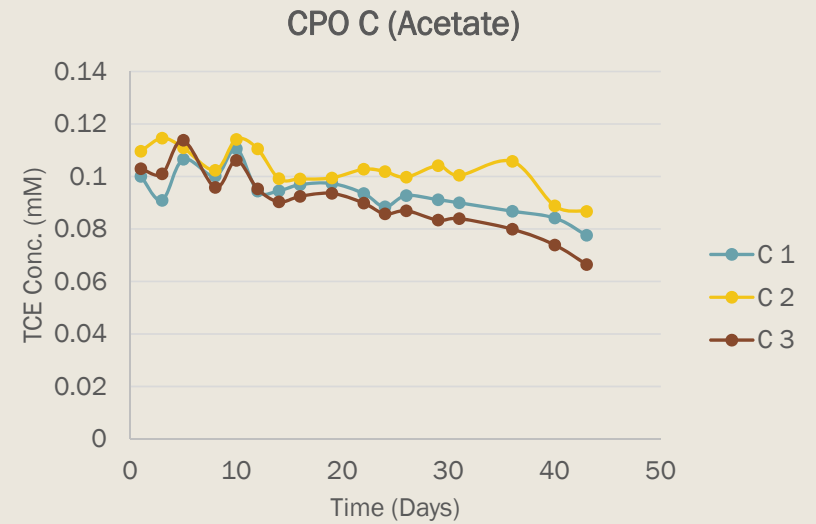
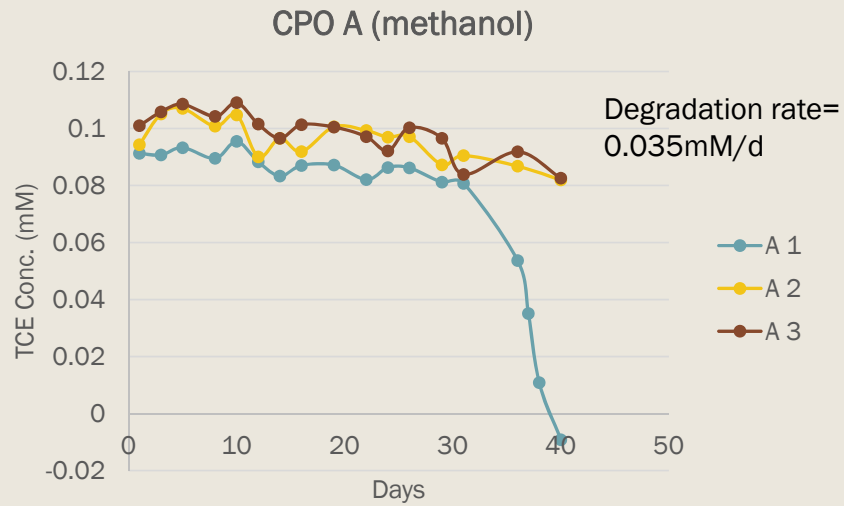


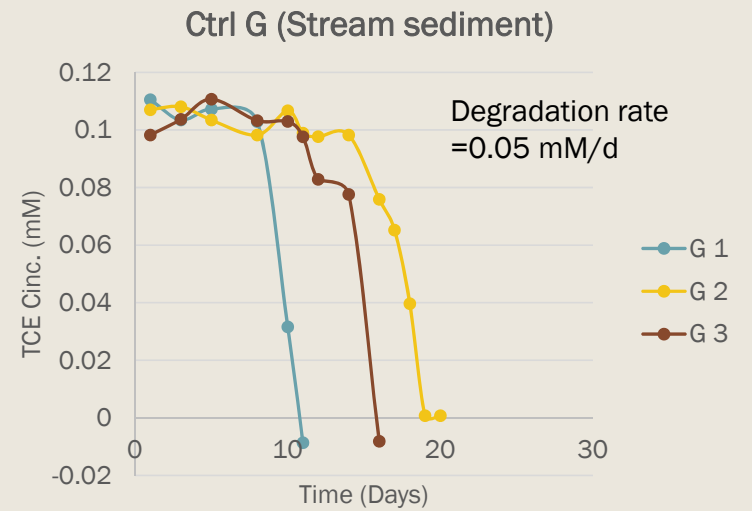
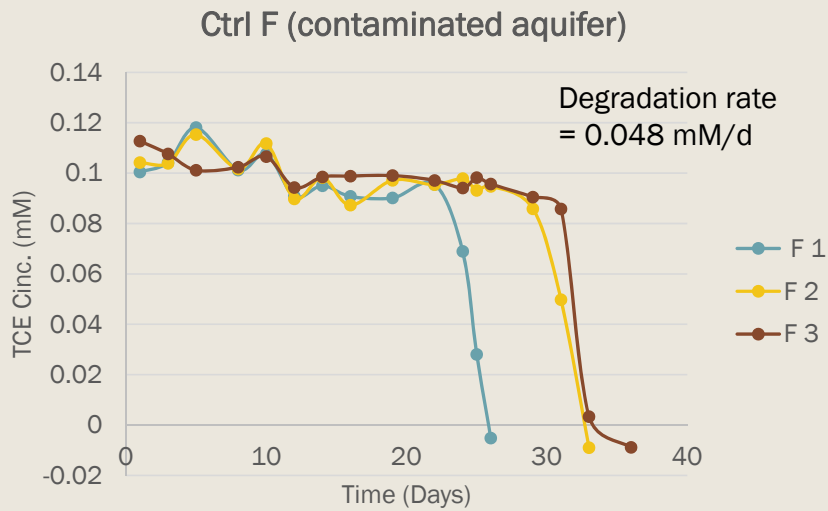
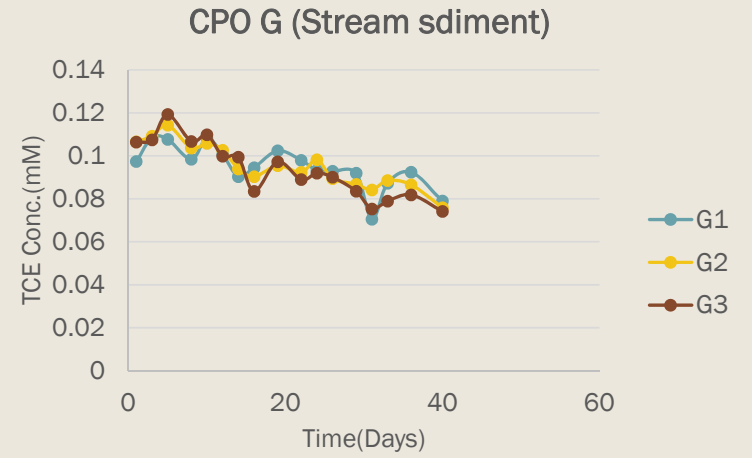
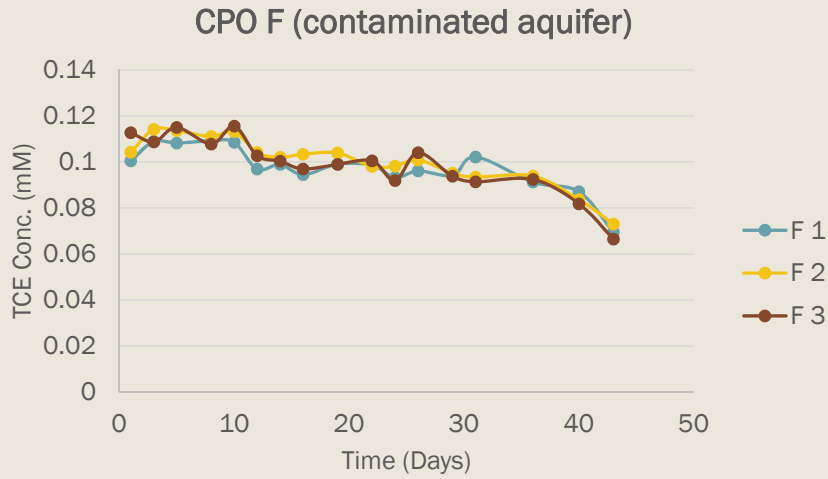
Degradation occurs within 20 days when co-amended with CPO-treated organic matter. Control extract also stimulates degradation sometime ~60 days. Soil without co-amendment fails to degrade (over several months).

Next question is ...

How will the TCE dechlorination experiment
being performed for various conditions
(carbon source, soil type, pH, redox)?

Microcosms setup					
No. of Microcosm	carbon source	soil	pH	Redox	Amendments
a	methanol	oak forest	7.0	Methanogenic	<p>Each microcosm will be amended with both CPO extract and Control extract.</p> <p>Condition (Methanol, oak, 7.0, methanogenic) and condition (molasses, oak, 7.0, methanogenic) also have (no-amendment controls).</p> <p>One autoclaved microcosm (methanol, oak, 7.0, methanogenic) is operated as another control.</p>
b	butyrate				
c	acetate				
d	molasses				
e	methanol	grass land	7.0		
f		contaminated aquifer			
g		stream sediment			
h		desert			
i	methanol	Oak forest	5.5		
j			8.5		
k	methanol	Oak forest	7.0	nitrate	
l				sulfate	
m				oxygen	





Thus far --

- CPO-produced organochlorides did stimulate the degradation of TCE through at least DCE in a uncontaminated soil, but inhibited TCE dechlorination in microcosms seeded with aquifer material undergoing in situ chlorinated ethane remediation, and seeded with sediments.
- Mechanism is not known, best guess
 - *CPO produced organochlorides can develop a dechlorinating community, but may be a competitive inhibitor with TCE, similar to that seen in many co-contaminated sites*
 - Thus, soils with sufficient communities may not get stimulated, but CPO produced organochlorides may help develop dechlorinating communities in soils in which dechlorinating communities are under-developed
 - Longer lag time in repeated experimental conditions with uncontaminated soil supports this – a higher dosage of CPO produced organochlorides was amended and thus take longer to dechlorinate, and thus inhibit TCE dechlorination longer,

Future work

- Microbial analysis to elucidate transcription of reductive dehalogenase genes, and growth of dechlorinators between CPO-produced OM reactor, and control, and with/without TCE.
 - *Determine mechanism of inhibition vs biostimulation*
- Toxicity assessments of CPO-reacted organic matter.

Thank you.

Question?