Case Studies on Practical Use of Stable Isotope Probing

Matt Burns
WSP USA Corp.
300 Trade Center, Suite 4690
Woburn, MA, 01801
781/933-7340
matt.burns@wspgroup.com

http://www.linkedin.com/in/mattburnswsp

Kerry Sublette
University of Tulsa
Keplinger Hall 321
918-631-3085
Kerry-sublette@utulsa.edu

http://ipec.utulsa.edu

providing management and consultancy services to the built and natural environment

15,000 people
300 offices
35 countries

WSP

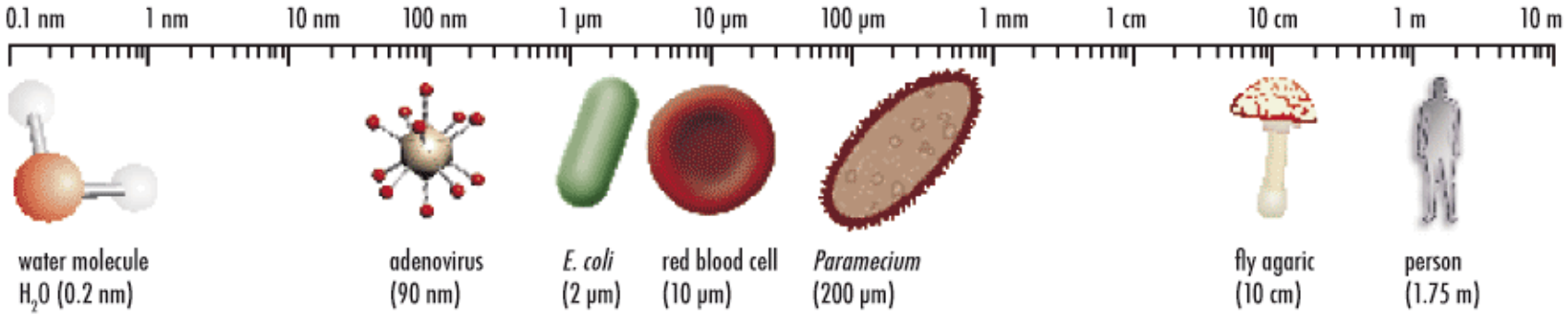
bioremediation

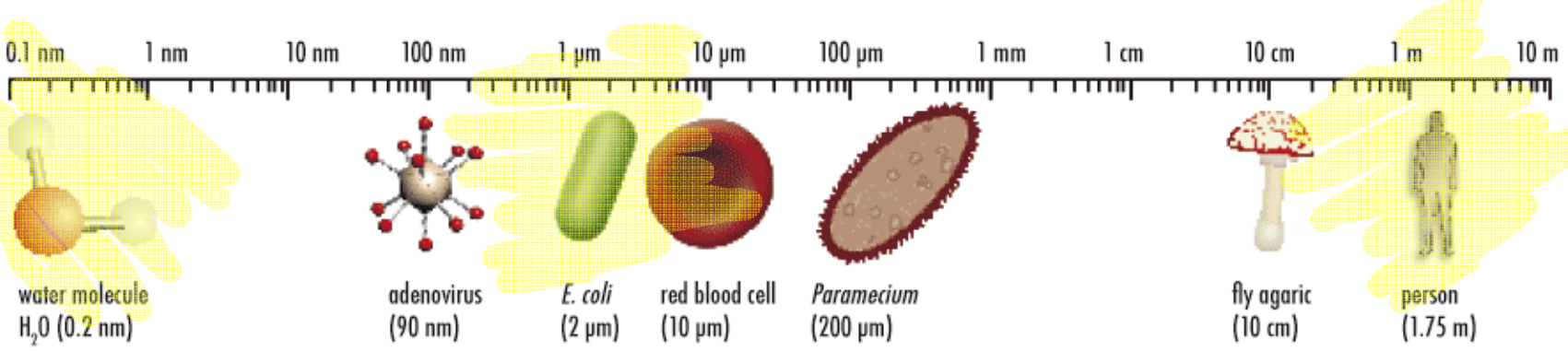
mechanisms of degradation are unseen and not as well understood as horsepower based remediation

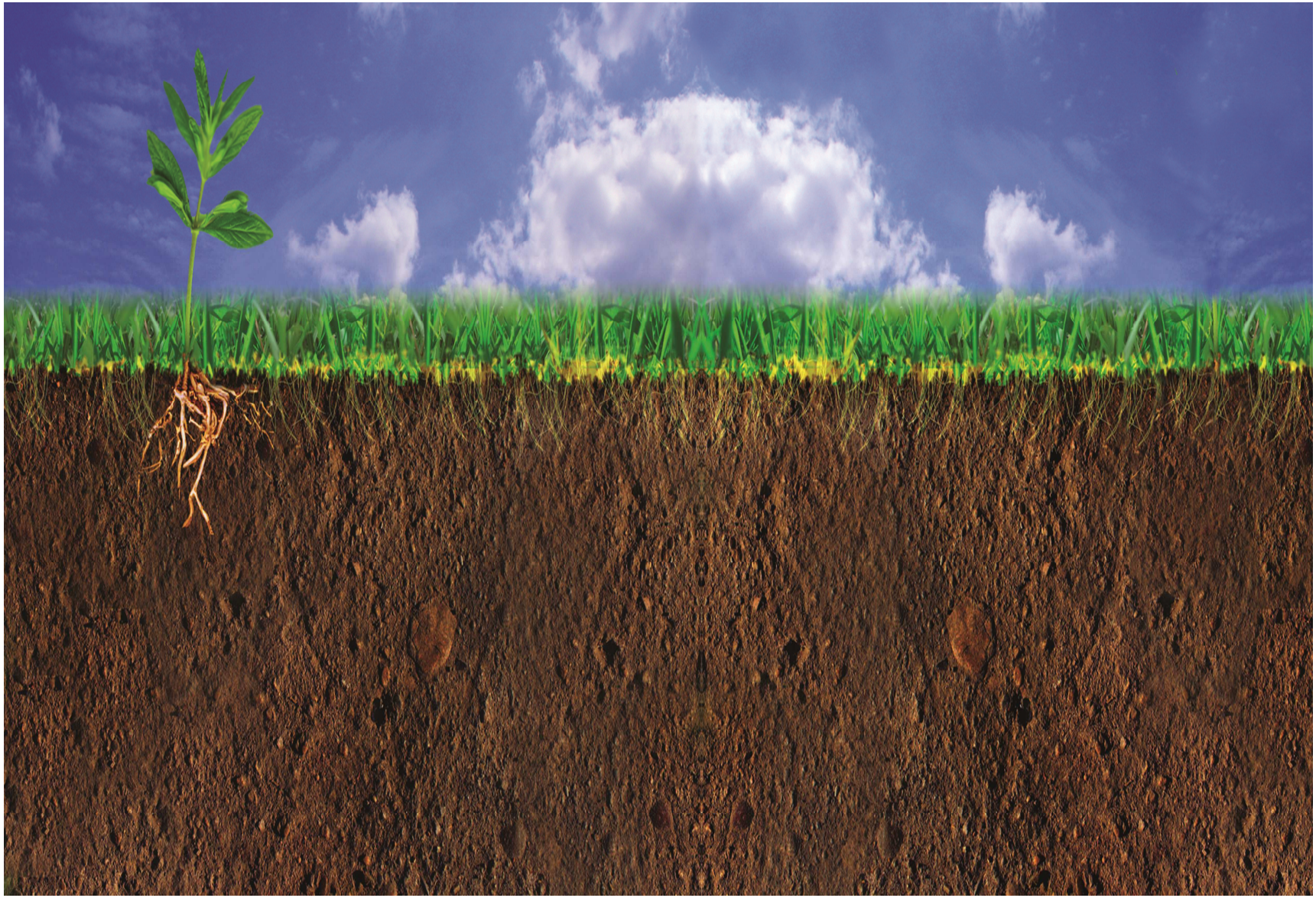

KOMATSU

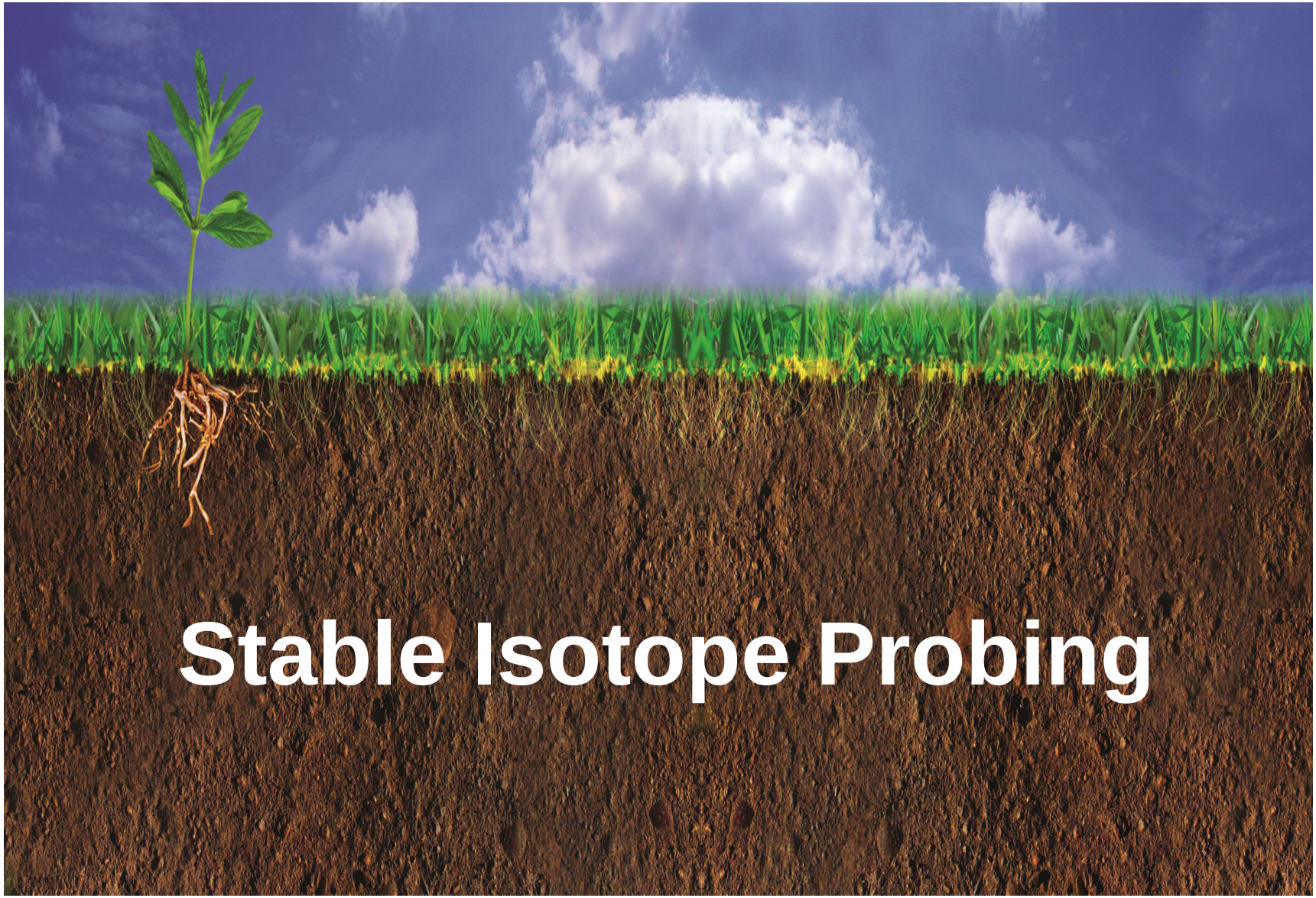
WSP


KOMATSU


0.1 nm
1 nm
10 nm
100 nm
1 μm
10 μm
100 μm
1 mm
1 cm
10 cm
1 m
10 m
water molecule
H_2O (0.2 nm)
adenovirus
(90 nm)
E. coli
(2 μm)
red blood cell
(10 μm)
Paramecium
(200 μm)
fly agaric
(10 cm)
person
(1.75 m)


0.1 nm
1 nm
10 nm
100 nm
1 μm
10 μm
100 μm
1 mm
1 cm
10 cm
1 m
10 m
water molecule
H_2O (0.2 nm)
adenovirus
(90 nm)
E. coli
(2 μm)
red blood cell
(10 μm)
Paramecium
(200 μm)
fly agaric
(10 cm)
person
(1.75 m)





Stable Isotope Probing

agenda

start with overview of SIP

agenda

start with overview of SIP

can't avoid the underlying science but will try to minimize

agenda

start with overview of SIP

can't avoid the underlying science but will try to minimize

see MI (microbe.com) recorded webinars by:

Mike Hyman
Frank Loeffler
Kirsti Ritalahti
Matt Burns

agenda

start with overview of SIP

can't avoid the underlying science but will try to minimize

see MI (microbe.com) recorded webinars by:

Mike Hyman
Frank Loeffler
Kirsti Ritalahti
Matt Burns

Pennsylvania case study

agenda

start with overview of SIP

can't avoid the underlying science but will try to minimize

see MI (microbe.com) recorded webinars by:

Mike Hyman
Frank Loeffler
Kirsti Ritalahti
Matt Burns

Pennsylvania case study

Michigan case study

agenda

start with overview of SIP

can't avoid the underlying science but will try to minimize

see MI (microbe.com) recorded webinars by:

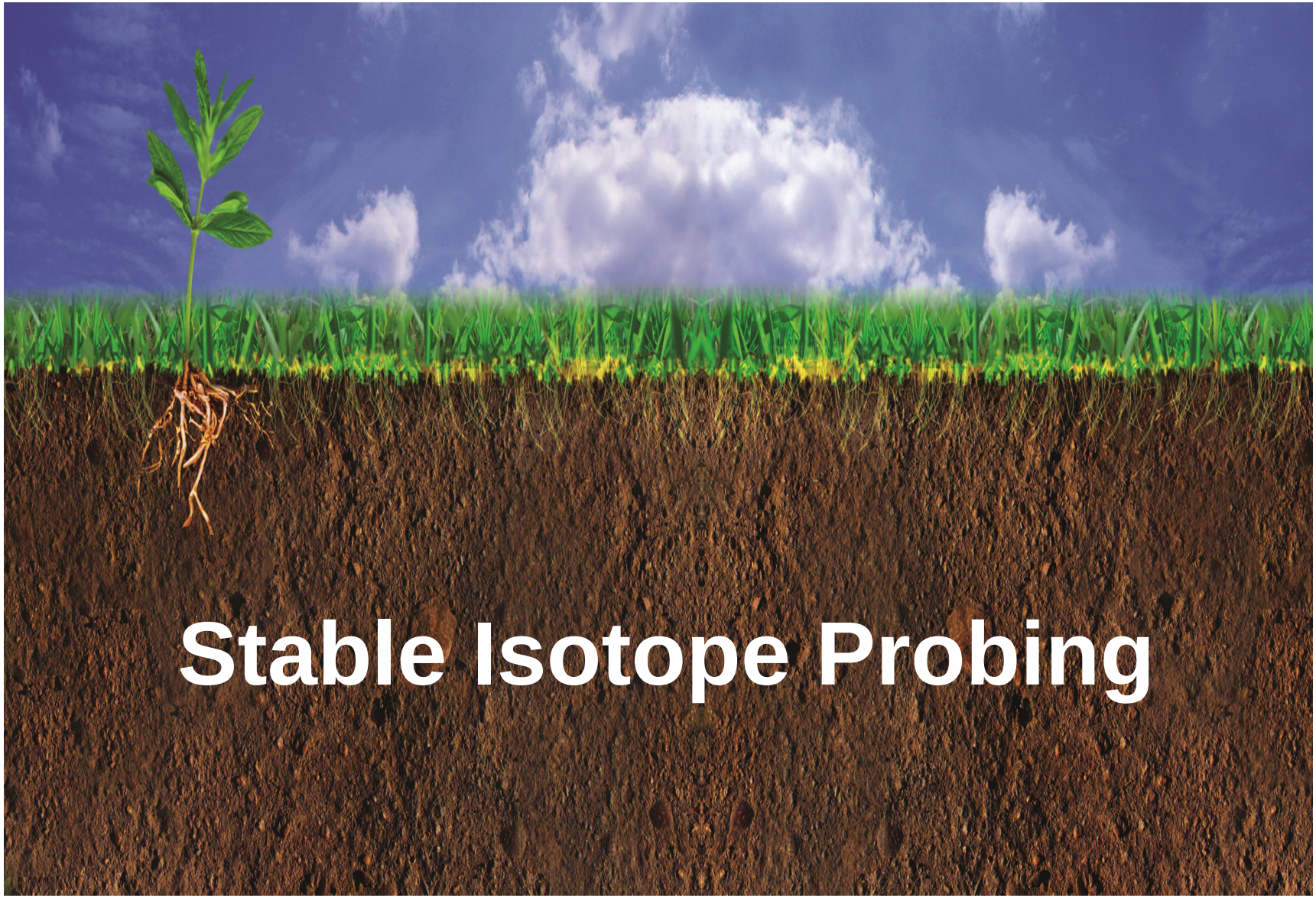
Mike Hyman
Frank Loeffler
Kirsti Ritalahti
Matt Burns

Pennsylvania case study

Michigan case study

another Michigan case study

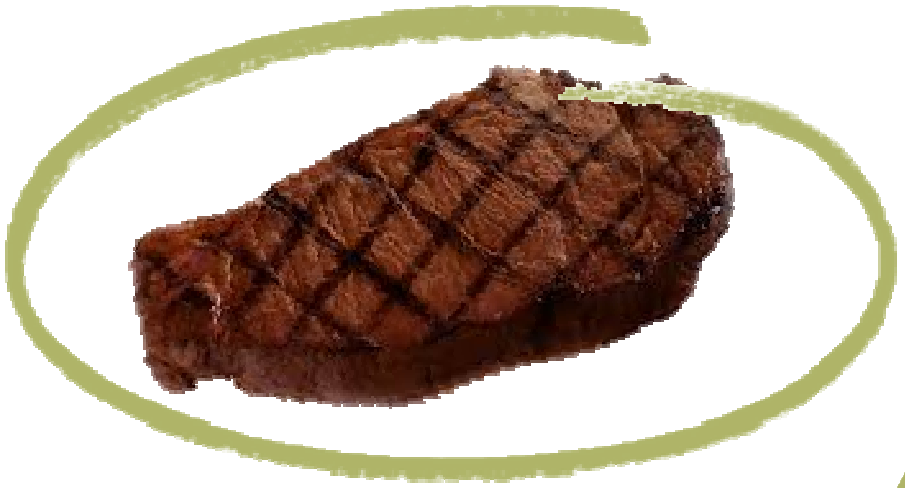
WSP



Stable Isotope Probing

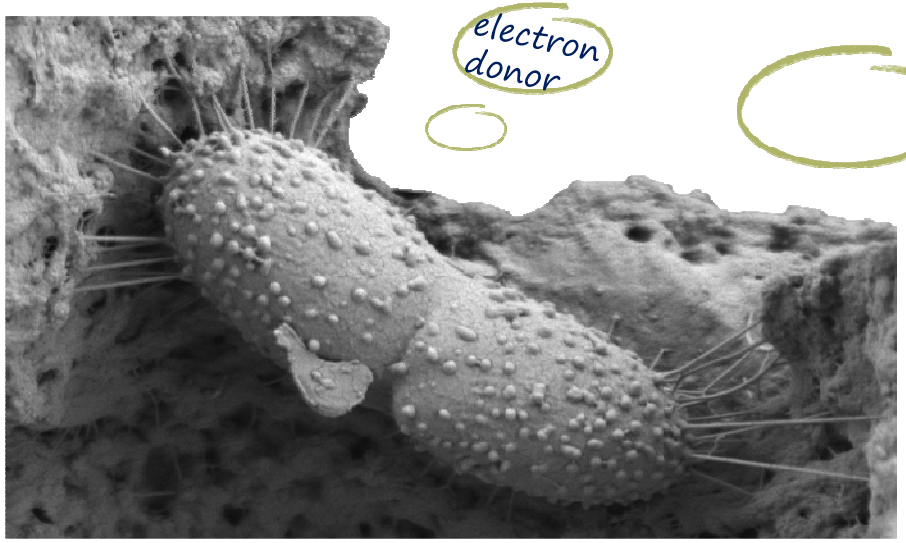
microbes are similar to us….

they eat and breathe too



electron donor

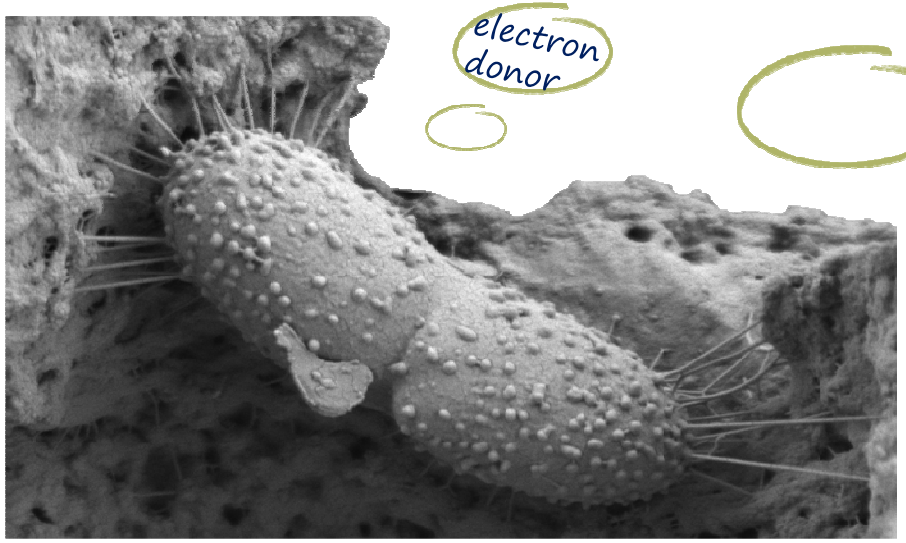
electron acceptor



as a group they can eat and breathe a lot more things

electron donor

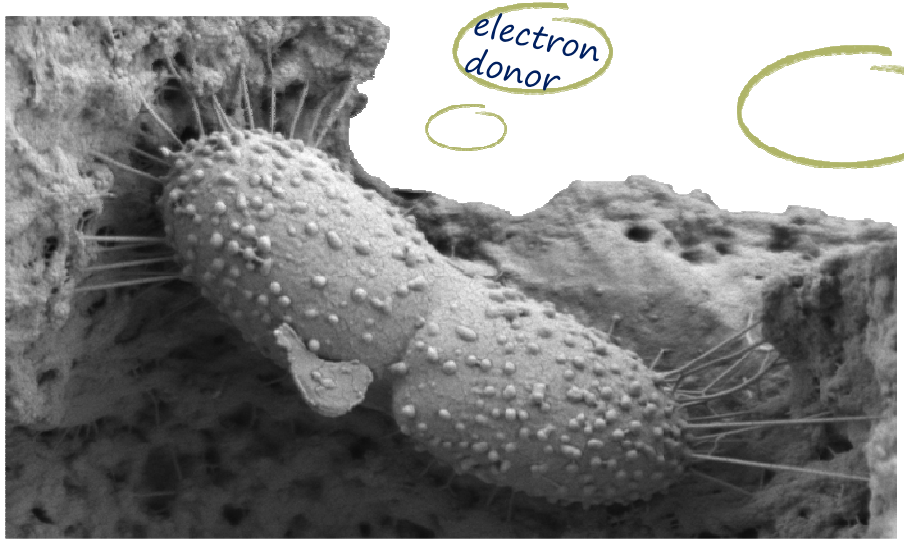
electron acceptor



as a group they can eat and breathe a lot more things

electron donor

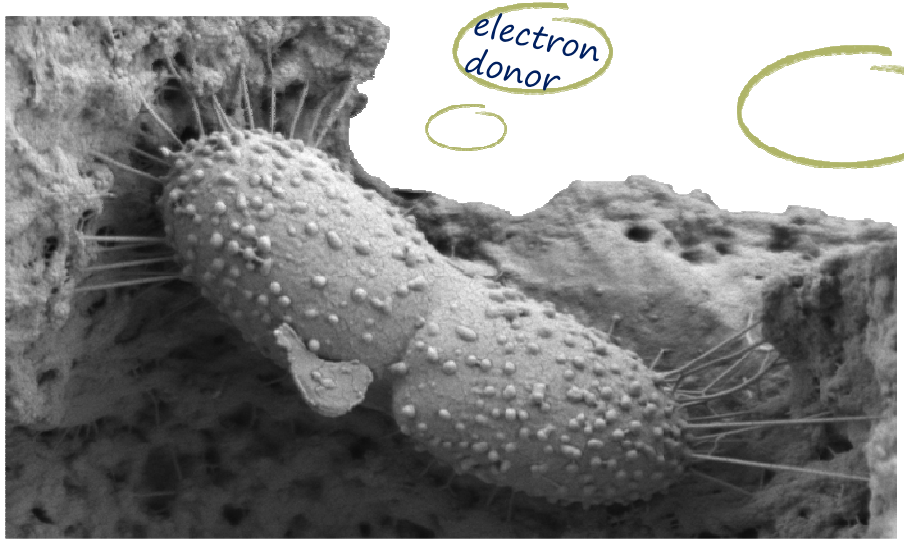
electron acceptor



as a group they can eat and breathe a lot more things

electron donor

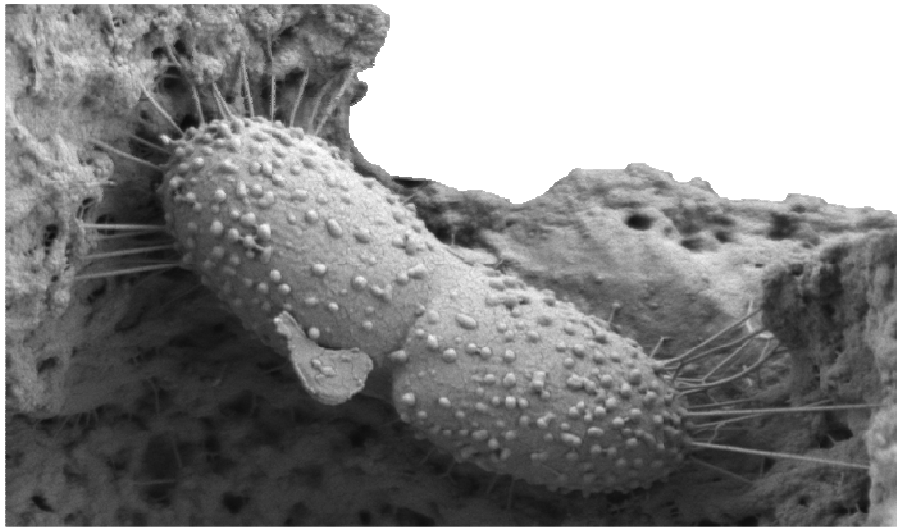
electron acceptor



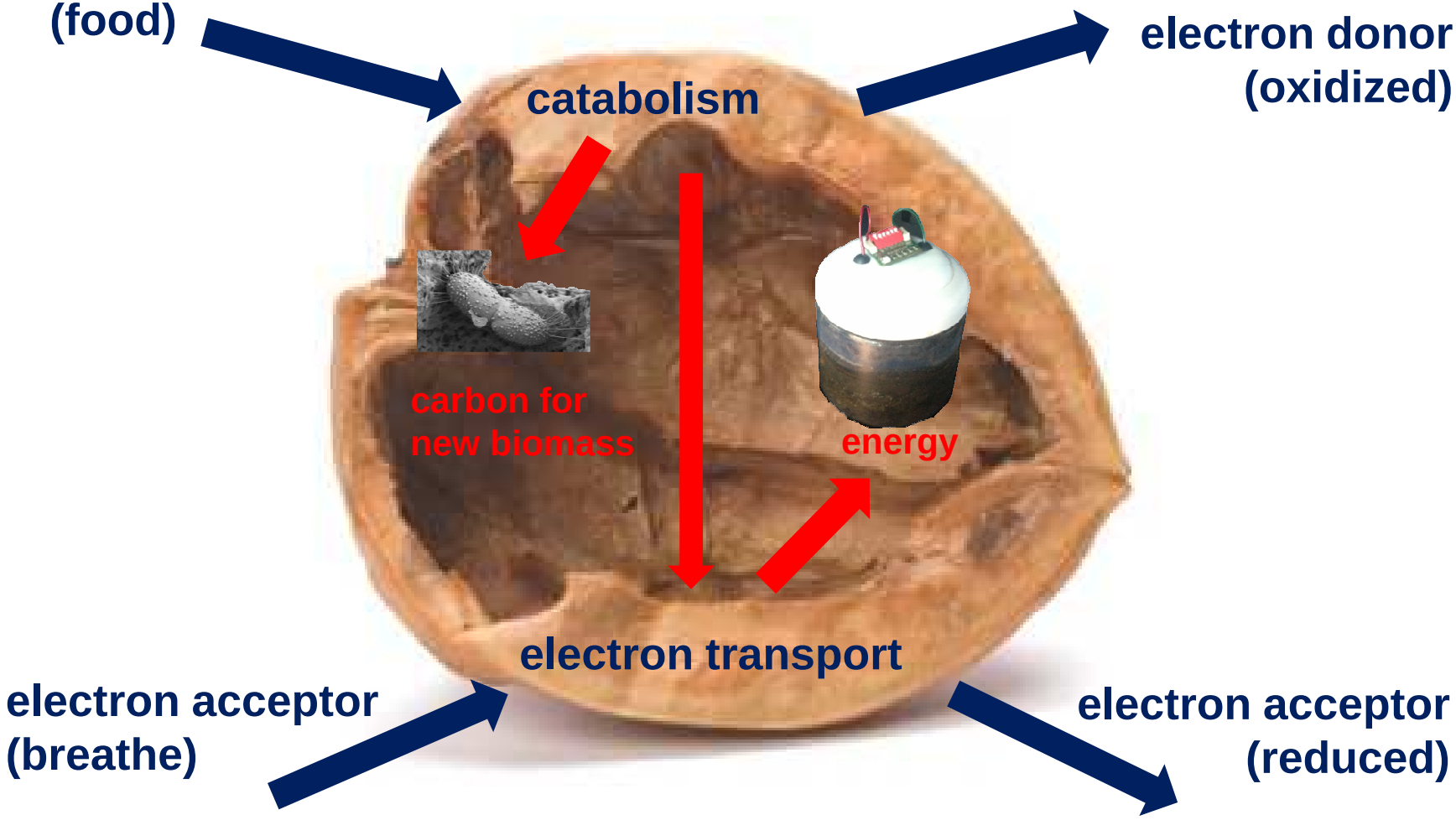
why all the eating and breathing

energy for cellular functions

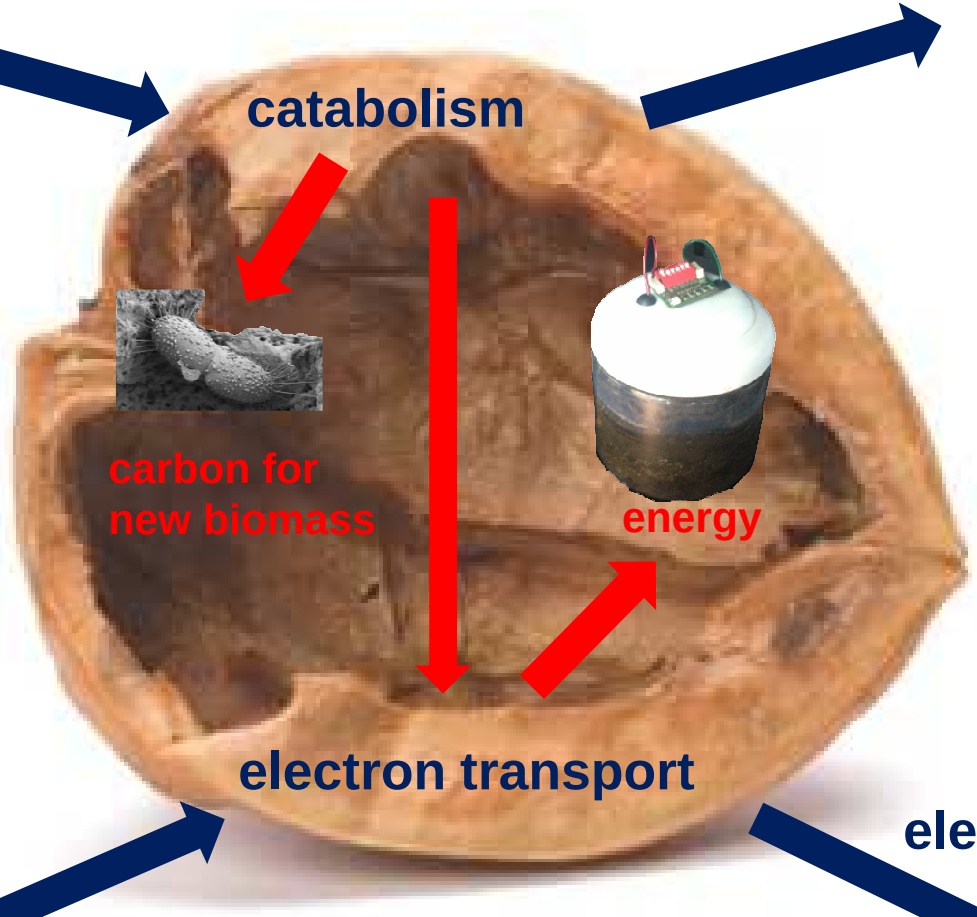
carbon for reproduction



SIP in a nut shell



SIP in a nut shell



electron donor
(oxidized)

electron acceptor
(reduced)

SIP in a nut shell

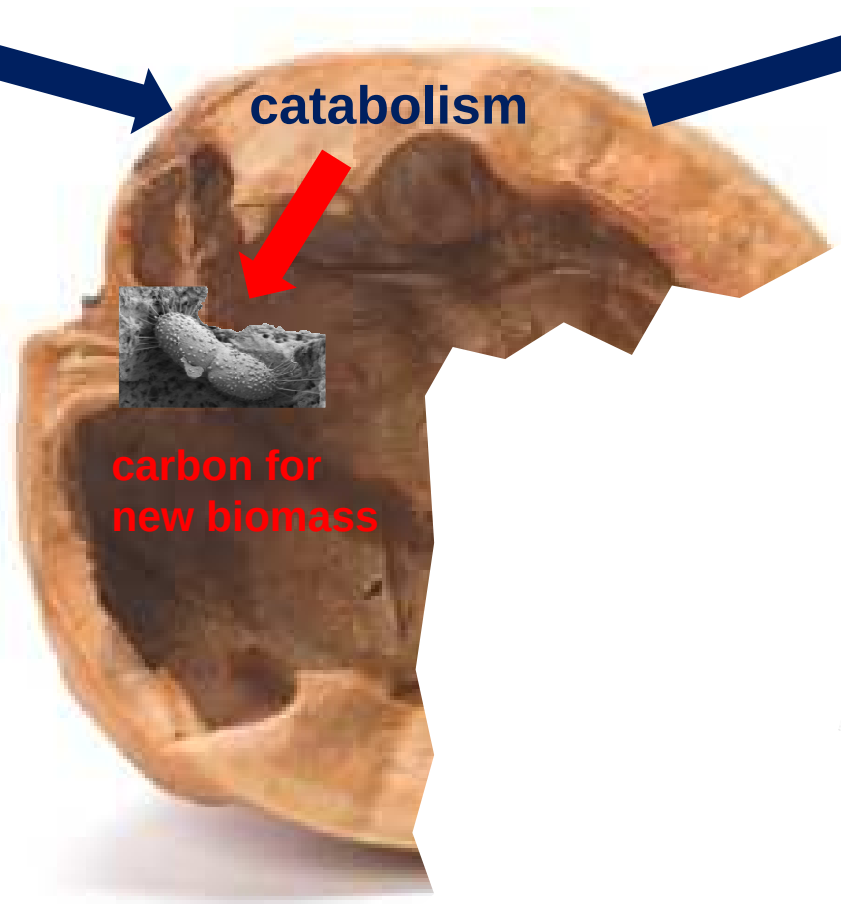
catabolism

carbon for new biomass

electron donor (oxidized)

SIP takes advantage of the electron donor processes

SIP in a nut shell



electron donor
(oxidized)

SIP takes advantage of the electron donor processes

SIP in a nut shell

electron donor (oxidized)

SIP takes advantage of the electron donor processes

SIP in a nut shell

catabolism

electron donor (oxidized)

SIP takes advantage of the electron donor processes

SIP in a nut shell

electron acceptor (reduced)

microbes use food to produce energy and make biomass

we can label the food and track its ultimate fate

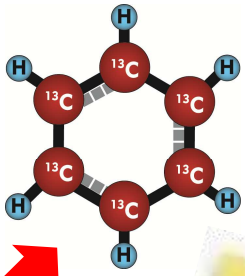

HELLO
my name is

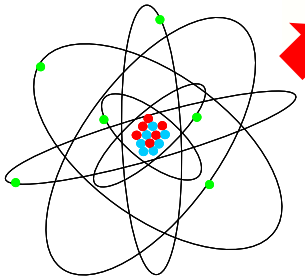
^{13}C Stable Isotope
Natural Abundance ~ 1%

HELLO

my name is

^{13}C enriched
(~ 10 – 100 %)
manufactured
contaminant



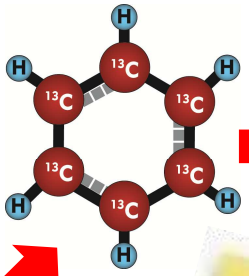


^{13}C Stable Isotope
Natural Abundance ~ 1%

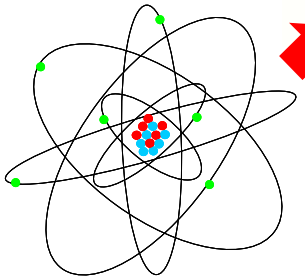
HELLO

my name is

^{13}C enriched
(~ 10 – 100 %)
manufactured
contaminant



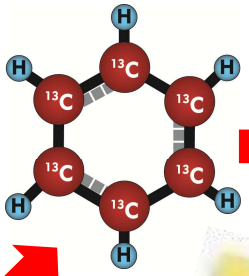
^{13}C enriched contaminant
loaded on a bio-trap
in situ microcosm

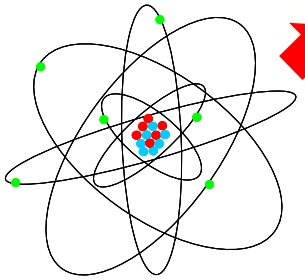


^{13}C Stable Isotope
Natural Abundance ~ 1%

HELLO
my name is

^{13}C enriched
(~ 10 – 100 %)
manufactured
contaminant





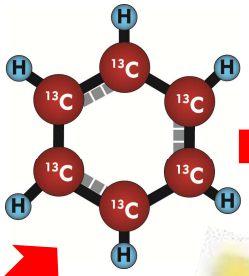
^{13}C Stable Isotope
Natural Abundance ~ 1%

^{13}C enriched contaminant
loaded on a bio-trap
in situ microcosm

incubate in site
well (30 to 90 days)

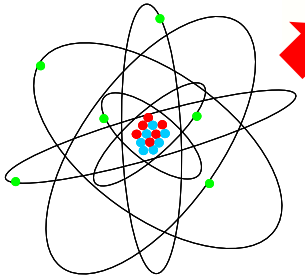
HELLO
my name is

^{13}C enriched (~ 10 – 100 %) manufactured contaminant



^{13}C enriched contaminant loaded on a bio-trap *in situ* microcosm

incubate in site well (30 to 90 days)



^{13}C Stable Isotope Natural Abundance ~ 1%

CSIA used to track ^{13}C enrichment in biomass and dissolved inorganic carbon

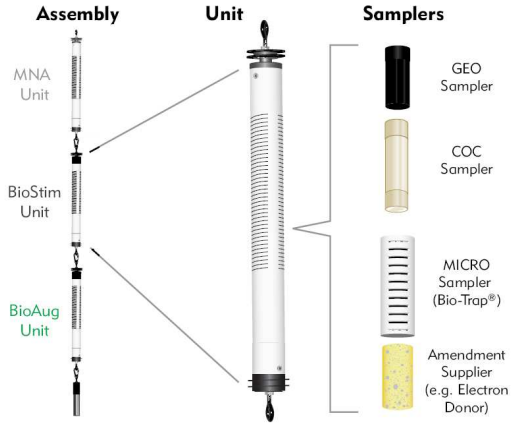
bio-trap samplers

bio-trap samplers

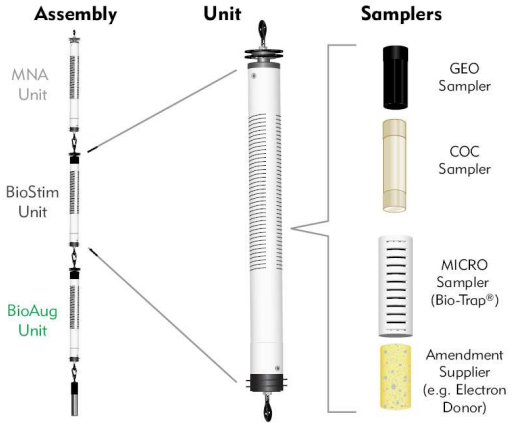
sterile growth medium for microbes

bio-trap samplers

bio-trap samplers



bio-trap samplers

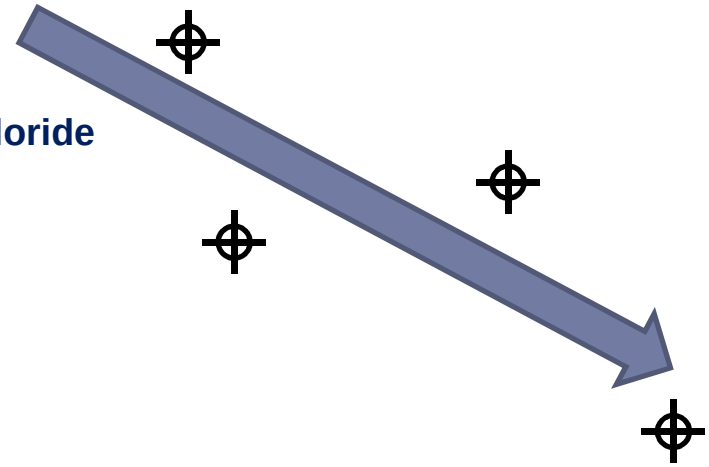


Pennsylvania case study

tear gas release site (CN)

chemical composition in groundwater varied with down gradient distance from source

source area



high concentrations

- chloroacetophenone
- chloropicrin
- benzene
- chloroform
  - methylene chloride

low concentrations

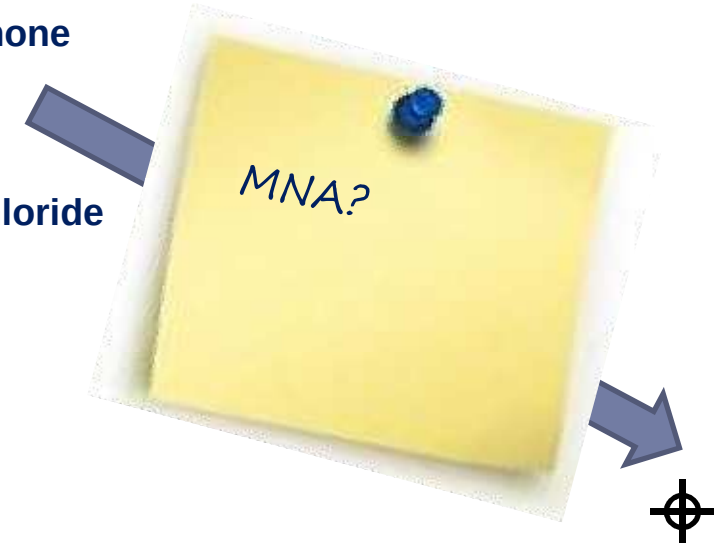
- benzene
- chloroform
  - methylene chloride
  - chloromethane

chemical composition in groundwater varied with down gradient distance from source

source area

high concentrations

- chloroacetophenone
- chloropicrin
- benzene
- chloroform
  - methylene chloride

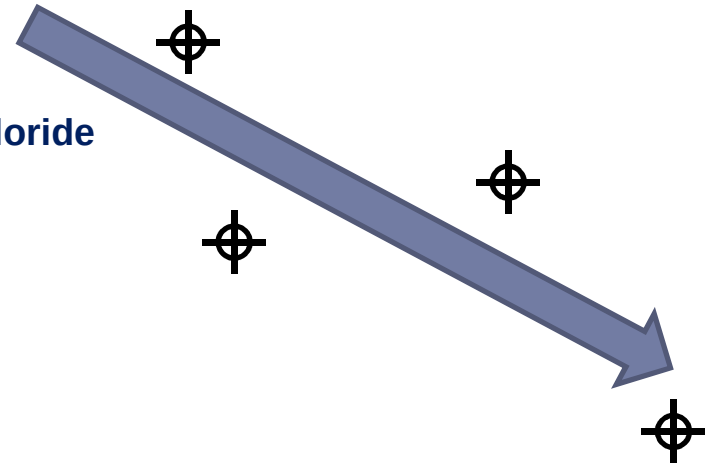


low concentrations

- benzene
- chloroform
  - methylene chloride
  - chloromethane

chemical composition in groundwater varied with down gradient distance from source

source area



high concentrations

- chloroacetophenone
- chloropicrin
- benzene
- chloroform
  - methylene chloride

low concentrations

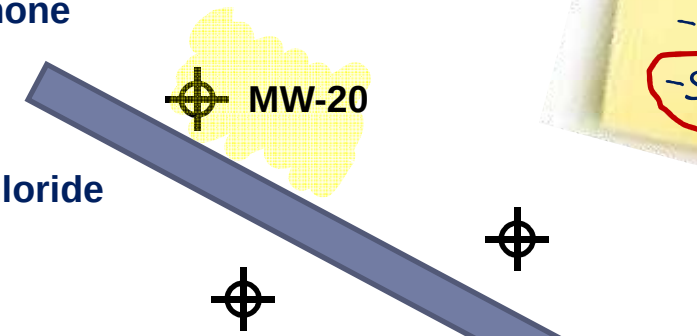
- benzene
- chloroform
  - methylene chloride
  - chloromethane

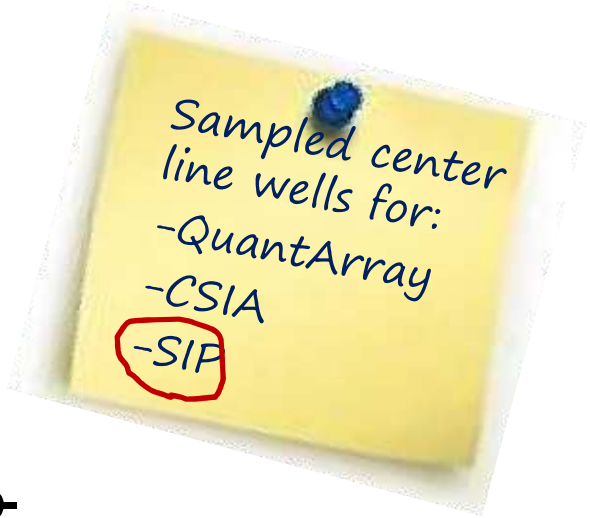
chemical composition in groundwater varied with down gradient distance from source

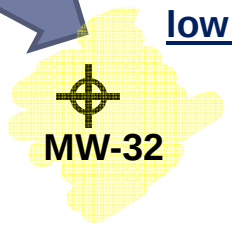
source area

<u>high concentrations</u>

- chloroacetophenone
- chloropicrin
- benzene
- chloroform
- methylene chloride



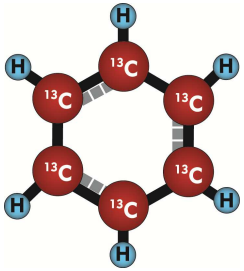




<u>low concentrations</u>

- benzene
- chloroform
- methylene chloride
- chloromethane

benzene natural attenuation



MW-32

135 ± 14 µg/bead

^{13}C Benzene

MW-20

135 ± 14 µg/bead

^{13}C Benzene

44 day incubation in site well

microbialinsights

WSP

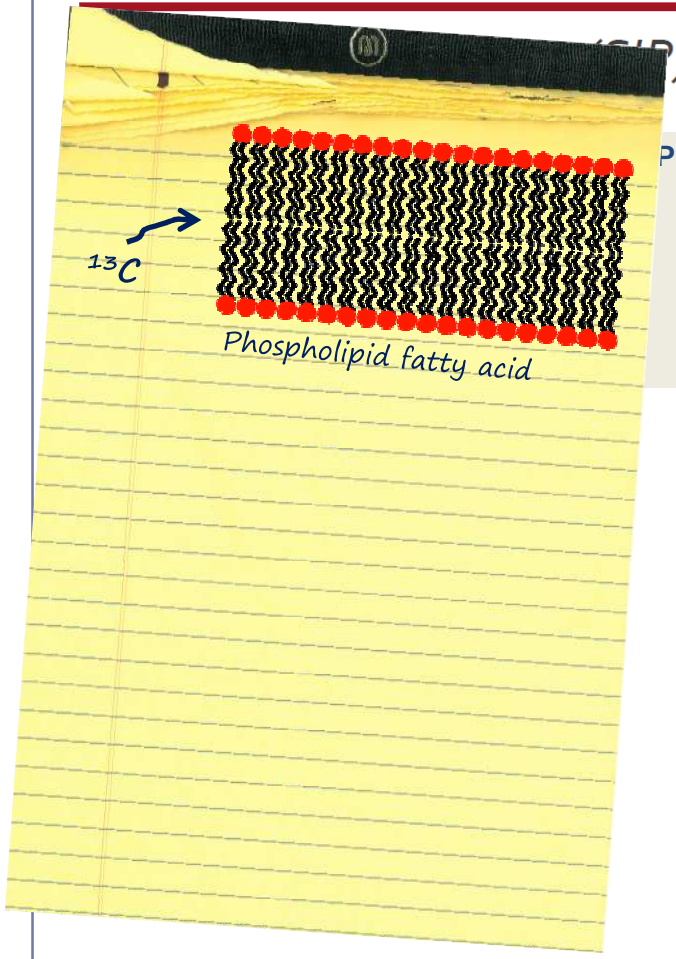
SITE LOGIC Report

Stable Isotope Probing (SIP) Study

	MW-20	MW-32
PLFA		
total biomass:	2.29E+04 cells/bead	2.43E+06 cells/bead
^{13}C enriched biomass:	1.73E+01 cells/bead	2.68E+04 cells/bead
average PLFA del ^{13}C:	4 ‰	138 ‰
maximum PLFA del ^{13}C:	4 ‰	675 ‰

SITE LOGIC Report

) Study

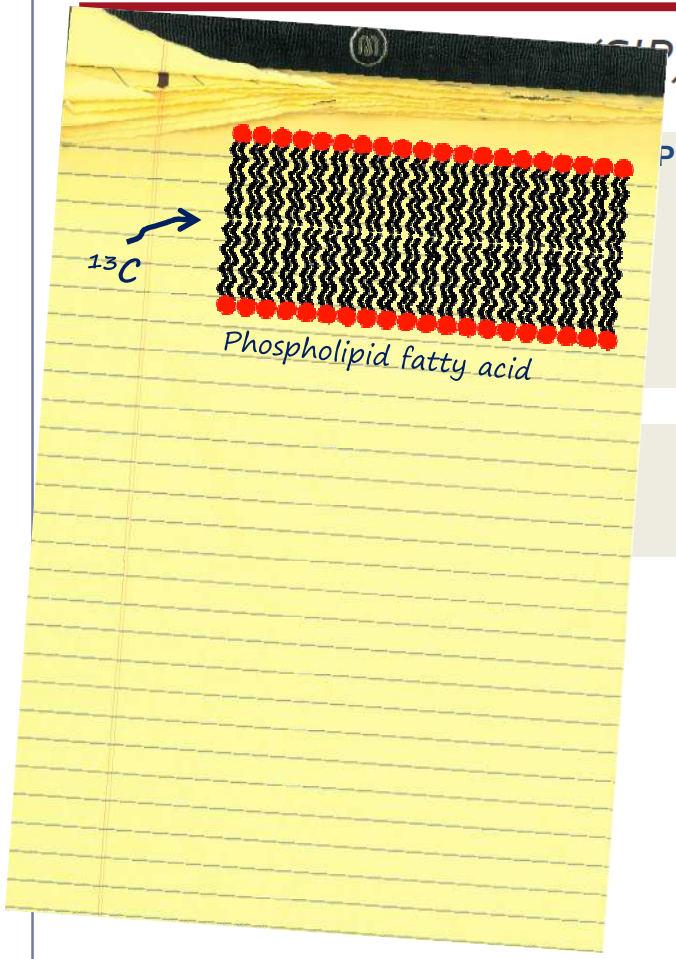


PLFA

	MW-20	MW-32
total biomass:	2.29E+04 cells/bead	2.43E+06 cells/bead
^{13}C enriched biomass:	1.73E+01 cells/bead	2.68E+04 cells/bead
average PLFA del ^{13}C:	4 ‰	138 ‰
maximum PLFA del ^{13}C:	4 ‰	675 ‰

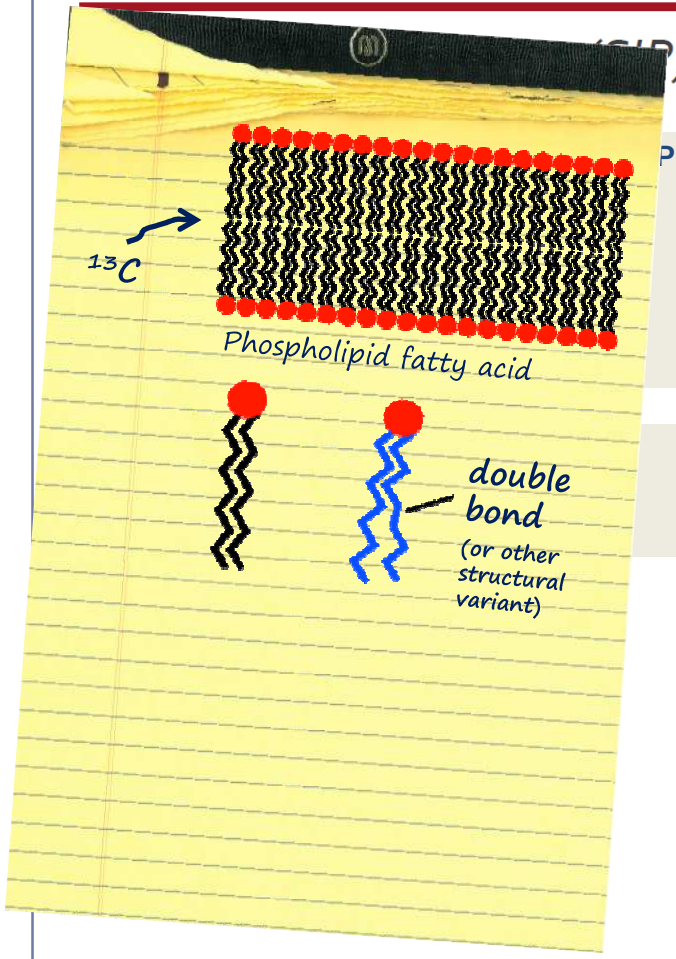
SITE LOGIC Report

) Study



	MW-20	MW-32
PLFA		
total biomass:	2.29E+04 cells/bead	2.43E+06 cells/bead
^{13}C enriched biomass:	1.73E+01 cells/bead	2.68E+04 cells/bead
average PLFA del ^{13}C:	4 ‰	138 ‰
maximum PLFA del ^{13}C:	4 ‰	675 ‰
DIC		
DIC del ^{13}C:	-20 ‰	10,921 ‰
percent ^{13}C DIC:	1.08 %	11.76 %

SITE LOGIC Report

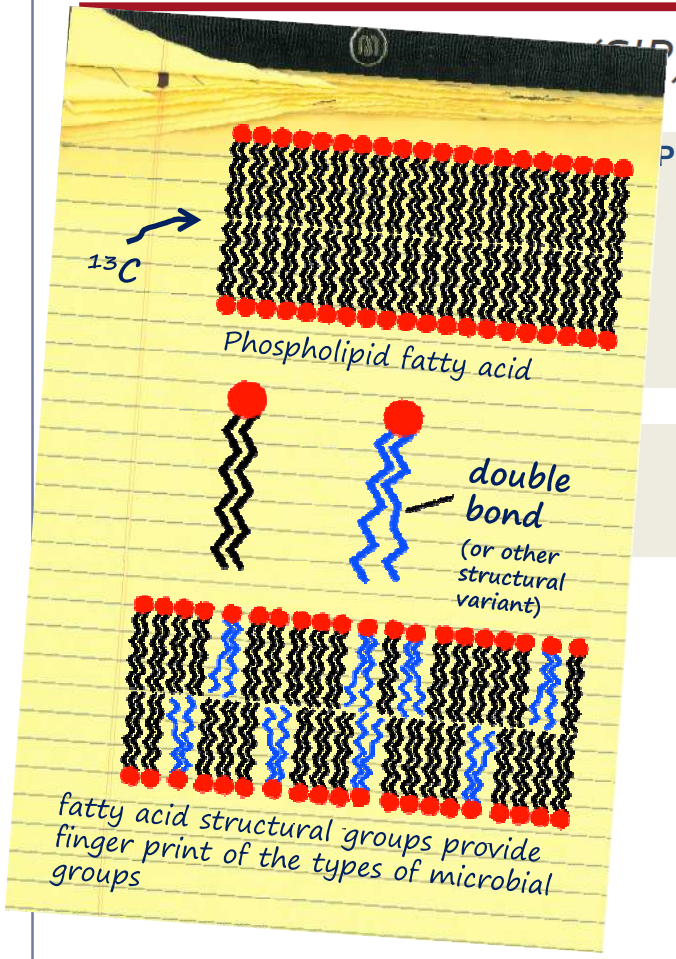


) Study

	MW-20	MW-32
PLFA		
total biomass:	2.29E+04 cells/bead	2.43E+06 cells/bead
^{13}C enriched biomass:	1.73E+01 cells/bead	2.68E+04 cells/bead
average PLFA del ^{13}C:	4 ‰	138 ‰
maximum PLFA del ^{13}C:	4 ‰	675 ‰
DIC		
DIC del ^{13}C:	-20 ‰	10,921 ‰
percent ^{13}C DIC:	1.08 %	11.76 %

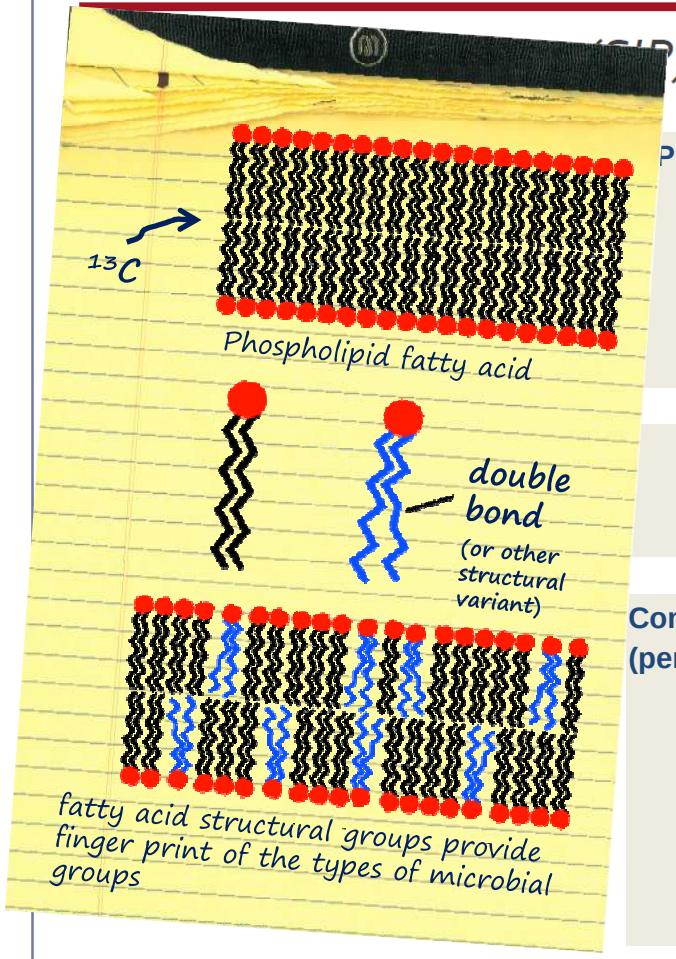
SITE LOGIC Report

) Study



	MW-20	MW-32
PLFA		
total biomass:	2.29E+04 cells/bead	2.43E+06 cells/bead
^{13}C enriched biomass:	1.73E+01 cells/bead	2.68E+04 cells/bead
average PLFA del ^{13}C:	4 ‰	138 ‰
maximum PLFA del ^{13}C:	4 ‰	675 ‰
DIC		
DIC del ^{13}C:	-20 ‰	10,921 ‰
percent ^{13}C DIC:	1.08 %	11.76 %

SITE LOGIC Report



) Study

	MW-20	MW-32
PLFA		
total biomass:	2.29E+04 cells/bead	2.43E+06 cells/bead
^{13}C enriched biomass:	1.73E+01 cells/bead	2.68E+04 cells/bead
average PLFA del ^{13}C:	4 ‰	138 ‰
maximum PLFA del ^{13}C:	4 ‰	675 ‰
DIC		
DIC del ^{13}C:	-20 ‰	10,921 ‰
percent ^{13}C DIC:	1.08 %	11.76 %
Community Structure (percent total PLFA)		
firmicutes (TerBrSats):	0.0	6.2
proteobacteria (Monos):	22.9	59.2
anaerobic metal reducers (brMonos):	0.0	1.1
actinomycetes (MidBrSat):	0.0	0.8
general (Nsat):	77.1	30.3
eukaryotes (Polynoics):	0.0	2.5

microbialinsights

SITE LOGIC Report

Stable Isotope Probing (SIP) Study

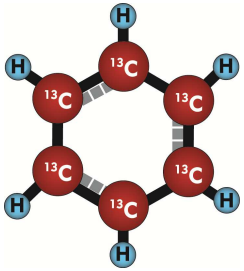
definitively identified biodegradation as mechanism of benzene attenuation at the site (downgradient area)

WSP

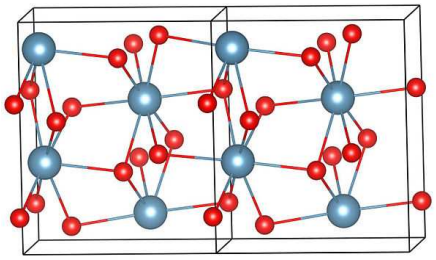
Michigan case study

gasoline release site

gasoline natural attenuation (benzene indicator)



MW-1
185 ± 12 µg/bead
^{13}C Benzene



Calcium Peroxide

MW-2
185 ± 12 µg/bead
^{13}C Benzene

82 day incubation
in site well

microbialinsights

WSP

SITE LOGIC Report

Stable Isotope Probing (SIP) Study

		MW-1	MW-2
PLFA	total biomass:	9.91E+05 cells/bead	3.83E+06 cells/bead
	^{13}C enriched biomass:	4.57E+04 cells/bead	2.37E+05 cells/bead
	average PLFA del ^{13}C:	864 ‰	4202 ‰
	maximum PLFA del ^{13}C:	4469 ‰	6971 ‰
DIC	DIC del ^{13}C:	2227 ‰	3,235 ‰
	percent ^{13}C DIC:	3.44 %	4.47 %

SITE LOGIC Report

Stable Isotope Probing (SIP) Study

		MW-1	MW-2
PLFA	total biomass:	9.91E+05 cells/bead	3.83E+06 cells/bead
	^{13}C enriched biomass:	4.57E+04 cells/bead	2.37E+05 cells/bead
	average PLFA del ^{13}C:	864 ‰	4202 ‰
	maximum PLFA del ^{13}C:	4469 ‰	6971 ‰
DIC	DIC del ^{13}C:	2227 ‰	3,235 ‰
	percent ^{13}C DIC:	3.44 %	4.47 %
Community Structure (percent total PLFA)	firmicutes (TerBrSats):	4.0	5.5
	proteobacteria (Monos):	76.4	73
	anaerobic metal reducers (brMonos):	0.5	4.1
	actinomycetes (MidBrSat):	0.0	0.05
	general (Nsat):	16.4	15.3
	eukaryotes (Polynoics):	2.7	1.6

SITE … t

Stable Is… …udy

		MW-1	MW-2
PLFA	total biomass:	9.91E+05 cells/bead	3.83E+06 cells/bead
	^{13}C enriched biomass:	4.57E+04 cells/bead	2.37E+05 cells/bead
	average PLFA del ^{13}C:	864 ‰	4202 ‰
	maximum PLFA del ^{13}C:	4469 ‰	6971 ‰
DIC	DIC del ^{13}C:	2227 ‰	3,235 ‰
	percent ^{13}C DIC:	3.44 %	4.47 %
Community Structure (percent total PLFA)	firmicutes (TerBrSats):	4.0	5.5
	proteobacteria (Monos):	76.4	73
	anaerobic metal reducers (brMonos):	0.5	4.1
	actinomycetes (MidBrSat):	0.0	0.05
	general (Nsat):	16.4	15.3
	eukaryotes (Polynoics):	2.7	1.6

microbialinsic

SITE … t

Stable Is… …udy

		MW-1	MW-2
PLFA	total biomass:	9.91E+05 cells/bead	3.83E+06 cells/bead
	^{13}C enriched biomass:	4.57E+04 cells/bead	2.37E+05 cells/bead
	average PLFA del ^{13}C:	864 ‰	4202 ‰
	maximum PLFA del ^{13}C:	4469 ‰	6971 ‰
DIC	DIC del ^{13}C:	2227 ‰	3,235 ‰
	percent ^{13}C DIC:	3.44 %	4.47 %
Community Structure (percent total PLFA)	firmicutes (TerBrSats):	4.0	5.5
	proteobacteria (Monos):	76.4	73
	anaerobic metal reducers (brMonos):	0.5	4.1
	actinomycetes (MidBrSat):	0.0	0.05
	general (Nsat):	16.4	15.3
	eukaryotes (Polynoics):	2.7	1.6
Contaminant Loss	^{13}C Benzene pre-deployment:	185±12 µg/bd	185±12 µg/bd
	^{13}C Benzene post-deployment:	130±11 µg/bd	144±11 µg/bd
	first order rate:	0.0043 day^{-1}	0.0031 day^{-1}

WSP

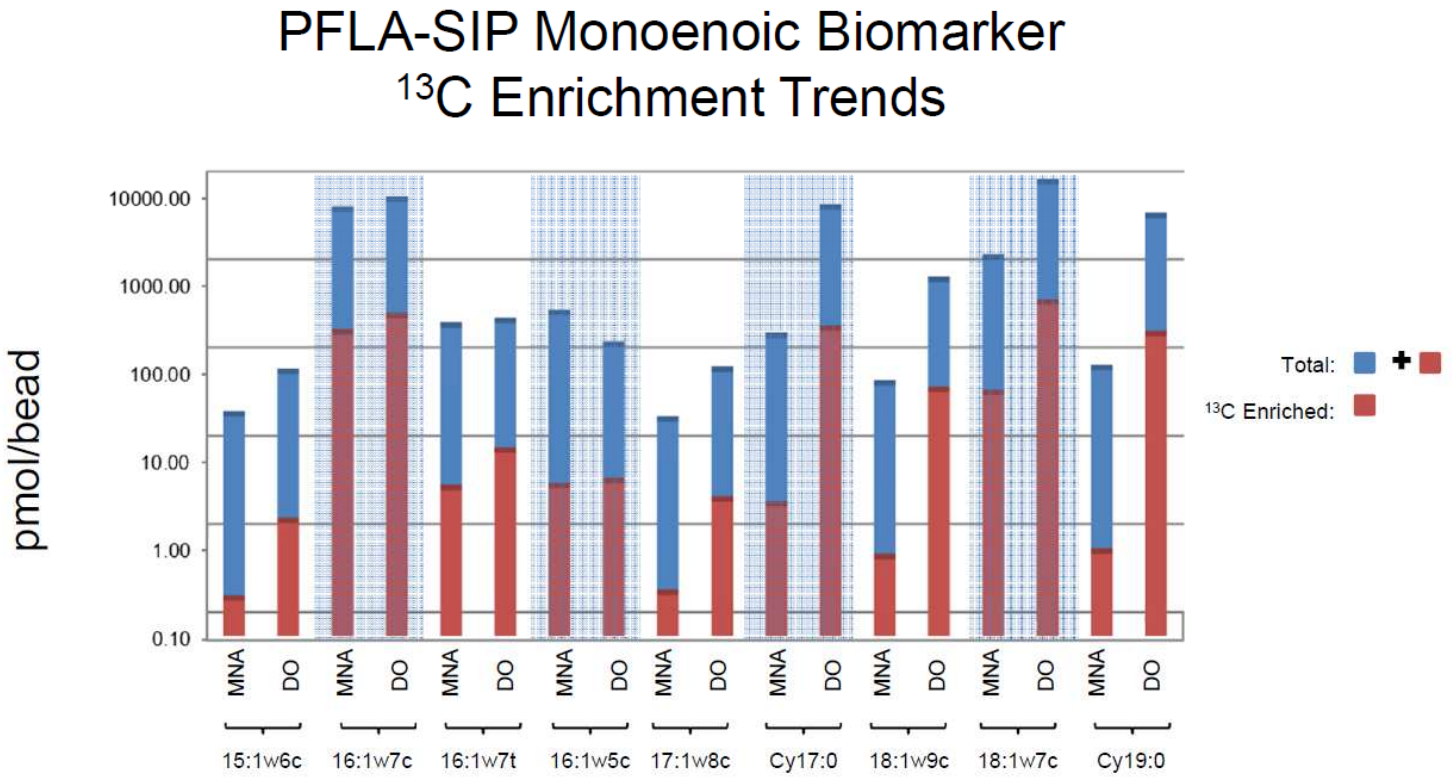
SITE … t

Stable Is… …udy

		MW-1	MW-2
PLFA	total biomass:	9.91E+05 cells/bead	3.83E+06 cells/bead
	^{13}C enriched biomass:	4.57E+04 cells/bead	2.37E+05 cells/bead
	average PLFA del ^{13}C:	864 ‰	4202 ‰
	maximum PLFA del ^{13}C:	4469 ‰	6971 ‰
DIC	DIC del ^{13}C:	2227 ‰	3,235 ‰
	percent ^{13}C DIC:	3.44 %	4.47 %
Community Structure (percent total PLFA)	firmicutes (TerBrSats):	4.0	5.5
	proteobacteria (Monos):	76.4	73
	anaerobic metal reducers (brMonos):	0.5	4.1
	actinomycetes (MidBrSat):	0.0	0.05
	general (Nsat):	16.4	15.3
	eukaryotes (Polynoics):	2.7	1.6
Contaminant Loss	^{13}C Benzene pre-deployment:	185±12 µg/bd	185±12 µg/bd
	^{13}C Benzene post-deployment:	130±11 µg/bd	144±11 µg/bd
	first order rate:	0.0043 day^{-1}	0.0031 day^{-1}

SITE LOGIC Report

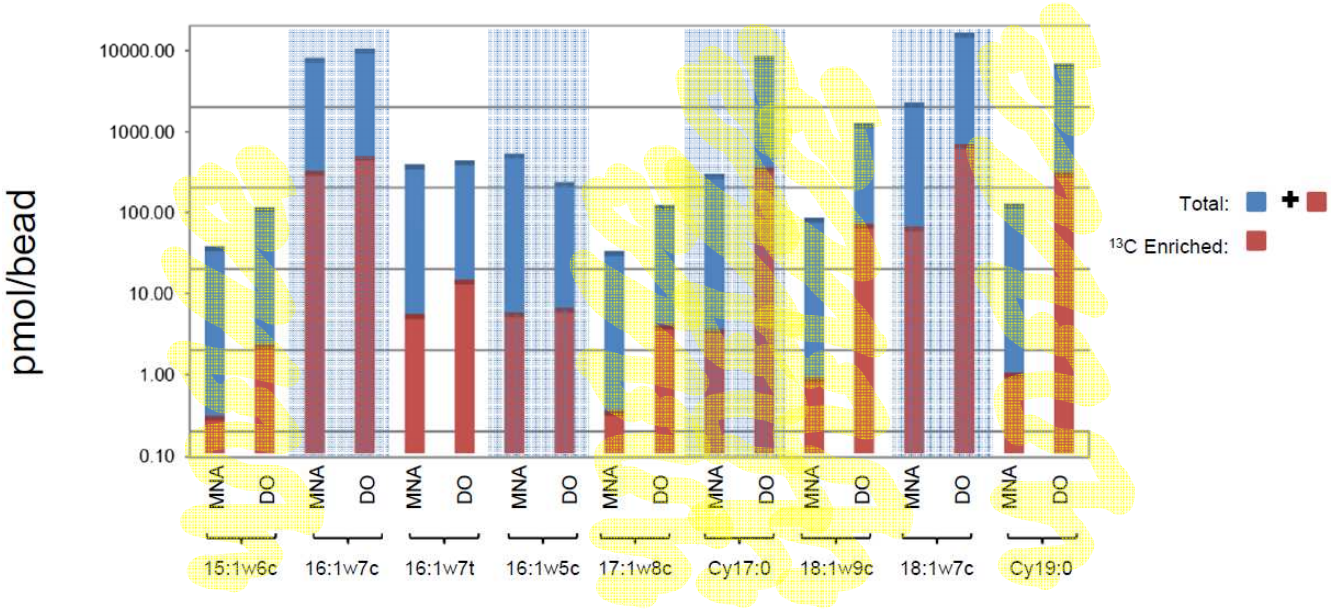
Stable Isotope Probing (SIP) Study



SITE LOGIC Report

Stable Isotope Probing (SIP) Study

PFLA-SIP Monoenoic Biomarker
^{13}C Enrichment Trends



PFLA Monoenoic Biomarkers

SITE LOGIC Report

Stable Isotope Probing (SIP) Study

quantified expected full-scale performance of two remedial options

Michigan case study

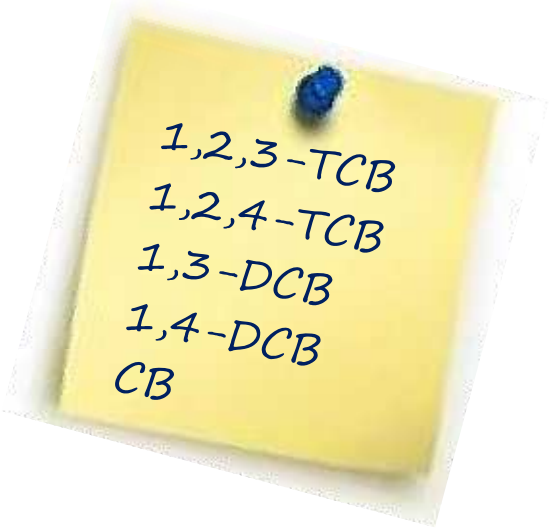
Michigan case study

excavated PCB-affected soil

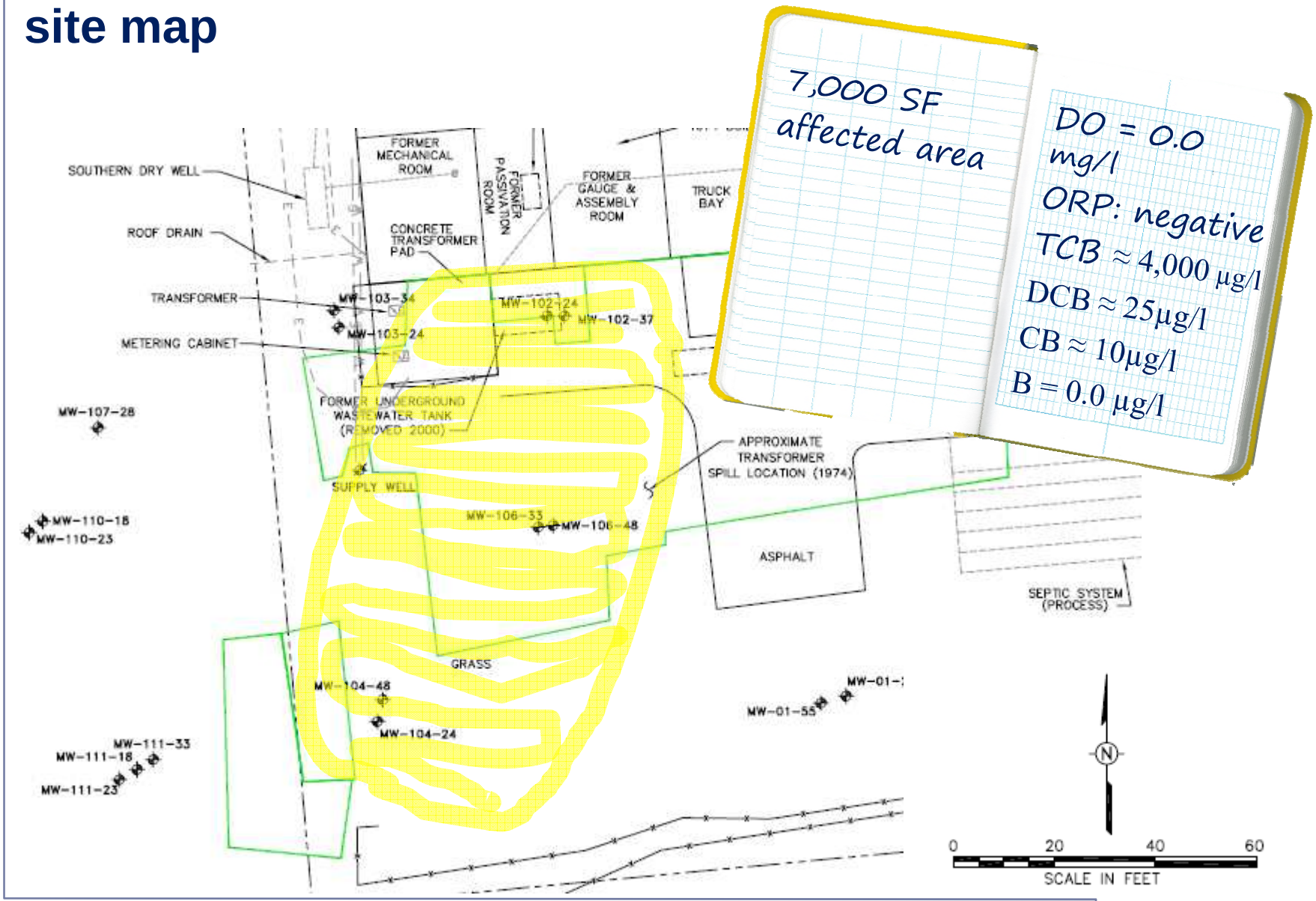
Excavation Stats

total: 2,180 tons
TSCA: 1,678 tons
max depth: 19 ft

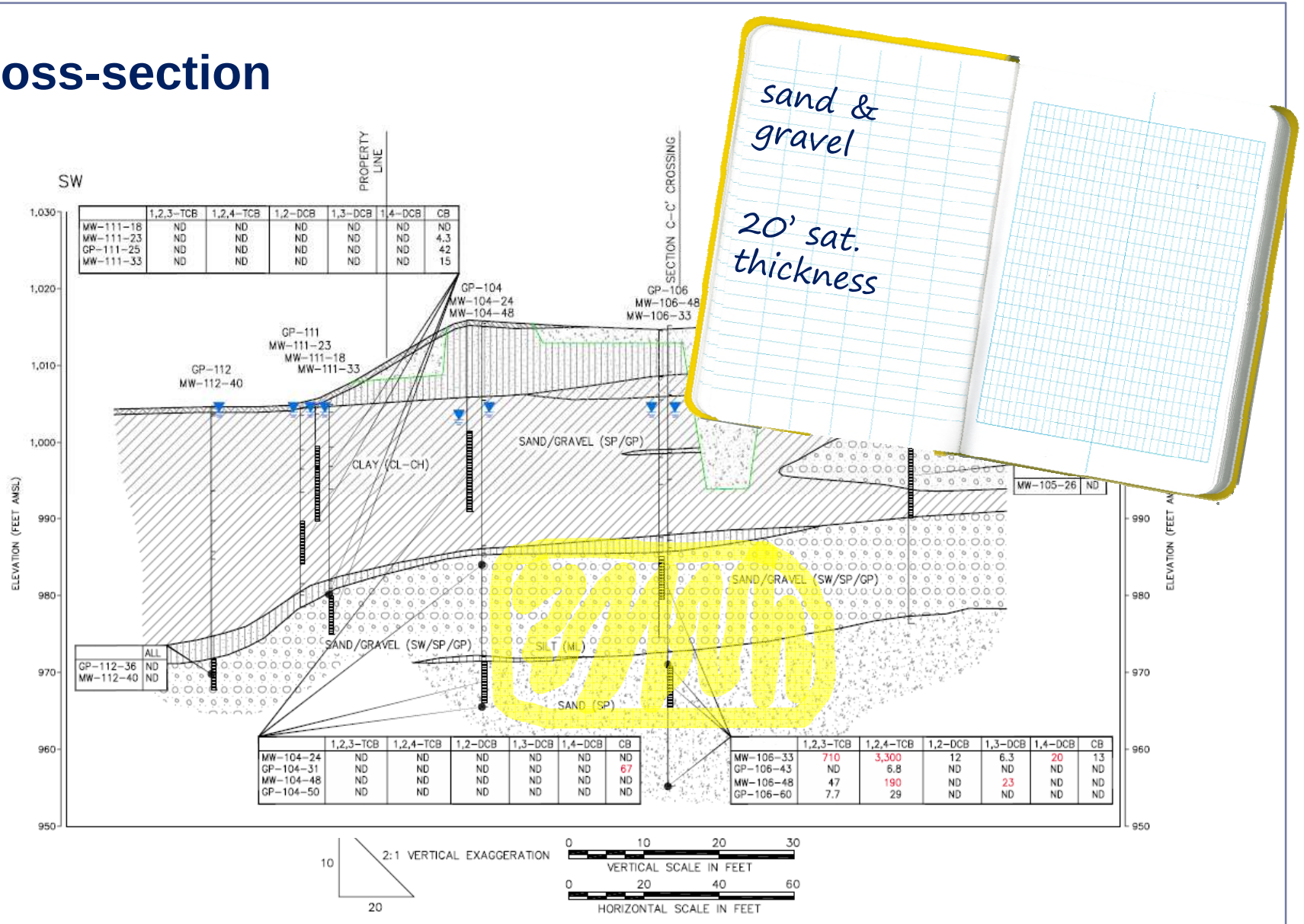
left with mixed chlorobenzenes in groundwater



site map


7,000 SF affected area
DO = 0.0 mg/l
ORP: negative
TCB ≈ 4,000 µg/l
DCB ≈ 25µg/l
CB ≈ 10µg/l
B = 0.0 µg/l
FORMER MECHANICAL ROOM
FORMER PASSIVATION ROOM
FORMER GAUGE & ASSEMBLY ROOM
TRUCK BAY
SOUTHERN DRY WELL
ROOF DRAIN
CONCRETE TRANSFORMER PAD
TRANSFORMER
METERING CABINET
MW-103-34
MW-103-24
MW-102-24
MW-102-37
MW-107-28
FORMER UNDERGROUND WASTEWATER TANK (REMOVED 2000)
SUPPLY WELL
APPROXIMATE TRANSFORMER SPILL LOCATION (1974)
MW-110-18
MW-110-23
MW-106-33
MW-106-48
ASPHALT
SEPTIC SYSTEM (PROCESS)
GRASS
MW-104-48
MW-104-24
MW-01-55
MW-111-33
MW-111-18
MW-111-23
N
0
20
40
60
SCALE IN FEET

cross-section



	1,2,3–TCB	1,2,4–TCB	1,2–DCB	1,3–DCB	1,4–DCB	CB
MW–111–18	ND	ND	ND	ND	ND	ND
MW–111–23	ND	ND	ND	ND	ND	4.3
GP–111–25	ND	ND	ND	ND	ND	42
MW–111–33	ND	ND	ND	ND	ND	15

	ALL
GP–112–36	ND
MW–112–40	ND

	1,2,3–TCB	1,2,4–TCB	1,2–DCB	1,3–DCB	1,4–DCB	CB
MW–104–24	ND	ND	ND	ND	ND	ND
GP–104–31	ND	ND	ND	ND	ND	67
MW–104–48	ND	ND	ND	ND	ND	ND
GP–104–50	ND	ND	ND	ND	ND	ND

	1,2,3–TCB	1,2,4–TCB	1,2–DCB	1,3–DCB	1,4–DCB	CB
MW–106–33	710	3,300	12	6.3	20	13
GP–106–43	ND	6.8	ND	ND	ND	ND
MW–106–48	47	190	ND	23	ND	ND
GP–106–60	7.7	29	ND	ND	ND	ND

	MW–105–26	ND

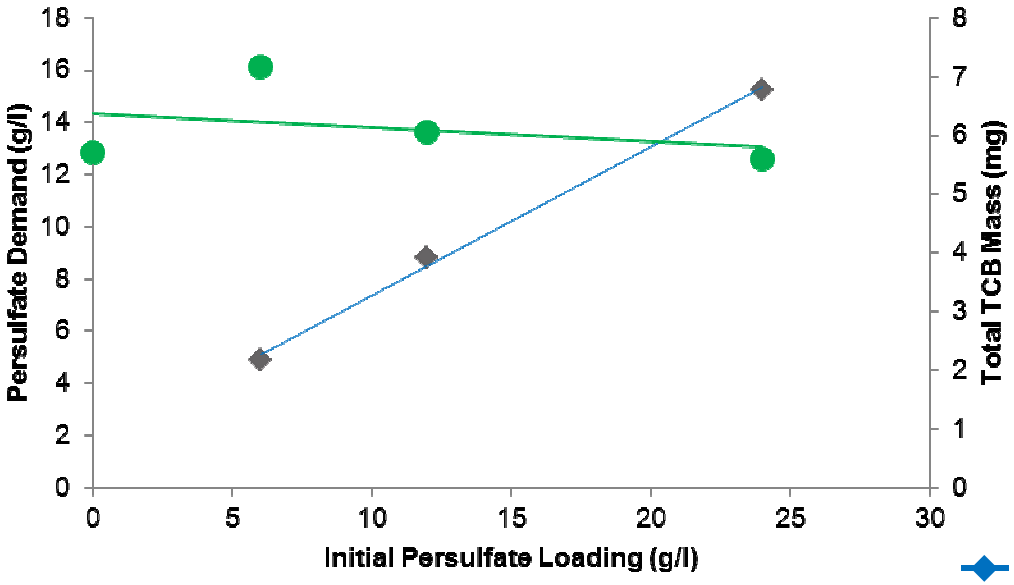
sand & gravel

20' sat. thickness

GW

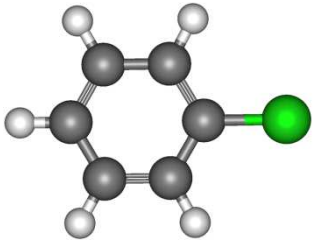
WSP

7-Day Activated Persulfate Study



- 7 day persulfate demand
- Total chlorobenzenes (soil and water)

chlorobenzene biodegradation



EHC-M
(FMC)

MW-1

185 ± 12 µg/bead
^{13}C Benzene

126 day incubation
in site well

microbialinsights

WSP

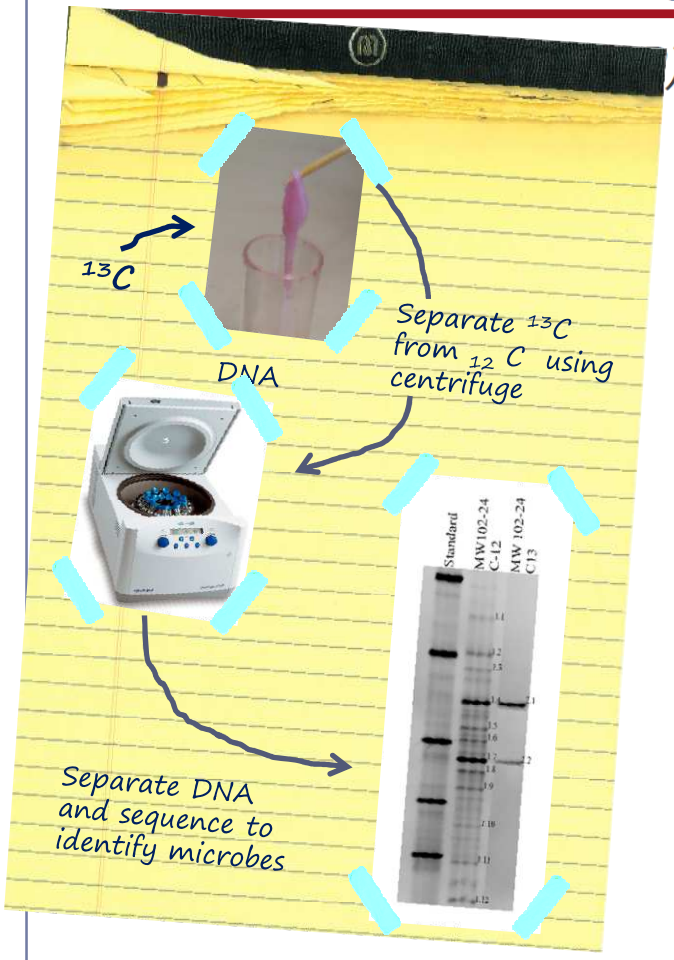
SITE LOGIC Report

Stable Isotope Probing (SIP) Study

MW-102

Contaminant Loss	^{13}C Benzene pre-deployment:	202±12 µg/bd
	^{13}C Benzene post-deployment:	156±11 µg/bd
	first order rate:	0.0021 day^{-1}
DIC	DIC del ^{13}C:	-35 ‰
	percent ^{13}C DIC:	1.06 %

SITE LOGIC Report



MW-102

Contaminant Loss	^{13}C Benzene pre-deployment:	202±12 µg/bd
	^{13}C Benzene post-deployment:	156±11 µg/bd
	first order rate:	0.0021 day^{-1}
DIC	DIC del ^{13}C:	-35 ‰
	percent ^{13}C DIC:	1.06 %

SITE LOGIC Report

Study

MW-102

Contaminant Loss	^{13}C Benzene pre-deployment:	202±12 µg/bd
	^{13}C Benzene post-deployment:	156±11 µg/bd
	first order rate:	0.0021 day^{-1}
DIC	DIC del ^{13}C:	-35 ‰
	percent ^{13}C DIC:	1.06 %

SITE LOGIC Report

Stable Isotope Probing (SIP) Study

identified unexpected pathway that can be enhanced

definitively demonstrate biodegradation

- evaluate inhibition
- quantifiably compare remedial options
- identify unexpected mechanisms

Case Studies on Practical Use of Stable Isotope Probing

Matt Burns
WSP USA Corp.
300 Trade Center, Suite 4690
Woburn, MA, 01801
781/933-7340
matt.burns@wspgroup.com

http://www.linkedin.com/in/mattburnswsp

Kerry Sublette
University of Tulsa
Keplinger Hall 321
918-631-3085
Kerry-sublette@utulsa.edu

http://ipec.utulsa.edu

providing management and consultancy services to the built and natural environment

15,000 people
300 offices
35 countries

WSP