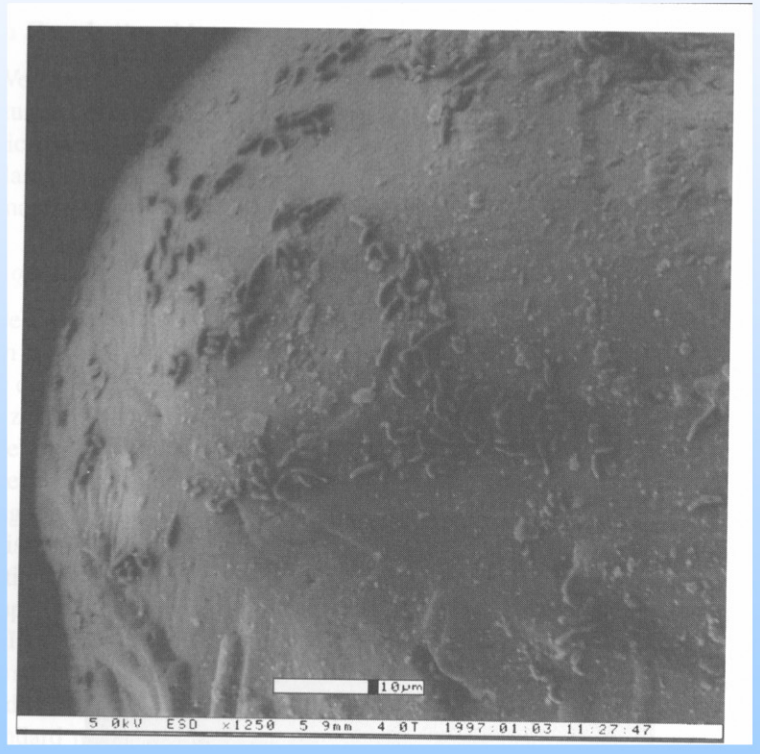
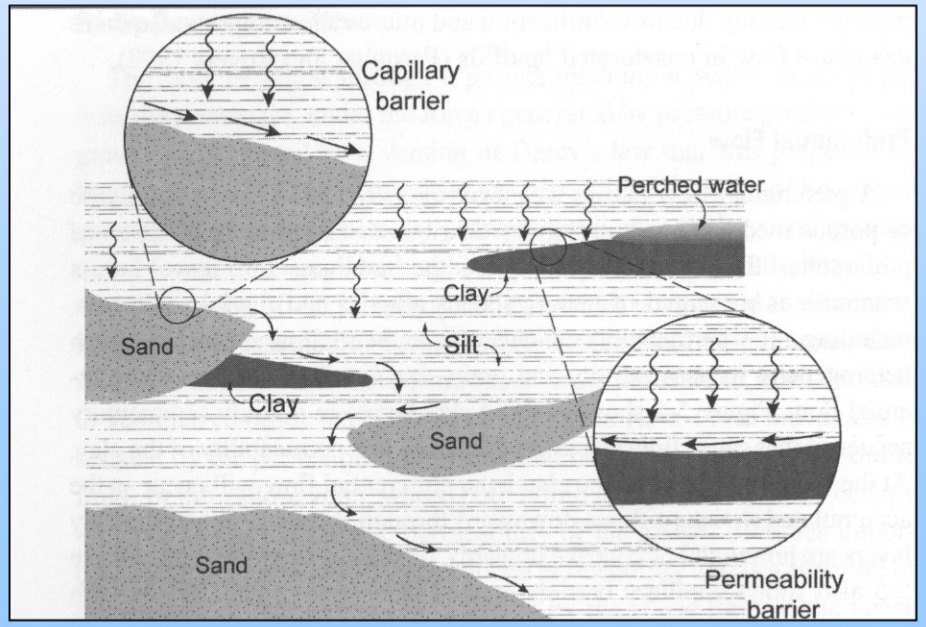
Microbial Communities in the Vadose Zone

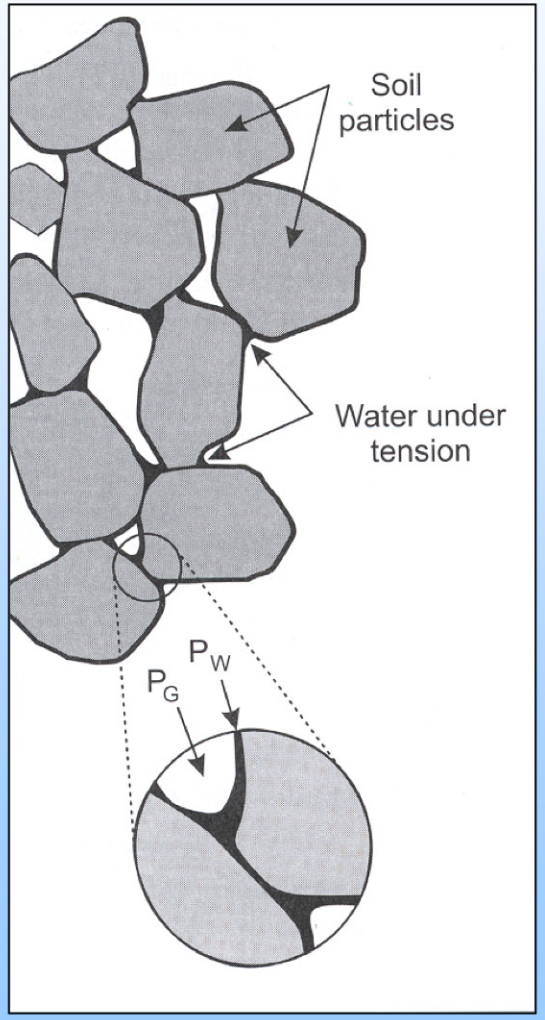


Fred Brockman
Pacific Northwest National Laboratory
April 18, 2005

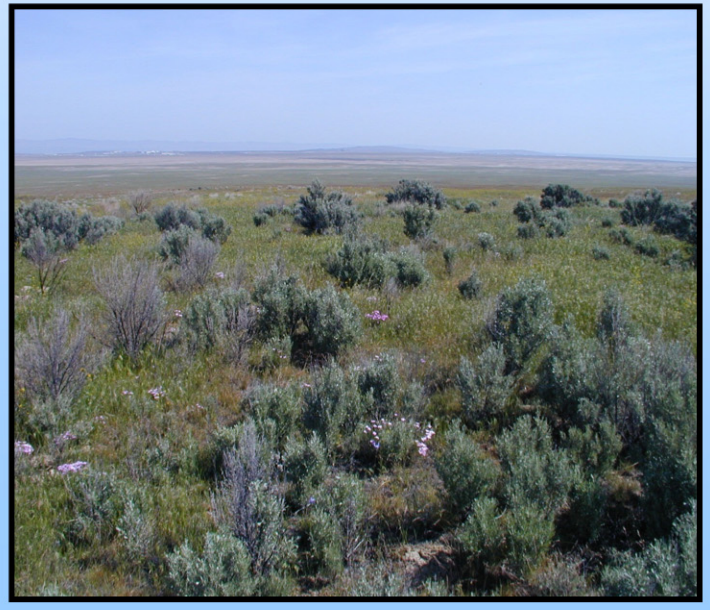
Unique Properties of Vadoze Zone

- Presence of gas phase
- Water is in thin films & wedges
- Variable water content
- Transient preferential flow
- Flow bypass

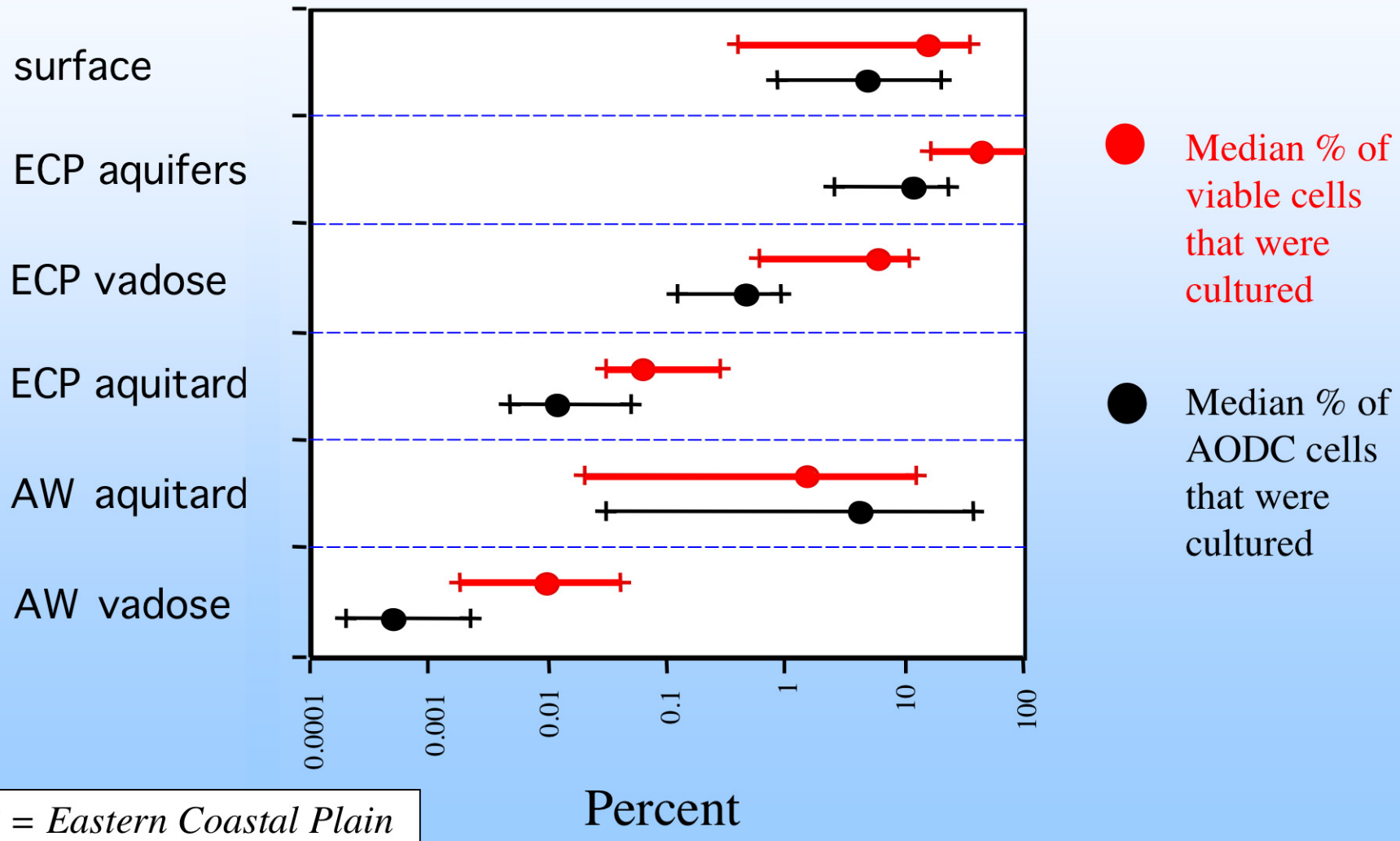




If you were a subsurface microbe, where would you want to live?



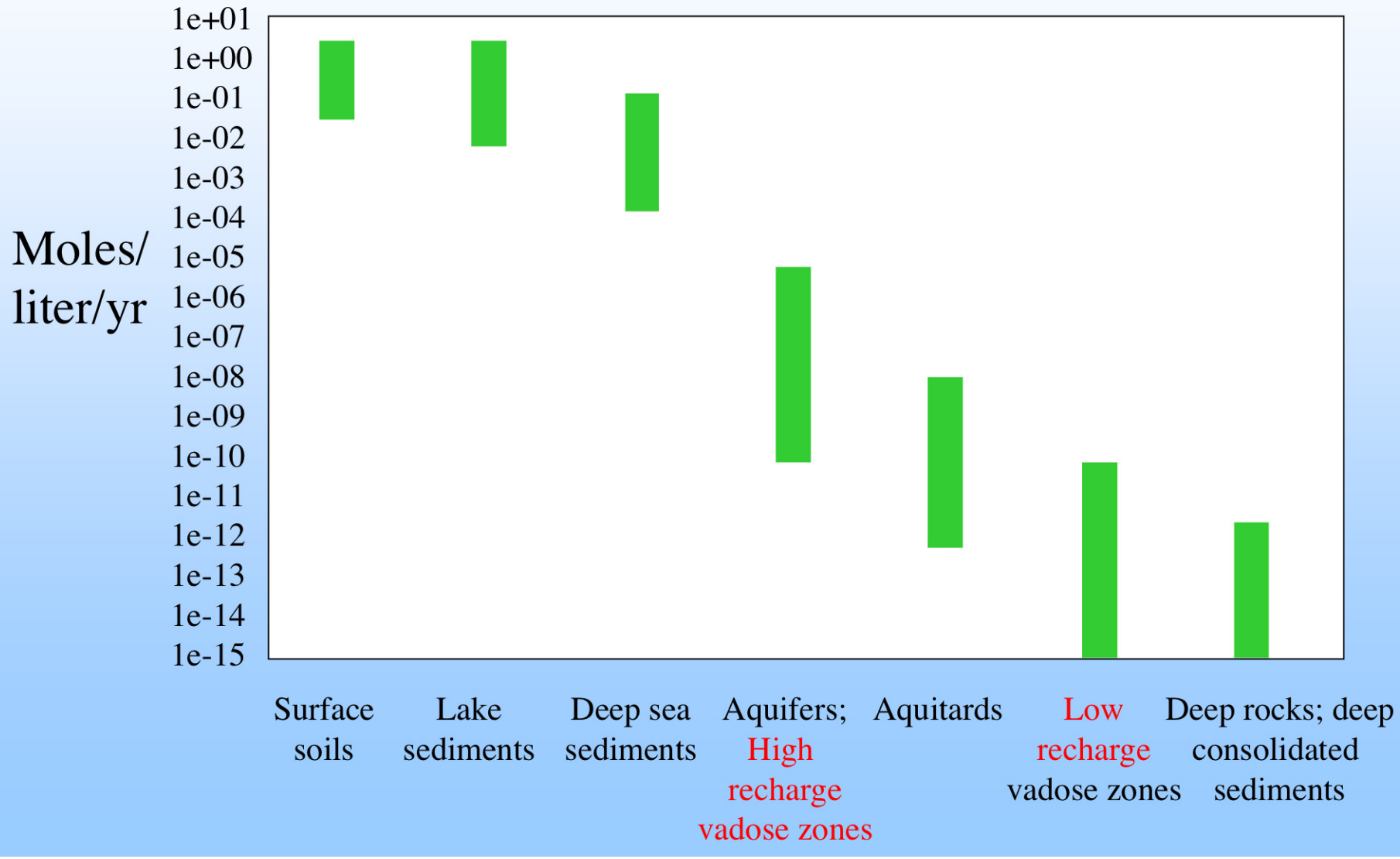
% Culturability in Different Settings



ECP = Eastern Coastal Plain
AW = Arid West

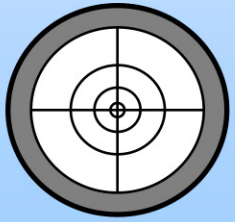
Relative Activity

In Situ CO_2 Production (after Kieft and Phelps, 1997)

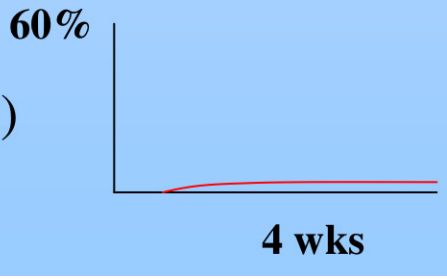


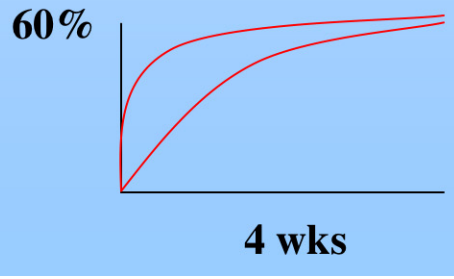
Hydrologic Controls (Climate) on Microbiological Properties in the Vadose Zone

	Low recharge	High recharge
Live cells/g	10^4-10^5	10^7
Cultured cells/g	$<10^1$-10^2	10^5-10^6
Spatial distribution	No activity in many 0.1 and 1 g samples; activity in some 10 g and most 100 g samples	All 0.1 g samples active

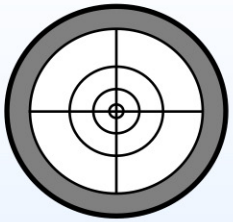


Activity (% mineralization)



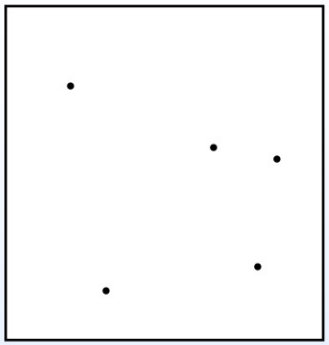


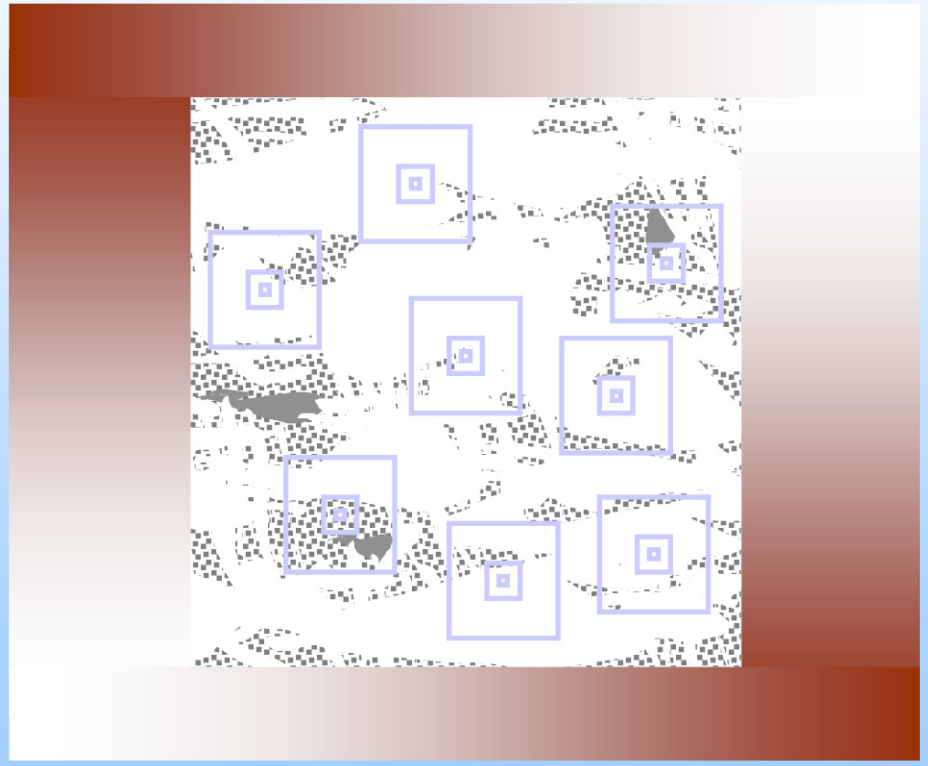
Spatial Heterogeneity



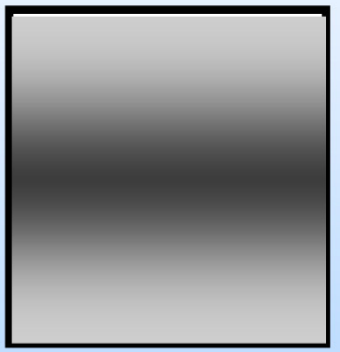
8 x 0.1 g
8 x 1 g
8 x 10 g
8 x 100 g

Widely dispersed microcolonies





Mixing zone-generated gradient



'Optimal', suboptimal, & excluding regions
- convective, diffusive, & bypass regions
- sediment chemistry

Heterogeneity exists at multiple scales
- *Important scale that is the one that dominates the behavior of the system*

What Do the Nondetects Mean?

Viable biomass at all sites = 10^3 - 10^4 cells/g (75 g)

^{14}C- mineralization

- **Detection requires ~ 10^5 cells/g**
- **Nondetects = inability to increase population 10- to 100-fold**

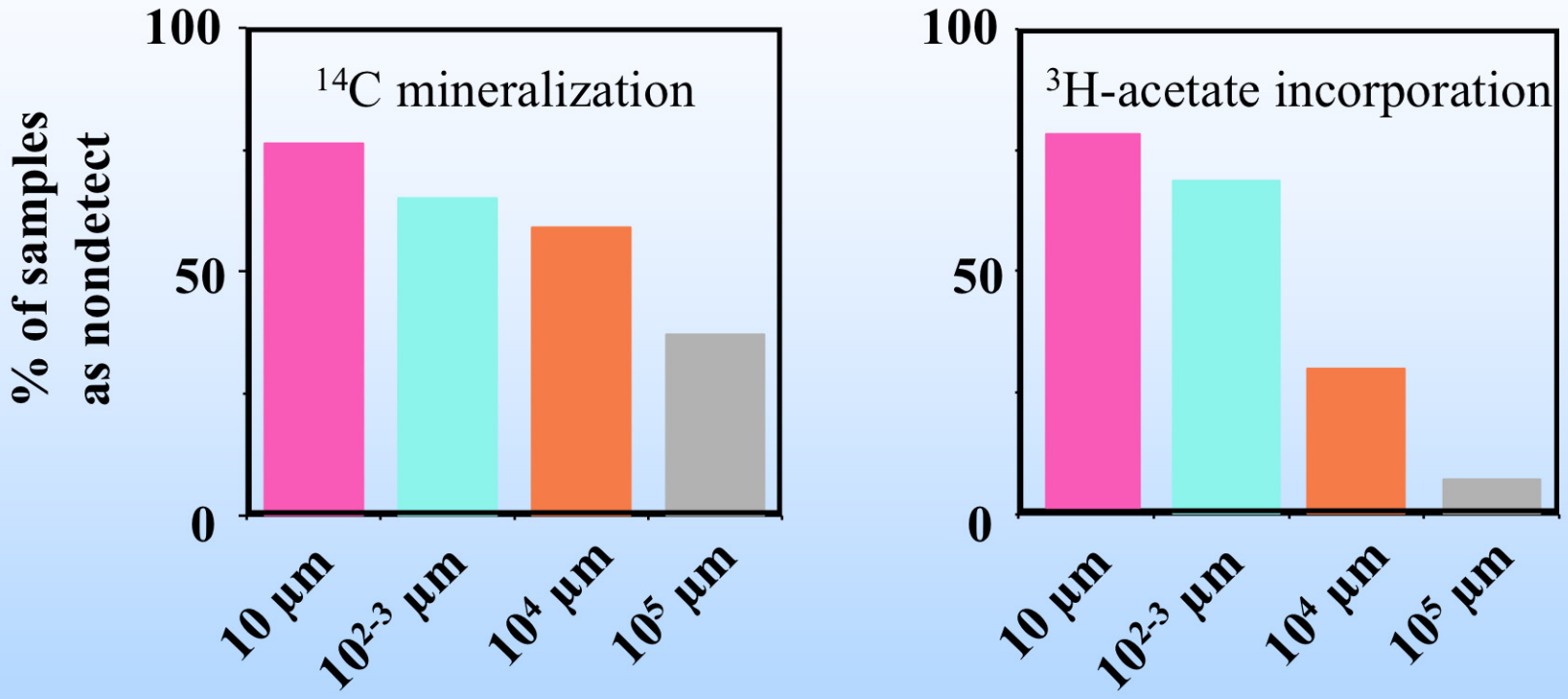
Nearly all non-mineralized substrate can be recovered by leaching/filtration

- **Not going to storage products**
- **Little to no colonization occurs**

More sensitive assay needed to detect growth

- **^{3}H-acetate incorp. into lipids can detect synthesis of 100s of cells/g**

Acetate Incorporation into Lipids



Local conditions supportive of growth become more rare as recharge decreases or age increases

Microorganisms at the low recharge sites appear to have experienced extensive local extinctions

Correlation to Hydrologic & Physical Properties

- Nondetects, variance, portion of variance not explained by spatial dependence, and averaging scale decrease:
  - with increasing recharge
  - With icreasing age of sediment and groundwater
- In low recharge areas, activity higher in fine-grained sediments
  - Higher connectivity of water increases nutrient capture area for isolated microbes
  - Organic carbon content positively correlated to smaller grain size
- In high recharge areas, activity higher in coarse grained, high permeability units
  - Higher flux of surface-derived nutrients

Community Composition

Shallow Eastern: Wide range of Gram negative and Gram positive

Shallow arid: Largely Actinobacteria and Firmicutes (Gram positives)
Gram negatives

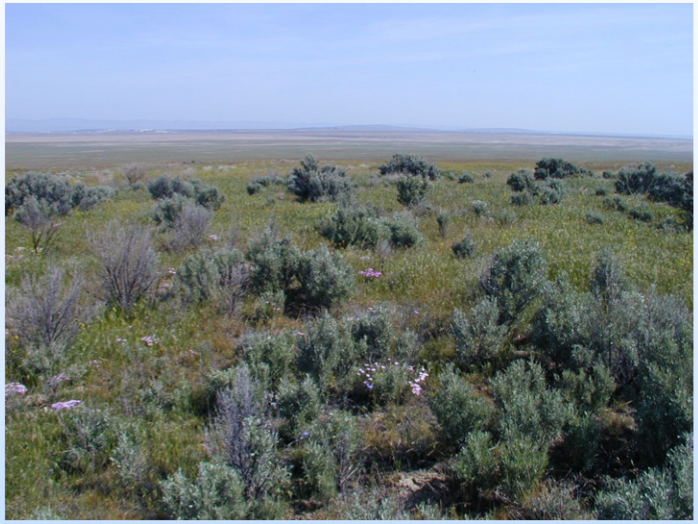
Deep arid: Largely Actinobacteria
Some Firmicutes and Sphingomonas
Gram negatives rare

Stimulation by organics in arid: Gram negatives esp. *Pseudomonas*

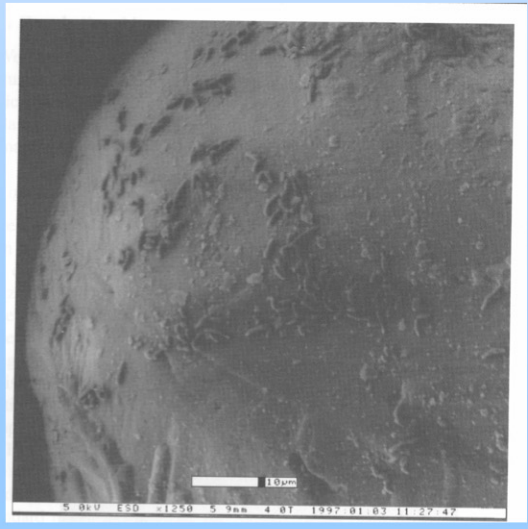
Stimulation by oxygen and mixing in arid: Largely Actinobacteria

Remediation Potential of Arid Sites

Hurdles:
- Patchy distribution
- Degraders present?

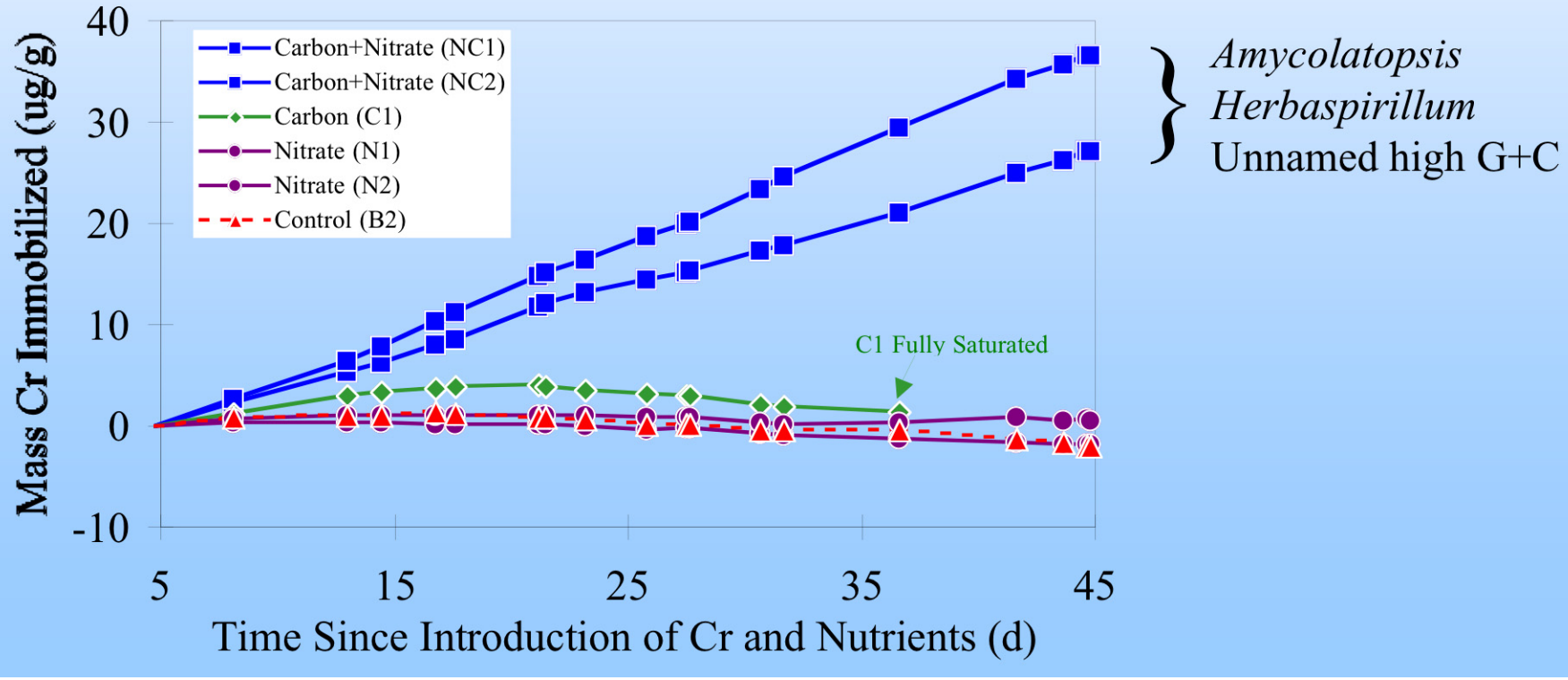


Requires:
- Resuscitation
- Orders of magnitude increase in biomass and activity
- Degraders must compete well in stimulated system



Chromium Bioremediation in Unsaturated Flow Columns

Cumulative Cr immobilized in the columns based on analysis of fluids and solids by FAA and XRF spectroscopies, respectively.



'Aerobic' Gaseous HC and CT Degradation in Deep Vadose Zone Sediments

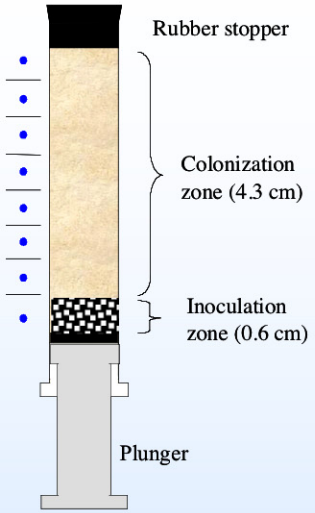
Hole 1, top panel; hole 2, bottom panel. Results are after 10 months of incubation.

Treatments	methane	ethane	propylene	propane	butane	carbon tetrachloride
NH_4NO_3/K_2HPO3, n=16	3	11	12	11	12	1
N_2O/TEP/TBP, n=9	0	0	1	0	0	0
no nutrient, n=12	1	1	1	0	1	0
# of positive bottles --->	4	12	14	11	13	1
% of positive bottles --->	11%	32%	38%	30%	35%	3%

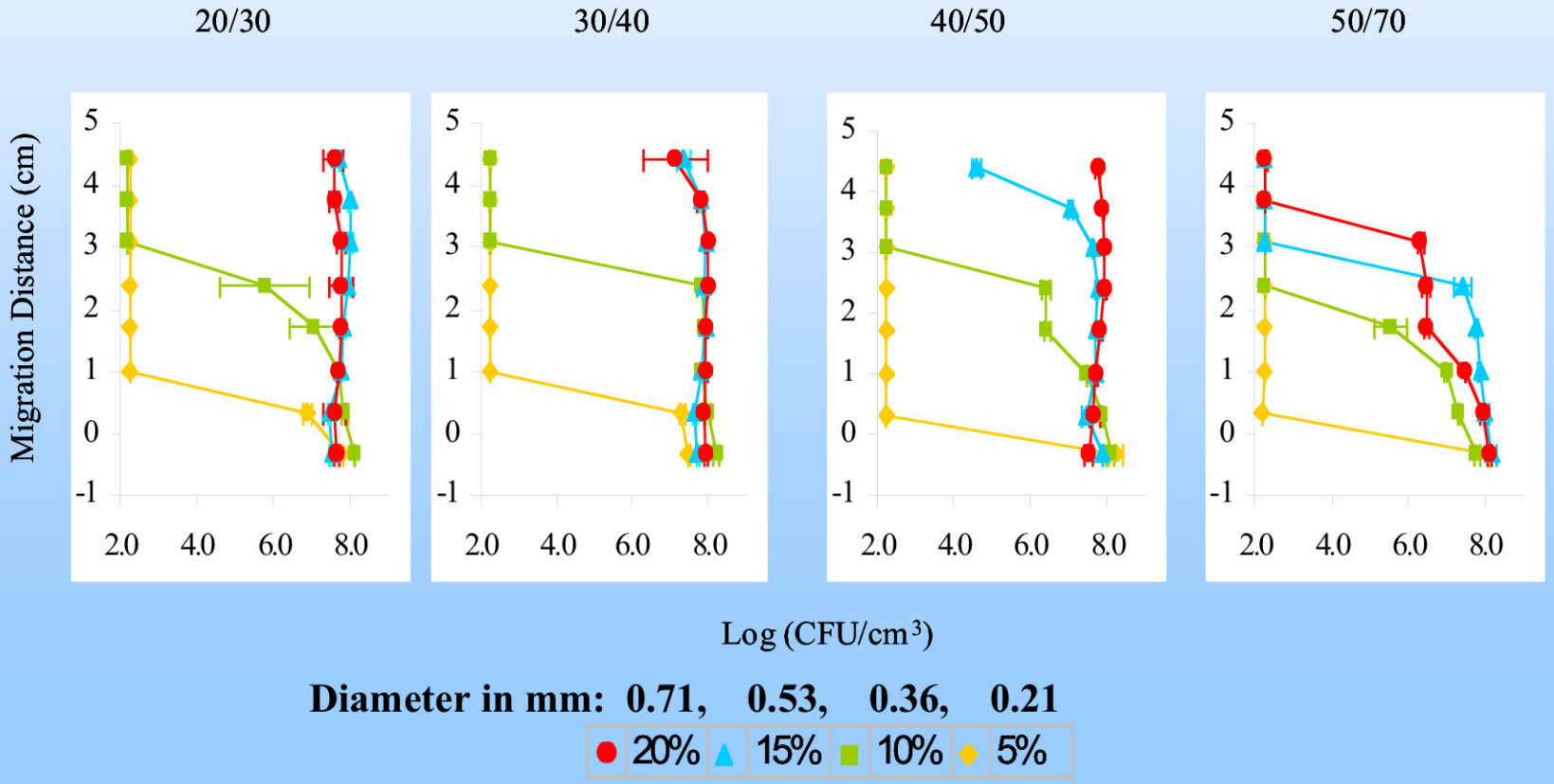
Treatments	methane	ethane	propylene	propane	butane	carbon tetrachloride
NH_4NO_3/K_2HPO3, n=16	2	10	13	9	10	2
N_2O/TEP/TBP, n=11	1	5	6	5	6	1
no nutrient, n=11	1	2	3	3	3	0
# of positive bottles --->	4	17	22	17	19	3
% of positive bottles --->	11%	45%	58%	45%	50%	8%

- 50% of positive samples removed >90% of one or more gases
- Generated populations of 10^7-10^8/g
- With addition of CT-degrading *Pseudomonas* and nitrate, CT removed in 20% of bottles

Colonization in Static Unsaturated Columns

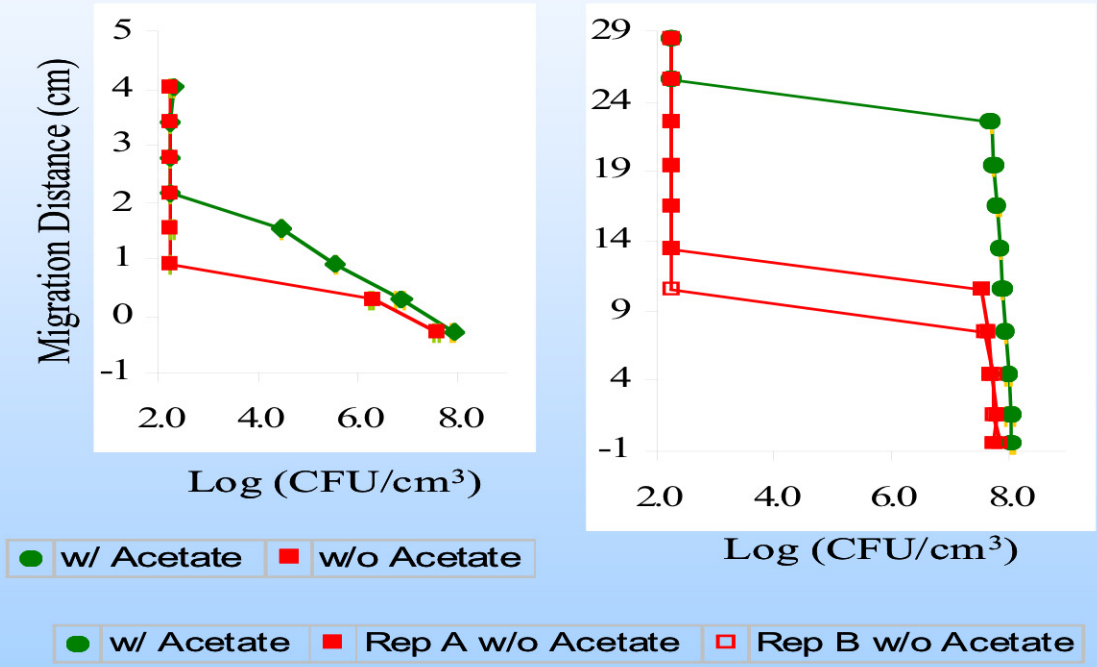


- Effect of sand size and vWC on bacterial movement after 24 hr in the absence of acetate as carbon source.
- Each panel shows bacterial profiles for the 4 sands.
- *Pseudomonas stutzeri* KC and *Pseudomonas fluorescens* HK44



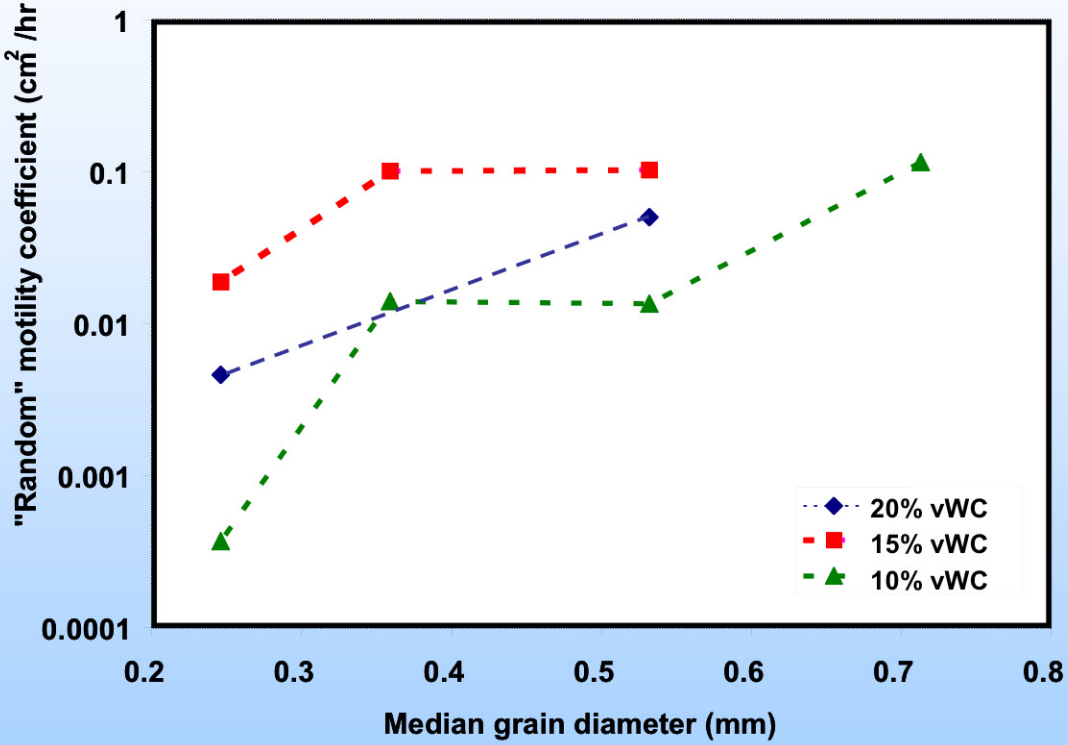
Acetate Driven Colonization in a Poorly-Sorted Non-Spherical Hanford Sand

20% vWC. Panel A, 24 hr; panel B, 7 days.



Modeling of Colonization

Random motility coefficients as a function of vWC and sand size.

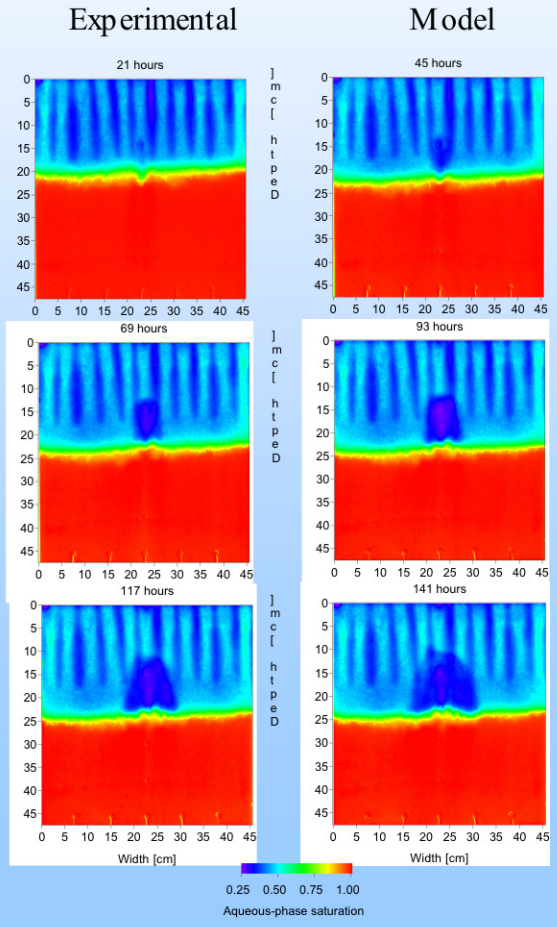


• Decreasing values at smaller grain diameters are due to decreasing minimum water film thickness in the connected pore water, which restrict bacterial motility.

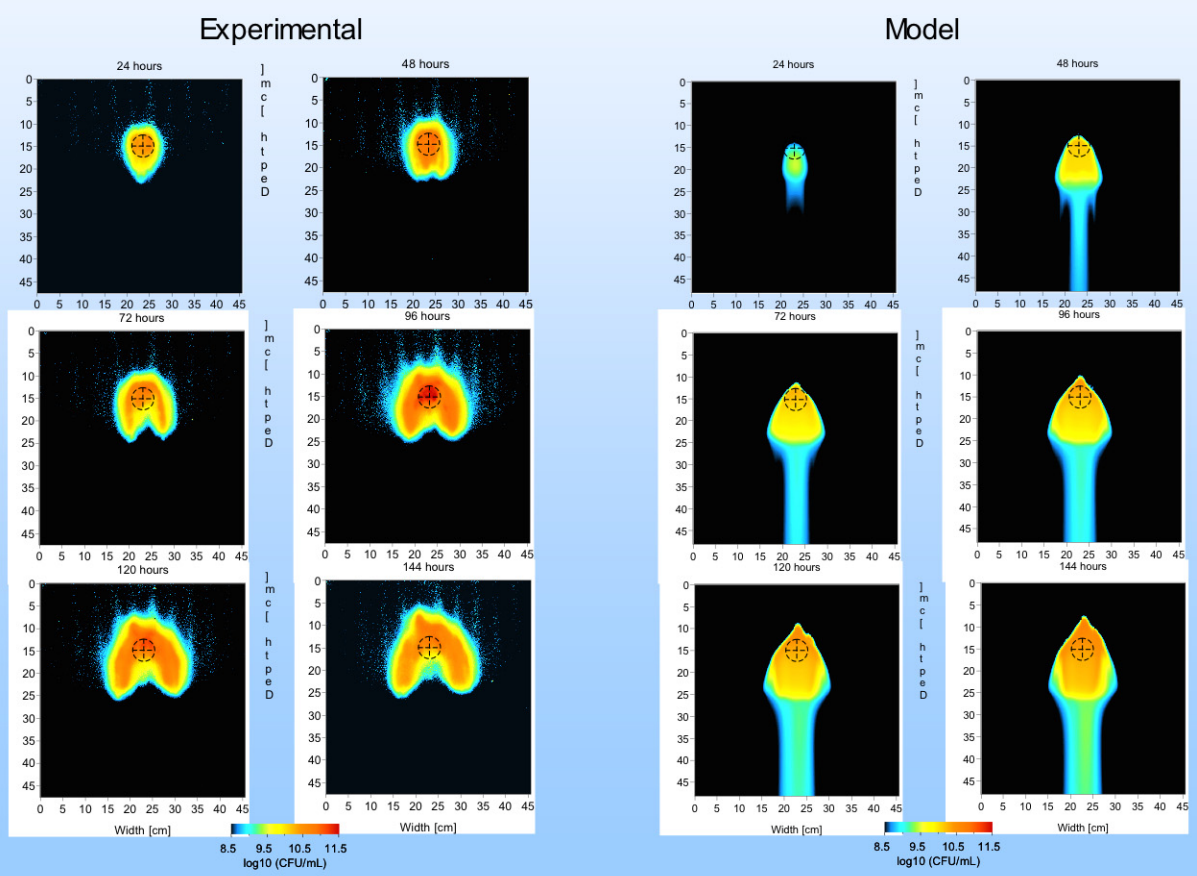
• The random motility coefficients (0.005 to 0.1 cm^2/hr, equal to 1.4×10^{-6} to 2.8×10^{-5} cm^2/sec) are in the same range as those found for saturated systems (3.5×10^{-6} to 3.5×10^{-5} cm^2/sec).

2-d Homogenous Unsaturated Flow

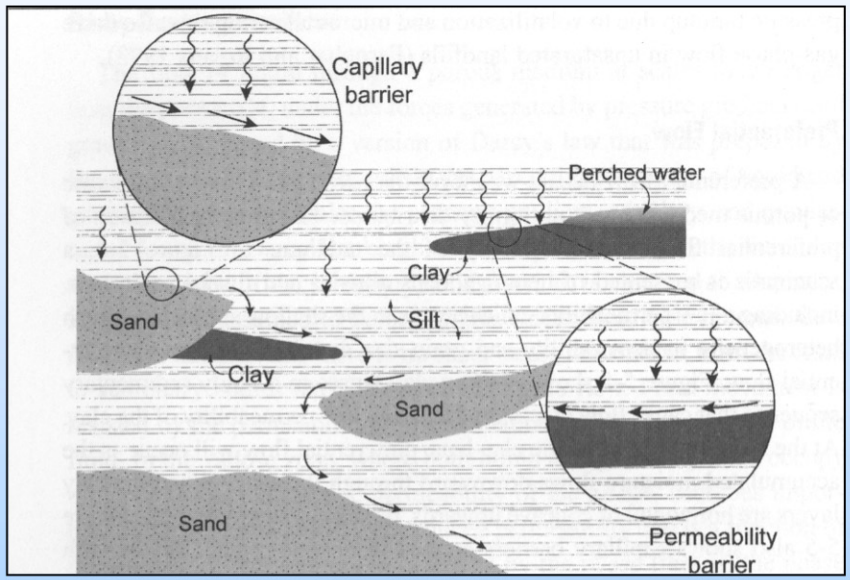
Water Saturation

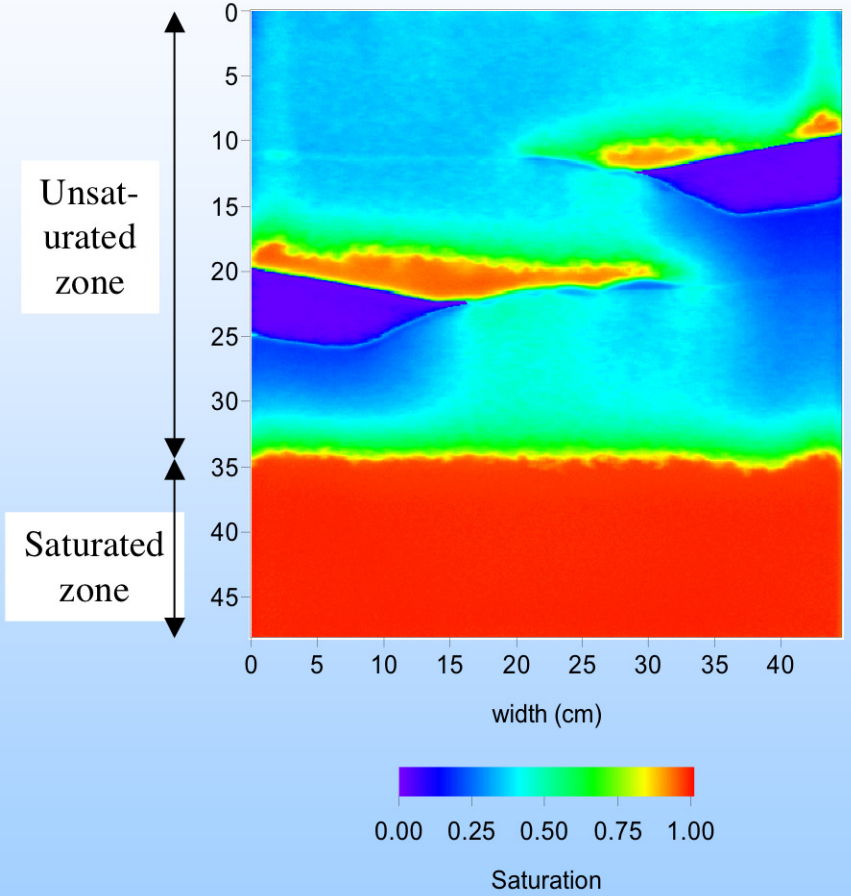


Aerobic biomass (lux)



2-d Heterogeneous Experiments

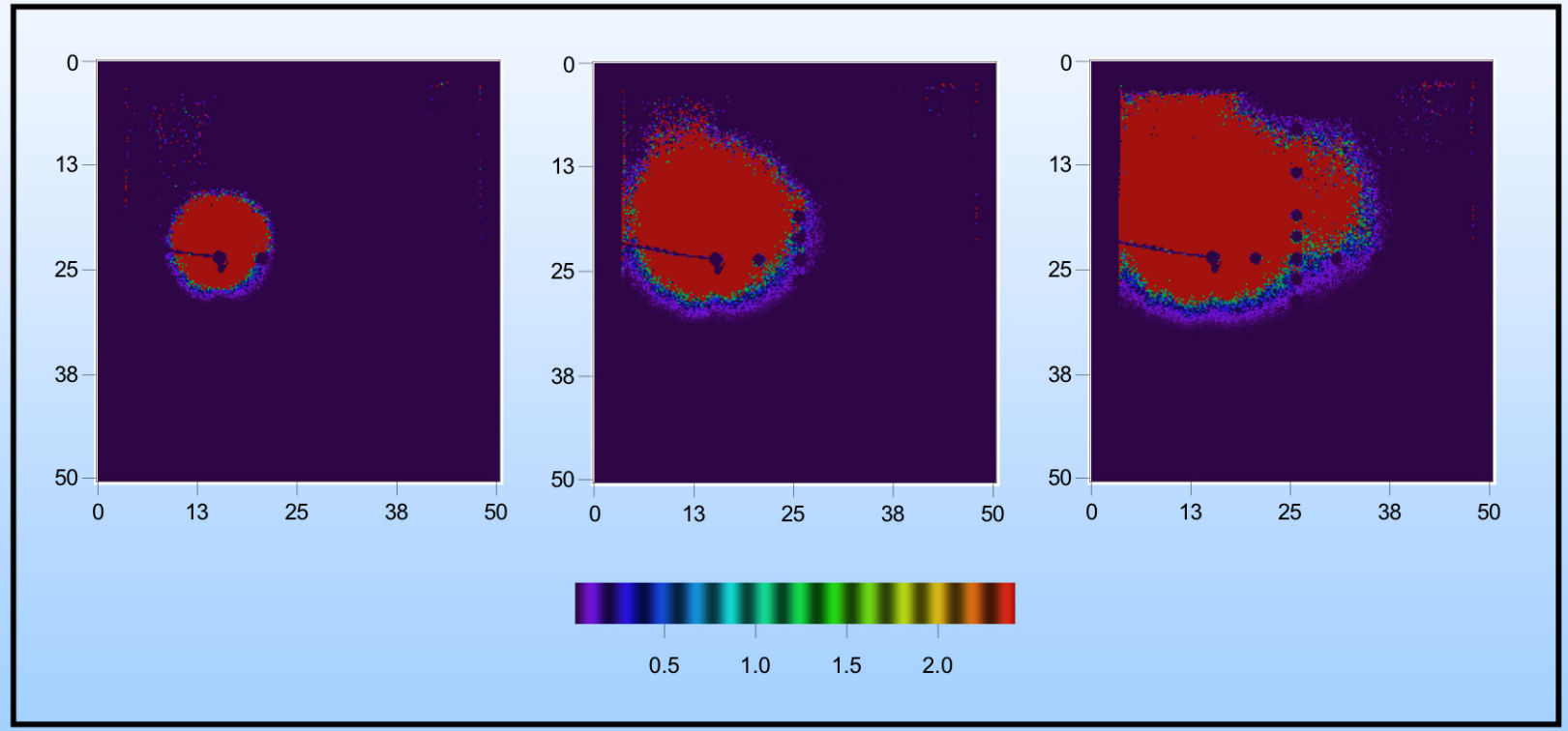




Physical heterogeneties (two coarse-grained sloping sand wedges in a chamber of finer sand, bright blue) cause locally variable water saturation during unsaturated water flow, which will impact the ability of microbes to colonize the vadose zone in response to nutrient delivery.

Real-time Monitoring of CO_2 Movement

(as a proxy for tracking movement of gaseous microbial nutrients)



Migration of carbon dioxide at the left-hand sparging stone at a constant rate of 110 mL/h. Image times are (L to R) 4.5 minutes, 10.5 minutes, and 20 minutes.

Support From

EMSP

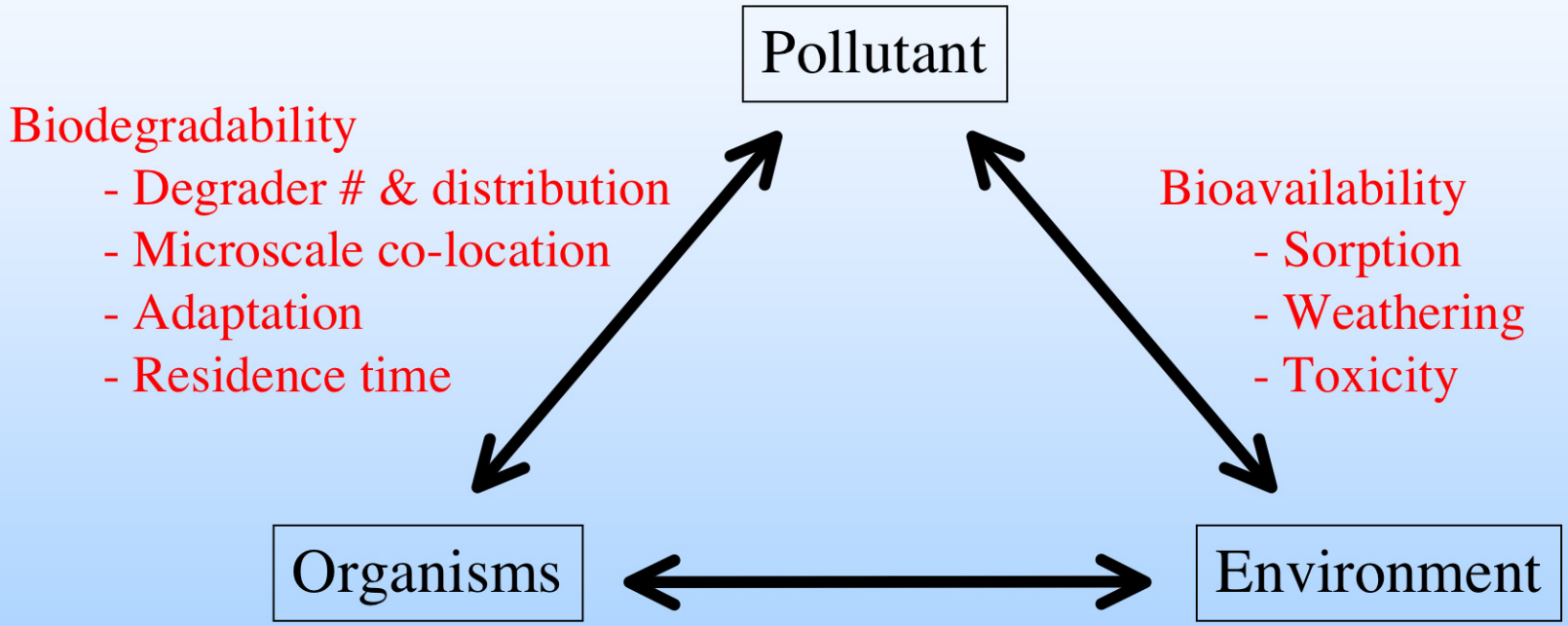
NABIR

Microbial Genome Program

EXTRAS FOLLOW

What Are The Major Controls?

(and How do they change with time?)



Habitability

- Levels of available nutrients
- Ability to colonize/adapt to change
- Functional diversity
- Functional redundancy

2. Why Do We Need to Know More?

Applied

- Geochemical characterization is less expensive than microbial
- Use of proxy information reduces future characterization needs & costs
- Enable predictive models for extrapolation to similar sites

Basic

- Verify/test ecological principles & conceptual models
- Our conceptual models don't explain important observations …
  - suggesting we don't understand some of the important controls
- We continue to discover microbes are highly versatile chemists
  - they 'understand' basic chemistry better than we do

Continuing Problems

Measurement

- Sample frequency
- Sample size
- Frequency and size of ‘hotspots’

Multiplicative effects between overlapping properties that are non-homogenously distributed (spatial heterogeneity)

Microbes can ‘run different programs’

Unknown physiologies

- Arise from poor ability to culture
- Knowledge best achieved by growth & experimentation

Poor understanding of impact of microbial interactions

- Anaerobic methane oxidation
- Anaerobic ammonia oxidation

Stove-piping of disciplines

Routes to Improved Prediction & Mapping of Microbial Properties

Development of a comprehensive conceptual model

- Hydrogeochemical categories approach
- Ecological principles, e.g., Threshholds
- Flow and transport codes coupled to thermodynamics
- Formulating and testing hypotheses

Inexpensive microscale sampling of groundwater for donors, acceptors, and micronutrients

Instrumented *in situ* microcosms

Greater use of geostatistics to evaluate spatial dependence

Technologies to culture novel microbes

Genomic sequencing, bioinformatics, and metabolic modeling

- Isolates
- Community DNA
- Community mRNA and proteomics

Transect Sampling

Regularly-spaced samples (every 5 cm)

50-100 samples

Intact, 10-15 g → Homogenized and 1 g assayed

100 nmoles each of ^{14}C-glucose & ^{14}C-acetate in 0.1 ml water

Incubated 4 wks

Fine-Scale Spatial Structure

50 1-g sample size at 0.33 cm intervals in core

100 nmoles each of ^{14}C-glucose &
^{14}C-acetate in 0.1 ml water

^{3}H-acetate into lipids (1 nmol in 0.1 ml water)
(synthesis of 100s of cells)

Incubated 4 wks (^{14}C), 1 d (^{3}H-gluc),
days to wks (^{3}H-acet)

Chronosequence Site

Aggrading landscape with many ancient buried soils

Known sediment and groundwater ages

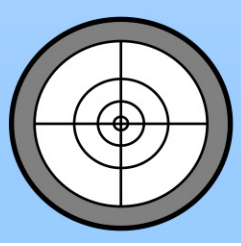
- **modern - 700,000 yrs (sed)**
- **0 - 900 yrs (gw)**

Hypothesis:
As sediment & groundwater age increases, microbial activity will become more discontinuous and its averaging scale will increase

Transect Results

(100 nmol each of glucose & acetate)

	Groundwater age (actual years)						
	1	60	130	200	90	625/ 675	725/ 900
	Sediment age (thousands of years)						
	Modern	**35**	**80**	**90**	**90**	**455/ 475**	**495/ 675**
Nondetects	**0%**	**0%**	**86%**	**54%**	**98%**	**95%**	**100%**
Mean	**37%**	**38%**	**0.03%**	**8.6%**	**0.01%**	**0.02%**	**0%**
St. dev.	**7%**	**31%**	**233%**	**149%**	**--**	**--**	**--**
Nugget ($\%^2$)	**3**	**60**	**--**	**115**	**--**	**--**	**--**
Averaging scale expmt.	**0.1 g**	**1 g**	**100 g**	**10 g**	**100 g**	**100 g**	**>100 g**

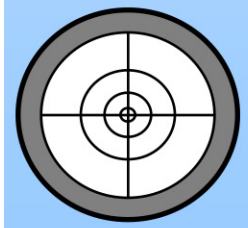


8 x 0.1 g
8 x 1 g
8 x 10 g
8 x 100 g

Discontinuity (nondetects, variance, and portion of variance not explained by spatial dependence) increases with age

Activity and Spatial Structure

	Extremely low	Low	High
	28 d	56 d	2 d (14 d)
Nondetects	76%	65%	0%
Mean of detects	2.5%	0.94%	7.8%
St. dev. of detects	360%	281%	124%
Nugget*	40%	43%	15%
Averaging scale experiment	100 g	100 g	0.1 g



8 x 0.1 g
8 x 1 g
8 x 10 g
8 x 100 g

Nondetects, variance, and portion of variance not explained by spatial dependence* decrease with increasing recharge