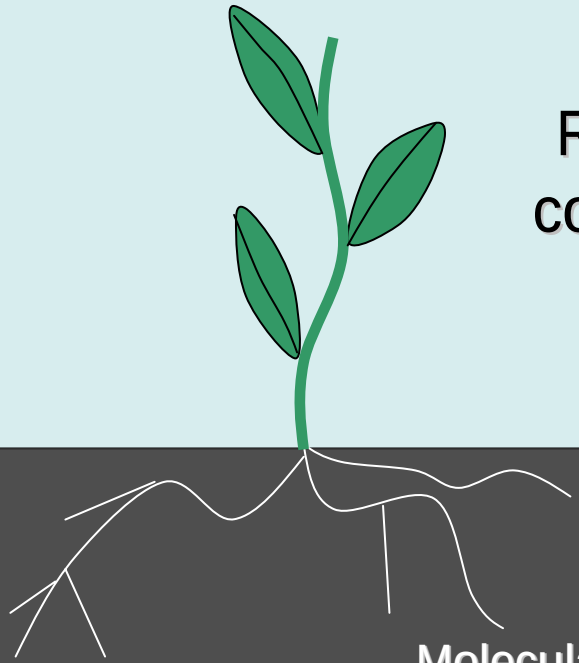
Soil microbial diversity in a mesquite savanna:

Response of bacterial and fungal communities to vegetation change



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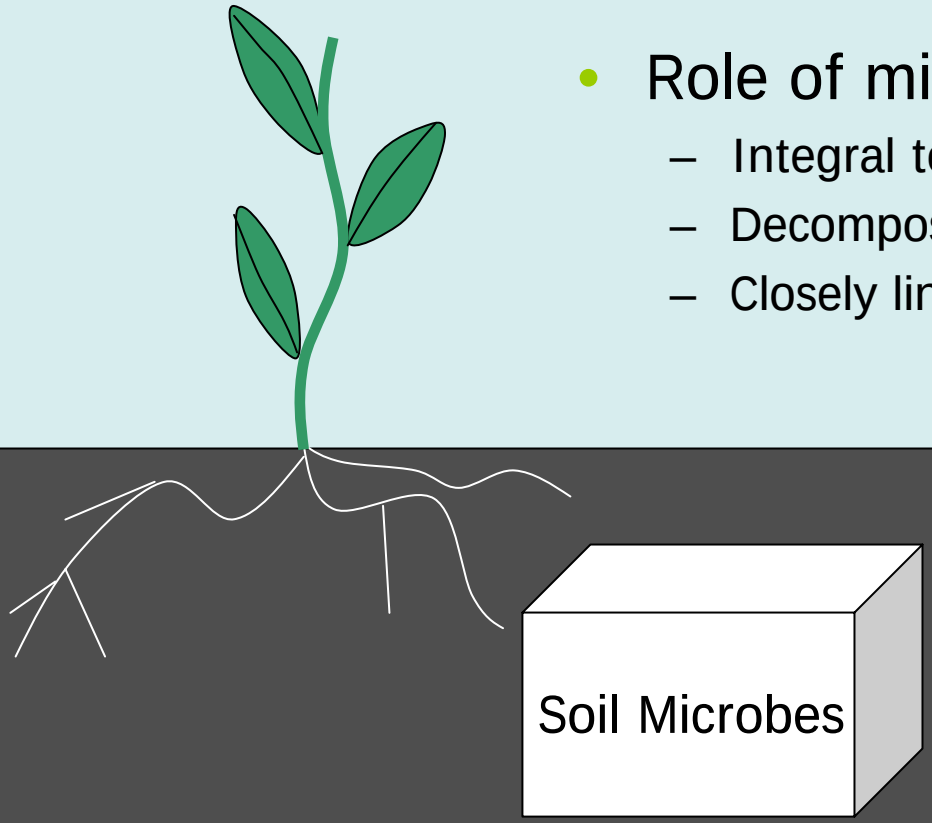
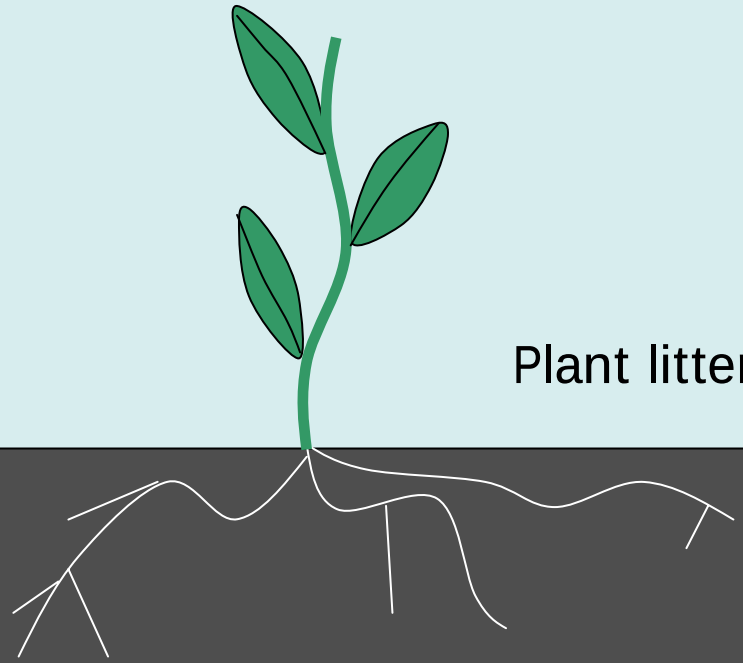
Scratching the Surface



- Role of microbial communities
  - Integral to ecosystem function
  - Decomposition and nutrient cycling
  - Closely linked to the plant community

- Detection & Characterization
  - Culturing and morphology
  - Substrate preferences
  - Biomass changes
  - Physiological measurements

The “Black Box”



Plant litter

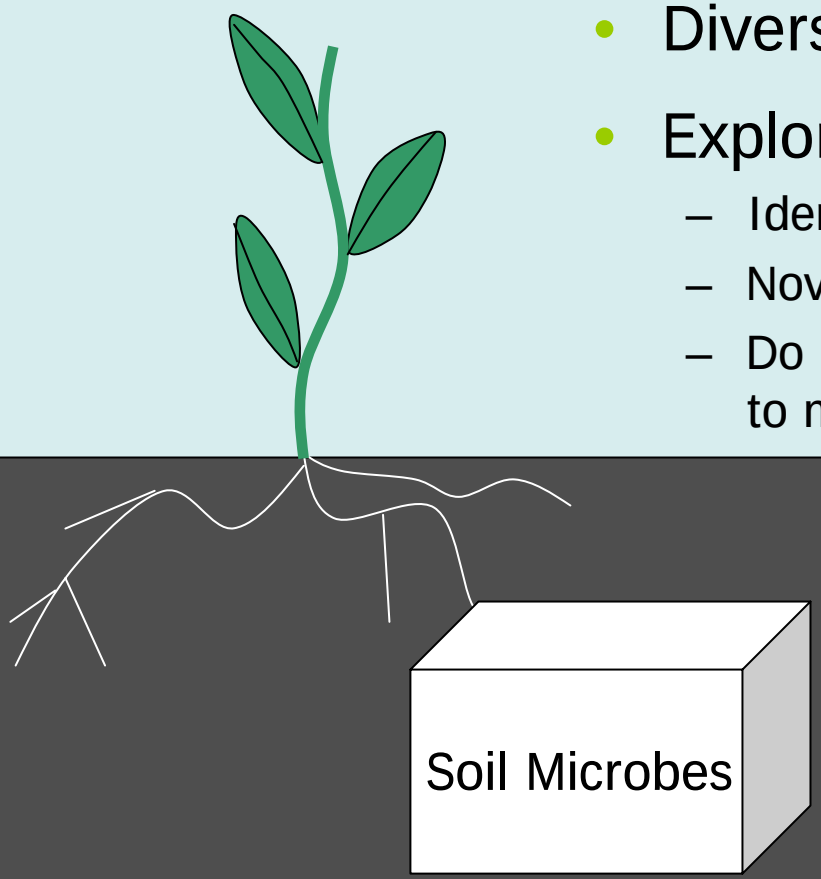
Soil respiration

Root tissue and exudates

Soil Microbes

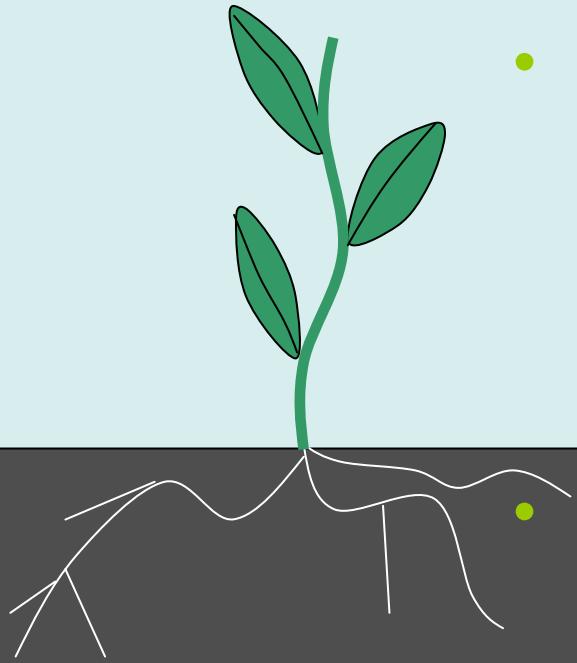
Mineralized nutrients
Undigested residues

Gaining New Perspectives



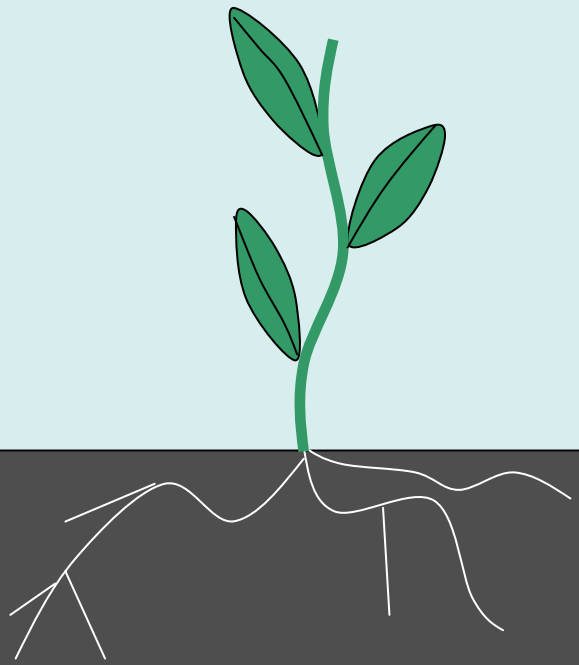
- Diversity
- Exploration
  - Identification of individuals
  - Novel associations and functions
  - Do macroscale ecological theories apply to microscale organisms?
- Monitoring
  - Specific associations with plant types
  - Seasonal variation
  - Sensitivity to management practices
- Function

Effects of Land Cover Change



- Woody encroachment of grassland systems
  - *Prosopis glandulosa* (honey mesquite)
  - Productivity rates
  - Tissue chemistry
  - Soil C and N concentrations
- Response of soil microbial communities?
  - Evidence for change in biomass and activity
  - Due to increased “supply”?
  - Altered composition? Function?

Looking below the surface

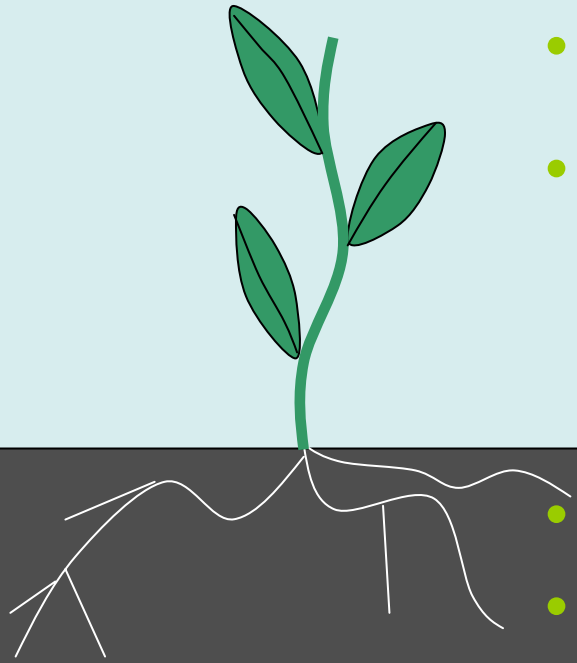


- Community Composition
  - Cloning and sequencing approach
  - Bacterial community
  - Fungal community
  - Four vegetation types
- Functional Gene Abundance
  - “Whole community” profile
  - Functional gene microarray
  - Potential to assess thousands of gene probes simultaneously

Study Site

W.T. Waggoner Ranch	
Location	Wilbarger County, TX
Mean annual precipitation	665 mm (peaks in May & Sept)
Mean annual temperature	16.1 °C
Soil type	Fine, mixed, superactive, thermic Vertic Paleutolls of the Tilman series
Soil texture	16% sand, 52% silt, 32% clay
Soil pH	7.00 – 7.21
Vegetation	Mesquite, C_3 and C_4 grasses

Assessing Community Composition



- Cloning and sequencing
- Ribosomal DNA is often used in the characterization of microbial communities
  - 16S ribosomal subunit for bacteria
  - 18S or 28S ribosomal subunit for fungi
- Avoids “culture bias”
- Well conserved targets
- Fine-scale identification is possible

Getting from Soil to Sequence

1. Extract community DNA from soil
   (Bacterial, fungal, plant, and invertebrate)
2. PCR-amplify targets of interest
   (16S for bacteria, 28S/ITS for fungi)
3. Clone into vector, then *E. coli*
4. Re-extract target DNA
5. Add fluorescent labels via PCR reaction
6. Sequence labeled products

Soil to Sequence…to Statistics

Multiple ways to describe communities

1. Richness
   - Number of species or OTUs
   - Estimated richness (extrapolation)

2. Structure
   - Species/OTUs and their relative abundances
   - Yue-Clayton similarity index (SONS)

3. Phylogenetic Relationships
   - Structure of evolutionary relationships
   - Parsimony test (TreeClimber)

Soil Community Comparisons

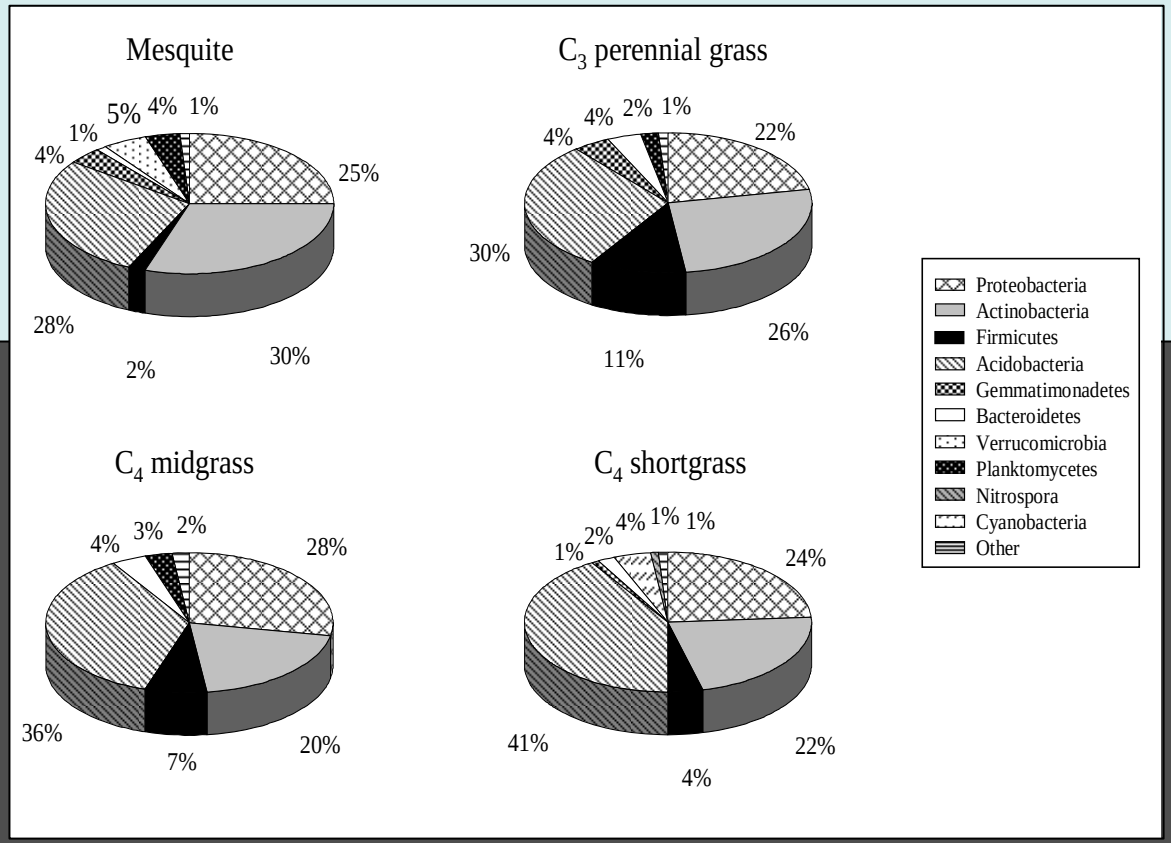
Bacterial Communities (16S rDNA)

Plant functional type	Library size	OTUs identified	Simpson (1/D)	Chao I estimated richness
Mesquite	83	71	227	341
C_3 perennial grass	54	47	179	184
C_4 shortgrass	75	54	75	162
C_4 midgrass	83	68	170	245

- 295 clones sequenced
- Species-rich and diverse
- Low-to-moderate degree of overlap shared among vegetation-based clone libraries

Soil Community Comparisons

Bacterial Communities (16S rDNA)



Soil Community Comparisons

Bacterial Community Parsimony Test

Vegetation pair	Parsimony score	Random vs. Directed
Whole community	146	0.078
M-C_3	37	0.960
M-C_4 Mid	46	0.093
M-C_4 Short	47	0.066
C_3-C_4 Mid	39	0.299
C_3-C_4 Short	43	0.670
C_4 Mid-C_4 Short	49	0.238

Do two or more communities share a common phylogenetic structure?

Soil Community Comparisons

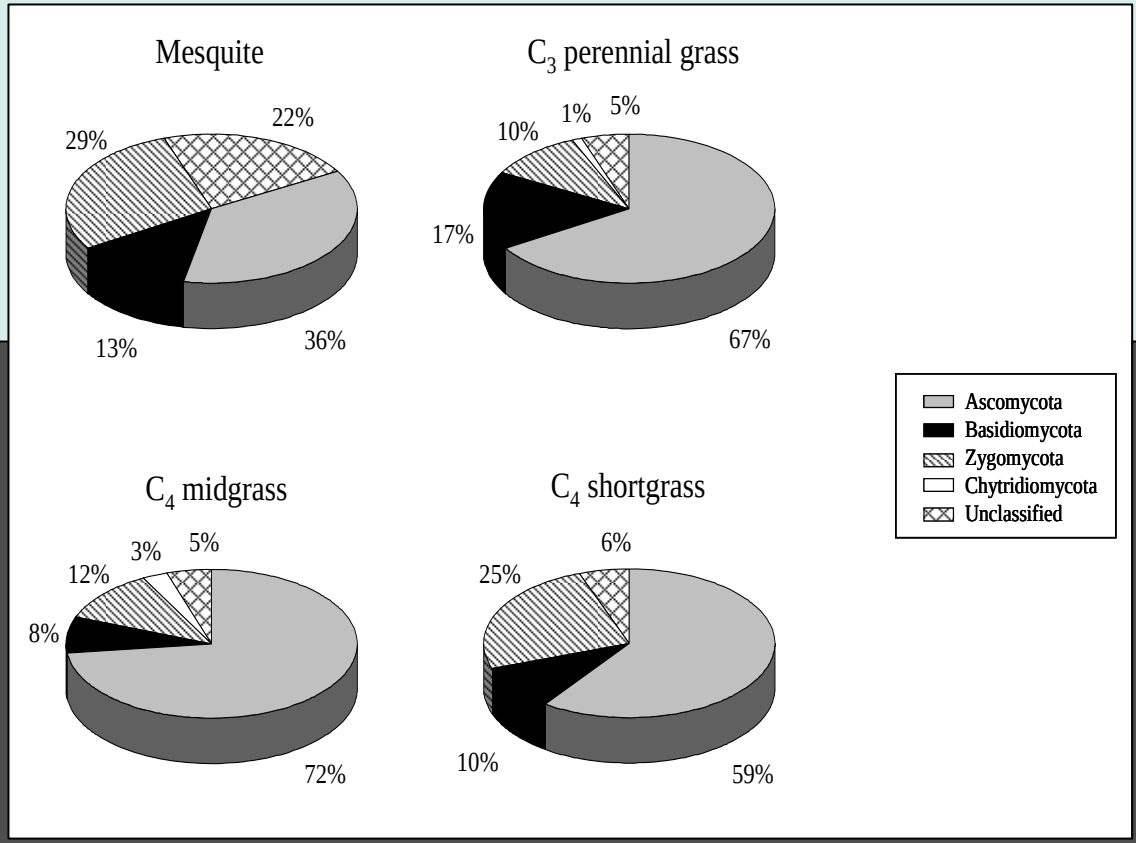
Fungal Communities (ITS/28S rDNA)

Plant functional type	Library size	OTUs identified	Simpson (1/D)	Chao I estimated richness
Mesquite	77	33	13	63
C_3 perennial grass	77	28	10	79
C_4 shortgrass	78	20	3	50
C_4 midgrass	72	22	8	33

- 304 clones sequenced (~76 per vegetation type)
- Less diverse than the bacteria
- Moderate degree of similarity shared among clone libraries

Soil Community Comparisons

Fungal Communities (ITS/28S rDNA)



Soil Community Comparisons

Fungal Community Parsimony Test

Vegetation pair	Parsimony score	Random vs. Directed
Whole community	135	0.045
M-C_3	37	0.037
M-C_4 Mid	36	0.033
M-C_4 Short	35	0.041
C_3-C_4 Mid	44	0.063
C_3-C_4 Short	41	0.056
C_4 Mid-C_4 Short	43	0.086

Do two or more communities share a common phylogenetic structure?

Soil Community Comparisons

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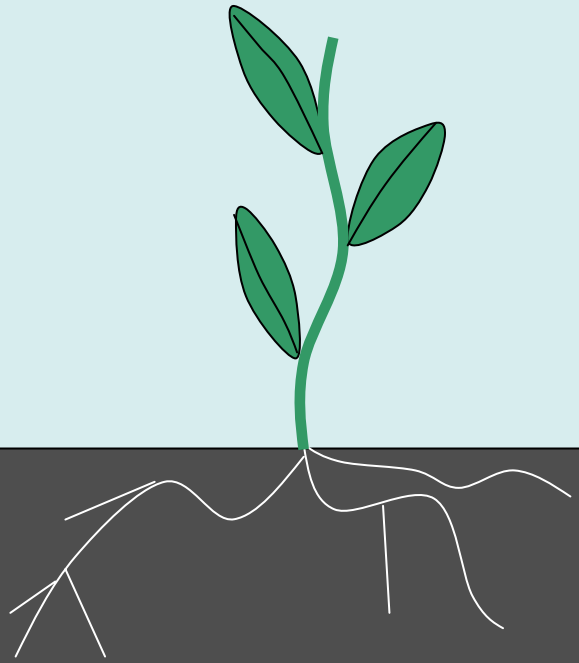
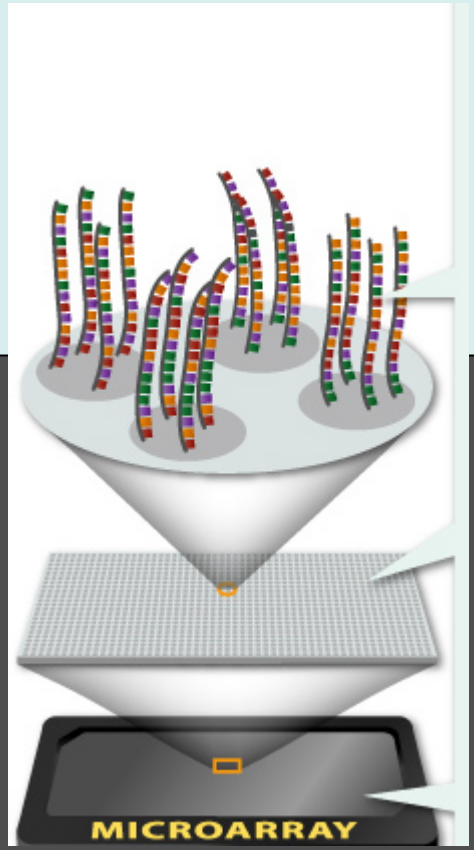
Soil Community Comparisons



- Bacterial Communities
  - Species-rich and diverse
  - Low degree of overlap shared among clone libraries
  - But…no statistically significant differences detected in community structure
- Fungal Communities
  - Less complex than the bacterial communities
  - Many “unclassified” sequences
  - Mesquite community differed significantly from each of the grass communities

Assessing Community Function

- Microarrays offer an alternative approach

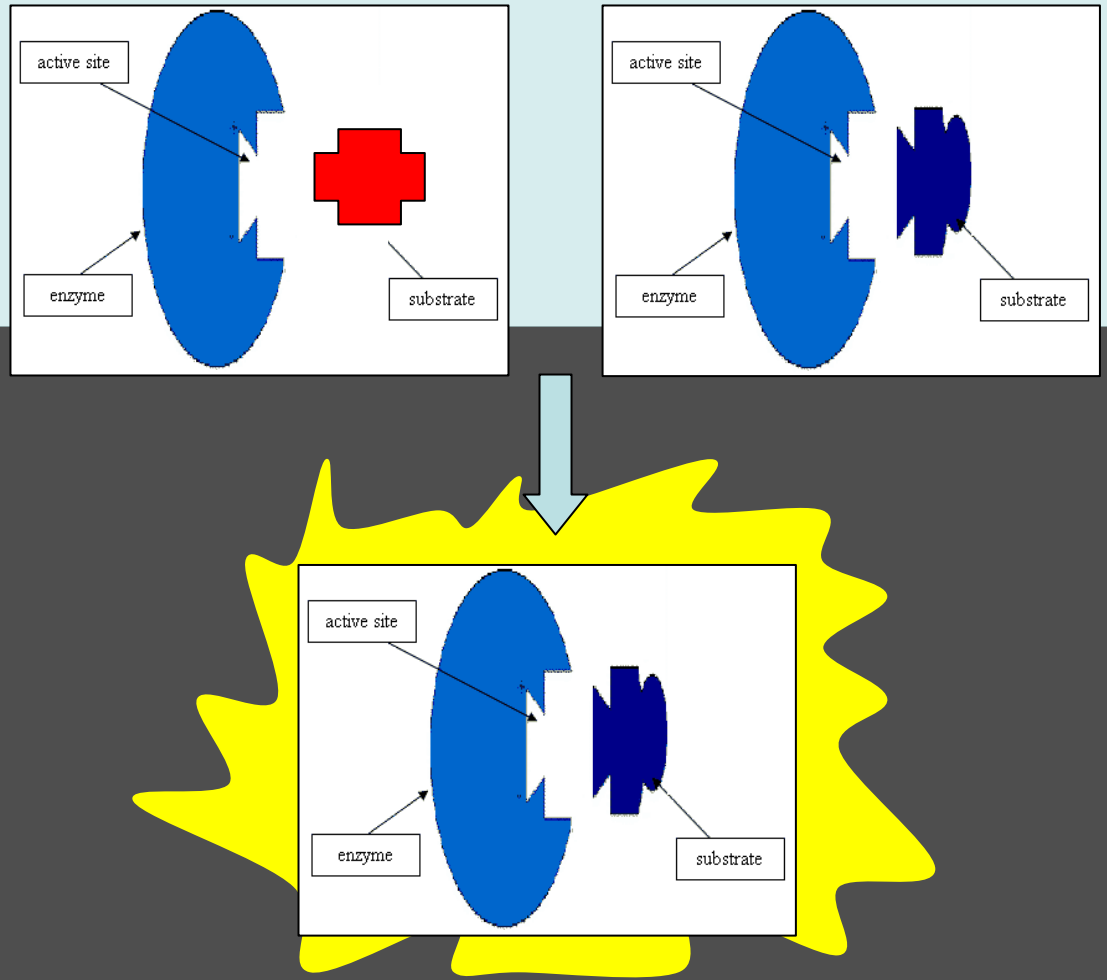


- High density collection of gene probes (thousands per array)
- Flexibility in design
- High throughput
- Simultaneous detection of multiple gene types

Microarray image from http://learn.genetics.utah.edu

Assessing Community Function

- Akin to the “Lock and Key” mechanism
- Sequence similarity



Enzyme image from http://regentsprep.org

Assessing Community Function

- GeoChip, a functional gene microarray (FGA)
- The most comprehensive FGA currently available
- Used to evaluate functional capacity
- Contains gene probes for the detection of:
  - Carbon degradation and fixation
  - Nitrogen cycling
  - Sulfur cycle
  - Metal resistance/reduction
  - Organic contaminant degradation

Assessing Community Function

Summary of FGA and Detection of Gene Probes

Gene function	# Genes	# Probes	# Detected
C degradation	980	1014	56
C fixation	376	428	14
Methane cycle	303	303	13
Nitrogen cycle	1988	2027	82
Sulfur cycle	627	646	20
Metals	1610	1933	128
Organic contaminants	2774	2774	178

Assessing Community Function

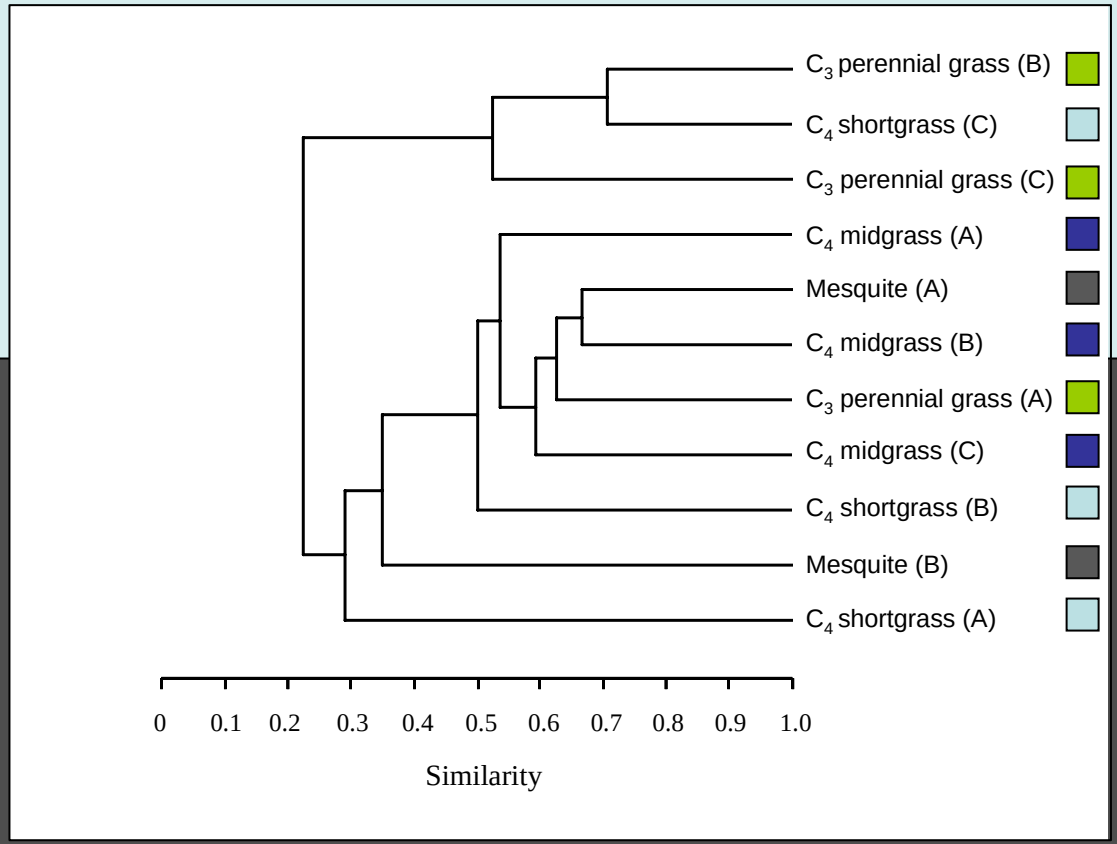
Analysis of Similarity
Comparing Gene Abundance Patterns

Vegetation type	Mesquite	C_3 perennial grass	C_4 shortgrass	C_4 midgrass
Mesquite				
C_3 perennial grass	0.083 (1.00)			
C_4 shortgrass	-0.250 (1.00)	-0.222 (1.00)		
C_4 midgrass	0.417 (0.60)	0.185 (1.00)	0.185 (0.58)	

- Relative signal intensity of detected gene probes
- R-values range from -1 to +1, p-values in parentheses

Assessing Community Function

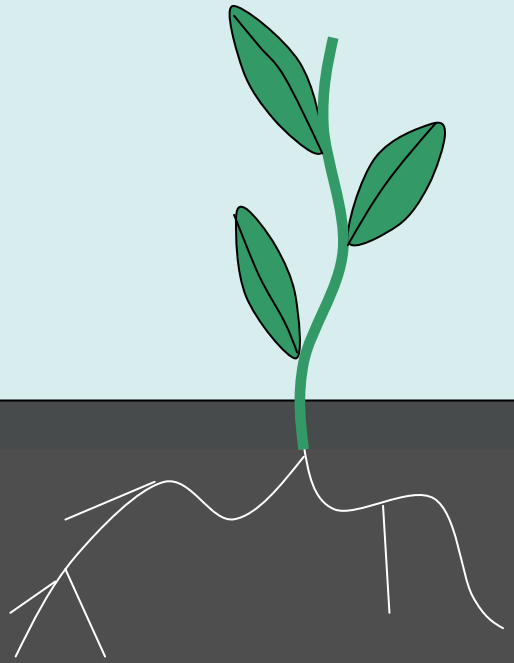
Hierarchical clustering of gene detection profiles



Assessing Community Function

- Subtle differences in this system, relative to other studies that have used this array
- Array is largely composed of bacterial genes (~98%)
- No significant differences in bacterial clone library composition
  - Functional redundancy?
  - Ecosystem complexity?
  - Array design?
  - Seasonal/temporal effects?

Summary



- Community composition
  - Bacterial communities diverse but not significantly different
  - Fungal communities differ strongly between grasses and mesquite
- Community function
  - Wide variety of functional genes detected
  - Gene detection profiles did not differ significantly
  - Redundancy
  - Bacterial vs. Fungal genes

More recently…..

- Working to improve the MixAlco Process
  - Characterizing a new microbial “black box”
  - Bioprospecting for new, pre-adapted, and optimized inoculum sources
  - Ecology of hypersaline microbial communities

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