

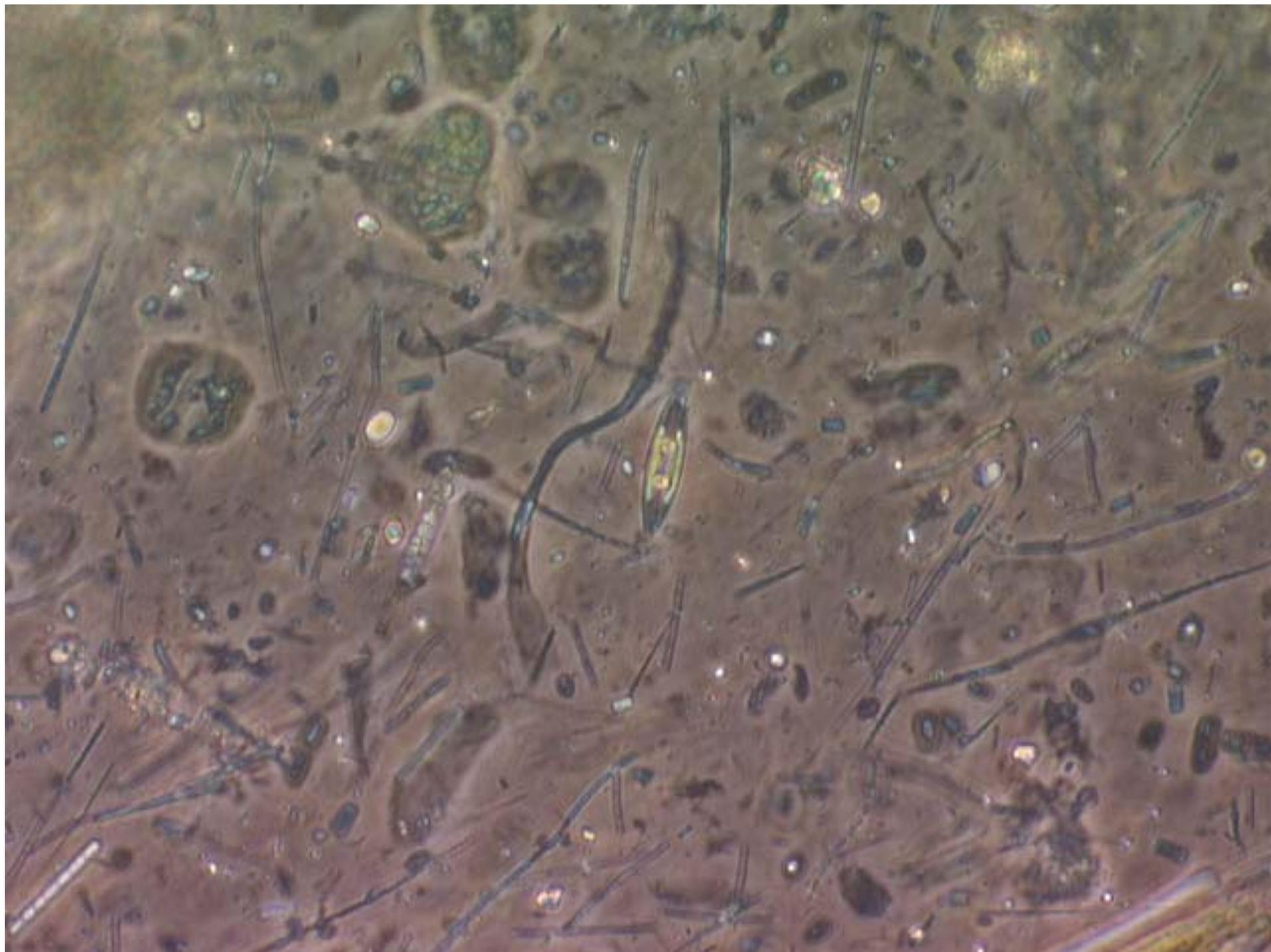
The PhyloChip: A complete microbial community approach to water quality research

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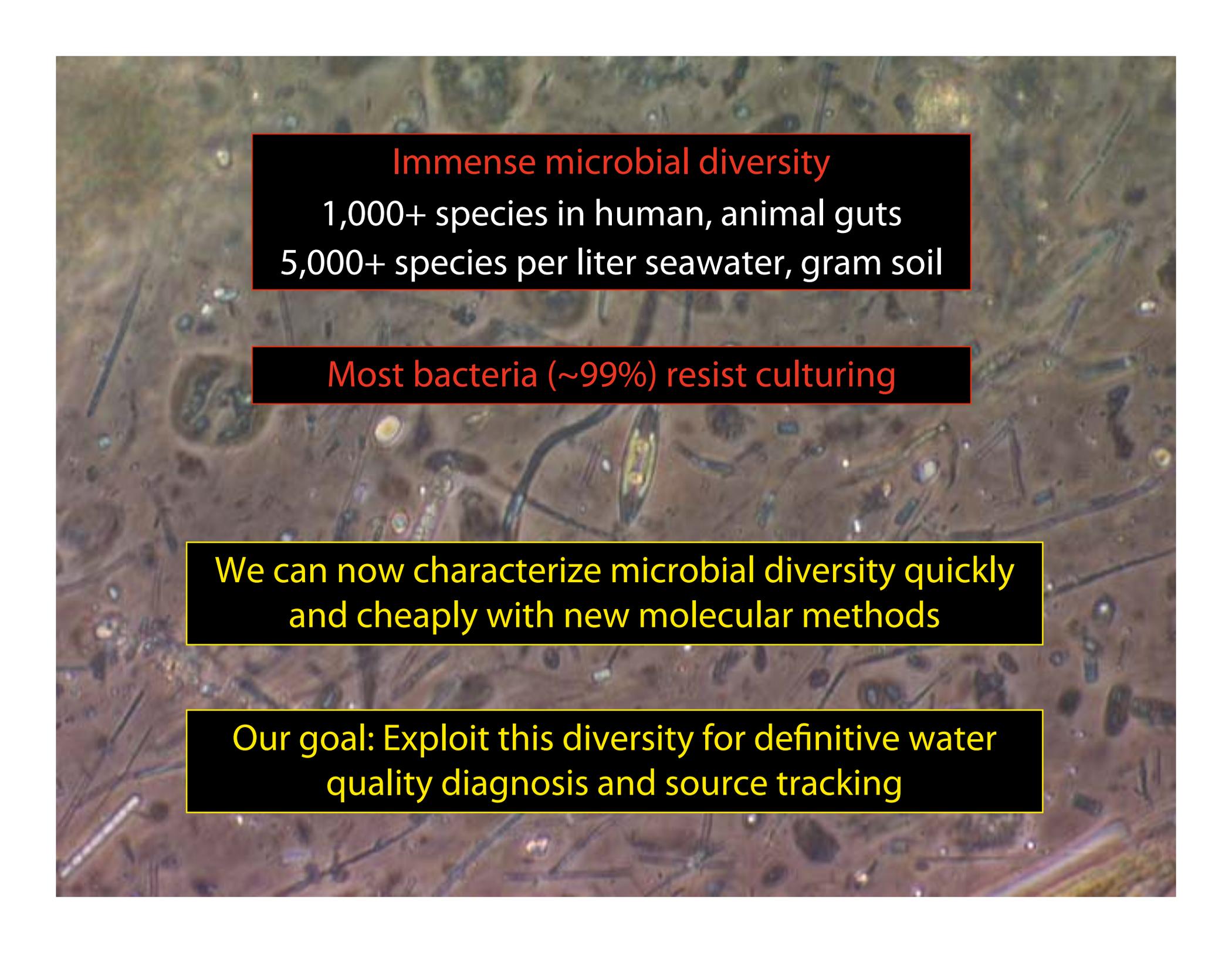


Immense microbial diversity

1,000+ species in human, animal guts

5,000+ species per liter seawater, gram soil

Most bacteria (~99%) resist culturing



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We can now characterize microbial diversity quickly and cheaply with new molecular methods

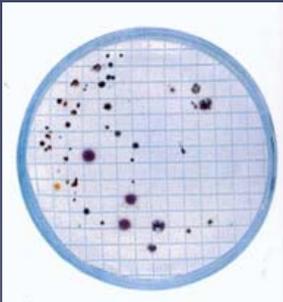
Our goal: Exploit this diversity for definitive water quality diagnosis and source tracking



Microbial community analysis: limited by our (lack of) power to observe

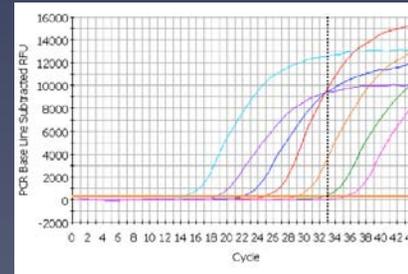
Culture methods

Restricted detection, must grow in captivity

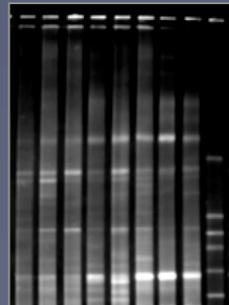


Culture-independent methods

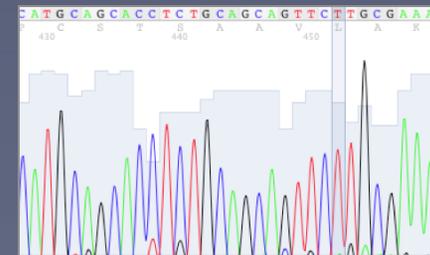
Restricted detection (Quantitative PCR)



Low resolution (RFLP, DGGE, PLFA)



Cost/labor prohibitive (DNA sequencing)



PhyloChip analysis of microbial communities



Detects 50,000 different bacteria and archaea in a single test

Comprehensive census of whole microbial community

Rapid, repeatable and standardized method

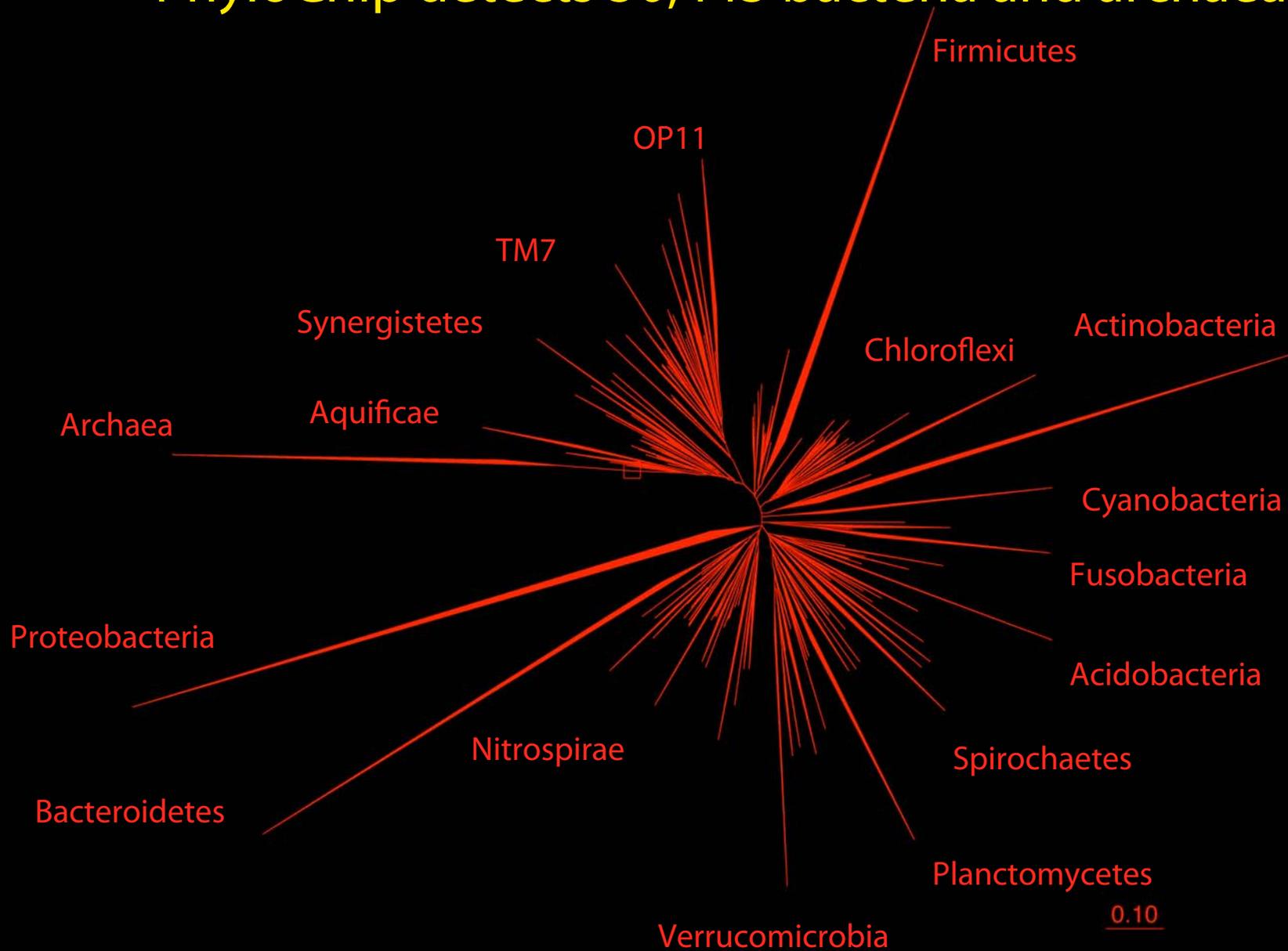
PhyloChip

comprehensive microbial census

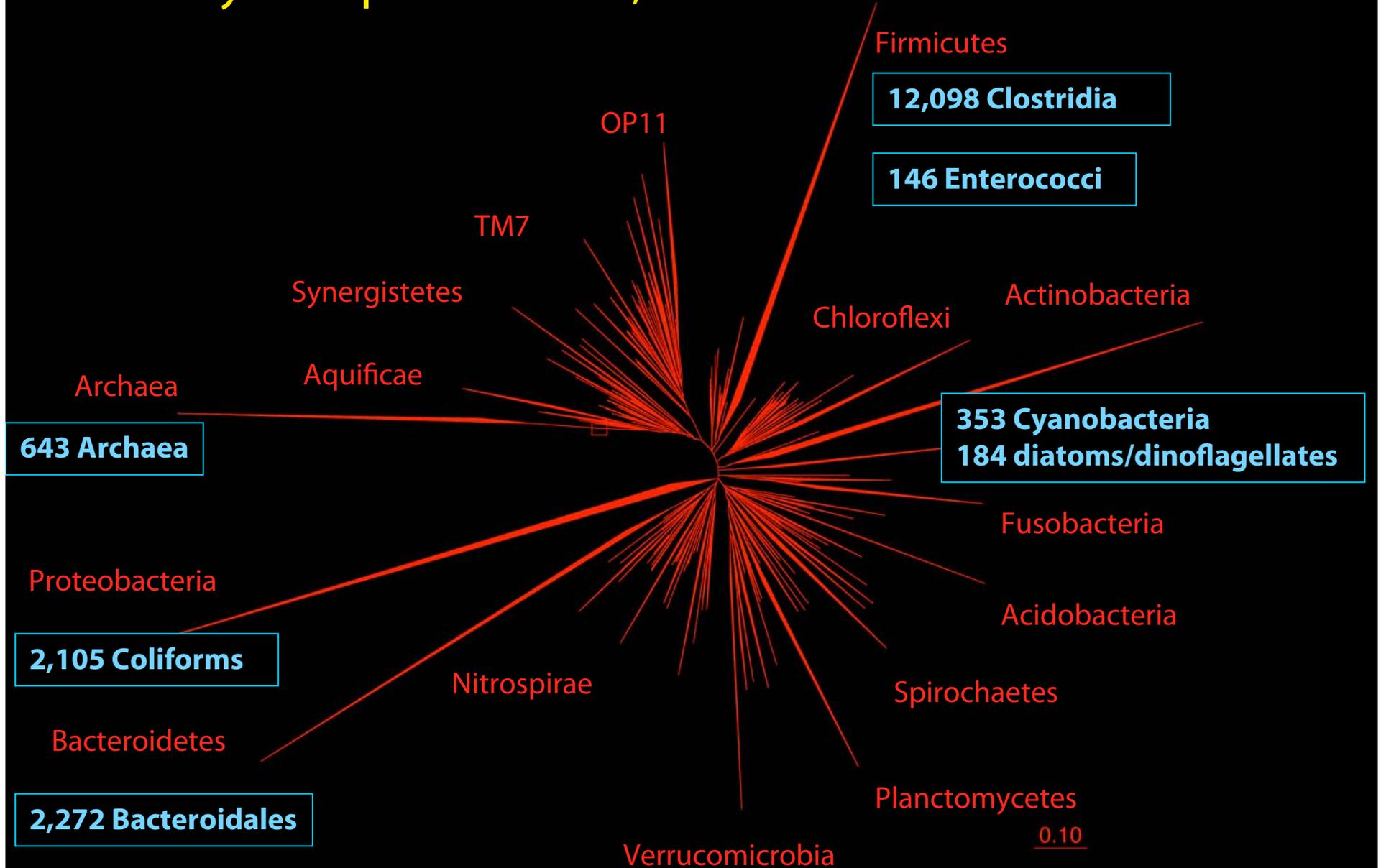


- High-density oligonucleotide gene microarray
- 1.1 million DNA probes for most known bacteria and archaea
- Based on entire 16S rRNA gene database (greengenes.lbl.gov)
- Analysis of entire pool of community DNA allows detection of very low abundance taxa, microbial community dynamics

PhyloChip detects 50,443 bacteria and archaea



PhyloChip detects 50,443 bacteria and archaea



Sample preparation



Extract DNA/RNA
from filtered sample



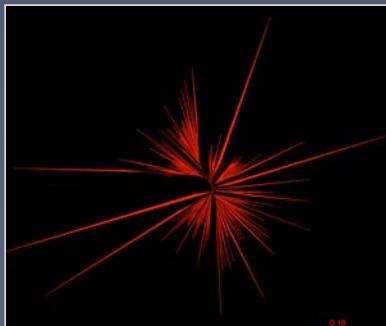
Amplify 16S rRNA
gene with PCR



Analyze
composition
amplified
DNA/RNA

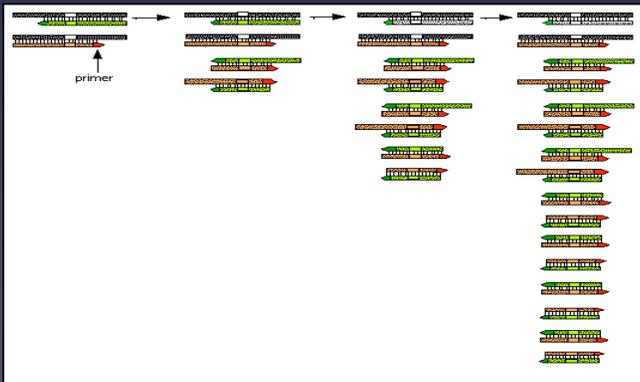


Profile of entire
community

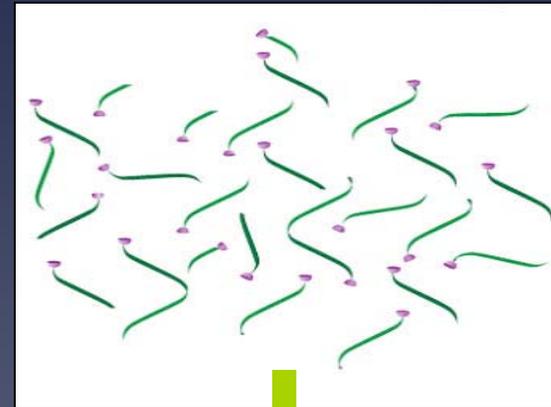


PhyloChip analysis

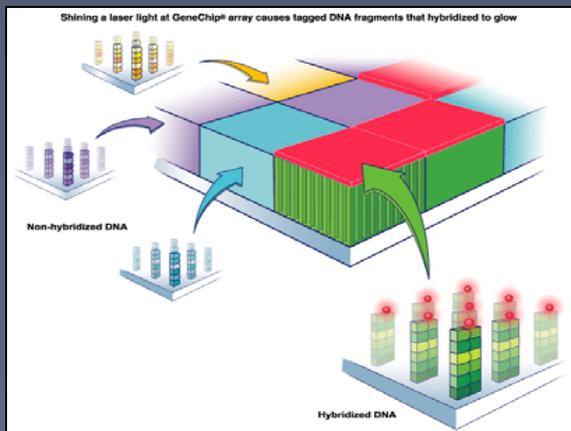
PCR amplification of community 16S rRNA genes



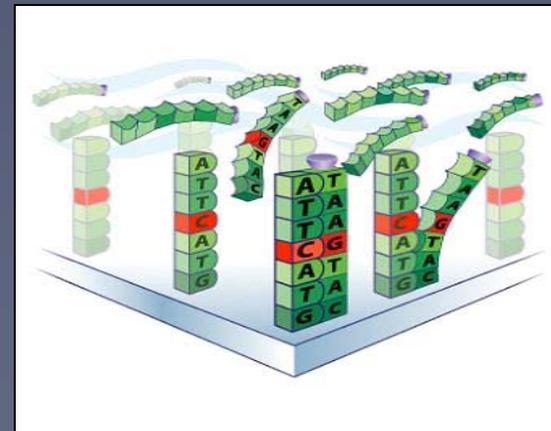
Fragment and biotin label

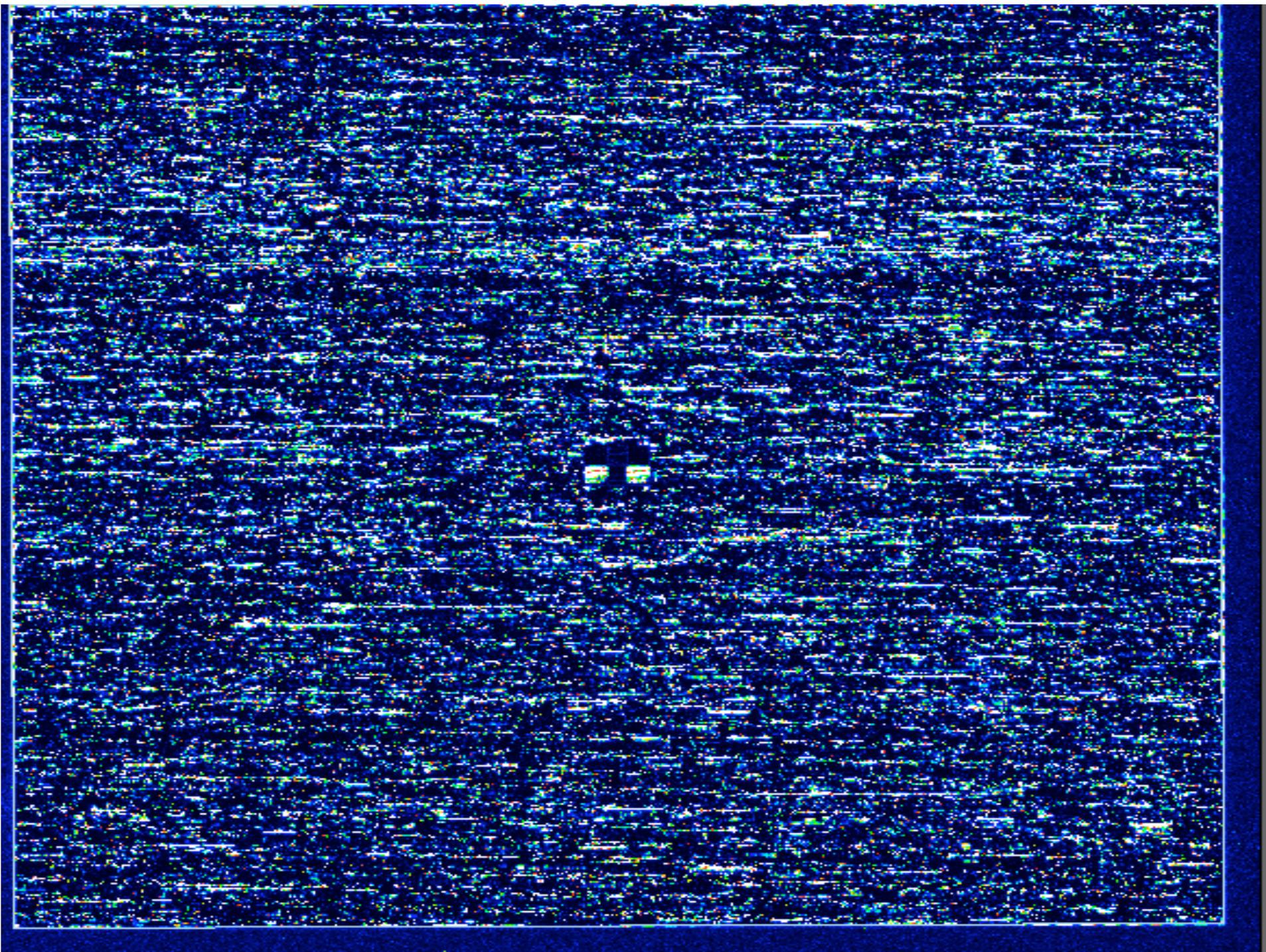


Wash, stain and scan

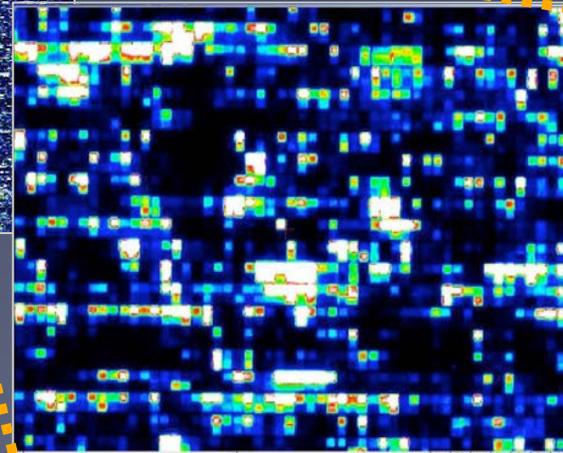
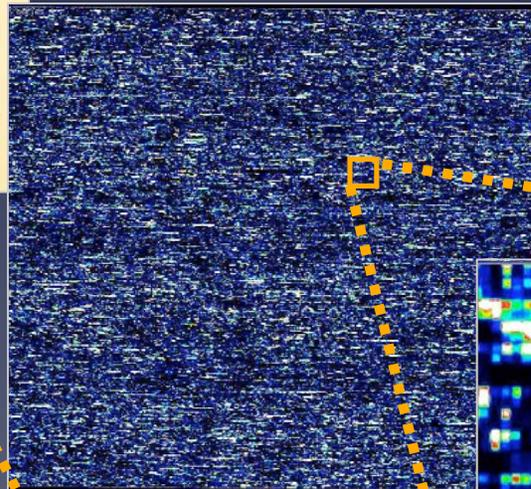


Hybridize to array





Location and intensity of fluorescence determines test results



Reveals occurrence and relative abundance 16S rDNA for 50,000 different bacteria and archaea

Clean Beaches Initiative



Goals

Better understand the fate of pollutant microbial communities in coastal waters

Identify key indicators of different sources of fecal contamination

Create more accurate, sensitive tests for routine testing and source tracking



PhyloChip water quality projects



- Fate of pollutant microbes: What happens to pollutant communities once they enter receiving waters?
- Source characterization: Which bacteria and archaea distinguish human and animal fecal sources?
- Variation in time and space: How do background microbial communities respond to changes in the coastal environment?
- Epidemiology: Which organisms are associated with human illness?

Field test:

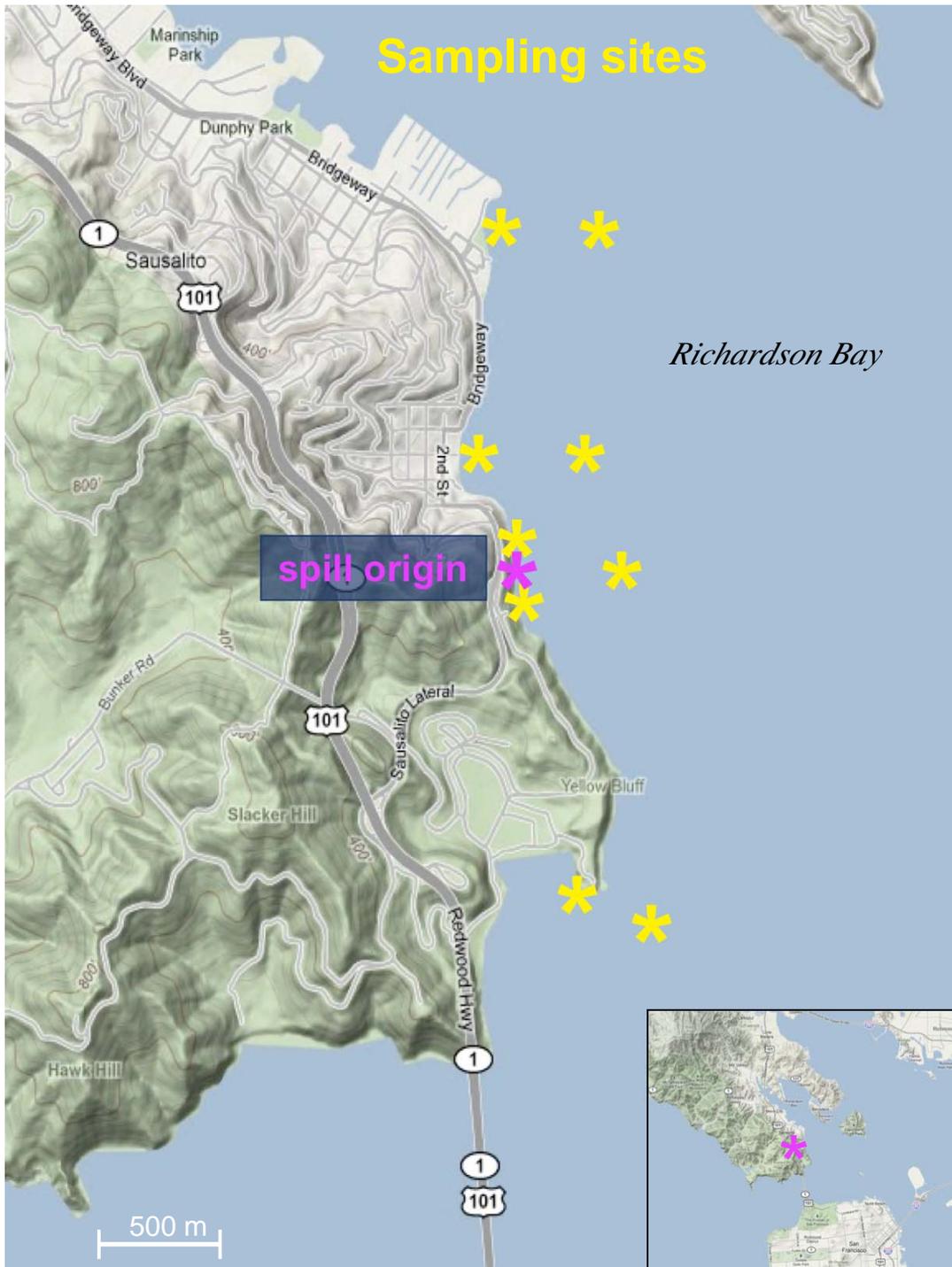
Tracking a 764,000
gallon sewage spill
in Richardson Bay



Kurt Rogers / The Chronicle



Kurt Rogers / The Chronicle



February 2009 spill at Sausalito treatment plant

Sampled 24, 48 and 72 hours after beginning of the spill

Smaller spill 10 days later

8 onshore/offshore locations

DNA analysis with PhyloChip, qPCR

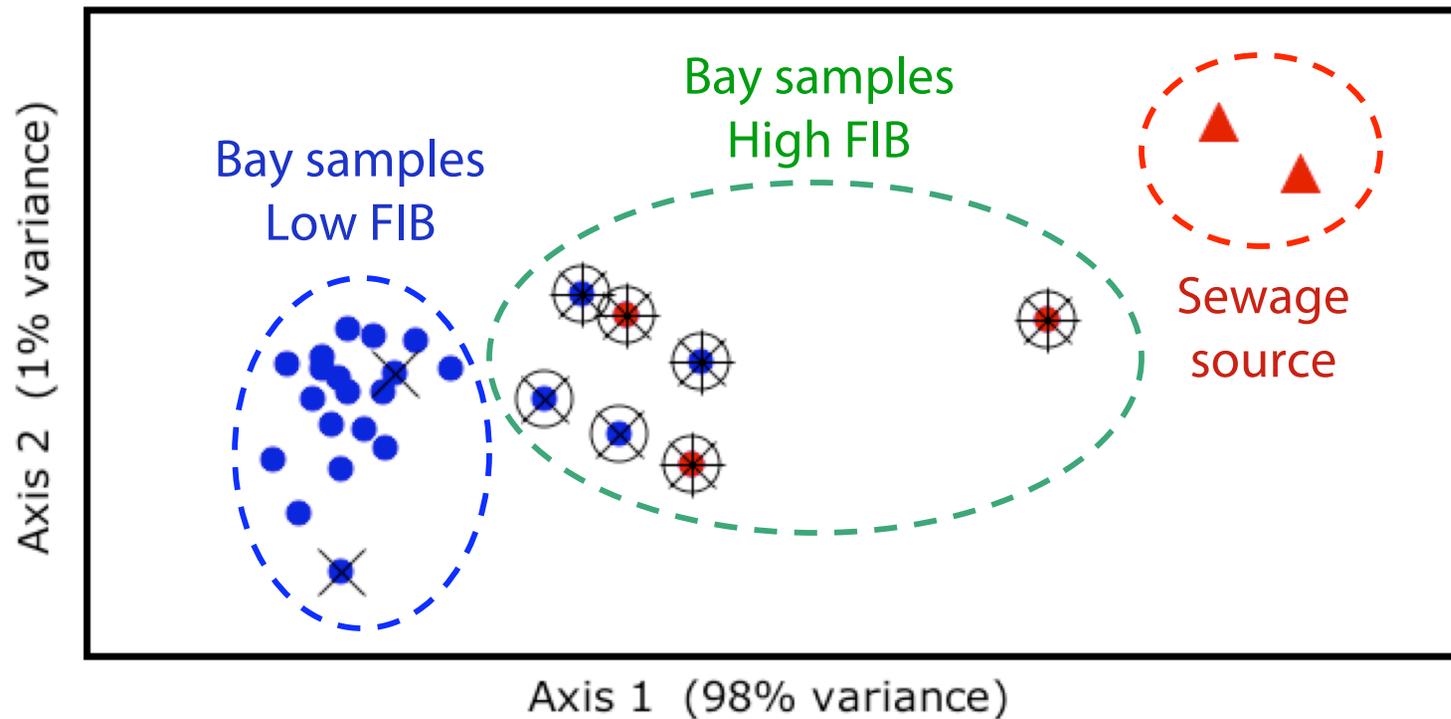
Fecal indicator tests (Enterococci, E. coli, total coliform)

11-day diffusion chamber experiment

PhyloChip Results

Community analysis of 24,748 detected taxa

Nonmetric Multidimensional Scaling ordination

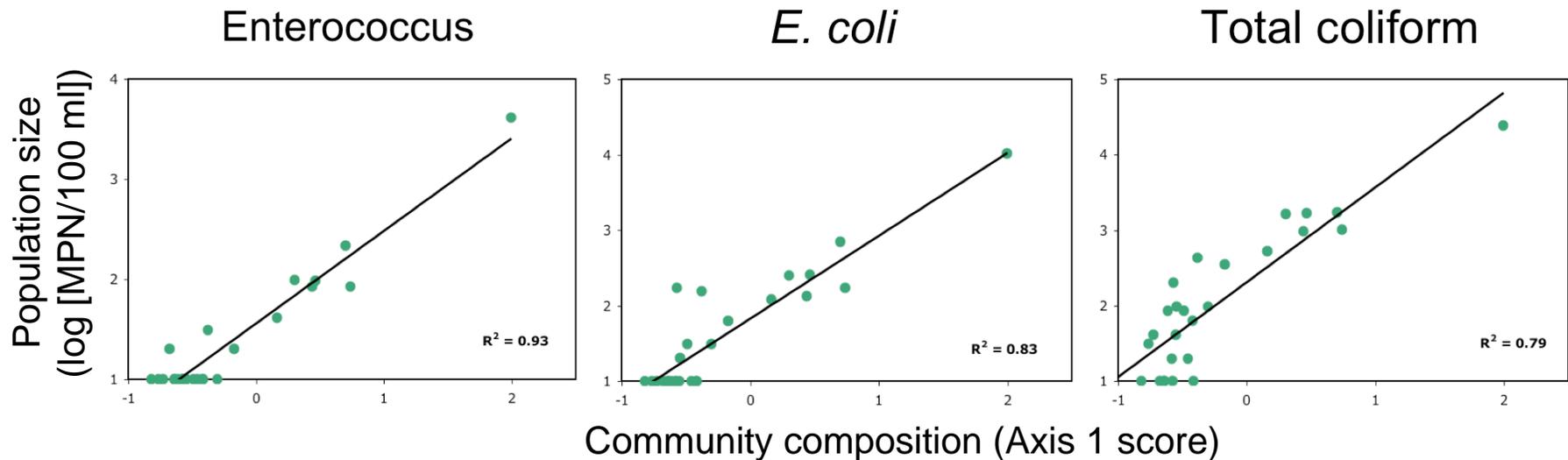


- Meets FIB criteria
- Exceeds FIB criteria
- ▲ Sewage source

- + High Coliform (>1000)
- High Enterococcus (>35)
- × High E.Coli (>126)

PhyloChip Results

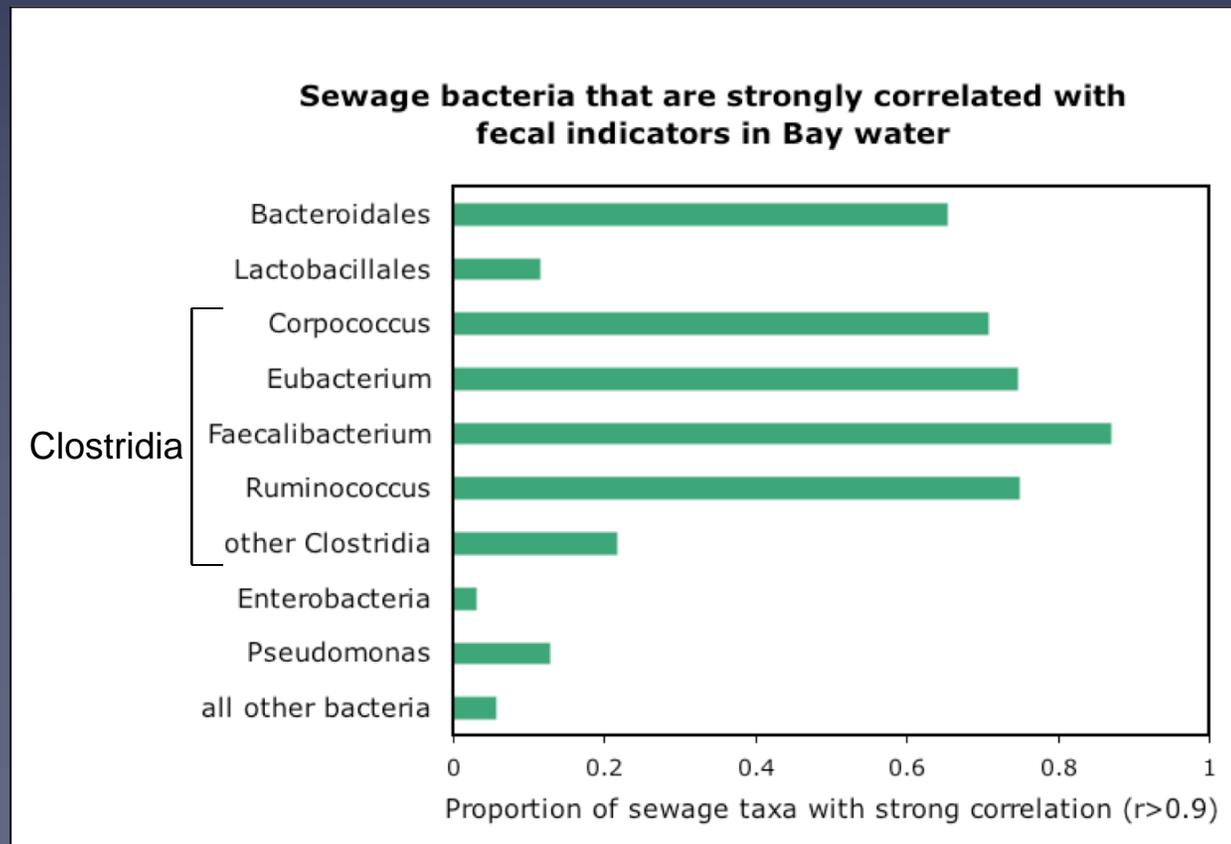
Microbial community composition strongly correlated with fecal indicator tests



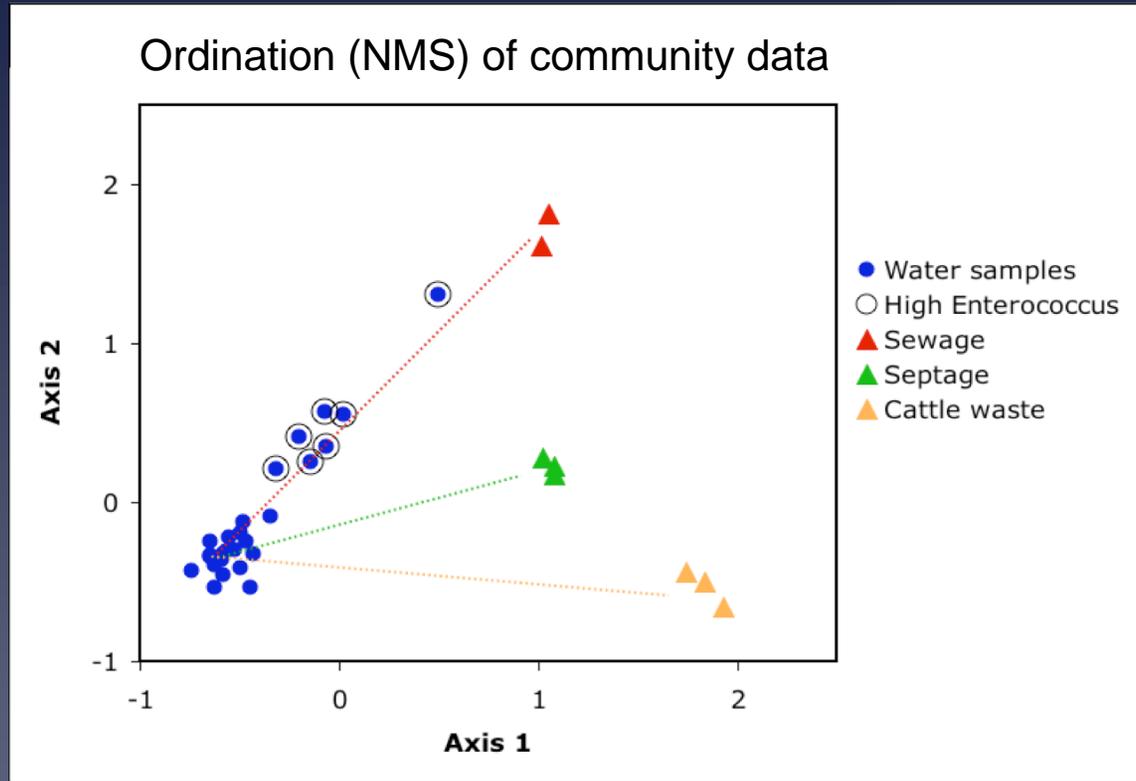
PhyloChip Results

Abundances of 4,625 different taxa found in sewage were strongly correlated ($r > 0.9$) with fecal indicators

Most correlated taxa were Bacteroidales and Clostridia



Polymicrobial Source Tracking



Water samples with high fecal indicators fall along a vector toward the known source community

Illustrates the power of community analysis with microarray to identify the cause of exceedences when the source is unknown

Diffusion chamber experiments

What is the fate of microbial communities from different fecal wastes in fresh and marine waters?

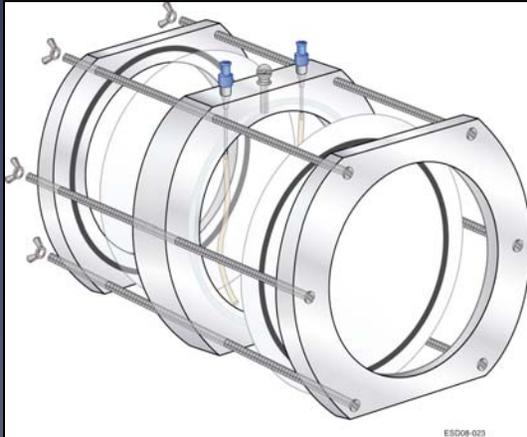


Photo credits: Janet Fang

A "cage" for microorganisms: holds captive anything greater than $0.2 \mu\text{m}$ in size. Permeable to water, molecules in solution

Monitor abundance and activity of all bacteria and archaea over time upon immersion into receiving waters

Experiment: fate of cattle waste and septage in fresh and marine waters

Freshwater
(Walker Creek)

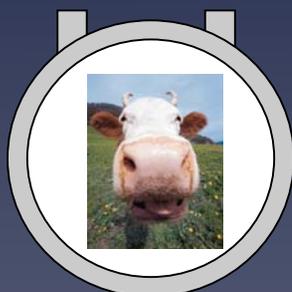


x 3



x 3

Saltwater
(Tomales Bay)



x 3



x 3

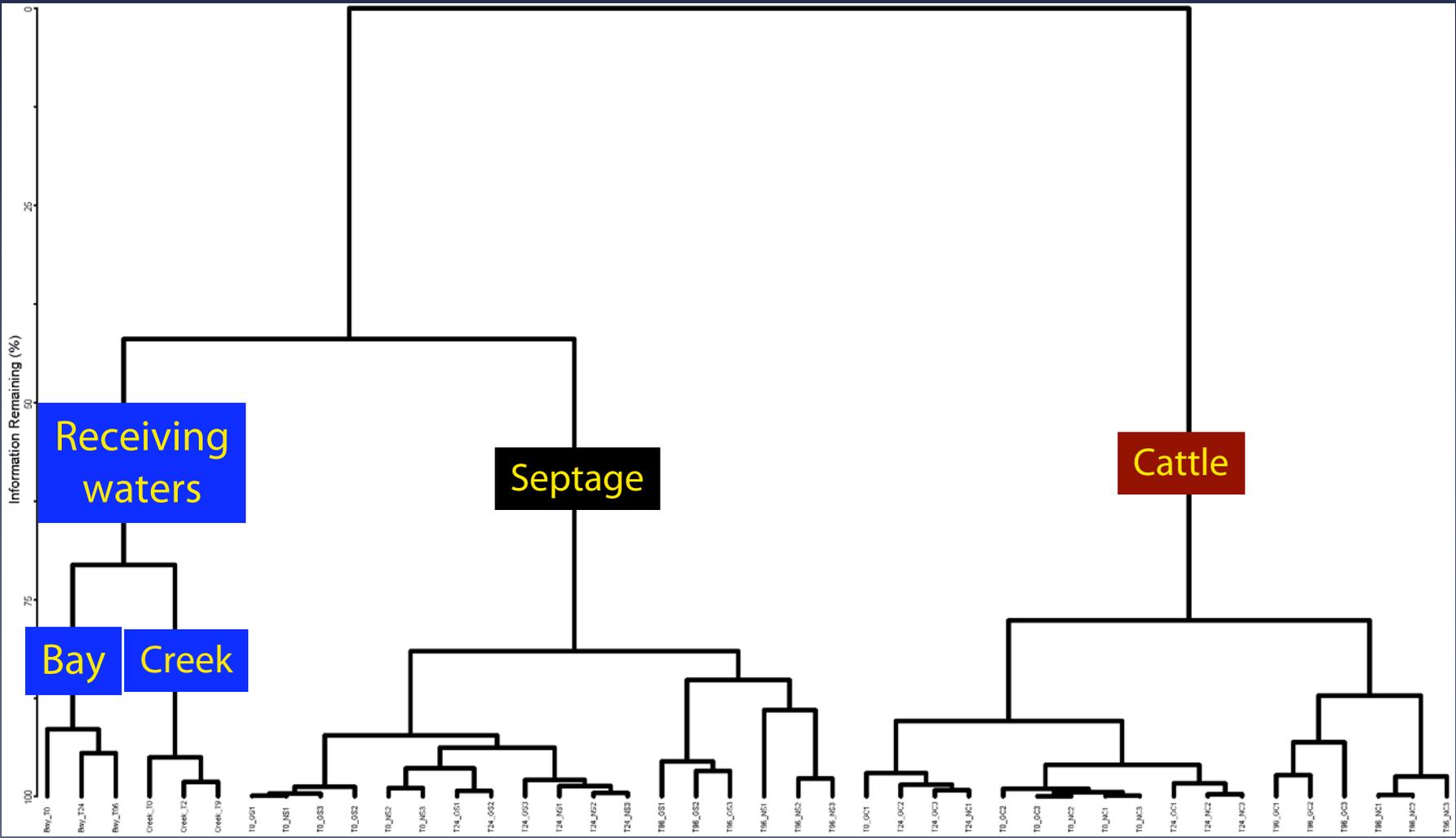


Sampled at 6 time points:
0, 1, 12, 24, 48, 96
hours after immersion

Vacuum filtration through 0.2 μm filters
Frozen on dry ice for DNA/RNA extraction
Unfiltered sample for direct counts

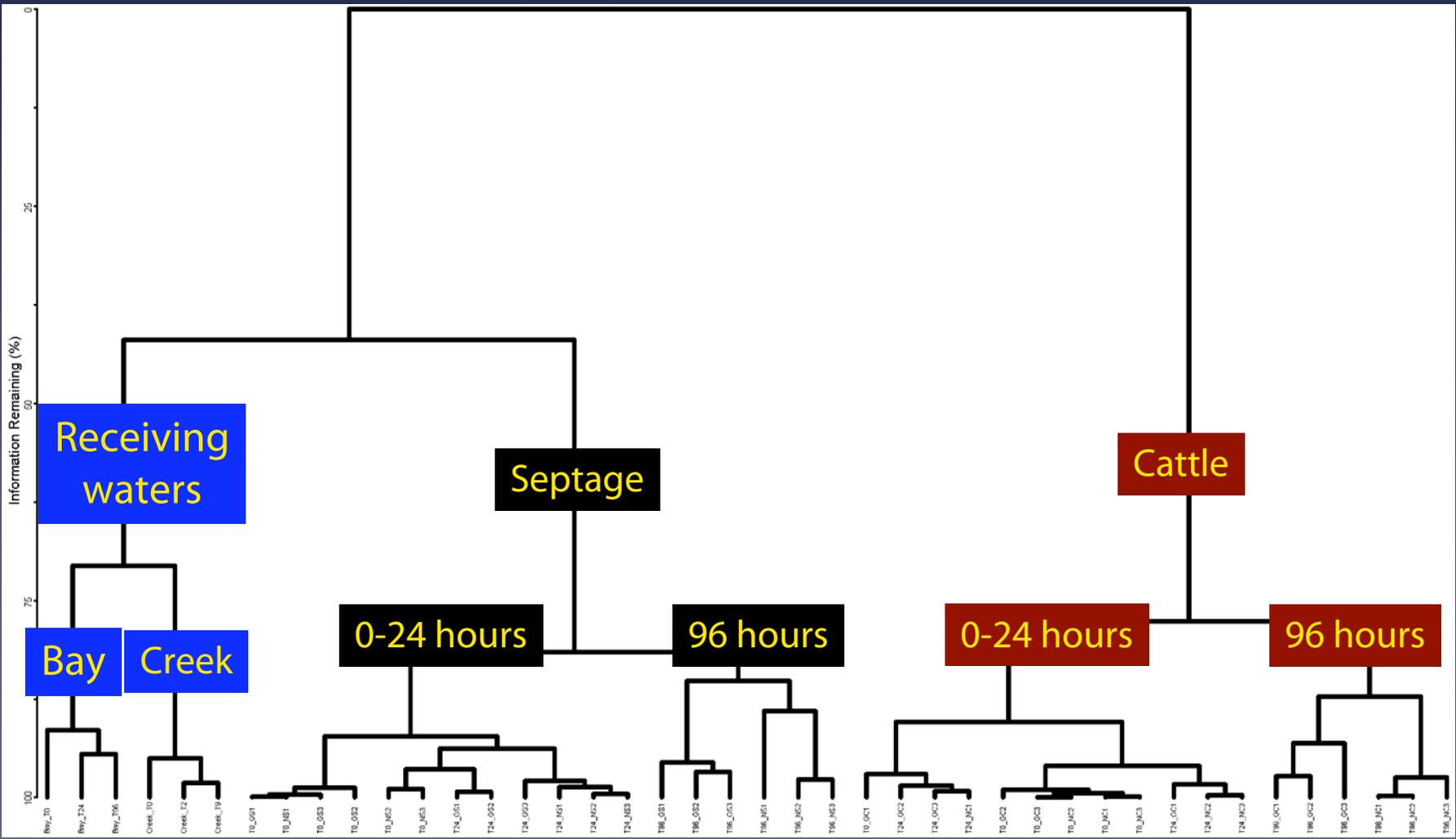
Comparison of diverse communities

Cluster analysis



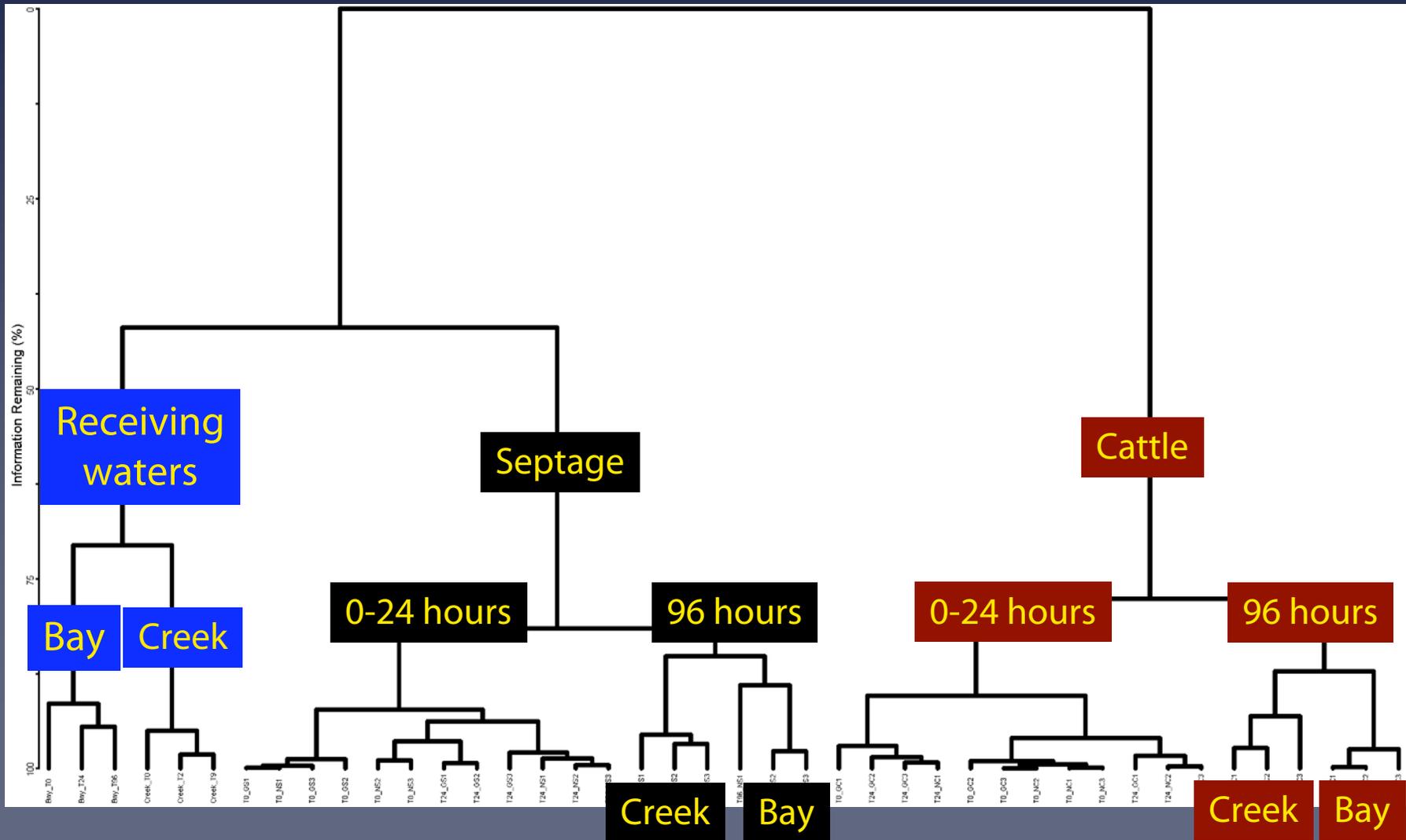
Comparison of diverse communities

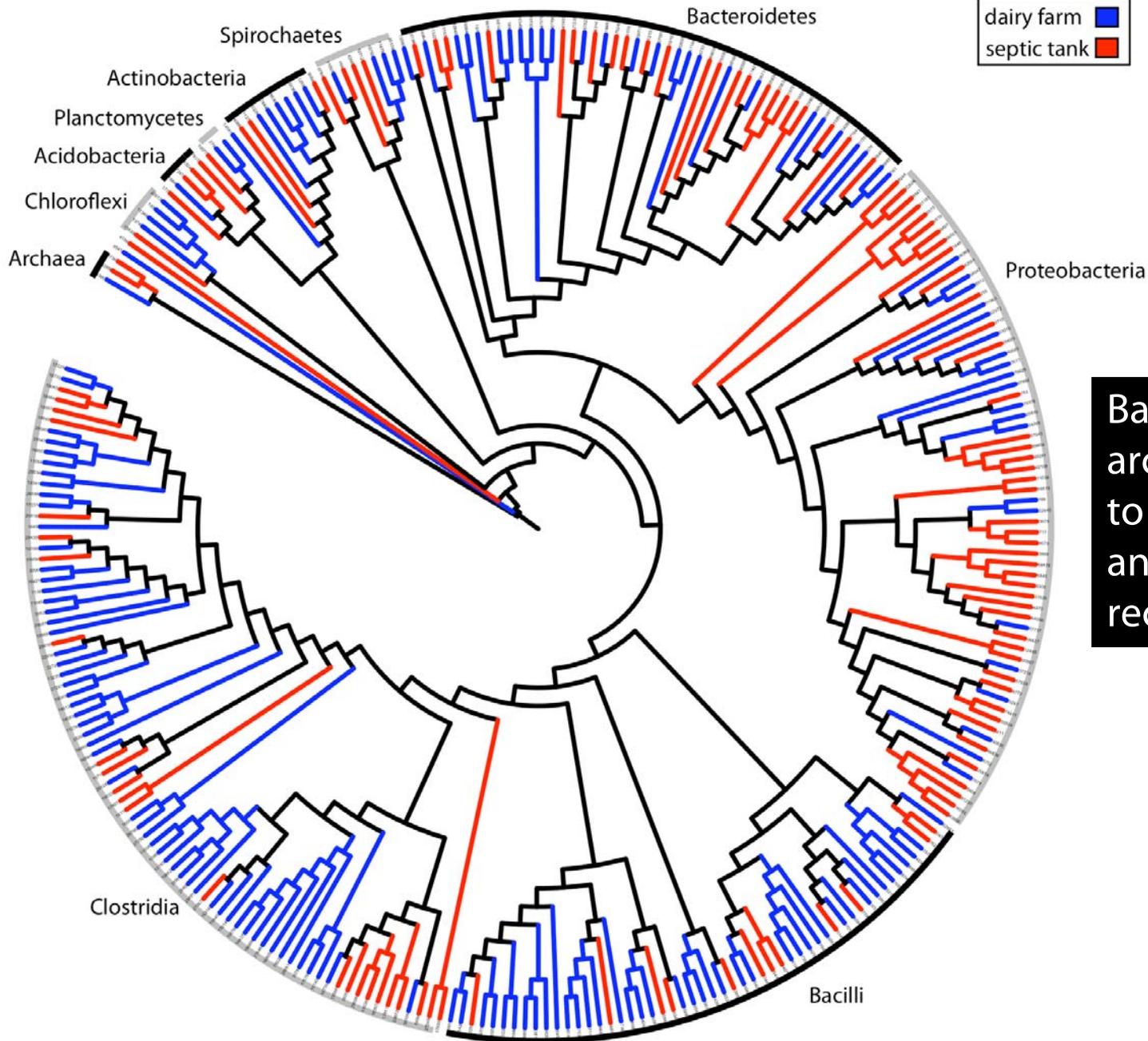
Cluster analysis



Comparison of diverse communities

Cluster analysis





Bacteria and archaea unique to each waste and absent from receiving waters

Effect of time in receiving waters on waste microbial communities

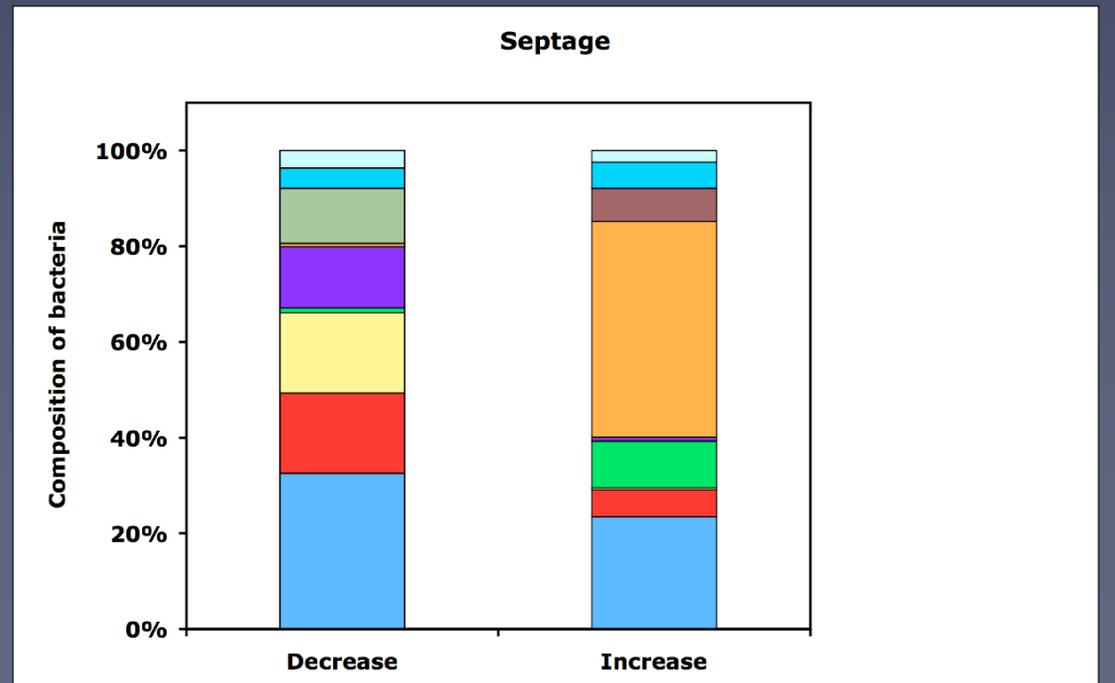
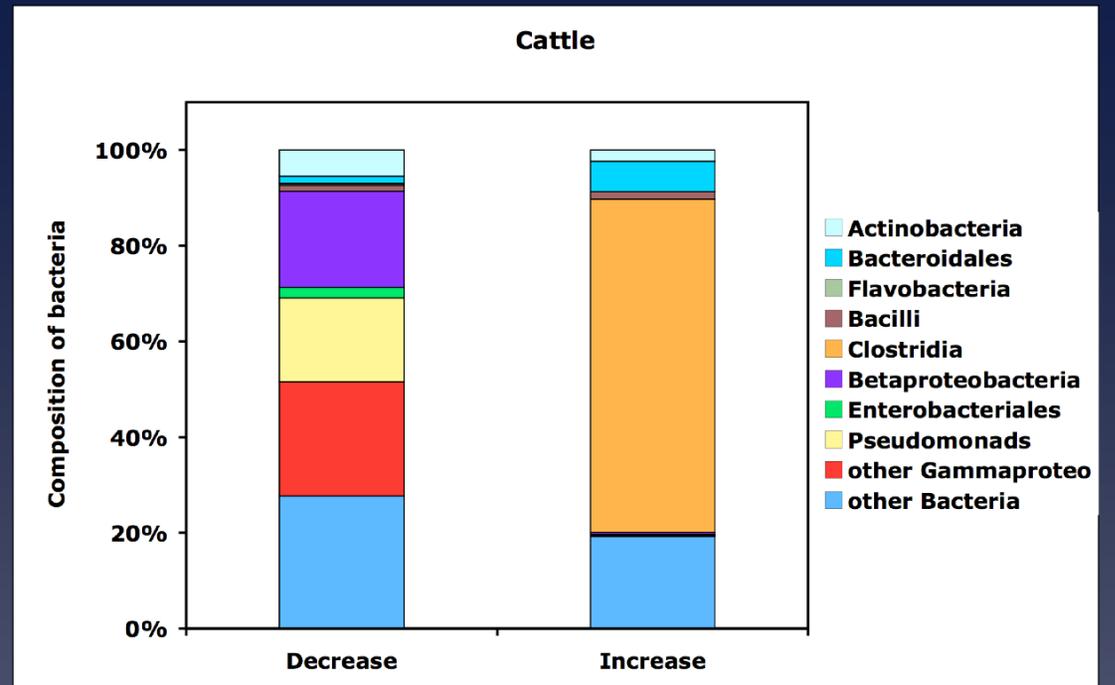
Four day immersion

Distinct differences in survivability among taxonomic groups

Most proteobacteria decrease in relative abundance in both wastes

Clostridia persist

Some bacilli (Enterococcus) and coliforms in septage persist



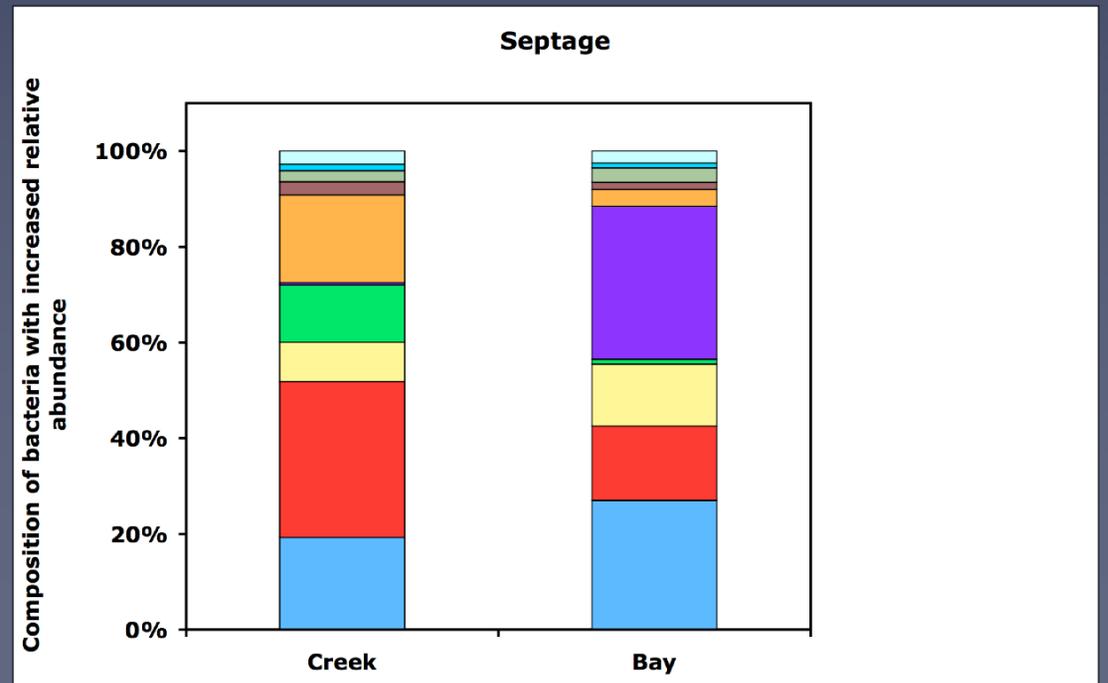
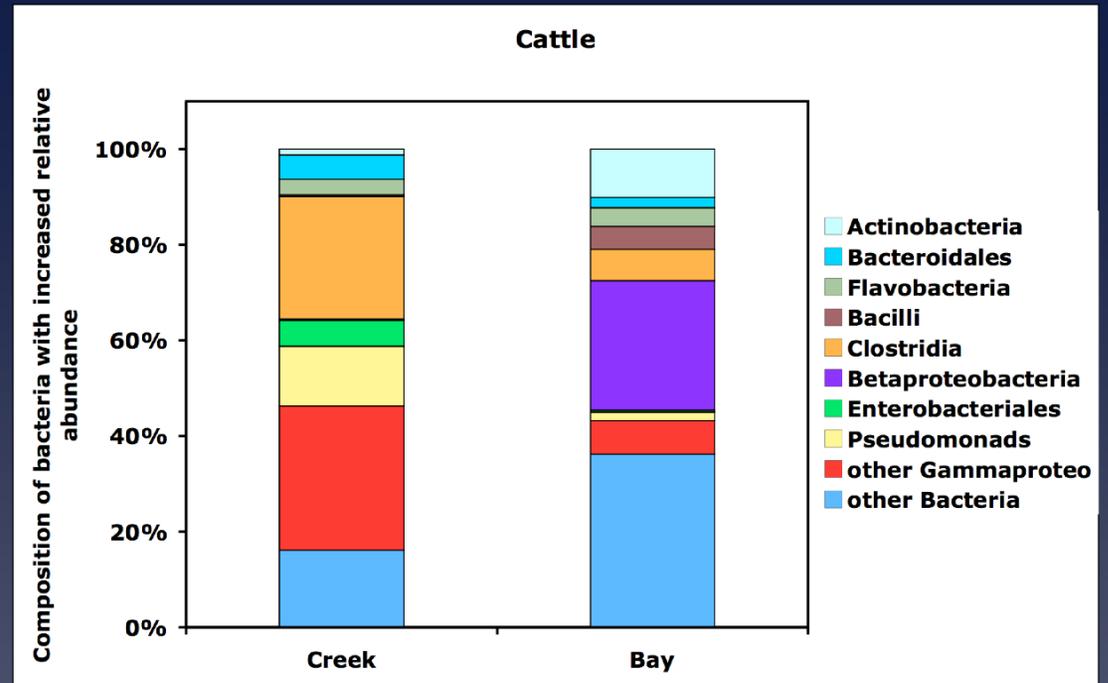
Composition of fecal bacteria that persist in creek versus bay water

Water type has a effects which fecal bacteria persist over time

Similar response of cattle and septage communities

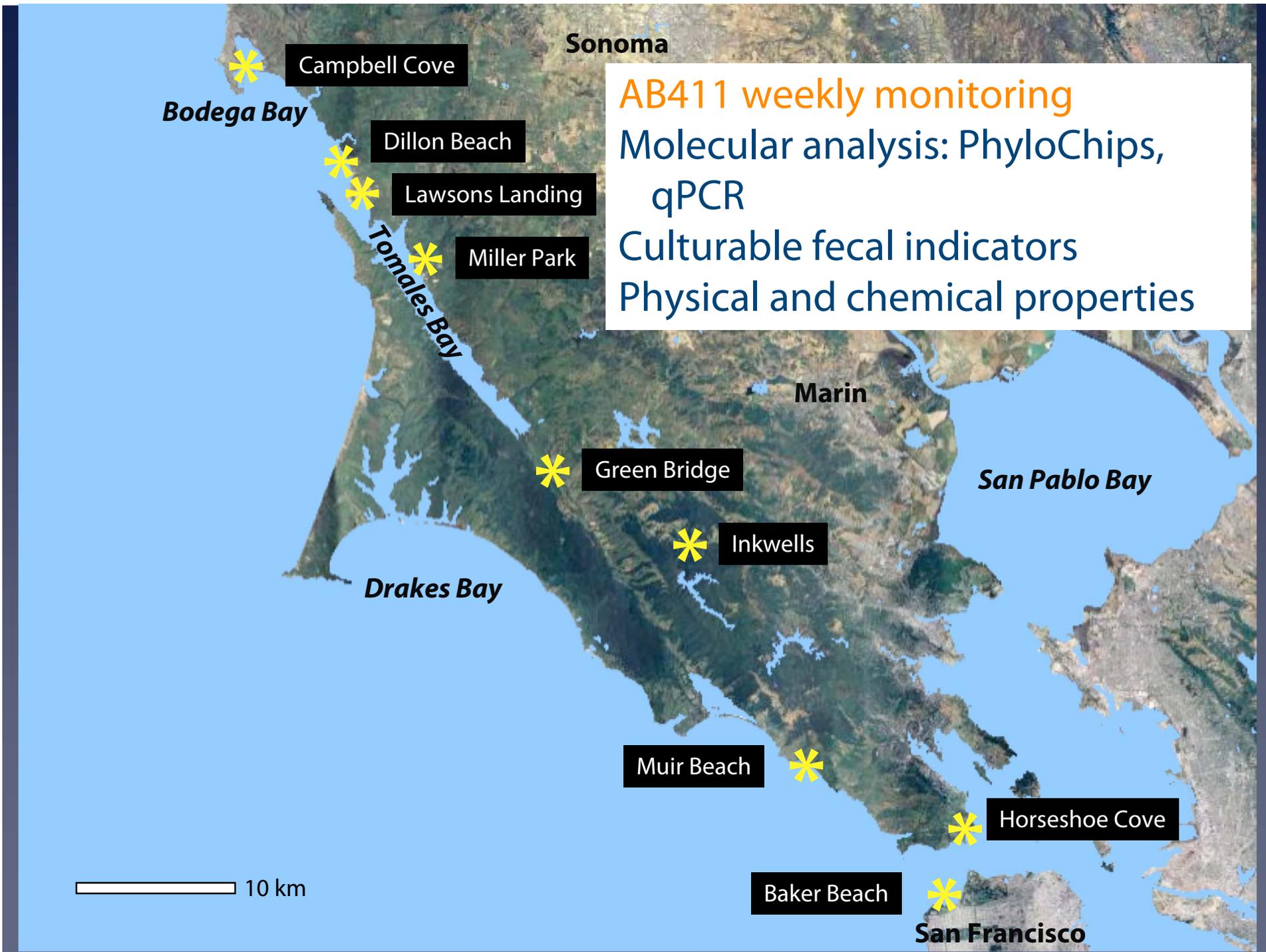
Clostridia, γ -proteobacteria, coliforms favored in creek

β -proteobacteria favored in Bay



Ongoing work: characterization of microbial communities in potential fecal sources





Summary

PhyloChip detects most known bacteria and archaea in a single test

Enables comprehensive, high resolution surveys of microbial communities

Opportunity to gather much needed information about microbial communities in pollutant sources and receiving waters

Potential to identify new sets of indicator taxa to better track coastal pollution

Thank You

Shariff Osman, Cindy Wu, Yvette Piceno, Bonita Lam,
Todd DeSantis

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Counties of Marin, Sonoma and San Francisco

California State Water Resources Board: Clean Beaches
Initiative

Rathmann Family Foundation

