The Impact of Climate Change on Soil Microbial Communities and Their Feedback to the Environment

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http://greengenes.lbl.gov

Outline

- Introduction: Microbes as climate change indicators
- Research objectives, hypothesis, and project design
- Part I: Changes in microbial communities in due to rainfall
- Part II: Possible implications of change in *Actinomycetales* on environmental health
- Part III: Possible implications for human health
- Summer research
- Conclusions
- Future work
- The value of my LBL experience

Microbes?

I thought climate change was all about oceans and Al Gore.

- Understanding ecosystem processes and interactions from the ground up.
- Climate change scenarios and predictions.
- Reactions to microbes in climate change conditions.

Research Objectives

- Brodie/Andersen lab:
  - Connect changes in microbial communities to changes in the ecosystem.

- Individual project:
  - Determine if bacteria populations significantly change between rainfall treatments.
    - From these, determine how many are in *Actinomycetales*.
  - Catalogue antibiotic resistance genes in *Actinomycetales* and screen soil

Conceptual Hypothesis Diagram

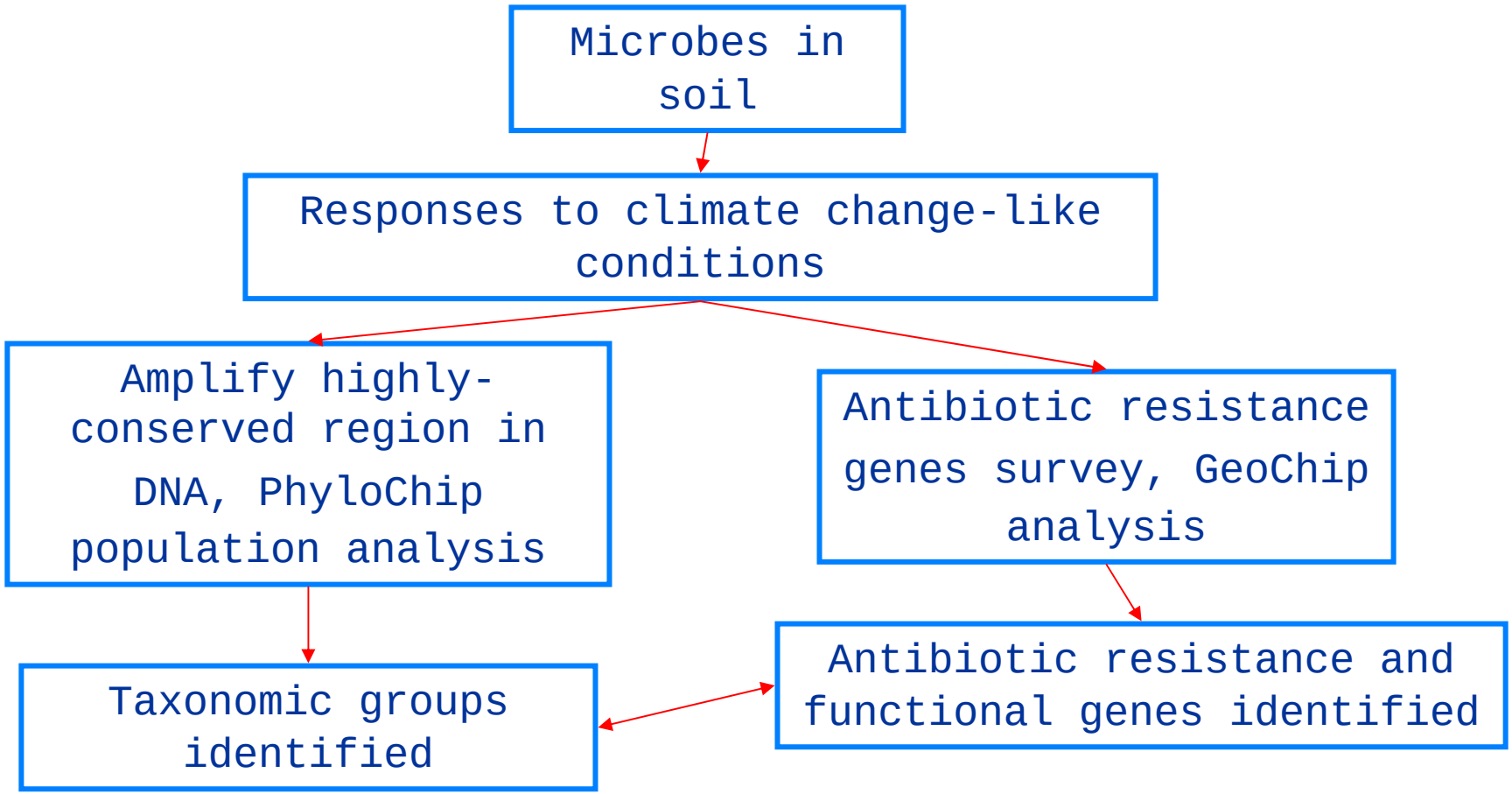
Rainfall influences activity of *Actinomycete* populations.

Actinomycetes are reservoirs of antibiotic resistance genes.

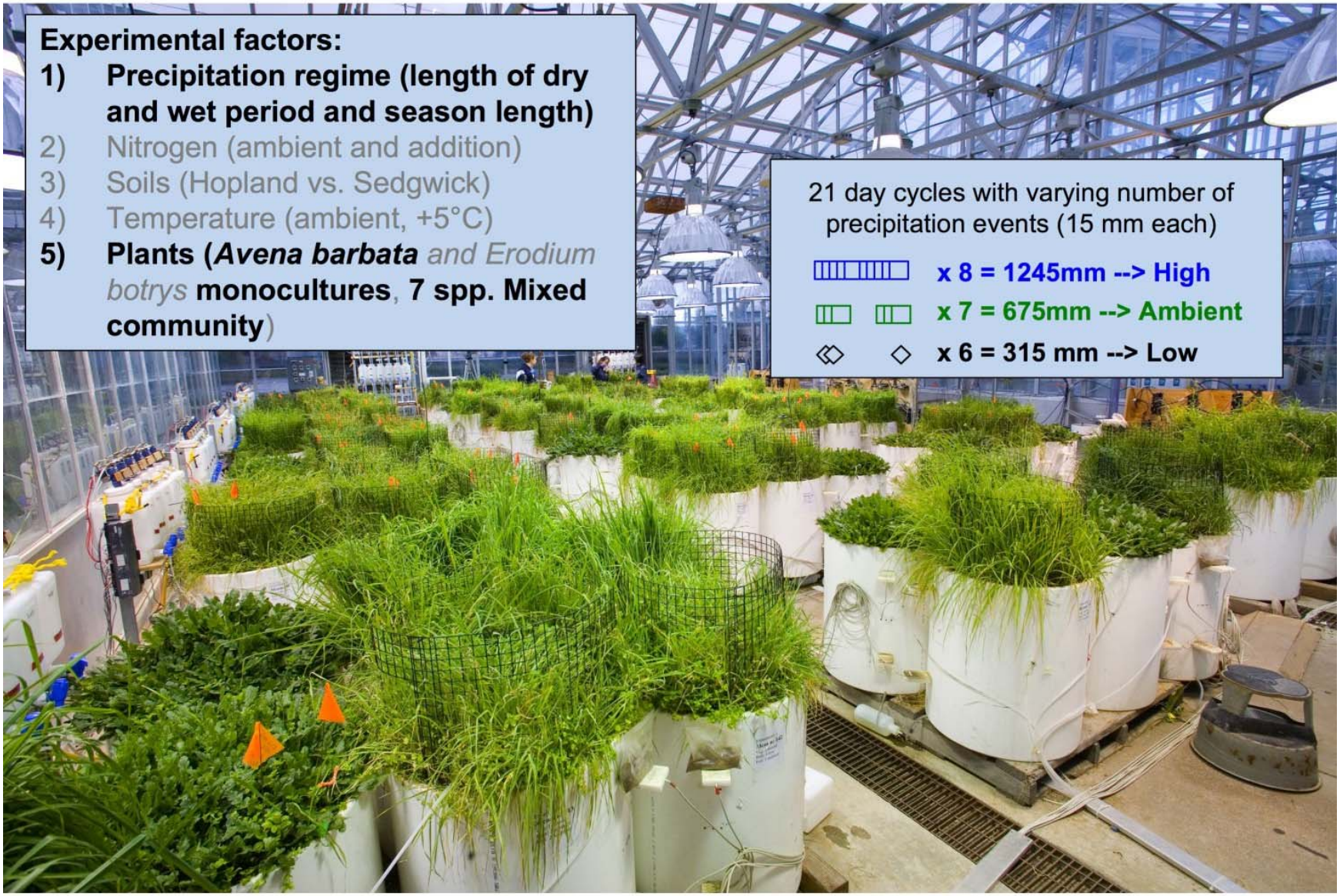
Under warm, dry conditions, *Actinomycetes* can become aerosolized.

Aerosolization of these bacteria could lead to a spread of antibiotic resistance genes.

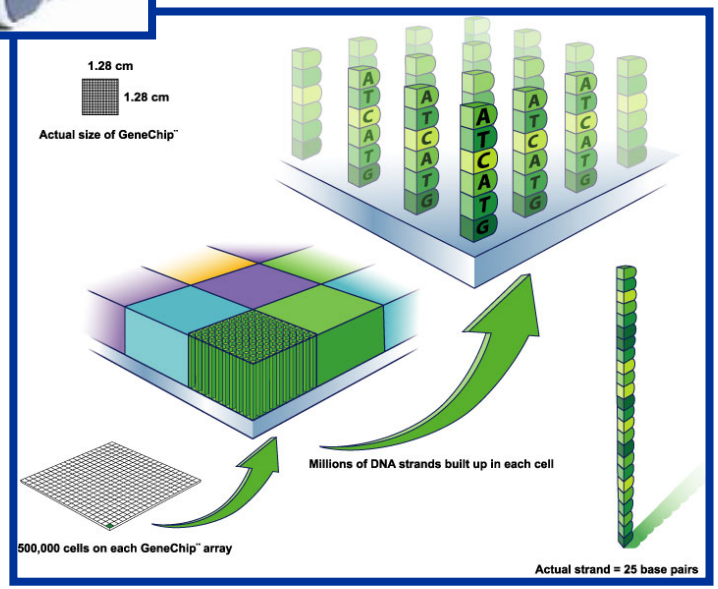
Part I: Changes in Microbial Communities due to Rainfall



Interdisciplinary Mesocosm Study



PhyloChip Microarray



- Multiple simultaneous hybridizing experiments.
- 500,000 unique probes (300,000 target 16S rRNA gene).
- ~ 9,000 taxa.
- Fluorescently labeled DNA in sample finds and hybridizes to its matching (complement)

Photos courtesy of Dr. Eoin L. Brodie, Lawrence Berkeley National Laboratory

Part I: Methods and Materials

Soil sample collection

1. Mesocosms were sampled at two time points during rainfall manipulation experiment (low, ambient, and high).

DNA extraction and microarray preparation

1. 16S rRNA gene from bacteria and archaea was amplified using primers 27F (bacteria), 4F (archaea), and 1492R (both)(Invitrogen, Carlsbad, CA).
2. DNA from samples was extracted using the PowerMax™ Soil DNA isolation kit (MoBio, Carlsbad, CA).
    1. Bacterial 16S was amplified in a 96-well PCR plate with a range of annealing temperatures between 48-58°C per row.
    2. PCR products were concentrated prior to microarray preparation.

Microarray preparation, washing, scanning, and analysis

1. Concentrated PCR product was fragmented, biotin labeled, and hybridized to 15 individual PhyloChips for 16 hours.
2. PhyloChips were washed, stained, and scanned.
3. Data was exported and statistically analyzed.

Data Analysis

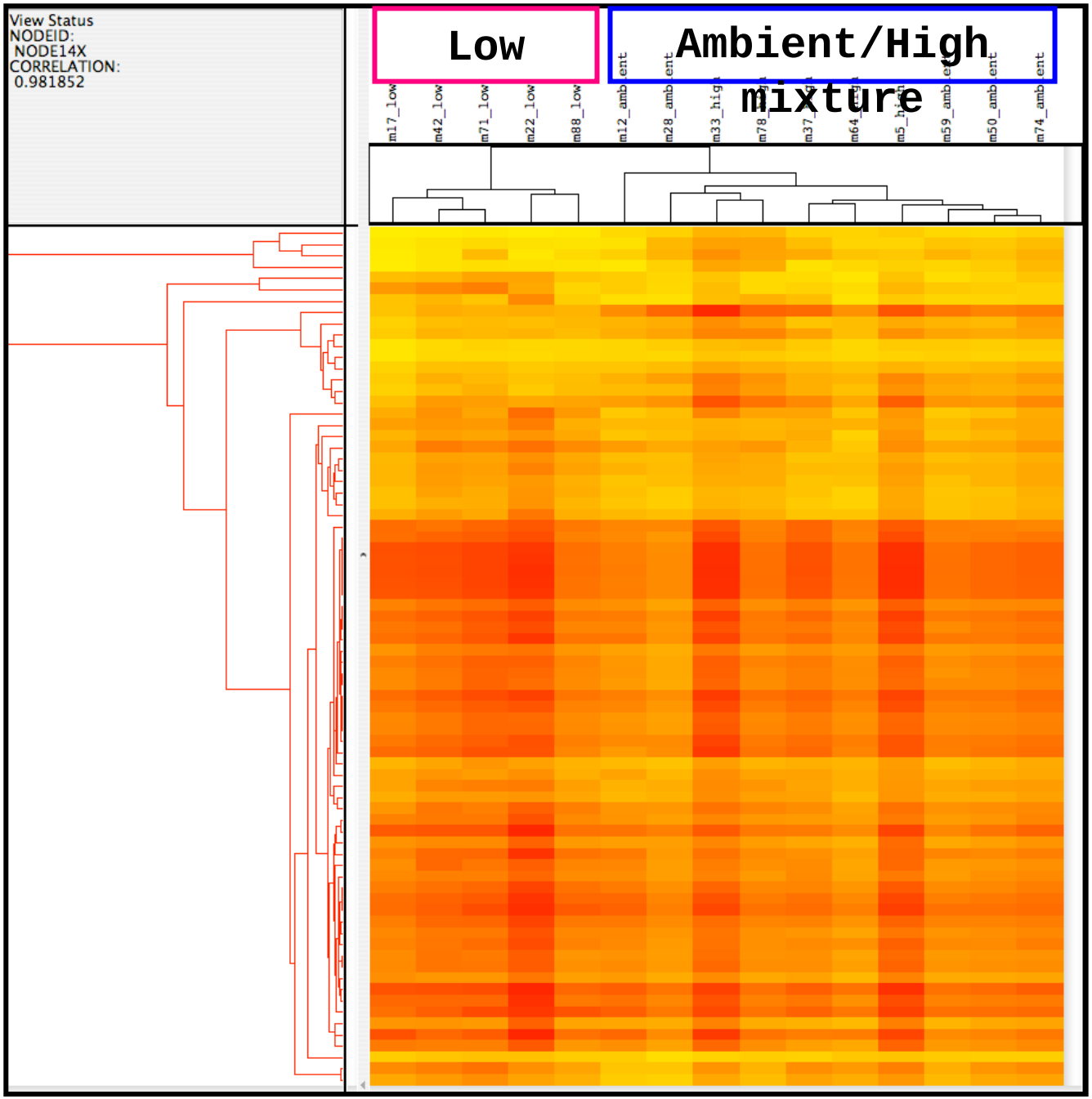
- To identify microbes that significantly changed in response to rainfall treatments, the following statistical analyses were performed:
  - 3-way raw intensity ANOVA (high/low/ambient).
  - 2-way ANOVAs (high/low, high/ambient, low/ambient).
- To visualize these changes, cluster analysis was performed using Cluster 3.0 and Java TreeView.

Results

- 2382 of 8741 taxa identified; 27.3% of all possible.

Table 1.1. ANOVA significance results showing *Actinomycete spp.* responses to variable rainfall.

Analysis type	Total Significant ($p<0.05$)	Percent *Actinomycetes spp.*
3-way ANOVA	39	12.8
2-way ANOVA *high/low*	38	5.26
2-way ANOVA *high/ambient*	23	0.00
2-way ANOVA *low/ambient*	76	27.6



- Bacteria communities, specifically populations of *Actinomycete spp.*, respond in significantly different ways to low versus ambient and high rainfall.

- In ANOVA low/ambient comparison, over one quarter of responsive bacteria were *Actinomycete spp.*

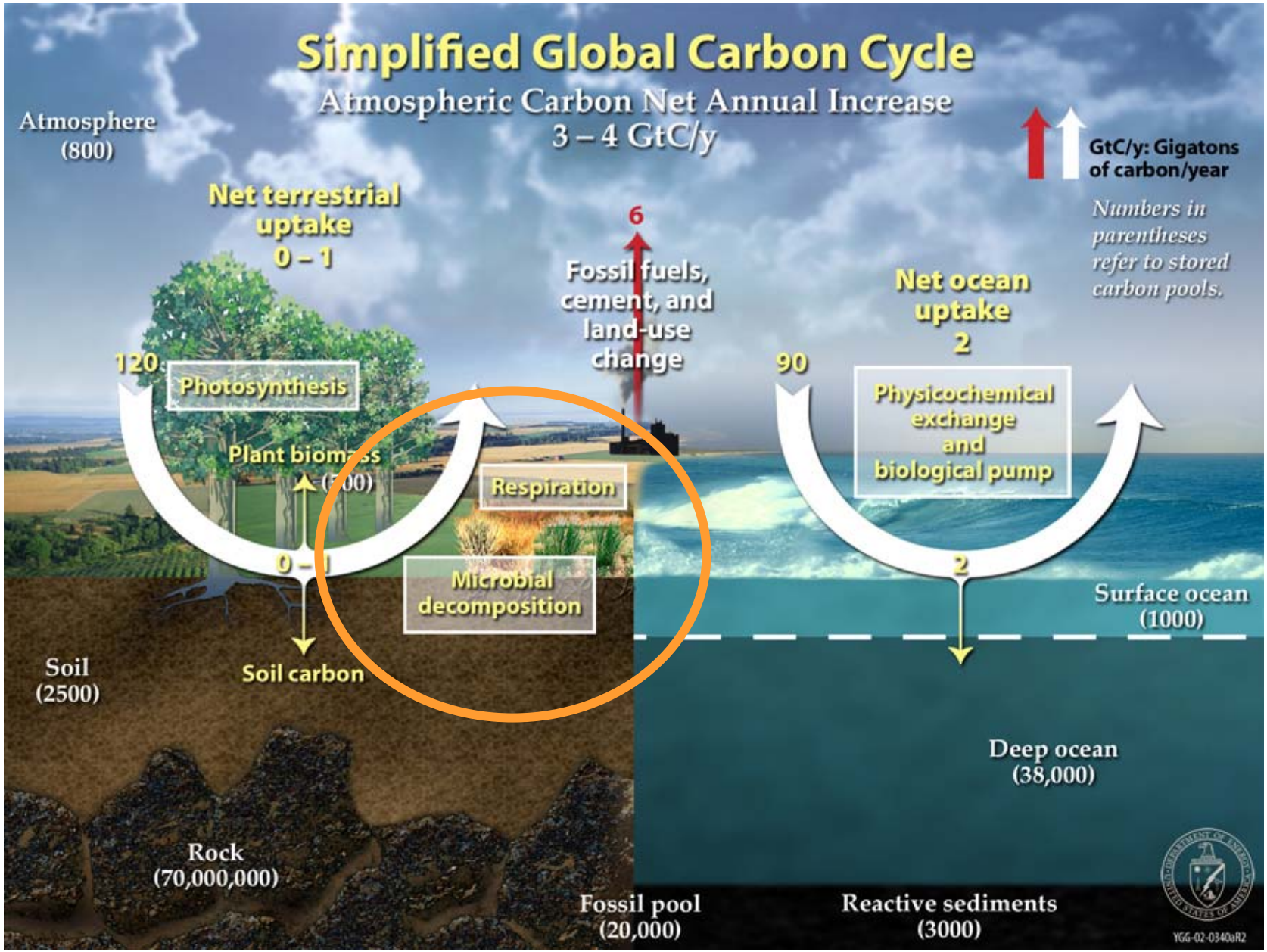


Image courtesy of Dr. Eoin L. Brodie, Lawrence Berkeley National Laboratory

Part II: Possible Implications of Change in *Actinomycetales* on Environmental Health

- Actinomycetes degrade cellulose and chitin in soil.
  - Carbon cycling implications:
    - Increased rainfall = lower *Actinomycete* abundance.
    - Decreased rainfall = higher *Actinomycete* abundance.
  - Depending on which climate change scenario is accurate, degradation of cellulose and chitin in the soil could either increase or decrease.
- GeoChip: Functional microarray analysis.
  - Did genes involved in cellulose and chitin degradation also increase or decrease?

Part III: Possible Implications for Human Health

- Aerosolization of *Actinomycetales* under warm, dry conditions (Brodie *et al.*, 2007).
- Antibiotic producers.
  - Proposed as origin of antibiotic resistance.
- Conditions that result in aerosolization of *Actinomycetes* could also result in aerosolization of antibiotic resistance genes.

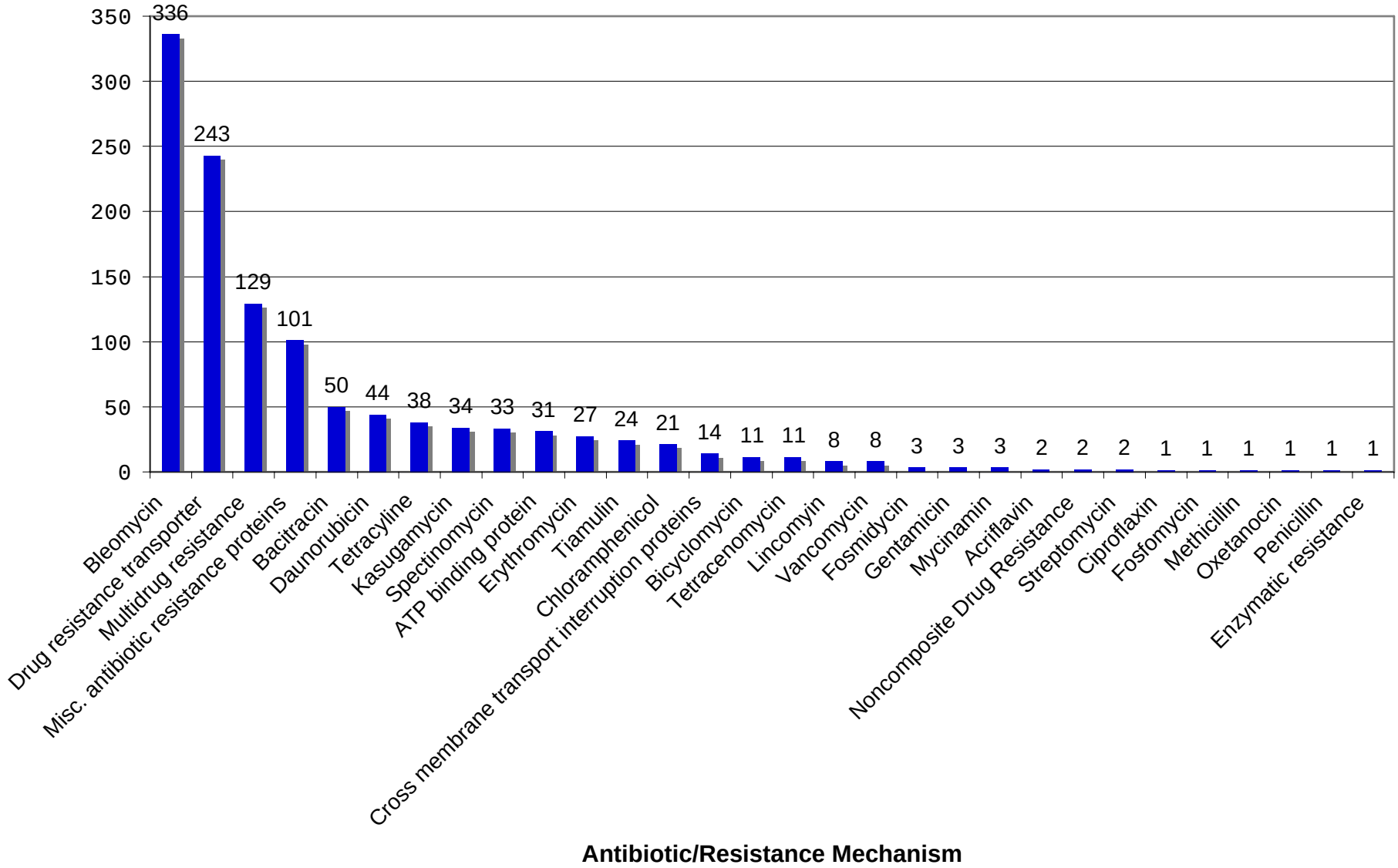
Questions We Are Asking

- Does climate change impact the aerosolization of *Actinomycetales*?
- Could climate change contribute to the spread of antibiotic genes?

My Summer Research

- Catalogued antibiotic resistance genes/proteins in *Actinomycetales.*
  - Literature searching.
  - NCBI database searching.
  - Key antibiotics:
    - Methicillin (MRSA)
    - Vancomycin *S. epidermidis* (VRSE) and Vancomycin Resistant Enterococcus (VRE)
- Primer discovery in literature and evaluation.
- Primer testing.
  - Primer-BLAST, Primer3.
- Screening of soil samples for antibiotic resistance genes.

Surveyed Antibiotic Resistance Mechanisms in Order Actinomycetales



- 22.7% of the *Actinomycetales* that responded significantly to low/ambient conditions have genes that confer antibiotic resistance.
  - 6.58% of all significantly-responding bacteria populations had antibiotic resistance genes.
- Proposed to have resistance to the following antibiotics:

Antibiotic/Mechanism	# of Proteins
Bacitracin	2
Bleomycin	18
Chloramphenicol	1
Daunorubicin	2
Erythromycin	1
Kasugamycin	2
Methicillin	1
Spectinomycin	1
Tiamulin	1
Drug resistance transporter	12
Misc. antibiotic resistance proteins	1
Multidrug resistance	1
TOTAL:	**43**

Table 1.2. Type and number of *Norcardiaceae* and *Nocardioidaceae* resistance mechanisms.

Conclusions

This 9-week investigation found:

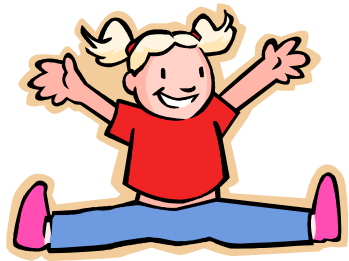
- Soil bacteria change in response to climate manipulations (rainfall).
- Of these soil bacteria, *Actinomycetes* constitute a group of organisms whose relative abundance significantly changes when climate is altered.
- Specific species of *Actinomycetes* detected by PhyloChip are known to contain antibiotic resistance genes.

Future Work

1. **Testing antibiotic susceptibility:**
    1. Isolate genes from soil samples.
    2. Transform genes into antibiotic susceptible organisms.
    3. Test for antibiotic resistance proving that genes confer resistance.
2. **Field component:**
    1. Integrate rainfall treatment in the field.
    2. Sample aerosols directly above plots.
3. **Chip information integration:**
    1. Integrate GeoChip (functional) and PhyloChip (phylogenetic) data.

The Value of My LBL Experience

- Molecular biology =



- Pursue PhD in microbial ecology.
- Exposure to and involvement in scientific advancements beyond the undergraduate mindset, classroom, and laboratory.
- 1+ papers!

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Questions?

At the molecular biology exhibit at San Francisco’s Exploratorium, August 2008.