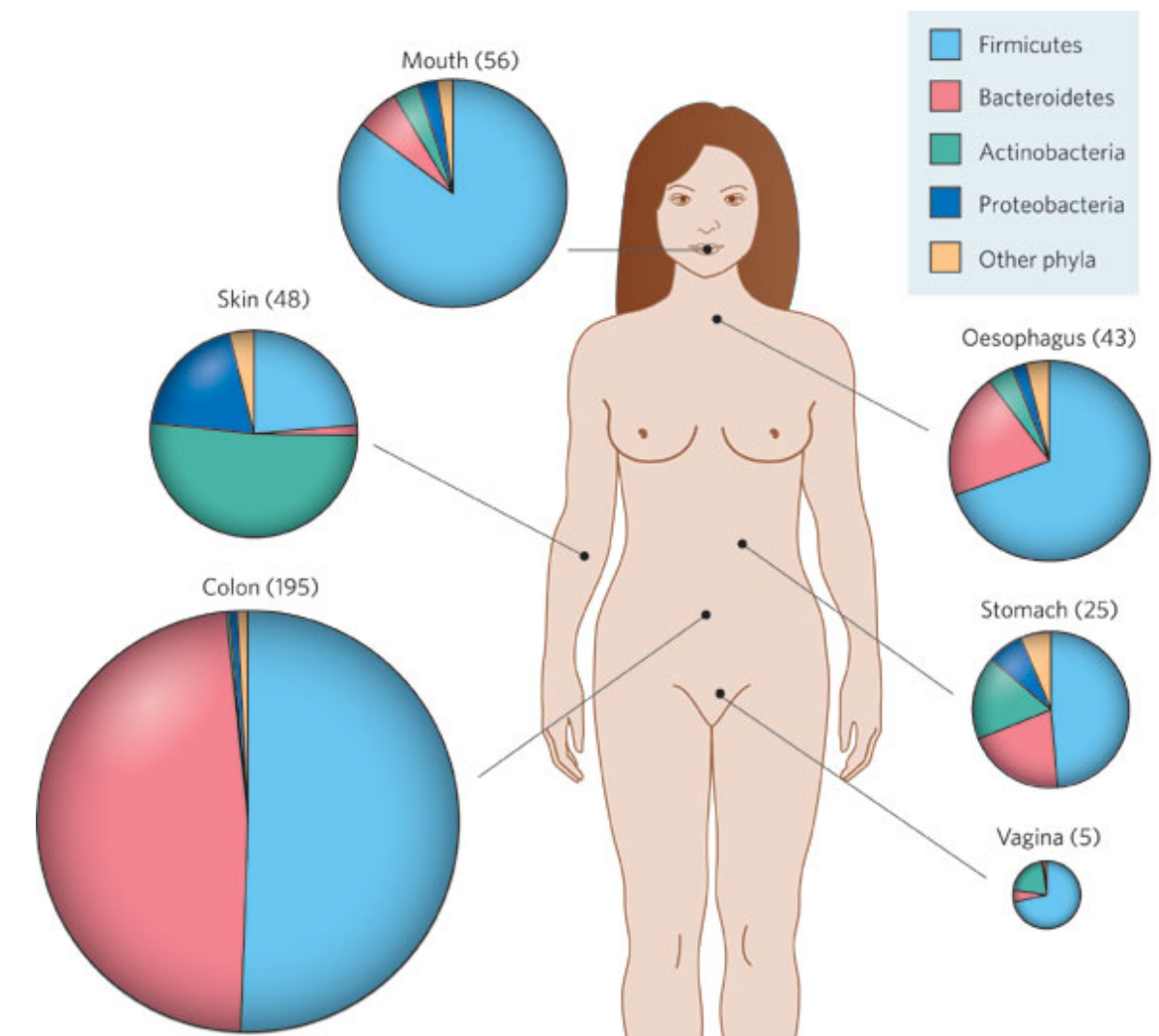


Stability and Resilience in the Human Microbiome: Response of Gut Microbiota to Colonic Lavage

Laurie Rumker, Dr. Les Dethlefsen, Dr. David Relman
Department of Microbiology and Immunology, Stanford University School of Medicine

Microbiota and the Human Body Ecosystem



Body Sites and Predominant
Taxa of the Microbiota

(Dethlefsen and Relman, 2007)

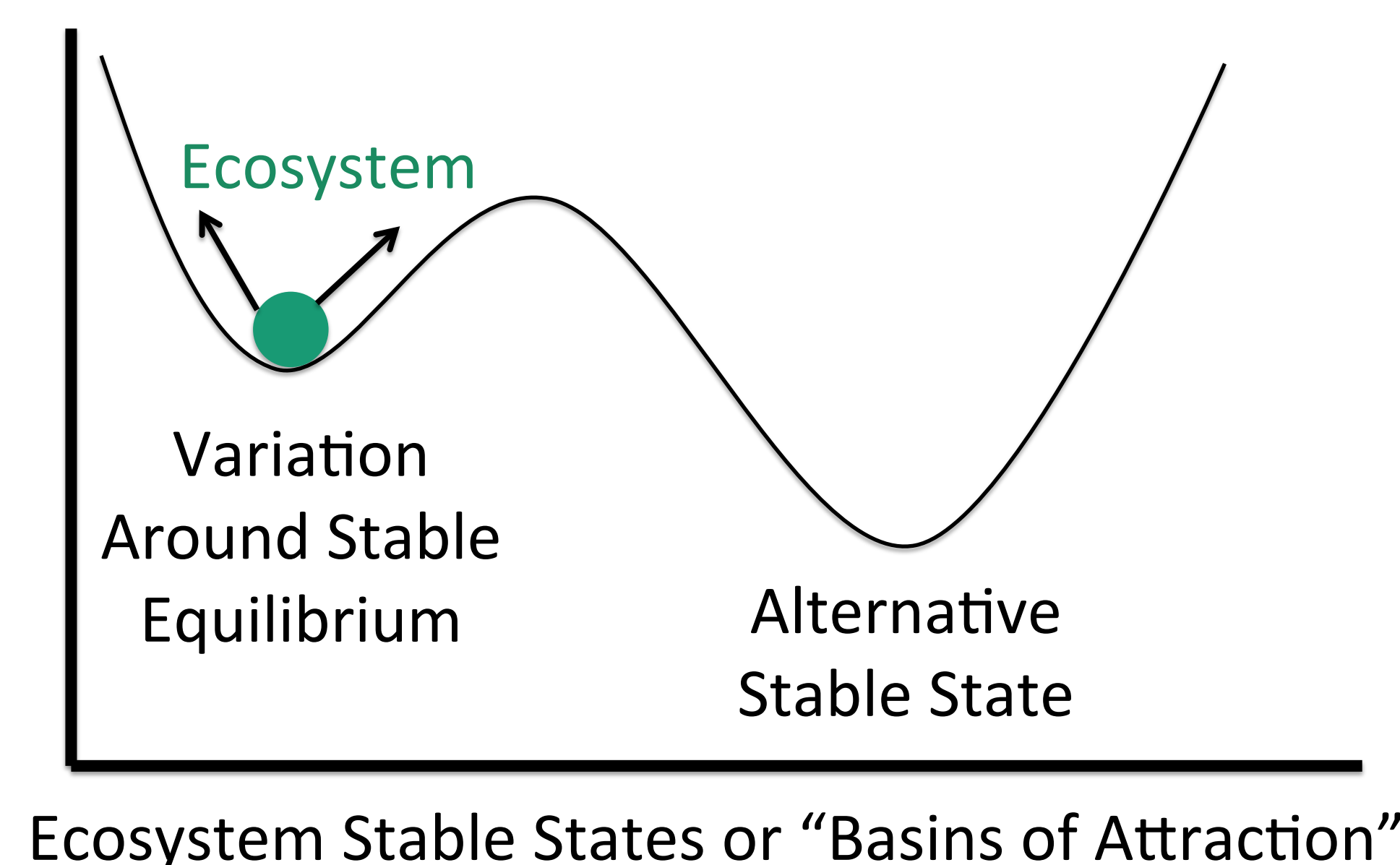
- Bacteria contribute 90% of cells, 99% of genes in human body
- Roles of microbiota in health:
 - Pathogen resistance
 - Metabolization
 - Xenobiotic modifications
 - Immune system regulation
- Contributes to malnutrition, obesity, heart disease, inflammatory bowel disease, Crohn's disease, many more

Research Questions

- How consistently does the microbiome retain its composition and activity over time?
- How easily can the health benefits conferred upon a host by the microbiota be lost due to a disturbance of the community?

Applying Macroecological Theory

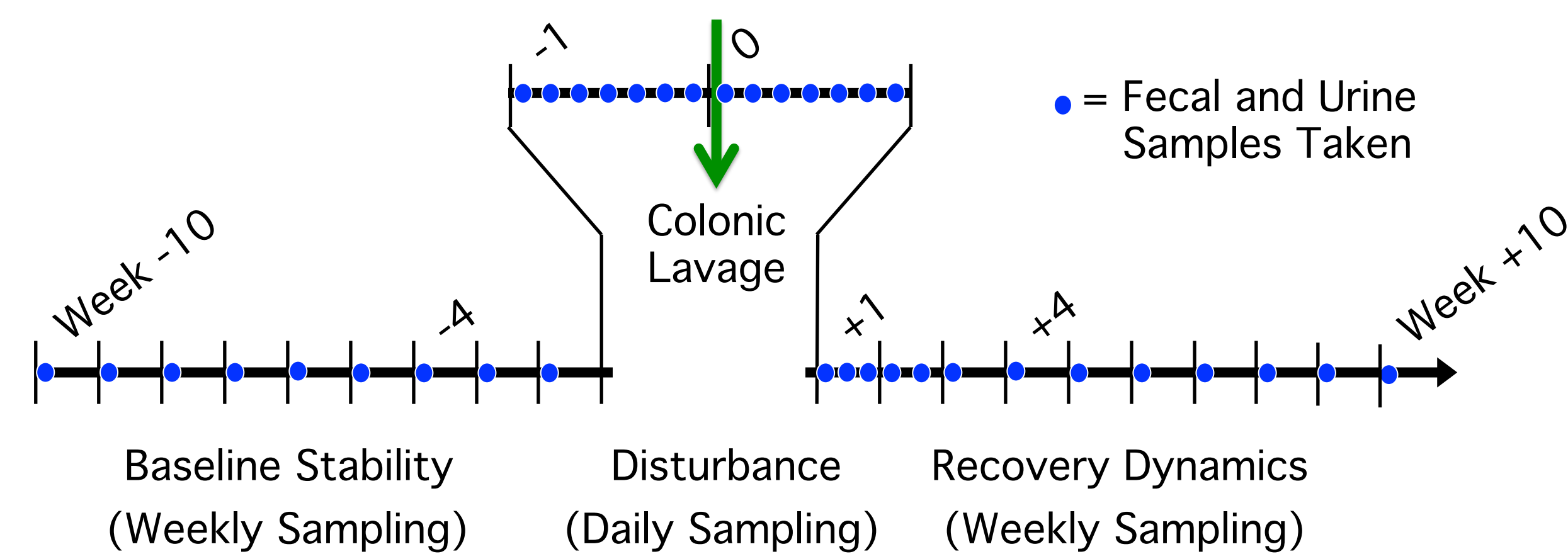
- Stability = ability of system to return to equilibrium quickly, with minimal deviation after temporary disturbance
- Equilibrium = set of defined values for relevant key variables
- Resilience = when challenged with disturbance, ecosystem ability to adapt and retain function, structure, identity, and feedbacks
- Disturbance could induce regime shift to new stable state



Ecosystem Stable States or "Basins of Attraction"

- Possible predictors of microbiome resilience:
 - Taxonomic diversity
 - Intermediate stability week-to-week or day-to-day
 - Functional redundancy (the Insurance Hypothesis)
 - Specific constellation of taxa

Experimental Design



- Study Subjects: 8 healthy volunteer adult participants
- Fecal and urine samples at each time point analyzed three ways:

16S rDNA Sequencing

- Marker gene amplified, sequenced
- Conserved identification tag
- Reveals changes in taxonomic composition, relative abundances

Shotgun Metagenomic Sequencing

- Fragmented, sequenced whole genome
- Reveals relative gene frequencies
- Computational reassembly associates genes with specific taxa

Metabolomic Mass Spectrometry

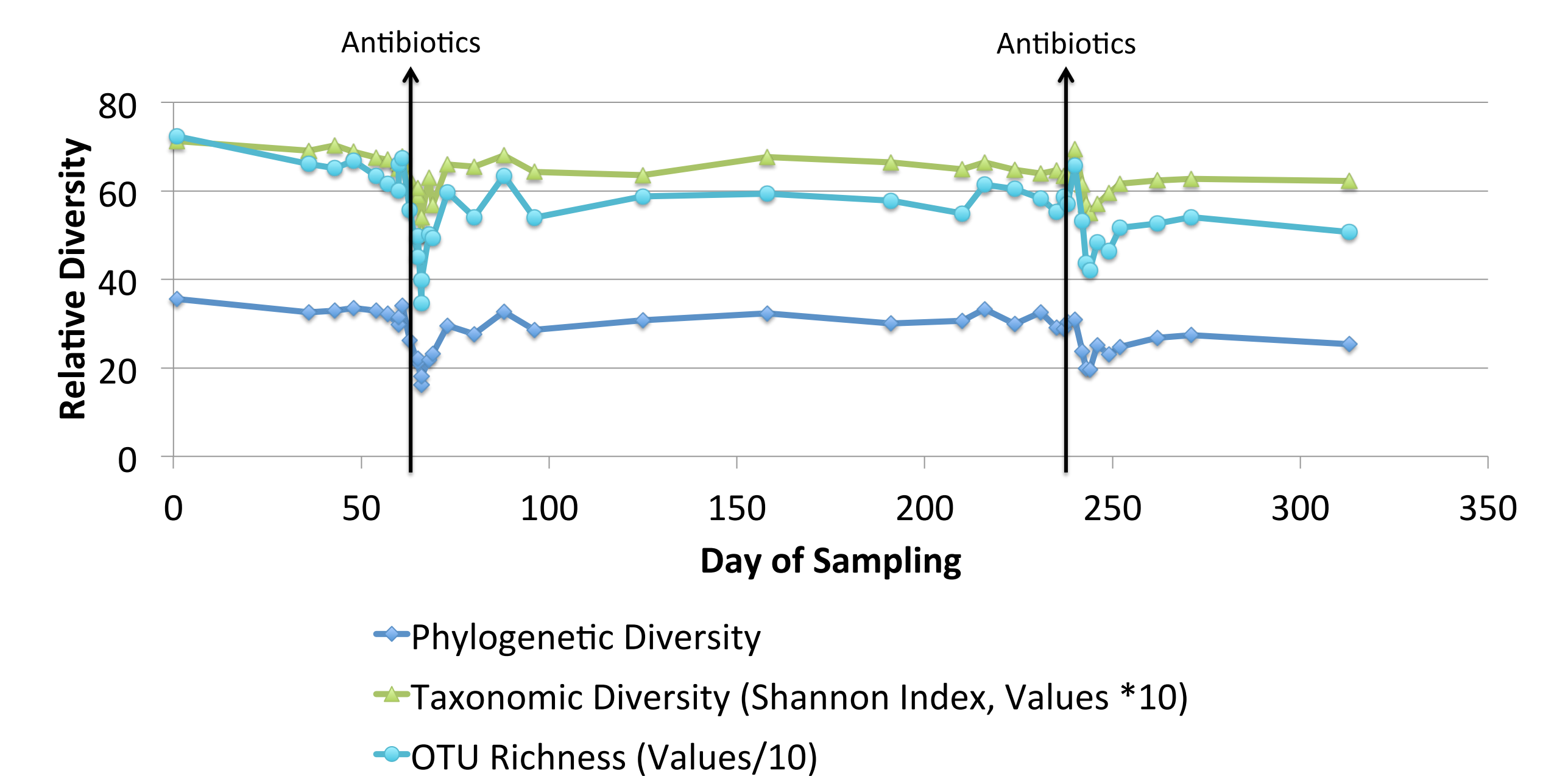
- Identifies small molecules produced
- (Analyses performed by collaborators at University College London)

Study Aims

- **Aim 1.** Compare routine unperturbed dynamics to varied responses to lavage; characterize effect of disturbance
- **Aim 2.** Evaluate application of ecological principles to human gut microbiota in context of colonic lavage disturbance
- **Aim 3.** Identify features characteristic of resilient communities

Proof of Concept: Antibiotic Disturbance

Fluctuations of α -Diversity Metrics in Response to an Antibiotic Series



- Antibiotic series triggered dramatic temporary decrease in α -diversity metrics based on 16S rDNA data (Dethlefsen, 2011)
- Present study expands upon this one with longer timescale, greater sampling depth, use of metagenomics and metabolomics
- Colonic lavage is less selective disturbance causing greater decrease in total biomass

Project Status

Design Study and Obtain IRB Approval

Recruit and Enroll Subjects

Subjects Sample and Cleanout

DNA Extraction from Fecal Samples

16S Sequencing

Metagenomic Sequencing and Reassembly

Send Metabolomics Urine and Fecal Samples

Data Processing with QIIME and Phyloseq

3 participants currently sampling, about to undergo cleanout, will complete sampling December 2014

5 participants' samples DNA extracted, 16S sequenced on HiSeq2500 with 150nt paired-reads, produced >98 million reads, metagenomics preparations ongoing

