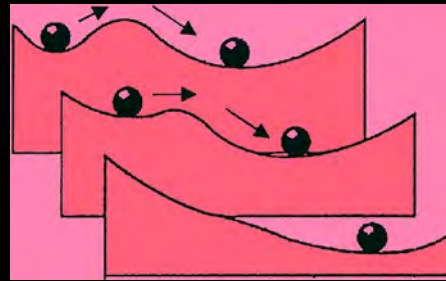


Diversity, Stability, and Resilience of the Human Microbiome



David A. Relman, Stanford University
"Human Microbiome Science: Vision for the Future"
Bethesda / July 24, 2013



Microbes as threats



Microbes as beneficial symbionts: co-evolution, co-adaptation, co-dependency



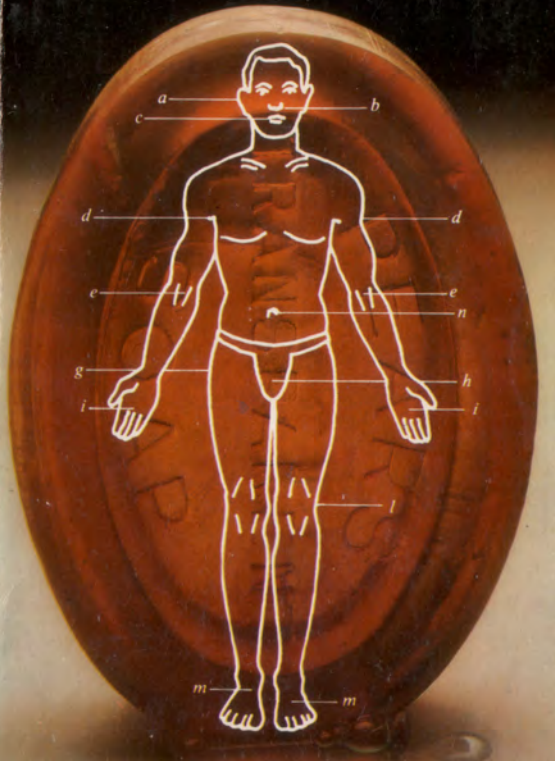
"I see it, but it scampers away from the light."

PALADIN

586 08085 6

Life on Man

Theodor Rosebury



1969

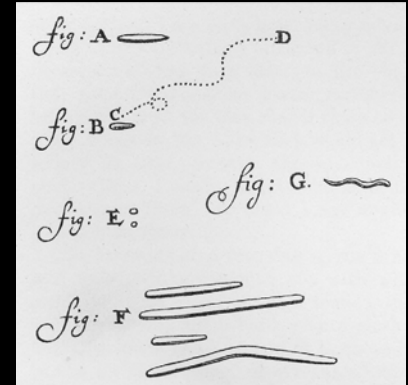
“We are becoming a nation of tubbed, scrubbed, deodorized neurotics....Traces remain of the puritan notion that our bodies, or parts of them, their functions and products are ugly, dirty...The attempt to kill the microbes that live on us normally is a mistake...The myth that germs and dirt are always are enemies is harmful and costly.”
 (“excretory prejudice”)

“Our feelings about feces and saliva have little or nothing to do with the microbes...and depend on ignorance...The connection with sex is undeniable”

“Faeces are the child’s first gift, the first sacrifice on behalf of his affection.” (Freud, *Complete Psychological Works*)

A bit of history

- Antony van Leeuwenhoek, 1683 ('animalcules')



- Razumov AS, 1932 ('Great plate anomaly')
- Dubos R *et al.*, 1965 (co-evolution)
- Moore W, 1975, 76; Savage DC, 1977 (cell counts, ecology)

Why interest in human microbiome?

- Human indigenous microbial communities as critical component of human biology ('Know thyself')
 - ★ Larger role in both health and disease than previously understood
 - ★ Determinant of individuality
 - ★ Emerging perspective: humans as "supraorganism"
- Novel approaches for therapy, prevention, dx?
- The time is "ripe" (tools, convergence of complementary disciplines, conceptual framework—e.g., community as unit of study)

Our 'extended self': human-microbe mutualism

(Based on cell counts, we are 10 parts bacterial, 1 part human...and based on numbers of unique genes, we are 150 parts bacterial, 1 part human...)

Our benefits

- Food digestion
- Nutrition (vitamins, energy)
- Xenobiotic processing
- Metabolic regulation, cometabolism
- Development: terminal differentiation of mucosa
- "Education", regulation of immune system
- Epithelial "homeostasis", barrier integrity
- Colonization resistance to pathogens

Our 'extended self': human-microbe mutualism

(Based on cell counts, we are 10 parts bacterial, 1 part human...and based on numbers of unique genes, we are 150 parts bacterial, 1 part human...)

Their benefits

- Nutrition
- Habitat
- Dispersal

Shouldn't we be focusing a bit more on their needs?

"Community as pathogen" ?

that is,

disease due to a
community disturbance

"pathogenic states"

Clinical problems associated with the indigenous microbiota

- Chronic periodontitis
- Crohn's disease & other IBD
- Irritable bowel syndrome
- Tropical sprue
- Antibiotic-associated diarrhea
- Pathogen invasion or bloom
- Bacterial vaginosis

Clinical problems associated with the indigenous microbiota

Cause, effect, neither?

Initiating or "propagating"?

Necessary, sufficient, neither?

- Antibiotic-associated diarrhea
- Pathogen invasion or bloom
- Bacterial vaginosis

Terms and Methods

- **Human Microbiota**—communities of microscopic living organisms found on, in humans (viruses, bacteria, archaea, eukarya)
- **Microbiome**—collective genomes of microbiota
- Phylogenetic or taxonomic composition (e.g., 16S rRNA gene-based surveys)
- **Metagenomics**—study of genetic material directly from environmental samples; genetic or **functional potential**

Questions



- Relationships between **diversity** and states of health (and disease)?
- How, and to what degree is microbiota **stable**, during adulthood?
- Is the microbiota/microbiome **resilient**?

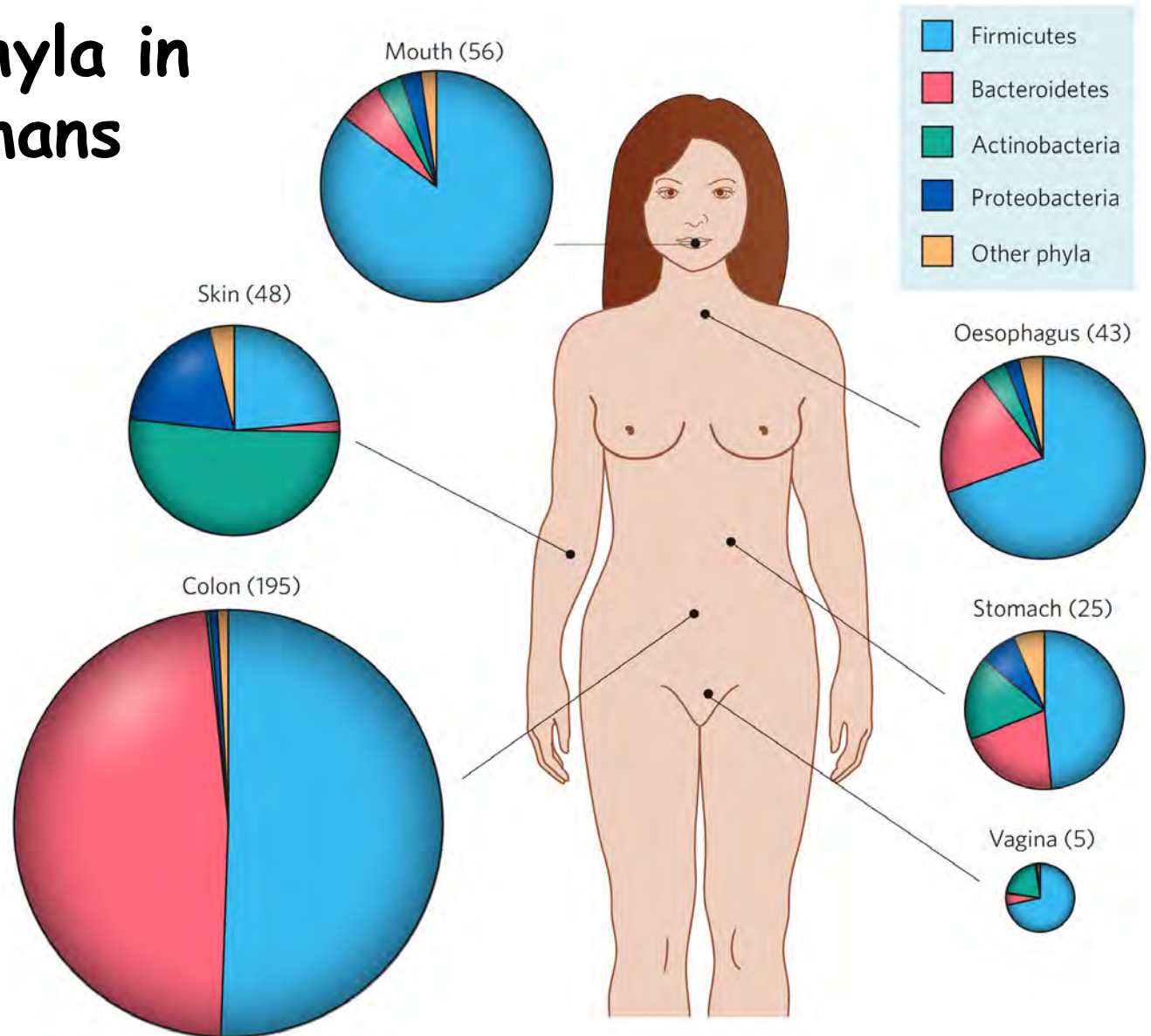
Diversity



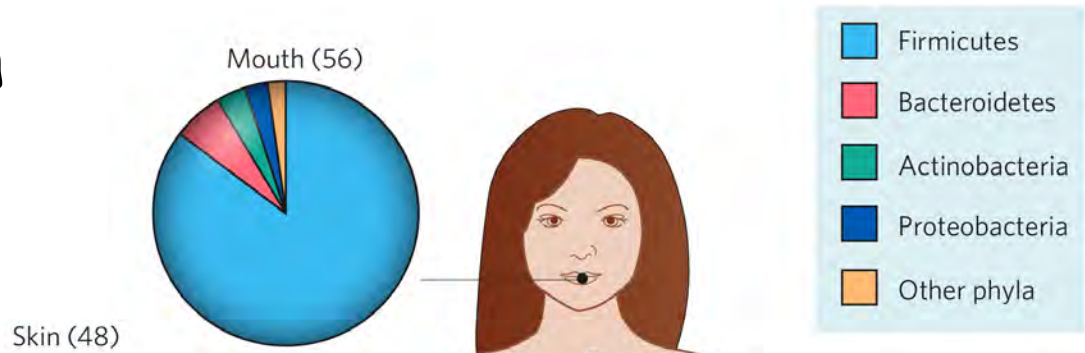
- How do we measure diversity? What aspects of diversity matter most?
 - At level of organism? Group?
 - ...Genes, pathways, products, activities?
 - Community-wide? Between communities?
Between populations of hosts?
- Importance of relative abundance?

Site-specific distributions of bacterial phyla in healthy humans

Size of circles is proportionate to average number of species-level phylotypes per individual (in parentheses)

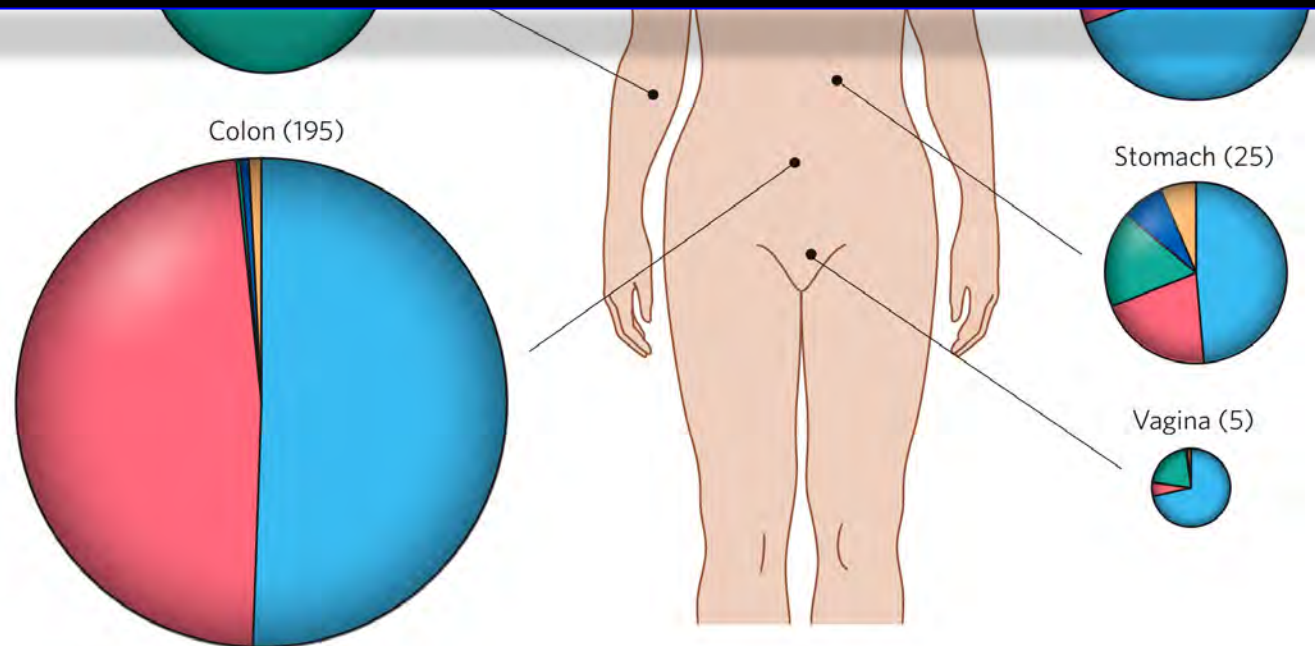


Site-specific distributions of bacterial phyla in healthy humans



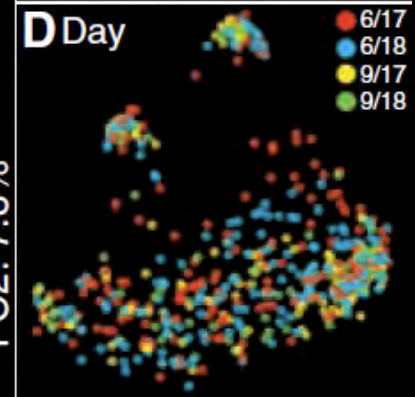
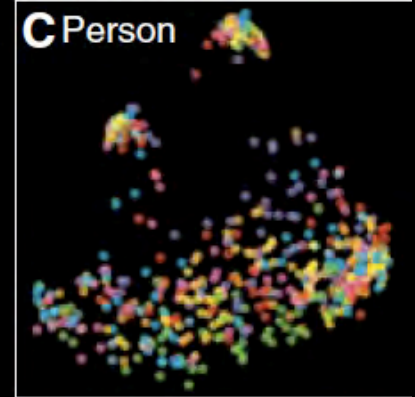
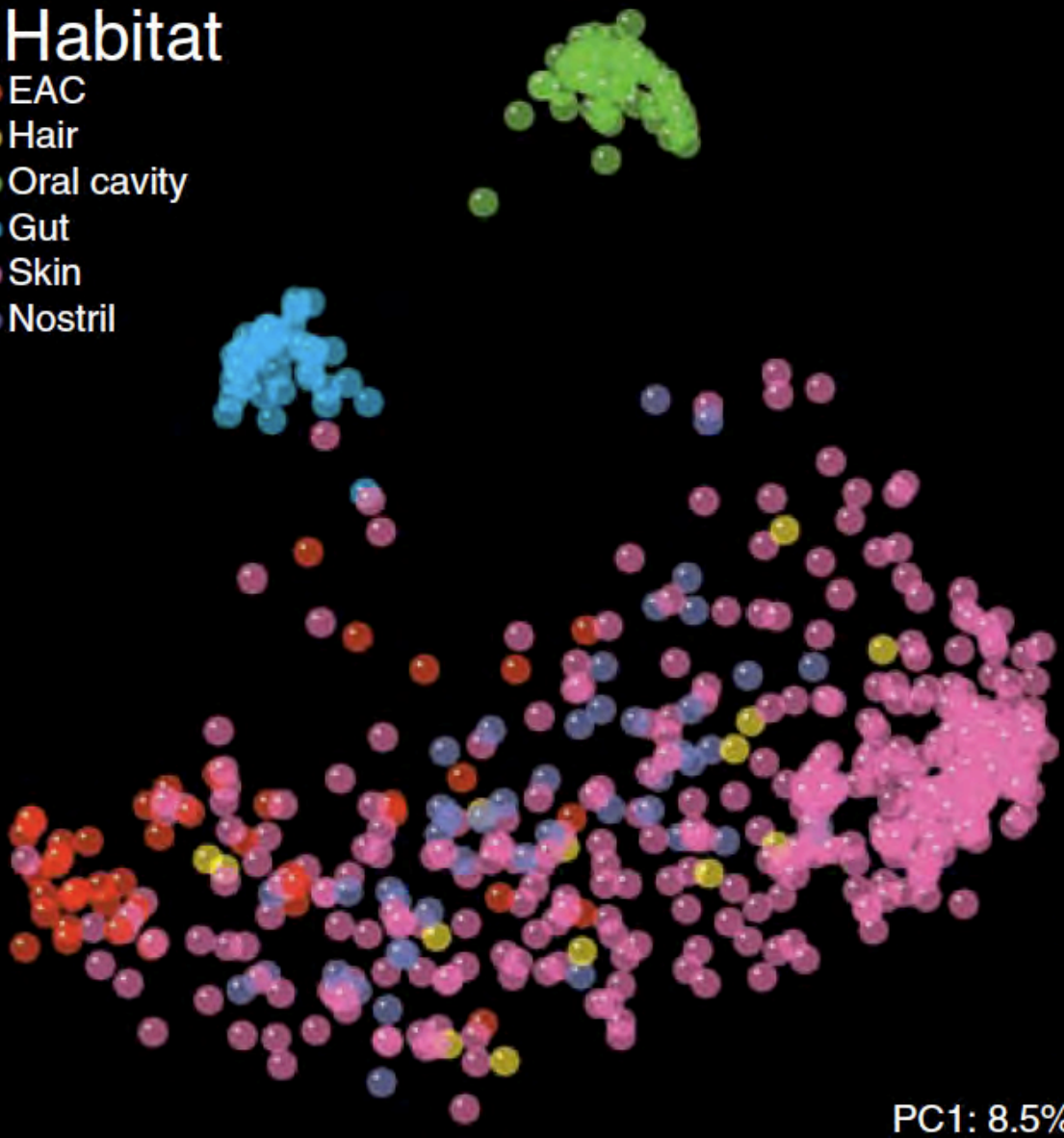
Everything is not everywhere!

Size of circles is proportionate to average number of species-level phylotypes per individual (in parentheses)



Habitat

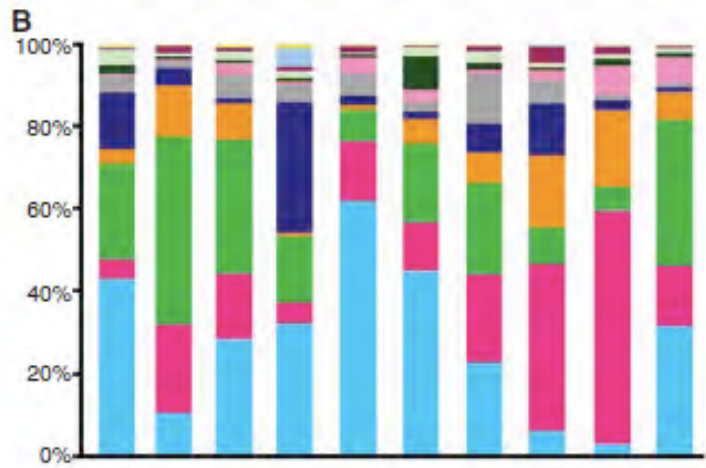
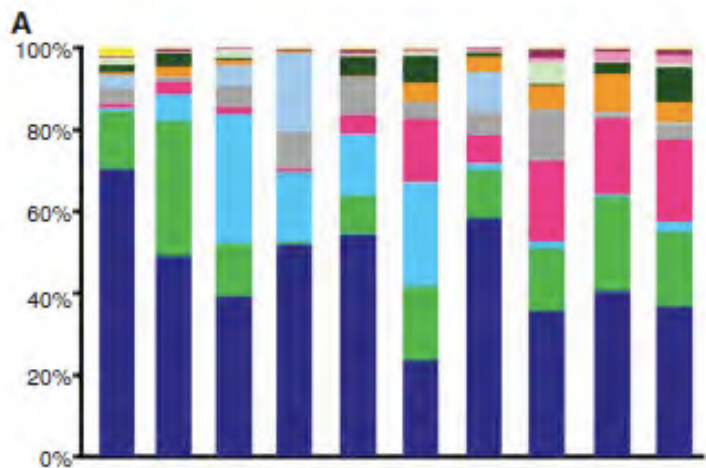
- EAC
- Hair
- Oral cavity
- Gut
- Skin
- Nostril



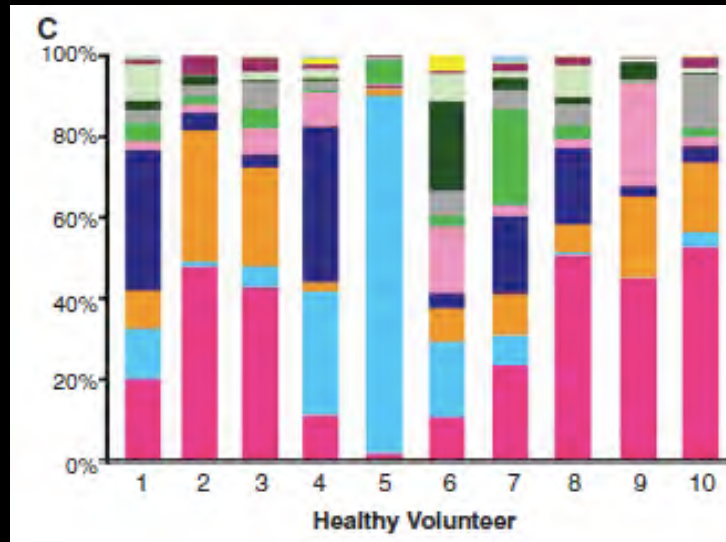
Costello E *et al.*, Bacterial community variation in human body habitats across space and time. *Science* 2009; 326:1694-1697

Skin microenvironments

Sebaceous



Moist



Dry

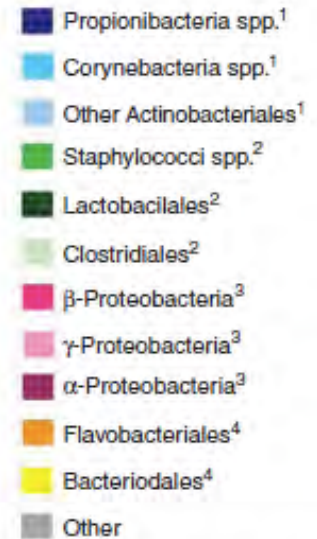
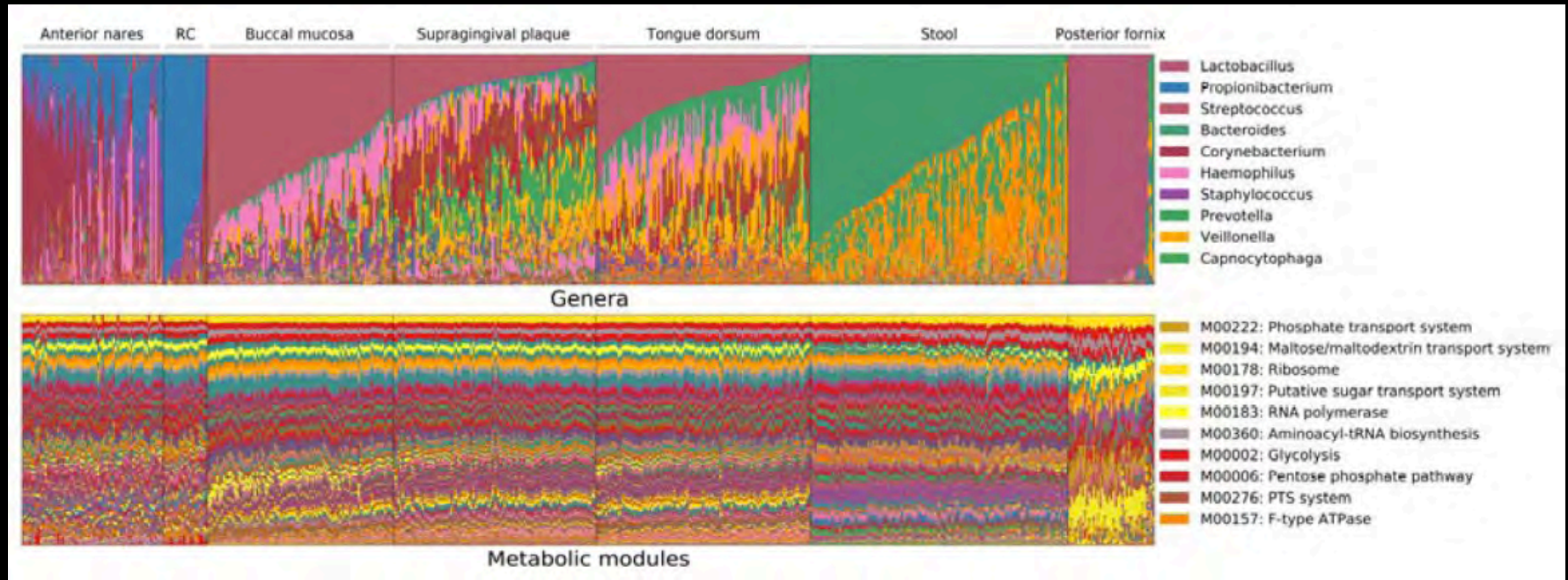


Fig. 1. The 20 skin sites and associated microbiota are representative of three microenvironments: (A) sebaceous, (B) moist, and (C) dry. The relative abundance of the most abundant bacterial groups associated with each microenvironment is depicted for each healthy volunteer. Superscripts indicate phylum: 1, Actinobacteria; 2, Firmicutes; 3, Proteobacteria; 4, Bacteroidetes.

Sources of variation in patterns of diversity

- Space (habitat, body site)
- Individual
- Health status
- Host genetics
- Environmental exposures
 - Diet
 - Chemical/drug/mechanical disturbance
 - Other aspects of lifestyle? (e.g. geography)
 - Other mammals/hosts
- Time (esp. early in life)

Greater evenness of functional gene profile than of taxonomic (genus) profile across individuals



Nature 486:207-214, (14 June) 2012

Stability

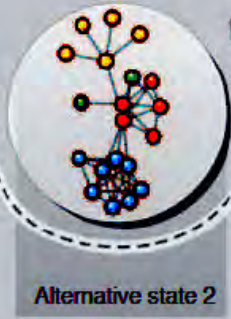


- How, and to what degree is microbiota stable, during adulthood?
- Relationship to age, immunocompetence?
- Determinants of stability?

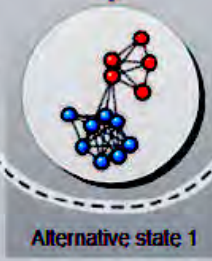
Community stability landscape

(A) Shift in "state" variables alters community directly

The community state landscape



Antibiotics, oral hygiene

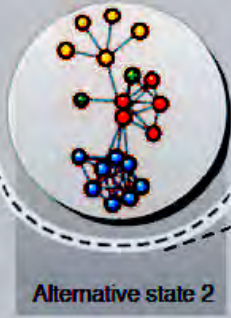


Shift in community state variables

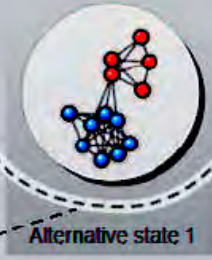


(B) Shift in environmental "parameters" alters the community indirectly

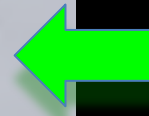
The community state landscape



Diet intervention, immunosuppressive drug



Shift in environmental parameters



Costello EK *et al.*,
Science **336**, 1255 (2012)

Stability during adulthood?

ARTICLE

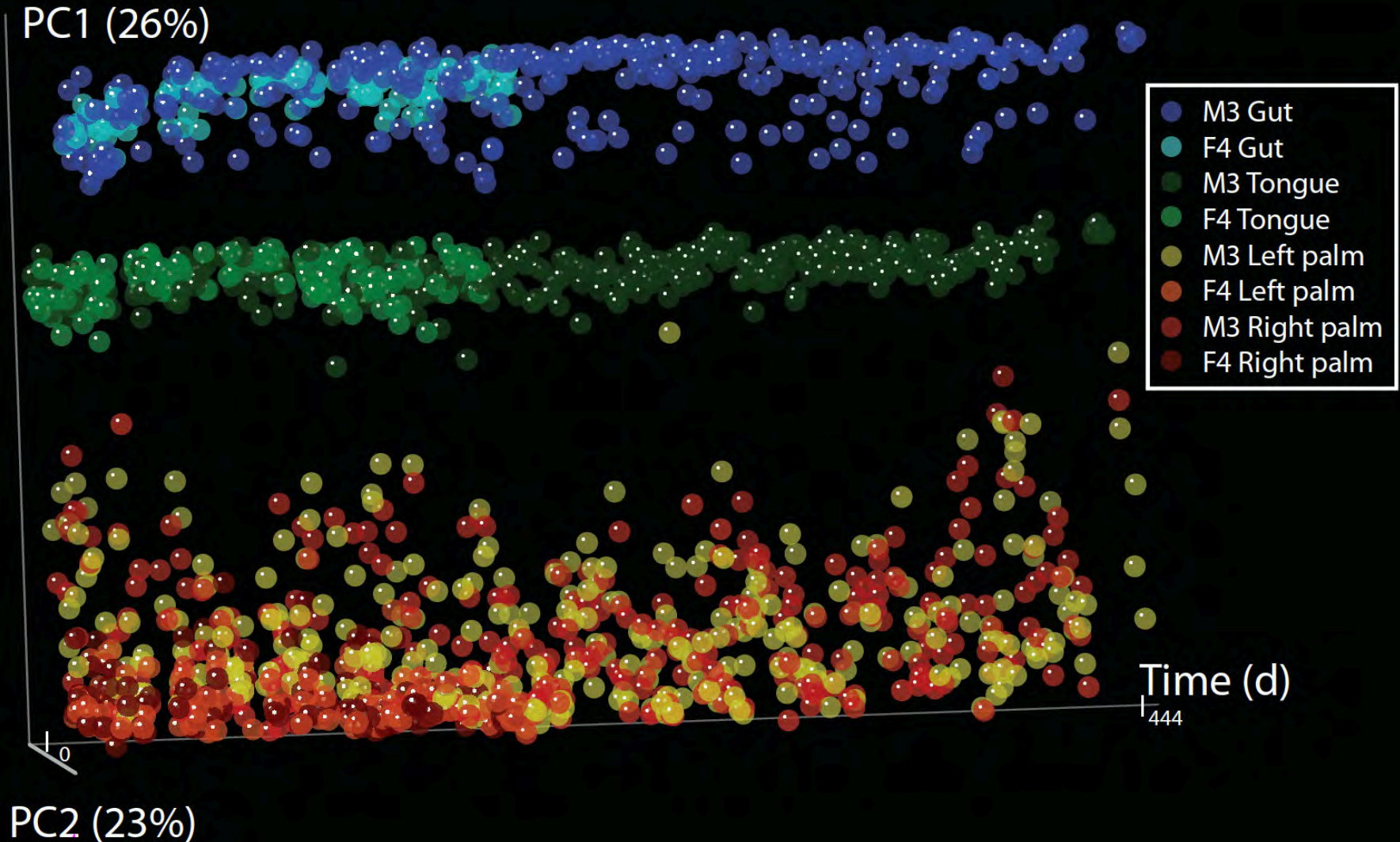
doi:10.1038/nature11234

Structure, function and diversity of the healthy human microbiome

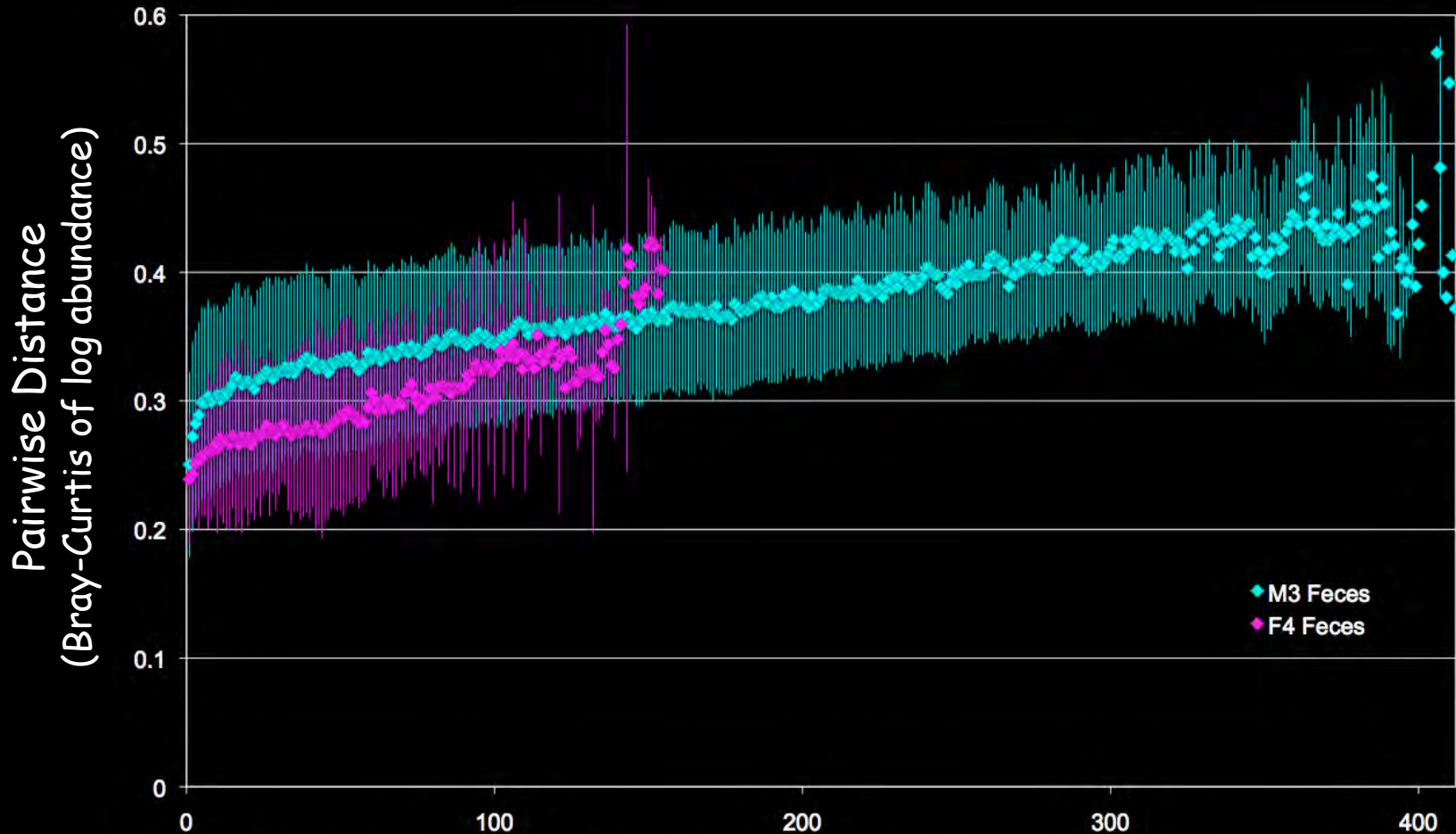
The Human Microbiome Project Consortium*

Only 2-3 time points

Two adults, daily sampling for 6 and 15 months



All pairwise stool sample comparisons as a function of temporal separation: drift?

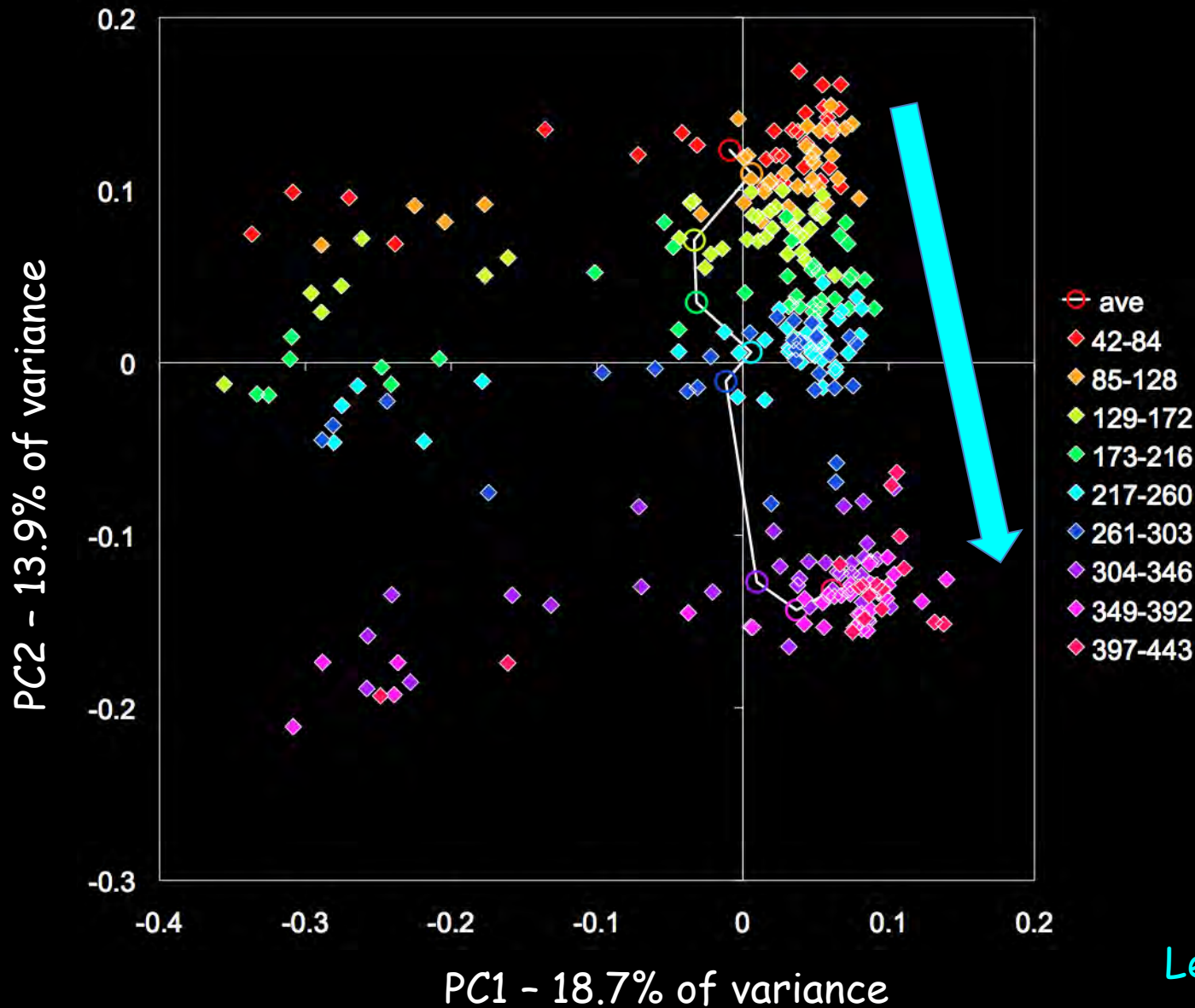


Interval (days)

Les Dethlefsen

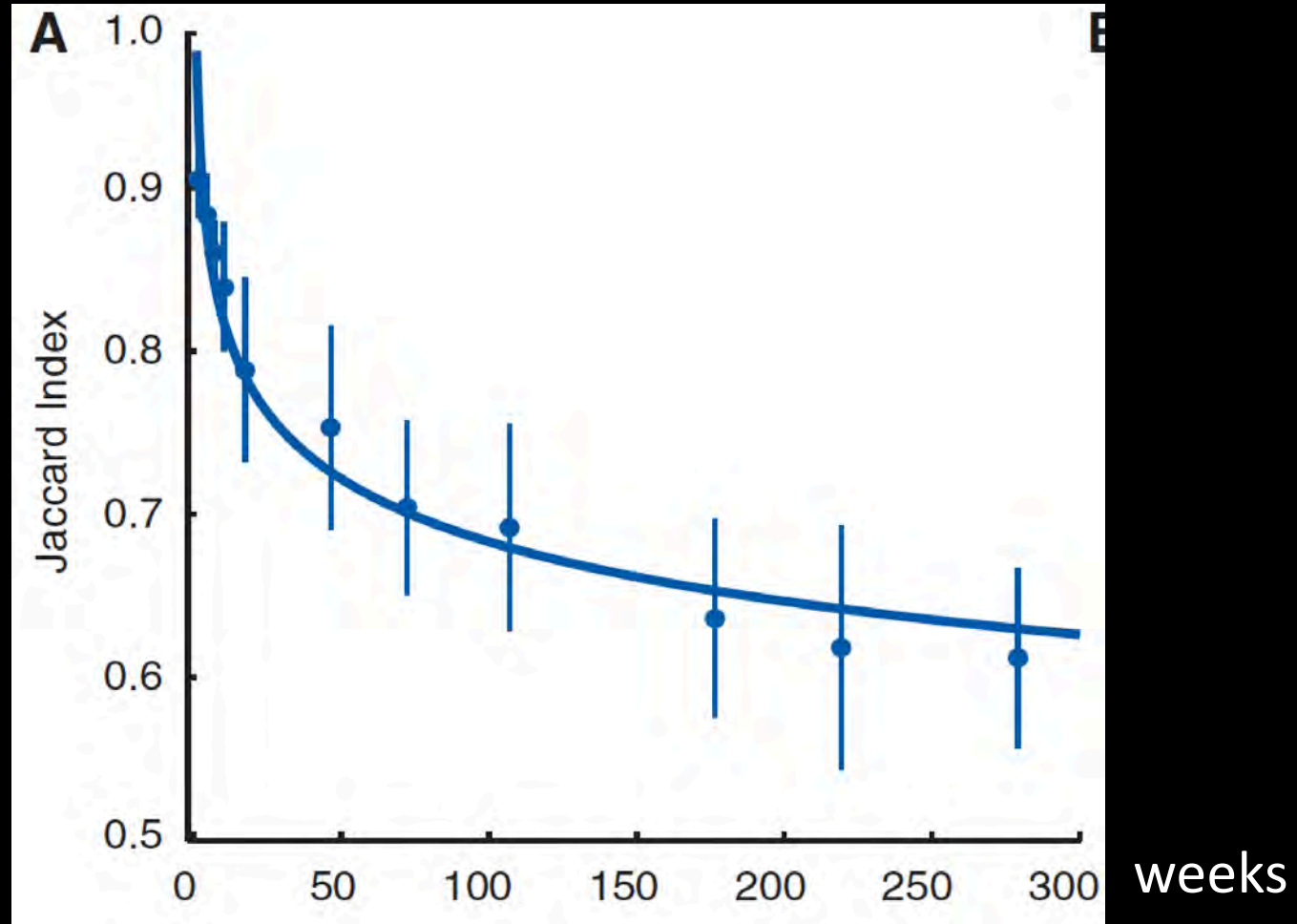
Ordination of Stool Samples

M3 fecal samples - PCoA of Bray-Curtis (log abundance)



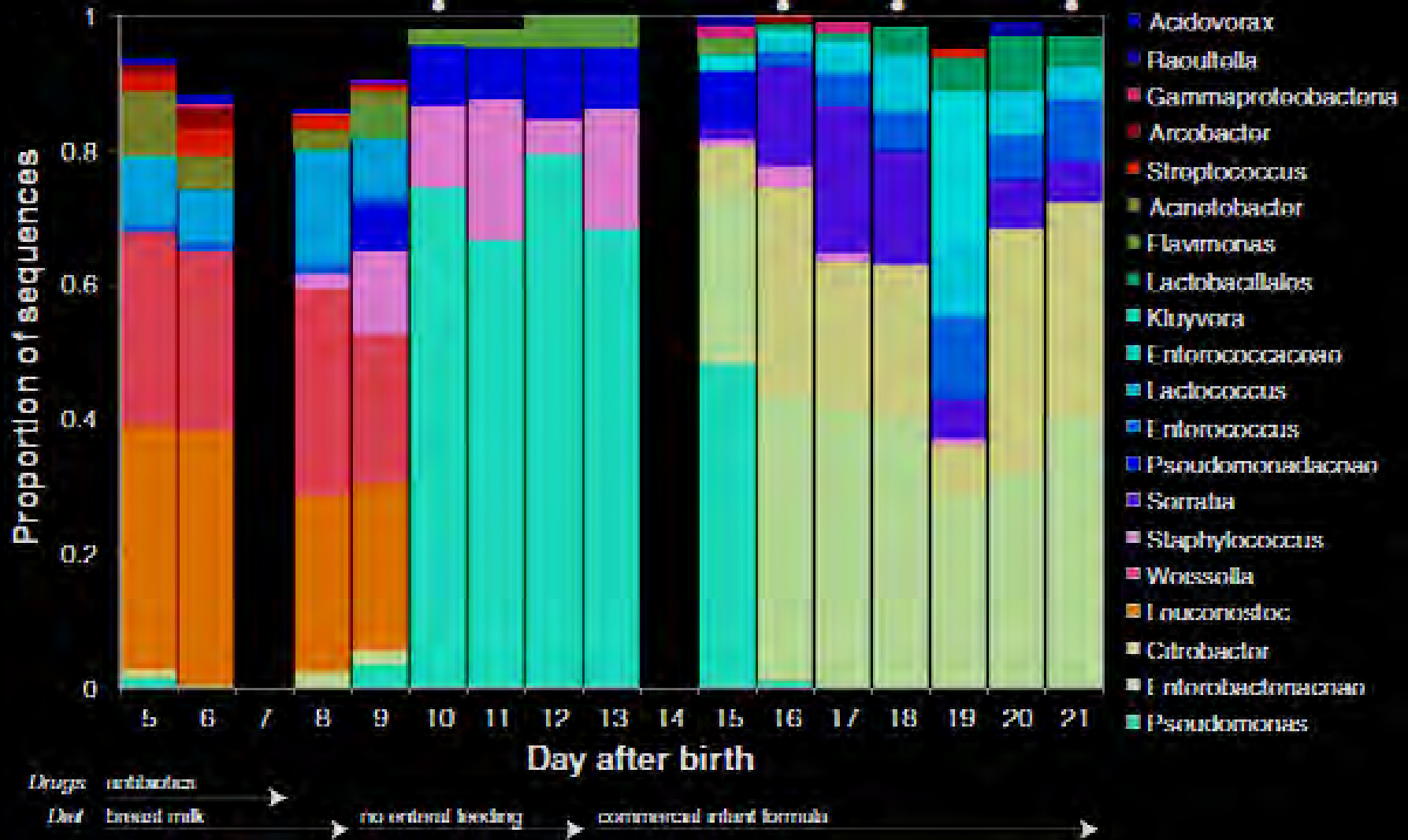
Les Dethlefsen

Fraction of shared 'strains'* within individuals (37) versus time interval



*100% similar over V1V2 16S rDNA

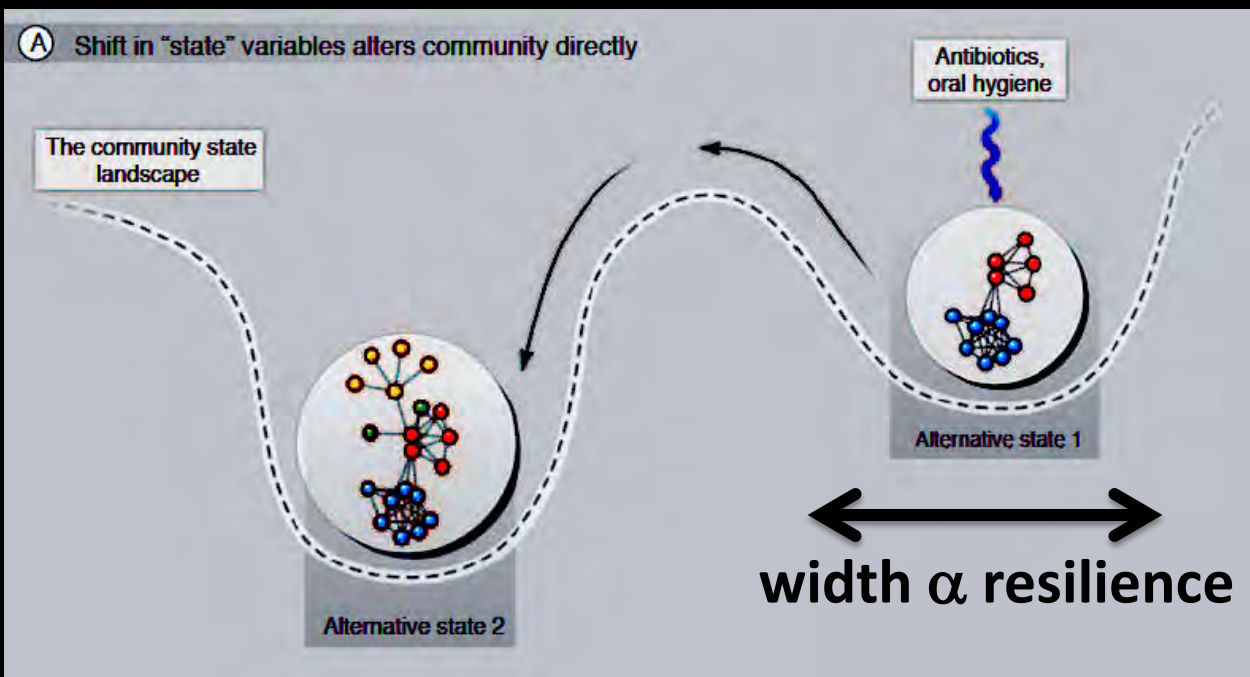
Population dynamics based on metagenomic profiling, and *Citrobacter* UC1CIT strain abundance



Disturbance



- Disturbances remove or kill some fraction of the community, creating opportunities for remaining community members or new colonists...effects directed at community and/or host
- Increasingly prominent in "modern" societies?



Resilience:
 capacity of ecosystem to absorb disturbance and retain same function(s)...

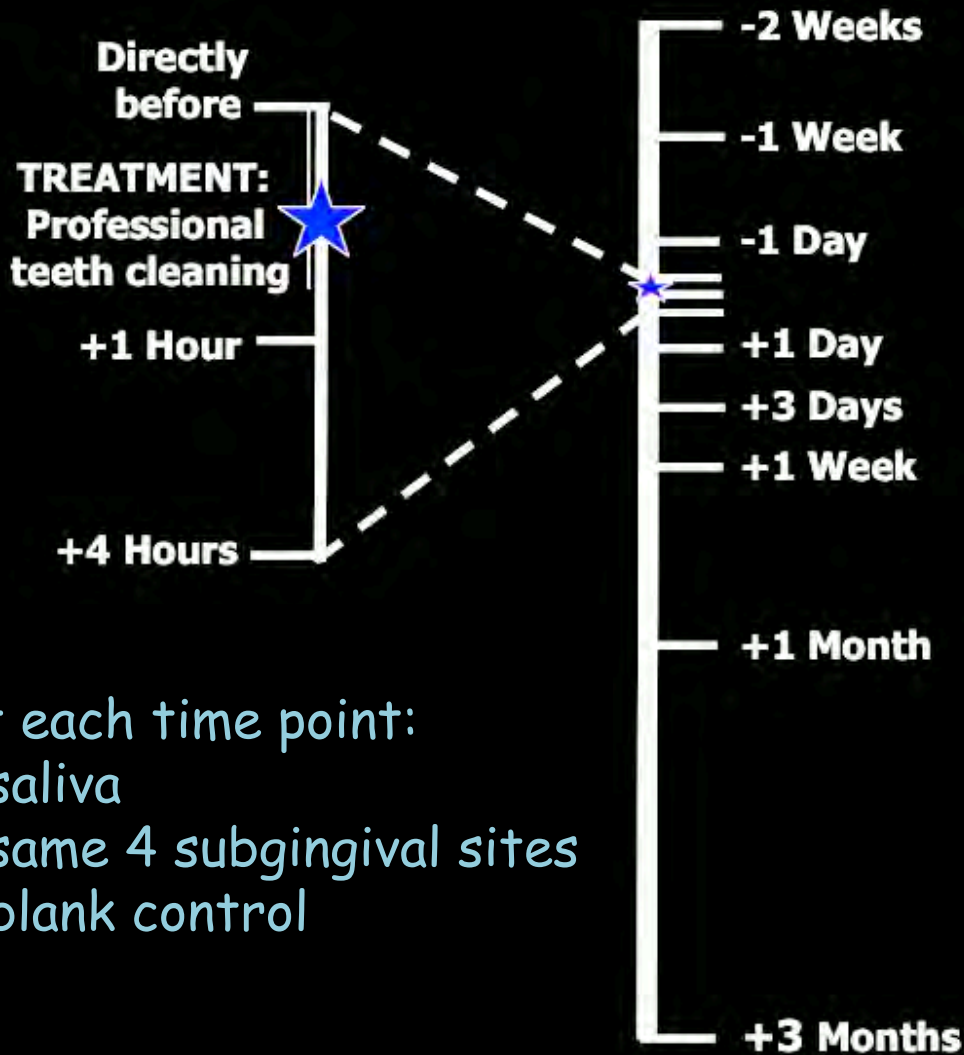


C. S. "Buzz" Hollings
 (1973)

<http://www.flickr.com/photos/sfupamr/5515528060/sizes/l/in/photostream/>

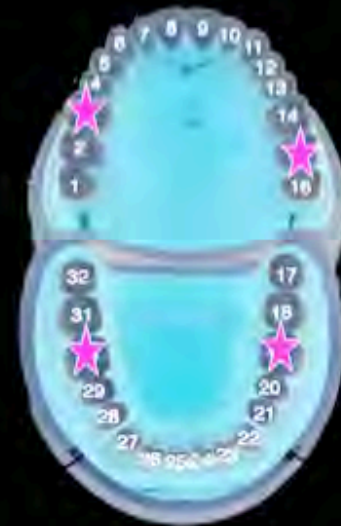
Costello EK *et al.*,
 Science **336**, 1255 (2012)

Time-course disturbance experiment



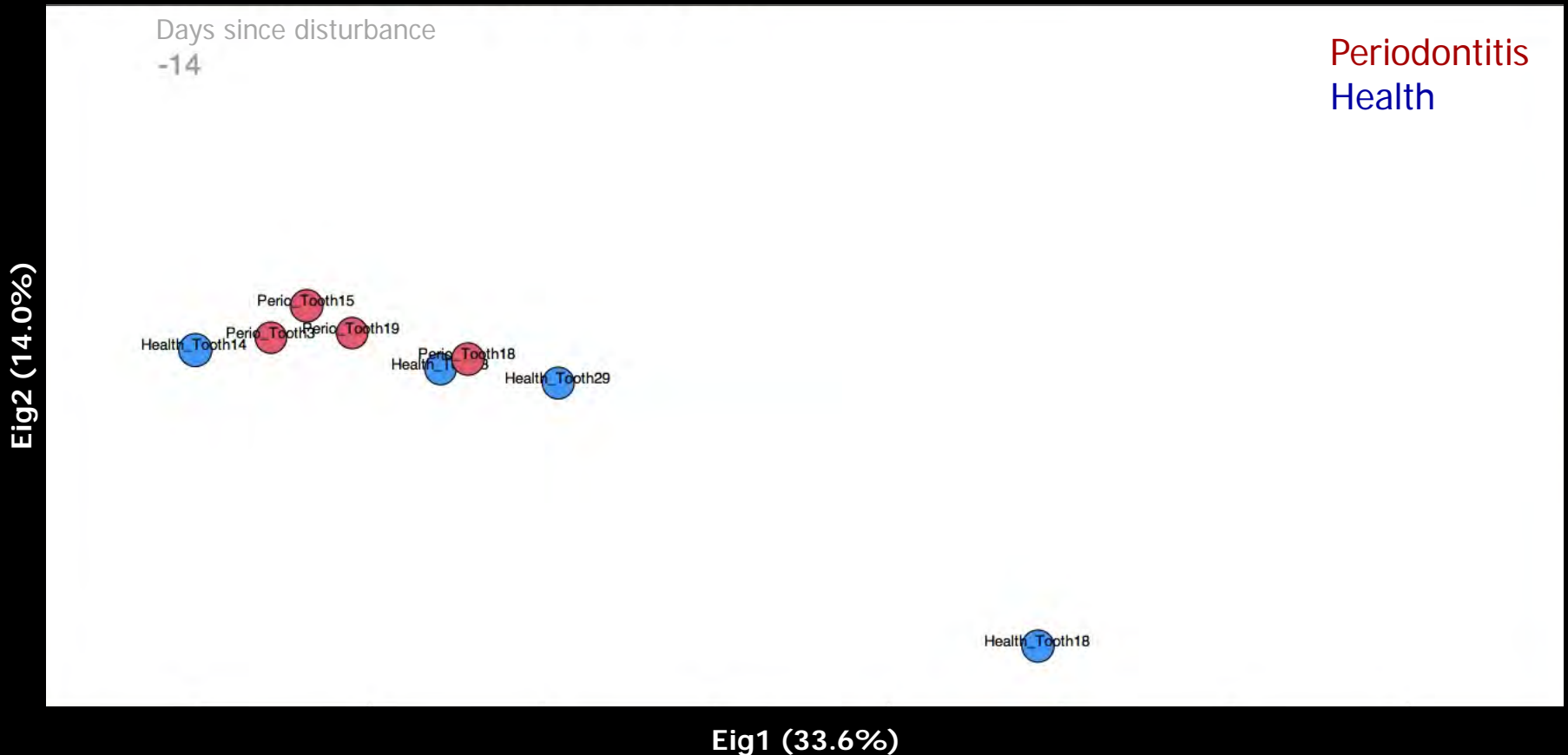
At each time point:
--saliva
--same 4 subgingival sites
--blank control

4 healthy mouths
4 moderate CP mouths



16S rDNA V3 tag
sequencing--
>2000 tags per sample

Tooth site-specific microbial community responses to mechanical disturbance



PCoA based on weighted Unifrac distance

Findings

- Conserved, as well as individualized responses
 - Site-specific bacterial diversity
 - Site-specific response to disturbance
- Larger response (displacement) at disease sites
- Rapid recovery (secondary succession)
- Predict subsequent health status at site?
(Disease is site-specific) Modify management?

Study design



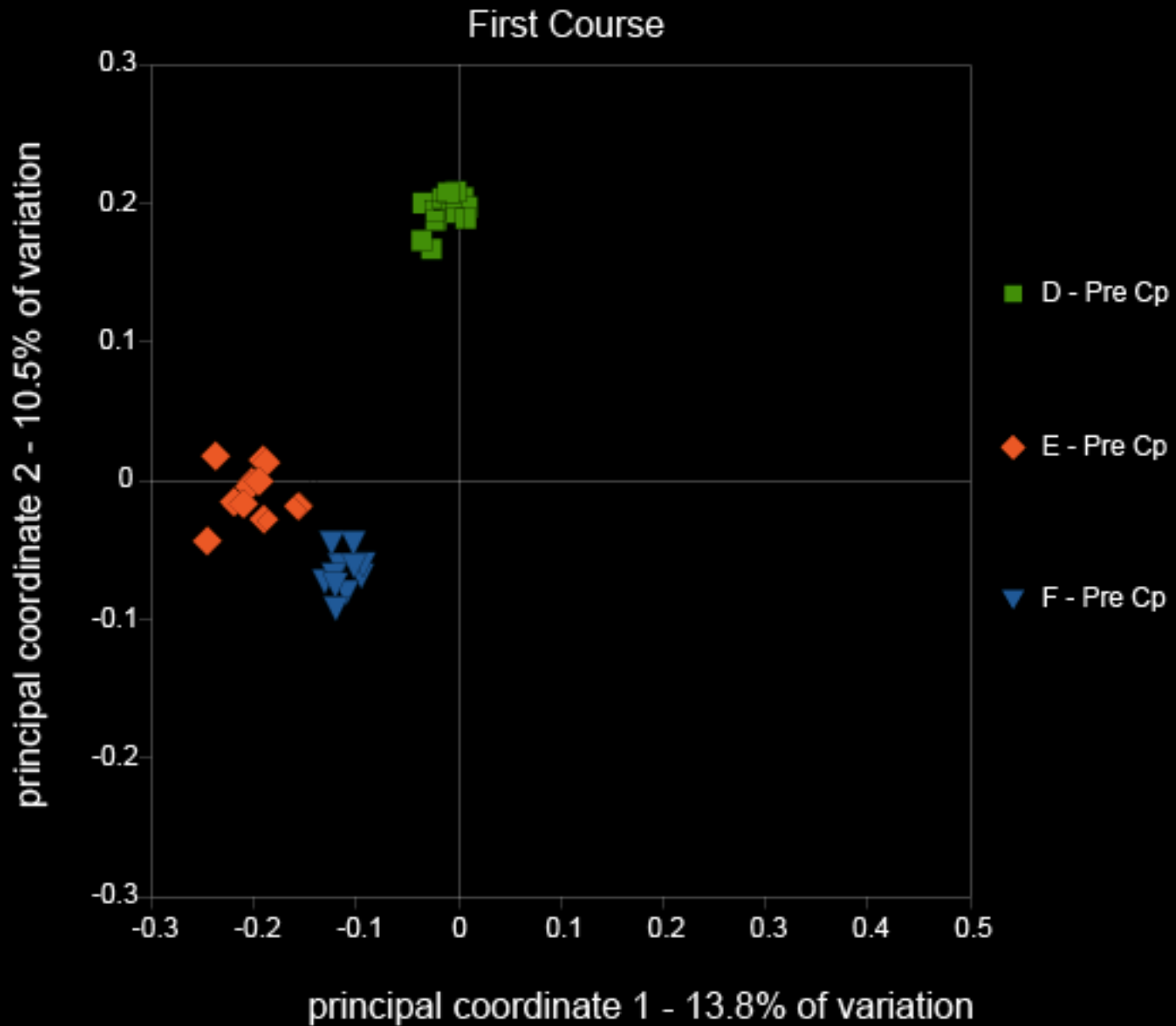
- Healthy subjects, no abx prev year
- Ciprofloxacin twice, 6 months apart (pulse perturbation)
- Stool samples over 10 months

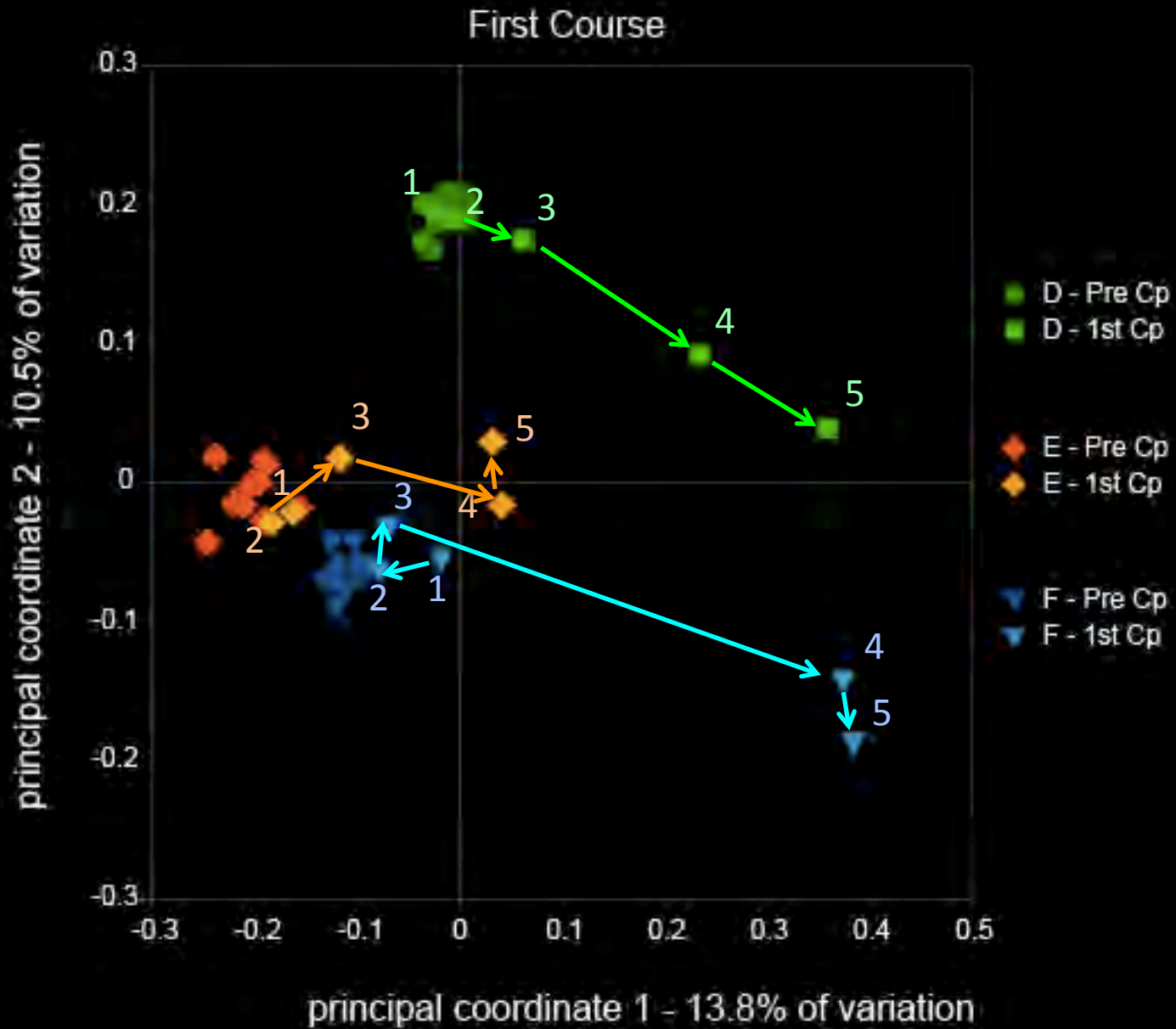
Dethlefsen et al. PLoS Biology 2008; 6:e280

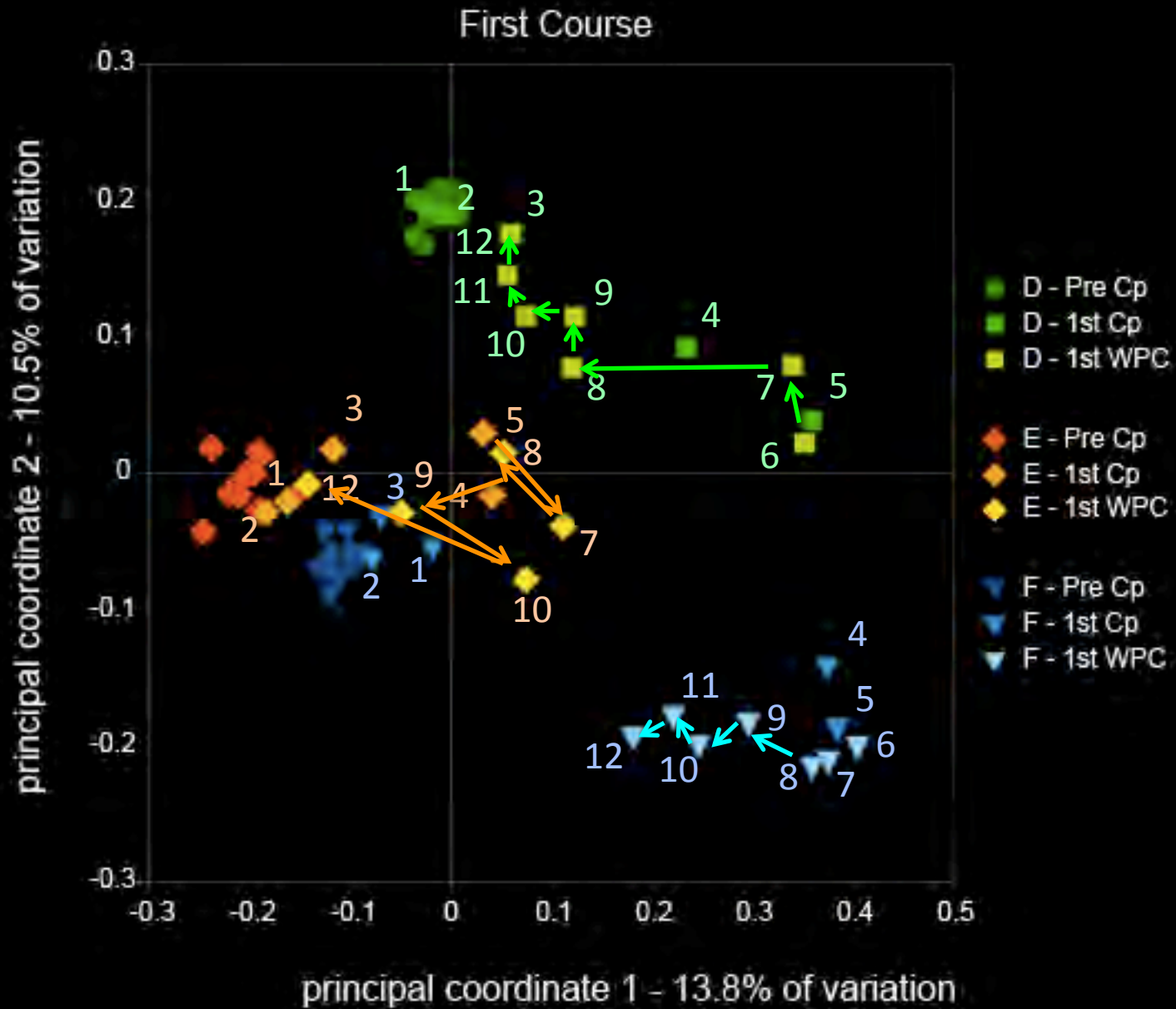
Dethlefsen L, Relman DA. PNAS 2011; 108:4554-61

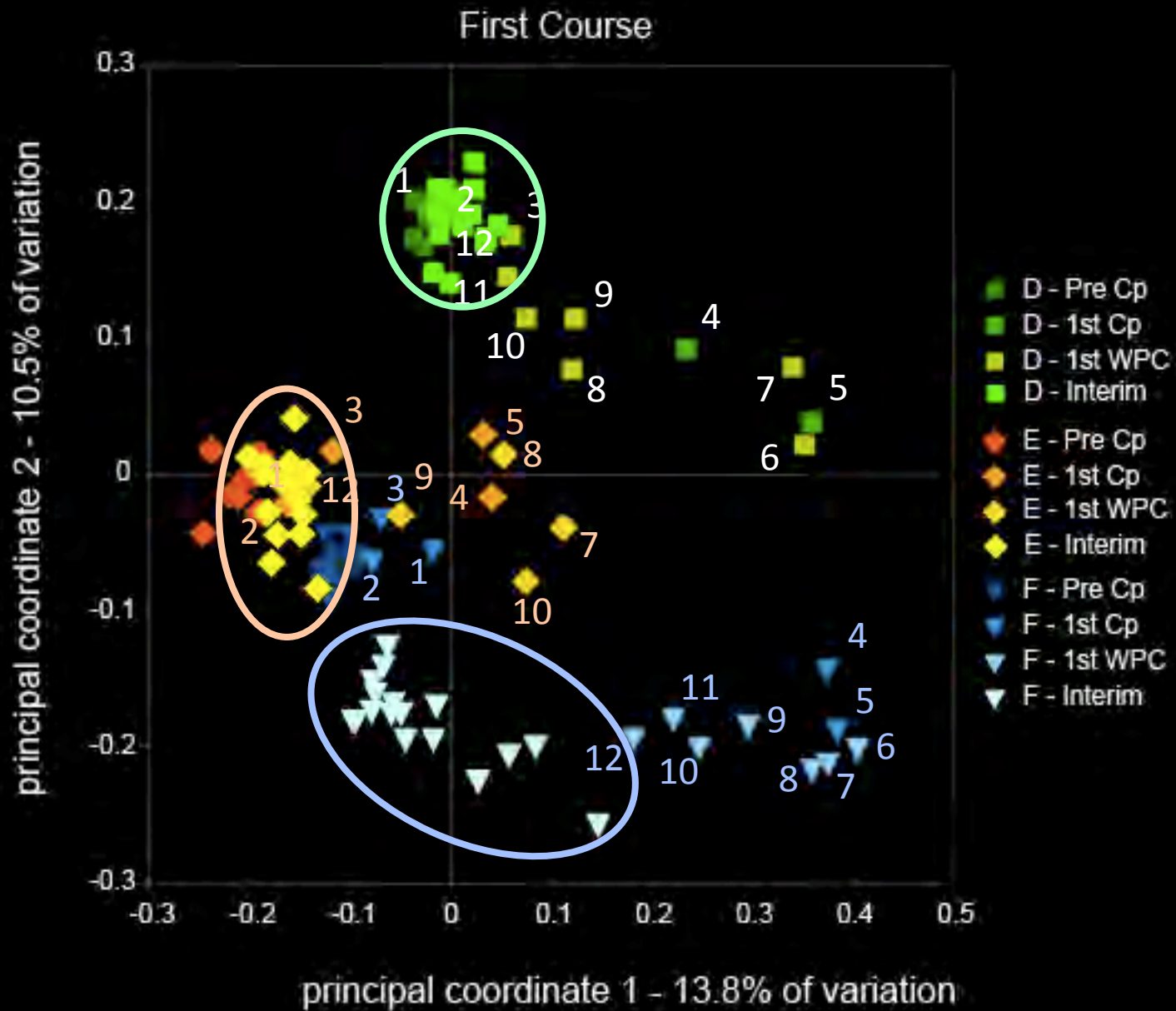


Les Dethlefsen

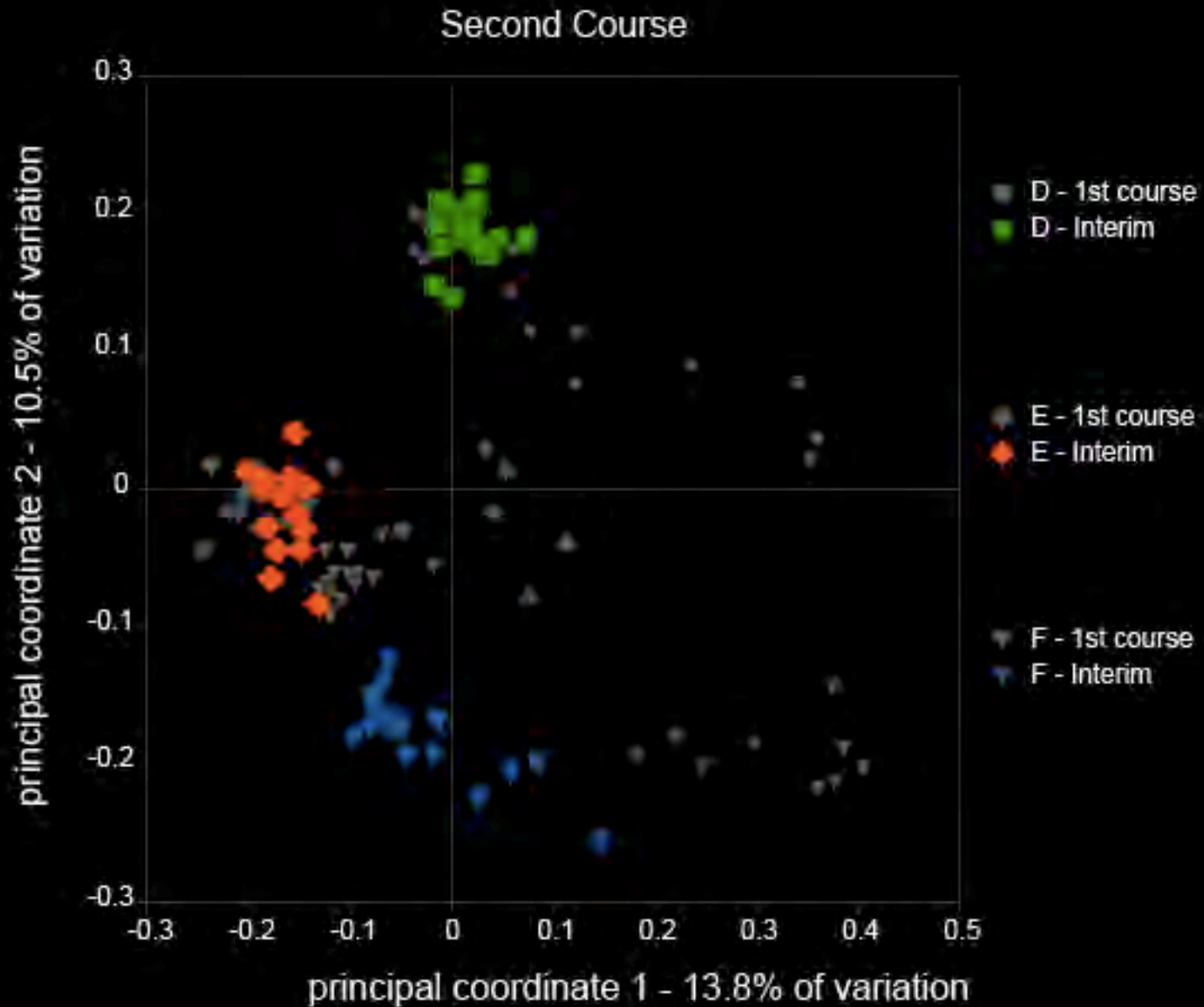


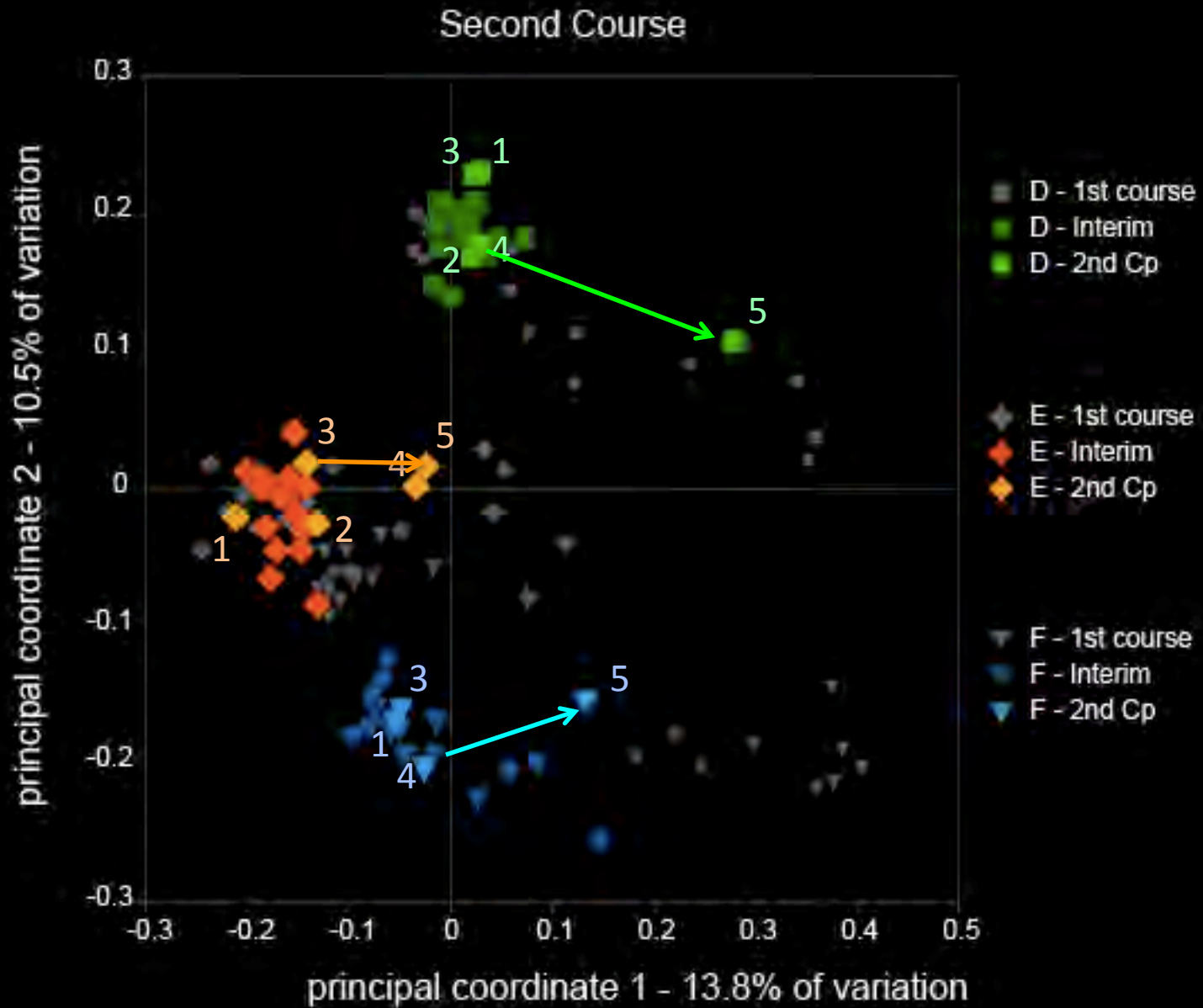


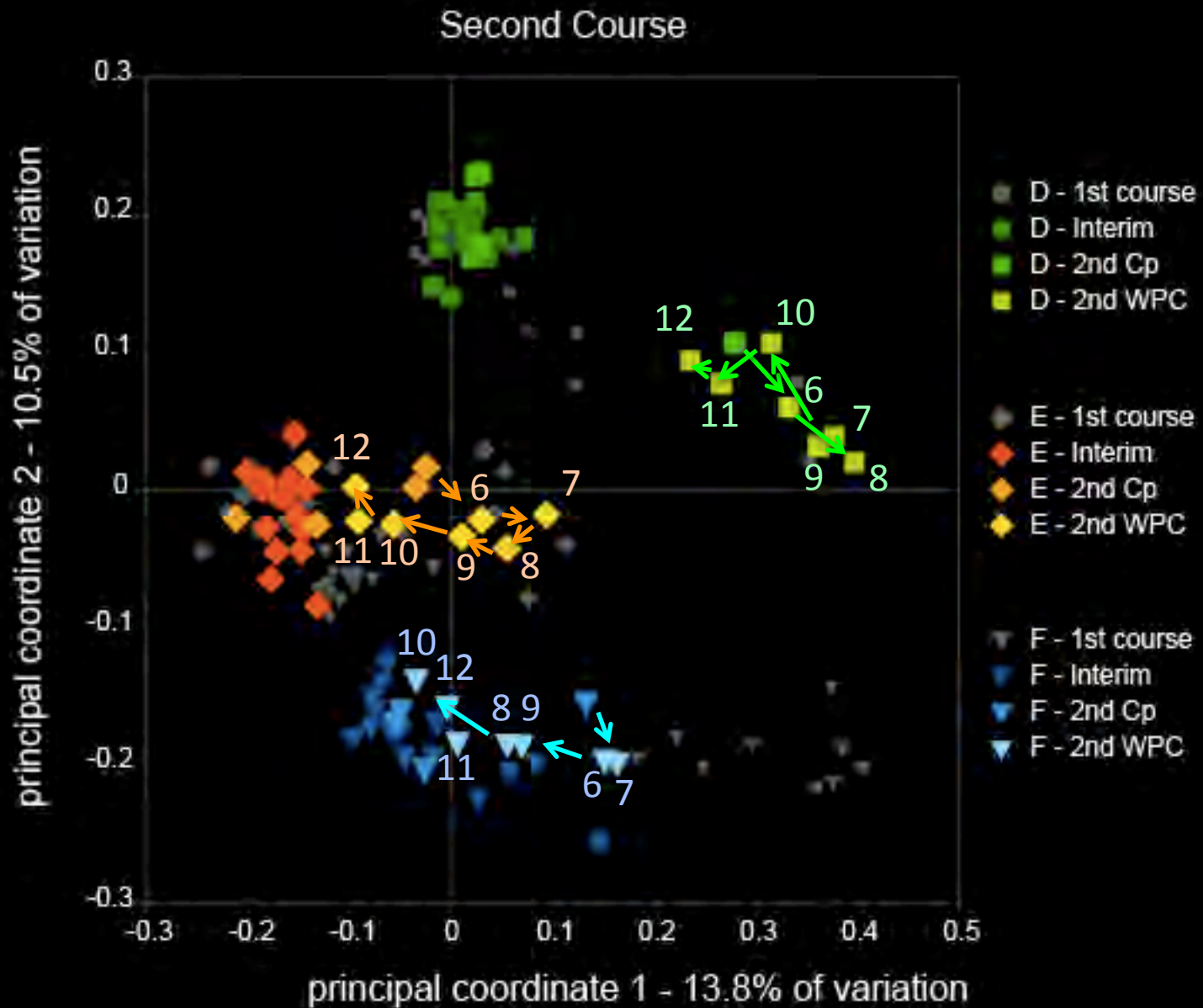


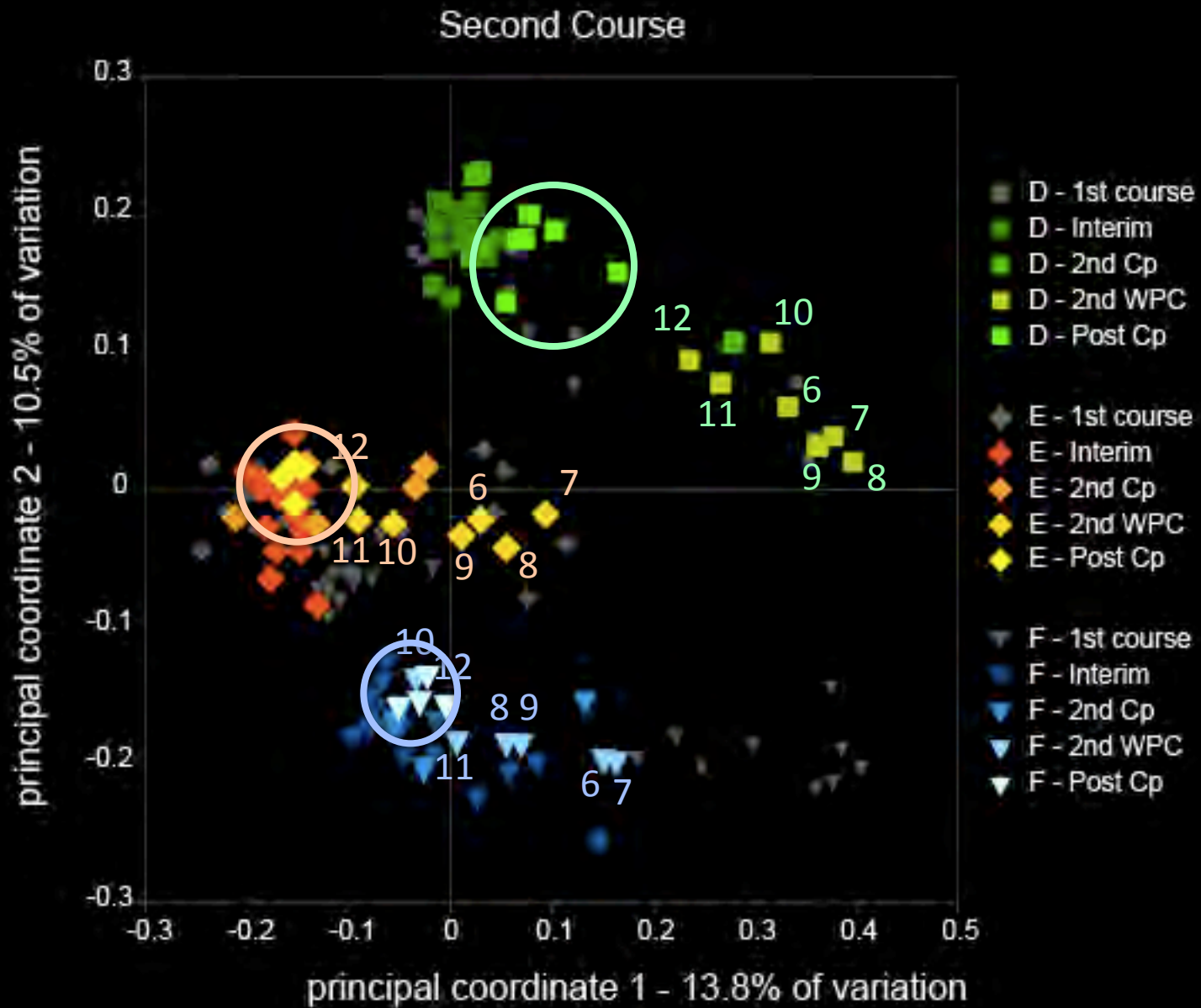


Shared, as well as individualized
responses





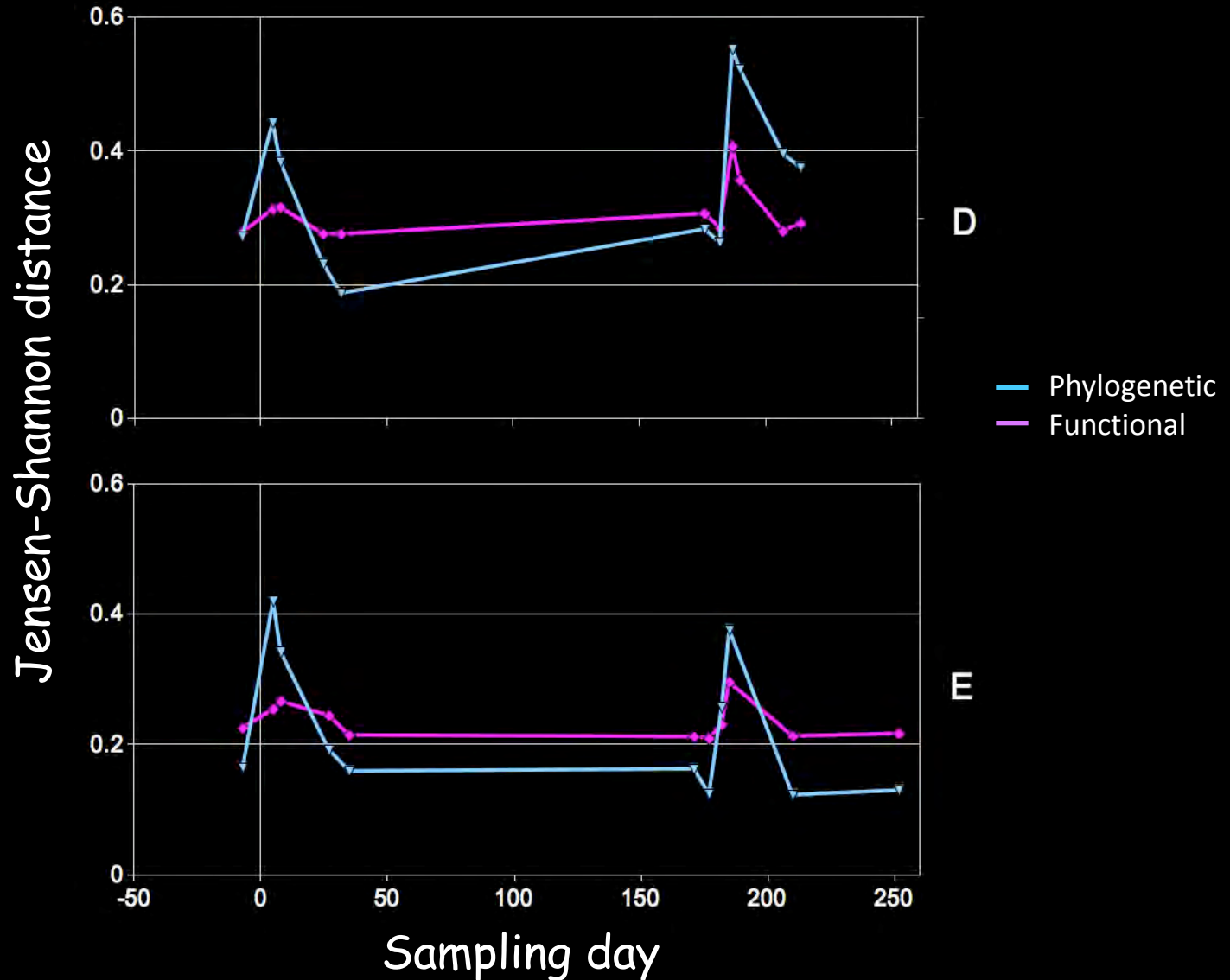




Community 'memory' ?

Compounded perturbations: alteration
of fitness landscape?

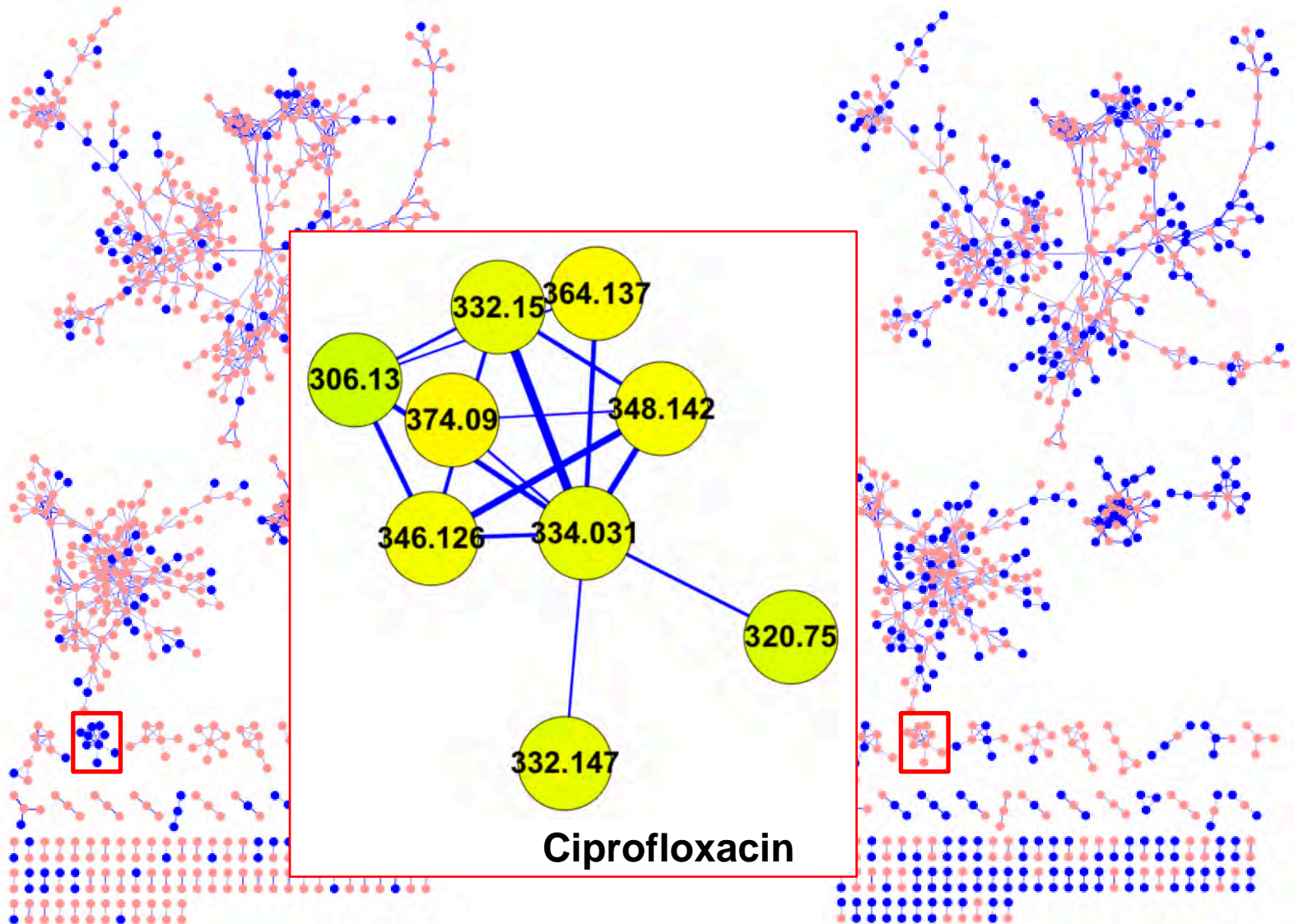
Effects of ciprofloxacin on taxonomic versus functional composition of distal gut microbiota



MS/MS network of subject D, second cipro course

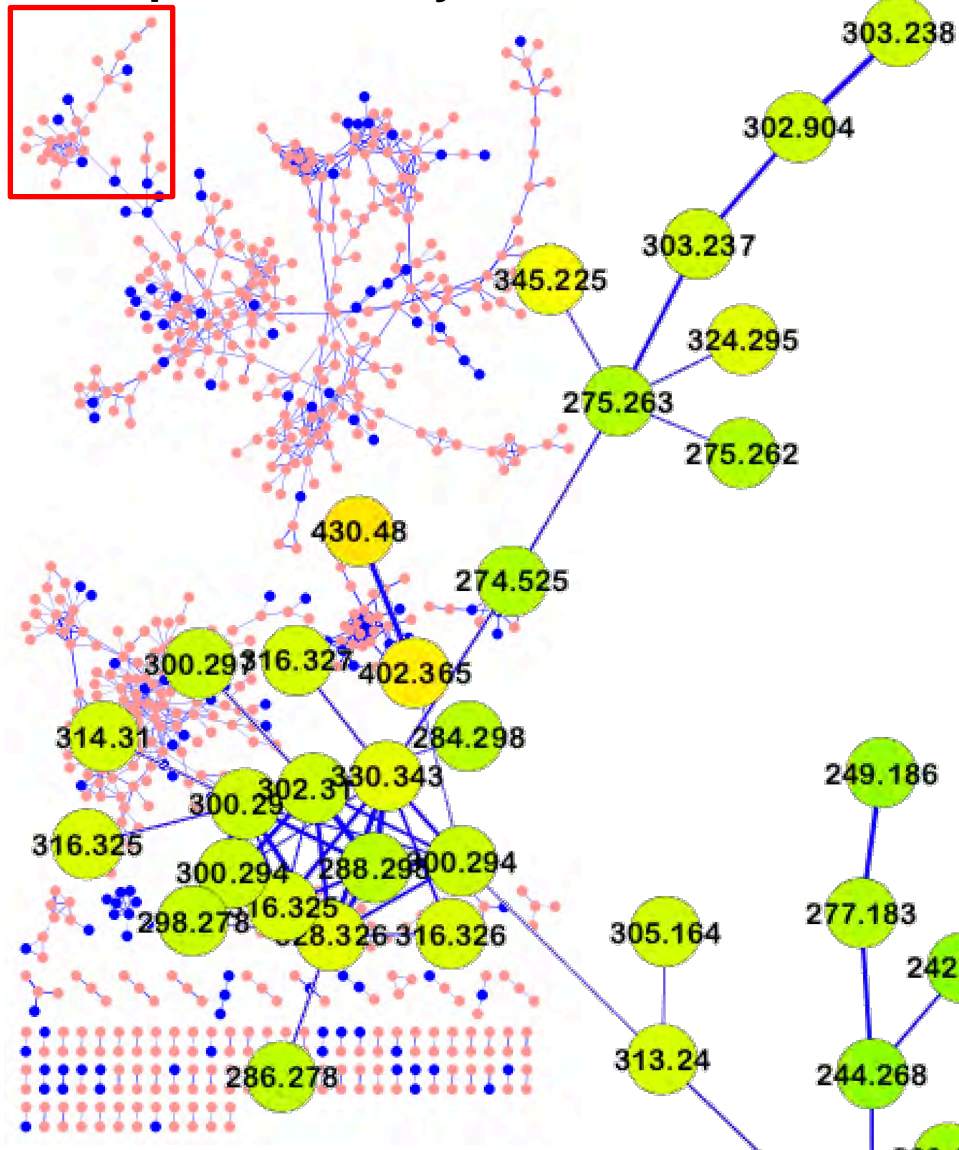
Ions only present on 4th day of cipro and 2 days after

Ions only present before cipro and weeks after cipro

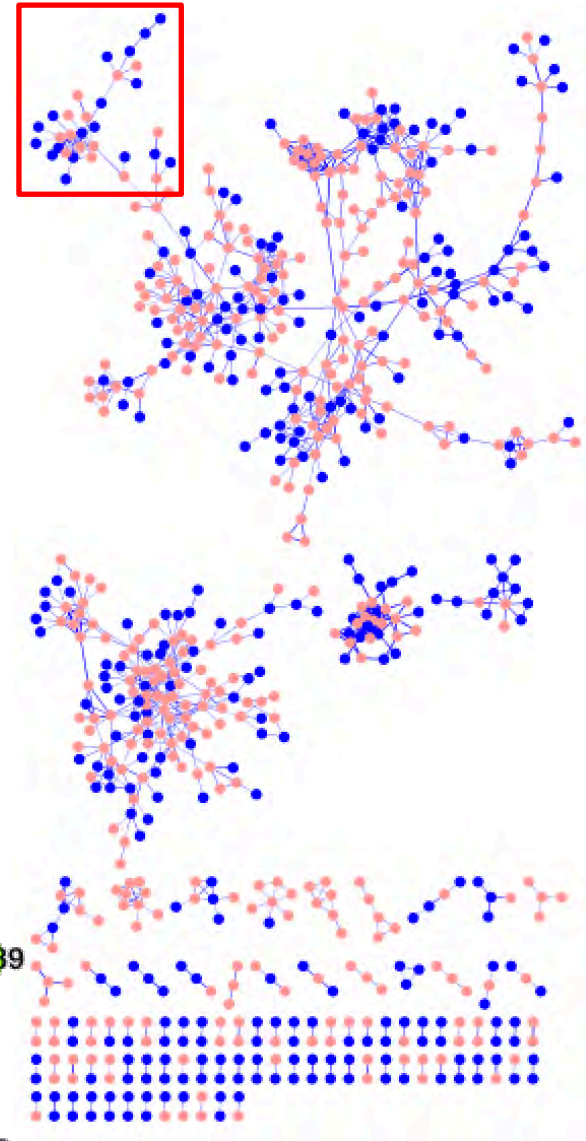


MS/MS network of subject D, second cipro course

Ions only present on 4th day of cipro and 2 days after



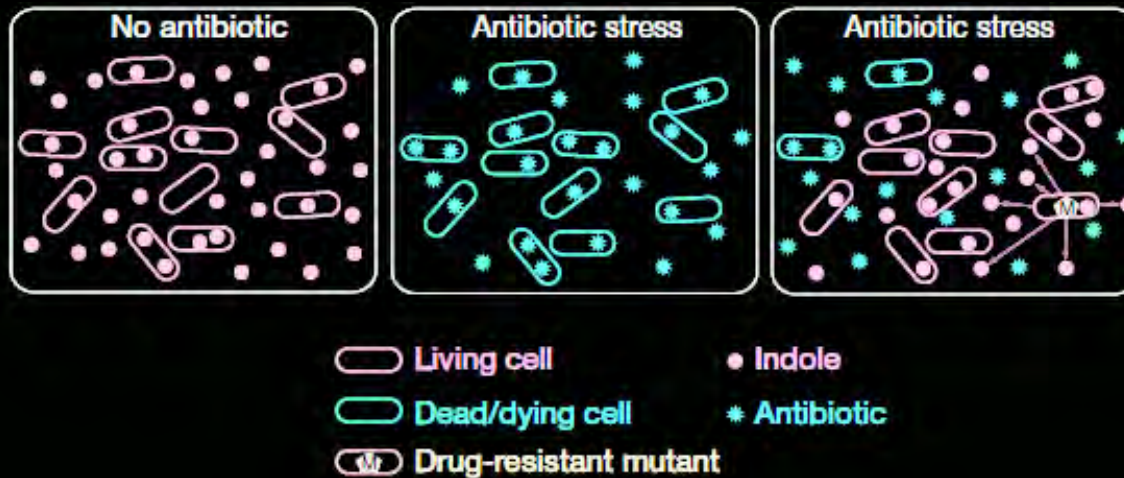
Ions only present before cipro and weeks after cipro



LETTERS

Bacterial charity work leads to population-wide resistance

Henry H. Lee^{1,2}, Michael N. Molla^{1,2}, Charles R. Cantor² & James J. Collins^{1,2,3}



Indole turns on drug efflux pumps and oxidative-stress protective mechanisms. Indole production comes at a fitness cost, made possible by drug resistance mutations unrelated to indole production.

Determinants of resilience ?

- ⇒ Species richness
- ⇒ Species evenness
- ⇒ Species interactions
(revealed by disturbance?)
- ⇒ Redundancy (insurance hypothesis)
- ⇒ Asynchronous responses by
different species in guild
- ⇒ Intrinsic versus extrinsic factors

Generic Indicators for Loss of Resilience Before a Tipping Point Leading to Population Collapse

Lei Dai,^{1*} Daan Vorselen,^{2*} Kirill S. Korolev,¹ Jeff Gore^{1†}

Theory predicts that the approach of catastrophic thresholds in natural systems (e.g., ecosystems, the climate) may result in an increasingly slow recovery from small perturbations, a phenomenon called critical slowing down. We used replicate laboratory populations of the budding yeast *Saccharomyces cerevisiae* for direct observation of critical slowing down before population collapse. We mapped the bifurcation diagram experimentally and found that the populations became more vulnerable to disturbance closer to the tipping point. Fluctuations of population density increased in size and duration near the tipping point, in agreement with the theory. Our results suggest that indicators of critical slowing down can provide advance warning of catastrophic thresholds and loss of resilience in a variety of dynamical systems.

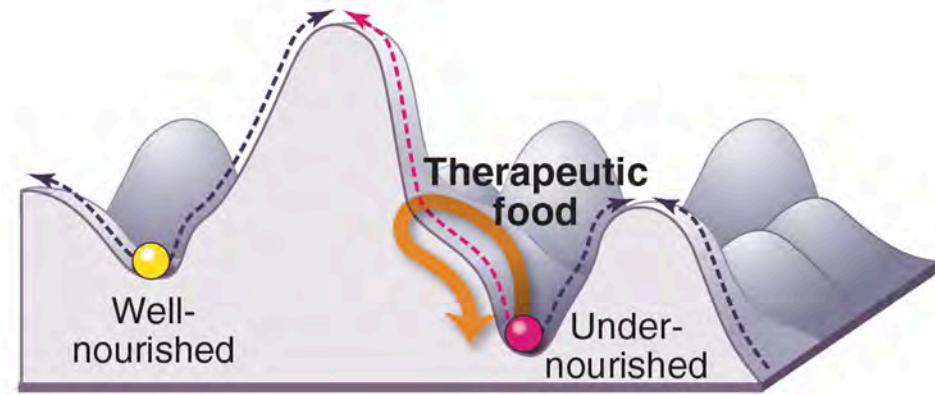
Science 2012 (June 1); 336:1175-1177

Gaps, Needs, Challenges

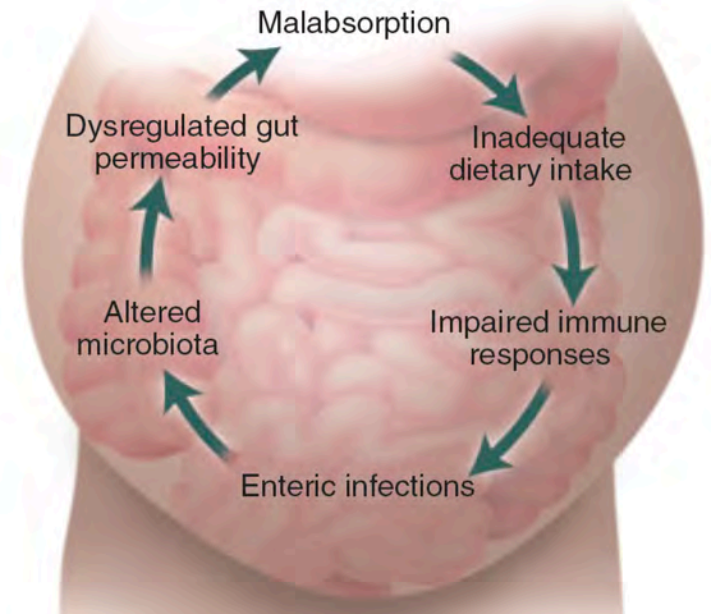


- Measuring function (ecosystem services)
- Stability of function with time
- Biological roles of low abundance members
- Host-microbiota interactions
- Describing the 'fitness landscape' of an individual (predictive understanding)
- Basis for ecosystem resilience?
- Strategies for maintenance and restoration of functions, resilience

Gut microbial ecosystem fitness landscape: undernutrition



Vicious cycle of undernutrition



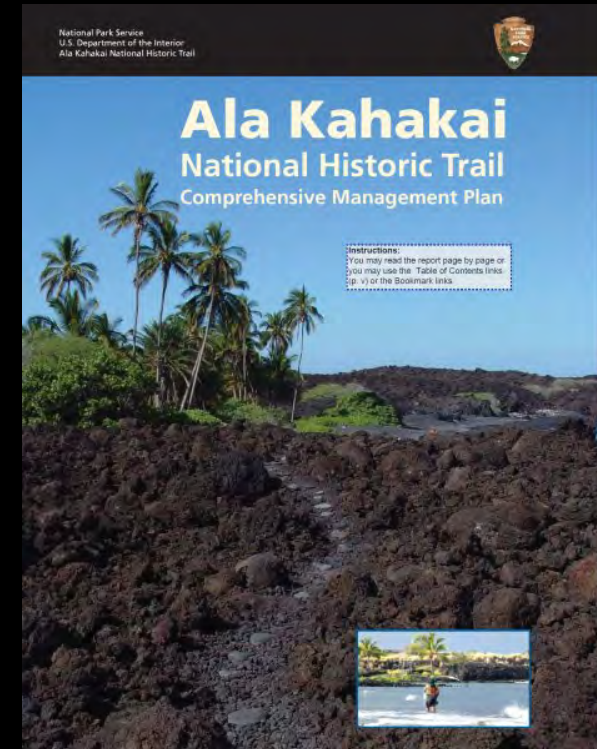
Science 339, 530 (2013)

Park Management Plan (for human microbial ecosystem)



- Habitat restoration
- Promotion of native species
- Targeted removal of invasive species (need precise diagnostics)

- Ecosystem service providers?
Community and host context?
- Adaptive management: system monitoring to inform decisions



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U. Colorado

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