Predictive models of microbiome dynamics

Designing bacterial cocktails to ameliorate enteric infections and to stimulate immune responses

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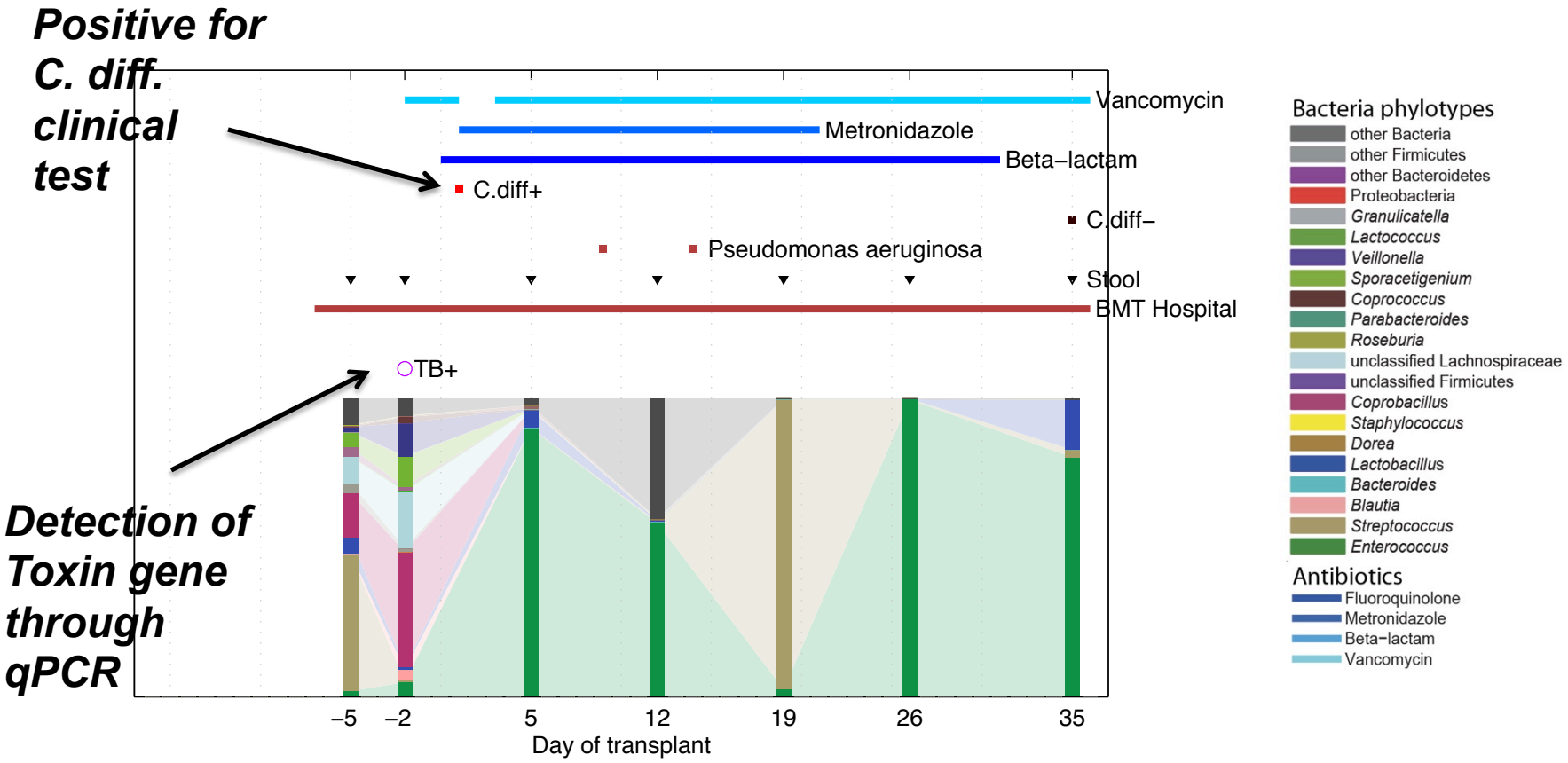
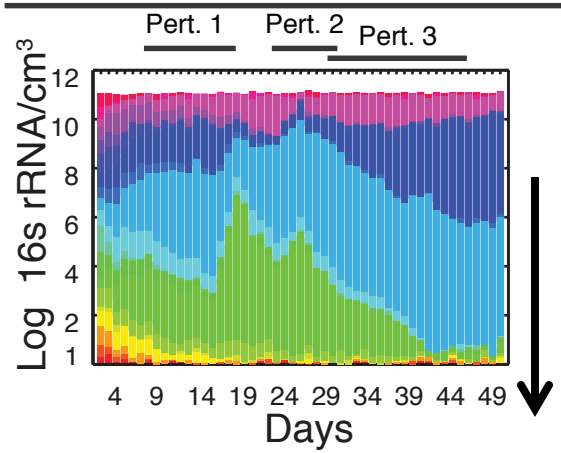
Antibiotic administration leads to enteric infections in BMT patients



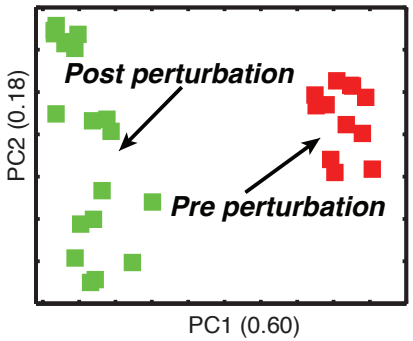
Building ecosystem dynamical models constrained on temporal observations

High-throughput community data and perturbation profiles



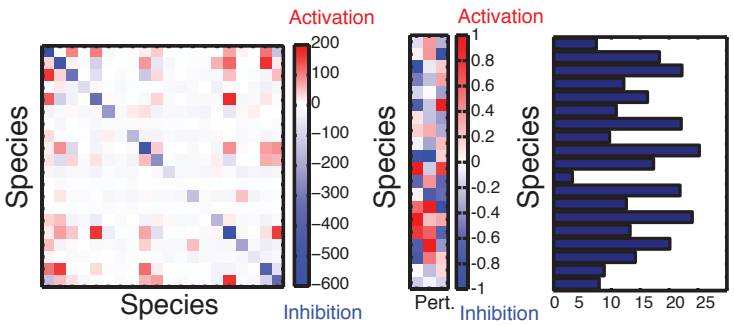
Cross-sectional analysis

Correspondence analysis

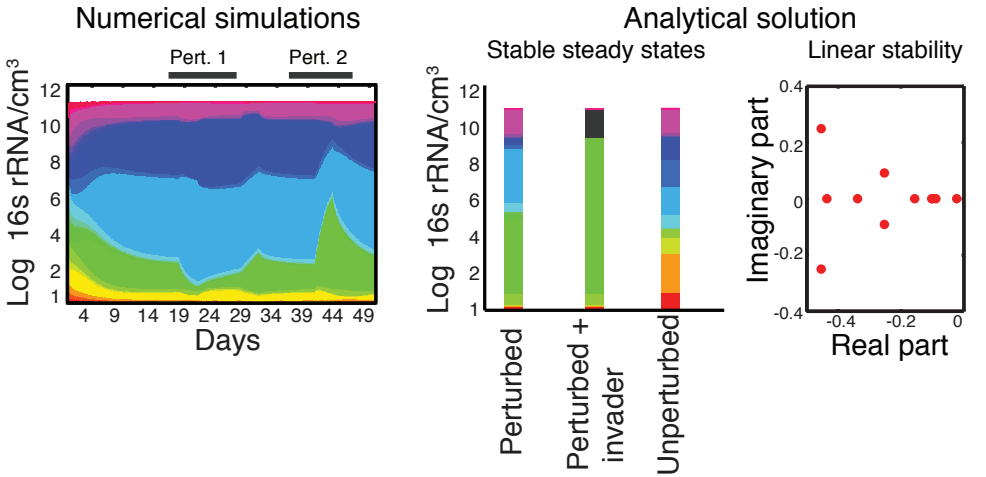


+ Statistical Tests
  (Kruskall–Wallis, Wilcoxon,…)
+ PCoA/NMDS
  (using Unifrac, Bray–Curtis, Euclidean)
+ Diversity Indices
  (Shannon, Chao,…)
+ Community Similarity Time Decay
+ Co-occurance (correlation) undirected networks

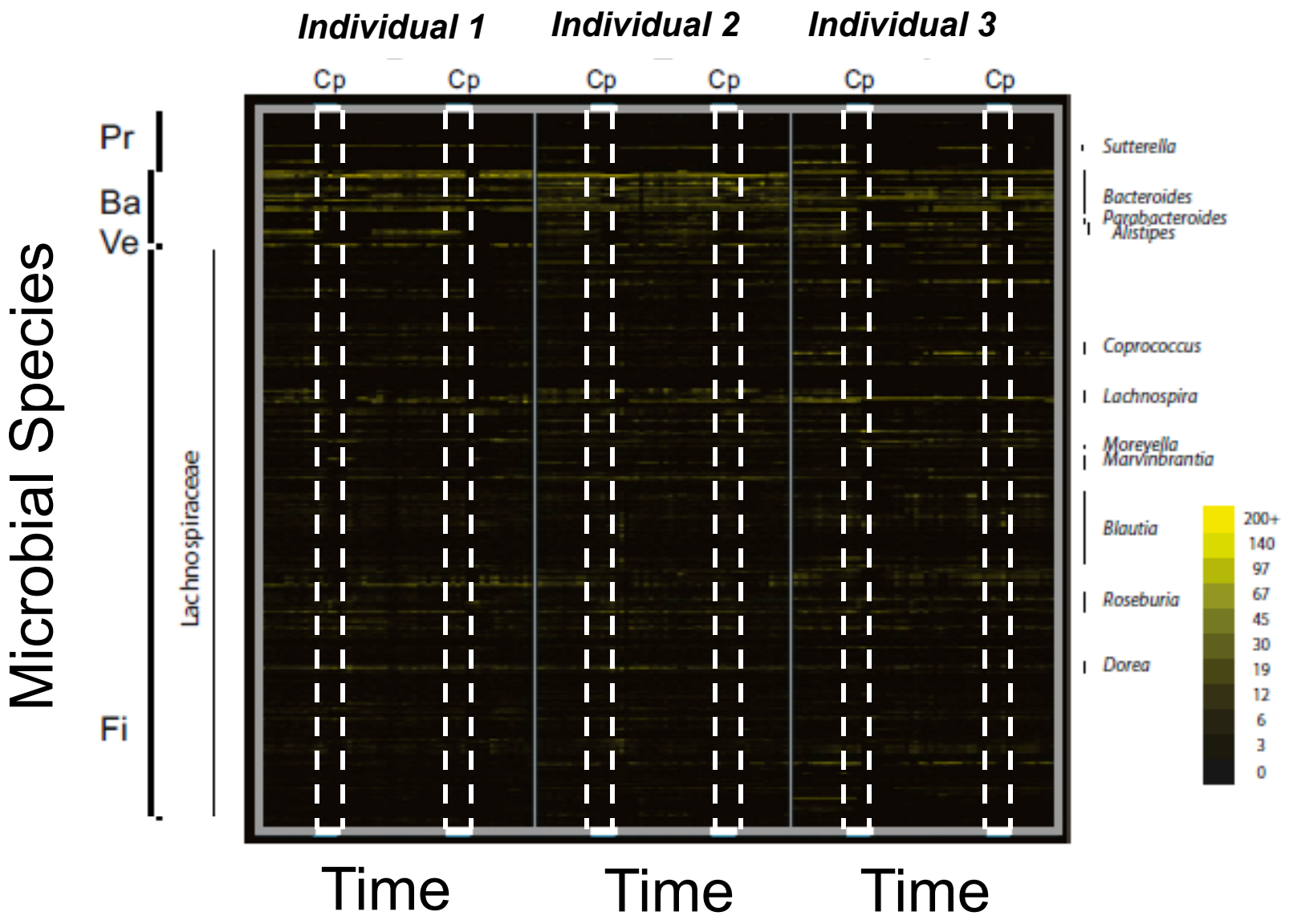
Community structure and response Inference



Ecosystem dynamics predictions



Microbiota treated with single antibiotic



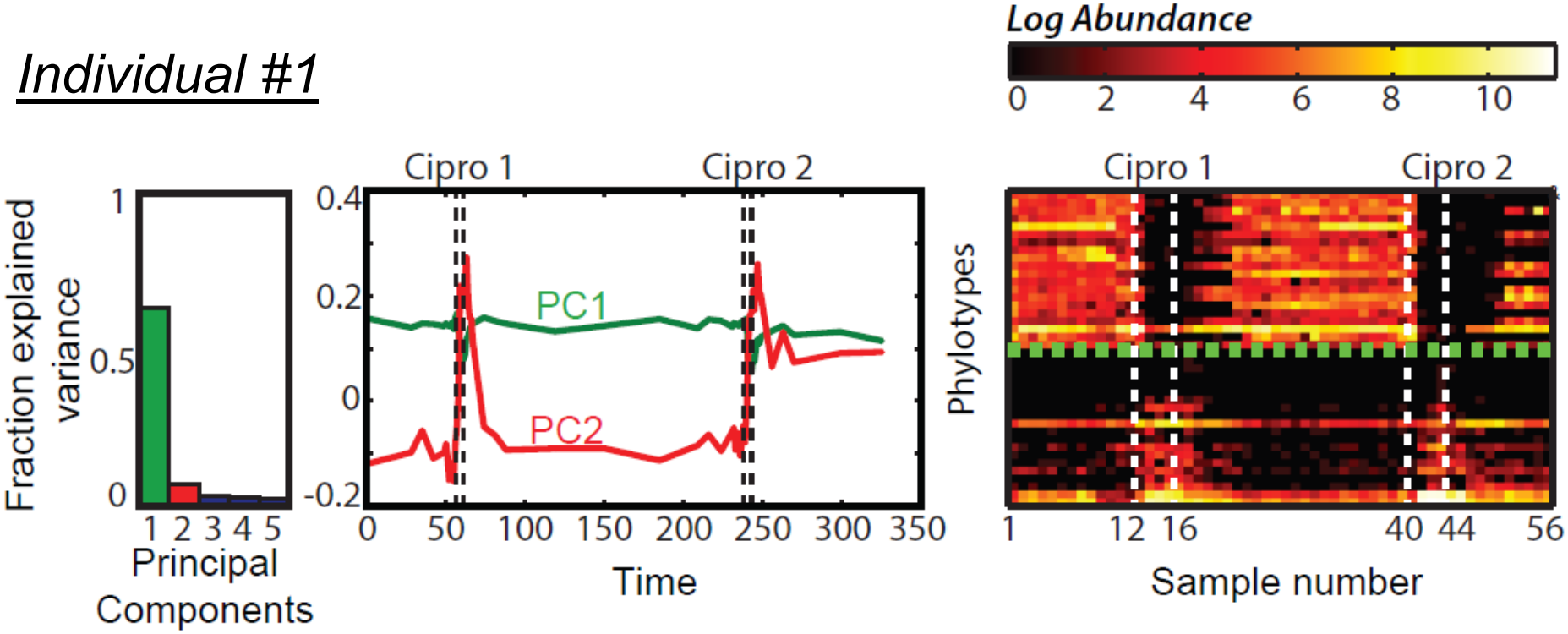
Dethlefsen & Relman (2011) PNAS

Microbes can be grouped according to antibiotic sensitivity

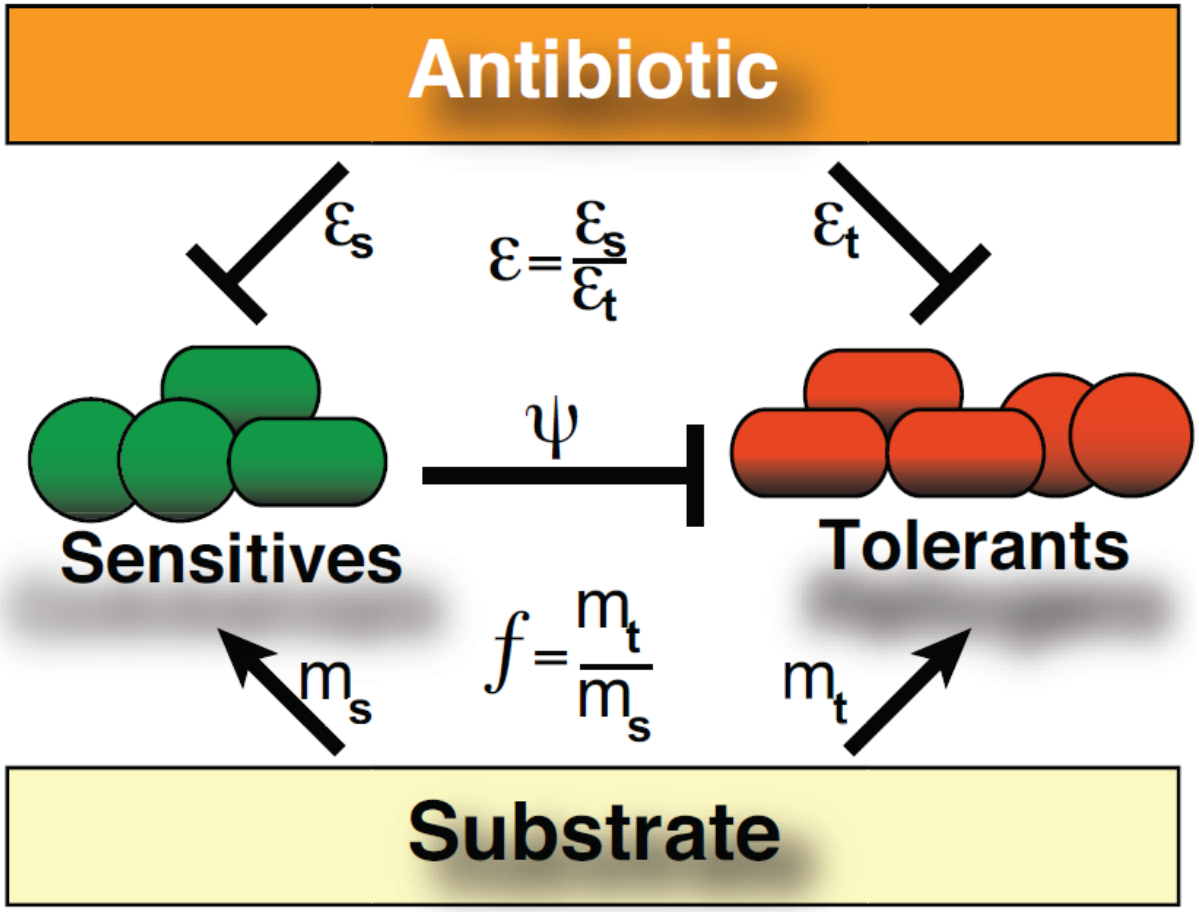
SVD on species correlation matrix:

- PC1 + PC2 > 70% Total information
- PC1 and PC2 have opposite stereotypical temporal behavior
- High Correlation PC1, Low correlation PC2 – antibiotic sensitives
- Low Correlation PC2, High correlation PC2 – antibiotic tolerants

Individual #1



A first minimal model to study microbiota dynamics



Bucci et al. (**2012**) *PLoS Comp. Bio.*

Microbiota modeled with four ordinary differential equations

Growth substrates

Inflow Dilution Consumption

$$\frac{dS}{dt} = 1 - S - \frac{m_s \rho_s}{S+a} S - \frac{m_t \rho_t}{S+a} S$$

Density of sensitives

Growth Antibiotic killing Dilution

$$\frac{d\rho_s}{dt} = \frac{m_s S}{S+a} \rho_s - \gamma A \rho_s - \rho_s$$

Density of tolerants

Growth Social pressure Dilution

$$\frac{d\rho_t}{dt} = \frac{m_t S}{S+a} \rho_t - \psi \rho_s \rho_t - \rho_t$$

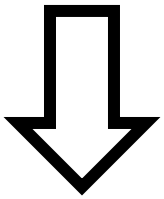
Antibiotic

Inflow Dilution

$$\frac{dA}{dt} = 1 - A$$

Microbiota modeled with two ordinary differential equations

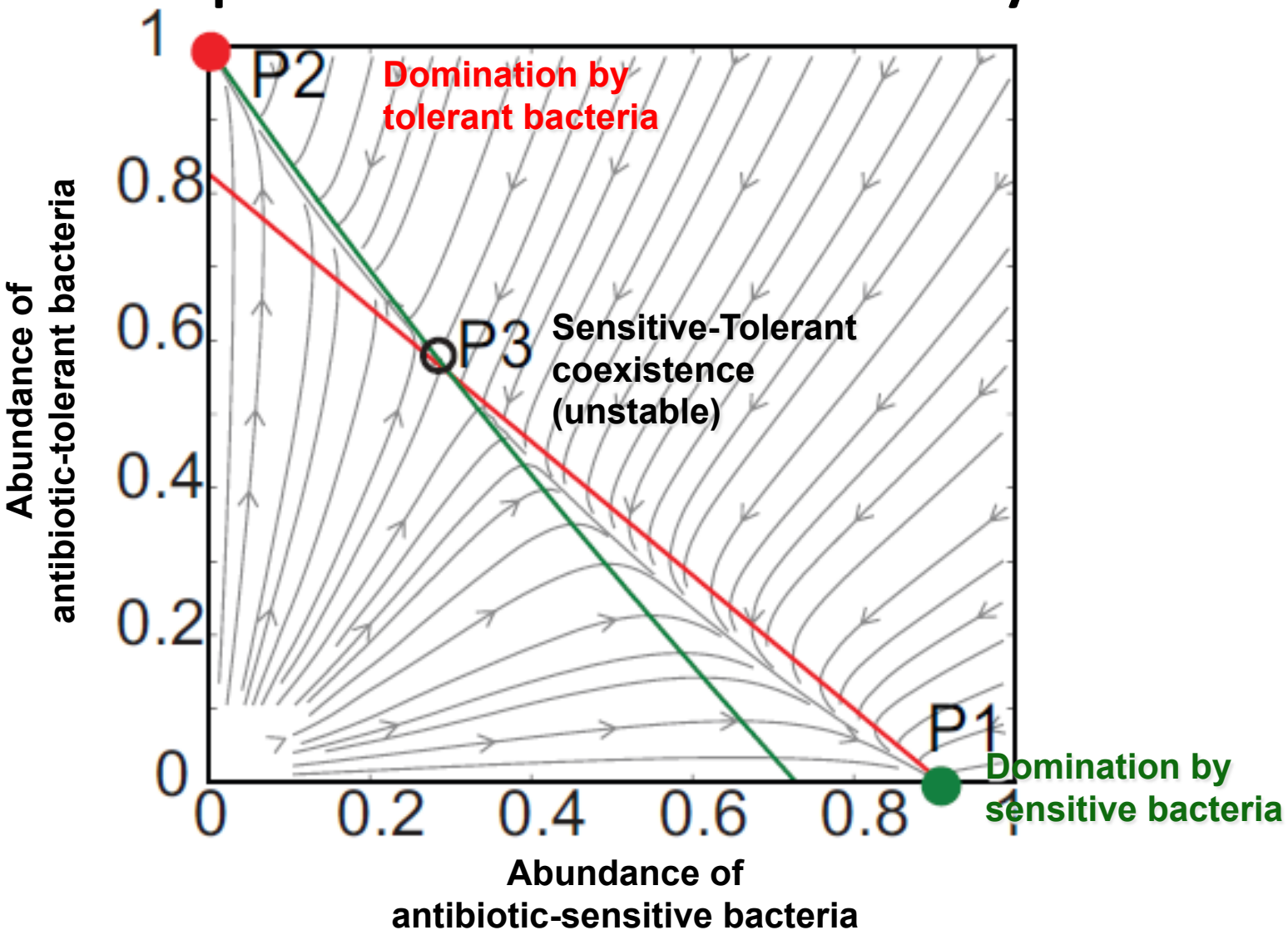
$$\frac{S}{S+a} = \frac{1}{m_s \rho_S + m_t \rho_t}$$



$$\frac{d\rho_s}{dt} = \frac{\rho_s}{\rho_s + f\rho_t} - \overbrace{(\gamma+1)}^{\varepsilon}\rho_s$$

$$\frac{d\rho_t}{dt} = \frac{f\rho_t}{\rho_s + f\rho_t} - (\psi\rho_s + 1)\rho_t$$

Antibiotic treatment and interactions produce multistability

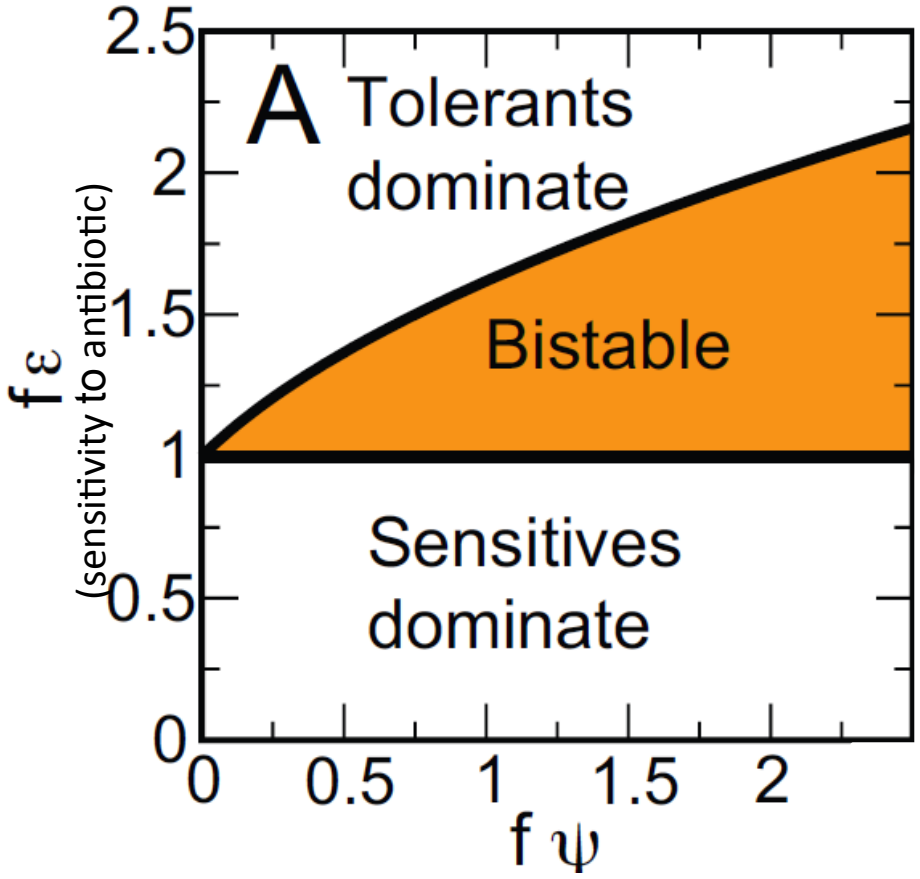


Bucci et al. (**2012**) *PLoS Comp. Bio.*

Model explains antibiotic-induced catastrophic shifts

Linear stability analysis:

-study stability of equilibrium points as function of parameters



What is the role of the external non-sterile environment?

- No influx of bacteria
  - Domination corresponds to competitor extinction
  - Impossible to revert to pre-treatment conditions

- Recovery time is finite and function of the level of isolation of the individual

- How external environment affects microbiota stability?

- Can we quantify the residence time in each microbiota state as a function of interactions, perturbations and exposure?

Microbiota modeled with two stochastic differential equations

Deterministic

Random noise

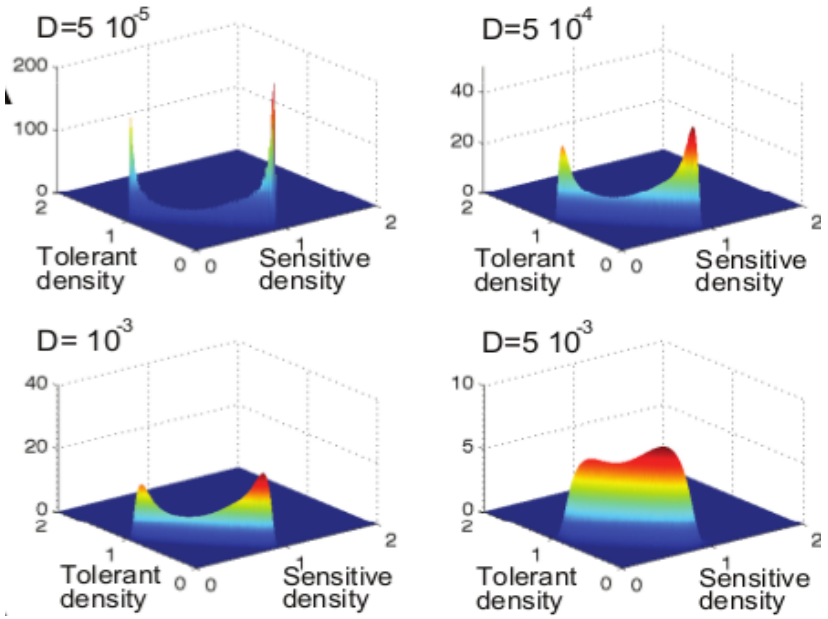
$$\frac{d\rho_s}{dt} = \frac{\rho_s}{\rho_s + f\rho_t} - \epsilon\rho_s + \xi_s(t) = F_s(\boldsymbol{\rho}) + \xi_s(t)$$

$$\frac{d\rho_t}{dt} = \frac{f\rho_t}{\rho_s + f\rho_t} - \psi\rho_s\rho_t - \rho_t + \xi_t(t) = F_t(\boldsymbol{\rho}) + \xi_t(t)$$

Analogy: over-damped system with additive noise in a non-conservative field of forces

Exposure to environment allows coexistence and reduces hysteresis

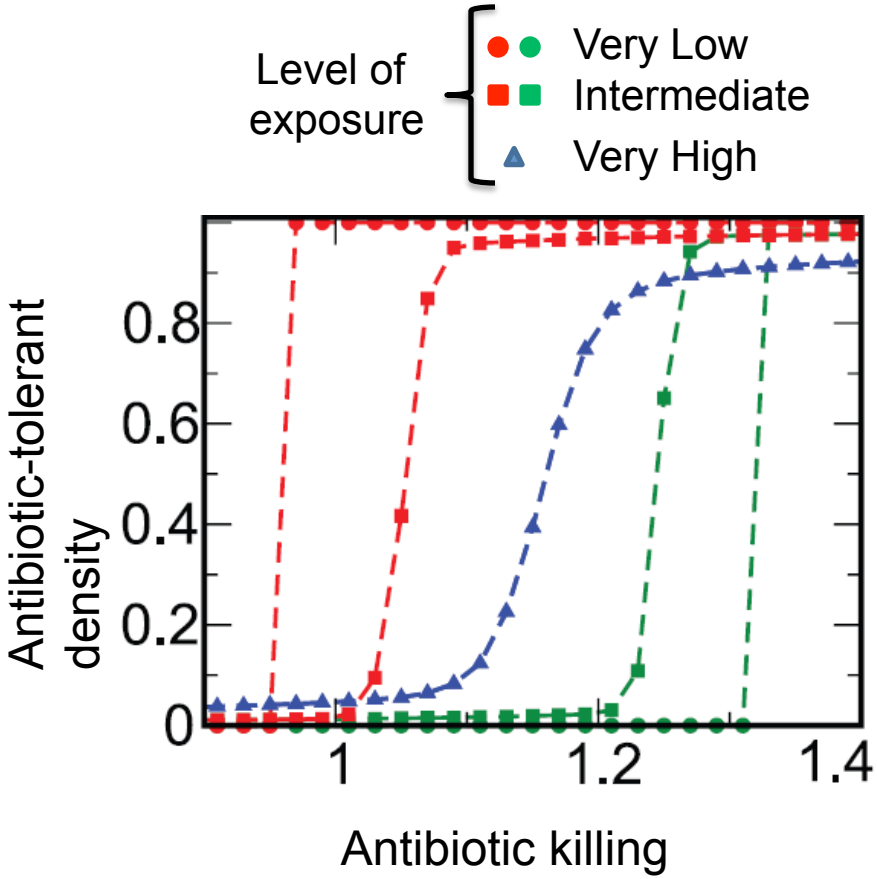
Fokker-Planck Equation



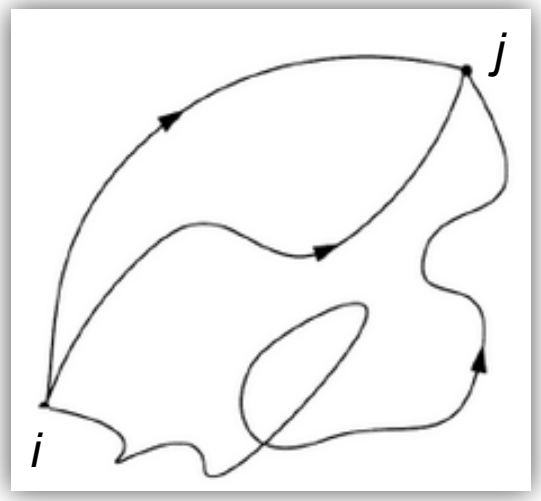
$$-\nabla \cdot (\mathbf{F}\, P_s) + \frac{D}{2}\nabla^2 P_s = 0$$

F: interactions + antibiotic effect

D: environmental exposure



Framework to quantify mean-residence domination time



Mean residence time in state *i*

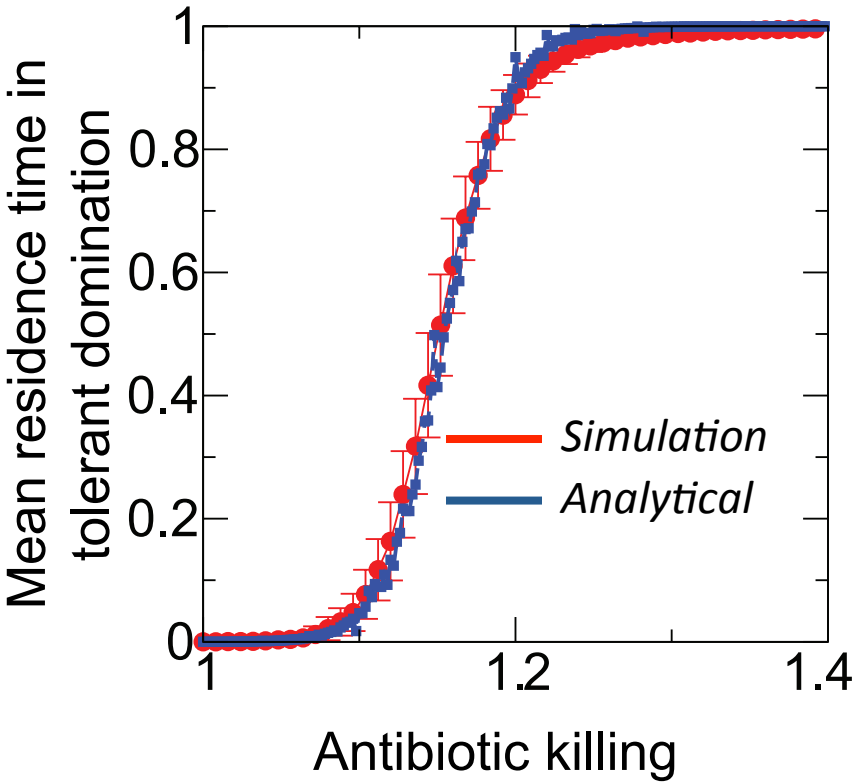
$$\pi_i = (1 + \mathcal{P}_{i\to j}/\mathcal{P}_{j\to i})^{-1}$$

Transition rate can be computed as

$$\mathcal{P}_{i\to j} \propto e^{-\frac{\mathcal{S}(\boldsymbol{\rho}^*)}{D}}$$

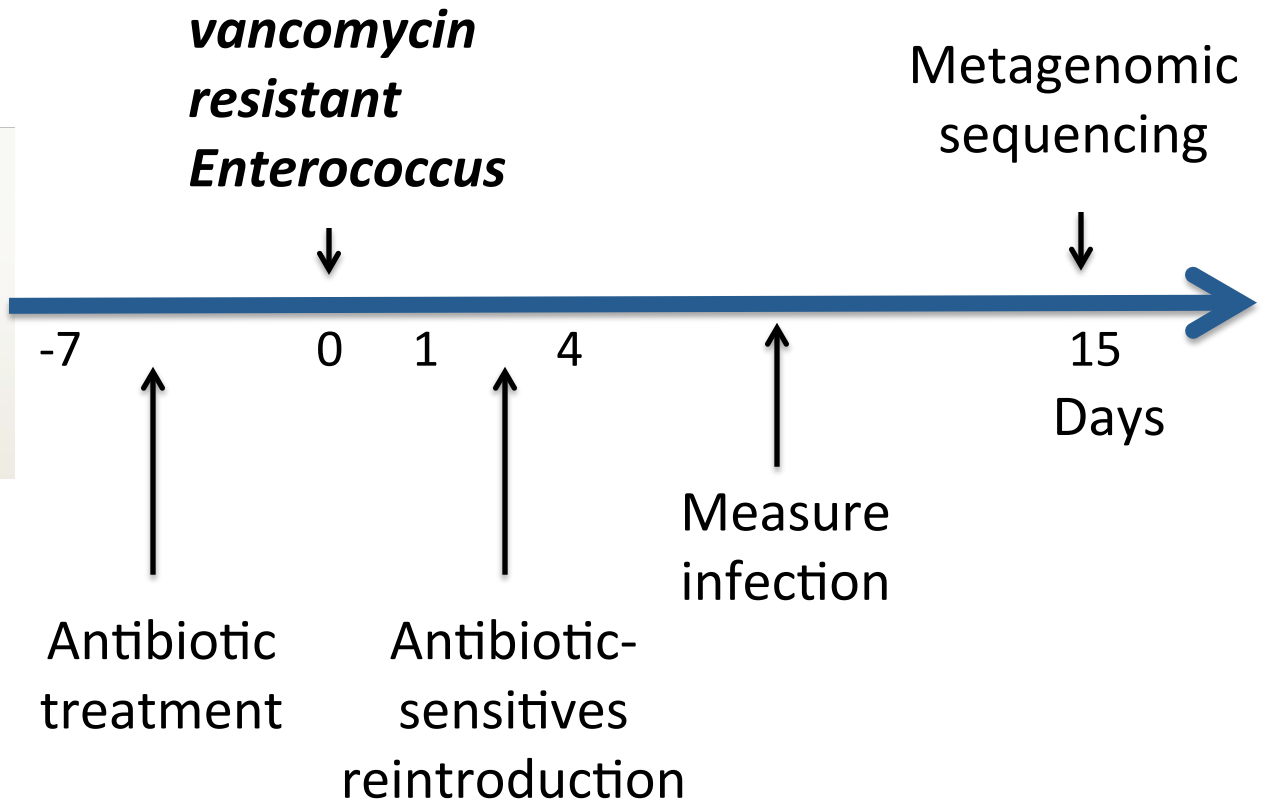
$$\mathcal{S}(\boldsymbol{\rho}^*) = \int_{t_i}^{t_f} dt' \ \left[|\dot{\boldsymbol{\rho}}^*(t')|^2 - \dot{\boldsymbol{\rho}}^*(t') \cdot \mathbf{F}(\boldsymbol{\rho}^*(t'))\right]$$

Computed by solving a system of II ordinary diff. equations



Bucci et al. (**2012**) *PLoS Comp. Bio.*

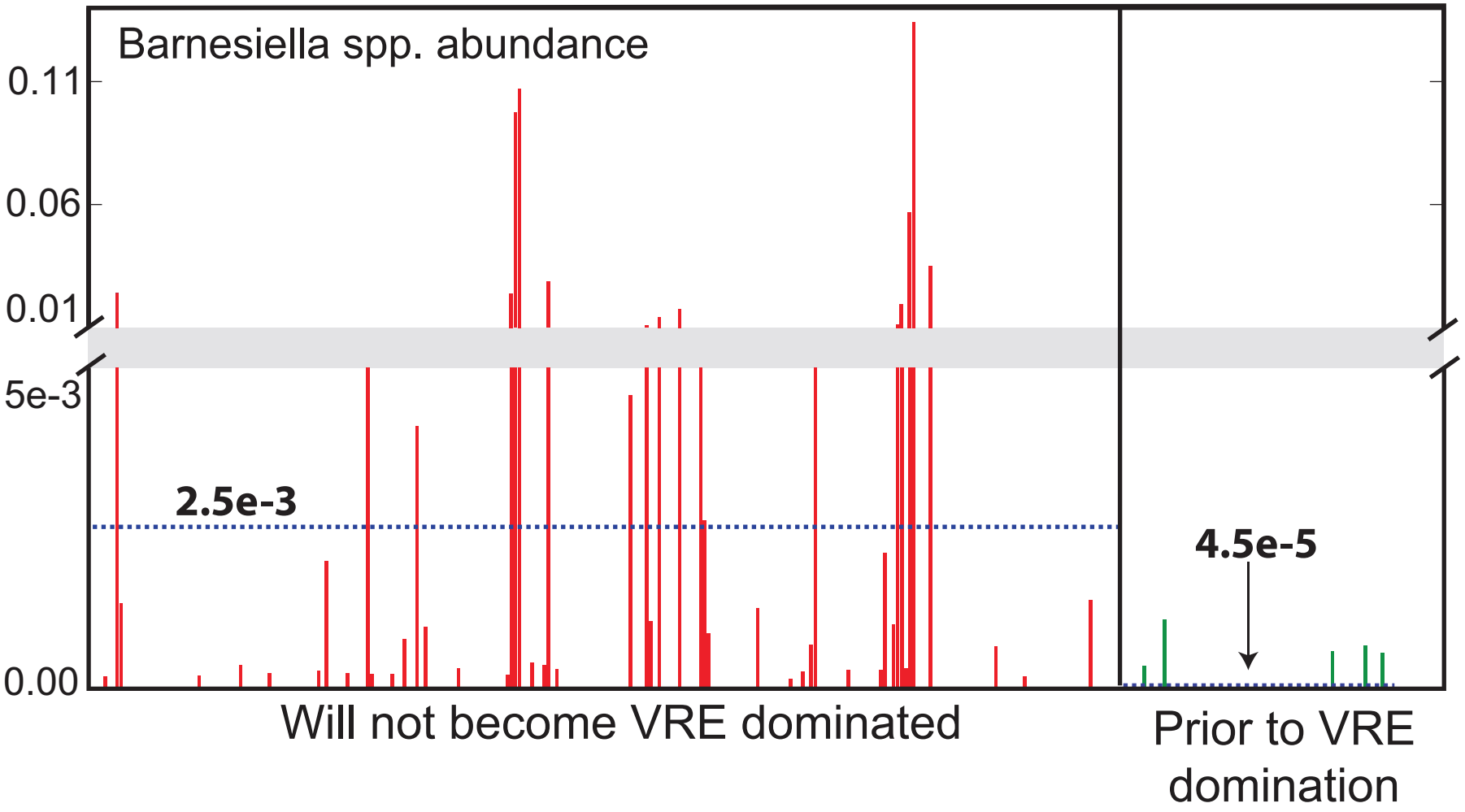
Reintroduction of antibiotic-sensitive microbes clears infection



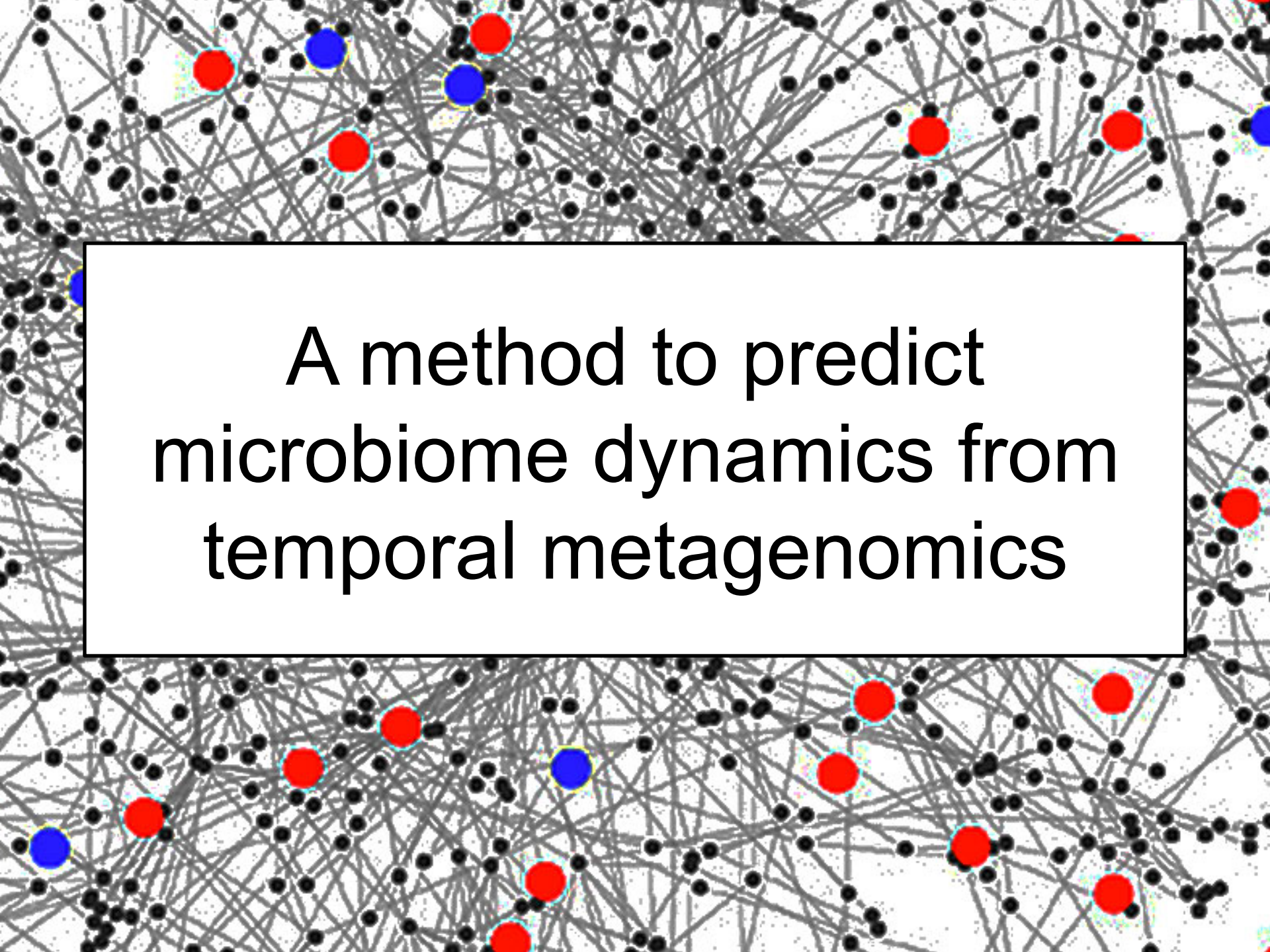
Barnesiella spp. strongly correlates with displacement of Vancomycin resistant Enterococcus

Ubeda, Bucci et al. (2013) *Inf. Immun*.

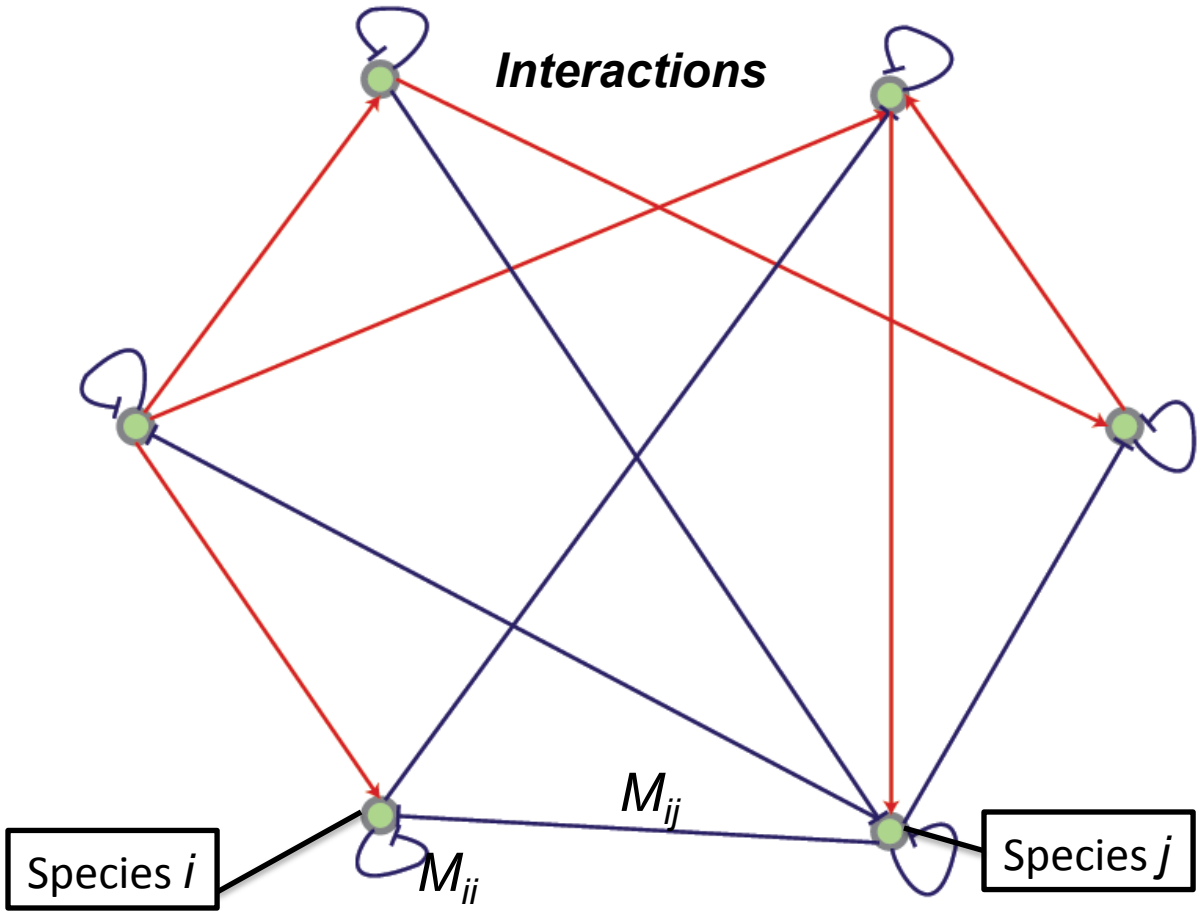
Barnesiella negatively correlates with VRE domination in patients



Ubeda, Bucci et al. (2013) *Inf. Immun.*

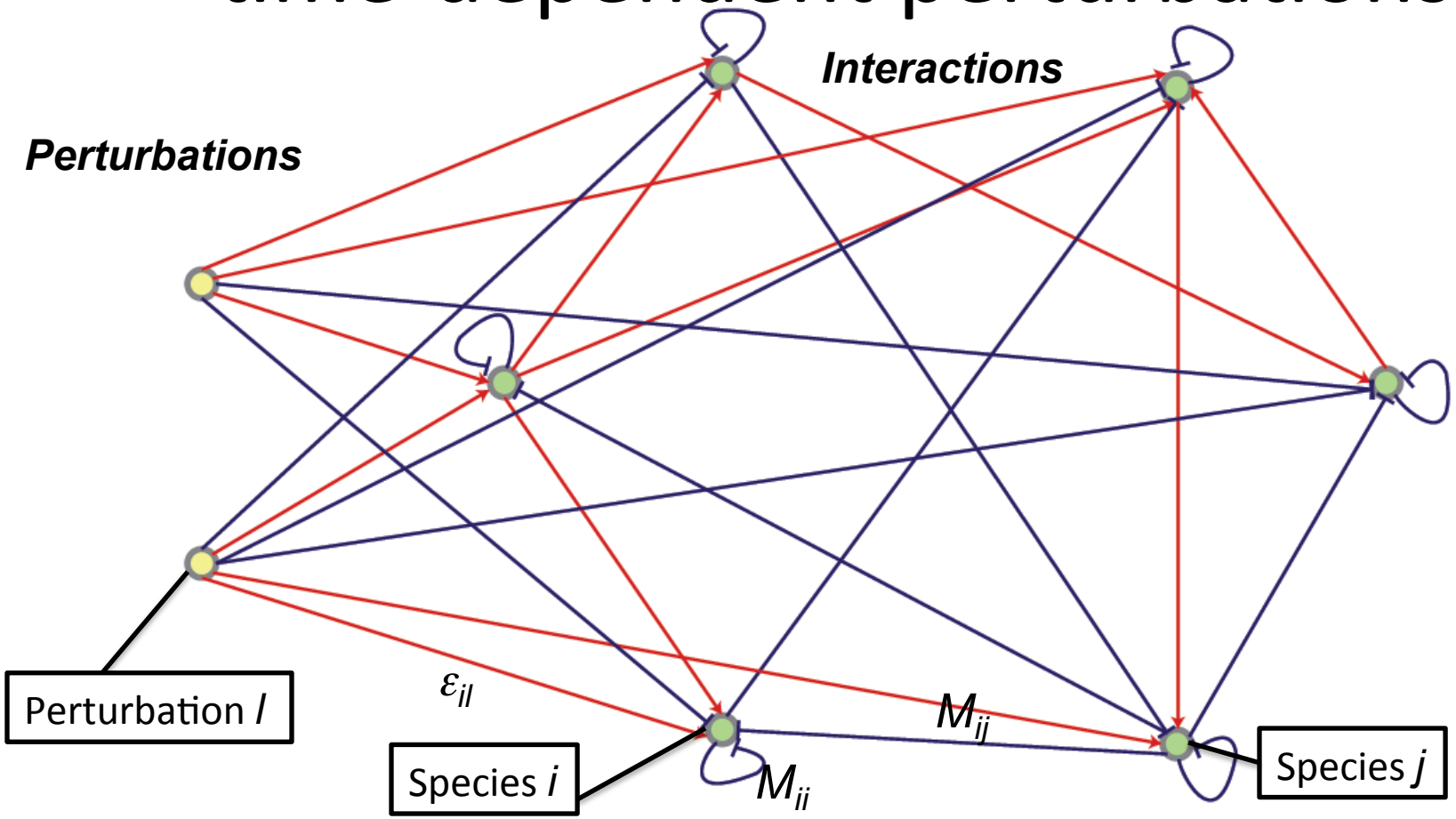

A method to predict
microbiome dynamics from
temporal metagenomics

Intestinal microbiota model under time-dependent perturbations



$$\frac{\mathrm{d}x_i(t)}{\mathrm{d}t} = \underbrace{\mu_i x_i(t)}_{\substack{\text{net} \\ \text{growth rate}}} + \underbrace{x_i(t) \sum_{j=1}^{L} M_{ij} x_j(t)}_{\text{interactions}}$$

Intestinal microbiota model under time-dependent perturbations



$$\frac{\mathrm{d}x_i(t)}{\mathrm{d}t} = \underbrace{\mu_i x_i(t)}_{\substack{\text{net} \\ \text{growth rate}}} + \underbrace{x_i(t)\sum_{j=1}^{L} M_{ij}x_j(t)}_{\text{interactions}} - \underbrace{x_i(t)\sum_{l=1}^{P}\varepsilon_{il}u_l(t)}_{\text{perturbations}}$$

Inference of microbiota ecology from metagenomics

- Fit the model to time-series data

- Defining:

$$F = \left(\frac{\Delta \ln x_i(t_k)}{\Delta t_k} \right)_{i,k} \qquad Y = \begin{pmatrix} (x_i(t_k))_{i,k} \\ (1)_k \\ (u_l(t_k))_{l,k} \end{pmatrix}$$

Linear Algebra inversion problem

$$F = \begin{pmatrix} M & \boldsymbol{\mu} & E \end{pmatrix} Y$$

Inference of microbiota ecology from metagenomics

- Matrix is not fully ranked
- Use least square minimization with Tikhonov regularization

$$\min\left\{\left\|\begin{pmatrix} M & \boldsymbol{\mu} & -E \end{pmatrix} Y - F\right\|_2^2 + \lambda_1 \left\|M\right\|_2^2 + \lambda_2 \left\|\boldsymbol{\mu}\right\|_2^2 + \lambda_3 \left\|E\right\|_2^2\right\}$$

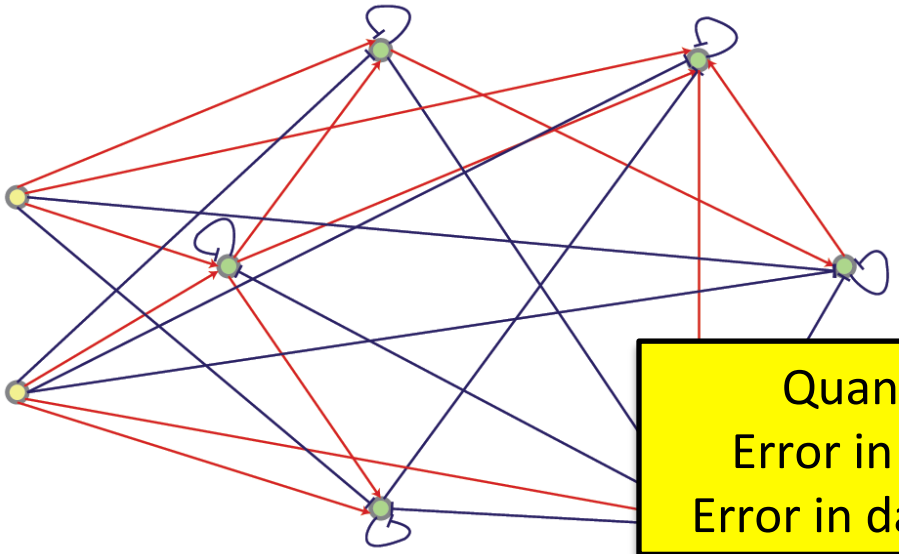
Regularization parameters

- Optimal regularization obtained by k-fold cross-validation

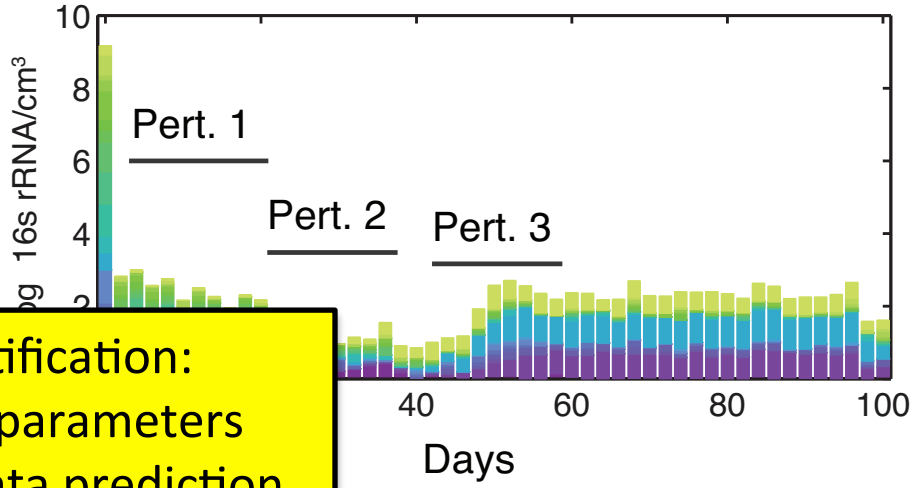
$$\left\|F^{\text{test}} - \begin{pmatrix} M & \boldsymbol{\mu} & E \end{pmatrix}_{\boldsymbol{\lambda}}^{\text{train}} Y^{\text{test}}\right\|_2^2$$

In silico inference testing

build a random (but known) network model

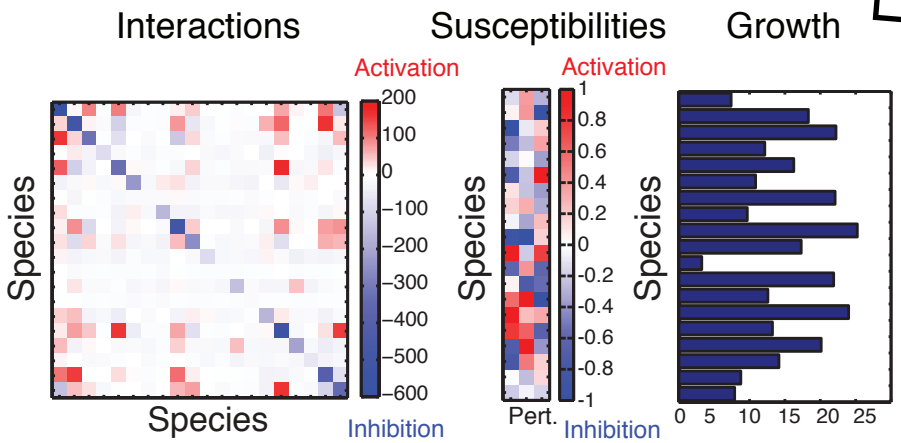


generate trajectories

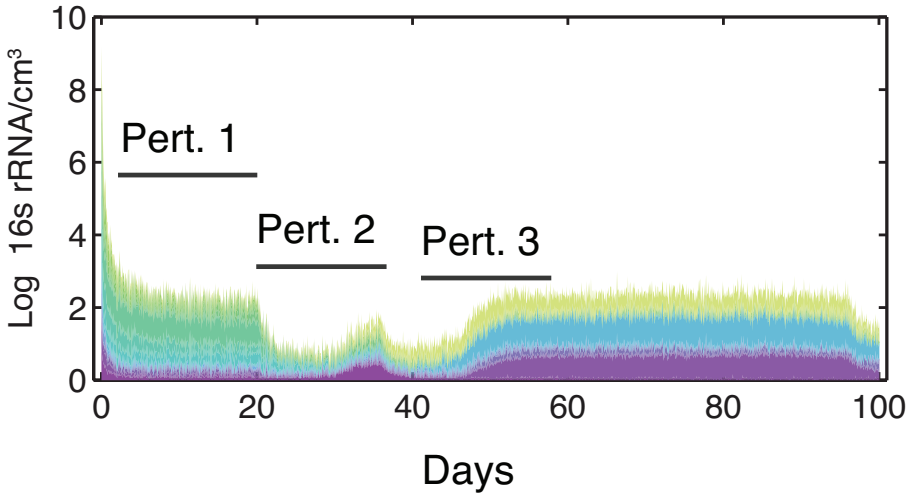


Quantification:
Error in parameters
Error in data prediction

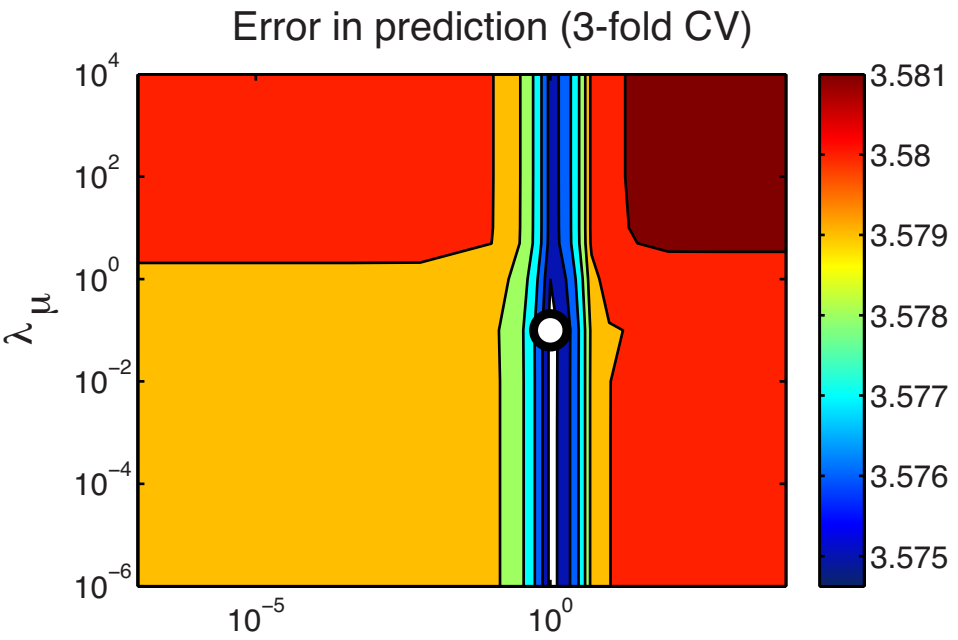
inference of model parameters

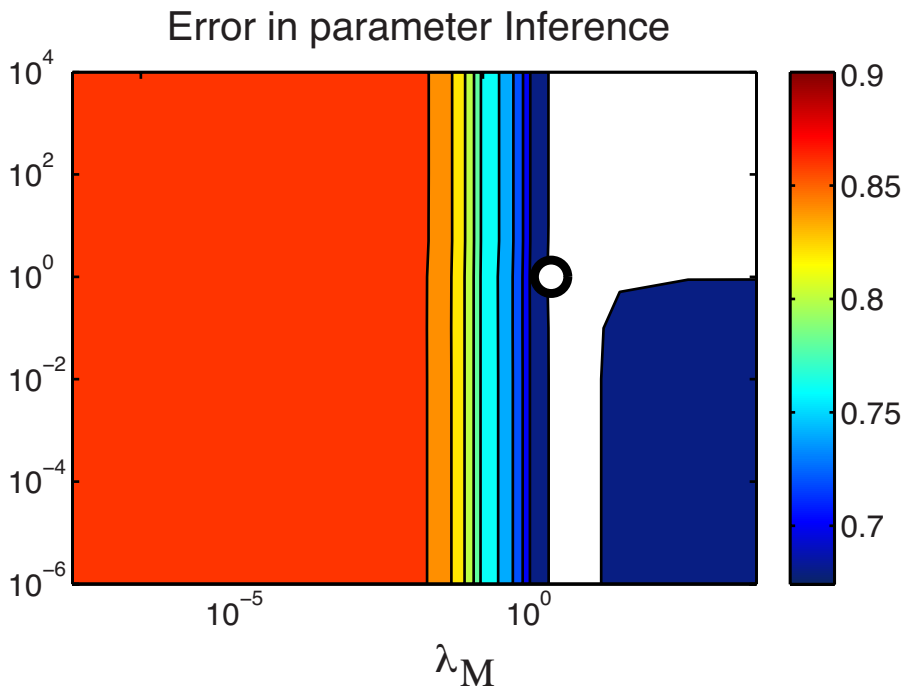


predictions of dynamics



Regularization minimizes error in parameters inference & data prediction





Network size: 20
Number replicated trajectories: 10
Number of randomnly administered perturbations: 3
Level of super-imposed white noise: 0.01
Results displayed for best λ_E=0.1

Stein, Bucci et al. (**2013**) *PLoS. Comp. Biol.*

Clostridium difficile: a low-abundant habitant of the human intestinal flora

- Gram positive bacteria of the genus *Clostridium*
- It normally reside in the human intestinal flora at very low abundances (2-5%)

Antibiotic administration cause *Clostridium difficile* infections

IMPORTANT WARNING:

Many antibiotics, including clindamycin, may cause overgrowth of dangerous bacteria in the large intestine. This may cause mild diarrhea or may cause a life-threatening condition called colitis (inflammation of the large intestine). Clindamycin is more likely to cause this type of infection than many other antibiotics, so it should only be used to treat serious infections that cannot be treated by other antibiotics. Tell your doctor if you have or have ever had colitis or other conditions that affect your stomach or intestines.

You may develop these problems during your treatment or up to several months after your treatment has ended. Call your doctor if you experience any of the following symptoms during your treatment with clindamycin or during the first several months after your treatment has finished: watery or bloody stools, diarrhea, stomach cramps, or fever.

Talk to your doctor about the risks of taking clindamycin.

http://www.nlm.nih.gov/medlineplus/druginfo/meds/a682399.html

- Prophylactic antibiotic administration increases the risk of *C. difficile* infections
- Symptoms range from diarrheas to pseudomebraneus colitis and toxic megacolon
- 3 million CDIs in US every year
- Immune compromised individual (e.g. BMT patients) are highly susceptible
- Treatment with Vancomycin (or Metronidazole) do worse → recurrent CDI and selection for other antibiotic-resistant bacteria (VRE)

CDI cleared by faecal transplant

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The New York Times

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When Pills Fail, This, er, Option Provides a Cure

By DENISE GRADY
Published: January 16, 2013 | 152 Comments

The treatment may sound appalling, but it works.


Enlarge This Image

Transplanting feces from a healthy person into the gut of one who is sick can quickly cure severe intestinal infections caused by a dangerous type of bacteria that antibiotics often cannot control.

A new study finds that such transplants cured 15 of 16 people who had recurring infections with Clostridium difficile bacteria, whereas antibiotics cured only 3 of 13 and 4 of 13 patients in two comparison groups. The treatment appears to work by restoring the gut's normal balance of bacteria, which fight off C. difficile.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*

Els van Nood, M.D., Anne Vrieze, M.D., Max Nieuwdorp, M.D., Ph.D., Susana Fuentes, Ph.D., Erwin G. Zoetendal, Ph.D., Willem M. de Vos, Ph.D., Caroline E. Visser, M.D., Ph.D., Ed J. Kuijper, M.D., Ph.D., Joep F.W.M. Bartelsman, M.D., Jan G.P. Tijssen, Ph.D., Peter Speelman, M.D., Ph.D., Marcel G.W. Dijkgraaf, Ph.D., and Josbert J. Keller, M.D., Ph.D.

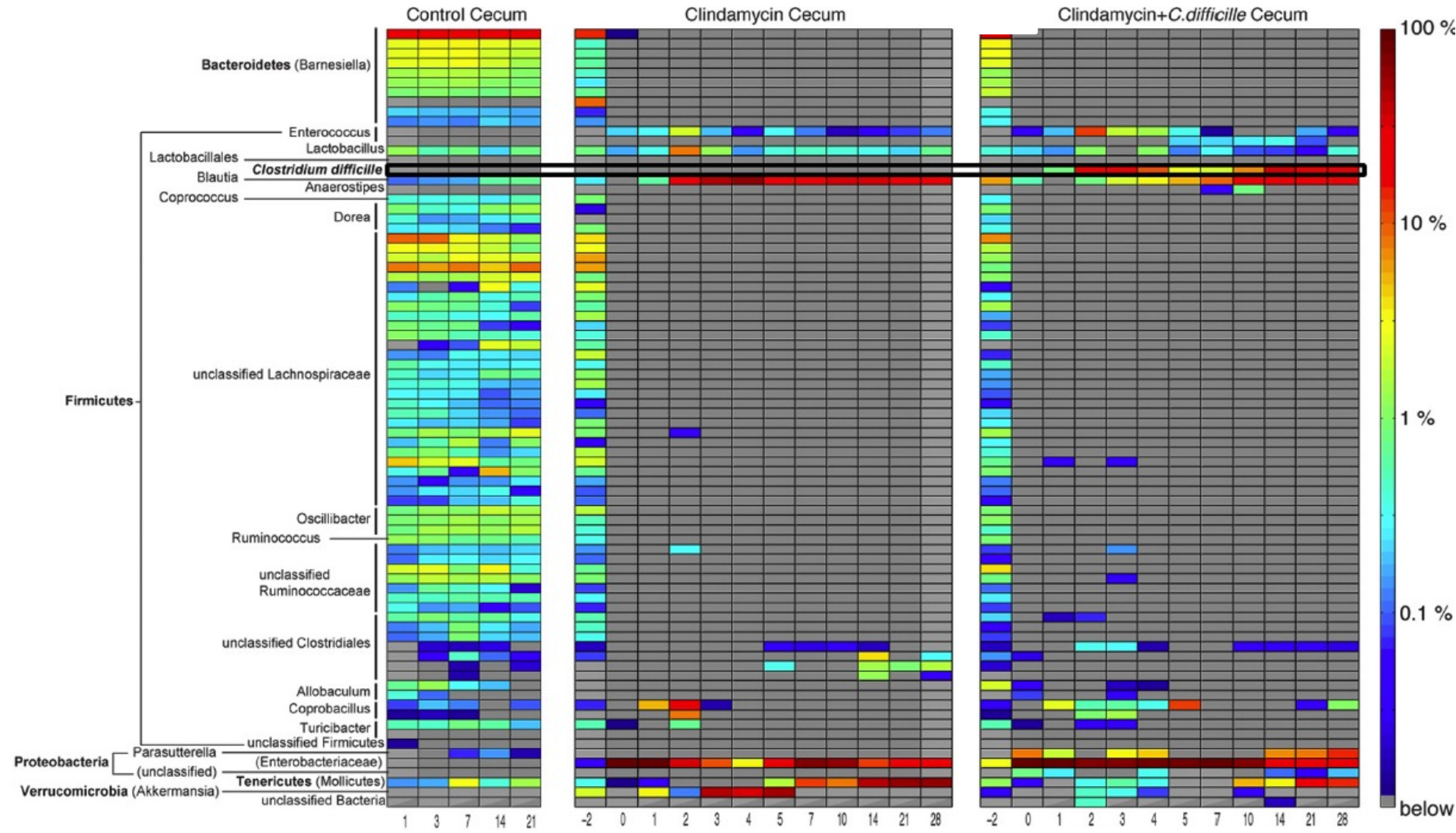
CONCLUSIONS

The infusion of donor feces was significantly more effective for the treatment of recurrent *C. difficile* infection than the use of vancomycin. (Funded by the Netherlands Organization for Health Research and Development and the Netherlands Organization for Scientific Research; Netherlands Trial Register number, NTR1177.)

What species protect from CDI?

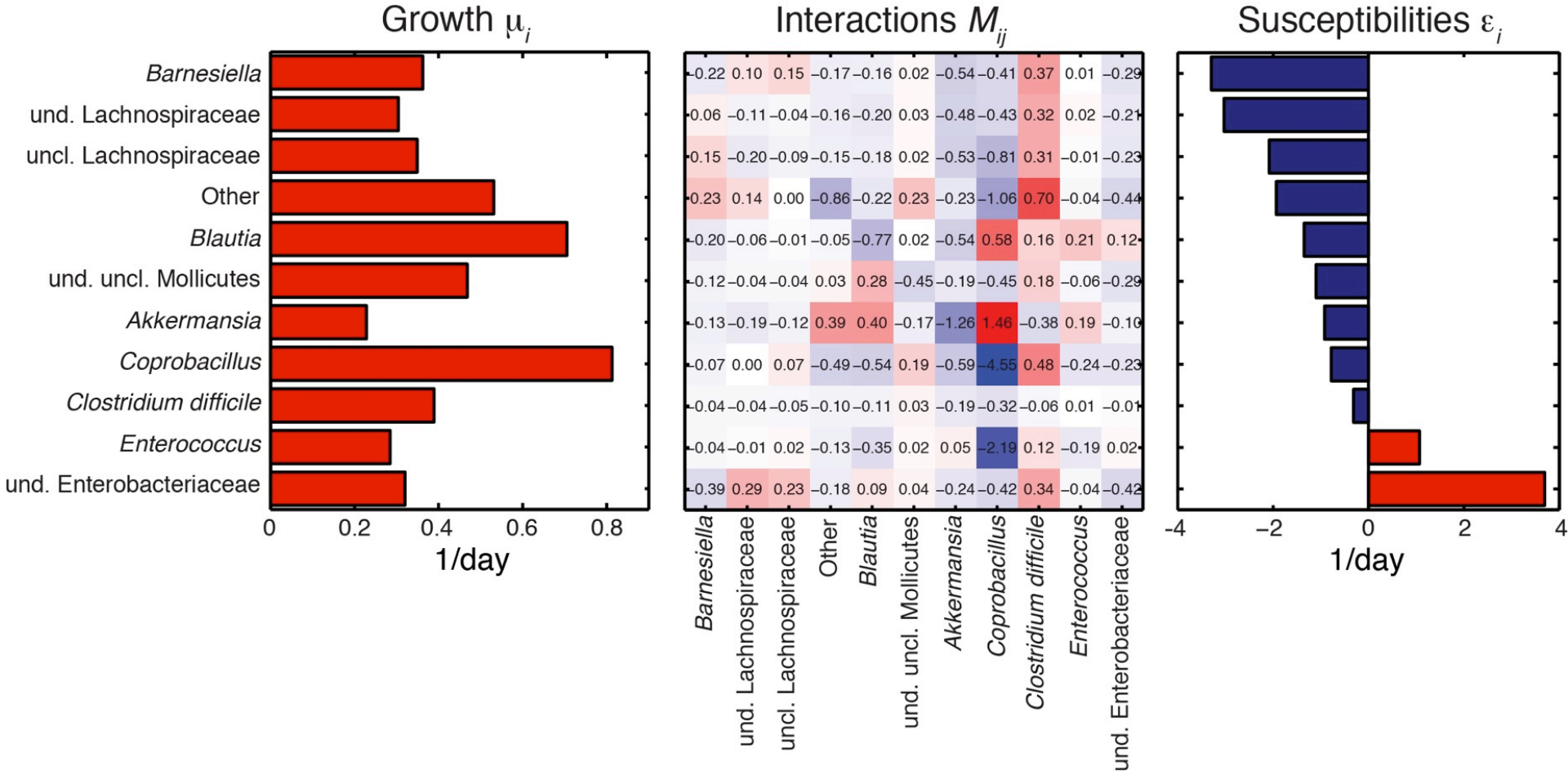
What species predispose to CDI?

Clindamycin disrupts microbiota and allows pathogen establishment



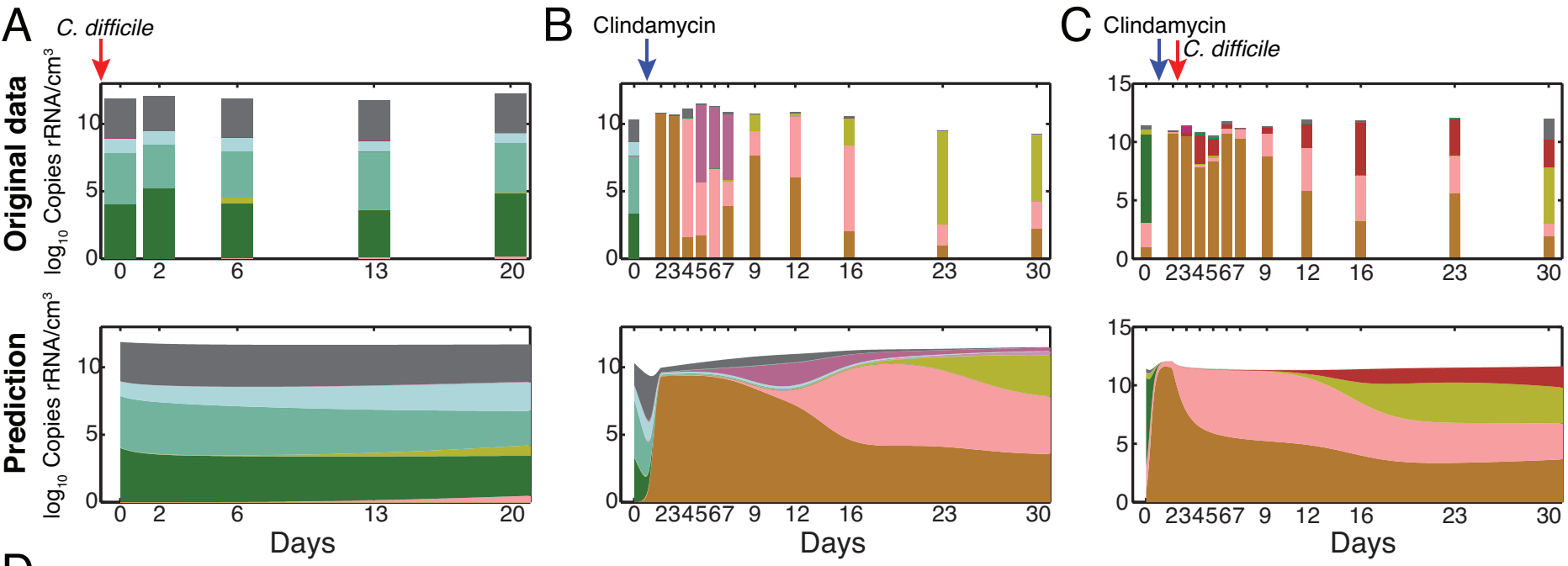
Buffie et al. (2011) *Inf. Imm.*

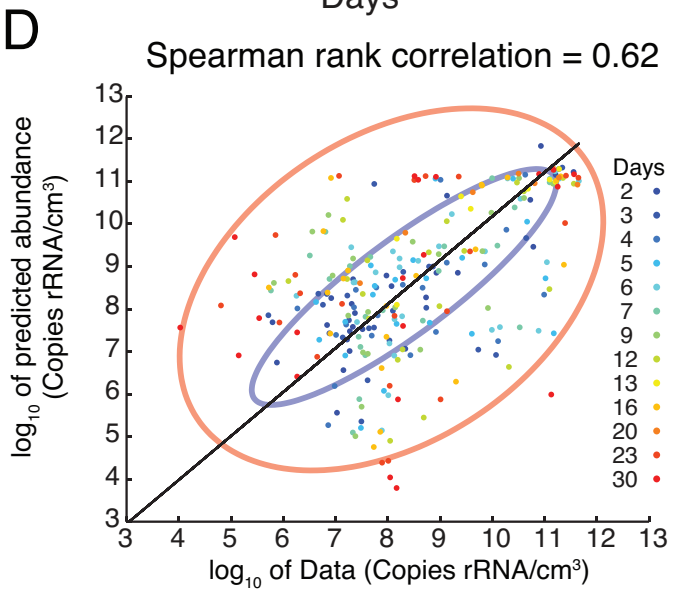
Inference of community structure and response to clindamycin



Stein, Bucci et al. (**2013**) *PLoS. Comp. Biol.*

Prediction of community dynamics



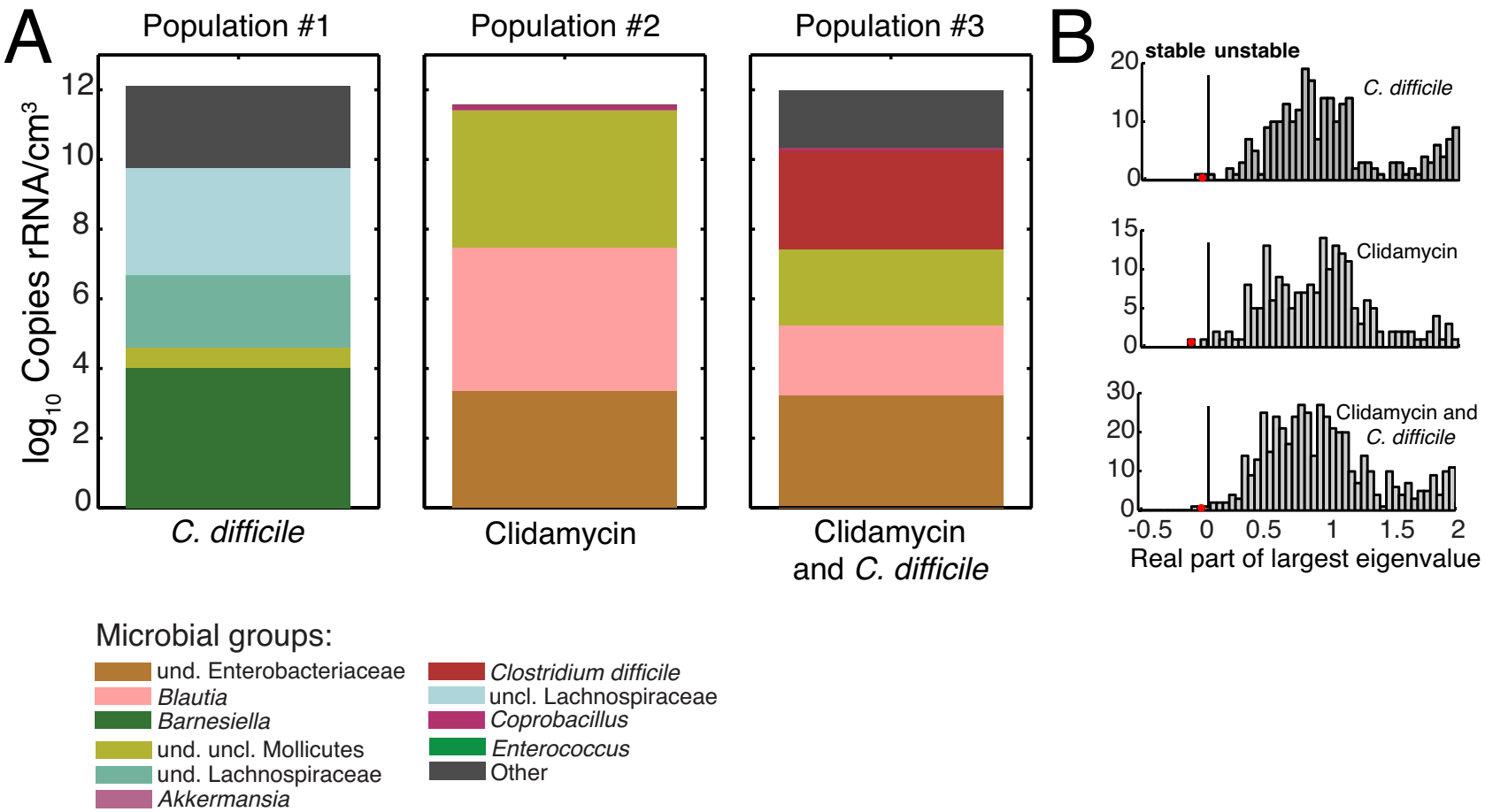


Microbial groups:

- und. Enterobacteriaceae
- *Blautia*
- *Barnesiella*
- und. uncl. Mollicutes
- und. Lachnospiraceae
- *Akkermansia*
- *Clostridium difficile*
- uncl. Lachnospiraceae
- *Coprobacillus*
- *Enterococcus*
- Other

Stein, Bucci et al. (**2013**) *PLoS. Comp. Biol.*

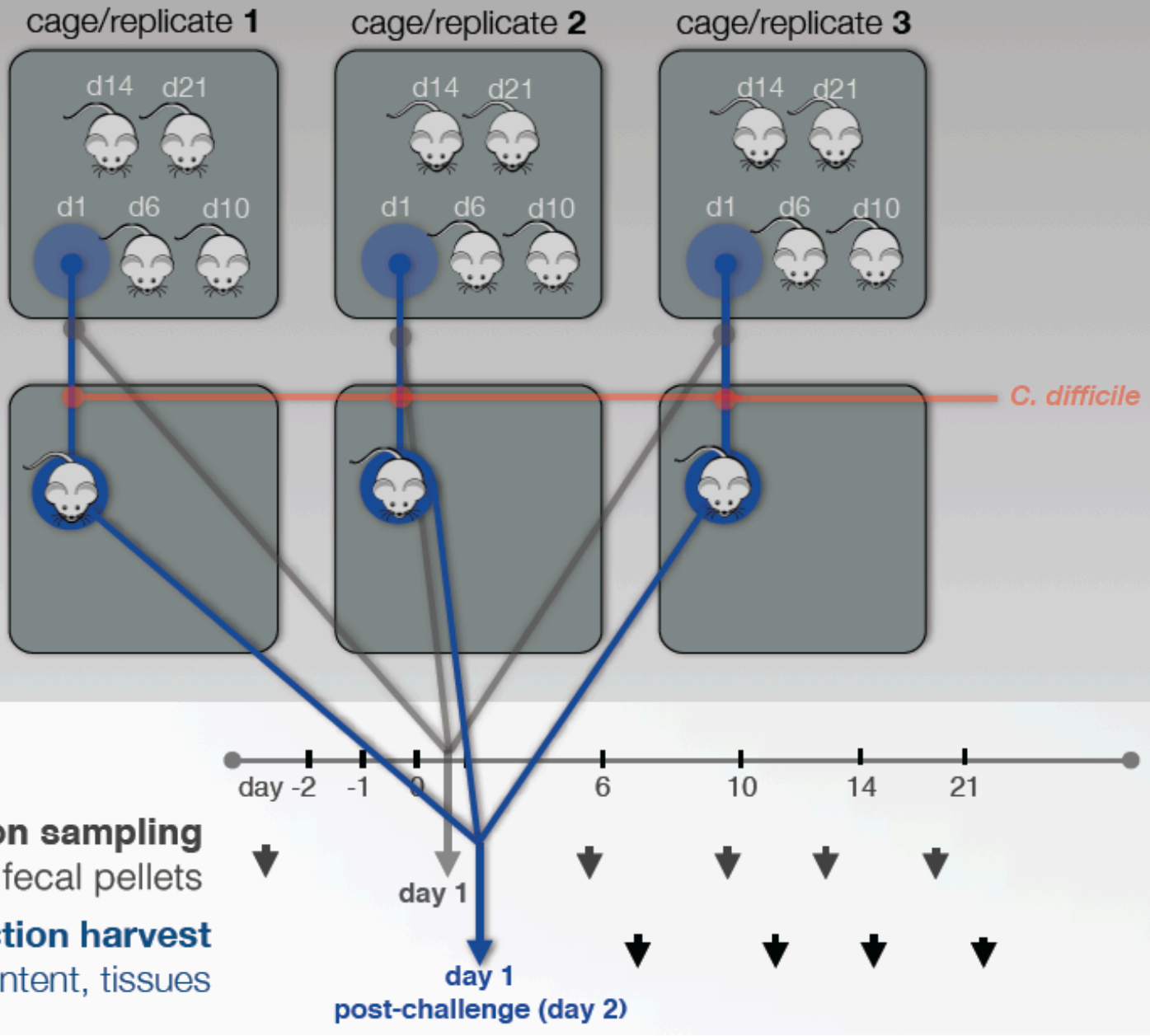
Stability analysis confirms alternative perturbation-dependent composition states



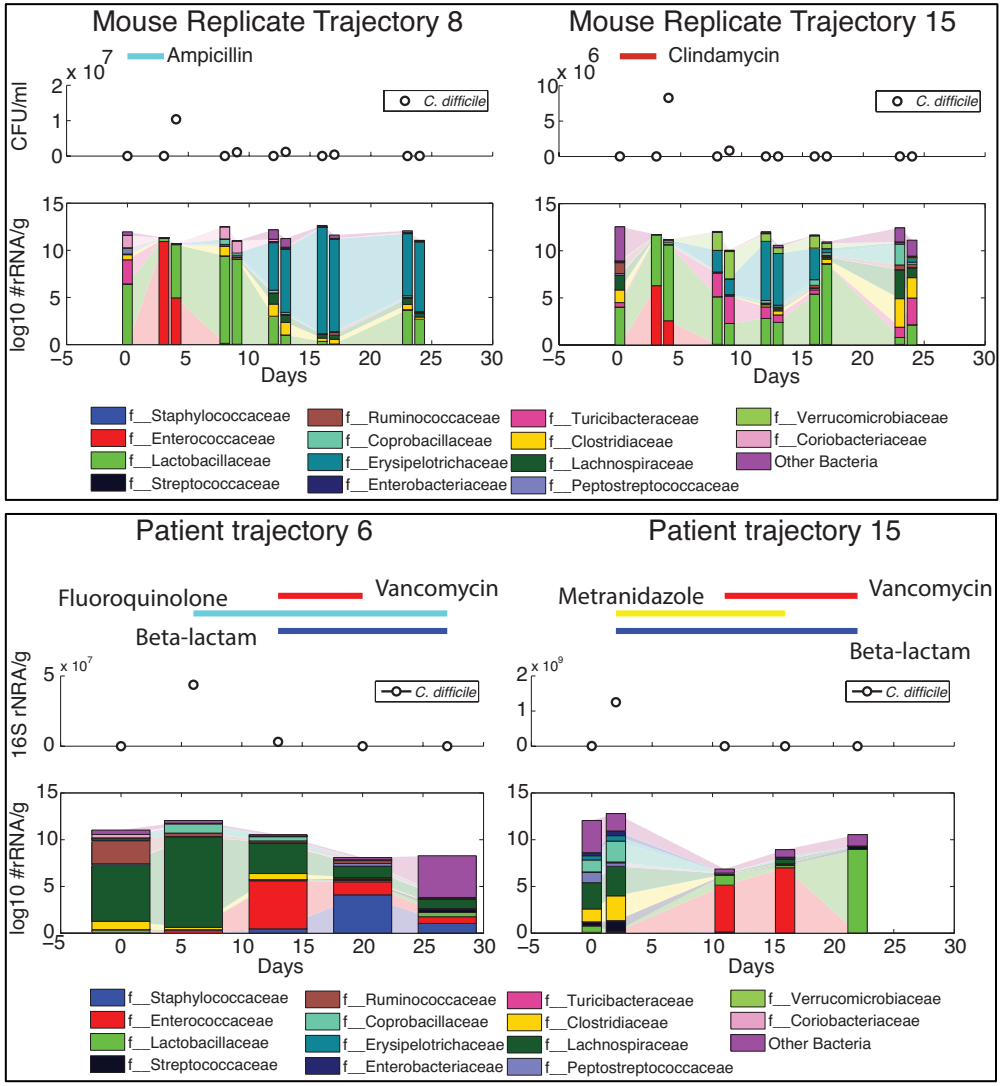
Stein, Bucci et al. (**2013**) *PLoS. Comp. Biol.*

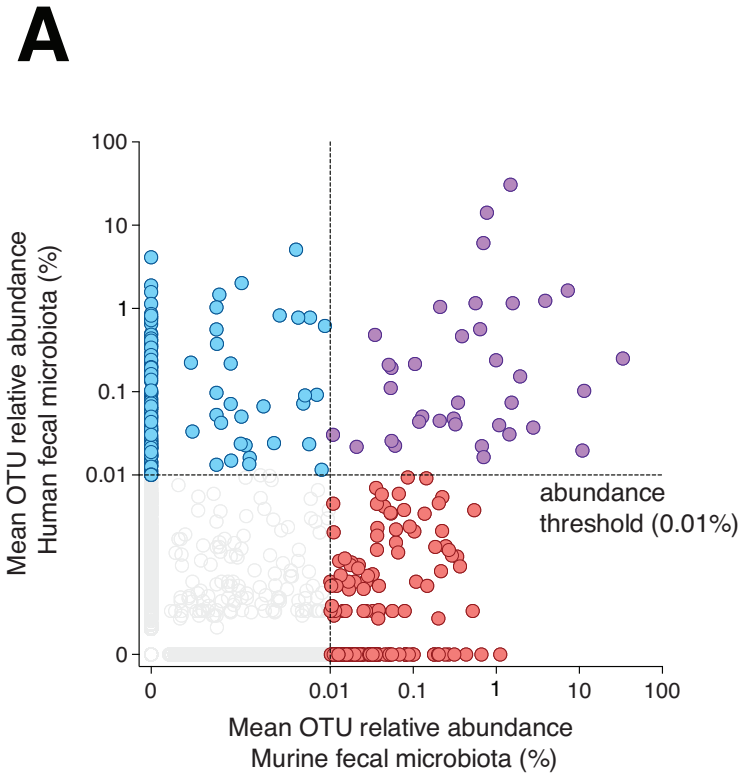
What mechanisms mediate protection from CDI ?

Independently-housed, longitudinally consistent



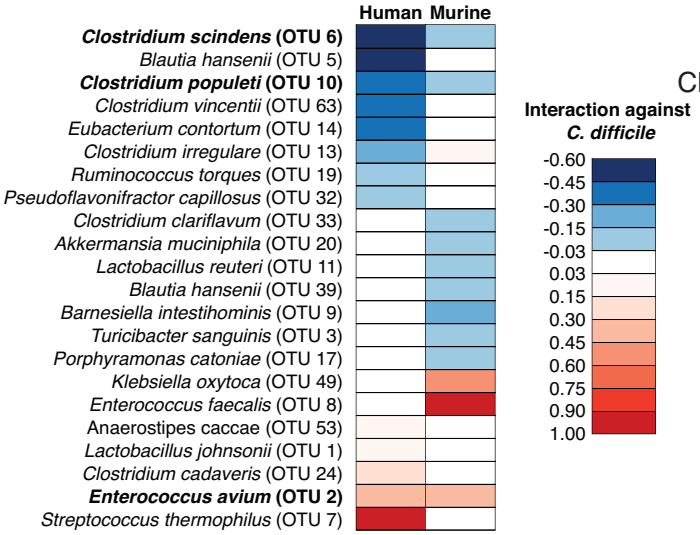
Combined mouse/human analysis to identify bacteria protective against *C. difficile*

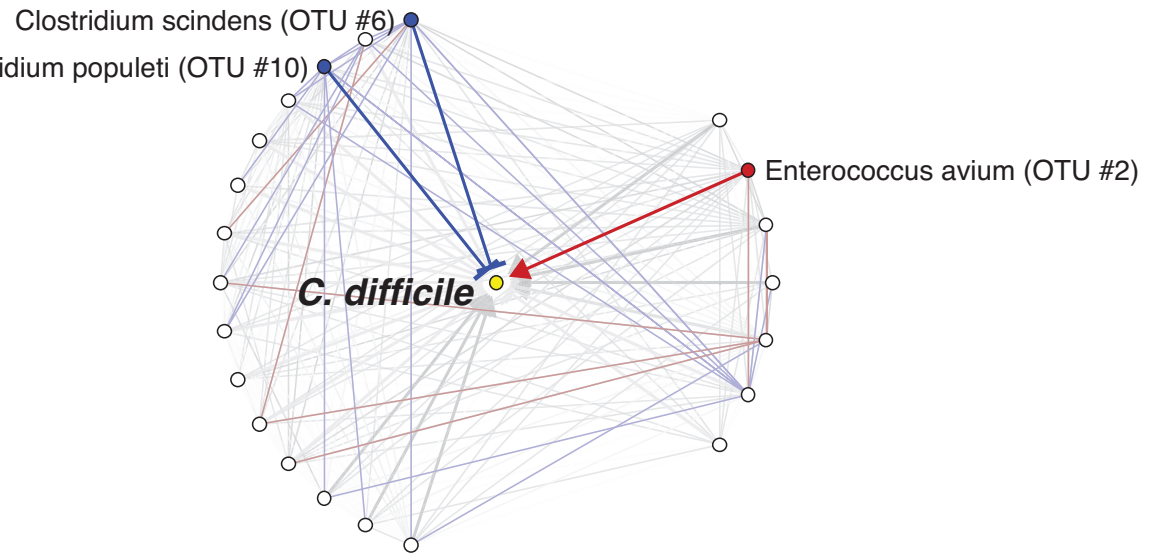




Buffie, Bucci et al. (**2014**) *Nature*

Common set of taxa with human–mouse conserved effects on *C. difficile*

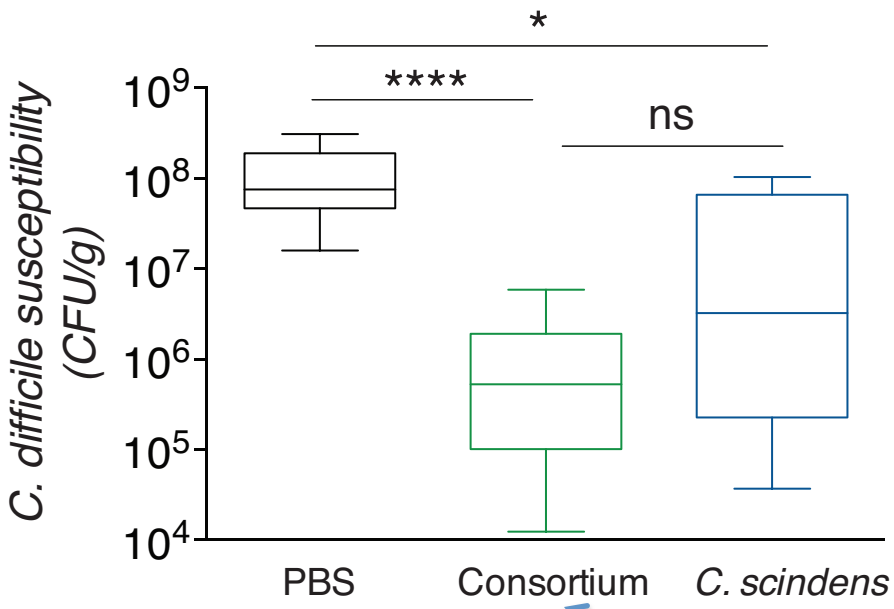


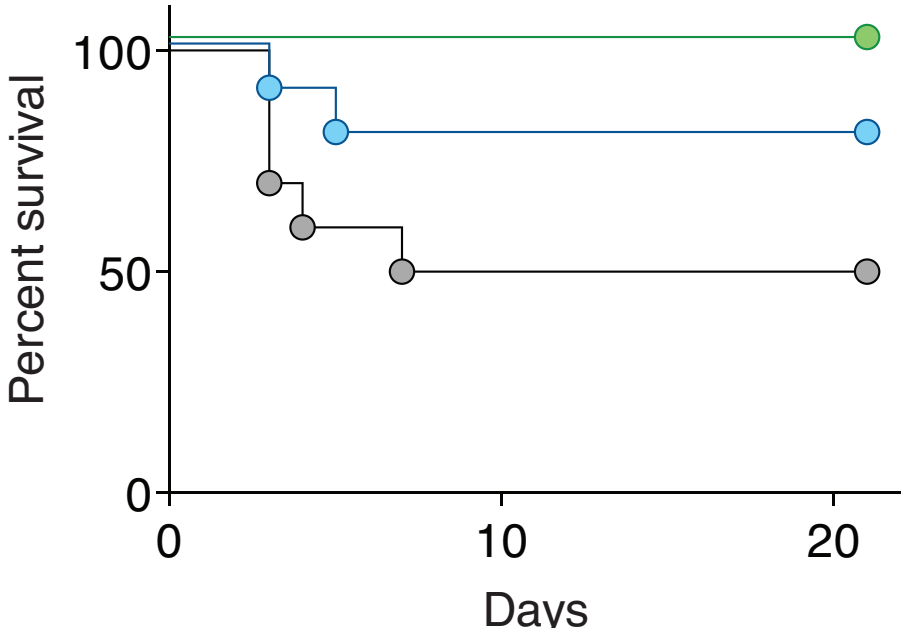


- Is *C. scindens* truly inhibitory?
- What mechanisms mediate this inhibition?

Buffie, Bucci et al. (**2014**) *Nature*

Transfer of resistance-associated intestinal bacteria increases resistance to CDI and survival





Transplant

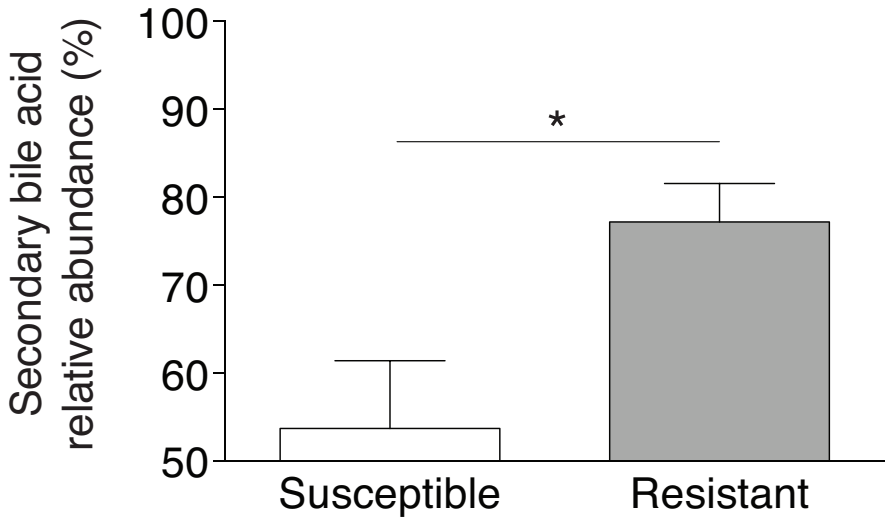
- PBS
- Consortium
- *C. scindens*

Consortium:

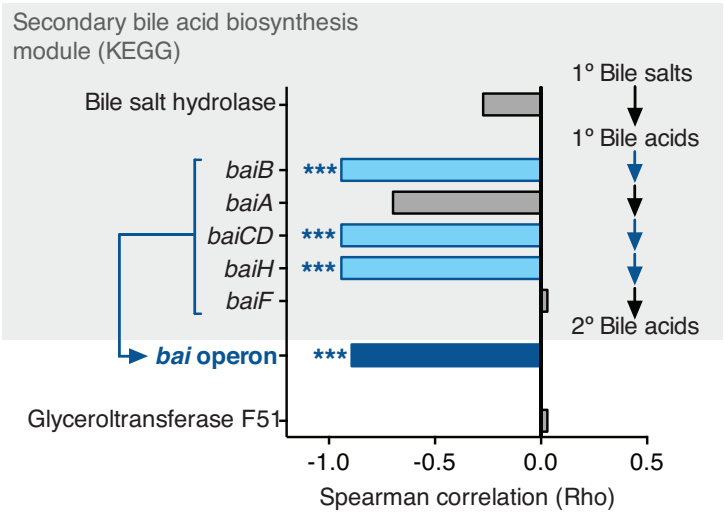
- *Barnesiella intestihominis* (OTU 9)
- *Blautia hansenii* (OTU 39)
- *Pseudoflavonifractor* capillosus (OTU 32)
- *Clostridium scindens* (OTU 6)

Buffie, Bucci et al. (**2014**) *Nature*

PICRUSt and shotgun genome seq. show negative correlation of secondary bile acids genes with CDI



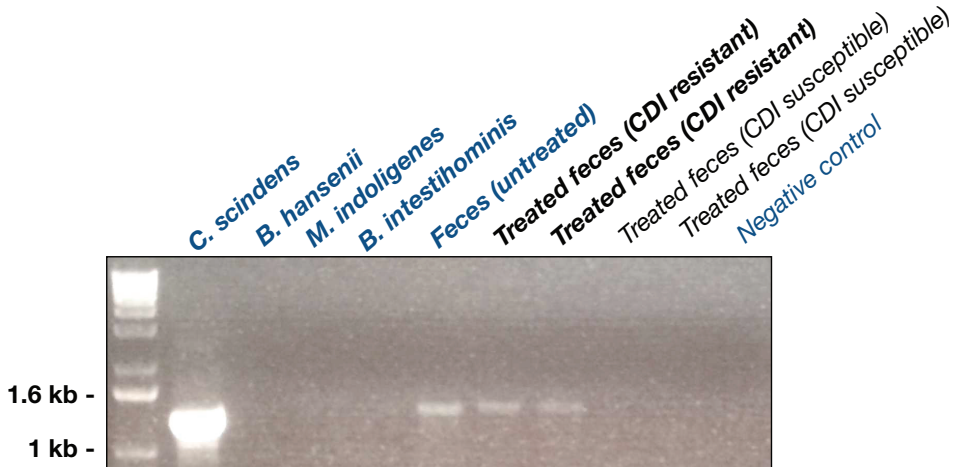
Secondary bile-acids genes depleted in CDI susceptible samples



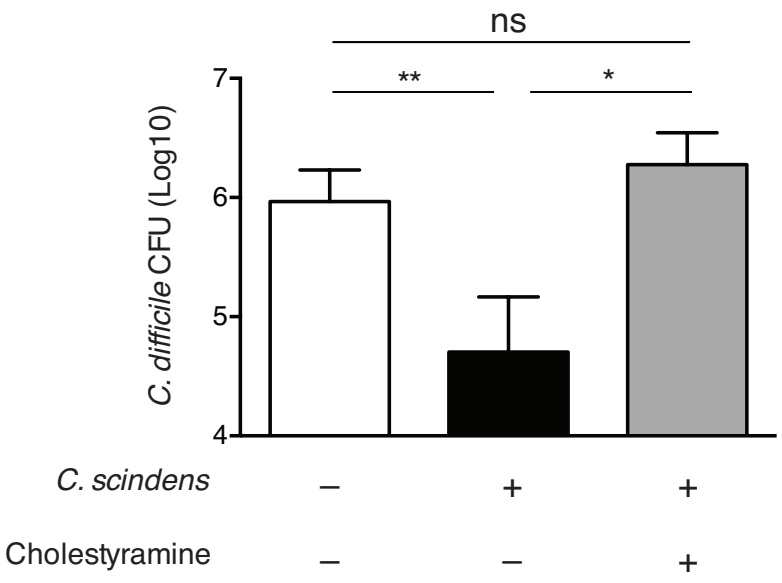
bai operon negatively correlates with *CDI*

Buffie, Bucci et al. (**2014**) *Nature*

C. scindens is the taxon harbouring gene for secondary bile acids production

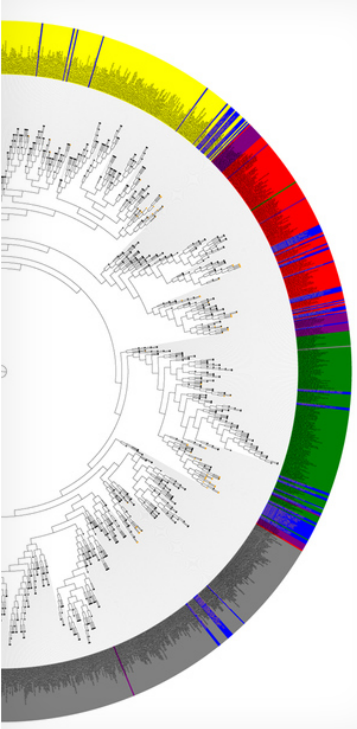


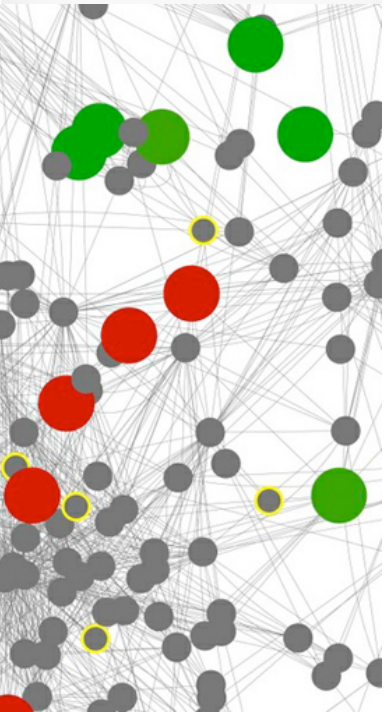
Detection of the 7-HSDH-encoding *baiCD* gene in bacterial isolates and intestinal microbiomes



Bile-acids sequestrants nullify *C. scindens* protective effect

Buffie, Bucci et al. (**2014**) *Nature*





Summary

- Intestinal microbiota has fundamental role in prevention against enteric infections (e.g. CDI)
- By combining mathematical modeling and time-series metagenomics we were able to determine a minimal consortium of bacteria that mediates CDI
- *C. scindens* plays a central role in this network. It is the only bacteria responsible for bile-acids production (mechanism of protection) through *baiCD* expression
- Better understanding of the ecology of *C. scindens* and of any functionally equivalent (e.g. bile-acids producers) microbes will open the way to new, effective and rationally-designed probiotic strategies