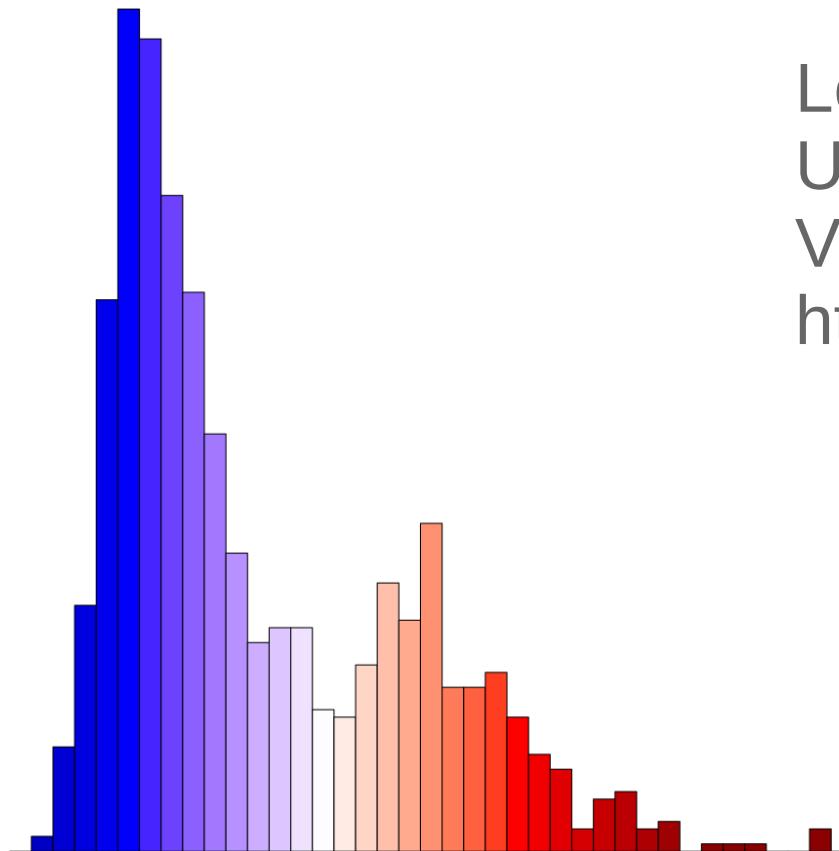


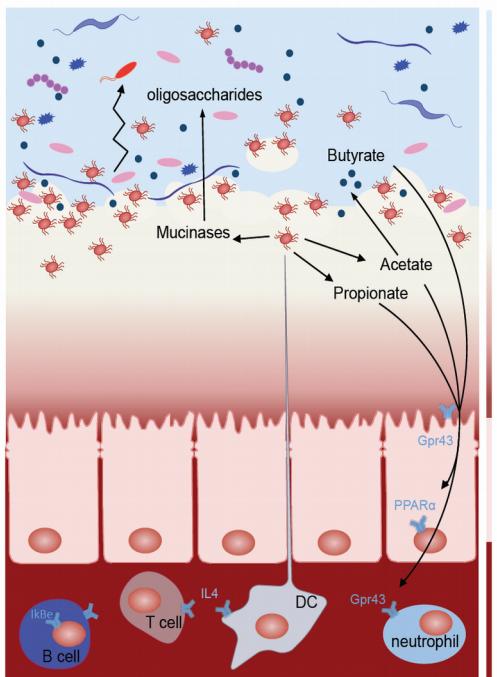
Population-level analyses of the intestinal microbiome and health



Leo Lahti, Doc.
University of Turku, Finland
VIB/KU Leuven, Belgium
<http://www.iki.fi/Leo.Lahti>

Gut microbiome: 300-400 m² surface area

- ~ bacterial cells outnumber our own
- ~ 100 billion bacteria / gram in the gut
- ~ 10,000 gut bacterial species
- ~ 10M uniq genes (500x human genome)
- ~ Plastic, varies in time



Brussel central square flower carpet



HITChip Atlas: Gut microbiota high-throughput profiling 10,000 samples ~ 5000 individuals ~ 1000 phylotypes

Highly standardized & reproducible platform

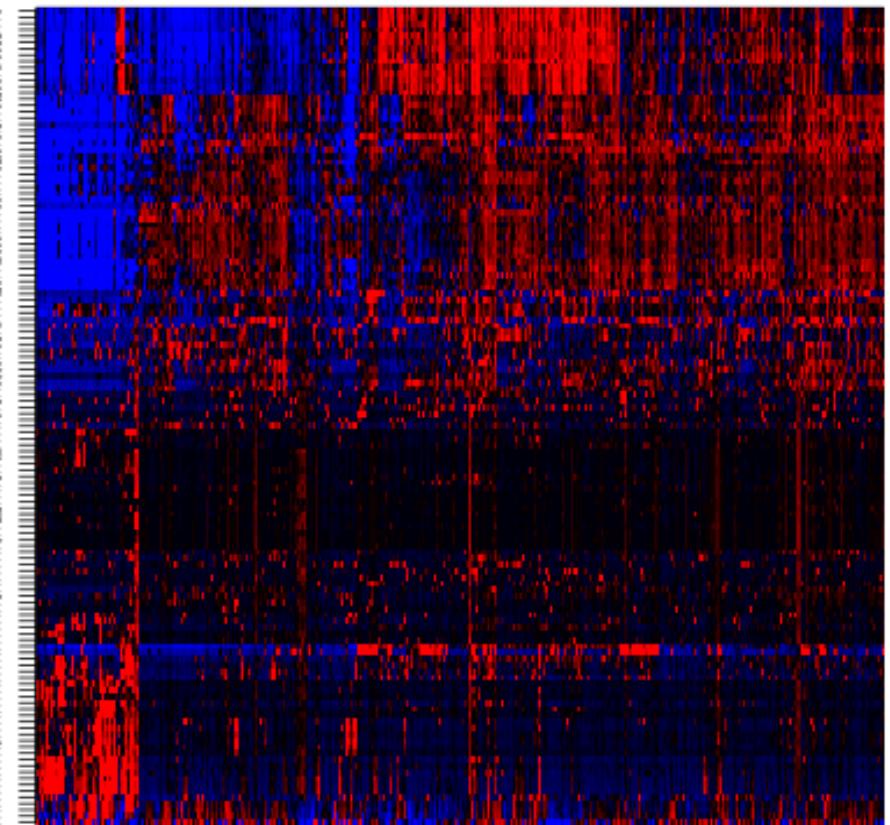
Deep phylotype level profiling

Open data & source code: Lahti et al. Nature Comm. 2014



1000 bacterial phylogenotypes

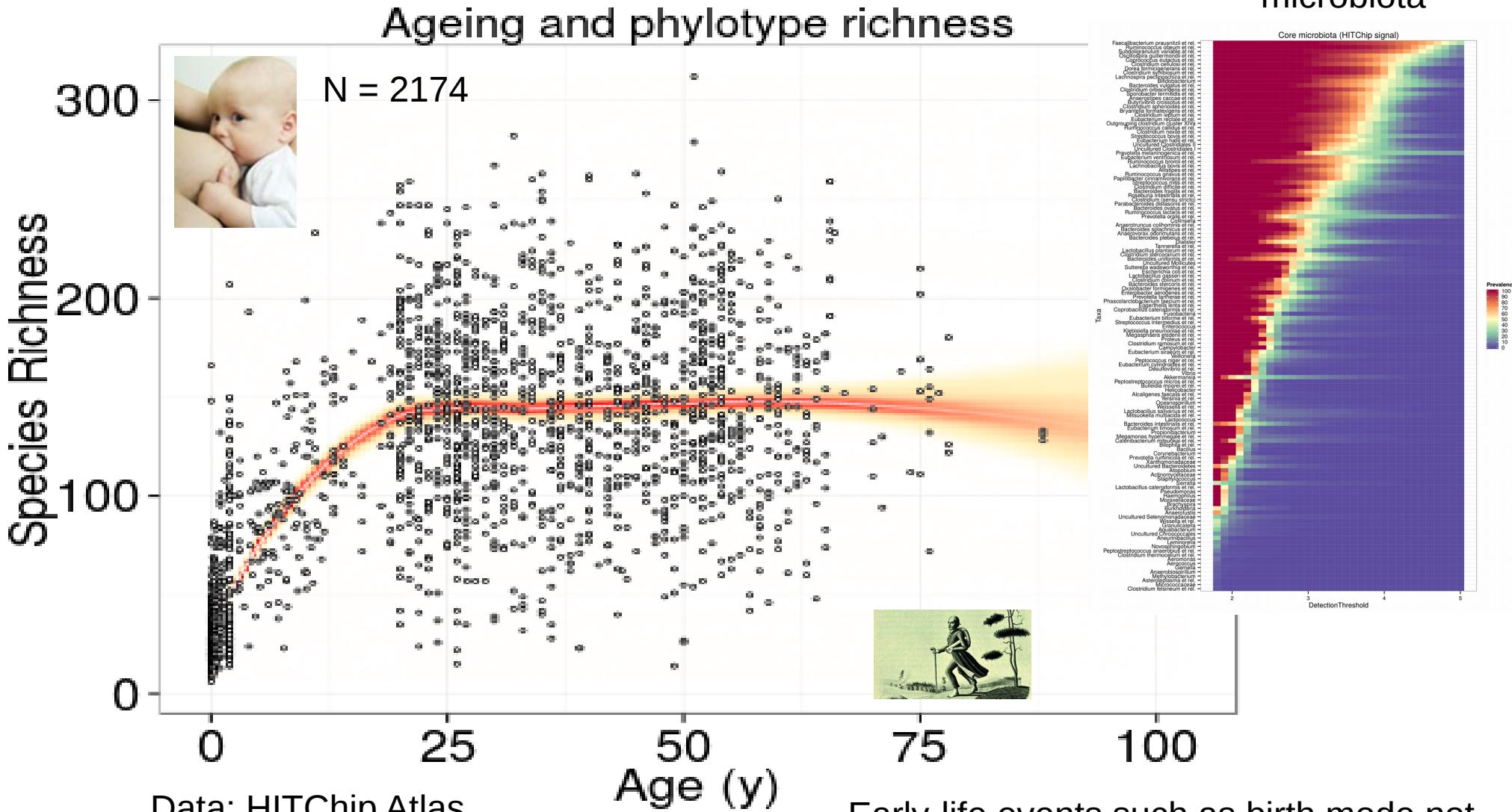
$N \sim 10,000$



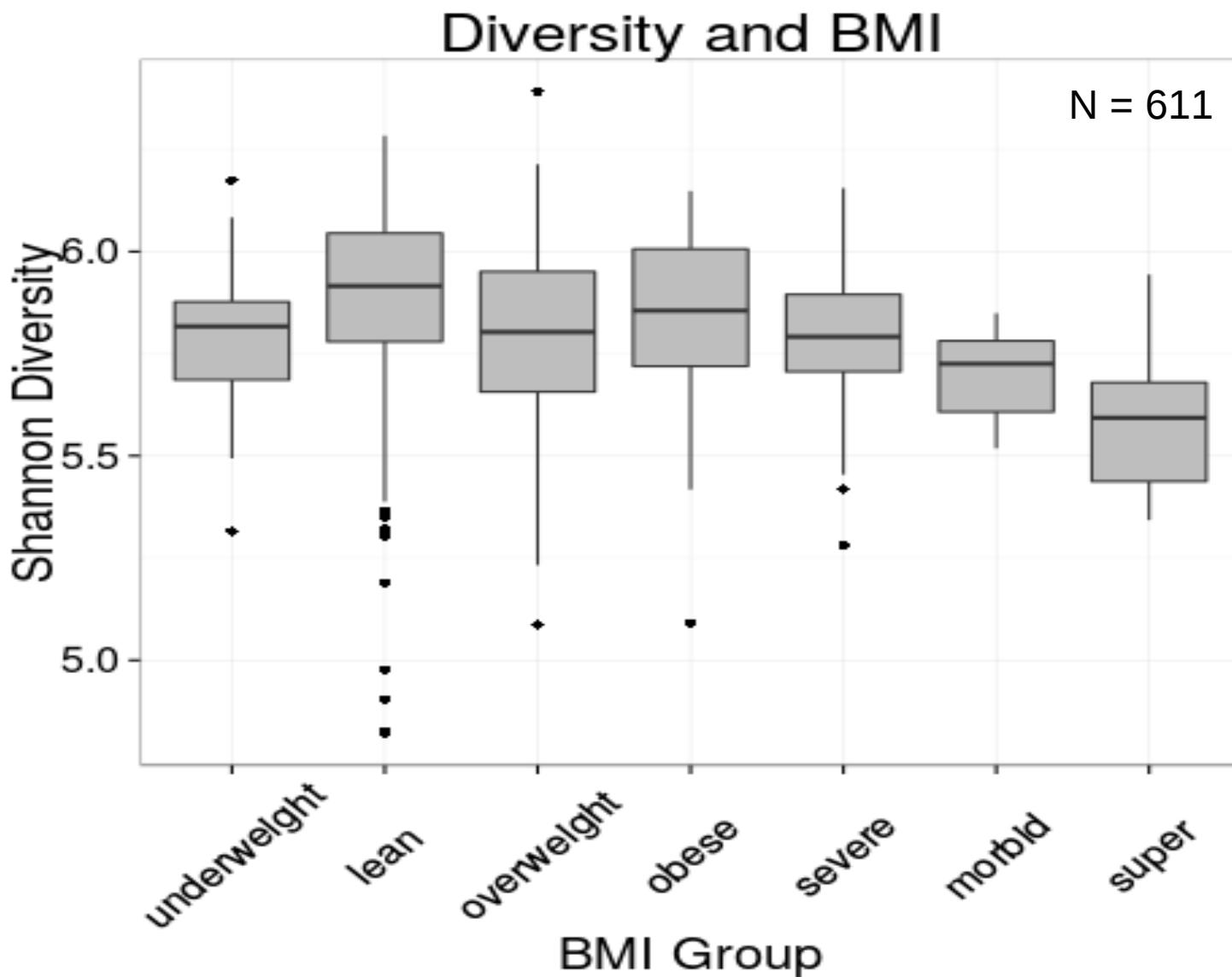
<http://microbiome.github.io>

Microbial composition is highly individual and variable !

~ hundreds of species per individual adult gut

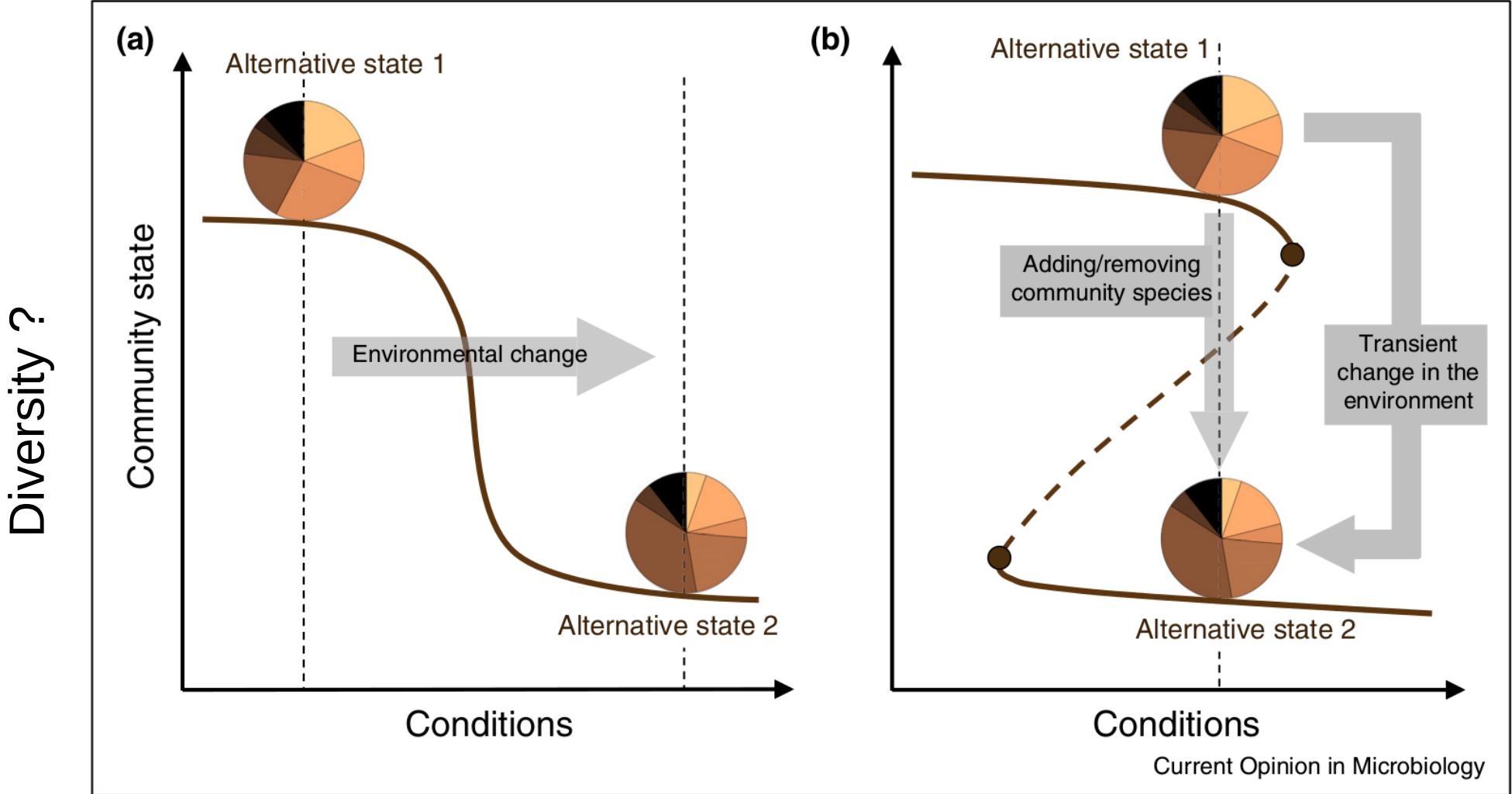


Microbial diversity decreases with obesity



State induced by external factors

Intrinsic stability: robust to external factors

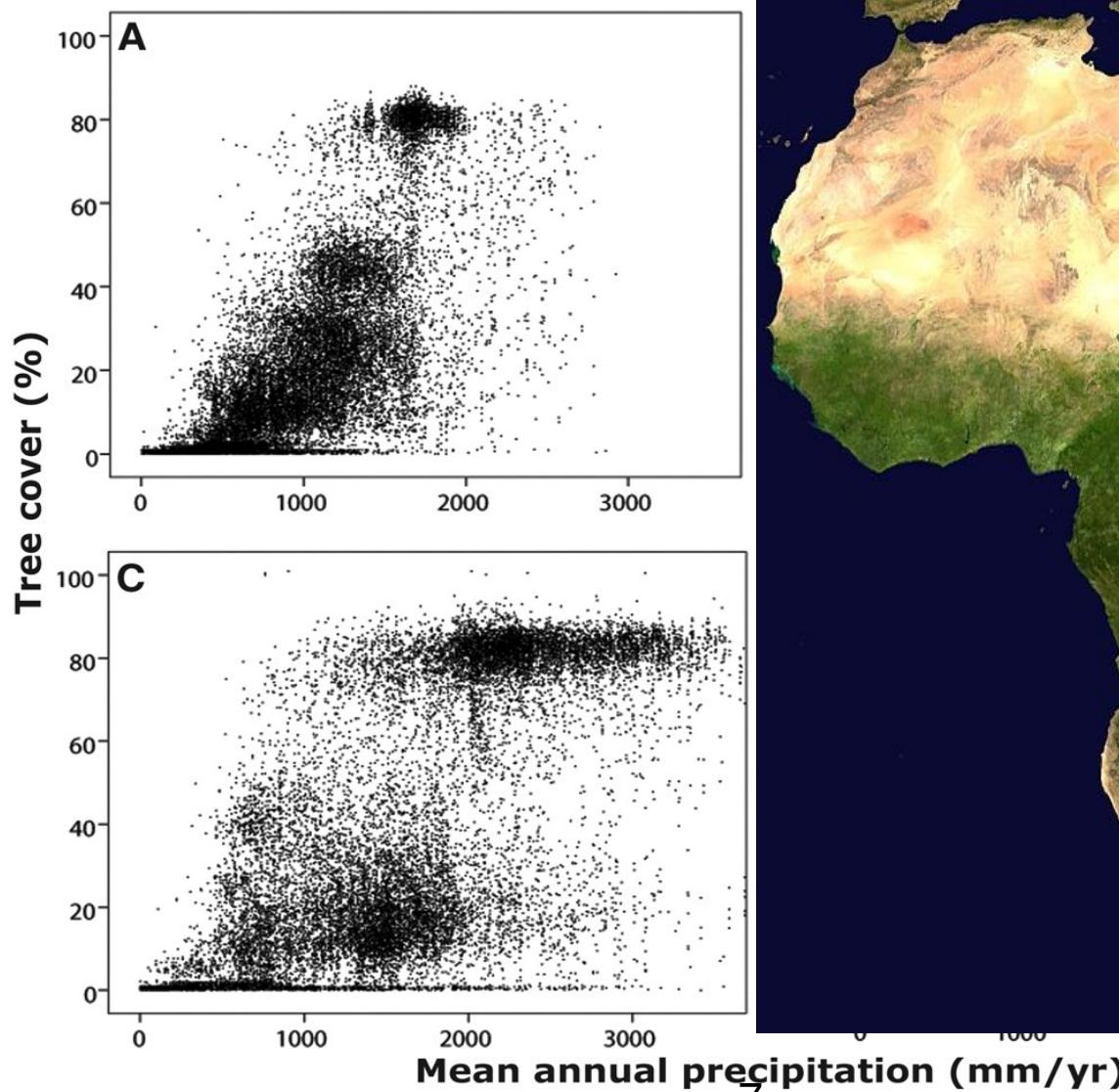


Environmental or host factor (e.g. body-mass index)

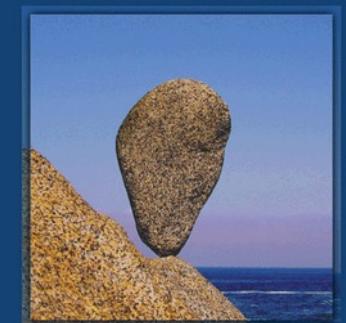
REPORT

Global Resilience of Tropical Forest and Savanna to Critical Transitions

Marina Hirota¹, Milena Holmgren^{2,*}, Egbert H. Van Nes¹, Marten Scheffer¹



Critical Transitions
in Nature and Society



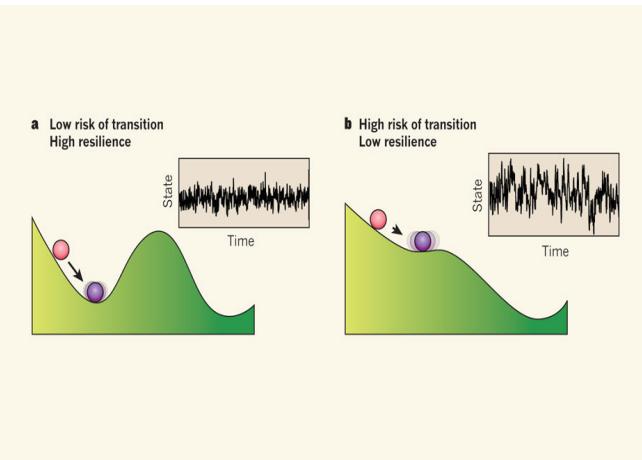
Marten Scheffer

PRINCETON STUDIES IN COMPLEXITY

Fecal transplant induced ecosystem level state shift

Efficient treatment in *C. difficile* infected patients.
Shift from Bacilli- and Proteobacteria-dominated state to a healthy state dominated by *Bacteroides* and many butyrate producing taxa

Better than antibiotics !



Patient gets donor microbiota -> Donor farms!?

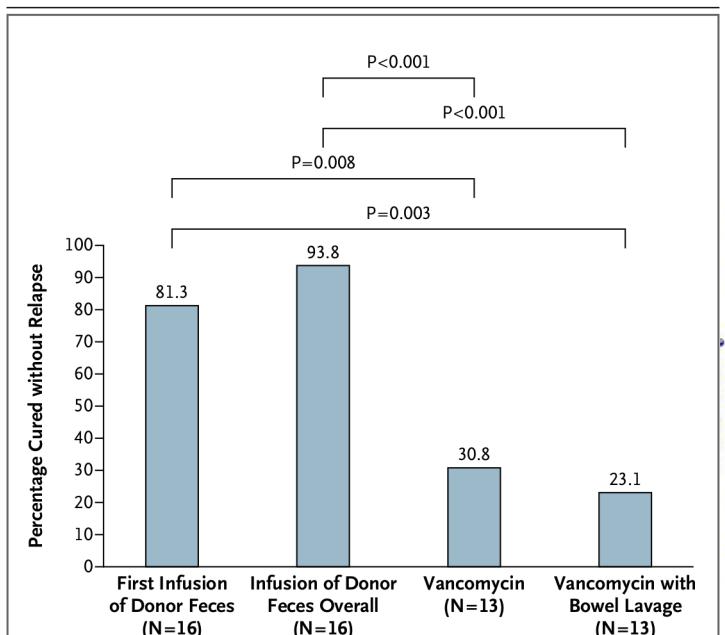
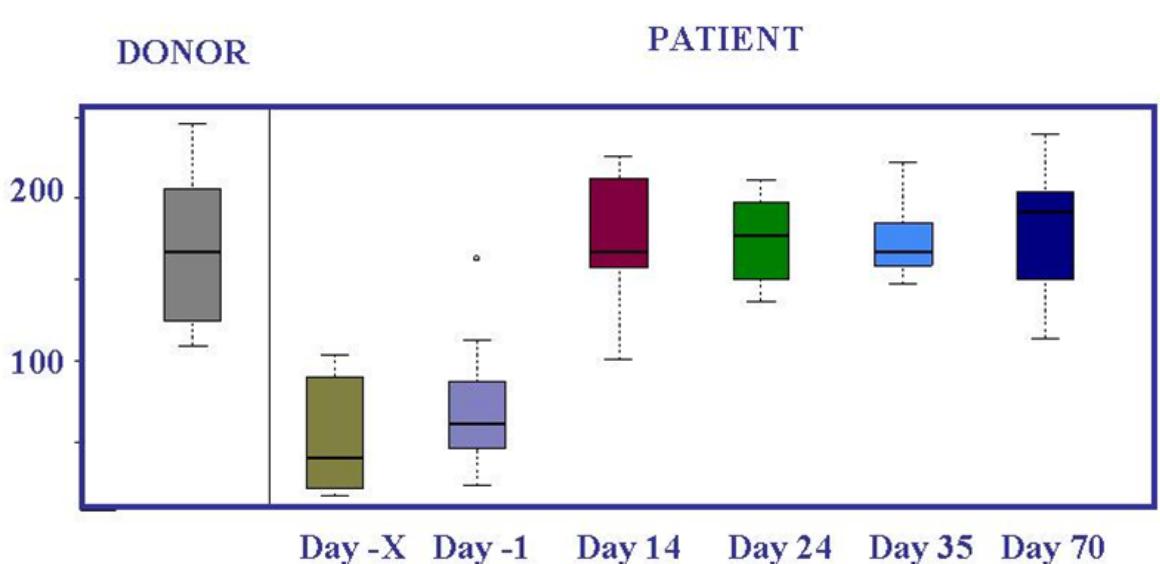


Figure 2. Rates of Cure without Relapse for Recurrent *Clostridium difficile* Infection.

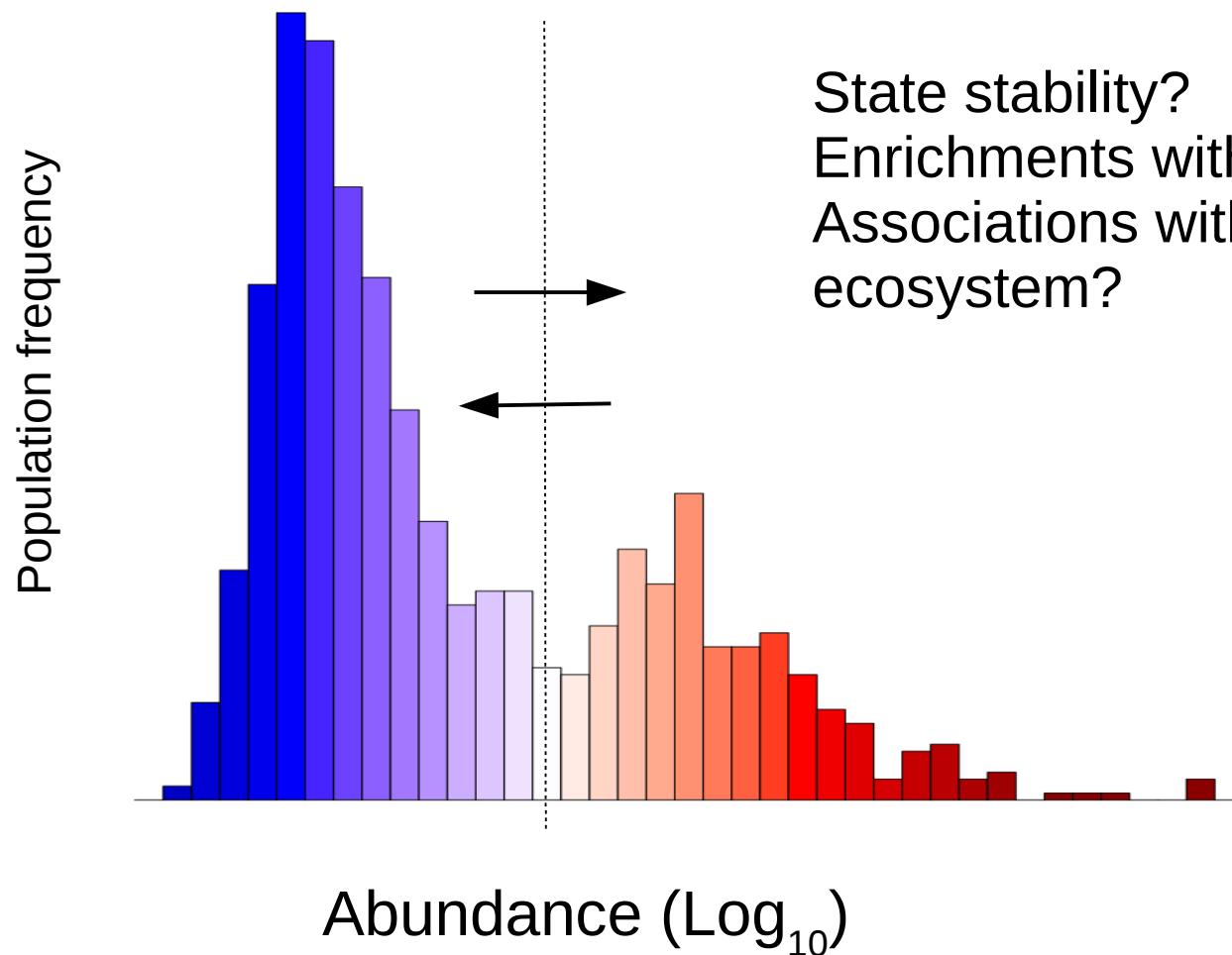
Show are the proportions of patients who were cured by the infusion of donor feces (first infusion and overall results), by standard vancomycin therapy, and by standard vancomycin therapy plus bowel lavage.



Els van Nood et al., NEJM 368(5) 2013
Fuentes et al. ISME J, 8:1621-33, 2014

Alternative states in bacterial abundance ?

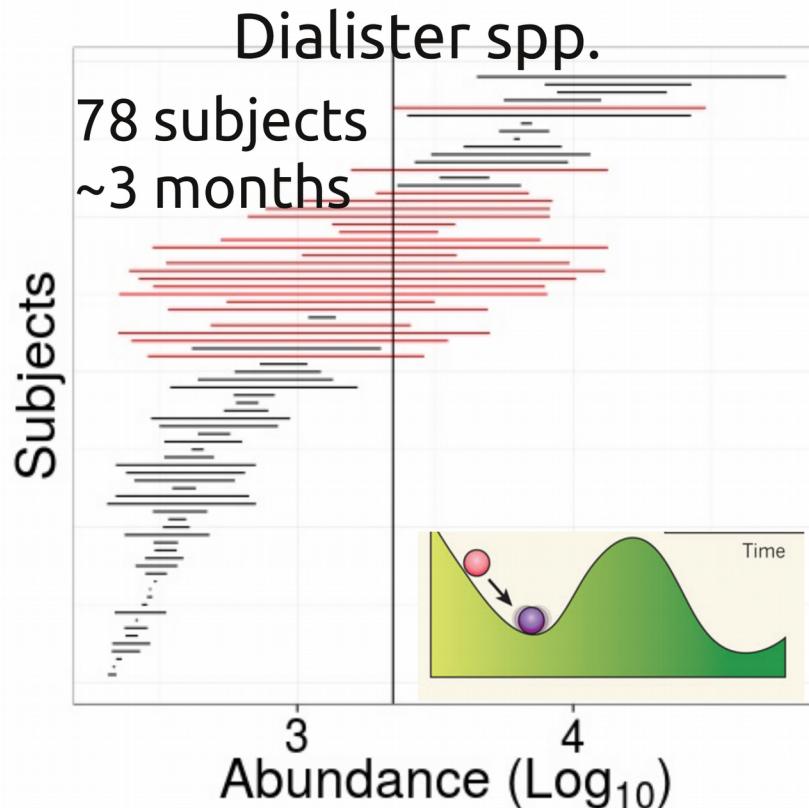
Dialister spp. (N = 1006)



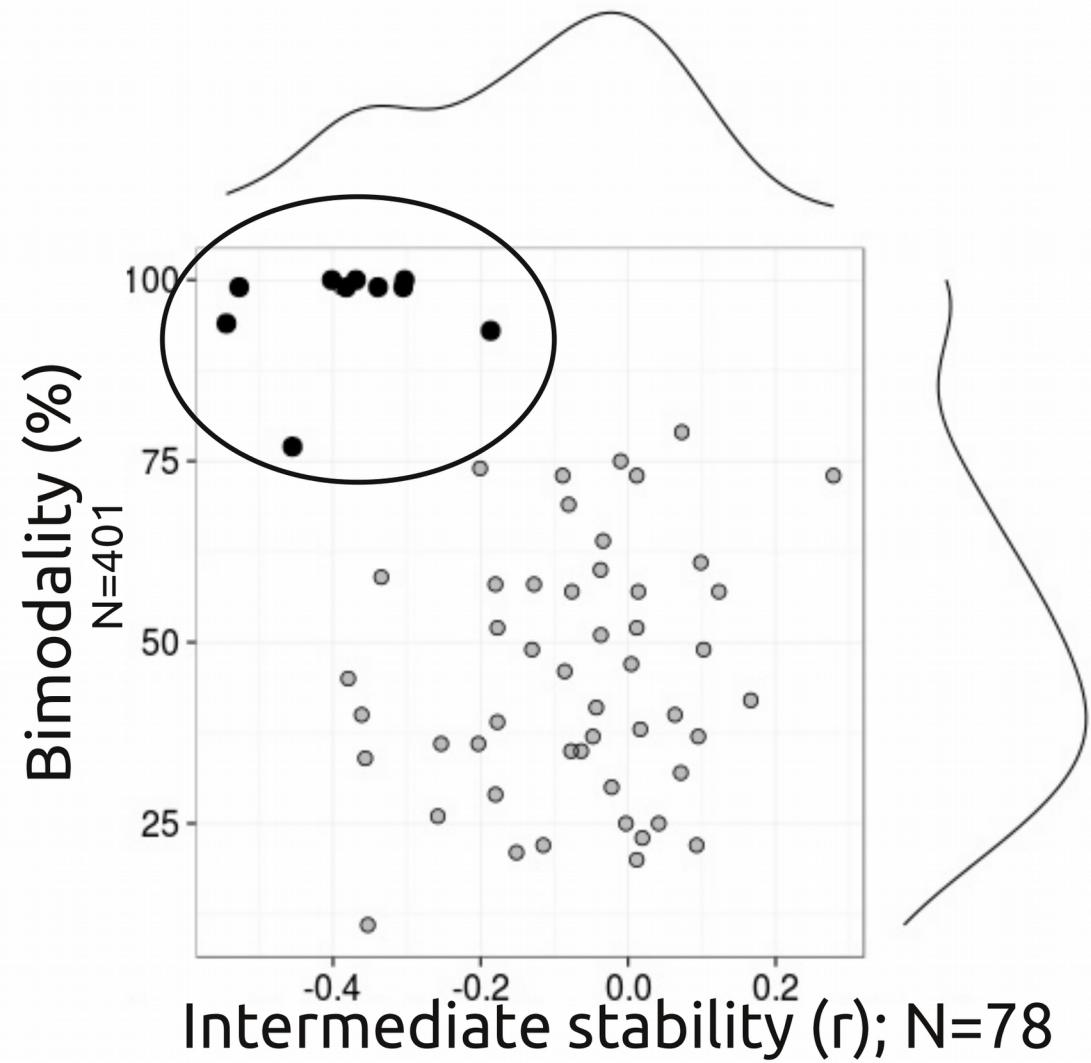
State stability?
Enrichments with diet / health / etc.?
Associations with the overall ecosystem?

Confirming bi-stability with temporal analysis

-> alternative attractors divided by a critical unstable tipping point

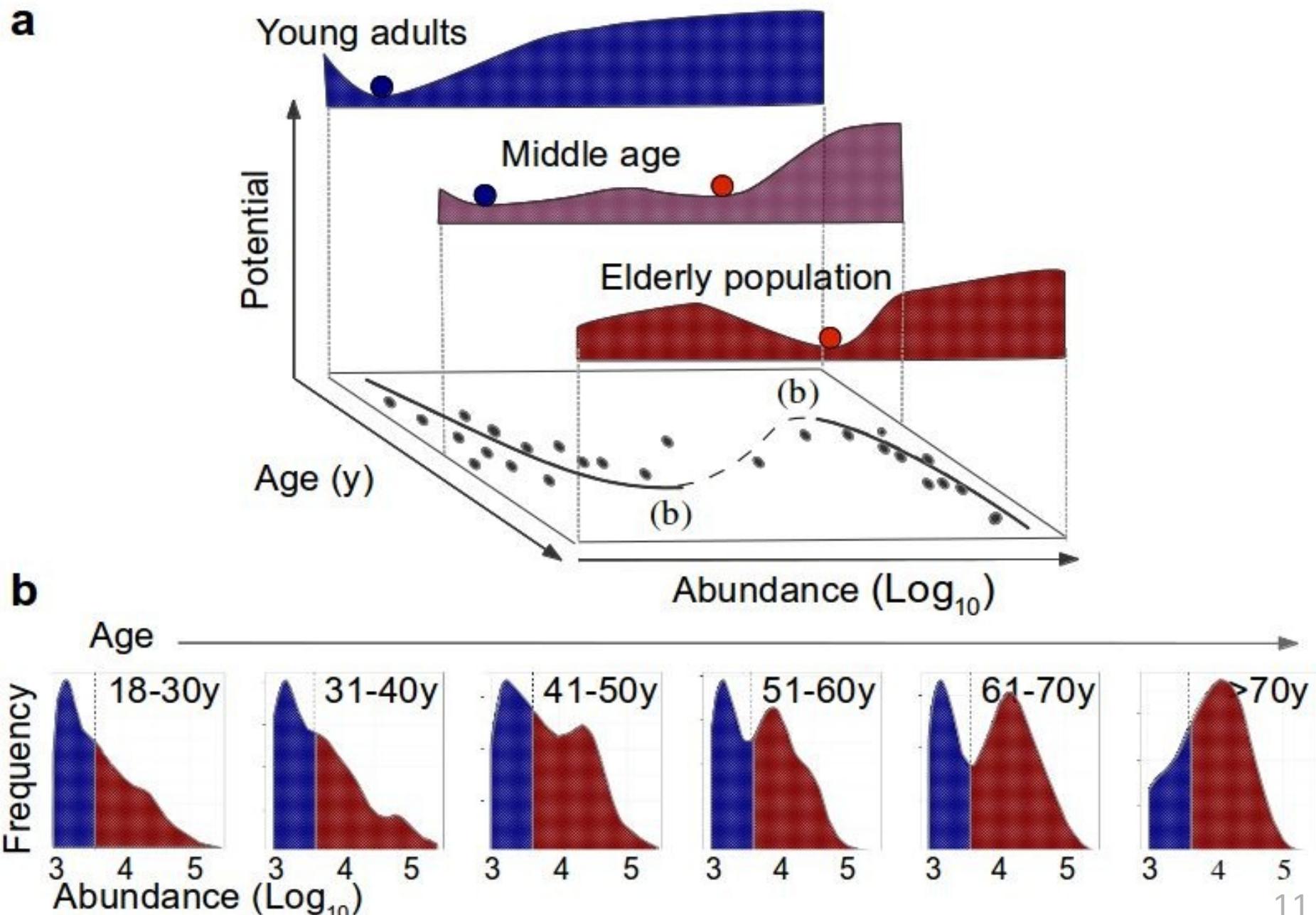


State transitions indicate instability in intermediate abundance range



Lahti et al. Nat. Comm. 5:4344, 2014

Tipping elements of the human gut microbiota: Uncultured Clostridiales I

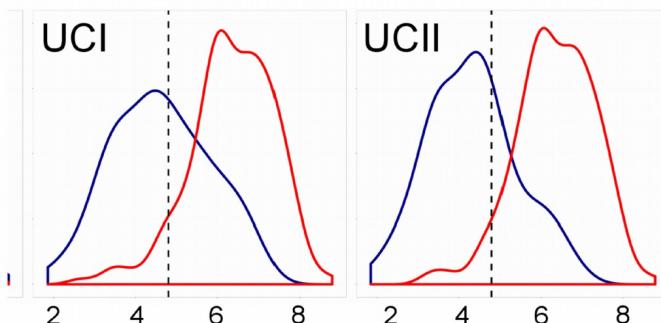


Effect on the overall ecosystem

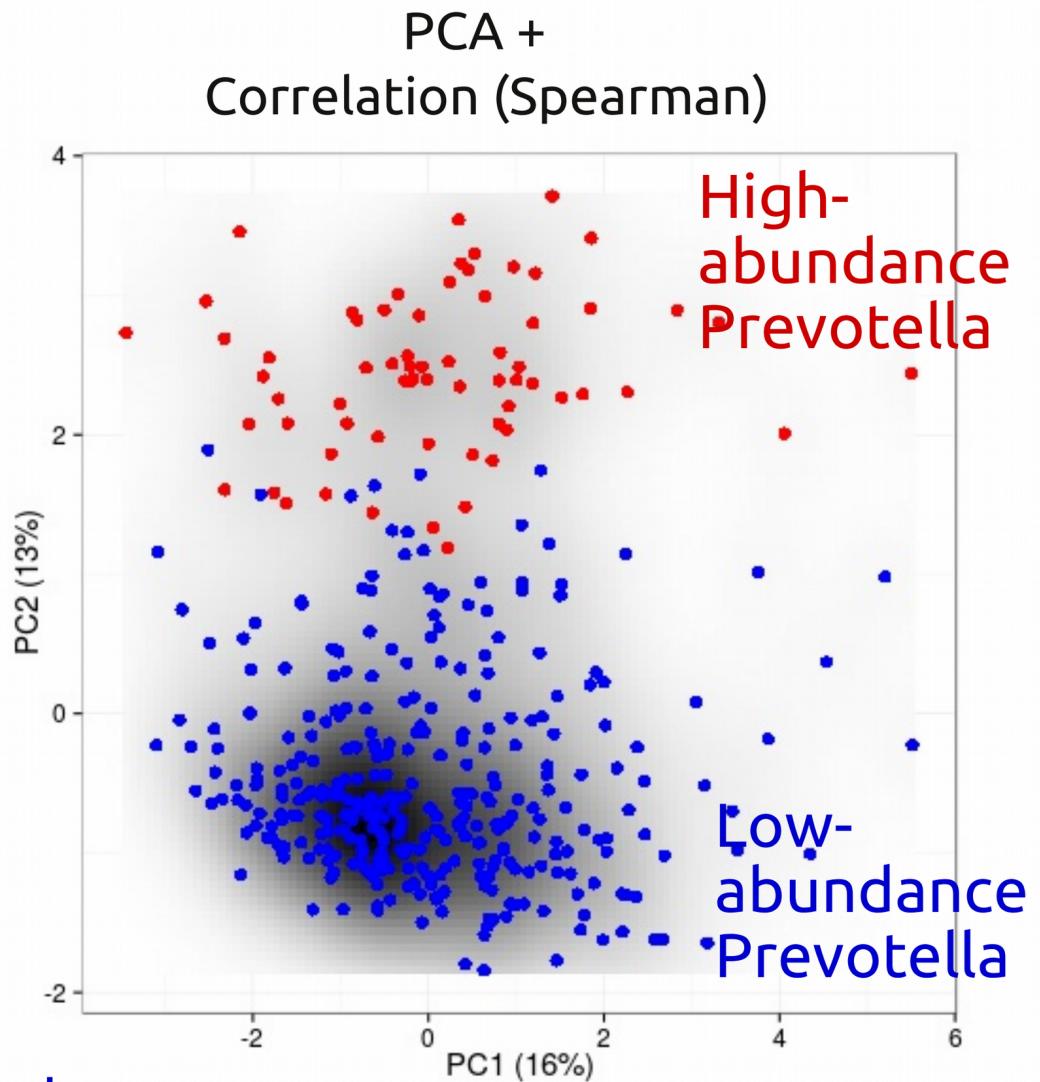
Prevotella states seen at ecosystem level

High ~10% relative abundance !

Other bi-stable taxa:
<2% relative abundance,
no ecosystem-level switches are visible



Uncultured Clostridiales associated with overall metagenomic richness



N = 401 western adults

Lahti et al. Nat. Comm. 5:4344, 2014

Health associations of bi-stable tipping elements

Health status	Bimodal group	Enriched state	Compromised (%)	Controls (%)	FDR (%)
Severe obesity (n=136)	UCI	Low abundance	29	55	<0.1
Severe obesity	UCII	Low abundance	38	61	<0.1
IBS (n=106)	UCII	Low abundance	50	61	1
MetS (n=66)	<i>B.fragilis</i> group	High abundance	89	78	<0.1
MetS	<i>Prevotella</i> group	Low abundance	11	22	11
MetS	<i>Dialister</i>	High abundance	36	28	13

- Causality: drivers or passengers?
- Diagnostic & prognostic indicators ?
- Targets for manipulation?
- Early warnings signals of state shift ?

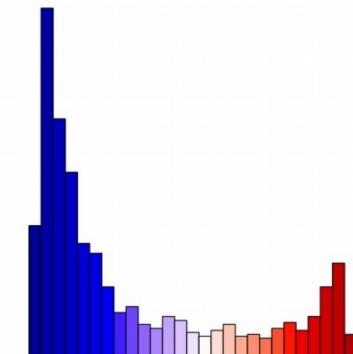
Received 23 Jan 2014 | Accepted 9 Jun 2014 | Published 8 Jul 2014

DOI: 10.1038/ncomms5344

OPEN

Tipping elements in the human intestinal ecosystem

Leo Lahti^{1,2}, Jarkko Salojärvi^{1,*}, Anne Salonen^{3,*}, Marten Scheffer⁴ & Willem M. de Vos^{1,2,3}



Early warning signals to predict state shifts ?

Early warning signals for a critical transition in a time series generated by a model of a harvested population⁷⁷ driven slowly across a bifurcation.

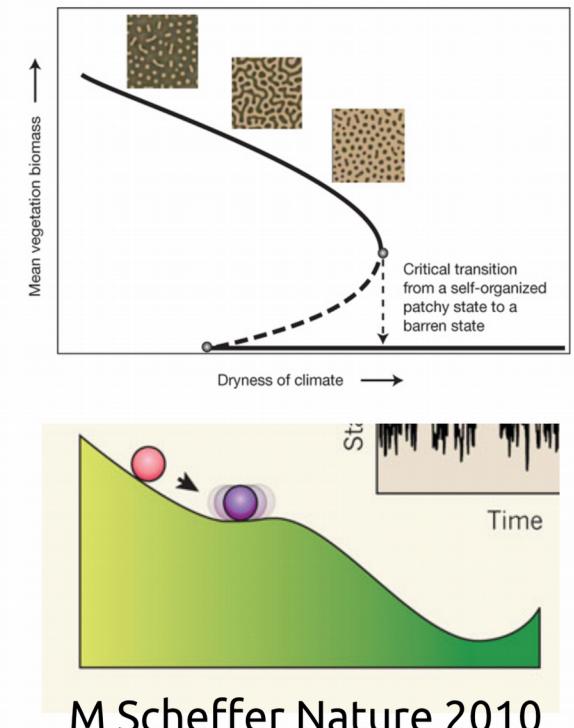
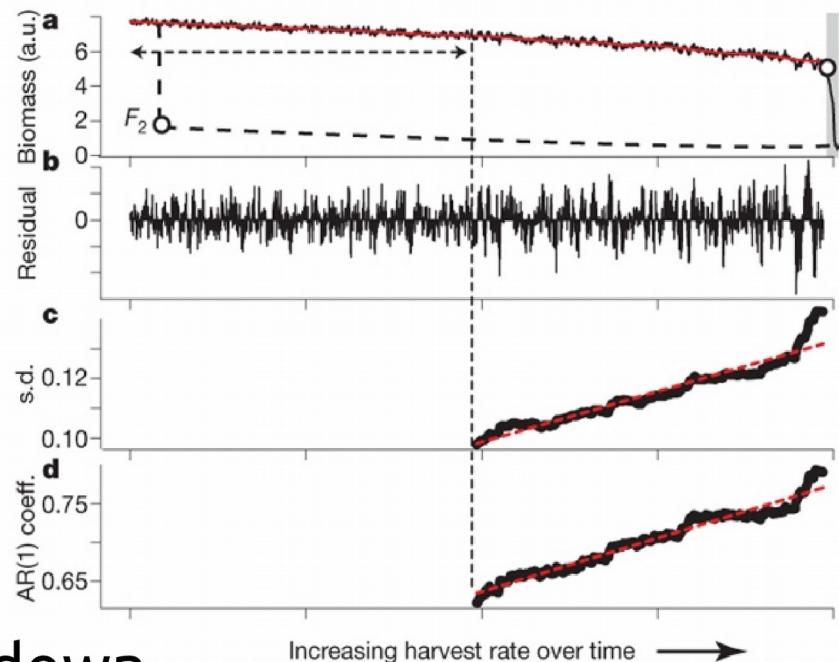
Autocorrelation

Variance

Skewness

Flickering

Critical slowing down



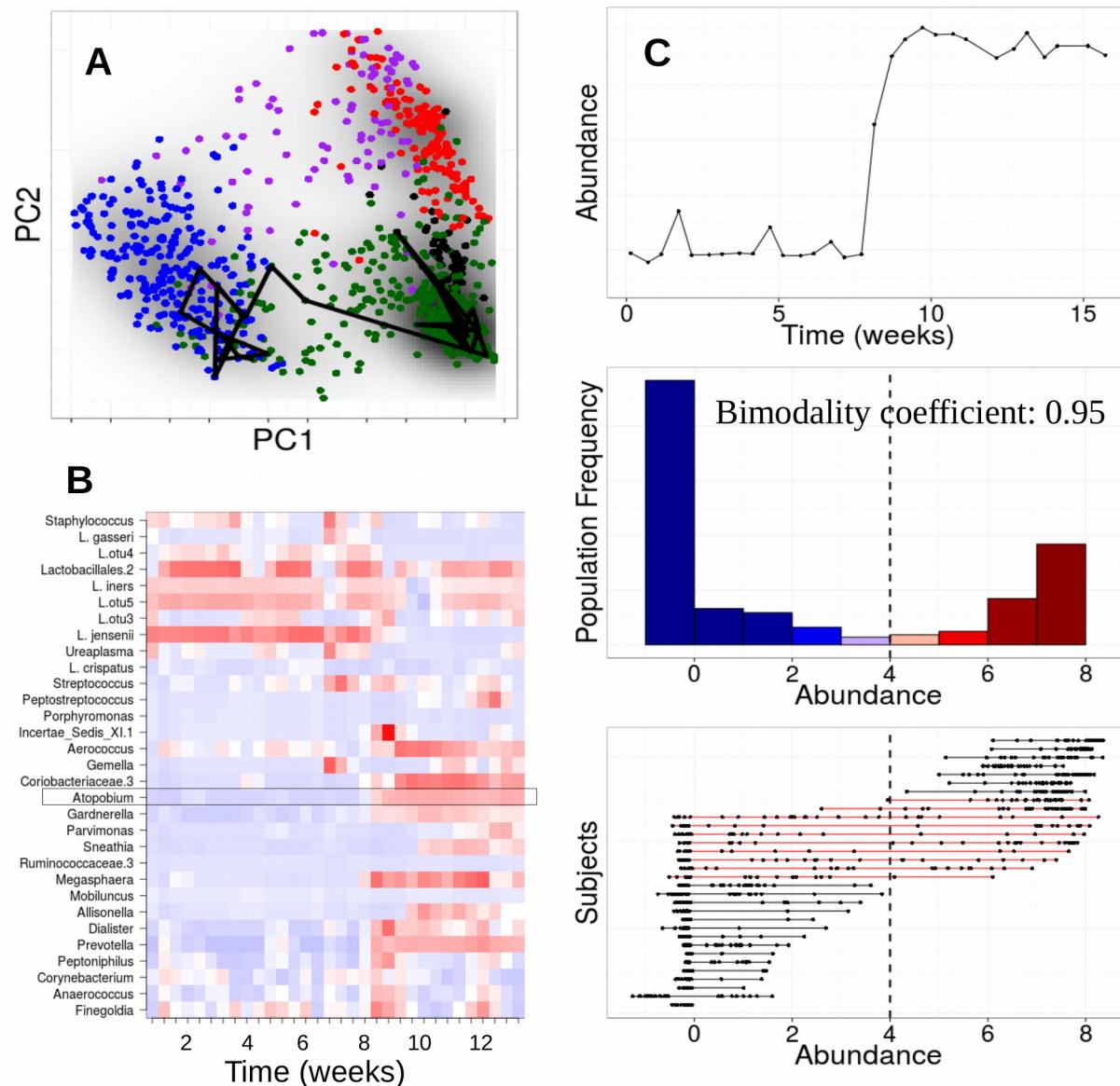
M Scheffer Nature 2010

M Scheffer et al. *Nature* **461**, 53-59 (2009) doi:10.1038/nature08227

CRAN: earlywarnings R package
V. Dakos & L. Lahti

nature

Community-level dynamics in vaginal microbiota

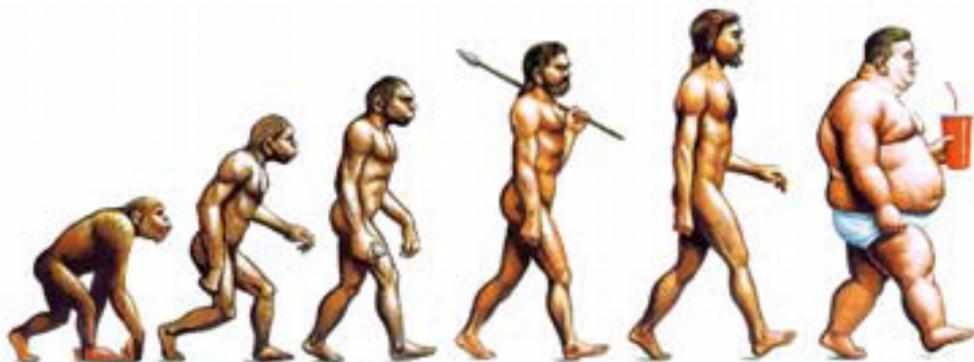


Data: Gajer et al. 2012

Figure: Faust et al. Curr. Op. Microbiol. 2015

Bowel cancer risk may be reduced by rural African diet, study finds

Tests on subjects who swapped a fatty, meat-heavy diet for foods rich in beans and vegetables found a drop in biological markers for cancer in just two weeks



Diet swap – Two weeks ?

nature
COMMUNICATIONS

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NATURE COMMUNICATIONS | ARTICLE



Fat, fibre and cancer risk in African Americans and rural Africans

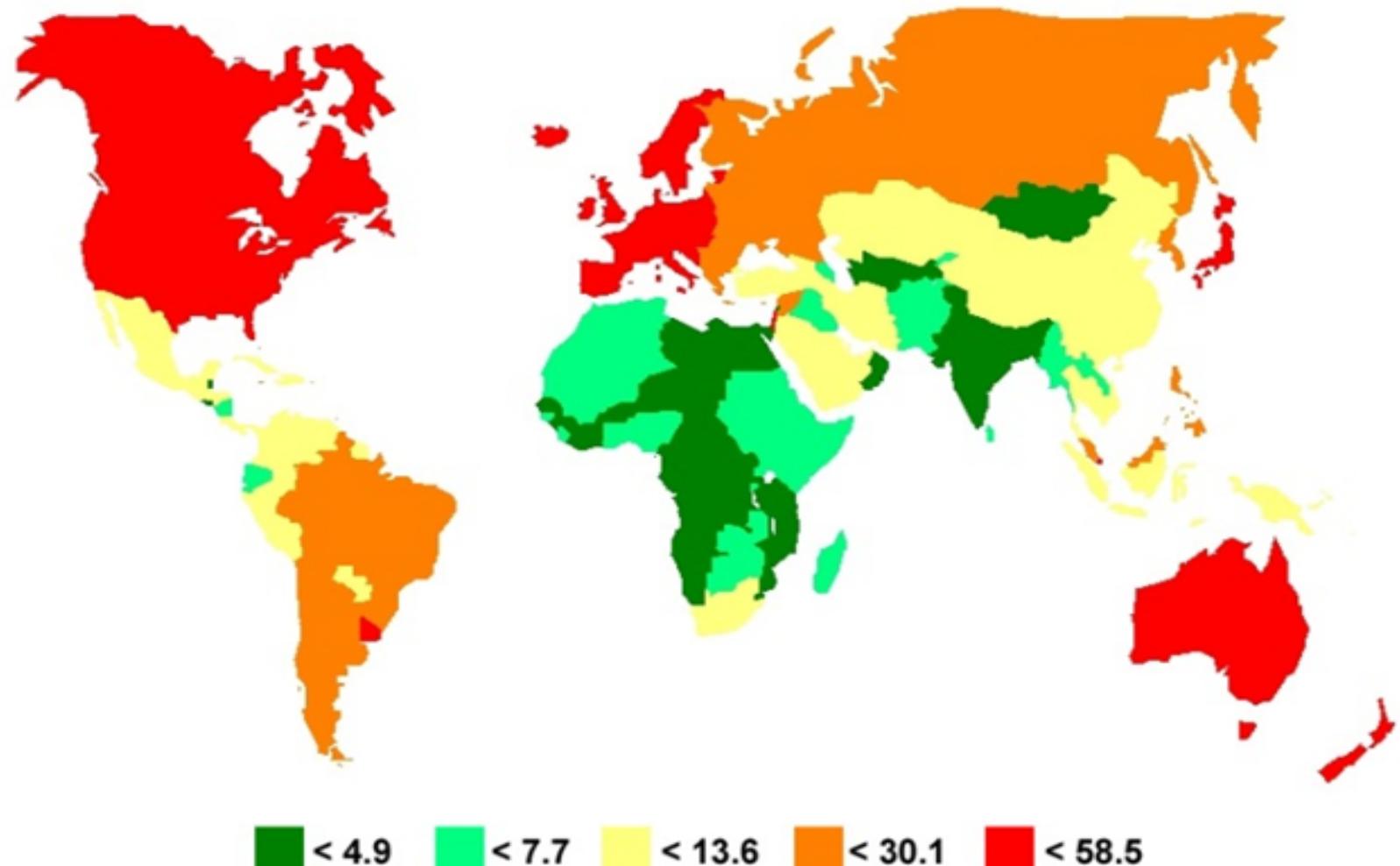
Stephen J. D. O'Keefe, Jia V. Li, Leo Lahti, Junhai Ou, Franck Carbonero, Khaled Mohammed, Joram M. Posma, James Kinross, Elaine Wahl, Elizabeth Ruder, Kishore Vipperla, Vasudevan Naidoo, Lungile Mtshali, Sebastian Tims, Philippe G. B. Puylaert, James DeLany, Alyssa Krasinskas, Ann C. Benefiel, Hatem O. Kaseb, Keith Newton * et al.

[Affiliations](#) | [Contributions](#) | [Corresponding author](#)

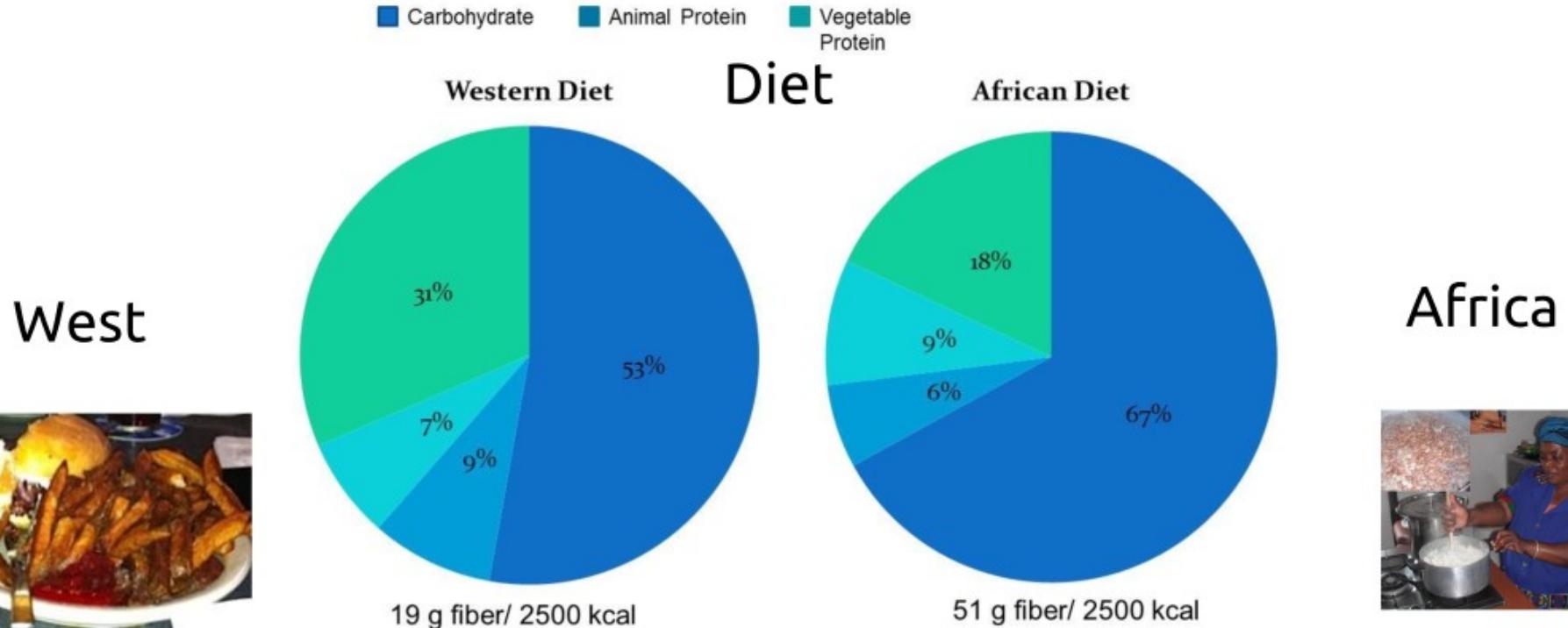
Nature Communications 6, Article number: 6342 | doi:10.1038/ncomms7342

Received 23 May 2014 | Accepted 20 January 2015 | Published 28 April 2015

Colon cancer prevalence



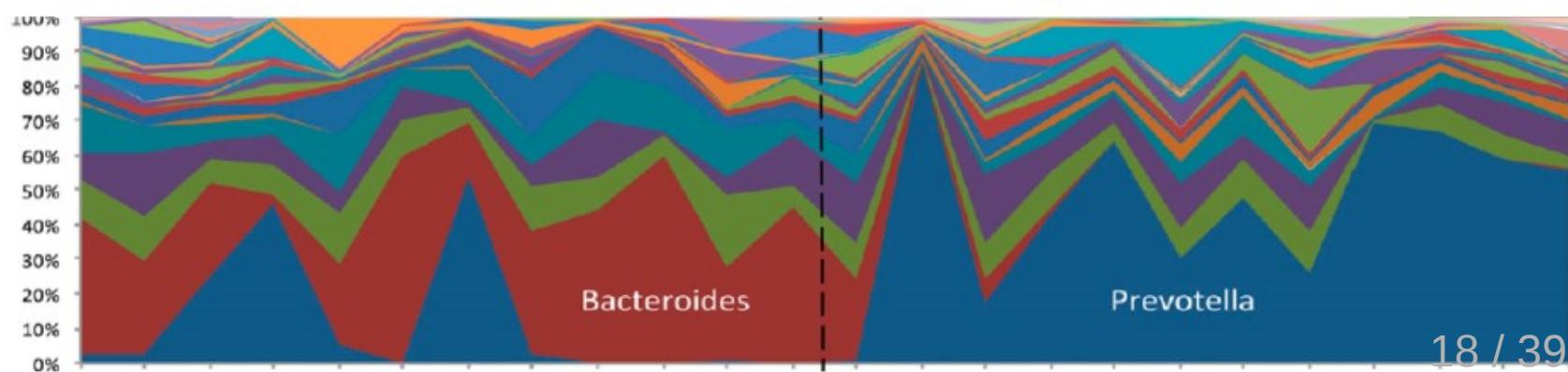
Colon cancer rates per country



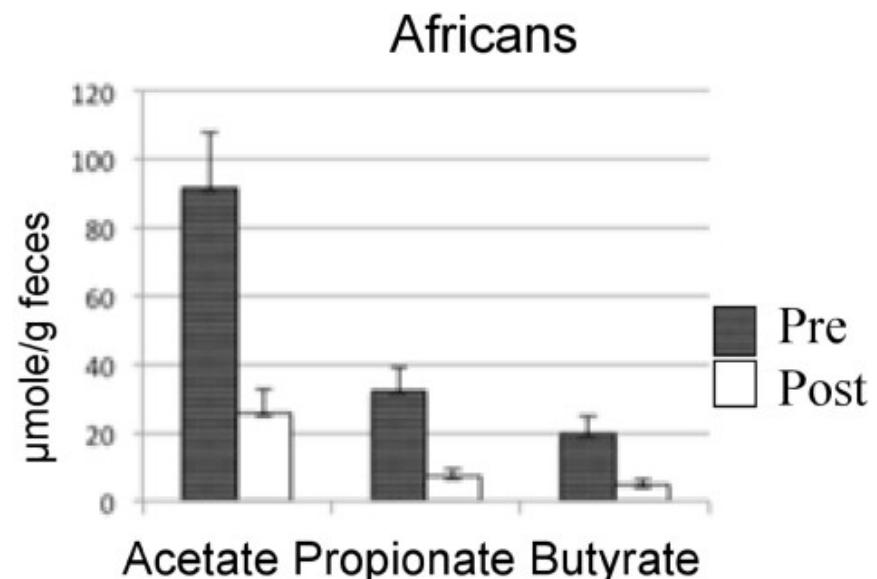
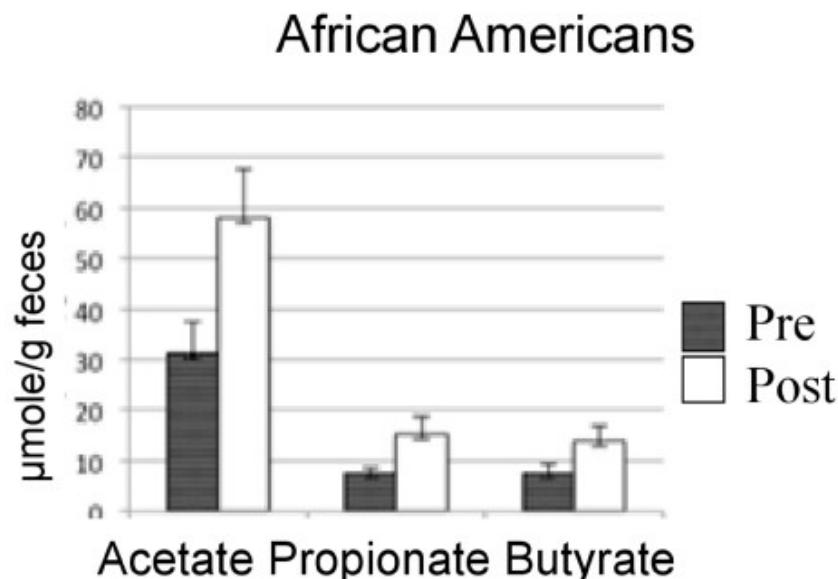
Microbiota composition

Colon cancer rates:
 -Africans: <10:100,000
 -African Americans: >65:100,000

Ou et al. Am J Clin Nutr.
 Jul 2013; 98(1): 111–120



Impact diet exchange on SCFA

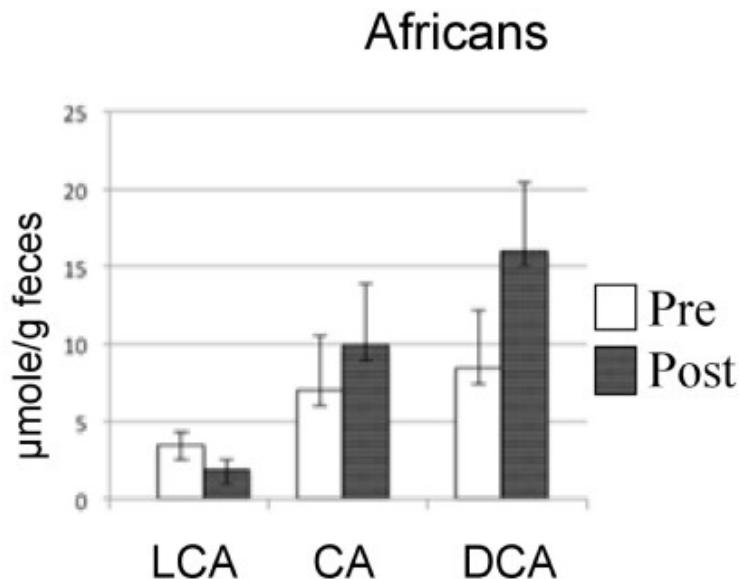
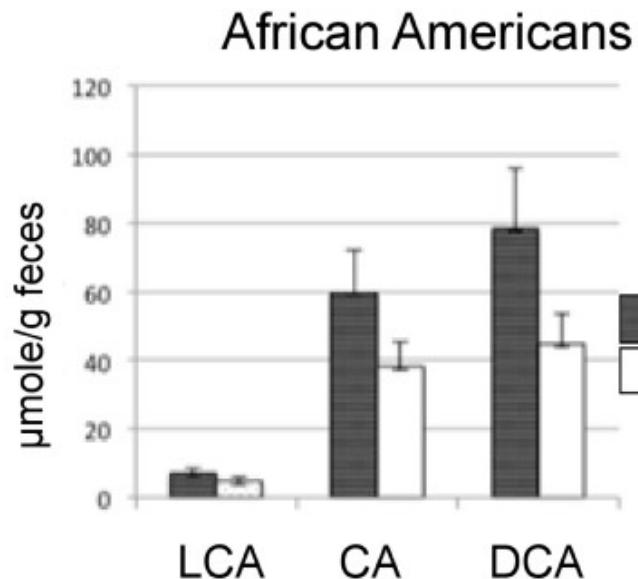


Reciprocally increased SCFA production with African diet: reported anti-inflammatory and anti-carcinogenic properties !

O'Keefe et al.
Nat. Comm. 2015

See also
Louis et al. Nat. Rev.
Microbiol Sept 2014

Impact diet exchange on bile acids



LCA: Lithocholic Acid, CA: Cholic Acid, DCA: Deoxycholic Acid

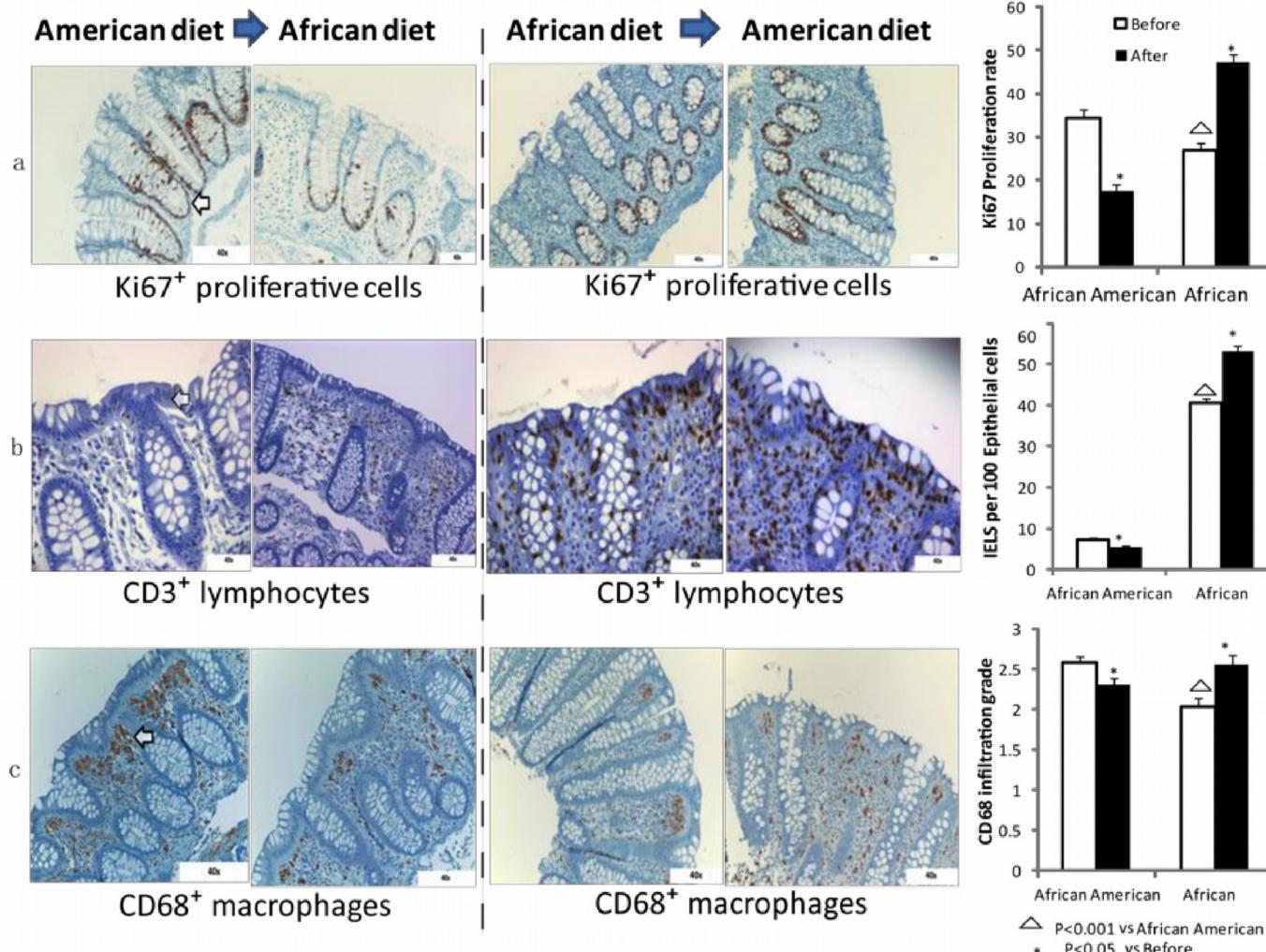
Primary and secondary bile acids reciprocally increased with American diet: reported pro-inflammatory and carcinogenic properties !

See also
Louis et al. Nat. Rev.
Microbiol Sept 2014

O'Keefe et al.
Nat. Comm. 2015

Diet swap (2 weeks) and colon cancer risk

Reciprocal impact on epithelial biomarkers for colon cancer risk seen already after two weeks !





Fiber rich vegetarian food



Finnish Savo
people?



African Zulu
(< 5:100,000)



African
americans
(65:100,000)

Meat and fat



Alaska Yupiks
>100:100,000



Stephen O'Keefe

"African" vegetarian diet – Two weeks

- + Inflammation-reducing microbes
- + Short-chain fatty acids
- Inflammation-promoting microbes
- Bile acids
- Cancer biomarkers (inflammation, cell proliferation)
- Role of bistable bacteria ?

O'Keefe et al.
Nat. Comm. 2015

Confounding variables: stool consistency showed the largest effect size on microbiota variation

RESEARCH | RESEARCH ARTICLES

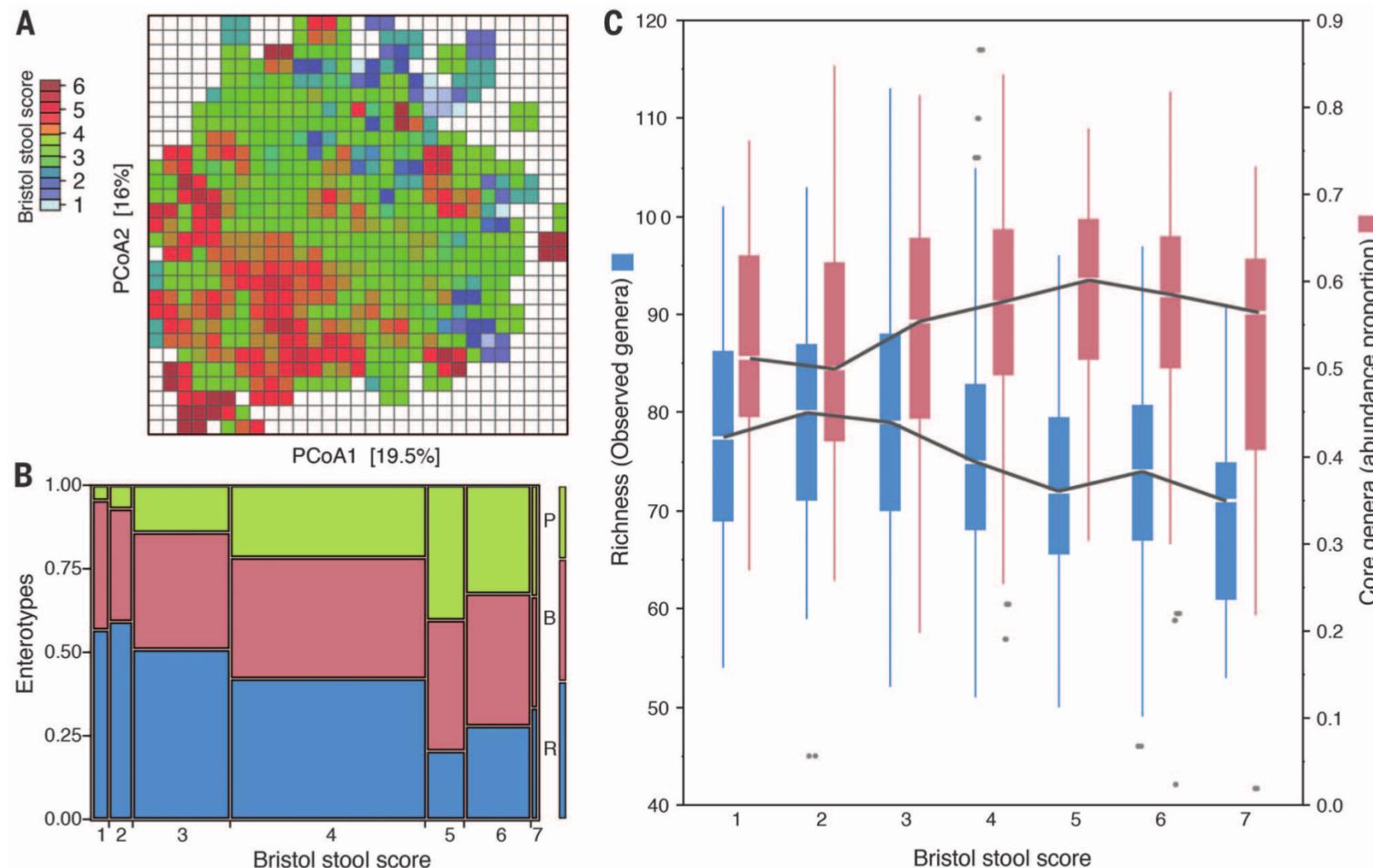
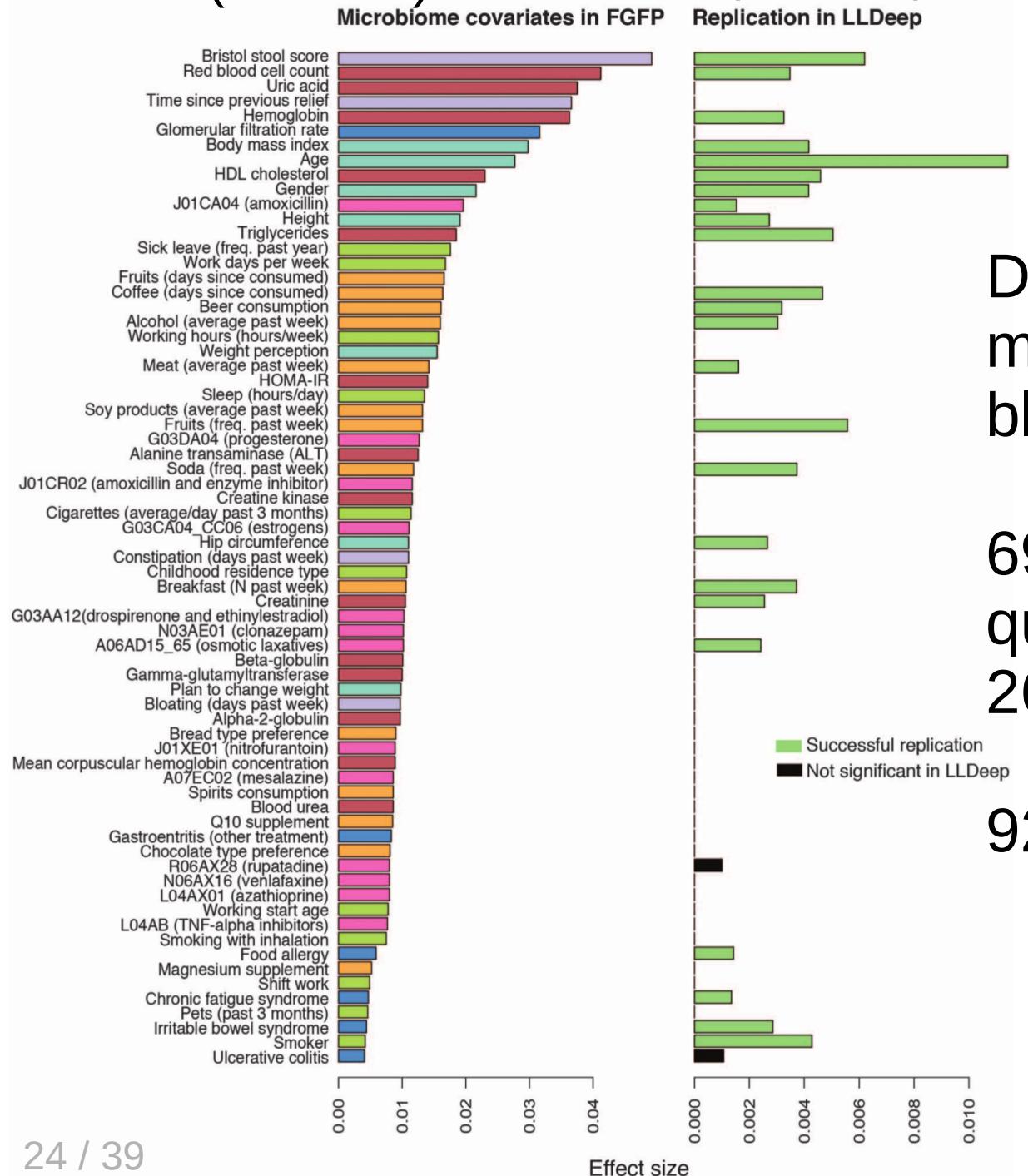


Fig. 4. BSS score association to microbiota variation. (A) BSS score variation across the FGFP cohort, as represented on the genus-level PCoA ordination (Bray-Curtis dissimilarity). Each cell is colored according to median BSS score of individual samples allocated to the cell coordinates. (B) Enterotype distribution over BSS scores [JSD enterotyping (18)] showing an increase in *Prevotella* individuals with looser stool consistency. (C) Median differences in abundance of the core microbiota (FGFP genus-level core at 99%) and in observed genus richness across BSS score.

Flemish Gut Flora (N=1106)

Dutch LifeLines-DEEP (N=1135)



Determinants of microbiota composition ?

Diet, health, lifestyle, medication, host variables, blood, bowel habits..

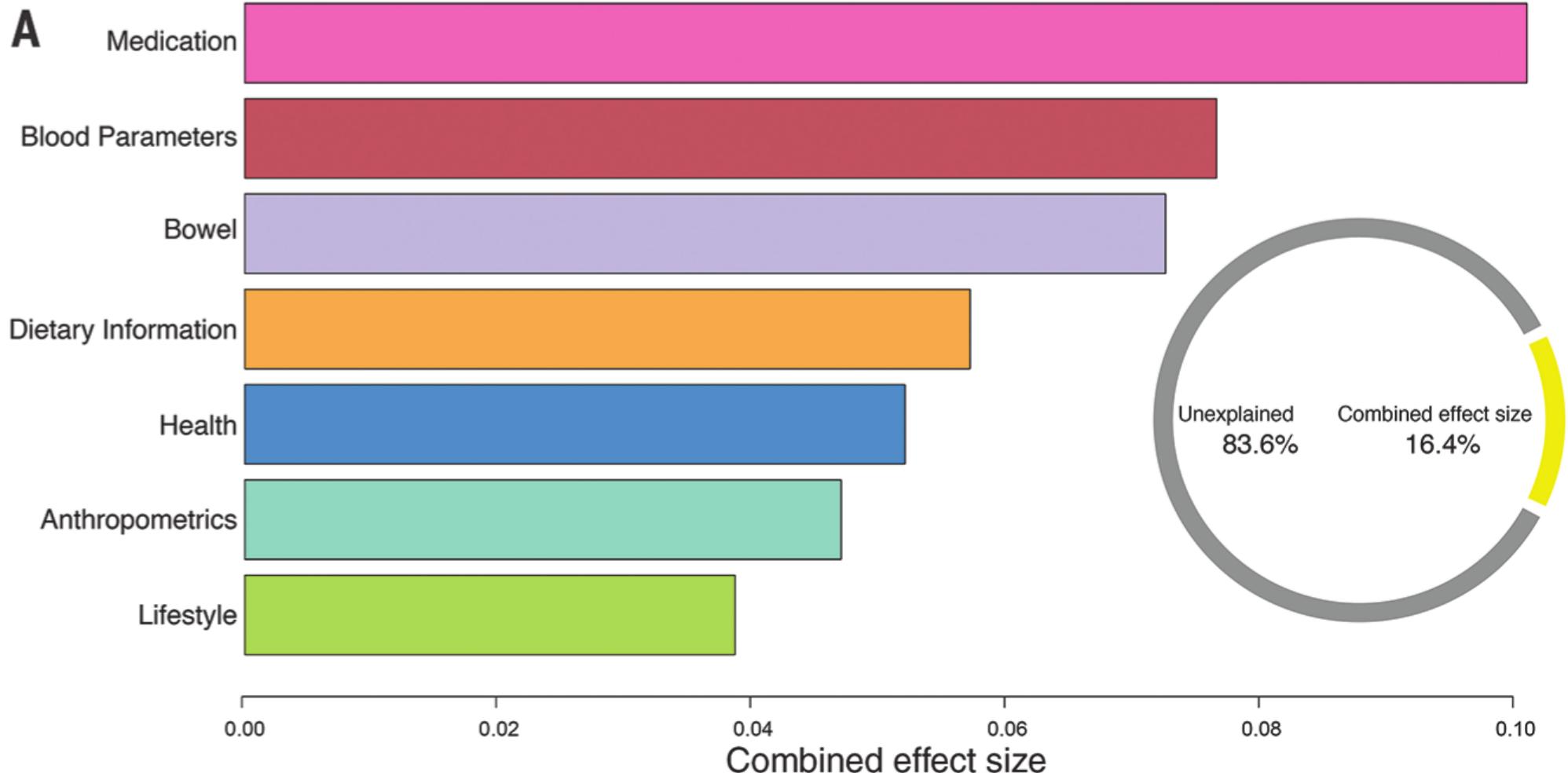
69 covariates (clinical & questionnaire) in FGFP;
26 shared with DEEP

92% replication rate

Falony et al. Science 352, 2016.

Total explained variation with recorded variables 16.4%

Proposed disease marker genera associated to host covariates and medication - inclusion in study design is essential !



Thank You !

Anne Salonen
Jarkko Salojärvi
Karoline Faust
Jonna Jalanka
Philippe Puylaert
Stephen O'Keefe
Erwin Zoetendal
Marten Scheffer
Jeroen Raes
Willem M de Vos

Univ. Turku, Dpt.
Mathematics & Statistics

Univ. Helsinki, Dpt.
Veterinary Biosciences

Wageningen University, Lab.
Of Microbiology, Netherlands

VIB/KU Leuven, Center for
Biol. Of Disease, Belgium



<http://microbiome.github.io>



- Population-level analysis of gut microbiome variation.
Falony et al. *Science* 352(6285):560-4, 2016
- Fat, Fiber and Cancer Risk in African, Americans and Rural Africans. O'Keefe et al. *Nat. Comm.* 6:6342, 2015
- Metagenomics meets time series analysis: unraveling microbial community dynamics. Faust, Lahti et al. *Current Opinion in Microbiology* 15:56-66 2015
- Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. Salonen et al. *ISME Journal* 8:2218-30, 2014
- Tipping elements in the human intestinal ecosystem.
Lahti et al. *Nat. Comm.* 5:4344, 2014
- Associations between the human intestinal microbiota, *Lactobacillus rhamnosus* GG and serum lipids indicated by integrated analysis of high-throughput profiling data.
Lahti et al. *PeerJ* 1:e32, 2013

Core microbiota, healthy western adults (n=456)

Top-10 genus-level groups

Faecalibacterium prausnitzii et rel.
Ruminococcus obeum et rel.
Subdoligranulum variable at rel.
Dorea formicigenerans et rel.
Oscillospira guillermondi et rel.
Lachnospira pectinoschiza et rel.
Bifidobacterium
Clostridium symbiosum et rel.
Clostridium orbiscindens et rel.
Clostridium sphenoides et rel.

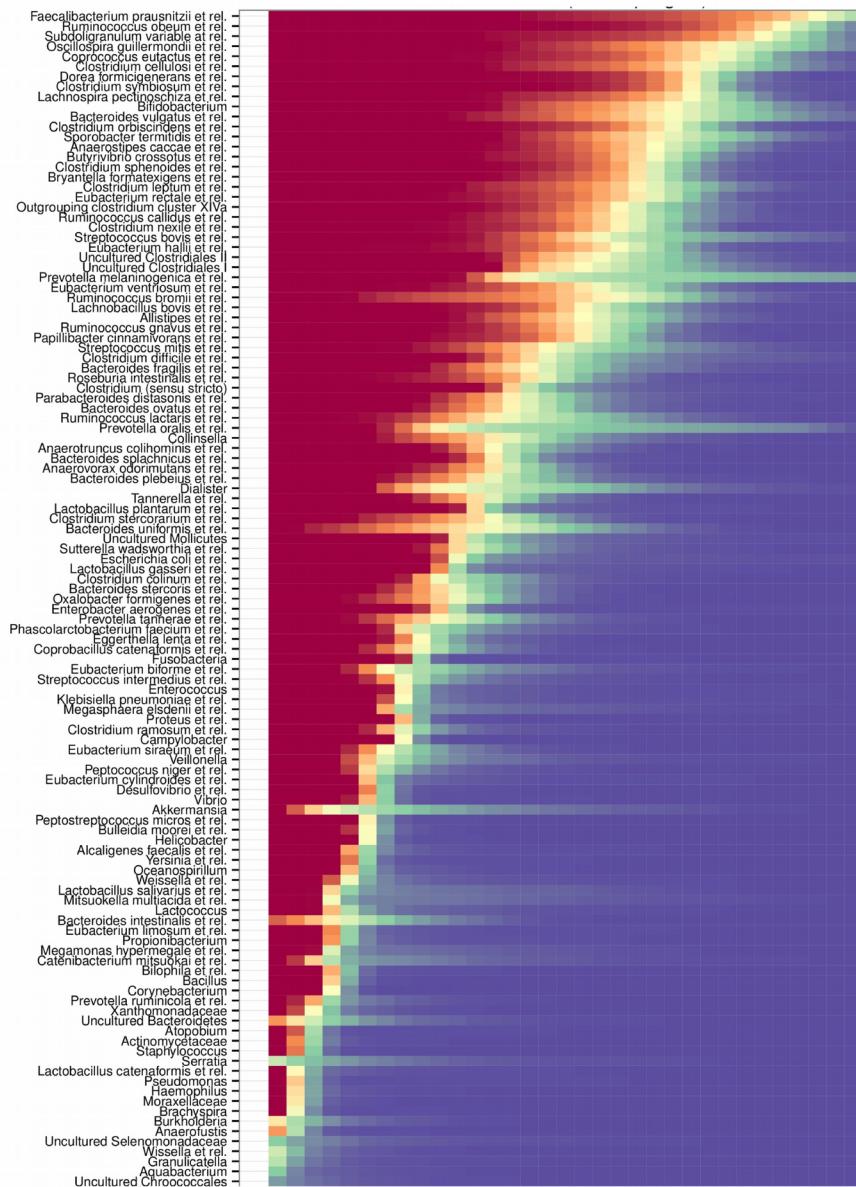
Jalanka-Tuovinen et al. (2011) PLoS One

6:e23035

Salonen et al. (2012) Clinical microbiology and
infection 18:16–20.

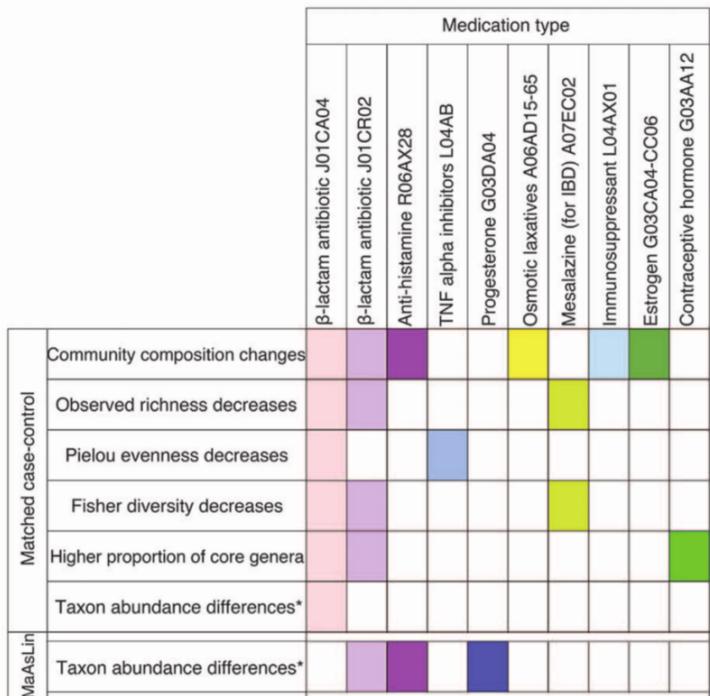


Code: [microbiome.github.com](https://github.com/microbiome)



Detection threshold (abundance)

A



*Decreased in medicated *Increased in medicated

Butyrivibrio	Anaerostipes
Coprococcus	Bacteroides
Prevotella	Collinsella
uncl. Bdellovibrionaceae	Flavonifractor
uncl. Clostridiaceae	Parabacteroides
uncl. Clostridiales	
uncl. Desulfovibrionaceae	
uncl. Gracilibacteraceae	
uncl. Lachnospiraceae	
uncl. Prevotellaceae	
uncl. Ruminococcaceae	
uncl. Veillonellaceae	
	uncl. Enterobacteriaceae
	Eggerthella
	Flavonifractor
	Parasutterella
	uncl. Eubacteriaceae

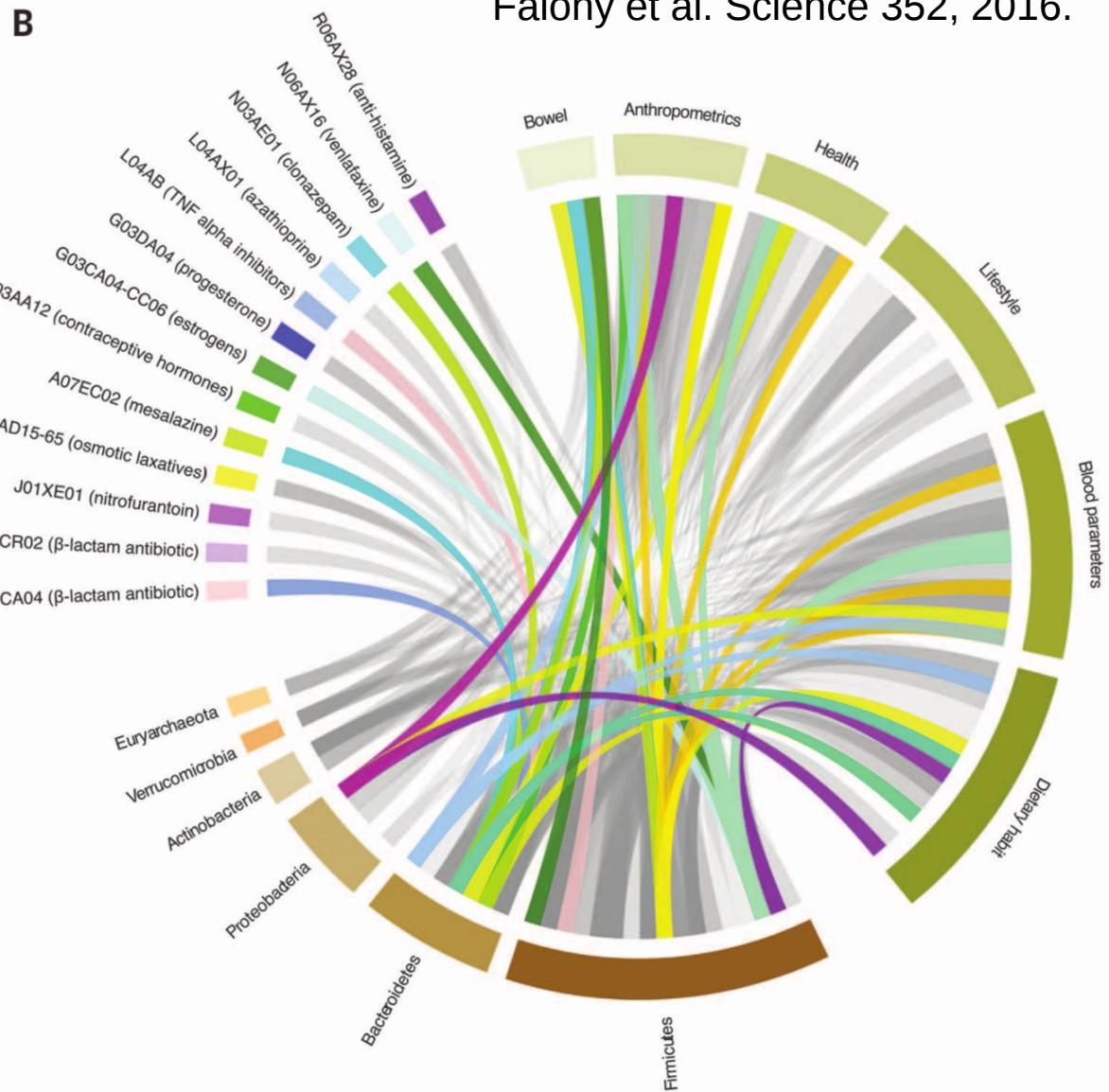
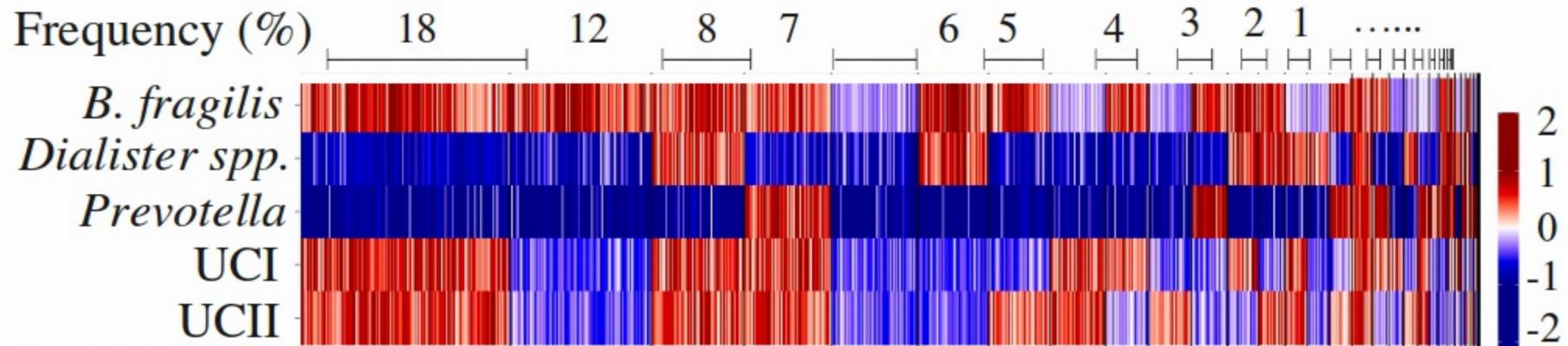


Fig. 5. Drug interactions in the FGFP. (A) Overview of the association between different types of medication and microbiome composition. Colored boxes (color coding according to medication) represent a significant result in the matched case-control (FDR<5%) or boosted additive general linear modeling (FDR<10%, table S11) analyses. The effect (decrease/increase) of medication on genera abundances is specified. (B) Circos plot showing correlations between covariates and genus abundances (FDR<10%) interacting with drugs. Genera are grouped at phylum level; ribbons represent genus-phenotype associations and are colored according to the confounding medication (gray indicates nonconfounded).

Independent 'tipping elements' of the intestinal microbiota ?

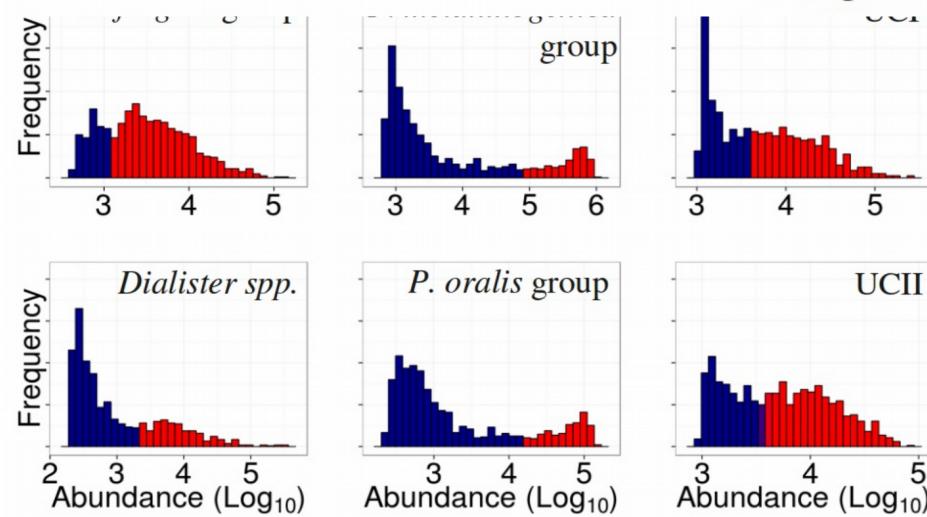
Uncorrelated state switches -> various combinations !



Lahti et al. Nat. Comm. 5:4344, 2014

Subjects (n=1006)

-> Ecosystem states as specific combinations of bi-stable taxa?

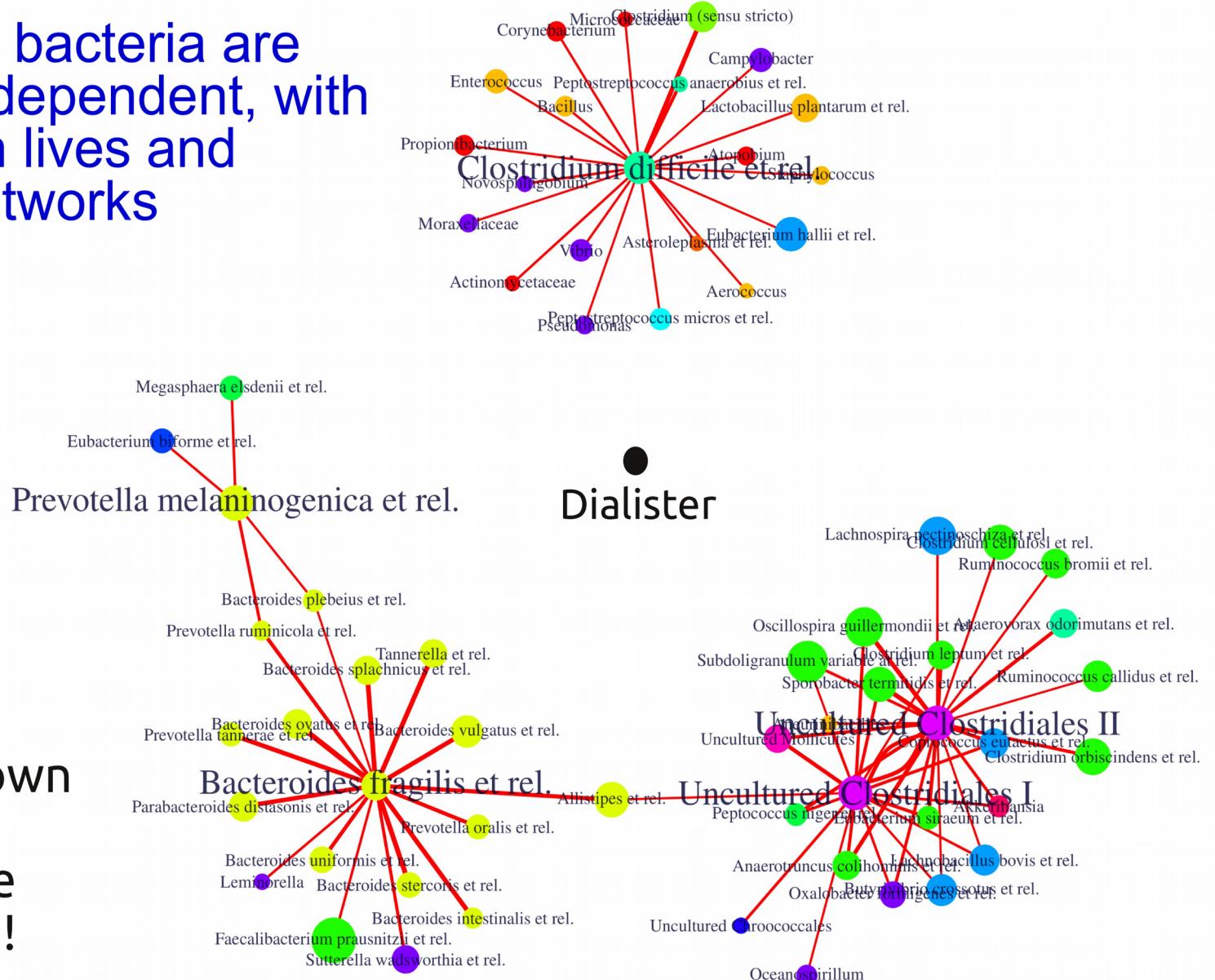


[http://commons.wikimedia.org
/wiki/File:RF-remote-control-dip-switch-calculator.png](http://commons.wikimedia.org/wiki/File:RF-remote-control-dip-switch-calculator.png)

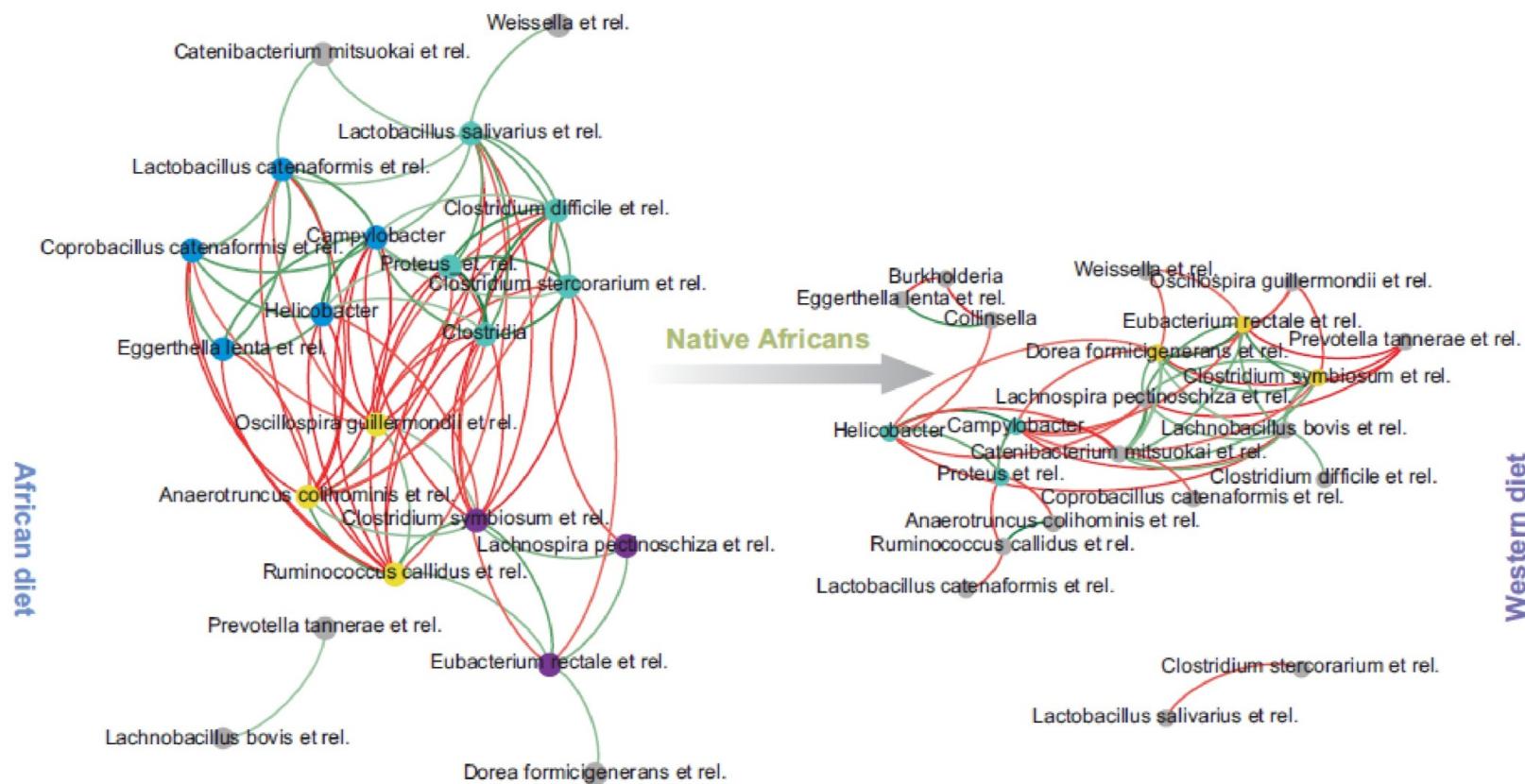
Bi-stable bacteria are rather independent, with their own lives and social networks

$|r| > 0.33$ shown

Only positive correlations !



Westernization of African communities?



Short-term diet shift

- > Limited impact on microbiota composition
- > Large impact on microbiota activity
- > Can microbiota interactions explain the differences ?

Enterotype hypothesis

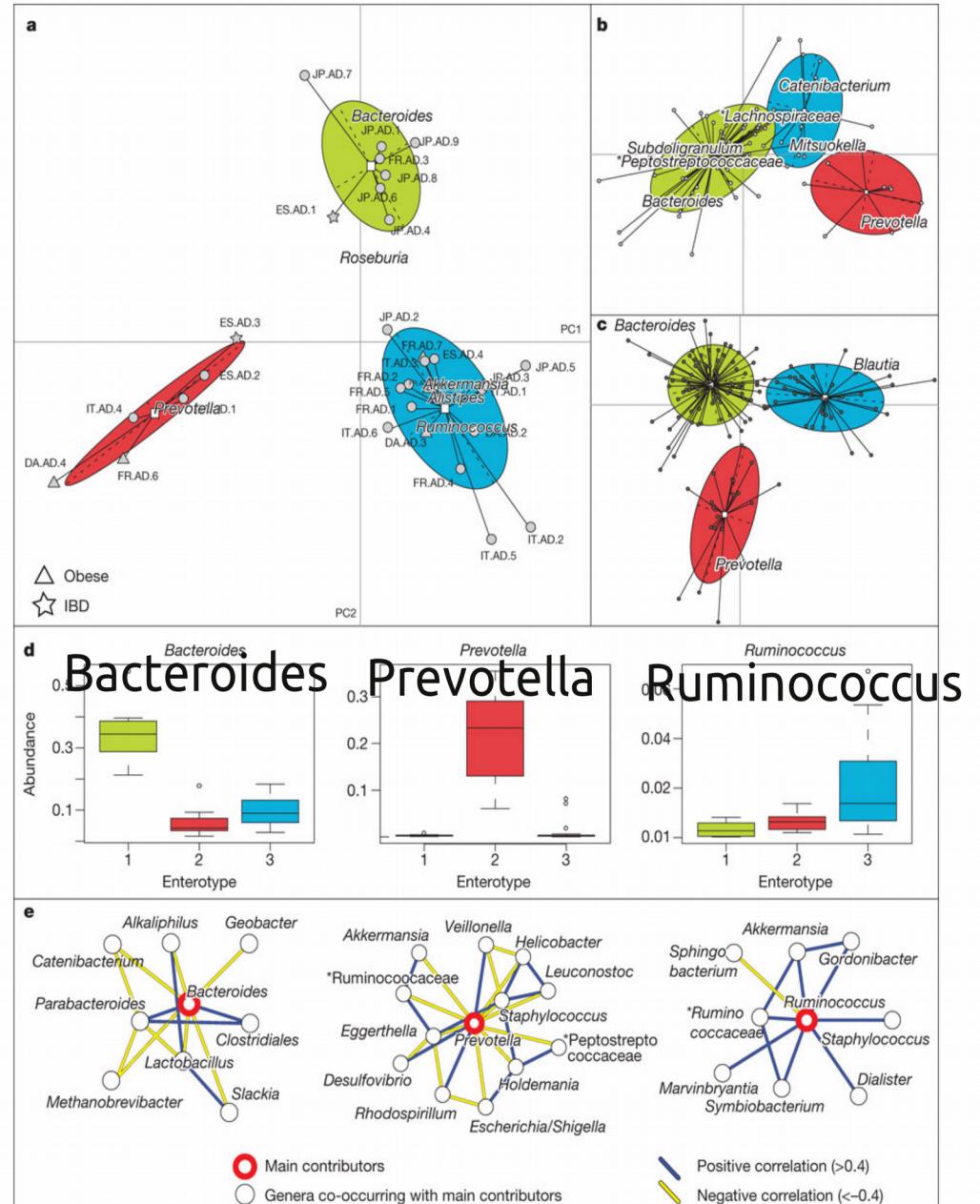
Three universal community types / cores:

-> alternative stable states of the human intestinal ecosystem ?

Not explained by nationality, diet, or other such factors

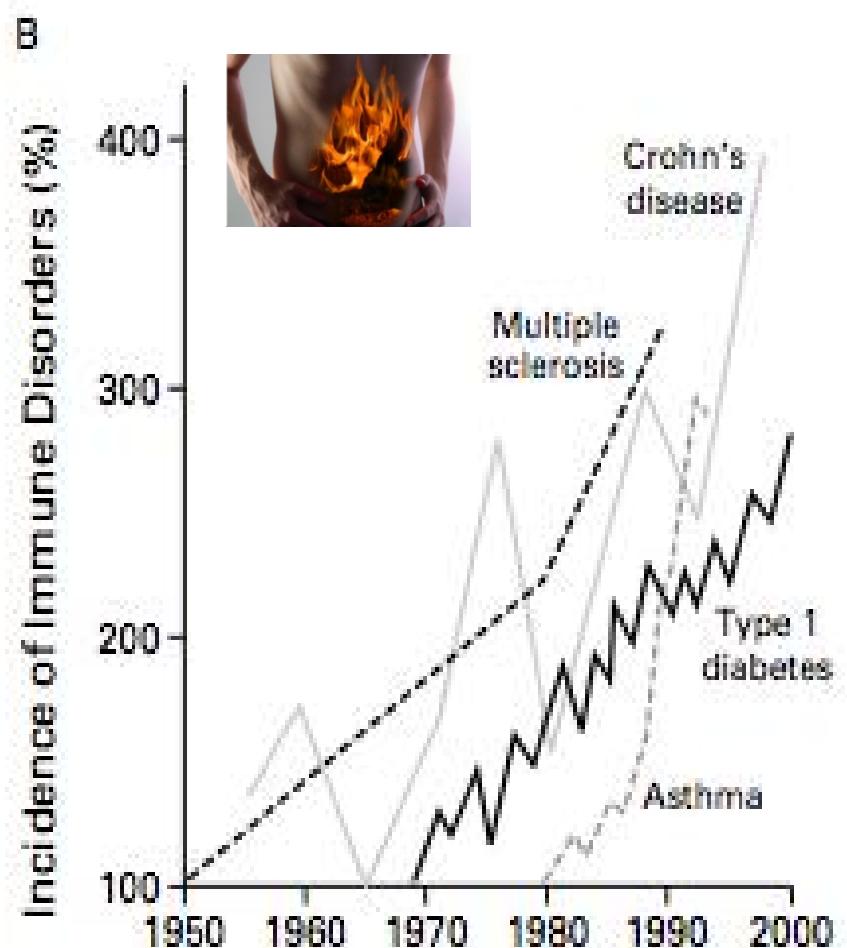
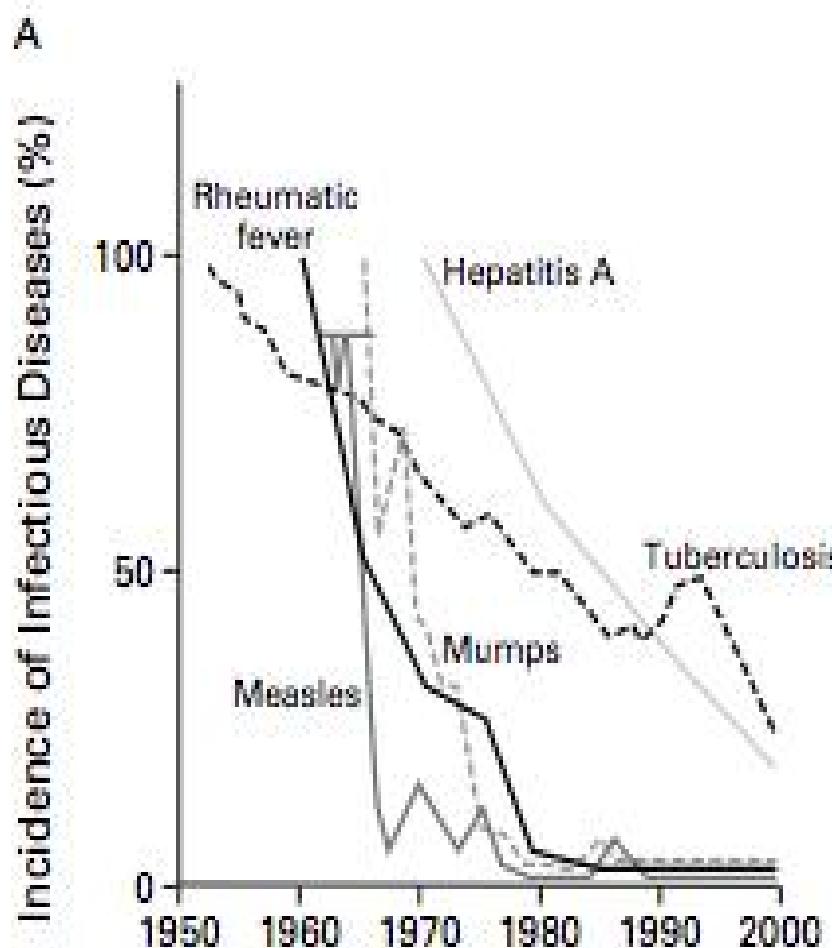
Also reported in
Mouse & Chimpanzee

- Wu et al. Science 2011
- Moeller et al. Nat. Comm. 2012
- Wang et al. PNAS 2014



Arumugam et al. Nature 2011

Many diseases linked to gut microbiota becoming more prevalent in the past decades !



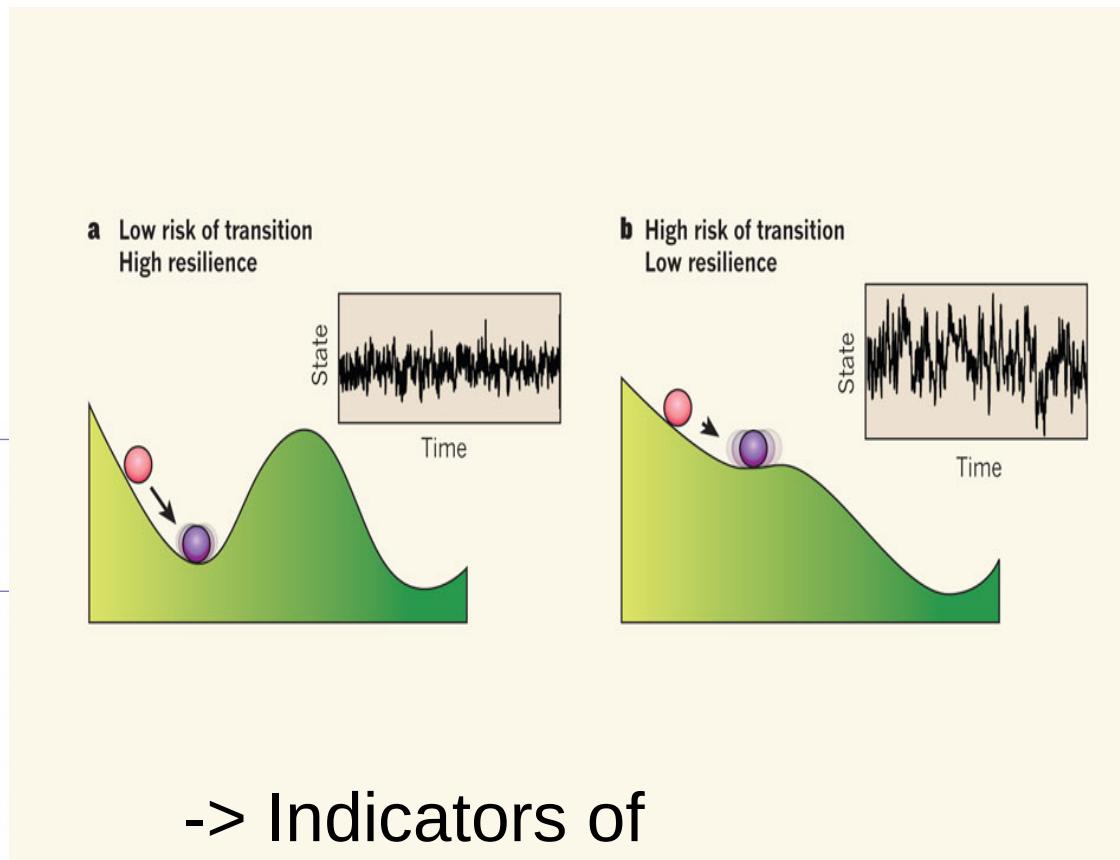
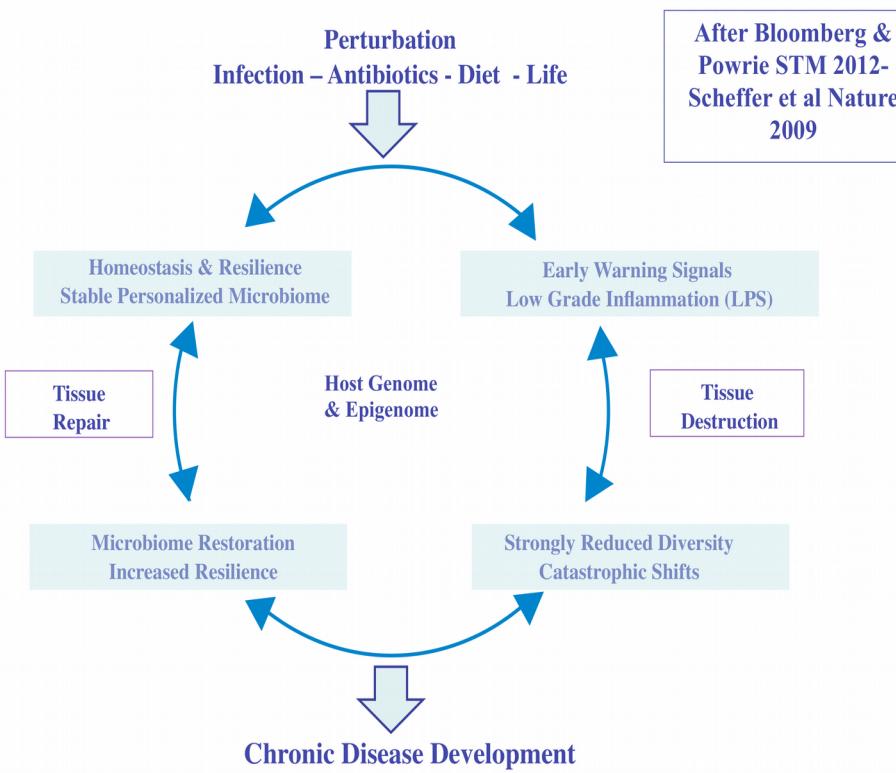
Microbiota manipulation

- Diet
- Life style (stress, sleep, exercise)
- Antibiotics
- Probiotics
- Prebiotics
- Fecal transplant



How do resilient, stable alternative states emerge?

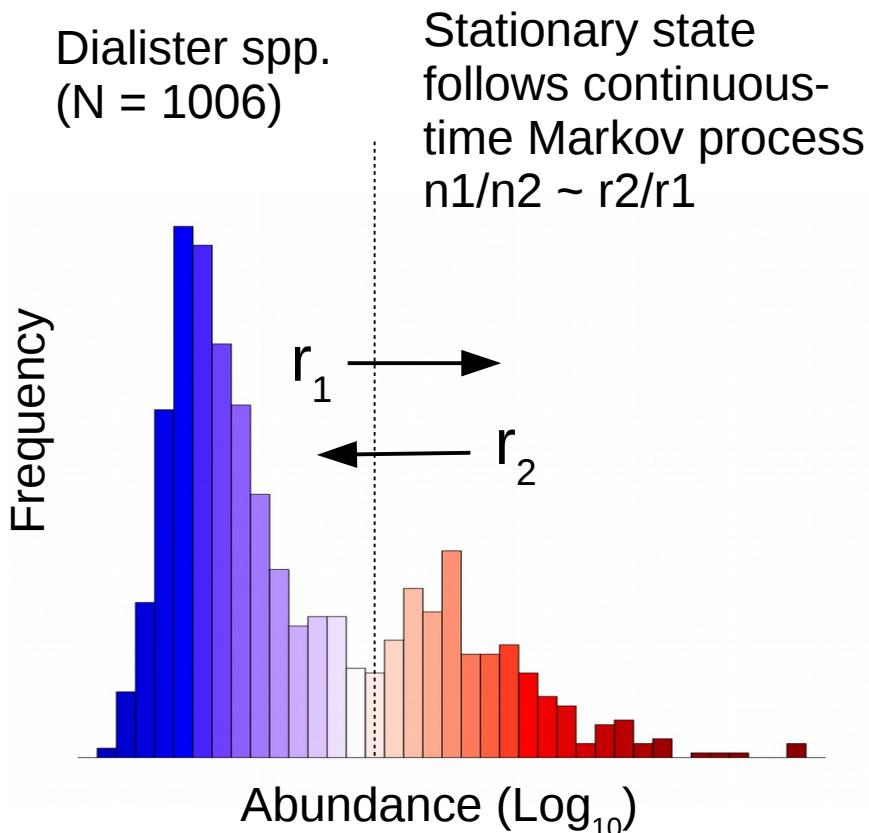
- Competition
- Mutualism
- Feed-back loops



-> Indicators of community state & health status?

-> Robust targets for therapeutic manipulation ?

Detecting bimodality: Potential analysis & Fokker-Planck dynamics



State stability?
Enrichments with diet / health / etc.?
Associations with the overall ecosystem?

System state described by

$$Dz = -U(z)dt + \sigma dW$$

Potential coupled with observation density:

$$U(z) = -\sigma^2 \log P(z)/2$$

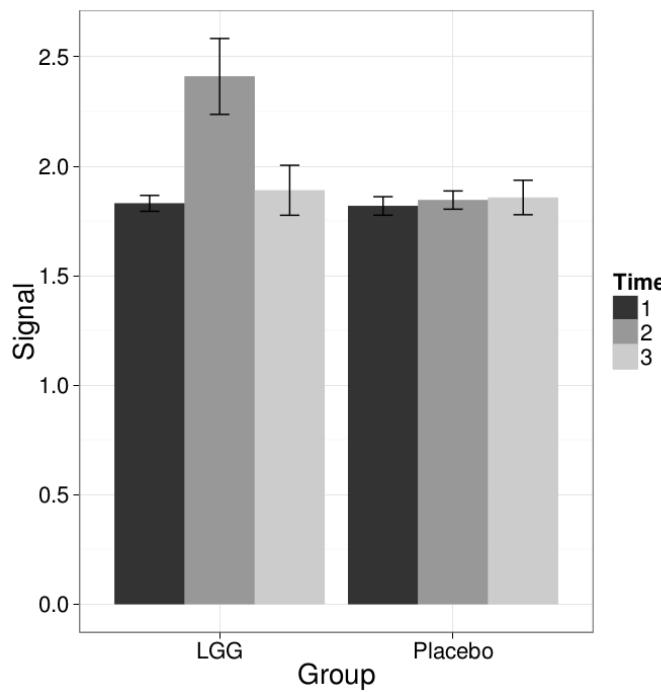
+ bootstrapping etc. to control sampling fluctuations



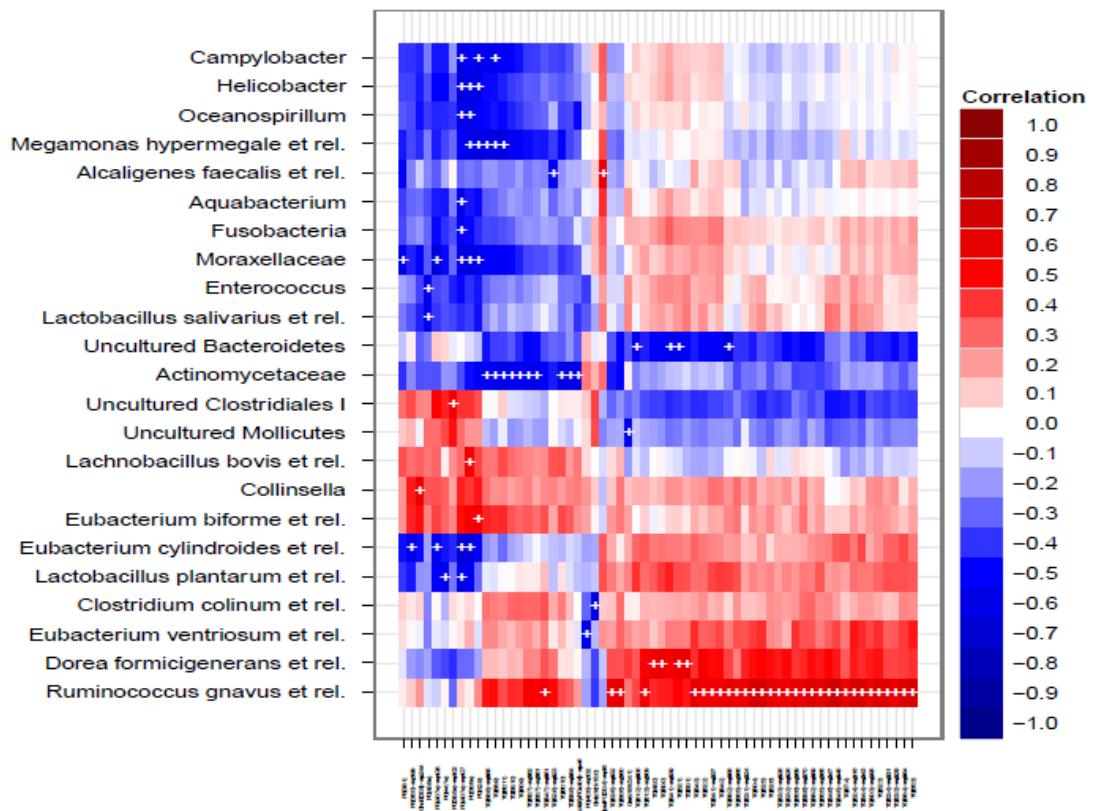
CRAN: [earlywarnings](#)

Hirota et al. Science 2011
Livina et al. Clim Past. 2010
Lahti et al. Nat. Comm 2014 36

Probiotic intervention (*L. rhamnosus* GG) 3-week follow-up → No global effect on microbiota composition



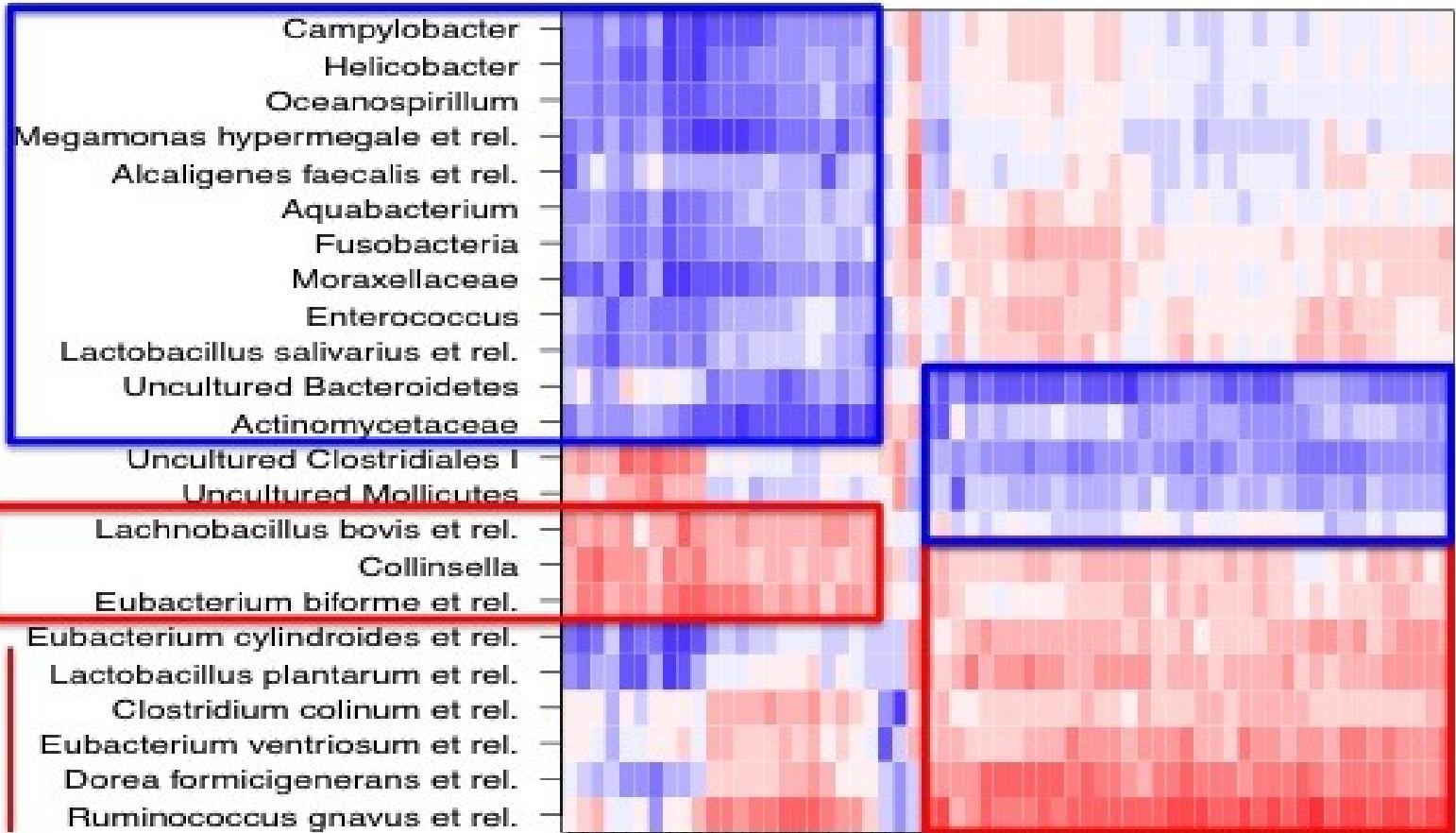
Correlations microbiota & serum lipids



Gut microbiota and blood serum lipid links

Metabolic rather than phylogenetic clusters which are likely to indicate functional links

Potential pathogens

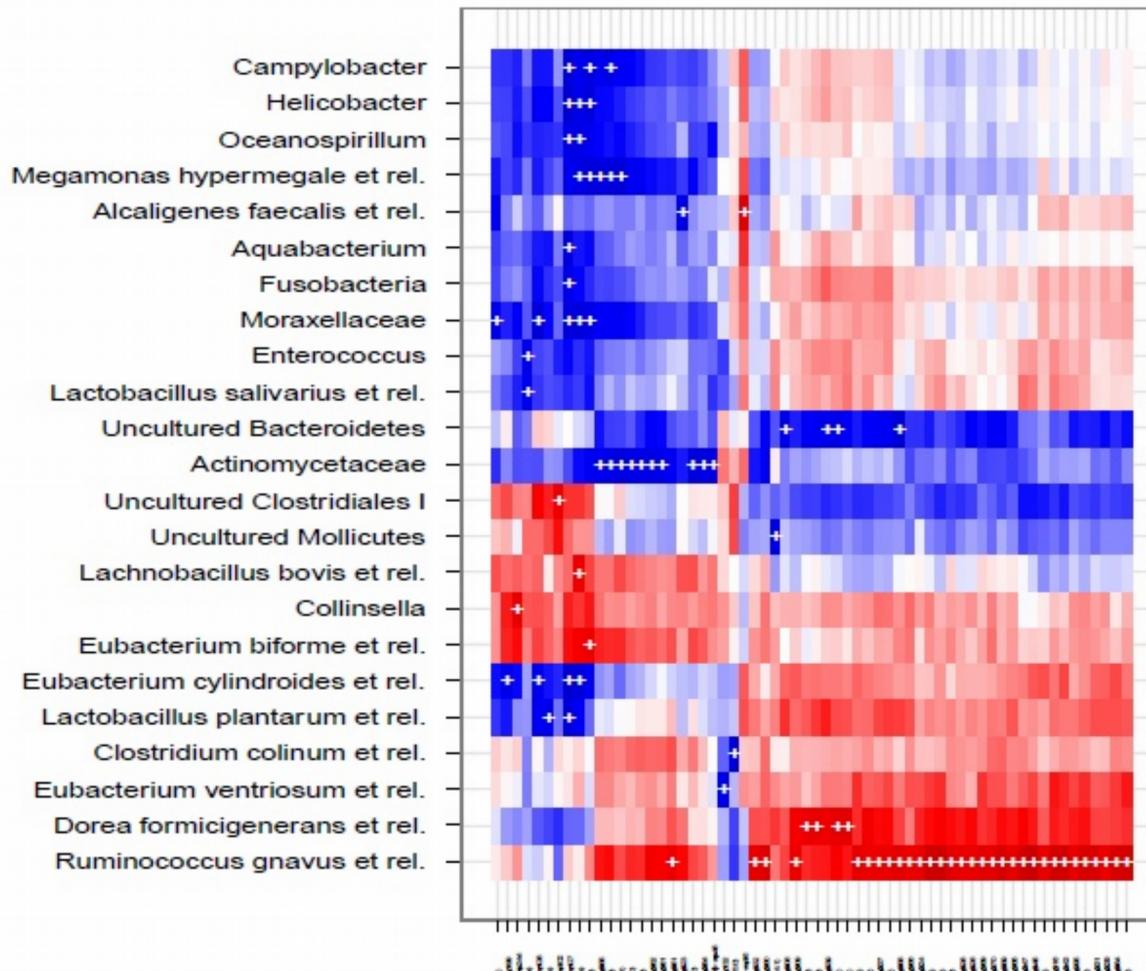


Lahti et al. PeerJ 32, 2013

$Q < 0.05$

Red = positive correlation Blue = negative correlation

Microbiota & serum lipid associations



Serum lipids high-throughput assay



PeerJ

Lahti et al.
PeerJ 1:e32, 2013

Positive:
Collinsella
~ serum cholesterol

Negative:
Actinomycetaceae
~ triglycerides
Proteobacteria
~ ether
phosphatidylcholines

Firmicutes, Actinobacteria,
Proteobacteria involved in
metabolism of dietary and
endogenous lipids?