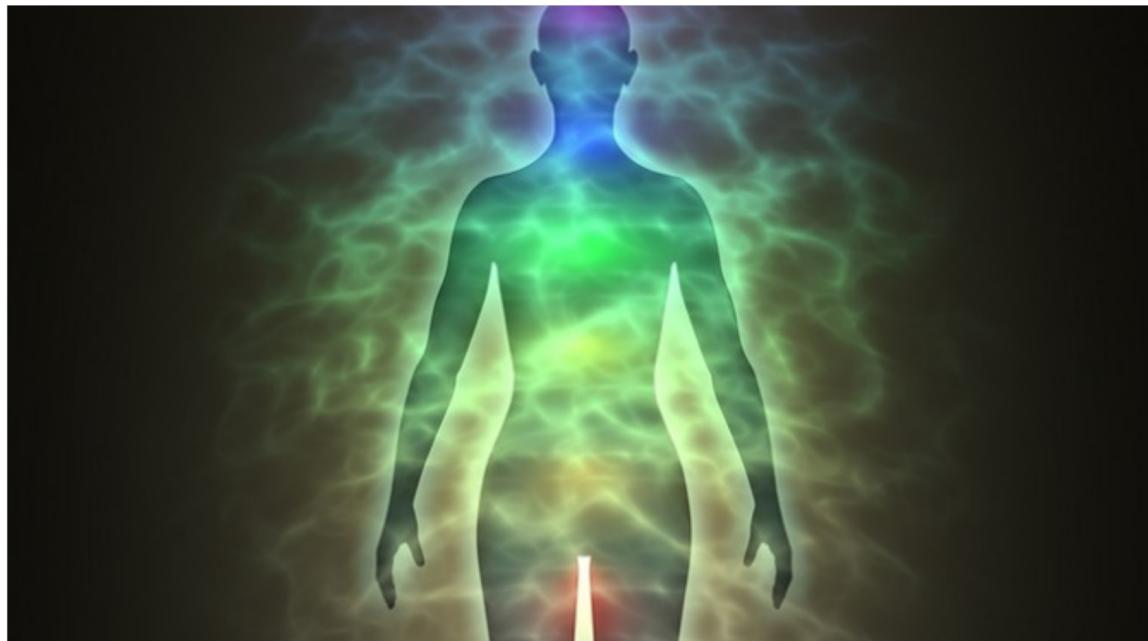


The human microbiome: from individuals to populations

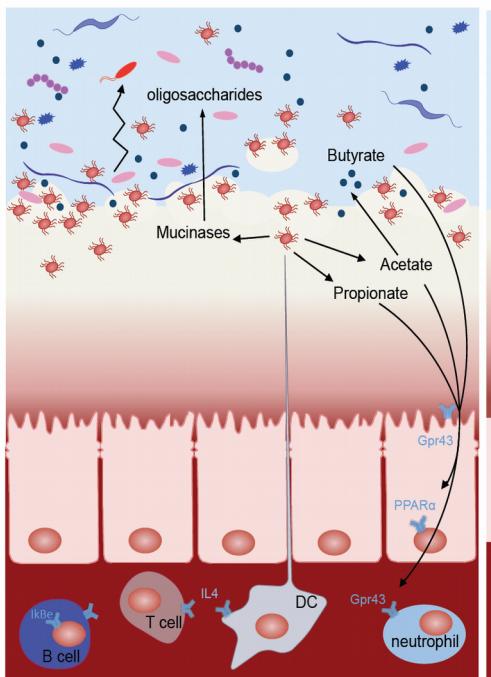
Leo Lahti | leo.lahti@iki.fi | <http://www.iki.fi/Leo.Lahti>
University of Turku, Finland & VIB/KU Leuven, Belgium



Emitting over 10 million biological particles per hour – personal cloud from cradle to grave. Meadows et al. PeerJ 2015 – Metcalf et al. Science 2015

Gut microbiome inhabits 300-400 m² intestinal surface

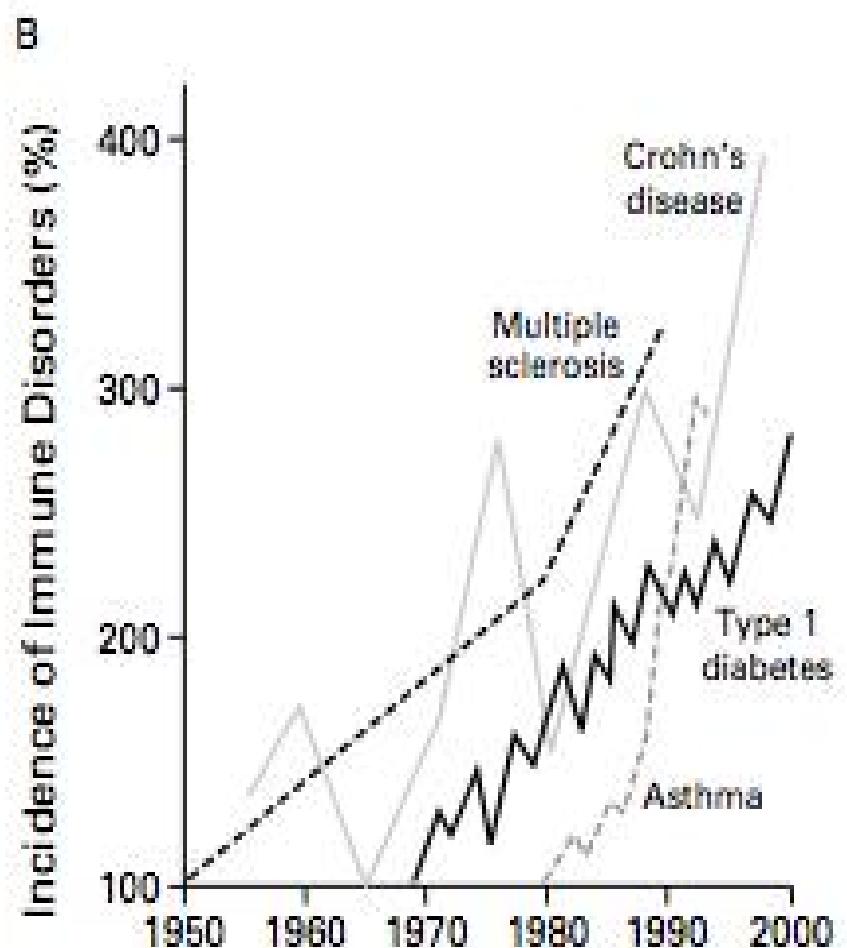
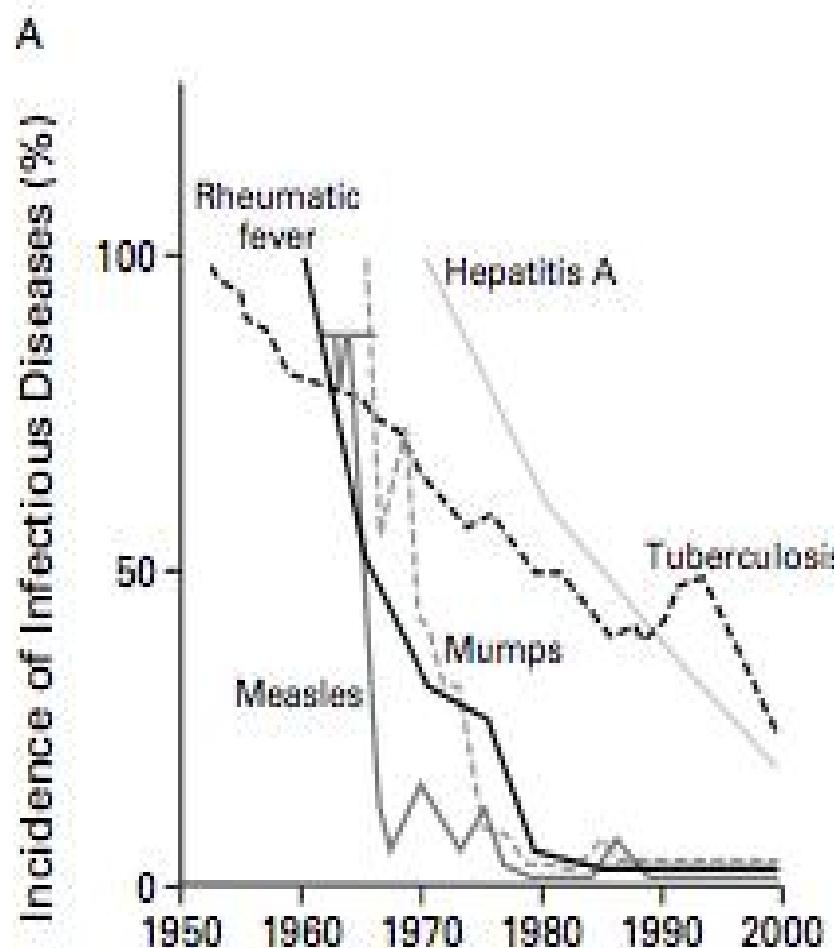
- ~ bacterial cells outnumber our own
- ~ 100 billion bacteria / gram in the gut
- ~ 10,000 gut bacterial species
- ~ 10M uniq genes (500x human genome)
- ~ Plastic, varies in time



Brussel central square flower carpet



Diseases linked to gut microbiome have become more prevalent !



The promise of the microbiome field: diagnostics & cure for all major diseases on the planet



Reality check: We don't even know what a healthy flora means!

Microbiome state-of-the-art:

MetaHIT, HMP + specific lab studies combined 2-3000 individuals,
still biased cut of the population



Genetics: 10.000-100.000s
individuals profiled

Variation in clinically relevant population = unknown
Temporal variation = largely unknown
Factors influencing gut flora composition = largely unknown
Effect environment = unknown

Clinical end points for functional foods, pre-/pro/synbiotics, pharma-/nutriceutical interventions etc are *unknown*

HITChip Atlas: Gut microbiota high-throughput profiling
10,000 samples ~ 5000 individuals ~ 1000 phylotypes
-> Microbial composition is individual and variable !

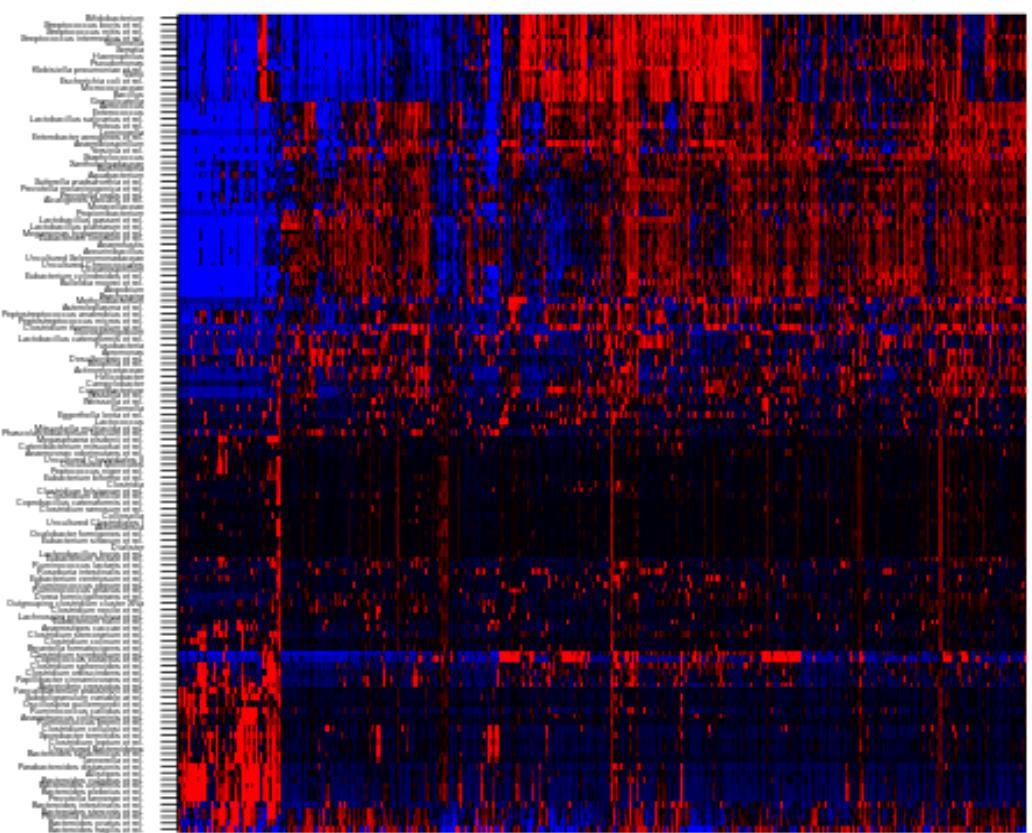
Highly standardized & reproducible platform

Deep phylotype level profiling

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



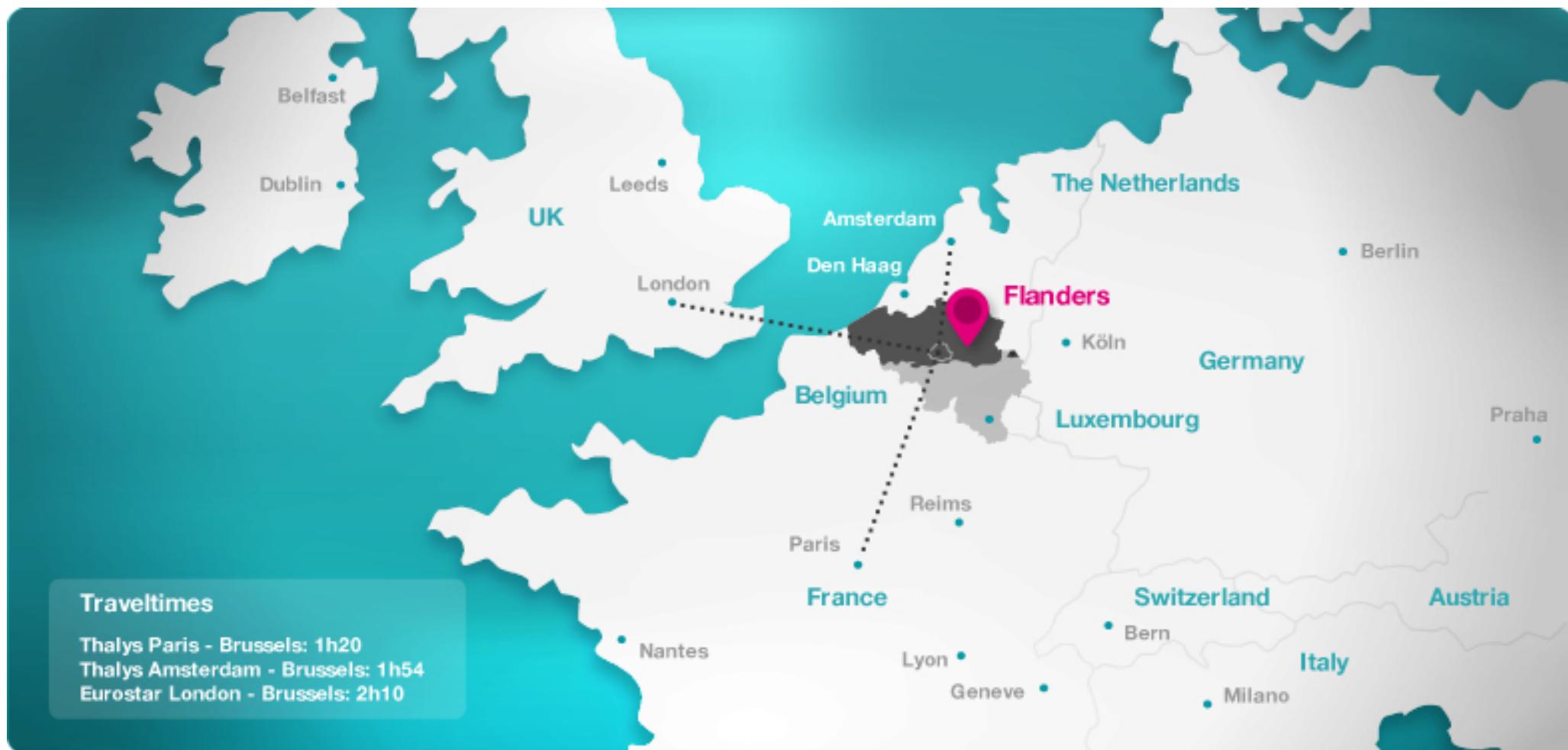
1000 bacterial phyotypes



<http://microbiome.github.io>

Standardized – cost efficient – accurate at 0.1% relative abundance
Rajilic-Stojanovic et al. Env. Microbiol. 2009

Flemish gut flora project: longitudinal study of +-5000 volunteers spread over a confined geographic region



FGFP sample collection

- Collection of faecal, blood (GP) and saliva samples

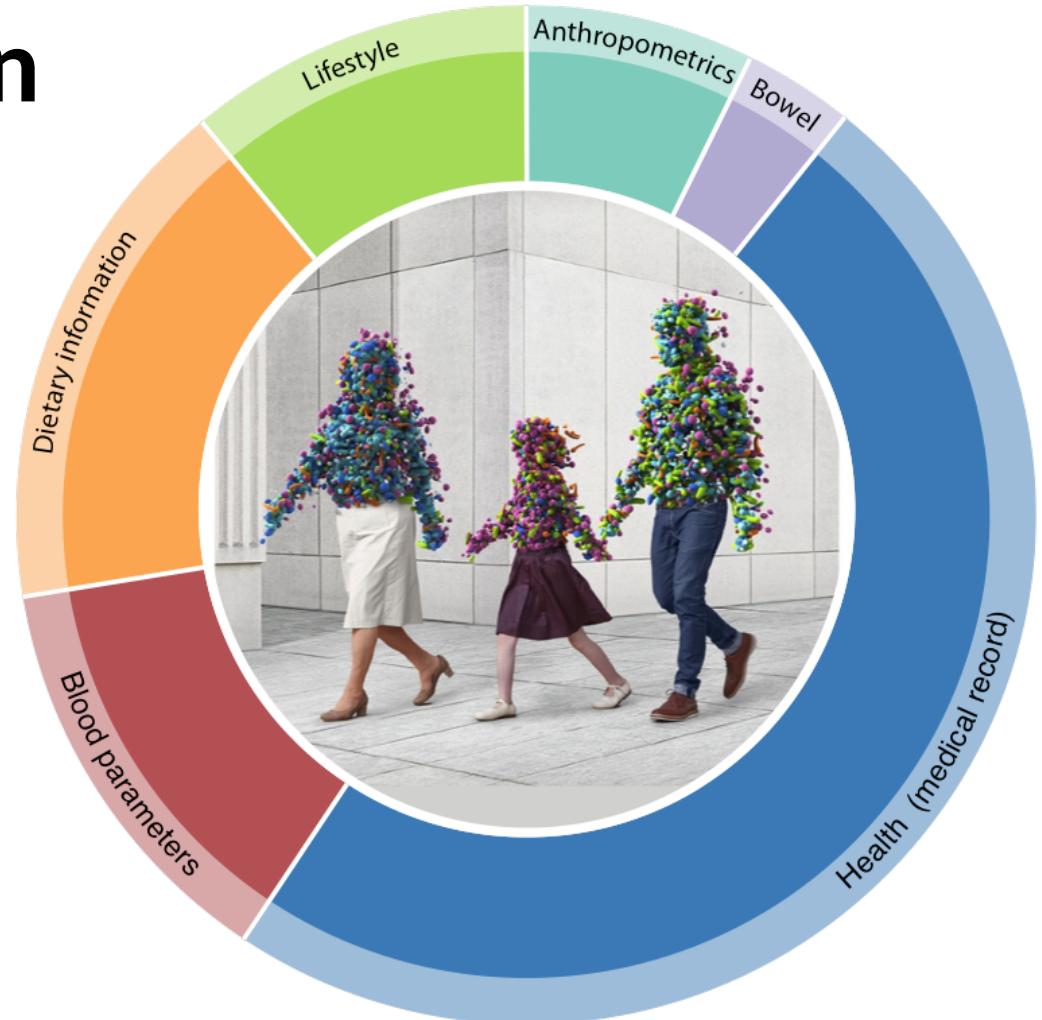
- Questionnaires:

- Self-reported health
- Detailed health (GP)
- Diet (incl probiotics, drugs)
- Wellbeing/QoL
- Hygiene
- Bowel habit/Bristol scale
- Travel, Stress etc

- Blood analysis: metabolic (e.g. glucose, HDL/LDL, triglycerides, insulin,...) and immunological/inflammatory readouts (cell counts, interleukins, CRP,...)

- Secured database, patient encoding

**Current status:
3400 sample sets collected**



Het Vlaams Darmflora Project
Handleiding voor staalafname

Lees heel de handleiding voor je aan de staalafname begin!

Vraag je steeds op in een schriftje, niet bijgevende serie posities van je darm kan je gebruiken om alles proberen te houden. Als alleen een paar stukjes van de steiger, maar dat is ook prima. De belangrijkste dingen zijn de grootte, het uiterlijk, Let op: het is erg belangrijk dat de steigering niet in contact met water komt.

Het Vlaams Darmflora-project
Stoelgangscore

Via onderstaande foto's en omschrijvingen kan je de steigeren en scoren tussen 1 en 7 groen. Schrijf deze code op de steigeren en voer dit bij de hand wanneer je de online registratie invult (zie de link in de eerste vragenlijst met als titel 'Stoelgangscore').

Score	Omschrijving
1	Steile, hoge krents. Moeilijke steigerig.
2	Samengeklitte krents.
3	Wenkelinge steigerig. Steigerig van mesche.
4	Wenkelige steigerig. Zacht en glad van mesche.
5	Zachte, makkelijke steigerig. Dodegrond, steiger contenten.
6	Zachte tot zeer zachte steigerig. Oudsteigende contenten.
7	Wenkelige steigerig, geen structurele samenting. Gekleed vloestand.

Het Vlaams Darmflora-project
Staanarmkaart

Wat kan ik nu in de keuze stellen en steigeren? Hierbij staan bij de hand 4 vragen over de online registratie invult (zie de link in de eerste vragenlijst met als titel 'Stoelgangscore').

Naam: _____
Voornaam: _____
Datum standaard: _____

Tijdstip staalname:

Vrije oefening: Minder dan 6 uur geleden
 Tussen 6 en 12 uur geleden
 Tussen 12 en 18 uur geleden
 Tussen 18 en 24 uur geleden
 Tussen 24 en 30 uur geleden
 Tussen 36 en 48 uur geleden
 Langer dan 48 uur geleden

Omschrijf je staaltype: 1 2 3 4 5 6 7

Het Vlaams Darmflora-project
Staanarmkaart

Wat kan ik nu in de keuze stellen en steigeren? Hierbij staan bij de hand 4 vragen over de online registratie invult (zie de link in de eerste vragenlijst met als titel 'Stoelgangscore').

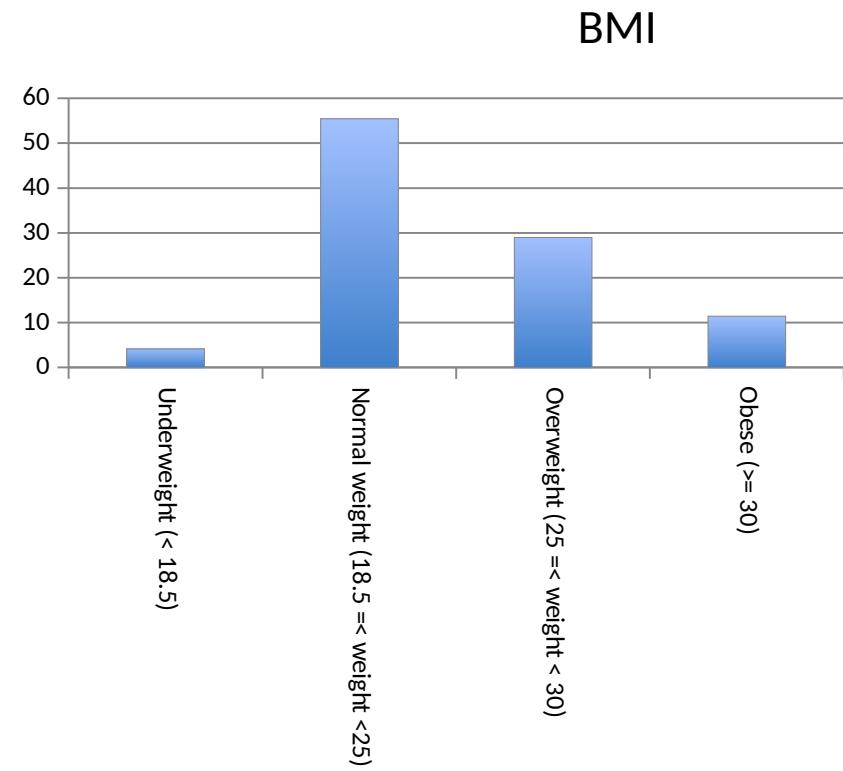
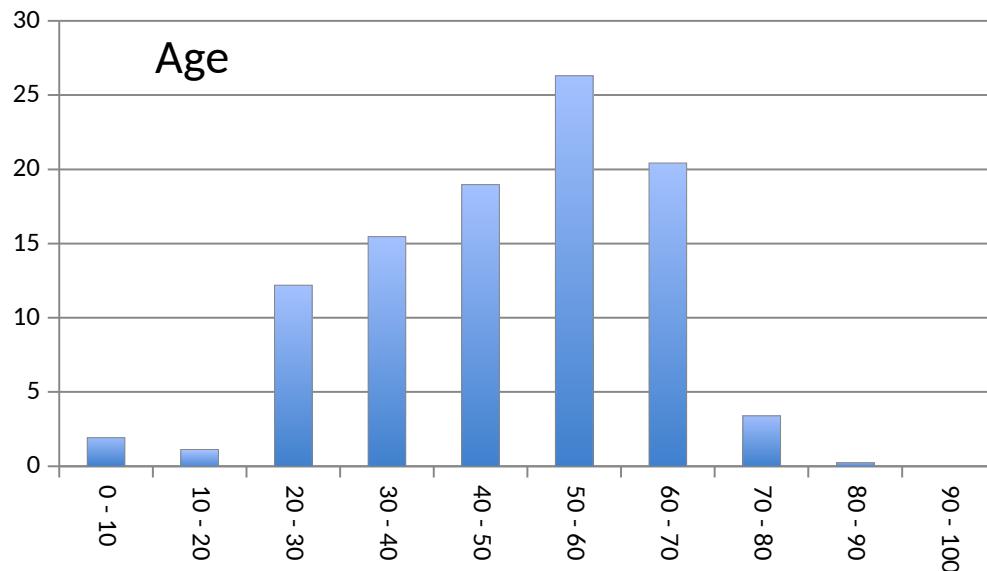
Naam: _____
Voornaam: _____
Datum standaard: _____

Tijdstip staalname:

Vrije oefening: Minder dan 6 uur geleden
 Tussen 6 en 12 uur geleden
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 Tussen 24 en 30 uur geleden
 Tussen 36 en 48 uur geleden
 Langer dan 48 uur geleden

Omschrijf je staaltype: 1 2 3 4 5 6 7

Population statistics in Flemish Gut Flora Project



40/60 M/F ratio

98.5% of Western or Eastern European ethnicity; 96.8% born in Belgium

Dedicated reference sequence database for the gut microbiome outperforms generic ones (Silva, Greengenes)

Improved sensitivity and coverage in gut profiling

Other body sites to be done

Included in light-weight OTU clustering pipeline Lotus:
<http://psbweb05.psb.ugent.be/lotus/>

Ritari et al. BMC Genomics (2015) 16:1056
DOI 10.1186/s12864-015-2265-y

BMC Genomics

RESEARCH ARTICLE

Open Access



Improved taxonomic assignment of human intestinal 16S rRNA sequences by a dedicated reference database

Jarmo Ritari^{1*}, Jarkko Salojärvi¹, Leo Lahti^{1,2} and Willem M. de Vos^{1,2,3}

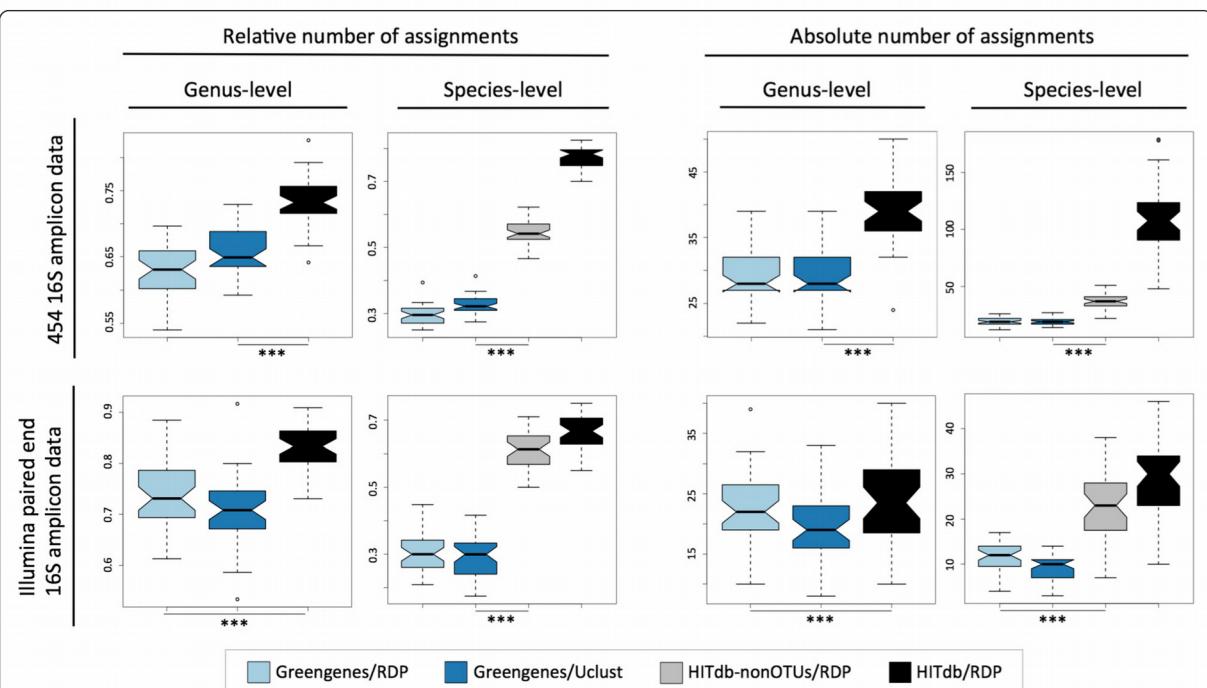
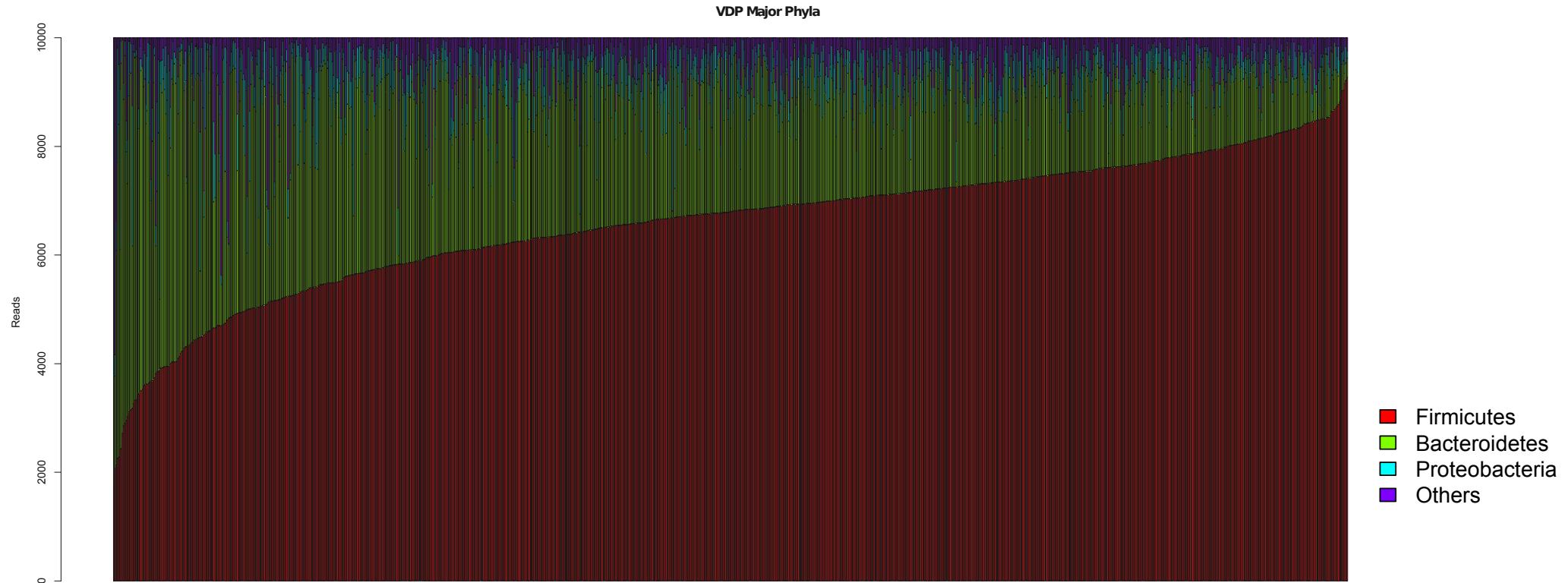


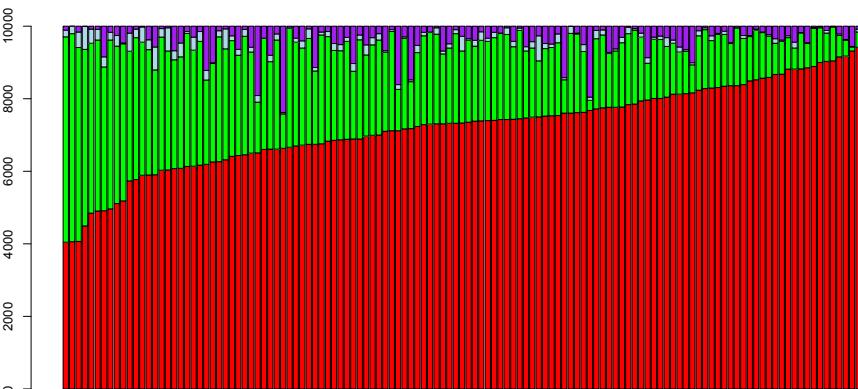
Fig. 4 Comparison between Greengenes and HITdb using data from biological samples. The used data set is indicated in rows, and relative and absolute numbers of assignments at genus and species levels in columns. At species level, the results for HITdb additionally show the biological species only (i.e. without OTUs) for easier comparison with Greengenes. *** $p < 0.001$. $n = 119$ and $n = 40$ samples for 454 and Illumina data sets, respectively

Good news: the Flemish flora is pretty ‘normal’

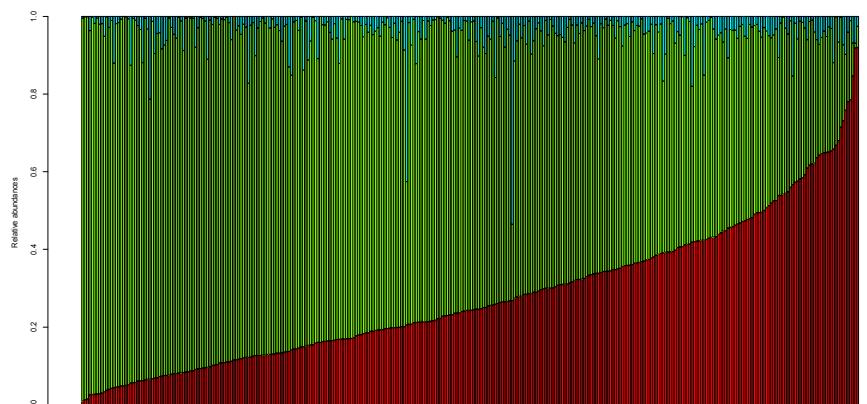


Flemish gut: 1,000 individuals, MiSeq pair-end sequenced V4 region, 10,000 reads per sample,
Firmicutes averaging 54%

Yatsunenko et al 2012 study, 125 individuals, Miseq sequenced V4
region, 10,000 reads, Firmicutes 72%



HMP, 214 individuals, 454 sequenced V4 region, 1,000 reads,
Firmicutes 24%



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 guillermondii et rel.
Lachnospira pectinoschiza et rel.
Bifidobacterium
Clostridium symbiosum et rel.
Clostridium orbiscindens et rel.
Clostridium sphenoides et rel.

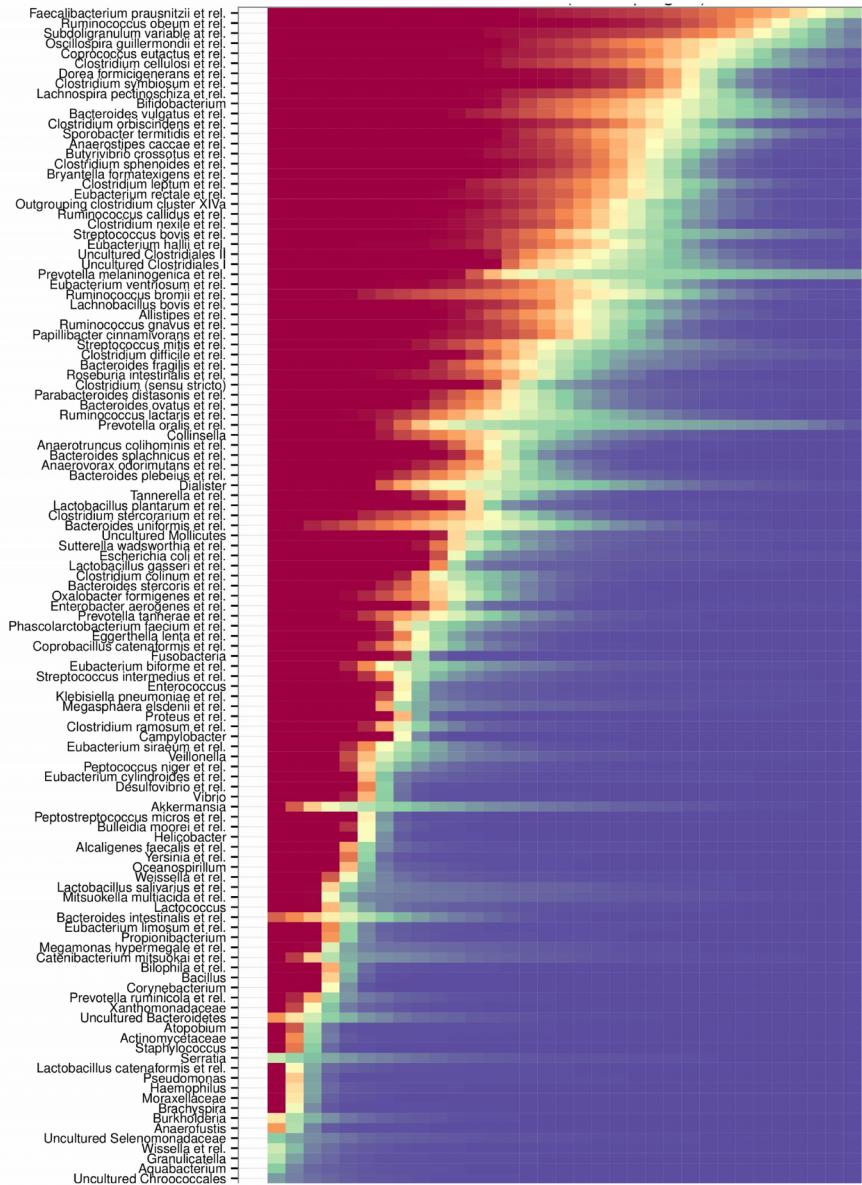
Jalanka-Tuovinen et al. (2011) PLoS One

6:e23035

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



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



Detection threshold (abundance)

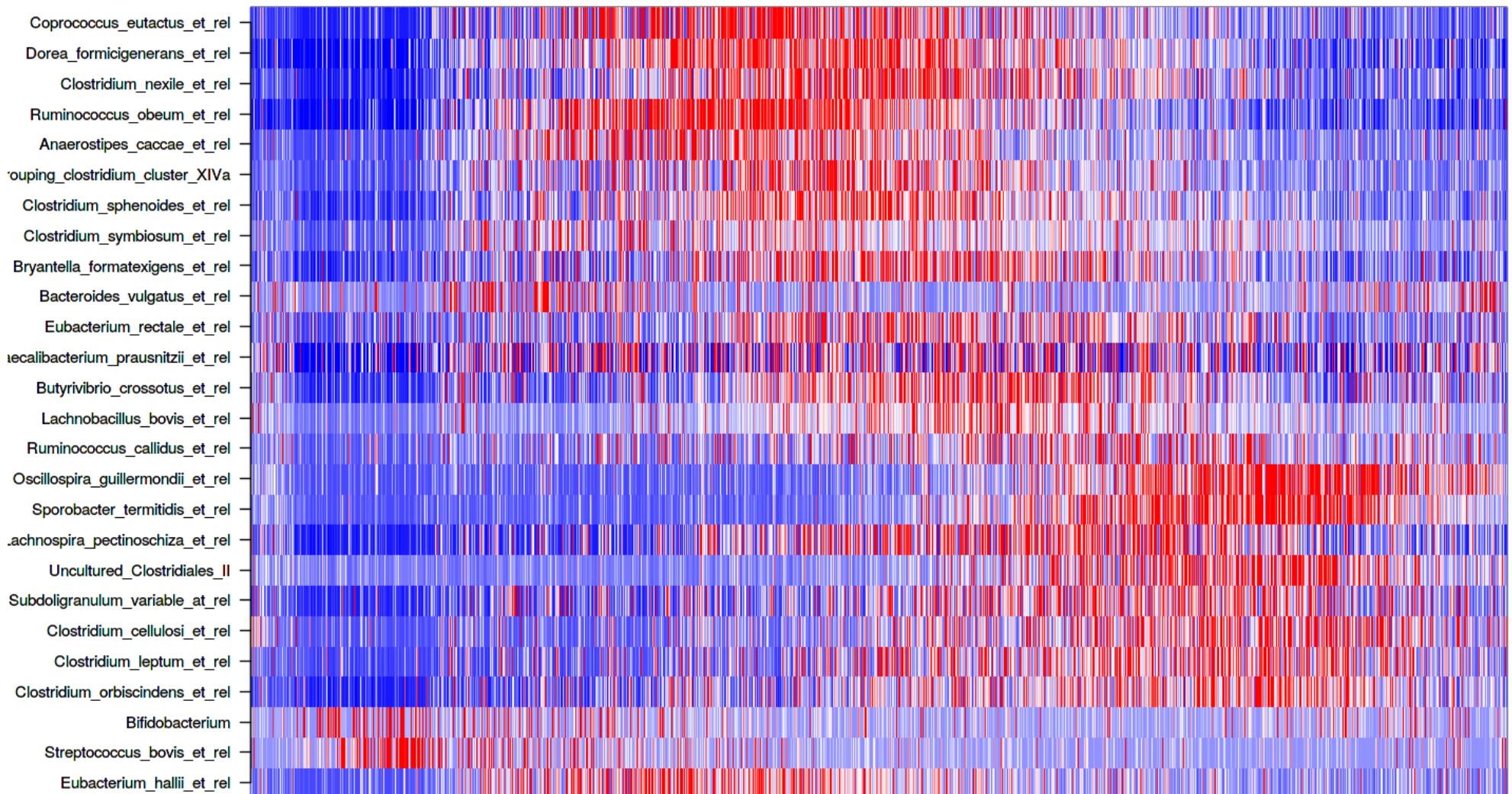
HITChip Atlas. Shetty et al. FEMS Microbiol. Reviews 2017, fuw045.
Data from Lahti et al. Nat Comm, 2014.

Abundances of the shared core taxa are highly individual

Z-score across subjects: **red – high abundance & blue – low abundance**

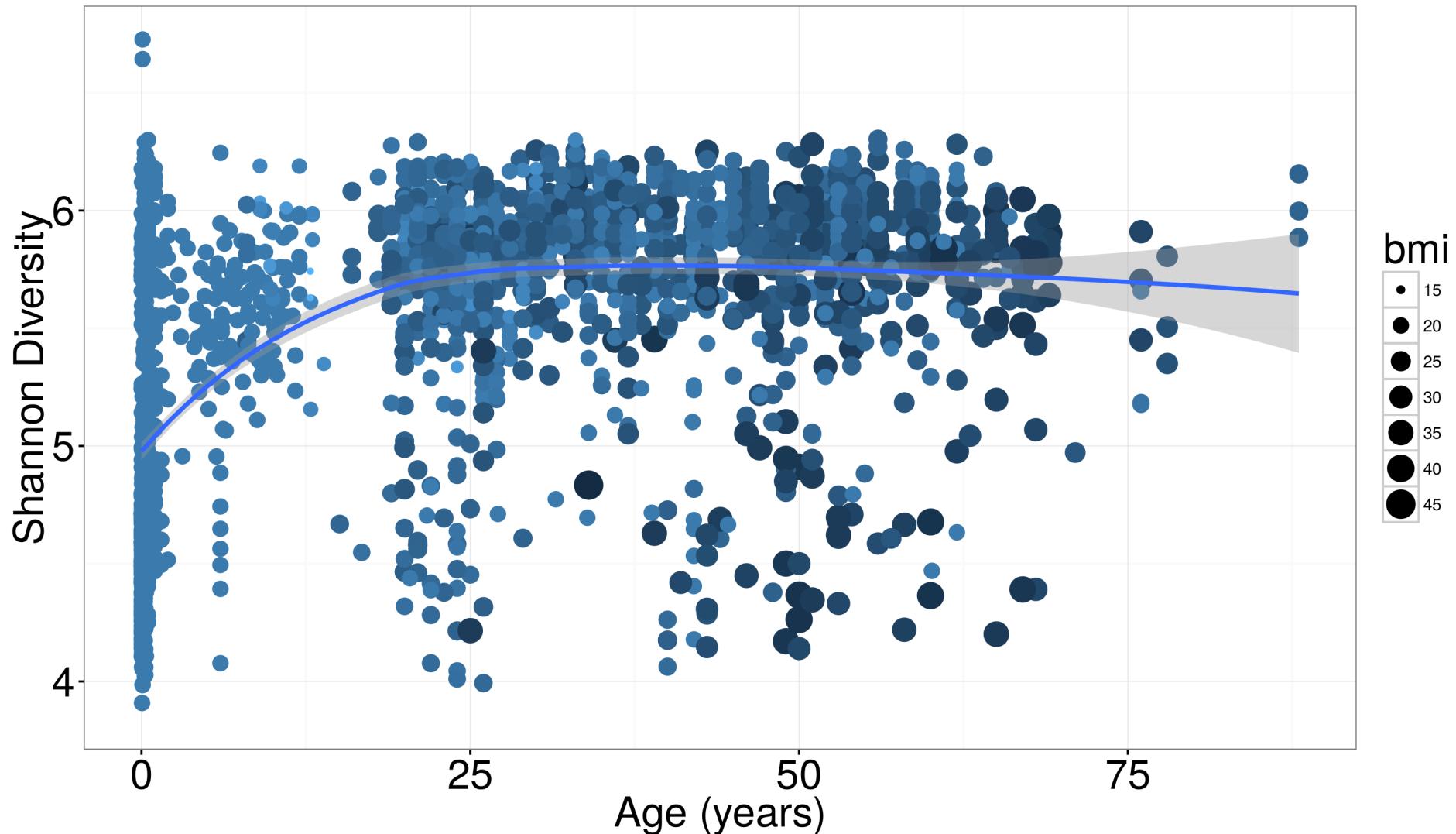
Core microbiota shows remarkable variation across population.

(HITChip Atlas; N = 5005)



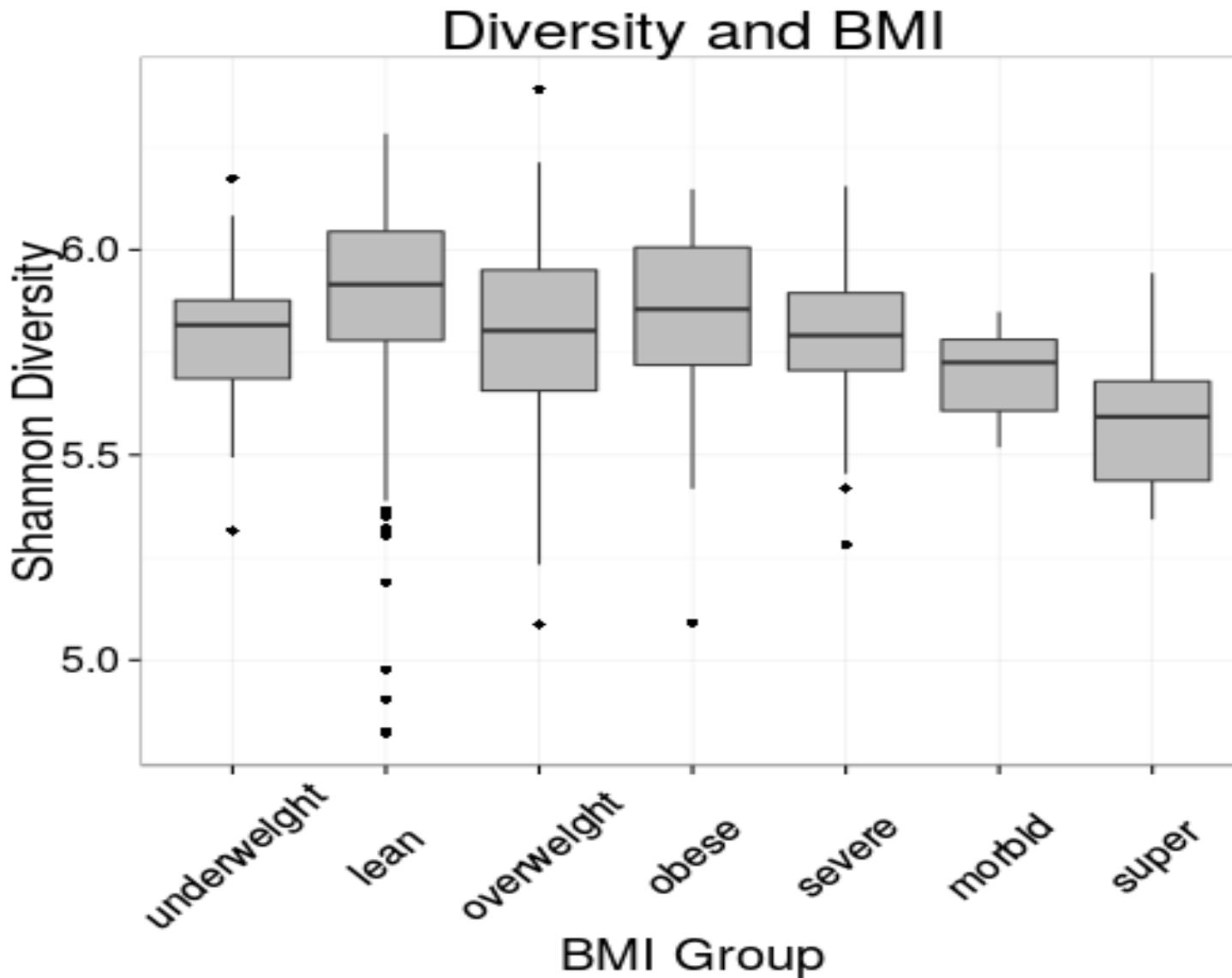
Microbiome diversity and age: healthy & normal obese subjects

N = 2363



Data: HITChip Atlas;
healthy fecal RBB samples

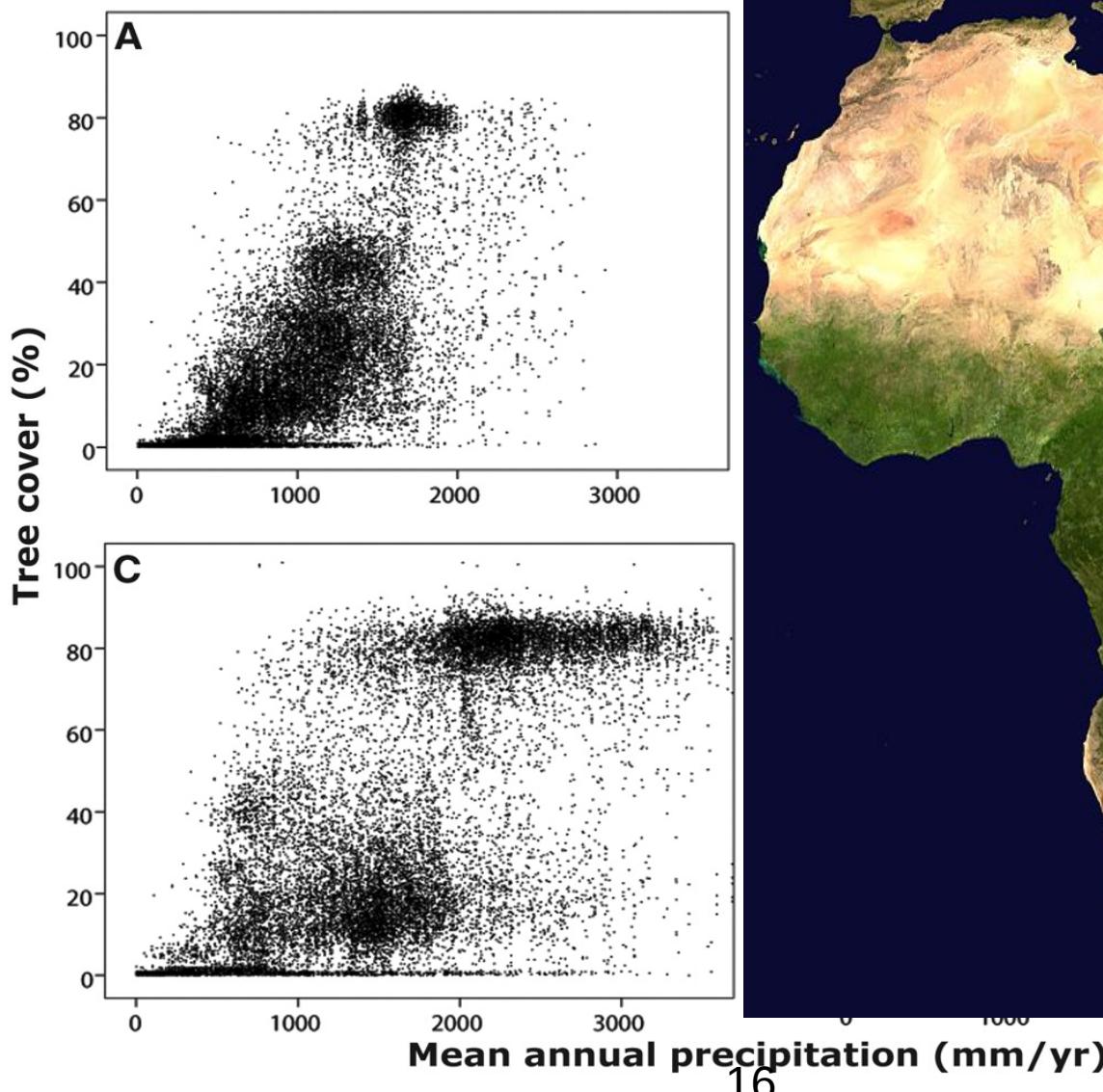
Microbial diversity decreases with obesity



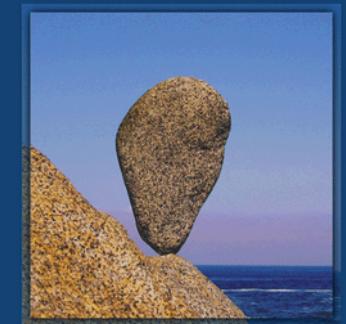
REPORT

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

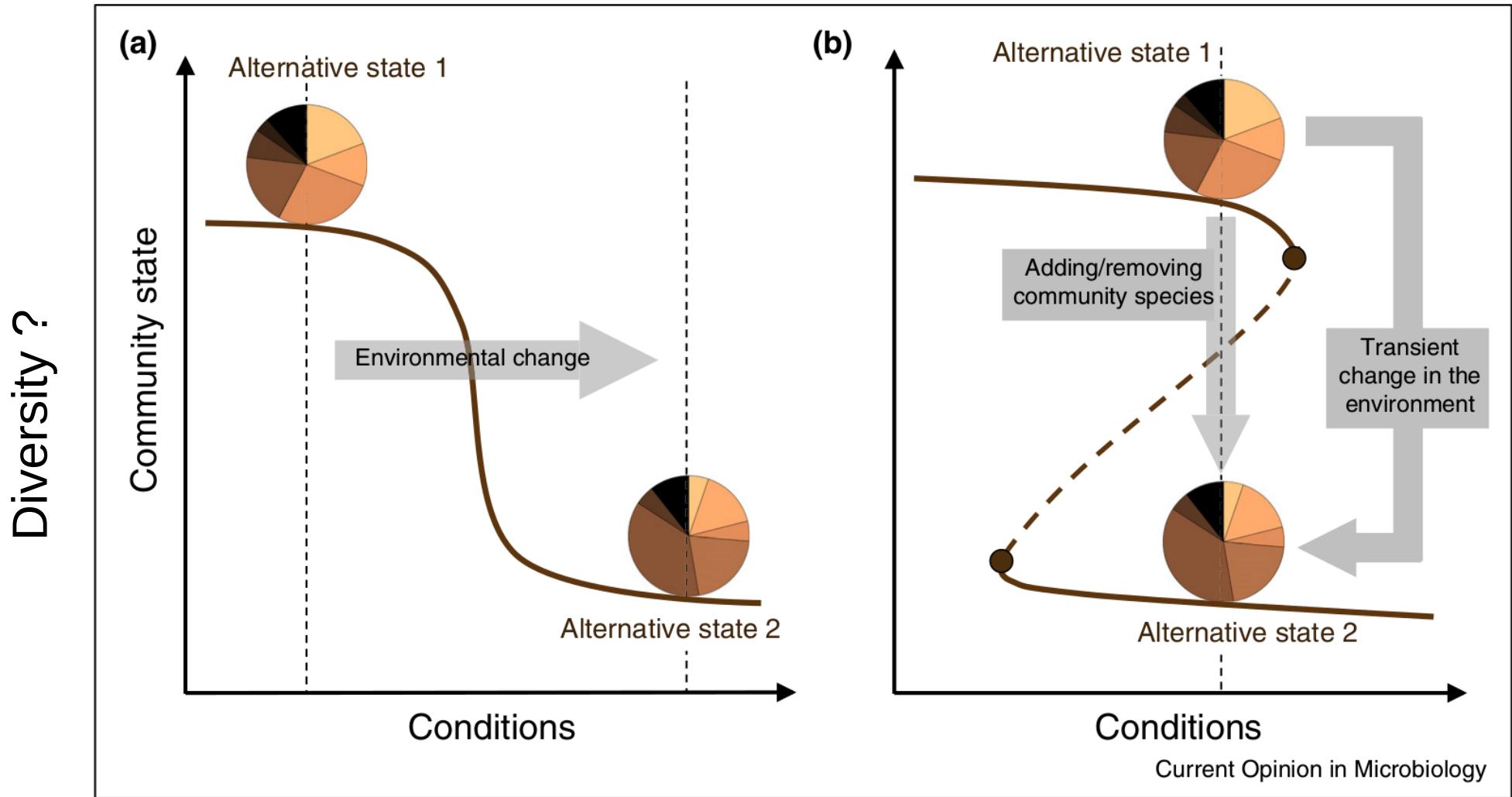


Marten Scheffer

PRINCETON STUDIES IN COMPLEXITY

Alternative (transient?) states are induced by external factors

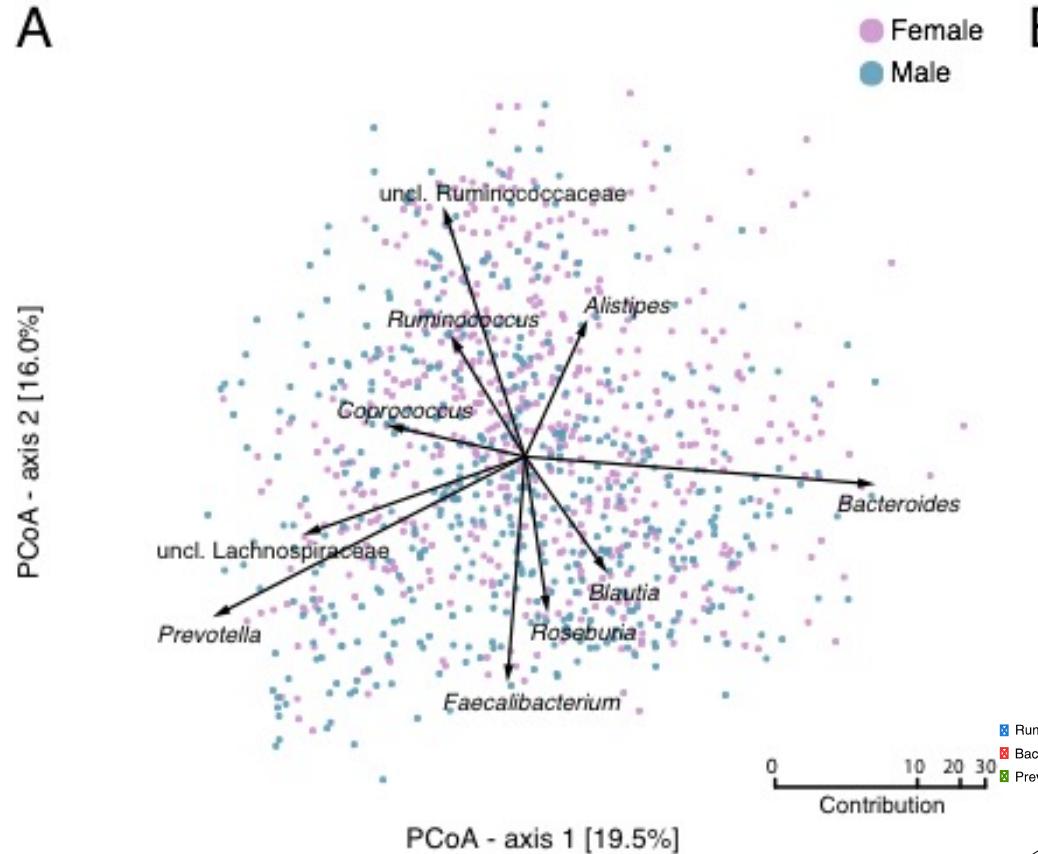
Alternative stable states are resilient to external factors



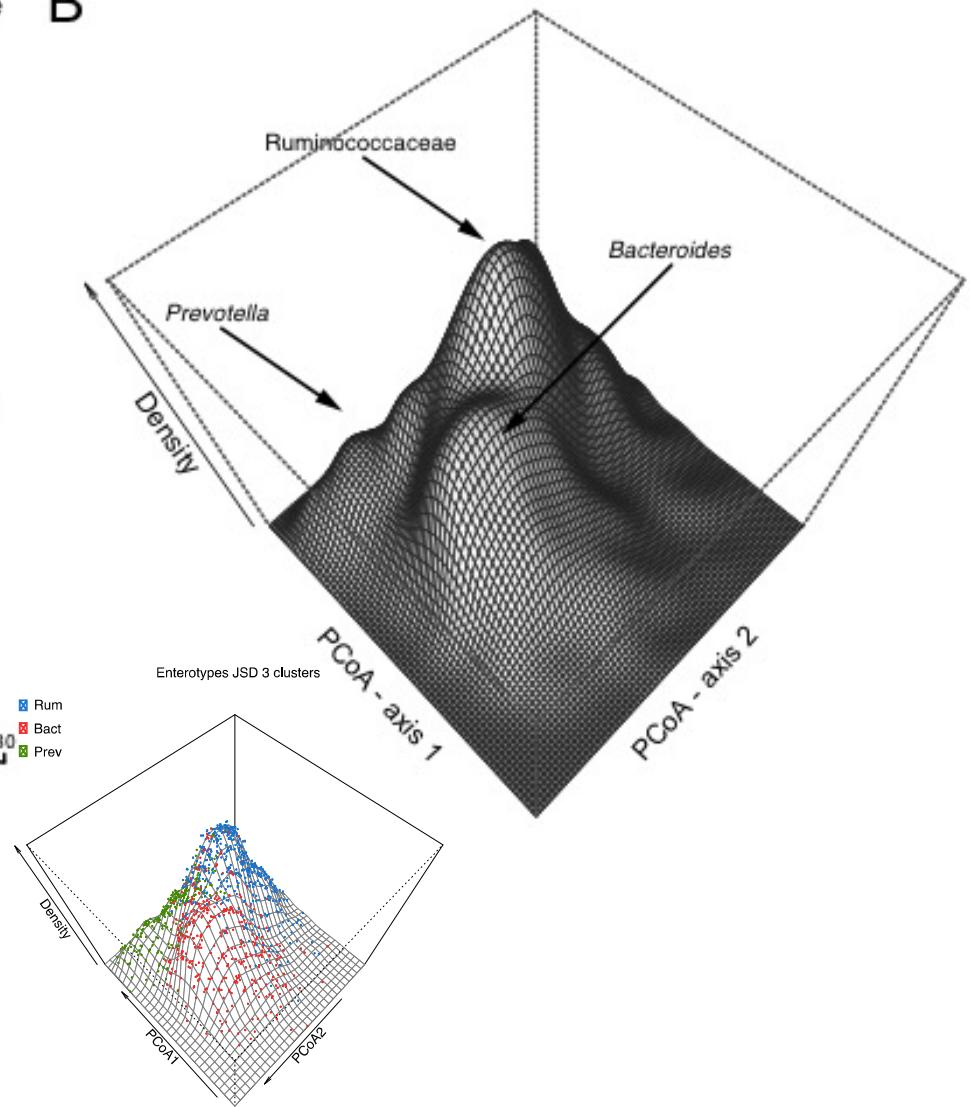
Environmental or host factor (e.g. body-mass index)

Enterotype landscape of the Flemish Population shows density peaks of 'preferred' ecosystem constellations

A



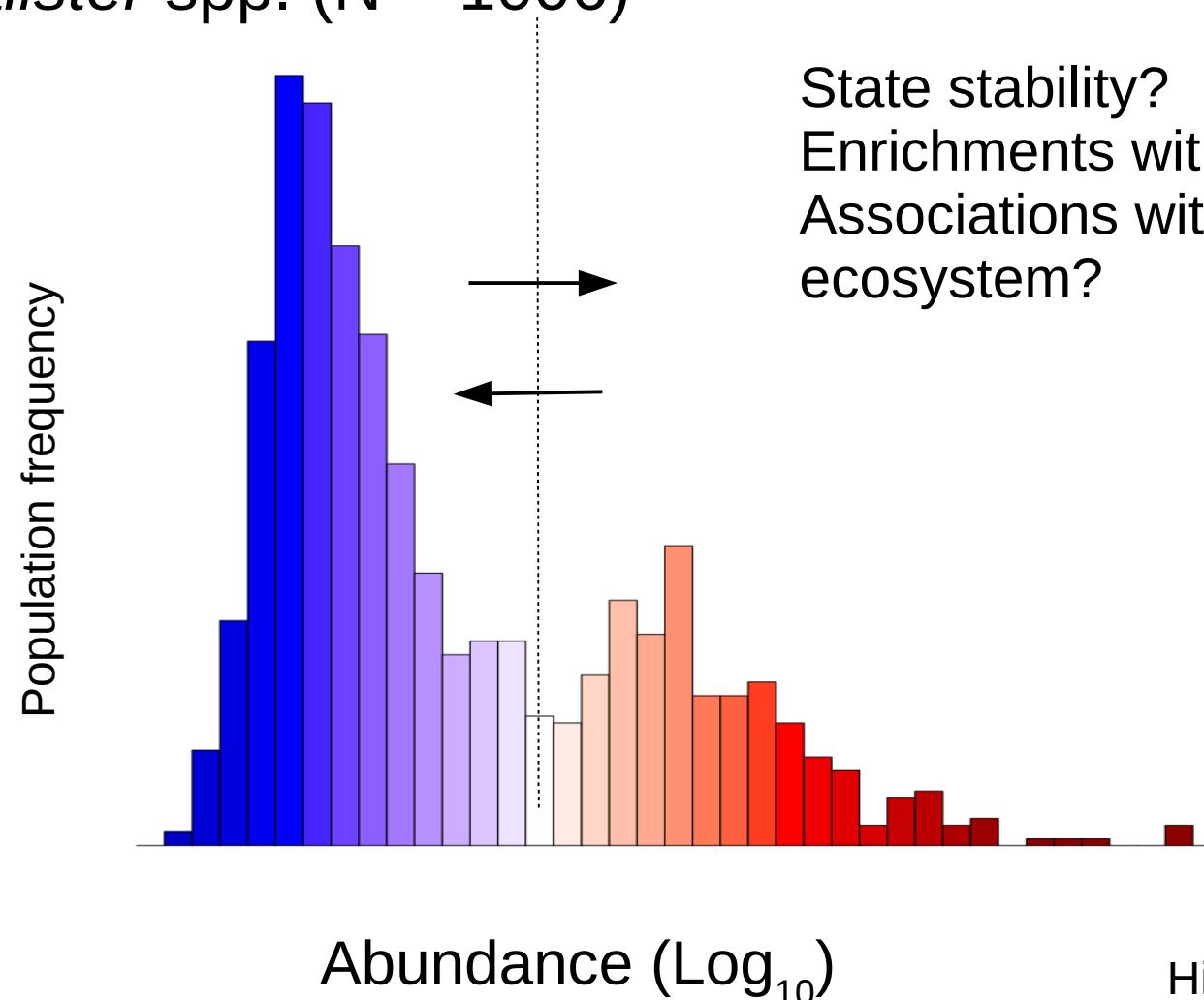
B



See also: Shetty et al. FEMS Microbiol. Reviews
2017, fwu045

Alternative states in specific bacterial groups ?
→ Bimodal population distribution is one indicator

Dialister spp. (N = 1006)

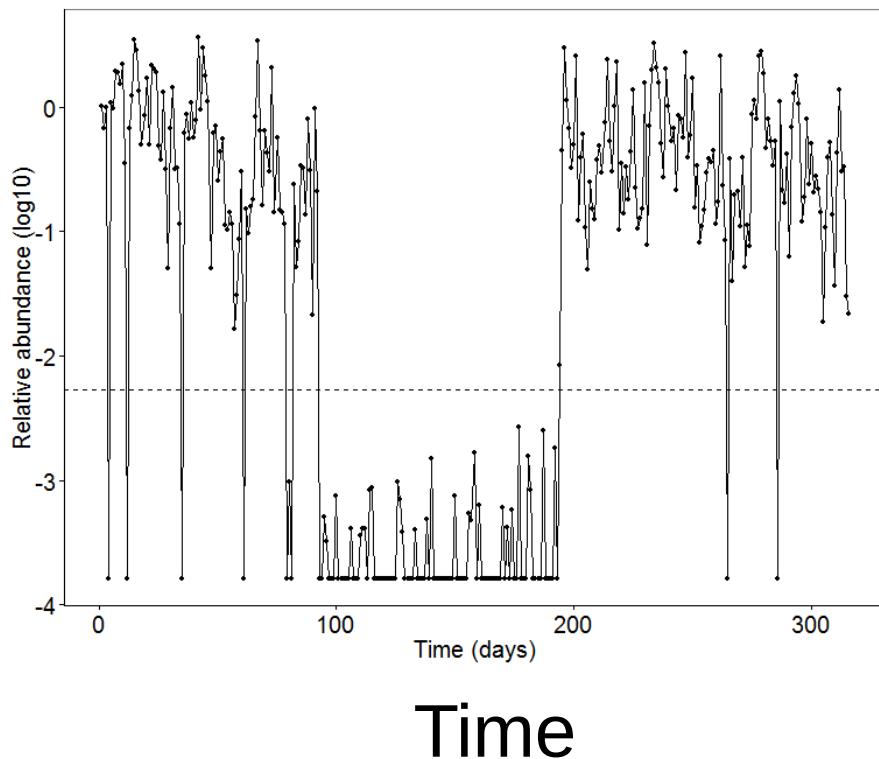


State stability?
Enrichments with diet / health / etc.?
Associations with the overall
ecosystem?

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

Detecting a tipping point: temporal instability at intermediate abundances is another key indicator of bistability

One long time series:
Lachnospiraceae

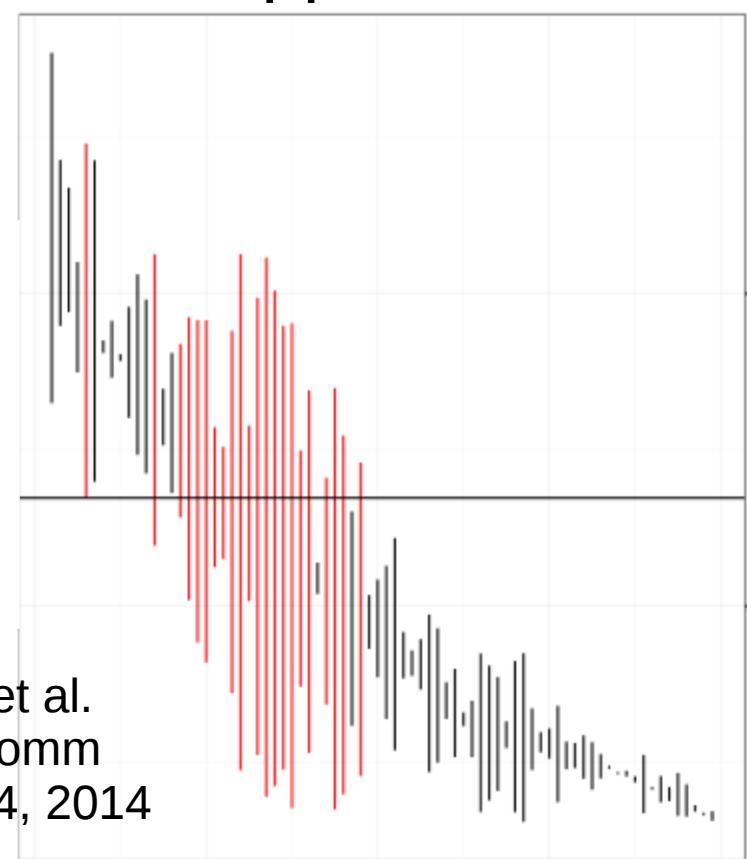


Data: David et al. 2014; Figure: T Blake 2015

Many short time series:
Dialister spp.

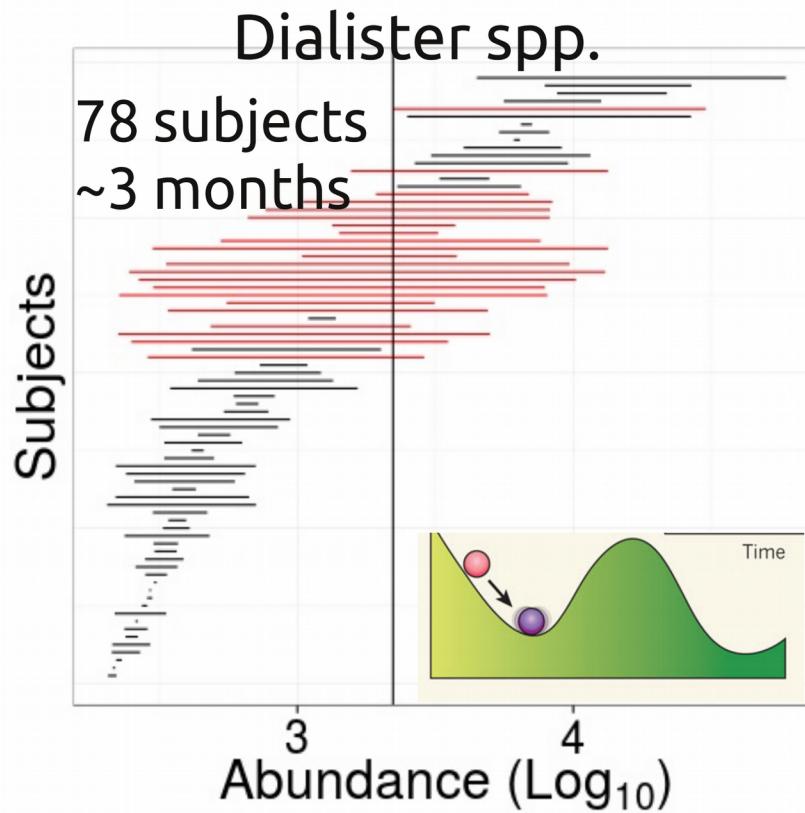
Abundance

Lahti et al.
Nat Comm
5:4344, 2014

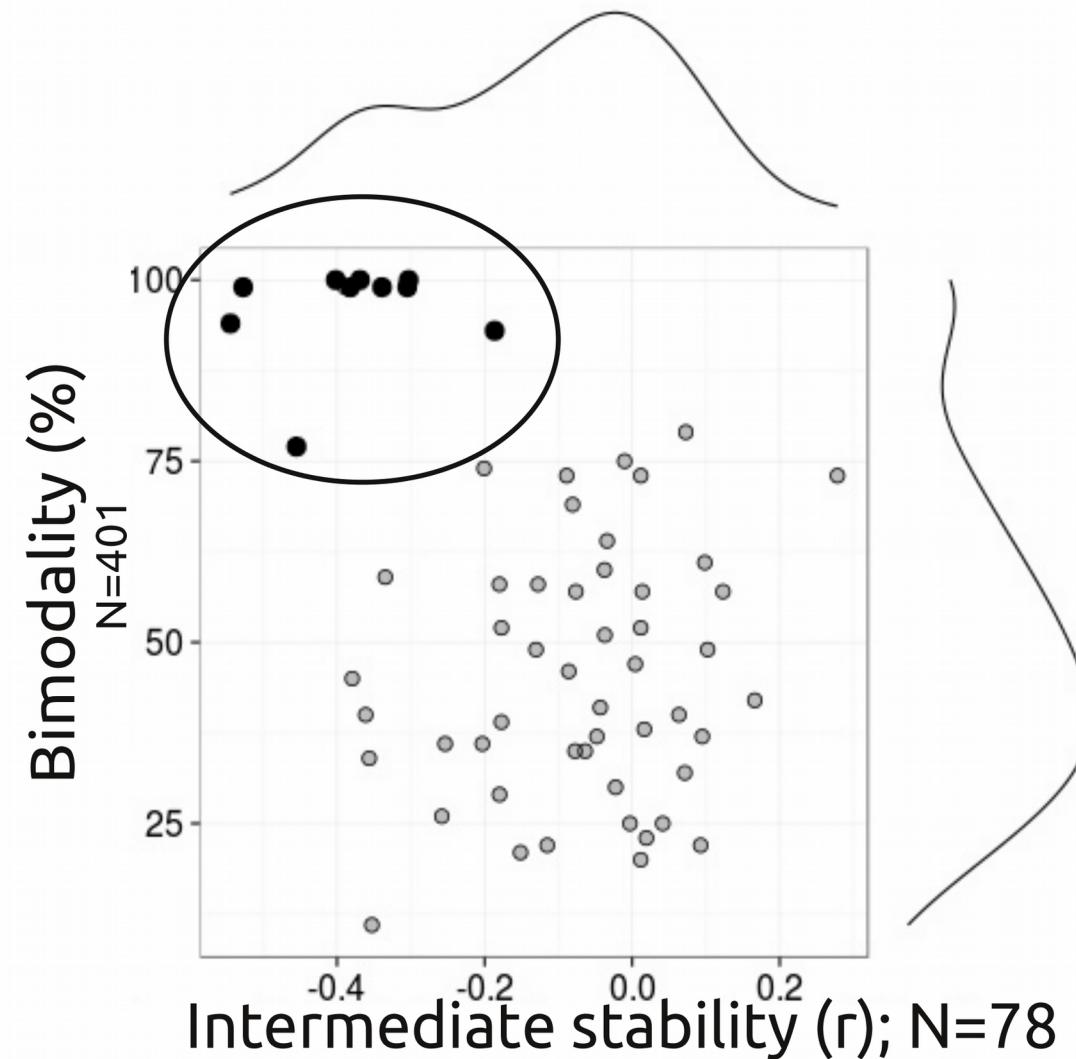


Confirming bi-stability with temporal analysis

-> alternative attractors divided by a critical unstable tipping point



State transitions indicate instability in intermediate abundance range



Bi-stable taxa:

Prevotella groups
(*oralis* & *melaninogenica*)

Dialister spp.

Uncultured Clostridiales I-II

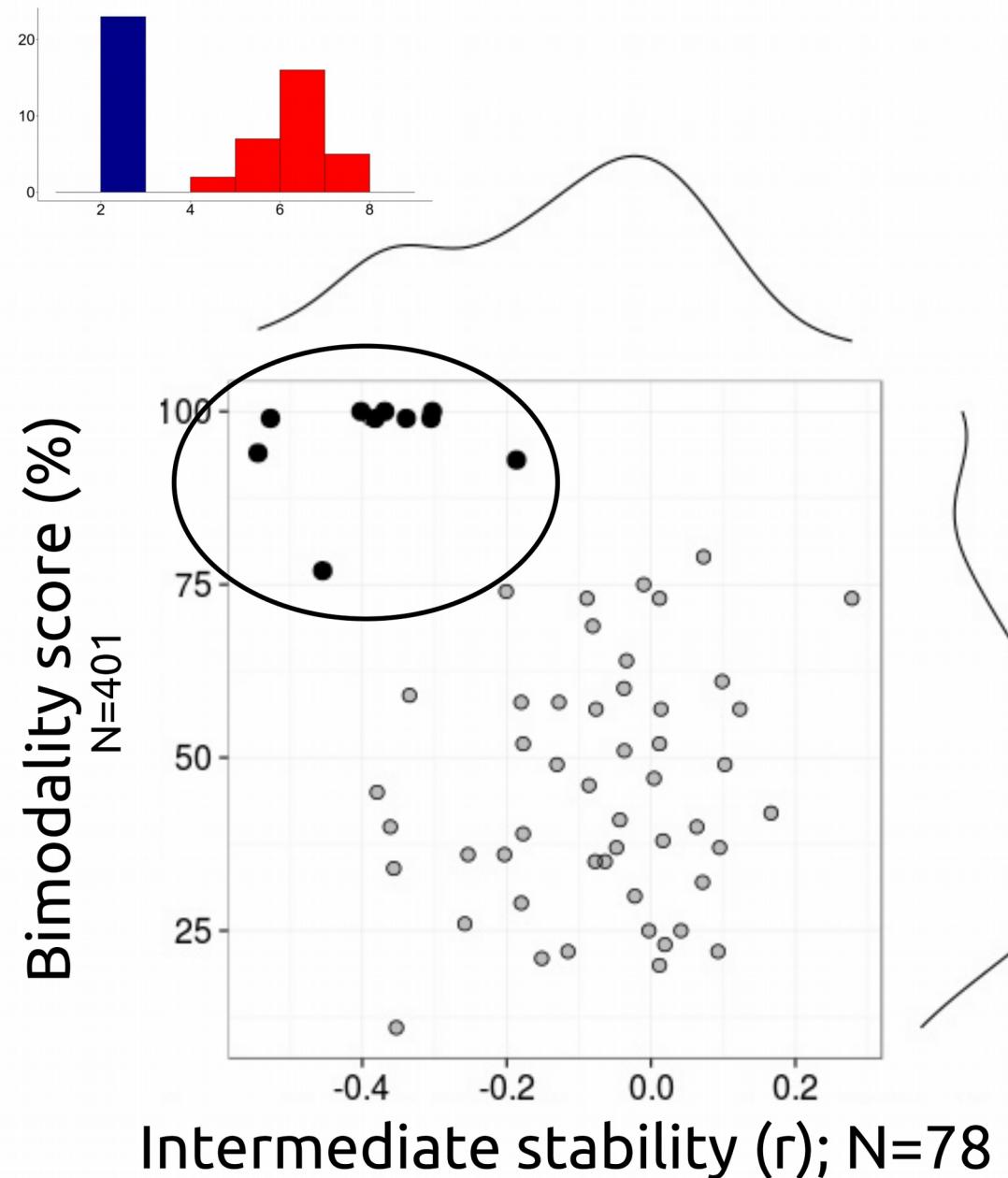
B. fragilis group

Uncultured Mollicutes

Clostridium groups
(*difficile*, *colinum*,
sensu stricto)

Lactobacillus plantarum

+ methanogenic archaea



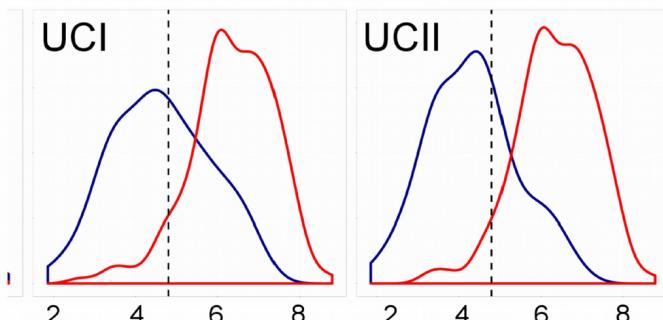
Lahti et al. Nat. Comm. 5:4344, 2014

Effect on the overall ecosystem

Prevotella states seen at ecosystem level

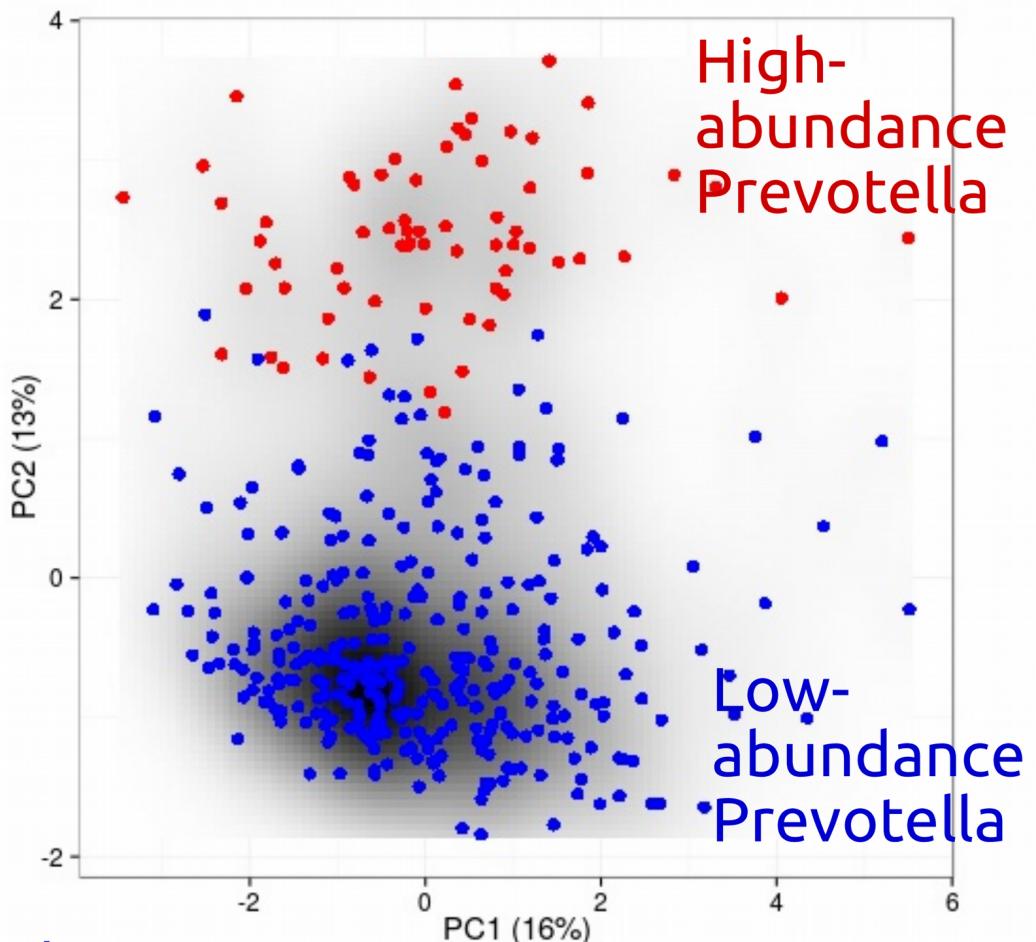
High ~10% relative abundance !

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



Uncultured Clostridiales associated with overall metagenomic richness

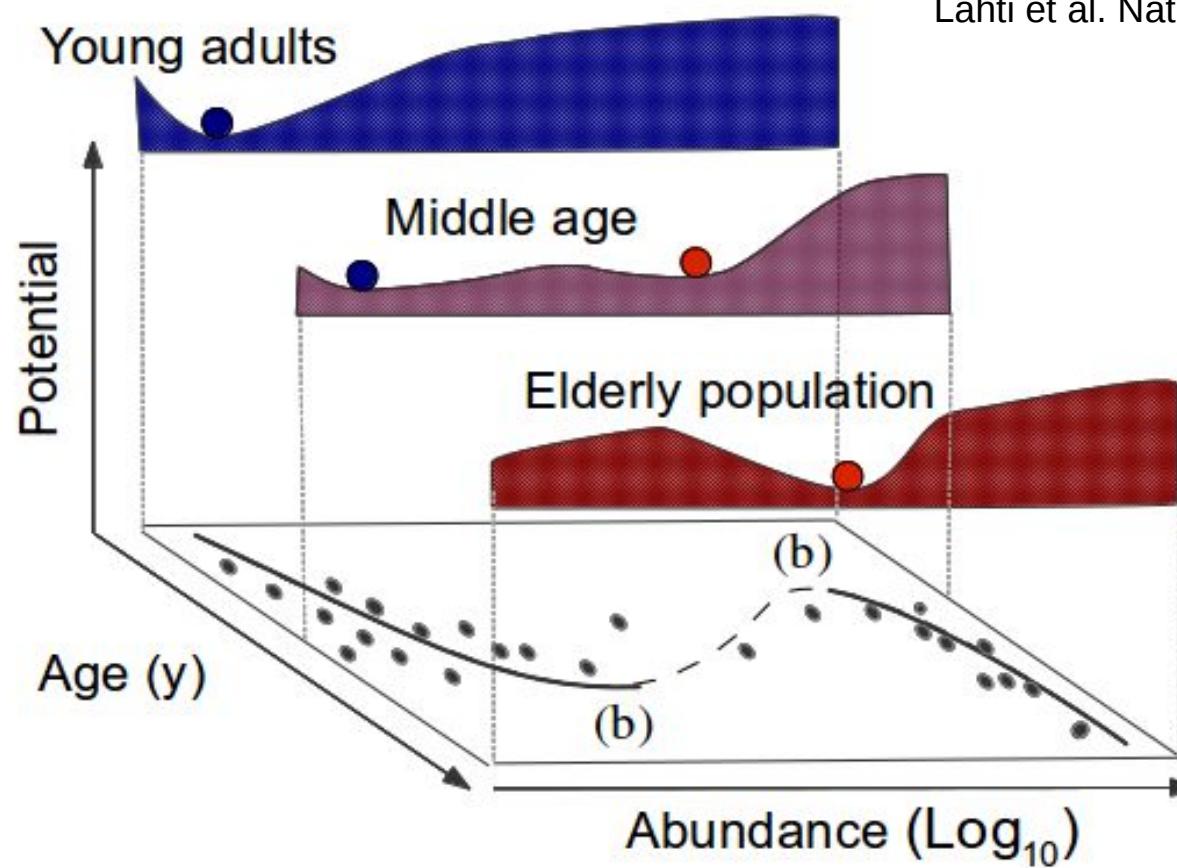
PCA +
Correlation (Spearman)



N = 401 western adults

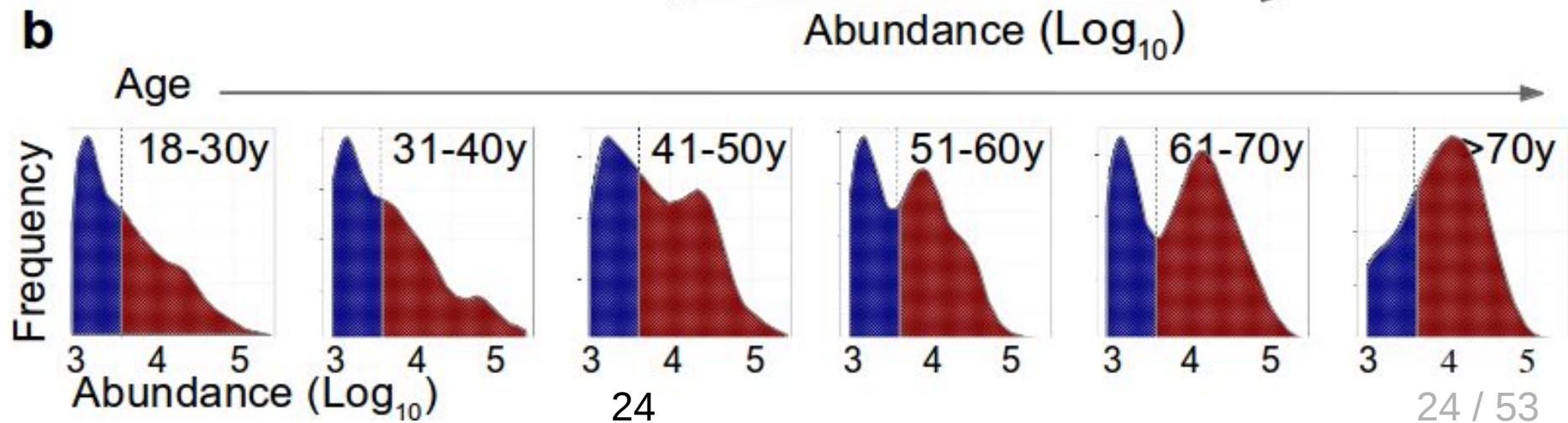
Tipping elements of the human gut microbiota: Uncultured Clostridiales I

a



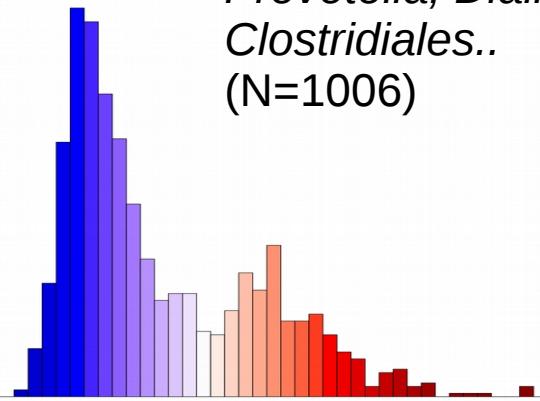
Lahti et al. Nat. Comm. 5:4344, 2014

b

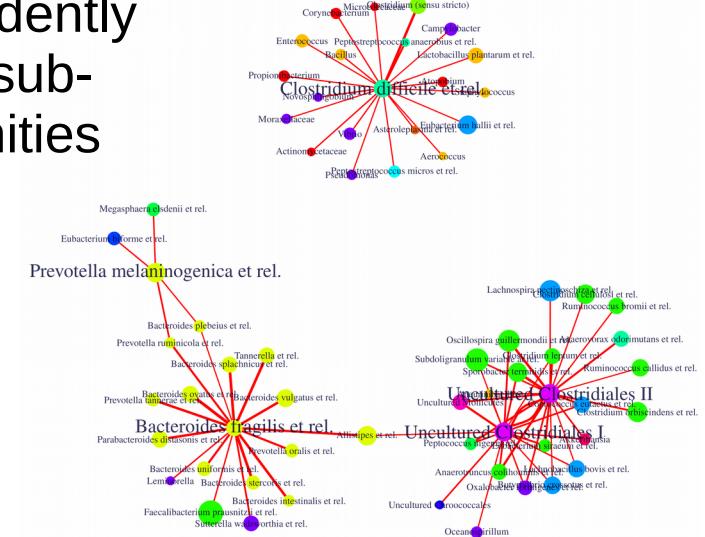


Subject stratification into community types based on simple tipping elements could reduce complexity

Bistable taxa:
Prevotella, *Dialister*,
Clostridiales..
(N=1006)

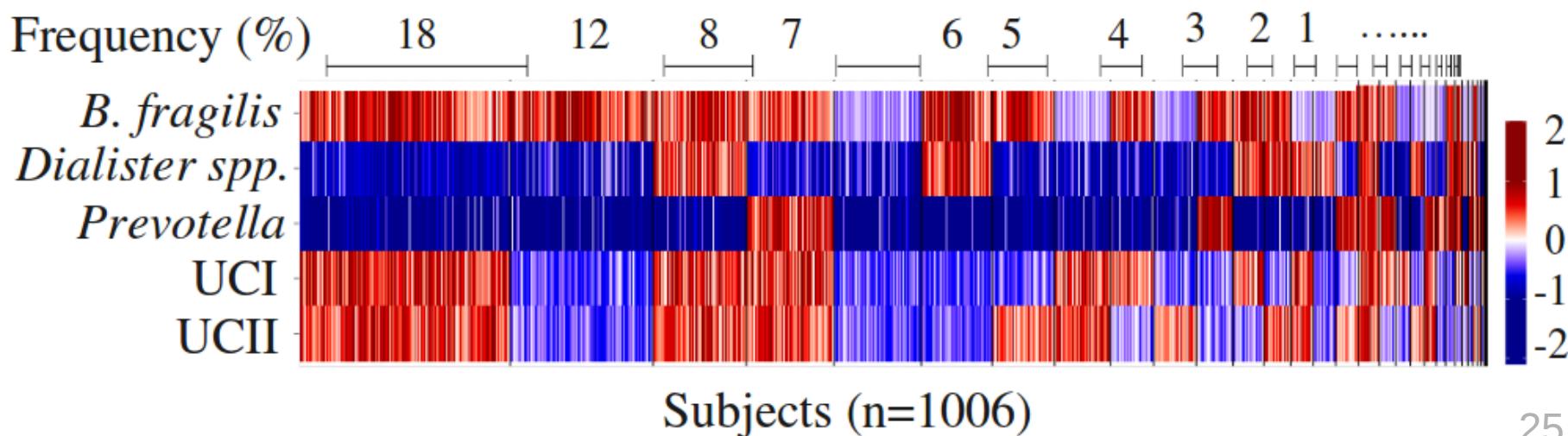


Independently varying sub-communities



Ecosystem states are rich combinations

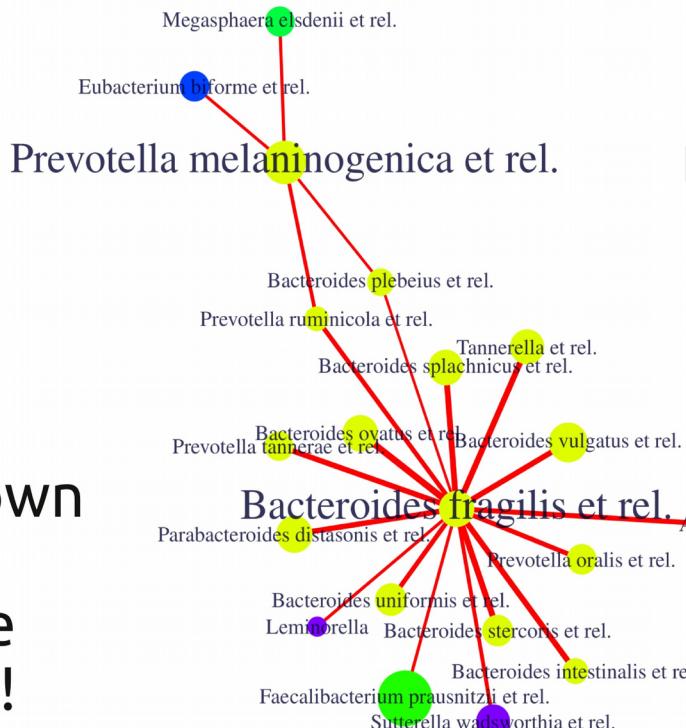
of independent tipping elements ? Lahti et al. Nat. Comm. 2014



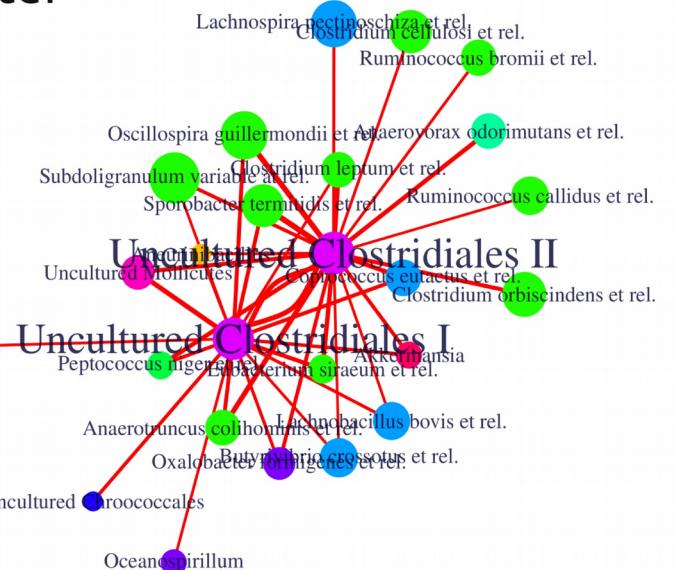
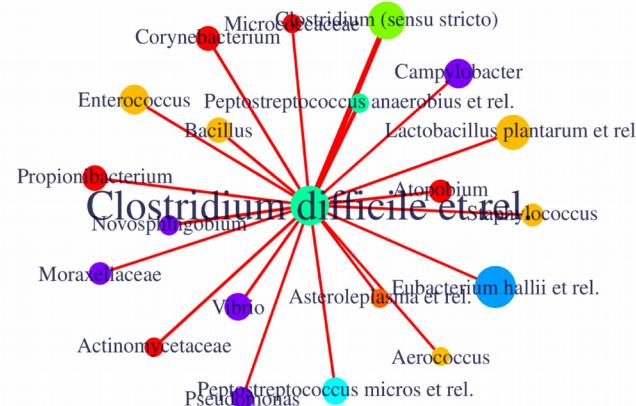
Bi-stable bacteria are rather independent, with their own lives and social networks

$|r| > 0.33$ shown

Only positive correlations !



Dialister



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 ?

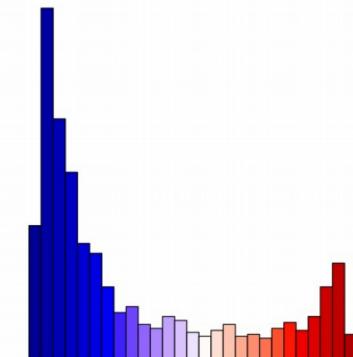
Received 23 Jan 2014 | Accepted 9 Jun 2014 | Published 8 Jul 2014

DOI: 10.1038/ncomms5344

OPEN

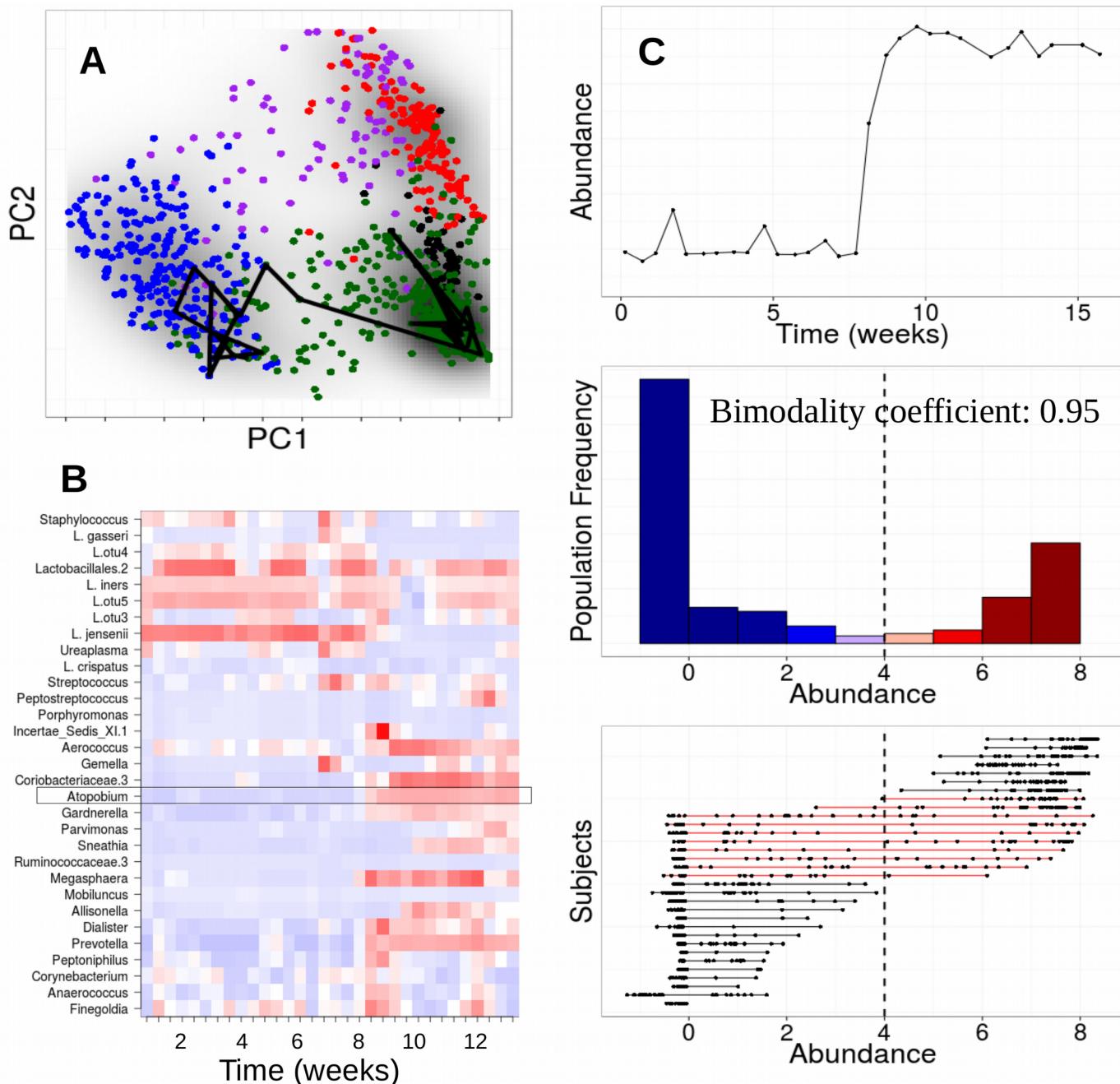
Tipping elements in the human intestinal ecosystem

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

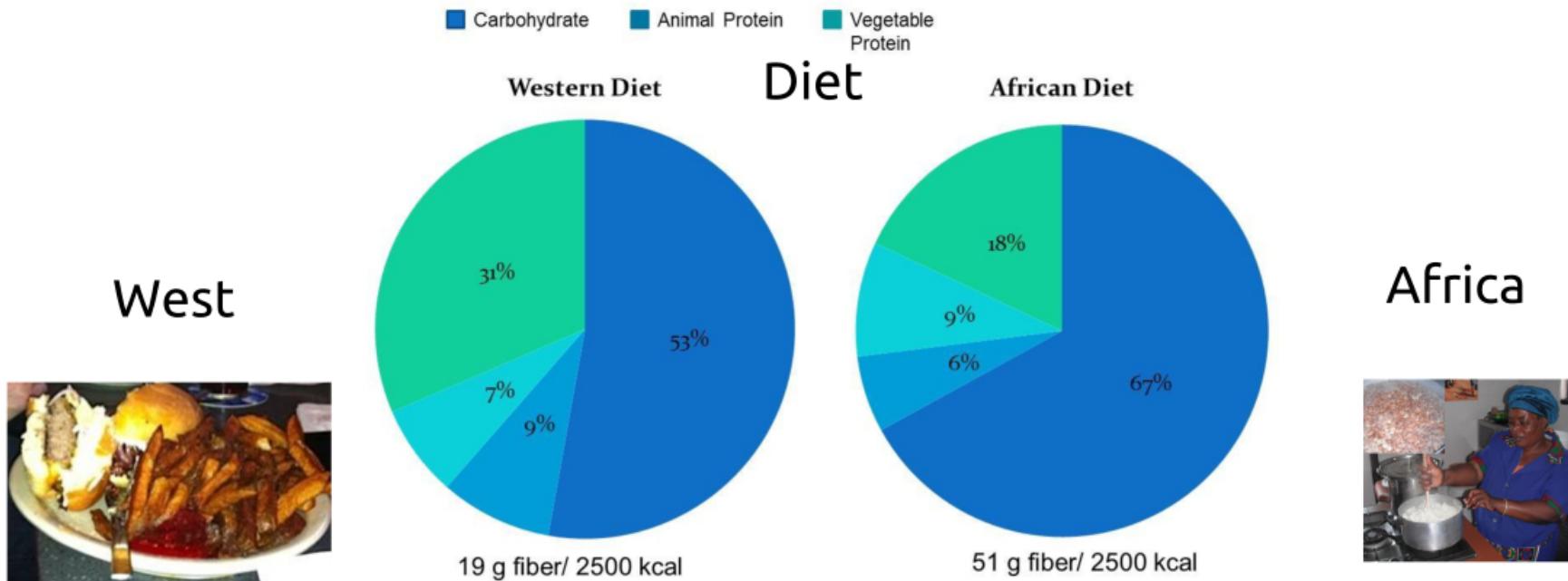


Community states and dynamics in normal vaginal microbiome of reproductive-age women

Figure: Faust et al. Curr.
Op. Microbiol. 2015
Data: Gajer et al. 2012



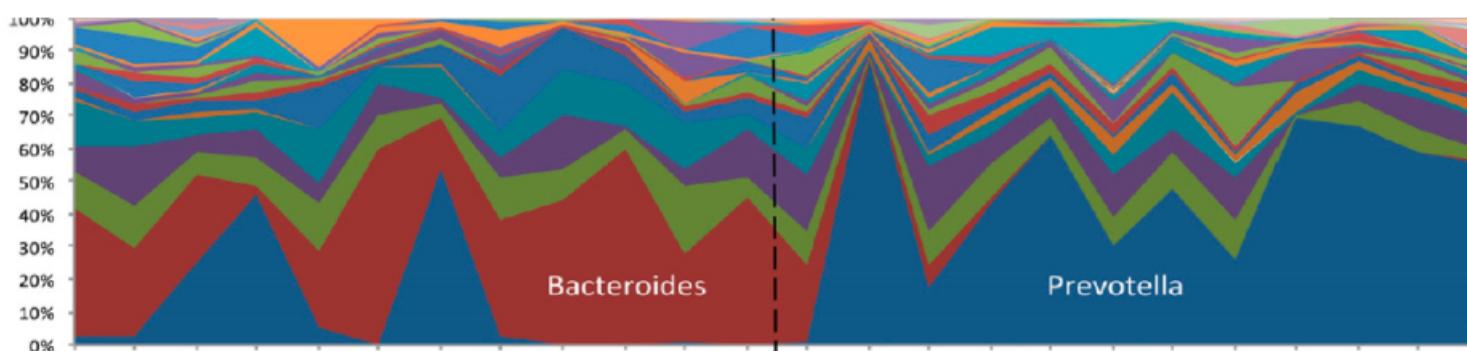
Diet is a major driver of gut microbiome composition



Microbiota composition

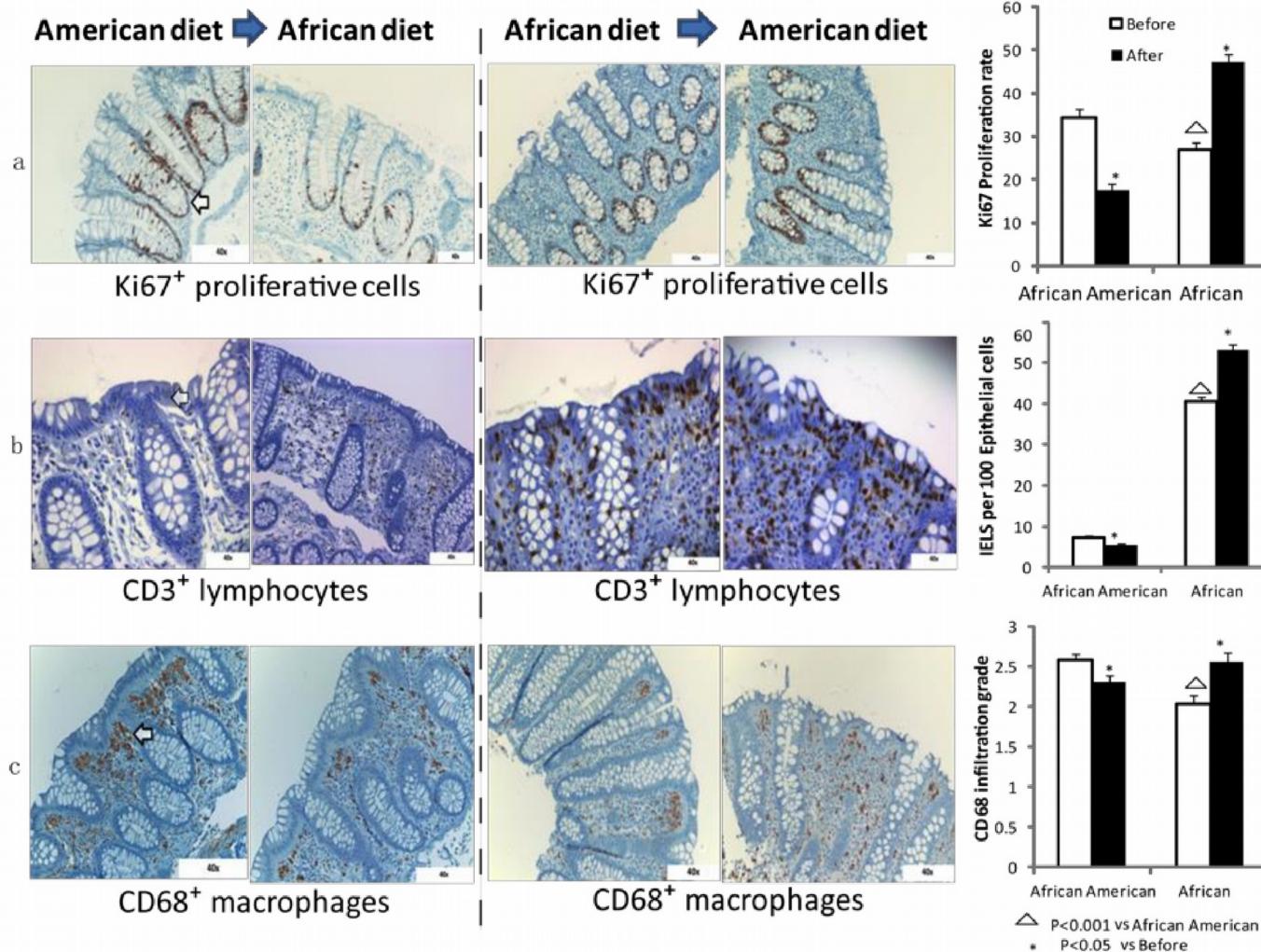
Colon cancer rates:
-Africans: <10:100,000
-African Americans: >65:100,000

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

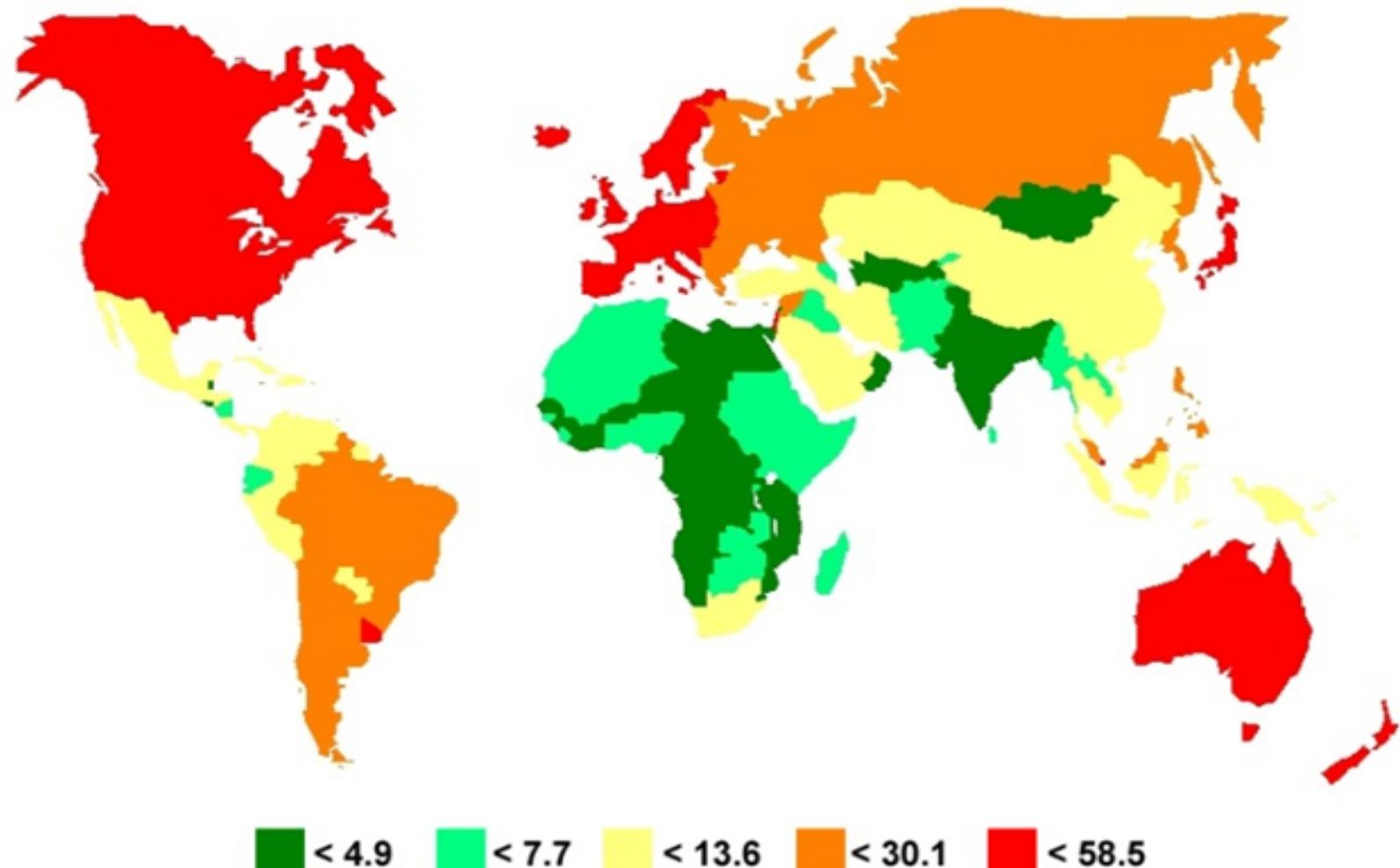


Diet swap (2 weeks) and colon cancer risk

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

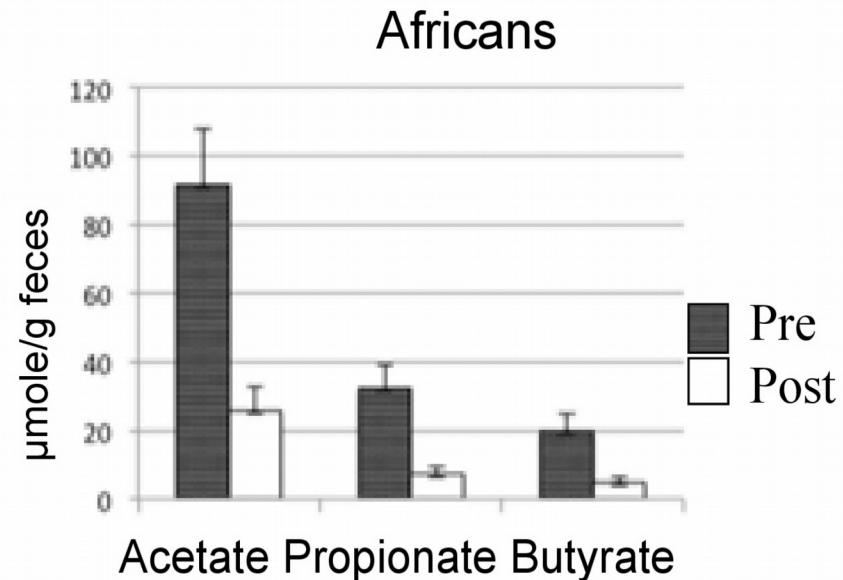
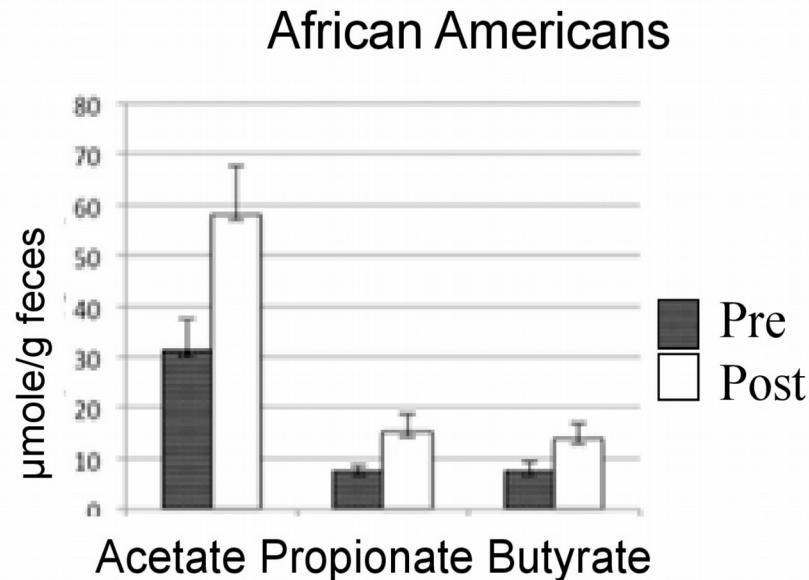


Colon cancer prevalence



Colon cancer rates per country

Impact diet exchange on SCFA

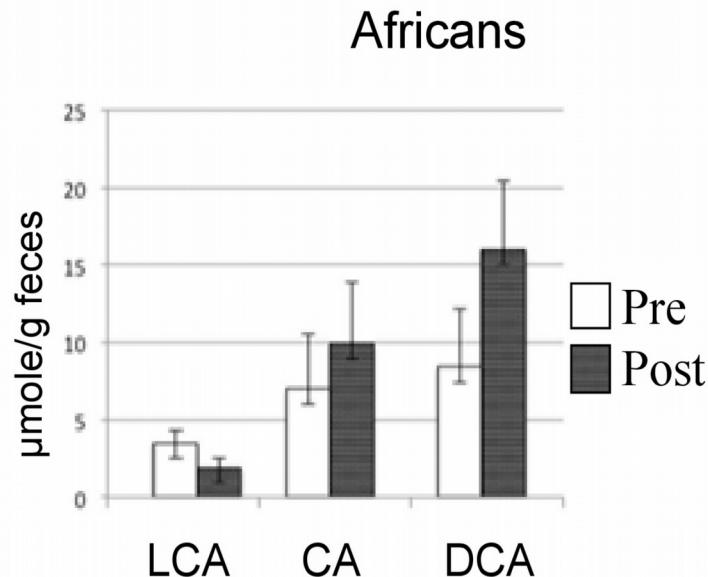
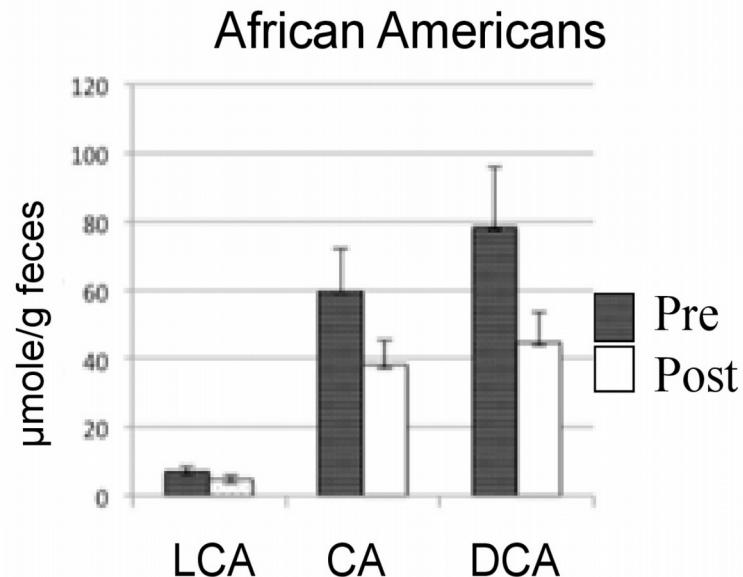


Reciprocally increased SCFA production with African diet: reported anti-inflammatory and anti-carcinogenic properties !

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

O'Keefe et al. Nat. Comm. 6:6342, 2015

Impact diet exchange on bile acids



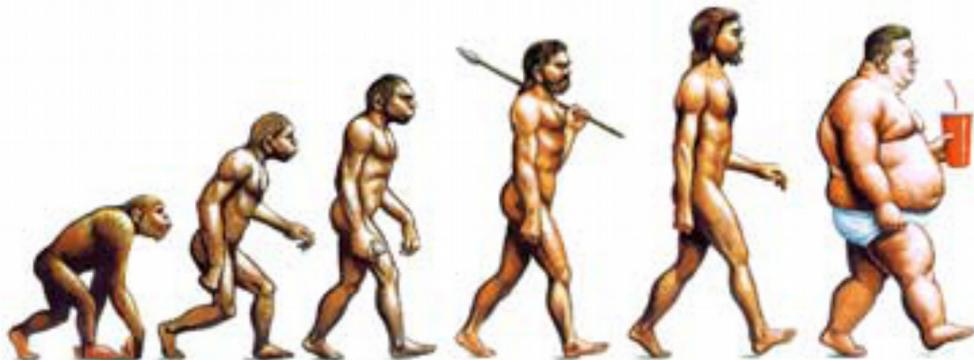
LCA: Lithocholic Acid, CA: Cholic Acid, DCA: Deoxycholic Acid

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

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 ?



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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

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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)

Challenge for manipulation:
- individual responses !

Responders vs.
non-responders
- personalized treatment ?

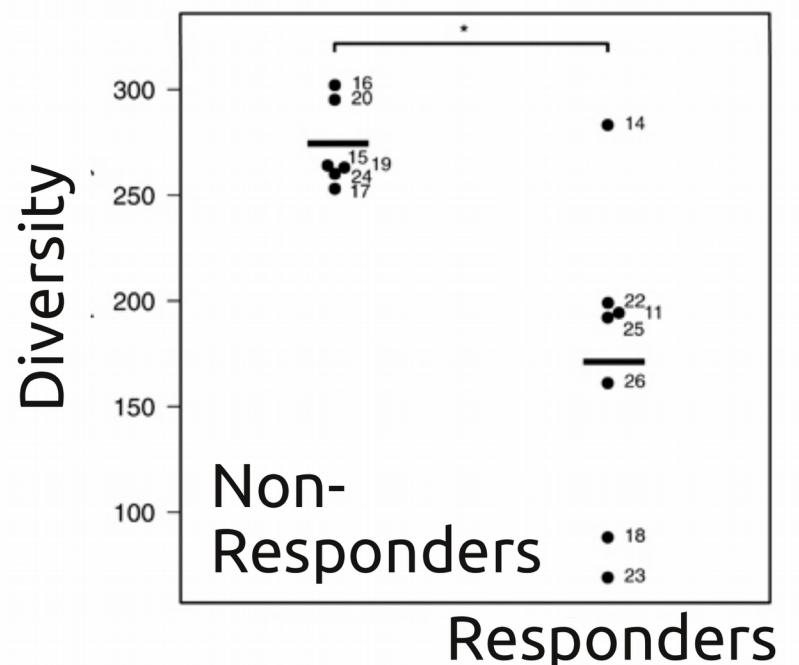
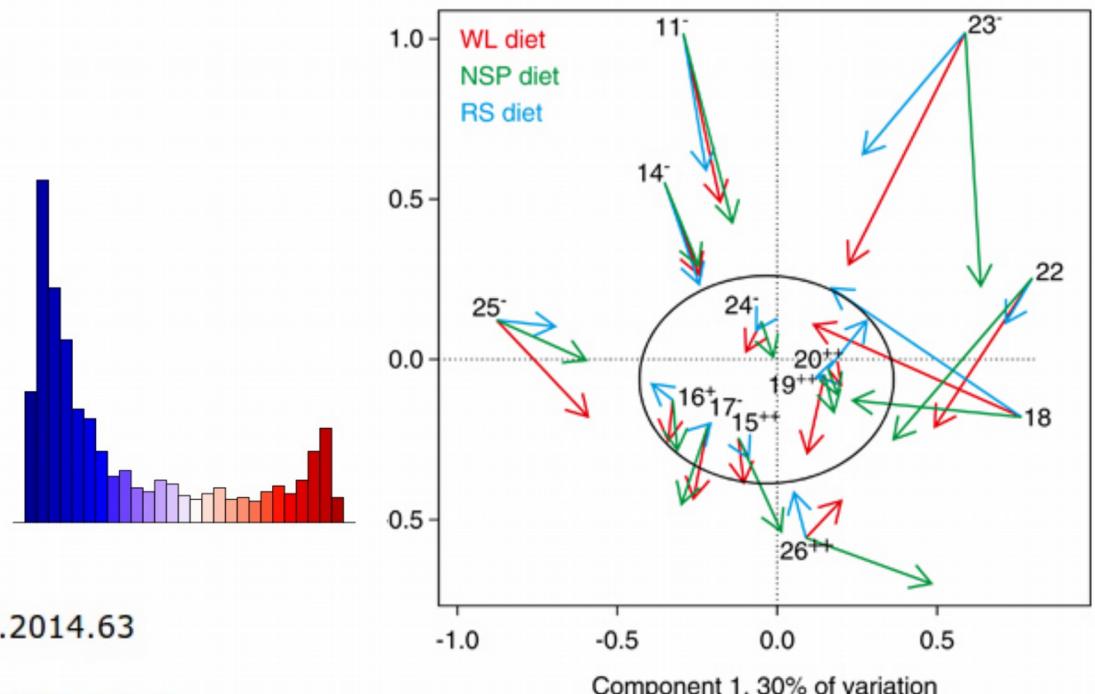
The ISME Journal , (24 April 2014) | doi:10.1038/ismej.2014.63

Impact of diet and individual variation on
intestinal microbiota composition and
fermentation products in obese men

Anne Salonen, Leo Lahti, Jarkko Salojärvi, Grietje Holtrop, Katri Korpela,
Sylvia H Duncan, Priya Date, Freda Farquharson, Alexandra M Johnstone,
Gerald E Lobley, Petra Louis, Harry J Flint and Willem M de Vos

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

Korpela et al. PLoS ONE 9(3): e90702, 2014



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.

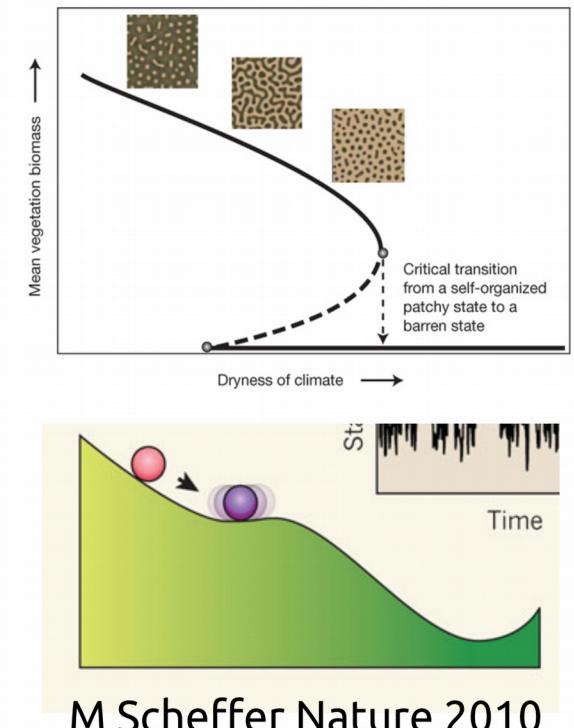
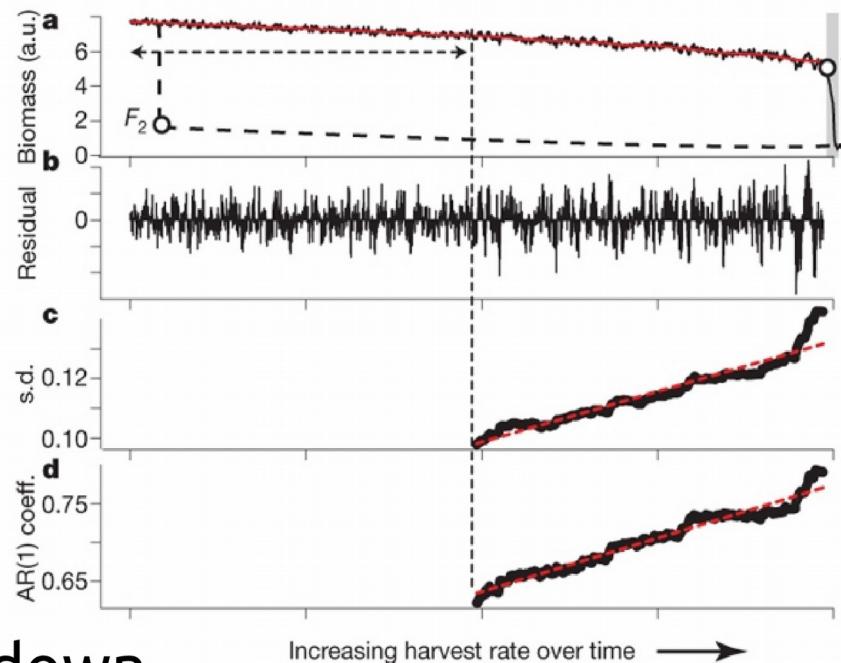
Autocorrelation

Variance

Skewness

Flickering

Critical slowing down

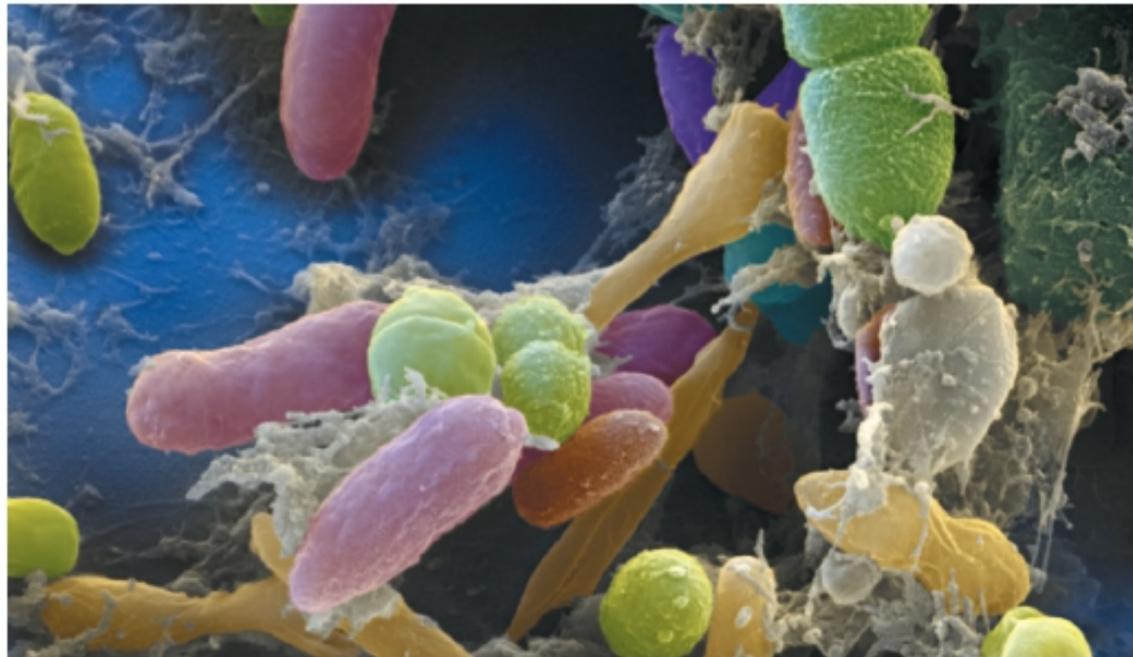


M Scheffer Nature 2010

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

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

nature



A scanning electron micrograph of bacteria in human faeces, in which 50% of species originate from the gut.

Microbiome science needs a healthy dose of scepticism

To guard against hype, those interpreting research on the body's microscopic communities should ask five questions, says William P. Hanage.

Comment August 2014 Nature

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

RESEARCH | RESEARCH ARTICLES

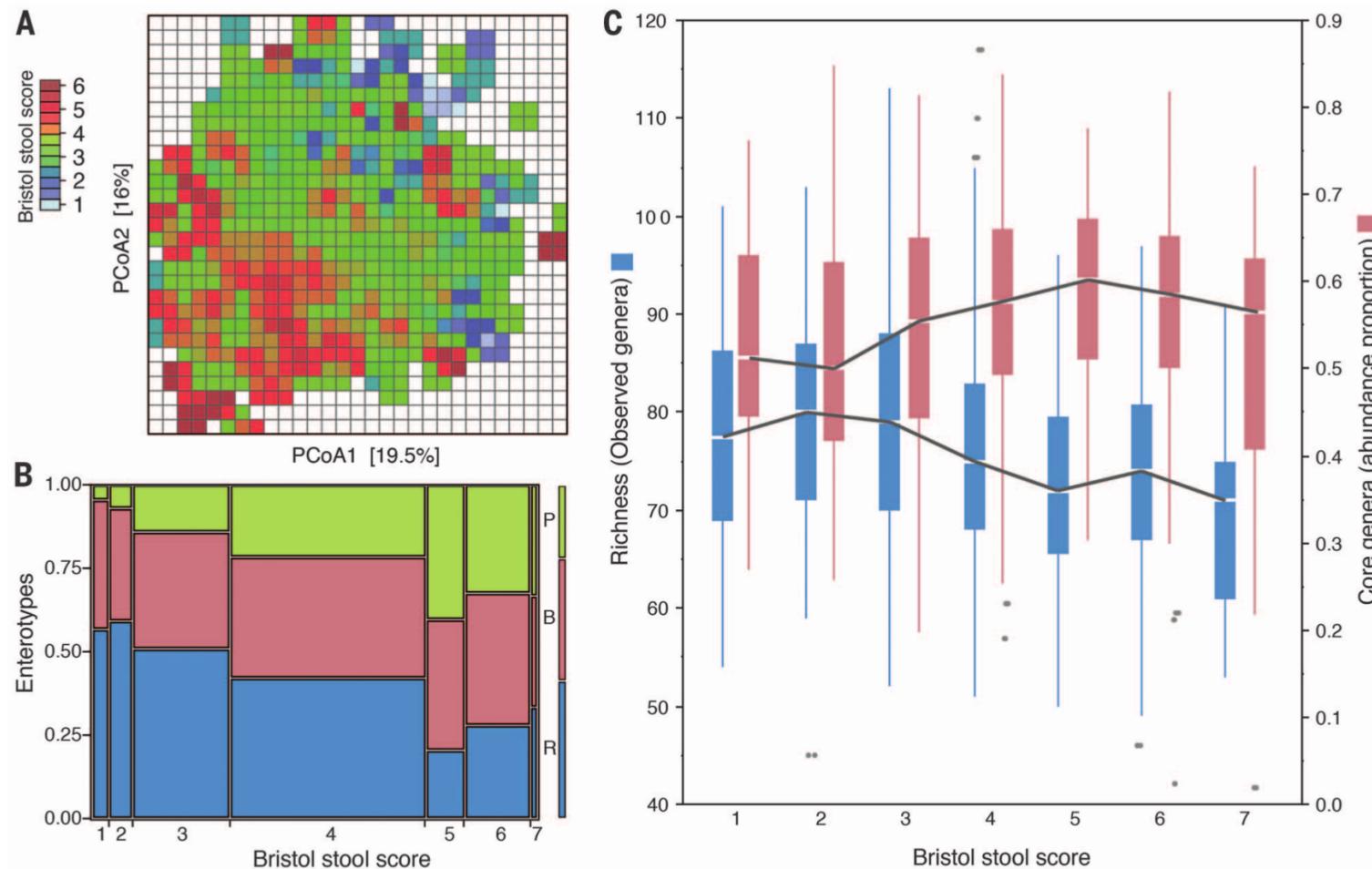
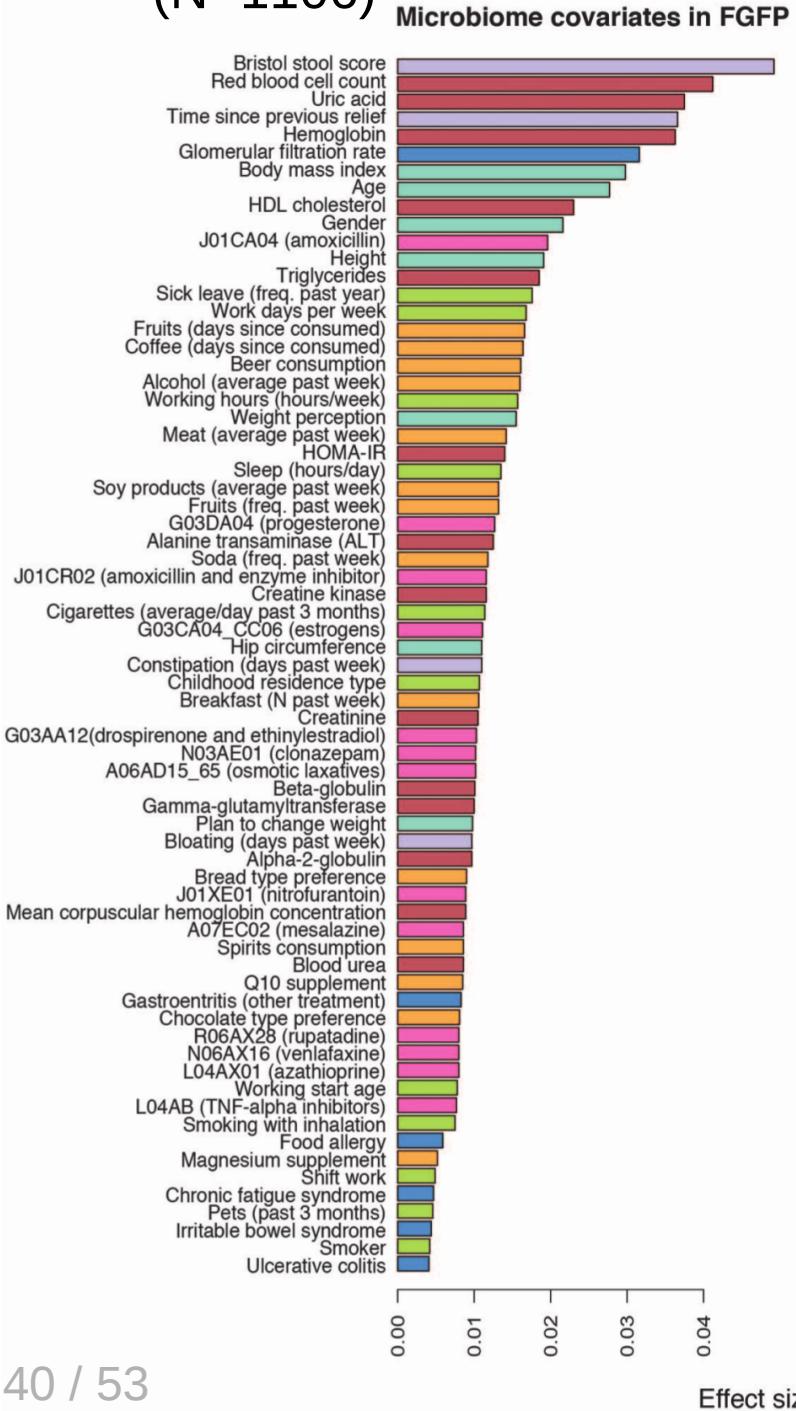


Fig. 4. BSS score association to microbiota variation. (A) BSS score variation across the FGFP cohort, as represented on the genus-level PCoA ordination (Bray-Curtis dissimilarity). Each cell is colored according to median BSS score of individual samples allocated to the cell coordinates. (B) Enterotype 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) Replication in LLDeep

Determinants of microbiota composition ?

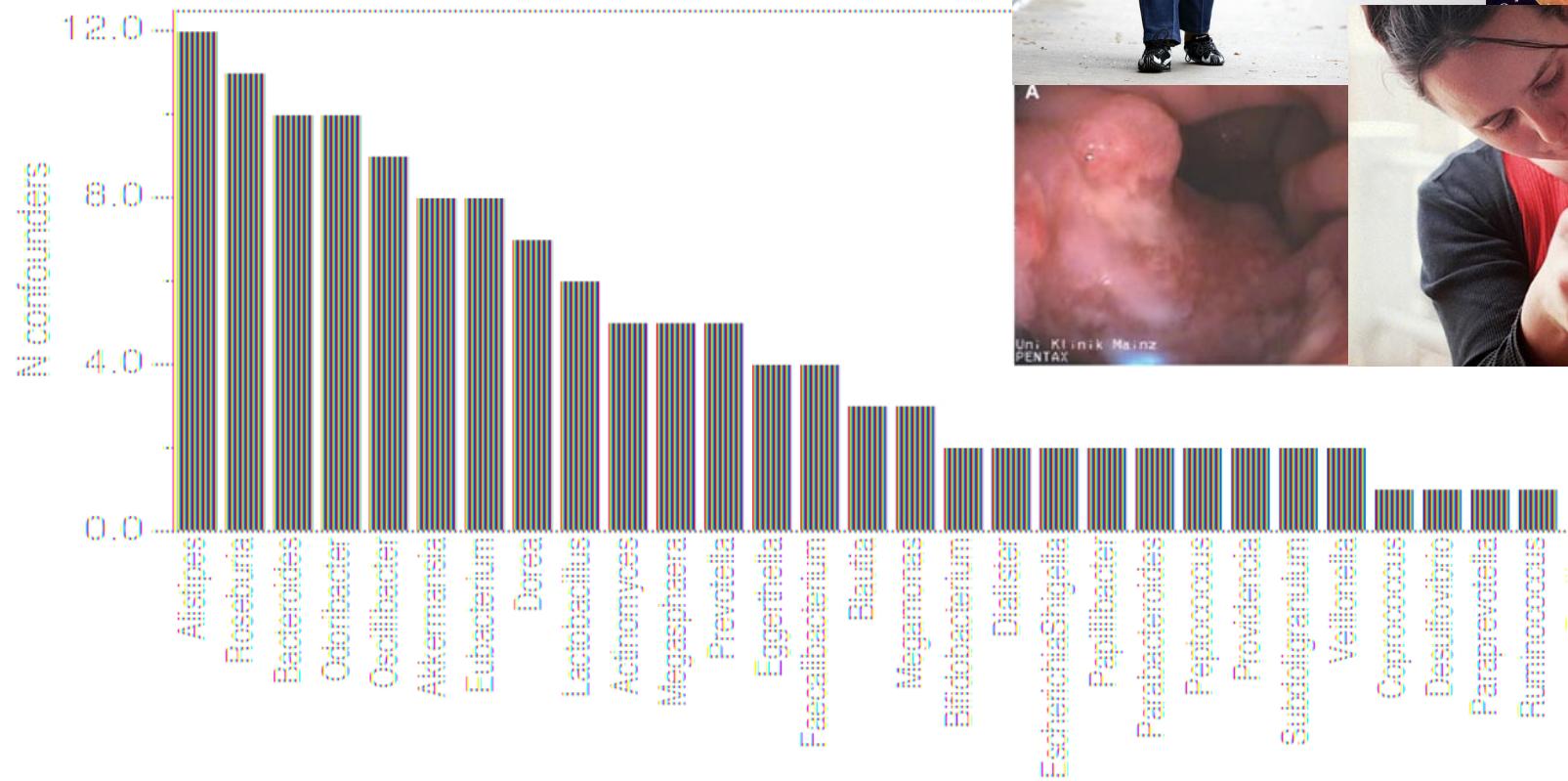
Diet, health, lifestyle,
medication, host variables,
blood, bowel habits..

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

92% replication rate !

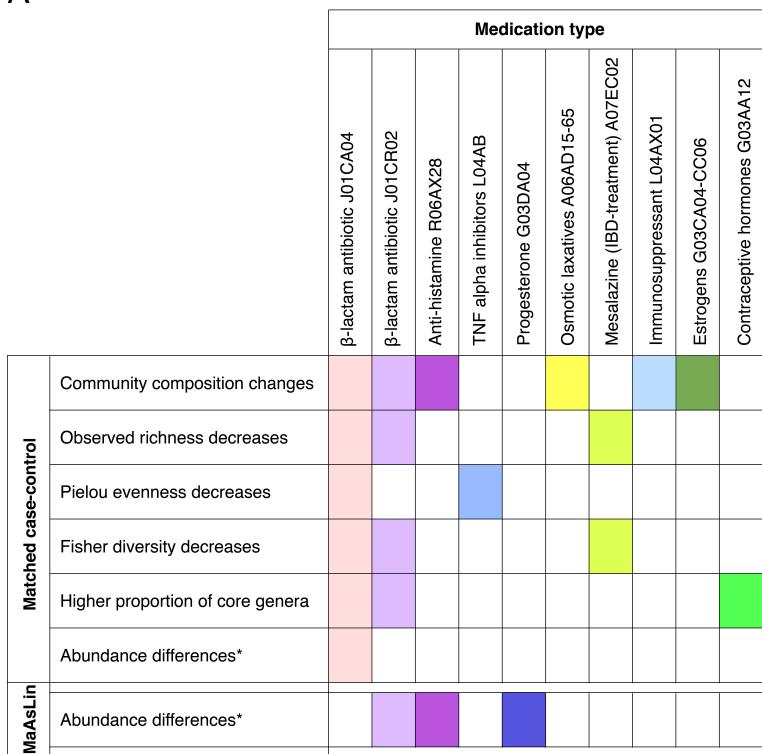
Falony et al. Science 352, 2016.

Majority of genera thus far associated to disease are also confounded by other covariates, incl drugs



Identification of microbiota-drug associations

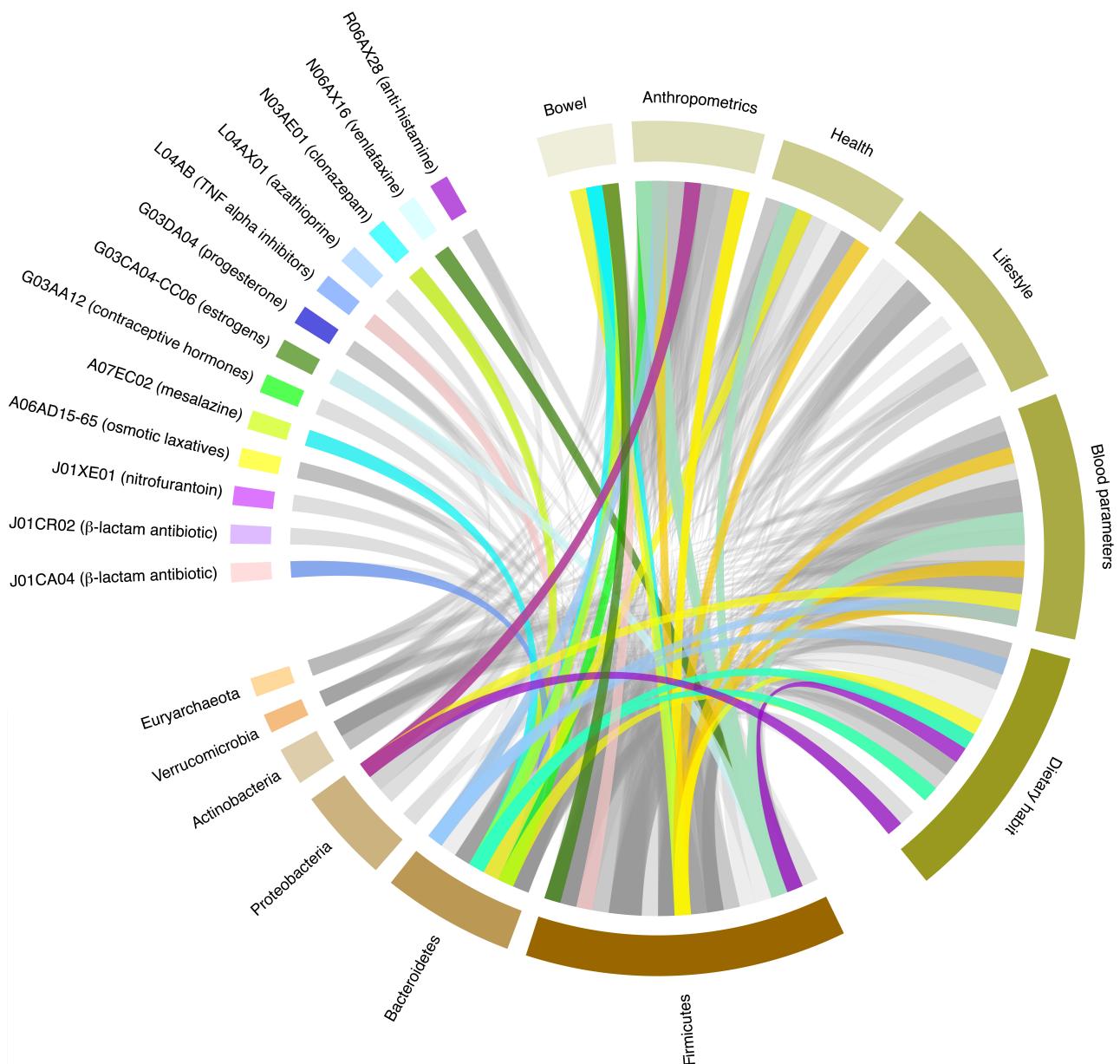
A



Direct associations

e.g. Antibiotics, laxatives,
Immunosuppressants, Hormones

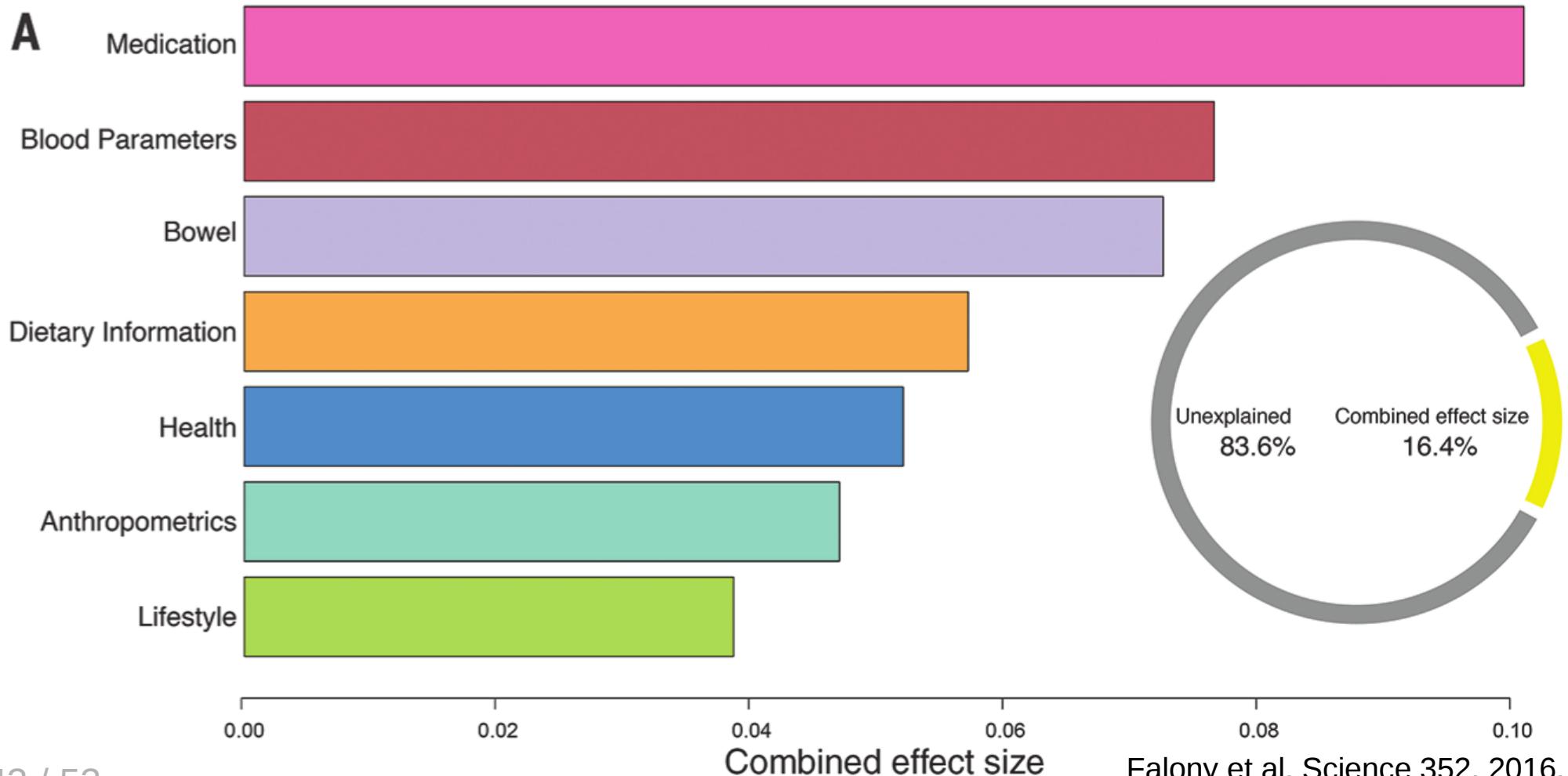
B



Total explained variation: 16.4%

(Flemish Gut Flora Project)

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



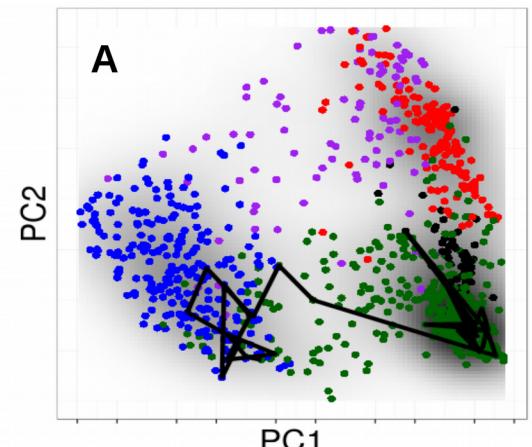
Summary

Individuality and temporal dynamics best understood in the context of larger populations

Majority (>80%) of total variation in healthy microbiome composition still not explained

Standardization, study design and confounder analysis remain essential yet easily overlooked

Open data, analysis & visualization tools:
<http://microbiome.github.io>



Thank You !

Materials at: <http://www.iki.fi/Leo.Lahti>

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

Intestinal microbiome landscaping: Insight into community assemblage and implications for microbial modulation strategies. Shetty, SA et al. *FEMS Microbiology Reviews* 2017, fuw045

Population-level analysis of gut microbiome variation. Falony et al. *Science* 352(6285):560-4, 2016

Metagenomics meets time series analysis: unraveling microbial community dynamics. Faust, Lahti et al. *Current Opinion in Microbiology* 15:56-66 2015

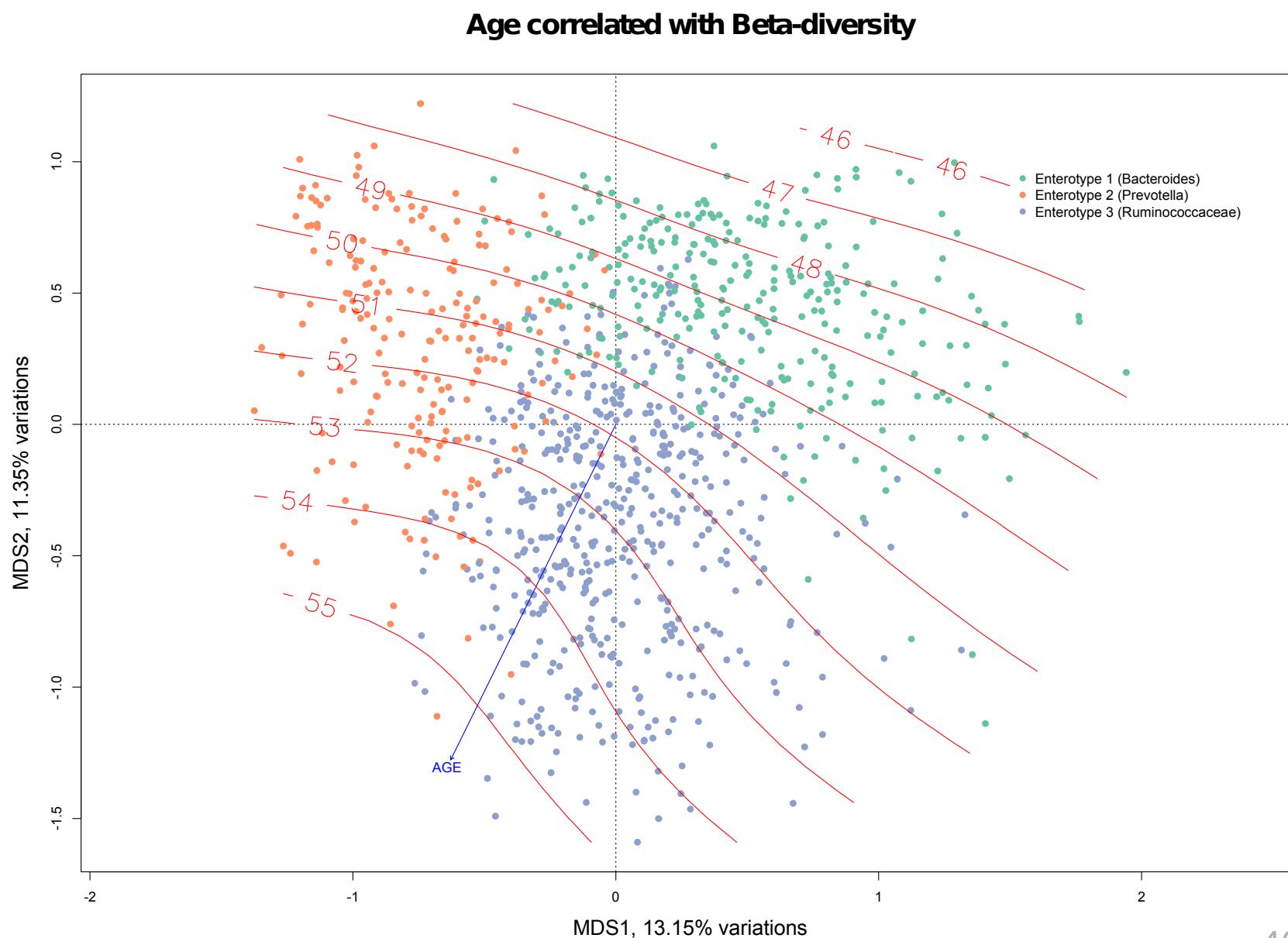
Improved taxonomic assignment of human intestinal 16S rRNA sequences by a dedicated reference database Ritari et al. *BMC Genomics* 16:1056, 2015.

Fat, Fiber and Cancer Risk in African, Americans and Rural Africans. O'Keefe et al. *Nat. Comm.* 6:6342, 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

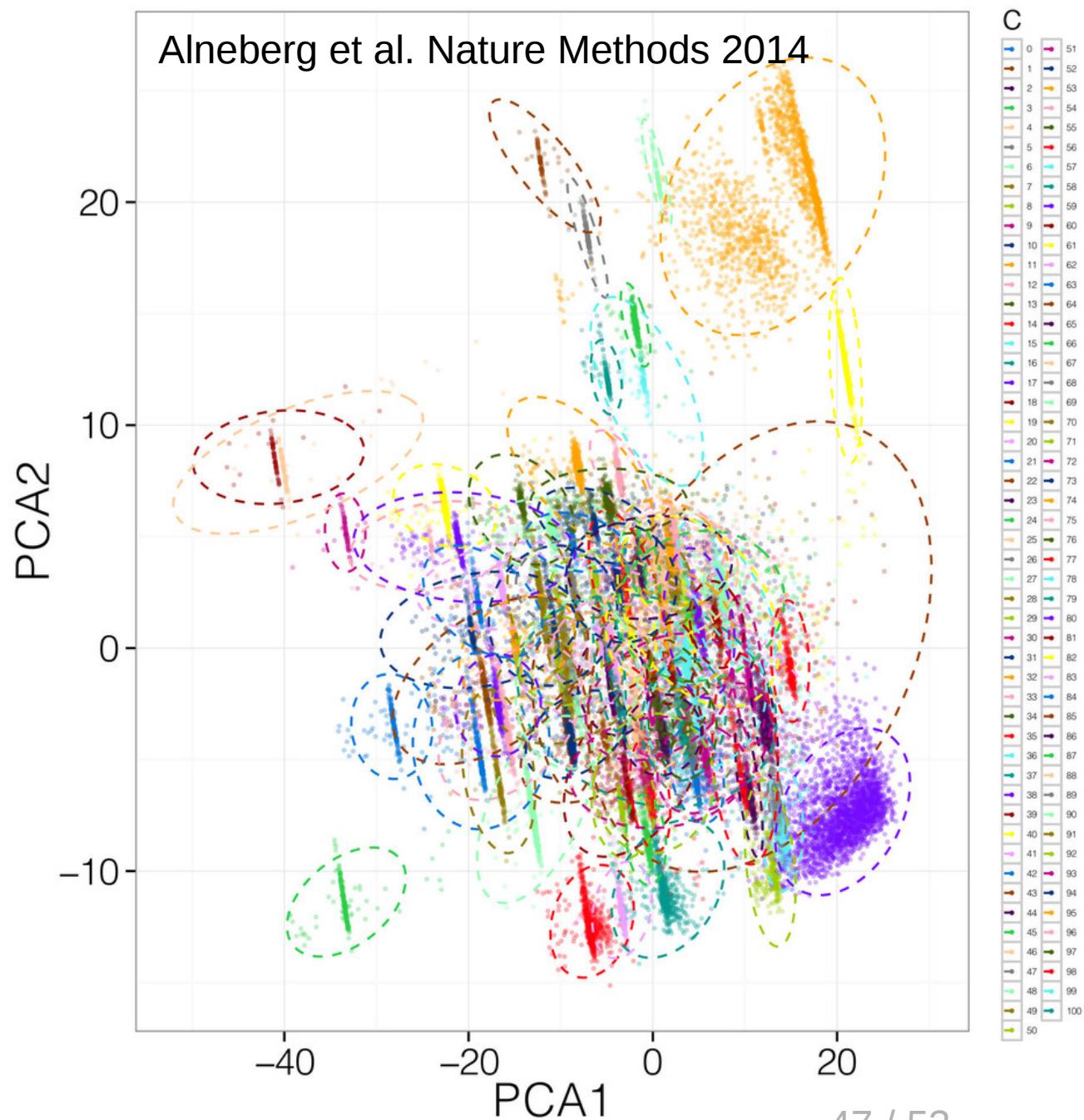
Tipping elements in the human intestinal ecosystem. Lahti et al. *Nat. Comm.* 5:4344, 2014

VDP Cohort: age is a major factor shaping community composition (even within only the adult population)



Clustering contigs by coverage and composition (CONCOCT)

Variational Dirichlet Process multivariate mixture models work very well in practical applications with high-dimensional data



Community types as species combinations (HITChip Atlas)

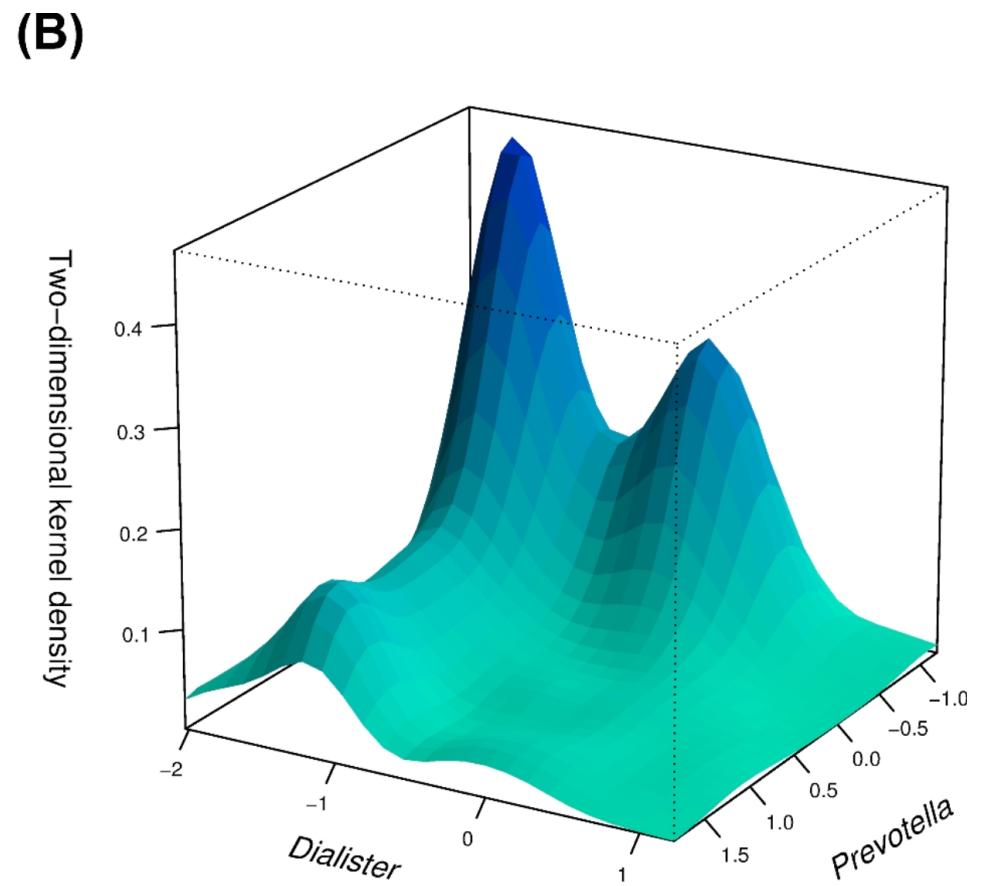
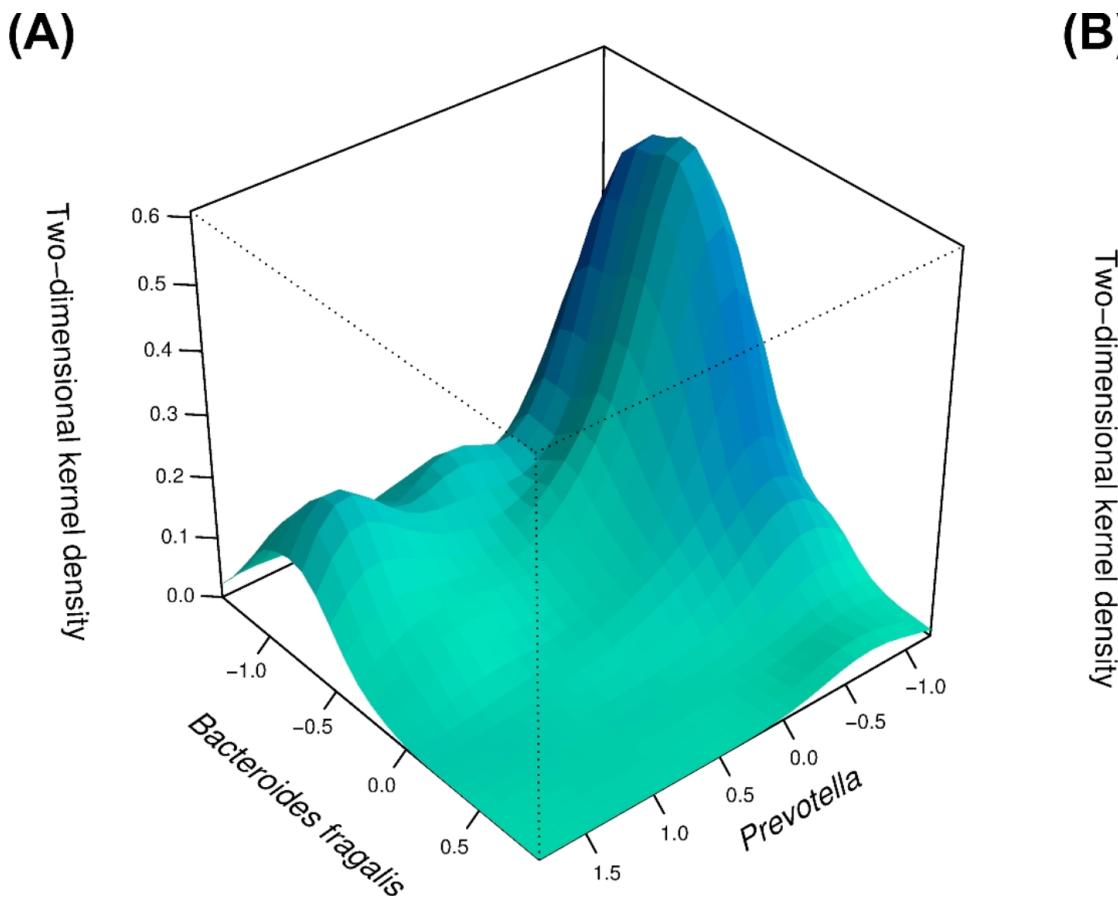


Figure: Intestinal microbiome landscaping: insight in community assemblage and implications for microbial modulation strategies. Shetty et al. 2017 FEMS Microbiol Rev. 2017 – Data: Lahti et al. Nat Comm 5:4344, 2014

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 !

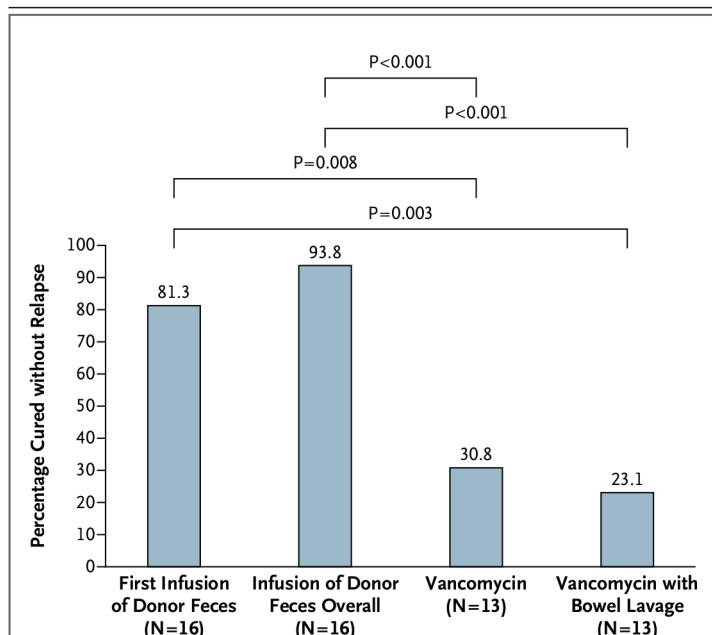
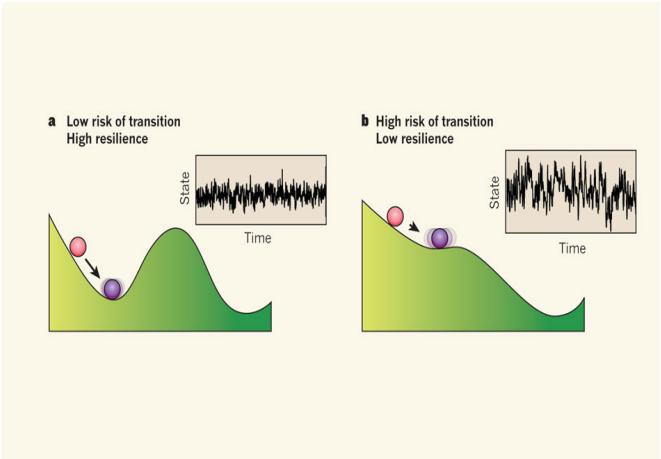
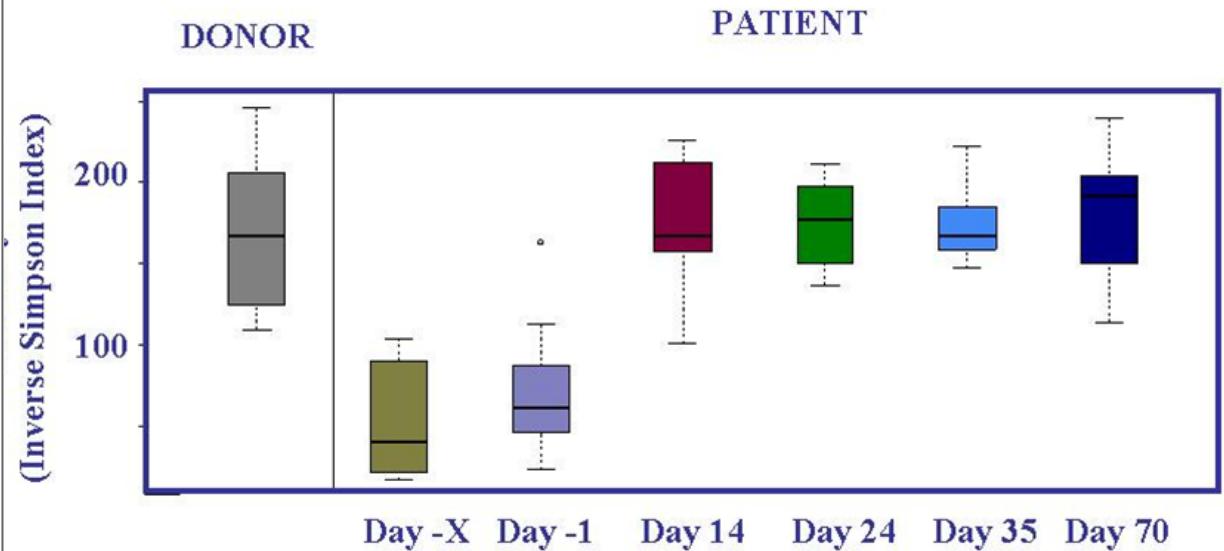


Figure 2. Rates of Cure without Relapse for Recurrent *Clostridium difficile* Infection.

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.



Patient gets donor microbiota -> Donor farms!?

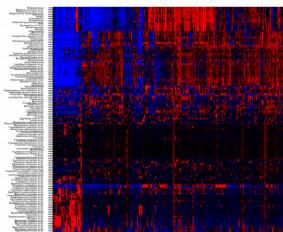


Els van Nood et al., NEJM 368(5) 2013
Fuentes et al. ISME J, 8:1621-33, 2014

Data availability: large & standardized population cohorts

HITChip Atlas (microarrays):

- > 10,000 samples
- > 5,000 subjects
- > 1,000 phylotypes (~0.1%)
- versatile geography
- highly reproducible (>98%)
- long follow-ups (10+ y)
- open data: Lahti et al. 2014



Flemish Gut Flora (NGS):

- > 5,000 samples
- > 1,000 subjects
- > 7,000 OTUs
- normal Flemish population
- dense time series
- open data: Falony et al. 2016



Challenges for data availability:

- Protocol & metadata standardization
- Custom tools & reproducible workflows
- Ethical considerations

Analysis & visualization tools: <http://microbiome.github.io>

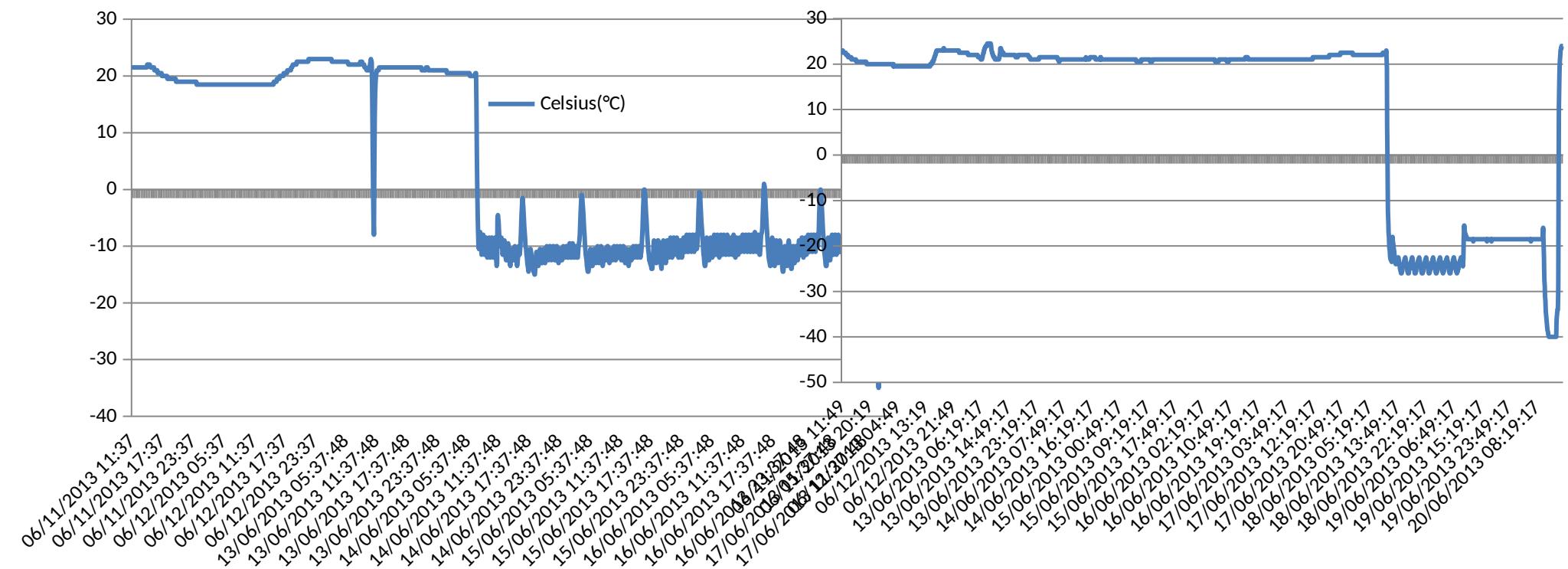
Other collections: MetaHIT, Human Microbiome Project, American Gut...

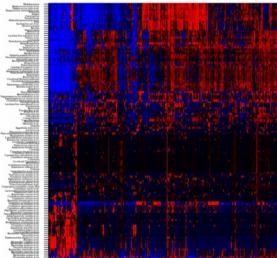
Strongly controlled logistics - burden but necessary

Extraction protocols
Sample storage and transport



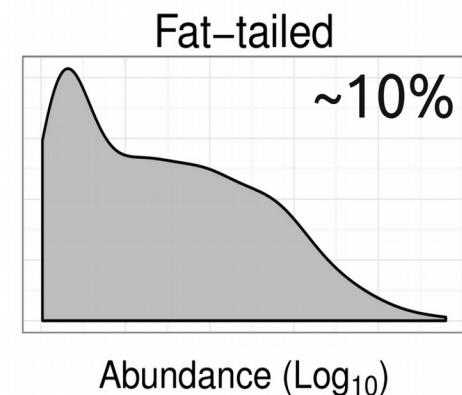
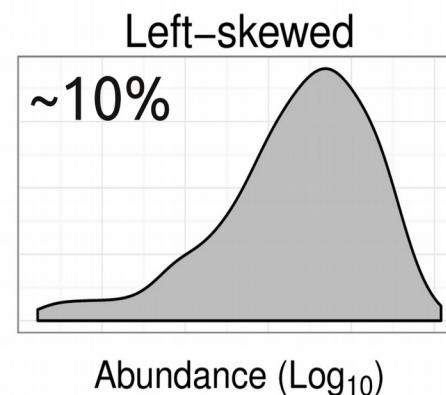
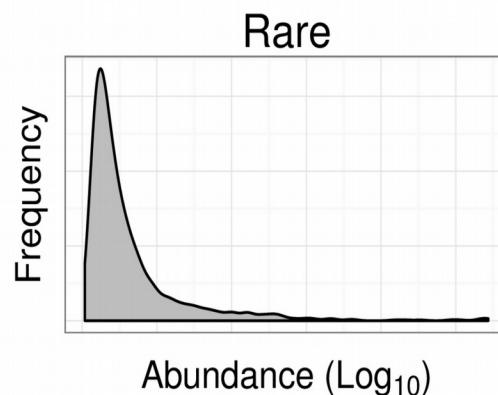
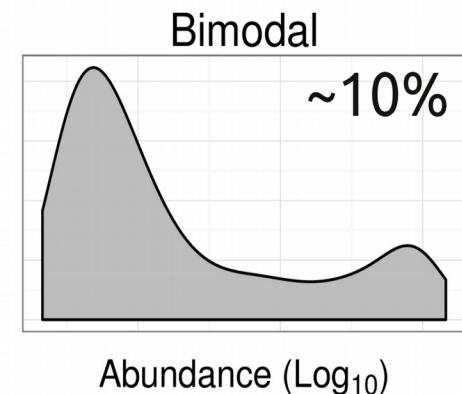
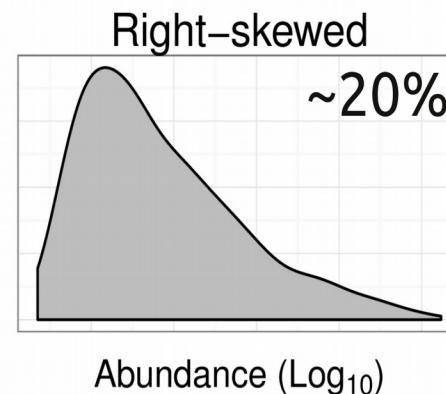
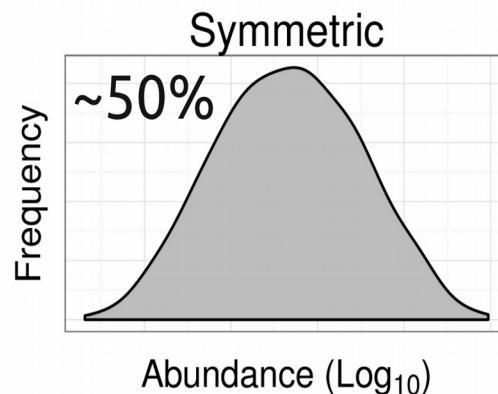
QC matters: Temperature logging of samples





Bacterial 'abundance types' in 1000 western adults:

~% indicates proportion among prevalent taxa



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