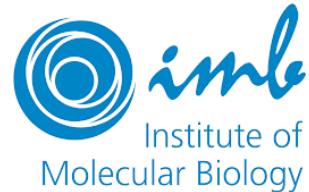


# Microbiome analyses for clinical research



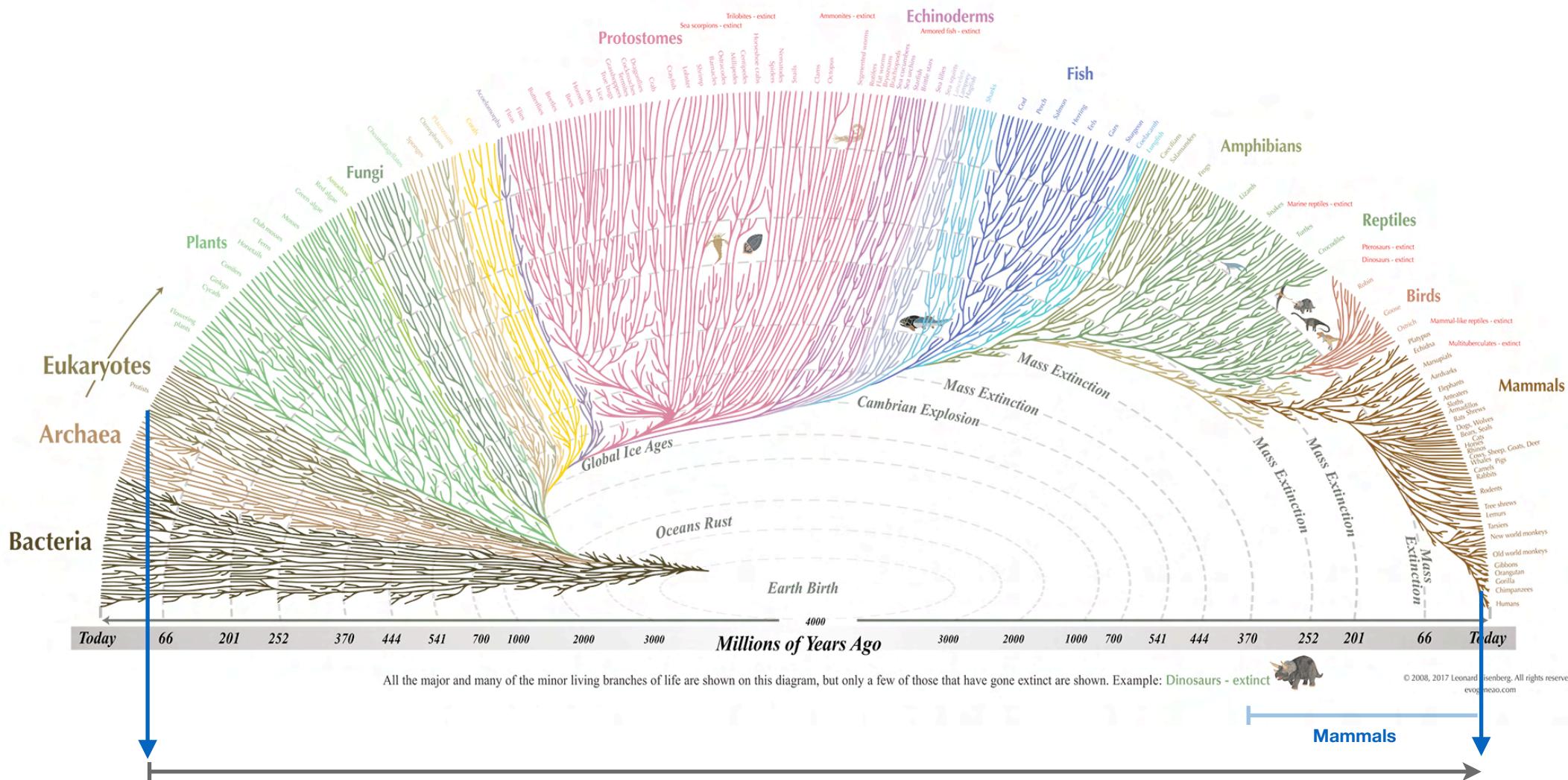
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MAINZ



**Sara Vieira-Silva**  
University Medical Center - JGU Mainz, DE  
Institute of Molecular Biology (IMB), Mainz, DE

# Human-microbiota coevolution



**(co-)evolution of eukaryotic-microbial interactions**

>300M years of mammalian immune system - microbiota interactions

©2008, 2017 Leonard Eisenberg.  
evogeneao.com

# Mucosal tissues in humans

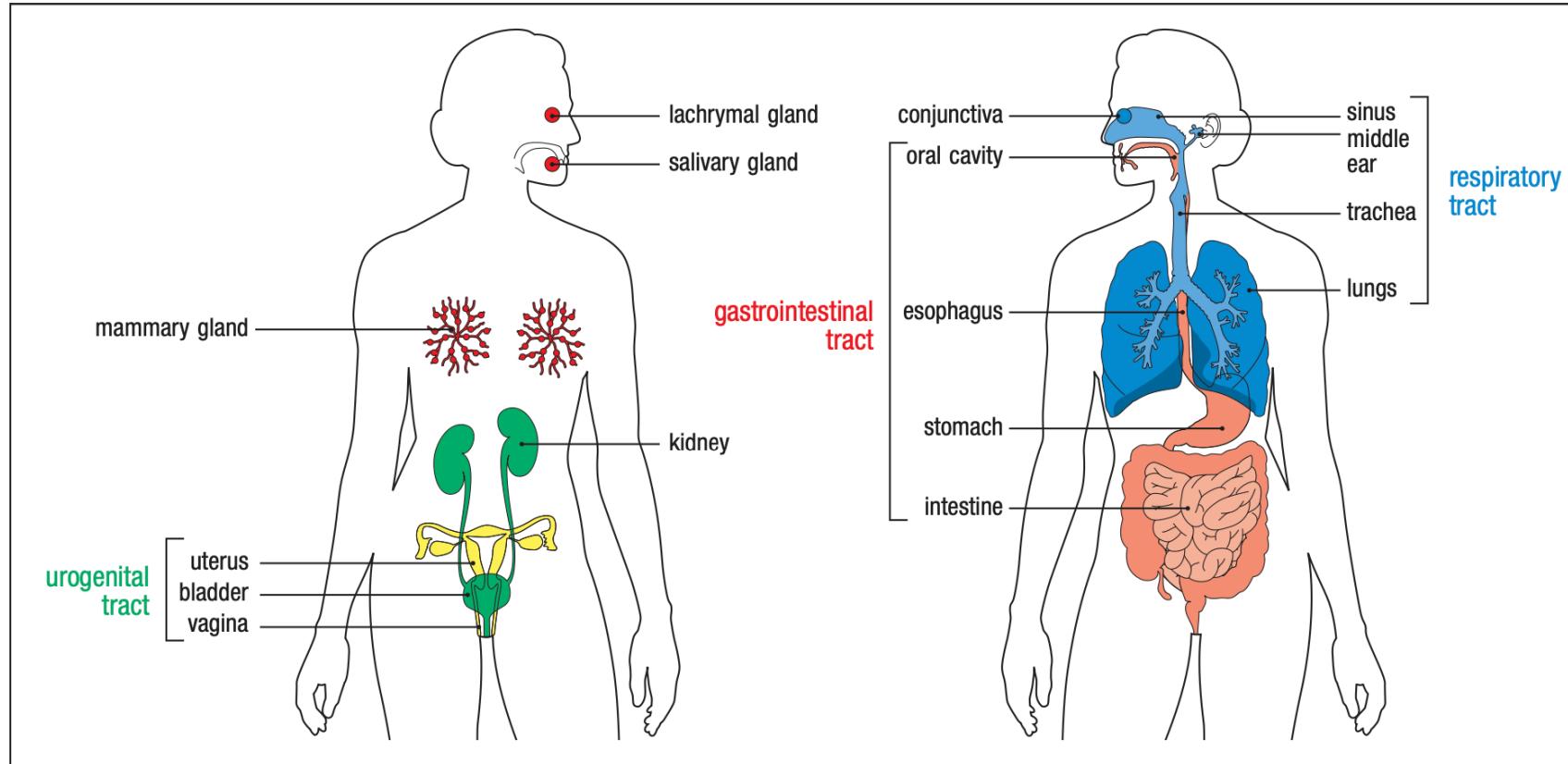


Figure 12.1 Immunobiology, 9th ed. (© Garland Science)

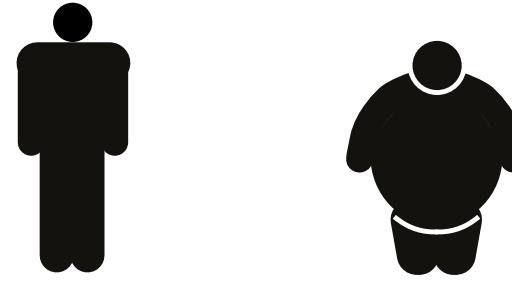
**Epithelial surfaces with a mucus layer**  
**Controlled exposure for selective permeability**  
Skin:  $2 \text{ m}^2$   
Mucosa:  $400 \text{ m}^2$

## Why?

1. Nutrition / breathing
2. Reproduction
3. Sensing

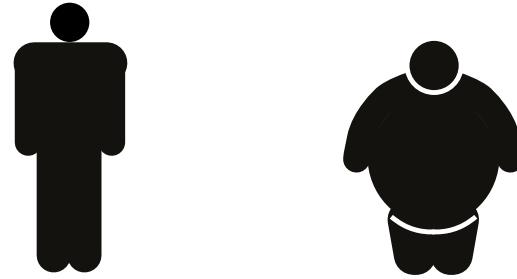
# Definition of dysbiosis

- Alteration associated to disease as compared to non-disease (**defunct**)



# Definition of dysbiosis

- Alteration associated to disease as compared to non-disease (**defunct**)



Assumption: all patients have (a degree) of dysbiosis

# Definition of dysbiosis

- Alteration associated to disease as compared to non-disease (**defunct**)
- Causally linked to disease, in individuals where it establishes, by contributing/sustaining a pathomechanism or failing to perform essential functions.

# Definition of dysbiosis

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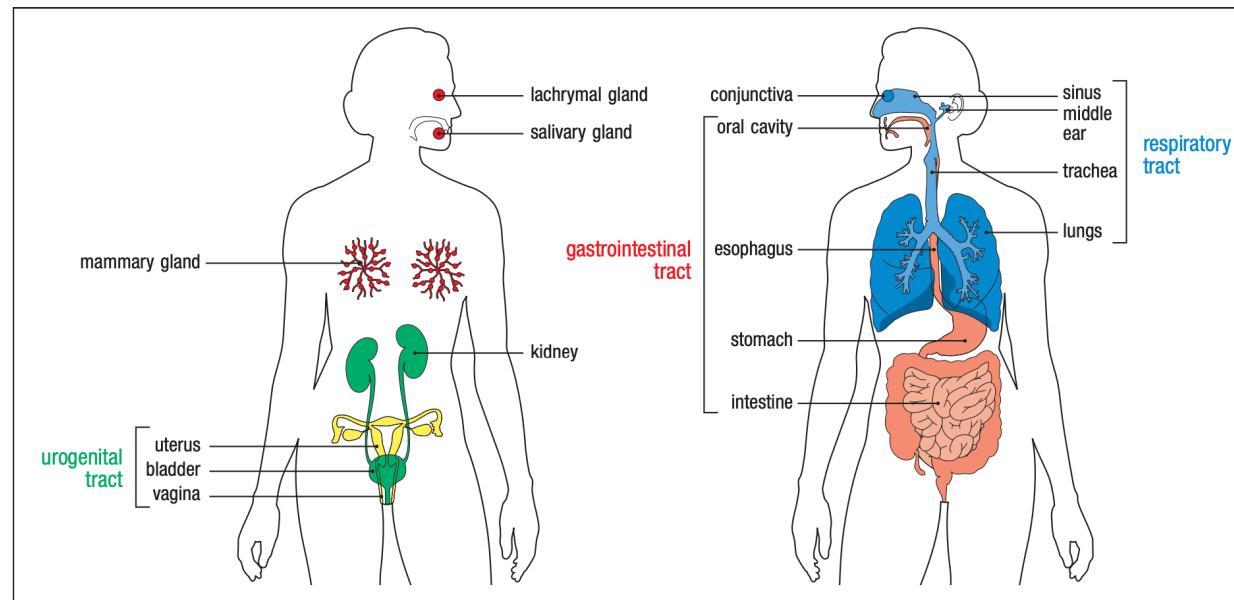


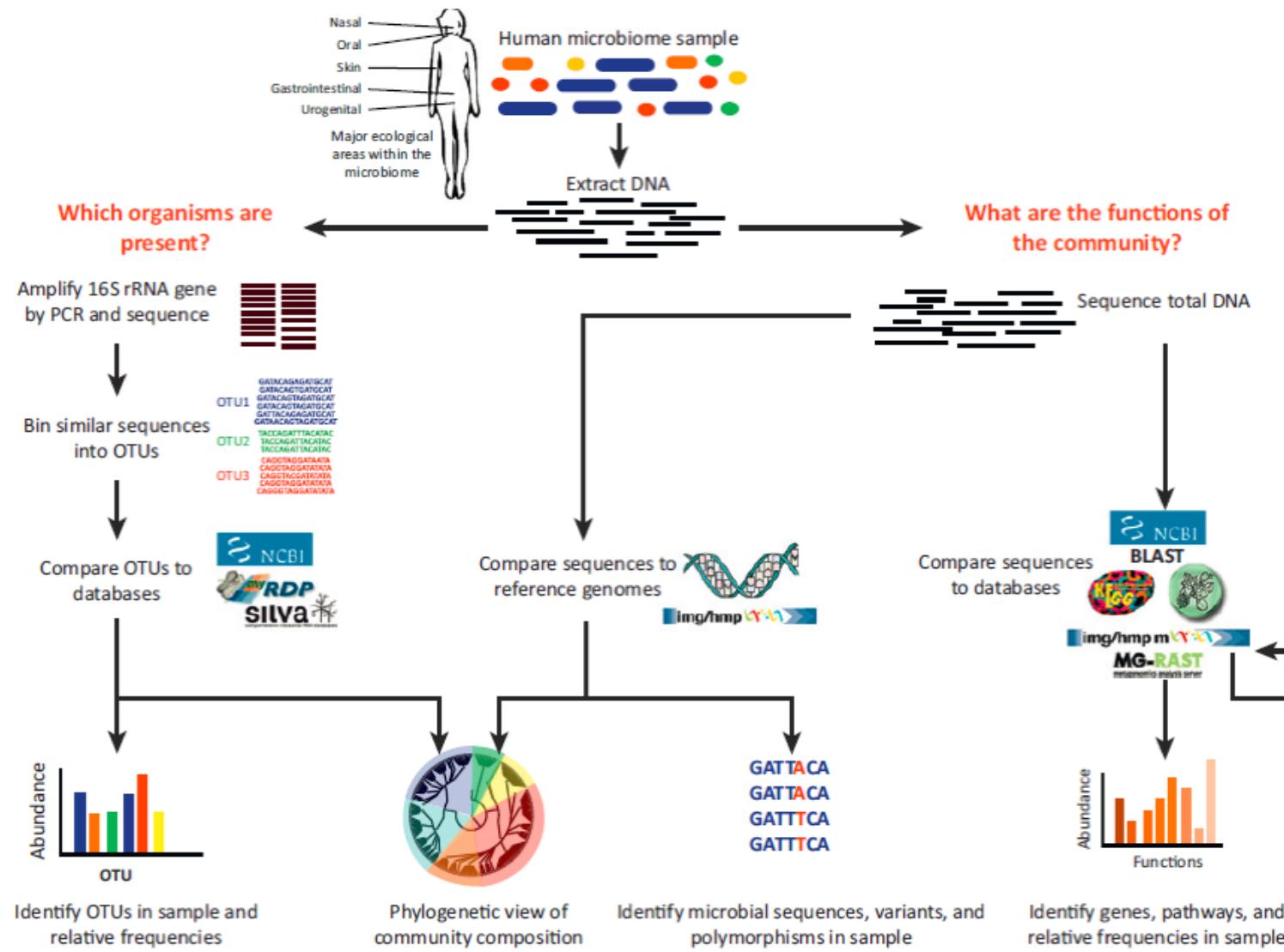
Figure 12.1 Immunobiology, 9th ed. (© Garland Science)

## Barrier function

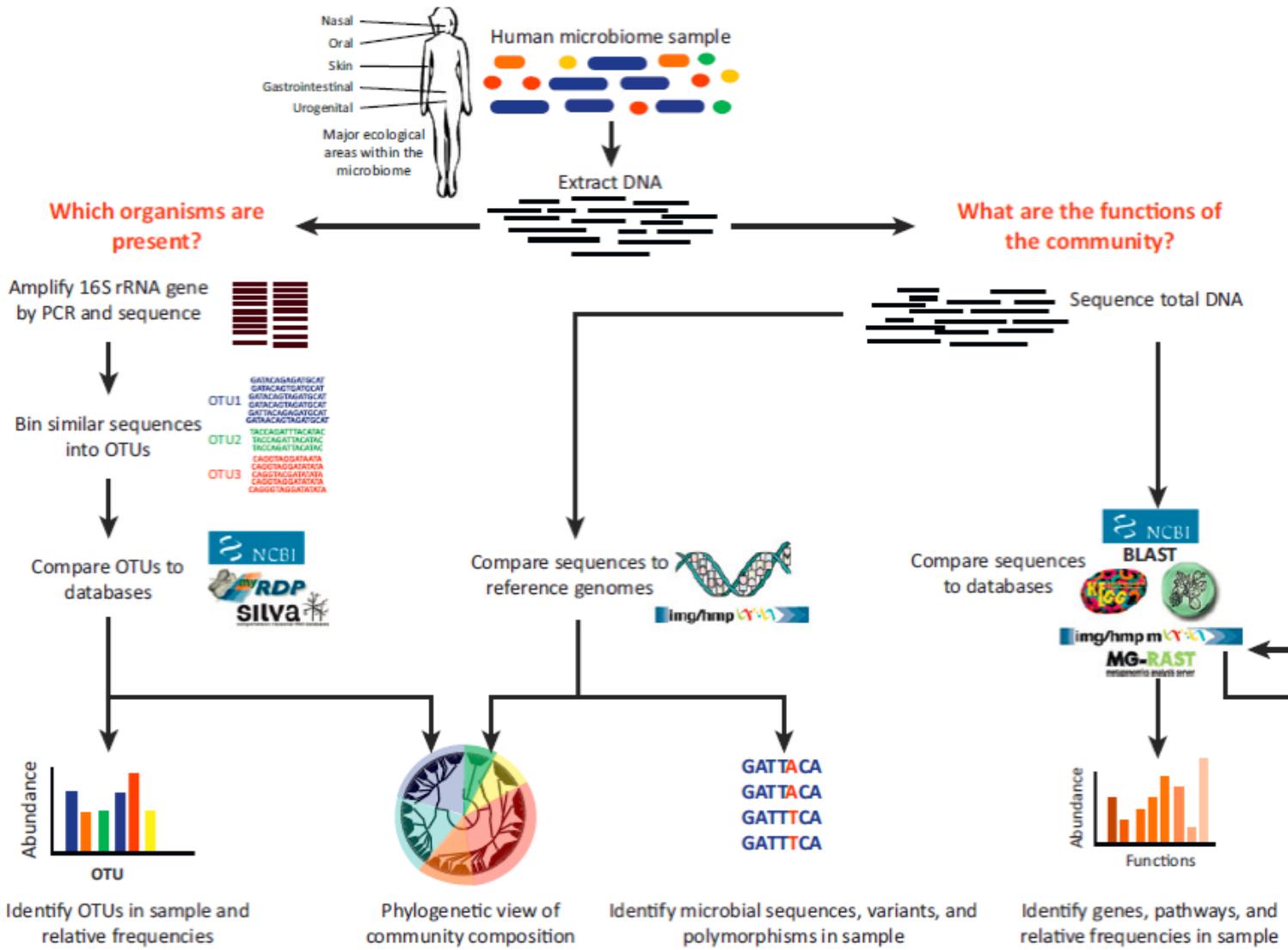
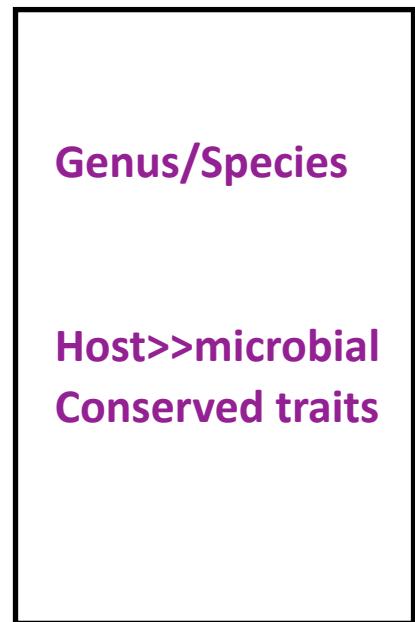
Gut dysbiosis ≠ Vagina dysbiosis

# How to probe host-associated microbiomes

## Metagenomics - amplicon/WGS; metaT; metabolomics



# How to probe host-associated microbiomes



Strain tracking  
Functions  
Traits

Host<<microbial  
Mobile traits

# Metagenomics for clinical application

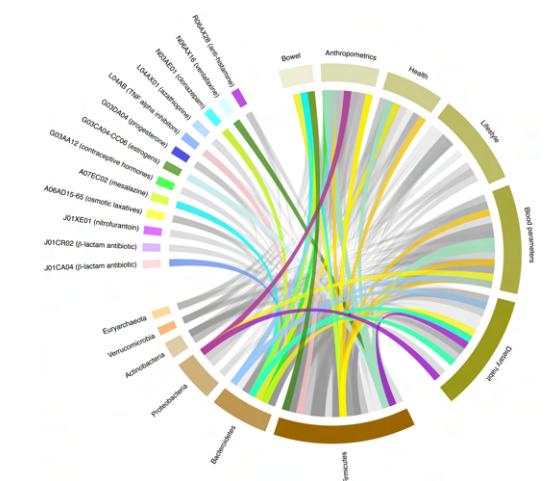
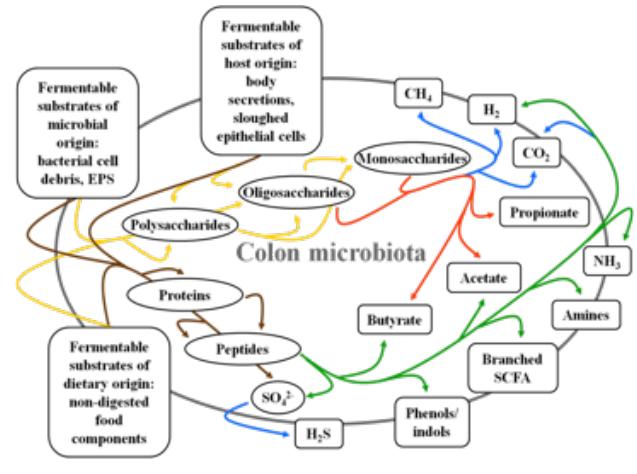
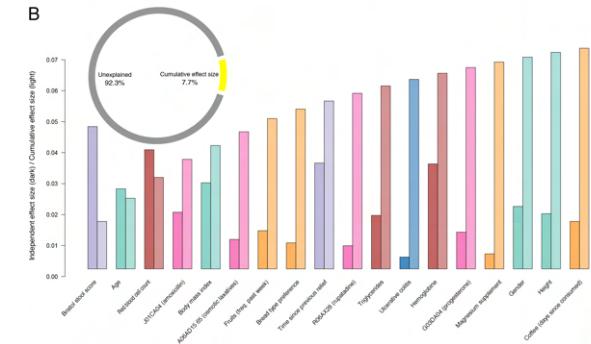
1. Fundamental/mechanistic insight for therapeutical translation

2. Diagnostic biomarker discovery

3. Risk prediction & prevention

4. Prediction of intervention response

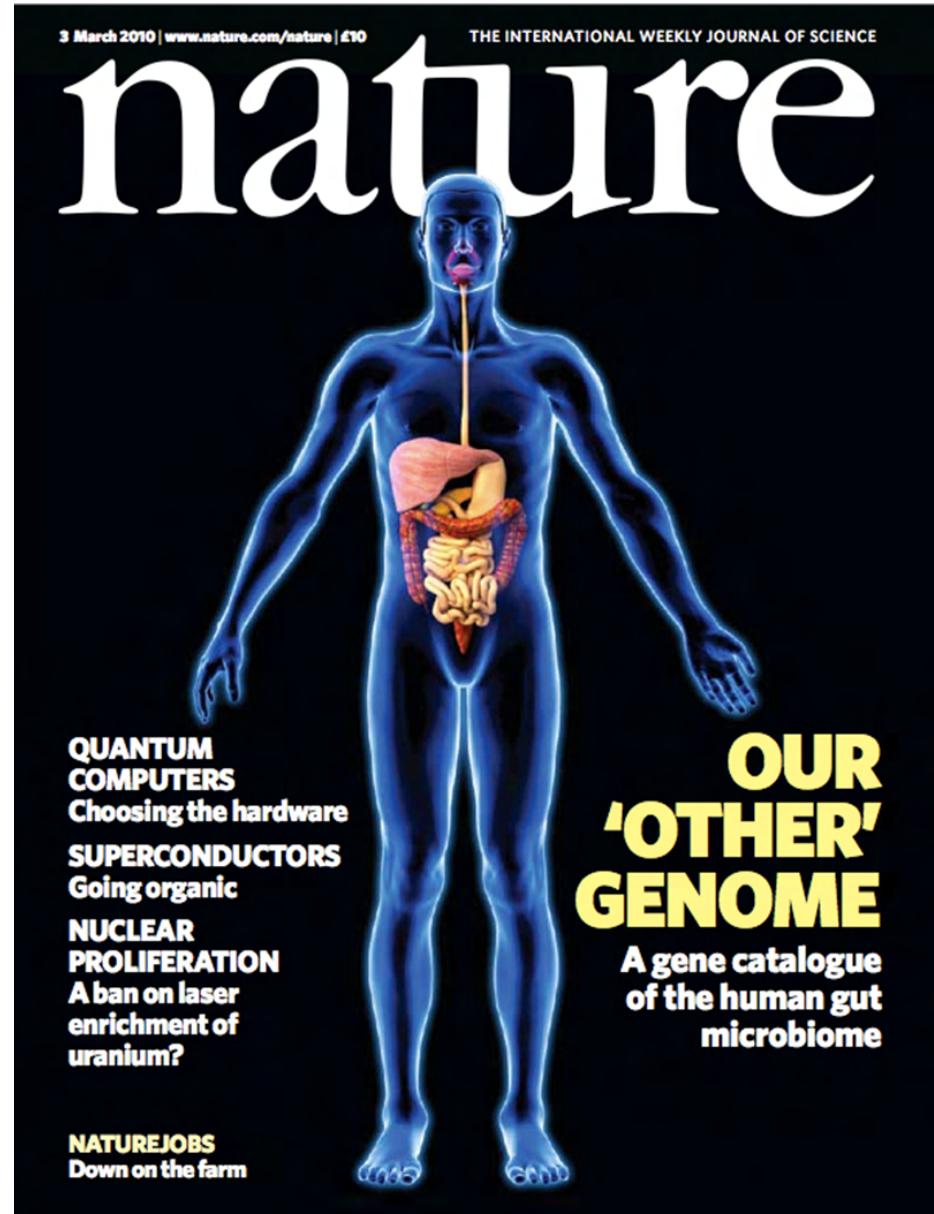
5. Stratification of patient cohorts



# The human gut microbiota

Densest and most metabolically active community

- produces essential nutrients,
- detoxifies xenobiotics
- is key for immune maturation and modulation
- and pathogen exclusion

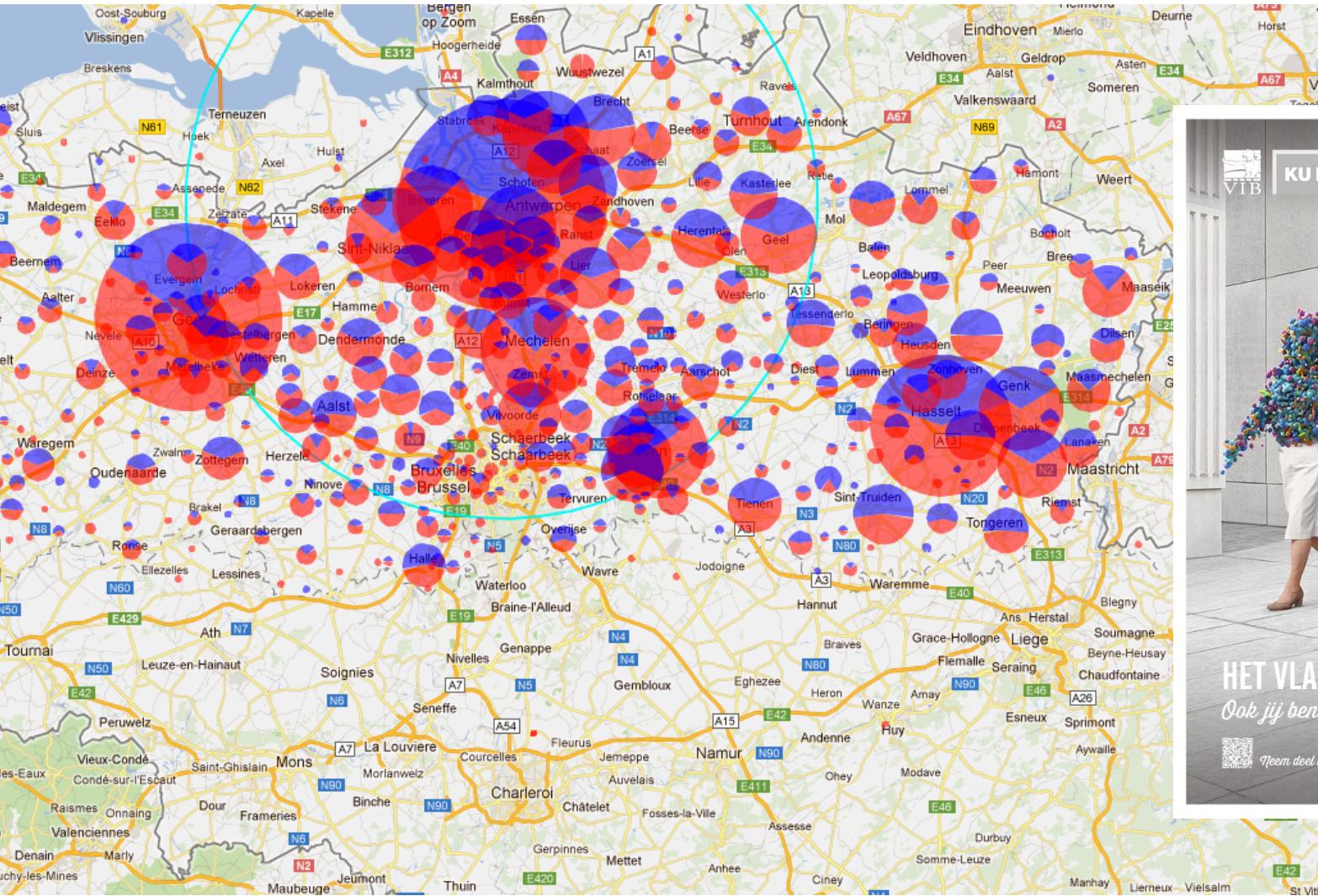




## Towards clinical microbiome research

- Study the boundaries of **healthy microbiota variation**
- Refine the approach to and the definition of **dysbiosis**
- Identify microbiome alterations associated to pathomechanisms and medication

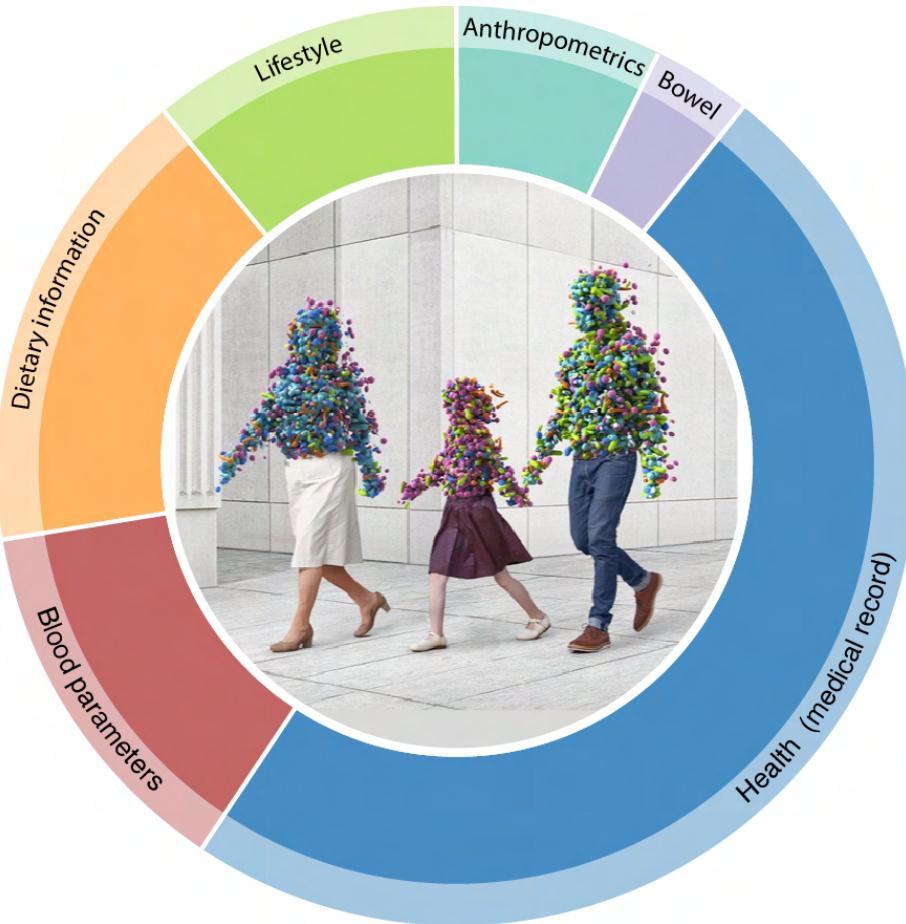
# **Gut microbiota variation in health**



# Studying health-associated microbiota variation

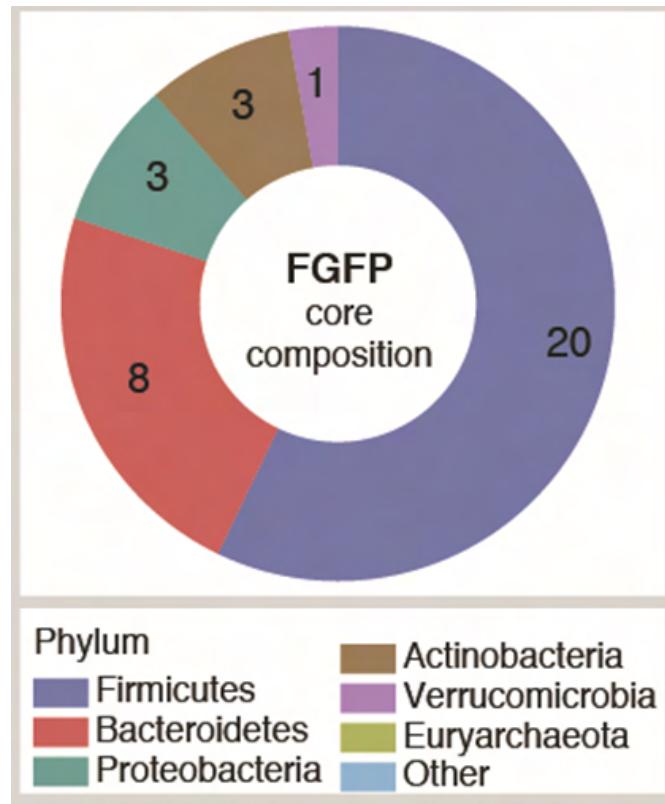
# The Flemish Gut Flora Project

Cross-sectional gut microbiome monitoring effort  
(3400 participants)



Metadata examples	Distributions
Volunteers N (1106)	
Gender	females (607) males (499)
Age	range [19:85] median (53)
Bristol Stool Score	score[1:2] (103) score[3:5] (824) score[6:7] (179)
BMI CVD risk	range [16:52] median (24) range [0:18] median (1)
CRP GFR HOMA-IR	normal (1018) elevated (88) low (424) normal (682) range [0:20] median (2)
Vegetarian Beer drinker	yes (80) no (1026) yes (754) no (352)
Pet owners Smoking Sleeping hrs	yes (471) no (635) current (102) never (547) range [4:15] median (7)

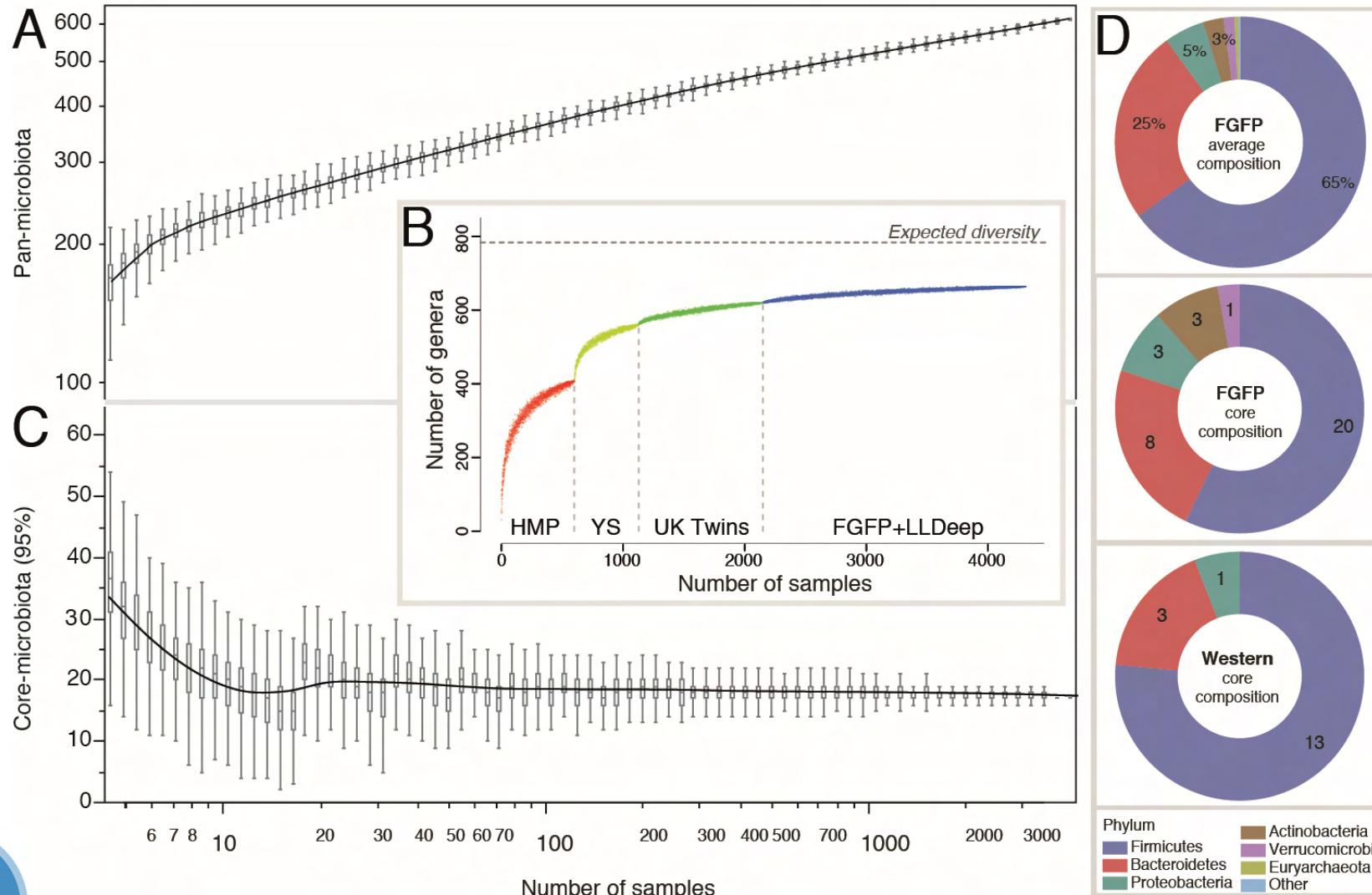
Falony et al., Science 2016



- Home sampling protocol
- Collection of frozen material
- Cold chain management

# Did we catch them all?

Combining worldwide sampling efforts - 3,948 samples, 664 genera...



Current estimate: pan-microbiota of the Western population counts  $\pm 850$  genera

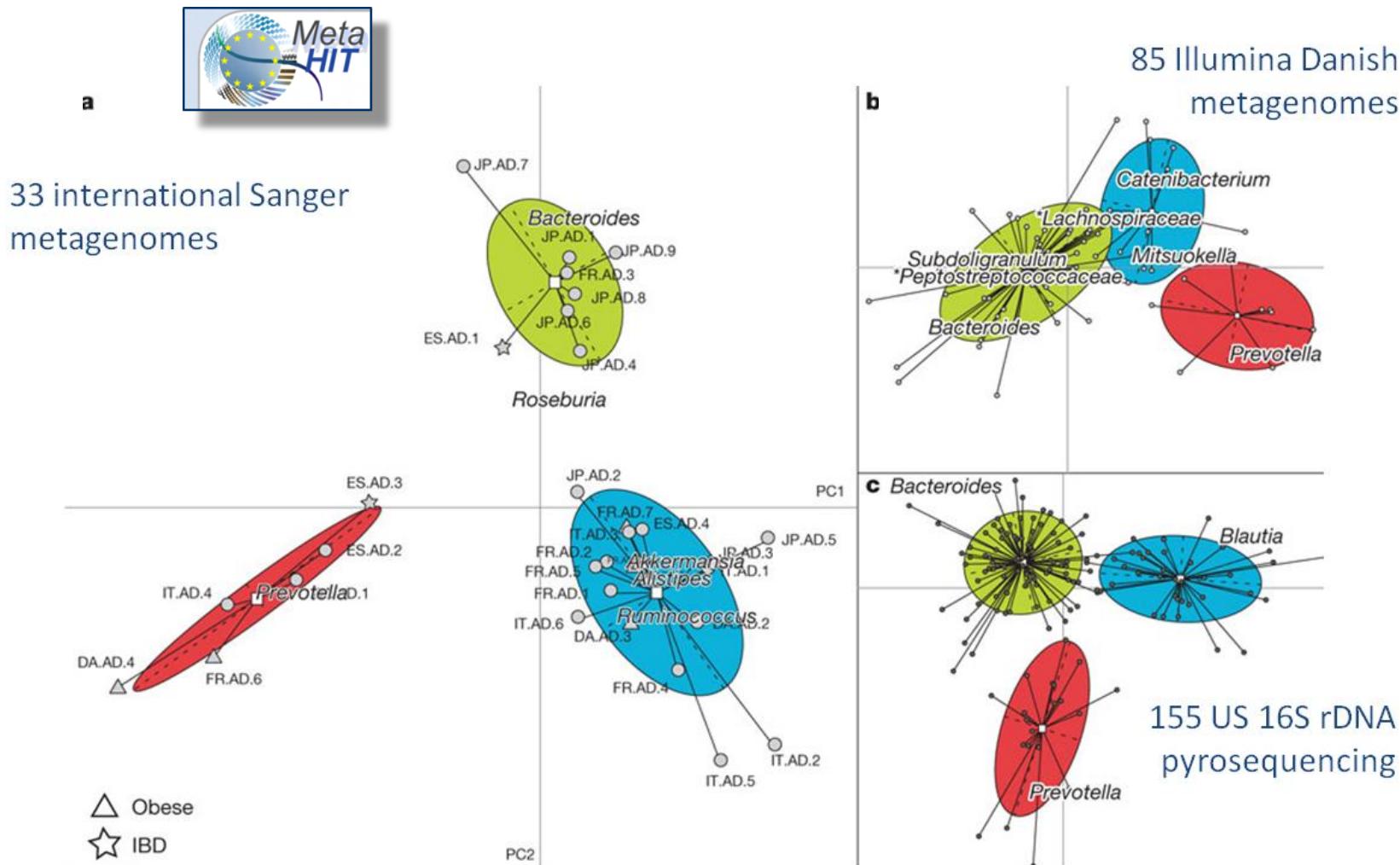
... and counting (est. 40,000 samples required to cover full diversity)



# **Gut microbiota variation in health**

## **Community-typing**

# Introducing enterotypes



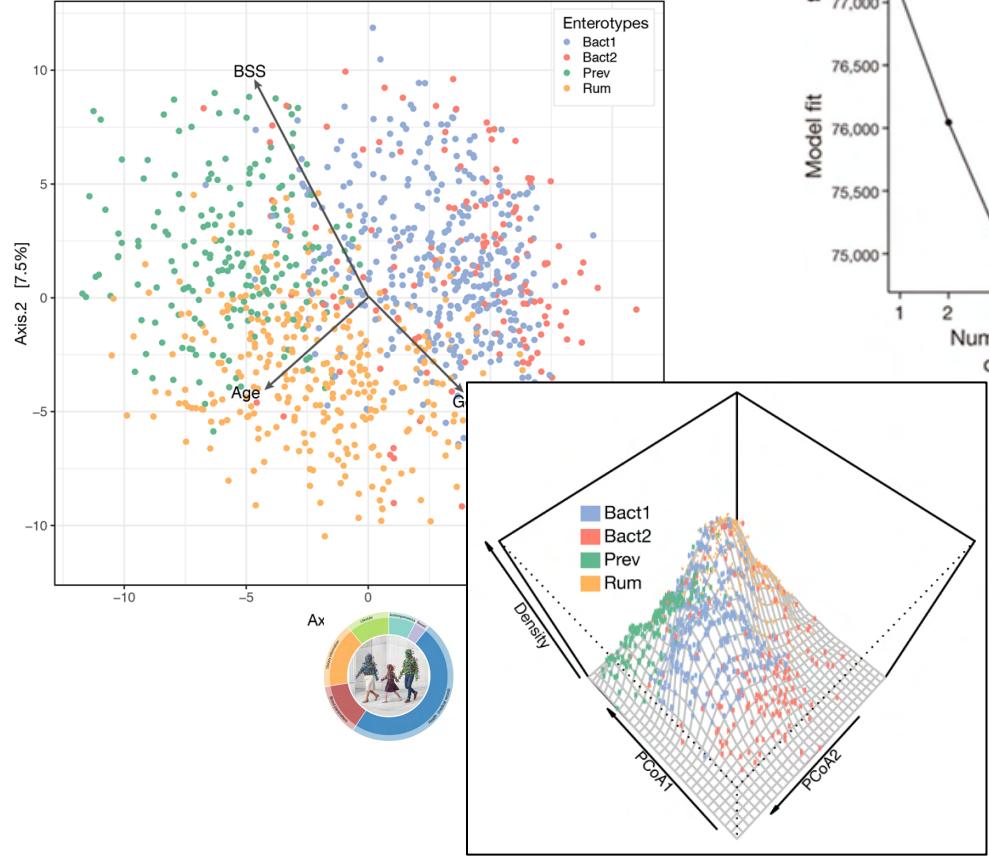
## Clustering on principal coordinates analysis

- Poor stability
- Poor reproducibility
- No apparent link with metadata

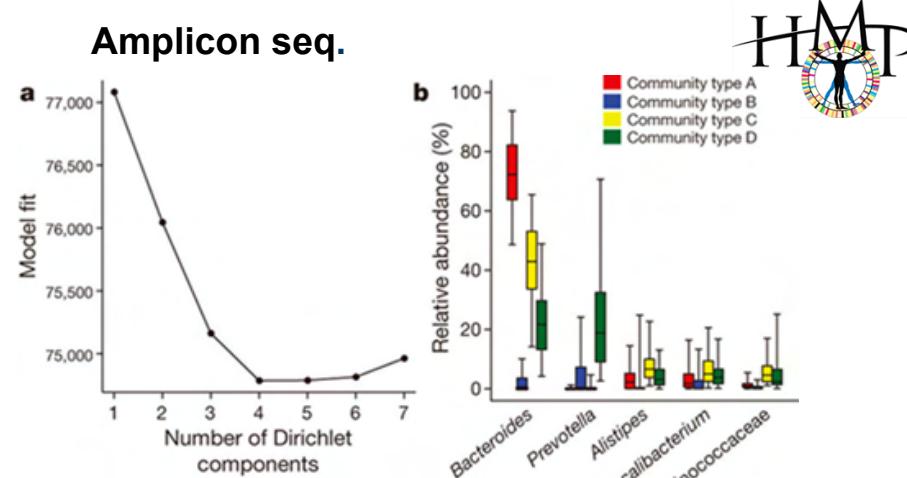
# Compositional approaches consistently identify four enterotypes in Western populations

Stable, reproducible, and associated to host metadata

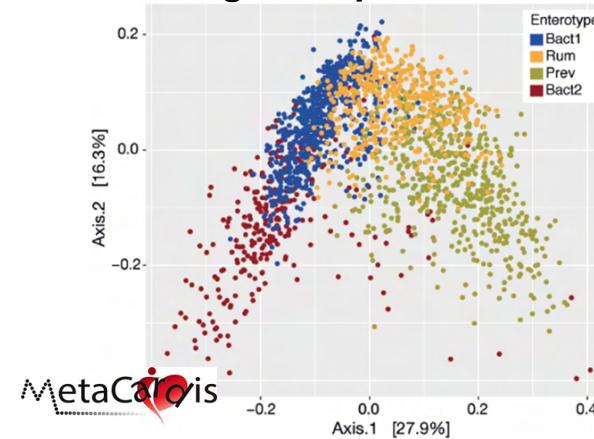
Amplicon seq.



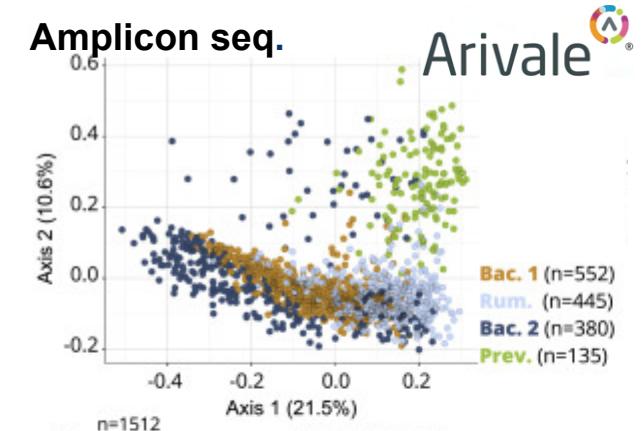
Amplicon seq.



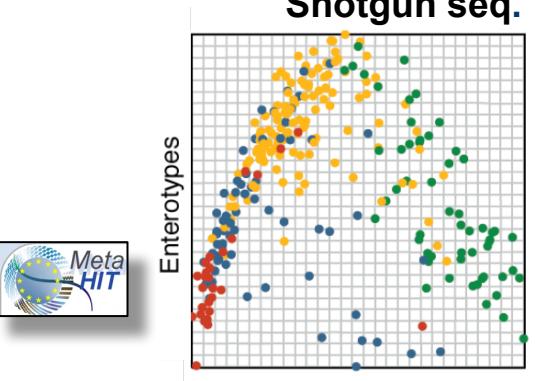
Shotgun seq.



Amplicon seq.



Shotgun seq.



Wilmanski et al., Med 2022

Ding et al., Nature 2014

Falony et al., Science 2016

Vieira-Silva et al., Nature 2020

Brial et al., Gut 2021

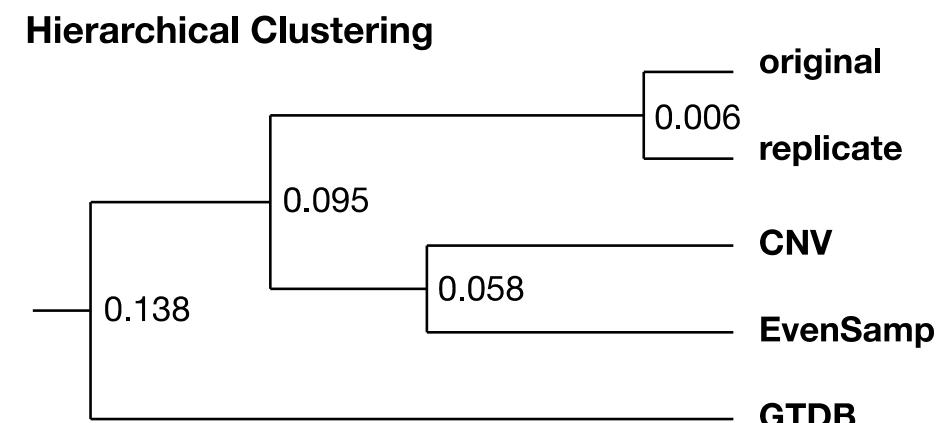
Community typing using Dirichlet-multinomial mixtures

Holmes et al., PLoS One 2012

# Testing enterotyping stability

- Original [RDP\_10K\_noCNV]
- Replicate [Rep\_RDP\_10K\_noCNV]
- CNV [RDP\_10K\_CNV]
- GTDB [GTDB\_10K\_CNV]
- EvenSampDepth [RDP\_EvenSamplingDepth\_CNV]

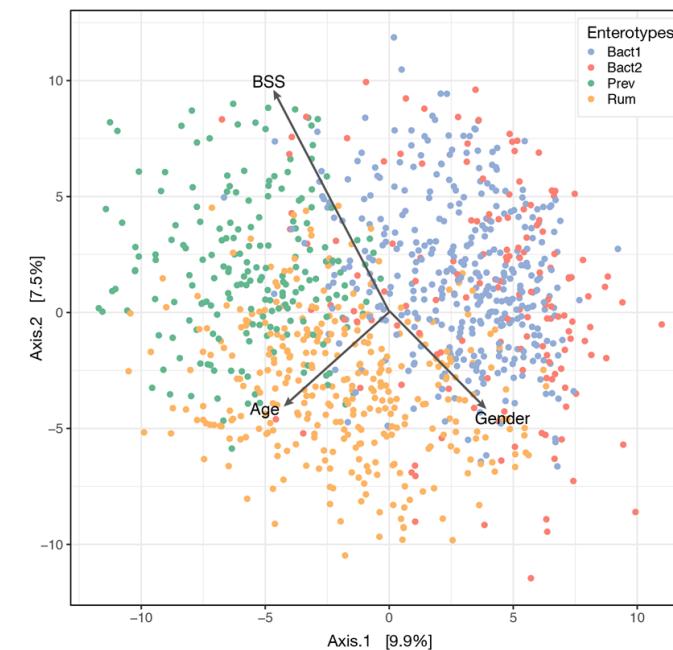
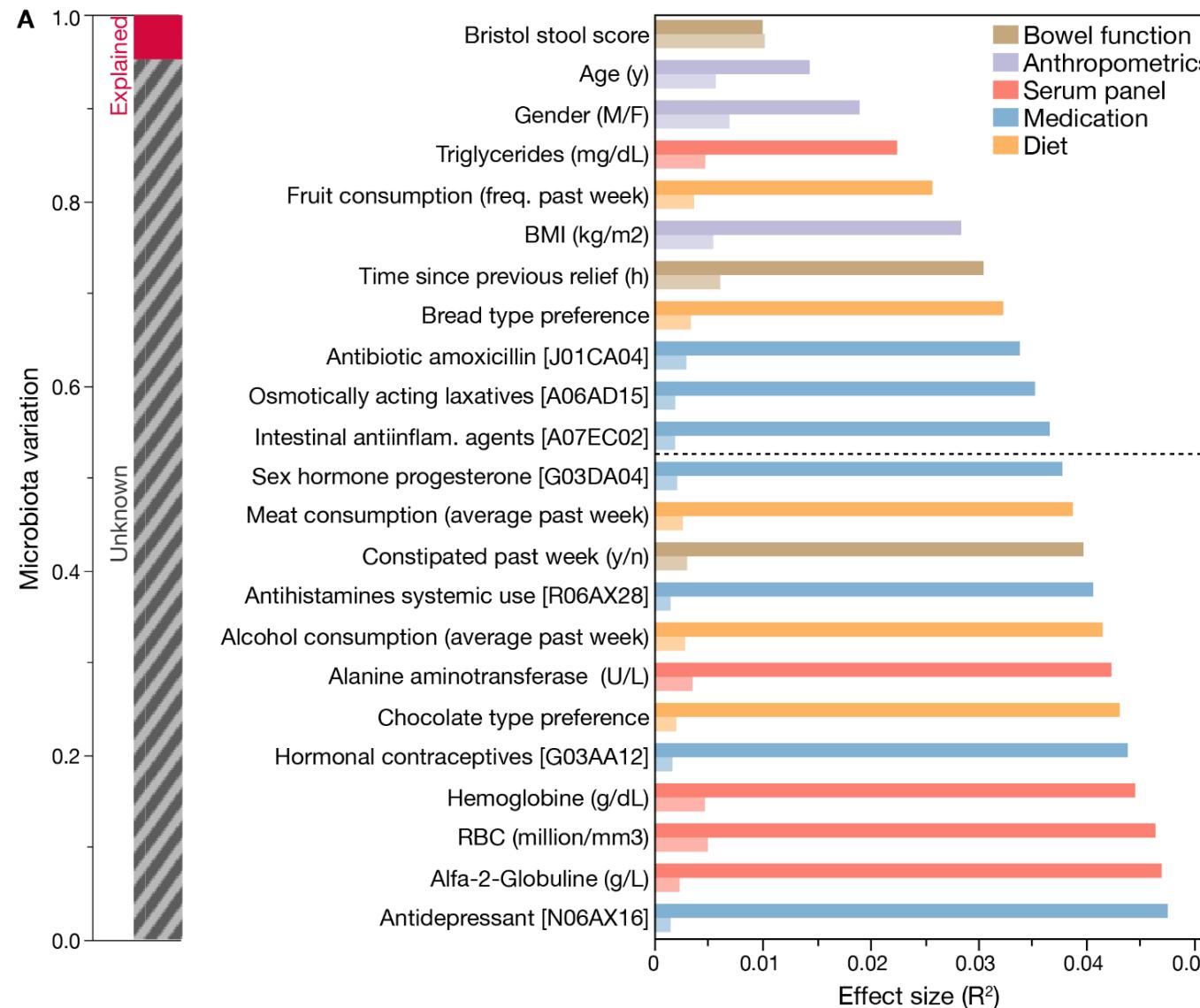
Comparison	Matches N	Match %
original_vs_replicate	2901	99.40%
original_CNV	2739	93.80%
EvenSeqDepth_EvenSampDepth	2750	94.20%
RDP_vs_GTDB	2592	88.80%



# **Gut microbiota variation in health**

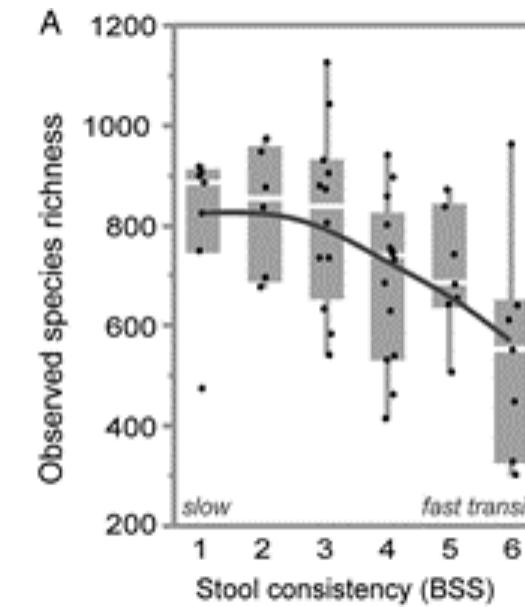
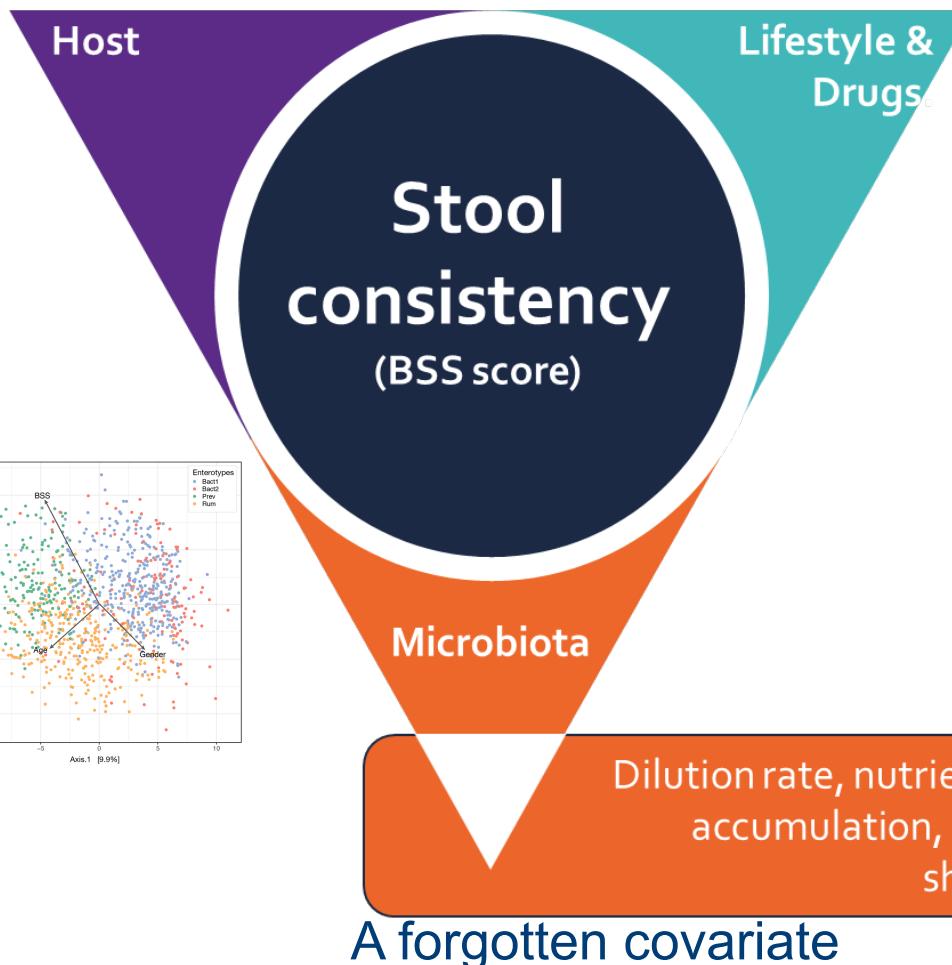
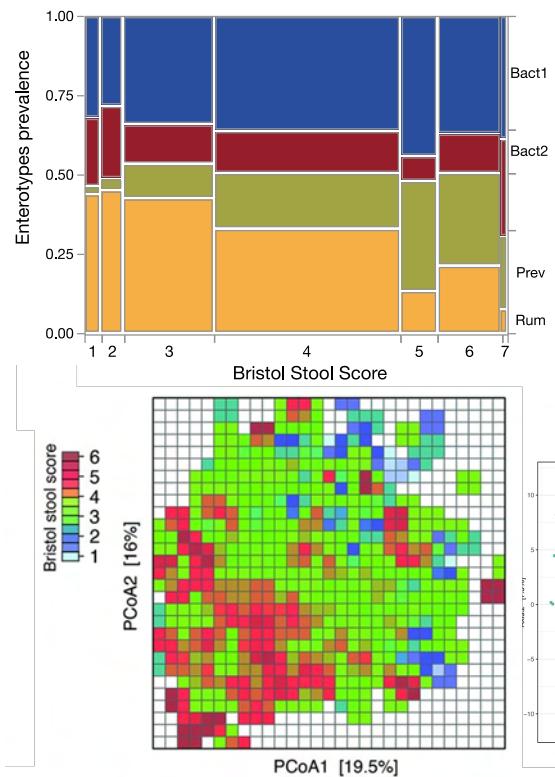
## **Partitioning healthy microbiome variation**

# A large fraction of microbiome variation remains unexplained



- **1106 samples**
- **503 metadata variables**
- **69 microbiome covariates**
- **11 non-redundant covariates**

# Stool consistency is associated with microbiota richness and enterotypes



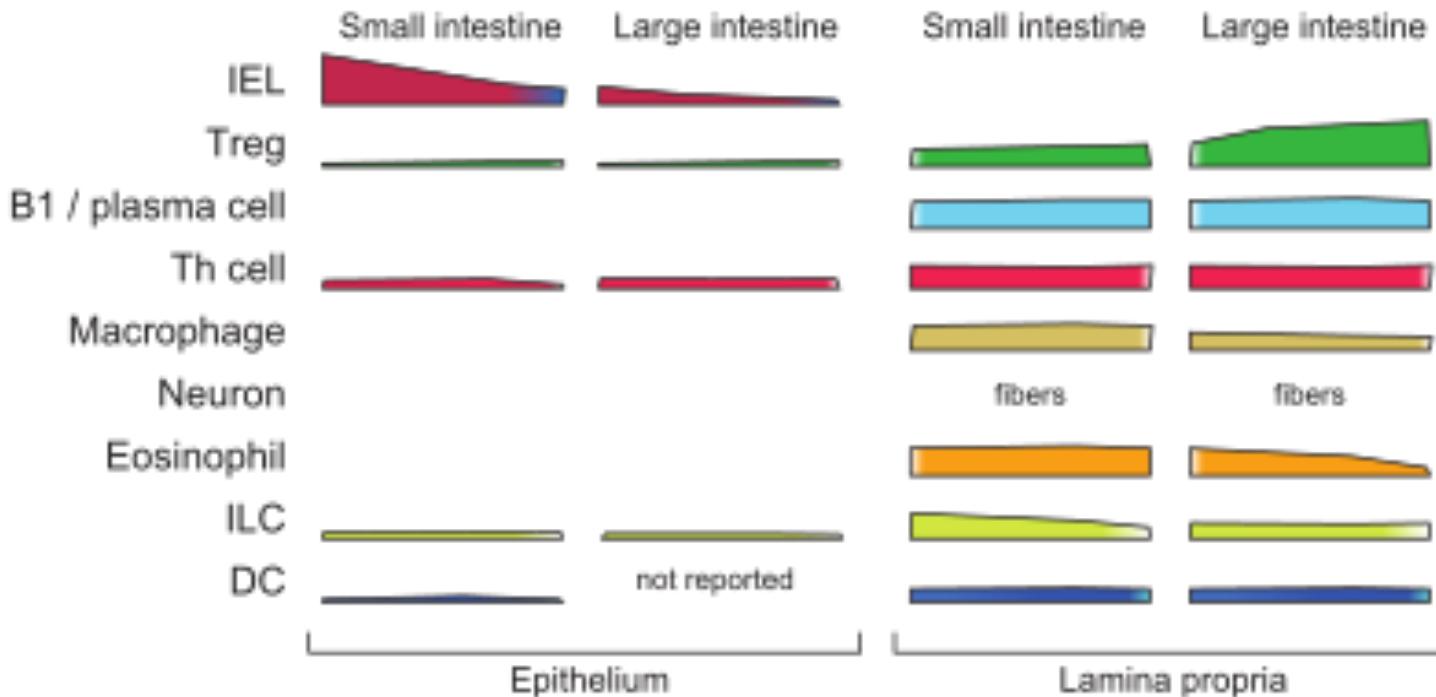
Vandeputte *et al.*, Gut 2015  
Falony *et al.*, Science 2016  
Falony *et al.*, ABC 2019

R enterotype – high richness – hard stools

# **Gut microbiota variation in health**

## **Functional annotation of bioactive metabolism**

# Microbiota-mediated immunodampening



<https://doi.org/10.1038/nature11551>

## 1. Barrier integrity and pO<sub>2</sub>

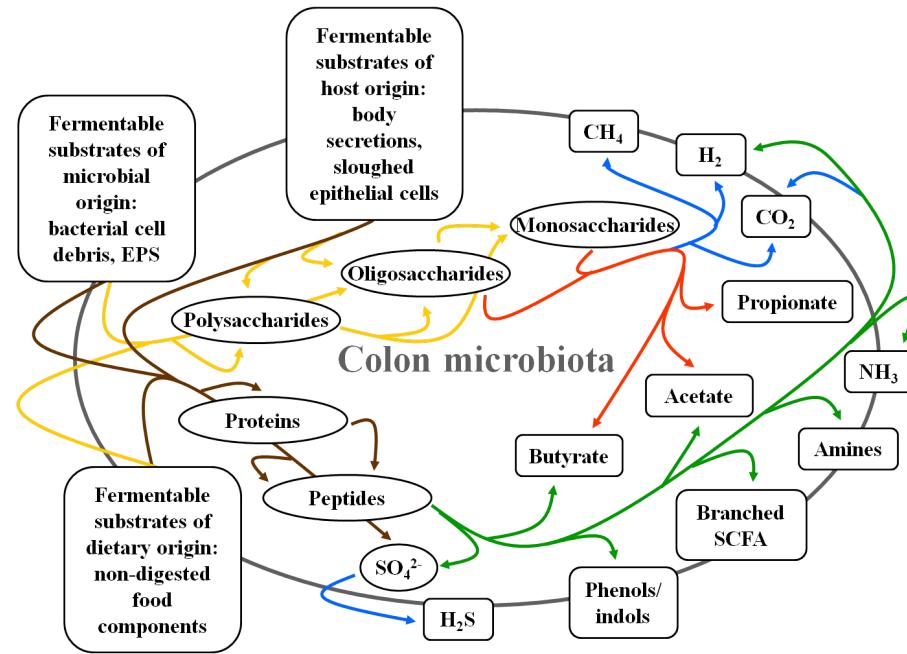
- Indigestible fibres fermentation end-products (SCFA): primary source of energy for EC

## 2. Treg expansion

- Antigen-specific: capsular PSA by *B. thetaiotaomicron*
- Non-specific: short chain fatty acids (SCFA)

# Metabolic modules translate metaG data into functional traits

## Gut-specific metabolic annotation framework (GMM)



Gwen Falony

Vieira-Silva *et al*, Nature Microbiology 2016  
Vieira-Silva *et al*, Nature Nature 2020

## Gut-brain axis modules (GBM)

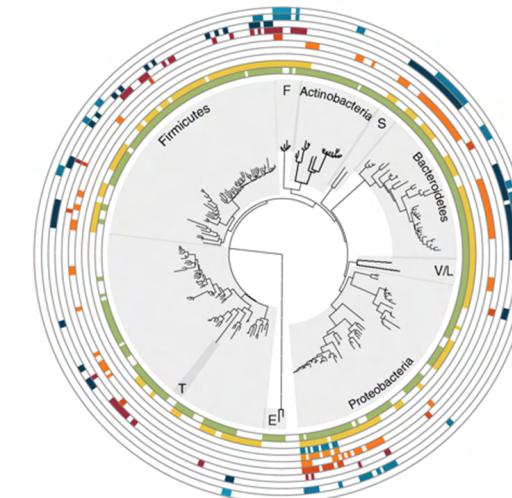
### The neuroactive potential of the human gut microbiota in quality of life and depression

Mireia Valles-Colomer, Gwen Falony, Youssef Darzi, Ettje F. Tigchelaar, Jun Wang, Raul Y. Tito, Carmen Schiweck, Alexander Kuriashikov, Marie Joossens, Cisca Wijmenga, Stephan Claes, Lukas Van Oudenhove, Alexandra Zhernakova, Sara Vieira-Silva & Jeroen Raes

*Nature Microbiology* 4, 623–632 (2019) | Cite this article

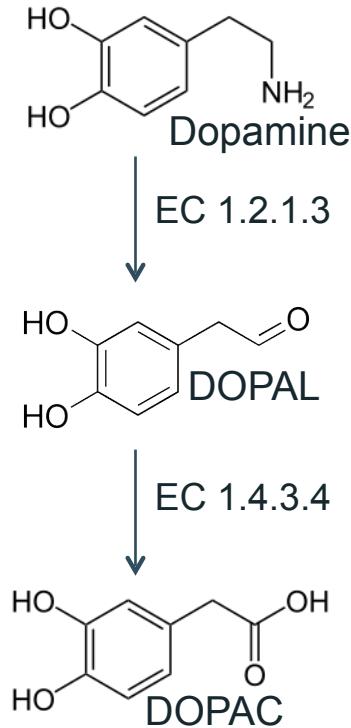
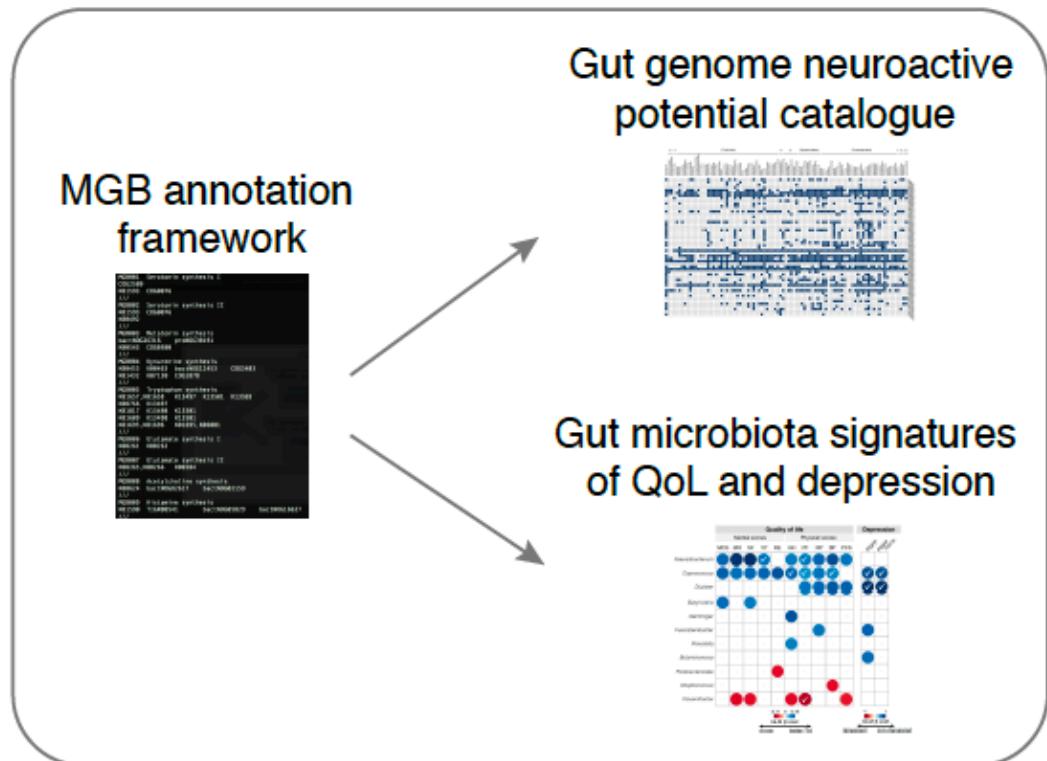
53k Accesses | 364 Citations | 1811 Altmetric | Metrics

Valles-Colomer *et al*, *Nature Microbiology* 2019



# Metabolic modules (GMMs)

**Module** = set of biochemical steps that follow the synthesis/ degradation of **bioactive compounds** by the gut microbiota (KEGG syntax)



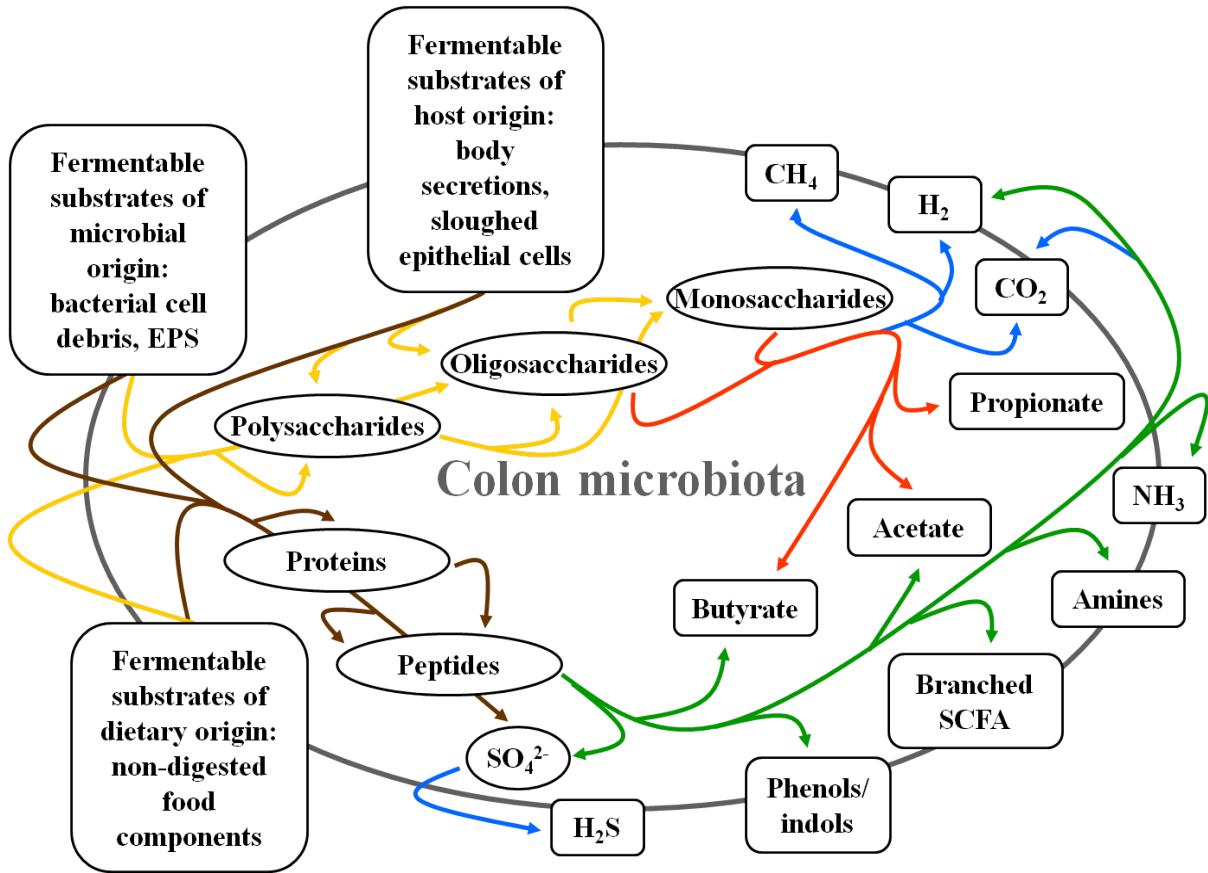
MGB024 DOPAC synthesis  
cpd [Dopamine] [DOPAC]  
K00274 K00276 K13371, K13372  
K00128 K00129 COG1012

<http://www.raeslab.org/software/gbms.html>

# Functional characterization of fecal microbiota

## Gut-specific annotation framework

Manually compiled set of pathway modules that captures microbial ‘food chain’



- Based on public databases, literature and genomic data
- Constructed according to Kegg module syntax
- Including relevant Kegg, Metacyc, and SEED pathways
- Covering several anaerobic fermentation processes
- Representing pathways as they occur in the gut ecosystem context – not generic
- Focusing on information relevant to microbiota studies – less noise

■ polysaccharide degradation ■ carbohydrate metabolism

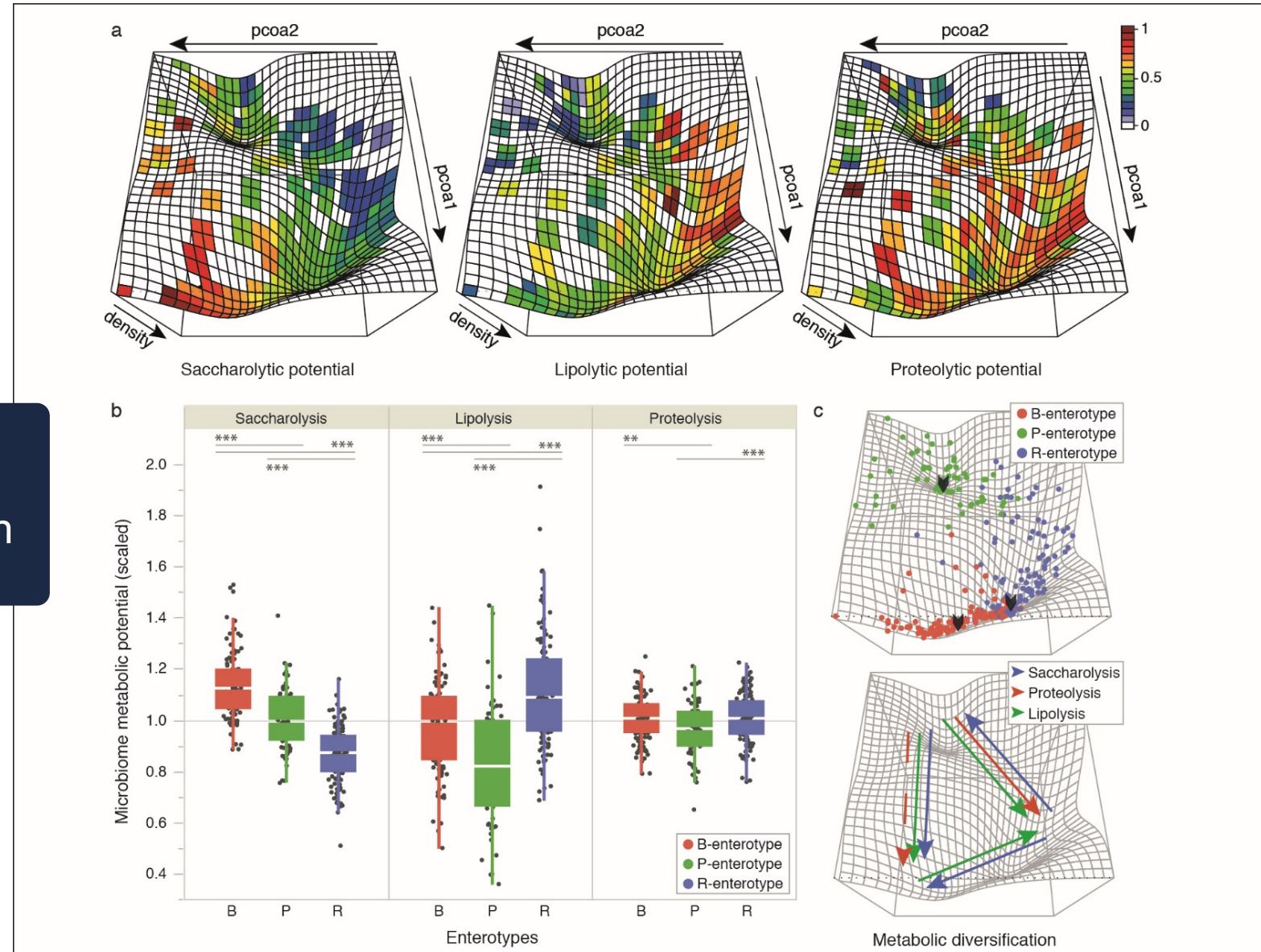
■ protein degradation ■ amino acid metabolism ■ gas production

# Enterotype stratification is linked to microbial substrate preference

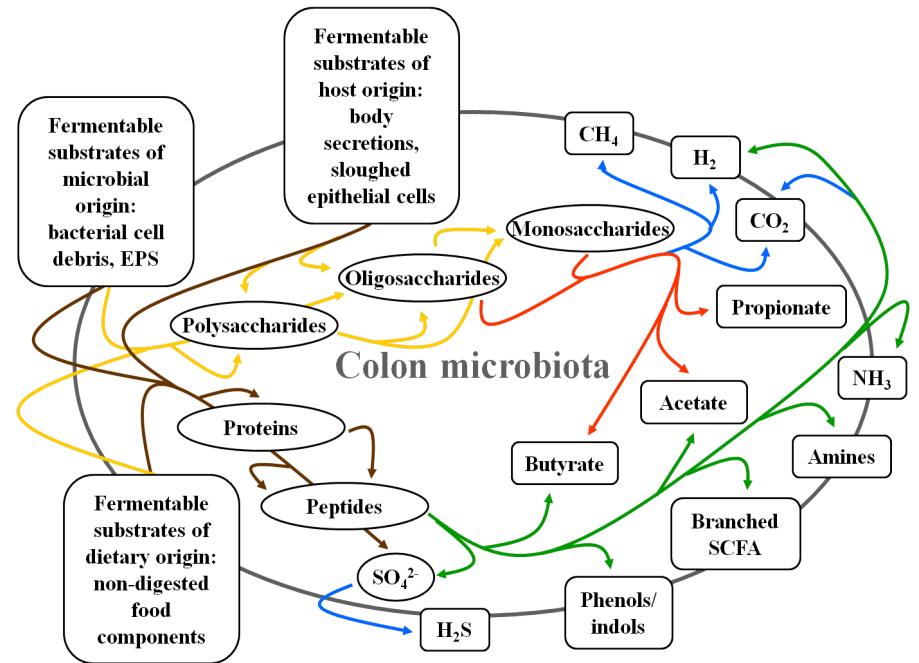
R enterotype – high richness –  
hard stools - proteolytic fermentation



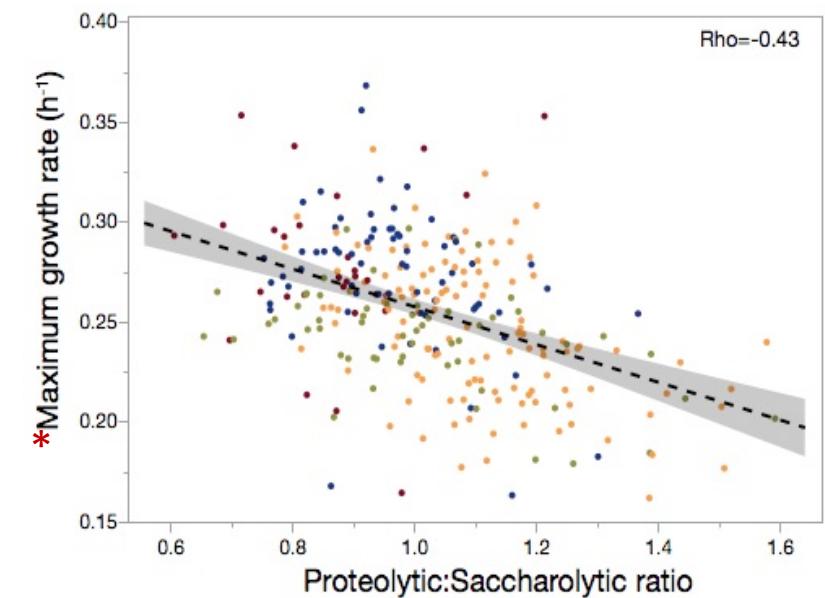
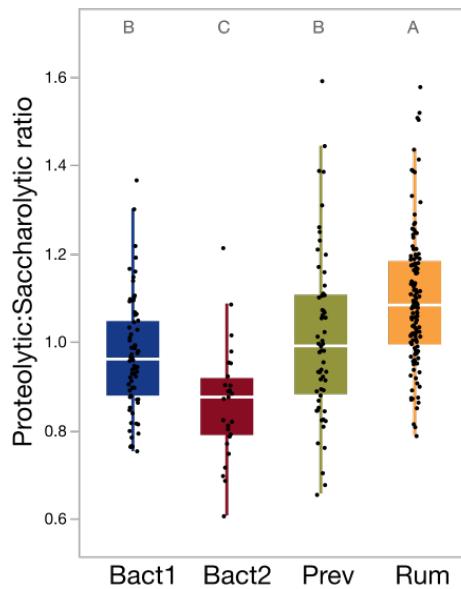
Vieira-Silva *et al*, Nature Microbiology 2016



# Transit time shapes community metabolic strategy



Gut-specific metabolic annotation framework (GMM)

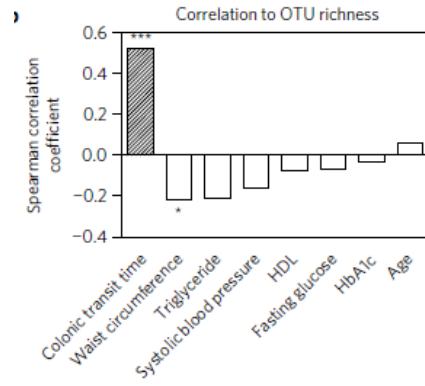
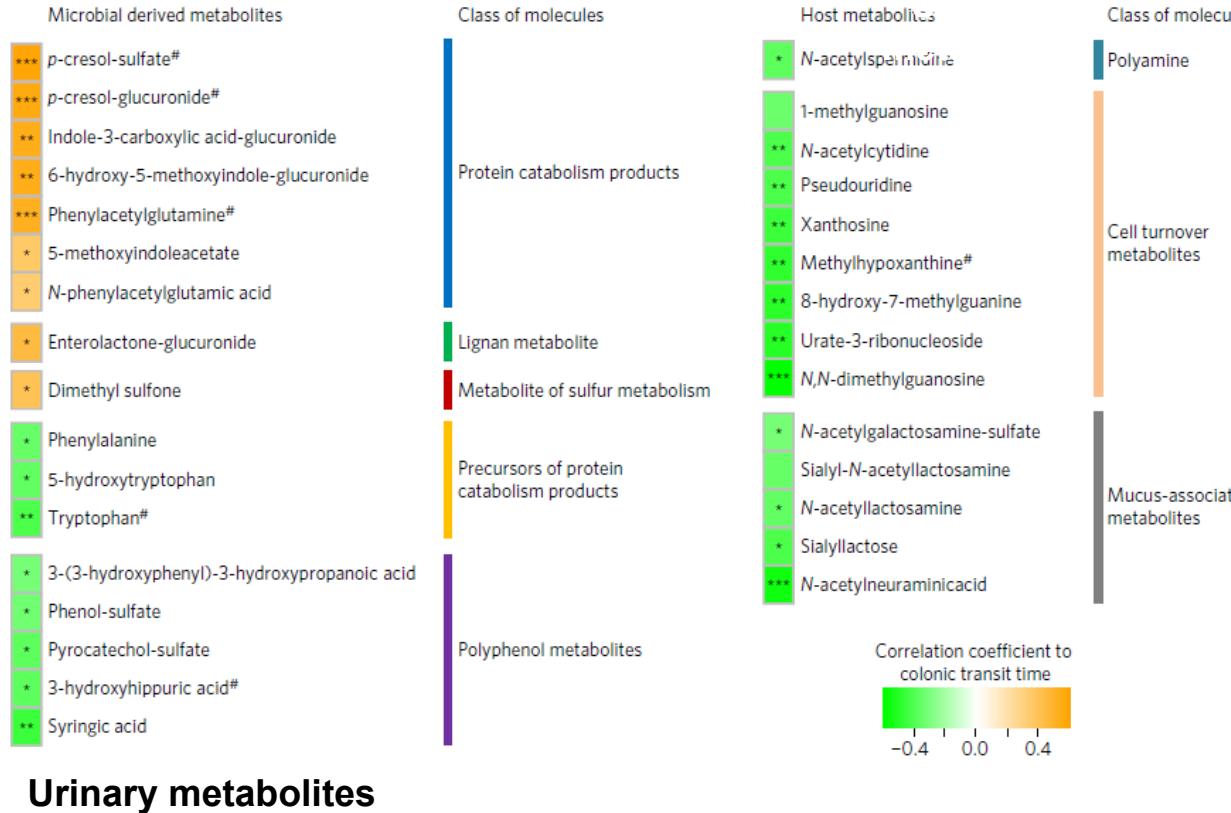


\* predicted from genomic imprint (codon usage bias)

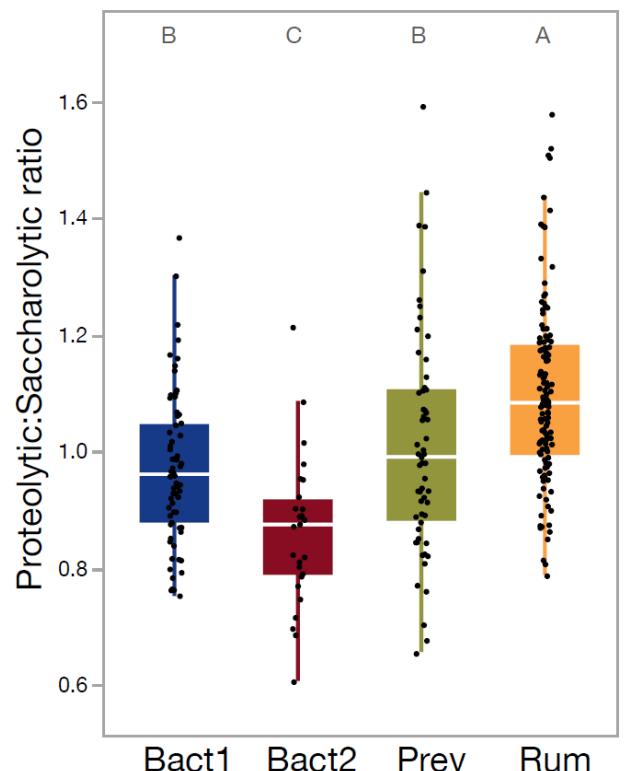
R enterotype – high richness – hard stools – slow bacterial growth – proteolytic fermentation

Vieira-Silva & Rocha, PLoS Genetics 2010  
Vieira-Silva *et al.*, Nature Microbiology 2016

# Slow transit, increased proteolytic fermentation



## Metabolic potential

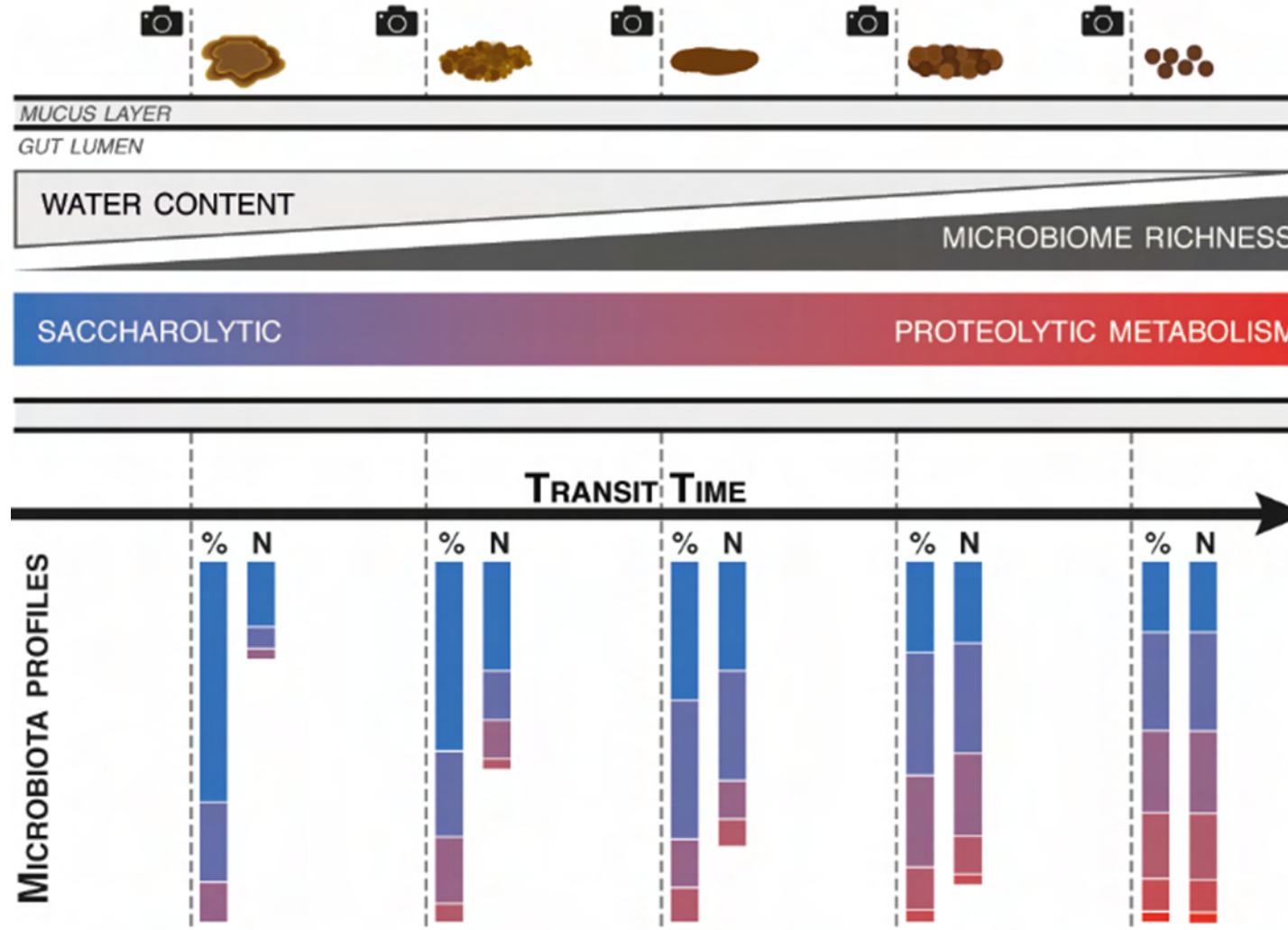


R enterotype – high richness – hard stools – slow growth – proteolytic fermentation – slow transit

# **Gut microbiota variation in health**

## **Sketching up a model of microbiota variation within health**

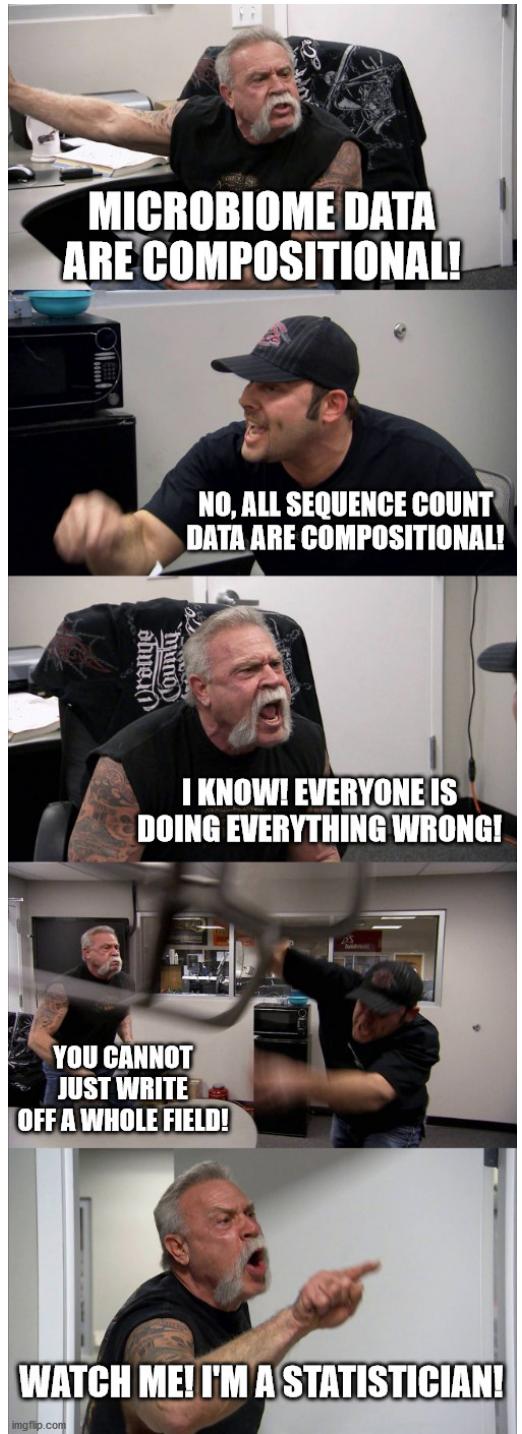
# Interpretation of microbiome results requires understanding of microbiota ecology



Richness is as a marker of ecosystem development

# **Gut microbiota variation in health and disease**

## **Quantitative microbiota profiling**



# Microbiome data are compositional

## Microbiome Datasets Are Compositional: And This Is Not Optional

Gregory B. Gloor<sup>1\*</sup>, Jean M. Macklaim<sup>1</sup>, Vera Pawlowsky-Glahn<sup>2</sup> and Juan J. Egozcue<sup>3</sup>

<sup>1</sup> Department of Biochemistry, University of Western Ontario, London, ON, Canada, <sup>2</sup> Departments of Computer Science, Applied Mathematics, and Statistics, Universitat de Girona, Girona, Spain, <sup>3</sup> Department of Applied Mathematics, Universitat Politècnica de Catalunya, Barcelona, Spain

- Loss information on extent and directionality of microbiome variation
- Introduction of a negative correlation bias between microbiome features Vandeputte *et al.*, Nature 2017  
Llorens-Rico *et al.*, Nature Communications 2021



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MAINZ



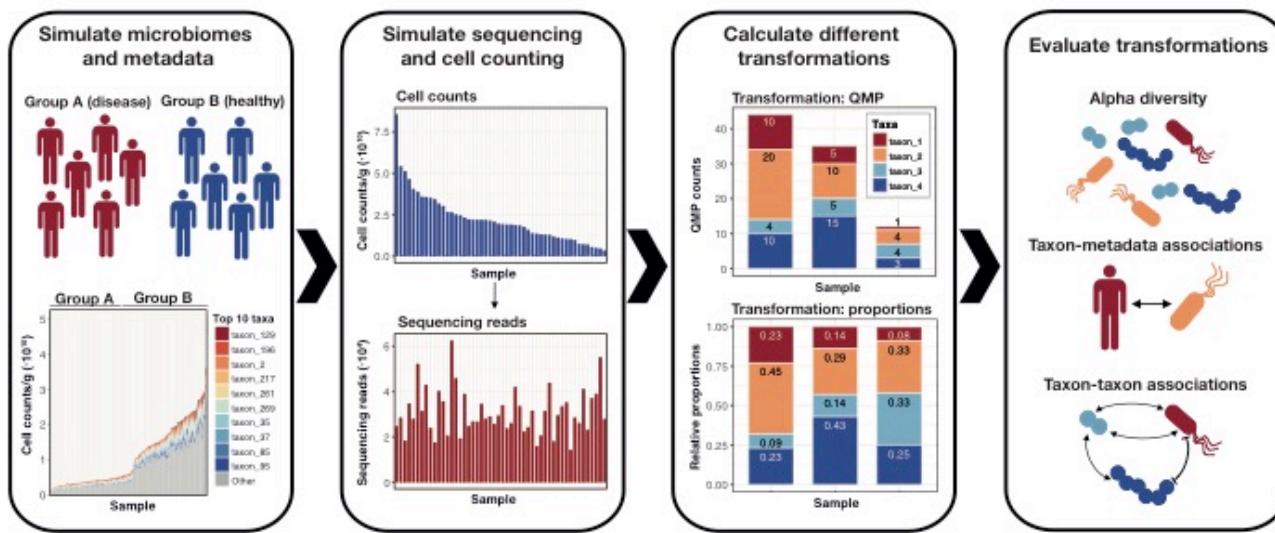
@biomickwatson

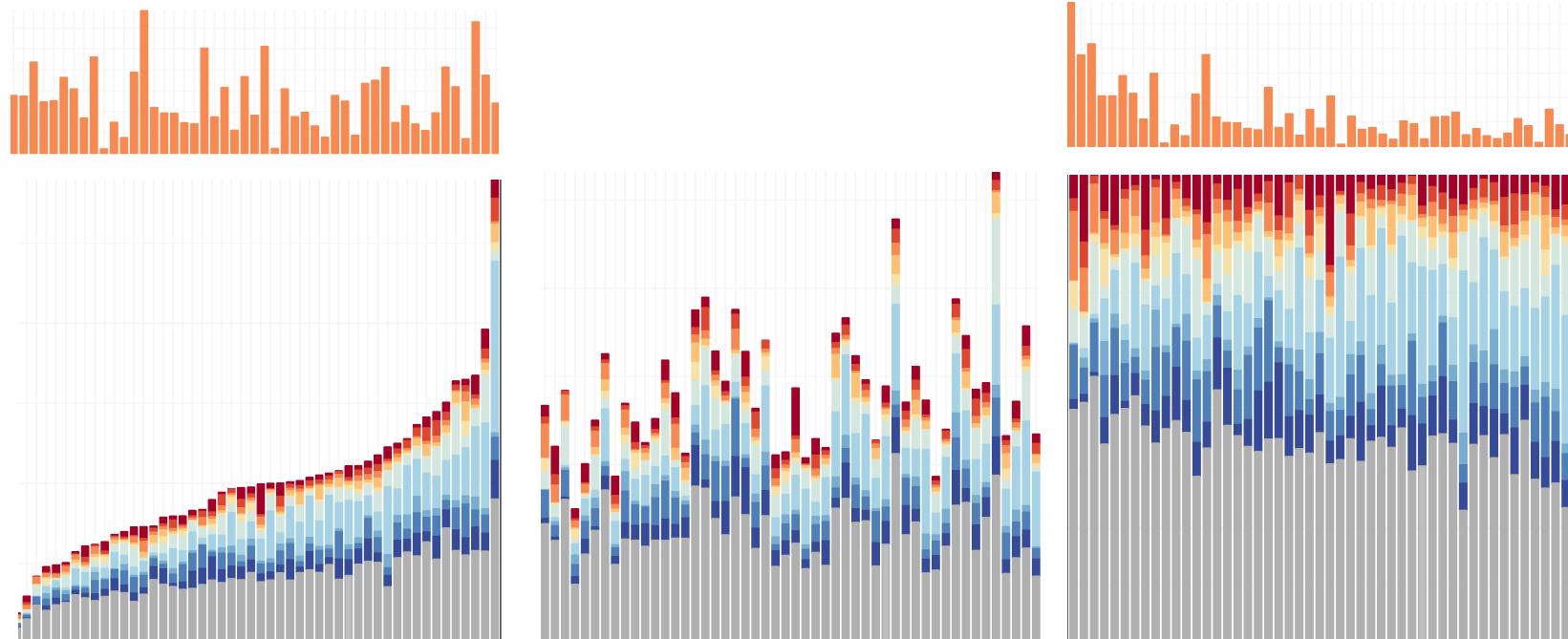
# Don't have access to QMP, what is the best alternative?

## Article

Benchmarking microbiome transformations favors experimental quantitative approaches to address compositionality and sampling depth biases

Lloréns-Rico, Vieira-Silva *et al*, Nature Communications, 2021





Community  
(cells)

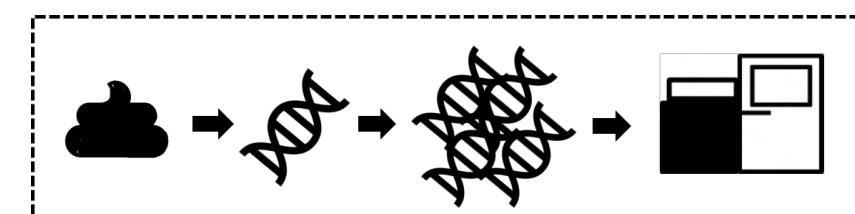
Sequences  
(reads)

Relative  
Microbiome  
Profiles  
(proportions)

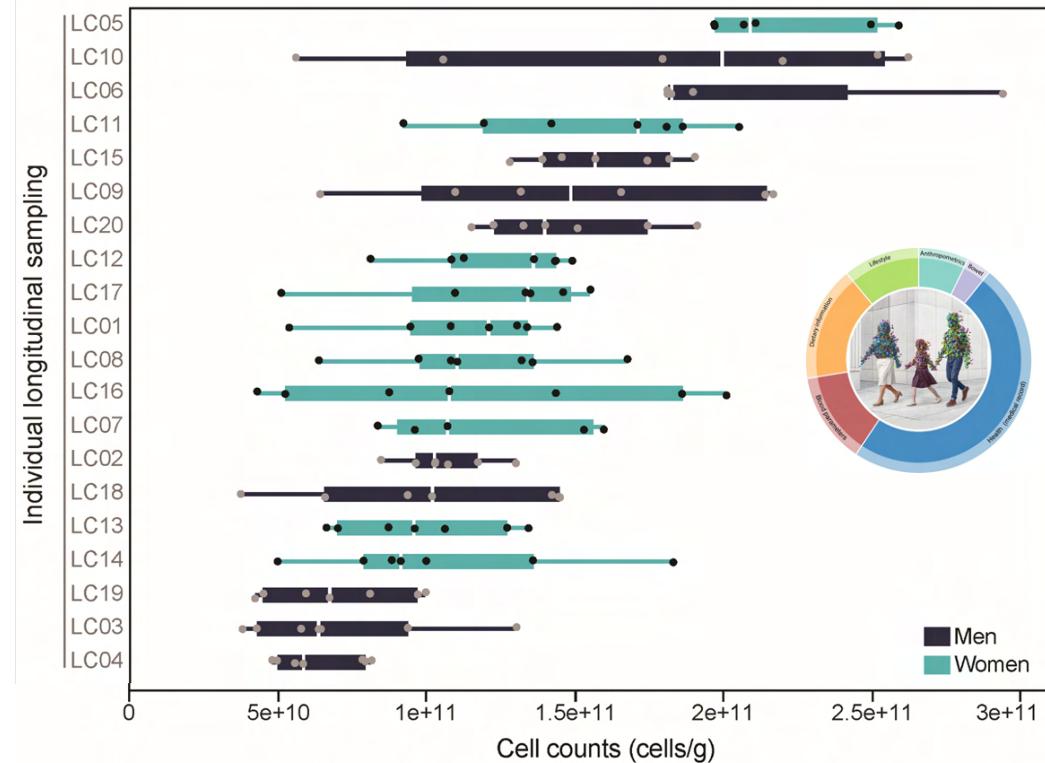
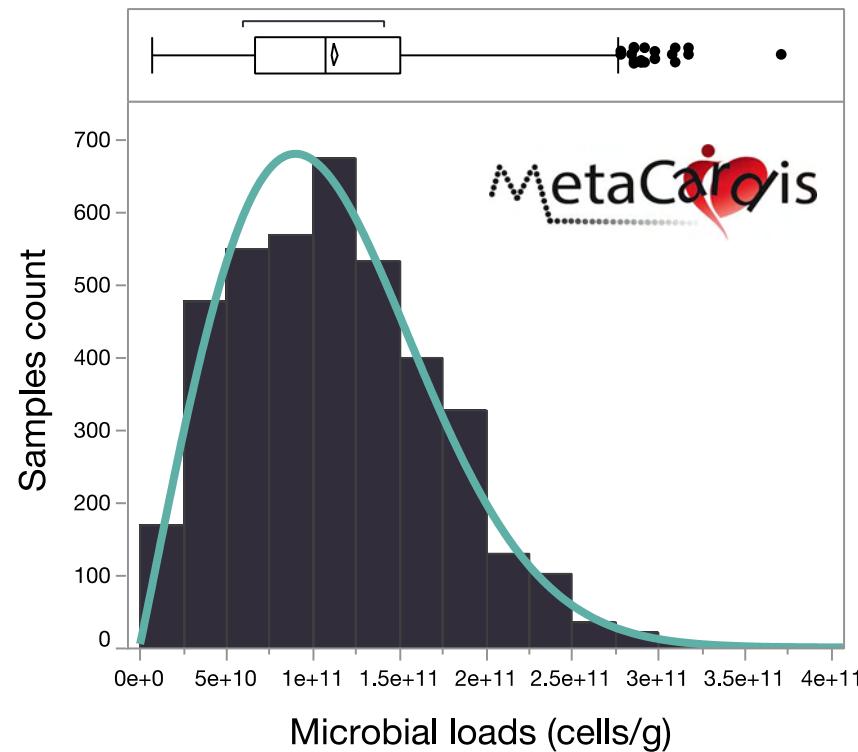
Vandeputte *et al.*, Nature 2017

Llorens-Rico *et al.*, Nature Communications 2021

Standard sequencing workflows do not conserve a link between the cell density of the sampled community and the amount of sequencing data generated.



# Substantial variation in microbial loads in feces



- 10-fold variation in 40 healthy individuals (Vandeputte et al., Nature 2017)
- 50-fold variation in clinical cohorts (Vieira-Silva et al., Nature 2020)
- One-week sampling reveals similar variation over time (Vandeputte et al., Nature 2017)

# Quantitative microbiome profiling

Parallelization of metagenomics and microbial load measurement

nature

Published: 01 November 2017

## Quantitative microbiome profiling links gut community variation to microbial load

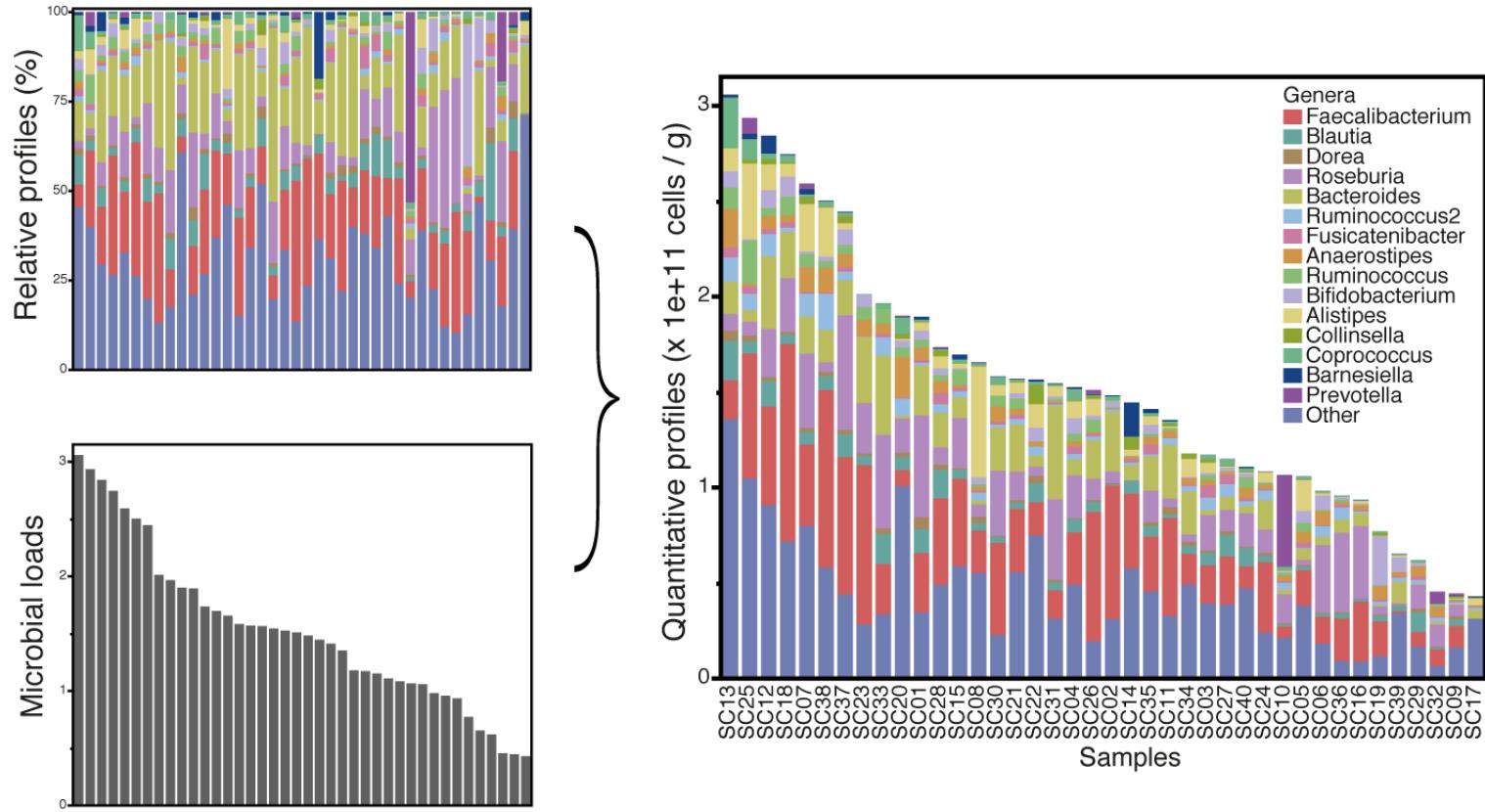
Doris Vandeputte, Gunter Kathagen, Kevin D'hoe, Sara Vieira-Silva, Mireia Valles-Colomer, João Sabino, Jun Wang, Raul Y. Tito, Lindsey De Commer, Youssef Darzi, Séverine Vermeire, Gwen Falony & Jeroen Raes

Nature 551, 507–511(2017) | Cite this article

13k Accesses | 275 Citations | 407 Altmetric | Metrics

### Abstract

Current sequencing-based analyses of faecal microbiota quantify microbial taxa and metabolic pathways as fractions of the sample sequence library generated by each analysis<sup>1,2</sup>. Although these relative approaches permit detection of disease-associated microbiome variation, they are limited in their ability to reveal the interplay between microbiota and host health<sup>3,4</sup>. Comparative analyses of relative microbiome data cannot provide information about the extent or directionality of changes in taxa abundance or

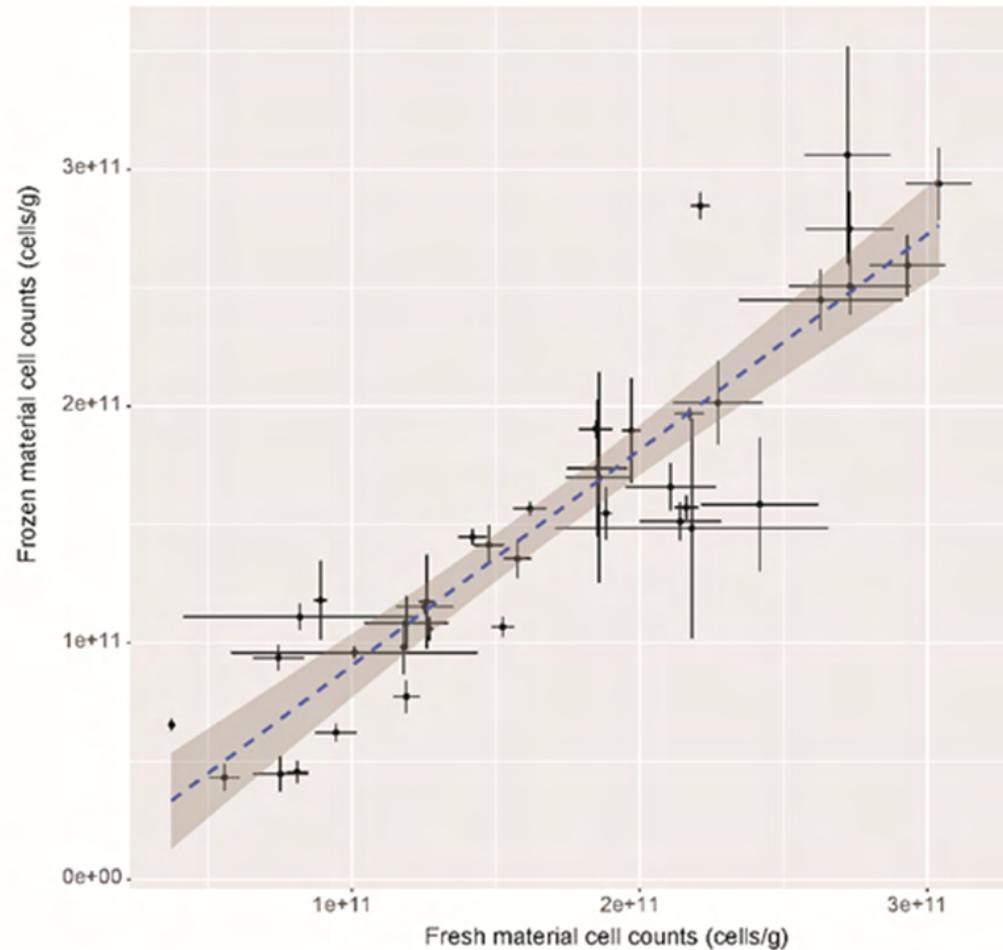


Vandeputte et al., Nature 2017  
Vieira-Silva et al., Nature 2020

# Cytometry-based quantification of microbial load

# micro-organisms/g fecal material

Vandeputte et al., Nature 2017

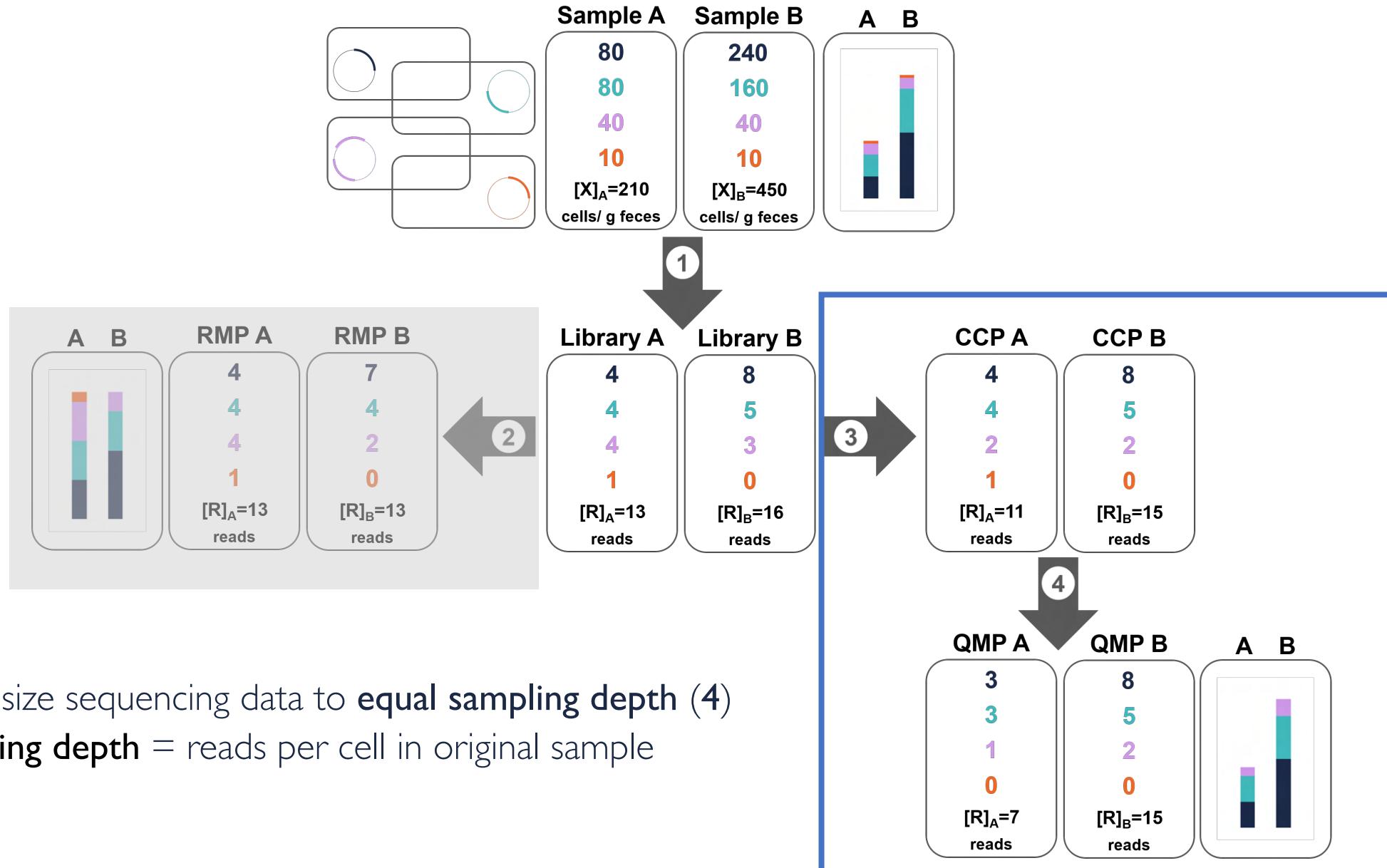


Fresh and frozen counts are correlated

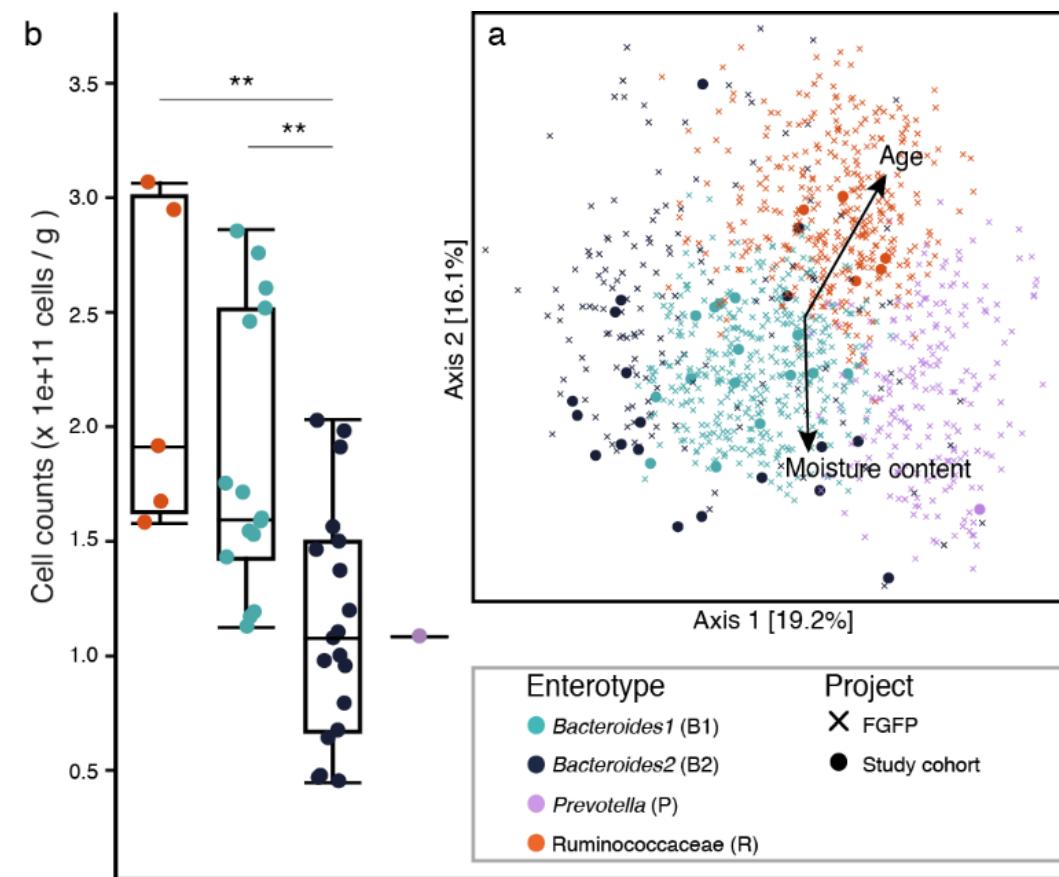


Alternative strategies: use of internal standards (bacterial cells), quantification of DNA post extraction.

# Quantitative microbiome profiling - computation

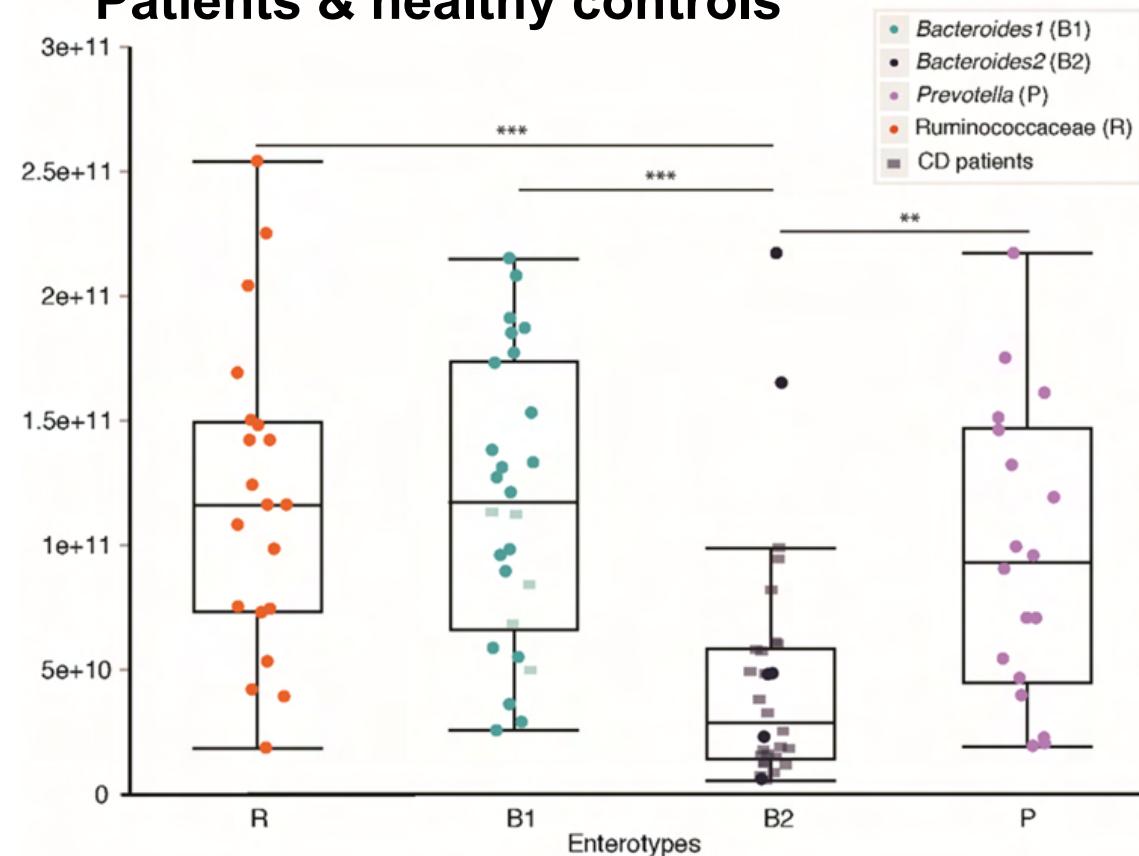


# Cell counts contribute to enterotype differentiation



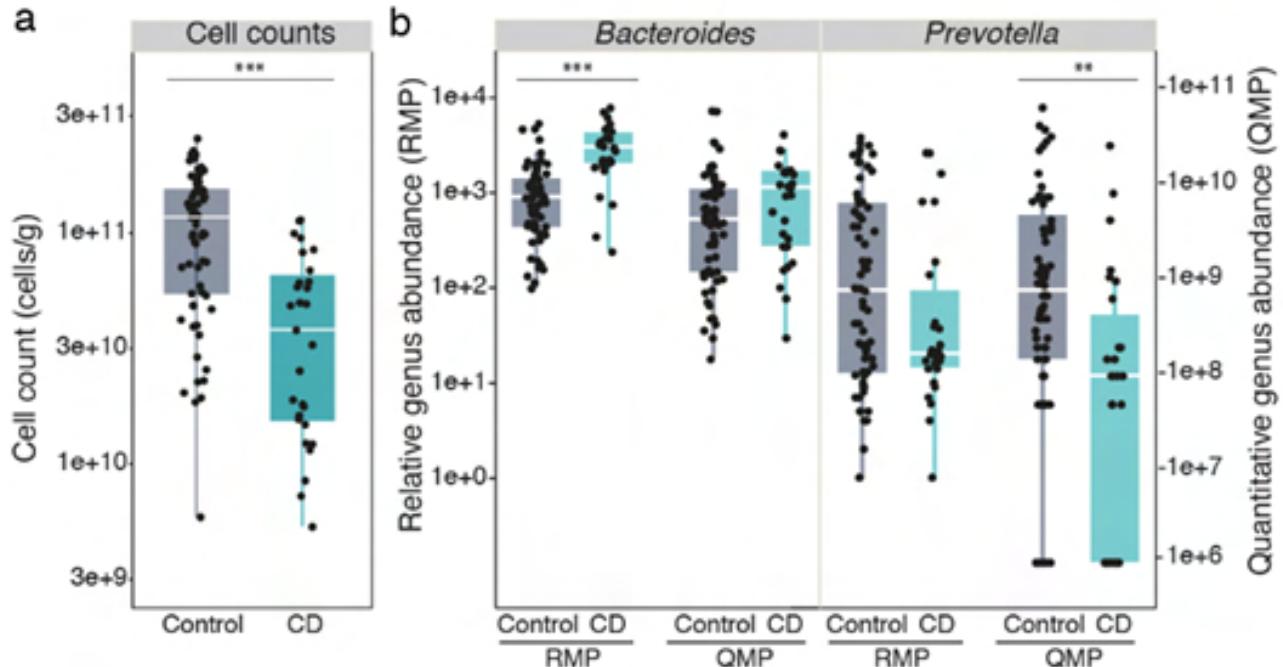
Healthy individuals

## Patients & healthy controls

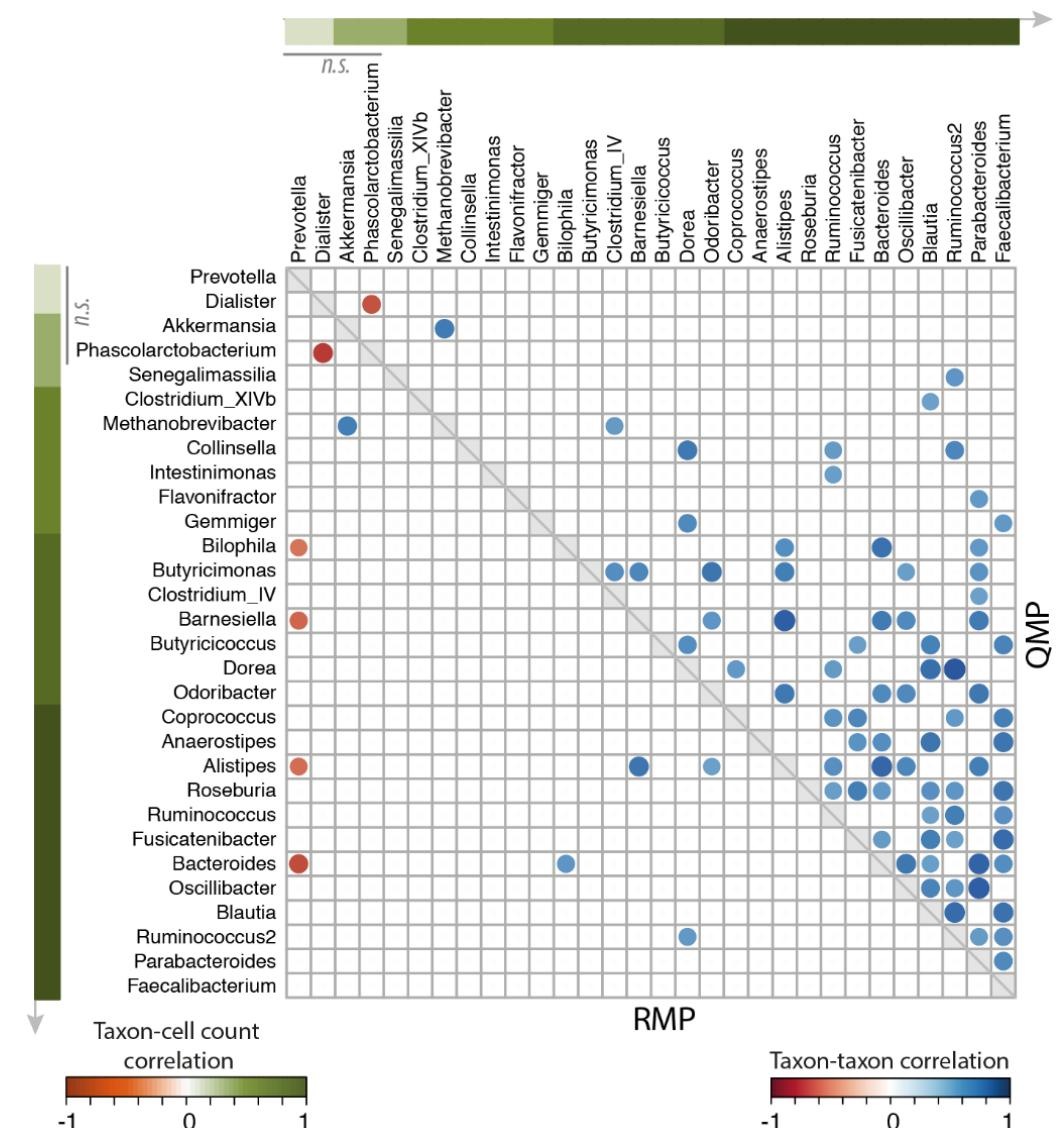


R enterotype – high richness – hard stools – slow growth – proteolytic fermentation – slow transit – high microbial load

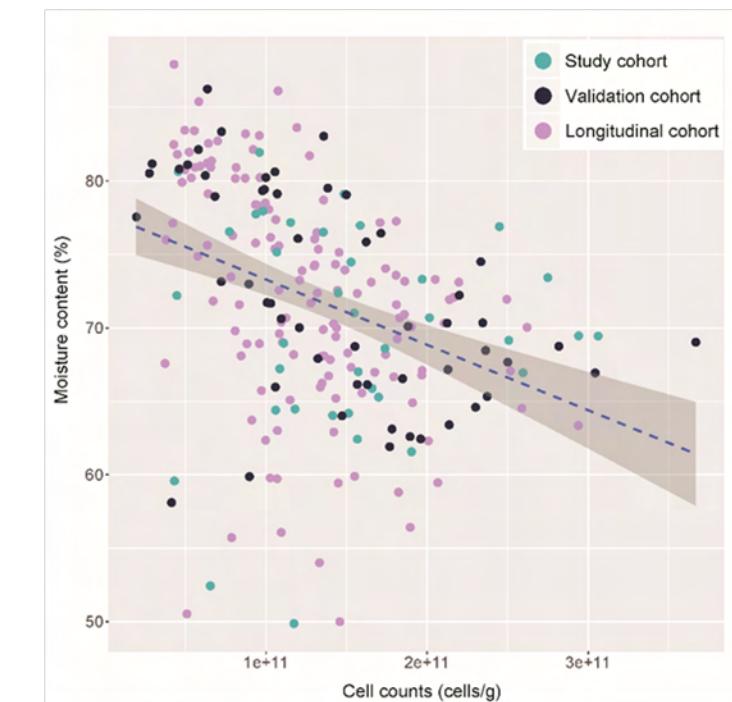
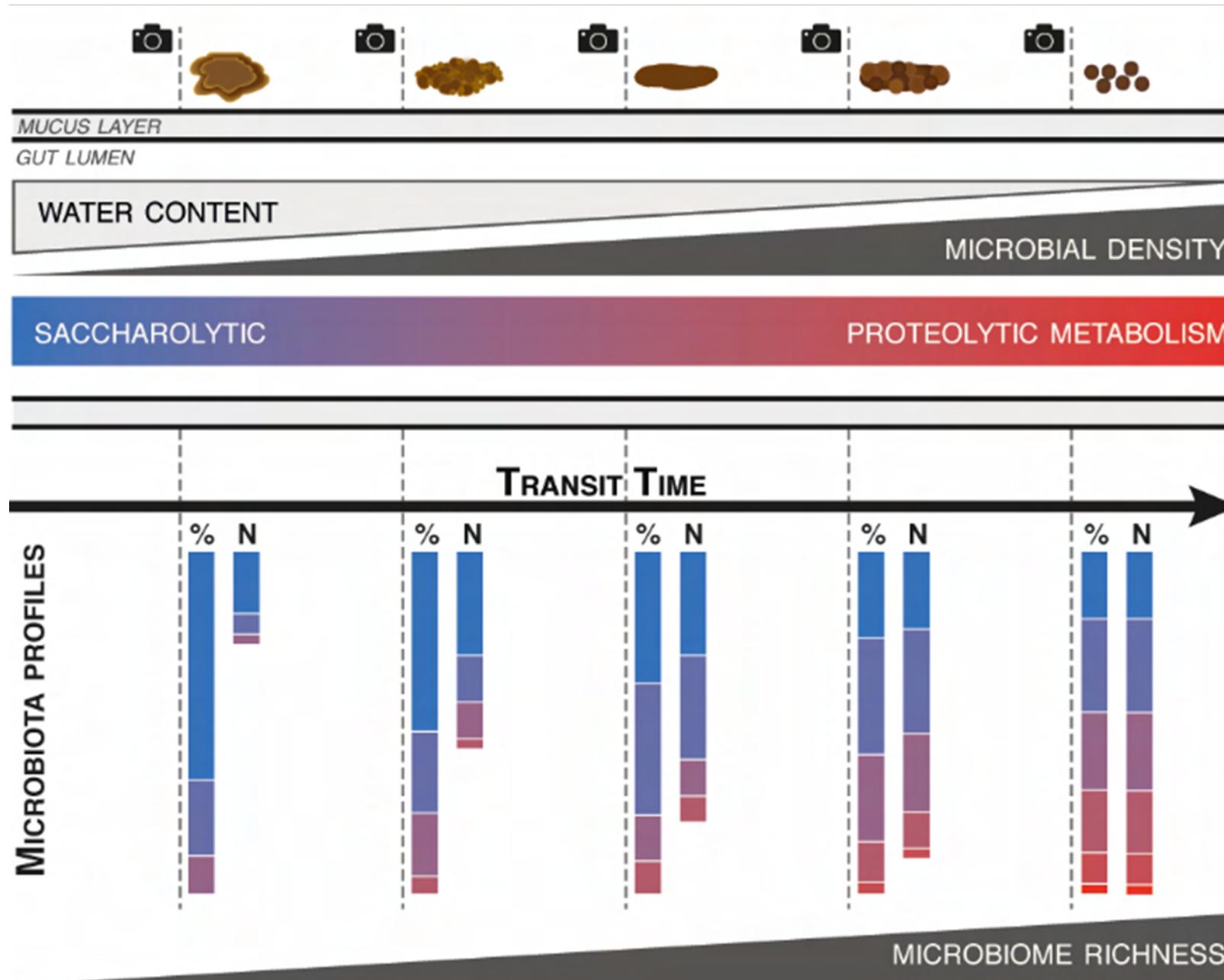
# RMP vs. QMP: implications



Cell counts are a key feature of a disease-associated microbiota



Relative data induce a clear negative correlation bias



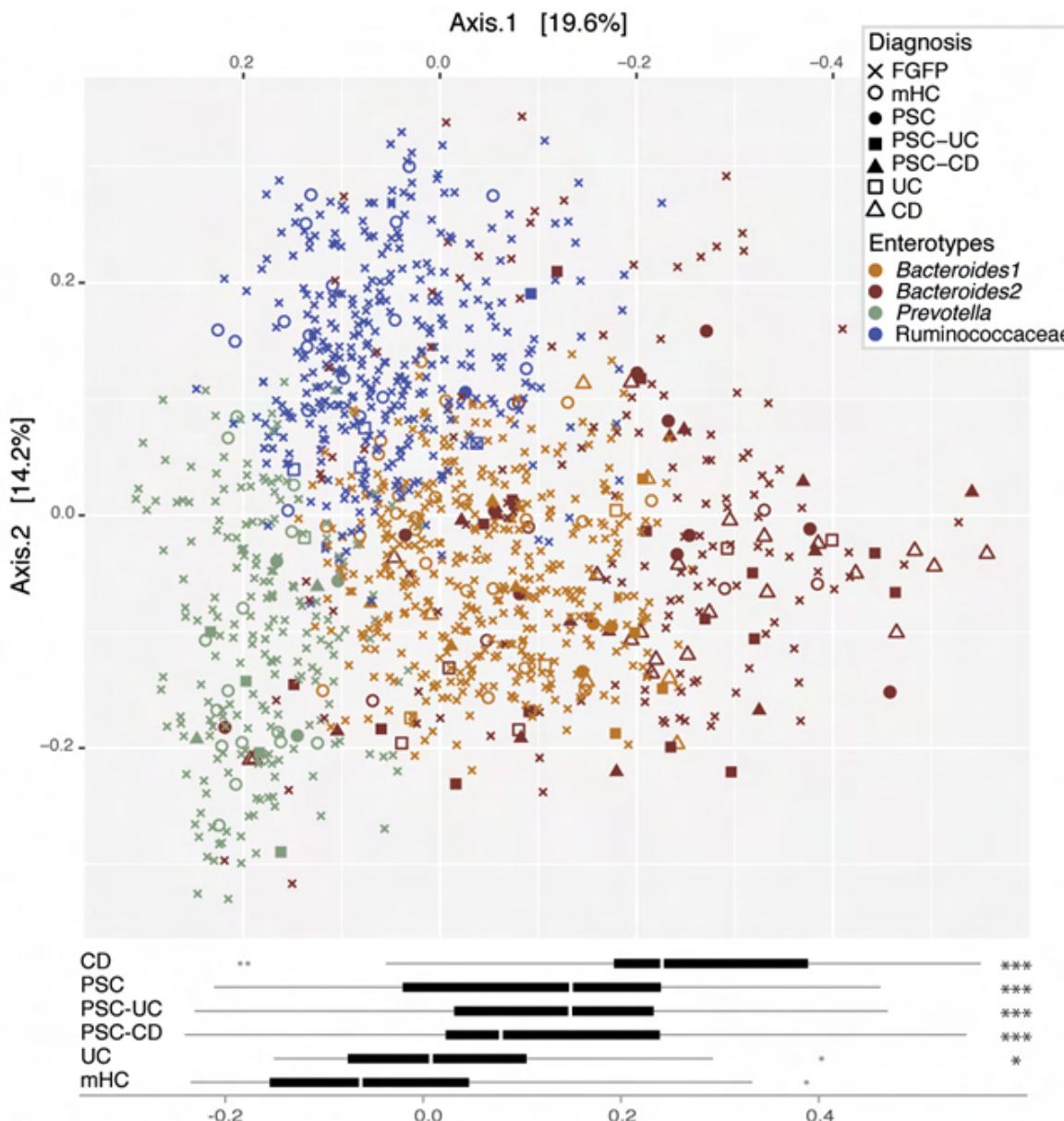
Richness as a marker of ecosystem development

# **Gut microbiota variation in disease**

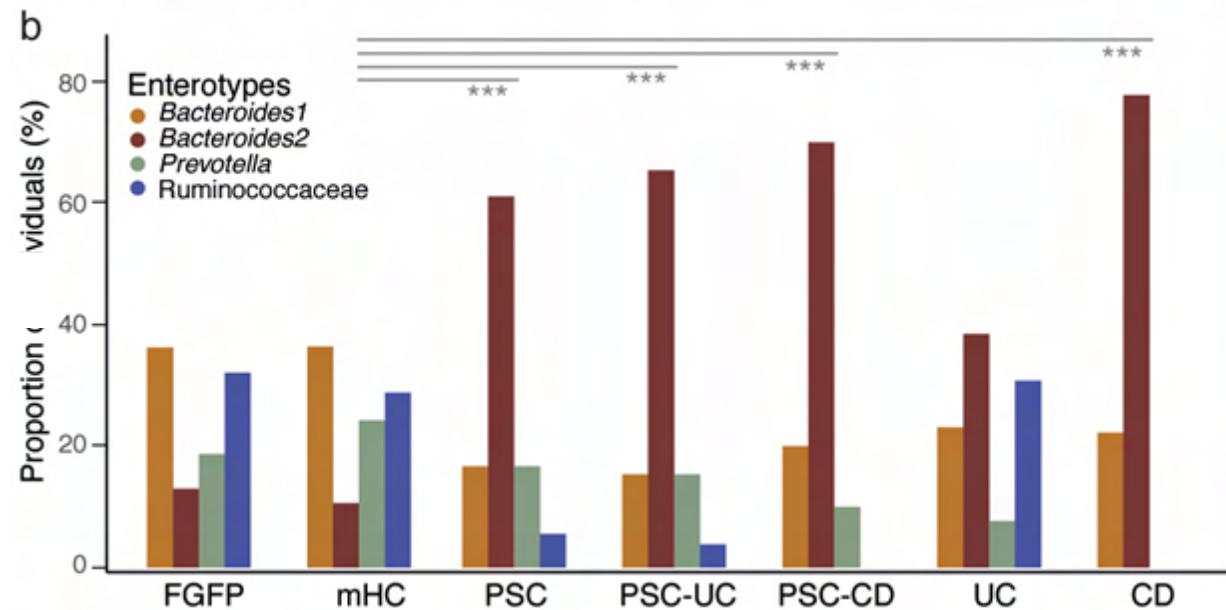
## **Re-defining gut dysbiosis**

# Defining dysbiosis

Vandeputte et al., Nature 2017  
Vieira-Silva et al., Nature Microbiology 2019



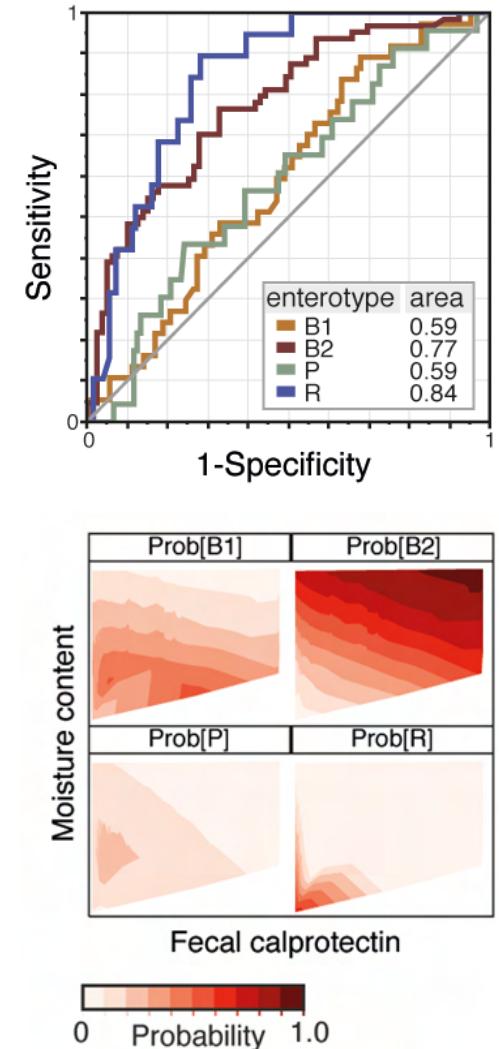
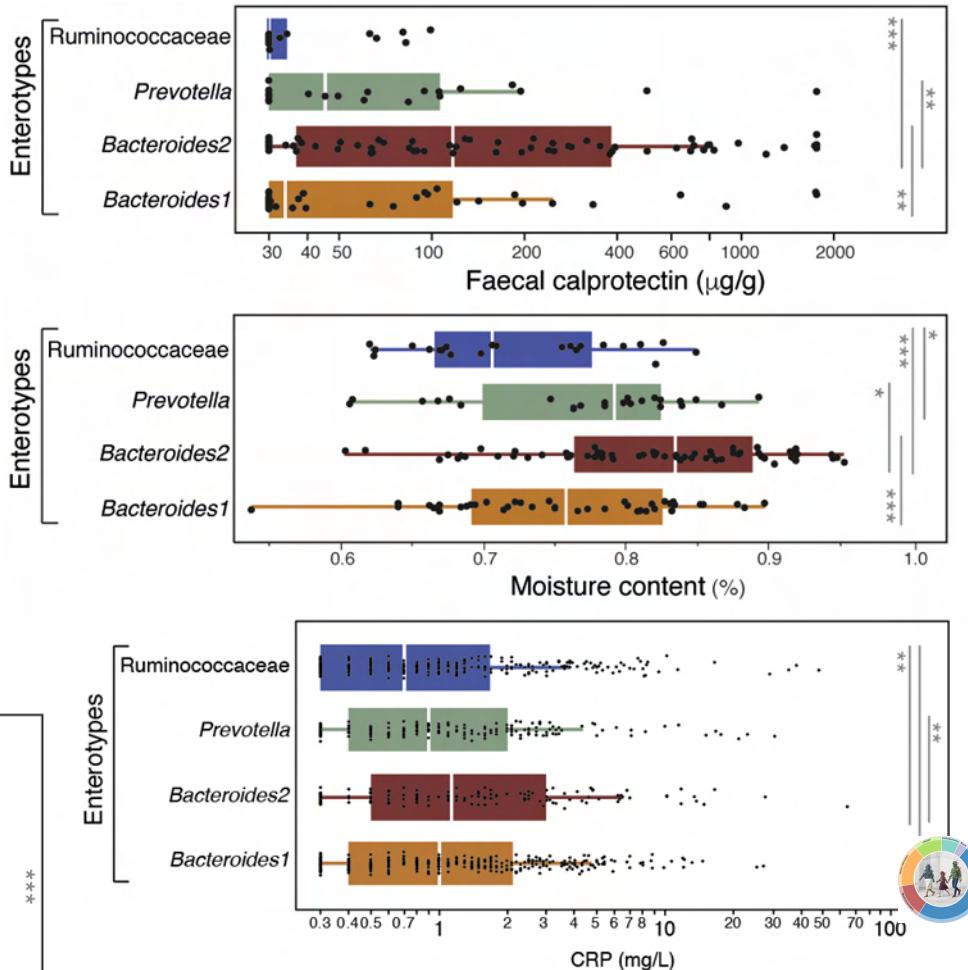
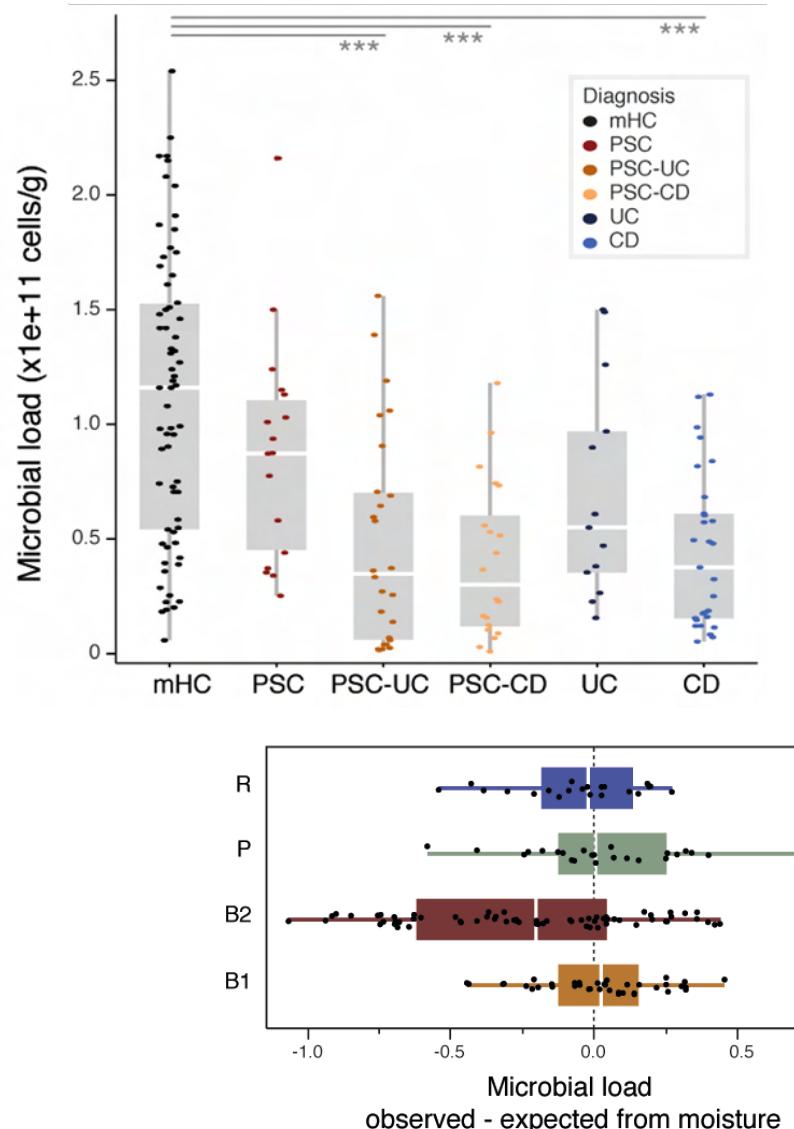
## Bact2: disease-associated low cell count enterotype



- Low microbial density
- Low species/functional diversity
- Low functional redundancy
- Low abundances of health associated bacteria (e.g. butyrate producers)

# Not just loose stools

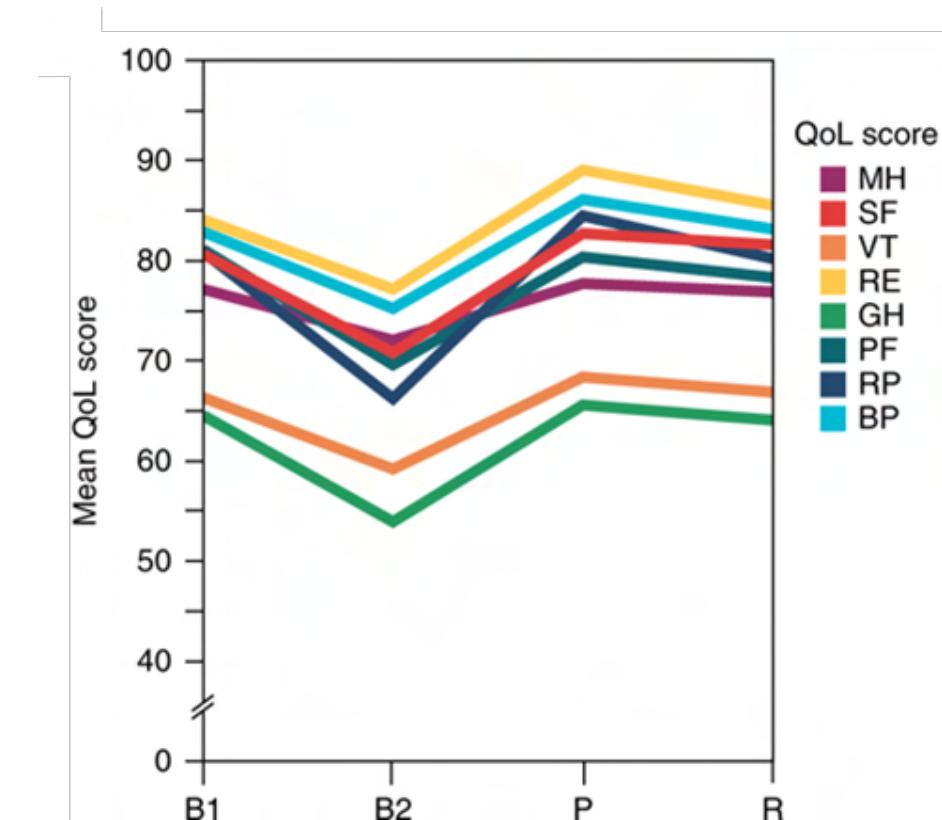
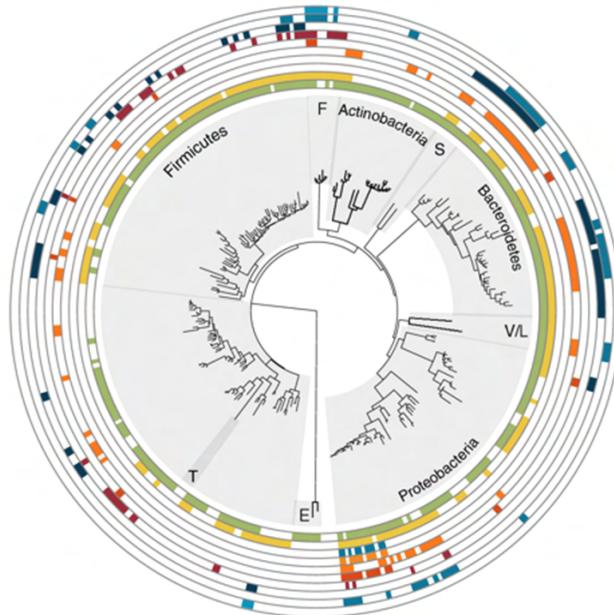
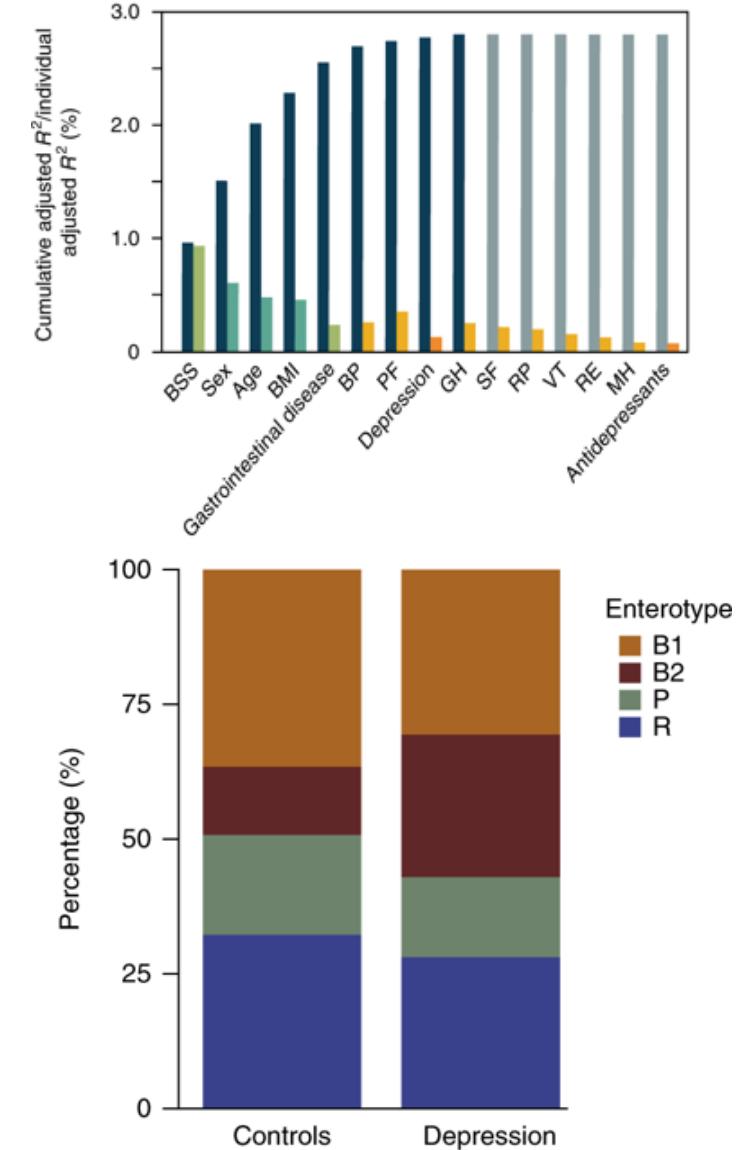
Bact2 is defined by increased moisture and inflammation



# The microbiota in quality of life and depression

## A link with Bact2 prevalence

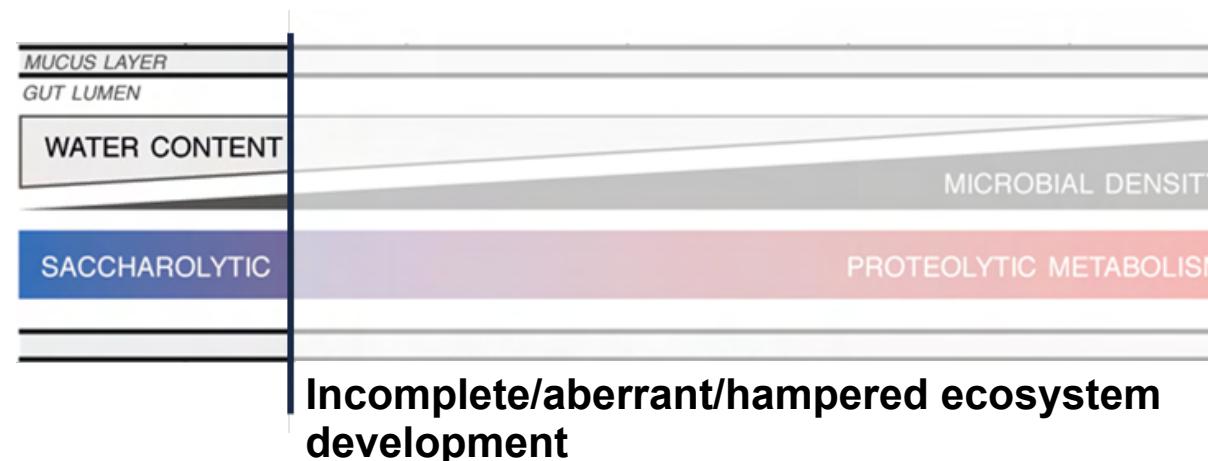
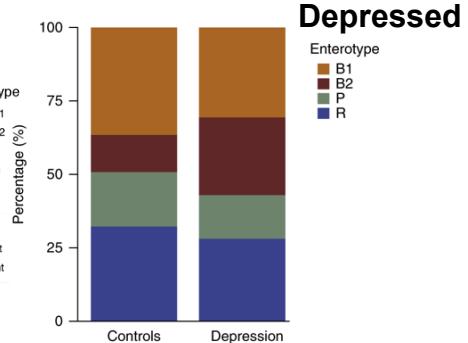
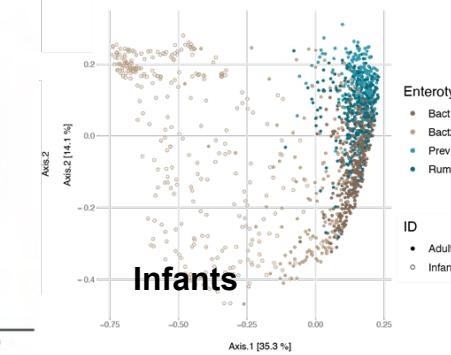
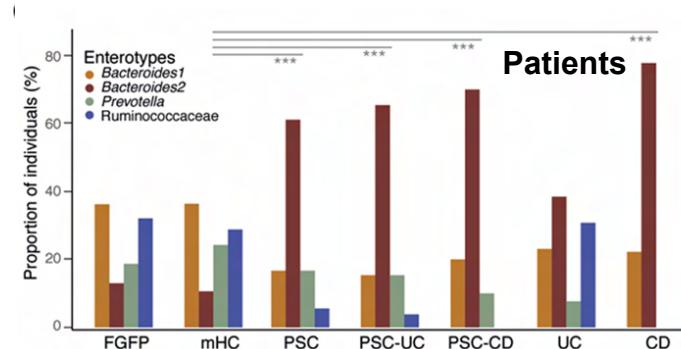
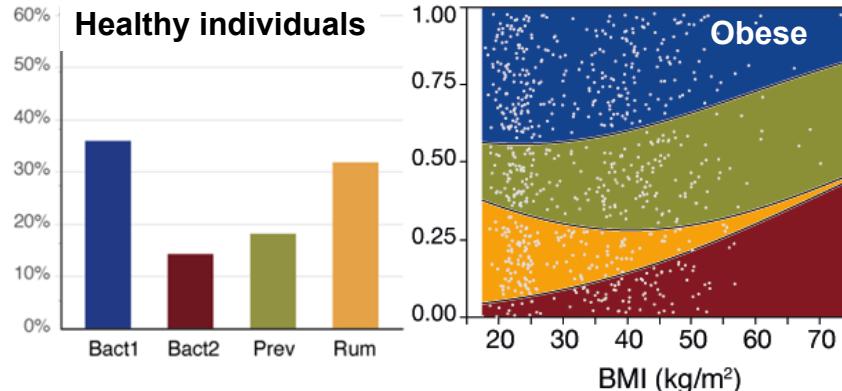
Valles-Colomer *et al.*, Nature Microbiology 2019



High Bact2 prevalence is linked to lower quality of life and depression, independent from inflammation

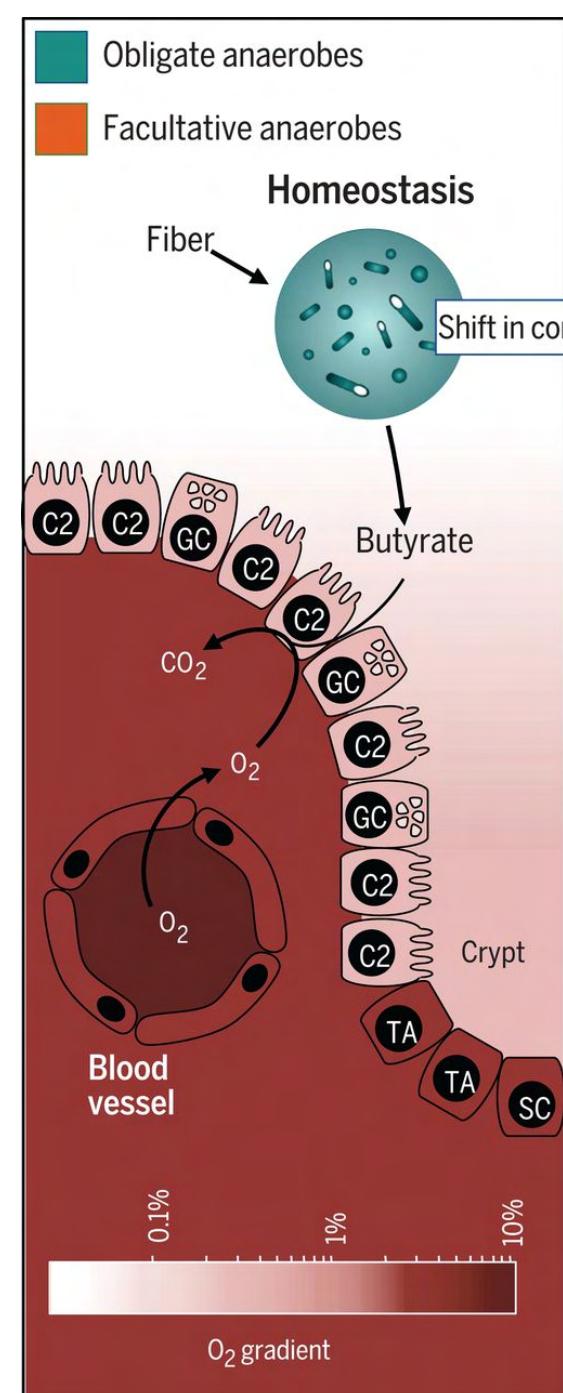
# Bact2 dysbiosis is not limited to disease

## Low cell count, high moisture, low abundance of butyrate producers



Falony *et al.*, Science 2016  
Vieira-Silva *et al.*, Nature 2020  
Beller *et al.*, 2021

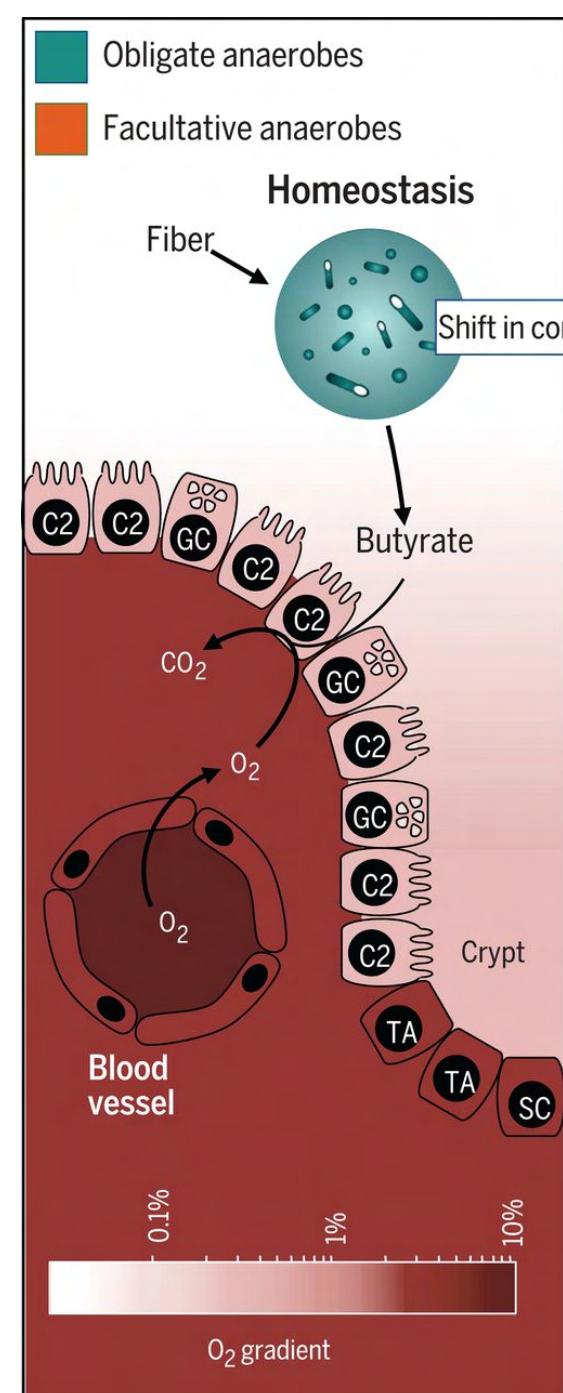
Dysbiosis of the gut microbiota: an ecosystem that fails to perform its commensal role and contributes to disease onset or progression by increasing host susceptibility toward disease development or by (co-)initiating or sustaining physiological responses affecting host health.



**Events leading to the development of gut ecosystem dysbiosis act through a primary disruption of colonocyte-butyrogen symbiosis**

- Lifestyle/dietary habits
- Infection
- Inflammation
- Antibiotic treatment
- ...

## The many roads to dysbiosis

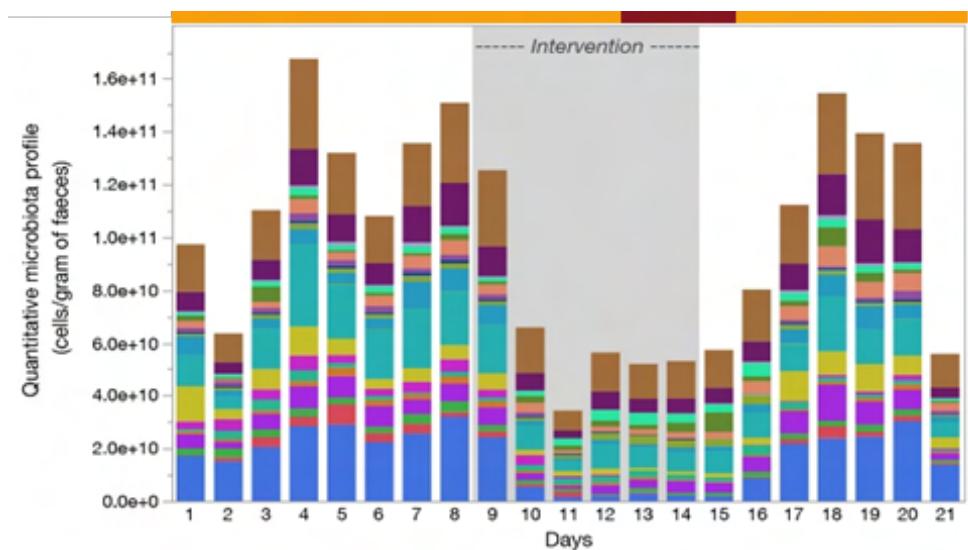


Events leading to the development of gut ecosystem dysbiosis act through a primary disruption of colonocyte-butyrigen symbiosis

- Lifestyle/dietary habits
- Infection
- Inflammation
- Antibiotic treatment
- ...

## The many roads to dysbiosis

### Dietary intervention



# **Gut microbiota variation in disease**

## **Quantifying the contribution of dysbiosis to disease**

# MetaCardis – probing the role of the microbiota in cardiovascular disease development



<http://www.metacardis.net/>

European consortium: 14 partners in 6 countries

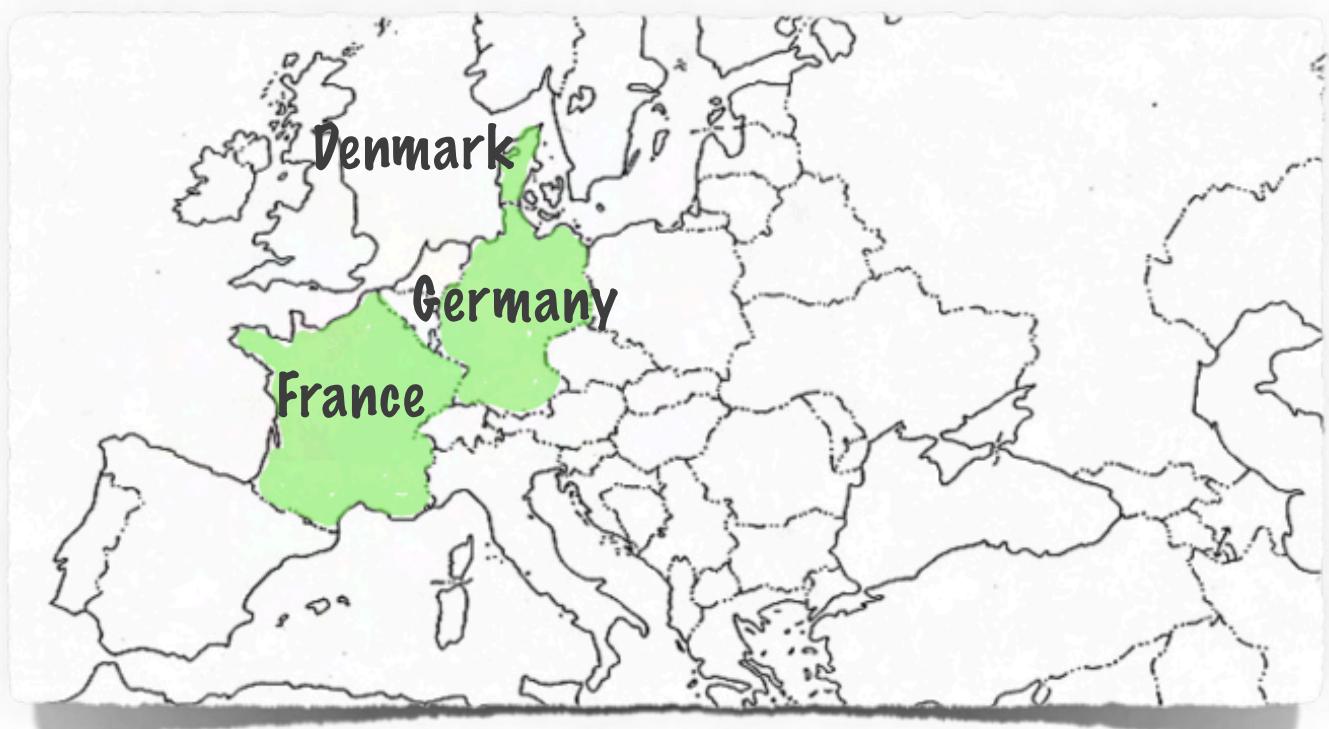
Multicenter recruitment: France, Germany, Denmark

N=3000 cross-sectional cohort

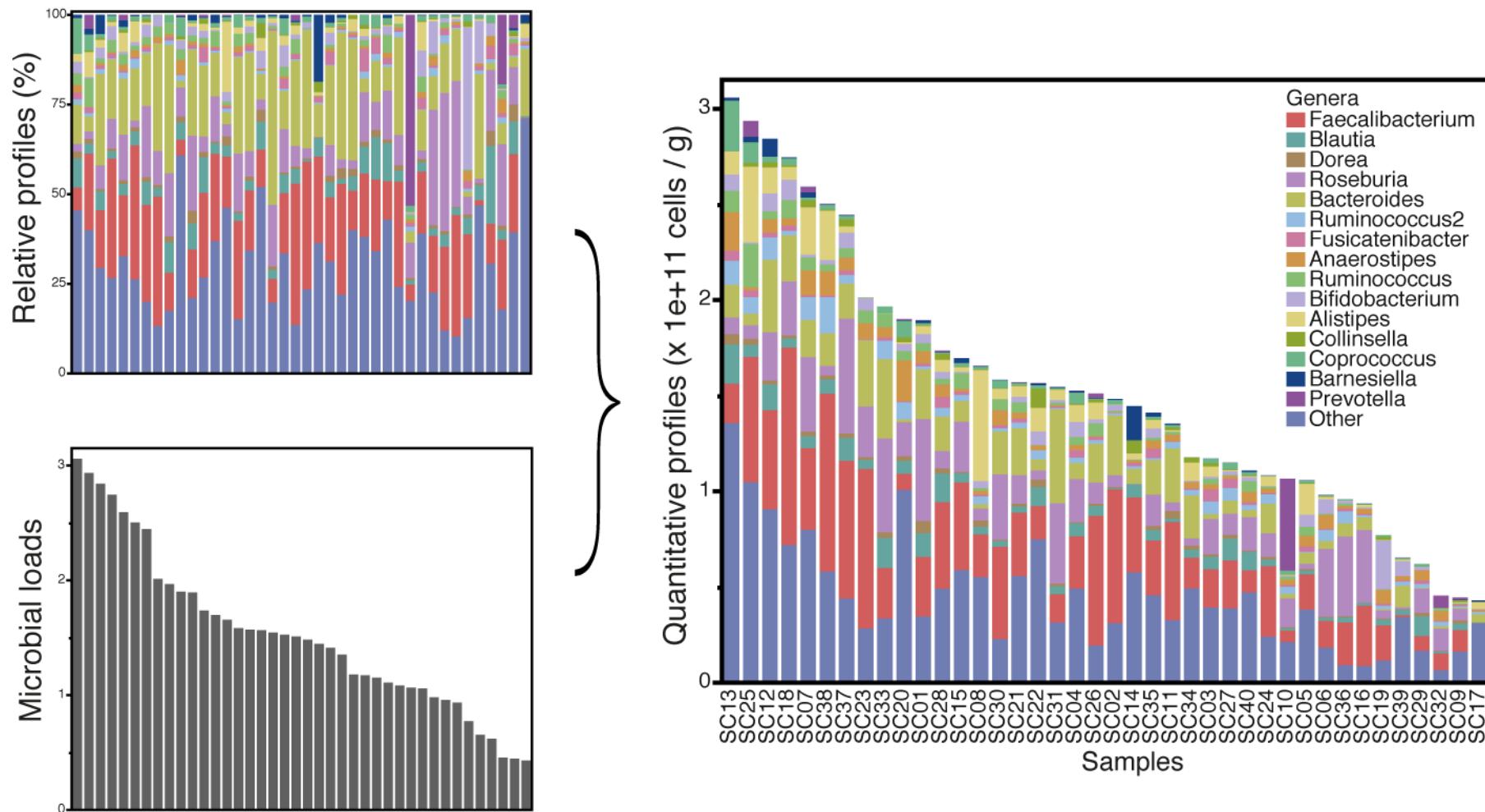
Clinical enrichment  
Multi-omics profiling



Karine Clément  
Oluf Pedersen  
Michael Stumvoll



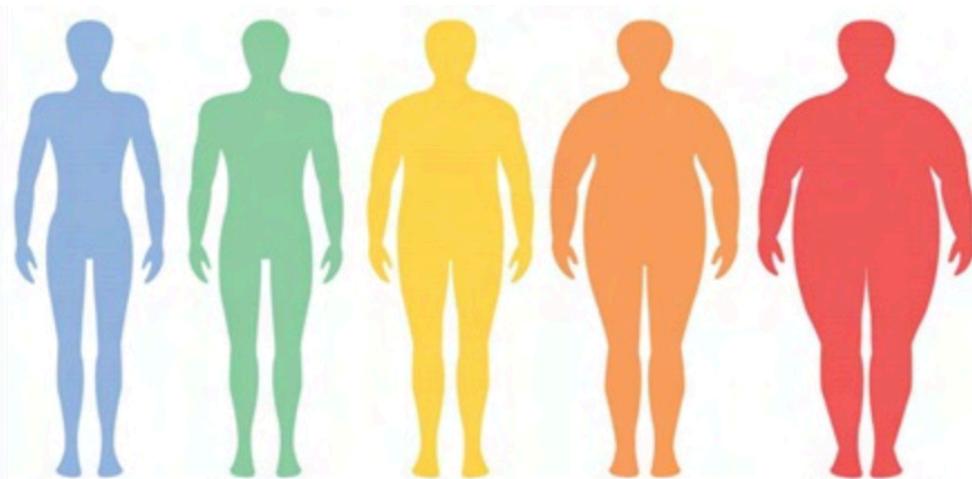
# Quantitative microbiome profiling



# MetaCardis – probing the role of the microbiota in cardiovascular disease development



The MetaCardis **Body Mass Index Spectrum (BMIS)** sub cohort (N=888):  
covering a range of BMI with no other progression of cardiometabolic disease  
without progression to diabetes

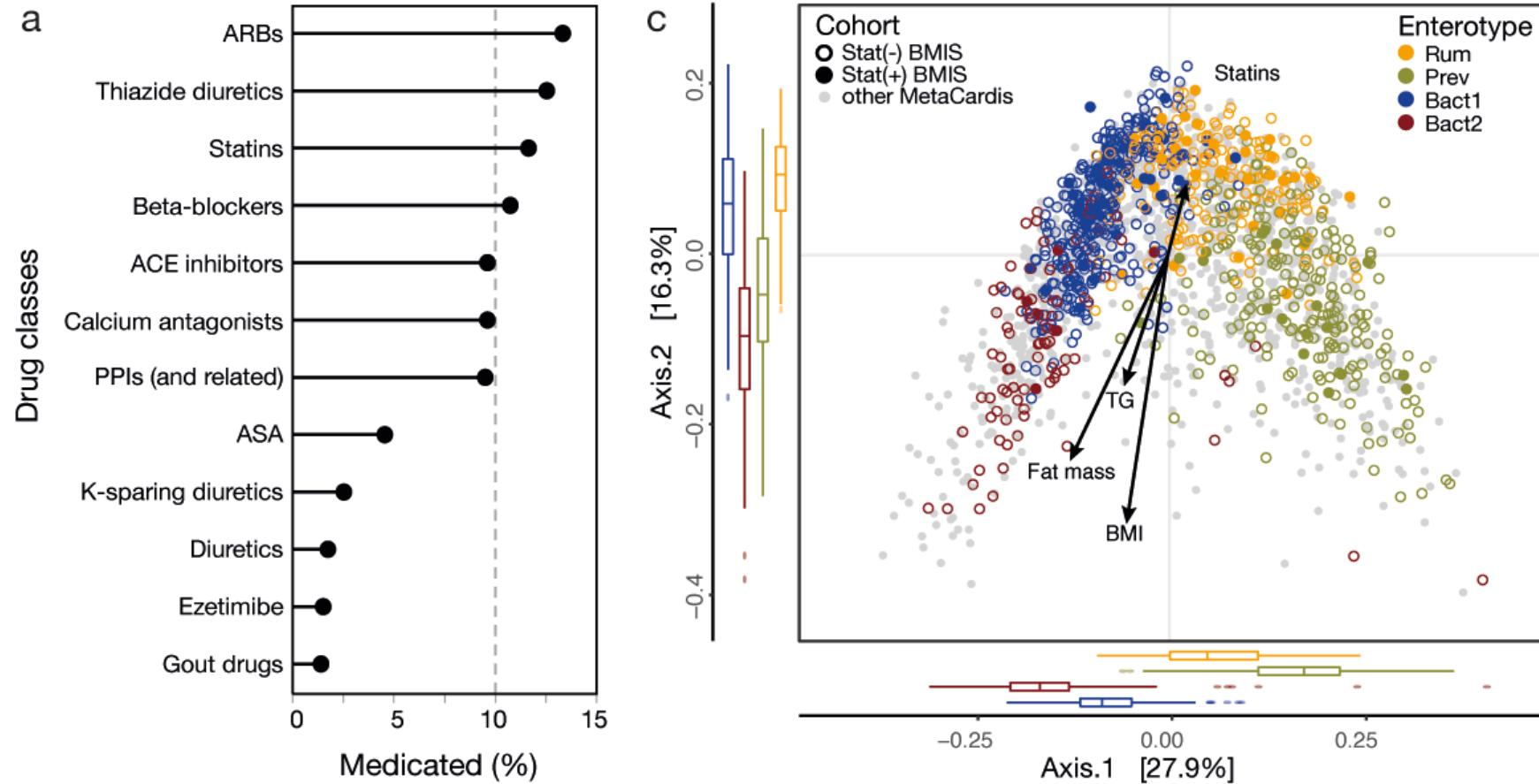




## Towards quantitative clinical microbiome research

1. Quantitative assessment of a pathomechanism
2. Stratification of patients with microbiota involvement
3. Quantification of microbiome contribution to disease risk
4. Identification of modulators of microbiota-disease interaction

# Characterization of microbiome variation in BMIS cohort

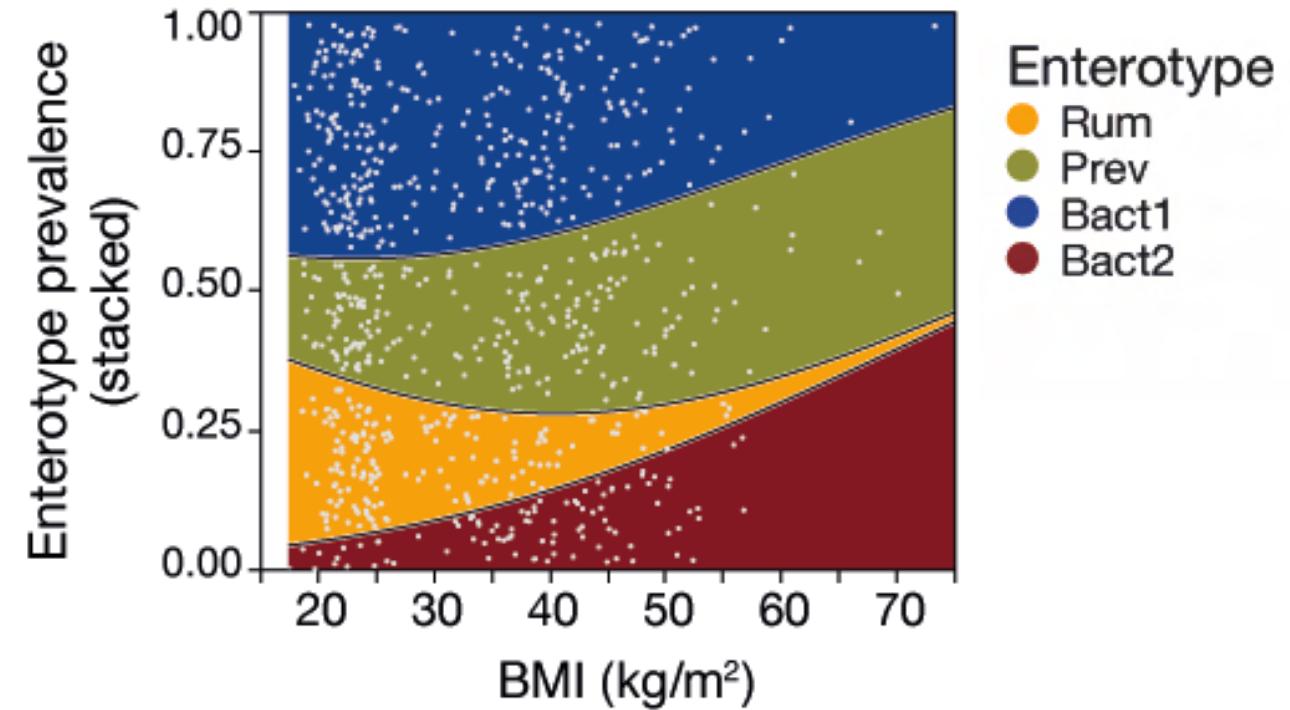
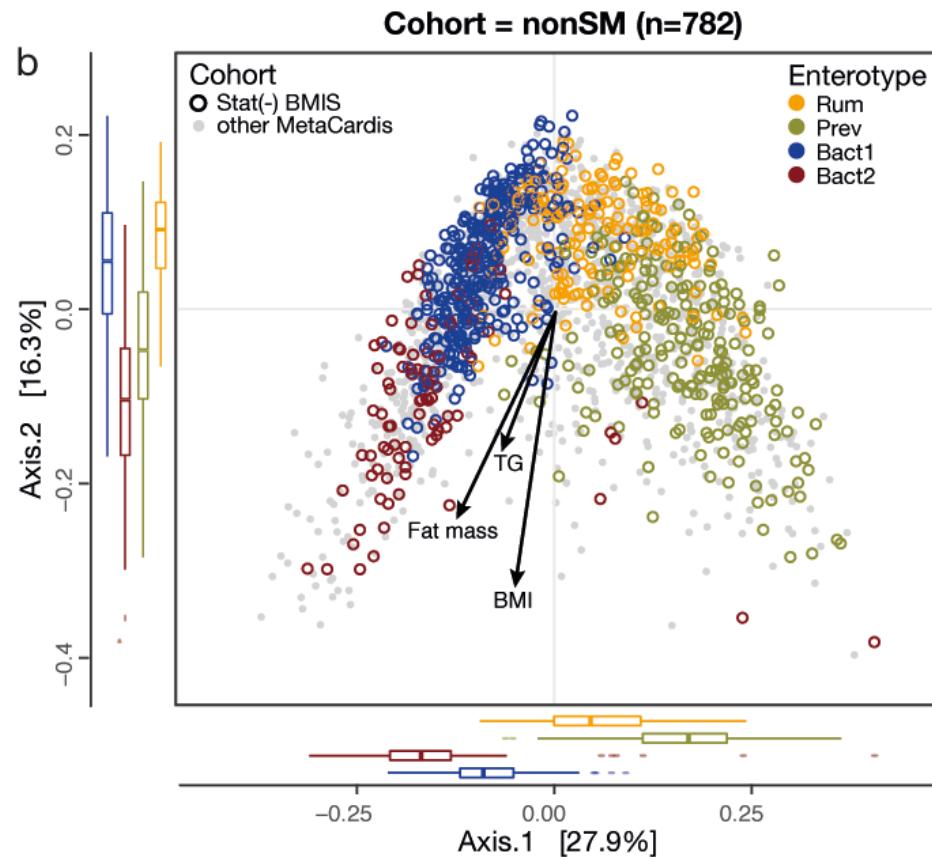


Statin intake has a significant correlation with microbiome variation

MetaCardis

Vieira-Silva *et al.*, Nature 2020

# The Bact2 enterotype prevalence increases with obesity



Dysbiosis prevalence increases with BMI

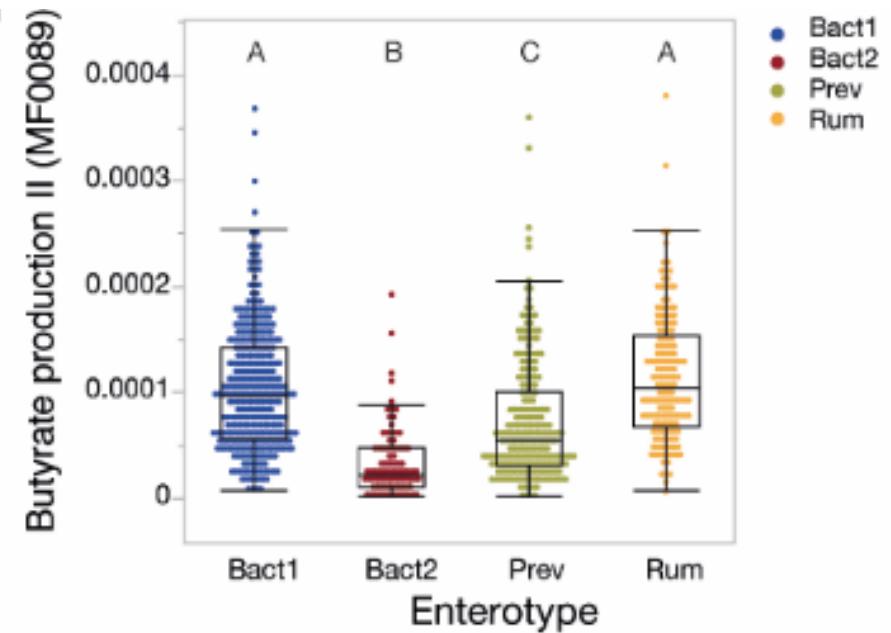
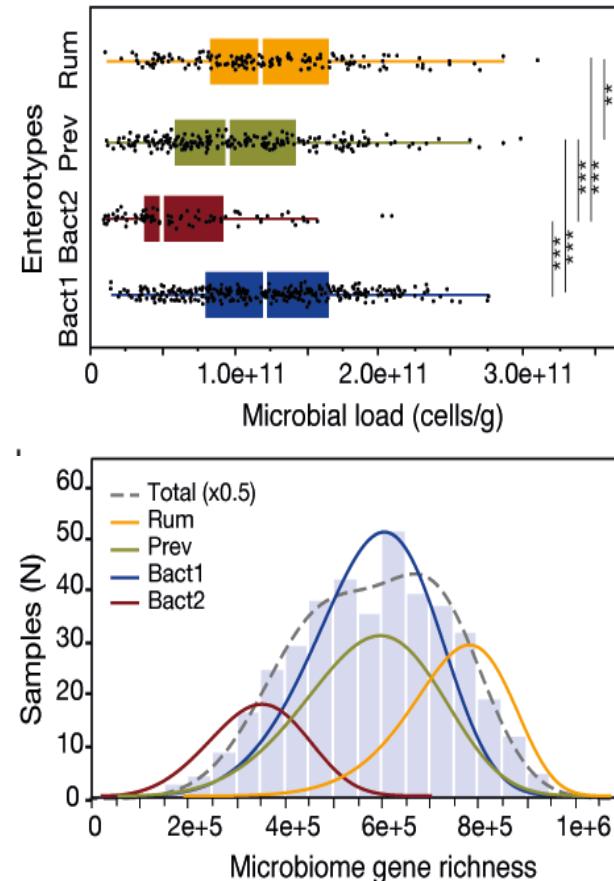
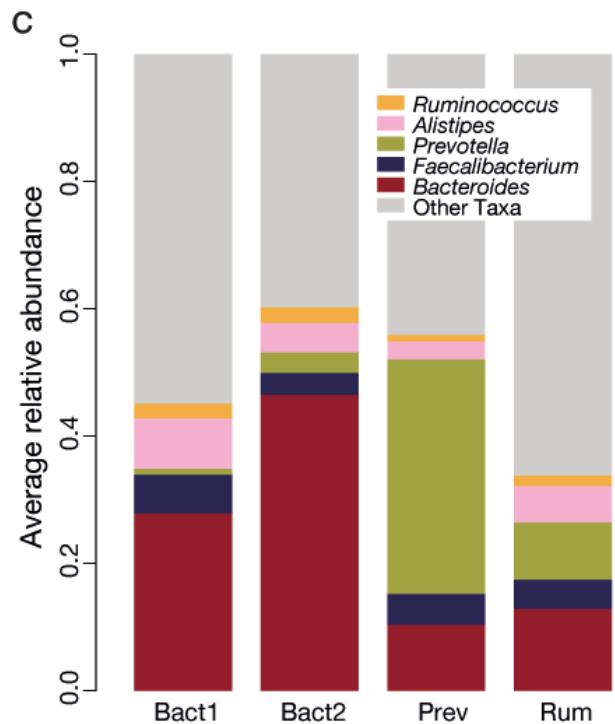
MetaCardis

Vieira-Silva *et al.*, Nature 2020

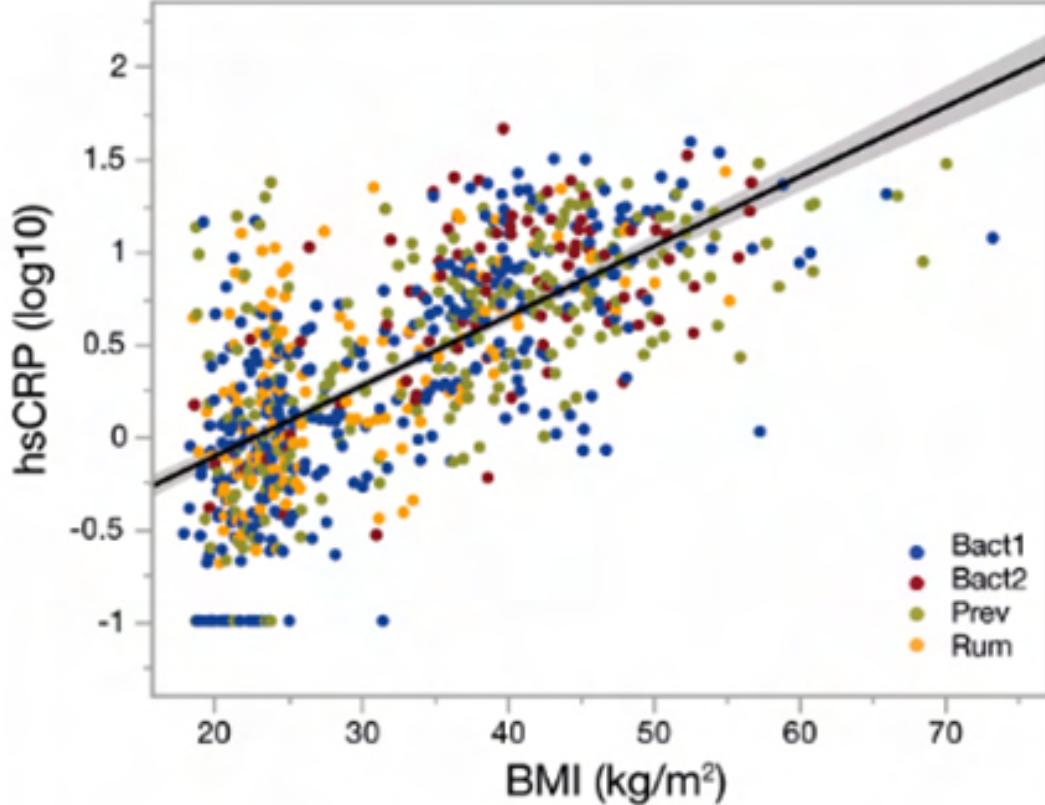
# Bact2 has the hallmarks of a disturbed ecosystem

Bact2 has low microbial load, species and functional diversity, and functional redundancy

Host-microbiota interaction through butyrate production is challenged.



# Contribution of dysbiosis in obesity-associated inflammation

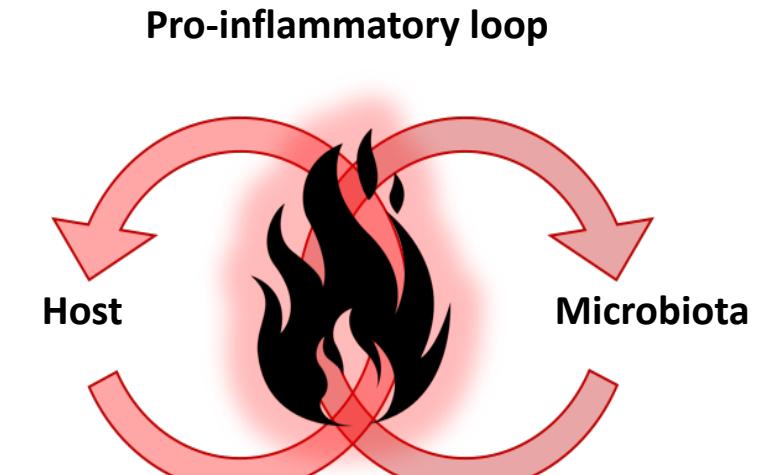
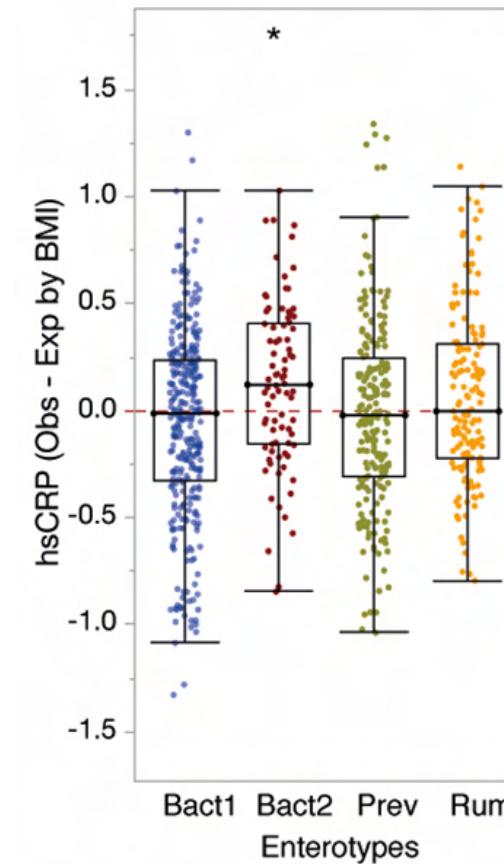
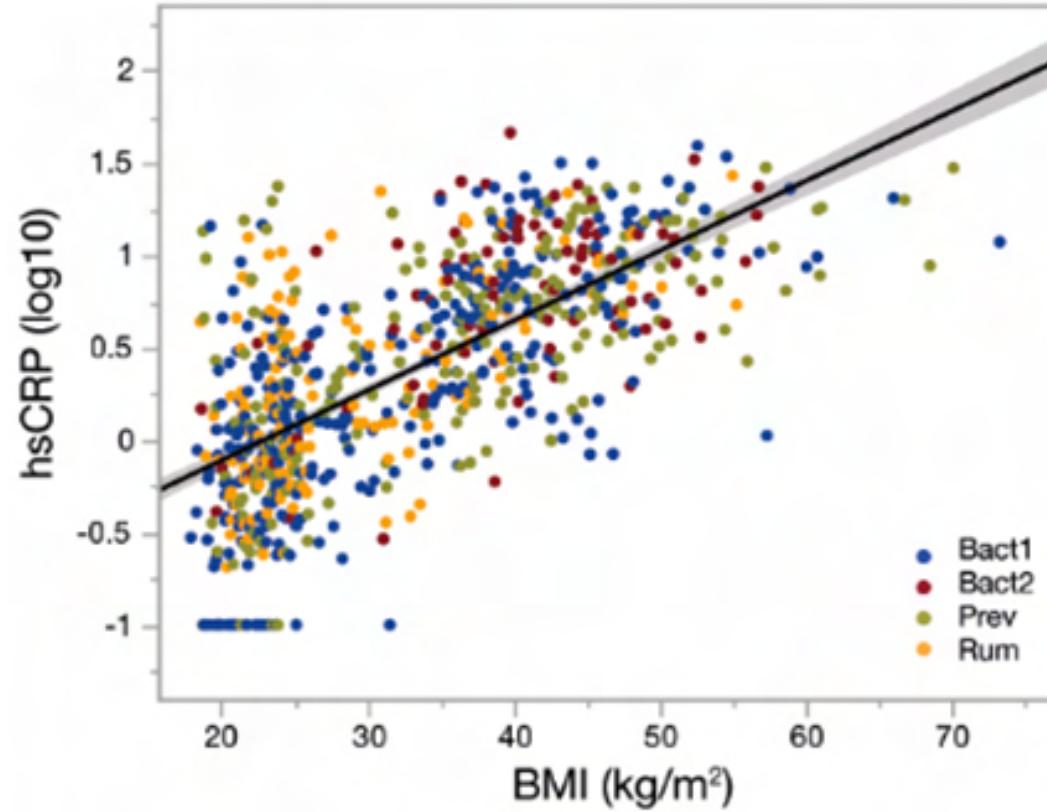


Inflammation correlates with BMI

MetaCardis

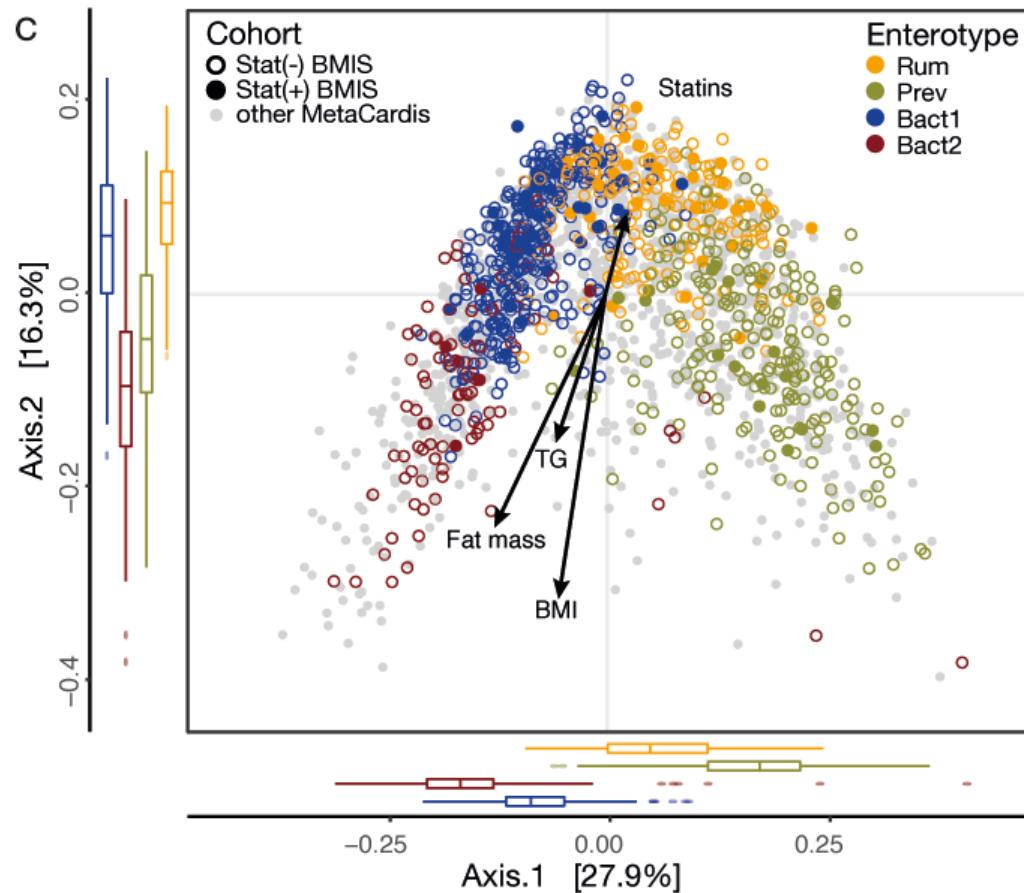
Vieira-Silva *et al.*, Nature 2020

# Contribution of dysbiosis in obesity-associated inflammation

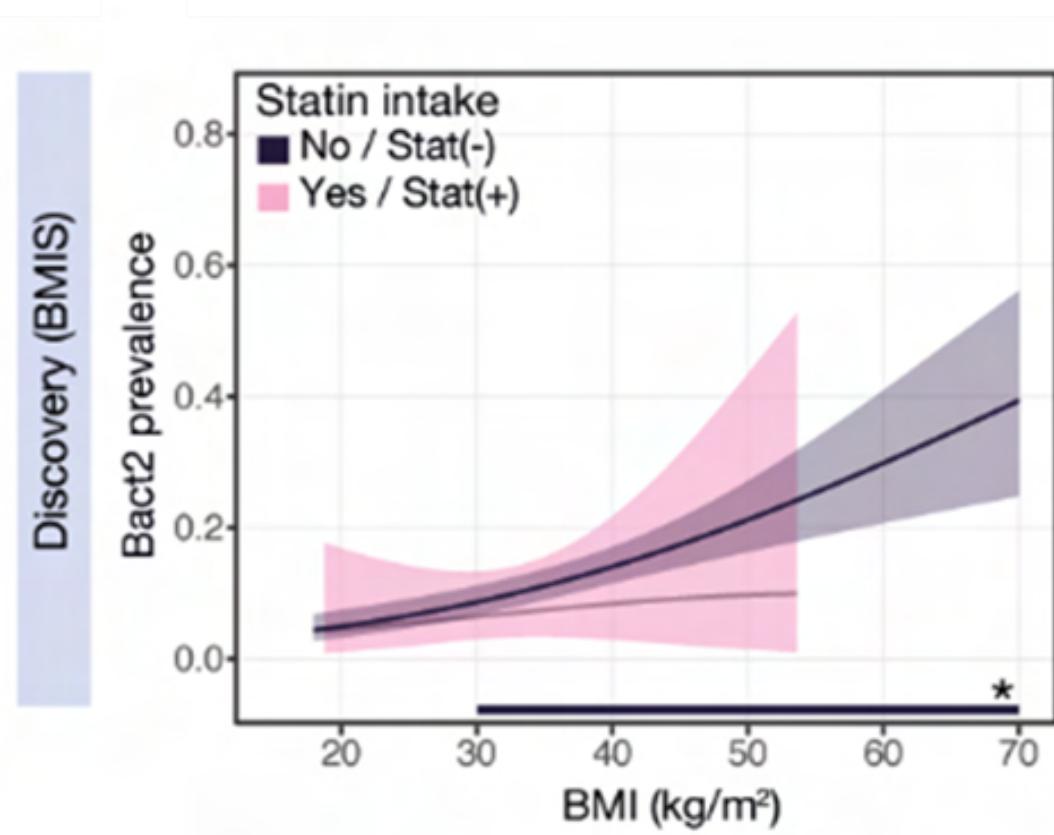
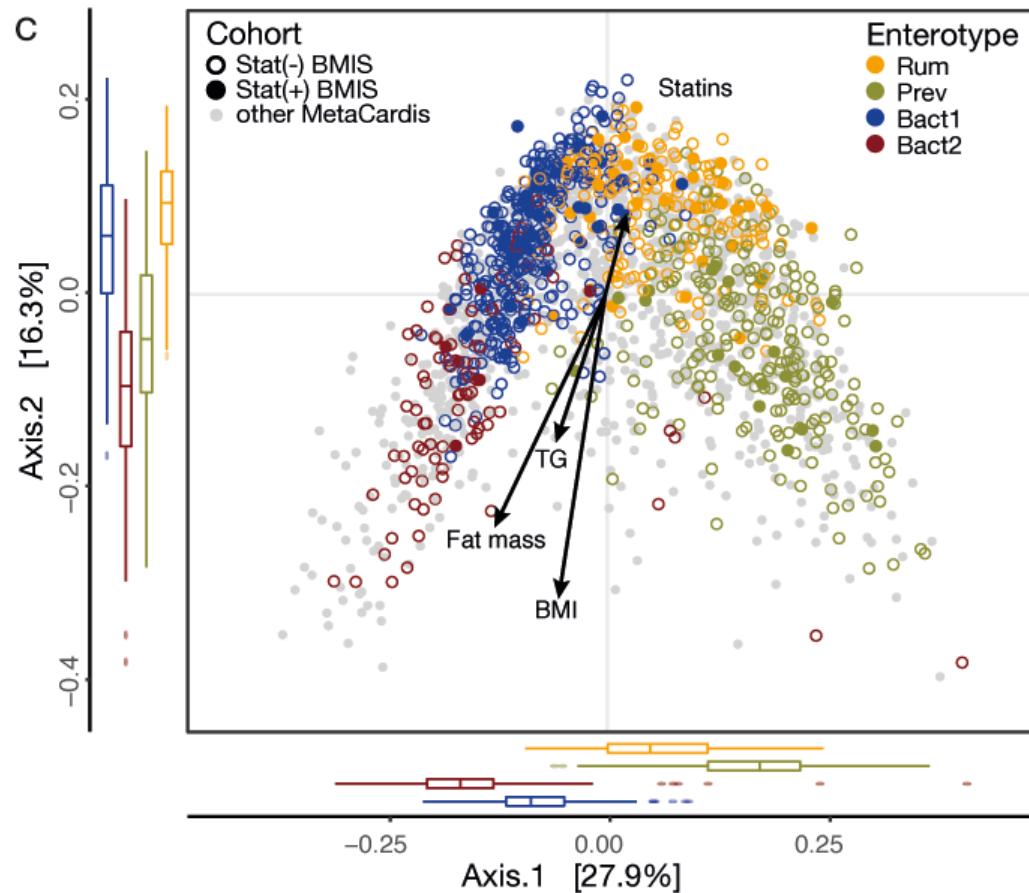


The inflammatory tone of Bact2-hosts exceeds levels expected by BMI

# But what about statin-treated individuals?



# Modulation of microbiota-disease interaction

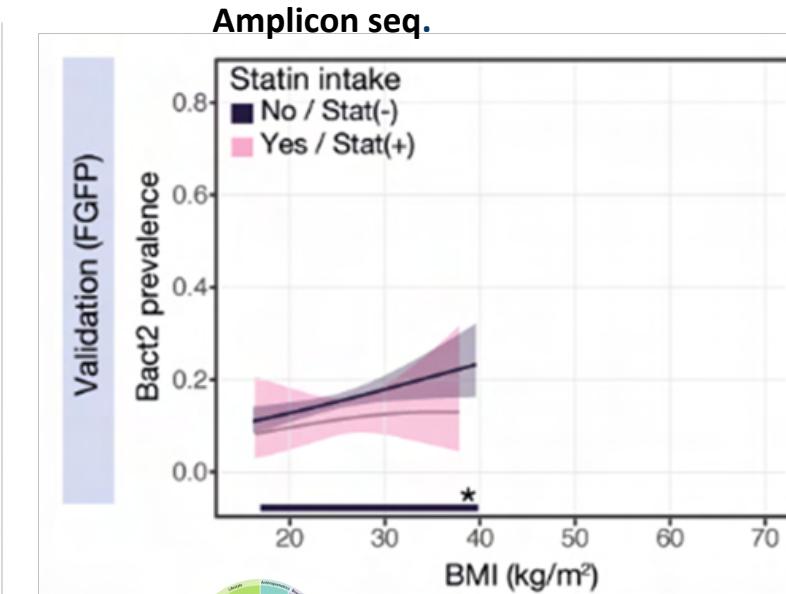
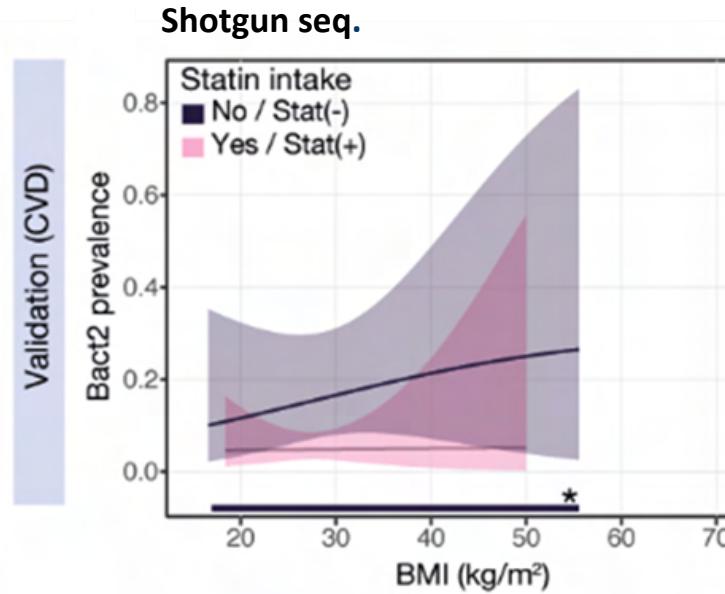
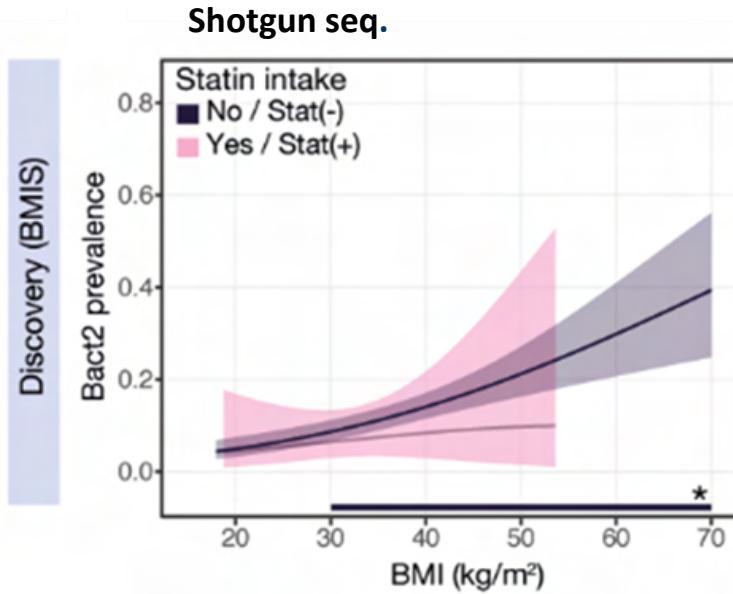


Increased Bact2 prevalence with increasing BMI is mitigated in statin-treated individuals

MetaCardis

Vieira-Silva *et al.*, Nature 2020

# Modulation of microbiota-disease interaction



Flemish Gut Flora Project

Validation in different cohorts

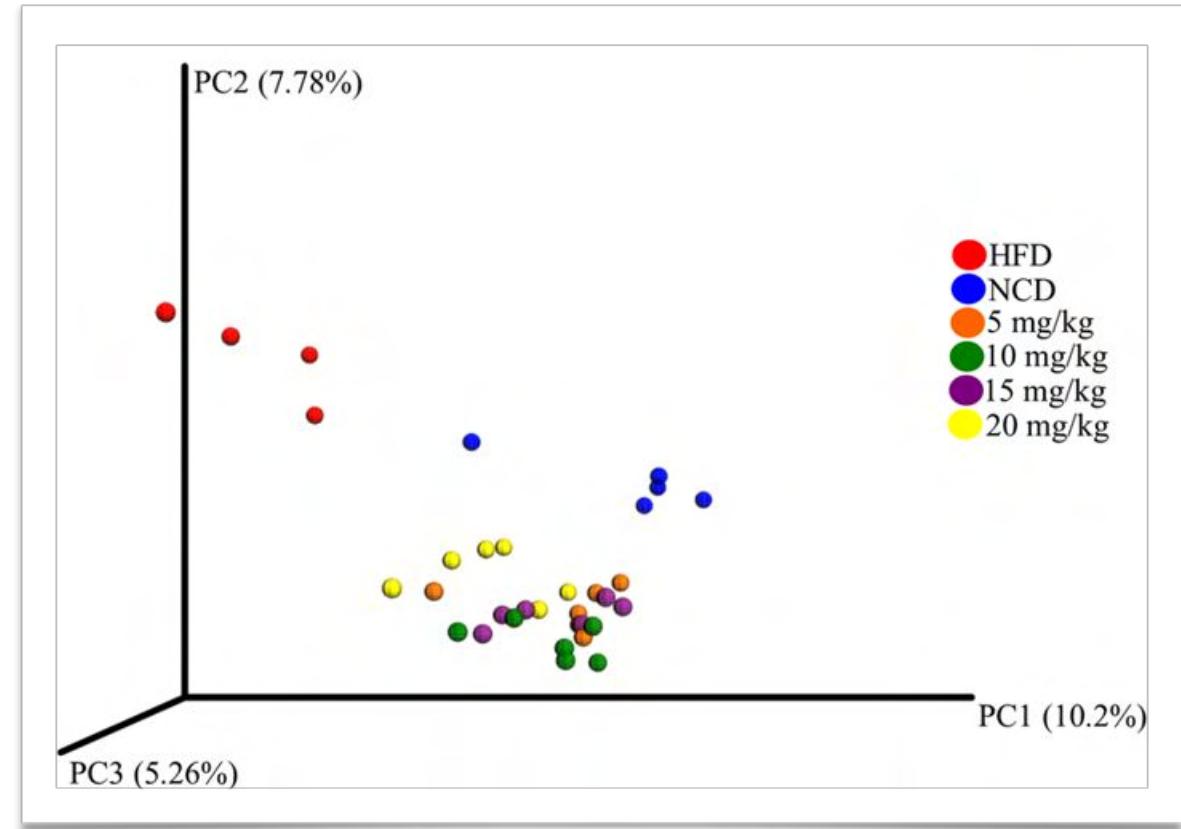
MetaCaroris

Vieira-Silva *et al.*, Nature 2020

# Supporting evidence in statin intake trial in rats

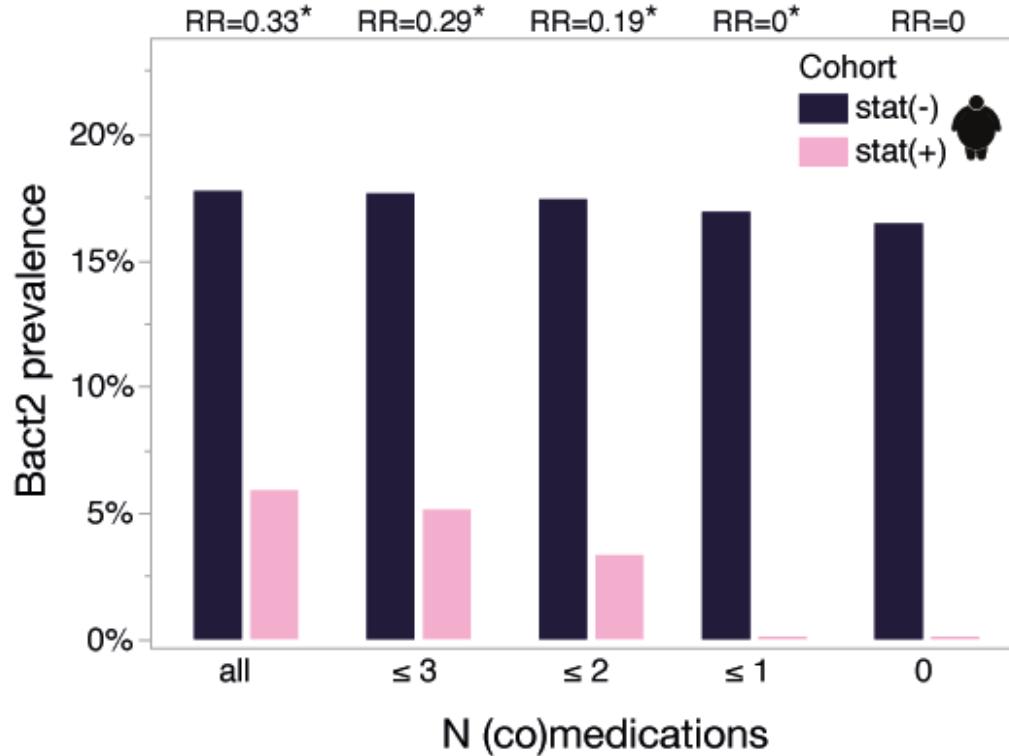
'Overall, bacterial community composition was altered, and diversity of gut microbiota increased with atorvastatin treatment in HFD group. Reversion in relative abundance of specific dominant taxa was observed with drug treatment to HFD rats.'

Khan et al. 2018, Scientific Reports



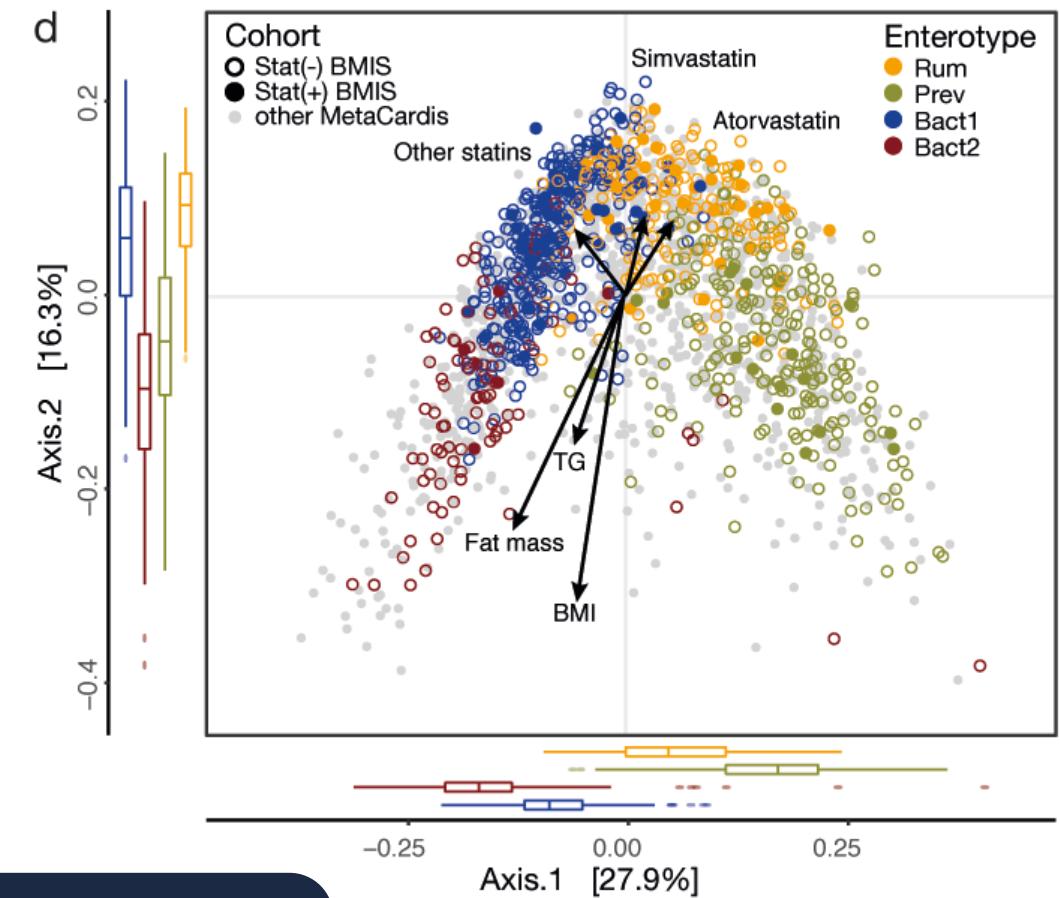
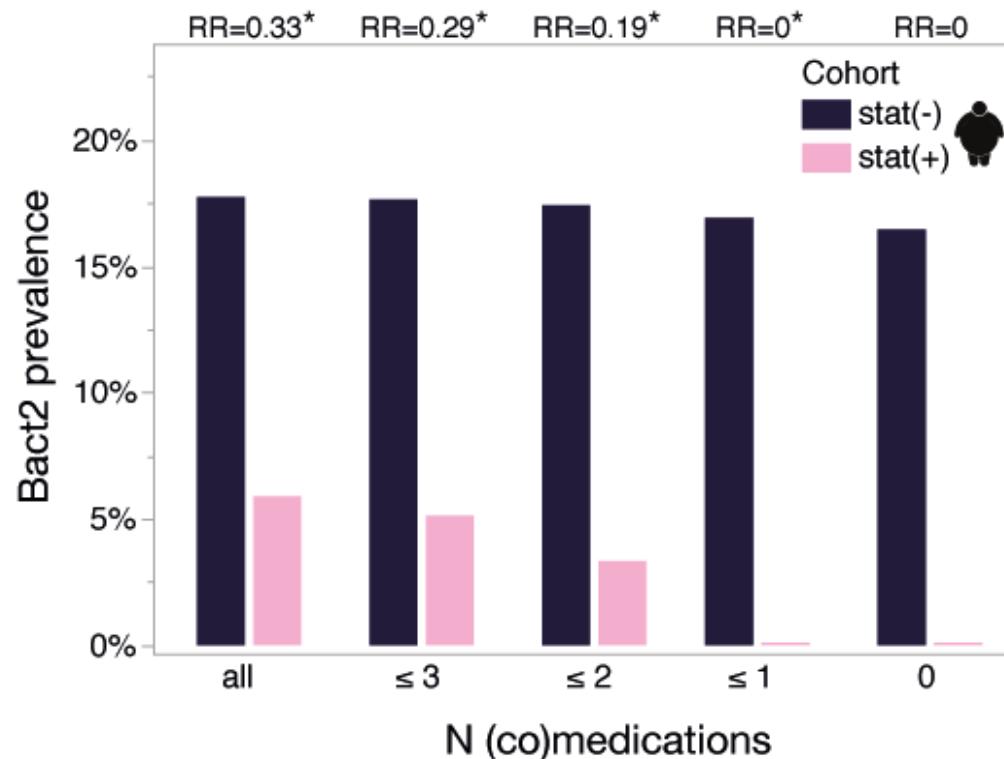
# Confounding by indication?

Co-medication does not drive the statin effect



- Without co-medication, Bact2 prevalence in the Stat(+) BMIS cohort is close to 0%

# Co-medication does not drive the statin effect



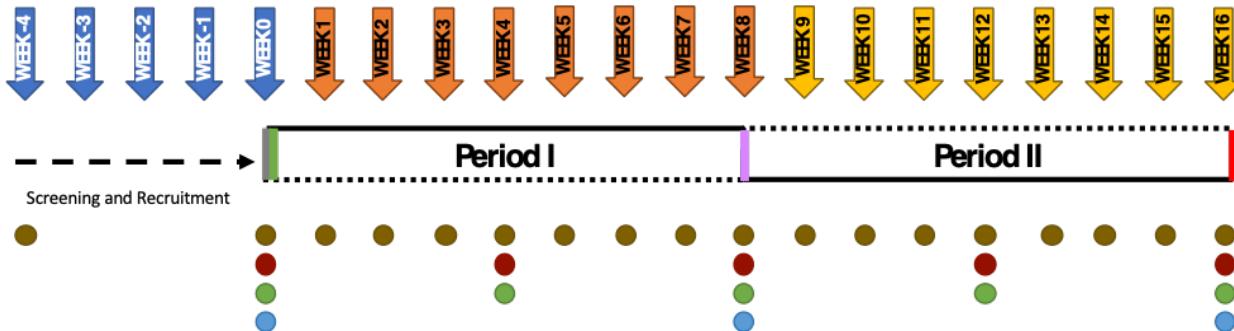
- Without co-medication, Bact2 prevalence in the Stat(+) BMIS cohort is close to 0%
- Effect on microbiome is not limited to a single class of statins

MetaCardis

Vieira-Silva *et al.*, Nature 2020

# Statin-mediated gut microbiota modulation: from correlation to causation

Randomized, double-blind, cross-over phase III trial



Séverine Vermeire, MD & PhD  
Principal investigator



Mechanism of action?

I USED TO THINK  
CORRELATION IMPLIED  
CAUSATION.



THEN I TOOK A  
STATISTICS CLASS.  
NOW I DON'T.



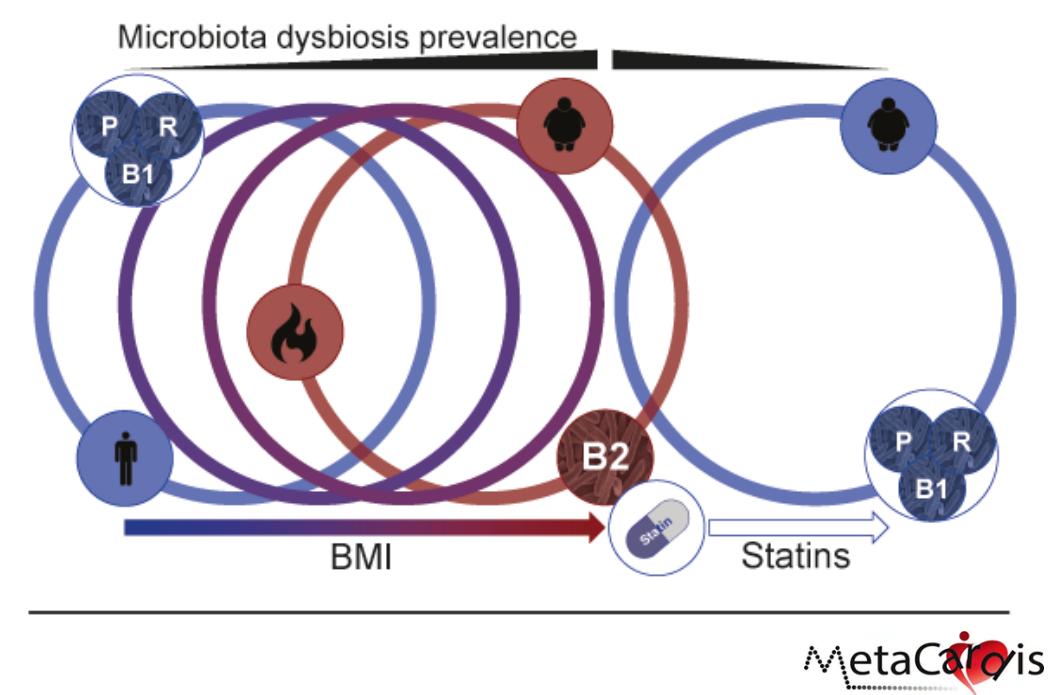
SOUNDS LIKE THE  
CLASS HELPED.  
WELL, MAYBE.



# Take home messages

## Towards quantitative clinical microbiome research

- Quantitative assessment of a pathomechanism
- Stratification of patients with microbiota involvement
- Quantification of microbiome contribution to disease risk
- Identification of modulators of microbiota-disease interaction



- The dysbiotic Bact2-enterotype contributes to the inflammatory pathomechanism in obesity
- Statins repurposing has potential for microbiota modulation