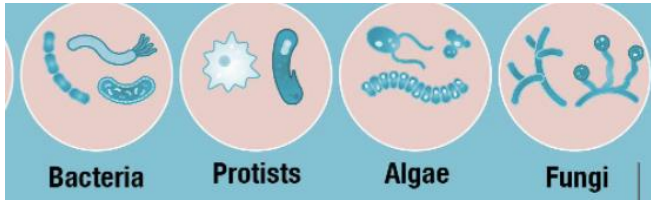


# Introduction to microbiome studies: Basic concepts and applications

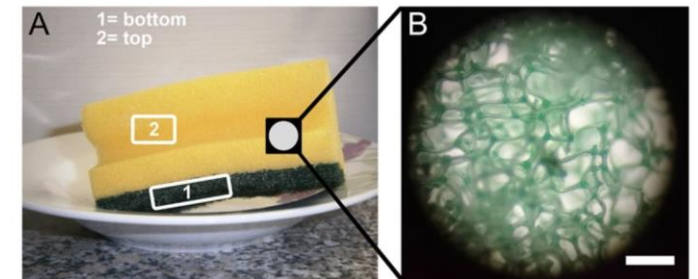
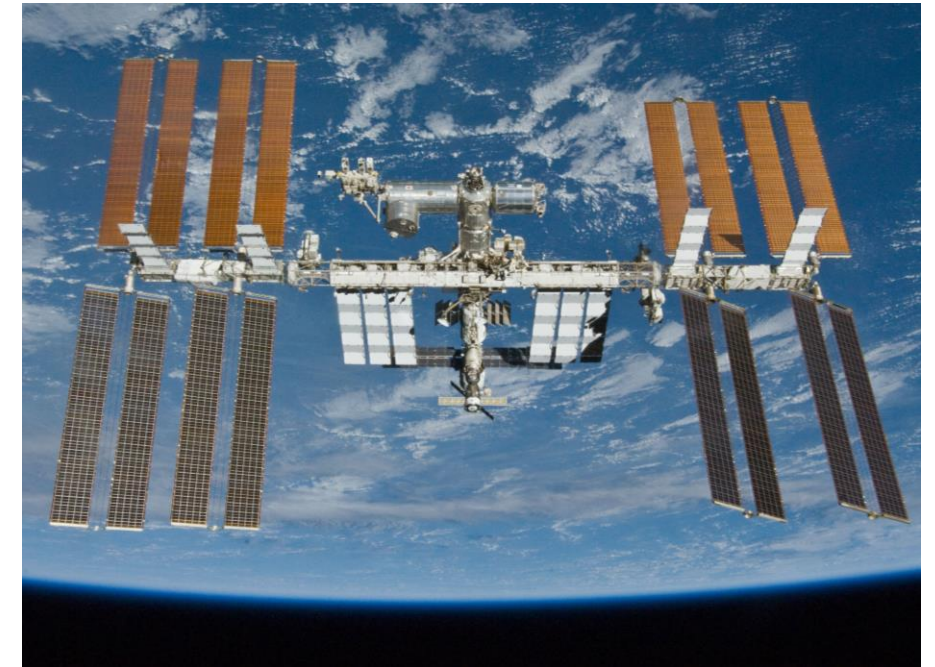
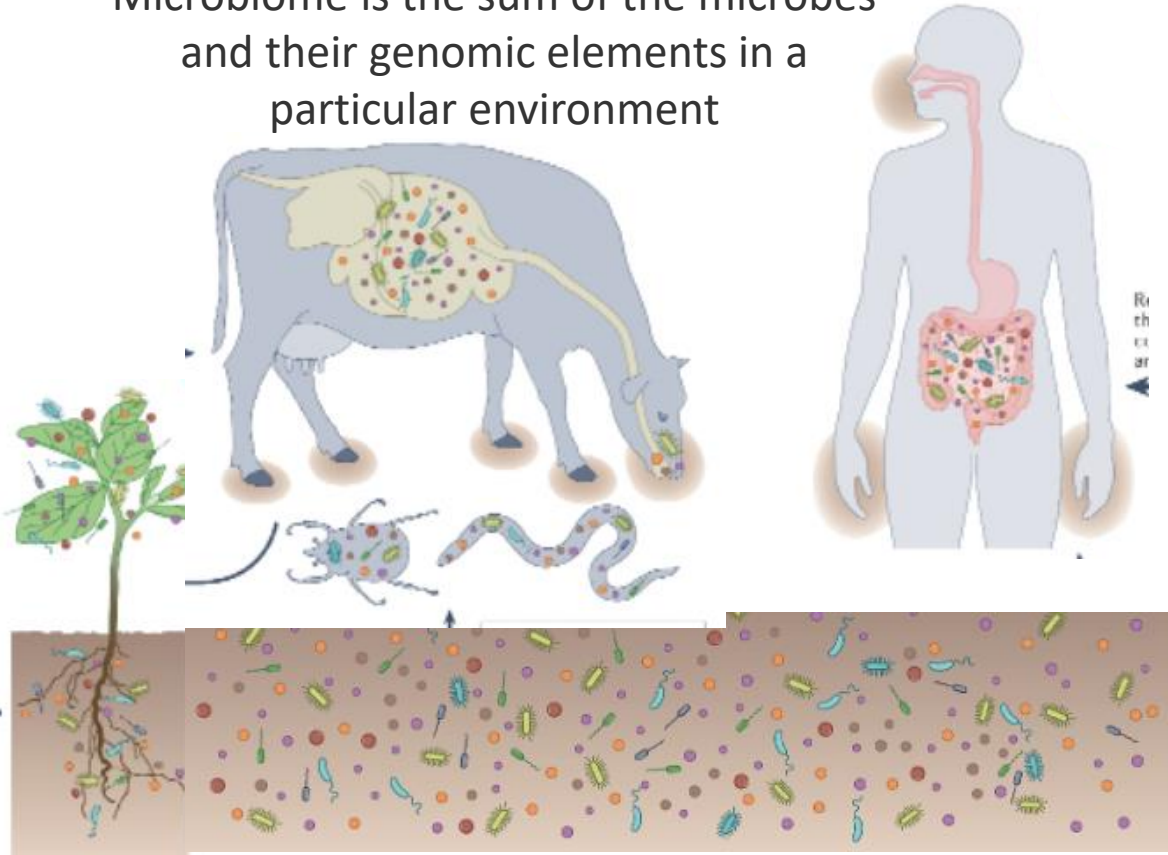
Blastocystis COST Action Training School: Blastocystis and the Gut Microbiome

# What is microbiome?



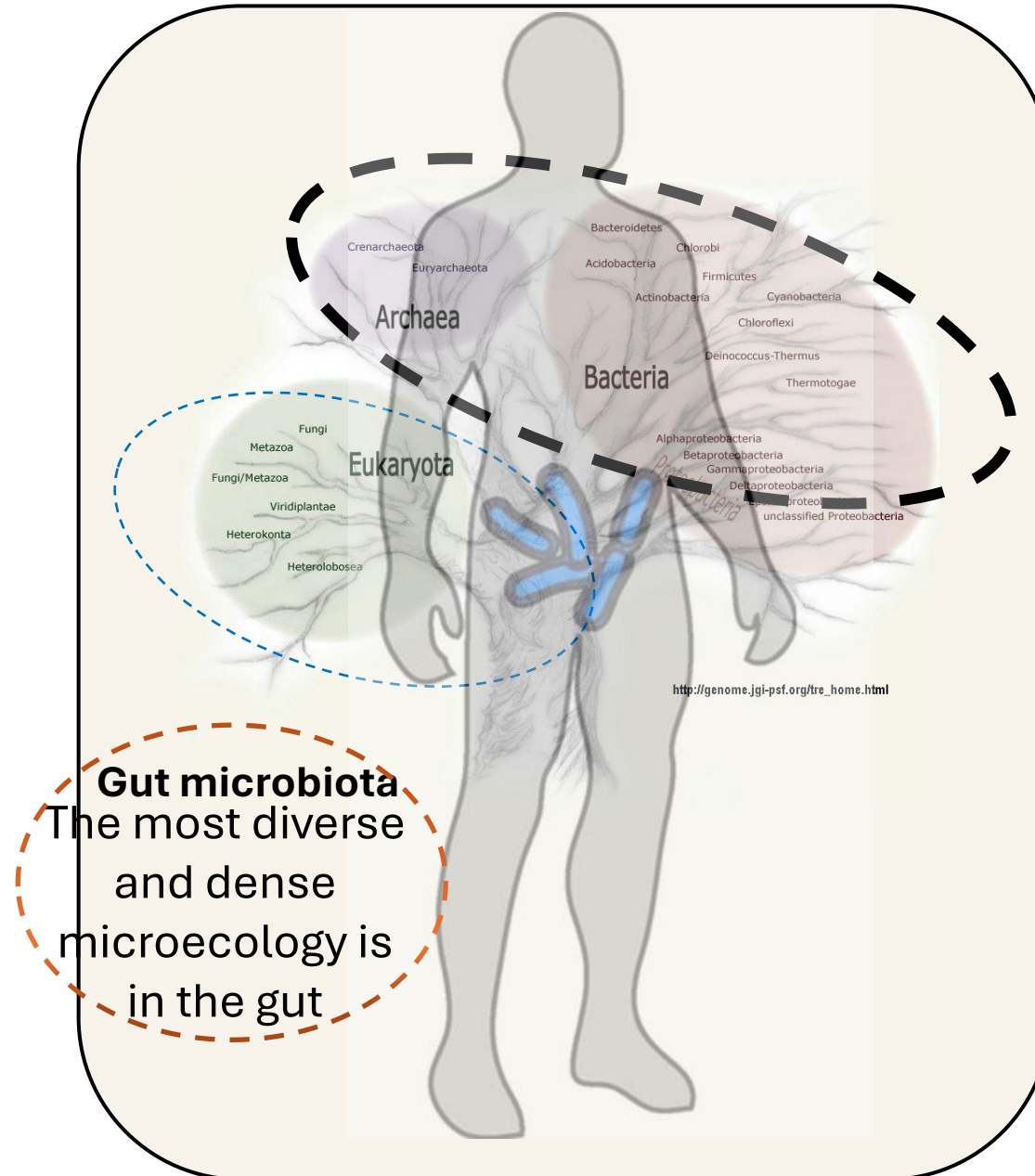
Microbiota is all microbes living in an environment

Microbiome is the sum of the microbes and their genomic elements in a particular environment



# The human microbiome

**Human cell /  
microorganisms  
cell  
50 / 50**



**95% of our  
bacteria are  
located in  
the GI tract**

# The human microbiome in health and disease



MetaHIT  
(Metagenomics of Human  
Intestinal Tract)

2008



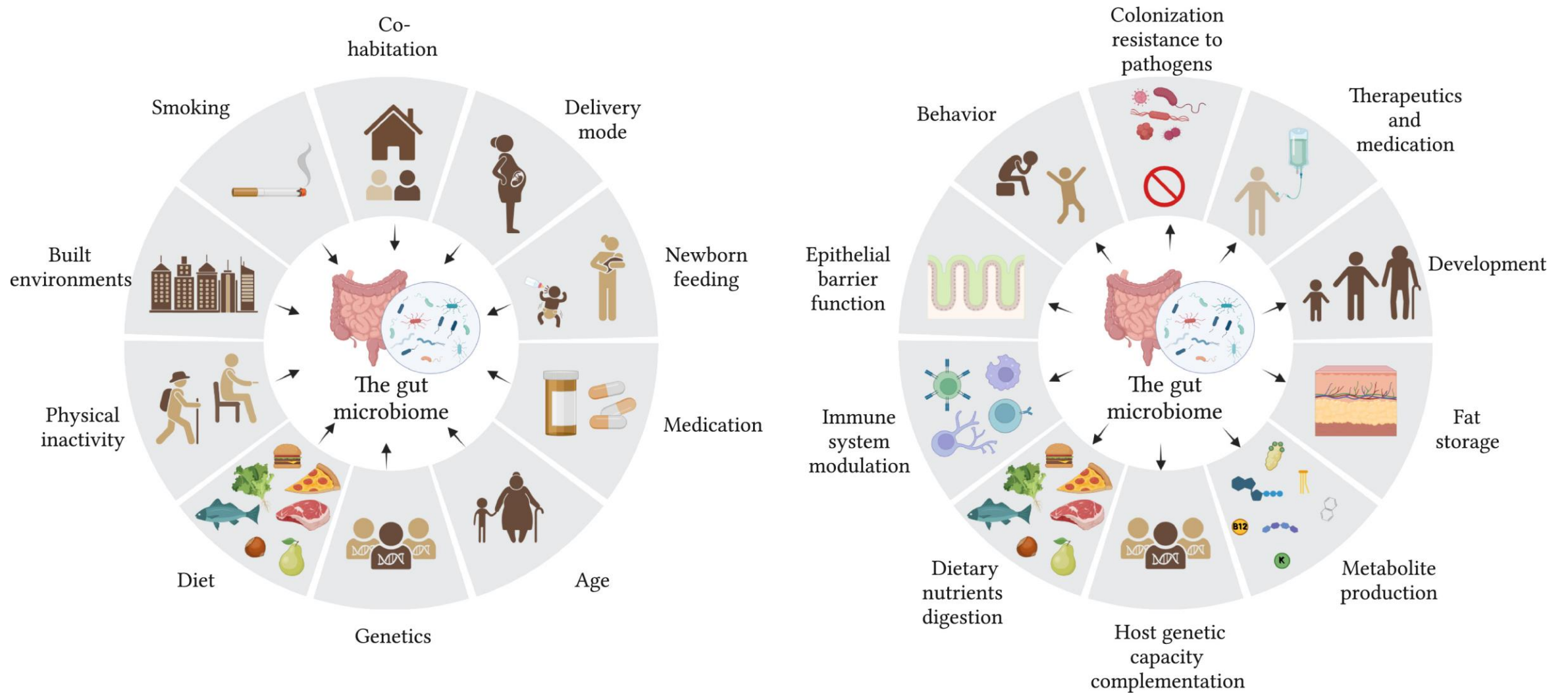
HMP  
(The NIH Human  
Microbiome Project)

2007



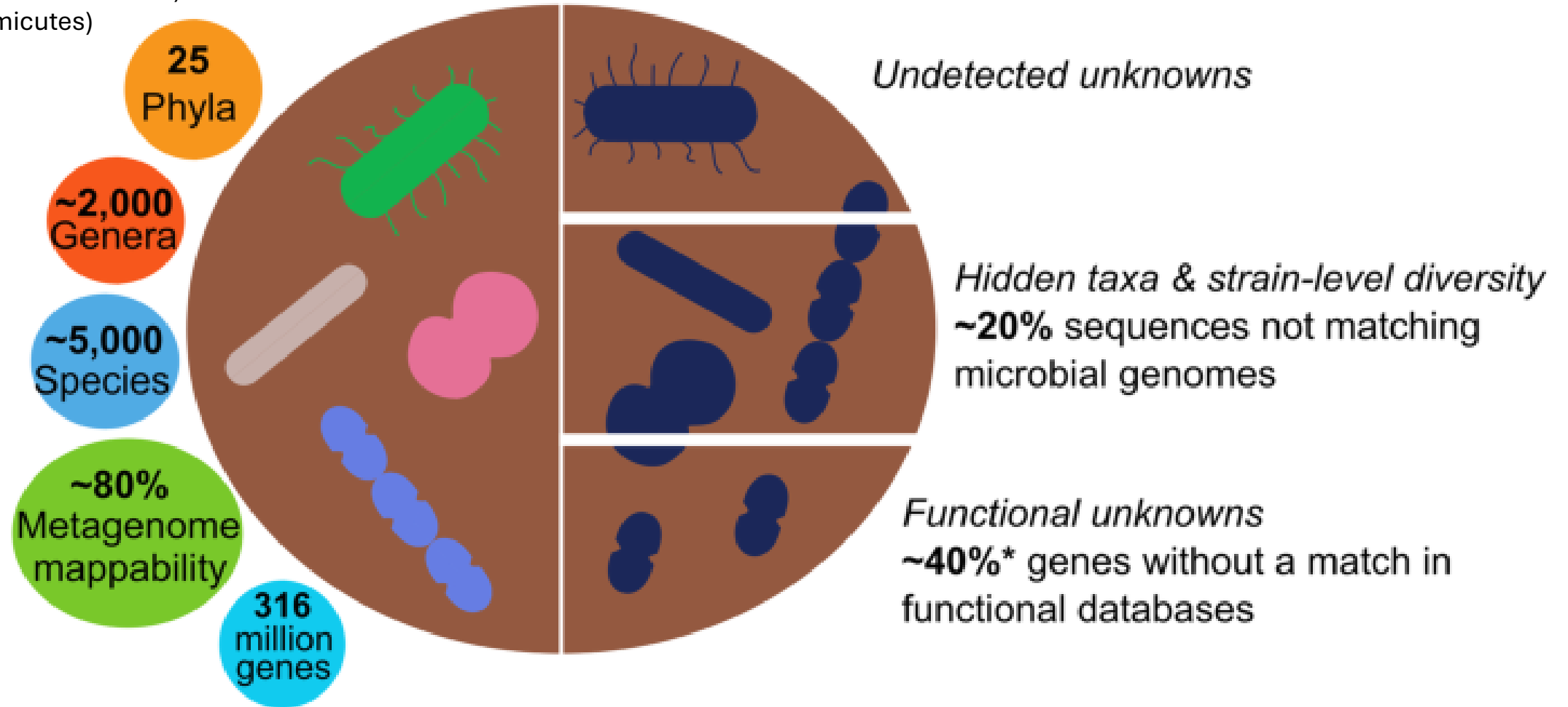
**Healthy gut microbiome boundaries?**

# The human microbiome in health and disease



# Human microbiome knowns

Actinomycetota (or Actinobacteria)  
Bacteroidota (or Bacteroidetes)  
Bacillota (or Firmicutes)  
Proteobacteria



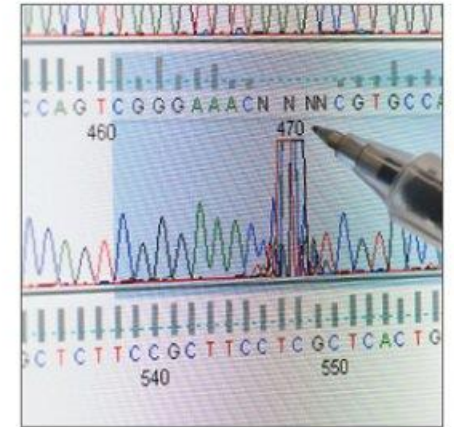
# Back in the days....



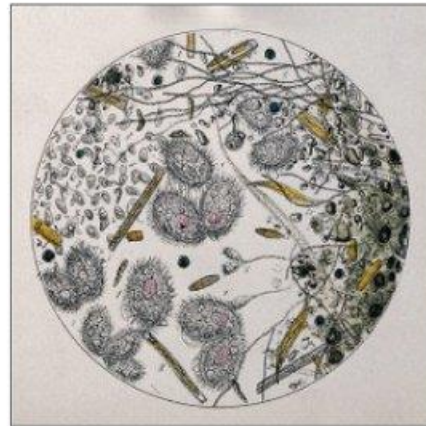
**mid-1600s:**  
First microbes  
described



**1800s – Present:**  
Culture, staining,  
and microscopy  
used to study  
microbes that can  
be cultured



**circa 1600:**  
Microscope  
invented



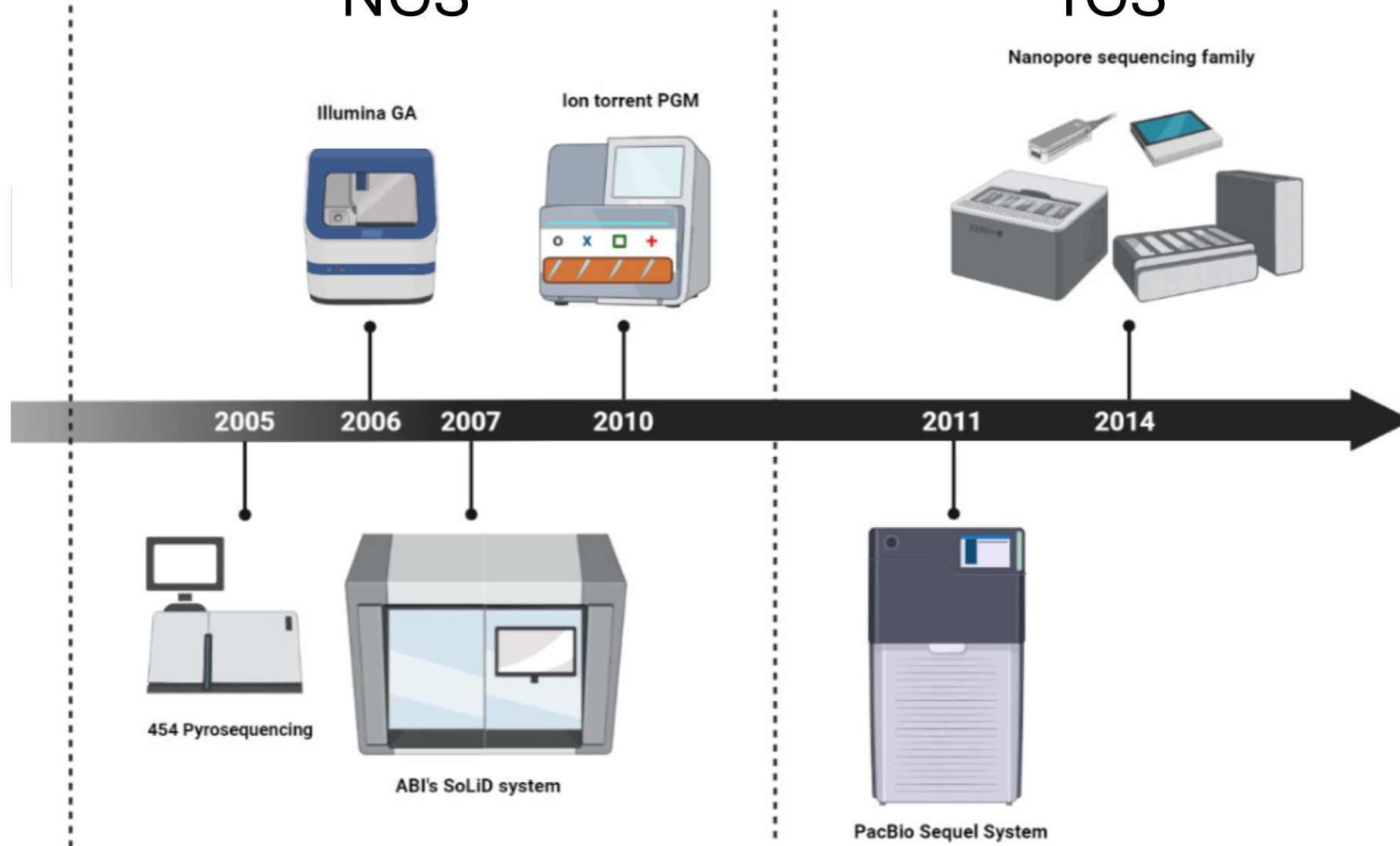
**1800s:**  
Connection  
made between  
microbes and  
disease



**1990s:**  
DNA sequencing  
becomes available,  
allowing study of  
microbes that cannot  
be cultured

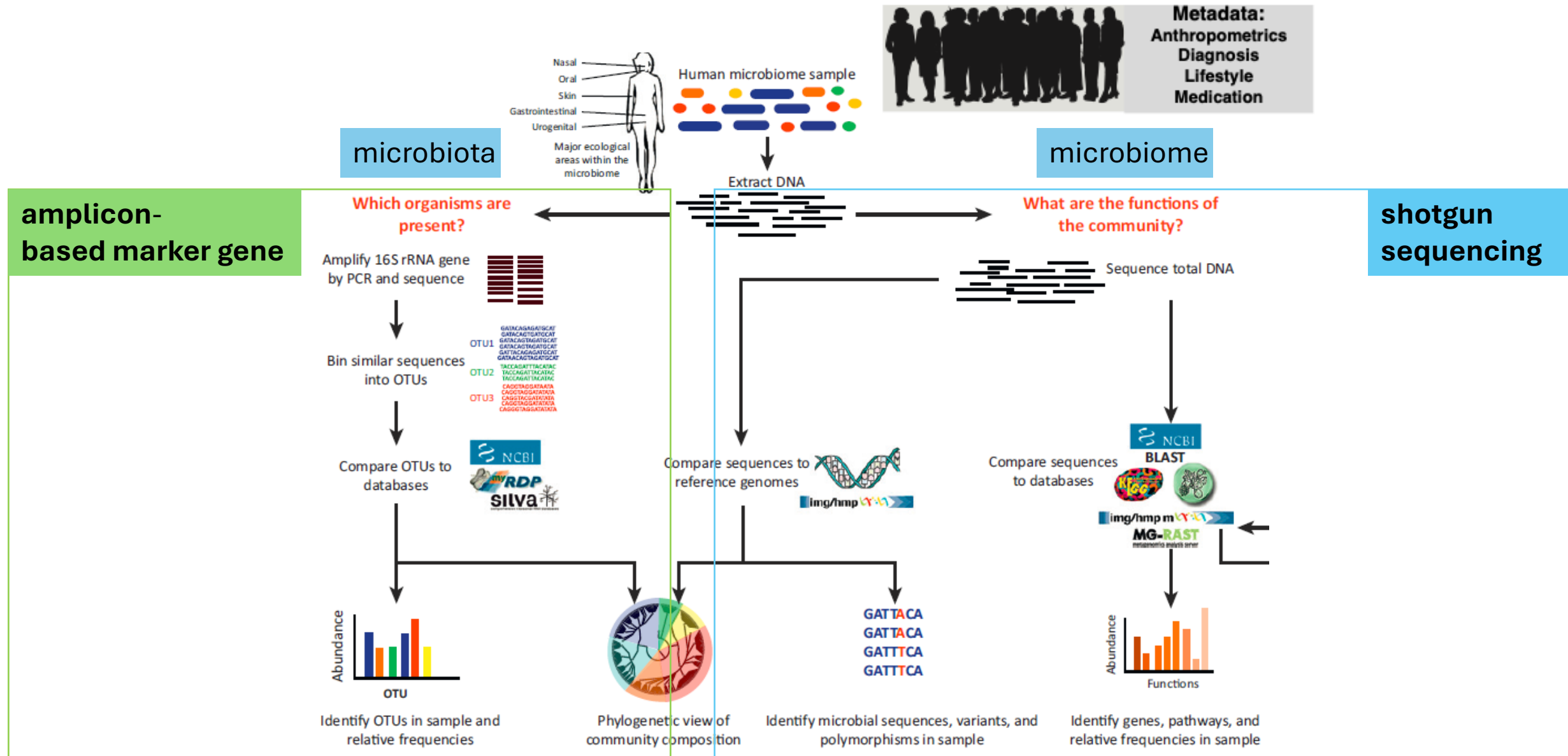
# NGS

# TGS





# How to study human microbial communities?



# How to study human microbial communities?

**FEMS MICROBIOLOGY REVIEWS**  
High-quality reviews from leaders in the field

[FEMS Microbiol Rev.](#) 2017 Aug; 41(Suppl 1): S154–S167.

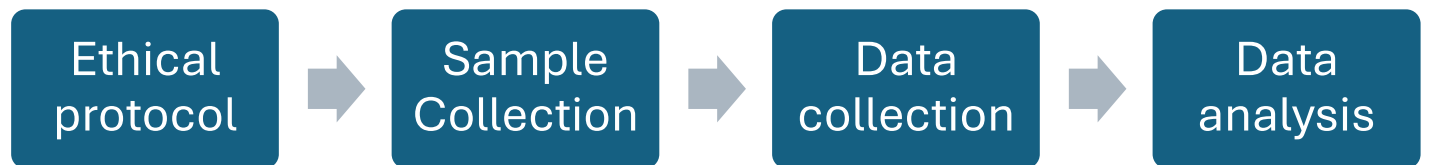
Published online 2017 Jun 30. doi: [10.1093/femsre/fux027](https://doi.org/10.1093/femsre/fux027)

PMCID: PMC7207147

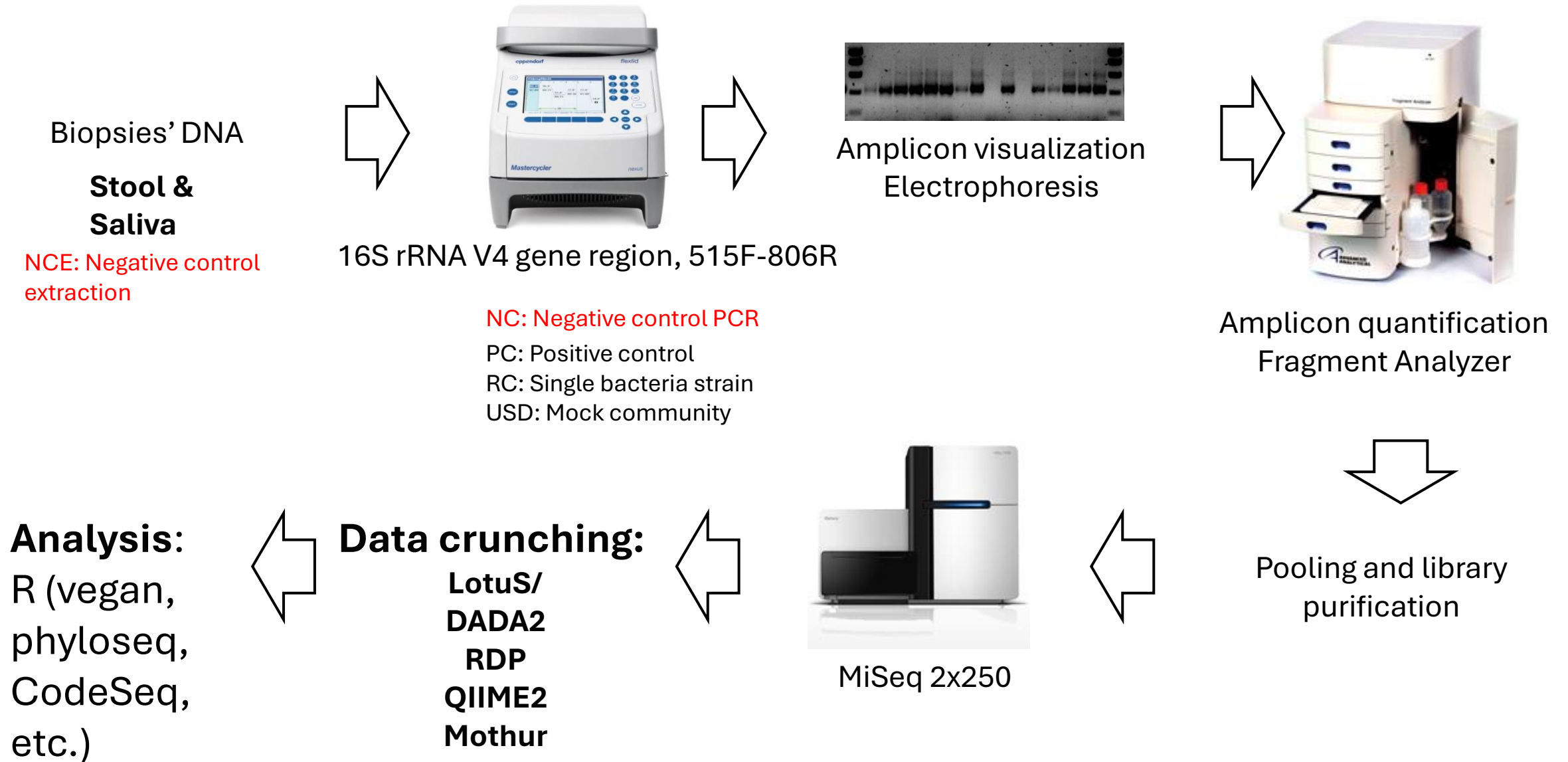
PMID: [28830090](https://pubmed.ncbi.nlm.nih.gov/28830090/)

## Practical considerations for large-scale gut microbiome studies

[Doris Vandeputte](#),<sup>1,2,3</sup> [Raul Y. Tito](#),<sup>1,2,3</sup> [Rianne Vanleeuwen](#),<sup>4</sup> [Gwen Falony](#),<sup>1,2,3</sup> and [Jeroen Raes](#)<sup>1,2</sup>

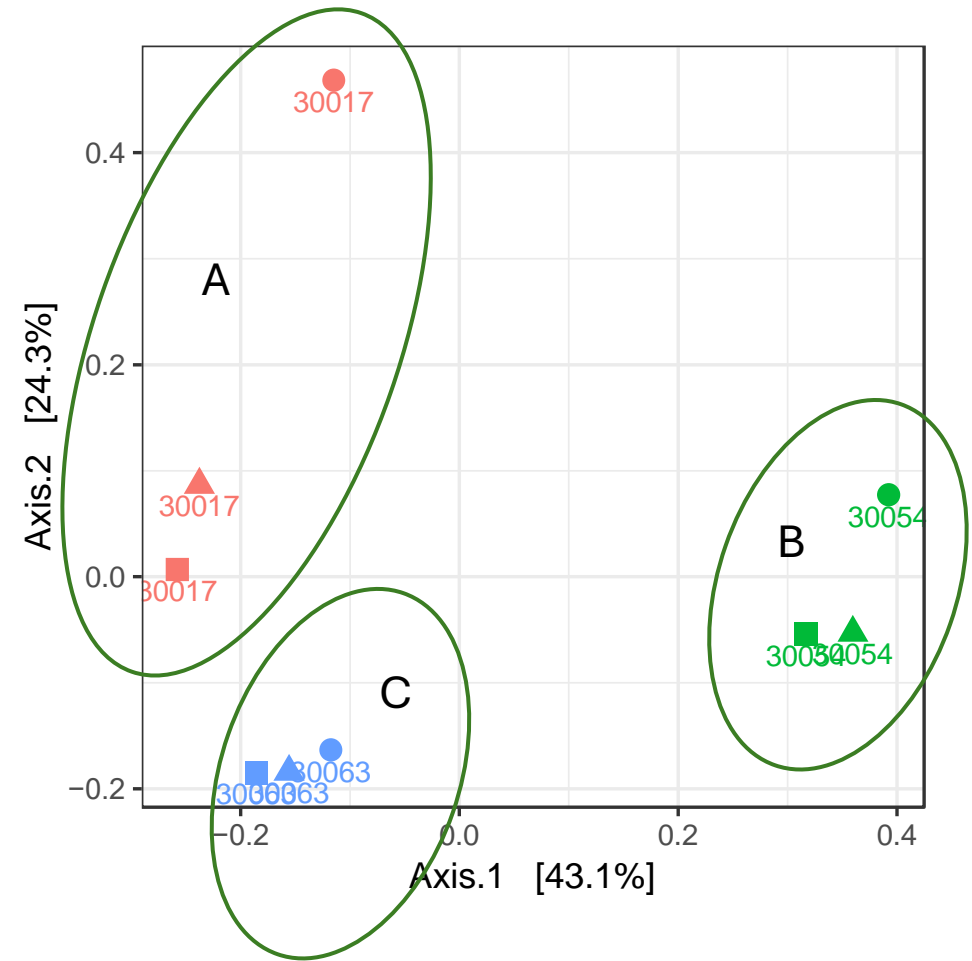
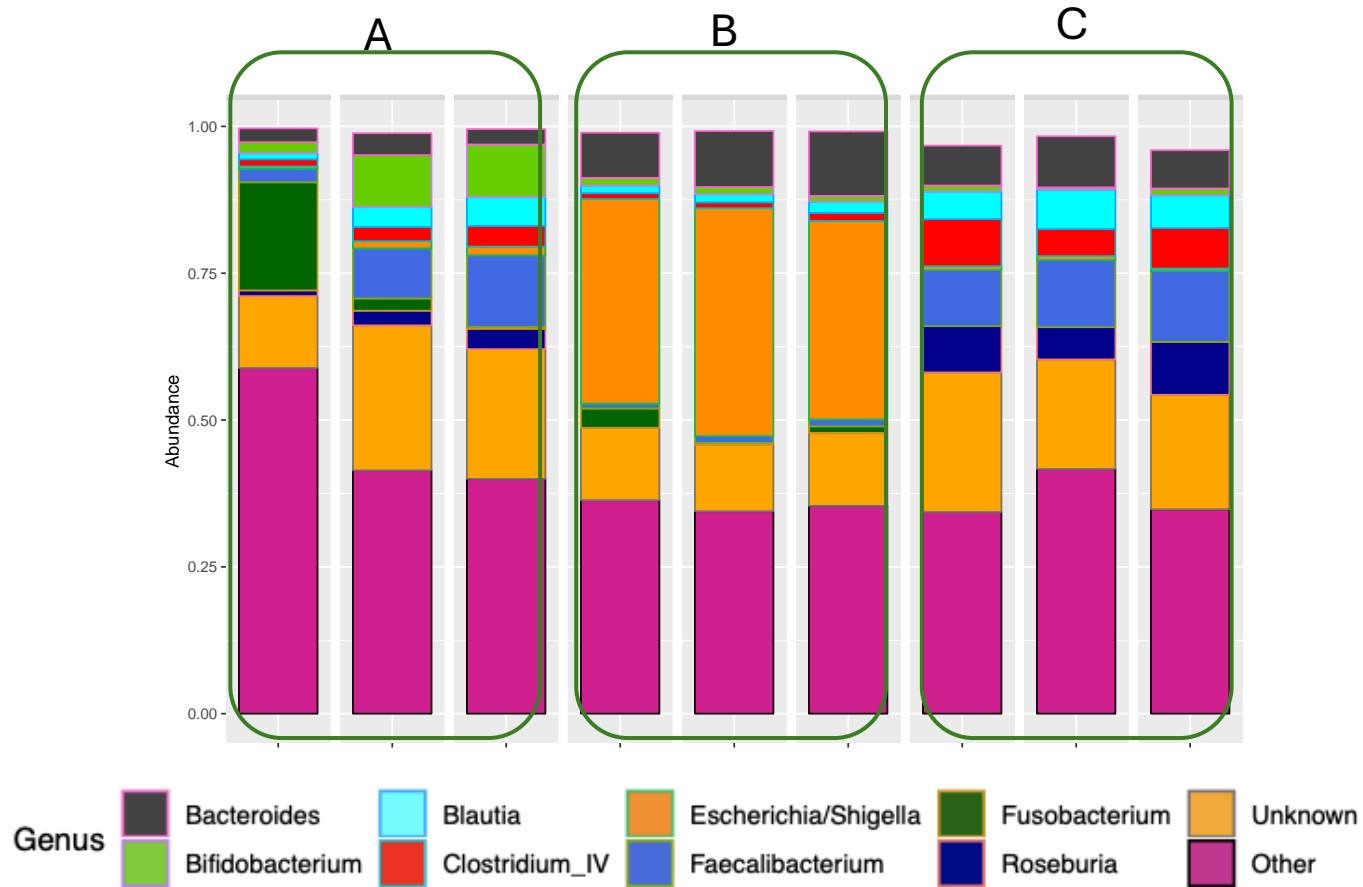


# Methodology / 16S rRNA gene amplicon sequencing

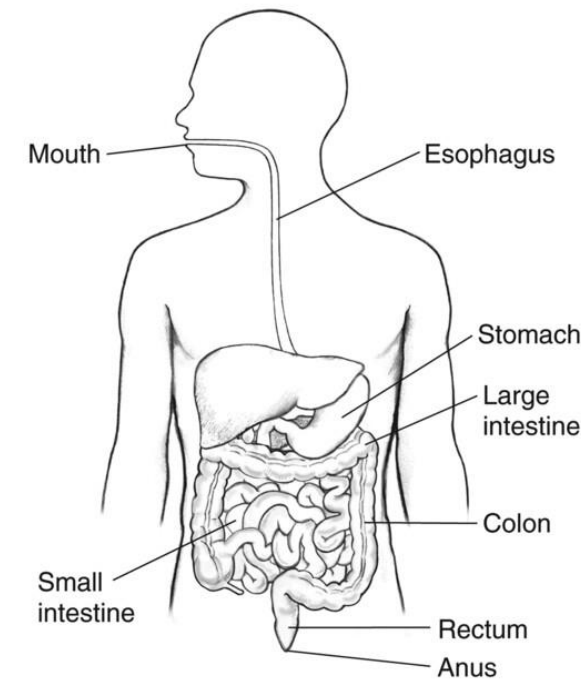
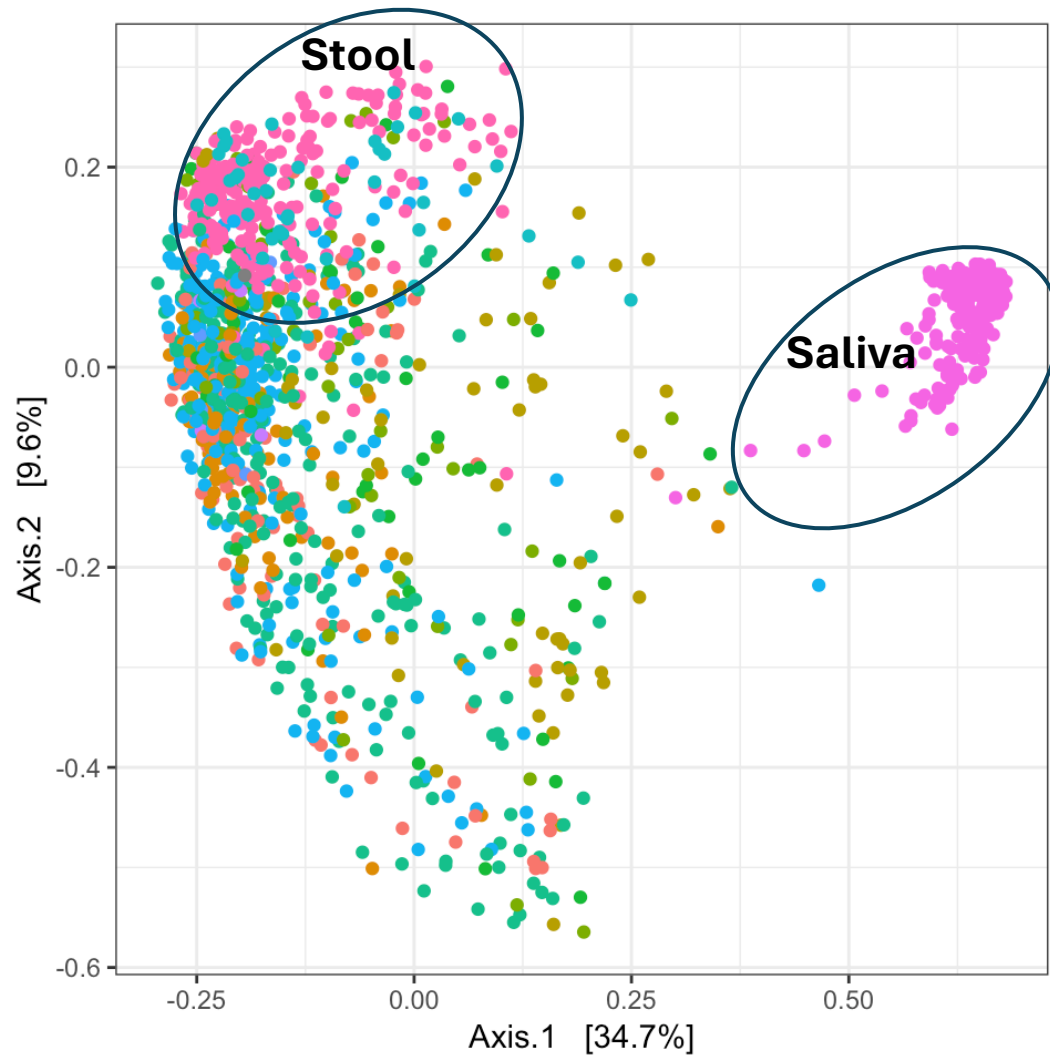


# Microbial profiles

Nine microbial profiles using 16S rRNA V4 gene marker



# Diversity of intestinal microbial profiles



Bray-Curtis distance dissimilarity

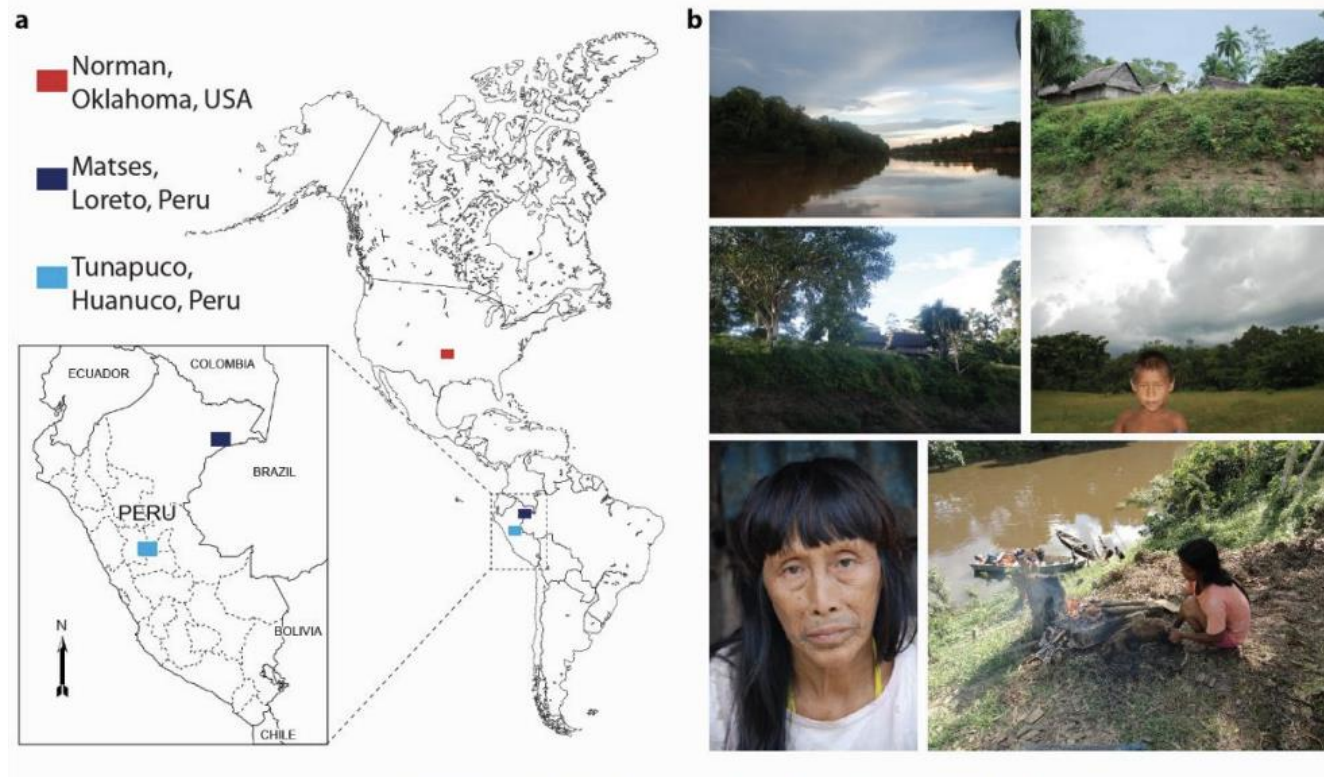
Ordination was performed at the genus level, 10000 reads per sample

# Studies

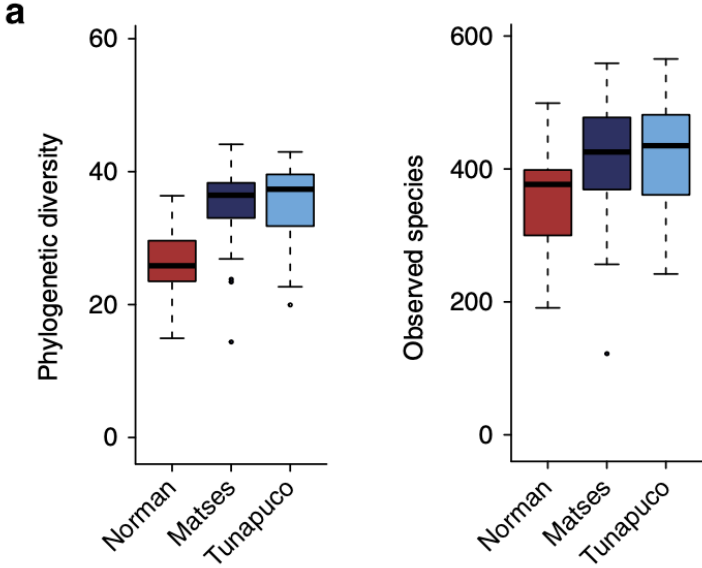
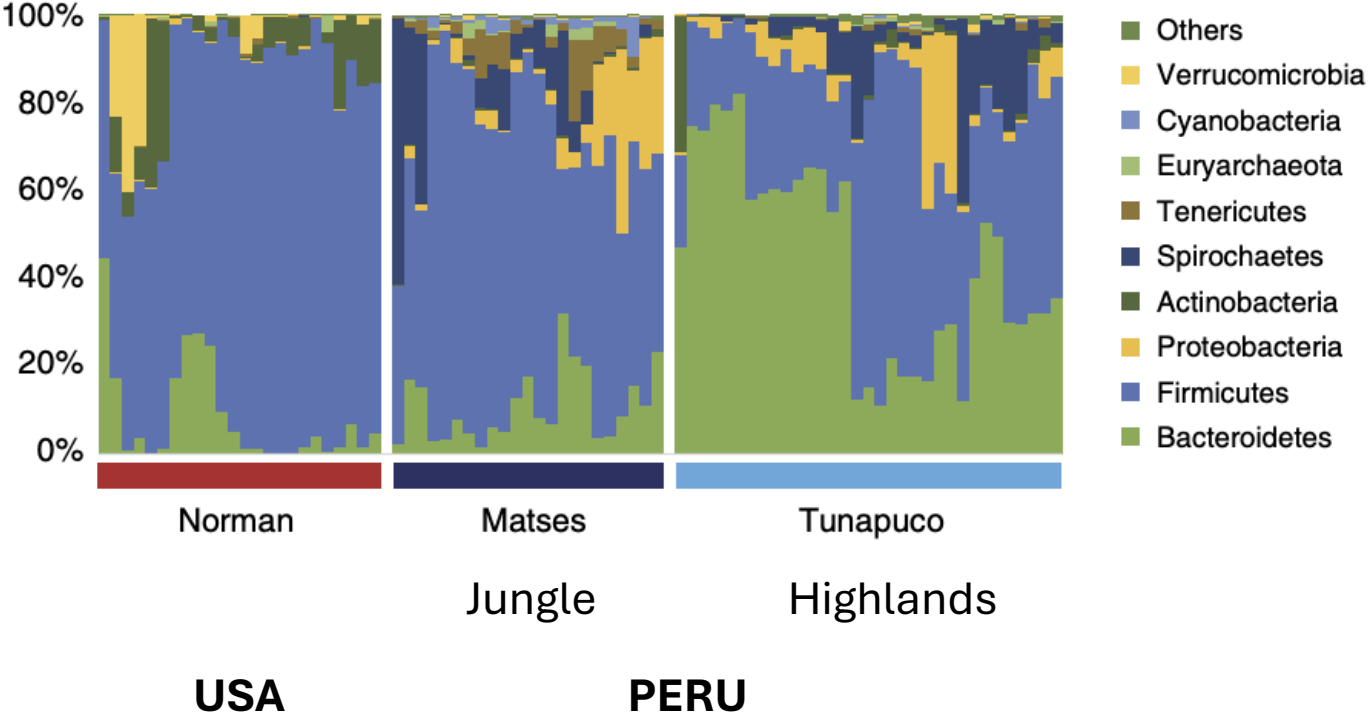
- Bacteria archaea focus

# Subsistence strategies in traditional societies distinguish gut microbiomes

Alexandra J. Obregon-Tito<sup>1,2,3,\*</sup>, Raul Y. Tito<sup>1,2,\*</sup>, Jessica Metcalf<sup>4</sup>, Krithivasan Sankaranarayanan<sup>1</sup>, Jose C. Clemente<sup>5</sup>, Luke K. Ursell<sup>4</sup>, Zhenjiang Zech Xu<sup>4</sup>, Will Van Treuren<sup>4</sup>, Rob Knight<sup>6</sup>, Patrick M. Gaffney<sup>7</sup>, Paul Spicer<sup>1</sup>, Paul Lawson<sup>1</sup>, Luis Marin-Reyes<sup>8</sup>, Omar Trujillo-Villarreal<sup>8</sup>, Morris Foster<sup>9</sup>, Emilio Guija-Poma<sup>2</sup>, Luzmila Troncoso-Corzo<sup>2</sup>, Christina Warinner<sup>1</sup>, Andrew T. Ozga<sup>1</sup> & Cecil M. Lewis<sup>1</sup>



# Distinct gut microbial communities among different lifestyles





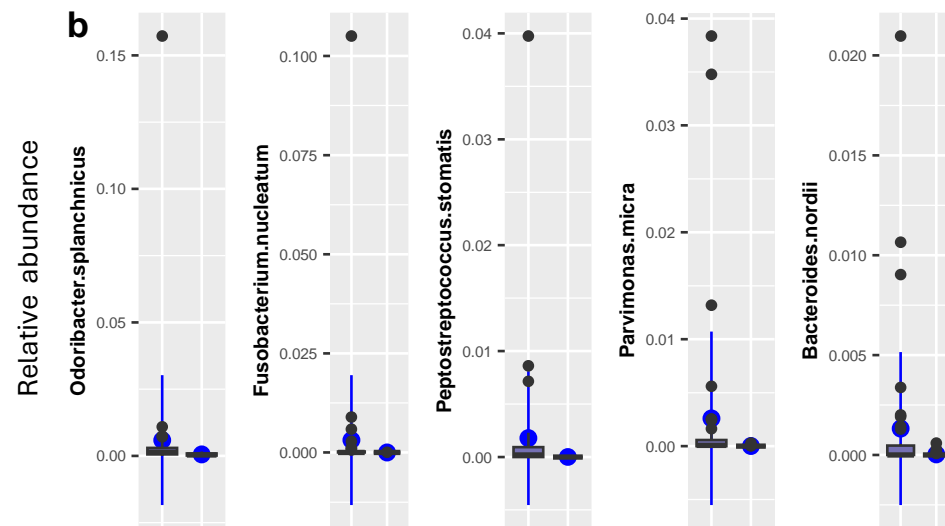
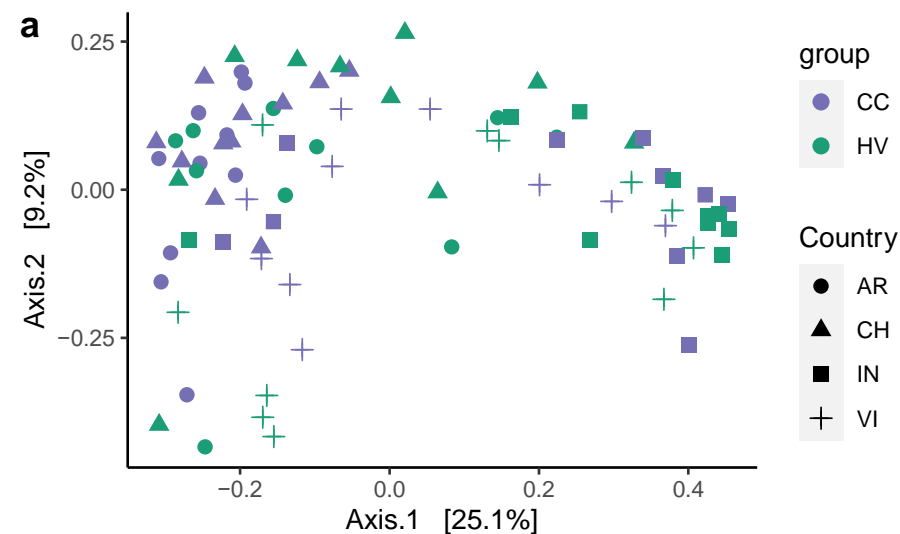
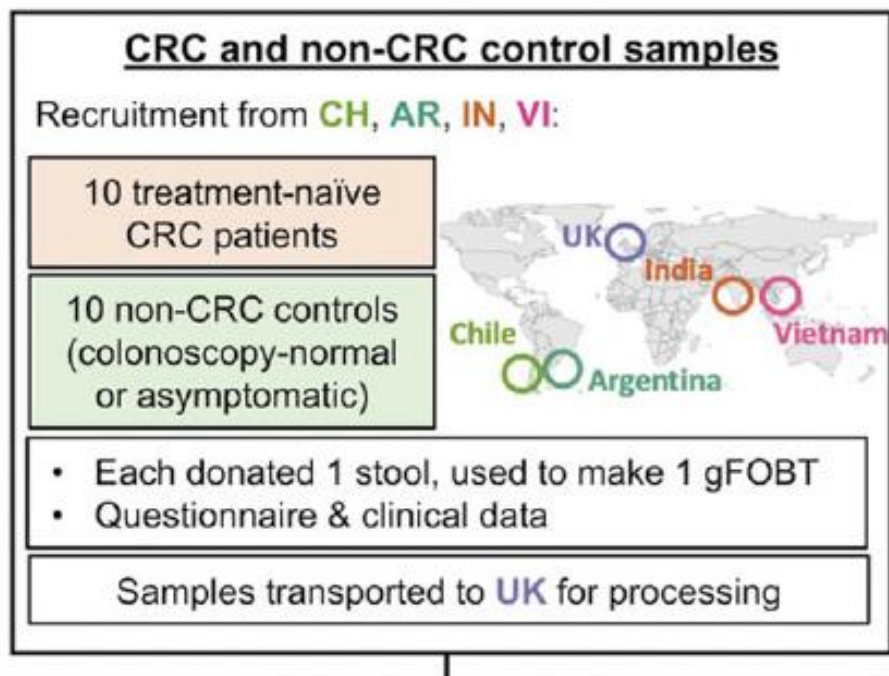
RESEARCH

Open Access

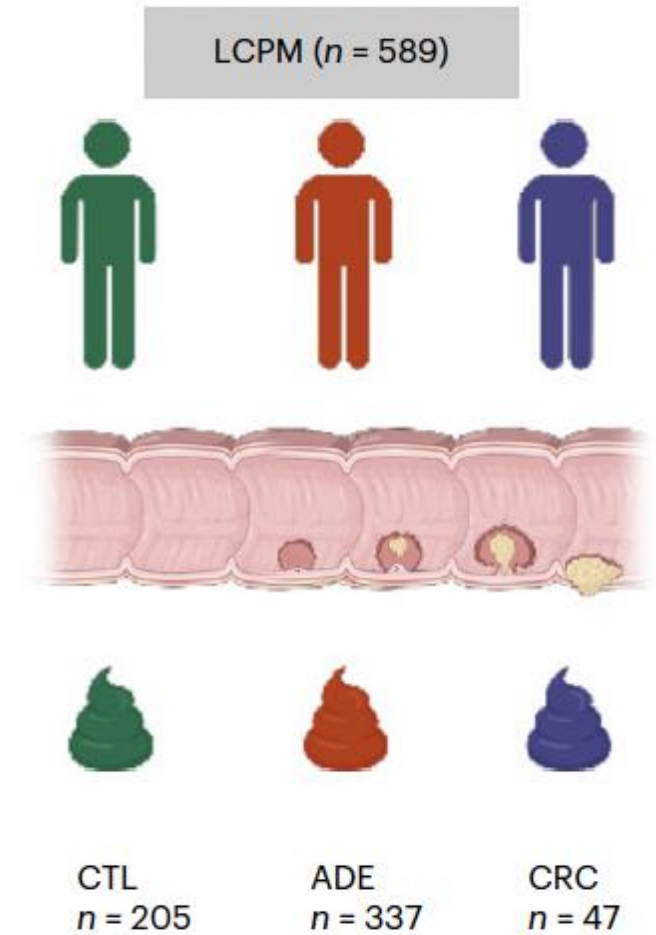
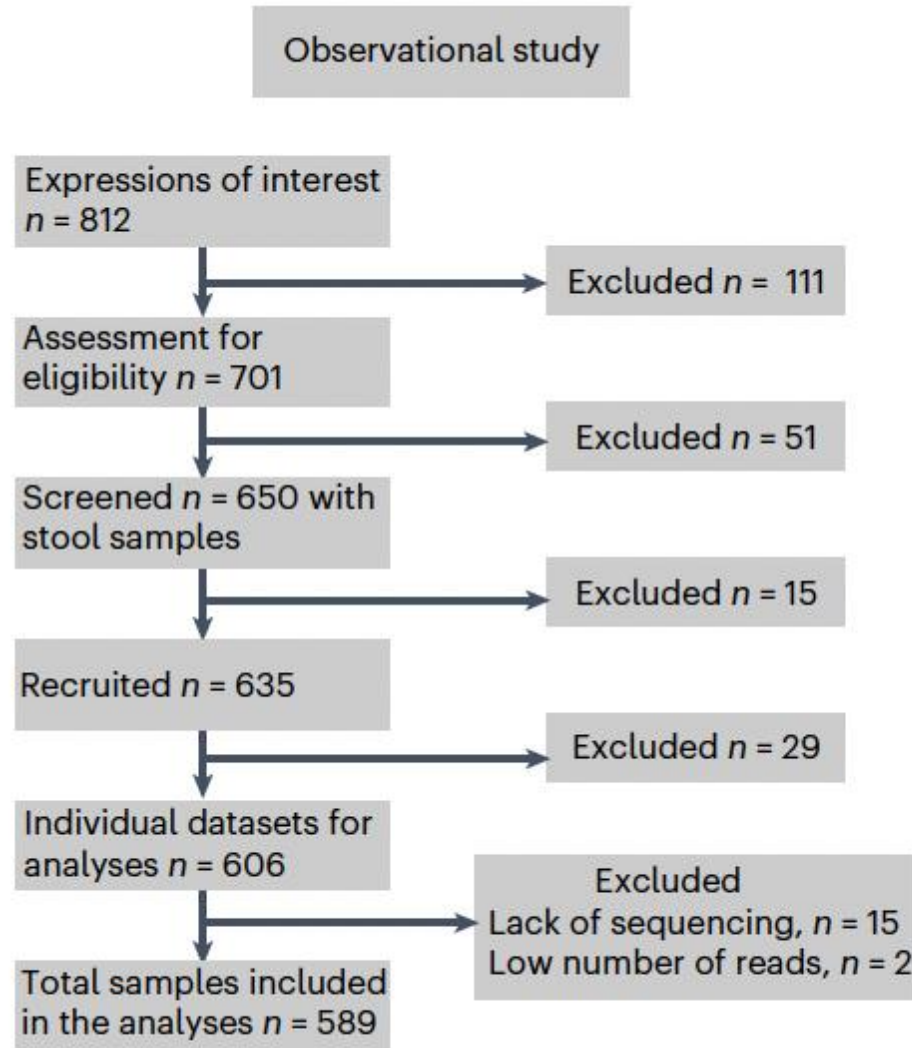
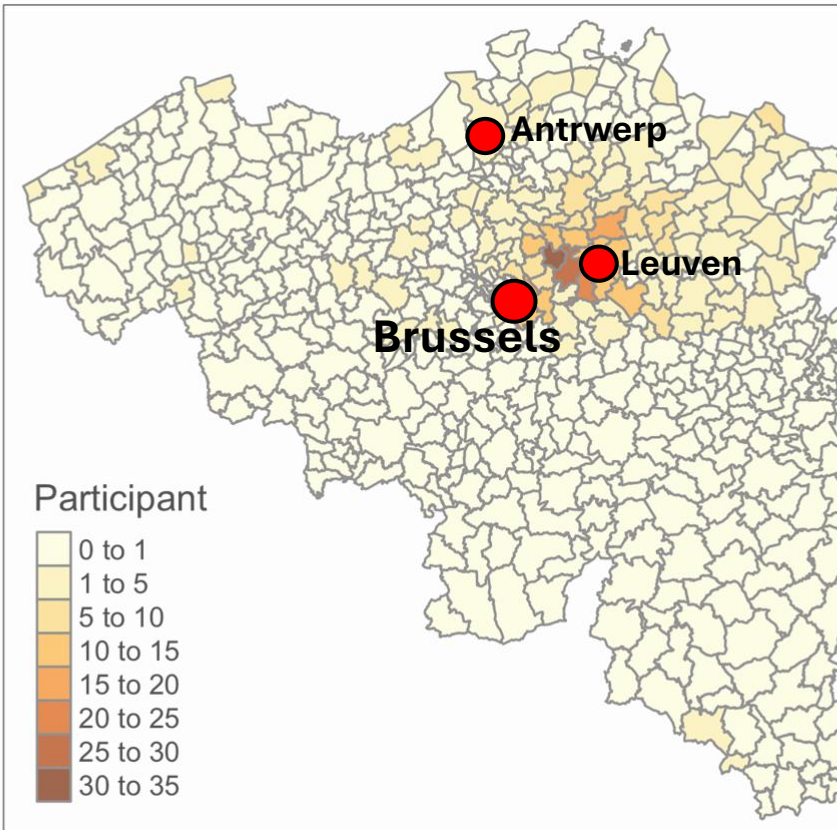


# The colorectal cancer-associated faecal microbiome of developing countries resembles that of developed countries

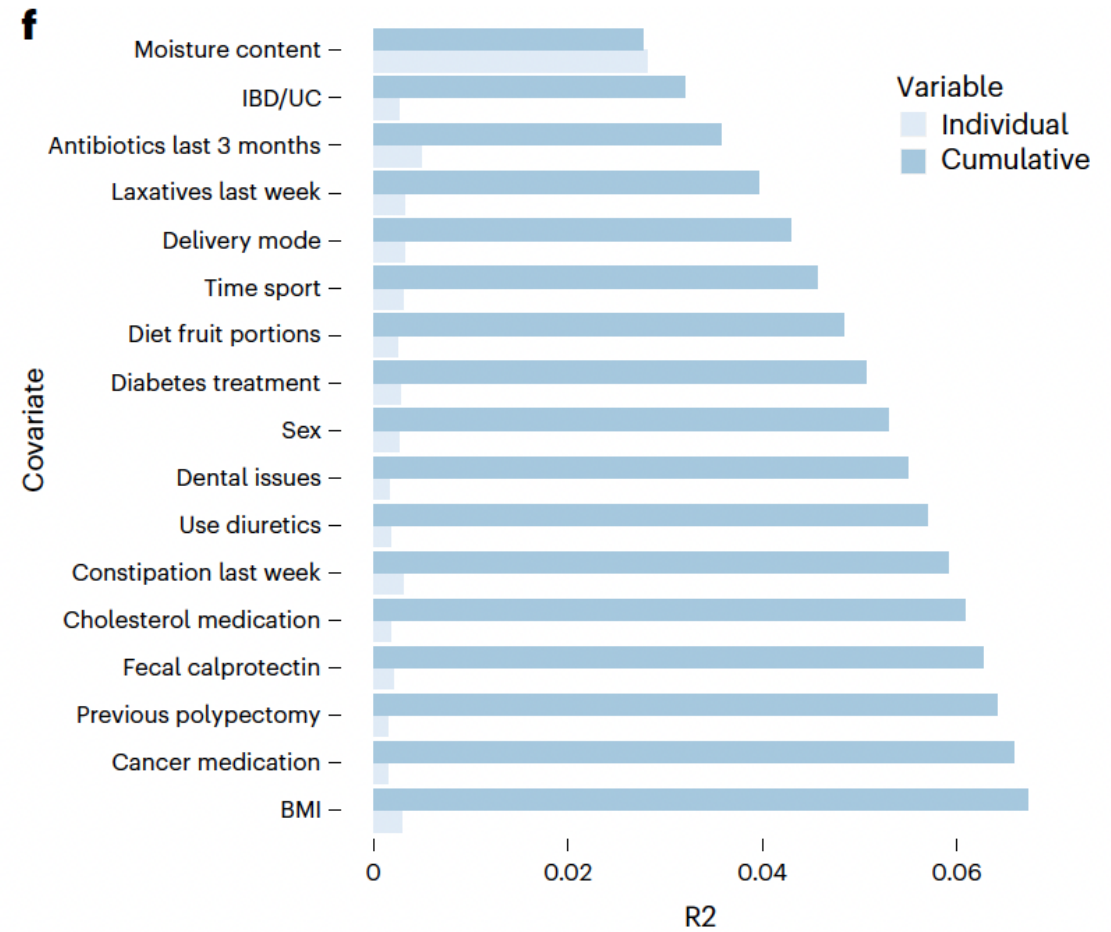
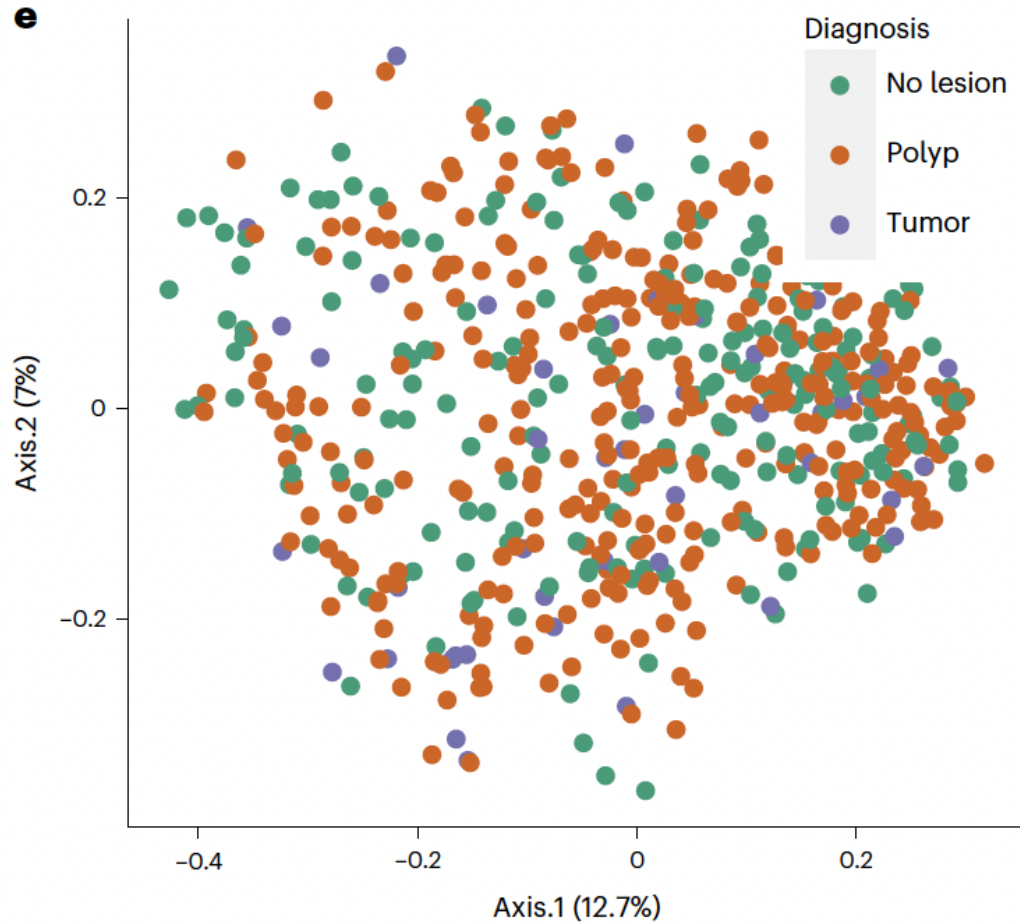
Caroline Young<sup>1\*</sup>, Henry M. Wood<sup>1</sup>, Ramakrishnan Ayloor Seshadri<sup>2</sup>, Pham Van Nang<sup>3</sup>, Carlos Vaccaro<sup>4</sup>, Luis Contreras Melendez<sup>5</sup>, Mayilvahanan Bose<sup>2</sup>, Mai Van Doi<sup>3</sup>, Tamara Alejandra Piñero<sup>4</sup>, Camilo Tapia Valladares<sup>5</sup>, Julieta Arguero<sup>4</sup>, Alba Fuentes Balaguer<sup>1</sup>, Kelsey N. Thompson<sup>6</sup>, Yan Yan<sup>6</sup>, Curtis Huttenhower<sup>6</sup> and Philip Quirke<sup>1</sup>



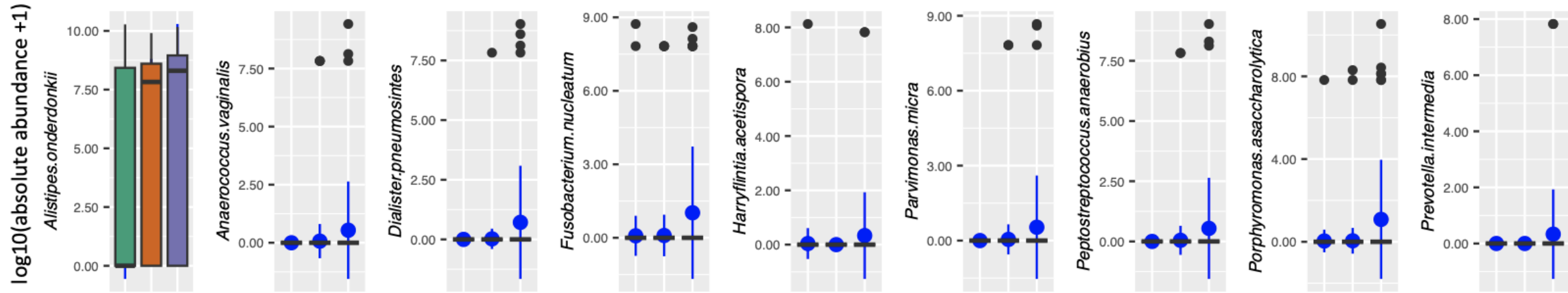
# Colorectal cancer and stool microbial communities in Flanders



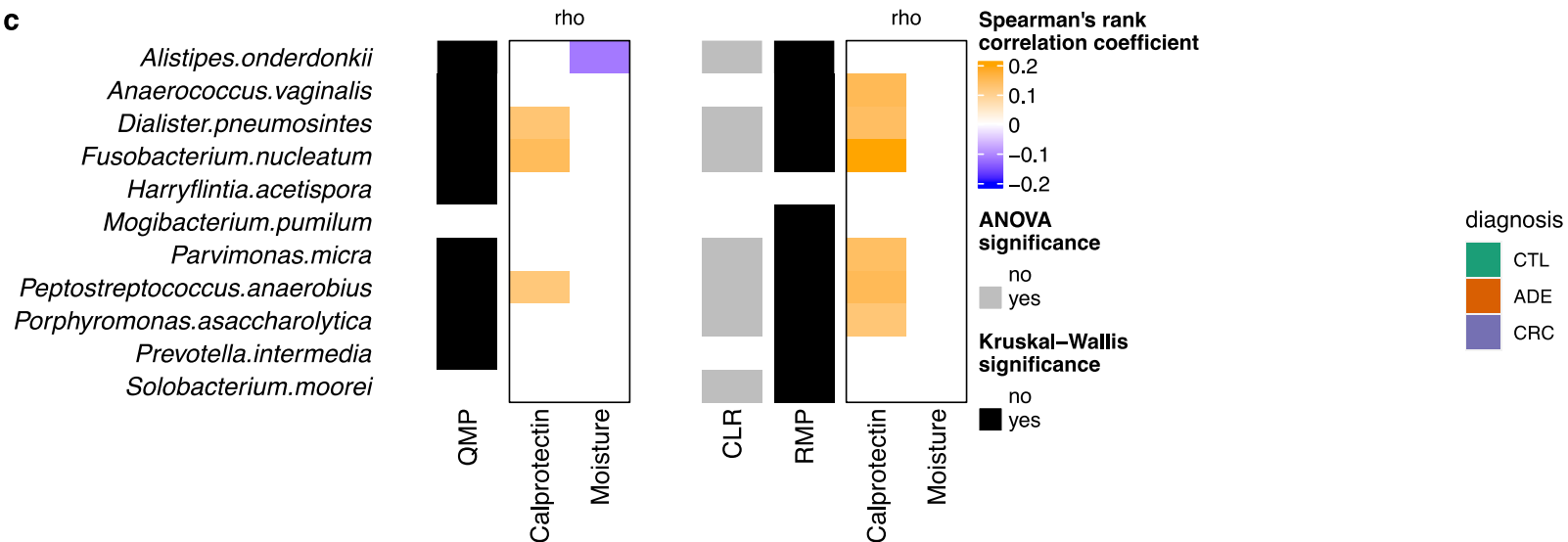
# Microbiota covariates explain close to 7% of the microbial community



# Microbial biomarkers in colorectal cancer progression



**c**



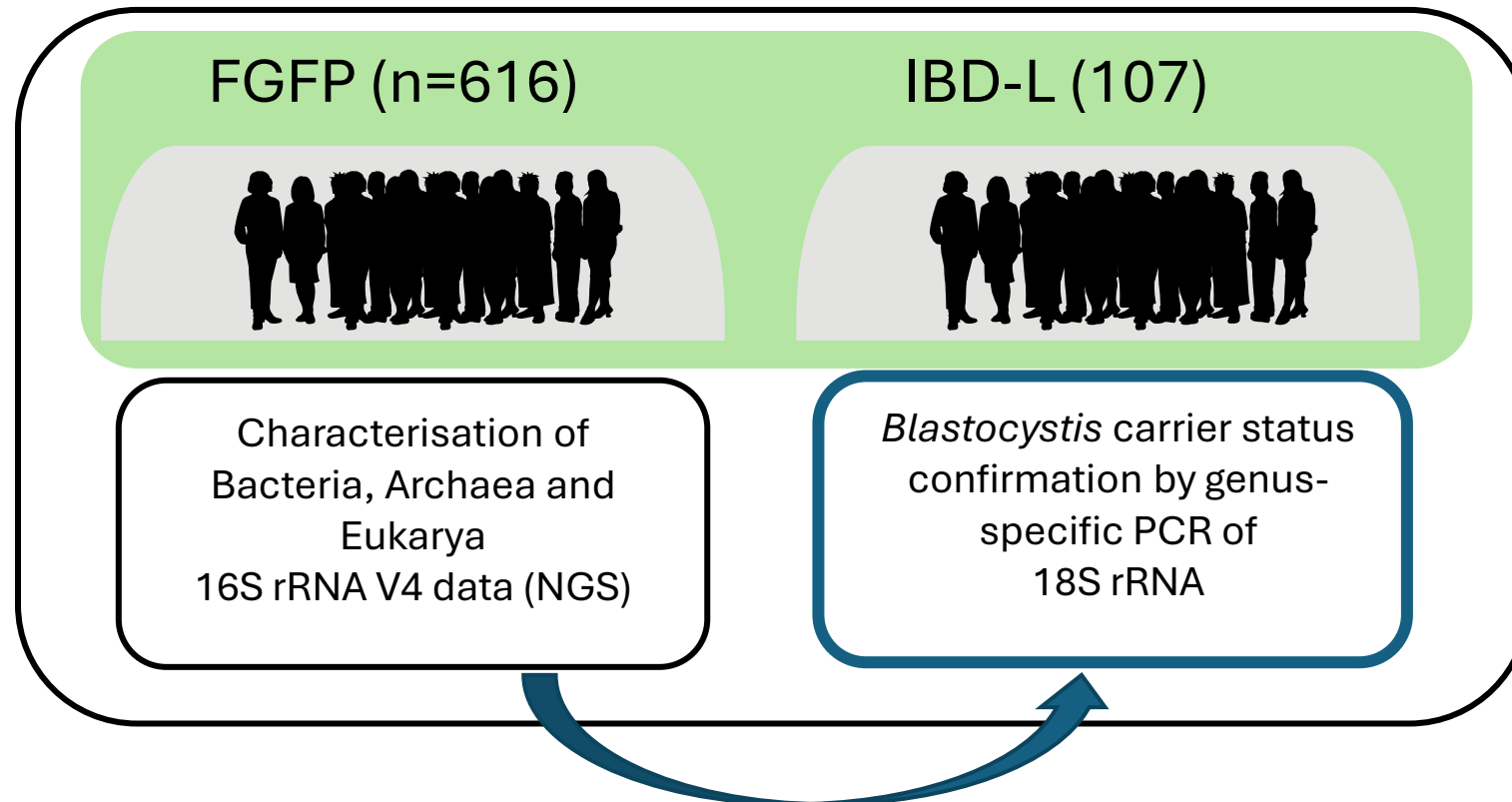
# Studies

- Multi-domain: the other players

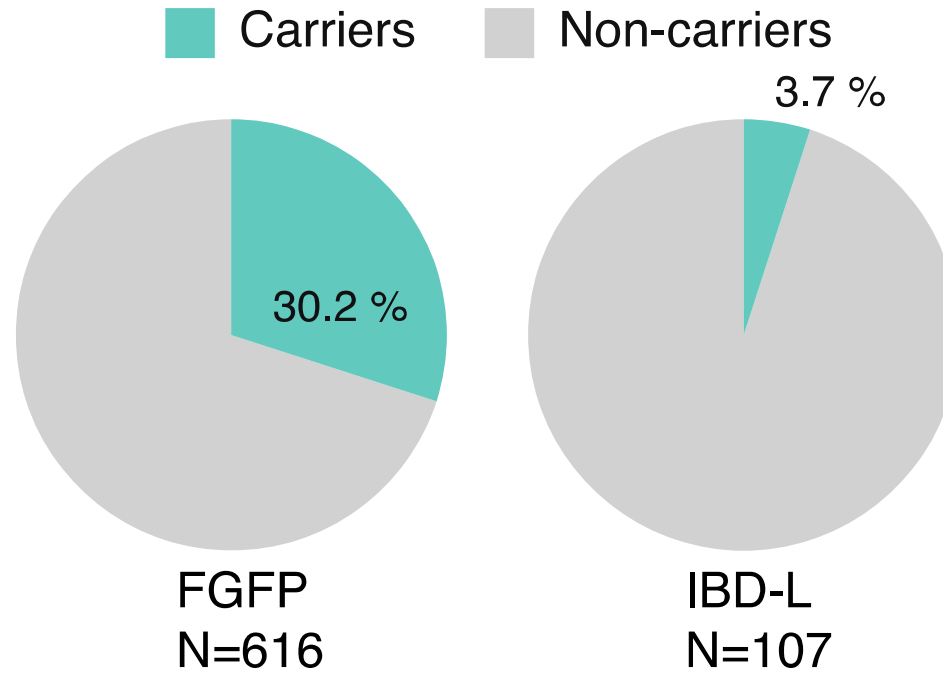


# Population-level analysis of *Blastocystis* subtype prevalence and variation in the human gut microbiota

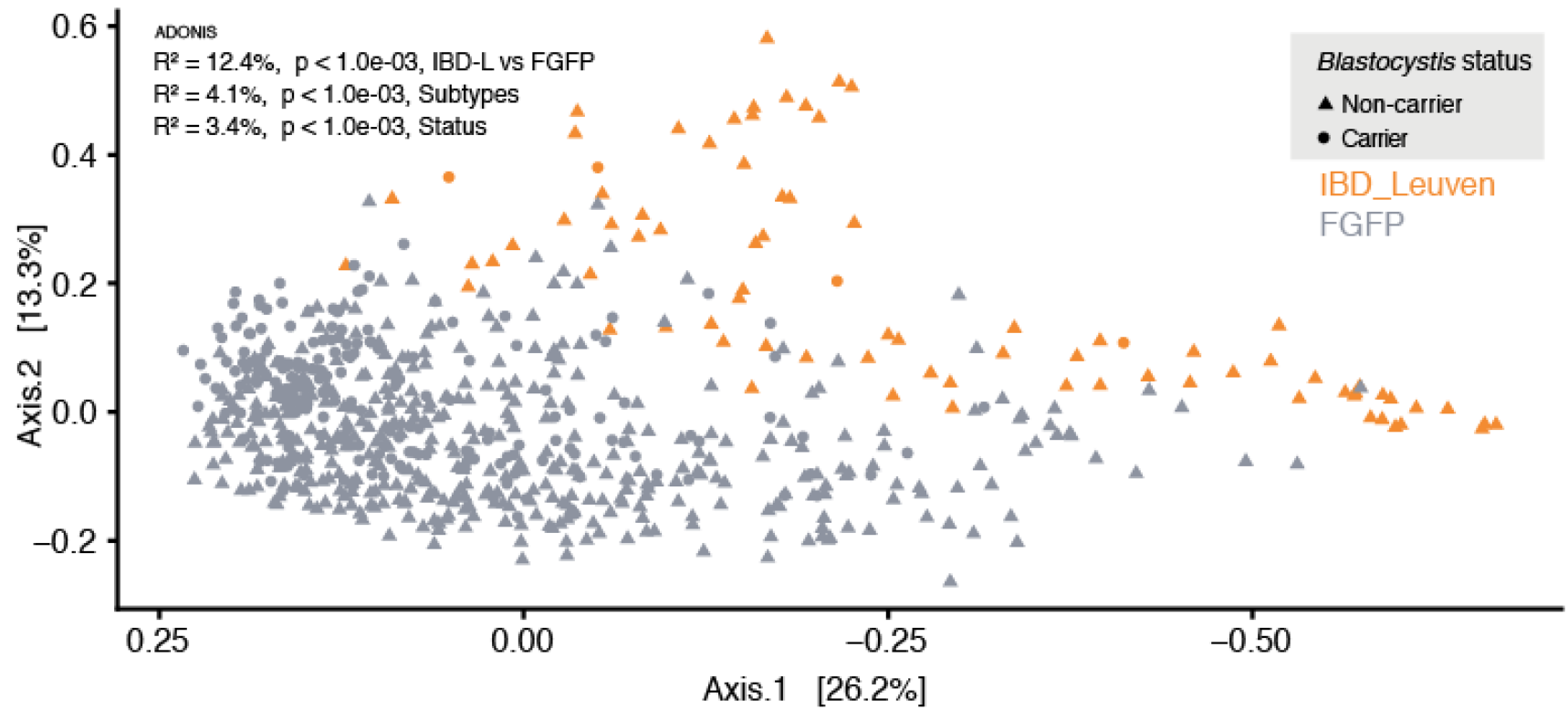
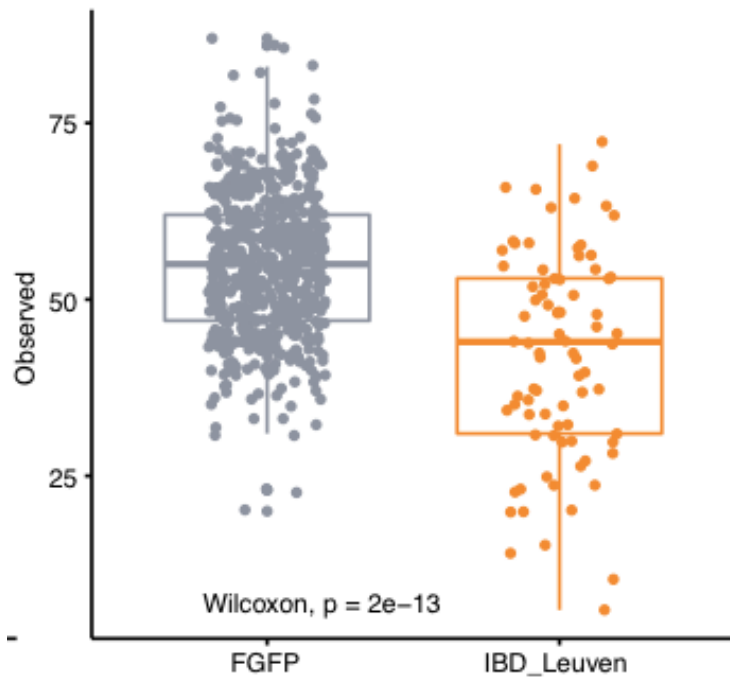
Raul Y Tito,<sup>1,2,3</sup> Samuel Chaffron,<sup>4</sup> Clara Caenepeel,<sup>5</sup> Gipsi Lima-Mendez,<sup>1,2</sup>  
Jun Wang,<sup>1,2</sup> Sara Vieira-Silva,<sup>1,2</sup> Gwen Falony,<sup>1,2</sup> Falk Hildebrand,<sup>6</sup> Youssef Darzi,<sup>1,2</sup>  
Leen Rymenans,<sup>1,2</sup> Chloë Verspecht,<sup>1,2</sup> Peer Bork,<sup>6,7,8</sup> Severine Vermeire,<sup>5</sup>  
Marie Joossens,<sup>1,2</sup> Jeroen Raes<sup>1,2</sup>



# IBD-L and FGFP exhibit distinct Blastocystis prevalence



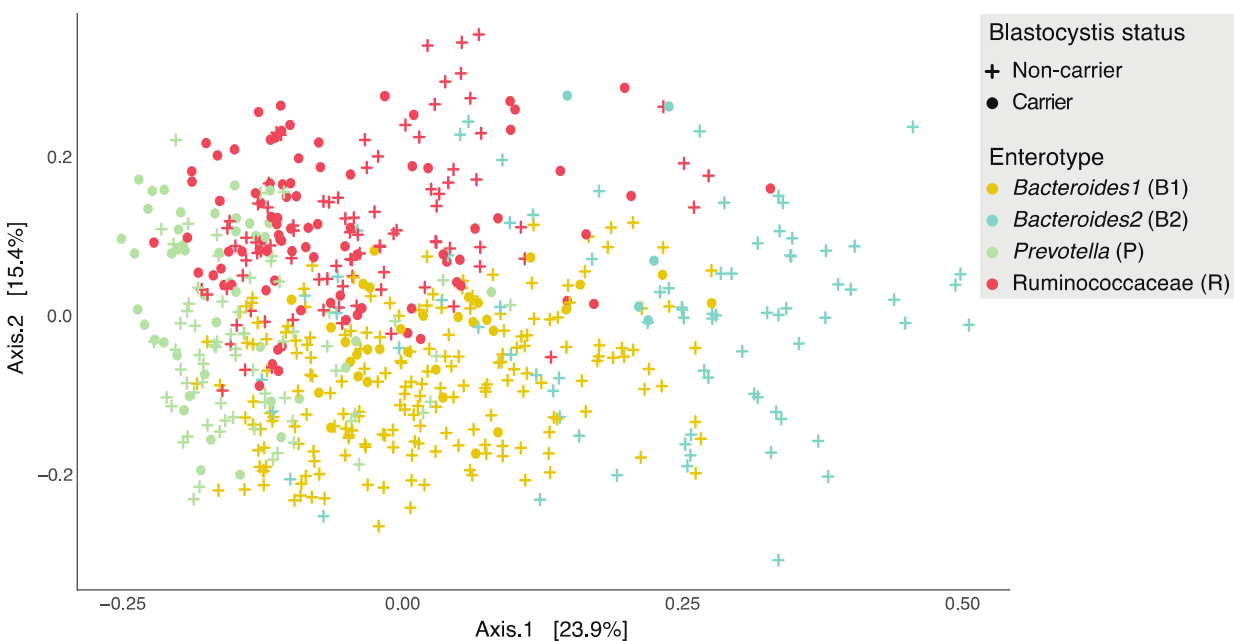
# IBD-L and FGFP exhibit distinct microbial profiles





# Blastocystis status has the highest explanatory power in inter-individual variation of microbiota composition

11 % of inter-individual microbiota variation explained



RESEARCH ARTICLE

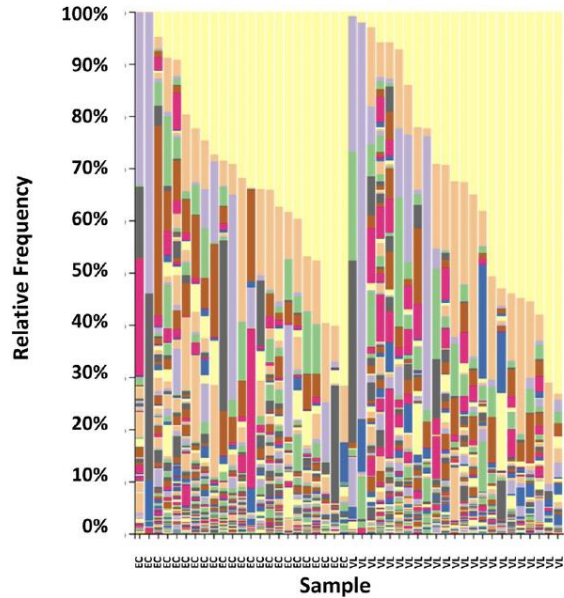
# Meta-taxonomic analysis of prokaryotic and eukaryotic gut flora in stool samples from visceral leishmaniasis cases and endemic controls in Bihar State India

Rachael Lappan<sup>1</sup>, Cajsa Classon<sup>2</sup>, Shashi Kumar<sup>3</sup>, Om Prakash Singh<sup>3</sup>, Ricardo V. de Almeida<sup>4</sup>, Jaya Chakravarty<sup>3</sup>, Poonam Kumari<sup>3</sup>, Sangeeta Kansal<sup>3</sup>, Shyam Sundar<sup>3,†</sup>, Jenefer M. Blackwell<sup>1,5,†\*</sup>

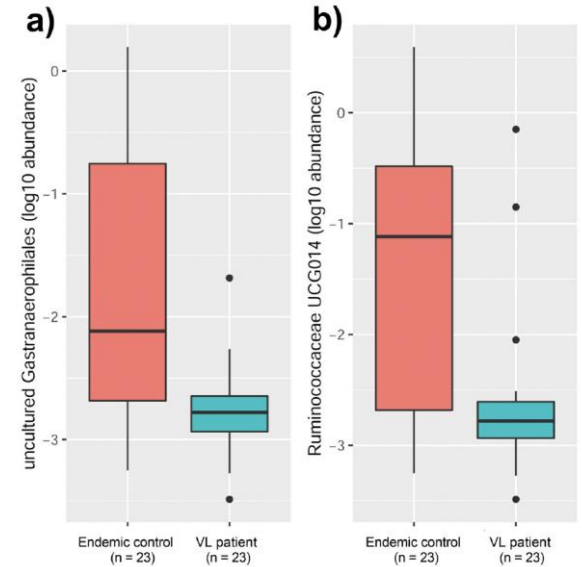
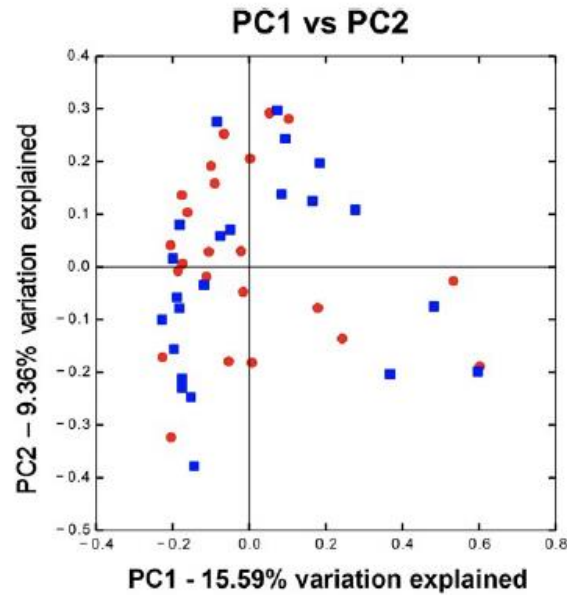
**1** Telethon Kids Institute, The University of Western Australia, Nedlands, Western Australia, Australia, **2** Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solna, Sweden, **3** Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, **4** Departamento de Bioquímica, Centro de Biotecnologías, Universidade Federal do Rio Grande do Norte, Natal, Brazil, **5** Department of Pathology, University of Cambridge, Cambridge, United Kingdom



a) Relative abundance 16S by EC vs VL



a) PCoA Bray-Curtis 16S EC vs VL



## Take home message

- Multiple variables contribute, confound and compound microbial diversity (potential for spurious associations).
- The field is moving towards a more inclusive (i.e. multidomain) but refined and detailed analysis.
- Extensive metadata is required for improved analysis.

# Questions