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The U.S. Government's Global Hunger & Food Security Initiative

Legumes and the microbiota in young Malawian children

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2017 Grain Legumes Conference
Ouagadougou, Burkina Faso, August 14, 2017

Legume Innovation Lab

Feed the Future Innovation Lab for Collaborative Research on Grain Legumes

USAID
FROM THE AMERICAN PEOPLE

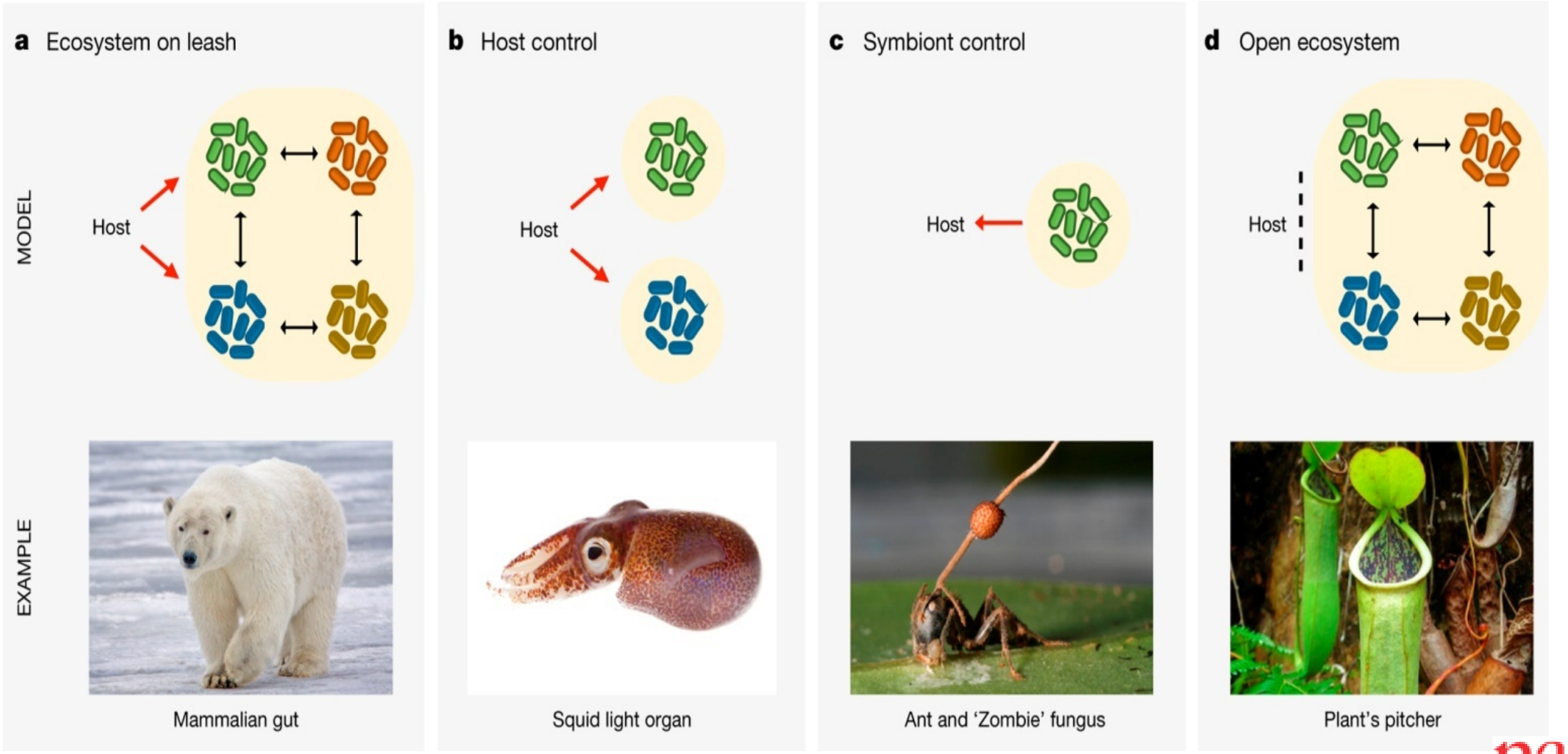
Microbiome

Microbiome is a complex and dynamic system in which species are in continual turnover.

Humans carry massive and diverse communities of symbiotic microbes and they colonizes almost every surface of our body.

Picture source– www.readworks.org

Models of host–microbiome interaction



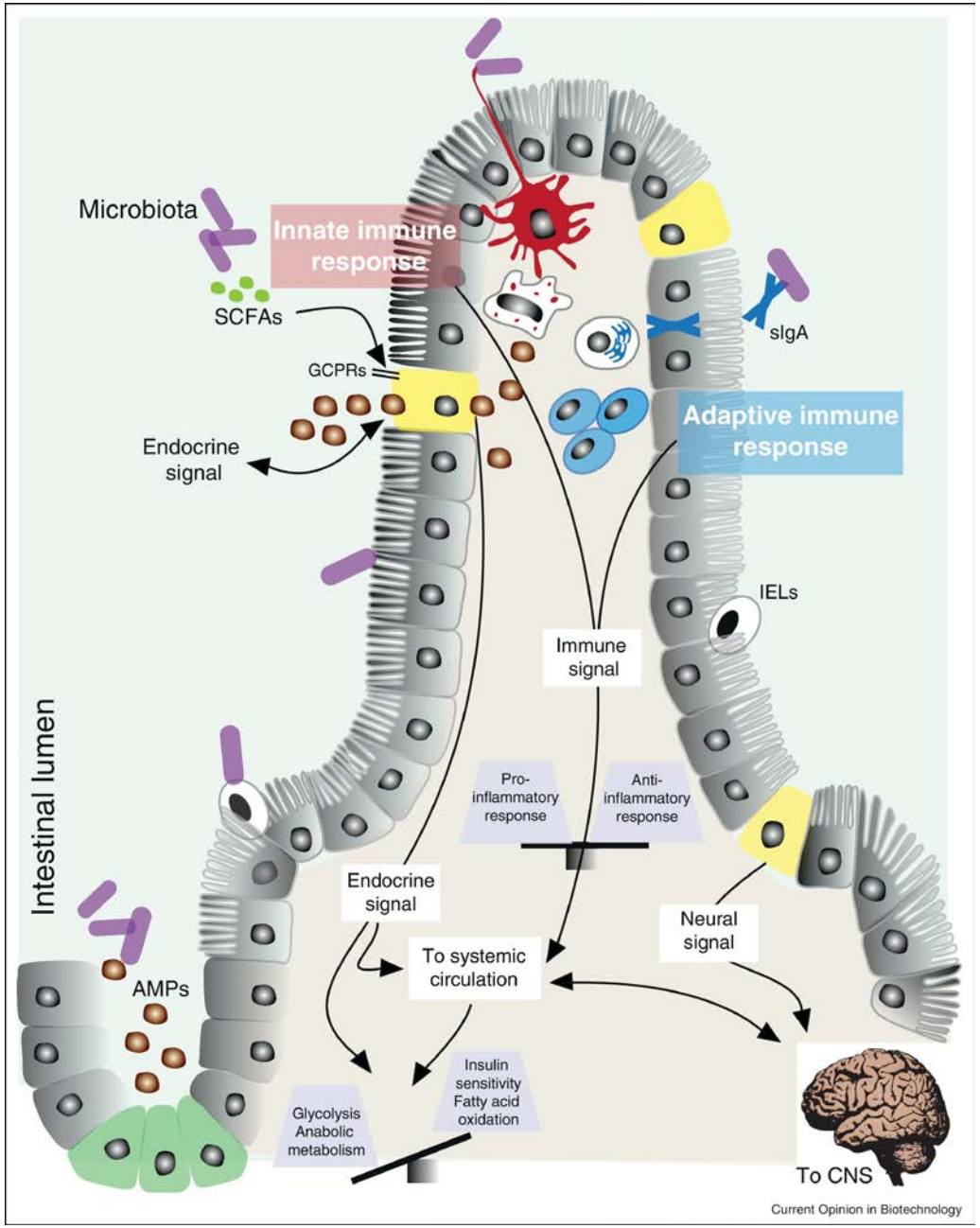
K R Foster *et al. Nature* **548,** 43–51 (2017) doi:10.1038/nature23292

nature

Crosstalk between the microbiota and local and systemic host processes

Microbiota impacts:

- **Nutrition.**
- **Tissue and immune development.**
- **Pathogen resistance.**
- **And even our mental health.**

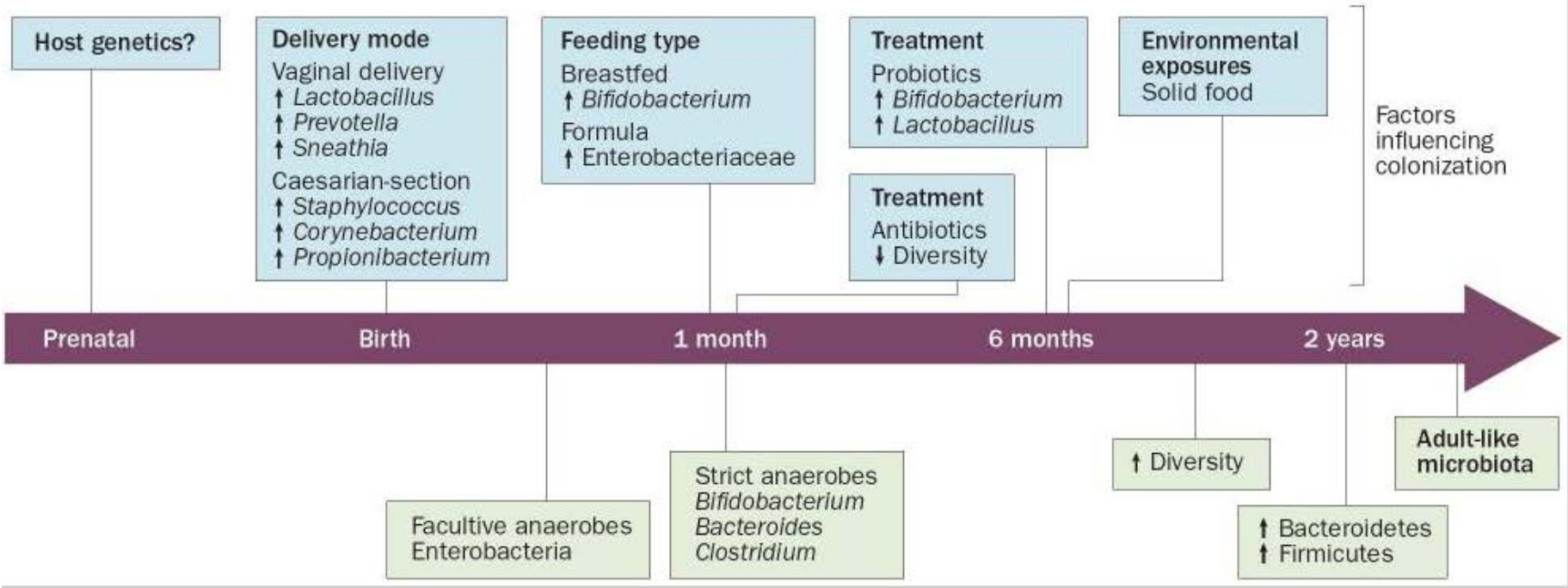


Development of gut microbiota

a

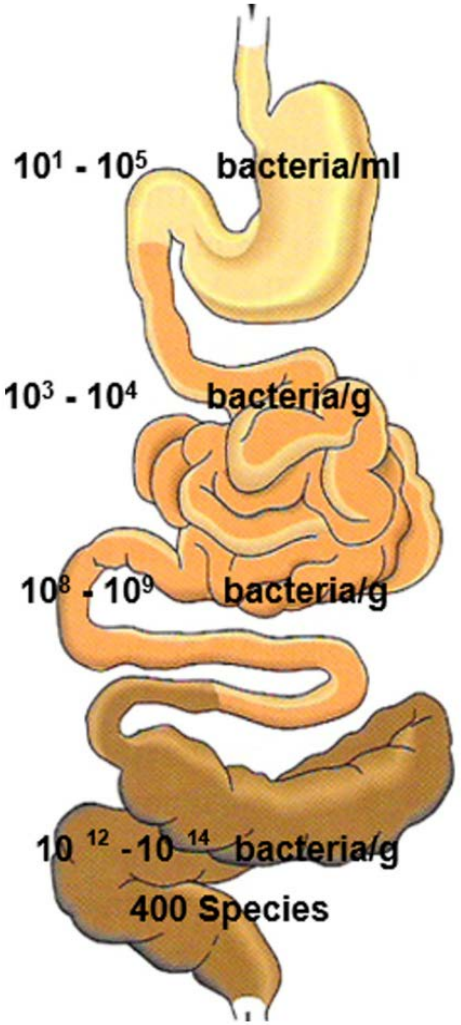
Intestinal location	Stomach	Duodenum	Jejunum	Ileum	Colon
Microbes/gram	1×10^{1}	1×10^{3}	1×10^{4}	1×10^{7}	1×10^{12}
Composition	*Lactobacillus* *Helicobacter* *Veillonella*	*Streptococcus* *Lactococcus* *Staphylococcus*	*Lactobacillus* *Streptococcus* *Enterococcus*	SFB Enterobacteriaceae *Bacteroides* *Clostridium*	*Bacteroides* *Clostridium* Lachnospiraceae Proteobacteria Actinobacteria Prevotellaceae

b



Verdu et al., (2015) Nat Rev Gastroenterol Hepatol. 2015 Sep; 12(9): 497–506.

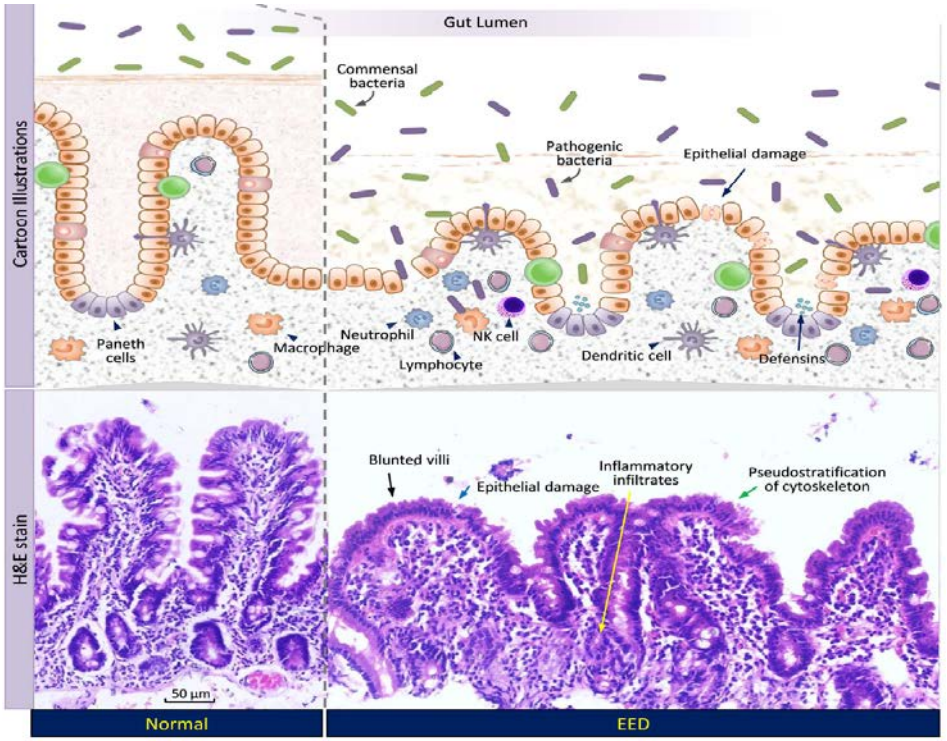
Positive effects of bacterial colonization of the intestine



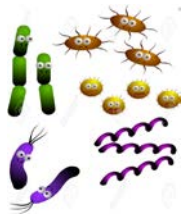
- Synthesis and excretion of supplementary vitamins, e.g., vitamin K and vitamin B12 (the amount of this supplementary "intake" derived by microbial production, however, is not sufficient to cover the necessary total amount)
- Prevention of colonization by pathogens by competition for attachment or nutrients
- Metabolomic activities (production of growth factors for epithelial cells, secretion of peroxides or bacteriocidins to inhibit other bacteria)
- Stimulation of the development of lymphoid structures (Peyer's patches) and T cell differentiation, correction of T cell imbalances
- Induction of production of cross-reactive antibodies (IgA, secreted into the gut lumen).

Biedermanan and Rogler. Eur J Pediatr (2015) 174:151–167

Biological perturbations in EED



BACTERIA



Low abundance

- *Succinivibrio*
- *Klebsiella*
- *Clostridium_XI*

High abundance

- *Megasphaera*
- *Mitsuokella*
- *Suterella*

SERUM METABOLITES

Low circulating

- *Phosphatidylcholines*
- *Sphingomyelins*
- *Tryptophan*
- *Ornithine*
- *Citrulline*
- *Carnitine*

High circulating

- *Glutamate*
- *Taurine*
- *Serotonin*

STOOL

KEGG Signalling Pathways

- *NF-kappa B signaling pathway*
- *Toll-like receptor signaling pathway*
- *NOD-like receptor signaling pathway*
- *Chemokine signaling pathway*
- *TNF signaling pathway*
- *Osteoclast differentiation*
- *B-cell receptor signalling pathway*
- *FC gamma R-mediated phagocytosis*
- *NK cell mediated cytotoxicity*
- *T-cell receptor signalling pathway*

GeneGO Canonical Pathways

- *Inegrin inside-out signalling in neutrophils*
- *Inhibition of neutrophil migration by proresolving lipid mediators in COPD*
- *Transcription regulation of granulocyte development*
- *Lipoxin inhibitory action on fMLF induced neutrophil chemotaxis*
- *G-CSF induced myeloid differentiation*
- *Chemokines and adhesion*

Transcripts

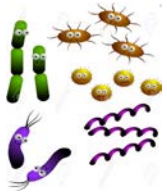
ARRB2
CXCR2
LYN, CLEC7A
NCF2, FCGR2A
FCGR3B, BCL2A1
SOCS3, IFITM1
FCER1G,
PIK3AP1

Host Response

- *Recruitment of diverse immunologically active cells*
- *Enhanced anti-viral defenses*
- *Chronic inflammation*
- *Compromised Mucus layer*
- *Compromised cell adhesion and increased permeability*
- *Apoptosis*

Feces analysis methods

BACTERIA



STOOL

Nucleic acid Extraction/purification

Metabolite Extraction/Lyophilize

16S rRNA sequencing N=2317

PCR amplify 16S rRNA gene

↓

Sequence

↓

Group sequences into OTUs
Compare OTU sequences to databases

↓

Identification of:

- Species
- Relative abundance of species within sample

Total microbiome DNA sequencing N=1189

Next generation sequence of total DNA from the microbiome samples

↓

Filter host DNA sequences
Compare microbial sequences to database and reference genomes

↓

Identification of:

- Species
- Relative abundance of species within sample
- Genes
- Variants
- Polymorphisms
- Functional information

Untargeted metabolites analysis N=1189

Reconstitution

↓

LC-MS/MS analysis

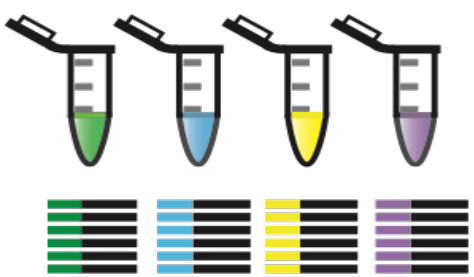
↓

Data analysis

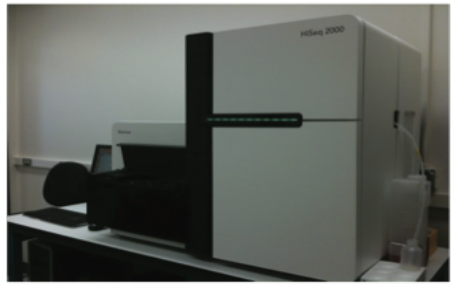
↓

Visualize/report data

Overview of 16S data generation, processing and analysis using QIIME



Multiplex Thousands of Samples
with Error-Correcting Barcodes



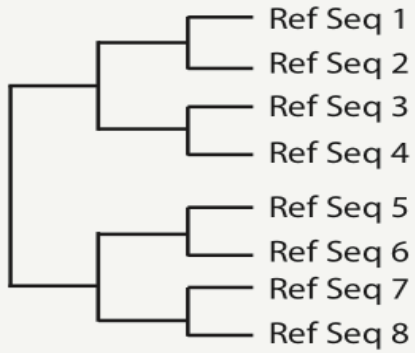
Pool Samples and Sequence

qiime
www.qiime.org

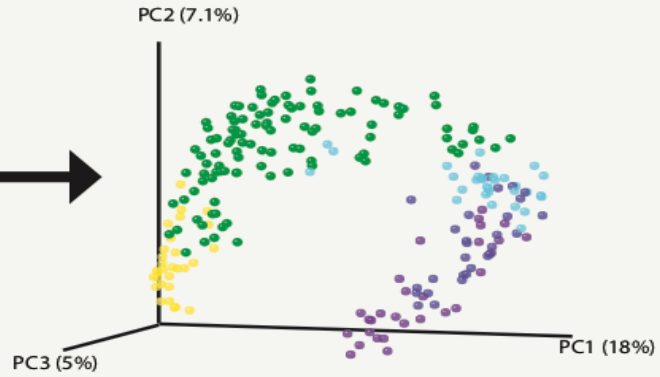
Process and Analyze Samples

>GCACCTGAGGACAGGCATGAGGAA...
>GCACCTGAGGACAGGGGAGGAGGA...
>TCACATGAACCTAGGCAGGACGAA...
>CTACCGGAGGACAGGCATGAGGAT...
>TCACATGAACCTAGGCAGGAGGAA...
>GCACCTGAGGACACGCAGGACGAC...
>CTACCGGAGGACAGGCAGGAGGAA...
>CTACCGGAGGACACACAGGAGGAA...
>GAACCTTCACATAGGCAGGAGGAT...
>TCACATGAACCTAGGGGCAAGGAA...
>GCACCTGAGGACAGGCAGGAGGAA...
>GAACCTTCACATAGGCAGGAGGAT...

Assign Sequences to Samples



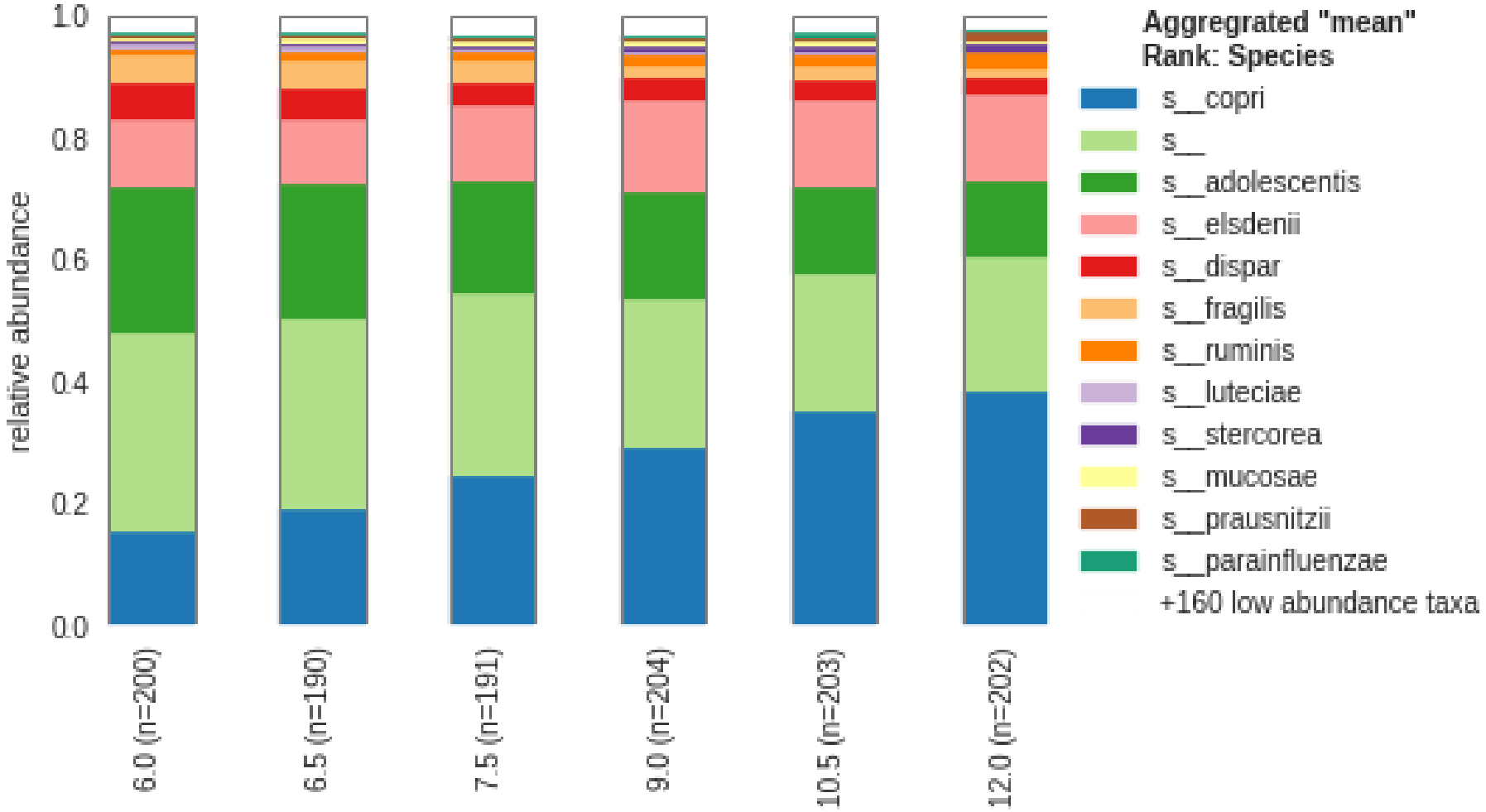
Assign Millions of Sequences to
Clusters of Closely Related Organisms



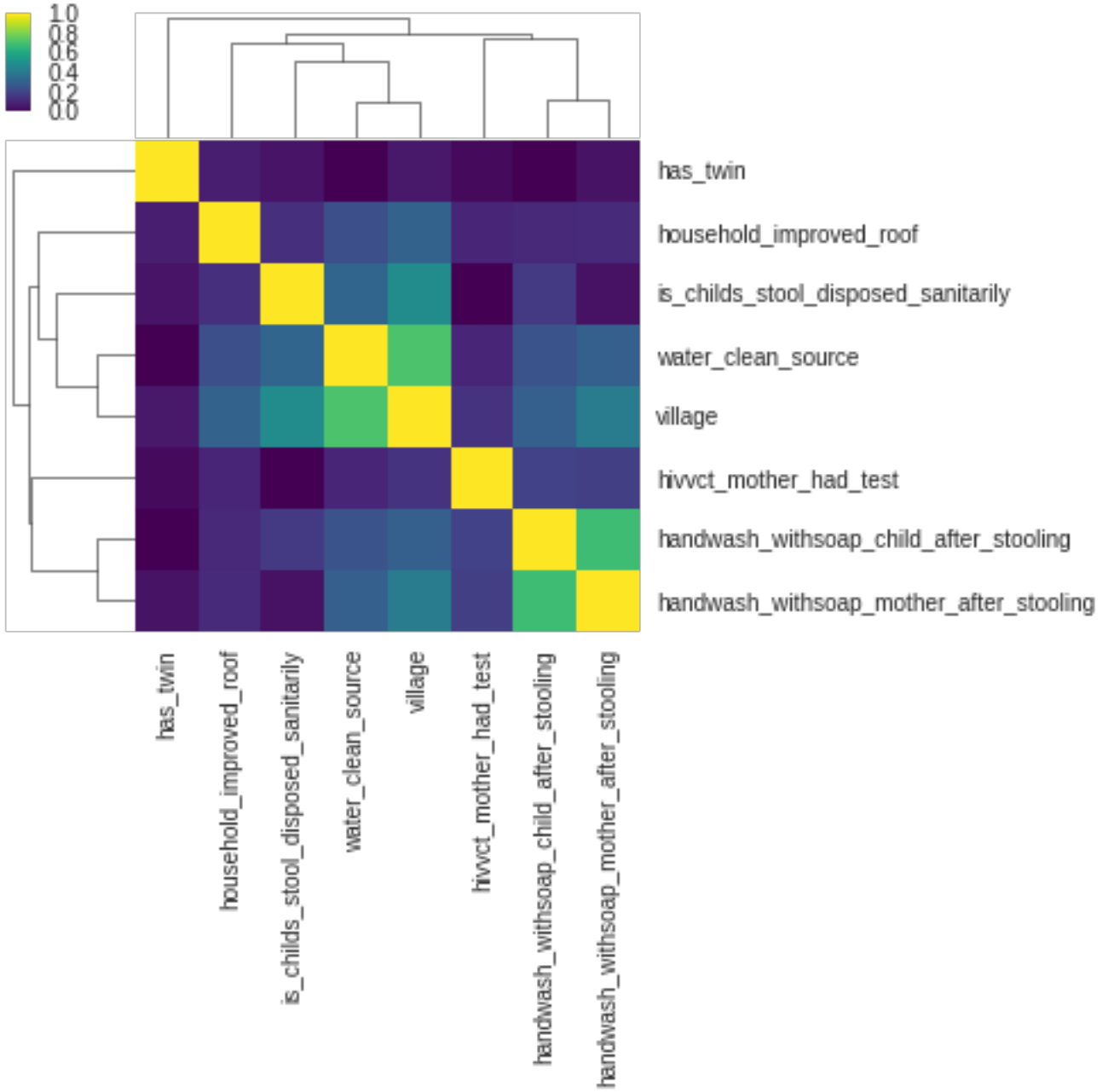
Compare Samples Visually and Statistically

http://figshare.com/articles/Overview_of_data_generation_processing_and_analysis_using_QIIME_/902219

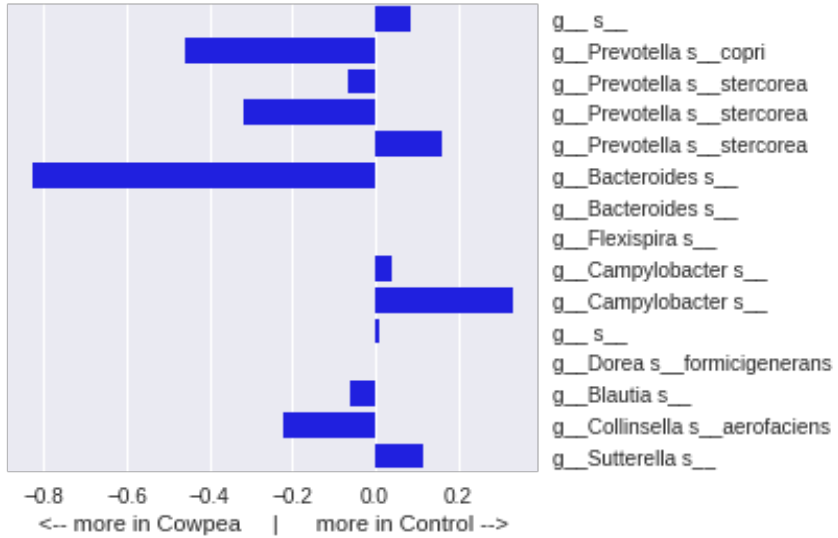
Effect of age on microbiota composition

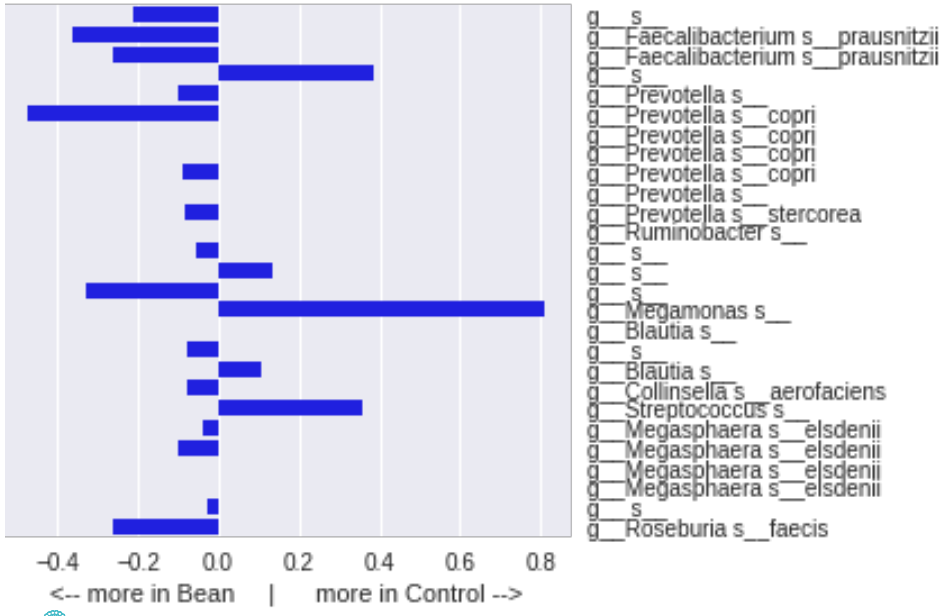


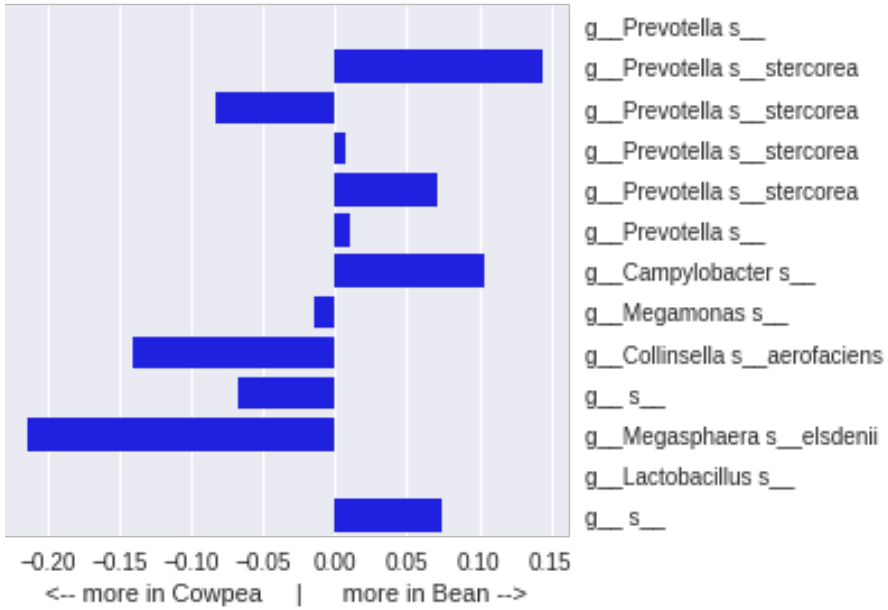
Sanitation practices affect microbiota composition



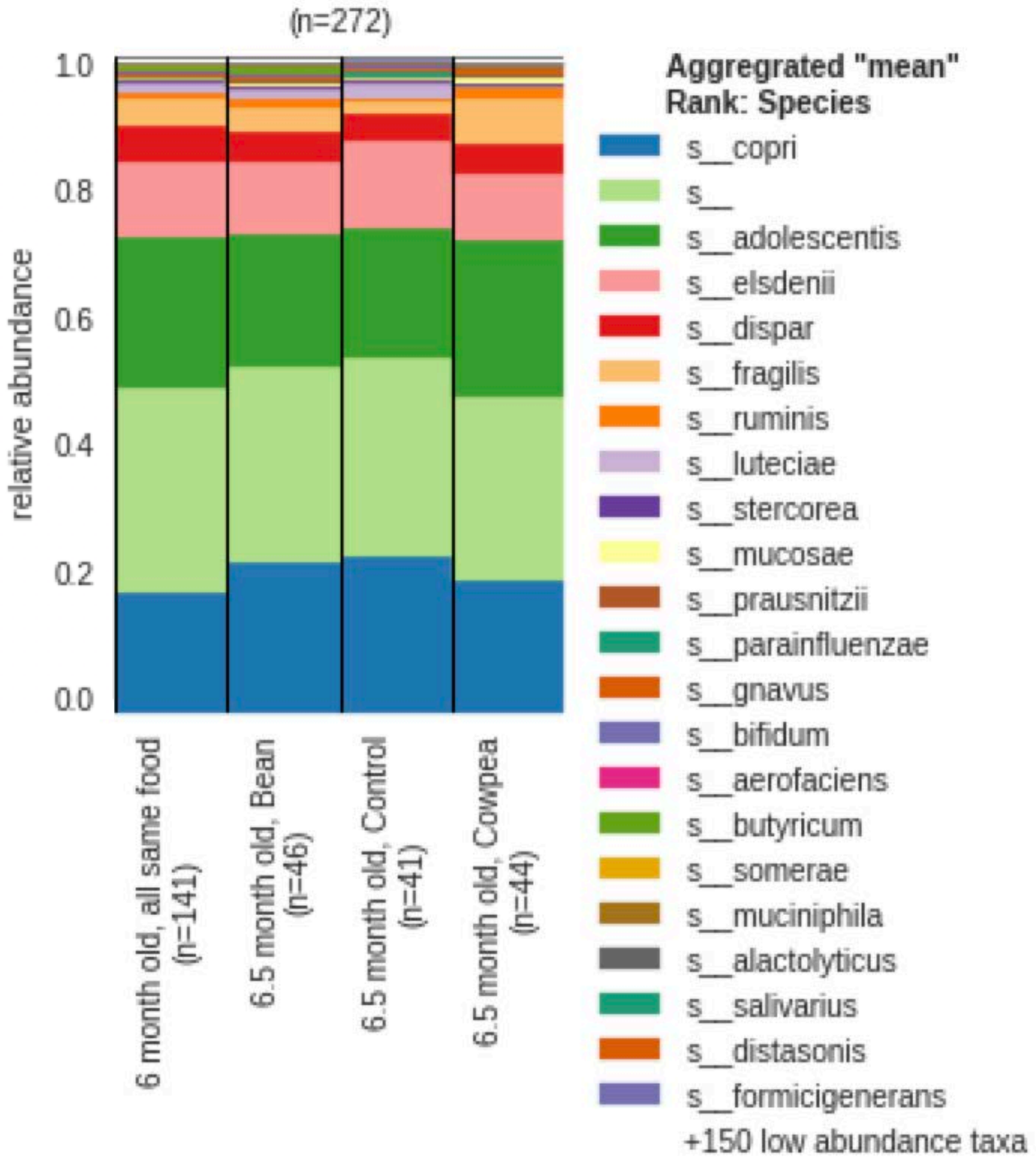
Diet and microbiota abundance



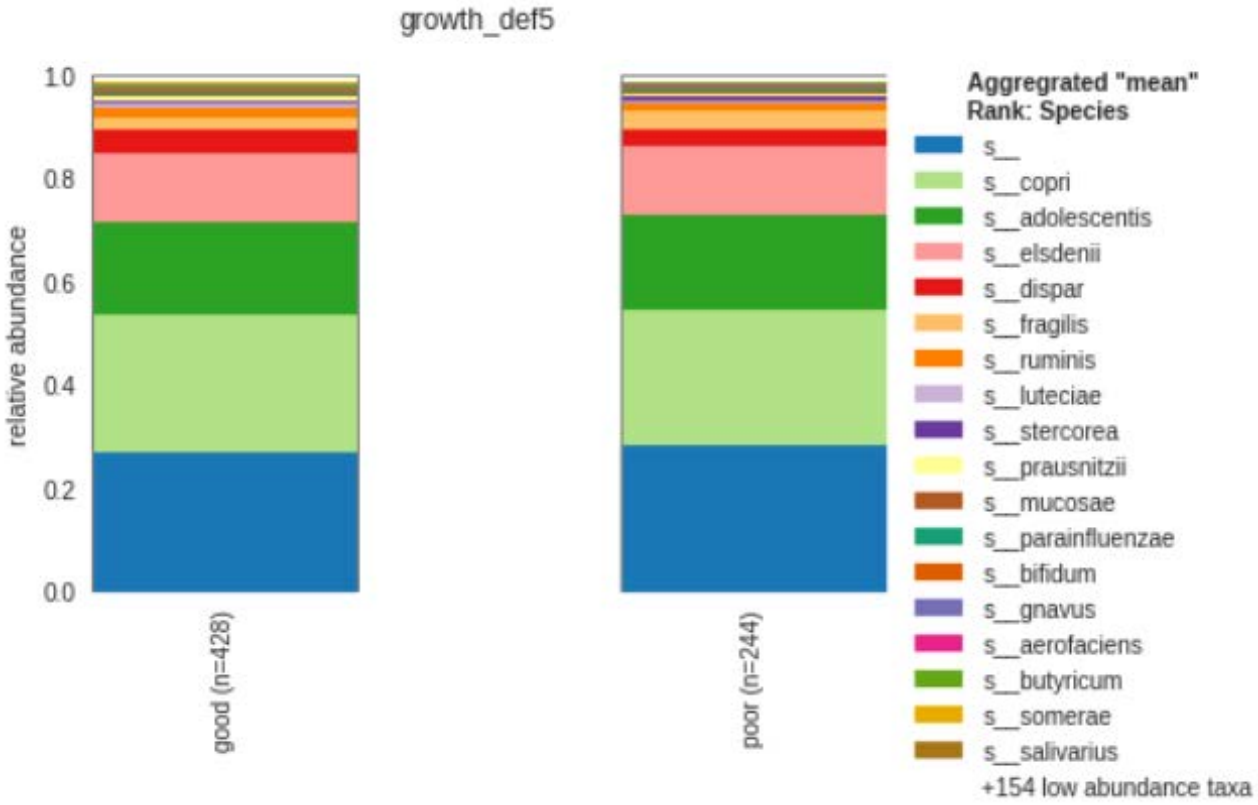


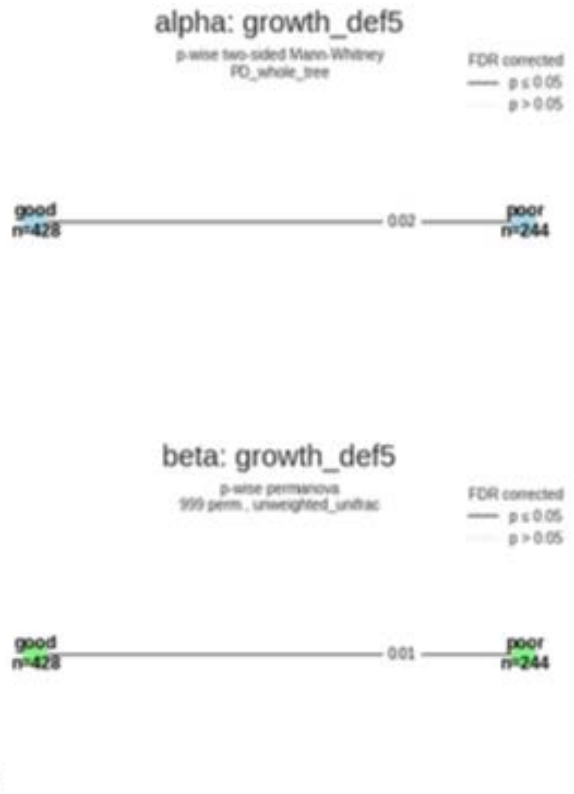


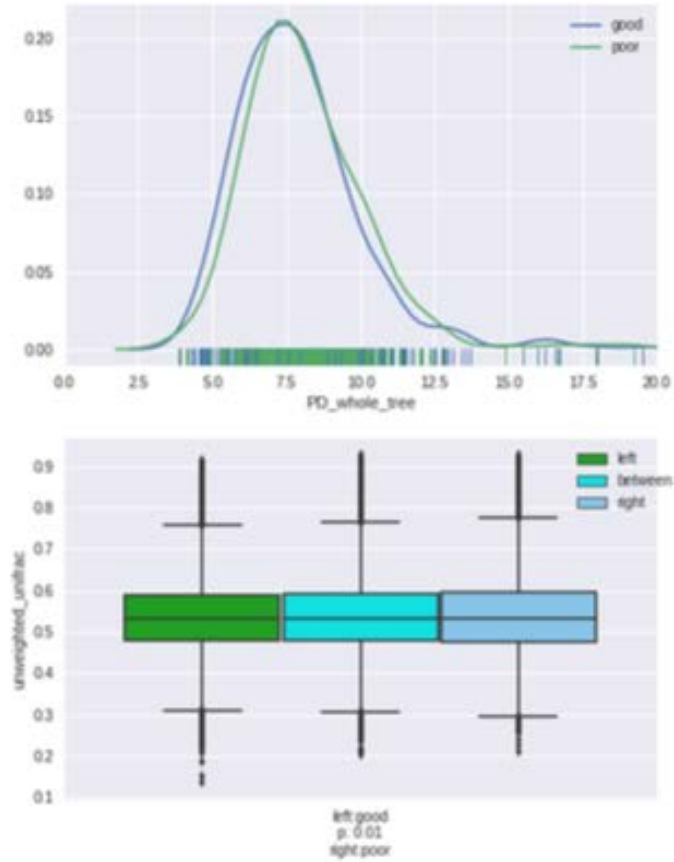
Combined effect of age and diet on microbiota composition



Growth and microbiota population

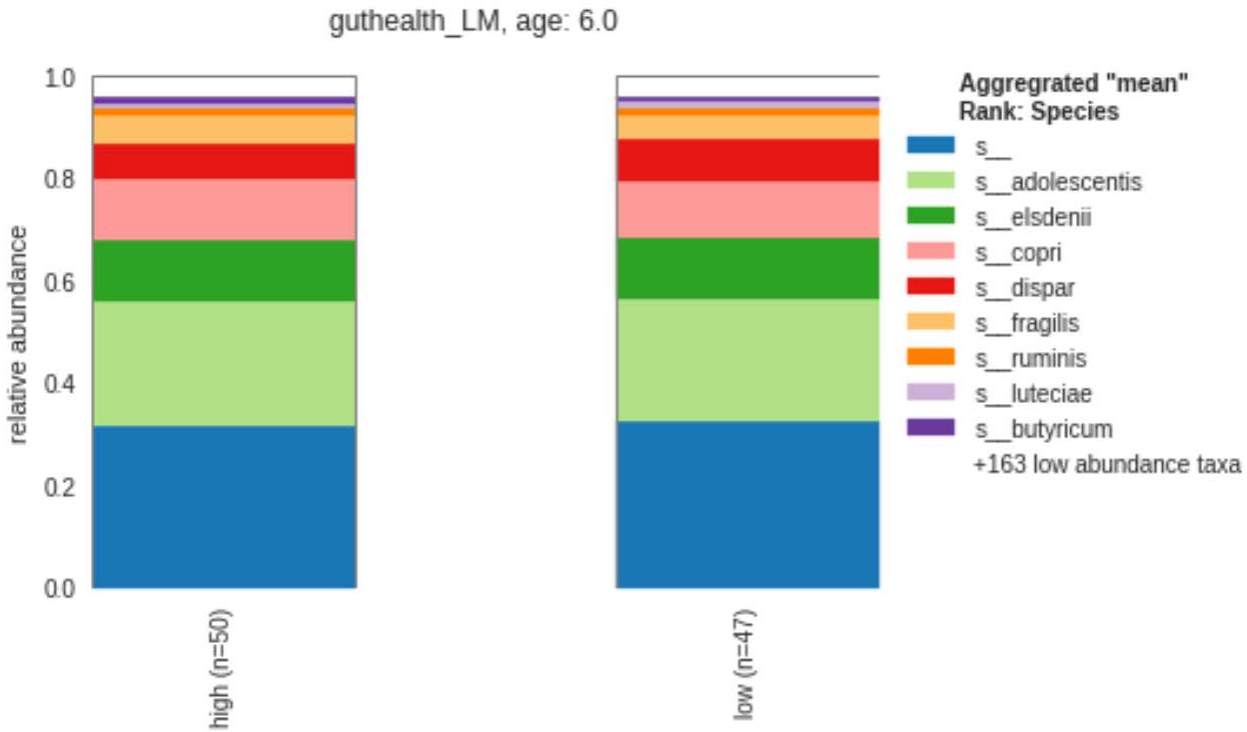


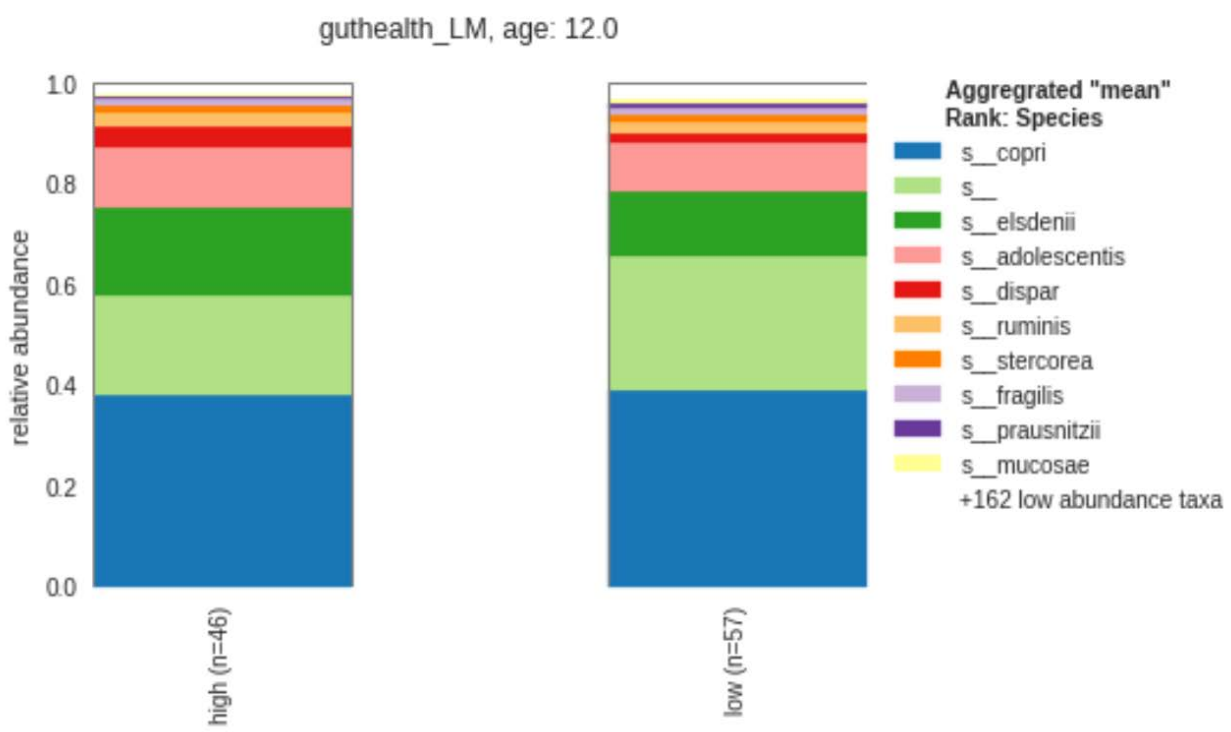




Good growers $dLAZ_{24\ wk} < -0.25$
Poor growers $dLAZ_{24wk} > -0.75$
Exclusions - kids all the time good or poor growers.

Gut health and microbiota diversity

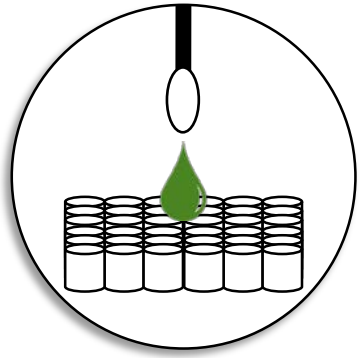




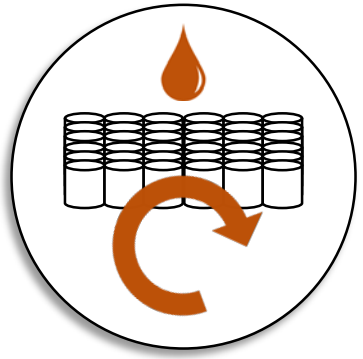
Untargeted Metabolomics Workflow

Samples

Extraction
EtOH-Water (9:1)

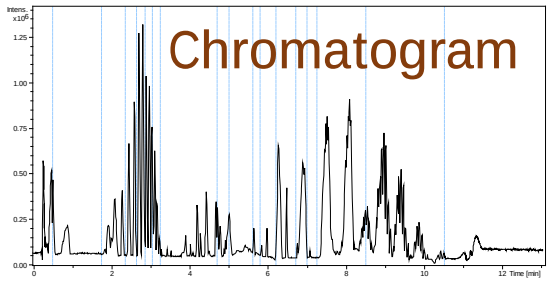


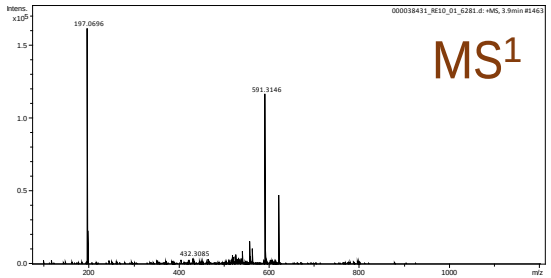
Resuspension
MeOH-Water (1:1)

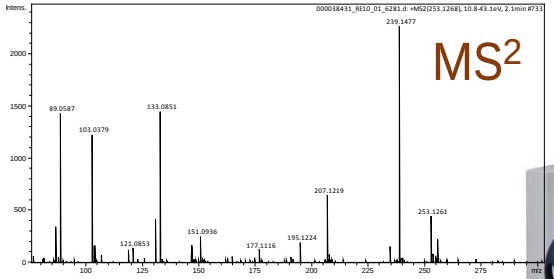


UHPLC - ESI - qToF

Data dependent acquisition



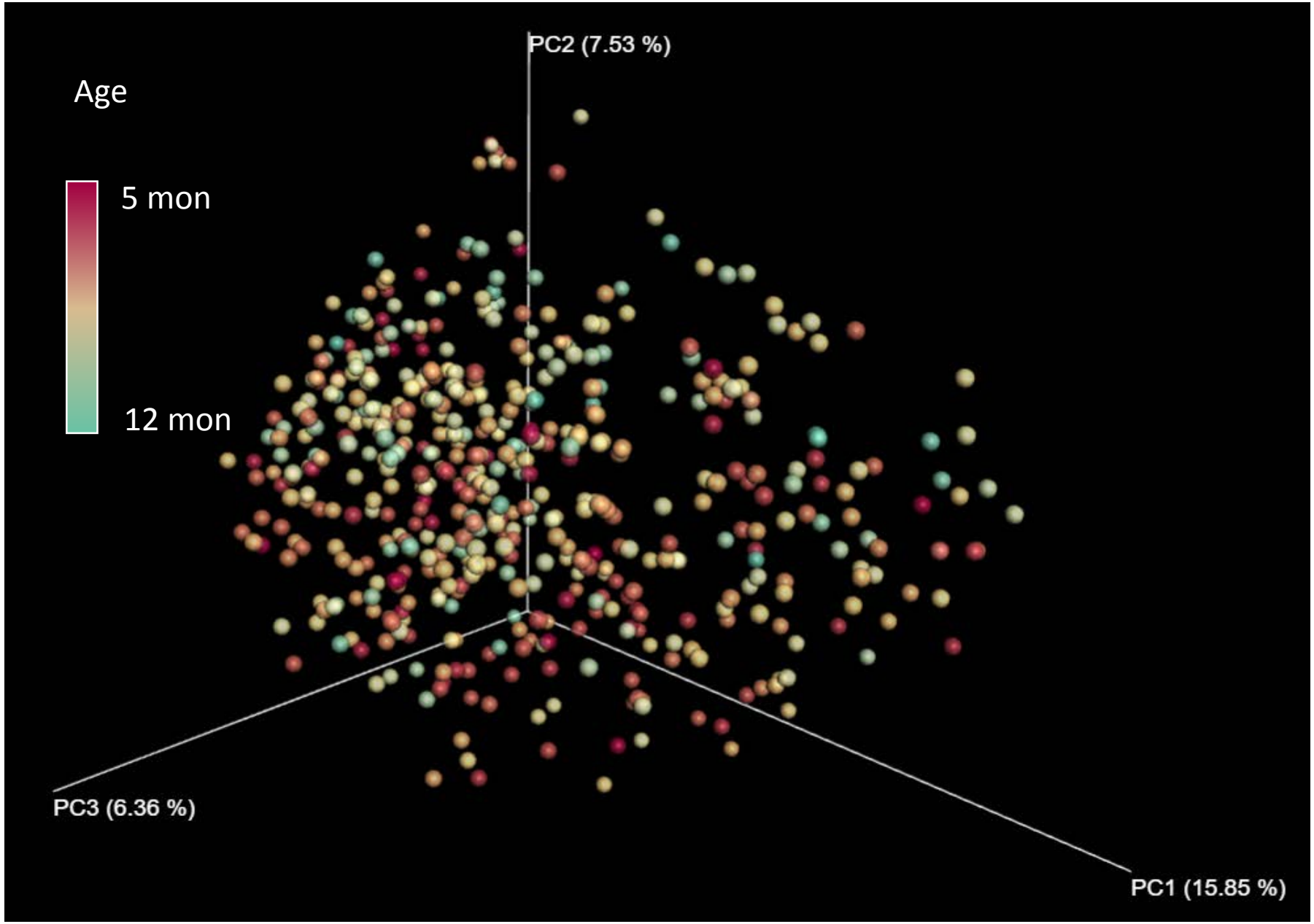




Multivariate Statistics (PCoA, Emperor) – Rob Knight (UCSD)

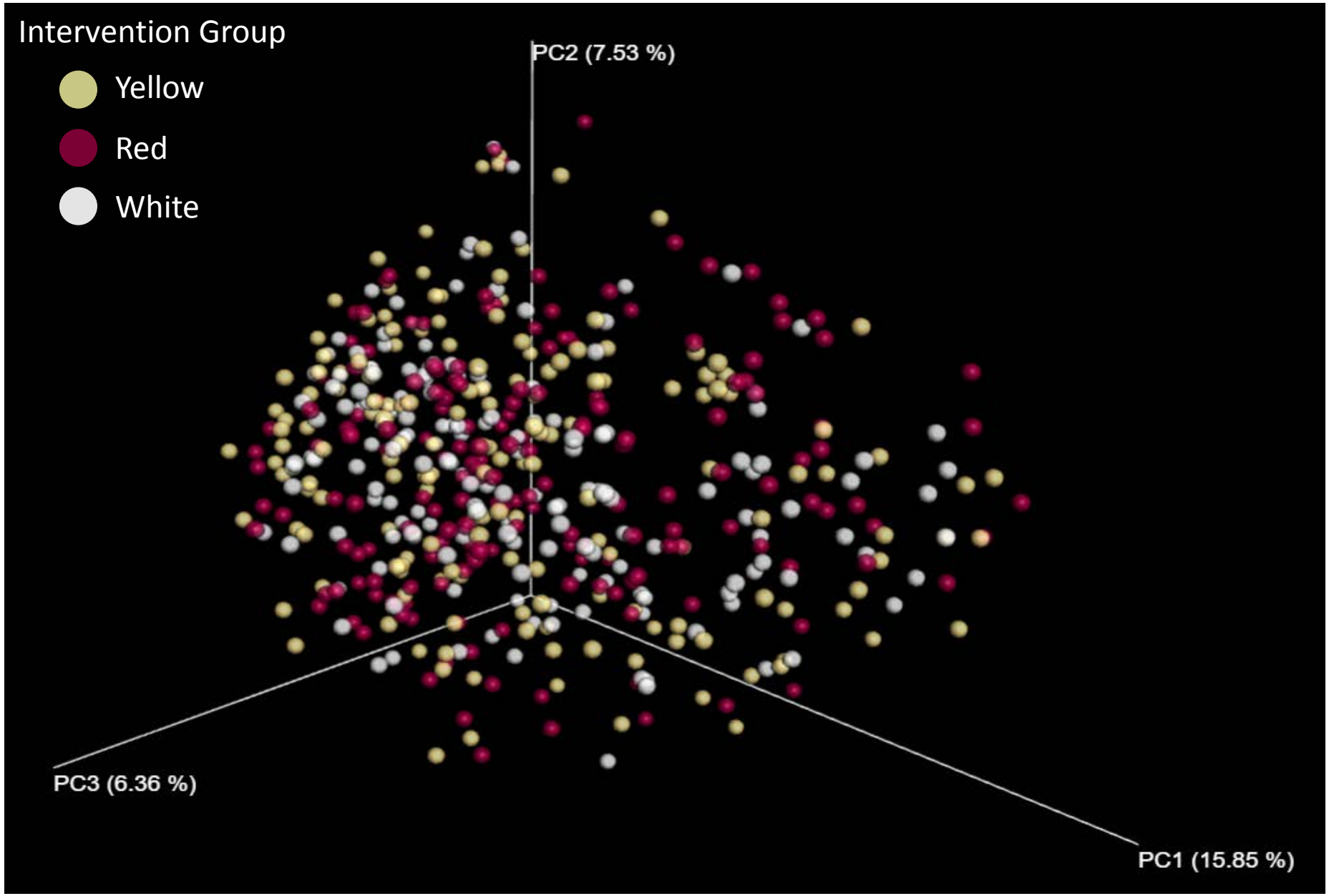
Molecular Networking and Annotation (GNPS) – Pieter Dorrestein (UCSD)

Metabolite composition by age



PCoA, Emperor

Metabolite composition by intervention

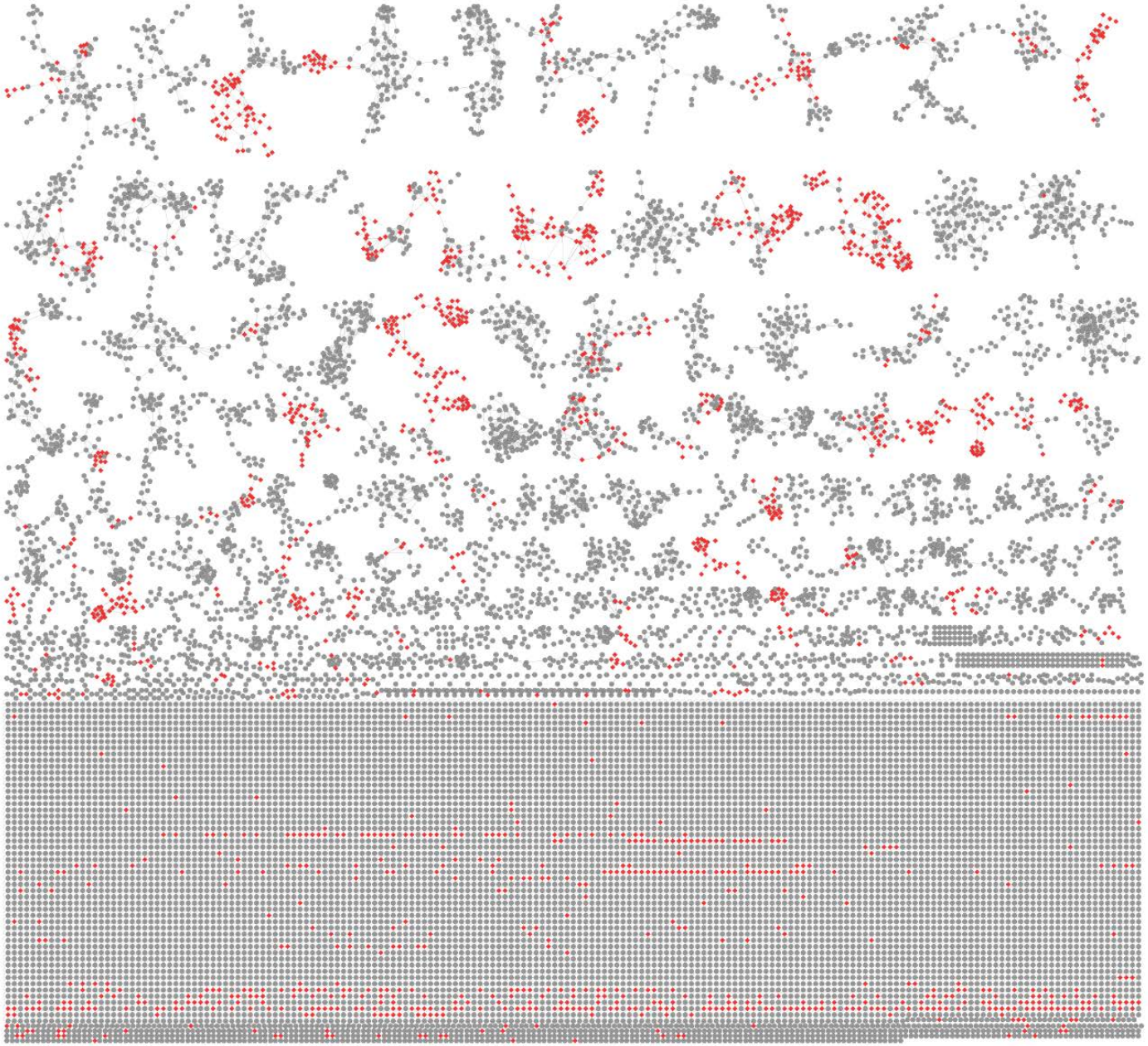


PCoA, Emperor

Yellow - common bean
Red - cowpea
White - control

Molecular Networking, GNPS

- Feces are very chemically complex, but current database information limits how many of these compounds can be identified.
- Each node (circle) represents a unique molecule
- “Clusters” of nodes are chemically related
- Red have putative IDs, grey does not have putative IDs
- Detect expected compounds as: lipids, small peptides, bile acids, bile pigments, drugs (antibiotics and antimalarial), and what appears to be molecules from food



CONCLUSIONS

- Fecal microbiota matures over time.
- Sanitation practices influences significantly the microbiota diversity.
- Microbiota do not change dramatically during the food supplementation.
- Growth affects the general microbiota profile but not specific species significantly.
- Gut health (L:M) change the microbiota diversity at 9 and 12 months.
- Further analysis of microbiota, metagenomics and metabolites will help us to better understand the effect of the different diets on Malawian young kids.

Acknowledgements

❑ Manary Lab

- Sophia Agapova
- Kevin Stephenson
- William Cheng
- Mark J. Manary

❑ UCSD - University California of San Diego

❑ Knight lab
- Greg Humphrey
- Gail Ackerman
- Stefan Janssen
- Rob Knight

❑ Dorrestein lab
- Alan Jarmusch
- Fernando Vargas
- Pieter Dorrestein

❑ WUSM

❑ Pediatrics
- Indi Trehan.

❑ University of Malawi

- Kenneth Maleta
- Chrissie Thakwalakwa
- Teresa Ngoma
- Oscar Divala
- Yankho Kaimila

❑ Baylor College of Medicine

- Robert Shulman.

❑ Funding

- USAID.

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Feed the Future Innovation Lab for Collaborative Research on Grain Legumes