

Transitional Metagenomes: the role of globalization in shaping the human gut microbiota



Carlotta De Filippo

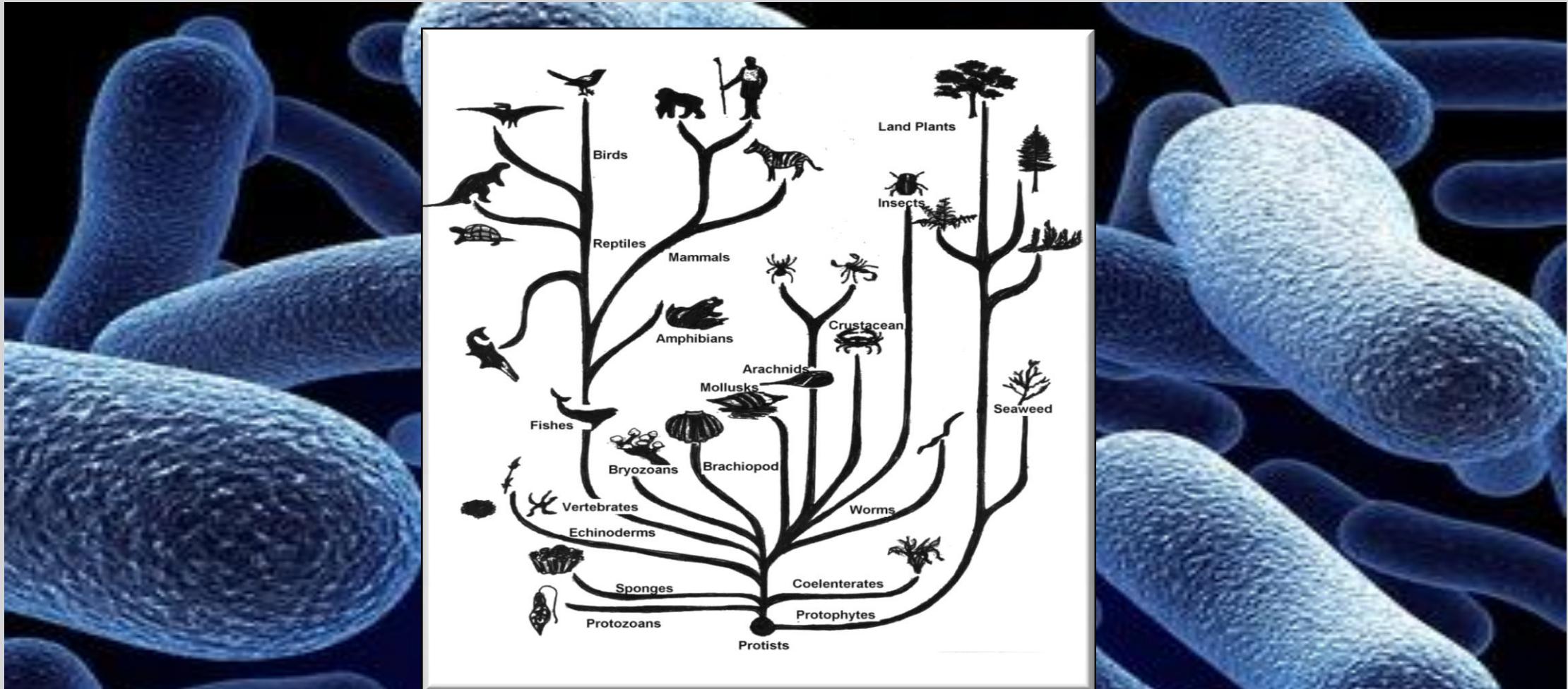
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Consiglio Nazionale delle Ricerche
Istituto di Biologia e Biotecnologia
Agraria



Symbiosis parallels organismal complexity

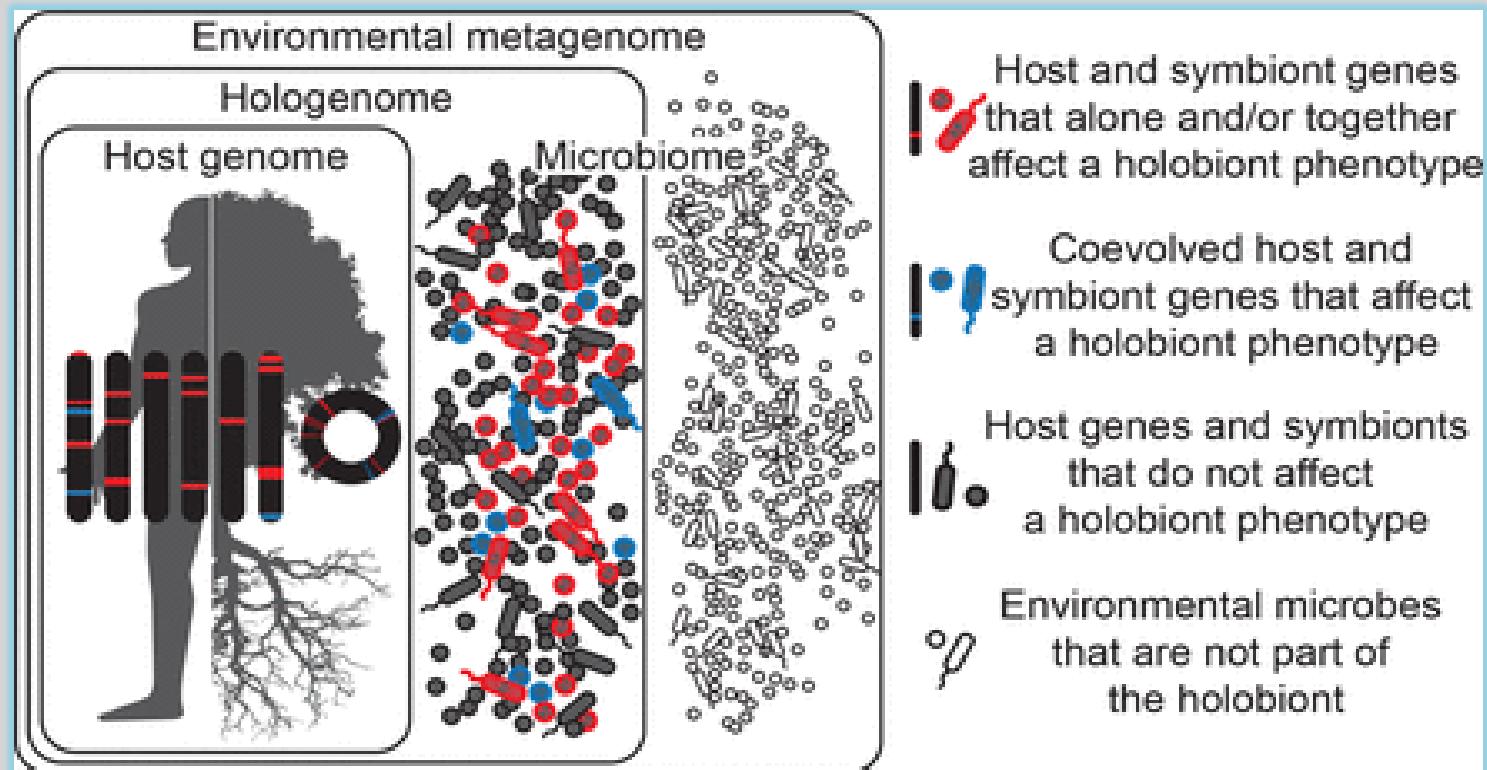


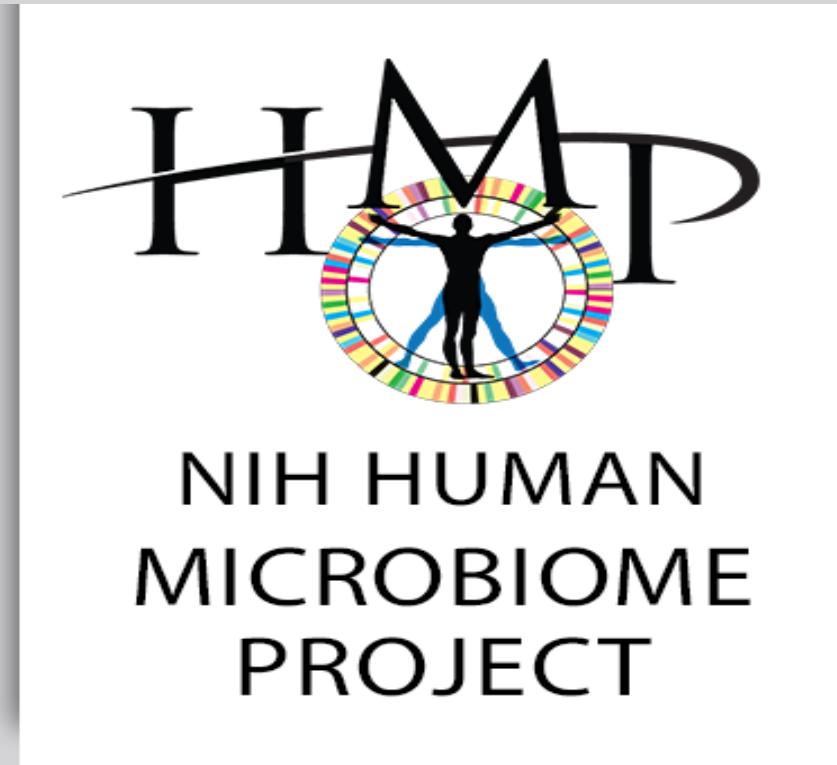
Microbes have co-evolved with plants and vertebrates over the millennia, so normal functioning of these complex systems depends on the presence of symbionts. This interaction is mediated by environmental factors, nutrients, diet, soil.

The hologenome

Hologenome encompasses the genomes of the host and all of its microorganisms (holobiont).

Microorganisms in the environment are not part of the holobiont but can affect the complexity and stability of hologenome.



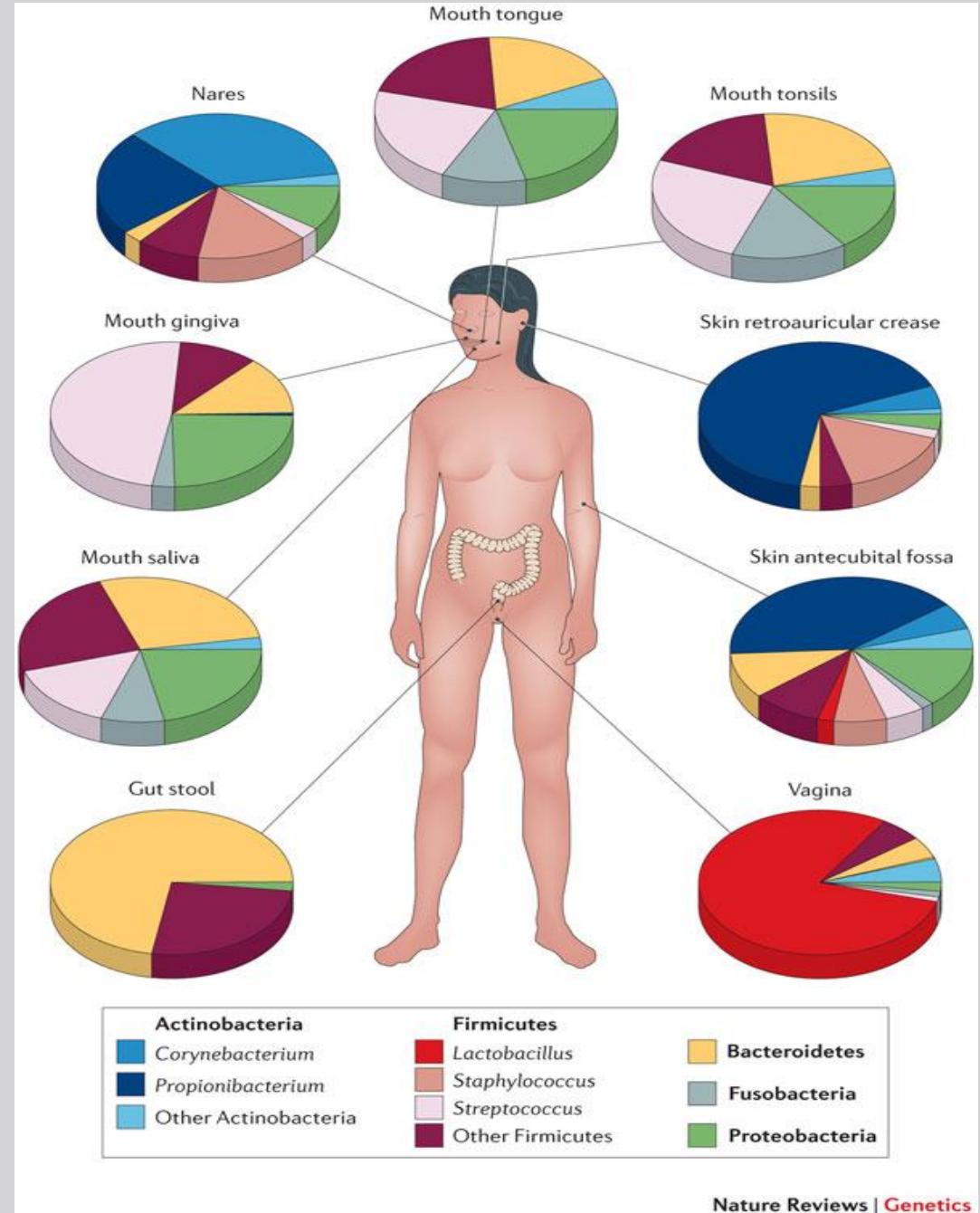


NIH HUMAN MICROBIOME PROJECT

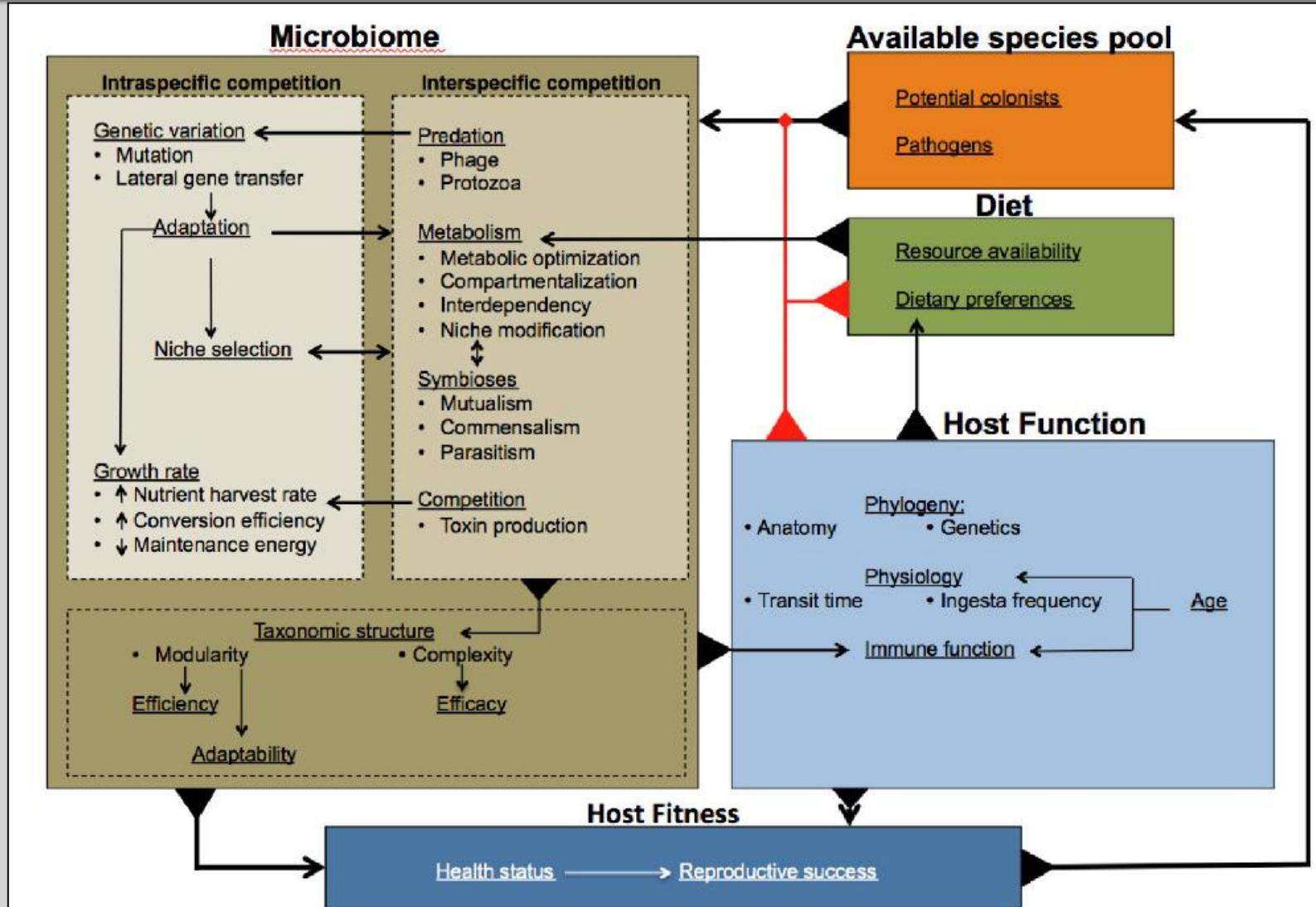
The Human Microbiome Project (HMP) funded by the US National Institutes of Health identified the sequencing of uncultivated bacteria as a crucial unmet goal.

Lasken & McLean. *Nature Reviews Genetics*, 2014

<http://www.hmpdacc.org/>

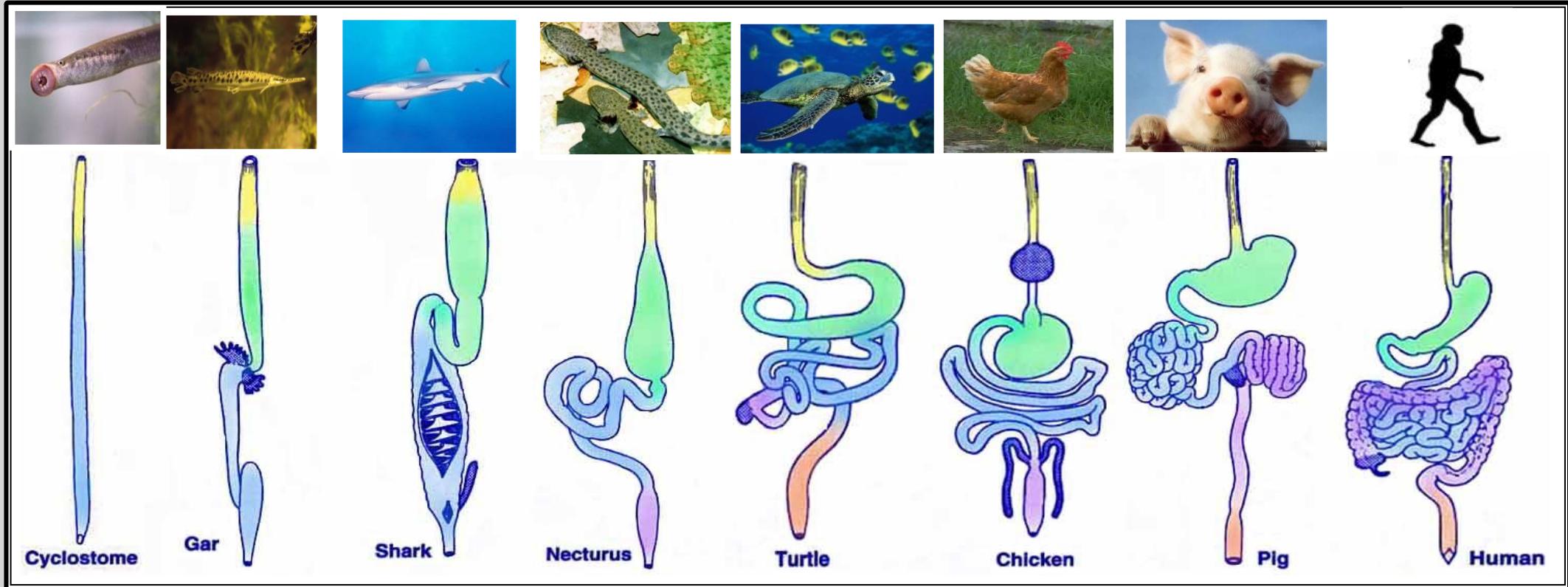


The complexity of selective forces acting on the microbiome



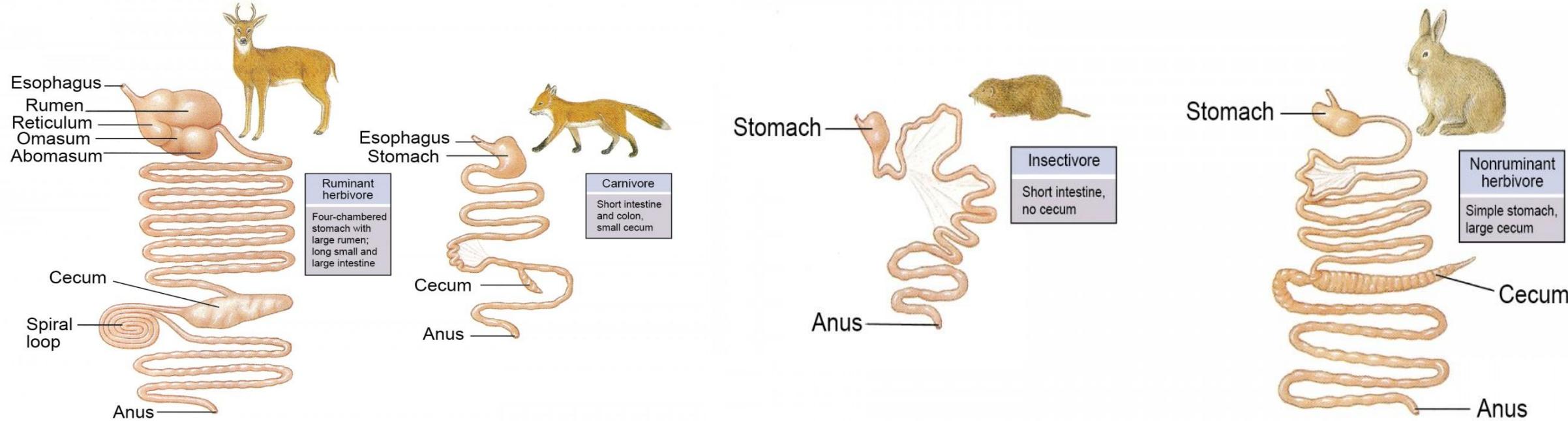
Yeoman et al., Entropy
2011, 13:570-594

Microbiota co-evolution



During evolution changes in the length and compartmentalization of the gastrointestinal tract in vertebrates have enabled vertebrates to occupy diverse habitats and exploit different feeding strategies

Comparison of the Digestive Systems of a ruminant Herbivore (Deer) and Carnivore (Fox) and non ruminant Herbivore (Rabbit) and Carnivore (Shrew)



Long small and large intestine helps ruminant herbivores to extract energy and nutrients from their food. The caecum is a very large structure, filled with symbiotic bacteria that produce **cellulase**.

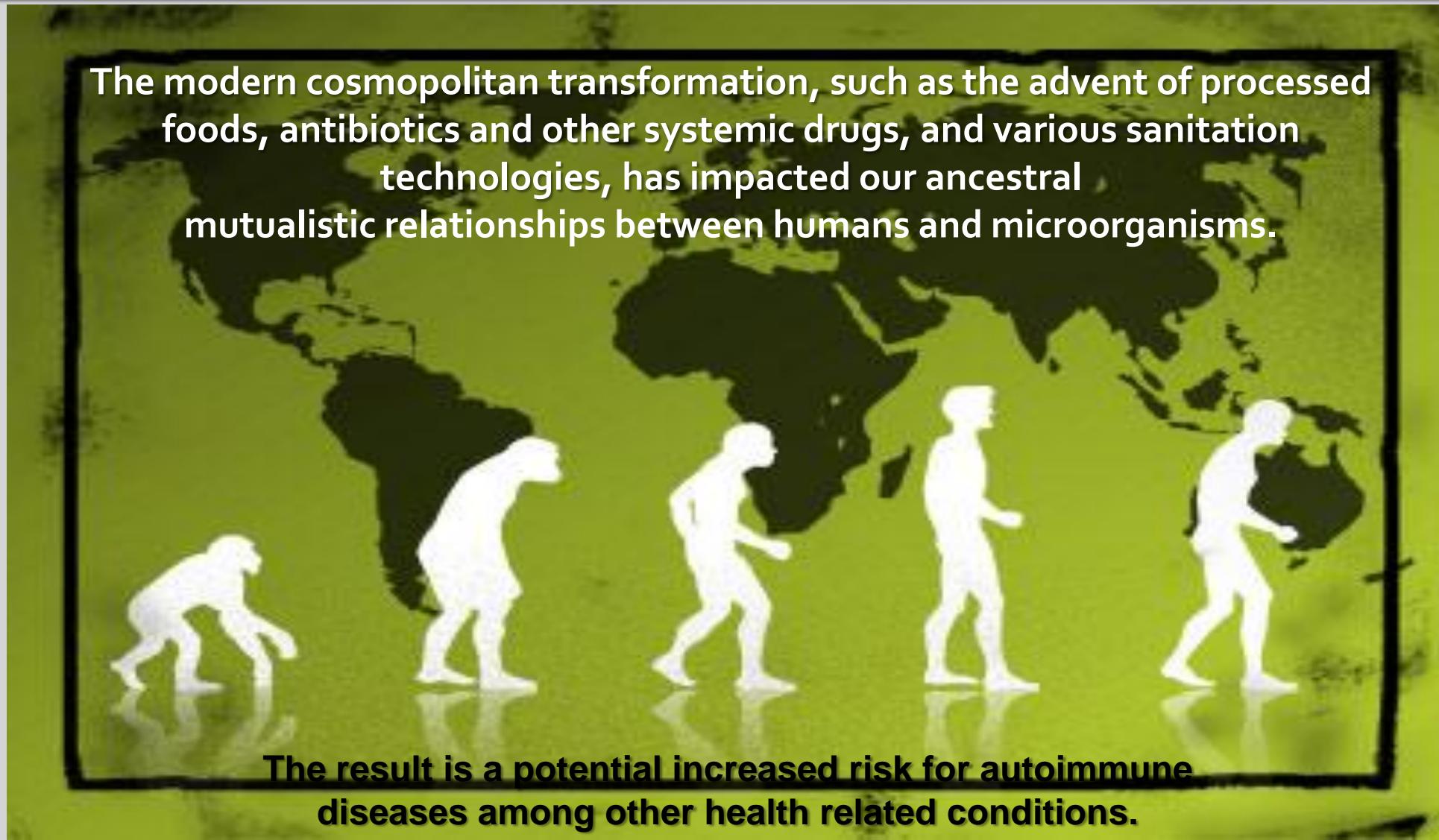
Carnivore, a fox, which has a more simply digestive tract with small caecum.

Small carnivore, a shrew, which has a very short digestive tract with no caecum.

Rabbits are herbivores without rumen, with large caecum that contains a population of symbiotic microorganisms that produce **cellulase**.

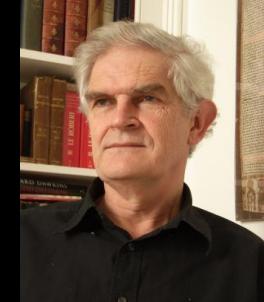
Microbiota Co-evolution

The evolution of our gut microbiota



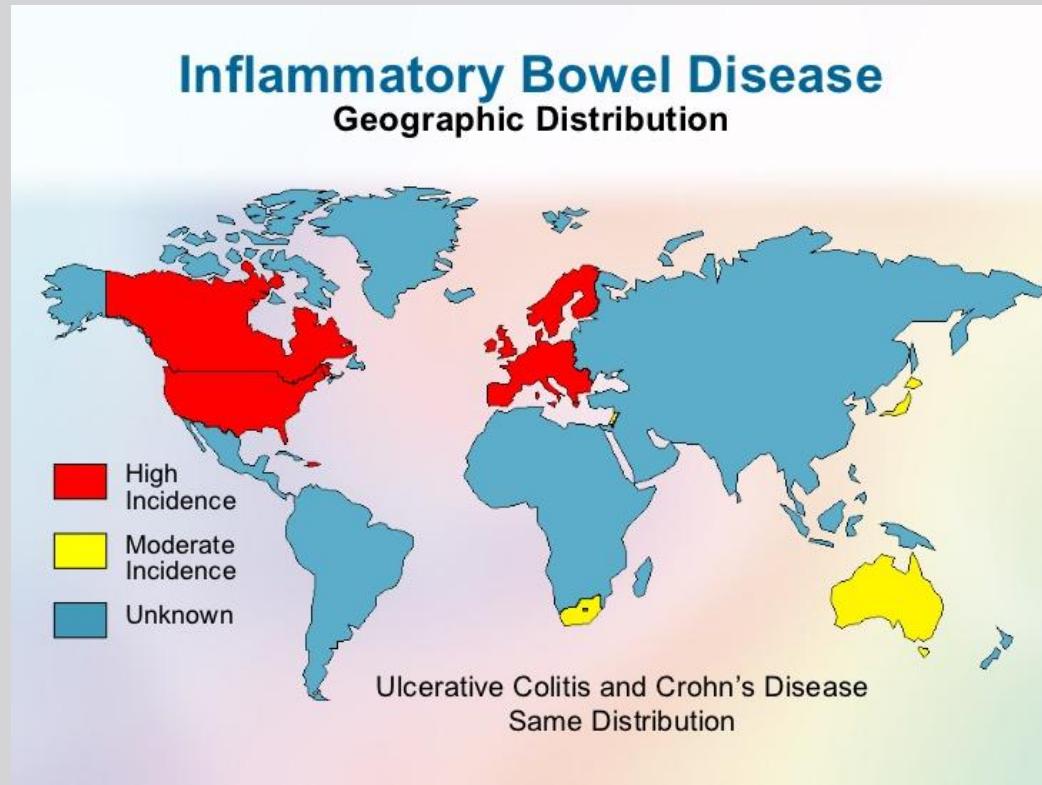
The Hygiene Hypothesis and Darwinian Medicine by Graham Rook

(University College, London, 2009)



"The hygiene hypothesis hit the headlines in 1989 when **David Strachan** observed that hay fever is less common in children with older siblings, suggesting that multiple childhood cross-infections from dirty older brothers might protect from allergic disorders."

The implication was that elimination of these infections by modern hygiene might be responsible for the increased prevalence of allergies and autoimmune diseases in developed countries.



The lower incidence of **Inflammatory Bowel Disease** in Africa and Asia reflects both genetic and environmental factors.



The Increasing Incidence of Immune Regulatory Disorders

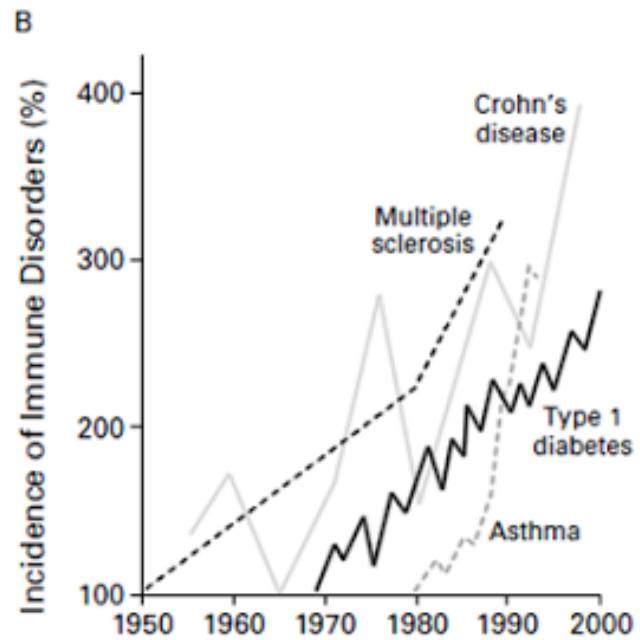
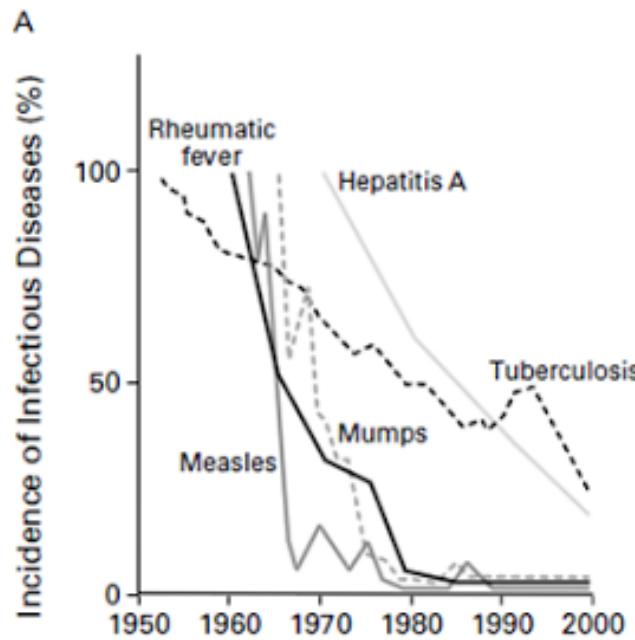


Figure 1. Inverse Relation between the Incidence of Prototypical Infectious Diseases (Panel A) and the Incidence of Immune Disorders (Panel B) from 1950 to 2000.

From Bach JF. N Engl J Med. 2002)

Our modern sterile, or clean, environment without parasitic and other infections have left our immune systems immature and uneducated.

*The lack of infections have left our immune systems prone to develop **autoimmunity**.*

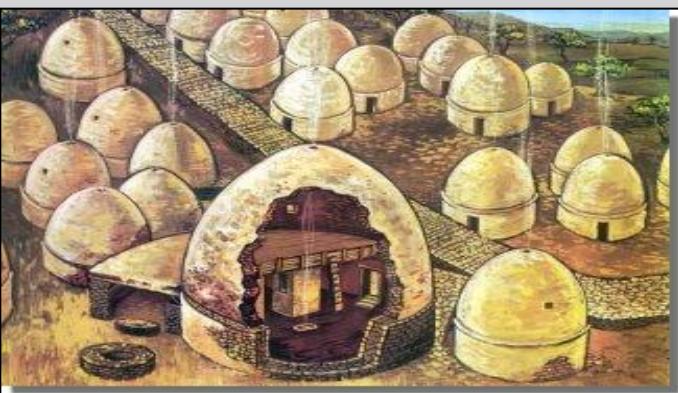


These organisms ("Old Friends") are depleted from the modern urban environment.

The Neolithic revolution



Neolithic grind stone for processing grain



The NEOLITHIC AGE (10.000 years ago) is identified with the advent of crop cultivation and domestication of animals.



Humans permanently start to reside in the same area, abundantly providing for themselves on farms



Concentration of large populations on limited areas



Emerging human diseases

The profound changes in diet and lifestyle conditions that began with the so called “**Neolithic revolution**” with the introduction of agriculture and animal husbandry (~10,000 years ago) occurred too recently on an evolutionary time scale for the human genome to adapt.

The western world



Controlled Infectious Diseases



New diseases

- Allergy
- Obesity, Diabetes, metabolic syndromes
- Autoimmune disorders

“Hygiene hypothesis”



In This Issue

The effect of diet on human gut microbial composition

The trillions of microbes that inhabit the human gut are considered an essential “organ” that helps to digest food, protect against pathogens, and limit inflammation, but researchers do not yet fully understand how environment and diet affect the gut’s microbial ecology. Carlotta De Filippo et al. (pp. 14691–14696) used rDNA sequencing and biochemical analysis to compare the fecal microbiota of 15 children, aged 1 to 6 years, from a rural African village with a similar population of children from Florence, Italy. The researchers found that the African children had a lower proportion of microbes associated with obesity in adults, and greater abundance of fatty acids known to protect against inflammation. The African children’s diet, which may resemble human diets shortly after the birth of agriculture, consisted mainly of cereals, legumes, and vegetables, whereas the Italian children ate higher quantities of meat, fat, and sugar. Only children who were still breast-feeding harbored bacterial compositions that resembled children from the other geographical group, indicating that diet may supersede factors such as ethnicity, sanitation, geography, or climate, according to the authors. They suggest that diets common to industrialized nations may reduce microbial richness, potentially contributing to a rise in allergic and inflammatory diseases in the last half-century. — J.M.



Millet and sorghum grain and flour.



Boulpon, a typical Burkina Faso rural village



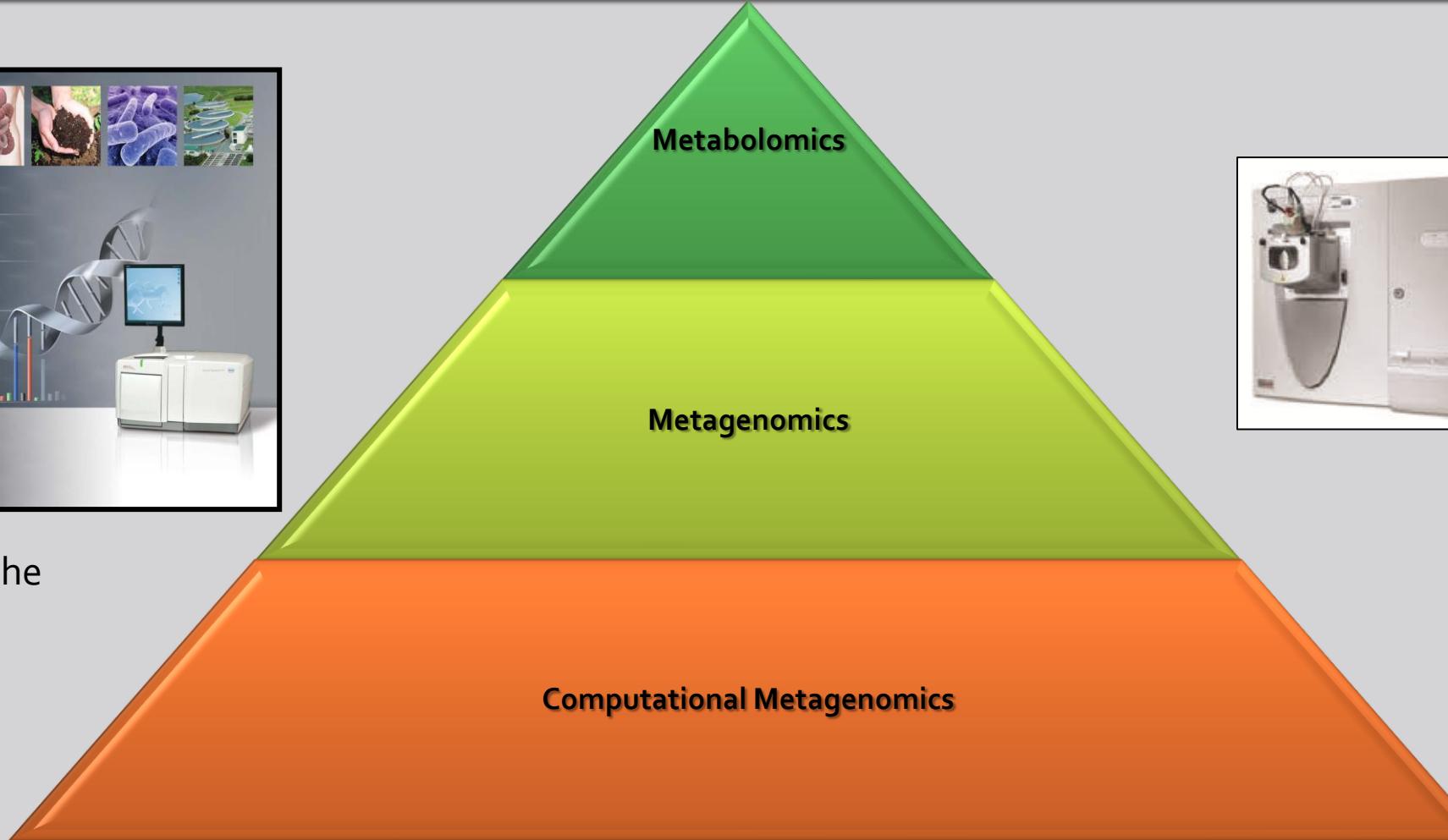
- How is **bacterial diversity** partitioned within and between the Burkina rural village and a typical westernized population (Italy)?
- Is there a possible correlation between bacterial diversity and **diet**?
- Is there a differential distribution of well known **bacterial pathogens** in the African children with respect to the Italians?



Experimental Approaches to study microbial communities



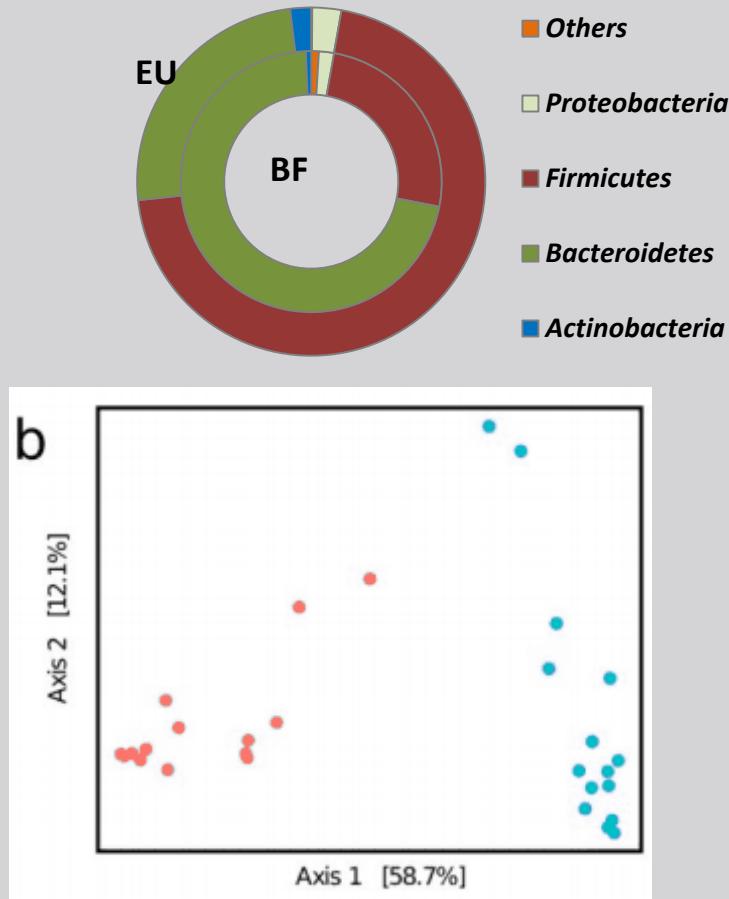
454 Roche



Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

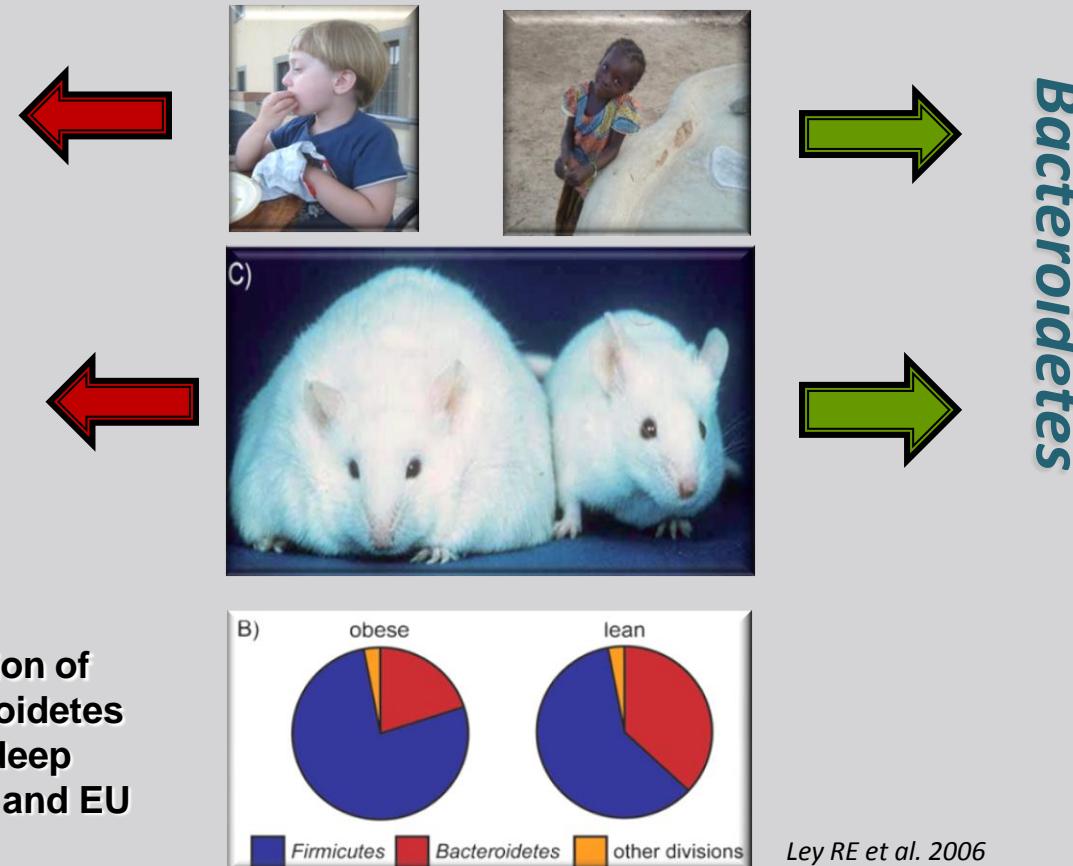
Carlotta De Filippo^a, Duccio Cavalieri^a, Monica Di Paola^b, Matteo Ramazzotti^c, Jean Baptiste Poulet^d, Sébastien Massart^d, Silvia Collini^b, Giuseppe Pieraccini^e, and Paolo Lionetti^{b,1}

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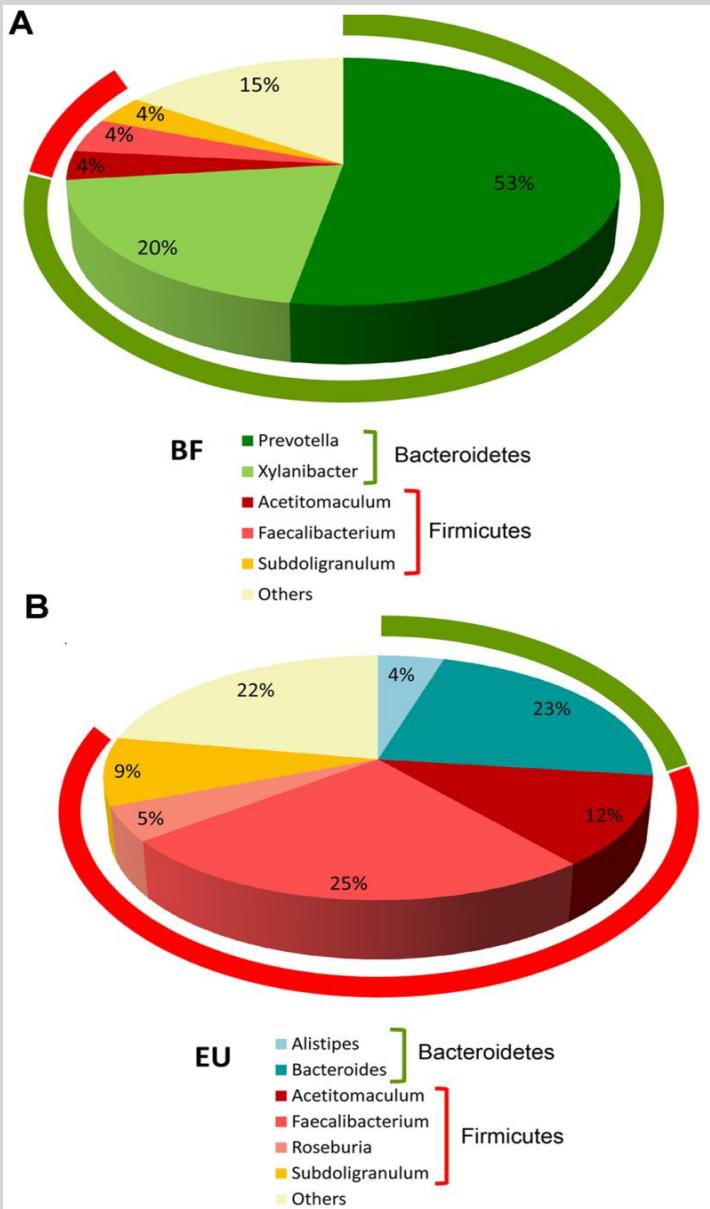


Firmicutes

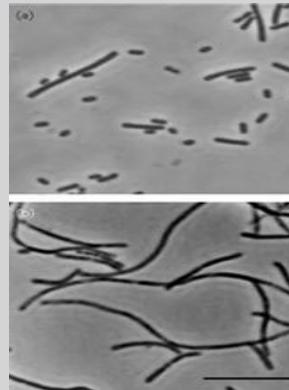
Differential distribution of Firmicutes and Bacteroidetes phyla delineates a deep difference between BF and EU



Ley RE et al. 2006

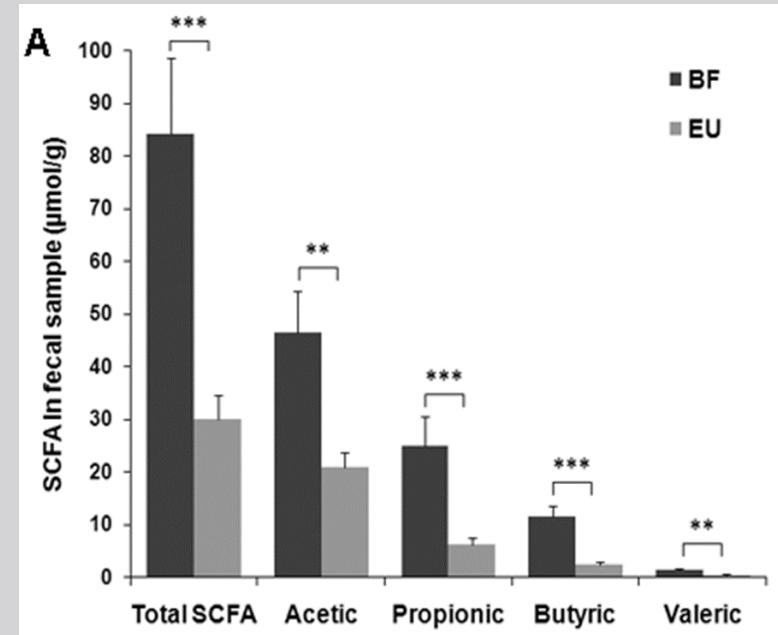


The presence of ***Xylanibacter*** and ***Prevotella*** and ***Treponema*** is a consequence of fiber rich diet, maximizing the possibility to extract metabolic energy from ingested plant polysaccharides. A possible symbiosis?



Xylanibacter

Prevotella



Complete linkage hierarchical clustering produced a net separation of BF and EU populations



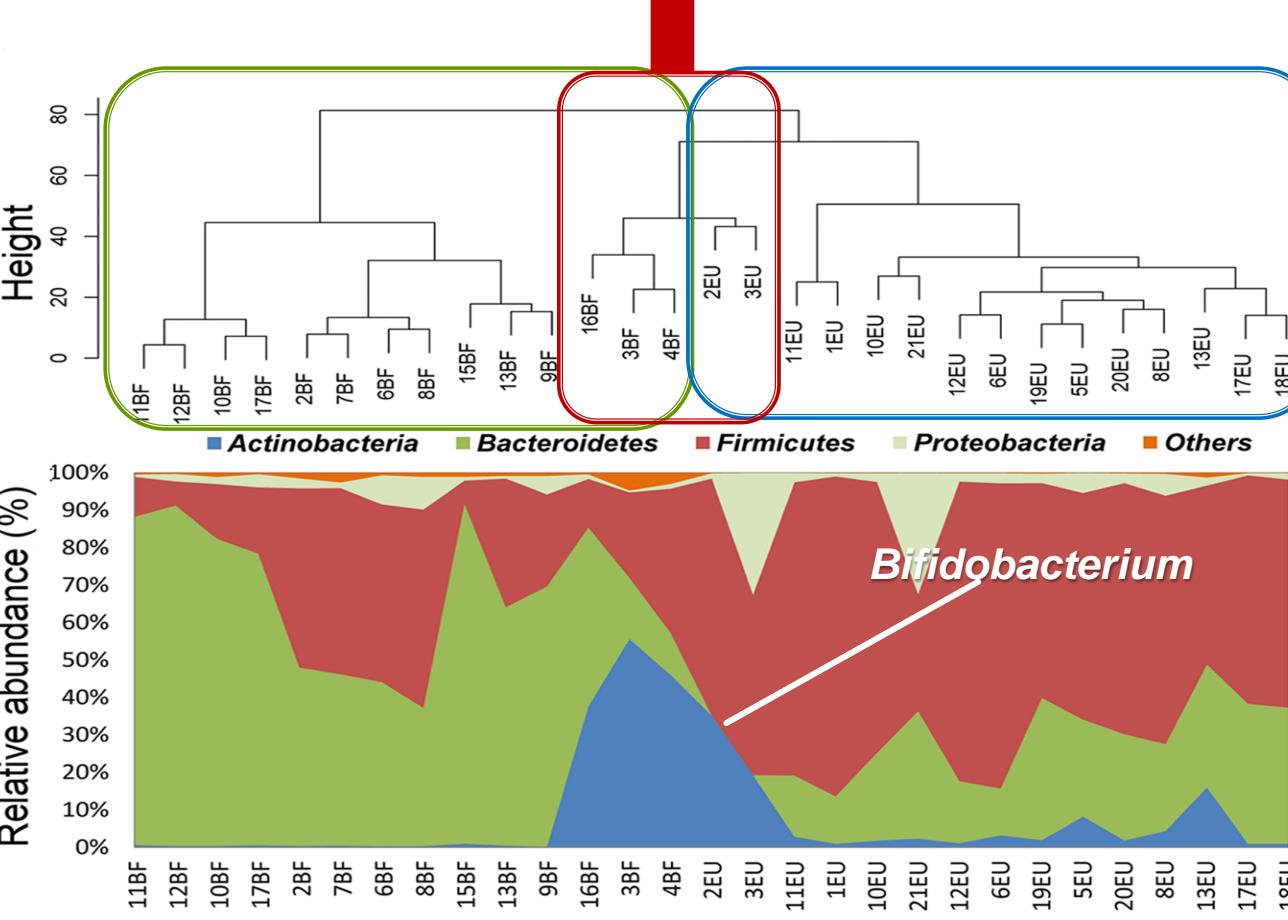
Bacteroidetes



Youngest children still
breast fed



C



Firmicutes

Clear indication of the **dominant role of diet** over the others variables in shaping the gut microbiota composition.

Human gut microbiome viewed across age and geography

Tanya Yatsunenko¹, Federico E. Rey¹, Mark J. Manary^{2,3}, Indi Trehan^{2,4}, Maria Gloria Dominguez-Bello⁵, Monica Contreras⁶, Magda Magris⁷, Glida Hidalgo⁷, Robert N. Baldassano⁸, Andrey P. Anokhin⁹, Andrew C. Heath⁹, Barbara Warner², Jens Reeder¹⁰, Justin Kuczynski¹⁰, J. Gregory Caporaso¹¹, Catherine A. Lozupone¹⁰, Christian Lauber¹⁰, Jose Carlos Clemente¹⁰, Dan Knights¹⁰, Rob Knight^{10,12} & Jeffrey I. Gordon¹

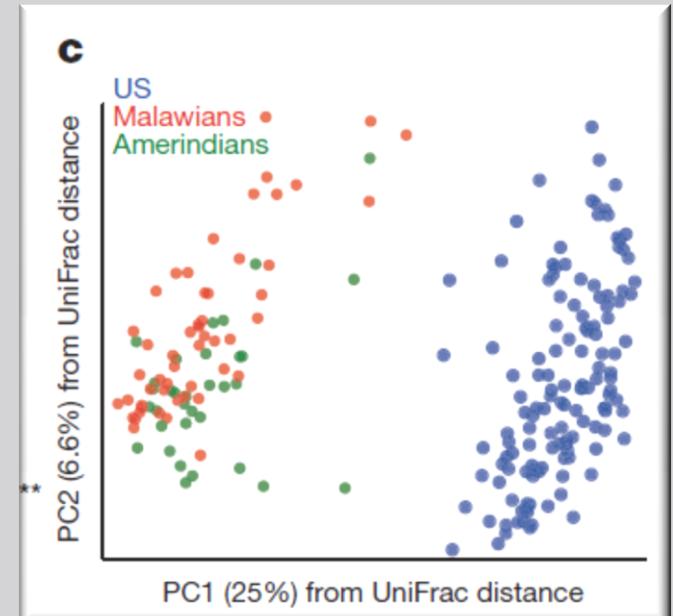
- 531 individuals (151 families):
- 115 individuals (34 families) from **Malawi** - 4 rural communities
- 100 individuals (19 families) from **Venezuela** - Amerindians residing in 2 villages
- 316 individuals (98 families) from the **United States**

Age and geography/cultural traditions primarily explain the variation in the data set, in which US microbiota clustered separately from non-US microbiota along principal coordinate 1

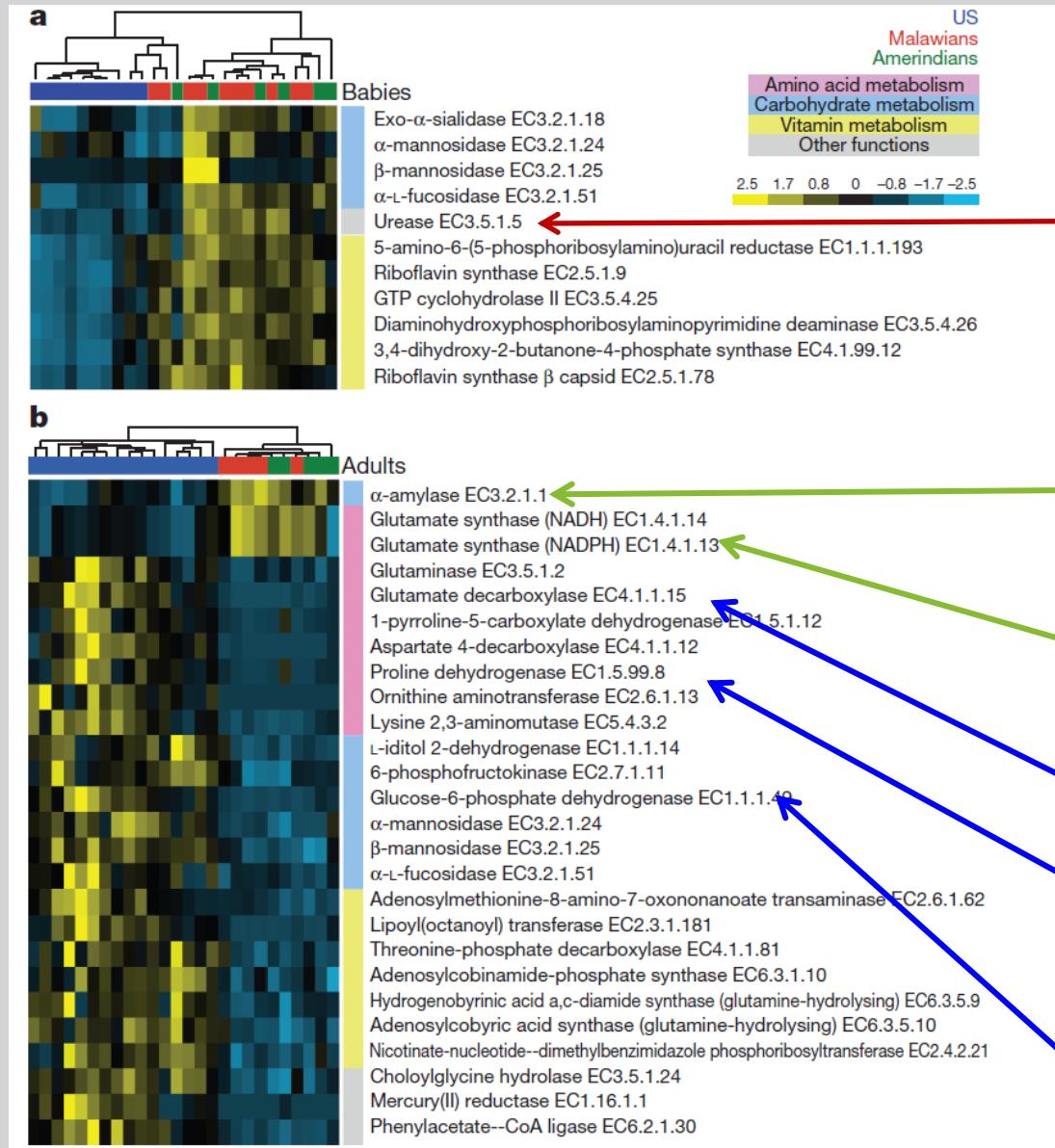
Yatsunenko *et al.*, *Nature* 2012



PCoA of unweighted UniFrac distances for the fecal microbiota of adults.



Differences in the functional profiles of fecal microbiomes in the 3 study populations.



Urease releases ammonia that can be used for microbial biosynthesis of essential and nonessential amino acids.

Urease has a crucial involvement in nitrogen recycling, particularly when **diets are deficient in protein**.

Under conditions in which dietary nitrogen is limiting, the ability of the microbiome to use **urea** would presumably be advantageous to both the microbial community and host.

a-amylase which participates in the degradation of starch, was overrepresented in the Malawian and Amerindian microbiomes, reflecting their **corn-rich diet**.

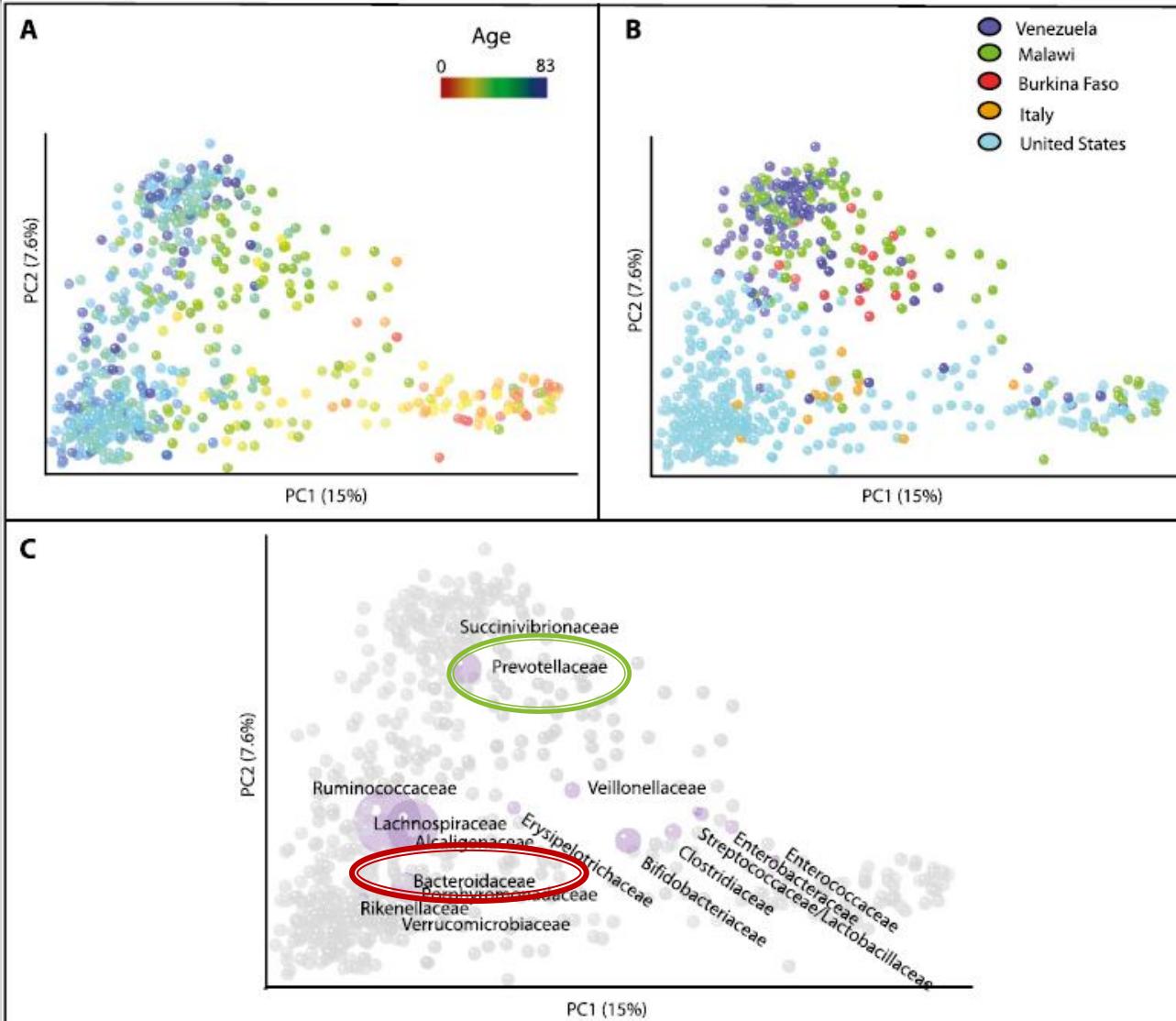
Glutamate synthase have higher proportional representation in **Malawian and Amerindian** adult microbiomes and are also higher in herbivorous mammalian microbiomes.

Degradation of glutamine was overrepresented in **US** as well as carnivorous mammalian microbiomes.

ECs involved in the **degradation of other amino acids** were overrepresented in adult **US** fecal microbiomes: aspartate; proline, ornithine and lysine.

Overrepresentation in adult **US** ECs involved in the catabolism of **simple sugars**.

Meta-analyses of studies of the human microbiota



Taxa analysis indicated that this difference was associated with an enrichment of **Prevotellaceae** in the adults from rural cultures and **Bacteroidaceae** in adults from Western cultures.

This observation is consistent with the independent reports of enrichment of **Prevotella** in fecal samples from individuals living in non-Western societies in two of the studies included in this analysis

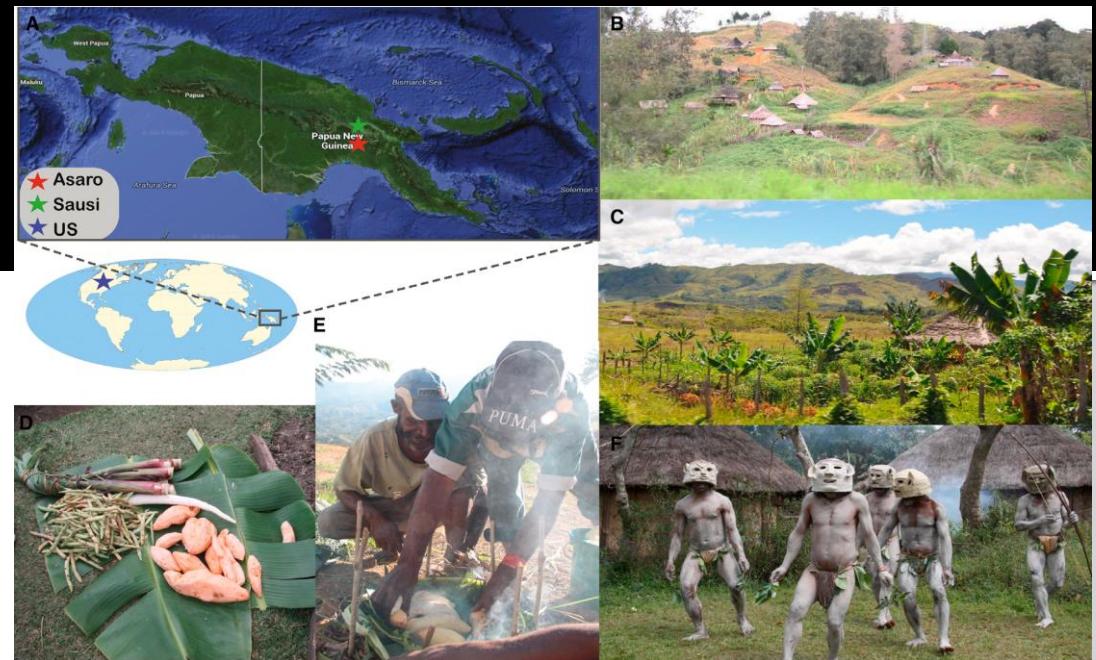
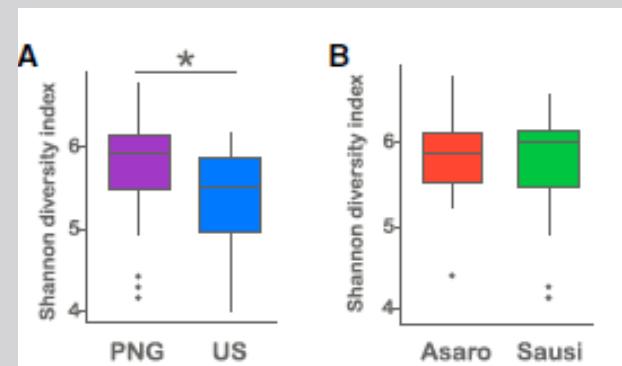
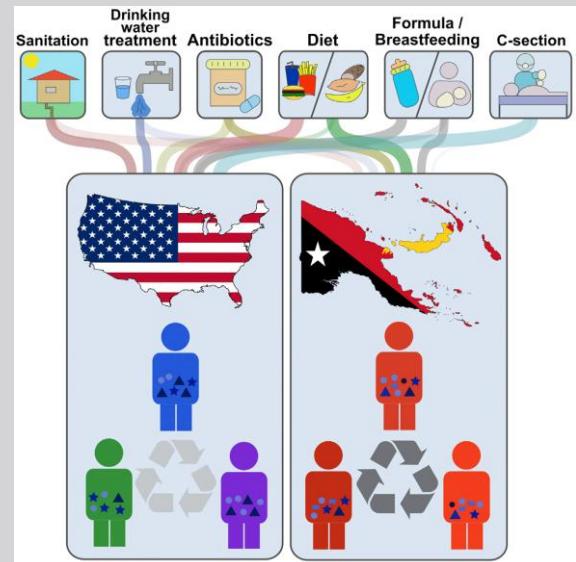
Lozupone et al., Genome Res, 2013

The PNG Fecal Microbiota has higher biodiversity and lower Inter-individual variation

Cell Reports
Article

The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes

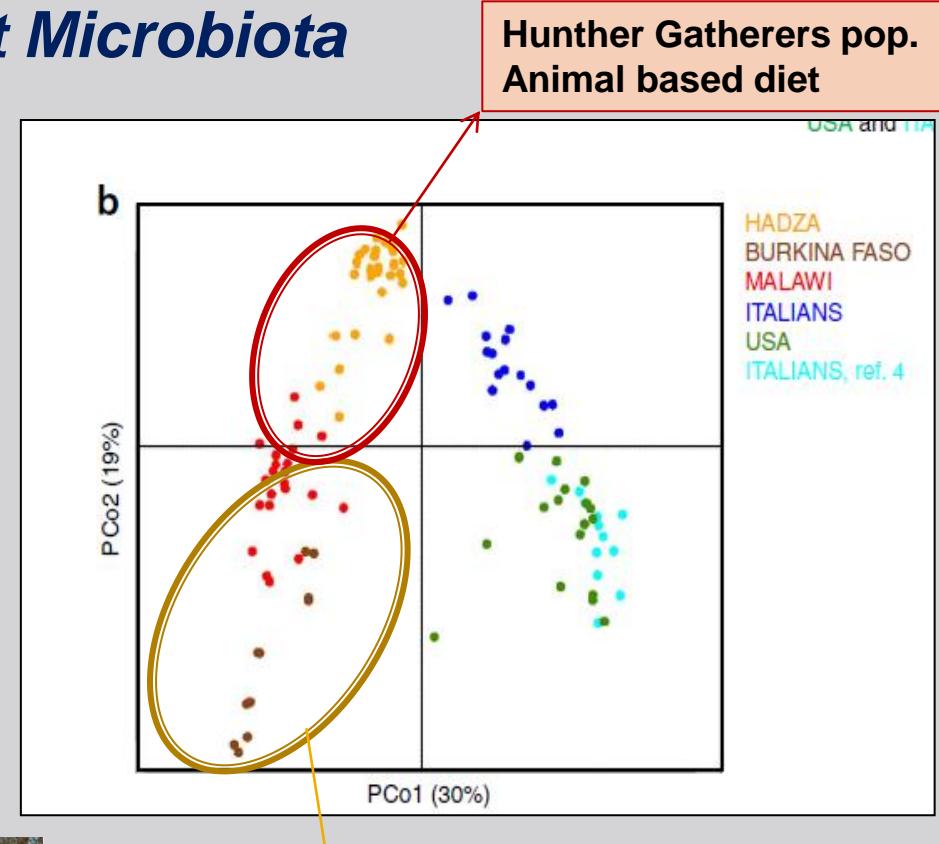
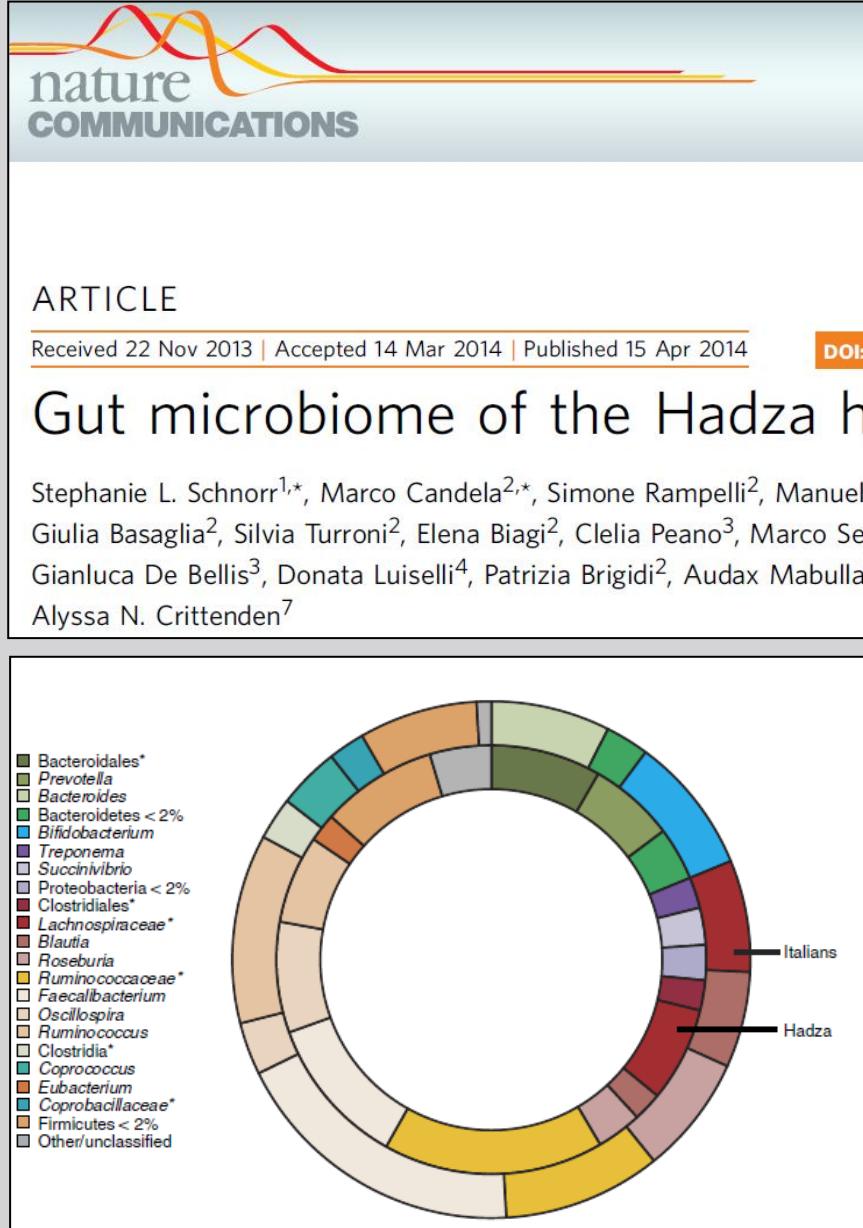
Inés Martínez,^{1,2} James C. Stegen,³ María X. Maldonado-Gómez,² A. Murat Eren,⁴ Peter M. Siba,⁵ Andrew R. Greenhill,^{5,6,*} and Jens Walter^{1,2,7,*}



- The fecal microbiota in PNG is more diverse but less individualized than in the US
- Most bacterial species are shared among PNG and the US, but abundance profiles differ
- Impact of lifestyle on ecological assembly processes might explain these patterns
- Westernization may decrease bacterial dispersal rates, altering microbiota structure

Martinez et al., Cell Reports 2015

Hunter-Gatherers Gut Microbiota

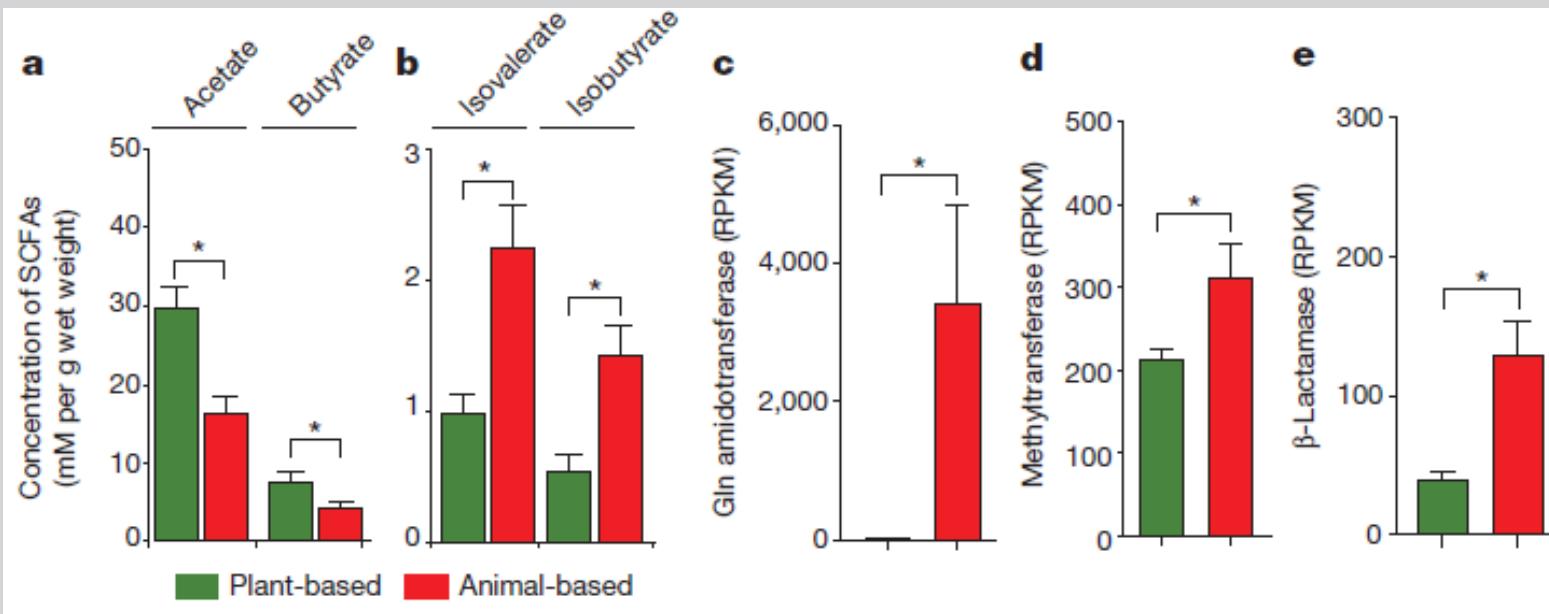


Comparison of gut microbiome relative abundance among populations reflects subsistence patterns.

Schnorr et al., 2014

Diet rapidly and reproducibly alters the human gut microbiome

Lawrence A. David^{1,2†}, Corinne F. Maurice¹, Rachel N. Carmody¹, David B. Gootenberg¹, Julie E. Button¹, Benjamin E. Wolfe¹, Alisha V. Ling³, A. Sloan Devlin⁴, Yug Varma⁴, Michael A. Fischbach⁴, Sudha B. Biddinger³, Rachel J. Dutton¹ & Peter J. Turnbaugh¹



Foodborne microbes from both diets transiently colonized the gut, including bacteria, fungi and even viruses.

David et al., Nature 2014

The animal-based diet increased the abundance of bile-tolerant microorganisms (*Alistipes*, *Bilophila* and *Bacteroides*) and decreased the levels of Firmicutes that metabolize dietary plant polysaccharides (*Roseburia*, *Eubacterium rectale* and *Ruminococcus bromii*).

Effect of diet and environment on gut microbiota in African children shifting from rural to urban areas

BR (Burkina Faso- rural village)



BT (Burkina Faso-Nanoro Town)



BC (Capital City of Burkina Faso)



EU (Florence-Italy)



Comparative study of gut microbiota composition of:

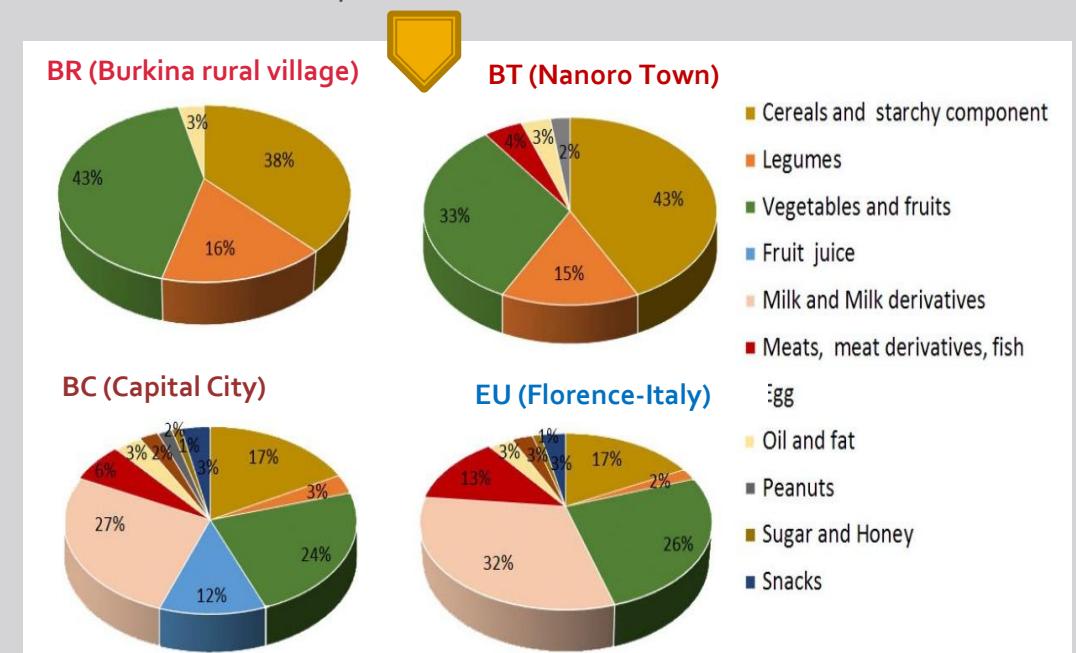
- **African children** living in rural/urban areas (N=24) with different levels of urbanization and socio-economic conditions
- **Italian children** (N=13), as a representative of western and urbanized population.

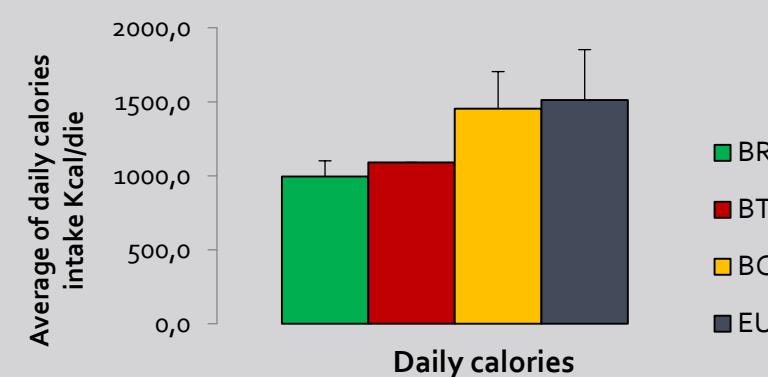
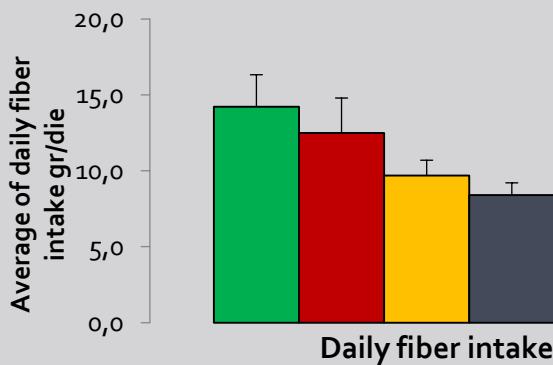
De Filippo et al., Frontiers in Microbiology 2017

Traditional Burkinabè food

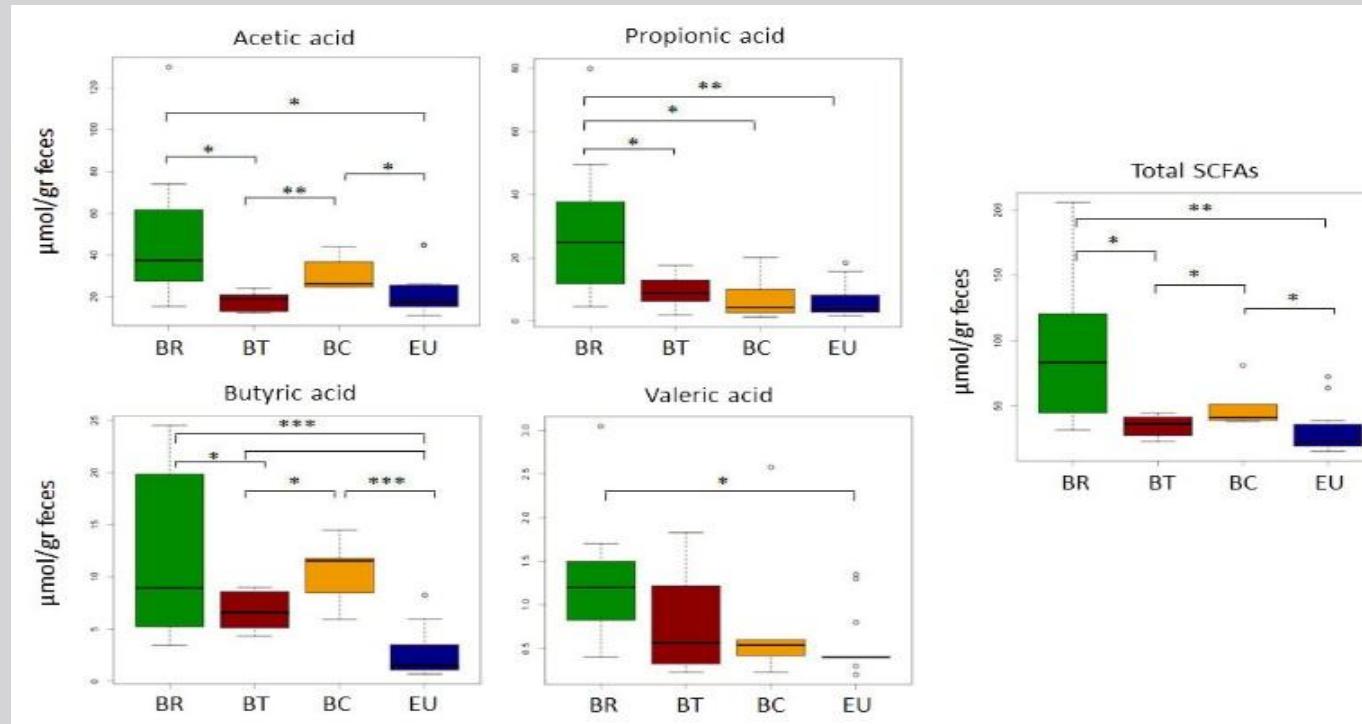


Moving people from a rural to an urban environment, we observed a gradual enrichment in food variety, with increase in animal-derived products (meat, fish and milk-derived food), processed and refined foods, and consequent increase in fat and animal protein and reduction in fiber intake.





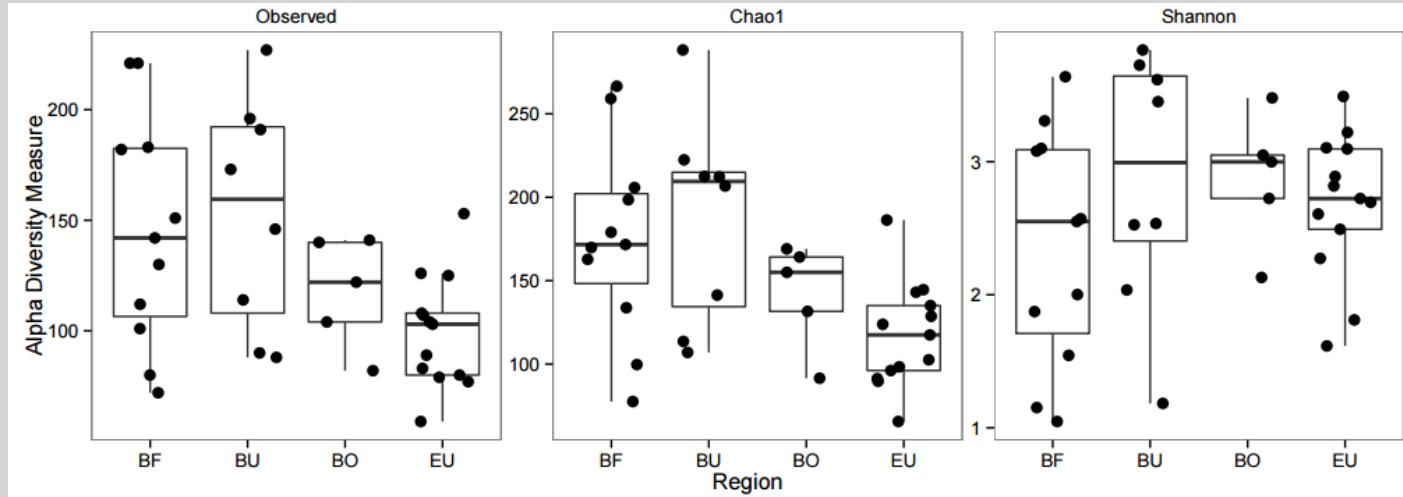
Daily fiber intake is inversely correlated with calories intake



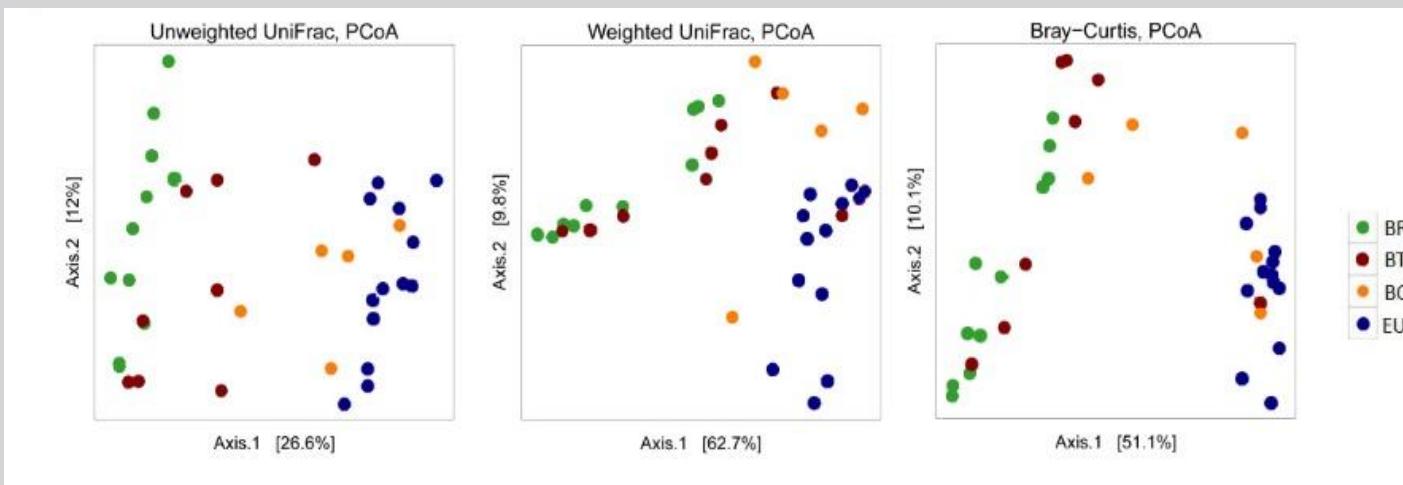
Daily fiber intake correlated with levels of fecal short chain fatty acids (SCFAs)

Quantification of SCFAs levels in fecal samples by SPME-Gas-Chromatogray-Mass Spectrometry (Mean values (\pm SEM) are plotted).

The Impact of urbanization on gut microbiota diversity

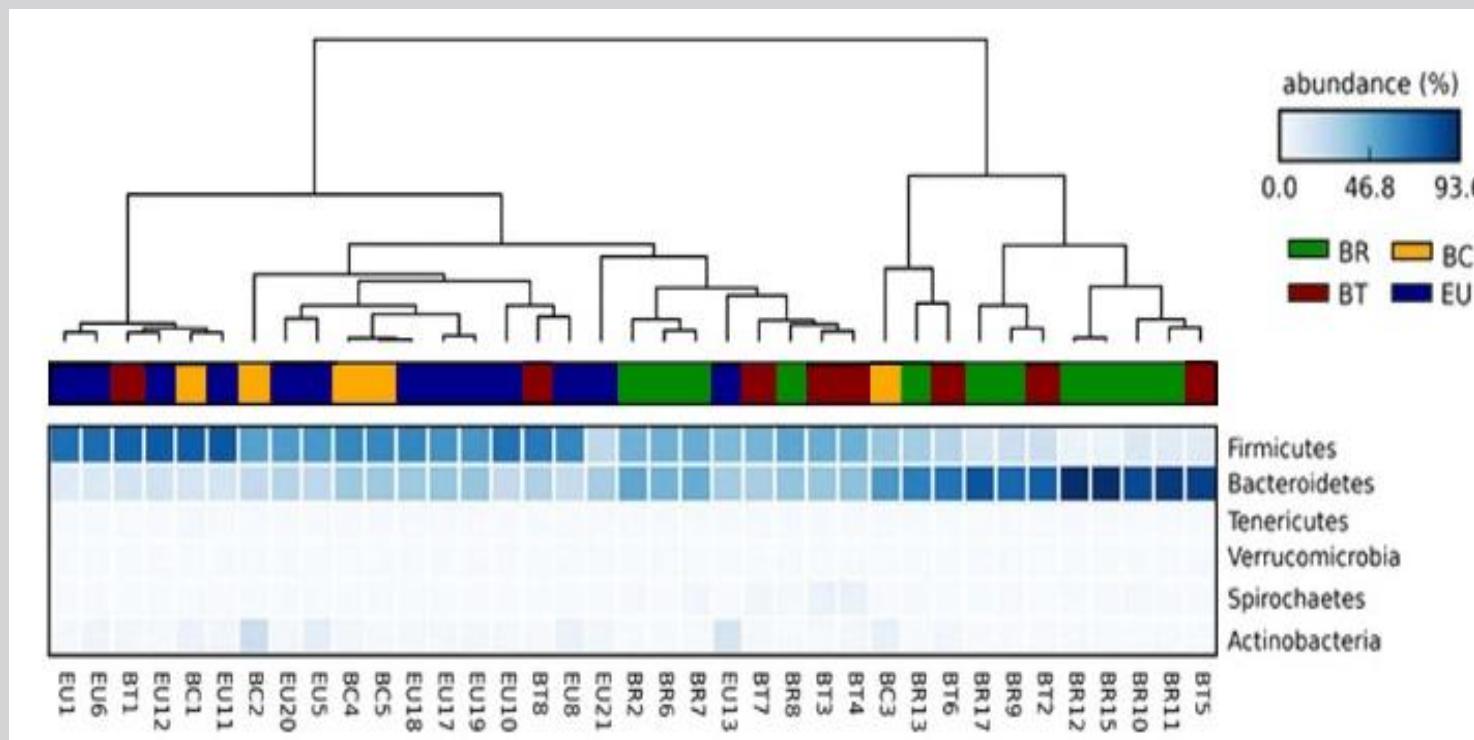


Alpha-diversity:
bacterial richness



Beta diversity:
microbiota variability among the populations

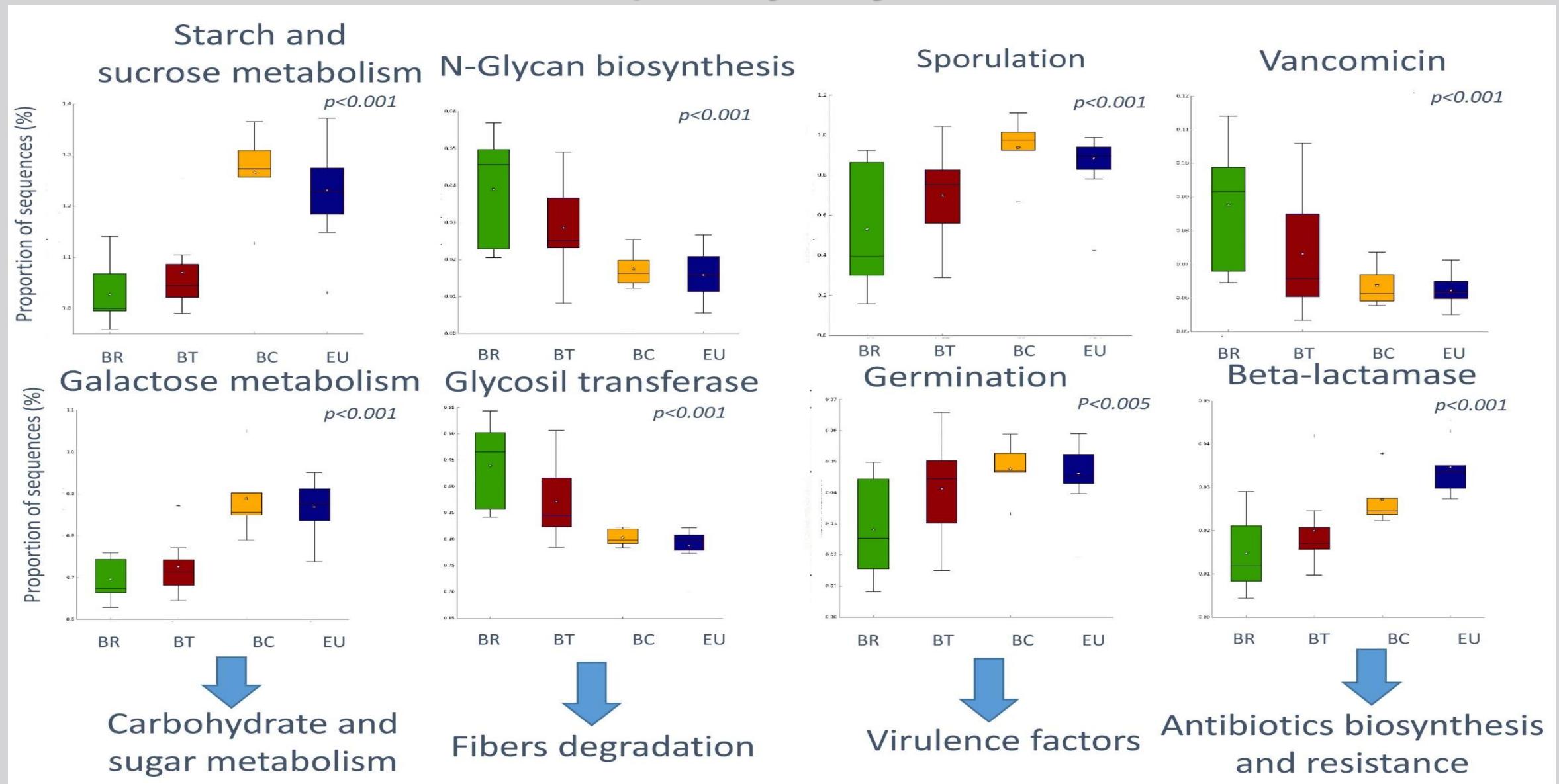
Taxonomic changes as an effect of diet and of migration from rural to urban environments



Dendrogram used to cluster microbiotas of African and Italian children, based on phyla abundances reported in the heatmap.

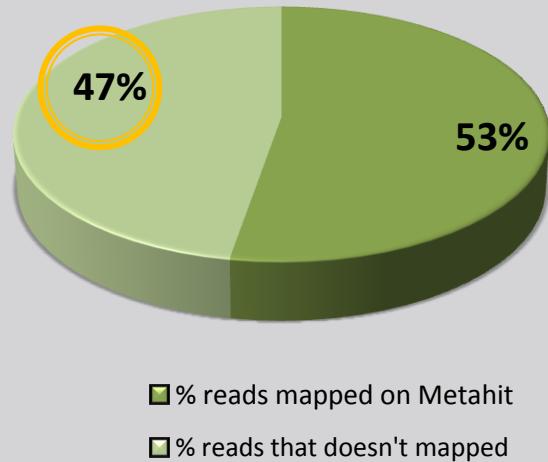
PICRUSt analysis_prediction of bacterial metabolic functions

KEGG pathway and function

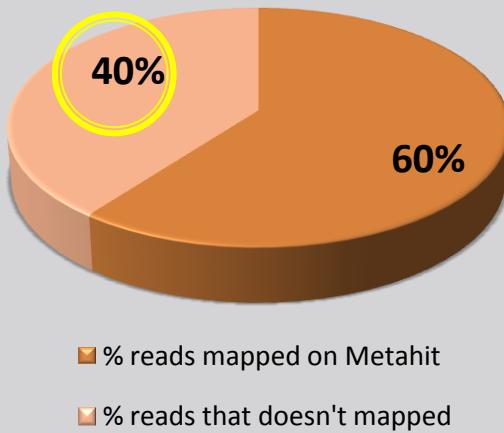


Percentage of reads generated with Illumina Genome Analyzer HiSeq 2000 mapped on MetaHIT

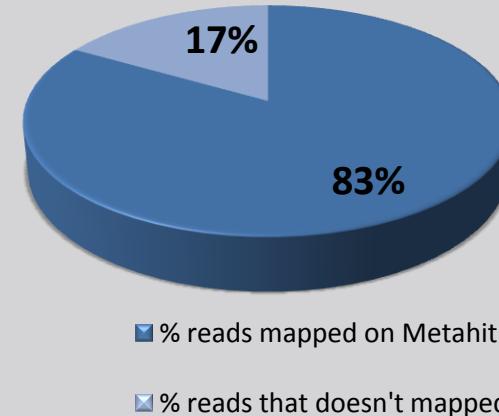
Rural Burkina Faso



Urban Burkina Faso



European



In BF Rural and BF Urban groups, respectively 47% and 40% of the bacterial sequences are "unknown", it is not mapped to the MetaHIT database.

Instead almost all of the microbiome of EU (83%) is known and map versus MetaHIT database.

It's reasonable to define enterotypes or we have yet to explore the rural populations of all of the world?

To what extent the Western lifestyle has led to the loss of biodiversity of microbial communities in symbiosis with the human body?

The most **dramatic change** to the gut microbiome in the human ancestral line has been the **modern transformation** of the human condition in cosmopolitan populations.

Tito RY et al., PLoS ONE, 2012

How the **pressures of westernization** are changing the microbial parts of our genetic landscape changes that potentially mediate the suite of pathophysiological states correlated with westernization.

Finally, given the need for global policies about **sustainable agriculture and improved nutrition**, it will be important to understand how we can match these policies not only to our varying cultural traditions but also to **our varied gut microbiomes**.

Yatsunenko et al., Nature 2012

The lessons learned from the BF children's microbiota prove the importance of sampling and **preserving microbial biodiversity** from regions where the effects of globalization on diet are less profound.

De Filippo et al., PNAS 2010

We further suggested possible explanations linking diet and lifestyle with microbial community functions and, finally, we showed that studying gut bacterial communities across regional cohorts with distinctive **socio-cultural features** extends our knowledge of the landscape of healthy human gut microbiome diversity.

Tyakht et al., Nat Comm 2013

Clearly, the characterization of the gut microbiota in non-westernized populations provides information that might aid in the development of **strategies to reintroduce bacterial lineages that have been eradicated in westernized human populations**. Studies such as ours are timely, as human populations that live a non-westernized lifestyle are in decline.

Martinez et al., Cell Rep 2015

How Environmental pressure can be responsible of loss of function or gain of function of gut microbiota?

SCIENTIFIC REPORTS

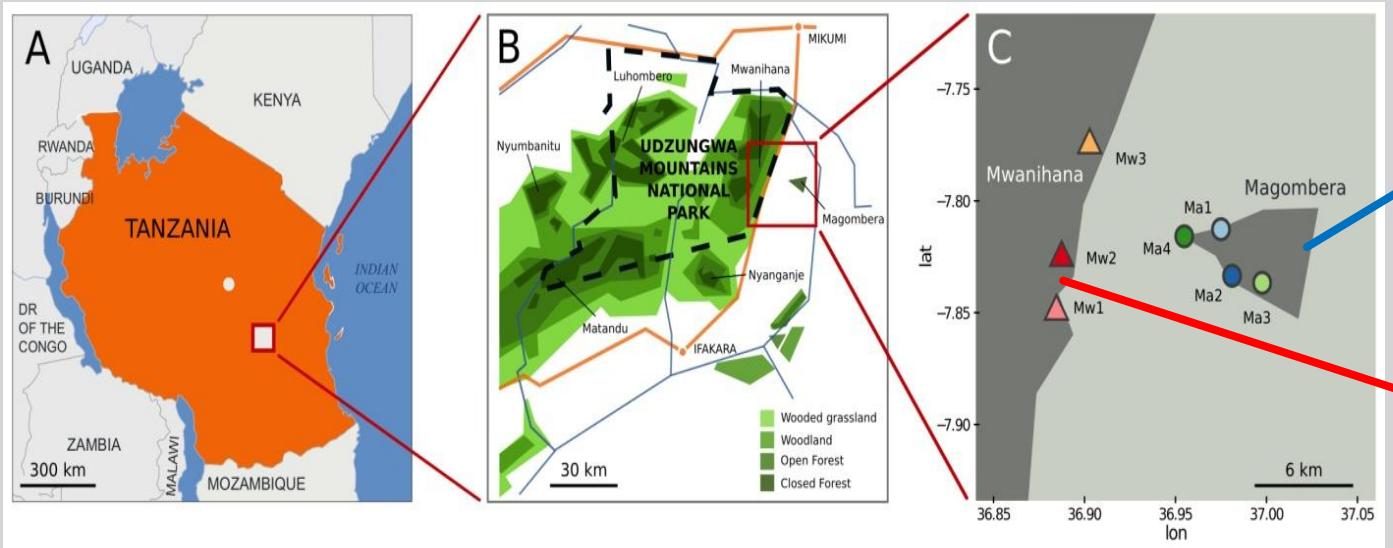


OPEN

Habitat fragmentation is associated to gut microbiota diversity of an endangered primate: implications for conservation

Claudia Barelli^{1,2,*}, Davide Albanese^{4*}, Claudio Donati⁴, Massimo Pindo⁵, Chiara Dallago⁵, Francesco Rovero¹, Duccio Cavalieri¹, Kieran Michael Tuohy⁶, Heidi Christine Hauffe¹ & Carlotta De Filippo^{6,7}

Received: 18 June 2015
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This primate species provides an excellent model for investigating variation in gut microbial communities across host habitats because it has a specialized diet, making it particularly sensitive to habitat degradation.

Barelli et al., *Scient Rep* 2015

*Red colobus monkeys (*Procolobus gordoni*) living in the Udzungwa Mountains (Tanzania) are arboreal forest dwellers.*



Magombera forest _ Disturbed

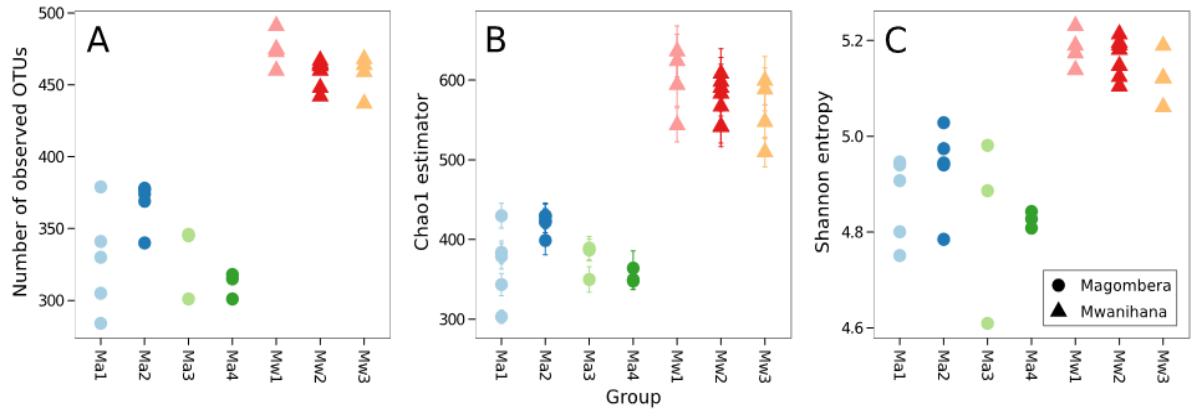


Mwanihana forest _ Undisturbed

Gut microbiota profiles differ between disturbed (*Magombera*) and un-disturbed (*Mwanihana*) forests

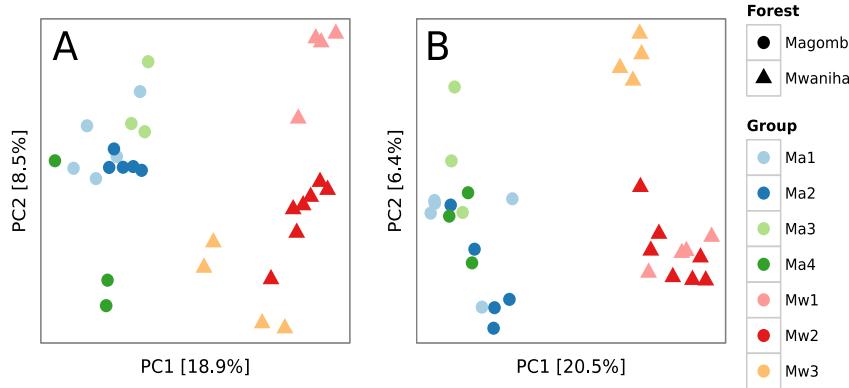
Barelli et al., *Scient Rep* 2015

Measures of α -diversity

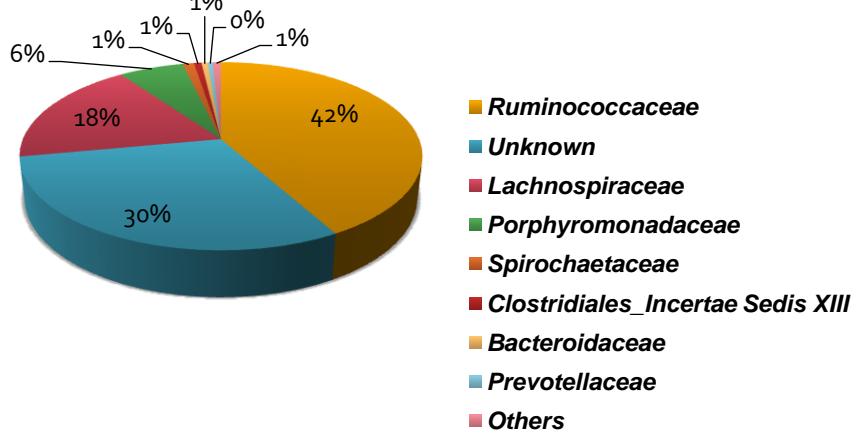


Microbiota richness and diversity are reduced in Magombera forest, likely as result of dietary changes enforced by human disturbance and habitat degradation.

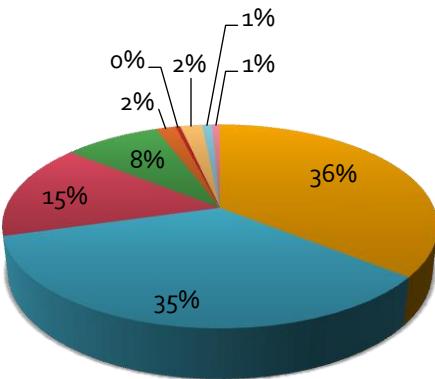
Measures of β -diversity



Magombera

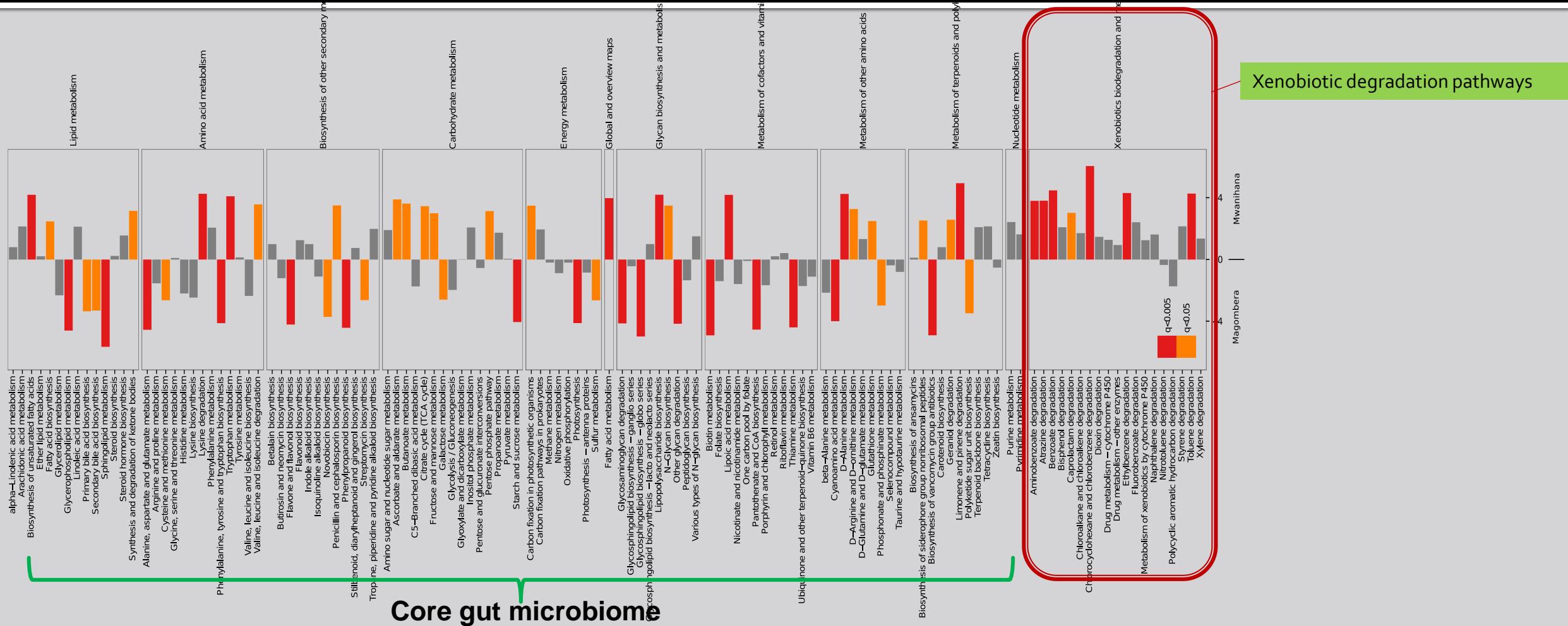


Mwanihana

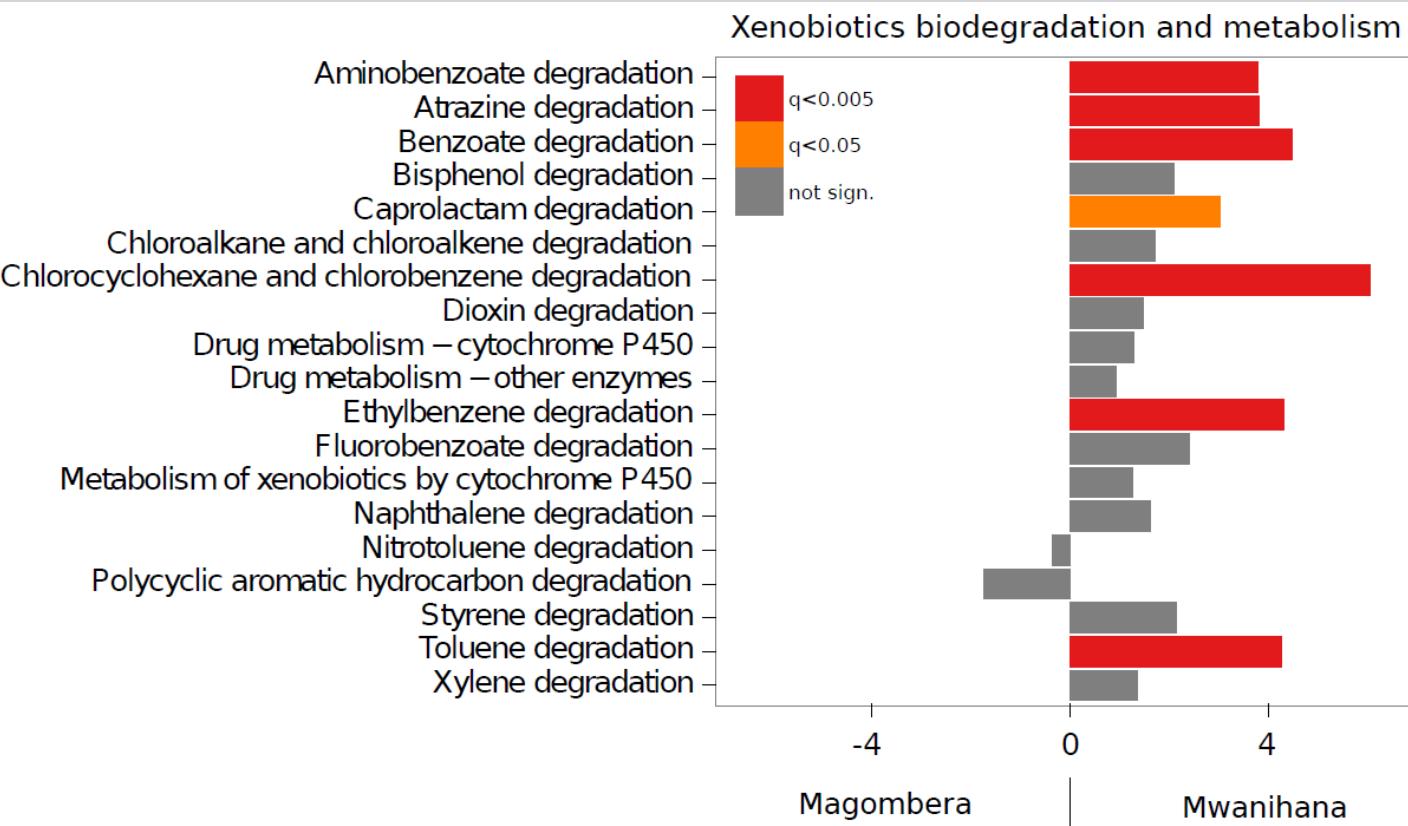


Relative abundance of **Bacteroidaceae**, **Prevotellaceae** and **Ruminococcaceae** were significantly different between social groups inhabiting the two forests

The fiber-rich diet of colobus monkeys has resulted in a gut microbiome enriched in metabolic functions such as carbohydrate metabolism



Lost of metabolic functions in disturbed forest: as the environment can influence the gut microbiota metabolic potentiality



The xenobiotic degradation pathways are significantly over-represented in samples from Mw forest.



Presence of plant species in Mw rich in potentially **harmful tannins** (i.e., *Antiaris toxicaria welwitschii*) is among the most frequently eaten plant species in Mw).

The microbial community involved in **detoxification** of potentially toxic compounds present in Mw red colobus has been **lost** in Ma, where, despite the small geographical distance, these plant species are no longer present.

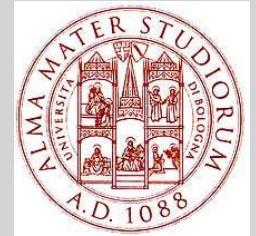
The diet-microbiota interaction: how globalization and sanitation are affecting biodiversity of human microbiota



*“Whether thinking about the impact of humanity’s discovery of fire and cooking food, our formation of communities with domestication of animals and crops, our current exploding populations **migrating from rural to urban settings**, or **our increasing hygiene and use of antibiotics**, we are prompted to consider that there is **another dimension to our human evolution** and human condition.*

*This other dimension, involving the collection of gut microbial species (microbiota) and their genes (microbiomes) is affecting our concepts of “**healthy**” of disease risk, and of how Western life-styles are affecting us.”*

Population level Metagenomic Variation in rural communities



Exploration of the microbiota of diverse human populations (including traditional societies such as hunter-gatherers), studies of ancient samples derived from coprolites and mummified or fossilized hominins, and investigations into our great-ape relatives will together provide a picture of how the microbiota has shaped and has been shaped by our natural history.

Strati *et al.* *Microbiome* (2016) 4:41
DOI 10.1186/s40168-016-0185-y

RESEARCH

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Altered gut microbiota in Rett syndrome

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Strati *et al.* *Microbiome* (2017) 5:24
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Microbiome

RESEARCH

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New evidences on the altered gut microbiota in autism spectrum disorders

Francesco Strati^{1,2}, Duccio Cavalieri³, Davide Albanese¹, Claudio De Felice⁴, Claudio Donati¹, Joussef Hayek^{5,6}, Olivier Jousson², Silvia Leoncini⁵, Daniela Renzi⁷, Antonio Calabro⁷ and Carlotta De Filippo^{8*}

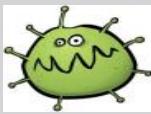
Alteration of Fecal Microbiota Profiles in Juvenile Idiopathic Arthritis. Associations with HLA-B27 Allele and Disease Status

Monica Di Paola¹, Duccio Cavalieri², Davide Albanese³, Maddalena Sordo³, Massimo Pindo³, Claudio Donati³, Ilaria Pagnini⁴, Teresa Giani⁴, Gabriele Simonini^{1,4}, Alessia Paladini⁴, Paolo Lionetti¹, Carlotta De Filippo^{5*} and Rolando Cimaz^{1,4*}

Age and Gender Affect the Composition of Fungal Population of the Human Gastrointestinal Tract

Francesco Strati^{1,2}, Monica Di Paola³, Irene Stefanini¹, Davide Albanese¹, Lisa Rizzetto¹, Paolo Lionetti³, Antonio Calabro⁴, Olivier Jousson², Claudio Donati¹, Duccio Cavalieri^{1,5*} and Carlotta De Filippo^{6*}

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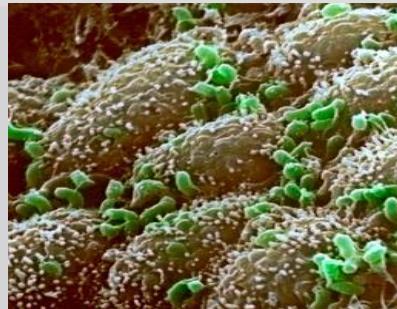
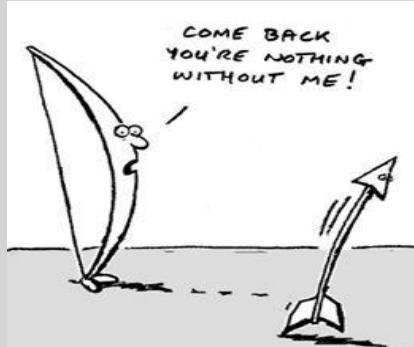
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