# Gut microbiome of the Hadza hunter-gatherers

Stephanie L. Schnorr et al. (2014) Nature Communications 5, Article number: 3654 doi:10.1038/ncomms4654

DEPT MEETING
3 June 2014
M. UMEZAKI

**ABSTRACT.** Human gut microbiota directly influences health and provides an extra means of adaptive potential to different lifestyles. To explore variation in gut microbiota and to understand how these bacteria may have co-evolved with humans, here we investigate the phylogenetic diversity and metabolite production of the gut microbiota from a community of human hunter-gatherers, the Hadza of Tanzania. We show that the Hadza have higher levels of microbial richness and biodiversity than Italian urban controls. Further comparisons with two rural farming African groups illustrate other features unique to Hadza that can be linked to a foraging lifestyle. These include absence of *Bifidobacterium* and differences in microbial composition between the sexes that probably reflect sexual division of labour. Furthermore, enrichment in *Prevotella*, *Treponema* and unclassified *Bacteroidetes*, as well as a peculiar arrangement of *Clostridiales* taxa, may enhance the Hadza's ability to digest and extract valuable nutrition from fibrous plant foods.

# Introduction

Host — Microbiome

Supra-organisms

Natural Selection

Adaptation

Previous studies showed:

Rural:
Higher biodiversity and enrichment of Bacteroidetes and Actinobacteria

Urban:
Oveall reduction in microbial diversity

Gut microbiota in Hunter gathers?

# Objectives

To compare
(1) phylogenetic diversity,
(2) taxonomic relative abundance
(3) the short-chain fatty-acid (SCFA) profile
of the Hadza microbiome with those of urban living Italian adults.

And compare these data with previously published data on two different rural African groups from Burkina Faso (BF) and Malawi to identify GM features unique to the Hadza lifestyle.

This study presents the first characterization of a forager GM through work with the Hadza hunter-gatherers, and will allow us to understand how the human microbiota aligns with a foraging lifestyle, one in which all human ancestors participated before the Neolithic transition.

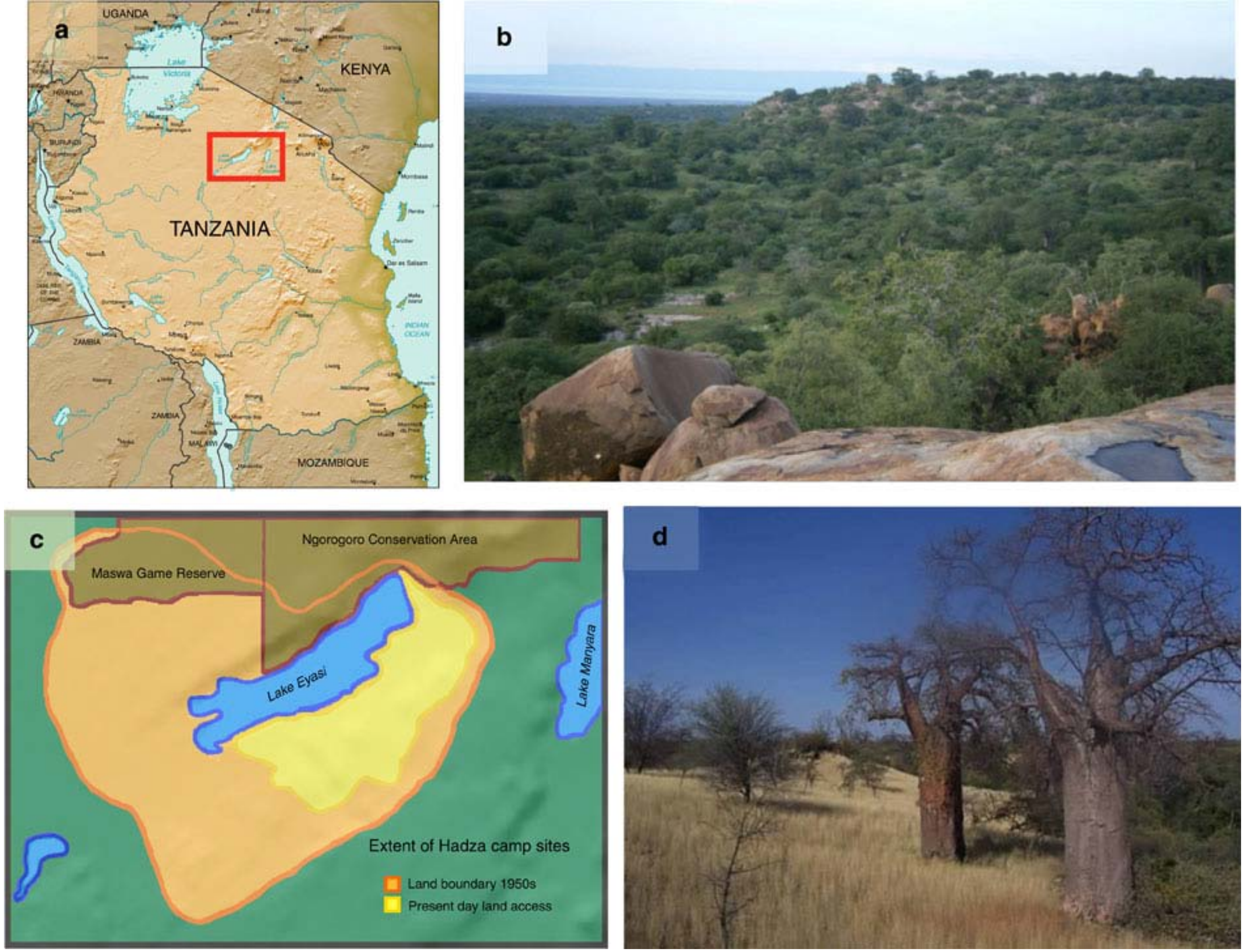


**Figure 1: Location and scenery of Hadza land in Tanzania, Africa.** In deep bush camps, hunting and gathering still make up the majority (>90%) of subsistence. (**a**) Location of Hadza land in northern Tanzania; (**b**) top of a rock ridge near Sengele camp overlooking a lush landscape in between two phases of the rainy season; (**c**) extent of the land surrounding Lake Eyasi where Hadza make their camp sites, orange border denotes land area in 1950s and area in yellow shows the reduced area Hadza occupy today; (**d**) view of baobab trees within Hadza land during the early dry season. Photo **a** modified from the CIA World Factbook. Photos **b** and **d** by SL Schnorr and AN Crittenden.

# Fecal samples:

Hadza (n=27), 8-70 years, mean 32 years, from two different camps (Jan 2013)
Italian (n=16), 20-40 years, mean 32 years, Bologna

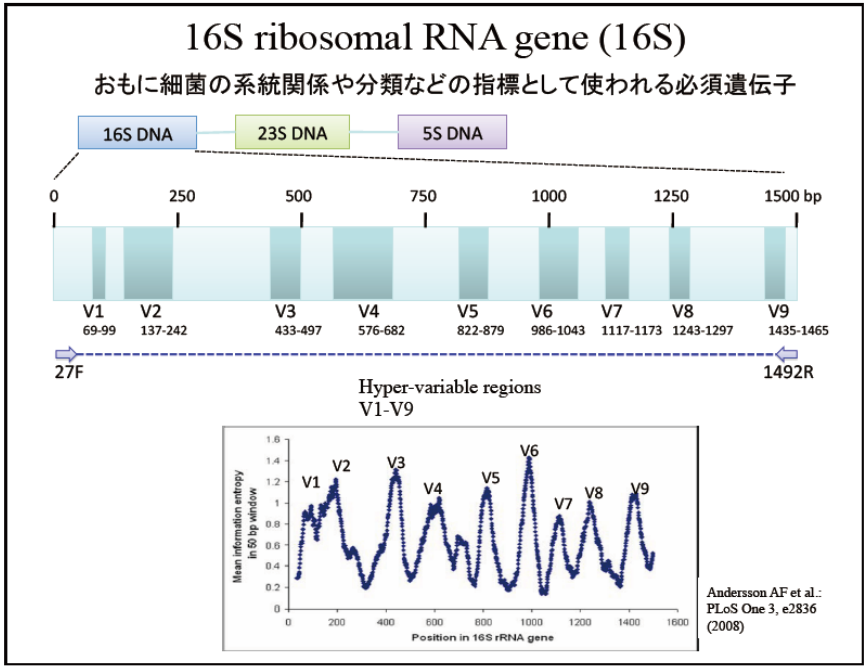
| | |
|---|---|
| Collection and storage | Samples were submerged in 30 ml of 97% ethanol for 24–36 h, after which the ethanol was carefully poured out and the remaining solid material was transferred to 50 ml tubes containing silica beads (Sigma 10087). |
| DNA extraction | QIAamp DNA Stool Mini Kit (QIAGEN). Final DNA concentration was determined by using NanoDrop ND-1000 (NanoDrop Technologies). |
| PCR amplification of V4 region of 16S rDNA gene | The primer set 520F (50-AYTGGGYDTAAAGNG-30) and 802R (50-TACNVGGGTATCTAATCC-30) (with Y.C/T, D.A/G/T, N.any base, V.A/C/G) |

# Phylogenetic Analysis

Identification with the database

Clustering

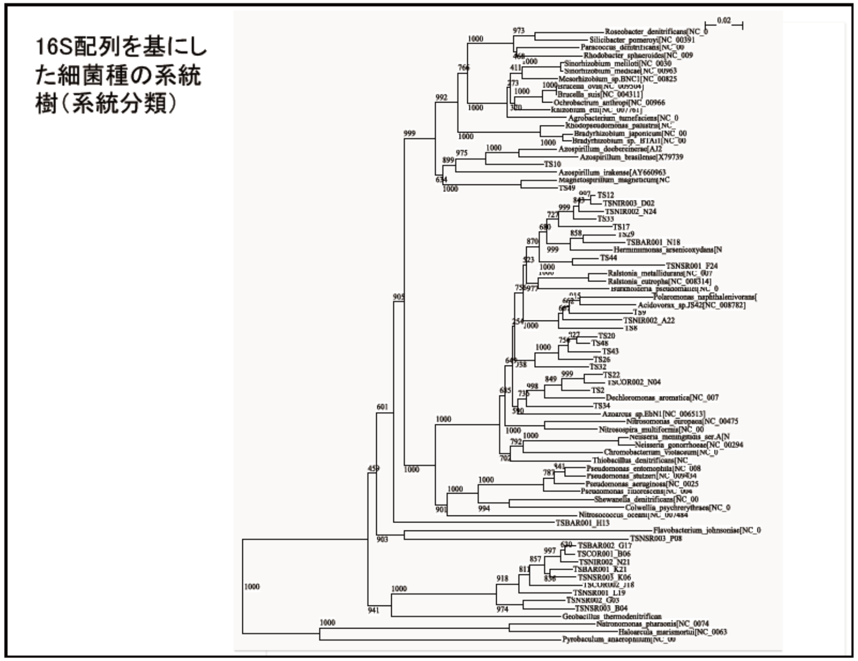
Specific sequences for each species



16S ribosomal RNA gene (16S)

おもに細菌の系統関係や分類などの指標として使われる必須遺伝子

Andersson AF et al.: PLoS One 3, e2836 (2008)

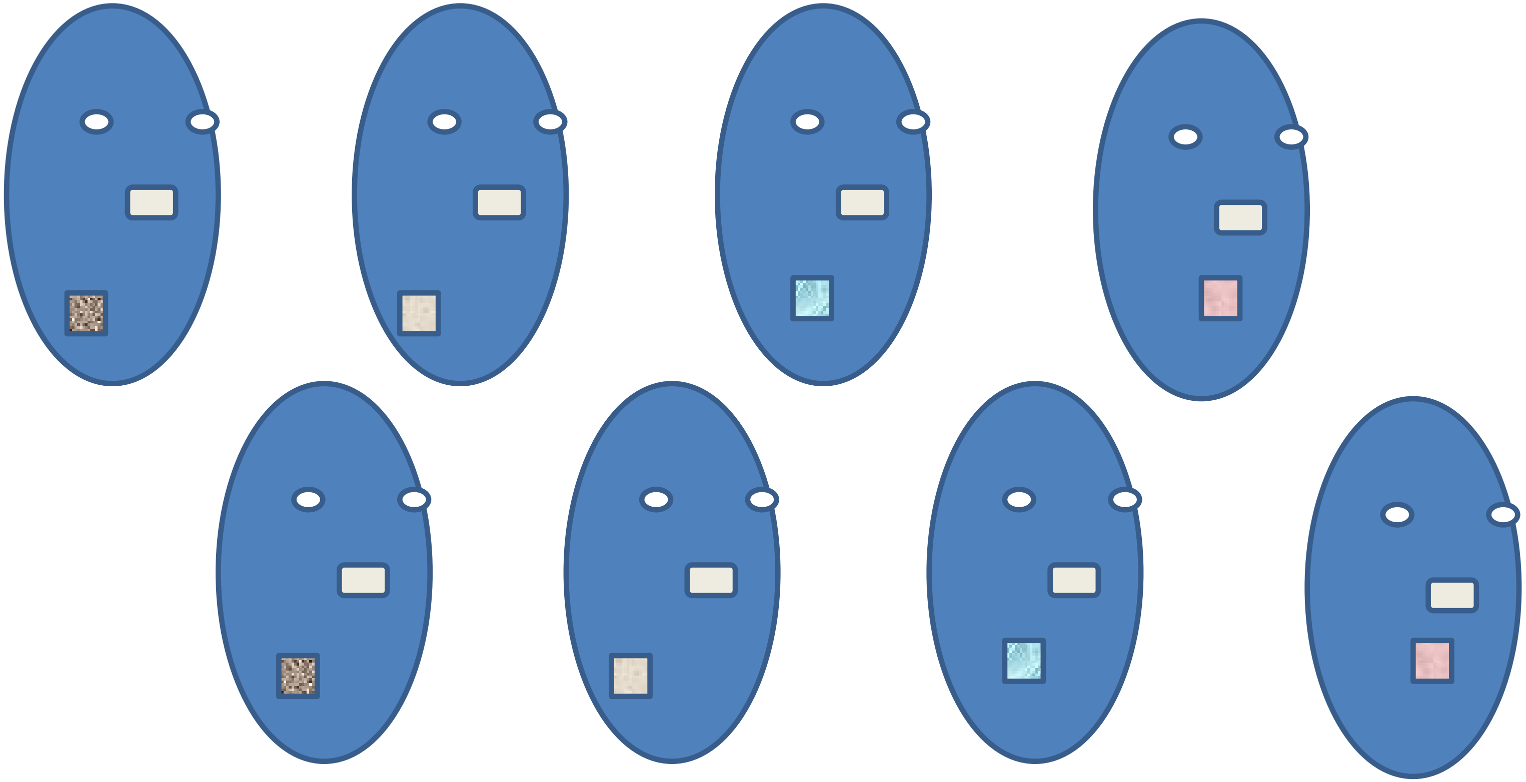


16S配列を基にした細菌種の系統樹（系統分類）

Operational Taxonomic Unit (OUT)

An operational definition of a species or group of species often used when only DNA sequence data is available.

Typically using rDNA and a percent similarity threshold for classifying microbes within the same, or different, OTUs



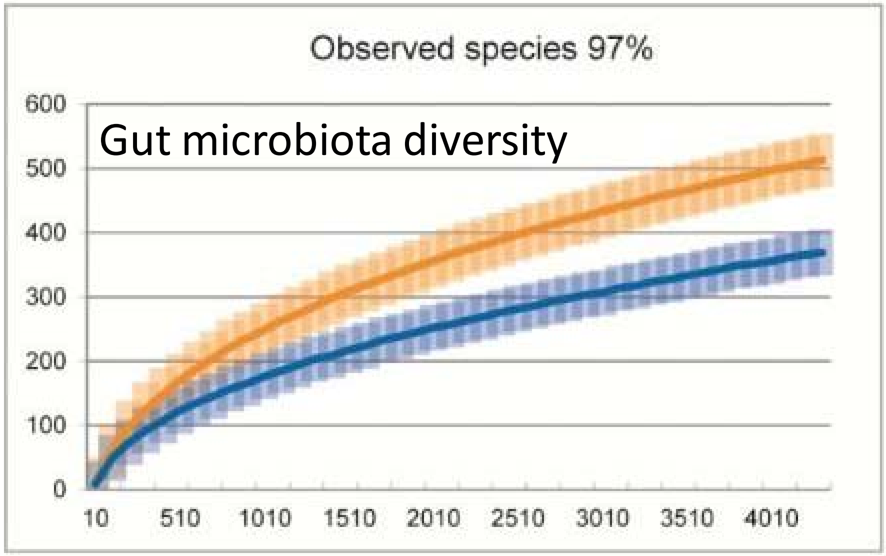
Database

- 🟫 ASD
- ⬜ ADS
- 🟦 WER
- 🟥 CFG

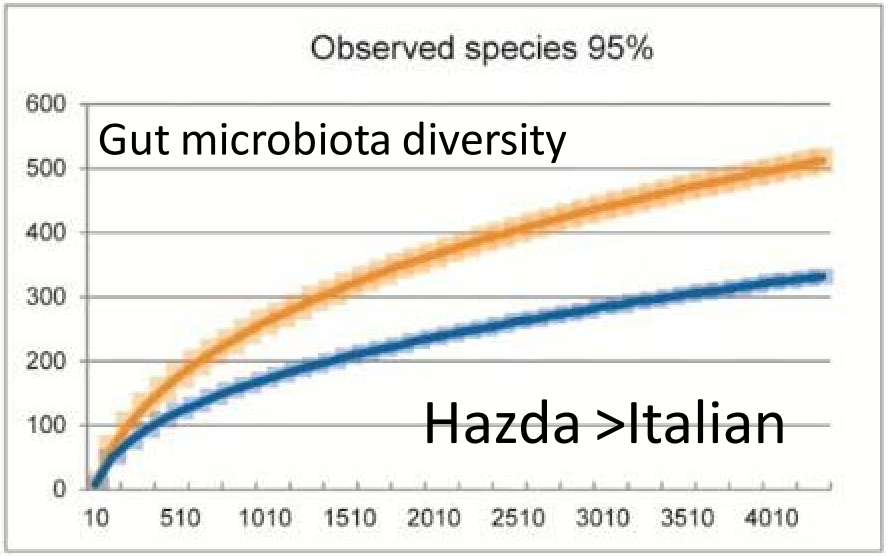
| | | |
|---|---|---|
| 🟫 | ASD | 2 |
| ⬜ | ADS | 2 |
| 🟦 | WER | 2 |
| 🟥 | CFG | 2 |

# Gut microbiota diversity

- 309,952 high-quality reads in total
- 7208 reads / subject in avetage
- 11,967 OUT at 97% identity



Gut microbiota diversity



Gut microbiota diversity

Hazda >Italian

One of 8 panels in S-Fig 3.

Phylum (門)

Microbiota relative abundance

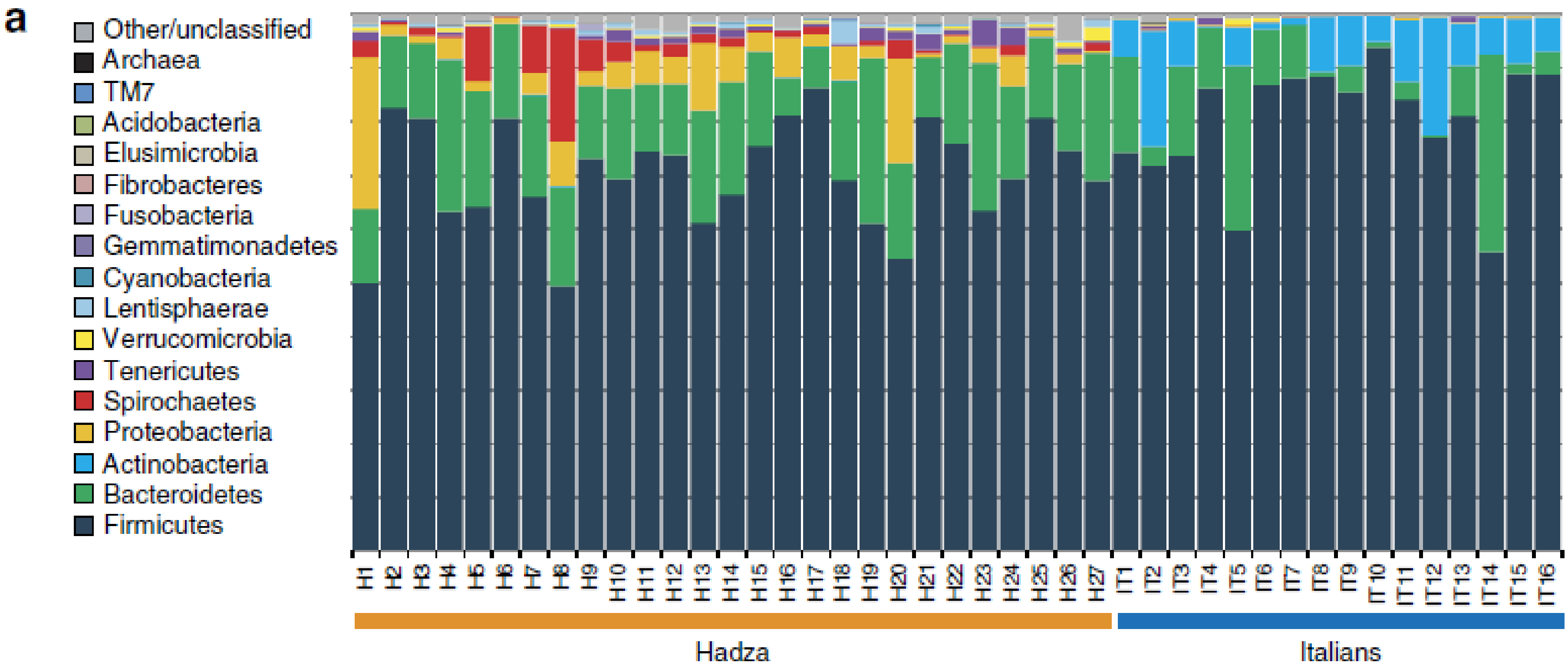
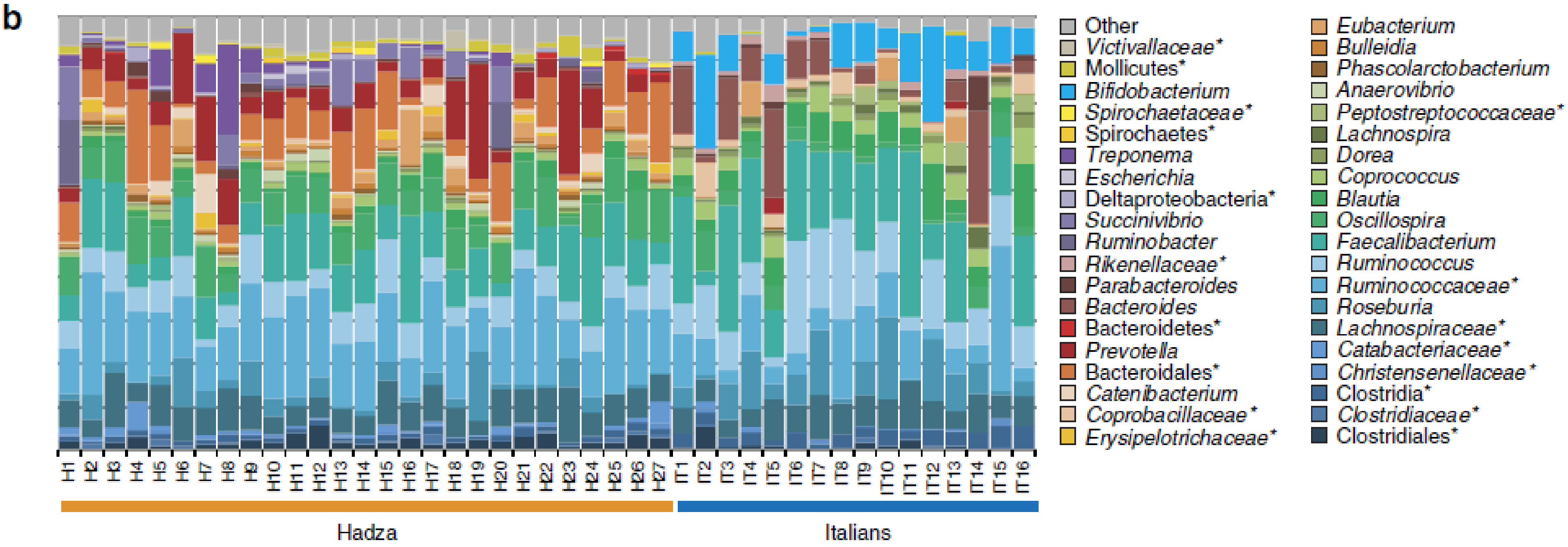


Figure 2 | Bacterial relative abundance of Hadza and Italian subjects. 16S rDNA gene survey of the faecal microbiota of 27 Hadza (H1-H27) and 16 Italian (IT1-IT16) adults. Relative abundance of (a) phylum and (b) genus-classified faecal microbiota is reported. Histograms are based on the proportion of OTUs per subject. Colours were assigned for all phyla detected, and for genera with a relative abundance Z1% in at least 10% of subjects.

# Composition at phylum level

- Firmicutes (72±1.9%), Bacteroidetes (17±1.1%), Proteobacteria (6±1.2%), Spirochaetes (3±0.9%), with 2% of phylum level OTUs remaining unclassified.



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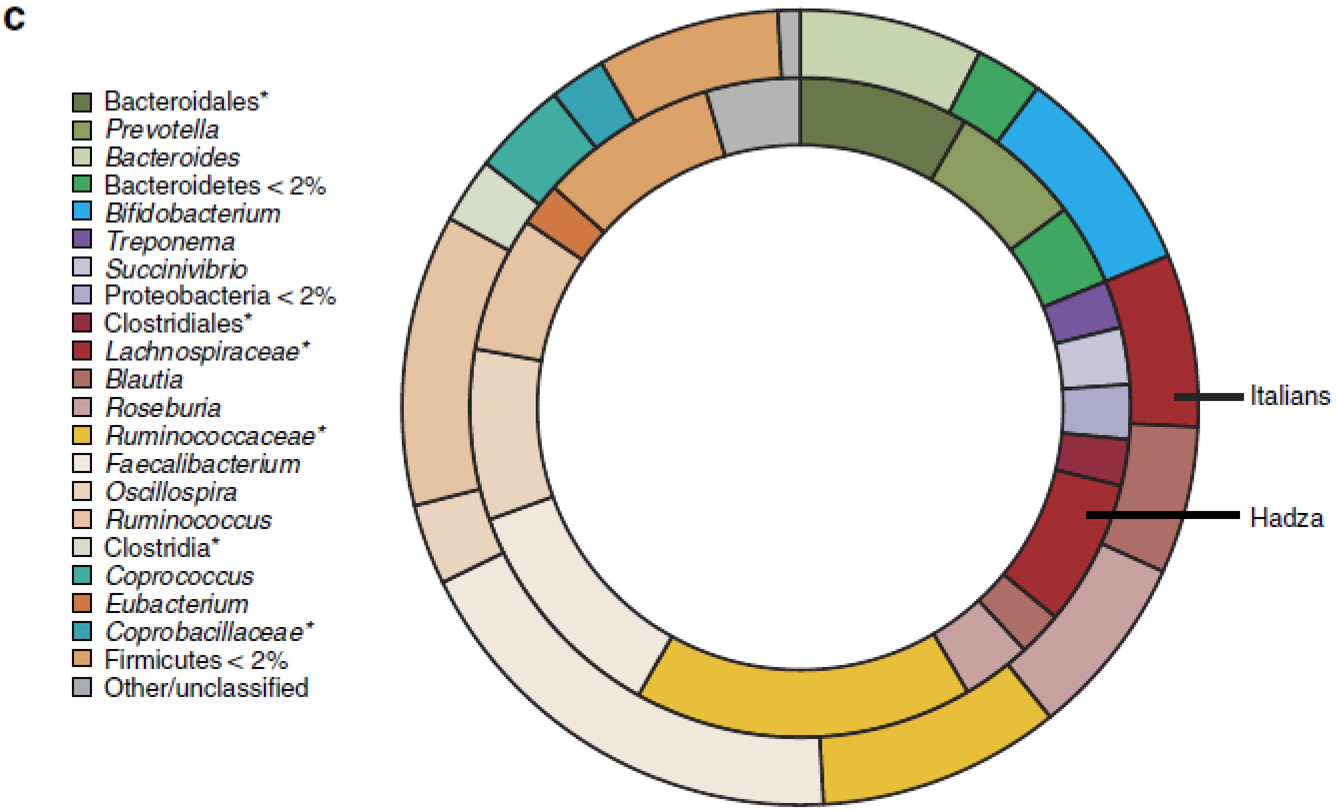
# Composition at genus level

Ruminococcaceae (34%), Lachnospiraceae (10%), Prevotellaceae (6%), Clostridiales Incertae Sedis XIV (3%), Succinivibrionaceae (3%), Spirochetaceae (2%) and Eubacteriaceae (2%).

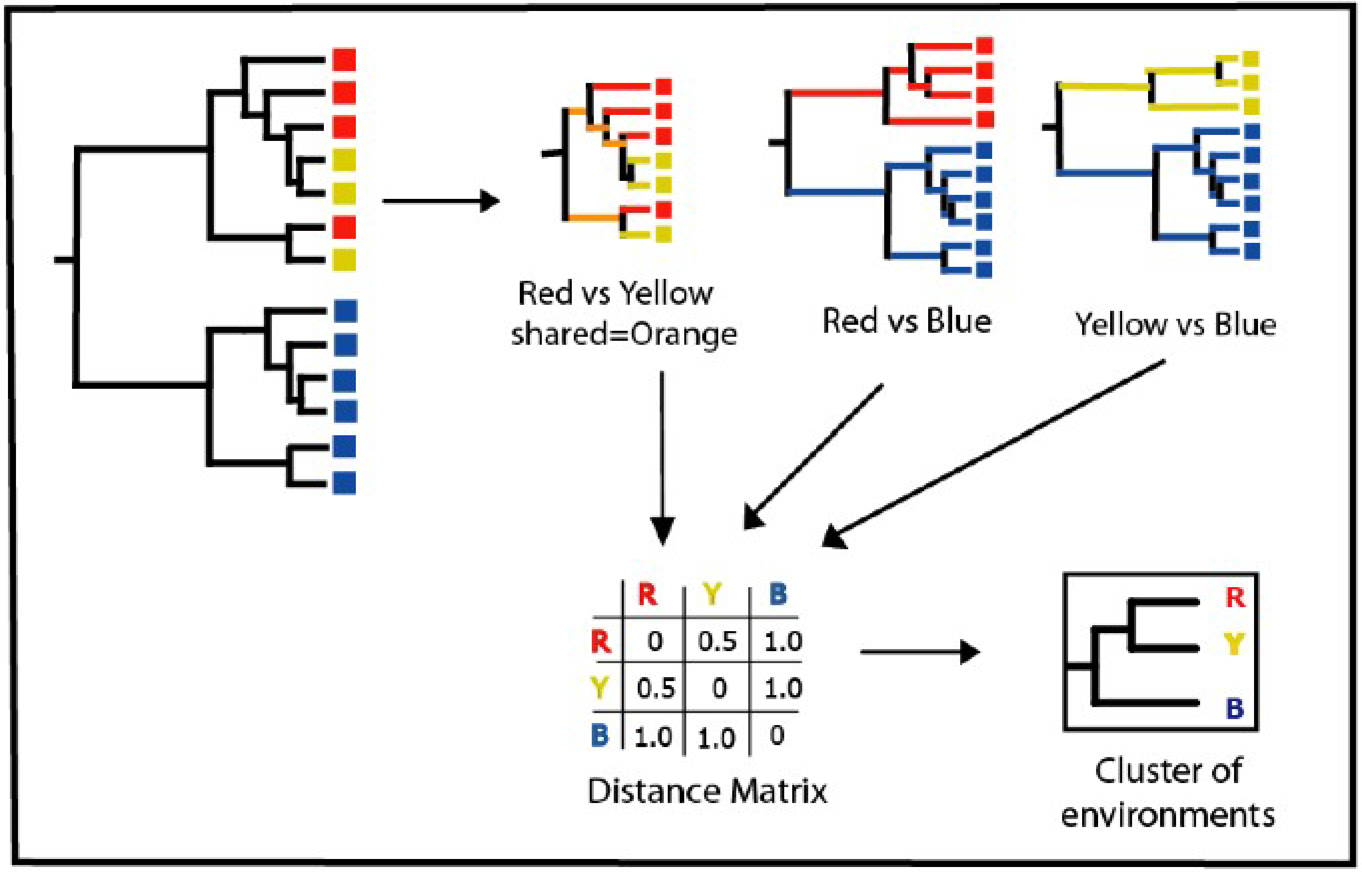
*A large number of taxa, the majority belonging to Bacteroidetes, Clostridiales, Bacteroidales and Lachnospiraceae, are unassigned at the level of family and genus, together representing 22% of the total community.



(c) Donut charts summarizing genera relative abundance for Italians (outer donut) and Hadza (inner donut). Genera were filtered for those with =>2% of total abundance in at least 10% of subjects. *denotes unclassified OTU reported at higher taxonomic level.

# UniFrac Distance

Lozupone C. and R. Knight. 2005. Appl. Environ. Microbiol. 71:8225-35



|   | R | Y | B |
|---|---|---|---|
| R | 0 | 0.5 | 1.0 |
| Y | 0.5 | 0 | 1.0 |
| B | 1.0 | 1.0 | 0 |

Distance Matrix

http://bmf.colorado.edu/unifrac/help.psp

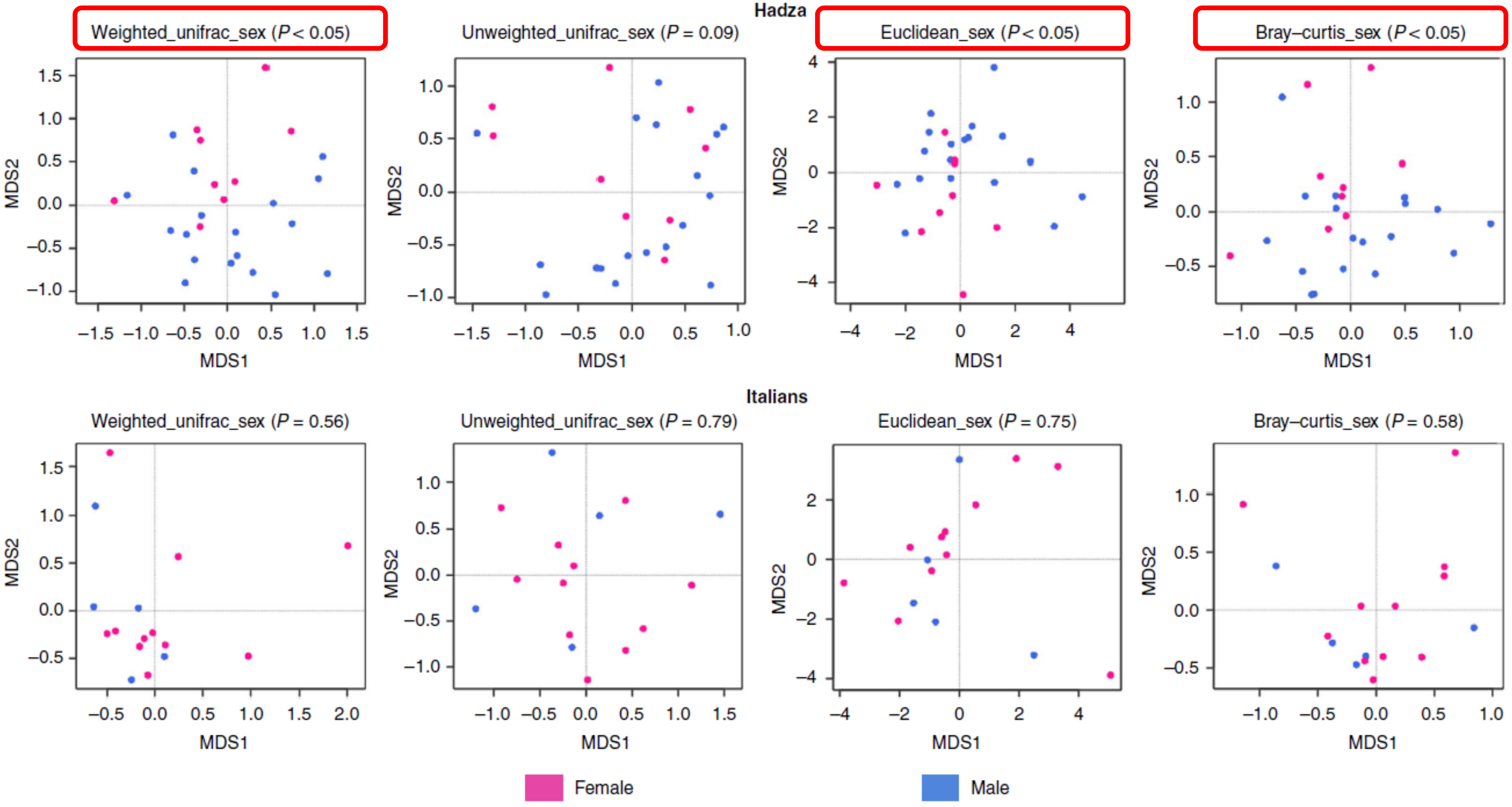
# Gut microbiota diversity within Hazda

- Camp: No
- Sex: Yes (see the next slide)

*Treponema*: women > men

*Eubacterium* and *Blautia*: men> women

- Sexual division of labor and sex difference in diet
- *Treponema: opportunistic pathgens.* Includes proficient cellulose and xylan hydrolyzers. *Degraded fibre?*



Weighted unifrac: community structure: consider number of reads
Unweighted unfrac: community members: ignore number of reads

Principle Coordinate analysis

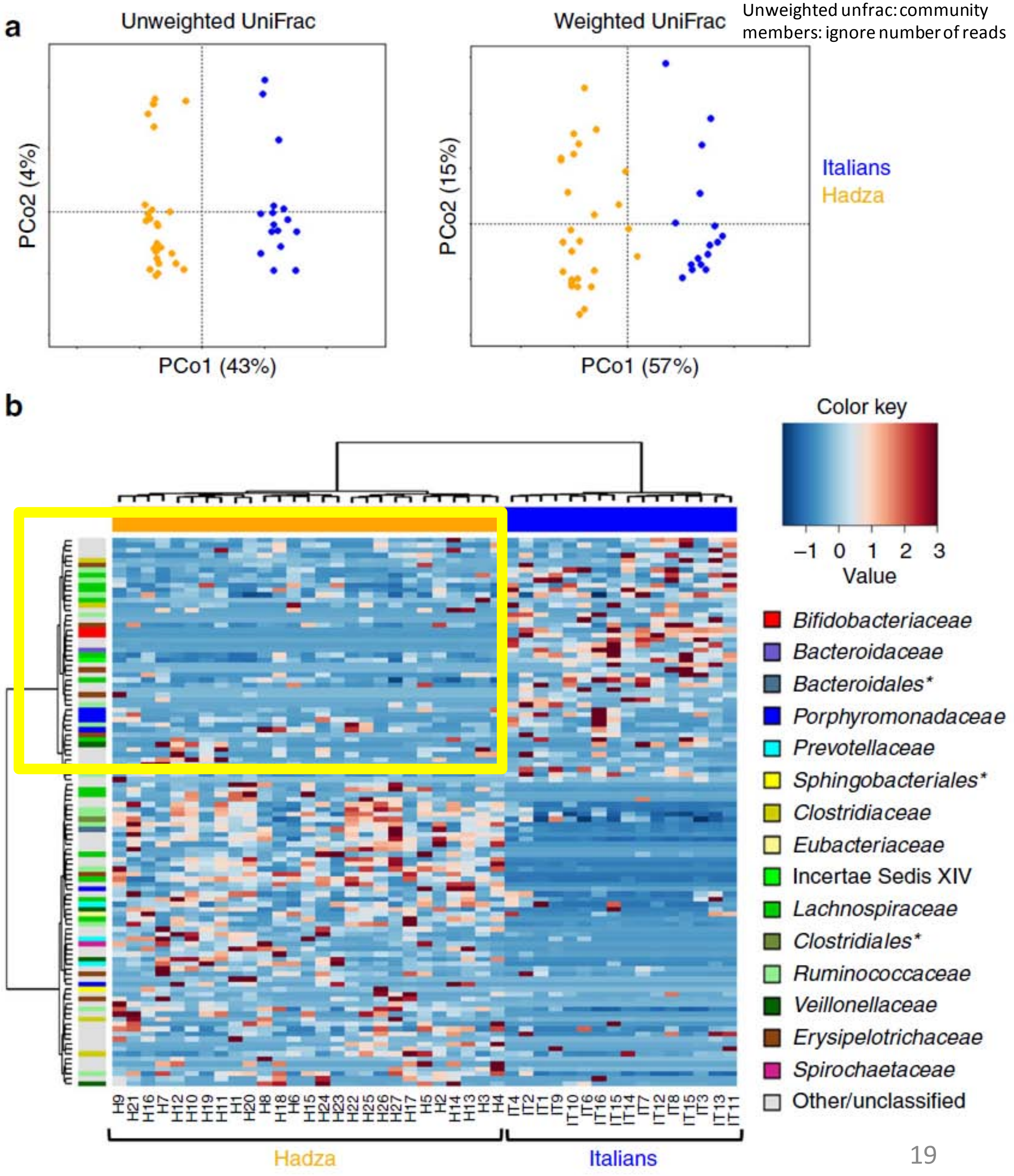
Figure 3 | Sex difference in GM structure among Hadza and Italians. PCoAs based on unweighted and weighted UniFrac distances as well as Euclidean and Bray–Curtis distances show patterns of separation by sex within each subject cohort and their respective P-values. Significance was calculated by permutation test with pseudo F-ratio. Pink, females; blue, males.

# Comparison between Hazda and Italian

- Mean value of unweighted UniFrac distances:
- → within-group variability of taxonomic diversity: Hazda < Italian

Weighted unifrac: community structure: consider number of reads
Unweighted unfrac: community members: ignore number of reads

Figure 4 | GM phylogenetic difference between Hadza and Italian subjects. (a) Unweighted and weighted UniFrac distance PCoA of the faecal microbiota from 27 Hadza (orange dots) and 16 Italians (blue dots). (b) hierarchical Ward-linkage clustering based on the Spearman correlation coefficients of genus proportion. Genera were filtered for subject prevalence of at least 30% within a population. Subjects are clustered on top of the panel and colour-coded as in a. Genera (110) clustered by the vertical tree are colour coded by family assignment. * denotes unclassified OTU reported at higher taxonomic level.



# Gut microbiota differed between Hazda and Italian

Phylum level:

- Higher abundance of Bacteroidetes and a lower abundance of Firmicutes in Hazda
- Proteobacteria↑ and Spirochaetes↑ in Hazda (rare in Italian)
- Actinobacteria↑ in Italian (absent in Hazda)

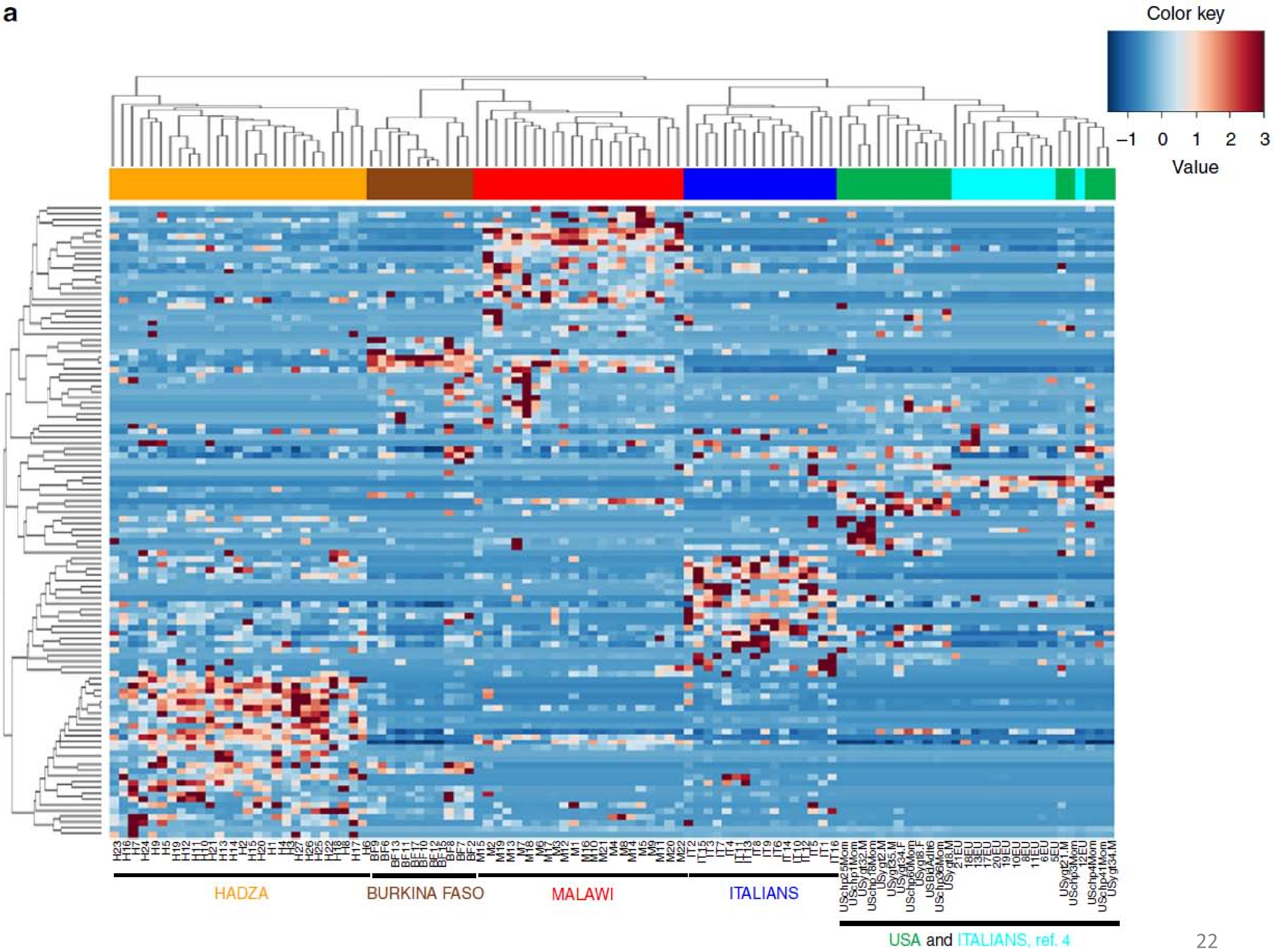
Genus level:

- *Prevotella, Eubacterium, Oscillibacter, Butyricicoccus, Sporobacter, Succinivibrio and Treponema: enriched in Hazda*
- *Bifidobacterium, Bacteroides, Blautia, Dorea, unclassified Lachnospiraceae, Roseburia, Faecalibacterium, Ruminococcus and unclassified Erysipelotrichaceae*: depleted in Hazda

- Absence of *Bifidobacterium*: confirmed by qPCR.

# Comparison with previous studies

- Children (5-6 years), Burkina Faso
- Chidlren (3-6 years), Italy
- Adults (20-44 years), Malawi
- Adults (24-40 years), US

a
Color key
−1
0
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3
Value
H23
H16
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H9
H5
H19
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M11
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M22
IT2
IT15
IT3
IT7
IT4
IT11
IT13
IT8
IT9
IT6
IT14
IT10
IT12
IT5
IT1
IT16
USchp25Mom
USchp1Mom
USygt32.M
USchp18Mom
USygt2.M
USygt35.M
USygt34.F
USchp60Mom
USygt8.F
USBdAdlt6
USchp36Mom
USygt8.M
21EU
18EU
13EU
17EU
20EU
19EU
10EU
8EU
11EU
6EU
5EU
USygt21.M
USchp3Mom
12EU
USchp4Mom
USchp41Mom
USygt34.M
HADZA
BURKINA FASO
MALAWI
ITALIANS
USA and ITALIANS, ref. 4

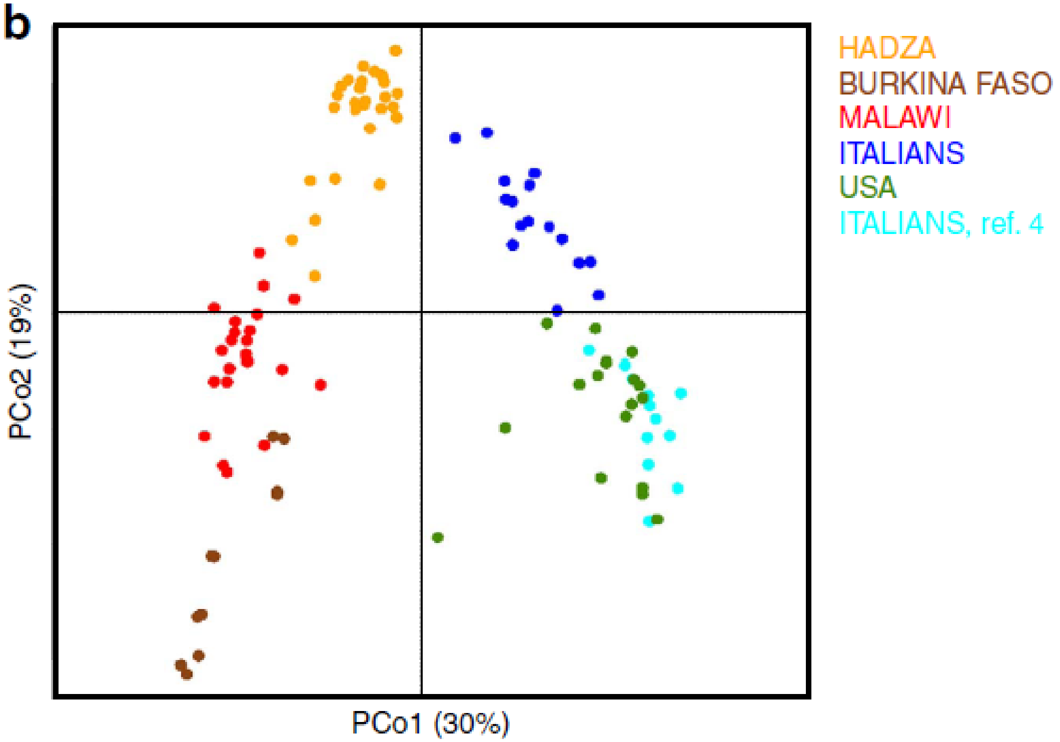
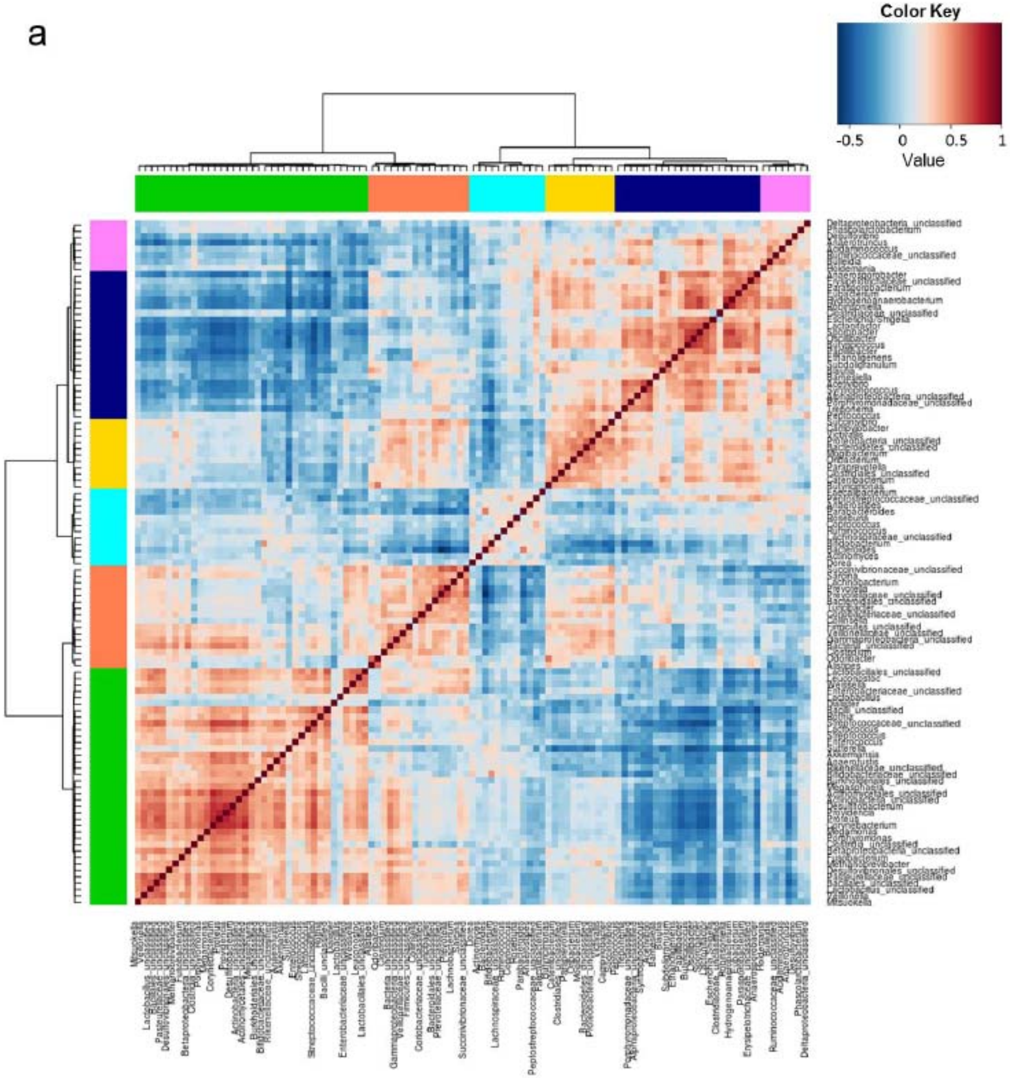
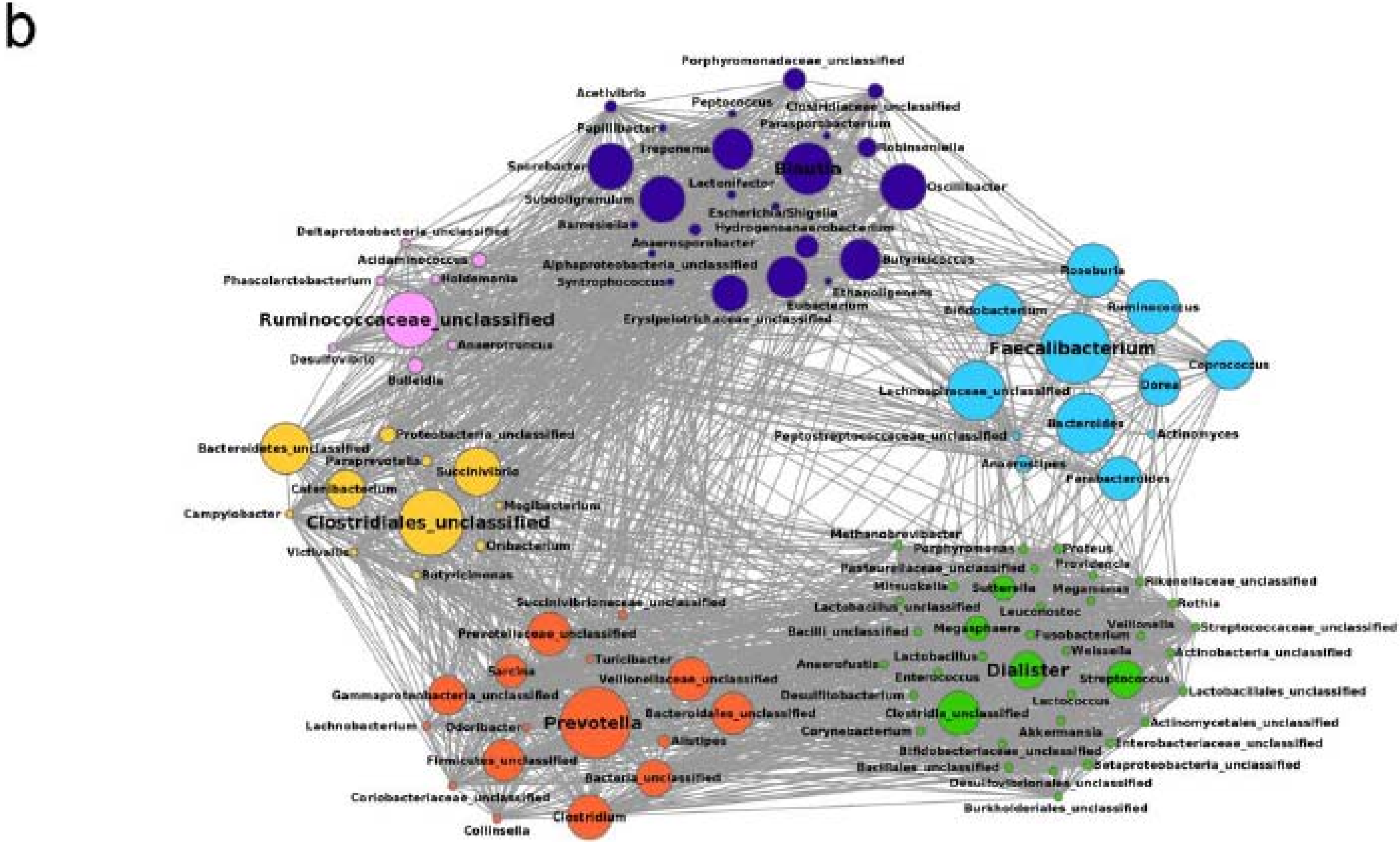


Figure 5 | Comparison of GM relative abundance among populations reflects subsistence patterns. (a) Hierarchical clustering based on Eisen's formula of correlation similarity metric of bacterial genus proportion and average linkage clustering. Genera were filtered for subject prevalence of at least 30% of samples. Subjects are clustered in the top of panel and colour-coded orange (Hadza), brown (BF), red (Malawi), blue (Italian adult controls from this study), green (US adults from ref. 9) and cyan (Italian children from ref. 4). Genera (107) are visualized and clustered by the vertical tree. (b) PCoA based on Bray–Curtis distances of the relative abundance of GM genera of each population.

# Identification of six co-abundance groups

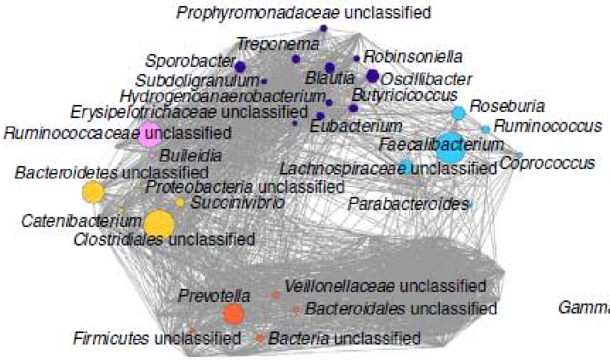


Sup-Fig 5

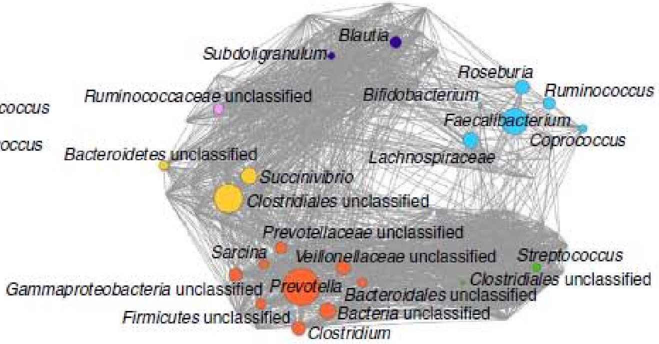

b
Porphyromonadaceae_unclassified
Acetivibrio
Peptococcus
Clostridiaceae_unclassified
Parasporobacterium
Papillibacter
Treponema
Robinsoniella
Sporobacter
Blautia
Lactonifactor
Oscillibacter
Subdoligranulum
Escherichia/Shigella
Hydrogenoanaerobacterium
Barnesiella
Anaerosporobacter
Alphaproteobacteria_unclassified
Butyricicoccus
Syntrophococcus
Ethanoligenens
Eubacterium
Erysipelotrichaceae_unclassified
Deltaproteobacteria_unclassified
Acidaminococcus
Phascolarctobacterium
Holdemania
Ruminococcaceae_unclassified
Anaerotruncus
Desulfovibrio
Bulleidia
Roseburia
Bifidobacterium
Ruminococcus
Faecalibacterium
Coprococcus
Dorea
Lachnospiraceae_unclassified
Bacteroides
Peptostreptococcaceae_unclassified
Actinomyces
Anaerostipes
Parabacteroides
Bacteroidetes_unclassified
Proteobacteria_unclassified
Paraprevotella
Succinivibrio
Catenibacterium
Megibacterium
Campylobacter
Clostridiales_unclassified
Oribacterium
Victivallis
Butyricimonas
Succinivibrionaceae_unclassified
Prevotellaceae_unclassified
Turicibacter
Sarcina
Veillonellaceae_unclassified
Gammaproteobacteria_unclassified
Bacteroidales_unclassified
Lachnobacterium
Odoribacter
Prevotella
Alistipes
Firmicutes_unclassified
Bacteria_unclassified
Coriobacteriaceae_unclassified
Clostridium
Collinsella
Methanobrevibacter
Porphyromonas
Proteus
Pasteurellaceae_unclassified
Providencia
Mitsuokella
Sutterella
Megamonas
Rikenellaceae_unclassified
Lactobacillus_unclassified
Leuconostoc
Rothia
Bacilli_unclassified
Megasphaera
Fusobacterium
Veillonella
Streptococcaceae_unclassified
Lactobacillus
Weissella
Actinobacteria_unclassified
Anaerofustis
Dialister
Enterococcus
Streptococcus
Desulfitobacterium
Lactococcus
Lactobacillales_unclassified
Clostridia_unclassified
Actinomycetales_unclassified
Corynebacterium
Akkermansia
Enterobacteriaceae_unclassified
Bifidobacteriaceae_unclassified
Betaproteobacteria_unclassified
Bacillales_unclassified
Desulfovibrionales_unclassified
Burkholderiales_unclassified

Sup-Fig 5

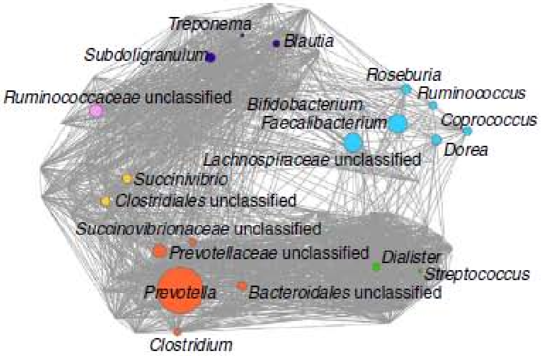
HADZA



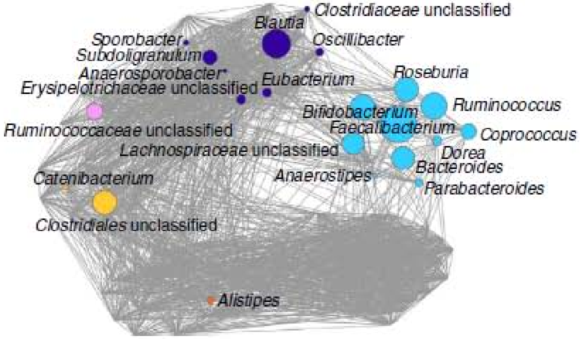
MALAWI



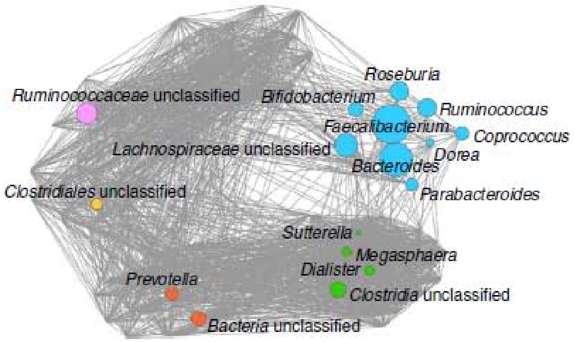
BURKINA FASO



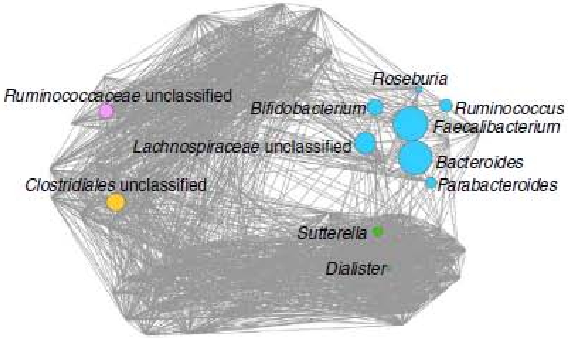
ITALIANS



USA



ITALIANS, ref. 4



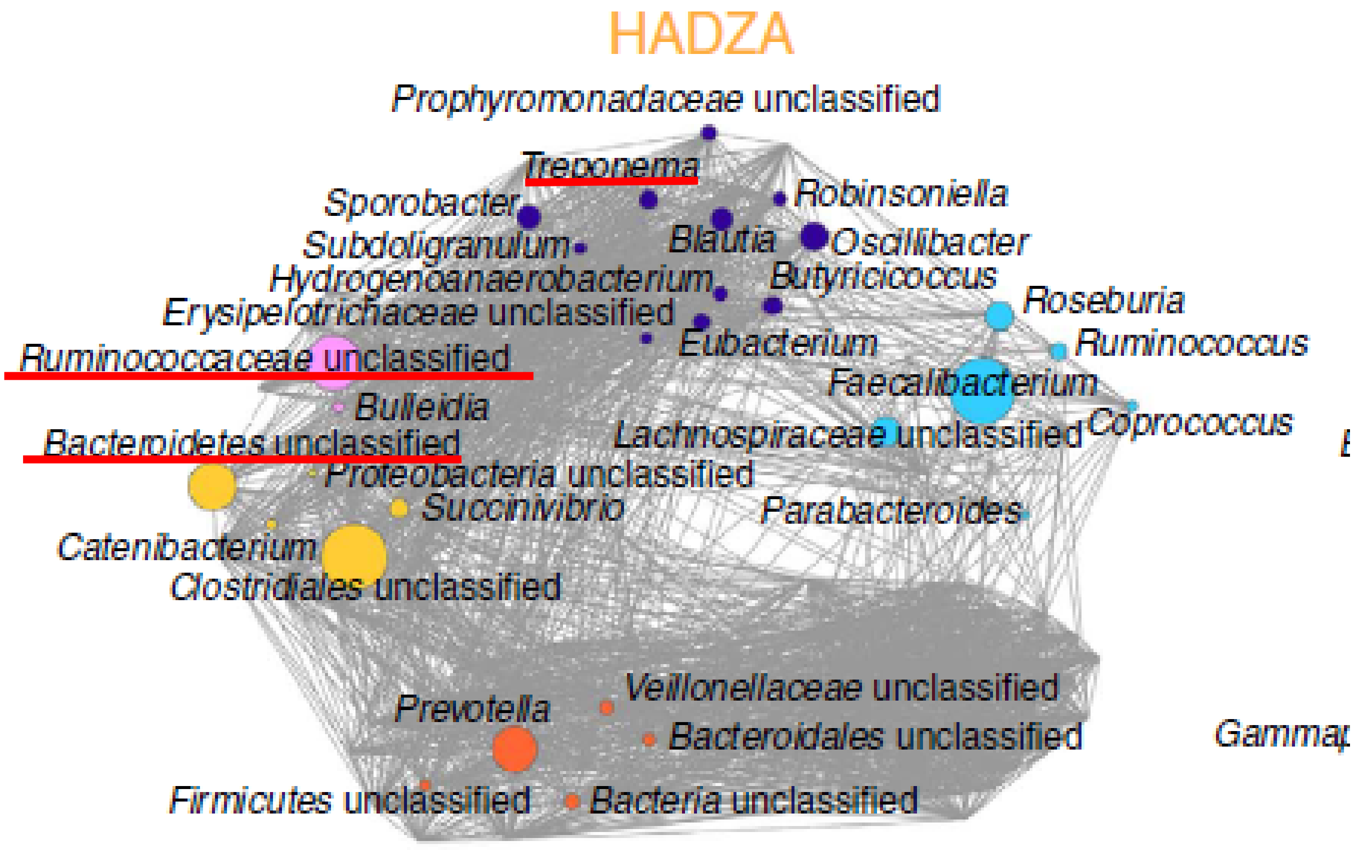


Figure 6 | Distinct bacterial co-abundance groups (CAGs) define each population. Wiggum plots indicate pattern of variation of the six identified CAGs in Hadza, Malawi, BF and western controls. CAGs are named with the name of the most abundant genera and are colour coded as follows: Faecalibacterium (cyan), Dialister (green), Prevotella (orange), Clostridiales_unclassified (yellow), Ruminococcaceae_unclassified (pink) and Blautia (violet). Each node represents a bacterial genus and its dimension is proportional to the mean relative abundance within the population. Connections between nodes represent positive and significant Kendall correlation between genera (FDRo0.05).

transferred to the liver where it serves as a precursor for hepatic gluconeogenesis

*Bifidobacterium, Bacteroides, Blautia, Faecalibacterium* and *Ruminococcus* are positively correlated with butyrate.

*Bifidobacterium, Blautia* and *Lachnospiraceae* show a significant negative correlation with propionate, while *Prevotella* demonstrates a positive correlation.

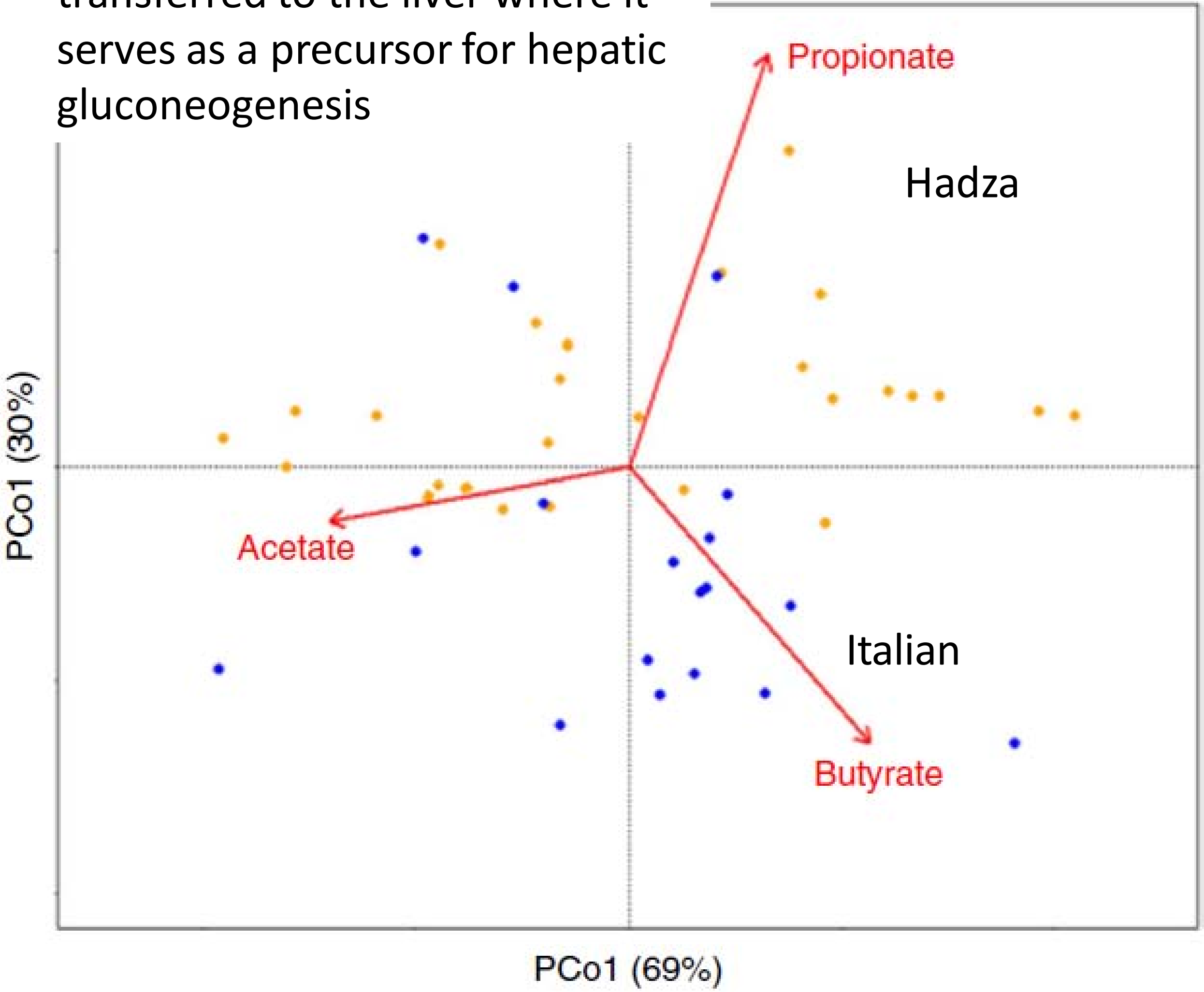


Figure 7 | Comparison of metabolite production between Hadza and Italian samples. PCoA based on Euclidean distances of the profiles of SCFA relative abundance in Hadza (orange) and Italians (blue). Vectors show propionate, butyrate and acetate abundance.

# Discussion

## GM structure as an adaptation to the Hadza foraging lifestyle

H & I : fibre-degrading Firmicutes—for example, members of *Lachnospiraceae, Ruminococcaceae, Veillonellaceae, Clostridiales Incertae Sedis* XIV and *Clostridiaceae*

H: Xylan degrading *Prevotella* and *Treponema*, and unclassified members of Bacteroidetes, Clostridiales and *Ruminococcaceae*. → glycan-degrading ability

Sup-Fig 1: digestible fraction is composed of largely water, simple sugars, starch and soluble fibre.

# Sex-related divergence in Hadza GM structure

Women consume more plant foods than men
Need energy for reproduction.

*Treponema*: women > men,
which could buffer women from nutritional deficiencies/

Lower prevalence of infection to *Treponema*
in women than in men (1966/67 survey)
→ immunoregulation of Treponema pathogens in women

## Discussion

The absence of Actinobacteria, particularly *Bifidobacterium* in Hadza GM

Breastfeeding in infants.
Dominant in the first few months after birth
Livestock animals

Cf. similar findings among vegans and Koreans.

Isolation?
A new equilibrium that is beneficial and symbiotic to the Hadza living environment.

# Discussion

Westernization and GM
-reduction in GM diversity and stability

Sterilization and limited environmental exposure
→ change in GM → change in health status

Unclassified bacteria

# Future studies

Infant GM
Conparative study with *Pan*
Gnotobiotic mice

Host — Microbiome

Supra-organisms

Natural Selection
Adaptation