

Supplementary Fig. 1 Abundance distributions for control subjects Logarithmic abundance distributions across 46 control subjects (*n*=92 samples) from dietary intervention trials (Methods) show indications of bimodality. These control subjects received placebo, and were controlled for other external factors including probiotics and antibiotics use. The *B. fragilis* group is an exception with no indications of bimodality.

Supplementary Fig. 2 State frequency and stability (a) State frequency (fraction of subjects associated with the state) among the 1,006 western adults (horizontal axis) correlates with short-term stability of the state in the 78 follow-up subjects (vertical axis). The stability is quantified by the fraction of subjects remaining in their original state over a three-month interval, as estimated by Kaplan-Meier survival analysis (Methods). All bi-stable groups appear more stable than expected by random state allocation between the consecutive time points (gray line), the *Prevotella* group (♦) being the most stable (>97%). Symbols: *B. fragilis* group (\triangle) , *Dialister* spp. (\blacksquare) , *Prevotella* group (\blacklozenge) , UCI (\lozenge) , UCII (\lozenge) ; the blue and red color indicate the low- and high-abundance states, respectively. **(b)** State mixing within each bistable group compared to the expected mixing; the mixing is defined as the overall fraction of subjects who exhibit a state switch during the study interval. Since the observed state mixing could reflect natural, continuous fluctuations in bacterial abundance rather than abrupt shifts between contrasting stable states, we compared the mixing rates in the bi-stable taxa (black dots) to the 54 other prevalent, unimodal taxa (boxplots) with simulated low- and highabundance states constructed based on the same population frequencies than in the corresponding bi-stable group. The *P. oralis* and *P. melaninogenica* groups appear the most stable, with less mixing than in any other group (100%). Also *Dialister* spp. (83%), UCI (93%) and UCII (78%) exhibit less mixing than most unimodal groups based on analogous, simulated tipping points. The *B. fragilis* group (35%) is an exception that appears less stable than most other taxa.

Supplementary Fig. 3 Temporal dynamics of a bi-stable abundance distribution The logarithmic abundance distribution for *Dialister* spp. exhibits two peaks of low and high abundance with the estimated state frequencies of n_1 =72% and n_2 =28% across the 1,006 western adults, respectively. In stationary state, the flow between the two states is balanced, and the ratio of the switching rates *r* should be inversely related to state frequencies *n* (r_1/r_2) n_2/n_1) assuming a stationary continuous-time Markov process. Hence the more frequent states are relatively more stable in the stationary state, while the absolute switching rates determine the overall mixing between the states during a given time interval. The tipping point (dashed line) marks the intermediate region between the alternative states of low and high abundance. In bi-stable systems the observations around this region have reduced stability¹.

Supplementary Fig. 4 Phylotype abundances for selected genus-like groups Logarithmic abundance distributions for the three most prevalent phylotypes within the genus-like groups that show indications for bi-stability (main Fig. 4). Prevalent phylotypes based on our criteria (see Methods) are identified within the *Dialister* spp*.,* relatives of *P. oralis and P. melaninogenica*, UCI and UCII groups. Abbreviations: Uncultured ('U.'); bacteria ('b.').

Supplementary Fig. 5 Abundance distributions for the phylotypes within *Dialister* **spp.** Strong bimodality in the abundance distributions is observed in three phylotypes within the *Dialister* spp. (Uncultured bacterium clones Eldhufec093 and Eldhufec089; and *D. invisus*).

Supplementary Fig. 6 Alternative states and gene richness Total gene count for 255 subjects (39-72 years) in a metagenomic sequencing study², where a bimodal gene count was reported; the corresponding threshold between the low and high gene count is indicated by the vertical dashed line. The total gene count distributions are illustrated separately for the subjects associated with the low- (blue) and high- (red) abundance state of each bi-stable group. The metagenomic gene count is significantly associated with all bi-stable groups (*P*<0.01; Wilcoxon test) except the *B. fragilis* group (*P*=0.2). *Dialister* spp*.* is associated with the low gene count²; the *Prevotella*, UCI and UCII groups are associated with high gene count.

Supplementary Fig. 7 Community-level variation (a) Visualization of the two main axes of Principal Component Analysis based on the 130 genus-like groups across the 401 samples extracted with the mechanical lysis. The gray shading indicates the density of the data points. The second principal axis, driven by the *Prevotella* group (relatives of *P. melaninogenica* and *P. oralis*), explains 13% of the overall variance in the data. **(b)** Visualization of the same data based on Principal Coordinates Analysis with un-weighted UniFrac distances. The blue and red colors indicate subjects with the low and high abundance state of the *Prevotella* group based on our analysis, respectively.

- Left-skewed (4) *Butyrivibrio crossotus et rel.*; *Faecalibacterium prausnitzii et rel.*; *Lachnospira pectinoschiza et rel.*; *Subdoligranulum variable et rel.*
- Right-skewed (16) *Anaerotruncus colihominis et rel.*; *Bacteroides splachnicus et rel.*; *Bacteroides stercoris et rel.*; *Clostridium (sensu stricto)*; *Clostridium colinum et rel.; Clostridium difficile et rel.*; Collinsella; *Eubacterium biforme et rel.*; *Lactobacillus plantarum et rel.*; *Parabacteroides distasonis et rel.*; *Prevotella tannerae et rel.*; *Ruminococcus lactaris et rel.*; *Streptococcus bovis et rel.*; *Streptococcus mitis et rel.*; *Sutterella wadsworthia et rel.*; Uncultured Mollicutes
- Bimodal (6) *Bacteroides fragilis et rel.*; *Dialister* spp.; *Prevotella melaninogenica et rel.*; *Prevotella oralis et rel.*; Uncultured Clostridiales I; Uncultured Clostridiales II
- Rare (70) Actinomycetaceae; Aerococcus; Aeromonas; *Alcaligenes faecalis et rel.*; Anaerobiospirillum; Anaerofustis; Aneurinibacillus; Aquabacterium; *Asteroleplasma et rel.*; Atopobium; Bacillus; *Bacteroides intestinalis et rel.*; *Bilophila et rel.*; Brachyspira; *Bulleidia moorei et rel.*; Burkholderia; Campylobacter; *Catenibacterium mitsuokai et rel.*; *Clostridium felsineum et rel.*; *Clostridium ramosum et rel.*; *Clostridium thermocellum et rel.*; *Coprobacillus catenaformis et rel.*; Corynebacterium; *Desulfovibrio et rel.*; *Eggerthella lenta et rel.*; *Enterobacter aerogenes et rel.*; Enterococcus; *Escherichia coli et rel.*; *Eubacterium cylindroides et rel.*; *Eubacterium limosum et rel.*; *Eubacterium siraeum et rel.*; Fusobacteria; Gemella; Granulicatella; Haemophilus; Helicobacter; *Klebisiella pneumoniae et rel.*; *Lactobacillus catenaformis et rel.*; *Lactobacillus gasseri et rel.*; *Lactobacillus salivarius et rel.*; Lactococcus; Leminorella; *Megamonas hypermegale et rel.*; *Megasphaera elsdenii et rel.*; Methylobacterium; Micrococcaceae; *Mitsuokella multiacida et rel.*; Moraxellaceae; Novosphingobium; Oceanospirillum; *Peptococcus niger et rel.*; *Peptostreptococcus anaerobius et rel.*; *Peptostreptococcus micros et rel.*; *Phascolarctobacterium faecium et rel.*; *Prevotella ruminicola et rel.*; Propionibacterium; *Proteus et rel.*; Pseudomonas; Serratia; Staphylococcus; *Streptococcus intermedius et rel.*; Uncultured Bacteroidetes; Uncultured Chroococcales; Uncultured Selenomonadaceae; Veillonella; Vibrio; *Weissella et rel.*; *Wissella et rel.*; Xanthomonadaceae; *Yersinia et rel.*

Supplementary Table 1 Bacterial abundance types Characteristic abundance types of the

130 genus-like bacterial groups quantified by the phylogenetic HITChip microarray. The genus-like groups (>90% sequence similarity in the 16S rRNA gene) are referred to as *type* species and relatives, the latter being shortened as "*et rel.*"³. The symmetric, skewed, bimodal, and rare abundance types are illustrated in main Fig. 2.

Supplementary Table 2 Phylotype-level characterization of the bimodal groups

Cultivated species and uncultured phylotypes (≥98% 16S rRNA gene sequence similarity) that constitute the six bimodal genus-like phylogenetic groups targeted by the HITChip microarray³. The right-most column lists the corresponding NCBI accession numbers.

Supplementary Table 3 Phylogenetic characterization of the UCI and UCII groups

Ribosomal Database Project $(RDP)^4$ alignment for the 16S rRNA target sequences of Uncultured Clostridiales I and II (UCI and UCII) detected with the HITChip microarray (October 2013). The RDP match score is 1 for all HITChip phylotypes except the UCII Uncultured bacterium clone Eldhufec333 (0.963). All sequences belong to the domain Bacteria. The RDP sequences are grouped according to their Phylum, Class, Order, Family and Genus; these are indicated up to the highest accessible taxonomic level, separated by semicolon.

Supplementary Table 4 Associations between health status and the bimodal taxa The

column ('Enriched state') indicates the state associated with the disease. The fourth ('Compromised') and fifth ('Controls') columns show the proportion of subjects in the highabundance state in the compromised and the healthy controls, respectively. To control for differences in subject characteristics or sample treatment between the two groups, we estimated the significance based on multiple logistic regression corrected for age, sex, bodymass index, and DNA extraction method using the log-ratio test followed by Benjamini-Hochberg correction. The associations with FDR<20% are shown.

Supplementary Notes

Supplementary Note 1 Phylogenetic analysis of the Uncultured Clostridiales I-II For the uncultured UCI and UCII groups that do not include any cultured representatives we performed a 16S rRNA target gene sequences alignment against the RDP database⁴ to update their phylogenetic assignments. The majority (19/30) of the phylotypes within the UCI group were identified as members of family Ruminococcaceae, and 12 could be further assigned to genus *Acetivibrio* (Supplementary Table 3). Uncultured bacterium clones Eldhufec308 and Eldhufec312 exhibited clear bimodality (Supplementary Fig. 4). Similar abundance distributions characterized also the phylotypes within the UCII group, which could not be identified further down than order Clostridiales.

Supplementary Note 2 Cross-hybridization control Cross-hybridization can reduce the accuracy of observations in microarray analyses. We controlled this based on pre-calculated cross-alignment tables between the taxonomic groups targeted by the HITChip microarray. Cross-hybridization was negligible (<10%) between the bimodal groups and other taxa. The *B. fragilis* group, targeted by 40 probes, was an exception with 43% of shared probes with the *B. ovatus* group. Since this could potentially contribute to the bimodality of the *B. fragilis* abundance distribution, we investigated the 16 probes that were specific for the *B. fragilis* group*.* Bimodal abundance patterns were detected in 25% of the unique probes, suggesting that this group may contain both bimodal and smoothly varying higher-level phylotypes. The highly correlated *P. oralis* and *P. melaninogenica* groups had 13-26% shared probes. The correlation between these two groups remained high (0.81) after excluding the shared probes, however, confirming positive association. To avoid potential biases associated with different DNA extraction methods, the correlations between phylogenetic groups were calculated based on the 401 samples analysed with the mechanical lysis (see Methods).

Supplementary Note 3 HITChip phylogeny The HITChip phylogeny is binned at three taxonomic levels based on 16S sequence similarity, roughly corresponding to species/phylotype (98% 16S sequence similarity; *n*=1033) and genus (90%; *n*=130) levels, that are further classified into 23 higher-level groups including 10 phyla. In the present work we primarily focus on the genus level, which is less prone to cross-hybridization between closely related targets than the higher-resolution phylotype-level analysis.

Supplementary Note 4 Community diversity We quantified the overall community diversity by the Shannon index of the probe-level HITChip data (3631 probes targeting 1033 phylotypes) within the subset of 255 samples that were also analyzed for metagenomic richness (gene count)².

Supplementary Note 5 Bimodality significance As the standard Potential Analysis approach does not provide p-values for the observed bimodal patterns, we derived empirical pseudo-pvalues for each taxon as the fraction of bootstrap samples that did not support the bimodality to obtain the following estimates of false discovery rates⁵: *Prevotella* <2%; *Dialister* spp., UCI and UCII 10%; and *B. fragilis* 25%.

Supplementary Note 6 DNA extraction bias When all samples with varying DNA extraction methods were included, we observed bimodal patterns also in bifidobacteria and ruminococci that thus appear to be confounded by different extraction methods, highlighting the necessity to take the extraction method into account in meta-analyses that integrate data across independent studies.

Supplementary Note 7 Kaplan-Meier analysis This is an approximation, however, as the changes in bacterial abundance are continuous and reversible unlike the life/death processes, and natural continuous fluctuations in bacterial abundance may induce some observed state shifts.

Supplementary Note 8 Community-level analysis A variety of methodologies have been suggested to detect community-level multimodality in microbiota profiling data. Such analysis can be sensitive to the choice of dissimilarity measure, clustering method, and the approach for validating the number of clusters⁶. Popular dissimilarity measures include Jensen-Shannon divergence, beta diversity measures, and correlation analyses. Partition Around Medoids (PAM) has been frequently used as a clustering method, and common methodologies for cluster number validation include the Kalinski-Harabasz, Silhouette width, and the Prediction Strength Index^{6,7}.

Supplementary References

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