PERSPECTIVE
https://doi.org/10.1038/s41564-017-0072-8

nature microbiology

Corrected: Publisher correction

Enterotypes in the landscape of gut microbial community composition

Paul I. Costea[1], Falk Hildebrand[1,2,3], Manimozhiyan Arumugam[4], Fredrik Bäckhed[5,6], Martin J. Blaser[7], Frederic D. Bushman[8], Willem M. de Vos[9,10], S. Dusko Ehrlich[11,12], Claire M. Fraser[13], Masahira Hattori[14], Curtis Huttenhower[15], Ian B. Jeffery[16], Dan Knights[17,18], James D. Lewis[19], Ruth E. Ley[20], Howard Ochman[21], Paul W. O'Toole[16], Christopher Quince[22], David A. Relman[23,24,25], Fergus Shanahan[16], Shinichi Sunagawa[1,26], Jun Wang[5,27,28,29,30], George M. Weinstock[31], Gary D. Wu[32], Georg Zeller[1], Liping Zhao[33], Jeroen Raes[2,3,34*], Rob Knight[35,36,37,38*] and Peer Bork[1,39,40*]

Population stratification is a useful approach for a better understanding of complex biological problems in human health and wellbeing. The proposal that such stratification applies to the human gut microbiome, in the form of distinct community composition types termed enterotypes, has been met with both excitement and controversy. In view of accumulated data and re-analyses since the original work, we revisit the concept of enterotypes, discuss different methods of dividing up the landscape of possible microbiome configurations, and put these concepts into functional, ecological and medical contexts. As enterotypes are of use in describing the gut microbial community landscape and may become relevant in clinical practice, we aim to reconcile differing views and encourage a balanced application of the concept.

The human body is colonized by trillions of microorganisms that contribute to our health and wellbeing. Different communities of microorganisms inhabit various anatomical regions (Fig. 1). Inter-individual variation at each of these body sites is considerable, but the separation among sites within individuals remains apparent[1] (Fig. 1). The most densely populated habitat is the gut, with an estimated 0.15 kg of microbial biomass[2]. The gut harbours hundreds of bacterial and archaeal species, with Firmicutes and Bacteroidetes as dominant phyla[1,3–5]. Considerable variation in microbiota composition has been described among individuals, for example in the US National Institute of Health Human Microbiome Project (HMP)[1], the European Metagenomics of the Human Intestinal Tract project (MetaHIT)[3,6] and multiple other population studies[7,8]. The gut microbial ecosystem shows a succession of

[1]European Molecular Biology Laboratory, Heidelberg, Germany. [2]VIB Center for Microbiology, VIB, Belgium. [3]Laboratory of Microbiology, Vrije Universiteit Brussel, Brussels, Belgium. [4]The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [5]Wallenberg Laboratory, Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. [6]Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark. [7]New York University Langone Medical Center, New York, NY, USA. [8]Department of Microbiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. [9]RPU Immunobiology, Department of Bacteriology & Immunology, University of Helsinki, Helsinki, Finland. [10]Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands. [11]Metagenopolis, Institut National de la Recherche Agronomique, Jouy en Josas, France. [12]King's College London, Centre for Host-Microbiome Interactions, Dental Institute Central Office, Guy's Hospital, London, UK. [13]Institute for Genome Sciences at the University of Maryland School of Medicine, Baltimore, MD, USA. [14]Graduate School of Advanced Science and Engineering, Waseda University, Tokyo, Japan. [15]Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA. [16]APC Microbiome Institute, University College Cork, Cork, Ireland. [17]Biotechnology Institute, University of Minnesota, Saint Paul, MN, USA. [18]Department of Computer Science and Engineering, University of Minnesota, Minneapolis, MN, USA. [19]Center for Clinical Epidemiology and Biostatistics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [20]MPI Department of Microbiome Science, Tübingen, Germany. [21]Department of Integrative Biology, University of Texas, Austin, TX, USA. [22]Warwick Medical School, University of Warwick, Coventry, UK. [23]Department of Microbiology and Immunology, Stanford University, Stanford, CA, USA. [24]Department of Medicine, Stanford University, Stanford, CA, USA. [25]Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA. [26]Department of Biology, Institute of Microbiology, ETH Zurich, Zurich, Switzerland. [27]Department of Biology, University of Copenhagen, Copenhagen, Denmark. [28]Princess Al Jawhara Albrahim Center of Excellence in the Research of Hereditary Disorders, King Abdulaziz University, Jeddah, Saudi Arabia. [29]Macau University of Science and Technology, Avenida Wai long, Taipa, Macau, China. [30]Department of Medicine and State Key Laboratory of Pharmaceutical Biotechnology, University of Hong Kong, Pokfulam, Hong Kong. [31]The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA. [32]Division of Gastroenterology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [33]Ministry of Education Key Laboratory for Systems Biomedicine, Shanghai Centre for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, China. [34]Department of Microbiology and Immunology, Rega Institute KU Leuven, Leuven, Belgium. [35]Department of Computer Science, University of Colorado, Boulder, CO, USA. [36]Biofrontiers Institute, University of Colorado, Boulder, CO, USA. [37]Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO, USA. [38]Howard Hughes Medical Institute, University of Colorado, Boulder, CO, USA. [39]Max-Delbrück-Centre for Molecular Medicine, Berlin, Germany. [40]Molecular Medicine Partnership Unit, Heidelberg, Germany. Paul I. Costea and Falk Hildebrand contributed equally to this work. *e-mail: jeroen.raes@kuleuven.be; robknight@ucsd.edu; bork@embl.de

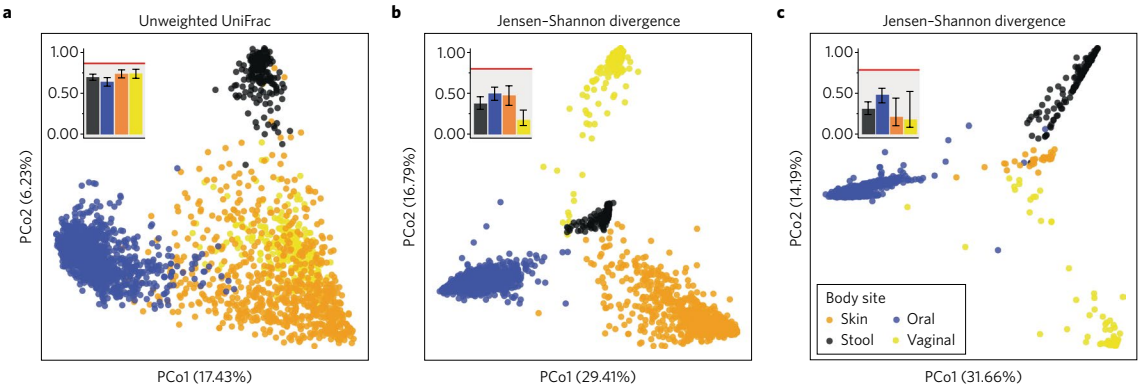

Fig. 1 | The microbiota of distinct body locations within the healthy human is separable at the genus level. a–c, Using 2381 HMP samples profiled with 16S rRNA, we illustrate the degree of separation between body sites using different distance measures and taxonomic resolutions: unweighted UniFrac at the operational taxonomic unit (OTU) level (**a**), Jensen–Shannon divergence at the genus level (OTUs belonging to the same genus are added up together) (**b**) and Jensen–Shannon divergence at the OTU level (**c**). Shown are the first two principal coordinates (PCo1 and PCo2) of a principle coordinate analysis (PCoA) for each, as well as a summary of the distances within and between body sites in the top left of each plot. Median inter-sample distances (error bars ranging from the 25th to 75th quantile) compared to the median between all body sites (red line) illustrate the ability to capture similarities and differences between these biomes, albeit with different effectiveness. We note that the silhouette index (a measure of clustering strength) in the case of unweighted UniFrac suggests a clustering into only three types, with an absolute value of ~0.2 (Supplementary Fig. 4).

different microbiota stages: community composition changes rapidly in early childhood, stabilizes in adults and deteriorates in old age[8,9]. There is no simple description of this complex landscape across large populations and geographies, in part because some taxa vary monotonically among individuals while most others show bimodal or more complex distributions[10] (Fig. 2a). Given the importance and complexity of the gut ecosystem, there is great interest in identifying compositional patterns and their underlying rules, as they may help us understand human health and disease states. A classification based on compositional patterns would potentiate microbiota-based diagnostics, therapies or prevention of disease, with implications for personalized treatment through nutritional, microbial and pharmaceutical interventions. Such patterns of microbial composition could be used to stratify populations, similarly to the molecular subtyping commonly used in cancer research, where, for example, breast cancer subclasses based on gene expression patterns are clinically relevant[11,12]. However, in other cases, such as colorectal cancer, the determination and usefulness of such classifications remains unclear[13], highlighting the fact that molecular stratification is not actionable in all situations.

Reproducible patterns of variation in the microbiota—for example, the proportions of major taxa such as *Bacteroides* and *Prevotella*—have been observed in the adult human gut (Fig. 2a and Supplementary Fig. 1). When separated into clusters, these variations were termed enterotypes[14] and proposed as a useful method to stratify human gut microbiomes. Later, other studies found stratification in other ecosystem types, such as the vagina[15] and other body sites[16–18]. However, due to the nature of clustering in the gut, the number or even existence of different community types has been a topic of heated debate after the publication of the original work[14].

Here we assess gut microbial community composition and test the different hypotheses using three of the largest available metagenomic datasets, which include data from three continents (from HMP, MetaHIT and a Chinese type II diabetes study)[1,6,19]. We perform a refined meta-analysis and propose a modified concept of enterotypes, with the goal of reconciling divergent viewpoints. The results illustrate the advantages and disadvantages of clustering and other stratification approaches. We find that the gut microbial composition is structured and that clustering can provide useful insights into some microbiome datasets, even when not strongly supported statistically. This approach does not diminish the need to pursue other analyses and avenues for interpretation, since broad community-wide stratification captures only some of the dimensions of microbiota complexity.

Recurrent compositional patterns in the gut microbiome

From the survey of the three large datasets mentioned above, it can be seen that groups of samples tend towards preferred genus level composition (Supplementary Fig. 2), as was also reported in the original study[14]. That is, some configurations of relative microbial abundance occur more frequently than others. This can be observed by calculating distances between samples and investigating the resulting clustering, as well as by directly observing the complex abundance distributions of some gut microbial taxa (Fig. 2). This preference for specific microbial community profiles is modest, resulting in higher sample density around the preferred constellations, but with a considerable proportion of samples falling between them. This makes it hard to describe these preferential microbial compositions mathematically or determine the number of such densely populated areas, prompting an alternative description of this space as consisting of gradients[16]. However, it is important to characterize these local optima of community compositions to understand the mechanisms responsible for these ecological constraints and community properties.

In 2011, clustering human faecal metagenomic samples from three countries (Denmark, Spain and the United States) based on their taxonomic composition—using three sequencing technologies (Illumina, 454 and Sanger), as well as 16S rRNA gene profiling data—resulted in the proposal of three enterotypes. They were described as being "densely populated areas in a multidimensional space of community composition", and were independent of age, gender, cultural background and geography[14]. An investigation of the properties of each enterotype found networks of co-occurring microorganisms centred around one indicator (driver) taxon, that is, the taxon correlating best to that given enterotype: enterotype 1, here denoted ET B for clarity, has *Bacteroides* as its best indicator; enterotype 2, here ET P, is driven by *Prevotella*, a genus whose abundance is inversely correlated with *Bacteroides*; and enterotype 3, here ET F, is distinguished by an overrepresentation of Firmicutes, most prominently *Ruminococcus*[14]. Analyses were performed at genus level, where

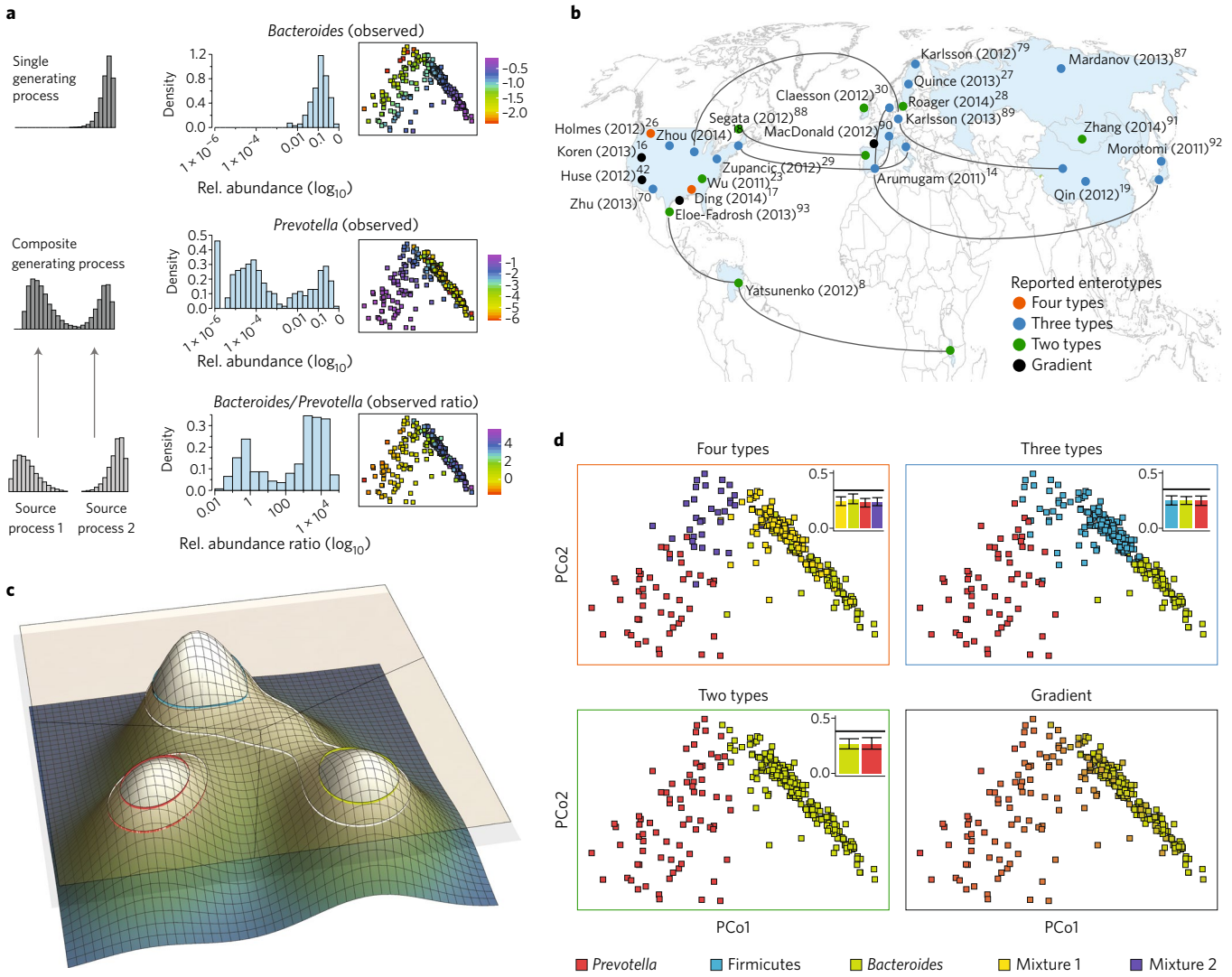

Fig. 2 | Stratification of the microbial composition landscape of the human gut microbiome. a, Abundance distributions of prevalent microbial genera of the human gut are often complex. Theoretical beta distributions (left) were compared with observed distributions (middle) and the observed abundance plotted in enterotype space (right) of key enterotype taxa or ratios thereof, based on 278 MetaHIT samples[6]. While *Bacteroides* abundance distribution is close to log-normal in the three large-scale datasets studied, that of *Prevotella* is bimodal, suggesting that the observed values are perhaps better explained by a mixture of two distributions, generated by two distinct processes, one of which corresponds to a dominating role in the community, while the other to a low-abundance state. **b**, Geographical distribution of studies that report enterotypes (Supplementary Table 1), coloured according to the number of microbial clusters reported. Map locations indicate the country from which samples were collected. Links between locations represent samples belonging to a single study. Overrepresentation of Western countries is a well-known bias and probably misses a portion of variation in other human societies. **c**, Schematic representation of the simulated microbial composition landscape with three density peaks, modelled as multivariate normal distributions, each representing an enterotype and drawn out of scale to make the concept more accessible. This figure illustrates how segmentation of this space by clustering with different parameters would result in different numbers of clusters (three and two here) and in differential coverage of individuals (represented by intersecting planes). The top-most overlay presents the discretizing segmentation, which splits the space into three zones. **d**, Projection onto a set of 278 Danish samples[6] of the three most frequent enterotype classification schemes based on different methods, including the *Prevotella*/*Bacteroides* gradient. This shows a split into a gradient or two, three (distance-based clustering) or four enterotypes (Dirichlet multinomial mixture models, DMMs). The local structure is preserved regardless of the method applied, and *Prevotella* (ET P) remains separated, suggesting the methods mostly differ in dividing the area between ET B and ET F. Additionally, the top right of each PCoA with a number of clusters greater than or equal to two shows the distance within a cluster (coloured accordingly) compared to the median distance between the clusters (black line), showing that for all cases the distances within are smaller than between; bar height is the median distance and the whiskers represent the 25th and 75th quantile. It should be noted that a 'horseshoe effect' can occur in ordinations, particularly if samples contain non-overlapping compositions[86], which is not the case in the datasets analysed here.

microbial ecological niches are hypothesized to be most clearly reflected[20], notwithstanding functional heterogeneity of some genera (such as streptococci, which groups deadly pathogens with common commensals and useful food-fermenting species). Species- and strain-level variations are neglected, although they can contribute to functional differences between individuals that are important in a clinical context[21,22].

Although much of the discussion emphasized the existence of three enterotypes, the original definition had made clear that they are not discrete, and that clustering is just one way to define them

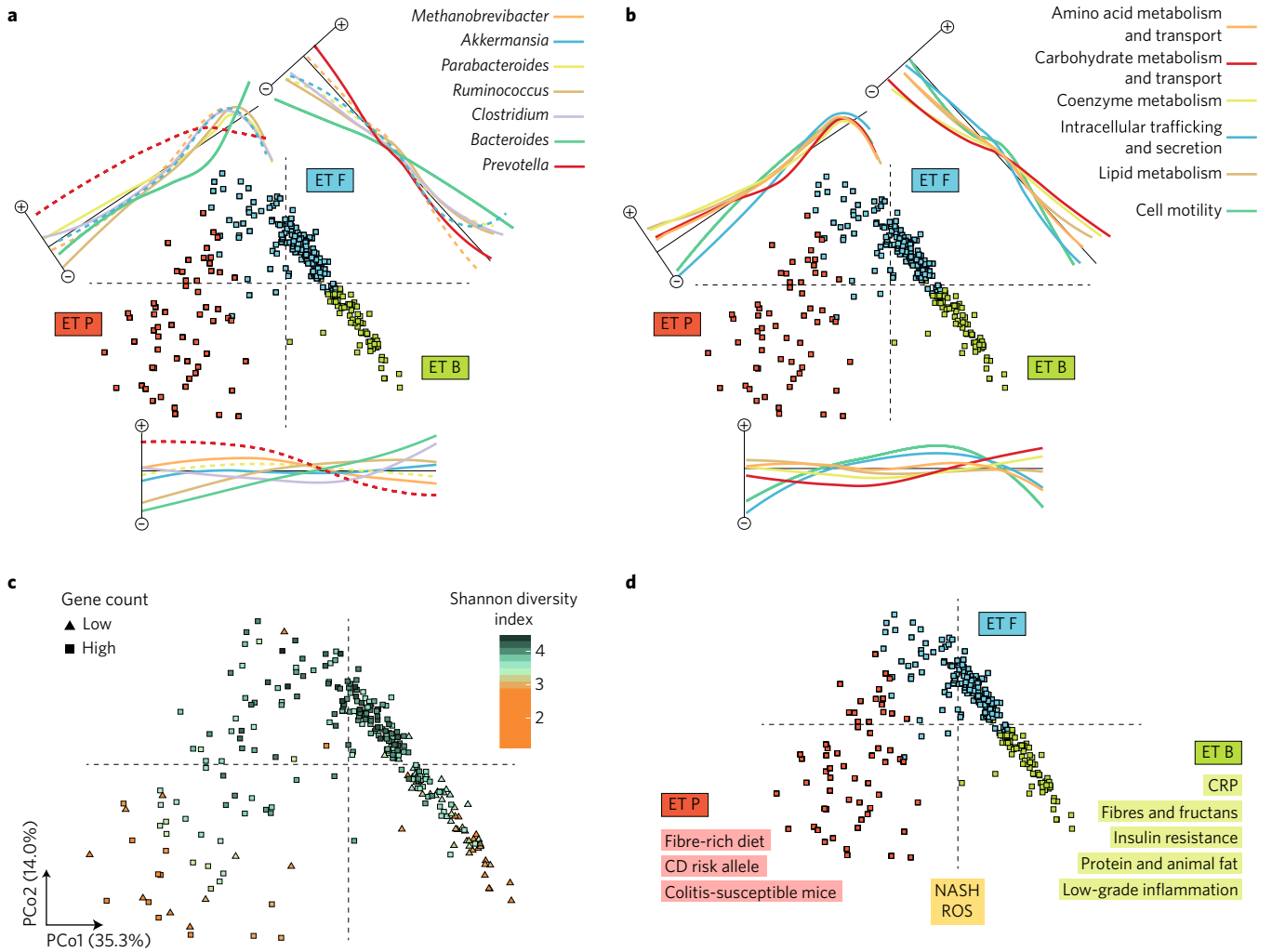

Fig. 3 | The microbiota of human faecal samples has local substructure. Ordination of 278 MetaHIT[6] samples on Jensen–Shannon distance transformed space. **a**, The log-transformed relative abundance of the most significantly differing genera. On the adjacent floating axis, the projected abundance changes between the respective community types are shown. Bimodal abundance profiles (dotted lines, dip test P value < 0.05) as well as gradual abundance changes (solid lines) can be identified, supporting a gradient or cluster model, respectively. **b**, Abundance changes of selected clusters of orthologous group (COG) categories were projected onto the ordination, illustrating that functional composition differs between enterotypes. **c**, Metagenomic operational taxonomic unit (mOTU)-level Shannon diversity index and gene richness (low gene count is considered for subjects with less than 480,000 genes (according to ref. [6]); all other subjects have high gene count) are significantly different between enterotypes (Supplementary Fig. 8), mostly following gradual changes over the whole enterotype space. **d**, A summary of the diseases and dietary constituents that have been associated with *Prevotella*-, Firmicutes- or *Bacteroides*-enriched gut communities (Supplementary Table 5). CD, Crohn's disease; CRP, C-reactive protein. For orientation, a three-enterotype model is illustrated by colour in **a**,**b**,**d**.

and stratify samples to reduce complexity (see Fig. 3a comparing clustering and genera abundances). There are limitations to this operational definition, and although the resulting stratification only partially reflects the more complex structure within the population space, the definition has been used to demonstrate that such stratification can be useful in analysing microbiome data.

Some later studies replicated enterotypes in new datasets to different extents, both in the numbers of enterotypes and the strength of the statistical support; whereas others reported finding no structure (Fig. 2 and Supplementary Table 1). For example, a large-scale, diet-focused study in a US cohort[23] reported support for two attractors, one of which shared similar dominant taxa with ET P, while the other was a merge of ETs F and B. Analysis of the HMP 16S rRNA data[18], a meta-analysis of four metagenomics datasets[24] and a population-wide Flemish study[7] showed a preference for three enterotypes, similar to the originally proposed ones. A study of individuals from Venezuelan and Malawian rural areas and US metropolitan areas emphasized the importance of *Prevotella* and *Bacteroides* as driving taxa, as well as a strikingly different composition in infants, with their communities mostly containing *Bifidobacteria* and Proteobacteria[8]. The establishment of an enterotype-like structure has been estimated to occur between the age of 9 and 36 months in humans[25], highlighting the need for caution when extrapolating overall community patterns from a limited sampling of the world population at different ages.

Departing from the clustering approach, Holmes et al.[26] propose an alternate approach to identify structure. Their method identifies a generative model for each possible state and determines how each explains the observed data, focusing on the actual genera abundances rather than the distances. Using this approach (Dirichlet multinomial mixture models; DMMs) they reported that the data from the original study most likely results from four generative

processes (loosely referred to here as 'clusters'). Two of the clusters resembled ET B and ET P, while a third showed an increased prevalence of *Ruminococcus* and other Firmicutes genera, which are usually lowly abundant in the gut microbiome. The last cluster had a high fraction of unidentified taxa. DMMs have also been used to identify three optimal clusters in a healthy Swedish cohort, again showing compositions similar to ET B and ET P, with one additional cluster dominated by unknown taxa[27]. A further study that applied the same method to the HMP 16S rRNA data found that the gut microbiome is best approximated with four similar models[17]. When applying DMMs to the MetaHIT metagenomics dataset, we identified four groups. Two of these are overlapping with ET B and ET P, while the other two are a more complex mixture (Fig. 2 and Supplementary Fig. 3). While DMMs represent a statistically more rigorous approach, further research is needed to determine if the distributional assumptions of generative models hold on microbiome data.

The three dominant gut taxa that contribute to enterotype clustering (*Prevotella*, *Bacteroides* and *Ruminococcaceae*) have been shown to have the largest variance in terms of relative abundance, despite being core taxa[7]. Therefore, it is not surprising that an ensemble-based network approach recovered them as hubs of three co-occurrence network clusters and showed that their abundances are mutually negatively correlated[1]. This negative correlation was also shown with qPCR data of 35 signature taxa[28]. Three distinct networks were found in adult Amish individuals[29], with the dominant genera in these networks largely overlapping with the driver taxa of the original enterotypes. Similarly, six species co-abundance groups (CAGs) were reported in a dataset consisting of Irish adults and elderly individuals, with healthy hosts mostly possessing networks that correspond to the original three enterotypes[9,30]. Thus, independent of clustering and modelling approaches, bacterial co-abundance networks provide a species network that may underline the fundamental properties of these preferred community profiles. Theoretical studies show that enterotype-like structures can be an emerging feature of communities over a wide range of species interaction strengths[31].

Enterotype-like structures have also been reported in several animal studies, although their gut composition is distinct from that of humans. In mouse gut microbiomes, obtained from hosts living under controlled experimental conditions[32], clustering showed a clear compositional stratification, while in animals living in the wild (mice[33], primates[34,35] and pigs[36,37]) clustering was considerably weaker. This is suggestive of preferred community states emerging more clearly when no external factors influence the microbiome. The concept of enterotypes is therefore not anthropocentric and can be defined in animals as well[38], which has led to the speculation that enterotypes have existed before the pan–human split[39]. As gut commensals are mostly evolving in competition with each other, under the restraints of the host organism[40], enterotypes might represent optimized states of symbiont compositions, which represent local optima in community effectiveness that are still compatible with the restraints imposed by the host.

Challenges in defining microbial community types

Assessing clustering in faecal microbiota profiles is non-trivial, given that demonstration of alternate states is debated in disciplines from ecology to philosophy[16,41]. Given the nature of enterotype clustering, and additional factors including multiple choices for taxonomic levels, distance metrics, clustering algorithms and cluster optimality scores, it is not surprising that analysis can yield different numbers of clusters (Supplementary Fig. 4 and Supplementary Information), even on the same dataset[16]. Some have therefore argued that there is little support for enterotypes in the data[8,16,23,29,42,43]. However, separating samples by body site (skin, stool, vaginal and oral) using the same methods also has little statistical support (Supplementary Fig. 4), even though this separation is widely accepted in the scientific community.

Regardless of clustering support or modelling assumptions (Supplementary Information), an analysis of the three largest public datasets, backed by reports from the literature (Supplementary Table 1), reveals that the local substructure is always similar—that is, a three-cluster model finds *Bacteroides*, *Prevotella* and Firmicutes-dominated clusters, and a two-cluster model separates *Prevotella*-driven samples from the rest. Partitioning of the gut microbiota is thus stable in the sense that related cluster compositions are recovered, reconciling many studies and supporting the existence of preferred community compositions.

There is certainly agreement that there are distinct areas within the complex microbial composition landscape in which the respective gut communities show biological differences[44]. The concept of enterotypes can help capture such differences, although defining meaningful and robust boundaries remains a challenge. This is analogous to clustering of macrobiomes, which faces similar problems despite the recognition of separate types of environments. For example, treeless, savannah and forest ecosystems in sub-Saharan Africa could equally be represented as a gradient in response to mean precipitation[45] or as contrasting stable states[46].

Given the practical challenges in accurately determining gut community structure, such as overcoming batch effects, considering confounders (Supplementary Fig. 5) and accounting for temporal variation, an objective number of stable states is difficult to determine. Still, in the (mostly Western) subjects studied cross-sectionally, *Bacteroides* and *Prevotella* act as the driving taxa that explain inter-individual differences, and delineate the main sources of variation, regardless of the technique employed. The extremes of the enterotype space are substantially different in microbial composition and diversity, and these are discussed in the following sections in terms of their function, ecology and disease. While three enterotypes may not always be the best explanation of the data, it is the model that has been used most and that provides the framework that we use below.

Functional and ecological context of enterotypes

Differences in taxonomic composition suggest that enterotypes may differ in functional and ecological properties. Analysis of the three large datasets revealed significant functional variation associated with microbial composition (Fig. 3b). Indeed, when gene types are considered, most KEGG (Kyoto Encyclopedia of Genes and Genomes) orthologues (KOs)[47] and non-supervised orthologous groups (NOGs)[48] differ in abundance among the three enterotypes (64% and 77%, respectively; false discovery rate (FDR) < 0.1). The same is true for eggNOG functional categories, where 23 out of 25 are significantly different (Supplementary Fig. 6). Other models choosing two or four enterotypes show similar broad functional differences (Supplementary Table 3), with some differences highly relevant to gut carbon metabolism. For example, it has been shown on several occasions that either ET P[23,29,49,50] or *Prevotella* (when no enterotype was reported) was enriched in individuals with non-Western and/or fibre-rich diets[8,51–53]. This association can be better understood in light of functional differences, as *Prevotella* hydrolases are specialized in the degradation of plant fibres[54], and an overall decreased lipolytic and proteolytic fermentation potential has been reported for the whole ET P community[44]. Conversely, ET B has been associated with diets enriched in animal proteins and saturated fats[23,53], in line with a large proportion of *Bacteroides*-specific carbohydrate-active enzymes (CAZymes) (50%)[55] being specialized for animal carbohydrates (Supplementary Table 2). Further, we find enzymes specific to carbohydrate metabolism overrepresented in ET B (Supplementary Table 3), corroborating recent research showing increased saccharolytic as well as proteolytic potential[44]. While some of the functional differences between enterotypes can be

attributed to the driver genera, others emerge only after imposing structure on the variation space.

The observed functional differences between enterotypes support the notion that they have varying community properties, such as richness, diversity and temporal stability. Such characteristics are relevant from an ecological perspective, where theory predicts a higher diversity in dynamic systems such as the gut, with nutrient availability and type fluctuating over time[56]. Using 16S amplicon sequencing, richness differences between three enterotypes were first shown in an Amish population[29], with a cluster similar to ET B having the lowest richness, as has been recently confirmed in a large population-wide study[7]. Our analysis of the three large datasets used here replicated these differences, with ET B having the lowest and ET F having the highest taxonomic as well as functional richness (Supplementary Figs. 7 and 8). Community diversity, as measured by the Shannon diversity index, is also highest in ET F in all datasets, while ET B and ET P are similarly decreased in diversity (Supplementary Fig. 8). Moreover, such differences go hand in hand with differences in stool consistency[57] and/or transit time[58]—with slow-transit-associated ETs also showing a higher relative ratio of proteolytic over saccharolytic potential[44] and proteolysis-derived metabolites[58].

Gut community composition in healthy adults in many studies does not change substantially over long time periods[23,29,59], indicative of a generally stable ecosystem and enterotype stability. There are, however, important exceptions. Our analysis of the HMP metagenomic time-series dataset, containing individuals sampled more than six months apart, reveals significant stability in all three enterotypes, although 16% of individuals switched putative enterotypes between visits (Supplementary Fig. 9). This suggests that, at least for some individuals, gut microbial types are relatively fluid and do not have discrete boundaries (Supplementary Fig. 9). These observations could be explained through alternative models of gut community dynamics: (1) the existence of preferred community compositions (that is, enterotypes; Supplementary Fig. 10A); or (2) individual-specific attractors that exist mostly due to temporal autocorrelation of that individual's gut community[60] (Supplementary Fig. 10B). Disentangling these models requires information about the response of the microbial community to different perturbations, thereby allowing us to determine if individuals are more likely to maintain/return to their original composition or maintain/return to their enterotype. Unfortunately, only limited data on gut community perturbations—such as antibiotics, faecal microbiota transplantation and diet—are available, many of which were not considered in the enterotype framework, making it difficult to draw conclusions about which steady-state model is correct. Short-term therapeutic antibiotic treatment was shown to induce substantial, partially recoverable shifts in the gut microbiota of humans[61,62], suggesting little resistance to such a dramatic disruption. Indeed, antibiotic treatment can lead to a complete deterioration of the community and subsequent pathogen invasion (for example, *Clostridium difficile*[63]), effectively resulting in failure to recover the original community state.

Dietary interventions, which cause considerably less perturbation to the microbial ecosystem of the human gut, may thus be better suited for investigating community resilience. The effects of such interventions, with significant compositional changes, have been observed within four days and could cause an enterotype shift[23,64]. However, after about ten days, enterotypes appeared to be stable[23], suggesting a tendency of recovering the original state. Stability was also observed in a six-month intervention, using the ratio of *Prevotella* to *Bacteroides* (obtained by qPCR) as a proxy for enterotype assignments[28]. These results suggest that there are limitations on how much an individual's microbiome may be perturbed by short-term dietary interventions, and support enterotype resilience. In contrast, long-term perturbations have a more profound effect, with dietary modulation over the period of a year having a strong impact on the Bacteroidetes/Firmicutes ratio[23,65], potentially leading to enterotype switches. As enterotypes were generally stable over time and no follow-up studies exist for the long-term interventions, no approximation of their resilience either in terms of overall community resemblance or enterotype assignment can be derived from the available data. There are, however, indications that enterotypes may vary in their recovery after intervention, with ET F estimated to have the lowest overall bacterial growth rate[44], possibly resulting in a delayed return to equilibrium.

Although it is not yet possible to predict how particular perturbations will modify the microbiota, it is possible that different microbiome configurations, including those stratified as enterotypes, might allow stratified treatment and diet recommendations in the future. Modulation of the gut microbiome is particularly relevant for diseases, where the challenge is to shift the microbiome back to a healthy pre-disease state in a given individual.

Clinical relevance of enterotypes

A simple classification scheme of gut community structure by enterotypes has the potential to be clinically useful. First, it can help in diagnosis, contributing to the identification of a disease state in an individual. Second, it can serve as an indicator of the risk or susceptibility of developing certain conditions. Third, the stratification may be a useful biomarker for changes that occur during disease progression. Fourth, given that the gut microbiota influences xenobiotic metabolism, it may be that different enterotypes are associated with different pharmacokinetics and dynamics of drug metabolism[66–69]. Thus, enterotyping may guide treatment options and help in understanding different treatment responses.

Several associations between enterotypes (or their main taxonomic drivers) and human disease phenotypes have been reported (Fig. 3d). For example, an increase of *Bacteroides* or ET B itself, which tends towards lower overall diversity (Supplementary Figs. 7 and 8), has been linked to nonalcoholic steatohepatitis (NASH)[70], colorectal cancer[49,71,72], caeliac disease[73], immune senescence and constant low-grade inflammation[6,30]. Reanalysis of the MetaHIT dataset found lymphocyte counts and C-reactive protein to be significantly increased in ET B compared to ET F (FDR < 0.1), with ET F samples on average lower in insulin resistance index (HOMA IR) and insulin levels (FDR = 0.107 for both) (Supplementary Table 4). Increased *Prevotella* abundance has been linked to long-term antibiotic usage[74], rheumatoid arthritis[75], type II diabetes[76] and HIV[77], although the latter is enriched in one of the risk groups (that is in men who have sex with men[78]), which might confound the reported association. Lastly, ET F has been linked to high microbiota diversity and decreased host inflammatory status, and has only been associated with an increased risk of atherosclerosis[79]. Given the multitude of associations to different disease phenotypes, an enterotype classification by itself may not be sufficiently specific as a standalone diagnostic marker of any disease[80], but may be able to indicate an increased risk of some. Enterotype associations within groups of healthy individuals at risk of certain conditions are rare, and it remains unclear if enterotype classifications might be useful as prognostics for disease development. In one example, increased prevalence of ET P had been reported in healthy individuals who had the heterozygous form of a Crohn's disease (CD) risk allele[27], while in the MetaHIT cohort, there is a significant enrichment of CD patients in ET B—implying that inflammation shifts community states to these two enterotypes and thus indicating an increased risk for inflammatory bowel disease.

Finally, it is possible that some diseases will have different aetiologies, depending on enterotype. Stratification could allow discovery of these underlying signals, thereby eliminating part of the large variation observed in microbial communities between individuals that may be irrelevant to the disease itself. In one mouse study, for

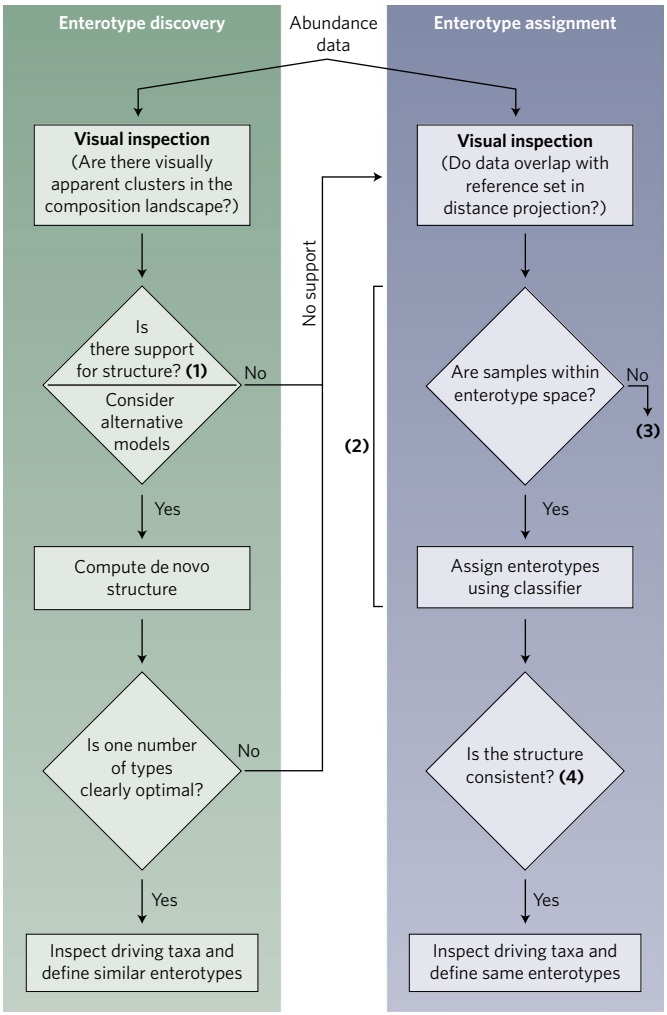

Fig. 4 | Determination of enterotype structure. A flow diagram of recommended steps for determining enterotype assignment based on microbial abundance data. Two main routes to obtain enterotype assignments are depicted: de novo identification (enterotype discovery, left) and enterotype assignment based on a reference dataset (right). The suitability of existing models imposed on the data to describe the composition landscape (1) can be assessed by either determining the existence of cluster structure using one of the proposed clustering strength measures (Supplementary Fig. 4), or by using a DMM modelling framework[26]. Other models might also be useful in capturing the structure in the data, although an exact implementation is not yet available. Determining whether samples are within the enterotype space (2) is based on similarity in composition to adult human stool samples from the HMP1 and MetaHIT6 studies. This suitability check and a respective classifier are available online (http://enterotypes.org). There are many explanations for the different compositional structures (3); for example, they may come from non-Western individuals, or from infants. Technical issues such as DNA extraction, PCR primers and/or bioinformatics preprocessing may skew the analysis. The consistency of the separation (4) obtained from the classifier may be determined using a silhouette index.

example, such stratification allowed discovery of genotype–microbiome and cage–microbiome associations[32]. Similarly, stratifying human patients into eight microbial clusters helped identify medical parameters that correlated with microbial composition[30], and microbial stratification significantly improved accuracy in classifying *C. difficile*-associated diarrhoea[81]. Although there are currently no long-term data, responses to diet and drugs as well as the impact of intestinal physiology and lifestyle are also likely to differ depending on the position of an individual in the compositional landscape. Thus, stratification represents an entry point into various clinically relevant areas. It can be implemented largely independently of a gradient- or cluster-centric view, analogous to the body mass index where defined cutoffs are an important guide to patient disease risk[82].

Towards guidelines for rational enterotyping

For enterotyping to be useful, standardization is essential. In addition to the technical challenges mentioned above, an inherent property of clustering is that assignment of single samples depends on which other samples are analysed at the same time. An enterotype defined this way makes comparisons across studies difficult. For example, if the majority of samples in a single study are ET B or ET F and only a few are ET P, the optimal cluster score might indicate two or even one cluster(s). Nevertheless, these few ET P samples may be identified based on the knowledge that similar samples have been clustered in other datasets. Combining data from multiple studies is often challenging, because differences in DNA extraction methods, sample handling, sequencing technology, primer choice (for 16S rRNA gene amplification) and data processing (for example, 16S rRNA clustering, copy number correction and chimaera reduction) influence the proportions of bacteria detected and lead to biases in detecting enterotype clusters[83]. Extreme rigour is needed in standardizing these steps, perhaps in conjunction with artificial 'mock' communities that span a large proportion of the phylogenetic spectrum of microorganisms found in the gut, and enable comparability between standard and clinical samples. Furthermore, there is a need for more longitudinal studies involving larger population cohorts across multiple continents to identify additional confounding factors. Indeed, several consortia such as the International Human Microbiome Standards (IHMS)[84], the Microbiome Quality Control Project (MBQC)[85] and the Genomic Standards Consortium (GSC) are already trying to set standards for metagenomics and identify sources of variation.

We propose a classification procedure that circumvents many of the problems outlined above while also providing more comparable results (Fig. 4). While we do not want to limit other explorations of the data or novel analysis options, alternative schemes should at least be compared with the results from the procedure described here. Based on the MetaHIT dataset[6], we have trained a classifier at genus level on taxonomic and functional features that recovers putative clustering observed in the Chinese type II diabetes study[19] and in the HMP1 dataset (Supplementary Fig. 11), available online (http://enterotypes.org). If the results of de novo clustering differ from the classifier results, we recommend caution in directly comparing the stratification outcome to the enterotypes described in this meta-analysis. Moreover, this approach also defines an enterotyping space, by determining which samples are compositionally similar to a reference set. This could be used to define the boundaries of 'normal' gut communities and identify individuals outside of them, serving as a health indicator. Unusual disease states have been previously reported: for example, by using a model of six-species communities, networks resembling the three enterotypes were most strongly overrepresented in healthy patients, whereas two new states were overrepresented in frail, elderly patients[9]. Another case reported a new enterotype H, enriched in Enterobacteriaceae[70]. The above classifier would consider samples from this 'enterotype' compositionally dissimilar to those present in large datasets, and they would thus be labelled as being outside the enterotyping space. The individuals with this unusual composition frequently suffered from obesity, NASH, and high blood ethanol and reactive oxygen species (ROS) levels[70], suggesting this unusual compositional state to be dysbiotic.

Whether used for disease state identification, prospective stratification or flagging technical issues, standardized enterotyping will ensure comparability across a wide range of studies and facilitate our understanding of the role and importance of enterotypes.

Conclusions

Identification and characterization of the major patterns related to human gut microbiota configurations remains challenging. Given an array of available approaches, each with their advantages and caveats, the number of recovered enterotype states and their statistical support can vary. With more standardization, control of sample processing and data analysis, increased concordance among different studies can be expected. Enterotype attribution can be further refined by the addition of a wider range of samples and contextual information, extending beyond the industrialized world to better represent the global human population. For now, however, we here propose a way of restricting the enterotyping space, allowing for the detection of samples that are outside of it.

Independent of the many difficulties outlined above, multiple studies have reported enterotypes with similar compositional properties albeit with varying statistical support (Fig. 2). While clearly not discrete and confounded by various factors, they differ in taxonomic, functional and ecological properties, and can be accurately recovered across large datasets (Supplementary Fig. 11). They represent a way of capturing preferred microbial compositions in the human gut and thus appear to be useful stratifiers in many settings.

Relying solely on enterotype classifications can obscure potentially important microbial variation, and therefore should not replace direct clinical associations and expert statistical analysis with microbial species and functions where possible. However, enterotypes may still be relevant in various clinical settings, ranging from direct disease associations to prospective study stratification, or even personalized dietary interventions or other gut modulation treatments. We believe, despite our still limited knowledge, that enterotypes can be a useful tool for studying the human microbial community landscape.

Received: 15 August 2017; Accepted: 27 October 2017;
Published online: 18 December 2017

References

1. Huttenhower, C. et al. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
2. Sender, R., Fuchs, S. & Milo, R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* **14**, e1002533 (2016).
3. Qin, J. et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).
4. Turnbaugh, P. J. et al. A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484 (2009).
5. Faith, J. J. et al. The long-term stability of the human gut microbiota. *Science* **341**, 1237439 (2013).
6. Le Chatelier, E. et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541–546 (2013).
7. Falony, G. et al. Population-level analysis of gut microbiome variation. *Science* **352**, 560–564 (2016).
8. Yatsunenko, T. et al. Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227 (2012).
9. Jeffery, I. B., Claesson, M. J., O'Toole, P. W. & Shanahan, F. Categorization of the gut microbiota: enterotypes or gradients? *Nat. Rev. Microbiol.* **10**, 591–592 (2012).
10. Lahti, L., Salojärvi, J., Salonen, A., Scheffer, M. & de Vos, W. M. Tipping elements in the human intestinal ecosystem. *Nat. Commun.* **5**, 4344 (2014).
11. Sørlie, T. et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl Acad. Sci. USA* **98**, 10869–10874 (2001).
12. Sorlie, T. et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc. Natl Acad. Sci. USA* **100**, 8418–8423 (2003).
13. Yamauchi, M. et al. Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal versus distal colorectum. *Gut* **61**, 847–854 (2012).
14. Arumugam, M. et al. Enterotypes of the human gut microbiome. *Nature* **473**, 174–180 (2011).
15. Ravel, J. et al. Vaginal microbiome of reproductive-age women. *Proc. Natl Acad. Sci. USA* **108**, 4680–4687 (2011).
16. Koren, O. et al. A guide to enterotypes across the human body: meta-analysis of microbial community structures in human microbiome datasets. *PLoS Comput. Biol.* **9**, e1002863 (2013).
17. Ding, T. & Schloss, P. D. Dynamics and associations of microbial community types across the human body. *Nature* **509**, 357–360 (2014).
18. Zhou, Y. et al. Exploration of bacterial community classes in major human habitats. *Genome Biol.* **15**, R66 (2014).
19. Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55–60 (2012).
20. Dethlefsen, L., Eckburg, P. B., Bik, E. M. & Relman, D. A. Assembly of the human intestinal microbiota. *Trends Ecol. Evol.* **21**, 517–523 (2006).
21. Schloissnig, S. et al. Genomic variation landscape of the human gut microbiome. *Nature* **493**, 45–50 (2013).
22. Zhu, A., Sunagawa, S., Mende, D. R. & Bork, P. Inter-individual differences in the gene content of human gut bacterial species. *Genome Biol.* **16**, 82 (2015).
23. Wu, G. D. et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**, 105–108 (2011).
24. Karlsson, F. H., Nookaew, I. & Nielsen, J. Metagenomic data utilization and analysis (MEDUSA) and construction of a global gut microbial gene catalogue. *PLoS Comput. Biol.* **10**, e1003706 (2014).
25. Bergstrom, A. et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. *Appl. Environ. Microbiol.* **80**, 2889–2900 (2014).
26. Holmes, I., Harris, K. & Quince, C. Dirichlet multinomial mixtures: generative models for microbial metagenomics. *PLoS ONE* **7**, e30126 (2012).
27. Quince, C. et al. The impact of Crohn's disease genes on healthy human gut microbiota: a pilot study. *Gut* **62**, 952–954 (2013).
28. Roager, H. M., Licht, T. R., Poulsen, S. K., Larsen, T. M. & Bahl, M. I. Microbial enterotypes, inferred by the *Prevotella-to-Bacteroides* ratio, remained stable during a 6-month randomized controlled diet intervention with the new nordic diet. *Appl. Environ. Microbiol.* **80**, 1142–1149 (2014).
29. Zupancic, M. L. et al. Analysis of the gut microbiota in the Old Order Amish and its relation to the metabolic syndrome. *PLoS ONE* **7**, e43052 (2012).
30. Claesson, M. J. et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature* **488**, 178–184 (2012).
31. Gibson, T. E., Bashan, A., Cao, H.-T., Weiss, S. T. & Liu, Y.-Y. On the origins and control of community types in the human microbiome. *PLoS Comput. Biol.* **12**, e1004688 (2016).
32. Hildebrand, F. et al. Inflammation-associated enterotypes, host genotype, cage and inter-individual effects drive gut microbiota variation in common laboratory mice. *Genome Biol.* **14**, R4 (2013).
33. Wang, J. et al. Dietary history contributes to enterotype-like clustering and functional metagenomic content in the intestinal microbiome of wild mice. *Proc. Natl Acad. Sci. USA* **111**, 2703–2710 (2014).
34. Moeller, A. H. et al. Chimpanzees and humans harbour compositionally similar gut enterotypes. *Nat. Commun.* **3**, 1179 (2012).
35. Moeller, A. H. et al. Stability of the gorilla microbiome despite simian immunodeficiency virus infection. *Mol. Ecol.* **24**, 690–697 (2015).
36. Mach, N. et al. Early-life establishment of the swine gut microbiome and impact on host phenotypes. *Environ. Microbiol. Rep.* **7**, 554–569 (2015).
37. Ramayo-Caldas, Y. et al. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. *ISME J.* **10**, 2973–2977 (2016).
38. Li, J. et al. Two gut community enterotypes recur in diverse bumblebee species. *Curr. Biol.* **25**, R652–R653 (2015).
39. Moeller, A. H. et al. Rapid changes in the gut microbiome during human evolution. *Proc. Natl Acad. Sci. USA* **111**, 16431–16435 (2014).
40. Foster, K. R., Schluter, J., Coyte, K. Z. & Rakoff-Nahoum, S. The evolution of the host microbiome as an ecosystem on a leash. *Nature* **548**, 43–51 (2017).
41. Scheffer, M. & Carpenter, S. R. Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends Ecol. Evol.* **18**, 648–656 (2003).
42. Huse, S. M., Ye, Y., Zhou, Y. & Fodor, A. A. A core human microbiome as viewed through 16S rRNA sequence clusters. *PLoS ONE* **7**, e34242 (2012).
43. Gorvitovskaia, A., Holmes, S. P. & Huse, S. M. Interpreting *Prevotella* and *Bacteroides* as biomarkers of diet and lifestyle. *Microbiome* **4**, 15 (2016).
44. Vieira-Silva, S. et al. Species–function relationships shape ecological properties of the human gut microbiome. *Nat. Microbiol.* **1**, 16088 (2016).
45. Sankaran, M. et al. Determinants of woody cover in African savannas. *Nature* **438**, 846–849 (2005).
46. Staver, A. C., Archibald, S. & Levin, S. Tree cover in sub-Saharan Africa: rainfall and fire constrain forest and savanna as alternative stable states. *Ecology* **92**, 1063–1072 (2011).
47. Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).

48. Huerta-Cepas, J. et al. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res.* **44**, 286–293 (2016).
49. Ou, J. et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am. J. Clin. Nutr.* **98**, 111–120 (2013).
50. Nakayama, J. et al. Diversity in gut bacterial community of school-age children in Asia. *Sci. Rep.* **5**, 8397 (2015).
51. Smith, M. I. et al. Gut microbiomes of Malawian twin pairs discordant for Kwashiorkor. *Science* **339**, 548–554 (2013).
52. De Filippo, C. et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl Acad. Sci. USA* **107**, 14691–14696 (2010).
53. David, L. A. et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).
54. Purushe, J. et al. Comparative genome analysis of *Prevotella ruminicola* and *Prevotella bryantii*: insights into their environmental niche. *Microb. Ecol.* **60**, 721–729 (2010).
55. Cantarel, B. L. et al. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* **37**, D233–D238 (2009).
56. Pereira, F. C. & Berry, D. Microbial nutrient niches in the gut. *Environ. Microbiol.* **19**, 1366–1378 (2017).
57. Vandeputte, D. et al. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut* **65**, 57–62 (2016).
58. Roager, H. M. et al. Colonic transit time is related to bacterial metabolism and mucosal turnover in the gut. *Nat. Microbiol.* **1**, 16093 (2016).
59. Caporaso, J. G. et al. Moving pictures of the human microbiome. *Genome Biol.* **12**, R50 (2011).
60. Gibbons, S. M., Kearney, S. M., Smillie, C. S. & Alm, E. J. Two dynamic regimes in the human gut microbiome. *PLoS Comput. Biol.* **13**, e1005364 (2017).
61. Voigt, A. Y. et al. Temporal and technical variability of human gut metagenomes. *Genome Biol.* **16**, 73 (2015).
62. Dethlefsen, L. & Relman, D. A. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc. Natl Acad. Sci. USA* **108**, 4554–4561 (2011).
63. Van Nood, E. et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N. Engl. J. Med.* **368**, 407–415 (2013).
64. Kovatcheva-Datchary, P. et al. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of *Prevotella*. *Cell Metab.* **22**, 971–982 (2015).
65. Ley, R. E., Turnbaugh, P. J., Klein, S. & Gordon, J. I. Human gut microbes associated with obesity. *Nature* **444**, 1022–1023 (2006).
66. Haiser, H. J. et al. Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science* **341**, 295–298 (2013).
67. Liang, X. et al. Bidirectional interactions between indomethacin and the murine intestinal microbiota. *eLife* **4**, e08973 (2015).
68. Spanogiannopoulos, P., Bess, E. N., Carmody, R. N. & Turnbaugh, P. J. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. *Nat. Rev. Microbiol.* **14**, 273–287 (2016).
69. Forslund, K. et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* **528**, 262–266 (2015).
70. Zhu, L. et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* **57**, 601–609 (2013).
71. Zeller, G. et al. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol. Syst. Biol.* **10**, 766 (2014).
72. Sobhani, I. et al. Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS ONE* **6**, e16393 (2011).
73. De Palma, G. et al. Intestinal dysbiosis and reduced immunoglobulin-coated bacteria associated with coeliac disease in children. *Microbiology* **10**, 63 (2010).
74. Jernberg, C., Löfmark, S., Edlund, C. & Jansson, J. K. Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology* **156**, 3216–3223 (2010).
75. Scher, J. U. et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife* **2**, e01202 (2013).
76. Larsen, N. et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE* **5**, e9085 (2010).
77. Lozupone, C. A. et al. Alterations in the gut microbiota associated with HIV-1 infection. *Cell Host Microbe* **14**, 329–339 (2013).
78. Noguera-Julian, M. et al. Gut microbiota linked to sexual preference and HIV infection. *EBioMedicine* **5**, 135–146 (2016).
79. Karlsson, F. H. et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nat. Commun.* **3**, 1245 (2012).
80. Knights, D. et al. Rethinking 'enterotypes'. *Cell Host Microbe* **16**, 433–437 (2014).
81. Schubert, A. M. et al. Microbiome data distinguish patients with *Clostridium difficile* infection and non-*C. difficile*-associated diarrhea from healthy controls. *mBio* **5**, e01021-14 (2014).
82. Flegal, K. M., Kit, B. K., Orpana, H. & Graubard, B. I. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA* **309**, 71–82 (2013).
83. Wu, G. D. et al. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags. *Microbiology* **10**, 206 (2010).
84. Morton, J. T. et al. Uncovering the horseshoe effect in microbial analyses. *mSystems* **2**, e00166–16 (2017).
85. Costea, P. et al. Towards standards for human fecal sample processing in metagenomic studies. *Nat. Biotechnol.* **35**, 1069–1076 (2017).
86. Sinha, R. et al. Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium. *Nat. Biotechnol.* **35**, 1077–1086 (2017).
87. Mardanov, A. V. et al. Metagenomic analysis of the dynamic changes in the gut microbiome of the participants of the MARS-500 experiment, simulating long term space flight. *Acta Naturae* **5**, 116–125 (2013).
88. Segata, N. et al. Metagenomic microbial community profiling using unique clade-specific marker genes. *Nat. Methods* **9**, 811–814 (2012).
89. Karlsson, F. H., Nookaew, I. & Nielsen, J. Metagenomic data utilization and analysis (MEDUSA) and construction of a global gut microbial gene catalogue. *PLoS Comput. Biol.* **10**, e1003706 (2014).
90. MacDonald, N. J., Parks, D. H. & Beiko, R. G. Rapid identification of high-confidence taxonomic assignments for metagenomic data. *Nucleic Acids Res.* **40**, e111 (2012).
91. Zhang, J. et al. Mongolians core gut microbiota and its correlation with seasonal dietary changes. *Sci. Rep.* **4**, 5001 (2014).
92. Morotomi, N. et al. Evaluation of intestinal microbiotas of healthy japanese adults and effect of antibiotics using the 16S ribosomal RNA gene based clone library method. *Biol. Pharm. Bull.* **34**, 1011–1020 (2011).
93. Eloe-Fadrosh, E. A. et al. Impact of oral typhoid vaccination on the human gut microbiota and correlations with *S. typhi*-specific immunological responses. *PLoS ONE* **4**, e62026 (2013).

Acknowledgements

The authors are grateful to the members of the Bork group at EMBL for discussions and assistance. The research leading to these results has received funding from EMBL, the VIB, the Rega institute for Medical Research, the European Research Council via the CancerBiome project (project reference 268985), MicrobesInside (250172) and the European Community's Seventh Framework Programme via the MetaHIT (HEALTH-F4-2007-201052), the METACARDIS project (FP7-HEALTH-2012-INNOVATION-I-305312), the European Union's Horizon 2020 research and innovation programme (Marie Sklodowska-Curie grant 600375), Metagenopolis grant ANR-11-DPBS-0001 and the IHMS project (FP7-HEALTH-2010-single-stage-261376).

Author contributions

P.B., R.K. and J.R. conceived the review. P.I.C., F.H. and G.Z. performed data analysis. F.H., P.I.C., J.R. and P.B. performed the literature research, with input from all co-authors. P.I.C., F.H., S.S., R.K., J.R. and P.B. wrote the manuscript with contributions from M.A., F.B., M.J.B., F.D.B., W.M.d.V., S.D.E., C.m.F., M.H., C.H., I.B.J., D.K., J.D.L., R.E.L., H.O., P.W.O., C.Q., D.A.R., F.S., J.W., G.M.W., G.D.W., G.Z. and L.Z.

Competing interests

The authors declare no competing financial interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41564-017-0072-8.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.R. or R.K. or P.B.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.