

Diversity, stability and resilience of the human gut microbiota

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Trillions of microbes inhabit the human intestine, forming a complex ecological community that influences normal physiology and susceptibility to disease through its collective metabolic activities and host interactions. Understanding the factors that underlie changes in the composition and function of the gut microbiota will aid in the design of therapies that target it. This goal is formidable. The gut microbiota is immensely diverse, varies between individuals and can fluctuate over time — especially during disease and early development. Viewing the microbiota from an ecological perspective could provide insight into how to promote health by targeting this microbial community in clinical treatments.

Most gut microbes are either harmless or of benefit to the host. The gut microbiota protects against enteropathogens^{1,2}, extracts nutrients and energy from our diets^{3,4}, and contributes to normal immune function⁵. Disruptions to the normal balance between the gut microbiota and the host have been associated with obesity^{6,7}, malnutrition⁸, inflammatory bowel disease (IBD)^{9,10}, neurological disorders¹¹ and cancer¹². Understanding how the gut microbiota affects health and disease requires a shift in focus from individual pathogens to an ecological approach that considers the community as a whole.

The first step in understanding the symbiotic relationship between gut microbes and their host is to characterize the baseline healthy microbiota and the differences that are associated with disease. Large-scale projects such as the European Metagenomics of the Human Intestinal Tract (MetaHIT)¹³ and the US Human Microbiome Project (HMP)¹⁴ have made substantial progress towards this goal. Once the healthy composition and functional states of gut microbiota are understood, the features that, when disrupted, are associated with disease can be determined. However, the complexity of the microbiota, and the variation between and within individuals complicates the definition of what this ideal state may be within a population or an individual.

Ecological principles can aid in understanding the host–microbe interactions and specific functions of the gut microbiota. Improvements in sequencing methods and technologies such as proteomics and metabolomics, coupled with metabolic network modelling^{15,16}, show how host and environmental factors can affect gut microbial ecology over a lifetime. The composition, diversity and function of gut microbial communities have the potential to inform personalized nutritional and drug treatment strategies (Fig. 1). In this Review, we summarize the progress that has been made towards characterizing the diversity and function of microbial communities in the healthy human gut, describe the ways in which this ecosystem can go awry and discuss the prospects for restoring a degraded ecosystem.

Taxonomic diversity in the healthy gut

Until the advent of higher-throughput sequencing, even basic questions — such as how many different species there are, how diverse their genetic make-up is, which features are ubiquitous and which

are unique to an individual, and whether community function can be predicted from species present — remained unanswered.

Culture-based studies suggest that all healthy adults share most of the same gut bacterial species — a core microbiota. For example, *Escherichia coli* can be isolated from most people. However, culture-independent sequencing studies (Fig. 2) have demonstrated a vast microbial diversity that is highly variable over time and across populations. Each of us harbours more than 1,000 ‘species-level’ phylotypes: clusters of sequences that have as much diversity in their small subunit ribosomal RNA genes as named species¹⁷. Most of these phylotypes are bacteria belonging to just a few phyla. In adults, Bacteroidetes and Firmicutes usually dominate the microbiota, whereas Actinobacteria, Proteobacteria and Verrucomicrobia, although found in many people, are generally minor constituents¹⁸ (Fig. 3). Methanogenic archaea (mainly *Methanobrevibacter smithii*), eukaryotes (mainly yeasts) and viruses (mainly phage) are also present¹⁹. Despite the consistency of these main components, their relative proportions and the species present vary markedly across individuals (Figs 3 and 4). Attempts to find a core set of species-level phylotypes in the adult microbiota have identified several main players, including *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Bacteroides uniformis*¹³, but in some individuals even these can represent less than 0.5% of the microbes present²⁰. As the data set expands to include developing countries and a broader age range^{4,21}, the concept that there is a core set of species in the microbiota is becoming more unlikely.

Functional diversity in the healthy gut

Knowing the composition of the microbial community alone does not necessarily lead to an understanding of its function. Functional information comes, in part, from studying cultured isolates that have well-characterized genome content and *ex vivo* phenotypes, and from sequencing community DNA. Functional screening by shotgun metagenomics relies on the sequencing of the total microbial community DNA, including from uncultured members, and matching the sequences to known functional genes (Fig. 2). Identifying the genes involved in specific metabolic pathways can predict functional capabilities, but without messenger RNA, protein and metabolite profiling these are still predictions. The process of mapping short gene

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sequence fragments to organisms and functions is improving as more human gut microbial genomes are sequenced and annotated²², and as more complementary ‘omics’ data sets become available^{23,24}.

Despite the highly divergent compositions of gut microbiota across individuals, the functional gene profiles are quite similar (Fig. 4). This similarity was reported in a study of 18 females who shared more than 93% of the enzyme-level functional groups, but few genus-level phylotypes²⁰, and was confirmed in much larger populations in HMP and MetaHIT data^{13,14}.

The gut microbiota all contain pathways for central, carbohydrate and amino-acid metabolism²⁰. However, not all pathways are represented, and grouping genes into broad functional categories can conceal the finer differences in function between individuals. Variable functions that are restricted to a species or strain — including pathogenicity islands, vitamin and drug catabolism, motility and nutrient transporters — are possible targets for personalized diets and therapeutic strategies.

Many genes are expressed only under specific conditions. Shotgun sequencing approaches that measure levels of mRNA (metatranscriptomics) or shotgun proteomics (metaproteomics) may uncover functional variation with factors such as disease or diet that DNA studies overlook. For example, genes involved in carbohydrate metabolism and energy generation have been found to be expressed as proteins at higher levels than predicted from metagenomic data, underscoring the importance of these processes in the gut²³.

The observation that we share a functional core microbiome but not a core microbiota can be related to macroecosystems. For instance, rainforests that look the same and have many of the same functions can be composed of different species that have independently evolved. The challenge of ecological studies of the gut is to understand which members of the community have similar functional niches and which ones can be substituted for one another. Although taxonomic composition is far more variable than functional gene composition, at a general process level at least, several studies have shown a correlation between the two^{4,14,25}. Functional gene composition variation that is not well explained by taxonomic variation can indicate that phylogenetically unrelated taxa have converged on similar functions and may be crucial for identifying and understanding functional components associated with altered physiological states (Fig. 2).

Factors that drive normal variation

Having established that the healthy gut microbiota is highly variable, we must next understand why it varies, so that this information can be used to tailor therapies or clinical trials. For example, the extent to which members of the same family harbour similar microbes will determine whether the family history of microbiota-driven disease is informative. How much the microbiota varies with age or pregnancy should be taken into account when designing cohorts. The sensitivity of the microbiota to external factors, such as diet, will inform strategies for treating microbiome-linked diseases.

Age

Marked changes in the gut microbiota occur early on in life, with an increase in diversity and stability over the first three years^{4,26,27} (Fig. 5). The maturation of the human microbiota is an example of ecological succession^{4,26,27}. After the initial colonization, communities undergo consecutive changes in composition and function until a relatively stable climax community is established.

The microbiota in infants is relatively volatile. Variation in microbial communities and functional gene repertoires between individuals is greater in infants than in adults. This has been seen in rural communities in Malawi and Venezuela, and in US metropolitan populations of European and African descent⁴. However, infant microbiomes do share characteristic properties across individuals and populations, both compositional (many bifidobacteria, and

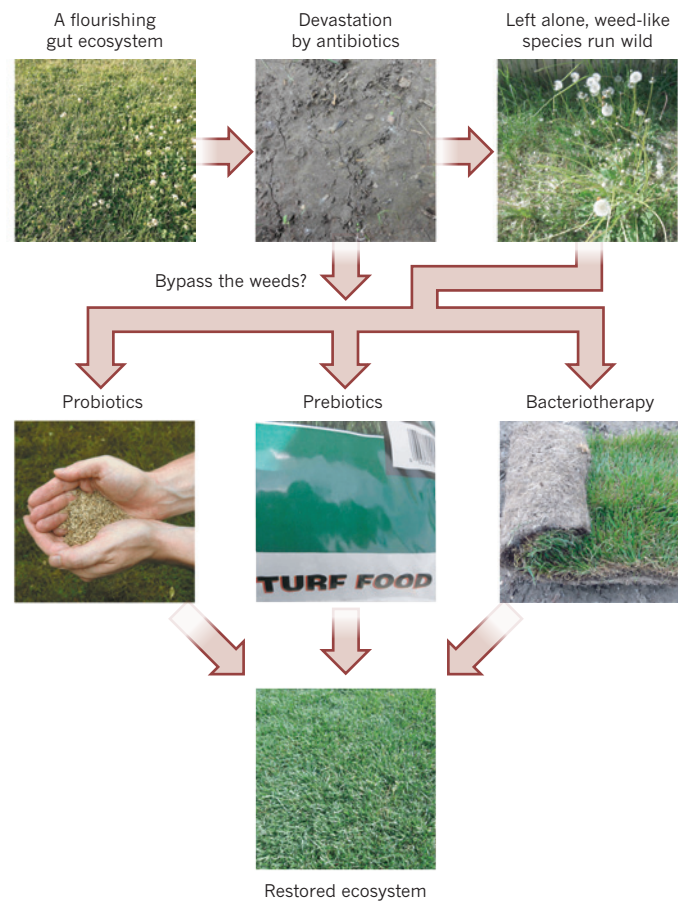
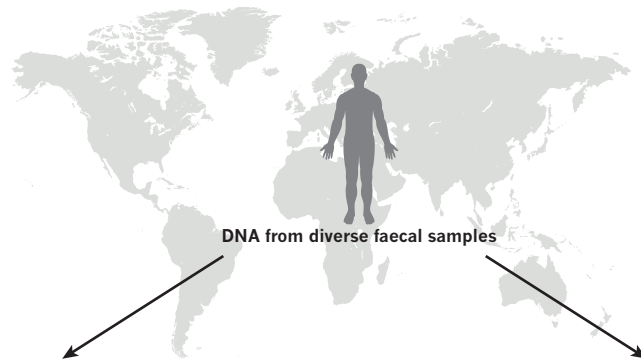


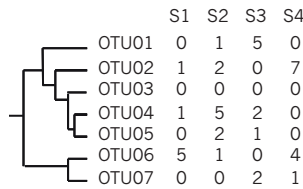
Figure 1 | Maintaining our gut microbial lawn. In some ways, maintaining a healthy microbiota is like lawn care: severe interventions can take the ecosystem back to bare earth, so it has to be re-established. Although many people recover naturally, this is not guaranteed, and weed-like species that are adapted to perturbed ecosystems often run wild. Several strategies can be used to restore the ecosystem: reseeding with a few well-defined ‘good’ microbes (probiotics), adding compounds that are thought to specifically promote the growth of beneficial microbes (prebiotics) and transplanting the entire microbial ecosystem, for example from a stool sample (faecal bacteriotherapy). The weed stage could be bypassed by administering the treatment immediately after the disturbance before the weeds can establish themselves. An additional strategy (not shown) is to use specific drugs, such as narrow-spectrum antibiotics, that target undesirable members of the microbial community. Although we are beginning to learn what a healthy microbial community looks like, and to recognize signs of weed species, our understanding of which strategies for altering the microbiota work best, and predicting which will work for a given individual, is still in its infancy.

lower species richness than in adults) and functional (more genes that encode enzymes involved in folate biosynthesis⁴).

The microbiota of infants is affected by antibiotic use, whether they were breastfed and the method of delivery, although whether differences in the microbiota in early life affect the composition of the adult microbiota is not well understood^{28,29}. However, differences in composition driven by factors in infancy may affect susceptibility to immunological diseases into adulthood, such as asthma and atopic diseases²⁹. A possible mechanism for this susceptibility has been demonstrated in germ-free mice that accumulate invariant natural-killer T (iNKT) cells in the colonic lamina propria and lungs. The mice were protected from the associated pathology of this accumulation if colonization with a conventional microbiota occurred in neonatal, but not adult germ-free mice, indicating that infancy is a crucial time for contact with the microbiota⁵. Identifying the components of the human microbiota that shape our immune system in early life is now needed.



SSU ribosomal RNA targeted gene sequencing



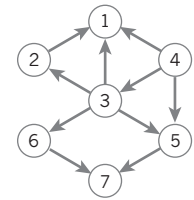
Cultured isolates from faecal samples

↓
Genome sequencing

Genome sequences relate functions to species

Shotgun metagenomic sequencing

	S1	S2	S3	S4
Gene 1	0	1	5	0
Gene 2	1	2	0	7
Gene 3	0	0	0	0
Gene 4	1	5	2	0
Gene 5	0	2	1	0
Gene 6	5	1	0	4
Gene 7	0	0	2	1

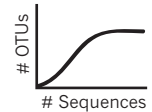


Alpha diversity

Which microbiota are the most compositionally diverse, with the most phylotypes or longest branch length?

If additional taxa (compositional diversity) do not increase the number of functions (functional diversity), there is functional redundancy.

Which microbiota are the most functionally diverse, with the most gene families?

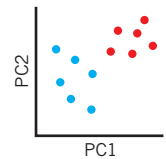


Beta diversity

Which factors, such as age, culture or disease state, correlate with differences in the overall composition of phylotypes or phylogenetic lineages?

Beta diversity plots on the basis of taxonomic and functional composition are usually highly correlated. Therefore, functional genes have considerable phylogenetic signal.

Which factors, such as age, culture or disease state, correlate with differences in the overall composition of functional genes?



Machine learning or classical statistics

Are there phylotypes or collections of phylotypes that discriminate or significantly differ between the microbiota of individuals in different groups – diets or disease states?

Functional differences are often reflective of taxonomic differences. Selected functions that cannot be explained by selected taxonomic groups indicate potential functional convergence.

Are there particular gene families or functions that discriminate or significantly differ between the microbiomes of individuals in different groups – diets or disease states?

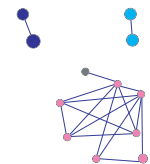


Co-occurrence

Which phylotypes co-distribute across people? Positive correlations are driven by shared environmental preferences, symbiotic or syntrophic relationships. Negative correlations are driven by divergent environmental preferences or competitive relationships.

Performing co-occurrence analyses on species with complete genomes can allow for further exploration of the driving factors of positive or negative correlations. For example, genes or functions that are shared by phylogenetically unrelated bacteria that co-occur can indicate potential environmental driving factors.

Which genes co-distribute across people? Positive correlations will largely be driven by genes that co-occur in the same genome (phylotype). Genes with high rates of horizontal gene transfer and strong environmental selection may co-distribute independently.



Metabolic network modelling

What metabolic activities are predicted for a collection of phylotypes?

Metabolic predictions can, in principle, be made for collections of phylotypes if associated reference genome sequences are available.

What overall metabolic activities are predicted for microbiota? What spectrum of metabolites would microbiota produce from a defined diet? Promising for designing personalized therapeutic diets, but has challenges.

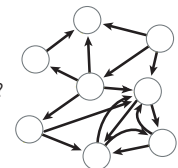


Figure 2 | Tools for evaluating microbiota diversity. Targeted sequencing of phylogenetically informative genes (usually small subunit (SSU) ribosomal RNA) or random sequencing of all genes (shotgun metagenomic sequencing) can be used to assess DNA extracted from faecal samples. Genome sequences from cultured isolates link these two data sets by indicating which species contain which genes, and therefore functions. Shotgun metagenomic data will become substantially more useful as the number of reference genomes continues to increase with additional strain sequencing efforts. SSU rRNA gene sequences are related to each other in the form of phylogenetic trees because related phylotypes (clusters of similar sequences defined by sequence similarity) generally have more similar functional attributes. Genes that

encode proteins that perform known enzymatic reactions are related using metabolic networks, because genes that participate in the same metabolic pathway can work together to produce a phenotype. Data from SSU rRNA targeted gene and shotgun metagenomic sequencing can be analyzed by the same suite of techniques (detailed in the table and with illustrations to the right). Combining analysis results from targeted and shotgun sequencing data from the same samples, and sometimes using genome sequences to relate the two, can provide additional insight (middle column) Combining targeted sequencing with mRNA, protein and metabolite level analyses will be crucial for moving from *in silico* predictions to direct measurements of expressed community properties. OTU, operational taxonomic unit.

Genetics, environment and diet

How factors such as the environment and diet shape the human gut microbiota is still unclear, partly because these factors are often confounded. Twins and mother-daughter pairs have more similar microbiota compositions than unrelated individuals, suggesting that there could be a genetic influence over the microbiota^{9,20}. However, monozygotic and dizygotic adult twins have equally similar microbiota, suggesting environment rather than genetics may drive familial similarities²⁰.

Populations can be separated by characteristic differences in the gut microbiota. For example, Italian children have a different microbiota from children from rural Africa³⁰, and both children and adults from the United States have a very different microbiota from populations in Malawi and the Amazonas state of Venezuela⁴ (Fig. 5). Although genetically different, these populations also differ in other factors that could affect the microbiota, such as environmental exposures, provision of adequate sanitation and levels of cleanliness, diet and antibiotic use. Cultural factors, especially diet, may be crucial in shaping the gut microbiota. The ratio of two of the main genera of gut bacteria, *Prevotella* and *Bacteroides*, correlates well with the overall pattern of diversity across healthy adults (see discussion of enterotypes later)^{31,32}. Levels of *Prevotella* were enriched in children from a rural African village who had a high-fibre diet³⁰, and in children and adults from Malawi and Venezuela whose diet was dominated by plant-derived polysaccharide foods such as maize and cassava. By contrast, the microbiota of those in the United States had more *Bacteroides*⁴. For healthy adults in the United States, differences in long-term diet between individuals also was correlated with these bacteria. *Bacteroides* was associated with a long-term diet rich in animal protein, several amino acids and saturated fats, and *Prevotella* was associated with carbohydrates and simple sugars³². The relative importance of changes in the microbiota as a result of diet, compared with other factors that affect health, remains a subject of active investigation.

Understanding how cultural traditions affect the microbiota should highlight the factors that result in the marked differences in the incidence of diseases associated with microbiota. For example, the incidence of IBD and other allergies is greater in industrialized, Western, societies than in agrarian cultures³³. Understanding these associations will require expanded studies that sample a greater number of populations and control the confounding factors. This may come in the form of a human microbiome diversity project that parallels the Human Genome Diversity Project³⁴. Especially valuable will be populations in which variables that correlate with microbiota diversity — such as history of antibiotic use, diet and environmental exposures — can be measured. Comparisons with environmental exposures may require sampling from the environment as well as from individuals. Because exposure to a microbiota in early life is particularly relevant to the development of immunological disease⁵, prospective studies that include infants or even prenatal studies, will be especially valuable. When determining disease aetiology, immigrant populations can provide an opportunity to disentangle host genetics, geography and culture because the incidence of some diseases matches that present at the destination rather than at the origin³⁵.

Microbiota variability, and human health and medicine

Understanding the compositional and functional differences in the gut microbiota can lay the foundation to relate these differences to human health. Differences in the microbiota and the microbiome could help to explain the variation in the gut metabolic processes of individuals, including the metabolism of drugs and food^{11,36}. Many of these metabolic pathways are outside the common functional core, so they can underlie host-specific responses. For example, the health benefits of diets rich in soya, such as improvements in vasomotor symptoms, osteoporosis, prostate cancer and cardiovascular disease, have been attributed to (S)-equol produced from the soya isoflavone daidzein by bacterial rather than human enzymes³⁷. Only 25–30% of

the adult population of Western countries produce (S)-equol with a diet of soya-rich foods, compared with 50–60% of adults from Japan, Korea or China³⁸. The cancer-protective effects of soya beans that have been described in Asian populations may therefore not be true of Western populations. Similarly, the gut microbiota can indirectly determine whether the widely used analgesic paracetamol will be metabolized to paracetamol sulphate or paracetamol glucuronide, potentially altering its efficacy and toxicity³⁶. The microbes mediate this metabolic phenotype by producing the compound *p*-cresol, which competes with paracetamol for human enzymes that catalyse sulphonation³⁶. Substantial differences in the gut microbiota between populations and concomitant effects on drug metabolism will necessitate population-specific drug trials for examining effectiveness and toxicity. Understanding how the microbiota varies across the population, and correlating this variability with specific microbial functions, is emerging as a component of personalized medicine.

Stable configurations of healthy microbiota

The landscape of stable states for the human gut is still unknown. Samples obtained over time from the same individual are more similar to one another than those obtained from different individuals, suggesting that each person has a relatively distinct, stable community^{20,39–42}. This temporal stability could be related to the concept that stable equilibrium states exist for the microbiota, for which disturbances, stochasticity and temporal dynamics of individual microbes produce change, but the community is still drawn to a central attractor^{43,44}.

Clinically, it would be convenient if a few clearly differentiable stable states could be used to stratify the gut microbiome across the human population. For example, if microbes in one state were known to metabolize a drug into harmful metabolites, a simple test could avoid complications for the patient, as in the case of paracetamol and liver toxicity³⁶. It would be especially helpful if the same states could be applied across the board so that, for example, the same community features that correlated with the generation of toxic metabolites from a drug also correlated with obesity.

Enterotypes propose this concept. The human microbiome has been described to form three distinct host–microbial symbiotic states driven by groups of co-occurring species and genera, which are characterized by a relatively high representation of *Bacteroides*, *Prevotella* or *Ruminococcus*³¹. Although the overall clustering structure, which was based on 33 faecal shotgun metagenomes, was not statistically significant, the three enterotype clusters described the real data better than randomly generated data sets³¹. Similar patterns were reported after existing 16S rRNA data from 154 individuals from the United States²⁰ and metagenomic data from 85 individuals from Denmark¹³ were reanalysed. However, subsequent studies of 98 healthy adults from the United States³², 531 healthy infants, children and adults from Malawi, Venezuela and the United States⁴, and 250 healthy adults from the United States¹⁴ failed to show the same pattern. Variation among adults in these populations was, however, associated with a trade-off between *Prevotella* and *Bacteroides*, suggesting an important role for these taxa, or taxa that co-distribute with them, in structuring the microbiota. When infants were included in the analysis, variation was also associated with a trade-off between the infant-associated *Bifidobacterium* genus and lineages that were common only to adults⁴ (Fig. 5). The number of unique configurations that form functional, stable communities may be large and may not be easy to classify into a manageable number of distinct ‘types’. Considering this concept further in more extensive data sets made up of different ages, cultural traditions or geographic locations, and physiological or disease states will be important.

Differences in the microbiota with disease

The composition of the microbiota differs with the physiological state of the host. People who are obese harbour fewer types of microbes in their gut than people who are lean, and have a significantly different abundance of specific taxa and functional genes^{6,7,45}. Classification

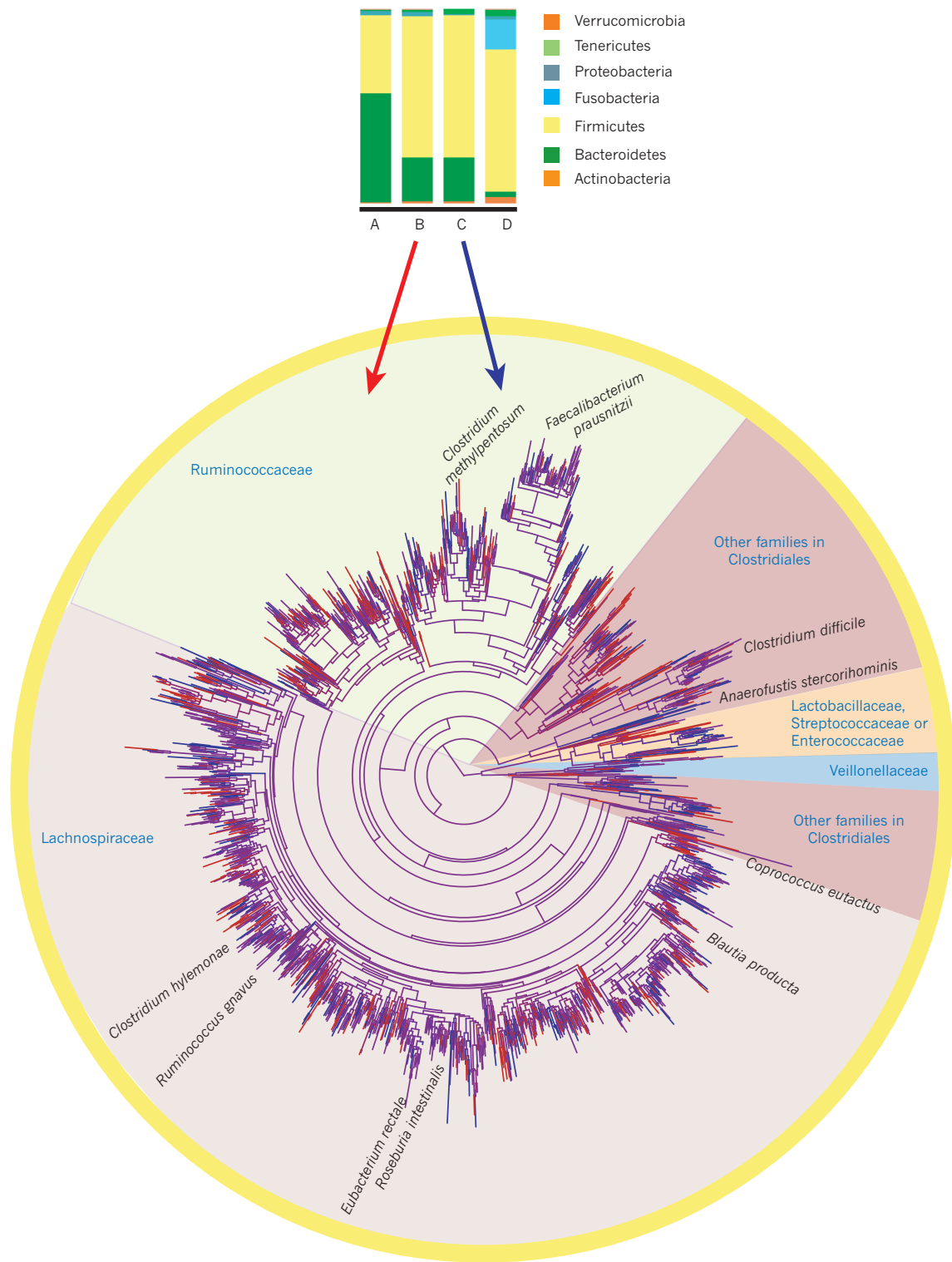


Figure 3 | Diversity of the human microbiota at different phylogenetic scales. The human microbiota is remarkably varied within and between individual hosts. Although this complexity can be simplified by evaluating communities at higher taxonomic levels, the species within each phylum have different biological properties, and significant changes detected at higher taxonomic levels are probably driven by only a subset of the species in those higher taxa. Using the 16S rRNA sequence data of four of the adults from the United States in ref. 4, the high diversity and variability among individuals, and the degree to which taxonomic grouping at high levels can mask this diversity is shown. Phylum level diversity can have a marked variation even across healthy adults in the same population.

Individual A has an unusually high proportion of Bacteroidetes; individual D has an unusually high proportion of Fusobacteria; and individuals B and C are dominated by Firmicutes and Bacteroidetes, which is the more typical phylum-level distribution for this cohort. However, even B and C differ at finer scales. The tree depicts the phylogenetic relationships between species-level phylotypes in just the most diverse of the phyla, Firmicutes, in individuals B and C. Branches specific to individual B are red; branches specific to individual C are blue; and shared branches are purple. Each individual has many unique phylotypes not found in the other. As described in many surveys of the human gut^{14,18,40}, the Ruminococcaceae and Lachnospiraceae families are particularly rich in phylotypes.

of people as lean or obese can be made solely on the basis of their gut microbiota with 90% accuracy^{46,47}, but they do not separate into distinct microbiota-based clusters on commonly used principal coordinates plots, which are used to identify statistical differences between groups. Thus, multiple statistical techniques are needed to show fully the differences in the microbiota between different physiological states (Fig. 2).

Some differences in the microbiota can contribute directly to disease states. Gnotobiotic mice that were raised germ-free then colonized with the microbiota from an obese mouse gained fat more rapidly than those colonized with the microbiota of a lean mouse^{7,45}. A phenotype can emerge from different compositional backgrounds, which may indicate that specific components of the microbiota can exert large effects or that many different changes can lead to the same functional result.

Differences in faecal microbial community diversity, composition and function have also been correlated with Crohn's disease⁹, ulcerative colitis¹⁰, irritable bowel syndrome (IBS)⁴⁸, *Clostridium difficile*-associated disease (CDAD)⁴⁹ and acute diarrhoea⁵⁰. Sometimes, the nature of the microbiota deviation from health is consistent across individuals with the same disease. For instance, a twin study of IBD found marked and reproducible deviations in patients with ileal Crohn's disease relative to the controls, and more subtle, but characteristic, changes in patients with colonic Crohn's disease⁵¹, and specific functional differences were also observed from metabolic profiling of the same samples²⁴. Other diseases are associated with marked deviations from health that are inconsistent across individuals. For instance, individuals with recurrent CDAD had a phylum-level diversity that was very different from controls but not similar to each other⁴⁹. Many disease studies are confounded by extensive use of treatments, such as antibiotics, that may obscure true disease-associated changes, highlighting the urgent need for prospective longitudinal studies that establish cause and effect.

Parallels between host physiological states

Studies of the microbiota often target one specific disease or state, but comparisons of the microbiota across many diseases can show common changes in the gut environment. Disturbed mucous layers that line the intestinal cell wall and concomitant inflammation are seen in individuals with IBD, coeliac disease, HIV enteropathy, acute diarrhoea, diverticulosis, carcinoma and IBS⁵². Given these parallels, an increase or decrease in abundance of similar microbes across different disturbances might be expected⁵³, but elucidation of these differences may require detailed biogeographical studies along the length of the gut — once safe and reliable means for such comprehensive sampling are developed.

Perturbed adult gut microbial communities are intriguingly similar to infant gut microbial communities. Both systems may represent successional communities in which the same opportunistic or fast-growing species can predominate⁵³. For instance, *C. difficile* is a normal gut resident that can cause disease when antibiotics compromise the stable adult gut communities; it also colonizes 2–65% of infants, although most infants are asymptomatic^{54,55}. *Clostridium bolteae* and *Clostridium symbiosum* are also associated with a disturbed gut and systemic infection, and are found in the infant gut⁵³. The microbiota of individuals with ileal Crohn's disease can also resemble that of infants: both have increased levels of *Ruminococcus gnavus* and Enterobacteriaceae in their stools, and an under-representation of the genera that are prevalent in healthy adults, including *Faecalibacterium* and *Roseburia*⁵¹. These examples show the importance of understanding whether generally opportunistic members of the gut microbiota have a selective advantage during early succession or disruption caused by disease, and therefore whether they are the side effects of disease rather than causal agents.

Resilience of stable states

Resilience is the amount of stress or perturbation that a system can tolerate before its trajectory changes towards a different equilibrium state⁵⁶. Macroecosystem studies of human interference such as

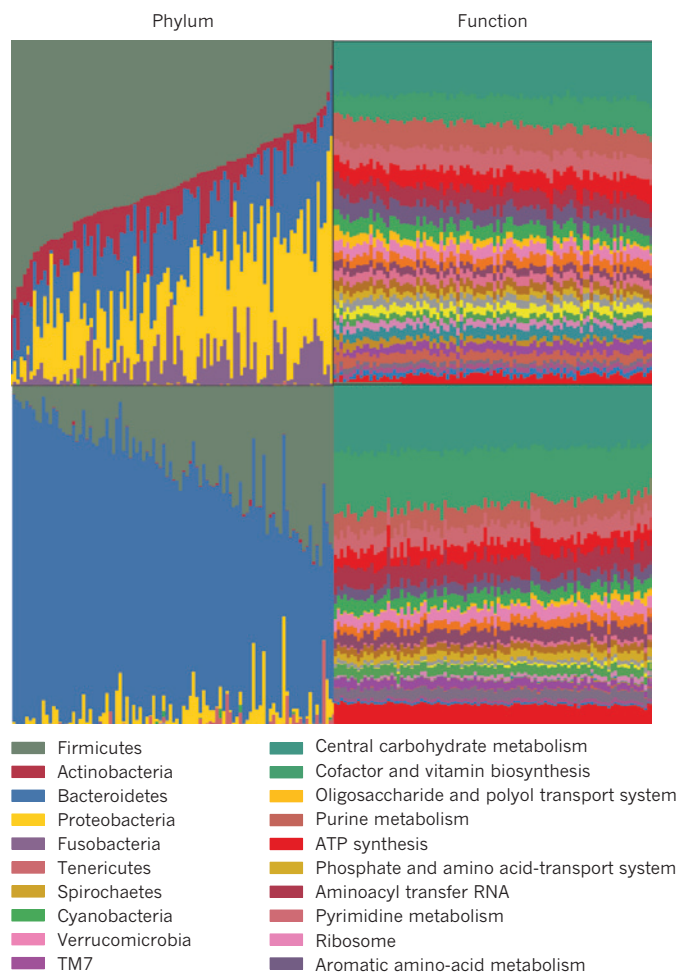


Figure 4 | Functional redundancy. The functional redundancy in microbial ecosystems may mirror that in macroecosystems. As shown in the HMP data set¹⁴, oral communities (top panels) and faecal communities (bottom panels) analysed using 16S rRNA (coloured by microbial phyla, left panels) show tremendous abundance diversity. The same samples analysed by shotgun metagenomics (panels on right) have remarkably similar functional profiles. Reprinted with permission from ref. 14.

resource exploitation, pollution, land-use change and global warming have shown how ecological systems can be transformed into less productive or less desirable states⁵⁶. For example, in a tropical lake community, a regime in which submerged plants dominate is preferred to one with extensive free-floating plant cover because dense mats of floating plants create anoxic conditions that reduce animal biomass and diversity. However, pollution can cause floating plants to predominate because they are better at competing for light and can exclude submerged plants when the nutrient load is high. By understanding the environmental drivers of conversion between states, interventions can be used to induce a regime change. For example, harvesting the floating plants once can induce a permanent shift to the submerged-plant-dominant state, but only if the nutrient loading is not too high⁵⁷.

Environmental studies can provide examples of the microbial response to perturbations, and perhaps an insight into how the gut microbiota might react. During the Deepwater Horizon oil spill in the Gulf of Mexico, the microbial community structure and functional gene repertoire in the deep-sea oil plume shifted, transiently, to an enrichment of microbes that were capable of hydrocarbon-degradation^{58,59}. This is similar to the effect of an extreme dietary change on the gut microbiota of mice that were moved from a low-fat, plant-rich diet to a high-fat, high-sugar diet⁷. In both cases, the

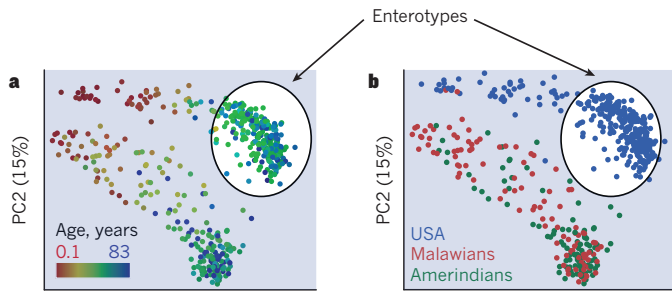


Figure 5 | Human microbial diversity and enterotypes. Enterotypes³¹ were determined when evaluating only adults from the United States and Europe (circled in white). By including children from the United States and children and adults from developing countries, the picture of human-associated microbiota diversity greatly expands. The relationship between the microbiota of 531 healthy children and adults from Malawi, Amazonas state of Venezuela (Amerindians) and the United States was evaluated using sequences from the 16S rRNA gene in faecal samples and a principle coordinate analysis of unweighted UniFrac distances (adapted with permission from ref. 4). **a**, Infants differentiate strongly from adults, and **b**, adults from the United States have a distinct composition from those of Malawi and Venezuela, indicating the diversity differences are mainly owing to age and culture.

microbial communities shifted substantially, probably because of the exposure to new substrates that provided a selective advantage to certain members of the community. Whether a particular disturbance will disrupt a stable state depends on the resilience of the community to a type of perturbation.

Dietary changes

Understanding the resilience of the gut microbiota is crucial for determining the efficacy of therapeutic diets. Consuming a carbohydrate- or fat-restricted low-calorie diet for a year⁶, or a high-fat and a low-fibre, or low-fat and high-fibre diet for 10 days³² can induce statistically significant changes in the gut microbiota. However, these changes in species and gene content are small compared with the baseline variations that occur between individuals. Long-term dietary surveys and cross-cultural comparisons suggest that changes to diet might lead to regime changes over longer periods of time^{4,32}, perhaps by eroding the landscape of alternative stable states to allow changes that short-term ‘nudges’ cannot.

Antibiotics

Antibiotic administration can move the microbiota to alternative stable states. In healthy volunteers who were given two courses of ciprofloxacin in a 10-month period, the faecal microbiota reached a stable state similar to, yet distinct from, the pretreatment state⁴¹. However, the magnitude of disturbance that occurred after ciprofloxacin treatment, and the speed and extent of recovery to the pre-ciprofloxacin state, suggested that resilience of the microbiota varies across individuals and between ciprofloxacin treatments in the same individual⁴¹.

Long-term studies of the microbiota after antibiotic treatment indicate that post-antibiotic equilibrium states are themselves resilient. For example, clindamycin treatment affected *Bacteroides* in the gut for up to 2 years after treatment had finished⁶⁰. Similarly, three individuals with dyspepsia treated for 1 week with a combination of metronidazole, clarithromycin and omeprazole had a shift in their microbiota state that persisted for up to 4 years without additional antibiotic treatment⁴². In both cases, a significant increase in the antibiotic-resistance genes persisted for years^{42,60}, suggesting that the post-disturbance state would probably be more resilient to the same disturbance again because a greater proportion of the microbiota would be resistant. This is consistent with a case from the study of healthy volunteers given the two courses of ciprofloxacin⁴¹. Initial recovery of the microbiota was slow and incomplete, stabilizing in a different interim state, but after a second treatment, recovery was relatively quick. But in the same study, a different case had the opposite

response, in which an essentially complete recovery occurred after the first ciprofloxacin treatment, but stabilized to a distinct state after the second treatment, suggesting that the initial antibiotic treatment decreased resilience⁴¹, and that the resilience of the microbiota to future antibiotic treatments can vary considerably between individuals.

New species invasion

Resilience of the microbiota to challenge with exogenous microbes is also important. The gut microbiota generally shows colonization resistance, in which the native microbiota prohibits harmful (pathogenic)^{1,2,61} and potentially beneficial (probiotic)⁶² microbes from establishing. Challenging the gut microbiota with a foreign microbiota rather than with specific pathogens or probiotics has suggested the native community may be less resilient to colonization by exogenous microbes than previously thought. When 14 conventionally raised Lewis rats were force fed with pooled microbiota from Sprague-Dawley and Wistar strain rats, the microbiota diversity of the Lewis rats increased substantially and changed to resemble that of the Sprague-Dawley and Wistar rats, and phylotypes established from the donors persisted for up to 3 months after transplantation⁶³. In contrast to ecological theory — that disturbances eliminating native species facilitate the establishment of exotic species⁶⁴ — transplantation after the resident microbiota have been depleted with two broad-spectrum antibiotics reduced rather than promoted the establishment of the donor microbiota⁶³.

The success of microbiota transplantation in the treatment of recurrent CDAD provides further support of the plasticity of an established gut microbiota when challenged with a complex one. Bacteriotherapy⁶⁵ involves introducing a faecal sample from a healthy donor as a homogenate by injection into the caecum using a colonoscope. In one case study, the symptoms of a patient with recurrent CDAD were resolved after faecal transplantation from a healthy donor. Before treatment, the recipient had a disturbed microbiota with *Veillonella* and *Streptococcus* predominating, whereas the donor microbiota was dominated by *Bacteroides*. A month after treatment, the patient had no *C. difficile* in stool samples, but the donor species were present, indicating that the donor microbiota persisted over this interval. Faecal transplantation for CDAD is becoming increasingly common and seems to be highly effective. A survey of 317 patients across 27 case series reported disease resolution in 92% of cases, with a single treatment being sufficient in 89% of cases⁶⁶. The success of bacteriotherapy suggests that a previous course of antibiotic treatment reduces the resident microbiota to the extent that a donor microbiota can establish itself. However, because faecal transplantation is not a first-line therapy, there are no data on its effectiveness without antibiotics (A. Khoruts, personal communication). Understanding the successional processes and which microbial taxa allow a healthy microbiota configuration to be established in faecal transplant recipients will require further studies. These studies will allow the development of a standardized way of formulating and analysing the donor specimen, design and interpretation of dosing studies, and analysis of short- and long-term safety.

Mechanisms for resilience

Understanding how the microbiota forms a stable, resilient state would allow strategies to be devised to increase the resilience of healthy states or decrease the resilience of unhealthy states. For example, a healthy state with a high resilience to exogenous microbes might mean one person at a dinner party escapes food poisoning but their companions with lower resilience fall ill. A degraded microbiota with high resilience can contribute to chronic diarrhoea or inflammation, as is inherent with CDAD, IBD and IBS.

Species and functional response diversity

The aspects of diversity that are crucial for conferring resilience in macroecosystems are probably the same features that are important in microbial ecosystems. One such parameter is the number of species present in a given system, or species richness. (In

culture-independent studies, this number depends on the number of sequences collected per sample.) Species-rich communities are less susceptible to invasion because they use limiting resources more efficiently, with different species specialized to each potentially limiting resource⁶⁴. An excess of nutrients in water, or eutrophication, often decreases ecosystem diversity because a small number of species overgrow and outcompete everything else, with a concomitant decrease in resilience⁶⁷. In a similar way, decreased diversity has been linked to obesity and to a diet that is high in fat and sugar compared with one that is low in fat and plant-based^{4,7}. Whether this decrease in microbiota diversity results in a decrease in resilience is not known. Low microbiota diversity also correlates with IBD⁵¹ and recurrent CDAD⁴⁹, but the effects on microbiota resilience are not known.

Functional response diversity is the degree to which species in a community that contribute to the same ecosystem function vary in their sensitivity to ecosystem changes⁶⁸. High functional response diversity may, for instance, allow a species that is relatively rare but functionally similar to fill a niche when an abundant species is compromised by an environmental disturbance⁶⁸. In a macroecology example, a compromised coral reef that was in a healthy state changed to an unhealthy, algal-dominated state only when both the algal-grazing fish were overfished and the sea urchins that had increased in numbers to fill this niche were compromised by a pathogen⁶⁸. The same principle is also likely to apply to the gut microbiota. Following antibiotic treatment, a previously rare microbe may increase in abundance to fill a niche that had been dominated by a microbe with higher antibiotic sensitivity, leading to persistence of the same stable state but with a decreased resilience because of the decrease in functional redundancy.

Human-gut-adapted bacteria are likely to have high functional response diversity because phylogenetically disparate microbes often perform similar metabolic functions. For example, methanogenic Archaea, sulphate-reducing bacteria and phylogenetically diverse acetogens in humans and mice all consume hydrogen generated by other microbes during fermentation⁶⁹. Butyrate producers of the Clostridiales order can have different ecological strategies, such as adaptation to different stages of community succession, oxygen tolerance and substrate preference. The abundance of *Anaerostipes caccae* peaks in infancy, whereas *Eubacterium hallii* and *R. intestinalis* are more abundant in adults. *A. caccae* is able to survive 10–60-minute periods of exposure to air better than *E. hallii* or *R. intestinalis*⁷⁰. Furthermore, *E. hallii* but not *F. prausnitzii* can use lactate as a substrate⁷¹. Thus, butyrate production in the gut can also continue through different successional and metabolic states.

Competition and feedback loops

The densely populated gut environment means that microbes compete to use the same resources or inhibit each other directly using antimicrobial products. Phylogenetically related bacteria could be expected to compete because of their overlapping functional roles, habitats or both, yet contrary to this expectation, phylogenetically similar species tend to appear in the same samples⁷². For example, analysis of bacteria with complete or draft genome sequences from 124 individuals from Europe found that the abundance of related Enterobacter species, including *E. coli*, *Salmonella enterica*, *Citrobacter koseri* and *Enterobacter cancerogenus* were positively correlated across individuals⁵³. These related species may share environmental preferences so that they are selected for simultaneously. The abundance of closely related species can also predict the susceptibility to intestinal colonization by both pathogenic and commensal bacteria in mice⁷³.

Feedback loops can either stabilize or destabilize the microbiota (Fig. 6). Stable physiological states are preserved by negative feedback, in which a change to the gut environment results in opposing changes that maintain homeostasis. This feedback is likely to be controlled by a tight interplay between microbial metabolic activities

and host pathways. For example, microbial metabolites might induce changes in the expression of the host pathways that control gut retention time so that it rises above or below the optimum, causing diarrhoea or constipation. This deviation probably induces host signalling pathways to correct it. This is similar to body temperature regulation, in which a rise above the optimum induces thermoregulatory mechanisms such as sweating, and a fall induces mechanisms such as shivering. Stabilizing the physiological parameters that are controlled by negative feedback from the host could therefore promote resilience of the microbiota.

Negative-feedback loops that promote microbiota resilience could also operate independently of the host. For example, if the abundance of a particular microbe exceeds a certain threshold, this would result in a change in the gut environment that would decrease the growth of that microbe relative to other species. These feedback loops may involve the accumulation of a phage specific to that microbe or the accumulation of a specific toxic metabolite. Negative feedback is thought to promote high ecosystem diversity, which can promote resilience⁷⁴.

Positive feedback is thought to induce an ecosystem change because a difference from a point in one direction produces further change in the same direction. However, positive-feedback mechanisms could support stability at the individual microbe level or of the microbial consortia that promote each other's growth. Metabolic activities or interactions with host pathways in microbes may induce a physiological state that favours their growth over the growth of potential competitors, promoting resilience. The microbiota is likely to contain both important functional drivers of physiological status and microbes that co-occur with these drivers because they are able to thrive in such an environment. Invasion by microbes that do not thrive in that particular physiological state would be prevented, for example if a microbiota containing functional drivers of a low inflammatory state resists colonization by pathogens, and microbiota with functional drivers of a relatively high inflammatory state resists colonization of beneficial microbes (Fig. 6).

Positive- and negative-feedback loops are also likely to have a role in destabilizing the microbiota during regime changes, such as during succession in early development and following a disturbance (Fig. 6). Negative feedback, whereby an organism's activity alters the environment so that fitness is decreased, can induce a directional change when microbes induce a physiological state that favours their competitors. For instance, the higher redox potential in the gut of infants is probably one of the factors that explains the relative success of facultative anaerobes such as *E. coli* or some *Lactobacillus* in early development⁷⁵, but the reduction of oxygen that results from their metabolism favours their eventual replacement by a consortium dominated by strict anaerobes.

The importance of feedback for successional changes is also likely to involve a complex interplay between the microbiota and its host. For example, the same change in redox potential that directly affects microbial fitness can also affect the expression of host factors in the gut epithelium, such as hypoxia-inducible factor, in early development and inflammatory diseases of the gut⁷⁶. Manipulating positive and negative feedback at the level of the host, the individual microbes and the entire gut ecosystem will be essential to maintaining healthy stable states and switching from an unhealthy to a healthy state.

Prospects

High-throughput sequencing of samples suggests that the microbiota of each person has some resistance to perturbation, but that this resistance can be overcome by diet, drugs, prebiotics or probiotics. Dietary changes may alter the regime in the gut over long time periods. The surprising success of whole community transplants in healthy rats and in humans with CDAD shows that exogenous microbes can colonize the gut even with resistance from an entrenched microbiota. However, which microbes will be the best colonizers is unknown; neither do we know how a particular microbial

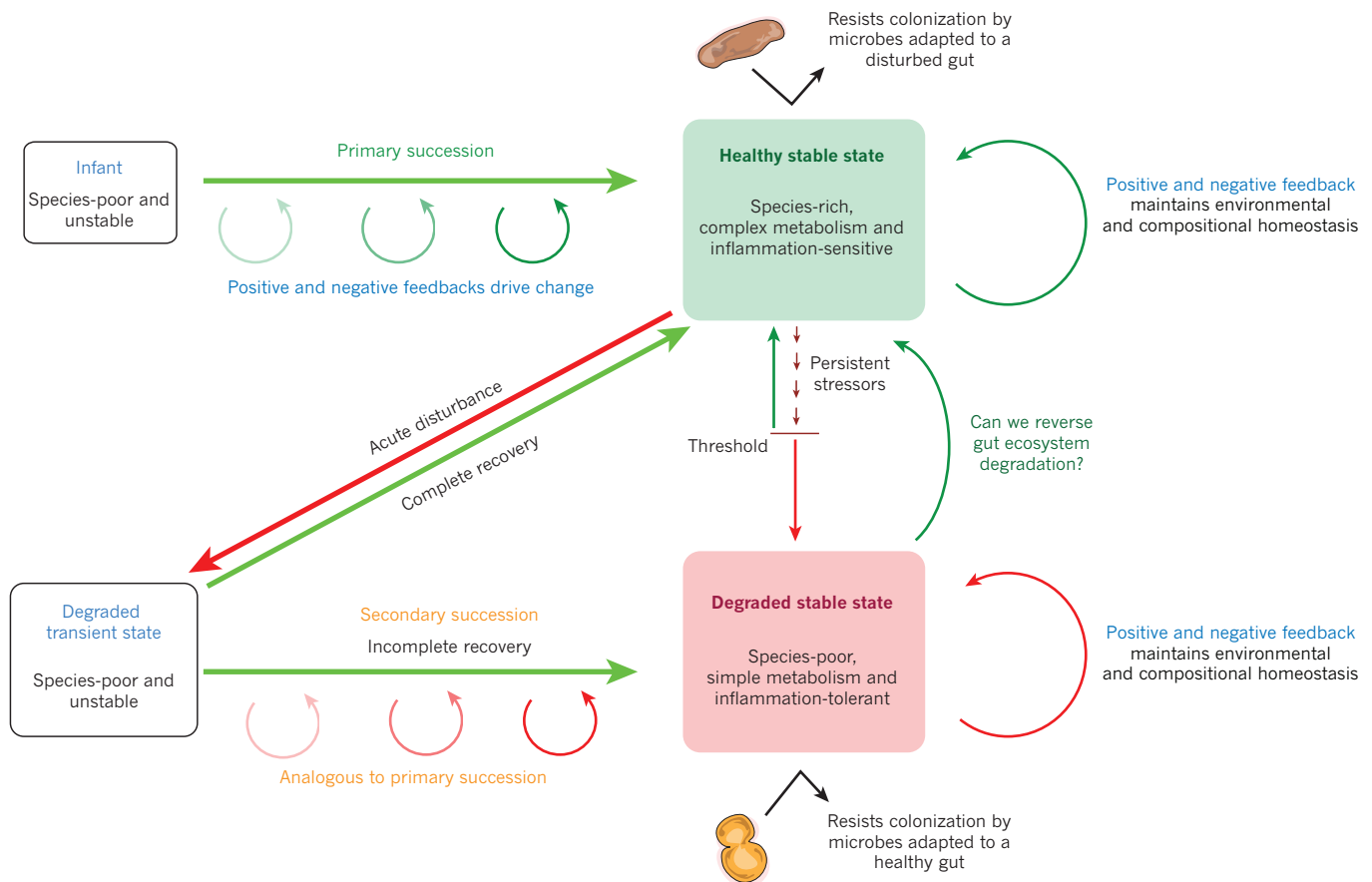


Figure 6 | Compositional transitions in the human gut microbiota.

Primary succession of the gut microbiota during early development involves a systematic turnover of species until a stable adult state is reached. Positive- and negative-feedback loops probably have a role in driving primary succession and in conferring resilience to healthy stable equilibrium states. Acute disturbances, such as antibiotic administration, are often followed by an unstable state that progresses to a stable state through secondary succession. In some cases, a complete recovery occurs, in which the stable state highly resembles the pre-disturbance state, but sometimes the post-recovery stable state is distinct. Post-disturbance stable states may be both degraded and resilient; for example, IBS that forms after an initial acute disturbance of the microbiota from an enteropathogen can persist in some individuals for years and even decades⁷⁸. Resilience

configuration and its functional attributes change in response to dietary components or exogenous microbes. We need to learn what conditions promote the health of desired species and exclude the undesirable ones, just as a gardener would exclude weeds.

The landscape of stable states for the microbiota and its implications for resilience is an important research direction. Small perturbations, such as short-term dietary changes, may allow the microbiota to return to its original state, but larger disturbances, such as antibiotic administration, could cause movement into a different state. The long-term implications for health are not yet well understood. Furthermore, changes to the stable equilibrium of the gut microbiota through long-term changes, such as inflammation, diet or repeated antibiotic administration, could make new states reachable with smaller perturbations. Host genetics, the factors that affect the microbiota early in development, diet and long-term drug administration may all contribute to individual differences. Consequently, the general landscape and the current community state could be important for determining individual responses to an intervention.

How resilient the microbiota is to diet, disturbance by antibiotics and challenge by exogenous microbes will have important implications for health care. The degree to which repeated applications of

of degraded states is probably driven by unique positive- and negative-feedback loops that occur both in concert with and independently of the host. Degradation to a stable state may also occur as a result of persistent stressors, such as poor diet, that slowly degrade resilience of a healthy state until a threshold is passed so that new feedback mechanisms are needed to maintain community composition and stability. Developing therapies that encourage transition from degraded to healthy stable states, or complete recovery to a healthy stable state following disturbance, may involve identifying species or combinations of species, and drivers of feedback loops. Whether interventions are more effective early in succession, when communities are more unstable but possibly stochastic, or later in succession, when convergence to the end point is more certain but the trajectory may be more difficult to change, will be important to investigate.

broad-spectrum antibiotics degrade the microbiota and its ability to function as a healthy system would need to be studied, especially in children, in whom the development of interactions between the microbiota and host is crucial⁵. However, identifying suitable controls is challenging given the large variation in and between individuals during early development. Regime change is not always instigated by acute disturbances and can occur gradually, as indicated by studies of resilience to dietary changes. Individuals whose microbiota has been degraded by long-term consumption of a high-fat and high-sugar diet may need long-term dietary changes to restore their microbiota to a healthy state.

The lower taxonomic diversity in individuals from Western cultures who have a high-fat and high-sugar diet raises the concern that global trends in diet could result in important microbial symbionts being lost from the broader population, possibly leading to the extinction of bacterial species that can provide important health benefits. Maintaining a collection of cultures from individuals in the developing world, and specifically from agrarian cultures, could help to preserve potentially important components of the microbiota.

Microbes often produce unique states in the gut through collective activities and cooperative metabolism; understanding associations

between disease states and sets of species rather than single taxa will therefore be important⁵³. A central problem of culture-independent metagenomic analyses is that identified phylotypes, or collections of these, often represent species for which there is little biological knowledge. Gut microbial ecology has come full circle, with the development of methods for culturing most of the diversity present in a community being given increasing attention so that the contribution that community members make can be further explored. The human faecal microbiota is largely composed of readily cultured bacteria⁷⁷. *In vitro* experiments with these isolates will be extremely valuable for exploring the metabolic attributes of a particular microbe or set of microbes, and the genes involved. However, because microbiota composition and function are also controlled by feedback from the host, *in vivo* studies in gnotobiotic mice will be particularly valuable. Indeed, the component of the microbiota that can be cultured has similar colonization dynamics, biogeographical distribution and response to dietary perturbations as the full microbiota, when transplanted into gnotobiotic mice⁷⁷. A translational science pipeline is developing in which particular phylotypes and their interactions that are important for health or that contribute to disease can be identified on the basis of distribution patterns with disease. Furthermore, the biological attribute that may be driving these patterns can be predicted from the expression or prevalence of functional genes in whole communities and in genomes. Cultures and animal models can be used to test and verify these hypotheses before human application. The tools are now in place and although the path will be difficult, the direction is clearer. ■

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