

2

Microbiota

2.1 Trophic levels in macroecology

A classical macroecological community takes in energy from the sun and accumulates biomass in each trophic level (Fig. 2.1). The sun is the only energy source in such a classical system, and this energy is only captured by photosynthetic microorganisms and plants. Each trophic level of animals is added on, as a predator in the food chain. Maintaining overall biodiversity cannot replace the management of particular ecosystem functions [1].

Terms like microbiota, microflora (Box 1.2, Chapter 1), and diversity have been popular ever since the revival of studies on commensal microbes almost two decades ago. We, however, remain humble for how little we understand the ecology of the human microbiome. Besides scavenging on host molecules and undigested food, members of the human microbiome can be complete or partial chemoautotrophs, e.g., to survive with CO₂ fixation, anaerobic nitrate respiration. The exchange of molecules or electrons between microbes and with their host has also largely escaped direct measurements, waiting for further breakthroughs in technology.

2.2 Microbiome stability, diversity, and richness

By analogy to macroecological systems, a more diverse microbiome is likely more stable, which is presumably healthy. α -diversity within each sample, often according to the Shannon index, takes into account species richness and evenness, so that both the number of taxa and their relative abundance distribution are considered. Yet, low-abundance microbes may have a good niche or a steady source, to make us not worried about their extinction.

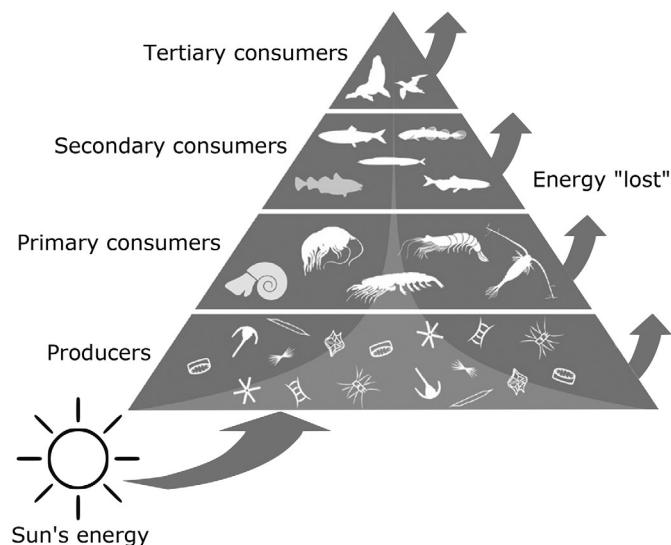


Fig. 2.1 An example of trophic levels in macroecology. Energy from the sun is converted into biomass by producers (plants). Each layer of consumers feeds on the previous layer. Credit: From Fig. 1 of Eddy T.D., Bernhardt J.R., Blanchard J.L., et al. *Energy flow through marine ecosystems: confronting transfer efficiency*. *Trends Ecol Evol* 2021;36(1):76–86. doi:10.1016/j.tree.2020.09.006. The sun and the arrows have been added to emphasize energy.

For bacteria, much of the functional characteristics are maintained down to the genus level ([Chapter 1](#), [Fig. 1.10](#), more on taxonomy in [Chapter 5](#)) [2]. It has been shown with soil and leaf bacteria that the family-level composition would converge under a single nutrient source, regardless of initial concentrations; the variation can be large at the species level, and rare species could co-exist [3]. While cooperation can be efficient, theoretical analyses indicate that competition, rather than cooperation, increases the stability of an ecosystem and is better for the host [4]. Nonspecific cross-feeding networks, instead of pairwise interactions, could help stabilize the competitive ecosystem [3]. Spatial structure that weakens the interaction between species also improves community stability [4]. Bacteriophages could appear to co-exist with their target bacteria due to spatial heterogeneity in the mouse gut [5].

In the human fecal microbiome from developed countries, having a more diverse fecal microbiome usually means having more *Firmicutes* instead of *Bacteroidetes* or *Actinobacteria*, or *Proteobacteria*. Genera in the *Firmicutes* phylum are responsible for many fermentation processes in the gut, and both nonspecific cross-feeding and spatial segregation may be involved. In preagricultural populations such as those in modern-day Amazon, and Hadza hunter-gathers, how *Spirchaetes* ([Box 2.1](#), [Fig. 2.2](#)), *Verrumicrobia*, or other taxa contribute to the higher

Box 2.1 *Treponema*, the preagricultural “enterotype”?

Prevotella (P. copri) has become known as the gut bacterium that is rarer than *Bacteroides* spp. in people in developed countries, while its relative abundance can be ~ 50% in some people in developing countries [110–112].

Treponema spp., however, are likely fundamental in the gut of hunter-gathers. *Treponema* spp. have also been found in the gut of nonhuman primates such as gorillas and baboons [113]. *Treponema* spp. were among the gut bacteria that showed seasonal fluctuations in humans and gorillas [114,115], consistent with increased consumption of dietary fibers. Xylan cellulose-degrading *Treponema* spp. are found in the hindgut of termites, whereas bacteria of the *Bacteroidetes* phylum are mainly responsible for the hydrolysis of xylan in the human gut and in the cow rumen [116]. Cooking followed by cooling slows the digestion of high-amylase starch (i.e., more resistant starch), and high-amylase starch enriches for *Treponema*, concomitant with reduction in the level of *Prevotella* [117,118].

The *Spirochaetes* phylum that contains the *Treponema* genus is present in many animals [113,119]. *Spirochaetes* appeared heritable in metagenomic data from the TwinsUK cohort, but given the fewer than 10 genes detected for this phylum in this modern cohort, we were not confident about the result that points to human genetic variations that help maintain this phylum in the gut [120].

The gut *Treponema* spp. are not the famous periodontal pathogen *T. denticola*, or the sexually transmitted *T. pallidum*. The species *T. succinifaciens* has been detected in multiple rural populations nowadays as well as in Mexico over 1000 years ago [96]. *T. succinifaciens* could not ferment amino acids, but is capable of fixing CO₂ to produce succinate [121]. Acetate is produced through reductive acetogenesis, from H₂ and CO₂. Feeding is a major contributor to the circadian rise in CO₂ level [122]. *Prevotella* spp. and *Bacteroides* spp. produce succinate in addition to propionate, and the succinate could be used for intestinal gluconeogenesis [123,124].

diversity in the gut microbiome remain unclear. Such closely interacting groups of people may have a lower β -diversity among their gut microbiome, i.e., more similar between individuals (Fig. 2.3, Box 2.2), young and old [6,7].

Richness at the gene or genus level only counts the number of genes or genera in each sample. An increase in richness could be due to microbes of low abundance in the sample, with minimum effect on the α -diversity (which takes into account evenness among taxa). And the detection of low abundance microbes depends on a sufficient amount of sequencing (Chapter 3).

For the gut (fecal) microbiome, inflammatory bowel diseases and obesity are known for having a lower α -diversity (Table 2.1). Colorectal cancer, and possibly other diseases with constipation, can show a higher richness in the gut microbiome (Table 2.1), containing low abundance bacteria from the mouth or other body sites. For the teeth and the vagina, a low diversity is considered healthy, in comparison to periodontitis and bacterial vaginosis, respectively (Table 2.1). Low diversity types

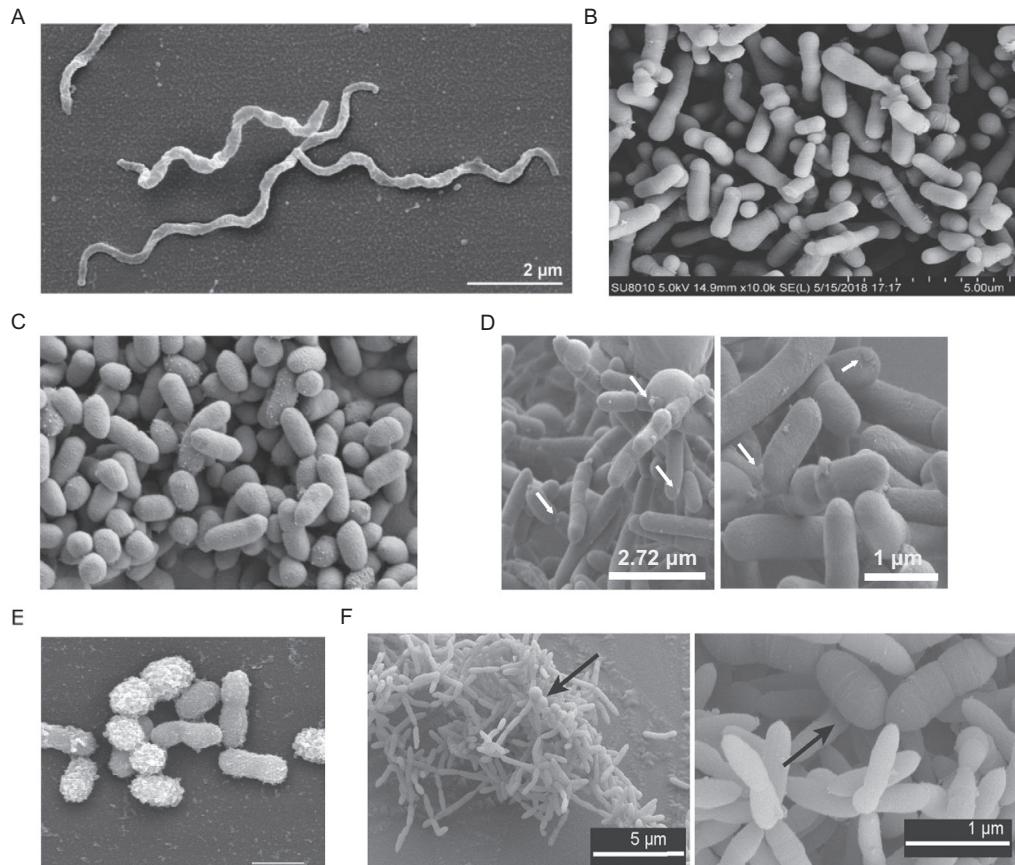


Fig. 2.2 Scanning electron micrographs showing the shape of some human commensal bacteria and archaea. (A) *Treponema succinifaciens*. (B) *Bifidobacterium animalis* subsp. *lactis*. (C) *Bacteroides thetaiotaomicron*. (D) *Faecalibacterium prausnitzii*. Arrows indicate cell wall extensions, like "swellings." (E) *Akkermansia muciniphila*. (F) Cocultures of *Christensenella minuta* and the archaea *Methanobrevibacter smithii*. Arrows indicate *Methanobrevibacter smithii*. Credit: (A) Han C, Gronow S, Teshima H, Lapidus A, Nolan M, Lucas S, et al. Complete genome sequence of *Treponema succinifaciens* type strain (6091). Stand Genomic Sci 2011;4:361–70. <https://doi.org/10.4056/sigs.1984594>. (B) Fig. 3C of Liu W, Chen M, Duo L, Wang J, Guo S, Sun H, et al. Characterization of potentially probiotic lactic acid bacteria and bifidobacteria isolated from human colostrum. J Dairy Sci 2020;103:4013–25. <https://doi.org/10.3168/jds.2019-17602>. (C) Fig 1A of Stentz R, Horn N, Cross K, et al. Cephalosporinases associated with outer membrane vesicles released by *Bacteroides* spp. protect gut pathogens and commensals against β -lactam antibiotics. J Antimicrob Chemother. 2015;70(3):701–709. <https://doi.org/10.1093/jac/dku466>. (D) Miquel S, Martín R, Rossi O, Bermúdez-Humarán L, Chatel J, Sokol H, et al. *Faecalibacterium prausnitzii* and human intestinal health. Curr Opin Microbiol 2013;16:255–61. <https://doi.org/10.1016/j.mib.2013.06.003>. (E) Derrien M, Belzer C, de Vos WM. *Akkermansia muciniphila* and its role in regulating host functions. Microb Pathog 2017;106:171–81. <https://doi.org/10.1016/j.micpath.2016.02.005>. (F) Ruaud A, Esquivel-Elizondo S, de la Cuesta-Zuluaga J, Waters JL, Angenent LT, Youngblut ND, et al. Syntrophy via interspecies H₂ transfer between *christensenella* and *methanobrevibacter* underlies their global cooccurrence in the human gut. MBio 2020;11. <https://doi.org/10.1128/mBio.03235-19>.

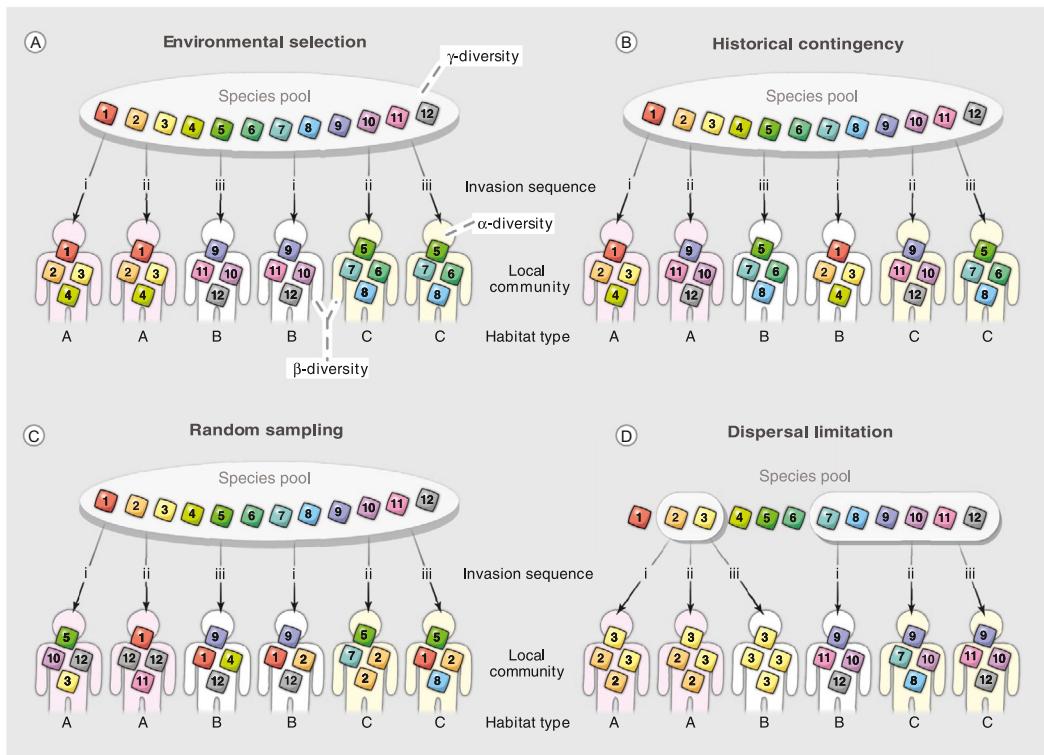


Fig. 2.3 Scenarios for assembly of the human microbiome. Alternative community assembly scenarios could give rise to the compositional variations observed in the human microbiota. Each panel shows the assembly of local communities in different habitat types from a pool of available species. (A–C) Each local community has access to all available colonists, but the order of invasion varies. In (A), local species composition is determined primarily by environmental selection: Regardless of invasion order, habitats with initially similar conditions select for similar assemblages. In (B), the opposite is true: Regardless of initial habitat conditions, historical contingencies (i.e., differences in the timing and order of species invasions) determine assemblage composition. In (C), neither habitat nor history matter: Local communities assemble via random draws from the species pool. (D) Dispersal barriers result in local communities that assemble from different species pools. For each of the pools, local communities may assemble as in (A), (B), or (C). The meaning of three different diversity measures is shown in (A): γ -diversity refers to the “regional” species pool (i.e., the total diversity of the local communities connected via dispersal); β -diversity refers to the differences between local communities (species turnover); and α -diversity refers to the diversity within a local community. Although multiple scenarios are likely to apply to any real-world setting, one may dominate. For example, differences between body habitats may be best explained by environmental selection, differences between siblings for the same habitat may be best explained by historical contingency, differences between monozygotic twins prior to weaning highlight the role of stochasticity, and differences between neonates born by cesarean section versus vaginal delivery are likely to be explained by dispersal limitation. Credit: Fig. 1 of Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. The application of ecological theory toward an understanding of the human microbiome. *Science* 2012;336:1255–62. <https://doi.org/10.1126/science.1224203>. Adapted by Costello, Alm from Chase JM. *Oecologia* 2003;136:489, and Fukami T. In: Verhoef HA, Morin PJ, editors. *Community ecology: processes, models, and applications*. Oxford: Oxford Univ. Press; 2010. p. 45–54.

Box 2.2 Some thoughts from Antoni van Leeuwenhoek on the individual differences in mucosal lining when studying ginger water

In a long letter written on October 9th, 1676 to the Royal Society of London, he included these interest thoughts, "Thus it may also occur that one body has greater interior heat or movement than another, or that some bowels are covered with soft thin mucus, others with thick and still others with hard or stiff mucus; consequently one person will be purged by very mild, another by strong and drastic medicines. But I am also ready to believe that there are extremely small, sharp particles which penetrate the mucus together with the food and are not operative till they enter the globules of the bowels, etc. You will excuse my once more speaking about things of which I have no knowledge, but please remember who and what I am" [8]. The letter was not fully published in the Royal Society version [9].

of the human vagina contain over 90% of a single *Lactobacillus* species. Only the human vagina and not other primates' vagina is so dominated by Lactobacilli [10], which is an intriguing evolutionary phenomenon. According to in vitro fermentation studies, monocultures could lead to significant deviations from a neutral pH, which would be attenuated by the presence of a community [3]. The low-diversity human vaginal has a pH < 4.5 (glycogen fermentation by Lactobacilli) during reproductive years (Chapter 1, Fig. 1.7), but a pH ~ 7 in postmenopausal women, who support a smaller number of vaginal bacterial cells [11-13].

2.3 De novo assembly of microbiota and robustness against invasions

Infant receives microbes from the mother through the fecal-oral route and through the reproductive tract, and then through breast milk, skin contact, etc. (Fig. 2.4, more in Chapter 8). Microbes, including spore-forming bacteria, present in the environment from family members and pets could also colonize the infant. They likely contribute to the increasing gut microbiome diversity after cessation of breastfeeding [35], a weaning response together with vitamin A and SCFAs (short-chain fatty acids) that also signal the development of the mucosal immune cells [36]. At least for mice, the introduction of solid foods in a critical time window before weaning reduces the risk of allergy [36,37]. In addition to historical contingency and succession of the microbes themselves (Fig. 2.3), babyhood is a key stage for immune development throughout the body, which has a major impact on which of the existing and newly acquired microbes are going to stay. Recent Metagenome-Genome-wide Association Studies (M-GWAS)

Table 2.1 Diseases and microbiome diversity.

Condition	Sample	Microbiome diversity compared to healthy controls	Microbiome richness compared to healthy controls	Reference
Obesity	Feces	Decreased	Lower in some ⁺	[14–16]
Crohn's disease	Mucosal biopsies; feces	Decreased	Decreased	[17–21]
Ulcerative colitis	Feces	Decreased in severe cases	Decreased in severe cases	[20,22]
Colorectal cancer	Rectal mucosal biopsies; feces	No difference	Increased*	[23,24]
Schizophrenia	Feces	Increased	Increased	[25]
Major depressive disorder	Feces	No difference	NA	[26]
Breast cancer	Feces	Increased in premenopausal cases	Increased in postmenopausal cases	[27]
Indirect [†] breastfeeding	Breast milk	Lower	Lower	[28]
Melanoma	Tumor	Not mentioned	Increased	[29]
Periodontal disease	Dental plaque	Increased	Increased	[30]
Bacterial vaginosis	Vaginal swab	Increased	Increased	[31]
Preterm birth	Vaginal swab	Increased	Increased	[32]
Male infertility	Semen	Increased	NA	[33]

This is not meant to be an exhaustive list, and only serves to show the range of possibilities. Microbiome diversity was compared at the species or genus level, unless otherwise noted. +, Gene richness from metagenomics confirmed by Human intestinal tract chip (HITChip) against ribosomal 16S DNA. *, Lower in [34] potentially due to cleansing for colonoscopy. †, i.e., pump instead of a direct manual expression of breast milk; Diversity according to inverse Simpson Index instead of Shannon index.

Credit: Huijue Jia.

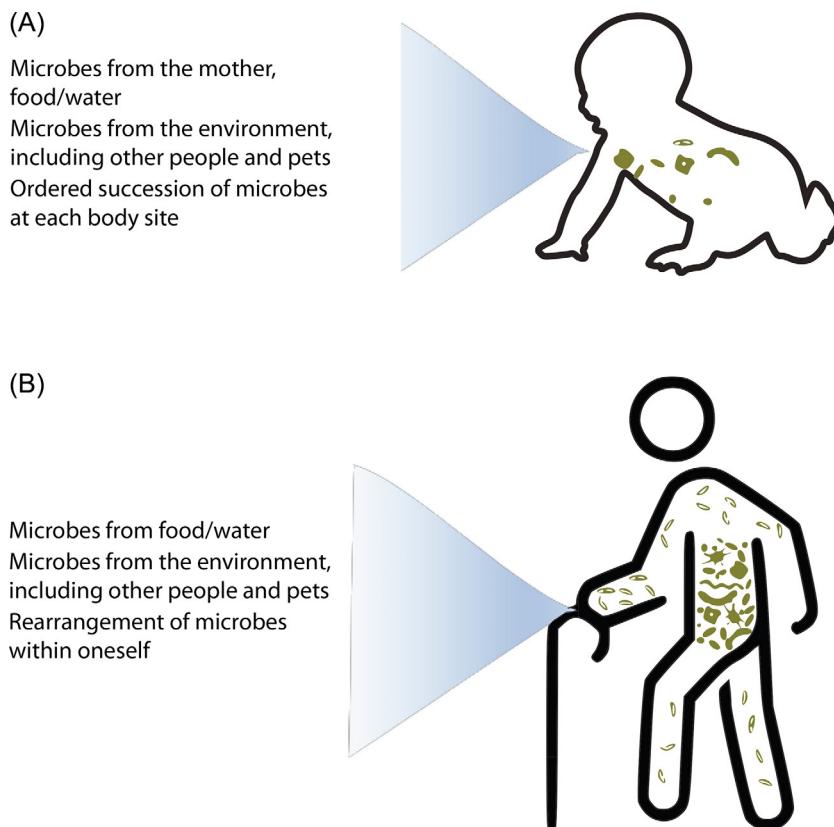


Fig. 2.4 Sources of microbes are selectively incorporated into ones' commensal community. (A) Infant. (B) Old age. Credit: Huijue Jia.

studies have not enrolled infants or children, but it would be a good idea to beware of the genetic influence (Box 2.3).

It appeared to be part of the healthy aging process to have an increasing richness in the gut microbiome, while dominant taxa became not as dominant [38–40]. Yet we do not know whether the numbers change due to a weaker immune system (e.g., fewer antibodies IgA as well as weaker peristalsis to keep the gut bacteria around, Section 2.6), or the microbes are more at different places throughout the aging human body (Fig. 2.4). The vaginal, gut, and oral microbiome show some correlation with hormones [12,41]. Waning of a dominant species could unmask low-abundance species. It will be a difficult decision for the aging immune system whether to try to eliminate an incoming microbe. Some of the fecal microbiome biomarkers for diseases such as colorectal cancer, liver and cardiovascular diseases may be of oral origin, and their abundance in the gut show some associations with human genetic variations [42].

Box 2.3 Prominent human genes associated with the fecal microbiome

LCT

Lactose-resistant individuals could have more *Bifidobacterium* if they ingest lactose, e.g., milk. The GWAS association between *LCT* and *Bifidobacterium* is the strongest signal in multiple European cohorts, while being undetectable in Chinese, and intermediate in a cohort from Israel [42,125]. Other bacteria, such as the Firmicutes *Negativibacillus*, and *Ruminococcus* spp. UBA3855, are also associated with *LCT* [126]. Serum level of the tryptophan metabolite indolepropionate has been reported to associate with nonpersistent *LCT*, possibly through *Bifidobacterium*. Indolepropionate associated with dietary fiber and reduced risk of Type 2 diabetes, and dietary fiber positively associated with Firmicutes such as *Butyrivibrio*, *Ruminococcus*, *Eubacterium*, and the Actinobacteria *Cellulomonas* in the same study [127].

Both historically and in modern days, various animals including camels and horses have served as sources of milk, all with different glycan structures and concentrations [128,129]. According to archeological evidence, consumption of milk products was well documented in Neolithic times, as early as 6000 years ago, 2000 years before the emergence of the lactose-persistent *LCT* allele [130]. The enzyme encoded by *LCT*, lactase phlorizin hydrolase (purified from sheep small intestine), has been shown to hydrolyze flavonoids and isoflavone that are common in plants [131]. Yet, spreading of the lactose-persistent *LCT* alleles is mostly attributed to pastoral populations [128].

ABO

Fecal microbial associations with the ABO loci also reached study-wide significance in European cohorts [126,132]. ABO histo-blood groups and *FUT2* secretor status associated with fecal relative abundances of *Bacteroides* spp. and *Faecalibacterium* spp., with implications for inflammatory bowel diseases and beyond [132]. Population differences in ABO blood group distribution can be rather large. For example, in malaria endemic regions blood group O can take up over 50% of the population [133,134]. Blood group B lowers susceptibility to *Vibrio cholera* infection [135]. The stomach cancer pathogen, *Helicobacter pylori* strains may differentially bind ABO blood groups [133,136].

2.4 Types of habitats for the skin microbiome

Although microbes are also found below the epidermis (Chapter 1, Fig. 1.6), we know too little about these, and are only talking about the skin microbiome on the surface of our body here. The major types of habitats include sebaceous (oily, waxy), moist, foot, and dry, corresponding to very different communities (Fig. 2.5). For both males and females, skin pH becomes less acidic with age [43,44]. High amounts of sebum secretion from adolescence until middle ages create a unique niche for *Cutibacterium acnes* (renamed from *Propionibacterium acnes*). So whenever we see *Cutibacterium acnes* DNA in the upper reproductive tract or in the brain [45–47], we get curious about what

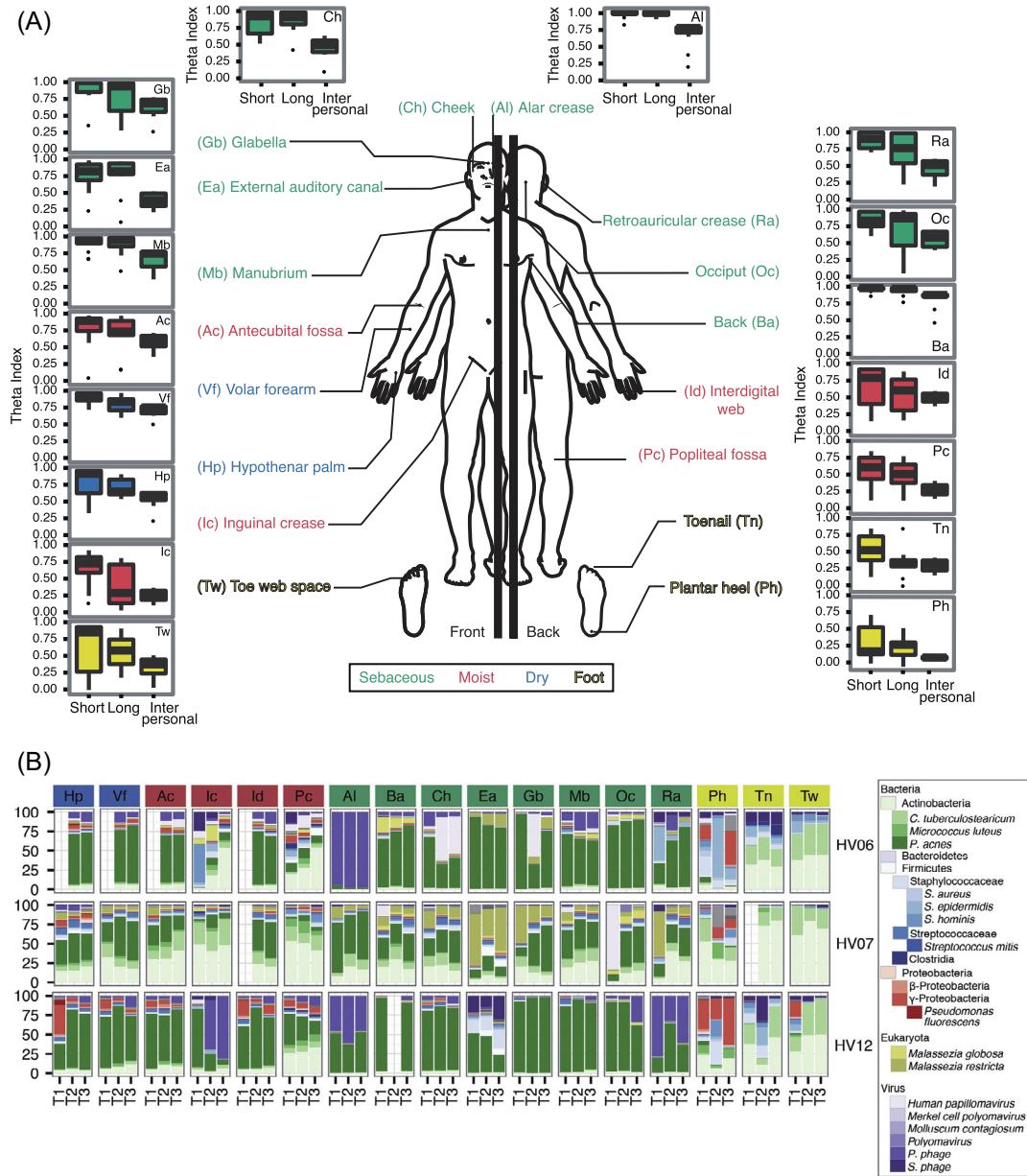


Fig. 2.5 Types of the skin microbiome and their temporal stability. (A) The 17 selected skin sites sampled in the study, and their location on the human body. These sites represent four microenvironments: sebaceous (green), dry (blue), and moist (red), and foot (black). For all sites, boxplots of Yue-Clayton theta indices calculate the similarity between samples in the time series, aggregated by the skin site characteristic. “Long” duration indicates > 1 year between samplings; “Short” duration averages a month. In comparison, “Interpersonal” values show the average distance between individuals. Black lines indicate median, boxes show first and third quartiles. Panels are color coded by site characteristic. For all intra- versus inter-individual comparisons, $P < .05$ except for the Id ($P = .24$); for all long versus short comparisons, $P > .05$ except for the Ic ($P = .04$). (B) Relative abundances of the most common skin bacteria, fungi, and viruses are shown for three representative individuals. T1, T2, and T3 indicate the order in the time series. Credit: From Fig. S1A and Fig. 2A of Oh J, Byrd AL, Park M, Kong HH, Segre JA. Temporal stability of the human skin microbiome. Cell 2016;165:854–66. <https://doi.org/10.1016/j.cell.2016.04.008>.

lipids it might be eating there, or maybe it has been killed by its phage. *Staphylococcus epidermidis*, and multiple species of *Corynebacterium* could be found in all types of skin sites. Besides phages, polyomavirus such as the potentially oncogenic Merkel cell polyomavirus, and papillomaviruses are also detected rather often [48]. The fungi *Malassezia* can be in hair follicles as well as on the epidermis [48]. Dry sites such as the forearms cannot sustain a high microbial biomass, and the skin microbiome tends to be diverse and changeable [49,50].

The fecal microbiome does not fit Hubbell's neutral theory, but some vaginal, skin, and respiratory microbial communities apparently do [51,52]. Neutral theory, in contrast to the more traditional niche theory, emphasizes random sampling from a source community (Fig. 2.3), followed by stochastic growth and death dynamics in competition for local space.

Chemicals stay along for longer than we think, possibly more so when they are not yet a good food for the microbiome. In a study which asked the two volunteers to avoid showering and application of hygiene or beauty products for 3 days, most of the known metabolites (no more news on whether some of the unknowns are microbial metabolites) annotated to ingredients of personal hygiene or beauty products [53]. For example, the surfactants C12 lauryl ether sulfate and cocoamidopropylbetaine, the sunscreen components avobenzone and octocrylene [53].

Worked sample 2.1

What do you think happen to the skin microbes during perspiration (sweating)?

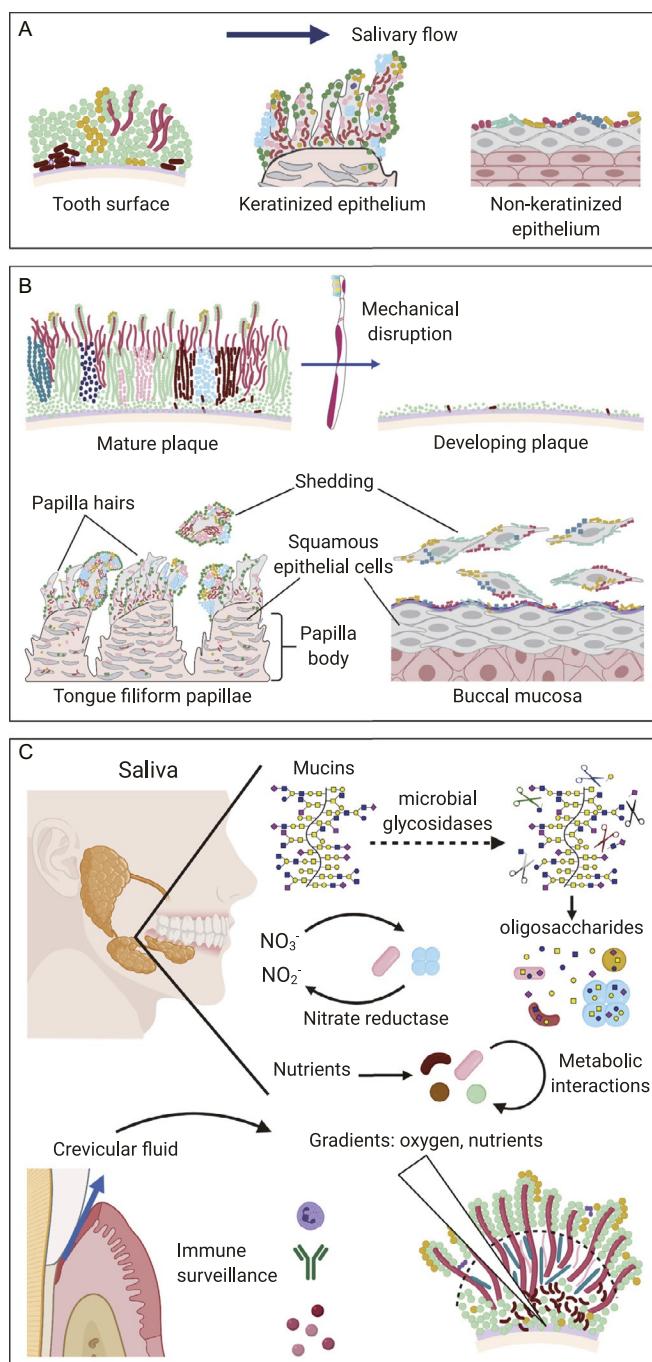
How might the assembly and maintenance of the skin microbiome be different for different body sites?

According to the taxonomic composition and functional capacity in the skin microbiome, what personal habits we may be able to guess back?

2.5 Forces shaping the oral microbiome

Microbes directly or indirectly adhere to the teeth, and mucosal or keratinized epithelia in the mouth (Fig. 2.6). They are challenged daily with oral hygiene, eating, drinking, and perhaps many other processes (as we heard from Antoni van Leeuwenhoek already in Chapter 1, Box 1.1). Yet, despite daily and monthly (menstrual cycle) fluctuations in microbial biomass, the composition of the oral microbiome is surprisingly stable for each individual [54]. In the absence of fermentable carbohydrates, *Streptococcus* of the *Streptococcus mitis* group bind saliva-coated/protected teeth; *Streptococcus* of the *Streptococcus mutans* group ferments sugar and produce lactic acid, pioneering the

Fig. 2.6 Selective forces for microbes in the oral cavity. (A) Flow and adhesion. (B) Shedding and colonization. (C) Host and microbe. The host and the microbial community exert mutual reciprocal influences on each other through binding interactions, immune surveillance, and gradients of nutrients and solutes. The host secretes salivary mucins, which are complex glycoproteins that support the growth of mixed syntrophic communities of microbes that possess glycosidases capable of releasing oligosaccharides from mucins. Secretion of nitrate and other nutrients into saliva by the host, and release of crevicular fluid from the gingival crevice into the mouth, could also serve to foster the growth of particular microbes, while immune surveillance limits the growth of others. Microbial metabolism, in turn, can generate strongly localized gradients of oxygen and nutrients. The positioning of microbes at favorable locations within these gradients can lead to metabolic interaction and spatial structure within the microbial community. Credit: From Fig. 4 of Mark Welch JL, Ramírez-Puebla ST, Borisoff GG. Oral microbiome geography: micron-scale habitat and niche. *Cell Host Microbe* 2020;28:160–68. <https://doi.org/10.1016/j.chom.2020.07.009>.



establishment of an acidic environment in the development of dental caries [55] (more on other diseases in [Chapter 4](#)). Different salivary glands are responsible for different ingredients in saliva, e.g., parotid saliva contains a large amount of amylase, lesser amounts of lysozyme, and no mucins, while submandibular/sublingual saliva is rich in mucins, cystatins, and lysozyme, but has less amylase [56]. Antibody-producing plasma cells activated in other mucosal sites are recirculated to lymphoid tissues (e.g., tonsils) in the oral cavity, and the IgA (Immunoglobulin A) and IgG (Immunoglobulin G) secreted can bind many oral bacteria. Chewing could induce gingiva-resident T helper 17 (Th17) cells [57]. Other than subgingival plaques from periodontal pockets, the oral microbiome mostly contains aerobes or facultative anaerobes (so it is probably a good idea for the bacteria to go to the lungs when the mouths are shut during sleeping [58]).

Direct visualization of intact structures from the tongue dorsum revealed bacteria domains that expand or shrink relative to their neighbors as they grow more layers ([Fig. 2.7](#)) [59]. Differences in growth rates, more shedding into the flow, or attacks by host defense systems could lead to such local dynamics. Remember the number question from [Chapter 1](#), and we hope to have a detailed census for the microbial habitats in the mouth. Other than a major increase or decrease in the ecological niche for a given microbe ([Figs. 2.8 and 2.9](#)), scuffles with neighbors would maintain long-term stability in the relative abundances (proportions).

2.6 A stable gut microbiome

2.6.1 Adhesion

The fecal microbiome is not an *in situ* community, so it takes some imagination and simulations to get a sense of how everyone can stay in the gut ([Figs. 2.10 and 2.11](#)) and show up in good proportions in feces every day. Adhesion to the favorite mucin glycan, holding onto glycans or other macromolecules from neighboring microbes, or getting other help from the human host?

Immunoglobulin A (IgA), the major type of mucosal antibodies traditionally known for its ability to remove pathogens by forming large aggregates, often binds weakly to many commensal bacteria, which helps retain them in the gut [60–63], and potentially in the oral cavity if it is not cleaved by *Streptococcus* protease [64]. High-avidity IgA (antibody) against *Salmonella enterica* subspecies enterica serovar Typhimurium prevented separation of daughter cells after division [65]. I would guess that aggregation by IgA depends on a relatively high fraction of dividing bacteria. IgA secretion takes place in the small intestine, but IgA could be precipitated from fecal samples, along with

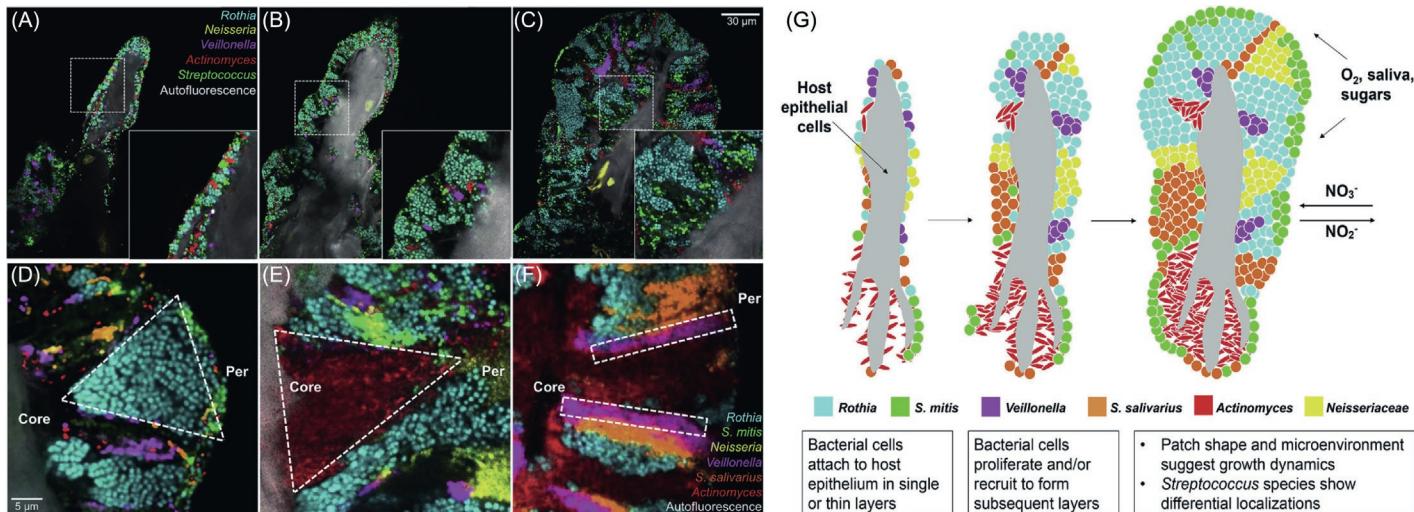


Fig. 2.7 Examples of microbiota dynamics in the tongue dorsum inferred from the difference in width between the inside (core) and the outer (periphery) layers. Host epithelial cells (autofluorescence) are surrounded by a growing number of oral bacterial cells. (A–F) Gradations in biofilm thickness and shape of clonal domains suggest biofilm growth and selective advantage. (A) A thin biofilm is composed of small clusters of cells from each bacterial taxon. (B) A thicker biofilm showing the expansion of the facultative anaerobe *Rothia* and the beginnings of expansion of anaerobes *Veillonella* and *Actinomyces*. (C) A mature structure showing well-defined domains. (D) Increasing width of a clonal domain toward the perimeter suggests a selective advantage toward the periphery. (E) Decreasing width toward the perimeter suggests a disadvantage at the periphery or selective advantage in the interior. (F) Constant width suggests neither selective advantage nor disadvantage with respect to neighboring taxa. (G) Inferred development of the tongue dorsum consortia. The study only focused on this kind of intact shape. The subjects sampled themselves under supervision by gently scraping the dorsal surface of the tongue from back to front using a ridged plastic tongue scraper. In this model, bacterial cells colonize host epithelial cells sparsely. As bacteria proliferate, layers of cells appear in a patch-like structure. Some *Streptococcus mitis* cells form a thin coat on the surface. Domain formation is dependent on neighbors and the microenvironment. Some nutrients may be gained from host epithelial material and other nutrients, O_2 , and NO_3^- from the oral cavity by saliva. Credit: From Fig. 6 and Fig. 7 of Wilbert SA, Mark Welch JL, Borisy GG. Spatial ecology of the human tongue dorsum microbiome. Cell Rep 2020;30:4003–15.e3. <https://doi.org/10.1016/j.celrep.2020.02.097>.

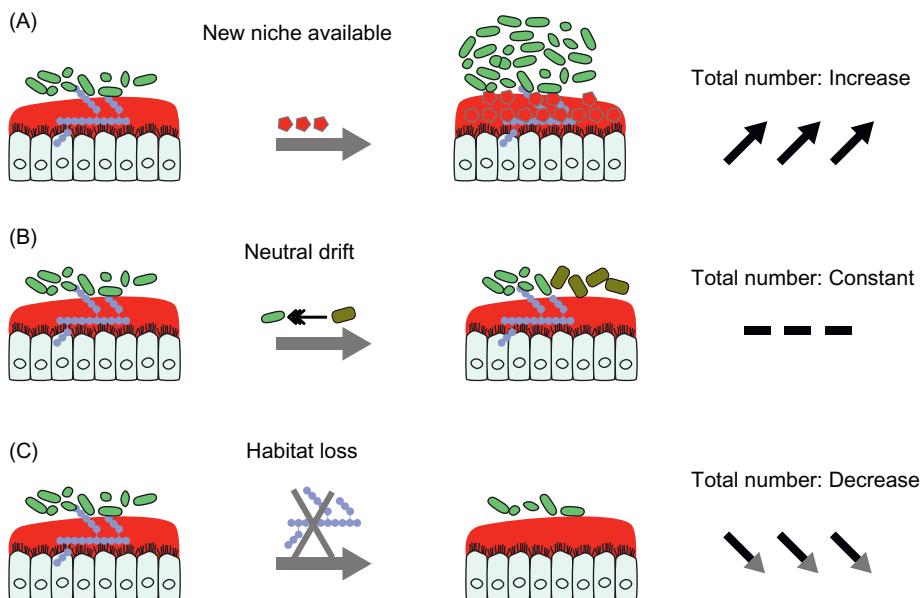


Fig. 2.8 Changes in the abundance of bacteria from an ecological point of view. (A) Availability of previously nonexistent or inaccessible niche. (B) The total capacity does not change, but replacement by other bacteria nearby or from a source community led to dynamics in the number of bacteria. (C) Habitat for bacteria is lost due to remodeling by the host or by other microbes. Competition for food sources or competition for attachment could also be reasons. Credit: Huijue Jia.

intestinal bacteria including species of the *Firmicutes* phylum and *Bifidobacterium* of the *Actinobacteria* phylum [61,63,66]. Bacteria such as *Escherichia coli* are overrepresented in feces from individuals with IgA deficiency, concomitant with the underrepresentation of bacteria such as the *Lachnospiraceae* and *Ruminococcaceae* families [63,67,68]. Lactobacillaceae and Enterobacteriaceae dominate the small intestine [69], and mice experiments have shown small intestinal IgA binding to Enterobacteriaceae and other *Proteobacteria* (e.g., *Acinetobacter*), and *Bifidobacterium* [61].

Note that the IgAs are also a source of complex glycan that can be foraged by bacteria such as *Bacteroides* spp. [70], and the altered glycan might then influence IgA binding to other bacteria.

2.6.2 Peristalsis

Peristaltic mixing not only sets the transit time, but also makes sure that a portion of the microbes stays on the proximal side of the squish (Figs. 2.11 and 2.12). One human peculiarity is that the ascending colon is really vertical as we stand. According to simulation, the ascending colon is the major bioreactor (Fig. 2.12), as remnants of food

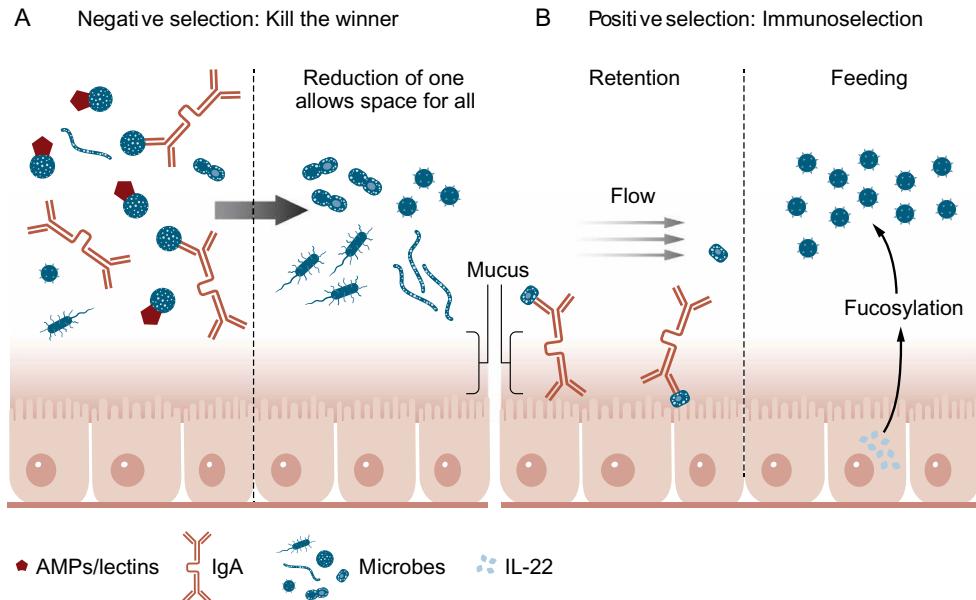


Fig. 2.9 Immunological control of microbial diversity. (A) Negative selection: kill the winner. Studies in macroecosystems demonstrate that predation increases biodiversity by serving to control particularly abundant and well-adapted species. Controlling the population growth of these species can liberate niches and resources that allow other organisms to thrive. The immune system can control gut microbial communities via negative selection through multiple mechanisms. AMPs can mediate direct killing, and IgA can cause aggregation and elimination of specific organisms, as well as downregulation of proteins involved in bacterial motility, invasion, or toxicity, such as flagellin. (B) Positive selection: immunoselection. The immune system might also serve to select particular organisms for residence within the gut. In addition to mediating negative selection, IgA may also help retain specific bacterial taxa by promoting retention of slow-growing species in the mucus or by enabling residence in protected niches, such as the colonic crypt. The immune system can also support the survival and growth of specific taxa by inducing luminal deposition of specific nutrients; for example, interleukin-22 (IL-22) induces epithelial fucosylation, which nourishes particular beneficial bacterial taxa. Credit: Fig. 3 of Round JL, Palm NW. Causal effects of the microbiota on immune-mediated diseases. *Sci Immunol* 2018;3:eao1603. <https://doi.org/10.1126/sciimmunol. eao1603>.

from the small intestine enter the colon [71]. Many animals, including mice, have a large cecum that keeps microbes. It is speculated that microbes from the human appendix might also help recover the microbiota after major disturbances [72]. Yet, some of the microbes may come from further up in the digestive tract, or be present locally on the colonic mucosa (Fig. 2.11C, Chapter 1, Fig. 1.9), and the lymphatic system might carry some microbes from other body sites. Remnants of food are wrapped with mucus (heavily glycosylated proteins that constitute the mucus hydrogel) in the ascending colon and more mucus toward the end of the colon [73]. There are subtle differences between

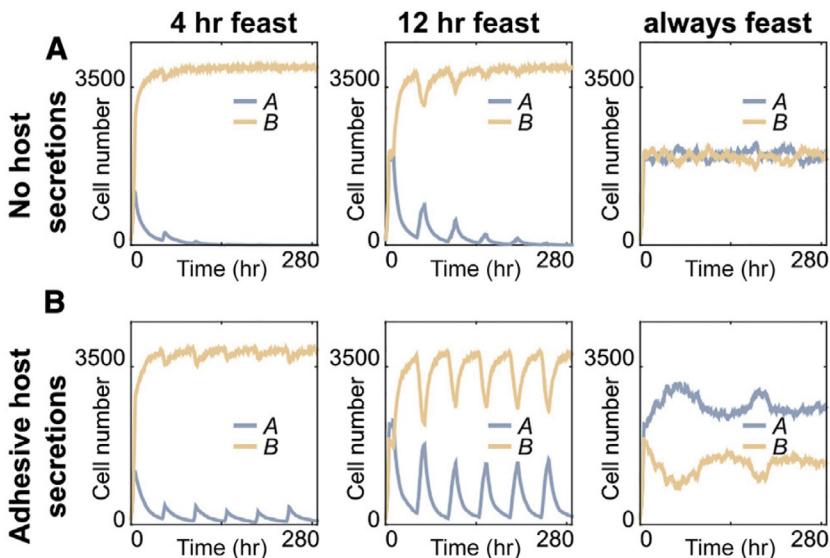


Fig. 2.10 Adhesive host secretions promote microbiota stability in fluctuating environments. Adhesive host secretions prevent the loss of specialist species whose host is only available periodically. McLoughlin et al. simulated two genotypes, a generalist species B that can consume lumen nutrients, which are available at all times. A second nutrient is only available periodically and is the exclusive nutrient source for specialist species A. Simulation results from 4 and 12 h feast durations per 48 h period are shown; “always feast” is a control where the second nutrient is also available at all times. Without adhesive host secretions, genotype A is lost from the community if “feast” periods are rare and short. When the host secretes an adhesion-promoting factor that creates a horizontal region in which genotype A resists displacement better than genotype B, both genotypes can be maintained even when the environment fluctuates. The variability in the “always feast” condition is due to stochastic fluctuations in population size. Our results are robust to changes in duration and periodicity of feasts. Credit: Fig. 3 of McLoughlin K, Schluter J, Rakoff-Nahoum S, Smith AL, Foster KR. Host selection of microbiota via differential adhesion. *Cell Host Microbe* 2016;1–10. <https://doi.org/10.1016/j.chom.2016.02.021>.

the epithelial side and the lumen. *Bacteroides fragilis* has been shown to express more of a sulfatase and glycosyl hydrolase when in mucin or in tissue compared to in the lumen [74]. *Bacteroides thetaiotaomicron* increased N-linked glycosidase transcription in mucus, while *Escherichia coli* showed a difference in the expression of iron acquisition pathway [75].

According to simulation with the standard western diet, microbes would likely spread out in order along the proximal colon, Bacteroidetes (*Bacteroides thetaiotaomicron*) before Firmicutes (*Eubacterium rectale*) [71]. Methane is mainly produced in the distal colon (Box 2.4) [76]. If the diet contains more fiber, the fermentation process could

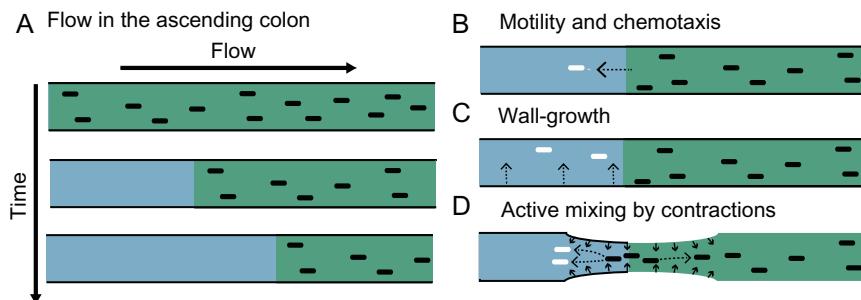


Fig. 2.11 Washout by flow and possible counteracting factors. The movement of luminal content down the colon has a mean velocity of $\sim 20 \mu\text{m/s}$ in the proximal colon of adult humans. (A) Flow alone leads to emptying of channel content over time. Additional factors are required to counteract this washout and help to maintain a stable bacterial density over time. Such factors may include: (B) active motility by bacteria to swim toward the nutrient source; (C) wall growth; and (D) peristaltic mixing, with backflow generated by contractions of colonic walls. Credit: Fig. 1 of Cremer J, Segota I, Yang C, Arnoldini M, Sauls JT, Zhang Z, et al. Effect of flow and peristaltic mixing on bacterial growth in a gut-like channel. Proc Natl Acad Sci U S A 2016;113:11414–419. <https://doi.org/10.1073/pnas.1601306113>.

go all the way from the ascending colon to the rectum (Chapter 8, Table 8.4) [77]. Interestingly, mice fed with a chow diet have circadian dynamics of *Bacteroidetes*, *Firmicutes*, and *Verrumicrobia*, without limiting the time of food availability [78] (Box 2.4). Although microbial growth rates in feces have been studied in metagenomic data of premature infants, *Citrobacter rodentium* infection, inflammatory bowel diseases (IBD), and diabetes patients [79–81], the author tends to believe that in healthy adults, much of the community is not growing, by the time the feces reaches the rectum. The *Firmicutes* (together with the methanogen which consumes H_2) perhaps more readily take advantage of each feeding (Fig. 2.13) [78,82]. The traditional agriculture use of human and animal feces might be a more fulfilling cycle for the microbes, $< 100 \text{ g/day}$ from a modern human [83] (Box 2.5).

2.7 “Enterotypes” and the Serengeti rules?

The controversial concept of “enterotypes” is essentially unsupervised clustering of fecal microbiome data. The statistically optimal number of clusters depends on the cohort [85]. For urban East Asian populations, and for less studied rural populations such as those in Africa, the *Firmicutes* (including the leanness-associated *Christensenella*) and methanogenic archaea (Box 2.4) (*Methanobrevibacter smithii*, Fig. 2.2) fermentation chain appear not as substantial as for northern European populations [86–88], and the colonic pH is likely not as acidic [69,71] (Fig. 2.12D). *Firmicutes*

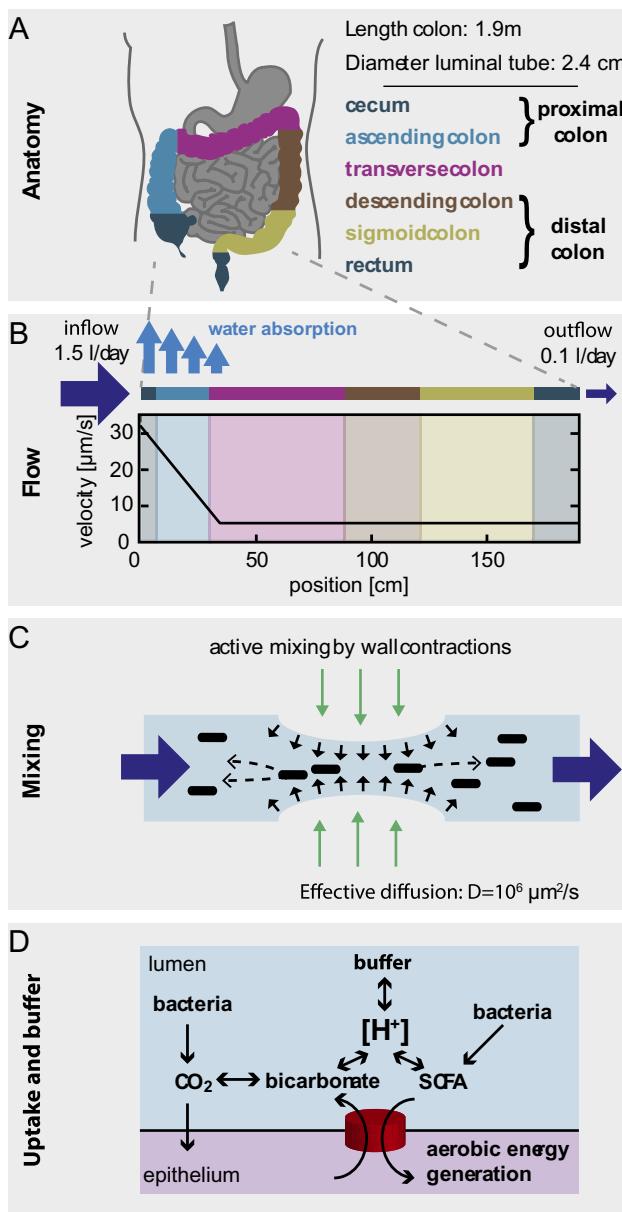


Fig. 2.12 Physiological parameters of the human colon. (A) Anatomical dimensions. Based on measurements of human colonic anatomy during the autopsy, X-ray and CT imaging using contrast media, and magnetic resonance tomography imaging, Cremer et al. derived operational numbers for the lengths, surface areas, and luminal diameters of the different colonic segments. (B) Luminal flow. About 1.5 L of fluid reaches the proximal colon every day. The epithelium absorbs most of this volume, and only 100–200 mL/day exit the colon as feces. This continuous water uptake along the colon leads to a steep gradient in luminal flow rates. We calculated an average flow velocity of about 30 μm/s at the beginning of the colon that drops to about 5 μm/s by the end of the ascending colon. (C) Mixing of luminal contents. Contractions of the intestinal walls can generate local mixing. Based on data on the mixing of radiolabeled dyes in the large intestine, we derive that the measured distributions can be approximated by an effective diffusion constant of $D \sim 10^6 \mu\text{m}^2/\text{s}$, a value orders of magnitude higher than molecular diffusion. (D) Epithelial SCFA uptake, bicarbonate excretion, and buffer chemistry. Bacterial fermentation leads to the production of SCFAs, which are taken up by the gut epithelium and contribute to the host's energy intake. SCFA uptake is coupled to the excretion of bicarbonate, which, in equilibrium with CO_2 and other luminal components, buffers the luminal acidity ($\text{pH} = -\log[\text{H}^+]$). All calculations are based on the measured characteristics of epithelial transporters and the buffer capacity of the lumen. Credit: Fig. 2 of Cremer J, Arnaldini M, Hwa T. Effect of water flow and chemical environment on microbiota growth and composition in the human colon. Proc Natl Acad Sci U S A 2017;114:6438–43. <https://doi.org/10.1073/pnas.1619598114>.

of the Clostridiales order suppress intestinal vitamin A production in mice, possibly finishing up a weaning reaction that induces T regulatory cells (Treg), and fecal Clostridiales species correlated with plasma vitamin A in adult humans [36,41,89,90]. But we are not aware of studies that link population differences in vitamin A levels to gut microbiome composition. The abundance of *Firmicutes* also relates to

Box 2.4 Methanogenic archaea in the human microbiome (metabolism of hydrogen, and TMAO)

Methanobrevibacter smithii reduces enteric hydrogen produced by fermentation into methane (e.g., *Christensenella* in Fig. 2.2). Its abundance is significantly higher in Europeans than in East Asians [86]. Methane production mainly takes place in the distal colon and has been linked to constituting conditions of irritable bowel disease (IBD), colon cancer, etc. [76].

Methanobrevibacter is also found in ruminants and other animals [119,137]. *Methanobrevibacter ruminantium* M1 from the cow rumen encodes an adhesin that binds hydrogen-producing microorganisms such as the rumen protozoa (including the genera *Epidinium caudatum* and *Entodinium* spp.), the bacterium *Butyrivibrio proteoclasticus* [138].

Methanomassiliicoccus luminyensis, a methanogen not as famous as *Methanobrevibacter smithii*, has been shown to utilize hydrogen to reduce methyl compounds including trimethylamine (TMA) [139]. This TMA-depleting function could potentially decrease the fishy odor (can also be due to cadaverine or putrescine [140]) in bacterial vaginosis (BV), or prevent trimethylamine N-oxide (TMAO)-facilitated atherosclerosis [141,142]. However, TMA and TMAO are high in sea fish [143]; TMA is excreted from urine and sweat [144]; Adult male mice fed with an indirect TMAO inhibitor had fewer victories in social dominance tests regardless of social rank [145], implying its evolutionary advantage in reproductive years. In mice, repression of the trimethylamine oxidation enzyme flavin-containing monooxygenase 3 (FMO3) in the liver is male-specific, and TMA is recognized by the olfactory receptor, trace amine-associated receptor 5 (TAAR5) as a species-specific attraction signal [146,147].

the feeding cycle (Fig. 2.13). According to the *gyrB* gene (DNA gyrase subunit B) in *Bacteroidaceae*, *Bifidobacteriaceae*, and the *Firmicute* family Lachnospiraceae, co-speciation pattern in humans, chimpanzees and gorillas were found to be strongest for *Bacteroidaceae*, followed by *Bifidobacteriaceae* (Box 2.3, yet, the not-so-healthy vaginal bacterium *Gardnerella vaginalis* (now *Bifidobacterium vaginalis*), oral-gut *Bifidobacterium dentium* also belong to this family), and weakest in Lachnospiraceae [91]. These results are also consistent with interindividual transmission of spore-forming *Firmicutes* [86,92,93], instead of more vertical transmission of *Prevotella*, *Bacteroides*, and *Bifidobacterium*.

In analogy to animals on the Serengeti savannah [94], the most abundant taxa are at the bottom of the ecosystem (where there is the largest amount of energy harvested from the sun, Fig. 2.1). The *Prevotella* spp. vs *Bacteroides* spp. patterns are visible in East Asian samples [95] (Fig. 2.14; Not *Treponema*, Box 2.1). Fecal samples from Mexico both now and over 1000 years ago (coprolites) showed more *P. copri* than samples from the United States and from Europe [96,97]. Many of the well-established vaccines were more effective in *Bacteroides*-dominated than in *Prevotella*-dominated populations [98]; Laboratory mice were also *Bacteroides* and *Firmicutes*-dominated, if a *Prevotella*-dominated model is not chosen intentionally [99,100].

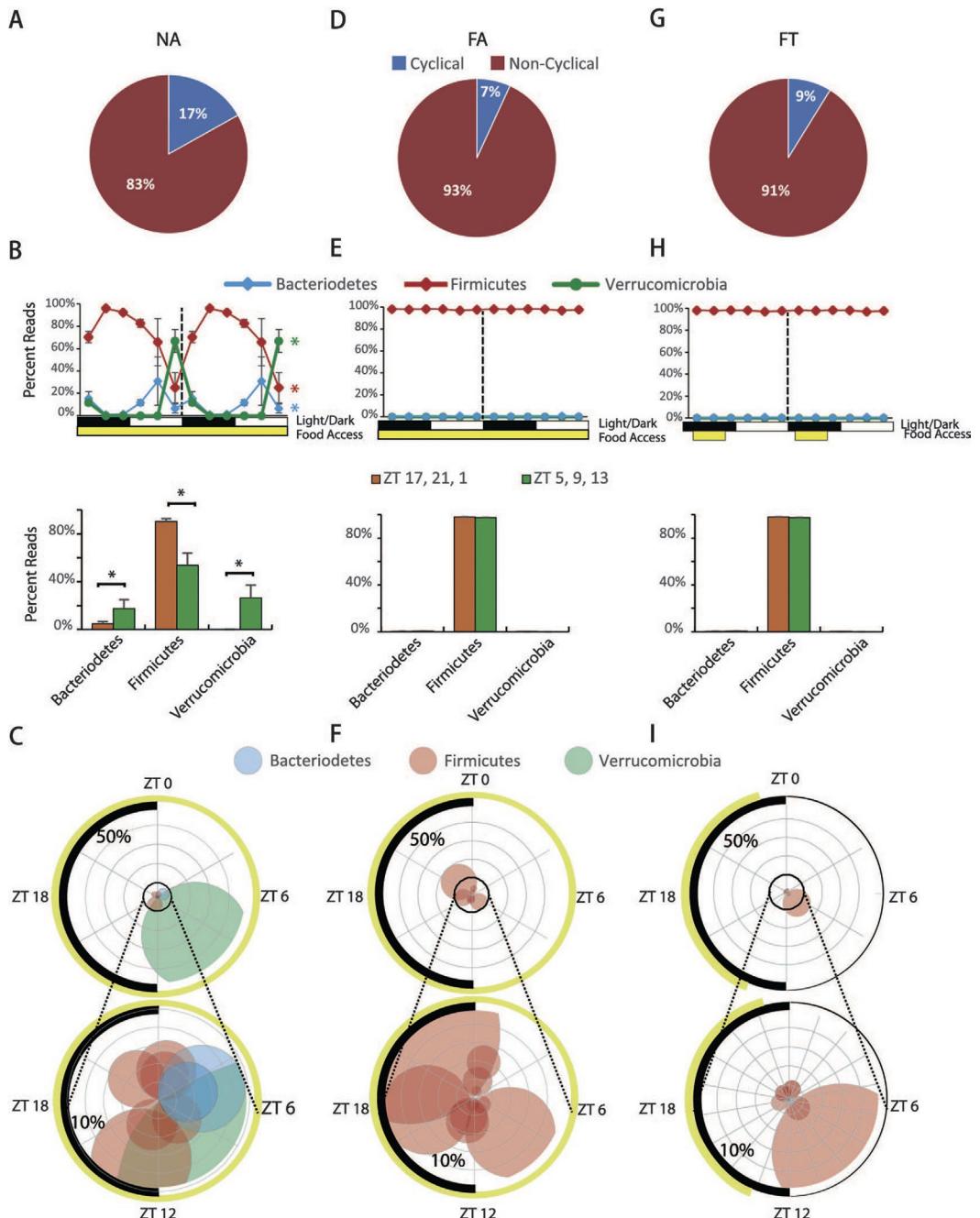


Fig. 2.13 Diurnal rhythms of gut microbiome phyla in mice from different feeding conditions. NA mice had ad libitum access to normal chow. FA mice had ad libitum access to HFD. FT mice had 8-h access (ZT 13–21) to HFD. (A) Pie chart showing the percentage of cycling and noncycling OTUs (across all conditions) in NA mice ($n=18$). (B) Upper double-plot line graph—where the second cycle is a duplicate of the first cycle following the dashed line—shows

(Continued)

Fig. 2.13, cont'd the average percent read (\pm SEM) of the three most predominant phyla at each time point ($n=3$ per time point). Black and white boxes indicate light off and light on, respectively. The yellow box shows when mice had access to food. Colored asterisks at the end of lines in the line graph show which phyla were cycling based on JTK analysis (that is $ADJ.P < 0.05$ and $BH.Q. < 0.05$). Since it takes > 1 h for a food bolus to reach the cecum [84], lower bar graphs show the average percent reads (\pm SEM, $n=9$) for the dark/active feeding phase (ZT 17, 21, and 1), and the light/inactive fasting phase (ZT 5, 9, and 13) * $P < .05$. (C) The top 10 OTUs (based on percent reads) are depicted in a polar plot. The radian indicates the phase of the OTU's peak, the distance from the center is the average percent read across all time points, and the radius of each point indicates the amplitude of cycling. The colors of the circles indicate the phylum of the OTU: Firmicutes (pink), Bacteroidetes (blue), and Verrucomicrobia (green). The black arc on the left side of the plot indicates the light/dark cycle. The yellow arc depicts access to food. The bottom polar plot shows a magnified view of the inner ring (10%) of the top polar plot. These descriptions also apply to panels for FA mice (D–F) and FT mice (G–I). Credit: From Fig. 2 of Zarrinpar A, Chaix A, Yoosheph S, Panda S. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. Cell Metab 2014;20:1006–17. <https://doi.org/10.1016/j.cmet.2014.11.008>.

People with higher relative abundances of *Bacteroides* tend to be free of worms and protists [101–103]. Human genetics also contributes to abundance differences in *Prevotella* spp. vs *Bacteroides* spp. [42,104]. Instead of harvesting energy from the sun and build up the trophic levels, the human microbiome, if not just living there and being a chemoautotroph, harvest energy from host molecules or from feeds such as food, drink, and medication. *Bacteroides* spp., especially *Bacteroides thetaiotaomicron*, are known for extracellular digestion of complex glycans from mucin, IgA, and from the diet [70,105–108]. Before these chopped-up glycans are transported for further metabolism intracellularly, there could be opportunities for scavenging by other bacteria, including many *Firmicutes*. *Bacteroides* spp. stayed together in a 15-species mix gavaged into gnotobiotic mice, and image analyses indicated a positive correlation between the numbers of *Bacteroides cellulolyticus* and *Bacteroides vulgatus*, whereas the numbers of *Bacteroides cellulolyticus* and *Ruminococcus torques* showed a negative correlation in $19 \times 19 \mu\text{m}$ grid squares [109]. Extracellular digestion of inulin has been shown to increase the fitness of *Bacteroides ovatus* owing to reciprocal benefits when it feeds other gut species such as *Bacteroides vulgatus* [108]. We are currently limited by technology to examine such local cooperation between members of the human gut microbiome. Simple communities from insects provide a nice example of the genomic and metabolic complementarity in shaping the spatial organization of a microbial community (Fig. 2.15).

Worked sample 2.2

When do you think we will be able to visualize all the microbes in a well-preserved sample, along with their complete genomic or transcriptomic information?

What questions do you think can be addressed with such information?

Box 2.5 Circadian rhythm in the gut microbiome and their products

Lack of microbiota (i.e., germfree mice) induces a prediabetic state due to overproduction of corticosterone in the ileum [148]. The nuclear receptors ROR α activator and RevErb α repressor together generate a circadian rhythm in the intestinal epithelial expression of TLRs (Toll-like receptors). Microbes signal through TLRs to induce rhythmic JNK and IKK β activities, which prevents RevErb α activation by PPAR α . The arrhythmic gut microbiome has recently been shown in T2D and obese patients [149].

If the *Verrucomicrobia* in Fig. 2.13 is indeed the mucin-degrading *Akkermansia*, a probable explanation for its rise toward the end of the day (remember that mice are active at night) for mice on a chow diet would be that, after consuming everything from food, the gut microbiome sustains on host mucin. The mucin instead of diet would not be so favorable for maintaining a large population of *Firmicutes* before feeding time [78,149,150]. Butyrate peaked immediately after sleeping [151]. The acetate or propionate-producing *Akkermansia muciniphila* increases thermogenesis but decreased with colder temperature [152,153]. The bacterium is well adapted to the loose outer mucus layer in the gut lining and can utilize nanomolar concentrations of oxygen in the presence of carbon dioxide [154]. The oxygen level in the body is also circadian [122,155]. *A. muciniphila* was more abundant in people with conditions such as Alzheimer's disease, atherosclerotic cardiovascular disease and schizophrenia [25,156–158].

Fecal samples from volunteers are typically taken in the morning, without or with breakfast. A sampling of the gut microbiome many times a day in human volunteers has been technically challenging [159]. The above-mentioned T2D study carefully recorded the time of defecation, which explained some variations in the gut microbiome [149]. Fecal bacteria that showed altered circadian oscillations in the T2D patients included *Akkermansia*, *Roseburia*, *Bifidobacterium longum*, *Faecalibacterium prausnitzii*, etc.

Histone deacetylase 3 (HDAC3) is rhythmically expressed in epithelial cells of the small intestine [160]. HDAC3 drives the expression of genes in nutrient transport and lipid metabolism, and activates estrogen-related receptor α , which promotes lipid absorption. Histone methylation in intestinal epithelial cells also showed rhythmicity [78].

Polyamines (putrescine, spermidine, and spermine) contribute to the normal circadian clock; the cycle lengthens with age with declining polyamine levels [161]. A diet deficient in polyamines did impact the diurnal oscillation of the liver transcriptome [78].

Besides, mice fed with a high-fat diet showed hydrogen sulfite (H_2S) production from dsrAB (dissimilatory sulfite reductase) after feeding, which would be inflammatory in the gut [151].

The lung microbiome may also be circadian, with more microbial migration from the mouth overnight, and slowed immigration and enhanced elimination during the day [58].

Independent of a host animal, some microbes may encode their own clock genes. Some *Cyanobacteria* have the complete set of *KaiA*, *KaiB*, *KaiC* genes for circadian oscillations; Clock systems with only *KaiB* and *KaiC*, or only *KaiC* also have time-keeping functions and have been found in some *Proteobacteria* and *Cyanobacteria* [162]. The *Proteobacteria* species *Klebsiella aerogenes* (used to be *Enterobacter aerogenes*) has a circadian pattern of swarming and motility in vitro, which showed enhanced robustness after the addition of the hormone melatonin [163,164]. Besides, the rhythm was impacted by temperature. This illustrates likely modes of receiving circadian clues from the host animal. *Bacillus subtilis* does not encode KaiABC, but has photoreceptors and Per-Arnt-Sim (PAS) domains; *Bacillus subtilis* shows a 24-h clock controlled by light or temperature [165].

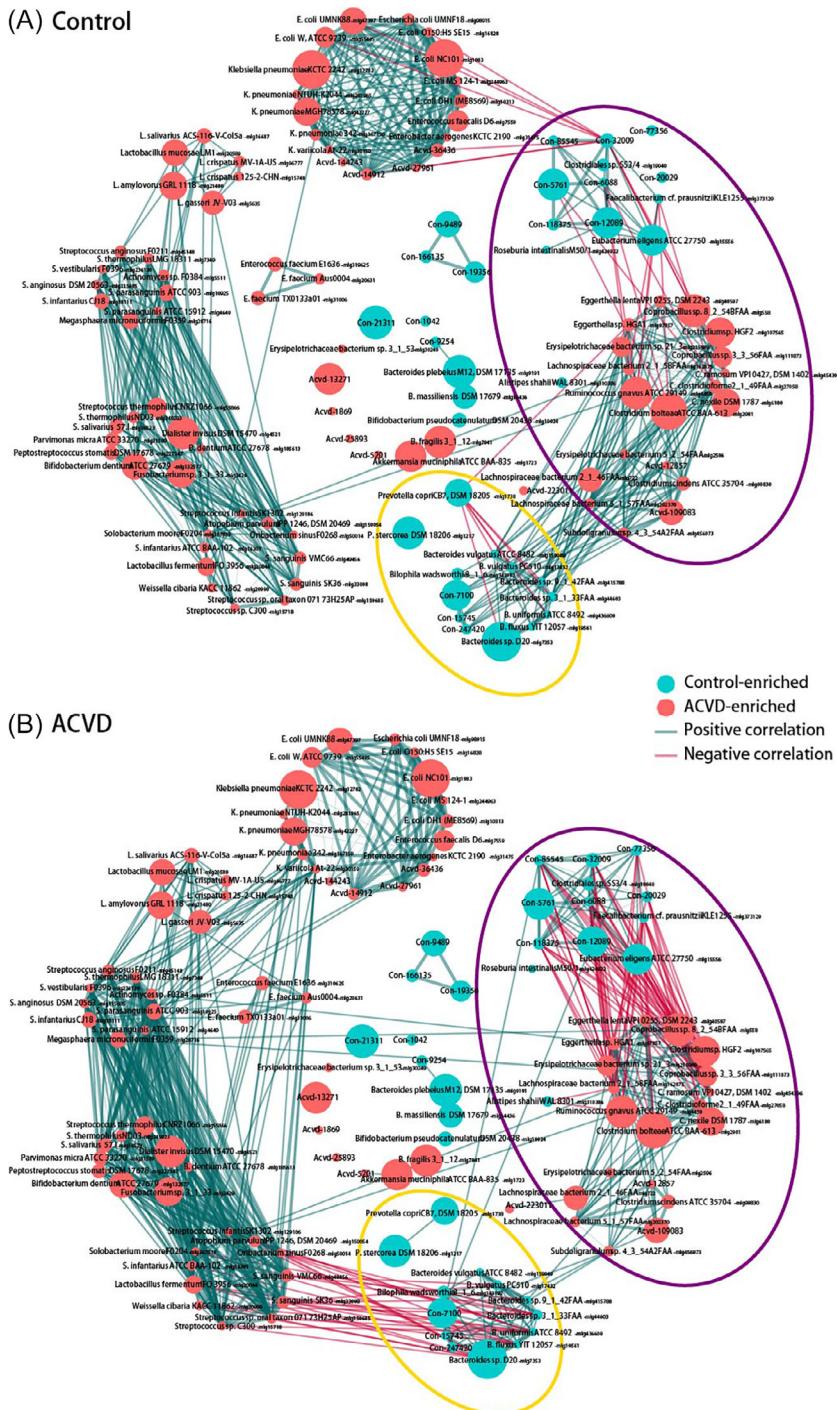


Fig. 2.14 See legend in next page

Fig. 2.14, Cont'd Fecal microbial species show patterns of abundance covariations in healthy individuals and in patients such as those with atherosclerotic cardiovascular diseases (ACVD). The *yellow circle* highlights *Bacteroides* spp. and *Prevotella* spp. More on compositional data in Chapter 5. The *purple circle* highlights members of the Firmicutes phylum that likely scavenge metabolites including glycans from the *Bacteroides* spp. (A) Correlations between species in 187 healthy controls. The *circles* are colored according to Wilcoxon rank-sum test $q < 0.05$ between their relative abundance in controls and in ACVD patients (*cyan circles*, controlled enriched; *red*, ACVD enriched). *Green lines*, Spearman's correlation coefficient > 0.3 ; *Red lines*, Spearman's correlation coefficient < -0.3 . (B) Correlations between species in 218 ACVD patients. Two hundred and five of the patients had stable angina, 8 had unstable angina, and 5 had an acute myocardial infarction (AMI). Credit: The *yellow and purple circles* are added onto Fig. 2 of Jie Z, Xia H, Zhong S-L, Feng Q, Li S, Liang S, et al. The gut microbiome in atherosclerotic cardiovascular disease. Nat Commun 2017;8:845. <https://doi.org/10.1038/s41467-017-00900-1>.

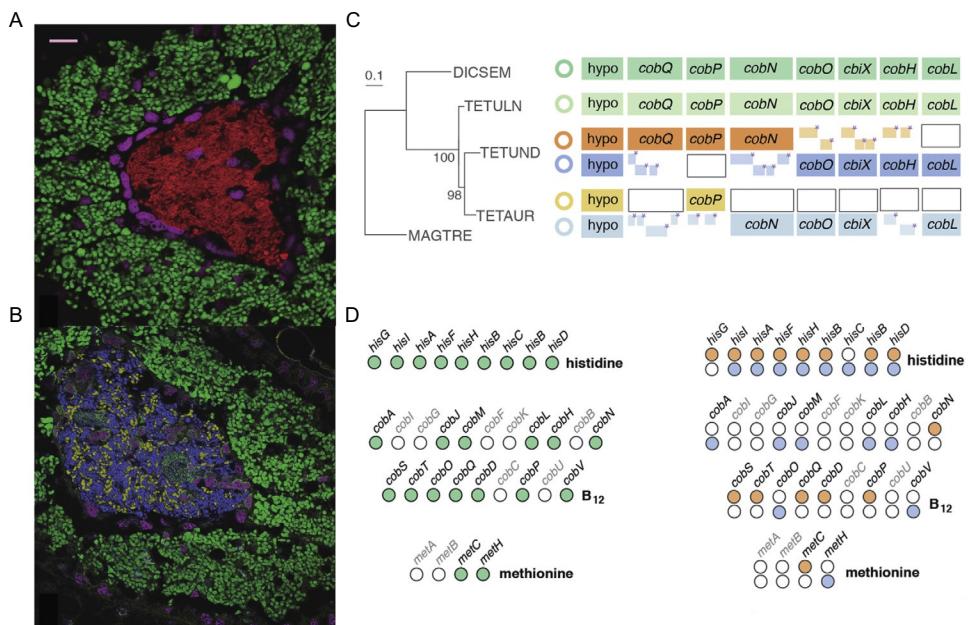


Fig. 2.15 Symbiosis *in situ*. Having information on the spatial organization and the genomes (metabolic potential) of microbial communities allowed researchers to discover interdependence between TETUND1 and TETUND2 (*Tettigades undata* chromosome 1 and 2) at the metabolic level, both are intracellular *Candidatus Hodgkinia cicadicola* bacteria that live inside cicada insects from the genus *Tettigades*. (A) FISH against rRNA to distinguish between the endosymbionts *Candidatus Sulcia muelleri* (green) from *Hodgkinia* (red). (B) FISH against genomes to distinguish among the three bacteria, *Sulcia* (green), TETUND1 (yellow) and TETUND2 (blue). (C and D) Genomic divergence of TETUND1 (orange) and TETUND2 (blue) to have complementary functions. The cicada *Diceroprocta semicincta* (DICSEM) only have *Sulcia* and one *Hodgkinia* (green). Credit: Cropped from Fig. 4, Fig. 1, Fig. 2 of Van Leuven JT, Meister RC, Simon C, McCutcheon JP. Sympatric speciation in a bacterial endosymbiont results in two genomes with the functionality of one. *Cell* 2014;158:1270–80. <https://doi.org/10.1016/j.cell.2014.07.047>.

2.8 Summary

This chapter brings in concepts from macroecology regarding diversity and trophic levels. Microbial diversity or richness could appear higher in some diseased conditions, especially when going beyond the gut microbiome. Single species dominance in the vaginal microbiome becomes more diverse in the majority of postmenopausal women (metagenomic applications in [Chapter 8](#)), as the pH is no longer maintained as acidic (attributed to glycogen fermentation into lactic acid by Lactobacilli). Lipids, microelements, and other ingredients feed a stable skin microbiome, which also tends to be acidic, before sebum secretion wanes with age. Spatial segregation and nonspecific cross-feeding are key to microbiome stability. Mucins, immunoglobulins, or even glycans expressed as blood group antigens, help microbes with localization and food. For the gut microbiome, the primary bioreactor is the ascending colon, while the microbes might spread out along the length of the colon, fermenting what they can ferment. In infants, the microbiome at multiple body sites recruit more members to reach new states; whereas in the elderly, ineffective containment of microbes likely lead to their spatial spread and shift in composition. Human genetics, historical contingency, and circadian rhythms ([Box 2.5](#)) all contribute to what we currently see in the microbiome, together with more studied factors such as nutrients and immune responses. Trophic levels in a human microbiome site may need to consider different scenarios of physiological states, during which different molecules are available, and different functions may be evolutionarily prioritized.

Worked sample 2.3

Try to draw a few scenarios for tropic levels in the human microbiome. For example, the gut microbiome on a fiber-rich diet, similar to the cow rumen [\[119\]](#); the gut microbiome when starved overnight, grazing on mucin and potentially other host molecules.

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