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Timescales of gut microbiome dynamics

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Vast communities of microorganisms inhabit the gastrointestinal tracts of humans and other animals. Understanding their initial development, fluctuations in composition, stability over long times, and responses to transient perturbations – in other words their dynamics – is important both for gaining basic insights into these ecosystems and for rationally manipulating them for therapeutic ends. Gut microbiome dynamics, however, remain poorly understood. We review here studies of gut microbiome dynamics in the presence and absence of external perturbations, noting especially the long timescales associated with overall stability and the short timescales associated with various underlying biological processes. Integrating these disparate timescales, we suggest, is an important goal for future work and is necessary for developing a predictive understanding of microbiome dynamics.

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Introduction

It is nowadays well appreciated that the legions of microbes resident in the gastrointestinal tracts of humans and other animals play major roles in development, health, and disease. Because a wide range of disorders are correlated with differences in gut microbial community composition, intentionally altering the composition of the intestinal flora is a major aim of many research strategies that share the ultimate goal of developing effective therapies. So far, progress toward this end has been quite limited. Treatments such as fecal microbiome transplantation ameliorate *Clostridium difficile* infection [1], but the underlying mechanisms remain largely mysterious and hard to generalize to other diseases. Similarly, microbiome perturbation using probiotic supplementation or diet changes remains inconsistently effective, hard to predict, and contentious [2,3••].

Understanding how to alter the composition of the gut microbiota requires, almost tautologically, an understanding of how the gut microbiota changes over time. Natural timescales for fluctuations should give a sense of the stability or instability of the microbiome, and timescales for responses to perturbations should inform strategies to maximize or minimize the system's adoption of new steady-state dynamics. In contrast to characterizations of community membership in different hosts at a given time, however, characterizations within a single host over a range of times are relatively few. Nonetheless, recent studies have provided some sense of the stability and response times of the gut microbiome in humans and other animals. In this review we summarize existing data on the dynamics of vertebrate gut microbiomes, discuss likely mechanisms governing dynamical timescales, and provide suggestions to guide future work.

Stability of the gut microbiota

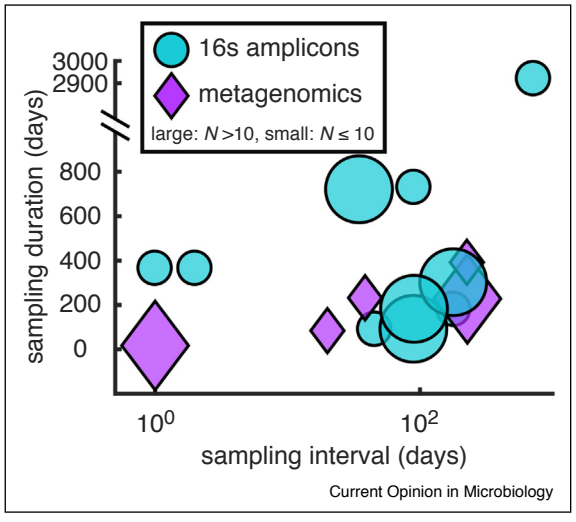
The considerable majority of experiments on gut microbiomes are cross-sectional in nature, meaning that multiple individuals are sampled at a single timepoint. A handful of longitudinal studies, however, in which data from the same individual are collected and compared over time, reveal a picture of rough stability of the human gut microbiota in adults over timescales of months and years. These studies typically make use of fecal samples, most often analyzed by sequencing 16S ribosomal RNA (rRNA) genes for taxonomic identification of bacteria, and especially in recent years by metagenomic sequencing to identify all genes present [4]. Longitudinal studies of the human fecal samples date back two decades, to work from Zoetendal *et al.* using electrophoresis of PCR products to assess the bacterial communities of two individuals [5]. Samples separated by half a year showed similar patterns, qualitatively indicating stability over this interval. Several studies since then have also demonstrated stability over timescales of months or years, roughly a dozen of which are noted in Table 1 and Figure 1. In most, the authors examined only a few individuals, over a duration of one year or less, sometimes as a control study against which to contrast some perturbation of the microbiome. Notable exceptions include Faith *et al.*, in which samples from 37 subjects were collected at approximately three week intervals for six years [6], Rajilić-Stojanović *et al.*, which followed four individuals for eight years and one for twelve years [7], Mehta *et al.*, in which two pairs of samples six months apart were obtained from 308 individuals [8], and the

Table 1

Characteristics of selected longitudinal studies of human gut microbiomes

Paper/year	Method (16s or metagenomics)	Number of individuals	Sampling interval (days)	Sampling duration (days)	Similarity metric used
Zoetendal *et al.* (1998) [5]	16s	2	180	180	Qualitative
Costello *et al.* (2009) [80]	16s	9	190 (median 45.5)	90	UniFrac distance
Duytschaever *et al.* (2011) [81]	16s	2	90	730	Correlation of DGGE densitometric curves
Claesson *et al.* (2011) [82]	16s	26	90	90	UniFrac distance
Caporaso *et al.* (2011) [12]	16s	2	1	365	UniFrac distance
Schloissnig *et al.* (2013) [10] (data from Ref. [9])	Metagenomic	43	37–378 (median 228)	37–378 (median 228)	Fixation index, allele sharing
Faith *et al.* (2013) [6]	16s	37	1–2074 (median 35)	37–2074 (median 719)	Jaccard index
Rajilić-Stojanović *et al.* (2013) [7]	16s	5	30–2920 (median 720)	2920	Pearson similarity of phylogenetic microarrays
David *et al.* (2014) [13]	16s	2	1	365	Jensen–Shannon distance
Voigt *et al.* (2015) [83]	Metagenomic	7	2–733 (median 226)	7–733 (median 392)	Rank correlation; hierarchical clustering
Li *et al.* (2016) [84]	Metagenomic	5	2–42 (median 20)	84	Euclidean distance of log-abundance, Jaccard index
Moss *et al.* (2017) [41]	Metagenomic	1	1–190 (median 38.5)	232	Chao, Bray–Curtis
Lloyd-Price *et al.* (2017) [11•]	Metagenomic	265	Irregular (median ~180)	Irregular (median ~300)	Jaccard index
Mehta *et al.* (2018) [8]	Metagenomic	308	2180 (median 91)	180	Jaccard Index, Bray–Curtis
Johnson *et al.* (2019) [33••]	Metagenomic	34	1	17	Aitchison's distances

Figure 1



Diversity of sampling schemes in selected longitudinal studies of human gut microbiomes.
Each marker corresponds to a study in Table 1. We plot here the median sampling interval (frequency of microbiome sequencing; logarithmic scale) and sampling duration (length of study) for each study. Values were estimated from publications and should be considered approximate. Circles represent studies that used 16s amplicon sequencing to identify microbial taxa; diamonds represents studies that included shotgun metagenomic sequencing. Large markers represent studies that examined more than 10 subjects; small markers, 10 or fewer.

original and the expanded Human Microbiome Project [9,10,11•], with data from 265 individuals over one year. Sampling intervals for studies lasting months or more have typically been weeks or longer. Notably, Caporaso *et al.* [12] and David *et al.* [13] each obtained daily stool samples over a one year period, though each from only two individuals, enabling the detection of rapid changes in composition. We plot in Figure 1 the sampling intervals and total durations for the studies noted in Table 1.

A consistent finding among these investigations is that the composition of the fecal microbiome, and by assumption the gut microbiome, is fairly stable over timescales of many months or years, with smaller intra-individual variation than inter-individual variation. Of course, assessing stability requires quantification of microbiome similarity and its temporal trends, and for this a diverse set of analysis methods, some quite opaque, have been used. For similarity itself it is common to calculate the Jaccard Index of the taxonomic units found in the sets being compared (e.g. [6,8,11•]); this is the number of items found in both sets divided by the number found in either. It is also common to calculate the Bray–Curtis dissimilarity, which incorporates abundance rather than only presence/absence into the measure. Even samples from the same individual at the same time will not be identical due to the noise associated with every aspect of sampling and processing; this technical dissimilarity is surprisingly rarely quantified or presented in microbiome

studies (see Ref. [11•] for a clear example). It is not obvious how to characterize the time-dependence of whatever similarity measure is used, a point to which we will return below, but studies to date show slow declines over months and years, never reaching complete dissimilarity, and similarity values for comparisons of samples from the same person that are consistently above those from inter-individual samples, leading to an overall assessment of long-term stability at least of a 'core' set of microbial groups.

It is worth cautioning, however, that these and many other conclusions are based on assessing fecal samples, which may not be an unbiased sample of intestinal contents, especially for microbes that maintain close proximity to epithelia. As part of an excellent recent study, Zmora *et al*. found strong differences between human stool and intestinal samples, the latter obtained invasively, the magnitude of which was greater for comparison to the upper gastrointestinal tract than the lower [3••]. Zmora *et al*. also noted differences in microbial colonization of mucosal and lumenal regions, in both humans and mice. Though daunting to perform, longitudinal studies of directly sampled intestinal contents would give unparalleled insights into the dynamics of the microbiome. It is also worth noting that the majority of longitudinal studies assess gut microbiome composition through sequencing of the 16s rRNA gene and therefore have limited taxonomic resolution, typically to the level of genus. The degree to which patterns of stability also occur at the strain or species level is less clear, though continued advances in metagenomic techniques [14–18] render this question increasingly tractable. The decreasing cost of DNA sequencing continues to enhance the feasibility and prevalence of metagenomic studies, a trend which should continue into the foreseeable future.

One might expect that the relative ease of experiments involving mice and other non-human animals would have resulted in an abundance of long-term microbiome sampling studies. This is, however, not the case. In mice, the existing data suggest stability at least on the order of weeks [19–23]. Notably, Schloss *et al*. found considerable variation in the nine days post weaning, but stability over the period from 141 to 150 days [23], Schulfer *et al*. found community compositions that were stable over many weeks except when perturbed by radical changes of diet [19], and Sarma-Rupavtarm *et al*. found that all strains of the altered Schaedler flora remained in mice three years after their colonization [24]. A handful of longitudinal studies have been done on other vertebrates, for example rufous mouse lemurs [25] and captive cheetahs [26]. The latter, with bimonthly sampling over three years, found considerable stability, with about 65% of groups persisting over one year. To our knowledge, there are no densely sampled, multi-year longitudinal studies of the gut microbiome in any non-human animal.

Instability of the gut microbiota

Though stable long-term behavior may suggest sluggish dynamics, gut microbial communities can exhibit rapid changes, both autonomously and in response to perturbations. Observations in model animals show major, self-driven changes taking place within less than one day. Thaiss *et al*. sampled the mouse microbiome every four or six hours, uncovering strong diurnal rhythms in bacterial abundance and localization, as well as in host transcription [27]. In zebrafish, live imaging shows-specific bacterial populations collapsing by orders of magnitude due to intestinal transport and expulsion from the gut, followed by periods of regrowth, with average collapse intervals on the order of hours [28–30].

In humans, studies of fecal samples of subjects experiencing a wide range of stimuli, including diet changes [13,31,32,33••], antibiotics [34–38], osmotic shocks [39,40], and fecal microbiome transplants [41] show marked changes in community composition occurring within a few days or even within one day, the limit of these studies' temporal resolution. Antibiotic treatments in particular, even in healthy individuals, have been shown to induce large changes in gut microbiota composition within one day [34]. Interestingly, the recovery of the microbiota to baseline composition following antibiotics, typically measured through diversity metrics, appears to vary in duration between people but can be as long as months, and some people never fully recover [34]. Recently, Johnson *et al*. tracked microbiome composition daily for over two weeks in 34 individuals, while also collecting detailed information about diet [33••]. Surprisingly, the microbiota from two people with near-constant diets, subsisting almost wholly on fixed meal-replacement beverages, were as variable as those of other subjects, suggesting that community variation on daily timescales is an intrinsic feature of the host–microbe system [33••]. Studying individuals who immigrated from Thailand to the United States, Vangay *et al*. found a steady shift in community composition over the course of years, with differences from the initial state evident within six to nine months, but monthly sampling prevents finer-scale assessment of temporal shifts [42•].

The initial development of the gut microbiome provides additional relevant timescales. In humans, the membership and stability of the gut microbiota in children up to at least age five do not match those of adults [43], and the compositions show considerable temporal variation over timescales of months [43,44]. The recent, large TEDDY (The Environmental Determinants of Diabetes in the Young) study followed over 900 children between 3 and 46 months of age with stool sampling approximately monthly, revealing stability after about 31 months, high variability before 14 months, and a transitional period in between [45••]. A 2019 study of about 300 young children similarly showed far greater changes in microbial diversity in the first year compared to the third [46].

Determinants of timescales for the gut microbiota

Empirically observed timescales characterize gut microbiome dynamics, ranging from fast responses on the order of days to slow changes on the order of months or years, must somehow be set by the timescales of underlying biological processes that influence the gut and its residents. These processes include microbial growth, intestinal transport, circadian and seasonal rhythms, bacterial evolution and horizontal gene transfer, and colonization and transmission. We will comment on aspects of each of these.

Perhaps the most fundamental timescales of intestinal population dynamics are set by the division rates of the microbes themselves. These are difficult to determine from *in vitro* studies, both because of the challenge of culturing many intestinal species, but also because the microbes' phenotypic state may be very different in the gut than in culture. Various techniques make measurements of growth rates inside vertebrate hosts possible, however. In larval zebrafish, the animals' transparency enables imaging-based quantification of populations and growth rates [28,30,47,48]. In other hosts, metagenomic sequencing can be used to infer *in vivo* growth rates, either relative to *in vitro* rates or absolutely if chromosome replication speeds are known beforehand, making use of the coverage patterns that result from capturing microbes at different points in their replication cycles [49,50]. With this approach, Korem *et al.* found that for several bacterial species, differences in growth rates correlated with indicators of inflammatory bowel disease and type II diabetes in human subjects, while differences in abundances did not [49].

The gastrointestinal tract is a dynamic environment that mixes and transports a vigorous flux of food, microbes, and waste. Mechanical contractions occur on the order of ten times per minute in humans, and propagation of contents corresponds to transit times on the order of several hours. Transit times are very different across different animal species, due both to the wide range of sizes spanned and to differences in anatomy and physiology. The consequences of this for microbiome composition remain poorly understood.

Circadian and seasonal cycles can influence the gut microbiome in a variety of ways. The circadian clock drives periodicity of host metabolic and immune pathways that, together with the diurnal rhythm of feeding, couple to microbial metabolism and dictate an approximately 24-h periodicity for the microbiome [51,52]. Seasonal variation in available foods as well as changes in temperature and weather drive periodic microbiome dynamics in many animals, including various human populations, the latter attributed primary to diet [53•,54]. Notably, the microbial taxa with the greatest seasonal variation in the intestinal microbiomes of Hazda hunter-gathers are those that differ most between traditional and modern human groups, suggesting that industrialization alters temporal features of gut communities along with their mean compositions [53•].

The rate of microbial evolution in the human gut microbiome will depend on the nature and strength of selective pressures in the intestine, which remain poorly known. Recent studies suggest that evolution in the gut occurs through processes that span a range of timescales, including selective sweeps that occur on timescales shorter than a few months [15]. In very recent work, Zhao *et al.* use metagenomic methods track the gut resident *Bacteroides fragilis* in individual humans over two years, resolving variation arising from unique founder populations, selective sweeps, and signatures of adaptive evolution well within this sampling duration [55••]. The rapidity of evolutionary dynamics in the gut suggests that host-specific adaption likely cannot be neglected in predictive models of the gut microbiome.

Transmission of microbes between adult animals can in principle be determined by a variety of processes of microbial dispersal from hosts, bacterial behaviors in the environment, intake of bacteria by hosts, and the establishment of successful colonies, all of which are so far minimally characterized for gut microbes. The resulting overall timescales for microbial exchange are, however, beginning to be quantified. In mice, co-housing experiments show timescales of days to a few weeks for microbial transmission and mixing [56–58]. Differences in gut communities of co-housed and solitary zebrafish over a span of three weeks similarly suggest days-to-weeks transmission timescales [59]. In humans, controlled co-housing studies, without confounding by diet and other environmental aspects, are more difficult to perform. Turroni *et al.* examined the gut microbiota of six 'crewmembers' in a 520-day ground-based space-flight simulation, finding very slight convergence of the microbial communities over time [60]. Yassour *et al.* tracked mother–infant pairs over the first few months of time, finding clear signatures of transmission over this timescale for some microbial strains, though a clear picture of transmission dynamics is yet to emerge [61].

Mathematical and experimental models

The fast timescales associated with responses of gut microbiota to perturbations and the slow timescales associated with overall stability are likely intertwined, together determining the population dynamics of gut microbial communities through means that are largely unknown. Mathematical models have the potential to provide insights into the coupling of timescales. Most dynamical modelling efforts to date, however, have ignored all intrinsic temporal factors other than bacterial growth rates. With the aim of inferring interactions between bacterial species from time-series data of

relative fecal abundances, researchers have typically tied abundance changes to altered growth rates via, for example, generalized Lotka–Volterra models [62,63]. While it is possible that all determinants of microbial dynamics can be mapped onto effective growth rate parameters, regardless of their true nature, this has not been established. It would be interesting to see, for example, if the long timescales of microbiota recovery measured following antibiotic treatments [34], are consistent with the timescales that emerge from growth-focused models, or if it is necessary to invoke other physiological processes. We note that despite nearly a decade of work on inferring microbial interaction networks from sequencing data [62–66], we are not aware of any network predictions that have been successfully experimentally tested. Complex networks themselves are not necessarily intractable; within-organism gene regulatory networks are now routinely modeled and rigorously assessed with perturbation-based methods [67,68]. Implementing similar approaches for gut microbiome networks will no doubt yield important insights into microbiome dynamics and stability.

An exceptional example of a modeling study that looks beyond growth to explicitly consider transport and mixing of fluid, bacteria, and nutrients, as well as pH-dependent growth, is Ref. [69], in which Cremer *et al.* incorporate physical and physiological parameters into a simple, robust, spatially resolved model of the human colon. They find that experimental observations of bacterial densities, spatial profiles of pH and short-chain fatty acid concentration, and ratios of two key bacterial species can be explained by their model, generating mechanistic insights into intestinal population dynamics. The idea that fast physical processes can dramatically impact the composition of the gut microbiota is also supported by multiple lines of experimental, observational, and theoretical evidence [70•,71–73]. For example, experiments in zebrafish show that stochastic expulsion of bacteria by intestinal transport can generate large variation in observed abundances [28,29], and that sublethal antibiotic perturbations can amplify this variation [30]. An interesting avenue for further theoretical research would be to investigate the effect of these physical processes on the stability and long-term dynamics of a microbiota with strong inter-species interactions.

An impediment to better, more predictive models of the gut microbiome is the lack of quantitative understanding of the underlying dynamical processes. It is, in other words, hard to imagine that a realistic, prediction-enabling computational model of the gut could exist before understanding rates of transport, transmission, evolution, and so on. To this end, there is a strong need for controlled, quantitative experiments. Methods for studying model organisms, such as gnotobiotic [74,75], imaging [48,76], genetic [77], and genomic [14,49,50] techniques already offer powerful, and underused, approaches for studying gut microbiota dynamics, and continue to advance. Non-living mimics, that is, 'guts-on-a-chip,' which can include cultured host cells [78] and peristalsis-like flows [70•] enable exquisite experimental control, and are increasingly realistic. Coupling human-mimicking anaerobic bioreactors with dense sampling and rigorous modeling not only is providing insights into community dynamics, but provides valuable assessments of sources of variation such as biological stochasticity and technical reproducibility [79].

Connecting models, whether computational or experimental, to data from humans and other animals will also require deeper insights into what temporal metrics are important to quantify in microbiome studies. To date, studies of microbiota stability over time report a wide array of different similarity measures (Table 1), making comparison difficult even between studies. Moreover, there is a striking lack of discussion of the forms that similarity functions might be expected to take. The observation, for example, of exponential decay of similarity metrics with time, or particular autocorrelation features, or decay to a nonzero floor, could suggest or rule out various dynamical models. Increased cross-talk between theory and experiment will be essential to cultivate predictive models.

To conclude, we emphasize that understanding the dynamics of the gut microbiota will be crucial for guiding the development of targeted therapies. Given the strong coupling between microbiota dynamics and composition – and by proxy, function – the timescales of treatments will likely be linked to their efficacies. For example, to shift microbial compositions to a new state, is a pulsed perturbation optimal, potentially jarring the system from one equilibrium to another, or are gradual shifts, for example through slow diet changes, more effective? Similarly, how frequently should markers of health be assessed, and when will indicators of desired or unwanted changes appear? These questions are analogous to long-standing issues in ecological management, control theory, and other well-studied fields. While casting therapies involving the gut microbiome in the framework of control theory may seem a lofty goal, it is an important one if we are to guide research toward a predictive and practical understanding of our microbiota.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- • of special interest
- •• of outstanding interest

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