Gut Microbes 5:2, 1–6; March/April 2014; © 2014 Landes Bioscience

Metagenomic systems biology and metabolic modeling of the human microbiome

From species composition to community assembly rules

Roie Levy[1] and Elhanan Borenstein[1,2,3,*]

[1]Department of Genome Sciences; University of Washington; Seattle, WA USA; [2]Department of Computer Science and Engineering; University of Washington; Seattle, WA USA; [3]Santa Fe Institute; Santa Fe, NM USA

The human microbiome is a key contributor to health and development. Yet little is known about the ecological forces that are at play in defining the composition of such host-associated communities. Metagenomics-based studies have uncovered clear patterns of community structure but are often incapable of distinguishing alternative structuring paradigms. In a recent study, we integrated metagenomic analysis with a systems biology approach, using a reverse ecology framework to model numerous human microbiota species and to infer metabolic interactions between species. Comparing predicted interactions with species composition data revealed that the assembly of the human microbiome is dominated at the community level by habitat filtering. Furthermore, we demonstrated that this habitat filtering cannot be accounted for by known host phenotypes or by the metabolic versatility of the various species. Here we provide a summary of our findings and offer a brief perspective on related studies and on future approaches utilizing this metagenomic systems biology framework.

Keywords: metabolic modeling, reverse ecology, species interaction, computational model, community assembly, habitat filtering

*Correspondence to: Elhanan Borenstein; Email: elbo@uw.edu

Submitted: 12/03/2013; Revised: 02/13/2014; Accepted: 02/17/2014; Published Online: 02/20/2014

http://dx.doi.org/10.4161/gmic.28261

Levy R, Borenstein E. Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. Proc Natl Acad Sci U S A 2013; 110:12804-9; PMID:23858463; http://dx.doi.org/10.1073/pnas.1300926110

Introduction

The human microbiome plays a critical role in maintaining the health of its host, contributing to energy harvest,[1] innate immunity,[2] and infection resistance,[3-5] and microbiome dysbiosis has been implicated in a number of diseases[6-9]. Recent years have seen a surge of interest in understanding how changes in the microbiome influence, or are influenced by, changes in host health, lifestyle, and physiology. In order to address these questions, numerous studies have been performed to characterize host-associated microbial communities and to identify factors that impact the composition of these communities. Specifically, comparative metagenomics approaches have been commonly used to assess variation across individuals, across anatomical sites, and between health and disease.[6,10-12] Comparing communities across different host states has shown, for example, that obese and lean microbiomes differ in composition and capacity for nutrient harvest[7,13] and that the microbiota of healthy individuals can be distinguished from the microbiota of individuals with colitis or Crohn disease.[6] Similarly, experiments using germ-free mouse models have shown that diet is a strong determinant of community composition[14] and that the microbiome undergoes marked shifts during diet-induced obesity.[15]

Comparative metagenomic studies, however, cannot usually reveal the underlying ecological forces that drive observed shifts in community structure, and several other approaches, often integrating additional information, have been proposed to confirm such assembly forces. One such study, for example, used operational taxonomic units (OTUs) not

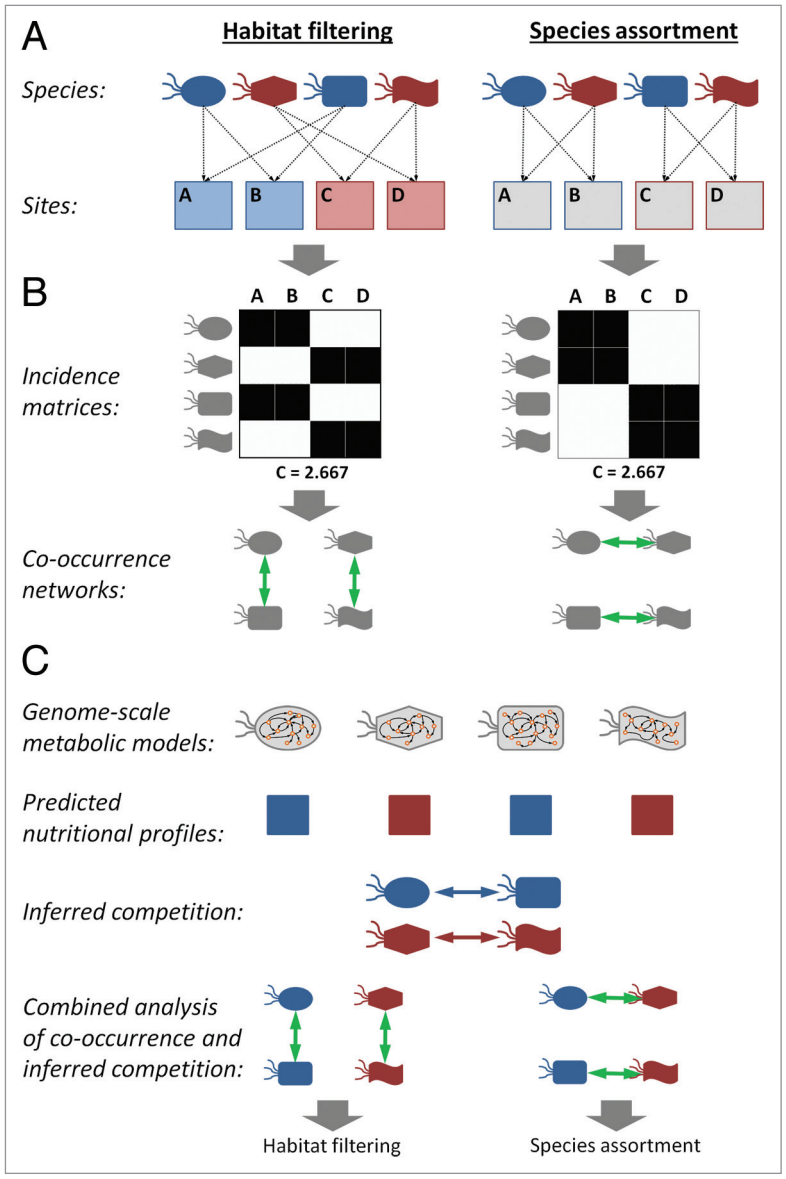

Figure 1. Habitat filtering and species assortment can produce similar community structures but can be distinguished by using genome-scale models to predict species interaction. (**A**) When habitat filtering dominates assembly, species tend to occur in their preferred habitats (indicated by color), even when competitors co-occur. In contrast, when community assembly is governed by species assortment, competitors exclude one another from the same habitat. (**B**) Without information about species interaction, habitat filtering and species assortment induce an equivalent community structure (as represented by either the species incidence matrix and checkerboard score or by the species co-occurrence network), making it challenging to distinguish between these two structuring forces. (**C**) Genome-scale metabolic models can be used to predict the nutritional profile of each species (indicated again by color) and, consequently, the competition between species. Combining these predicted interactions with information about species co-occurrence reveals the dominant assembly force; co-occurrence of competitors indicates habitat filtering whereas exclusion of competitors indicates species assortment.

only to identify member species but also to infer evolutionary distances among various community members.[16] A null-model analysis then revealed that abundant OTUs in the vertebrate gut clustered with rare OTUs in sequence space, a characteristic of non-neutrally assembled communities. Investigating microbial co-occurrence patterns can further provide clues as to the non-neutral forces that structure a community. For example, an examination of oral communities from 10 individuals revealed that segregation is more apparent at the level of genera than species, indicating that structuring forces may act differently across different taxonomic levels.[11] Within the gut, co-occurring microbes further group into phylogenetically related clusters[6] and may partition individuals into specific enterotypes.[17] More generally, across the human microbiome, co-occurrence patterns are largely constrained by anatomical site,[12] suggesting that species are adapted to specific niches.[18] On a more global scale, literature-driven co-occurrence analysis has demonstrated the influence of competitive metabolic interactions on lifestyle and on ecological strategy.[19] Yet, while these studies provide evidence of the existence of ecological forces that structure the community and that give rise to non-random composition patterns, they do not point to specific ecological assembly dynamics and cannot clearly distinguish between alternative assembly rules.[20]

As a specific example, consider the checkerboard incidence pattern often observed in naturally occurring communities, including those that inhabit the human body.[11] A checkerboard pattern refers to the tendency of certain taxa to exclude one-another from shared habitats and is seen as an indicator of non-neutral assembly.[21] Such a pattern, however, can be the outcome of two distinct niche processes (see, for example, **Fig. 1A**). In the species assortment model, first proposed by Diamond,[20] competition leads to forbidden pairs of taxa which cannot co-exist within one site. Alternatively, a habitat filtering model supposes that habitats with differing environmental features support non-overlapping sets of taxa.[22] Without additional information about species interaction, taxon incidence or co-occurrence data alone may often be insufficient to determine which of these two forces underlies the observed community structure (**Fig. 1B**).

To address this challenge, we recently presented a study[23] aiming to quantify community-level assembly rules and to determine the relative roles of habitat filtering[22,24] vs. species assortment[20] in forming patterns of microbial co-occurrence across the

human microbiome. In this study we supplemented comparative metagenomics and null-model analysis such as those described above with a systems-biology approach and with metabolic modeling, treating microbiome species not only as amorphous members of a complex community but rather as constituents which interact in a definite manner. Specifically, we utilized whole genome sequence information to model the metabolic networks of hundreds of species inhabiting the human body (with a focus on the intestine) and used these models to determine the nutritional profile of each species. We then analyzed these nutritional profiles to infer the level of competition and complementarity between species, providing a proxy for metabolic interactions. Combining these predicted interactions with species abundance data obtained through shotgun metagenomic community profiling allowed us to distinguish communities structured via alternative assembly processes (**Fig. 1C**). Here we review our results, discuss their relation to other studies, and provide perspective on future work.

Results and Discussion

Our study utilized data from the recently published gut microbiome gene catalog, generated by the international MetaHIT consortium.[6] This data set represents a deep profile of the intestinal microbiome and allowed us to examine a number of host states that may be relevant to community assembly. In all, fecal samples from 124 adult individuals from Denmark and Spain were profiled using Illumina-based shotgun metagenomic sequencing. Individuals were either lean or obese and were either healthy or diagnosed with inflammatory bowel disease (IBD). For a subset of these individuals, an enterotype classification[17] (clustering of community composition into a limited number of steady-states) was also available. Shotgun reads were aligned to a large set of reference genomes to estimate the relative abundance of each genome and to determine the species composition in each sample. We then used these estimated abundances to calculate pairwise species co-occurrence scores, identifying pairs of species that tend to co-occur vs. those that tend to exclude one another.

A crucial feature of our analysis was the use of whole genome sequence information to model the metabolic network of each microbial species and to ultimately predict metabolic interactions between species. As described above, this approach was intended to supplement standard null-model analyses that are commonly performed to determine the existence of non-neutral structure in a meta-community[11,16] and to allow us to differentiate between alternative forces that can give rise to such structure.[20,22,24,25] Given the metabolic networks of the various species, we employed a previously introduced reverse ecology algorithm[26-28] to determine each species' nutritional profile: the set of compounds in its metabolic network that it cannot synthesize from other precursors and that allow synthesis of all other compounds. This set was previously shown to accurately represent the set of nutrients each species extracts from its environment and to successfully serve as a proxy for the biochemical niche of each species.[26] Calculating the pairwise overlap between these nutritional profiles, we then defined a metabolic competition index, representing an upper limit for the level of competition one species may be expected to experience in the presence of another. We similarly used these nutritional profiles to define a metabolic complementarity index, representing the potential for cohabiting species to reduce niche competition by synthesizing, rather than acquiring, required nutrients.

Notably, a few features of these interaction indices make them especially favorable for modeling and studying pairwise metabolic interactions. Most importantly, in contrast to simple set-similarity coefficients (e.g., Jaccard index), the defined competition and complementarity indices were not necessarily symmetric, accurately capturing the potentially unbalanced impact of ecological interactions; if, for example, one organism's nutritional profile is a subset of another organism's significantly larger profile, competition would impact more heavily the first organism (since it cannot utilize alternative nutrients). Moreover, the definition of these indices also accounts for the nutritional flexibility of generalist species and controlling for nutritional profile size in our downstream analysis did not change any of the observed patterns.

Calculating the interaction indices and comparing them with the species co-occurrence scores discussed above, we found that species tend to co-occur most frequently with species with which they most strongly compete. Put differently, our finding implies that the various species inhabiting each individual all utilize relatively similar nutritional niches, whereas species with markedly different nutritional requirements tend to be found in different hosts. This pattern suggests that species in the gut do not competitively exclude one another but are rather filtered by the environment, and while species interaction may still play some role in structuring the composition of species in the gut, our finding indicates that habitat filtering is the dominant factor governing community assembly in the gut microbiome.

As mentioned above, the MetaHIT data set was chosen for its coverage of diverse host phenotypes. Central to the habitat filtering model is the idea that habitats are distributed along some environmental axes (e.g., pH, sucrose availability, etc.). Environmental parameters then form a stability landscape, wherein each species finds an optimal point.[29] Given the ability to distinguish the intestinal community of individuals with a disease from controls[6] or to divide samples into enterotypes[17] solely based on community composition, it is reasonable to assume that partitioning samples according to these attributes would potentially highlight underlying environmental differences that drive habitat filtering. It is possible, for example, that the gut environment of obese individuals or those with IBD is abundant in a given nutrient, strongly selecting for species that rely on this nutrient for growth. When considering only these samples, environmental variation will be restricted, and, if this variation indeed contributes to habitat filtering, one would expect a commensurate reduction in the observed habitat filtering pattern. Surprisingly, however, when partitioning along body

mass index (BMI), IBD, nationality, or enterotype, each a promising candidate for driving habitat filtering and for serving as an environmental filtering axis, no reduction in signal was found and in each subset of samples species still tended to co-occur with their strongest competitors.

As proposed in our study, this suggests that as-yet-to-be-determined environmental features (e.g., host genotype, pH, etc.) define crucial habitat filtering axes and contribute most strongly to community assembly. One obvious environmental factor that could play a major role in filtering species in the gut is diet. Diet, for example, was shown to be a successful predictor of community composition in mice.[14] More recently, additional studies have further strengthened this proposition. David et al. have shown that a significant dietary shift in humans leads to reproducible changes in the composition of the intestinal community.[30] Among the most affected taxa, a clear association can be made as to the influence of particular macromolecules. Furthermore, a meta-transcriptomic analysis demonstrated that microbial metabolic activity similarly shifted with diet, suggesting that diet is a major, and perhaps the predominant, environmental factor that acts on the intestinal microbiota. Ridaura et al. additionally demonstrated that the microbiota transplant-induced development and rescue of an obesity-like phenotype in mice was diet dependent.[31] Future studies and additional meta-omic analyses (e.g., including meta-metabolomics data) may be able to provide more details on such environmental factors.

Importantly, our framework also allowed us to control for phylogeny. Phylogenetic clustering is often considered the best evidence of habitat filtering. Directly controlling for phylogenetic effects using several methods, we observed no loss in signal, in spite of a previously reported tendency for related species toward similar nutritional profiles.[26]

Having examined potential axes of habitat filtering and the role of phylogeny, we turned to assess the impact of scale. To this end, we used data obtained by the NIH Human Microbiome Project (HMP) survey.[12,32] In contrast to the MetaHIT data set, all HMP samples were obtained from healthy individuals, but a total of 18 body sites were included. These HMP samples first allowed us to validate our main results with an independent data set. It further allowed us to demonstrate the applicability of our hypothesis to the microbiome at large and to explore observed assembly rules at varying scales. Specifically, our finding that comparing across all body sites in aggregate reveals a strong signature of habitat filtering is in accord with a recent study reporting that increasing meta-community scale makes local communities appear clustered.[33] We further demonstrated that even when body sites are considered separately, each local site appears habitat-filtered in almost all cases (and see also our discussion[23]). Notably, sampling method also plays an important role in determining the biogeographic scale of the study. Our analysis (as well as many other analyses of the gut microbiome) assumes that fecal samples can provide an accurate representation of the underlying community composition in the gut. Nonetheless, much evidence shows that the microbial community is not evenly distributed along the gastrointestinal tract[10,34] or between the lumen and epithelial surface,[10] and indeed, spatial heterogeneity leads to significant differences in niches and their occupancy.[5] In this sense fecal sample profiling provides, at best, an estimate of the average species composition along the gut, ignoring any spatial heterogeneity and local scale variability. It is therefore not yet clear how the availability of fine-resolution data, describing the species composition of "micro-habitats" in the gut, may alter our perspective of community assembly rules.

While our analysis considered only nutrient utilization and production, many other factors may clearly contribute to community assembly. Our modeling framework ignores, for example, cell-cell signaling and bacteriocin production, and it still remains to be seen whether factors such as these, rather than nutrient utilization, dominate community assembly. A recent study performed in germ-free mice identified, for example, a genetic locus mediating invasion resistance.[35] Interestingly, the locus appears to be responsible for oligosaccharide import, and the ability to resist invasion by a competing strain was accordingly determined by the ability to exploit a given set of resources. Similarly, the expansion of the pathogens *S. enterica* and *C. difficile* following antibiotic administration was shown to be dependent on their reliance on available sialic acid,[36] supporting the hypothesis that community abundance is controlled by limiting substrates.[37]

Such studies demonstrate the importance of nutrient utilization and niche effects in assembling the intestinal community. Yet, under the assumption of limiting substrates, the tendency of competing species to co-occur seems somewhat paradoxical. Recent work has shown that not only do competitive interactions dominate pairwise interactions of naturally co-occurring microbes,[38] higher order positive effects, which could lessen competition's deleterious effects,[39] are rare at best. Nonetheless, many other factors may play a role, including spatial heterogeneity,[40,41] environmental stochasticity,[42] and the selective influence of the host.[1,43,44] Furthermore, it should be noted that the levels of metabolic competition observed using our metrics do not, in general, approach complete niche overlap.

Interestingly, one of our interaction indices, namely the metabolic competition index, shares conceptual similarity with another test for habitat filtering, termed convex hull volume.[24] In this test, the species in each community are represented as points in an n-dimensional trait-space. Communities structured by habitat filtering then occupy a relatively narrow region of this trait-space. The various compounds identified by our reverse ecology framework can similarly be conceived as representing the various axes of such a trait-space, and our finding that co-occurring species have more similar profiles is consequently analogous to finding a community with a small convex hull volume. A critical difference, however, is that the various compounds in each nutritional profile are linked through the organism's metabolic network and are thus not independent of one another and do not necessarily represent orthogonal axes. Nonetheless, this method suggests

a potential approach by which additional ecological trait information can be combined with our modeling framework.

As our appreciation for the complexity of the human microbiome grows, researchers are moving beyond descriptive studies of this system and are focusing on understanding mechanisms of community assembly. Germ-free host systems, in particular, provide an exciting opportunity to study the interplay of diet, microbe interactions, genetics, and environmental factors in shaping the microbiome's structure and function.[14,35,45] Supplementing such experimental studies with in silico modeling of microbial interactions can offer valuable insights into the mechanisms, dynamics, and robustness of community assembly. The framework presented in our study, combining metagenomic analysis with genome-scale metabolic modeling, represents an important step in this direction.

Clearly, such a framework relies heavily on the availability of fully sequenced genomes to construct genome-scale models. Thanks to the efforts of consortia such as the HMP and the MetaHIT, as well as numerous independent groups, genome sequence information is now available for many of the species most abundant within the human microbiome. Efforts to sequence and to explore the metabolic potential of poorly characterized clades through single-cell genomics will further allow us to model species from diverse environments.[46] Such initiatives can be further complemented by the development of computational tools that integrate available genomic and metagenomic data for inferring the genomic content of yet-uncharacterized species. A recently introduced method, for example, integrates variation in gene and taxonomic composition across multiple samples to deconvolve metagenomes into taxa-specific gene profiles, providing an assembly-free reconstruction of the genomic content of microbiome taxa.[47] Another recently introduced computational platform, PICRUSt, couples whole genome sequence information with a reference taxonomic tree to predict the gene contents of OTUs with no representative genomes.[48] Such computational methods—associating OTUs whose abundances in the community were assayed through 16S surveys with their predicted gene content—are especially relevant to our framework and can be used to reconstruct the metabolic models of various species in the community. These methods are especially important for studying less well-characterized ecosystems, where OTU information may be available but genomic information is scarce.

Ultimately, however, the goal of our study, and of other efforts to model various aspects of this complex system,[13,49,50] is to develop a comprehensive, predictive, and systems-level model of the microbiome.[51,52] Clearly, this goal is extremely challenging. Few biological systems approach the complexity of the human microbiome: a web of hundreds of interacting species, each encoding a unique set of metabolic capabilities,and all functioning within a complex host environment with potential interplay between the microbiome and host genetics, physiology, and lifestyle. Temporal dynamics, spatial heterogeneity across multiple scales, and strain-level variation[53,54] further challenge current efforts to construct such a complete modeling framework. Considering this complexity and our limited understanding of this system, it is perhaps not surprising that microbiome research is witnessing an exciting synthesis of experimental, computational, and analytic approaches. Metagenomic systems biology—the coupling of metagenomic data analysis with computational systems biology and modeling techniques—is likely to play a central role in this synthesis.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Acknowledgments

R.L. is supported by a National Science Foundation Graduate Research Fellowship under Grant DGE-0718124. This work was supported in part by New Innovator Award DP2 AT007802-01 to E.B.

References

1. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005; 307:1915-20; PMID:15790844; http://dx.doi.org/10.1126/science.1104816
2. Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol 2004; 5:104-12; PMID:14691478; http://dx.doi.org/10.1038/ni1018
3. Barthel M, Hapfelmeier S, Quintanilla-Martínez L, Kremer M, Rohde M, Hogardt M, Pfeffer K, Rüssmann H, Hardt WD. Pretreatment of mice with streptomycin provides a Salmonella enterica serovar Typhimurium colitis model that allows analysis of both pathogen and host. Infect Immun 2003; 71:2839-58; PMID:12704158; http://dx.doi.org/10.1128/IAI.71.5.2839-2858.2003
4. Croswell A, Amir E, Teggatz P, Barman M, Salzman NH. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric Salmonella infection. Infect Immun 2009; 77:2741-53; PMID:19380465; http://dx.doi.org/10.1128/IAI.00006-09
5. Britton RA, Young VB. Interaction between the intestinal microbiota and host in Clostridium difficile colonization resistance. Trends Microbiol 2012; 20:313-9; PMID:22595318; http://dx.doi.org/10.1016/j.tim.2012.04.001
6. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, et al.; MetaHIT Consortium. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010; 464:59-65; PMID:20203603; http://dx.doi.org/10.1038/nature08821
7. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444:1027-31; PMID:17183312; http://dx.doi.org/10.1038/nature05414
8. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. A core gut microbiome in obese and lean twins. Nature 2009; 457:480-4; PMID:19043404; http://dx.doi.org/10.1038/nature07540
9. Grice EA, Snitkin ES, Yockey LJ, Bermudez DM, Liechty KW, Segre JA; NISC Comparative Sequencing Program. Longitudinal shift in diabetic wound microbiota correlates with prolonged skin defense response. Proc Natl Acad Sci U S A 2010; 107:14799-804; PMID:20668241; http://dx.doi.org/10.1073/pnas.1004204107
10. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. Science 2005; 308:1635-8; PMID:15831718; http://dx.doi.org/10.1126/science.1110591
11. Bik EM, Long CD, Armitage GC, Loomer P, Emerson J, Mongodin EF, Nelson KE, Gill SR, Fraser-Liggett CM, Relman DA. Bacterial diversity in the oral cavity of 10 healthy individuals. ISME J 2010; 4:962-74; PMID:20336157; http://dx.doi.org/10.1038/ismej.2010.30
12. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012; 486:207-14; PMID:22699609; http://dx.doi.org/10.1038/nature11234

13. Greenblum S, Turnbaugh PJ, Borenstein E. Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. Proc Natl Acad Sci U S A 2012; 109:594-9; PMID:22184244; http://dx.doi.org/10.1073/pnas.1116053109
14. Faith JJ, McNulty NP, Rey FE, Gordon JI. Predicting a human gut microbiota's response to diet in gnotobiotic mice. Science 2011; 333:101-4; PMID:21596954; http://dx.doi.org/10.1126/science.1206025
15. Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 2008; 3:213-23; PMID:18407065; http://dx.doi.org/10.1016/j.chom.2008.02.015
16. Jeraldo P, Sipos M, Chia N, Brulc JM, Dhillon AS, Konkel ME, Larson CL, Nelson KE, Qu A, Schook LB, et al. Quantification of the relative roles of niche and neutral processes in structuring gastrointestinal microbiomes. Proc Natl Acad Sci U S A 2012; 109:9692-8; PMID:22615407; http://dx.doi.org/10.1073/pnas.1206721109
17. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto J-M, et al.; MetaHIT Consortium. Enterotypes of the human gut microbiome. Nature 2011; 473:174-80; PMID:21508958; http://dx.doi.org/10.1038/nature09944
18. Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J, Huttenhower C. Microbial co-occurrence relationships in the human microbiome. PLoS Comput Biol 2012; 8:e1002606; PMID:22807668; http://dx.doi.org/10.1371/journal.pcbi.1002606
19. Freilich S, Kreimer A, Meilijson I, Gophna U, Sharan R, Ruppin E. The large-scale organization of the bacterial network of ecological co-occurrence interactions. Nucleic Acids Res 2010; 38:3857-68; PMID:20194113; http://dx.doi.org/10.1093/nar/gkq118
20. Cody M. Diamond J. Ecology and Evolution of Communities. Belknap Press of Harvard University Press; 1975.
21. Stone L, Roberts A. The checkerboard score and species distributions. Oecologia 1990; 85:74-9; http://dx.doi.org/10.1007/BF00317345
22. Weiher E, Clarke PGD, Keddy PA. Community assembly rules, morphological dispersion, and the coexistence of plant species. Oikos 1998; 81:309-22; http://dx.doi.org/10.2307/3547051
23. Levy R, Borenstein E. Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. Proc Natl Acad Sci U S A 2013; 110:12804-9; PMID:23858463; http://dx.doi.org/10.1073/pnas.1300926110
24. Cornwell WK, Schwilk LDW, Ackerly DD. A trait-based test for habitat filtering: convex hull volume. Ecology 2006; 87:1465-71; PMID:16869422; http://dx.doi.org/10.1890/0012-9658(2006)87[1465:ATTFHF]2.0.CO;2
25. Emerson BC, Gillespie RG. Phylogenetic analysis of community assembly and structure over space and time. Trends Ecol Evol 2008; 23:619-30; PMID:18823678; http://dx.doi.org/10.1016/j.tree.2008.07.005
26. Borenstein E, Kupiec M, Feldman MW, Ruppin E. Large-scale reconstruction and phylogenetic analysis of metabolic environments. Proc Natl Acad Sci U S A 2008; 105:14482-7; PMID:18787117; http://dx.doi.org/10.1073/pnas.0806162105
27. Janga SC, Babu MM. Network-based approaches for linking metabolism with environment. Genome Biol 2008; 9:239; PMID:19040774; http://dx.doi.org/10.1186/gb-2008-9-11-239
28. Levy R, Borenstein E. Reverse Ecology: from systems to environments and back. Adv Exp Med Biol 2012; 751:329-45; PMID:22821465; http://dx.doi.org/10.1007/978-1-4614-3567-9_15
29. 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; PMID:22674335; http://dx.doi.org/10.1126/science.1224203
30. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014; 505:559-63; PMID:24336217; http://dx.doi.org/10.1038/nature12820
31. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013; 341:1241214; PMID:24009397; http://dx.doi.org/10.1126/science.1241214
32. Human Microbiome Project Consortium. A framework for human microbiome research. Nature 2012; 486:215-21; PMID:22699610; http://dx.doi.org/10.1038/nature11209
33. O'Dwyer JP, Kembel SW, Green JL. Phylogenetic diversity theory sheds light on the structure of microbial communities. PLoS Comput Biol 2012; 8:e1002832; PMID:23284280; http://dx.doi.org/10.1371/journal.pcbi.1002832
34. Zhang Z, Geng J, Tang X, Fan H, Xu J, Wen X, Ma ZS, Shi P. Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota. ISME J 2013; PMID:24132077; http://dx.doi.org/10.1038/ismej.2013.185
35. Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. Nature 2013; 501:426-9; PMID:23955152; http://dx.doi.org/10.1038/nature12447
36. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature 2013; 502:96-9; PMID:23995682; http://dx.doi.org/10.1038/nature12503
37. Freter R, Brickner H, Botney M, Cleven D, Aranki A. Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora. Infect Immun 1983; 39:676-85; PMID:6339388
38. Foster KR, Bell T. Competition, not cooperation, dominates interactions among culturable microbial species. Curr Biol 2012; 22:1845-50; PMID:22959348; http://dx.doi.org/10.1016/j.cub.2012.08.005
39. Roberts A, Stone L. Advantageous indirect interactions in systems of competition. J Theor Biol 2004; 228:367-75; PMID:15135035; http://dx.doi.org/10.1016/j.jtbi.2004.01.013
40. Tilman D. Competition and biodiversity in spatially structured habitats. Ecology 1994; 75:2-16; http://dx.doi.org/10.2307/1939377
41. Nava GM, Friedrichsen HJ, Stappenbeck TS. Spatial organization of intestinal microbiota in the mouse ascending colon. ISME J 2011; 5:627-38; PMID:20981114; http://dx.doi.org/10.1038/ismej.2010.161
42. Chase JM. Stochastic community assembly causes higher biodiversity in more productive environments. Science 2010; 328:1388-91; PMID:20508088; http://dx.doi.org/10.1126/science.1187820
43. Schluter J, Foster KR. The evolution of mutualism in gut microbiota via host epithelial selection. PLoS Biol 2012; 10:e1001424; PMID:23185130; http://dx.doi.org/10.1371/journal.pbio.1001424
44. Johansson ME, Larsson JM, Hansson GC. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proc Natl Acad Sci U S A 2011; 108(Suppl 1):4659-65; PMID:20615996; http://dx.doi.org/10.1073/pnas.1006451107
45. Mahowald MA, Rey FE, Seedorf H, Turnbaugh PJ, Fulton RS, Wollam A, Shah N, Wang C, Magrini V, Wilson RK, et al. Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proc Natl Acad Sci U S A 2009; 106:5859-64; PMID:19321416; http://dx.doi.org/10.1073/pnas.0901529106
46. Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng J-F, Darling A, Malfatti S, Swan BK, Gies EA, et al. Insights into the phylogeny and coding potential of microbial dark matter. Nature 2013; 499:431-7; PMID:23851394; http://dx.doi.org/10.1038/nature12352
47. Carr R, Shen-Orr SS, Borenstein E. Reconstructing the genomic content of microbiome taxa through shotgun metagenomic deconvolution. PLoS Comput Biol 2013; 9:e1003292; PMID:24146609; http://dx.doi.org/10.1371/journal.pcbi.1003292
48. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013; 31:814-21; PMID:23975157; http://dx.doi.org/10.1038/nbt.2676
49. Thiele I, Heinken A, Fleming RM. A systems biology approach to studying the role of microbes in human health. Curr Opin Biotechnol 2013; 24:4-12; PMID:23102866; http://dx.doi.org/10.1016/j.copbio.2012.10.001
50. Shoaie S, Karlsson F, Mardinoglu A, Nookaew I, Bordel S, Nielsen J. Understanding the interactions between bacteria in the human gut through metabolic modeling. Sci Rep 2013; 3:2532; PMID:23982459; http://dx.doi.org/10.1038/srep02532
51. Greenblum S, Chiu H-C, Levy R, Carr R, Borenstein E. Towards a predictive systems-level model of the human microbiome: progress, challenges, and opportunities. Curr Opin Biotechnol 2013; 24:810-20; PMID:23623295; http://dx.doi.org/10.1016/j.copbio.2013.04.001
52. Borenstein E. Computational systems biology and in silico modeling of the human microbiome. Brief Bioinform 2012; 13:769-80; PMID:22589385; http://dx.doi.org/10.1093/bib/bbs022
53. Morowitz MJ, Denef VJ, Costello EK, Thomas BC, Poroyko V, Relman DA, Banfield JF. Strain-resolved community genomic analysis of gut microbial colonization in a premature infant. Proc Natl Acad Sci U S A 2011; 108:1128-33; PMID:21191099; http://dx.doi.org/10.1073/pnas.1010992108
54. Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, Waller A, Mende DR, Kultima JR, Martin J, et al. Genomic variation landscape of the human gut microbiome. Nature 2013; 493:45-50; PMID:23222524; http://dx.doi.org/10.1038/nature11711