Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Systems Biology

Robustness of microbiome function

Kiseok Keith Lee[1], Yeonwoo Park[3] and Seppe Kuehn[1,2]

Abstract

Microbial communities perform metabolic processes that sustain life on Earth and promote human health. Microbial consortia sustain these functions in the face of constant structural and environmental perturbations. How do complex communities robustly sustain their functional properties despite perturbations? Most studies of functional robustness in the microbiome have been limited to biodiversity and functional redundancy, the idea that there are multiple members of the community that can sustain a specific function. Here, we propose that ideas from other complex biological systems may be applied to deepen our understanding of microbiome robustness. By surveying the causes of functional robustness in a variety of biological systems, including proteins and cells, and discussing how they can be applied to the microbiome, we build conceptual and experimental frameworks for understanding the functional robustness of microbial communities. We hope that these insights might help better predict and engineer microbiome function.

Addresses

[1] Department of Ecology and Evolution, The University of Chicago, Chicago, IL, 60637, USA

[2] Center for the Physics of Evolving Systems, The University of Chicago, Chicago, IL, 60637, USA

[3] Center for RNA Research, Institute for Basic Science, Seoul, 08826, South Korea

Corresponding author: Lee, Kiseok Keith (kiseoklee@uchicago.edu)

Current Opinion in Systems Biology 2023, **36:**100479

This review comes from a themed issue on **Systems Ecology & Evolution (2023)**

Edited by **Edo Kussell** and **Nobuto Takeuchi**

For a complete overview see the Issue and the Editorial

Available online 14 September 2023

https://doi.org/10.1016/j.coisb.2023.100479

Keywords

Robustness, Evolvability, Microbiome, Ecosystem function, Adaptive landscape, Neutral space, Functional redundancy, Environmental perturbation.

Introduction

Complex ecosystems across biological scales have persisted in the face of perturbations, including environmental shifts, invasions, mutations, and stochastic fluctuations in both the internal makeup and external circumstances. Not only have ecosystems maintained their core functions, such as the carbon and nitrogen cycling that sustains life on Earth, but they have also adapted to diverse niches. Such resilience is surprising because a complex system, consisting of many mutually dependent interacting parts, might be sensitive to perturbing any of its many parts or interactions. Therefore, it is important to ask why natural ecosystems are functionally robust. The advent of high-throughput methods for characterizing the structure and function of microbial communities offers an unprecedented opportunity to elucidate the robustness of microbiome function.

Robustness and its mechanisms have been extensively studied in macromolecules, gene regulatory networks, metabolic networks, and organismal development, but little is known about the extent and causes of robustness in microbiome function. We argue that adapting knowledge from other complex systems can foster new ideas and experiments to elucidate microbiome robustness, which would ultimately help engineer robust microbial communities with practical applications and predict the response of global microbiome function to climate change. To that end, we begin with a definition of robustness and its theoretical and practical importance. We review how microbiome robustness has been studied, and then explore mechanisms of robustness in other complex systems and how they might be applied to the microbiome.

Why study robustness?

Robustness is the persistence of an attribute in the face of perturbations. Similar concepts include buffering, canalization, homeorhesis, tolerance, resistance, or resilience. Robustness in the microbiome can be studied from both structural and functional perspectives. Structure refers to the taxonomic or genomic composition of the microbial community; a microbial community is structurally robust when its composition remains stable amidst perturbations. This review focuses on functional robustness because it is pertinent to engineering microbiome function. Here, we define microbiome function as a community-level metabolic trait that emerges from the collective activity and interactions of individuals, such as changes in microbial biomass, metabolic rate, or metabolite flux. Under this definition, function is not always equal to the potential to carry out a function (genes present). A microbial

community is functionally robust when it continues to retain its metabolic function in the face of environmental and structural perturbations. Environmental perturbations encompass changes in the abiotic environment, such as temperature, pH, carbon and nitrogen resources, oxygen level, and moisture. Structural perturbations include changes in the abundance of microbial species, immigration (invasion) or emigration, and genetic alterations through mutation, horizontal gene transfer, and recombination.

Studying robustness is important for both basic and applied science. How are key metabolic functions, such as nutrient cycling and metabolite flux in the gut and rhizosphere, sustained in the face of environmental fluctuations? Answering this question requires understanding what structural properties make complex systems more or less robust and how robustness emerges over time. Robustness is also key to understanding the relationship between microbiome structure and function [1]. Recent studies have uncovered tremendous structural variations across natural microbial communities, but it is unknown whether these structural variations imply functional variation, or whether they are neutral variations among functionally equivalent communities [2]. The answer depends on the degree of functional robustness because a robust system tolerates more variation. Robustness is also related to the ability of the microbiome to adapt to new environments—its evolvability [3,4]. Robustness and evolvability appear antagonistic at first, but theoretical and empirical evidence from across biological scales show that robustness promotes evolvability [3]. This is because robustness permits neutral variations to accumulate (also called cryptic genetic variation [5]), which serve as a source for innovation in new environments.

With respect to applications, engineering functional robustness is a key step in microbiome technologies. One of the biggest hurdles to microbiome-based therapeutics such as probiotics is ensuring a consistent effect across the spectrum of preexisting microbiota, dietary factors, drug usage history, and other patient-specific conditions. Because we cannot yet engineer robust microbial communities, current strategies utilize natural consortia that appear to be functionally robust for unknown reasons. One such example is the newly FDA-approved fecal microbiome transplant Rebyota. Likewise, a principal challenge in microbial solutions for agricultural applications is engineering robustness to heterogeneous field conditions. Furthermore, given the role of microbial communities in global carbon and nitrogen cycling, understanding robustness is essential for predicting and potentially mitigating the consequences of climate change.

How can we study robustness in microbial communities?

Rigorous investigation of robustness requires the microbial function and types of perturbations to be precisely defined and quantified. Examples of quantifiable functions include changes in total microbial biomass [6,7] and the consumption rate of electron acceptors or carbon sources [8–12] (Table 1). When studying host-associated microbial communities, microbiome function can be indirectly measured through the host's phenotype, such as its health state [13,14], resistance to pathogen invasion [15], plant growth, or lifespan/fecundity [16], although the exact microbial role in determining host phenotype may be unknown.

For perturbations, we can either perturb the environment or the structure of the microbiome. A common strategy to induce structural perturbation is to manipulate the relative or absolute taxonomic abundance by building synthetic communities from isolated strains [8,9,17–19]. High-throughput methods such as microfluidics and robotics allow a large structural variation space to be mapped, although exhaustive exploration is typically prohibitive. Another possibility is to alter the genetic makeup of the community using in-situ genome engineering tools, such as CRISPR- [20–22] and phage-based methods [23]. Although promising, these molecular methods have not yet been employed to study microbiome robustness.

The bottom-up approaches like building synthetic communities are limited in that they cannot approximate the complexity found in natural microbiomes. Although large-scale synthetic communities such as 119-strain hCom2 [15] have successfully recapitulated some aspects of natural complexity, such as robust pathogen resistance, they are still too simple compared with natural communities that typically consist of hundreds to thousands of strains. An alternative top-down approach is to take natural communities in their full complexity and indirectly perturb their structural composition by changing the environment.

For environmental perturbation, the conventional approach relies on studying the response of communities to natural variations in the environment. The best examples are microbiome surveys accompanied by environmental metadata, which have been performed for soil [11,26], ocean [27], plant [28], and human [29] microbiota (Table 1). While this approach preserves the complexity of natural communities, it is limited in scale and can only examine perturbations that nature has applied. Also, since different environmental factors change in a correlated fashion, it is hard to disentangle the effects of the many environmental variables,

Table 1

Quantifiable microbiome functions and methods of structural and environmental perturbation.

(1) Measurable microbiome function	Non-host associated	• Microbial biomass [6,7,24] • Metabolite consumption/production flux [8–12] • Antibiotic resistance [25]
	Host-associated	• Human health [13,14], pathogen resistance [15] • Plant growth, health • Fly's fecundity and life span [16]
(2-1) Methods for structural perturbation	Synthetic community approaches [8,9,17–19]	
	In-situ microbiome engineering	• Phage-based [23] • CRISPR-based [20–22]
(2-2) Methods for environmental perturbation	Naturally occurring environmental variation	• Soil [11,26] • Ocean [27] • Plant [28] • Human [29] • Microbial mats [30]
	Controlled lab or in-situ environmental perturbation	• Soil [31–34] • Ocean [6,12] • Plant [28,35] • Mouse [13] • Human [14,36]

limiting causal statements that can be made. Without in situ or laboratory experiments, microbiome function can only be inferred by mapping DNA sequences to functional ortholog databases or by analyzing the abundance of particular functional genes, such as antibiotic-resistance genes. These limits can be overcome in a hybrid approach that performs controlled laboratory experiments on sampled natural communities [6,12–14,28,31–33,36,37] (Table 1). Functional measurements on samples in nature can be made alongside induced environmental perturbations in a controlled laboratory setting.

An open question is how robustness to structural and environmental perturbations are interrelated. In other biological systems, the two types of robustness are typically correlated. Proteins that are robust to mutations are also robust to temperature and chemical fluctuations because both types of robustness can be conferred by the same mechanism—enhanced thermodynamic stability [2]. Likewise, the structural and environmental robustness of gene regulatory networks and metabolic pathways can also have similar mechanistic bases [2]. This implies that a general understanding of microbiome robustness could be achieved regardless of the particular choice of perturbation.

Current state of knowledge

Mechanisms for robustness in microbiome function remain under-explored. Most studies of microbiome robustness have focused on structural robustness [30,32,38]. Global microbiome surveys and large-scale perturbation experiments generally lack functional measurements [30,33,39], and studies that do obtain functional data have overlooked the question of robustness and evolvability [9,18,40,41]. The few studies that address functional robustness have largely focused on functional redundancy [42–45] and biodiversity as possible mechanisms [24,46–49] (Table 2). Therefore, there is a need for a comprehensive review of mechanisms underlying functional robustness in other complex systems, which can serve as guiding hypotheses for studying the robustness of microbiome function. The remainder of this review presents conceptual frameworks and mechanisms of robustness established from other biological systems and discusses their potential application to the microbiome.

Mechanisms of robustness in other biological systems

Conceptual frameworks to study robustness have been established by a large body of work on macromolecules, gene regulatory networks, and metabolic pathways. These frameworks can be classified broadly as (1) adaptive landscape thinking, which encompasses mechanisms such as functional redundancy, distributed function, and plasticity, and (2) network structure thinking, which includes mechanisms such as modularity (Table 2). Lastly, there is (3) theoretical ecology thinking, which is derived from studies of macro-ecosystems.

Adaptive landscape thinking

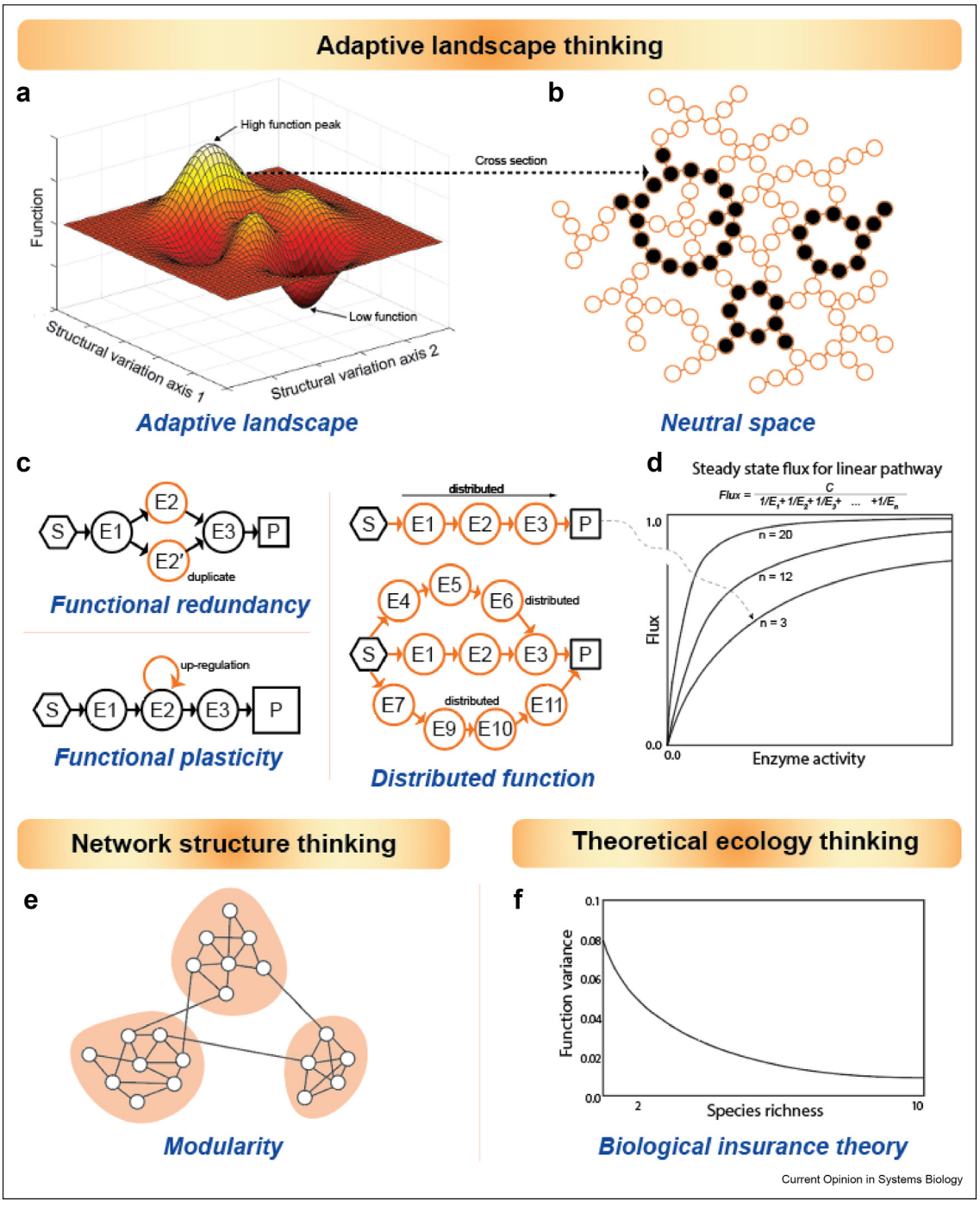
Adaptive landscape thinking has recently been applied to microbial community function [1]. An adaptive landscape (Figure 1a) is a mapping from the space of all possible

Table 2

Mechanisms of robustness in a variety of biological systems.

Mechanism of robustness		RNA	Protein	Genetic network	Protein interaction/metabolic network	Communities
Adaptive landscape thinking	Large neutral space	• Ribozyme [67,68]	• Fluorescent proteins [5,69] • Beta-lactamase [63]	• Feed forward signaling network [70] • Gene regulatory networks [71]	• Human protein interaction network [72]	• *E. coli* populations [73]
	Functional Redundancy	• Stem and loop (secondary) RNA structure [2]	• Serine protease family [62] • PDZ family [58] • Beta-lactamase [63]	• Yeast genetic network [54] • Yeast ribosomal regulation network [74]	• Human metabolism [75] • Yeast metabolic network [76] • Carotenoid network [77]	• Soil microbiome [52] • Ocean microbiome [44] • Human gut [45] • Meta-study [42]
	Distributed function	• Stem and loop (secondary) RNA structure [2]	• Serine protease family [62] • PDZ family [58] • Beta-lactamase [63]	• Fly segmentation and cooperativity [78]	• *E. coli* genome-scale metabolic network [79] • In silico evolved metabolic networks [80]	
	Functional plasticity			• Aphid colonizing gene cluster [81] • Macrophage polarization [82] • *E. coli* synthetic genetic network [83]	• Oyster ATP, lipid metabolism [84] • *E. coli*, Yeast, Human metabolic network [85]	
Network structure thinking	Modularity	• Ribozyme [67]	• Serine protease family [62] • PDZ family [58] • Beta-lactamase [63] • Protein solved tertiary structures [86]	• Embryonic development [4,87] • Gene activity phenotype [57] • Yeast ribosomal regulation network [74]	• Ribosomal protein network [88] • In silico metabolic network [89]	• Microarthropod metapopulation [55] • Human gut microbiome [14] • Soil microbiome [61]
Theoretical ecology thinking	Biological insurance theory (Biodiversity)					• Synthetic bacterial community [24] • Aquatic microbiome [46,47] • Soil microbiome [48,49]

Figure 1



Conceptual frameworks for studying robustness and known mechanisms of robustness. (a) In this schematic adaptive landscape, the two horizontal axes represent structural variations and the vertical axis represents function. **(b)** A neutral network is a group of variants with the same functionality. Here, each node represents a structural variant of the system (e.g., a genotype or a microbial community with a particular composition), colored black when their functionality is identical. Edges indicate that the two nodes are related by a point mutation or by a change in the presence or absence of a single taxon. All structural states that correspond to this flat area of the functional landscape are functionally identical but structurally distinct. **(c)** Metabolic networks illustrating functional redundancy, distributed function, and functional plasticity. S, substrate; E, enzymes; P, product. **(d)** Flux (amount of input substrate converted into output product per unit time) as a function of enzyme activity in a linear metabolic pathway. E, enzyme; C, a constant; *n*, number of enzymes (adapted from Ref. [2]). **(e)** Co-occurrence network with three modules illustrating modularity (adapted from Ref. [50]). Each node represents a microbial taxon, and the edge indicates that the two nodes are highly correlated in their abundance in a pool of samples. **(f)** Relationship between variation in function and species richness (number of species present in a community). This diagram illustrates how biodiversity can reduce functional variance, thus increasing the system's robustness to environmental fluctuations (adapted from Ref. [51]).

structural variations to function. For a simple DNA molecule of two nucleotides, the space of possible variations can be drawn on a two-dimensional plane, each axis representing the four possible bases at a site. The function of each genotype can then be plotted as the height. A real adaptive landscape is high-dimensional and cannot be directly visualized, but it offers a powerful way to conceptualize how the structure—function mapping shapes robustness and evolvability. Evolution under natural selection can be conceptualized as climbing the landscape; mutations randomly propose points in the landscape to move to, and natural selection preferentially drives the system to points with higher fitness. If the landscape has a single peak, selection would drive the system towards the peak. If there are many peaks (a rugged landscape), evolution could get stuck in a local optimum. In a rugged landscape, the outcome of evolution is sensitive to the starting point and particular variations sampled during evolution. Once the system reaches a peak, it could remain in that peak or fall because of structural or environmental perturbations. If the system retains its function despite perturbations, we can call the system robust.

The structure of a microbial community with n possible species can be described by a vector recording the abundance or presence/absence of each species: $X = (X_1, X_2, X_3, \ldots, X_n)$. This n-dimensional vector describes the space of all possible structural variations, which can be mapped to a function of interest: $F(X)$. Structural perturbations change the coordinates of the system in this space, potentially changing the functional level. Environmental perturbations can change the coordinates by altering the structural composition X, or change how the structure maps to function (the function F), in which case the function of the consortium will no longer be the same despite having the same structure.

What makes a microbial community robust? In the adaptive landscape language, a system is robust when it has a large neutral space. A neutral space is a collection of structural variations with the same functionality. It can be visualized as a horizontal slice of the landscape (Figure 1b). A system at the peak is robust if the peak is broad. In macromolecules, the space of possible genotypes can be arranged on a graph, where each node represents a genotype, and two nodes are connected if they are related by a point mutation. We can then speak of a "neutral network"—a network of connected nodes with the same functionality (Figure 1b). For the microbiome, each node can represent a community with a particular species presence/absence: e.g., $X_i = (0, 1, 1, 0)$. Two nodes are connected if they can be interconverted by adding or subtracting a single species: e.g., $X_j = (1, 1, 1, 0)$. Thus, we can quantify robustness by observing the number of nodes in a system that are connected in the neutral network. If the current node is surrounded by k layers of neutral nodes, it would be robust to perturbations affecting up to k species. In summary, a large neutral network confers robustness to structural perturbations and therefore also to environmental perturbations that do not dramatically alter the landscape itself.

Causes of large neutral space in microbial communities

Three major mechanisms can expand the neutral space: (1) functional redundancy, (2) distributed function, and (3) plasticity. Functional redundancy refers to two or more parts of a system independently performing the same function [2], which allows the parts to change freely as long as one of them remains functional. Functional redundancy has been the predominant focus of studies in microbiome robustness [42—45,52], where it can be defined as the fraction of community members performing overlapping functions. It is typically measured by reconstructing the genomic content of each taxon through metagenomic or metatranscriptomic sequencing, and then functionally annotating the genomes and characterizing the degree of predicted functional overlap between taxa. This approach has revealed that there exists a large overlap of functions performed by community members that potentially cause structural or functional robustness [43,44,52].

Two questions remain, however. First, does the same functional category indicate true functional equivalence? Current gene ontology terms may not be sharp enough to distinguish subtle but consequential functional differences. Fundamentally, gene ontology terms cannot be a substitute for the collective function of the microbial consortium because it is often unclear how gene-level functions manifest at the community level. One way to address this limitation is to experimentally characterize and compare individual taxa. Second, is functional redundancy the major mechanism of robustness? Functional redundancy is widespread in eukaryotic gene regulatory networks, with up to 50% of genes in a genome having paralogs [2,53]. However, when thousands of genes were individually knocked out in yeast, as many as 40% of the genes with weak or no fitness effect were single-copy genes [54]. This indicates that gene-level redundancy is only partly responsible for organismal robustness. Microbiome function may similarly possess other sources of robustness.

A much less explored alternative mechanism in the context of microbiome is distributed function. A function is distributed when many parts contribute to it with different roles [2]. To demonstrate how a distributed function can lead to robustness, let us consider a linear metabolic pathway (Figure 1c) whose function can be quantified as the amount of substrate converted into product per unit time (flux). Under the Michaelis—Menten kinetics, the flux is a hyperbolic

function of each enzyme's activity. The more enzymes are involved in the pathway, the more concave the activity-flux relationship becomes (Figure 1d), which causes the flux to be more robust to changes in the activity of individual enzymes. The hyperbolic activity-flux relationship is prevalent across many organisms [2]. More complex metabolic pathways can evolve further robustness by having multiple independent sub-paths to the same product, with each sub-path distributed across multiple enzymes (Figure 1d). Compared to a microbial community where one generalist species performs an entire chain of metabolic reactions, distributed function through multiple specialist species may exhibit greater robustness.

Functional plasticity is a third mechanism for larger neutral space (Figure 1c). A system is functionally plastic if its components can adjust to environmental perturbations to maintain the overall functional output. In gene regulatory networks, feedback at multiple levels, including chromatin remodeling, transcriptional output, and post-transcriptional control, ensures robust execution of the genetic program. Each network can function in a wider range of environments, which allows different networks to be joined in a neutral network. A microbiome would be functionally plastic if the overall metabolic trait can be maintained not by changing its composition but through composition-independent mechanisms such as changing gene expression levels and controlling enzyme activities. Combining meta-transcriptomics, metabolomics, and proteomics has the potential to provide a powerful means to characterize the functional plasticity of the microbiome.

Network structure thinking

Network structure thinking promotes the search for simplicity within a complex structure. This approach differs from adaptive landscape thinking, which emphasizes the positive effects of complexity on the size of the neutral network. A biological system can be represented as a network where each node corresponds to a component (e.g., a microbial taxon) and a link denotes an interaction. This is distinct from the neutral network, where nodes represent structural variants of a system (e.g., a microbial community with a particular composition) rather than its components. Simple patterns in the network structure, such as modularity, can provide robustness against perturbations.

Modularity and robustness

In a modular system, interactions are concentrated on parts within a group or module, with parts in different modules acting largely independently of each other [4,50,55–57] (Figure 1e). Modularity can enhance robustness by limiting the number of system parts affected by perturbations within particular modules. Modularity can also facilitate evolvability [58] by allowing independent structural changes without disrupting other adapted modules [56]. Modularity is thought to evolve by selection for robustness in fluctuating environments [57] or selection for functional specialization [59]. Modularity has been observed in macromolecules, gene regulatory networks, and metabolic pathways, where it reduces the fragility of the system to perturbations and minimizes adaptive trade-offs between parts (Table 2).

In the microbiome, modularity can be analyzed by detecting pairwise correlations of microbial taxa abundance, generating a co-occurrence network [60]. This method has been employed to show that modular structures increase the robustness of the microbiome against perturbations [61]. Another approach is to look for parts (or the low-dimensional space of components) that co-vary under perturbations. In proteins, modules of amino acid positions, called "protein sectors," were found to be functionally sensitive to mutations, while non-sector parts were functionally neutral and tolerant to environmental (ligand) changes [58,62,63]. This approach was applied to the gut microbial communities at various stages of infant development, where a co-varying group of microbial species, dubbed an "ecogroup," was found to enable the microbiome to adapt to perturbations [14]. Furthermore, with network time-series data, frameworks from temporal networks can be utilized to study robustness [64]. These approaches suggest the value of searching for simplicity in complex microbiome structures.

Theoretical ecology thinking

The relationship between species diversity and ecosystem function has been studied in theoretical ecology under the name of biological insurance theory (Table 2). Species diversity can be closely related to functional redundancy or distributed function, but this theory can help us understand diversity from a different angle. Species diversity can buffer ecosystem function against environmental perturbation [65] because the differential responses of species reduce the variability of the system (called the buffering or portfolio effect) [51] (Figure 1f). Different response times to perturbation also stabilize the ecosystem function against perturbation. Dispersal of species through space provides another layer of robustness, called spatial insurance [66]. Experiments and field studies have demonstrated that a greater number of species confers robustness to environmental perturbations in many ecological communities, including the microbiome [24,47,48] (Table 2). To better understand the causes of robustness, we must go beyond the simple number of species and investigate the detailed mechanisms enabled by diversity. We can begin by examining the role of differential functional/temporal responses and spatial dispersal.

Conclusion

The mechanisms of robustness in microbiome function can be better understood by leveraging knowledge from other complex biological systems, including what we have termed adaptive landscape thinking, network structure thinking, and theoretical ecology. Precise functional measurements and laboratory-controlled structural and environmental perturbations, including synthetic community experiments, are necessary to acquire data suitable for testing hypotheses about the mechanisms of robustness. Combining perspectives and experimental strategies from a variety of biological fields will help enhance our understanding and engineering of robust microbial communities.

Declaration of competing interest

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.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work was supported by the National Science Foundation Division of Emerging Frontiers EF 2025293 (S.K, K.K.L) and the National Science Foundation Division of Molecular and Cellular Biosciences MCB 2117477 (S.K, K.K.L).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. * Sanchez A, Bajic D, Diaz-Colunga J, Skwara A, Vila JCC, Kuehn S: **The community-function landscape of microbial consortia**. *Cell Syst* 2023, **14**:122–134.
This review explains in detail how landscape thinking can be applied to the microbiome.

2. Wagner A: *Robustness and evolvability in living systems: Princeton university press*. 2005.

3. Wagner A: **Robustness and evolvability: a paradox resolved**. *Proc Royal Soc B* 2008, **275**:91–100.

4. Kirschner M, Gerhart J: **Evolvability**. *Proc Natl Acad Sci USA* 1998, **95**:8420–8427.

5. * Zheng J, Payne JL, Wagner A: **Cryptic genetic variation accelerates evolution by opening access to diverse adaptive peaks**. *Science* 2019, **365**:347–353.
Authors use directed evolution experiments with *E. coli* to show how large neutral space can lead to evolvability. This suggests a positive relationship between robustness and evolvability.

6. Pontrelli S, Szabo R, Pollak S, Schwartzman J, Ledezma-Tejeida D, Cordero OX, Sauer U: **Metabolic cross-feeding structures the assembly of polysaccharide degrading communities**. *Sci Adv* 2022, **8**, eabk3076.

7. Ostrem Loss E, Thompson J, Cheung PLK, Qian Y: **Venturelli OS: carbohydrate complexity limits microbial growth and reduces the sensitivity of human gut communities to perturbations**. *Nat Ecol Evol* 2023, **7**:127–142.

8. ** Gowda K, Ping D, Mani M, Kuehn S: **Genomic structure predicts metabolite dynamics in microbial communities**. *Cell* 2022, **185**:530–546.
This study showed that it is possible to map structure to function. This is related to adaptive landscape thinking. The authors also demonstrated that metabolite dynamics, or flux, can be used as a function.

9. ** Clark RL, Connors BM, Stevenson DM, Hromada SE, Hamilton JJ, Amador-Noguez D, Venturelli OS: **Design of synthetic human gut microbiome assembly and butyrate production**. *Nat Commun* 2021, **12**:3254.
This study used butyrate production as a function. With regression and Lotka-Volterra models, they succeeded in mapping the community to function. This shows the importance of having precise functional measurements.

10. De Jesús Astacio LM, Prabhakara KH, Li Z, Mickalide H: **Kuehn S: closed microbial communities self-organize to persistently cycle carbon**. *Proc Natl Acad Sci USA* 2021, **118**, e2013564118.

11. Bahram M, Espenberg M, Pärn J, Lehtovirta-Morley L, Anslan S, Kasak K, Kõljalg U, Liira J, Maddison M, Moora M: **Structure and function of the soil microbiome underlying N2O emissions from global wetlands**. *Nat Commun* 2022, **13**:1430.

12. Nguyen TTH, Zakem EJ, Ebrahimi A, Schwartzman J, Caglar T, Amarnath K, Alcolombri U, Peaudecerf FJ, Hwa T, Stocker R, *et al.*: **Microbes contribute to setting the ocean carbon flux by altering the fate of sinking particulates**. *Nat Commun* 2022, **13**:1657.

13. Gibson TE, Kim Y, Acharya S, Kaplan DE, Dibenedetto N, Lavin R, Berger B, Allegretti JR, Bry L, Gerber GK: **Intrinsic instability of the dysbiotic microbiome revealed through dynamical systems inference at scale**. *bioRxiv* 2021, https://doi.org/10.1101/2021.1112.1114.469105.

14. ** Raman AS, Gehrig JL, Venkatesh S, Chang H-W, Hibberd MC, Subramanian S, Kang G, Bessong PO, Lima AAM, Kosek MN: **A sparse covarying unit that describes healthy and impaired human gut microbiota development**. *Science* 2019, **365**:140.
This study finds a group of species that confers robustness and evolvability under perturbations. This is connected to modularity because they were trying to detect a group of species that is less correlated with the rest of the community.

15. * Cheng AG, Ho P-Y, Aranda-Díaz A, Jain S, Feiqiao BY, Meng X, Wang M, Iakiviak M, Nagashima K, Zhao A, *et al.*: **Design, construction, and in vivo augmentation of a complex gut microbiome**. *Cell* 2022, **185**:3617–3636.
Authors succeed in constructing a synthetic community that is resistant to invasion. This shows that to some degree synthetic community construction can recapitulate robustness.

16. Gould AL, Zhang V, Lamberti L, Jones EW, Obadia B, Korasidis N, Gavryushkin A, Carlson JM, Beerenwinkel N, Ludington WB: **Microbiome interactions shape host fitness**. *Proc Natl Acad Sci USA* 2018, **115**, E11951–E11960.

17. Gopalakrishnappa C, Gowda K, Prabhakara KH, Kuehn S: **An ensemble approach to the structure-function problem in microbial communities**. *iScience* 2022, **25**:103761.

18. Baranwal M, Clark RL, Thompson J, Sun Z, Hero AO, Venturelli OS: **Recurrent neural networks enable design of multifunctional synthetic human gut microbiome dynamics**. *Elife* 2022, **11**, e73870.

19. Skwara A, Lemos-Costa P, Miller ZR, Allesina S: **Modelling ecological communities when composition is manipulated experimentally**. *Methods Ecol Evol* 2023, **14**:696–707.

20. Rubin BE, Diamond S, Cress BF, Crits-Christoph A, Lou YC, Borges AL, Shivram H, He C, Xu M, Zhou Z, *et al.*: **Species- and site-specific genome editing in complex bacterial communities**. *Nat Microb* 2022, **7**:34–47.

21. Marsh JW, Ley RE: **Microbiome engineering: taming the untractable**. *Cell* 2022, **185**:416–418.

22. Jin W-B, Li T-T, Huo D, Qu S, Li XV, Arifuzzaman M, Lima SF, Shi H-Q, Wang A, Putzel GG, *et al.*: **Genetic manipulation of gut microbes enables single-gene interrogation in a complex microbiome**. *Cell* 2022, **185**:547–562.

23. Voorhees PJ, Cruz-Teran C, Edelstein J, Lai SK: **Challenges & opportunities for phage-based in situ microbiome engineering in the gut**. *J Contr Release* 2020, **326**:106–119.

24. Awasthi A, Singh M, Soni SK, Singh R, Kalra A: **Biodiversity acts as insurance of productivity of bacterial communities under abiotic perturbations**. *ISME J* 2014, **8**:2445–2452.

25. Lamberte LE, van Schaik W: **Antibiotic resistance in the commensal human gut microbiota**. *Curr Opin Microbiol* 2022, **68**:102150.

26. Zhong Y, Yan W, Canisares LP, Wang S, Brodie EL, Schrodt F: **Alterations in soil pH emerge as a key driver of the impact of global change on soil microbial nitrogen cycling: evidence from a global meta-analysis**. *Global Ecol Biogeogr* 2022, **00**: 1–21.

27. Paoli L, Ruscheweyh H-J, Forneris CC, Hubrich F, Kautsar S, Bhushan A, Lotti A, Clayssen Q, Salazar G, Milanese A, *et al.*: **Biosynthetic potential of the global ocean microbiome**. *Nature* 2022, **607**:111–118.

28. Gao M, Xiong C, Gao C, Tsui CKM, Wang M-M, Zhou X, Zhang A-M, Cai L: **Disease-induced changes in plant microbiome assembly and functional adaptation**. *Microbiome* 2021, **9**:187.

29. Rao C, Coyte KZ, Bainter W, Geha RS, Martin CR, Rakoff-Nahoum S: **Multi-kingdom ecological drivers of microbiota assembly in preterm infants**. *Nature* 2021, **591**:633–638.

30. De Anda V, Zapata-Peñasco I, Blaz J, Poot-Hernández AC, Contreras-Moreira B, Gonzalez-Laffitte M, Gamez-Tamariz N, Hernandez-Rosales M, Eguiarte LE, Souza V: **Understanding the mechanisms behind the response to environmental perturbation in microbial mats: a metagenomic-network based approach**. *Front Microbiol* 2018, **9**:2606.

31. Fernández-Calviño D, Bååth E: **Growth response of the bacterial community to pH in soils differing in pH**. *FEMS Microbiol Ecol* 2010, **73**:149–156.

32. Xun W, Liu Y, Li W, Ren Y, Xiong W, Xu Z, Zhang N, Miao Y, Shen Q, Zhang R: **Specialized metabolic functions of keystone taxa sustain soil microbiome stability**. *Microbiome* 2021, **9**:35.

33. * Chase AB, Weihe C, Martiny JBH: **Adaptive differentiation and rapid evolution of a soil bacterium along a climate gradient**. *Proc Natl Acad Sci USA* 2021, **118**, e2101254118.
This study demonstrates how one can perturb the environment in the field. Although the authors don't investigate functional robustness, they observe the structural adaptation of the microbial communities to the new environment.

34. Goldford JE, Lu N, Bajić D, Estrela S, Tikhonov M, Sanchez-Gorostiaga A, Segrè D, Mehta P, Sanchez A: **Emergent simplicity in microbial community assembly**. *Science* 2018, **361**:469–474.

35. Van Der Voort M, Kempenaar M, Van Driel M, Raaijmakers JM: **Mendes R: impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression**. *Ecol Lett* 2016, **19**:375–382.

36. Creswell R, Tan J, Leff JW, Brooks B, Mahowald MA, Thieroff-Ekerdt R, Gerber GK: **High-resolution temporal profiling of the human gut microbiome reveals consistent and cascading alterations in response to dietary glycans**. *Genome Med* 2020, **12**:59.

37. Ossowicki A, Raaijmakers JM, Garbeva P: **Disentangling soil microbiome functions by perturbation**. *Environ Microbiol Rep* 2021, **13**:582–590.

38. Oña L, Kost C: **Cooperation increases robustness to ecological disturbance in microbial cross-feeding networks**. *Ecol Lett* 2022, **25**:1410–1420.

39. Mancuso CP, Lee H, Abreu CI, Gore J, Khalil AS: **Environmental fluctuations reshape an unexpected diversity-disturbance relationship in a microbial community**. *Elife* 2021, **10**, e67175.

40. ** Muller E, Algavi YM, Borenstein E: **A meta-analysis study of the robustness and universality of gut microbiome-metabolome associations**. *Microbiome* 2021, **9**:203.
Authors perform a meta-analysis of the available microbiome and metabolomics data. Here, the metabolomic profiles are the functions. Although they focused on mapping structure to function and making predictions, one can approach this data from a different angle by inquiring about functional robustness.

41. * Rothschild D, Leviatan S, Hanemann A, Cohen Y, Weissbrod O, Segal E: **An atlas of robust microbiome associations with phenotypic traits based on large-scale cohorts from two continents**. *PLoS One* 2022, **17**, e0265756.
This study maps the structure to function by using a large dataset of the human gut microbiome. This dataset can be used to ask about robustness.

42. Biggs CR, Yeager LA, Bolser DG, Bonsell C, Dichiera AM, Hou Z, Keyser SR, Khursigara AJ, Lu K, Muth AF, *et al.*: **Does functional redundancy affect ecological stability and resilience? A review and meta-analysis**. *Ecosphere* 2020, **11**, e03184.

43. Shade A: **Microbiome rescue: directing resilience of environmental microbial communities**. *Curr Opin Microbiol* 2023, **72**:102263.

44. * Royalty TM, Steen AD: **Functional redundancy in ocean microbiomes controls trait stability**. *bioRxiv* 2021, https://doi.org/10.1101/2021.1106.1118.448980.
In marine microbial communities, the authors were able to quantify functional redundancy by mapping genes to their KEGG Orthologs (KOs). They conclude that functional redundancy inferred from KOs leads to functional robustness.

45. * Tian L, Wang X-W, Wu A-K, Fan Y, Friedman J, Dahlin A, Waldor MK, Weinstock GM, Weiss ST, Liu Y-Y: **Deciphering functional redundancy in the human microbiome**. *Nat Commun* 2020, **11**:6217.
In the human gut microbiome, the authors infer functional redundancy by constructing bipartite networks of genomic content in the microbiome to quantify the overlapping functions among taxa. They found that higher functional redundancy correlates with stronger resistance to the invasion of donor microbiota.

46. Leary DJ, Petchey OL: **Testing a biological mechanism of the insurance hypothesis in experimental aquatic communities**. *J Anim Ecol* 2009, **78**:1143–1151.

47. Bouvier T, Venail P, Pommier T, Bouvier C, Barbera C, Mouquet N: **Contrasted effects of diversity and immigration on ecological insurance in marine bacterioplankton communities**. *PLoS One* 2012, **7**, e37620.

48. * Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MGA: **Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning**. *Nat Commun* 2019, **10**:4841.
This study investigates how the diversity of the soil microbial community affects ecosystem function related to nutrient cycling. They report that communities with lower diversity perform fewer functions.

49. Chen Q-L, Ding J, Zhu Y-G, He J-Z, Hu H-W: **Soil bacterial taxonomic diversity is critical to maintaining the plant productivity**. *Environ Int* 2020, **140**:105766.

50. Newman MEJ: **Modularity and community structure in networks**. *Proc Natl Acad Sci USA* 2006, **103**:8577–8582.

51. Yachi S, Loreau M: **Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis**. *Proc Natl Acad Sci USA* 1999, **96**:1463–1468.

52. Chen H, Ma K, Lu C, Fu Q, Qiu Y, Zhao J, Huang Y, Yang Y, Schadt CW, Chen H: **Functional redundancy in soil microbial community based on metagenomics across the globe**. *Front Microbiol* 2022, **13**:878978.

53. Rubin GM, Yandell MD, Wortman JR, Gabor GL, Miklos, Nelson CR, Hariharan Ik, Fortini ME, Li PW, Apweiler R: **comparative genomics of the eukaryotes**. *Science* 2000, **287**: 2204–2215.

54. Wagner A: **Robustness against mutations in genetic networks of yeast**. *Nat Genet* 2000, **24**:355–361.

55. Gilarranz LJ, Rayfield B, Liñán-Cembrano G, Bascompte J, Gonzalez A: **Effects of network modularity on the spread of perturbation impact in experimental metapopulations**. *Science* 2017, **357**:199–201.

56. * Hernández U, Posadas-Vidales L, Espinosa-Soto C: **On the effects of the modularity of gene regulatory networks on phenotypic variability and its association with robustness**. *Biosystems* 2022, **212**:104586.
This study shows computationally how modularity in gene regulatory networks can enhance functional robustness.

57. Espinosa-Soto C: **On the role of sparseness in the evolution of modularity in gene regulatory networks**. *PLoS Comput Biol* 2018, **14**, e1006172.

58. McLaughlin Jr RN, Poelwijk FJ, Raman A, Gosal WS, Ranganathan R: **The spatial architecture of protein function and adaptation**. *Nature* 2012, **491**:138–142.

59. Espinosa-Soto C, Wagner A: **Specialization can drive the evolution of modularity**. *PLoS Comput Biol* 2010, **6**, e1000719.

60. Matchado MS, Lauber M, Reitmeier S, Kacprowski T, Baumbach J, Haller D, List M: **Network analysis methods for studying microbial communities: a mini review**. *Comput Struct Biotechnol J* 2021, **19**:2687–2698.

61. Hernandez DJ, David AS, Menges ES, Searcy CA, Afkhami ME: **Environmental stress destabilizes microbial networks**. *ISME J* 2021, **15**:1722–1734.

62. Halabi N, Rivoire O, Leibler S, Ranganathan R: **Protein sectors: evolutionary Units of three-dimensional structure**. *Cell* 2009, **138**:774–786.

63. Stiffler MA, Hekstra DR, Ranganathan R: **Evolvability as a function of Purifying selection in TEM-1 β-Lactamase**. *Cell* 2015, **160**:882–892.

64. Li A, Cornelius SP, Liu YY, Wang L, Barabási AL: **The fundamental advantages of temporal networks**. *Science* 2017, **358**:1042–1046.

65. * Loreau M, Barbier M, Filotas E, Gravel D, Isbell F, Miller SJ, Montoya JM, Wang S, Aussenac R, Germain R: **Biodiversity as insurance: from concept to measurement and application**. *Biol Rev* 2021, **96**:2333–2354.
This review explains how biodiversity can increase the functional robustness of a community, using biodiversity insurance theory.

66. Loreau M, Mouquet N, Gonzalez A: **Biodiversity as spatial insurance in heterogeneous landscapes**. *Proc Natl Acad Sci USA* 2003, **100**:12765–12770.

67. Hayden EJ, Bendixsen DP, Wagner A: **Intramolecular phenotypic capacitance in a modular RNA molecule**. *Proc Natl Acad Sci USA* 2015, **112**:12444–12449.

68. Rotrattanadumrong R, Yokobayashi Y: **Experimental exploration of a ribozyme neutral network using evolutionary algorithm and deep learning**. *Nat Commun* 2022, **13**:4847.

69. Zheng J, Guo N, Wagner A: **Selection enhances protein evolvability by increasing mutational robustness and foldability**. *Science* 2020, **370**, eabb5962.

70. Fernández P, Solé RV: **Neutral fitness landscapes in signalling networks**. *J R Soc Interface* 2007, **4**:41–47.

71. Yang C-H, Scarpino SV: **A family of fitness landscapes modeled through gene regulatory networks**. *Entropy* 2022, **24**:622.

72. Burke DF, Bryant P, Barrio-Hernandez I, Memon D, Pozzati G, Shenoy A, Zhu W, Dunham AS, Albanese P, Keller A, *et al.*: **Towards a structurally resolved human protein interaction network**. *Nat Struct Mol Biol* 2023, **30**:216–225.

73. Karve S, Wagner A: **Environmental complexity is more important than mutation in driving the evolution of latent novel traits in E. coli**. *Nat Commun* 2022, **13**:5904.

74. Tanay A, Regev A, Shamir R: **Conservation and evolvability in regulatory networks: the evolution of ribosomal regulation in yeast**. *Proc Natl Acad Sci USA* 2005, **102**:7203–7208.

75. Gómez-Romero L, López-Reyes K, Hernández-Lemus E: **The large scale structure of human metabolism reveals resilience via extensive signaling crosstalk**. *Front Physiol* 2020, **11**:588012.

76. Harrison R, Papp B, Pál C, Oliver SG, Delneri D: **Plasticity of genetic interactions in metabolic networks of yeast**. *Proc Natl Acad Sci USA* 2007, **104**:2307–2312.

77. Badyaev AV, Morrison ES: **Emergent buffering balances evolvability and robustness in the evolution of phenotypic flexibility**. *Evolution* 2018, **72**:647–662.

78. Burz DS, Rivera-Pomar Rolando, Jäckle Herbert, Steven D: **Hanes: cooperative DNA-binding by Bicoid provides a mechanism for threshold-dependent gene activation in the Drosophila embryo**. *EMBO J* 1998, **17**:5998–6009.

79. Aguilar-Rodríguez J, Wagner A: **Metabolic determinants of enzyme evolution in a genome-scale bacterial metabolic network**. *Genome Biol Evol* 2018, **10**:3076–3088.

80. Soyer OS, Pfeiffer T: **Evolution under fluctuating environments explains observed robustness in metabolic networks**. *PLoS Comput Biol* 2010, **6**, e1000907.

81. Mathers TC, Chen Y, Kaithakottil G, Legeai F, Mugford ST, Baa-Puyoulet P, Bretaudeau A, Clavijo B, Colella S, Collin O, *et al.*: **Rapid transcriptional plasticity of duplicated gene clusters enables a clonally reproducing aphid to colonise diverse plant species**. *Genome Biol* 2017, **18**:27.

82. Liu SX, Gustafson HH, Jackson DL, Pun SH, Trapnell C: **Trajectory analysis quantifies transcriptional plasticity during macrophage polarization**. *Sci Rep* 2020, **10**:12273.

83. Nagy-Staron A, Tomasek K, Caruso Carter C, Sonnleitner E, Kavčič B, Paixão T, Guet CC: **Local genetic context shapes the function of a gene regulatory network**. *Elife* 2021, **10**, e65993.

84. Li A, Li L, Zhang Z, Li S, Wang W, Guo X, Zhang G: **Noncoding variation and transcriptional plasticity promote thermal adaptation in oysters by altering energy metabolism**. *Mol Biol Evol* 2021, **38**:5144–5155.

85. Klein B, Holmér L, Smith KM, Johnson MM, Swain A, Stolp L, Teufel AI, Kleppe AS: **A computational exploration of resilience and evolvability of protein–protein interaction networks**. *Commun Biol* 2021, **4**:1352.

86. Rorick MM, Wagner GP: **Protein structural modularity and robustness are associated with evolvability**. *Genome Biol Evol* 2011, **3**:456–475.

87. Ciliberti S, Martin OC, Wagner A: **Innovation and robustness in complex regulatory gene networks**. *Proc Natl Acad Sci USA* 2007, **104**:13591–13596.

88. Timsit Y, Sergeant-Perthuis G, Bennequin D: **Evolution of ribosomal protein network architectures**. *Sci Rep* 2021, **11**:625.

89. Hintze A, Adami C: **Evolution of complex modular biological networks**. *PLoS Comput Biol* 2008, **4**:e23.