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Incorporating functional trade-offs into studies of the gut microbiota

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Trade-offs constrain evolution through genetic linkages and environmental limitations, impacting organismal physiology, morphology, and behavior. They are likely to also play a role in modulating functions of the microbiota, but previous research has not included tests of trade-off theory. Here, we review broadly how gut microbial functions are typically studied and outline evolutionarily-informed mechanisms to improve such research. These include measuring a diverse set of functions, with a focus on changes in host phenotype; more explicitly articulating the selective forces relevant to the microbiota; and using functionally relevant models. We present dietary intervention as a case study where trade-offs are likely to be relevant and discuss how the health effects of the modern human diet could be better understood in light of trade-offs. Appreciating microbial functional trade-offs as well as host trade-offs will be necessary to design effective interventions targeting the microbiota and, more generally, to understand the evolution of host-microbe interactions.

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Introduction

Animals aim to optimize their growth and reproductive success under environmental constraints. Traits typically cannot increase without consequent decreases in others: for example, higher fecundity is associated with reduced longevity, body size, and/or offspring size [1,2]. These trade-offs play a fundamental role in the study of animal physiology, morphology, and behavior, and they provide a framework for understanding how traits evolve. The gut microbiota are an integral part of animal biology, increasingly recognized for their role in host health and disease. Gut microbiota effects are felt on metabolism [3], immunity [4], behavior [5], and other diverse physiological traits [e.g. Refs. 6,7]. Evolutionary theory indicates that beneficial functions are likely promoted by host evolution to control the microbiota, but the microbiota are subject to selection from the environment and interactions with competitors as well as host control [8••]. The balance of these differing selective pressures likely underlie variability in microbial behavior, and understanding them within the evolutionary framework of trade-offs is necessary to direct efforts to maintain beneficial functions.

Even when selection is not expected to act directly on some traits, microbial functions may be lost or gained under changing conditions due to trade-offs experienced at the host and the microbial level [9]. Many microbial functions, whether conferred by a single taxon or a community, behave in a non-independent manner just as host phenotypes are tied through genetic linkages, pleiotropy, and developmental constraints. The increase in one function often comes at the cost of another. These costs can come from the reallocation of resources between traits, the increased risk of mortality that comes with acquiring additional resources to support extended traits, or the incompatibility between specialized and generalist traits [9]. If the cost is too high, the enhancement of a function will never be selected for. Understanding the trade-offs between microbial functions will be necessary to understand what is possible in the gut environment and thus how changes in selective pressure will actually play out. To date, however, trade-offs have not been explicitly considered in studies of gut microbiota function.

In this review, we advocate for explicit consideration of functional trade-offs in the design of host microbiota studies as a way to increase our understanding of gut microbiota functioning. We first review methods for studying microbial function and note the traditional absence of trade-offs in these studies. We then provide suggestions on how to design future work on the microbiota to consider trade-offs, presenting dietary intervention as a case study where such an evolutionary perspective would be helpful. Finally, we close with thoughts on the implications for understanding human health.

Methods for studying microbiota functions

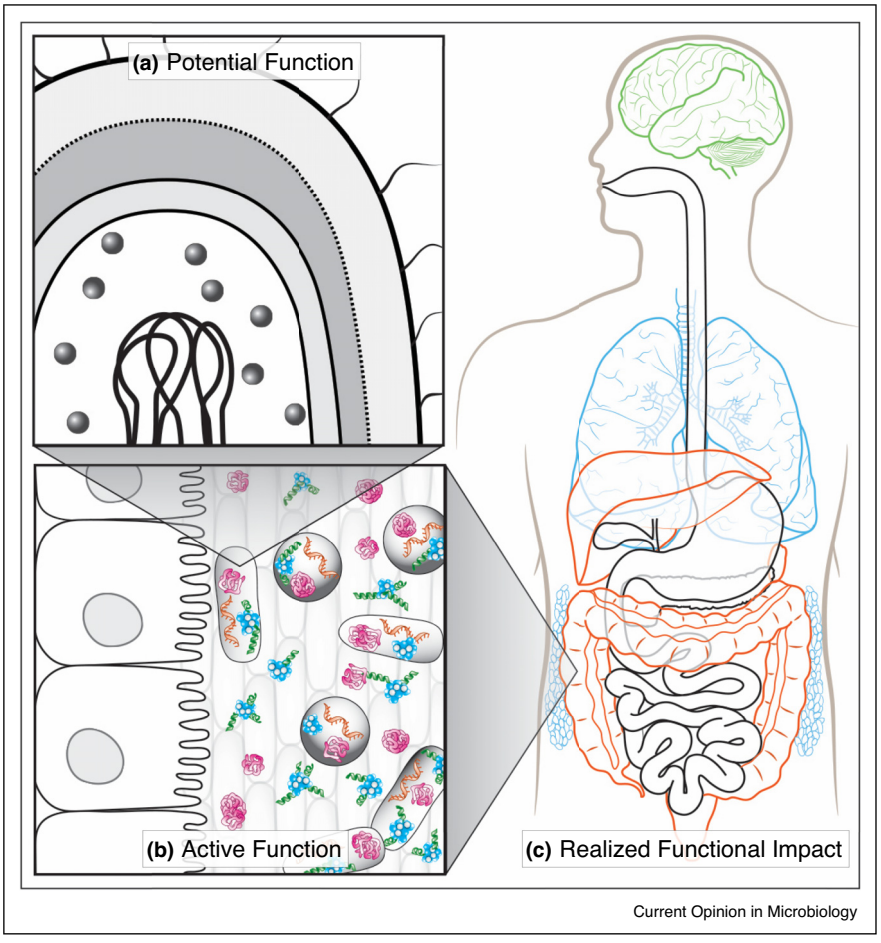
An extensive review of gut microbial functioning is beyond the scope of this paper (for a recent treatment see Ref. [10•]). Here, instead, we consider representative reports of different approaches to study functioning of the

gut microbiota. These studies can be broken up by the type of methods used, but also the scales considered — there are different spatial and temporal scales, of course, but also different scales of influence. These scales of influence include, what we term, potential function, active function, and realized functional impact (Figure 1).

Studies of potential function typically use genomic approaches, both untargeted shotgun metagenomics and targeted PCR or whole-genome sequencing techniques, to characterize the functional genes carried by the microbiota. Previous work has highlighted the types of roles that gut microbes can play, including carrying out specific metabolic and signaling pathways as well as producing antibiotics and other compounds [11–14]. It has also been noted that potential function is much more highly conserved than microbial composition between individuals [15,16]. These studies are limited, however, by our ability to annotate genes with known functions and, oftentimes, they are focused on overly general groupings of functions or pathways (e.g. carbon metabolism). Improvements are being made to annotation and analytical platforms [e.g. Refs. 17,18], but ultimately these types of studies will be incapable of discerning what microbial genes are active and what the implications of their activity are.

To assess active function, one can instead utilize genomic or molecular approaches which focus on actual microbial behavior, including transcriptomics, proteomics, and metabolomics. For example, alterations in microbial metabolism have been found in response to diet variation [19], infant development [20], and drug treatment [21,22]. Individual microbial taxa can have measurable effects on the fecal metabolome [23] and produce specialized metabolites that interact with host G-protein coupled receptors [24•]. These approaches have the advantage of only capturing active processes, but they still have

Figure 1



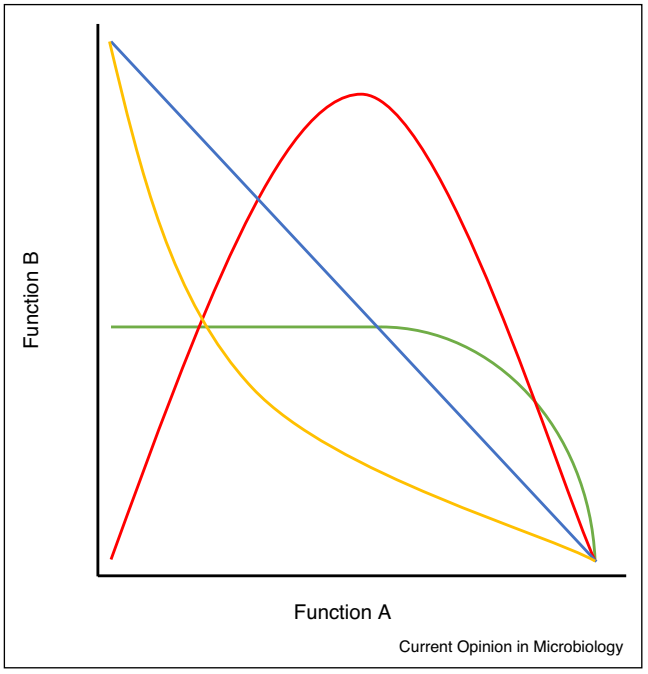
Scales at which to study gut microbiota function.
(a) Studies of potential function aim to identify functional genes carried by the microbiota. **(b)** Studies of active function capture the behavior of the microbiota through measuring transcripts (red), metabolites (green and blue), and proteins (pink). **(c)** Studies of realized functional impact measure the effect of the microbiota on those host including changes in host gene expression (red), physiology (blue), and behavior (green).

interpretive limitations. Transcriptomics, like metagenomics, can only assess annotated functions [25] and may not map directly onto protein expression, while metabolomic and proteomic screens are limited by the lack of comprehensive standards and the fact they cannot necessarily distinguish between host-sourced and microbially-sourced molecules [26]. Here, too, improvements are being made in molecular and analytical methods [17,27,28], but the fundamental question of how microbial behavior translates into host fitness remains unclear. Such biological inference cannot be drawn at the level of nucleic acids or molecules alone and must accompany measures of host physiological outputs.

The final scale of function — realized functional impact — deals with this fundamental question by focusing on host phenotype. Phenotypic measurements might include host gene expression, host metabolomics and proteomics, physiological measurements like body condition or immunity, and/or host behavior. To ascribe host phenotypic changes to microbiota composition typically requires gnotobiotic studies [29]. For example, the canonical role of the microbiota in mediating host obesity was demonstrated when germfree mice colonized with the microbiota of an obese mouse gained more weight and had increased adiposity relative to mice colonized with the microbiota of a lean donor [30]. Obesogenic traits can also be conferred by colonizing germfree mice with the gut microbiota of pregnant women [31] or non-hibernating brown bears [32], two other groups associated with weight gain normally. Gnotobiotic studies have also been used to document effects of the microbiota on diverse host phenotypes ranging from immune system maturation [33] to locomotion [34•]. Germfree colonization studies like these cannot be performed with most hosts (although a relatively diverse set of germfree model hosts are available including mice, pigs, zebrafish, fruit flies, and *Caenorhabditis elegans*) and host expression and physiology are not necessarily maintained when the microbiota of other hosts are colonized into germfree mice [33]. In these cases, comparative work may identify association between microbiota composition and a host phenotype and generate hypotheses for studying mechanism in more experimentally tractable organisms with their evolutionarily matched microbiota.

Support for host or microbial trade-offs can be found at each of these scales by identifying alleles, expression pathways, or phenotypes that show a negative relationship within some regime or that are tied across environmental gradients [35] (Figure 2). But such analyses require measuring multiple functions at once, ideally across a gradient of natural variation (Figure 2). Comparisons between functions are standard for shotgun approaches, but often rare for host phenotypic assays where diverse expertise and techniques are required to cover multiple functional classes. One notable exception

Figure 2



Models of potential functional trade-offs.
Function A increases at the expense of function B in a direct, linear fashion (blue); function B decreases rapidly with small increases of A when A is low, but decreases more slowly with small increase of A when A is high (yellow); function B is independent of function A until a critical threshold, after which function B decreases with additional increases in A (green); function A and B increase together until a critical threshold after which function A decreases with additional increases in the function B (red).

highlights how important such diverse functional comparisons can be. Gould *et al.* recently showed that a diverse microbiota will decrease fruit fly longevity but increase fecundity, resulting in an overall improvement in host fitness [36••]. Similar comprehensive analyses have not been conducted with other organisms, although there is evidence that germfree mice also live longer than conventional mice [37]. Ultimately, to demonstrate trade-offs and understand their mechanism requires performing experimental interventions altering the selective landscape and then tracking changes in multiple functions.

Designing studies to test for functional trade-offs

Studying trade-offs in gut microbiota function will require both practical and intellectual adjustments to current approaches. The most notable of these is the need to measure manifold functions, representing different types and scales of functioning, in the same study. Whenever possible, measurements of host phenotype — what we term here realized functional impact — should be collected as not all changes in microbial activity or potential

function will impact the host. Collecting diverse phenotypic datasets will benefit from more extensive collaboration of researchers with varied expertise, particularly drawing from those with backgrounds in immunology, metabolism, and animal behavior. In some cases, tradeoffs expected from mathematical modeling of biological function [38] may not be observed; this can reveal incomplete understanding of the compensatory mechanisms and feedbacks between host and microbiota. Explicit inclusion of null results will be key to demonstrate where multiple functions have been analyzed and trade-offs or selection were not observed, allowing us to iterate on and improve our mathematical and conceptual models of these systems.

As an extension, we need to develop a more general and mechanistic understanding of the conditions which select for different microbial functions. Some selective forces are intuitive, like diet impacting metabolism. Others may be less obvious. And others may be noticeable only when they are removed. Disturbance may lessen host control over the microbiota, for example contributing to the adverse metabolic effects of jet lag induced microbial shifts [39]. Disturbance may also knock out requisite members of the community and thus their function [40], potentially explaining the loss of colonization resistance following antibiotic treatment [41,42]. Alternatively, new environmental conditions could select for novel taxa or functions in the gut, for example leading to protein rather than carbohydrate metabolism under high protein diets [43]. Overall host health is ultimately a product of the array of microbial functions, so diverse pathways and the conditions which promote them must be studied in concert. Whenever possible, such selective forces should be studied along gradients rather than categorically, to ensure that the shape of the relationship between functions and environmental conditions is fully captured (Figure 2). Changes in these conditions, whether through natural disturbance or intentional manipulation, are often the best opportunities to study the effect of the microbiota as they will result in observable changes in functioning.

It is worth noting that many studies of the forces influencing microbial function in nature have focused on laboratory strains of model organisms, and for good reason. Model organisms provide efficiency and ease of study, with advantages such as germfree lines and advanced genomic tools. However, we must recognize that the laboratory setting is devoid of the selective agents that historically shaped host-microbe interactions in the wild (Figure 3). This absence can leave signatures on microbial assemblage or function [44,45••,46] only recognizable in comparison to more evolutionarily relevant conditions. Going forward, we will continue to rely upon laboratory models but changes to husbandry practice which recapitulate natural conditions may significantly increase translational accuracy [47].

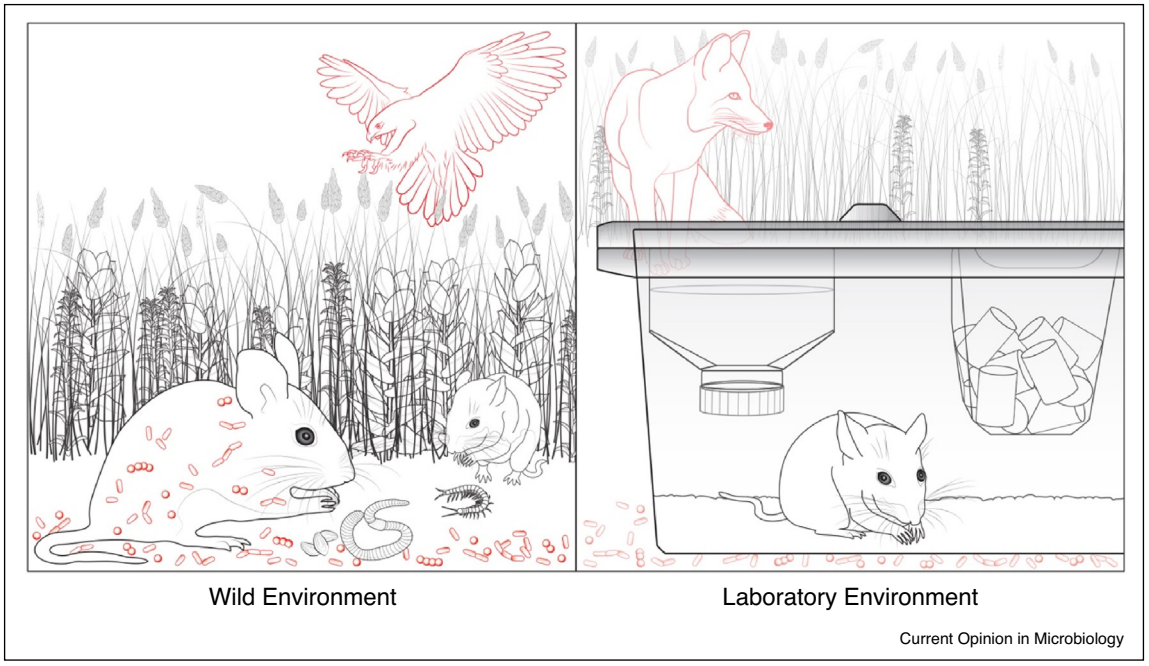
More generally, researchers should be careful when choosing experimental models. Realism of models should be prioritized in any attempts to understand how functional traits have arisen or are supported. As function is often the emergent effect of species interactions [36••] and different functions are carried by different organisms [23], monocolonized animals rarely display the full array of microbial functions [48]. Even high complexity human-designed communities may be insufficient to recapitulate natural dynamics. Specific pathogen free (SPF) animals, for instance, have microbiota chosen for the absence of certain bacteria rather than the presence of certain functions, and it is unknown if they represent normal functioning [49]. Moreover mismatch between host and microbiota (e.g. humanized mice) may result in loss of function even when the microbial community is intact [33], and the conditions of captivity may also alter microbial functioning [50,51•]. There is still a need for simple systems to allow for observational studies to describe patterns and experiments to identify the processes driving microbial function [52,53]. Eventually, experiments with animals with their naturally occurring microbiota [45••], kept in as close to their natural environments as possible (e.g. diet [50], light cycles [39]) will be necessary for studies of the evolution and persistence of microbial functioning. Altogether, more intentionality in observing and quantitatively modeling trade-offs is necessary. To the greatest extent possible, one should consider the evolutionary context of host-microbe interactions when designing studies and choosing functional metrics.

Dietary manipulation as case-study

Diet is expected to act as a selective force on the functional capabilities and activity of gut microbial communities [54]. Experimental or natural dietary variation can result in rapid shifts in composition and function [43,55], and over evolutionary time scales diet has been found to be a major structuring force of the microbiota [56–58]. Diet manipulation studies are therefore ripe for exploring the effects of varying selection and functional trade-offs. They are experimentally tractable, and they involve identifiable direct and indirect selective forces that provide specific testable hypotheses. Diet treatments can be modeled directly at different levels of granularity ranging from the macromolecule and element [59•] through to culture-specific dietary practices [43,60]. More generally, diet can be thought of as a model for other interventions (e.g. drug treatment, infection) that may alter the selective forces acting on gut microbial functioning.

To date, most research on diet and its impacts on the gut microbiota has focused on microbial metabolic functions, explored with various methods including metagenomics, metatranscriptomics, and host phenotyping. For example, absence of complex carbohydrates from the diet can result in loss of the pathways necessary to metabolize them [61], while amino acid catabolism expression is

Figure 3



Laboratory mice are isolated from the selective environment of the wild.
Factors that shape the microbiota of wild mice, including pathogen exposure, stressors like predators, and a complex diet and environment, are absent in the laboratory where animals are physically isolated from their nature. The absence of these features likely shapes the microbiota of laboratory mice in unrecognized ways.

enriched with the consumption of a protein rich diet [43]. These functional shifts suggest a potential trade-off between saccharolytic fermentation and proteolytic fermentation [62]. As noted above, the microbiota of obese humans [30] and pregnant women [31] have higher energy harvesting capacity, resulting in increased adiposity of the host. Such obesogenic microbiota also commonly arise under high fat, Western diets [63].

The impact of dietary selection on other microbial functions remains more unclear, although such impacts are certainly possible. Dietary sugars have recently been shown to alter gut colonization by commensals [64], while increased dietary zinc or iron intake has been found to induce pathogenesis [65]. Dietary enrichment can protect against stress-induced microbial shifts and cognitive impairments [66]. In contrast, high fat diet consumption has been associated with increased gut inflammation via microbial pathways [67], reduced gut barrier function [68], and higher susceptibility to *Listeria* infection [69]. It is important to note, however, that diet changes can impact the host immunology directly independent of microbiota responses [70•].

Research has not previously approached the question of how diet as a selective force modulates gut microbial functions and their trade-offs or whether these phenomena explain the health impacts tied to diet-associated microbial shifts. Increases in energy harvest may potentially improve other microbial or host functions, by providing more resources overall [71]. Alternatively, improvements or specializations in metabolic functioning may come at the expense of other microbial functions as microbes conferring those functions are outcompeted. To test these alternatives, experiments measuring an array of metabolic functions in response to diet manipulations are still necessary.

Implications of an evolutionary perspective for understanding human health

In closing, we discuss how incorporating the evolutionary principle of trade-offs may lead us to a better understanding of the effects of the modern human diet and its associated gut microbiota on human health. The Western industrialized human diet and its effects on the gut microbiota have been tied to a growing list of negative host health outcomes including obesity, metabolic syndrome, and cardiovascular disease [4,72,73]. But, such associations do not necessarily mean that the modern Western human gut microbiota are maladaptive, although that is often suggested [74,75].

Introduction of the Western diet might have selected for a community equipped to digest it but with negative

consequences due to trade-offs. One might expect a trade-off to exist between immunological functions and metabolic functions, such that diet-induced shifts would result in loss of immune traits [76•]. The rise of inflammation-associated bacteria under high protein diets [43] and the increased susceptibility to infection under high fat diets [69] could reflect the trade-off between metabolic and immunological functions. The acquisition of additional nutrients may be worth these potentially costly immunological deficits in many environmental conditions, and thus evolution likely promoted the shift to a metabolically complementary microbiota despite the associated trade-offs. Alternatively, changes in the modern environment — most notably improvements in health care — correlated with diet shifts may have led to a reduction in the strength of selection on immunological functions, independent of their relationship to metabolic functions. Experiments explicitly testing for trade-offs under varying diets are necessary to disentangle these hypotheses.

Undernutrition, regardless of microbiota composition, is likely less of a problem for most people consuming a contemporary Western diet compared to traditional populations, although malnutrition is still rampant. Today, gaining too many nutrients or calories can be a pressing problem in these populations. The question then becomes what changes to selective forces could reduce metabolic functioning while promoting more immediately beneficial microbial traits.

Dietary changes alone are insufficient to explain shifts towards a Western gut microbial composition in recent immigrants [60], indicating that functional differences between Western and non-Western populations likely are shaped not just by diet, but by many selective forces acting on specific populations. For example, familial (or vertical) transmission of strains may lead to inheritance of traits within kin groups, but it is unclear whether there are differences in the extent to which vertically versus horizontally acquired strains affect host phenotype. In part, this knowledge gap arises from difficulty in determining the origin of particular microbial strains, but technical advances in metagenomics and single-cell sequencing are quickly closing this gap. In the accompanying review in this issue, Kuthyar *et al.* [77] propose non-human primates as models for studying social (or horizontal) transmission of microbes, potentially allowing for discerning the extent to which familial acquisition versus social acquisition alter host phenotype. Under host-relevant evolutionary timescales, selective forces may act on vertical transmission of particular microbial functions.

Altogether, an evolutionarily informed framework for understanding the microbiota illustrates how conditions often seen to be maladaptive (such as the Western gut) may be the result of selection promoting certain functions (an actually adaptive outcome) that led to loss of others. Identifying these trade-offs and selective conditions will be necessary for designing intelligent interventions to promote human health via the gut microbiota. More broadly, explicitly studying the forces shaping microbial function will provide greater insight into the role of the gut microbiota in host biology and evolution, and so research should be designed with trade-offs in mind.

Conflict of interest statement

Nothing declared.

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