Into the wild: microbiome transplant studies need broader ecological reality

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Abstract

Resident gut microbial communities (microbiomes) profoundly shape the ecology and evolution of multicellular life. Interactions between host and microbiome appear to be reciprocal, and ecological theory is now being applied to better understand how hosts and their microbiome shape each other. However, the ecological processes that underlie reciprocal host-microbiome interactions may be obscured by current highly-controlled transplantation experiments, creating a need for a broad array of transplant studies to understand host-microbiome reciprocity. Using a directed review, we surveyed the breadth of ecological reality in the current literature on gut microbiome transplants with non-human recipients. For 55 studies, we categorized 9 key experimental conditions, including host taxon match and donor environment, that impact the ecological reality (EcoReality) of the transplant. Using these categories, we rated the EcoReality of each transplant. Encouragingly, the breadth of EcoReality has increased over time, but some components of EcoReality are relatively unexplored including recipient host environment and microbiome state. Our novel conceptual framework maps out the landscape of possible EcoReality, assisting future researchers to incorporate fundamental ecological processes in their transplant experiments. Broadening EcoReality in the microbiome literature is necessary to fully understand the reciprocal interplay between hosts and their microbiome.

A Quest for Ecological Reality

We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.
T.S. Eliot - Little Gidding (1)

Far from passive passengers, resident microbial communities (microbiomes) are integral to the basic biological functioning of multicellular life. This revelation, ushered in by advances in sequencing and computing technology, is grounded in a growing understanding that microbiomes profoundly shape their host's biology, influencing factors such as immunity (2), adiposity (3), thermogenesis (4), hormonal regulation (5), physiological development (6), memory (7), and behaviour (8). To date, biomedical research on human or laboratory rodent microbiomes has been instrumental in advancing our understanding of how the microbiome affects its host. However, there remains ample room for contributions by comparative animal physiologists, ecologists, and evolutionary biologists to fill knowledge gaps in our understanding of host-microbiome evolution and the interactions which underlay these partnerships (9).

Recently, researchers have started to appreciate the intertwining nature of host-microbiome interactions. Evidence is mounting that hosts can shape the composition of their microbiome community (10), and that microbiomes can influence their host's behaviour (8) and physiology (5). Based on differing cases of how host and microbiome might interact, Foster et al. (11) proposed four distinct models: 1) 'host control', in which the host unilaterally governs the composition of its microbiome; 2) 'symbiont control', in which the microbiome shapes the host phenotype; 3) 'open ecosystem', in which the host and microbiome do not interact; and 4) 'ecosystem on a leash', in which the host influences the microbiome by selecting upon microbial function rather than for specific microbial taxa. These connections can be so intimate that some researchers (12,13) proposed that a host and its associated microorganisms are a single biological entity—termed the 'holobiont'—on which selection acts, challenging notions of organismal individuality. Using this holobiont perspective, Alberdi et al. (14) posited that the microbial component of the holobiont, with its greater mutability compared to the host genome, may be an important mechanism facilitating host adaptation to rapid environmental change. Therefore, understanding the interplay between the host and the microbiome is crucial for addressing both fundamental and applied questions about the microbiome.

Host-microbiome interactions are shaped by ecological and evolutionary processes (15,16). Because host-microbiome interactions are potentially reciprocal, these processes act on three levels: the assembly and dynamics of the microbiome, the influence of the host on the microbiome, and the influence of the microbiome on the host. Microbiome assembly is governed by a variety of factors including environmental filtering, priority effects, random sampling, and dispersal limitation (16,17). The within-microbiome community dynamics are influenced by new invasions, competition, mutualisms, and other interactions (15). A host's actions can also shape their associated microbiomes. For example, the host's social behaviour can impact microbial dispersal (18). Conversely, the dynamics of the microbiome can impact the host; the change in microbiome community composition leading to *Clostridium difficile* colonization and pathogenicity is a classic example in humans (19). Evolutionary processes also occur in tandem with all the ecological processes mentioned previously because of the short timescales associated with microbial turnover relative to microbial evolutionary rates (17). Consequently, considering the ecological processes that underlie host-microbiome interactions is critical for making sense of the reciprocity between the host and its microbiome.

The most convincing evidence for host-microbiome interactions has been gleaned through microbiome transplantation studies. In these studies, researchers experimentally translocate microbial species or communities from donor hosts or external substrates to recipient hosts. Such studies have proven an invaluable and widely used tool for experimentally probing the host-microbiome relationship. The outcomes of microbiome transplants on the host and the resultant microbiome are likely shaped by the donor's and recipient's host physiology as well as the same long-established ecological processes (drift, dispersal, competition, etc.) that influence reciprocal host-microbiome interactions in nature. However, these ecological processes may be obscured by highly-controlled conditions employed by researchers leading to blind-spots in our understanding of reciprocal host-microbiome interactions. In a sense, these highly-controlled conditions are not ecologically realistic (which we term EcoReal, see Box 1 for a full definition) in that they do not match what the host plus its microbiome would experience in a wild ecosystem. For example, the use of germ-free recipients may preclude competition between introduced and resident microbes (20), and isolated laboratory conditions may limit the potential for microbial dispersal from influencing the composition of the resulting microbiome (21). Thus, there is a trade-off: highly-controlled experiments are designed to reduce ecological complexity to isolate mechanisms of interest, but they cannot simultaneously capture the full suite of mechanisms likely at play. The tradeoffs of laboratory approaches and the need for comparison to studies that use ecologically realistic conditions have long been recognized by comparative animal physiologists (22), though to date there does not seem to have been a similar recognition in microbiome research. Specifically, the breadth of EcoReality in microbiome transplant studies has not been examined, meaning such an evaluation remains an exciting potential avenue for future work.

Here, we probe the current EcoReality of microbiome transplantation studies. Our work here is not unlike Hanage's (23) questioning of the reality and applicability of biomedical microbiome studies. By taking advantage of the recent explosion of studies conducting microbiome transplants, we evaluate whether the current microbiome transplant literature limits opportunities for ecological processes to influence study outcomes. We investigated two key questions: 1) How EcoReal are the experimental conditions in the current microbiome transplant literature? and 2) does the literature currently cover the full potential range of EcoReality? Using long-established ecological concepts, we categorized microbiome transplantations into different experimental conditions which can impact the EcoReality of the transplant (Figure 1 and Box 2). Using this framework, we scored the EcoReality of microbiome transplant studies that used non-human recipients. We show that, overall, the breadth of EcoReality of the present microbiome transplant literature has increased over time. However, EcoReality has been constrained by hosts bred and kept in lab conditions, and with transplants into germ-free recipient hosts. Importantly, we provide a conceptual framework, illustrated in Figure 1, to help broaden the range of EcoReality in transplant experiments and to facilitate comparisons between transplants of varying EcoReality.

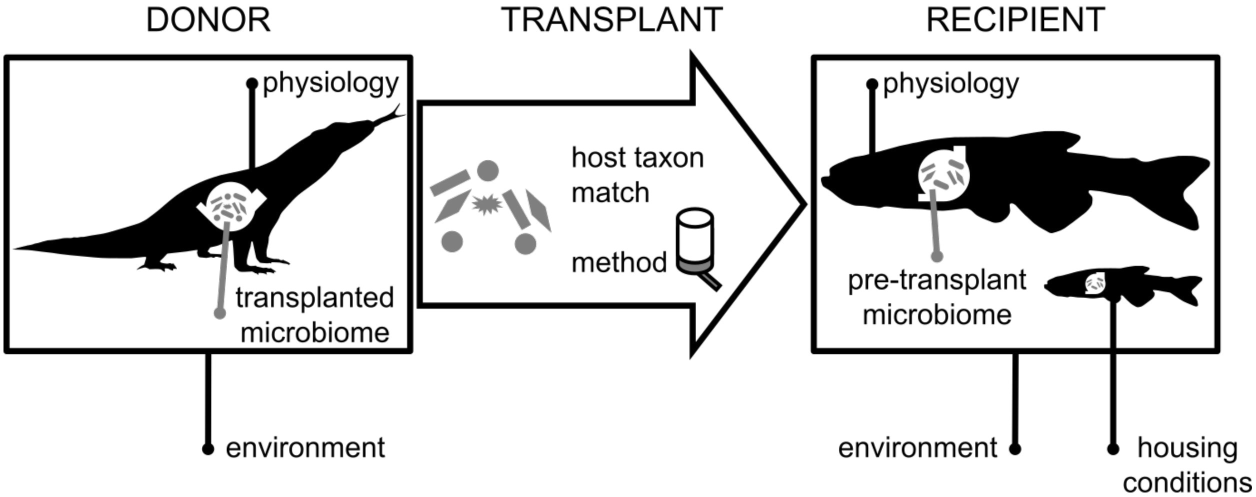

Figure 1: Conceptual framework of all the experimental conditions in a microbial transplant where EcoReality can vary. See Box 2 for explanations for each experimental condition.

Box 1. Key terms and definitions

Term	**Definition**
Transplant Instance	A transplant of a microbial strain or community from its native host or substrate to a different host population. A given study can involve multiple transplant instances, which are delineated based on non-substitutability of host populations or of transplant parameters.
Experimental Conditions	A decision or step in a transplant instance where there is the potential for variation in ecological reality.
Level of EcoReality	The degree to which an experimental condition matches the conditions that a host-microbiome interaction would experience in a wild ecosystem. Each experimental condition possesses its own intrinsic EcoReality. Each transplant instance and article can also be assigned an EcoReality score.

Box 2. Ecological reasoning for each experimental conditions within a transplant

Experimental condition	**Reasoning**
Taxon Match	Organisms can become locally adapted to their environment (24). Local adaptation of a microbial species to a given host may mean it is not adapted to hetero-specific hosts and will perform poorly after transplantation (25).
Donor & Recipient Environment	The wider species pool from which a local external environment gets its species from can influence community assembly and dynamics (21). Compared to field conditions, laboratory conditions likely possess smaller microbial pools, especially if laboratory conditions are sterile.
Donor & Recipient Physiology	An individual's physiological context is an important filter in community assembly and changes to this internal environment can affect species interactions (21). A host's physiology is the *de facto* environment of inhabitant microbes, and changes or dysregulation in the host may disrupt associations between host and the microbes that persist under homeostatic physiological conditions.
Transplanted Microbiome	The interactions within an invading community, including predation or mutualism, can impact whether colonisation is successful or not (20). Thus, a full community microbiome transplantation may differ significantly from the transplantation of a single microbe.
Transplant Method	Species have different dispersal abilities (26) and local environments filter species from the wider species pool (27). Active transplantations may circumvent differing dispersal abilities of microbial species and may undermine host filtering of the microbial community. Furthermore, active transplant methods can stress the host thereby changing host physiology and disrupting endogenous microbial communities (28).
Recipient Pre-transplant Microbiome	High species diversity in a community is predicted to reduce niche opportunities and to increase invasion resistance (20). Germ free or antibiotic perturbed recipients are likely to have lower invasion resistance than recipients with intact microbiomes.

Housing conditions	Dispersal between patches is an integral ecological process which can maintain stable populations or can rescue extirpated populations (21,29). Recipient host cohabitation allows for further transmissions of the microbiome.

Lay of the land

Literature Search

We conducted a directed review of the existing literature on gut microbiome transplants, finishing on October 26th 2018. We conducted our literature search in three stages. First, to gauge the extent of the current literature, we did a preliminary search of gut microbiome transplant studies using both Google Scholar and Web of Science (University of Guelph subscription). Based on this preliminary search, we conducted a more methodical search using both Google Scholar and Web of Science. Search terms can be found in the Supporting Information (SI) section of Greyson-Gaito et al. (30). We then sought additional publications through searching the citations of papers already collected using the Web of Science citations tool. We retained only those studies that conducted at least one gut microbiome transplant into a non-human recipient organism. To ensure our findings were generalizable to ecological and evolutionary frameworks across a broad range of taxa and ecosystems, we excluded studies focused on a single human disease, *C. difficile*.

Literature Evaluation

For each study that met our criteria, we determined the number of transplant instances, which we defined as the transfer of a microbial strain or community from its native host or substrate to a different host population (see Box 1). We used transplant instances as our unit of focus because many studies contained multiple transplant instances which sometimes differed substantially in EcoReality (e.g. Seedorf et al. (31)). For studies that had sequential transplants (i.e., transplant from donor to a first recipient, which then was the donor for a second recipient, e.g. Seedorf et al. (31)), we used only the first phase of the transplant experiment.

We identified nine key experimental conditions in a transplant where variation in EcoReality might substantially affect the outcome of the experiment: host taxon match, donor environment, donor physiology, transplanted microbiome, transplant method, recipient pre-transplant microbiome, recipient environment, recipient physiology, and recipient housing conditions (see Figure 1 & Box 2). Each experimental condition was given an ordinal data scale (see SI in Greyson-Gaito et al. (30)) based on the range of observed and possible levels for that condition, with one always representing the lowest level of EcoReality. For each transplant instance, we characterized the level of EcoReality in each of the 9 experimental conditions. To ensure consistent evaluation methods, EcoReality scores for each transplant instance were determined independently by two co-authors (separate pairs of co-authors randomized per paper). The co-author pairs then compared their scores and agreed upon the final transplant EcoReality scores.

To determine the overall standardized EcoReality score of a transplant instance, we divided each score by its corresponding maximum potential EcoReality score and then added the scaled scores for each experimental condition. Thus all experimental conditions were equally weighted in the overall calculation of standardized EcoReality.

We separated lab rodents from other animals in our results for each experimental condition because the ecology, physiology and genetics of lab-reared, inbred rodent models are heavily modified from wild-type rodents and other wild animals in ways that may affect our understanding of reciprocal host-microbiome interactions (for example Newman et al. (22)).

Literature EcoReality patterns

Our literature search returned 55 articles that met our criteria for inclusion. These articles ranged from having one to 13 transplant instances with an average of 2.91 transplant instances per article and a total of 160 from all articles. There was a clear shift over time in the number of articles using microbiome transplants. Notably, there were 20 articles in the first 10 years of our search period in comparison to almost 40 articles during 2015-2018 (SI Figure 1, (30)). This increase coincided with a switch from mainly lab rodent studies to a more diverse group of donor hosts (Figure 2A, around 2013), and later also to more diverse recipient hosts (Figure 2B, around 2016).

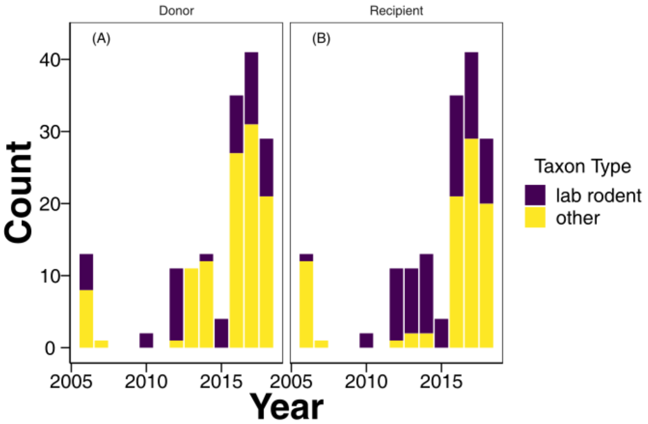

Figure 2: Number of transplant instances over time where the donor or recipient animal was either a lab rodent (mouse or rat) or another animal.

The transplant conditions Donor and Recipient Physiology had the highest EcoReality with average scores of 1.8 out of 2 (Figure 3C & H). Taxon match (score 1.6 out of 2, Figure 3A), transplanted microbiome (score 2.5 out of 3, Figure 3D), transplant method (score 1.7 out of 2, Figure 3E), and housing condition (score 1.5 out of 2, Figure 3I) were moderately EcoReal. Donor environment (score 2.4 out of 5, Figure 3B), recipient environment (score 1.6 out of 5, Figure 3G), and recipient microbiome (score 1.8 out of 3, Figure 3F) had the lowest EcoReality. Breaking EcoReality into recipient lab rodents and other animals, we see that active transplant methods (score of 1) were used more for lab rodents, and passive transplant methods (score of 2) were used more for other animals (Figure 3E). Interestingly, there were fewer transplants with germ-free recipient lab rodents than germ-free recipient other animals (score of 1) (Figure 3F). This pattern was driven by bees (19 out of 85 transplant instances from five articles) and zebrafish (14 out of 85 transplant instances from two articles). Overall, most transplants were performed with matching (score of 2, Figure 3A) wild-type, non-diseased donor and recipient hosts (score of 2, Figures 3C & H) using passive transplant methods (score of 2, Figure 3E) of whole microbial communities (score of 3, Figure 3D) and with a mixture of individual (score of 1, Figure 3I) and cohousing (score of 2, Figure 3I) of recipient hosts. However, transplants were mostly in sterile or normal lab conditions (score of 1 & 2, Figures 3B & G) with germ-free recipient hosts (score of 1, Figure 3F).

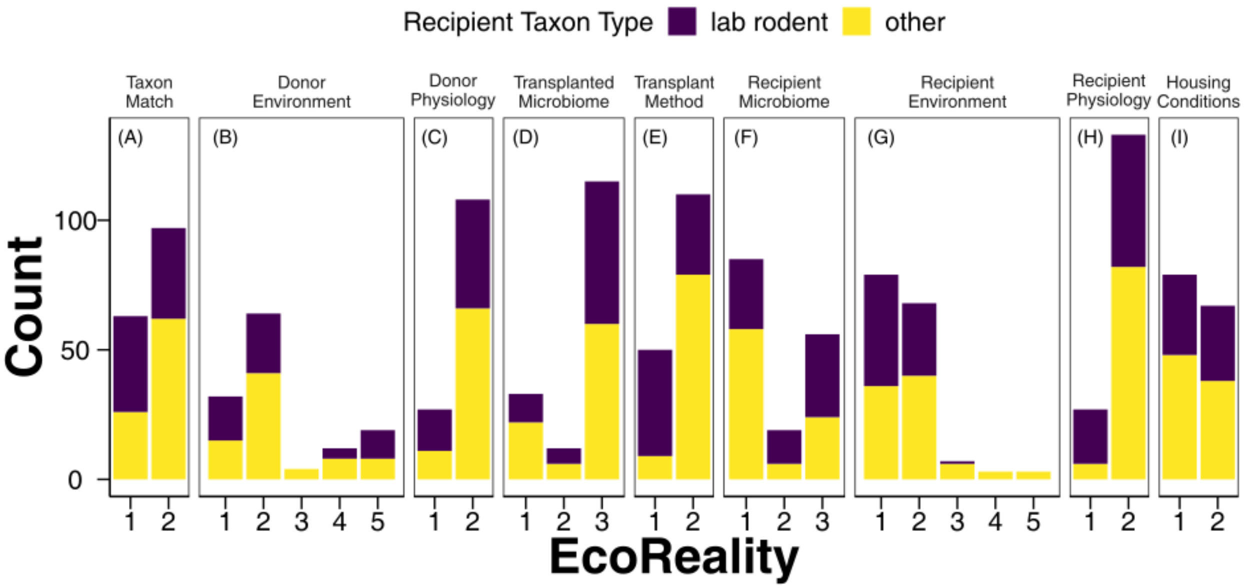

Figure 3: Number of transplant instances in each experimental condition, separated into whether the recipient animal was a lab rodent or another animal. The X-axis is the level of EcoReality, with 1 always the lowest EcoReality. The levels are explained in our Supporting Information on GitHub (30).

Although the maximal EcoReality score increased, increasing the breadth of EcoReality studied, the maximal EcoReality score was still below the theoretical maximum average standardized EcoReality score of 9 possible outlined in our framework (Figure 4).

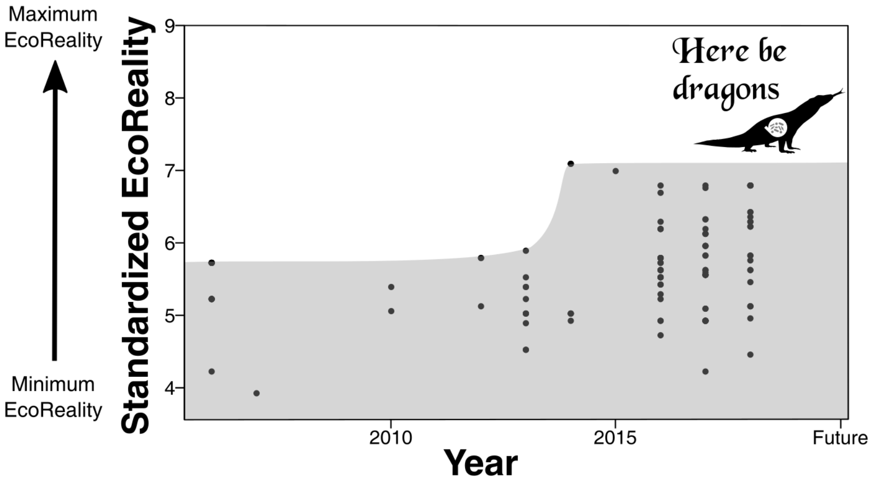

Figure 4: Standardized EcoReality score for each transplant instance.The grey area identifies the zone of EcoReality that has been studied in the literature, and the "Here be Dragons" area is the unexplored zone of EcoReality that is bound at the top by the theoretical maximum standardized EcoReality score of 9.

Here Be Dragons!

The burgeoning field of microbiome research is integrating the traditionally disparate disciplines of ecology, evolution, and physiology, which examine distinct but interrelated processes at different scales. Yet, these interrelated processes across scales are inherent in host-microbiome relationships (e.g. Stothart et al. (32)), and thus further integration of ecology, evolution, and physiology with microbiology will be crucial for unlocking important insights about the interactions between hosts and their microbiome. As microbiome research expands further to include ecological processes that are well established in traditional ecosystems, studies that can capture these processes will be necessary. Here, we expand on the insights from foundational highly-controlled experiments that identified key mechanisms in host-microbiome interactions. We surveyed the state of the microbiome transplant literature and identified gaps in how well ecological processes are captured in transplants, what we term as ecological reality, (i.e., EcoReality, see Box 1). Our results are promising; the breadth of EcoReality is increasing over time in transplant experiments (Figure 4), but there are still some key gaps in the types of studies conducted on host-microbiome interactions (Figure 3). We suggest that a critical step in understanding reciprocal host-microbiome interactions includes explicitly designing a broader array of studies that can evaluate the role of various ecological processes that are known to shape traditional ecological systems.

Our evaluation of the microbiome transplantation literature revealed broadening

EcoReality in experimental procedures. Lately, there has been a sharp increase in taxonomic diversity of both donor and recipient hosts (Figure 2). Transplants often used passive transplantation methods with wild-type non-diseased donors as well as a mixture of individual and cohabitation housing conditions (Figure 3). Finally, the maximal EcoReality score of microbiome transplant studies has increased over time (Figure 4). These results are encouraging because they suggest that researchers are building on the initial flurry of highly-controlled transplant experiments and designing diverse studies that differ in their degree of EcoReality in several of the categories we examined. Continuing to broaden EcoReality will be essential for understanding the ecological and evolutionary processes at work in reciprocal host-microbiome interactions.

However, our results show that the current literature lacks EcoReality in two key areas: host environment and the state of the recipient microbiome (Figure 3). Although the environment of the donor hosts was on average more EcoReal than the environment of the recipient hosts, the EcoReality of the donor and recipient host's environments was generally low. Most studies that we evaluated used laboratory settings that exclude the chance for hosts to encounter the broader microbial species pool in the environment (18,21). Laboratory conditions can also either increase or decrease conspecific interactions relative to what would be observed in nature, thus affecting the dispersal of microbes between hosts (18). Furthermore, laboratory conditions may be obscuring feedbacks between the host and its microbiome that can impact diet and habitat choice (18). The second key area lacking EcoReality is the state of the recipient microbiome where most recipient hosts were germ-free. Although some animals naturally start out with germ-free gastrointestinal tracts (e.g., newly eclosed worker bees (33)) or do not have a resident microbiome (34), most animal species host substantial microbial communities (35). Germ-free gastrointestinal tracts may lack key biotic processes such as predation, competition, and facilitation, which are important filters in classic ecological communities that act to mediate incoming species (20,36). Overall, neglecting natural environments and intact recipient microbiomes risks constraining the fundamental processes that impact reciprocal host-microbiome interactions.

Consequently, we are advocating for more breadth in EcoReality in microbiome transplant experiments. This breadth includes the already-common highly controlled laboratory transplants, which offer critical points of comparison, and provide focused understanding of particular mechanisms. The wider breadth of EcoReality for which we are advocating for requires that we venture into the largely untested realm of highly EcoReal experimental conditions (Figure 4), despite the logistical challenges likely associated with wild conditions. There are many ways in which we might venture beyond our present frontier. For example, using wild caught animals that are either allowed to roam freely or are housed in outdoor enclosures, and to use recipient animals with intact microbiomes, rather than germ free microbiomes (Figure 3). We also suggest identifying and filling in the major phylogenetic gaps in the tree of life for the donor and recipient host taxa. Overall, we call for an even spread of studies dealing with all permutations of EcoReality in each experimental condition. We hope researchers will use and adapt our conceptual breakdown (Figure 1) in their own systems to help identify where EcoReality can and cannot be increased and, where appropriate, consider how constrained EcoReality may impact their conclusions. Likewise, we encourage researchers to report the methodological details pertaining to each experimental condition we have identified. Other research fields have undergone a similar stage of self-reflection, identifying key issues and biases, which then precipitated new conceptual frameworks and methodologies (37). We hope that our literature evaluation and conceptual framework will precipitate such a self-reflection stage in host-microbiome science and precipitate the design of studies that can evaluate the role of many ecological processes.

Our literature evaluation suggests that we may understand only a small subset of possible reciprocal host-microbiome interactions impacting our ability to assess the conservation potential of the microbiome. Because we are presently likely constraining fundamental ecological and evolutionary processes, host-microbiome studies may be biased towards results that indicate a strong role of the microbiome on the host. Yet researchers have already made strong and general assertions about the role of the microbiome in the biology of the host. Due to the large effects of the microbiome on its host and its mutability, Alberdi et al. (14) argued that the microbiome could act as an additional axis of ecological adaptation for hosts. If the microbiome does act as an additional axis, conserving microbial diversity and using bioaugmentation tools (probiotic therapy and transplantation of microbiomes) would then be critical tools for animal conservation (38,39). We caution that experimental protocols which lack EcoReality might lead us to overestimate the capacity for microbiome variation to shape host phenotypes in nature by biasing our understanding of the host-microbiome relationship towards models of symbiont control (11). We suspect that a full reckoning of the spectrum of EcoReality in microbiome transplant studies will uncover more examples of the ‘ecosystem on a leash’ model (11), which posits an important but more limited reciprocity between the host and the ‘ecosystem’ of the microbiome. These sorts of nuanced interactions may or may not include the large microbiome effects which underpin the ecological adaptation and conservation arguments above. Thus, we may not yet have the level of understanding about reciprocal host-microbiome interactions that is required to know the role of the microbiome in host adaptation or to confidently inform conservation efforts. Moving forward, we assert that a consideration of EcoReality is required in the design and interpretation of every study that explores how the host-microbiome relationship impacts ecological adaptation.

Microbiome research has undoubtedly fascinated biologists across disciplines, prompting advances in both pure and applied research and raising questions about some of the most fundamental ideas in biology (13). Yet, the lay of the land in terms of ecological reality of this rapidly growing research area was unexplored. Our objective here—to survey the breadth of EcoReality in the microbiome transplant literature and identify key areas lacking EcoReality—was not unlike a fact finding mission expanding the map of our understanding of reciprocal host-microbiome interactions. We recommend a full, extended journey into the wilds to round out the literature's coverage of the landscape of possible EcoReality. Charting all territories, from highly controlled lab studies to free-ranging organisms, is necessary to fully comprehend the interplay between microbiomes and their hosts.

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Author contributions

All authors conceived of and produced the directed review. CJGG wrote the first draft and all authors contributed to editing the manuscript.

Data accessibility

The data, supporting information, and R script for this manuscript can be found on Zenodo/Github (30). The full list of transplant studies used in this article can be found in the dataset on Zenodo/GitHub, and (10,25,31,33,40–90) in the bibliography below.

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