Into the wild: microbiome transplant studies need broader ecological reality

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Abstract

Resident gut microbial communities (microbiomes) have profound impacts on the ecology and evolution of multicellular life, shaping host physiology, behaviour, and community interactions. We are beginning to understand that ecological theories can be applied to the interactions between hosts and their microbiomes. However, the ecological processes that govern host-microbiome interactions may be obscured by current experimental protocols that rely on highly controlled transplantation of microbiomes. We surveyed current studies that used gut microbiome transplants with non-human recipients, and categorized the 9 key experimental conditions that impact the ecological reality (EcoReality) of the transplant. Using these categories, we rated the EcoReality of all transplants and assessed the breadth of EcoReality in the microbiome transplant literature. Encouragingly, we found an increase in EcoReality over time, but EcoReality was still lacking in the host environment and in the state of the recipient host microbiome. From this process, we have created a novel conceptual framework for future researchers to adapt as necessary to incorporate fundamental ecological processes in their transplant experiments and employ broader ranges of EcoReality.

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 basic biological functioning of multicellular life and challenge notions of organismal individuality. 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, including 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.

Recently, an appreciation of how the intertwining nature of host-microbiome interactions has developed. Foster et al. (9) proposed four distinct frameworks for these interactions: 1) 'host control', in which the host unilaterally governs the composition of its microbiome, 2) 'symbiont control', in which the microbiome shapes the global 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. Regardless of the interaction, these connections can be so intimate that some researchers (10,11) proposed that a host and its associated microorganisms are a single biological entity, or 'holobiont', and the unit on which selection acts. Using this holobiont perspective, Alberdi et al. (12) 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. This makes understanding the interplay between the host and the microbiome important from both a fundamental and an applied perspective.

Animal microbiomes are specious communities, and thus are shaped by the interactions between constituent microbes and processes at larger scales including the host itself, which in turn affect host-microbiome interactions. Environmental filtering, priority effects, random sampling, and dispersal limitation have been suggested as key between community factors governing microbiome assembly (13,14). Furthermore from a metacommunity perspective, hosts can be thought of as habitat patches, and therefore microbial dispersal between hosts shape microbiomes too (15,16). Additionally, due to the short timescales associated with microbial turnover relative to microbial evolutionary rates, evolutionary processes occur in tandem with ecological processes (17). Although there are clear differences in scale between macroecology and microbiomes (16,18,19), overall, ecological processes, which also interact with evolutionary processes, shape the microbiome. Thus considering the ecological conditions that microbiomes experience is critical for a complete understanding of host-microbiome interactions.

Much of our current understanding of host-microbiome interactions has been gleaned through microbiome transplant studies, which experimentally translocate microbial species or communities from donor hosts or external substrates to recipient hosts. The outcomes of microbiome transplants are likely to be shaped by donor and recipient host physiology and the same ecological processes (drift, dispersal, competition etc.) that govern macroscopic ecosystems. Although transplants have proven an invaluable and widely used tool for experimentally probing the host-microbiome relationship with high precision, the ecological processes that govern host-microbiome interactions in nature may be obscured by highly controlled transplantation methodologies that are not ecologically realistic (which we term EcoReal, see Box 1 for a full definition). For example, the use of germ-free recipients or cultured microbiomes in transplants may restrict the opportunity for key ecological processes like competition and dispersal to influence the resulting microbiome composition. The limitations of laboratory approaches lacking ecological consideration have long been recognized by comparative animal physiologists (20); however, blind-spots in our understanding of host-microbiome interactions introduced by transplantation experiments lacking EcoReality has, until now, not been examined and remains an exciting potential avenue for future work.

The recent explosion of studies conducting microbiome transplants allows us to evaluate whether the current microbiome transplant literature limits or removes opportunities for ecological processes to influence study outcomes, and to highlight how future studies can address any fundamental knowledge gaps. Here, we probe the current EcoReality of microbiome transplantation studies, not unlike Hanage's (21) questioning of the reality and applicability of biomedical microbiome studies. We investigated three key questions: 1) How EcoReal are the experimental conditions in the current microbiome transplant literature? 2) Are experimental conditions increasing in EcoReality over time? and 3) does the literature currently cover the full potential range of EcoReality? Using macro-ecological theory, 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. Overall, the EcoReality of the present microbiome transplant literature has increased over time, but 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, as illustrated in Figure 1, to emphasize the importance of considering ecological processes in experimental design and to explore the wild frontiers of host-microbiome interactions.

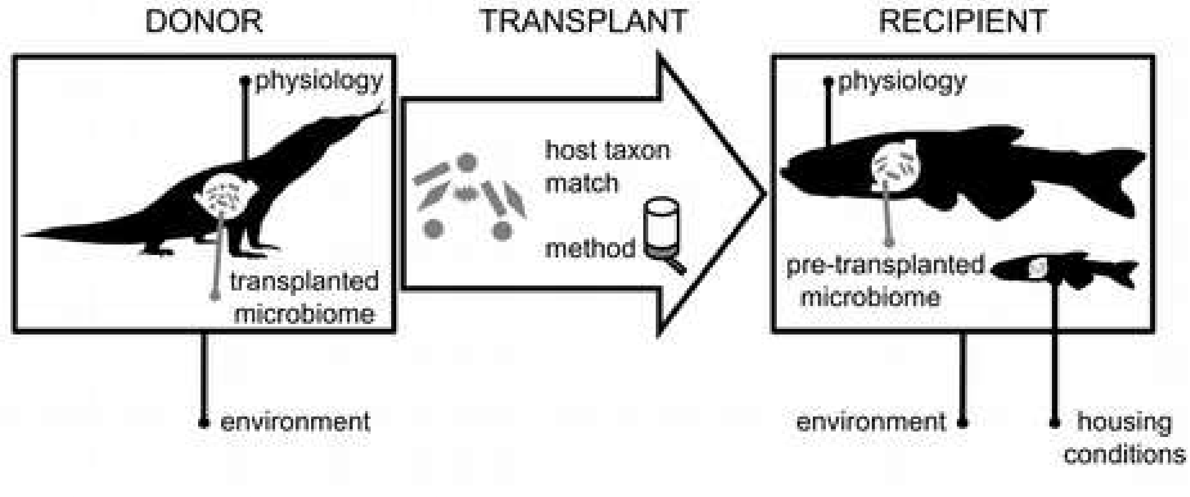

Figure 1: Conceptual framework of all the experimental conditions in a microbial transplant where EcoReality can vary. See Box 2 for explanations for each experimental condition.

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 conditions	**Reasoning**
Taxon Match	Organisms can become locally adapted to their environment (22). Local adaptation of a microbial species to its host may mean it is not adapted to hetero-specific hosts and performs poorly after transplantation.
Donor & Recipient Environment	The wider species pool from which a local environment gets its species from can influence community assembly and dynamics (23). Compared to field conditions, laboratory conditions likely possess smaller microbial pools, especially if laboratory conditions are sterile.
Donor & Recipient	The local environment is an important filter in community

Physiology	assembly and changes to this environment can affect species interactions (23). 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 (24). Thus, a full community microbiome transplantation may differ significantly from the transplantation of a single microbe.
Transplant Method	Species have different dispersal abilities (25) and local environments filter species from the wider species pool (26). 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 (27).
Recipient Pre-transplanted Microbiome	High species diversity in a community is predicted to reduce niche opportunities and to increase invasion resistance (24). Germ free or antibiotically 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 (23,28). 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. (29). We then sought additional publications through "forward snowballing" (i.e. 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, such as *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. (30)). 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. (30)), 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 taxonomic match, donor environment, donor physiology, transplanted microbiome, transplant method, recipient microbiome, recipient environment, recipient physiology, and housing conditions of the recipient (see Box 2). Each experimental condition was given an ordinal data scale (see SI in Greyson-Gaito et al. (29)) 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. EcoReality scoring for each transplant instance was conducted by two of the co-authors (separate pairs randomized per paper) to ensure consistent evaluation methods.

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 gave each article an overall EcoReality score using the average of its transplant instances' EcoReality scores.

The literature is dominated by studies using lab-reared, inbred rodent models for biomedical research, the ecology, physiology and genetics heavily modified from wild-type rodents in ways that may affect our understanding of host-microbiome interactions (for example Newman et al. (20)). Thus, we separated lab rodents from other animals in our results for each experimental condition.

Literature EcoReality patterns

Our literature search returned 53 articles that met our criteria for inclusion. These articles ranged from having one to 13 transplant instances with an average of 2.85

transplant instances per article and a total of 151 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, (29)). 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).

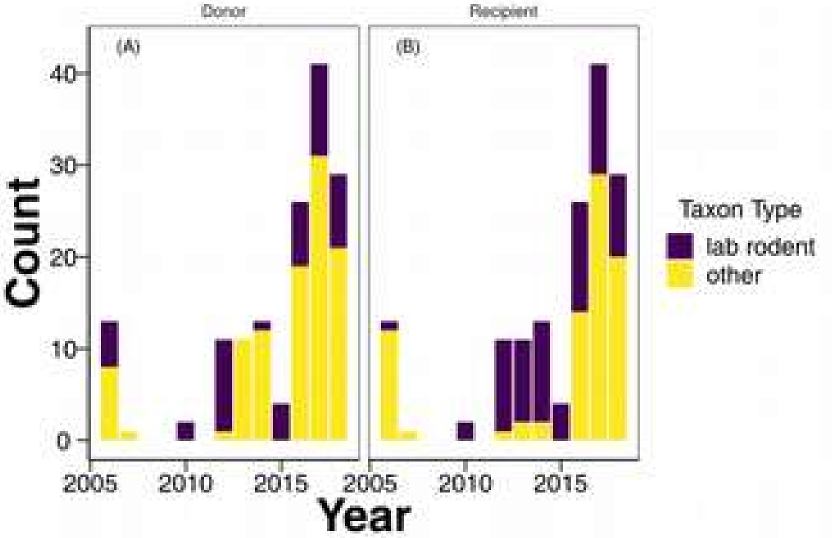

Figure 2: Number of transplant instances over time where the donor or recipient animal was either a lab rodent (mouse or rat) or another animal.

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 were used more for lab rodents and passive transplant methods were used more for other animals (Figure 3E). Interestingly, there were more other animals than lab rodents who were germ-free (Figure 3F). This pattern was driven by bees (19 out of 83 transplant instances from five articles) and zebrafish (14 out of 83 transplant instances from two articles). Overall, most transplants were performed with matching wild-type, non-diseased donor and recipient hosts using passive transplant methods and with a mixture of individual and cohousing of recipient hosts. However, transplants were mostly in lab conditions with germ-free recipient hosts.

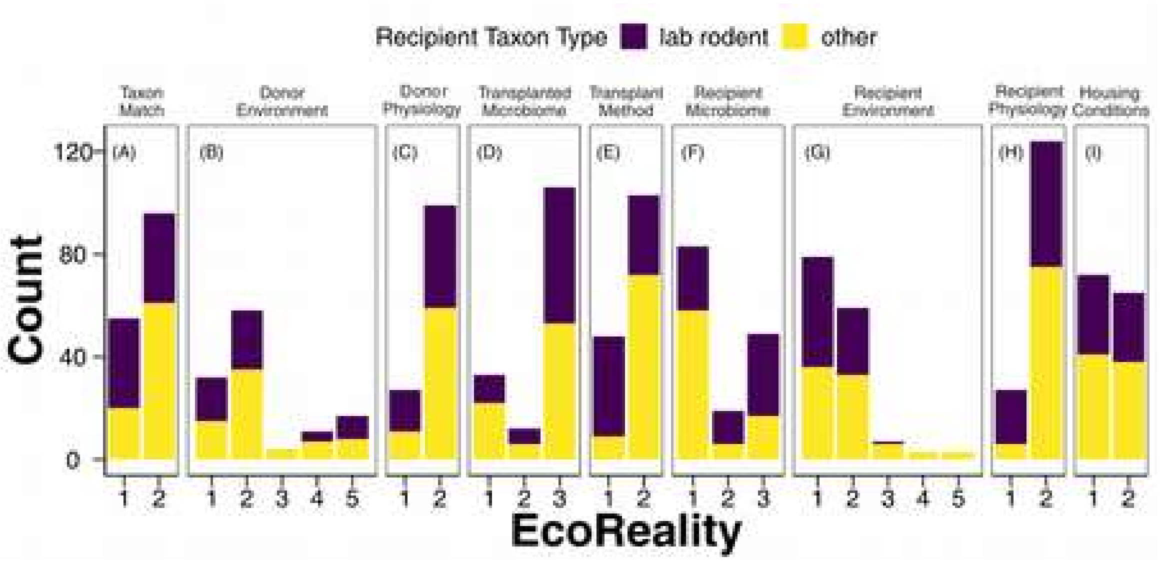

Figure 3: Number of transplant instances in each experimental condition, separated into whether the recipient animal was a lab rodent or another animal. The X-axis is the level of EcoReality, with 1 always the lowest EcoReality. The levels are explained in our Supporting Information on GitHub (29).

Finally, we found that studies did increase in average standardized EcoReality scores in recent years (Figure 4). While this improved the breadth of EcoReality studied, the maximum EcoReality score was still below the theoretical maximal average standardized EcoReality score of 9 possible with our methodology.

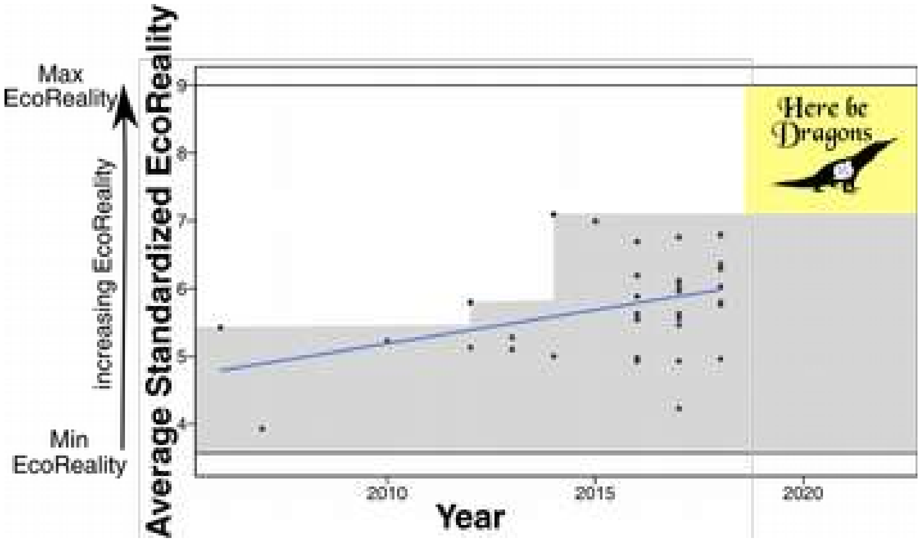

Figure 4: Average standardized EcoReality score for each article. Each point is for a single article. The blue line is the line of best fit from a least squares regression. The grey zone identifies the zone of EcoReality that is studied in the literature, and the yellow “Here be Dragons” zone is bound at the top by the theoretical maximum average standardized EcoReality score of 9.

Here Be Dragons!

Traditionally disparate, the disciplines of ecology, evolution, and physiology are being integrated in the burgeoning field of microbiome research. However, the potential this field holds can only be met with a rich understanding of how each of these disciplines contributes to the interactions between host and microbiome. By surveying the state of the literature on microbiome transplant experiments and identifying gaps in ecological reality (i.e., EcoReality, Box 1), we are taking a critical step in ensuring that our understanding of host-microbiome interactions includes the various ecological processes that are known to shape traditional ecological systems.

Our evaluation of the microbiome transplantation literature revealed both broadening EcoReality in experimental procedures and some key knowledge gaps that will need to be addressed. Encouragingly, transplants often used wild-type non-diseased donors as well as a mixture of individual and cohabitation housing conditions. Furthermore, there was a sharp increase in taxonomic diversity of both donor and recipient hosts, and where non-lab rodent animals were used, passive transplantation methods predominated. Although average EcoReality of microbiome transplant studies has been increasing over time, most studies have used sterile lab conditions where the recipient hosts were either germ free or antibiotically perturbed, highlighting two areas where EcoReality can be increased. If we are to understand the ecological and evolutionary processes at work in host-microbiome interactions, exploring the largely uncharted space of EcoReal experimental conditions is essential.

The current literature lacks EcoReality most often in two key areas: host environment and the state of the recipient microbiome. Although the environment of the donor hosts was on average more EcoReal than the environment of the recipient hosts, in general, the EcoReality of the donor and recipient host's environments was low. Most studies that we evaluated used laboratory settings which excludes the chance for hosts to encounter the broader microbial species pool in the environment (16,23). 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 (16). Furthermore, there are likely feedbacks between the host and its microbiome that can impact diet and habitat choice, further obscuring natural conditions with laboratory conditions (16). 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 (31)) or do not have a resident microbiome (32), most animal species host substantial microbial communities (33). Germ-free gastrointestinal tracts may lack key biotic processes such as predation and competition, which are important filters in classic ecological communities that act to mediate incoming species (34). Classic ecological theory would predict that a microbial species or community may colonise a germ free gut successfully where they would never have been able to under natural conditions (24). Overall, neglecting natural environments and intact recipient microbiomes risks constraining the fundamental processes that impact host-microbiome interactions, suggesting that we are sampling a sliver of the total range of host-microbiome interactions which occur in free-living systems.

Given that we may understand only a small subset of possible host-microbiome interactions, curbing the expectations of the efficacy of microbiome applications to ecological adaptation and conservation might be prudent. Due to the large effects of the microbiome on its host and its mutability, Alberdi et al. (23) 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 (35). However, 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 (Foster et al. (9)). We suspect that a full reckoning of the spectrum of EcoReality in microbiome transplant studies would uncover more examples of nuanced host-microbiome interactions, including the ‘ecosystem on a leash’ type interactions. These nuanced interactions may or may not include the large microbiome effects which underpin the ecological adaptation and conservation arguments above. Consequently, 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 and conservation.

To be clear, we are not advocating for moving entirely out of the lab; laboratory studies offer a critical point of comparison and can play a key role in identifying host-microbiome systems that might be worth pursuing in a wilder but more logistically challenging contexts. Nevertheless, we advocate for increasing the range of experimental conditions and crossing our present frontier into highly EcoReal experimental conditions with a variety of animal species. We could prescribe specific changes to transplant procedures, but we could not possibly cover all permutations of microbiome transplant studies here. Therefore, we urge researchers to 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 be impacting their conclusions. Likewise, we encourage researchers to report the methodological details pertaining to each experimental condition we have identified. Some recommendations are to use wild caught animals and where possible allow them to roam freely, or failing that, house them in outdoor enclosures. These experiments should also seek to use recipient animals with intact microbiomes, rather than germ-free recipients. When germ-free recipients are of the greatest utility, researchers could consider the EcoReality of these hosts, for example, developing a germ-free mouse model more closely aligned with the phylogenetic history of the donors. Finally, the current literature has scratched the surface of recipient and donor host taxa, and so we suggest identifying and filling in major phylogenetic gaps in the tree of life. Other research fields have undergone a similar stage of self-reflection, identifying key issues and biases, which then precipitated new conceptual frameworks and methodologies (36). We hope that our critiques and conceptual framework will precipitate such a self-reflection stage in the host-microbiome research field.

Microbiome research has excited biologists because it spans disciplines and promises to help advance both pure and applied biology. Our objective here--to survey the extent of EcoReality in the microbiome transplant literature and identify key areas lacking EcoReality--is not unlike a fact finding mission expanding the map of our understanding of 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 remote 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 Github (29). The full list of transplant studies used in this article can be found in the dataset on GitHub, and (15,30,31,37–59,59–86) in the bibliography below.

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