

1 **Into the wild: microbiome transplant** 2 **studies need broader ecological reality**

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30 ecosystem on a leash

37 **Abstract**

38

39 Gut microbial communities (microbiomes) profoundly shape the ecology and
40 evolution of multicellular life. Interactions between host and microbiome appear to be
41 reciprocal, and ecological theory is now being applied to better understand how hosts
42 and their microbiome influence each other. However, some ecological processes that
43 underlie reciprocal host-microbiome interactions may be obscured by the current
44 convention of highly-controlled transplantation experiments. Although these approaches
45 have yielded invaluable insights, there is a need for a broader array of approaches to
46 fully understand host-microbiome reciprocity. Using a directed review, we surveyed the
47 breadth of ecological reality in the current literature on gut microbiome transplants with
48 non-human recipients. For 55 studies, we categorized 9 key experimental conditions
49 that impact the ecological reality (EcoReality) of the transplant, including host taxon
50 match and donor environment. Using these categories, we rated the EcoReality of each
51 transplant. Encouragingly, the breadth of EcoReality has increased over time, but some
52 components of EcoReality are still relatively unexplored, including recipient host
53 environment and microbiome state. The conceptual framework we develop here maps
54 the landscape of possible EcoReality to highlight where fundamental ecological
55 processes can be considered in future transplant experiments.

56

57 **A Quest for Ecological Reality**

58

59 ***We shall not cease from exploration***

60 ***And the end of all our exploring***

61 ***Will be to arrive where we started***

62 ***And know the place for the first time.***

63 **T.S. Eliot - Little Gidding (1)**

64

65 Far from passive passengers, resident microbial communities (microbiomes) are
66 integral to the basic biological functioning of multicellular life. This revelation, ushered in
67 by advances in sequencing and computing technology, is grounded in a growing
68 understanding that microbiomes profoundly shape their host's biology, influencing
69 factors such as immunity (2), adiposity (3), thermogenesis (4), hormonal regulation (5),
70 physiological development (6), memory (7), and behaviour (8). To date, highly-
71 controlled experiments with laboratory rodent microbiomes have provided foundational
72 and indispensable knowledge on host-microbiome interactions. Furthermore, these
73 initial experiments have set the stage for integrative contributions by comparative
74 animal physiologists, ecologists, and evolutionary biologists to fill knowledge gaps in our
75 understanding of host-microbiome evolution and the interactions which underlay these
76 partnerships (9).

77

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

96

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

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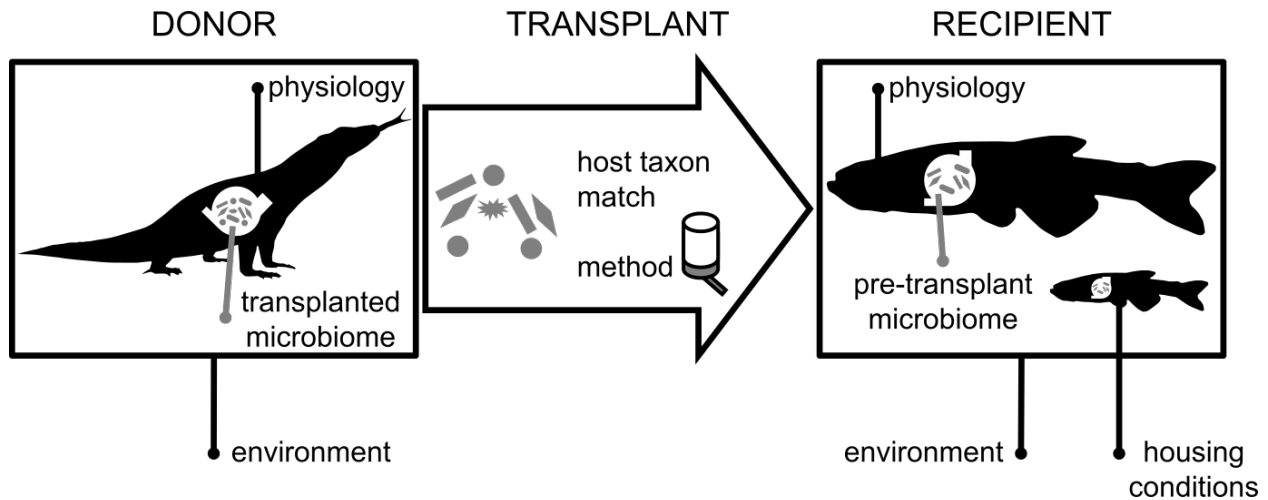
115 The most convincing evidence for host-microbiome interactions has been
116 gleaned through microbiome transplantation studies. In these studies, researchers
117 experimentally translocate microbial species or communities from donor hosts or

118 external substrates to recipient hosts. Highly-controlled transplantation studies have
119 been and will continue to be invaluable to experimentally probe the host-microbiome
120 relationship. However, there is a trade-off: highly-controlled experiments isolate
121 mechanisms of interest, but they cannot simultaneously capture the full suite of
122 ecological processes (drift, dispersal, competition, etc.) that influence reciprocal host-
123 microbiome interactions in nature. For example, the use of germ-free recipients may
124 preclude competition between introduced and resident microbes (20), and isolated
125 laboratory conditions may limit the potential for microbial dispersal from influencing the
126 composition of the resulting microbiome (21). How researchers weigh this trade-off
127 depends on the research question of interest. If a researcher's goal is to understand the
128 effect of specific microbes on host physiology, or to develop applications for human
129 health and domestic animal production, controlled experimental conditions are
130 preferred. In contrast, when examining the role and consequences of reciprocal host-
131 microbiome interactions in ecological and evolutionary contexts (e.g., fitness effects,
132 intergenerational microbial transmission, speciation, species persistence, etc.),
133 ecological complexity needs to be considered (22). Therefore, a comparison of highly-
134 controlled transplants and ecologically realistic (which we term EcoReal, see Box 1 for a
135 full definition) transplants that match what the host plus its microbiome would
136 experience in a wild ecosystem is required. The trade-offs of laboratory approaches and
137 the need for comparison to studies that use ecologically realistic conditions have long
138 been recognized by comparative animal physiologists (23), though to date there does
139 not seem to have been a similar recognition in microbiome research. Specifically, the
140 breadth of EcoReality in microbiome transplant studies has not been examined,
141 meaning such an evaluation remains an exciting potential avenue for future work.

142

143 Here, we probe the current EcoReality of microbiome transplantation studies.
144 Our work here is not unlike Hanage's (24) questioning of the reality and applicability of
145 biomedical microbiome studies. By taking advantage of the recent explosion of studies
146 conducting microbiome transplants, we evaluated whether the current microbiome
147 transplant literature limits opportunities for ecological processes to influence study
148 outcomes. We investigated two key questions: 1) how EcoReal are the experimental
149 conditions in the current microbiome transplant literature? and 2) does the literature
150 currently cover the full potential range of EcoReality? Using long-established ecological
151 concepts, we categorized microbiome transplantations into different experimental
152 conditions which can impact the EcoReality of the transplant (Figure 1 and Box 2).
153 Using this framework, we scored the EcoReality of microbiome transplant studies that
154 used non-human recipients. We show that, overall, the breadth of EcoReality of the
155 present microbiome transplant literature has increased over time. However, EcoReality
156 has been constrained by hosts bred and kept in lab conditions, and with transplants into
157 germ-free recipient hosts. Importantly, we provide a conceptual framework, illustrated in
158 Figure 1, to help broaden the range of EcoReality in transplant experiments and to

159 facilitate comparisons between transplants of varying EcoReality.
 160



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

165

166 **Box 1.** Key terms and definitions

167

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 can also be assigned an EcoReality score.

168

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

Experimental condition	Reasoning Ecological theory + application to microbiome
Taxon Match	Organisms can become locally adapted (25). 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 (26).
Donor & Recipient Environment	During community assembly, the local environment acts as a filter, incorporating species from a wider species pool (21). From the microbiome’s perspective, the host’s physiology and the external environment are one intertwined environment. Therefore, the external environment can affect microbiome dynamics in two ways: indirectly through impacting the host physiology (27), and directly through the wider microbial species pool that the host and its microbiome has access to.
Donor & Recipient Physiology	The local environment acts as a filter in community assembly (21). For this experimental condition, we define physiology as physiological states that would occur regardless of the external environmental context (e.g. gene knock-out, disease-state). We differentiate intrinsic physiology from mutable host physiological responses to the external environmental context. Although these indirect environmental effects acting through host physiology are relevant (27), they are captured by the ‘Environment’ experimental condition. A host’s physiology is the <i>de facto</i> 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 monoculture at artificially high densities.
Transplant Method	Species have different dispersal abilities (27) and local environments filter species from the wider species pool (28). 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 (29).
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 (21,30). Recipient host cohabitation allows for further transmissions of the microbiome.

170

171 **Lay of the land**

172

173 *Literature Search*

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

187

188 *Literature Evaluation*

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

197 We identified nine key experimental conditions in a transplant where variation in
198 EcoReality might substantially affect the outcome of the experiment: host taxon match,
199 donor environment, donor physiology, transplanted microbiome, transplant method,
200 recipient pre-transplant microbiome, recipient environment, recipient physiology, and
201 recipient housing conditions (see Figure 1 & Box 2). Each experimental condition was
202 given an ordinal data scale (see SI Table S1) based on the range of observed and
203 possible levels for that condition, with one always representing the lowest level of
204 EcoReality. Our goal was to maintain similar resolution for each highly dimensional
205 experimental condition within our framework. For example, Taxon Match could have
206 included more levels to capture phylogenetic distance, geographic distance, and
207 feeding relationships between host and recipient (32). However given the variation in
208 scale, generalization across host taxa would have then been difficult. The levels in each
209 experimental condition were based on likely conditions found in the wild. For example,
210 with respect to the Transplanted Microbiome experimental condition, a single bacterial
211 strain at high densities entering a host in the wild is less likely than invasion by mixed
212 communities. For each transplant instance, we characterized the level of EcoReality in
213 each of the 9 experimental conditions. To ensure consistent evaluation methods,
214 EcoReality scores for each transplant instance were determined independently by two
215 co-authors (separate pairs of co-authors randomized per paper). The co-author pairs
216 then compared their scores and agreed upon the final transplant EcoReality scores.

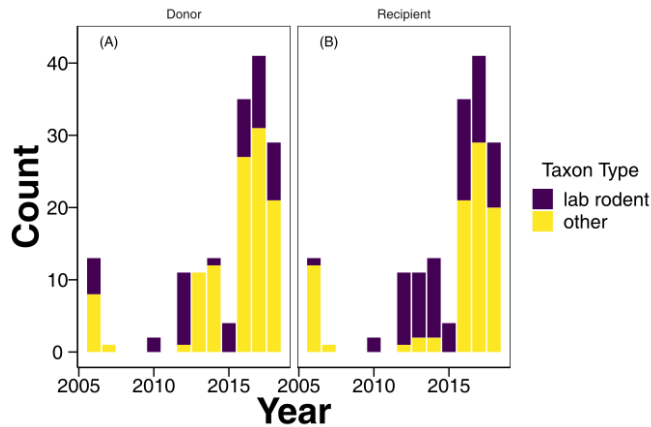
217 To determine the overall standardized EcoReality score of a transplant instance,
218 we divided each score by its corresponding maximum potential EcoReality score and
219 then added the scaled scores for each experimental condition. Thus all experimental
220 conditions were equally weighted in the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

221 We separated lab rodents from other animals in our results for each experimental
222 condition because the ecology, physiology and genetics of lab-reared, inbred rodent
223 models are heavily modified from wild-type rodents and other wild animals in ways that
224 may affect our understanding of reciprocal host-microbiome interactions (for example
225 Newman et al. (23) and Walter et al. (33)).

226 227 *Literature EcoReality patterns*

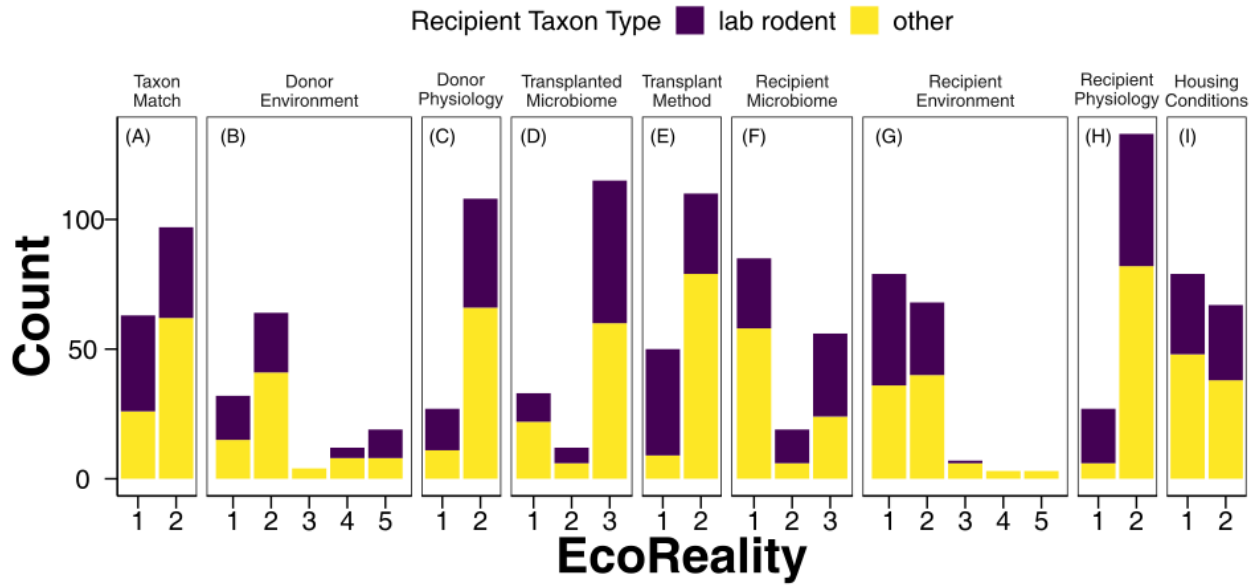
228 Our literature search returned 55 articles that met our criteria for inclusion. These
229 articles ranged from having one to 13 transplant instances with an average of 2.91
230 transplant instances per article and a total of 160 from all articles. There was a clear
231 shift over time in the number of articles using microbiome transplants. Notably, there
232 were 20 articles in the first 10 years of our search period in comparison to almost 40
233 articles during 2015-2018 (SI Figure 1). This increase coincided with a switch from
234 mainly lab rodent studies to a more diverse group of donor hosts (Figure 2A, around
235 2013), and later also to more diverse recipient hosts (Figure 2B, around 2016).

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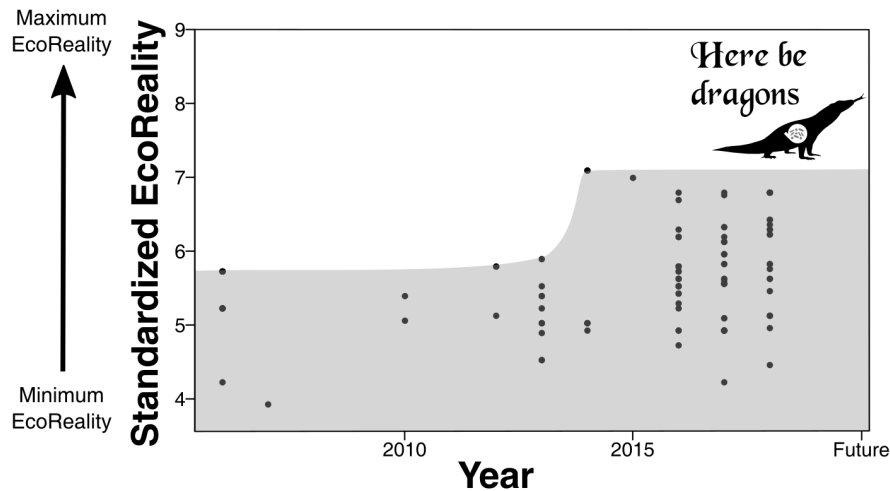
237
 238 Figure 2: Number of transplant instances over time where the donor or recipient animal
 239 was either a lab rodent (mouse or rat) or another animal.
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241 The transplant conditions Donor and Recipient Physiology had the highest
 242 EcoReality with average scores of 1.8 out of 2 (Figure 3C & H). Taxon match (score 1.6
 243 out of 2, Figure 3A), transplanted microbiome (score 2.5 out of 3, Figure 3D), transplant
 244 method (score 1.7 out of 2, Figure 3E), and housing condition (score 1.5 out of 2, Figure
 245 3I) were moderately EcoReal. Donor environment (score 2.4 out of 5, Figure 3B),
 246 recipient environment (score 1.6 out of 5, Figure 3G), and recipient microbiome (score
 247 1.8 out of 3, Figure 3F) had the lowest EcoReality. Breaking EcoReality into recipient
 248 lab rodents and other animals, we see that active transplant methods (score of 1) were
 249 used more for lab rodents, and passive transplant methods (score of 2) were used more
 250 for other animals (Figure 3E). Interestingly, there were fewer transplants with germ-free
 251 recipient lab rodents than germ-free recipient other animals (score of 1) (Figure 3F).
 252 This pattern was driven by bees (19 out of 85 transplant instances from five articles)
 253 and zebrafish (14 out of 85 transplant instances from two articles). Overall, most
 254 transplants were performed with matching (score of 2, Figure 3A) wild-type, non-
 255 diseased donor and recipient hosts (score of 2, Figures 3C & H) using passive
 256 transplant methods (score of 2, Figure 3E) of whole microbial communities (score of 3,
 257 Figure 3D) and with a mixture of individual (score of 1, Figure 3I) and cohousing (score
 258 of 2, Figure 3I) of recipient hosts. However, transplants were mostly in sterile or normal
 259 lab conditions (score of 1 & 2, Figures 3B & G) with germ-free recipient hosts (score of
 260 1, Figure 3F).



261
 262 Figure 3: Number of transplant instances in each experimental condition, separated into
 263 whether the recipient animal was a lab rodent or another animal. The X-axis is the level
 264 of EcoReality, with 1 always the lowest EcoReality. The levels are explained in Table S1
 265 of our Supporting Information.
 266

267 Although the maximal EcoReality score increased, increasing the breadth of
 268 EcoReality studied, the maximal EcoReality score was still below the theoretical
 269 maximum standardized EcoReality score of 9 possible outlined in our framework (Figure
 270 4).



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

288 **Here Be Dragons!**

289

290 The burgeoning field of microbiome research is integrating the traditionally
291 disparate disciplines of ecology, evolution, and physiology, which examine distinct but
292 interrelated processes at different scales. Yet, these interrelated processes across
293 scales are inherent in host-microbiome relationships (e.g. Stothart et al. (34)), and thus
294 further integration of ecology, evolution, and physiology with microbiology will be crucial
295 for unlocking important insights about the interactions between hosts and their
296 microbiome. As microbiome research expands further to include ecological processes
297 that are well established in traditional ecosystems, studies that can capture these
298 processes will be necessary. Here, we expand on the insights from foundational highly-
299 controlled experiments that identified key mechanisms in host-microbiome interactions.
300 We surveyed the state of the microbiome transplant literature and identified gaps in how
301 well ecological processes are captured in transplants, what we term as ecological
302 reality, (i.e., EcoReality, see Box 1). Our results are promising; the breadth of
303 EcoReality is increasing over time in transplant experiments (Figure 4), but there are
304 still some key gaps in the types of studies conducted on host-microbiome interactions
305 (Figure 3). We suggest that a critical step in understanding reciprocal host-microbiome
306 interactions includes explicitly designing a broader array of studies that can evaluate the
307 role of various ecological processes that are known to shape traditional ecological
308 systems.

309

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

322

323 However, our results show that the current literature lacks EcoReality in two key
324 areas: host environment and the state of the recipient microbiome (Figure 3). Although
325 the environment of the donor hosts was on average more EcoReal than the
326 environment of the recipient hosts, the EcoReality of the donor and recipient host's
327 environments was generally low. Most studies that we evaluated used laboratory
328 settings that exclude the chance for hosts to encounter the broader microbial species

329 pool in the environment (18,21). Laboratory conditions can also either increase or
330 decrease conspecific interactions relative to what would be observed in nature, thus
331 affecting the dispersal of microbes between hosts (18). Furthermore, laboratory
332 conditions may be obscuring feedbacks between the host and its microbiome that can
333 impact diet and habitat choice (18). The second key area lacking EcoReality is the state
334 of the recipient microbiome where most recipient hosts were germ-free. Although some
335 animals naturally start out with germ-free gastrointestinal tracts (e.g., newly eclosed
336 worker bees (35)) or do not have a resident microbiome (36), most animal species host
337 substantial microbial communities (37). Germ-free gastrointestinal tracts may lack key
338 biotic processes such as predation, competition, and facilitation, which are important
339 filters in classic ecological communities that act to mediate incoming species (20,38).
340 Overall, neglecting natural environments and intact recipient microbiomes risks
341 constraining the fundamental processes that impact reciprocal host-microbiome
342 interactions.

343
344 Consequently, we advocate for more breadth in EcoReality in microbiome
345 transplant experiments. This breadth includes highly-controlled laboratory transplants,
346 which offer critical points of comparison, and provide a focused understanding of
347 particular mechanisms. The wider breadth of EcoReality for which we are advocating for
348 requires that we venture into the largely untested realm of highly EcoReal experimental
349 conditions (Figure 4), despite the logistical challenges likely associated with wild
350 conditions. There are many ways in which we might venture beyond our present
351 frontier. For example, using wild-caught animals that are either allowed to roam freely or
352 are housed in outdoor enclosures, and to use recipient animals with intact microbiomes,
353 rather than germ-free microbiomes (Figure 3). We also suggest identifying and
354 addressing the major phylogenetic gaps in the tree of life for the donor and recipient
355 host taxa. Overall, we call for a balance of studies dealing with all permutations of
356 EcoReality in each experimental condition. We hope researchers will use and adapt our
357 conceptual framework (Figure 1) in their own systems to incorporate EcoReality and,
358 where appropriate, consider how constrained EcoReality may impact their conclusions.
359 Likewise, we encourage researchers to report the methodological details pertaining to
360 each experimental condition we have identified. We hope that our literature evaluation
361 and conceptual framework will stimulate new avenues of collaborative research that will
362 evaluate the role of ecological processes in host-microbiome interactions.

363
364 Our literature evaluation suggests that we may understand only a small subset of
365 possible reciprocal host-microbiome interactions impacting our ability to assess the
366 conservation potential of the microbiome. Because we are presently likely constraining
367 fundamental ecological and evolutionary processes, host-microbiome studies may be
368 biased towards results that indicate a strong role of the microbiome on the host. Yet
369 researchers have already made strong and general assertions about the role of the

370 microbiome in the biology of the host. Due to the large effects of the microbiome on its
371 host and its mutability, Alberdi et al. (14) argued that the microbiome could act as an
372 additional axis of ecological adaptation for hosts. If the microbiome does act as an
373 additional axis, conserving microbial diversity and using bioaugmentation tools
374 (probiotic therapy and transplantation of microbiomes) would then be critical tools for
375 animal conservation (39,40). We caution that experimental protocols that lack
376 EcoReality might lead us to overestimate the capacity for microbiome variation to shape
377 host phenotypes in nature by biasing our understanding of the host-microbiome
378 relationship towards models of symbiont control (11). We suspect that a full reckoning of
379 the spectrum of EcoReality in microbiome transplant studies will uncover more
380 examples of the ‘ecosystem on a leash’ model (11), which posits an important but more
381 limited reciprocity between the host and the ‘ecosystem’ of the microbiome. These sorts
382 of nuanced interactions may or may not include the large microbiome effects which
383 underpin the ecological adaptation and conservation arguments above. Thus, we may
384 not yet have the level of understanding about reciprocal host-microbiome interactions
385 that is required to know the role of the microbiome in host adaptation or to confidently
386 inform conservation efforts. Moving forward, we assert that a consideration of
387 EcoReality is required in the design and interpretation of every study that explores how
388 the host-microbiome relationship impacts ecological adaptation.

389

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

401

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412

413 **Author contributions**

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

416

417 **Data accessibility**

418 The supporting information is included with this paper. The supporting information plus
419 the data, and R script for this manuscript can be found on Zenodo/Github (41). The full
420 list of transplant studies used in this article can be found in the dataset on
421 Zenodo/GitHub (41), and (10,26,31,35,42–92) in the bibliography below.

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673

Supporting Information

Into the wild: microbiome transplant studies need broader ecological reality

Authors

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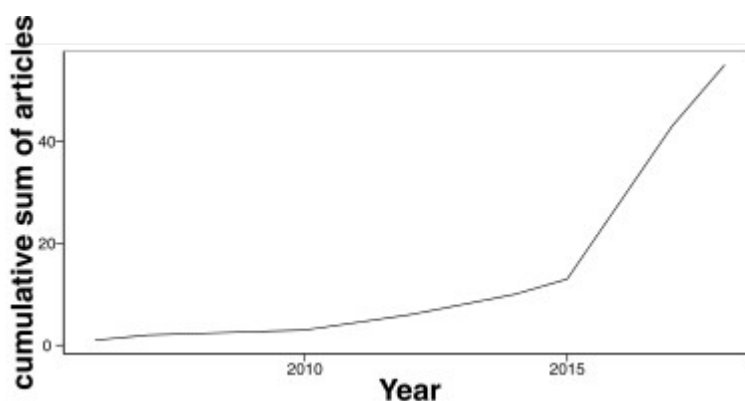
Search terms used for methodological literature search

- Google scholar
 - (transplant* AND microbio) (gut OR fecal OR feces OR gastrointestinal OR gastro-intestin* OR faecal OR faeces OR forces OR faecal) -human -patient -"homo sapiens" -man -woman -child*
- Web of Science
 - ((TOPIC:(transplant) AND TOPIC: (microbio))AND (TOPIC: (((((((gut OR fecal) OR feces) OR gastrointestinal) OR gastro-intestin) OR faecal) OR faeces) OR foeces) OR faecal) NOT TOPIC:((((human OR patient) OR homo sapiens) OR man) OR woman) OR child*)))

Table S1 Ordinal data scale (EcoReality score) for each experimental condition

Experimental Condition	Ordinal Data Scale
Taxon Match	1 = Mismatch (different species) 2 = Match (same species)
Donor Environment	1 = Lab animal host in sterile lab 2 = Lab animal host in non-sterile lab 3 = Captive bred wildlife (multiple generations bred in captivity) 4 = Wildlife brought into captivity (no generations bred in captivity) 5 = Free-ranging wildlife (capture and release)
Donor Physiology	1 = Gene knockout or disease harbouring (non-microbial) 2 = Wildtype non-diseased
Transplanted Microbiome	1 = Single strain 2 = Consortium, mixture of select strains

	3 = Whole community (no sorting or altering of community sampled for transplantation)
Transplant Method	1 = Active (microbiome sample forcefully added to recipient gut e.g., by a suppository or oral gavage) 2 = Passive (microbiome sample passively given to recipient e.g., mixed into food)
Recipient Microbiome	1 = Germ-free 2 = Antibiotic perturbed/pathologic 3 = Whole community (no experimental alteration of community)
Recipient Environment	1 = Lab animal host in sterile lab 2 = Lab animal host in non-sterile lab 3 = Captive bred wildlife (multiple generations bred in captivity) 4 = Wildlife brought into captivity (no generations bred in captivity) 5 = Free-ranging wildlife (capture and release)
Recipient Physiology	1 = Gene knockout or disease harbouring (non-microbial) 2 = Wildtype or non-diseased
Housing Conditions	1 = Housed singly (after microbiome transplantation) 2 = Co-housed (after microbiome transplantation with either other replicates in the experiment or with individuals of the same species that were not replicates. Co-housing could also have been used as the method of transplantation)



SI Figure 1 Cumulative sum of articles from our directed review between 2006 and 2018.

Data accessibility

The data, the above supporting information, and the R script for this manuscript are in a repository on GitHub. This repository can be cloned or downloaded straight from Github (https://github.com/cgreysongaito/Intothewild_Microbiome) or from Zenodo (<https://doi.org/10.5281/zenodo.2652255>).

Folder and file structure of Github repository (Intothewild_Microbiome)

- data
 - EcoRealTable_2019-10-09_Data.csv
- figs
 - 2019-10-09 CountAnimals.pdf – Figure 2 in manuscript
 - 2019-10-09 Eco-realityComparisons.pdf – Figure 3 in manuscript
 - 2019-10-09 Eco-realityAverageStandardOverTime.pdf – Figure 4 in manuscript
 - 2019-10-09 CumulativeSumArticles.pdf – Supporting Information Figure 1
- .gitignore – File containing files or folders that git should ignore
- IntotheWild_Microbiome_Greyson-Gaito_etal_2019.R – R script for analysis and figure creation
- SupportingInformation_Intothewild_GreysonGaitoetal.pdf – Supporting information (search terms, ordinal data scales, figure)
- LICENSE – Mozilla Public License 2.0
- README.md – Important information
- meta_transplant_microbiome.Rproj – R Project to increase ease of use

Instructions for use

- Download the whole repository (either by forking and cloning or by downloading a ZIP folder)
- In RStudio, open the project called meta_transplant_microbiome.Rproj and open the file IntotheWild_Microbiome_Greyson-Gaito_etal_2019.R
 - If not using RStudio, open the file IntotheWild_Microbiome_Greyson-Gaito_etal_2019.R and edit the path to the data file called Microbiome_Literature_Summaries.csv to whatever path is required on your computer.
- Run the script in RStudio or however you normally run R scripts