Understanding How Microbiomes Influence The Systems They Inhabit: Moving From A Correlative To A Causal Research Framework

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Abstract

Translating the ever-increasing wealth of information on microbiomes (environment, host, or built environment) to advance the understanding of system-level processes is proving to be an exceptional research challenge. One reason for this challenge is that relationships between characteristics of microbiomes and the system-level processes they influence are often evaluated in the absence of a robust conceptual framework and reported without elucidating the underlying causal mechanisms. The reliance on correlative approaches limits the potential to expand the inference of a single relationship to additional systems and advance the field. We propose that research focused on how microbiomes influence the systems they inhabit should work within a common framework and target known microbial processes that contribute to the system-level processes of interest. Here we identify three distinct categories of microbiome characteristics (microbial processes, microbial community properties, and microbial membership) and propose a framework to empirically link each of these categories to each other and the broader system level processes they affect. We posit that it is particularly important to distinguish microbial community properties that can be predicted from constituent taxa (community aggregated traits) from and those properties that are currently unable to be predicted from constituent taxa (emergent properties). Existing methods in microbial ecology can be applied to more explicitly elucidate properties within each of these categories and connect these three categories of microbial characteristics with each other. We view this proposed framework, gleaned from a breadth of research on environmental microbiomes and ecosystem processes, as a promising pathway with the potential to advance discovery and understanding across a broad range of microbiome science.

Current Approaches Linking Microbial Characteristics and Ecosystem Processes

Virtually all ecosystem processes are influenced by microorganisms, and many processes are carried out exclusively by microorganisms. This has sometimes led to the assumption that a better description of the microbiome (including its associated transcripts, proteins, and metabolic products) should lead to a better understanding and predictions of system level processes. However, such justifications assume that measurable characteristics of the microbiome (e.g. 16S rRNA gene libraries, metagenomes, enzymatic activities) can inform our ability to better understand and predict system-level processes. Unfortunately, additional information about the microbiome does not always provide a clearer understanding of ecosystem processes beyond what can be predicted by environmental factors alone[1,2].

Two recent meta-analyses[3,4] suggest that research at the intersection of ecosystem science and microbial ecology often relies on assumed relationships between microbiome characteristics and ecosystem processes and often do not test to see if those relationships are present. The first, an examination of 415 studies, found little evidence that protein-encoding genes (sometimes referred to as "functional genes") or gene transcripts correlate with associated biogeochemical processes[3]. Although all studies attempted (or presumed) to link microbial genes or transcripts with function, only 14% measured both the abundance of genes or transcripts and the corresponding process. When the relationship between microbial characteristic and ecosystem process was measured and tested only 38% exhibited a positive correlation[3]. This result was consistent whether functional gene or transcript abundance was used as the response variable.

49 The second study compiled a separate dataset of 148 studies that
50 examined microbial membership and ecosystem processes in response to
51 experimental manipulations[4]. Whereas 40% of included studies reported
52 concomitant changes in microbial membership and an ecosystem process, only one
53 third of those cases reported the relationship between microbial membership and an
54 ecosystem process. Interestingly, of the 53 studies that posed a hypothesis
55 about links between microbial membership and ecosystem processes, more than
56 half (53%) did not report testing for a statistical link between membership and
57 processes[4].
58 Microbiomes are the engines that power system-level processes[5]. However the
59 meta-analyses described above illustrate that the current approach to study the links
60 between microbiome characteristics and ecosystem processes are not well
61 formulated and relationships between microbiome characteristics and system level
62 processes are implied yet rarely tested. When linkages are tested, significant
63 correlations between microbiome characteristics and ecosystem processes are
64 sometimes present, but more frequently not[3,4]. One reason for the ambiguity
65 between microbiome characteristics and system level processes is that many studies
66 are conducted in the absence of a conceptual framework that illustrates how different
67 measurable microbial characteristics relate to one another and to the system level
68 process of interest.
69 ***Challenges in Linking Microbial Characteristics and Ecosystem Processes***
70 Current conceptual research frameworks that attempt to link microbial information
71 to a system-level process often do not effectively align with the methods being
72 applied or the data those methods generate. For example, environmental factors act
73 on the physiology of individual organisms, which alters their competitive ability,

abundance, and ultimately their contribution to an ecosystem process. However, designing an observational study or experiment from this framework assumes that environmental and microbial characteristics are measurable across multiple categories of ecological organization (i.e., individuals, populations, and communities) at the temporal and spatial scales at which they influence system level processes (Figure 1a). In addition, the relationships between environmental variables and microbial characteristics can be decoupled in both time and space[4], and are often non-linear[6]. Recent immigration, phenotypic plasticity, disequilibrium between the environment and the extant microbiome at the time of sampling, functional redundancy, and dormancy can all mask the relationship between measurable microbial characteristics and the processes microorganisms influence (Figure 1b).[4,7,8,9] As micrometer scale characteristics of microbiomes (10^{-6} m) are scaled to the level of ecosystems (m to km), we assume that our conceptual understanding is also scalable. However, each of the aforementioned confounding factors aggregate over multiple orders of magnitude often masking the very relationships we seek to elucidate (Figure 1b). To formalize how measurable microbiome characteristics are linked with system-level processes we have conceptually defined the intersection of microbial and ecosystem ecology and identified three categories of microbial characteristics to illustrate how they interact with each other and may contribute to an ecosystem process (Figure 2).

Mapping Ecosystem Processes to Microbial Characteristics

Defining the ecosystem process, its critical sub-processes, and the known phylogenetic distribution of the metabolic pathways that drive those sub-processes creates an explicit conceptual pathway that links the ecosystem process to the microorganisms that contribute to it. Ecosystem processes are defined as qualitative

99 changes in a pool, or a flux from one pool to another (e.g., NH_4^+ to NO_3^-, or dissolved
100 organic matter to CO_2). The first step to understand how a microbiome influences an
101 ecosystem process is to define the ecosystem process of interest and each sub-
102 process that contributes to it (i.e., the set of constituent reactions that combine to
103 determine a net flux). Ecosystem processes are composites of complementary or
104 antagonistic sub-processes, carried out by phylogenetically and metabolically
105 diverse microorganisms[10]. For example, net ecosystem productivity (NEP) is the
106 balance between antagonistic processes of C-fixation and C-mineralization. Each
107 sub-process of NEP can be further partitioned into a series of metabolic pathways
108 (e.g., chemoautotrophic nitrification and photoautotrophic C-fixation or heterotrophic
109 fermentation and aerobic respiration). Partitioning each ecosystem process in this
110 hierarchical manner can continue until the sub-processes maps directly to specific
111 microbial metabolic pathways (e.g., acetoclastic methanogenesis). Subsequently,
112 each of these metabolic pathways can be categorized as either phylogenetically
113 broad or narrow[11]. Broad processes are phylogenetically common (i.e., widely
114 distributed among taxa), whereas narrow processes are phylogenetically conserved
115 (i.e., limited to a specific subset of taxa). For example, denitrification and
116 photosynthesis are phylogenetically broad processes, while both methanogenesis
117 and methanotrophy are currently thought to be phylogenetically narrow processes
118 (with at least one notable exception[12]).
119 The second step is to identify the controls or constraints on each constituent sub-
120 process. For example, the kinetics of a single metabolic pathway in a model
121 organism may help us understand the rate limiting steps of a narrow process, but
122 insights from model organisms are much less likely to capture the full spectrum of
123 responses of a broad process where phenotypic variation among phylogenetically

124 diverse organisms is likely to be much greater[13,14]. Once the ecosystem process has
125 been conceptually partitioned into its component parts and their primary controls, a
126 concerted approach can be applied to investigate how characteristics of the
127 microbiome influence the ecosystem process of interest within the complexity of a
128 natural environment.

129 ***Categories of Microbial Characteristics***

130 The contribution of the microbiome to ecosystem processes is exerted through
131 aggregate community properties that are shaped by both microbial membership and
132 environmental factors. Most current studies rarely articulate how measurements of
133 microbial characteristics differ in their specificity (i.e., the level of phylogenetic
134 resolution), precision (i.e., the ability of the method to repeatedly describe the
135 characteristic of interest), or context (i.e., how a characteristic relates to other
136 microbial characteristics or the ecosystem). To aid in designing experiments that link
137 microorganisms to the processes they influence we propose three distinct categories
138 of microbial characteristics: 1) microbial processes, 2) microbial community
139 properties, and 3) microbial membership (Figure 2). This proposed framework allows
140 the researcher to clearly identify how different measurements used to characterize a
141 microbiome interact with each other, and to identify the potential of each
142 characteristic to elucidate the microbial contribution to the system level process. All
143 measurable characteristics of microbial communities (e.g., abundance of cells,
144 sequence of genes, transcripts, or proteins; enzyme expression or activity) can be
145 placed within one of the above categories. This conceptual structure that orientates
146 each microbial category within a broader context creates the opportunity to improve
147 the design of observational and experimental studies in microbiome research.

148 *Microbial Processes* - Microbial processes are the collective metabolisms of the
149 microbiome that contribute to changes in pools and fluxes of elements or compounds
150 (i.e., Figure 2, Letter K). This is the category of microbial information that can most
151 readily be incorporated into system-level models because many microbial processes
152 represent the key sub-processes that contribute to a particular ecosystem process
153 (e.g., methanogenesis + methanotrophy ≈ methane efflux). Commonly measured
154 microbial processes in ecosystem science include nitrogen fixation, denitrification,
155 nitrification, phosphorus uptake and immobilization, carbon fixation, and organic
156 carbon mineralization. The rates of many microbial processes can be approximated
157 through physiological assays (e.g., biological oxygen demand to estimate microbial
158 respiration), and while they do not open the "black box" of the microbial community,
159 they do directly quantify the microbial contribution (or potential contribution) to
160 changes in resources moving through the box. Microbial processes can be
161 distinguished from other microbial characteristics because they are all rates (i.e.,
162 have time in the denominator) and require a bioassay to estimate.
163 Assays used to estimate microbial processes are often logistically challenging,
164 require manipulations that inevitably deviate from *in situ* conditions, and often
165 depend on the environment from which the microbiome was sampled. For example,
166 the relationship between temperature and microbial processes such as enzyme
167 activity and phosphorus use efficiency vary across latitudinal gradients[15] and among
168 seasons[16], respectively. Thus, observations of the effect of temperature on either
169 enzyme activity or phosphorus use efficiency depend on where (e.g., at what
170 latitude) and when (e.g., during which season) they were measured. In the absence
171 of an understanding of the underlying physiological mechanism (e.g., the
172 physiological change that allows a community to perform differently at different

173 temperatures), the relationship between and environmental driver (e.g., temperature)
174 and a microbial process must be measured through a direct assay at each location
175 and at each time. This limits the inference possible from relying on measurements of
176 microbial processes alone to understand the microbial contribution to an ecosystem
177 process.
178 *Microbial Community Properties* - Microbial community properties include a broad
179 set of microbial characteristics such as community biomass or biomass elemental
180 ratios (e.g., biomass C:N or C:P ratios) and the majority of phylogenetically
181 undifferentiated aggregate sequence based measurements (e.g., gene abundance,
182 metagenomes, transcriptomes). Microbial community properties (Figure 2) represent
183 an integrated characteristic of the microbiome that has the potential to predict or at
184 least constrain the estimates of microbial processes. For example, microbial
185 community biomass C:N (a community property) has been shown to indicate a
186 microbiome's potential to mineralize or immobilize N in terrestrial[17], freshwater[18] and
187 marine[19] ecosystems.
188 Microbial community properties can be separated into two categories, emergent
189 properties and community aggregated traits. It is generally agreed that emergent
190 properties refer to a quality of the whole that is unique and distinguishable from the
191 additive properties of its constituents. Whereas in some cases, emergent properties
192 may be predicted from their constituent parts, for example, prediction of the physical
193 properties of carbon polymers is possible based on the atomic organization of
194 carbon atoms within each structure[20], in microbial ecology our understanding of the
195 constituent parts and their interactions are more often than not insufficient to predict
196 emergent properties. Here we use emergent properties as it has been defined
197 previously in ecology[21]: "An emergent property of an ecological unit is one which is

198 wholly unpredictable from observation of the components of that unit", which is also
199 consistent with its contemporary use in microbial ecology[22].
200 Emergent properties of microbiomes influence important ecosystem processes. For
201 example, a series of experiments in flow-through flumes mimicked development and
202 metabolism of stream biofilms[23]. Transient storage (i.e., an increase in residence
203 time of the water and its solutes near the biofilm relative to the flow around it)
204 increased as the microbial biofilm density increased.[23] In this case biofilm density
205 was an emergent property of the microbiome and transient storage was the microbial
206 process it mediated. Another example of an emergent property is the distribution of
207 traits that influences key microbial processes. Trait based approaches have a rich
208 history in ecology and have been increasingly applied to address questions in
209 multiple areas of microbial ecology.[24] For example, uptake of an organic substrate
210 can often involve the expression of multiple genes, differing among individual
211 organisms, all capable of performing uptake of the organic substrate, albeit with
212 differences in the underlying efficiency. The distribution of the expression of these
213 functional gene variants generates a trait distribution that is an emergent property of
214 the microbiome. That emergent trait distribution determines the overall performance
215 of the microbiome for that function (i.e., the uptake of the organic substrate), but it
216 cannot be predicted from the presence of the organisms conferring that trait using
217 current methods.[25] While characterization of emergent properties may improve the
218 understanding of microbial processes (Figure 2, Letter G) currently they most often
219 cannot be estimated or predicted on the basis of the constituent taxa (i.e.
220 membership) alone (Figure 2, Letter F), and thus must remain as an intermediary
221 between environmental drivers (Figure 2, Letter C) and microbial processes (Figure
222 2, Letter G).

223 Unlike emergent properties, community aggregated traits can potentially be
224 estimated from characteristics of their constituents and provide a pathway to link
225 microbial community membership to the community properties that drive microbial
226 processes (Figure 2, Letter E).[26] For example, community aggregated traits may
227 include commonly measured community properties such as functional gene
228 abundance as estimated from qPCR (e.g., *pmo*A which encodes a subunit of the
229 enzyme involved in methane oxidation, can be used to estimate potential for
230 methanotrophy and as a phylogenetic marker for methanotrophs)[27]. A recent
231 perspective article that discussed the role of community aggregated traits in
232 microbial ecology noted a series of additional putative community aggregated traits
233 (e.g., maximum growth rate, dormancy, osmoregulation) that could be inferred from
234 metagenomic data of the extant community.[26]
235 Understanding which community properties can be predicted by membership is a
236 critical research question and an important step in understanding how the
237 microbiome contributes to system level processes. Whether or not a community
238 property is likely to be an emergent property or a community aggregated trait is an
239 exciting area of research and provides an important framework to advance research
240 at the microbial-ecosystem nexus. New approaches, like studying higher-level
241 interactions in ecological communities could help understand how a microbiome's
242 constituents interact to from emergent properties.[28] This is not a trivial task, yet a
243 suite of existing methods, discussed below, provides the ability to directly pursue this
244 challenge.
245 *Microbial Community Membership* **-** Although the now commonplace analysis of
246 community membership by sequencing phylogenetic markers (e.g., ITS regions or
247 regions of the16S rRNA and 18S rRNA genes) or suites of phylogenetically

248 conserved protein sequences identifies constituent microbial taxa, the direct coupling
249 of microbial phylogeny to physiology and ecology remains elusive (Figure 2, Letter
250 H).[29,30,31] In general the paucity of associated physiological data or information on
251 population phenotypes that accompany phylogenetic analyses limits the system-level
252 inference that is possible from analyses of community membership. Even when the
253 physiology of an organism is known, it appears many metabolic pathways are
254 phylogenetically broad, and that any given microbiome will contain a diverse set of
255 microorganisms with the genes that encode many of the same common microbial
256 metabolic pathways, often referred to as "functional redundancy"[32]. There also
257 appears to be no consistent phylogenetic resolution at which specific microbial
258 metabolic pathways are constrained[32]. This constrains our ability to attribute
259 microbial processes to community membership of even relatively simple
260 environmental consortia. Whereas it is clear that microbial populations are not
261 randomly distributed in space and time[31], and that some microbial traits are
262 conserved at coarse taxonomic scales, [24,33,34] the physiological mechanisms
263 underlying non-random distributions of microbial taxa across environmental
264 gradients are often unknown. This limited understanding of the metabolism of most
265 bacterial phyla is one thing that currently prevents linking a microorganism's
266 abundance in an environment to its role in a related microbial process.

267 ***A Path Forward***

268 We suggest that a challenging but necessary step for microbiome science is to
269 move away from identifying correlative relationships between characteristics of the
270 microbiome and system level processes, and towards identifying more causative and
271 mechanistic relationships. The conceptual diagram (Figure 2) is a road map to
272 organize and link the diverse suite of measurable microbial characteristics that are

273 currently available to researchers. Figure 2 does not represent how these
274 components necessarily interact in the environment; rather it is a map that identifies
275 potential links between measurable microbial and system-level characteristics that
276 can help structure our exploration of how microorganisms influence the systems they
277 inhabit. Ecosystem ecology has traditionally been confined to interactions between
278 environmental parameters and ecosystem processes (depicted within the horizontal
279 arrow, Figure 2). Similarly, microbial ecology (depicted within the vertical arrow,
280 Figure 2) has historically focused on phylogenetically undefined aspects of microbial
281 communities (e.g., bacterial abundance) and microbial processes (e.g., bacterial
282 production), or the physiology of microbial isolates (e.g., sulfate reducing bacteria) or
283 the collective physiology of highly reduced communities with known membership
284 (e.g., waste water treatment microbiome). The routine inclusion of sequence-based
285 approaches in studies of environmental microorganisms has led to an increasingly
286 detailed description of the world's microbiomes and an increasing interest in how
287 constituents of those microbiomes interact to influence the system as a whole.
288 Currently, researchers use a range of approaches for attempting to link
289 characteristics of the microbiome to ecosystem processes. Direct connections
290 between microbial membership and ecosystem processes (Figure 2, Letter I), or
291 community properties and ecosystem processes (Figure 2, Letter J), have proven
292 difficult to establish.[3,4] We propose 1) identifying which microbial processes are likely
293 to contribute to ecosystem-level pools and fluxes *a priori* (Figure 2, Letter K), 2)
294 determining which microbial community properties best describe and predict these
295 microbial processes (Figure 2, Letter G), and 3) identifying whether the community
296 properties that best describe each process are a community aggregated trait or a
297 emergent property (Community Properties, Figure 2). If the community property is

298 likely to be a community aggregated trait then exploring the link between microbial
299 membership and community properties may lead to further understanding and
300 perhaps an enhanced predictive power (Figure 2, Letter E). However, if the
301 community property is likely an emergent property elucidating the microbial
302 membership that contributes to the emergent property is, given the current
303 understanding, unlikely to improve understanding of the drivers of that community
304 property (Figure 2, Letter F). Formalizing microbiome research into a structured,
305 conceptual framework should help the research community better focus on potential
306 links between microbiome characteristics and system-level processes that are most
307 likely to be detected empirically. This approach will also allow researchers working in
308 different systems to test the same pathways among defined microbiome
309 characteristics and thus increase the possibility of understanding the causal
310 mechanism (or absence of causality) for observed correlations. Thus, future
311 research endeavors will be most powerful if they focus on elucidating connections
312 through the complete path of microbial ecology (Figure 2, blue arrow, Letters E, F,
313 and G) and not direct connections between microbial membership or community
314 properties and ecosystem processes (Figure 2, Letters I and J).

315 ***Applying and Testing the Proposed Framework***

316 Applying and testing the proposed framework will depend on the ability to more
317 robustly characterize each category of microbial characteristics and to directly
318 measure the arrows that connect each category (Figure 2). Both labeling/sorting
319 approaches and phenotypic description of isolates provide an opportunity to better
320 understand how microbial membership contributes to community properties (Figure
321 2, Letter E or F). Labeling and cell sorting approaches (e.g., fluorescent in situ
322 hybridization (FISH) coupled with flow cytometry cell sorting,[35] or immunocapture,

323 such as with bromodeoxyuridine, BrdU)[36] provide powerful tools to constrain the
324 complexity of the microbiome and directly test hypotheses that link membership to
325 community properties or microbial processes. For example, a study of an Arctic
326 Ocean bacterial community labeled the actively growing component of the
327 community using BrdU and then separated those populations from the rest of the
328 community using an immunocapture technique to better understand the portion of
329 the microbiome that was contributing to secondary production.[36] In addition,
330 physiological studies of isolates that are representative of important community
331 properties have the potential to advance understanding of the role of phenotypic
332 plasticity in structuring how constituent populations do or do not contribute to
333 community properties (Figure 2, Letter E)[13]. Detailed studies of isolates of common
334 environmental OTUs have demonstrated immense variation within a given OTU (i.e.,
335 "microdiversity") that in part explains the challenge of linking membership to a
336 community property[14]. For example, work on *Prochlorococcus* has led to a better
337 understanding of how ecotypes within a single taxonomic unit (OTU) can lead to
338 specialization in temperature, and substrate affinity[37]. OTUs that form a substantial
339 portion of the microbiome's sequence abundance provide potential candidates for
340 further investigation of possible phenotypic plasticity and or microdiversity[14]. For
341 example, a single phylotype of the class *Spartobacteria* within the phyla
342 *Verrucomicrobia* was found to be present in a broad range of soil ecosystems and
343 comprised as much as 31% of all 16S rRNA gene sequences returned from prairie
344 soils[38], making it an excellent candidate for targeted isolation and physiological
345 studies. Studies of environmental isolates are essential in building a broader
346 understanding of how community membership does or does not contribute to
347 community properties (Figure 2, Letters E and F).

348 In addition to a better description of each category of microbial characteristics, an
349 important step in moving from a correlative and descriptive approach to a causative
350 and mechanistic approach comes in measuring the arrows represented by letters in
351 Figure 2. There is a suite of powerful methods already being employed in microbial
352 ecology that can actively measure many of the arrows illustrated in Figure 2,
353 including: stable isotope probing of mixed communities[39], single cell methods that
354 can assay cells in the physiological state they occur in in the environment, and
355 labeling individual cells with stable isotopes for single cell analyses[40]. Studies that
356 use stable isotope probing or any form of tracking isotopically labeled substrates into
357 a population have been successful in linking microbial membership to microbial
358 processes (Figure 2, Letter H). For example, a study of a Scottish peatland used SIP
359 to reveal that a single species of *Desulfosporosinus* was most likely responsible for
360 the totality of sulfate reduction within the peatland even though it only comprised
361 0.006% of the retrieved 16S rRNA gene sequences[41]. In addition to this example of
362 using SIP to link microbial membership and microbial processes (Figure 2, Letter H),
363 there is a suite of cultivation independent techniques (such as Raman
364 microspectroscopy (MS), NanoSIMS, or energy dispersive spectroscopy, EDS) that
365 complement sequence-based microbiome analyses by reporting on the physiological
366 and phenotypic characteristics of individual cells *in situ*. [40,42,43] Both Raman MS and
367 NanoSims can be coupled with a range of *in situ* hybridization techniques (e.g.,
368 fluorescent in situ hybridization, FISH) to identify which populations are contributing
369 to community properties (Figure 2, Letter E) or microbial processes (Figure 2, Letter
370 H). For example, a study of a microbial consortia from the Sippewissett Salt Marsh
371 on the coast of Massachusetts, USA used a combination of FISH and NanoSIMs to
372 confirm a syntrophic association between a population of autotrophic purple sulfur

373 bacteria and heterotrophic sulfate reducing bacteria (SRB)[44]. These existing
374 methods of confirmatory ecophysiology allowed for direct measurements of the
375 arrows connecting membership with microbial processes (in this case both carbon
376 fixation and sulfate reduction, Figure 2, Letter H) in a stable microbial consortium.
377 These cultivation independent approaches also create the potential to begin to
378 determine which community properties are emergent properties, and which are
379 community aggregated traits. For example, microbial community biomass
380 stoichiometry (e.g., biomass C:N or C:P) cannot currently be predicted (or even
381 constrained) from a list of its constituent taxa (Figure 2, Letter F). However, microbial
382 biomass stoichiometry is a community property with power to predict the microbial
383 contribution to nutrient cycling (Figure 2, Letter G).[17-19] Energy dispersive
384 spectroscopy (EDS) has the power to measure the C:N:P of individual bacterial cells
385 growing *in situ* (i.e. not in culture)[43]. The potential to couple EDS analysis with a
386 phylogenetic label presents the opportunity to assay mixed microbial communities
387 and assess the link between phylogenetic identity and biomass stoichiometry under
388 natural conditions[45]. This approach would provide a direct link between community
389 membership and a community property (e.g., biomass C:N, Figure 2, Letter E), that
390 influences an important microbial process (i.e., nutrient recycling). These
391 approaches applied in concert with sequence-based analyses have the potential to
392 empirically link the categories of microbial information defined here (Figure 2) with
393 the processes they influence, moving microbiome science from a descriptive and
394 correlative approach to a mechanistic and causative approach.

395 ***Designing microbiome research***

396 The framework presented here provides one approach to formalize inquiry across
397 microbiome science and encourages empirical linkages between the presence of

organisms in a system and the processes that characterize that system. Whereas we draw examples from environmental microbiomes and the ecosystems they inhabit, this structured approach has the potential to benefit the analysis of microbiomes associated with other systems such as host organisms and those of the built environment. As important as establishing causal links among microbial membership, community properties, microbial processes, and ecosystem processes, is determining when these links are unlikely to be present. Research that indiscriminately seeks to identify correlations, which places all microbial characteristics on an equal plane and does not explicitly recognize the relationships between microbial characteristics, are likely to continue to yield conflicting and ambiguous results that not only fail to provide new insight into ecosystem processes, but also blur the connections that do exist. We suggest that rather than looking for linkages among microbiome membership and system-level processes in every study, research efforts would benefit from strategically targeting the linkages and processes for which an *a priori* understanding of microbial physiology should allow us to improve our understanding of the ecosystem process.

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Figures

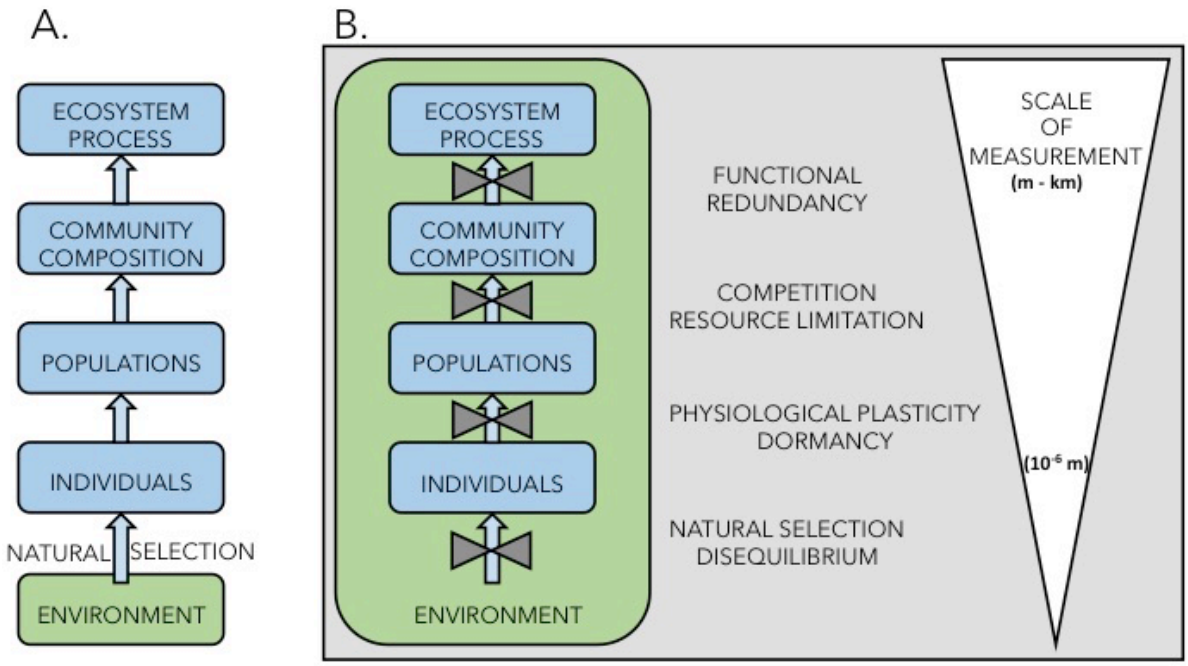

Figure 1 Diagram of microbial-ecosystem linkages A) how linkages are commonly conceptualized across levels of ecological organization and B) the series of ecological phenomena that create challenges when attempting to link metrics from one level of ecological organization to the other.

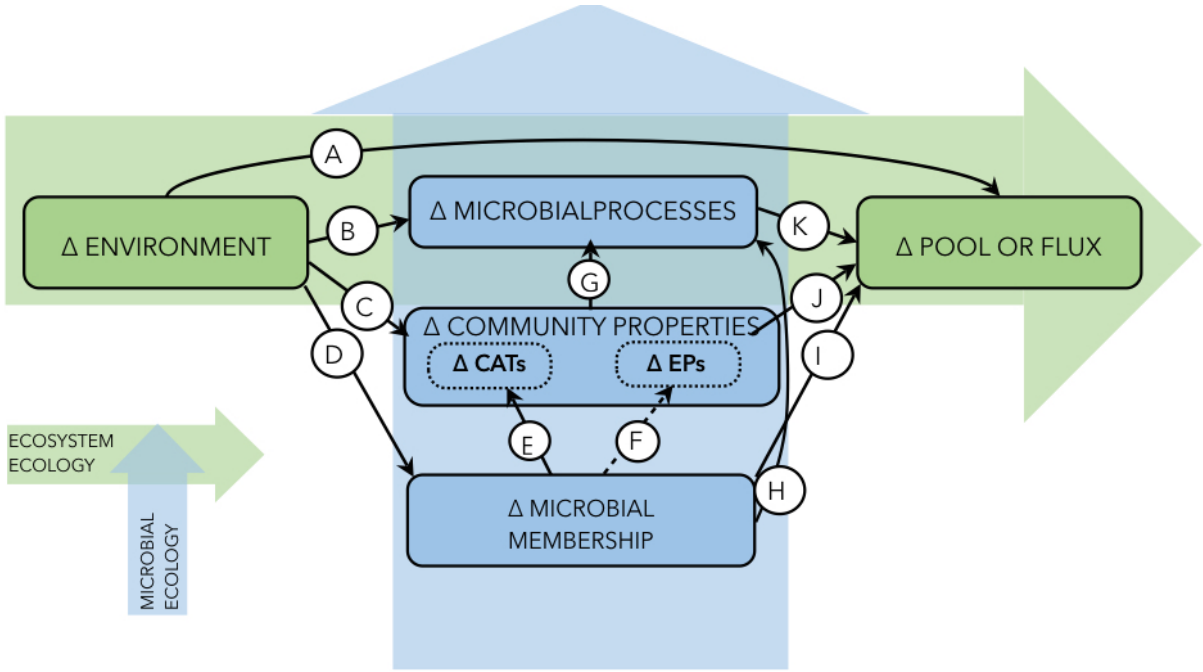

Figure 2 A conceptual map of the intersection between microbial (vertical) and ecosystem (horizontal) ecology illustrating each of the three categories of microbial characteristics (microbial processes, community properties, and microbial membership) as defined in the text. We argue for an increased focus on studies that elucidate pathways E, F, and G. In addition, we note that pathways I and J are less likely to effectively incorporate microbiome characteristics into system-level science. The delta symbol in each category indicates an emphasis on how changes within a category may lead to a change in a connected category. The dotted arrow for letter F denotes that many emergent properties cannot currently be linked to membership and is an important area for active research. CAT = community aggregated trait, EP=emergent property.

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