Understanding How Microbiomes Influence the Systems they Inhabit: Insight from Ecosystem Ecology

Authors: Hall, E.K.[1*], Bernhardt, E.S[2], Bier, R.L.[2], Bradford, M.A.[3], Boot, C.M.[1], Cotner, J.B.[4], del Giorgio, P.A.[5], Evans, S.E.[6], Graham E. B [2,7.8], Jones, S.E.[9], Lennon, J.T.[10], Locey, K.[10] Nemergut, D.[2], Osborne, B.[11], Rocca, J.D.[1,2], Schimel J.S.[12], Waldrop, M.P.[13], Wallenstein, M.W.[1]

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Affiliations: [1]Colorado State University, Fort Collins CO (ed.hall@colostate.edu, claudia.boot@colostate.edu, matt.wallenstein@colostate.edu) [2]Duke University, Durham, N.C. (ebernhar@duke.edu, ebgraham2@colorado.edu, diana.nemergut@duke.edu) [3]Yale University, New Haven, CT (mark.bradford.yale@gmail.com) [4]University of Minnesota, Saint Paul, MN (cotne002@umn.edu) [5]Université du Québec à Montréal, Montréal, CA (del_giorgio.paul@uqam.ca) [6]Michigan State University, Hickory Corners, MI (evanssar@gmail.com) [7]Institute of Arctic and Alpine Research, University of Colorado at Boulder [8] Pacific Northwest National Laboratory, Richland, WA, USA [9]Notre Dame University, South Bend, IN (sjones20@nd.edu) [10]Indiana University, Bloomington, IN (lennonj@indiana.edu, ken@weecology.org) [11]Brown University, Providence, Rhode Island (brooke_osborne@brown.edu) [12]University of California, Santa Barbara, Santa Barbara, CA (josh.schimel@lifesci.ucsb.edu) [13]U.S. Geological Survey, Menlo Park, CA (mwaldrop@usgs.gov) Biological Sciences Division,

* **corresponding author**: Dr. Ed Hall, Natural Resource Ecology Laboratory, Department of Ecosystem Science and Sustainability, Campus Delivery 1499, Colorado State University, Fort Collins, CO 80523, ed.hall@colostate.edu, 970-491-2162

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Abstract

The well-documented significance of microorganisms to the function of virtually all ecosystems has led to the assumption that more information on microbiomes will improve our ability to understand and predict system-level processes. Notably, the importance of the microbiome has become increasingly evident in the environmental sciences and in particular ecosystem ecology. However, translating the ever-increasing wealth of information on environmental microbiomes to advance ecosystem science is proving exceptionally challenging. One reason for this challenge is that correlations between microbiomes and the ecosystem processes they influence are often reported without the underlying causal mechanisms. This limits the predictive power of each correlation to the time and place at which it was identified. In this paper, we assess the assumptions and approaches currently used to establish links between environmental microbiomes and the ecosystems they influence, propose a framework to more effectively harness our understanding of microbiomes to advance ecosystem science, and identify key challenges and solutions required to apply the proposed framework. Specifically, we suggest identifying each microbial process that contributes to the ecosystem process of interest *a priori.* We then suggest linking information on microbial community membership through microbial community properties (such as biomass elemental ratios) to the microbial processes that drive each ecosystem process (e.g. N - mineralization). A key challenge in this framework will be identifying which microbial community properties can be determined from the constituents of the community (community aggregated traits, CATs) and which properties are unable to be predicted from a list of their constituent taxa (emergent properties, EPs). We view

25 this directed approach as a promising pathway to advance our understanding of how
26 microbiomes influence the systems they inhabit.

Current approaches in linking microbial characteristics and ecosystem processes

27 Recently there has been a broad call, including the National Microbiome Initiative
28 led by the executive branch of the United States Federal Government, for a
29 coordinated effort to evaluate the role of microorganisms in all environments[1,2] .
30 Coordinating efforts to explore microbiomes and their functioning across such a
31 broad range of systems is exciting and ambitious and holds the potential to transform
32 societies approach many of the most important challenges we currently face[3].
33 However, advances in this direction require an assessment of our progress to date
34 and an attempt to identify the most promising paths forward.
35 In most ecosystems, many processes are carried out primarily by microorganisms,
36 and virtually all processes are influenced by microorganisms. Therefore it is common
37 to apply high-resolution analytical techniques to better describe microbial
38 communities, assuming that greater resolution of the community, (including its
39 associated transcripts, proteins, and metabolic products) should lead to better
40 predictions of ecosystem processes. However, such justifications assume that
41 microbial metrics (e.g. 16S rRNA gene libraries, metagenomes, enzymatic activities)
42 will improve our ability to understand, model, and predict ecosystem processes. This
43 assumption may not necessarily be valid, because microbial information may not
44 provide additional explanatory power for understanding ecosystem process rates
45 beyond what can be predicted by environmental factors alone[4,5] . In addition, when
46 correlations between microbial information and an ecosystem process are identified
47 the underlying causal associations may remain difficult to resolve and may not be
48 causal at all—e.g. the microbiome and the ecosystem may be responding in concert

49 to a common underlying driver; limiting the predictive ability of each result across
50 additional systems.
51 Two recent meta-analyses suggest that the current research approach at the
52 intersection of ecosystem and microbial ecology has the potential to be better
53 focused to more effectively achieve the anticipated insights into how microbiomes
54 influence ecosystems[6,7]. The first meta-analysis evaluated studies that related the
55 relative abundance of protein encoding gene copy or transcript abundance to
56 associated biogeochemical processes[6]. Of 416 identified studies that attempted to
57 address the correlation between the relative abundance of a protein-encoding gene
58 (or transcript) and an ecosystem process, only 56 measured both genes or transcript
59 copy number and the corresponding process. Within these 56 studies, 14% of the
60 observations showed a significant negative correlation between gene copy number
61 and process rate, 38% had a significant positive relationship, and 48% had no
62 significant relationship. Thus, the effect size for the relationship between gene copy
63 number and process rate had an approximately normal distribution with a mean near
64 zero[6]. The second meta-analysis evaluated links between microbial community
65 composition and ecosystem processes in response to an experimentally-induced
66 disturbance in the environment (e.g. drought, warming, nutrient addition)[7]. Whereas
67 40% of published papers reported concomitant changes in microbial community
68 structure and ecosystem function, only about a third of those cases (only 12% of
69 total studies) attempted to identify a statistical relationship between community
70 composition and an ecosystem process. Interestingly, many of the studies that did
71 not measure both community composition and a corresponding ecosystem process
72 still framed their study in the microbial structure-ecosystem function framework[7].

These meta-analyses illustrate that links between microbial characteristics and ecosystem processes are often assumed to be present but are rarely tested. When linkages are explicitly tested, connections between microbial structure and ecosystem processes are more often than not weak or non-existent[6]. These findings suggest that our current approach to linking microbial metrics to ecosystem function should be refocused with more attention paid to empirically identifying explicit linkages between microbial characteristics and the ecosystem processes that they influence.

Linking microbial characteristics and ecosystem processes

A key challenge in linking microbial information to an ecosystem process is that conceptual research frameworks often do not align directly with the available methods or the data they generate. For example, environmental factors act on the physiology of individual organisms, which alters their competitive ability, relative abundance, collective physiology, and ultimately their contribution to ecosystem processes (Figure 1a). However, designing an observational study or experiment from this conceptual framework (Figure 1a) assumes that environmental metrics can be empirically linked to measureable microbial characteristics across multiple categories of ecological organization (i.e. individuals, populations, and communities) at the appropriate temporal and spatial scales. Yet in nature, relationships between environmental variables and microbial characteristics are dynamic and non-linear[8], simultaneously affected by a plethora of biotic and abiotic variables[9,10] and decoupled in both time and space[7]. Each of these aspects of microbial-environment interactions obscures the relationships among microbial characteristics collected at each level of ecological organization and the ecosystem processes they affect (Figure 2b). To address this challenge we propose a framework that explicitly

identifies the ecosystem process of interest and how it relates to microbial characteristics. The proposed framework illustrates the relationship among different categories of microbial characteristics and conceptually defines their contribution to ecosystem processes.

Identifying the Ecosystem Process The first step to understand how microorganisms influence an ecosystem process is to define each of its sub-processes, the set of constituent reactions that combine to dominate the net flux of the ecosystem process of interest. Ecosystem processes are defined as a change in a pool size or a flux from one pool to another (e.g. NH_4^+ to NO_3^-, or dissolved organic matter mineralization to CO_2). Few, if any of these processes are carried out by a single physiological pathway or a single organism. Rather, ecosystem processes are aggregate processes consisting of complementary or antagonistic sub-processes carried out by a breadth of phylogenetically diverse microorganisms[11]. For example, net ecosystem productivity (NEP) is the balance between C-fixation and C-mineralization. Each sub-process of NEP can be further partitioned into a series of metabolic pathways (e.g. heterotrophic fermentation and aerobic respiration or chemoautotrophic nitrification and photoautotrophic C-fixation). Partitioning each ecosystem process in this hierarchical manner can continue until the sub-process maps directly to specific microbial metabolic pathways (e.g. acetoclastic methanogenesis). Subsequently each of these metabolic pathways can be categorized as either broad or narrow[12]. Broad processes are phylogenetically common (i.e. widely distributed among taxa), whereas narrow processes are phylogenetically conserved (i.e. limited to a specific subset of taxa). For example, denitrification is broad, while both methanogenesis and methanotrophy are narrow (with some notable exceptions[13]).

The second step is to identify the controls or constraints on each constituent sub-process. For example, kinetics of a given metabolic pathway in a model organism may help understand the rate limiting steps of a narrow process, but insights from model organisms are much less likely to be useful for a broad process where phenotypic variation among phylogenetically diverse organisms should be much greater. Defining the ecosystem process, its critical sub-processes, and the known phylogenetic distribution of the metabolic pathways that drive those sub-processes in this manner creates an explicit conceptual pathway that directly links the ecosystem process to the microorganisms that influence them. Once this conceptual pathway has been identified a concerted empirical approach can be applied to investigate how the microbiome influences the ecosystem process of interest.

Understanding the relationship between categories of microbial characteristics

Categories of microbial characteristics At present, researchers are measuring a wide variety of characteristics of microbial communities (e.g. sequence or relative abundance of genes, transcripts or proteins, enzyme expression, and process rates). Much of that work does not clearly articulate how these measurements differ in their specificity, precision, or linkage among each other or how they inform the microbial contribution to ecosystem processes. We propose that by categorizing microbial characteristics into three distinct categories, 1) microbial processes, 2) microbial community properties, and 3) microbial community membership (Figure 2), we can frame how different metrics interact with each other and how they can elucidate the microbial contribution to an ecosystem process.

Microbial Processes Microbial processes are the collective physiology of the microbial community that drives changes in pools and fluxes of important elements

147 or compounds[14] (Figure 2). They are the level of microbial information that can most
148 readily be incorporated into ecosystem-level models because many of these
149 processes are the sub-processes that contribute to an overall pool or flux. Examples
150 include nitrogen fixation, denitrification, nitrification, phosphorus uptake and
151 immobilization, primary production, respiration, and carbon use efficiency. The rates
152 of many microbial processes can be estimated through physiological assays, and
153 while they do not open the "black box" of the microbial community, they do directly
154 quantify the microbial contribution to the transformation of substrates moving through
155 the box.

156 However, physiological assays to estimate microbial processes are often
157 logistically challenging, require experimental manipulations that inevitably deviate
158 from the *in situ* conditions, and often depend on the environment in which they were
159 measured. For example, the relationship between microbial process rates and
160 temperature vary across geographical temperature gradients[15] (enzyme activity) and
161 among seasons[16] (phosphorus use efficiency, PUE). Thus observations of the effect
162 of temperature on either enzyme activity or PUE are time and place dependent.
163 Therefore, without an underlying physiological mechanism, to accurately quantify the
164 microbial process the relationship between temperature and community physiology
165 must be measured through a direct assay at each location and at each time.
166 Because of these limitations, a microbial community property that can be measured
167 *in situ* or collected and preserved in the field for later analysis in the laboratory has
168 several advantages over using bioassays to directly measure microbial processes. A
169 community property may include characteristics of community biomass such as
170 elemental ratios (biomass C:N or C:P ratios) that estimate potential to mineralize or
171 immobilize limiting nutrients, or the relative abundance of genes that encode for an

172 known physiology or physiological response (such as the relative abundance of cold
173 shock proteins to estimate cold tolerance)[17]. We refer to these *in situ* measurements
174 that allow estimation of microbial processes as microbial community properties
175 (hereafter community properties, Figure 2). Community properties represent an
176 integrated characteristic of the extant microbial community that has the potential to
177 estimate the microbial process of interest.

178 *Microbial Community Properties*

179 Microbial community properties can be separated into two categories, emergent
180 properties (EPs) such as biofilm thickness, which cannot be determined from the
181 properties of their constituent populations[18], and community aggregated traits
182 (CATs) such as nitrification potential, which can be estimated from community
183 membership or at least characteristics such as relative gene abundance (e.g.
184 AmoA), of those members[17].

185 The potential importance of EPs to influence ecosystem processes was
186 demonstrated in series of experiments conducted in flow-through flumes that
187 mimicked development and metabolism of stream biofilms[19]. Both transient storage
188 (i.e. an increase in residence time of the water and its solutes near the biofilm
189 relative to the flow around it) and the biofilm community's ability to use arabinose
190 relative to glucose increased as the microbial biofilm density increased and porosity
191 decreased. Microbial biofilm thickness and density are both EPs that affected the
192 important ecosystem processes of hydrological transient storage and substrate use
193 specificity[19]. In this case biofilm thickness was affected by physical factors (i.e. flow)
194 but biofilm thickness may also be influenced by other environmental characteristics
195 such as P availability[20].

196 While EPs are powerful metrics for understanding ecosystem processes (Figure 2,
197 Letter F or H) they cannot, by definition, be estimated from a list of constituent taxa
198 or characteristics of those taxa (Figure 2, Letter E) and thus must remain as an
199 intermediary between environmental drivers such as flow or P availability (Figure 2,
200 Letter C) and ecosystem processes.

201 Unlike EP's CATs can be estimated from characteristics of their constituents and
202 provide one pathway to link microbial community membership to the community
203 properties that drive ecosystem processes[17] (Figure 2, Letter E and F). Microbial
204 community biomass stoichiometry (e.g. biomass C:N or C:P) is one example of a
205 putative CAT that has been shown to be a useful predictor of nutrient immobilization
206 or mineralization during litter decomposition[21], and can predict both respiration and
207 N-mineralization better than microbial biomass alone[22]. In a study of soil microcosms
208 amended with organic carbon and reactive N, the relationship between the resource
209 C:N and microbial biomass C:N was able to better predict whether C would be
210 respired or immobilized relative to microbial biomass alone[22]. Biomass stoichiometry
211 has been shown to differ among phylogenetically different organisms. For example,
212 at the broadest level microbial biomass C:N differs between fungi and bacteria[24], and
213 has been shown to be variable among a wide range of taxa grown on the same
214 media[25,26]. Thus community biomass stoichiometry has the potential to be empirically
215 deconstructed into the biomass stoichiometry of its constituent taxa[27], linking
216 community membership and a community property (e.g. biomass C:N) with the
217 power to estimate an important microbial process (e.g. N-mineralization).

218 *Microbial Community Membership*

219 Although analysis of community membership by sequencing phylogenetic marker
220 genes provides organism identity, the direct coupling of microbial phylogeny to its

221 physiology and ecology is often weak[28] (Figure 2, Letter G). For example, most
222 organic carbon molecules can be metabolized by a phylogenetically diverse suite of
223 organisms and denitrification is also a phylogenetically broad process. The result is
224 that with the exception of some specialists (e.g. nitrifiers), we can infer very little
225 about the function of microbial communities from a list of their constituent taxa.
226 Whereas it is clear that microbial populations are non-randomly distributed in space
227 and time[29] and some microbial traits appear to be conserved at coarse taxonomic
228 scales[30,31,32] the underlying physiological mechanisms for phylogenetic sorting
229 across environmental gradients is often unknown. This prevents an explicit link
230 between the organism's relative abundance and their role in the collective
231 community physiology that influences ecosystems. The paucity of associated
232 physiological data that accompanies phylogenetic sequence data in most studies
233 limits the ecological insight from phylogenetic analyses and constrains our ability to
234 attribute microbial processes to community membership even of well-defined
235 consortia.
236 In addition to the paucity of ecological inference provided by an analysis of
237 community membership, community analysis using current methods has two
238 exceptional challenges that need to be addressed in order to gain insight from
239 community membership to drivers of ecosystem processes. First, bulk extraction of
240 DNA from environmental samples, often the first step in analysis of microbial
241 communities, may or may not represent the extant and active microbial community at
242 the time of sampling. In microbial ecology, unlike in plant or animal ecology, the
243 number, biomass, and identity of different populations cannot be assessed with
244 confidence[33,34]. When DNA is extracted from the environment the presence or
245 relative abundance of a given sequence is not necessarily proportionate to the

246 absolute abundance or biomass of that organism within the community. One reason
247 for this is that the extracted nucleic acid may not have been contained within a
248 microbial cell at the time of extraction. This idea has been well established[35] and the
249 proportion of extracellular DNA is known to vary among ecosystems[36] (e.g. there
250 may be more DNA in sediment than the water column). It is clear that a portion of
251 sequences derived from any environmental sample are not from intact cells[37,38,39].
252 Even when specific phylotypes can be empirically linked to intact cells (e.g. using *in*
253 *situ* hybridization), the viability and metabolism of that cell typically remains
254 unknown. Because of this, the presence of a sequence within a "community" does
255 not indicate the associated organism is participating in the microbial process of
256 interest, and if active, it does not indicate that that the organism's contribution is
257 proportionate to the relative abundance of its DNA sequence. While these facts are
258 readily acknowledged, they can be very challenging to address, and therefore are
259 often overlooked or ignored as a problematic but non-addressable constraint,
260 something of an inconvenient truth of microbial ecology.
261 The second major challenge in linking relative abundance of microbial populations
262 with microbial processes is that the diversity, growth rate, and metabolic complexity
263 of environmental microorganisms are orders of magnitude greater than all other
264 organisms. The result is that when scaling from individuals through populations to
265 microbial communities, the obfuscating factors described in Figure 1b introduce and
266 ultimately accumulate more uncertainty at higher levels of ecological organization,
267 relative to the same analyses applied to macroorganism-ecosystem linkages. This
268 uncertainty is further confounded because community composition and processes
269 are routinely measured at different spatial scales[40]. Translating microbial
270 measurements to an ecosystem flux typically requires linking measurements from

271 microbial physiology (10^{-12} m), to microbial process measurements (10^{-1} or 10^{-2} m),
272 to ecosystem process measurements (m to 10^{3} m). This enormous scale (15 orders
273 of magnitude) over which to interpolate data, raises challenges analogous to
274 mechanistically analyzing the global carbon cycle, i.e. linking experiments on grams
275 of carbon in soils (e.g. g C per g dry weight) to global fluxes of Petagrams (10^{15} g).
276 However, unlike global C cycle research, microbial-to-ecosystem research often
277 addresses these in a descriptive manner using correlative approaches, without the
278 rigor and quantitative modeling approaches typically applied to global
279 biogeochemical cycles[41]. Each of these challenges must be effectively addressed in
280 order to rigorously incorporate the growing wealth of information on microbiomes to
281 system level processes.

282 ***A Way Forward at the Intersection of Microbial and Ecosystem Science***

283 The conceptual diagram presented here (Figure 2) provides a road map for
284 organizing and linking the diverse suite of microbial characteristics that are
285 commonly measured. Ecosystem ecology has traditionally been confined to the
286 interactions depicted within the horizontal arrow, moving from environmental
287 parameters to ecosystem processes (Letter A, Figure 2). As the role of the
288 microbiome has come to the forefront of environmental sciences it is clear that
289 microbial ecology has a great deal to contribute. However, microbial ecology has
290 traditionally been confined to interactions depicted within the vertical arrow, moving
291 from individuals (microbial community membership) to process rates (microbial
292 processes) of populations or more recently communities (Figure 2). The excitement
293 to integrate microbial metrics into ecosystem science has led to a range of novel
294 approaches of linking characteristics on the microbiome to ecosystem processes.
295 Direct connections between microbial community membership and ecosystem

296 processes (Figure 2, Letter J) or community properties and ecosystem processes
297 (Figure 2, Letter I) are almost exclusively correlative in nature. Whereas many of
298 these results are intriguing, the relationships discovered across these pathways, in
299 the absence of the defining physiological mechanism, are often restricted to the time
300 and place they are identified. Because of this, moving from correlative,
301 phenomenological approaches to a causative and mechanistic understanding is a
302 challenging but necessary step for microbiome science.
303 Because microbial processes can be estimated by community properties (e.g. N
304 mineralization and biomass C:N), understanding the drivers of community properties
305 is a way to more explicitly link environmental microorganisms with the ecosystem
306 processes they control. We propose that identifying which community properties best
307 describe microbial processes (Figure 2, Letter F), then identifying whether or not the
308 community properties that best describe each process are a CAT (Letter E, Figure 2)
309 or an EP (Figure 2 Letter C) provides clear pathway to understand whether
310 environmental drivers or microbial drivers dominate ecosystem processes. Currently,
311 many microbial community properties are EPs (i.e. cannot be predicted from their
312 constituent members or their characteristics). Understanding when community
313 properties can be predicted by membership is an important and open research
314 direction. Distinguishing which community properties represent aggregated traits
315 (CATs) and which are actually EPs may be an essential link in advancing our ability
316 to apply microbial information to ecosystem science. This is not a trivial task,
317 however a suite of existing methods already provides the ability to directly pursue
318 this challenge.
319 *Applying the proposed framework*

320 Understanding the principal drivers of community properties will require a series of
321 complementary approaches applied in concert, including; stable isotope probing of
322 cultured isolates and mixed communities, single cell methods that can assay cells in
323 the same physiological state they occur in in the environment, sorting of complex
324 communities into subsets of populations or consortia for further investigation, and
325 physiological assays of isolates grown in culture. Studies that use either labeled
326 substrates or single cell techniques (or both) have been successful in linking
327 community composition and process rates. One example, stable isotope probing
328 (SIP, in which an isotopically labeled element (or elements) from a defined substrate
329 can be tracked into microbial biomass) is a method used to identify which organisms
330 may be participating in an ecosystem process of interest[42]. For example, a study of
331 sulfate reduction in a Scottish peatland revealed that a single species of
332 *Desulfosporosinus* was most likely responsible for the totality of sulfate reduction
333 within the peatland even though it only comprised 0.0006% of the retrieved
334 sequences[43]. In this case the *Desulfosporosinus* species represented the only
335 known sulfate reducer within the community and thus the kinetics of this organism
336 seemingly defined the kinetics of sulfate reduction in the entire system. Whereas this
337 is a single example of using confirmative ecophysiology to link categories of
338 microbial information (pathway G in Figure 2), there is a suite of culture-free
339 techniques (such as Raman microspectroscopy (MS), NanoSIMS, or X-ray
340 microanalysis, XRMA) that complement sequence-based microbiome analysis by
341 reporting on the physiological and compositional characteristics of individual cells *in*
342 *situ*[27, 44,45]. All three methods (Raman MS, NanoSIMS and XRMA) can be coupled
343 with phylogentic labels (*in situ* hybridization) that can visualize identification of
344 phylotypes simultaneously with macromolecular (Raman MS), isotopic (NanoSIMS),

345 and elemental composition (XRMA). In addition, both Raman MS and NanoSIMS
346 can trace substrates that are labeled with stable isotopes into cells, providing the
347 ability to identify populations that are participating in specific metabolic pathways
348 within complex communities. These powerful approaches applied in concert with
349 sequence analysis have the potential to empirically link the categories of microbial
350 information defined here (Figure 2). These methods also have the greatest potential
351 to begin to unravel, which community properties are EPs, and which are CATs.
352 In addition to direct visualization of individuals cells from mixed populations or
353 consortia using single cell approaches, there are abundant examples of
354 immunocapture (e.g. bromodeosyuridine, BrdU)[46] or other labeling and cell sorting
355 approaches (eg. fluorescent in situ hybridization(FISH) coupled with flow cytometry
356 cell sorting, FACS)[47] that provide powerful tools to constrain the complexity of
357 microbial communities and link community membership to microbial characteristics
358 that influence ecosystem processes. Labeling and sorting techniques allow the cells
359 that incorporate a labeled substrate or can be targeted with a stain or fluorescent
360 reporter to be separated from the broader community and then assayed for
361 membership or for biomass composition. For example, a study of a North Atlantic
362 bacterial community labeled the actively growing component of the community using
363 BrdU and then separated those populations from the rest of the community using an
364 immune capture technique[46]. Similarly, cells can be labeled with phylogenetic probes
365 (e.g. FISH) that fluorescent at different wavelengths and can be separated from the
366 general community using a flow cytometry to only select those cells that reported for
367 the phylogenetic label[47].
368 In addition to these approaches for assaying natural communities, there is a need
369 for broader representation of cultured taxa that better represent physiologies that are

370 similar to those phyla found in the environment. For example, microbial community
371 carbon use efficiency (the amount of carbon allocated to biomass production relative
372 to carbon respired or stored) is a central parameter in many ecosystem level carbon
373 cycling models. Differences in CUE among phyla depend largely on the relative
374 plasticity of growth rate, carbon storage capacity, and maintenance respiration. *E.*
375 *coli*, the "poster bacterium" for physiological assays is unique both in the plasticity of
376 its growth rate and its capacity to store energy as organic carbon and thus not
377 necessarily informative to develop a better understanding of CUE[48]. *Streptococcus*
378 *Bovis* has proven to as a more appropriate organism to study energy cycling in
379 bacteria[48]. Physiological studies of isolates from a broader distribution of
380 representative phyla are key to advancing our understanding of environmental
381 microbiomes. However, it is unlikely that information about specific phenotypes
382 estimated in isolation from pure-culture studies can be directly used to estimate
383 community properties because of the plasticity of organismal physiology and
384 because of the prevalence of competitive interactions when isolates are grown in co-
385 culture with even one other organism[49]. Therefore studies of isolates grown in
386 culture would provide more powerful information if they reported the plasticity of a
387 given phenotype, rather than only the phenotype under a single set of environmental
388 conditions. For example, a recent study of 24 freshwater bacterial isolates showed
389 differences in phenotypic plasticity in the biomass stoichiometry of the taxa studied[26].
390 Some of the taxa had highly variable biomass C:P ratios whereas others
391 demonstrated a high degree of homeostasis in their C:P ratios. This allowed the
392 authors to hypothesize how communities or consortia with homeostatic phenotypes
393 would respond to environmental drivers compared to communities or consortia
394 composed of populations with more plastic phenotypes.

395 Whereas linking microbial membership to system level processes is an exceptional
396 challenge the tools and approaches to address this challenge already exist. These
397 existing and established microbiological methods both relatively novel (NanoSIMS)
398 and foundational in the field (physiological assays of isolates) can be applied in
399 concert to begin to parse the exceptional complexity of environmental microbiomes.

400 ***Designing microbiome research to maximize insights into system-level***
401 ***processes***

402 The meta-analyses discussed above clearly illustrate that a more directed
403 approach to microbiome research is necessary. We suggest that rather than looking
404 for linkages among microbial community membership and system-level processes in
405 every study, research efforts would benefit from strategically targeting the linkages
406 and processes for which an *a priori* understanding of microbial physiology should
407 allow us to improve our understanding of the ecosystem process. These cases may
408 be identified first by noting patterns in which environmental factors explain little of the
409 variability in an ecosystem process, or where system-level responses deviate
410 significantly from rates that are predicted from environmental factors alone. These
411 deviations could include spatial or temporal heterogeneity in an ecosystem response
412 where the environmental characteristics do not have the same level of heterogeneity.
413 For example, the discovery of novel microbial metabolic pathways (i.e. annamox)
414 has helped explain otherwise puzzling chemical transformations, such as the
415 oxidation of ammonium under anoxic conditions[50]. Such advances are most likely to
416 be cases where the microbial process of interest can be directly linked to a
417 phylogenetically constrained group (i.e. a narrow process) and where the system-
418 level behavior of the process reflects the organismal-level physiological controls,
419 such as N-sensitivity of methane monooxygenase[51].

The framework presented here provides one approach to formalize inquiry across microbiome science and encourage 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, the framework presented here should also benefit the analysis of microbiomes associated with other systems such as host organisms and those of engineered environments. We assert that this framework provides an important and straightforward starting point as the global research community aims to undergo one of the most exciting concerted efforts in the microbial sciences to date.

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Figures

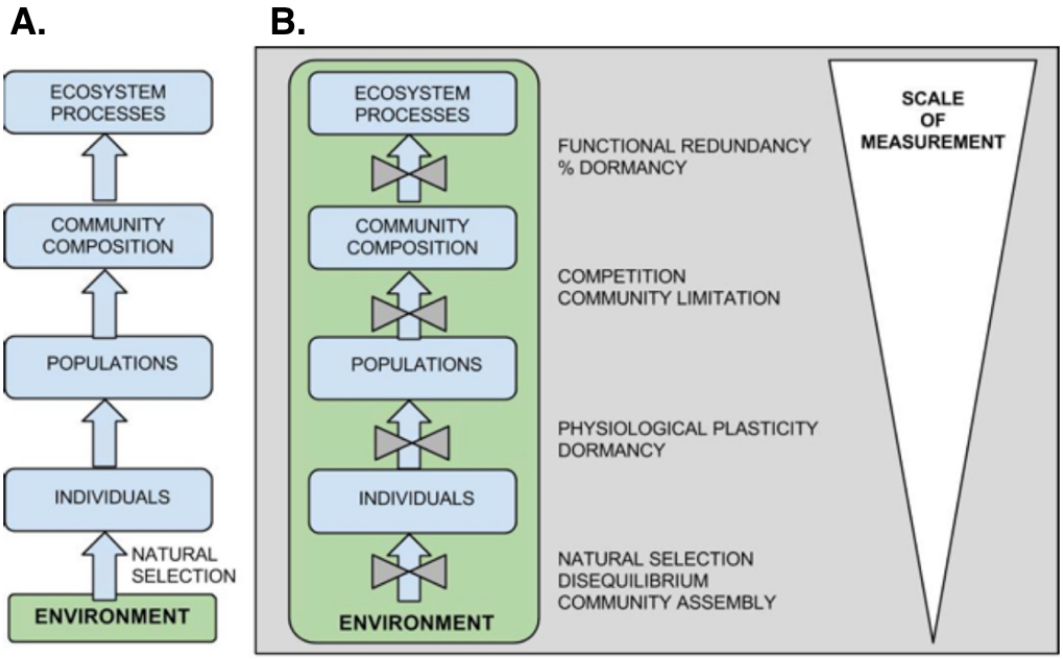

Figure 1 Diagram of microbial-ecosystem linkages A) how linkages are commonly conceptualized across levels of ecological organization and B) the series of environmental filters that create challenges when attempting to link metrics from one level of ecological organization to the other.

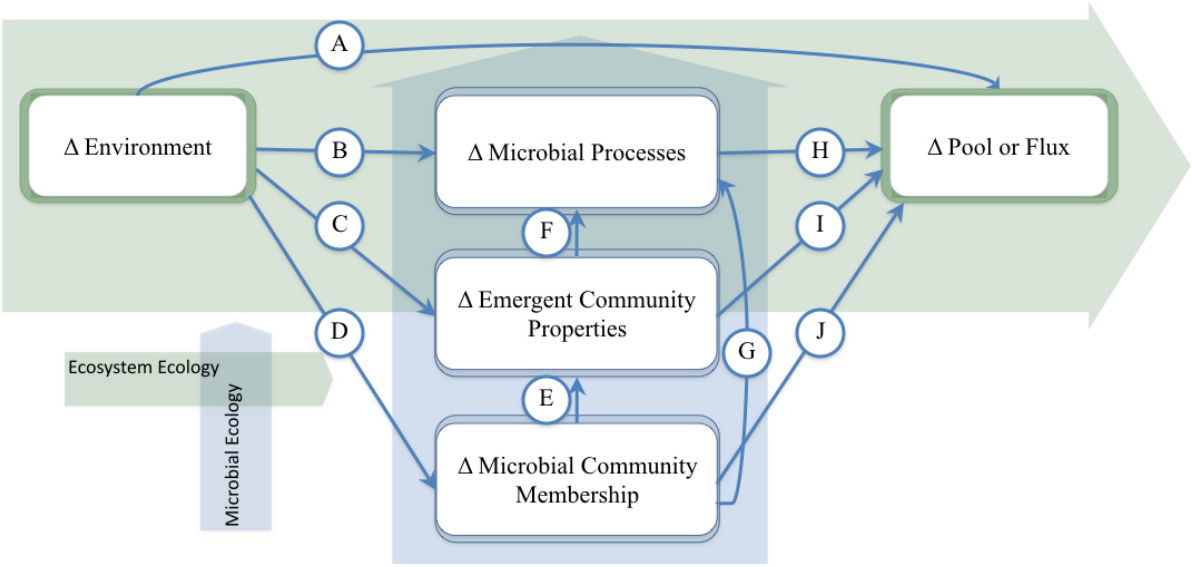

Figure 2 Reframing how we study microbial-ecosystem linkages. Shown is the intersection between microbial (vertical) and ecosystem (horizontal) ecology with each of the three categories of microbial information (microbial processes, emergent community properties, and microbial community membership) as defined in the text. We argue for an increased focus on studies that elucidate pathways E, F and H. In addition we note that pathways G, J and I are less likely to effectively incorporate microbial information into ecosystem 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.

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