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

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1 **Abstract**

2 The well-documented significance of microorganisms to the function of virtually all

3 ecosystems has led to the assumption that more information on microbiomes will

4 improve our ability to understand and predict system-level processes. Notably, the

5 importance of the microbiome has become increasingly evident in the environmental

6 sciences and in particular ecosystem ecology. However, translating the ever-

7 increasing wealth of information on environmental microbiomes to advance

8 ecosystem science is proving exceptionally challenging. One reason for this

9 challenge is that correlations between microbiomes and the ecosystem processes

10 they influence are often reported without the underlying causal mechanisms. This

11 limits the predictive power of each correlation to the time and place at which it was

12 identified. In this paper, we assess the assumptions and approaches currently used

13 to establish links between environmental microbiomes and the ecosystems they

14 influence, propose a framework to more effectively harness our understanding of

15 microbiomes to advance ecosystem science, and identify key challenges and

16 solutions required to apply the proposed framework. Specifically, we suggest

17 identifying each microbial process that contributes to the ecosystem process of

18 interest *a priori.* We then suggest linking information on microbial community

19 membership through microbial community properties (such as biomass elemental

20 ratios) to the microbial processes that drive each ecosystem process (e.g. N -

21 mineralization). A key challenge in this framework will be identifying which microbial

22 community properties can be determined from the constituents of the community

23 (community aggregated traits, CATs) and which properties are unable to be

24 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].

73   These meta-analyses illustrate that links between microbial characteristics and

74   ecosystem processes are often assumed to be present but are rarely tested. When

75   linkages are explicitly tested, connections between microbial structure and

76   ecosystem processes are more often than not weak or non-existent[6]. These findings

77   suggest that our current approach to linking microbial metrics to ecosystem function

78   should be refocused with more attention paid to empirically identifying explicit

79   linkages between microbial characteristics and the ecosystem processes that they

80   influence.

Linking microbial characteristics and ecosystem processes

81   A key challenge in linking microbial information to an ecosystem process is that

82   conceptual research frameworks often do not align directly with the available

83   methods or the data they generate. For example, environmental factors act on the

84   physiology of individual organisms, which alters their competitive ability, relative

85   abundance, collective physiology, and ultimately their contribution to ecosystem

86   processes (Figure 1a). However, designing an observational study or experiment

87   from this conceptual framework (Figure 1a) assumes that environmental metrics can

88   be empirically linked to measureable microbial characteristics across multiple

89   categories of ecological organization (i.e. individuals, populations, and communities)

90   at the appropriate temporal and spatial scales. Yet in nature, relationships between

91   environmental variables and microbial characteristics are dynamic and non-linear[8],

92   simultaneously affected by a plethora of biotic and abiotic variables[9,10] and

93   decoupled in both time and space[7]. Each of these aspects of microbial-environment

94   interactions obscures the relationships among microbial characteristics collected at

95   each level of ecological organization and the ecosystem processes they affect

96   (Figure 2b). To address this challenge we propose a framework that explicitly

5

97   identifies the ecosystem process of interest and how it relates to microbial

98   characteristics. The proposed framework illustrates the relationship among different

99   categories of microbial characteristics and conceptually defines their contribution to

100   ecosystem processes.

101   *Identifying the Ecosystem Process* The first step to understand how microorganisms

102   influence an ecosystem process is to define each of its sub-processes, the set of

103   constituent reactions that combine to dominate the net flux of the ecosystem process

104   of interest. Ecosystem processes are defined as a change in a pool size or a flux

105   from one pool to another (e.g. NH_4^+ to NO_3^-, or dissolved organic matter

106   mineralization to CO_2). Few, if any of these processes are carried out by a single

107   physiological pathway or a single organism. Rather, ecosystem processes are

108   aggregate processes consisting of complementary or antagonistic sub-processes

109   carried out by a breadth of phylogenetically diverse microorganisms[11]. For example,

110   net ecosystem productivity (NEP) is the balance between C-fixation and C-

111   mineralization. Each sub-process of NEP can be further partitioned into a series of

112   metabolic pathways (e.g. heterotrophic fermentation and aerobic respiration or

113   chemoautotrophic nitrification and photoautotrophic C-fixation). Partitioning each

114   ecosystem process in this hierarchical manner can continue until the sub-process

115   maps directly to specific microbial metabolic pathways (e.g. acetoclastic

116   methanogenesis). Subsequently each of these metabolic pathways can be

117   categorized as either broad or narrow[12]. Broad processes are phylogenetically

118   common (i.e. widely distributed among taxa), whereas narrow processes are

119   phylogenetically conserved (i.e. limited to a specific subset of taxa). For example,

120   denitrification is broad, while both methanogenesis and methanotrophy are narrow

121   (with some notable exceptions[13]).

122   The second step is to identify the controls or constraints on each constituent sub-

123   process. For example, kinetics of a given metabolic pathway in a model organism

124   may help understand the rate limiting steps of a narrow process, but insights from

125   model organisms are much less likely to be useful for a broad process where

126   phenotypic variation among phylogenetically diverse organisms should be much

127   greater. Defining the ecosystem process, its critical sub-processes, and the known

128   phylogenetic distribution of the metabolic pathways that drive those sub-processes in

129   this manner creates an explicit conceptual pathway that directly links the ecosystem

130   process to the microorganisms that influence them. Once this conceptual pathway

131   has been identified a concerted empirical approach can be applied to investigate

132   how the microbiome influences the ecosystem process of interest.

133   ***Understanding the relationship between categories of microbial***

134   ***characteristics***

135   *Categories of microbial characteristics* At present, researchers are measuring a wide

136   variety of characteristics of microbial communities (e.g. sequence or relative

137   abundance of genes, transcripts or proteins, enzyme expression, and process rates).

138   Much of that work does not clearly articulate how these measurements differ in their

139   specificity, precision, or linkage among each other or how they inform the microbial

140   contribution to ecosystem processes. We propose that by categorizing microbial

141   characteristics into three distinct categories, 1) microbial processes, 2) microbial

142   community properties, and 3) microbial community membership (Figure 2), we can

143   frame how different metrics interact with each other and how they can elucidate the

144   microbial contribution to an ecosystem process.

145   *Microbial Processes* Microbial processes are the collective physiology of the

146   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].

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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

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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].

18

420   The framework presented here provides one approach to formalize inquiry across

421   microbiome science and encourage empirical linkages between the presence of

422   organisms in a system and the processes that characterize that system. Whereas we

423   draw examples from environmental microbiomes and the ecosystems they inhabit,

424   the framework presented here should also benefit the analysis of microbiomes

425   associated with other systems such as host organisms and those of engineered

426   environments. We assert that this framework provides an important and

427   straightforward starting point as the global research community aims to undergo one

428   of the most exciting concerted efforts in the microbial sciences to date.

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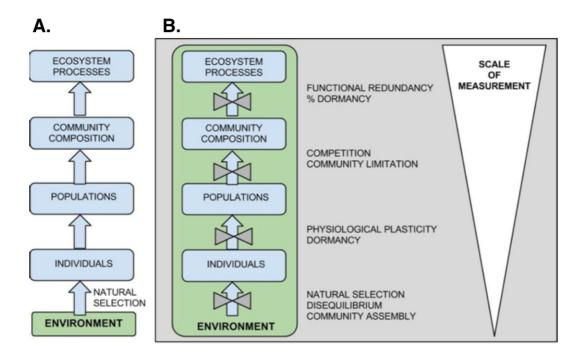
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Figures

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.
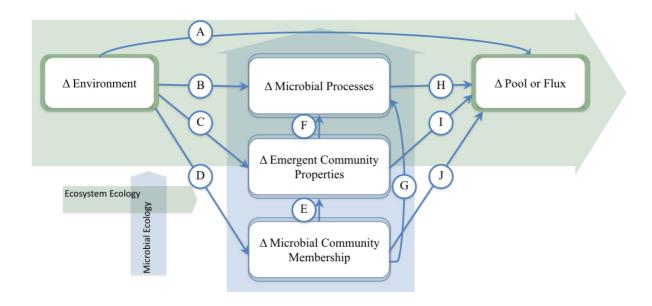
Figure 2 Reframing how we study microbial-ecosystem linkages. Shown is the intersection between microbial (vertical) and ecosystem (horizontal) ecology with each of the three categories of microbial information (microbial processes, emergent community properties, and microbial community membership) as 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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