

1 Perspectives Paper:

2 A horizon scan of priorities for coastal marine microbiome research

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44

45 **Abstract**

46 Research into the microbiomes of natural environments is changing the way ecologists and
47 evolutionary biologists view the importance of microbes in ecosystem function. This is
48 particularly relevant in ocean environments, where microbes constitute the majority of
49 biomass and control most of the major biogeochemical cycles, including those that regulate
50 the Earth's climate. Coastal marine environments provide goods and services that are
51 imperative to human survival and well-being (e.g. fisheries, water purification), and emerging
52 evidence indicates that these ecosystem services often depend on complex relationships
53 between communities of microorganisms (the ‘microbiome’) and their hosts or environment
54 – termed the ‘holobiont’. Understanding of coastal ecosystem function must therefore be
55 framed under the holobiont concept, whereby macroorganisms and their associated
56 microbiomes are considered as a synergistic ecological unit. Here we evaluated the current
57 state of knowledge on coastal marine microbiome research and identified key questions
58 within this growing research area. Although the list of questions is broad and ambitious,
59 progress in the field is increasing exponentially, and the emergence of large, international
60 collaborative networks and well-executed manipulative experiments are rapidly advancing
61 the field of coastal marine microbiome research.

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64 **Keywords:** bioremediation, core microbiome, dysbiosis, functional diversity, environmental
65 stress, evolution, holobiont, microbial ecology

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70 **Background**

71 Coastal marine ecosystems provide a range of ecologically and economically important
72 ecosystem services, including habitat provisions, nutrient cycling, coastal protection and
73 fisheries enhancement¹. The health and services of these ecosystems are inherently linked to
74 the microorganisms residing in these ecosystems (e.g. pollution remediation, disease and drug
75 discovery²⁻⁴). As we increase our understanding of the importance of coastal marine
76 microorganisms and their genetic makeup (i.e. the microbiome, see Box 1), the number of
77 research articles describing the distribution, structure, and function of microbiomes
78 associated with coastal marine ecosystems has flourished (Supplementary Figs. 1 and 2). The
79 ecosystem services are largely attributed to the habitat-forming organisms, such as corals,
80 sponges, macroalgae, seagrasses, mangroves and saltmarshes, which form the foundation of
81 these ecosystems. Furthermore, due to the reliance of coastal marine ecosystem health on
82 these habitat-forming organisms, the field has realized the importance of understanding the
83 macroorganisms and their microbiomes as a synergistic ecological unit (i.e. holobiont, see
84 Box 1). As a result, there has been a relative surge in host-associated microbiome research in
85 recent years (Supplementary Fig. 1) aimed at identifying how microbiomes influence host
86 phenotype, physiology, and development⁵⁻⁷. Although our understanding of several
87 fundamental concepts in coastal marine microbial ecology has increased^{7,8}, coastal
88 microbiome research -- particularly in the context of holobionts -- is still in its infancy,
89 especially relative to other microbiome fields, such as the human microbiome. A large
90 number of open questions currently limits our capacity to assess how microbial processes
91 influence the ecology of these environments, both under contemporary conditions and under
92 future environmental change. Therefore, there is a clear need to prioritize and define key
93 questions for future research that will allow for better assessments of how microbial
94 processes truly influence the ecology and health of coastal marine environments.

95

96 [suggested Box 1 placement]

97

98 **Evaluating the state of the science**

99 To evaluate the current state of coastal marine microbiome research, we surveyed the current
100 literature, then ‘horizon scanned’ with experts in the field to identify major research gaps, in
101 order to determine where future challenges lie and ultimately progress this field of research
102 (see Box 2 for description of the approach and limitations). For the literature search, we
103 focused on six key holobionts that form the foundation of these coastal ecosystems - corals,
104 sponges, macroalgae, seagrasses, mangroves and saltmarshes. We also considered the
105 microbiomes of sediments and the water column within coastal marine ecosystems. The key
106 findings from the literature survey include identification of areas of progress, as well as
107 holobiont systems that need more attention (Supplementary Figs. 1 and 2). For example,
108 research on seawater- and sediment-associated microbiomes has dominated coastal marine
109 microbiome literature to-date (consistently $\geq 50\%$ of the total number of studies), while host-
110 associated microbiome research is steadily increasing and has generally focused on coral and
111 sponge holobionts (Supplementary Fig. 1). In the last five years, however, the diversity and
112 quantity of microbiome and holobiont research has incrementally increased with the inclusion
113 of macrophyte-associated microbiome studies, although mangrove- and saltmarsh-associated
114 microbiome research is still nascent (Supplementary Fig. 1). Additionally, the methodologies
115 used to describe coastal marine microbiomes has diversified over time from predominantly
116 microscopy, cell counts, and community fingerprinting techniques, to sequencing-dominated
117 technologies (Supplementary Fig. 1). The literature survey also identified geographic
118 hotspots and gaps in microbiome studies (Supplementary Fig. 2). The coastlines of Australia,
119 Europe, the northern Mediterranean Sea, the Red Sea and US are relatively well-sampled in

120 multiple ecosystem types, while there are clear regional gaps for host-associated microbiome
121 studies along the South American, African and northern Asian coastlines. Some of the well-
122 studied regions are dominated by studies on specific host-associated microbiomes
123 (Supplementary Fig. 2). For instance, seagrasses have been heavily studied in the temperate
124 US, while the biodiversity hotspots in the Indo-Pacific have been dominated by studies on
125 coral- and sponge-associated microbiomes (Supplementary Fig. 2).

126

127 The horizon scan resulted in 108 questions key to progressing coastal marine microbiome
128 research. Nearly half of the questions (~50) directly or indirectly concerned host-associated
129 microbiomes, with the remaining covering a range of fundamental microbiome ecology or
130 methodological topics independent of a specific ecosystem, host or substrate. In assessing the
131 literature and identifying priority research questions via the horizon scanning exercise (see
132 Box 2 for the methodology used), we outline seven microbiome research themes relevant to
133 deciphering the role of microbiomes within coastal marine ecosystems. The themes begin
134 with microbiome questions, followed by host-microbiome themes, and lastly questions
135 concerning microbiomes and holobionts in the environment (Box Diagram 1). While some of
136 the themes are holobiont-centric, we do not focus on one particular holobiont system. Rather
137 the themes represent general concepts that can be applied to multiple substrate- or host-
138 associated, or free-living microbiome systems. Therefore, we have provided a diversity of
139 references to support the presented themes throughout, with the aim to create a
140 comprehensive vision that may unify the strategy of research on coastal marine microbiomes.

141

142 [suggested Box 2 placement]

143

144 **Key research themes in coastal marine microbiome research**

145

146 ***Microbiome***

147

148 *Theme 1: How can community structure be matched to microbiome function?*

149 In coastal marine ecosystems, enormous microbial diversity has been revealed via, for
150 instance, phylogenetic analyses of the 16S rRNA gene (e.g. ⁸⁻¹⁰). However, it is important to
151 define the function of a microbiome in order to understand how it is likely to influence its
152 host and the ecosystem¹¹. Currently, the best way to directly determine the function of the
153 entire microbiome is via metagenomic and metatranscriptomic sequencing¹²⁻¹⁶. The recent
154 availability of many genome reconstruction or binning approaches¹⁷ offers a greater capacity
155 to obtain near-whole genomes out of metagenomes, allowing a better understanding of the
156 function of the microbiome members. However, our ability to successfully annotate
157 functional genes within metagenomic and metatranscriptomic datasets remains outstripped by
158 the availability of sequencing data itself. For example, extensive sequencing of the global
159 ocean microbiome found that 40% of core orthologous genes were of unknown function¹⁸.

160

161 Another approach to link diversity with function is to identify the ‘core microbiome’, or the
162 persistent and functionally essential members of host-associated microbiomes, possibly a key
163 determinant of host well-being and therefore overall ecosystem functioning and health (Fig.
164 1)^{8,19}. For example, conserved bacterial taxonomic groups, which constitute the coral core
165 microbiome, play a critical role in the success of the coral-zooxanthellae symbiosis¹⁹. Other

166 organisms, such as the green seaweed *Ulva mutabilis*, require a core set of functions from
167 their microbiome, rather than the presence of specific taxa²⁰.

168 Connecting diversity with function drives central ecological questions such as: (1) How does
169 microbial community diversity influence functional aspects (e.g. resilience) of the host,
170 microbiome and environment; and (2) How can we define the function of a coastal
171 microbiome? Yet despite substantial effort in recovering metagenomes and
172 metatranscriptomes from dominant marine hosts, there remain significant challenges in
173 demonstrating causation between shifts in the microbiome and shifts in host health due to
174 reduced capabilities to manipulate microbiomes in the field¹⁸ (e.g. manipulative field
175 experiments, see Box 3). We recognize this as a particular challenge for microbial ecologists.
176 Therefore, we identify several questions that we hope will move the field forward and lead
177 into innovative approaches that determine the functional roles of coastal marine microbiomes,
178 and thereby resolve the relationship between microbiome diversity and their functions:

- 179 ● How can novel techniques, e.g. single cell raman spectroscopy²¹, be applied to
180 complex microbiomes and holobionts to interrogate microbial functions in situ?
- 181 ● How can intensive efforts for isolating coastal and host-associated microbes (i.e.
182 ‘culturomics’²²) open the door for tracking the function of candidate genes and
183 investigating homology, predictability and certainty of curated gene function?
- 184 ● How does identifying the core microbiome (taxa or set of functions, e.g. depicted in
185 Fig. 1b-c, respectively) offer valuable insights towards advancing in-depth
186 identification and experimental manipulation?

187

188 [suggested placement for Figure 1]

189

190 *Theme 2: At which spatial and temporal scales do the microbiomes of coastal organisms*
191 *change?*

192

193 Host-associated microbiomes are highly dynamic communities that change at both small (i.e.
194 ecological and physiological) and large (i.e. evolutionary and geographical) timescales.

195 Substantial variability on very small spatial scales (i.e. within host) can be driven by host
196 provisions, such as nutrient and oxygen availability^{10,23}, as well as by trophic- and quorum
197 sensing-related interactions among members of the microbiomes within a physical niche (e.g.
198 ^{24,25}). Hosts also differ in microbiome community structure depending upon host distribution

199 in a population (e.g. center vs. edge of a seagrass meadow²⁶), and microbiomes on host
200 species can also vary across large environmental gradients²⁷. However, for some holobionts

201 such as seaweeds, geographical variability in surface-associated microbiomes is relatively
202 low even at continental scales, relative to other factors such as host health condition⁹. Short-
203 term temporal variability can also be surprisingly consistent, with predictable successional

204 patterns over periods of days to weeks occurring in the epiphytic bacterial communities of
205 macroalgae²⁸, corals²⁹ and sponges³⁰. However, evidence for the scales at which coastal

206 marine microbiomes shift in time and space, and the apparent drivers behind these shifts, is
207 often conflicting. Studies showing host-specificity and stability in the microbiome over time
208 and location^{10,28,31,32} contradict studies that suggest that microbial communities are highly

209 dependent on the host physiological or environmental conditions^{9,33,34}. These conflicting
210 patterns prevent us from making generalizations about the stability or variability of coastal

211 host-associated microbial communities (e.g. Theme 5).

212

213 At evolutionary time scales, there is little doubt that hosts and their associated microbiomes

214 influence each others' evolution, and indeed at very large time scales, these interactions are

215 the basis for fundamental macroevolutionary events³⁵. For individual marine systems,
216 however, the details of how hosts and their microbial associates affect each other on shorter
217 evolutionary time scales is limited, and whether or not these effects are broadly reciprocal
218 (i.e. coevolutionary). To date, there is limited evidence among benthic marine hosts for
219 coevolution with their microbiome^{34,36}, both because of the multiple interplaying factors that
220 ultimately influence evolutive patterns, and because of the challenges in demonstrating
221 formal coevolutionary relationships³⁷. In some instances broad taxa of marine hosts and their
222 microbiota appear to reciprocally evolve in response to one another³⁸, but in others selective
223 effects appear to be limited to the host and individual members of its microbiome rather than
224 the entire microbial community³⁹. Further complexities in teasing out the evolution of
225 members in a holobiont include both internal microenvironments of the host that act like
226 discrete coevolving ecosystems⁴⁰, as well as the disparate evolutionary timescales that
227 influence the host and the diversity of the microbiome members⁴¹. Evolutionary patterns
228 within holobiont spatial niches/compartments have been shown for Scleractinian corals and
229 their microbiomes, whereby the ecological relatedness of host-associated microbial
230 communities parallels the phylogeny of related host species, and therefore evolutionary
231 changes in the host associate with ecological changes in the microbiota⁴². This
232 coevolutionary pattern, or phylosymbiosis was strongest in the coral skeleton compared to the
233 coral tissue and mucus⁴³. Although many coral-associated bacteria were host-specific, only a
234 select minority of coral-associated bacterial families showed co-phylogenetic signals
235 consistent with long-term host-microbe co-diversification⁴³.

236

237 Here, we outline key questions to progress our understanding of the scales at which
238 microbiomes shift:

239

240 ● What are the implications of disparate evolutionary timescales between the host and
241 its microbiota?

242 ● How does the resolution at which we study microbiomes influence how we interpret
243 differences in their composition and function at spatial and temporal scales?

244 ● Do the holobiont members differentiate between beneficial and detrimental
245 relationships in order to selectively favor the transmission of mutualistic partners
246 between generations, and if so, what are the mechanisms?

247

248 To improve our understanding of the temporal and spatial dynamics of host-associated
249 microbiomes, a structured approach to characterize spatial and temporal variation at multiple
250 scales for both taxonomic and functional characteristics is needed. Future research should
251 focus not only on descriptive studies, but also on perturbation experiments to assess resilience
252 and stability under the context of variable systems⁴⁴⁻⁴⁶. Investigating microbiome evolution is
253 inherently challenging, therefore clearly defining the boundaries of the question (e.g.
254 phylogenetic vs functional level or the whole microbiome vs individual members), as well as
255 identifying the limitations of what can be tested is necessary. Additional reflection on
256 (co)evolution in systems other than coastal marine ecosystems may provide insight that could
257 progress these questions. Examples include the formation of niches by symbiotic microbiota⁴⁰
258 and the broader literature on geographic aspects of coevolution (e.g. ⁴⁷).

259

260

261 ***Microbiome and Host***

262

263 *Theme 3: How are host-microbiome interactions formed?*

264 Several studies have established that most benthic organisms, including seagrasses¹⁰, corals³¹
265 and macroalgae⁴⁸, carry microbiomes that are distinct from the surrounding sediment or
266 seawater. Yet, the timing and underlying mechanisms of microbiome acquisition (either host-
267 directed selection, or microbe-direct colonization) remain largely unresolved. Chemical
268 signaling, specifically secondary metabolites produced by host species independently or in
269 response to environmental or microbial cues, or signaling from microbial taxa that have
270 already colonized the host, have been suggested to be important factors in both host defense
271 against pathogenic microbes and microbiome colonization. For example, the pathogen *Vibrio*
272 *coralliilyticus* has been shown to be attracted to corals that increase their production of the
273 sulfur compound dimethylsulfoniopropionate (DMSP) under heat stress⁴⁹. Conversely, in
274 seaweeds such as *Lobophora variegata*, secondary metabolite production acts as a defense
275 strategy by preventing colonization of pathogenic microbes, such as saprophytic marine
276 fungi⁵⁰.

277

278 In addition to host-microbe interactions, some studies have suggested a role for microbe-
279 microbe interactions in determining microbiome composition, including lottery models⁵¹ and
280 symbiotic modes of interaction³¹. It has also been shown that microbiome composition is
281 affected by host condition (e.g. seaweed⁹; corals⁴⁹), as well as environmental conditions (see
282 Theme 5). Although there are few global census studies of the microbiome of particular
283 marine species, recent studies in seagrasses^{10,33} suggest that microbial functions and
284 microbiome composition are also affected by geographic location, indicating an influential
285 role of the environment in shaping microbiome composition. Taken together, we hypothesize
286 that the active role of the host in determining microbiome composition lies along a
287 continuum, ranging from being determined by host condition to being determined by
288 environmental factors, which no doubt affect host condition. Where the system lies within the

289 continuum is largely determined by host species. Additional studies in coastal marine
290 ecosystems are needed to elucidate further:

- 291 ● What are the differing selection strategies between host species that determines
292 whether the microbiome is shaped more by the environment or by the host?
- 293 ● What are the chemical pathways or specific processes by which a host attracts specific
294 microbes, e.g. as observed in the model organism *Arabidopsis* microbiome⁵²?
- 295 ● Does the host species dynamically change its selection strategies as a function of
296 microbial colonization, or changing environmental conditions?

297

298 To address these questions, controlled experiments in mesocosm systems are needed for the
299 use of model organisms and standardized initial conditions. Tuneable manipulation of
300 environmental parameters, addition of other microbial species, comparing a variety of host
301 genotypes, and characterizing host exudate composition, could elucidate mechanistic
302 interactions between host and microbiome, and discern the conditions under which a
303 mechanism can be expected to occur. Some studies have made use of mesocosm
304 systems^{44,53,54}, and we expect even further advances from the use of controlled systems.

305

306

307 *Theme 4: To what extent is the resilience and health of the holobiont determined by the*
308 *structure and function of its microbiota?*

309 The importance of microbes to the health of plants and animals is now well accepted. The
310 microbial components of the holobiont can aid in digestion, provide essential vitamins and
311 nutrients, protect from invading pathogenic organisms and stimulate developmental
312 processes^{7,55,56}. Therefore, any disturbance to the host microbiome are likely to result in a

313 breakdown of holobiont function (or dysbiosis), which can manifest itself as disease.
314 Analogous disease concepts have been proposed for chronic conditions in humans, including
315 common periodontal and gastrointestinal disorders, which are thought to result from a
316 disturbance to the natural microbiota rather than infection by a singular pathogen^{57,58}. While
317 less well understood for marine holobionts, microbial dysbiosis may also play a role in
318 diseases, for example, the bleaching diseases of invertebrates and seaweeds (e.g. see recent
319 reviews^{59,60}). However, with some exceptions⁶¹, the majority of these observations are based
320 on correlative data, and the extent to which disease is a direct result of microbial dysbiosis
321 remains an important research question. To fully appreciate the role of microbial dysbiosis
322 we need to understand the core components of a healthy microbiome and identify those
323 beneficial consortia that offer holobiont resilience. Importantly, given the capacity of
324 microbes to rapidly respond, adapt and evolve to environmental conditions, the host
325 microbiome is also likely to be instrumental in assisting the adaptation of higher organisms to
326 future climate conditions or other anthropogenic stressors⁶².

327

328 Structure and function of the microbiota within a holobiont can have important links to
329 broader scale holobiont health and resilience. These connections are likely to aid in
330 identifying core microbiome members and their corresponding functions essential for
331 holobiont health (i.e. Theme 1). As we move to a changing climate, several key questions
332 remain:

- 333 ● How do the interactions among microbiomes, within or across different niches of the
334 same host affect host, resilience and homeostasis?
- 335 ● What are the criteria to designate specific taxa as beneficial core microbiome
336 members or sentinels of dysbiosis in marine organisms?

337 • To what degree are members of the transient microbiome a source of functional
338 redundancy and thus providing resilience during environmental change?
339
340 Looking to the future, having sound knowledge and access to culturable, beneficial members
341 of the core microbiota will have applied uses; for example, as biomarkers for the early
342 detection of host stress or for the development of probiotic consortia that can be used to
343 support aquaculture and marine restoration programs (Theme 6). However, taxa not
344 considered part of the core microbiome under current conditions may become more important
345 (core) under future conditions.

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349 *Microbiome, Holobiont & the Environment*

350

351 *Theme 5: What is the role of the tripartite interaction, host-microbiome-environment, on*
352 *holobiont resilience?*

353

354 Holobionts living in the dynamic ocean-land interface of coastal ecosystems⁶³ can be exposed
355 to substantial diel changes in temperature, salinity, tidal levels, light, oxygen, and nutrients⁶⁴.
356 Their resilience and adaptation is at least partially influenced by the microbiomes that
357 modulate the environmental conditions to which they are exposed⁶⁵. The environment can act
358 as a source for holobiont microbiota that in turn are shaped by strong selective forces driven
359 by the host biology and behaviours. For example, fiddler crabs carapace and gut⁶⁶ are
360 colonized by different pools of microbial colonists that are taken up from the environment,

361 but the burrowing and filter feeding behaviors of the crabs finely select such colonists from
362 the sediment after strongly reconditioning its geochemical properties⁶⁷.

363

364 The effects of either short- or long-term environmental changes on host-microbiome
365 interactions are inherently complex and thus difficult to predict⁶⁸. The intrinsic environmental
366 variability, for instance linked to seasonal changes⁶⁹, perturbation events⁷⁰, or a combination
367 of these⁷¹, strongly influence microbiome diversity and functionality. Environmental stressors
368 that can interact in opposing, additive or synergistic ways to influence hosts, microbiomes
369 and their interactions, can lead to positive, negative or neutral impacts on them⁷².

370

371 Using as an example thermal stress, frequently investigated in coastal marine microbiome
372 research, we should consider that all organisms, whether microbial or macroscopic, have
373 optimal thermal tolerance thresholds⁷³. Thermal stress has been correlated with functional
374 and/or structural shifts in microbiomes of corals⁷⁴, sponges⁷⁵ and oysters⁷⁶, among others.
375 Higher temperatures can induce virulence in otherwise commensal microbes⁷⁷, and/or
376 decrease the host chemical defences, with continued stress leading to the break-down of
377 symbioses, the introduction of new microbes (e.g. opportunistic pathogens) and, eventually,
378 deterioration of the host^{61,75}.

379

380 The ecological interactions within and among holobionts can also be indirect, for instance,
381 microbiome recruitment by one host that may be affected by the exudates of another nearby
382 host^{23,78}. Host proximity may affect microbiome compositions, such as for algal turfs on the
383 surface of *Porites* coral that were associated with increased alpha diversity of coral surface
384 microbes, particularly of pathogenic bacterial taxa⁷⁹. Host coexistence may also provide a
385 more suitable habitat, e.g. seagrasses in anoxic sediments are favored by the aerobic sulfide-

386 oxidizing bacterial symbionts associated with benthic burrowing bivalves, which detoxify the
387 anoxic sediment⁸⁰.

388

389 Such tripartite interactions are highly complex and challenging to investigate in ‘real-world’
390 scenarios. We envisage the following research questions as priorities for the future research
391 on coastal marine microbiomes:

392

- 393 ● How do environmental changes and stressors shape the functional redundancy of
394 coastal microbiomes?
- 395 ● What are the environmental factors that determine and select microbiome members as
396 beneficial or harmful for a host?
- 397 ● What are the chemical signals and how do they modulate the ecological interactions
398 of microbiomes within and between holobionts and microbiomes?

399

400 Investigations using real-world scenarios like those on combined multi-stressors, such as
401 heat, pH (ocean acidification), and oxygen availability, are still rare^{68,81}. However, in order to
402 address the above question, such approaches are essential to build more ecologically reliable
403 models on how host-microbiome interactions respond and adapt to changes⁸². Additionally,
404 the holobiont approach sets a research framework, to comprehensively explore the adaptive
405 and evolutionary patterns of organismal resilience and ecological function, in response to the
406 critical challenges imposed by multiple combined environmental changes^{83,84}.

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410 *Theme 6: How can we ‘manage’ microbiomes in the coastal environment and in association*
411 *with hosts?*

412

413 Management or manipulation of microbial functions and communities are well-established
414 techniques in bioremediation of terrestrial and aquatic ecosystems – for instance, those
415 impacted by hydrocarbons and toxins contamination⁸⁵. Principal approaches involve either
416 biostimulation (the process of ‘activating’ indigenous microbes via, for example, nutrient
417 amendments) or bioaugmentation (the process of inoculating the ecosystem with non-
418 indigenous microbes that have desired metabolic properties⁸⁵), which have both been applied
419 at ecosystem scales^{86,87}. While these approaches have been less well-tested for marine
420 systems, biostimulation strategies have been applied to deal with oil spills in ocean waters
421 (e.g. Exxon Valdes or Deep Horizon disasters), primarily by supplying growth limiting
422 nutrients, such as phosphate, to the site of contamination⁸⁶. Biostimulation and
423 bioaugmentation have also been used to accelerate degradation of polycyclic aromatic
424 hydrocarbons in marine coastal sediments⁸⁸.

425

426 Host-associated microbial communities can also in principle be managed, as exemplified over
427 the last few years by the development of sophisticated pre- and probiotic strategies for
428 disease prevention and health improvement of humans, plants and some aquaculture
429 species⁸⁹⁻⁹¹. The advances in these hosts are facilitated in an increasingly detailed
430 understanding of microbial diversity and functional processes, but such information is sparse
431 for most natural marine hosts, thus preventing rational designs of pre- and probiotic
432 strategies.^{62,92} Taken together, we envisage the following questions to be areas of increasing
433 research:

434

- 435 ● What are the key conditions to establish microbial-driven bioremediation processes in
436 the coastal environment?
- 437 ● How does microbial manipulation in the early lifecycle stages of a host influence the
438 performance and health of more mature host stages?
- 439 ● What is the role of microbial communities in facilitating the restoration of key hosts
440 in impacted marine habitats?

441

442 With the increasing urbanization of our coastlines and the increasing need to use polluted
443 sites for recreational, private or commercial purposes, microbial-driven bioremediation will
444 be one of the key tools to tackle this issue. Engagement and involvement of the local and
445 regional stakeholders in the early stages of research will be essential for successful
446 implementation. Additionally, as there is an increasing interest in developing probiotics or
447 improving microbial symbiont function of important habitat-forming holobionts, global
448 networks or initiatives, such as the Beneficial Microorganisms for Marine Organisms
449 (BMMO), are powerful tools to progress this work, as has been shown for corals^{62,92}.

450

451

452 *Theme 7: To what extent are coastal marine holobionts and their interactions with the*
453 *environment relevant to human health and well-being?*

454 Coastal environments, including their associated biota, are the principal interface for human
455 exposure to marine microbiomes and these interactions can sometimes have detrimental
456 impacts on human health (Fig. 2). Human pathogens present within marine microbiomes
457 include both indigenous marine microbes and enteric microbes that are exogenously
458 introduced to coastal habitats via sewage and urban storm-water⁹³ (Fig. 2). The microbiomes
459 of benthic marine flora and fauna often display a high representation of marine pathogens - in

460 particular members of the *Vibrio* genus⁹⁴. Several species within this genus are highly
461 virulent and dangerous human pathogens, and in the USA alone are cumulatively responsible
462 for health costs exceeding \$250 million yr⁻¹⁹⁵. In addition to native marine microbes, enteric
463 pathogens that become, at least transiently, incorporated into marine microbiomes following
464 exposure to coastal pollution also pose a significant health risk. Indeed, due to (i) the
465 preference for coastal and estuarine habitats among many native marine pathogens⁹⁴ and (ii)
466 the regular exposure of coastal flora and fauna to human waste streams, the microbiomes of
467 coastal organisms represent potentially important hotspots and reservoirs of human
468 pathogens⁹³ (Fig. 2). On the other hand, there is recent evidence that some marine macro-
469 organisms, specifically seagrasses, may act as effective natural filtration systems that remove
470 human pathogens from coastal ecosystems, potentially through the production of biocides by
471 the plant or its microbiome⁹⁶ (Fig. 2).

472

473 As the global human population rapidly increases its dependence and impact upon coastal
474 environments⁹⁷, it is imperative that we develop an understanding of the potential human
475 health consequences of increasing contact with marine microbiomes. This is particularly true,
476 given that there is emerging evidence that climate change and the anthropogenic degradation
477 of coastal habitats are enhancing the occurrence and virulence of dangerous human pathogens
478 within these ecosystems⁹⁸ (Fig. 2). Within this specific context, we identify several key
479 questions that remain unanswered, including:

480

- 481 • Are potential human pathogens persistent or ephemeral members of the microbiomes
482 of coastal organisms?
- 483 • To what extent are environmental change and degradation enhancing the occurrence,
484 persistence and virulence of human pathogens within coastal microbiomes?

485 • To what degree do enteric pathogens introduced to coastal microbiomes via human
486 waste streams influence the health of the benthic coastal macro-organisms?

487

488 New analytical approaches for interpreting microbiome data provide several opportunities to
489 answer these questions. For instance the detection of novel “indicator” organisms⁹⁹ or
490 genes¹⁰⁰ within microbiome data-sets delivers potentially powerful capacity to detect
491 environmental perturbations and human contamination within coastal waters that goes far
492 beyond standard indicators of human contamination (i.e. Faecal Indicator Bacteria [FIB]),
493 which are often limited in sensitivity and explanatory power¹⁰¹. The analysis of coastal
494 microbiome data also provides a facility to detect novel or emerging pathogens that are
495 missed by standard FIB monitoring approaches^{102,103}. Finally, the application of analytical
496 approaches such as SourceTracker¹⁰⁴ and random forest analyses¹⁰⁵ allow for microbiome
497 data to be directly used as a sensitive new tool for tracking sources of contamination or
498 signals of environmental change.

499

500 [suggested placement for Figure 2]

501 [suggested placement for Box 3]

502

503 **Synthesis and Outlook**

504 Coastal marine microbiome research represents a direct pathway to understanding how
505 microbes affect – both positively and deleteriously – the coastal ecosystems on which human
506 populations so heavily rely. The themes and questions presented here, summarized in a
507 conceptual framework (Fig. 3), include resolving the spatial, temporal, and evolutionary
508 scales at which the holobionts and microbiomes function, resolving how holobionts change in
509 response to environmental stimuli and each other, and determining the scope for how

510 microbiomes can be managed. Summarizing the future of coastal microbiome research
511 through the horizon scan and literature survey has identified two overarching concepts
512 common across the themes that reflect the current state of the science, as well as how we
513 envision the science will progress: microbiome function and utilizing manipulative
514 approaches.

515

516 Defining microbiomes, either functionally or within the framework of a core microbiome,
517 was a fundamental concept shared by all the themes. As outlined in Theme 1 and the
518 literature survey, the field has made large strides in how we define microbiomes via
519 taxonomic descriptions from amplicon sequencing. For some holobionts and ecosystems like
520 mangrove and saltmarshes, gathering basic information on what microbiota are present and
521 how they may be functioning is still lacking and would benefit from global-scale initiatives,
522 such as recent efforts for seagrasses and sponges^{10,106}. Conversely, the microbiome and
523 holobiont systems that already have solid taxonomic foundations are looking to investigate
524 how the microbiota function, alone and together with their hosts, in coastal marine
525 ecosystems in order to answer the pressing ecological questions presented.

526

527 Such investigations, as shown throughout the themes, are inherently complex, whereby the
528 questions and concepts presented in one theme relied on the understanding of another theme.
529 For example, teasing apart the relationship between microbiome and host health and
530 resilience (Theme 4) depends on the temporal scale (Theme 2) and environmental conditions
531 (Theme 5) that influence the interactions, but each of these themes in themselves also
532 influence how microbiota are selected and form holobionts (Theme 3). The ever-changing
533 nature of the ecological processes that influence the microbiomes and holobionts in the
534 natural environment necessitates manipulative experimental approaches in order to tease

535 apart the questions presented. In some cases, such as the evolutionary, multi-stressor or
536 management questions, highly controlled experiments are the best options currently available
537 to progress the respective themes. Here, the use of model organisms may provide insight, for
538 example, on selection mechanisms between host and microbe (Theme 3), and microbiome-
539 driven restoration (Theme 6). The large national and international collaborations or
540 consortium efforts that have produced the descriptive data on environmental microbiomes to-
541 date, may be equally useful in progressing hypothesis-driven questions through concerted
542 manipulative experimental approaches¹⁰⁷, e.g. temperature effects on holobiont resilience at
543 the biogeographic limits of the host (Themes, 5, 4 and 2, respectively) or how holobionts can
544 act as sources or sinks of pathogenic microbiota under various point source or diffuse
545 pollution scenarios (Theme 7). In summary, although the list of research themes we present
546 here is broad and ambitious, the ongoing collaborative networks along with well-executed
547 hypothesis-driven manipulative experiments are significantly progressing the definition and
548 functional relationship between the core microbiome and host, illuminating global
549 microbiome biogeography, and identifying key regional- and global-scale environmental
550 influences on coastal marine microbiomes and holobionts.

551

552 [suggested placement for Figure 3]

553

554 Data availability: The original questions for the horizon scan are available in the
555 supplementary materials.

556

557 Code availability: The code used to extract literature from databases is available in the
558 supplementary materials.

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892

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894

895 Figure Legends

896

897 **Fig. 1:** Determining microbiome contribution to coastal ecosystem health. **a.** This paper on
898 coastal marine microbiomes highlights six key holobionts that form the foundation of coastal
899 ecosystems: corals, macroalgae, seagrasses, mangroves (sponges and saltmarshes not shown
900 here). **b.** The core microbiome concept allows the identification of both persistent microbial
901 phylotypes, and **c.** core functional roles played by microbes within holobionts, seawater and
902 the sediment. Different microbes can constitute persistent microbiomes across varying
903 spatial, temporal, organism and ecosystem scales (intersection in Venn diagram, **b**).
904 However, these ubiquitous microbial communities are likely to present functional redundancy
905 across coastal environments (similar relative abundance in functional genes, **c**), playing
906 crucial roles in the functioning and health of coastal marine ecosystems. OTU = operational
907 taxonomic unit, KEGG = Kyoto Encyclopedia of Genes and Genomes. Photo credits: coral
908 reef: Alexander J. Fordyce; mangrove: Michael Bradley; seagrass and macroalgae:
909 Pommeyrol Vincent and Ethan Daniels, respectively / Shutterstock.

910

911 **Fig. 2:** Conceptual design of the potential relationships between coastal marine microbiomes
912 and humans. (1) Coastal pollution, including stormwater and sewage effluence, introduce
913 potentially pathogenic microbiota to coastal marine ecosystems. (2) Endemic marine
914 pathogens, including *Vibrio* and toxic cyanobacteria, persist in coastal marine ecosystems. (3)
915 Coastal aquaculture species can become contaminated by endemic and introduced pathogens,
916 which can both (i) cause mortality, e.g. oysters, and (ii) have health implications for human
917 consumers. (4) Endemic pathogens, e.g. *Vibrio*, can cause holobiont disease. (5) According to
918 Lamb et al.⁹⁶, coastal macrophytes may act as natural pathogen filters, buffering the impact
919 of pathogens for humans and coastal marine ecosystems. Climate change and CO₂-mediated

920 pollution also represent major impacts that humans have on coastal microbiomes. These
921 impacts, for example, may result in increased occurrence and virulence of pathogens like
922 *Vibrio* via the warming of sea surface temperatures (2), and increased coastal pollution due to
923 the greater frequency of storm events (1). The industry and global warming symbols are
924 courtesy of the Integration and Application Network, University of Maryland Center for
925 Environmental Science (ian.umces.edu/symbols/).

926

927 **Fig. 3:** A conceptual diagram depicting several of the major research themes in coastal
928 marine microbiome research. The diagram highlights several interactions that occur at
929 multiple spatial levels. **a.** Using the macroalgae *Ulva* sp. as an example, the inset highlights
930 six host-microbiome interactions and associations in relation to the ‘baseline’ holobiont
931 (center host + microbiome). **b, c.** More broadly, the holobiont-scale of interactions and
932 associations can also apply to large-scale or ecosystem-level scenarios, whereby the
933 holobiont interacts with environmental microbiomes (e.g. sediments/substrates, seawater) and
934 neighboring inter- and intra-species holobionts, while also being influenced by environmental
935 or climatic conditions (not depicted here).

936

937 Boxes

938 **Box 1: Key Definitions**

939 **Dysbiosis:** An imbalance or disruption of the normally beneficial symbiotic relationship
940 between the host and its associated microbiota. A dysbiotic microbiota may result in poor
941 host health and/or reduced capacity for resistance to environmental perturbation.

942 **Holobiont:** An ecological unit formed by a host and its associated microbiome(s).

943 **Horizon scanning:** A technique used to systematically identify the gaps, challenges and
944 opportunities in a field with the aim to outline future priorities and is often employed by
945 eliciting the perspectives of experts in the field.

946 **Metagenomics:** The study of microbial community structure, function and interactions
947 through the sequencing and analysis of genetic material directly extracted from the
948 environment.

949 **Metatranscriptomics:** The study of the expressed genes in an environment or holobiont.

950 **Microbiome:** The sum of the microbial consortia (and their genetic material) in an
951 environment. The microbiome typically includes a diversity of prokaryotes (bacteria and
952 archaea), eukaryotes (fungi and protozoa) and viruses.

953 **Operational taxonomic unit (OTU):** Marker genes from multiple individuals that were
954 clustered/grouped on the basis of sequence similarity to represent a taxonomic group.

955 **Phenotype:** The observable characteristics of an organism, influenced by genetics and the
956 environment.

957 **Phylogenetic marker genes:** A genetic marker whose sequence is used to delineate
958 taxonomic and evolutionary relationships. Examples are the 16S rRNA gene in
959 prokaryotes and 18S rRNA gene and ITS (internal transcribed spacer regions) in
960 eukaryotes.

961 **Quorum sensing:** The ability to regulate gene expression in response to changes in cell-
962 population density through the production and detection of specific chemical signal
963 molecules (autoinducers) within or among populations.

964

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969 **Box 2: Literature Search and Horizon Scan Methodology**

970 **Approach:** First, we surveyed the literature to identify the breadth of research to-date and
971 evaluate the state of understanding in the field of coastal, estuarine, and marine microbiome
972 research. A detailed description of the literature search, including the script that was used,
973 can be found in Supplementary Methods. In brief, the SCOPUS database was used for
974 searching, with keywords including: seagrass, mangrove, saltmarsh, macroalgae, coral,
975 sponge, seaweed, seawater and sediment. This resulted in 671 publications, after irrelevant
976 publications were excluded. We then manually scanned these publications to extract
977 information on methods, host type, and GPS location of sampling site. The content of these
978 papers was then used quantitatively and qualitatively in this paper to report on the state of the
979 science.

980
981 Second, we used a modified horizon scanning method¹⁰⁸ to identify key questions in coastal
982 marine microbiome research. This approach uses expert solicitation to provide strategic
983 foresight into the key research gaps for future research; an approach that has been historically
984 underutilized in environmental science¹⁰⁹, yet can provide a powerful approach to prioritizing
985 a research agenda when appropriately structured^{110,111}. Briefly (more details provided in
986 Supplementary Data 1), the approach involved asking experts in the field of microbiome
987 research to freely contribute what they considered to be the most pending questions (10
988 maximum) in coastal and estuarine microbiome science – i.e. what are the major research
989 gaps and where do the challenges lie? Experts were initially selected from within the lead
990 and last author’s research networks and based on who the lead/last author perceived to be at
991 the forefront of the research field through the aforementioned literature search. To this end,
992 the selection of authors could be considered haphazard.

993
994 A total of 34 experts were approached by email, spanning 12 institutions from Australia,
995 Europe, Saudi Arabia and the United States (US). Of the invited experts, 28 experts (84%)
996 contributed responses (after two email reminders) and submitted 108 questions collectively.
997 A workshop was held at Deakin University (Melbourne, Australia) on 6-7 July 2017 to refine
998 the questions based on the voting process as described in Sutherland et al. ¹⁰⁸. The questions
999 were grouped into major research themes and are presented herein to discuss the research
1000 gaps in the context of the questions that underpinned them. Therefore, the themes presented
1001 below are not in a particular order of importance; they are arranged in a way that starts with

1002 microbiome-centric concepts, to relationships between microbiome and host, and lastly to
1003 broader interactions within and across microbiomes, the holobiont and the environment (Box
1004 Diagram).

1005

1006 [suggested Box Diagram placement. Caption below]

1007 Box Diagram: Conceptual design depicting the seven themes resulting from the horizon
1008 scanning exercise.

1009

1010 **Limitations:** Horizon scanning approaches have limitations, which, most importantly,
1011 include the risk of questions being inherently biased by the interests of the researchers¹⁰⁸. To
1012 limit this bias, we made an effort to solicit questions from researchers across a wide range of
1013 sub-fields, including plankton, sediment/substrate, seagrass, seaweed, coral, sponge and
1014 mangrove microbiomes. Additionally, while the solicited scientists work internationally, we
1015 were aware of potential biogeographic biases that could influence the questions. Therefore, in
1016 the original request for questions, we asked the scientists to keep their questions global in
1017 order to avoid national- or regional-specific topics.

1018

1019

Box 3: Methodological challenges

1. Molecular Approaches

DNA and RNA sequencing has been increasingly used as the preferred technique in coastal marine microbiome studies (Supplementary Fig. 1), yet several challenges have the potential to limit the production of reliable datasets. Such molecular challenges are broadly found in microbiome research, so we outline two current challenges in coastal marine microbiome research and suggest promising techniques that could help overcome these issues:

Challenges

- Host genome contamination in (meta)genomic studies on host-associated microbiomes

Potential Solutions & Benefits

- Pre-and post-sequencing removal of contaminating host cells and DNA sequences via
 - Physical removal of host tissue, e.g. centrifugation, Percoll separation
 - *In-silico* removal of well-curated host DNA sequences post-sequencing
 - Removal of methylated eukaryotic host DNA*¹¹²
 - Host-specific blocking primers

Bioinformatic challenges

- The level of taxonomic resolution needed in order to address questions on microbial composition and function
- The arbitrarily defined 97% sequence similarity designation of operational taxonomic units (OTUs)
- Employ amplicon sequencing approaches using universal primers as a first step (e.g. optimal gene segments V3 and V4), with added approaches, such as meta-‘omics’, for a more comprehensive understanding on microbial dynamics and functional roles.
- Use the most up-to-date and statistically valid methods for inferring the highest taxonomic resolution, e.g.¹¹³⁻¹¹⁵. Benefits include
 - Higher resolution profiles of microbial communities in a unit
 - Directly comparable between datasets
 - Genotype discrimination could also be improved by longer sequences

2. Manipulative Experiments

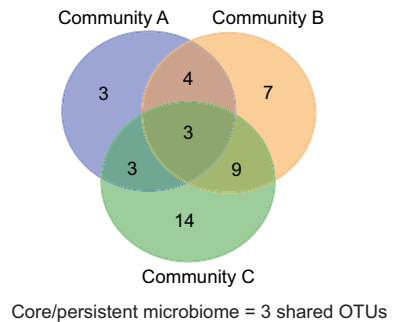
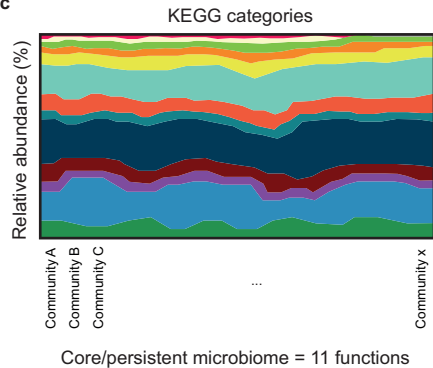
Laboratory manipulative experiments are key to addressing hypotheses in many of the research themes addressed here. Yet, while field microbiome experiments are essential to answer questions under natural, real-world conditions, field manipulations of host-microbiome interactions in coastal marine ecosystems are rare^{116,117}. Challenges include the logistics of excluding prokaryotes in the environment e.g. sterilization or antibiotics, but, for holobionts that are easily transportable, could be overcome with antibiotic treatment in the laboratory before field deployment¹¹⁸. Such a combination of innovatively designed laboratory and field experiments likely hold the key to teasing out important microbiome and holobiont interactions. Experiments that exclude or add specific microbes, resources, or isotope tracers would be useful in understanding functions and fine-scale interactions (e.g. beneficial microbiota⁵³), while the manipulation of environmental conditions could be used to simulate climate change, stress, or pollution scenarios (e.g. adding oil-degrading and plant growth promoting bacteria to oil spills in mangrove forests^{54,119}). Additionally, large simulator laboratories and *in situ* manipulative experiments could be a potential middle ground for testing hypotheses that have inherent field challenges⁴⁴.

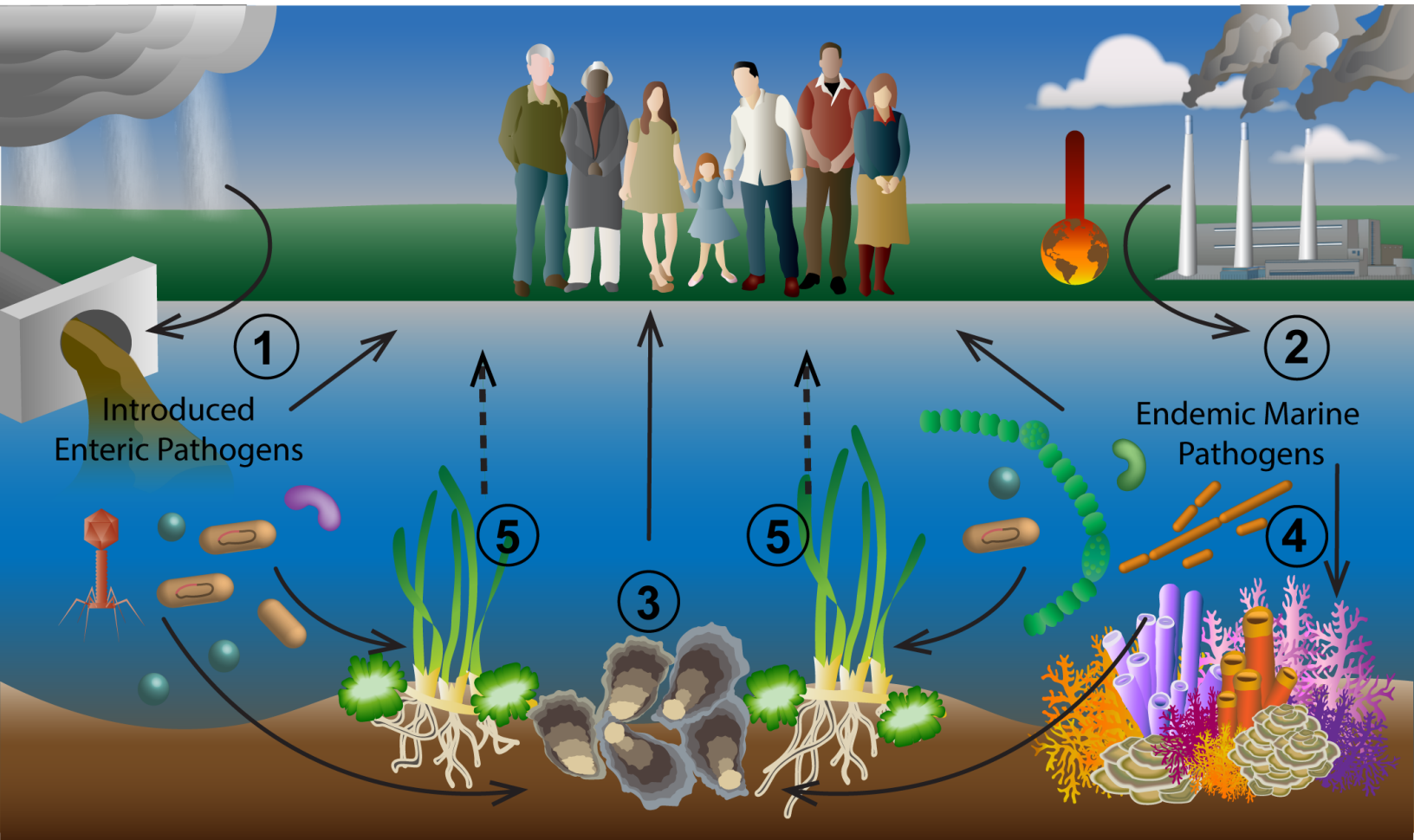
3. Coastal Marine Microbiome Management

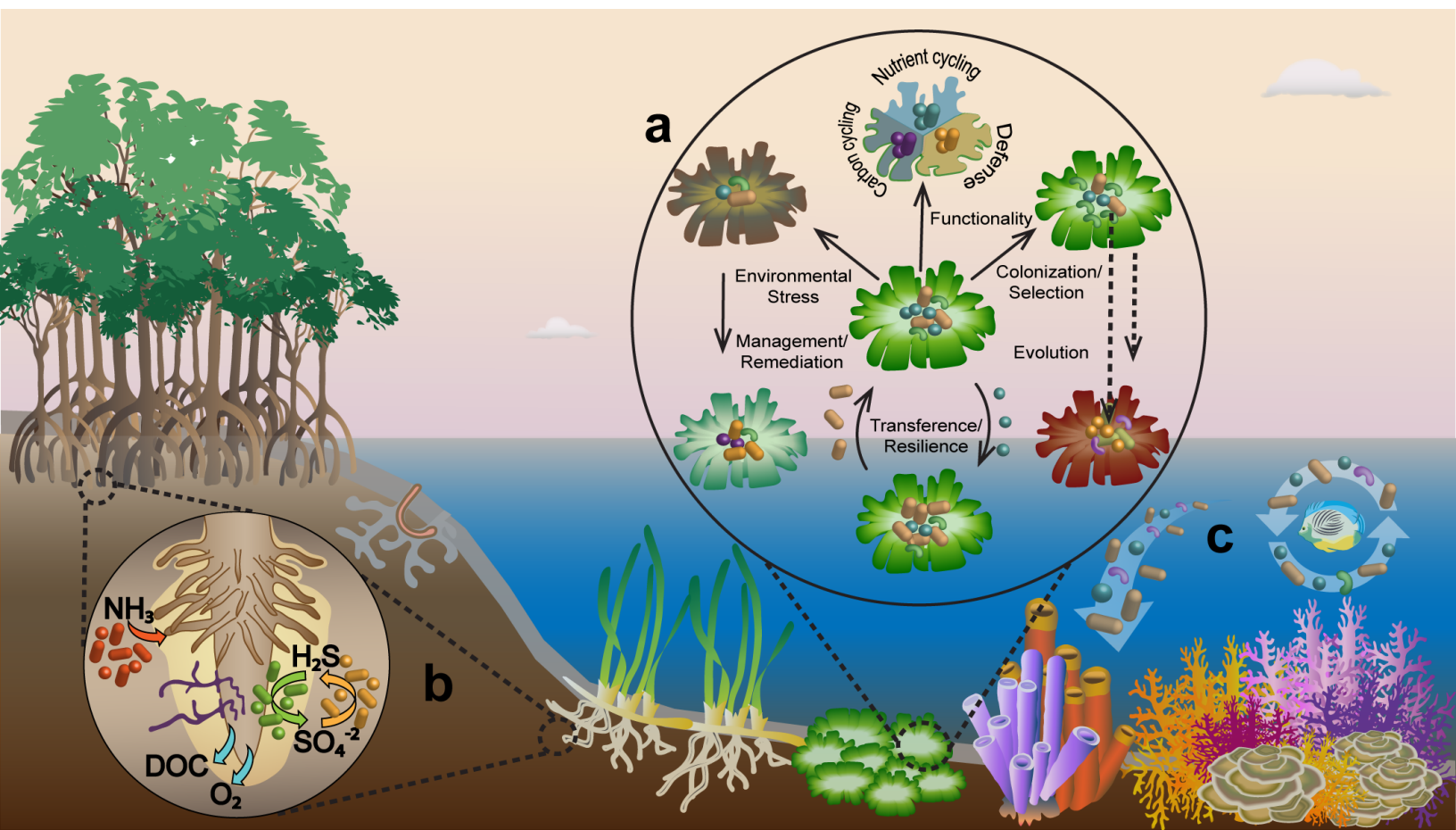
We discussed in Themes 4 and 6 the possibilities of managing and manipulating microbes in coastal marine ecosystems to aid in pollution and eutrophication remediation, restoration, disease management and enhancing host health and growth. With the current momentum in this space, we predict that several challenges and questions will arise, such as, ‘Can a managed or manipulated microbiome outlive its function, and if so, what impacts does this have on the microbiome, holobiont or ecosystem?’, ‘Is there a way to ‘stop’ a microbial function or remove a community once a particular job has been done?’, and ‘How resilient would a managed or manipulated microbiome be to disturbances and how would they be monitored to know if a desirable or undesirable outcome is achieved?’. Lastly, we predict that one of the key challenges will be to incorporate applied microbiome research into local, regional and national policy and methodology. As Bourlat et al.¹²⁰ also outlined, we suggest that stakeholders need to be identified and engaged at the state and national levels early on in the research.

*This will lead to the removal of any methylated DNA including that from protists and occasionally fungi, depending on the methylation rate.

1020

a**b****c**





Microbiome

1: Defining
2: Scale

Microbiome & Host

3: Assembly and disassembly
4: Resilience

Holobiont & Environment

5: Tripartite interactions
6: Management
7: Human connection