Perspectives Paper:

A horizon scan of priorities for coastal marine microbiome research

Stacey M. Trevathan-Tackett[1*], Craig Sherman[1], Megan J. Huggett[2,3], Alexandra H. Campbell[4,5], Bonnie Laverock[6], Valentina Hurtado-McCormick[7], Justin Seymour[7], Alana Firl[8], Lauren Messer[9], Tracy Ainsworth[4,10], Karita L. Negandhi[11], Daniele Daffonchio[12], Suhelen Egan[4], Aschwin H. Engelen[13], Marco Fusi[12,14], Torsten Thomas[4], Laura Vann[8], Alejandra Hernandez-Agreda[10,15], Han Ming Gan[1], Ezequiel M. Marzinelli[4,16,17], Peter D. Steinberg[4,16,17], Leo Hardtke[7], Peter I. Macreadie[1]

[1] Deakin University, Geelong, Centre for Integrative Ecology, School of Life and Environmental Sciences, Burwood and Waurn Ponds Campuses, VIC, 3130 Australia
[2]Centre for Marine Ecosystems Research and Centre for Ecosystem Management, School of Science, 270 Joondalup Dr, Edith Cowan University, Joondalup 6027 WA Australia
[3]School of Environmental and Life Sciences, The University of Newcastle, 10 Chittaway Rd, Ourimbah 2258 NSW Australia
[4]Centre for Marine Bio-Innovation, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney NSW 2052, Australia
[5]Faculty of Science, Health, Education & Engineering, University of the Sunshine Coast, QLD 4556, Australia
[6]School of Science, Auckland University of Technology, Private Bag 92006, Auckland, New Zealand
[7]Climate Change Cluster, University of Technology Sydney, 15 Broadway, Ultimo, NSW 2007, Australia
[8]Genome Center, University of California, Davis, California 95616

[9]Australian Centre for Ecogenomics, University of Queensland, St Lucia, Brisbane, QLD, 4072, Australia

[10]Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD, 4811, Australia

[11]Department of earth and planetary sciences, Macquarie University, North Ryde, NSW 2109, Australia

[12]King Abdullah University of Science and Technology (KAUST), Red Sea Research Center (RSRC), Thuwal 23955-6900, Saudi Arabia

[13]Centre for Marine Sciences (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

[14]School of Applied Sciences, Edinburgh Napier University, Edinburgh, UK

[15]The College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, QLD, 4811, Australia

[16]Sydney Institute of Marine Science, 19 Chowder Bay Rd, Mosman, NSW 2088, Australia

[17]Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore 637551

*Corresponding Author. E-mail: s.trevathantackett@deakin.edu.au

Abstract

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

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

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Background

Coastal marine ecosystems provide a range of ecologically and economically important ecosystem services, including habitat provisions, nutrient cycling, coastal protection and fisheries enhancement[1]. The health and services of these ecosystems are inherently linked to the microorganisms residing in these ecosystems (e.g. pollution remediation, disease and drug discovery[2-4]). As we increase our understanding of the importance of coastal marine microorganisms and their genetic makeup (i.e. the microbiome, see Box 1), the number of research articles describing the distribution, structure, and function of microbiomes associated with coastal marine ecosystems has flourished (Supplementary Figs. 1 and 2). The ecosystem services are largely attributed to the habitat-forming organisms, such as corals, sponges, macroalgae, seagrasses, mangroves and saltmarshes, which form the foundation of these ecosystems. Furthermore, due to the reliance of coastal marine ecosystem health on these habitat-forming organisms, the field has realized the importance of understanding the macroorganisms and their microbiomes as a synergistic ecological unit (i.e. holobiont, see Box 1). As a result, there has been a relative surge in host-associated microbiome research in recent years (Supplementary Fig. 1) aimed at identifying how microbiomes influence host phenotype, physiology, and development[5-7]. Although our understanding of several fundamental concepts in coastal marine microbial ecology has increased[7,8], coastal microbiome research -- particularly in the context of holobionts -- is still in its infancy, especially relative to other microbiome fields, such as the human microbiome. A large number of open questions currently limits our capacity to assess how microbial processes influence the ecology of these environments, both under contemporary conditions and under future environmental change. Therefore, there is a clear need to prioritize and define key questions for future research that will allow for better assessments of how microbial processes truly influence the ecology and health of coastal marine environments.

[suggested Box 1 placement]

Evaluating the state of the science

To evaluate the current state of coastal marine microbiome research, we surveyed the current literature, then 'horizon scanned' with experts in the field to identify major research gaps, in order to determine where future challenges lie and ultimately progress this field of research (see Box 2 for description of the approach and limitations). For the literature search, we focused on six key holobionts that form the foundation of these coastal ecosystems - corals, sponges, macroalgae, seagrasses, mangroves and saltmarshes. We also considered the microbiomes of sediments and the water column within coastal marine ecosystems. The key findings from the literature survey include identification of areas of progress, as well as holobiont systems that need more attention (Supplementary Figs. 1 and 2). For example, research on seawater- and sediment-associated microbiomes has dominated coastal marine microbiome literature to-date (consistently $\geq 50\%$ of the total number of studies), while host-associated microbiome research is steadily increasing and has generally focused on coral and sponge holobionts (Supplementary Fig. 1). In the last five years, however, the diversity and quantity of microbiome and holobiont research has incrementally increased with the inclusion of macrophyte-associated microbiome studies, although mangrove- and saltmarsh-associated microbiome research is still nascent (Supplementary Fig. 1). Additionally, the methodologies used to describe coastal marine microbiomes has diversified over time from predominantly microscopy, cell counts, and community fingerprinting techniques, to sequencing-dominated technologies (Supplementary Fig. 1). The literature survey also identified geographic hotspots and gaps in microbiome studies (Supplementary Fig. 2). The coastlines of Australia, Europe, the northern Mediterranean Sea, the Red Sea and US are relatively well-sampled in multiple ecosystem types, while there are clear regional gaps for host-associated microbiome studies along the South American, African and northern Asian coastlines. Some of the well-studied regions are dominated by studies on specific host-associated microbiomes (Supplementary Fig. 2). For instance, seagrasses have been heavily studied in the temperate US, while the biodiversity hotspots in the Indo-Pacific have been dominated by studies on coral- and sponge-associated microbiomes (Supplementary Fig. 2).

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

[suggested Box 2 placement]

Key research themes in coastal marine microbiome research

Microbiome

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

In coastal marine ecosystems, enormous microbial diversity has been revealed via, for instance, phylogenetic analyses of the 16S rRNA gene (e.g. [8-10]). However, it is important to define the function of a microbiome in order to understand how it is likely to influence its host and the ecosystem[11]. Currently, the best way to directly determine the function of the entire microbiome is via metagenomic and metatranscriptomic sequencing[12-16]. The recent availability of many genome reconstruction or binning approaches[17] offers a greater capacity to obtain near-whole genomes out of metagenomes, allowing a better understanding of the function of the microbiome members. However, our ability to successfully annotate functional genes within metagenomic and metatranscriptomic datasets remains outstripped by the availability of sequencing data itself. For example, extensive sequencing of the global ocean microbiome found that 40% of core orthologous genes were of unknown function[18].

Another approach to link diversity with function is to identify the ‘core microbiome’, or the persistent and functionally essential members of host-associated microbiomes, possibly a key determinant of host well-being and therefore overall ecosystem functioning and health (Fig. 1)[8,19]. For example, conserved bacterial taxonomic groups, which constitute the coral core microbiome, play a critical role in the success of the coral-zooxanthellae symbiosis[19]. Other organisms, such as the green seaweed *Ulva mutabilis*, require a core set of functions from their microbiome, rather than the presence of specific taxa[20].

Connecting diversity with function drives central ecological questions such as: (1) How does microbial community diversity influence functional aspects (e.g. resilience) of the host, microbiome and environment; and (2) How can we define the function of a coastal microbiome? Yet despite substantial effort in recovering metagenomes and metatranscriptomes from dominant marine hosts, there remain significant challenges in demonstrating causation between shifts in the microbiome and shifts in host health due to reduced capabilities to manipulate microbiomes in the field[18] (e.g. manipulative field experiments, see Box 3). We recognize this as a particular challenge for microbial ecologists. Therefore, we identify several questions that we hope will move the field forward and lead into innovative approaches that determine the functional roles of coastal marine microbiomes, and thereby resolve the relationship between microbiome diversity and their functions:

- How can novel techniques, e.g. single cell raman spectroscopy[21], be applied to complex microbiomes and holobionts to interrogate microbial functions in situ?
- How can intensive efforts for isolating coastal and host-associated microbes (i.e. ‘culturomics’[22]) open the door for tracking the function of candidate genes and investigating homology, predictability and certainty of curated gene function?
- How does identifying the core microbiome (taxa or set of functions, e.g. depicted in Fig. 1b-c, respectively) offer valuable insights towards advancing in-depth identification and experimental manipulation?

[suggested placement for Figure 1]

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

Host-associated microbiomes are highly dynamic communities that change at both small (i.e. ecological and physiological) and large (i.e. evolutionary and geographical) timescales. Substantial variability on very small spatial scales (i.e. within host) can be driven by host provisions, such as nutrient and oxygen availability[10,23], as well as by trophic- and quorum sensing-related interactions among members of the microbiomes within a physical niche (e.g. [24,25]). Hosts also differ in microbiome community structure depending upon host distribution in a population (e.g. center vs. edge of a seagrass meadow[26]), and microbiomes on host species can also vary across large environmental gradients[27]. However, for some holobionts such as seaweeds, geographical variability in surface-associated microbiomes is relatively low even at continental scales, relative to other factors such as host health condition[9]. Short-term temporal variability can also be surprisingly consistent, with predictable successional patterns over periods of days to weeks occurring in the epiphytic bacterial communities of macroalgae[28], corals[29] and sponges[30]. However, evidence for the scales at which coastal marine microbiomes shift in time and space, and the apparent drivers behind these shifts, is often conflicting. Studies showing host-specificity and stability in the microbiome over time and location[10,28,31,32] contradict studies that suggest that microbial communities are highly dependent on the host physiological or environmental conditions[9,33,34]. These conflicting patterns prevent us from making generalizations about the stability or variability of coastal host-associated microbial communities (e.g. Theme 5).

At evolutionary time scales, there is little doubt that hosts and their associated microbiomes influence each others' evolution, and indeed at very large time scales, these interactions are the basis for fundamental macroevolutionary events[35]. For individual marine systems, however, the details of how hosts and their microbial associates affect each other on shorter evolutionary time scales is limited, and whether or not these effects are broadly reciprocal (i.e. coevolutionary). To date, there is limited evidence among benthic marine hosts for coevolution with their microbiome[34,36], both because of the multiple interplaying factors that ultimately influence evolutive patterns, and because of the challenges in demonstrating formal coevolutionary relationships[37]. In some instances broad taxa of marine hosts and their microbiota appear to reciprocally evolve in response to one another[38], but in others selective effects appear to be limited to the host and individual members of its microbiome rather than the entire microbial community[39]. Further complexities in teasing out the evolution of members in a holobiont include both internal microenvironments of the host that act like discrete coevolving ecosystems[40], as well as the disparate evolutionary timescales that influence the host and the diversity of the microbiome members[41]. Evolutionary patterns within holobiont spatial niches/compartments have been shown for Scleractinian corals and their microbiomes, whereby the ecological relatedness of host-associated microbial communities parallels the phylogeny of related host species, and therefore evolutionary changes in the host associate with ecological changes in the microbiota[42]. This coevolutionary pattern, or phylosymbiosis was strongest in the coral skeleton compared to the coral tissue and mucus[43]. Although many coral-associated bacteria were host-specific, only a select minority of coral-associated bacterial families showed co-phylogenetic signals consistent with long-term host-microbe co-diversification[43].

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

- What are the implications of disparate evolutionary timescales between the host and its microbiota?
- How does the resolution at which we study microbiomes influence how we interpret differences in their composition and function at spatial and temporal scales?
- Do the holobiont members differentiate between beneficial and detrimental relationships in order to selectively favor the transmission of mutualistic partners between generations, and if so, what are the mechanisms?

To improve our understanding of the temporal and spatial dynamics of host-associated microbiomes, a structured approach to characterize spatial and temporal variation at multiple scales for both taxonomic and functional characteristics is needed. Future research should focus not only on descriptive studies, but also on perturbation experiments to assess resilience and stability under the context of variable systems[44-46]. Investigating microbiome evolution is inherently challenging, therefore clearly defining the boundaries of the question (e.g. phylogenetic vs functional level or the whole microbiome vs individual members), as well as identifying the limitations of what can be tested is necessary. Additional reflection on (co)evolution in systems other than coastal marine ecosystems may provide insight that could progress these questions. Examples include the formation of niches by symbiotic microbiota[40] and the broader literature on geographic aspects of coevolution (e.g. [47]).

Microbiome and Host

Theme 3: How are host-microbiome interactions formed?

Several studies have established that most benthic organisms, including seagrasses[10], corals[31] and macroalgae[48], carry microbiomes that are distinct from the surrounding sediment or seawater. Yet, the timing and underlying mechanisms of microbiome acquisition (either host-directed selection, or microbe-direct colonization) remain largely unresolved. Chemical signaling, specifically secondary metabolites produced by host species independently or in response to environmental or microbial cues, or signaling from microbial taxa that have already colonized the host, have been suggested to be important factors in both host defense against pathogenic microbes and microbiome colonization. For example, the pathogen *Vibrio coralliilyticus* has been shown to be attracted to corals that increase their production of the sulfur compound dimethylsulfoniopropionate (DMSP) under heat stress[49]. Conversely, in seaweeds such as *Lobophora variegata,* secondary metabolite production acts as a defense strategy by preventing colonization of pathogenic microbes, such as saprophytic marine fungi[50].

In addition to host-microbe interactions, some studies have suggested a role for microbe-microbe interactions in determining microbiome composition, including lottery models[51] and symbiotic modes of interaction[31]. It has also been shown that microbiome composition is affected by host condition (e.g. seaweed[9]; corals[49]), as well as environmental conditions (see Theme 5). Although there are few global census studies of the microbiome of particular marine species, recent studies in seagrasses[10,33] suggest that microbial functions and microbiome composition are also affected by geographic location, indicating an influential role of the environment in shaping microbiome composition. Taken together, we hypothesize that the active role of the host in determining microbiome composition lies along a continuum, ranging from being determined by host condition to being determined by environmental factors, which no doubt affect host condition. Where the system lies within the continuum is largely determined by host species. Additional studies in coastal marine ecosystems are needed to elucidate further:

- What are the differing selection strategies between host species that determines whether the microbiome is shaped more by the environment or by the host?
- What are the chemical pathways or specific processes by which a host attracts specific microbes, e.g. as observed in the model organism *Arabidopsis* microbiome[52]?
- Does the host species dynamically change its selection strategies as a function of microbial colonization, or changing environmental conditions?

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

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

The importance of microbes to the health of plants and animals is now well accepted. The microbial components of the holobiont can aid in digestion, provide essential vitamins and nutrients, protect from invading pathogenic organisms and stimulate developmental processes[7,55,56]. Therefore, any disturbance to the host microbiome are likely to result in a breakdown of holobiont function (or dysbiosis), which can manifest itself as disease. Analogous disease concepts have been proposed for chronic conditions in humans, including common periodontal and gastrointestinal disorders, which are thought to result from a disturbance to the natural microbiota rather than infection by a singular pathogen[57,58]. While less well understood for marine holobionts, microbial dysbiosis may also play a role in diseases, for example, the bleaching diseases of invertebrates and seaweeds (e.g. see recent reviews[59,60]). However, with some exceptions[61], the majority of these observations are based on correlative data, and the extent to which disease is a direct result of microbial dysbiosis remains an important research question. To fully appreciate the role of microbial dysbiosis we need to understand the core components of a healthy microbiome and identify those beneficial consortia that offer holobiont resilience. Importantly, given the capacity of microbes to rapidly respond, adapt and evolve to environmental conditions, the host microbiome is also likely to be instrumental in assisting the adaptation of higher organisms to future climate conditions or other anthropogenic stressors[62].

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

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

- To what degree are members of the transient microbiome a source of functional redundancy and thus providing resilience during environmental change?

Looking to the future, having sound knowledge and access to culturable, beneficial members of the core microbiota will have applied uses; for example, as biomarkers for the early detection of host stress or for the development of probiotic consortia that can be used to support aquaculture and marine restoration programs (Theme 6). However, taxa not considered part of the core microbiome under current conditions may become more important (core) under future conditions.

Microbiome, Holobiont & the Environment

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

Holobionts living in the dynamic ocean-land interface of coastal ecosystems[63] can be exposed to substantial diel changes in temperature, salinity, tidal levels, light, oxygen, and nutrients[64]. Their resilience and adaptation is at least partially influenced by the microbiomes that modulate the environmental conditions to which they are exposed[65]. The environment can act as a source for holobiont microbiota that in turn are shaped by strong selective forces driven by the host biology and behaviours. For example, fiddler crabs carapace and gut[66] are colonized by different pools of microbial colonists that are taken up from the environment, but the burrowing and filter feeding behaviors of the crabs finely select such colonists from the sediment after strongly reconditioning its geochemical properties[67].

The effects of either short- or long-term environmental changes on host-microbiome interactions are inherently complex and thus difficult to predict[68]. The intrinsic environmental variability, for instance linked to seasonal changes[69], perturbation events[70], or a combination of these[71], strongly influence microbiome diversity and functionality. Environmental stressors that can interact in opposing, additive or synergistic ways to influence hosts, microbiomes and their interactions, can lead to positive, negative or neutral impacts on them[72].

Using as an example thermal stress, frequently investigated in coastal marine microbiome research, we should consider that all organisms, whether microbial or macroscopic, have optimal thermal tolerance thresholds[73]. Thermal stress has been correlated with functional and/or structural shifts in microbiomes of corals[74], sponges[75] and oysters[76], among others. Higher temperatures can induce virulence in otherwise commensal microbes[77], and/or decrease the host chemical defences, with continued stress leading to the break-down of symbioses, the introduction of new microbes (e.g. opportunistic pathogens) and, eventually, deterioration of the host[61,75].

The ecological interactions within and among holobionts can also be indirect, for instance, microbiome recruitment by one host that may be affected by the exudates of another nearby host[23,78]. Host proximity may affect microbiome compositions, such as for algal turfs on the surface of *Porites* coral that were associated with increased alpha diversity of coral surface microbes, particularly of pathogenic bacterial taxa[79]. Host coexistence may also provide a more suitable habitat, e.g. seagrasses in anoxic sediments are favored by the aerobic sulfideoxidizing bacterial symbionts associated with benthic burrowing bivalves, which detoxify the anoxic sediment[80].

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

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

Investigations using real-world scenarios like those on combined multi-stressors, such as heat, pH (ocean acidification), and oxygen availability, are still rare[68,81]. However, in order to address the above question, such approaches are essential to build more ecologically reliable models on how host-microbiome interactions respond and adapt to changes[82]. Additionally, the holobiont approach sets a research framework, to comprehensively explore the adaptive and evolutionary patterns of organismal resilience and ecological function, in response to the critical challenges imposed by multiple combined environmental changes[83,84].

Theme 6: How can we 'manage' microbiomes in the coastal environment and in association with hosts?

Management or manipulation of microbial functions and communities are well-established techniques in bioremediation of terrestrial and aquatic ecosystems – for instance, those impacted by hydrocarbons and toxins contamination[85]. Principal approaches involve either biostimulation (the process of 'activating' indigenous microbes via, for example, nutrient amendments) or bioaugmentation (the process of inoculating the ecosystem with non-indigenous microbes that have desired metabolic properties[85]), which have both been applied at ecosystem scales[86,87]. While these approaches have been less well-tested for marine systems, biostimulation strategies have been applied to deal with oil spills in ocean waters (e.g. Exxon Valdes or Deep Horizon disasters), primarily by supplying growth limiting nutrients, such as phosphate, to the site of contamination[86]. Biostimulation and bioaugmentation have also been used to accelerate degradation of polycyclic aromatic hydrocarbons in marine coastal sediments[88].

Host-associated microbial communities can also in principle be managed, as exemplified over the last few years by the development of sophisticated pre- and probiotic strategies for disease prevention and health improvement of humans, plants and some aquaculture species[89-91]. The advances in these hosts are facilitated in an increasingly detailed understanding of microbial diversity and functional processes, but such information is sparse for most natural marine hosts, thus preventing rational designs of pre- and probiotic strategies.[62,92] Taken together, we envisage the following questions to be areas of increasing research:

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

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

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

Coastal environments, including their associated biota, are the principal interface for human exposure to marine microbiomes and these interactions can sometimes have detrimental impacts on human health (Fig. 2). Human pathogens present within marine microbiomes include both indigenous marine microbes and enteric microbes that are exogenously introduced to coastal habitats via sewage and urban storm-water[93] (Fig. 2). The microbiomes of benthic marine flora and fauna often display a high representation of marine pathogens - in particular members of the *Vibrio* genus[94]. Several species within this genus are highly virulent and dangerous human pathogens, and in the USA alone are cumulatively responsible for health costs exceeding \$250 million yr^{-1} [95]. In addition to native marine microbes, enteric pathogens that become, at least transiently, incorporated into marine microbiomes following exposure to coastal pollution also pose a significant health risk. Indeed, due to (i) the preference for coastal and estuarine habitats among many native marine pathogens[94] and (ii) the regular exposure of coastal flora and fauna to human waste streams, the microbiomes of coastal organisms represent potentially important hotspots and reservoirs of human pathogens[93] (Fig. 2). On the other hand, there is recent evidence that some marine macro-organisms, specifically seagrasses, may act as effective natural filtration systems that remove human pathogens from coastal ecosystems, potentially through the production of biocides by the plant or its microbiome[96] (Fig. 2).

As the global human population rapidly increases its dependence and impact upon coastal environments[97], it is imperative that we develop an understanding of the potential human health consequences of increasing contact with marine microbiomes. This is particularly true, given that there is emerging evidence that climate change and the anthropogenic degradation of coastal habitats are enhancing the occurrence and virulence of dangerous human pathogens within these ecosystems[98] (Fig. 2). Within this specific context, we identify several key questions that remain unanswered, including:

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

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

New analytical approaches for interpreting microbiome data provide several opportunities to answer these questions. For instance the detection of novel “indicator” organisms[99] or genes[100] within microbiome data-sets delivers potentially powerful capacity to detect environmental perturbations and human contamination within coastal waters that goes far beyond standard indicators of human contamination (i.e. Faecal Indicator Bacteria [FIB]), which are often limited in sensitivity and explanatory power[101]. The analysis of coastal microbiome data also provides a facility to detect novel or emerging pathogens that are missed by standard FIB monitoring approaches[102,103]. Finally, the application of analytical approaches such as SourceTracker[104] and random forest analyses[105] allow for microbiome data to be directly used as a sensitive new tool for tracking sources of contamination or signals of environmental change.

[suggested placement for Figure 2]
[suggested placement for Box 3]

Synthesis and Outlook

Coastal marine microbiome research represents a direct pathway to understanding how microbes affect – both positively and deleteriously – the coastal ecosystems on which human populations so heavily rely. The themes and questions presented here, summarized in a conceptual framework (Fig. 3), include resolving the spatial, temporal, and evolutionary scales at which the holobionts and microbiomes function, resolving how holobionts change in response to environmental stimuli and each other, and determining the scope for how microbiomes can be managed. Summarizing the future of coastal microbiome research through the horizon scan and literature survey has identified two overarching concepts common across the themes that reflect the current state of the science, as well as how we envision the science will progress: microbiome function and utilizing manipulative approaches.

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

Such investigations, as shown throughout the themes, are inherently complex, whereby the questions and concepts presented in one theme relied on the understanding of another theme. For example, teasing apart the relationship between microbiome and host health and resilience (Theme 4) depends on the temporal scale (Theme 2) and environmental conditions (Theme 5) that influence the interactions, but each of these themes in themselves also influence how microbiota are selected and form holobionts (Theme 3). The ever-changing nature of the ecological processes that influence the microbiomes and holobionts in the natural environment necessitates manipulative experimental approaches in order to tease apart the questions presented. In some cases, such as the evolutionary, multi-stressor or management questions, highly controlled experiments are the best options currently available to progress the respective themes. Here, the use of model organisms may provide insight, for example, on selection mechanisms between host and microbe (Theme 3), and microbiome-driven restoration (Theme 6). The large national and international collaborations or consortium efforts that have produced the descriptive data on environmental microbiomes to-date, may be equally useful in progressing hypothesis-driven questions through concerted manipulative experimental approaches[107], e.g. temperature effects on holobiont resilience at the biogeographic limits of the host (Themes, 5, 4 and 2, respectively) or how holobionts can act as sources or sinks of pathogenic microbiota under various point source or diffuse pollution scenarios (Theme 7). In summary, although the list of research themes we present here is broad and ambitious, the ongoing collaborative networks along with well-executed hypothesis-driven manipulative experiments are significantly progressing the definition and functional relationship between the core microbiome and host, illuminating global microbiome biogeography, and identifying key regional- and global-scale environmental influences on coastal marine microbiomes and holobionts.

[suggested placement for Figure 3]

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

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

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Correspondence and requests for materials should be addressed to S.T.T.

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Figure Legends

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

Fig. 2: Conceptual design of the potential relationships between coastal marine microbiomes and humans. (1) Coastal pollution, including stormwater and sewage effluence, introduce potentially pathogenic microbiota to coastal marine ecosystems. (2) Endemic marine pathogens, including *Vibrio* and toxic cyanobacteria, persist in coastal marine ecosystems. (3) Coastal aquaculture species can become contaminated by endemic and introduced pathogens, which can both (i) cause mortality, e.g. oysters, and (ii) have health implications for human consumers. (4) Endemic pathogens, e.g. *Vibrio*, can cause holobiont disease. (5) According to Lamb et al. [96], coastal macrophytes may act as natural pathogen filters, buffering the impact of pathogens for humans and coastal marine ecosystems. Climate change and CO_2-mediated

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

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

Boxes

Box 1: Key Definitions

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

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

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

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

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

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

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

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

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

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

Box 2: Literature Search and Horizon Scan Methodology

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

Second, we used a modified horizon scanning method[108] to identify key questions in coastal marine microbiome research. This approach uses expert solicitation to provide strategic foresight into the key research gaps for future research; an approach that has been historically underutilized in environmental science[109], yet can provide a powerful approach to prioritizing a research agenda when appropriately structured[110,111]. Briefly (more details provided in Supplementary Data 1), the approach involved asking experts in the field of microbiome research to freely contribute what they considered to be the most pending questions (10 maximum) in coastal and estuarine microbiome science – i.e. what are the major research gaps and where do the challenges lie? Experts were initially selected from within the lead and last author's research networks and based on who the lead/last author perceived to be at the forefront of the research field through the aforementioned literature search. To this end, the selection of authors could be considered haphazard.

A total of 34 experts were approached by email, spanning 12 institutions from Australia, Europe, Saudi Arabia and the United States (US). Of the invited experts, 28 experts (84%) contributed responses (after two email reminders) and submitted 108 questions collectively. A workshop was held at Deakin University (Melbourne, Australia) on 6-7 July 2017 to refine the questions based on the voting process as described in Sutherland et al. [108]. The questions were grouped into major research themes and are presented herein to discuss the research gaps in the context of the questions that underpinned them. Therefore, the themes presented below are not in a particular order of importance; they are arranged in a way that starts with microbiome-centric concepts, to relationships between microbiome and host, and lastly to broader interactions within and across microbiomes, the holobiont and the environment (Box Diagram).

[suggested Box Diagram placement. Caption below]

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

Limitations: Horizon scanning approaches have limitations, which, most importantly, include the risk of questions being inherently biased by the interests of the researchers[108]. To limit this bias, we made an effort to solicit questions from researchers across a wide range of sub-fields, including plankton, sediment/substrate, seagrass, seaweed, coral, sponge and mangrove microbiomes. Additionally, while the solicited scientists work internationally, we were aware of potential biogeographic biases that could influence the questions. Therefore, in the original request for questions, we asked the scientists to keep their questions global in order to avoid national- or regional-specific topics.

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	Potential Solutions & Benefits
• Host genome contamination in (meta)genomic studies on host-associated microbiomes	• Pre-and post-sequencing removal of contaminating host cells and DNA sequences via o Physical removal of host tissue, e.g. centrifugation, Percoll separation o *In-silico* removal of well-curated host DNA sequences post-sequencing o Removal of methylated eukaryotic host DNA*[112] o Host-specific blocking primers
Bioinformatic challenges	
• The level of taxonomic resolution needed in order to address questions on microbial composition and function	• 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.
• The arbitrarily defined 97% sequence similarity designation of operational taxonomic units (OTUs)	• Use the most up-to-date and statistically valid methods for inferring the highest taxonomic resolution, e.g. [113-115]. Benefits include o Higher resolution profiles of microbial communities in a unit o Directly comparable between datasets o Genotype discrimination could also be improved by longer sequences

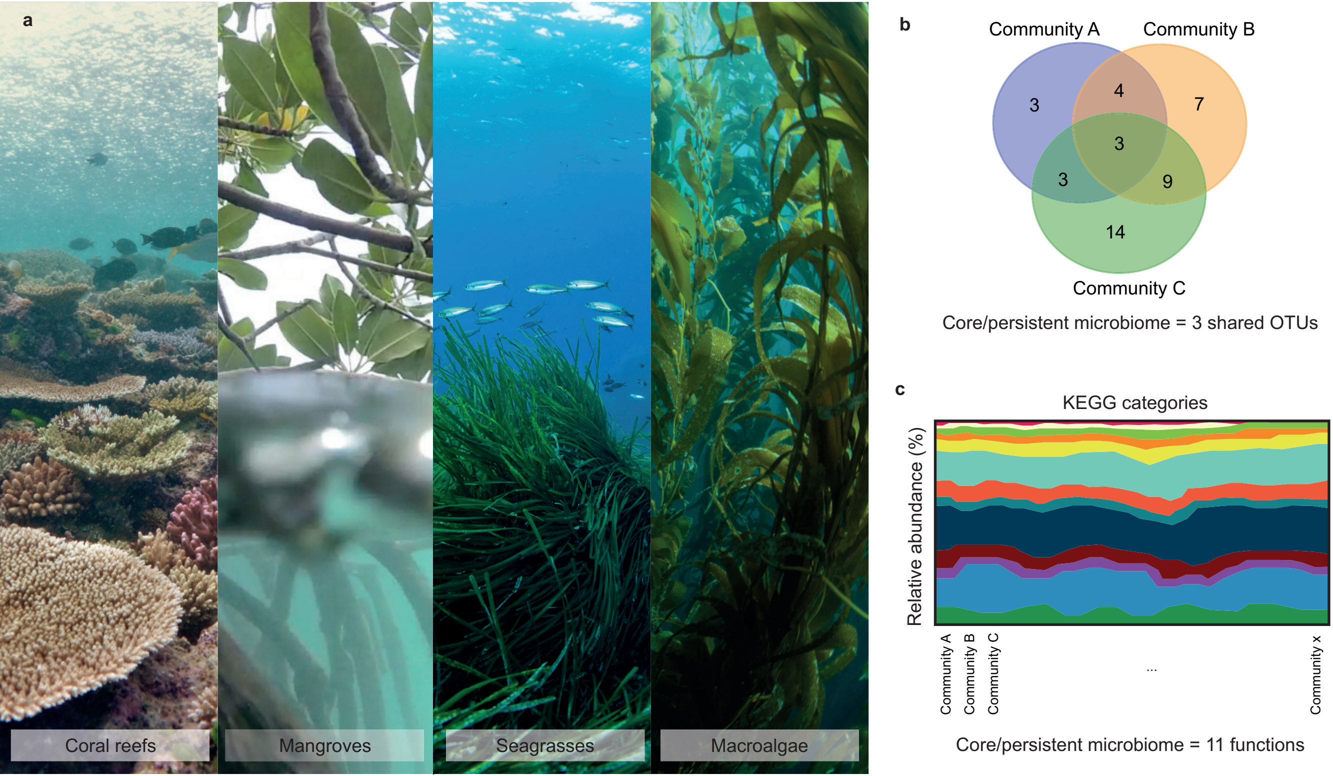
2. Manipulative Experiments

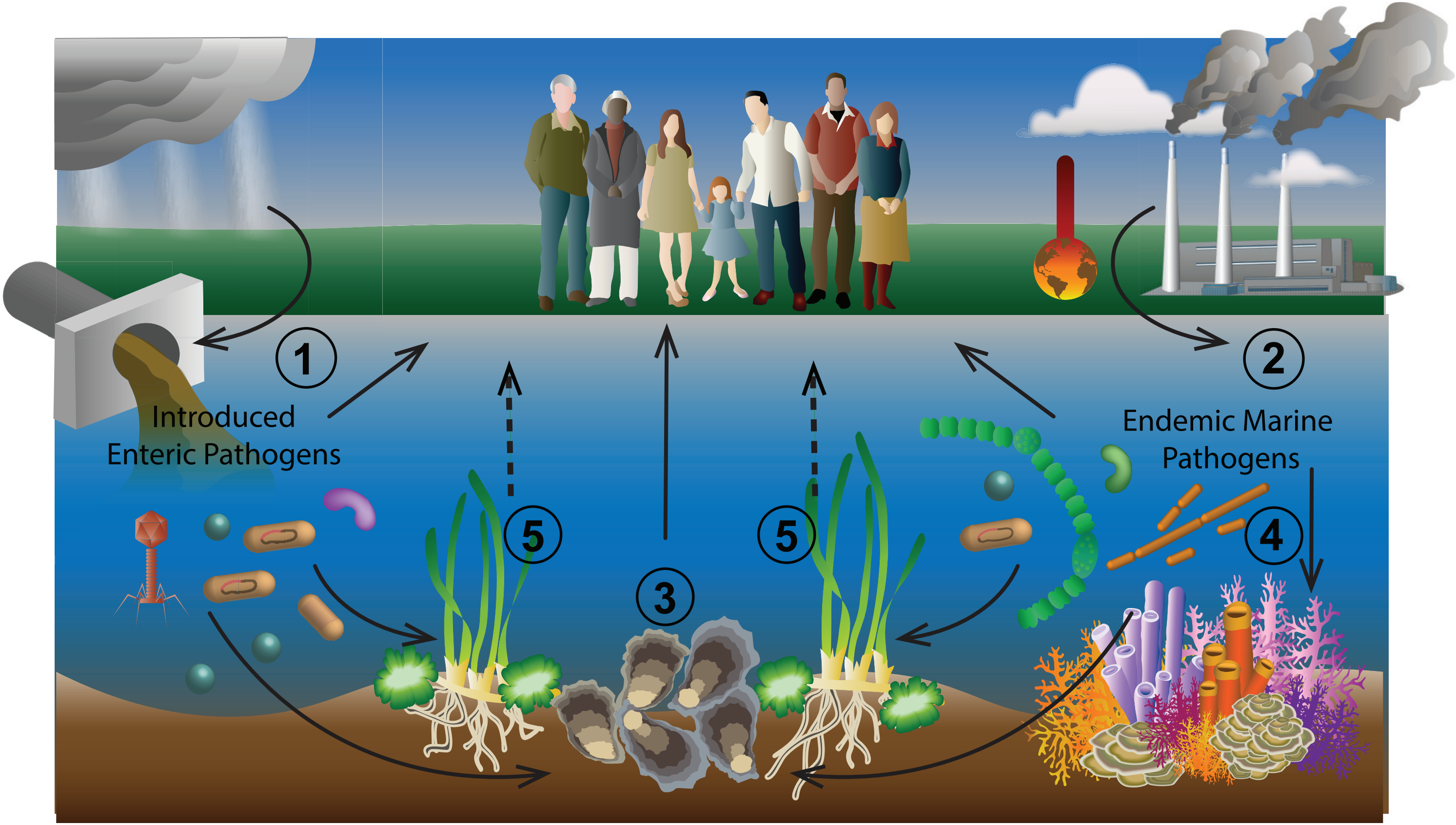
Laboratory manipulative experiments are key to addressing hypotheses in many of the research themes addressed here. Yet, while field microbiome experiments are essential to answer questions under natural, real-world conditions, field manipulations of host-microbiome interactions in coastal marine ecosystems 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 deployment[118]. 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 microbiota[53]), 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 challenges[44].

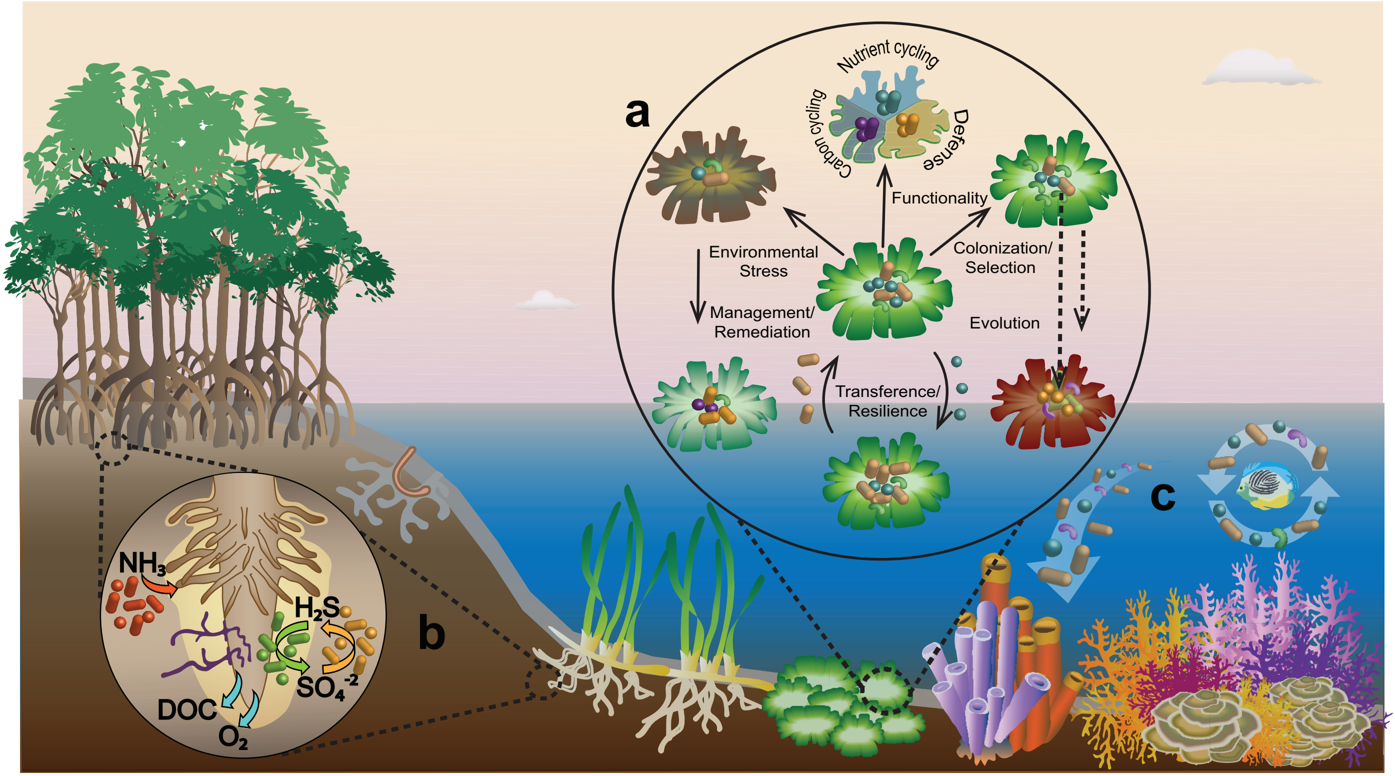
3. Coastal Marine Microbiome Management

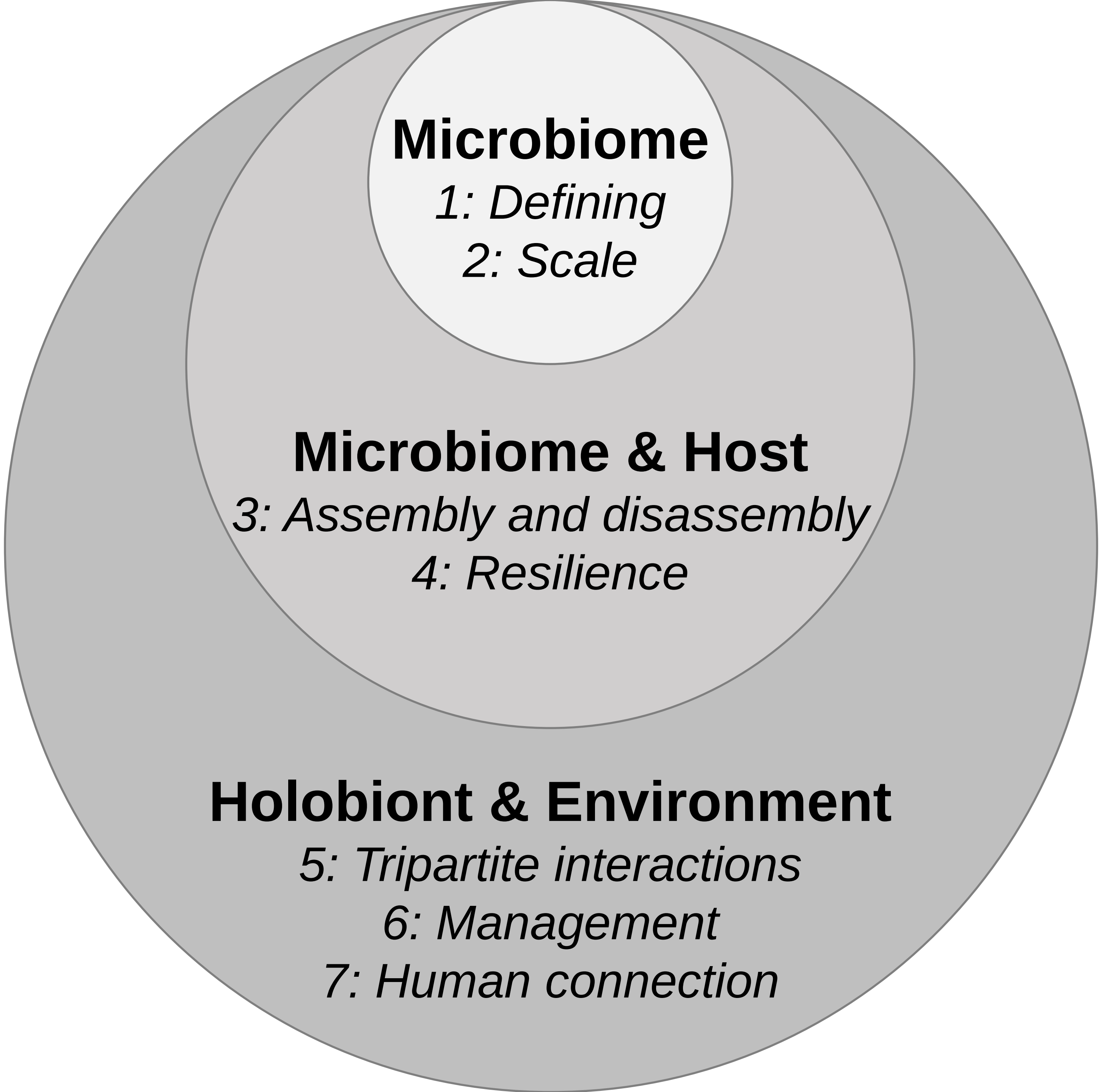
We discussed in Themes 4 and 6 the possibilities of managing and manipulating microbes in coastal marine ecosystems to aid in pollution and eutrophication remediation, restoration, disease management and enhancing host health and growth. With the current momentum in this space, we predict that several 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.*


a
Coral reefs
Mangroves
Seagrasses
Macroalgae
b
Community A
Community B
3
4
7
3
3
9
14
Community C
Core/persistent microbiome = 3 shared OTUs
c
KEGG categories
Relative abundance (%)
Community A
Community B
Community C
...
Community x
Core/persistent microbiome = 11 functions


①
Introduced
Enteric Pathogens
②
Endemic Marine
Pathogens
③
④
⑤
⑤


a
Nutrient cycling
Carbon cycling
Defense
Functionality
Environmental Stress
Colonization/ Selection
Management/ Remediation
Evolution
Transference/ Resilience
b
NH_3
H_2S
SO_4^{-2}
DOC
O_2
c



Microbiome

1: Defining

2: Scale

Microbiome & Host

3: Assembly and disassembly

4: Resilience

Holobiont & Environment

5: Tripartite interactions

6: Management

7: Human connection