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# Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems

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## Keywords

coral holobiont, coral microbiome, coral hologenome, cumulative stressors, holobiont resilience

## Abstract

Corals are fundamental ecosystem engineers, creating large, intricate reefs that support diverse and abundant marine life. At the core of a healthy coral animal is a dynamic relationship with microorganisms, including a mutually beneficial symbiosis with photosynthetic dinoflagellates (*Symbiodinium* spp.) and enduring partnerships with an array of bacterial, archaeal, fungal, protistan, and viral associates, collectively termed the coral holobiont. The combined genomes of this coral holobiont form a coral hologenome, and genomic interactions within the hologenome ultimately define the coral phenotype. Here we integrate contemporary scientific knowledge regarding the ecological, host-specific, and environmental forces shaping the diversity, specificity, and distribution of microbial symbionts within the coral holobiont, explore physiological pathways that contribute to holobiont fitness, and describe potential mechanisms for holobiont homeostasis. Understanding the role of the microbiome in coral resilience, acclimation, and environmental adaptation is a new frontier in reef science that will require large-scale collaborative research efforts.

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## INTRODUCTION

Healthy corals are the basis of a productive and sustainable reef ecosystem, providing ecosystem goods upon which more than 500 million people rely, supplying a fundamental source of protein for many marine nations through fisheries, and generating substantial gross domestic product earnings through tourism-based industries (81). Although coral reefs have only a small spatial footprint, covering 280,000 km<sup>2</sup> in tropical and subtropical waters, their topographic complexity and unique primary production support approximately 25% of all known marine species (63). However, coral reefs are also critically threatened habitats, experiencing significant global declines through a combination of local impacts, including poor water quality, changed land management practices, and overfishing; and global climate shifts, such as increased sea surface temperature and ocean acidification (28, 36, 55).

### Symbionts:

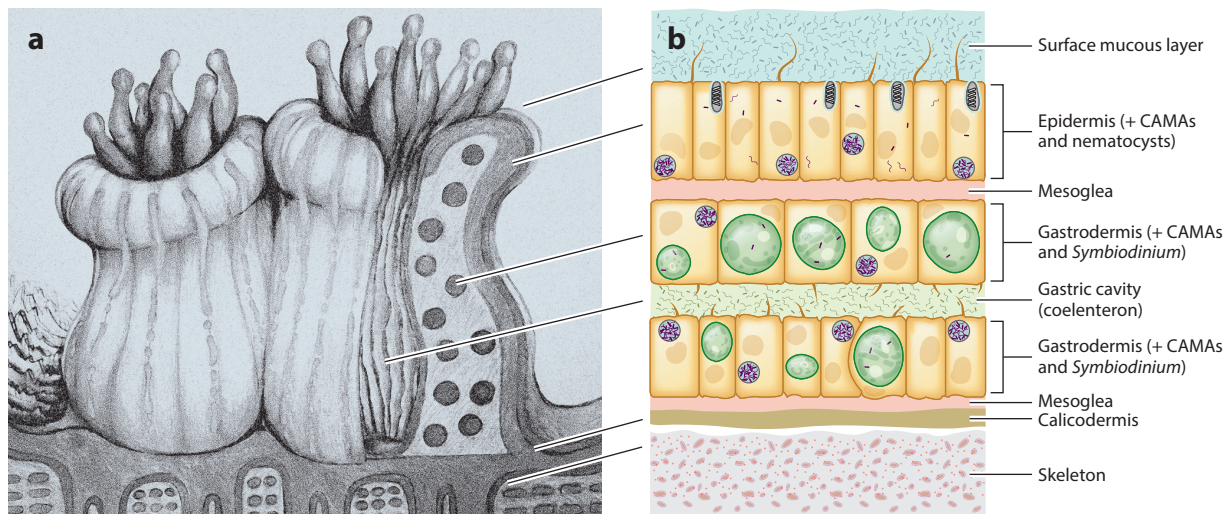
organisms that live in close association in a relationship that is not necessarily mutually beneficial

### *Symbiodinium*:

the genus of endosymbiotic dinoflagellates that reside within the endoderm of tropical cnidarians, including corals

## THE CORAL HOLOBIONT

The increasing research focused on improving the resilience of coral reefs to environmental stress requires an intricate understanding of the factors underpinning healthy reef ecosystems. As reported for other holobiont systems (e.g., humans, termites, hydra, squid, and sponges) (20, 39, 45, 79, 132, 139), coral-associated microorganisms are not only critical to host fitness and survival, but also sensitive to environmental perturbation and the physiological status of their host. The best-known coral symbionts are dinoflagellates, within the genus *Symbiodinium*. High



**Figure 1**

(a) A schematic representation of a coral polyp and (b) its detailed microstructure representing microhabitats including the surface mucopolysaccharide layer (SML), tissue layers (epidermis, mesoglea, gastrodermis), gastric cavity (coelenteron), calicodermis, and skeleton. *Symbiodinium* spp. are represented as large green cells within the gastrodermis layers. Coral nematocysts are represented as gray elongated structures in the epidermis. Bacterial colonization is represented by an abundant community in the SML; occasional coral-associated microbial aggregates (CAMAs) within the epidermis and gastrodermis layers; an abundant community in the gastric cavity; rare and highly specific intracellular associations with *Symbiodinium* cells (green); and a unique mix of bacteria, fungi, and filamentous algae in the coral skeleton. Note that the abundance and localization of microbial assemblages within the coral holobiont differ across coral species.

densities ( $>10^6 \text{ cm}^{-2}$ ) of these obligate photosymbionts occur in the coral gastroderm (**Figure 1**), where they can provide  $>90\%$  of a coral's nutritional requirements (87). More than 100 species types within nine evolutionary lineages (clades) of *Symbiodinium* are known to form associations with corals (103), and symbiont identity as well as ambient environmental conditions ultimately determine the amount of carbon and nitrogen translocated to the coral host (26, 131, 153). While other recent reviews have concentrated primarily on this component of the holobiont (26), here we instead focus on the prokaryotic microbiome.

### Taxonomic Overview of Coral-Associated Microbial Communities

Coral-associated microorganisms may be commensal, mutualistic, or pathogenic, with estimated densities of  $1 \times 10^2$  to  $>1 \times 10^6$  cells per square centimeter of host tissue (46) and diversities exceeding thousands of unique operational taxonomic units (OTUs) in some host species (15, 86, 127). Extensive phylogenetic surveys of coral microbiomes have revealed that the dominant symbionts reside within the *Proteobacteria* (particularly *Gamma*- and *Alphaproteobacteria*) as well as *Actinobacteria*, *Bacteroidetes* (especially *Flavobacteria*), and *Cyanobacteria* (15, 56, 73, 113, 127).

### Species Specificity in Coral Microbiomes

A considerable body of research has shown that coral microbial communities are often specific to host species (73, 113) and that within a species, microbial communities can be highly stable across biogeographies and under different environmental conditions (127). However, a number of

**Coral microbiome:** the entire microbial community (and associated genes) that resides on or within a coral

**Core microbiome:**

a subset of the microbiome that is shared among multiple individuals of the same taxa

**Mutualism:**

the relationship between organisms existing in close association such that each benefits from the activity of the other

**Coral holobiont:**

the unit of biological organization composed of the coral host and its microbiota

recent studies provide compelling evidence that coral-microbe interactions are not always uniform over the coral colony (113, 128) and are instead influenced by a range of factors, including host physiological characteristics (86), location within the coral substructure (tissue, gastric cavity, mucus, skeleton) (3, 128), life history stage (119), and the physical environment (56, 73, 113). Broad-scale profiling studies therefore likely oversimplify coral-microbe associations, and high-resolution investigations with a view to understanding the spatial, functional, and environmental dynamics of the core microbiome are instead required (3). Importantly, studies have clearly shown that low-abundance phylotypes can form physiologically significant interactions with their host (29); hence, it is essential to define mechanisms for analyzing the core coral microbiome (2), that is, species that are persistent either temporally or among different environments or locations (76), irrespective of their relative abundance. This core species approach was recently employed by the Human Microbiome Project to identify stable and consistent associations that correlate with human health (132).

A recent meta-analysis of microbiomes from diverse coral species identified 7 core bacterial phylotypes (primarily members of the *Actinobacteria* and *Ralstonia*) which were localized within specific niche habitats (i.e., mucus, gut, skeleton) (2). In addition, a notable component of the core coral microbiome is affiliated with the *Endozoicomonas* genus (*Gammaproteobacteria* class, *Haemellaceae* family), which can be as much as 90% of the microbiome (10, 11, 89) and inhabits a wide range of coral species in addition to other marine invertebrates (12, 49). This putative symbiont is thought to reside within the coral endoderm (11, 89) (**Figure 1**) and appears to play an important role in host fitness (11, 89, 90). The core coral microbiome is likely determined by the host (although the mechanisms that facilitate this selection are still to be determined) and has been suggested to enable metabolic adaptation to local environmental conditions (2, 60).

### Poorly Understood Members of the Coral Holobiont

While coral microbiome analyses have focused predominantly on bacteria, an array of less-studied microorganisms also associate with corals. Archaea are a minor yet functionally important component, as they are thought to recycle nutrients for the benefit of the host. *Crenarchaeota* are the most commonly reported archaeal phylum, contributing to nitrogen recycling and ammonia removal through nitrification, denitrification (122), and ammonia oxidation (140). Archaeal sequences related to Marine Group II *Euryarchaeota*, anaerobic methanotrophs, and anaerobic nitrate reducers have also all been reported from corals (122, 141), although to date, no studies have confirmed the physiological activity of archaea within the holobiont.

In terms of eukaryotic symbionts, endolithic fungi are also proposed to undertake nitrogen cycling in the holobiont, including reduction of nitrate/nitrite to ammonia and assimilation of ammonia for biosynthesis (140). In addition, the single-cell eukaryotic alveolates (a group comprising the ciliates, dinoflagellates, apicomplexans, and chromerids) are known to form intimate associations with corals (57). The apicomplexans are primarily parasitic protists, although recent surveys have identified abundant apicomplexan-related sequences in a wide range of healthy coral species (57), indicating a potential mutualism. In particular, the apicomplexan-like *Chromera velia*, which shares a common ancestor with dinoflagellates (82), associates with adult scleractinian corals across a broad geographic range and can also form endosymbiotic partnerships with coral larvae (25). However, the beneficial and/or detrimental role of apicomplexans within the coral holobiont is yet to be determined.

Ciliates are common coral associates thought to opportunistically feed on coral-associated bacteria and provide a top-down control on specific microbial populations. In some cases, ciliates are associated with a shift to a disease state, particularly when the coral host becomes

compromised (59). For instance, skeleton-eroding band (SEB) syndrome is widespread throughout the Indo-Pacific and is characterized by a speckled black band composed of the empty loricae (shell-like housings) of the foliicolinid ciliate *Halofolliculina corallasia* (95). Similarly, brown band (BrB) syndrome is widespread in the Indo-Pacific; the lesion is populated by the ciliate *Porpostoma guamensis*, which ingests intact *Symbiodinium* spp., contributing to the visible symptoms of BrB (75). The role of other predatory microorganisms in top-down control of coral microbiomes is uncertain, despite their significant potential to influence the structure, stability, and function of coral-associated microorganisms. However, recent research has identified that *Halobacteriovorax*, a genus within the *Bdellovibrio* and like organism (BALO) group, preys on common coral pathogens, including *Vibrio*, and may play a role in preventing secondary colonization by other potentially pathogenic bacteria (143).

Virus-like particles (VLPs) have been observed in a broad range of scleractinian coral species, their associated microbial communities (77, 149), and their algal endosymbionts (24), and recent metagenomic studies have documented a high diversity of DNA and RNA viruses associated with corals (145). While the functional repertoire of coral viruses is still uncertain, they likely play critical roles in structuring prokaryotic communities (9), contributing to nutrient recycling and mediating transfer of genetic material that may confer benefits to their hosts (134). However, environmental stress commonly triggers latent viruses to enter a lytic cycle, a scenario demonstrated in the sea anemone *Anemonia viridis*, where heat and ultraviolet light increased the VLPs and concomitant degeneration of *Symbiodinium* spp. (148). In corals, environmental stressors can induce the production of herpes-like viruses (135), and specific viral groups have been associated with diseased and bleached corals (125). Temperature-induced latent infection has also recently been suggested to confer virulence to specific coral pathogens (144).

## Establishment and Maintenance of Symbiosis Within the Coral Holobiont

For maintenance of the coral holobiont, inheritance of symbionts needs to occur either directly via parental gametes (i.e., vertical transmission) or through faithful acquisition from the surrounding environment (i.e., horizontal transmission), or a mixture of both strategies. Corals can reproduce both sexually and asexually, and while little is known about the extent of vertical versus horizontal symbiont transmission in corals, there is some evidence that species that undertake internal fertilization and brood larvae tend to transmit at least some of their symbionts vertically, whereas species that rely on external fertilization most likely acquire their symbionts horizontally (5, 119). This early acquisition and maintenance of bacteria may protect coral larvae through production of antibiotics and provision of nutrients (67, 119), although this has yet to be empirically tested.

Coral-associated bacteria exhibit significant levels of chemotaxis toward chemicals released from the coral, suggesting that chemotaxis plays an important role in the establishment and maintenance of specific coral-microbe interactions (130). Once a microbe has found its host via chemotaxis, pattern-recognition molecules termed microbe-associated molecular patterns (MAMPs) along with pattern-recognition receptors (PRRs) in the host can initiate signaling pathways that ultimately eliminate pathogens or lead to tolerance of mutualists (93). MAMPs comprise peptidoglycans, glycans, and lipopolysaccharides present on the surface of microbes, and lectins are also involved in the cell recognition process for *Symbiodinium* spp. (26, 142). The C-type lectin protein millectin, the first coral PRR identified, might recognize both mutualistic and pathogenic microorganisms, since it can bind *Symbiodinium* spp. as well as the coral bacterial pathogen *Vibrio coralliilyticus* (65, 66). In general, however, the molecular mechanisms that allow for the establishment, recognition, and maintenance of specific symbionts within the coral holobiont are currently unknown, although this is a major focus of current research programs (136).

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### Endosymbionts:

smaller symbiotic partners living inside the host organism's tissues or cells and establishing endosymbiosis

### Coral bleaching:

loss of the intracellular endosymbiotic *Symbiodinium* cells from the coral tissues due to loss of pigmentation

### Latent infection:

asymptomatic infection by a dormant pathogenic virus capable of manifesting symptoms under certain conditions

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**Coral microhabitats:**

individual compartments of the coral host (SML, tissues, skeleton, gastric cavity) that provide niche environments for the microbiota

**Opportunistic pathogen:**

an organism that takes advantage of a host with a compromised immune system and/or an altered microbiome

## CORAL MICROHABITATS

Coral microbiome research has traditionally analyzed composite coral samples without distinguishing between microhabitats, such as the surface mucopolysaccharide layer (SML), epidermis, gastrodermis, gastric cavity, and coral skeleton. However, human microbiome research has clearly revealed that different parts of the body are unique microbial landscapes (132). Coral microbiome research incorporating high-resolution partitioning is therefore needed to fully understand how interactions between these microhabitats contribute to holobiont health.

### Coral Mucus

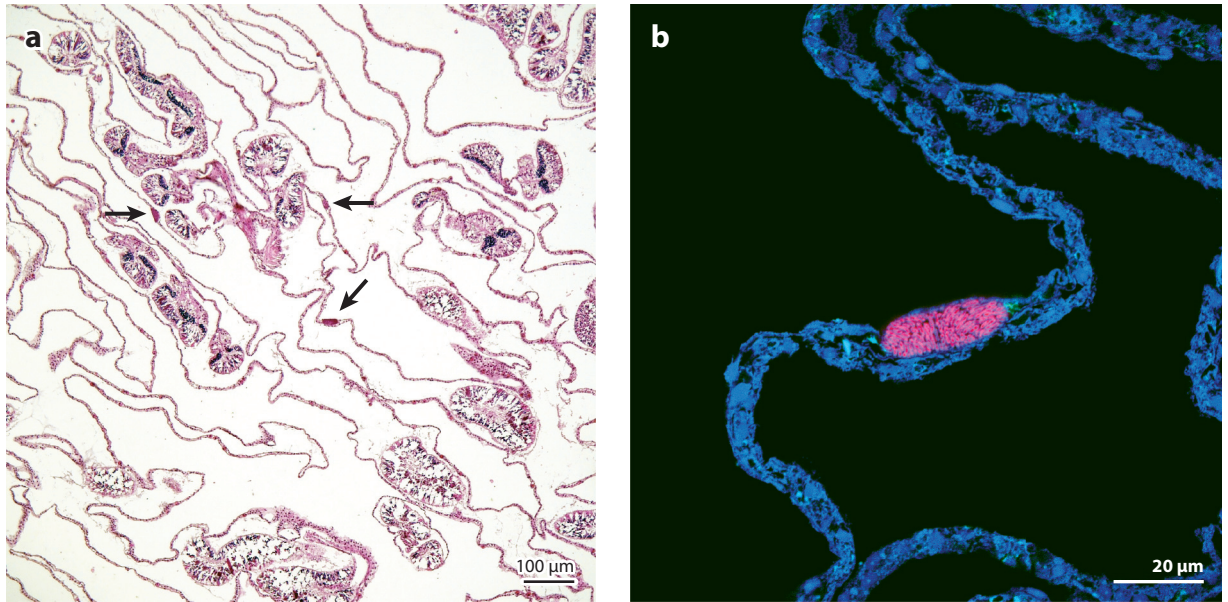
The coral SML is in part produced by endosymbiotic *Symbiodinium* spp. and is composed of glycoproteins containing enzyme-rich polypeptides and sulphated oligosaccharides attached via *O*-glycosidic linkages (19, 21). Enriched in nitrogen and organic matter, the SML supports a microbial community of  $10^6$ – $10^8$  cells per milliliter (46) and provides the coral with nutritional benefits (147), a protective barrier against pathogens through production of antibacterial compounds (112), and protection against solar radiation via production of ultraviolet light-absorbing pigments (110). Microbes within the mucus aid the coral through niche occupation and active exclusion of detrimental microbial communities. Importantly, however, pathogenic bacteria can be attracted to the SML via chemically induced chemotactic signaling, with the coral pathogen *Vibrio coralliilyticus* able to use chemotaxis and chemokinesis to target the SML of *Pocillopora damicornis* (47). Commensal and pathogenic bacteria can also use glycosidases and glucosaminidases to metabolize the SML glycoprotein, and interestingly, native coral microbiomes can inhibit glycosidase activity in the putative coral pathogen for white pox, *Serratia marcescens*, thereby inhibiting pathogen growth (64). Regular shedding of the SML is not only critical to coral reef nutrient cycling (46) but also vital for coral health, as it facilitates removal of sediments and opportunistic pathogens and replenishes the SML microbiome (19, 21).

### Coral Tissues

Unlike the dynamic microbial communities associated with the SML, microorganisms inhabiting the epithelium and gastrodermal tissues likely represent highly specific symbiotic associations (**Figure 1**). Although methodological limitations have hindered investigation of these tissue-associated microorganisms, visualization of coral tissue using transmission electron microscopy, light microscopy, and fluorescence in situ hybridization has revealed a generally low microbial abundance with cells clustered in coral-associated microbial aggregates (CAMAs) resembling bacteriocytes (99, 151) (**Figure 2**). These CAMAs have now been identified in healthy tissue of over 24 coral species spanning a wide geographic range (151). Microorganisms affiliated with *Ralstonia* (2), *Actinobacteria* (2), and *Endozoicomonas* species (11, 89) have been detected within coral tissue. *Actinobacteria* affiliated microorganisms were not only found in gastrodermal tissue layers but also observed within the endosymbiotic *Symbiodinium* cells potentially contributing to the metabolic repertoire of the photosymbionts (2). Cyst-like aggregations of *Endozoicomonas* cells have been observed at the interface of the epidermis and gastrodermis tissues of *Stylophora pistillata* across a wide biogeography (89), and evidence for their role in the core microbiome is further provided by the dominance of different *Endozoicomonas* ecotypes across a broad range of coral species (10, 86).

### Gastric Cavity

The coral gastric cavity is a primitive gastrovascular system used for digesting plankton and detritus, which can supplement nutrition obtained from *Symbiodinium*-derived photosynthates.



**Figure 2**

Visualization and localization of coral-associated microbial aggregates (CAMAs) within the gastrodermis tissues of healthy *Acropora hyacinthus* corals. (a) A hematoxylin and eosin-stained histological section of healthy tissue showing three CAMAs, marked by arrows. (b) Specific fluorescence in situ hybridization detection of bacteria within the CAMA using Cy3-labeled probes targeting bacteria (EUB-338).

Although coral heterotrophy has been widely reported, the processes involved in gastric digestion and the role of the gut microbiome are poorly understood (54). In *Galaxea fascicularis*, the gastric cavity maintains a chemical environment with extremely low oxygen concentrations and high concentrations of inorganic nutrients and vitamin B<sub>12</sub>, essential for both host and endosymbionts (1). Vitamin B<sub>12</sub> was present only at trace levels in the surrounding seawater, indicating it is synthesized by gastric cavity bacteria in the semiclosed gastrovascular system (1). As in higher organisms, the coral gastric microbiome appears to perform functions including catabolizing ingested organic matter and recycling nutrients. Corals are known to continuously transport organic mucus containing trapped particles into the gastric cavity, which would maintain the symbiotic associations of the gastrovascular microbiome (1, 21).

### Coral Skeleton

The large structural habitat of the calcium carbonate coral skeleton provides a unique habitat for an array of microorganisms including filamentous algae (120), bacteria (154), and fungi (14). In some instances, these endolithic microorganisms are thought to contribute to holobiont fitness. For instance, endolithic chlorophytes of the genus *Ostreobium* translocate photoassimilates to bleached coral tissues, which can sustain the coral holobiont during times of temperature-induced stress (42). However, in other instances, endolithic microorganisms are associated with diseased states such as the diverse assemblages of endolithic sulphate-reducing bacteria (SRB) that form black skeletal bands (154). Similarly, while fungi are believed to be important members of the coral endolithic assemblages when in a state of equilibrium, they can become detrimental in times of

**Holobiont fitness:** ability of the coral holobiont to survive and reproduce, passing on its genetic content to the next generation

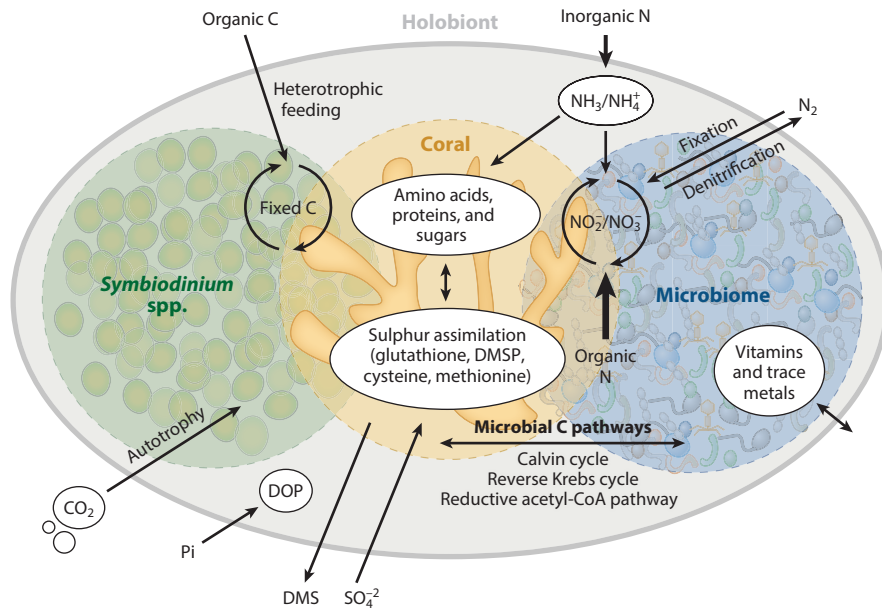
stress, penetrating the skeleton (euendolithic), parasitizing the algae, and exploiting mineralizing organic matter (50).

**Euendolithic:**

characteristic of an organism that exhibits boring activities when living within the skeleton of corals

**FUNCTIONAL ROLE OF MICROBES WITHIN THE CORAL HOLOBIONT**

*Symbiodinium* spp. translocate photosynthates and essential nutrients to the coral animal and in exchange are provided a niche habitat and return transfer of essential nutrients (87). The existence of this tripartite relationship between the coral, its symbiotic *Symbiodinium* spp., and the associated microbiome is well established (Figure 3), although the functional interactions and controlling mechanisms between any two components of the holobiont are only now being elucidated. Many of the purported interactions involve cycling of essential nutrients such as carbon, nitrogen, sulphur, and phosphate in addition to essential passage of trace metals, vitamins, and other cofactors. Although the proposed symbiotic functions are extensive, examples of specific microbial symbionts being unequivocally assigned functional roles are rare. Many coral microbial symbionts are so far recalcitrant to cultivation, and the lack of a tractable host cellular assay or axenic models for



**Figure 3**

Schematic illustration of the major nutrient pathways and functional interactions facilitated by the tripartite relationship between corals, *Symbiodinium* spp., and the associated microbiome. These interactions and metabolic pathways occur across all three components of the coral holobiont. For example, fixed carbon from autotrophic *Symbiodinium* spp. is passed to both the coral animal and the coral microbiome. Similarly, carbon derived from heterotrophic coral feeding can supplement *Symbiodinium* requirements and potentially be utilized by the microbiome. Heterotrophic feeding also contributes trace nutrients, vitamins, and metals to the holobiont, which may be further supplemented by microbiome production. Fixed nitrogen from diazotrophs contributes to satisfying the nitrogen requirements of *Symbiodinium* spp. DMSP produced by both the coral and the *Symbiodinium* spp. is potentially another major driver structuring the holobiont microbiome. Abbreviations: DMS, dimethylsulphide; DMSP, dimethylsulfoniopropionate; DOP, dissolved organic phosphorus; Pi, orthophosphate.



experimental manipulation is a major constraint to unequivocally linking microbial symbiont identity and function. Single-cell and meta-omic sequencing approaches need to be coupled with physiological experiments to provide greater insight into the metabolic pathways operating within the coral holobiont (**Figure 4**).

## Carbon Cycling

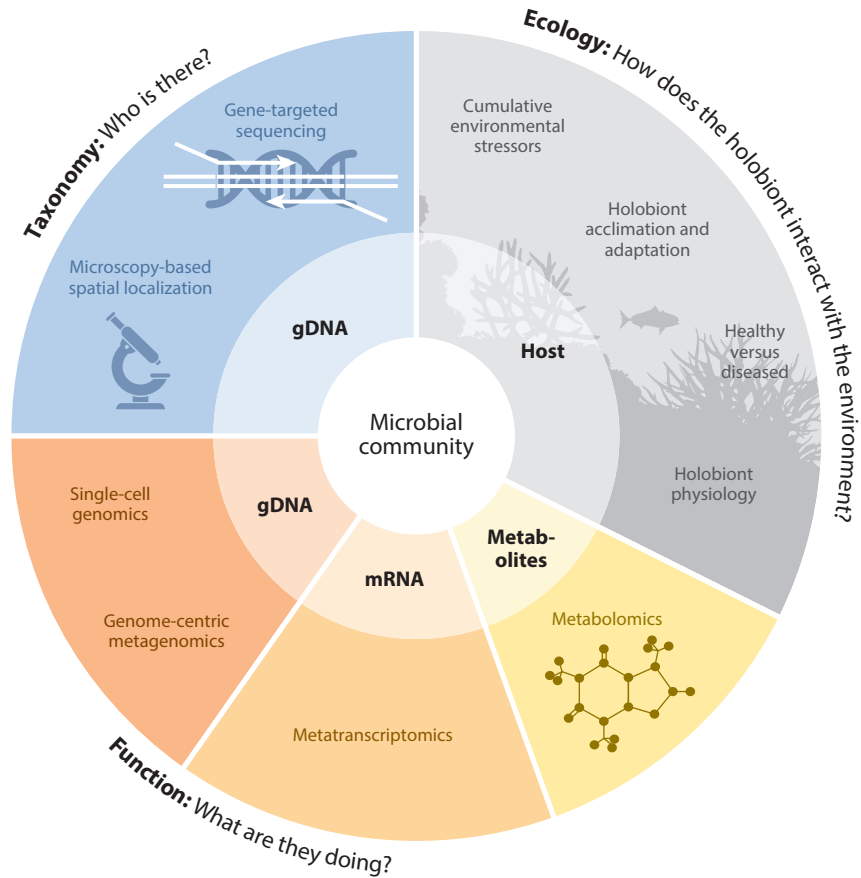
It is generally accepted that healthy scleractinian corals can acquire their carbon requirements, up to 60%, through heterotrophy via predation of zooplankton and/or up to 100% through photoautotrophy via photosynthetically fixed carbon translocated from *Symbiodinium* spp. (37, 96). Acquisition patterns can dramatically change during holobiont stress, such as coral bleaching (6). Recent functional gene analysis of coral microbiomes also identified carbon fixation (via the Calvin cycle, a reductive acetyl-CoA pathway, and the reverse Krebs cycle) and carbon degradation pathways of bacterial and archaeal origin (61), indicating that microorganisms transform and passage nutrients within the different components of the coral holobiont (**Figure 3**). Metagenomic analyses of coral microbiomes have also identified a large number of genes putatively involved in processing sugars and proteins along with their associated transporters (140). In particular, enrichment in carbohydrate utilization pathways in SML-associated microbiomes is consistent with the role of the SML in cilia-derived feeding (19). This process not only captures new carbon in the form of prey that can be digested through gut processing (including microbial metabolism) but also recycles a significant amount of energy expended on mucus production (up to 45% of fixed carbon) (19). Despite these insights, direct evidence supporting shared metabolic pathways within the holobiont is still limited, and reconstruction of microbial genomes is urgently required to better define symbiont physiologies and identify codependency of key carbon metabolisms.

Importantly, nutrient interactions within the holobiont can dramatically change during periods of stress, with coral microbiomes at elevated temperatures showing an increase in genes associated with metabolism of fatty acids, protein, and carbohydrates (72, 129). This is likely due to a stress-induced shift in microbiome composition (17) toward an opportunistic community that takes advantage of the destabilization in carbon utilization pathways. Host production of mucus is also influenced by environmental factors that can further shift the balance of carbon cycling within the holobiont. For example, mucus production increases under moderate temperature stress, likely buffering the holobiont and moderating homeostasis (101). However, under extreme temperature stress, corals experience bleaching and destabilization of the holobiont, which is further compounded by compositional changes in mucus structure and depletion of reserves of mucus, which directly affects carbon cycling (44, 150).

## Nitrogen Cycling

Nitrogen is limited in coral reef ecosystems (37), and corals have therefore evolved a number of strategies to derive nitrogen from organic (e.g., heterotrophic feeding) and inorganic sources. For instance, *Symbiodinium* spp. possess RUBISCO and nitrate reductase enzymes enabling them to convert inorganic substrates into organic forms of available nitrogen (153). While nitrogen supplementation is required to sustain coral growth (38), excessively high concentrations can also destabilize the coral-*Symbiodinium* symbiosis (146). Coral-associated microorganisms therefore undertake nitrogen regulation within the holobiont via processes such as nitrogen fixation, nitrification, and denitrification (22, 23, 61, 69, 70, 105, 122, 140) (**Figure 3**).

Diazotrophs, defined as bacteria and archaea capable of fixing nitrogen, can additionally supplement the coral holobiont with nitrogen in the form of ammonia (NH<sub>3</sub>). However, nitrogen



**Figure 4**

Multidisciplinary approaches for studying different aspects of the coral holobiont. (*Center*) The microbial community is obtained from different coral microhabitats (surface mucopolysaccharide layer, tissues, gastric cavity, and skeleton), each with a distinct microbiome composition and a unique set of environmental conditions. The derived DNA, RNA, proteins, and metabolites can be isolated and studied with meta-omic analyses. (*Gray*) The coral holobiont interacts with a range of different, often concurrent, environmental stressors (e.g., temperature, pH, sedimentation, competition) that directly affect the physiology of the coral host and influence the composition and function of the associated coral microbiomes. These environmental conditions influence health outcomes (e.g., disease states) and may drive holobiont physiology, acclimation, and adaptation. (*Blue*) Community-profiling approaches that target DNA gene markers (e.g., 16S rRNA, 18S rRNA, and *nifH*) facilitate the reconstruction of microbial phylogenies and can reveal the relative abundance of individual taxa. Microscopy approaches such as fluorescence in situ hybridization and transmission electron microscopy can spatially locate bacteria and viruses within the holobiont microstructure. (*Orange, yellow*) Genomic sequencing of single microbial cells provides insights into the genetic repertoire of specific symbionts or pathogens of interest. Genome-centric sequencing provides information on particular functional clades within the holobiont, and metagenomic sequencing combined with binning and genome reconstruction can reveal the functional potential of entire holobiont communities. Metagenomic sequencing has proven to be challenging for corals, owing to the complexity of genetic material supported by the holobiont. Metatranscriptomics and metabolomics provide further information on microbial activity within the holobiont. Together, these approaches can be used to construct interaction networks among taxa, genes, metabolites, or proteins to provide a comprehensive understanding of the coral holobiont and the central role of microorganisms in coral health. Abbreviation: gDNA, genomic DNA.

fixation requires the nitrogenase enzyme with most of the enzyme complexes irreversibly inactivated by oxygen; hence, unique biochemical and physiological mechanisms that segregate aerobic and anaerobic conditions within the holobiont are required. Diverse diazotroph assemblages, particularly within the *Rhizobiales*, occur in both adult and larval coral life stages, likely forming part of the core coral microbiome (67, 69, 119). Endosymbiotic cyanobacteria related to *Synechococcus* spp. and *Prochlorococcus* spp. also form part of coral microbiomes (71) with the products of their N<sub>2</sub> fixation taken up by the *Symbiodinium* hosts (43, 70). Diazotroph-mediated nitrogen fixation can provide ~11% of the *Symbiodinium* nitrogen requirements within the coral holobiont, sustaining productivity when external sources of nitrogen are scarce (22).

Nitrification is conducted by nitrifying bacteria and archaea that oxidize NH<sub>3</sub> into nitrite (NO<sub>2</sub><sup>-</sup>) and NO<sub>2</sub><sup>-</sup> into nitrate (NO<sub>3</sub><sup>-</sup>) under aerobic conditions, using autotrophy to acquire carbon. Although nitrifying bacteria and archaea are abundant associates of corals (61, 122, 152), their role in holobiont function is largely undescribed. *Symbiodinium* spp. prefer nitrogen in the form of ammonium; hence, the conversion of nitrogen into less-appetizing by-products through nitrification may play a critical role in regulating *Symbiodinium* populations within the holobiont (105). In fact, a low concentration of dissolved inorganic nitrogen in coral tissue has been hypothesized to control *Symbiodinium* growth rates (37), and coral-associated bacteria can compete effectively with *Symbiodinium* spp. for host-generated ammonia (105). Denitrifying and ammonia-oxidizing microorganisms have also been detected in coral microbiomes (152) and are thought to remove nitrogen from the coral host, through the coupling of nitrification and denitrification (122).

A combination of sequencing-based studies and powerful imaging approaches such as nanoscale secondary ion mass spectrometry (nanoSIMS) is also starting to provide valuable insights into shared nitrogen cycling pathways in coral holobionts (23, 68, 97). For example, ammonium assimilation from <sup>15</sup>N-labeled ammonium chloride (<sup>15</sup>NH<sub>4</sub>Cl) has been observed in cells of both the coral host and its associated *Symbiodinium* spp. (98), and a potential role of bacteria in nitrogen acquisition for coral larvae has been revealed (23, 68).

## Sulphur Cycling

Sulphur is essential for all life, being a key constituent in proteins, coenzymes and metalloproteins, while also being assimilated (mainly through dissolved sulphate) into organic sulphur-containing molecules (123). In the coral holobiont, a large component of the sulphur pool is assimilated into cysteine and methionine and subsequently converted to the highly stable and soluble dimethylsulfoniopropionate (DMSP) (126, 133). DMSP is a vital link in the transfer of sulphur from the oceans to the atmosphere via its breakdown to dimethylsulphide (DMS), which delivers biogenically derived sulphate aerosols into the marine boundary layer (123). Corals are one of the largest producers of DMSP in the marine environment primarily owing to the high concentration of *Symbiodinium* spp. within the coral tissues (133), although recent research has demonstrated that the coral animal can also synthesize this compound (107). DMSP can function as an osmolyte and/or cryoprotectant (126), and its breakdown products, acrylate, DMS, and dimethylsulfoxide (DMSO), have potential functions in the stress responses owing to their antioxidant properties (30, 108, 107).

Coral-derived DMSP is an abundant organic carbon source for microorganisms and likely contributes to the structuring of associations within the holobiont. For example, a metasurvey of coral-associated microorganisms identified a number of bacteria capable of DMSP metabolism, including the putative core symbiont *Endozoicomonas* spp. (106), although not all ribotypes appear to have this potential (90). Uptake of <sup>34</sup>S by *Symbiodinium* spp. as inorganic sulphate, biosynthesis

and exudation of DMSP, and its subsequent uptake and degradation by bacteria were recently confirmed in nanoSIMS experiments, highlighting the close interactions between different members of the coral holobiont and confirming bacterial derivation of substrates directly from *Symbiodinium* spp. It is possible that by-products of bacterial metabolism are similarly passed back to the algal partner, as recently demonstrated for other marine algal/bacterial interactions (4, 34). Within the coral SML, DMSP likely mediates the interplay of corals and their associated microbes. For example, putatively symbiotic bacteria such as *Rhizobiales* and *Roseobacter* spp. display chemotaxis toward DMSP (117) and are known to associate with corals during early life stages (67, 119). Thus, the production of DMSP in coral larvae may play a role in mediating initial acquisition of their bacterial symbionts. Similarly, DMSP may act as a chemoattractant for coral pathogens, as evidenced by the coral pathogen *Vibrio coralliilyticus*, displaying strong chemotaxis toward this compound (47). However, the complex role of DMSP in the coral holobiont is further highlighted by the recent observation that a common coral-associated bacterium, *Pseudovibrio* sp. P12, is able to produce the antimicrobial sulphur-containing compound tropodithietic acid (TDA), likely derived from DMSP catabolism, indicating the potential of DMSP-metabolizing microbes to contribute to coral disease prevention (108).

Within the coral holobiont, glutathione may be used as an additional or alternative source of organic sulphur (140), and inorganic sulphur can also be cycled via sulphate-reducing microorganisms (61). In particular, sulphate-reducing bacteria (SRB) are involved in anaerobic respiration, oxidizing organic compounds or molecular hydrogen while reducing sulphate to hydrogen sulphide, although some SRB also play a role in anaerobic methane oxidation (88). Endolithic SRB have been observed within healthy corals (154) and disease lesions such as black band disease, where they contribute to toxic microenvironments (reviewed in 116). However, despite the genomic potential for sulphate reduction, empirical evidence demonstrating activity of SRB within the holobiont is still lacking. Genomic potential for anaerobic processes involving methane, hydrogen sulphide, or other organic compounds highlights the need to better define spatial differentiation of these functions.

### Other Key Nutrient Pathways

Phosphorus is another element essential for life and a key determinant of marine productivity, linked closely with carbon and nitrogen cycling (35). The most bioavailable form of phosphorus is orthophosphate (Pi), though concentrations can be very low on oligotrophic coral reefs. Interestingly, a recently described function for sponge symbionts is the microbial production and storage of polyphosphate granules, which may protect the sponge holobiont against periods of phosphate deprivation (157), though it is still unknown whether a similar mechanism exists in corals. Dissolved organic phosphorus and in particular phosphonate-P may be recycled by coral-associated microorganisms to supply phosphorus to the coral holobiont. In particular, coral-associated *Vibrio* spp. are known to contain phosphonate-degrading gene pathways (48), and an increased abundance of these microorganisms in bleached corals has been linked to the increased bioavailability of phosphonate-P, which is likely due to the breakdown of membrane lipids (17). Excessive nitrogen in the holobiont can also result in phosphate starvation and further increase the susceptibility of corals to bleaching caused by light- and heat-induced environmental stress (146).

To maintain holobiont fitness, essential nutrients, cofactors, and metals must be either acquired from the environment or derived from associated microorganisms. A mixed heterotrophic feeding strategy likely enables the coral to acquire many of these trace elements, including essential polyunsaturated fatty acids (PUFAs), sterols, and cholesterol (100) as well as metal cofactors such as zinc (41) directly from their food. In addition, photoheterotrophy from *Symbiodinium* spp.

contributes to the accumulation, regulation, and passage of PUFAs, lipids, sterols, and trace metals within the holobiont. Corals may also regulate their microbial communities to derive essential vitamins such as B<sub>12</sub> (1). As corals are unable to synthesize these essential vitamins, microbially mediated synthesis likely places a selective advantage on their maintenance, thereby contributing to stability of the holobiont.

## IMPACT OF ENVIRONMENTAL STRESS ON THE CORAL HOLOBIONT

### Thermal Stress

The thermal sensitivity of the coral holobiont is well established, with expulsion of *Symbiodinium* spp. at higher temperatures causing widespread coral bleaching and extended immune system suppression (6, 102). The ecological and physiological impacts of coral bleaching have been extensively studied and are reviewed elsewhere (18). However, in a pioneering study of coral-bacteria interactions, Rosenberg and colleagues demonstrated that *Vibrio shiloi* infection of *Oculina patagonica* caused seasonal coral bleaching (reviewed in 114). This finding precipitated experimental research, field studies, and coral microbiome meta-analyses, all of which demonstrated the sensitivity of coral-associated bacterial communities to elevated seawater temperature (17, 72, 86, 127, 129). Most studies report that destabilization of the coral holobiont due to thermal stress is concomitant with a microbial community shift toward opportunistic microorganisms and/or potential pathogens, such as *Vibrio* spp. (72, 112, 129), although mechanistic studies attributing cause and effect are often lacking. Lysis of *Symbiodinium* spp. by proviruses has also been suggested as an alternative cause of coral bleaching in some instances, although this has yet to be experimentally confirmed (134, 144). Defining the role and contribution of the coral microbiome and virome to bleaching and disease is an urgent priority for the field.

### Ocean Acidification

The impact of ocean acidification on calcifying corals has been extensively investigated; however, its effects on host-associated microbiomes are relatively underexplored (reviewed in 94). Studies on pelagic microbes suggest that global biogeochemical cycles will be fundamentally altered by ocean acidification, including increased primary production and nitrogen fixation in some scenarios (74). However, the functional mechanisms driving these changes remain uncertain, and microbial adaptation to ocean acidification may influence the predicted biogeochemical effects (58). Microbiome studies exposing corals to conditions of ocean acidification have reported increases in virulence-associated genes (129) and shifts in the community composition, including increases in opportunistic pathogens such as *Vibrionaceae* and *Alteromonadaceae* (80) and a loss of putatively symbiotic *Endozoicomonas* spp. (84). However, not all species are equally sensitive to ocean acidification. Recent experimental research demonstrated that *Acropora millepora* and *Seriatopora hystrix* can maintain stable microbial communities at elevated pCO<sub>2</sub>, although it was noteworthy that *Endozoicomonas* was particularly sensitive to reduced pH (138). Interestingly, some reef sponges are known to increase their cyanobacterial photosymbionts, which may give them a competitive advantage over corals under future ocean acidification scenarios (13, 84).

### Sedimentation and Nutrients

Changing land practices that increase runoff, sediments, and nutrients play a major role in compromising reef health (32, 33, 36). Sediment accumulation, especially organic-rich sediments, can suffocate coral tissues and allow rapid growth of heterotrophic microorganisms that contribute to the formation of anoxic zones and high concentrations of toxic sulphide, likely through the activity

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**Provirus:** a virus genome integrated into the DNA of a host cell (in bacteria, the prophage form of a bacteriophage)

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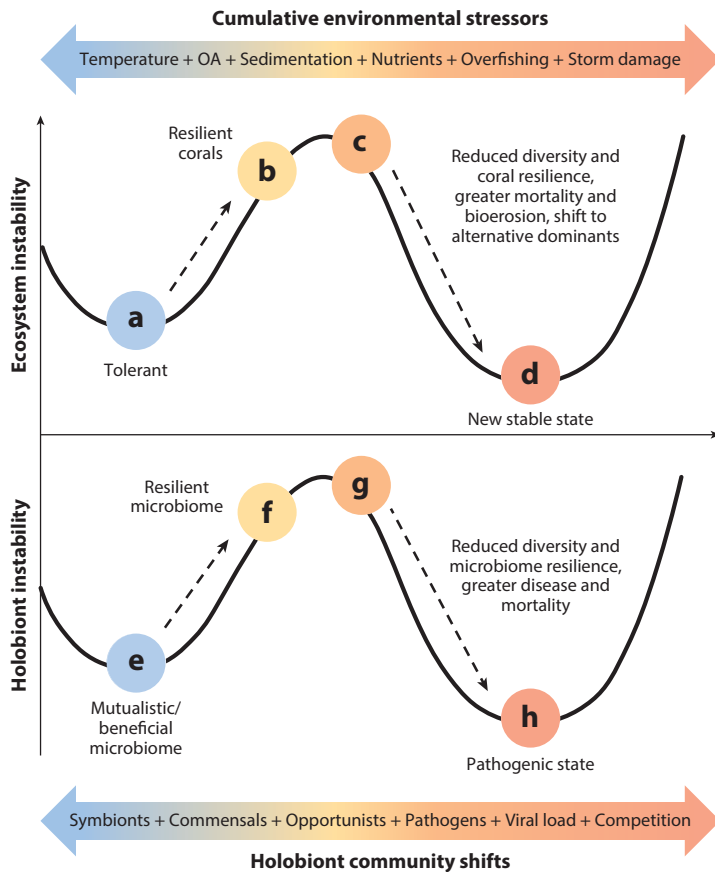
**Equilibrium of coral holobiont:** ability of the coral holobiont to regulate variables so that internal conditions remain stable

of SRB (137). Sediment plumes from dredging activity (104) have been implicated in increased coral disease, and although the cause-effect pathway for this phenomenon has not yet been defined, mechanisms are likely to include compromised immune function, declines in energy reserves due to light attenuation, and shifts in bacterial and viral communities. Dissolved organic carbon associated with elevated sediment loads can accelerate the growth of microbes within the coral SML and disrupt the microbiome composition, eventually leading to coral mortality (62). Release of dissolved nutrients by algae can also stimulate microbial activity (8, 124), and chemical toxicity from algal exudates can affect coral-associated microbial communities (85, 109) and promote the invasion of opportunistic pathogens and coral mortality (7). In particular, a dissolved organic matter (DOM) feedback mechanism involving reciprocal microbial and macroalgal growth is thought to shift the microbial equilibrium of the coral holobiont, resulting in declines in coral fitness (7, 8).

### Cumulative Impacts and Alternative Holobiont States

Cumulative coral reef stress occurs when one or more pressures and/or their interactions sum or multiply over time and/or in space. Microorganisms rapidly respond to changing environmental conditions and can therefore mitigate or exacerbate host and/or ecosystem resilience. Reef microbiomes clearly reflect human impacts associated with land use and fishing, with higher cumulative impacts driving microbial communities toward heterotrophy (32, 60). For instance, nutrient addition and overfishing reduce the abundance of herbivores and contribute to increased algal growth (33, 115). Algal exudates are rich in dissolved sugars, which can rapidly stimulate microbial growth, resulting in localized hypoxia and a community dominated by opportunistic microorganisms, including putative pathogens (91). A three-year field study showed that overfishing and nutrient pollution destabilized the coral microbiome, elevated putative pathogen load, increased disease prevalence, and resulted in higher coral mortality, with these effects further exacerbated by the cumulative stress of higher seawater temperatures (156). Ecosystem microbialization scores were recently proposed to measure these impacts (52, 78), and initial analyses suggest that human impacts are altering reef energy budgets by shifting the allocation of metabolic energy from macroorganisms to microorganisms (32, 53, 78). Such shifts were reported across 60 reefs from three ocean basins, with turf and macroalgae-dominated reefs exhibiting higher abundances of copiotrophic organisms, which reduced the availability of energy to higher trophic levels through inefficient remineralizing of the available organic nutrients (52). Correlation of community metabolic potential with geographic location also indicates some adaptation of microbiomes, with functional capacity seemingly driven by local biogeochemistry (60).

It is well established that cumulative disturbance events may shift ecosystems with low resilience toward an alternative stable state, from which it may be difficult to return (118) (**Figure 5**). However, environmentally induced changes in the microbiome may enable adaptation of the coral holobiont (16, 111), ultimately influencing coral reef ecosystem resilience. Many studies have documented environmental stress-associated shifts in the coral microbiome, and in at least some instances, the coral microbiome has been shown to return to its original healthy state after the stressor is removed (17). Based on these and other well-studied holobiont models (e.g., hydra, squid, termites, plants, rhizospheres, sponges), the coral microbiome should be considered a symbiotic continuum with variable levels of stable equilibrium. Environmental drivers can cause shifts in symbiosis from healthy mutualistic relationships to those that are more pathogenic and detrimental to the host (**Figure 5**). Some host-microbiome communities may be more stable than others, owing to factors such as higher diversity and functional redundancy, and these organisms are likely to prevail, influencing overall coral ecosystem structure and function.



**Figure 5**

(*Top*) Cumulative stressors can shift the stable equilibrium of reef benthos from a coral-dominated community to reefs dominated by organisms such as macroalgae or sponges. (a) Cumulative stressors (e.g., elevated sea surface temperature, ocean acidification, sediments, nutrients, overfishing, storm damage) initially select for resilient coral species. If stressors are below a critical threshold (intensity or duration), it is possible for an ecosystem to recover and return to the original stable community state. (b,c) However, once a tipping point is reached, there is (d) reduced species diversity, less resilience, and higher mortality, and potentially shifts to alternative dominant species. (*Bottom*) The concept of reef resilience applies equally to the coral microbiome. (e) Under low pressure, the microbiome can buffer environmental change. (f,g) However, with increasing pressure, the initially stable microbial community shifts from mutualism to pathogenesis. Once this tipping point is reached, (h) destabilization of the holobiont drives further changes in the microbiome, manifesting in coral disease, reduced fitness, and mortality.

### SUMMARY POINTS

1. Corals harbor a highly diverse microbial community with species-specific core members that are spatially partitioned across coral microhabitats.
2. The functional contribution of the coral microbiome to holobiont fitness is poorly understood but appears to be intricately linked to the passage and cycling of essential nutrients, including carbon, nitrogen, sulphur, phosphorus, and vitamins.

3. Environmental stress can alter the coral microbiome, disrupting the holobiont equilibrium, shifting defensive mechanisms and nutrient cycling pathways that contribute to bleaching and disease susceptibility.
4. Coral reef microbiomes are central to reef resilience, as they can buffer or exacerbate cumulative impacts via their role in holobiont fitness as well as by modifying energy flow within the ecosystem.
5. Combining (meta)genomic/transcriptomic sequencing with advanced imaging and analytical techniques will provide unique insights into the location, diversity, and physiology of microorganisms within the coral holobiont (**Figure 4**).
6. Large-scale community sequencing initiatives targeting the host, *Symbiodinium* spp., and the microbiome will provide unprecedented insights into shared metabolic pathways, co-evolution and the genomic interplay between the various components of the holobiont, the molecular mechanisms underpinning the establishment and maintenance of the holobiont, and the potential role of the microbiome in holobiont acclimation/adaptation to environmental change (**Figure 4**).

## FUTURE ISSUES

1. Establishing long-term coral microbiome baselines: Lack of datasets describing long-term coral-microbe dynamics significantly hinders our ability to rapidly predict how environmental change will affect the coral holobiont. Establishing coral microbiome baselines at key global sites would enable the identification of microbial indicators (species or functions) of environmental perturbation and/or coral health. Sensitive microbial indicators derived from these baselines could be incorporated into reef-monitoring programs to provide an early warning system for declines in coral health that enable early management interventions.
2. Defining mechanisms that underpin the maintenance of holobiont interactions: While little is known about how the coral holobiont is established and maintained, a number of potential molecular mechanisms for host-symbiont interactions have been discovered in other reef invertebrates, ranging from quorum sensing (155) to symbiont proteins containing eukaryotic domains (40, 92). Domains found in the eukaryotic-like proteins mediate protein-protein interactions for a suite of biological processes, including those that are responsible for establishing an intracellular lifestyle. Insights into the molecular mechanisms mediating coral-microbe interactions will likely be achieved by current and future single-cell and (meta)genomic/transcriptomic sequencing efforts.
3. Determining the extent of functional equivalence and functional redundancy in coral microbiomes: The extent of functional redundancy within a microbiome may influence holobiont vulnerability to environmental change. Basal reef species such as sponges host phylogenetically distinct microbiomes that share core symbiotic functions (40). These core microbial functions are provided by analogous enzymes and biosynthetic pathways, with symbiont communities in divergent hosts evolving different genomic solutions to undertake the same function or to occupy the same niche (40). While 16S rRNA surveys have confirmed that different coral species also host phylogenetically distinct microbial



populations, our understanding of functional equivalence and functional redundancy in coral microbiomes is currently hindered by a lack of adequate genomic datasets.

4. Defining the extent of coevolution in coral holobionts: Little is known about the degree to which closely or distantly related coral species share symbiont communities. Signals of codivergence should be evident if corals rely predominantly on vertical transmission (although it is important to note that greater microbiome similarity between closely related host species does not necessarily imply that hosts and symbionts have coevolved; 83). Insights into coevolution will be achieved by large-scale community-sequencing efforts targeting the genomes of all holobiont members as well as research that better defines symbiont transmission strategies.
5. Combining molecular sequence data with experimental research: While greater genomic resources will undoubtedly yield unique insights into the likely molecular pathways employed by both host and symbionts, it is essential for coral holobiont researchers to rapidly move from describing functional potential to conducting hypothesis-driven experimental research that can confirm putative symbiont physiologies and determine shared metabolic pathways in coral holobionts.
6. Defining how host innate immunity influences the coral microbiome: Insights into the coral immune system have resulted from comparison of coral genome sequence data with other model invertebrates (45, 121). However, while it is clear that the coral immune system plays an important role in maintaining symbionts and eliminating potential pathogens, our understanding of the interplay between host immunity and the microbiome under different environments is limited. The immune system is sensitive to environmental change, and experimental studies with holobiont sequencing that take host physiology into account are required to reveal drivers of compromised health.
7. Epigenetic mechanisms for coral adaptation: Increased thermal tolerance observed in several bleaching-sensitive coral taxa has provided evidence that at least some coral species may be able to acclimatize or adapt to climate change (51). While current estimates suggest that the rate of climate change is outpacing corals' capacity to evolve, research has demonstrated that nongenetic responses (epigenetic mechanisms that modify regulation of gene transcription, such as DNA methylation and histone modifications) can sometimes be passed on from one generation to the next (31) and even potentially drive rapid adaptation (27). The role of epigenetic modifications in the maintenance of coral holobionts and the adaptation of corals to environmental change is a priority for future research.
8. Evaluating the extent to which coral microbiomes enhance environmental adaptation of the holobiont: Environmentally induced fluctuations in the microbiome can have significant functional consequences for the holobiont phenotype, upon which selection can likely act (16). For instance, environmentally induced changes in microbial abundance and community shifts within the coral holobiont may be akin to gene duplication events, thereby introducing or removing raw genetic material into/from the coral hologenome. Random nucleotide changes, recombination events, gene duplications and/or losses, and horizontal gene transfer are all potential mechanisms that could produce genetic variation in the coral holobiont, although there is currently limited evidence for horizontal gene transfer events in available coral genome sequence data.

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**Coral hologenome:**

the complete genetic content of the coral holobiont: host nuclear genome, *Symbiodinium* genomes, and genomes of all microbiome members

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## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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