

Minireview

New directions in coral reef microbial ecology

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Summary

Microbial processes largely control the health and resilience of coral reef ecosystems, and new technologies have led to an exciting wave of discovery regarding the mechanisms by which microbial communities support the functioning of these incredibly diverse and valuable systems. There are three questions at the forefront of discovery: What mechanisms underlie coral reef health and resilience? How do environmental and anthropogenic pressures affect ecosystem function? What is the ecology of microbial diseases of corals? The goal is to understand the functioning of coral reefs as integrated systems from microbes and molecules to regional and ocean-basin scale ecosystems to enable accurate predictions of resilience and responses to perturbations such as climate change and eutrophication. This review outlines recent discoveries regarding the microbial ecology of different microenvironments within coral ecosystems, and highlights research directions that take advantage of new technologies to build a quantitative and mechanistic understanding of how coral health is connected through microbial processes to its surrounding environment. The time is ripe for natural resource managers and microbial ecologists to work together to create an integrated understanding of coral reef functioning. In the context of long-term survival and conservation of reefs, the need for this work is immediate.

Introduction

A healthy coral reef functions as a finely tuned microbially driven system that excels at capturing and recycling

nutrients in oligotrophic waters and its intricate three-dimensional structure allows for niche partitioning that supports astounding biodiversity and productivity. Individual reef-building corals embody this paradigm—animals living symbiotically with algae, viruses, bacteria, archaea and protists distributed in spatially distinct patterns to function as holobionts (Knowlton and Rohwer, 2003; Ainsworth *et al.*, 2010). The great complexity of coral reef microbial ecology has until recently resisted functional, mechanistic and system-based analyses (Wild *et al.*, 2011). Novel molecular, biochemical and imaging advances have opened up the field and promise exciting fundamental discoveries. The time is ripe to deepen our understanding of these ‘captive’ systems in intimate contact with the pelagic realm of the sea, and discover the mechanisms by which they exist as an integrated, microbially supported system within the surrounding water and sediment environments.

There are three overarching questions currently at the forefront of discovery in coral reef microbiology. What are the mechanisms underlying coral reef health and resilience? How do environmental and anthropogenic pressures affect reef ecosystem function? What is the ecology of microbial diseases of corals? The rapid decline of coral reefs globally creates a pressing need to answer these questions (Hughes *et al.*, 2010). The goal is to understand the functioning of coral reefs as integrated systems from microbes and molecules to regional and ocean-basin scale ecosystems to enable accurate predictions of ecosystem resilience and responses to environmental perturbations such as climate change and eutrophication.

The sediment, coral and water environments of a reef are often studied in isolation of each other. A key component for answering all three of the driving questions is to uncover and quantify the mechanisms that tie these environments together into a single functioning ecosystem. For instance, we know that reef sediments generally have 10 000 times more bacteria than the surrounding seawaters ($\sim 10^9$ cells cm^{-3} sediment versus $\sim 10^5$ cells ml^{-1} seawater), and that coral-associated bacterial abundances are reported to range widely from approximately 1×10^2 to 6×10^7 cells per cm^2 (Coffroth, 1990; Koren and Rosenberg, 2006). However, the mechanisms within these different niches by which bacterial growth rates or

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community composition, for example, may aid in maintaining the stability and health of the coral holobiont have not yet been explored. Fortunately, new tools are now available that allow for a quantitative microbial ecology approach to discovering the mechanisms underlying these interactions and determining their relative roles in ecosystem function. The goal of this review is to outline recent discoveries in microbial ecology of the different environments within coral reef ecosystems, and to highlight research directions that, with the help of coral reef managers, will build a quantitative and mechanistic understanding of how holobiont health and resilience is connected to the microbial ecology of the environment in which it lives.

The coral holobiont

Reef-building corals are covered in a surface mucus layer composed of proteins, lipids, and polysaccharides (Brown and Bythell, 2005; Tremblay *et al.*, 2011). Glycoproteins, in particular, are likely to be the mucus constituent responsible for its gelling properties and function (Jatkar *et al.*, 2010). The microbial communities inhabiting this surface region and the coral tissue below it have been studied more than any other bacterial niche of the reef environment. These communities are thought to be an important component of a coral's ability to adapt to environmental change (Reshef *et al.*, 2006), and perhaps to function as a single unit together with the coral animal and its other symbionts on which evolutionary selection acts (Rosenberg *et al.*, 2007). Two recent articles (Ainsworth *et al.*, 2010; Mouchka *et al.*, 2010) offer an overview of the immense diversity and the specificity of coral–bacteria associations, the onset of these associations (also see Sharp *et al.*, 2010), and many of the roles we currently understand microbes to play in coral health. These roles include subsidizing the host nutrient budget through nitrogen, carbon, and sulfur cycling, and assisting in disease resistance through the secretion of antimicrobial compounds to exclude colonization by exogenous bacteria. Additionally, Bourne and colleagues recently reviewed much of the research on coral diseases, and showed that while the ecology of a handful of pathogens has been elucidated, the study of most coral diseases will benefit from an integrated understanding of coral reef microbial ecology and access to new technologies and concepts from the biomedical field (Bourne *et al.*, 2009, also see Mao-Jones *et al.*, 2010). Thus, we will focus our discussion of the holobiont on discoveries made since the publication of those articles.

A number of recent advances have been made by applying the most current technologies available to the study of the holobiont. This exciting progress has opened new doors for mechanistically linking microbial

ecology interactions within the holobiont to changes in the surrounding environments. For instance, pyrosequencing has enabled the discovery of 17 metalloprotease genes in the genome of the coral pathogen *Vibrio coralliititicus* that are potentially involved in its virulence (de O Santos *et al.*, 2011), as well as substantially higher levels of diversity (Shannon Index of 6.71) and seasonal variability in bacterial community composition associated with a scleractinian coral than previously recognized (Chen *et al.*, 2011). Further, reef-associated algae have been identified as potential reservoirs of coral disease-associated bacteria (Barott *et al.*, 2011). Ultra-performance liquid chromatography – tandem quad mass spectrometry (UPLC-MS/MS) was recently used to identify different responses in cyanotoxin production by several strains of black band disease (BBD)-associated cyanobacteria, often found to co-occur in an infection, to variations in environmental conditions (Stanic *et al.*, 2011). Quantitative and real-time polymerase chain reaction (qPCR and RT-PCR) techniques have also been applied to the coral holobiont to elucidate functional changes in the microbial members of the community over the course of disease progression (Bourne *et al.*, 2011). Uncovering the mechanisms of reef function with the aid of these new technologies is becoming ever more accessible, and the insights gained are now recognized as necessary for understanding the resilience of the ecosystems and developing effective conservation strategies. One fundamental research objective is to use these new technologies to spatially resolve where different microbial processes are taking place within the holobiont and what mechanisms mediate these processes. We know that bacteria can have spatially defined distributions within the holobiont (e.g. skeleton, gastrodermis, epidermis and surface mucus layer), but we know very little about how that distribution affects their function and their interactions with other members of the holobiont.

Mucus as a connection and source of structure

Within the holobiont, the mucus itself is an important energetic and ecological link between the coral animal and the surrounding water and sediment environments in which it lives (Rasheed *et al.*, 2004; Wild *et al.*, 2004a,b; 2005; Naumann *et al.*, 2009; Mayer and Wild, 2010). Mucus can play a role in structuring the coral-associated microbial communities while attached to the coral (Ritchie, 2006), and once dissociated from the coral, can select for otherwise rare microbial members of the water community (Allers *et al.*, 2008). Microbial ecologists have proposed consideration of the marine environment as a size continuum of organic matter, ranging from truly dissolved organic molecules to large particles of detritus visible to

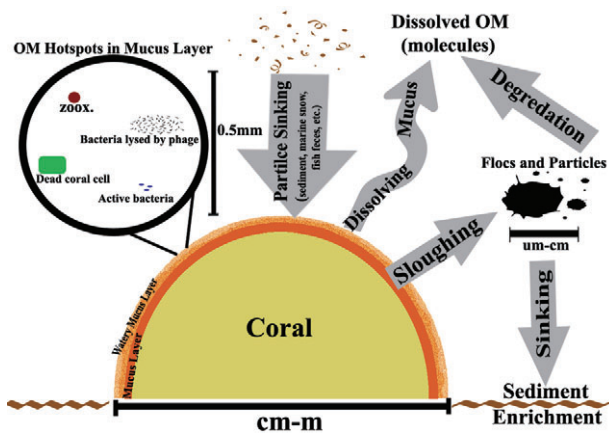


Fig. 1. Coral in the context of the marine organic matter continuum (of both size and phase).

the naked eye (Azam and Malfatti, 2007). This concept can be applied to the corals themselves, which might be envisioned as essentially a heterogenous organic matter continuum extending through the organic matter mucus into the environment (Fig. 1).

This framework allows the path of mucus to be followed as it morphs through a variety of microscale microbial habitats embedded in the organic matter continuum, connecting the microbial communities of the holobiont to those in the surrounding seawater and sediments, and providing a variety of physical architectures for microbial interaction. For instance, as mucus sloughs off from the coral into the surrounding water it can dissolve (van Duyl and Gast, 2001) (potentially fuelling water column microbial production), floc (to be eaten by larger detritivores or degraded by microbes), or sink (to be consumed by sediment microbial communities) (Vacelet and Thomassin, 1991; Huettel *et al.*, 2006; Wild *et al.* 2006; Naumann *et al.*, 2009; Mayer and Wild, 2010) (Fig. 2). In the context of studying the mechanisms of health and resilience of coral reefs, quantifying the magnitude and variability of these fluxes, and how they respond to perturbation is a major research subject. Clarifying the role of microscale architecture in these mechanisms is also important: if the spatial distribution of organic matter in the water column changes, would the distribution of coral-associated microbiota also change?

Coral reef waters

Researchers have explored the microbial processes occurring in a variety of reef water habitats – everywhere from remote atoll lagoons (Sorokin, 1978; Yoshinaga *et al.*, 1991; Torreton and Dufour, 1996a,b; Torreton *et al.*, 2000; Rochelle-Newall *et al.*, 2008) to the water overlaying heavily anthropogenically influenced coastal reefs

(Gast *et al.*, 1999; van Duyl *et al.*, 2002; Garren *et al.*, 2008; Hoch *et al.*, 2008). A common theme that appears across these diverse habitats is that the benthos influences the water column environment, and it does so at several different scales.

At the scale of individual colonies, a coral influences the niche structure for microbes at the millimetre to centimetre scale. Corals can release large quantities of dissolved organic carbon [DOC; 2–25 μM (mg protein)⁻¹ day⁻¹] and dissolved organic nitrogen [DON; 0.5–3 μM (mg protein)⁻¹ day⁻¹] (Ferrier-Pages *et al.*, 1998; Naumann *et al.*, 2010), and water in contact with the coral surface can be enriched in labile DOC, have significantly higher bacterial specific growth rates than surrounding water, and have elevated oxygen concentrations compared with water overlaying algal-dominated substrate (van Duyl and Gast, 2001; Wild *et al.*, 2010; Tanaka *et al.*, 2011). Some coral species have been shown to consume bacterioplankton directly as a source of phosphorus, nitrogen and up to 20% of their carbon demand (Johannes *et al.*, 1972; Sorokin, 1973a,b). This is consistent with observations that cavities in the reef framework can have 29% less bacterioplankton and 15% less DOC than surround reef water (de Goeij and van Duyl 2007). Furthermore, 12-cm-long transects from the surface of corals have shown an average of a twofold increase in bacterial abundance and a 3.5-fold increase in virus-like particle (VLP) abundance in the 4 cm of water nearest to the surface (as compared with the abundances 8–12 cm away) (Seymour *et al.*, 2005). High DNA (HDNA) bacterial cells, a measure of the proportion of actively dividing cells, were also most abundant in the 4 cm closest to the coral surface (Seymour *et al.*, 2005) and were 10% higher above live coral sur-

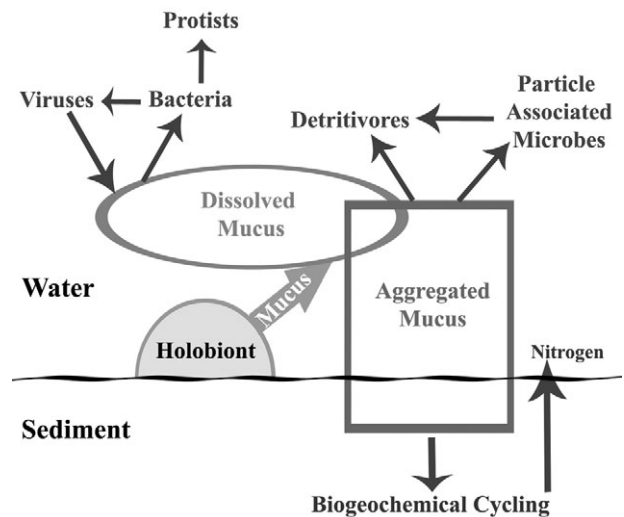


Fig. 2. Mucus is an important physical connection among the holobiont, water column and sediment microbial microenvironments within a coral reef ecosystem.

faces than in water 1 m away despite the fact that total bacterial abundances were lower (Patten *et al.*, 2006). The physiological state of the coral colony can influence these small-scale habitats. For instance, VLPs can be 30% more abundant in the 12 cm above diseased corals compared with healthy and dead colonies (Patten *et al.*, 2006).

At the larger scale of a single reef, phytoplankton and bacteria abundances in the water column can be depleted by the filter feeding action of the benthos (Linley and Koop, 1986; Yahel *et al.*, 1998; 2006; Gast *et al.*, 1999; Genin *et al.*, 2009) and the size structure of phytoplankton communities can be shifted towards larger cells by size-selective filter feeding (van Duyl *et al.*, 2002). **The concentrations of DOC and bacterial cells can become depleted over the scale of several kilometres even on a rapidly flushed reef (Nelson *et al.*, 2011).** Phytoplankton, bacteria and virus blooms can all be stimulated by coral mass spawning events (Patten *et al.*, 2008; Wild *et al.*, 2008), as can changes in bacterial community composition (Aprill and Rappe, 2011). The physical oceanography of a reef can also drastically influence the observed nutrient levels and fluxes. An internal tidal bore, for instance, can increase nutrient concentrations 10- to 40-fold relative to non-bore conditions (Leichter *et al.*, 2003).

Evidence suggests that nitrogen metabolism and production rates of bacterioplankton in reef waters are sensitive to eutrophication (Hoch *et al.*, 2008). However, in general, the large-scale mechanisms controlling nutrient fluxes and their relationship to microscale processes that may be critical for individual coral health remain to be identified. For instance, it would be useful to know if there are microscale hotspots of nutrient cycling and regeneration that influence the large-scale patterns we observe or if spatial heterogeneity plays a role in regulating the flux of nutrients from the water column into the coral holobiont, perhaps by the action of coral-associated bacteria and archaea.

Reef sediments

In shallow reef habitats, the water column is usually well mixed and sediment resuspension can shape the microbial seascape and create interaction opportunities among the benthic, water column and coral microbial communities (Yahel *et al.*, 2002; Rasheed *et al.*, 2004). Reef sediments are generally well colonized [on the order of 1 to 2×10^9 cells cm^{-2} (Wild *et al.*, 2006)] by a diverse community of microbes (Hewson and Fuhrman, 2006; Pringault *et al.*, 2008; Rusch *et al.*, 2009; Gaidos *et al.*, 2011). The microbial communities in reef sediments play an important role in benthic-pelagic coupling through the degradation of sloughed coral mucus and nitrogen cycling

(Gaidos *et al.*, 2011). This mucus traps organic particles, bacteria and picoplankton in the water column, and eventually settles on the sediments where up to 7% can be degraded per hour (Wild *et al.*, 2004b; Huettel *et al.*, 2006; Naumann *et al.*, 2009; Mayer and Wild, 2010). At Heron Island (Great Barrier Reef, Australia), researchers found that the total production from benthic diatoms, dinoflagellates and cyanobacteria in the sediments is of the same order of magnitude as the production by corals themselves (Werner *et al.*, 2006), and that those sediments were both net calcifying and nitrogen fixing.

A significant amount of nitrogen is thought to be exported from calcareous reef sediments to the rest of the ecosystem through grazing and resuspension of benthic microalgae that readily take up N fixed in the sediments (Miyajima *et al.*, 2001). All benthic environments, such as sand, coral rubble, live coral and cyanobacterial mats on coral reefs that have been examined, were found to be active in N_2 -fixation (Larkum *et al.*, 1988; Shashar *et al.*, 1994; Casareto *et al.*, 2008; Charpy *et al.*, 2010). For example, the sandy bottom of a lagoon in the Red Sea provided 70% of the nitrogen fixation to the surrounding fringing reef (Shashar *et al.*, 1994) and endolithic algae living in coral rubble generated enough nitrogen to support up to 28% of primary production on the reef at La Reunion Island in the Indian Ocean (Casareto *et al.*, 2008).

It has been shown that anthropogenic enrichment of reef sediments, in this case from fish farms, can saturate the ability of the microbial communities to metabolize organic material, and shift the sediment environment to one that is dominated entirely by anaerobic metabolism (Holmer *et al.*, 2003). The spatial resolution of microscale niches within the sediments has rarely been examined, and thus our mechanistic understanding of the coupling between benthic and pelagic processes remains at a larger, coarser scale than is needed for accurate predictions of ecosystem responses to perturbation.

One example of a specific mechanism that allows interaction among water column, coral mucus and sediment microbial communities is the process of mucus bundle formation in response to sedimentation (Smriga, 2010). When sediment is deposited on some coral species, such as *Montipora aequituberculata* and *Acropora microphthalma*, mucus is exuded that entrains the sediment and is released from the coral. These bundles then behave as particles in the water column that can continue to entrain other particles or plankton, be consumed by detritivores, or sink onto the benthos (perhaps another coral, an alga or sediment). This is just one example of the tight coupling that can exist among the various reef microscale habitats. The grazing and subsequent defecation patterns of mobile reef organisms (such as fish and invertebrates) provide another such mechanism (Johannes *et al.*, 1972; Smriga *et al.*, 2010).

In thinking about the various connections among the microbial habitats discussed thus far, it is important to consider the time component and the role sediments might play as an archive of anthropogenic influence. For instance, Cu and Zn contamination in reef sediments can reach high levels from a single ship grounding (Negri *et al.*, 2002; Jones, 2007) such that small amounts of the sediment are toxic to coral larvae and inhibit settlement (Negri *et al.*, 2002; Smith *et al.*, 2003). **The selection pressure of these metals can shift sediment bacterial community composition, and increase the incidence of antibiotic resistance** (Nogales *et al.*, 2011). This metal signature may persist in the sediments and/or the associated bacterial communities for many years to come with unexplored consequences for overall ecosystem function. This is an example of why we need to understand the propagation of sublethal perturbations through the ecosystem to be able to predict the responses and resilience of coral reefs to future change.

Towards a mechanistic and quantitative understanding of reef microbial ecology

Given the strong coupling in coral reef ecosystems, it is not possible to clearly separate benthic and pelagic processes, and thus we need to understand the holobiont within the continuum of benthic and pelagic environments. In a similar context, each of these environments encompasses many different microscale niches that can change the dynamics of benthic-pelagic interactions depending on the physical processes and various types of external forcing at play. In the pelagic environment, for example, we know that organic matter aggregates such as marine snow and phytoplankton are point sources of high concentrations of organic matter that vary on the millimetre scale, and are profoundly important for the functioning of ocean basin scale pelagic marine ecosystems (Azam, 1998; Seymour *et al.*, 2000; Kiorboe and Jackson, 2001; Long and Azam, 2001). Similarly, we can consider how microscale architecture and its heterogeneous distribution may regulate some mechanisms of microbial interactions. Spatial heterogeneity within coral microbial communities has been documented on the scale of centimetres (Rohwer *et al.*, 2001), but has rarely been investigated on a smaller scale. For example, one potential mechanism to consider is local organic matter enrichment within the coral mucus layer by expelled zooxanthellae (Paul *et al.*, 1986; Jones and Yellowlees, 1997; Baghdasarian and Muscatine, 2000; Wild *et al.*, 2005; Garren and Azam, 2010), **which could act as a hotspot for microbial growth, as in the case of rapid and profuse colonization by *Vibrio cholerae* of the marine dinoflagellate *Lingulodinium polyedrum*** (Mueller *et al.*, 2007). Heterogeneity in the microscale physical architecture of the coral mucus layer may

contribute to spatial variation in microbial interactions such as bacteria-bacteria antagonism (Ritchie, 2006; Rypien *et al.*, 2010), nitrogen cycling (Beman *et al.*, 2007; Siboni *et al.*, 2008; Olson *et al.*, 2009), or pathogen survival (Looney *et al.*, 2010).

A parallel continuum of size must be layered into our understanding as organic matter, the fuel for heterotrophic microbial processes, is considered within the spatial continuum of the reef environment. One of the next challenges for the field is to elucidate and quantify the mechanistic connections among these microbial communities within this multidimensional, multi-scale framework to attain a cause-and-effect understanding of how a reef ecosystem functions. The technology is now available to begin adding multiple spatial and time scale contexts to fundamental questions of coral reef ecosystem function such as how growth of associated microbial communities is regulated, why we see certain dominances in a given niche, what the primary sources of bacterial mortality are under specific environmental conditions, and how all of these mechanisms relate to the health or disease state of a reef (see Fig. 3). For example, new developments in confocal imaging technology have enabled the visualization of the natural coral-microbial assemblage *in situ*, and micro-scale ecological interactions can now be observed in real time (M. Garren and F. Azam, submitted).

Concluding remarks

As we consider questions that are currently driving the field of coral microbial ecology forward, it is an opportune moment to reflect on the progress that has been made in recent history. **In 2003, Knowlton and Rohwer wrote an article in *The American Naturalist* entitled 'Multispecies microbial mutualisms on coral reefs: the host as a habitat' (Knowlton and Rohwer, 2003).** They outlined the then most current understanding of bacterial and archaeal associations with corals, and concluded that 'we know almost nothing about the role of non-eukaryotic microbes in healthy coral' (p. S54). Among the research questions that they suggested to drive the field forward were: (i) What is the scope of diversity for coral-associated bacteria and archaea, and how is it patterned in space and time? (ii) Which of the many bacterial and archaeal associates of corals are true mutualists, and what roles do they play? (iii) How important are bacterial and archaeal communities to the health of coral reefs, and are they being disrupted by anthropogenic stress?

The field has made substantial progress on these questions in the past 8 years, and yet there remains much to be learned by revisiting these same questions and taking advantage of significant advances in genomics and imaging technologies. As discussed earlier

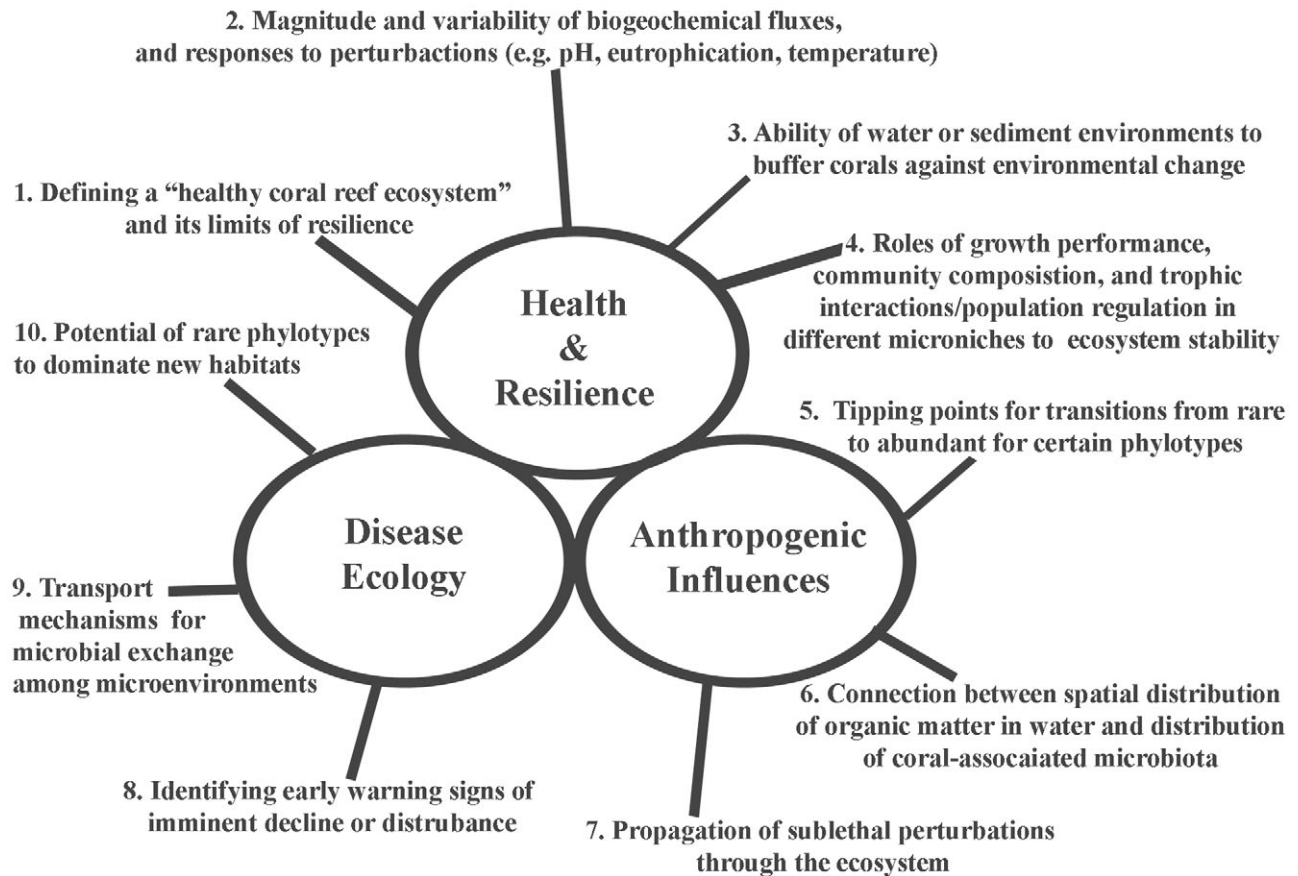


Fig. 3. Important research problems in coral reef microbial ecology.

and recently reviewed by others (Bourne *et al.*, 2009; Ainsworth *et al.*, 2010; Mouchka *et al.*, 2010), the scope of diversity of bacteria is much clearer than it was before, and we have more information about patterns of distribution and diversity through space and time. However, the same cannot be said for the archaeal communities, and there remain many levels of spatial and time scales on which we do not understand bacterial behaviours. Further, and critically, our understanding of *in situ* microbial activity and interactions is still quite incomplete. It has become clear that studying the tropical reef environment requires some modifications of methods from traditional temperate marine microbial ecology to accurately quantify essential parameters (e.g. Torretón and Dufour, 1996a for FDDC conversion to specific growth rate, μ ; Wild *et al.*, 2006; Garren and Azam, 2010 for enumeration of bacterial cells), and these breakthroughs are helping to constrain and quantify critical pieces of the puzzle such as bacterial abundance, growth rates, and production rates (Table 1).

And thus, while the questions we highlight (Fig. 3) address some of the current gaps in our understanding of reef microbial ecology, many are questions that have

been asked previously. The reason to revisit them is that new technologies (including NanoSIMS, super resolution microscopy, high-speed imaging techniques, confocal Raman microspectroscopy, next-generation sequencing, and microfluidics) are becoming accessible to marine microbial ecologists that can help answer the questions in a quantitative and mechanistic way (Table 2). We further stress the need to develop *in situ* microscopy. In this new era of microbial ecology, some researchers are considering phage therapy as a potential response to global increases in coral disease (Efrony *et al.*, 2007; 2009) and the ability to employ the rapidly advancing concepts and techniques from biomedicine is ever more accessible (Bourne *et al.*, 2009). Studies of coral reef ecology and microbial diseases of corals would also benefit by learning from the highly dynamic field of human health considerations of microbial pathogenesis and the ecology of microbial diseases. Indeed, future discoveries in coral-microbe interactions may 'return the favours' through potential applicability to human-microbe interactions. The time is ripe for microbial ecologists to discover and create an integrated and mechanistic understanding of coral reef functioning; however, they will need the help of natural

Table 1. Reported values showing the range of measurements for bacterial abundance, growth rates and primary production made in coral reef environments.

Sample type	Location	Bacteria abundance ($\times 10^5$ cells ml ⁻¹)	Bacterial growth rate (μ ; day ⁻¹)	Primary production	Growth and production methods	Source
Epilithic and endolithic <i>Cyanobacteria</i> on coral rubble	Sesoko Island, Okinawa, Japan (May 2007)	–	–	134.6 \pm 55.2 nmol C μ g Chl-a ⁻¹ day ⁻¹	¹³ C Tracer following Hama <i>et al.</i> (1993)	Casareto <i>et al.</i> (2008)
Reef water (overlying, crevice and bottom)	Curacao	3–8	0.12–0.48	–	Tritiated Leucine incorporation	Gast <i>et al.</i> (1998)
Reef water	Florida Keys, USA	4	1.1 \pm 0.2	24.0 \pm 4.8 μ g C l ⁻¹ day ⁻¹	¹⁴ C HCO ₃ ; ¹⁴ C leucine	Hoch <i>et al.</i> (2008)
Atoll lagoon water	Tuamotu Archipelago, French Polynesia	6 (Takapoto) to 18 (Tikehau)	0.72–4.01	–	Tritiated Thymidine incorporation	Torretton and Dufour (1996b)
Reef water	Barrier Reef, Belize	3.5	0.53	1.1 μ g C l ⁻¹ day ⁻¹ (cyanobacteria only)	Seawater culture technique	Herndl (1991)
Atoll lagoon water	12 Atolls in the Tuamotu Archipelago, French Polynesia	2.2 (Tekokota) to 20.7 (Hiti)	0.07 (Marokau) to 1.54 (Reka-Reka)	1 (Tekokota) to 91 (Reka-Reka) μ g C l ⁻¹ day ⁻¹	Tritiated Thymidine incorporation; ¹⁴ C HCO ₃	Torretton <i>et al.</i> (2002)
Reef waters around remote atolls	Northern Line Islands	0.72 (Kingman) to 8.4 (Kiritimati)	–	–	–	Dinsdale <i>et al.</i> (2008)
Reef waters (near fish farms)	Bolinao, Philippines	15.1–74.3	0.23–0.26	–	Frequency of dividing-divided cells (conversion to μ following Torretton and Dufour, 1996a)	Garren <i>et al.</i> (2009)
Reef waters	Marshall, Gilbert and Hawaiian Islands; Great Barrier Reef	9.5 (Majura Atoll) to 39.5 (Butaritari Atoll)	0.30 (Butaritari) to 0.65 (Kaneohe Bay, Oahu)	4.1 (Majura Atoll) to 67 (Heron Island, GBR) μ g C l ⁻¹ day ⁻¹	¹⁴ C labelling for both bacteria and phytoplankton production	Sorokin (1973a)
Atoll lagoon water	Majero Atoll	8–14	0.24–0.85	–	Tritiated thymidine and tritiated leucine incorporation	Yoshinaga <i>et al.</i> (1991)
Reef waters	New Caledonia	5.5–7.4	0.07–0.34	5.6–12.2 μ g C l ⁻¹ day ⁻¹	Tritiated Thymidine incorporation; ¹⁴ C HCO ₃	Torretton <i>et al.</i> (2010)

Table 2. Technologies now available to marine microbial ecologists that may help in the investigation of some important research problems in coral reef microbial ecology (depicted in Fig. 3). References to recent reviews are provided for further reading.

Technology	Benefits	Potential applications to coral microbial ecology problems depicted in Fig. 3	Recent reviews
NanoSIMS	High spatial resolution (to 50 nm) and sensitivity (p.p.m.) for elemental, isotopic and molecular composition of a surface	#2 (biogeochemical flux) #7 (sublethal perturbations)	Wagner (2009)
Super resolution microscopy	Capture images with a higher resolution than the diffraction limit	#6 (spatial distributions)	Haagensen <i>et al.</i> (2011)
High speed imaging techniques (e.g. atomic force microscopy, laser scanning confocal, etc.)	New developments in traditional imaging techniques allow living samples to be imaged in real time	#4 (growth rates and community composition) #5 (tipping points) #6 (spatial distributions) #9 (transport and exchange)	Haagensen <i>et al.</i> (2011)
Confocal raman microscopy	Provides spatially explicit information on chemical bonds and can be done on unfixed samples	#2 (biogeochemical flux) #3 (buffering perturbations) #6 (spatial distributions)	Petry <i>et al.</i> (2003); Wagner (2009)
Next Generation sequencing	Cost effective high throughput DNA, RNA and protein sequences	#1 (defining healthy reef) #8 (early warning signs) #10 (rare phylogeny dynamics)	Gilbert and Dupont (2011)
Microfluidics	Ability to constrain microenvironments and test microbial responses	#4 (growth rates and community composition) #3 (buffering perturbations) #10 (rare phylogeny dynamics)	Ahmed <i>et al.</i> (2010)

resource managers working in coral reef ecosystems. In the context of long-term survival and conservation of coral reef ecosystems, the need for this work is immediate.

There are powerful and logistically simple ways now possible in which resource managers and microbial ecologists might collaborate to greatly advance our understanding of coral reef microbial ecology and microbial pathogenesis. Most coral reef managers and researchers lack access to specialized technologies in field settings around the world. However, it could be straightforward to collect samples during regular monitoring routines and send them to specialized laboratories for analyses and archiving. For instance, coral and environmental samples for analyses of microbial abundance, community composition and gene expression can be preserved (even at room temperature) and sent to specialized central laboratories. Dramatic decrease in the cost and increase in the speed of the -omic analyses makes practical such previously prohibitive approaches as metagenomic and metatranscriptomics to monitoring and research. We envision a limited number of centres that analyse and archive the samples, by methods that are inter-calibrated among the centres. Importantly, this will enable the scientific community to obtain geographically broad and long time series, critical for understanding the effects of climate change on the health of coral reefs. **It would be most valuable to create an organized and well-documented archive of samples from around the globe accessible to coral reef researcher worldwide.** The emerging data associated with the samples should also be made freely accessible. Interrogation of such archived samples would further deepen our knowledge of corals and their environments by technologies currently not envisioned and for testing hypotheses not yet formulated.

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