Coral Microbiomes as Bioindicators of Reef Health

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Abstract Coral reefs are currently in steep decline worldwide due to changes in climate and anthropogenic activity. Given reefs' key roles as centers of biodiversity and the variety of services they provide for humans, it is imperative that we develop reef management strategies that are sensitive to environmental changes and that allow timely interventions in response to specific threats. The use of bioindicators has been demonstrated as an effective way to monitor a broad range of ecosystems, and coral microbiomes show immense potential as bioindicators for coral reefs. Given the decline of coral reefs worldwide, and the diversity of species that are currently under threat, coral microbiomes can provide much-needed insights and information for the purposes of reef conservation and protection.

1 Introduction

Coral reefs are often compared to tropical rainforest ecosystems for their high species richness and complexity (Mulhall 2008). Containing one-quarter to one-third of all marine species, coral reefs provide a milieu of economic and ecological services to humans, including fisheries and coastal protection (Hoegh-Guldberg et al. 2007; Plaisance et al. 2011). Due to a combination of overexploitation, pollution, and global climate change, these ecosystems have undergone significant degradation in recent decades (Parry et al. 2007). Given their immense value and threatened status, conservation of coral reefs has emerged globally as a pressing management concern. Monitoring potential stressors (e.g., increased temperature, sedimentation, nutrients)

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and assessing their influence on ecosystem status and function form the basis of effective adaptive natural resource management (Holling 1978). However, the sporadic and heterogeneous nature of anthropogenic pressures combined with the remoteness of many reef systems presents a significant challenge to traditional monitoring methods. Bioindicators hold the promise of providing highly sensitive, time-integrated measures linking changes in water quality to the condition and function of reef ecosystems (Cooper et al. 2009; Fabricius et al. 2012; Leite et al. 2018). In this chapter, we highlight the potential of coral microbiomes as novel bioindicators of reef health.

Many reef monitoring programs aim to document changes in environmental condition and quantify the impact of these changes on the diversity, health, and function of reef-dwelling organisms (Rogers et al. 1994). This information is critical for understanding the role of specific human activities in reef declines and informing the effective management of ongoing and proposed projects. Reef monitoring programs often employ direct measurement of fish, coral, and/or non-coral invertebrate health and abundance and collect data on environmental parameters (e.g., temperature, salinity, and turbidity). These methods require highly trained SCUBA divers to conduct repeated underwater surveys often at multiple reef locations, which can be time-consuming and extremely costly. For example, the Australian Institute of Marine Science's Long-Term Monitoring Program has cost an estimated AUD$50 million over 27 years (AIMS 2013). This detailed monitoring provides invaluable documentation of coral decline on the Great Barrier Reef. It also serves as a unique resource to assess the causes of this decline and assess the effectiveness of protection measures. However, direct measurements of environmental conditions are limited in their ability to provide holistic information on reef health. Direct environmental measurements, for example, can only capture the state of an ecosystem at the time of sampling. The significant costs and logistical challenges (e.g., extreme weather) associated with delivering teams of specialist divers to often remote reefs limit the frequency of in situ monitoring (Holt and Miller 2011). Sporadic, infrequent sampling is likely to miss rare or highly variable impacts and/or responses and limits the utility of such monitoring as early-warning management triggers (Fabricius et al. 2012). Inferences of ecosystem or organismal health based on direct environmental measurements are currently limited by an imperfect understanding of the complex relationships between environmental and biotic variability (Fabricius et al. 2012). It is worth noting that these monitoring methods are not able to determine the quality of the reef ecosystem as a whole or to fully assess the presence or extent of changes in the surrounding environment. In order to continue protecting and conserving coral reefs in the future, it is imperative to develop monitoring methods that are more sensitive to changes in reef ecosystems and organismal responses to these changes.

2   Bioindicators of Coral Reef Ecosystem Health

In contrast to traditional environmental monitoring, which often relies upon direct measurement of environment parameters (e.g., temperature, salinity, nutrients, pollutants, turbidity), bioindicator-based monitoring quantifies specific biological processes, species, and/or communities as proxies for environmental condition. Embracing the view that the biota itself is the best predictor of how ecosystems respond to disturbance, researchers and managers working in terrestrial, freshwater, and marine ecosystems increasingly employ bioindicators for environmental monitoring (Bispo et al. 2009; Phillips and Rainbow 2013; Thakur et al. 2013). While monitoring whole-community dynamics – rather than a subset of bioindicators – can be informative, it is simply not feasible in high diversity systems like coral reefs (Bouchet 2006). Focusing on a subset of species satisfies the established criteria for effective bioindicators – specificity, monotonicity, variability, practicality, and ecological relevance (see Box 1) – maximizing sensitivity while reducing costs.

Box 1 Microbiomes Meet the Criteria for Useful Bioindicators
While the criteria for adequate bioindicators can vary by environment and need, universal standards for useful bioindicators have been prescribed. These include an organism's ability to exhibit biological responses that are specific to one stressor or environmental change, termed *specificity*; its *monotonicity*, which dictates that the size of an organism's response to a stressor or change should be proportional to both the intensity and the duration of said stressor or change; an organism's (or community of organisms') *variability*, that is, its ability to remain relatively consistent in the absence of changes or stressors; the *practicality* of a bioindicator, which hinges on the feasibility, cost-effectiveness, and the ease of measurement of an organism's biological responses; and finally that a bioindicator must be *relevant* and exhibit stress responses that are both ecologically relevant as well as relevant to the general public in order to facilitate the communication of results (Cooper et al. 2009).

Microbiomes meet these criteria for useful bioindicators. Microbial responses have been shown to be specific to certain changes in the environment, particularly with regard to environmental pollution. For example, studies on soil microbiomes have shown them to be highly responsive to lead pollution even at low concentrations, as well as to copper and zinc (Nwuche and Ugoji 2008; Sobolev and Begonia 2008). Shifts in microbial diversity have also been observed in aquatic environments in response to increased pCO_2, suggesting that microorganisms could be used to detect increases in the acidification of the water column (Liu et al. 2010). A study conducted by Cooper et al. (2009) on potential bioindicators also found that microbes ranked well in both monotonicity and variability as bioindicators, indicating that their response to disturbance is proportional to the strength of the disturbance and

Box 1 (continued)

that they show low variability in the absence of stress (Cooper et al. 2009). Microorganisms are also a quite practical option: they tend to be abundant in all environments and are easily accessible and testable, which allows for cost- and time-effective sampling that can yield quick results for the purposes of environmental assessment (Parmar et al. 2016). Finally, microbial responses are relevant at ecological scales, and associated changes are relevant to the public. This is because microorganisms are key players in a variety of different biogeochemical cycles, such as the carbon and nitrogen cycles, that are integral for the continued survival of an ecosystem (Bloem and Breure 2003). As aggregate microbiomes, they can also have large impacts on the health and function of their host, which makes them particularly important in ecosystems such as coral reefs in which their hosts are also keystone species. Responses to changes in their ecosystem can take the form of changes in community diversity and overall function, which can have serious implications for the natural cycles of an ecosystem as well as for organisms with which they have symbiotic relationships.

Despite their vast potential, only a handful of studies have explored the potential of bioindicators in reef monitoring (Cooper et al. 2009; Leite et al. 2018). Studies examining the suitability of different marine fauna (e.g., invertebrates, fishes, and marine plants and algae) as bioindicators of water quality and reef health have consistently highlighted coral physiology, health, and benthic cover as promising options (Cooper et al. 2009; Fabricius et al. 2012). Measurements of coral health, function, and diversity have been employed as bioindicators, providing information on environmental changes at different temporal scales and impact intensities (Fabricius et al. 2012). As the backbone of reef ecosystems, corals are intrinsically tied to the fluxes and changes in reef health, and as a result they display a range of measureable responses to changes in the environment. Measurements of coral symbiont photophysiology, for example, provide information on environmental disturbances at the colony level within a short time frame (with responses ranging from seconds to days) (Cooper et al. 2009; Jones et al. 2003). Coral disease, another common bioindicator, provides information at intermediate time scales, with diseases becoming visually evident days to weeks after disturbance (Cooper et al. 2009). Coral diseases have been tied to changes in a multitude of environmental conditions, including increases in temperature, algal contact, nutrient enrichment, sedimentation, and turbidity (Bruno et al. 2007; Harvell et al. 2007; Nugues et al. 2004; Vega Thurber et al. 2014; Pollock et al. 2014). Changes in hard coral taxonomic richness and cover have been employed to demonstrate prolonged impacts over time frames ranging from months to years and serve as community-level bioindicators for chronic declines in water quality (Cooper et al. 2009; De'ath and Fabricius 2010).

Despite their immense value, several constraints limit the utility of coral-related factors as effective bioindicators. Direct reef monitoring methods, such as coral disease detection and observance of community-level changes, lack the sensitivity to detect sublethal levels of stress and/or identify stress prior to the macroscopic manifestation of bleaching, disease, or population declines. One of the main goals of reef monitoring is to detect harmful changes in ecosystem health before they cause damage to the health of keystone reef organisms. Since the visible signs of coral diseases and bleaching can take days to weeks to appear, by the time they are observed, the most effective window for adaptive management interventions may have passed (Gil-Agudelo et al. 2004; Gladfelter 1982). While coral disease and taxonomic richness can be useful indicators of chronic and/or extreme water quality changes, their potential for rapid adaptive management is limited. Ultimately, an adequate reef bioindicator must be both intimately affiliated with the coral species that form the basis of these ecosystems and provide broad-range responses that span time scales and impact levels.

3 Microorganisms as Bioindicators

The abundance, diversity, and sensitivity of coral-associated microbial communities and their intimate ties with the water column and keystone reef species make coral microbiomes ideal targets for bioindicator development. Microorganisms have been successfully utilized as bioindicators in diverse ecosystems, including soils, rivers, and even the human body, but the potential application of microbes as reef bioindicators has only just begun to be explored (Glasl et al. 2017; Leite et al. 2018; Peixoto et al. 2017). The extensive literature on microbial bioindicators and the responses of coral microbiomes to environmental change provides an ideal base for the establishment of coral-associated microbes as bioindicators in reef ecosystems (see Box 2 and Table 1).

> **Box 2 Microorganisms and Microbiomes Have Been Used as Bioindicators in Diverse Ecosystems**
> Microorganisms are well-established bioindicators in both aquatic and terrestrial ecosystems and have proven successful for the purposes of monitoring environmental health. Microbial bioindicators are particularly common in studies focusing on soil health, as soil microbial communities are often good indicators of changes in soil quality. This is because of their involvement in many key cycling processes in the soil as well as their sensitivity to alterations in the ecosystem, which results in quick responses and even responses that precede environmental changes, further establishing their potential to act as early-warning systems of soil changes (Nielsen et al. 2002). Researchers seeking to identify the best indicators for soil health consistently include

Box 2 (continued)

microorganisms, and their functions, near the top of their lists. For example, studies using diversity analyses have found that bacteria and archaea in soil were the best indicators for the monitoring of carbon, nitrogen, and phosphorus cycling, as well as for soil biodiversity and the availability of appropriate habitats for sustaining biodiversity (Bispo et al. 2009; Mendes et al. 2016; Stone et al. 2016). Microbial parameters have also been used to detect and monitor soil pollution as an alternative to more expensive and long-term field experiments, due to the fact that microorganisms can be highly responsive to certain pollutants even at low concentrations (Brookes 1995; Nwuche and Ugoji 2008; Sobolev and Begonia 2008).

Monitoring efforts focused on aquatic environments have also benefitted from the use of microbial bioindicators. Though microbial bioindicators are more established in certain ecosystems and monitoring situations than others, their sensitivity to changes in water quality has led researchers to call for their use in broad contexts, from wetland monitoring to the detection of mining pollutants in rivers (Sims et al. 2013; Yergeau et al. 2012). Organisms such as ciliates, with over 300 species having established roles in river and lake monitoring efforts, are an excellent example of the variety of microorganisms available for the purposes of indicating changes in an aquatic environment (Foissner and Berger 1996).

The use of microbial bioindicators to determine organismal health has also been growing in popularity, particularly in the context of human health. Studies on the gut microbiome have found that it can be affected by a variety of factors including diet, and the microbiome's responses can be used as indicators for changes in the health of an organism. This concept was highlighted by a study on gut microbiota that found that structural shifts in the microbial composition of mice's guts can be used as bioindicators to monitor the health of organisms exposed to carcinogens (Wei et al. 2010). Microbial bioindicators are also beginning to gain traction in the realm of disease and preventative care: a recent study on leukemia patients found that microbiome diversity before chemotherapy treatment is linked to risk of infection during the treatment (Galloway-Peña et al. 2016). The authors suggest that the composition of the microbiome could therefore be used as an indicator of infection risk and help to mitigate infections in leukemia patients undergoing chemotherapy (Galloway-Peña et al. 2016).

Microorganisms are widely applicable as biological indicators for different environmental and even organismal changes. With continued study of their function and roles in ecosystem health, microorganisms will be able to be integrated with common monitoring systems for the purposes of providing a wider and more accurate range of information on environmental shifts and ecosystem response.

Table 1 Specific microbial responses have been detected within coral hosts as the result of environmental stress

Stressor	Coral species	Microbial response	Source
Thermal changes	*Acropora muricata*	Shift towards *Verrucomicrobiae-* and *α-Proteobacteria*-dominated community	Lee et al. (2015)
Pollution/ proximity to shore	*Orbicella faveolata, Porites astreoides; Orbicella annularis*	Increase in bacterial diversity	Morrow et al. (2012); Klaus et al. (2007)
Pathogens	*Diploria strigosa, Siderastrea siderea; Orbicella faveolata*	Increase in *α-Proteobacteria*, decrease in β- and γ-*proteobacteria;* increase in diversity and *Rhodobacterales*	Cárdenas et. al. (2012); Sunagawa et al. (2009)
Eutrophication	*Acropora hemprichii*	Increase in diversity	Jessen et al. (2013)
Salinity	*Fungia granulosa*	Increase in abundance of *Rhodobacteraceae*	Röthig et al. (2016)

Microorganisms' sensitivity and ability to show marked shifts in community composition and abundance in response to specific stressors highlight their potential as indicators of changes in reef ecosystems as well as in coral host health

Microorganisms and microbiomes meet the established criteria for ideal environmental indicators (see Box 1). In the context of reef health, using microorganisms as bioindicators can provide valuable insights into both biological and environmental changes. Microorganisms are present not only in the sediment and water column but also in association with keystone organisms such as sponges or corals (Bourne and Webster 2013). These intimate connections could allow for a more thorough analysis of the state of a coral reef than would coral health assessments on their own. Several microbe-focused monitoring techniques currently employed on reefs rely on microorganisms residing in the water column and benthos and can provide information on changes in water quality and temperature. Tools such as Autonomous Reef Monitoring Structures (ARMS) capitalize on the wealth of bioindicators present in the water column and the reef ecosystem, serving as long-term collection devices for a wide variety of marine species, including microbial ones. ARMS allow for the monitoring of marine diversity in reef environments by analyzing the communities of invertebrates and algae that colonize three-dimensional structures deployed on the benthic substrate, which are used as proxies for overall biodiversity (Knowlton et al. 2010). Coupled with molecular tools, ARMS can also be useful for determining overall reef bacterial diversity by focusing on colonizing bacteria and, in turn, determining fluxes in microbial diversity over time and with changes in the environment. Colonizing bacteria tend to form biofilms on the structure. Biofilms, microbial assemblages that are attached to surfaces and enclosed in extracellular polymeric substance matrices, often serve as bioindicators in aquatic environments, providing information on water quality due to their ability to absorb heavy metals and their sensitivity to changes in their environment (Burns and Ryder 2001; Donlan 2002; Mages et al. 2004). Biofilms have been implemented successfully in reef

systems as well, showing changes in the diversity of the biofilm's microbial community alongside a water quality gradient as well as in correlation to terrestrial runoff (Kriwy and Uthicke 2011; Witt et al. 2012). Though biofilms collected by ARMS can serve as useful bioindicators for the purposes of assessing reef water quality as well as determining coral health if they respond to similar disease-causing or coral health-altering factors, their functional disconnect from corals themselves puts them as a disadvantage and limits their ability and scope as bioindicators.

Foraminifera, a group of eukaryotic microbes that are highly responsive to fluxes in the nutrient content of the water column, provide a good example of microorganisms successfully used as bioindicators for reef health (Hallock et al. 2003). Foraminifers are particularly suitable for reef monitoring since their water quality requirements are similar to those of reef-building corals (Hallock et al. 2003). Hallock et al. (2003) developed the FORAM Index, a comprehensive and easily implemented procedure allowing resource managers to collect foraminifers and use simple abundance and diversity analyses to detect and quantify environmental change and assess the water column's suitability for reef growth (Hallock et al. 2003). While this method remains limited due to its focus on one phylum, the FORAM Index is an excellent example of the immense potential of microbial species as bioindicators. For this reason, it serves as a stepping stone toward similar systems and indexes that are able to integrate information from a variety of different microorganisms and provide a more holistic picture of reef health.

While both biofilms collected by ARMS and the FORAM Index have been relatively successful, there is still a need for a bioindicator that can be used as a proxy for both coral and reef ecosystem-wide health. In this context, coral microbiomes show substantial promise in providing more accurate measures for environmental changes and health. Coral microbiota are assemblages of bacteria, archaea, and microscopic eukaryotes that sustain long-term symbioses with corals, collectively comprising the coral holobiont (Ainsworth et al. 2010; Knowlton and Rohwer 2003; Rohwer et al. 2002). Coral-associated microorganisms provide a variety of services for corals, ranging from antibiotic production to the metabolism of coral waste products (Ainsworth et al. 2010; Glasl et al. 2017; Rohwer et al. 2002). One important criterion of an effective bioindicator is *specificity* in its response to changes and stressors in the environment. Microorganisms satisfy this criterion well. Many studies have shown that the microorganisms that inhabit corals are sensitive and have specific reactions to environmental changes; the diversity and community composition of a reef's microbiome will often change in response to stress (Fig. 1). Common stressors such as increases in temperature, variations in the water's nutrient content, and contact with algae have been shown to increase microbial diversity within corals (see Table 1) (Jessen et al. 2013; Morrow et al. 2012; Zaneveld et al. 2016). Environmental changes can also alter microbial community structure in the coral holobiont, usually eliciting an increase in beta diversity (variability) and affecting the abundance of specific microbial taxa (Klaus et al. 2007; Lee et al. 2016; Röthig et al. 2016; Zaneveld et al. 2017). *Endozoicomonas*, a bacterium thought to be beneficial to corals, appears particularly sensitive to environmental changes, decreasing in abundance during periods of stress
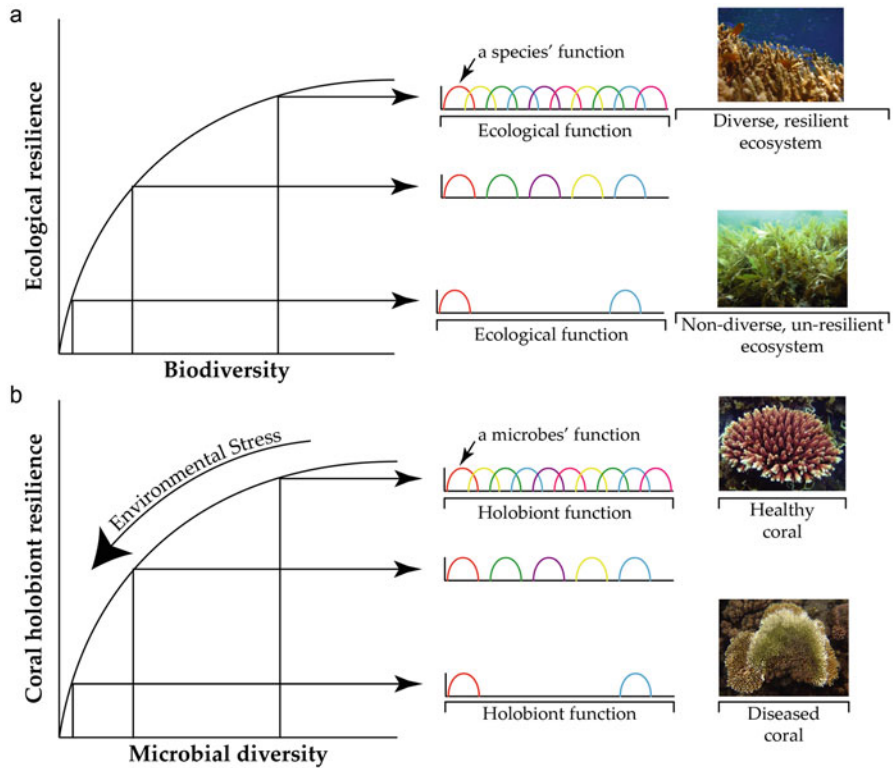
Fig. 1 (**a**) The "rivet hypothesis" is an ecological theory positing that biodiversity in an ecosystem creates functional redundancy and complementarity due to the limited number of niches available (Ehrlich and Ehrlich 1981). As a result of this functional overlap, biodiverse ecosystems tend to be more resilient to change, given that the loss of one or two species will not affect the ecosystem severely. (**b**) The structure and function of microorganisms and the microbial community within the coral holobiont could potentially mirror that of individual species within a biodiverse system, with the multitude of species and consequent overlap in function making the system more resilient. However, coral microbiomes can also be subjected to and respond to environmental stressors, potentially in ways that can reduce diversity and, in turn, coral host resiliency

(McDevitt-Irwin et al. 2017). Studies have also shown that despite their ability to form long-term stable symbioses with corals, microbial communities vary in their resistance and resilience based on the length and impact of environmental disturbances, thereby meeting the criteria for both *variability* and *monotonicity* (Glasl et al. 2017). It is worth noting that not all coral microbiomes can serve as adequate bioindicators. Coral microbiomes vary by species, regions, temperatures, and depths (Apprill et al. 2016). To serve as suitable indicators for changes in reef ecosystems, specific coral-associated microorganisms (and/or the combined microbiome) must have intermediate resiliency: too much sensitivity or too much resistance will not provide useful data on water quality and reef changes.

Using coral microbiomes as proxies for reef health has many advantages over traditional monitoring methods and the use of corals themselves in the context of *practicality*, including ease of sampling, availability, and an abundance of data generated. Coral microbiomes can be sampled simply by extracting a small section of a coral, in the form of tissue, mucus, skeleton, or all three, which is a relatively cheap and rapid process. Mucus in particular can be sampled without inflicting significant harm on the coral, and mucus-associated bacteria show greater responses to environmental changes than those associated with the skeleton or tissue (Pollock et al., in review). Each sample yields a wealth of data, providing information on microbial diversity, community structure, abundance, and function, all of which can be used as proxies for overall reef health. Microbiomes are also undoubtedly *relevant* as bioindicators, due to their intimate connection to reefs and, in turn, their importance for overall reef and ecosystem health. While microbial samples from the water column and sediments can serve as adequate indicators of environmental changes, the microbiome of corals themselves can serve as an even more accurate proxy for environmental health. Their intimate connection with the reef system, the specificity of their responses to change, and their potential to provide predictive snapshots of a reef ecosystem's health all point to their potential for successful implementation as bioindicators.

4   Tools Available for Microbiome Bioindicator Monitoring

As the field of microbiology, particularly microbial ecology, continues to grow, so does the list of tools available for the analysis of microbial communities, which currently include cultivation, 16S and 18S tag sequencing, metagenomics, and metatranscriptomics (Fig. 2). Historically, studies using bacteria for monitoring of environmental health relied heavily on cultivation techniques (Cardenas and Tiedje 2008). For example, culture-based detection of fecal coliforms is a well-established and widely employed bioindicator of human fecal contamination in water bodies (Tan et al. 2015). Studies focusing on microbial communities in reef ecosystems have used cultivation techniques for the purposes of taxonomic identification of microbes present in a sample and genetic sequence retrieval (Haygood et al. 1999). Culture-based methods are relatively cheap and straightforward, and they allow for the targeting of specific bacteria for growth and analysis via specialized media. This is beneficial when targeting a particular bioindicator species for analyses of presence and abundance. However, in order for this method to be successful, the microorganisms chosen for analysis must be good indicators, and the specificity of this method does not allow for the discovery of other potential bioindicators. Furthermore, given that only around 1% bacteria are cultivable, the pool of potential culture-based bioindicators is quite limited. Culture-based methods also cannot provide information on environmental processes in which the microorganisms being cultured may be involved or on their function and contribution to an ecosystem.
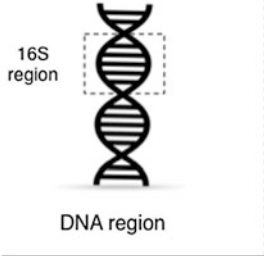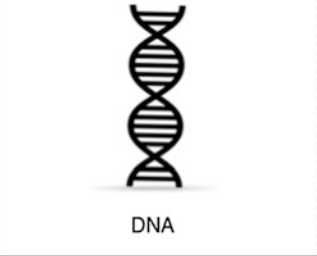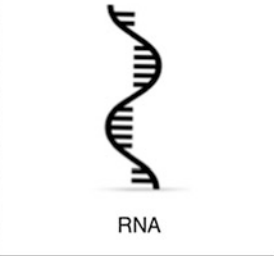
16S sequencing	**Metagenomics**	**Metatranscriptomics**
"Who is there?"	*"What can they do?"*	*"What are they doing?"*

16S region		
DNA region	DNA	RNA

Advantages:	**Advantages:**	**Advantages:**
Relatively inexpensive Phylogenetic data	Assessment of entire holobiont Information on functional potential	Information on realized function, gene expression, response to changes
Disadvantages:	**Disadvantages:**	**Disadvantages:**
Low phylogenetic resolution Poor information on microbial function	Large amount of host genetic material can swamp microbial sequences	Relatively expensive Still a developing field

Fig. 2 Summary of the advantages and disadvantages of different sequencing tools available for analysis of microbial samples. Each tool can serve to answer different questions regarding the microbial community present in a sample and provide different levels of information

Amplicon-based sequencing of phylogenetically conserved genetic regions is often preferred over culture-based techniques due to the large amount of information it can provide on microbial life in an ecosystem. The processes of ribosomal DNA tag sequencing (e.g., 16S, 18S, and ITS) from coral tissue samples allow for the targeting of a particular genetic region for amplification and subsequent sequencing in order to identify the organisms present in a sample. The small subunit rRNA gene, also known as 16S in prokaryotes, is about 1,500 base pairs in length and is highly conserved, enabling amplification across most bacterial taxa (Janda and Abbott 2007). The eukaryotic counterpart is the 18S rRNA gene, which has similar features and can be used for the identification of microbial eukaryotes (Tan et al. 2015). In contrast, the ITS2 (internal transcribed spacer 2) region can be used for more targeted identification of *Symbiodinium*, a genus of symbiotic algae that form integral symbioses with corals (LaJeunesse 2001). Due to their low cost and high data yield, tag sequencing techniques have emerged as a cornerstone of microbiome analysis. These cultivation-independent methods are particularly popular in microbiome analyses of coral samples, and have been used to identify bacteria associated with specific species of coral, determine how bacterial communities change when corals are diseased, and analyze the link between bacterial communities and environmental stress (Babu et al. 2004; Ben-Haim et al. 2003; Bourne and Munn 2005; Cooney et al. 2002; Sunagawa et al. 2009; Ziegler et al. 2017).

High-throughput tag sequencing techniques allow for the discovery of new potential indicators and analysis of indicative bacterial responses to changes in the environment and stress, such as shifts in overall diversity and community structure. While this method is an improvement from culturing, in that it provides information on all bacteria present in a sample rather than a small subset, it still has its drawbacks; this method does not allow direct assessment of bacterial function, nor does it provide a high level of phylogenetic resolution. It also does not account for the possibility that bacteria grouped into a single operational taxonomic unit (OTU) (i.e., microbes with similar sequences) may actually be acting as separate functional entities in the coral holobiont.

To answer questions about microbial functional potential, researchers have turned to metagenomics. While tag sequencing targets a relatively short stretch of the microbial genome, metagenomic approaches provide data on the entire genetic repertoire present in a given sample (Cardenas and Tiedje 2008). Metagenomic approaches, therefore, provide insights into the functional potential of all organisms within a sample (Cardenas and Tiedje 2008; Streit and Schmitz 2004). Unlike 16S or 18S sequencing, which only characterizes either prokaryotes or eukaryotes, respectively, metagenome analysis characterizes all genes in a sample regardless of their phylogenetic origins (Ainsworth et al. 2010). Metagenomic analyses have been used to detect taxonomic, functional, and metabolic shifts in stressed and diseased corals (Kimes et al. 2010; Littman et al. 2011; Vega Thurber et al. 2009). For example, metagenomic approaches have uncovered that increases in temperature, excess nutrients, carbon loading, and reduced pH can lead to the increase of microbial genes involved in virulence, stress resistance, and sulfur metabolism (Vega Thurber et al. 2009). By providing a more holistic perspective on the genetic potential of the coral microbiome, metagenomic analyses can increase the accuracy of microbial indicators as well as the scope of detectable responses. Advances in metagenome analysis software have expanded the accessibility of metagenomics tools, allowing for rapid identification of draft genomes and intuitive inference of microbial population dynamics (Eren et al. 2015). Metagenomic approaches also come with their own technical challenges, as metagenomes extracted from coral samples tend to be comprised of mostly host DNA. To be able to apply metagenomics to coral microbiome analyses effectively, since full genomes are available for only a handful of coral species, techniques must be developed to remove coral host signal. Metagenome analyses only reveal the genetic potential of microorganisms; genetic data gleaned from metagenomes can provide predictions of function, but cannot confirm that a microorganism is, in fact, performing that function, due to lack of protein and transcriptomic evidence.

More recent advances have led the field of microbiome monitoring toward metatranscriptomics tools and methods for the purposes of confirming microbial function and metabolism. While metagenomics tools only describe the functional potential of a microorganism, metatranscriptomics (i.e., sequencing of RNA to infer gene expression) provides information on realized function (Martinez et al. 2016). Gene expression elucidates function as well as interactions between microorganisms and their environment or host (Martinez et al. 2016). Using metatranscriptomes,

researchers across disciplines have been able to answer important questions about host-symbiont-environment interactions by identifying and quantifying stress-related function by revealing cross talk among hosts and their symbionts during environmental changes (Luo et al. 2015; Moitinho-Silva et al. 2014). This information is particularly relevant in the context of microbes as bioindicators, given their intimate connection with environmental changes and stressors and the multitude of changes in function and gene expression that could serve as indicators for environmental perturbation. Metatranscriptomic sequencing of white plague-diseased corals revealed "stress signatures" in the bacterial community, in this case characterized by an increased abundance of proteins associated with DNA repair as well as an enrichment of genes associated with virulence and antibiotic resistance (Daniels et al. 2015). Researchers also observed metabolic shifts, specifically toward glucogenesis, ammonia assimilation, and sulfur assimilation (Daniels et al. 2015). Such results provide insight to differences in microbial function during periods of stress and disease and suggest that these changes may also have downstream effects on coral and/or ecosystem health. While this remains an emerging field, it holds the promise of elucidating the complex interactions that occur in the microbiome and strengthening the potential of coral microbiomes as bioindicators of reef health (Cardenas and Tiedje 2008).

5   Challenges and Future Directions

Despite the promise they show as bioindicators, the study of microorganisms still presents significant challenges. While the abundance of microbes in reef ecosystems provides many advantages, it can also complicate interpretation of the cause-and-effect relationship between shifts and environmental changes. While a shift in diversity, function, structure, or abundance might be directly related to a specific stressor or change, it could also result from a number of other factors. Coral species often differ significantly in their microbial assemblages and responses to ecosystem changes, providing different "normal" microbial baselines and bioindicative responses. Corals also contain PCR inhibitors, which can make amplicon-based analyses challenging. Host genetic sequences can also be substantially more abundant than microbial sequences, particularly in metagenomic studies. This can make it difficult to target and separate out microbial sequences for analysis. These hurdles hinder our ability to create microbial baselines, determine microbial specificity to environmental changes, and properly analyze microbial data, all of which are necessary when dealing with bioindicators. As a result, they represent significant roadblocks in the process of developing microbial bioindicator-based monitoring systems.

In addition to these technical challenges, an incomplete yet growing knowledge of the ecological and biological underpinnings of microbiomes limits our ability to effectively use microbiomes as bioindicators. Though microbiomes have been studied extensively in diverse systems, research on the microorganisms associated

with corals is still in its infancy. Limited understanding of baseline diversity, community structure, and function across different coral clades and species prevents the development of robust bioassays. Many innovative projects are attempting to tackle this problem by establishing "core" microbial members of the coral holobiont, information that is necessary when attempting to study and connect environmental changes to shifts in the coral microbiome (Ainsworth et al. 2015). Other studies are targeting specific microbial responses to stressors, using tools such as tag sequencing, metagenomics, and metatranscriptomics in order to draw clearer and more specific connections between stresses and microbial stress responses (Lee et al. 2016; Mouchka et al. 2010; Vega Thurber et al. 2009). Others still are attempting to identify the specific location of different microbial members within coral micro-habitats (e.g., physiologically distinct compartments such as the skeleton, mucus, and tissue), in order to gain a better understanding of the functions of specific microorganisms within the coral host (Ainsworth et al. 2015). Glasl et al. (2017) outline the importance of such research for management efforts that seek to incorporate microorganisms as bioindicators and provide a detailed list of steps and measures that must be taken to successfully implement microbes as bioindicators.

The staggering rate of advancement in the field of genomics, particularly single-cell genomics (considered a new frontier in the "omics" fields), has great potential to expand our understanding of the roles of specific microorganisms in the coral holobiont (Wang and Bodovitz 2010). Current approaches to study the function of entire microbial communities (such as metagenomics or metatranscriptomics) are unable to provide functional information for potentially key microorganisms which may be less abundant and thus may receive lower or no coverage within the metagenome. Single-cell genomics can fill this gap, as it can be used to elucidate a fuller picture of a single uncultivated microorganism by providing its complete genome and thus full functional potential, and a small number of studies have already begun to implement this tool in both sponge and coral systems (Hentschel et al. 2012; Kamke et al. 2013; Pernice et al. 2012; Siegl et al. 2011). Similarly, proteomic approaches are beginning to gain traction, delving deeper into the roles of proteins in host-microbe interactions (de O Santos et al. 2011). Machine learning, another technological advancement referring to the development of algorithms to identify patterns and make predictions based on existing data, has also recently been applied to biological sciences and has great potential as a way to recognize previously invisible patterns in many different types of microbiome datasets (Tarca et al. 2007).

While a substantial gap remains between our current understanding of coral microbiomes and the knowledge base required to systematically and effectively employ microorganisms as bioindicators, continued technological advances and innovative studies are rapidly narrowing this gap.

6 Conclusion

As coral reefs continue to decline worldwide, it is imperative that we develop robust monitoring methods for the rapid identification of environmental changes driving coral loss. Such monitoring methods must be highly sensitive and provide information on whole ecosystem health in the face of changes in water quality and stressors. Given their intimate connection to corals and their ability to respond to a diverse array of stressors, coral microbiomes hold great potential as bioindicators of reef health. A system of microbial bioindicators would beautifully complement current reef monitoring methods that focus more on coral colony and macroorganismal health. Microbial bioindicators' sensitivity to alterations in the environment also shows that they have the potential to provide specific information on ecosystem changes that could serve as an early-warning system for effective adaptive management. Such a breakthrough could revolutionize reef conservation, as well as conservation efforts in other systems that aim to employ microorganisms for the development of more sensitive and accurate monitoring systems. Ultimately, the use of microbial bioindicators for the purposes of monitoring reef ecosystem health would aid conservation efforts significantly by providing more accurate information on the fluxes and changes occurring in the reef environment, allowing for more informed management decisions in the future.

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