Implications of Ocean Acidification for Marine Microorganisms from the Free-Living to the Host-Associated

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Anthropogenic CO_2 emissions are causing oceans to become more acidic, with consequences for all marine life including microorganisms. Studies reveal that from the microbes that occupy the open ocean to those intimately associated with their invertebrate hosts changing ocean chemistry will alter the critical functions of these important organisms. Our current understanding indicates that bacterial communities associated with their host will shift as pH drops by another 0.2–0.4 units over the next 100 years. It is unclear what impacts this will have for host health, however, increased vulnerability to disease seems likely for those associated with reef corals. Natural CO_2 seeps have provided a unique setting for the study of microbial communities under OA *in situ*, where shifts in the bacterial communities associated with corals at the seep are correlated with a decline in abundance of the associated coral species. Changes to global biogeochemical cycles also appear likely as photosynthesis and nitrogen fixation by pelagic microbes becomes enhanced under low pH conditions. However, recent long-term studies have shown that pelagic microbes are also capable of evolutionary adaptation, with some physiological responses to a decline in pH restored after hundreds of generations at high pCO_2 levels. The impacts of ocean acidification (OA) also will not work in isolation, thus synergistic interactions with other potential stressors, such as rising seawater temperatures, will likely exacerbate the microbial response to OA. This review discusses our existing understanding of the impacts of OA on both pelagic and host-associated marine microbial communities, whilst highlighting the importance of controlled laboratory studies and *in situ* experiments, to fill the current gaps in our knowledge.

Keywords: ocean acidification, microorganisms, invertebrates, coral, biogeochemical cycle, holobiont, CO_2 Seeps, adaptation

INTRODUCTION

Since the industrial revolution, the oceans have absorbed ∼30% of human generated CO_2 emissions, leading to a reduction of seawater pH by 0.1 units (i.e., more acidic) and equating to a 26% increase in the concentration of hydrogen ions (Royal Society, 2005; Doney, 2010; Rhein et al., 2013). To put this into perspective, the current average pH of surface seawater is now ∼8.1 (Doney, 2010; Rhein et al., 2013) and seawater pH has

not dropped below this level in the last 23 million years (Pearson and Palmer, 2000). Furthermore, current emissions modeling data predict an alarming decline in surface ocean pH by a further 0.2–0.4 units by 2100 (Rhein et al., 2013). Such a shift in the chemistry of the oceans will have profound effects on the organisms that inhabit our marine ecosystems, from the microorganisms through to the largest of the marine mammals (Guinotte and Fabry, 2008). Given ocean pH varies with season, depth, and productivity, some marine organisms may already be experiencing pH values below levels predicted for the next century (Joint et al., 2011). However, it is the long-term, persistent decline in global ocean pH, termed ocean acidification (OA), which is of particular concern and has gained considerable attention over the last decade (**Figure 1**). Calcifying organisms are a focal point of research efforts because as the partial pressure of carbon dioxide ($p\text{CO}_2$) rises, the subsequent concentration of carbonate ions and the saturation state of calcium carbonate (CaCO_3) minerals declines, making it more difficult for marine organisms to build shells and skeletons (Orr et al., 2005; Doney, 2010; Erez et al., 2011). Comparative studies on the effects of OA on marine microorganisms have lagged far behind in comparison to the speed at which these changes are occurring (**Figure 1**).

This review explores how a further reduction in ocean pH may influence processes governed by marine microbes, within both host-associated and pelagic environments. Tropical reef invertebrates are a specific focus, given that coral holobionts have emerged as model habitats for studying microbial influences on host health. Additionally, we highlight volcanically driven CO_2 seeps as ideal experimental settings to assess the long-term effects of high $p\text{CO}_2$ and reduced pH on microbial processes. Seep habitats are compared and contrasted with studies conducted under more controlled manipulative conditions that may include additional forces such as temperature within mesocosm experiments. Lastly, we examine the potential for OA to act in synergy with other environmental stressors, causing small and large-scale changes in marine microbial processes.

MARINE MICROBES INFLUENCE GLOBAL PROCESSES AND HOST FITNESS THROUGH SMALL-SCALE INTERACTIONS

The microorganisms inhabiting our oceans are central to key global processes, such as carbon, nitrogen and sulfur cycling, as well as the exchange of trace gases that have direct impacts on local climate (Moran et al., 2003; Webster and Hill, 2007; Raina et al., 2009, 2010, 2013; Doney, 2010). Bacteria dominate the oceans in terms of abundance, diversity, and metabolic activity (Azam et al., 1983; Giovannoni and Stingl, 2005), and it is in the oceans that approximately half of the global primary production occurs (Field et al., 1998). Heterotrophic and autotrophic bacteria both utilize and contribute substantially to marine primary production, and thus are not only heavily involved in the cycle of carbon, but form the basis of marine ecosystem function (Azam et al., 1983; Pomeroy et al., 2007). Single cell cyanobacteria, for example, have a major impact on the global carbon cycle, with *Synechococcus* and *Prochlorococcus* contributing up to 50%

of the fixed carbon in marine systems (Partensky et al., 1999), while heterotrophic bacteria rapidly respire about 50% of the fixed carbon in marine systems (Giovannoni and Stingl, 2005). Cyanobacteria are also heavily involved in the global nitrogen cycle, with both *Trichodesmium* and unicellular diazotrophs making significant contributions to N_2 fixation in marine systems (Capone et al., 1997; Montoya et al., 2004; Welsh et al., 2008). This important role allows inert N_2 to become readily available for use in biological systems thereby supporting marine productivity, and is especially significant in oligotrophic waters such as coral reefs, allowing them to be among the most productive ecosystems in the ocean (Larkum et al., 1988).

Coral reefs occupy less than 1% of the Earth's marine environment, however they are of immense value as they support ∼25% of the marine biodiversity and millions of people around the globe depend on them for their livelihood (Moberg and Folke, 1999). Recent declines in the health of coral reefs are predicted to cause great social, environmental, and economic consequences (White et al., 2000; Jones G. P. et al., 2004; Hoegh-Guldberg et al., 2007), making them a necessary habitat in which to study the effects of OA. Scleractinian corals build the foundations of these reefs and rely on favorable carbonate ion and aragonite (a mineral form of calcium carbonate) concentrations to lay down their skeletons. Since elevated CO_2 reduces both the aragonite and carbonate ion concentrations in seawater, coral reefs are predicted to be the biggest losers in an acidified ocean, greatly reducing the growth of all organisms with calcium carbonate skeletons (Doney et al., 2009; Fabricius et al., 2011). On coral reefs, microorganisms provide essential roles in nutrient cycling, which contributes to ecosystem productivity in nutrient poor waters (see Bourne and Webster, 2013). Microbial communities in reef sediments are responsible for the metabolism of organic matter that is used by the surrounding reef ecosystem (Bourne and Webster, 2013), and benthic nitrogen-fixing bacteria are responsible for an important source of internal nitrogen on coral reefs (Shashar et al., 1994; Cardini et al., 2014).

In addition to the roles microorganisms perform in ecosystem-wide geochemical and nutrient cycling, a wide range of reef invertebrates, including cnidarians, sponges, molluscs, and echinoderms also rely on complex associations with microbes to maintain their health (Lesser and Blakemore, 1990; Rosenberg et al., 2007; Roeselers and Newton, 2012; Webster and Taylor, 2012). Perhaps one of the best studied of these relationships involves the association between scleractinian corals and their microbes, a metacommunity known as the holobiont (Rohwer et al., 2002). The holobiont includes the coral animal, endosymbiotic photosynthetic dinoflagellates (*Symbiodinium*), and diverse members of the Bacteria, Archaea, and Protista, as well as fungi and viruses (Rohwer et al., 2002; Rosenberg et al., 2007). It has been established for some time that Symbiodinium provide an energy source for their hosts through photosynthesis, but more recently Symbiodinium have also been shown to influence and potentially regulate the intracellular pH of corals (Venn et al., 2009).Bacteria associated with corals have been shown to be diverse, though the functional roles are poorly understood and only recently have some roles been elucidated (**Figure 2**).
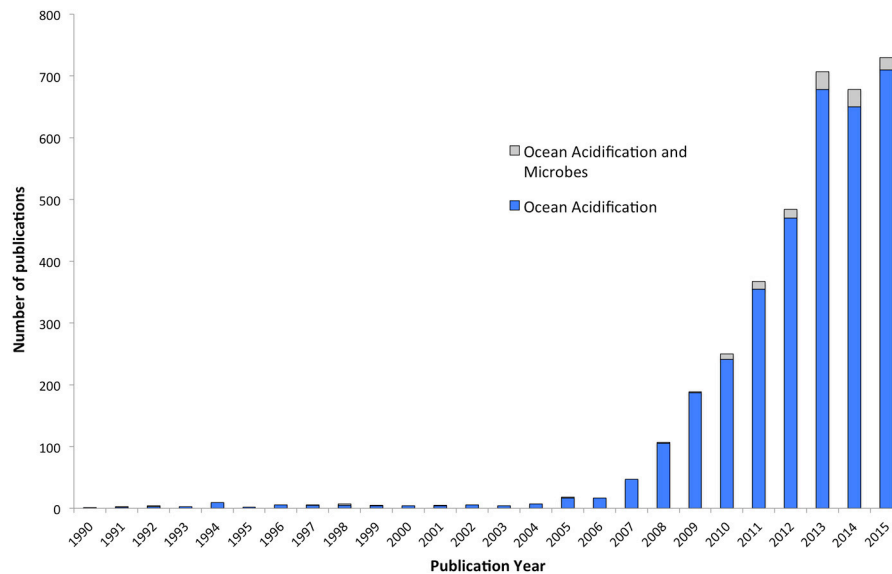
FIGURE 1 | Number of publications based on the key words [ocean acidification] and [ocean acidification and microbes] or [ocean acidification and microorganisms] or [ocean acidification and bacteria], as searched through the Web of Science database. Research on OA has increased dramatically in the past decade, however, little research has been directed toward the implications of OA for microbes.

Examples of the roles coral-associated bacteria perform include nitrogen-fixation (Lesser et al., 2004; Lema et al., 2012, 2014b), which allows their host to exploit inorganic nitrogen, enabling them to thrive in tropical waters where nitrogen is the most limiting nutrient to primary productivity (D'Elia and Wiebe, 1990). Efficient internal recycling of nitrogen with the *Symbiodinium* is believed to help sustain a healthy symbiosis (Cardini et al., 2015; Rädecker et al., 2015), while carbon-fixing cyanobacteria have been said to provide an additional energy source when *Symbiodinium* densities are low (Fine and Loya, 2002). High levels of dimethylsulfoniopropionate (DMSP), produced by both the coral host and *Symbiodinium*, are passaged to the surrounding water and likely provide an ideal nutrient source for coral-associated bacteria (Raina et al., 2009, 2010, 2013). Bacterial metabolism of DMSP is then proposed to provide antioxidants that protect the coral tissues against harmful reactive oxygen species (Sunda et al., 2002; Yost et al., 2010). Coral-associated bacteria not only produce nutrients utilized by the coral and *Symbiodinium*, but also protect the coral from pathogens through the production of potent antibiotics and by maintaining a niche otherwise available to opportunistic microbes (Ritchie, 2006). Functions of associated prokaryotes are not limited to corals with many other coral reef invertebrates benefitting from these microbial relationships (Duperron et al., 2009; Schmitt et al., 2012; Webster and Taylor, 2012). However, the majority of studies examining host-associated microbial communities on coral reefs remain largely descriptive. There is a need for further research into the functional roles of host-associated bacteria, the effects of OA on microbial metabolic processes, and how subsequent community shifts might affect overall host fitness.

IMPLICATIONS OF OA FOR HOST-ASSOCIATED MARINE MICROORGANISMS

Marine invertebrates, like all marine organisms, have evolved in environments rich in microorganisms, and have formed specific mutualistic relationships that persist through generations (McFall-Ngai, 1999). Sponges harbor dense microbial communities and in some cases this may comprise up to 40% of the sponges' total biomass (Webster and Hill, 2007; Webster and Taylor, 2012). However, despite the ecological importance of sponges on reefs, very little research has focused on how the associated microbial communities of sponges will be influenced by low pH (Bell et al., 2013). A limited number of studies suggest that sponge populations will undergo profound changes under low pH conditions (Goodwin et al., 2014; Morrow et al., 2014), and are one group of marine invertebrates that may benefit from projected climate change and OA scenarios. Thus, it has been argued that coral reefs may become sponge-dominated reefs under future ocean conditions (Bell et al., 2013). Shifts in the associated microbial communities are thought to be one of the mechanisms driving these changes at the sponge community level (see later section CO_2 seeps: a window into the future). Such changes in microbial communities have been found following other environmental stressors, such as elevated temperature and heavy metals, where shifts in the normally stable microbial communities have been linked with a decline in sponge health (Webster et al., 2001, 2008; Fan et al., 2013). However to date, no studies have examined the effects of pH on sponge-associated microbial communities under controlled laboratory conditions.
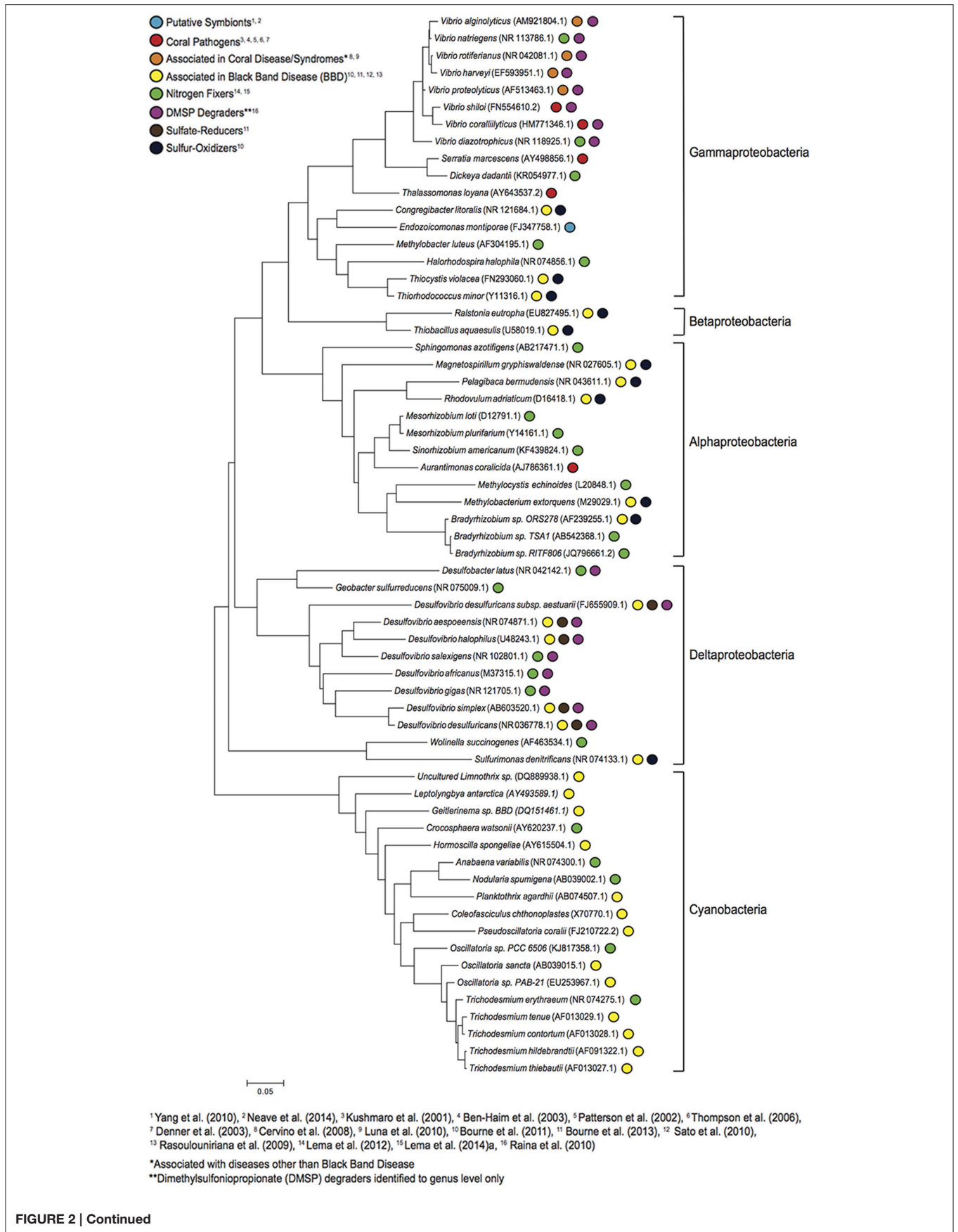
FIGURE 2 | Continued

Other filter feeding invertebrates, such as bivalves, also host symbiotic bacteria, and of particular interest are those that host chemosynthetic bacteria. Identified in five bivalve families thus far, chemosynthetic bacteria have the ability to convert CO_2 or methane (CH_4) into organic compounds, providing the host with nutrients and allowing it to survive in a variety of habitats, including those as acidic and harsh as hydrothermal vents (Roeselers and Newton, 2012). Under future OA conditions, it may be symbioses such as these that will become advantageous in a high CO_2 world. However, not all microorganisms associated with invertebrates are beneficial, and through filter feeding, bivalves may accumulate large numbers of pathogenic bacteria (Asplund et al., 2014). While such accumulations of pathogens can usually occur without the host becoming affected, the blue mussel pathogen *Vibrio tubiashii* was shown to be more infectious under low pH conditions (Asplund et al., 2014). With the enormous range of invertebrate-microbial associations that may be influenced by OA, there is an urgent need to address the paucity of knowledge in this field of research.

Changes in host-microbial associations (e.g., community shifts) are often considered detrimental to the host, although they may provide a means for rapidly adapting to environmental change, as suggested by the coral probiotic hypothesis and hologenome concept (Reshef et al., 2006; Zilber-Rosenberg and Rosenberg, 2008). The hologenome concept considers the collective genomic content of the host and microbial constituents as a single unit of evolutionary selection. This concept has gained recent popularity (Bordenstein and Theis, 2015), but is contested by others, who suggest we consider that microorganisms can maintain intimate associations with their host over significant time-frames without undergoing natural selection to primarily benefit the host (Moran and Sloan, 2015). Evidence that microbial communities overlap among host species and may differ with geographic location (Littman et al., 2010), and season (Li et al., 2014), suggests that microbial communities do shift to some extent to suit the surrounding environment, which supports the probiotic hypothesis. However, if the host and its associated microbes function as a single unit of selection (i.e., a hologenome), one would expect a spatially and temporally stable microbiome that is ubiquitously associated with its host species. Considering this, current evidence suggests that host species and their associated microorganisms are better represented as holobionts or metaorganisms, rather than as hologenomes. Understanding the coevolution of corals and their associated microbiomes is a current research focus, which will help to further establish the appropriateness of these alternative models (Bordenstein and Theis, 2015; Moran and Sloan, 2015).

On tropical coral reefs, the coral holobiont is emerging as a model system for exploring host-microbial interactions (Bourne and Webster, 2013), and much of the remaining research exploring the impact of OA on host-associated microbes has been focused here. Using aquaria-based experiments, coral-associated bacterial communities have been shown to change in response to decreasing pH, with potential shifts toward a more disease-associated state (Vega Thurber et al., 2009; Meron et al., 2011; Webster et al., 2013a). In *Acropora eurystoma*, changes to bacterial communities were documented in the tissues, skeleton, and mucus of colonies exposed to pH 7.3 for 10 weeks, compared with those exposed to ambient pH 8.2 (Meron et al., 2011). One notable change was an increase in bacteria belonging to *Vibrionaceae* and *Alteromonadaceae*, which have both previously been associated with stressed or diseased corals (Bourne et al., 2008; Sunagawa et al., 2009; **Figure 2**). An increase in antibacterial activity was also seen at low pH and was attributed mostly to an increase in *Vibrio* sp., suggesting a mechanism for host invasion (Meron et al., 2011). Disease-associated microbes were also detected when colonies were exposed to pH levels 1.4 units below ambient (8.1), including bacteria related to the phylum *Bacteroidetes* and disease-associated fungi (Vega Thurber et al., 2009). Vega Thurber also detected an increase in genes encoding for resistance to antibiotics and toxins, as well as an increase in virulence genes, which may have facilitated the increase in disease-associated microbes at low pH. However, it should be noted that the pH conditions used in studies by Meron et al. (2011) and Vega Thurber et al. (2009) were below what is expected over the next 100 years and may represent unrealistic OA conditions.

A similar trend was again detected in *A. eurystoma*, along with foraminifera and crustose coralline algae (CCA), when pH was reduced from 8.1 to 7.9 (Webster et al., 2013a). After 6 weeks exposure to low pH, microbial profiles were significantly different to those at pH 8.1, and in the case of *A. eurystoma,* included a loss of sequences that were affiliated with the *Proteobacteria* and previously shown to be associated with healthy corals (Raina et al., 2009; Sato et al., 2010; Sunagawa et al., 2010). Significant shifts in the microbial biofilm associated with CCA as a result of reduced pH has also correlated with a reduced ability of CCA to induce coral larval settlement, which is an additional concern for the stability and/or recovery of reefs globally (Webster et al., 2013b). Taken together, the above results suggest an increase in microbial instability with impacts for many coral reef organisms, potentially leading to heightened disease susceptibility due to a shift in their normal microbiome, antibacterial, and virulence activity.

Not only is the microbial community associated with marine invertebrates likely to shift under future OA conditions, but the functional roles these communities perform also appear to be influenced. For the first time, evidence has shown that nitrogen (N_2) fixation by coral-associated bacteria can be affected by OA (Rädecker et al., 2014). Under high pCO_2 conditions (1080 μatm), N_2 fixation associated with the *Seriatopora hystrix* holobiont was significantly reduced by 53% compared with ambient pCO_2 conditions (486 μatm). This is in contrast to the planktonic cyanobacterium, *Trichodesmium,* where N_2 fixation

has shown to become enhanced under high pCO_2/low pH conditions (Levitan et al., 2007; Liu et al., 2010). Why N_2 fixation appears to decline in the coral holobiont under high pCO_2 conditions is currently unknown, however it is suggested this may be caused by an energy trade-off within the holobiont due to the increased energy demand by calcification under high pCO_2 conditions (Rädecker et al., 2014).

Whilst the above short-term laboratory studies provide important preliminary insight into how microbial communities and their functions may change under future OA conditions, care must be taken when interpreting conclusions due to potential pitfalls related to organism handling, experimental design, and time-scale. A recent review of OA studies showed that in 465 studies published between 1993 and 2014, 95% of these either used inappropriate experimental design or data analysis, or did not provide enough methodological details to determine the precise design (Cornwall and Hurd, 2016). For instance, some experiments were limited in aquaria or mesocosm replication, leading to pseudoreplication among replicates. Many laboratory studies also occured over short time periods (days to weeks), when realistically the effects of OA on marine organisms will occur over much longer more subtle time scales. Thus there is a push for long-term, highly replicated, mesocosm studies to better simulate the potential implications of OA on coral reef ecosystems (Leblud et al., 2014). In the meantime, additional research endeavors have utilized unique marine environments where the pH is naturally lower than ambient ocean conditions due to upwelling or CO_2 seepage into the sediments and water column. In these areas, organisms are already living unmanipulated under high pCO_2/low pH conditions that are often representative of predicted levels for the next century.

CO_2 SEEPS: A WINDOW INTO THE FUTURE

Volcanic CO_2 seeps provide a natural and unique experimental setting to assess the long-term effects of OA on marine organisms *in situ* (Hall-Spencer et al., 2008; Fabricius et al., 2011, 2014; Borell et al., 2014; Morrow et al., 2014). In Papua New Guinea (PNG), for example, the D'Entrecastraux Islands of the Milne Bay province contain reefs where volcanic seeps release 99% pure CO_2 into the surrounding sediments and sea water. At these seeps, pH is ~7.8 (however varies with intensity and proximity to the seep) and reflects what is predicted for the next century (**Figure 3**). Compared with benthic invertebrate communities on nearby reefs that are exposed to the same temperatures, currents and light, seep communities show dramatic changes in community structure, abundance and diversity (Fabricius et al., 2011, 2014; Morrow et al., 2014). The mechanism(s) underlying these community changes at high pCO_2 sites is unclear however, recent evidence suggests shifts in host-associated microbial communities at low pH sites may contribute to host fitness and success (Morrow et al., 2014).

To investigate the effects of elevated pCO_2 on host-associated microbes, Morrow et al. (2014) examined the bacterial communities associated with the corals *Acropora millepora* and *Porites cylindrica* at a CO_2 seep in PNG. Both corals were less abundant at the CO_2 seep, and were found to host
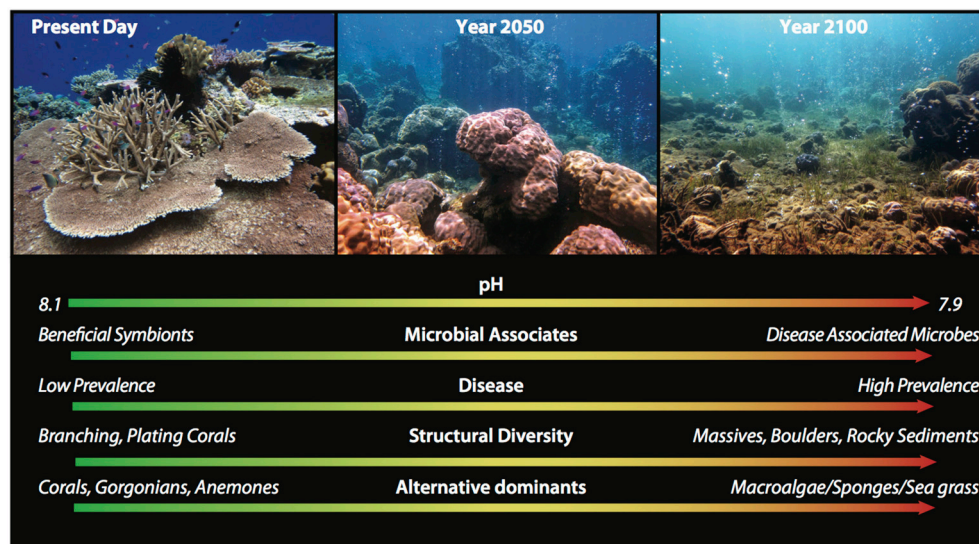

FIGURE 3 | Photos, taken along a $pCO2$/pH gradient in Papua New Guinea (year 2014), and text depict three potential scenarios for coral reefs under present day and future (year 2050 and 2100) OA conditions if we continue on current predicted CO_2 emissions trajectories. Present Day illustrates a healthy coral reef with high structural complexity and diversity that is likely colonized by beneficial microbial associates and experiences a low incidence of disease. As pCO_2 increases, scenarios for years 2050 and 2100 illustrate the successional degradation of coral reef heterogeneity (structural diversity), destabilization of microbial associations and increase in disease, transitioning to an alternative stable state composed of competitive dominants (e.g., sponges, macroalgae) and/or sediment/rubble. Arrows from green to red indicate the positive to negative changes a coral reef might experience over time. (Photo credit: K. Morrow).

distinctly different microbial communities compared with corals at control sites. This was primarily driven by a decrease in *Gammaproteobacteria* at the seep site, specifically a reduction in the relative abundance of *Endozoicomonas* spp. in *A. millepora* corals. This putative symbiont has been found in high abundance in other coral species (Morrow et al., 2012; Bayer et al., 2013), and is thought to contribute to the nutritional requirements and defense system of their invertebrate hosts (Yang et al., 2010; Neave et al., 2014; **Figure 2**). In a similar study, two Mediterranean coral species, *Balanophyllia europaea,* and *Cladocora caespitosa*, were transplanted along a pH gradient at a CO_2 vent in the Gulf of Naples, Italy. After 7 months, neither species demonstrated a significant shift in microbial community composition along the pH gradient of 7.3–8.1 (Meron et al., 2012). However, changes in the relative abundance of bacteria in the class *Gammaproteobacteria* were apparent in the tissues of both corals increasing in *C. caespitosa* and decreasing in *B. europaea* at low pH. An increase in bacteria related to the *Bacteroidetes* were also found in *B. europaea* corals, a phylum also found in higher abundance within the lesions of black band disease (Barneah et al., 2007). However, the corals transplanted in this study do not normally occur in the area of the vent, and therefore may not illustrate the microbial community associated with corals adapted to living at lower pH conditions over extended time periods. Furthermore, no data were provided for bacterial communities associated with corals prior to transplantation from control to seep sites. Nevertheless, it shows potential resilience of some coral-microbial associations, while others may be more susceptible to reduced pH.

Research at CO_2 seeps has also shown that coral physiology can be influenced by elevated pCO_2 levels, which may indirectly influence the associated microbial community. For instance, at the PNG CO_2 seeps, *Acopora millepora* was found to have an increase in net photosynthesis rates and a decrease in dark calcification rates at the seep (862 μatm) in comparison to the control site (323 μatm; Strahl et al., 2015). A healthy coral host produces a complex mixture of metabolites as a result of normal cellular homeostasis that can drive shifts in coral-associated microbial communities (Raina et al., 2010; Nelson et al., 2013). Common marine bacteria such as the Bacteroidetes, Altermonadaceae, and Vibrionaceae are able to colonize such surfaces and take advantage of the enriched organic matter (Dang and Lovell, 2016). However, corals that are sensitive to changes in surrounding pH will divert resources away from key metabolic processes, thereby changing the amount and composition of the exudates released and subsequently influencing the associated microbial consortia. As discussed previously, the microbial community associated with *A. millepora* was different at the seep site compared to the control (Morrow et al., 2014). It may be that the changed physiology of the host, in addition to the direct effects of elevated pCO_2 and lowered pH, influences the associated microbial community. Future research would benefit from correlative studies that examine the impact of host physiology on host-associated microbial communities, particularly when environmental conditions appear to have disparate impacts on host physiology. For example, a small

number of coral and sponge species appear to be more tolerant of high pCO_2/low pH conditions in PNG and provide an ideal system for examining these changes (Fabricius et al., 2011; Strahl et al., 2015).

Seagrasses and sponges have also been shown to host significantly different microbial communities at CO_2 seep sites, when compared with control sites of ambient pH. Seagrasses host epiphytic biofilms, which are known to affect seagrass physiology, as well as their interactions with other reef organisms (Sand-Jensen, 1977; Bengen et al., 2011). Evidence from CO_2 seeps in PNG suggests that at low pH these communities show a pronounced shift in both prokaryotic and eukaryotic assemblages when compared with control sites (Hassenruck et al., 2015). Specifically, increases in bacteria associated with coral diseases (Fusobacteria and Thalassomonas) were found at the seep site, raising concerns about the increase in susceptibility of disease on coral reefs in a high CO_2 environment, as well as the potential for seagrasses to act as reservoirs for coral pathogens (Sweet et al., 2013). Sponges, like corals, also consist of a very different community at CO_2 seep sites compared with nearby reefs of ambient pH (Fabricius et al., 2011; Goodwin et al., 2014; Morrow et al., 2014). At a PNG CO_2 seep, both *Cinachyra* sp. and *Coelocarteria singaporensis* sponges are significantly more abundant at the seep site, while other species, such as *Stylissa massa*, are less abundant (Morrow et al., 2014). An analysis of the microbial communities associated with sponges located at both seep and ambient control sites revealed significant shifts in associated microbial communities (Morrow et al., 2014). Most notably, the two more abundant sponge species at the seep site hosted significantly more Cyanobacteria-related bacteria in the genus *Synechoccocus*, a photosymbiont that is known to contribute up to 50% of the carbon demand of sponges (Erwin and Thacker, 2008). This illustrates how a drop in pH can potentially benefit the host through a shift in associated microbes that provide additional nutritional resources. Thus, although many organisms will be detrimentally impacted by future OA conditions, some hosts may be able to take advantage of their microbial partners ability to adapt more readily to shifting environmental conditions, which may lead to fundamental changes in ecosystem structure. Moreover, host physiology will likely cause pH gradients on the surface or within tissues of the host (Kühl et al., 1995; De Beer and Larkum, 2001), and may be contrasted between CO_2 producing organisms (e.g., through respiration) and CO_2 consuming organisms (e.g., through photosynthesis), resulting in differential responses of host-microbial communities to reduced pH. Likewise, the effect of host physiology on host pH may represent a key difference in the response of host-associated microbial communities to OA, compared to free-living microbial communities.

LIKELY RESPONSES OF FREE-LIVING MARINE MICROORGANISMS TO OA

The response of pelagic marine microorganisms to OA, and subsequent effects on biogeochemical processes, are critical questions with global implications. Pelagic marine microbes are

naturally exposed to fluctuating pH, and populations in some environments already experience pH levels lower than what is predicted for the rest of the century, suggesting that some microbes may already possess the means to cope with rapid changes in pH (Joint et al., 2011). However, short-term studies have shown significant changes in calcification rates, growth rates, and primary production in coccolithophores at high pCO$_2$ levels (Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008; Langer et al., 2009). Initial reports stated that calcification was likely to decrease in coccolithophores with increasing CO$_2$ concentrations (Riebesell et al., 2000; Zondervan et al., 2001), however further studies have shown that consequences are not so clear. Following contradictory results of increasing calcification with increasing CO$_2$ in *Emiliania huxleyi* (Iglesias-Rodriguez et al., 2008), it was highlighted that *E. huxleyi* shows strain specific responses to increases in ocean pCO$_2$ concentrations, which likely explains much of the variation in results with studies using different cultures (Langer et al., 2009). Furthermore, this demonstrated that short-term culture-based studies are not sufficient to draw conclusions on how microorganisms will respond to OA.

Most marine microbes have short generation times, from hours to days under favorable conditions, in addition to highly versatile metabolic repertoires, which provides significant potential for acclimation and/or adaptation to decreasing pH over large temporal scales. This potential was realized when Lohbeck et al. (2012) tested for evolutionary adaptation in the globally distributed coccolithophore, *E. huxleyi,* over ∼500 generations. Cultures were grown under three different pCO$_2$ conditions (400, 1100, and 2200 μatm), and it was found that those grown under high pCO$_2$ conditions (2200 μatm) showed significantly higher rates of growth and particulate inorganic carbon (PIC) production than those grown under ambient (400 μatm) conditions, when exposed to high pCO$_2$ conditions. Further evidence of evolutionary adaptation was seen in the physiological response of the coccolithophore *Gephyrocapsa oceanica* (Jin et al., 2013). While low-level adaptation was discovered in terms of growth, cells showed a striking adaptation in elemental composition. Cell content and production rates for both particulate organic carbon (POC) and particulate organic nitrogen (PON) shifted back to ambient pCO$_2$ values (390 μatm) after ∼670 generations under high pCO$_2$ conditions (1000 μatm). Overall, the above results suggest acclimation of growth, calcification and photosynthetic carbon acquisition in coccolithophores to the heightened pCO$_2$ levels.

Marine microorganisms contribute to the carbon cycle via photosynthesis and the production of a calcium carbonate shell (CaCO$_3$). Therefore, understanding their ability to adapt to OA will have important implications. For example, if coccolithophores, the main calcifying phytoplankton, increase in calcification with OA, this would likely shift the ocean carbonate system toward a higher seawater pCO$_2$ concentration and promote CO$_2$ outgassing, since the calcification reaction produces CO$_2$. On the other hand, a more likely decline in coccolithophore calcification could lead to a negative feedback on atmospheric CO$_2$ concentrations through reduced CO$_2$ production (Zondervan et al., 2001; Iglesias-Rodriguez et al.,

2008; Hutchins, 2011). There is still no consensus as to how these microorganisms will respond to OA however, a crucial clue may lie in ancient marine sediment cores, which show a decrease in coccolith mass as CO$_2$ increases (Beaufort et al., 2011). Secondly, changes to the photosynthetic capacity in microorganisms due to reduced pH could also lead to profound changes in the carbon cycle. Cyanobacteria, for instance, contribute up to 50% of the fixed carbon in marine systems and thus represent a critical component of the global carbon cycle (Partensky et al., 1999). Although physiological responses to changes in pH are not uniform across cyanobacterial species, many show an increase in photosynthetic ability with increases in pCO$_2$(Hutchins et al., 2007; Kranz et al., 2009; also see review Liu et al., 2010). Since the photosynthesis reaction uses CO$_2$, this may lead to an additional negative feedback on CO$_2$ concentrations.

The nitrogen cycle is another biogeochemical cycle where marine microbes have a central role (Ward, 2000), and has a high potential to be fundamentally altered by OA. As the pH of seawater decreases, more ammonia molecules (NH$_3$) are converted to ammonium ions (NH$_4^+$) causing a shift in the equilibrium. This means less oxidation of ammonia to nitrite (NO$_2^-$) by ammonia-oxidizing microbes, and therefore less oxidation of nitrite to nitrate (NO$_3^-$) and an overall reduction of marine nitrification (Webster and Hill, 2007). Furthermore, denitrification, another step in the nitrogen cycle controlled by bacteria (Capone, 2000), may also be reduced because of the decline of nitrite and nitrate ions, ultimately causing a build-up of fixed nitrogen, with potential eutrophication implications (Webster and Hill, 2007). Lastly, N$_2$ fixation by *cyanobacteria* in the genus *Trichodesmium* have consistently been shown to increase with elevated pCO$_2$ (Levitan et al., 2007; Liu et al., 2010). This group contributes ∼50% of the total marine fixed N$_2$ (Capone et al., 2005), thus greater available nitrogen coupled with decreased denitrification, could further add to the build-up of fixed nitrogen in the ocean (**Figure 4**).

Experiments using microcosms and mesocosms have also proved useful in understanding the community effects of OA on pelagic marine microbes. Collectively, these studies have illustrated that elevated pCO$_2$ and declining ocean pH can disrupt the community equilibrium, leading to further changes in biogeochemical cycles as new species become dominant (Riebesell et al., 2007; Liu et al., 2010; Krause et al., 2012, 2013; Bowen et al., 2013). For instance, Riebesell et al. (2007) used mesocosms to demonstrate multiple shifts in the dominant phytoplankton taxa as a consequence of increased CO$_2$, and showed a net increase in inorganic carbon consumption by the community. Changes in community structure of ammonia-oxidizing bacteria (AOB) have also been shown in response to decreasing pH, owing to the increase in relative abundance of the *Nitrosomonas ureae* clade (Bowen et al., 2013). This group is able to switch to urea as an energy source, which may underpin its success at low pH levels where the availability of NH$_3$ is decreased (Pommerening-Roser and Koops, 2005; **Figure 4**). Whether or not these changes in community structure have the ability to offset potential alterations to the nitrogen cycle is yet to be elucidated. Moreover, these studies have
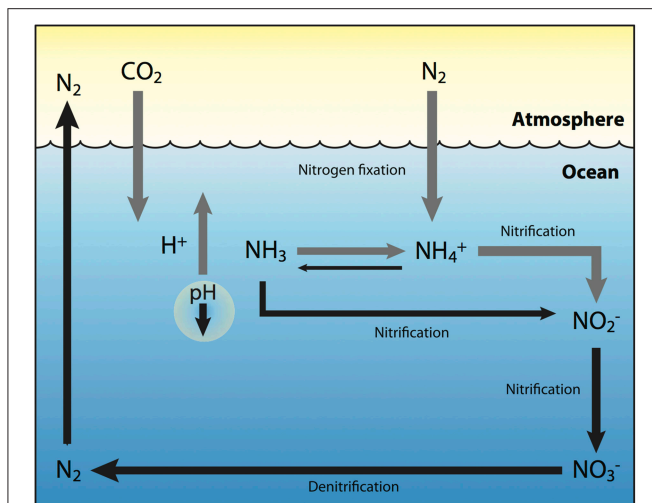
FIGURE 4 | Changes to the marine nitrogen cycle under future ocean acidification conditions. As additional CO_2 is absorbed by the ocean and reacts with water molecules (H_2O), carbonic acid is formed and broken down into both bicarbonate (H_2CO_3) and hydrogen (H^+) ions (gray arrow), which reduces bioavailable carbonate and decreases ocean pH (black arrow). This is likely to enhance nitrogen fixation by diazatrophs (gray arrow), which utilize H^+ ions to convert N_2 into bioavailable forms such as ammonia (NH_3) and ammonium ions (NH_4^+). However, as pH decreases, and more ammonia molecules are converted to ammonium ions (gray arrow), there is less oxidation of ammonia to nitrite (NO_2^-), resulting in an overall decrease in nitrification and denitrification (black arrows). This in turn would lead to a further build up of fixed nitrogen in the ocean, with the potential consequence of eutrophication. Gray arrows represent an increase while black arrows represent a decrease in the associated process.

occurred over days to weeks and therefore assess the short-term "shock-effects" on these communities, highlighting the need for more long-term studies to fully comprehend the effect of OA on pelagic microbes (Liu et al., 2010; Godbold and Calosi, 2013).

Long-term, *in situ* microbial time-series studies provide a powerful alternative to mesocosm studies for investigating marine microbial processes and dynamics. Such "microbial observatories" have changed the way we understand microbial involvement in biogeochemical cycles and enabled the discovery of novel microorganisms (such as *Prochlorococcus* spp.) and metabolic pathways (Steinberg et al., 2001; Karl and Church, 2014). For instance, the Hawaiian Ocean Time-series (HOT) was influential in recognizing the role of marine microbes in the global carbon cycle (Karl and Church, 2014), while the Bermuda Atlantic Time-series has illustrated that biogeochemical variability is ultimately tied to larger-scale climatic changes (Steinberg et al., 2001). Moreover, the Western Channel Observatory (WCO) located in the English Channel has shown seasonal variation in the structure and dynamics of microbial communities and linked this with environmental variables, including temperature and nutrients (Gilbert et al., 2009, 2012). Recently, Sunagawa (2015) utilized 7.2 terabases of metagenome data sequenced from 243 *Tara* Ocean samples collected across the globe and determined that temperature was the strongest factor in shaping pelagic microbiome composition. While these

observations have yet to address the issue of OA and marine microbes, they have no doubt provided a valuable baseline to which future microbial dynamics and processes can be compared. The establishment of new time-series programs in other waters around the globe will vastly improve our understanding of how marine microorganisms are influenced by OA (as well as other key environmental variables) and contribute to a potentially instrumental global collaboration (Gilbert et al., 2010).

SYNERGISTIC ENVIRONMENTAL STRESSORS: TEMPERATURE, POLLUTION, AND OA

The close coupling of OA with other anthropogenically-driven environmental stressors, notably ocean warming, means that OA will not occur in isolation (Boyd, 2011). A warming ocean is one of the most widely documented outcomes resulting from the continued global emission of climate gases (including CO_2 and methane) (Domingues et al., 2008; Rhein et al., 2013), and like OA will affect marine microbes globally. Although, the joint effects of increased ocean pCO_2 and temperature on the growth and physiology of marine microbes is still comparatively unknown, results from Fu et al. (2007) suggest the effects are synergistic, with increased pCO_2 causing greater changes at higher temperatures. Growth rates of *Synechococcus* were only slightly enhanced under high pCO_2 conditions but were doubled when combined with elevated temperature. Furthermore, photosynthetic efficiency and maximum carbon fixation were both greatest when the stressors were combined. This may be explained through the enzymatic dark reactions of photosynthesis, which are sensitive to both CO_2 and temperature (Beardall and Raven, 2004; Fu et al., 2007). However these responses were not consistent when studied in *Prochlorococcus*, further highlighting differences among cyanobacterial species. Likewise, coccolithophores also demonstrate synergistic responses under increased temperature and pCO_2. The optimum CO_2 levels for growth, organic carbon fixation and calcification have been shown to shift toward higher pCO_2 concentrations with increasing temperature (Sett et al., 2014). This combined response may explain the increase in coccolithophore occurrence in the North Atlantic. Since the 1960's, the continuous plankton recorder of the North Atlantic has shown an order of magnitude increase in coccolithophore abundance (from ~2 to 20%), with increases in CO_2 and temperature anomalies tracked through the Atlantic Multidecadal Oscillation identified as the highest probable environmental drivers of the change (Rivero-Calle et al., 2015). Thus OA, when combined with increasing temperatures, may not be as detrimental to this group of microbes as previously thought. Furthermore, coccolithophores hold a rapid adaptive potential to future ocean conditions. In a study of ecological relevance, Schlüter et al. (2014) showed that *E. huxleyi* underwent evolutionary adaptation after one year of being exposed to high temperatures and CO_2. While cells had evolved to become smaller in size and contained less carbon, organic carbon cell

quotas (PIC:POC) had been restored to initial levels owing to faster growth and increased production, with calcification rates also being partially restored.

The combined effects of increased CO_2 and temperature on host-associated bacteria are also poorly understood. An increase in ocean temperature has been shown to cause a shift in the microbial community associated with corals and sponges (Bourne et al., 2008; Webster et al., 2008; Littman et al., 2010; Lee et al., 2015; Tracy et al., 2015), and new evidence suggests the effects of ocean pH and temperature are interactive. A recent laboratory study that incorporated variable pH and temperature conditions, demonstrated that the microbiomes of two Pacific corals species were fairly tolerant of elevated pCO_2 (738–835 µatm)/reduced pH (7.9) and elevated temperature (31°C) (Webster et al., 2016). However, they detected an interactive effect between stressors (pH and temperature), with pCO_2 causing more divergent community compositions at higher temperatures, although this was not significant. Additionally, it is noteworthy that they found that sequences related to the putative coral symbiont, *Endozoicomonas* sp., were significantly reduced in *A. millepora* corals exposed to experimental OA conditions. In the case of reef corals, future studies may benefit from studying how the microbiome will respond to low pH and elevated temperature when hosting particular strains of *Symbiodinium*. For example, juvenile colonies of *Acropora tenuis* hosting *Symbiodinium* clade C1 maintained a stable bacterial community through a thermal stress event, while those who hosted clade D underwent shifts in associated bacterial communities (Littman et al., 2010). Since different phylotypes of *Symbiodinium* also show differential responses to increased pCO_2 (Brading et al., 2011), it is possible that phylotypes that are more resistant to increases in pCO_2 may also provide some stability for the associated microbial community.

The combined effects of OA and warming on marine diseases are complex, and early evidence suggests that the effect of one stressor on disease susceptibility may counteract the effect of the other. For example, antibacterial activity in the blue mussel was reduced by around 40% at low pH (6.5), but when the temperature was increased from 12.5 to 17°C, antibacterial activity was restored to initial levels (Ellis et al., 2015). Conversely, the progression rate of coralline fungal disease (CFD) increased in crustose coralline algae (CCA) under elevated temperatures, however this rate increase was ameliorated under low pH (Williams et al., 2014). Reduced calcification of CCA was observed under low pH conditions, and it is possible this contributed to reduced disease progression. Many coral diseases are also known to display increased virulence under warmer ocean temperatures (Jones R. J. et al., 2004; Sato et al., 2009), and future research would benefit from studying pathogenesis under the combined effects of low pH and elevated temperature stress.

The interactive effects of OA and pollution on marine microbes are also complex and poorly understood, but are of high importance. Microbes are fundamental to environmental detoxification of pollutants and under low pH conditions

the chemistry of environmental pollutants will change with implications for the way they interact with marine organisms (Coelho et al., 2013). Toxicity of pollutants may also be indirectly affected through OA, given that as the composition of microbial communities change (Krause et al., 2012), so too may the detoxification processes provided by microbes. Metals that are involved in microbial growth and metabolism (e.g., iron) for example, may change in bioavailability with potentially harmful effects (Coelho et al., 2013). Coral-associated bacterial communities are sensitive to poor water quality (Bourne and Webster, 2013), sedimentation (Pollock et al., 2014), and elevated dissolved organic carbon (DOC). Higher DOC levels, correlated with human population growth and macroalgal overgrowth, are particularly detrimental and can cause microbial proliferation, oxygen depletion, and can disturb the holobiont equilibrium (Kline et al., 2006; Smith et al., 2006; Barott et al., 2011). However, the impact of these interactions under low pH conditions is still unknown.

CONCLUSIONS AND FUTURE DIRECTIONS

Anthropogenic CO_2 emissions have unequivocally led to decreasing pH in our oceans, and the resulting consequences for marine microorganisms and their hosts are only just beginning to emerge. Both the carbon and nitrogen cycles are likely to be fundamentally altered through increased primary production, changes to calcification, increased nitrogen fixation, and reduced nitrification. However, microbial responses to pH vary among species, and natural pH fluctuations may provide some resilience to physiological changes. Additional long-term studies or mathematical models that replicate long-term scenarios are clearly required to further understand whether keystone microorganisms or microbial communities can and will adapt to OA. The majority of studies suggest that reduced pH will result in a shift in the microbial communities associated with invertebrate hosts, with direct impacts on host health and disease susceptibility, although the magnitude of effects will vary among species. Comparisons between microbial communities associated with invertebrate "winners" and "losers" at CO_2 vent sites will provide important insights into future changes in acidifying oceans (Fabricius et al., 2011). Future studies should also focus on how the functional roles of host-associated microbes might be altered by OA, i.e., changes to photosynthetic capacity or nitrogen fixation. Finally, a better understanding of the synergistic effects of OA and other stressors is foremost. Microcosm and mesocosm experiments enable manipulative studies of the effects of multiple stressors on physiological processes in pelagic microbes (Coelho et al., 2013). For host-associated microbes, taking advantage of thermal anomalies at CO_2 vents may reveal insights into the combined effects of elevated temperature and OA *in situ* (Noonan and Fabricius, 2016). The potential for invertebrates to acquire new symbiotic microbes in an acidifying ocean, as well as adaptation of existing

ones must also be explored, as it is this relationship that will define the existence of marine invertebrates in a future ocean.

AUTHOR CONTRIBUTIONS

PO: Substantial contributions to the conception or design of the work; Drafting the manuscript; KM, DB, and BW: Substantial contributions to the conception or design of the work. Revising the manuscript critically for important intellectual content.

FUNDING

ACKNOWLEDGMENTS

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