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The Enzymology of Ocean Global Change

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Abstract

A small subset of marine microbial enzymes and surface transporters have a disproportionately important influence on the cycling of carbon and nutrients in the global ocean. As a result, they largely determine marine biological productivity and have been the focus of considerable research attention from microbial oceanographers. Like all biological catalysts, the activity of these keystone biomolecules is subject to control by temperature and pH, leaving the crucial ecosystem functions they support potentially vulnerable to anthropogenic environmental change. We summarize and discuss both consensus and conflicting evidence on the effects of sea surface warming and ocean acidification for five of these critical enzymes [carbonic anhydrase, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), nitrogenase, nitrate reductase, and ammonia monooxygenase] and one important transporter (proteorhodopsin). Finally, we forecast how the responses of these few but essential biocatalysts to ongoing global change processes may ultimately help to shape the microbial communities and biogeochemical cycles of the future greenhouse ocean.

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1. INTRODUCTION

Ocean biological productivity depends on a complex network of resources that includes major nutrients, trace elements, and organic growth factors. Equally important to planktonic food webs are physiochemical controls such as irradiance, salinity, oxygen, carbonate chemistry, and temperature. Continual temporal and spatial variability in these key environmental factors drives selection and leads to microbial adaptation, which in turn helps to shape ecological interactions such as competition, cooperation, and trophic relationships.

For marine microbes in today's ocean, however, the limits of this intrinsic flexibility relative to environmental change are being severely tested. The dissolution of a large fraction of anthropogenic CO_2 into the ocean has started a stepwise reaction leading to an accelerating drop in pH, a process known as ocean acidification (Orr et al. 2005). At the same time, sea surface temperatures continue to increase due to the heat-trapping properties of rising levels of atmospheric CO_2 (Bindoff et al. 2019). As we enter these uncharted waters, environmental research efforts have focused on the impacts of ocean warming and acidification on many species and groups of microbes (Hutchins & Fu 2017) or on a range of ecosystem dynamics and biogeochemical functions (Wannicke et al. 2018, Tagliabue et al. 2020).

The effects of a changing ocean environment are not necessarily decided primarily at the whole-organism or ecological level, though. Marine microbiologists are keenly aware that underpinning each and every physiological response to global change are the basic catalytic workhorses of all living cells: the enzymes. And in an ocean that is rapidly warming and acidifying, the abundance and activity of key enzymes that turn the wheels of the ocean carbon and nitrogen cycles are changing. Indeed, it has been known since the early days of biochemistry that rates of enzyme catalysis are often extraordinarily responsive to temperature and hydrogen ion concentration (pH) fluctuations. Enzyme activities typically increase in tandem with either temperature or pH up to some well-defined optimum threshold level, beyond which enzyme denaturation and inactivation occur relatively rapidly (Robinson 2015). In this review, we explore how a key set of enzymes with uniquely important influences on ocean biology and biogeochemistry may respond to a future warmer and more acidified ocean.

1.1. Ocean Acidification

A couple of decades ago, it was unimaginable that human activities could overwhelm the carbonate buffer capacity of the ocean. That type of event has happened only a few times in the history of the planet, usually associated with world-changing volcanic activity or massive methane releases (Bond & Grasby 2017). Paleoceanographic proxies indicate that oceanic pH has remained relatively uniform for at least 20 million years (**Figure 1*a***). Since the Industrial Revolution, the relative acidity of ocean water has increased by approximately 30% (0.1 pH units), and a further pH decline of 0.3–0.4 units is projected by the year 2100 (Steinacher et al. 2009) (**Figure 1*b***). By the end of the twenty-first century, the pH of the ocean will be the lowest in 40 million years (Pearson & Palmer 2000, Pelejero et al. 2010). Furthermore, the rate of acidification observed in the last 200 years is unprecedented in the last few million years (Raven et al. 2005) (**Figure 1*a***), making it more difficult for organisms to adapt to the new and rapidly changing environmental conditions.

The pH in the ocean is regulated by the carbonate buffering system, driven by differences in water temperature, salinity, partial pressure of CO_2 (pCO_2), alkalinity, and the concentrations of bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and Ca^{2+} ions (Zeebe & Wolf-Gladrow 2001). As more anthropogenic CO_2 dissolves in seawater, there is a subsequent increase in bicarbonate and hydrogen ions (protons, H^+), significantly decreasing both seawater pH and carbonate concentrations (**Figure 1*b***). Because calcifying organisms use calcium carbonate to build their shells

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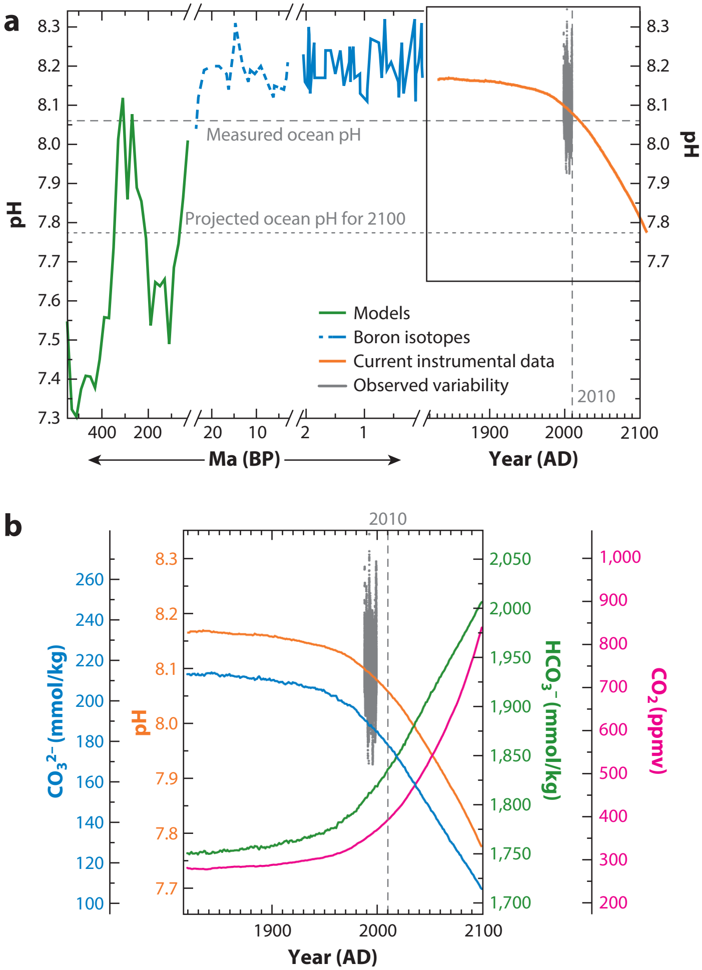

Figure 1

(*a*) Geological changes in pH obtained from models (*green line*), boron isotopes (*blue lines*), and current instrumental data (*orange line*). The cloud of gray points represents the variability observed in the ocean surface layer (50-m depth). The vertical dashed gray line indicates the year 2010, the horizontal dashed gray line indicates the measured ocean pH in that year, and the horizontal dotted gray line indicates the projected ocean pH in the year 2100. (*b*) Projected changes in surface-water dissolved inorganic carbon speciation in response to ocean acidification from 1800 to 2100 under a business-as-usual scenario for CO_2 emissions. As in panel *a*, the vertical dashed gray line indicates the year 2010, and the cloud of gray points represents the variability observed in the ocean surface layer (50-m depth). Abbreviations: BP, before present; ppmv, parts per million by volume. Figure adapted with permission from Pelejero et al. (2010).

or skeletons, ocean acidification makes the formation of those biomineral structures more costly due to the reduction in carbonate levels (Orr et al. 2005). It is not surprising, then, that many of the studies on the impact of ocean acidification have been on its devastating effect on the calcification and/or dissolution of the different types of carbonate precipitates in marine organisms (Hofmann et al. 2010). However, there are other potential biological effects based on biochemical inferences that have received less attention from the scientific community. For instance, it remains to be seen how the catalytic efficiency of different enzymes required for cellular metabolism and biogeochemical functions will actually respond to the increase in ocean acidity.

1.2. Ocean Warming

The ocean has stored ~90% of the excess solar heat retained by anthropogenic greenhouse gases, and consequently sea surface temperatures have already warmed by an average of 0.8–1.3° globally (NOAA 2016). Under current high-emission scenarios, we can expect a mean worldwide increase of at least another 2–4°C by the end of the century (Bindoff et al. 2019). Mean temperature increases are, however, likely to be less problematic for marine microbes than the rising frequency and intensity of damaging marine heat waves with a changing climate (Kling et al. 2020). Annual days of extreme ocean heating events are predicted to increase ~12 times by 2100, relative to the 1986–2005 period (Bindoff et al. 2019).

A major biological consequence of the warming that has happened already has been an average ~360-km poleward range shift over the past 70 years for many marine organisms (Poloczanska et al. 2013, Bindoff et al. 2019). Expansion into higher latitudes will help subtropical picocyanobacteria expand their global abundance by as much as 29% by 2100 (Flombaum et al. 2013). By contrast, some tropical marine N_2-fixing cyanobacteria face being completely excluded from their current lowest-latitude habitats by stressful levels of future warming (Thomas et al. 2012, Fu et al. 2014). Whether thermal adaptation will happen quickly enough to rescue these microbial climate refugees remains unknown (Hutchins & Fu 2017), but if so, it will necessarily involve the thermal recalibration of numerous vital enzyme systems.

The responses of enzymes to temperature have long been defined using the classic Arrhenius equation (Arrhenius 1889). In an Arrhenius plot, the natural log of the catalytic rate constant is graphed against the reciprocal of the temperature, allowing the enzyme's activation energy to be calculated from the slope. The activation energy represents the amount of energy required for the enzyme to form a transitional complex with its substrate, allowing the reaction to go to completion (Robinson 2015). Another fundamental thermal concept is the Q_{10}, which is usually defined as the fractional change in the catalytic rate with a 10°C change in temperature (James 1953). Although biological Q_{10} values are often said to be around 2, actually this value varies considerably (Robinson 2015). The empirical Arrhenius and Q_{10} approaches can be refined to better account for actual enzymatic reaction mechanisms (Robinson 2015, Mundim et al. 2020), but they can still provide a useful approximation of the thermal reactivity of enzymes in the context of environmental warming (Qin et al. 2014).

1.3. The Enzymes

The oceanic microbiome owes its existence to the activity of countless numbers of biochemically diverse enzymes and transport proteins. However, just a handful of specific biological catalysts play truly outsized roles in marine biology and biogeochemistry and have consequently received much attention from oceanographic researchers. These enzymes are critically important, because they largely determine the rates of crucial transformation reactions in the microbially mediated cycling of carbon and nitrogen in the ocean. Surface transporters are equally important, as they mediate chemical exchanges between the biota and the environment by translocating ions across cell membranes. This same set of biogeochemically prominent enzymes and membrane proteins will also be intimately involved in shaping how marine microbial populations will respond to the ever-accelerating warming and acidification of the ocean.

In this review, we examine the implications of ocean environmental change for five key types of enzymes and one transporter system: (*a*) carbonic anhydrase (CA), which is essential to ensure

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Table 1 Properties of five microbial enzymes and one transporter with pivotal roles in ocean biogeochemistry

	Reaction catalyzed	Marine microbes	Biogeochemical significance	Global change relevance
Carbonic anhydrase (CA)	$HCO_3^- + H^+ \rightarrow CO_2 + H_2O$	Photoautotrophic eukaryotes and some prokaryotes	Supplies CO_2 to support carbon fixation	Up- or downregulated by changing ocean pH and pCO_2
Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO)	$CO_2 \rightarrow$ 3-phosphoglycerate	Photoautotrophic eukaryotes and some prokaryotes	Carries out most global carbon fixation	Up- or downregulated by changing ocean pH and pCO_2
Proteorhodopsin	$H^+_{(intracellular)} \rightarrow H^+_{(extracellular)}$	Photoheterotrophic bacteria and some eukaryotic phytoplankton	Enables synthesis of ATP for growth and survival	Sensitive to pH changes
Nitrogenase	$N_2 + 8e^- + 16ATP + 8H^+ + 16H_2O \rightarrow 2NH_3 + 16ADP + 16H^+ + H_2$	Cyanobacteria, heterotrophic bacteria, and archaea	Provides a major source of new nitrogen to marine ecosystems	Activity increased by elevated pCO_2 and warming
Nitrate reductase (NR)	$NO_3^- + NADH + H^+ \rightarrow NO_2^- + NAD^+ + H_2O$	Phytoplankton, cyanobacteria, and heterotrophic bacteria	Assimilates NO_3^- to support growth	Sensitive to changing temperature
Ammonia monooxygenase (AMO)	$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$	Chemoautotrophic archaea (AOAs) or bacteria (AOBs)	Carries out the first step of nitrification	Subject to temperature control and affected by acidification

Abbreviations: AOA, ammonia-oxidizing archaeon; AOB, ammonia-oxidizing bacterium; pCO_2, partial pressure of CO_2.

an adequate supply of CO_2 for (*b*) ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the carbon-fixing enzyme found in virtually all phytoplankton; (*c*) proteorhodopsin, which is the most abundant microbial light-driven ion pump in the world ocean; (*d*) nitrogenase, which acts as a gateway between the vast reservoir of unavailable atmospheric N_2 gas and the insatiable need of marine life for the limiting nutrient nitrogen; (*e*) nitrate reductase (NR), which allows microbes to access the most abundant form of fixed nitrogen in the ocean to support their growth; and (*f*) ammonia monooxygenase (AMO), which catalyzes the first critical step in the oxidation of fully reduced ammonia (NH_3) to fully oxidized NO_3^-, which ultimately completes the marine nitrogen cycle during nitrification. The reactions that these biomolecules carry out, the microorganisms that have them, their biogeochemical significance, and their relevance to ocean acidification and rising temperatures are summarized in **Table 1**.

Of course, we cannot comprehensively cover global change impacts on all important enzymes and transporters in marine microbes, so we apologize in advance to readers whose favorites were necessarily omitted due to space limitations. For instance, we examine only relevant assimilatory forms of NR, not the equally important dissimilatory enzymes, nor do we discuss the important enzymes and transport systems involved in the utilization of other biologically limiting elements, such as phosphorus, silicon, and trace metals. For practical purposes, we focus exclusively on enzymatic responses of planktonic microbes in the water column, rather than trying to encompass the extensive body of literature on microbiological processes in marine sediments. Finally, although we concentrate on the effects of the two primary global change factors, temperature and acidification, interactions with many secondary environmental change processes, such as deoxygenation, stratification, and nutrient and trace element availability, are clearly important as well.

2. CARBONIC ANHYDRASE AND RUBISCO

Photosynthetic organisms use inorganic carbon for growth in the Calvin–Benson–Bassham cycle (Volokita et al. 1984, Raven et al. 2008), and its availability depends on seawater pH

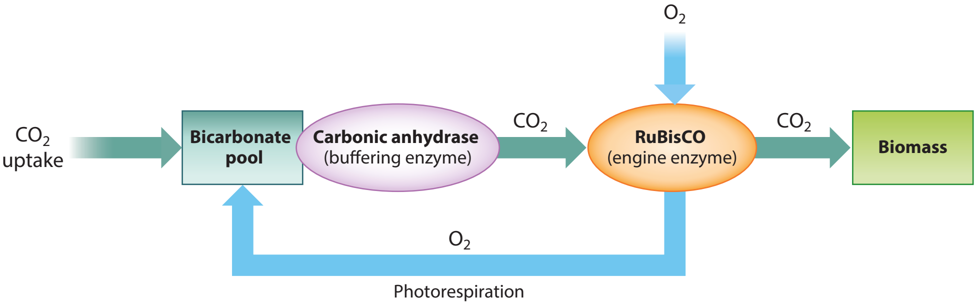

Figure 2

Functions of carbonic anhydrases and RuBisCO in the CO_2-concentrating mechanism in phytoplankton, which produces biomass from CO_2. The source of CO_2 for RuBisCO is the bicarbonate pool, through the activity and buffering properties of carbonic anhydrases. RuBisCO is then the engine enzyme that produces biomass from CO_2, but it also generates a feedback loop via its oxygenase reaction (photorespiration) to top up the bicarbonate pool when there is a reduction in the CO_2 supply. Abbreviation: RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase. Figure adapted with permission from Igamberdiev (2015).

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(Zeebe & Wolf-Gladrow 2001). As CO_2 is the chemical form of inorganic carbon utilized by the carboxylating enzyme RuBisCO (**Table 1**), the enzymatic conversion of HCO_3 to CO_2 becomes the rate-limiting step in photosynthesis. However, the CO_2-fixing RuBisCO has a slow rate of catalysis as well as low specificity for CO_2 (Igamberdiev 2015), catalyzing not only carboxylation but also oxygenation (photorespiration) reactions (**Figure 2**). This becomes problematic when the O_2/CO_2 ratio is high, as is the case today in aqueous solutions in equilibrium with an atmosphere that contains approximately 20 times more oxygen than carbon dioxide. It has been hypothesized that the evolution of a CO_2-concentrating mechanism (CCM) was indeed to overcome the catalytic rate limitation and enzymatic promiscuity of the photosynthetic engine enzyme RuBisCO, thus reducing the energetic costs caused by large photorespiratory fluxes in the present-day oxygenated atmosphere (Kaplan & Reinhold 1999) (**Figure 2**). The CCM in autotrophic phytoplankton uses CAs as fast-catalysis auxiliary buffering enzymes during carbon fixation. CAs are a superfamily of metalloenzymes that equilibrate the chemical reaction between CO_2, bicarbonate, and protons (DiMario et al. 2018). The metabolic function of the CAs in the RuBisCO reaction is then to provide an efficient and continuous supply of CO_2 from the bicarbonate pool (Amoroso et al. 1998, Reinfelder 2011) (**Figure 2**). However, the underlying biochemical mechanisms of the CCMs supporting high rates of photosynthesis in phytoplankton are not fully understood and are still being extensively investigated (e.g., Kustka et al. 2014, Poschenrieder et al. 2018).

2.1. Acidification

In the future greenhouse world, the impact of acidification on oxygenic photosynthesis might depend on how the catalytic efficiency of CAs is maintained, even more than the impact that high-CO_2, low-pH conditions might have on RuBisCO. Laboratory results have established that under fluctuating levels of ambient CO_2, CAs are the most upregulated proteins (Kustka et al. 2014). A decline in the efficiency of CAs could then lead to an increase in oxygenase activity (Badger et al. 1998), resulting in photorespiration at the expense of carbon fixation (Riebesell et al. 1993) (**Figure 2**). However, experimental results on the impact of ocean acidification on the physiology of single algal species or on the community composition of autotrophic phytoplankton have not been consistent, producing both positive and negative effects (e.g., Häder & Gao 2015)

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or even no detectable changes in growth rate (Crawfurd et al. 2011). Some short-term temporal and spatial fluctuations in the biological responses to acidification are expected, as phytoplankton bloom dynamics also influence local environmental pH values (Duarte et al. 2013). However, the differing responses of phytoplankton under high CO_2 levels and low pH suggest that compensatory physiological mechanisms may be at play, even if they have not been fully identified (e.g., Kustka et al. 2014).

Despite some of the conflicting results produced in different studies under reduced pH and high CO_2, there is empirical evidence suggesting that future declines in seawater pH under high CO_2 concentrations could overwhelm any compensating CA buffer mechanisms, potentially impacting the CCM in phytoplankton. For example, the catalytic activity of CAs depends on the physiological pH being optimum at more alkaline conditions (Donaldson & Quinn 1974). And although in vitro studies carried out to establish the kinetic properties of CAs have their limitations, mostly because those experiments do not account for any metabolic regulatory strategy, they provide a biochemical understanding of the problems that could arise at the protein level under high CO_2. A homeostatic balance maintains the internal pH for carboxylation reactions controlling the relative abundance of HCO_3^- and H_2CO_3 (Mangan et al. 2016). Cellular pH is controlled by the diffusive influx of inorganic carbon species driven by concentration gradients (increasing under high-CO_2 conditions) and the ATP-energy driven efflux of any excess. The active transport of inorganic carbon out of the cell has a high energetic cost, which may lead to a depletion of ATP needed for other physiological processes. Consistent with the pH dependence of CAs, model results indicate that an alkaline cytosolic pH reduces the energetic cost of the CCM (Mangan et al. 2016).

As for CAs, the activity and activation of RuBisCO are also pH controlled, increasing at higher pH values (Mott & Berry 1986). The catalytic properties of RuBisCO then depend on how well fluctuations in cellular pH are buffered by the activity of CAs (**Figure 2**). Any changes in the homeostatic mechanisms that keep cellular pH stable for RuBisCO to work could have global implications, as the two enzymes in the CCM system carry out most of the biological fixation in the carbon cycle. The pH dependence of the kinetic properties of these enzymes has not been fully explored, and it is clear that future investigations are required to establish activity thresholds, as well as how the cellular mechanisms responsible for pH homeostasis will respond to levels of atmospheric CO_2 not seen in millions of years.

2.2. Temperature

Rising temperatures present a two-pronged physiological challenge to marine autotrophs. First, warmer waters decrease the solubility of CO_2 and cause a concomitant reduction in HCO_3^- concentrations, increasing the activity of CAs (Shiraiwa & Miyachi 1985, Beardall et al. 1998). Second, warming increases the dissolved $[O_2]/[CO_2]$ ratio, favoring RuBisCO photorespiration over carboxylation (Galmés et al. 2019). Higher temperatures also have a direct impact on the catalytic functioning of RuBisCO. The maximum carboxylase turnover rate of RuBisCO increases with temperature, while the RuBisCO specificity factor decreases, making the oxygenase activity more favorable (Bernacchi et al. 2001, Galmés et al. 2015). However, differences in the thermal response of RuBisCO have been reported, even in closely related photosynthetic organisms (Perdomo et al. 2015, Galmés et al. 2016), suggesting that some basic aspects of the catalytic functioning of this enzyme are still unresolved. This high variability in temperature responses is not totally unexpected, as different lineages of photosynthetic organisms have divergent RuBisCO subtypes with a more than 20-fold range in their specificity factor, probably associated with their evolutionary traits (Badger et al. 1998, Tabita et al. 2008, Young et al. 2016, Young & Hopkinson 2017).

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3. PROTEORHODOPSINS

Proteorhodopsins are transmembrane photoactive proteins that function as light-driven proton pumps (Béjà et al. 2000), translocating ions across the cell membrane to enhance various metabolic reactions (**Table 1**). These reactions include ATP synthesis (Steindler et al. 2011), substrate uptake (Gómez-Consarnau et al. 2016), and ultimately the promotion of bacterial growth or survival (Gómez-Consarnau et al. 2010). They are typically classified as absorbing either green or blue wavelengths (Béjà et al. 2001), a feature that can be interconverted by a single point mutation (Man et al. 2003). Proteorhodopsin-based photoheterotrophy is the most widely distributed light-dependent metabolism in the surface ocean worldwide (Finkel et al. 2013) and a key determinant of the energy balance of the biosphere (Gómez-Consarnau et al. 2019).

Most studies on the effects of reduced pH and high temperature on marine heterotrophic bacteria have focused primarily on how those stressors influence species distributions, relative abundance, growth rate, and the activity of extracellular enzymes associated with the decomposition of organic matter (e.g., Grossart et al. 2006). As observed with autotrophic phytoplankton (e.g., Häder & Gao 2015), general trends in bacterial responses are not easy to elucidate (Liu et al. 2010, Joint et al. 2011, Newbold et al. 2012). The effects of acidification and warming on particular bacterial metabolisms of high ecological relevance, such as proteorhodopsin phototrophy, are still unclear. For example, no studies have established thresholds of ion transport in heterotrophs in response to lower pH and high temperature, making future projections about the respiration component of the carbon cycle very difficult.

3.1. Acidification

Heterotrophic bacteria indeed have a measurable functional response to elevated-CO_2, reduced-pH conditions, as reflected in their gene expression (Bunse et al. 2016). As might be expected, these transcriptional responses of bacteria to acid stress are mostly in their pH homeostasis genes, such as those encoding membrane transporters, respiration complexes, and proteorhodopsins (**Figure 3**). The metabolic strategies used by bacterioplankton to cope with acidification are taxon specific and congruent with their different life strategies (Bunse et al. 2016). This suggests that, as for phytoplankton, generalizations are difficult to make. Furthermore, the pH-induced response seems to occur only under low phytoplankton biomass, consistent with the alkalinization effect of photosynthesis (Duarte et al. 2013). The fact that the activity of primary producers buffers the responses of marine bacteria to acidification suggests a temporal and spatial component associated with biomass production.

Laboratory studies on the effects of reduced pH on membrane proteins acting as ion pumps have generated contrasting results, which are not easy to extrapolate to environmental conditions (e.g., Dioumaev et al. 2003, Lörinczi et al. 2009). For example, experiments have shown that the proton pump direction in proteorhodopsins is a function of external pH: outward at alkaline pH and inward under acidic conditions (Vogt et al. 2013). In addition to reversing the proton pathway, acidification shifts the light absorption properties by converting blue- into green-absorbing microbial rhodopsins (Yamada et al. 2012). While the biological relevance of these findings is still unclear because of the nonphysiological acidic conditions used in those experiments, the fact that a simple acidification triggers structural changes and a major functional conversion shows the complex pH dependence of proteorhodopsins. Furthermore, a decline of just a few tenths of a pH unit, even under alkaline conditions, causes a significant reduction in the proton transport activity of proteorhodopsins (Dioumaev et al. 2003), suggesting that acidification could have a profound effect on one of the most abundant microbial light-driven ion pumps in the world ocean.

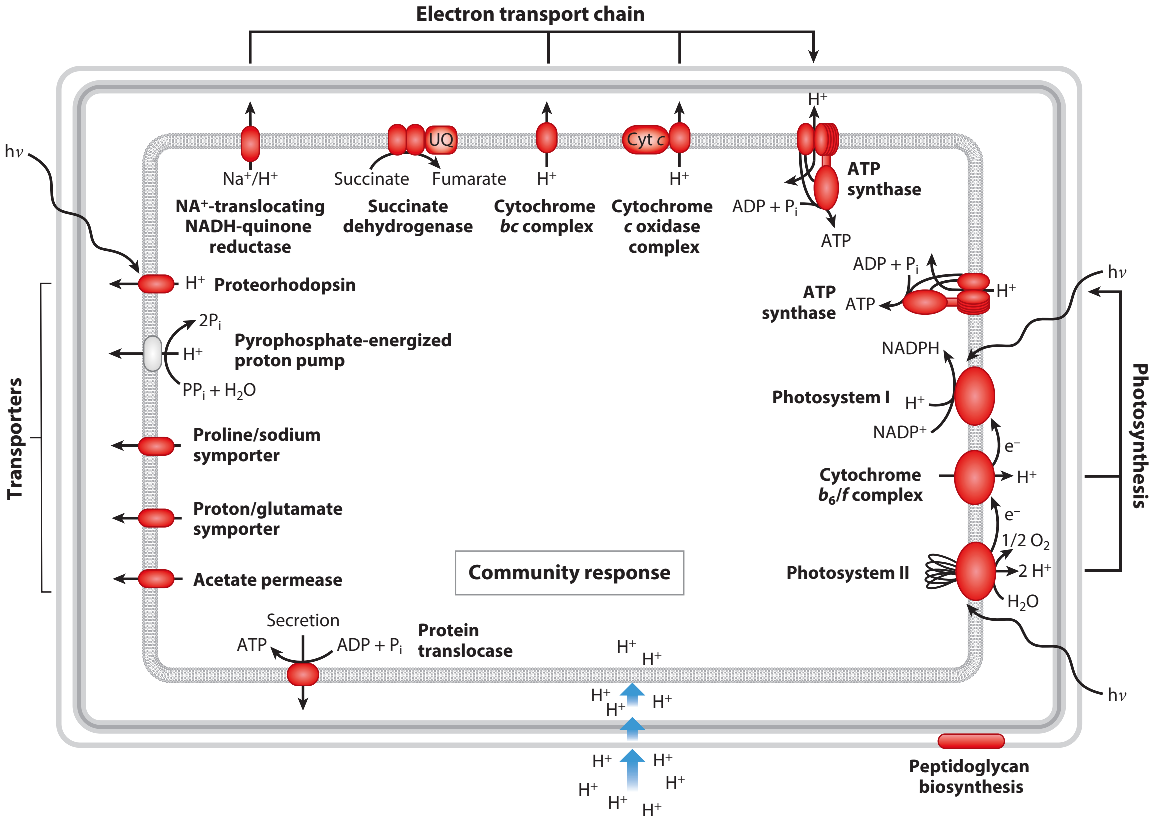

Figure 3

Molecular responses to acidification observed in different groups of bacteria. This review discusses the impact of acidification on proteorhodopsins, but low-pH conditions also affect other surface transporters as well as the electron transport chain and the synthesis of ATP during photosynthesis. Abbreviations: Cyt *c*, cytochrome *c*; P_i, inorganic phosphate; PP_i, inorganic pyrophosphate; UQ, ubiquinone. Figure adapted with permission from Bunse et al. (2016).

3.2. Temperature

Although only a few studies have dealt with the impact of temperature on proteorhodopsin activity, they consistently show that their function might be affected by warming. For instance, while pH can shift their absorption spectrum (Yamada et al. 2012), their photochemical cycle shows a linear positive dependence with temperature, regardless of their maximum absorption spectra, suggesting that these ion pumps are well adapted to large fluctuations in water temperature (Jung et al. 2008, Kim et al. 2020).

The different photocycling rates (and corresponding ion pumping activity) seem to be an evolutionary adaptation to different temperature gradients. Photoheterotrophs from cold environments where the metabolic energy requirements are low tend to have slow-photocycle proteorhodopsins, while faster photocycles can be found at higher temperatures, where overall metabolic rates are higher (Jung et al. 2008). The contrasting effects of increasing temperatures on proteorhodopsin activity compared with the CCMs discussed above suggest that future warming could expand the areas where photoheterotrophs may outcompete autotrophic phytoplankton.

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4. NITROGENASE

The nitrogenase enzyme catalyzes the thermodynamically unfavorable reduction of triple-bonded N_2 gas, thereby converting abundant but highly refractory atmospheric N_2 to biologically available NH_3 (**Table 1**). N_2 fixation provides an indispensable supply of the essential nutrient nitrogen to life on Earth and is an especially crucial source of nitrogen supporting open ocean food webs (Sohm et al. 2011). Nitrogenase is found in a wide phylogenetic array of marine prokaryotes, ranging from cyanobacteria to heterotrophic proteobacteria and archaea, but is wholly absent from eukaryotes (Zehr & Capone 2020).

Evolution has produced multiple nitrogenase orthologs, and the one most commonly found in marine microbes, such as cyanobacteria, contains molybdenum and iron cofactors at the active site (MoFe nitrogenase; Einsle & Rees 2020). Alternate forms utilize vanadium and iron (VFe nitrogenase) or iron alone for catalysis (Seefeldt et al. 2020). All of these isozymes also rely heavily on iron, largely in the form of FeS proteins, with a total of 26 iron ions required for each active enzyme complex, as well as additional iron for electron carriers, such as ferredoxin, that shuttle reductant to the reaction. This exceptionally large enzymatic iron requirement imposes a significant resource burden on marine N_2 fixers (diazotrophs), as vast swaths of the surface ocean (up to 40%; Moore et al. 2013) are seriously deficient in this micronutrient. Consequently, diazotrophs are often iron limited throughout much of the open ocean, and iron availability is a primary factor constraining global marine N_2 fixation (Hutchins & Boyd 2016).

The formidable activation energy barrier that must be overcome to break the N_2 triple bond requires diazotrophs to invest 16 ATPs in addition to 8 electrons for every N_2 molecule reduced to NH_3 (**Table 1**). Another consequence of this cumbersome and expensive catalytic reaction is that nitrogenase is among the slowest enzymes known, with an effective substrate turnover time of only ∼1 per second (Einsle & Rees 2020). To add to this list of limitations, nitrogenase is also easily inactivated by molecular O_2, and so diazotrophs must employ metabolic, structural, or temporal regulatory strategies to shield their nitrogenase when fixing N_2 in aerobic ocean environments (Sohm et al. 2011, Zehr & Capone 2020). All of these apparent drawbacks are clearly outweighed by the selective advantage of having access to a virtually unlimited supply of scarce nitrogen, and yet the benefits and trade-offs involved in relying on nitrogenase-mediated N_2 fixation may be fundamentally altered in a warmer and more acidic future ocean.

4.1. Temperature

The importance of N_2 fixation to agriculture has resulted in a large body of literature on nitrogenase kinetics and enzymology, but virtually no in vitro studies have examined nitrogenase originating from marine microbes. The enzyme activity and iron protein reduction rates of nitrogenase from the terrestrial bacterium *Azotobacter* typically exhibit a break in the slope of the Arrhenius plot between reaction rate and temperature. This nonlinear relationship suggests that nitrogenase may undergo an abrupt shift in conformation and/or electron transfer mechanism as temperature changes (Wilson et al. 2004). Little is known about likely direct thermal effects on marine nitrogenase activity, though, partly because the catalytic reaction mechanisms at the active site of the enzyme remain poorly understood (Einsle & Rees 2020, Seefeldt et al. 2020).

Almost all measurements of nitrogenase kinetics in the ocean are from in vivo assays of live diazotrophs and therefore integrate the influences of temperature along with other physiological and environmental factors affecting the enzymatic reaction rate. Both laboratory and field studies have suggested that future rising sea surface temperatures could increase the N_2 fixation rates of important diazotrophic groups, such as the dominant cyanobacterial genera *Trichodesmium* and *Crocosphaera*. Culture experiments show that these two groups each have their own characteristic

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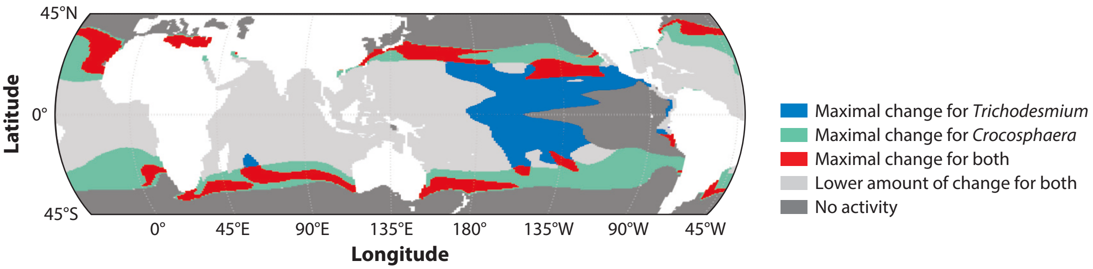

Figure 4

Modeled changes in global N_2 fixation rates for the diazotrophic cyanobacteria *Trichodesmium* (*blue*) and *Crocosphaera* (*teal*) and for both (*red*) between 2010 and 2100, as determined by warming-driven changes in cellular iron use efficiency. These three colored regions are areas where the changes in N_2 fixation are maximal (>75% quartile) for one or both of these diazotrophs; the light gray regions are areas where the changes are lower (<75% quartiles) for both diazotrophs; and the dark gray regions are areas where low temperatures or high nitrate levels (>5 μm) preclude the growth of both diazotrophs. Figure adapted with permission from Yang et al. (2021).

thermal response curve, with nitrogenase activity peaking at 24–28°C for *Trichodesmium* and 28–30°C for *Crocosphaera* (Fu et al. 2014). N_2 fixation rates drop off very quickly above these optimum temperatures, though, and it is thought that these cyanobacteria could even disappear from the warmest parts of the future tropical oceans due to thermal stress (Thomas et al. 2012, Fu et al. 2014). However, at the same time both genera may expand their current ranges into higher latitudes, and climate warming could also help to alleviate the prevailing iron limitation of N_2 fixation over large areas of the ocean (Jiang et al. 2018, Yang et al. 2021) (see the sidebar titled Ocean Warming, Iron Limitation, and Global N_2 Fixation Rates along with **Figure 4**).

Both *Trichodesmium* and *Crocosphaera* have global distributions that are positively correlated with temperature (Mazard et al. 2004, Langlois et al. 2008). The other dominant diazotrophic cyanobacterial group in the ocean is the uncultivated phytoplankton symbiont UCYN-A, which specializes in cooler waters (<23°C) (Moisander et al. 2010). It has been suggested that warmer temperatures particularly benefit marine N_2-fixing cyanobacteria due to increased respiration rates and decreased gas solubility, both of which would tend to alleviate O_2 inhibition of nitrogenase (Staal et al. 2003, Brauer et al. 2013, Inomura et al. 2019). In fact, the notable thermal sensitivity of nitrogenase-mediated nitrogen inputs may in turn intensify the temperature dependence of other key metabolic processes, such as respiration, photosynthesis, and biomass accumulation in aquatic ecosystems (Welter et al. 2015).

OCEAN WARMING, IRON LIMITATION, AND GLOBAL N_2 FIXATION RATES

Recent culture studies suggest that by the year 2100, ocean warming may allow the dominant marine cyanobacteria *Trichodesmium* and *Crocosphaera* to utilize the scarce iron supplies available to them much more efficiently to fix N_2. This large, thermally mediated increase in cellular iron use efficiency (moles of N_2 fixed per mole of cellular iron per hour) may thus partially relieve the basin-scale iron limitation of both of these diazotrophs, leading to greatly increased N_2 fixation rates across vast expanses of currently iron-starved oceanic regions, such as the South Pacific (**Figure 4**). It has been suggested that this enhancement in iron use efficiency may occur because warming leads to more rapid substrate turnover by the nitrogenase enzyme but without requiring any concomitant increase in the enzyme's catalytic iron content (Jiang et al. 2018, Yang et al. 2021).

4.2. Acidification

Direct effects of changing seawater carbonate chemistry on marine nitrogenase activity are likely minor. The rate constant for reduction of the iron protein of *Azotobacter* nitrogenase increases linearly but modestly as pH falls (approximately +20% from pH 8.1 to 7.8; Wilson et al. 2004), and VFe nitrogenase requires a carbonate ligand (Sippel & Einsle 2017), a chemical species that is declining precipitously with ocean acidification (Orr et al. 2005) (**Figure 1*b***). However, the intracellular location of the enzyme may largely buffer it from adverse effects of external acidification.

Nitrogenase is a very nonspecific enzyme, and in addition to N_2 can reduce many other substrates, including nitriles, acetylene, and azide. It also reduces substrates that are relevant to ocean environmental change, including H^+ and the greenhouse gases N_2O and CO_2 (Seefeldt et al. 2020). CO_2 reduction by bacterial iron-only nitrogenase produces $CH_4{}^+$, an even more potent greenhouse gas (Zheng et al. 2018), but this reaction could also potentially be harnessed for the direct microbial conversion of atmospheric CO_2 to hydrocarbon fuels (Fixen et al. 2016).

Like temperature, it seems likely that the major effects of acidification on marine N_2 fixation rates are indirect ones. A substantial body of research has shown that acclimation to projected end-of-the-century CO_2 concentrations of 700–1,000 ppm stimulates nitrogenase activity in nutrient-replete *Trichodesmium* and *Crocosphaera* cultures by up to 60% (Barcelos e Ramos et al. 2007; Hutchins et al. 2007, 2013; Levitan et al. 2007); a meta-analysis covering 47 individual experiments with these two groups found an average enhancement of ~29% ± 4% (Wannicke et al. 2018). This positive effect of acidification on marine N_2 fixation rates has been attributed to cellular energetic savings when CCMs are downregulated under high CO_2, but this stimulatory effect is largely negated under iron limitation (Fu et al. 2008, Walworth et al. 2016). These consensus findings have been disputed (Hong et al. 2017b), although methodological inconsistencies make meaningful comparisons with prior studies difficult (Hutchins et al. 2017). A long-term experimental evolution study with *Trichodesmium* showed that after several years of selection by high CO_2, upregulation of N_2 fixation rates transitions from being a reversible plastic response to being a constitutive adaptive response. These elevated rates then persist even if the cyanobacteria are subsequently returned to low-CO_2 conditions (Hutchins et al. 2015). This result implies that enduring changes in new nitrogen supplies from adapted diazotrophs may be one legacy of our acidification of the future ocean.

5. NITRATE REDUCTASE

Assimilatory NR is an enzyme that catalyzes the two-electron reduction of nitrate to nitrite (**Table 1**). Nitrite then undergoes a further six-electron reduction via nitrite reductase to ammonia and the glutamine synthetase/glutamate synthase pathway for incorporation into proteins. Nitrate uptake also requires ATP hydrolysis, and NR is usually the rate-limiting step for microbial nitrogen assimilation (Berges & Mulholland 2008). In eukaryotes like diatoms, NR is a dimer consisting of a molybdenum protein containing the active site, associated with heme iron and flavin (FAD-binding) electron transfer protein domains. Eukaryotic NR gene transcription is regulated by both internal and external cues and pools of nitrogen (Wang et al. 2018).

Most marine planktonic prokaryotes can utilize nitrate, with some exceptions, such as many strains of the ecologically dominant picocyanobacterium *Prochlorococcus* (Berube et al. 2019). Initial nitrate uptake is via an ATP-dependent permease enzyme (Lin & Stewart 1998). The cyanobacterial Nas NR system is a molybdenum protein that is quite divergent from that of eukaryotes, consisting of a monomer that accepts photosynthetically produced reductant from either flavodoxin or iron-containing ferredoxin (Flores et al. 2005). Heterotrophic bacterial assimilatory Nas NR systems are similar but often rely on NAD(P)H to supply reductants rather than proteins

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(Lin & Stewart 1998). NR functional gene diversity in the ocean has been documented and explored in detail for eukaryotes (Bhadury & Ward 2009, Ward & van Oostende 2016, Wang et al. 2018), cyanobacteria (Berube et al. 2019), and heterotrophic bacteria (Jiang et al. 2015, Jiang & Jiao 2016).

5.1. Temperature

Many studies have examined NR activity and nitrate uptake as a function of temperature in marine phytoplankton. One frequent observation is that NR activity in diatoms is often optimal at lower temperatures but falls off more quickly as temperature rises than it does in other phytoplankton groups (Lomas & Glibert 1999, Gao et al. 2000, Kudo et al. 2000). This trend would seem to put diatoms at an obvious disadvantage in a warming ocean. Using a cell-free NR extract from the diatom *Skeletonema costatum*, Gao et al. (2000) found that thermal inhibitory effects manifested through changes in the FAD domain and electron transfer reactions. However, there are many exceptions to this general rule about diatom NRs. In a temperate coastal diatom-dominated community, nitrate uptake rates did not differ across a thermal range relevant to global change (19–23°C) (Spackeen et al. 2017). In a polar diatom assemblage, though, the same magnitude of increase (0–4°C) increased nitrate uptake in the largest phytoplankton size class by two to three orders of magnitude (Spackeen et al. 2018). Thus, there are clearly major regime- and taxon-specific differences in the thermal sensitivity of eukaryotic nitrate acquisition systems that will need to be considered to make predictions about the effects of future ocean warming.

Gleich et al. (2020) grew the model diatom *Thalassiosira pseudonana* at 4°C, 17°C, and 28°C and found that while NR gene expression was lowest at 17°C, NR activity was often higher at this middle temperature. This observation lends support to suggestions that NR activity is regulated at the protein level (Gao et al. 2000, Berges et al. 2002). A thermal evolution experiment with the same diatom isolate selected at 16°C and 31°C for 450 generations showed no systematic differences in nitrate growth affinities (O'Donnell et al. 2018), raising the possibility that diatom NR systems may not have the capacity to adapt readily to the lower nitrate availabilities that will accompany warming temperatures (Hutchins & Fu 2017).

Marine heterotrophic bacteria can be very active in nitrate uptake (Bradley et al. 2010, Jiang & Jiao 2016), and their abundance and NR activity can be closely correlated to temperature (Jiang et al. 2015). However, heterotrophic bacteria have been less studied than marine photoautotrophs in this respect. At times, bacteria may directly compete with phytoplankton for nitrate, potentially tipping the balance between heterotrophy and autotrophy in marine ecosystems (Diner et al. 2016), so an understanding of how this relationship may change in a warming ocean is needed.

5.2. Acidification

Steadily rising seawater $p\mathrm{CO}_2$ and acidity will inevitably interact with microbial nitrate acquisition via NR, but the available studies on interactions with ocean acidification are sometimes contradictory. Li et al. (2020) grew the diatom *Nitzschia closterium* at three pH/$p\mathrm{CO}_2$ levels (8.10/400 ppm, 7.71/800 ppm, and 7.45/1,500 ppm) and found a stepwise reduction in NR activity with decreasing pH (**Figure 5*a***). Two studies using other model diatom species also showed that elevated $p\mathrm{CO}_2$ had either negative or negligible effects on NR activity (Shi et al. 2015, Hong et al. 2017a). Similarly, NR activity in freshwater green algae and cyanobacteria can be strongly inhibited by rising $p\mathrm{CO}_2$ (Xia & Gao 2005, Ma et al. 2019).

By contrast, NR activity levels increased linearly from pH 8.2 to 7.6 in the coccolithophore *Emiliania huxleyi* (Rouco et al. 2013) (**Figure 5*b***) and in a cultured dinoflagellate between 400 and

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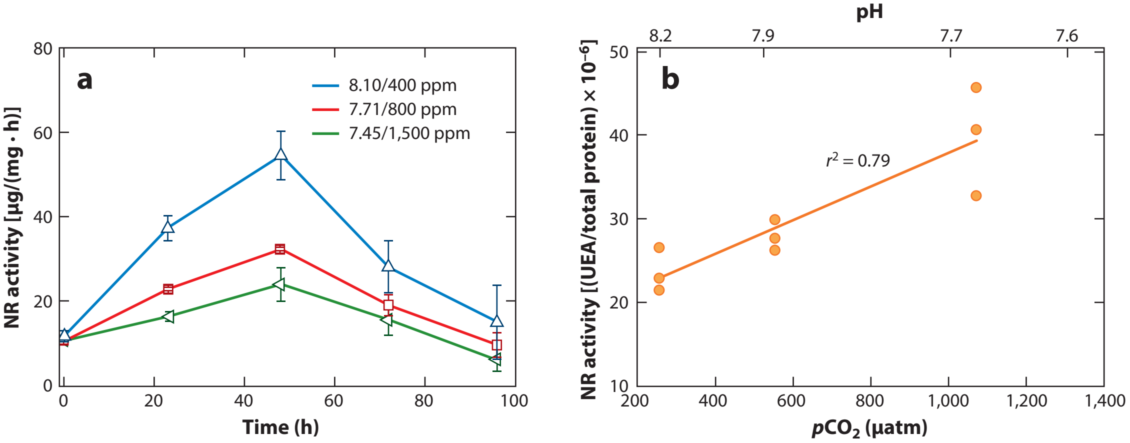

Figure 5

Contrasting responses of NR activity to global change–relevant changes in pH and $p\text{CO}_2$ for (*a*) the diatom *Nitzschia closterium* grown at three pH/$p\text{CO}_2$ levels (8.10/400 ppm, 7.71/800 ppm, and 7.45/1,500 ppm) during a 100-h incubation (NR activity units of micrograms of NO_2^- produced per milligram of algal cells per hour) and (*b*) the coccolithophorid *Emiliania huxleyi* grown at three pH/$p\text{CO}_2$ levels (8.18/250 ppm, 7.94/525 ppm, and 7.61/1,200 ppm) (NR activity measured as UAE divided by total algal protein, where 1 UAE reduces 1 μmol of NO_3^- to NO_2^-). Results from these experiments and others suggest that NR responses to ocean acidification may be species or group specific. Abbreviations: NR, nitrate reductase; $p\text{CO}_2$, partial pressure of CO_2; UEA, unit of enzyme activity. Panel *a* adapted with permission from Li et al. (2020); panel *b* adapted with permission from Rouco et al. (2013).

1,000 ppm $p\text{CO}_2$ (Pierangelini et al. 2017). Expression of genes involved in nitrate uptake and assimilation pathways is upregulated under low-pH, high-$p\text{CO}_2$ conditions in both the diatom *Chaetoceros muelleri* (Liang et al. 2020) and the chlorophyte *Coccomyxa subellipsoidea* (Peng et al. 2016). Like temperature, the effects of acidification on phytoplankton NR activity may be taxon specific, with contrasting trends that preclude making broad generalizations.

Despite the apparent collective ambiguity of these studies that measured NR enzyme activities under acidified conditions, the net effects of high CO_2 on experimental nitrate uptake rates are often positive in cultured diatoms and dinoflagellates (Shi et al. 2015, Hu et al. 2016, Li et al. 2016) and mixed phytoplankton communities (Liu et al. 2017, Spackeen et al. 2017). Insensitivity of nitrate uptake to acidification has sometimes been reported (Bellerby et al. 2008, Qu et al. 2018, Spackeen et al. 2018), but no marine field studies have reported NR inhibition by realistic low pH values. As for nitrogen fixation, downregulation of costly CCMs under ocean acidification may provide nitrate uptake with an energetic subsidy (Shi et al. 2015, Hong et al. 2017a, Wannicke et al. 2018).

6. AMMONIA MONOOXYGENASE

AMO is a trimeric protein containing both copper and nonheme iron that catalyzes the crucial first step in the chemoautotrophic process of nitrification (Simon & Klotz 2013). AMO oxidizes ammonia (NH_3) to hydroxylamine (NH_2OH_2) (**Table 1**), which is then further oxidized by other enzymes in one or two poorly understood steps to nitrite (NO_2^-). Incomplete nitrification is a major oceanic source of the greenhouse gas N_2O (Santoro et al. 2019).

Complete nitrification usually requires the coupling of two separate groups of organisms, with the initial oxidation of NH_3 via AMO being carried out by either marine Thaumarchaeota such as *Nitrosopumilus* and *Nitrosopelagicus* (Konneke et al. 2005, Santoro et al. 2015) or bacteria such as *Nitrosomonas* [ammonia-oxidizing archaea (AOAs) or bacteria (AOBs)]. Reductant originating from NH_3 oxidation is used to fix inorganic carbon via the Calvin–Benson–Bassham cycle (bacteria) or the 3-hydroxypropionate/4-hydroxybutyrate cycle (archaea). NO_2^- produced by AOAs or AOBs can subsequently be converted to fully oxidized NO_3^- by nitrite-oxidizing bacteria. Recent work has found that some *Nitrospira* species with a phylogenetically divergent AMO can fully oxidize NH_3 all the way to NO_3^- (van Kesse et al. 2015), but the importance of this complete ammonia oxidation (comammox) pathway in the ocean is currently unclear. The gene coding for one AMO subunit (*amoA*) has been widely applied as a functional biomarker for the abundance and activity of ammonia oxidizer clades in the ocean (Shiozaki et al. 2016, Carini et al. 2018). Field studies have shown that AOAs dominate ammonia oxidation at all depths throughout most of the open ocean, and indeed represent the most abundant microbial functional group below the euphotic zone (Bristow et al. 2015). AOBs are usually more abundant in shallow coastal waters and estuaries, or associated with marine particles (Dang & Chen 2017).

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6.1. Temperature

Published growth rates of AOA cultures are usually based on the rate at which NH_3 is converted to NO_2^-, and so actually represent substrate turnover rates of the AMO enzyme. Studies using this method have found that cultured AOAs often have relatively broad thermal response curves, spanning from 22°C to 33°C (Ahlgren et al. 2017). Reported Q_{10} values for three AOA cultures were between 2.49 and 2.89, suggesting considerable thermal sensitivity, and inferred enzymatic activation energies ranged from 64.2 to 78.3 kJ/mol (Qin et al. 2014). The first marine AOA culture was isolated from a tropical aquarium (Konneke et al. 2005), and its thermal optimum is ~32°C. Other AOA cultures obtained from coastal and open ocean regimes have thermal optima that range from 22°C to 34°C (Qin et al. 2014, Santoro et al. 2015, Bayer et al. 2016, Ahlgren et al. 2017). In many cases, these AMO thermal optima substantially exceed the annual peak temperatures where the cultures were collected, suggesting that AOAs could have considerable scope to benefit from future warming trends.

Some fieldwork also supports temperature as a major determinant of NH_3 oxidation rates in the ocean. One study found that nitrification rates in a eutrophic coastal bay were strongly correlated with temperature, with a Q_{10} of 2.2 (Zheng et al. 2017). In this study, the thermal optimum of the free-living nitrifying microbial community was ~24°C, but the optimum of particle-associated nitrifiers was ~29°C, suggesting taxonomic differences. Another coastal study found that temperature was second only to photoinhibition as a control on AOA distributions and activity (Liu et al. 2018). Summer NH_3 oxidation rates increased from 20°C to 30°C in a temperate estuary, while NO_2^- oxidation lagged as temperatures rose. The resultant decoupling of the two steps of nitrification led to accumulation of NO_2^-, a thermal mismatch that could be further exacerbated by future climate warming (Schaefer & Hollibaugh 2017). However, other field studies have found that NH_3 oxidation rates of natural AOA assemblages are relatively unresponsive to temperature changes (Baer et al. 2014, Horak et al. 2017), perhaps indicating community temperature resiliency due to coexisting thermal ecotypic diversity. If so, selection-driven thermal sorting could act to provide functional redundancy for the NH_3 oxidation niche as the ocean warms.

A recent coastal-to-offshore transect study showed that in nearshore waters where NH_3 oxidation was dominated by AOBs, rates increased with temperature up to ~10°C above ambient conditions. By contrast, rates of offshore AOA communities declined linearly with rising temperature, although this trend could be reversed by substrate additions, which shifted the thermal optimum of the assemblage to higher temperatures (Zheng et al. 2020). They calculated that sea surface temperature increases of 4°C by 2100 under current substrate conditions would enhance coastal AOB NH_3 oxidation rates by an average of 13% while inhibiting oceanic AOA rates by 27%. Eutrophication via atmospheric deposition of anthropogenic NH_3 could, however, help offshore AOAs to acclimate to warming, while nearshore NH_3 enrichment as temperatures rise would directly benefit AOBs, perhaps allowing them to extend their biogeographic niche seaward.

6.2. Acidification

The optimum pH levels for AMO activity in cultured AOAs are often much lower than those predicted under even the most pessimistic ocean acidification scenarios. AOA cultures have reported pH optima of only 6.8–7.3 (Qin et al. 2014, Bayer et al. 2016), representing seawater H^+ concentrations that are ∼6–20 times present-day values and 3–10-fold higher than even the elevated levels predicted for the year 2100 (Bindoff et al. 2019). Similarly, a compilation of estuarine measurements found that these NH_3 oxidation rates were negatively correlated with pH (Fulweiler et al. 2011). It has been suggested that this trend reflects an adaptation to localized low-pH conditions around the cells, produced by acidity generated during the NH_3 oxidation reaction (Bayer et al. 2016).

By contrast, fieldwork has suggested that ocean acidification could significantly reduce NH_3 oxidation rates in diverse marine environments. Experimental acidification at year-2100 levels using either CO_2 bubbling or acid additions at realistic substrate levels inhibited NO_2^- production by 8–38% in the Sargasso Sea, the North Pacific Central Gyre, and coastal California waters (Beman et al. 2011). Wannicke et al. (2018) collected published data from 55 pelagic experiments and found that ocean acidification reduced NH_3 oxidation rates by an average of ∼28%. This inhibition has been attributed to the protonation of NH_3 to NH_4^+ at low pH, since NH_3 has been presumed to be the sole chemical species oxidized by NH_3 oxidizers (Beman et al. 2011). It has been pointed out, though, that some AOAs likely utilize NH_4^+ as a substrate (Qin et al. 2014). It is also notable that the substrate for archaeal 3-hydroxypropionate/4-hydroxybutyrate cycle carbon fixation is carbonate ions (CO_3^{2-}), not molecular CO_2 (Santoro et al. 2019), and CO_3^{2-} ion concentrations are quickly dwindling as they become protonated to HCO_3^- with the progression of ocean acidification (Orr et al. 2005, Hofmann et al. 2010) (**Figure 1*b***). At present, there is a need to reconcile laboratory findings of low pH optima for AMO activity with contrasting field evidence that NH_3 oxidation is often inhibited by acidification.

7. CONCLUSIONS

A major unknown regarding the adaptability of microorganisms to the emerging greenhouse world ocean is the cellular energetic cost associated with maintaining current rates of carbon and nitrogen fixation and cycling, regardless of the physiological strategies being examined (e.g., oversynthesis of RuBisCO; Devos et al. 1998, Young et al. 2015). The questions therefore become, How much cellular energy are marine microbes currently allocating to counteract the physiological effects of simultaneous increases in temperature and decreases in pH, and how much will this energetic investment need to increase under more extreme future conditions? Enzymatic results obtained from in vitro experiments at 25°C are sometimes not easily extrapolated to the wide spectrum of cell types living in the many varied environments of the real ocean (Bar-On & Milo 2019). However, we currently lack sufficient fundamental information to predict how the combined effects of acidification and warming will affect the catalytic rates of many marine enzymes. As a result, basic information like threshold levels and rate limits is often not available, and so more mechanistic studies examining in vitro responses of crucial marine microbial enzymes to global change variables may be warranted.

These mechanistic insights could have major real-world consequences, such as determining whether the rate-limiting enzymatic steps may change for important carbon and nitrogen processing pathways under future ocean environmental conditions. Comparisons between in vitro and in vivo responses could also help to unravel often substantial uncertainties about direct

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versus indirect effects of temperature and pH shifts on enzyme kinetics. Microbial oceanographers understandably tend to focus on measuring enzyme rates in live microorganisms, rather than in cell-free enzyme extracts. In these studies, however, it is not always easy to distinguish the primary responses of catalytic mechanisms to warming and acidification from the secondary metabolic influences of a host of other environmental variables.

Of course, if we are interested in predicting the holistic responses of the marine microbiota to a changing ocean, it is precisely these types of complex, multivariate interactions that we must understand in order to put enzymatic responses into a realistic context. We have focused here on warming and acidification as the two main global change stressors, but their effects may be either amplified or diminished by other drivers, including deoxygenation, stratification, and changes in nutrient supplies. For instance, increasing expansion of suboxic zones into the euphotic zone in tandem with ongoing acidification (Hutchins & Fu 2017) could significantly lower seawater O_2/CO_2 ratios. This would strongly favor the RuBisCO carboxylation reaction and lessen the need for phytoplankton to rely on costly CAs, potentially favoring particular species that are able to tolerate these low-O_2 conditions (the picocyanobacterium *Prochlorococcus*, for instance; Lavin et al. 2010). Other examples of multiple global change driver feedbacks for biogeochemically crucial enzymes include the interactions between warming and iron availability for nitrogenase activity that are presented in the sidebar titled Ocean Warming, Iron Limitation, and Global N_2 Fixation Rates (Jiang et al. 2018, Yang et al. 2021) and between rising temperature and anthropogenic NH_3 enrichment for AMO activity that are discussed in Section 6.1 (Zheng et al. 2020). Clearly, there is a need for more field and laboratory work that takes on the challenge of determining how carefully considered, more complex anthropogenic change scenarios will affect key marine microbial enzyme systems.

Throughout this review, we have highlighted many inconsistent responses from various studies examining all five enzymes, as well as the one transporter system. These contrasting findings from different laboratories are especially obvious for acidification, but they are often evident for temperature as well. Frequently, these differences are dismissed as being just taxon-specific responses to stressors, and indeed it does make sense that enzymes in species with widely divergent evolutionary histories could react quite differently to increasing temperature and falling pH. This explanation is perhaps less convincing for differences within closely related clades, as most critical enzyme systems are likely to be highly conserved at this phylogenetic level. Marine microbiologists also need to be aware of another potential explanation for opposing findings—namely, spurious comparisons arising from unrecognized inconsistencies in experimental methods. When comparing results from different studies, misleading artifacts can result from even relatively modest differences in treatments and growth conditions. Mitigating this problem requires scrupulous reporting of precise and replicable experimental designs and the use of community-level intercomparison exercises and common analytical standards (Hutchins & Fu 2017, Boyd et al. 2018).

The wide range of observed responses to warming and acidification also raises the question of the role of enzymatic diversity in buffering climate change impacts on marine biological communities. We have pointed out the very large collective genetic and phenotypic diversity that exists in the ocean for the biogeochemically important enzymes discussed here. The branches of the tree of life have given rise to numerous orthologs, especially for ancient enzyme systems such as nitrogenase, which appeared early in the evolution of life on Earth (Einsle & Rees 2020). Over the course of their separate evolutionary pathways, some isozymes have developed differing optimal activity ranges for temperature or pH that can potentially increase the resiliency of mixed assemblages to anthropogenic global change (Kling et al. 2020).

Even fine-scale, intraspecific microdiversity may have significant global change implications. An example of a very small but potentially consequential genetic difference is the single nucleotide change that coverts blue- to green-absorbing proteorhodopsin, as mentioned in Section 3 (Man et al. 2003)—a shift that could select for a photoheterotrophic lifestyle in oligotrophic as opposed to eutrophic marine environments. This type of cryptic biochemical microdiversity could also help to provide important enzymatic pathways with robust functional redundancy, thus potentially allowing marine microbial populations to adapt to changing ocean temperatures and acidity.

Global warming and ocean acidification are considered major drivers behind some of Earth's greatest mass extinctions. In some other instances, though, abrupt changes in ambient temperature and pH have had relatively little effect on the biosphere (Bond & Grasby 2017). Therefore, we do not have a congruent biochemical mechanism that could explain the selective disappearance of certain taxonomic groups while leaving others unaffected. As we are currently facing some of the same environmental conditions implicated in past biosphere collapses, it is important that we attain a better understanding of the physiological capacity that some organisms have to buffer the combined effect of multiple environmental stressors acting simultaneously. A possible answer to the selective extinction riddle could lie in the plasticity of responses of different families of enzymes (e.g., homeostatic enzymes such as CAs) to rapid fluctuations in temperature and pH. Knowledge of those enzymatic thresholds in different groups of biogeochemically critical marine microorganisms could potentially help us to identify likely winners and losers in the strange new ocean of the future greenhouse world.

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SUMMARY POINTS

1. Global marine biological productivity and nitrogen fixation and utilization depend largely on the activity of a relatively small number of keystone microbial enzyme and surface transporter systems.
2. Because the activity of each of these biogeochemically critical catalytic biomolecules is influenced by temperature and pH, all of them are potentially affected by anthropogenic warming and ocean acidification.
3. Rising temperature makes CO_2 less soluble and thus favors upregulation of carbonic anhydrase (CA), while elevated CO_2 has the opposite effect, and excessive acidification could even overwhelm the buffering capacity of CA.
4. The activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) may decrease with acidification unless adequately buffered by CA, and warming tends to favor RuBisCO photorespiration over carboxylation reactions.
5. Proteorhodopsin proton pump activity may be impaired and even reverse direction due to acidification, while photocycling rates are likely to increase with warming.
6. Nitrogenase activity is generally promoted by higher temperatures up to a threshold level, and most studies indicate that N_2 fixation rates are also likely to increase with rising CO_2, unless iron is severely limiting.
7. In laboratory phytoplankton cultures, nitrate reductase (NR) activity is decreased or unaffected by warming, and the effects of acidification appear to be group specific, while the nitrate reduction rates of field assemblages often increase with rising CO_2.
8. The activity of ammonia monooxygenase (AMO) in the ocean often declines with acidification despite the low pH optima observed in laboratory cultures, and temperature strongly controls ammonia oxidation rates, while interactions between warming and substrate concentration may differ for nearshore bacteria and offshore archaea.

FUTURE ISSUES

1. What are the cellular energetic costs of coping with ocean acidification and warming for marine microbes, today and in the future?
2. How do temperature and pH affect the threshold levels, thermal limits, and catalytic mechanisms of marine microbial enzyme and transport systems?
3. In what ways will the entire complex of multiple global change variables interact to influence the activity and substrate promiscuity of key enzymes and transporters in oceanic microorganisms?
4. Can the broad genetic and phenotypic diversity of catalytic biomolecules in marine microbes help to buffer the effects of a changing ocean environment on essential biogeochemical functions?
5. Does the differential sensitivity of enzyme and transporter biochemistry to warming and acidification help to decide which groups of marine organisms will disappear or persist during past, present, and future mass extinction events?

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DISCLOSURE STATEMENT

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

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Errata

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