CONSEQUENCES OF HIGH CO_2 AND OCEAN ACIDIFICATION FOR MICROBES IN THE GLOBAL OCEAN

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EXECUTIVE SUMMARY

Human activities are altering the chemistry of the seas at a fundamental level. Atmospheric concentrations of carbon dioxide are increasing as a result of burning of fossil fuels. As CO_2 from the atmosphere dissolves in the surface ocean, seawater becomes more CO_2-rich and more acidic (increased hydrogen ion levels and lower pH), resulting in substantial changes in a host of chemical properties. The pH of the oceans has been relatively constant for more than 20 million years but is now changing very quickly and will decrease from on average pH 8.1 to pH 7.8 by the end of the century – probably at least a 10 times more rapid change than anything over that period of time.

A group of expert microbial oceanographers met at the Center for Microbial Oceanography and Education, Hawaii in February 2009 to assess the consequences of higher CO_2/lower pH for marine microbes – crucial regulators of the Earth System. It is widely recognized that marine microbes provide great benefit to human society, through essential ecological services, and maintain the health of the oceans. Microbial photosynthesis, and hence oxygen production, in the ocean is equivalent to that of plants on land. Microbes maintain the productivity of the oceans through nutrient cycling and nitrogen fixation. Microbial activity results in the production of biogases that affect the chemistry of the atmosphere, which in turn affects our planet's climate.

It is not known if microbes can or will adapt or evolve over the likely time-scale of ocean pH change. There are no robust predictions of how marine microbes will be affected by increased CO_2 and pH change or what the resulting impacts would be on marine ecology and biogeochemistry. Research investment should be expanded in laboratory and field experiments and models to make predictions and provide probability estimates of how microbial processes may be sensitive or insensitive to higher CO_2/lower pH. Better data and numerical models are required to inform policy makers on the issues that will arise as we tackle the consequences of rising atmospheric CO_2. Considerable investment is

required to design and carry out experiments to assess which ecological services provided by marine microbes might be at risk from ocean acidification.

It was the view of the experts that experimental approaches should rely heavily on existing environments where higher CO_2 and lower pH occurs naturally, such as zones of high respiration, particularly where respiration is much higher than primary production, and in cold polar seas, which have lower calcium carbonate saturation state. Freshwater lakes and estuarine waters offer exceptional opportunities since they are less well buffered than the oceans and experience daily to seasonal changes in hydrogen ion concentration that can be orders of magnitude greater than those projected for the oceans in the next century. Coastal and estuarine environments also experience substantial pH variations over short time and space scales. An important question is whether marine microbes have lost the metabolic flexibility of their freshwater counterparts because they have experienced relatively constant pH for 20 million years.

Our understanding of basic marine microbial physiology is inadequate to answer some important questions involving the consequences of ocean acidification. For example, most phytoplankton species regulate internal pH, which is generally maintained below the pH of seawater, but it is not known how well other marine microbes control pH, nor if a change in external pH will affect this process. Elevated CO_2 levels increase photosynthesis rates in some but not all microbial species, and laboratory studies suggest that marine nitrogen fixation may also be enhanced. Carbonate ions – the building block for calcium carbonate shells – will decline in a high-CO_2 world. The mechanisms involved in the biological formation of carbonate shells are not well understood, and there is conflicting evidence that shell formation rates could either increase or decrease under future elevated CO_2.

Ocean acidification will not occur in isolation to other consequences of CO_2-induced climate change and more local human perturbations to the marine environment such as nutrient overloading. Potential feedbacks and synergies are poorly understood, as are impacts on low oxygen zones, the oceanic reservoir of dissolved organic matter, and ocean carbon storage and sequestration.

Microbial diversity in the oceans is enormous and complex, with many thousands of bacterial species in every liter of surface seawater. Although it is highly unlikely that increased CO_2 and lower pH will result in species loss, alteration of pH may change the dominant species with subtle but potentially important consequences for biogeochemical processes and food webs. Since natural marine microbial communities are very complex, experiments to investigate higher CO_2 / lower pH should attempt to capture that complexity. The best approaches used to date have involved the use of large volume mesocosm experiments, but there are real challenges in using mesocosms for long time periods (> 1 month) or in the open ocean. A large investment in infrastructure will be required if the research community decides that such experimental approaches offer the best chance to understand how marine microbial assemblages will respond to higher CO_2 and lower pH changes.

PRIORITY QUESTIONS

It is the view of the expert group that the following are likely to be the most important questions to be answered in relation to the impact of higher CO_2 / lower pH on marine microbes. The following topics are discussed in greater detail in the report. We have attempted to indicate urgency and the likely scale of investment that will be required.

Problem or Question	Barriers to progress	Urgency and scale of investment
1. Agreement on best methods to manipulate seawater chemistry for biological incubations. Can specific changes/biological responses be isolated (e.g., pH versus pCO_2 vs. carbonate ion)?	Requires international agreement on the most appropriate methodologies. The European project EPOCA has made suggestions but these have yet to be accepted by all groups. (http://www.epoca-project.eu/index.php/Home/Guide-to-OA-Research/).	**Priority - 1:** Requires good international communication. *Cost ~ small- possibly to fund attendance at international workshops.*
2. Basic studies on how microbial physiology responds to pH change (e.g. internal cellular controls on pH) May require development of new techniques (e.g., single cell manipulation).	Few barriers. Laboratory experiments are feasible now using culture experiments; flow cytometry and encapsulation methods allow manipulation of single cells.	**Priority - 1:** *Scale:* 2-4 investigators. *Cost ~ $2 million over 3 years.*
3. Accessing genomic information of how natural populations respond to pH change using metagenomic and metatranscriptomics approaches.	A significant proportion of sequences have no known function - a problem that is not unique to marine microbiology.	**Priority - 1:** *Scale:* Cost of sequencing remains high; 2-4 investigators. *Cost ~ $2 million over 3 years.*
4. Single species studies on CO_2 / pH sensitivity across major groups (calcifiers, photosynthesizers; N_2-fixers; heterotrophic bacteria).	Laboratory experiments are feasible now using cultures. Some well-characterized laboratory cultures may not be representative of microbes in the natural environment.	**Priority – 1 - 2:** *Scale:* 2-4 investigators. *Cost ~ $2 million over 3 years.*
5. Comparison of ocean zones of high respiration (high natural pCO_2) and tropical versus polar (cold water seas).	Requires the organization of large, multi-institution cruises on ocean research vessels.	**Priority – 1 - 2:** *Scale:* 8-12 investigators; collaboration between research groups *Cost ~ $10 million over 3 years.*

6. Freshwater and estuarine microbes accommodate frequent and rapid natural pH change. Are marine microbes less adaptable to pH change?	Laboratory experiments are feasible now using culture experiments. However, grant-awarding bodies rarely fund projects with both marine and freshwater components.	**Priority - 2:** Requires a collaborative grant to a freshwater and a marine laboratory. *Scale:* 2-4 investigators. <u>*Cost ~ $2 million over 3 years.*</u>
7. What are the time scales of adaptation (evolution) to higher CO_2 / lower pH and can this be demonstrated in laboratory cultures?	Experiments are possible now using laboratory culture experiments. However, long-term culture experiments are technically demanding.	**Priority - 2:** Requires collaboration between individual research groups. *Scale:* 8-12 investigators. <u>*Cost ~ $10 million over 3 years.*</u>
8. How will complex natural assemblages respond to higher CO_2 / lower pH over time scales of years to decades?	Microcosm and mesocosm scale experiments have not been attempted over time scales of months to years but are technically feasible. They are probably only possible in coastal waters but should involve reference experiments in freshwaters.	**Priority – 2-3 but requires new methodological development:** *Scale:* 12-20 investigators - very labor intensive - investment in infrastructure required (probably at only one or two geographical locations) to allow collaborative experiments involving different laboratories. <u>*Cost ~ $5-10 million per year over 10+ years*</u>
9. How will open ocean ecosystems structure respond to higher CO_2/lower pH? Can mesocosm experiments be extended to the open ocean?	Requires the development of experimental systems that can be deployed from ocean research vessels. Technically very demanding (only one ocean facility presently under development, in Germany). Short-term (days to weeks) experiments are already possible; probably only feasible to do a small number of such experiments so they will require careful planning.	**Priority – 3 – 4 but requires methodological development:** *Scale:* ~20 investigators. <u>*Cost: Design and fabrication of ocean-going mesocosms ~ $5 million. Requires large ocean-going research vessels (additional cost). Staff costs $5-10 million per year over5+ years*</u>
10. Mesoscale CO_2-enrichment experiments (similar to iron-enrichment studies).	Technically difficult to disperse CO_2 at controlled concentrations over large areas of the ocean.	**Priority – 4** Not currently practical. Will require very large investment to develop infrastructure to release and maintain controlled amounts of CO_2. May be the only robust way to test and validate model prediction of the consequences of higher CO_2/lower pH. <u>*Cost ~ $10-15 million per experiment.*</u>

Contents of Report

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PART A: GENERAL INTRODUCTION

A.1 CARBON DIOXIDE DISSOLVES IN SEAWATER AND CHANGES ITS CHEMICAL PROPERTIES

Carbon dioxide dissolves readily in seawater. As the amount of CO_2 has increased in the atmosphere in the last 200 years due to human activity, more CO_2 has dissolved in the ocean and will continue to do so in the future. Even if atmospheric CO_2 were to stabilize, CO_2 will continue to increase in the mid-depth and deep ocean for several centuries because of the long-timescales of ocean circulation (>1000 years). Increasing dissolved CO_2 means that the pH – the measure of acidity or alkalinity of a solution – of the surface ocean is changing rapidly.

The chemistry is straightforward but not simple; CO_2 and water react to form carbonic acid – a weak acid:

$$[CO_2] + [H_2O] = [H_2CO_3]$$

Carbonic acid dissociates to hydrogen ions and bicarbonate ions:

$$[H_2CO_3] = [H^+] + [HCO_3^-]$$

The extra hydrogen ions combine with carbonate ions to form bicarbonate ions:

$$[H^+] + [CO_3^{2-}] = [HCO_3^-]$$

Therefore, increasing dissolved CO_2 in seawater results in higher concentrations of carbonic acid (H_2CO_3), hydrogen ions (H^+) and bicarbonate ions (HCO_{3-}) and fewer carbonate ions (CO_3^{2-}). Since pH is defined by the activity of hydrogen ions (and varies in the opposite direction), it is clear that an increase in H^+ must result in a decrease in pH. This is why the pH of the oceans is changing and why the process is referred to as ocean acidification. In addition, another aspect of climate change – increased ocean temperature – will also affect ocean pH because there is a temperature effect on solubility and the equilibrium constants of the CO_2 system.

Changing ocean pH, along with the CO_2-driven greenhouse effect of rising temperature due to the trapping of heat in the atmosphere, is therefore one of the major consequences of human activities that result from the burning of fossil fuels, production of cement and changed land use such as deforestation. Since atmospheric CO_2 concentrations are relatively evenly distributed and the atmosphere is in contact with the surface of the oceans, the effects on ocean chemistry are global. Although the phenomenon is usually referred to as "ocean acidification", the oceans will still be alkaline (pH > 7) even under the worst scenarios of anthropogenic production of atmospheric CO_2.[1]

The fact that seawater CO_2 concentrations will also be higher is as important as the pH change, since changing CO_2 affects multiple aspects of seawater chemistry, not just pH. Microbes will experience a range of different environmental perturbations in a high CO_2 world, and different parts of the microbial community may respond in different ways. In addition, climate change will result in higher surface temperatures in many regions of

[1] **The term 'ocean acidification'**
Perhaps it is unfortunate that the term 'ocean acidification' has been used to describe pH change since it has resulted in inaccurate reporting in the media. Headlines often say that the oceans are turning acid; they are not. There will be a change in pH – the scale that describes the range in acidity or alkalinity that ranges from 0 (maximum acid) to 14 (maximum alkaline). Technically, pH is the logarithm of hydrogen ion activity so a small change in the value of pH may actually be equivalent to a very large change in hydrogen ion concentration. The pH of the surface oceans is currently about pH 8.1 and has decreased from about pH 8.2 over the last 30 years. By the end of this century, the surface pH is expected to decrease to about pH 7.8, but surface waters will still be alkaline.

the ocean, stronger vertical stratification (and thus reduced nutrient supply), and altered frequency of storms, all of which will influence the environment in which marine microbes exist.

The chemistry of the CO_2 system (dissolved CO_2, bicarbonate and carbonate ions) may be clear, but the consequences for life in the oceans are far from understood. In particular, there is great uncertainty about how lower pH and higher CO_2 concentrations may affect marine microbes.

A.2 WHY ARE MICROBES IMPORTANT?

All life in the sea relies on marine microbes. In contrast to the terrestrial environment where the primary producers are higher plants, which are large (often very large) and live for many months or years, in the oceans the primary producers are microbes that live for only a day or so. Yet these microbial plants (phytoplankton and cyanobacteria) are extremely important for the planet since they produce about 50% of the total oxygen that is produced globally by photosynthesis each year. Bacteria and archaea are also very important, maintaining the productivity of the oceans by recycling nutrients that are required by the phytoplankton. Bacteria are involved in biogeochemical cycling of elements. Ocean productivity depends on a wide range of microbial species – a diversity that has only recently been revealed through the sequencing of DNA from the oceans. There is still a very poor understanding of the microbes that are responsible for these fundamentally important biogeochemical cycles that maintain the productivity of the oceans. The truth is that we can more readily chemically measure the result of microbial activity, such as release of greenhouse gases like methane or nitrous oxide to the atmosphere, than we can identify and describe the organisms involved in the process.

A.3 WHY IS OCEAN ACIDIFICATION AN URGENT PROBLEM?

The pH of the ocean has been relatively constant for many millions of years. The best estimates are that pH has not changed significantly for more than 20 million years – certainly much longer than the existence of *Homo sapiens* or indeed any earlier hominid species. Mankind has long relied on the oceans for food and, even today, most of the largest cities and other centers of population are located on the coast. Given that pH change in the surface ocean is currently very rapid, it is extremely important to be able to predict the consequences of pH change for the goods and services that mankind relies on the oceans to provide.

The rate of pH change is perhaps as important as the magnitude of change because pH is now probably changing more rapidly that at any time in the recent geological record. The best estimates are that pH will decrease by 0.2 or 0.3 pH units in less than 100 years. Previous pH changes of this magnitude have probably occurred over thousands or even tens of thousands of years. If change occurs slowly, organisms can adapt or evolve, thus prospering under the changed environmental conditions. Over long time-scales, the oceans will also be buffered by geological reactions involving dissolution of calcium carbonate. However, when change occurs over short time scales (and 100 years is a short timescale in terms of the history of the Earth), there are increased risks of species extinctions. How will marine ecosystems respond to the new conditions? In the context of this report, the big question is – will marine microbes be able to adapt and continue to provide the same functions as present that maintain the productivity of the oceans?

A.4 POLITICAL PRIORITIES FOR POLICY MAKERS AND GOVERNMENTS: CONSEQUENCES AND POSSIBLE SOLUTIONS

Given that man-made climate change will pose a number of problems for governments throughout the world, is it possible to scale the potential threat of ocean acidification? That is, how will ocean acidification compare with temperature increase or sea-level rise as problems that must be given priority in government planning? It is currently impossible to give an informed answer to that question – one that deals with likelihoods and probability – without more research.

The first priority is to determine if high CO_2 / pH change will alter ocean productivity and hence the provision of food. Current estimates from the FAO are that the oceans provide about 15% of global human protein, so any change in that productivity would be very important. The most immediate concerns are clearly fish and shellfish – the direct food items for people – but these resources are already under great pressure. Many of the world's fisheries are over-exploited, many severely so, due to overfishing. Will pH change add an additional stress that will impact on the ability of fisheries to recover? Will ocean acidification reduce or increase phytoplankton productivity? Will there be changes in the dominant phytoplankton groups that might affect food web dynamics, possibly by replacing nutritious phytoplankton with those that form harmful algal blooms? And will ocean acidification alter marine ecosystems in ways that reduce the ocean's ability to remove CO_2 from the atmosphere – a process that is currently slowing global warming? It is far from clear if phytoplankton will be affected by pH change. And conflicting results are coming from different studies. Yet the health and productivity of the planet depends on marine phytoplankton, and we urgently need to know if marine productivity is likely to change.

There is also uncertainty about other global services provided by marine microbes, such as nutrient recycling that maintains ocean productivity, the breakdown of anthropogenic contaminants, or the production of gases that are released to the atmosphere, hence changing atmospheric chemistry and influencing short-term weather as well as causing climate variability. Bacteria provide many of these services, and the effect of pH change on these micro-organisms is probably less well understood than for phytoplankton.

Current knowledge is probably inadequate to inform the crucial decisions and choices facing policy makers in the near future and better scientific understanding is required

Unfortunately, global scale problems are extremely complex and difficult to solve. While a number of different research groups around the world have been doing experiments, there has been no concerted focus on the problem. The easiest experiments to do are not necessarily those that provide the most insight. It may be necessary to conduct large, multidisciplinary, international experiments. Such experiments need to be prioritized, which is difficult given the fractured funding of scientific research. In addition, most existing conceptual and numerical models are inadequate because they do not adequately capture the complexity of the microbial world, nor parameterize the key metabolic functions. Currently, it is very difficult to ascribe uncertainty factors to any of the predictions on the consequences of pH change.

This report is an attempt to bring some structure to the problem, to highlight areas of ignorance and to indicate areas that should be priorities for study. The experimental approaches that are necessary to advance understanding will also be considered.

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PART B: MARINE MICROBES: WHY THEY ARE IMPORTANT AND HOW THEY MAY RESPOND TO OCEAN ACIDIFICATION

B.1 KEY MICROBIAL PROCESSES

As discussed above, microbes mediate all fundamental biogeochemical cycles in the oceans. Alteration of these cycles has potentially significant feedback on climate change (feedbacks that could both dampen or accelerate human perturbations to climate). Yet, some of the most important processes may not be readily amenable to experimental study, and the danger is that there will be too much focus on those processes and organisms that are currently experimentally tractable.

B.1.1 Key environmental services provided by marine microbes

Although little is known about the functional diversity of marine microbes – which species carry out what biogeochemical process – we know in general terms which are the important processes in terms of environmental services that microbes provide. These processes are summarized below, along with an assessment of knowledge of how the process is likely to be altered in higher CO_2 / lower pH conditions.

PROCESS	IMPORTANCE	STATE OF KNOWLEDGE
Primary productivity	The net rate of organic carbon production determines support for higher trophic levels. Elemental stochiometry and biochemical composition (e.g. protein, lipid and carbohydrate content) determine food quality. Estimates of primary production can be obtained on global scales through satellite remote sensing. Carbon sequestration to the deep ocean depends on biological export flux.	Some studies indicate increased production (or at least higher carbon content per cell) at high CO_2, others show little affect, possibly due to species differences.
Species composition of dominant primary producers	Different species have different roles in ecosystem dynamics. For example, size of phytoplankton results in different grazing efficiencies and hence may alter grazer population structure. Also of great interest are species that produce climate-active gases (e.g. N_2O, DMS and CH_4) or are involved in toxin production.	Too few robust studies to be able to predict how communities might change.
Sinking of particles and export rates of organic carbon	An essential process affecting atmospheric CO_2 concentrations that may alter ocean carbon storage through the activity of the biological carbon pump. It is linked to species composition (small phytoplankton cells do not sink rapidly).	There have been very few studies to determine how high CO_2 / lower pH will affect vertical export

	Biomineralization is a key to export because of "ballast" or the association of organic carbon with inorganic components of rapidly sinking particles; reduced calcification as a result of lower carbonate ion concentrations could result in reduce export. Elemental composition is also important; higher C/N effectively makes nutrients more efficient and would enhance ocean carbon sequestration; altered carbon export could also affect oxygen minimum zones.	of carbon.
Bacterial respiration and remineralization rates	The ultimate fate of a substantial fraction of the organic carbon in the oceans depends upon breakdown by heterotrophic bacteria. Changes in bacterial activity and growth efficiency can profoundly affect the oceanic net carbon balance.	Little is known about the sensitivity of heterotrophic metabolism to high CO_2 / lower pH or the effect of change in 'nutritional value' of organic matter.
Limits to ocean fertility	Microbes play a dominant role in the cycling of nutrients, particularly biologically available nitrogen which limits primary productivity in many oceanic provinces. Key processes that set the oceanic inventory of biologically available nitrogen in the oceans are nitrogen fixation and denitrification, plus anammox (anaerobic ammonium oxidation), leading to a loss of fixed nitrogen. Nitrification is also important for speciation of nitrogen in the sea.	The sensitivity of these processes and underlying key organisms to ocean acidification has begun to be addressed in preliminary experiments.
Trace gas production	Microbes drive the production and consumption of many other potent climate-active gases – methane, nitrous oxide, dimethylsulfide and organohalides. They have very important implications for potential climate-feedback mechanisms	Little research has been done on the effect of higher CO_2 and lower pH on net biogas production or consumption.
Trace metal availability	There is the potential for pH driven changes in dust and particle dissolution rates (and hence availability) for Fe and other metals that are known to exert a powerful control on marine microbial activity. A large fraction of dissolved trace metals in seawater are complexed by organic ligands, and pH changes could alter total metal concentrations and bioavailability.	Almost nothing is known, other than the effects on metal speciation.

Clearly, there is currently insufficient information about how these basic biogeochemical processes will respond to increased CO_2 / lower pH. Without more experimental data, it is not possible to provide robust, statistically relevant predictions of how marine microbial activity will change (if at all) by the end of the century. Nor is it possible to anticipate if marine microbial communities will adapt or acclimate to the new conditions.

B.2 Acclimation and/or Adaptation

In the last 5 years, as a result of the application of high throughput sequencing technologies, we now know that microbial diversity in the oceans is huge. There are many thousands of bacterial and archaeal ecotypes, but the role of most of these organisms in the marine ecosystem is not known. In the context of climate change, there are three possible scenarios.

Firstly, given the huge microbial diversity, it is reasonable to assume that microbes will always be present that are capable of carrying out fundamental biogeochemical processes in the ocean. Species composition may change and different microbes may come to dominate, but there will always be microbes present which will find the new environmental conditions favorable and be able to carry out key process such as nitrogen fixation.

Secondly, given their genetic plasticity and the prevalence of lateral gene transfer, it is entirely plausible that most marine microbes will be able to evolve by acquiring genes from other species. In this case, microbial assemblages may not change much and the same dominant bacteria and archaea will be present. However, their ecological fitness to the new environmental conditions will have been enhanced by lateral gene transfer, allowing the same species to survive.

A third possibility is that higher CO_2 and lower pH will cause diversity loss and even wipeout whole assemblages. This is unlikely to happen for heterotrophic bacteria, archaea and most phytoplankton. One possible exception amongst marine microbes is the coccolithophore group, whose calcite coccoliths (external carbonate shell plates) will dissolve more readily because calcium carbonate will be less saturated or even under-saturated at pH 7.8. Even though there are some “naked” coccolithophores without calcareous plates which may be unaffected by higher $CO_{2,}$, loss of coccolithophores with calcareous plates could still have major impacts on the oceans.

Acclimation – that is, adjustment of an organism in response to perturbations using existing cell machinery. This may involve changes in gene expression (e.g. changed expression of inorganic carbon concentrating mechanisms in response to changed inorganic carbon availability) but no change in the genetics of the organism. Acclimation can be rapid (minutes-hours), though a new steady state may not be achieved for several generations.

Accurate prediction of the ability to acclimate or even to detect acclimation already underway will be difficult because we do not currently have adequate descriptions of existing microbial diversity, either at the genotypic or phenotypic level. Although rapid progress is being made in describing natural assemblages in many different marine provinces, it is probably premature to try to define, let alone describe, species and strains that form key functional groups. Such comprehensive descriptions of microbial communities are a necessary precursor to monitoring and predicting the ability of different species, strains, or functional groups to acclimate to a changing environment of higher CO_2, lower pH and increasing water temperature.

Adaptation – that is, adjustment of an organism in response to perturbation using genetic change bought about through natural selection. This typically takes many hundreds of generations.

Acclimation experiments can readily be carried out on laboratory cultures, but there is a need to be able to make predictions about adaptation at the level of an assemblage or community. Adaptation rates for microbes may be faster than for multi-cellular marine organisms, even accounting for the differences in life space, because the likely mechanisms of adaptation for microbes are lateral gene transfer and viral infection. Experimental studies must try to incorporate the complexity of natural assemblages. It is unclear whether changing pH will influence the transfer of genetic information, other than by providing an environmental change that will act to select for biological adaptation and microbial evolution. Certainly, the response of viruses to pH change is particularly poorly understood and should be a priority for experiments on natural assemblages.

None of these effects will occur in isolation. At a time of rapid climate change, there will also be warmer surface seawater temperatures, enhanced vertical stratification and reduced nutrient supply, reduced Arctic sea-ice, altered freshwater inputs and the possibility of increased storm frequency and intensity. Many coastal waters are already experiencing the effects of elevated nutrients (nutrient eutrophication), and subsequent enhanced primary production, due to agriculture, land-use, nutrient runoff and atmospheric deposition. Respiration of the excess organic matter production can lead to localized regions with low oxygen, high CO_2 and low pH, with perturbations much larger than the more gradual acidification effects from rising atmospheric CO_2. All of these processes will influence microbial populations; it is not clear how these changes will interact with the way that marine microbes will respond to ocean acidification.

B.3 MICROBIAL PROVISION OF ECOSYSTEM SERVICES

B.3.1 Biogeochemistry and nutrient cycling

Changes in rates of microbially-mediated biogeochemical processes are of particular consequence for marine ecosystems, as they alter the relative balance of different compounds in the sea. For example, the forms and quantities in which the nitrogen is present are entirely controlled by microbial processes, through nitrogen fixation, nitrification, denitrification, and anaerobic ammonium oxidation (anammox). There is preliminary evidence that nitrogen-fixation rates by *Trichodesmium* are enhanced by higher pCO_2, and nitrification rates also appear to be sensitive. Experiments have shown either a reduction due to decreasing pH, enhancement due to higher pCO_2, or some combination of these effects.

N-cycling microorganisms clearly can respond to changes in pH and pCO_2, but exactly how the full N cycle may change is an open question. The two most salient questions with regard to how the N cycle responds to ocean acidification are whether it may be accelerated, decelerated, or temporarily thrown out of balance, and how the relative speciation of N may change—including the important greenhouse gas nitrous oxide (N_2O). These possible changes should be considered in the context of uncertainty that is still associated with many N cycle terms and processes. For example, the overall N budget and the sources and sinks for N_2O are not fully constrained. The recent discovery of anammox and archaeal nitrification illustrate our ignorance of basic biogeochemical processes.

Will ocean acidification alter the nitrogen cycle or temporarily throw it out of balance?
The major sources and sinks for nitrogen in the ocean are separated spatially: microbial N fixation is most active in surface waters of stratified gyres and anaerobic N loss occurs in oxygen minimum zones. If either process is enhanced or reduced, the other must respond to maintain balance, and it seems unlikely that this will occur instantaneously (but may occur over long time scales). Anammox bacteria could respond to changes in pH or pCO_2, while changes in oxygen concentrations may have the most direct effect on N loss processes; these changes may be a secondary effect of ocean acidification or a more direct result from climate-driven changes in circulation or stratification. Research into the response of N loss pathways to ocean acidification is therefore essential.

How might the relative speciation of N change?
Regardless of the overall rate at which N is fixed from the atmosphere and then returned, the form it occupies along the way may change with ocean acidification. Decreases in pH will drive the NH_3/NH_4^+ couple further towards NH_4^+. This may result in decreased NH_3 flux to the atmosphere through air-sea exchange. Within surface waters, biological responses to this change may be nonlinear, with different functional groups and species having different affinities and responses. Most importantly, ammonia oxidation, nitrifier-denitrification (a possible response to nitrite toxicity), denitrification, and anammox all produce N_2O. Yields of N_2O are typically small relative to overall rates, but are nonlinear – hence any change in process rates could produce a far greater change in N_2O production rates.

Another possibility with higher pCO_2 is long term siphoning of nutrients from the upper ocean into other storage reservoirs through carbon overproduction. There is evidence of dissolved organic carbon and nutrient accumulation in mid ocean gyres from the long-term oceanic observatories (HOT and BATS programs). These accumulations have never been explained beyond speculation that it might be associated with intensified stratification.

There is a need for focused studies to investigate the amount of production shunted into dissolved organic matter (DOM) and the nutrient elemental ratio of the DOM. This is fairly straightforward involving the determination of the range of DOM produced as a fraction of primary production, and the range of C/N, C/P and N/P ratios expected. Less clear are questions about changes in DOM bioavailability with altered pH, variations in surface charge of macromolecules and colloids, and aerogel formation and particle aggregation. All of these processes may impact on substrate availability to marine microbes.

Another chemical process whose sensitivity to acidification is poorly understood is the potential change in trace metal bioavailability, which exerts a powerful control on marine microbial activity. This could result from changes in the solubility of atmospheric aerosols deposited over the oceans. But there could also be changes in pH sensitivity of ligands that bind trace metals; ligand control is crucial and poorly understood. Our knowledge is also incomplete about the effect of pH change on the dissolution rate (and hence availability) of iron and other metals from sedimentary particles.

B.3.2 Consequences for biodiversity

It is critical to understand what effect ocean acidification will have on keystone microbes. Rapid change could drastically alter the presence or dominance of such species, with significant consequences for the entire microbial population in the short run. It may be possible to mine genomes and marine metagenomes for known pathways that are critical but lacking. For example, SAR11, an abundant group of bacteria in the ocean, lacks the ability to synthesize vitamins like cobalamin. This will be a very difficult task since demonstrating that something is missing is harder than looking for things that are present.

A major uncertainty is how microbial communities will behave in a high CO_2 ocean, particularly in relation to bacterial evolution. Lateral gene transfer (LGT) in the oceans could be crucial over very short periods of time (decades to centuries) but the process is poorly understood. While genome sequencing has allowed us to see that LGT does occur, we still have a very imperfect view of how it occurs. Will selective pressure be enough to fix new genes into the population in just a few decades? For example, if SAR11 in the ocean has a similar doubling time as it does in the laboratory, it may only double 12 times a year. It will take a while before a microbe acquiring perfect genes by LGT can repopulate the seas.

Although any changes in biodiversity within the bacteria and archaea may not significantly affect the services that these organisms provide, there is more potential for biodiversity loss within eukaryotic organisms and arguably a higher likelihood that these losses will affect biogeochemical processes in the ocean. Abrupt changes in other (principally terrestrial) ecosystems typically result in the loss of species richness and the appearance of weedy species. These changes have major effects on higher trophic levels, ecosystem stability and reduce the gene/species pool on which the processes of natural selection can act. So the consequences can be quite dramatic. In the case of ocean acidification, it is legitimate to be concerned about changes in community composition, e.g., relative abundances of coccolithophores and diatoms, and about the potential for loss of some of the more exotic and most highly adapted species of phytoplankton and zooplankton such as foraminifera, radiolarians etc. Biodiversity loss is typically accompanied by loss of ecosystem robustness, which could have major consequences for some ocean processes and lead to knock-on effects for higher trophic levels.

B.3.3 Food chain dynamics – the microbial loop

Fluxes through heterotrophic bacteria and the rest of the microbial loop are likely to be different in a high CO_2 world. As a first approximation, these fluxes follow the phytoplankton, but in fact they vary as a complex function of both abiotic and biotic factors. Why microbial loop fluxes vary as they do is not well understood. Consequently, it is not clear how heterotrophic bacteria and other components of the microbial loop will respond to rising CO_2 and decreasing pH in future oceans. Predicting this response is crucial because heterotrophic bacteria are major components of the marine biota and currently process about half of primary production in the oceans.

Previous work suggests several reasons why heterotrophic and photoheterotrophic microbes may be different in a high CO_2 world. Elevated CO_2 may stimulate the production of DOM. This potentially important experiment needs to be repeated for other algae and for more environmental conditions. In addition, the nature of the DOM needs to be examined. If the DOM is labile, then higher production will lead to higher heterotrophic bacterial production and respiration and in turn to more material being processed by the rest of the microbial loop. Depending on the ratio of C to other elements such as N and P, CO_2-enhanced production of labile DOM could lead to more uptake of inorganic nutrients (e.g. ammonium and phosphate) by heterotrophic microbes, which would have negative feedbacks on the phytoplankton community. Some of this DOM may not be used immediately by heterotrophic bacteria and would contribute to the storage of C in the DOM pool. Regardless of the lability of the DOM, any change in DOM production by phytoplankton would affect the production of particulate organic material by phytoplankton and the availability of that material and energy for herbivores and eventually higher trophic levels.

Some phytoplankton species are likely to thrive in a high CO_2 world while others will decline. This change in phytoplankton community structure is likely to have major impacts on many biogeochemical processes and on fluxes through the microbial loop, even if primary production rates remain unaffected. Because the relationships between phytoplankton community structure and bacterial community structure and fluxes are complex and not well-understood, how they may vary in a high CO_2 world is also unclear.

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PART C: EXPERIMENTAL APPROACHES TO INVESTIGATE THE CONSEQUENCES OF INCREASED CO_2 / DECREASED PH

C.1 EXISTING HIGH CO_2 / LOW PH ENVIRONMENTS PROVIDE OPPORTUNITIES TO TEST THE RESPONSE OF MICROBIAL ASSEMBLAGES

C.1.1 The oceans are very complex and show large natural seasonal changes and spatial variation in pH

There is a tendency to assume that there will be uniformity in the response of the oceans to high CO_2 but this is not the case. Carbon dioxide is dispersed much more rapidly in the atmosphere than in the oceans. While there is relatively direct coupling of the marine habitat to increased atmospheric CO_2 in the surface ocean, the dissolution of CO_2 from the atmosphere is influenced by factors such as sea surface temperature, salinity and surface roughness. Temperature modulates seawater carbonate chemistry; cold polar waters have lower saturation states for calcium carbonate and may be more susceptible to acidification. And biological processes result in large natural variations in pH and pCO_2. Anywhere that there is a phytoplankton bloom, there will be a change in pH because CO_2 is utilized and dissolved CO_2 concentrations decline. In a bloom, pH increases (less dissolved CO_2 means higher pH) which is the opposite of ocean acidification (increasing dissolved CO_2 results in lower pH). It is important to realize that marine organisms in the present-day ocean are continually exposed to natural variation in pH on a variety of space and time scales. There are also regions of particularly high CO_2/lower pH throughout the oceans. Regions of volcanic activity may release CO_2, with or without higher concentrations of sulfur gases. Of wider relevance are regions, often at the depth of the thermocline, where CO_2 concentrations are high because of the breakdown of organic matter by heterotrophic (mostly microbial) activity.

In some places, the surface euphotic zone can be affected by continuous or seasonal injection of mid-depth thermocline waters with high CO_2 and other nutrient concentrations. Where there is such upwelled water, the CO_2 and pH values today may reflect atmospheric equilibrium at the end of the century or later, hence providing experimental systems in which to study the effect of ocean acidification. Although these areas may show smaller relative changes in dissolved inorganic carbon (DIC), even a small amount of added anthropogenic carbon can substantially increase the partial pressure of CO_2 because of the reduced buffer factor at elevated DIC levels. Modeling is needed to estimate what CO_2 and pH conditions will prevail in the future in upwelled waters

Upwelling systems with CO_2-enriched waters support a number of functions include chemolithotropy (the anammox reaction, sulfide oxidation and some nitrification) and (for near-surface anoxic waters) anoxygenic photolithotrophy, as well as oxygenic photolithotrophy in very recently upwelled waters and oxygenic photolithotrophy in deep chlorophyll maxima coincident with oxygen minima/CO_2 maxima. The remaining oxygenic photolithotrophy, and perhaps some nitrification, occurs in waters more closely coupled to the atmosphere.

Coastal and estuarine systems already experience considerable variability in pH and carbonate saturation states. Low salinity coastal waters are often also less buffered than open-ocean surface waters and can exhibit the lower pH and carbonate undersaturation conditions that are likely to occur in the open-ocean in future decades. Estuaries provide a natural laboratory to investigate microbial responses to strong gradients in pH, pCO_2, and carbonate saturation state.

C.1.2 Comparison of marine and freshwater environments

Many freshwaters, especially productive lakes that stratify, experience pH change up to 2 pH units at time scales of days, and 4 pH units over a year. The range is even greater across a range of lakes, although here typically a distinct microbial community (certainly phytoplankton) is found at different sites.

Many studies have examined the effects of acidification on freshwater biota, including microbes and higher trophic levels and ecosystem processes. Almost all of these studies have focused on much lower pH-values than are relevant to the oceans (e.g. pH 4 to 6 versus 7.5 to 8), but the sensitivity of various processes may be common. A literature review should identify possible pH-sensitive processes (photosynthesis, nitrogen-fixation etc.) in freshwaters that might help identify which processes in the marine environment might also be sensitive to a reduction in pH.

Freshwater phytoplankton as a community within a lake has evolved to cope with or exploit these changes in pH. This includes genotypic and phenotypic plasticity within a species, seasonal shifts in species composition from within the local propagule pool and, for large or rapid changes, migration from neighboring habitats. In contrast, phytoplankton in the open oceans experience relatively low variability in pH and in CO_2 concentration and limited scope for migration from less affected habitats. An exception is estuarine and coastal zones where riverine freshwater inputs can dramatically alter carbonate chemistry (relative to marine conditions) on short spatial and temporal scales. Much could be learned by comparing phylogenetically-related species from marine, coastal, estuarine and freshwater environments (e.g. freshwater and marine diatoms) and other functional groups, including calcifiers (although rare, some are present in freshwaters) and nitrogen fixers. Such a comparison would help establish if there are fundamental differences between marine and freshwater systems and if the broad tolerance of freshwater species can be used as a proxy for the response of marine microbes to ocean acidification. It would also provide useful scientific information on evolution of tolerance to variability. These experiments will be scientifically challenging to carry out the experiments in an ecologically relevant way that takes multiple factor interaction into account.

C.1.3 Are marine ecosystems robust?

Elevated CO_2 and reduced pH will be accompanied by elevated temperature and altered ocean circulation patterns, with the likelihood of non-linear responses due to multiple stressors. The effects of these changes on microorganisms could range in mechanism and severity. Little is known about the potential effects on microbial communities. A subtle pH effect could potentially be enough to gradually affect species abundances or evolution. Of particular interest would be the existence of biological thresholds at the level of either populations or communities.

Increased CO_2 / lower pH could have wide ranging effects from causing species shifts to changes in interlinked ecosystem functions and parameters. A number of scenarios can be imagined. For example, if primary production increases, this could result in an increase in heterotrophic bacterial activity and/or biomass. On the other hand, depending on the species or functional groups selected by the changing environmental conditions, decomposition could become uncoupled from production, resulting in enhanced storage in surface water DOM or in organic matter in the deep ocean. Enhanced production and climate change could lead to more severe N limitation that would select for nitrogen-fixing microorganisms. This could lead to a shift in the elemental stoichiometry of organisms in the surface ocean and have downstream effects on nutrient limitation controls. Not enough is known either at the organism, assemblage or ecosystem level, to evaluate the consequences of ocean acidification.

C.2 Manipulative Experimental Approaches

C.2.1 Experimental design

Manipulations to investigate the effect of increased CO_2 / lower pH can be carried out in a variety of experimental designs, depending on the specific question to be answered. The approaches can basically be characterized as -

- Detailed autecological studies of key microbial species.
- Laboratory scale microcosm experiments with batch and chemostat cultures of selected species of marine organisms.

- Mesocosm experiments with sufficient volume (e.g. several m^3) to support complex food webs.
- Mesoscale experiments with changing pH/CO_2 concentrations in drifting patch experiments, followed by SF_6 tracers.
- Natural pH perturbations across pCO_2 gradients at sea, in regions of intense coastal upwelling, or in the vicinity of volcanic CO_2 seeps.

Some of these experimental approaches have been frequently used to address other questions and could be immediately employed. Others, such as sea-going mesocosm or mesoscale experiments are still at the development stage or present logistic challenges that will require considerable investment in infrastructure and multidisciplinary approaches. Finally, observing pH responses along natural pCO_2 gradients are conceptually difficult due to the complex and multivariate character of natural perturbations.

Whichever experimental approaches are used in the future, it is important that the research community achieves consensus on the best way to manipulate seawater pH. Since high and changing CO_2 concentration affects many aspects of seawater chemistry, not just pH, it is important to adequately mimic the future ocean in any manipulation experiments. There are two basic approaches, each of which has advantages and disadvantages. Bubbling with CO_2-enriched air will readily achieve the pCO_2 levels expected in the future. But bubbles may break fragile microbes by cavitation and hence change the microbial community. It may also aggregate portions of the DOM pool. Alternatively, the addition of mineral acids will change pH but, unless additional bicarbonate is also added, the CO_2 chemistry will not adequately reflect the future situation in the oceans. Manipulation of pH and CO_2 concentrations can be used to mimic the future ocean but such experiments also require careful measurement and monitoring of the whole CO_2 system (at least two of the following parameters – pCO_2, pH, total alkalinity or dissolved inorganic carbon) to determine how the system is responding. Also, ocean acidification will not occur in isolation and, wherever possible, it would be desirable to conduct multi-factorial experiments to distinguish between the effects of changing pH and pCO_2 and warming.

C.2.2 Autoecology

There are clear benefits from doing bottle experiments with laboratory cultures – only one species is investigated in each experiment and the growth and experimental conditions can be closely controlled. However, there are caveats; most commonly used cultures have been in culture collections for many years or decades and may be genetically very different from cells in the natural environment. Since few culture media have been designed to adequately maintain pH, and since the density of cells in culture collections is usually very high, the pCO_2 of the culture medium will often be greatly reduced, with a likely pH of 9 or more. That is, culture collection material has probably been maintained at much higher pH than is found in the sea. Single species cell cultures are also very artificial and have no cell-to-cell or inter-species interactions. These have been increasingly shown to be important through processes such as quorum sensing in bacteria and even cross-kingdom signaling. Nevertheless, there are basic physiological questions that can best be answered with single species cultures.

Energy cost benefit to cells of higher CO_2. Increases in extracellular CO_2 may be beneficial in terms of inorganic carbon uptake mechanism in autotrophic cells. Some data are available for phytoplankton but there are still major questions. For example, why do coccolithophores calcify? Why do some species have inorganic carbon concentrating mechanisms that are absent in other species? Mechanistic studies at the cellular level are required to elucidate this fundamental question.

Control of intracellular pH. Studies have demonstrated that eukaryotic organisms maintain internal pH at lower values than seawater. There have been fewer studies of bacteria and archaea, but it is a reasonable assumption that they also control intracellular pH. Studies are needed to determine if intracellular pH changes as a function of external CO_2/pH and if there is a metabolic cost; e.g. could a decrease in growth rate at high CO_2/low pH be related to a failure in intracellular pH regulation or to some other cause. Data on intracellular pH of autotrophs, with the values for intracellular inorganic carbon, would permit a more direct estimate of the capacity of cells to accumulate total inorganic carbon, and CO_2, during growth on high CO_2 concentrations. Such measurements would give information on the occurrence, and the regulation of inorganic carbon concentrating mechanisms that complements, and adds valuable additional information to, the more commonly measured relationship between photosynthetic rate and inorganic carbon concentration in the medium for organisms grown in different CO_2 regimes. These two measurements would also show the extent of any possibly inhibitory CO_2 build-up, and intracellular pH decrease, in organisms lacking autotrophic inorganic carbon assimilation and so with net CO_2 production.

What relatively simple additions to autecological studies would yield valuable functional information for a range of taxa? Work on cultures of autotrophs grown at different CO_2 concentrations (and hence pH values) could be combined with measurements of intracellular pH and inorganic carbon concentration. While the fluorescent methods for estimating intracellular pH are attractive, combination of intracellular pH measurements with estimates of the intracellular inorganic carbon concentration could use ^{14}C methods for both determinations.

C.2.3 Laboratory scale/microcosms

Whilst the volume of most laboratory culture is usually a few hundred milliliters, this volume is often not suitable for studies involving consortia or grazing – i.e. simple interaction studies at a reduced community level. Microcosm experiments, with volumes of a few liters have advantages. The disadvantages are similar to those of bottle experiments with single species cultures in that they usually involve experiments with cells that have been maintained in culture collections for a long time. Moreover, the isolation of microbial communities in bottles and microcosms greatly distorts light regimes and nutrient supply and eliminates turbulence and mixing from the experiment.

In addition, microcosm experiments could also be conducted for short time periods with wild/natural populations collected at sea to study spatial and seasonal variations. An advantage of microcosm experiments is that they can be used in rapid multi-factorial experimental design that could complement the mesocosm approach.

C.2.4 Mesocosms

Manipulations of pH in mesocosms, with volumes of 1 – 20 m^3, offer the best chance of developing a robust research program to investigate the effects of pH shift on microbial energy cycle in the ocean. Although these experiments do not fully address the fundamental challenges of time and space resolution, they do allow a much fuller examination of ecosystem level responses to perturbations. Mesocosm experiments have been done to examine the impacts of CO_2 / pH manipulations on primary productivity, heterotrophic activity and grazing – but almost exclusively in coastal environments. Existing efforts to develop, deploy, and operate such mesocosms at sea have involved large logistical problems and have had a low success rate to date. But, with appropriate investment, this methodology could be improved to allow routine, large-scale open-ocean experiments.

This development effort will not be easy, being a high risk – high reward activity. As the costs and the risks involved are rather high, it is unlikely that such projects will be undertaken without well expressed support from funding agencies. If successful, this effort will benefit not only the ocean acidification community, but also the oceanographic community at large by opening new avenue for experimentation at sea.

Besides the technical aspects of mesocosm development, a basic methodological problem needs to be addressed. What are appropriate controls for mesocosm experiments and how can controls maintain unperturbed conditions with respect to the manipulated variables? A mesocosm experiment can only run for a maximum of a few months before biofouling becomes a significant problem. Therefore, they are only appropriate for step-function manipulations, i.e. a single rather than gradual change. Although such changes in CO_2 concentration are highly unrealistic in terms of actual perturbation time-scales, they do provide 'worst-case' scenario information of the magnitude and direction of potential changes. If robust and reliable ocean-going mesocosm facilities can be developed, they should ideally be deployed in oceanic regions of greatly contrasting physical, chemical and biological characteristics – for example, open ocean gyres (subtropical and subpolar) vs. coastal upwelling regimes. Such information on the response of microbial assemblages in contrasting environments is needed in order to provide predictions on the global impact of increasing CO_2 concentration / decreasing pH.

C.2.5 Mesoscale experiments and comparison of natural variability in different environments

The mesoscale iron enrichment experiments (*in situ* patches tens of kilometers in size) demonstrated that the mechanisms and the extent of iron limitation can be evaluated at ecologically relevant scales. The logistics of a similar CO_2 enrichment would be much more difficult due to the scales and volumes of required CO_2 additions. Whereas iron addition experiments involved the addition of a few hundred kilograms of iron, CO_2 enrichment studies have the added problem of dispersing kilotons of CO_2 at constant target concentrations over appropriate patch scales. The lessons learned from the iron enrichment experiment, however, may be used to develop much smaller experiments, with more precise tracking and confinement of the enriched patch. Employing a suite of new, more sensitive instruments, and new observational platforms (gliders and small AUVs) to follow the chemical and biological evolution of the observed signals can make this task feasible. Moreover, satellite platforms can provide synoptic critical information, integrating over large spatial and temporal scales, not easily derived from ships, moorings and autonomous vehicles. Specifically, the ability to measure suspended calcium carbonate using satellite ocean color measurements will enhance our ability to detect the impact of large-scale ocean acidification on microbial communities within the surface ocean. There are many more regions in the ocean that are amenable pH mesoscale manipulations; there is no requirement for a particular type of water characteristics such as high nutrient / low chlorophyll (HNLC) systems in the case of iron enrichment. Among these locations, relatively quiescent regions can be found, with slow rates of patch advection and dissipation. Alternatively, local convergence zone, or anticyclonic eddies with well-defined central convergence region may be selected to maintain patch coherence. A very attractive aspect of such experiments is the possibility of assessing chemical and biological responses on the pCO_2 / pH gradients that will naturally develop across the enriched patch.

The existing spatial and temporal gradients of pH at the edges of coastal upwelling, seasonal discharge of high organic content / high pCO_2 rivers, upwelling in the Atlantic versus the Pacific Oceans or the natural CO_2 enrichment due to underwater volcano seeps all offer possibilities of observing biological responses to pH shift in existing natural environments. The confluence of a variety of factors (nutrients, trace metals, temperature, salinity) will require a system approach to these observations in order to deconvolute pH responses from other forcings. That may dictate particular patterns of sampling (e.g. a single transect across the pCO_2/pH may not be sufficient). The highly variable patterns of local volcanic CO_2 plumes may require establishing of observing infrastructure, with sensor density corresponding to spatial variability of local pCO_2 fields. Modeling will probably be required to adequately analyze and interpret the acquired data.

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PART D: BARRIERS TO PROGRESS IN RESEARCH ON THE EFFECT OF OCEAN ACIDIFICATION ON MARINE MICROBES

1. Current level of understanding is poor and the knowledge base is small. Experiments are difficult to compare because detailed information is not included in papers and is only revealed during discussions. This information includes, for example, details on experimental and manipulation techniques, time-course of carbonate chemistry in incubations, culture strains etc.

2. There is a need for better international co-ordination. There is one multi-national program in Europe (EPOCA, http://www.epoca-project.eu) but there are few opportunities for practical collaboration between the US and Europe because there are few international funding sources.

3. It would be very beneficial to compare the response of microbes from the ocean with those of coastal and estuarine systems and freshwater lakes that undergo rapid and large pH changes. However, funding agencies provide few opportunities to support research projects that have the goal of comparing microbes in freshwater and marine systems.

4. Methodological approaches for performing pH and CO_2 perturbations have been proved to be problematic. pH manipulations by acid addition alone are probably unacceptable, given that higher CO_2 concentrations will have effects other than pH change. If H^+ concentration is increased by the addition of acid, HCO_3^- must also be added to mimic the future ocean. Bubbling with CO_2-enriched air may cause cellular damage to fragile marine microbes due to cavitation. It would be useful to reach international agreement on appropriate methods for doing ocean acidification manipulation experiments.

5. Standardized techniques for chemical measurements of the ocean CO_2 system are required as are biological measurements of ocean acidification-sensitive variables (e.g. intracellular pH, nitrogen fixation, photosynthesis, calcification, etc.)

6. Large enclosure or mesocosm experiments are logistically difficult and potentially expensive. The research community needs to reach agreement on methodological approaches, such as how to perform the perturbations. The logistics of large scale enclosures makes it difficult to perform replication of large scale experimental treatments, and limits the number of treatments that can be performed at one time in one place. Lack of replication presents a difficult statistical problem.

7. Large-scale in situ?? experiments need to be performed in a wide range of habitats and seasons to facilitate scaling results globally. Again this will be expensive.

However, there are also opportunities as well as barriers. Metagenomics and metatranscriptomics technology has developed sufficiently to study how whole communities might respond to higher CO_2/ lower pH. But the sequencing costs are still high. There have been new instrumentation developments, such as for carbonate sensors, that will aid the control of experiments, and autonomous platforms for ocean measurement are become for prevalent.

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