

**MARINE MICROBIOLOGY INITIATIVE (MMI) EXPERT PANEL REPORT TO THE GORDON  
AND BETTY MOORE FOUNDATION**

October 6, 2017

Summary

A panel of seven experts met September 21-23, 2017, to review the progress, impact and vision of the Moore Foundation funded MMI, which is nearing the end of its second Phase (2011-2019) of funding. **The quality of science accomplished has been extraordinary** as witnessed by the large number of publications in top journals for all of science as well as in their field, their leadership and impact for all of microbial ecology and also extending to other areas of biology and ecosystem science, the development and use of impressive new methodologies, e.g. study of single cells, new chemistry-based methods to reveal cell functions, environmental sensing, new genetic tools for previously unstudied but important taxa, system modeling and more. Our report calls out many examples of important advances that contribute to the larger goals set by the MMI. In short, the science is of the very highest quality. The overall progress has been enabled by the synergy from the suite of different funding mechanisms including block funding to exemplary PIs, multidisciplinary projects and tool and method development, both more dynamic to solve current needs, and community resources, together providing for more than the sum of the parts.

The panel was asked to score MMI progress towards key challenges in the field, outlined in 2011 and revised in 2015 as eight goals to be accomplished by 2019. We scored progress towards those goals as excellent for three, one very good to excellent, two very good and two good. These were to be challenging goals and those with lower scores are especially challenging, i.e. stretch goals, and ones that continue to provide important guidance to the researchers. The panel expects significant [progress toward excellence in all of the stated goals but recognizes not all can be fully achieved by 2019 given their ambitious nature](#). Research progress has continued to accelerate from Phase I (2004-2011), through the first half of Phase II (2011-2015) and now in the last half of Phase II (2015-2019). Given their accumulated knowledge, the existing and new talent and the impressive methodology and experimental systems, the committee feels that MMI is poised to accomplish in a Phase III the challenge of integrating and scaling to better define and predict ocean processes. Hence we recommend that MMI **build upon the many advances achieved to understand and predict the cascading impact of microbial variability on marine ecosystems in space and time - past, present, and future - in order to understand ocean change**. Clearly, and despite the recent growth of the field, we are in an accelerating period of understanding diversity of genes, populations and communities and their habitats in the world's oceans.

## **INTRODUCTION**

The Expert Panel was asked to evaluate the Marine Microbiology Initiative (MMI) over the second phase of funding (2011-2019), concentrating on the 2015-2019 period. This evaluation concerns i) MMI scientific achievements, ii) evolution of the field and iii) perspectives on future high impact research opportunities in marine microbial ecology and related fields. Panel members were provided with a summary document of the MMI program background, a summary of the recent accomplishments of each of the funded projects, six appendices that provided more detailed information on the projects, and a bibliometric analysis and grantee survey by Science-Metrix. The Panel met at the Moore Foundation offices on September 21-23, 2017, where we discussed our findings and arrived at a consensus report. Our report is organized according to the three topic areas for which our comments were requested. We present our comments on the scientific achievements section according to the 2019 grand goals, followed by the eight subgoals; we also address three additional questions posed by the Foundation staff.

### **I. SCIENTIFIC ACHIEVEMENTS (Reported by goal)**

**Grand goal: Establish a new paradigm by uncovering the principles that (1) govern how microbes interact with one another and (2) influence the nature of microbial transformations of nutrients in marine microbial ecosystems**

The MMI Science Program initially targeted the knowledge gap in linking “who is out there” with “what they are doing” in the complex marine environment. The panel recognizes the immense challenge of deciphering the biology of key microbial agents in the sea, their specific functions, and rates of matter transformation. We congratulate the initiative on selecting an excellent group of Investigators (Approach A) to tackle the problem of identifying and quantifying important processes mediated by specific taxa from an enormous diversity of marine microbes and genes. By exemplary studies, the Investigators have significantly advanced knowledge, and developed methods and technologies to observe and quantify microbial processes across the entire scale – from the single cell to the ocean ecosystem. The initiative’s range of approaches and goals is unique in targeting a broad set of high risk/high award interdisciplinary studies. These include the biology and genetic accessibility of protists, computational models to address the diversity and role of marine microbes in the Ocean System, and an outstanding set of biological interaction systems across all three domains and their viruses. In particular, individual Investigator awards have made a difference in the capacity of the field to address the link between microbial identities, functions, and their environmental distribution. The multidisciplinary Projects (Approach B) provide an important and timely addition to MMI that are advancing the field with potential for considerable impact. Altogether, based on the range of exemplary studies advancing the toolbox to link organisms and matter transformations, the panel rates the progress towards the overarching goal for 2019 as **excellent**. Given all the breakthroughs in methods and research results, the panel feels that

MMI is well positioned to develop more quantitative approaches for linking genetic identity and biogeochemical transformations, kinetics and rates; and developing a more integrative knowledge that will allow researchers to address seamlessly all scales of microbial functions from genes to the ecosystem.

**Goal 1: Exemplar laboratory and field investigations of microbial interactions demonstrate their importance in understanding and ecosystem processes.**  
**Goal 4: Exemplar laboratory and field investigations at the single cell level reveal mechanistic understanding of microbial interactions and nutrient flow.**

MMI funding has resulted in significant advances in methods, including single cell methodologies and their use in combinations, and experimental chambers. The design, chemical detection, and characterization in both laboratory and field investigations have led to new understanding of microbial interactions, nutrient flow, and ecosystem processes. New strategies in single cell sorting were developed that rely on organism morphology (e.g. chitin cell wall, flagellum), on chemical fingerprint (single-cell Raman spectra), or on tagged proteins (bio-orthogonal non-canonical amino acid tagging; BONCAT-FACS). These sorting advancements, along with other single cell methods, led to the cultivation and sequencing of numerous un-cultured and under-sampled microbial groups (single cell eukaryotes, archaea, bacteria). Newly developed single cell methods that have led to better visualization and understanding of *in situ* microbial transformations and interactions include: Matrix Assisted Laser Desorption/Ionization used in combination with fluorescent *in situ* hybridization (MALDI-FISH); BONCAT-FACS sorting for nanoSIMS (see below), combining phageFISH and geneELISA with protein analyses (e.g. virus structural metaproteomic: LC-MS for protein quantification) and Raman-FISH. Results from the latter are leading to a re-evaluation of the impact of viruses on particle and carbon export.

Analytical advances have improved detection and characterization of molecular signals in seawater. Two classes of signals were identified and verified in the field, one of which plays a key role during viral infection. Co-culture experiments identified a bacteria-derived molecule that could increase cell size and chlorophyll *a*, while inhibiting cell division in a model diatom. Identification of these molecules highlights a new concept of “lateral” control on populations via chemical interactions and opens the field for further developments. Microfluidic devices (*in situ* chemotaxis assay) were developed and coupled with community diversity and activity measures to link microbial chemo-response in the environment to phylogeny at relevant spatiotemporal scales. A robust suite of sophisticated analytical methods was developed to measure and map (on a scale of microns to meters) end members and intermediates of the biological sulfur (S) cycle. Measurements were optimized for tracking nutrient flow within single cells; they can also be applied to pore waters. The project included an application of FISH-SIMS and multi-isotope S (<sup>33</sup>S and <sup>34</sup>S) phenotyping. Experiments using stable isotope probing (SIP) and labeled substrates (e.g. proteins from algal cultures,

lysates) applied in field experiments have led to new understanding of which microbes actively mediate transformations. Many represent uncultivated and rare classes of single cell eukaryotes, bacteria, and archaea. A sophisticated incubation chamber was deployed in the deep sea with  $^{13}\text{C}$  labeled substrates to study the microbial diversity, activity, and role of viruses at deep-sea vents. The project is at a 'proof of concept' stage, but holds promise to identify the impact of viruses on carbon flow in a hard-to-study environment. Another novel design will use BONCAT-FACS in concert with nanoSIMS to image environmental samples of microbial cells, grazers and viruses. Success of the latter will demonstrate a new approach to assessing top-down control while tracing nutrient flow (nitrogen and carbon substrates) across different trophic levels.

Overall **excellent progress** has been made in developing single cell methods to link metabolic activity to individual microbial cells. The detection, characterization of nutrients (e.g. dissolved organic matter), and lateral resolution (e.g. mm level) for visualizing substrate uptake and transfer has greatly improved. New and creative ways to sort populations using FACS were developed that are useful within and outside the field of microbial ecology. Results from laboratory and field experiments within goals 1 and 4 have led to deeper understanding of the microbial impact on nutrient flow and ecosystems and opened the field for future work and new questions.

**Goal 2. Experimental model systems for probing microbe–microbe interactions and nutrient flow enable generation of new ecological and evolutionary hypotheses for ocean investigation.**

The Experimental Model Systems (EMS) program of Approach D (New instrumentation, tools and technologies) was a particularly ambitious project within Goal 2. Classical genetic approaches can be applied to very few single cell eukaryotes. *Chlamydomonas* is the archetypal model organism, in which it has been possible to isolate mutants for decades; this has led to a vast literature of molecular genetic studies. However, equivalent progress has not happened with marine single cell eukaryotes, which lack tractable genetic systems. The EMS initiative was very high risk because it was not known if genetic systems could be developed in any of these marine organisms. Nevertheless, the ability to genetically manipulate these systems would be a major step forward and yield very high reward. Thirty-four teams were funded through the EMS portfolio. To date very promising progress has been made for diatoms (*Phaeodactylum tricorutum*, *Thalassiosira pseudonana*, *Cylindrotheca closterium*, *Skeletonema marinoi*), a choanoflagellate (*Sapingoeca rosetta*), two ciliates (*Euplotes focardii* and *E. crassus*), and the protalveolate *Perkinsus*.

MMI Investigator awards also contributed to Goal 2. A phage-host model system was used to investigate lytic phage infection efficiency and a *Micromonas* model system was employed to investigate genes of unknown function and vitamin B1 requirements. Multidisciplinary projects also mapped onto Goal 2, which included

work focused on a model system of *Synechococcus* and a heterotrophic bacterium; signal molecules in diatoms and coccolithophores; and a virus-bacterium-algal model system to investigate virus impact on microbial evolution.

Overall, **excellent progress** has been made towards the MMI goals. EMS is highlighted as a significant example of a high risk/high reward project that has the potential to develop significant new understanding in marine microbial ecology.

### **Goal 3. Computational models accommodate the complexity of microbial interactions and nutrient flow in the ocean.**

This goal is lofty and important given the central role that microbial processes play in biogeochemical cycles within the global ocean and indeed for the planet as a whole. The task is demanding because fully accommodating the immense complexity of microbial interactions will require massive investment and perhaps multiple generations. However, the goal of moving the needle on computational models on this topic has been possible.

MMI grantees have collectively made significant progress on computational modeling at multiple spatial scales, including efforts with 3-dimensional, relatively large-scale models, efforts on largely 1-dimensional (vertical) flux, and efforts on micro-scale processes. The larger scale models encompass population and community level issues whereas microscale studies focus on individual cell interactions. The development of a fully integrated model of microbial nutrient cycling in the oceans is very challenging because it cuts across many scales. Nonetheless the ground has been laid by MMI researchers, whose studies of diverse environments in a changing world similarly integrate across scales, whether in the context of nitrogen (N) cycling or carbon (C) export. The findings include a model showing sensitivity of N export from low oxygen zones to climate change and changes to N<sub>2</sub>O emissions with anoxic to hypoxic conditions. Another model shows the effect of oxygen limitation on the energetics of nitrogen fixation and the concentrations at which systems flip to anaerobic activity with major changes in N cycling. Additional work on genes to ecosystem modeling shows that virus abundance best predicts C export. These results, as well as a predictive model of the global distributions of marine microbes (based on potential function, protein expression patterns, and taxonomy) toward an Environmental Niche Atlas offer great promise in advancing global predictive capacity in biogeochemical cycles. At the other extreme (but equally important) spatial scale, microfluidic work on species interaction during and as blooms collapse shows promise in developing optimal foraging models of marine bacteria.

The individual projects have largely made significant progress, but the major challenge of nesting the many scales and variables of interest means that the goal is both attainable and unattainable. Nonetheless, this is largely an integration issue and one hardly unique to MMI.

Overall progress towards this goal **is very good**, which is not a criticism of the projects but rather the capacity of the overall program to tackle a massive challenge – modeling the role of marine microorganisms in the global ocean effectively. “Accommodating the complexity of all relevant scales in the ocean” is no small task!!!

**Goal 5. Exemplar field investigations of geochemical pathways (including the rates of geochemical transformations) use finely resolved taxonomic information to provide new insights into nutrient flow.**

MMI investigators have unraveled the activities of taxonomically and functionally different versatile organisms looking at single cells, a  $\mu\text{m}$ -scale ecosystem, and coastal ecosystems. MMI researchers confirm the need for new tools because using bulk geochemical measurements and 16S rRNA amplicon or metagenome sequencing in natural systems does not provide the precision necessary to assign function to individuals or guilds (e.g. predicting substrate utilization by individual populations). The development of new, and optimization of existing, micro-analytical methods led to groundbreaking research tracking the fate of C and N across different trophic levels; in microbial cells, coexisting viral populations, and eukaryotic grazers. Using high precision, high sensitivity measurements of sulfur-containing metabolic products have led to new levels of inquiry into near seafloor cycling of sulfur by microbial communities. Studies to ascertain the impact of virus infection on the C cycle in coastal systems revealed the surprising result that viral infection not only stimulates respiration but also can induce particle aggregation and massive downward vertical fluxes of both particulate organic and inorganic carbon, thus enhancing the biological pump efficiency. Development of a deep-sea incubation chamber enabled the investigation of biogeochemical cycling in a challenging and understudied system. Hydrothermal systems are a significant feature of the seafloor known to exert a dominant control on global ocean chemistry.

Linking taxonomically identifiable and functionally relevant units to rates of transformation and fluxes still remains elusive. **However, since 2011, MMI made very good progress towards achieving this goal.**

**Goals 6 and 8 were discontinued in 2015, and hence are not reported.**

**Goal 7. A conceptual framework exists to infer ecosystem scale processes from microscale measurements.** The panel recognizes the goal of scaling microbial information to infer ecosystem processes as highly meritorious, in fact a grand challenge. Its realization is, however, a major effort that requires coalescing of the tools, talents, and knowledge of the initiative, for which MMI is poised but would need more time and effort. A conceptual framework, the focus of this goal, is an important guiding step and more tractable. While this product was not explicit in the text we were provided, some projects demonstrate components. Some modeling projects more fully provide a framework, while others provide smaller-

scale information with context or inference of larger-scale ecosystem impact, as is noted under our Goal 3 response. In addition, several multi-disciplinary projects - perhaps a characteristic of their multidisciplinary nature - make or project ecosystem inferences.

The program lacks a more direct articulation of the conceptual framework as stated for this goal. **Progress towards this goal is good**, and could rank higher if the investigators were more explicit in stating their framework. Importantly, the synthesis activities encouraged in the final stages of Phase II funding would be a timely and strategic means for shaping this framework.

**Goal 9. Exemplar laboratory and field investigations of evolutionary processes and mechanisms demonstrate the importance of placing microbial interactions and nutrient flow in an evolutionary framework.** This goal calls for investigators to place field and laboratory investigations into an explicitly evolutionary context. This goal can be accomplished through consideration of evolutionary process, in population genetic analyses of laboratory or field-based experiments, or by evaluating research on model systems with reference to phylogeny and/or the geologic record. The benefit lies in providing a better understanding of the relevance of model systems for more general patterns of interaction and diversity in the oceans. To date, few MMI investigations have specifically provided evolutionary perspective, although nearly all have the capacity to illuminate the evolutionary basis of marine microbial ecology. Some detail physiological responses to environmental variability – phenotypic responses to be sure, but also the raw material for evolutionary change. Another team is working to identify genes involved in anammox metabolism and to understand their distribution among taxa and in space, research that will provide both new mechanistic molecular understanding of a key metabolism and illuminate the evolutionary history of its constituent genes. One researcher places her genetic research on the green phytoflagellate *Micromonas* in a phylogenetic framework, enabling her to interpret her findings more broadly in terms of green algal ecology and evolution. Among other discoveries, she has shown that the biosynthetic pathway for peptidoglycan, the cell wall material of cyanobacteria, occurs widely in green algae and is retained in several vascular plant lineages.

**The interim grade for progress towards this goal would be “good,”** but much more is promised by recent grants to talented evolutionary biologists who have committed to developing new genetic tools for research on marine single cell eukaryotes. In general, genetic specificity in dissecting ecological interactions among microorganisms provides fertile ground for evolutionary analysis.

**10. Exemplar laboratory and field investigations integrate highly resolved organic matter composition and microbial analyses to understand organic matter cycling.** Across all grant approaches funded, about 10% tackled this goal set specifically; and many more contributed knowledge to the goal eventually. Overall, in the first phase key innovations were made in protein and lipid profiling,

resolving sugar molecules in the black box of dissolved organic matter all via high-resolution mass spectrometry, but also in tackling organic-inorganic complexes and processes around sulfur and cobalt. Several Investigators specifically addressed this goal by developing methods and carrying out experiments and field studies. One Investigator, who did not address this goal specifically, has added substantial progress in cell-level metabolomics by MALDI-MS Imaging. The Multidisciplinary Projects also contribute considerably to goal 10, but their success cannot be fully evaluated yet. Three instrumentation proposals were dedicated to goal 10, all addressing highly relevant gaps. Overall, especially the linking of high-resolution organic and inorganic matter profiling with gene expression studies, and/or with visual identification of the key agents involved, has enabled new findings resulting in high impact papers. Substantial progress has also been made outside of MMI funding especially by geochemists addressing the high-resolution composition and origin of dissolved, gaseous and particular OM analyses (e.g. by FT-ICR-MS - Fourier transform ion cyclotron resonance: ultra-high-resolution direct laser-based analysis of lipid biomarkers, clumped isotopologues:  $^{14}\text{C}$ -dating of organic matter) – but rarely connect with microbiological analyses. In this regard, the MMI PIs have been more integrative and innovative than the field at large. In the framework of goal 10 they addressed chemical microenvironments of microbes, chemical cues for cell motility, cell-cell and cell-particle interactions, the composition of cell debris, the impact of viruses on organic matter cycling and new substrates fueling marine symbioses. Further progress could be made by clever experiments with complex enrichments and mesocosms, which allow for high-resolution sampling under defined conditions, including the dynamics in gene expressions and turnover rates of microbes, matter and minerals. Also, an evolving new field attempts to decipher the microbial metabolome, and which chemical signals are available for intra- and interspecies or even inter-domain communication.

**Overall we rate progress towards this goal as very good to excellent, it is clearly an area of growth.**

#### **Further Questions to the Panel**

##### **How would the panel characterize the quality of the science and scientists MMI has funded?**

MMI has identified exceptional research talent, supported the advancement of key technology and tools, solicited ideas from the international scientific community, and made strategic investments to break open “black boxes” in the field and take understanding of marine microbial communities to a new level. In short, the MMI program, through its suite of project types and exemplarily investigators, has hugely influenced the specific areas targeted and has had additional impact on microbial ecology in other environments and some other areas of contemporary biology and ecosystem science.



The MMI program exemplifies the Moore Foundation's goals to advance basic science through developing new technologies, supporting imaginative research scientists, and creating new collaborations at the frontiers of traditional scientific disciplines. MMI is a high impact program because i) the importance of tackling unknown diversity and functions of life in this large ecosystem of Earth (70% of the Earth's surface is covered by ocean, ii) microbial communities in the sea are critical for making the planet habitable and productive, yet had been poorly explored, and iii) of the quality and productivity of the research community it has supported. Notable evidence is the very large number of publications in top journals for all of science (e.g. Nature, Science, PNAS) as well as in the very top journals of this discipline (e.g. ISMEJ). Furthermore, marine microbiology, led by the MMI investigators, has pioneered single-cell quantitative approaches (e.g. nanoSIMS), metagenomic, and microbiome sciences, providing methods and guiding concepts to the human, terrestrial, and plant microbiome fields.

A comprehensive understanding of the evolution and activities of marine microbial species and guilds is essential for understanding their role in marine ecosystems and for understanding the multitude of mechanisms by which organisms on the planet thrive, and MMI researchers have advanced the field and its interdisciplinary methods substantially.

### **In what ways did (or didn't) MMI-supported researchers drive important new science forward?**

The Foundation defines its science philanthropy as investing in high-impact research and technology development to address important and emerging issues in marine microbiology that will leave an enduring legacy. The Phase II overarching goals were to establish a new paradigm for marine microbiology by supporting grantees to advance research frontiers and research projects. Specifically the program aimed to reveal the scientific principles and underlying mechanisms that govern how microbes interact with the environment and one another, and uncover the role that microbial species and guilds play in the transformation and flow of nutrients at many scales in the marine environment. Through a mix of initiatives, portfolios, "stand-alone" projects, and far-reaching, riskier projects, MMI researchers have driven their science and technologies to provide fundamentally new insights and tools, e.g. role of viruses, high resolution in chemistry, and new *in situ* genetic monitoring tools. In short, excellent progress has been made towards its goals articulated in 2011 and significant progress towards the 2019 goals as reframed in 2015. In the areas of theory, synthesis, and modeling of biological interactions, MMI goals have not been fully met. However, we note that developing conceptual frameworks, scaling processes and interactions from micron to ocean basin, and predictive modeling are often intractable goals for most environmental research. The MMI group, however, is well poised to make further advancements on these goals than was possible in the past because of their novel approaches and impressive tools, their increasingly comprehensive knowledge, and a growing synergy across the project portfolio.

## **Has MMI's approach been effective in supporting the field?**

MMI has employed multiple approaches to develop their strong, productive, and innovative portfolio. MMI investigators have contributed significantly to the steady advancement of the field of marine microbiology since the program began, through well-cited research that has shifted microbial research approaches, along with our understanding of the importance of microbes in marine systems, and the ability to simulate their responses to ocean dynamics. The Foundation's ability to take risks, make long-term and relatively large commitments and fund interdisciplinary teams of scientists that include international partners allows it to undertake challenges not accessible to most other science funding organizations. One undeniably unique contribution MMI has made in the field of Marine Microbiology is the investment in the development of tools, techniques, and computational resources that will increase the accessibility of systems limited by technology and open research in those areas. For example, MMI PIs have developed model systems that can be genetically manipulated to allow testing of hypotheses on the evolution and function of marine microbial species and communities, particularly for viruses and microeukaryotes. In addition, the MMI program officers adaptively manage their portfolio to create synergies between projects and enhance interdisciplinary research that has increased the impact and created synergies where the wholes exceed the sums of their parts. All of their efforts have added the final ingredient to a truly successful research endeavor that promotes communities of practice.

## **II. EVOLUTION OF THE FIELD**

The MMI was born out of a conviction that marine microbiology held great promise for transformation, and research over the past seven years, much of it conducted and catalyzed by MMI, has delivered handsomely on this promise. The field is currently undergoing rapid expansion, with new tool development, an influx of talent with new ideas, and an increasingly coherent research community of practice. Every discovery, however, raises important new questions, and the rapid acceleration of marine microbial ecology has therefore demonstrated just how much we've yet to learn, providing exciting opportunities for continuing research.

### **1. How has the field evolved since 2011?**

Advances in technology have provided sophisticated new methods for the detection, taxonomic resolution, and functional characterization of cells, at relatively low cost, providing unprecedented volumes of data on the distribution and interactions of microorganisms in the global ocean. Fluorescence-activated cell sorting enables microbiologists to sort mixtures of cells, one cell at a time, while meta-barcoding of global sample sets has vastly improved our understanding of marine microbial diversity and spatial relationships among populations. Indeed, since MMI began, the documented diversity of major branches of life within both the bacteria and archaea has doubled, while the TARA expedition has vastly

improved our understanding of single cell eukaryote diversity in the sunlit oceans. The sampling and processing of environmental DNA further documents organisms other methods cannot readily capture. At the same time, *in situ* and *in vitro* experimental studies together with metagenomics and transcriptomics have provided a sharply improved sense of the functional biology of marine microorganisms, facilitated meaningful ecological investigations, and generated new hypotheses. New molecular markers provide novel biogeochemical tools for paleoenvironmental reconstruction, while improved techniques for the chemical characterization of dissolved organic matter (stable isotope signatures,  $^{14}\text{C}$  dating of individual particles and dissolved organic carbon, orbital trap mass spectrometry) illuminate hitherto underappreciated transformations of marine matter and components of the marine carbon cycle.

Intensive field studies in microbial ecology have made great strides across a breadth of different habitats, including once difficult-to-access environments such as the deep-sea floor and the sediments beneath it. The grain of ecological analysis has also grown finer, down to the level of molecular interactions between cells and with viruses. Taking advantage of these advances, engineered model systems are now being developed and manipulated using new tools for genetic modification. Computational models are rapidly maturing and can now be connected to Earth System models. And our understanding of the diversity and function of viruses in the oceans has mushroomed, requiring us to think in new ways about ecology and evolution in the marine realm. In short, a new and vibrant field of marine microbial ecology has emerged, providing unprecedented opportunities to understand how microorganisms interact with cycle carbon, nitrogen, and other elements in the oceans, how those systems and their component species have evolved through time, and how microbial communities will respond to 21<sup>st</sup> century global change.

## **2. What factors are promoting or impeding further advances in the field of marine microbial ecology?**

### ***Promoting***

Over the last decade, advances in marine microbiology have paralleled and benefitted greatly from advances in technology, particularly in high-throughput sequencing. Very large, good quality data sets of marine microbes now exist and associated financial costs have declined significantly over the period. Development of bioinformatics tools has largely kept pace with the increase in sequence information. The European Commission and government agencies throughout the world have funded large research projects on marine microbial ecology, in addition to those supported by Moore Foundation; these projects have successfully brought creative people from diverse disciplines with varied skillsets together. Sampling of natural populations, often from ocean-going research ships, defines a major emphasis of marine microbial ecology. This working environment fosters close working relationships that have generated a culture facilitating interaction among groups. PIs are highly interdisciplinary, exposing all participants

to a wide range of techniques; one consequence is that many individual investigators now take a more integrative view of the marine microbial ecology. Sequencing is not the only technological advance and state-of-the art instrumentation has become sufficiently robust to take to sea on research ships, allowing measurements either *in situ*, or soon after sample retrieval. Genomics, and transcriptomics, both at the individual organism and assemblage level have resulted in very large databases that are generally accessible in public databases. Finally sophisticated instrumentation to both visualize and quantify the activities of microorganisms at the nano-meter scale have also become more common practice and have significantly advanced our understanding of how organisms interact with each other and their environment.

***Impeding:***

Nevertheless, progress has sometimes not been as rapid as it might be. Sampling the deep ocean relies on access to research ships, which are expensive with long lead- and planning-cycles that are not compatible with rapidly developing science. Ongoing developments in instrumentation for *in situ* sampling or long-term deployment require investments to increase availability to more research groups. Despite the availability of very large datasets to the community, data accessibility remains an issue when the owner of the data does not release it quickly. Furthermore, lack of knowledge of which samples are archived in some centers limits their utility, although we know these samples could be a significant resource that bypasses the time delays associated with getting ship-time. Bioinformatic analytical tools and pipelines may not be sufficiently 'user-friendly' and standardized to ensure uptake by the wider community. There are too few long-term time series of sequence data to allow robust predictions of the dynamics of microbial life in the future ocean. Acknowledging the increasing number of genome sequences, laboratory "weeds" often dominate these datasets, rather than the most abundant species in natural assemblages or those likely of ecological importance. Many genes code for unknown function (a problem not unique to marine microbiology). The focus on different habitats has led to a partial fragmentation in the field (e.g. deep sea, blue water, coastal). A dearth of cultures of the most abundant species in the ocean persists, and laboratory-based studies typically focus on organisms whose sequences are rare in natural assemblages. Few laboratory model systems exist to study the response behavior of marine microbes. The advancements in sequencing technology and culture-independent techniques have dramatically halted efforts to isolate new microorganisms. Finally, ecosystem models generally make little use of the knowledge and vast amount of information that now exists on marine microbes and their interactions.

**3. How can private philanthropy make a difference in marine microbial ecology?**

Private organizations generally seek mechanisms to support science that do not duplicate government efforts, by taking advantage of their independence and associated flexibility to maximize impact. In this sense, Moore has prioritized the promotion of higher risk science that promises high reward. Their independence

has allowed them to develop an integrative and visionary program that publicly funded programs typically cannot; the latter often fund a series of largely unrelated projects of shorter term (e.g. 3-year maximum) and with little potential for the whole to exceed the sum of its parts. Philanthropic agencies have flexibility to break down institutional barriers and geographic boundaries, with a nimbleness to respond to emerging opportunities and changing priorities. Their investment in staff trained in talent – and idea – assessment can make a difference in detecting and supporting outstanding talent in science. Government agencies must often limit funding to particular disciplines and, at least in the US, they lack any mechanism to support international collaboration. They also cannot switch priorities quickly or significantly because they must serve a wider constituency. The longer funding period typically often supported by philanthropy – or at least the flexibility to consider funding periods beyond 3 years – also allows for deeper investigation and more nuanced science where the researchers have the time to take more risk without fear of total project failure. Instrument development represents a specific area in which these added areas of flexibility (higher risk, international/cross disciplinary and/or international collaboration) add unique opportunities for philanthropic organizations to move the needle in a specific area of science. Although the Moore Foundation has considered these opportunities and niches carefully, MMI has potentially underutilized their flexibility in taking advantage of international collaboration opportunities and in the delivery of integrated products that communicate the vision and exceed the sum of the parts (i.e. synthesis). Furthermore, MMI could increase efforts in talent search among junior investigators, and reconsider a funding approach to stimulate careers of exciting young investigators as they did successfully earlier in the initiative though perhaps with some modifications.

### III. FUTURE

#### **1. The Big Question: The Vision**

Ocean environments vary greatly in space and time in terms of their productivity, biota and function, and microbes play a lead role in this variability. Microbes live at scales of microns yet drive ocean processes at regional and even global spatial scales, and at temporal scales from seconds to seasons to geological periods. Hence we recommend that MMI should **build upon the many advances achieved to understand and predict the cascading impact of microbial variability on marine ecosystems in space and time - past, present, and future - in order to understand ocean change.** Clearly, and despite the recent growth of the field, we are in an accelerating period of understanding diversity of genes, populations and communities and their habitats in the world's oceans. Major advances in understanding life in the global ocean will require integration from genes to cells to communities to ecosystems, each with its own sets of tools, experts, and biases. But it will also demand novel expertise on effective integration of those resources and opportunities across time and space, and require that the Moore Foundation continue to search out the talent to fill these gaps.

## 2. The Payoff

By linking microbial ecology to present and future ocean states (e.g. contribution to and feedback mechanisms to climate change) and conservation (e.g. baseline conditions, habitat assessment; healthy ocean ecosystem concepts) **we can dramatically improve how we, as a major component of ocean ecosystems, interact with and benefit from ocean biota.**

## 3. The pieces of the puzzle

**At the gene to cell level**, we can now resolve the function of genes and proteins in the marine environment by relating the content of the genome to the physical and chemical environment, opening up a plethora of opportunities. *We can continue to expand on single cell methods, genetic tools, cultivation, and enrichment approaches in order to gain mechanistic understanding of microbial communication and the importance of intracellular and extracellular features in the marine environment. Such efforts might also consider symbioses, how these systems evolved and how they work. Symbioses between single cell eukaryotes and single cell prokaryotes (bacteria, archaea) are reminiscent of ancient events; hence they hold great promise for studying organelle evolution and synthetic biology. It might also consider viruses as evolutionary drivers, shaping communities through natural selection on genomes at the species level (HGT) and at the community level (community structure).*

**At the community level**, we can apply new tools (e.g. stable isotopes, lipids, amino tracers), many enhanced in MMI, to examine interactions and biogeochemical linkages between microbes and macrobes, even as large as whales, and how microbes fundamentally drive secondary production in the ocean. *Tools developed by MMI can facilitate the use of and infuse new techniques into longer time series observations and investigations. Microbial ecologists could explore the use of environmental DNA or archived samples to reveal information about current and potential past community composition, species co-occurrence and environmental function, and discern persistent versus transient associations (e.g. microbial and consortia, symbiosis) in time and space. Although this approach offers opportunities across a wide range of environments, the deep sea offers a unique opportunity for addressing sophisticated questions in a relatively pristine habitat -- not unlike exploring a new planet. Autonomous observation systems are currently limited to depths less than 2000 m; however, new sensors – physical, biological, chemical – paired with significantly improved battery life will enable microbiologists and oceanographers to sample, via optical, chemical, acoustic, and molecular biological means, the presence or absence, function, and (in some instances) abundances of organisms in order to understand their interacting roles in deep-sea microbial communities. On the one hand we have already gone far beyond simple exploration (e.g. hydrothermal vents), on the other hand, much remains to be discovered in terms of unknown habitats, functions of life to tap into Earth's energy, and microbial interactions and behaviors.*

**At the ecosystem to global level,** MMI discoveries offer the opportunity to explore habitats, niches, and processes that may serve as analogues for early Earth, potentially illuminating aspects of both the origin of life and life beyond Earth. *We can build from these opportunities by incorporating theoretical ecology and evolution in studies of microbial ecology, adding increased rigor and specific scaling expertise to existing approaches.*

#### **IV. CONCLUDING REMARKS**

We compliment the MMI staff on a very well organized review, and for providing us with appropriate materials and at a manageable level of detail. We also appreciated the Science-Metrix summary information.

We were asked 'for the panel's perspectives on future funding opportunities', "what is the 'next big thing' in marine microbial ecology and related ocean, microbiology, and biological sciences disciplines?" The Foundation staff indicated that "Symbiosis" was a one topic under review. We provide our perspective and recommendation on this topic as Appendix A.

Submitted,

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## Appendix A: Consideration of a Focus on Symbiosis as a Research Theme External Panel Report

The Moore Foundation is actively considering ‘Symbiosis’ as a topic of future interest. While recognizing the considerable potential of the topic, the Panel cautions that it requires focus, since ‘Symbiosis’ covers a remarkably broad set of biological relationships. Some of the projects within and discoveries from the MMI are already very relevant advancements to the field of symbiosis research, and we encourage development of a unifying theme focusing on partnerships between single-cell eukaryotes and bacteria or archaea. This would require development of, for example, new models systems for studying microbial cell-to-cell interactions, and could include consideration of other relevant topics: organelle evolution, gene exchange and gene streamlining across partners.

Mutualistic relationships between large multicellular organisms (plants and animals) and fungal, bacterial, or archaeal symbionts are relatively well studied. In comparison, next to nothing is known about partnerships between single-cell eukaryotes and single cell prokaryotes (Bacteria or Archaea). Moreover many of these associations are widespread in marine and freshwater ecosystems, and drive biogeochemical cycles. The field of marine symbioses is wide open, and would be a natural progression from the current MMI program and it’s overarching goals of interactions between microorganisms and nutrient flow. Much attention in the study of planktonic partnerships has been on the symbiont side, highlighting streamlining of genomes, leaving the genome content of the single cell eukaryotic hosts largely unstudied; far less is known of host function.

A facet of ‘Symbiosis’, which is widespread in natural assemblages, is the concept of microbial consortia. In terms of ecological function, consortia of microorganisms are a special form of symbiosis, where the association of different organisms is of benefit to all. At its simplest, one microbe may partially metabolize a complex substrate, the product being utilized by another microbe, potentially leading to specific adaptations and eventually genome streamlining. The difference is that consortia represent a much looser association than most examples of ecto- and endosymbiosis with animal or plant hosts. This can be characterized as ‘functional symbiosis’ without the cellular integration of the participants into a holobiont. Microbial consortia also play a major role in the carbon cycle, especially in the dark ocean and so their study would simultaneously enhance our understanding of ecological relationships among microbes, the remineralization of particulate and dissolved organic carbon, and the microbiology of the poorly studied deep sea.

Modern-day examples of consorting pairs of diverse eukaryotes and cyanobacteria are reminiscent of ancient symbiotic events; this opens up a framework for many evolutionary relevant questions, such as organelle evolution. The advancement in single cell methods and high resolution / high throughput technologies including microfluidics mass spectrometry at cellular resolution; *in situ* microscopes, and novel types of enrichment cultures primes the field to tackle a wide array of microbial interactions. Key advances for the field would be in uncovering the genomic factors furthering symbiotic association; examples of cell-cell recognition, communication, cooperation as well as energy and matter exchange. *We strongly encourage an additional focus within MMI on consortia of marine (and freshwater) microbes to develop new concepts of functional symbiosis that would include the full range of interactions, from dynamic microbial associations to persistent, endosymbiosis.*