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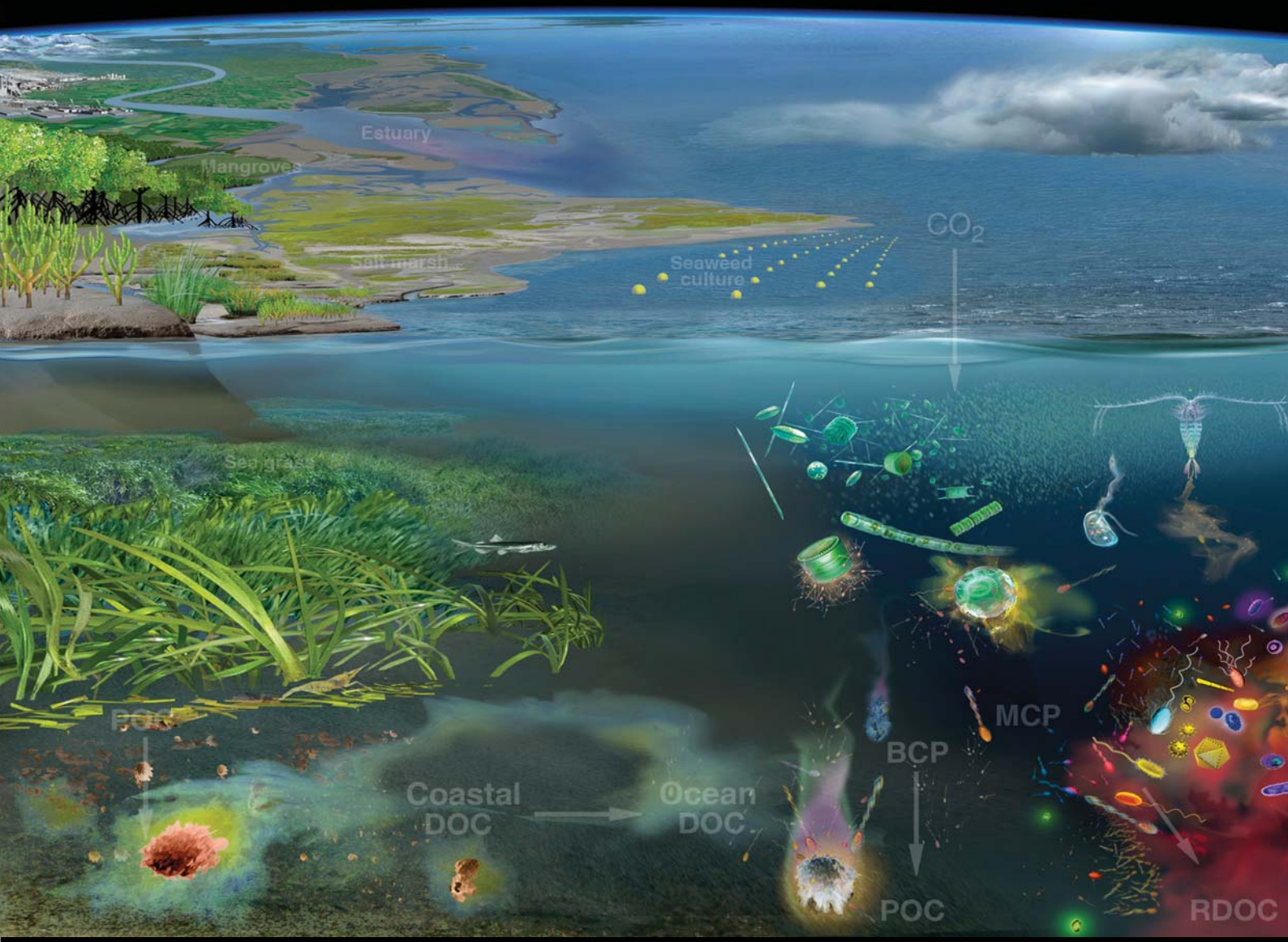
Special Topic: Marine Carbon Sequestration and Climate Change



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Special Topic: Marine Carbon Sequestration and Climate Change

Editorial for the special issue on marine carbon sequestration and climate change

Nianzhi Jiao[1,*,†], Zhengtang Guo[2,†,‡], Louis Legendre[3,†], Curtis Suttle[4,†], Richard Rivkin[5,†] and Farooq Azam[6,†]

In the 1980s, the realization that the ocean was potentially a major sink of atmospheric CO_2 stimulated the international research community under the auspices of the Scientific Committee of Oceanic Research (SCOR), International Council for Science (ICSU), to launch the multidisciplinary research program Joint Global Ocean Flux Study. Since then, the understanding of the biological, ecological, chemical and physical processes underlying the ocean carbon cycle and the ability to predict its sensitivity to global change have increased enormously. One piece of significant progress from the above international joint efforts is the understanding of the biological mechanism of ocean carbon sequestration known as the Biological Carbon Pump (BCP), which depends on the vertical transport of organic matter from surface waters to the deep ocean and the seabed. However, the BCP does not address the following enigma, which was proposed half a century ago: what were the formation mechanisms of the huge pool of refractory dissolved organic carbon (RDOC) in the ocean, which has an amount of carbon equivalent to the total inventory of atmospheric CO_2 and an average estimated residence time of 5000 years?

Recently, the recognition of a microbial carbon sequestration mechanism, the Microbial Carbon Pump (MCP), shed light on the enigma. The MCP operates independently of depth, as it depends on the microbial transformation of organic matter to RDOC, thus sequestering carbon throughout the water column. A working group under SCOR (2008–14) made significant progress in the MCP framework, followed by numerous studies with intriguing insights. Given the pressing need for knowledge on climate change and its regulation mechanisms, a joint working group was established under the auspices of the North Pacific Marine Science Organization (PICES) and the International Committee for Exploration of the Sea (ICES) in 2015 (WG 33 for PICES and WGCCBOCS for ICES) on the theme of climate change and the biologically driven ocean carbon sequestration. This theme was selected as the topic of the 1st Yanqi Lake Meeting (YLM)—a new international forum initiated by the Chinese Academy of Sciences (CAS) as a world-class platform for brainstorming at the frontiers of science and technological innovation.

The 1st YLM was held on 19–22 September 2017, at the Yanqi Lake Convention Center, Beijing, co-chaired by five academicians from China, Canada, Europe and the USA. Leaders from CAS, the Ministry of Science and Technology of China, the State Ocean Administration of China and the Intergovernmental Ocean Commission of UNESCO delivered addresses at the opening ceremony. Members of the PICES-ICES joint WG and 30 Chinese scholars and CAS members held in-depth discussions on various aspects of the MCP and the BCP. The editors of *National Science Review* (*NSR*) attended the 1st YLM, and invited six articles forming the present special issue:

- A *Highlight* on a Chinese research project on the MCP, whose outcomes are considered to have profound implications for the understanding of biologically driven ocean carbon storage.
- A *Perspective* paper that tackles the RDOC enigma in the MCP framework at both microscales (cellular, enzymatic and genetic) and macroscales (observation of microbial transformations of organic carbon in the field and under manipulated experimental conditions).
- A *Perspective* paper about blue carbon, which conventionally referred to coastal rooted plants and now includes the open-ocean carbon sequestered by the BCP and the MCP, whose amounts are much more significant at the global scale.
- A *Perspective* paper that proposes an implementation strategy to quantify the MCP and its sensitivity to global change, suggesting that the analytical tools and intellectual interaction between chemists and microbiologists are now at a stage where the societally driven question of marine carbon storage can, and must, become a high-priority research topic.
- A *Perspective* paper that proposes a modelling framework describing the formation of RDOC through the MCP. The

proposed formulation only uses two state variables and can be integrated in biogeochemical/ecosystem models with different levels of complexity.

- A *Review* paper on evolving paradigms in biological carbon cycling in the ocean, examining the MCP concept from theoretical and practical points of view with related concepts including the BCP, the Microbial Loop (ML) and the Virus Shunt (VS). The review highlights the importance of the MCP supported by data from advanced 'omics' and its inspiration of novel research, and advocates integrated approaches to promote future research on the MCP and its integration with the BCP, ML and VS.

This special issue is a collection of up-to-date syntheses on biological ocean carbon sequestration mechanisms and their relations with climate change. The MCP paradigm is shaping a new direction in ocean carbon-cycle research. Papers in this topic provide a benchmark for researchers and policy makers.

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RESEARCH HIGHLIGHT

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Special Topic: Marine Carbon Sequestration and Climate Change

A recent project shows that the microbial carbon pump is a primary mechanism driving ocean carbon uptake

Jing M. Chen[1,*], Louis Legendre[2] and Ronald Benner[3]

The microbial carbon pump (MCP) contributes to ocean carbon sequestration by converting reactive organic matter into recalcitrant dissolved organic carbon (RDOC) that can remain in seawater for thousands of years. The MCP is a potentially important ecosystem pathway operating in parallel with the well-known biological pump (BP), which turns atmospheric CO_2 into particulate organic matter that sinks to deep waters and the ocean bottom, where its carbon is sequestered. Since the MCP was proposed by Jiao *et al.* [1], it has become an important impetus for new research in the ocean carbon cycle (e.g. Legendre *et al.* [2]). A study of bacterial exometabolites recently showed that these dissolved molecules share many compositional and structural characteristics of recalcitrant DOC present in the deep ocean [3].

A project entitled 'Processes and mechanisms of carbon sequestration by the microbial carbon pump' was funded in 2013 by the Chinese Ministry of Science and Technology as part of the Key Global Change Research Program, which aimed to explore the detailed mechanisms and processes of the MCP. The project is led by Professor Nianzhi Jiao, who initially proposed the MCP idea. On 16 September 2017, a panel of international experts gathered in Qingdao, China, to evaluate the project and was impressed by several of its outcomes.

Among many important discoveries, this project substantiated the concepts of RDOCt (recalcitrant DOC in a specific environmental context) and RDOCc (recalcitrant DOC due to the extremely low concentration of its molecules), which unified the 'dilution hypothesis' and 'recalcitrance hypothesis' of RDOC [4]. A new modeling study concluded that a small pool of diluted DOC likely survives global ocean overturning along with a larger pool of recalcitrant DOC [5]. The findings of this project also emphasized (i) the active pathway of the MCP [6], (ii) the passive pathway of the MCP [7], (iii) archaeal community-mediated pathways of the ocean carbon cycle [8] and (iv) the role of the MCP in paleo oceans [9]. Modeling of MCP vs BP indicated that the importance of the MCP relative to BP may increase under global-warming scenarios [10].

These findings have laid solid foundations for addressing fundamental questions regarding the MCP. Particularly, it is important and extremely valuable to quantify the RDOC pool in modern and ancient oceans in order to better understand the coupling between the ocean carbon cycle and global climate in the Earth's history. The breadth and depth of this project, which was executed over the past five years, have highlighted the importance as well as the complexity of microbial processes associated with the MCP. The outcomes have profound implications for the understanding of biologically driven ocean carbon uptake and storage and thus potential ocean feedbacks to climate change. Though the project will end soon, the MCP has become a central paradigm that is shaping a new direction in ocean carbon cycle research.

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GEOSCIENCES

Special Topic: Marine Carbon Sequestration and Climate Change

Unveiling the enigma of refractory carbon in the ocean

Nianzhi Jiao[1,*], Ruanhong Cai[1], Qiang Zheng[1], Kai Tang[1], Jihua Liu[2,3], Fanglue Jiao[3], Douglas Wallace[3], Feng Chen[2,4], Chao Li[5], Rudolf Amann[6], Ronald Benner[7] and Farooq Azam[8]

INTRODUCTION

The ocean holds a tremendous reservoir of refractory dissolved organic carbon (RDOC) that plays an important role in carbon cycling and climate change [1]. However, the origin of the RDOC has been an enigma for half a century. This perspective is to address why the enigma deserves scientific efforts and illustrate a robust scheme—the Microbial Carbon Pump (MCP)—to unveil the enigma from molecular to ecosystem levels. Through generation of intrinsic RDOC ($RDOC_t$) under specific biotic and abiotic environmental conditions, as well as through derivation of diverse organic molecules at extremely low concentrations ($RDOC_c$), the MCP links the seemingly contrary 'intrinsic recalcitrance hypothesis' and 'dilution hypothesis' together, and provides a framework for testable hypotheses linking microbial activities with the behavior of organic compounds for future studies regarding carbon sequestration in the ocean.

A HALF-CENTURY ENIGMA

The enigma: how is the huge ocean organic carbon reservoir formed?

The ocean harbors a vast reservoir of RDOC that is equivalent in amount (670 Pg C) to the total inventory of CO_2 in the atmosphere [2]. The RDOC can be sequestered in the deep ocean for 4∼6 kyr [2], playing an important role in carbon cycling and climate change [1]. This carbon reservoir was recognized half a century ago and how it is formed has been an intriguing topic for research for decades [3–7]. Given its old age, the RDOC was once attributed to seabed seepage of organics, but later studies on oil decomposition, particularly the consequences of the Deepwater Horizon oil spill, showed that most of the spilled old organic carbon is actually labile for the microbes in the water column [8]. Furthermore, a substantial portion of the RDOC was found recently to have a modern radiocarbon age [9]. While radiocarbon age may not necessarily be a proper proxy of recalcitrance of organic matter, the recalcitrance itself could also be apparent. From this viewpoint, RDOC could be composed of diverse labile compounds at extremely low individual concentrations and thus inaccessible to microbes (i.e. the 'dilution hypothesis') [3–6], although dilute labile molecules appear to account for a relatively small portion [10,11]. These paradoxes show the complexity of the ocean RDOC and its cycling, and why its origin has remained an enigma for half a century [4,7].

A robust scheme: the MCP

The MCP conceptual framework was proposed to address the mechanisms involved in the formation of RDOC [12]. Three pathways of the MCP are identified: RDOC generated directly from microbial cell materials, RDOC derived from degradation of particulate organic matter and residual RDOC after microbial utilization of the bulk DOC [13]. All RDOC compounds can be classified into two types: $RDOC_t$ that is composed of compounds that are intrinsically refractory in a specific environmental context (including its biotic and abiotic aspects) and $RDOC_c$ that is composed of a tremendous diversity of labile compounds with extremely low individual concentrations, lying below their uptake thresholds [5]. Products from all three MCP pathways contribute to either $RDOC_t$ or $RDOC_c$ in all cases (Fig. 1).

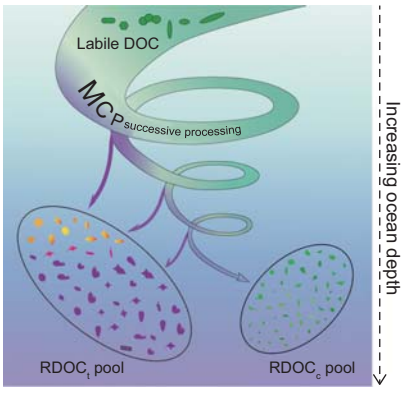

Figure 1. MCP successive processing of organic carbon generates $RDOC_t$ as well as $RDOC_c$ in the water column. The $RDOC_t$ pool is composed of those compounds that are refractory under a given environmental context including its biotic and abiotic aspects; RDOCc is composed of diverse labile compounds with extremely low individual concentrations below their uptake thresholds.

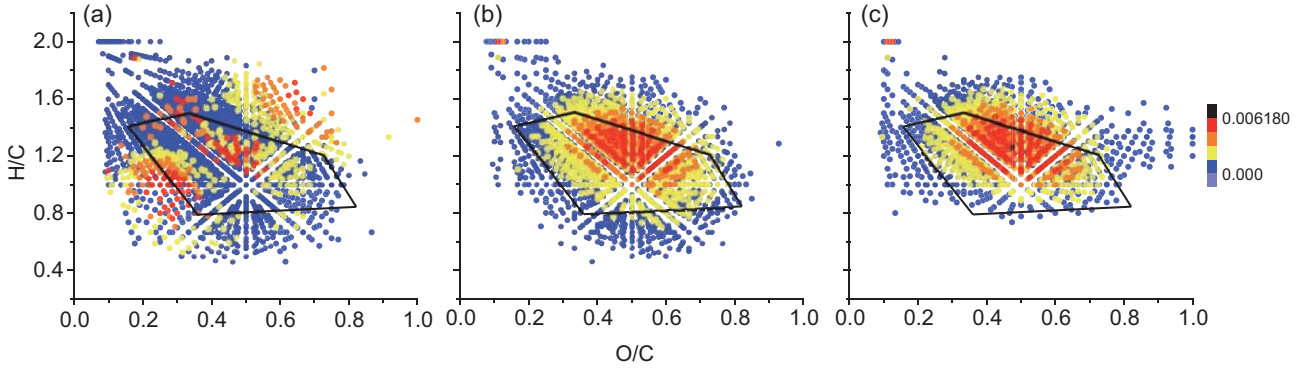

Figure 2. van Krevelen diagrams of molecular formulas derived from FT-ICR-MS results of samples from an incubation experiment conducted at the Aquatron Tower Tank (Dalhousie University, Canada). (a) initial (with addition of phytoplankton debris); (b) after 573-day incubation; (c) a deep-sea water sample (2000 m) from the South China Sea. Color bar indicates the normalized intensity obtained by FT-ICR-MS spectra; carboxyl-rich alicyclic molecules (CRAM) are located within the black polygons.

TACKLING THE ENIGMA IN THE MCP FRAMEWORK

Molecular characteristics and microbial origin of RDOC

Advances in analytical chemistry, such as ultra-high-resolution Fourier transform-ion cyclotron resonance-mass spectrometry (FT-ICR-MS), high field nuclear magnetic resonance (NMR) spectrometry and excitation emission matrix (EEM) fluorescence have helped to characterize molecular fingerprints and structures of organic compounds in the deep sea. Carboxyl-rich alicyclic molecules (CRAM), characterized by FT-ICR-MS and NMR as refractory components, are found to be widespread in the deep ocean [14]. A large suite of CHO-containing molecules with CRAM-like characteristics (located in the elemental ratio region known as the 'island of stability', IOS) are reported to have residence times that greatly exceed the oceanic mixing time in the deep Atlantic Ocean [15]. These CRAM-like components of RDOC appear to be oxidized molecules of microbial origin [16,17]. A long-term incubation experiment employing the Aquatron Tower Tank (>100 tons of water) showed robust evidence that phytoplankton debris (Fig. 2a) is effectively transformed to $RDOC_t$ during this experiment as indicated by the CRAM pattern (Fig. 2b). These, in turn, resemble the deep-sea sample from the South China Sea (Fig. 2c), indicating that deep-sea RDOC was generated through the MCP.

Observed microbial transformation of organic carbon in the field

Humic-like fluorescent dissolved organic matter (FDOM) is another indicator of the microbial transformation of organic matter, which can be readily recorded in field samples. The turnover time of FDOM is much longer than that of the dark global-ocean circulation [18], indicating that humic-like FDOM is a fraction of the RDOC. FDOM is produced *in situ* and accumulates in the interior of the ocean. Recent studies have provided new evidence that both cyanobacteria and heterotrophic bacteria contribute to deep-ocean FDOM [19]. These findings not only demonstrate the existence of the MCP, but also suggest rapid microbial modification of the organic carbon structure and its chemical complexity. That is why the dual concepts of $RDOC_t$ and $RDOC_c$ are necessary [5]. $RDOC_t$ varies significantly between surface and deep waters in terms of molecular structures, redox state, degradation state and contributions of diverse compound groups [11,15]. $RDOC_t$ in the deep ocean has been modified by microbial enzymatic processes and features relatively higher double-bond equivalent values and degradation state but lower H/C ratios, as well as higher degree of unsaturation plus rings in molecules [20]. In contrast, the majority of the molecular formulas enriched in surface seawater are classified either as highly unsaturated compounds or as unsaturated aliphatic compounds, which make a particularly important contribution at the depth of the chlorophyll maximum, suggesting that these compounds are a major fraction of labile phytoplankton exudates [11]. Only a tiny fraction of the deep-sea organic compounds, on the other hand, are unsaturated aliphatics [11], indicating the existence of $RDOC_c$ in the deep ocean. Compared to the sharp decline in labile DOC compounds with water depth, including unsaturated aliphatic compounds containing nitrogen, the $RDOC_t$ such as polycyclic aromatics (PCAs), highly aromatic compounds and highly unsaturated compounds increased with depth [11].

These spatial patterns help to reveal the active transformation of the MCP ongoing within the ocean's water column. The downward transport of young (recently formed) RDOC compounds from the surface ocean to depths by the ocean's 'mixed-layer pump' [21] as well as the supply of RDOC derived from decomposition of sinking organic particles explains why a considerable fraction of deep-ocean RDOC (10–30%) has a modern radiocarbon age [9]. On the other hand, there is also 'recent' formation of RDOC with old radiocarbon signatures ongoing

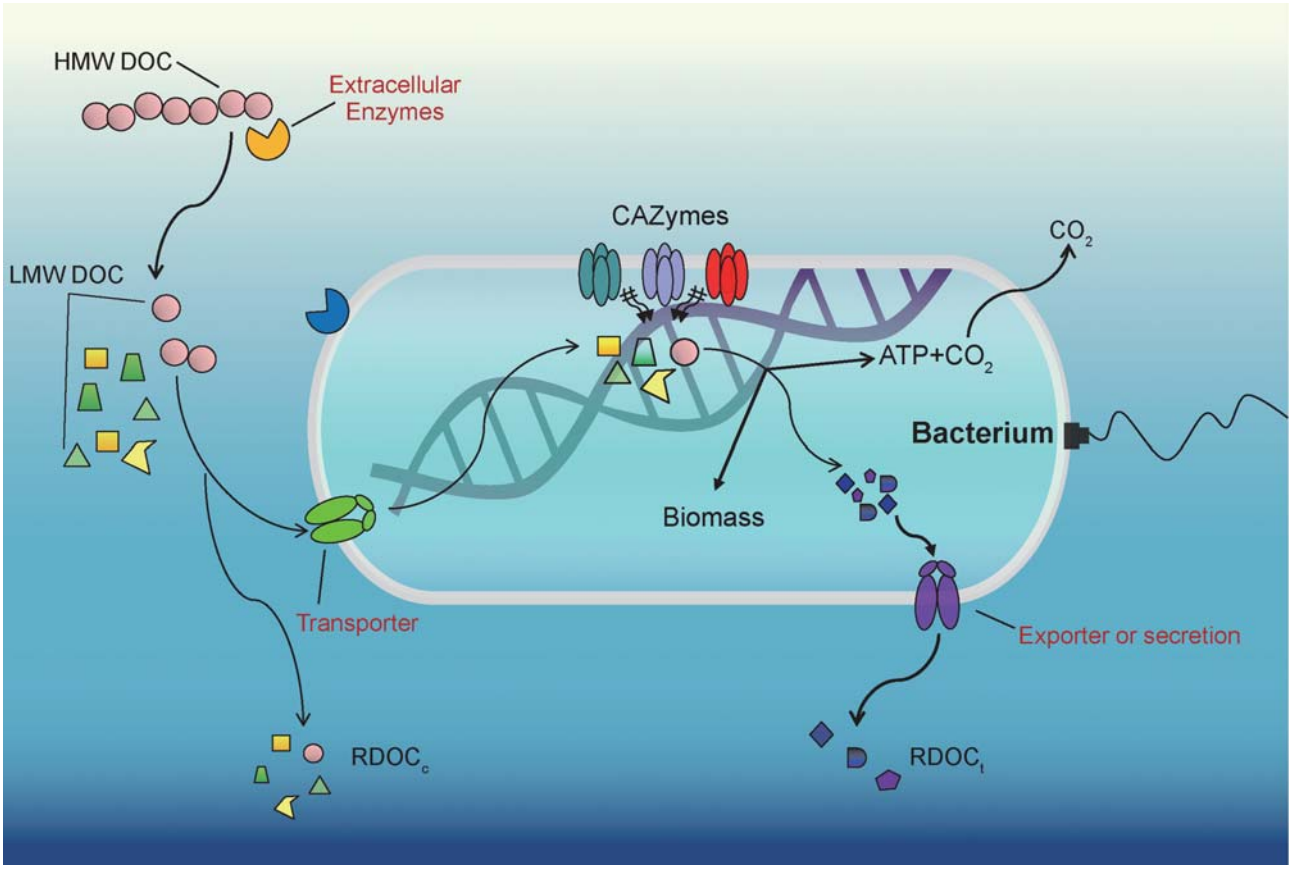

Figure 3. The processes of bacterial hydrolysis, transport, metabolism and secretion of DOC compounds. HMW DOC compounds are degraded by extracellular hydrolyses into LMW DOC molecules and part of them are then transported into the cell. There are three fates for intracellular metabolism of DOC: energy generation and CO_2 release; biosynthesis as cell biomass; and transformation and secretion to the environment, with part of it as $RDOC_t$ and $RDOC_c$. CAZymes, the carbohydrate-active enzymes and associated carbohydrate-binding modules involved in the synthesis and degradation of complex carbohydrates.

within the deep sea: even if natural hydrocarbon seepage, as well as methane, is labile and capable of rapid remineralization into CO_2 [22], subsequent chemosynthetic microbial activity could synthesize the aged CO_2 within deep-sea environments such as hydrothermal systems [23], thereby contributing to the RDOC pool via the MCP.

Mechanisms at the cellular, enzymatic and genetic levels

Marine microbiomes are diverse in terms of their community composition and metabolic potentials. In the upper ocean, ~20 Gt of carbon is fixed annually by phytoplankton and rapidly metabolized mostly by heterotrophic microbes. Different microbial groups (e.g. *Flavobacteriia*, *Roseobacter* and *Alteromonas*) display a successive order in utilization of the phytoplankton biomass composed primarily of polysaccharides and proteins [24] which consists of hundreds of different compounds [7]. Such successive mineralization processes make use of ectoenzymes, exoenzymes or alternative selfish-uptake mechanisms [24] generating specific $RDOC_t$ and $RDOC_c$ under corresponding specific environmental contexts [5]. The pathways and rates of microbial transformation determine the fate and the amount of carbon that is converted ultimately to CO_2 or RDOC [25]. Currently, the big data approaches of 'omics' (genomics, transcriptomics, proteomics and metabolomics) open up possibilities to study the transporters and enzymes involved in phytoplankton mineralization and RDOC generation in the natural environment (Fig. 3).

Genomic data analysis can provide information on which microbes utilize what DOC components and thus potentially discriminate $RDOC_t$, while metabolomics information analysis together with advanced FT-ICR-MS and NMR analysis offer an approach for the detection of molecular formulas and chemical structures of $RDOC_t$ and $RDOC_c$ [5]. Molecular mechanisms for DOC utilization vary among different bacterial groups. For example, ABC transporters responsible for the

uptake of low-molecular-weight (LMW) molecules are rich within the *Roseobacter* genomes, while TonB-dependent transporters for high-molecular-weight (HMW) molecules are abundant in the *Flavobacteriia* genomes [26]. To date, there is a wide variety of small molecules (∼14 000) in the biochemical network of microbes, and the HMW molecules derived from macromolecule metabolism (e.g. peptides and peptidoglycan) can also contribute greatly to metabolic diversity. However, there are ∼600 known transporter reactions, most of which are responsible for transporting a specific substrate or a group of substrates with similar chemical structures. Thus, many metabolites may have no corresponding transporters. Furthermore, some metabolites are produced by known metabolic reactions of an organism and have no reactions consuming it, accounting for ∼3% of the compounds derived from all pathway reactions of a microbe [27]. The aerobic anoxygenic phototrophic bacterium *Roseobacter denitrificans* OCh 114 produces oxidized carotenoids spheroiden-2-one and 2, 2'-dioxospirilloxanthin by spheroidene monooxygenase. It becomes obvious that the number of metabolites found in a single species exceeds the number of genes encoding enzymes involved in their uptake and further biotransformation. There are at least 100 000 different compounds in DOM in the deep ocean [28]. The huge gap between the diversity of enzymes and the diversity of DOC compounds likely facilitates the formation and stability of deep-sea $RDOC_t$. On the other hand, the affinity of transporters constants (*Ks*) varies greatly from millimolar to nanomolar, and are generally higher than the apparent picomolar concentrations of deep-sea compounds [28], consistently with the existence of $RDOC_c$.

FUTURE PERSPECTIVES

MCP sustains the global-ocean RDOC pool

Efficient microbial transformations of labile substrates into $RDOC_t$ have been demonstrated by both laboratory experiments and field observations [16,29]. A recent study estimates that the annual production of RDOC via the MCP ranges from 0.1 to 0.2 Pg C [30], which is in amount equivalent to 4–8% of the current net annual uptake of atmospheric CO_2 by the ocean. At least 25% of the oceanic RDOC is of bacterial origin [31]. Even with the lowest microbial transformation efficiency (<0.4% of the net marine community production is shunted to RDOC), the MCP can still sustain the ocean's huge global RDOC pool [32]. In addition to sequestration of carbon from the atmosphere, it has been suggested that chemosynthetic crustal microbial communities synthesize DOC from inorganic carbon contained in ridge-flank fluids. This may support an indigenous biosphere that can export substantial fixed carbon to the overlying water columns via the ridge-flank circulation [23]. Furthermore, the isotopic biogeochemical record suggests a huge DOC reservoir in the late Neoproterozoic oceans (≥2–3 orders of magnitude more abundant than at present) [1] when metazoans were not evolved but microbes thrived, suggesting that the MCP could have worked very efficiently in ancient times [5]. Recent studies further suggest that the proximal-to-distal marine redox gradient may have favored an intense MCP in shelf areas and the buildup of RDOC in deeper waters of the late Proterozoic oceans [33]. Taken together, the MCP is the principal mechanism generating and sustaining the tremendous oceanic RDOC reservoir.

Future studies

There is an urgent and overall need to better understand the impacts of global-scale environmental change, including ocean warming and ocean acidification on carbon cycling within the ocean. Improved understanding of the microbial processes responsible for transformations of organic carbon is central to this requirement, among all related issues. In order to advance understanding, in-depth and coordinated studies are particularly required using the following approaches:

Approach 1) Investigation of microbiomes in different, natural environments, including much better coverage of the deep ocean. The Global Ocean Sampling (GOS) expedition only sampled from the surface ocean and the ambitious Tara Oceans expedition collected microbial samples from only three depths (5, 70 and 600 m) [34]. The deeper-ocean microbes are reported to be abundant in metabolic genes related to the degradation of complex organic molecules but there is a lack of sufficient studies. Application of various omics technologies (i.e. metagenomics, metatranscriptomics and metaproteomics, and metabolomics) at all levels of the microbial community (i.e. virus, bacteria, archaea, phytoplankton and chemoautotrophs) is needed. In addition to surveys, these methods should also be applied at selected time-series locations in the coastal and open ocean, in order to identify how the metabolic capacity of the ocean's microbiome responds to temporal changes in environmental context.

Approach 2) Linkages between microbial metabolisms and the chemical structure of DOC compounds requires further investigation. In addition to bioassays on changes of DOC composition coupled with changes in bacterial communities, the combination of omics and FT-ICR-MS and NMR technologies offers the potential for new insights into mechanisms responsible for the formation of $RDOC_t$ and $RDOC_c$. This area of research is often restricted by technical limitations on DOC extraction and analysis. Coupling of various DOC extraction methods with promising new analytical chemistry approaches is urged to achieve comprehensive understanding on the still largely unknown fingerprints and structures of complex DOC. This is likely to benefit from coordinated experimentation and intercomparison of diverse analytical approaches by different groups working in this area.

Approach 3) A key requirement for making progress in understanding is the establishment and expansion

of long-term incubation studies employing large-scale facilities, such as the Aquatron Tower Tank (12 m in height, 3.8 m in diameter) or the MECS (marine environmental chamber system, designed as 50 m in height and 5 m in diameter) under controlled environmental conditions. These facilities offer a unique complement to field studies by allowing the deliberate creation of ocean-relevant physical/chemical/biological environmental context (e.g. depth profiles) for long-term experimentation. Long-term experiments under controlled yet realistic environmental conditions are required to provide data and insight for testing of theories and hypotheses regarding the effects of global environmental change on ocean carbon cycle. Together with time-series studies, such large-scale experimental facilities also provide opportunities for testing and inter-comparison of diverse and novel analytical and experimental approaches (see Approaches (1) and (2) above). By allowing control of euphotic/aphotic depths, compensation depths, stratification, nutrient gradients, oxygen levels and redox gradient, pH, etc., large-scale experimental facilities can enable focused and repeatable studies of the microbial processing of organic carbon and its role for the consequent transformation and sequestration of carbon within the ocean.

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ENVIRONMENT/ECOLOGY

Special Topic: Marine Carbon Sequestration and Climate Change

Blue carbon on the rise: challenges and opportunities

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BLUE CARBON ON THE RISE

Climate change is a global concern that requires urgent solutions. As a signatory to the Paris Agreement, China has committed to have its greenhouse gas emission reach a peak by the year 2030, which means that severe countermeasures for reducing emissions have to be put into practice. This is a hard mission given that development is still the top priority in the years to come for China. Under such circumstances, enhancing carbon sequestration becomes an effective approach to achieving the goal. While terrestrial green carbon sink is already in practice, the ocean carbon reservoir, containing 93% of global CO_2, as 50 and 20 times the carbon inventories of atmosphere and land, respectively, has great potential to expand. Each year, at least 25% of the anthropogenic CO_2 has been captured by marine ecosystems as blue carbon [1].

In 2009, the United Nations Environment Programme (UNEP), the Food and Agricultural Organization of the United Nations (FAO) and the Intergovernmental Oceanographic Commission (IOC) of the United Nations Educational, Scientific and Cultural Organization (UNESCO) jointly published the *Blue Carbon* report, pointing out that more than 55% of global primary production is blue carbon [2]. Moreover, the production efficiency of the coastal blue carbon biomes (mangroves, sea grass, salt marsh ecosystems, etc.) is much higher than that of the Amazon rain forest. However, these blue carbon biomes are being degraded and disappearing at rates 5–10 times faster than rainforests [3], thus protection and restoration are in urgent need.

Besides the visible coastal blue carbon, there are enormous invisible blue carbon biomes composed of tiny but extremely abundant microbes, including phytoplankton, bacteria, archaea and viruses, which contribute up to 95% of the world's blue carbon [4–6]. These microbes can interact with the visible blue carbon biomes, transforming their organic carbon into refractory forms, prolonging the residence time of the organic carbon in the ocean. The environmental issues we are facing in the coastal water today (such as eutrophication, hypoxia, acidification, etc.) also interact with the invisible blue carbon processes, but the mechanisms are not yet clear. Therefore, both visible and invisible blue carbon biomes should be taken into consideration for marine ecosystem services, and understanding their interactions and their relationship with environmental issues is critical for marine ecosystem management and sustainable development.

BLUE CARBON INITIATIVE AND PROGRESS

The IOC, Conservation International (CI) and the International Union for Conservation of Nature (IUCN) jointly launched the *Blue Carbon Initiative* in 2011, aimed at promoting the management of marine and coastal ecosystems through international cooperation, and maintaining carbon sink function in the mitigation of climate change. 'Blue Carbon Action' set up two working groups. One is the scientific group toward the establishment of blue carbon measurement and monitoring protocols, data acquisition and quality control, field survey handbooks as well as blue carbon conservation planning and management guidelines. The other is the policy working group toward integration of the blue carbon project into the *United Nations Framework Convention on Climate Change* and *Convention on Biological Diversity*, and the development of a program of financial support and other policies. Since then, 'Blue Carbon Action' has been in action in the protection and restoration of mangroves, wetlands, etc. in many regions and countries such as Australia, Dhabi, Indonesia, India, Kenya, Madagascar, Vietnam and the USA.

Significant progress has been made in coastal blue carbon research in recent years, such as the description of blue carbon status and carbon sequestration potentials at the global scale [7,8] and regional scale [8]; the assessment of impacts of wetland destruction and the proposal of corresponding policy [7]; the evaluation of the eco-value of blue carbon in sea grasses, salt marshes and mangroves (e.g. in Philip Bay and Western Harbor, Australia, with an estimate of the rooted plants to be 1.03 million tons of carbon and a price of $15.38 million) [9]; proposal of countermeasures for conservation and restoration of blue carbon (e.g. in Scotland, Columbia, Korea and other countries) [10,11]; as well as scenario simulation of blue carbon benefit function and market price [12]. In 2017, the Intergovernmental Panel on Climate Change (IPCC) launched the writing process of the Sixth Assessment Report on Climate Change, with a Special Report on the Ocean and Cryosphere in which blue carbon is included.

CHALLENGES AND OPPORTUNITIES

Although the original concept of blue carbon proposed in 2009 refers to the carbon that is captured by marine ecosystems covering both coastal and open

waters [3], practical research and development of blue carbon have predominantly involved macrofauna, such as mangrove, seagrass and salt marsh in the coastal zone. After years of promotion of the coastal blue carbon, the IUCN published the *Call for Action on Ocean Carbon* in 2014, which highlights the importance of ocean carbon sinks in the mitigation of climate change, and identifies the key components of blue carbon in open oceans, expanding the blue carbon from coastal zones to oceanic environments [13]. In fact, the invisible microbes are an essential part of blue carbon in the ocean, but have been largely overlooked so far. These microbes, including phytoplankton (microalgae), cyanobacteria, bacteria, archaea and viruses, are tiny but extremely abundant, contributing up to 90% of the marine biomass and 95% of the marine production [4–6]. Annually, over 360 billion tons of CO_2 is fixed by marine phytoplankton, 1.39% of which is transported down to the seafloor by the biological pump (BP) for long-term storage [14]. The rest of the fixed organic carbon is mainly respired into CO_2, but a small portion of the organic carbon is shunted through the microbial carbon pump (MCP) to biologically inaccessible phases, being either refractory or at extremely low concentrations [15]. The MCP is a major contributor to the tremendous marine refractory DOC (RDOC) reservoir, which is equivalent in amount to the total inventory of CO_2 in the atmosphere [16]. Paleoclimate studies show that there was an inextricable link between the RDOC pool and climate change [17,18]. The MCP effects exist in all water environments and even soil environments [19], connecting with the visible blue carbon ecosystem, as all the blue carbon macro-biomes (mangrove, sea grass, salt marsh, etc.) release DOC into the water, which can be further transformed by the MCP into RDOC. Such processes are influenced by environmental conditions and thus allow manipulations to pursue maximum outputs of the sum of the BP and MCP (Fig. 1).

LAND–SEA INTEGRATED COUNTERMEASURES FOR CARBON SEQUESTRATION AND SUSTAINABLE DEVELOPMENT OF THE COASTAL ECOSYSTEM

Chemical fertilizers have been excessively applied in farming for decades, especially in developing countries. Excessive nitrogen (N) and phosphorus (P) are then washed out into rivers and ultimately discharged into the coastal waters, causing eutrophication and algal blooms. Although algal blooms seemingly produce more organic carbon, this carbon is basically labile and can be respired rapidly. In addition, the labile DOC produced by primary producers has priming effects on the river discharged terrestrial RDOC, namely remobilizing RDOC for microbial uptake and respiration, which can create high CO_2 concentrations in the water, making the carbonate equilibrium system move toward proton generation causing acidification in ambient water, and excess CO_2 escape from water to atmosphere as outgassing. That is why productive estuarine and coastal waters are often sources rather than sinks of atmospheric CO_2. Meanwhile, this process consumes a large quantity of oxygen, resulting in hypoxia. Anoxic conditions could cause massive death of macro- and microbiomes, resulting in the breeding of anaerobic bacteria that transform organics into CH_4, H_2S, N_2O and other toxic substances, which in turn are destructive for the ecosystem. On top of that, excess discharge of nutrients (N, P) shapes the C/N and C/P elemental ratios in favor of remobilization of RDOC for respiration, lowering the MCP efficiency and carbon sequestration. Therefore, reducing terrestrial input of inorganic nutrients becomes a feasible countermeasure for the enhancement of carbon sequestration in coastal waters (Fig. 1). This idea is supported by a statistical data analysis of organic carbon versus nitrate in various natural environments [20] as well as by experimental results in estuarine and offshore waters, which found that, in all cases, if there are too many inorganic nutrients, there will be less organic carbon preserved in the environment. Therefore, land–ocean integrated management and engineering become necessary, such as reducing the application of chemical fertilizers in farming and eliminating sewage discharge into rivers so as to reduce the N, P inputs into the sea. Such eco-engineering is not aimed at changing the natural ecosystems, but rather protecting them by reducing eutrophication and red-tides occurrence while increasing carbon sequestration through the MCP. This idea also brings new policy as bonus-based carbon trade rather than penalty-based pollution policy, as is being used in many countries nowadays. The bonus-based policy would be such that any economy loss claimed due to the reduction of farming fertilization (should not be the case if fertilization is scientifically applied, though) and sewage work in the watersheds can be compensated by the eco-value or carbon price of the increment of carbon sequestration in the sea. Once a carbon-accounting system for the watershed-coastal-offshore environments is established, a blue carbon sequestration-based voluntary emission reduction trading mechanism could be easily developed.

BLUE CARBON STRATEGY IN CHINA

The China Seas include the Bohai Sea, the Yellow Sea, the East China Sea and the South China Sea, with coastlines of 18 000 km, stretching from the northern temperate zone to tropic zones. There are more than 1500 rivers to the China Seas, including the world third largest river, the Yangtze River to the East China Sea, the Yellow River carrying a huge amount of sediment to the Bohai Sea and the Pearl River to the South China Sea connecting the Tibetan plateau with the 'warm pool' in the West Pacific. Such rich habitats harbor great biodiversity and

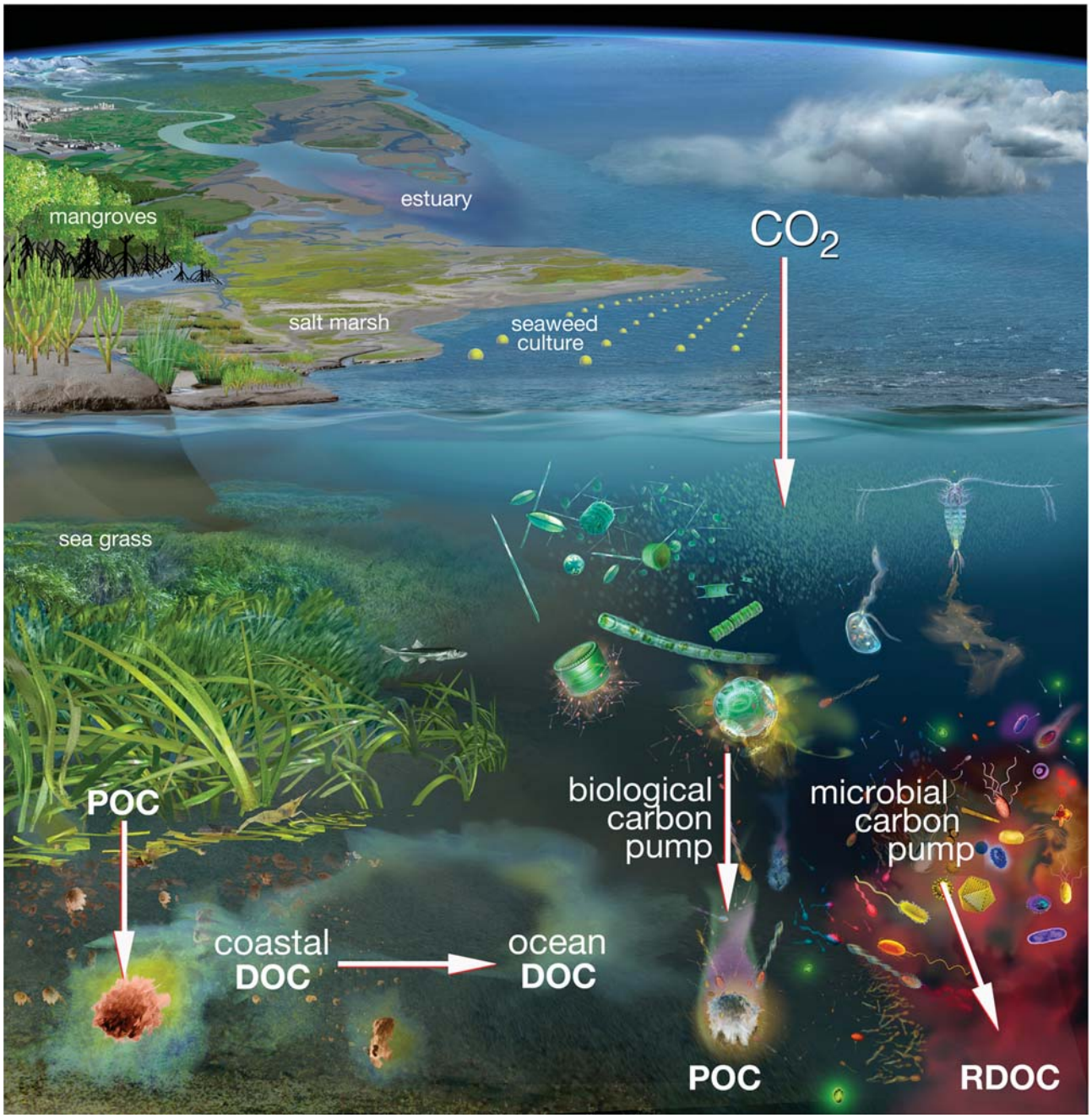

Figure 1. A demo of eco-engineering to make coastal waters into sinks rather than sources of atmospheric CO_2. In addition to the conservation and restoration of coastal blue carbon (mangroves, salt marshes, sea grasses, etc.), reducing terrestrial nutrient inputs could avoid the priming effects on organic carbon respiration and thus increase carbon sequestration in the ocean through both the BP and the MCP.

carbon-storage capacity. On the other hand, China's coastal zones are also highly inhabited, with most of them receiving severe anthropogenic impacts such as harbor construction, tidal zone reclamation, as well as mariculture. Discharges of nutrients and organic matter from rivers as well as sea-farming activities have fundamental impacts on the ecosystem's health and sustainability. Many of the estuarine waters are currently suffering from algal bloom, hypoxia and acidification caused by eutrophication and the following microbial processes as pointed out in the 'Challenges and Opportunities' section above.

Despite coastal rooted plants like mangroves, saltmarshes and sea grasses having high carbon production, their coverage in China's coastal areas is small and the annual buried amounts of organic matter from them are limited (Table 1). Meanwhile, their production of DOC has never been counted due

Table 1. Carbon sequestration fluxes of various ecosystems in China's seas. *The references were from [22].

Ecosystems	Burial flux ($Tg\ C\ yr^{-1}$)	DOC export flux ($Tg\ C\ yr^{-1}$)	References
Macrofauna			
Mangrove	0.04	–	Liu *et al.*, 2014; Wang *et al.*, 2016
Salt marsh	0.26	–	Wang *et al.*, 2016; Mei and Zhang, 2008; Suo *et al.*, 2010; Cao *et al.*, 2013; Zhou *et al.*, 2016
Seagrass	0.01	0.30	Zhou *et al.*, 2016; Jiang *et al.*, 2017; Zheng *et al.*, 2008; Krause-Jensen and Duarte, 2016; Gan *et al.*, 2016; Cao, 2017
Mariculture	0.14	0.50	Zhang *et al.*, 2017; Krause-Jensen and Duarte, 2016; Gan *et al.*, 2016; Cao, 2017; Bureau, MoAFA, 2017
Open waters (mainly microbes)			
Bohai Sea	2.0	1.51	Hu and Zhao, 2017; Hu *et al.*, 2016; Wei *et al.*, 2002; Liu *et al.*, 2015; Shang, 2011
Yellow Sea	3.6	13.20	Hu and Zhao, 2017; Hu *et al.*, 2016; Song *et al.*, 2017
East China Sea shelf	7.4	15∼35	Deng *et al.*, 2006; Yuan *et al.*, 2017; Hu and Zhao, 2017
South China Sea shelf	4.8	31.82	Hu and Zhao, 2017; Chen *et al.*, 2006; Hung *et al.*, 2007; Wu *et al.*, 2015, 2017

to lack of study. Take the case of DOC release from cultured seaweeds, for example (although cultivated seaweeds are not counted as blue carbon officially, its buried debris and derived RDOC do contribute to marine carbon sink). The total production of China's seaweeds culture is about 3.52 Tg C yr^{-1}. If 23–26% of this carbon is released as DOC [21], and assuming that only half of the DOC becomes refractory (in the water column, 77–94% of the bulk DOC is refractory [22]), a conservative estimate of the RDOC derived from seaweed culture would be 0.5 Tg C yr^{-1} [22], which is even higher than the total burial flux of organic carbon from the coastal blue carbon in China.

When looking at blue carbon on larger scales, terrestrial inputs of DOC to the coastal waters and marginal sea export of DOC to oceanic waters become essential for the mitigation of climate change. Each year, a great deal of DOC is discharged into China's seas by rivers. For example, the annual DOC flux of the Yangtze River to the East China Sea is 1.62 Tg C yr^{-1} [22], and the annual export flux of DOC from the East China Sea to the Western Pacific is 15–35 Tg C yr^{-1} [23]. Such DOC has been exposed to a variety of environmental conditions before it is exported and is supposed to be relatively refractory. If it keeps the recalcitrance under controlled nutrient inputs (as discussed in the above section) instead of being remobilized under eutrophic conditions and respired in the marine environments, it would be a substantial contribution to marine carbon sequestration [24]. Together with the BP flux on the continental shelf (7.4 Tg C yr^{-1}) [22], the East China Sea is actually a significant carbon sink. In fact, the total carbon export flux of China's continental shelves is up to 90 Tg C yr^{-1} (Table 1); this recognition changes the overall image of China's seas as 'sources' as perceived from the exchange of CO_2 between the atmosphere and sea surface. Such 'sources' are actually due to the respiration of terrestrial DOC and the outgassing of imported high-dissolved inorganic carbon (DIC) deep water from the Western Pacific [22]. It is therefore worth pointing out that, even if a marine region is the source of atmospheric CO_2, carbon sequestration could take place at the same time, just like the case of oceanic upwelling areas.

Based on the above understanding, an effective strategy for China's blue carbon project would be that, while deploying restoration of the visible coastal blue carbon (rooted plants), efforts should be made in invisible microbial carbon sequestration in the waters, especially estuarine and shelf regions. Only in that case will the blue carbon project become essentially meaningful for the mitigation of climate change. In practice, the following measures should be implemented: to establish long-term monitoring and observation networks covering representative sites in the river catchments, estuaries as well as shelf waters; to establish standard protocols for core measurements of different forms of blue carbon in various ecosystems; to establish the mechanisms of blue carbon eco-value assessment; to establish a carbon-accounting system for the watershed-coastal-offshore environments; to establish a land–ocean integrated compensation policy to promote blue carbon trading with the farming industry; to establish a framework for voluntary emission reduction trading with blue carbon for low carbon economy.

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GEOSCIENCES

Special Topic: Marine Carbon Sequestration and Climate Change

Modelling marine DOC degradation time scales

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INTRODUCTION

Marine dissolved organic carbon (DOC) is formed of a large number of highly diverse molecules. Depending on the environmental conditions, a fraction of these molecules may become progressively resistant to bacterial degradation and accumulate in the ocean for extended time scales. This long-lived DOC (the so-called recalcitrant DOC, RDOC) is thought to play an important role in the global carbon cycle by sequestering carbon into the ocean interior and potentially affecting the climate. Despite this, RDOC formation is underrepresented in climate models. Here we propose a model formulation describing DOC recalcitrance through two state variables: one representing the bulk DOC concentration and the other representing its degradability (κ) which varies depending on the balance between the production of 'new' DOC (assumed to be easily degradable) and bacterial DOC utilization assumed to leave behind more recalcitrant DOC. We propose this formulation as a means to include RDOC dynamics into climate model simulations.

Assessing the capacity of the ocean to store atmospheric CO_2 is one of the major challenges for oceanographers. Several physical and biological

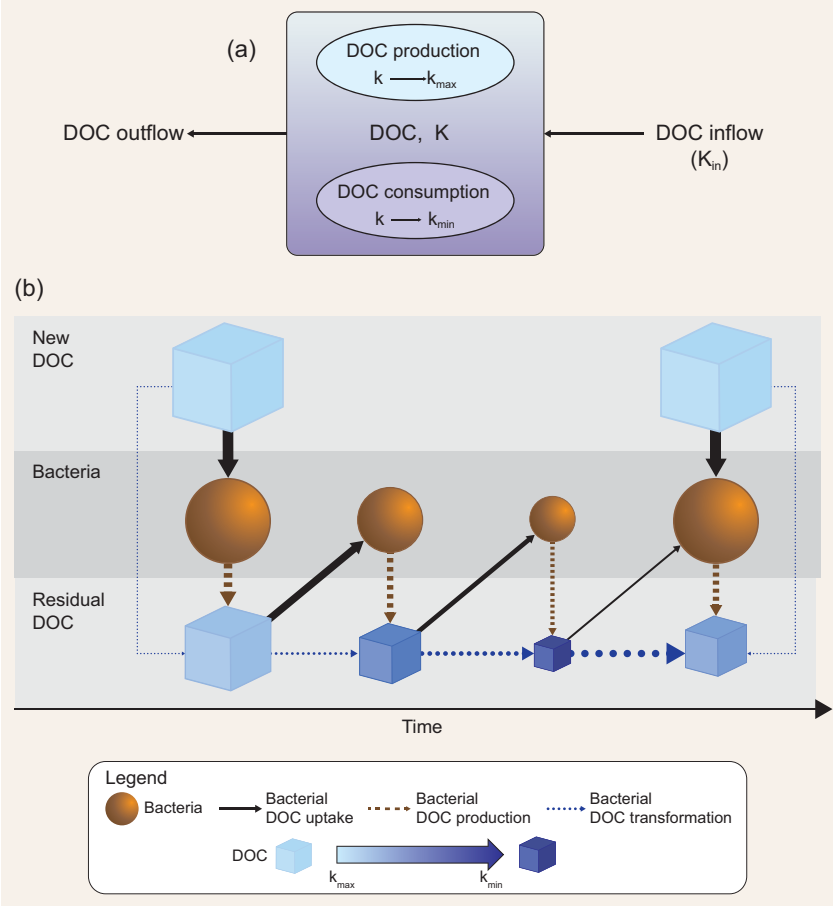

Figure 1. (a) Schematic representation of the model. DOC is the DOC concentration inside the box model; *k* is the DOC degradation function (see the main text for further explanation); DOC production is the DOC that is newly produced through primary production or other food web processes; DOC consumption is the DOC that is assimilated by bacteria. DOC production increases the value of *k* towards k_{max} while DOC consumption decreases the value of *k* towards k_{min}. The DOC transported inside the box (inflow) influences *k* depending on the degradation function associated with the incoming DOC (k_{in}) and on the magnitude of the flux (Equation 2.3, Table 1). Transported DOC can be expressed as an external forcing function if the model is used in a 'standalone' mode (e.g. the example reported in this paper) or through advective and/or diffusive fluxes from adjacent boxes if a 1D or 3D physical models are used. The export of DOC outside the box (outflow) does not affect *k* inside the box model. DOC has a concentration unit (e.g. mass per unit volume or area) while *k* is dimensionless. (b) Model functioning. Light-blue boxes indicate freshly produced, semi-labile DOC (i.e. with $k = K_{max}$). The degree of recalcitrance is represented by increasingly dark-blue colour. The interactions between bacteria and fresh DOC produce residual DOC with lower *k*. If the production of new DOC stops, DOC is biochemically altered and transformed and the value of *k* progressively decreases approaching k_{min}. If the production of fresh DOC starts again (or if fresh DOC is transported), *k* increases proportionally to the amount of the new DOC biologically produced and/or physically transported relative to the initial concentration of DOC (standing stock). Boxes and spheres represent pools (concentrations) while arrows indicate fluxes. Arrow widths represent the magnitude of the flux relative to the DOC pool.

mechanisms have been proposed to 'pump' CO_2 from the surface to the ocean interior, thus storing carbon for extended time frames [1,2]. Some of these mechanisms are driven by physical processes (i.e. the solubility pump) while others are the results of the interactions between biology (primary production, particle formation, prey–predators interactions) and physics (gravitational sinking, mixing, convection). The latter processes have collectively been termed the 'Biological Carbon Pump'. The recently proposed Microbial Carbon Pump (MCP) provides an additional carbon sequestration mechanism primarily due to biological drivers [3]. Indeed, the main process underpinning the MCP is the bacterially mediated transformation of labile (i.e. rapidly degradable) dissolved organic carbon (DOC) into recalcitrant (i.e. slowly degradable) DOC (RDOC), which may accumulate into the ocean at time scales ranging from months to millennia, in this latter case sequestering atmospheric CO_2 into stable long-lived organic molecules [4]. The production of RDOC is not directly affected by physical processes (mixing, sinking or thermohaline circulation) and its production is depth-independent—that is, it is active through the entire water column [2]. However, abiotic forcing such as vertical mixing and photo-degradation may also affect the RDOC fate and its spatial distribution, thus influencing the strength and the efficiency of the MCP.

Being the latest recognized mechanism of ocean carbon sequestration, the MCP is also the least well investigated and represented in marine ecosystem models. Generally, DOC is modelled by using up to three state variables, with each of them characterized by a constant degradation time scale [5]. This approach is not consistent with the prevailing idea that the recalcitrance of DOC is an environmentally dependent property [3] emerging from the repeated transformation and selective use of the labile organic carbon substrates by bacteria [6]. Some models have explicitly described the bacterially mediated transformation of DOC into RDOC; however, these studies do not consider the long-lasting fractions of RDOC and are not able to simulate RDOC accumulation on time scales that are longer than seasonal [7].

One of the main challenges with modelling DOC accumulation beyond the seasonal time scale is representing the turnover time of the various pools of RDOC that is formed of a large number of highly diverse molecules with a continuum spectrum of degradation rates [4]. Explicitly modelling such a wide diversity would end up in an unmanageable number of state variables,

Table 1. Model equations.[a]

	Model equations
1.DOC	$\frac{\partial DOC}{\partial t} = \frac{\partial DOC}{\partial t}\Big\vert^{Prod} - \frac{\partial DOC}{\partial t}\Big\vert^{Cons} + \frac{\partial DOC}{\partial t}\Big\vert^{Phys}$
1.1	$\frac{\partial DOC}{\partial t}\Big\vert^{Prod} = Const$
1.2	$\frac{\partial DOC}{\partial t}\Big\vert^{Cons} = L_k \cdot k \cdot DOC$
1.3	$\frac{\partial DOC}{\partial t}\Big\vert^{Phys} = Const$
2. k	$\frac{\partial k}{\partial t} = \frac{\partial k}{\partial t}\Big\vert^{Prod} - \frac{\partial k}{\partial t}\Big\vert^{Cons} + \frac{\partial k}{\partial t}\Big\vert^{phys}.$
2.1	$\frac{\partial k}{\partial t}\Big\vert^{Prod} = (k_{max} - k)\cdot \frac{\frac{\partial DOC}{\partial t}\Big\vert^{Prod}}{DOC^*}$
2.2	$\frac{\partial k}{\partial t}\Big\vert^{Cons} = (k - k_{min})\cdot \frac{\frac{\partial DOC}{\partial t}\Big\vert^{Cons}}{DOC^*}$
2.3	$\frac{\partial k}{\partial t}\Big\vert^{Phys} = (k_{in} - k)\cdot \frac{\frac{\partial DOC}{\partial t}\Big\vert^{Phys}}{DOC^*}$ if $\frac{\partial DOC}{\partial t}\Big\vert^{Phys} > 0$
2.3.1	$\frac{\partial k}{\partial t}\Big\vert^{Phys} = 0$ if $\frac{\partial DOC}{\partial t}\Big\vert^{Phys} < 0$
	Time integration
3	$DOC^{t+1} = DOC^t + \frac{\partial DOC}{\partial t}\cdot \Delta t$
4	$k^{t+1} = k^t + \frac{\partial k}{\partial t}\cdot \Delta t$

[a]The equations presented in this table refer to the simplified example reported in this paper (Figs 2 and 3), which assumes constant production of DOC, implicit bacterial uptake and a constant transport of DOC. However, the proposed formulations describing DOC degradability (k) are also meant to be implemented in more complex models that have DOC production, consumption and physical transport represented by more complex equations. *DOC concentration in the box model (Fig. 1a) is assumed to be always >0.

increasing the computational costs of the model and yielding a large number of at best poorly constrained parameters. This is an important limiting factor, especially when a simulation is run within a global ocean or Earth-system model. In this paper, we propose a conceptual framework capable of representing the continuum spectrum of DOC degradation rates in a tractable way (Fig. 1). The current formulation is meant to be generic and to be implemented in numerical models with different levels of complexity, from ecosystem models only accounting for implicit DOC remineralization to process models explicitly describing DOC–bacteria interactions.

A NEW MODELLING FRAMEWORK OF DOC DEGRADATION SCALES

We propose to model transformations of the DOC pools (Fig. 1 and Table 1) using one state variable representing the bulk DOC concentration and a degradation function $k(t)$. The use of a degradation function can have two different meanings. Depending on the model formulation, k can be (i) a function regulating the affinity of bacteria for a substrate, if bacteria biomass and DOC uptake are modelled explicitly [7], or (ii) a bulk rate constant representing DOC consumption in a model without explicit parameterization of the heterotrophic bacterial transformations of DOC [8]. In both cases, k describes the stability (i.e. resistance to degradation) of a one form of DOC (i.e. RDOC) with respect to another form of DOC (i.e. labile DOC) and ranges from a minimum (i.e. k_{min}) to a maximum (i.e. k_{max}) value. High k values imply high affinity by the bacteria for DOC or high consumption rate, while low k values indicate low affinity or low consumption rate. To give an example, a $k(t) = 0.01$ means that, at time t, RDOC is 100 times less susceptible to bacterial degradation (i.e. more stable) than labile DOC. While the degradation scale of labile DOC (assumed to be 1 d^{-1}) is used as reference in our formulation (see the parameter L_k in Equation 1.2 in Table 1), we set the upper limit of the degradation function k_{max} to a lower value as our formulation is specifically designed to assess DOC degradation at time scales much longer than daily (i.e. from years to longer). Consequently, k_{max} has a value of 0.01, implying a DOC consumption rate of 100 days. It should be also stressed that, in this paper, we assume that bacteria dominate environmental DOC degradation and transformations; consequently, k represents only the biologically mediated DOC consumption and transformation. However, $\frac{\partial DOC}{\partial t}|^{Conc}$ (Fig. 1, Equation 1.2) may also include abiotic processes in future model implementation. To explain model functioning and assumptions, we use a simple box model characterized by a concentration X of DOC with an associated degradation value equal to $k(t_0)$ (Fig. 1a).

This model can be either considered as a standalone box model or as a spatial unit (i.e. a subunit of a larger model grid) of a 3D domain. In this latter case, k will be dependent on space (x) and time (t) [i.e. $k = k(t, x)$]. DOC produced inside the box through primary production has associated degradation that is equal to k_{max}. This is consistent with previous findings suggesting that most of the DOC that is freshly produced by phytoplankton is degraded by bacteria within tens of days [9]. As a first approximation, here we do not consider other food web processes (e.g. grazing), which are also known to produce DOC [10]. However, the term $\frac{\partial DOC}{\partial t}|^{Prod}$ (Equation 1.1) may also include other DOC sources in future model implementation. The value of k inside the box model is affected by the newly produced DOC proportionally to the increase in DOC and the difference between k and k_{max} (Equation 2.1 in Table 1). Bacterial activity alters the DOC molecular structure and composition by removing specific components (i.e. chemical reactive groups or compounds or parts of them) and leaving behind biochemically altered material that becomes progressively more recalcitrant [6]. The residual DOC fraction resulting from the DOC–bacteria

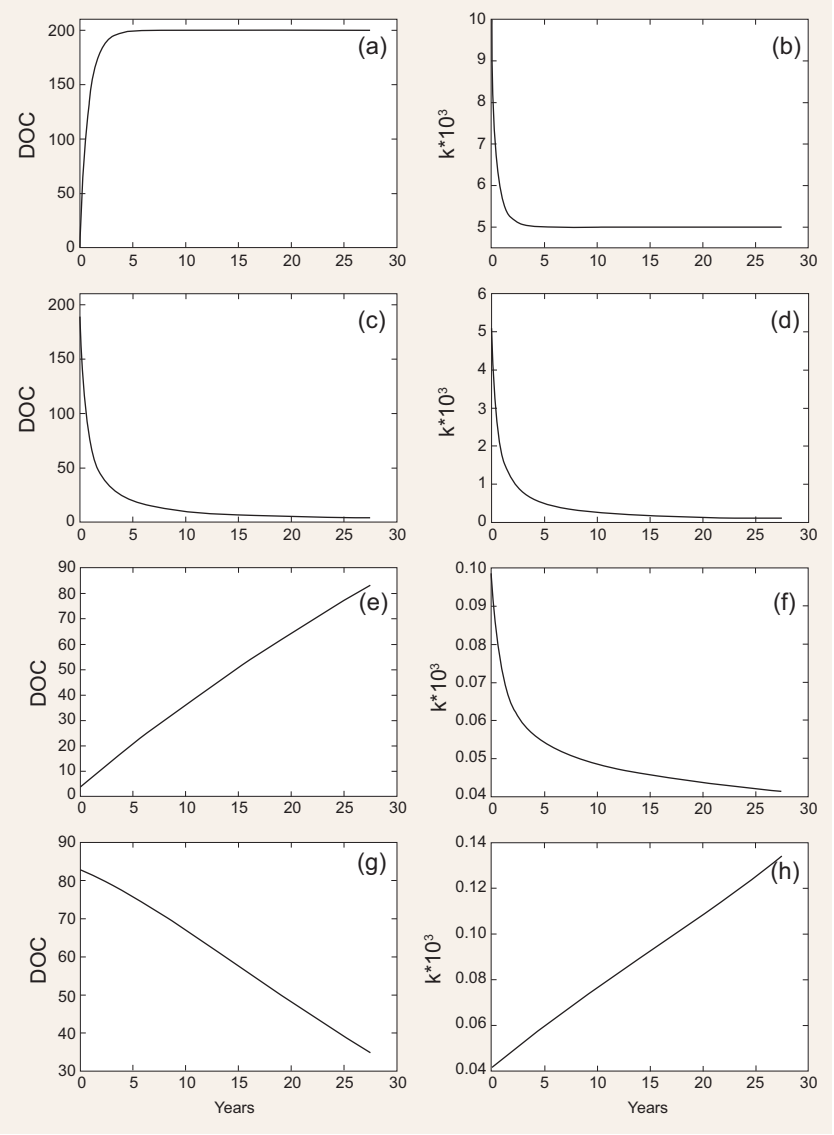

Figure 2. Model simulations. (a) and (b) Starting from low initial concentration (1 mg C m^{-3}) and a constant production rate of new DOC (1 mg C m^{-3} d^{-1}), the DOC concentration increases until reaching a steady state (i.e. consumption = production). Starting from a *k* value of K_{max}, the modelled value of *k* exponentially decreases as a result of DOC utilization by bacteria (Equation 2.2 in Table 1) until a steady state is reached. (c) and (d) If DOC production stops, the DOC pool decreases with a decrease in *k*. (e) and (f) If allochthonous DOC with a k_{in} that is similar to the local value of *k* is mixed with the DOC inside the box model, the (combined) DOC accumulates, while *k* continues to decrease due to bacterial DOC consumption (Equation 2.2 in Table 1). (g) and (h) When there is a slow production (0.001 mg C m^{-3} d^{-1}) of fresh DOC (i.e. with $k = K_{max}$) or fresh allochthonous DOC is transported inside the box (Fig. 1a) at the same rate (i.e. 0.001 mg C m^{-3} d^{-1}), *k* increases and DOC is consumed.

interactions also includes compounds derived from bacterial metabolism that are resistant to fast degradation [6]. Here, we thus assume that, every time DOC is assimilated/consumed, the remaining organic fraction becomes less biologically available (i.e. more degraded) and its degradation time scales increase with *k* approaching k_{min}. The decrease in *k* mimics the increased degradation state of DOC following bacteria utilization [6] and is dependent on the decrease in DOC concentration inside the box and on the difference between *k* and k_{min} (Fig. 1 and Table 1).

Ocean circulation and vertical turbulent mixing strongly affect DOC distributions. For example, DOC can be laterally transported or mixed within the water column [11]. Consequently, *k* is also affected by physical transportation of DOC. The DOC inflow into the box model implies a change in the local *k* (i.e. inside the box) value dependent on the degradability associated with the incoming DOC (k_{in}) and proportional to the magnitude of the DOC flux into the box (Fig. 1; Equation 2.3 in Table 1). If $k_{in} < k$, *k* will decrease; if $k_{in} > k$, *k* will increase. DOC outflow does not affect the value of *k* associated with the remaining DOC. It should be noted that our model does not explicitly represent the effect of environmental factors, such as temperature and nutrients, or grazer- and viral-mediate mortality on phytoplankton and bacterial processes. These effects, which potentially impact both DOC production and consumption [10], are routinely described in plankton models, and are therefore meant to be accounted for by the modelling framework in which the proposed formulation is implemented.

An example of how DOC and its associated degradation characteristics are dynamically modelled as a function of DOC production and consumption is given in Fig. 2. Under specific assumptions (see figure caption), the model can accumulate relatively labile DOC (i.e. k $\sim 10^{-3}$; Fig. 2a and b), generate a small amount of long-lasting DOC (k $\sim 10^{-4}$, Fig. 2c and d), accumulate DOC increasingly resistant to degradation (k $\sim 10^{-5}$, Fig. 2e and f) and degrade RDOC when fresh, labile DOC is produced or added to the system (Fig. 2g and h). This latter feature, mimicking the so-called 'priming effect' [12], is further explored in the simulations reported in Fig. 3. The rate of input of labile DOC (through production or transport) regulates both the rate of consumption of recalcitrant DOC initially present and its degradability. The consumption and degradability of recalcitrant DOC increase with the

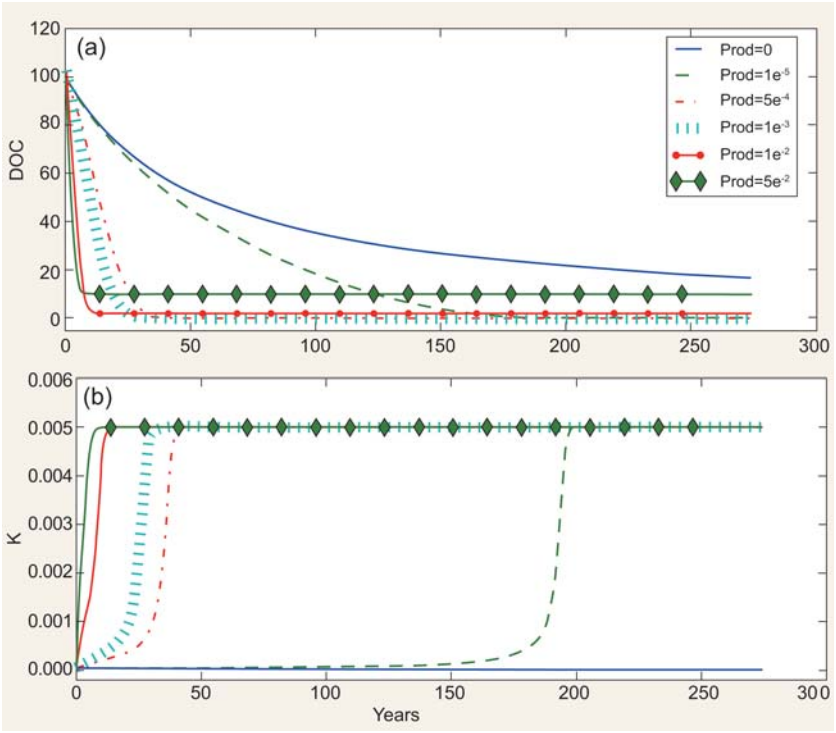

Figure 3. Effect of fresh DOC on recalcitrant DOC consumption. (a) Consumption of 'old' DOC (i.e. DOC with initial $k = 5 \cdot 10^{-5}$) at different production rates [prod (mg C m^{-3} d^{-1})] of 'new' DOC (i.e. DOC with K = k_{max}). (b) k dynamics at different production rates of 'new' DOC.

Table 2. Model parameters.

Parameter	Symbol	Value
Degradation rate of labile DOC	$L_k\ (d^{-1})$	1
Max degradation rate relative to L_k[a]	$k_{max}(adim)$	$1 \cdot 10^{-2}$
Min degradation rate relative to L_k[a]	$k_{min}(adim)$	$1 \cdot 10^{-7}$
k associated with the incoming DOC^	$k_{in}\ (adim)$	$1 \cdot 10^{-5}$
Model time step	Δt (sec)	900

[a]These parameters may assume slightly different meanings depending on the model used; see the main text for further explanations. k_{max} and k_{min} were estimated considering the orders of magnitude of the life times of semi-labile and refractory DOC, respectively [4]). DOC^: the value of this parameter refers to the example reported in Fig. 2e and f.

production of fresh DOC. More specifically, the model predicts that the time required for degrading half of the initial stock of DOC decreases from ∼50 to ∼5 years if the production of fresh DOC increases from $1 \cdot 10^{-5}$ to $5 \cdot 10^{-2}$mg $Cm^{-3}\,d^{-1}$. It needs to be stressed that this relationship and the patterns displayed in Fig. 2 are, at this stage of development, purely conceptual examples, as a quantitative validation against experimental data is still to be performed. Despite this, however, and although performed in a highly simplified theoretical frame, model simulations reproduce key aspects related to the MCP, such as (i) the coupling between DOC production and consumption observed in highly productive areas such as estuaries [13]; (ii) the decrease in DOC degradability when primary production is reduced or absent, as for example in the deep ocean [4]; and (iii) the increase in DOC degradability following the addition of freshly produced DOC [12].

TOWARDS MODELLING THE MCP

The general absence of RDOC and its dynamics in (most) marine ecosystem models may reflect the assumptions that the contribution of marine biota to global carbon sequestration is mainly through the biological carbon pump and that the majority of RDOC reacts at time scales (millennia) exceeding those investigated with current ecosystem and climate models. However, since the MCP is a ubiquitous process in the ocean, even small alterations in its functioning due to climate change could impact on global biogeochemical cycles on much smaller timescales [2,3]. For example, the projected increase in sea-water temperature, thermal stratification, mid-latitude oligotrophication, ocean acidification and increase in riverine discharge of both dissolved organic matter and nutrients are all factors expected to change the MCP-mediated RDOC production [3]. However, the amplitude and the direction (positive or negative) of the feedback are highly uncertain at this stage of understanding. For this reason, we are proposing a simple model that can be used to investigate these potentially important processes with a hypothesis-testing approach. The formulation we propose (Table 1) is computationally 'light' and can be applied to represent slowly degradable DOC in models with different complexity, including large-scale models that do not explicitly include bacteria. The next step in the development of our model will be to implement the formulation into a simple 3D ocean biogeochemistry model to assess whether the simulated variability of k is consistent, at global scales, with known properties of the DOC pool (e.g. k should be smaller in the deep layers where RDOC is dominant [4]). Furthermore, by comparing DOC simulation with existing large datasets [10], it will be possible to evaluate whether the

proposed k_{min} and k_{max} values (Table 2) provide the best fit with observed DOC.

Concomitantly with large-scale simulations, process-oriented experiments should be executed to evaluate whether the bacterially mediated transformation of the DOC pool simulated by the model (through the variability of k, Fig. 2) is quantitatively realistic. Mechanisms regulating DOC production from primary production are well investigated and constrained, and a set of established models is present in the literature [10]. As a consequence, DOC production ($\frac{\partial DOC}{\partial t}|^{Prod}$, in model equation, Table 1) can be represented in different ways, from simple empirical relationships [11] to more mechanistic, physiologically-based formulations [7]. In contrast to the relatively well-known processes leading to the production of DOC by the marine food web, the bacterially mediated biochemical transformation of DOC and the controlling factors that lead to the formation of RDOC are still largely unknown. For example, although some studies suggest that RDOC formation through the MCP can be enhanced by low inorganic nutrient concentrations [3], quantitative relationships between inorganic nutrient availability to bacteria and the production of RDOC still need to be established. This limited observation makes the modelled relationship between DOC consumption ($\frac{\partial DOC}{\partial t}|^{Cons}$ in Table 1) and DOC degradability (represented by k) highly uncertain and thus a challenge to incorporate into models.

The understanding of the mechanisms underpinning RDOC formation and accumulation has so far been limited by the difficulty in characterizing and quantitatively measuring RDOC (i.e. on a chemical structure basis). Although we are still far from a complete chemical characterization of RDOC, in recent years, state-of-the-art mass spectrometry techniques have allowd the identification of specific combinations of elements (in terms of C:H and C:O ratios) and molecular masses that characterize RDOC [15]. Such a 'chemical fingerprint' allows RDOC to be recognized in bacterial cultures and is observed to be produced ubiquitously by bacteria in remarkably short time frames (e.g. months [16]). Controlled, *ad hoc* performed experiments exploiting these techniques and specifically addressing microbial RDOC production starting from labile substrates (under different environmental conditions, e.g. temperature and nutrient concentrations) are required to iteratively calibrate, validate and refine our model. In addition to traditional, laboratory-based experiments, in the near future, model development will also benefit from newly designed studies performed with large-volume facilities [17] that may strategically combine the advantage of a controlled system with the realism of the dynamics observed within them. Only after a rigorous, experimentally based validation can our model be used for reliable (quantitative) prediction of MCP dynamics. Although the model is at an early stage of development, we propose that it is a means to include RDOC dynamics into climate model simulations. Such simulations will represent a powerful hypothesis-testing tool to complement experimental and field studies in the investigation of the role played by the MCP in ocean carbon sequestration in past, present and future oceans.

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GEOSCIENCES

Special Topic: Marine Carbon Sequestration and Climate Change

An implementation strategy to quantify the marine microbial carbon pump and its sensitivity to global change

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INTRODUCTION

The persistence of the vast pool of recalcitrant dissolved organic carbon (RDOC) in the deep ocean is fundamental to both global carbon cycling and global climate. Yet, quantitative understanding of the mechanisms that produce and utilize RDOC is still in its infancy. Moving from a conceptual framework to quantitative understanding in marine carbon cycling can take the international research community several decades—precious time that is not available in this era of anthropogenic climate change. This perspective paper sets out an implementation strategy to move efficiently from conceptual hypotheses of marine carbon storage to quantification of the capacity for marine carbon storage through the microbial carbon pump (MCP), its sensitivity to global change and potential for modification. The aim is to excite and facilitate the international scientific community to hasten this process via an internationally co-ordinated multidisciplinary research initiative.

The marine microbial carbon pump (MCP) [1] produces a concentration gradient of reactivity in dissolved organic carbon (DOC) from low concentrations of reactive fractions of DOC that have an average lifetime of less than 100 years ($DOC_{<100}$) to high concentrations of recalcitrant DOC (RDOC) fractions that persist for longer than 100 years ($DOC_{>100}$) [2]. This gradient is maintained against the continuous degradation of $DOC_{>100}$ by photochemical transformation in surface waters, elevated temperatures at deep hydrothermal vents and gradual microbial degradation throughout the water column [1–3]. The resultant pool of RDOC amounts to the sequestration of carbon in the ocean equivalent to the amount of carbon dioxide in the atmosphere. The magnitude of the MCP is determined as the rate of production of $DOC_{>100}$ in mol C m^{-2} y^{-1} [2]. Global warming, weakening thermohaline circulation, increasing UV radiation, ocean acidification, increasing oxygen minimum zones and increasing nutrient concentrations are all processes potentially affecting the magnitude of the MCP, leading to increased or decreased transformation of marine RDOC to atmospheric CO_2 with associated consequences for global climate [3,4].

In order to quantify the MCP, and understand its sensitivity to large-scale environmental change, the rates of production and loss of RDOC via each potential formation and loss pathway need to be determined and understood. This is a major challenge, likely beyond the ability of any individual team of researchers, given the complexity and diversity of the substances, processes and microbial organisms involved and the variety of controlling factors. However, the connectivity and biogeographical characterization of the ocean—a consequence of ocean circulation and mixing—allow a co-ordinated approach where results from multiple groups, working at different locations and times, could be compared and assembled in order to achieve progress.

Such a co-ordinated approach will require a multi-component programme of systematic experimentation linked with related conceptual and numerical modelling. The suggested programme components include: (i) determination of the chemical components of RDOC, including 'fingerprint' constituents to identify the presence of RDOC; (ii) a standardized and intercomparable programme of long-term microcosm incubation experiments conducted worldwide to determine loss of labile DOC, and net accumulation or loss of bulk RDOC, under specific environmental conditions; (iii) the concurrent determination and quantification of RDOC production and loss pathways; and (iv) quantification of the presence and activity of the responsible microbial organisms and their genetic potential. In order to test our understanding as well as the efficacy of our experimental protocols, a linked 'backbone' programme of larger-scale, controlled manipulation experiments and related, long-term time-series studies should be established (v). This should involve controllable meso- and macro-cosm facilities as well as in-situ time-series studies where the determination of rate processes, RDOC composition change (and associated proxies), microbial community composition and genetic potential can be examined repeatedly, under varying conditions. The programme needs to be structured within (vi) a framework of conceptual and numerical models and, crucially, in order to assess the global context of RDOC production and loss, (vii) large spatial-scale and long temporal-scale surveys of, at minimum, the fingerprints or proxies of RDOC accumulation will be required. The surveys will allow experimental findings to be parameterized in global biogeochemical models to explore the sensitivity of the MCP and associated carbon storage to environmental change over long timescales.

For each of these programme components, we describe here the principle of the approach, current limitations and future research requirements. This perspective paper will therefore provide a route by which to progress from the conceptual framework of the MCP, to the quantification of the microbial processes that produce and utilize RDOC, and the environmental, trophic and physiological influences on them.

Identification of the components of RDOC

The very nature of RDOC, in terms of its complexity, diversity, low concentration and longevity, means that identifying representative components of RDOC is a major analytical challenge.

A variety of methods are now available to extract low-molecular-weight, ^{14}C-depleted RDOC and nuclear magnetic resonance (NMR) spectrometry, gas chromatography–mass spectrometry (GC–MS) and Fourier transform ion cyclotron resonance mass spectrometry (FT–ICR–MS) can identify specific chemical fingerprints of this material, including carboxyl-rich alicyclic molecules (CRAM), material derived from linear terpenoids and a group of stable molecular formulae known as the 'island of stability'.

However, the comprehensive analysis of DOC and quantification of RDOC are still far from complete. Current techniques are limited in that existing isolation methods including solid phase extraction (SPE) can only isolate representative fractions of DOC, some of which are further discriminated during mass spectrometry analysis. In addition, direct injection of the DOC sample into the mass spectrometer cannot differentiate the numerous possibilities of isomer configuration for an assigned molecular formula, which therefore precludes the identification of an unambiguous chemical structure [5].

Future approaches should include the use of combined methods for DOC isolation and the development of new techniques to isolate the DOC (Fig. 1) [6]. Chromatographic analysis prior to ultra-high-resolution mass spectrometry could help to differentiate isomers, and subsequent analyses by a combination of FT–ICR–MS and high-field NMR would allow progress towards the ultimate goal of revealing the structures and concentration of representative RDOC molecules.

Incubation experiments to determine loss of labile DOC and accumulation of RDOC

Operationally, the reactivity of DOC is determined from the loss of DOC over a given period of time [7], with a biologically labile fraction (LDOC) defined as that which is degraded over hours to days, a semi-labile fraction (SLDOC) that is removed in weeks to months and a semi-refractory fraction (SRDOC) with turnover times of years to decades. Studies of the degradation of LDOC involve the incubation of seawater samples under controlled laboratory conditions for days to years, allowing the calculation of the DOC decay constant k_C over time [8].

However, there is no standard method by which to undertake these experiments and, since both the concentration and decay rate of DOC are dependent on time and incubation conditions such as temperature and nutrients, collation and comparison of the available data are problematic. Methodological differences include the method by which a diluted seawater sample is obtained, such as the composition and pore size of the filters used, the ratio of filtrate to inoculum, the incubation temperature, light and nutrient conditions, and the volume and time period of the incubation.

A comparison of methods and recommendations for standardization are required to advance this aspect of DOC reactivity research, following the approach taken recently by the research community investigating the impact of ocean acidification on marine plankton activity [9]. Once standardized methods are defined, systematic laboratory-scale bioassay experiments can be undertaken to assess the influence of environmental conditions such as temperature, light and nutrients on DOC reactivity, and incorporation of measurements of RDOC components or proxies of RDOC will allow the accumulation of RDOC to be determined alongside the loss of DOC.

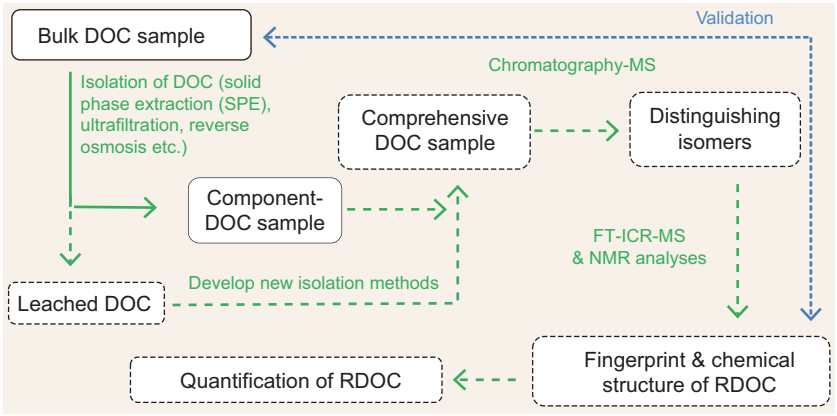

Figure 1. Schematic of an experimental procedure to chemically characterize RDOC. The solid lines and boxes indicate well-established analyses, while the dashed lines and boxes indicate those that are still to be explored.

Identification and quantification of RDOC production and loss pathways

The ocean reservoir of RDOC is determined by the net effect of its production and removal processes. Therefore, it is essential to investigate the mechanisms of both production and loss pathways in order to understand the influence of global climate change on ocean carbon sequestration.

Potential RDOC production pathways include phytoplankton excretion and autolysis, viral lysis, bacterial and archaeal transformation, and protistan and zooplankton grazing and excretion. A systematic approach is needed to determine the rate of production of 'model' RDOC compounds from each of these pathways. For example, a range of RDOC biomarker compounds that are only produced by heterotrophic bacteria, including muramic acid and D-enantiomers of amino acids, have been identified. Quantitative measurements of these bacterial biomarkers in seawater-dissolved organic matter indicate that about 25% of the carbon and 50% of the nitrogen is of bacterial origin [10]. Based on biomarker data and bioassay experiments, the rate of production of bacterial RDOC in the upper 200 m of the ocean is estimated to be 8–23 Tg C year^{-1} [11].

The persistence of RDOC in the ocean is well known, but the reasons for this persistence are still being explored [4,12]. Three broad areas of research are being investigated: (i) intrinsic properties of molecules, such as chemical composition and structure; (ii) environmental conditions, such as microbial community structure and exposure to solar radiation; and (iii) concentrations of molecules that are below a critical threshold for microbial utilization. Multiple and independent approaches are needed to delve deeper into the mysteries of RDOC removal from the ocean. For example, recent bioassay experiments have provided evidence that some RDOC is too diluted for microbial utilization [13] and that intrinsic properties of RDOC and environmental conditions limit its microbial utilization [3]. A modelling approach addressing these same questions provided independent evidence indicating the dilution of labile substrates likely accounts for a relatively small fraction of the RDOC in the deep ocean [14]. It appears that most RDOC persists in the deep ocean due to intrinsic properties and environmental conditions. Ocean mixing moves RDOC into different environmental conditions, and exposure to solar radiation and different microbial communities plays a critical role in its removal [3].

Stable isotope probing (SIP) [15] can be used to ascertain which organisms are responsible for DOC degradation. SIP tracks the incorporation of isotopically enriched substrates into the DNA, RNA or phospholipid fatty acids of these organisms. Combined with high-throughput sequencing, DNA/RNA SIP can identify which organisms are responsible for any observed biodegradation pathway with high phylogenetic resolution. Thus, incubation experiments with additions of ^{13}C-labelled labile DOC and subsequent ^{13}C- and ^{12}C-DNA analysis with terminal-restriction fragment length polymorphism (T-RFLP) and pyrosequencing can identify the microbial populations that incorporate the ^{13}C-labelled substrate. The molecular characteristics of the produced ^{13}C-labelled DOC (relatively more refractory) can be further determined using FT–ICR–MS, NMR or excitation emission matrix (EEM) fluorescence. Unfortunately, SIP techniques are not inherently quantitative. Substrate assimilation rates can be derived from changes in δ^{13}POC (particulate organic carbon) during concurrent incubations to help link the DOC-specific degradation rates to the activity of the microbial population.

Future work should use laboratory-controlled phytoplankton, zooplankton and bacterial cultures to produce DOC from each potential RDOC formation pathway to identify potential chemical biomarkers for each. The recent connection between RDOC fractions and precursors such as phytoplankton carotenoids lays the foundation for hypothesis testing of these potential transformation pathways. Long-term degradation incubations incorporating DNA/RNA SIP could then be conducted to assess the bioreactivity of DOC from each of these formation pathways.

Microbial community composition and genetic potential

The microbial transformation of short-lived particulate and dissolved organic carbon ($POC_{<100}$ and $DOC_{<100}$) to $DOC_{>100}$ is ultimately driven by the activity of microbial functional genes [16]. For example, genes for the ATP-binding cassette (ABC) transporter, including importers and exporters, are thought to be indicators of the capabilities of microbes to use or generate corresponding DOC compounds [16]. Therefore, a strategy to quantify the various processes

of the MCP could depend on the quantification of microbial functional genes.

The quantification methods (such as PCR, qPCR, omics) usually used in ecological studies are time-consuming and potentially incomplete when considering the extremely high diversity of microbial functional genes involved in MCP processes. Microarrays that contain numerous probes targeting hundreds or thousands of genes are a promising technique in this regard. The development of a functional gene microarray involves gene selection and sequence retrieval from a database, probe design and evaluation, and microarray fabrication.

GeoChip is one of the most comprehensive functional gene arrays for quantification of the functional diversity, metabolic potential/activity and dynamics of microbial communities [17]. GeoChip 4 contains ~82 000 probes covering >140 000 coding sequences from 410 functional gene families, many of which are encoding biogeochemically important functions, such as carbon fixation and carbon degradation. In addition, GeoChip 4 has the ability to analyse targeted functional gene families of microorganisms in all four domains (Bacteria, Archaea, eukaryotes and viruses). A recent case study performed in the East China Sea suggested a possible connection between GeoChip data and MCP processes such as metabolic preferences of microbes along an environmental gradient and the degradation of recalcitrant organic matter (e.g. aromatic carboxylic acid and chlorinated aromatics) at depth [18].

However, a major technical limitation of GeoChip and other functional gene microarrays is the inability to interrogate novel sequences and, therefore, find novel biogeochemical pathways. Combination with high-throughput metagenomic analysis may be an option. In the last decade, with the rapid progress in sequencing techniques, microbiologists have accumulated an unprecedented amount of genetic data (e.g. Tara Oceans Expedition (https://oceans.taraexpeditions.org/en/m/about-tara/), Global Ocean Sampling Expedition (GOS; http://www.jcvi.org/cms/research/projects/gos/overview/) and provided fundamental new understanding of biogeochemical cycles. This required the inclusion of probes for newly recognized functional genes and rapid updates of the microarray.

Assigning an exact function to a particular gene is a slow process, and significantly impedes the linkage between genetic sequence data and DOC transformation pathways [19]. Nevertheless, progress is being made. Recent studies have directly linked geochemical dynamics to the genetic composition of microbial communities [20] and the Tara Ocean microbial genomic data were successfully connected to carbon export in the oligotrophic ocean, which could even be predicted from a few bacterial and viral genes [21]. This progress bodes well for the future quantification of the MCP using microbial functional genes (Fig. 2).

Large-scale manipulation experiments

The global ocean is experiencing a slowdown in overturning circulation, increasing temperature, decreasing pH, increasing UV radiation, increasing dissolved CO_2, decreasing dissolved O_2 and changing inorganic and organic nutrient availability [22], with as-yet unknown consequences for ocean carbon storage. A combination of laboratory microcosm- (10^{-3} to 1 m^3), mesocosm- (1 to 10^3 m^3) and macrocosm- (>10^3 m^3) scale manipulation experiments are required to test hypotheses related to the individual and combined effects of these multiple drivers on the MCP [23]. Mesocosms deployed at sea and macrocosms on land have been used successfully to investigate the effects of ocean acidification and nutrient additions (via, for example, atmospheric dust deposition) on marine plankton activity [24]. The range of scale is required due to the trade-off between numerous replication and full factorial matrices available at the microcosm scale and improved representation of the complexity and depth distribution of the microbial community, including the potential formation of a pycnocline, at the macrocosm scale [23,25].

Future studies of the MCP should use at-sea mesocosms and on-land macrocosms to bridge the gap between data derived from microcosm bioassay experiments and in-situ measurements. A project to build a facility with 9 × 50 m tall macrocosms to allow experiments to be performed with three replicate controls and three replicates of each of two treatments is currently underway in Qingdao, China [25]. This advanced experimental system will allow the concurrent determination of microbial diversity, the chemical composition of DOC and the rate processes affecting the production and loss of RDOC over relevant space scales of euphotic zone depths and time scales of years, under two environmental treatments in a previously unobtainable statistically robust manner [23]. In addition to advances in process understanding, experiments conducted in meso- and macrocosms can be used as a basis for systematic comparison of methods used elsewhere (components (i) through (iv) above). The design of experiments should be linked directly, when possible, with hypotheses and process-related questions that emerge from field studies, including time-series

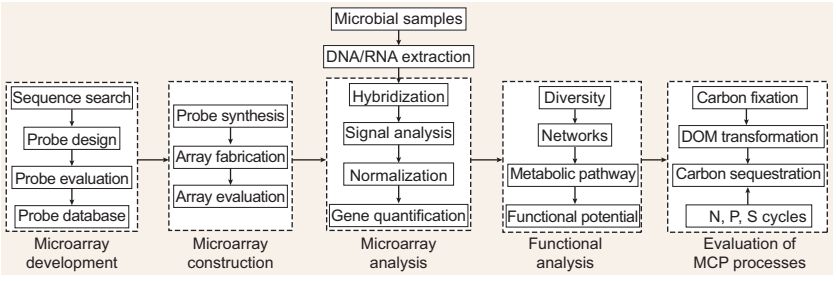

Figure 2. An experimental microarray procedure to link gene analysis with the quantification of MCP processes.

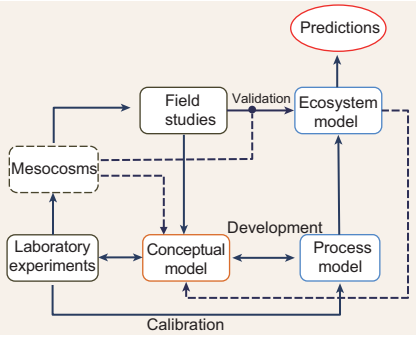

Figure 3. Integration of laboratory, field and numerical studies.

studies of naturally variable systems (component (vii) above).

Modelling framework

The dynamics underpinning the MCP are rarely considered in marine ecosystem models and this limits our capacity to predict carbon sequestration in future oceans [26]. While exploratory modelling studies on the MCP have been published recently [26,27], we need to embed these studies in a coherent framework if we want to have reliable MCP simulations. We propose that, in future projects, each aspect of the model development (formulation, calibration, validation) is carried out in co-ordination with empirical studies, in order to exploit the multiple feedbacks between data and simulations to iteratively refine the model and achieve reliable MCP predictions (Fig. 3). Laboratory experiments (i.e. microcosm and mesocosm batch cultures and chemostats) using the techniques described earlier are able to provide a detailed description of RDOC production and microbial consumption in a controlled environment. The understanding generated by these experiments allows the formalization of a conceptual model (i.e. a diagram describing the process being investigated). The conceptual model can be seen as the central hypothesis driving subsequent modelling steps. The conceptual model then needs to be 'translated' into a mathematical form (process model) and calibrated to reproduce the dynamics observed in the experiments. The development of the process model might highlight knowledge gaps and inform new, more informative, experimental designs. When the process model successfully reproduces the dynamics observed in the experiments, it is ready to be implemented in a full ecosystem model. Field studies (including incubation experiments) provide measurements (standing stock concentrations and rates) to scale the conceptual model up to the ecosystem level and to validate the ecosystem model. Eventually, after the ecosystem model is satisfactorily validated, it can be used for climate predictions. During the validation of the ecosystem model, it is possible to highlight knowledge gaps and inconsistencies which imply that the conceptual model should be revised and new experiments planned. Mesocosms and macrocosms represent a valuable compromise between the laboratory and the real environment, providing additional information to refine the conceptual model, inform the development of the process model and validate the ecosystem model.

Global and decadal scale perspective

In order to predict future changes in the capacity and efficiency of the MCP, global spatial-scale and decadal temporal-scale surveys of robust, high-throughput proxies of RDOC accumulation will be required alongside chemical fingerprints of RDOC transformation pathways, such as D-enantiomers of amino acids, and the more analytically challenging comprehensive chemical characterization of DOC. High-throughput proxies might include the fluorescence intensity of humic-like chromophoric dissolved organic material (CDOM), the production of which correlates with apparent oxygen utilization (i.e. microbial degradation of organic material) in the deep ocean.

Global and decadal monitoring of the MCP would require the development of sensors for components of the DOM pool (e.g. CDOM, fDOM, DOC, RDOC fingerprints) appropriate for deployment on a range of platforms including moorings, gliders, volunteer observing ships (such as ferries and container ships) and biogeochemical ARGO floats (http://www.argo.ucsd.edu/), as well as remote sensing algorithms for surface distributions. An international co-ordinated programme would be required, including standardized methods, inter-calibration exercises, and scientific training and capacity building. This should be embedded within established time-series stations such as the Hawaii Ocean Time-series (HOT; http://hahana.soest.hawaii.edu/hot/) and those within the OceanSITES (http://www.oceansites.org/index.html) worldwide network, as well as global shipboard surveys such as those co-ordinated within GO-SHIP (http://www.go-ship.org/index.html) and GEOTRACES (http://www.geotraces.org/) to ensure that appropriate biogeochemical, biodiversity and omics data are collected concurrently. The Global Ocean Observing System (GOOS; http://www.goosocean.org/) Biogeochemistry Panel has recognized the need for sustained ocean observations of DOC, designating it as an essential ocean variable (EOV) required for addressing societal drivers such as the role of biogeochemistry in climate and human impacts on ocean biogeochemistry. The future aspiration should be to designate RDOC or a proxy of DOC lability as an EOV.

Progress in MCP quantification will also rely on continued advances in quantification of the microbial processes related to DOC and RDOC production and transformation, including heterotrophic bacterial respiration, viral lysis and chemoautotrophy. Improvements in bioinformatics and data manipulation and visualization tools will enhance the opportunities for connections to be made between the molecular complexity of RDOC and meta-genomics, -proteomics and -transcriptomics [20].

Given the complexity and diversity of measurements involved (i–iii) and the value of repeated measurement and experimentation for model validation (vii), a logical starting point for a systematic MCP field study would be the addition of MCP appropriate measurements at the locations of already established moored- and ship-based multidisciplinary ocean time series that examine

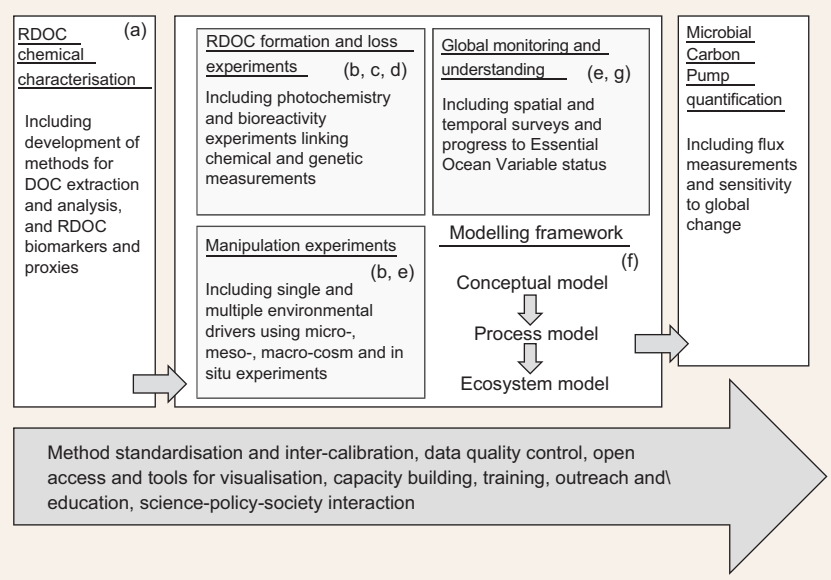

Figure 4. Schematic of a co-ordinated international research initiative linking each of these programme components (i–vii) with data-management, capacity-building and science–policy–society activities.

a diverse range of ocean ecosystems and biogeochemical conditions. Overall, MCP quantification would be most efficiently achieved through a co-ordinated international research initiative linking each of these programme components (i–vii) and including appropriate policies for method standardization and inter-calibration, data-management, capacity-building, outreach and education, and science–policy–society activities to address the societal driver of understanding the role of marine biogeochemistry in climate (Fig. 4).

CONCLUSION

Quantitative understanding of the mechanisms that produce and utilize RDOC is still in its infancy, despite their fundamental role in global climate. Significant progress towards quantification of the MCP requires an internationally co-ordinated multidisciplinary research initiative that interlinks in-situ time-series studies and micro- and macro-scale manipulation experiments within a robust conceptual to numerical modelling framework. The analytical tools and intellectual interaction between chemists and microbiologists are now at a stage where this societally driven question can, and must, become a high priority research topic.

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GEOSCIENCES

Special Topic: Marine Carbon Sequestration and Climate Change

Evolving paradigms in biological carbon cycling in the ocean

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ABSTRACT

Carbon is a keystone element in global biogeochemical cycles. It plays a fundamental role in biotic and abiotic processes in the ocean, which intertwine to mediate the chemistry and redox status of carbon in the ocean and the atmosphere. The interactions between abiotic and biogenic carbon (e.g. CO_2, $CaCO_3$, organic matter) in the ocean are complex, and there is a half-century-old enigma about the existence of a huge reservoir of recalcitrant dissolved organic carbon (RDOC) that equates to the magnitude of the pool of atmospheric CO_2. The concepts of the biological carbon pump (BCP) and the microbial loop (ML) shaped our understanding of the marine carbon cycle. The more recent concept of the microbial carbon pump (MCP), which is closely connected to those of the BCP and the ML, explicitly considers the significance of the ocean's RDOC reservoir and provides a mechanistic framework for the exploration of its formation and persistence. Understanding of the MCP has benefited from advanced 'omics' and novel research in biological oceanography and microbial biogeochemistry. The need to predict the ocean's response to climate change makes an integrative understanding of the BCP, ML and MCP a high priority. In this review, we summarize and discuss progress since the proposal of the MCP in 2010 and formulate research questions for the future.

Keywords: biological carbon pump, microbial loop, microbial carbon pump, ocean carbon cycle, global climate change

INTRODUCTION

The modern ocean accounts for ~50% of global photosynthesis, with its primary production of organic matter forming the core of the ocean carbon cycle. Thus the ocean has a major influence on the chemistry and redox status of the atmosphere through the net uptake of atmospheric CO_2 and net release of molecular oxygen. An early estimate showed that about 25% of the ocean's primary production was transported to the interior of the ocean (below the euphotic zone) via the biological carbon pump (BCP) [1]; later on, this number was changed to 10–15% for gravitational sinking with another 5% each for passive transport by water motion and active transport by vertical migrators [2]. Carbon transported to the deep ocean (>1000 m) is sequestered on timescales of >100 years up to 1000 years (i.e. the residence time of deep waters). About 0.3% of the ocean's primary production is buried in marine sediments [3,4], some of which eventually forms a major reservoir of organic matter that persists for hundreds of millions of years in rock formations (Fig. 1).

Since the industrial revolution, the ocean is estimated to have taken up approximately 25% of the anthropogenic CO_2 [5], resulting in ocean acidification with consequences for biogeochemical and climatological processes and the ocean carbon cycle [6,7,1,8]. Global warming and ocean acidification and their respective consequences influence the functioning of the BCP, a major pathway for

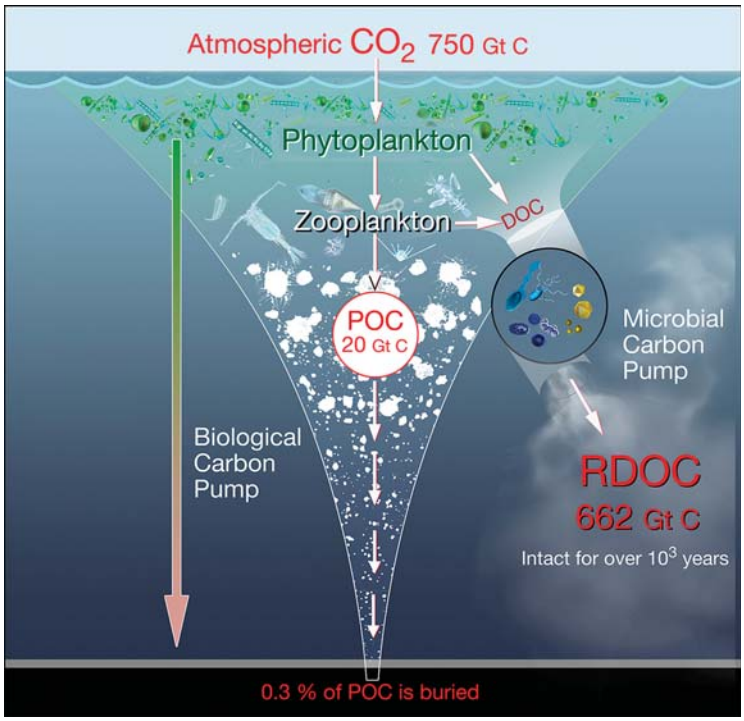

Figure 1. Cycling of biologically produced organic carbon (POC and DOC) in the ocean and links between the seafloor and the atmosphere: the BCP, which transports organic matter from the surface to the interior and floor of the ocean; the MCP, which converts parts of labile organic carbon into RDOC via microbial activities, mainly by heterotrophic archaea and bacteria, and associated viruses.

sequestering atmospheric CO_2 in the ocean. The microbial carbon pump (MCP) [9] provides an additional path for carbon sequestration within the marine ocean carbon cycle [10], which is intimately linked to climate change.

The BCP is the mechanism by which carbon-containing compounds are exported via biological processes from the surface to the deep ocean [11], whereas the MCP addresses the dissolved organic carbon (DOC) pool, specifically the recalcitrant (R) DOC (Fig. 1), which constitutes the majority of DOC and persists in the ocean for up to 4000–6000 years [12,13]. Hansell [13] defines recalcitrant dissolved organic carbon (RDOC) as 'DOC that is resistant to rapid microbial degradation and so has accumulated and is observable in the ocean'. Concentrations of DOC in the open ocean range from 360–960 μg/kg (or 30–80 μmol/kg) [14] with significant seasonal variation often seen in surface waters [15]. Accounting for a global ocean inventory of 662 Gt C, the huge DOC pool is almost equal to the carbon dioxide pool (750 Gt C) in the atmosphere. Therefore, the biogeochemical behavior of the DOC pool has important implications for the ocean carbon cycle and climate.

The MCP mediates the transformation of labile carbon to RDOC, which builds on elements of the previously recognized processes involved in ocean carbon cycling and storage [16], namely the BCP, microbial loop (ML) and viral shunt (VS). The functioning of the MCP also impacts nutrient stoichiometry when preferentially remineralizing N and P from dissolved organic matter (DOM). This DOM is produced via the VS [17,18] and other processes such as phytoplankton excretion and zooplankton sloppy feeding [19–24]. This recycling of nutrients enhances local primary production while enriching the remaining DOM in carbon, thus lowering its nutritional value.

The detailed processes of the MCP are currently not well understood. This is largely due to microbial complexity and the vast unresolved chemical structures of DOM compounds. Growing efforts have been devoted to using microbiological and geochemical tools to bridge the gap between microbial omics and organic carbon composition [25–27]. In this review, we discuss important aspects of the BCP, the ML and the MCP, and summarize progress that has been made concerning the MCP since Jiao *et al.* [9].

EVOLUTION OF OUR UNDERSTANDING OF THE MICROBIAL ROLE IN DOC GENERATION AND DEGRADATION

Understanding of the ocean's carbon cycle in the late twentieth century was largely promoted by the BCP (called 'soft tissue pump' in [28]) and the ML [29]. The term 'pump' was initially used to refer to the movement of carbon against a concentration gradient between the surface ocean and the deep ocean [28]. Both concepts find their roots in Dugdale and Goering [30], who recognized new (BCP) and regenerated (ML) production in the ocean.

The BCP begins in the euphotic zone where photoautotrophic organisms fix dissolved CO_2 to produce particulate organic carbon (POC) (Fig. 2). Particulate organic matter (POM) consists of both living and non-living components, and most of it is respired to CO_2 by metabolic processes in the epipelagic ecosystem. The subsequent export of a small fraction of the POM is carried out by gravitational flux, vertical migrations of zooplankton and physical subduction of water masses, which remove the organic matter to deeper regions where it accumulates or is respired. The respiratory CO_2 at depth is removed from contact with the atmosphere for a period corresponding to the residence time of deep waters, namely tens to hundreds of years below 100 m and thousands of years below 1000 m (Fig. 2). In addition, organic matter in particulate or dissolved form reaching the latter depth via the BCP

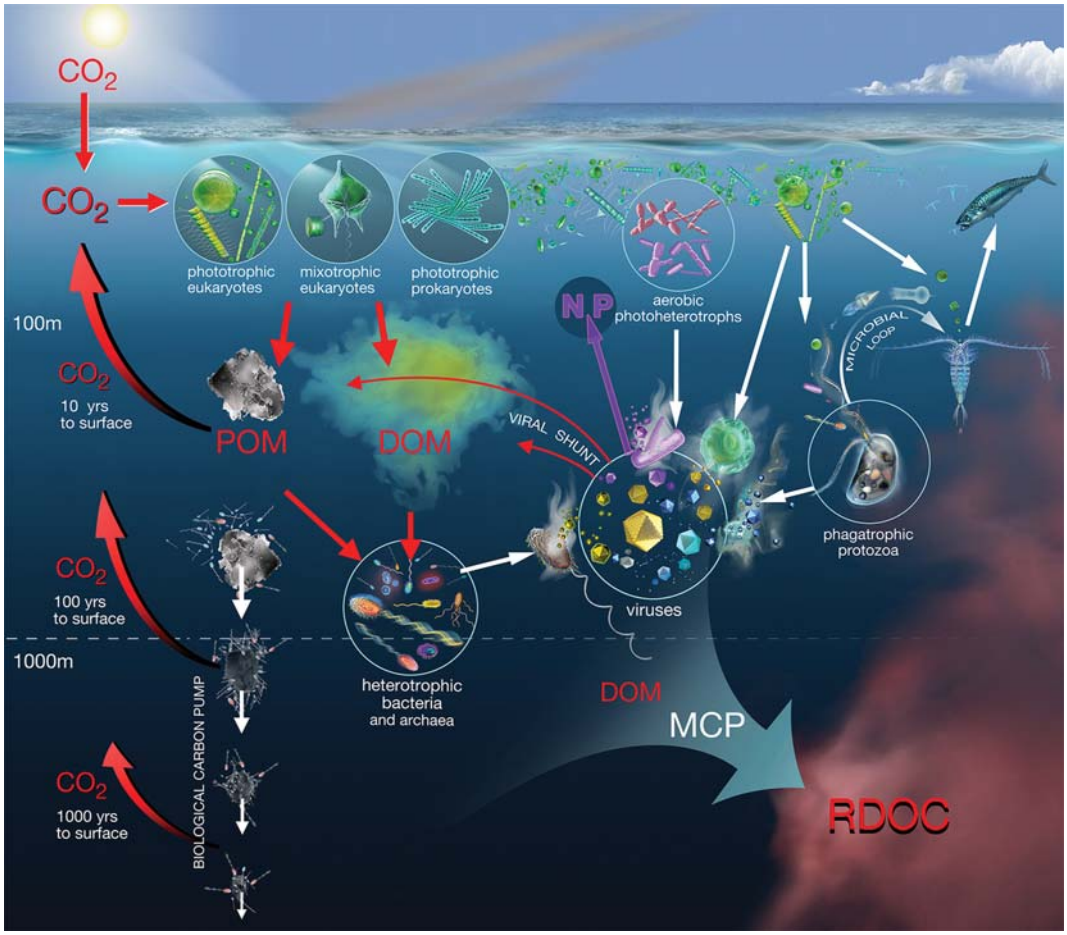

Figure 2. Schematic depiction of the BCP, the ML and the MCP. The remineralization length scale in the left part of the figure shows the return of respired CO_2 back to the surface, from three depth zones (modified from [31]).

should be considered as sequestered at the time scale of climate change.

Increasing atmospheric CO_2 concentration raises several questions: '*(1) Will the ocean continue to take up carbon? (2) At what rate? (3) For how long will the exported carbon remain removed from the atmosphere?* These questions address the functioning and efficiency of the future BCP. Global warming and past carbon sequestration (ocean acidification) will also change the BCP leading to the next question: *(4) How will the biological pump respond to the consequences of increased carbon input combined with warming?*' [31]. One scenario suggests that, in the coming decades, decreasing phytoplankton cell size will decrease the downward POC flux from the surface ocean, while changes in zooplankton community structure will decrease the downward POC flux in subsurface waters [32]. However, other predictions suggest alternative outcomes and the answers to these questions are still discussed controversially in the scientific community. A recent report on a transformative understanding of the ocean's BCP to the US National Science Foundation [33] recommended three major research directions addressing '*(i) food web regulation of export, (ii) the dissolved-particulate continuum, and (iii) variability of organic transport in space and time*'. Several large programs, such as the ongoing US-EXPORTS [2] and the UK COMICS [34] programs as well as many other efforts are currently focusing on the BCP.

Though many forms of vertical export can be related to BCP, it mainly focuses on particles that move downward through physical and biological forces (i.e. by gravity and transport by vertically migrating zooplankton). The ML, on the other hand, intimately links intricate interactions between microorganisms and their physical and chemical surroundings [29,35]. The ML focuses on carbon cycling in the water column where bacteria (actually referring to both bacteria and archaea), protozoa and viruses determine the fate of DOM [35]. It was estimated that bacteria could channel up to 50% of marine primary production into the ML, highlighting their importance in the ocean's carbon cycle [35,36]. Similarly, Legendre and Rivkin [37] found

Table 1. Definitions and major impacts of the BCP, ML and MCP.

Concept	Definition	Major impacts and focus	Reference
Biological pump	A complex ecosystem process that transports particulate organic carbon from the epipelagic zone to the deep interior of the ocean and further to the ocean floor	Sequestration of atmospheric CO_2 through vertical transportation of living biomass to marine sediments; focusing on sediment storage	[28,42]
Microbial loop	A 'feedback' pathway of loss of the primary production to the environment in the form of dissolved organic matter and the utilization of the latter by bacteria that feed the protozoa, which enter the food chain	The role of bacteria in sequestering nutrients from the environment, which are consumed by protozoa; focusing on organismal populations above thermocline	[29,35]
Microbial carbon pump	A conceptual framework for understanding the role of microbial processes in the production of recalcitrant dissolved organic matter in the ocean water column	Sequestration of atmospheric CO_2 through transformation of labile organic matter to recalcitrant organic matter; focusing on capacity of the ocean to store atmospheric CO_2	[9,50]

that heterotrophic microbes always dominate respiration in the euphotic zone, even when most particulate primary production is grazed by metazoans. The ML intertwines with the grazing food web and provides a mechanism to retain nutrients such as N and P in the highly stratified upper oligotrophic oceans by recycling them through pico-phytoplankton, bacteria and microzooplankton [29] (Fig. 2).

The MCP complements and connects the concepts of BCP and ML, additionally including the idea of the VS, into a more integrated concept of the cycling of biogenic carbon in the ocean. The VS, which refers to the release of carbon and nutrients back into the environment due to cell lysis, is tightly connected to the BCP, the ML and the MCP because cell lysis transforms living POM into DOM and nonliving POM [38,18]. As much as a quarter of the C fixed by phytoplankton is estimated to flow through the VS [18], thereby promoting ecosystem respiration [39]. The released DOM and POM are largely of bacterial origin and, hence, relative to bacterial requirements (because of the carbon required for respiration), have too little carbon relative to other nutrients. This shortage of carbon is exacerbated because of the recalcitrant nature (e.g. cell-wall material) of some of the carbon released by cell lysis. Therefore, as the lysis products are processed by the ML, the more accessible DOM is metabolized, releasing inorganic nutrients, altering pathways of nutrient cycling [40,41] and enriching the pool of less labile DOC. This process directly couples the VS to the ML and MCP, and has been termed the 'shunt and pump' [17].

The BCP, ML and MCP have distinct ecological or biogeochemical meanings (Table 1) and each has influenced multiple research disciplines (Table 2). These three concepts are fundamental in developing global biogeochemical and ecological models that rely on understanding organismal biology and the interactions between the POC and DOC pools (Fig. 3).

Several reviews provide thorough descriptions of the BCP and the ML (e.g. [36,42,43,31,11]). Here, we focus on recent progress concerning the MCP in the context of the BCP and ML.

PROGRESS ON THE MCP DURING THE LAST EIGHT YEARS

During the last eight years, our understanding of the MCP has advanced appreciably (e.g. [44–49,26,50,51]), specifically addressing some of the questions raised in Jiao *et al.* [9]. In particular, substantial progress has been made on the composition of recalcitrant DOM, the mechanisms of its formation, the nature of its interactions with ML biogeochemistry and the associated community shifts and trophic dynamics. There were also gains in our understanding of the microbial processing of DOM at various taxonomic and functional group levels (e.g. [52–54]) (Table 3). The state of the art of these topics will be discussed in the remainder of this review.

IDENTIFICATION AND QUANTIFICATION OF THE COMPOSITION OF RDOM

According to Hansell *et al.* [14], less than 1% of the DOC in the ocean is labile and 94% is refractory, while the remaining 5% is classified as semi-labile (note: [13] divided the DOC into labile,

Table 2. Impacts of the three original publications that defined the ML [29], BCP [28] and MCP [9] in different research disciplines, based on the definition of the disciplines in the Web of Science [v.5.29] Core Collection Result Analysis (http://apps.webofknowledge.com). Data were up to 6 July 2018. The percentage value for each discipline is the standardized percentage of the citations in a discipline (minimal 10 citations) vs. the total citations of BCP, ML or MCP since their publication.

Research discipline defined by Web of Science	ML % of 3051 citations since 1983	BCP % of 308 citations since 1985	MCP % of 357 citations since 2010
Microbiology, Biodiversity, Biotechnology	18	1	17
Environmental Sciences, Ecology	23	21	22
Geology Geochemistry Geophysics, Chemistry	3	27	23
Marine Freshwater Biology	32	8	9
Meteorology Atmospheric Sciences	0	12	1
Oceanography, Science Technology other topics	24	30	28

semi-labile, semi-refractory, refractory and ultra-refractory). Much of the RDOC production in the ocean can be attributed to microbial activities (e.g. [55]). Kaiser and Benner [56] estimated that 25% of the total organic carbon (including both POC and DOC) was of bacterial origin. Based on the estimates of Hansell *et al.* [14] and Kaiser and Benner [56], Benner and Herndl [57] calculated that about 10 Pg of semi-labile DOC and 155 Pg of refractory DOC are of bacterial origin. Hansell [13] calculated rates of DOC production for different fractions based on meridional DOC concentration gradients, with the production of RDOC having a rate of 0.043 Pg C/year, which is comparable to the higher end of the RDOC production estimated by Benner and Herndl [57]. Other authors have estimated RDOC production using different criteria. Legendre *et al.* [50] estimated a rate of 0.2 Pg C/year for production of RDOC in the world's oceans at all depths using the constraint of RDOC lifetime of $>$100 years, which is the minimum residence time for the ocean sequestration of carbon in the literature (the origin of the 100-year threshold is explained in [50]). Walker *et al.* [58] calculated production rates of low-molecular-weight DOM in the range of 0.11–0.14 Pg C/year as a proxy for RDOC production in the deep ocean. These numbers interestingly are comparable to earlier estimates from microbial incubation experiments (0.5–0.6 Pg C/year) [59].

Recent efforts to quantify the RDOC pool have been accompanied by progress in the identification of the molecular composition of RDOC and the microbial populations that are responsible for its production in the ocean water column. Microbial RDOC production will be the focus of the following sections, whereas RDOC turnover at deep-sea hydrothermal vents [60–62] and other processes will not be discussed.

Characterization of specific biochemicals in RDOM

Carbohydrates, amino acids and amino sugars

Early studies examined the composition of RDOC based on measurements of common biochemicals, such as carbohydrates, amino acids and lipids. Ogawa *et al.* [55] reported the transformation of labile substrates (D-glucose and D-glutamate) into refractory forms of hydrolysable neutral sugars, amino sugars and amino acids that persisted after 1 year in bioassay experiments. The concentrations of these compounds were later confirmed to be similar to those reported for natural deep ocean waters [63] and represented less than 2% of the total RDOC in low-molecular-weight DOC [16]. In particular, D-enantiomers of amino acids have been observed to contribute to the RDOC pool and are predominantly derived from bacterial sources [56,63]. The ratio of the D-amino acids vs. L-amino acids has been used as a proxy for the degree of recalcitrance, which increases dramatically from bulk POM to the refractory low-molecular-weight DOM [16] (Table 4).

Microbial lipids

Microbial lipids may be important compounds contributing to the RDOC pool in the ocean [64]. Some lipids are much more resistant to degradation than carbohydrates or proteins (hydrolysed to amino acids) [16] and can be preserved in sediments or rocks for hundreds of millions or billions of years [65–67]. Most studies of microbial lipids have been conducted in sediments or POM (e.g. [68–71]) because of the requirement for a large amount of organic material for lipid analysis. Selective accumulation of the refractory lipid-like material in the water column has been demonstrated by the increasing alkanes in the pyrolyzates of sinking POC as depth increased in the

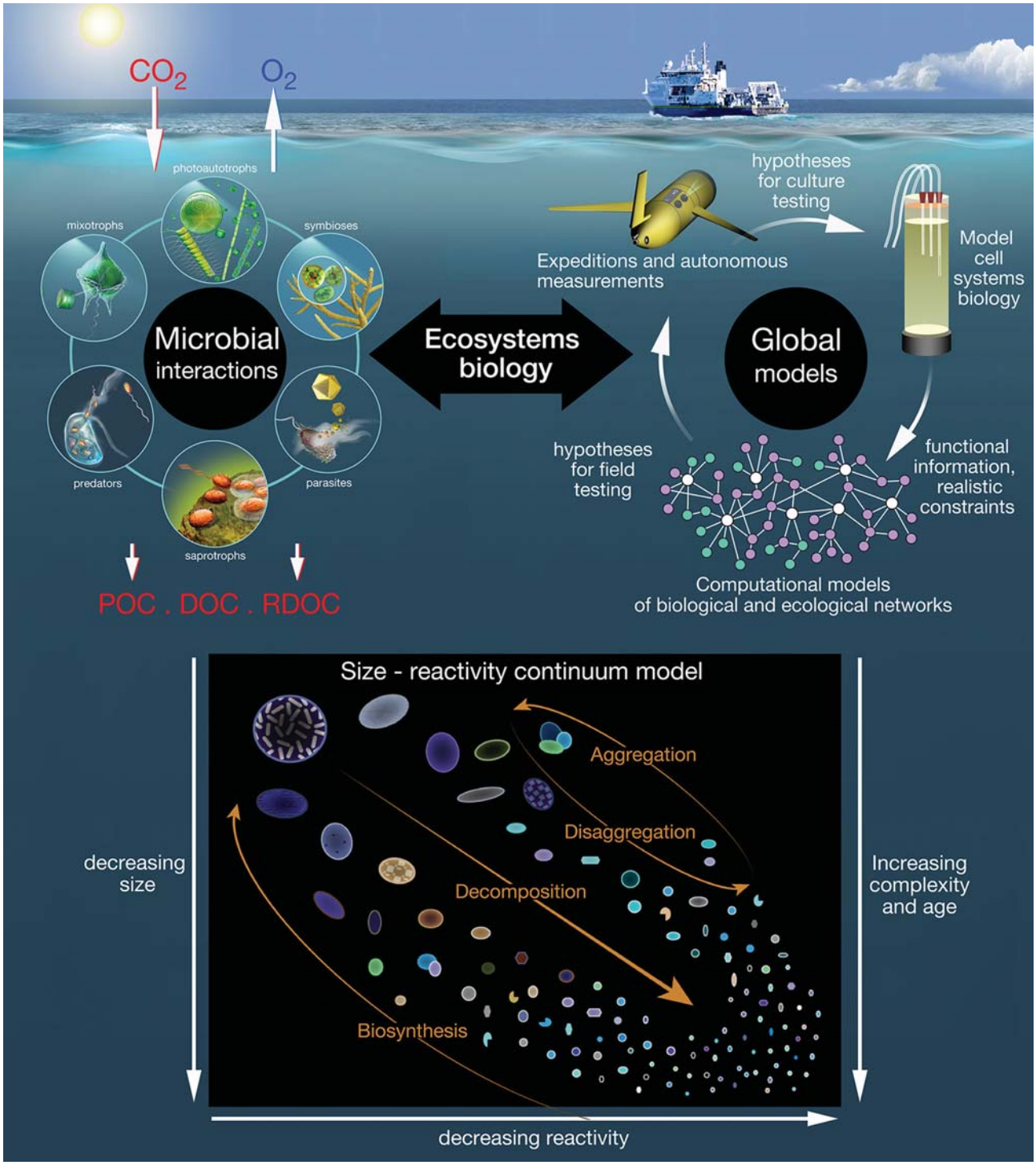

Figure 3. Global biogeochemical and ecological models rely on the present understanding of organismal biology and the interactions between the POM and DOM carbon pools. Modified from Worden *et al.* [116]. The inset panel is from Benner and Amon [16], showing a decreasing size and reactivity and an increasing complexity and age of organic molecules along the decomposition pathway. Small dissolved molecules comprise the bulk of RDOC.

Mediterranean Sea [72]. Alkanes from Proterozoic rocks were also identified as biomarkers of heterotrophic bacteria [66]. These biomarkers might have been derived from MCP activity that contributed to the large DOC pool that may have been 100–1000 times greater than in the modern ocean [73–75]. Lipid-like macromolecules in the deep ocean have similar radiocarbon ages and $\delta^{13}C$ values as the majority (~70%) of the uncharacterized acid-insoluble fraction, indicating that the bulk POC may be compositionally similar to the lipid-like macromolecules [64] (Table 4).

Table 3. Progress made over the past 8 years related to microbial carbon pump framework.

Question asked in Jiao *et al.* [9]	Progress made*	Note	Representative papers
What are the concentrations, compositions and spatiotemporal variations of recalcitrant dissolved organic matter (RDOM) in the ocean?	√√√√	Considerable progress has been made in addressing this question with the past 8 years (see Table 4 for more information)	[16,49,87]
Is the RDOM inventory currently changing and, if so, is the rate of change fast enough for RDOM to serve as an evolving reservoir for stored carbon?	√	The projected global warming is likely to enhance MCP, which may produce more RDOC in future ocean. However, current information is insufficient to make any concrete conclusion	[137,149]
Why do heterotrophic bacteria and archaea not degrade RDOM in 'microbial timeframes'?	√√	Degradation of RDOM by heterotrophic bacteria or archaea is constrained by a specific environment (RDOCt) or low concentration (RDOCc)	[46,47,110,111]
What are the structural and biochemical constraints on degradability?	√√√	Progress has been made in addressing this question with the past 8 years (see Table 4 for more information)	[16,26,87,49]
What environmental conditions make RDOM more or less degradable?	√√	The rate of RDOM degradation is affected by multiple variables, among which temperature, sunlight, pH, redox, nutrient availability and water movement all play a role	[46,111,150]
Can we predict the chemical composition of the degradation products?	√√	Several proxies have been developed for characterization of the composition of RDOC. However, the exact composition of RDOC is still largely unknown	[49,87,102,105]
What is the taxon-specific variation in the degradability of RDOM?	√√√	The best examples are copiotrophic organisms that prefer to degrade carbohydrates and oligotrophic organisms preferring to utilize nitrogen-containing compounds	[52,118]
Is ecosystem energy supply also a constraint on RDOM degradability?	√	Energy supply at the ecosystem level would be an ultimate constraint on RDOM production and degradation. This question is poorly addressed so far	[53,134]
How does organic-matter flux through the microbial loop affect RDOM lability?	√	The flux of labile DOM through the microbial loop can serve as a priming agent enhancing RDOM removal	[111]

*The level of progress made most (√√√√) or least (√) since Jiao *et al.* [9].

The greater ages of lipid-like material than carbohydrate- and protein-like substances were also observed in the DOM pool of the open Atlantic and Pacific Oceans [76]. In particular, the deep-water lipid extract was 13–14 kyr older than the corresponding protein- and carbohydrate-like components in the DOM. This lipid extract was also up to 1 kyr older than the high-molecular-weight DOM. However, the $\delta^{13}C$ values of the high-molecular-weight DOC were more similar to the carbohydrate- and protein-like substances than to the lipid extracts, in contrast to the observations of POC [64]. This suggests that deep-ocean POM and DOM have different origins, with the latter having undergone more extensive recycling [76] (Table 4).

Hwang *et al.* [64] and Loh *et al.* [76] did not identify specific lipid compositions in either the POM or DOM fractions. However, numerous studies focusing on POM have shown diverse lipid biomarkers from planktonic archaea, bacteria and phytoplankton [77–81,69,82,83]. In particular, crenarchaeol was identified as a major glycerol dialkyl glycerol tetraether (GDGT) biomarker for planktonic *Thaumarchaeota* that are present in the global ocean at

Table 4. Summary of compounds or proxies for description of recalcitrant-dissolved organic matter.

Compounds or proxy	Description or method	References
Amino sugars, amino acids	Total hydrolysable, accounts less than 5% of RDOM	[16,55]
D:L-amino acids	Ratio of D-enantiomers of amino acids vs. L-enantiomers of amino acids High D:L ratio in RDOM	[16,63]
Lipid-like macromolecules in POM	Acid-insoluble fraction	[64]
Lipid-like extracts in DOM	Organic solvent extracts, contributing to 0.1–0.3% of DOM_{HMW}	[76]
Glycerol dialkyl glycerol tetraethers, Crenarchaeol	Total lipid extracts in 0.2–0.7 μm fraction	[86]
Carotenoid degradation product	Solid-phase extracted (SPE) DOM using comprehensive gas chromatography coupled to mass spectrometry; contribute to ∼4% of total DOM	[87]
DOC:DON	The percentage of DOC mineralization is negatively correlated with the initial percentage of total fluorescence	[89]
TDAA (%DOC)	TDAA (%DOC) is defined as the ratio of carbon in total dissolved amino acids to the whole DOC. DOM is thought to be refractory when TDAA (%DOC) is less than 0.7%	[91,92]
% Humic-like fluorescent (F) DOM	The percentage of DOC mineralization is negatively correlated with the initial contribution of humic-like fluorescence to the total fluorescence	[89,96,97]
Humic-like FDOM	Humic-like FDOM (Ex = 320 nm, Em = 420 nm) are thought to be bio-refractory	[94]
% Protein-like FDOM	The percentage of DOC mineralization is positively correlated with the initial contribution of protein-like fluorescence to the total fluorescence	[89,96,97,151]
Specific UV absorbance (SUVA)	SUVA positively correlates with aromaticity and thus the recalcitrance of DOM	[89,95–97]
Size-age-reactivity continuum	Increasing decomposition along the flow of organic carbon from larger to smaller size classes results in greater chemical complexity, less biological reactivity and older radiocarbon ages of the organic matter	[16,58,152]
CRAM (carboxyl-rich alicyclic molecules)	CRAM are thought to be refractory and derived from terpenoids. They are defined with these criteria: DBE/C = 0.30–0.68; DBE/H = 0.20–0.95; DBE/O = 0.77–1.75. CRAM account for 8% of the RDOC in the ocean	[88]
IOS (island of stability)	IOS (falling into the area with the same criteria as CRAM) is thought to be the most stable combination of elements in a distinct window of H/C (1.17 ± 0.13), O/C (0.52 ± 0.10) and molecular mass (360 ± 28 and 497 ± 51 Da). IOS compounds contribute about 50% of SPE-DOM	[49]
Degradation Index (I_{DEG})	I_{DEG} was developed to compare the degradation state of marine SPE-DOM samples analysed with FT–ICR MS based on correlation between peak intensity and ^{14}C. $I_{DEG} = \Sigma(\text{magnitudes } NEG_{Ideg})/\Sigma(\text{magnitudes}(NEG_{Ideg} + POS_{Ideg}))$ Since higher I_{DEG} indicates older age, I_{DEG} could be positively related to DOM recalcitrance	[102]
Terrestrial indicator compounds	The 184 terrestrial formulae, identified in most river samples and ocean samples based on correlation between peak intensity and ^{13}C, are thought to be resistant to degradation. It contributes 2–3% to SPE-DOM in ocean samples	[105]

a total inventory of 10^{28} cells [84]. GDGTs can be preserved in sediments for millions of years [85] and can be a significant component of the lipids in the RDOC pool (Table 4). Because *Thaumarchaeota* cell size is small, they are more abundant in the DOM fraction (operationally defined as the fraction passing through a ~0.7-μm filter) than the particulate organic fraction [86]. Measurements of the dissolved phases of lipids give total GDGT abundance in the tens of nanograms per liter range [86]; however, once the organisms die, their core lipids may be incorporated into larger particles (0.7- to 60-μm size fraction) that can be more quickly transported into the deeper ocean and buried in marine sediments (Table 4). The same mechanism may apply to bacterial lipid accumulation in the POM fraction that is preserved in marine sediments. It is unknown, however, how much bacterial or archaeal lipids are actually present in the uncharacterized fraction of the RDOM because the uncharacterized RDOM is largely acid-insoluble and cannot be identified by regular gas chromatography or liquid chromatography mass spectrometry.

Carotenoid-degradation products

A recent report by Arakawa *et al.* [87] identified carotenoid-degradation products (CDP) to be a significant component of the aged DOM using solid-phase extraction and comprehensive gas chromatography coupled to mass spectrometry. The CDP are a subset of carboxyl-rich alicyclic molecules (CRAM) and have similar nuclear magnetic resonance spectra as CRAM [88]. However, the cyclic head groups and branched methyl side chains, with conjugated double bonds, are defining features of isoprenoids characteristic of numerous unique carotenoids that can be produced by plankton [87]. The CDP-rich DOM fraction was depleted in radiocarbon (^{14}C age >1500 years), indicating a possible long-term accumulation of CDP in the ocean. This was the first direct confirmation of these terpenoids accumulating in refractory DOM and may provide a distinct pathway for a single class of biosynthetic precursors to transform to refractory DOM [87] (Table 4). However, this pathway can be either biotic or abiotic and the role that microorganisms play in the transformation of carotenoids to RDOM is unknown.

Characterization of RDOM using proxies

DOC:DON ratio, TDAA (%DOC) and fluorescent DOM

Microorganisms preferentially utilize nitrogen-containing molecules. Thus the ratio DOC:DON could be used to indicate the bioavailability of DOM [89]. Jiao *et al.* [9] noted that DOC:DON (molar ratio) increased from 10.0 in surface labile DOM to 17.4 in deep-sea refractory DOM [90]. Similarly, DOC-normalized total dissolved amino acid (TDAA (%DOC)) may be an indicator of DOC lability [91,92]. Davis and Benner [91] observed that TDAA (%DOC) decreased from >20% in labile DOM to 0.7% in deep-ocean refractory DOM. Humic-like fluorescent DOM was also thought to be bio-refractory as revealed by its good correlation with apparent oxygen utilization in deep ocean water. This relationship is explained as the production of RDOC from *in situ* microbial degradation of more labile DOC at the expense of oxygen [93,94]. In addition to fluorescence, absorbance could also be used to infer DOM lability. Specific ultraviolet absorbance has been demonstrated to be a good indicator of aromaticity [95], which negatively correlates with the lability of DOM (or positively correlates with DOM recalcitrance) ([89,96,97]) (Table 4).

Coupling between molecular size and radiocarbon age of DOC

It has been observed that the distribution of total organic carbon in the global ocean is heavily skewed toward the nanometer size range [16]. A hypothesis is that bioavailability of the organic matter decreases with decreasing size and alteration of the organic molecules (Fig. 3 insert), meaning that smaller-sized classes of organic molecules are more slowly remineralized by microorganisms [98,16]. This has been confirmed by approaches coupling the chemical composition and radiocarbon content of marine organic matter in different size fractions ([76,58]. In Loh *et al.* [76], seawater from different depths of the central North Pacific and the Sargasso Sea region of the North Atlantic showed that the Δ^{14}C values ranged from –5 to –434‰ for high-molecular-weight DOM and from –210 to –539‰ for low-molecular-weight DOM, with the latter being older than the former by 1650–1850 kyr. The low-molecular-weight DOM was also the most abundant (77–95%) fraction of total DOM, consistent with the overall dominance of RDOM in the ocean [14]. Walker *et al.* [58] examined the C:N ratio and ^{14}C age of organic matter in different size classes from the coastal, surface and deep waters of the Pacific Ocean. In all three environments, larger particles were characterized by young ages and nitrogen enrichment and smaller molecules by older ages and nitrogen depletion. The size–age–composition relationship was also observed in marine sediments with pore water DOC being dominated by low-molecular-weight DOM [99].

In addition to the relationships between size, age and composition, a recent study observed declining concentrations of high-molecular-weight DOM correlated with increasing apparent oxygen utilization along the shallow overturning circulation cell of the Mediterranean Sea [93]. Decreases in high-molecular-weight DOM accounted for about 30% of DOM mineralization. The apparent low-molecular-weight DOM experienced little mineralization, indicating microbes primarily utilized high-molecular-weight molecules, whereas the smaller size classes resisted degradation and were the primary source of recalcitrant DOM in the deep ocean [93].

Characterization of RDOM composition using FT–ICR MS

It is well established that RDOM is composed of less than 10% of common biomolecules such as carbohydrates, amino acids or lipids (see discussion above). Proxies such as the DOC:DON ratio, TDAA (%DOC), fluorescent DOM or the size–age relationship provide insights about the composition and reactivity of DOM, but additional analytical approaches are needed to understand RDOM composition. One approach, Fourier transform ion cyclotron resonance mass spectrometry (FT–ICR MS), has gained popularity in recent years because it identifies thousands of molecular formulae, which can be further analysed in detail. FT–ICR MS was proposed over 20 years ago [100] and has been increasingly applied in the characterization of changes in DOM composition in both terrestrial and marine environments and along environmental gradients [49,88,101–106].

A number of proxies have been developed based on characterization of DOM using FT–ICR MS. CRAM are commonly believed to be refractory and occur as the most abundant components of DOM in the deep ocean. Using the FT–ICR MS technique, Hertkorn *et al.* [88] identified over 613 CRAM (Table 4), which can be constrained by the double bond equivalent (DBE) normalized to C (DBE/C = 0.30–0.68), H (DBE/H = 0.20–0.95) or O (DBE/O = 0.77–1.75) within the van Krevelen diagram. These compounds are characterized by abundant carboxyl groups and alicyclic rings commonly found in terpenoids that occur as membrane constituents or secondary metabolites in diverse prokaryotic and eukaryotic organisms [107]. Such findings can be linked to the GC/GC MS analysis of the CDP that can account for 4% of the RDOM component [87], which agrees with the estimate that CRAM account for 8% of the DOC [88]. Lechtenfeld *et al.* [49] further identified 361 most stable molecular formulae, called the 'island of stability' (IOS) (Table 4) within the CRAM domain [49] in the Atlantic and Southern Ocean waters. These molecules are deemed potential indicators of refractory DOM in the Southern Ocean; however, it is unknown whether the same IOS compounds exist in other oceanic environments.

Another proxy called the degradation index (I_{DEG}) was developed by Flerus *et al.* [102] to describe the degradation status of marine DOM analysed with FT–ICR MS from solid-phase extraction (SPE) samples (Table 4). I_{DEG} was calculated using 10 mass peak magnitudes that have either significant linear positive or negative correlation with the Δ^{14}C values of the SPE-DOM. The value of I_{DEG} ranges between 0 and 1 with higher I_{DEG} indicating older age and greater recalcitrance of the DOM. Analysis of seawater at 37° N and 14° W from the eastern Atlantic Ocean showed that I_{DEG} values increased from 0.756 at 400–500 m to 0.808 at 4000–5000 m, consistent with the notion that DOM from deeper water is more refractory than shallower water. Likewise, the I_{DEX} was developed based on the SPE-DOM samples from the Atlantic Ocean, which needs to be verified in other oceanic regions [102].

Lastly, Medeiros *et al.* [105] identified 184 molecular formulae (Table 4) using FT–ICR MS and used them to indicate riverine inputs in the deep North Atlantic and North Pacific Oceans. These compounds are most enriched in river water and correlated well with known terrigenous tracers in the deep ocean waters, based on which the authors concluded that terrigenous organic matter can be preserved in the deep ocean [105]. This observation is consistent with the deep-ocean distributions of dissolved lignin phenols—biomarkers derived from terrestrial plants [108].

FT–ICR MS and nuclear magnetic resonance spectroscopy have been used together to trace the source of deep-ocean RDOC from surface primary production. Zhao *et al.* [109] observed that cultured picocyanobacteria, *Synechococcus* and *Prochlorococcus*, released fluorescent DOM that underwent similar photo-degradation behavior when compared with deep-ocean fluorescent DOM (Table 4). Ultrahigh-resolution mass spectrometry and nuclear magnetic resonance spectroscopy revealed abundant nitrogen-containing compounds in *Synechococcus* DOM, which may originate from degradation products of the fluorescent phycobilin pigments. Their results suggested that picocyanobacteria are likely to be important sources of marine autochthonous fluorescent DOM, which may accumulate in the deep ocean as RDOC [109].

Proxies of RDOM in carbon cycle studies must be used with caution given the current constraints in

defining the composition and reactivity of RDOC. Jiao *et al.* [46] used the term RDOCt to describe RDOC compounds maintaining recalcitrance in a specific environmental context and used RDOCc to describe RDOC compounds being inaccessible to microbes due to their extremely low concentrations. It was debated whether low concentration of any DOC compound is the predominant reason for RDOC to remain recalcitrant in the ocean [47,110]. Recent evidence indicates that only a small fraction of RDOC molecules are too dilute for microbial utilization and that environmental conditions, including exposure to photochemical alterations in surface waters and varying microbial communities, are critical for the removal of RDOC from the ocean [111]. The size–age–composition relationship that organic-matter size is negatively correlated with radiocarbon age and carbon:nitrogen ratios also supports the dominant role of chemical composition (RDOCt) in determining the long persistence of the RDOC pool [58,112].

In addition, if the majority of deep oceanic DOC is RDOCc, namely the dilution hypothesis dominates deep oceanic DOC persistence, the $\Delta^{14}C$ in the deep ocean calculated from a mass balance model of deep oceanic diluted DOC would be difficult to reconcile with the observed $\Delta^{14}C$ (4000–6000 years) for deep oceanic DOC [113]. This is because, with this observed age constraint, the box model of diluted DOC in the deep ocean would result in either (i) labile DOC comprising a relatively large fraction of bulk DOC but with radiocarbon ages similar to or older than bulk radiocarbon ages or (ii) a smaller labile DOC pool with much younger radiocarbon ages; the latter would be most consistent with a variety of other observations [114].

MECHANISMS AND PROCESSES OF RDOC PRODUCTION

Studies on the MCP have attempted to address the grand challenges of dissecting the composition of the bulk RDOM and identifying the diverse microbial populations responsible for the fate and complexity of RDOM; both are still largely 'black boxes'. The research community has reached a consensus that in-depth and integrative characterization of both complex DOM compounds and microbial communities is a prerequisite for exploring the relationship between microbial community composition and the processing of DOM [27,115]. Hopes are high to unveil the intimate linkages between the two black boxes by using the advanced technologies provided by both genomics and bioinformatics, and by mass spectrometry capabilities [25,27,51,116]. Here we present some of the latest advances on focused groups of marine organisms as well as community shifts and trophic dynamics associated with RDOM production.

Carbon metabolism of known organisms

Bacterial metabolism of organic matter is constrained by their physiological capability and biochemical pathways for processing organic molecules. The most studied marine bacteria have been the 'eutrophic' *Roseobacter* clade and the 'oligotrophic' SAR11 clade of marine alphaproteobacteria [117]; both are numerically dominant and functionally important groups of marine bacteria [52]. These clades have distinct patterns of DOC utilization, with *Roseobacter* clade strains mostly taking up carbohydrates and SAR11 preferring nitrogen-containing DOC such as amino acids, which are attributed to different capabilities of ATP binding cassette transporters among these organisms [45,52,118]. Two other studied groups of marine bacteria are the *Gammaproteobacteria* and the *Cytophaga-Flavobacterium-Bacteroides*, which are known to be capable of metabolizing macromolecules through the TonB-dependent transporter proteins [52,118]. Cottrel and Kirchman [43] observed in estuarine and coastal environments that the *Cytophaga–Flavobacter* cluster showed overrepresentation in the assemblage consuming chitin, *N*-acetylglucosamine and protein but underrepresentation in the assemblage consuming amino acids. Tang *et al.* [119] demonstrated through multi-omics analysis and cultivation experiments that the *Bacteroidetes* strain *Gramella flava* JLT2011 (*Flavobacteria*) has the ability to grow on a wide range of polysaccharides such as xylan and homogalacturonan from pectin, which are operated by different polysaccharide utilization loci (PUL) or PUL-like systems. *Flavobacteria* have also been demonstrated to be a major contributor for the utilization of exopolysaccharides that represent an important source of organic carbon in marine ecosystems [120]. However, *Flavobacteria* could not completely utilize exopolysaccharides and fluorescent DOM (e.g. humic acid-like substances) produced during metabolism of exopolysaccharides, which may be refractory and may contribute to the carbon storage in the oceans [120]. While these model organisms provide specific knowledge of carbon compounds they metabolize, it is uncertain how these compounds can be identified in natural environments where complex community interactions occur (see below).

Carbon metabolism of natural populations

Studies using individual organisms under laboratory conditions often focus on limited substrates of known compositions. However, the situation is much more complex for natural populations regarding which bacteria may utilize which carbon compounds and whether such compounds in turn may affect specific bacterial community composition [121]. Multiple reports demonstrate that specific carbon compounds can select for particular species or groups of organisms under different environmental conditions [122]. For example, low-molecular-weight molecules (e.g. monomers amino acids, sugars, short chain fatty acids) can be easily transported across cell membranes and may be utilized by most heterotrophic bacteria or archaea. However, it has been demonstrated that different low-molecular-weight organic compounds stimulated growth of different types of bacteria, leading to the suggestion that changing composition of the DOC pool can selectively alter the community structure of bacterioplankton [121]. This is consistent with observations of the distribution of *Roseobacter* or SAR11 types of organisms selecting for different types of organic substrates (see above). However, it also has been demonstrated that it is the quantity and not the quality of phytoplankton-derived DOC that selects for different types of bacteria in a given range (10–100 μM) of substrate concentrations [54].

The importance of community composition for the fate of DOM has also been shown [53,115]. For example, in incubation experiments using only $<$1.0-μm microbial populations, DOM composition was dominated by compounds with lipid and peptide characteristics; whereas, in incubations with the presence of organisms larger than 1.0 μm, the DOM composition from the culture experiment was nearly identical to that in the natural water, indicating the role of larger microorganisms in constraining DOM composition in the marine environment [53]. These studies highlight the importance of both microbial community structure and composition or abundance of DOM in the marine system, which should allow distinction between RDOCt and RDOCc to better understand the MCP framework (see above).

The interplay between bacterial community and DOM composition is also examined by comparing particle-attached vs. free-living organisms using genomic tools [123–128]. Despite our awareness of the different ecological strategies of particle-associated and free-living microbes (e.g. [129]), we know little about the principles behind the phylogenetic differences and life strategies between free-living and particle-attached microbes in the marine environment [126,130]. Particle-associated microbes are capable of utilizing a variety of substrates under nutrient-rich conditions. Free-living heterotrophs, on the other hand, often face a massive pool of refractory dissolved organic molecules under oligotrophic conditions [130,131]. However, Zhang *et al.* [132] observed that the composition of POM was more strongly related to the free-living than to the particle-attached bacterial community, which indicates that POM composition may significantly influence the free-living bacterial community through the release of labile or semi-labile organic matter from particles contributing to the bioavailability of DOC [132]. The nutritional status of the environment may also affect the difference between particle-attached and free-living populations. For example, in the deep ocean when substrates (ammonia, for example) are scarce, particles provide concentrated life-supporting microenvironments. Microorganisms adapted to a particle-attached lifestyle show the dominance of extracellular hydrolytic enzymes; free-living bacteria, on the other hand, are characterized by hydrolytic enzymes typically bound to the cell surface [130]. In the eutrophic surface ocean and estuaries, substrates or nutrients are abundant and organisms were found to be similar between particle-attached and free-living populations [129,133].

Microbes–DOM interaction at the ecosystem level

The finding of Kujawinski *et al.* [53] that incubation experiments using the whole water community resulted in DOM composition similar to the natural water composition highlights the need to examine the microbes–DOM interaction at the ecosystem scale (Fig. 3). This is convincingly demonstrated by a long-term large volume ($>$100 tons) water column (12 m in depth) incubation, which showed solid evidence of the effective microbial transformation of organic matter from labile to refractory states [48]. Another study provides metagenomic evidence of system-level dynamics of microbes–DOM interactions, utilizing the Tara Ocean data that included comprehensive sequences of eukaryotic, prokaryotic and viral lineages from samples collected within the euphotic zone of ocean waters [134]. The increased carbon export in this water column was found to correlate not only with bacteria, particularly *Synechococcus*, but also several unicellular eukaryotic microorganisms including three *Rhizaria* lineages and three dinoflagellate lineages that have previously not been

believed to play important roles for carbon flux. Also important is the finding of a correlation between the abundance of *Synechococcus* phages and increased carbon export at depth, indicating that phage induced cell lysis promotes particle sinking through enhanced aggregate formation [17], thus increasing carbon export to the deep ocean [134]. The importance of viruses in deeper water is also highlighted by Zhang *et al.* [135], who considered viral particles as 'bottom-up' agents fueling the ML in the deep ocean.

Another comprehensive study [136] examined the genomic and transcriptional responses of microbial communities to high-molecular-weight DOM addition in samples from the surface ocean. These authors observed specific resource partitioning of DOM by the bacterial species *Idiomarina* and *Alteromonas* spp. that were most highly represented at the early time points and *Methylophaga* at the final point of the experiment. Their results demonstrated a temporal succession of taxa, metabolic pathways and chemical transformations associated with high-molecular-weight DOM turnover, suggesting that the cycling of marine DOM may require a coordinated and cooperative effort between different bacterial 'specialists'.

CASE STUDIES OF INTERACTIONS BETWEEN BCP, ML AND MCP

Case Study 1: MCP dynamics associated with upwelling activities

Jiao *et al.* [46] hypothesized that microbial activity plays a significant role in mediating the source and/or sink of CO_2 in a productive upwelling region. This hypothesis was tested by measuring multiple biogeochemical parameters at two cyclonic-eddy-induced upwelling sites in the western South China Sea, which allowed the formulation of a scenario model of MCP processes under different upwelling conditions.

In the western South China Sea, satellite altimetric data identified intensification of two cold-core cyclonic eddies, CE1 (decaying) and CE2 (growing), during sample collection [46]. In the case of the decaying eddy CE1 (modeling scenario 1, Fig. 4), no phytoplankton bloom occurred and *Prochlorococcus* dominated. The small-sized non-sinking organic particles favored the transfer of energy and organic matter through the ML pathway rather than through the BCP. The enhanced production of labile organic carbon due to upwelled nutrients and phytoplankton growth stimulated microbial respiration (e.g. net community respiration) and decreased POC flux, which suggested that the MCP is the prevailing mechanism for carbon sequestration. In the case of a growing eddy, CE2 (modeling scenario 2, Fig. 4), the rapid growth of phytoplankton caused enhancement of POC downward export flux, where the BCP was the prevailing mechanism for carbon sequestration. Further research is needed to validate these models for general applications.

Case Study 2: Modeling the MCP functions

Lu *et al.* [137] made an attempt to analyse the MCP-related variables and processes using a coupled physical-ecosystem model that used data collected in the South China Sea and assumed a constant annual production of RDOC of ~0.2 Pg C for global oceans [50]. They also ran the model with different scenarios simulating rising sea-surface temperature and compared the BCP and MCP rates and their relative contributions to carbon sequestration.

The model coupled a physical model from the operational Taiwan Strait Nowcast\Forecast system [138,139] and a biogeochemistry model based on the Carbon, Silicon, Nitrogen Ecosystem module [140], which was modified to incorporate an explicit RDOC pool and the MCP processes (Fig. 5). With the constraint of a bulk RDOC concentration of 40 μM [13], and the satellite-based value of primary production, this model estimated the ratio of MCP to BCP (at the depth of 1000 m) to be 1:6.08 in the South China Sea. The annual production rate of RDOC by the MCP averaged over the whole South China Sea domain was estimated to be 1.55 mg C m^{-2} d^{-1}. The BCP, on the other hand, sequestered 9.43 mg C m^{-2} d^{-1}.

FUTURE RESEARCH FOCI AND PROSPECTS

Jiao *et al.* [9] highlighted nine major questions regarding MCP processes, which have been addressed at different levels over the past 8 years (Table 3). There is an urgent need to better understand the impacts of global-scale environmental change, including ocean warming and acidification and related deoxygenation and changes in nutrients availability on carbon cycling in the ocean [141]. A central question is how microbial processes contribute to the transformation of organic carbon in the ocean. We advocate three approaches to promote future research in this direction in accordance with Jiao *et al.* [48].

First, we recommend increased investigation of microbiomes in different natural environments, including a much better coverage of the deep ocean. These studies should integrate various omics

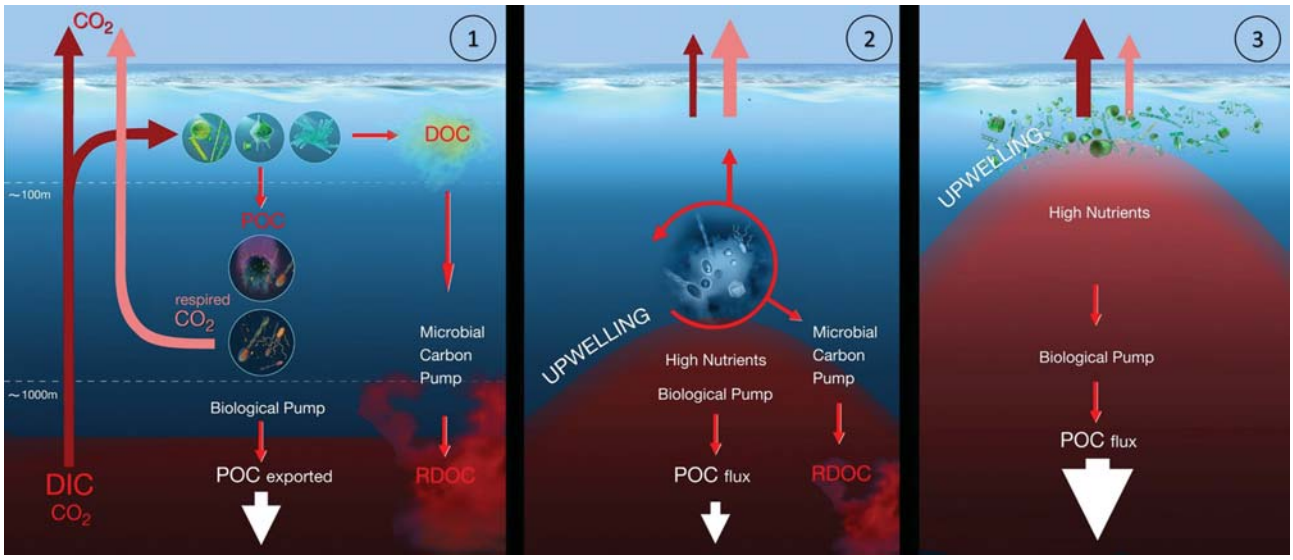

Figure 4. Scenario models for the effects of upwelling on ocean carbon uptake/outgassing dynamics (adopted and modified from [46]). (1) Functioning of the BCP and the MCP in a non-upwelling region of the ocean. (2) Dominance of the MCP in scenario 1 where the total upward CO_2 flux exceeds downward POC export flux: nutrients are injected only into the lower layer of the euphotic zone; *Prochlorococcus* is dominant; microbial respiration is enhanced; CO_2 outgassing exceeds POC export; the MCP is the prevailing mechanism for carbon sequestration. (3) Dominance of the BCP in scenario 2 where the downward POC flux exceeds the total upward CO_2 flux: nutrients are injected into the upper layer of the euphotic zone; diatoms are dominant; POC export exceeds CO_2 outgassing; the BCP is the prevailing mechanism for carbon sequestration.

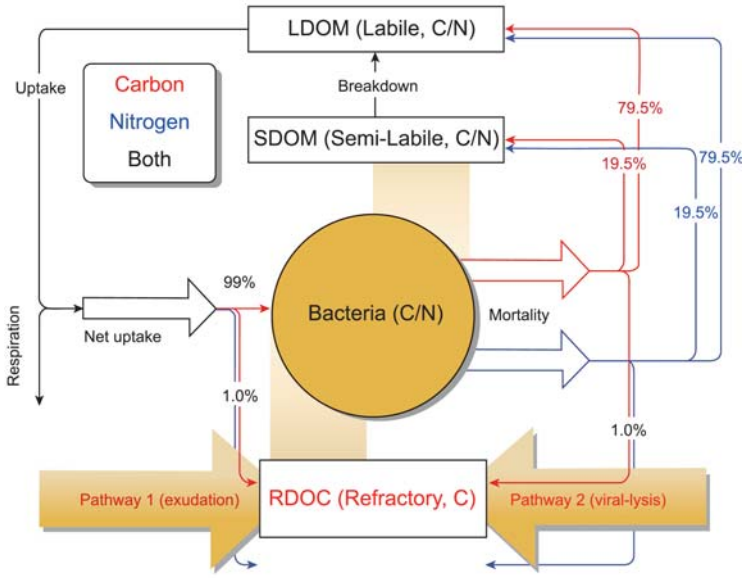

Figure 5. Schematic diagram of the MCP module (from [137]). The RDOC in the model is produced via two bacteria-related pathways: (1) direct exudation by bacteria and (2) passive release from viral lysis of microbial cells. The additional POC degradation pathway [9] is implicitly included by transforming from POC to labile/semi-labile organic carbon and then to RDOC via aforementioned two pathways (see [137] for detailed explanation).

approaches (i.e. metagenomics, metatranscriptomics, metaproteomics and metabolomics) at all levels of the microbial community (i.e. virus, bacteria, archaea, phytoplankton and zooplankton), as well as at selected time-series locations in the coastal and open ocean to identify how the metabolic capacity of the ocean's microbiome responds to spatial and temporal changes in an environmental context (e.g. [27,133]).

The second proposed approach is to strengthen the understanding of the connections between microbial metabolism and the chemical structure of DOC compounds (e.g. [51]). Bioassays of DOC composition coupled with changes in bacterial communities can now be conducted integrating omics and FT–ICR MS and NMR technologies, which offers the potential for new insights into mechanisms responsible for the formation of RDOCt and RDOCc. In particular, efforts are needed to fully examine the fate of DOM under different trophic conditions and at the ecosystem level [53,134,142].

The third proposed approach is to establish and expand long-term incubation studies employing large-scale facilities, such as the existing Aquatron Tower Tank (Dalhousie University, Canada) and the planned Marine Environmental Chamber System (Shandong University, China) under controlled environmental conditions. Using such facilities provides a unique complement to field studies by seeking to mimic ocean-relevant physical, chemical and biological environmental conditions (e.g. vertical stratification) and their variations for long-term experiments. Such experiments are required to provide unique data and insight for testing hypotheses regarding the effects of global environmental change on the ocean carbon cycle [143,144].

We also highlight the need to examine the role of planktonic archaea in the carbon cycle. These archaea, such as *Thaumarchaeota*, have been recognized to play an important role in the ocean carbon cycle [145]. Yet, the claim made 7 years ago that '*we are woefully unaware of DOM production (or assimilation) mechanisms in the Archaea*' [25] still holds true. The study of archaea is largely hampered by the difficulty of isolating strains from the ocean (e.g. MGII and MGIII). Hence, future efforts should include the development of new technologies for enrichment and isolation of these and other organisms, guided by genomic information [133,146].

The MCP has stimulated provocative and constructive discussions and studies on the processes and mechanisms of RDOC formation and preservation [26,47,111,112,147,148]. Increasing and synergistic efforts will continue to be made to gain further understanding of the ocean carbon cycle through an integration of the concepts of the BCP, ML, VS and MCP, particularly in the context of global ocean circulation (e.g. [111]).

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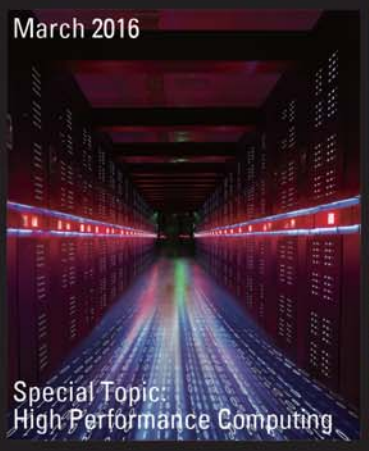

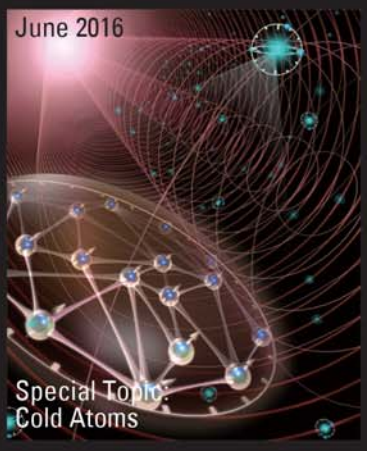

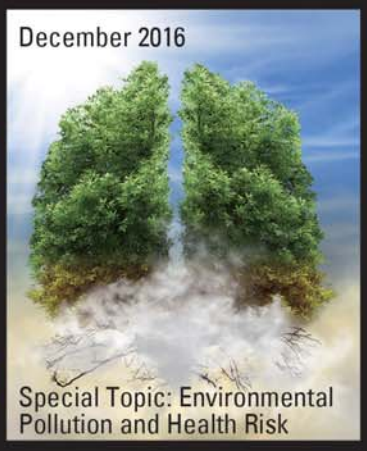

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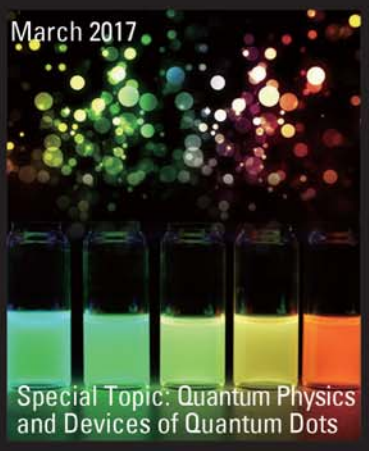

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