Proposal for a SCOR Working Group on

The Microbial Carbon Pump in the Ocean

Abstract

The utilization of labile dissolved organic carbon (DOC) and production of refractory DOC by heterotrophic prokaryotes can shape the chemical composition of organic carbon and thereby influence the residence time of carbon in the ocean. This process is analogous to the better-known "biological pump". To better understand the microbial processing of carbon and its possible impacts on oceanic carbon sequestration, microbiologists and biogeochemists need to work together to bridge the fields of microbial ecology and organic biogeochemistry. This working group (WG) will document the state of the art in microbial processing of organic carbon and acquire new insights through analyzing the available data on microbial biomass, production and diversity along with dissolved organic matter (DOM) data from a variety of marine environments. The goal of the WG is to identify priority scientific questions and the corresponding technical needs, and establish or standardize protocols for the observations of key microbial and DOM parameters, to advance interdisciplinary research on the microbial carbon pump in the ocean.

Rationale

The "biological pump", a key mechanism for atmospheric CO_2 fixation by the ocean, is based on particulate organic carbon (POC) transport from the surface to the deep ocean and burial in seafloor sediments. Recent studies have revealed that dissolved organic carbon (DOC) may also be a potential mechanism for carbon sequestration in the ocean. Besides the known physical and chemical processes transporting DOC from surface to deep sea, biological processes play a central role in DOC dynamics.

Labile DOC (LDOC) can be utilized by heterotrophic prokaryotes and then transported to higher trophic levels through the "microbial loop" (Azam et al., 1983), consequently forming sinking POC. Refractory DOC (RDOC), either left or produced by microbial processes, can remain in the water without returning to the atmosphere for up to thousands of years (Bauer et al., 1992). In contrast to the "sinking biological pump", RDOC does not sink, and can be coined "non-sinking biological pump" (Jiao, 2006). Since DOC is the largest organic carbon pool in the ocean, and RDOC is the majority of total DOC, the non-sinking biological pump is one of the keys in understanding the carbon sink of the ocean. As a major pathway to generate RDOC, microbial processes have been identified (Ogawa et al., 2001; Kawasaki and Benner, 2006). This RDOC production process can also be coined "microbial pump" (Jiao et al., 2007; Jiao et al., 2008).

Although this new research line is beginning to emerge, studies on the non-sinking microbial pump are still in its infancy compared with the well-documented sinking POC-based biological pump. Detailed information about interactions between microbial processes and organic carbon is required. Scientists from different disciplines need to work together on the interdisciplinary scientific questions and protocols to measure these newly recognized processes and parameters.

A SCOR working group on the microbial carbon pump would be the best forum to bring together outstanding scientists from marine microbial ecology and biogeochemistry, bridging the gaps between different disciplines. Such a WG will not only benefit the members in addressing scientific and technical aspects of the problem, but also benefit the general fields of biological and chemical oceanography, and global biogeochemical modeling, by producing new angles of view, new concepts, and new methods.

Scientific Background

The ocean acts as a global buffer system mitigating increases of anthropogenic CO_2. The known biological mechanism is the "biological pump", which is responsible for the transfer of particulate organic carbon (POC) from the surface to deep waters and even to the sediment, and thus can hinder carbon from returning to the atmosphere for hundreds and even thousands of years. In addition to the "sinking particle"-based biological pump, recent studies have revealed that dissolved organic carbon (DOC) is another potential mechanism that needs to be studied regarding carbon cycling in the ocean.

The DOC pool in the ocean is estimated to be 700 Gt (Ogawa and Tanoue, 2003), the second largest carbon reservoir in the ocean and approximately equivalent to the carbon stock of atmospheric CO_2 (~750 Gt) or terrestrial biomass (~600 Gt) (Hedges, 1992). However, DOC consists of a plethora of different compounds with different availability to microbial degradation. Total DOC can be roughly divided into LDOC that is readily utilized by heterotrophic microorganisms and RDOC that is resistant to biological decomposition.

DOC does not sink except for the portion adsorbed to sinking particles. The fate of much of the LDOC is to be taken up by heterotrophic prokayrotes and transformed into particulate organic carbon (POC) through microbial loop and then transported to upper trophic levels (Azam et al., 1983). However, RDOC will stay in water until being further decomposed by non-biological processes, like photochemical degradation (Benner and Biddanda, 1998). Approximately 650 Gt of the DOC in the ocean is RDOC (Ogawa and Tanoue, 2003) accounting for the majority of marine DOC (more than 90%). Compared with inorganic carbon storage, RDOC is much less sensitive to environmental changes, and there are no chemical equilibrium limitations for increase of RODC in the water. With a turnover time of about 4000-6000 years (Bauer et al., 1992), RDOC is the most persistent carbon form and could be one of the keys to the understanding of carbon sequestration in the ocean.

Although the precise mechanism for generation of RDOC is still unclear, microbial processes are known to play an important role in generating RDOC (Kawasaki and Benner, 2006). Microbial activities pump a fraction of the available organic carbon pool from low-concentration bioactive LDOC pool to the high-concentration RDOC pool, a process coined "microbial pump" (Jiao, 2006). There are at least two major consequences of microbial pump processes. First, it pumps organic carbon from low concentrations to high concentrations and keeps the organic carbon in the ocean for a longer time. Second, the microbial pump shapes the chemical composition of DOC and alters the ratio of carbon to other elements, such as nitrogen and phosphorus. Stoichiometric analyses of marine organic matter have shown that the C:N:P ratio of RDOM (3511:202:1) is quite different from LDOM (199:20:1) and POM (106:16:1) (Hopkinson Jr and Vallion, 2005). That means that the microbial pump sequesters more carbon relative to nitrogen and phosphorus from the active organic matter pool into inert organic matter. In contrast to the biological pump accomplished by sinking particles, the microbial pump is a "non-sinking biological pump". Quantification of the relative importance of the two paths is mandatory for a better understanding of the mechanisms controlling carbon cycling and sequestration of CO_2 by the ocean.

Two major disciplines are involved in studying the microbial carbon pump: microbial ecology and organic biogeochemistry. In the past few years, substantial progress has been made in both of these fields. Some new techniques have been introduced:

- Micro-FISH (Microautoradiography and Fluorescent In Situ Hybridization) for simultaneous observation of abundance and carbon uptake rate of major bacterial groups (Cottrell and Kirchman, 2003), addressing "who are out there and what they are doing" simultaneously.
- CARD-FISH (Catalysed Reported Deposition Fluorescence *in situ* Hybridization) and rolling circle PCR-FISH for in situ identification of environmental microbes and for simultaneous detection of mRNA and rRNA in environmental bacteria, thereby linking the identification of single cells to the expression of particular functional genes (Pernthaler and Amann, 2004).
- Micro-CARD-FISH (Microautoradiography and Catalyzed Reporter Deposition Fluorescence *in situ* Hybridization Combined with Microautoradiography) for determination of specific carbon metabolism of different functional groups of bacteria. Applications to meso- and bathypelagic realm of the ocean have revealed that archaea play a significant role in deep sea carbon cycling (Herndl et al., 2005).
- TIREM (Time Series Observation-Based Infrared Epifluorescence Microscopy) for accurate quantification of aerobic anoxygenic photosynthetic bacteria (AAPB) (Jiao et al., 2006), resulting recognition of distinct global distribution pattern of AAPB against theoretical speculations (Jiao et al., 2007) and consequently evoking further studies on the role of AAPB in energy flow and carbon cycling in the surface ocean.
- HTC (DOC), HPAEC-PAD (amino Sugars) to document chemical dynamics of marine DOM when exposed to microbial transformation (Benner and Opsahl, 2001; Benner and Kaiser, 2003)
- HPAEC-PAD (neutral sugars), HPLC (amino acids) to profile bioreactivity of marine DOM (Amon et al., 2001) (Sempéré and Kawamura, 1996; Sempéré et al., 2003; Sempéré and Kawamura, 2003).
- Fluorescence spectroscopy and parallel factor analysis (PARAFAC) for the detection of optically active component of marine Chromophoric DOM (CDOM) (Stedmon et al., 2003)
- SPE-DOM (solid-phase extraction of dissolved organic matter) for extraction of DOM in seawater (Dittmar et al., 2008).

A perfect combination of technique development and scientific theory can be found in the case of the "Microbial Loop" and microscale interactions of bacteria with organic matter and its influence on carbon export flux in the ocean (Azam and Worden, 2004).

In spite of the above achievements, there are still great needs for interdisciplinary interactions between scientists studying microbial processes and organic carbon cycling. New theories and concepts could be generated and validated through exchange and interactions between scientists from different disciplines and new techniques could be worked out or standardized for comparable observations. A SCOR working group would bring together scientists with the expertise required to address the newly merged scientific questions, and to work out protocols for measurement of the newly recognized processes and parameters.

Relevance to Other Activities of SCOR or Other International Organization

SCOR has a long history of support of research in ocean carbon cycling. Relevant past SCOR Working Groups that are related to the present one include

- WG 62: Carbon Budget of the Ocean;
- WG 79: Variations in Carbon Dioxide and the Carbon Cycle
- WG 116: Sediment Trap and ^{234}Th Methods for Carbon Export Flux Determination

WGs 62 and 79 worked on CO_2 budgets and dynamics and WG116 worked on sinking POC-based biological pump. The outputs of these WGs provide an important basis for the proposed WG on the microbial carbon pump. The proposed WG will extend the topic of carbon cycling and deepen our understanding of the mechanisms. Another distinct feature of the proposed WG is the interdisciplinary interactions among scientists from microbial ecology and biogeochemistry, which will bridge the gaps between different fields that hinder progress on this topic, and will hopefully bring breakthroughs in new angles of view, new concepts, and new methods.

Terms of Reference

- Summarize representative microbial data on biomass, production and diversity as well and DOC data along environmental gradients, establish the current state of knowledge and identify essential scientific questions regarding the role of microbial processing in carbon cycling in the ocean;
- Assess the available techniques for quantifying functional groups of prokaryotes and different types of DOC, document state-of-the-art techniques and parameters addressing microbial processing of organic carbon, and establish/standardize key protocols for the essential observation/measurements;
- Convene International Workshop(s) and publish a special volume in an internationally recognized peer-reviewed journal, or a protocol book (practical handbook) by a major publisher on measurements of the key parameters related to microbial processing of carbon in the ocean.
- Make recommendations for future research related to the microbial carbon pump in the ocean, toward development of a large-scale interdisciplinary research project.

Working Group Membership

Full Members

Nianzhi Jiao (China) – Co-Chair – Marine Microbial ecology
Farooq Azam (USA) – Co-Chair –Microbial Oceanography
Gerhard J. Herndl (Netherlands) – Microbial Oceanography
Ronald Benner (USA) – Marine Biogeochemistry
Bernhard Fuchs (Germany) – Molecular Microbiology
Colin Stedmon (Denmark) –Marine biogeochemistry
Michal Koblizek (Czech) –Marine microbial ecology
Susan Ziegler (Canada)–Marine biogeochmistry
Ingrid Obernosterer (France) – Marine microbial ecology
Gerhard Kattner (Germany) –Marine Biogeochemistry

Associate Members

David L. Kirchman (USA) – Marine Microbial ecology
Simon Meinhard (Germany) – Marine Microbiology
Feng Chen (USA) – Marine Virus and Molecular Ecology
Richard Sempéré (France) – Marine Biogeochemistry
Tron Frede Thingstad (Norway)- Mcirobial ecology
Danyun Ou (China) – Marine Microbial Ecology
Steven W. Wilhelm (USA) – Virus Ecology
Paul Harrison (Canada) – Phytoplankton Ecology
Rainer Amon (USA) – Marine Biogeochemistry
Sang-Jin Kim (Korea) –Microbiology and Genomics
Nagappa Ramaiah (India) – Microbiology and Marine Ecosystem
Carol Robinson (UK) Marine microbiology

Key items to examine and corresesoponding members

The following issues would fall within the Terms of Reference of the proposed working group and would be the focus of the WG. （Necessary sampling for methodological intercalibrations and field observations will be carried out with samples from a cruise to the West Pacific Warm Pool supported by the Minisity of Science and Technology of China (MOST) and the National Natural Science Foundation of China (NSFC) (see “Operation Mode and Timeline” item 4 and 5 ）

1. Discrimination and quantification of functional microbial groups
   - Flow Cytometry (FCM) recognition, sorting, and enumeration of functional microbial groups on board and in lab (Jiao)
   - Time-series observation-based infrared epifluorescence microscopy (TIREM) analysis of aerobic anoxygenic photosynthetic bacteria (AAPB) (Jiao)
   - Catalyzed reported deposition fluorescence in situ hybridization (CARD-FISH) for discrimination of functional microbial groups and the application of Nano-SIMS (Fuchs/Herndl)
   - *in situ* dual fluorescence monitoring of bacterial chlorophyll and chlorophyll−−

(Koblizek)

2. DOC analysis methodology
   - Solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater, especially from the deep sea (Kattner)
   - DOC composition bioassay method-sugars, dicarboxylic acids, amino acids (Obernosterer)
   - CDOM extraction, measurement and indications (Stedmon)

3. Contribution of bacteria to the marine DOM transformation
   - Bacterial colonization and enzyme action affect aggregation potential with consequences for carbon export flux (Azam)
   - Shaping of marine DOM composition under exposure to microbial transformation (Sempéré/Obernosterer)
   - Bioreactivity of marine DOM to natural microbial community (Benner)

4. Carbon metabolism of functional groups of microorganisms
   - Microautoradiography and fluorescent in situ hybridization (Micro-FISH) for simultaneous observation of abundance and carbon uptake rate of major bacterial groups (Kirchman)
   - Archaeal carbon fixation, Micro-CARD-FISH for determination of specific carbon metabolism of different functional groups of bacteria (Herndl)
   - Stable isotopes in combination with microbial assay studies toward the role and fate of marine and terrestrially derived DOM in the ocean (Ziegler).
   - Virus-host interaction and the role of virus in the ocean carbon cycle (Chen)
   - Selective use of carbon sources by functional groups of prokaryotes as seen from Biolog bioassay (Ou /Jiao) and by Nano-SIMS (Fuchs)
   - Bacterial utilization of phytoplankton exudation as carbon or energy source (Harrison/Jiao)

<u>Prospective Products:</u>

1. A few review papers by the whole group or subgroups on the following topics
   1) Microbial pumping process of carbon in the ocean
   2) DOC (including CDOM) measurements
   3) Carbon metabolism of functional groups of microorganism
2. A handbook of practical protocols for observation of marine microorganisms and microbial processing of carbon in the ocean. The following items should be included:
   1) Flow cytometry analysis and sorting
   2) Specialized/modified epifluorescence microscopy
   3) Atomic force microscopy
   4) Fluorescence probing techniques (Micro-CARD-FISH, Card-FISH)
   5) Isotopic tracing techniques
   6) Functional gene probing
   7) DOC extraction approaches

8) Bioassay on microbial shaping of DOM composition
9) Bioassay on microbial carbon utilization spectrum
10) Bioreactivity of environmental DOM to natural microbial community
11) Interaction between phytoplankton and bacterioplankton (carbon / electron donor test)
12) Virus-Host interaction system (carbon lysis bioassay)
13) Bacterial colonization/aggregation test system
14) Carbon metabolism at community and functional groups (Micro-CARD-FISH)
15) Application of genomics and proteomics to microbial carbon use

3. A special volumes of a major international peer-reviewed journal for papers presented at the WG workshop(s). (The group will decide at its second meeting whether or not to develop a workshop with its final meeting).

Operation Mode and Timeline of the Working Group

1. Since its initiation last year, interactions among the potential members at individual level has been going well, and **a group of interest has been gradually formed naturally,** Continuing discussions are taking place this year:
   - Exchange ideas between potential members at individual level by emails (Jan-Apr, 2008);
   - Partial group discussion and planning at the 10th International Estuarine Biogeochemistry Symposium (Xiamen, May 18-22, 2008);
   - Partial group discussion among prospective members at ASLO meeting (Canada, June 8-13, 2008) and AOGS meeting (Korea, June 16-20, 2008);
   - Partial group study at summer school in Xiamen and Qingdao, China (July 8-22, 2008).
   - Partial group members meet in Xiamen for preparation of ISME-12 round table session (see 2) (China, Aug 7-11, 2008).
2. A **round table session** as **a pilot WG meeting at the ISME-12 conference** (International Symposium for Microbial Ecology) (Australia, 2008.8.12-17) has been approved officially by the ISME-12: Four WG members are invited speakers: Farooq Azam (USA), Nianzhi Jiao (China), Gerhard Herndl (The Netherland), and Feng Chen (USA).
3. Partial group members meet at the "Xiamen Ocean Festival" （Nov.2-8, 2008）for exchange and preparation of sampling on a cruise to the West Pacific Warm Pool (see 4).
4. **Cruise opportunity** will be provided by Chinese research projects for necessary field sampling and methodological intercalibrations. The most recent one will be a 30-day cruise along environmental gradients from the Yangtze Rive estuary to the the Kuroshio Currents and the West Pacific Warm Pool (so called "the engine of climate changes") which will be carried out from November to December,2008. Once-a-year oceanic cruises to the Pacific and Indian Oceans (R/V Ocean No.1, 3800 ton) are another opportunities (Participation of scientists outside China need to go through official formalities for approval).
5. **Support from inside China**. The idea of this SCOR WG was approved and recommended by the Chinese SCOR Committee at the last annual meeting (Suzhou, Sept. 15-26, 2007). The NSFC and the State Key Lab for Marine Environmental Science will provide partial financial

support (200K-300K RMB) for the WG related academic activities and WG meeting/workshops held in China.

6. **The first official WG meeting**, will be taking place in Xiamen, China in early 2009. A detailed WG four-year work plan will be made in accordance with the assignments of the above-listed missions to each member. Face-to-face interactions between members with microbiology and biogeochemistry expertise would take place on the hot topics regarding the role of microbial processes in carbon cycling in the ocean. A review paper will be initiated during this meeting.
7. **The second official WG meeting** will be held approximately one year after the first one targeting on examination of implementation of the assignments and problems-solving toward the planed outputs. The group will decide at this meeting whether or not to develop a **workshop** with the final meeting for a special volume of a major international peer-reviewed journal.
8. **The final meeting** will be convened in the last year (early 2012) to complete the group's work.

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