Review

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.

Received: 16-May-2018; Revised: 28-Jun-2018; Accepted: 03-Jul-2018

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) (Falkowski et al., 2000); 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 (Siegel et al., 2016). 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 (Dunne et al., 2007; Ridgwell and Arndt, 2015), 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 (Le Quéré et al., 2018), resulting in ocean acidification with consequences for biogeochemical and climatological processes, and the ocean carbon cycle (Falkowski et al., 2000; Cai, 2011; Bauer et al., 2013; Laruelle et al., 2018). Global warming and ocean acidification and their respective consequences influence the functioning of the BCP, a major pathway for sequestering atmospheric CO_2 in the ocean. The microbial carbon pump (MCP) (Jiao et al. 2010) provides an additional path for carbon sequestration within the marine ocean carbon cycle (Stone, 2010), 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 (Sarmiento and Gruber, 2006), 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 (Bauer et al., 1992; Hansell, 2013). Hansell (2013) defines 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) (Hansell et al., 2009) with significant seasonal variation often seen in surface waters (Copin-Montégut and Avril, 1993). 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 (Benner and Amon, 2015), 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 (Wilhelm and Suttle, 1999; Suttle, 2007) and other processes such as phytoplankton excretion and zooplankton sloppy feeding (Roy et al., 1989; Ducklow et al., 1995; Biddanda and Benner, 1997; Strom et al., 1997; Arrigo, 2007; Moller 2007). 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 use microbiological and geochemical tools to bridge the gap between microbial omics and organic carbon composition (Kujawinski, 2011; Lechtenfeld et al., 2015; Moran et al., 2016). 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. (2010).

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

Understanding of the ocean's carbon cycle in the late 20th century was largely promoted by the biological carbon pump (called "soft tissue pump" in Volk and Hoffert, 1985) and the microbial loop (Azam et al., 1983). 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 (Volk and Hoffert, 1985). Both concepts find their roots in Dugdale and Goering (1967), 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 nonliving 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, i.e. 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 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*?" (Passow and Carlson, 2012). 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 (Boyd, 2015). However, other predictions suggest alternative outcomes and the answers to these questions are still discussed controversially in the scientific community. In a recent report on a transformative understanding of the ocean's BCP to the US National Science Foundation, Burd et al. (2016) 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, e.g. the ongoing US -EXPORTS (Siegel et al., 2016) and the UK COMICS (Sanders et al., 2016) 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 (Azam et al., 1983; Azam, 1998). 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 dissolved organic matter (Azam, 1998). It was estimated that bacteria could channel up to 50% of marine primary production into the microbial loop, highlighting their importance in the ocean's carbon cycle (Azam, 1998; Fenchel, 2008). Similarly, Legendre and Rivkin (2008) found 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 (Azam et al. 1983) (Fig. 2).

The MCP complements and connects the concepts of BCP and ML, additionally including the idea of the viral shunt, into a more integrated concept of the cycling of biogenic carbon in the ocean. The viral shunt, 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 particulate organic matter (POM) into DOM and non-living POM

(Wilhelm and Suttle 1999; Suttle, 2005). As much as a quarter of the C fixed by phytoplankton is estimated to flow through the VS (Wilhelm and Suttle, 1999), thereby promoting ecosystem respiration (Fuhrman, 1999). 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 (Weitz et al., 2015; Shelford and Suttle, 2018), 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' (Suttle, 2007).

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. Kirchman, 2000; Sarmiento and Grüber, 2006; Fenchel, 2008; Honjo et al., 2008; Passow and Carlson, 2012). 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. Jiao and Zheng, 2011; Jiao et al., 2014, 2015; Lechtenfeld et al., 2014, 2015; Legendre et al., 2015; Zhang, 2016; Chen et al., 2018; Jiao et al., 2018a), specifically addressing some of the questions raised in Jiao et al. (2010). In particular, substantial progress has been made on composition of recalcitrant DOM, the mechanisms of its formation, the nature of its interactions with microbial loop 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., Dang and Jiao, 2014; Kujawinski et al., 2016; Sarmento et al., 2016) (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. (2009) 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: Hansell, 2013 divided the DOC into labile, semi-labile, semi-refractory, refractory and ultra-refractory). Much of the RDOC production in the ocean can be attributed to microbial activities (e.g., Ogawa et al., 2001). Kaiser and Benner (2008) 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. (2009) and Kaiser and Benner (2008), Benner and Herndl (2011) calculated that about 10 Pg of semi-labile DOC and 155 Pg of refractory DOC are of bacterial origin. Hansell (2013)

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 (2011). Other authors have estimated RDOC production using different criteria. Legendre et al. (2015) 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 Legendre et al., 2015). Walker et al. (2016a) 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) (Brophy and Carlson, 1989).

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 (Hawkes et al., 2015; Lang et al., 2006; Walter et al., 2018) 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. (2001) 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 one year in bioassay experiments. The concentrations of these compounds were later confirmed to be similar to those reported for natural deep ocean waters (Kaiser and Benner, 2009) and represented less than 2% of the total RDOC in low-molecular-weight DOC (Benner and Amon, 2015). In particular, D-enantiomers of amino acids have been observed to contribute to the RDOC pool and are predominantly derived from bacterial sources (Kaiser and Benner, 2008, 2009). 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 (Benner and Amon, 2015) (Table 4).

Microbial lipids. Microbial lipids may be important compounds contributing to the RDOC pool in the ocean (Hwang et al., 2003). Some lipids are much more resistant to degradation than carbohydrates or proteins (hydrolyzed to amino acids) (Benner and Amon, 2015) and can be preserved in sediments or rocks for hundreds of millions or billions of years (Logan et al., 1995; Summons et al., 1999; Brocks et al., 2003). Most studies of microbial lipids have been conducted in sediments or POM (e.g., Zhang et al., 2002, 2003; Pancost and Sinninghe Damsté, 2003; Wakeham et al., 2003) 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 Mediterranean Sea (Peulvé et al., 1996). Alkanes from Proterozoic rocks were also identified as biomarkers of heterotrophic bacteria (Logan et al., 1995). 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 (Rothman et al., 2003; Ridgwell, 2011; Tziperman et al., 2011). 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 (Hwang et al., 2003) (Table 4).

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 (Loh et al., 2004). 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 (Hwang et al., 2003). This suggests that deep ocean POM and DOM have different origins, with the latter having undergone more extensive recycling (Loh et al., 2004) (Table 4).

Hwang et al. (2003) and Loh et al. (2004) 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 (Sinninghe Damsté et al., 2002; Wakeham et al., 2003, 2007; Ingalls et al., 2006; Turich et al., 2007; Schubotz et al., 2009; Wei et al., 2011; Schouten et al., 2012). 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 a total inventory of 10^{28} cells (Karner et al., 2001). GDGTs can be preserved in sediments for millions of years (Kuypers et al., 2001) 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 dissolved organic matter fraction (operationally defined as the fraction passing through a ~0.7 μm filter) than the particulate organic fraction (Ingalls et al., 2012). Measurements of the dissolved phases of lipids give total GDGT abundance in the tens of nanograms per liter range (Ingalls et al., 2012); 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. (2017) 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 (Hertkorn et al. 2006). 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 (Arakawa et al., 2017). 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 (Arakawa et al., 2017) (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 (Fellman et al., 2008). Jiao et al. (2010) noted that DOC:DON (molar ratio) increased from 10.0 in surface labile DOM to 17.4 in deep sea refractory DOM (Hopkinson and Vallino, 2000). Similarly, DOC-normalized total dissolved amino acid (TDAA (%DOC)) may be an indicator of DOC lability (Davis and Benner, 2007; Shen et al., 2015). Davis and Benner (2007) 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 (Yamashita and Tanoue, 2008; Martínez-Pérez et al., 2017). 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 (Weishaar et al., 2003), which negatively correlates to the lability of DOM (or positively correlates to DOM recalcitrance) (Fellman et al., 2008; Fellman et al., 2009a,b) (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 (Benner and Amon, 2015). 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 size classes of organic molecules are more slowly remineralized by microorganisms (Amon and Benner, 1996; Benner and Amon, 2015). This has been confirmed by approaches coupling the chemical composition and radiocarbon content of marine organic matter in different size fractions (Loh et al., 2004; Walker et al., 2016a, b). In Loh et al. (2004), 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 (Hansell, 2009). Walker et al. (2016a) 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 (Burdige and Gardner, 1998).

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 (Martínez-Pérez et al., 2017). 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 (Martínez-Pérez et al., 2017).

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 analyzed in detail. FT-ICR MS was proposed over 20 years ago (Kujawinski et al., 2002) and has been increasingly applied in the characterization of changes in DOM composition in both terrestrial and marine environments and along environmental gradients (Koch et al., 2005; Hertkorn et al., 2006; Sleighter et al., 2008; Kujawinski et al., 2009; Flerus et al., 2012; Lechtenfeld et al., 2014; D'Andrilli et al., 2015; Medeiros et al., 2017).

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. (2006) 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 (Ourisson et al., 1987). Such findings can be linked to the GC/GC MS analysis of the carotenoid degradation products that can account for 4% of the RDOM component (Arakawa et al., 2017), which agrees with the estimate that CRAM account for 8% of the DOC (Benner, 2002; Hertkorn et al., 2006). Lechtenfeld et al. (2014) further identified 361

most stable molecular formulae, called the "island of stability" (IOS) (Table 4) within the CRAM domain (Figure 8 of Lechtenfeld et al., 2014) 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. (2012) to describe the degradation status of marine DOM analyzed 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 $\Delta^{14}C$ values of the SPE-DOM. The value of I_{DEG} ranges between 0–1 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 (Flerus et al., 2012).

Lastly, Medeiros et al. (2017) 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 (Medeiros et al., 2017). This observation is consistent with the deep-ocean distributions of dissolved lignin phenols, biomarkers derived from terrestrial plants (Hernes and Benner, 2006).

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. (2017) 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 (Zhao et al., 2017).

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. (2014) 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 (Arrieta et al., 2015; Jiao et al., 2015). 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 (Shen and Benner, 2018). 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 (Walker et al., 2016a; Amon et al., 2016).

In addition, if the majority of deep oceanic DOC is RDOCc, i.e., 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 (Wilson and Arndt, 2017). This is because with this observed age constraint, the box model of diluted DOC in the deep ocean would result in either (1) labile DOC comprising a relatively large fraction of bulk DOC but with radiocarbon ages similar to or older than bulk radiocarbon ages or (2) a smaller labile DOC pool with much younger radiocarbon ages; the latter would be most consistent with a variety of other observations (Hansell et al., 2012).

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 are prerequisites for exploring the relationship between microbial community composition and the processing of DOM (Logue et al., 2016; Moran et al., 2016). 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 (Kujawinski, 2011; Worden et al., 2015; Moran et al., 2016; Zhang et al., 2016). 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 (Giovannoni, 2017); both are numerically dominant and functionally important groups of marine bacteria (Dang and Jiao, 2014). 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 (Jiao and Zheng, 2011; Tang et al., 2012; Dang and Jiao, 2014). 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 (Tang et al., 2012; Dang and Jiao, 2014). Cottrel and Kirchman (2000) 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. (2017) 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 (Zhang et al., 2015). 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 (Zhang et al., 2015). 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 (Gómez-Consarnau et al., 2012). Multiple reports demonstrate that specific carbon compounds can select for particular species or groups of organisms under different environmental conditions (Rosselló-Mora et al., 2008). 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 (Gómez-Consarnau et al., 2012). 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 dissolved organic carbon that selects for different types of bacteria in a given range (10-100 μM) of substrate concentrations (Sarmento et al., 2016).

The importance of community composition for the fate of DOM has also been shown (Kujawinski et al., 2016; Logue et al., 2016). 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 (Kujawinski et al., 2016). 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 (Crump et al., 1998; DeLong et al., 1993; Moeseneder et al., 2001; Ghiglione et al., 2007; Zhang et al., 2007; Eloe et al., 2011; Tarn et al., 2016). Despite our awareness of the different ecological strategies of particle-associated and free-living microbes (e.g., Dang and Lovell, 2016), we know little about the principles behind the phylogenetic differences and life strategies between free-living and particle-attached microbes in the marine environment (Moeseneder et al., 2001; Herndl and Reinthaler, 2013). 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 (Lauro et al., 2009; Herndl and Reinthaler, 2013). However, Zhang et al. (2016) 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 semilabile organic matter from particles contributing to the bioavailability of dissolved organic carbon (Zhang et al., 2016). 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 (Herndl and Reinthaler, 2013). 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 (Dang and Lovell, 2016; Xie et al., 2018).

Microbes-DOM interaction at the ecosystem level. The finding of Kujawinski et al. (2016) 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 (Jiao et al., 2018a). A 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 (Guidi et al., 2016). 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 (Suttle, 2007), thus increasing carbon export to the deep ocean (Guidi et al., 2016). The importance of viruses in

deeper water is also highlighted in Zhang et al. (2014), who considered viral particles as 'bottom-up' agents fueling the microbial loop in the deep ocean.

Another comprehensive study (McCarren et al., 2010) 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 1. MCP dynamics associated with upwelling activities

Jiao et al. (2014) 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 (Jiao et al., 2014). 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. (2018) made an attempt to analyze 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 (Legendre et al. 2015). 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 (Jiang et al., 2011; Lin et al., 2016) and a biogeochemistry model based on the Carbon, Silicon, Nitrogen Ecosystem module (Xiu and Chai, 2014), 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 (Hansell, 2013), and the satellite-based value of primary production, this model estimated the ratio of MCP to BCP (at the depth of 1,000 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. (2010) highlighted nine major questions regarding MCP processes, which have been addressed at different levels over the past eight 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 (Jiao et al., 2018b). 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. (2018a).

Firstly 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 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., Moran et al., 2016; Xie et al., 2018).

The second proposed approach is to strengthen the understanding of the connections between microbial metabolism and the chemical structure of DOC compounds (e.g., Zhang et al., 2016). 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 (Guidi et al., 2016; Kujawinski et al., 2016; Osterholz et al., 2016).

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 (Legendre et al., 2017; Robinson et al., 2018).

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 (Dang and Chen, 2017). Yet, the claim made 7 years ago that "*..we are woefully unaware of DOM production (or assimilation) mechanisms in the Archaea*" (Kujawinski, 2011) 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 (Zhang et al., 2015; Xie et al., 2018).

The MCP has stimulated provocative and constructive discussions and studies on the processes and mechanisms of RDOC formation and preservation (Jiao et al., 2015; Lechtenfeld et al., 2015; Wilson and Arndt, 2017; Zark et al., 2017; Lønborg et al., 2018; Shen and Benner, 2018). 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., Shen and Benner, 2018).

ACKNOWLEDGEMENTS

Writing of this review benefited greatly from discussions by the PICES-ICES WG members and other participants at the MCP Workshop held in Qingdao and the Yanqi Lake Conference on Global Climate Change held in Beijing, both in September 2017. We thank Penghui Li, Yuwu Jiang, Yuan Shen, and Nannan Wang for their contribution and constructive comments that improved the quality of the manuscript. This MCP project was supported by the State Key R&D project of China grant Nos. 2018YFA0605802 and 2016YFA0601101 (CZ), the National Natural Science Foundation of China (NSFC) grant Nos. 41530105 (CZ), 91428308 (CZ), 91751207 (NJ), 91428308 (NJ) and 41676122 (HD), the CAS-NSFC project Nos. L1624030 and 2016ZWH008A-008 (NJ), the Fundamental Research Funds for the Central Universities 20720170107 (NJ), the Gordon and Betty Moore Foundation award No. 4827 (FA), the NSF grant No. OCE-1538602 (UP), the UK Natural Environment Research Council (NERC) National Capability on Marine Modelling (LP), the UK NERC grant No. NE/R000956/1 (CR), and the Leverhulme Trust grant No. RPG-2017-089 (CR).

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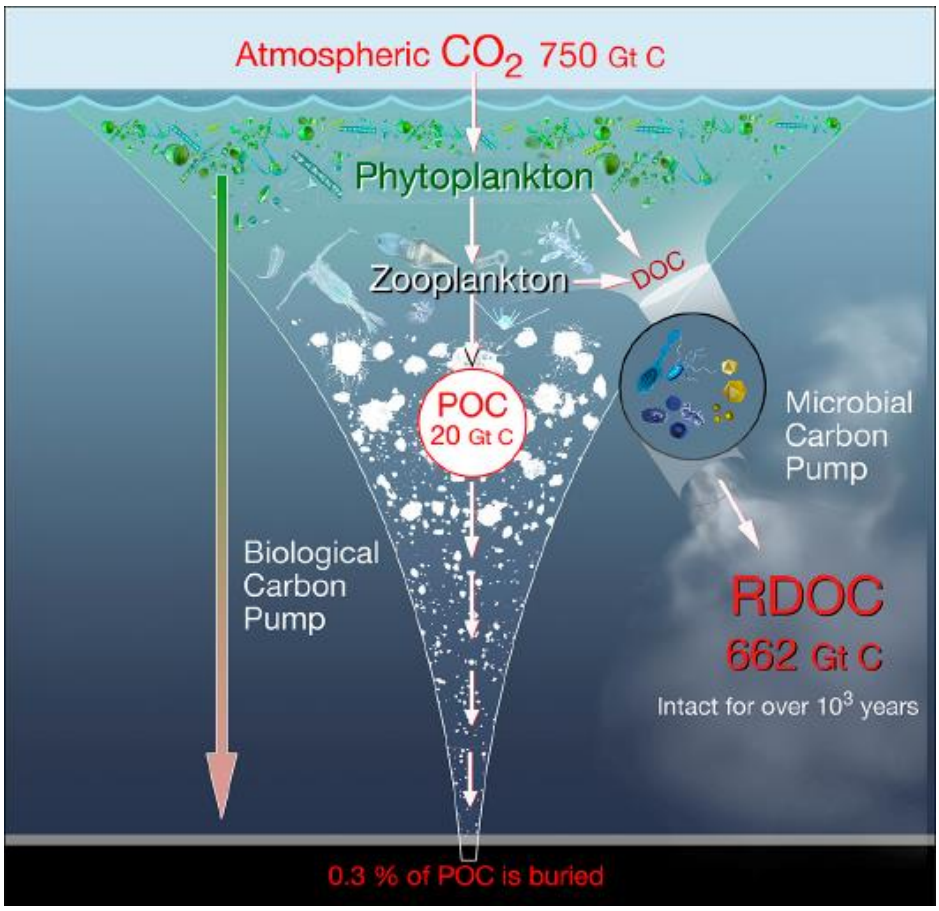

Figure 1. Cycling of biologically produced organic carbon (POC and DOC) in the ocean and links between the seafloor and the atmosphere: The BCP, which transports organic matter from the surface to the interior and floor of the ocean; the MCP, which converts parts of labile organic carbon into RDOC via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

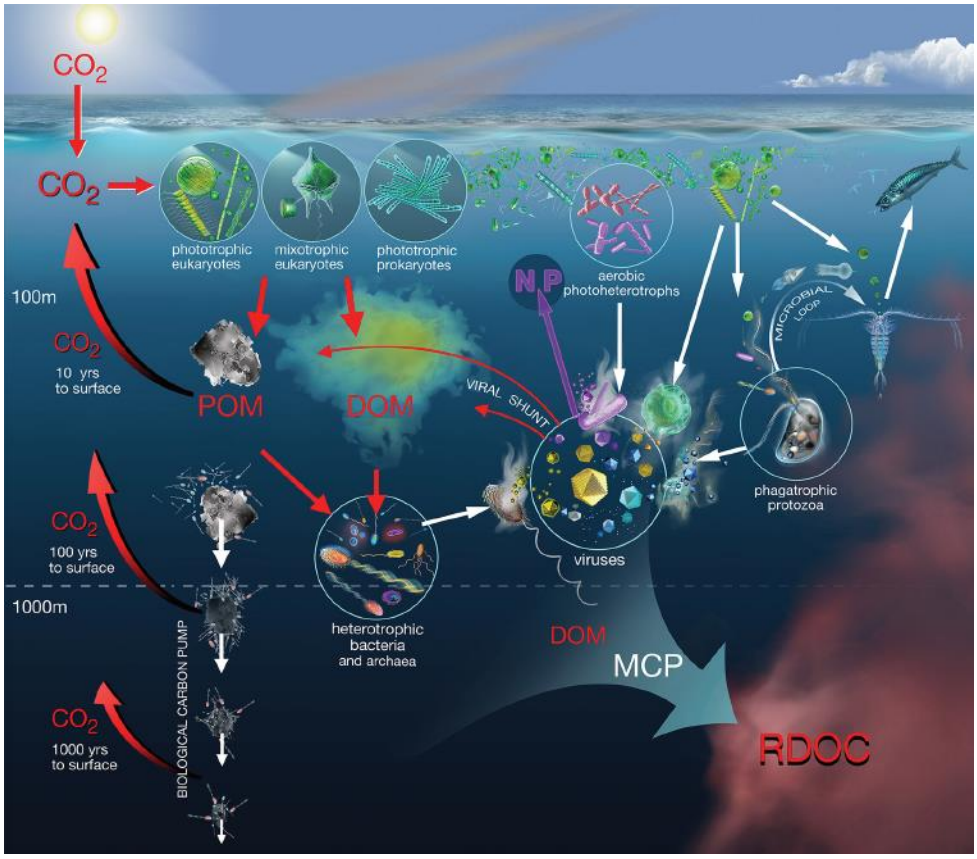

Figure 2. Schematic depiction of the BCP, the ML, and the MCP. The remineralization length scale in the left part of the figure shows the return of respired CO_2 back to the surface, from three depth zones (modified from Passow and Carlson, 2012).

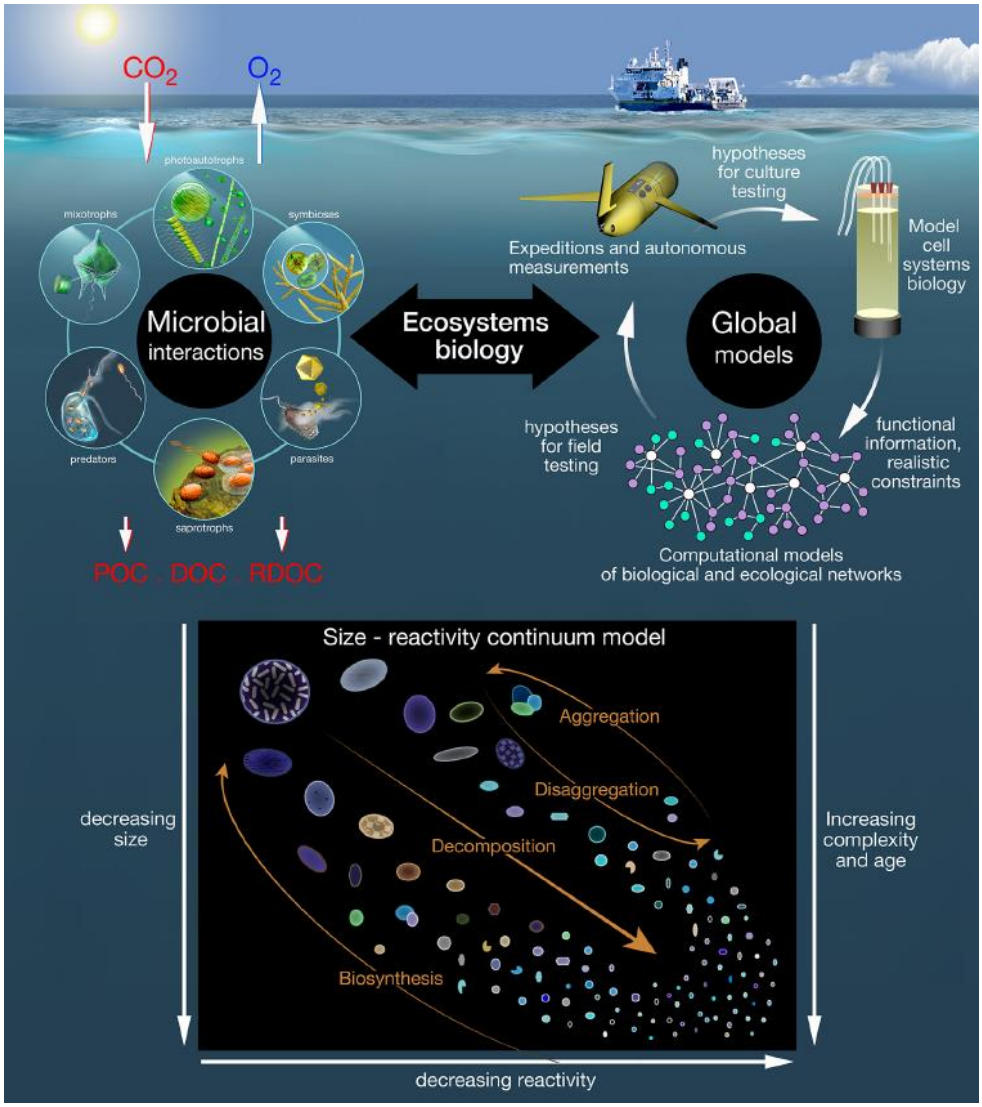

Figure 3. Global biogeochemical and ecological models rely on the present understanding of organismal biology and the interactions between the POM and DOM carbon pools. Modified from Worden et al. (2015). The inset panel is from Benner and Amon (2015), showing a decreasing size and reactivity and an increasing complexity and age of organic molecules along the decomposition pathway. Small dissolved molecules comprise the bulk of RDOC.

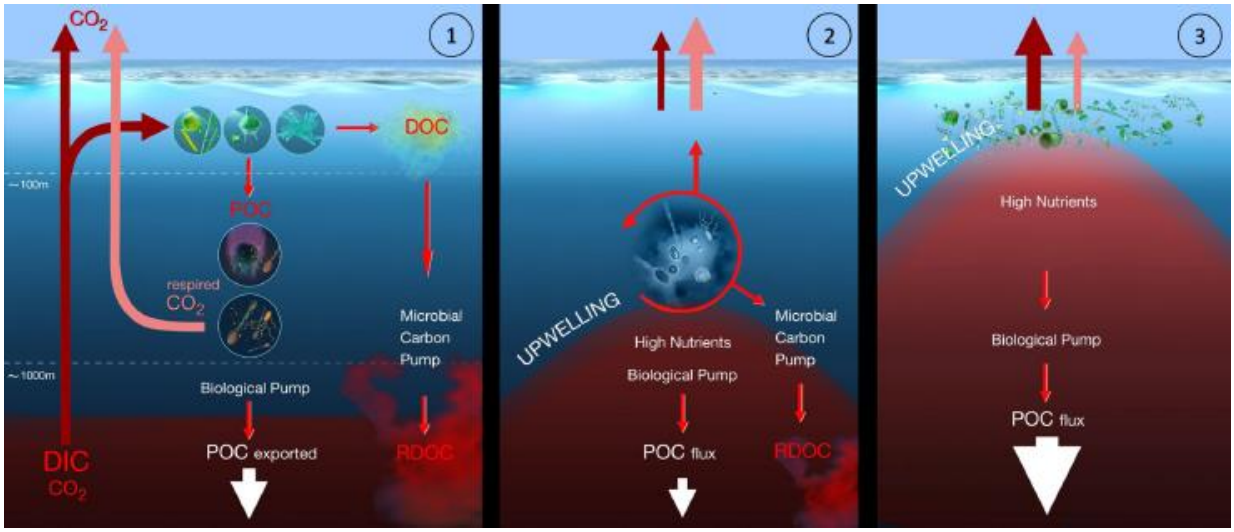

Figure 4. Scenario models for the effects of upwelling on ocean carbon uptake/outgassing dynamics (adopted and modified from Fig. 7 of Jiao et al., 2014). **(1)** Functioning of the BCP and the MCP in a non-upwelling region of the ocean. **(2)** Dominance of the MCP in scenario 1 where the total upward 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.

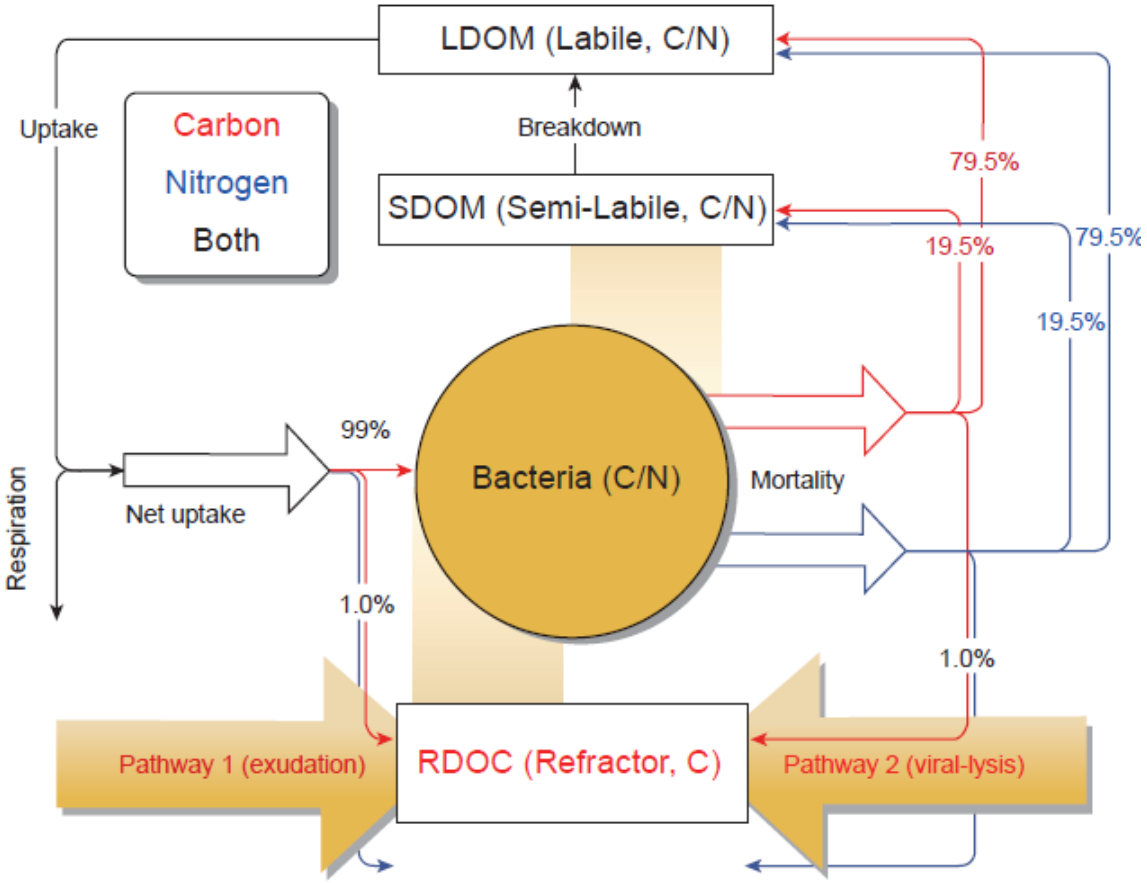

Figure 5. Schematic diagram of the MCP module (Lu et al., 2018). Schematic diagram of the MCP module (from Lu et al., 2018). The RDOC in the model is produced via two bacteria-related pathways: (1) direct exudation by bacteria, and (2) passive release from viral lysis of microbial cells. The additional POC degradation pathway (Jiao et al. 2010) is implicitly included by transforming from POC to labile/semi-labile organic carbon and then to RDOC via aforementioned two pathways (see Lu et al., 2018 for detailed explanation).

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	Volk and Hoffert, 1985; Honjo et al., 2008
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	Azam et al., 1983 Azam, 1998
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	Jiao et al., 2010 Legendre et al., 2015

Table 2: Impacts of the three original publications that defined the ML (Azam et al., 1983), BCP (Volk and Hoffert, 1985) and MCP (Jiao et al., 2010) 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 was up to July 6, 2018. The % 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	BCP	MCP
	% of 3051 citations since 1983	% of 308 citations since 1985	% 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

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

Question asked in Jiao et al. (2010)	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 eight years (see Table 4 for more information)	Arakawa et al., 2017; Benner and Amon, 2015; Lechtenfeld et al., 2014
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	Polimene et al., 2016; Lu et al., 2018
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)	Jiao et al., 2014a; Arrieta et al., 2015; Jiao et al., 2015; Shen and Benner, 2018
What are the structural and biochemical constraints on degradability?	√ √ √	Progress has been made in addressing this question with the past eight years (see Table 4 for more information)	Arakawa et al., 2017; Benner and Amon, 2015; Lechtenfeld et al., 2014; Lechtenfeld et al., 2015
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	Jiao et al., 2014b; Shen and Benner, 2018 Thingstad et al., 1997
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	Arakawa et al., 2017; Lechtenfeld et al., 2014; Flerus et al., 2012 ; Medeiros et al., 2017
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	Tang et al., 2012; Dang and Jiao, 2014
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	Guidi et al., 2016; Kujawinski et al., 2016
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	Shen and Benner, 2018

*The level of progress made most (√ √ √ √) or least (√) since Jiao et al. (2010).

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	Ogawa et al., 2001; Benner and Amon, 2015
D:L amino acids	Ratio of D-enantiomers of amino acids vs. L-enantiomers of amino acids, High D:L ratio in RDOM	Kaiser and Benner, 2009; Benner and Amon, 2015
Lipid-like macromolecules in POM	Acid-insoluble fraction	Hwang et al., 2003
Lipid-like extracts in DOM	Organic solvent extracts, contributing to 0.1-0.3% of DOM_{HMW}	Loh et al., 2004
Glycerol dialkyl glycerol tetraethers, Crenarchaeol	Total lipid extracts in 0.2-0.7 μm fraction	Ingalls et al., 2012
Carotenoid degradation product	Solid-phase extracted (SPE) DOM using comprehensive gas chromatography coupled to mass	Arakawa et al., 2017

	spectrometry; contribute to ~4% of total DOM	
DOC:DON	The percentage of DOC mineralization is negatively correlated with the initial percentage of total fluorescence.	Fellman et al., 2008
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%.	Davis and Benner 2007; Shen et al., 2015
% 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.	Fellman et al., 2008; Fellman et al., 2009a; Fellman et al., 2009b
Humic-like FDOM	Humic-like FDOM (Ex = 320 nm, Em = 420 nm) are thought to be bio-refractory.	Yamashita and Tanoue, 2008
% Protein-like FDOM	The percentage of DOC mineralization is positively correlated with the initial contribution of protein-like fluorescence to the total fluorescence.	Fellman et al., 2008; Fellman et al., 2009a; Fellman et al., 2009b; Cory and Kaplan, 2012
Specific UV absorbance (SUVA)	SUVA positively correlates with aromaticity and thus the recalcitrance of DOM.	Weishaar et al., 2003; Fellman et al., 2008; Fellman et al., 2009a; Fellman et al., 2009b
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.	Benner and Amon, 2015; Walker et al., 2016a,b
CRAM Carboxyl-rich alicyclic molecules (CRAM)	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.	Hertkorn et al., 2006
IOS Island of stability (ISO)	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.	Lechtenfeld et al., 2014
Degradation Index (I_{DEG})	I_{DEG} was developed to compare the degradation state of marine SPE-DOM samples analyzed 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	Flerus et al., 2012

	positively related to DOM recalcitrance.	
Terrestrial indicator compounds	The 184 terrestrial formulas, 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.	Medeiros et al., 2017