



Perspectives on the microbial carbon pump with special reference to microbial respiration and ecosystem efficiency in large estuarine systems

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Abstract. Although respiration-based oxidation of reduced carbon releases CO₂ into the environment, it provides an ecosystem with the metabolic energy for essential biogeochemical processes, including the newly proposed microbial carbon pump (MCP). The efficiency of MCP in heterotrophic microorganisms is related to the mechanisms of energy transduction employed and hence is related to the form of respiration utilized. Anaerobic organisms typically have lower efficiencies of energy transduction and hence lower efficiencies of energy-dependent carbon transformation. This leads to a lower MCP efficiency on a per-cell basis. Substantial input of terrigenous nutrients and organic matter into estuarine ecosystems typically results in elevated heterotrophic respiration that rapidly consumes dissolved oxygen, potentially producing hypoxic and anoxic zones in the water column. The lowered availability of dissolved oxygen and the excessive supply of nutrients such as nitrate from river discharge lead to enhanced anaerobic respiration processes such as denitrification and dissimilatory nitrate reduction to ammonium. Thus, some nutrients may be consumed through anaerobic heterotrophs, instead of being utilized by phytoplankton for autotrophic carbon fixation. In this manner, eutrophied estuarine ecosystems become largely fueled by anaerobic respiratory pathways and their efficiency is less due to lowered ecosystem productivity when compared to healthy and balanced estuarine ecosystems. This situation may have a negative impact on the ecological function and efficiency of the MCP which depends on the supply of both organic carbon and metabolic energy. This review presents our current un-

derstanding of the MCP mechanisms from the view point of ecosystem energy transduction efficiency, which has not been discussed in previous literature.

1 Introduction

The microbial carbon pump (MCP) is a recently proposed biological mechanism for explaining the dynamics of dissolved organic carbon (DOC) transformation and sequestration in the ocean, which involves the production of recalcitrant DOC (RDOC) from labile DOC (LDOC) via microbial processing (Jiao et al., 2010). Millennial mean ages of marine DOC have been observed throughout the water column except in surface water (Loh et al., 2004; Hansell, 2013) and it has been estimated that approximately 155 Pg (10¹⁵ g) of RDOC are currently sequestered via MCP (Benner and Herndl, 2011). In addition to aquatic ecosystems, soil and sediment microbial communities may play similar roles in RDOC production and sequestration (Benner, 2011; Liang and Balser, 2011). The MCP potential of organic carbon sequestration on a global scale is likely to have profoundly impacted the Earth's carbon cycle and potentially also its climate (Wang et al., 2014).

Carbon sequestration by the formation of RDOC is a basic ecosystem property in marine systems in a manner that is similar to what has been described for soil environments (Schmidt et al., 2011). Abiotic and biotic factors that influence the structure, composition and functions of

an ecosystem may also influence the functioning and efficiency of the MCP. Marine microbes differ substantially from one another in their genetic potential, gene expression, and thus their ability to utilize specific DOC compounds, with some microbes being generalists and others being specialists (Gómez-Consarnau et al., 2012). Changes in the abundance or composition of the DOC pool may act as selective pressures that structure the natural microbial communities in these systems (Gómez-Consarnau et al., 2012; Nelson and Carlson, 2012; Nelson et al., 2013). On the other hand, changes in the composition and structure of microbial communities may also impact the abundance and composition of the marine DOC pool, leading to the accumulation of different RDOC compounds with varying ages of persistence in distinct environments.

Heterotrophic bacteria and archaea play a dominant role in the MCP process (Jiao et al., 2010; Benner and Herndl, 2011), which putatively involves three distinct mechanisms, including (1) the active mode pertaining to the release of RDOC via direct microbial secretion or environmental production through extracellular enzymatic activities, (2) the passive mode pertaining to the release of RDOC via grazing and viral lysis, and (3) the threshold mode pertaining to the retention of environmental DOC at low concentration due to its low metabolic efficiency (Jiao and Azam, 2011; Kattner et al., 2011). The synthesis and secretion of extracellular products including enzymes may consume respiratory metabolic energy in heterotrophic microbes. Grazing and viral lysis release LDOC into the environment, stimulating microbial respiration and thus the active mode of the MCP process. In the threshold mode, a DOC substrate must first be sensed or recognized at or above a threshold concentration before it can be utilized, likely at the expense of additional metabolic energy (the cost of synthesis of additional sensory gene products). Thus, energy metabolism seems to be essentially linked to the MCP processes.

Under natural environmental conditions, most of the environmental LDOC that is taken up by microbes is used for respiration (del Giorgio et al., 1997; Brune et al., 2000; Cotner and Biddanda, 2002; del Giorgio and Duarte, 2002; Carlson et al., 2007; Karl, 2007; Robinson and Ramaiah, 2011; Ducklow and Doney, 2013), which likely results in the simultaneous production and secretion of by-product and/or waste-product chemical compounds (often in the form of RDOC). The respiration process not only participates in the MCP via direct production of respiratory RDOC products but also provides metabolic energy to fuel the ecosystem for running MCP processes.

Microorganisms utilize a variety of respiration systems, including both aerobic and anaerobic pathways with distinctly different energy transduction efficiencies, for energy conservation (Burgin et al., 2011; Wright et al., 2012). In general, anaerobic respiration generates less metabolic energy than aerobic respiration (Burgin et al., 2011; Wright et al., 2012). Furthermore, some microbes may harbor sev-

eral different respiration pathways. For example, *Paracoccus denitrificans*, a common environmental bacterium, employs the cytochrome *aa₃* oxidase as the terminal enzyme in its respiratory chain in the presence of high oxygen concentrations to operate an energetically efficient electron-transfer pathway. However, in the presence of low oxygen concentrations, this bacterium mainly employs the high-affinity cytochrome *cbb₃* oxidase (Richardson, 2000). Further, under anoxic conditions, *Paracoccus denitrificans* switches to an anaerobic respiration pathway that employs respiratory enzymes capable of reducing nitrate, nitrite, nitric oxide and nitrous oxide (Richardson, 2000). Thus, even for the same bacterium, different energy transduction efficiencies may pervade under distinct redox conditions. Given that the MCP is mainly fueled by respiratory metabolic energy, the efficiency of the MCP for DOC transformation and sequestration may be better understood by considering contrasting environments such as oxic, suboxic/hypoxic and anoxic marine waters and sediments that harbor different microbial communities with distinctly different energy conservation efficiencies.

2 Linkages of respiratory energy metabolism to MCP

All microorganisms carry out respiration to generate adenosine-5'-triphosphate (ATP) and reducing equivalents, except for obligate fermenters which rely on substrate-level phosphorylation (Carlson et al., 2007). ATP molecules produced by energy transduction processes such as respiration play critical roles in cellular carbon metabolism. ATP, along with proton-motive force (*pmf*) or sodium-motive force (*smf*) in certain microbes (Mulkidjanian et al., 2008), provides the metabolic energy for various essential cellular processes. These include (1) motility and chemotaxis in sensing, signaling and response to environmental cues such as utilizable LDOC substrates; (2) uptake, utilization and transformation of metabolic substrates such as LDOC; (3) biosynthesis and storage of cellular products, some of which may be converted into RDOC once released into environment; (4) DNA replication and cell reproduction; (5) secretion of extracellular compounds such as toxins, metabolic products, by-products and waste products, some of which may be RDOC; and (6) biosynthesis, modification and activity regulation of enzymes (including extracellular enzymes) and other proteins for carrying out the abovementioned processes (Fig. 1). Thus, the MCP-related microbial processes may be fueled in several ways by respiration-generated metabolic energy (in the form of ATP, *pmf* or *smf*).

2.1 Environmental DOC substrate sensing – a critical step of the MCP

In nature, microorganisms often encounter frequently changing or dynamic physicochemical and nutritional conditions. Microorganisms have evolved the mechanisms and

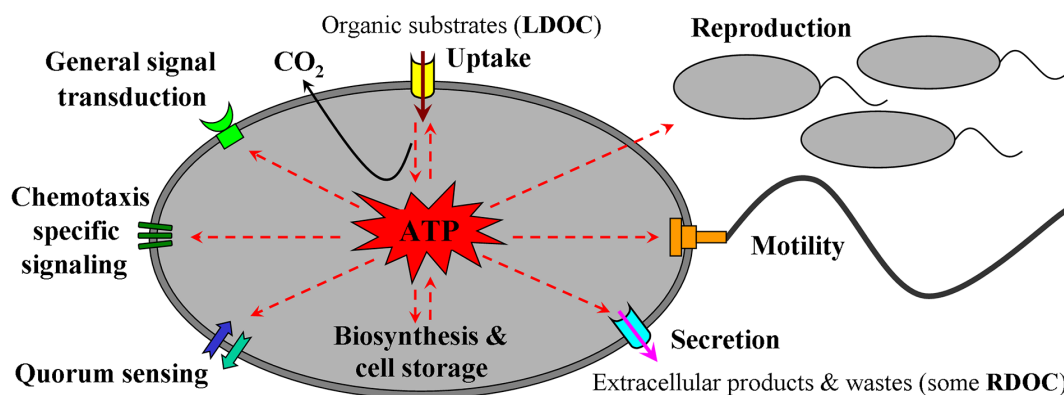


Figure 1. Schematic of metabolic energy transduction by microbial respiration and certain MCP-related cellular processes. These processes are fueled by respiration-generated ATP, which is consumed through the sensing, uptake, transformation and storage of environmental LDOC substrates and the secretion of extracellular products and waste materials that may be related to RDOC production.

machinery to sense and adapt to the changing extracellular conditions (Stocker, 2012). For an environmental LDOC substrate to be taken up and utilized, it needs to first be sensed and recognized by a microbe, resulting in the expression of cross-membrane transporters. Sensing may be the necessary first step for the MCP, i.e., for microbial uptake and transformation of environmental LDOC.

2.1.1 Two-component signal transduction

The two-component signal transduction systems (TCS) are present in more than 95 % and 50 % of currently sequenced bacterial and archaeal genomes, respectively (Wuichet et al., 2010). A single bacterial cell may contain up to hundreds of TCS (Laub and Goulian, 2007). Some microbial TCS can sense simple organic compounds such as sugars and organic acids in the environment, triggering the activation of specific transporter systems for uptake (Galperin, 2010). These TCS may participate directly in MCP for environmental LDOC uptake, utilization and transformation. To the extent that the rate of RDOC formation depends on the ability of microorganisms to sense organic compounds and respond to their presence at a metabolic level (Ogawa et al., 2001), the TCS may enhance the functionality and efficiency of the marine MCP for RDOC production and sequestration.

2.1.2 Chemotaxis

Chemotaxis systems are among the most thoroughly studied TCS (Nixon et al., 1986). In natural aquatic environments, many physicochemical and nutrient conditions are highly dynamic at the microscale. Chemotaxis provides chemotactic bacteria with a competitive advantage relative to non-chemotactic populations, enabling the uptake of nutrients and metabolic substrates that would otherwise be unattainable. Thus, chemotaxis may not only facilitate the microbial loop that channels more carbon into the organic particulate phase (Azam et al., 1983) but also enhance the MCP functioning

and efficiency for RDOC production and sequestration in the ocean (Ogawa et al., 2001).

In contrast to the stimulating effect of chemotactic attractants on some microbial populations, repellent chemicals can drive chemotactic microorganisms away from a given environment. Thus, organic chemotactic repellents may constitute an important source of RDOC or at least environmental context-specific RDOC (RDOC_{context}) (Jiao et al., 2014, this issue) and therefore may contribute to the sequestration of organic carbon in the ocean.

2.1.3 Quorum sensing

Microbes utilize quorum sensing (QS) for population density-dependent sensing, signaling and response, which may include synchronized production and secretion of toxins, extracellular polysaccharides (EPS), pigments, siderophores, biosurfactants and exoenzymes (West et al., 2012). The production or secretion of these compounds may contribute to the production and sequestration of RDOC or RDOC_{context} in the ocean.

QS is typically activated when high microbial density is reached. In marine environments, high microbial population densities are often achieved via the formation of biofilm communities which may live on surfaces or on suspended particles, detritus, aggregates and marine snows (Azam and Long, 2001; Simon et al., 2002). Particle-associated microbes play important roles in the biogeochemical cycling of C, N and S, in estuarine, coastal and deep-sea environments (Aristegui et al., 2002; Dang and Lovell, 2002; Bochdansky et al., 2010; Eloë et al., 2011; Smith et al., 2013). Particle-associated communities contribute to the degradation of particulate organic matter (POM) and may account for as much as 90 % of the total water column heterotrophic bacterial activity (Crump et al., 1999; Turley and Stutt, 2000; Simon et al., 2002). Particle-associated microbes provide LDOC substrates through the enzymatic decomposition of biopolymers

(Cho and Azam, 1988). Biopolymer degradation may also produce RDOC directly (Jiao and Azam, 2011).

Particle-associated bacteria have been found to produce acylated homoserine lactones (AHLs), the major type of QS autoinducers (Gram et al., 2002). AHLs have also been identified in marine surface-associated microbial communities (Decho et al., 2009; Huang et al., 2009). The bacterium *Kordia algicida* relies on QS-dependent excretion of an algicidal protein to suppress activity of marine diatoms such as *Skeletonema costatum*, *Thalassiosira weissflogii* and *Phaeodactylum tricorutum* (Paul and Pohnert, 2011). Algicidal bacteria, such as *K. algicida*, may contribute not only to the termination of algal blooms, but also to the release of algal particulate organic carbon (POC) and DOC, thus influencing the MCP process. QS may influence the activity of extracellular hydrolytic enzymes on marine particles (Hmelo et al., 2011). By participating in the regulation of POC degradation and DOC production, microbial QS may impact the marine biological pump (BP) (Hmelo et al., 2011) and the MCP as well.

2.1.4 Interwoven networks of cellular sensing, signaling and response

Bacteria and archaea employ complicated regulatory networks for extracellular resource utilization to achieve optimal growth and maintenance. It has been found that the addition of high-molecular-weight (HMW) dissolved organic matter (DOM) to marine samples significantly stimulates microbial community gene expression of TCS, chemotaxis and motility (McCarren et al., 2010). It has also been found that some simple sugars such as glucose are not utilized by oligotrophic bacteria inhabiting the Sargasso Sea (Nelson and Carlson, 2012). The lack of a suitable glucose-sensing membrane apparatus and thus the incapability to recognize or sense glucose may be the cause of this phenomenon.

The sensing of LDOC represents a little-explored area of research that is ripe for study and will enhance our understanding of the MCP. The microbial sensing and response network, fueled primarily by respiratory processes as described above, may have profound impacts on the sequestration of organic carbon in the ocean.

2.2 Metabolic energy-fueled transmembrane transportation and secretion

Heterotrophic microbes are the most important consumers of marine organic matter (OM), driving the microbial loop and MCP (Azam et al., 1983; Azam and Malfatti, 2007; Jiao et al., 2010), primarily through the utilization of metabolic energy-driven transport systems for the uptake of organic substrates. Bacteria and archaea also utilize energy-driven transport systems for the secretion of extracellular enzymes and compounds (including certain RDOC) to meet their metabolic needs.

2.2.1 ATP binding cassette transporters

The ATP binding cassette (ABC) transporters constitute the most common and versatile transport systems in microorganisms (Lee et al., 2007; George and Jones, 2012; Rinta-Kanto et al., 2012). Some ABC importers may have broad substrate specificity, although most ABC importers exhibit high substrate specificity (Couñago et al., 2012). High-affinity substrate-binding proteins that specifically associate with their ligands, together with their cognate ABC importers, facilitate the unidirectional translocation of specific substrates (Couñago et al., 2012). This mechanism results in specific substrate uptake of environmental DOC compounds. Due to the specificity of ABC importers, certain DOC compounds may not be taken up and utilized by a microbial assemblage and may result in accumulation in the environment.

Various “omic”-based approaches have provided further insights into organic carbon cycling in natural systems. The “eutrophic” *Roseobacter* clade, a numerically dominant and functionally important group of marine alphaproteobacteria, harbors diverse carbohydrate-related ABC importer genes, implying their involvement in the carbohydrate-related DOC utilization or transformation (Poretsky et al., 2010; Jiao and Zheng, 2011). By contrast, the “oligotrophic” SAR11 clade, another important group of marine alphaproteobacteria, harbors a great deal more ABC importer genes for the uptake of amino acids and other nitrogenous compounds than for the uptake of carbohydrates (Sowell et al., 2009, 2011; Poretsky et al., 2010; Jiao and Zheng, 2011; Zeigler Allen et al., 2012; Ottesen et al., 2013). Many common carbohydrate compounds cannot be utilized by SAR11 isolates (Schwalbach et al., 2010). The contrasting ABC importer machineries associated with the *Roseobacter* clade and the SAR11 clade may be closely related to their distinct niches and ecophysiological adaptations. Their different environmental DOC utilization profiles may also exemplify the potential for RDOC_{context} accumulation in different marine habitats.

Bacteria and archaea harbor diverse ABC exporters important in the secretion of extracellular enzymes, polysaccharides, toxins, antimicrobial agents and other compounds (Binet et al., 1997; Omori and Idei, 2003; Davidson and Chen, 2004; Dawson et al., 2007; Cuthbertson et al., 2009; Lalithambika et al., 2012). The association of genes encoding biopolymer degradation enzymes with ABC exporters, usually within the same operon, facilitates efficient and tightly controlled secretion of extracellular enzymes (Omori and Idei, 2003). Thus, ABC exporters participate in POC decomposition and DOC production, contributing to the MCP process and forming a linkage between the POC-based BP and the RDOC-based MCP. ABC transporters are also involved in the secretion of recalcitrant EPS and capsular polysaccharides and the extrusion of various waste products, toxins and antimicrobial compounds, which may persist as RDOC in the environment (Jiao and Zheng, 2011).

A number of bacteria use the type VI secretion system (T6SS), which may be partially ATP-dependent, to kill other bacteria that coexist in the same microhabitat (Basler et al., 2013; Casabona et al., 2013). Enzymes, such as muramidases, lipases and phospholipases that hydrolyze bacterial peptidoglycans or disrupt cell membrane integrity were identified as the relevant effectors delivered by the T6SS (Russell et al., 2011, 2013; Dong et al., 2013). The T6SS-mediated antibacterial activities result in the release of cellular LDOC and some RDOC (such as certain refractory bacterial cell wall components) into the environment from lysed bacteria, contributing to the functioning of the MCP. More than a quarter of bacteria studied have been found to harbor the T6SS (Bingle et al., 2008), indicating its importance in bacterial survival and competition (Schwarz et al., 2010) and its potential role in mediating carbon cycling in natural environments.

2.2.2 TonB-dependent transporters

TonB-dependent transporters (TBDT) play a role in microbial uptake of ion complexes (such as Fe, Ni, Co and Cu), vitamin B₁₂, vitamin B₁, heme, carbohydrates, lipids, aromatic hydrocarbons and/or their breakdown products (Schauer et al., 2008; Miller et al., 2010; Noinaj et al., 2010; Dupont et al., 2012). Bacteroidetes are particularly rich in carbohydrate-assimilation TBDT (Blanvillain et al., 2007; González et al., 2008; Hehemann et al., 2010; Tang et al., 2012) and biopolymer degradation enzymes (McBride et al., 2009; Hehemann et al., 2010; Qin et al., 2010; Thomas et al., 2012; Mann et al., 2013). Many Bacteroidetes glycoside hydrolase genes are organized in polysaccharide utilization loci, usually clustered with genes for TonB-dependent receptors, SusD-like proteins, sensors/transcription factors, transporters and frequently with genes for sulfatases (McBride et al., 2009; Hehemann et al., 2010; Qin et al., 2010; Thomas et al., 2012; Fernández-Gómez et al., 2013; Mann et al., 2013). This indicates coordinated degradation, transportation and utilization of extracellular polysaccharides and their breakdown products. Environmental sequences of Bacteroidetes TBDT-related proteins, genes and their transcripts have been frequently found in abundance in metaproteomic, metagenomic and metatranscriptomic sequences from various ocean habitats, especially in coastal waters (Morris et al., 2010; Ottesen et al., 2011; Tang et al., 2012). Bacteroidetes also harbor diverse genes for the degradation of proteins, chitin and bacterial cell wall peptidoglycans (Cottrell and Kirchman, 2000; McBride et al., 2009; Qin et al., 2010; Gómez-Pereira et al., 2012; Mann et al., 2013). Thus, Bacteroidetes have been regarded as specialists for degradation of HMW biomacromolecules in both the particulate and dissolved fraction of the marine OM pool (Bauer et al., 2006). They thus are likely to contribute substantially to the transformation of POC to DOC, HMW DOC to low-molecular-weight

(LMW) DOC, and the accumulation of certain RDOC in the ocean.

Certain Proteobacteria subgroups are also rich in TBDT (Blanvillain et al., 2007; Tang et al., 2012). Genomic and metagenomic studies have identified Gammaproteobacteria, particularly in the Alteromonadales order, harboring diverse TBDT (Tang et al., 2012). The addition of HMW DOM to marine microbial communities could significantly stimulate the expression of TBDT-related genes in *Alteromonas* and *Idiomarina* (McCarren et al., 2010), suggesting a role of TBDT in environmental DOM uptake and assimilation (Tang et al., 2012). Alteromonadales harbor diverse extracellular hydrolytic enzymes and prefer living in a marine particle-associated lifestyle (Ivars-Martinez et al., 2008; Thomas et al., 2008; Oh et al., 2011), thus contributing to POC degradation and fueling of the marine microbial loop and MCP (Azam and Long, 2001; Jiao et al., 2010).

SAR86, another dominant marine Gammaproteobacteria subgroup, also harbor abundant TBDT, which may be involved in the uptake and metabolism of large polysaccharides and lipids (Dupont et al., 2012; Ottesen et al., 2013). SAR86 may also degrade peptidoglycans, producing D-amino acids as by-products (Dupont et al., 2012). However, unlike particle-associated Bacteroidetes and Alteromonadales, SAR86 bacteria are predominantly free-living (Dupont et al., 2012). Niche differentiation between SAR86 and Alteromonadales or Bacteroidetes may therefore facilitate resource partitioning, exemplifying the basic principle of resource-driven competition and coexistence in the ocean.

Environmental LDOC substrate uptake constitutes a critical step in the MCP process. If an environmental LDOC substrate cannot be taken up by any microbe in an ecosystem, it is likely to persist as RDOC in the environment. The microbial substrate sensing and uptake mechanisms are useful to consider when characterizing the potential recalcitrance of DOC compounds in natural environments. Furthermore, the MCP efficiency is intrinsically related to the microbial energy metabolic efficiency in substrate sensing, transportation and transformation.

3 Biogeochemical linkage of microbial respiration and MCP in estuaries – aerobic vs. anaerobic respirations and related shift of estuarine ecosystem functioning

Due to anthropogenic impacts and intense interactions between the terrestrial and marine compartments of the Earth system, estuaries represent some of the most complex and dynamic ecosystems on Earth. Rivers discharge large amounts of terrigenous materials, such as nutrients, organic matter, suspended particles, wastes and pollutants, into estuaries and coastal seas. The flow of material and energy through the estuarine system in the land–ocean continuum strongly impacts the metabolism and functioning of the in

situ ecosystem, which, in turn, determines the net autotrophic or heterotrophic status of the coastal system and its role in the global carbon cycle. In the past, large river estuaries sustained high marine productivity and fisheries; however, at present, many estuaries and their associated coastal seas have been experiencing frequent and intense environmental and ecological perturbations, including eutrophication, blooms of harmful phytoplankton and gelatinous zooplankton, hypoxia, anoxia and seawater acidification (Anderson et al., 2002, 2012; Xian et al., 2005; Paerl et al., 2006; Breitburg et al., 2009; Rabalais et al., 2009, 2010; Condon et al., 2011; Duarte et al., 2013). The input of terrigenous nutrients and OM, mainly from crop fertilizer applications and wastewater discharge contributes to the deterioration of the estuarine ecosystems worldwide.

Estuaries mediate a large fraction of the global carbon cycle due to anthropogenic eutrophication (Doney, 2010). Excessive riverine supply of nutrients and OM strongly stimulates microbial respiration that may rapidly consume dissolved O_2 in impacted seawater and sediments, producing hypoxic and even anoxic zones near estuaries. Estuarine waters are also characterized by high turbidity caused by input of riverine suspended particles, creating low-light habitats and suboxic and anoxic microhabitats with varying microscale redox and nutrient gradients (Stocker, 2012). O_2 -limited or O_2 -depleted conditions enhance the metabolic activities of anaerobic bacteria and archaea, which divert the flow of available energy away from higher trophic levels (Diaz and Rosenberg, 2008). The difference in energetic efficiency of metabolism between anaerobic respiration and aerobic respiration appears to be consistent with research suggesting that hypoxic and anoxic zones are usually a net source of CO_2 (Doney et al., 2009; Cai et al., 2011; Melzner et al., 2013). Thus, eutrophied estuaries are usually net heterotrophic in nature and can become acidified due to CO_2 production (Frankignoulle et al., 1998; Cai, 2011).

3.1 Varying MCP efficiency in distinct estuarine environments

Due to the intrinsic connection of respiratory energy transduction and MCP functioning, anaerobic and aerobic microbes are likely to have distinctly different DOC processing efficiencies and thus different contributions to RDOC production and sequestration in the ocean. In hypoxic and anoxic seawater and sediments, the flow of energy typically follows a well-defined sequence of redox reactions determined by the amount of free energy extractable from each reaction (Wright et al., 2012). For microbial respiration, free oxygen is the most favorable electron acceptor while sulfate is utilized as an electron acceptor only after nitrate, nitrite, manganese oxides and iron oxides are exhausted. This sequential order not only defines specific metabolic niches and biogeochemical potentials spanning oxic, suboxic and anoxic environmental conditions (Wright et al., 2012), but also determines the net

energetics of community metabolism. For example, different forms of anaerobic respiration, such as denitrification, manganese oxide reduction, dissimilatory nitrate reduction to ammonium (DNRA), iron oxide reduction and sulfate reduction, have distinctly different and sequentially decreasing respiratory energy transduction efficiencies (Burgin et al., 2011; Lam and Kuypers, 2011; Wright et al., 2012), which may greatly impact MCP carbon sequestration efficiency in the ocean (Fig. 2). Although estuarine hypoxia and anoxia have already been recognized as a major global environmental problem with significant deleterious effects (Diaz and Rosenberg, 2008), the continually expanding estuarine and coastal hypoxic zones may interact with the expanding open-ocean oxygen minimum zones (OMZs), which may potentially give rise to even more severe environmental and ecological consequences (Gilly et al., 2013). However, there is currently no research that links in situ microbial community respiration and energy transduction efficiency with the functionality and efficiency of MCP in different environmental statuses of an estuarine ecosystem.

3.2 A putative mechanism of the MCP for RDOC production in estuaries

It has been suggested that microbial enzymatic activity plays an important role in the formation of small-molecule RDOC (Amon and Benner, 1996; Ogawa et al., 2001). Nonspecific or promiscuous enzymatic activities may produce abnormal organic molecules that may no longer be recognizable as substrates and thus no longer utilizable by microbes (O'Brien and Herschlag, 1999; Ogawa et al., 2001). This mechanism could be responsible for much of the detrital carbon that is sequestered in the ocean (Ogawa et al., 2001). Steep physicochemical gradients and fluctuations of environmental conditions exist in estuarine systems. Rivers may also transport heavy metals, toxic organic compounds and other antimicrobial substances to estuaries. The harmful and varying environmental conditions may represent physiological stressors to estuarine microbes. Under such conditions, microbes may be prone to produce more abnormal compounds caused by suboptimal or even disrupted enzymatic synthesis or transformation of metabolic compounds. Whether this scenario represents a realistic MCP mechanism of enhanced RDOC production in the estuarine microbiota remains unsolved and warrants further investigation.

3.3 Negative impacts of eutrophication on the estuarine MCP efficiency

Although many questions remain concerning the MCP efficiency and capacity for carbon sequestration in estuaries, the multitude of harmful environmental effects caused by escalated anthropogenic activities and global climate change have been established with high certainty. Estuarine hypoxia and repletion of nutrients such as nitrate originating

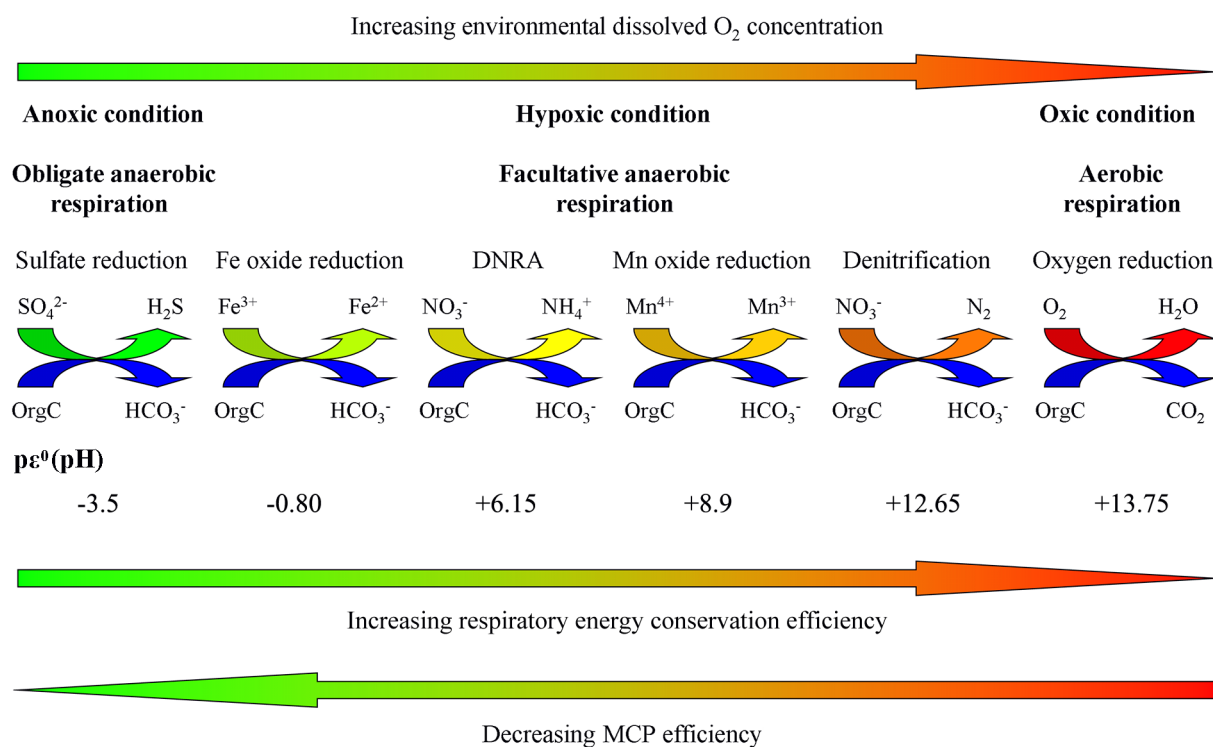


Figure 2. Schematic of microbial aerobic and anaerobic respiration pathways and their potentially different contribution to cellular metabolic energy transduction and MCP efficiencies. $p\varepsilon^0$ (pH) values refer to the electron activity for unit activities of oxidants and reductants at neutral pH (Nealson and Saffarini, 1994).

anthropogenically from soil and river systems may stimulate enhanced anaerobic respiration such as denitrification, which may increase the production and release of N₂O and other greenhouse gases (Naqvi et al., 2000; Wright et al., 2012). Nitrogenous nutrients, such as nitrate, nitrite and ammonium, are also consumed to produce N₂ by marine anaerobic ammonium-oxidizing bacteria in suboxic and anoxic aquatic and sediment environments, and are likely coupled to respiratory DNRA or denitrification (Jetten et al., 2009; Lam et al., 2009; Zehr, 2009). In certain coastal OMZs, there exists a cryptic S cycle, coupled to intensified denitrification and organic carbon mineralization processes (Thamdrup et al., 2010). Many heterotrophic microbes in general also assimilate nitrate and ammonium for biomass production (Cabello et al., 2004; Luque-Almagro et al., 2011; Zehr and Kudela, 2011). Thus, estuarine nutrients such as nitrate may be consumed mainly by heterotrophic microorganisms (especially by anaerobes) rather than being utilized by phytoplankton for carbon fixation and primary production. In this situation, the ecological function of the estuarine ecosystem is altered and the ecological efficiency is lowered, as less metabolic energy and fixed carbon can be produced through anaerobic pathways when compared to aerobic or phototrophic pathways. This may also negatively influence the ecological efficiency of MCP for carbon sequestration. In line with this logic, it has been found that short-term nutrient disturbances such

as those caused by wind-driven upwelling, forest fires and desert dust depositions can stimulate coastal microbial respiration significantly and thus shift coastal ecosystems strongly towards net heterotrophy (Bonilla-Findji et al., 2010). On the global scale, it has been found that the ecosystem organic carbon pool exhibits consistent and negative correlations with nitrate accrual along a hydrologic continuum from soils, through freshwater systems and coastal margins, to the open ocean (Taylor and Townsend, 2010). Another study has shown that the increase of nitrogen deposition in soils may negatively influence the terrestrial MCP for RDOC sequestration (Liang and Balser, 2012). Due to the increased overloading of nitrogenous nutrients from anthropogenic sources, nutrient eutrophication may also negatively influence the efficiency of the marine MCP for RDOC production and sequestration in estuarine environments.

4 Conclusions and perspectives

The MCP provides a fundamental framework for designing new studies aimed at improving the understanding of carbon sequestration mechanisms different from that of the BP (Jiao et al., 2010), which is more distinct in estuarine and coastal seas where light availability is limited but nutrients and DOC are replete. However, impacts induced by

anthropogenic perturbations and climate change may alter the efficacy of the MCP in the estuarine and coastal environments.

The incorporation of respiration into the MCP theoretical framework provides the basis from which marine carbon cycling and sequestration can be understood and evaluated in terms of energy flow and budget. This is important as both MCP and BP may have the potential to enhance carbon sequestration in the ocean. However, any strategy targeting climate change mitigation must not harm the environment and ecosystem (Lawrence, 2002; Glibert et al., 2008; Lampitt et al., 2008; Smetacek and Naqvi, 2008). Through a consideration of respiratory CO₂ emission and metabolic energy transduction, the efficiency of the MCP in different environments or ecosystems, such as the estuarine, coastal, continental shelf and open-ocean areas, and in the distinctly different oxic, suboxic and anoxic water zones can be compared.

It has been hypothesized that reduction of the discharge of excessive terrigenous nutrients and OM into the estuarine and coastal seas may enhance the MCP efficacy (Jiao et al., 2011). Thus, reducing anthropogenic inputs may not only mitigate various environmental and ecological problems but also enhance carbon sequestration in estuaries. The integrated consideration of marine microbial community respiration and MCP functioning may help to develop engineering strategies to enhance carbon sequestration in the ocean and to mitigate anthropogenic impacts on the estuarine and coastal environments.

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References

- Amon, R. M. W. and Benner, R.: Bacterial utilization of different size classes of dissolved organic matter, *Limnol. Oceanogr.*, 41, 41–51, 1996.
- Anderson, D. M., Glibert, P. M., and Burkholder, J. M.: Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences, *Estuaries*, 25, 704–726, 2002.
- Anderson, D. M., Cembella, A. D., and Hallegraeff, G. M.: Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management, *Annu. Rev. Mar. Sci.*, 4, 143–176, 2012.
- Aristegui, J., Duarte, C. M., Agustí, S., Doval, M., Alvarez-Salgado, X., and Hansell, D. A.: Dissolved organic carbon support of respiration in the dark ocean, *Science*, 298, 5600, doi:10.1126/science.1076746, 2002.
- Azam, F. and Long, R. A.: Sea snow microcosms, *Nature*, 414, 495–498, 2001.
- Azam, F. and Malfatti, F.: Microbial structuring of marine ecosystems, *Nat. Rev. Microbiol.*, 5, 782–791, 2007.
- Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, F.: The ecological role of water-column microbes in the sea, *Mar. Ecol.-Prog. Ser.*, 10, 257–263, 1983.
- Basler, M., Ho, B. T., and Mekalanos, J. J.: Tit-for-tat: type VI secretion system counterattack during bacterial cell-cell interactions, *Cell*, 152, 884–894, 2013.
- Bauer, M., Kube, M., Teeling, H., Richter, M., Lombardot, T., Allers, E., Würdemann, C. A., Quast, C., Kuhl, H., Knaust, F., Woebken, D., Bischof, K., Musmann, M., Choudhuri, J. V., Meyer, F., Reinhardt, R., Amann, R. I., and Glöckner, F. O.: Whole genome analysis of the marine *Bacteroidetes* “*Gramella forsetii*” reveals adaptations to degradation of polymeric organic matter, *Environ. Microbiol.*, 8, 2201–2213, 2006.
- Benner, R.: Biosequestration of carbon by heterotrophic microorganisms, *Nat. Rev. Microbiol.*, 9, 75, doi:10.1038/nrmicro2386-c3, 2011.
- Benner, R. and Herndl, G. J.: Bacterially derived dissolved organic matter in the microbial carbon pump, in: *Microbial Carbon Pump in the Ocean*, edited by: Jiao, N., Azam, F., and Sanders, S., Science/AAAS, Washington DC, 46–48, 2011.
- Binet, R., Létoffé, S., Ghigo, J. M., Deleplaire, P., and Wandersman, C.: Protein secretion by Gram-negative bacterial ABC exporters – a review, *Gene*, 192, 7–11, 1997.
- Bingle, L. E., Bailey, C. M., and Pallen, M. J.: Type VI secretion: a beginner’s guide, *Curr. Opin. Microbiol.*, 11, 3–8, 2008.
- Blanvillain, S., Meyer, D., Boulanger, A., Lautier, M., Guynet, C., Denancé, N., Vasse, J., Lauber, E., and Arlat, M.: Plant carbohydrate scavenging through TonB-dependent receptors: a feature shared by phytopathogenic and aquatic bacteria, *PLoS One*, 2, e224, doi:10.1371/journal.pone.0000224, 2007.
- Bochdansky, A. B., van Aken, H. M., and Herndl, G. J.: Role of macroscopic particles in deep-sea oxygen consumption, *P. Natl. Acad. Sci. USA*, 107, 8287–8291, 2010.
- Bonilla-Findji, O., Gattuso, J.-P., Pizay, M.-D., and Weinbauer, M. G.: Autotrophic and heterotrophic metabolism of microbial planktonic communities in an oligotrophic coastal marine ecosystem: seasonal dynamics and episodic events, *Biogeosciences*, 7, 3491–3503, doi:10.5194/bg-7-3491-2010, 2010.
- Breitburg, D. L., Hondorp, D. W., Davias, L. A., and Diaz, R. J.: Hypoxia, nitrogen, and fisheries: Integrating effects across local and global landscapes, *Annu. Rev. Mar. Sci.*, 1, 329–349, 2009.
- Brune, A., Frenzel, P., and Cypionka, H.: Life at the oxic-anoxic interface: microbial activities and adaptations, *FEMS Microbiol. Rev.*, 24, 691–710, 2000.
- Burgin, A. J., Yang, W. H., Hamilton, S. K., and Silver, W. L.: Beyond carbon and nitrogen: how the microbial energy economy couples elemental cycles in diverse ecosystems, *Front. Ecol. Environ.*, 9, 44–52, 2011.
- Cabello, P., Roldan, M. D., and Moreno-Vivian, C.: Nitrate reduction and the nitrogen cycle in archaea, *Microbiology*, 150, 3527–3546, 2004.
- Cai, W. J.: Estuarine and coastal ocean carbon paradox: CO₂ sinks or sites of terrestrial carbon incineration?, *Annu. Rev. Mar. Sci.*, 3, 123–145, 2011.
- Cai, W. J., Hu, X. P., Huang, W. J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., Chou, W. C., Zhai, W. D., Hollibaugh, J. T., Wang, Y. C., Zhao, P. S., Guo, X. H., Gundersen, K., Dai, M. H., and Gong, G. C.: Acidification of subsurface coastal waters enhanced by eutrophication, *Nat. Geosci.*, 4, 766–770, 2011.

- Carlson, C. A., del Giorgio, P. A., and Herndl, G. J.: Microbes and the dissipation of energy and respiration: from cells to ecosystems, *Oceanography*, 20, 89–100, 2007.
- Casabona, M. G., Silverman, J. M., Sall, K. M., Boyer, F., Couté, Y., Poirel, J., Grunwald, D., Mougous, J. D., Elsen, S., and Attree, I.: An ABC transporter and an outer membrane lipoprotein participate in posttranslational activation of type VI secretion in *Pseudomonas aeruginosa*, *Environ. Microbiol.*, 15, 471–486, 2013.
- Cho, B. C. and Azam, F.: Major role of bacteria in biogeochemical fluxes in the ocean's interior, *Nature*, 332, 441–443, 1988.
- Condon, R. H., Steinberg, D. K., del Giorgio, P. A., Bouvier, T. C., Bronk, D. A., Graham, W. M., and Ducklow, H. W.: Jellyfish blooms result in a major microbial respiratory sink of carbon in marine systems, *P. Natl. Acad. Sci. USA*, 108, 10225–10230, 2011.
- Cotner, J. B. and Biddanda, B. A.: Small players, large role: Microbial influence on biogeochemical processes in pelagic aquatic ecosystems, *Ecosystems*, 5, 105–121, 2002.
- Cottrell, M. T. and Kirchman, D. L.: Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter, *Appl. Environ. Microbiol.*, 66, 1692–1697, 2000.
- Couñago, R. M., McDevitt, C. A., Ween, M. P., and Kobe, B.: Prokaryotic substrate-binding proteins as targets for antimicrobial therapies, *Curr. Drug Targets*, 13, 1400–1410, 2012.
- Crump, B. C., Armbrust, E. V., and Baross, J. A.: Phylogenetic analysis of particle-associated and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean, *Appl. Environ. Microbiol.*, 65, 3192–3204, 1999.
- Cuthbertson, L., Mainprize, I. L., Naismith, J. H., and Whitfield, C.: Pivotal roles of the outer membrane polysaccharide export and polysaccharide copolymerase protein families in export of extracellular polysaccharides in gram-negative bacteria, *Microbiol. Mol. Biol. Rev.*, 73, 155–177, 2009.
- Diaz, R. J. and Rosenberg, R.: Spreading dead zones and consequences for marine ecosystems, *Science*, 321, 926–929, 2008.
- Dang, H. Y. and Lovell, C. R.: Seasonal dynamics of particle-associated and free-living marine *Proteobacteria* in a salt marsh tidal creek as determined using fluorescence *in situ* hybridization, *Environ. Microbiol.*, 4, 287–295, 2002.
- Davidson, A. L. and Chen, J.: ATP-binding cassette transporters in bacteria, *Annu. Rev. Biochem.*, 73, 241–268, 2004.
- Dawson, R. J., Hollenstein, K., and Locher, K. P.: Uptake or extrusion: crystal structures of full ABC transporters suggest a common mechanism, *Mol. Microbiol.*, 65, 250–257, 2007.
- Decho, A. W., Visscher, P. T., Tomohiro, J. F., He, K. L., Przekop, K. M., Norman, R. S., and Reid, R. P.: Autoinducers extracted from microbial mats reveal a surprising diversity of N-acylhomoserine lactones (AHLs) and abundance changes that may relate to diel pH, *Environ. Microbiol.*, 11, 409–420, 2009.
- del Giorgio, P. A. and Duarte, C. M.: Respiration in the open ocean, *Nature*, 420, 379–384, 2002.
- del Giorgio, P. A., Cole, J. J., and Cimleris A.: Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems, *Nature*, 385, 148–151, 1997.
- Doney, S. C.: The growing human footprint on coastal and open-ocean biogeochemistry, *Science*, 328, 1512–1516, 2010.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: The other CO₂ problem, *Annu. Rev. Mar. Sci.*, 1, 169–192, 2009.
- Dong, T. G., Ho, B. T., Yoder-Himes, D. R., and Mekalanos, J. J.: Identification of T6SS-dependent effector and immunity proteins by Tn-seq in *Vibrio cholerae*, *P. Natl. Acad. Sci. USA*, 110, 2623–2628, 2013.
- Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., Carstensen, J., Trotter, J. A., and McCulloch, M.: Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH, *Estuar. Coast.*, 36, 221–236, 2013.
- Ducklow, H. W. and Doney, S. C.: What is the metabolic state of the oligotrophic ocean? A debate, *Annu. Rev. Mar. Sci.*, 5, 525–533, 2013.
- Dupont, C. L., Rusch, D. B., Yooseph, S., Lombardo, M. J., Alexander Richter, R., Valas, R., Novotny, M., Yee-Greenbaum, J., Selengut, J. D., Haft, D. H., Halpern, A. L., Lasken, R. S., Nealson, K., Friedman, R., and Craig Venter, J.: Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage, *ISME J.*, 6, 1186–1199, 2012.
- Eloe, E. A., Shulse, C. N., Fadrosch, D. W., Williamson, S. J., Allen, E. E., and Bartlett, D. H.: Compositional differences in particle-associated and free-living microbial assemblages from an extreme deep-ocean environment, *Environ. Microbiol. Rep.*, 3, 449–458, 2011.
- Fernández-Gómez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S. G., González, J. M., and Pedrós-Alió, C.: Ecology of marine *Bacteroidetes*: a comparative genomics approach, *ISME J.*, 7, 1026–1037, 2013.
- Frankignoulle, M., Abril, G., Borges, A., Bourge, I., Canon, C., Delille, B., Libert, E., and Théate, J. M.: Carbon dioxide emission from European estuaries, *Science*, 282, 434–436, 1998.
- Galperin, M. Y.: Diversity of structure and function of response regulator output domains, *Curr. Opin. Microbiol.*, 13, 150–159, 2010.
- George, A. M. and Jones, P. M.: Perspectives on the structure-function of ABC transporters: the Switch and Constant Contact models, *Prog. Biophys. Mol. Biol.*, 109, 95–107, 2012.
- Gilly, W. F., Beman, J. M., Litvin, S. Y., and Robison, B. H.: Oceanographic and biological effects of shoaling of the oxygen minimum zone, *Annu. Rev. Mar. Sci.*, 5, 393–420, 2013.
- Glibert, P. M., Azanz, R., Burford, M., Furuya, K., Abal, E., Al-Azri, A., Al-Yamani, F., Andersen, P., Anderson, D. M., Beardall, J., Berg, G. M., Brand, L., Bronk, D., Brookes, J., Burkholder, J. M., Cembella, A., Cochlan, W. P., Collier, J. L., Collos, Y., Diaz, R., Doblin, M., Drennen, T., Dyhrman, S., Fukuyo, Y., Furnas, M., Galloway, J., Granéli, E., Ha, D. V., Hallegraeff, G., Harrison, J., Harrison, P. J., Heil, C. A., Heimann, K., Howarth, R., Jauzein, C., Kana, A. A., Kana, T. M., Kim, H., Kudela, R., Legrand, C., Mallin, M., Mulholland, M., Murray, S., O'Neil, J., Pitcher, G., Qi, Y., Rabalais, N., Raine, R., Seitzinger, S., Salomon, P. S., Solomon, C., Stoecker, D. K., Usup, G., Wilson, J., Yin, K., Zhou, M., and Zhu, M.: Ocean urea fertilization for carbon credits poses high ecological risks, *Mar. Pollut. Bull.*, 56, 1049–1056, 2008.
- Gómez-Consarnau, L., Lindh, M. V., Gasol, J. M., and Pinhassi, J.: Structuring of bacterioplankton communities by specific dis-

- solved organic carbon compounds, *Environ. Microbiol.*, 14, 2361–2378, 2012.
- Gómez-Pereira, P. R., Schüler, M., Fuchs, B. M., Bennke, C., Teeling, H., Waldmann, J., Richter, M., Barbe, V., Bataille, E., Glöckner, F. O., and Amann, R.: Genomic content of uncultured *Bacteroidetes* from contrasting oceanic provinces in the North Atlantic Ocean, *Environ. Microbiol.*, 14, 52–66, 2012.
- González, J. M., Fernández-Gómez, B., Fernández-Guerra, A., Gómez-Consarnau, L., Sánchez, O., Coll-Lladó, M., Del Campo, J., Escudero, L., Rodríguez-Martínez, R., Alonso-Sáez, L., Latasa, M., Paulsen, I., Nedashkovskaya, O., Lekunberri, I., Pinhassi, J., and Pedrós-Alió, C.: Genome analysis of the proteorhodopsin-containing marine bacterium *Polaribacter* sp. MED152 (Flavobacteria), *P. Natl. Acad. Sci. USA*, 105, 8724–8729, 2008.
- Gram, L., Grossart, H.-P., Schlingloff, A., and Kjøboe, T.: Possible quorum sensing in marine snow bacteria: Production of acylated homoserine lactones by *Roseobacter* strains isolated from marine snow, *Appl. Environ. Microbiol.*, 68, 4111–4116, 2002.
- Hansell, D. A.: Recalcitrant dissolved organic carbon fractions, *Annu. Rev. Mar. Sci.*, 5, 421–445, 2013.
- Hehemann, J. H., Correc, G., Barbeyron, T., Helbert, W., Czjzek, M., and Michel, G.: Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota, *Nature*, 464, 908–912, 2010.
- Hmelo, L. R., Mincer, T. J., and Van Mooy, B. A. S.: Possible influence of bacterial quorum sensing on the hydrolysis of sinking particulate organic carbon in marine environments, *Environ. Microbiol. Rep.*, 3, 682–688, 2011.
- Huang, Y. L., Ki, J. S., Lee, O. O., and Qian, P. Y.: Evidence for the dynamics of Acyl homoserine lactone and AHL-producing bacteria during subtidal biofilm formation, *ISME J.*, 3, 296–304, 2009.
- Ivars-Martinez, E., Martin-Cuadrado, A. B., D'Auria, G., Mira, A., Ferreria, S., Johnson, J., Friedman, R., and Rodriguez-Valera, F.: Comparative genomics of two ecotypes of the marine planktonic copiotroph *Alteromonas macleodii* suggests alternative lifestyles associated with different kinds of particulate organic matter, *ISME J.*, 2, 1194–1212, 2008.
- Jetten, M. S., Niftrik, L. V., Strous, M., Kartal, B., Keltjens, J. T., and Op den Camp, H. J.: Biochemistry and molecular biology of anammox bacteria, *Crit. Rev. Biochem. Mol. Biol.*, 44, 65–84, 2009.
- Jiao, N. and Azam, F.: Microbial carbon pump and its significance for carbon sequestration in the ocean, in: *Microbial Carbon Pump in the Ocean*, edited by: Jiao, N., Azam, F., and Sanders, S., Science/AAAS, Washington, DC, 43–45, 2011.
- Jiao, N. and Zheng, Q.: The microbial carbon pump: from genes to ecosystems, *Appl. Environ. Microbiol.*, 77, 7439–7444, 2011.
- Jiao, N., Herndl, G. J., Hansell, D. A., Benner, R., Kattner, G., Wilhelm, S. W., Kirchman, D. L., Weinbauer, M. G., Luo, T., Chen, F., and Azam, F.: Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean, *Nat. Rev. Microbiol.*, 8, 593–599, 2010.
- Jiao, N., Tang, K., Cai, H., and Mao, Y.: Increasing the microbial carbon sink in the sea by reducing chemical fertilization on the land, *Nat. Rev. Microbiol.*, 9, 75, doi:10.1038/nrmicro2386-c2, 2011.
- Jiao, N., Robinson, C., Azam, F., Thomas, H., Baltar, F., Dang, H., Hardman-Mountford, N. J., Johnson, M., Kirchman, D. L., Koch, B. P., Legendre, L., Li, C., Liu, J., Luo, T., Luo, Y.-W., Mitra, A., Romanou, A., Tang, K., Wang, X., Zhang, C., and Zhang, R.: Mechanisms of microbial carbon sequestration in the ocean – future research directions, *Biogeosciences Discuss.*, 11, 7931–7990, doi:10.5194/bgd-11-7931-2014, 2014.
- Karl, D. M.: Microbial oceanography: paradigms, processes and promise, *Nat. Rev. Microbiol.*, 5, 759–769, 2007.
- Kattner, G., Simon, M., and Koch, B. P.: Molecular characterization of dissolved organic matter and constraints for prokaryotic utilization, in: *Microbial Carbon Pump in the Ocean*, edited by: Jiao, N., Azam, F., and Sanders, S., Science/AAAS, Washington, DC, 60–61, 2011.
- Lalithambika, S., Peterson, L., Dana, K., and Blum, P.: Carbohydrate hydrolysis and transport in the extreme thermoacidophile *Sulfolobus solfataricus*, *Appl. Environ. Microbiol.*, 78, 7931–7938, 2012.
- Lam, P. and Kuypers, M. M. M.: Microbial nitrogen cycling processes in oxygen minimum zones, *Annu. Rev. Mar. Sci.*, 3, 317–345, 2011.
- Lam, P., Lavik, G., Jensen, M. M., van de Vossenberg, J., Schmid, M., Woebken, D., Gutiérrez, D., Amann, R., Jetten, M. S., and Kuypers, M. M.: Revising the nitrogen cycle in the Peruvian oxygen minimum zone, *P. Natl. Acad. Sci. USA*, 106, 4752–4757, 2009.
- Lampitt, R. S., Achterberg, E. P., Anderson, T. R., Hughes, J. A., Iglesias-Rodriguez, M. D., Kelly-Gerreyn, B. A., Lucas, M., Popova, E. E., Sanders, R., Shepherd, J. G., Smythe-Wright, D., and Yool, A.: Ocean fertilization: a potential means of geoengineering?, *Philos. T. R. Soc. A*, 366, 3919–3945, 2008.
- Laub, M. T. and Goulian, M.: Specificity in two-component signal transduction pathways, *Annu. Rev. Genet.*, 41, 121–145, 2007.
- Lawrence, M. G.: Side effects of oceanic iron fertilization, *Science*, 297, 5589, doi:10.1126/science.297.5589, 2002.
- Lee, S. J., Böhm, A., Krug, M., and Boos, W.: The ABC of binding-protein-dependent transport in Archaea, *Trends Microbiol.*, 15, 389–397, 2007.
- Liang, C. and Balsler, T. C.: Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy, *Nat. Rev. Microbiol.*, 9, 75, doi:10.1038/nrmicro2386-c1, 2011.
- Liang, C. and Balsler, T. C.: Warming and nitrogen deposition lessen microbial residue contribution to soil carbon pool, *Nat. Commun.*, 3, 1222, doi:10.1038/ncomms2224, 2012.
- Loh, A. N., Bauer, J. E., and Druffel, E. R.: Variable ageing and storage of dissolved organic components in the open ocean, *Nature*, 430, 877–881, 2004.
- Luque-Almagro, V. M., Gates, A. J., Moreno-Vivián, C., Ferguson, S. J., Richardson, D. J., and Roldán, M. D.: Bacterial nitrate assimilation: gene distribution and regulation, *Biochem. Soc. Trans.*, 39, 1838–1843, 2011.
- Mann, A. J., Hahnke, R. L., Huang, S., Werner, J., Xing, P., Barbeyron, T., Huettel, B., Stüber, K., Reinhardt, R., Harder, J., Glöckner, F. O., Amann, R. I., and Teeling, H.: The genome of the alga-associated marine flavobacterium *Formosa agariphila* KMM 3901^T reveals a broad potential for degradation of algal polysaccharides, *Appl. Environ. Microbiol.*, 79, 6813–6822, 2013.

- McBride, M. J., Xie, G., Martens, E. C., Lapidus, A., Henrissat, B., Rhodes, R. G., Goltzman, E., Wang, W., Xu, J., Hunnicutt, D. W., Staroscik, A. M., Hoover, T. R., Cheng, Y. Q., and Stein, J. L.: Novel features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* as revealed by genome sequence analysis, *Appl. Environ. Microbiol.*, 75, 6864–6875, 2009.
- McCarren, J., Becker, J. W., Repeta, D. J., Shi, Y., Young, C. R., Malmstrom, R. R., Chisholm, S. W., and DeLong, E. F.: Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea, *P. Natl. Acad. Sci. USA*, 107, 16420–16427, 2010.
- Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., and Körtzinger, A.: Future ocean acidification will be amplified by hypoxia in coastal habitats, *Mar. Biol.*, 160, 1875–1888, 2013.
- Miller, T. R., Delcher, A. L., Salzberg, S. L., Saunders, E., Detter, J. C., and Halden, R. U.: Genome sequence of the dioxin-mineralizing bacterium *Sphingomonas wittichii* RW1, *J. Bacteriol.*, 192, 6101–6102, 2010.
- Morris, R. M., Nunn, B. L., Frazar, C., Goodlett, D. R., Ting, Y. S., and Roco, G.: Comparative metaproteomics reveals ocean-scale shifts in microbial nutrient utilization and energy transduction, *ISME J.*, 4, 673–685, 2010.
- Mulkidjanian, A. Y., Dibrov, P., and Galperin, M. Y.: The past and present of sodium energetics: may the sodium-motive force be with you, *Biochim. Biophys. Acta*, 1777, 985–992, 2008.
- Naqvi, S. W., Jayakumar, D. A., Narvekar, P. V., Naik, H., Sarma, V. V., D'Souza, W., Joseph, S., and George, M. D.: Increased marine production of N₂O due to intensifying anoxia on the Indian continental shelf, *Nature*, 408, 346–349, 2000.
- Nealson, K. H. and Saffarini, D.: Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation, *Annu. Rev. Microbiol.*, 48, 311–343, 1994.
- Nelson, C. E. and Carlson, C. A.: Tracking differential incorporation of dissolved organic carbon types among diverse lineages of Sargasso Sea bacterioplankton, *Environ. Microbiol.*, 14, 1500–1516, 2012.
- Nelson, C. E., Goldberg, S. J., Wegley Kelly, L., Haas, A. F., Smith, J. E., Rohwer, F., and Carlson, C. A.: Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages, *ISME J.*, 7, 962–979, 2013.
- Nixon, B. T., Ronson, C. W., and Ausubel, F. M.: Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntxB* and *ntxC*, *P. Natl. Acad. Sci. USA*, 83, 7850–7854, 1986.
- Noinaj, N., Guillier, M., Barnard, T. J., and Buchanan, S. K.: TonB-dependent transporters: regulation, structure, and function, *Annu. Rev. Microbiol.*, 64, 43–60, 2010.
- O'Brien, P. J. and Herschlag, D.: Catalytic promiscuity and the evolution of new enzymatic activities, *Chem. Biol.*, 6, R91–R105, 1999.
- Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., and Benner, R.: Production of refractory dissolved organic matter by bacteria, *Science*, 292, 917–920, 2001.
- Oh, C., De Zoysa, M., Kwon, Y. K., Heo, S. J., Affan, A., Jung, W. K., Park, H. S., Lee, J., Son, S. K., Yoon, K. T., and Kang, D. H.: Complete genome sequence of the agarase-producing marine bacterium strain s89, representing a novel species of the genus *Alteromonas*, *J. Bacteriol.*, 193, 5538, doi:10.1128/JB.05746-1, 2011.
- Omori, K. and Idei, A.: Gram-negative bacterial ATP-binding cassette protein exporter family and diverse secretory proteins, *J. Biosci. Bioeng.*, 95, 1–12, 2003.
- Ottesen, E. A., Marin, R. 3rd, Preston, C. M., Young, C. R., Ryan, J. P., Scholin, C. A., and DeLong, E. F.: Metatranscriptomic analysis of autonomously collected and preserved marine bacterioplankton, *ISME J.*, 5, 1881–1895, 2011.
- Ottesen, E. A., Young, C. R., Eppley, J. M., Ryan, J. P., Chavez, F. P., Scholin, C. A., and DeLong, E. F.: Pattern and synchrony of gene expression among sympatric marine microbial populations, *P. Natl. Acad. Sci. USA*, 110, E488–E497, 2013.
- Paerl, H. W., Valdes, L. M., Peierls, B. L., Adolf, J. E., and Harding, L. W. Jr.: Anthropogenic and climatic influences on the eutrophication of large estuarine ecosystems, *Limnol. Oceanogr.*, 51, 448–462, 2006.
- Paul, C. and Pohnert, G.: Interactions of the algicidal bacterium *Kordia algicida* with diatoms: regulated protease excretion for specific algal lysis, *PLoS One*, 6, e21032, doi:10.1371/journal.pone.0021032, 2011.
- Poretzky, R. S., Sun, S., Mou, X., and Moran, M. A.: Transporter genes expressed by coastal bacterioplankton in response to dissolved organic carbon, *Environ. Microbiol.*, 12, 616–627, 2010.
- Qin, Q. L., Zhang, X. Y., Wang, X. M., Liu, G. M., Chen, X. L., Xie, B. B., Dang, H. Y., Zhou, B. C., Yu, J., and Zhang, Y. Z.: The complete genome of *Zunongwangia profunda* SM-A87 reveals its adaptation to the deep-sea environment and ecological role in sedimentary organic nitrogen degradation, *BMC Genomics*, 11, 247, doi:10.1186/1471-2164-11-247, 2010.
- Rabalais, N. N., Turner, R. E., Díaz, R. J., and Justić, D.: Global change and eutrophication of coastal waters, *ICES J. Mar. Sci.*, 66, 1528–1537, 2009.
- Rabalais, N. N., Díaz, R. J., Levin, L. A., Turner, R. E., Gilbert, D., and Zhang, J.: Dynamics and distribution of natural and human-caused hypoxia, *Biogeosciences*, 7, 585–619, doi:10.5194/bg-7-585-2010, 2010.
- Richardson, D. J.: Bacterial respiration: a flexible process for a changing environment, *Microbiology*, 146, 551–571, 2000.
- Rinta-Kanto, J. M., Sun, S., Sharma, S., Kiene, R. P., and Moran, M. A.: Bacterial community transcription patterns during a marine phytoplankton bloom, *Environ. Microbiol.*, 14, 228–239, 2012.
- Robinson, C. and Ramaiah, N.: Microbial heterotrophic metabolic rates constrain the microbial carbon pump, in: *Microbial Carbon Pump in the Ocean*, edited by: Jiao, N., Azam, F., and Sanders, S., Science/AAAS, Washington, DC, Science/AAAS, 52–53, 2011.
- Russell, A. B., Hood, R. D., Bui, N. K., LeRoux, M., Vollmer, W., and Mougous, J. D.: Type VI secretion delivers bacteriolytic effectors to target cells, *Nature*, 475, 343–347, 2011.
- Russell, A. B., LeRoux, M., Hathazi, K., Agnello, D. M., Ishikawa, T., Wiggins, P. A., Wai, S. N. and Mougous, J. D.: Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors, *Nature*, 496, 508–512, 2013.
- Schauer, K., Rodionov, D. A., and de Reuse, H.: New substrates for TonB-dependent transport: do we only see the “tip of the iceberg”?, *Trends Biochem. Sci.*, 33, 330–338, 2008.
- Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J.,

- Manning, D. A., Nannipieri, P., Rasse, D. P., Weiner, S., and Trumbore, S. E.: Persistence of soil organic matter as an ecosystem property, *Nature*, 478, 49–56, 2011.
- Schwalbach, M. S., Tripp, H. J., Steindler, L., Smith, D. P., and Giovannoni, S. J.: The presence of the glycolysis operon in SAR11 genomes is positively correlated with ocean productivity, *Environ. Microbiol.*, 12, 490–500, 2010.
- Schwarz, S., Hood, R. D., and Mougous, J. D.: What is type VI secretion doing in all those bugs?, *Trends Microbiol.*, 18, 531–537, 2010.
- Simon, M., Grossart, H. P., Schweitzer, B., and Ploug, H.: Microbial ecology of organic aggregates in aquatic ecosystems, *Aquat. Microb. Ecol.*, 28, 175–211, 2002.
- Smetacek, V. and Naqvi, S. W.: The next generation of iron fertilization experiments in the Southern Ocean, *Philos. T. R. Soc. A*, 366, 3947–3967, 2008.
- Smith, M. W., Zeigler Allen, L., Allen, A. E., Herfort, L., and Simon, H. M.: Contrasting genomic properties of free-living and particle-attached microbial assemblages within a coastal ecosystem, *Front. Microbiol.*, 4, 120, doi:10.3389/fmicb.2013.00120, 2013.
- Sowell, S. M., Wilhelm, L. J., Norbeck, A. D., Lipton, M. S., Nicora, C. D., Barofsky, D. F., Carlson, C. A., Smith, R. D., and Giovannoni, S. J.: Transport functions dominate the SAR11 metaproteome at low-nutrient extremes in the Sargasso Sea, *ISME J.*, 3, 93–105, 2009.
- Sowell, S. M., Abraham, P. E., Shah, M., Verberkmoes, N. C., Smith, D. P., Barofsky, D. F., and Giovannoni, S. J.: Environmental proteomics of microbial plankton in a highly productive coastal upwelling system, *ISME J.*, 5, 856–865, 2011.
- Stocker, R.: Marine microbes see a sea of gradients, *Science*, 338, 628–633, 2012.
- Tang, K., Jiao, N., Liu, K., Zhang, Y., and Li, S.: Distribution and functions of TonB-dependent transporters in marine bacteria and environments: implications for dissolved organic matter utilization, *PLoS One*, 7, e41204, doi:10.1371/journal.pone.0041204, 2012.
- Taylor, P. G. and Townsend, A. R.: Stoichiometric control of organic carbon-nitrate relationships from soils to the sea, *Nature*, 464, 1178–1181, 2010.
- Thamdrup, B., De Brabandere, L., Dalsgaard, T., DeLong, E. F., Revsbech, N. P., and Ulloa, O.: A cryptic sulfur cycle in oxygen-minimum-zone waters off the Chilean coast, *Science*, 330, 1375–1378, 2010.
- Thomas, F., Barbeyron, T., Tonon, T., Génicot, S., Czjzek, M., and Michel, G.: Characterization of the first alginolytic operons in a marine bacterium: from their emergence in marine *Flavobacteriia* to their independent transfers to marine *Proteobacteria* and human gut *Bacteroides*, *Environ. Microbiol.*, 14, 2379–2394, 2012.
- Thomas, T., Evans, F. F., Schleheck, D., Mai-Prochnow, A., Burke, C., Penesyan, A., Dalisay, D. S., Stelzer-Braid, S., Saunders, N., Johnson, J., Ferreira, S., Kjelleberg, S., and Egan, S.: Analysis of the *Pseudoalteromonas tunicata* genome reveals properties of a surface-associated life style in the marine environment, *PLoS One*, 3, e3252, doi:10.1371/journal.pone.0003252, 2008.
- Turley, C. M. and Stutt, E. D.: Depth-related cell-specific bacterial leucine incorporation rates on particles and its biogeochemical significance in the Northwest Mediterranean, *Limnol. Oceanogr.*, 45, 419–425, 2000.
- Wang, P., Li, Q., Tian, J., Jian, Z., Liu, C., Li, L., and Ma, W.: Long-term cycles in the carbon reservoir of the Quaternary ocean: a perspective from the South China Sea, *Natl. Sci. Rev.*, 1, 119–143, 2014.
- West, S. A., Winzer, K., Gardner, A., and Diggle, S. P.: Quorum sensing and the confusion about diffusion, *Trends Microbiol.*, 20, 586–594, 2012.
- Wright, J. J., Konwar, K. M., and Hallam, S. J.: Microbial ecology of expanding oxygen minimum zones, *Nat. Rev. Microbiol.*, 10, 381–394, 2012.
- Wuichet, K., Cantwell, B. J., and Zhulin, I. B.: Evolution and phyletic distribution of two-component signal transduction systems, *Curr. Opin. Microbiol.*, 13, 219–225, 2010.
- Xian, W., Kang, B., and Liu, R.: Jellyfish blooms in the Yangtze Estuary, *Science*, 307, 5706, doi:10.1126/science.307.5706.41c, 2005.
- Zehr, J. P.: New twist on nitrogen cycling in oceanic oxygen minimum zones, *P. Natl. Acad. Sci. USA*, 106, 4575–4576, 2009.
- Zehr, J. P. and Kudela, R. M.: Nitrogen cycle of the open ocean: from genes to ecosystems, *Annu. Rev. Mar. Sci.*, 3, 197–225, 2011.
- Zeigler Allen, L., Allen, E. E., Badger, J. H., McCrow, J. P., Paulsen, I. T., Elbourne, L. D., Thiagarajan, M., Rusch, D. B., Nealson, K. H., Williamson, S. J., Venter, J. C., and Allen, A. E.: Influence of nutrients and currents on the genomic composition of microbes across an upwelling mosaic, *ISME J.*, 6, 1403–1414, 2012.