Eco-DAS X
Symposium Proceedings

Eco-DAS X Chapter 4, 2014, 48–68

A more inclusive loop: Examining the contribution of five bacterial specialists to nutrient cycling and the microbial loop

Erin R. Graham[1], Huan Chen[2], Claudia Dziallas[3], Beatriz Fernández-Gómez[4], and John B. Kirkpatrick[5]*
[1]Temple University, Department of Biology, 1900 N. 12th St., Philadelphia, PA USA
[2]National High Magnetic Field Laboratory, 1800 E. Paul Dirac Dr., Tallahassee, FL USA
[3]University of Copenhagen, Marine Biological Section, Strandpromenaden 5, 3000 Helsingor, Denmark
[4]Department of Marine Biology and Oceanography, Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain
[5]University of Rhode Island, Graduate School of Oceanography, 215 South Ferry Road, Narragansett, RI USA

Abstract

Marine microbes have been studied for centuries. However, only in the past four decades have microbes been recognized as drivers of energy and nutrient cycles in the ocean. Several pivotal publications in the mid- to late-twentieth century affirmed the role of microbes in primary production and consumption of dissolved organic matter. These findings supported the hypothesis that microbes provide a link in the food web between phytoplankton, dissolved nutrients, and zooplankton. This concept became known as the microbial loop. More recent discoveries have identified additional contributors to the loop, such as novel phototrophic bacteria, archaea and viruses, yet the role of many microbial specialists in nutrient cycling remains unclear. In this chapter, we summarize the history and development of the microbial loop concept, and discuss five bacterial groups whose contribution to the microbial loop has largely been overlooked in the literature. We propose a modified loop that integrates processes performed by nitrogen-fixing bacteria, particle- and organism- associated bacteria, bacterial symbionts, Flavobacteria, and predatory bacteria, and conclude that the microbial loop must continue to evolve as the ecology of additional microbial specialists is revealed.

Section 1. Introduction

Prokaryotic microbes have the highest abundance and biomass of all organisms on our planet. The number of bacteria on earth by far exceeds the number of stars in the universe (Curtis and Sloan 2004; Pomeroy et al. 2007). The ocean alone contains an estimated 10^{29} bacteria (Whitman et al. 1998); thus, billions of microbes are present in each liter of seawater. Consequently, it is not surprising that bacteria play a critical role in biogeochemical cycling in all ecosystems. Prokaryotic microbes fulfill an enormous diversity of functions (Harwood 2008), affecting a wide range of matter cycles, such as nitrogen fixation and phosphate storage (Davelaar 1993; Falkowski et al. 1998; Gruber and Galloway 2008). Bacteria function in degradation and transformation of organic matter, providing inorganic nutrients for phytoplankton, and bacteria serve as a food source for bacteriovores. In addition, marine bacteria, along with eukaryotic microbes, contribute to about half of all primary production on the planet (Arrigo 2005).

Over the past four decades, research in microbial ecology has begun to reveal the diversity of microbes in the food web, and the breadth and complexity of their interactions and metabolic processes that drive biogeochemical cycles. These new discoveries changed the way we think about trophic interactions in the ocean, and drove the transformation of the classic linear food chain into a multidirectional food web that highlights the role of microbes in trophic dynamics and nutrient flux. This new food web also includes a remineralization and recycling pathway for dissolved organic material known as the microbial loop.

In this chapter, we will outline the evolution of the microbial loop and discuss the bacterial groups and processes that

*Corresponding author: E-mail: erin.graham@oregonstate.edu;
Current address: USDA-ARS, Hatfield Marine Science Center, 2030 SE Marine Science Dr., Newport, OR USA

Acknowledgments

The authors would like to thank Paul Kemp and Lydia Baker for their leadership, guidance, and valuable suggestions throughout the creative and editorial process. We would also like to Sharon Sakamoto, the Center for Microbial Oceanography: Research and Education (C-MORE), the University of Hawai`i School of Ocean and Earth Science and Technology (SOEST) and the UH Department of Oceanography for organizing and supporting the Eco-DAS symposium, and the National Science Foundation for funding this event.

Publication was supported by NSF award OCE08-12838 to P.F. Kemp

ISBN: 978-0-9845591-4-5, DOI: 10.4319/ecodas.2014.978-0-9845591-4-5.48

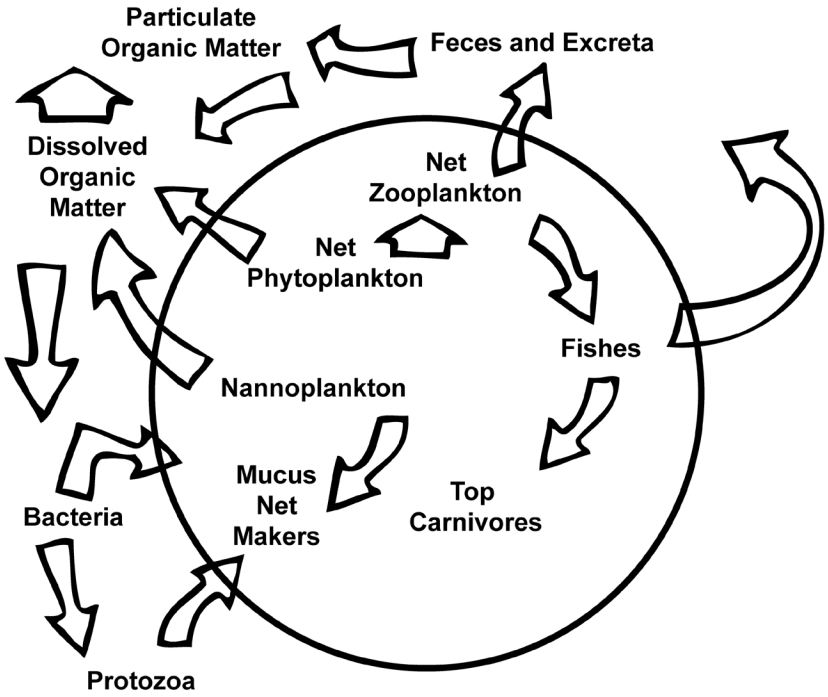

Fig. 1. The ocean's food web conceived by Pomeroy (1974). The classical food chain paradigm is contained inside of the circle, whereas pathways described in his "new paradigm" are outside the circle. Reprinted from Pomeroy (1974) with permission from Oxford University Press.

have defined the microbial loop to date. We will then present several additional bacterial groups that are absent from most microbial loop models, despite their importance in nutrient cycling. The goal of this chapter is not to present an inclusive list of all microbes (or even all bacterial groups) that contribute to the microbial loop, but rather to highlight the contribution of five overlooked bacterial groups that perform important functions in the microbial loop.

Section 2. History of the microbial loop

Marine microbes were studied as early as the late 1600s by the Dutch lensmaker Antony van Leeuwenhoek. However, the field of marine microbiology really began to develop following the establishment of several marine laboratories and teaching facilities in the late 1800s (see Karl and Proctor 2007). At the turn of the twentieth century, several scientists including Henry Bryant Bigelow, Selman Waksman, Cornelis B. van Niel, and Claude ZoBell published works that suggested that bacteria play a role in marine biogeochemical cycling, though it took several more decades of experimental evidence to support these ideas (see Karl and Proctor 2007 and Sherr and Sherr 2008 for reviews).

Prior to the 1970s, the accepted paradigm for nutrient flux in the ocean was a linear food chain: 1) marine plants and phytoplankton conduct primary production, 2) zooplankton consume phytoplankton, 3) zooplankton are consumed by predators, and 4) predators are consumed by higher predators. This traditional food chain, often called the "grazing food chain," assumed that the bulk of primary production is consumed by herbivorous zooplankton as particulate matter, and little is dissolved and used by microbes. It also assumed that size is the best proxy for identifying the key players in the food chain. In other words, larger organisms such as fish and mammals are the biggest consumers of energy in the ocean. Until the 1970s, few studies offered accurate estimates of microbial abundance in the ocean. In addition, many researchers assumed that the majority of bacteria in the water column are free-living, or live independent of other particles and organisms (see Grossart 2010) and are metabolically inactive (Azam 1998; Pomeroy et al. 2007). Thus, the traditional grazing food chain model grossly underestimated the impact of bacteria and other microbes (particularly nanoplankton) on nutrient cycling.

In 1974, Lawrence Pomeroy introduced a "new paradigm" for the ocean's food chain in which microbes emerged as major contributors to carbon, energy, and nutrient cycling. Influenced by the work of his colleagues who demonstrated that microbes are essential players in the food web of salt marshes (see Sherr and Sherr 2008), Pomeroy suggested that microbes are not only major primary producers, but are also the leading consumers of dissolved organic material in the ocean (Fig. 1; Pomeroy 1974). He cited multiple studies that

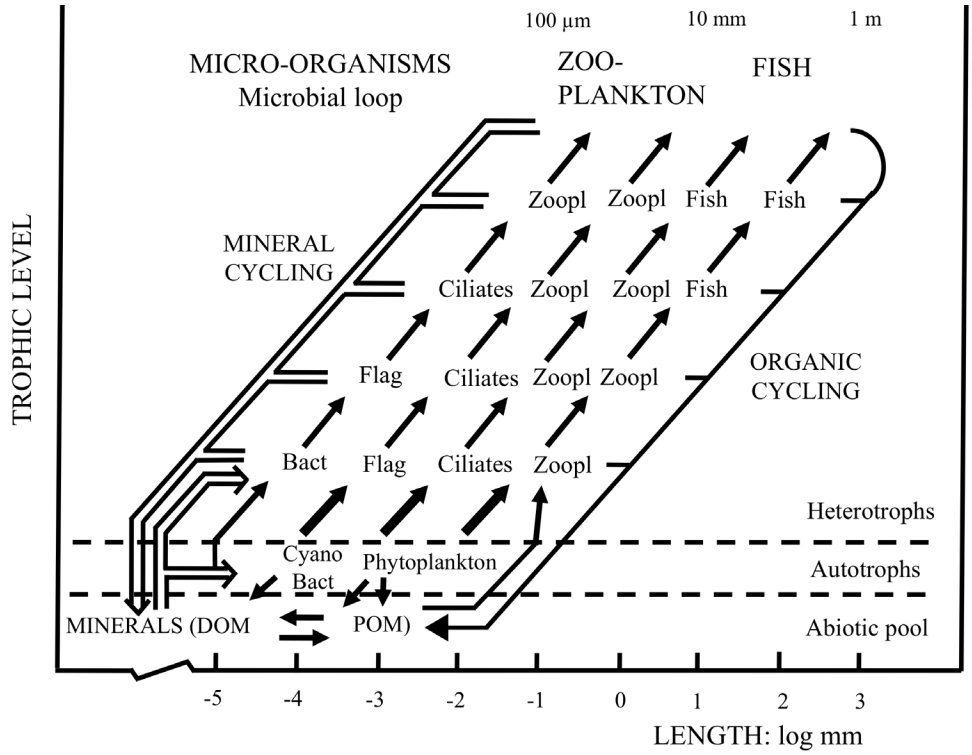

Fig. 2. The microbial loop as presented by Azam et al. (1983). Solid arrows represent energy flow and materials, and open arrows represent material flow only. Reprinted with permission from Inter-Research.

supported his hypothesis, and credited new techniques for measuring photosynthesis and respiration for revealing the abundance and significance of microbes in photosynthesis and metabolism (Pomeroy 1974).

Additional methodological advances for quantifying bacteria and measuring bacterial growth rates, combined with the discovery that the majority of bacteria in the water column are metabolically active (see Fenchel 2008), further supported the hypothesis that microbes contribute to ocean metabolism. In 1983, Azam and colleagues expanded on the idea of Pomeroy's new paradigm by describing a "microbial loop" in which dissolved organic matter released by phytoplankton (and to a lesser extent by animals) is consumed by bacteria. Bacteria are consumed by protozoa, which are then consumed by microzooplankton that are part of the traditional grazing chain (Azam et al. 1983) (Fig. 2). The microbial loop concept identified the critical role of bacteria in recycling organic matter and returning it to the grazing food chain.

The microbial loop describes several bacterial processes that connect microbes to the rest of the producers and consumers in the ocean's food web. First, bacteria remineralize nitrogen and phosphorus and supply it to phytoplankton for primary production. Phytoplankton are eventually consumed by zooplankton, which are consumed by higher metazoans. Second, autotrophic bacteria take up dissolved inorganic materials to produce organic matter. Finally, dissolved organic matter assimilated by bacteria enters the food chain again when bacteria are consumed by heterotrophic protists. These microbial cycles occur throughout the ocean, but are particularly important in oligotrophic waters lacking upwelling and seasonal nutrient inputs (see Fenchel 2008). In oligotrophic environments, the primary function of micobes in the loop is to remineralize dissolved organic matter and promote primary production (Fenchel 2008).

Conceptual diagrams of the microbial loop have been published since Azam and colleagues' 1983 version (see Sherr and Sherr 2008 for review), and some recent versions include additional microbial groups now recognized as major players (Fig. 3). For example, it was revealed that phototrophic prokaryotes like *Synechococcus* sp. and *Proclorococcus* sp. cyanobacteria are the greatest contributors to photosynthesis in the ocean (see DeLong and Karl 2005). Also, studies showed that viruses outnumber bacteria in the water column, and promote the release of organic material by lysing bacteria and other host cells (see Fenchel 2008). Marine microbes using novel forms of phototrophy have also recently been acknowledged as contributors to the microbial loop (Delong and Karl 2005; Giovannoni and Stingl 2005). For example, aerobic anoxigenic photoheterotrophs (AAPs) in the genus *Roseobacter* comprise up to 25% of marine bacteria in some locations (Lami et al. 2007; Brinkhoff et al. 2008). Finally, mixotrophic eukaryotes that combine phagotrophy and photosynthesis are

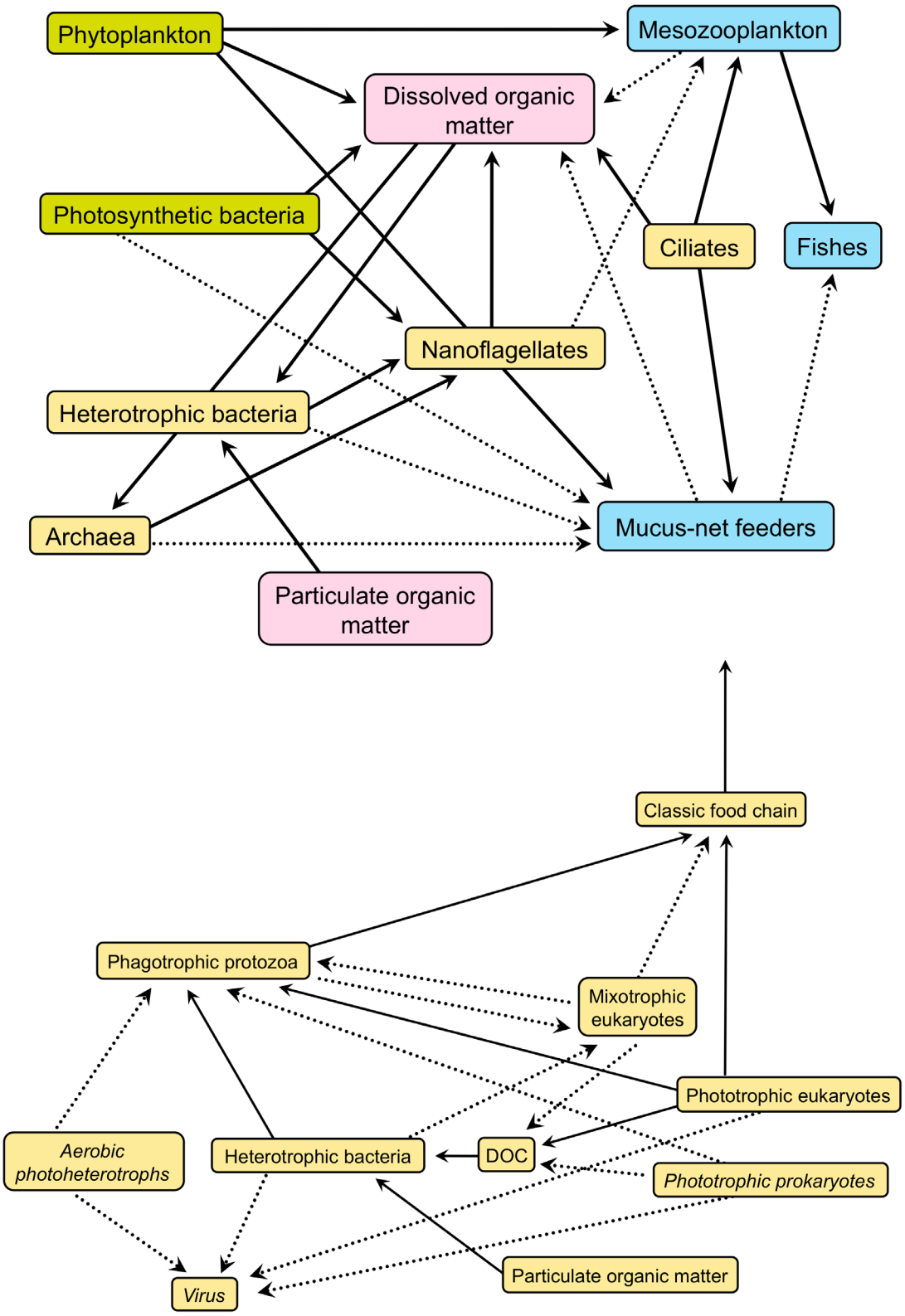

Fig. 3. Current views of the ocean's food web and microbial loop. Top) Solid arrows represent major fluxes of carbon and energy whereas dotted lines represent minor pathways. Green (autotrophic) and orange (heterotrophic) boxes represent organisms in the microbial loop. Reprinted from Pomeroy et al. (2007). Bottom) The microbial loop presented by Azam et al. (1993) redrawn with additions (dashed arrows). DOC is dissolved organic matter. Reprinted from Fenchel (2008) with permission from Elsevier.

now recognized as commonly occurring and perhaps ubiquitous organisms in the loop (Sanders 1991; Zubkov and Tarran 2008; Flynn et al. 2013).

Some microbial loops now include the organisms mentioned above, however, several important contributors remain excluded from most microbial loop diagrams. Moreover, modern loop diagrams continue to overlook important concepts, such as the idea that many bacteria are members of complex aggregates or form tight associations with particles or organisms (Grossart 2010). For the remainder of this chapter, we will describe five ubiquitous groups of bacteria that have been overlooked in microbial loop schematics, despite the important functions that they perform.

Section 3. Free-living, nitrogen-fixing bacteria

Nitrogen fixation, or the process by which dissolved N_2 is "fixed" into NH_3 by a series of microbially mediated steps, is the dominant process on Earth that makes atmospheric nitrogen available for biosynthesis. N-fixing organisms, or diazotrophs, are found among bacteria and archaea, and can include both heterotrophic and autotrophic species. Bacteria and archaea that are unable to fix atmospheric nitrogen, as well as all eukaryotic organisms, must obtain nitrogen (for proteins, nucleic acids, and other compounds) from inorganic "fixed" sources, such as NO_3^- or NH_4^+, dissolved or particulate nitrogen, or from diazotrophic symbionts.

In the marine environment, significant research has focused on phototrophic cyanobacterial diazotrophs. Breaking the triple bond to convert N_2 into NH_3 requires a large energy investment, and so it is not surprising that some diazotrophs inhabit sunlit waters, where abundant light energy is available. Phototrophic diazotrophs are particularly common study organisms due to their accessibility and visibility. For example, the model organism *Trichodesmium* is not only culturable, but it forms visible multi-cellular colonies which have been noted throughout history, including by Darwin:

> "when not far distant from the Abrolhos Islets, my attention was called to a reddish-brown appearance in the sea. The whole surface of the water, as it appeared under a weak lens, seemed as if covered by chopped bits of hay, with their ends jagged. These are minute cylindrical confervæ, in bundles or rafts of from twenty to sixty in each. Mr. Berkeley informs me that they are the same species (*Trichodesmium erythræum*) with that found over large spaces in the Red Sea, and whence its name of Red Sea is derived. Their numbers must be infinite. In almost every long voyage some account is given of these confervæ." (Darwin 1845)

Phototrophic diazotrophs are ubiquitous in the marine environment; however, there are many other diazotrophs. The following paragraphs will summarize recent research that has highlighted the role of some of these "other" diazotrophs.

In the presence of available nitrogen (NH_4^+, NO_3^-), fixation is not expected, as all diazotrophs studied to date are capable of acquiring fixed nitrogen from their environment. Studies of model organisms in culture have described strong controls on enzymatic machinery and repression in the presence of fixed nitrogen such as NH_4^+ (*see* Leigh and Dodsworth 2007). However, several studies have reported measurable fixation in the presence of nitrate (NO_3^-) (Voss et al. 2004; Holl and Montoya 2005). Whereas somewhat surprising, NO_3^- requires significant reduction before nitrogen atoms can be incorporated into biological macromolecules. Therefore, fixation in the presence of NO_3^- could be due to the relatively small difference in energetic cost.

Although fixation in the presence of NO_3^- can be reasonably explained, fixation in the presence of NH_4^+ is more baffling, considering the ease at which NH_4^+ can be assimilated and used. In laboratory studies, NH_4^+ was shown to be a strong repressor of nitrogen fixation (Leigh and Dodsworth 2007), however, *in situ* nitrogen fixation has been measured in lakes with NH_4^+ concentrations exceeding 10 mM (Halm et al. 2009). Nitrogen fixation has also been documented in oxygen minimum zones with active denitrification, NO_2^- availability, and low NH_4^+ (Fernandez et al. 2011; Jayakumar et al. 2012; Farnelid et al. 2013).

Sediment may not be expected to host significant N fixation (Thamdrup and Daalsgard 2008), as this environment is generally either high in energy flux and dissolved NH_4^+ (near-shore environments) or low in both (e.g., gyre sediments). However, fixation has been documented in a coastal embayment with NH_4^+ ranging upwards of 100 mM. (Fulweiler et al. 2008). Organic fluxes that stimulate denitrification initially have been shown to shift toward increasing N fixation over time (Fulweiler et al. 2013). This sediment-based fixation has been confirmed and linked in part to anthropogenic forcing (Brown and Jenkins 2014). N fixation associated with intertidal mats has been previously documented (Steppe and Paerl 2002). In light of studies like these, which include many groups associated with anoxia, we begin to make more sense of the broad polyphyletic nature of N fixation.

Despite the discovery of N fixation in these and other environments (e.g., methane seeps and hydrothermal vents (Mehta and Baross 2006; Dekas et al. 2009), the extent of diazotroph diversity remains largely unknown. It is difficult to identify heterotrophic diazotrophs, primarily because of polyphyly and the sensitivity of molecular biology techniques that rely on degenerate primers and nested PCR. Molecular biology techniques (Zehr and Turner 2001) are so sensitive that the *nifH* gene for nitrogenase reductase can be amplified from commercial reagents (Zehr et al. 2003). This has cast a shadow on reports of N fixation in unusual environments. However, corroboration of diazotrophy in heterotrophic bacteria using other methods such as ^{15}N-N_2 incubations can validate DNA- or RNA-based findings.

The discovery of diazotrophs in unconventional environments raises questions about natural selection. For microbiologists, an interesting question is *why* does fixation occur in the presence of small amounts of fixed N? Are thresholds of ambient nitrogen required to regulate gene expression simply higher than assumed? Are diazotrophs performing fixation in the presence of fixed nitrogen actually located in microniches that have low organic N compared with the larger ambient environment? Finally, could there be a benefit to fixing nitrogen even in the presence of dissolved inorganic nitrogen (DIN), such as H_2 production? Questions remain about the importance of N fixation in global nutrient cycles, the variety of organisms that conduct fixation, and the selective pressures that have resulted in the widespread use of the nitrogenase complex in bacteria and archaea. For biogeochemists and modelers, overlooked N fixation constitutes a sneaky feedback loop to primary productivity, which can upset mass and especially isotope budgets.

To what extent does N fixation in out-of-sight environments affect the microbial loop? N fixation can somewhat offset denitrification in environments where there is co-occurrence (e.g., Fulweiler et al. 2013). Heterotrophic and other "dark" microbes could contribute more to primary producers (as per the open arrows in Fig. 2) than previously thought. In the photic zone, N fixation of any kind could more directly stimulate photosynthesis, thereby increasing uptake of inorganic carbon while increasing output of organic carbon. Additional biomass encourages the growth and proliferation of herbivores, which can affect multiple trophic levels. In dark environments, diazotrophs may also be influencing population densities and/or activities of chemoautotrophs (e.g., Dekas et al. 2009). Another possibility is that diazotrophs below the photic zone combined with denitrifiers and anammox organisms may represent their own partially closed microbial loop, where N is fixed and denitrified. As N fixation is revealed in more environments, the total impact of diazotrophs on the microbial loop will become more clear.

Section 4. Particle- and organism-associated bacteria

In general, aquatic microbes can be free-living, particle- or organism-associated, or generalists, which switch between free-living and associated (Grossart et al. 2006). For decades, research has focused primarily on free-living and pathogenic bacteria, despite the evidence that particle-associated bacteria can have higher growth rates and metabolic activities in comparison to free-living bacteria (Middelboe et al. 1995; Grossart and Simon 1998). Indeed, particle-associated bacteria can be up to 100 times more abundant in coastal and estuarine environments, and much higher in total volume than free-living bacteria (Griffith et al. 1990; Crump et al. 1999; Simon et al. 2002). Thus, particle-associated bacteria are ubiquitous organisms in the water column, and in some environments, their role in cycling matter and energy may exceed the contribution of their free-living counterparts. In addition, the diversity of particle-associated bacteria, combined with their relatively short life span, encourages genetic exchange and evolution that may lead to species that fill additional niches in the microbial loop.

One way that associated bacteria contribute to nutrient cycling is by creating areas of concentrated nutrients that can be assimilated by other organisms. When associated bacteria attach to abiotic surfaces or organisms, some increase their metabolic activity immediately (Grossart and Simon 2007), which was also shown in experiments with bacterial isolates from marine snow (Grossart et al. 2007). These isolates increased protease activity 10-20 times after attachment. This increased activity, or biodegradation, leads to an accumulation of small molecules and nutrients in the ambient water called a plume (Smith et al. 1992). When bacteria attach to moving particles such as sinking debris or motile organisms, this nutrient plume travels behind the particle or organism (Kiorboe and Thygesen 2001). The nutrient-rich environment of these plumes may attract chemotactic bacteria and promote an increase in their cell-specific production rates (Kiorboe and Jackson 2001).

Associated bacteria also cycle nutrients acquired from phototrophic organisms that exude and excrete nutrients themselves. The area surrounding phototrophic organisms that is rich in exudation products is called a phycosphere (Cole 1982, Fig. 4). It is common for these areas to be densely populated by bacteria (Grossart 1999) because they offer abundant nutrients and refuge from predators. For example, cyanobacteria exude sugars and other organic compounds (Amemiya et al. 1990), which can attract bacterial cells during bloom events (Cole 1982). In these associations, the growth stage of the phototrophic host seems to be crucial for the number of attached bacteria (Grossart et al. 2001; Maruyama et al. 2003), and more bacterial cells attach to phytoplankton in stationary and declining blooms.

Associated bacteria can have several effects on their phototrophic hosts. For example, associated bacteria can inhibit growth of cyanobacterial hosts (Salomon et al. 2003; Berg et al. 2009), thereby decreasing the number of toxic cyanobacterial cells and reducing the effect of cyanotoxins on the food web (Dziallas and Grossart 2011). Associated bacteria can also cause lysis of the host (Cole 1982), releasing additional nutrients into the water column. However, phytoplankton hosts can also benefit from their associated bacteria by using growth factors, vitamins, and other exudates released by the associated bacteria (Cole 1982). In addition, bacterial respiration can increase the carbon supply for host photosynthesis and provide an area of reduced oxygen for nitrogen fixation by some cyanobacteria. On the other hand, these nitrogen-fixing cyanobacteria can pass ammonium to the attached or associated bacteria in their microenvironment (Ploug et al. 2011).

Bacteria may also associate with crustacean zooplankton (Fig. 5, see reviews by Grossart and Tang 2010 and Tang et al.

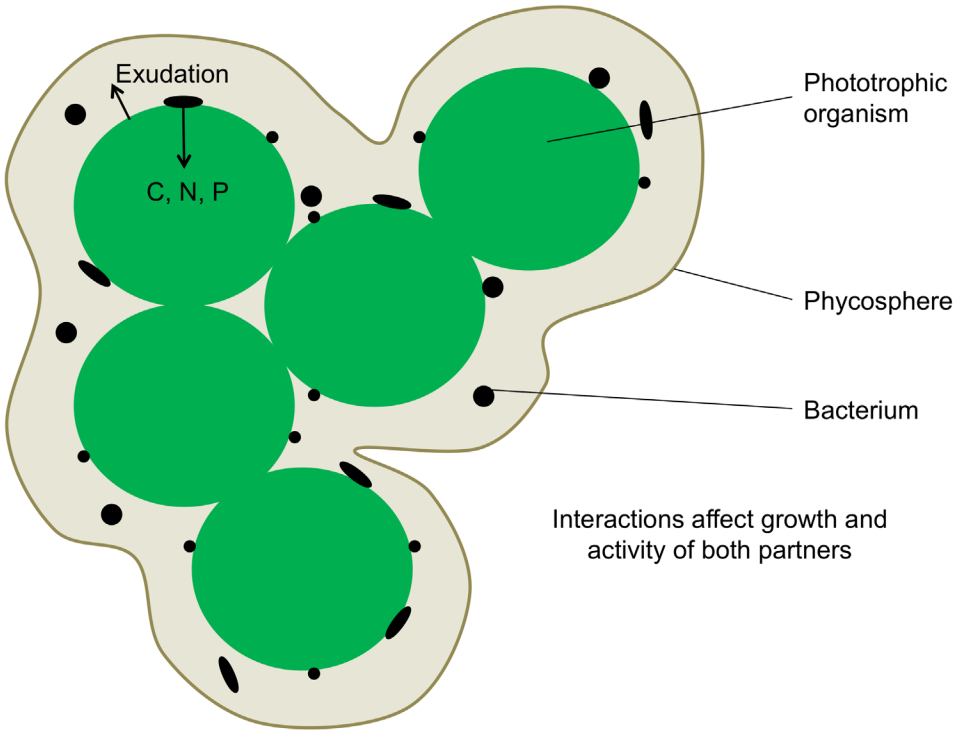

Fig. 4. Associated microbes with cyanobacteria or algae including obligate particle-attached bacteria and generalist bacteria that can enter and exit the phycosphere (see also Bell and Mitchell 1972).

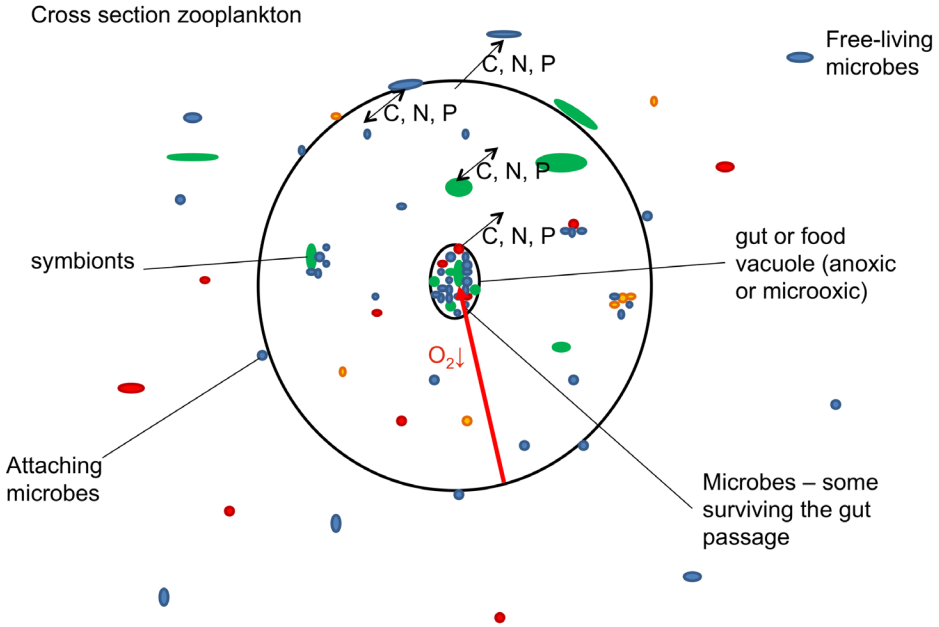

Fig. 5. Associated microbes with zooplankton. The oxygen concentration affects the production and degradation of specific compounds—e.g., an anoxic environment in the gut enables methanogenesis or ammonification.

2010). Zooplankton ingest a high number and variety of microorganisms. Some of these microorganisms survive the gut passage and establish themselves as associated bacteria inside of their zooplankton predators, persisting on high amounts of nutrients in the gut (Peterson et al. 1978; Pedrosalio and Brock 1983; Stukel et al. 2013). Zooplankton can also serve as

conveyor belts for microorganisms that attach to the carapace in one water layer, travel with the zooplankton, and detach in another (Grossart et al. 2010). Furthermore, zooplankton can provide a refuge for associated bacteria in hostile situations, such as in ballast water treatment systems (Tang et al. 2011). In fact, some *Vibrio* species have increased tolerance against stressors such as thermal stress and fluctuating pH when attached to zooplankton (Castro-Rosas and Escartin 2002). Finally, microorganisms can grow inside or on fecal pellets where they benefit from the locally concentrated nutrients (Tang 2005).

Associated bacteria alter the food web in several ways. First, nutrient plumes created by biodegradation activities of associated bacteria can promote proliferation of other microorganisms, which in turn, can increase population densities of heterotrophic organisms and have additional bottom-up effects. Second, associated bacteria can control the duration and extent of population blooms of cyanobacteria and eukaryotic phototrophic hosts by altering their growth. Because autotrophic population density is directly linked to overall primary production and predation by zooplankton, bottom-up control of autotrophic populations by associated bacteria impacts the entire food web. Finally, the nutrient-rich environments provided by eukaryotic host organisms can increase growth and dispersal rates of the associated bacteria themselves, ultimately increasing the microbial population in the ocean, as well as gene exchange between populations, and thus their evolution by such changed distribution patterns.

Section 5. Symbiotic bacteria

Some bacteria establish more permanent symbiotic relationships within (endobiotic) or on (epibiotic) host organisms. Symbiotic relationships with bacteria are found in organisms including (but not limited to) sponges, ascidians, mollusks, bivalves, protists, and cnidarians. Despite being tighly associated with a host, bacterial symbionts can have several effects on the microbial loop. First, bacterial symbionts reduce heterotrophic feeding in their host by providing organic carbon and other nutrients, or in some cases, by being digested by their host. This increases plankton available to other heterotrophs. Also, although much of the organic carbon synthesized by symbionts is translocated to their hosts or used for symbiont growth, a small portion may be leaked from the association as dissolved inorganic carbon (DOC), thereby contributing to the DOC pool. Symbiotic associations have several effects on nitrogen in the food web as well. Some symbionts, such as those in cyanobacteria-ascidian mutualisms, recycle nitrogen (ammonia) from the host organism that would have been released to the environment. Symbiont nitrification reduces the overall amount of ammonia in the system, and in some cases, releases significant amounts of nitrite—enough to sustain 50% to 120% primary productivity of the reef (Corredor et al. 1988). In addition, nitrification reduces the need for host organisms to acquire nitrogen from the ambient water, leaving more available for other organisms. Some symbionts even provide their hosts with additional nitrogen through amino acids (Parry 1985), and some cyanobacteria symbionts fix atmospheric nitrogen (Wilkinson and Fay 1979), not only adding additional nitrogen to the system, but further reducing the need for their hosts to acquire nitrogen from the water.

Ascidians (tunicates) in the genera *Didemnum*, *Lissoclinum*, *Trididemnum*, and *Diplosoma* harbor cyanobacterial symbionts from several genera: *Prochloron* (Prochlorales) (Hirose et al. 1996), which is unique for containing both chlorophylls *a* and *b*, chlorophyll *a*–containing *Synechocysis* (Chroococcales), and the chlorophyll d–containing *Acaryochloris* (Lopez-Legentil et al. 2011). *Prochloron* symbionts are usually located extracellularly in the cloacal cavity of their ascidian hosts, in the outer surface and tunic, in amongst zoids, or embedded in the cloacal test or on the surface of a colony (Kott 1983; Hirose et al. 2009).

Although *Prochloron* are typically not intracellular, they can translocate up to 51% of their photosynthetically fixed carbon to their host. Fixed carbon translocated from *Prochloron* can fulfill 12% to 56% of the host's respiratory demands (Alberte et al. 1987; Olson 1985). In some tunicate species, more than half of their daily carbon gain comes from photosynthesis rather than from filter feeding, and photosynthetic carbon can exceed or meet carbon lost to host respiration (Koike and Suzuki 1996; Koike et al. 1993). In addition, a small amount of organic carbon (less than 10%) can be leaked from the ascidian colony to the surrounding water (Griffiths and Thinh 1983; Pardy and Lewin 1981). Finally, amino acids synthesized by *Prochloron* may provide some nitrogen for the ascidian host (Parry 1985).

Whereas *Prochloron* may translocate carbon (and potentially nitrogen) to its host, evidence shows that the ascidians may provide nitrogen to *Prochloron* in the form of ammonia (Donia et al. 2011), and additional nitrogen may be supplied by filtration (Koike et al. 1993). Also, a recent study identified genes required to convert ammonia to glutamine, and genes for nitrate in *Prochloron*; however, no nitrogenases or nitrogen-fixing genes were detected (Donia et al. 2011).

Sponges (Porifera) also maintain bacterial photosymbionts. Sponges harboring photosynthetic bacterial symbionts are diverse and abundant in both temperate and tropical regions (Lemloh et al. 2009; Steindler et al. 2002), and can make up as much as 90% of sponge species in some areas (Usher 2008). *Synechococcus* are the dominant symbiont of sponges, however, cyanobacteria from the genera *Aphanocapsa* and *Oscillatoria*, and Prochlorales symbionts including *Prohloron* and *Synechocystis* have been identified (see Usher 2008). Sponge bacterial symbionts are usually intercellular, inhabiting the surface, ectosome, and/or intercellular mesohyl, and can be found surrounding or within sponge archeocytes (Erwin et al. 2012).

Cyanobacterial symbionts transfer photosynthetically fixed carbon to their sponge hosts in the form of glycerol and

organic phosphate (Wilkinson and Fay 1979). In addition, they may transfer glycogen stored in granules to the host (Erwin et al. 2012). Sponge symbionts can fulfill 100% of their host's respiratory carbon requirement (Wilkinson 1983). In addition, some fixed carbon from these associations is released as DOC and contributes to the nutrition of the surrounding reef (Wilkinson 1983).

Symbionts of cyanosponges also fix atmospheric nitrogen (Wilkinson and Fay 1979; Wilkinson et al. 1999) and provide their hosts with a large portion of their nitrogen demand (Mohamed et al. 2008). Sequence data reveal that nitrogen-fixing bacterial symbionts in sponges are unique among marine nitrogen-fixing bacteria, and transcript levels indicate that nitrogen fixation may be occurring simultaneously with photosynthesis (Mohamed et al. 2008). Sponge symbionts are also nitrifiers. Nitrification by the Caribbean cyanosponge *Chondrilla nucula* is over twice the rate of the highest benthic nitrification, and enough to contribute to an estimated 50% to 120% of nitrogen needed for reef productivity (Corredor et al. 1988). In addition to aerobic ammonia-oxidizing bacteria (AAOB) and nitrite-oxidizing bacteria (NOB) responsible for nitrification, anaerobic ammonia-oxidizing bacteria (AnAOB) were also recently identified in marine sponges (Mohamed et al. 2009).

Nitrogen-fixing cyanobacteria have been identified in corals that have algal symbionts (zooxanthellae) as well. These bacterial symbionts live in close association with the zooxanthellae, and may provide them with fixed nitrogen (Lesser et al. 2004, 2007). There is also evidence that additional symbionts (both bacterial and archael) perform nitrification, denitrification, and ANAMMOX in corals (see Fiore et al. 2010; Yang et al. 2013).

Bacterial symbionts are present in a number of protists (see Gast et al. 2009). *Synechococcus* sp. and *Prochloroccocus* sp. have been reported in radiolarians, and in a number of dinoflagellates, where they are thought to be nitrogen-fixing symbionts (Foster et al. 2006; Farnelid et al. 2010; Yuasa et al. 2012). Phototrophic as well as diazotrophic cyanobacterial symbionts have been reported in diatoms, where these symbionts contribute to a large portion of nitrogen fixation (see Fiore et al. 2010). Moreover, several of these diatom-diazotroph associations (DDAs) bloom simultaneously with phytoplankton in the North Pacific Subtropical Gyre (Villareal et al. 2012) and may encourage phytoplankton blooms elsewhere. Finally, ciliate species host a number of bacterial (and eukaryotic) symbionts that are phototrophic and chemosynthetic (see Dziallas et al. 2012) but symbionts may also be phagocytized and serve as food directly (Ott et al. 2004).

Chemosynthetic bacterial symbionts are ubiquitous in a variety of deep sea invertebrates that inhabit hydrothermal vents, cold seeps, whale falls, and other unique environments (reviewed by Dubilier et al. 2008). Associations with chemosynthetic bacteria also occur in some shallow-water environments. In these associations, the host organism gains organic carbon, either by translocation of fixed carbon from symbiont to host, or by direct digestion of the symbionts (Cavanaugh et al. 2006). Most deep sea bacterial symbionts are thiotrophic Gammaproteobacteria, although thiotrophic Epsilonproteobacteria have been isolated from two vent organisms (see Dubilier et al. 2008). Thiotrophic, or sulfur-oxidizing symbionts use energy acquired from reducing sulfur to fix inorganic carbon (usually carbon dioxide) to organic carbon that can be translocated to their hosts. Several families of deep sea bivalves have thiotrohic symbionts. Solemyid clams rely on obligate symbionts to provide over 90% of organic carbon (Conway et al. 1989), and lucinid, vesicomyid, and thyasirid bivalves also gain a portion of their energy from sulfur-oxidizing symbionts in their gills, although symbiosis is not found in all thyasirids (see Duperron et al. 2013). Mytilid mussels not only host thiotrophs, but methanotrophic, or methane-oxidizing symbionts, as well as methyltrophs, Bacteriodetes, and hydrocarbon-degrading bacterial symbionts (see Duperron et al. 2013). In the deep sea, methanotrophic symbionts are also found in some species of sponges, polycheates, and snails (see Dubilier et al. 2008).

As seen in these examples, bacterial symbiosis occurs in both benthic and pelagic habitats from surface waters to the bottom of the sea. Bacterial symbionts occupy a different niche from their free-living counterparts by conferring portions of carbon and nitrogen compounds to their hosts. Nevertheless, these compounds remain in the microbial loop when they are leaked by the symbiosis, and by the simple fact that these symbionts are still performing the function of transforming and remineralizing nutrients, and synthesizing organic carbon, albeit if only for a specific host. As additional symbioses are revealed, closer examination of the importance of bacterial symbionts on nutrient flux should be considered, as their role is likely greater than currently recognized.

Section 6. Particle-attached marine Bacteroidetes (Flavobacteria)

Flavobacteria are marine members of the phylum Bacteroidetes, which constitute the most abundant group of bacteria in the ocean following Proteobacteria and cyanobacteria (Glöckner et al. 1999; Kirchman 2002; Amaral-Zettler et al. 2010). In coastal areas alone, they represent between 10% and 30% of the total bacterial abundance (Alonso-Saez and Gasol 2007). Bacteroidetes are globally distributed (Pommier et al. 2007), and it is possible to find them in a variety of marine environments including coastal and pelagic waters, in sediments and sea ice, and surrounding hydrothermal vents (Brinkmeyer et al. 2003; Alonso et al. 2007; Pommier et al. 2007). This group of marine bacteria has recently received attention for their unique physiology and contribution to nutrient cycling.

Flavobacteria have a specialized role in dissolved organic matter (DOM) uptake and degradation. The microbial loop

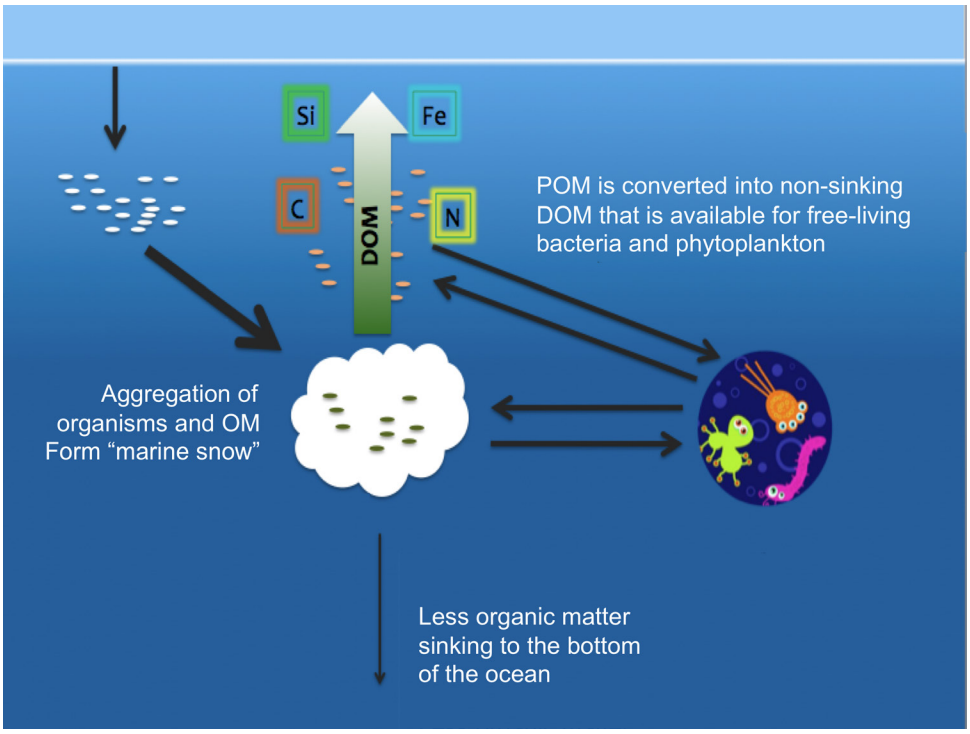

Fig. 6. Suggested role of Flavobacteria in recycling marine snow among other bacteria colonizing these aggregates.

depends on DOM uptake by heterotrophic bacteria, which mineralizes about 50% of primary production (Ducklow 2000). Flavobacteria play a unique part in the microbial loop in that they are especially proficient at degrading high molecular weight (HMW) compounds of DOM, such as cellulose and chitin (Cottrell and Kirchman 2000b; Kirchman 2002; Cottrell et al. 2005), and polymeric organic matter (POM) (Riemann et al. 2000; Pinhassi et al. 2004). Thus, they occupy a different niche than Proteobacteria and cyanobacteria, which prefer monomeric compounds.

Flavobacteria have a major effect on colonizing OM particles and marine snow (macroscopic detrital aggregates that fall to the ocean floor), delivering large inputs of organic matter to organisms in the aphotic zone (Fig. 6). Several studies have revealed that Bacteroidetes are the dominant group of microorganisms attached to marine snow (Alldredge et al. 1986; Delong et al. 1993). Because Flavobacteria are capable of degrading these aggregates, it is hypothesized that less organic matter will sink to the bottom of the ocean, and more small particles will be released to the water column, becoming available for free-living bacteria (Azam and Malfatti 2007). Thus, Flavobacteria are major recyclers of organic matter and play a substantial role in energy flux and ecosystem structuring.

Several studies have demonstrated how Flavobacteria use HMW-DOM in aquatic environments. A study in the Mediterranean Sea showed that Flavobacteria dominated the particle-attached fraction in coastal waters and accounted for a sizable percentage of the total bacterial assemblage, whereas α-Proteobacteria represented the majority of free-living bacteria (Crespo et al. 2013). Microautoradiography and fluorescence *in situ* hybridization (MICRO-FISH) experiments showed Flavobacteria as the primary group consuming HMW compounds including chitin, *N*-acetylglucosamine (NAG), and protein in marine waters (Cottrell and Kirchman 2000a). In another study, population densities of Flavobacteria correlated with phytoplankton blooms, indicating the potential role of Flavobacteria in processing bloom-associated DOM (Pinhassi et al. 2004). Also, microcosm experiments reported enrichment of Flavobacteria on organic matter particles (Pedrotti et al. 2009), and they have been observed in the phycosphere of nanoplankton cells in the Atlantic Ocean (Gomez-Pereira et al. 2010). Indeed, there is a growing amount of experimental evidence that supports the role of Flavobacteria as HMW-DOM specialists.

In addition to experimental evidence, genomic analysis has identified genes that supported the specialized role of Bacteroidetes in processing HMW-DOM. The first marine Bacteroidetes genome sequenced, that of *Gramella forsetii* KT0803 (Bauer et al. 2006), revealed a 3.8 Mb genome with a relatively large number of degradative enzymes such us glycoside hydrolases, proteases, and adhesion proteins for attaching to surfaces, suggesting an adaptation for degrading HMW compounds (Fig. 7). These traits were also observed in *Polaribacter* sp. MED152 (Gonzalez et al. 2008), *Dokdonia* sp. MED134 (Gonzalez et al. 2011), and in uncultured Bacteriodetes from the North Atlantic Ocean (Gomez-Pereira

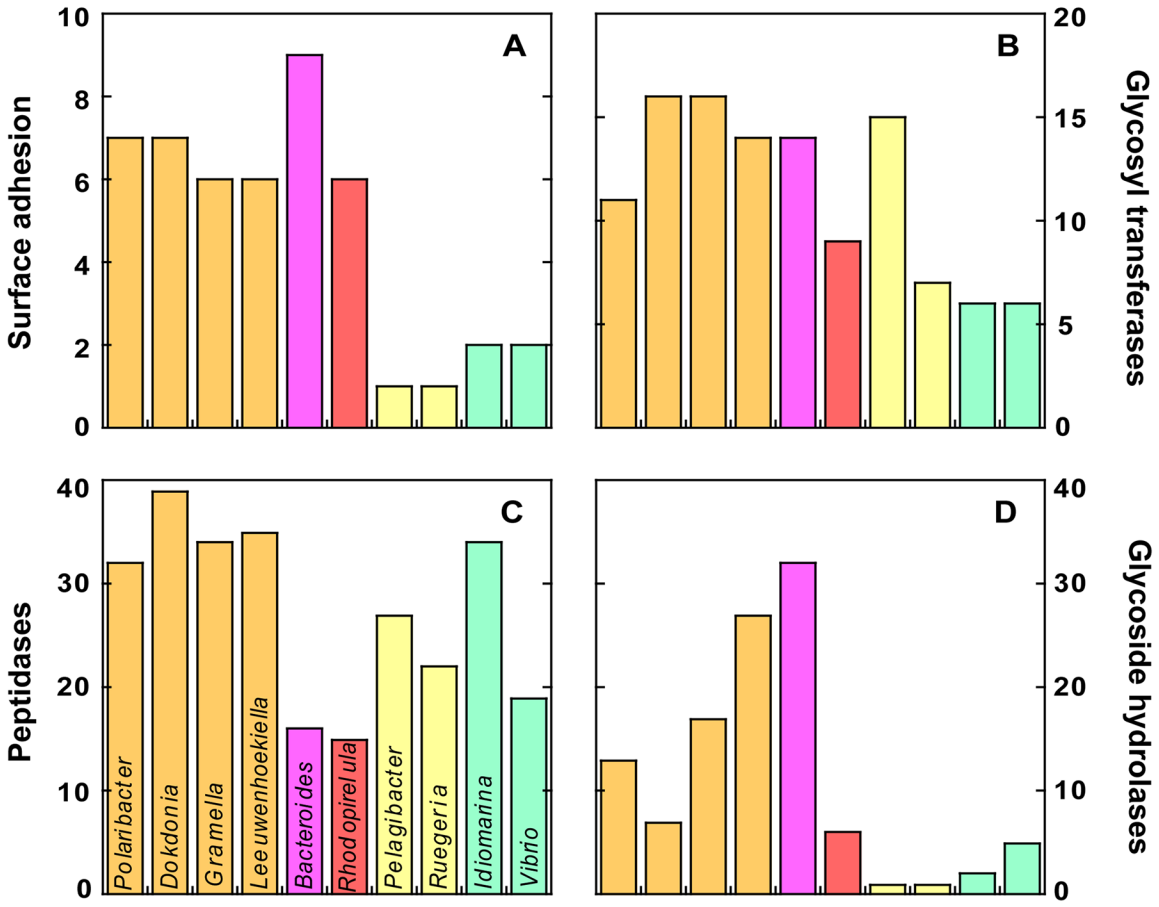

Fig. 7. Numbers of different enzymes per megabase of genome for a selection of bacteria: Marine Bacteroidetes (orange) *Polaribacter* sp. MED152, *Dokdonia* sp. MED134, *Gramella forsetii*, and *Leeuwenhoekiella blandensis* MED217; *Bacteroides thetaiotaomicron* (pink); *Rhodopyrelula baltica* (maroon); the Alphaproteobacteria (yellow) *Pelagibacter ubique* and *Ruegeria pomeroyi*; and the Gammaproteobacteria (green) *Idiomarina loihiensis* and *Vibrio parahaemolyticus* (Fernandez-Gómez et al. 2013). Reprinted with permission from Nature Publishing Group.

et al. 2013). Marine Bacteroidetes genomes are enriched with proteases compared with glycoside hydrolases, suggesting a predilection for degrading proteins (Fig. 7) (Fernandez-Gomez et al. 2013). Moreover, a comparative genomic analysis confirmed that degradative capabilities are a common characteristic among Flavobacteria (Fernandez-Gomez et al. 2013).

Although it is not totally understood, Bacteroidetes may have a specialized mechanism for using polymers that involves gliding over their surfaces. For example, *Polaribacter* sp. MED152 (Gonzalez et al. 2008) and *Dokdonia* sp. MED134 (Gonzalez et al. 2011) have a complete set of genes involved in gliding motility, which would be beneficial in the exploration of solid surfaces. This is also the predicted mechanism that *C. hutchinsonii* (Xie et al. 2007) and *F. johnsoniae* (Braun et al. 2005) use to attach to and degrade cellulose and chitin, respectively. Finally, compared with other bacteria, Bacteroidetes possess a low number of monomer transporters, indicating that Bacteroidetes use only a small number of monomeric carbon compounds (Fernandez-Gomez et al. 2013).

Further evidence supports a tight coupling between surface adhesion and degradation in Bacteroidetes. Polymeric organic matter can be bound by outer membrane complexes formed by SusC, a ligand-gated channel (TonB-dependent transporter), and SusD, an outer membrane protein (SusCD complexes). These complexes function as polysaccharide binding entities in Bacteroidetes to aid in polymer degradation (Anderson and Salyers 1989; Shipman et al. 2000; Blanvillain et al. 2007). In these complexes, hydrolytic enzymes are located on the surface of the bacterium, in addition to outer membrane proteins that bind polysaccharides to the bacterial surface (SusD) (Fig. 8) (Reeves et al. 1997). This is clear evidence that adhesion and degradation are tightly coupled for efficient use of polymers. In fact, TonB-dependent transporters are, together with ABC transporters, the most abundant type of transporters in *Polaribacter* sp. MED152, representing 3.9% of the total amount of transporters (Gonzalez et al. 2008).

It is clear that marine Bacteroidetes have evolved several mechanisms to efficiently use HMW compounds. As

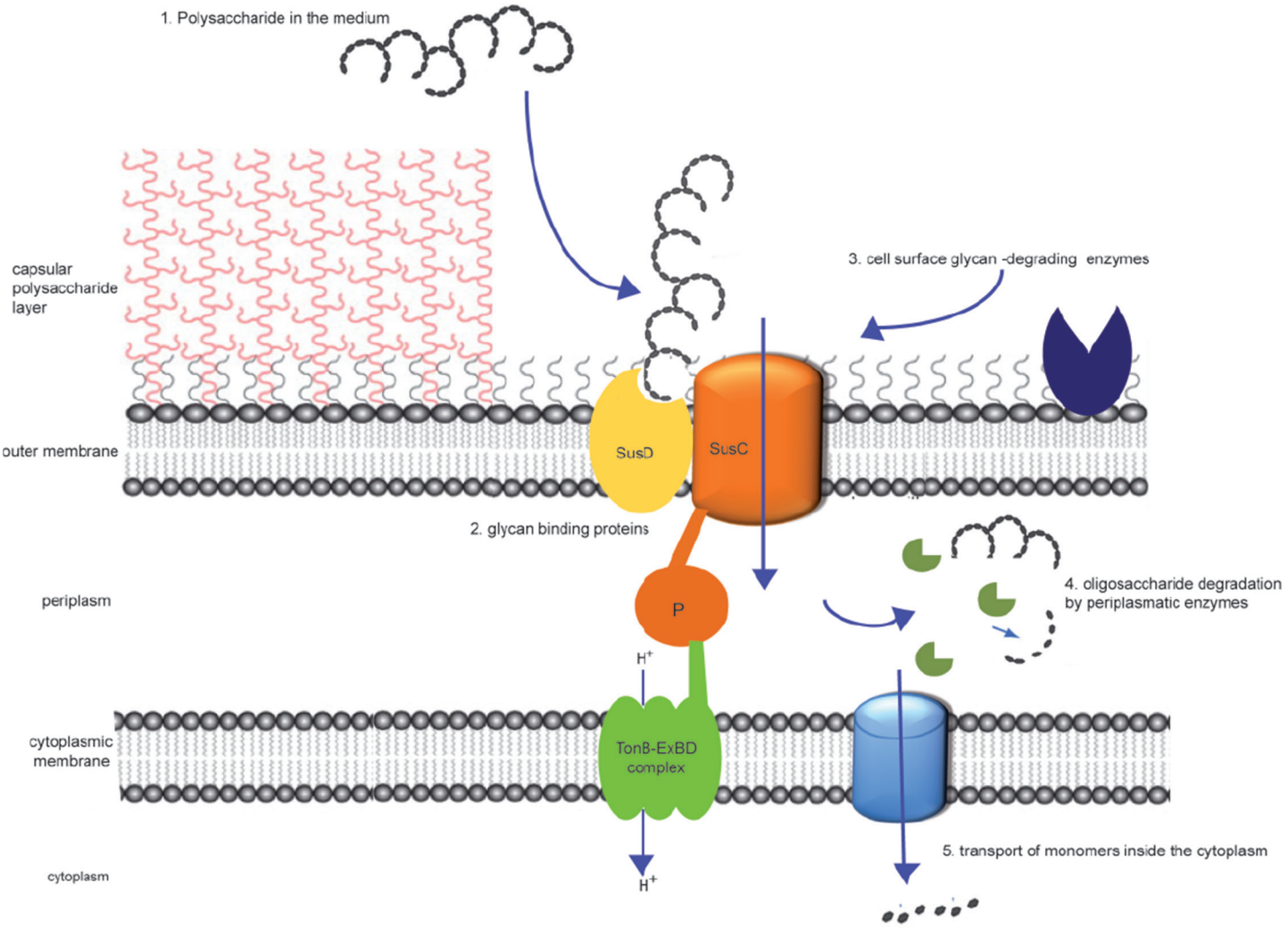

Fig. 8. Simplified model for polysaccharide processing based on Sus System in *B. thetaiotaomicron*. Reprinted from Martens et al. (2009).

mentioned, the contribution of Flavobacteria to nutrient cycling and the microbial loop is through their ability to break down large organic aggregates into small organic material that can be used by other bacteria and pelagic organisms. The ability of Bacteroidetes to process HMW compounds allows them to occupying a niche that Proteobacteria do not, and therefore, their role in the microbial loop should be distinguished.

Section 7. Predatory prokaryotes

Microbial predators including protists, bacteriophages (phages), and predatory prokaryotes acquire energy and key biomolecules by preying on living bacterial cells, which are the most abundant organisms in the ocean. Therefore, microbial predators are often the primary organisms controlling energy flows and nutrient cycling in aquatic systems (Jurkevitch 2007b). Phagotrophic protists, especially bacterivorous nanoflagellates, were once considered the primary cause of bacterial mortality (Pace 1988) and nutrient cycling in aquatic systems (Hahn and Hofle 2001). More recently, however, studies have focused on the importance of phages (Winter et al. 2005; Rohwer and Thurber 2009). Although there are a significant number of reports on protistan and viral predation, studies on bacterial predation by bacteria are rather limited, and many aspects of their lifestyle remain enigmatic (Jurkevitch 2007a). Predatory bacteria play an essential role in the aquatic food web as they provide an alternative pathway to recycle nutrient in bacteria (Fig. 9). Rather than directly released to the DOM pool by viruses, the nutrients of susceptible bacteria are retained inside the predatory prokaryotes. This section will highlight what is known about prokaryotic predators, and discuss their roles in the microbial loop.

Both Gram-positive and Gram-negative predatory prokaryotes have been discovered and described, although examples of Gram-positive predatory bacteria are relatively rare. The most widely-known Gram-positive predators include *Streptoverticillium,* which preys on *Micrococcus*

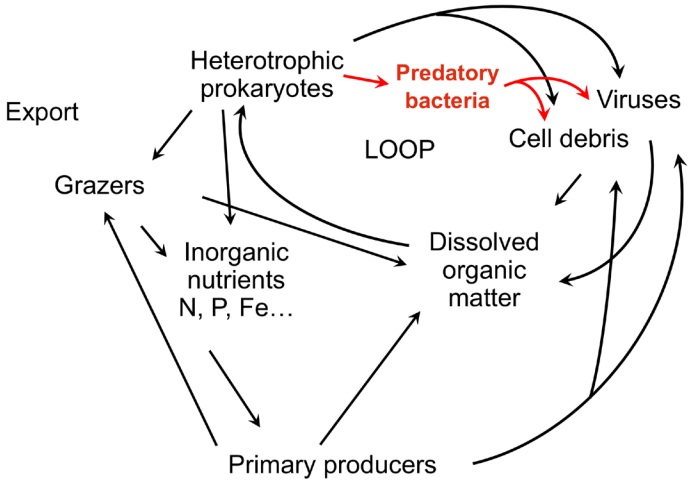

Fig. 9. Conceptual diagram of an aquatic food web from Fuhrman (1999). The alternative pathway to recycle nutrient through predatory bacteria is highlighted in red. Reprinted with permission from Nature Publishing Group.

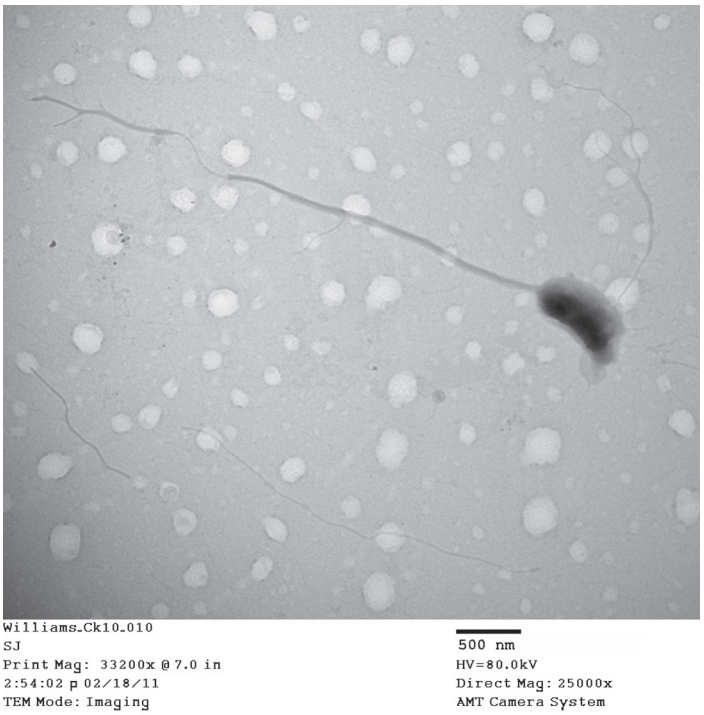

Fig. 10. Electron micrograph of negatively stained *Bacteriovorax marinus* SJ, a saltwater strain of BALOs. (H. Chen unpubl. data).

luteus (Casida 1980), and *Agromyces ramosus,* which attacks and destroys a variety of Gram-negative and Gram-positive soil bacteria, as well as yeast cells (Casida 1983; Byrd et al. 1985). *Streptoverticillium* uses slender filaments of mycelium to search for prey cells under nutritionally poor conditions, and lyses them using a diffusible lytic agent without requiring direct contact. Predation by *A. ramosus*, on the other hand, does not involve diffusible factors and requires the predator to be in close vicinity of the prey. Neither predator is an obligate predator though.

Gram-negative predators are primarily members of Proteobacteria, and possess diverse morphologies and predatory mechanisms. Four basic strategies have been described for predation by these predators: "wolfpack" group predation, epibiotic attachment, direct cytoplasmic invasion, and periplasmic invasion (Martin 2002). So far, the only known example of prokaryotic predators capable of periplasmic invasion is a unique group of obligate predators collectively named *Bdellovibrio* and like organisms (BALOs)(Fig. 10).

BALOs are the only known predatory bacteria that possess a life cycle alternating between an extracellular free-living phase and an intraperiplasmic phase, during which they invade the periplasmic space of prey bacteria, resulting in the death and lysis of the prey and release of new progeny (Fig. 11) (Rendulic et al. 2004). It is reported that up to 80% of marine bacteria are susceptible to predation by BALOs (Rice et al. 1998).

BALOs have largely been excluded from bacterial predators as a group, although their importance has been revealed in a number of studies (Schoeffield and Williams 1990; Rice et al. 1998; Chauhan et al. 2009; Chen et al. 2011). The fact that BALOs' abundance in nature is typically magnitudes lower than phages does not necessarily suggest BALOs play less of a role in bacterial mortality. For example, phages may multiply exponentially without causing additional bacterial lysis due to their large burst size (on average 24 but as high as 50) (Parada et al. 2006). In contrast, BALOs' burst size is reported to be between 4 and 6 (Varon and Shilo 1969). Second, phages can remain stable for years without the support of prey, whereas BALOs generally lose viability within several hours if prey is not available. Also, phages typically prey on a few select bacterial species, whereas BALOs prey on a wide range of Gram-negative bacteria (Schoeffield and Williams 1990). Finally, phages attack rapidly growing and dominant bacterial strains in aquatic ecosystems (Robb and Hill 2000), whereas BALOs are able to efficiently prey on bacteria in the stationary growth phase.

Which predator will dominate in a particular environment depends on several abiotic and biotic factors: nutrient availability, temperature, light, and salinity, to name a few. When predicting the biogeography of BALOs and phages, open oceans favor predation by BALOs which prey efficiently on bacteria in stationary growth phases in low nutrient environments. In these environments, however, BALOs have fewer opportunities for finding prey, and risk mortality when prey is not available. Areas where plankton blooms and nutrient inputs occur can promote bacterial growth and predation by both phages and BALOs, and even cause competition between the two. A recent investigation found that both predators can survive in the same bacterial cell and successfully reproduce themselves (Chen and Williams 2012). This is an especially

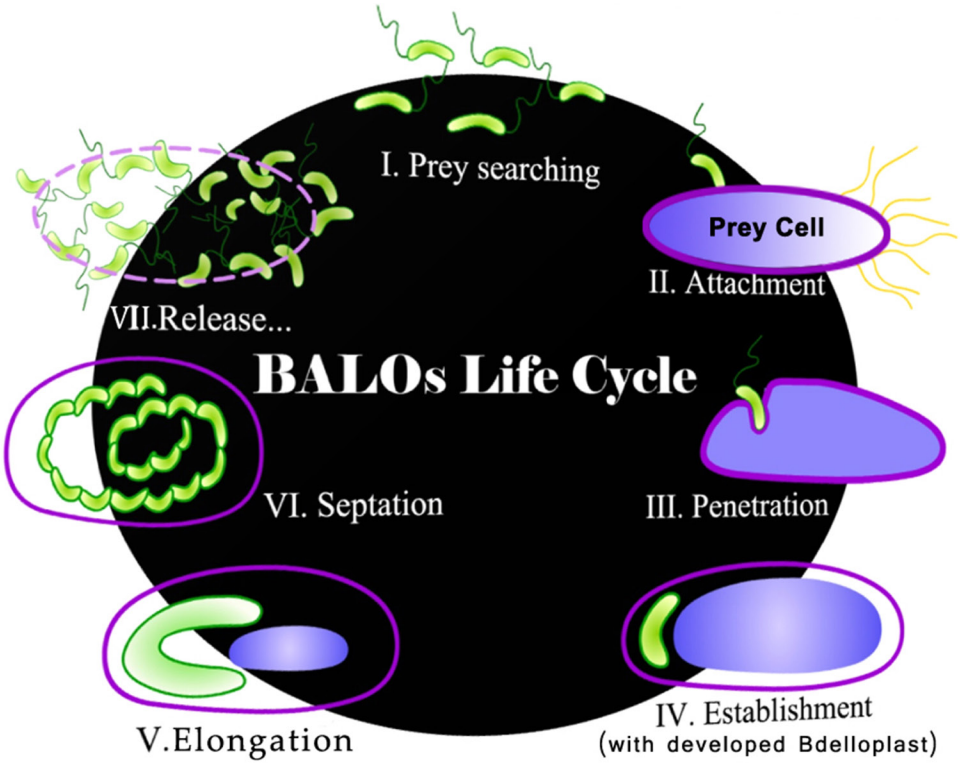

Fig. 11. Diagram depicting the dual phase life cycle of BALOs that consists of an extracellular free-living component in which the predator searches for a susceptible prey bacterium, and an intraperiplasmic stage occurring inside the prey. The predator penetrates through the outer membrane of the prey cell, lodges within the periplasmic space, and feeds on prey macromolecules. It forms an osmotically stable structure termed a bdelloplast that prevents invasion by other predator cells. The BALO subsequently reproduces and lyses the prey cell and releases new progenies into the environment.

valuable mechanism when the prey is in short supply, and the survival of the predators may be at stake.

Evidence strongly suggests that BALOs exert a potential sideways control on nutrient cycling, such that BALO predation sequesters nutrients from bacteria that would have been released into the environment for higher trophic levels. In this way, BALOs alter the structure, function, and dynamics of bacterial communities, and subsequently influence nutrient cycling within the microbial loop (Chauhan et al. 2009; Davidov and Jurkevitch 2004). Both bacteriophages and BALOs ultimately lyse their bacterial prey, however, these two predators process cellular material differently. Whereas phages do not use much of their prey's cytoplasmic material, releasing most into the ambient water as DOM, BALOs typically consume their prey's DOM, leaving little to be released.

Despite the wide prey range and distribution of BALOs, protistan grazing and selective viral lysis continue to be considered the major top down factors controlling bacterial mortality and shaping community structure in aquatic systems (Bouvier and Del Giorgio 2007; Jurgens and Matz 2002; Pernthaler 2005). Although substantial progress has been made in the 50 years since their discovery, BALOs' potential to control other environmental bacteria still remains elusive, due to a lack of research. Until recently, no published report had directly compared the effects of BALOs to phage and protistan predators. Improved investigation of BALOs' role in nature is a premise to better understanding factors controlling bacterial mortality and nutrient cycling within the microbial loop.

In conclusion, BALOs play a significant role in bacterial mortality and in shaping microbial communities. Whether BALOs play a larger role than other bacterial predators depends on environmental conditions and the type of bacterial community present. Given the fact that most bacteria are in oligotrophic environments and persist primarily in a slow or non-growing state (Kolter et al. 1993), it is reasonable to assume that BALOs are the prominent predators of bacteria in the ocean and play the greatest role in bacterial mortality. The control that BALOs exert on microbial populations supports the importance of BALOs in nutrient cycling.

Section 8. Conclusion

In this chapter, we have shown that in addition to the roles described by the current microbial loop, bacteria occupying other niches make significant contributions to nutrient cycling in the ocean. Free-living diazotrophs fix dissolved atmospheric nitrogen and supply usable nitrogen for other organisms in the food web. Particle- and organism-associated bacteria create nutrient plumes of DOM through their

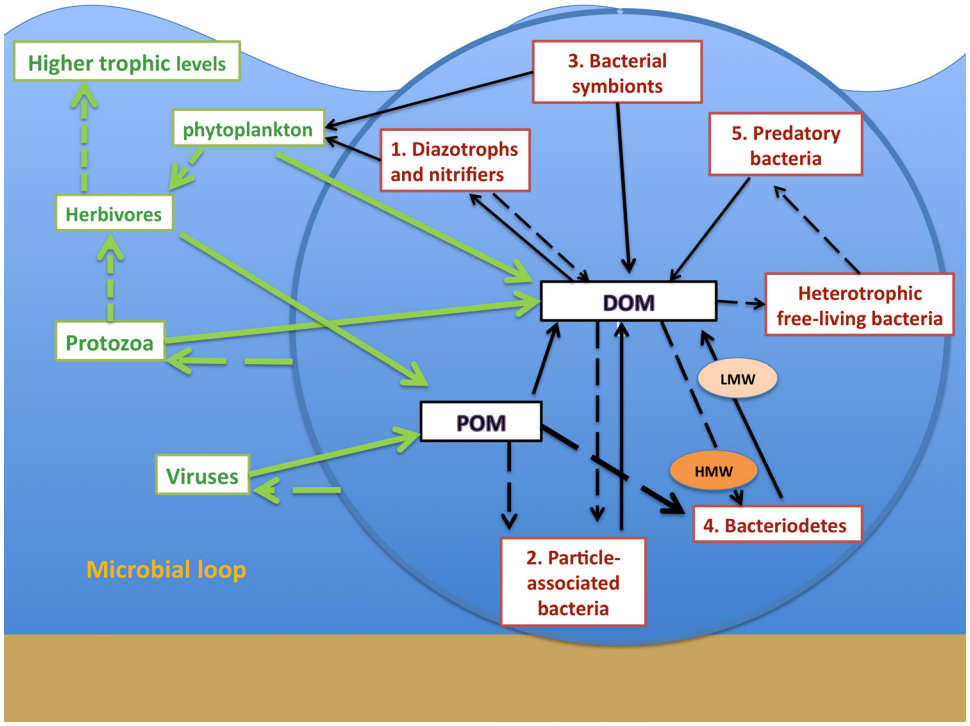

Fig. 12. A modified microbial loop that illustrates the processes of the bacterial specialists discussed in this chapter. Dotted lines represent consumption by the organisms at the end of the arrow. Solid lines represent contribution. LMW = low molecular weight; HMW = high molecular weight; DOM = dissolved organic matter; POM = particulate organic matter.

processing of POM. They impart bottom-up control on phototrophic bacteria and eukaryotes by enhancing carbon supply for their hosts and promoting production, or by stifling host proliferation and causing cell lysis. Bacterial symbionts reduce the need for heterotrophic feeding by their hosts and contribute nitrogen to the environment through nitrification or nitrogen fixation. Bacteriodetes transform high molecular weight aggregates into usable DOM, and finally, BALOs prey on bacteria, controlling bacterial populations and affecting the release of DOM into the water column.

Each of these bacterial specialists contributes to nutrient cycling in the ocean by fulfilling roles not currently recognized in the microbial loop concept. To illustrate and summarize some of the key roles we have described, we present a modified microbial loop, which includes a "bacterial loop" that links the specialized roles of bacteria to the traditional microbial loop and grazing food chain (Fig. 12). Whereas this revised loop illustrates how the major groups of bacteria contribute to nutrient cycling based on the current body of knowledge, it most certainly does not represent all bacterial species and their contributions. As we have seen over the past three decades, the microbial loop must continue to evolve as new microbial specialists and new niches are revealed. What is permanent, however, is the importance of microbes in cycling nutrients in the ocean, and the importance of the pivotal research that led to, and will continue to support the microbial loop.

Authors' contribution

This chapter was written as a collaboration between all authors. With the exception of the first author, all authors are listed alphabetically and contributed equally to this publication.

References

Alberte, R., L. Cheng, and R. Lewin. 1987. Characteristics of Prochloron Ascidian Symbioses. 2. Photosynthesis-irradiance relationships and carbon balance of associations from Palau, Micronesia. Symbiosis 4:147-170.

Alldredge, A. L., J. J. Cole, and D. A. Caron. 1986. Production of heterotrophic bacteria inhabiting macroscopic organic aggregates (marine snow) from surface waters. Limnol. Oceanogr. 31:68-78 [doi:10.4319/lo.1986.31.1.0068].

Alonso, C., F. Warnecke, R. Amann, and J. Pernthaler. 2007. High local and global diversity of Flavobacteria in marine plankton. Environ. Microbiol. 9:1253-1266 [doi:10.1111/j.1462-2920.2007.01244.x].

Alonso-Saez, L., and J. M. Gasol. 2007. Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in northwestern Mediterranean coastal waters. Appl. Environ. Microbiol. 73:3528-3535 [doi:10.1128/AEM.02627-06].

Amaral-Zettler, L., and others. 2010. A global census of marine microbes, p. 221-245. *In* Life in the world's oceans.

Wiley-Blackwell.
Amemiya, Y., K. Kato, T. Okino, and O. Nakayama. 1990. Changes in the chemical composition of carbohydrates and proteins in surface water during a bloom of microcystis in Lake Suwa. Ecol. Res. 5:153-162 [doi:10.1007/BF02346987].
Anderson, K. L., and A. A. Salyers. 1989. Genetic-evidence that outer-membrane binding of starch is required for starch utilization by bacteroides-thetaiotaomicron. J. Bacteriol. 171:3199-3204.
Arrigo, K. R. 2005. Marine microorganisms and global nutrient cycles. Nature 437:349-355 [doi:10.1038/nature04159].
Azam, F. 1998. Microbial control of oceanic carbon flux: The plot thickens. Science 280:694-696 [doi:10.1126/science.280.5364.694].
———, T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyerreil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10:257-263 [doi:10.3354/meps010257].
———, and F. Malfatti. 2007. Microbial structuring of marine ecosystems. Nature Rev. Microbiol. 5:782-791 [doi:10.1038/nrmicro1747].
Bauer, M., and others. 2006. Whole genome analysis of the marine Bacteroidetes 'Gramella forsetii' reveals adaptations to degradation of polymeric organic matter. Environ. Microbiol. 8:2201-2213 [doi:10.1111/j.1462-2920.2006.01152.x].
Bell, W., and R. Mitchell. 1972. Chemotactic and growth responses of marine bacteria to algal extracellular products. Biol. Bull. 143:265-277 [doi:10.2307/1540052].
Berg, K. A., and others. 2009. High diversity of cultivable heterotrophic bacteria in association with cyanobacterial water blooms. Isme J. 3:314-325 [doi:10.1038/ismej.2008.110].
Blanvillain, S., and others. 2007. 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].
Bouvier, T., and P. Del Giorgio. 2007. Key role of selective viral induced mortality in determining marine bacterial community composition. Environ. Microbiol. 9:287-297 [doi:10.1111/j.1462-2920.2006.01137.x].
Braun, S. T., L. M. Proctor, S. Zani, M. T. Mellon, and J. P. Zehr. 1999. Molecular evidence for zooplankton-associated nitrogen-fixing anaerobes based on amplification of the nifH gene. FEMS Microbiol. Ecol. 28:273-279 [doi:10.1111/j.1574-6941.1999.tb00582.x].
Braun, T. F., M. K. Khubbar, D. A. Saffarini, and M. J. Mcbride. 2005. Flavobacterium johnsoniae gliding motility genes identified by mariner mutagenesis. J. Bacteriol. 187:6943-6952 [doi:10.1128/JB.187.20.6943-6952.2005].
Brinkhoff, T., H. A. Giebel, and M. Simon. 2008. Diversity, ecology, and genomics of the *Roseobacter clade*: a short overview. Arch. Microbiol. 189:531-539 [doi:10.1007/s00203-008-0353-y].
Brinkmeyer, R., K. Knittel, J. Jurgens, H. Weyland, R. Amann, and E. Helmke. 2003. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl. Environ. Microbiol. 69:6610-6619 [doi:10.1128/AEM.69.11.6610-6619.2003].
Brown, S. M., and B. D. Jenkins. 2014. Profiling gene expression to distinguish the likely active diazotrophs from a sea of genetic potential in marine sediments. Environ. Microbiol. 16:3128-3142 [doi:10.1111/1462-2920.12403].
Byrd, J. J., L. R. Zeph, and L. Casida Jr. 1985. Bacterial control of *Agromyces ramosus* in soil. Can. J. Microbiol. 31:1157-1163 [doi:10.1139/m85-218].
Casida, L. E., Jr. 1980. Bacterial predators of *Micrococcus luteus* in soil. Appl. Environ. Microbiol. 39:1035-1041.
Casida, L. E. 1983. Interaction of *Agromyces ramosus* with other bacteria in soil. Appl. Environ. Microbiol. 46:881.
Castro-Rosas, J., and E. F. Escartin. 2002. Adhesion and colonization of Vibrio cholerae O1 on shrimp and crab carapaces. J. Food Protect. 65:492-498.
Cavanaugh, C. M., Z. P. McKiness, I. L. G. Newton, and F. J. Stewart. 2006. Marine chemosynthetic symbioses. *In* The prokaryotes, pp. 475-507. Springer [doi:10.1007/0-387-30741-9_18].
Chauhan, A., J. Cherrier, and H. N. Williams. 2009. Impact of sideways and bottom-up control factors on bacterial community succession over a tidal cycle. Proc. Nat. Acad. Sci. U.S.A. 106:4301-4306 [doi:10.1073/pnas.0809671106].
Chen, H., R. Athar, G. Zheng, and H. N. Williams. 2011. Prey bacteria shape the community structure of their predators. ISME J. 5:1314-1322 [doi:10.1038/ismej.2011.4].
———, and H. N. Williams. 2012. Sharing of prey: Coinfection of a bacterium by a virus and a prokaryotic predator. mBio 3 [doi:10.1128/mBio.00051-12].
Cole, J. J. 1982. Interactions between bacteria and algae in aquatic ecosystems. Ann. Rev. Ecol. Syst 13:291-314 [doi:10.1146/annurev.es.13.110182.001451].
Conway, N., J. McDowell Capuzzo, and B. Fry. 1989. The role of endosymbiotic bacteria in the nutrition of *Solemya velum*: evidence for a stable isotope analysis of endosymbionts and host. Limnol. Oceanogr. 34:249-255 [doi:10.4319/lo.1989.34.1.0249].
Corredor, J. E., C. R. Wilkinson, V. P. Vicente, J. M. Morell, and E. Otero. 1988. Nitrate release by Caribbean reef sponges. Limnol. Oceanogr. 33:114-120 [doi:10.4319/lo.1988.33.1.0114].
Cottrell, M. T., and D. L. Kirchman. 2000a. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl. Environ. Microbiol. 66:5116-5122 [doi:10.1128/AEM.66.12.5116-5122.2000].
———, and ———. 2000b. 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 [doi:10.1128/AEM.66.4.1692-1697.2000].
———, L. A. Waidner, L. Y. Yu, and D. L. Kirchman. 2005.

Bacterial diversity of metagenomic and PCR libraries from the Delaware River. Environ. Microbiol. 7:1883-1895 [doi:10.1111/j.1462-2920.2005.00762.x].

Crespo, B. G., T. Pommier, B. Fernandez-Gomez, and C. Pedros-Alio. 2013. Taxonomic composition of the particle-attached and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA. MicrobiologyOpen 2:541-552 [doi:10.1002/mbo3.92].

Crump, B. C., E. V. Armbrust, and J. A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. Appl. Environ. Microb. 65:3192-3204.

Curtis, T. P., and W. T. Sloan. 2004. Prokaryotic diversity and its limits: microbial community structure in nature and implications for microbial ecology. Curr. Opin. Microbiol. 7:221-226 [doi:10.1016/j.mib.2004.04.010].

Darwin, C. R. 1845. Journal of researches into the natural history and geology of the countries visited during the voyage of H.M.S. Beagle round the world, under the Command of Capt. Fitz Roy, R.N. 2d edition. John Murray.

Davelaar, D. 1993. Ecological significance of bacterial polyphosphate metabolism in sediments. Hydrobiologia 253:179-192 [doi:10.1007/BF00050737].

Davidov, Y., and E. Jurkevitch. 2004. Diversity and evolution of Bdellovibrio-and-like organisms (BALOs), reclassification of *Bacteriovorax starrii* as *Peredibacter starrii* gen. nov., comb. nov., and description of the *Bacteriovorax-Peredibacter* clade as Bacteriovoracaceae fam. nov. Int. J. Syst. Evol. Microbiol. 54:1439-1452 [doi:10.1099/ijs.0.02978-0].

Dekas, A. E., R. S. Poretsky, and V. J. Orphan. 2009. Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. Science 326:422-426 [doi:10.1126/science.1178223].

Delong, E. F., D. G. Franks, and A. L. Alldredge. 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. 38:924-934 [doi:10.4319/lo.1993.38.5.0924].

———, and D. M. Karl. 2005. Genomic perspectives in microbial oceanography. Nature 437:336-342 [doi:10.1038/nature04157].

Donia, M. S., and others. 2011. Complex microbiome underlying secondary and primary metabolism in the tunicate-Prochloron symbiosis. Proc. Nat. Acad. Sci. U.S.A. 108:E1423-E1432 [doi:10.1073/pnas.1111712108].

Dubilier, N., C. Bergin, and C. Lott. 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat. Rev. Microbiol. 6:725-740 [doi:10.1038/nrmicro1992].

Ducklow, H. 2000. Bacterial production and biomass in the oceans. Microb. Ecol. Oceans 1:85-120.

Duperron, S., S. M. Gaudron, C. F. Rodrigues, M. R. Cunha, C. Decker, and K. Olu. 2013. An overview of chemosynthetic symbioses in bivalves from the North Atlantic and Mediterranean Sea. Biogeosciences 10:3241-3267 [doi:10.5194/bg-10-3241-2013].

Dziallas, C., and H. P. Grossart. 2011. Increasing oxygen radicals and water temperature select for toxic microcystis sp. PLOS One 6(9):e25569 [doi:10.1371/journal.pone.0025569].

———, M. Allgaier, M. T. Monaghan, and H. P. Grossart. 2012. Act together—implications of symbioses in aquatic ciliates. Front. Microbiol. 3:288 [doi:10.3389/fmicb.2012.00288].

Erwin, P. M., S. Lopez-Legentil, and X. Turon. 2012. Ultrastructure, molecular phylogenetics, and Chlorophyll a content of novel cyanobacterial symbionts in temperate sponges. Microb. Ecol. 64:771-783 [doi:10.1007/s00248-012-0047-5].

Falkowski, P. G., R. T. Barber, and V. Smetacek. 1998. Biogeochemical controls and feedbacks on ocean primary production. Science 281:200-206 [doi:10.1126/science.281.5374.200].

Farnelid, H., W. Tarangkoon, G. Hansen, P. J. Hansen, and L. Riemann. 2010. Putative N-2-fixing heterotrophic bacteria associated with dinoflagellate-Cyanobacteria consortia in the low-nitrogen Indian Ocean. Aquat. Microb. Ecol. 61:105-117 [doi:10.3354/ame01440].

———, and others. 2013. Active nitrogen-fixing heterotrophic bacteria at and below the chemocline of the central Baltic Sea. ISME J. 7:1413-1423 [doi:10.1038/ismej.2013.26].

Fenchel, T. 2008. The microbial loop—25 years later. J. Exp. Mar. Biol. Ecol. 366:99-103 [doi:10.1016/j.jembe.2008.07.013].

Fernandez, C., L. Farias, and O. Ulloa. 2011. Nitrogen fixation in denitrified marine waters. PLOS One 6(6):e20539 [doi:10.1371/journal.pone.0020539].

Fernandez-Gomez, B., and others. 2013. Ecology of marine Bacteroidetes: a comparative genomics approach. ISME J. 7:1026-1037 [doi:10.1038/ismej.2012.169].

Fiore, C. L., J. K. Jarett, N. D. Olson, and M. P. Lesser. 2010. Nitrogen fixation and nitrogen transformations in marine symbioses. Trends Microbiol. 18:455-463 [doi:10.1016/j.tim.2010.07.001].

Flynn, K. J., and others. 2013. Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. J. Plankton Res. 35:3-11 [doi:10.1093/plankt/fbs062].

Foster, R. A., E. J. Carpenter, and B. Bergman. 2006. Unicellular cyanobionts in open ocean dinoflagellates, radiolarians, and tintinnids: ultrastructure characterization and immuno-localization of phycoerytherin and nitrogenase. J. Phycol. 42:453-463 [doi:10.1111/j.1529-8817.2006.00206.x].

Fuhrman, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature 399:541-548 [doi:10.1038/21119].

Fulweiler, R. W., S. W. Nixon, B. A. Buckley, and S. L. Granger. 2008. Net sediment N-2 fluxes in a coastal marine system-experimental manipulations and a conceptual model. Ecosystems 11:1168-1180 [doi:10.1007/

s10021-008-9187-3].
———, S. Brown, S. Nixon, and B. Jenkins. 2013. Evidence and a conceptual model for the co occurrence of nitrogen fixation and denitrification in heterotrophic marine sediments. Mar. Ecol. Progr. Ser. 482:57-68 [doi:10.3354/meps10240].
Gast, R. J., R. W. Sanders, and D. A. Caron. 2009. Ecological strategies of protists and their symbiotic relationships with prokaryotic microbes. Trends microbiol. 17:563-569 [doi:10.1016/j.tim.2009.09.001].
Giovannoni, S. J., and U. Stingl. 2005. Molecular diversity and ecology of microbial plankton. Nature 437:343-348 [doi:10.1038/nature04158].
Glöckner FB.M. Fuchs and R. Amann. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl Environ Microbiol 65: 3721–3726.
Gomez-Pereira, P. R., B. M. Fuchs, C. Alonso, M. J. Oliver, J. E. E. Van Beusekom, and R. Amann. 2010. Distinct flavobacterial communities in contrasting water masses of the North Atlantic Ocean. ISME J. 4:472-487 [doi:10.1038/ismej.2009.142].
———, G. Kennaway, B. M. Fuchs, G. A. Tarran, and M. V. Zubkov. 2013. Flow cytometric identification of Mamiellales clade II in the Southern Atlantic Ocean. FEMS Microbiol. Ecol. 83:664-671 [doi:10.1111/1574-6941.12023].
Gonzalez, J. M., and others. 2008. Genome analysis of the proteorhodopsin-containing marine bacterium Polaribacter sp MED152 (Flavobacteria). Proc. Nat. Acad. Sci. U.S.A. 105:8724-8729 [doi:10.1073/pnas.0712027105].
———, and others. 2011. Genomics of the proteorhodopsin-containing marine flavobacterium dokdonia sp. strain MED134. Appl. Environ. Microbiol. 77:8676-8686 [doi:10.1128/AEM.06152-11].
Griffith, P. C., D. J. Douglas, and S. C. Wainright. 1990. Metabolic-activity of size-fractionated microbial plankton in estuarine, nearshore, and continental-shelf waters of Georgia. Mar. Ecol. Prog. Ser. 59:263-270 [doi:10.3354/meps059263].
Griffiths, D. J., and L. V. Thinh. 1983. Transfer of photosynthetically fixed carbon between the prokaryotic green-alga prochloron and its ascidian host. Aust. J. Mar. Fresh Res. 34:431-440 [doi:10.1071/MF9830431].
Grossart, H. P. 1999. Interactions between marine bacteria and axenic diatoms (Cylindrotheca fusiformis, Nitzschia laevis, and Thalassiosira weissflogii) incubated under various conditions in the lab. Aquat. Microb. Ecol. 19:1-11 [doi:10.3354/ame019001].
———. 2010. Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed. Environ. Microbiol. Rep. 2:706-714 [doi:10.1111/j.1758-2229.2010.00179.x].
———, and M. Simon. 1998. Bacterial colonization and microbial decomposition of limnetic organic aggregates (lake snow). Aquat. Microb. Ecol. 15:127-140 [doi:10.3354/ame015127].
———, L. Riemann, and F. Azam. 2001. Bacterial motility in the sea and its ecological implications. Aquat. Microb. Ecol. 25:247-258 [doi:10.3354/ame025247].
———, T. Kiorboe, K. W. Tang, M. Allgaier, E. M. Yam, and H. Ploug. 2006. Interactions between marine snow and heterotrophic bacteria: aggregate formation and microbial dynamics. Aquat. Microb. Ecol. 42:19-26 [doi:10.3354/ame042019].
———, and M. Simon. 2007. Interactions of planktonic algae and bacteria: effects on algal growth and organic matter dynamics. Aquat. Microb. Ecol. 47:163 [doi:10.3354/ame047163].
———, K. W. Tang, T. Kiorboe, and H. Ploug. 2007. Comparison of cell-specific activity between free-living and attached bacteria using isolates and natural assemblages. FEMS Microbiol. Lett. 266:194-200 [doi:10.1111/j.1574-6968.2006.00520.x].
———, and K. W. Tang. 2010. aquaticmicrobial.net. Commun. Integrat. Biology 3:491-494 [doi:10.4161/cib.3.6.12975].
———, C. Dziallas, F. Leunert, and K. W. Tang. 2010. Bacteria dispersal by hitchhiking on zooplankton. Proc. Nat. Acad. Sci. U.S.A. 107:11959-11964 [doi:10.1073/pnas.1000668107].
Gruber, N., and J. N. Galloway. 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451:293-296 [doi:10.1038/nature06592].
Hahn, M. W., and M. G. Hofle. 2001. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol. Ecol. 35:113-121 [doi:10.1111/j.1574-6941.2001.tb00794.x].
Halm, H. and others. 2009. Co-occurrence of denitrification and nitrogen fixation in a meromictic lake, Lake Cadagno (Switzerland). Environ. Microbiol. 11:1945-1958 [doi:10.1111/j.1462-2920.2009.01917.x].
Harwood, C. a. M. B. 2008. Uncharted microbial world: microbes and their activities in the environment. Colloquium Reports. American Society for Microbiology.
Hirose, E., T. Maruyama, L. Cheng, and R. A. Lewin. 1996. Intracellular symbiosis of a photosynthetic prokaryote, Prochloron sp, in a colonial ascidian. Invertebr. Biol. 115:343-348 [doi:10.2307/3227023].
———, A. T. Oka, and M. Hirose. 2009. Two new species of photosymbiotic ascidians of the genus Diplosoma from the Ryukyu Archipelago, with partial sequences of the COI gene. Zool. Sci. 26:362-368 [doi:10.2108/zsj.26.362].
Holl, C. M., and J. P. Montoya. 2005. Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph Trichodesmium (Cyanobacteria). J. Phycol. 41:1178-1183 [doi:10.1111/j.1529-8817.2005.00146.x].
Jayakumar, A., M. M. D. Al-Rshaidat, B. B. Ward, and M. R. Mulholland. 2012. Diversity, distribution, and expression of diazotroph nifH genes in oxygen-deficient waters of the Arabian Sea. FEMS Microbiol. Ecol. 82:597-606

[doi:10.1111/j.1574-6941.2012.01430.x].
Jurgens, K., and C. Matz. 2002. Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. Antonie Van Leeuwenhoek 81:413-434 [doi:10.1023/A:1020505204959].
Jurkevitch, E. 2007a. A brief history of short bacteria: a chronicle of Bdellovibrio (and like organisms) research, pp. 1-9. *In* E. Jurkevitch (ed.), Predatory prokaryotes. Springer.
———. 2007b. Predatory behaviors in bacteria-diversity and transitions. Microbe 2:67.
Karl, D. M., and L. M. Proctor. 2007. Foundations of microbial oceanography. Oceanography 20:16-27 [doi:10.5670/oceanog.2007.44].
Kiorboe, T., and G. A. Jackson. 2001. Marine snow, organic solute plumes, and optimal chemosensory behavior of bacteria. Limnol. Oceanogr. 46:1309-1318 [doi:10.4319/lo.2001.46.6.1309].
———, and U. H. Thygesen. 2001. Fluid motion and solute distribution around sinking aggregates. II. Implications for remote detection by colonizing zooplankters. Mar. Ecol. Prog. Ser. 211:15-25 [doi:10.3354/meps211015].
Kirchman, D. L. 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments. FEMS Microbiol. Ecol. 39:91-100 [doi:10.1111/j.1574-6941.2002.tb00910.x].
Koike, I., M. Yamamuro, and P. C. Pollard. 1993. Carbon and nitrogen budgets of 2 ascidians and their symbiont, prochloron, in a tropical seagrass meadow. Aust. J. Mar. Fresh Res. 44:173-182.
———, and T. Suzuki. 1996. Nutritional diversity of symbiotic ascidians in a Fijian seagrass meadow. Ecol. Res. 11:381-386 [doi:10.1007/BF02347795].
Kolter, R., D. A. Siegele, and A. Tormo. 1993. The stationary phase of the bacterial life cycle. Ann. Rev. Microbiol. 47:855-874 [doi:10.1146/annurev.mi.47.100193.004231].
Kott, P. 1983. Two new genera of didemnid ascidians from tropical Australian waters. The Beagle: Records of the Museums and Art Galleries of the Northern Territory 1:13-19.
Lami, R., and others. 2007. High abundances of aerobic anoxygenic photosynthetic bacteria in the South Pacific Ocean. Appl. Environ. Microbiol. 73:4198-4205 [doi:10.1128/AEM.02652-06].
Leigh, J. A., and J. A. Dodsworth. 2007. Nitrogen regulation in bacteria and archaea. Ann. Rev. Microbiol. 61:349-377 [doi:10.1146/annurev.micro.61.080706.093409].
Lemloh, M. L., J. Fromont, F. Brummer, and K. M. Usher. 2009. Diversity and abundance of photosynthetic sponges in temperate Western Australia. BMC Ecol. 9:4 [doi:10.1186/1472-6785-9-4].
Lesser, M. P., C. H. Mazel, M. Y. Gorbunov, and P. G. Falkowski. 2004. Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science 305:997-1000 [doi:10.1126/science.1099128].
———, et al. 2007. Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral Montastraea cavernosa. Mar. Ecol. Prog. Ser. 346:143-152 [doi:10.3354/meps07008].
Lopez-Legentil, S., B. Song, M. Bosch, J. R. Pawlik, and X. Turon. 2011. Cyanobacterial diversity and a new acaryochloris-like symbiont from Bahamian Sea-Squirts. PLOS One 6(8):e23938 [doi:10.1371/journal.pone.0023938].
Martens, E. C., N. M. Koropatkin, T. J. Smith, and J. I. Gordon. 2009. Complex glycan catabolism by the human gut microbiota: The bacteroidetes sus-like paradigm. J. Biol. Chem. 284:24673-24677 [doi:10.1074/jbc.R109.022848].
Martin, M. O. 2002. Predatory prokaryotes: an emerging research opportunity. J. Mol. Microbiol. Biotechnol. 4:467-478.
Maruyama, T., K. Kato, A. Yokoyama, T. Tanaka, A. Hiraishi, and H. D. Park. 2003. Dynamics of microcystin-degrading bacteria in mucilage of Microcystis. Microb. Ecol. 46:279-288 [doi:10.1007/s00248-002-3007-7].
Mehta, M. P., and J. A. Baross. 2006. Nitrogen fixation at 92 degrees C by a hydrothermal vent archaeon. Science 314:1783-1786 [doi:10.1126/science.1134772].
Middelboe, M., M. Sondergaard, Y. Letarte, and N. H. Borch. 1995. Attached and free-living bacteria—Production and polymer hydrolysis during a diatom bloom. Microb. Ecol. 29:231-248 [doi:10.1007/BF00164887].
Mohamed, N. M., A. S. Colman, Y. Tal, and R. T. Hill. 2008. Diversity and expression of nitrogen fixation genes in bacterial symbionts of marine sponges. Environ. Microbiol. 10:2910-2921 [doi:10.1111/j.1462-2920.2008.01704.x].
———, K. Saito, Y. Tal, and R.T. Hill. 2009. Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. ISME J. 4:38-48 [doi:10.1038/ismej.2009.84].
Olson, P., J. Porter. 1985. In situ measurement of photosynthesis and respiration in the ascidian-Prochloron symbiosis *Didemnum molle*. Proceedings of the 5th International Coral Reef Congress, Tahiti, 27 May-1 June 1985 5:257-262.
Ott, J., M. Bright, and S. Bulgheresi. 2004. Marine microbial thiotrophic ectosymbioses. Oceanogr. Mar. Biol. Ann. Rev 42:95-118 [doi:10.1201/9780203507810.ch4].
Pace, M. L. 1988. Bacterial mortality and the fate of bacterial production. Hydrobiologia 159:41-49 [doi:10.1007/BF00007366].
Parada, V., G. J. Herndl, and M. G. Weinbauer. 2006. Viral burst size of heterotrophic prokaryotes in aquatic systems. J. Mar. Biol. Assoc. U.K. 86:613-621 [doi:10.1017/S002531540601352X].
Pardy, R. L., and R. A. Lewin. 1981. Colonial ascidians with prochlorophyte symbionts—Evidence for translocation of metabolites from alga to host. Bull. Mar. Sci. 31:817-823.
Parry, D. L. 1985. Nitrogen assimilation in the symbiotic marine-algae prochloron Spp. Mar. Biol. 87:219-222 [doi:10.1007/BF00397797].
Pedrosalio, C., and T. D. Brock. 1983. The impact of zooplankton feeding on the epilimnetic bacteria of a eutrophic lake.

Freshw. Biol. 13:227-239 [doi:10.1111/j.1365-2427.1983.tb00673.x].

Pedrotti, M., S. Beauvais, M.-E. Kerros, K. Iversen, and F. Peters. 2009. Bacterial colonization of transparent exopolymeric particles in mesocosms under different turbulence intensities and nutrient conditions. Aquat. Microb. Ecol. 55:301 [doi:10.3354/ame01308].

Pernthaler, J. 2005. Predation on prokaryotes in the water column and its ecological implications. Nat. Rev. Microbiol. 3:537-546 [doi:10.1038/nrmicro1180].

Peterson, B. J., J. E. Hobbie, and J. F. Haney. 1978. Daphnia grazing on natural bacteria. Limnol. Oceanogr. 23:1039-1044 [doi:10.4319/lo.1978.23.5.1039].

Pinhassi, J., and others. 2004. Changes in bacterioplankton composition under different phytoplankton regimens. Appl. Environ. Microbiol. 70:6753-6766 [doi:10.1128/AEM.70.11.6753-6766.2004].

Ploug, H., B. Adam, N. Musat, T. Kalvelage, G. Lavik, D. Wolf-Gladrow, and M. M. M. Kuypers. 2011. Carbon, nitrogen, and O_2 fluxes associated with the cyanobacterium *Nodularia spumigena* in the Baltic Sea. ISME J. 5:1549-1558 [doi:10.1038/ismej.2011.20].

Pomeroy, L. R. 1974. Oceans food web, a changing paradigm. Bioscience 24:499-504 [doi:10.2307/1296885].

———, P. J. I. Williams, F. Azam, and J. E. Hobbie. 2007. The microbial loop. Oceanography 20:28-33 [doi:10.5670/oceanog.2007.45].

Pommier, T., and others. 2007. Global patterns of diversity and community structure in marine bacterioplankton. Mol. Ecol. 16:867-880 [doi:10.1111/j.1365-294X.2006.03189.x].

Reeves, A. R., G. R. Wang, and A. A. Salyers. 1997. Characterization of four outer membrane proteins that play a role in utilization of starch by Bacteroides thetaiotaomicron. J. Bacteriol. 179:643-649.

Rendulic, S., P. Jagtap, A. Rosinus, M. Eppinger, C. Baar, and C. Lanz. 2004. A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303:689-692 [doi:10.1126/science.1093027].

Rice, T. D., H. N. Williams, and B. F. Turng. 1998. Susceptibility of bacteria in estuarine environments to autochthonous *Bdellovibrios*. Microb. Ecol. 35:256-264 [doi:10.1007/s002489900081].

Riemann, L., G. F. Steward, and F. Azam. 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl. Environ. Microb. 66:578-587 [doi:10.1128/AEM.66.2.578-587.2000].

Robb, F. T. and R.T. Hill. 2000. Bacterial viruses and hosts: influence of culturable state, pp.199-208. *In* Nonculturable microorganisms in the environment. Springer US [doi:10.1007/978-1-4757-0271-2_11].

Rohwer, F., and R. V. Thurber. 2009. Viruses manipulate the marine environment. Nature 459:207-212 [doi:10.1038/nature08060].

Salomon, P. S., S. Janson, and E. Graneli. 2003. Molecular identification of bacteria associated with filaments of Nodularia spumigena and their effect on the cyanobacterial growth. Harmful Algae 2:261-272 [doi:10.1016/S1568-9883(03)00045-3].

Sanders, R. W. 1991. Mixotrophic protists in marine and fresh-water ecosystems. J. Protozool. 38:76-81 [doi:10.1111/j.1550-7408.1991.tb04805.x].

Schoeffield, A. J., and H. N. Williams. 1990. Efficiencies of recovery of *Bdellovibrios* from brackish- water environments by using various bacterial species as prey. Appl. Environ. Microbiol. 56:230-236.

Sherr, E., and B. Sherr. 2008. Understanding roles of microbes in marine pelagic food webs: a brief history, pp.27-44. *In* Microbial ecology of the oceans, 2nd ed. John Wiley & Sons [doi:10.1002/9780470281840.ch2].

Shipman, J. A., J. E. Berleman, and A. A. Salyers. 2000. Characterization of four outer membrane proteins involved in binding starch to the cell surface of Bacteroides thetaiotaomicron. J. Bacteriol. 182:5365-5372 [doi:10.1128/JB.182.19.5365-5372.2000].

Simon, M., H. P. Grossart, B. Schweitzer, and H. Ploug. 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquat. Microb. Ecol. 28:175-211 [doi:10.3354/ame028175].

Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Intense hydrolytic enzyme-activity on marine aggregates and implications for rapid particle dissolution. Nature 359:139-142 [doi:10.1038/359139a0].

Steindler, L., S. Beer, and M. Ilan. 2002. Photosymbiosis in intertidal and subtidal tropical sponges. Symbiosis-Rehovot 33:263-274.

Steppe, T. F., and H. W. Paerl. 2002. Potential N-2 fixation by sulfate-reducing bacteria in a marine intertidal microbial mat. Aquat. Microb. Ecol. 28:1-12 [doi:10.3354/ame028001].

Stukel, M. R., M. Décima, K. E. Selph, D. A. Taniguchi, and M. R. Landry. 2013. The role of Synechococcus in vertical flux in the Costa Rica upwelling dome. Progr. Oceanogr. 112-113:49-59 [doi:10.1016/j.pocean.2013.04.003].

Tang, K., V. Turk, and H. P. Grossart. 2010. Linkage between crustacean zooplankton and aquatic bacteria. AME Special 4 61:261-277 [doi:10.3354/ame01424].

Tang, K. W. 2005. Copepods as microbial hotspots in the ocean: effects of host feeding activities on attached bacteria. Aquat. Microb. Ecol. 38:31-40 [doi:10.3354/ame038031].

———, C. Dziallas, and H. P. Grossart. 2011. Zooplankton and aggregates as refuge for aquatic bacteria: protection from UV, heat and ozone stresses used for water treatment. Environ. Microbiol. 13:378-390 [doi:10.1111/j.1462-2920.2010.02335.x].

Thamdrup, B., and T. Daalsgard. 2008. Microbial ecology of the oceans. John Wiley & Sons.

Usher, K. M. 2008. The ecology and phylogeny of cyanobacterial symbionts in sponges. Mar. Ecol. Evol. Persp.

29:178-192 [doi:10.1111/j.1439-0485.2008.00245.x].
Varon, M., and M. Shilo. 1969. Interaction of Bdellovibrio bacteriovorus and host bacteria. II. Intracellular growth and development of Bdellovibrio bacteriovorus in liquid cultures. J. Bacteriol. 99:136-141.
Villareal, T. A., C. G. Brown, M. A. Brzezinski, J. W. Krause, and C. Wilson. 2012. Summer diatom blooms in the North Pacific subtropical gyre: 2008-2009. PLOS One 7(4):e33109 [doi:10.1371/journal.pone.0033109].
Voss, M., P. Croot, K. Lochte, M. Mills, and I. Peeken. 2004. Patterns of nitrogen fixation along 10°N in the tropical Atlantic. Geophys. Res. Lett. 31 [doi:10.1029/2004GL020127].
Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: The unseen majority. Proc. Nat. Acad. Sci. U.S.A. 95:6578-6583 [doi:10.1073/pnas.95.12.6578].
Wilkinson, C., and P. Fay. 1979. Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. Nature. 279:527-529 [doi:10.1038/279527a0].
Wilkinson, C. R. 1978. Microbial associations in sponges.1. Ecology, physiology and microbial-populations of coral-reef sponges. Mar. Biol. 49:161-167 [doi:10.1007/BF00387115].
———. 1983. Net primary productivity in coral-reef sponges. Science 219:410-412 [doi:10.1126/science.219.4583.410].
———, R.E. Summons and E. Evans. 1999. Nitrogen fixation in symbiotic marine sponges: ecological significance and difficulties in detection. Mem Queensl Mus 44:667–673.
Winter, C., A. Smit, G. J. Herndl, and M. G. Weinbauer. 2005. Linking bacterial richness with viral abundance and prokaryotic activity. Limnol. Oceanogr. 50:968-977 [doi:10.4319/lo.2005.50.3.0968].
Xie, G., and others. 2007. Genome sequence of the cellulolytic gliding bacterium *Cytophaga hutchinsonii*. Appl. Environ. Microbiol. 73:3536-3546 [doi:10.1128/AEM.00225-07].
Yang, S., W. Sun, F. Zhang, and Z. Li. 2013. Phylogenetically diverse denitrifying and ammonia-oxidizing bacteria in corals *Alcyonium gracillimum* and *Tubastraea coccinea*. Mar. Biotech. 15:540-551 [doi:10.1007/s10126-013-9503-6].
Yuasa, T., T. Horiguchi, S. Mayama, A. Matsuoka, and O. Takahashi. 2012. Ultrastructural and molecular characterization of cyanobacterial symbionts in Dictyocoryne profunda (polycystine radiolaria). Symbiosis 57:51-55 [doi:10.1007/s13199-012-0174-2].
Zehr, J. P., and P. J. Turner. 2001. Nitrogen fixation: Nitrogenase genes and gene expression. Method Microbiol. 30:271-286 [doi:10.1016/S0580-9517(01)30049-1].
———, L. L. Crumbliss, M. J. Church, E. O. Omoregie, and B. D. Jenkins. 2003. Nitrogenase genes in PCR and RT-PCR reagents: implications for studies of diversity of functional genes. Biotechniques 35:996-1013.
Zubkov, M. V., and G. A. Tarran. 2008. High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. Nature 455:224-U248 [doi:10.1038/nature07236].