


## Review

Biological interactions with *Prochlorococcus*:  
implications for the marine carbon cycleLanlan Cai,<sup>1,2</sup> Haofu Li,<sup>1,3</sup> Junwei Deng,<sup>1</sup> Ruiqian Zhou,<sup>1</sup> and Qinglu Zeng <sup>1,3,4,\*</sup>

The unicellular picocyanobacterium *Prochlorococcus* is the most abundant photoautotroph and contributes substantially to global CO<sub>2</sub> fixation. In the vast euphotic zones of the open ocean, *Prochlorococcus* converts CO<sub>2</sub> into organic compounds and supports diverse organisms, forming an intricate network of interactions that regulate the magnitude of carbon cycling and storage in the ocean. An understanding of the biological interactions with *Prochlorococcus* is critical for accurately estimating the contributions of *Prochlorococcus* and interacting organisms to the marine carbon cycle. This review synthesizes the primary production contributed by *Prochlorococcus* in the global ocean. We outline recent progress on the interactions of *Prochlorococcus* with heterotrophic bacteria, phages, and grazers that multifacetedly determine *Prochlorococcus* carbon production and fate. We discuss that climate change might affect the biological interactions with *Prochlorococcus* and thus the marine carbon cycle.

## Introduction

The unique biological features and ecological importance of *Prochlorococcus* [1–4] have been widely recognized since its discovery in 1988 [5]. This tiny cyanobacterium is the smallest (0.6–1 μm) and most abundant known oxygenic photoautotroph (see Glossary) on Earth [1,2]. *Prochlorococcus* dominates the euphotic zone of the low-nutrient (oligotrophic) oceans between 45°N and 40°S where the whole population accounts for more than half of gross primary production (GPP) in certain regions [2,6]. *Prochlorococcus* has adapted to oligotrophic oceans by reducing cell and genome sizes to minimize resource requirements [2]. Its high surface-to-volume ratio also provides competitive advantages in nutrient uptake and light-harvesting [1–3]. The ecological success of *Prochlorococcus* is further magnified by the extensive collective genetic and physiological diversity, which facilitates niche expansions and carbon fixation of the whole population along the euphotic zones [1–3,7–10].

Our understanding of *Prochlorococcus* has progressed considerably, especially with the rapid advances in sequencing technologies, global-scale oceanic surveys, and ecosystem models [11–14]. Many aspects of *Prochlorococcus* biology, physiology, and ecology have been discussed in the most recent review in 2015, with a comprehensive summary of the diversity and function of *Prochlorococcus* at that time [1]. Research in the past decade shows the importance of biological interactions between *Prochlorococcus* and the surrounding organisms in the marine food web and global biogeochemical cycles [13–19]. For example, some co-occurring heterotrophic bacteria tend to enhance the growth of *Prochlorococcus*, which in turn provides organic carbon to support the heterotrophic bacteria, forming mutualistic interactions that drive marine carbon cycling [14,20–22]. Interactions with heterotrophic bacteria can also lead to the aggregation and export of *Prochlorococcus* cells as particulate organic matter (POM) to the deep ocean, which is a key process of the marine carbon cycle [23]. Meanwhile, lysis by viruses (cyanophages) and predation by protists both mediate the fate of *Prochlorococcus*-fixed carbon, with viruses

## Highlights

*Prochlorococcus* is the smallest and most abundant photoautotroph on Earth, contributing significantly to global CO<sub>2</sub> fixation. Under climate change, ocean warming and reduced nutrients are expected to benefit *Prochlorococcus*, increasing its distribution and global abundance.

The presence of helper heterotrophic bacteria facilitates the growth and environmental adaptability of *Prochlorococcus*, which in turn provide organic carbon and nutrients to heterotrophic bacteria, forming mutualistic interactions that drive marine carbon cycling.

The fate of *Prochlorococcus* is mainly controlled by two top-down drivers, including viral lysis and protist grazing. Viruses primarily recycle *Prochlorococcus*-fixed carbon within the microbial loop via the viral shunt, and predatory protists transfer *Prochlorococcus* biomass to higher trophic levels through the grazing food chain.

*Prochlorococcus* phages inhibit carbon fixation of the infected host cells while maintaining the light reactions of photosynthesis and the pentose phosphate pathway to maximize energy and materials for nucleotide biosynthesis which is a bottleneck in phage replication.

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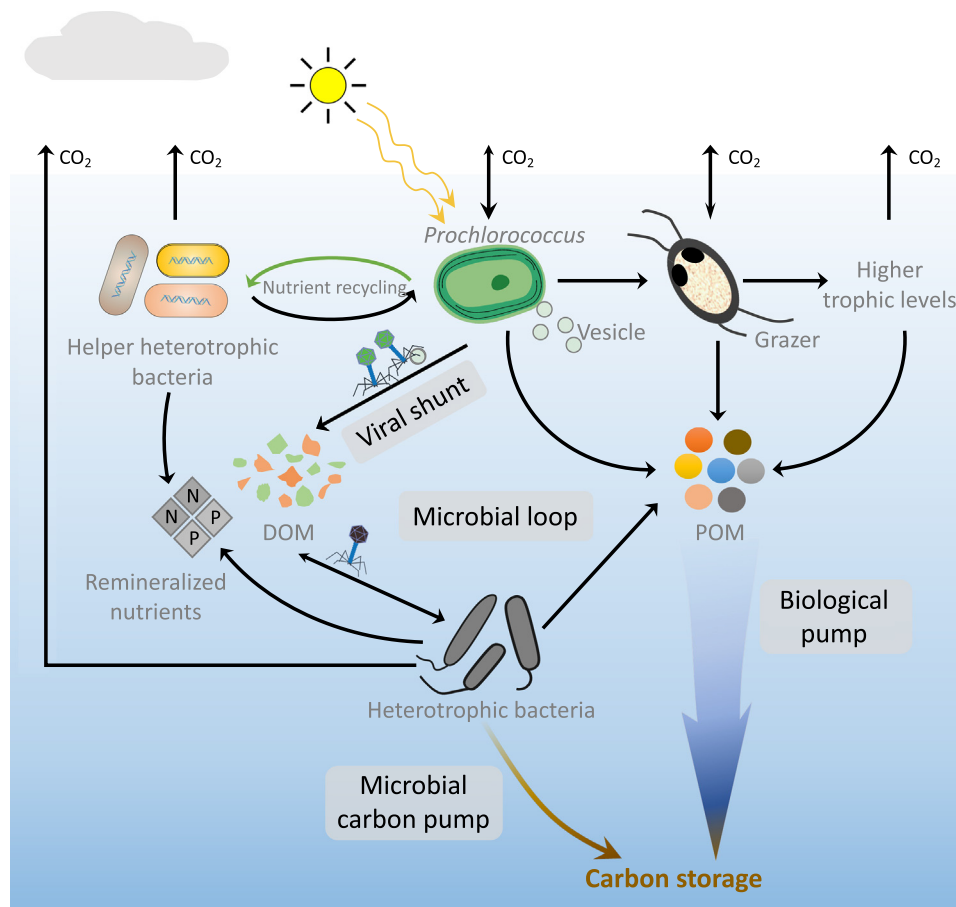
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primarily recycling carbon within the **microbial loop** via **viral shunt** [24,25], and predatory protists transferring *Prochlorococcus* carbon (biomass) to higher trophic levels through grazing food chain (Figure 1) [18,19,26]. Hence, the amount and flow of *Prochlorococcus* biomass are in part controlled by biological interactions, which could have important implications for marine ecosystem functions such as primary production and the marine carbon cycle.

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In this review, we first present an overview of the contribution of *Prochlorococcus* to marine primary production. Next, we synthesize recent advances in the interactions of *Prochlorococcus*

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Figure 1. The biological interactions of *Prochlorococcus* with some key players in the ocean and their roles in the marine carbon cycle. In the ocean, *Prochlorococcus* (and other phytoplankton) converts  $CO_2$  into organic carbon by photosynthesis, which forms the base of marine food webs. Helper heterotrophic bacteria facilitate the growth of *Prochlorococcus* and promote nutrient recycling in the euphotic zone. A fraction of *Prochlorococcus* is captured by grazers and the *Prochlorococcus* carbon is further transferred to higher trophic levels through the grazing food chain. Viral lysis of *Prochlorococcus* shunts the flow of carbon and nutrients away from higher trophic levels toward the pool of dissolved organic matter (DOM) that can be reused by the microbial community. Microbes take up and transform a part of labile dissolved organic carbon into recalcitrant forms that resist further degradation and can be stored in the ocean for long periods, a process known as the microbial carbon pump. Grazing by zooplankton and/or aggregation of *Prochlorococcus* cells into particles contribute to the pool of particulate organic matter (POM) that can be exported from the surface oceans to depth via the biological pump. *Prochlorococcus* also releases extracellular vesicles that may play important roles in the interaction with other organisms. The sizes of organisms are not to scale.

with heterotrophic bacteria, phages, and grazers that influence the production and fate of carbon fixed by *Prochlorococcus* (Figure 1). Extracellular vesicles (EVs) released by *Prochlorococcus*, which play an increasingly recognized role in interacting with surrounding organisms [27,28], are also discussed. Finally, we highlight some promising topics that should be specifically targeted in future studies in order to have a complete understanding of how *Prochlorococcus* cells contribute to the marine carbon cycle.

### Contribution of *Prochlorococcus* to marine primary production

The marine ecosystem is responsible for approximately 50 petagrams of carbon per year in **net primary production (NPP)**, an amount rivalling that on land [29]. In the oceans, photosynthesis is mainly conducted by phytoplankton, among which *Prochlorococcus* numerically dominates the vast oligotrophic areas and significantly contributes to carbon fixation [3,6,30]. The abundance of *Prochlorococcus* typically ranges from  $10^3$  to  $10^5$  cells/ml in the sunlit zones of the subtropical and tropical oceans, with an estimated global abundance of  $2.9 \times 10^{27}$  cells [6]. Under climate change, future oceans are predicted to be warmer, more acidic, and reduced in nutrient supply from deep waters due to ocean stratification, a scenario that is generally thought to favor the growth of small picophytoplankton because of their high nutrient uptake rates [31,32]. *Prochlorococcus* distribution is projected to expand poleward under climate change, and its global abundance is estimated to increase by 29% at the end of the 21st century [6], implying a more important role for *Prochlorococcus* in the future ocean [32].

The astronomical global abundance makes *Prochlorococcus* a major contributor to oceanic primary production and a key player in global biogeochemical cycles. Field investigations revealed that the contribution of *Prochlorococcus* to marine primary production differs seasonally and geographically. In the surface water of the equatorial Pacific, *Prochlorococcus* production varied between 174 and 620 mg C m<sup>-2</sup> d<sup>-1</sup>, accounting for 5–39% of GPP [33,34], while the contribution accounted for up to 82% of GPP at Station ALOHA in the subtropical North Pacific Ocean [34]. In the Sargasso Sea, the contribution of *Prochlorococcus* to primary production ranged from 10 to 20% in surface waters and to 80% at depth in summer, while the contribution ranged from 28 to 38% in winter with no clear vertical trend [35]. In the subtropical and tropical northeast Atlantic Ocean, direct flow cytometric sorting coupled with radioactive tracer measurements showed that the whole *Prochlorococcus* population accounted for 20–83% of total carbon fixation, although the cell-specific CO<sub>2</sub> fixation rate of *Prochlorococcus* ( $1.2 \pm 0.6$  fg C cell<sup>-1</sup> h<sup>-1</sup>) was lower than its closest relative *Synechococcus* ( $9.5 \pm 4.3$  fg C cell<sup>-1</sup> h<sup>-1</sup>) [30]. Based on the cell-specific carbon fixation rates and the predicted global abundance, *Prochlorococcus* is estimated to fix ca. 4 Gt of organic carbon annually and contribute to 8.5% of marine NPP on a global scale [6]. However, it should be noted that there is a high uncertainty in the estimated global primary productivity of *Prochlorococcus*, as the carbon fixation rates used in the estimations were only from the surface water of limited locations and times [6]. The carbon fixation rate of *Prochlorococcus* could vary with environmental conditions (e.g., light and nutrient availability) [36]. Naturally, *Prochlorococcus* has to cope with fluctuating environmental conditions, including changes in nutrients, light, and CO<sub>2</sub>/O<sub>2</sub> concentrations, which results in the appearance of different **ecotypes** [7]. *Prochlorococcus* comprises the high-light (HL) and low-light (LL) adapted ecotypes, each exhibiting specific environmental niches along the water column [7,8]. Gradients of environmental parameters (e.g., light intensity) provoke adaptive modifications in the photosynthetic apparatus of different ecotypes [4,37]. The carbon fixation rates of *Prochlorococcus* ecotypes with various photosynthetic performances could also differ (see **Outstanding questions**). Thus, a detailed characterization of the carbon fixation rates for different *Prochlorococcus* ecotypes, in conjunction with their abundances in the ocean [8,10], would enable a more precise estimation of the global primary production contributed by *Prochlorococcus*.

### Glossary

**Auxiliary metabolic genes (AMGs):** virally encoded genes that are presumably derived from hosts and reprogram host metabolism during viral infection, most likely resulting in increased viral reproduction.

**Biological pump:** the set of biologically mediated processes by which inorganic carbon is fixed into organic compounds via photosynthesis and then transported from the upper ocean to the depths.

**Carboxysome:** a family of polyhedral bacterial microcompartments that encapsulate the CO<sub>2</sub>-fixing enzyme RuBisCO and carbonic anhydrase to increase carbon fixation efficiency.

**CO<sub>2</sub>-concentrating mechanism (CCM):** the strategy used by cyanobacteria and some chemoautotrophs for elevating the concentration of CO<sub>2</sub> around the active site of RuBisCO to enhance CO<sub>2</sub> capture and fixation.

**Dissolved organic matter (DOM):** the fraction of organic matter dissolved in water, operationally defined as that which can pass through a filter typically with a pore size of 0.45 μm.

**Ecotypes:** genetically and physiologically distinct clades of one population, each of which adapts specifically to its ecological niche.

**Euphotic zone:** the part of the water column extending from the surface to the depth that receives enough sunlight for photosynthesis.

**Extracellular vesicle (EV):** lipid-bound particles containing proteins, nucleic acids, and other molecules released from cells into extracellular environments.

**Gross primary production (GPP):** the total amount of biomass or carbon produced by primary producers.

**Microbial carbon pump:** mechanisms of microbial transformation of labile dissolved organic carbon to recalcitrant dissolved organic carbon, which is resistant to biological degradation and thus can persist in the water column for long periods.

**Microbial loop:** trophic pathways of nutrient cycling and energy flow through microbial components of aquatic communities.

**Net primary production (NPP):** the amount of biomass or carbon retained in an ecosystem, that is, the difference between gross primary production and respiratory costs.

### Interactions of *Prochlorococcus* with heterotrophic bacteria

Microbial phototroph–heterotroph interactions are considered as a major engine of carbon cycling that regulates the balance between CO<sub>2</sub> fixation, **rem mineralization** of organic carbon back to CO<sub>2</sub>, and carbon storage in the ocean [38]. Interactions between *Prochlorococcus* and heterotrophic bacteria keep nutrients recycling in the euphotic zone, contribute to marine carbon sequestration, and maintain ecosystem diversity and structure [11,13–15,20–22,39–47].

During adaptation to oligotrophic environments, *Prochlorococcus* has evolved a streamlined genome of only ~2000 genes [1–3]. Due to the loss of some metabolic genes, *Prochlorococcus* appears to become dependent on several types of heterotrophic bacteria (e.g., *Alteromonas*, *Marinobacter*, and *Thalassospira*) [48] for growth and environmental adaptability [47,49]. The best-known example is the beneficial relationship between *Prochlorococcus* and *Alteromonas macleodii*, a heterotrophic bacterium commonly coisolated with *Prochlorococcus* [14,15,21,22,46]. The growth of *Prochlorococcus* strains of multiple ecotypes (e.g., HL-adapted strains MED4, MIT9312, MIT0604, and LL-adapted strain NATL2A) can be enhanced in terms of longevity and cell density in the stationary phase by the presence of *Alteromonas* [14]. The existence of *Alteromonas* also facilitates the survival of *Prochlorococcus* under the stresses of extended darkness [45,46], elevated CO<sub>2</sub> [21], temperature extremes [22], and nutrient starvation [14,20]. These effects are primarily attributed to the ability of heterotrophs to reduce the levels of toxic reactive oxygen species (ROS, e.g., hydrogen peroxide) that *Prochlorococcus* cannot detoxify due to the lack of genes encoding catalase [15,21,22,46,49]. Furthermore, incubation experiments suggest that *Prochlorococcus* might simultaneously benefit from other yet-to-be-determined factors provided by *Alteromonas*, as *Prochlorococcus* grew better in coculture with *Alteromonas* than it did with a ROS scavenger (sodium pyruvate) [13,15,22,45,46]. One possibility is that the accumulation of photosynthate may become toxic to *Prochlorococcus*, and heterotrophs can consume photosynthate and release remineralized nutrients to their autotrophic partners. This phenomenon has been observed in *Synechococcus*–heterotroph systems [50], and it remains to be tested in *Prochlorococcus*–heterotroph systems. It is worth noting that, although synergistic relationships are commonly found in *Prochlorococcus*–heterotroph, no clear impact or antagonistic behaviors leading to growth inhibition do exist in some *Prochlorococcus* strains when cocultured with heterotrophic bacteria [14,39,47]. For example, coculture with four strains of the *Rhodobacter* clade can strongly inhibit the growth of both HL-adapted strain MED4 and LL-adapted strain MIT9313 [39], and the presence of *Alteromonas* strains can delay the growth of MIT9313 [14,39,47].

From the heterotroph's perspective, heterotrophic bacteria benefit from the photosynthetically fixed organic carbon and some essential organic compounds provided by *Prochlorococcus* [13,15,40,42,43]. A large number of bacteria, such as the SAR11 clade, which is the most abundant group of marine heterotrophic bacteria, cannot synthesize B vitamins that are important for the activity of several enzymes and numerous vital metabolisms [51,52]. In contrast, some *Prochlorococcus* strains (e.g., NATL2A and MIT9313) have complete pathways for vitamin synthesis, potentially serving as vitamin producers for heterotrophs in the oligotrophic oceans [13,15,41,49,51,52]. Furthermore, coculture experiments confirmed that the release of carbon-rich compounds by *Prochlorococcus* MIT9313 fulfills the unique nutrient (glycine) requirement for SAR11 growth [13]. Under laboratory conditions, more than 90% of the carbon fixed by axenic *Prochlorococcus* MIT9312 was released as exudates [44]. A large fraction of *Prochlorococcus*-derived organic matter can be rapidly used by marine bacteria within minutes to weeks [25]. During utilization of organic matter, heterotrophs convert organic carbon back into CO<sub>2</sub> and regenerate inorganic nutrients (e.g., NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, and iron) that are essential for the growth of phytoplankton (including *Prochlorococcus* and other phototrophs) [38,50]. Thus, the metabolic exchange between *Prochlorococcus* and heterotrophic bacteria keeps nutrients (e.g., carbon,

**Photoautotroph:** a photosynthetic organism (such as a plant or a cyanobacterium) that utilizes light energy to convert CO<sub>2</sub> into organic compounds.

**Remineralization:** the breakdown or the transformation of organic matter into inorganic forms.

**Viral shunt:** virus infection leads to the lysis of host cells and release of cellular content, thus shunting the flow of carbon and nutrients away from higher trophic levels toward the pool of dissolved organic matter.

nitrogen, phosphate, and metals) continually circulating in the euphotic zones. Furthermore, marine heterotrophs take up a part of labile dissolved organic carbon (LDOC) released by *Prochlorococcus* and transform it into recalcitrant forms (RDOC) [25] through processes that are collectively known as the **microbial carbon pump** [53]. RDOC can resist microbial decomposition and might persist in the water for centuries to millennia, thus contributing to long-term carbon storage in the ocean [53].

*Prochlorococcus* cells are usually assumed to contribute little to POM flux due to their small size and low sinking rate [54]. However, the presence of heterotrophic bacteria was recently found to enhance the aggregation of *Prochlorococcus* by stimulating the production of transparent exopolymer particles (TEPs) [23]. TEPs are a group of sticky organic substances consisting mainly of acidic polysaccharides [55]. The increased TEP production and aggregation would facilitate the export of *Prochlorococcus* to the deep ocean via the **biological pump** [56]. In addition, aggregation of *Prochlorococcus* into suspended particles may enhance the grazing by zooplankton, thus increasing the flux of *Prochlorococcus* biomass to the grazing food chain. Future studies should be conducted to determine the extent of the enhancement so that such data can be incorporated into biogeochemical models.

Besides the central role in biogeochemical cycling, the interactions between *Prochlorococcus* and heterotrophic bacteria have significant impacts on the composition and dynamics of the marine microbial community. For instance, the diel transcriptional oscillations of different heterotrophic bacterial groups in the field often mirror those of *Prochlorococcus* [57,58], possibly due to the dependence of heterotrophic bacteria on *Prochlorococcus* photosynthate for growth. Furthermore, Calfee *et al.* noted that *Prochlorococcus* promotes the competition of *Alteromonas* against rival phytoplankton (e.g., *Synechococcus*) by providing *Prochlorococcus* exudates to *Alteromonas* under nutrient-limited conditions [42]. The indirect interactions are supposed to contribute, in part, to the competitive fitness of *Prochlorococcus* in the oligotrophic oceans [42]. Indeed, metabolic models suggested that the metabolic rate of *Prochlorococcus* and its excretion of organic carbon have steadily increased over evolutionary time, which is estimated to stimulate the positive feedback from microbes and consequently increase the total biomass and stability of the entire ecosystem [59].

### Infection of *Prochlorococcus* by cyanophages

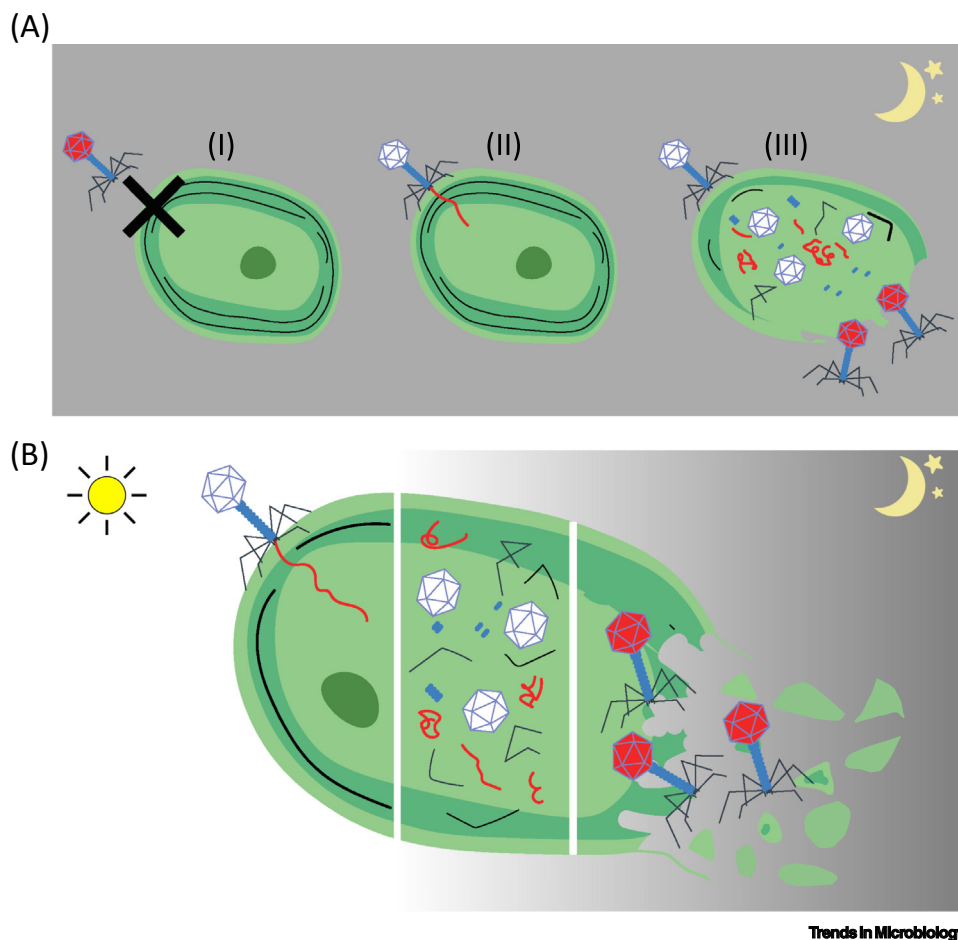
The metabolic status and population dynamics of *Prochlorococcus* in the ocean are significantly affected by 'top-down' factors, including viral infection and protist grazing (see the next section) [1]. Viruses infecting *Prochlorococcus*, together with those infecting *Synechococcus*, are termed cyanophages [60–62]. Cyanophages are ubiquitous and constitute a significant proportion of the marine viral community, accounting for up to 21% of the total double-stranded viruses in certain oligotrophic regions [60,61]. Cyanophages play essential roles in manipulating *Prochlorococcus* community composition and carbon fixation mainly through virus-induced mortality of host cells [60] and cyanophage-encoded genes potentially involved in host metabolism [63].

The most direct impact of virus infection on marine primary production is to enhance the mortality of *Prochlorococcus* cells. Although there are several reproduction strategies of viruses, all the currently known *Prochlorococcus* phages employ lytic life cycles that consist of attaching themselves to host cells, invading, replicating in, and eventually lysing the host cells [62,64–66]. In the North Pacific Ocean, cyanophages killed 0.35–31% of *Prochlorococcus* cells every day [60,61], significantly reducing primary production and diverting the flow of nutrients away from higher trophic levels toward the pool of **dissolved organic matter (DOM)**. Viral lysis of *Prochlorococcus* releases a diverse range of DOM (namely, vDOM), including carbohydrates, amino acids, and

lipids [24,25]. Viral lysis seems to alter the chemical composition of *Prochlorococcus*-released DOM [25]. Compared to *Prochlorococcus* exudates, vDOM is more labile and easier to be consumed by the heterotrophic bacteria, providing a significant fraction of nutrients for the growth of the surrounding microbial community (e.g., sustaining up to 33% of the bacterial carbon demand in the North Pacific Ocean) [25,60]. Notably, *Prochlorococcus* cells are also likely to take up vDOM. It was recently found that the CO<sub>2</sub> fixation rate of *Prochlorococcus* MIT9313 decreased by 16% but the respiration rate increased by 51% after adding vDOM to the culture [24]. Indeed, many pieces of evidence suggest that the natural *Prochlorococcus* population employs a mixotrophic lifestyle, by which *Prochlorococcus* both conducts photosynthesis and takes up organic carbon to enhance its fitness in oligotrophic oceans [12,67]. The responses of *Prochlorococcus* to vDOM may have a considerable negative impact on marine carbon fixation given the sheer abundance of *Prochlorococcus* and frequent viral lysis in the ocean. The supply of vDOM to *Prochlorococcus* might also deepen the distribution of *Prochlorococcus* to where not enough light is available for obligate photoautotrophy, thus contributing to the ecological success of *Prochlorococcus*.

Another way *Prochlorococcus* phages influence host photosynthesis and the marine carbon cycle is through the expression of host-like metabolic genes, termed **auxiliary metabolic genes (AMGs)** [68]. To reproduce, viruses must hijack the cellular machinery of their hosts and redirect host metabolism toward the production of progeny viruses [64]. Most cyanophages carry AMGs, which are supposed to originate from the hosts and reprogram host metabolism during infection [65,69–71]. These host-derived AMGs not only promote the evolution and diversification of both *Prochlorococcus* and cyanophages [69,70] but also significantly influence global biogeochemical cycles [16,72]. For example, cyanophages can inhibit the carbon fixation (Calvin cycle) of the infected host cells while maintaining the light reactions of photosynthesis and the pentose phosphate pathway (PPP), likely via the expression of AMGs [73–75]. Almost all T4-like *Prochlorococcus* phages carry a Calvin cycle inhibitor gene *cp12*, the host homolog of which is used to shut off the Calvin cycle and direct carbon flux toward the PPP [75,76]. In cyanobacteria, the PPP metabolizes the glycolytic metabolites, yielding ribose 5-phosphate as a precursor for the synthesis of nucleic acids [75,77]. Different AMGs involved in the PPP (e.g., *talC*, *zwf*, *gnd*) were identified in several *Prochlorococcus* phages and demonstrated to be expressed in the infected *Prochlorococcus* MED4 cells, although only the function of TalC was studied [75,76]. These cyanophage AMGs are proposed to redirect the use of NADPH, ATP, and ribose 5-phosphate away from carbon fixation toward nucleotide biosynthesis, which is a rate-limiting step in the phage replication process [75]. By assuming that 1–60% of cyanobacteria are infected by cyanophages each day and cyanophages normally have latent periods of 6–15 h, Puxty and colleagues estimated that between 0.02 and 5.39 petagrams of carbon are lost annually due to cyanophage-induced inhibition of carbon fixation [72]. Noteworthy, the function of cyanophage CP12 has not been experimentally verified, and cyanophages with and without *cp12* may have different infection kinetics. Future laboratory and field experiments are needed to reveal the function of cyanophage-encoded AMGs and give a more accurate estimation on how cyanophages influence the carbon fixation of *Prochlorococcus* cells in various oceanic regions.

Unlike the light-insensitivity of phages infecting heterotrophs, light has a crucial role in governing the life cycle of cyanophages [17,78,79]. Three diel-dependent life history traits have been observed in *Prochlorococcus* phages (Figure 2A), with some unable to adsorb in the dark, some able to adsorb but not replicate in the dark, and some able to reproduce although with decreased phage progeny under total darkness [78]. In the oceans, cyanophages that can infect host cells only in the light could synchronize their infections to the daily light–dark cycle, which is supported



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**Figure 2.** Light availability affects the interaction of *Prochlorococcus* with cyanophages. (A) Three diel-dependent life history traits have been observed in *Prochlorococcus* phages [78], with some unable to adsorb in the dark (I), some able to adsorb but not replicate in the dark (II), and some able to reproduce but with decreased phage progeny under total darkness (III). (B) The synchronized infection of *Prochlorococcus* cells due to the light-dependent phage infection [17].

by field observations of rhythmic expression of cyanophage genes [17,80]. Synchronized infection of *Prochlorococcus* cells may result in the rhythmic release of vDOM (Figure 2B) and subsequent diurnal cycling of organic carbon in the ocean via the viral shunt. Differential infection of host cells over light–dark cycles may be an ecological strategy by which cyanophages specialize in infection at separate times of the day (namely, diel niche partitioning), thereby minimizing competition for limited resources (such as host cells) in the environment. It will be particularly interesting to examine the impacts of diel-dependent life history traits on cyanophage fitness and *Prochlorococcus* carbon fixation in the ocean, as well as the contribution of the rhythmic release of vDOM to the marine food web.

### Predation of *Prochlorococcus* by grazers

The predator–prey relationship between grazers and *Prochlorococcus* is another potentially important top-down regulator for controlling the *Prochlorococcus* population. So far, investigations and data on the grazing mortality of *Prochlorococcus* remain scarce, and consequently, the extent to which the grazing process regulates *Prochlorococcus* in the field remains unclear.

Regardless of this knowledge gap, it is generally recognized that grazers can influence both the population dynamics and metabolic status of *Prochlorococcus*, thus affecting marine primary productivity and carbon cycles.

Research regarding the grazing impact on *Prochlorococcus* mainly focuses on the identification of potential predators consuming *Prochlorococcus* [18,19,26,81–83]. Based on the RNA-Stable Isotope Probing technique (RNA-SIP), Frias-Lopez *et al.* showed that Prymnesiophyceae, Dictyochophyceae, *Bolidomonas*, and Dinoflagellata are the four major groups that predate *Prochlorococcus* and *Synechococcus* in the surface waters of the Pacific Ocean [81]. A combination of flow cytometric cell sorting and dual tyramide signal amplification fluorescence *in situ* hybridization provided direct visual evidence that Prymnesiophyceae and Chrysophyceae feed on *Prochlorococcus* in the Atlantic Ocean [82]. A recent culture-based study isolated 32 *Prochlorococcus*-consuming flagellates in diverse classes, including dictyochophytes, haptophytes, chrysophytes, bolidophytes, dinoflagellates, and chlorarachniophytes, from the North Pacific Subtropical Gyre [19]. Culture-independent methods (RNA-SIP and 18S rRNA amplicon analyses) further showed that rather than previously thought predators (e.g., dinoflagellates), choanoflagellates along with an uncultivated Radiolarian and some uncultured stramenopiles are actually the most active consumers of *Prochlorococcus* in the North Pacific Ocean [26]. These active grazers of *Prochlorococcus* are usually overlooked because of their low amplicon abundances relative to the total eukaryotic community [26]. Furthermore, a special form of grazing on *Prochlorococcus* was recently reported from a 1.3  $\mu\text{m}$  nano-haptophyte alga (*Braarudosphaera bigelowii*) [18], which is slightly bigger than *Prochlorococcus*. To address the space limitations within the cell, this tiny algal grazer seems to specifically select the ball-shaped *Prochlorococcus* as prey and hold *Prochlorococcus* in their open hemispheric cytostomes like a plug. Interestingly, it is proposed that the captured *Prochlorococcus* cells continue to fix  $\text{CO}_2$  and provide organic carbon to the algal grazers as a temporary chloroplast substitute [18].

Most of the identified grazers of *Prochlorococcus* are mixotrophic, being able to get nutrients from both photosynthesis and phagocytosis [18,19,26,81,82]. It is estimated that these mixotrophs would consume 26–50% of *Prochlorococcus* production daily in the upper euphotic waters of the North Pacific Ocean [19]. However, these diverse mixotrophic protists may vary their trophic strategies and grazing abilities under different environmental conditions (e.g., nutrient availability and light intensity), which could result in distinct grazing pressures on *Prochlorococcus* and impacts on carbon flux. Further identification of the trophic strategies and feeding mechanisms of these mixotrophs is essential for predicting how predators control marine primary production and mediate the carbon flux from *Prochlorococcus* biomass to higher trophic levels.

### Extracellular vesicles released by *Prochlorococcus* cells

**Extracellular vesicles (EVs)** are small (approximately 50–250 nm in diameter) membrane-limited particles containing lipids, proteins, and genetic materials that are secreted into the extracellular environments by cells [84]. Many Gram-negative bacteria continually produce EVs during growth, which have been known to play multifunctional roles in DNA transfer, phage interception, cell detoxification, and interactions with surrounding organisms [84]. EV production by *Prochlorococcus* was first characterized by Biller *et al.*, who showed that spherical, membrane-enclosed vesicles with a diameter of 70 to 100 nm were released by *Prochlorococcus* under both continuous light and diel light–dark cycling conditions [27]. In laboratory cultures, EV production rates differed among *Prochlorococcus* strains, with 1.2 to 7.7 EVs being produced per cell per generation [85]. *Prochlorococcus*-derived EVs contain lipopolysaccharides, DNA, RNA, and a variety of proteins, including nutrient transporters, proteases, porins, and hydrolases [27,28]. The massive release of *Prochlorococcus*-derived EVs with diverse contents was



proposed to have key roles in the marine carbon cycle, gene transfer, microbial communication, predator defense, and mediation of extracellular biogeochemical reactions [28,85,86]. Purified *Prochlorococcus* vesicles could be used as the sole carbon source to support the growth of marine heterotrophs *Alteromonas* and *Halomonas* [27,28]. Via EV production, roughly  $10^4$  to  $10^5$  tonnes of carbon fixed by *Prochlorococcus* were estimated to be exported into the ocean daily, constituting a notable proportion of nutrients for surrounding heterotrophs in the oligotrophic ocean [27], which likely, in turn, facilitate *Prochlorococcus* and contribute to nutrient recycling through the microbial loop. In addition, it was suggested that *Prochlorococcus* vesicles could be employed as key mechanisms of horizontal gene transfer among marine organisms, and serve as a decoy for cyanophages to prevent phage infection since cyanophages can attach to EVs [27]. Recently, Biller *et al.* have revealed that the secretion of EVs from *Prochlorococcus* varies in response to changes in abiotic factors, such as temperature and light intensity [85]. Whether or not – and if so, to what extent – the release of EVs by *Prochlorococcus* can be affected in the community context has not yet been examined. Considering the potential role of EVs in microbial communication, it is highly possible that the characteristics (such as number, size, and content) of EVs secreted by *Prochlorococcus* may vary in the processes of their interactions with other organisms.

### Concluding remarks and future perspectives

*Prochlorococcus* has the smallest size and simplest photosynthetic apparatus, and a high ability to fully use the available light, making it one of the most efficient photosynthetic organisms on Earth [2,4,36]. Given the substantial contribution of *Prochlorococcus* to global primary production, it is necessary to uncover the photosynthetic characteristics (e.g., organization of photosynthetic complexes, CO<sub>2</sub> uptake and fixation mechanisms) of *Prochlorococcus*, especially ecotypes with different photosynthetic adaptability. During the long geological history of dramatic O<sub>2</sub> increases and CO<sub>2</sub> decreases, cyanobacteria have developed effective **CO<sub>2</sub>-concentrating mechanisms (CCMs)** to improve carbon fixation performance (for details, see reviews [87–89]). CCM is a complicated biochemical process involving multiple protein complexes working together in a specialized microcompartment called **carboxysome** [88]. *Prochlorococcus* has a highly concise CCM, appearing to contain the minimal set of components required for a functional CCM [36]. In the past few years, significant efforts have been made to improve our understanding of the functions and mechanisms of carboxysomes; however, most of the studies were conducted using  $\alpha$ -carboxysomes of chemoautotrophs [90]. Whether or not carboxysomes of *Prochlorococcus* follow an assembly process like that of chemoautotrophs remains unclear. Deciphering the mechanistic and structural features of carboxysome is of critical importance for understanding the effect of compartmentalization on CO<sub>2</sub> fixation in *Prochlorococcus* cells, which will shed light on prokaryotic evolution, CO<sub>2</sub> fixation, and the marine carbon cycle.

The growth and loss of *Prochlorococcus* are tightly coupled, resulting in a relatively stable *Prochlorococcus* population in the ocean [1,60]. It is now well recognized that the presence of certain heterotrophic bacteria improves the growth and environmental adaptability of *Prochlorococcus* [14,15,21], yet the underlying mechanisms remain unclear. Studies have shown ecotype-specific responses of *Prochlorococcus* when cocultured with different heterotrophic bacteria [13,14,47]. In-depth studies to characterize the responses of different heterotrophic bacteria and *Prochlorococcus* ecotypes would provide important information about the underpinnings of *Prochlorococcus*–heterotroph interactions and how the interactions mediate *Prochlorococcus* production and carbon cycles.

As one of the major top-down factors, cyanophages directly reduce primary production through the lysis of *Prochlorococcus* cells [60,66,74]. Meanwhile, cyanophages encode genes that

### Outstanding questions

How do photosynthetic apparatus and carbon fixation rates vary with *Prochlorococcus* ecotypes for the adaptation to different environmental conditions?

Besides reducing the oxidative stress of *Prochlorococcus* by heterotrophic bacteria and possible metabolic exchanges between them, are there any other underlying mechanisms that heterotrophic bacteria may influence the growth of *Prochlorococcus* and thus the marine carbon cycle?

Recent advances in high-throughput sequencing technologies have identified a large number of cyanophage-encoded AMGs based on sequence similarity, yet only a few of them have been experimentally confirmed to be functional. Do these phage genes play roles in modulating *Prochlorococcus* metabolism during infection, and how do they affect carbon fixation of *Prochlorococcus*?

It is still unclear which top-down factors predominate in affecting the activity and dynamics of the *Prochlorococcus* population. The study on grazing is now largely lagging behind that of viral lysis. How and to what extent do grazers control *Prochlorococcus* populations and contribute to marine carbon cycles?

What are the potential combined effects of global climate change on *Prochlorococcus* and its biological interactions with surrounding organisms, and what are the cascading effects on primary production and carbon cycling on a global scale?

reprogram host carbon metabolism, thus altering carbon and nutrient cycling in marine ecosystems [63,72,75]. Recent advances in high throughput sequencing and new bioinformatic tools have greatly facilitated the detection of cyanophage-encoded AMGs from global-scale oceanic metagenomic datasets [64,69,91]. Most AMGs were assigned based on their sequence similarity to the annotated genes in host genomes. However, a large number of *Prochlorococcus* genes remain unannotated, thus excluding the discovery of uncharacterized AMGs. Furthermore, despite the expanding inventory of cyanophage-encoded AMGs, only a few of them have been experimentally confirmed to be functional. Further work to test the activities and ecological roles of cyanophage-encoded AMGs is not only critical for understanding cyanophage–*Prochlorococcus* interactions but also an essential step for an accurate estimation of the role of cyanophages in mediating *Prochlorococcus* production and carbon flux.

Global climate change is altering the biological community dynamics in the ocean [6,31]. The global abundance of *Prochlorococcus* is projected to increase due to its expanded distribution over this century [6], but how *Prochlorococcus* might contribute to primary production in the context of global change remains an open question. We can expect that the physiology of *Prochlorococcus* could be significantly affected by climate change factors. For instance, decreased expressions of carbon fixation operon were observed in both HL-adapted strain MED4 and LL-adapted strain MIT9312 in response to rising CO<sub>2</sub> [21,40]. MIT9312 showed a longer lag phase and greater die-offs under elevated CO<sub>2</sub>, largely attributable to increased oxidative stress [21]. The presence of helper heterotrophic bacteria can partially alleviate the oxidative stress but to a lesser extent compared to under ambient CO<sub>2</sub> [21]. Thus, global change may elicit changes in the carbon fixation efficiency of *Prochlorococcus* cells and their biological interactions with other organisms, which would alter the amount of carbon fixed in the ocean and the flux of carbon mediated by microbial interactions. Studying the individual and combined effects of global change factors (e.g., warming, elevated CO<sub>2</sub>, and reduced nutrients) on *Prochlorococcus* and its biological interactions is critical for our understanding of the response of marine microbes and marine ecosystems to future climate changes.

Besides its ecological implications, the synthetic biology of *Prochlorococcus* is of great interest. The small genome size and vast genetic diversity make *Prochlorococcus* an attractive organism as chassis for enhancing CO<sub>2</sub> fixation and photosynthesis-based production of hydrocarbon molecules. Over the past decade, *Prochlorococcus* has been reported to produce a broad range of valuable metabolites, including methane [92], isoprene [93], iodomethane [94], methyl iodide [95], and alkanes [96]. In fact, a recent study screening the metabolic potential of cyanobacteria for biofuel production identified *Prochlorococcus* strains as top candidates [97]. Recently, a genome-scale metabolic model of *Prochlorococcus* MED4 has been established, enabling dynamic allocation of carbon storage towards metabolic production [98]. Nevertheless, engineering *Prochlorococcus* for increased carbon fixation and biotechnological applications is a challenging future option that requires intensive research efforts.

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### Declaration of interests

No interests are declared.

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