environmental microbiology

Environmental Microbiology (2019) **00**(00), 00–00 doi:10.1111/1462-2920.14513

Transcriptomic responses of the marine cyanobacterium *Prochlorococcus* to viral lysis products

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Summary

Viral infection of marine phytoplankton releases a variety of dissolved organic matter (DOM). The impact of viral DOM (vDOM) on the uninfected co-occurring phytoplankton remains largely unknown. Here, we conducted transcriptomic analyses to study the effects of vDOM on the cyanobacterium *Prochlorococcus*, which is the most abundant photosynthetic organism on Earth. Using *Prochlorococcus* MIT9313, we showed that its growth was not affected by vDOM, but many tRNAs increased in abundance. We tested tRNA-gly and found that its abundance increased upon addition of glycine. The decreased transcript abundances of N metabolism genes also suggested that *Prochlorococcus* responded to organic N compounds in vDOM. Addition of vDOM to *Prochlorococcus* reduced the maximum photochemical efficiency of photosystem II and CO_2 fixation while increasing its respiration rate, consistent with differentially abundant transcripts related to photosynthesis and respiration. One of the highest positive fold-changes was observed for the 6S RNA, a noncoding RNA functioning as a global transcriptional regulator in bacteria. The high level of 6S RNA might be responsible for some of the observed transcriptional responses. Taken together, our results revealed the transcriptional regulation of *Prochlorococcus* in response to viral lysis products and suggested its metabolic potential to utilize organic N compounds.

Introduction

As the foundation of the ocean food web (Falkowski, 2012), phytoplankton contribute to about one half of global primary production (Field *et al*., 1998). A significant proportion of phytoplankton are infected by viruses (Proctor and Fuhrman, 1990; Fuhrman, 1999) and release a variety of dissolved organic matter (DOM) upon cell lysis (Kujawinski, 2011; Lønborg *et al*., 2013; Zhao *et al*., 2017; Ma *et al*., 2018), including carbohydrates, amino acids and lipids (Benner and Amon, 2015). Viral lysis products have been thought to be primarily consumed by heterotrophic microorganisms (Gobler *et al*., 1997; Fuhrman, 1999; Azam and Malfatti, 2007; Haaber and Middelboe, 2009; Jiao *et al*., 2010; Sheik *et al*., 2014), whereas their impact on uninfected phytoplankton has historically been ignored.

The unicellular picocyanobacteria *Prochlorococcus* and *Synechococcus* are the numerically dominant phytoplankton, and they are responsible for a vast majority of primary production in many oligotrophic regions (Liu *et al*., 1997; Partensky *et al*., 1999; Scanlan and West, 2002). The presence of viruses was reported to have a positive effect on the growth of *Synechococcus* in the ocean, suggesting that marine *Synechococcus* might benefit from the presence of viral lysis products (Weinbauer *et al*., 2011). Indeed, there is evidence that phytoplankton are capable of taking up organic compounds. *Prochlorococcus* and *Synechococcus* can take up amino acids (Zubkov *et al*., 2003; Mary *et al*., 2008; Gomez-Pereira *et al*., 2013; Bjorkman *et al*., 2015),

Received 17 August, 2018; accepted 19 December, 2018. *For correspondence. E-mail zeng@ust.hk; Tel. 852-23588701; Fax 852-36934766.

dimethylsulfoniopropionate (Vila-Costa *et al*., 2006) and glucose (Gomez-Baena *et al*., 2008; Munoz-Marin Mdel *et al*., 2013). Natural *Prochlorococcus* populations showed rapid transcriptional responses to DOM derived from *Prochlorococcus* exudates, providing evidence that *Prochlorococcus* might use these organic compounds (Sharma *et al*., 2014). However, key regulators of these transcriptional responses have remained unidentified.

A widely conserved global transcriptional regulator in bacteria is the 6S RNA. In model bacteria such as *Escherichia coli* and *Bacillus subtilis*, the 6S RNA is well known to respond to changes in nutrient supply (Steuten *et al*., 2014; Wassarman, 2018). In *E. coli*, 6S RNA accumulates as the culture enters the stationary phase of growth and binds to the σ^{70} RNA polymerase (Wassarman and Storz, 2000). The association of 6S RNA with the σ^{70} RNA polymerase inhibits transcription at many σ^{70}-dependent promoters that are used in exponential growth and activates some σ^{S}-dependent promoters that are used in stationary phase (Trotochaud and Wassarman, 2004). The regulatory mechanism involves promoter mimicry, as the RNA polymerase carrying the housekeeping sigma factor σ^{70} binds to 6S RNA that mimics an open promoter complex instead of binding the respective promoter elements (Barrick *et al*., 2005; Cavanagh and Wassarman, 2014; Steuten *et al*., 2014). In cyanobacteria, the 6S RNA was initially named Yfr7 and its coding gene *ssrS* is identified in all the sequenced marine cyanobacterial genomes (Axmann *et al*., 2005). In *Synechococcus* sp. PCC 6301, 6S RNA levels change with growth, possibly due to differences in the nutrient status (Watanabe *et al*., 1997), although in contrast to observations in *E. coli* and *B. subtilis,* 6S RNA was abundant in exponential phase and reduced in stationary phase. Similarly, the accumulation of 6S RNA in *Prochlorococcus* MED4 was reported to be cell cycle-dependent and light-dependent (Axmann *et al*., 2007). Finally, genetic studies of the 6S RNA in *Synechocystis* sp. PCC 6803 and in vivo pull-down studies of the RNA polymerase complex demonstrated its involvement in the recovery from nitrogen depletion by accelerating the switch from alternative group 2 σ factors SigB, SigC and SigE to SigA-dependent transcription (Heilmann *et al*., 2017).

Prochlorococcus and *Synechococcus* coexist in many regions of the world's oceans, although their distributions are not identical (Partensky *et al*., 1999). *Prochlorococcus* and *Synechococcus* have been shown to be actively infected by viruses (cyanophages) (Sullivan *et al*., 2003). In this study, we asked if the released viral lysis products could affect the uninfected neighbouring cyanobacterial cells. We addressed this question by adding viral lysis products of *Synechococcus* WH8102 to *Prochlorococcus* MIT9313. These two model cyanobacterial strains were both isolated from the North Atlantic Ocean and are among the first marine cyanobacteria that have complete genomes sequenced (Palenik *et al*., 2003; Rocap *et al*., 2003). After addition of viral lysis products, we used RNA-seq to analyse the transcriptomic responses of *Prochlorococcus* MIT9313 cells, focusing on genes related to translation, photosynthesis and nitrogen metabolism. We also measured whether viral lysis products affected the growth and photosynthesis of *Prochlorococcus* MIT9313. Furthermore, we found evidence that the 6S RNA may play a role in regulating the transcriptional responses of *Prochlorococcus* MIT9313 to viral lysis products.

Results and discussion

Prochlorococcus *growth is not affected by viral lysis products*

We generated viral lysis products (vDOM) by infecting axenic *Synechococcus* WH8102 cultures with cyanophage S-ShM2, which does not infect *Prochlorococcus* MIT9313 (Sullivan *et al*., 2003). Recently, infection of *Synechococcus* WH7803 by cyanophage S-SM1 was shown to release abundant dissolved organic nitrogen compounds, including peptides derived from the major light-harvesting protein phycoerythrin (Ma *et al*., 2018). In our experiments, we also found that the concentrations of total combined amino acids in vDOM (> 10 kDa dissolved protein fraction after protease digestion) were much higher than those of the natural seawater-based Pro99 growth medium (Supporting Information Table S1). In addition to vDOM-specific organic nitrogen compounds, viral lysis of *Synechococcus* WH7803 was found to release abundant lipids, proteins and pigments that were also released by exudation and mechanical cell lysis (Ma *et al*., 2018). Therefore, the vDOM we used in this study did not only contain viral lysis–specific compounds, but also contained common compounds that are released by various mechanisms.

To study whether vDOM affected the growth of uninfected cyanobacterial cells, vDOM was added to mid-log axenic *Prochlorococcus* MIT9313 cultures at a volume/volume ratio of 1:4. The control cultures were amended with the Pro99 growth medium, which is based on natural seawater and contains DOM (see Materials and Methods). After vDOM amendment, the cultures continued exponential growth for up to 3 days, with growth rates indistinguishable from the control cultures (Fig. 1A). In our experiments, *Prochlorococcus* MIT9313 was grown in nutrient-replete conditions and this may explain why vDOM addition did not promote its growth. Our results were consistent with a recent study showing that the exponential growth of marine *Synechococcus* in nutrient-replete conditions was not affected after DOM was added at an amount similar to what we used here (Christie-Oleza *et al*., 2017).

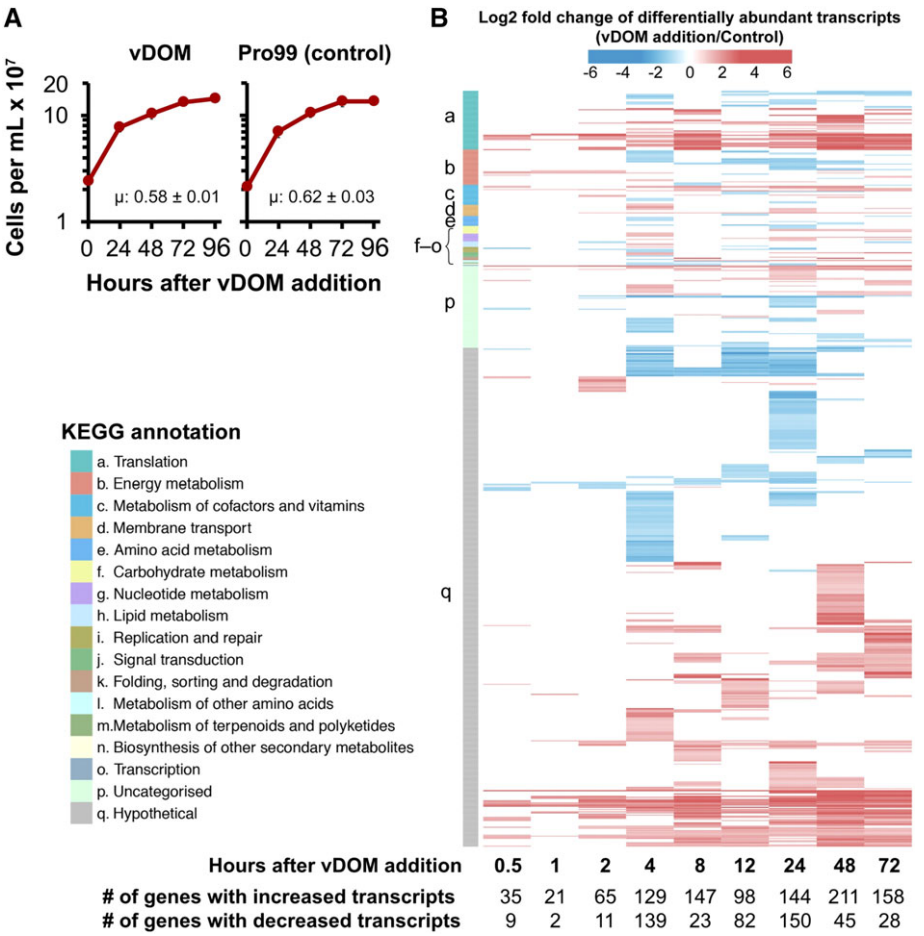

Fig. 1. Growth and transcriptomic responses of *Prochlorococcus* after vDOM addition.
A. vDOM or the Pro99 growth medium (control) was added to log-phase *Prochlorococcus* MIT9313 cultures. Cell concentrations were determined by flow cytometry and were used to calculate the growth rate (μ). Data points and errors are means and standard deviations of three independent cultures respectively.
B. Genes with differentially abundant transcripts at 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h after vDOM addition to *Prochlorococcus* MIT9313 cells. Differentially abundant transcripts were identified by normalizing their abundances in the vDOM amended cultures to those in the control cultures amended with the Pro99 growth medium (fold change ≥ 2 in either direction and an adjusted p value < 0.1 at least at one time point). The colour bar represents log2 fold change of differentially expressed transcripts (vDOM addition/control). In the head map, a red line indicates significantly increased transcripts, a blue line indicates significantly decreased transcripts and a white line indicates nondifferentially abundant transcripts. Genes are grouped under KEGG (Kyoto Encyclopedia of Genes and Genomes) functional categories. The numbers of genes with differentially abundant transcripts at each time point are shown below the heat map.

An overview of the transcriptomic responses of Prochlorococcus *to vDOM*

The unchanged growth rate of *Prochlorococcus* MIT9313 after vDOM addition inspired us to wonder whether it showed any transcriptional responses. To address this question, we employed RNA-seq to analyse the transcriptomes of *Prochlorococcus* MIT9313 cells taken at 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h after amendments with vDOM or the Pro99 growth medium (control). Genes with differentially abundant transcripts (Supporting Information Table S2) were identified by comparing transcript abundances in vDOM amended cultures to those of the control cultures (fold change ≥ 2 in either direction and an adjusted p value < 0.1 at least at one time point; see Materials and Methods). Differentially abundant transcripts appeared as early as 0.5 h, indicating a rapid transcriptional response of *Prochlorococcus* cells to vDOM addition (Fig. 1B).

To resolve the biological functions of differentially abundant transcripts, we grouped them into functional categories (KEGG in Fig. 1B and COGs in Supporting Information Fig. S1). Other than genes of unknown function, the most abundant functional category was translation (tRNA and ribosomal protein genes) and the second most abundant was energy metabolism (photosynthesis and respiration genes; Fig. 1B and Supporting Information Fig. S1). After examining genes in the functional categories membrane transport and amino acid metabolism (Fig. 1B), we found that genes related to nitrogen (N) metabolism were especially affected. In the following sections, we present our detailed analysis of the genes with differentially abundant transcripts.

tRNA and ribosomal protein genes

After vDOM addition, 31 out of 43 tRNA genes showed increased transcript abundances (Supporting Information Fig. S2A). Not much is known about the transcriptional regulation of tRNA genes in cyanobacteria, but in *E. coli* cells tRNA abundances increased after amino acids were added (Dong *et al*., 1996). The tRNA aminoacylation level (charging level) in *E. coli* is also positively correlated with amino acid concentrations in the growth medium (Dittmar *et al*., 2005). To test whether amino acids can increase the tRNA abundances, we added glycine to *Prochlorococcus* MIT9313 and used quantitative reverse transcription PCR (RT-qPCR) to detect the transcript

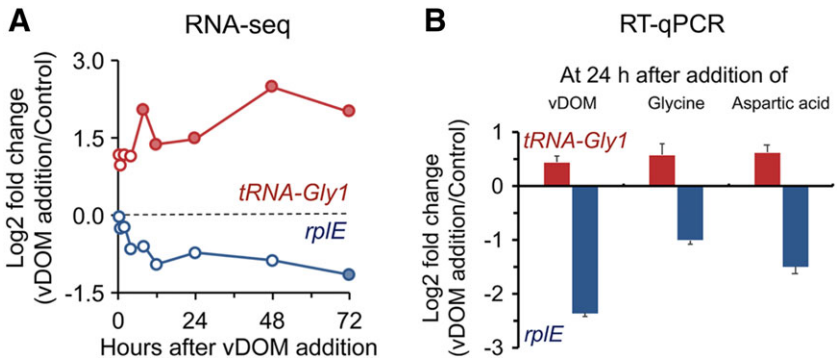

Fig. 2. Transcript abundances of *Prochlorococcus* tRNA and ribosomal protein genes.
A. RNA-seq data showing the transcript abundances of the tRNA gene *tRNA-Gly1* and the ribosomal protein gene *rplE* after vDOM was added to *Prochlorococcus* MIT9313 cells. Transcript abundances in the vDOM amended cultures were normalized to those amended with the growth medium Pro99. A dotted line indicates log2 fold change = 0. Filled symbols indicate adjusted *p* values < 0.1.
B. RT-qPCR data showing the transcript abundances of *tRNA-Gly1* and *rplE* at 24 h after vDOM, glycine (800 μM) and aspartic acid (800 μM) were added to *Prochlorococcus* MIT9313 cells. Data are mean ± SEM. from four biological replicates.

levels of *tRNA-Gly1*. Glycine did not affect the growth rate of *Prochlorococcus* MIT9313 (Supporting Information Fig. S3), which was previously observed in *Prochlorococcus* PCC 9511 (Rippka *et al*., 2000). However, similar to vDOM (Fig. 2A), glycine addition increased the transcript abundances of *tRNA-Gly1* (Fig. 2B), suggesting that amino acids are one of the compounds in vDOM that affected the expression of tRNA genes. To test whether tRNA genes can be responsive to noncognate amino acids, we added aspartic acid and found that this noncognate amino acid can also increase the transcript abundances of *tRNA-Gly1* (Fig. 2A) while did not change the growth rate of *Prochlorococcus* MIT9313 (Supporting Information Fig. S3). Moreover, we found that the tRNA abundances for 16 amino acids increased, while those for aspartic acid, histidine and tryptophan did not change significantly, and those for isoleucine decreased (Supporting Information Fig. S2A). This may be caused by the availability of these amino acids in vDOM and/or the cellular requirements for different amino acids.

Out of the 57 annotated ribosomal protein genes, five showed increased transcript abundances after vDOM addition, while 14 showed decreased abundances (e.g. *rplE*, Fig. 2A; Supporting Information Fig. S2B). Amino acids might also be responsible for this transcriptional response, since addition of glycine and aspartic acid decreased the *rplE* mRNA abundance (Fig. 2B). Our interpretation of the transcriptional responses of ribosomal protein genes is that vDOM and amino acids are unlikely to cause a dramatic change in the number of ribosomes per cell, since the growth rate of *Prochlorococcus* MIT9313 was not affected (Fig. 1A and Supporting Information Fig. S3). Recently, the relative transcript abundances of ribosomal proteins were used to assess the in situ growth rates of marine bacteria (Gifford *et al*., 2013), and it remained to be tested whether this method could be affected by the DOM concentration at the sampling site.

Photosynthesis and respiration genes

Photosynthetic and respiratory electron transport chains are important for the energy metabolism of cyanobacteria (Vermaas, 2001), and many of the respective genes showed differentially abundant transcripts after vDOM addition, but did not show a clear trend (Fig. 3A). For the light reactions of photosynthesis, transcripts of photosystem II (PSII), photosystem I (PSI) and chlorophyll binding/metabolism showed mixed patterns (some increased and some decreased; Fig. 3A). Conversely, five ATP synthase genes and one Calvin cycle gene *gap2* showed decreased transcript abundances (Fig. 3A), suggesting that *Prochlorococcus* cells might generate less ATP and fix less CO_2. For respiration, one NADPH dehydrogenase (NDH) gene *ndhA* had decreased transcript abundances (Fig. 3A), while the succinate dehydrogenase (SDH) gene *sdhB* had increased transcript abundances (Fig. 3A). Cytochrome *b*6/*f* genes also showed mixed transcriptional patterns (Fig. 3A). In summary, although many photosynthesis and respiration genes showed differentially abundant transcripts, their transcriptional patterns did not clearly reveal how the photosynthesis and the respiration of *Prochlorococcus* were affected by vDOM. Hence, we set out to directly measure the photosynthesis and the respiration of *Prochlorococcus* MIT9313 after vDOM addition.

For photosynthesis, we measured the maximum photochemical quantum yield (F_v/F_m) of PSII, which reveals the potential ability of PSII to transform absorbed photons

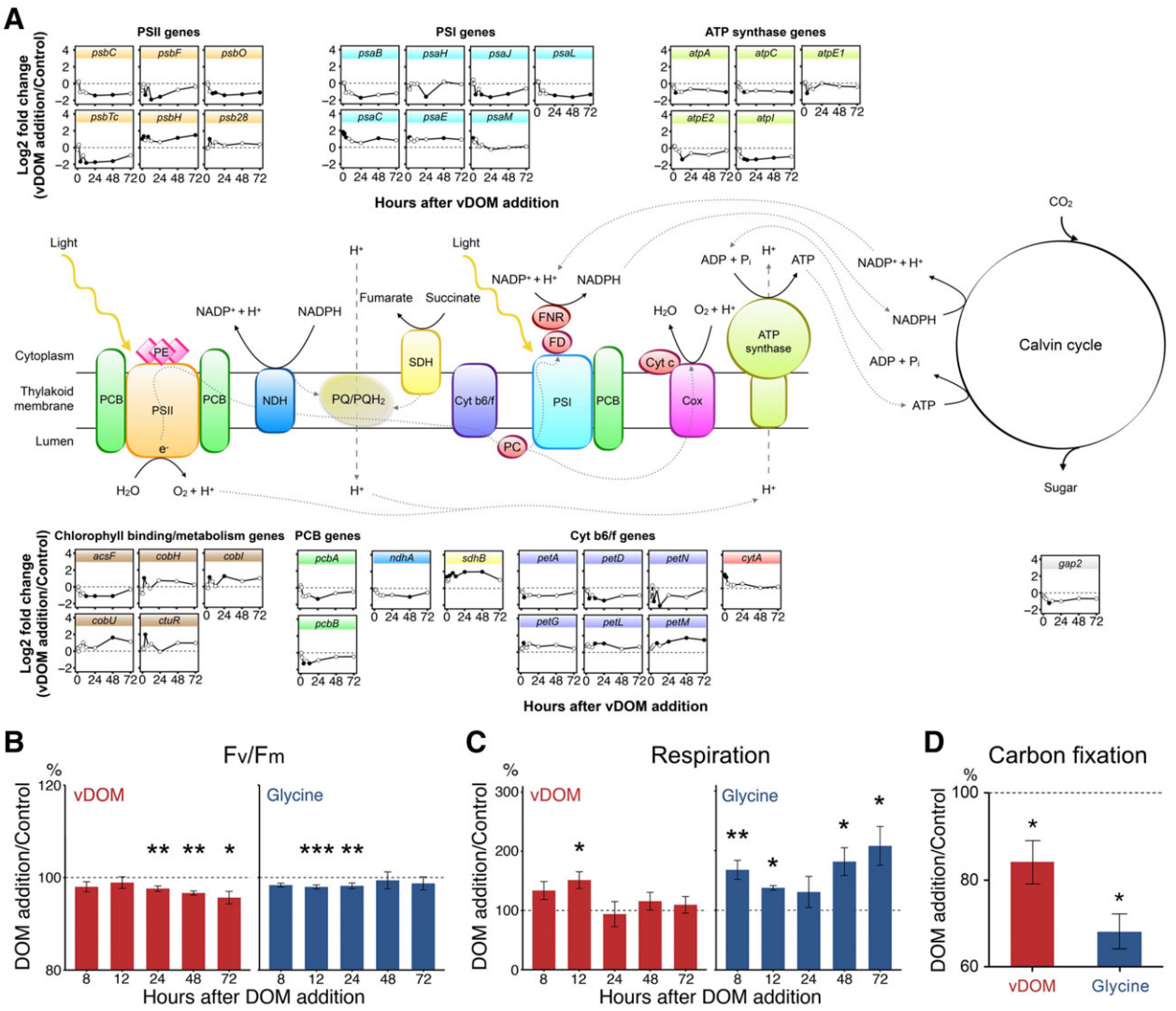

Fig. 3. Photosynthesis and respiration of *Prochlorococcus* after DOM addition.
A. A Schematic shows photosynthetic and respiratory electron transport chains, together with the Calvin cycle. Graphs show log2 fold changes of transcripts (vDOM addition/control) at different time points after vDOM addition. In each graph, a dotted line indicates log2 fold change = 0, and filled symbols indicate adjusted *p* values < 0.1. Abbreviations: PCB, chlorophyll *a*/*b*-binding protein; PE, phycoerythrin; PSII, photosystem II; PSI, photosystem I; PQ/PQH_2, plastoquinone/plastoquinol (oxidized/reduced); NDH, NADPH dehydrogenase; SDH, succinate dehydrogenase; Cyt *b*6/*f*, cytochrome *b*6/*f* complex; FD, ferredoxin; FNR, ferredoxin-$NADP^+$ reductase; PC, plastocyanin; Cox, cytochrome *c* oxidase.
B–D. Mid-log *Prochlorococcus* MIT9313 cultures were amended with vDOM, glycine (800 μM) or the growth medium Pro99 (control). After DOM addition, maximum photochemical efficiency of PSII (F_v/F_m) (B), respiration (C) and carbon fixation rate (D) were measured and normalized to those of the control cultures. Photosynthetic carbon fixation rates were calculated by adding $NaH^{13}CO_3$ into the cultures and measuring intracellular ^{13}C over time (Supporting Information Fig. 4) (Hama *et al*., 1983). Dotted lines indicate normalized measurement = 1. Data shown are mean ± SEM from five (B and C) or three (D) biological replicates. Asterisks denote significant changes after DOM addition compared with the control cultures (**p* < 0.05, ***p* < 0.01, ****p* < 0.001, One-way ANOVA with post hoc Dunnett's test).

to chemical energy. F_v/F_m of *Prochlorococcus* MIT9313 decreased by approximately 3% from 24 h after vDOM addition (Fig. 3B). Despite the slight decrease of F_v/F_m, the carbon fixation rate of *Prochlorococcus* cells decreased by approximately 16% after vDOM addition (Fig. 3D and Supporting Information Fig. S4). In contrast to photosynthesis, the respiration of *Prochlorococcus* MIT9313 cells was transiently enhanced by approximately 51% at 12 h after vDOM addition (Fig. 3C). Similar to vDOM, glycine slightly reduced the F_v/F_m of *Prochlorococcus* cells by approximately 2% (Fig. 3B). However, glycine reduced the carbon fixation rate by approximately 32% (Fig. 3D) and increased the respiration by up to approximately 108% at 72 h (Fig. 3C). The more significant effects of glycine than vDOM on respiration and carbon fixation rate (Fig. 3C and D) might be due to the higher DOM concentration of glycine. Although, *Prochlorococcus* has been shown to take up amino acids (Zubkov *et al*., 2003; Mary *et al*., 2008; Gomez-Pereira

et al., 2013; Bjorkman *et al*., 2015), as far as we know, our study showed for the first time that amino acids can reduce the carbon fixation of *Prochlorococcus.* With decreased carbon fixation and increased respiration, *Prochlorococcus* was supposed to accumulate less organic carbon and in that case its growth should have been inhibited. However, our results showed that *Prochlorococcus* MIT9313 maintained its growth rate after vDOM and glycine addition (Fig. 1A and Supporting Information Fig. S3). One plausible explanation is that *Prochlorococcus* assimilates DOM compounds, which is consistent with the expression of tRNA genes (Fig. 2) and is also consistent with the ability of *Prochlorococcus* to assimilate organic compounds (Zubkov *et al*., 2003; Vila-Costa *et al*., 2006; Gomez-Baena *et al*., 2008; Mary *et al*., 2008; Gomez-Pereira *et al*., 2013; Munoz-Marin Mdel *et al*., 2013; Bjorkman *et al*., 2015).

Photosynthesis genes and F_v/F_m of *Prochlorococcus* MIT9313 were previously shown to be affected under N starvation (Tolonen *et al*., 2006). F_v/F_m of *Prochlorococcus* MIT9313 decreased by more than 30% under N starvation (Tolonen *et al*., 2006), and the majority of the photosynthesis genes showed decreased transcript abundances, comparing with the mixed responses we saw after vDOM addition (Supporting Information Table S3). The growth of *Prochlorococcus* MIT9313 stopped under N starvation (Tolonen *et al*., 2006) and 47 out of 57 ribosomal protein genes showed decreased transcript abundances, while only 14 showed decreased transcript abundances after vDOM addition (Supporting Information Table S3). Thus, comparing with the severe responses of *Prochlorococcus* MIT9313 cells under N starvation (Tolonen *et al*., 2006), the moderate transcriptional responses of photosynthesis and ribosomal protein genes after vDOM addition seemed to agree with the slight decrease of F_v/F_m and the unaffected growth rate.

Nitrogen metabolism genes

After vDOM addition, several genes related to N metabolism showed decreased transcript abundances (Fig. 4). These genes include the global N regulator PII gene *glnB*, the glutamate dehydrogenase gene *gdhA* and transporter genes for ammonium (*amt1*) and urea (*urtA*, *urtB*; Fig. 4A and B). As a nitrogen scavenging response, these genes show increased transcript abundances during N starvation of *Prochlorococcus* (Tolonen *et al*., 2006). Consistently, Sharma *et al*. observed decreased transcript abundances for ammonium and urea transporter genes when they added DOM derived from *Prochlorococcus* exudates to natural *Prochlorococcus* populations (Sharma *et al*., 2014). The authors of this study suggested that the DOM source they used contained labile organic nitrogen compounds that could be assimilated by *Prochlorococcus* (Sharma *et al*., 2014). The similar transcriptional responses of these transporter genes in our study might also be due to the organic nitrogen compounds in vDOM.

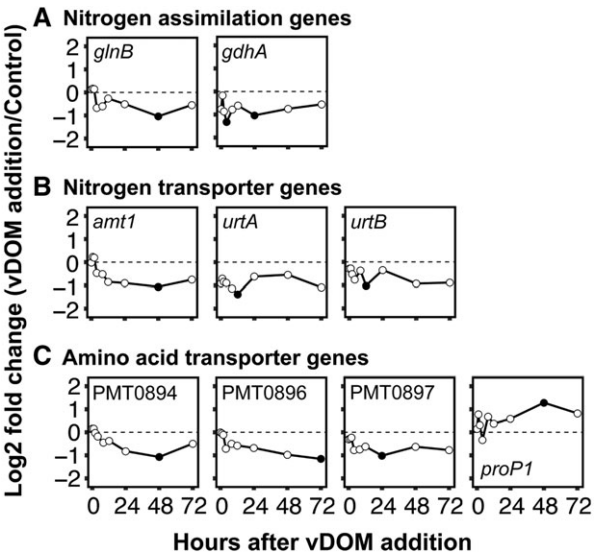

Fig. 4. Transcript abundances of nitrogen metabolism genes. RNA-seq data showing the transcript abundances of genes for nitrogen assimilation (A), nitrogen transporters (B) and amino acid transporters (C). Transcripts of the DOM amended cultures are normalized to those of the control cultures amended with the growth medium Pro99. A dotted line indicates log2 fold change = 0. Filled symbols indicate adjusted *p* values < 0.1.

Three amino acid transporter genes (PMT0894, 0896 and 0897) also showed decreased transcript abundances after vDOM addition (Fig. 4C). Although the transcriptional regulation of these genes in cyanobacteria is less clear, it has been shown in *E. coli* (Chubukov *et al*., 2014) and *Streptococcus pneumoniae* (Kloosterman and Kuipers, 2011) that bacteria downregulate the transcription of amino acid transporter genes when amino acids are abundant in the environment (Chubukov *et al*., 2014). This is consistent with abundant amino acids in vDOM (Supporting Information Table S1). Curiously, an amino acid transporter gene *proP* showed increased transcript abundances (Fig. 4C). *E. coli* upregulates *proP* transcription in high osmolality environments to enhance its uptake of proline and glycine betaine to avoid dehydration (Kempf and Bremer, 1998). As an adaptation to saline environments, *Prochlorococcus* MIT9313 has been found to accumulate glycine betaine and sucrose as the major compatible solutes (Klahn *et al*., 2010). Hence, it is possible, that the upregulated ProP system contributes to the pool of intracellularly accumulated solutes when glycine betaine becomes available in the environment, for example, due to viral lysis of the surrounding bacteria.

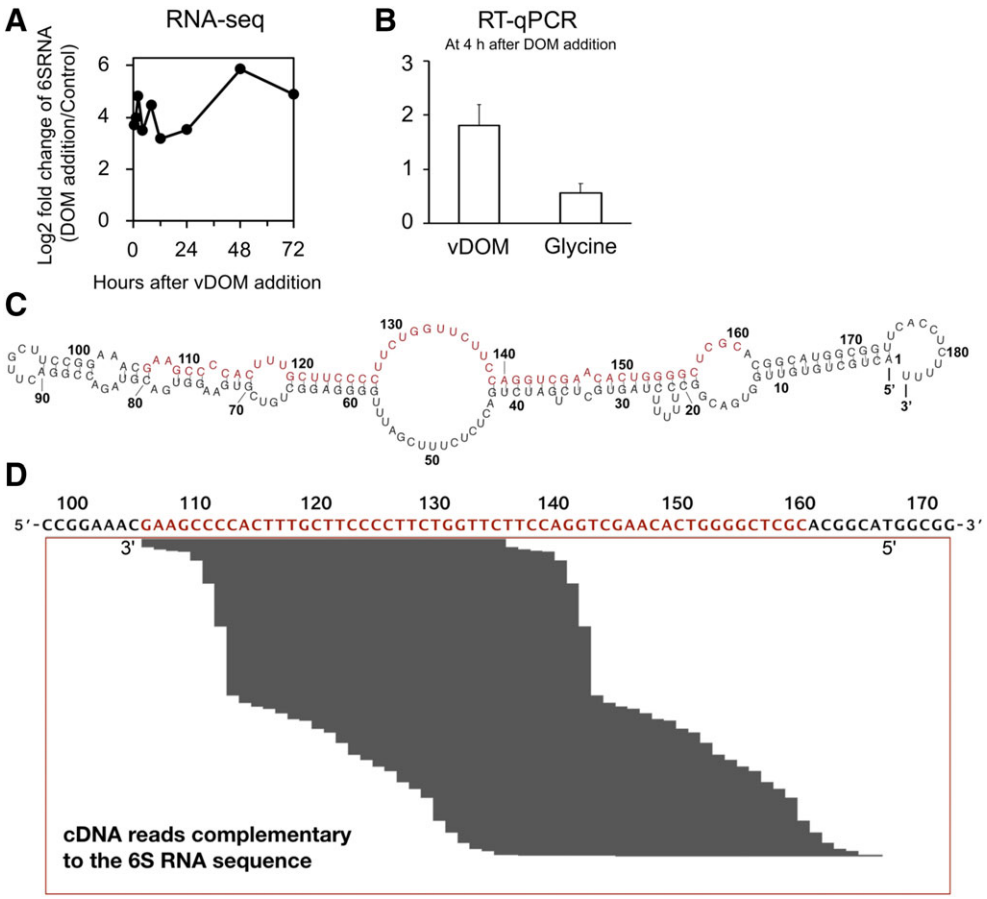

Fig. 5. 6S RNA abundance of *Prochlorococcus* MIT9313 and its predicted secondary structure.
A. RNA-seq data showing log2 fold change of 6S RNA after vDOM addition (vDOM addition/control). All the data have adjusted *p* values < 0.1.
B. RT-qPCR data showing log2 fold change of 6S RNA (DOM addition/control) at 4 h after vDOM or glycine (800 μM) was added to *Prochlorococcus* MIT9313 cells. Data are means ± SEM from three biological replicates.
C. The secondary structure of *Prochlorococcus* MIT9313 6S RNA was predicted using the RNAfold webserver with default settings (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) (Hofacker, 2003).
D. cDNA reads of the putative pRNA that are complementary to the cDNA sequence of 6S RNA. The top sequence shows partial cDNA sequence of 6S RNA. The bottom reads are from the RNA-seq library (0.5 h, vDOM amended sample) that are complementary to the cDNA sequence of 6S RNA. Reads were extracted using SAMtools (Li *et al*., 2009) and visualized using the Integrative Genomics Viewer (http://igv.org/). Red letters in (C) and (D) indicate the 6S RNA sequences that have a coverage of above 10 by the complementary pRNA reads in all the vDOM amended samples.

The global transcriptional regulator 6S RNA

Our RNA-seq data showed that the abundances of 6S RNA (gene ID PMT_ncRNA_Yfr7) increased approximately 13 fold at 0.5 h after vDOM addition (Fig. 5A). The 6S RNA exhibited the highest positive fold-change of all genes during some of the sampling points and remained highly expressed over the entire 72 h experimental period (Fig. 5A and Supporting Information Table S2). To confirm the RNA-seq results, we used RT-qPCR to show that both vDOM and glycine can increase the abundances of 6S RNA in *Prochlorococcus* MIT9313 cells (Fig. 5B).

The predicted secondary structure of *Prochlorococcus* MIT9313 6S RNA contains a central single-stranded bulge within a mostly double-stranded molecule (Fig. 5C), which is conserved in cyanobacteria and *E. coli* (Rediger *et al*., 2012). The 6S RNA structure mimics the melted promoter DNA and is shown in *E. coli* (Wassarman and Saecker, 2006) and *Prochlorococcus* MED4 (Rediger *et al*., 2012) to inhibit transcription by competing with promoter DNA binding to the RNA polymerase holoenzyme. With a conserved secondary structure (Axmann *et al*., 2007), the highly expressed 6S RNA of *Prochlorococcus* MIT9313 should be able to bind to RNA polymerase to regulate gene expression after vDOM addition. When 6S RNA binds to RNA polymerase, it has been shown in *E. coli* and *B. subtilis* that 6S RNA is used by RNA polymerase as a template for the transcription of product RNA (pRNA) (Wassarman and Saecker, 2006; Beckmann *et al*., 2012; Cavanagh *et al*., 2012). Consistently, we also identified putative pRNA reads from the RNA-seq data that

are complementary to the 6S RNA sequence (Fig. 5D), providing strong evidence that the 6S RNA of *Prochlorococcus* MIT9313 can bind to its RNA polymerase. Interestingly, in vivo pull-down experiments of the RNA polymerase complex in the model cyanobacterium *Synechocystis* sp. PCC 6803 indicated that high levels of 6S RNA promote the recruitment of the housekeeping σ factor SigA, and the dissociation of alternative group 2 σ factors (Heilmann *et al*., 2017). *Prochlorococcus* MIT9313 harbours seven different sigma 70-type sigma factors (compared to five in *Synechocystis* sp. PCC 6803) and one type 3 alternative sigma factor (Scanlan *et al*., 2009). Therefore, we speculated that some of the differentially expressed *Prochlorococcus* MIT9313 genes might be attributed to sigma factor replacement mediated by the 6S RNA.

Conclusions

Viral lysis of phytoplankton is estimated to release 6%–26% of photosynthetically fixed organic carbon into the marine DOM pool (Wilhelm and Suttle, 1999). Using RNA-seq analysis, our study showed rapid transcriptomic responses of *Prochlorococcus* MIT9313 to this DOM pool. The transcriptional responses of tRNA and N metabolism genes both suggested that *Prochlorococcus* MIT9313 may take up organic nitrogen compounds in vDOM, which is consistent with previous studies that *Prochlorococcus* can take up amino acids (Zubkov *et al*., 2003; Mary *et al*., 2008; Gomez-Pereira *et al*., 2013; Bjorkman *et al*., 2015). A recent study has shown that cyanophage infection of *Synechococcus* WH7803 releases abundant peptides derived from degradation of phycoerythrin (Ma *et al*., 2018), providing potential inducing compounds for the transcriptional responses of *Prochlorococcus* cells. In addition, our study showed for the first time that vDOM addition reduced the carbon fixation of *Prochlorococcus* MIT9313 by approximately 16%, which was also reduced by amino acid addition (~32%). This effect might be important for marine carbon cycling, considering that *Prochlorococcus* is the dominant primary producer in many oligotrophic oceans. Furthermore, our study showed that the 6S RNA was highly expressed after vDOM addition and we suggested that this global transcriptional regulator may regulate the transcriptional responses of *Prochlorococcus* MIT9313 to the availability of organic nutrients.

In this study, we used the DOM source produced by viral lysis of *Synechococcus*. It remains to be explored whether DOM sources generated by other processes (e.g. exudation and grazing) have similar or different effects on *Prochlorococcus* cells. It should be noted that our study was conducted using the low light–adapted *Prochlorococcus* strain MIT9313 under nutrient-replete conditions. Thus, our study might only represent oceanic regions where *Prochlorococcus* cells are not severely nutrient-limited because of rapid nutrient recycling (Vaulot *et al*., 1995; Liu *et al*., 1997). Future experiments with different *Prochlorococcus* ecotypes under nutrient-limited conditions are needed, since these ecotypes have different regulatory networks (Martiny *et al*., 2006; Tolonen *et al*., 2006) and their natural distributions are shaped by various environmental factors (Johnson *et al*., 2006).

Materials and methods

Cultivation of Prochlorococcus *and* Synechococcus

Axenic *Prochlorococcus* MIT9313 and *Synechococcus* WH8102 cultures were grown in polycarbonate bottles (Nalgene) with the Pro99 medium (Moore *et al*., 2002), which was based on natural seawater from Port Shelter, Hong Kong. *Prochlorococcus* and *Synechococcus* cultures were incubated at 24 °C under continuous cool white light at 15–20 and 30 μmol quanta m^{-2} s^{-1} respectively. Bulk culture chlorophyll fluorescence was monitored using a fluorometer (10-AU model, Turner Designs). For cell counting, 200 μl culture was preserved with 1% Glutaraldehyde solution (50 wt.% in H_2O, SIGMA) and stored at −80 °C until use. Preserved cells were counted using a flow cytometer (BD FACSCalibur) with the ModfitLT software.

Axenicity tests

Prochlorococcus and *Synechococcus* cultures were routinely tested for axenicity (no contamination of heterotrophs) by inoculating them in three purity broths ProAC (Morris *et al*., 2008), MPTB (Saito *et al*., 2002) and ProMM (Berube *et al*., 2015). The cyanobacterial cultures were considered axenic when no purity broth became turbid within 1 week at room temperature. Axenicity was also tested by flow cytometry and epifluorescence microscopy. In axenic cultures, all of the SYBR-Gold staining cells were *Prochlorococcus* or *Synechococcus* (determined by their autofluorescence), and no other SYBR-Gold staining cells were observed.

Preparation of viral lysis products (vDOM)

vDOM was generated by infecting early-log *Synechococcus* WH8102 cultures (~3×10^7 cells per mL) with 1/10 cyanophage S-ShM2 lysate (volume/volume) until cultures became clear. Lysates were filtered through a 0.2 μm filter to remove cell debris and then were stored in acid-washed glassware at 4 °C in the dark for several days before they were used in vDOM addition experiments.

Measurements of dissolved organic carbon and dissolved nitrogen

The dissolved organic carbon and dissolved total nitrogen (inorganic and organic) concentrations of the DOM sources were measured and analysed using an automated Shimadzu TOC analyser (TOC-V_{CPH} and Autosampler ASI-V, Shimadzu, Japan) according to the manufacturer's instruction. Dissolved organic carbon concentrations in vDOM and Pro99 were 682.95 ± 14.88 and 367.22 ± 5.24 μM respectively. Dissolved total nitrogen concentrations in vDOM and Pro99 were 804.59 ± 14.64 and 870.54 ± 12.18 μM respectively.

Measurements of total combined amino acids

Dissolved proteins in vDOM were concentrated using a centrifugal filter unit (10 kDa cutoff, Amicon). The resulting protein concentrates from 50 ml vDOM were dissolved in approximately 300 μl ultrapure water, and were hydrolyzed using 5 μg protease XIV (Sigma) at 37 °C for 16 h as described previously (Liu *et al*., 2016). Protein hydrolysates were then derivatized with 9-fluorenylmethyl chloroformate (Fmoc-Cl) and individual amino acid concentrations were determined by high performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) following published protocols (Buha *et al*., 2011; Liu *et al*., 2016). The concentrations of amino acids in the Pro99 growth medium were measured using the same method and were all below the detection limit (Supporting Information Table S1).

vDOM addition into Prochlorococcus *MIT9313 cultures*

In the oceans, approximately 15% of cyanobacteria are infected by cyanophages at any given time (Proctor and Fuhrman, 1990), and hence approximately 85% uninfected cyanobacteria are exposed to the DOM released by the infected cells. To mimic this effect, vDOM was added to mid-log phase *Prochlorococcus* MIT9313 cultures at a volume/volume ratio of 1:4 and the cultures were then incubated under continuous light. In the control cultures, the growth medium Pro99 was added at a 1:4 ratio.

RNA-seq library construction

With two biological replicates for each DOM amendment, cultures were collected at 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h after DOM addition. About 40–80 ml *Prochlorococcus* culture was spun down at 15 000 g for 15 min at 4 °C, and cell pellets were flash frozen in liquid nitrogen and stored at −80 °C. The mirVana RNA isolation kit (Ambion) was used to extract total RNA from cell pellets and the Turbo DNA-free kit (Ambion) was used to remove residual genomic DNA. Total RNA was concentrated with the RNA Clean & Concentrator-5 kit (Zymo Research), 150 ng total RNA was then fragmented into 60–200 nt by magnesium catalysed hydrolysis (40 mM Tris-Acetate, pH 8.1, 100 mM KOAc and 30 mM MgOAc) for 4 min at 83 °C, and purified with the RNA Clean & Concentrator-5 kit. As we described previously (Lin *et al*., 2016), strand-specific RNA-seq libraries were constructed with a dUTP second-strand marking protocol, while16S and 23S cDNA molecules were degraded by a duplex-specific nuclease (DSN) treatment. The DSN treatment has been shown to reduce the abundances of 16S and 23S reads in the total cDNA pool, without significantly affecting the expression levels of other genes (Yi *et al*., 2011). After DSN treatment, Illumina sequencing primers with barcodes were used to amplify the libraries. Equal amounts of barcoded libraries were pooled within one lane, and paired-end sequencing was done by Illumina HiSeq 2000 (49 nt for insert +6 nt for barcode).

Data availability

The raw reads of RNA-seq data have been submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under project number PRJEB22768 (ERP104472). Sample accession numbers are listed in Supporting Information Table S4.

RNA-seq data analysis

After Illumina sequencing, reads were separated based on their barcodes and their quality was assessed by FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc). The resulting clean reads were mapped against the *Prochlorococcus* MIT9313 genome using the Burrows-Wheeler Aligner (BWA) (Li and Durbin, 2009). HTSeq (Anders *et al*., 2015) was then used to calculate the number of reads perfectly aligning to the sense and antisense strands of ORFs, rRNAs, tRNAs and intergenic regions. For reads spanning two ORFs, they were counted once for each ORF. Reads that mapped to the 16S and 23S rRNA genes were removed manually from the total reads before further analysis. Among the 2765 annotated genes, over 98.8% were found to be transcribed in at least one sample, and 93.2% of the transcribed genes had a sequencing depth of more than 10 times, which indicated a thorough coverage. For each sample, the numbers of mapped reads per gene are listed in Supporting Information Table S5. The two biological replicates at each time point were highly reproducible (Pearson's R value > 0.95 at most time points, Supporting Information Table S4).

Identification and functional categorization of genes with differentially abundant transcripts

The DESeq2 package (Love *et al*., 2014) in R (www.R-project.org) was used to identify genes with differentially abundant transcripts with default parameters. For each sample, DESeq2 first normalized the number of reads per gene to the total number of mapped non-rRNA reads in that sample. DESeq2 then compared the normalized gene expression levels in the vDOM amended samples to those in the control samples amended with the growth medium Pro99. Adjusted *p* values were calculated by DESeq2 using the Benjamini-Hochberg procedure and adjusted *p* values < 0.1 were considered by DESeq2 as statistically significant (Love *et al*., 2014). To identify genes of biological relevance, we considered transcripts as differentially abundant after DOM amendment if transcript abundances showed a fold change ≥ 2 in either direction and an adjusted *p* value < 0.1. Genes with differentially abundant transcripts are listed in Supporting Information Table S2.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database of *Prochlorococcus* MIT9313 was fetched on-line (www.kegg.jp/kegg-bin/get_htext?htext=pmt00001) (Kanehisa *et al*., 2017). The Clusters of Orthologous Groups (COGs) were acquired by mapping *Prochlorococcus* MIT9313 protein sequences against the NCBI COG database (www.ncbi.nlm.nih.gov/COG/) (Tatusov *et al*., 1997). In total, 1108 COG-assigned proteins were found, with an E-value cutoff of 0.01. Higher categories of KEGG and COG classes were used to cluster the differentially abundant transcripts using the pheatmap package in R (CRAN.R-project.org/package= pheatmap). In brief, genes were first grouped under KEGG (Fig. 1B) or COG (Supporting Information Fig. S1) categories. Genes within each category were then hierarchically clustered with pheatmap.

Quantitative reverse transcription PCR

In Fig. 2B, vDOM, glycine (diluted in Pro99), aspartic acid (diluted in Pro99) or Pro99 was added to mid-log *Prochlorococcus* MIT9313 cells at a volume/volume ratio of 1:4. The final glycine and aspartic acid concentrations were both 800 μM. At 24 h after DOM addition, cells were collected by centrifugation. The relative transcript abundances of *tRNA-Gly1* and *rplE* genes were measured by quantitative reverse transcription PCR (RT-qPCR) following a previous protocol (Lin *et al*., 2016). Briefly, after RNA extraction and reverse transcription, cDNA copies were quantified using a QuantiTect SYBR Green PCR Kit (QIAGEN) with 0.5 μM forward and reverse primers (see Supporting Information Table S6 for primer sequences). The relative transcript abundances of *tRNA-Gly1* and *rplE* genes were normalized to those of the host *rnpB* gene (Lindell *et al*., 2007; Zeng and Chisholm, 2012).

In Fig. 5B, vDOM (volume/volume ratio 1:4) or glycine (final concentration 800 μM) was added to mid-log *Prochlorococcus* MIT9313 cells. At 4 h after DOM addition, the abundances of 6S RNA were measured by RT-qPCR using primers listed in Supporting Information Table S6 and normalized to the *rnpB* transcripts.

Carbon fixation rate

vDOM, glycine (diluted in Pro99) or Pro99 was added to mid-log *Prochlorococcus* MIT9313 cells at a volume/volume ratio of 1:4 (this was also done before the measurements of F_v/F_m and respiration). The final glycine concentration was 800 μM. Immediately after DOM amendments, freshly prepared 1 M $NaH^{13}CO_3$ was added to the cultures at a final concentration of 6 mM. At 0, 4, 8, 12 and 24 h after $NaH^{13}CO_3$ addition, 50 ml culture was centrifuged at 4 °C at 20 000 g for 5 min. Cell pellets were washed twice with 50 ml Milli-Q water to remove extracellular $NaH^{13}CO_3$. Cell pellets were then transferred to a 1.5 ml Eppendorf tube and subjected to freeze-dry for 1–2 h. The freeze-dried pellet was washed with 200 μl hydrochloric acid (5%) for less than 5 min to remove intracellular inorganic carbon. Acid was removed after centrifugation and the cell pellet was washed twice by ultrapure water (200 μl water for the first wash and 400 μl for the second). Cell pellets were freeze-dried, weighted and wrapped in tin capsules. ^{13}C and total carbon measurements were done by the Stable Isotope Facility of the University of California at Davis.

Photosynthetic carbon fixation rate was calculated based on the intracellular ^{13}C measurements (Hama *et al*., 1983). Briefly, the photosynthetic carbon fixation rate P (fg C cell^{-1} h^{-1}) can be obtained using the equation below:

$$P = \frac{\Delta C}{t} = \frac{C \cdot (a_{es} - a_{ns})}{t \cdot (a_{ic} - a_{ns})}$$

Here, a_{es}, a_{ns} and a_{ic} are the atom percentages of ^{13}C over total carbon in the experimental samples, natural organic samples and natural total inorganic carbon respectively. C is the particulate organic carbon (POC) in the experimental samples (fg C cell^{-1}), ΔC is POC increase during $NaH^{13}CO_3$ incubation (fg C cell^{-1}) and t is the incubation time in hours. After addition of vDOM, glycine or Pro99, intracellular organic carbon of *Prochlorococcus* MIT9313 increased linearly throughout the experimental period (Supporting Information Fig. 4). Carbon fixation rates were estimated by linear-fitting the calculated photosynthetic production against incubation time (Supporting Information Fig. 4). The estimated carbon fixation rates in our study (0.52–0.62 fg C cell^{-1} h^{-1}) are comparable with

those of *Prochlorococcus* lab cultures and field populations using similar methods (Partensky *et al*., 1999).

Photosystem II photochemical efficiency

The maximum PSII photochemical efficiency of mid-log *Prochlorococcus* MIT9313 was measured using a fluorometer (PSI FL 3500, Photon Systems Instruments, Czech Republic) with the fast repetition rate (FRR) fluorescence technique (Kolber *et al*., 1998). Prior to each measurement, 2 ml culture was loaded into a 10 × 10 mm cuvette and dark-adapted for 10 min, and the FRR induction was driven by a train of 40 × 1.2 μs flashlets (625 nm, ~100 000 μmol photons m^{-2} s^{-1}). The resulting FRR induction curves were then analysed in R with a published model (Kolber *et al*., 1998) to derive F_0, the base line fluorescence of cells after 10 min darkness, and F_m, the maximal fluorescence with all PSII closed respectively (van Kooten and Snel, 1990). The maximum photochemical quantum yields of PSII (F_v/F_m) was then calculated by $(F_m - F_0)/F_m$. All measurements were conducted at room temperature under the dark.

Measurement of respiration rate

The respiration rate was obtained by measuring the oxygen consumption of *Prochlorococcus* MIT9313 in the dark, using a FireSting Optode sensor controlled through the Oxygen Logger software (PyroScience, Germany). Prior to measurements, 10 ml culture was loaded into a chamber in a customized acrylic vial, which is connected to a circulating thermostatted bath (Cole-Parmer, USA) to maintain the growth temperature. A magnetic stir bar was put into the chamber to remove air bubbles. The oxygen concentration in the cultures was continuously monitored (1-s interval) in the dark for 5 min at growth temperature. Oxygen removal rates were calculated in R by linear-fitting oxygen concentration (μmol O_2 L^{-1}) against elapsed time (s). Respiration rates (fg O_2 $cell^{-1}$ h^{-1}) were then calculated by normalizing the oxygen removal rate to total number of cells (cells per 10 ml).

Acknowledgements

This study is supported by grants to Qinglu Zeng from the National Natural Science Foundation of China (Project No. 41776132 and 91851112) and the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. 16102317). This study is partially supported by grants to Yu-Zhong Zhang from the National Natural Science Foundation of China (Project No. 31630012 and U1706207). We thank Dr. Yehui Tan and Jiaxing Liu in the South China Sea Institute of Oceanology and Dr. Dinghui Zou in the South China University of Technology for their experimental assistance.

Author contributions

Q.Z. and X.F. designed the project; X.F. performed the experiments and data analysis, with assistance from other authors; Y.L. measured DOC, DN, *tRNA*, *rplE* and 6S RNA expression; Y.Z. and W.C. did amino acid measurements; Y.C. measured *tRNA* and *rplE* expression; R.L. measured growth rates and did axenicity tests; G.L. measured F_v/F_m and respiration; Q.-L.Q. and Y.-Z.Z. analysed genes related to amino acid uptake; Q.Z., X.F. and W.R.H. wrote the manuscript with contributions from all authors.

Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Concentrations of total combined amino acids.
Table S2. Prochlorococcus MIT9313 genes with differentially abundant transcripts after vDOM addition.
Table S3. Responses of energy metabolism and translation genes of Prochlorococcus MIT9313 after vDOM addition and N starvation.
Table S4. RNA-seq libraries constructed in this study.
Table S5. Number of mapped reads per gene.
Table S6. qPCR primers used in this study.
Appendix S1. Supplementary Figures.