Metatranscriptomics of the prokaryotic community in response to atmospheric deposition in the Western North Pacific Ocean

Shangjin Tan [1], **Shun Yan Shun Yan** [1], **Tung-Yuan Ho** [2], **Hongbin Liu** [Corresp. 1]

[1] Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong, China

[2] Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan

Corresponding Author: Hongbin Liu
Email address: liuhb@ust.hk

Atmospheric deposition represents a major vector of both macro- and micro-nutrients to the oligotrophic open oceans, potentially imposing a profound impact on the functioning of the microbial community. Whereas responses of the prokaryotes to atmospheric deposition are being studied at the community level, corresponding functional changes are essentially unknown. Here we used metatranscriptomic approaches to elucidate taxonomic and functional profiles of the prokaryotic community in response to Asian aerosols in the Western North Pacific Ocean. While *Bacteria* were downrepresented, *Virus* and *Archaea* were overrepresented in the aerosol treatment compared to the control. Within *Bacteria*, transcripts related to *Cyanobacteria*, including *Prochlorococcus*, *Trichodesmium* and *Synechococcus*, decreased dramatically, whereas transcripts related to *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes* showed differential increases in the aerosol treatment. Nutrients and organic matters were enriched as evidenced by an overexpression of transporters for amino acids and utilization of various carbohydrates and a down-expression of transcripts related with phosphorus metabolism. Increased expression included transcripts involved in tricarboxylic acid cycle, pentose phosphate pathway, glycolysis and gluconeogenesis. Unexpectedly, the expression of transcripts associated with Fe metabolism suggested that Fe limitation was intensified. Transcripts associated with N fixation declined, corresponding to the decline of diazotroph-related transcripts. This result is against the paradigm of Fe fertilization from atmospheric deposition but may represent an extreme case that Fe was scavenged after aerosol addition. Negative effects included impaired sugar utilization and the stimulation of oxidative stress and heavy metal toxicity. All these changes lead the community to an energy-conserving lifestyle and promoted motility, chemotaxis and interspecies competition and interaction. The results provide new insights into the biogeochemical impacts of atmospheric deposition in the Western North Pacific Ocean.

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Shangjin Tan[1], Shun-Yan Isaac Cheung[1], Tung-Yuan Ho[2], Hongbin Liu[1*]

[1] Division of Life Science, Hong Kong University of Science and Technology, Hong Kong SAR, China

[2] Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan

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* Corresponding author: E-mail liuhb@ust.hk; Tel. (+852) 23587341; Fax (+852) 23581552.

Address: CYT5004, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

Abstract

Atmospheric deposition represents a major vector of both macro- and micro-nutrients to the oligotrophic open oceans, potentially imposing a profound impact on the functioning of the microbial community. Whereas responses of the prokaryotes to atmospheric deposition are being studied at the community level, corresponding functional changes are essentially unknown. Here we used metatranscriptomic approaches to elucidate taxonomic and functional profiles of the prokaryotic community in response to Asian aerosols in the Western North Pacific Ocean. While *Bacteria* were downrepresented, *Virus* and *Archaea* were overrepresented in the aerosol treatment compared to the control. Within *Bacteria*, transcripts related to *Cyanobacteria*, including *Prochlorococcus*, *Trichodesmium* and *Synechococcus*, decreased dramatically, whereas transcripts related to *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes* showed differential increases in the aerosol treatment. Nutrients and organic matters were enriched as evidenced by an overexpression of transporters for amino acids and utilization of various carbohydrates and a down-expression of transcripts related with phosphorus metabolism. Increased expression included transcripts involved in tricarboxylic acid cycle, pentose phosphate pathway, glycolysis and gluconeogenesis. Unexpectedly, the expression of transcripts associated with Fe metabolism suggested that Fe limitation was intensified. Transcripts associated with N fixation declined, corresponding to the decline of diazotroph-related transcripts. This result is against the paradigm of Fe fertilization from atmospheric deposition but may represent an extreme case that Fe was scavenged after aerosol addition. Negative effects included impaired sugar utilization and the stimulation of oxidative stress and heavy metal toxicity. All these changes lead the community to an energy-conserving lifestyle and promoted motility,

chemotaxis and interspecies competition and interaction. The results provide new insights into the biogeochemical impacts of atmospheric deposition in the Western North Pacific Ocean.

Keywords: metatranscriptomics, aerosol, bacterial community, nutrient enrichment, SAR11, Western North Pacific Ocean.

Introduction

Among the factors controlling microbial abundance, activity and community composition in the ocean, nutrient availability has been recognized as the most important one (*Arrigo, 2005*). Roughly 80% of the global surface water is considered under nutrient limitation (*Longhurst, 1998*). The primary macronutrients, nitrate and phosphate, are depleted in 60% of the global oceans, leading to the low standing stock of phytoplankton and other organisms (*Antoine, André & Morel, 1996*). Limitation by micronutrients, such as Fe, Co, Zn, Cu, Ni and Cd, has also been observed in many surface waters (*Morel, Milligan & Saito, 2003*). Phytoplankton and productivity in the low-latitude oligotrophic oceans are typically limited by nitrogen (*Moore et al., 2008*). Picophytoplankton dominate both biomass and productivity in nutrient-limited oligotrophic regions due to their high surface-to-volume ratio that makes them more competitive to assimilate nutrients at low concentrations (*Emilio Fernández, 2003*). On the other hand, iron limitation has been proposed as the main cause for the high-nutrient, low chlorophyll regions in the subarctic Pacific Ocean, equatorial Pacific Ocean and Southern Ocean (*Jickells et al., 2005*).

Atmospheric deposition is increasingly being recognized as an important source of nutrients, including N, P, Fe and other elements, to the open ocean (*Duce & Tindale, 1991; Duce et al., 2008*). Experimental and field studies attempting to assess the impacts of atmospheric deposition on carbon biogeochemistry have been conducted, which demonstrated the fertilizing effects of aerosol deposition on phytoplankton, as well as the dynamics of microbial food web and carbon flux. Atmospheric nutrient deposition has been shown to directly stimulate phytoplankton growth and primary production in the South China Sea (*Guo et al., 2012*), Mediterranean Sea (*Bonnet et al., 2005; Herut et al., 2005*), North Atlantic Ocean (*Marañén et al., 2010*) and North Pacific Ocean. Mesocosms (e.g., *Rahav et al., 2016*) and on-board

71 incubation experiments (e.g., *Langlois et al., 2012*) have shown that N_2 fixation can be strongly
72 promoted by dust deposition by providing Fe and P. Field observation also found a coincidence
73 between the increase of *Trichodesmium* abundance and dust deposition (*Karl et al., 2002*).

74 Prokaryotes, namely *Bacteria* and *Archaea*, are a critical component of the microbial
75 food web with cyanobacteria performing as the main primary producer and heterotrophic
76 prokaryotes involved in the remineralization of elements and conversion of nutrients into
77 biomass (*Azam et al., 1983*). Heterotrophic bacteria are often considered as the best competitor
78 for phosphorus uptake. It is estimated that bacteria are responsible for 20-85% of the total Fe
79 uptake by the community (*Tortell, Maldonado & Price, 1996*). However, to date, only a small
80 number of studies have attempted to address the effects of atmospheric deposition on
81 prokaryotes. Generally, aerosol deposition is considered beneficial to the bacterioplankton
82 community in terms of the promotion of metabolic activities (*Herut et al., 2005; Pulido-Villena,*
83 *Wagener & Guieu, 2008; Lekunberri et al., 2010; Marañén et al., 2010; Guo et al., 2013;*
84 *Pulido-Villena et al., 2014; Guo et al., 2016*). Yet decoupling has been frequently observed
85 between the activity and the abundance. While bacterial production and respiration show a
86 powerful response, bacterial abundance remains relatively unchanged (*Lekunberri et al., 2010;*
87 *Marañén et al., 2010; Guo et al., 2013; Pulido-Villena et al., 2014; Guo et al., 2016*). Similarly,
88 as to the community composition, RNA-based community profiling shows that the metabolically
89 active community is more sensitive than the total community in response to aerosol addition
90 (*Van Wambeke et al., 2009; Laghdass et al., 2011; Guo et al., 2016*). Therefore, more insights
91 could be gained into the functional changes and their resulting ecological impacts by looking at
92 the RNA pool owing to its fast response to environmental perturbations and the close reflection
93 of cellular metabolic activities. Metatranscriptomics involves the isolation and sequencing of

environmental mRNAs from a complex of microbial assemblages, providing extensive information on both taxonomic affiliation and functions.

Large quantities of aerosols from East Asia are transported eastward, mixed with anthropogenic pollutants and then spread over the Western North Pacific Ocean (*Young et al., 1991; Zhang et al., 1993; Kim et al., 2014a; Martino et al., 2014*). In summer seasons, the Western North Pacific Ocean is characterized by stratification and a low primary production. Nutrients are rarely supplied by water column mixing. Atmospheric input represents the dominant nutrient source, supplying iron, nitrogen and other elements (*Duce et al., 2008; Kim et al., 2014b; Martino et al., 2014*). Therefore, this season of the year appears to be an ideal time to study the biogeochemical effects of atmospheric depositions on the surface water.

We reported here results from an on board aerosol addition microcosm experiment conducted during a cruise to the Western North Pacific Ocean (WNPO) in July 2013. We applied metatranscriptomic approaches to elucidate taxonomic and functional profiles of the prokaryotic community in response to atmospheric deposition. This study provides valuable information on the dominant metabolic processes and changes in biogeochemistry-related processes after atmospheric deposition.

Materials & Methods

Aerosol collection

Fine aerosol particles ($PM_{2.5}$) were collected at the roof of a building at the Hong Kong University of Science and Technology, which is located in an area with a relatively small population and low level human activities to reduce the influence of regional aerosols. Sampling was conducted during a sunny day using a high-volume sampler at a flow rate of 1130 L/min for 24 h onto a quartz filter (#2500 QAT-UP, Pall Life Science, Ann Arbor, MI, USA). Filters were stored under -20 ºC.

The aerosol composition was measured as described before (*Guo et al., 2012*). Inorganic nutrients were measured following the colorimetric method (Knap *et al.* 1996) using a Skalar autoanalyzer (Skalar Analytical B.V., Breda, Netherlands).

Seawater sampling and incubation experimental setup

The field incubation study was conducted on board R/V Ocean Research V at a Taiwan GEOTRACES station (23.50 N, 123.00 E, 150 km off Taiwan) in the Western Philippine Sea in July 2013 (*Liao, Yang & Ho, 2017*). Seawater was collected at 10 m depth with trace metal-clean Teflon-coated GO-FLO bottles mounted on a trace metal clean rosette (General Oceanic, Florida, FL, USA). The salinity, fluorescence and dissolved oxygen data obtained by CTD and related sensors were further calibrated by manual methods at a land-based laboratory. Seawater samples were also collected for major nutrient and dissolved trace metal concentration measurement. Detailed sampling information is described in *Liao, Yang & Ho (2017*). Nutrient samples were frozen in liquid N_2 and were brought back to our land-based laboratory for further processes.

Major nutrients were determined by standard methods adapted for a flow injection analyzer (*Pai, Yang & Riley, 1990b; Pai, Yang & Riley, 1990a*). Dissolved trace metal concentrations in seawater samples were measured by using chelating resin pre-concentration method and high resolution ICPMS (Element XR, Thermo Fisher Scientific, USA). The analytical procedures of the methods were described in details in Ho *et al.* (2010) and *Wang, Lee & Ho (2014*).

After pre-filtered through a 200-μm mesh to remove mesozooplankton, the seawater was dispensed into 6 acid-washed 20 L transparent polycarbonate microcosms. Three of the carboys were immediately amended with 0.2 mg/L aerosol to simulate a high-flux dust event (0.1-0.5 mg/L) (*Zhang et al., 1993*). The aerosol collection filter was cut into pieces with 4 mg aerosols and added to the incubations. The carboys were softly shaken to help aerosols to dissolve and mix the water. The other 3 unamended treatments were kept as the control. The bottles were capped, sealed and incubated in tanks at ~60% ambient light density to mimic the *in situ* light condition. Temperature was controlled by a running seawater system with water collected from the sea surface. After a 2-day incubation, subsamples were used for the following measurements: bacterial abundances and total RNA extraction.

Abundance of prokaryotic cells

For the enumeration of prokaryotic cell abundance, 1.8 ml seawater was taken from each incubation and the initial seawater, fixed with 0.5% (final concentration) seawater buffered paraformaldehyde (pH 7.2), flash frozen by liquid nitrogen and stored at -80 ºC before analyses. The abundances of total prokaryotic cells as well as the picocyanobacteria, *Prochlorococcus*, *Synechococcus* were determined using a Becton-Dickinson FACSCalibur Flow Cytometer. *Prochlorococcus* and *Synechococcus* were discriminated based on the side scattering and the

auto-fluorescence (*Olson, Zettler & DuRand, 1993*). Total cell abundance was enumerated after the samples were stained by 0.01% SYBR Green I followed with an incubation for 60 min (*Guo et al., 2013*).

RNA extraction and sequencing

Seawater was filtered onto 0.22 μm polycarbonate membranes using a peristaltic pump. The membranes were soaked in the RNAlater solution (Ambion, Austin, Texas, USA) and stored under -80ºC before analyses. Total RNA was extracted using the TRIzol reagent (Ambion, Austin, Texas, USA) in combination with the PureLink™ RNA Mini Kit (Ambion, Austin, Texas, USA). Genomic DNA was removed by digestion with Turbo-DNA Free DNase (Ambion, Austin, TX, USA). Due to low RNA yields, RNA samples from the control or treatment were pooled and subjected to library preparation. The prokaryotic libraries were prepared by using the SMARTer Universal Low Input RNA Kit (TaKaRa, Otsu, Japan) and the TruSeq RNA Library Prep Kit (Illumina, San Diego, CA, USA). Sequencing was conducted using the Illumina Miseq 250PE platform at the Macrogen Inc. (South Korea).

Bioinformatic analyses

Quality check of the paired-end sequence reads was performed using FastQC v0.11.3 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were quality trimmed using Trimmomatic (*Bolger, Lohse & Usadel, 2014*), with short reads (length <100 bp) removed and adaptor sequences and low-quality trailing bases (Phred score <10) trimmed off. Removal of

rRNA sequences from the datasets was done using the SortMeRNA with the default rRNA databases including 5S, 5.8S, 16S, 23S, 18S and 28S rRNAs (*Kopylova, Noé & Touzet, 2012*).

The resulting sequences in each dataset were aligned using BLASTX against the NCBI nr database (April 28, 2016) with an evalue cutoff of 1E-5. Taxonomic and functional assignments were obtained by parsing the BLASTX results using the lowest common ancestor algorithm in MEGAN6 (*Huson et al., 2016*) with the default setting for all parameters. Taxonomic classification was done by mapping the BLASTX results against the NCBI taxonomy tree. Functional profiling was carried out by mapping the BLASTX results against the SEED (*Overbeek et al., 2005*) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (*Kanehisa & Goto, 2000*) classification tree.

Sequences reported here were deposited in the GenBank through the sequence read archive and can be retrieved under the following accession number PRJNA371359.

Statistical analyses

Multiple comparisons of bacterial abundances, including heterotrophic bacteria, *Prochlorococcus* and *Synechococcus*, across the initial, control and aerosol treatment were performed using analysis of variance (ANOVA) when they comply with normal distribution. If not, the generalized linear model in the R packages, robust and multcomp (*Hothorn, Bretz & Westfall, 2008*), were used. Tests of normality and equal variance were conducted using the Shapiro-Wilk test and the Bartlett's test, respectively. All analyses were conducted in the R software (*Team, 2014*). The Bonferroni correction method was used to correct the *P* values.

To identify if microbial taxa or functions significantly upregulated or downregulated in the aerosol treatment, gene counts were first normalized by dividing the gene number of individual taxa or functional category by total number of gene hits in each metatranscriptomic dataset to account for different sequencing efforts. Then two-sample comparison was carried out using the two-sided Fisher's exact test with the Benjamini-Hochberg False Discovery Rate (FDR) for multiple test correction method and a q-value <0.05 in the STAMP v2.1.3 software (*Parks et al., 2014*).

204

205 **Results and Discussion**

206 Limited studies have examined functional changes of marine phytoplankton in response
207 to atmospheric deposition (*Van Wambeke et al., 2009; Laghdass et al., 2011; Guo et al., 2016*).
208 To the best of our knowledge, this is the first study using a metatranscriptomic approach to
209 examine prokaryotic community activities in response to aerosol addition, carried out in the
210 summer time, when the water column is supposed to be highly stratified and undergoes nutrient
211 limitation (*Odate, 1996*), with which we expected to observe the strongest response.

212

213 **Initial environmental features and aerosol composition**

214 The nutrient concentration in the surface water (10 m depth) used in this experiment was
215 characterized by low nitrogen species (NO_3^-+NO_2^-, 0.13 μM) and phosphate (under detection
216 limit, 0.01 μM) (Table 1). The concentrations of dissolved trace metals were similar to what
217 were observed in the North Pacific Ocean (*Bruland, 1980*). The total dissolved concentrations of
218 Mn, Zn, Cu, Co, Ni, and Cd in the top 200 m of the sampling stations generally ranged from
219 0.50-3.0, 0.50-2.0, 0.50-1.0, 0.010-0.020, 1.8-2.1, and 0.010-0.050 nM, respectively (Ho et al. in
220 preparation).

221 The aerosol used in this experiment was composed of a large amount of macronutrients
222 and trace metals. The dominant components were the sulfate, reactive nitrogen (NO_3^-, NO_2^- and
223 NH_4^+) and carbon, constituting 25.0%, 17.1% and 16.7% of the aerosol mass, respectively (Table
224 2). Though not measured phosphate constitutes a small portion according to our previous studies
225 having aerosol samples collected at the same site (*Guo et al., 2012*). Overall, the aerosol

composition was similar with our previous collections in Hong Kong. We have also collected aerosol samples from a remote place in Taipei (Taiwan), which is located in the western Pacific Rim. The elemental composition of the Hong Kong aerosol overall was similar to those collected in Taipei (Table S2), except for a lower concentration of NO_3^-+NO_2^- (4.4% versus 12.5%) and Fe (0.432% versus 0.960%) and a higher concentration of Zn (0.307% versus 0.149%) and Pb (0.071% versus 0.036%). Generally, the Hong Kong aerosol is representative of aerosols settled to the WNPO.

Response of prokaryotic cell abundance

Although with a statistically significant increase, heterotrophic prokaryotic abundance exhibited a minor change with aerosol addition (Fig. 1). Similarly, previous reports have shown that bacterial abundance does not show marked changes to aerosol additions (*Herut et al., 2005; Marañén et al., 2010; Laghdass et al., 2011; Guo et al., 2012; Pulido-Villena et al., 2014; Guo et al., 2016*). However, bacterial activities, such as bacterial production and respiration, have been shown to exhibit clear responses (*Pulido-Villena, Wagener & Guieu, 2008; Marañén et al., 2010; Pulido-Villena et al., 2014; Guo et al., 2016*). This decoupling in the responses between the abundance and activity has been suggested because of enhanced grazing pressure by ciliates (*Herut et al., 2005*) and heterotrophic nanoflagellates (*Guo et al., 2012*).

Prochlorococcus abundance was ~12 times as numerous as *Synechococcus* in the initial environment, but decreased close to the abundance of *Synechococcus* in the aerosol treatment. The abundance of *Synechococcus* decreased by 21.5% in the treatment (Fig. 1). Generally, *Prochlorococcus* tend to decrease with the addition of dusts, while variable responses have been observed for *Synechococcus* (*Herut et al., 2005; Marañén et al., 2010; Guo et al., 2012; Guo et*

249 *al., 2013*). With the nutrients provided by aerosol, *Prochlorococcus* and *Synechococcus* have
250 been observed with promoted cellular carbon and Chl *a* contents (*Guo et al., 2012*). Such a
251 pronounced physiological state is suggested to be a reason for increased grazing pressure (*Herut*
252 *et al., 2005; Guo et al., 2012*). In addition, the abundance of *Prochlorococcus* decreased by
253 28.3% in the control compared to the initial (Fig. 1), while the abundance of *Synechococcus* was
254 not significantly different between the control and the initial.

255

256 **Overview of the metatranscriptomic libraries**

257 A total of 8,295,242 and 9,351,598 reads were generated for the metatranscriptomes of
258 the control and the aerosol treatment, respectively. Of the quality reads, 8.6% (control) and 9.6%
259 (aerosol) of the reads were mRNA, which is not surprising as rRNA depletion was not applied to
260 the samples because of low RNA yields. The percentages of significant hits in the NCBI nr
261 database were 25.6% and 25.9% for the control and aerosol treatment, respectively. The
262 remaining reads likely represent novel genes or poorly conserved regions of known genes
263 (*Poretsky et al., 2009*). Of these reads, 41.7% (control) and 34.6% (aerosol) were mapped to
264 subsystems based on SEED classification. Similarly, 47.9% (control) and 50.4% (aerosol) were
265 assigned to KEGG functional categories (Table S1).

266

267 **Response of the taxonomic composition**

268 Within the mRNA pool, the domain *Bacteria* was dominant in both the control and
269 aerosol treatment, followed by *Eukaryota*, *Virus* and *Archaea* (Fig. 2A). The relative abundance
270 of bacterial sequences decreased in the aerosol treatment, while the eukaryotic sequences

increased, corresponding to a decline in the abundances of *Prochlorococcus, Synechococcus* (Fig. 1) and *Trichodesmium* (Fig. S1) and an increase in the picoeukaryote abundance (data not shown). Though rare, archaeal and viral sequences were also detected and both increased the relative abundance. As the focus of this study is on the prokaryotic community, eukaryotic sequences are excluded from further analyses. Viral sequences were also included given the importance of viral lysis in controlling bacterial abundance.

Within the domain *Bacteria*, typically abundant phyla in the open ocean such as *Proteobacteria* (42.4%) and *Cyanobacteria* (42.0%) were highly represented (Fig. 2B) in the control. *Bacteroidetes* also presented in a high abundance (11.5%). Aerosol addition resulted in a dramatic change in abundances of bacterial phyla. In general, cyanobacterial taxa decreased the relative abundance whereas heterotrophic bacterial taxa increased the relative abundance. Out of 18 phyla showing significant (q<0.05) changes, 8 phyla (only *Cyanobacteria* with a >1% relative abundance) decreased and 10 phyla (*Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Firmicutes* with a >1% relative abundance) increased. Further discussion is made below about the 3 most abundant phyla.

Cyanobacteria

Cyanobacteria were dominated in the control by *Prochlorococcus* (18.6%), *Trichodesmium* (9.8%), and *Synechococcus* (2.7%), which were often found dominant in the open ocean (Odate 1996). Corresponding to flow cytometry results, the relative abundance of *Prochlorococcus* (5.7%) and *Synechococcus* (2.3%) both decreased in the aerosol treatment (Fig. S1). Unexpectedly, two diazotrophic cyanobacteria, *Trichodesmium* (0.5% in the aerosol treatment) and *Crocosphaera* (0.14% and 0.02% in the control and treatment, respectively), were

294 also reduced with aerosol addition, which is opposite to the main fertilization scenario of dusts
295 on diazotrophs. Enhanced *Trichodesmium* abundance has been observed to be correlated with
296 dust deposition (*Karl et al., 2002*). At the molecular level, total and *Trichodesmium nifH* gene (a
297 marker gene encoding the nitrogenase reductase) abundance increased after Saharan dust
298 addition (*Langlois et al., 2012; Rahav et al., 2016*). In the contemporary ocean, diazotrophs are
299 generally assumed to be limited by iron (Fe) (*Karl et al., 2002*) due to a high Fe demand of the
300 N_2 fixing enzyme nitrogenase and photosynthetic apparatus. The decline in diazotrophic
301 *Cyanobacteria* may be due to similar reasons for *Prochlorococcus* and *Synechococcus*.

302

303 ***Proteobacteria***

304 Signatures of *Alphaproteobacteria* and *Gammaproteobacteria* were the most abundant
305 proteobacterial taxa in both the control and aerosol treatment, and both increased the
306 representation with aerosol addition (Fig. 2B). Among them, *Alphaproteobacteria* was
307 dominated by the genus *Pelagibacter* (belonging to SAR11 cluster) and *Puniceispirillum*
308 (SAR116 cluster); *Gammaproteobacteria* showed high abundances of *Alteromonas*,
309 *Pseudoalteromonas* and *Vibrio* (Fig. S1). SAR11 dominates nutrient-limited ocean surface
310 waters, enabled by a small streamlined genome. Owing to its high-affinity nutrient acquisition
311 system (*Alonso & Pernthaler, 2006*), SAR11 could benefit from the nutrients introduced from
312 aerosol addition (*Hill, Zubkov & Purdie, 2010; Guo et al., 2016*). Accordingly, SAR11
313 contributed a large amount of transcripts in nitrogen metabolism (1.91% in control and 2.62% in
314 aerosol treatment), and exhibited an increased in carbohydrate metabolism and a decrease in P
315 metabolism. *Puniceispirillum marinum* was first reported to be stimulated by aerosol addition
316 and linked to atmospheric deposition in this study. Genomic sequencing showed that

317 *Puniceispirillum marinum* has a small and streamlined genomes such as that of *Pelagibacter*
318 *ubique* (*Oh et al., 2010*). *Alteromonas*, *Pseudoalteromonas* and *Vibrio* have been found to
319 respond positively to dust addition (*Langlois et al., 2012; Guo et al., 2013*).
320 *Gammaproteobacteria* is regarded as opportunisitic, responsive to environmental changes and
321 nutrient enrichment (*Allers et al., 2007*). Therefore this positive response may reflect increased
322 nutrient availability, which is supported by an increase in branched-chain amino acid ABC
323 transporters and carbohydrate metabolism and a decrease in a range of genes involved in N and P
324 metabolisms (Fig. 4C and Table S5).

325

326 ***Bacteroidetes***

327 *Bacteroidetes* have been thought to play a role in the cycling of organic matter related to
328 phytoplankton blooms (*Teeling et al., 2012*). *Flavobacteria* have been reported to substantially
329 incorporate *Synechococcus* exudates (*Nelson & Carlson, 2012*). The most dominant family,
330 *Flavobacteriaceae*, and genus, *Fluviicola*, both increased the relative abundance (from 5.9% to
331 6.2% and from 0.15% to 0.36%, respectively) with aerosol addition. Although *Fluviicola* are
332 rarely isolated from seawater, versatile abilities have been shown to utilize dissolved organic
333 matter (DOM) (*Woyke et al., 2011*). Therefore, it seems *Bacteroidetes* may benefit from aerosol
334 addition by assimilating exudates from senescent cyanobacterial cells and/or stimulated
335 picoeukaryotes (data not shown). Additionally, *Bacteroidetes* showed a slight decrease in
336 transcripts associated with P metabolism (Fig. 4C and Table S5). Members of *Bacteroidetes* (e.g.
337 *Flavobacteriaceae* and *Tenacibaculum*), which have been shown to benefit from P addition
338 (*Lekunberri et al., 2010*), also increased the relative abundance significantly in aerosol treatment
339 in this study, indicating increased P availability.

340

341 ***Archaea and Virus***

342 Responses of *Archaea* to atmospheric deposition have rarely been studied. Low
343 representation of archaeal transcripts is consistent with previous field surveys (*DeLong et al.,*
344 *2006*). The archaeal groups were dominated by *Euryarchaeota* (97.4% and 96.2% of archaeal
345 reads in the control and treatment, respectively) (Fig. 2C), particularly by Marine Group II (MG
346 II), which is ubiquitous in marine environments.

347 *Virus*-related transcripts increased from 1.4% in the control to 2.0% in aerosol treatment
348 (Fig. 2A), indicating promoted viral lysis. Because relative abundance is used in this study, an
349 increase in viral transcripts could be due to the decrease in cyanobacterial transcripts. A 0.3%
350 increase was seen when cyanobacterial transcripts were removed, confirming stimulated viral
351 transcripts. An increase in viral abundance has been observed to coincide with a decrease in
352 bacterial abundance (*Pulido-Villena et al., 2014*). Viral control of increased bacterial abundance
353 in response to dust addition has been suggested (*Pulido-Villena et al., 2014*). After aerosol
354 addition, the dominant taxa, the relative abundance of *Phycodnaviridae*, *Mimiviridae*, and
355 *Siphoviridae* increased, whereas *Myoviridae* decreased (Fig. 2D). *Myoviridae* were mainly
356 composed of members of *Cyanophage*, *Prochlorococcus* phage, and *Synechococcus* phage. A
357 decrease of *Myoviridae*-related transcripts corresponded to the decrease of cyanobacterial species
358 (Fig. 1). The decline in *Prochlorococcus* phage implies that promoted viral lysis may not be the
359 most likely reason for the decrease of *Prochlorococcus* abundance. Phytoplankton phages
360 dominated in *Phycodnaviridae*- and *Mimiviridae*-affiliated transcripts, corresponding to the
361 stimulated growth of eukaryotic cells (data not shown).

362

363 **Response in microbial functions**

364 **Global pattern in bacterial functional activities**

365 In general, functional categorization of transcripts in the control versus aerosol treatment
366 indicated stimulated bacterial metabolism as well as negative effects. A total of 39 subsystems
367 were identified in both datasets. The greatest number of transcripts were in the subsystem of
368 protein metabolism (10.2% and 10.9% in the control and aerosol treatment, respectively),
369 followed by virulence, disease and defense (8.2% and 9.6%) and carbohydrates (8.5% and 8.3%)
370 (Fig. 3). The dominance of transcripts belonging to these categories was consistent with the
371 metatranscriptomes of other marine surface waters (*Poretsky et al., 2009*). In total, there were 23
372 subsystems exhibiting significantly differential expression. Aerosol addition resulted in an
373 enrichment of 14 subsystems, such as protein metabolism, virulence, and amino acids. The other
374 9 subsystems that were negatively impacted were related to cofactors, photosynthesis, phages,
375 etc. (Fig. 3 and Table S2).

376

377 **Membrane transporters**

378 The most highly expressed subsystems of membrane transporters fell into the Ton and
379 Tol transport system (1.6% and 2.3% in the control and aerosol treatment, respectively) and
380 ATP-binding cassette (ABC) transporters (0.31% and 0.33%). Significant differences were found
381 in 5 categories: Ton and Tol transport systems, branched-chain amino acid ABC transporter,
382 alkylphosphonate ABC transporter, HtrA and Sec secretion, and widespread colonization island
383 (Fig. 4A). TonB-dependent receptor dominated the Ton and Tol transport systems. Involvement
384 of TonB-dependent receptor in Fe metabolism is discussed below. TonB transporters are also

responsible for a range of carbohydrates, such as biopolymers (*Schauer, Rodionov & de Reuse, 2008*), which is evident by the presence of TonB transporters identified in the context for biopolymers. Similarly, TonB transporters have been shown to be involved in decomposing phytoplankton biomass (*Teeling et al., 2012*). An increased (from 0.13% to 0.19%) representation of branched-chain amino acid ABC transporter in aerosol treatment compared to the control (Fig. 4A) suggests increased availability of branched-chain amino acids after aerosol addition (*Rinta-Kanto et al., 2012*). Down representation (~3 times lower) was seen in alkylphosphonate ABC transporters, including an ATP-binding polypeptide (*phnC*), a periplasmic binding protein for phosphonate (*phnD*), and a membrane-spanning transporter polypeptide (*phnE*) (Fig. 4A and Table S3). Phosphonates can be produced by many marine organisms (*Villarreal-Chiu, Quinn & McGrath, 2012*). It has been shown that *Trichodesmium erythraeum* IMS 101 contain a level of phosphonate as high as 10% of total P in cells (*Dyhrman et al., 2009*). Thus, the down representation of alkylphosphonate ABC transporters indicates not only cyanobacteria, such as *Trichodesmium*, as an important source of phosphonate but phosphonate as an important P resource. A 5-fold increase in the HtrA and Sec secretion in the aerosol treatment may suggest a stress of misfolded proteins (*Gullón, Vicente & Mellado, 2012*), which may be caused by oxidative stress and heavy metal toxicity (discussed below) as supported by a decrease in nickel and cobalt transporters (Table S3) and an increase in Co/Zn/Cd resistance genes (Table S9). An increase in the widespread colonization island indicates higher activity of colonization and biofilm (*Tomich, Planet & Figurski, 2007*), which is a common feature formed under a stressful condition.

Iron acquisition and metabolism

After aerosol addition, we observed significant changes in 4 subsystems: iron acquisition (21.7% increase compared to the control), transport of iron (71.5% decrease), iron metabolism (64.5% increase), and heme and hemin uptake and utilization systems (53.5% increase) (Fig. 4B). The iron acquisition subsystem was dominated by TonB-dependent receptors, which increased the representation (from 1.05% to 1.31%) in parallel with ferrous iron (Fe^{2+}) transport protein B (*feoB*) (0% to 0.005%) after aerosol addition (Table S4). TonB is commonly thought to be associated with iron and vitamin uptake (*Schauer, Rodionov & de Reuse, 2008*). The expression of both TonB-dependent receptors (*Lim, 2010*) and *feoB* (*Rong et al., 2008*) have been shown to be induced by Fe limitation. However, ferric iron (Fe^{3+}) ABC transporters were largely unchanged, indicating Fe^{3+} ABC transporters are not important as such. Modulated by cellular Fe level, iron-induced regulator (*Irr*) is degraded when sufficient Fe is available (*Hamza et al., 1998*), and increased by 1.5 folds in the aerosol treatment. Fe metabolism was dominated by the Fe^{3+} siderophore transport system, *ExbB* and *TonB*, highly expressed under Fe-limited conditions (*Higgs, Larsen & Postle, 2002*), which was observed with a 4.4-fold and 1.6-fold increase, respectively (Table S4). It has been hypothesized that marine microorganisms make use of siderophores to access Fe (*Granger & Price, 1999*). All the results lead us to propose that aerosol addition resulted in increased Fe limitation, even though Fe constituted as a major component (Table 2). This finding is against the paradigm of Fe fertilization from atmospheric deposition (*Duce & Tindale, 1991; Jickells et al., 2005*), which increases primary production and nitrogen fixation. Similarly, mesocosm experiments in an oligotrophic environment showed that dust addition resulted in a decrease in dissolved Fe, possibly scavenged by fast-sinking particles and organic matters produced by bacteria and phytoplankton (*Ye et al., 2011; Wuttig et al., 2013*). The fractional mean residence time of Fe in the surface waters decreased with the increase

in dust fluxes (*Croot, Streu & Baker, 2004*). Consistent with our study, aerosol was added to represent a high-flux event. Senescence of cyanobacteria may lead to the release of organic matter-rich cellular contents. Thus, both particle and organic matter scavenging may possibly be the reasons for Fe limitation. Fe limitation may be one of the most important reasons for the dramatic decrease of cyanobacterial taxa.

Phosphorus metabolism

In the aerosol treatment the significantly differentially changed subsystems were high-affinity phosphate transporter and control of the Pho regulon, P uptake, and phosphate metabolism, all of which were down-expressed (Fig. 4C). This pattern still holds true when cyanobacterial transcripts were excluded. The most depleted transcripts belonged to the phosphate-binding (*pstS*) and ATP-binding (*pstB*) components of the high affinity phosphate transporter (Table S5), which are up-regulated under P limitation (*Dyhrman, Ammerman & Mooy, 2007*). Consistent with the decrease in alkylphosphonate ABC transporters, down-representation was also found in *phnIL*, constituting the core components for phosphonate catabolism (Table S5). Also down-expressed were transcripts encoding the phosphate transport system regulatory protein (*phoU*) and the response regulator in two-component regulatory system (*phoQ*) (Table S5), which are a global regulatory mechanism involved in bacterial metabolism of inorganic phosphorus (Pi) (*Villarreal-Chiu, Quinn & McGrath, 2012*). Genes of the Pho regulon and phosphonate metabolism are induced by Pi limitation but repressed under Pi-replete conditions (*Villarreal-Chiu, Quinn & McGrath, 2012*). All the results together point to a relieve of P limitation possibly direct through P enrichment from aerosol addition though P is

typically low in East Asia aerosols (*Guo et al., 2012; Martino et al., 2014*) and indirectly through excretion of P and degradation of phosphonate from senescent cyanobacterial cells.

Nitrogen metabolism

Subsystems significantly depleted in the aerosol treatment were nitrogen fixation (from 1.04% to 0.01%), nitrogen fixation with *nifL* (from 1.15% to 0.13%), and dissimilatory nitrite reductase (from 0.01% to 0.001%) while nitric oxide synthase was significantly enriched (from 0.02% to 0.05%) (Fig. 4D). The dramatic decrease in nitrogen fixation transcripts echoed the marked decline in the relative abundance of diazotrophic transcripts as shown in rRNA and mRNA transcripts (Fig. 2 and S1). Nitric oxide synthase is responsible for the synthesis of nitric oxide, a widespread signalling agent. Enrichment of nitric oxide synthase is an implication that the bacterial community is experiencing stresses (*Crane, Sudhamsu & Patel, 2010*), which corresponds to the increased oxidative stress and heavy metal toxicity (Fig. 6A and B and Tables S8 and S9). The genes for ammonium transporter (*amt*) and glutamine synthase and glutamate synthase (*GOGAT*) were among the most highly transcribed nitrogen metabolism genes in both datasets (Table S6), indicating the importance of ammonium as a nitrogen nutrient. Nitrogen two-component systems typically sense nitrogen limitation via intracellular glutamine concentrations (*van Heeswijk, Westerhoff & Boogerd, 2013*). Both *amt* and *GOGAT* are typical signatures for nitrogen limitation, ready to be actively expressed upon nitrogen starvation (*van Heeswijk, Westerhoff & Boogerd, 2013*). Significant overrepresentation of *amt*, nitrogen regulation protein I (*NRI*) and *GOGAT* in the aerosol treatment (Table S6) is in line with N contribution from nitrogen fixation and/or may suggest that aerosol addition drove the bacterial community into an increased nitrogen limitation, which is probably caused by the decline in

diazotrophs (Fig. S1) and/or P (Fig. 4C and Table S5) and carbon (Fig. 5 and Table S7) enrichment. Similarly, bacterial community can be driven into nitrogen limitation as a consequence of elevated concentrations of DOM (*McCarren et al., 2010*) and glucose (*Martínez-García et al., 2013*).

Carbohydrate metabolism

Of the 106 subsystems related to carbohydrate metabolism, 44 subsystems were found to be significantly differentially expressed, with 67.7% transcripts down-expressed (Fig. 5). The most highly down-expressed subsystems were identified in sugar utilization, Calvin-Benson-Bassham cycle (CBB cycle, carbon fixation), photorespiration, glycolysis and gluconeogenesis, Entner-Doudoroff pathway, pentose phosphate pathway (PPP). However, when cyanobacterial transcripts were removed, the last 3 categories increased in the aerosol treatment and the decrease in sugar utilization was relieved, indicating a stimulation in broad cellular metabolisms and a large investment to these 3 categories by *Cyanobacteria*. While as the major category sugar utilization decreased, some carbohydrate metabolisms, such as the utilization of fructose, L-arabinose, lactate, melibiose and sucrose, the metabolism of mannose, sucrose, glycerate and glycerol, galactose degradation, and starch biosynthesis, increased to different degrees (Table S7).

The major overexpressed subsystems were associated with tricarboxylic acid cycle (TCA cycle), serine-glyoxylate cycle, methylcitrate cycle and Rubisco shunt, contributed mainly by members from *Alteromonas*, *Pseudoalteromonas*, *Pelagibacteraceae*, and *Halieaceae* (Table S7). The TCA cycle makes use of carbohydrates, fats and proteins to generate energy, precursors of some amino acids and NADH that can be used in many biochemical pathways. The serine-

499 glyoxylate cycle is involved in the utilization of simple carbon source for the synthesis of
500 carbohydrates when glucose are not available (*Lorenz & Fink, 2002*). The methylcitrate cycle is
501 a common pathway in microorganisms required for growth on fatty acids (*Eoh & Rhee, 2014*).
502 The Rubisco shunt is a process that converses carbohydrates to acetyl-CoA with an increased
503 efficiency relative to glycolysis (*Schwender et al., 2004*).

504 Upon glucose amendment, glucose, sucrose, and N-acetylglucosamine-specific
505 components of the phosphoenolpyruvate phosphotransferase system (PTS) were highly enriched
506 in bacterioplankton (*Martínez-García et al., 2013*). Sugars are the major bioreactive component
507 of phytoplankton leachate (*Opsahl & Benner, 1997*). Transporters of carbohydrates and PPP can
508 be significantly induced by phytoplankton blooms (*Rinta-Kanto et al., 2012; Teeling et al.,*
509 *2012*). Additionally, eukaryotic phytoplankton was stimulated as evidenced by a significant
510 increase in picoeukaryote abundance and concentrations of Chl *a* and *b* and fucoxanthin (a
511 marker pigment of diatom, another primary producer in the open ocean) (data not shown). All
512 these results suggest that *Cyanobacteria* in this region are the major source of carbohydrates and
513 metabolic intermediates for the biosynthesis of heterotrophic bacteria, that heterotrophic bacteria
514 may benefit more through the direct addition of limiting nutrients and less through the excretion
515 of carbohydrates from senescent cyanobacterial cells and/or from stimulated eukaryotic
516 phytoplankton, and that heterotrophic bacteria actively resort to alternative biochemical
517 pathways for the syntheses of energy, NADH and metabolic intermediates when external sources
518 of carbohydrates are running down.

519 Additionally, fructose bisphosphate aldolase (FBA) is a key enzyme involved in
520 glycolysis, gluconeogenesis, and CBB cycle. Fe stress can shift a pairwise substitution of class I
521 FBA for class II FBA (*Lommer et al., 2012*). Aerosol addition resulted in a decrease from 1.7 to

0.6 in the ratio of class II/class I FBA (*Cyanobacteria* removed), corroborating the aggravation of Fe limitation (Table S7).

Stress responses

Among the most abundant categories were chaperones (*DnaJ* and *DnaK*) and proteases (ATP-dependent *clp* protease) (Fig. 6A and Table S8), corroborating prior reports on marine bacterioplankton (*Vila-Costa et al., 2010*). Some genes involved in the defense against oxidative stress were found with a significant increase in the aerosol treatment, including twitching motility protein (*pilT*), sarcosine oxidase (*soxA*), glutamate cysteine ligase (*gcl*), phosphomannomutase (*algC*), RNA polymerase sigma factor *rpoH*, NADH pyrophosphatase (*nudC*), rubrerythrin (*Rbr*), Fe-S oxidoreductase-like protein in the rubrerythrin cluster (*glpC*), and various polyol ABC transporter (*smoK*) (Fig. 6A and Table S8), indicating elevated oxidative stress. Oxidative stress by heavy metals from aerosols has suggested to impact on marine microorganisms (*Mann et al., 2002; Paytan et al., 2009; Jordi et al., 2012*). An increase in *smoK* (Fig. 6A and Table S8) reflects oxidative stress caused by selenate and/or selenite (*Bebien et al., 2001*).

Toxicity imposed by heavy metals such as Co, Zn and Cd was evidenced by an increase in resistance genes including *czcB*, *czcB*, *czcA* and a probable Co/Zn/Cd efflux system membrane fusion protein (Fig. 6B and Table S9), which are associated with an active efflux of heavy metals. The expression of *czcA* is induced by Co, Zn and Cd (*Große et al., 1999*). Cadmium can be used to replace Zn as a metal centre in Zn proteins or in Cd-specific proteins replacing Zn proteins (*Lee & Morel, 1995*). However, it is one of the most toxic metals at a high concentration, inhibiting phytoplankton growth (*Payne & Price, 1999; Miao, Wang & Juneau,*

545 *2005; Quan et al., 2016*). It is suggested that Cd from atmospheric deposition is involved in
546 phytoplankton community succession due to the varying sensitivity of phytoplankton species
547 (*Quan et al., 2016*). Zinc may inhibiting cell growth rate by affecting the photosynthetic electron
548 transport chain (*Miao, Wang & Juneau, 2005*). While Co is an essential component of vitamin
549 B_{12}, excess Co has been shown to inhibit chlorophyll biosynthesis (*Csatorday, Gombos &*
550 *Szalontai, 1984*). Metal toxicity has been reported on a range of marine phytoplankton,
551 butpicophytoplankton, such as *Prochlorococcus* and *Synechococcus*, are the most sensitive
552 (*Payne & Price, 1999; Miao, Wang & Juneau, 2005; Quan et al., 2016*). It is suggested that this
553 is due to the higher surface-to-volume ratio and thus a higher capability for metal incorporation
554 (*Miao, Wang & Juneau, 2005*). Results of gene expression, together with the sharp decrease in
555 the abundance of picocyanobacteria, especially *Prochlorococcus*, in aerosol amended mesocosm
556 (Fig. 1) suggest that heavy metals, such as Co, Zn and Cd, from atmospheric deposition could
557 impose a toxic effect on the bacterial community. This result is consistent with increased protein
558 disfolding (Fig. 4A and Table S3) and oxidative stress (Fig. 6A and Table S8). In addition, we
559 cannot rule out toxicity imposed by other heavy metals, such as Pb, which is also a major
560 component of our aerosol sample (Table 2). Although not measured in this study, Cu is expected
561 to be a major component of the aerosol sample according to our previous studies (*Guo et al.,*
562 *2012*). It is estimated that the concentration of Cu added to the incubation was ~0.9 μg per mg of
563 Chl *a*, lying within the threshold concentration of toxicity (*Paytan et al., 2009*). Aerosol
564 deposition has been suggested to be toxic to phytoplankton owing to Cu concentrations (*Paytan*
565 *et al., 2009; Jordi et al., 2012*). Cu toxicity to *Prochlorococcus* and *Synechococcus* has been
566 found in natural communities (*Mann et al., 2002; Hill, Zubkov & Purdie, 2010*). Unexpectedly,
567 genes associated with Cu homeostasis exhibited no significant changes, except that the blue

copper oxidase gene *cueO* decreased (Fig. 6B and Table S9), indicating no significant Cu toxicity. This could be because of the slow dissolution rate (*Mackey et al., 2015*) and/or scavenging processes.

Also significantly increased were transcripts corresponding to resistance to antibiotics (beta-lactamase; DNA gyrase, *gyrB*; acriflavin resistance protein) and involved in the expression of virulence factors (glycerol-3-phosphate acyltransferase, *GPAT*; succinate dehydrogenase flavoprotein subunit, *sdhA*; DNA-directed RNA polymerase beta' subunit, *rpoC*; translation elongation factor G) (Fig. 6B and Table S9). This result suggests stimulated interspecies competition and interactions under a stressful condition, consistent with the overrepresentation of the widespread colonization island (Fig. 4A).

Respiration

Bacterial respiration appeared to be stimulated as we observed a significant increase in aerobic respiratory complexes, I (NADH-ubiquinone oxidoreductase), II (succinate dehydrogenase), III (ubiquinol: cytochrome c oxidase), and IV (cytochrome c oxidase) (Fig. 7A and Table S10). A pronounced stimulation of bacterial respiration by dust deposition has been well reported (*Pulido-Villena, Wagener & Guieu, 2008; Lekunberri et al., 2010; Pulido-Villena et al., 2014*) and suggested due to both direct and indirect effects of dusts amendments (*Pulido-Villena et al., 2014*). In this study, bacterial respiration seems more stimulated directly and less indirectly (as discussed in carbohydrate metabolism). In addition, respiration would be stimulated for the maintenance of basic cellular machineries under oxidative stress and heavy metal toxicity. An increase was also seem in categories such as anaerobic respiration, mainly sulfate reduction, and Na^+-translocating NADH-quinone oxidoreductase (Fig. 7A and Table

591 S10), involved in an energy-saving way of flagellar rotation, nutrient transport and growth
592 (*Barquera, 2014*), indicating a shift in the bacterial community to a facultative anaerobic and
593 energy-conserving lifestyle when external DOC concentration is reduced.

594

595 **Motility and chemotaxis**

596 Significant overexpression was found in extensive genes related to motility and
597 chemotaxis, including gliding (*pilT*), flagellin protein (*flaG*), flagellar motor switch proteins
598 (*fliM* and *fliN*), flagellar basal-body rod protein (*flgB*), flagellar hook (*flgE*) and hook-associated
599 proteins (*flgK* and *fliD*), and flagellar biosynthesis protein (*flhB*). In addition, the flagellar sensor
600 histidine kinase (*fleS*) and flagellar synthesis regulator (*fleN*) were also significantly enriched as
601 were some other chemotaxis regulator components (*cheY* and *cheW*) (Fig. 7B and Table S11).
602 Consistent with a similar increase observed after DOC addition (*McCarren et al., 2010;*
603 *Martínez-García et al., 2013*) and a phytoplankton bloom (*Rinta-Kanto et al., 2012*), this result
604 indicates an adaptive lifestyle to a changing environment of increased nutrients from aerosol
605 addition (Fig. 4A and C and Tables 2, S3 and S5), reduced organic nutrients due to the
606 senescence of cyanobacterial cells (Fig. 5 and Table S7), and stressful conditions (Fig. 6A and B
607 and Tables S8 and 10). Interesting, also significant was the increase of a negative regulator of
608 flagellin synthesis (*flgM*) (Fig. 7B and Table S11), which prevents the expression of flagellin
609 genes when basal body, hook, or switch proteins are defective (*Gillen & Hughes, 1991*), which
610 may be caused by deteriorating Fe limitation (*McCarter & Silverman, 1989*), oxidative stress or
611 heavy metal toxicity.

612

Functions of *Archaea* and *Virus*

Only highly expressed archaeal and viral genes were discussed to gain insights into the ecological functions. *Archaea* exhibited both positive and negative responses to aerosol addition. Transcripts that were found to increase fell into categories including TCA (succinyl-CoA ligase, *SUCLA2*), glycolysis and gluconeogenesis (phosphoenolpyruvate synthase, *ppsA*), cell division (cell division protein, *ftsZ*), and metabolisms of fatty acids (aldehyde dehydrogenase B, *aldB*) and nucleotides (*de novo* purine biosynthesis and pyrimidine conversion) (Table S12). An increase was also found in oxygen response (thioredoxin reductase, *TrxR*). On the other hand, cell wall (peptidoglycan biosynthesis, *mraY*) and protein metabolism (translation elongation factor 2) were found down-represented (Table S12).

Viral transcripts were mainly associated with P acquisition (*pstS*), PS II (*psbAD*), heat shock protein 60 family chaperone GroEL, and an iron-sulfur cluster assembly protein *sufB* (involved in electron transfer under stress) (Table S13), indicating active involvement of viruses in vital microbial functional activities. Abundant genes also included major capsid proteins, ribonucleotide reductase and guanosine monophosphate synthetase (Table S13). This high abundance of viral structural genes and genes associated with nucleotide metabolism suggests active viral production, likely leading to cell lysis of host cells. This active viral lysis could be induced by pseudolysogeny, which may resolve into active production of viruses upon nutrient replenishment (*Ripp & Miller, 1997*). In this case nutrients can be introduced directly from aerosol addition and indirectly from exudates from senescent cyanobacterial cells.

Conclusions

This paper provides the first comprehensive experimental metatranscriptomic analysis of the effects of atmospheric deposition on the prokaryotic community in the open ocean, demonstrating a clear response in metabolic pathways. In general, both positive and negative responses were evident. This antagonistic effects may be one reason explaining the decoupling of bacterial abundance and metabolism under the influence of atmospheric deposition. The transcriptional profile implies an enrichment in a range of inorganic and organic nutrients, including amino acids, phosphorus, and various carbohydrates, which stimulated metabolisms, such as TCA, PPP, glycolysis and gluconeogenesis. Nutrients might be derived from both direct aerosol addition and exudation from senescent cyanobacterial and eukaryotic phytoplankton. Contrary to the main Fe fertilization scenario, Fe limitation was strengthened, probably through scavenging via sinking particles and cellular exudates. Negative effects included decreased sugar utilization and increased oxidative stress and heavy metal toxicity. All of these environmental changes lead the prokaryotic community to an energy-conserving lifestyle and promoted motility, chemotaxis and interspecies competition and interaction.

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655

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Data Accessibility

All raw sequence data used in this study have been deposited in the GenBank through the sequence read archive and can be retrieved under the following accession number PRJNA371359.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Figure 1(on next page)

Cell abundance

Figure 1 Abundances of heterotrophic prokaryotes, *Prochlorococcus* and *Synechococcus*. Error bars represent the standard deviation of the measurements. Different letters on the bars indicate statistical differences among the treatments ($P < 0.05$) while the same letters indicate there are no statistical differences ($P > 0.05$).

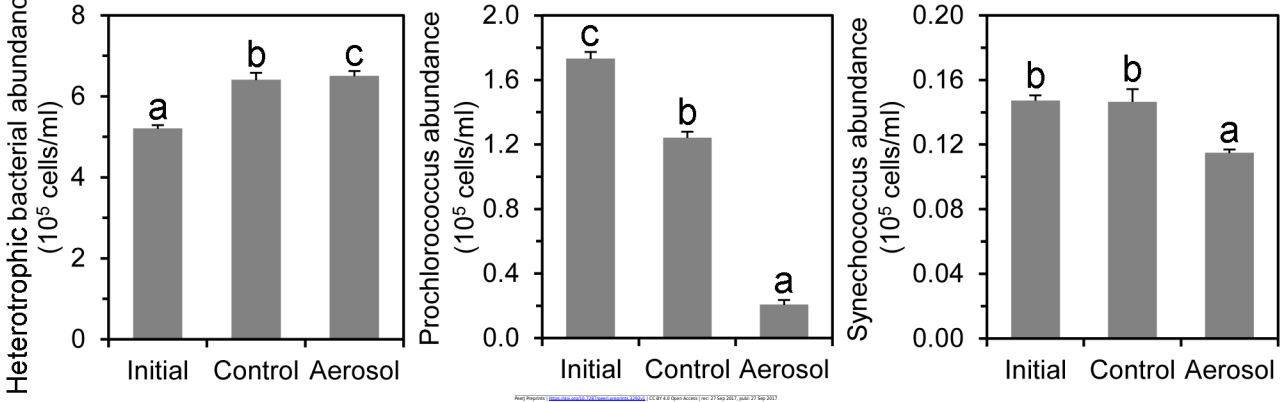

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Figure 2(on next page)

Taxonomic profile of the microbial community.

Figure 2 Taxonomic profile of the microbial community. Overview of sequences from taxonomic domains (A). Taxonomic classification of bacterial phyla (proteobacterial classes) representing >1% of total reads in at least one dataset (B), all archaeal phyla (C) and viral families using mRNA reads (D).

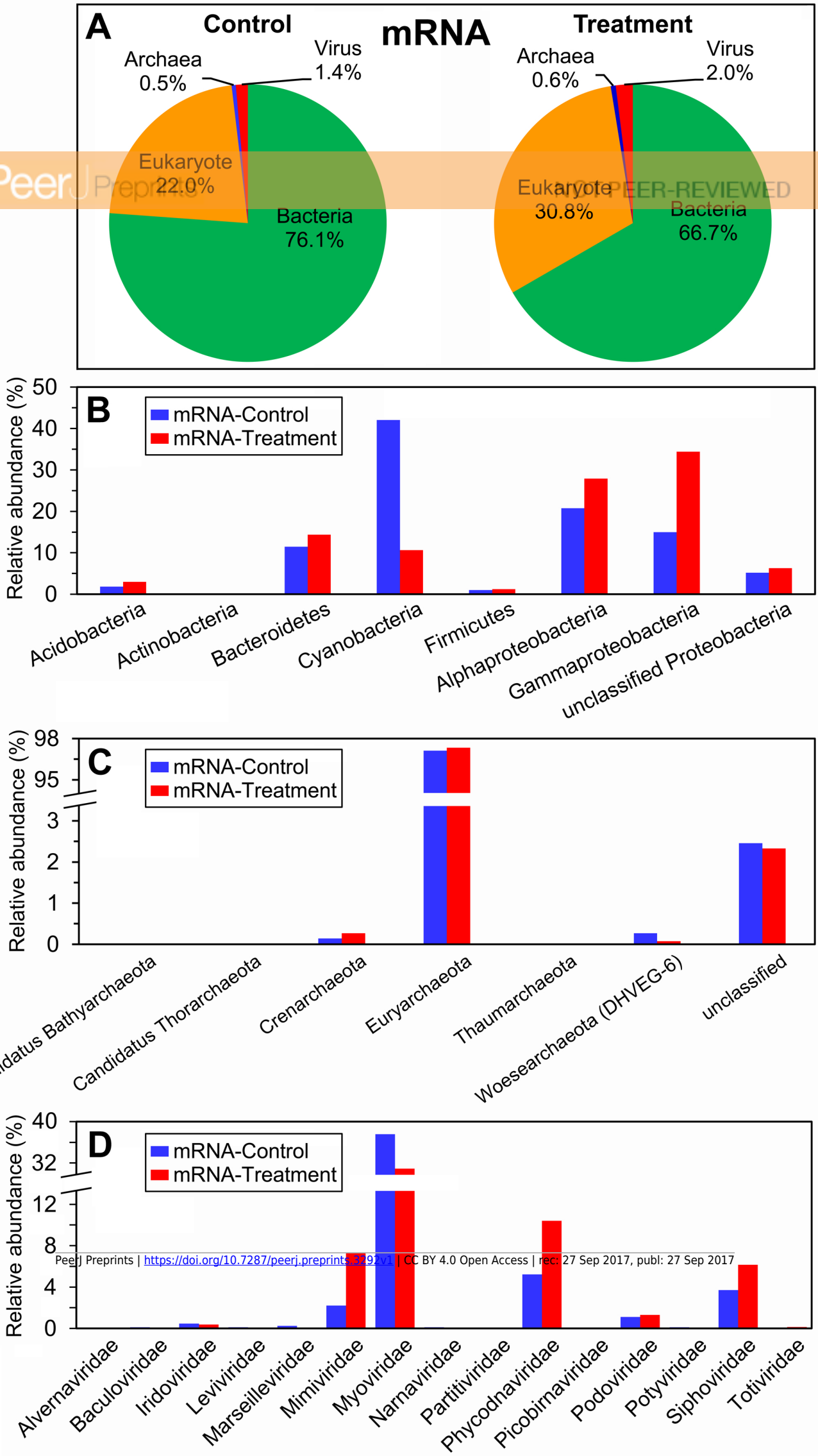

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Figure 3(on next page)

Significantly expressed functional profiles.

Figure 3 Significantly (*p*-corrected value <0.05) expressed functional profiles, SEED subsystems, of the bacterial community. The subsystems are sorted from top to bottom based on high to low relative abundance.

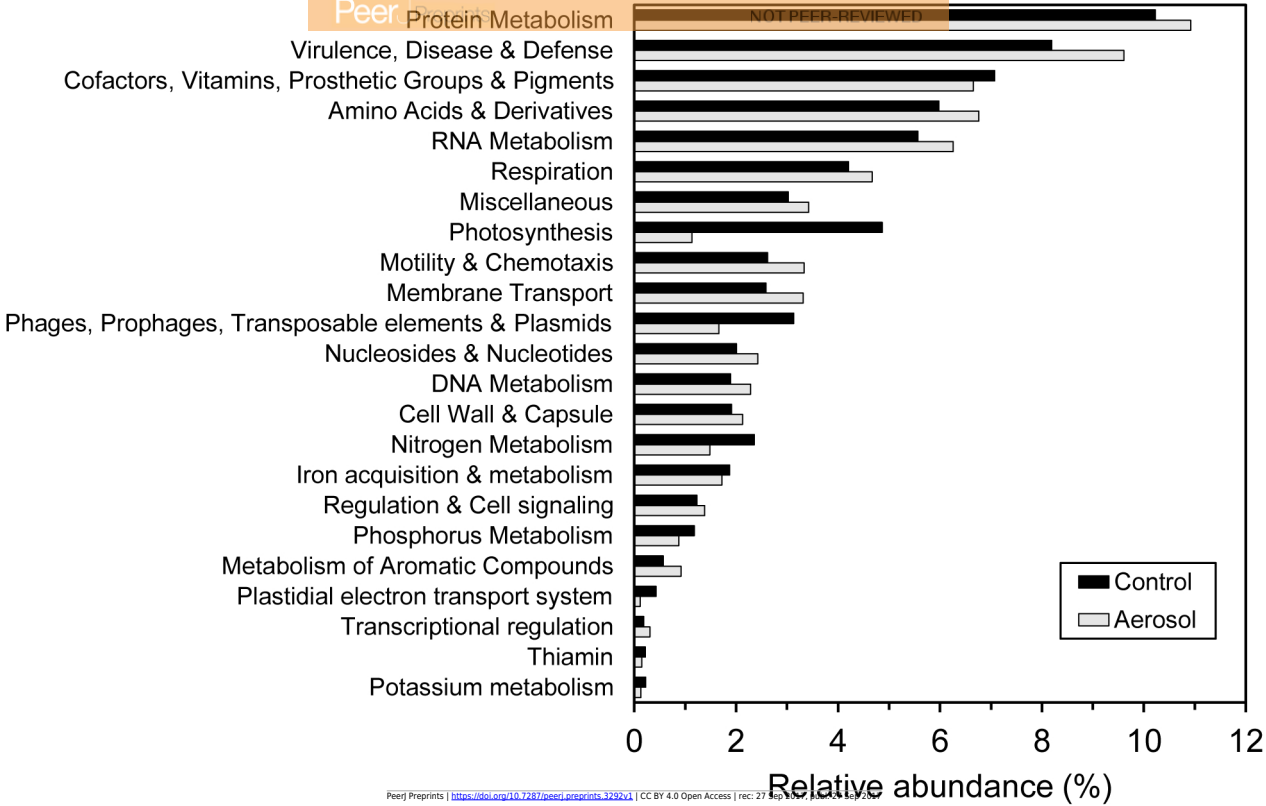

Figure 4(on next page)

Significantly expressed SEED subsystems in membrane transporters, iron acquisition and metabolism, phosphorus metabolism, and nitrogen metabolism.

Figure 4 Significantly (*p*-corrected value <0.05) expressed SEED subsystems in membrane transporters (A), iron acquisition and metabolism (B), phosphorus metabolism (C), and nitrogen metabolism (D). The subsystems are sorted from top to bottom based on high to low relative abundance.

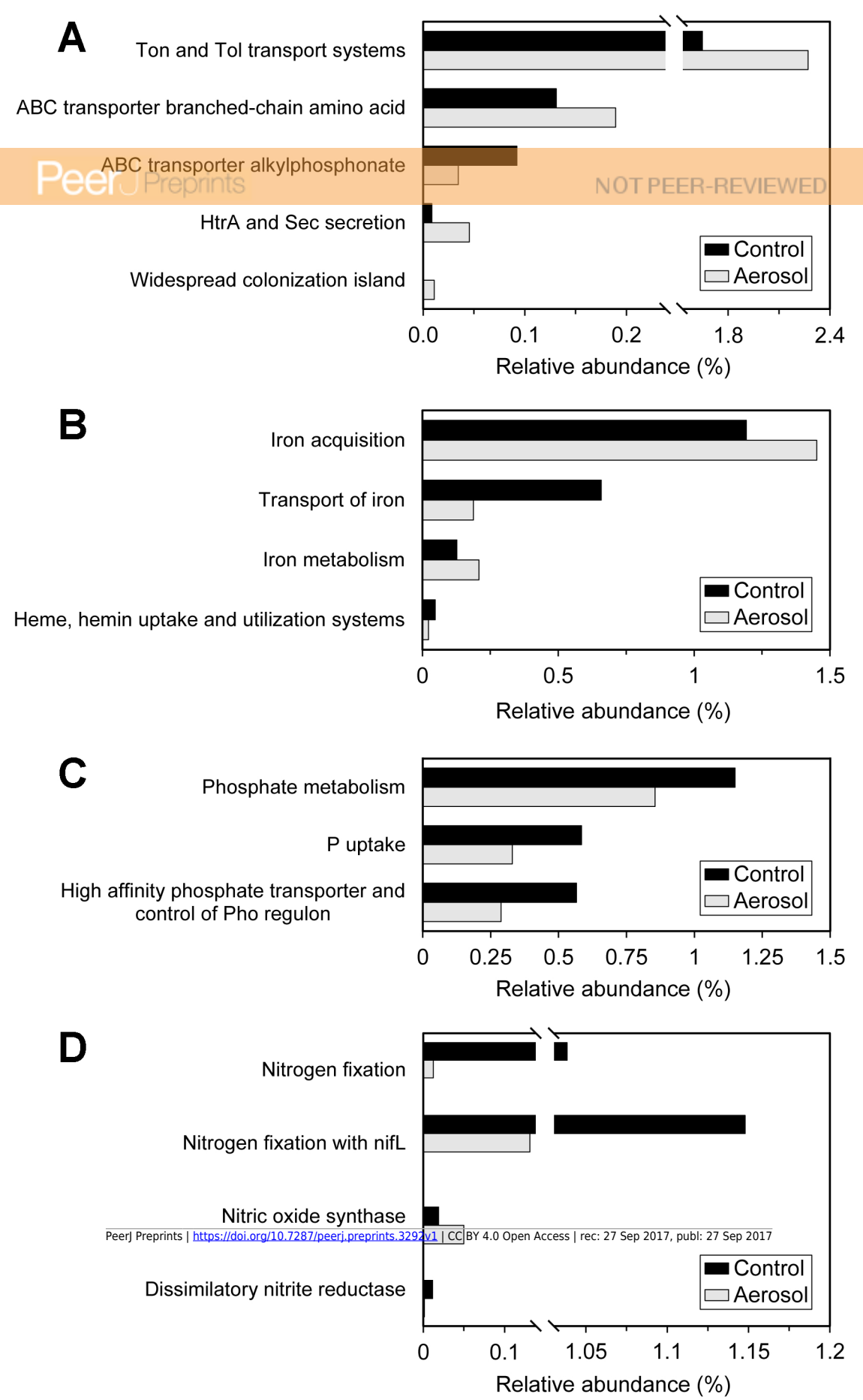

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Figure 5(on next page)

Significantly expressed subsystems in carbohydrate metabolism.

Figure 5 Significantly (*p*-corrected value <0.05) expressed SEED subsystems in carbohydrate metabolism. The subsystems are sorted from top to bottom based on high to low relative abundance.

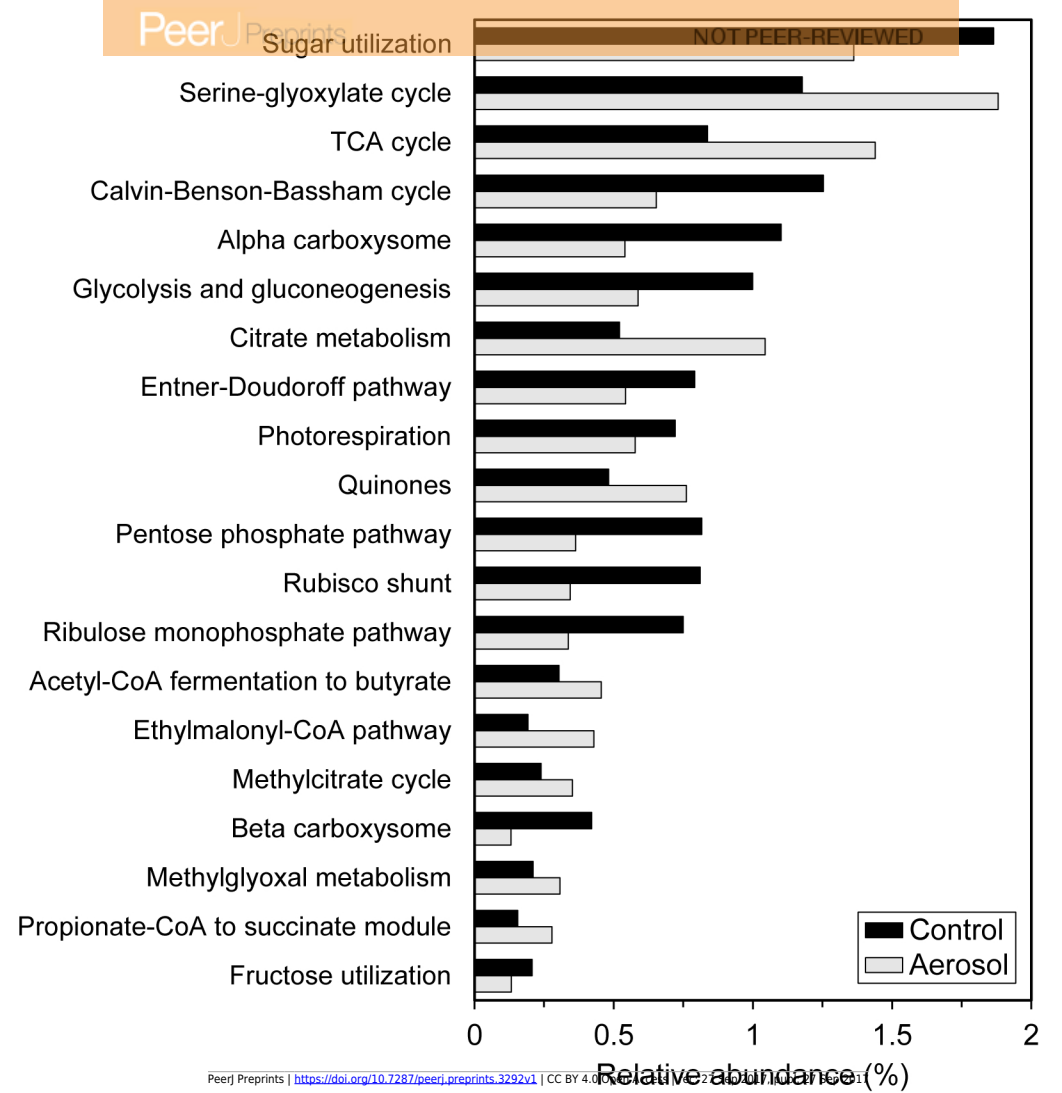

Figure 6(on next page)

Significantly expressed SEED subsystems in stress response and virulence.

Figure 6 Significantly (*p*-corrected value <0.05) expressed SEED subsystems in stress response (A) and virulence (B). The subsystems are sorted from top to bottom based on high to low relative abundance.

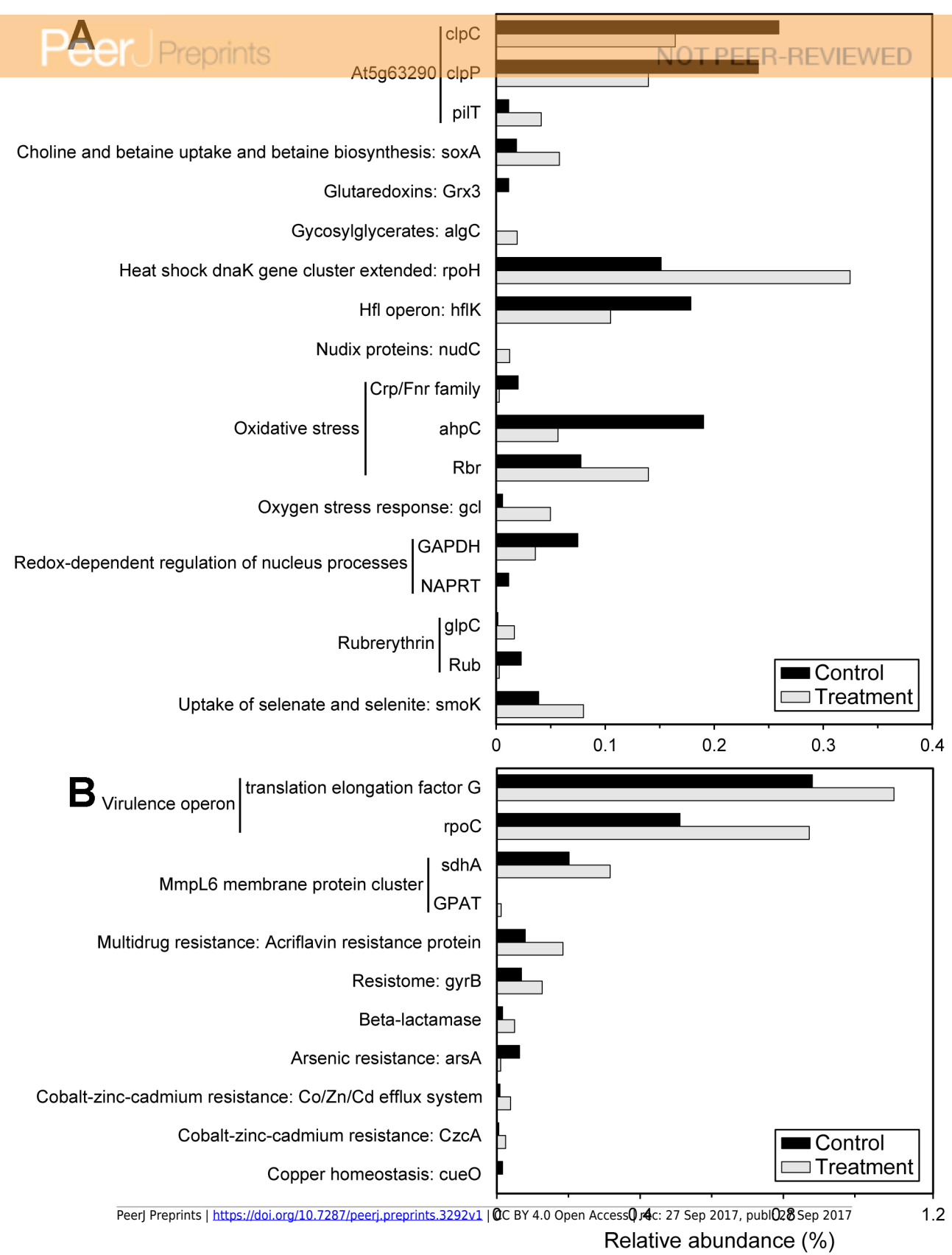

Figure 7 (on next page)

Significantly expressed subsystems in respiration and motility and chemotaxis.

Figure 7 Significantly (*p*-corrected value <0.05) expressed SEED subsystems in respiration (A) and motility and chemotaxis (B). The subsystems are sorted from top to bottom based on high to low relative abundance.

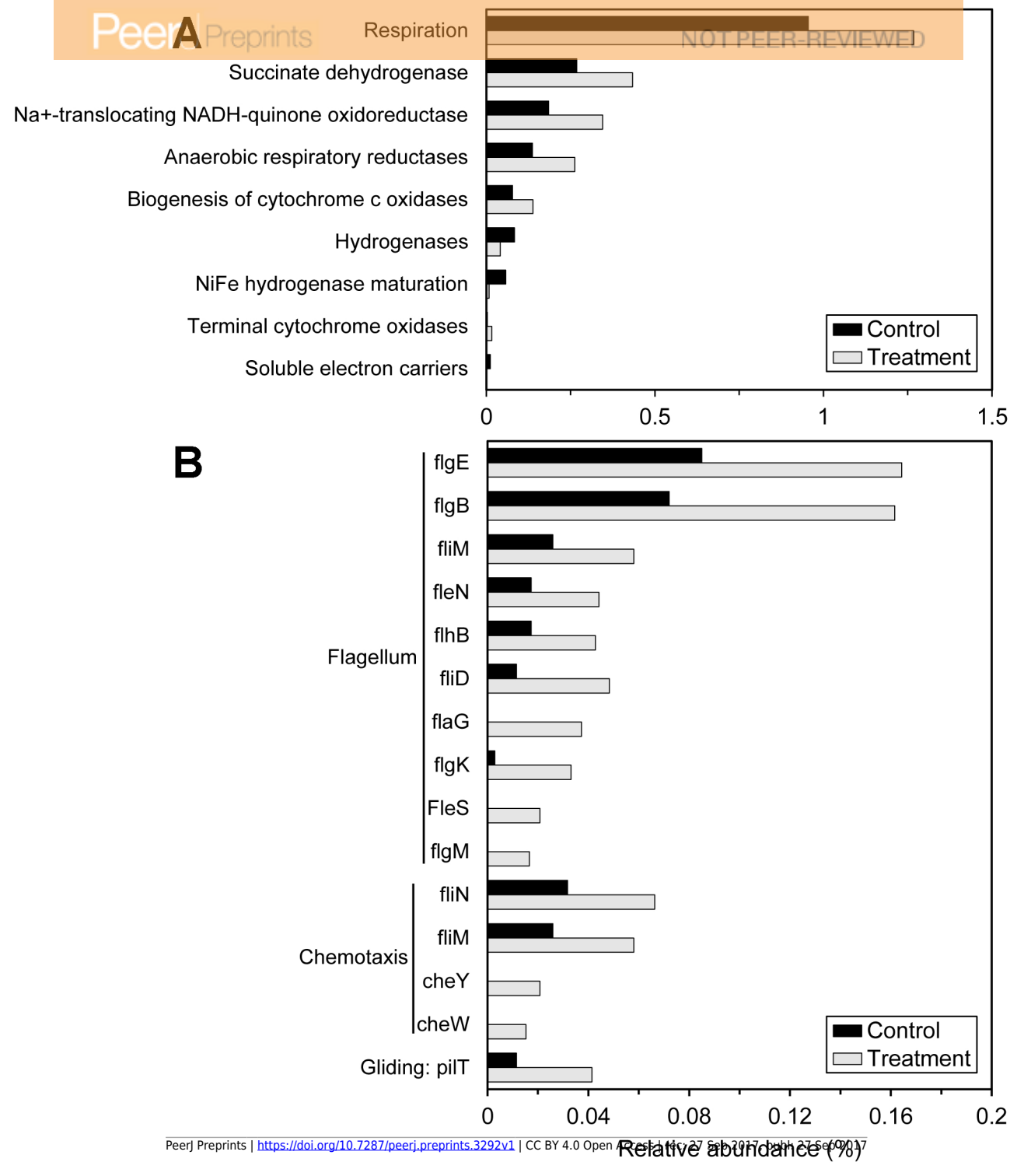

Table 1(on next page)

The initial chemical properties of the surface water.

Table 1 The initial chemical properties of the surface water used in the incubation experiment.

1 Table 1 The initial chemical properties of the surface water used in the incubation experiment.

Temp. (℃)	Chlorophyll a (μg/L)	Salinity	DO (μM)	NO_3^-+NO_2^- (μM)	PO_4^{3-} (μM)	Mn (nM)	Fe (nM)	Co (nM)	Ni (nM)	Cu (nM)
29.30	0.16	34.29	215.49	0.13	0.01	2.321	0.355	0.006	1.963	0.745

2

Table 2(on next page)

Mass percentage of the aerosol components.

Table 2 Mass percentage of the aerosol components in every gram of aerosol samples. OC: organic carbon; EC: elemental carbon.

1 Table 2 Mass percentage of the aerosol components in every gram of aerosol samples. OC: organic carbon; EC: elemental carbon.

Species	NH_4^+	NO_3^-+NO_2^-	OC	EC	SO_4^{2-}	Na^+	K^+	Cl^+	Fe	Ca^{2+}	Zn	Ti	Mn	Pb	V
%	6.1	4.4	9.3	1.0	15.4	0.393	0.568	0.249	0.432	0.273	0.307	0.040	0.027	0.071	0.010

2