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

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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 bacterial responses to atmospheric deposition are being studied at the community level, corresponding functional changes are essentially unknown. Here we conducted a microcosm experiment coupled with metatranscriptomic analyses to elucidate taxonomic and functional profiles of the bacterial community in response to East Asian aerosols in the Western North Pacific Ocean (WNPO). While the abundance of heterotrophic bacteria showed a minor change, cyanobacterial cell count number decreased dramatically, with *Prochlorococcus* and *Synechococcus* counts reduced by 83.2% and 21.5% in the aerosol treatment in relation to the control. Expression of transcripts related with *Prochlorococcus*, *Synechococcus*, *Trichodesmium* and *Crocosphaera* both were lower in the treatment (5.7%, 2.3%, 0.5% and 0.02%, respectively) than in the control (18.6%, 2.7%, 9.8% and 0.14%, respectively). Aerosol addition led to an increase in transcripts involved in iron metabolism (*tonB*, *feoB*, *irr*, *exbB*), indicating Fe limitation. Heavy metal toxicity was evidenced by an elevated expression of resistance genes, such as *czcC*, *czcB*, *czcA* and a probable Co/Zn/Cd efflux protein, and a range of genes functioning against oxidative stress. Our findings provide insights into an inhibitory effect of high-flux East Asian aerosols on cyanobacteria in the WNPO likely due to Fe scavenging and heavy metal toxicity.

1. Introduction

Among the factors controlling microbial abundance, activity and community composition in the ocean, nutrient availability has been recognized as one of the most important (Arrigo, 2005). Atmospheric deposition is increasingly being considered as an important source of nutrients to the open ocean (Duce et al., 2008). Experimental and field studies have been conducted to demonstrate the fertilizing effects of aerosol deposition on phytoplankton, as well as the dynamics of microbial food web and carbon flux (Bonnet et al., 2005; Herut et al., 2005; Marañén et al., 2010; Guo et al., 2012). Bacteria are a critical component of the microbial food web with cyanobacteria performing as the main primary producer and heterotrophic bacteria involved in the remineralization of elements and conversion of inorganic nutrients and dissolved organic matters into biomass (Azam et al., 1983). However, to date, only a few studies have attempted to address the effects of atmospheric deposition on bacteria. Generally, while bacterial metabolism such as production and respiration show a marked response, bacterial abundance remains relatively unchanged (Lekunberri et al., 2010; Marañén et al., 2010; Guo et al., 2013; Pulido-Villena et al., 2014; Guo et al., 2016). Similarly, RNA-based community profiling shows that the metabolically active bacteria are more sensitive than the total community in response to aerosol addition (Van Wambeke et al., 2009; Laghdass et al., 2011; Guo et al., 2016). Therefore, more insights could be gained into the functional changes and their resulting ecological impacts if looking at the RNA pool owing to its fast response to environmental perturbations and the close reflection of cellular metabolic activities.

Every year large quantities of aerosols from East Asia are transported eastward and spread out in the Western North Pacific Ocean (WNPO). Anthropogenic pollutants are picked up when they cross over industrial regions (Young et al., 1991; Zhang et al., 1993; Kim et al., 2014a; Martino et al., 2014). In summer seasons, the WNPO is characterized by stratification and a low primary production. Nutrients are rarely supplied by water column mixing. Atmospheric input represents the dominant nutrient source (Duce et al., 2008; Kim et al., 2014b; Martino et al., 2014). Therefore, this season of the year is an ideal time to study the biogeochemical effects of atmospheric depositions on the surface water.

Metatranscriptomics involves the isolation and sequencing of environmental mRNAs from a complex of microbial assemblages, providing extensive information on both taxonomic affiliation and functions. We reported here results from an on-board aerosol addition microcosm experiment conducted during a cruise to the WNPO in July 2013. We applied metatranscriptomic approaches to elucidate the taxonomic and functional profiles of the bacterial community in response to atmospheric deposition. This study provides valuable information on the dominant metabolic processes and changes in

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biogeochemistry-related processes after atmospheric deposition.

2. Materials & methods

2.1. Aerosol sample

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. 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 °C. The aerosol composition was measured as described (Guo et al., 2012).

2.2. Experimental setup for incubation

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. 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). Seawater samples were also collected for the measurement of the concentration of major nutrients and dissolved trace metals. Nutrient samples were frozen in liquid N_2 and brought back to our land-based laboratory for further processing. The concentration of major nutrients was determined by standard methods adapted for a flow injection analyzer (Pai et al., 1990). Dissolved trace metal concentrations were measured by using the chelating resin pre-concentration method and a high resolution ICPMS (Element XR, Thermo Fisher Scientific, USA) (Wang et al., 2014a).

After being pre-filtered through a 200-μm mesh, the seawater was dispensed into 6 acid-washed 20 L transparent polycarbonate carboys. 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 carboys were softly shaken to help aerosols 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, subsampling was conducted for bacterial abundance and total RNA extraction.

2.3. Microbial abundance

1.8 mL seawater was taken from the initial seawater and from each incubation, fixed with 0.5% (final concentration) seawater-buffered paraformaldehyde (pH 7.2), flash frozen by liquid N_2 and stored at −80 °C before analyses. The abundance of total bacteria as well as the picocyanobacteria, *Prochlorococcus* and *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 et al., 1993). Total bacterial abundance was enumerated following the method of Guo et al. (2013). Samples were first stained by 0.01% (final concentration) SYBR Green I for 60 min.

2.4. 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 −80°C 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 the Turbo-DNA Free DNase (Ambion, Austin, TX, USA). Due to insufficient RNA yields, RNA samples from the control and treatment were separately pooled and subjected to sequencing. cDNA synthesis was conducted by using a SMARTer universal low input RNA kit (Takara, Otsu, Japan). Prokaryotic libraries were prepared by using a 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).

2.5. Bioinformatic analyses

Quality check was performed using the FastQC v0.11.3 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were quality trimmed using Trimmomatic (Bolger et al., 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 (Kopylova et al., 2012).

The resulting sequences were mapped using BLASTX against the NCBI nr database (April 28, 2016) with an e-value 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 default settings. 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 and Goto, 2000) functional categories.

Sequences reported here were deposited in the GenBank through the sequence read archive under the accession number PRJNA371359.

2.6. Statistical analyses

Multiple comparisons of cell abundance 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 et al., 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 were significantly upregulated or downregulated in the aerosol treatment, gene counts were first normalized by dividing the gene number of each taxa or functional category by the 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).

3. Results and discussion

3.1. Initial environmental features and aerosol composition

The nutrient concentration in the surface water (10 m depth) used in this experiment was characterized by low nitrogen species ($NO_3^- + NO_2^-$, 0.13 μM) and phosphate (under detection limit, 0.01 μM) (Table 1). The concentrations of dissolved trace metals were similar to what were observed in the North Pacific Ocean (Bruland, 1980). The total dissolved concentrations of Mn, Zn, Cu, Co, Ni, and Cd in the top 200 m of the sampling stations generally ranged from 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 preparation).

The aerosol used in this experiment was composed of a large amount of macronutrients and trace metals. The dominant components were the sulfate, reactive nitrogen (NO_3^-, NO_2^- and NH_4^+) and carbon, constituting 25.0%, 17.1% and 16.7% of the aerosol mass, respectively (Table 2). Trace metals included iron (Fe) (0.432%), zinc

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

Temp. (°C)	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

(Zn) (0.307%) and manganese (Mn) (0.027%). Overall, the aerosol composition was similar to our previous aerosol collections at the same site (Guo et al., 2012). 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 S1), except for a slightly lower concentration of NO^{3-} + NO^{2-} (4.4% in Hong Kong versus 12.5% in Taipei) and Fe (0.432% versus 0.960%) and a minor 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.

3.2. Response of bacterial abundance

Compared to the control (6.4×10^5 cells/mL), heterotrophic bacterial abundance in the aerosol treatment (6.5×10^5 cells/mL) exhibited a minor change though statistically significant ($P < .05$) (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). The abundance of *Prochlorococcus* and *Synechococcus* decreased respectively by 83.2% and 21.5% in the aerosol 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 al., 2013).

3.3. Overview of the metatranscriptomic libraries

A total of 8,295,242 and 9,351,598 reads were generated for the metatranscriptomes of the control and the aerosol treatment, respectively. Of the quality reads, 8.6% (control) and 9.6% (aerosol) of the reads were mRNA, which is not surprising as rRNA depletion was not applied. The percentages of significant hits in the NCBI nr database were 25.6% and 25.9% for the control and aerosol treatment, respectively. Of these reads, 41.7% (control) and 34.6% (aerosol) were mapped to subsystems based on SEED classification (Table S2).

Due to low RNA yields, three replicates for the control and treatment were pooled. So there were no replicates for metatranscriptomic dataset, making it impossible to analyse variance between replicates or for robust statistical analyses of differences between experimental treatment and control. Along with low sequencing depth, we only focus on dominant and apparent changes with certainty, such as the decrease in cyanobacterial expression and functions of iron metabolism and heavy metal toxicity.

3.4. Response of taxonomic composition

Within the mRNA pool, the domain *Bacteria* was dominant in both the control and aerosol treatment. The relative abundance of bacterial sequences was lower in the aerosol treatment than in the control (Fig. 2A). This result corresponds to a decline in cyanobacterial abundance in the aerosol treatment compared to the control (Fig. 1). Among bacterial phyla, sequences from the phylum *Cyanobacteria* showed a significantly ($q < 0.05$) reduced representation in the treatment (10.6%) compared to the control (42.0%) (Fig. 2B). Consistent with flow cytometry results, the relative abundance of sequences belonging to *Prochlorococcus* and *Synechococcus* both were lower in the aerosol treatment (5.7% and 2.3%, respectively) than in the control (18.6% and 2.7%, respectively) (Fig. 2C). Unexpectedly, a decrease was also found in two diazotrophic cyanobacteria, *Trichodesmium* (9.8% and 0.5% in the control and treatment, respectively) and *Crocosphaera* (0.14% and 0.02%) (Fig. 2C), which have been shown to be strongly promoted by dust deposition (e.g., Karl et al., 2002).

3.5. Expression of iron acquisition and metabolism

After aerosol addition, we observed significant changes in 4 subsystems: iron acquisition (by 21.7% increase compared to the control), transport of iron (by 71.5% decrease), iron metabolism (by 64.5% increase), and metabolism of heme and hemin (by 53.5% decrease) (Fig. 3A). The iron acquisition subsystem was dominated by TonB-dependent receptors, which increased from 1.05% in the control to 1.31% in the aerosol treatment in parallel with an increase in transcripts related with the ferrous iron (Fe^{2+}) transport protein B (*feoB*) (0% to 0.005%) (Table S3). TonB is commonly thought to be associated with iron and vitamin uptake (Schauer et al., 2008). The expression of both TonB-dependent receptors for Fe transport (Lim, 2010) and protein FeoB (Rong et al., 2008) have been shown to be induced by Fe limitation. Within the category transport of iron, significantly changed genes were *psbC* (chlorophyll *a*/b binding protein, decreased by 88.3%) and *irr* (iron-induced regulator, increased by 1.5 fold). PsbC protein (CP43) is a component of the photosystem II core complex. The downregulation of *psbC* transcripts is probably because of the decrease of cyanobacteria. Modulated by cellular Fe level, iron-induced regulator (Irr) is degraded when sufficient Fe is available (Hamza et al., 1998). Fe metabolism was dominated by the Fe^{3+} siderophore transport system, *exbB* and *tonB*, which are highly expressed under Fe-limited conditions (Higgs et al., 2002). The expression of *exbB* and *tonB* were induced by aerosol addition with a 4.4-fold and 1.6-fold increase, respectively (Table S3). It has been hypothesized that marine microorganisms make use of siderophores to access Fe (Granger and Price, 1999). Cyanobacterial transcripts (54.1%) constituted a major portion of transcripts involved in heme and hemin metabolism in the control. When cyanobacterial transcripts were removed from analyses, the number of transcripts involved in heme and hemin metabolism was comparable in the control (0.045%) and in the treatment (0.044%) (data not shown). Thus, the decreases of transcripts involved in heme and hemin metabolism could be due to the decline of cyanobacterial cell number.

All the results lead to the proposal that aerosol addition increased the degree of Fe limitation, even though Fe constituted as a major aerosol component (Table 2). Increased Fe limitation was echoed in transcripts involved in carbohydrate metabolism. Fructose bisphosphate aldolase (FBA) is a key enzyme involved in glycolysis,

Table 2
Mass percentage of the aerosol components in every gram of aerosol samples.

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

OC: organic carbon; EC: elemental carbon.

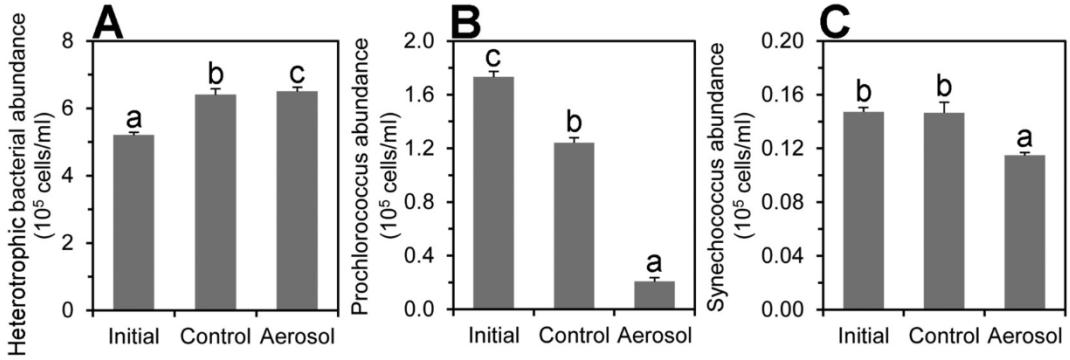

Fig. 1. Abundances of heterotrophic bacteria, *Prochlorococcus* and *Synechococcus*. Error bars represent the standard deviation of the measurements. Different letters on the bars indicate statistical differences between the groups ($P < .05$) while the same letters indicate that there are no statistical differences ($P > .05$).

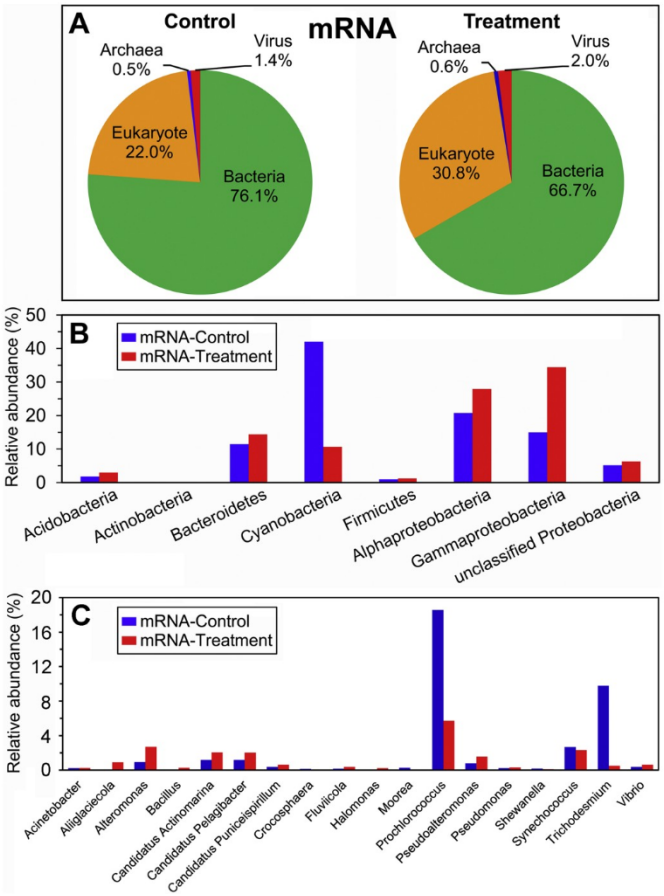

Fig. 2. Taxonomic profile of the microbial community. Overview of sequences from taxonomic domains (A). Taxonomic classification of bacterial phyla (proteobacterial classes) representing > 1% of total reads in at least one dataset (B) and top 10 bacterial genera (C).

gluconeogenesis, and CBB cycle. Fe stress can shift a pairwise substitution of class I 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-related transcripts (*Cyanobacteria* removed) (Table S4). Increased Fe limitation is against the paradigm of Fe fertilization from atmospheric deposition to the world ocean (Duce and Tindale, 1991; Jickells et al., 2005). Similar with our results, mesocosm experiments in an oligotrophic environment showed that dust addition resulted in a decrease in dissolved Fe concentration, which was suggested due to possible scavenging by fast-sinking particles and organic matter 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 et al., 2004). Aerosol was added to represent a high-flux event in our study, and our results were consistent with these earlier findings. The majority (> 99.9%) of dissolved Fe in the surface ocean exists in a non-bioavailable form, chelated by organic ligands (Granger and Price, 1999). Senescence of cyanobacteria may lead to the release of organic matter-rich cellular contents. Organic matter (9.3% by mass) was major aerosol chemical component. Thus, both particle and organic matter scavenging may possibly be a reason for increased Fe limitation. Diazotrophs have a high requirement for Fe due to an additional demand of Fe as a component in the nitrogenase enzyme complex. Thus, Fe limitation may be one of the reasons for the dramatic reduced representation of cyanobacterial taxa, particularly for *Trichodesmium* and *Crocosphaera*. It should be pointed out that the decline of *Trichodesmium* must be interpreted with caution because they are easily subjected to sampling bias owing to the filamentous and colony-forming features.

3.6. Expression of stress responses

Toxicity imposed by heavy metals such as Co, Zn and/or Cd was evidenced by an increased expression of resistance genes including *czcD* (by 0.003%), *czcB* (by 0.003%), *czcA* (by 0.02%) and a probable Co/Zn/Cd efflux protein (by 0.03%) (Fig. 3B and Table S5), 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 is one of the most toxic metals at a high concentration, inhibiting phytoplankton growth (Payne and Price, 1999; Miao et al., 2005; Quan et al., 2016). It is suggested that Cd from atmospheric deposition is involved in phytoplankton community succession due to the varying sensitivity of phytoplankton species (Quan et al., 2016). Zinc may inhibiting cell growth rate by affecting the photosynthetic electron transport chain (Miao et al., 2005). While Co is an essential component of vitamin B_{12}, excess Co has been shown to inhibit chlorophyll biosynthesis (Csatorday et al., 1984). Metal toxicity has been reported on a range of marine phytoplankton. Picophytoplankton, such as *Prochlorococcus* and *Synechococcus*, are the most sensitive ones (Payne and Price, 1999; Miao et al., 2005; Quan et al., 2016). It is suggested that this is due to the higher surface-to-volume ratio and thus a higher capability for metal incorporation (Miao et al., 2005). Additionally, diazotrophic cyanobacteria, such as *Trichodesmium* and *Crocosphaera*, have been shown to be sensitive to toxicity of heavy metals, such as Cu, in some cases even more sensitive than picocyanobacteria (Lopez et al., 2019).

Heavy metal toxicity is supported by a significant elevated representation of genes related with defense against oxidative stress, including twitching motility protein (*pilT*, by 0.03%), sarcosine oxidase (*soxA*, by 0.04%), glutamate cysteine ligase (*gcl*, by 0.04%), phosphomannomutase (*algC*, by 0.02%), RNA polymerase sigma factor *rpoH* (by 0.17%), NADH pyrophosphatase (*nudC*, 0.01%), rubrerythrin (*rbr*, 0.06%), Fe–S oxidoreductase-like protein in the rubrerythrin cluster

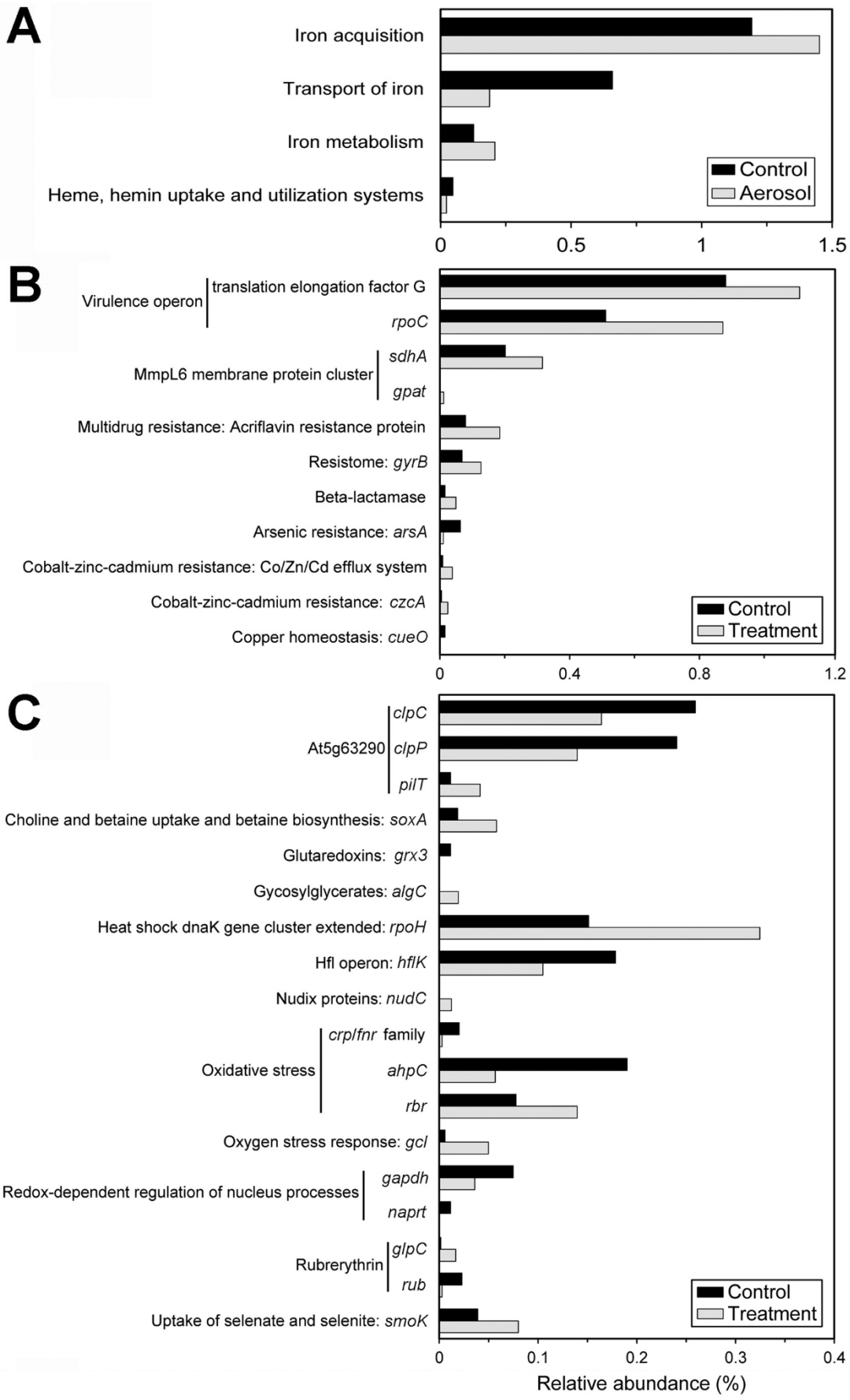

Fig. 3. Significantly (p-corrected value < 0.05) expressed SEED subsystems in iron acquisition and metabolism (A), virulence (B) and stress response (C).

(*glpC*, by 0.02%), and various polyol ABC transporter (e.g., *smoK*, by 0.04%) (Fig. 3C and Table S6). The expression of these genes were suggested to be induced by diverse environmental stresses, such as oxidation and heavy metal stresses (Huang et al., 2014; Wang et al., 2014b; Coolen and Orsi, 2015). Oxidative stress by heavy metals from aerosols has been suggested to inhibit phytoplankton growth in oceanic regions, such as the Sargasso Sea, the Red Sea and the Mediterranean Sea (Mann et al., 2002; Paytan et al., 2009; Jordi et al., 2012).

Results of gene expression, together with the sharp decline in the abundance of picocyanobacteria, particularly *Prochlorococcus*, in the aerosol amended microcosm (Fig. 1), suggest that heavy metals, such as Co, Zn and Cd, from atmospheric deposition could impose a toxic effect on the bacterial community. In addition, we cannot rule out toxicity imposed by other heavy metals, such as Pb, which is also a major component of our aerosol sample (Table 2). Although not measured in this study, Cu is expected to be a major component of the aerosol sample according to our previous studies (Guo et al., 2012). Aerosol deposition has been suggested to be toxic to phytoplankton owing to Cu concentrations (Paytan et al., 2009; Jordi et al., 2012). Unexpectedly, genes associated with Cu homeostasis exhibited no significant changes, except that the blue copper oxidase gene *cueO* decreased from 0.16% in the control to 0.001% in the treatment (Fig. 2B and Table S5), indicating no significant Cu toxicity. This could be because of the slow dissolution rate (Mackey et al., 2015) and/or scavenging processes.

4. Conclusions

This paper provides the first experimental metatranscriptomic analysis of the effects of atmospheric deposition on the bacterial community in the open ocean. We found East Asian aerosol at a high flux could impose an inhibitory effect on cyanobacteria, particularly *Prochlorococcus*, *Synechococcus*, *Trichodesmium* and *Crocosphaera*, in terms of cell abundance and gene expression. Functional analyses based on metatranscriptome showed that the negative impact is likely to be a joint effect of Fe limitation and heavy metal toxicity. Fe scavenging by aerosol addition is against the paradigm of Fe fertilization from atmospheric deposition to the world ocean. Due attention should be paid to the unique features of aerosols from the East Asia, where there is a number of fast-developing countries with an increasing emission of a large quantity of anthropogenic pollutants.

5. Compliance with ethical standards

The authors declare that there is no conflict of interest.

This study involved no human participants or animals. No field permit was required to access the field sites.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margen.2019.01.008.

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