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Characteristics of bacterial community in fog water at Mt. Tai: similarity and disparity under polluted and non-polluted fog episodes

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Abstract:

Bacteria, widely distributed in atmospheric bioaerosols, are indispensable components in fog/clouds and play an important role in atmospheric hydrological cycle. However, limited acknowledge is acquired about bacterial community structure and function, especially for the increasing air pollution events in North China Plain. Here we presented a comprehensive characterization of bacterial community composition and function, variation and environmental influence on fog water collected at Mt. Tai under polluted and non-polluted fog episodes from 24 Jul to 23 Aug 2014. Using the Miseq 16S rRNA gene sequencing, the facts that fog water harbored a highly diverse bacterial community and the predominant phyla of Proteobacteria, Bacteroidetes, Cyanobacteria and Firmicutes were investigated. The presence of bacterial taxa survived in low temperature, radiation and poor nutrients conditions were encountered in fog water, suggesting the well adaption to extreme environment. Bacterial gene

functions predicted from 16S rRNA gene using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) suggested the pathways relating to metabolism and disease infections are significantly correlated to the predominant genera. The abundant genera *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas*, and *Empedobacter* originated from a wide range of habitat included cloud condensation nuclei and ice nuclei active species, opportunistic pathogenic and functional species, suggesting the bacterial ecological and healthy importance in fog water should be concerned. Clustering analysis including hierarchical cluster (Hcluster) and principal coordinate analysis (PCoA) indicated a significant disparity between polluted and non-polluted samples. Linear discriminant analysis effect size (LEfSe) demonstrated that the polluted fog samples were enriched with potential pathogens. The non-polluted samples had more diverse ecological function groups. Community structure discrepant performed by redundancy analysis (RDA) indicated $PM_{2.5}$ have negative impact on bacteria, playing vital role in shaping microbial community structure. $PM_{2.5}$ was possibly associated with different origins and pathways of air mass using source tracking by the backward trajectory and wind analysis, mainly related to the long-term transport. This work furthered our understanding of bacterial ecological characteristics in the atmospheric aqueous phase, highlighted the potential influence of environmental variables on bacterial community over fog process, which will provide fundamental acquaintance of bacterial community response in fog water under increasing pollution stress.

Key words： fog water, 16r RNA gene, PICRUST, LefSe, $PM_{2.5}$

1. Introduction

Fog is the near-surface cloud and aerosol system compose of tiny droplets suspended in the atmosphere. In the atmosphere, pollutants attached to particles could be dissolved or incorporated into fog droplets, which may induce complex impacts on environment security and human health. Over the past decades, studies on fog/cloud water have mainly focused on the physicochemical properties (Aikawa et al., 2001; Boris et al., 2015; Fernández-González et al., 2014). Recently, with the in-depth understanding of the characteristics of fog, studies on bioaerosols in fog/clouds have been in the ascendant.

Studies have showed that living microorganisms, including bacteria, fungi and yeasts, are present in fog or clouds (Burrows et al., 2009). As the first study on biological particles in fog water, Fuzzi et al (1997) suggest the bacterial replication in foggy days. Afterwards, with the development of detection techniques, microorganisms in fog/cloud water have been systematically studied (Amato et al., 2007c; Delort et al., 2010; Vaïtilingom et al., 2012). Combined with the field investigations and lab experiments, diverse bacterial communities are retrieved, and the bacterial metabolism active in fog/cloud water are further demonstrated. In atmospheric aqueous phase, microorganisms can act as cloud condensation nuclei (CCN) and ice nuclei (IN), which have potential impact on cloud formation and precipitation processes (Amato et al., 2015; Bauer et al., 2003; Mortazavi et al., 2015). Moreover, microorganisms in fog/cloud water are available to metabolize organic carbon compounds (degrading organic acids, formate, acetate, lactate, succinate), associate with carbon recycling (Amato et al., 2007a; Vaïtilingom et al., 2010) and influence photochemical chemical reactions (Vaïtilingom et al., 2013), involve in the nitrogen cycling (mineralization and nitrification) (Hill et al., 2007), and therefore participate in a series of complex and diverse biochemical metabolic activities.

Fog occurrence is a complex process, in contaminated area, fog typically contains numerous pollutants, e.g., sulfate and nitrate ions, organic carbon compounds, and bacteria (Badarinath et al., 2007; Després et al., 2012; Fernández-González et al., 2014; Mohan & Payra, 2009). Emissions and resuspension of bacteria by wind erosion or splashing water from various terrestrial environments into atmosphere recruit diverse airborne bacteria, which possibly involve opportunistic and functional

bacteria. During fog process, these bacteria attached to particles or incorporated in fog droplets will be deposited back to land via dry or wet deposition, which may induce health risks through microbial pathogens dispersion and potential effect on the diversity and function of aquatic or terrestrial ecosystems. Therefore, to evaluate the potential ecological functional bacteria in fog water have been an urgent issue, especially for the polluted fog episodes.

It is noteworthy that atmospheric microorganisms are subject to a wide range of environmental condition including the meteorological factors and aerosols physiochemical composition (Womack et al., 2010). Community structure and function are closely related to the environmental characteristics in atmosphere and geomorphic characteristics (Dong et al., 2016; Gao et al., 2016). For instance, studies about inhalable bioaerosols in particulate matter suggest environmental parameters including temperature, relative humidity, PM_{10}, $PM_{2.5}$ and particle size have significant impact on the composition and dynamic of microbial communities (Adhikari et al., 2006; Bowers et al., 2013). However, due to the paucity of detailed and comprehensive studies of atmospheric bacterial composition, the understanding of the dynamic of bacterial community remains incomplete, particularly in the North China Plain. As an intensive agricultural and economic region in China, the North China Plain has been suffering serious air pollution in recent years, e.g., the severe fog and haze pollution in Beijing, Ji'nan in January 2013 (Huang et al., 2014; Wang et al., 2014). During polluted fog process, how bacterial community varied and which environmental factor play decisive role in shaping bacterial community structure are still scarcely studied.

In the present work, typical fog episodes under polluted and non-polluted weather were collected on the summit of Mt. Tai in North China Plain. To understand the bacterial community structure and function, the Miseq 16S rRNA gene sequencing was performed, and PICRUSt predictive function was applied to examine the metabolic and ecological function. Analysis of similarities (ANOSIM) and linear discriminant analysis effect size (LEfSe) were executed to clarify the discrepant bacterial taxa. Moreover, RDA analysis was applied to identify the pivotal environmental factor influencing bacterial community. Air mass back trajectory and wind analysis were conducted to definitude the most likely source and transmission paths of pollutants and bacteria.

2. Material and methods

2.1 Sample collection

Fog samples were collected using the Caltech Active Strand Cloud water Collector (CASCC2) with a droplet size cut of 3.5 μm on the summit of Mt. Tai (36°18′ N, 117°13′ E, and 1534 m a.s.l) (Guo et al., 2012). The flow rate was 24.5 m^3 min^{-1} and fog water was collected on the strings flows down to Teflon bottles.

The polluted fog episodes were defined according to the 24 h concentration of WHO air quality guideline (25 μg/m^3) and this standard has been applied by Australia, New Zealand and European Union. During a fog episode, the average $PM_{2.5}$ concentration higher than 25 μg/m^3 was classified as polluted. Seven fog episodes including thirteen samples were detected over the whole sampling period (from 24 July to 23 August 2014), including 10 polluted and 3 non-polluted fog episodes (Table 1). The samples for microbial community investigation were stored with dry ice in transit and then frozen at -80°C in laboratory until further analysis.

In fog water, the pH and conductivity was detected with a Multi pH/COND/TEMP 6350 hand held Meter immediately after sampling. Hourly data, e.g., meteorological parameters, and $PM_{2.5}$ were measured to evaluate the air quality during fog episodes (Table 1). The meteorological parameters including atmospheric visibility, temperature, relative humidity, wind direction, wind speed were measured with an automatic meteorological station (PC-4, JZYG, China) *in situ*. The mass concentration of $PM_{2.5}$ was measured using a Model 5030 SHARP monitor (SHARP 5030, Thermo Fisher Scientific, Massachusetts, USA). To determine the most likely source region for air mass of fog episodes, the 24-h back trajectory analysis was performed using the Hybrid Single-Particle Lagrangian Integrated Trajectories (HYSPLIT) model (http://ready.arl.noaa.gov/HYSPLIT.php). Moreover, the wind rose diagram of study area (origin, version 9.0, Origin Lab Corporation, Northampton, MA) during fog process were analyzed to clarify the predominant wind direction and wind speed.

2.2 DNA Extraction and PCR Amplification

Genomic DNA was extracted in triplicate with the FastDNA spin kit for soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's directions. The

concentration of DNA was determined spectrophotometrically (Nano-Drop 2000, Thermo, Wilmington, Delaware, USA).

The designed primer sets with the V3-V4 region of 16S rRNA gene (338F-806R) (Masoud et al., 2011), adapter and barcodes were selected in the illumina Miseq sequencing. For each sample, a 25-μL PCR mix was prepared containing 10 μL of 5x Buffer, 1μL of dNTP (10mM), 1 U Phusion High-Fidelity DNA polymerase, 20 ng of template DNA, 1 μL of each 10 μM modified primer, with double-distilled water until 25 μL. PCR was performed at 94°C for 2 min; 25 cycles of 94 ℃ for 30 s, 56 ℃ for 30 s and 72 ℃ for 30 s; 72 ℃ for 5 min; and hold at 10 ℃.

The PCR products were separated by 2% agarose gel electrophoresis and purified with the nucleic acid purification Kit (AxyPrepDNA, Axygen, USA). Purified PCR products were quantified using a Qubit 3.0 fluorometer (Invitrogen, Carlsbad, CA) and then mixed to equal concentration. For each sample, 4 μL of 10 nM pooled DNA was denatured with 1 μL of 0.2 N NaOH at room temperature. Finally, the Illumina paired-end sequencing was performed on a MiSeq platform (Illumina, Inc., San Diego, CA). After sequencing, two FASTQ files (read 1 and read 2) for each sample were generated on the MiSeq reporter software automatically. Raw 16S rRNA gene sequences are available at the Sequence Read Archive (SRA) under accession number SRX1904235.

2.3 Illumina high-throughput sequencing and analyzing

Raw sequences were processed using the QIIME package (Kuczynski et al., 2011). The PE reads were firstly merged with overlap greater than 10 bp. Then, the adapter, barcodes and primers were removed from the merged sequences. Subsequently, the trimmed sequences with length shorter than 200 bp, quality score lower than 25, homologous longer than 8 bp, containing ambiguous characters were screened. Finally, chimeric sequences were distinguished using the Usearch61 algorithm and removed from the dataset. Optimized sequences were clustered into OTUs at the threshold of 97% similarity with the usearch61 algorithm. Single OTUs were removed and taxonomy was assigned to each OTU using the Ribosomal Database Project (RDP) classifier in QIIME, with a minimum confidence cutoff of 80% against the Silva reference database (silva 119, http://www.arb-silva.de/) to the genus level. Subsequently, we focused on the bacterial genera including species known or

suspected to be opportunistic pathogen and performed a systematic literature review to identify potential pathogenic bacteria in water habitats (Bibby et al., 2010; Guo & Zhang, 2012; Luo & Angelidaki, 2014).

To acquire bacterial community function, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) was performed. The PICRUST can be used to predict the metabolic function pathway from corresponding bacteria and archaea and provide a community's functional capabilities based on the 16S rRNA gene sequence not (Langille et al., 2013). PICRUST has been used in bacterial diversity and function analysis (Corrigan et al., 2015; Wu et al., 2016). In the present, the phylogenetic and functional capacities for the bacteria in fog water are of great interest to help understanding their roles in atmosphere, ecosystem and health. Bacterial metagenome predicted from 16S rRNA gene-based microbial compositions using the PICRUSt algorithm, and functional inferences were made against with the Kyoto Encyclopedia of Gene and Genomes (KEGG) annotated databases. Spearman's correlation coefficients were estimated for each pairwise comparison of genus and KEGG pathway counts. Selected KEGG pathways relating to metabolism and disease infection and predominant genera are included in the heatmap. Correlation is significant at P-value of less than 0.05 and 0.01.

Alpha diversity was assessed by examining the rarefaction curves, shannon-wiener curves and rank-abundance curves calculated with Mothur (v.1.34.0; http://www.mothur.org) (Schloss et al., 2009) and visualized in R project (v.3.1.3; https://www.r-project.org/). Community richness estimators including the observed OTUs, nonparametric Chao1, ACE, and community diversity estimators including Shannon and Simpson indexes were also calculated with Mothur. Moreover, the Good's coverage was used to evaluate the sequencing depth.

Principal component analysis (PCA) was carried out to visualize the changes in bacterial community between polluted and non-polluted fog samples. The PCA plots were constructed based on Bray-Curtis similarity index calculated with the abundance of OTUs using the BIODIVERSITYR package (Kindt & Coe, 2005) in R. The difference in OTU composition for samples collected in polluted and non-polluted fog episodes was tested by the analysis of similarity (ANOSIM) (Clarke, 1993). ANOSIM was performed with the VEGAN package in R. Linear discriminant analysis effect size (LEfSe, http://www.huttenhower.sph.harvard.edu/galaxy/) was applied to identify differentially abundant bacterial taxa associated with the polluted and non-polluted

fog episodes at genus or higher taxonomy levels (Segata et al., 2011). All statistical tests were two-sided, and a P-value of less than 0.05 was considered statistically significant.

2.4 Intercation between bacterial community structure and environmental variables

Relationship between bacterial community structure and environmental variables was first performed using a detrended correspondence analysis (DCA) to estimate the length of the gradient. The resulting length (0.99) indicates a linear model was appropriate, hence RDA was subsequently performed. RDA was elaborated with the predominant bacteria data matrix and the environmental data matrix including $PM_{2.5}$ mass concentration, meteorological conditions, water pH and electric conductivity (Anderson & Willis, 2008). Interset correlations were used to determine the most important environmental variables in determining the community structure. The cumulative fit per species as fraction of variance was performed to determine the importance for the ordination space and select the species most associated with environmental factors. Analysis was performed in Canonical Community Ordination for windows (Canoco, v 4.5).

3. Results and discussions

3.1 Microbial community in fog water

Information on bacterial community in fog/cloud droplets are scarce, our study provided comprehensive investigation of bacterial community under both polluted and non-polluted fog episodes. From the 13 samples collected during 7 fog episodes, a total of 232148 high quality sequences were obtained after quality filtering and OTUs ranged from 975 to 1258 (Table 2). This value was similar with the previous sequence-based survey of atmospheric bacteria in dust storm (OTUs, 1214) (Katra et al., 2014), and bacteria in rain water in July (OTUs, 1542) (Cho & Jang, 2014). Identification of OTUs at different taxonomic levels yielded 359 species, 411 genera, 152 families, 70 orders, 38 classes and 26 phyla. Across all samples, Proteobacteria was the dominant phylum, followed by Bacteroidetes, Cyanobacteria, Firmicutes, Deinococcus-Thermus, Actinobacteria and Nitrospirae (Figure 1). Bacterial

community structure is similar to few other studies explored bacterial diversity in fog/cloud samples, the aforementioned phyla contained a series of genera participate in the atmospheric hydrological cycle (Amato et al., 2007b; Delort et al., 2010). They are predominant taxa in clouds at a high elevation determined by sanger sequencing and tagged pyrosequencing (Bowers et al., 2009), and which are also the typical culturable heterotrophic bacteria from clouds broadly distributed in aquatic and terrestrial habitats (Amato et al., 2005; Kourtev et al., 2011). In the present study, Figure S2 shows the dominant genera collected during fog process. The predominant genera from Proteobacteria (including *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas*, *Sphingomonas*, *Massilia*, *Delftia*, *Brevundimonas*), Firmicutes (*Bacillus*) and Bacteroidetes (*Empedobacter*) were similar across all samples. The identified genera in fog water were similar to the limited data descripted microorganisms in fog/cloud water. Fuzzi et al. (1997) investigated bacteria in fog droplets in a highly polluted area and found the predominant genera from *Pseudomonas*, *Bacillus* and *Acinetobacter*. Amato et al (2007b) observed more diverse genera from the phylum of Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes, which mainly belonging to *Pseudomonas*, *Sphingomonas*, *Staphylococcus*, *Streptomyces* and *Arthrobacter*. Ahern et al (2006) investigated bacterial community in clouds collected in Scotland and found the dominant species were from *Pseudomonas* and *Acinetobacter*.

Bacterial community function based on inferred metagenomes using the PICRUSt algorithm. After PICRUST analysis, pathways with participants less than 10% were removed, leaving 225 non-human-gene KEGG pathways. These predominant pathways showed in Figure S3 were mainly related to Amino Acid Metabolism, Carbohydrate Metabolism, Cell Motility, Cellular Processes and Signaling, Energy Metabolism, Enzyme Families, Folding, Sorting and Degradation, Membrane Transport, Nucleotide Metabolism, Nucleotide Metabolism, Replication and Repair, Signal Transduction, Transcription, Translation. Besides the pathways associated with microbial physiological metabolism, we focused on the microbial pathways of metabolic processes in a variety of environments. Fog/cloud droplets contains carbon and nitrogen sources, which could be available medium for microbial activity in the atmosphere. The predicted function of metabolism was likely attributed to the bacterial gene from the identified taxa (Figure 2). Previous studies have demonstrated that atmospheric bacterial community contained a metabolically diverse group found

in a wide range of water/soil habitats. For example, *Acinetobacter*, the most abundant genera widely distributed in land or ocean, was positively associated with the biodegradation, leaching and removal of several organic and inorganic man-made hazardous wastes (Abdelelhaleem, 2003). *Stenotrophomonas* and *Pseudomonas*, positively correlation with carbohydrate metabolism and glycan biosynthesis and metabolism, are well-known for the striking capability to utilize numerous carbon sources, have been widely utilized in the degradation and transformation of complex organic compounds in a wide range of habitats (Boonchan et al., 1998; Stanier et al., 2010). Moreover, predicted functions associated with human disease are especially concerned. For instance, some species from *Acinetobacter*, were positively associated with infection disease (Nemec et al., 2001). *Empedobacter* from Bacteroidetes widely distributed in water habitats, are human clinical origins, certain species from *Empedobacter* are ranked as potential pathogens (Hugo et al., 2005).

In fog water, a variety of genera adapt to harsh environments were also identified. *Sphingomonas*, the ability to survive in low concentrations of nutrients has been reported, which can metabolize a series of carbon compounds, events toxic compounds (Xu et al., 2006). Similar to *Sphingomonas*, members of *Brevundimonas* are well known for their ability to withstand extreme harsh environment (Kopcakova et al., 2014). The spore forming bacteria *Bacillus* included in the phylum Firmicutes are commonly airborne bacteria found in bioaerosol, cloud water and rainwater and could survive in cold environment (Despr és et al., 2012). Similar to *Bacillus*, some strains of *Pseudomonads* found in Antarctic environments revealed the cold adaption (Bozal et al., 2003). Certain *Pseudomonads* species found in cloud water were psychrophiles, they faster growth at 5 ℃ than at high temperature (17 ℃ or 27 ℃) (Amato et al., 2007b). Members of *Deinococcus* from Deinococcus-Thermus are available to withstand extreme radiation conditions that could potentially adapt to the cloud environment (Mattimore & Battista, 1996).

The identification of microorganisms active at barren nutrition, low temperatures and radiation environment encountered in clouds is not surprising since similar bacteria have been recovered and demonstrated to be active in harsh environments. Their adaption to the specific environments in fog/cloud water with the potential role in the nucleation, metabolism of organic pollutants, demonstrated the potential importance in participation and influence atmospheric biochemistry cycle.

3.2 Implications in human health and ecosystem

Bacteria in fog/cloud water have been discovered for decades but detailed information on community composition and potential function in atmosphere, ecosystem and human beings is severely limited. Bioaerosols in fog/cloud have been complex assemblages of airborne and exogenic microorganisms, likely emissions and resuspension from various terrestrial environments, e.g., soil, water, plants, animals or human beings. In the atmosphere, fog/clouds may be favorable niche for bacteria and these bacteria could thrive and influence cloud processes by acting as cloud condensation nuclei and ice nuclei. Bacteria including pathogenic or beneficial species can also be attached to particles or incorporated into water droplets of fog/clouds. During fog or rain process, they can be deposited back to land via deposition and possibly induce infections to human health and impose effect on the diversity and function of aquatic and terrestrial ecosystems (Kaushik & Balasubramanian, 2012; Simmons et al., 2001; Vaďilingom et al., 2012) (Figure 3 and Table 3).

Atmospheric bacteria are efficient cloud condensation nuclei by offering cell surface for the condensation of water vapour (Mohler et al., 2008). The hygroscopic growth of bacteria below water saturation and critical supersaturations have been observed for some bacterial species, e.g. Bauer et al. (2003) found that *Brevundimonas diminuta* was activated at ＜0.1% supersaturation (Bauer et al., 2003). In addition, various strains of *Pseudomonas*, *Rhodococcus* and *Bacillus* found in cloud water samples could produce biosurfactants and act as cloud condensation nuclei (Delort et al., 2010). They may form cloud droplets combined with aerosol particles at lower supersaturations and quickly grow to large droplet sizes, thereby facilitating rain formation (Mohler et al., 2007). Moreover, *Pseudomonas* was responsible for inducing ice nucleation at a temperature warmer than usual (Amato et al., 2015). Simulations of cloud forming conditions carried out in a cloud chamber suggest that *Pseudomonas* cells first acted as CCN, then induced freezing, and ice nucleation process (Mohler et al., 2008). In addition to *Pseudomonas*, some other bacteria from *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Sphingomonas*, and *Stenotrophomonas* sp. (Table 3), were shown to be ice nucleation active bacteria (Mortazavi et al., 2008). Gaining an understanding of possible role in cloud condensation and ice nucleation processes might open a new sight of bacterial communities influence on meteorology

and climate change.

In addition to the bacteria survive in cold environments and act as efficient cloud condensation nuclei or ice nuclei, the presence of microorganisms in fog/cloud may play vital role in atmospheric biochemistry. The detection of microorganisms in cloud water associated with biotransformation of organic compounds raised a general understanding of the potential role in atmospheric chemistry. The identified species from the genera of *Rhodococcus*, *Sphingomonas*, *Delftia*, *Comamonas* (Table 3) were mainly participated in the biodegradation of organic compounds. Excessive studies have illustrated their capability of metabolism of hydrocarbon compounds, even toxic pollutants, e.g., aromatic compounds (Bock et al., 1996; Busse et al., 2003; Geng et al., 2009; Goyal & Zylstra, 1996). Two strains from *Stenotrophomonas* (*S. rhizophila*) and *Phyllobacterium* (*P. myrsinacearum*) are typical rhizospheric microorganisms, which were typically dispersed into atmosphere from soil. As plant-associated strains, *S. rhizophila* fulfill plant-protective roles and have been safely applied in biotechnology (Alavi et al., 2013). *P. myrsinacearum* is a predominant rhizospheric bacterium, which has been utilized in plant growth promotion and biological control of soil-borne diseases due to its capability of azotification (Gonzalezbashan et al., 2000). The methylotrophic bacteria *Methylobacterium* (*M. aquaticum* and *M. adhaesivum*) are typically inhabit in soil and water. Previous studies have demonstrated the carbon fixing function in ecosystem (Gallego et al., 2006; Gallego et al., 2005). Similar to *Methylobacterium*, *Cyanobacterium* sp., widely distributed in soil, water, and various arid environments, have excellent nitrogen and carbon fixing ability (Jha et al., 2004). Fog water seems to harbor highly diverse bacterial communities in ecosystem, which may be the atmospheric mixing of diverse point sources origins in the rhizosphere, soil and water and possibly participate in the biodegradation of organic compounds in fog water.

In addition, after sequencing, bacterial genera containing potential pathogens were especially concerned. By blast with the reference pathogen database, sequences high similar with potential pathogens were identified. In the present study, the presence of potential pathogen sequences indicated occasional distribution and dispersion of pathogens in fog water (Table 3). The identified opportunistic pathogens from *Empedobacter*, e.g., *E. brevis*, can be easily isolated from clinical resources, which may be associated with eye infections (Bottone et al., 1992). Occurrence of *Staphylococcus equorum* in fog water can be expected since *Staphylococcus* are

frequently isolated from airborne samples (Seo et al., 2008). They can reside on the skin and mucous membranes of humans and induce severe infections (Novákov á et al., 2006). Similarly, species from *Brevundimonas* (*B. vesicularis* and *B. diminuta*) can induce virulent infections, often associated with nervous system or bacteraemia (Gilad et al., 2009; Han & Andrade, 2005). Besides that, the pathogenic strains from *Acinetobacter* (*A. schindleri*) and *Moraxella* (*M. osloensis*) are associated with skin and wound infections, bacteremia and pneumonia (Banks et al., 2007; Nemec et al., 2001).

Previous studies on potential pathogens are mostly focused on the atmospheric particulate matter ($PM_{2.5}$ and PM_{10}) (Cao et al., 2014; Creamean et al., 2013), rain water (Kaushik & Balasubramanian, 2012; Simmons et al., 2001), and indicated that health risk-related bacteria in atmospheric samples should be concerned. For fog water, studies of health risks to individuals are typically focused on the chemical characteristic, e.g., the low pH (acid fog) (Hackney et al., 1989), PAH (Ehrenhauser et al., 2012), etc. Limited literature discussed the microorganism in fog/cloud water suggested potential pathogens in fog/cloud water (Va ïtilingom et al., 2012), they find potential plant pathogens such as *Pseudomonas syringae* and *Xanthomonas campestris* and suggest these living species of plant pathogens could then infect new hosts through precipitation. Possibly, greater survival of human pathogens may be supported in the atmosphere. Fog/cloud and rain process are part of atmospheric life cycle and dispersal pathway for some pathogenic bacteria. Studies of the airborne dispersal of pathogenic bacteria, e.g., *Neisseria meningitides*, *Staphylococcus aureus* from dust samples from Kuwait and *Pseudomonas aeruginosa* in USA Virgin Islands have indicated the spread of specific human, animal and plant diseases over long term transport in atmosphere (Griffin, 2007; Griffin et al., 2003; Griffin et al., 2006). However, detailed health risk-oriented studies induced by pathogenic microorganisms should be deeply conducted and prudently assessed. Further study depending on the culture-dependent method and biochemical experiments will perform to check the pathogenicity.

3.3 Disparity between polluted and non-polluted fog episodes

Although the predominant bacteria are similar between polluted and non-polluted fog episodes, significant disparity within bacterial taxa are also identified. ANOSIM

analysis suggest that the OTUs from polluted samples were grouped into one large cluster, and separated from the non-polluted clusters (ANOSIM comparison, R =0.579, P=0.024, ＜0.05). Cluster analysis including PCoA and Hcluster indicated a highly similar community composition in polluted samples, regardless of the fog episodes (Figure S4). Cluster analysis based on the relative abundance of genera showed similar clustering patterns (Figure S5), and the polluted samples also shared high similarity in their bacterial community structure.

To distinguish indicator speices within the polluted and non-polluted fog episodes, LEfSe is performed, which showed statistically significant differences. A total of 70 bacterial groups were distinct using the default logarithmic (LDA) value of 2. Cladograms show taxa with LDA values higher than 3.5 for clarity (Figure 4). Consequently, 8 and 19 represent bacterial taxa in polluted and non-polluted fog episodes were detected.

In polluted fog episodes, most enriched bacteria were ranked as opportunistic pathogens, such as Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Stenotrophomonas, Haliscomenobacter, and Saprospiraceae. Like Proteobacteria, the Gram-negative Gammaproteobacteria contains a series of ecologically and medically important bacteria, e.g., the pathogenic Enterobacteriaceae, Vibrionaceae and Pseudomonadaceae. The Xanthomonadales from this phylum has been reported to cause disease in plants (Saddler & Bradbury, 2005). Certain species from *Stenotrophomonas* (Gammaproteobacteria, Xanthomonadales) are associated with multiple human infections. The Saprospiraceae, a family within the phylum Bacteroidetes, include the genus *Haliscomenobacter*, are typical planktonic bacteria isolated from aquatic environments, such as marine, freshwater and activated sludge. The notable ability for the hydrolysis and utilization of complex carbon compounds has been illustrated (McIlroy & Nielsen, 2014).

In comparison, the majority of indicator species in the non-polluted samples are from Bacteroidetes, Firmicutes, Alphaproteobacteria, Betaproteobacteria and Deinococcus-Thermus. An important biomarker from Bacteroidetes is Flavobacteriia, relative study has illustrated the marine sources for Flavobacteria, most of Flavobacteria sequences searched by NCBI blast are mainly from marine sources, i.e., algae, oysters and sea cucumbers (Cho & Jang, 2014). The genera *Empedobacter* was abundant across all samples, which are included in the family Flavobacteriaceae. As

mentioned above, *Empedobacter* and *Staphylococcus* **(**Firmicutes) are potential pathogens and resistant to a wide range of antimicrobials (Hugo et al., 2005; Trilla & Miro, 1995). Phyllobacteriaceae (Alphaproteobacteria) are typical rhizobia. Similar with Phyllobacterium, members of Oxalobacteraceae (Betaproteobacteria) are rhizosphere microorganisms involved in the biological nitrogen fixation (Donn et al., 2015). The Burkholderiales (Betaproteobacteria) commonly found in water and soil are involved in the biodegradation of various aromatic compounds (Pérez-Pantoja et al., 2012). Deinococci, from the phylum of Deinococcus-Thermus could resistant to extreme radiation and survive in extremes of heat and cold (Griffiths & Gupta, 2007). Although bacteria with potential function of nitrogen fixing (Phyllobacterium from Alphaproteobacteria) and degradation of organic compounds (Burkholderiales from Betaproteobacteria) were distinguished both in polluted and non-polluted episodes, a significant distinct was observed. By comparison, potential pathogens were significant groups in the polluted samples, whereas a diverse ecological function group was identified in the non-polluted samples originated from a wide range of habitat. Ecologically meaningful distinguish of bacterial groups under polluted and non-polluted conditions is essential for understanding the structure and function, and which provide a general understanding of bacterial metabolism in fog water.

3.4 Environmental factors shaping bacterial community structure

To clarity the vital environmental factor in shaping bacterial community structure, RDA was performed to discern the genus-level structure with the selected environmental factors (Figure 5). The first two axes explained 73.3% of the accumulated variance in the species-environment relation. Interset correlations showed $PM_{2.5}$ was the most important environmental variable structuring the bacterial community (axis 1, -0.328); in turn, temperature registered the highest value for axis 2 (0.368) (Table S1). Cumulative fit indicated that the predominant genera affiliated with *Acinetobacter*, *Empedobacter*, *Phyllobacterium*, *Aeromonas* and *Prevotella* displayed strong correlations with axis 1, *Streptococcus*, *Stenotrophomonas*, *Brevundimonas*, *Deinococcus* and *Pseudomonas* were notable genera highly correlations with axis 2 (Table S2). As aforementioned in section 3.3, the above bacteria were metabolically diverse groups found in various habitats.

Of the atmospheric environmental characteristics measured, $PM_{2.5}$ was the best

predictor of diversity variability of bacterial community structure and strongly correlated with represent bacterial genera. Bacterial community composition was highly variable under $PM_{2.5}$ mass concentration in this study, which was consistent with previous studies that $PM_{2.5}$ was important environmental factor shaping the variation of community composition (Cao et al., 2014). Moreover, statistical analysis, e.g., correlation or multiple linear regression, indicated that $PM_{2.5}$ exhibited a negative correlation with airborne bacteria in haze days (Gandolfi et al., 2015; Gao et al., 2015), whereas in another study, spearman correlation analysis showed $PM_{2.5}$ exhibited a significant positive correlations with the airborne microbe concentration (Dong et al., 2016). Previous study has suggested that nutrients are related to the distribution of bacteria in water habitats (Newton et al., 2011). Possibly, the inorganic and organic compounds in particulate matter ($PM_{2.5}$) can be available nutrients for microbial growth. However, aggregation of harmful substances such as heavy metals, polycyclic aromatic hydrocarbons would be toxic for bacteria under high $PM_{2.5}$ mass concentration. Since the $PM_{2.5}$'s two-sided influences on bacterial growth, bacterial community from polluted and non-polluted samples was significantly correlated with $PM_{2.5}$ mass concentration.

The identified taxa either from polluted or non-polluted samples were typically found in soil, water, plant or human skins. These bacterial groups aerosolized and dispersed into atmosphere either from local regional emissions or long-term transport. Source tracking analysis by backward trajectory indicated that air mass of polluted fog episodes came largely from northern and western China, moved east through Shanxi, Henan, Hebei province to the study area, or from outer Mongolia, crossing the Jingjinji area to Mt.Tai, The passed areas were notable as heavy industry region with frequent coal mining activities and serious pollution. Moreover, the large population and agricultural activities resulted in the numerous pathogenic microorganisms from human or animal fecal dispersed in the atmosphere. Moreover, a small part from southern China, and the passed region were rich of water resources, e.g., Dongting Lake, Huaihe river, Yangtze river etc. The aquatic bacteria such as *Haliscomenobacter* dispersed in the atmosphere typically derived from the evaporation of lakes and rivers water (Figure 6). In contrary, air mass of non-polluted fog episodes originated mostly from the southern China, the marine sources bacteria (Flavobacteria) indicated the release of prokaryotes into atmosphere from sea-air interactions and airborne marine bacteria can be transported to inland through

long-term transport.

In the sampling site (the sumit of Mt.Tai, 1534 m a.s.l), local anthropogenic pollution might be minimized and air pollution is mainly influenced by long term transport. Wind rose diagram suggest the predominant north wind during polluted fog episodes and wind speeds ranged 1.2-1.6 m/s, whereas in non-polluted fog episodes it was mainly south wind with higher wind speed (2.1-3.5 m/s). Wind direction and speed are important meteorological factors influencing fog formation (Fu et al., 2014). Recent studies also indicate the variation of bacterial concentration and community structure conducted by wind (Evans et al., 2006; Jones & Harrison, 2004). In the present study, air mass from the contaminated area through long-term transport combined with lower wind speeds, largely reduce the diffusion rate of pollutants and thus lead to the sustained high $PM_{2.5}$ during polluted fog episodes. Whereas in the non-polluted fog episodes, higher wind speed was beneficial to the diffusion of pollutants and resulted in the lower $PM_{2.5}$ mass concentration, which finally led to the significant discrepancy of bacterial community structure. However, further research still needed to address the detailed interaction between bacterial community and environmental factors, and to understand the mechanism that how chemical composition influence microbial community.

4. Conclusion

The composition and potential function of microbial communities in atmospheric water phase (fog and clouds) remained rarely studied. Using the 16S rRNA gene sequencing, this work provided a thorough investigation on bacterial ecological diversity under polluted and non-polluted fog episodes and revealed a highly diverse bacterial community harbored in fog water. Correlation analysis for the predominant genera and PICRUST function predication enhanced the understanding of the distribution of bacteria and their potential involvements in atmosphere, ecosystem and human health. The identification of bacteria survive in barren nutrition, low temperatures and radiation environment encountered in fog/cloud water demonstrated bacterial active in harsh atmospheric environments. They may act as efficient cloud condensation nuclei or ice nuclei, associate with biogeochemical cycling (nitrogen/carbon cycling) and microbial degradation of organic compounds in fog/clouds and spreading of specific human, animal and plant diseases by potential

pathogens. Moreover, community disparity between polluted and non-polluted fog episodes suggest $PM_{2.5}$ seems a pivotal variable in shaping bacterial community, which is likely to provide a more comprehensive understanding of the factors controlling the atmospheric water biodiversity under environmental stress. These results provide a basic understanding of mechanism of bacterial community response and metabolism in polluted weather for further study.

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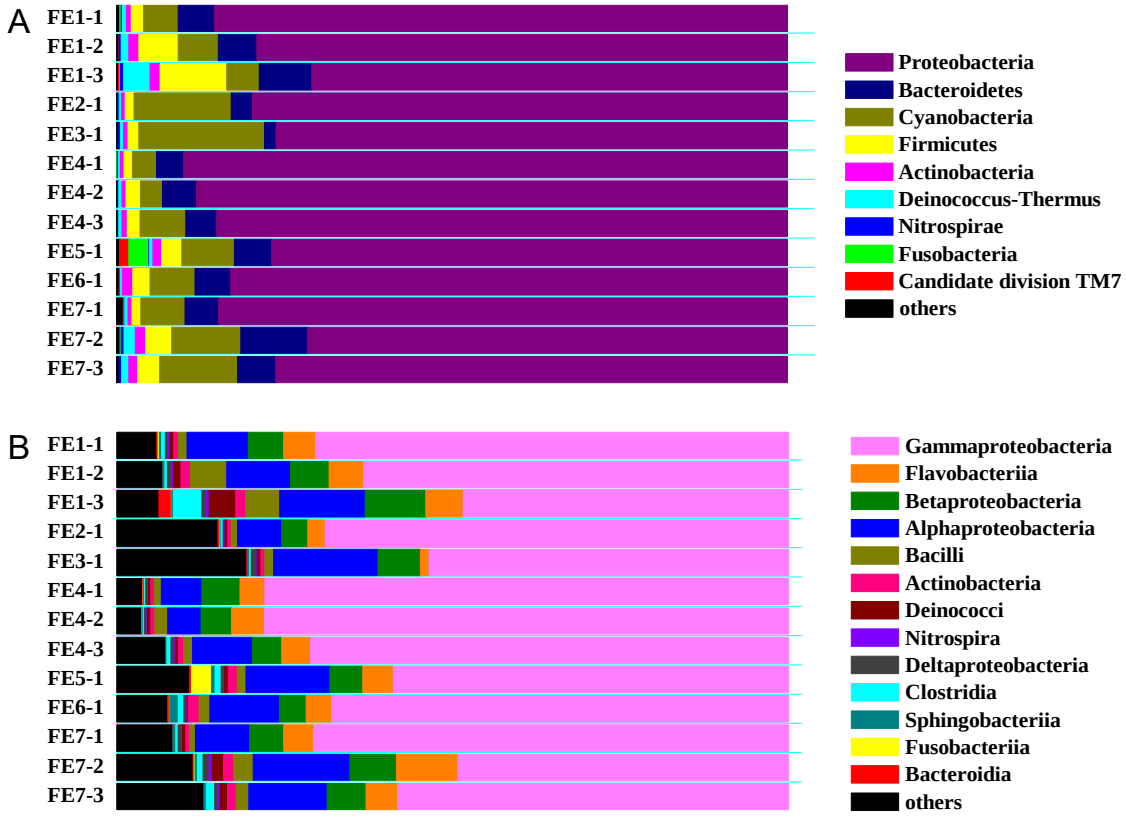

Figure 1 Bacterial community variation for fog episodes at the phylum (A) and class (B) level. FE refers to the fog episodes. Bar graphs for each sample represent the percentage of taxa assigned to each phylum with 80% bootstrap confidence. Taxonomic summary of the most abundant taxa (more than 1%) across all fog samples are indicated in the figure.

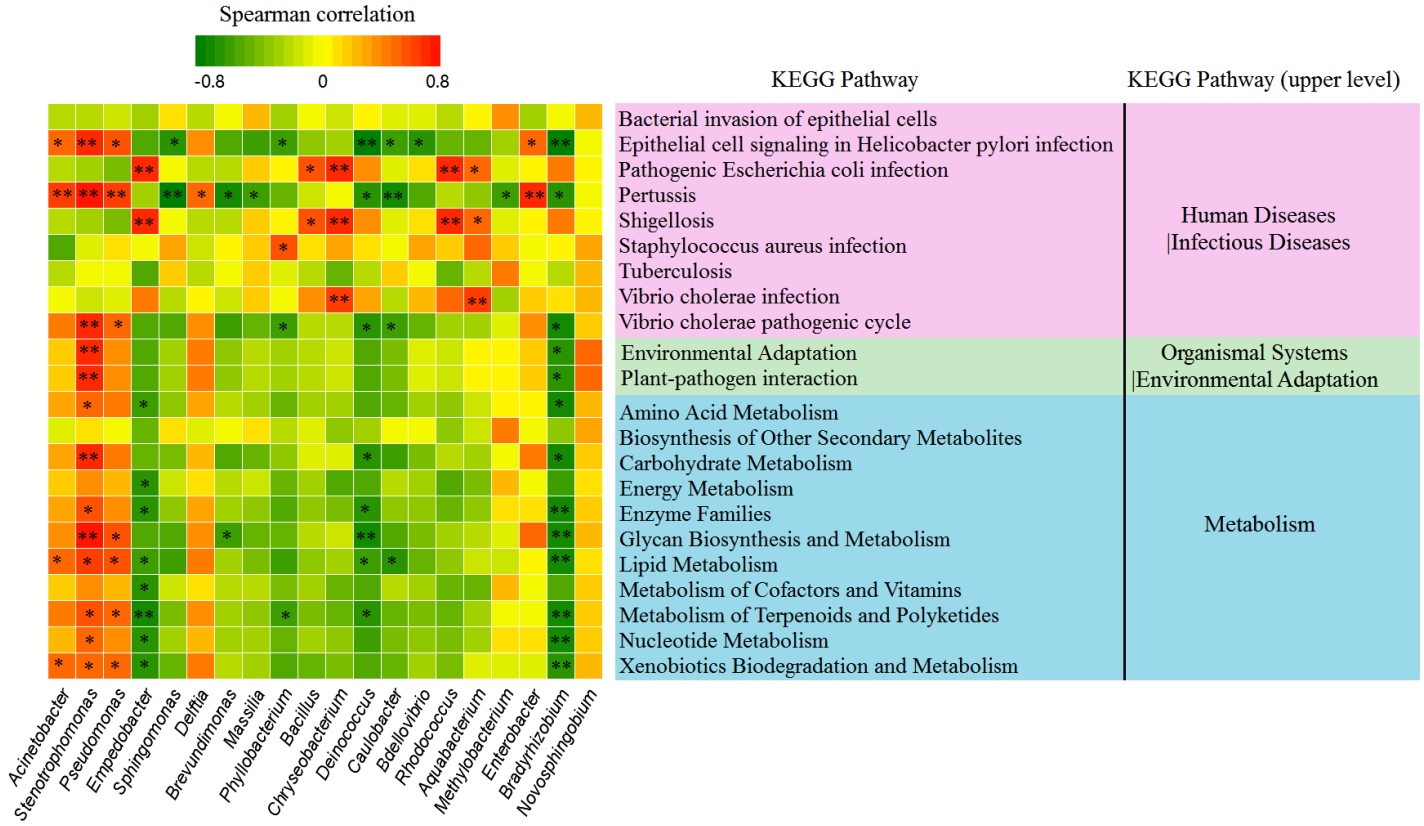

Figure 2 Bacterial taxa are related to KEGG functional pathways. Bacterial gene functions were predicted from 16S rRNA gene-based microbial compositions using the PICRUSt algorithm to make inferences from KEGG annotated databases. Spearman's correlation coefficients were estimated for each pairwise comparison of genus and KEGG pathway counts. Selected KEGG pathways relating to metabolism and disease infection and predominant genera are included in the heatmap. Red color refers to the positive correlation, and green indicates a negative correlation. Correlation is significant at *P < 0.05, **P < 0.01.

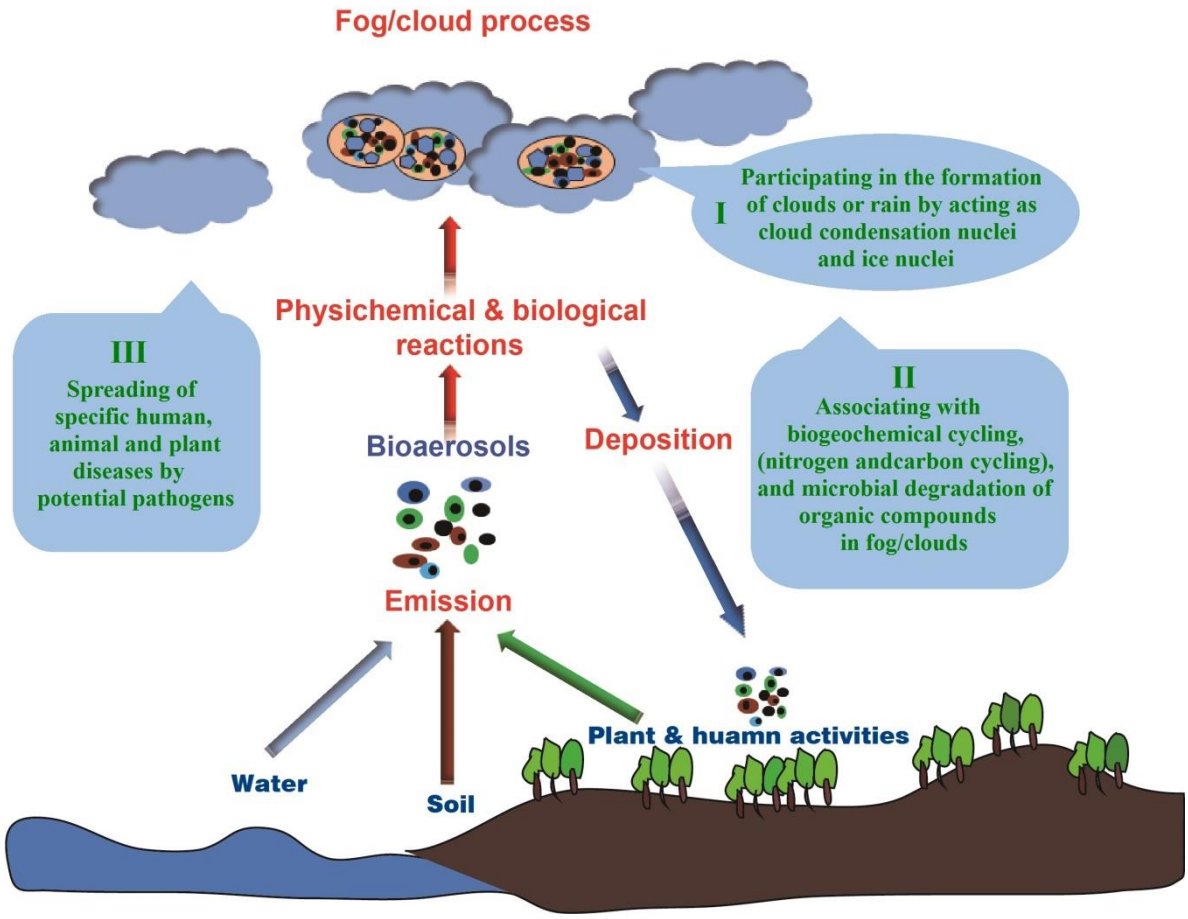

Figure 3 Schematic representation of bioaerosols life cycle and potential influence on atmosphere, ecosystem and human health, modified from Poeschl (Poeschl, 2006). The predominant bacteria species with potential functions are indicated in the figure.

Bioaerosols emissions and resuspension from various terrestrial environments, e.g., soil, water, plants, animals or human beings, may include pathogenic or functional species. These bacteria can be attached to particles or incorporated into water droplets of clouds/fog. Certain species can serve as biogenic nuclei for Cloud Condensation Nuclei (CCN) and Ice Nuclei (IN), which induce rain formation, precipitation, and wet deposition of gases and particles. For the potential pathogens and functional bacteria, during fog process, they can be deposited back to land via deposition and possibly induce infections to human health and impose effect on the diversity and function of aquatic and terrestrial ecosystems.

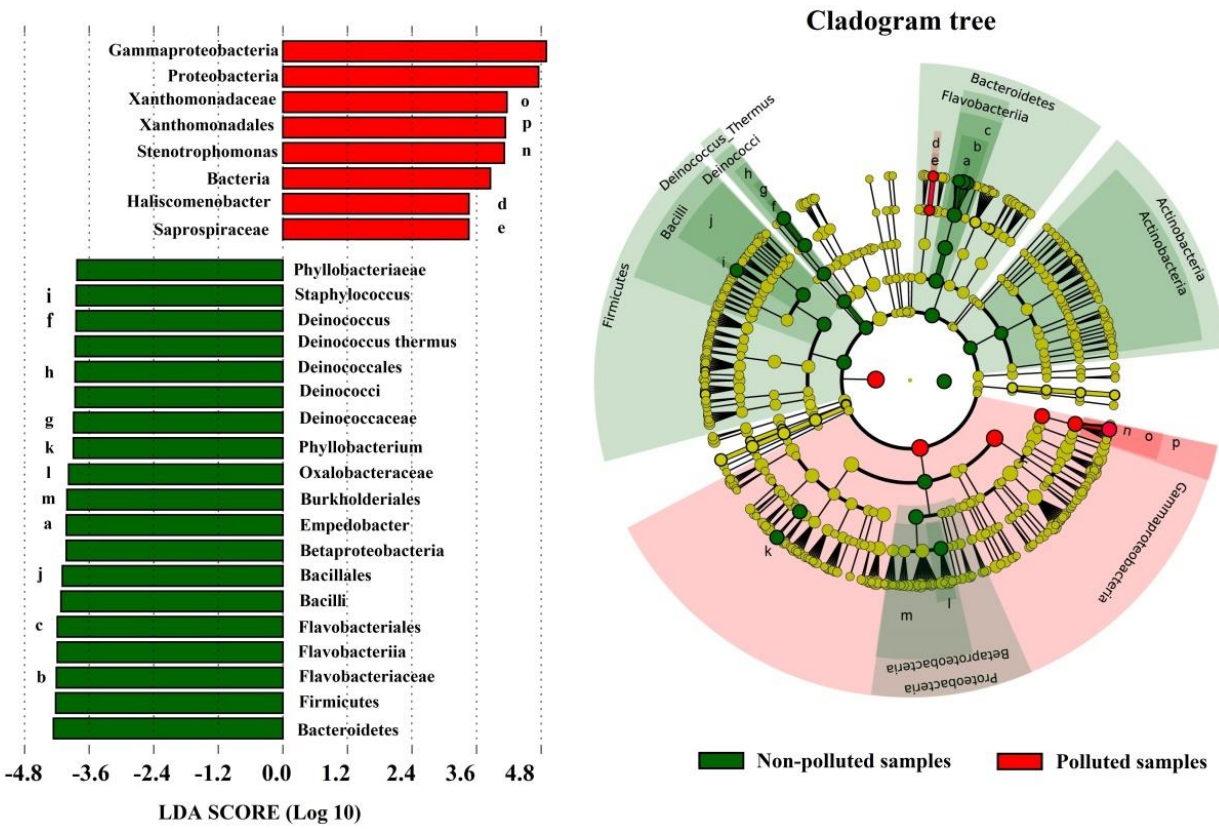

Figure 4 Bacterial taxa significantly differentiated between the polluted and non-polluted fog episodes identified by linear discriminant analysis coupled with effect size (LEfSe). The LDA effect sizes (left) were calculated using the default parameters. The taxonomic cladogram (right) with LDA values higher than 3.5 comparing all bacterial taxa and significantly discriminant taxon nodes are colored and branch areas are shaded according to the highest-ranked variety for that taxon. Taxa with significant difference in polluted and non-polluted fog episodes are indicated in red and green circles, respectively. The bacterial taxa with nonsignificant differences are represented as yellow circles and the diameter of the circles are proportional to relative abundance.

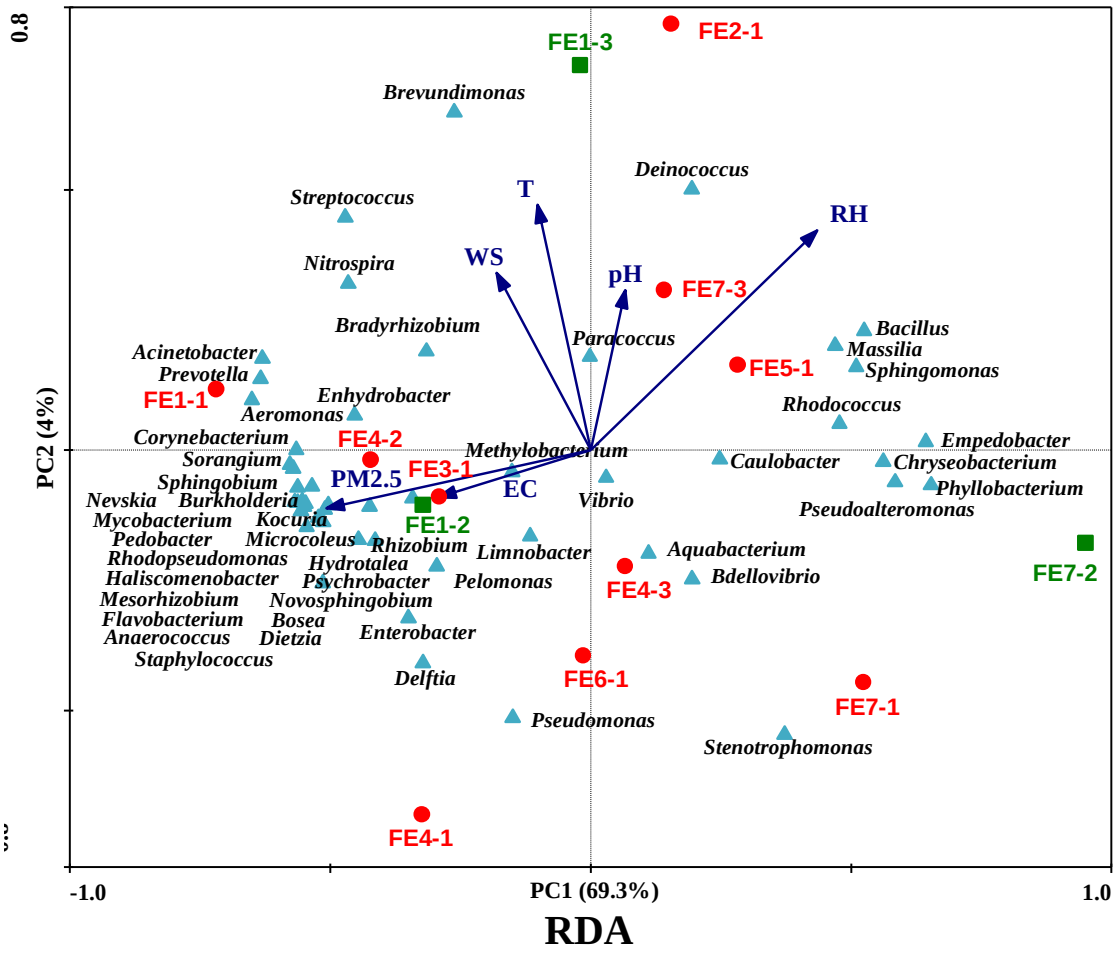

Figure 5 Biplot of the environmental variables and genus-level community structure using a redundancy analysis (RDA) model, describing the variation in bacterial community explained by environmental variable. FE refers to fog episodes. Polluted fog episodes are indicated in red circle, and non-polluted fog episodes are green squares. Species data are listed in Table S2. The selected environmental variables are significant ($P < 0.05$) using Monte Carlo permutation testing. Species are labeled with triangle, the closer of which indicates existence in similar environment. Environmental variables are showed by arrows; the relative length is positive correlation with the importance in influencing bacterial community structure. The angle between the arrow and the ordination axis suggest the variable response respect to the RDA gradient. The two axes explain 73.3% of the variability. For bacteria, $PM_{2.5}$ seems to be the most important environmental variable shaping the community structure.

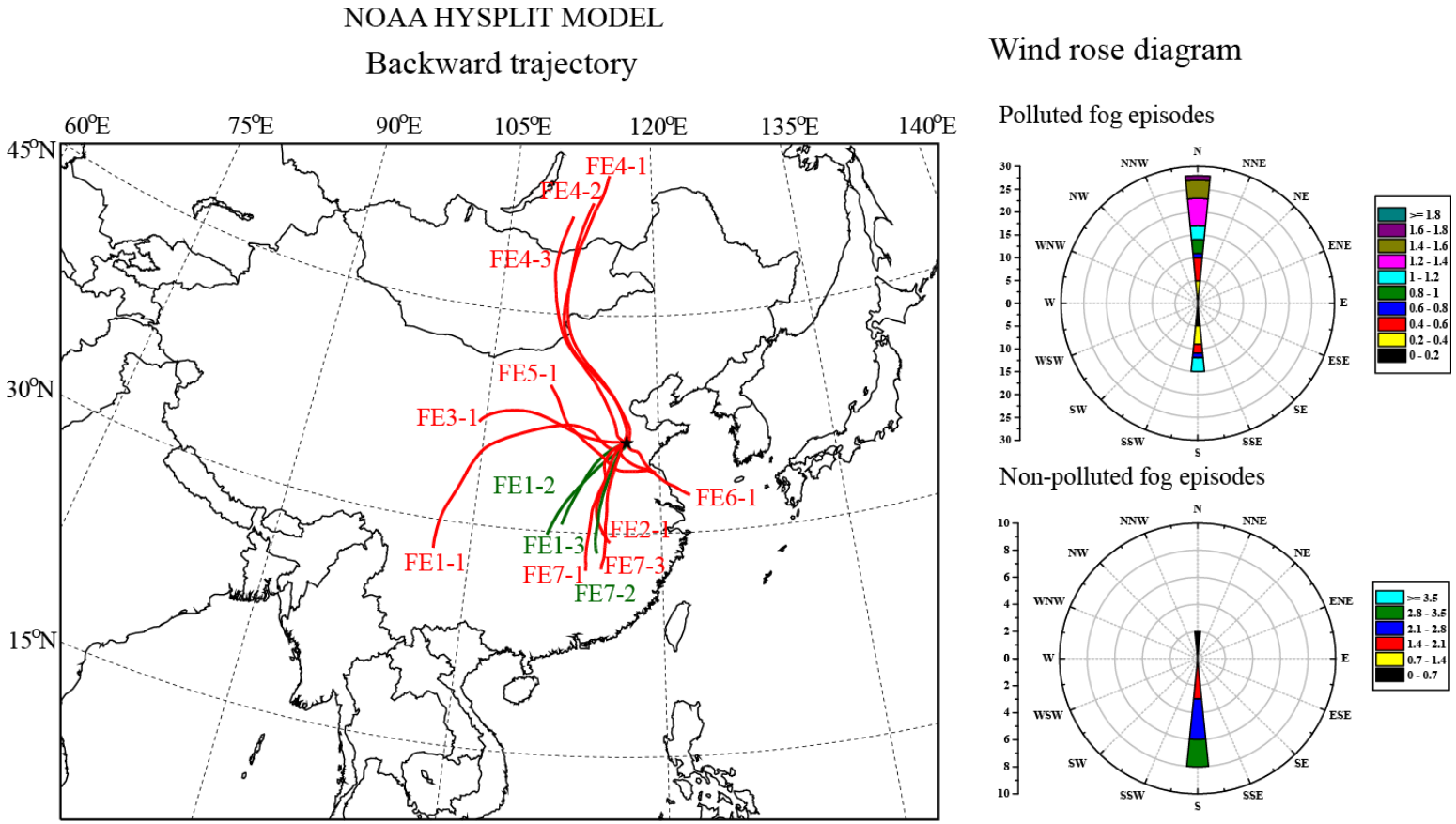

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Figure 6 Air mass transport pathways for the fog episodes using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model. 24-hour backward trajectories were calculated for air parcels arriving at the summit of Mt. Tai (36°18′ N, 117°13′ E, and 1534 m a.s.l). FE refer to fog episodes. The polluted fog episodes are indicated in red lines, and green lines are non-polluted fog episodes. Wind Rose Diagram to quantitative analysis of wind speed and wind direction during sampling time. The frequency of winds is plotted by wind direction, the color bands show wind speed range. The direction of the longest spoke shows the wind direction with the greatest frequency.

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Table 1 Description fog episodes at Mt.Tai, China

Fog episodes	Data	Samples	Start time (BJT)	Stop time (BJT)	Duration (h)	$PM_{2.5}$ [a] ($\mu g \cdot m^{-3}$)	$PM_{2.5}$ [b] ($\mu g \cdot m^{-3}$)	pH	EC ($\mu S \cdot cm^{-1}$)	Pollution
FE1	24 Jul 2014	FE1-1	8:50	15:30	6:40	105.07	49.89	4.03	583	A
		FE1-2	15:30	17:30	2:00	22.35		4.32	219.2	B
		FE1-3	17:30	22:51	5:21	14.66		5.74	104.4	B
FE2	5 Aug 2014	FE2-1	6:45	9:17	2:32	30.36	54.84	5.80	275.7	A
FE3	5 Aug - 6 Aug 2014	FE3-1	19:05	4:01	8:56	42.25	18.19	5.10	501	A
FE4	14 Aug - 15 Aug 2014	FE4-1	22:41	0:44	2:03	42.69	48.47	6.36	170.4	A
		FE4-2	0:44	5:06	4:22	47.98		5.34	86.34	A
		FE4-3	5:06	6:03	0:57	36.88		4.89	64.95	A
FE5	17 Aug 2014	FE5-1	10:10	11:18	1:08	63.18	69.54	5.20	120.5	A
FE6	17 Aug - 18 Aug 2014	FE6-1	22:18	1:25	3:07	54.33	53.70	3.80	321.8	A
FE7	23 Aug 2014	FE7-1	2:30	4:38	2:08	30.45	48.83	4.38	356.2	A
		FE7-2	4:38	6:21	1:43	23.39		5.01	207.5	B
		FE7-3	6:21	9:20	2:59	41.60		5.74	187.6	A

FE refers to fog episode. BJT refers to Beijing Time, which equals UTC + 8. EC refers to the electric conductivity.

The A, B refers to the the polluted and non-polluted samples based on the WHO 24-hr average standard $PM_{2.5}$ mass concentration ($PM_{2.5}$ = 25 $\mu g \cdot m^{-3}$), respectively. The $PM_{2.5}$ [a] refers to the average value during a fog process.

Daily average concentration is indicated with $PM_{2.5}$ [b].

Table 2 Summary of bacterial diversity and richness of fog water

Sample ID	Reads	OTUs	Ace	Chao1	Coverage	Shannon	Simpson
Polluted fog episodes							
FE1-1	18213	975	1835	1491	0.9761	3.9418	0.0646
FE2-1	19914	1125	1756	1684	0.9744	3.9582	0.0630
FE3-2	18199	1022	2082	1582	0.9734	3.9749	0.0647
FE4-1	18350	941	1828	1461	0.9762	3.6041	0.0953
FE4-2	17707	967	1522	1427	0.9752	3.6748	0.0902
FE4-3	17397	981	2091	1611	0.9725	3.8074	0.0832
FE5-1	16384	1132	1814	1790	0.9676	4.3173	0.0546
FE6-1	16896	1186	1997	1872	0.9657	4.1268	0.0666
FE7-1	16350	1103	2501	1795	0.965	3.9040	0.0810
FE7-3	18122	1258	1958	1999	0.9686	4.3776	0.0531
Non-polluted fog episodes							
FE1-2	18702	1184	1841	1730	0.9719	4.1919	0.0620
FE1-3	17662	1173	1689	1687	0.9732	4.7067	0.0327
FE7-2	18252	1150	1732	1673	0.9729	4.3709	0.0426
Aerosol (Katra et al., 2014)	4020	1412		2142	0.8300		
Bioaerosol (Madsen et al., 2015)						2.64-3.05	0.816-0.922
Rain water in July (Cho & Jang, 2014)	3055	1542	13083	6387			
$PM_{2.5}$ in summer (Franzetti et al., 2011)		2222		4036			
TSP annual (Bertolini et al., 2013)	271587	765-26,187		107		2.40	

The diversity indexes including OTUs, ace, Chao1, coverage, shannon, simpson were defined at 97% sequence similarity. FE refers to fog episodes. TSP refers to the total suspended particulate matter.

Table 3 The identified bacterial species in fog water samples correlation with the potential ecological function

Genus	Identified species	Habitats	Ecological role	Reference
Acinetobacter GP	*A. schindleri*	soil/water	CNN or IN; Opportunistic pathogens	(Mortazavi et al., 2008; Nemec et al., 2001)
Bacillus FR	*B. anthracis*	soil/water/air	CNN or IN; Opportunistic pathogens	(Makino & Cheun, 2003; Mortazavi et al., 2008)
Brevundimonas BP	*B. diminuta*	soil/water	CNN	(Bauer et al., 2003; Han & Andrade, 2005)
	B. vesicularis	soil/water	Opportunistic pathogens	(Gilad et al., 2009)
Caulobacter AP	*Caulobacter. sp.*	water	Metabolism/Biodegradation	(Nakamura et al., 2007)
Chryseobacterium BA	*C. aquaticum*	soil/water	Protect and promote plants growth	(Gandhi et al., 2009)
	C. jejuense	soil/water		(Ben Abdeljalil & Vallance, 2016)
Clostridium FR	*C. tertium*	soil/gut	Opportunistic pathogens	(Miller et al., 2001)
Comamonas BP	*C. testosteroni*	soil/water	Metabolism/Biodegradation	(Goyal & Zylstra, 1996)
Cyanobacterium CY	*Cyanobacterium sp.*	soil/water	Carbon and nitrogen fixing	(Jha et al., 2004)
Deinococcus DT	*D. aquatilis*	soil/water	Extremophiles, radiation-resistant	(Kämpfer et al., 2009)
Delftia BP	*D. tsuruhatensis*	soil/water	Metabolism/Biodegradation	(Geng et al., 2009)
Empedobacter BA	*E. brevis*	soil/water/plant	Opportunistic pathogens	(Bottone et al., 1992)
Methylobacterium AP	*M. aquaticum*	water	Methylotrophic, carbon fixing	(Gallego et al., 2005)
	M. adhaesivum	soil/water		(Gallego et al., 2006)
Moraxella GP	*M. osloensis*	soil/animal	Opportunistic pathogens	(Banks et al., 2007)
Novosphingobium AP	*N. aromaticivorans*	soil/water	Metabolism/Biodegradation	(Bell & Wong, 2007)
Staphylococcus GP	*S. equorum*	soil/water/clinic	Opportunistic pathogens	(Novákováet al., 2006)
Phyllobacterium AP	*P. myrsinacearum*	soil/plant	Rhizosphere bacteria, nitrogen fixation	(Gonzalezbashan et al., 2000)
Pseudomonas GP	*P. psychrotolerans*	soil/water	Extremophiles, psychrotolerant	(Hauser et al., 2004)
	P. geniculate	soil/water/plant	Metabolism/Biodegradation	(Gopalakrishnan et al., 2015; Liu et al., 2014)
	P. putida	water/soil	Protect and promote plants growth	(Meziane et al., 2005; Reardon et al., 2000)

Table 3 (Continued)

Genus	Identified species	Habitats	Ecological role	Reference
			CNN or IN	(Amato et al., 2015; Joly et al., 2013)
Rhodococcus AC	*R. ruber*	soil/water	Metabolism/Biodegradation	(Bock et al., 1996)
Sphingomonas AP	*S. faeni*	soil/water	CNN or IN; psychrotolerant	(Ponder et al., 2005)
	S. kaistensis	soil/water	Metabolism/Biodegradation	(Busse et al., 2003)
	S. leidyi	soil/water		(Glaeser & Kämpfer, 2014)
Stenotrophomonas GP	*S. rhizophila*	soil/water/plant	CNN or IN; Rhizosphere bacteria	(Mortazavi et al., 2008; Wolf et al., 2002)

CNN and IN refers to the bacteria participating in the formation of clouds or rain by acting as cloud condensation nuclei (CNN) and ice nuclei (IN).

Abbreviates are as followed: AP, Alphaproteobacteria; BP, Betaproteobacteria; GP, Gammaproteobacteria; AC, Actinobacteria; BA, Bacteroidetes; CY, Cyanobacteria; DT, Deinococcus-Thermus; FR, Firmicutes.

Biodegradation refers to the bacteria associated with the biodegradation of organic compounds, even toxic pollutants, e.g., aromatic compounds.

The initial submitted manuscript

Characteristics of bacterial community in fog water at Mt. Tai: similarity and disparity under polluted and non-polluted fog episodes

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Abstract:

Bacteria, widely distributed in atmospheric bioaerosols, are indispensable component in fog water system and play an important role in atmospheric hydrological cycle. However, little is known about the bacterial community dynamics and ecological function, especially under the increasing serious air pollution events in North China Plain. Here we have a comprehensive characterization of bacterial community structure, variation and environmental influence about fog water collected at Mt. Tai under polluted and non-polluted fog episodes from 24 Jul to 23 Aug 2014. Using the Miseq 16S rRNA gene sequencing, the facts that fog water harbored a highly diverse bacterial community and the predominant phyla of Proteobacteria, Bacteroidetes, Cyanobacteria and Firmicutes were investigated. The abundant genera *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas*, and *Empedobacter* originated from a wide range of habitat included opportunistic pathogenic and functional species, suggesting the

bacterial ecological and healthy importance in fog water should be concerned. Clustering analysis including hierarchical cluster (Hcluster) and principal coordinate analysis (PCoA) indicated a significant disparity between polluted and non-polluted samples. Potential pathogens were significant group in the polluted samples, whereas a more diverse ecological function group of bacteria were identified in the non-polluted samples using linear discriminant analysis effect size (LefSe). Community structure discrepant performed by redundancy analysis (RDA) indicated $PM_{2.5}$ have negative impact on bacteria, playing vital role in shaping microbial community structure. $PM_{2.5}$ was possibly associated with different origins and pathways of air mass using source tracking by the backward trajectory and wind analysis, mainly related to the long-term transport combing with local regional emission processes. This work furthered our understanding of bacterial ecological characteristics in the atmospheric aqueous phase, highlighted the potential influence of environmental variables on bacterial community over fog process, which will provide fundamental acquaintance of bacterial community response in fog water under increasing pollution stress.

Key words： fog water, bacterial diversity, community disparity, $PM_{2.5}$

1. Introduction

Fog is the near-surface cloud and aerosol system composed of tiny droplets suspended in the atmosphere. In the atmosphere, numerous pollutants could be dissolved or suspended in fog, which may induce complex effects on environment security and human health. Over the past decades, studies on fog/cloud water are mainly focused on the physicochemical properties (Aikawa et al., 2001; Boris et al., 2015; Fernández-González et al., 2014). Recently, with the in-depth understanding of the characteristics of fog, bioaerosols in fog have been the upcoming focus.

Studies have showed that living microorganisms, including bacteria, fungi and yeasts, are present in fog or clouds (Burrows et al., 2009). As the first study on biological particles in fog water, Fuzzi et al (1997) suggest the bacterial replication in foggy days. Afterwards, with the development of detection techniques, microorganisms in fog/cloud water are more systematically studied (Amato et al., 2007c; Delort et al., 2010; Vaïtilingom et al., 2012). Combined with the field investigations and lab experiments, diverse bacterial communities are identified, and the bacterial metabolically active in fog/cloud water are also demonstrated. In atmospheric aqueous phase, microorganisms can act as cloud condensation nuclei and ice nucleation, which have potential impact on precipitation processes (Amato et al., 2015; Mortazavi et al., 2015). Moreover, microorganisms in fog/cloud water are available to metabolize organic carbon compounds and influence photochemical chemical reactions (Vaïtilingom et al., 2013), involve in the nitrogen cycling (mineralization and nitrification) (Hill et al., 2007), degrade organic acids (formate, acetate, lactate, succinate) and associate with carbon recycling (Amato et al., 2007a; Vaïtilingom et al., 2010), and therefore participate in a series of complex and diverse biochemical metabolic activities.

A fog occurrence is a complex process, in contaminated area, fog typically contains numerous pollutants, e.g., sulfate and nitrate ions, organic carbon compounds, and bacteria(Badarinath et al., 2007; Després et al., 2012; Fernández-González et al., 2014; Mohan & Payra, 2009). Emissions and resuspension of bacteria by wind erosion or splashing water from various terrestrial environments into the atmosphere recruit diverse airborne bacteria, which possibly involve opportunistic and functional bacteria. During fog process, these bacteria attached to particles or incorporated in fog water

will be deposited back to the land via dry or wet deposition processes, which may induce human risks through microbial pathogens dispersion and potential effect on the diversity and function of aquatic and terrestrial ecosystems. Therefore, to evaluate the potential ecological functional bacteria in fog water is urgent, especially for the polluted fog episodes.

It is noteworthy that airborne bacterial communities are closely related to environmental characteristics (Gao et al., 2016), and meteorological factors are often correlated with the observed bacterial community structure (Dong et al., 2016). For instance, studies about the relationships between ambient inhalable airborne and environmental parameters suggest temperature, relative humidity, PM_{10}, $PM_{2.5}$ and particle size have significant impact on the composition and dynamic of microbial communities (Adhikari et al., 2006; Bowers et al., 2013). However, due to the paucity of detailed and comprehensive studies of atmospheric bacterial composition, the understanding of the dynamic of bacterial community remains incomplete, particularly in the North China Plain. The North China Plain is the most important agricultural and economic region in China, which has been suffering serious air pollution events in recent years, e.g., the severe fog and haze pollution in Beijing during January 2013 (Huang et al., 2014). During a polluted fog process, how bacterial community varied and which environmental factor play decisive role in shaping bacterial community structure are still unclear.

In the present work, typical fog episodes under polluted and non-polluted weather were collected in the summit of Mt. Tai in North China Plain. To understand the dynamic of bacterial community, the Miseq 16S rRNA gene sequencing was performed, and analysis of similarities (ANOSIM) and linear discriminant analysis effect size (LEfSe) were executed to clarify the discrepant bacterial taxa. Moreover, RDA analysis was applied to identify the pivotal environmental factor influencing bacterial community. Air mass back trajectory and wind direction and speed analysis were selected to definitude the most likely source and transmission paths of pollutants and bacteria.

2. Material and methods

2.1 Sample collection

Fog samples were collected using the Caltech Active Strand Cloud water Collector

(CASCC2) with a droplet size cut of 3.5 μm at the summit of Mt. Tai (36°18′ N, 117°13′ E, and 1534 m a.s.l) (Guo et al., 2012). The flow rate was 24.5 $m^3 min^{-1}$ and fog water was collected on the strings flows down to Teflon bottles. The collected samples were stored at 4°C until analysis.

In fog water, the pH and conductivity was detected with a Multi pH/COND/TEMP 6350 hand held Meter immediately after sampling. Hourly data, e.g., meteorological parameters, and $PM_{2.5}$ were measured to evaluate the air quality during fog episodes. The meteorological parameters including atmospheric visibility, temperature, relative humidity, wind direction, wind speed were measured with an automatic meteorological station (PC-4, JZYG, China) *in situ*. The mass concentration of $PM_{2.5}$ was measured using a Model 5030 SHARP monitor (SHARP 5030, Thermo Fisher Scientific, Massachusetts, USA). To determine the most likely source region for air mass of fog episodes, the 24-h back trajectory analysis was performed using the Hybrid Single-Particle Lagrangian Integrated Trajectories (HYSPLIT) model (http://ready.arl.noaa.gov/HYSPLIT.php). Moreover, the wind rose diagram of study area (origin, version 9.0, Origin Lab Corporation, Northampton, MA)) during fog process were utilized to clarify the predominant wind direction and wind speed.

2.2 DNA Extraction and PCR Amplification

Genomic DNA was extracted in triplicate with the FastDNA spin kit for soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's directions. The concentration of DNA was determined spectrophotometrically (Nano-Drop 2000, Thermo, Wilmington, Delaware, USA).

The designed primer sets with the V3-V4 region of 16S rRNA gene (338F-806R) (Masoud et al., 2011), adapter and barcodes were selected in the illumina Miseq sequencing. For each sample, a 25-μL PCR mix was prepared and contained 10 μL of 5x Buffer, 1μL of dNTP (10mM), 1 U Phusion High-Fidelity DNA polymerase, 20 ng of template DNA, 1 μL of each 10 μM modified primer, with double-distilled water until 25 μL. PCR was performed at 94°C for 2 min; 25 cycles of 94 ℃ for 30 s, 56 ℃ for 30 s and 72 ℃ for 30 s; 72 ℃ for 5 min; and hold at 10 ℃.

The PCR products were separated by 2% agarose gel electrophoresis and purified with the nucleic acid purification Kit (AxyPrepDNA, Axygen, USA). The purified

PCR products were quantified using a Qubit 3.0 fluorometer (Invitrogen, Carlsbad, CA) and then mixed to the equal concentration. For each sample, 4 μL of 10 nM pooled DNA was denatured with 1 μL of 0.2 N NaOH at room temperature. Finally, the Illumina paired-end sequencing was performed on a MiSeq platform (Illumina, Inc., San Diego, CA). After sequencing, two FASTQ files (read 1 and read 2) for each sample were generated on the MiSeq reporter software automatically. Raw 16S rRNA gene sequences are available at the Sequence Read Archive (SRA) under accession numbers SRX1904235.

2.3 Illumina high-throughput sequencing and analyzing

Raw sequences were processed and analyzed using the QIIME package (Kuczynski et al., 2011). The PE reads were firstly merged with overlap greater than 10 bp. Then, the adapter, barcodes and primers were removed from the merged sequences. Subsequently, the trimmed sequences with length shorter than 200 bp, quality score lower than 25, homologous longer than 8 bp, contained ambiguous characters were screened. Finally, chimeric sequences were identified using the Usearch61 algorithm and removed from the dataset. The optimized sequences were clustered into OTUs at the threshold of 97% similarity with the usearch61 algorithm. Single OTUs were removed and taxonomy was assigned to each OTU using the Ribosomal Database Project (RDP) classifier in QIIME, with a minimum confidence cutoff of 0.8 against the Silva reference database (silva 119,http://www.arb-silva.de/) to the genus level.

Alpha diversity was assessed by examining the rarefaction curves, shannon-wiener curves and rank-abundance curves calculated with Mothur (v.1.34.0; http://www.mothur.org) (Schloss et al., 2009) and visualized in R project (v.3.1.3; https://www.r-project.org/). Community richness estimators including the observed OTUs, nonparametric Chao1, ACE, and community diversity estimators including Shannon and Simpson indexes were also calculated with Mothur. Moreover, the Good's coverage was used to evaluate the sequencing depth.

Differences between polluted and non-polluted samples were tested by ANOSIM (Clarke, 1993). The ANOSIM R statistic is calculated on the basis of difference in mean ranks between and within groups. Linear discriminant analysis effect size (LEfSe, http://www.huttenhower.sph.harvard.edu/galaxy/) was applied to identify differentially abundant bacterial taxa associated with the polluted and non-polluted

fog episodes at genus or higher taxonomy levels (Segata et al., 2011).

2.4 Intercation between bacterial community structure and environmental variables

To determine the relationship between bacterial community structure and environmental variables, a detrended correspondence analysis (DCA) was first performed to estimate the length of the gradient. The resulting length (0.99) indicates a linear model was appropriate, hence RDA was subsequently performed. RDA was elaborated with the predominant bacteria data matrix and the environmental data matrix (Anderson & Willis, 2008). Interset correlations of this analysis were used to determine which environmental variables were the most important in determining the community structure. The cumulative fit per species as fraction of variance of species was performed to determine the importance of a species for the ordination space and which species were most associated with environmental factors. Analysis was performed in Canonical Community Ordination for windows (Canoco, v 4.5).

3. Results and discussions

3.1 Microbial community in fog water

Seven fog episodes from 24 July to 23 August 2014 were observed. Detail information was summarized in Table 1. Fog episodes can be classified as polluted and non-polluted according to the average $PM_{2.5}$ mass concentration.

Information on the bacterial community of fog water has been very scarce, our study provided comprehensive investigation of bacterial community under both polluted and non-polluted fog episodes. From the 13 samples collected during 7 fog episodes, a total of 232148 high quality sequences were obtained after quality filtering and OTUs ranged from 975 to 1258 (Table 2). This value was similar with the previous sequence-based survey of atmospheric bacteria (OTUs, 1214) (Katra et al., 2014). Identification of OTUs at different taxonomic levels yielded 359 species, 411 genera, 152 families, 70 orders, 38 classes and 26 phyla.

Rarefaction curves of observed OTUs continued to rise with increasing numbers of sequences (Figure S1), suggesting further sequencing will yield more species. However, the average Good's coverage of 13 samples was 97.2% (Table 2), indicating

a comprehensive sampling of the dominant microbial groups. Moreover, the Shannon-wiener and species accumulation curves reached plateau indicating a sufficient sequencing. For the Rank-abundance curves, the wide horizontal range and smooth curves reflect the rich abundance and even species distribution. The richness estimators Chao1 predicted 1491-1999 OTUs. Chao1 estimator for the polluted samples (1671) was similar to the non-polluted samples (1696). Diversity estimators Shannon and Simpson indexes fluctuated between polluted and non-polluted samples. Bacterial diversity was higher in non-polluted samples (polluted, 3.94; non-polluted, 4.42).

Across all samples collected from the 7 fog episodes, Proteobacteria was the dominant phylum, followed by Bacteroidetes, Cyanobacteria, Firmicutes, Deinococcus-Thermus, Actinobacteria and Nitrospirae (Figure 1). The bacterial community structure is similar to few other studies explored the bacterial diversity in cloud/fog samples, the aforementioned phyla contained a series of genera participate in the atmospheric hydrological cycle (Amato et al., 2007b; Delort et al., 2010). They are predominant taxa in clouds at a high elevation determined by sanger sequencing and tagged pyrosequencing (Bowers et al., 2009), and which are also the typical culturable heterotrophic bacteria from clouds broadly distributed in aquatic and terrestrial habitats (Amato et al., 2005; Kourtev et al., 2011). In the present study, Figure 2 shows the dominant genera collected during fog process. For the 7 fog episodes, the predominant genera from Proteobacteria were similar, including *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas*, *Sphingomonas*, *Massilia*, *Delftia*, *Brevundimonas*. These bacteria contained a metabolically diverse group found in a wide range of water/soil habitats. For instance, *Acinetobacter*, the most abundant genera widely distributed in land or ocean, are contribute to the biodegradation, leaching and removal of several organic and inorganic man-made hazardous wastes (Abdelelhaleem, 2003). *Stenotrophomonas* and *Pseudomonas*, which are well-known for the striking capability to utilize numerous carbon sources, have been widely utilized in the degradation and transformation of complex organic compounds in a wide range of habitats (Boonchan et al., 1998; Stanier et al., 2010). *Sphingomonas*, has reported the ability to survive in low concentrations of nutrients, metabolize a series of carbon compounds, events toxic compounds (Xu et al., 2006). Similar to *Sphingomonas*, members of *Brevundimonas* are well known for their ability to withstand extreme harsh environment (Kopcakova et al., 2014). *Massilia*, isolated

from air samples, could participate in the biodegradation and transport of Phenanthrene (Gu et al., 2016). *Empedobacter* from Bacteroidetes are widely distributed in water habitats, since the human clinical origins, *Empedobacter* are ranked as potential pathogens (Hugo et al., 2005). *Bacillus* included in the phylum Firmicutes commonly found in soil and water, are also found in air samples (Suominen et al., 2001). Similar to *Pseudomonads*, some strains of *Bacillus* could produce biosurfactants that can act as cloud condensation nuclei (Delort et al., 2010). Moreover, members of *Deinococcus* from Deinococcus-Thermus are well known for their ability to withstand extreme radiation conditions that could potentially adapt to the cloud environment (Mattimore & Battista, 1996). The identification of bacteria adapt to specific environments in fog/cloud water (low temperature, harsh nutrition and high radiation environment) with the potential role in the nucleation, metabolism of organic pollutants, demonstrated the potential importance of participation and influence the atmospheric biochemistry cycle.

3.2 Implications in human health and ecosystem

Bioaerosols have been complex assemblages of airborne and exogenic microorganisms, many of which likely emissions and resuspension from various terrestrial environments, e.g., soil, water, plants, animals or human beings. In the atmosphere, bacteria including pathogenic or beneficial species can be attached to particles or incorporated into water droplets of clouds/fog. During fog process, they can be deposited back to land via deposition and possibly induce infections to human health and impose effect on the diversity and function of aquatic and terrestrial ecosystems (Figure 3).

In the present study, the presence of potential pathogen sequences indicated occasional distribution and dispersion of pathogens in fog water. The levels of opportunistic pathogens found in polluted fog episodes are comparable to non-polluted samples (Table 3). The identified opportunistic pathogens, e.g., *Empedobacter brevis*, can be easily isolated from clinical resources, which may be associated with eye infections (Bottone et al., 1992). Occurrence of *Staphylococcus equorum* in fog water can be expected since *Staphylococcus* are frequently isolated from airborne samples (Seo et al., 2008). As important pathogens, they can reside on the skin and mucous membranes of humans and induce server infections (Novákov á

et al., 2006). Similarly, the *Brevundimonas vesicularis* and *Brevundimonas diminuta* can induce virulent infections, often associated with nervous system or bacteraemia (Gilad et al., 2009; Han & Andrade, 2005). Besides that, the *Acinetobacter schindleri* and *Moraxella osloensis* are associated with skin and wound infections, bacteremia and pneumonia (Banks et al., 2007; Nemec et al., 2001). Previous studies has showed health risk-related bacteria in atmospheric samples, including rainwater (Cho & Jang, 2014), which can be part of atmospheric life cycle and dispersal pathway for some pathogenic bacteria. Possibly, greater survival of human pathogens may be supported in the atmosphere. Since the dispersion of these opportunistic pathogens via aerosol and fog droplets will cause infection of skin tissue and internal organs, pathogens in fog water need special attention.

The identified ecological function bacteria mainly participated in the biodegradation of organic compounds, such as *Rhodococcus ruber*, *Sphingomonas faeni*, *Delftia tsuruhatensis*, *Comamonas testosterone* (Table 3). Excessive studies have illustrated their capability of metabolism of hydrocarbon compounds, even toxic pollutants, e.g., aromatic compounds (Bock et al., 1996; Busse et al., 2003; Geng et al., 2009; Goyal & Zylstra, 1996). *Stenotrophomonas rhizophila* and *Phyllobacterium myrsinacearum* are two typical rhizospheric microorganisms. As plant-associated strains, S. rhizophila fulfill plant-protective roles and have been safely applied in biotechnology (Alavi et al., 2013). *Phyllobacterium myrsinacearum*, which is a predominant rhizospheric bacterium, its capability of azotification has made the utilization in plant growth promotion and biological control of soil-borne diseases (Gonzalezbashan et al., 2000). In addition to the potential impact on human health and ecosystem, there are extremophiles, e.g., *Deinococcus aquatili*, which is radiation-resistant and well adapted to the harsh atmospheric conditions (Kämpfer et al., 2009), *Pseudomonas psychrotolerans*, a psychrotolerant bacterium, could grow at 4^{o}C (Hauser et al., 2004). Overall, fog water seems to harbor highly diverse bacterial communities in ecosystem, which may be the atmospheric mixing of diverse point sources origins in the rhizosphere, soil and water and possibly participate in the biodegradation of organic compounds in fog water.

3.3 Disparity between polluted and non-polluted fog episodes

Although the predominant bacteria are similar between polluted and non-polluted fog

episodes, significant disparity within bacterial taxa are also identified. ANOSIM analysis suggest that the OTUs of the polluted samples were grouped into one large cluster, and were distantly related to the non-polluted clusters (ANOSIM comparison, R =0.579, P＜0.05). Cluster analysis including PCoA and Hcluster indicated that the bacterial communities in polluted samples, regardless of the fog episodes, were highly similar (Figure S2). Cluster analyses based on the relative abundance of genera showed similar clustering patterns (Figure 4), and the polluted samples also shared high similarity in their bacterial community structure.

To find specialized bacterial groups within the polluted and non-polluted fog episodes, LEfSe is performed, which showed statistically significant differences. A total of 70 bacterial groups were distinct using the default logarithmic (LDA) value of 2. Cladograms show taxa with LDA values higher than 3.5 for clarity (Figure 5). Consequently, 8 and 19 differentially represent bacterial taxa in polluted and non-polluted fog episodes were detected.

In polluted fog episodes, most indicated bacteria were ranked as opportunistic pathogens, such as Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Stenotrophomonas, Haliscomenobacter, and Saprospiraceae. Like Proteobacteria, the Gram-negative Gammaproteobacteria contains a series of ecologically and medically important bacteria, e.g., the pathogenic Enterobacteriaceae, Vibrionaceae and Pseudomonadaceae. The Xanthomonadales from this phylum has been reported to cause disease in plants (Saddler & Bradbury, 2005). Certain species from Stenotrophomonas (Gammaproteobacteria, Xanthomonadales) have been associated with a variety of infections in humans. The Saprospiraceae, a family within the phylum Bacteroidetes, include the genus *Haliscomenobacter*, are typical planktonic bacteria isolated from aquatic environments, such as marine, freshwater and activated sludge. The notable ability for the hydrolysis and utilization of complex carbon compounds has been illustrated (McIlroy & Nielsen, 2014).

In comparison, the majority of detected taxa in the non-polluted samples are from Bacteroidetes, Firmicutes, Alphaproteobacteria, Betaproteobacteria and Deinococcus-Thermus. An important biomarker from Bacteroidetes is Flavobacteriia, relative study has illustrated the marine sources for Flavobacteria, most of Flavobacteria sequences searched by NCBI blast are mainly from marine sources, i.e., algae, oysters and sea cucumbers (Cho & Jang, 2014). The genera *Empedobacter* was

abundant across all samples, which are included in the family Flavobacteriaceae. As mentioned above, *Empedobacter* and *Staphylococcus* **(**Firmicutes) are potential pathogens and resistant to a wide range of antimicrobials (Hugo et al., 2005; Trilla & Miro, 1995). Phyllobacteriaceae (Alphaproteobacteria) are typical rhizobia. Similar with Phyllobacterium, members of Oxalobacteraceae (Betaproteobacteria) are rhizosphere microorganisms involved in the biological nitrogen fixation (Donn et al., 2015). The Burkholderiales (Betaproteobacteria) commonly found in water and soil are involved in the biodegradation of various forms of aromatic compounds (Pérez-Pantoja et al., 2012). Another important group of indicator bacteria was Deinococci from the phylum of Deinococcus-Thermus, which include many resistant species, as well as several thermophiles, could metabolize toxic materials, resistant to extreme radiation and survive in extremes of heat and cold (Griffiths & Gupta, 2007). Although bacteria with the potential function of nitrogen fixing (Phyllobacterium from Alphaproteobacteria) and degradation of organic compounds (Burkholderiales from Betaproteobacteria) were distinguished both in polluted and non-polluted episodes, a significant distinct was observed. By comparison, potential pathogens were significant groups in the polluted samples, whereas a diverse ecological function group of bacteria were identified in the non-polluted samples originated from a wide range of habitat. Ecologically meaningful distinguish of bacterial groups under polluted and non-polluted conditions is essential for understanding the structure and function of bacterial communities, and which provide a general understanding of the metabolism of bacteria in fog water.

3.4 Environmental factors shaping bacterial community structure

To clarity the vital environmental variable in shaping bacterial community structure, RDA was performed to discern the genus-level structure with the selected environmental factors (Figure 6). The first two axes explained 73.3% of the accumulated variance in the species-environment relation. Interset correlations showed $PM_{2.5}$ was the most important environmental variable structuring the bacterial community (axis 1, -0.328); in turn, temperature registered the highest value for axis 2 (0.368) (Table S1). Cumulative fit indicated that the predominant genera affiliated with groups from *Acinetobacter*, *Empedobacter*, *Phyllobacterium*, *Aeromonas* and *Prevotella* displayed strong correlations with axis 1, *Streptococcus*,

Stenotrophomonas, *Brevundimonas*, *Deinococcus* and *Pseudomonas* were the notable genera highly correlations with axis 2 (Table S2). As aforementioned in section 3.3, the above bacteria were metabolically diverse groups found in various habitats.

Of the atmospheric environmental characteristics measured, $PM_{2.5}$ was the best predictor of variability in diversity levels within the dominant genera and strongly correlated with represent bacterial genera. The composition of bacterial communities was highly variable under $PM_{2.5}$ mass concentration in this study, which was consistent with the previous study that $PM_{2.5}$ was important environmental factor shaping the variation of community composition (Cao et al., 2014). Moreover, statistical analysis, e.g., correlation or multiple linear regression, indicated that $PM_{2.5}$ exhibited a negative correlation with airborne bacteria during (Gandolfi et al., 2015; Gao et al., 2015) whereas in another study, spearman correlation analysis showed $PM_{2.5}$ exhibited a significant positive correlations with the airborne microbe concentration during hazy days (Dong et al., 2016). Previous study has suggest that nutrients are related to the distribution of bacteria in water habitats (Newton et al., 2011). Possibly, the inorganic and organic compounds in particulate matter ($PM_{2.5}$) can be available nutrients for microbial growth. However, aggregation of harmful substances such as heavy metals, polycyclic aromatic hydrocarbons would be toxic for bacteria under high $PM_{2.5}$ mass concentration. Since the $PM_{2.5}$'s two-sided influence on bacterial growth, bacterial community both under polluted and non-polluted samples were significantly correlated with $PM_{2.5}$ mass concentrations.

The identified taxa either from polluted or non-polluted samples were found in soil, water, plant or human skins. These bacterial groups are aerosolized and dispersed in the air, and partly from local regional emissions or long-term transport. Source tracking analysis by the backward trajectory indicated that the air mass of polluted fog episodes came largely from northern and western China, moved east through Shanxi, Henan, Hebei province to the study area, or from outer Mongolia, crossing the Jingjinji area to Mt.Tai, The passed areas were notable as heavy industry region with frequent coal mining activities and serious pollution. Moreover, the large population and agricultural activities resulted in the numerous pathogenic microorganisms from human or animal fecal dispersed in the atmosphere. Moreover, a small part from southern China, and the passed region were rich of water resources, e.g., Dongting Lake, Huaihe river, Yangtze river etc. The aquatic bacteria such as *Haliscomenobacter* dispersed in the atmosphere typically derived from the

evaporation of lakes and rivers water (Figure 7). In contrary, air mass of non-polluted fog episodes originated mostly from the southern China, the marine sources bacteria (Flavobacteria) indicated the release of prokaryotes into the air from sea-air interactions and airborne marine bacteria can be transported to inland through long-term transport.

In addition, wind rose diagram suggest the predominant north wind during polluted fog episodes and wind speeds ranged 1.2-1.6 m/s, whereas in non-polluted fog episodes it was mainly south wind with higher wind speed (2.1-3.5 m/s) than the polluted fog episodes. Wind direction and speed are important meteorological factors influencing fog formation (Fu et al., 2014). Recent studies also indicate the variation of bacterial concentration and community structure conducted by wind (Evans et al., 2006; Jones & Harrison, 2004). In the present study, air mass from the contaminated area through long-term transport or local regional emission combined with lower wind speeds, largely reduce the diffusion rate of pollutants and thus lead to the sustained high $PM_{2.5}$ during polluted fog episodes. Whereas in the non-polluted fog episodes, higher wind speed was beneficial to the diffusion of pollutants and resulted in the lower $PM_{2.5}$ mass concentration, which finally led to the significant discrepancy of bacterial community structure. However, further research still needed to address the detailed interaction between bacterial community and environmental factors, and understanding the mechanism that how chemical composition influence microbial community.

4. Conclusion

In summary, this work on fog water provided a thorough investigation on bacterial ecological diversity under polluted and non-polluted fog episodes, enhanced understanding the distribution and dispersion of bacteria and their potential involvements in ecosystem variation and human health. To some degree, $PM_{2.5}$ seams a pivotal variable in shaping bacterial community, which is likely to provide a more comprehensive understanding of the factors controlling the atmospheric water biodiversity under environmental stress. These results provide a basic understanding of mechanism of bacterial community response and metabolism in polluted weather for further study.

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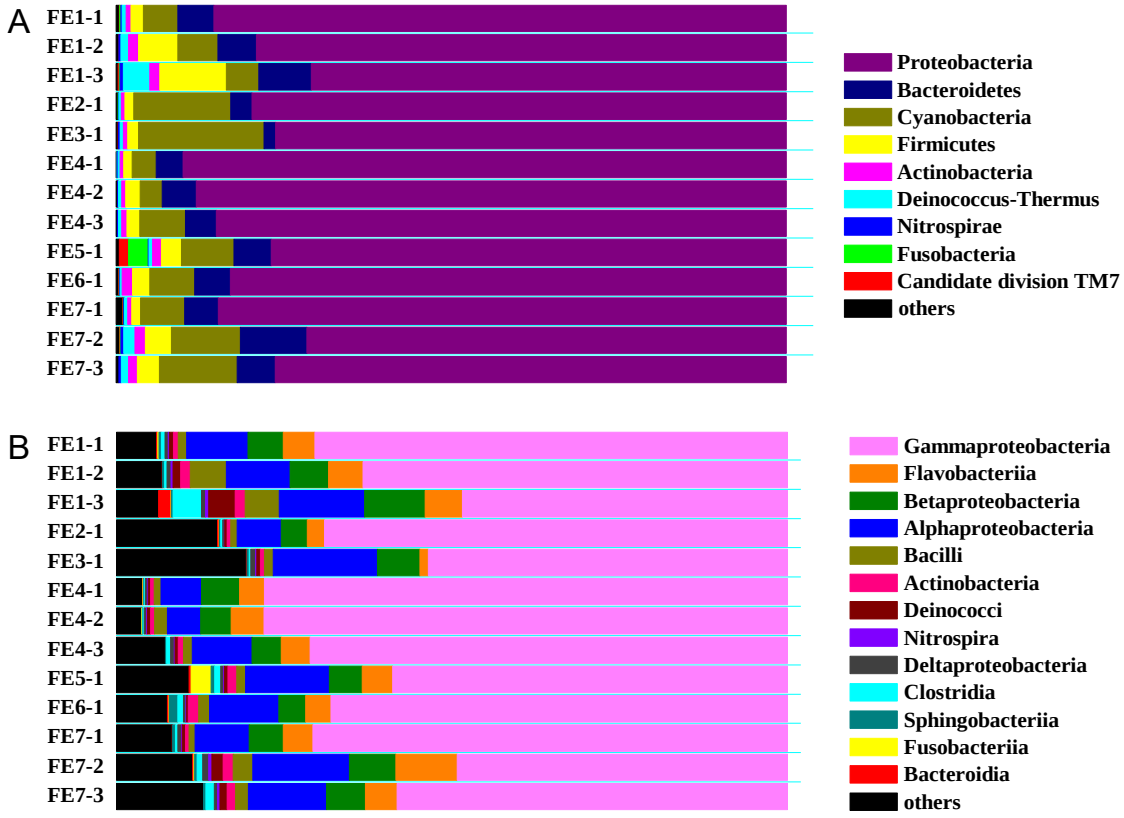

Figure 1 Bacterial community variation for the fog episodes at the phylum and class level. Predominant taxa higher than 1% are indicated in the bar graphs.

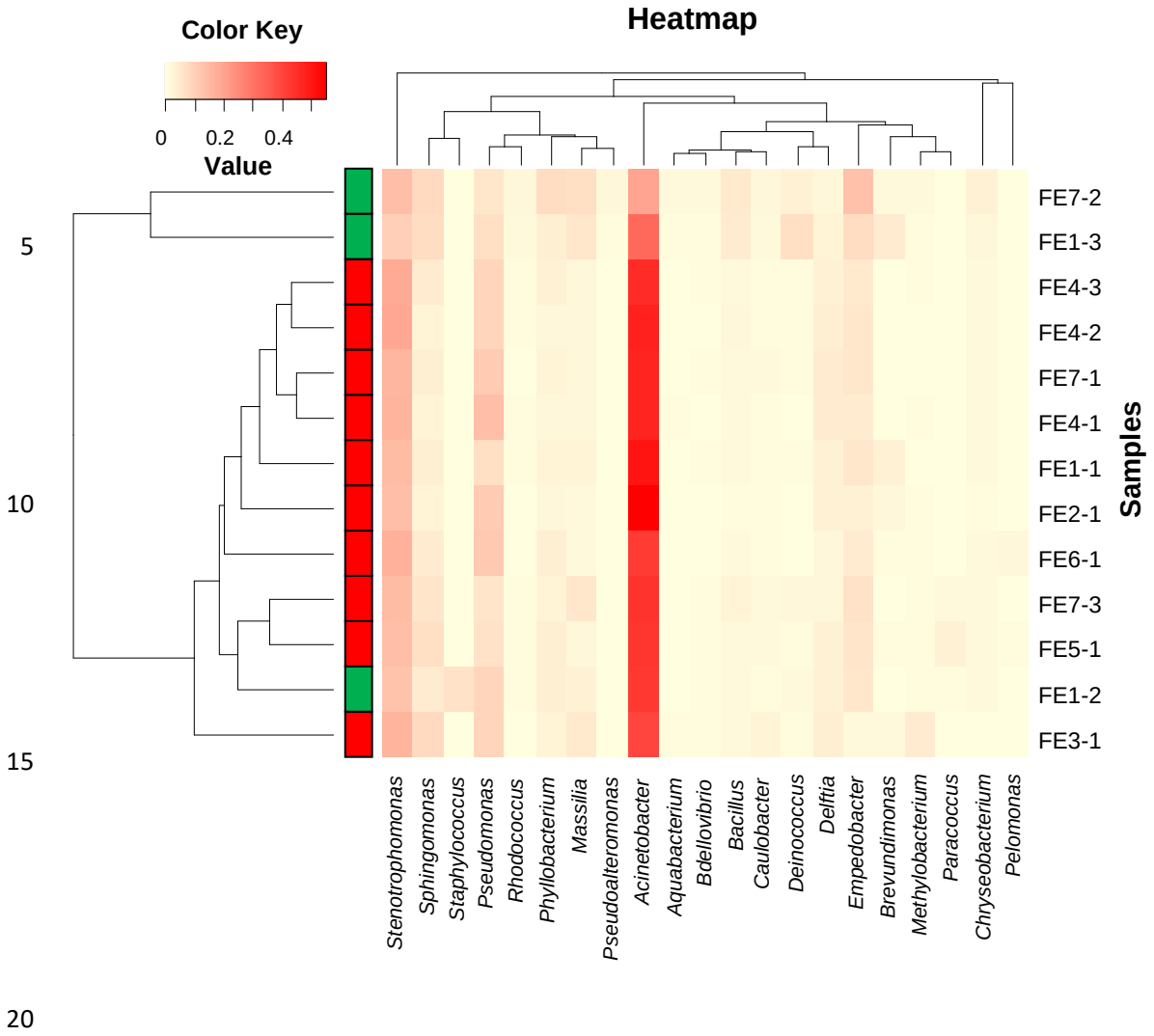

Figure 2 Hierarchically clustered heatmap of the predominant bacterial genus distribution under polluted and non-polluted fog episodes. Polluted fog water samples are indicated by red square, non-polluted samples are green.

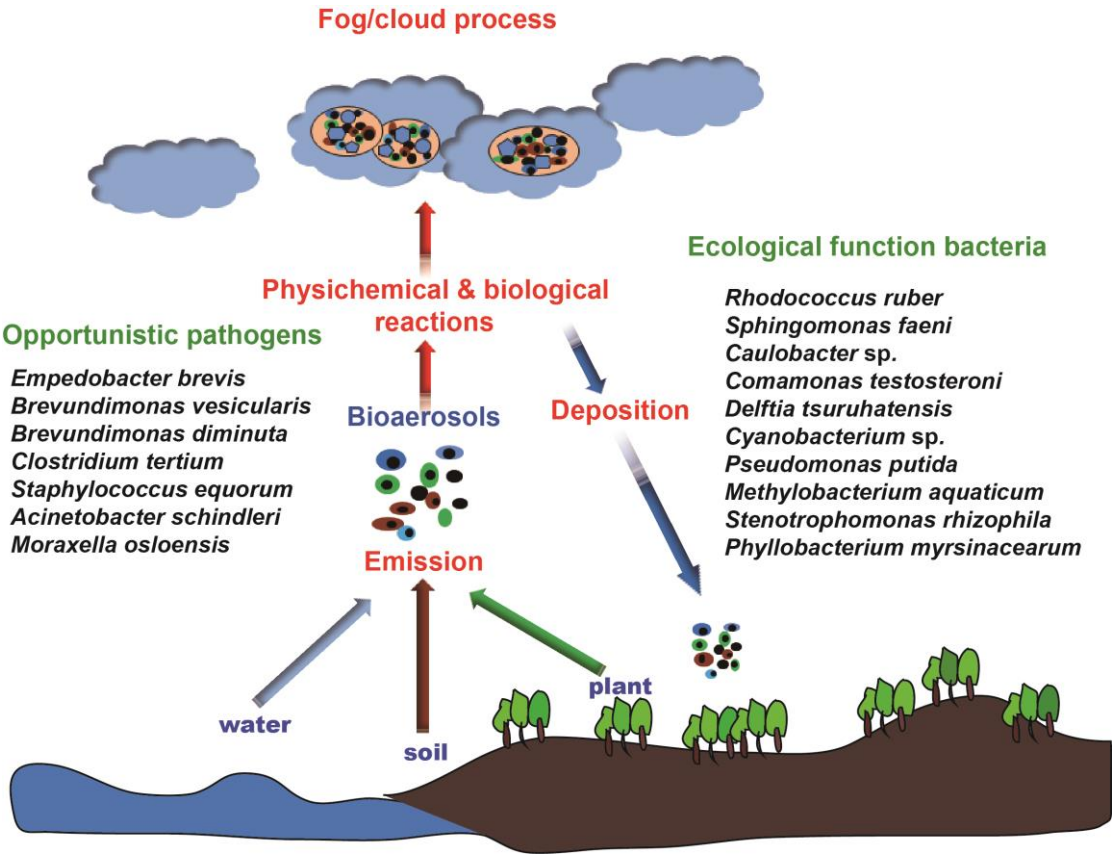

Figure 3 Schematic representation of the life cycle and potential influence on the ecosystem of bioaerosols in the atmosphere, modified from Poeschl (Poeschl, 2006). The predominant identified bacteria species with potential ecological functions are indicated in the figure.

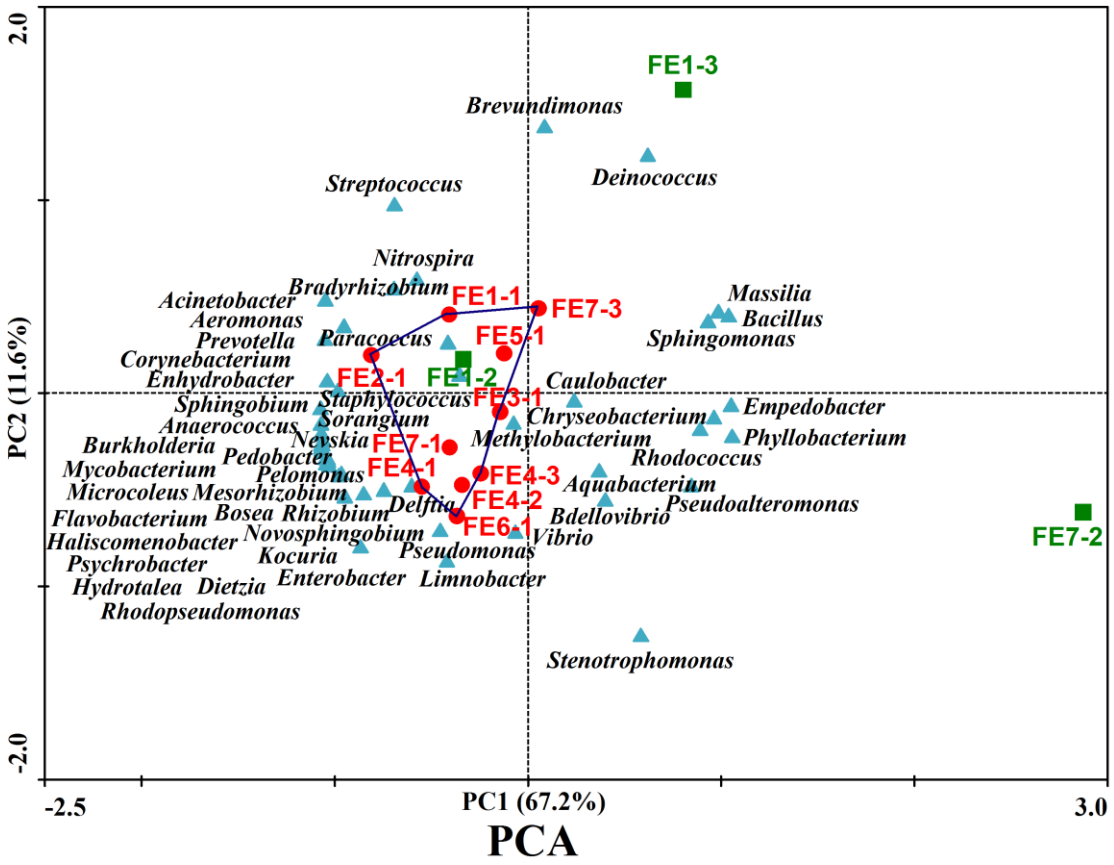

Figure 4 Principal component analysis shows the bacterial community variability between polluted and non-polluted fog episodes. Samples in the same group indicate the cluster similarity.

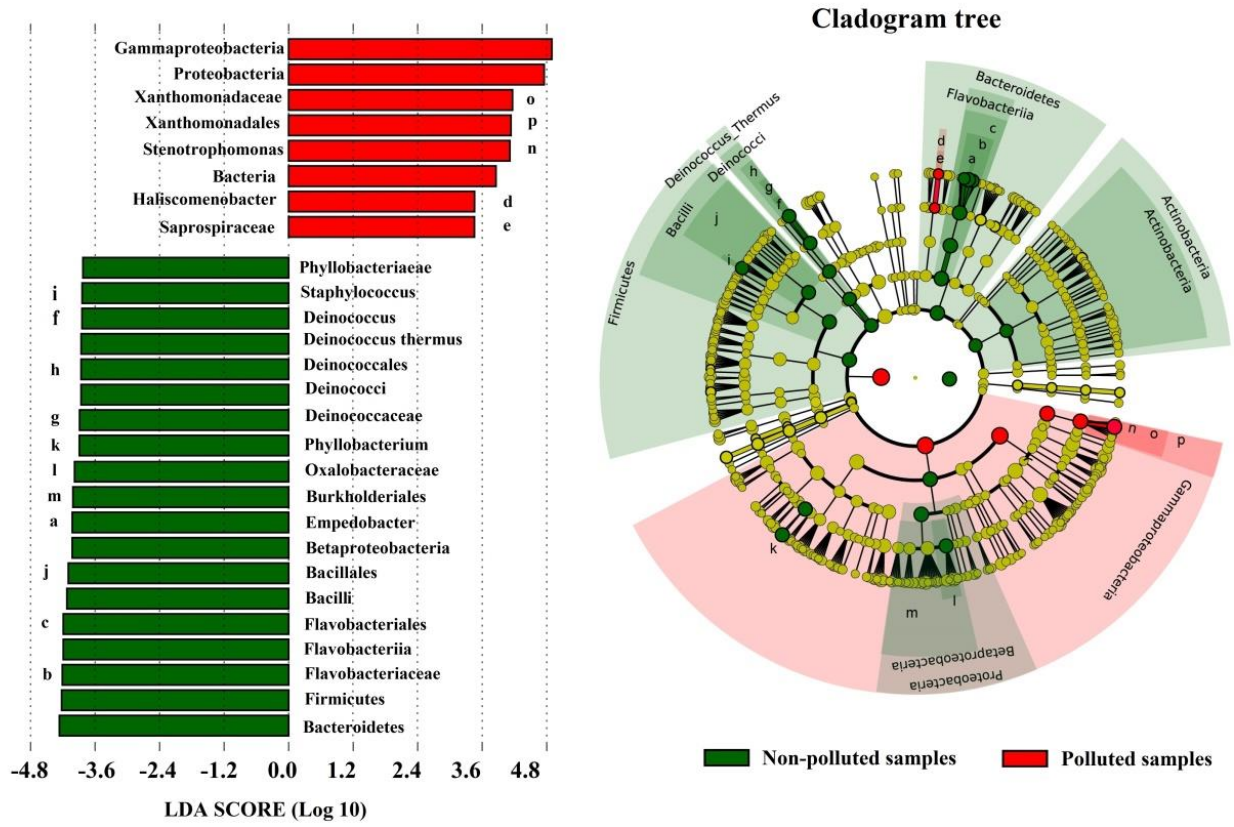

Figure 5 Cladogram of phylogenetic relationships of bacterial lineages associated with polluted and non-polluted fog episodes; taxa with LDA values higher than 3.5 by LEfSe are displayed. Differences are represented with different color (red indicating polluted fog episodes, green non-polluted fog episodes, and yellow nonsignificant).

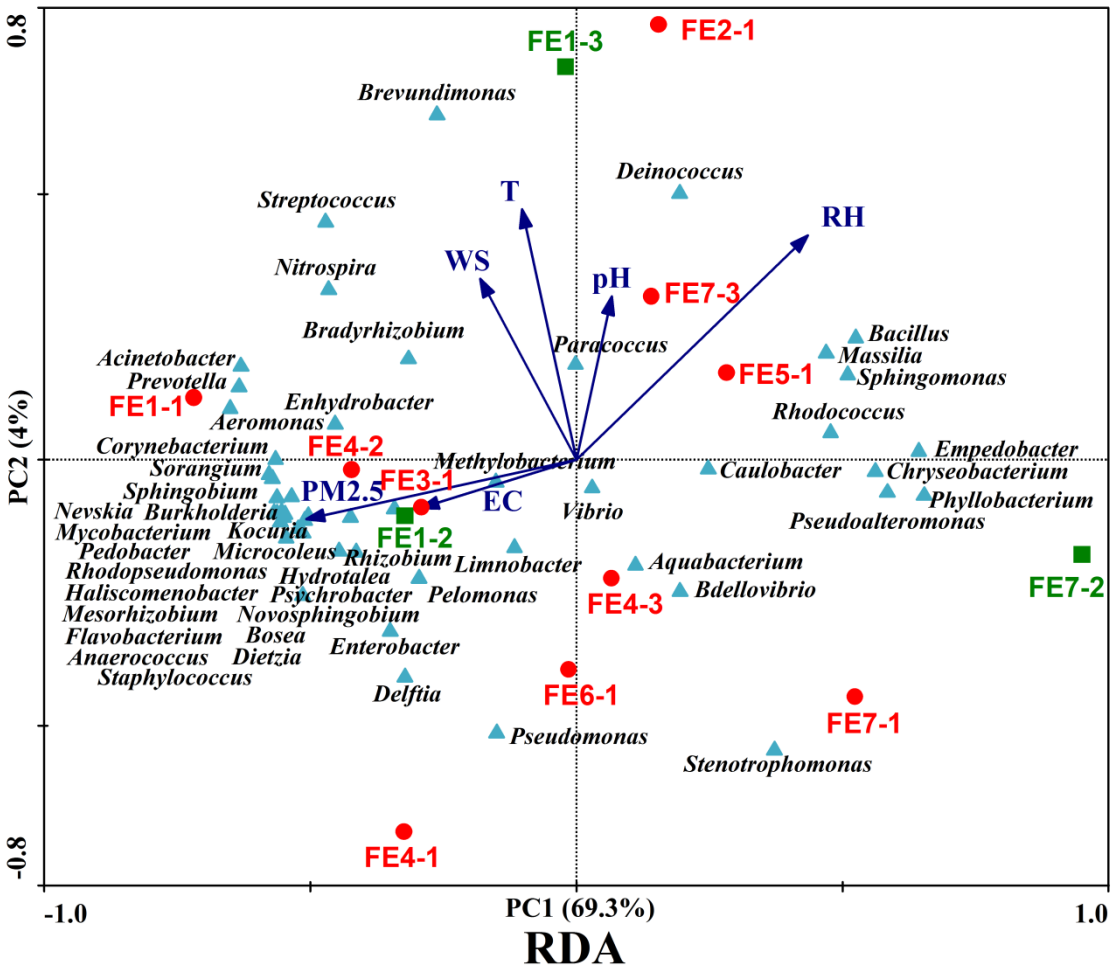

Figure 6 Biplot of the environmental variables and predominant genera using a redundancy analysis (RDA) model. Species data are listed in Table S2. The selected environmental variables are significant ($P < 0.05$) using Monte Carlo permutation testing. Species are labeled with triangle, the closer of which indicates existence in similar environment. Environmental variables are showed by arrows; the relative length is positive correlation with the importance in influencing bacterial community structure. The angle between the arrow and the ordination axis suggest the variable response respect to the RDA gradient.

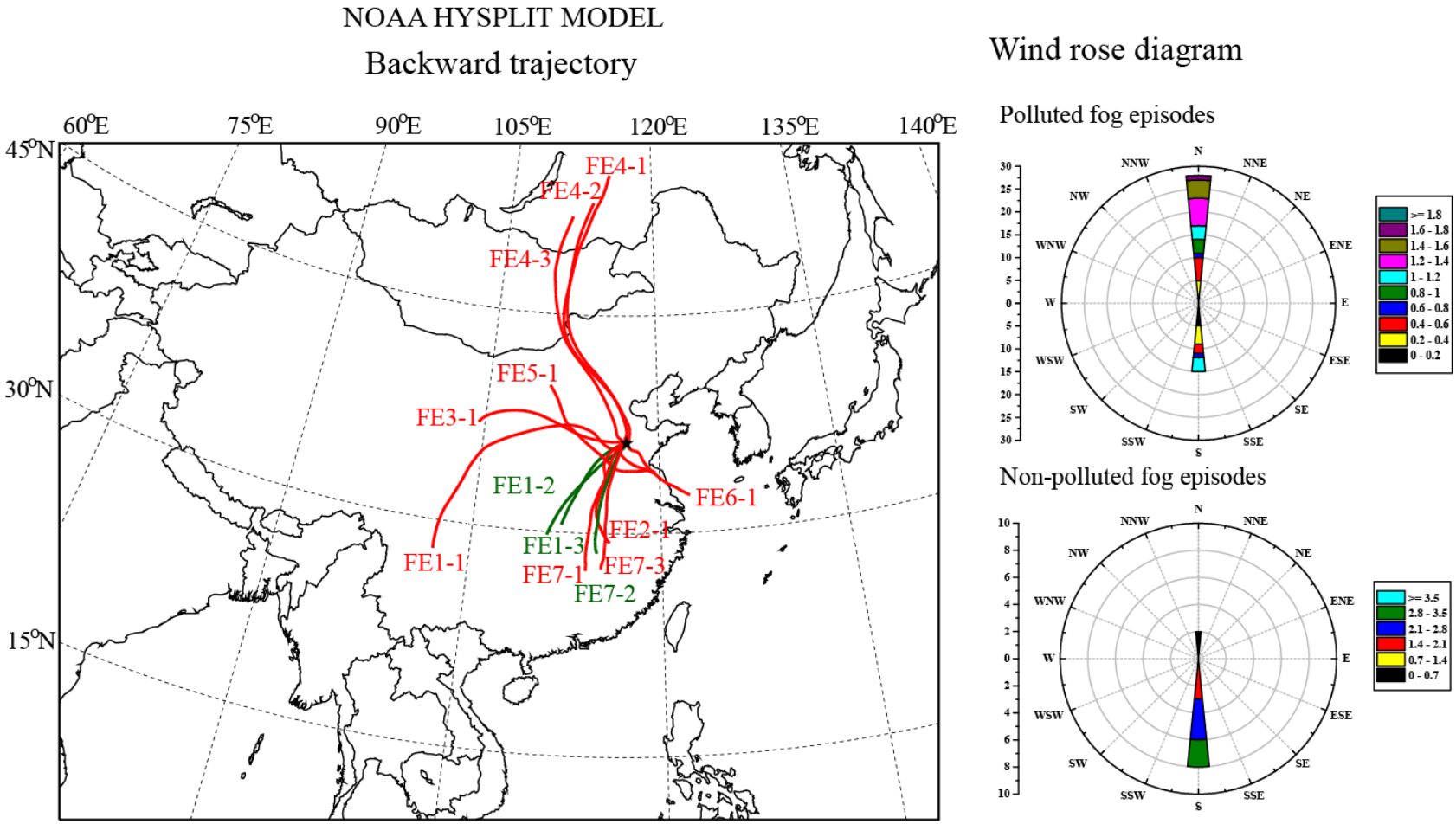

Figure 7 Air mass transport pathways for the fog episodes using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model. 24-hour backward trajectories were calculated for air parcels arriving at 1534 m above sea level. Wind Rose Diagram of Study Area during sampling time.

Table 1 Description fog episodes at Mt.Tai, China

Fog episodes	Data	Samples	Start time (BJT)	Stop time (BJT)	Duration (h)	$PM_{2.5}$ ($\mu g \cdot m^{-3}$)	pH	EC ($\mu S \cdot cm^{-1}$)	Pollution
FE1	24 Jul 2014	FE1-1	8:50	15:30	6:40	105.07	4.03	583	A
		FE1-2	15:30	17:30	2:00	22.35	4.32	219.2	B
		FE1-3	17:30	22:51	5:21	14.66	5.74	104.4	B
FE2	5 Aug 2014	FE2-1	6:45	9:17	2:32	30.36	5.80	275.7	A
FE3	5 Aug - 6 Aug 2014	FE3-1	19:05	4:01	8:56	42.25	5.10	501	A
FE4	14 Aug - 15 Aug 2014	FE4-1	22:41	0:44	2:03	42.69	6.36	170.4	A
		FE4-2	0:44	5:06	4:22	47.98	5.34	86.34	A
		FE4-3	5:06	6:03	0:57	36.88	4.89	64.95	A
FE5	17 Aug 2014	FE5-1	10:10	11:18	1:08	63.18	5.20	120.5	A
FE6	17 Aug - 18 Aug 2014	FE6-1	22:18	1:25	3:07	54.33	3.80	321.8	A
FE7	23 Aug 2014	FE7-1	2:30	4:38	2:08	30.45	4.38	356.2	A
		FE7-2	4:38	6:21	1:43	23.39	5.01	207.5	B
		FE7-3	6:21	9:20	2:59	41.60	5.74	187.6	A

BJT refers to Beijing Time, which equals UTC + 8. EC refers to the electric conductivity.
The A, B refers to the the polluted and non-polluted samples based on the WHO 24-hr average standard $PM_{2.5}$ mass concentration ($PM_{2.5}$ = 25 $\mu g \cdot m^{-3}$), respectively.

Table 2 Summary of bacterial diversity and richness of fog water

Sample ID	Reads	OTUs	Ace	Chao1	Coverage	Shannon	Simpson
FE1-1	18213	975	1835	1491	0.9761	3.9418	0.0646
FE1-2	18702	1184	1841	1730	0.9719	4.1919	0.0620
FE1-3	17662	1173	1689	1687	0.9732	4.7067	0.0327
FE2-1	19914	1125	1756	1684	0.9744	3.9582	0.0630
FE3-2	18199	1022	2082	1582	0.9734	3.9749	0.0647
FE4-1	18350	941	1828	1461	0.9762	3.6041	0.0953
FE4-2	17707	967	1522	1427	0.9752	3.6748	0.0902
FE4-3	17397	981	2091	1611	0.9725	3.8074	0.0832
FE5-1	16384	1132	1814	1790	0.9676	4.3173	0.0546
FE6-1	16896	1186	1997	1872	0.9657	4.1268	0.0666
FE7-1	16350	1103	2501	1795	0.965	3.9040	0.0810
FE7-2	18252	1150	1732	1673	0.9729	4.3709	0.0426
FE7-3	18122	1258	1958	1999	0.9686	4.3776	0.0531
Aerosol (Katra et al., 2014)	4020	1412		2142	0.8300		
Rain water in July (Cho & Jang, 2014)	3055	1542	13083	6387			
$PM_{2.5}$ in summer (Franzetti et al., 2011)		2222		4036			

The diversity indexes including OTUs, ace, Chao1, coverage, shannon, simpson were defined at 97% sequence similarity.

Table 3 The predominate bacterial species identified in fog water samples

Species	Habitat	Ecological roles	Polluted (%)	Non-polluted (%)
Empedobacter brevis[BA]	soil/water/plant	Opportunistic pathogens	2.8796	4.8345
Brevundimonas vesicularis[BP]	soil/water	Opportunistic pathogens	0.2126	0.8271
Brevundimonas diminuta[BP]	soil/water	Opportunistic pathogens	0.2811	0.0579
Clostridium tertium[FR]	soil/faeces	Opportunistic pathogens	0.0269	0.0055
Staphylococcus equorum[GP]	soil/water/annimal	Opportunistic pathogens	0.0044	0.0089
Acinetobacter schindleri[GP]	soil/water	Opportunistic pathogens	0.0084	0.0238
Moraxella osloensis[GP]	soil/animal	Opportunistic pathogens	0.097	0.0962
Rhodococcus ruber[AC]	soil/water	Biodegradation	0.3314	0.6226
Chryseobacterium aquaticum[BA]	soil/water	Protect and promote plants growth	0.0307	0.073
Chryseobacterium jejuense[BA]	soil/water	Protect and promote plants growth	0.6698	1.0107
Deinococcus aquatilis[DT]	soil/water	Extremophiles, radiation-resistant	0.0193	0.036
Novosphingobium aromaticivorans[AP]	soil/water	Biodegradation	0.2178	0.2007
Sphingomonas faeni[AP]	soil/water	Biodegradation	0.0659	0.1799
Sphingomonas kaistensis[AP]	soil/water	Biodegradation	0.0529	0.0417
Sphingomonas leidyi[AP]	soil/water	Biodegradation	0.0305	0.0149
Methylobacterium aquaticum[AP]	water	Methylotrophic, carbon fixing	0.0915	0.0955
Methylobacterium adhaesivum[AP]	soil/water	Methylotrophic, carbon fixing	0.0574	0.0808

Caulobacter sp.[AP]	water	Biodegradation	0.6916	0.7264
Phyllobacterium myrsinacearum[AP]	soil/plant	Rhizosphere bacteria, nitrogen fixation	1.3408	2.3989
Comamonas testosteroni[BP]	soil/water	Biodegradation	0.0788	0.0502
Delftia tsuruhatensis[BP]	soil/water	Biodegradation	1.9164	1.3085
Cyanobacterium sp.[CY]	soil/water	Carbon and nitrogen fixing	1.8229	0.1444
Pseudomonas geniculata[GP]	soil/water/plant	Biodegradation	0.0516	0.0293
Pseudomonas putida[GP]	water/siol	Biodegradation/protect and promote plants growth	0.0263	0.0457
Pseudomonas psychrotolerans[GP]	soil/water	Extremophiles, psychrotolerant	0.0324	0.0217
Stenotrophomonas rhizophila[GP]	soil/water/plant	Rhizosphere bacteria, plant-protective	0.6016	1.1263

Abbreviates are as followed: AP, Alphaproteobacteria; BP, Betaproteobacteria; GP, Gammaproteobacteria; AC, Actinobacteria; BA, Bacteroidetes; CY, Cyanobacteria; DT, Deinococcus-Thermus; FR, Firmicutes. Biodegradation refers to the bacteria associated with the biodegradation of organic compounds, even toxic pollutants, e.g., aromatic compounds.