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

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Abstract:

Bacteria are widely distributed in atmospheric aerosols and are indispensable components of clouds, playing an important role in the atmospheric hydrological cycle. However, limited information is available about the bacterial community structure and function, especially for the increasing air pollution in the North China Plain. Here, we present a comprehensive characterization of bacterial community composition, function, variation and environmental influence for cloud water collected at Mt. Tai from 24 Jul to 23 Aug 2014. Using Miseq 16S rRNA gene sequencing, the highly diverse bacterial community in cloud water and the predominant phyla of Proteobacteria, Bacteroidetes, Cyanobacteria and Firmicutes were investigated. Bacteria that survive at low temperature, radiation, and poor nutrient conditions were found in cloud water, suggesting adaptation to an extreme environment. The bacterial gene functions predicted from the 16S rRNA gene using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) suggested that the pathways related to metabolism and disease infections were significantly correlated with the predominant genera. The abundant genera *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas*, and *Empedobacter* originated from a wide range of habitats including cloud condensation nuclei and ice nuclei active species, opportunistic pathogens and functional species, demonstrating the importance of bacterial ecology and health in cloud water. Cluster 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 potential pathogens were enriched in the polluted cloud samples whereas the diverse ecological function groups were significant in the non-polluted samples. Discrepant community structure determined by redundancy analysis (RDA) indicated that the major ions in cloud water and $PM_{2.5}$ in the atmosphere have negative impact on bacteria, playing a vital role in shaping microbial community structure. The major ions might provide nutrition to bacteria and directly influence the bacterial community, whereas $PM_{2.5}$ in air has an indirect impact on bacterial community structure. During wet deposition, soluble particulate matter was dissolved in water droplets resulting in elevated concentration in cloud water. $PM_{2.5}$ was possibly associated with different origins and pathways of air mass as determined using source tracking by the backward trajectory and wind analysis, mainly related to long-term transport. This work enhanced our understanding of the characteristics of bacterial ecology in the atmospheric aqueous phase, highlighting the potential influence of environmental variables on the bacterial community in cloud processes. It may provide fundamental information of the bacterial community response in cloud water under increasing pollution. However, due to the limited sample size (13 samples) collected at the summit of Mt. Tai, these issues need in-depth discussion. Further studies based on an annual series of field observation experiments and laboratory simulations will continue to track these issues.

Key words:cloud water, 16r RNA gene, function prediction, major ions, $PM_{2.5}$

1. Introduction

Clouds are the aerosol system composed of tiny droplets suspended in the atmosphere. In the atmosphere, pollutants attached to particles can be dissolved or incorporated into cloud droplets, which may have complex effects on environment security and human health. Over the past decades, studies on 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 cloud characteristics, studies on bioaerosols have been on the rise.

Living microorganisms, including bacteria, fungi and yeasts have been shown to be present in clouds (Burrows et al., 2009). In the first study on biological particles in fog/cloud water, Fuzzi et al (1997) suggested bacterial replication on foggy days. Later, 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 field investigations and laboratory experiments, diverse bacterial communities have been retrieved, and the bacterial metabolism active in cloud water has been further demonstrated. In the 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 cloud water are available to metabolize organic carbon compounds (degrading organic acids, formate, acetate, lactate, and succinate) and are associated with carbon and nitrogen recycling (Amato et al., 2007a; Hill et al., 2007; Vaïtilingom et al., 2010). They can also influence photochemical reactions (Vaïtilingom et al., 2013) and participate in a series of complex and diverse biochemical metabolic activities.

Cloud occurrence is a complex process. In contaminated areas, clouds typically contains numerous pollutants such as 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). As an intensive agricultural and economic region in China, the North China Plain has been affected by severe air pollution in recent years, for instance, the severe fog and haze pollution in Beijing, Ji'nan in January 2013 (Huang et al., 2014; Wang et al., 2014). Mt. Tai (36°15′ N, 117°06′ E, and 1534 m a.s.l), the highest mountain in the North China Plain, is frequently attacked by cloud episodes (Guo et al., 2012a; Liu et al., 2012). Emissions and resuspension of bacteria by wind erosion or splashing water from various terrestrial environments into the atmosphere recruits diverse airborne bacteria, which possibly include opportunistic and functional bacteria. During the cloud formation process, these bacteria attached to particles or incorporated in cloud droplets will be deposited back to land via dry

or wet deposition. Accumulating literature indicates that bacteria in cloud/fog water droplets have a potential effect on the diversity and function of atmospheric and terrestrial ecosystems (Delort et al., 2010; Vaïtilingom et al., 2013), even inducing health risks through microbial pathogens dispersion (Vaïtilingom et al., 2012). Previous studies have examined the bacterial community in rain or snow (Cho & Jang, 2014; Mortazavi et al., 2015). They also focus on the bacteria associated with CNN/IN, potential pathogens and biochemical reactions. Therefore, evaluation of the potential ecologically functional bacteria in cloud water has been an urgent issue, especially for polluted cloud episodes.

Notably, atmospheric microorganisms are subject to a wide range of environmental conditions including meteorological factors and the physiochemical composition of aerosols (Womack et al., 2010). Community structure and function are closely related to the environmental characteristics in atmosphere and the geomorphic characteristics (Dong et al., 2016; Gao et al., 2016). For instance, studies about inhalable bioaerosols suggest that environmental parameters including temperature, relative humidity, PM_{10}, $PM_{2.5}$ and particle size have significant impact on the composition and dynamics of microbial communities (Adhikari et al., 2006; Bowers et al., 2013). However, owing to the paucity of detailed and comprehensive studies on atmospheric bacterial composition, our understanding of the bacterial community dynamics remains incomplete. During the polluted cloud process, the bacterial community variation, and the environmental factors playing a decisive role in shaping bacterial community structure are still scarcely studied.

In the present study, samples from typical cloud episodes under polluted and non-polluted weather conditions were collected on the summit of Mt. Tai in the North China Plain. To understand the bacterial community structure and function, 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 factors influencing the bacterial community. Air mass back trajectory was conducted to define the most likely source and transmission paths of the pollutants and bacteria.

2. Material and methods

2.1 Sample collection

Cloud water samples were collected using the Caltech Active Strand Cloud water Collector (CASCC) on the summit of Mt. Tai (36°15′ N, 117°06′ E, and 1534 m a.s.l). The collector was cleaned prior to each cloud event and kept closed prior to cloud interception to ensure not to be contaminated. The collector will be activated by a sensor only when cloud formed in the ambient air. Cloud water was

aspirated through a Teflon duct at a rate of 24.5 m^3 min^{-1} by a fan situated at the rear of collector. Water was collected from the Teflon strands, through Teflon tube and down into Teflon bottles. The theoretical 50% cut-off size was equivalently drop diameter of 3.5 μm.

To avoid artificial and instrumental contamination, the Teflon tube and the polyethylene bottles were pretreated with anhydrous ethanol and washed 3 times using the sterilized ultrapure water. Before sampling, the collector was washed with the sterilized deionized distilled water filtered through 0.22 μm membrane. The sterilized dd-H_2O was sprayed into the collector and the collected water sample was as the blank.

To distinguish the polluted and non-polluted cloud episodes, we firstly checked the air pollution condition according to the 24 h WHO air quality guideline ($PM_{2.5}$ concentrations, 25 μg/m^3). This standard has been applied in Australia, New Zealand and European Union. A cloud episode with the average $PM_{2.5}$ concentration higher than 25 μg/m^3 was considered as polluted. Further definition of cloud water was combined with the major ions in water droplets, which provide deep insight into pollution levels. Therefore, in the present study, cloud episodes under high $PM_{2.5}$ concentration and high concentration ions in cloud water were classified into polluted episodes.

After adjustment, seven cloud episodes including 13 samples were obtained during the whole sampling period (from 24 July to 23 August 2014), including 11 polluted and 2 non-polluted cloud water samples (Figure S1). 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 cloud water, the pH and conductivity was detected with a Multi pH/COND/TEMP 6350 hand held Meter immediately after sampling. The major inorganic ions (Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+) were quantified using the ion-chromatography system (Dionex ICS-90). Hourly data, e.g., meteorological parameters, and $PM_{2.5}$ were measured to evaluate air quality during cloud episodes (Table 1). The meteorological parameters including air temperature, relative humidity, wind direction, and wind speed were measured with an automatic meteorological station (PC-4, JZYG, China) *in situ*. The $PM_{2.5}$ mass concentration 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 cloud 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 during cloud process was drawn to clarify the predominant wind direction and wind speed (origin, version 9.0, Origin Lab Corporation, Northampton, MA).

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 manufacturer's directions. The concentration of DNA was determined spectrophotometrically (Nano-Drop 2000, Thermo, Wilmington, Delaware, USA). To check sample contamination, DNA was extracted through an identical extraction procedure for the blank samples. These blanks were PCR amplified together with the DNA samples extracted from cloud water samples. For the blank, no obvious bands and target fragment was detected by examination of electrophoretic gel images.

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 mixture 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 packages (Kuczynski et al., 2011). The pair-end reads were firstly merged with overlap length 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 representative 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, 2012b; Luo & Angelidaki, 2014).

5   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 (Langille et al., 2013; Corrigan et al., 2015; Wu et al., 2016). In the present study, the phylogenetic and functional

10   capacities for the bacteria in cloud water are of great interest to help understanding their roles in atmosphere, ecosystem, and health. Bacterial community functional profiles were predicted from 16S rRNA gene using the PICRUSt program and annotated against with the Kyoto Encyclopedia of Gene and Genomes (KEGG) database. Spearman's correlation coefficients were calculated to link the pairwise comparison of KEGG pathway and genus. Selected KEGG pathways related to metabolism

15   and disease infection, and predominant genera are included in the heatmap. Correlation is significant at P-value 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 were visualized in R project (v.3.1.3; https://www.r-project.org/). Community richness

20   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.

Hierarchical cluster (Hcluster) and Principal coordinate analysis (PCoA) were performed to visualize the changes in bacterial community for the collected samples. Hcluster and PCoA plots were

25   constructed depending on Bray-Curtis similarity index calculated with the abundance of OTUs using the Biodiversity package (Kindt & Coe, 2005) in R. The difference in OTU composition for samples collected in polluted and non-polluted cloud episodes was tested by the analysis of similarity (ANOSIM) (Clarke, 1993). ANOSIM was implemented with the VEGAN package in R. Linear discriminant analysis effect size (LEfSe, http://www.huttenhower.sph.harvard.edu/galaxy/) was

30   applied to identify differentially abundant bacterial taxa associated with polluted and non-polluted cloud episodes at genus or higher taxonomy levels (Segata et al., 2011). For all statistical tests, the P-value less than 0.05 and 0.01 was considered significant.

2.4 Interaction between bacterial community structure and environmental variables

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

3. Results and discussions

3.1 Definition of polluted and non-polluted cloud episodes

In the present study, we first defined cloud water samples according to the air pollution conditions. In the condition of air pollution, the collected cloud water was considered as polluted sample. However, in Hcluster and PCoA analysis (Figure S3), sample CE1-2 (the non-polluted sample) was separated from other non-polluted samples but closed to the polluted samples. Reclassification of cloud water samples were combined with the major ions in water droplets. By checking the major ions, we observed that although the $PM_{2.5}$ concentration for sample CE1-2 was low, a relative high major ions concentration was detected (Figure S1). Therefore, we categorized sample CE1-2 as polluted sample. The cluster and PCoA analysis also confirmed the reclassification (Figure S4).

Although the predominant bacteria are similar between polluted and non-polluted cloud episodes, significant disparity are also identified. ANOSIM analysis suggest that OTUs from polluted samples were grouped into one large cluster, and separated from the non-polluted clusters (ANOSIM comparison, R =0.683, p<0.05). Cluster analysis including PCoA and Hcluster indicated a highly similar community composition in polluted samples, regardless of the cloud episodes (Figure S3). Principal component analysis based on the relative abundance of genera showed similar clustering patterns (Figure S4), and the polluted samples also shared high similarity in the bacterial community structure.

3.2 Microbial community in cloud water

Information on bacterial community in fog/cloud droplets are scarce, our study provided comprehensive investigation of bacterial community. From the 13 samples collected during 7 cloud episodes, a total of 232148 high quality sequences were obtained after quality filtering, and OTUs ranged from 975 to 1258 (Table 2). This was similar with other sequence-based survey such as the atmospheric bacteria in dust storm (1214, Katra et al., 2014) and bacteria in rain water in July (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). These taxa are predominant bacteria in clouds 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 S5 shows the dominant genera collected during cloud 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 were also similar to other studies of microorganisms in fog/cloud water. Fuzzi et al. (1997) investigated bacteria in fog droplets in a highly polluted area and found the predominant genera were *Pseudomonas*, *Bacillus* and *Acinetobacter*. Amato et al (2007b) observed more diverse genera from the phylum of Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes, 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 *Pseudomonas* and *Acinetobacter*.

In the bacterial community, the aforementioned taxa contained a series of species participating in the atmospheric hydrological and biochemical cycle (Amato et al., 2007b; Delort et al., 2010). Community function analysis estimated with PICRUSt algorithm confirmed the viewpoint. After PICRUSt analysis, pathways with participants less than 10% were removed, leaving 225 non-human-gene KEGG pathways. These predominant pathways 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 (Figure S6). Besides the pathways associated with microbial physiological metabolism, we focused on the pathways of microbial metabolism in a variety of environments. Fog/cloud

droplets contains carbon and nitrogen compounds, which could be available substrate for microbial growth 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 from 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 correlated with carbohydrate metabolism and glycan biosynthesis and metabolism, are well-known for the striking capability to utilize numerous carbon sources. They 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 cloud water, a series 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 variety of carbon compounds, even toxic compounds (Xu et al., 2006). Similar to *Sphingomonas*, members of *Brevundimonas* are well known to withstand extreme harsh environment (Kopcakova et al., 2014). The spore forming bacteria *Bacillus* from Firmicutes are commonly found in bioaerosol, cloud water, rainwater and could survive in cold environment (Despré́s 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 in cloud water were psychrophiles, they grow faster 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).

Although most bacterial ecophysiological role in biogeochemical cycles is generally established based on soils and water habitats, information about bacterial activity in cloud water is available. The identification of microorganisms in barren nutrition, low temperatures and radiation environment encountered in clouds is expected since similar bacterial species have been retrieved and proved to be active in harsh environments. Their adaption to the specific environments in fog/cloud water with potential role in the nucleation, metabolism of organic pollutants, demonstrated the potential importance in atmospheric biochemistry cycle.

3.3 Implications in human health and ecosystem

Bacteria in fog/cloud water have been discovered for decades but detailed information on community composition and potential ecophysiological role 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/cloud or rain process, they can be deposited back to land via deposition and possibly cause human infections and affect the diversity and function of aquatic/terrestrial ecosystems (Kaushik & Balasubramanian, 2012; Simmons et al., 2001; Vaïtilingom et al., 2012) (Figure 3 and Table 3).

Atmospheric bacteria are efficient cloud condensation nuclei, and water vapour can be condensed on bacterial cell surface (Mohler et al., 2008). The hygroscopic growth of bacteria below water saturation and supersaturations has been observed for some species, e.g. Bauer et al. (2003) found that *Brevundimonas diminuta* was activated at ＜0.1% supersaturation. Strains from *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 size droplets and facilitate rain formation (Mohler et al., 2007). Moreover, *Pseudomonas* could induce ice nucleation at a warmer temperature than usual (Amato et al., 2015). Simulations experiments about cloud forming suggest that *Pseudomonas* was first acted as CCN, then induced freezing and ice nucleation process (Mohler et al., 2008). In addition to *Pseudomonas*, other bacteria from *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Sphingomonas*, and *Stenotrophomonas* sp. (Table 3), were ice nucleation active (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, microorganisms living in fog/cloud may play a vital role in atmospheric biochemistry. The detection of bacteria in cloud water associated with biotransformation of organic compounds raised a general understanding of the potential role in atmospheric chemistry. The identified species from *Pseudomonas*, *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). Cloud 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 cloud water.

Besides that, bacterial genera containing potential pathogens were especially concerned after sequencing. 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 cloud 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). Occurrences of *Staphylococcus equorum* in cloud 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 (Nováková 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). Moreover, 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 cloud/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 (Vaïtilingom et al., 2012). They find potential plant pathogens

such as *Pseudomonas syringae* and *Xanthomonas campestris* and suggest these living 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 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.

3.4 Disparity between polluted and non-polluted cloud episodes

To distinguish indicator species within the polluted and non-polluted cloud episodes, LEfSe was 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 cloud episodes were detected.

In polluted cloud episodes, most enriched bacteria were ranked as opportunistic pathogens, such as Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Stenotrophomonas, Moraxellaceae and *Acinetobacter*. 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. *Moraxella* form Moraxellaceae (Gammaproteobacteria) has been reported to associate with septic arthritis of the ankle (Banks et al., 2007). As previous mentioned, species from the genus of *Acinetobacter* are opportunistic pathogens and cause severe clinical infections (Nemec et al., 2001).

In comparison, the majority of indicator species in the non-polluted samples are from Bacteroidetes, Firmicutes, Betaproteobacteria and Deinococcus-Thermus. An important biomarker from Bacteroidetes was 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* (Firmicutes) are potential pathogens and resistant to a wide range of antimicrobials (Hugo et al., 2005). Clostridiales (Clostridia) and *Bacillus* (Bacillaceae) are two represent biomarkers from Firmicutes. As ubiquitous in nature, these two groups contain some medically significant species (Miller et al., 2001; Makino & Cheun, 2003). Moreover, their specific physiological characteristics (produce a variety of enzymes and metabolites) and excellent ability to decomposition of organic matter have made the widely utilization in biotechnology and fermentation industry (Doi et al., 1993; Łoś, et al., 2010). 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).

By comparison, potential pathogens were significant groups in the polluted samples, whereas diverse ecological function groups were identified in the non-polluted samples originated from a wide range of habitats. Ecologically meaningful distinguish of bacterial groups under polluted and non-polluted conditions is essential for understanding the structure and function which provided a general understanding of bacterial metabolism in cloud water.

3.5 Environmental factors shaping the bacterial community structure

To clarity the vital environmental factor in shaping the bacterial community structure, RDA was performed to discern the genus-level structure with the selected environmental factors (Figure 5). The first two axes explained 65.9% of the accumulated variance in the species-environment relation. Interset correlations showed major ions and $PM_{2.5}$ was the most important environmental variables structuring the bacterial community (axis 1, major ions, -0.436; $PM_{2.5}$, -0.367); in turn, wind speed and temperature registered the high value for axis 2 (wind speed, -0.509; temperature, -0.494) (Table S1).

Cumulative fit indicated that the predominant genera affiliated with *Acinetobacter*, *Bacillus*, *Corynebacterium*, *Phyllobacterium*, *Pseudoalteromonas* and *Rhodococcus* displayed strong correlations with axis 1. *Empedobacter*, *Hydrotalea*, *Paracoccus*, *Pelomonas*, *Pseudomonas* and *Stenotrophomonas* 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 and certain genera included potential pathogens.

Of the environmental characteristics measured, major ions in cloud water and PM$_{2.5}$ were the crucial predictors of diversity variability of bacterial community structure. These two parameters were strongly correlated with the represent bacterial genera. As indicated in Figure S1, significant positive correlation was observed between major ions (x) and PM$_{2.5}$ (y) (y=0.00477x+5.324, p<0.01,R^2=0.757). Relevant studies suggested that bacterial community was highly variable under different PM$_{2.5}$ mass concentration (Cao et al., 2014). 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). Possibly, the inorganic and organic compounds in particulate matter (PM$_{2.5}$) can be available nutrients for microbial growth in air. However, aggregation of harmful substances such as heavy metals, polycyclic aromatic hydrocarbons under high PM$_{2.5}$ mass concentration would be toxic for bacteria.

During cloud process, most atmospheric particles (including PM$_{2.5}$) are scavenged in cloud water. In polluted air, high PM$_{2.5}$ concentration resulted in the elevated water soluble inorganic ions in cloud droplets. Therefore, similar trends were observed between major ions and PM$_{2.5}$ concentration. In cloud water, the major water soluble ions and microorganisms co-exist in the same microenvironment. Major ions could provide available nutrition for bacterial growth and duplication. Previous study has suggested that these nutrients are related to the distribution of bacteria in water habitats (Newton et al., 2011). Meanwhile, PICRUSt analysis in section 3.2 discovered a series of metabolic pathway involved in the bacterial basic physiological activities and carbon, nitrogen, sulfur metabolism (Figure S6). For example, magnesium and calcium are involved in a series of physiological activities (e.g., signal regulation, transmembrane transport), the sulfate, nitrate and ammonium can be available substrates for bacterial growth (Fagerbakke, et al., 1999; Fiermonte, et al., 2004; Michiels et al., 2002). Therefore, major ions were important environmental factor shaping community structure in cloud water. PM$_{2.5}$ played an indirect role on bacterial community by influencing the concentration of major ions in water droplets.

The identified taxa either from polluted or non-polluted samples were typically found in soil, water, plant or human beings. 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 cloud 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 (Figure 6). The passed areas were notable 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 air. In contrary, air mass of non-polluted cloud episodes originated mostly from the southern China, and the passed region were rich of water resources, e.g., Dongting Lake, Huaihe river, Yangtze river etc. The marine sources bacteria (Flavobacteria, significant biomarker in non-polluted cloud water samples by LefSE, Figure 4) dispersed in the atmosphere typically derived from the evaporation of lakes and rivers water. These bacteria mainly originated from sea-air interactions, and the marine bacteria can be transported to inland through long-term transport.

In the sampling site (the summit 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 west wind during polluted cloud episodes and wind speeds ranged 1.2-1.4 m/s, whereas in non-polluted cloud episodes it was mainly southwest wind with higher wind speed (2.4-3 m/s) (Figure 7). Wind direction and speed are important meteorological factors influencing fog/cloud 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 addition, wind and $PM_{2.5}$ distribution graph during the whole sampling time (from 24 July to 23 August 2014) (Figure 7C) indicates that $PM_{2.5}$ concentration was high under low wind speed, whereas $PM_{2.5}$ was lower with high wind speed. 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 cloud episodes. In polluted air, the soluble composition in $PM_{2.5}$ was accumulated in atmosphere and could be dissolved in cloud water droplets during wet deposition. Therefore, the concentration of water soluble ions increased under high $PM_{2.5}$ concentration, which has directly influence on microbial community. Whereas in the non-polluted cloud 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 need to address the detailed interaction between bacterial community and environmental factors, and to understand the mechanism of bacterial community response to chemical composition in clouds.

4 Conclusion

The composition and potential function of microbial communities in the atmospheric water phase (fog and clouds) remained rarely studied. Using 16S rRNA gene sequencing, this study present a comprehensive investigation of bacterial ecological diversity under polluted and non-polluted cloud

episodes and revealed a highly diverse bacterial community harbored in cloud water. Correlation analysis for the predominant genera and PICRUSt function predication enhanced our understanding of the distribution of bacteria and their potential involvements in the atmosphere, ecosystem and human health. Identification of bacteria surviving in poor nutrition, low temperatures and radiation environments encountered in fog/cloud water demonstrated bacterial activity in harsh atmospheric environments. They may act as efficient cloud condensation nuclei or ice nuclei, associate with biogeochemical cycling (nitrogen/carbon cycling), 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 cloud episodes suggested major ions in cloud water seem to be pivotal in shaping bacterial communities. $PM_{2.5}$ had a potential impact on bacterial community structure by influencing the major ions in water droplets, which is likely to provide a more comprehensive understanding of atmospheric microbial biodiversity under environmental stress. These results provide a basic understanding of the mechanism of bacterial community response and metabolism in polluted weather for further studies. However, due to limited sampling size and collected volume, the aforementioned focus needs further discussion. Continuous annual observation and culture-dependent experiments will be performed to target the detailed functions of the atmospheric bacterial community.

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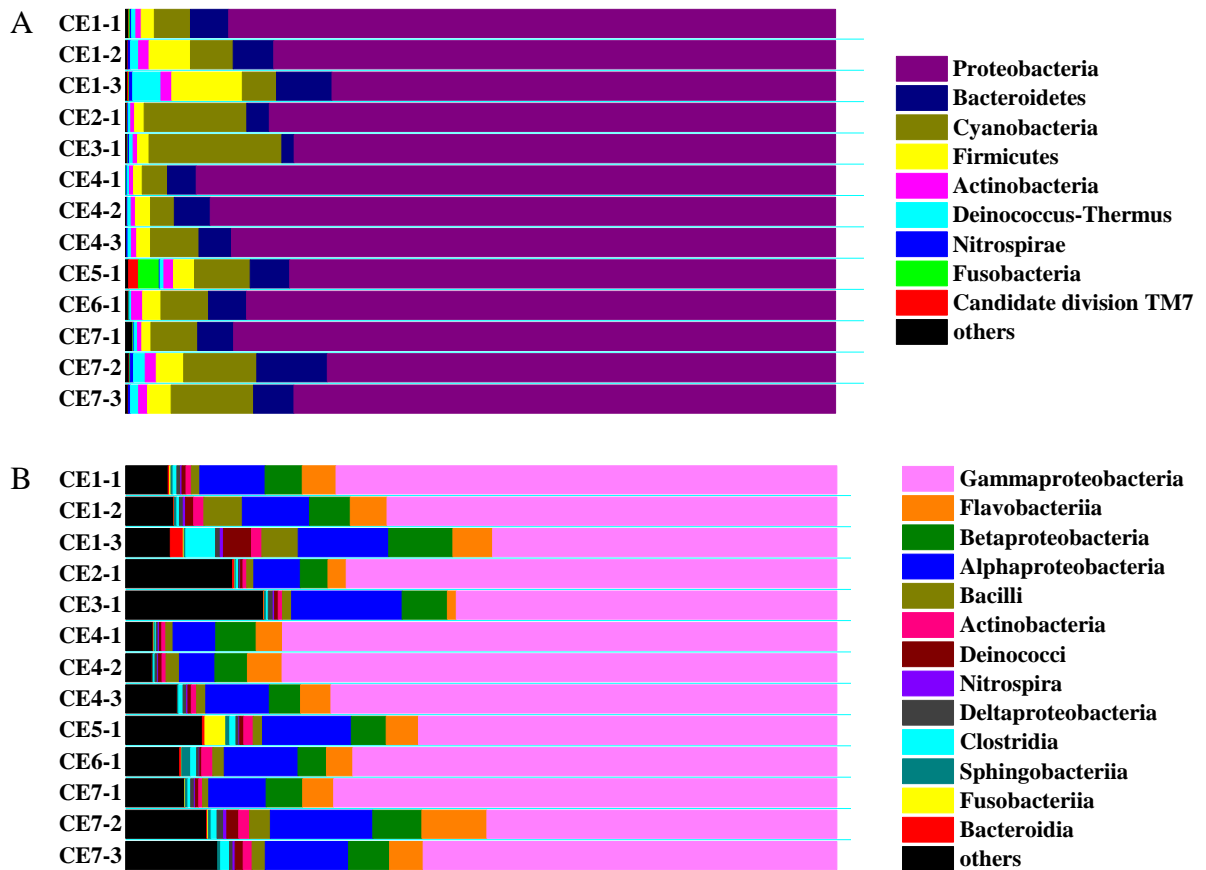
Figure 1 Bacterial community variation for cloud episodes at the phylum (A) and class (B) level. CE refers to the cloud episodes. Bar graphs for each sample represent the percentage of taxa assigned to each phylum with 80% bootstrap confidence. Taxonomic summary of the most abundant taxa (more than 1%) across all cloud samples are indicated in the figure.
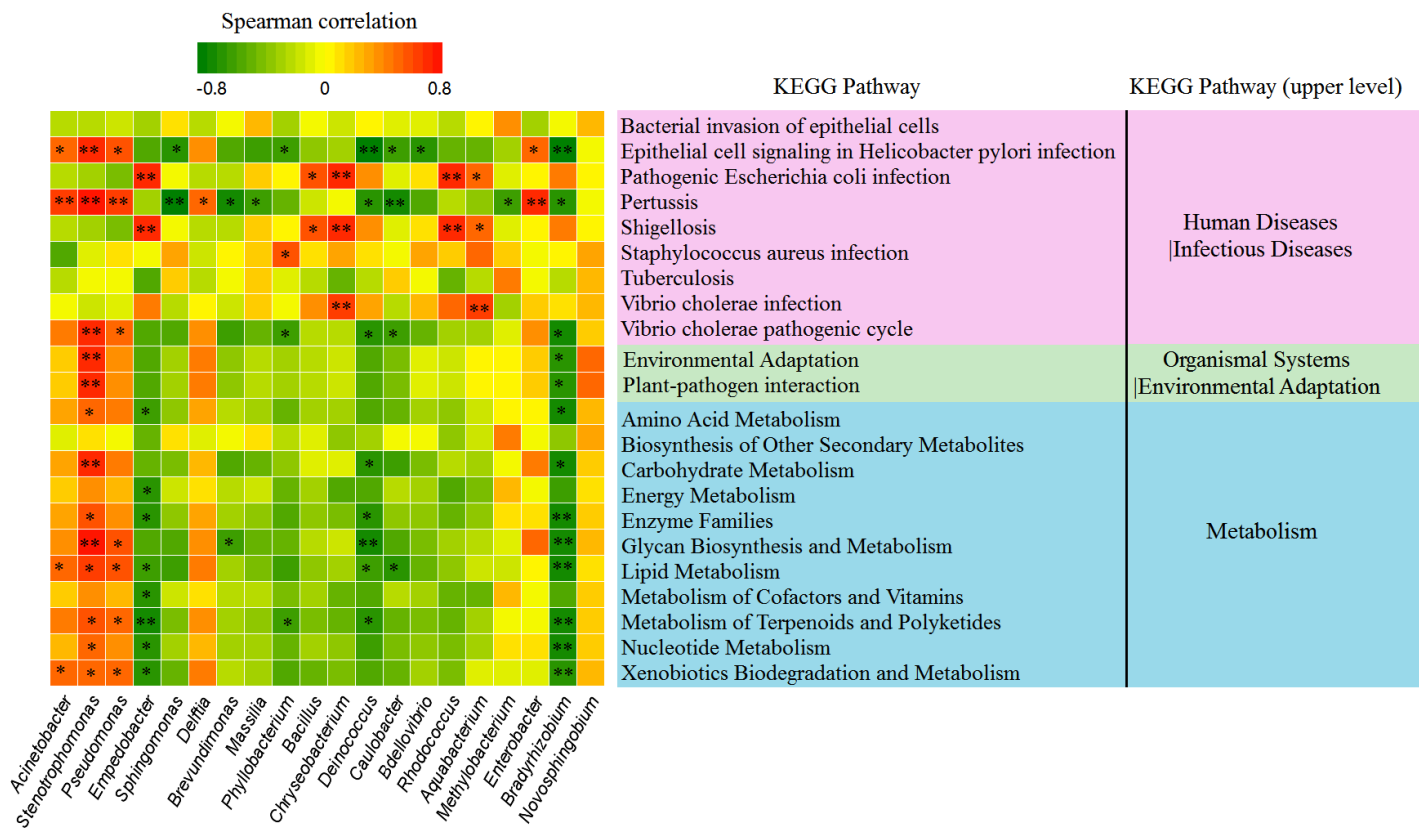
Figure 2 Bacterial taxa are related to KEGG pathways. Bacterial gene functions were predicted based on 16S rRNA gene sequences using the PICRUSt algorithm and annotated from KEGG databases. Spearman's correlation coefficients were calculated for each pairwise comparison of genus and KEGG pathway. Selected KEGG pathways related 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 $*P < 0.05$, $**P < 0.01$.
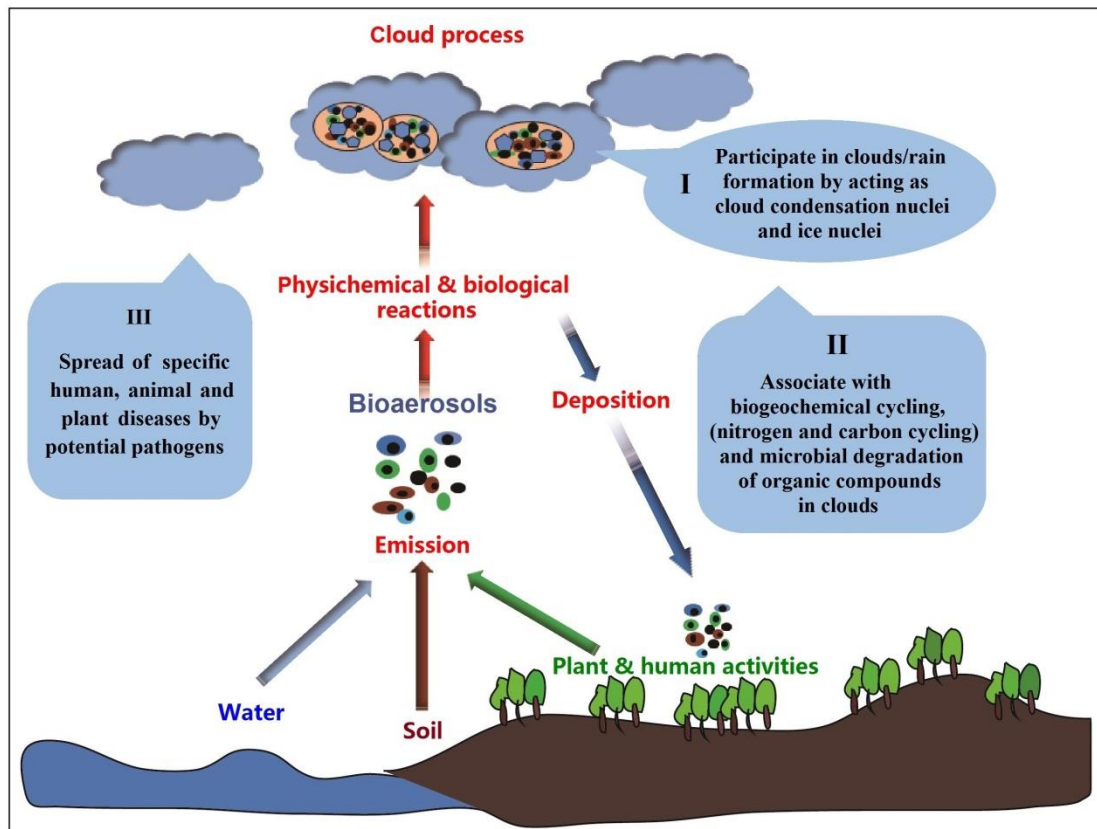
Figure 3 Schematic representation of bioaerosols life cycle and potential influence on atmosphere, ecosystem and human health, modified from Poeschl (2006). The predominant bacterial species with potential functions are indicated in the figure. Bioaerosols emissions and resuspension from various terrestrial 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 cloud process, they can be deposited back to land via deposition and possibly induce human infections and affect the diversity and function of aquatic and terrestrial ecosystems.
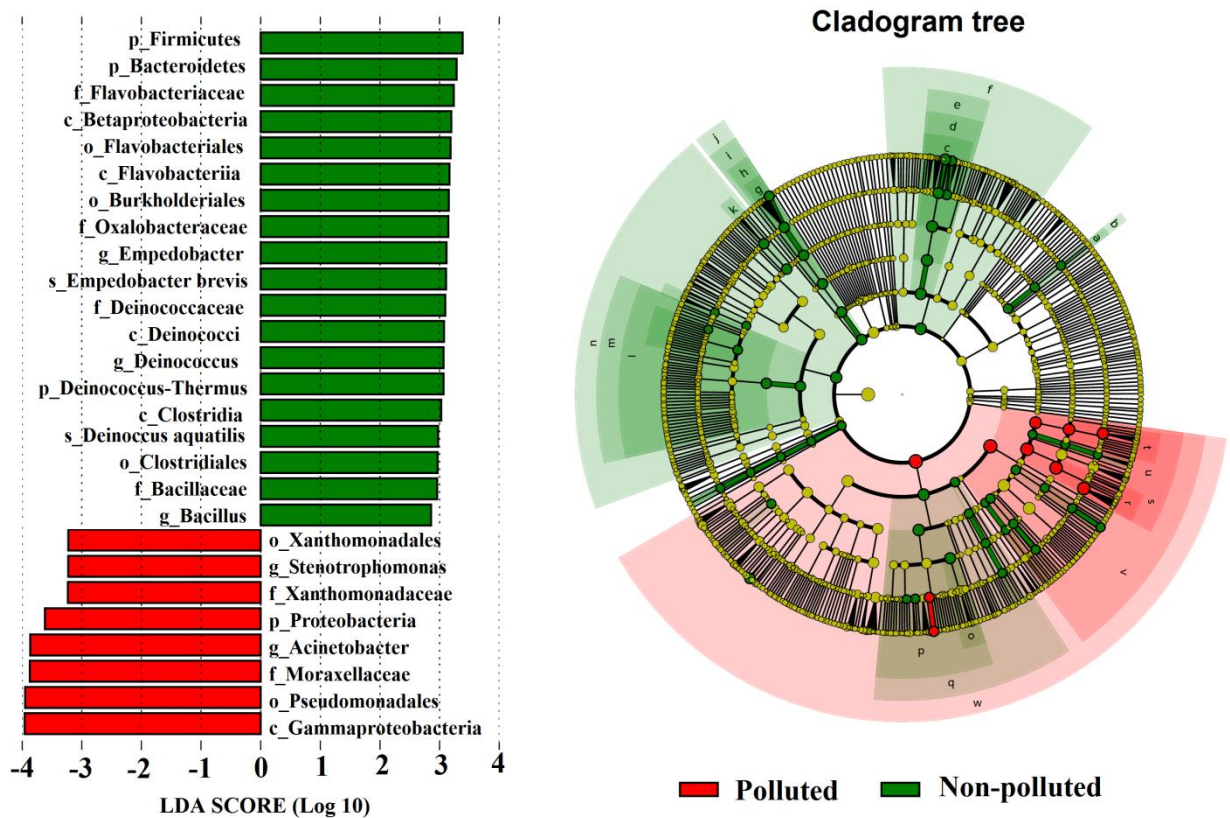
Figure 4 Distinct bacterial taxa between polluted and non-polluted cloud episodes identified by linear discriminant analysis coupled with effect size (LEfSe). The LDA effect sizes (left) were calculated using the default parameters. The taxonomic cladogram (right) was visualized with LDA values higher than 3.5 comparing all bacterial taxa. The significantly distinct taxon nodes are colored in red and green for polluted and non-polluted cloud episodes, respectively. Bacterial taxa with nonsignificant differences are indicated with yellow circles and circle diameter is proportional to relative abundance. The abbreviation in the cladogram tree: a: g_Rhodococcus, b: f_Nocardiaceae, c: f_Flavobacteriaceae, d: o_Flavobacteriales, e: c_Flavobacteriia, f: p_Bacteroidetes, g: f_Deinococcaceae, h: o_Deinococcales, i: c_Deinococci, j: p_Deinococcus-Thermus, k: f_Bacillaceae, l: o_Clostridiales, m: c_Clostridia, n: p_Firmicutes, o: f_Oxalobacteraceae, p: o_Burkholderiales, q: c_Betaproteobacteria, r: f_Moraxellaceae, s: o_Pseudomonadales, t: f_Xanthomonadaceae, u: o_Xanthomonadales, v: c_Gammaproteobacteria, w: p_Proteobacteria.
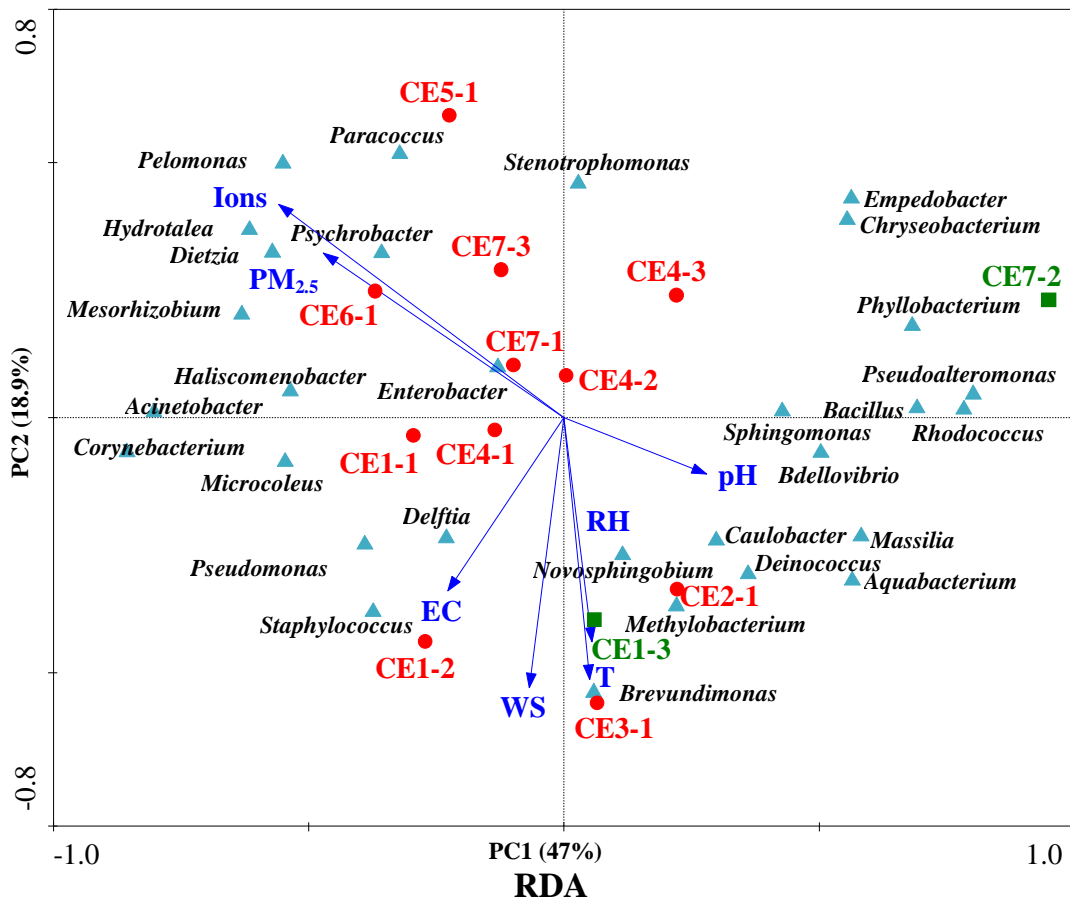
Figure 5 Biplot of the environmental variables and genus-level community structure using a redundancy analysis (RDA) model, describing the variation in bacterial community explained by environmental variable. CE refers to cloud episodes. Polluted episodes are indicated in red circle, and non-polluted episodes are green squares. Species data are listed in Table S2. The selected environmental variables are significant ($p<0.05$) using Monte Carlo permutation test. 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 65.9% of the variability. For bacteria, major ions in cloud water seem to be the most important environmental variable in shaping community structure.
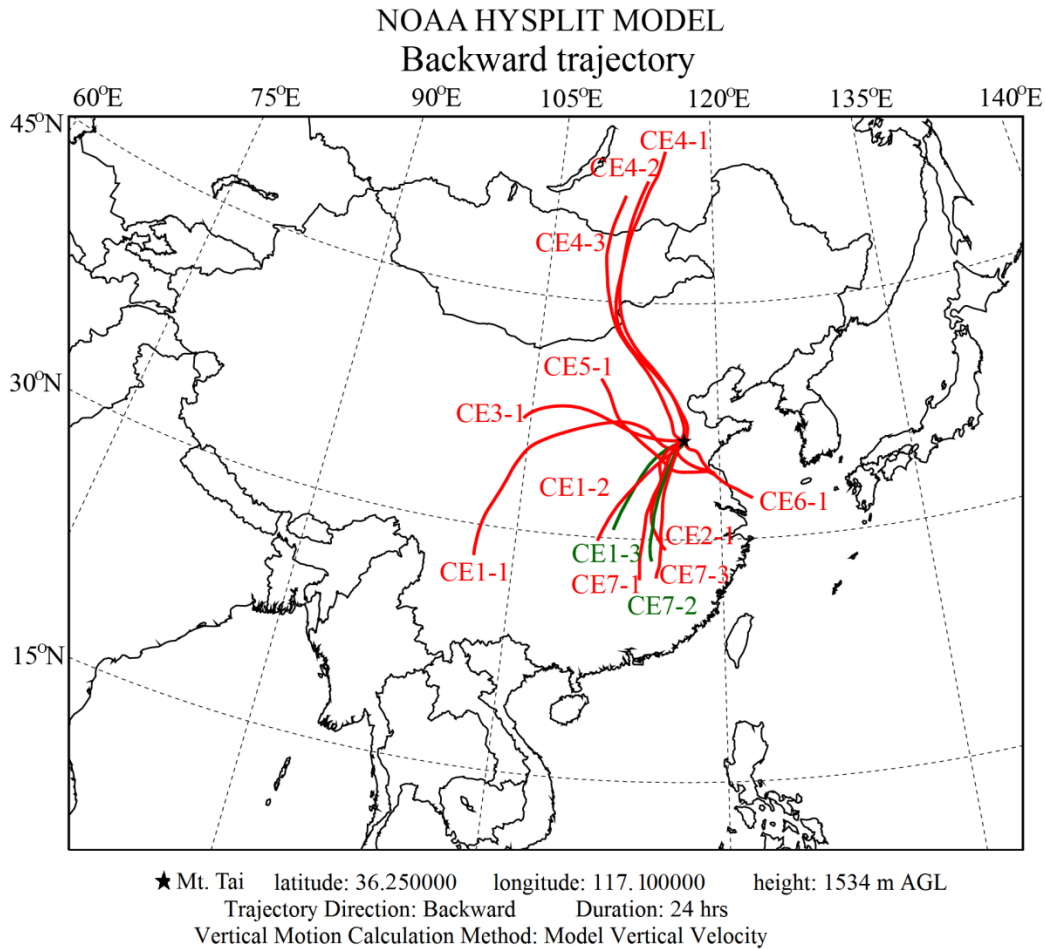
Figure 6 Air mass transport pathways for the cloud episodes using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model. 24-hour backward trajectories were calculated for air parcels arriving at the summit of Mt. Tai (36°15′ N, 117°06′ E, and 1534 m a.s.l). CE refers to cloud episodes. The polluted episodes are indicated in red lines, and green lines are non-polluted episodes.
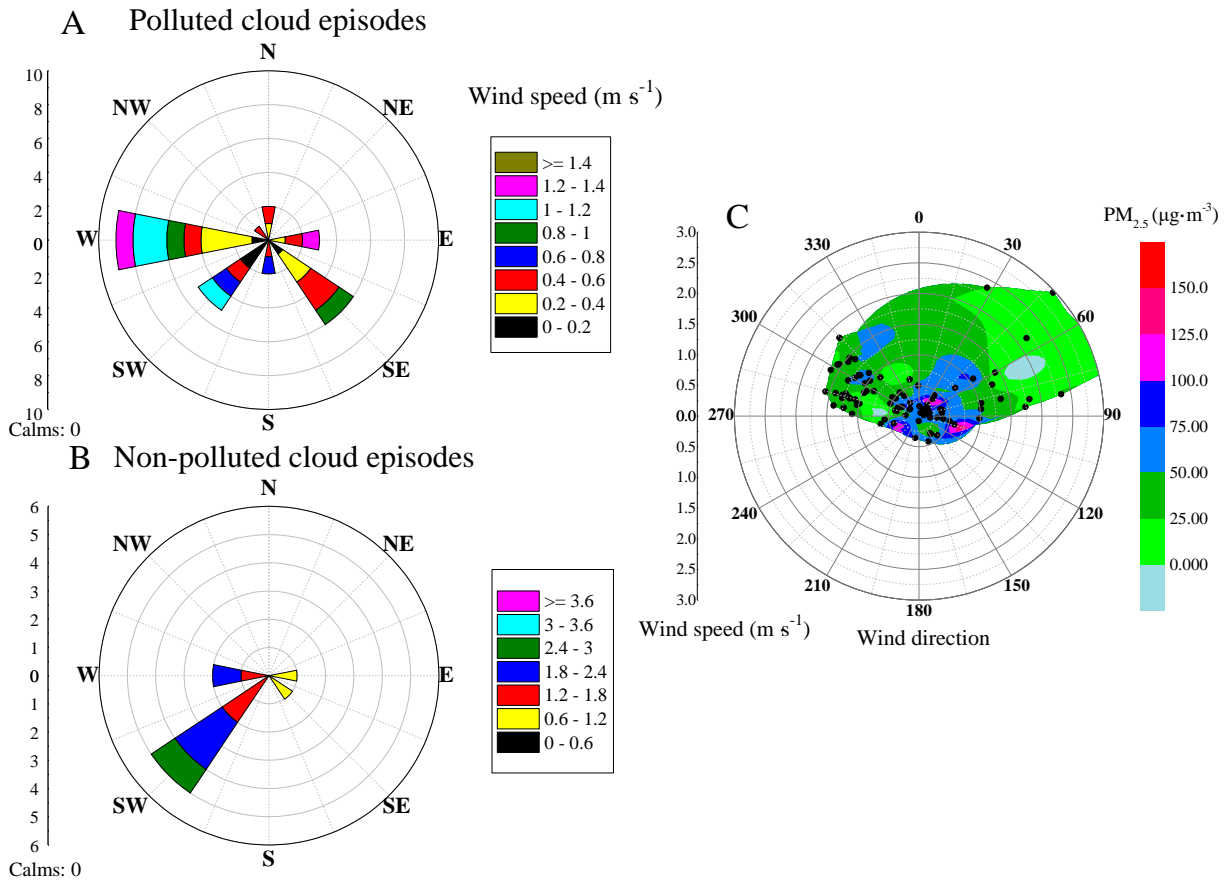
Figure 7 Wind Rose Diagram to quantitative analysis of wind speed and wind direction during sampling time between polluted (A) and non-polluted cloud episodes (B). The frequency of winds is indicated by wind direction. Wind speed range is labeled with color bands. Wind direction with the greatest frequency is shown with the direction of the longest spoke. Figure 7 (C) indicates distribution of wind speed during the whole sampling time (from 24 July to 23 August 2014) and the correlation with $PM_{2.5}$ concentration. As shown in the figure, $PM_{2.5}$ concentration was high under lower wind speed, whereas $PM_{2.5}$ was lower when wind speed was high.

Table 1 Description cloud episodes at Mt. Tai, China

Data	Samples	Start time (BJT)	Stop time (BJT)	Duration (h)	$PM_{2.5}$ [a] ($\mu g \cdot m^{-3}$)	LWC ($g\ m^{-3}$)	pH	EC ($\mu S \cdot cm^{-1}$)	OC ($mg\ L^{-1}$)
24 Jul 2014	CE1-1	8:50	15:30	6:40	105.07	0.21	4.03	583	ND
	CE1-2	15:30	17:30	2:00	22.35	0.23	4.32	219.2	ND
	CE1-3	17:30	22:51	5:21	14.66	0.24	5.74	104.4	ND
5 Aug 2014	CE2-1	6:45	9:17	2:32	30.36	0.22	5.80	275.7	ND
5 Aug - 6 Aug 2014	CE3-1	19:05	4:01	8:56	42.25	0.10	5.10	501	ND
14 Aug - 15 Aug 2014	CE4-1	22:41	0:44	2:03	42.69	0.02	6.36	170.4	BDL
	CE4-2	0:44	5:06	4:22	47.98	0.03	5.34	86.34	0.04
	CE4-3	5:06	6:03	0:57	36.88	0.02	4.89	64.95	BDL
17 Aug 2014	CE5-1	10:10	11:18	1:08	63.18	0.39	5.20	120.5	0.11
17 Aug - 18 Aug 2014	CE6-1	22:18	1:25	3:07	54.33	0.10	3.80	321.8	0.02
23 Aug 2014	CE7-1	2:30	4:38	2:08	30.45	0.20	4.38	356.2	0.03
	CE7-2	4:38	6:21	1:43	23.39	0.22	5.01	207.5	0.15
	CE7-3	6:21	9:20	2:59	41.60	0.21	5.74	187.6	0.21

CE refers to cloud episode. BJT refers to Beijing Time, which equals UTC + 8. LWC refers to the cloud liquid water content.

EC refers to the electric conductivity. OC refers to the organic carbon in cloud water.

ND means not detected due to instrument failure. BDL means below detection limitation.

Table 2 Summary of bacterial diversity and richness of cloud water

Sample ID	Reads	OTUs	Ace	Chao1	Coverage	Shannon	Simpson
Polluted cloud episodes							
CE1-1	18213	975	1835	1491	0.98	3.94	0.065
CE1-2	18702	1184	1841	1730	0.97	4.19	0.062
CE2-1	19914	1125	1756	1684	0.97	3.96	0.063
CE3-2	18199	1022	2082	1582	0.97	3.97	0.065
CE4-1	18350	941	1828	1461	0.98	3.60	0.095
CE4-2	17707	967	1522	1427	0.98	3.67	0.090
CE4-3	17397	981	2091	1611	0.97	3.81	0.083
CE5-1	16384	1132	1814	1790	0.97	4.32	0.055
CE6-1	16896	1186	1997	1872	0.97	4.13	0.067
CE7-1	16350	1103	2501	1795	0.97	3.90	0.081
CE7-3	18122	1258	1958	1999	0.97	4.38	0.053
Non-polluted cloud episodes							
CE1-3	17662	1173	1689	1687	0.97	4.71	0.033
CE7-2	18252	1150	1732	1673	0.97	4.37	0.043
Aerosol (Katra et al., 2014)	4020	1412		2142	0.83		
Bioaerosol (Madsen et al., 2015)						2.64-3.05	0.82-0.92
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.

CE refers to cloud episodes. TSP refers to the total suspended particulate matter.

Table 3 The identified bacterial species in cloud water samples correlated 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	(Nováková et 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)

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.

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