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Profile of Inhalable Bacteria in $PM_{2.5}$ at Mt. Tai, China: Abundance, Community, and Influence of Air Mass Trajectories

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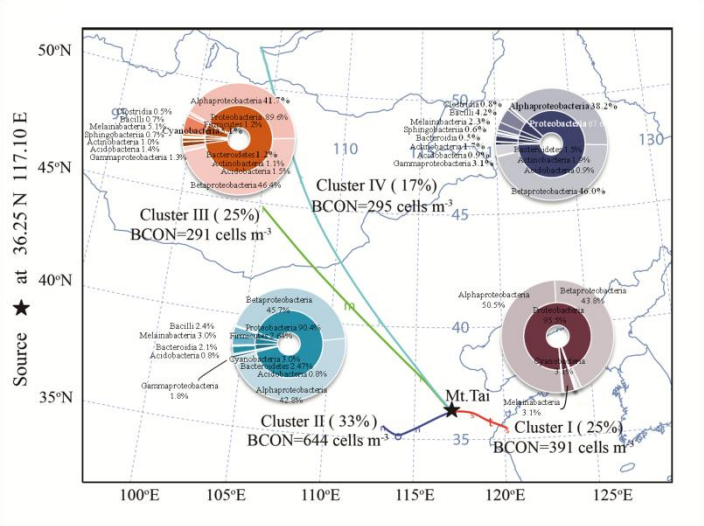
Highlights

1. $PM_{2.5}$ samples exhibited a high microbial biodiversity and complex chemical composition
2. Relationships between bacterial populations and PM composition were investigated.
3. The highest bacterial concentration and more potential pathogens were recorded under the influence of local air pollutants.
4. The long-range transported air mass exhibited highest richness and diversity with an increased abundance of dust-borne bacteria.

Graphical abstract



Keywords

Bioaerosol, PM, Air mass origin, Diversity

Abstract

Bacteria are ubiquitous in the near-surface atmosphere where they constitute an important component of aerosols with the potential to affect climate change, ecosystems, atmospheric process and human health. Limitation in tracking bacterial diversity accurately has thus far prevented the knowledge of airborne bacteria and their pathogenic properties. We performed a comprehensive assessment of bacterial abundance and diverse community in $PM_{2.5}$ collected at Mt. Tai, via high-throughput sequencing and real-time PCR. The samples exhibited a high microbial biodiversity and complex chemical composition. The dominating populations were gram-negative bacteria including *Burkholderia*, *Delftia*, *Bradyrhizobium*, and *Methylobacterium.* The PM mass concentration, chemical composition, bacterial concentration and community structure varied under the influence of different air-mass trajectories. The highest mass concentration of $PM_{2.5}$ (61 μg m^{-3}) and major chemical components were recorded during periods when marine southeasterly air masses were dominant. The local terrestrial air masses from Shandong peninsula and its adjacent areas harbored highest bacterial concentration loading (602 cells m^{-3}) and more potential pathogens at the site. In contrast, samples influenced by the long-distance air flow from Siberia and Outer Mongolia were found to have a highest richness and diversity as an average, which was also marked by the increase of dust-associated bacteria (*Brevibacillus* and *Staphylococcus*). The primary research may serve as an important reference for the environmental microbiologist, health workers, and city planners.

1. Introduction

The particulate matter with aerodynamic diameters of ≤2.5 μm ($PM_{2.5}$) is known as a major health risk for the spread of asthma, stroke burden, and chronic diseases such as nasopharyngitis, bronchitis, and pneumonia[1-3]. Assessment of air pollution exposure for the global burden of disease revealed that about 29.2% of stroke burden was associated with inhaled particulate matters at a high concentration[4]. The environmental health scholars considered that the toxic effect of $PM_{2.5}$ on the respiratory tract and lungs mainly induced by the suspension and reaction of gases, heavy metals, mineral element, and toxic organic matters (cancerogenic substance) in ambient air[5, 6]. In addition to these components, there is increasing evidence that pathogenic and allergenic microorganisms (bacteria, eukaryotes, archaea, and viruses) attached to the particles, may deposit in the epithelial cells through respiration, and thus causing various ailments in humans and livestock[7-10]. The pathogenic species (e.g. adenovirus, influenza, SARS, and rhinovirus) and their components (e.g. endotoxins, glucans, and mycotoxins) widely distributed in near-surface atmosphere bring a great threat to the ecosystem balance[11-13].

Bacteria, approximately 10^4 cells m^{-3} and 0.1 μg m^{-3} in PM, mainly originated from the emission of soil, vegetation, surface of rock, human and animals (excrements and debris from skin and hair), precipitation (surface snow and ice core), and ocean[14]. Investigations on the ambient bacteria experienced a recent growth in interest with the aerosol community. To our knowledge, the first study on airborne microorganisms was reported in the mid-19th century[15]. The occurrence of high throughput sequencing offers a unique possibility to examine the bacterial, viral, and eukaryotic diversity in the aerosol. While the understanding of airborne microbe is still being developed due to current challenges, e.g., low density of airborne microbes, variability in airborne microbial community structure, and the lack of unified regulations for the research. However, in spite of this, this method has been applied to study the airborne bacteria and the associated pathogens in Japan, USA, Korea, and China in the last few years[7, 16-18]. Franzetti et al. (2011)[19] described the significant seasonal variation of bacterial community associated with PM_{10} and $PM_{2.5}$ based on the Roche 454 sequencing. Based on the high-throughput sequencing, Bower et al. (2013)[20] found the significant seasonal variability of Actinobacteridae and Pseudomonadales across $PM_{2.5}$ and $PM_{2.5-10}$ in Greeley, CO. Cao et al. (2014)[21] developed a comprehensive framework of the microbial community in $PM_{2.5}$ and PM_{10} under sever smog events by metagenomics. These findings mainly dressed the scanty composition community of bacteria in the urban air, the identification for the inhaled microbes and their pathogenic members at high altitudes were incomplete. Airborne bacteria with active metabolic have been reported in the mountains and the upper troposphere[16,22]. These species dissolving into cloud droplets proposed an important impact on

the regional precipitation distribution, biogeochemical cycling of elements in ecosystem processes, and cloud chemistry by acting as cloud condensation nucleation or ice nucleation[23,24]. Thus it is necessary to further the understanding for the profile of airborne bacterial community structure at high elevations.

Here, we expound on our initial efforts to advance the knowledge of airborne bacteria over Mt. Tai, the highest site at the North China Plain. It is located on the transport path of the Asian continent outflow and be affected by air mass from the Asian dust area. Mt. Tai is a tilted fault block mountain with height increasing from the north to the south, facing to the East China Sea and back on the yellow river to the west. In this region, the airborne bacterial populations in cloud system and fungal populations in particles have been reported before[24-26]. The preponderant gram-negative bacteria inhabited in the cold and leaf-surface environments which were involved in the cloud condensation/ice nuclei formation and pathogenicity, demonstrating their importance in ecology, epidemiology and human health[24-25]. The pioneering studies were lack of a comprehensive and compound research on the bacteria in particles. The chemical composition in particles have been proved to be related to the variable airborne microbe. Further, they also highlighted the crucial influence of environmental factors humidity, and temperature on the bacterial community in cloud water and fungal community in particles. Generally the chemical composition of particles may be influenced by different air mass origin with the input of local aerosol or long-range transported aerosol. Whether the different air trajectories do play a role in shaping bacterial community of particles still remains a question. In this study, we aim to: (1) profile the bacterial abundance and community of $PM_{2.5}$ at Mt. Tai, China, (2) to elucidate the environmental variables (temperature, humidity, $PM_{2.5}$, $PM_{2.5}$ chemistry), and the air mass trajectories during sampling time, (3) to estimate whether the different air mass trajectories effect on the bacterial characteristics.

2. Materials and method

2.1 Aerosol collection and DNA Extraction

Fine particulate matter ($PM_{2.5}$) were sampled on quartz fiber filters (PALL, NY, U.S. 88mm) with a middle-volume sampler (TH-100A, Wuhan Tianhong Instruments Co., Ltd., China) and a flux speed of 100 L min^{-1}. The blank filters were obtained by placing a sterilized quartz microfiber filter inside of the sampler without operation. The sampling campaigns were carried out in 8~13 day periods in summer, autumn, and winter during July 2014 to August 2015. The sampler was located at the summit of Mt. Tai (36.25^{o}N and 117.10^{o}E, 1534 m above sea level), a typical tourist area in the North China Plain (Figure 1). The geographical characteristics of the sampling site have been previously described[25,26]. Each

collection period lasted 23 h. Before sampling, all the filters were baked at a Muffle furnace at 500°C for 5 h and then placed into sterilized aluminum foil before deposited into a sealed bag. To avoid the contamination, the sampling filter holder and materials used for changing filters were treated with 75% ethanol before sampling every day. After sampling, the samples were stored at -80 ^{o}C until the next analysis. The environmental factors including temperature, relative humidity, visibility, water-soluble inorganic ions (NO_3^-, SO_4^{2-}, NH_4^+, Ca^{2+}, Na^+, Mg^{2+}, and Cl^-), meteorological data, the mass concentration of $PM_{2.5}$, DNA extraction procedure and PCR amplification have been described before[26]. The final product was ready for resolving for the abundance and community of airborne bacteria by real-time quantitative PCR and Illumina Miseq sequencing.

2.2 Quantification of 16S rRNA gene copy number

The bacterial abundance was determined by the quantification of the copy numbers of the gene 16S rRNA. This value is not directly related to bacterial number due to the presence of multiple ribosomal operons in the bacterial genomes; however, it can be used to look at relative shifts in microbial biomass as the bias in copy number is likely constant across samples. The V4-V5 hypervariable regions were PCR-amplified with universal primers 515F (5'-TGCCAGCMGCCGCGGTAA-3') and 926R (5'-CGTCAATTCMTTTGAGTTT-3'). The real-time quantitive PCR was performed in a total volume of 25 μL reaction mixture including 12.5 Ml TransStart Green qPCR SuperMix (Transgene, AQ101-03), 1μL forward primer, 1μL reverse primer, 5 μL DNA, and 5.5 μL double distilled H_2O. The amplification was run on a BioRad CFX96 Real-Time PCR system under the following conditions: 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s, with acquisition of the fluorescence at the end of each 72 °C elongation step. The deionized water was employed as the negative controls and was pipetted into the wells in a 96-well microplate with the DNA extracted from the samples. All the samples and controls were tested in triplicate in each run. Based on the average rRNA gene copy number (3.98), the bacterial cell concentration (cells m^{-3}) was calculated using the method described before[27-29].

2.3 Sequence Analyses

A DNA sequencing library was constructed on the basis of the TruSeq DNA PCR-Free Sample Prep Kit (Illumina) and was sequenced by the MiSeq reagent kit v2 (Illumina) on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA). The raw sequences were analyzed using standard Mothur software. Variable sequence numbers (7044 to 32,293) were obtained after the quality control of raw reads by removing the chimeric sequences, primers, barcodes, and low-quality reads (average quality score<20) using FASTX-ToolKit. We

normalized the sequences number to 7044 for the subsequent analysis. The Operational Taxonomic Units (OTUs) were defined using a distance-based cutoff method by Usearch (version 7.1; http://drive5.com/uparse/). The alpha-diversity estimators Chao1 and Shannon indexes, which indicated the richness and diversity of the bacterial community, were calculated by QIIME (version 1.17; http://qiime.org/scripts/assign_taxonomy.html) after removing the low-abundance OTUs (<0.01%). The taxonomic classification was aligned to the Silva database (http://www.arb-silva.de/) using uCLUST (http://www.drive5.com/uclust/downloads1_2_22q.html) at different similarity levels to determine the diverse bacteria at phylum, class, order, family, genus, and species levels. The sequences obtained in this study have been deposited in the Sequence Read Archive (SRA) under accession numbers SRR51461. The potential pathogenic sequences corresponding to human health were screened referenced to the previous database[30-33]. The human pathogenic bacteria list, including species names and the diseases caused, was used to find the potential pathogens sequences from total sequences. We selected the operational taxonomic units (OTUs) belonged to these genera mentioned in the reference list. The nearest phylogenetic species were obtained by NCBI BLAST. Finally, the bacterial species belonging to the reference list were determined as the potential pathogens in $PM_{2.5}$.

2.4 Statistical Analyses

The difference in bacterial concentration across different air mass trajectories were assessed by an ANOVA test, followed by post-hoc tests. The ANOVAs test was also conducted to determine whether season had a significant impact on the relative abundances of specific microbial groups. The weighted UniFrac analysis was employed to estimate the significant differences of the microbial community among three seasons. The relationship between the airborne microbial concentrations and environmental factors including PM concentrations and chemical compositions was assessed with a Pearson correlation by SPSS 16.0. The results that Bonferroni adjusted P-value for each pairwise comparison higher than 0.05 were considered to be significant. The correlation coefficient between NMDS plot and environmental elements was calculated via metadata analysis with Mothur software. Statistical values, including Pearson and Spearman correlation coefficients and independent t-tests, were calculated using R software with the R package.

3. Results and discussion

3.1 Bacterial abundance in $PM_{2.5}$

During the sampling period, the $PM_{2.5}$ mass concentration, ranging from 3 to 111 μg m^{-3} with a mean value of 40 μg m^{-3} at Mt. Tai, were much lower than that in summer 2006 (123 μg m^{-3})[32] and comparable with that in summer 2007 (59 μg m^{-3})[30] that conducted at the same site. Table 1 summarizes the corresponding bacterial concentrations in a different size. The bacterial concentration of $PM_{2.5}$ varied from 40 to 1728 cells m^{-3} with an average of 490 cells m^{-3} at Mt. Tai. It was much lower than those TSP from the high-altitude air, urban Japan, Korea, and Italy, $PM_{2.5}$ and PM_1 from China. The lower bacterial concentration were found to be correlated with $PM_{2.5}$ mass concentration ($r = 0.526$, $p < 0.05$), SO_4^{2-} ($r = 0.549$, $p < 0.01$), NO_3^- ($r = 0.458$, $p < 0.05$), NH_4^+ ($r = 0.570$, $p < 0.01$), K^+ ($r = 0.508$, $p < 0.01$), Na^+ ($r = 0.585$, $p < 0.01$), Mg^{2+} ($r = 0.608$, $p < 0.01$), and Ca^{2+} ($r = 0.432$, $p < 0.05$) significantly based on the Spearman rank correlations. The ambient pollutants attached to the particles may provide nutrients (sulfur, nitrogen, and ammonia) and thus affect the bacterial characteristics. In general, the airborne bacteria, ranging between 10 and 10^7 cells m^{-3}, varied in a degree with many aspects[11,23,29,34], like sampling time, site (altitude, temperature, humidity, etc.), sample type (TSP, PM_{10}, $PM_{2.5}$, PM_1, cloud/fog water, etc.), and analysis methods (culture methods, bio-molecular technology, and quick estimation model)[14,35,36]. And these indexes effectively represented the situation of bacterial community on particles under different conditions. Borodulin et al. (2005)[37] observed the varied concentration of culturable microorganisms for about more than two orders of magnitude over an altitude range of 0.5~7 km in southwestern Siberia. They found the overall bacterial concentration in the atmosphere where not only dependent on altitude but also related to the cell size of bacteria and particulate matters. Different bacteria have respective sizes ranging from 0.1~5 μm, and each bacterium has a particular shape feature that plays an important role in the adhesion on PM[3]. Since the study environments were breeding settings in which bacteria had better conditions to grow and propagate, the bacteria concentration may decrease with decreasing particulate size[38]. Hence more high-throughput sequencing researches for the bacterial community distribution were vital to understanding their diversity fully.

3.2 Inhalable bacterial community in $PM_{2.5}$

After quality control and normalization, a number of OTUs ranged from 237 to 1579 were detected. The alpha diversity estimators were summarized in Table 2. Similar with Despr´es et al. (2007)[54], the airborne bacteria at Mt. Tai commonly exhibited lower richness and

diversity compared with those from urban, marine, sand, and dust sites. The diversity estimators can reflect the state of the bacteria and their relationship to the environment, and microbial resources developments, ecosystem functioning, global change and human health are all closely related to microbial diversity. In addition to the changing sampling sites, this discrepancy may be caused by the different analysis platform (Miseq sequencing, pyrosequencing, and clone library). Using the same method (Miseq Sequencing), TSP seems to have higher OTUs, chao1, and Shannon indexes than $PM_{2.5}$ and PM_1, indicating more and various bacterial species in the larger particulate matter. We attribute this increasement to the larger surface area of TSP for bacterial adhesion. As mentioned above, the size of a single bacterial cell is smaller than particles, there is a mass of nutrients and bacterial slime coat on the bacteria that make the bacterial size bigger but still smaller than particles, so there were more bacteria presenting in particles with a larger size. Actually different bacteria had respective variation tendency for particle sizes, although there were some differences on size distribution, bacteria mainly attached to particles with two peaks (1.1~2.1 μm and >7.1 μm)[55]. Except for the bacterial size, the structure of bacterial community can also exert a certain extent influence on the diversity characteristics.

At Mt. Tai, the diverse bacterial community including 40 phyla, 78 classes, 154 orders, 286 families, 724 genera was classified in $PM_{2.5}$. About 97.82% of OTUs belongs to the phylum Proteobacteria, Cyanobacteria, Firmicutes, and Bacteroidetes. The co-occurrence network were used to reveal the complex bacterial community structure based on the top 100 OTUs (Figure 2). The species of dominant phyla (Proteobacteria, Actinobacteria, Cyanobacteria, Firmicutes, Acidovacteria, Fusobacteria, and Bacterioidetes) were similar in USA[7,56], Italy[34], and East Asian Regions[15,21,48,49,51,56], but the relative abundance were different. Furthermore, the microbiome is a dynamic aspect of the near-surface layer among extreme weather incidents such as dust, haze, rain, and tropical storms. Proteobacteria members were the dominant species in the atmosphere over mountains and from the troposphere. The relative abundance of this bacterium (91.42%) were richer than that in the dust (69.0%), non-dust (53.5%)[18], and haze (16.5%)[27] days. It was dominated by a few taxa throughout the whole sampling period, with a marked prevalence of Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. At higher taxonomic levels, a bacterium in the nitrogen fixing, gram-negative pathogenic bacteria genus *Burkholderia* appeared to be the most abundant. Bowers et al. (2009)[57] also showed the dominant Burkholderiales in particles collected at the top of Mt. Werner near Steamboat Springs CO, USA at 3220 m above sea level via tagged pyrosequencing. The preponderant gram-negative bacteria in $PM_{2.5}$ were different with Fang et al. (2007)[58] who reported the 80%~86% culturable bacteria in outdoors environment were gram-positive. They considered the gram-negative bacteria cannot adapt to the higher air

pollutants concentration, stronger solar radiation, and complicated meteorological environment outdoors. We speculate this gap attributed to the different analysis platform. The Illumina Miseq sequencing method gained the comprehensive airborne bacteria no matter culturable or unculturable. Actually, the proportion of culturable bacteria is very small. Another reason is that those gram-negative bacteria in the atmosphere lose cultivability after aerosolization[59]. Hence the majority of airborne bacteria outdoors were gram-negative.

Out of all the gram-negative species, eight opportunistic pathogens were determined based on the human pathogenic bacteria list as described before. *Acinetobacter baumannii*, primarily associated with hospital-acquired infections, commonly found in water and soil. Over the past decades, reports on community-acquired *A. baumannii* infections (wound infection, ventilator-associated pneumonia, and bloodstream infection) have increased and attracted public attention[60-63]. An outbreak of soil-borne bacteria *Pseudomonas putida* was involved in clinic infections in patients with medical devices and immunosuppressed hosts [64-65]. Besides, five gram-positive potential pathogens were detected including *Bacillus subtilis*, *Rhodococcus equi*, *Streptococcus anginosus*, *Bacillus pumilus*, *and Rhodococcus fascians*. Some strains of *B. subtilis* cause food poisoning through the ropy spoilage of bread and rots in potatoes[66-67]. *R. equi*, mainly thrive on water and soils, were connected with pneumonia in immunocompromised patients and animal hosts[68-69]. *S. anginosus* were able to cause diseases including bloodstream infections, pyogenic liver abscess, brain and liver abscesses[70-71]. These bacterial species suspending in the atmosphere diffuse downwind, yielding a threat to the health of plants and animals in local and downstream area.

Other than the pathogenicity, the diverse bacteria demonstrates a diverse metabolism in the atmospheric process and ecosystem interactions. The bacteria with ice nucleation activity suspending in the atmosphere can take part in the cloud chemistry via act as cloud condensation or ice nuclei. During the same project, we captured some typical cloud events (Event F and G)[24], incidentally. The special terrain of Mt. Tai is conducive to the formation of orographic clouds. The tiny droplets could dissolve or adsorb the particles suspending in the atmosphere and then accumulated into the cloud water. Though the same sampling site, the dominant bacterial species varied from *Burkholderia* in $PM_{2.5}$ to the preponderance of *Pseudomonas* in cloud water. The distant discrepancy of dominating bacterial distribution also found between $PM_{2.5}$ of Mt. Tai and PM_1 of Ji'nan (Figure 3A). As to this phenomenon, different bacterial suitable inhabits and growing media (water, soil, atmosphere, etc.) may be important factors. Additionally, Liu et al. (2018)[72] considered the relative humidity, wind scale, CO, and SO_2 were the main factors, which affected the diversity of total bacteria and the proportion of pathogenic bacteria. The PM's chemical components serve as the

nutritional ingredient, coupled with surrounding environments such as temperature, humidity, wind direction, and atmospheric pressure seems to shift on the bacterial growth and metabolism in atmosphere[3,10,73]. The chemical and biological data were jointly processed and verified that the varied NH_4^+, NO_3^-, Mg^{2+}, Ca^{2+}, Cl^- and Na^+ explained about 53.7%~63.1% of the variance of species-environment correlations[74]. The chemical composition of $PM_{2.5}$ correlated with the air mass back trajectories. Air mass originating from Mongolia region had strong correlations with dust pollution, along with high concentration of Ca^{2+} in ambient $PM_{2.5}$. Local emission and air pollutants were more favorable for formation of secondary pollutants (SO_4^{2-}, NH_4^+, and NO3$^-$) and reactions between particulate species. We also detected the obvious correlation between airborne bacterial community and Ca^{2+}, Mg^{2+}, and temperature at Mt. Tai, while the influence of atmospheric circulation using the back-trajectory analysis was not evaluated fully. Therefore how does the air mass trajectories vary and whether the air mass trajectories evoked the change of concentration and structure of bacterial community is worth discussing.

3.3 Cluster analysis of backward trajectory and $PM_{2.5}$ chemistry

At Mt. Tai, the air mass back trajectories have been categorized into four types, typified by the examples in Figure 4, based on trajectories terminating at the site on the day of measurements. One trajectory per day was, in our experience, adequate to represent the flow patterns which tended to change over timescales of a few days. Inevitably, there were a few days on which assignment of trajectory type was difficult, but the predominant type from the four synoptic hours was adopted in these circumstances. The four types of back trajectory were as follows:

Cluster I (25%): The air mass had passed across the marginal seas in the western Pacific Ocean and had been in contact with the land immediately prior to arrival in the study area. Broadly speaking, the highest mass concentration of $PM_{2.5}$ (61 μg m^{-3}) and major water inorganic ions were recorded followed the decreasing order: $SO_4^{2-} > NO_3^- > Cl^- > Na^+ > Ca^{2+} > K^+ > NH_4^+ > Mg^{2+}$. In addition to the secondary inorganic ions, Cl^- (4.35 μg m^{-3}) and Na^+ (3.73 μg m^{-3}) can be assumed mostly to originate from sea salt aerosols. The Cl^-/Na^+ molar ratios obtained (0.75) was lower than that of bulk seawater (1.18)[75], suggesting that the chlorine loss of marine aerosols during transport to some extent. These ions are proved to play essential roles in atmospheric processes. e.g., cloud formation, visibility degradation, solar radiation, acidification of cloud, rain, fog and haze formation and served as the nutrients for the growth and development of airborne bacteria[24, 45].

Cluster II (33%): The air mass had come in from a generally western direction bringing it across Shandong peninsula and its adjacent areas, a typical densely polluted areas of northern China. Influenced by the local terrestrial air flow, the average of $PM_{2.5}$ mass was 37 μg m^{-3}. The secondary inorganic ions, i.e., SO_4^{2-} (9.92 μg m^{-3}), NO_3^- (4.23 μg m^{-3}), and NH_4^+ (2.29 μg m^{-3}), which mostly originated from the transformation of their precursors (SO_2, NO_2, and NH_3) were found as the dominant species, followed by Cl^- (0.75 μg m^{-3}), K^+ (0.63 μg m^{-3}), Ca^{2+} (0.51 μg m^{-3}), Na^+ (0.31 μg m^{-3}), and Mg^{2+} (0.07 μg m^{-3}). In order to investigate the continental influence, non-sea-salt sulfate (nss-SO_4^{2-}) concentrations are estimated as: $\mathrm{nss-SO_4^{2-}}\ (\mu g\ m^{-3}) = [SO_4^{2-}] - 0.245 \times [Na^+]$. The mass fraction of nss-SO_4^{2-} ranged from 1.17 μg m^{-3} to 18.89 μg m^{-3} (mean 9.85 μg m^{-3}), accounted for 99.02% of total sulfate. SO_4^{2-} mainly originates from anthropogenic and biogenic sources, while nss-SO_4^{2-} originates mainly from stationary sources. We often use the mass ratio of [NO_3^-]/[nss-SO_4^{2-}] as an indicator of the relative importance of stationary versus mobile sources of sulfur and nitrogen in the atmosphere. High [NO_3^-]/[nss-SO_4^{2-}] mass ratios represent the predominance of mobile sources over stationary sources. Due to the extensive use of sulfur-containing coal, the average ratio [NO_3^-]/[nss-SO_4^{2-}] mass ratios in China were ascribed lower value[76]. The measured [NO_3^-]/[nss-SO_4^{2-}] mass ratios at Mt. Tai (0.25) was higher or comparable with those reported in other cities, e.g., 0.17 in Hongkong[77], 0.20 in Guangzhou[78] and 0.35 in Qingdao[79]. However, the ratio was lower than those cities where the use of coal was limited, for example, Los Angeles (2.0)[80] and Seoul (0.73)[81]. The result implied that stationary source emissions were likely more important than mobile sources at Mt. Tai under the influence of local terrestrial air flow.

Cluster III (25%) and *Cluster IV* (17%): The air mass arrived from a northwesterly sector outer Mongolia and having crossed dust area (Inner Mongolia and Shanxi Province) and high anthropogenic emission area (Henan and Shandong Province). This type air mass moves quickly and the corresponding mass concentration of coarse particulate matter was usually high. Typically, these westerly and northwesterly trajectories had not been in contact with any land for several days prior to arriving at the site.

3.4 Influence of air mass trajectories on bacterial populations and community

Obviously, no obvious variation for the bacterial concentration was observed under the influence of air mass trajectories. The terrestrial air masses from the Shandong peninsula and its adjacent areas exhibited a little higher bacterial concentration loading (644 cells m^{-3}) and more potential pathogens. While the bacterial concentration of PM influenced by the marine air mass was lower, though the highest chemical composition (Figure 4). In contrast, samples

influenced by the long-distance air flow from Siberia and Outer Mongolia were found to have a highest richness and diversity as an average (Table 2). The effects of long-transported air parcels on the aerosol particles and its composition and sources have been discussed in previous studies[82-85]. The Asian dust particles blow from these areas in the westerly wind and play an important role in the leeward ecosystem. The bacterial community at phyla, class, and order levels under the influence of four air mass trajectories was shown in Figure 5. However, the bacterial abundance made no significant difference among the top 20 OTUs (Figure 3B), suggesting the stable community structure of airborne bacteria. Yet, some non-dominant bacterial groups that exhibited pronounced changes in relative abundance across the different air mass trajectories (Figure 6) can be linked to specific source environments of $PM_{2.5}$. The long-range transported air parcels seem to be able to harbor diverse and dynamic microbial populations[53]. Kakikawa et al., (2008) verified the injection of dustborne bacteria (*Brevibacillus*, *Staphylococcus*, *Rhodococcus*, *Delftia*, *Pseudomonas*, and *Agrobacterium tumefaciens*) into the atmosphere during the dust event[86]. Maki et al., (2017) also observed the variable microbial community in response to the Asian dust events[22]. The number of microbial particles at high altitudes (>1200m) increased about 5.5-folds. The corresponding bacterial community was predominantly composed of natural-sand/terrestrial bacteria during dust events instead of anthropogenic/terrestrial bacteria, marine bacteria, and plant-associated bacteria during non-dust events. The prevalent existence of marine bacteria in cloud droplets, the air masses suspended from the Yellow Sea over the marine environment harbored more proteobacterial populations, *Pandoraea*, and *Phyllobacterium* associated with plant bodies or surface. The prevalence of *Pandoraea* had a significant positive relationship with the highest content of SO_4^{2-}(**p<0.01). Consistent with these findings, the long-range transported westerly air mass increase the diversity and richness of airborne bacterial community, with the increase of some dustborne species such as genus *Brevibacillus*, *Staphylococcus*, and *Anoxybacillus* (Figure 6A). The Bacilli members obtained from mineral dust particles over downwind areas were positively correlated to temperature and Cl^- in $PM_{2.5}$ (**p<0.01), excepting the obvious effect of humidity and temperature. They showed a stronger ability to resist ultraviolet light and be able to survive in the atmosphere by the formation of resistant endospores. The natural dust particles from dust areas can be transported to the free troposphere and vertically mixed with the anthropogenic particles during the transportation processes. Together, the airborne bacteria community at Mt. Tai contains a mixture of dust-borne bacteria, terrestrial bacteria, marine bacteria, and others. The community of dominant bacteria in $PM_{2.5}$ was relatively stable, and little affected by the sampling environment. In other words, ambient bacterial community structure were stable in atmosphere. Four air mass trajectories from various origin environments may lead to the discrepant composition in $PM_{2.5}$,

and then driving the shift on the pronounced changes observed in some non-dominant bacterial groups.

4. Conclusions

In this study, 16S rRNA gene sequencing was conducted to study the bacterial communities at Mt. Tai's $PM_{2.5}$ pollutants from July 2014 to August 2015. Results demonstrated the lower bacterial concentration and diverse bacterial community in $PM_{2.5}$ compared with some aforementioned studies. We also detected thirteen potential pathogenic species, such as *A. baumannii*, *P. putida*, *B.subtilis*, and *R. equi* which effect on the immunocompromised populations and livestock. In future studies, we intend to analyze the changing bacterial community structure affected by different air mass trajectories, combined with the meteorological data, PM mass concentration, water soluble ion and other physical and chemical properties. The highest mass concentration of $PM_{2.5}$ and major chemical components was recorded during periods when marine southeasterly air masses were dominant. The local terrestrial air masses from Shandong peninsula and its adjacent areas harbored highest bacterial concentration loading and more potential pathogens at the site. In contrast, samples influenced by the long-distance air flow from Siberia and Outer Mongolia were found to have a highest richness and diversity as an average, which was also marked by the increased abundance of dust-associated bacteria (*Brevibacillus* and *Staphylococcus*). We conclude that the air flow from different origin environments may lead to the discrepant chemical composition in $PM_{2.5}$, and then driving the shift on the pronounced changes observed in some non-dominant bacterial groups. We hope our research can provide for some reference value in the global air microbial propagation and air microbial research at higher elevation site.

Conflict of interest

The authors declare that they have no competing financial interests.

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Table 1 Bacterial abundance in PM with different size fraction.

Type	Site	Abundance Range	Average	Method	Reference
		Cells m^{-3}	Cells m^{-3}		
TSP	High-altitude air, Caribbean Sea	3.0×10^{3}~9.0×10^{4}	5.1×10^{3}	q*PCR*	[39]
TSP	High-altitude air, Japan	1.0×10^{3}~2×10^{5}		q*PCR*	[40]
TSP	Toyama, Japan	1.1×10^{3}~1.3×10^{5}	1.8×10^{4}	q*PCR*	[41]
TSP	Seoul, Korea	5.2×10~4.3×10^{3}		q*PCR*	[29]
PM_{10}	Milan and Venice, Italy	2.4×10^{2}~$4.9\times10^{5*}$	$3.0\times10^{4*}$	q*PCR*	[42]
$PM_{2.5}$	BTH, China	8.0×10~$5.8\times10^{3**}$	$1.1\times10^{3**}$	Culture method	[43]
$PM_{2.5}$	BTH, China	4.8×10^{4}~ 2.6×10^{5}	1.2×10^{5}	q*PCR*	[44]
PM_{1}	Ji'nan, China	1.8×10^{4}~2.6×10^{4}	2.2×10^{4}	q*PCR*	[27]
CW***	Mt.Tai, China	5.8×10^{4}~1.6×10^{5}	1.2×10^{5}	q*PCR*	[45]
$PM_{2.5}$	Mt.Tai, China	4.0×10~1.728×10^{3}	4.9×10^{2}	q*PCR*	This study

* Copies m^{-3}; ** CFU m^{-3}; *** Cloud Water

Table 2 Statistical summary of alpha diversity estimators from different sample type and study environments.

Environment	Sample Type	OTUs	Chao1	Shannon	Method	Reference
Urban/Terrestrial	$PM_{2.5}$	2457	4915	5.89	Miseq Sequencing	[46]
Urban/Terrestrial	$PM_{2.5}$	552	571	3.80	Miseq Sequencing	[44]
Urban/Terrestrial	$PM_{0.56\text{-}1}$	222	272	2.44	Miseq Sequencing	[27]
Dust/Terrestrial	$PM_{2.5}$	62	200	3.51	Clone Library	[47]
Dust/Terrestrial	TSP		270		Pyrosequencing	[48]
Dust/Terrestrial	Dust	869	1485		Pyrosequencing	[49]
Dust/Terrestrial	PM_{10}	733	1265		Pyrosequencing	[18]
Sand/Terrestrial	Sand	1445	1858	5.99	Pyrosequencing	[50]
Marine/Coastal	TSP	753			Pyrosequencing	[51]
Marine/Coastal	TSP	5015		7.66~9.28	Miseq Sequencing	[52]
High altitude	TSP	410			Pyrosequencing	[22]
High altitude	TSP	314	241		Miseq Sequencing g	[53]
High altitude	CW*	664	1302	3.80	Miseq Sequencing	[24]
High altitude	$PM_{2.5}$	580	1282	3.01	Miseq Sequencing	This Study
Cluster I	$PM_{2.5}$	491	1151	2.67		
Cluster II	$PM_{2.5}$	567	1322	2.93		
Cluster III	$PM_{2.5}$	677	1397	3.09		
Cluster IV	$PM_{2.5}$	625	1234	3.63		

* Cloud Water

List of Figures

Figure 1 The geographic situation of Mt. Tai in the North China Plain.

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Figure 3 The principal component analysis showed the overall distribution pattern of A bacterial communities in different air samples and B profiles among four air mass cluster.

Figure 4 Cluster analysis of air-mass trajectories and corresponding chemical composition and bacterial abundance in $PM_{2.5}$

Figure 5 Bacterial community at phyla, class, order levels under the influence of four air mass trajectories.

Figur 6 Variance analysis of genera species based on the Kruskal–Wallis rank sum test (A) and the Spearman's rank correlations with environmental factors (B; $*p < 0.05$; $**p < 0.01$).

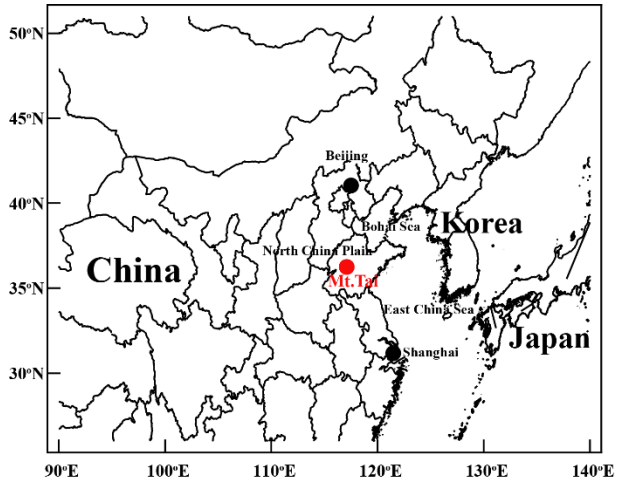

50°N
45°N
40°N
35°N
30°N
90°E
100°E
110°E
120°E
130°E
140°E
Beijing
Bohai Sea
Korea
North China Plain
China
Mt.Tai
East China Sea
Japan
Shanghai

Figure 1

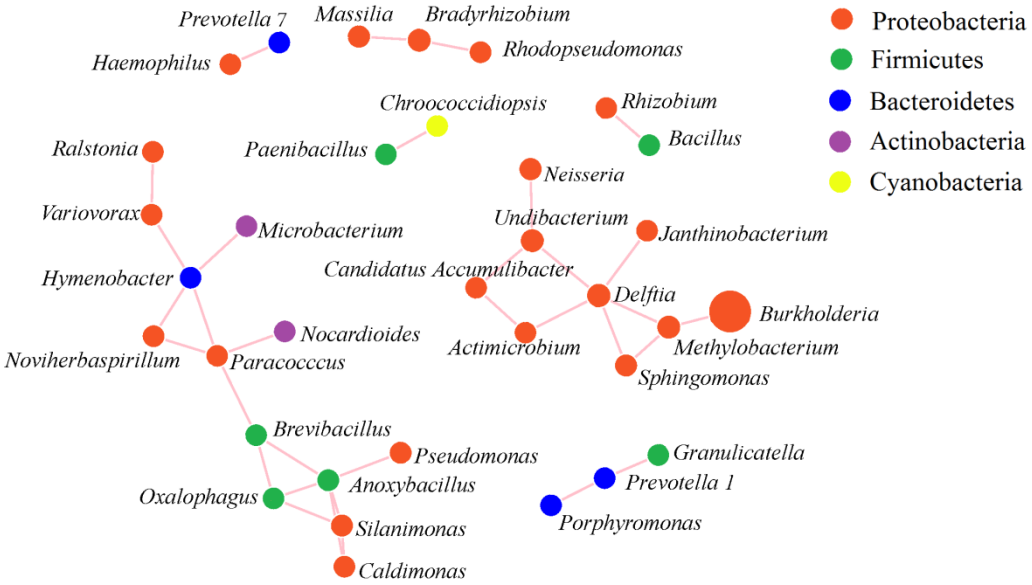

Prevotella 7
Massilia
Bradyrhizobium
Haemophilus
Rhodopseudomonas
Chroococcidiopsis
Rhizobium
Bacillus
Ralstonia
Paenibacillus
Neisseria
Variovorax
Microbacterium
Undibacterium
Janthinobacterium
Hymenobacter
Candidatus Accumulibacter
Delftia
Burkholderia
Nocardioides
Actimicrobium
Methylobacterium
Noviherbaspirillum
Paracocccus
Sphingomonas
Brevibacillus
Pseudomonas
Granulicatella
Anoxybacillus
Prevotella 1
Oxalophagus
Porphyromonas
Silanimonas
Caldimonas
Proteobacteria
Firmicutes
Bacteroidetes
Actinobacteria
Cyanobacteria

Figure 1

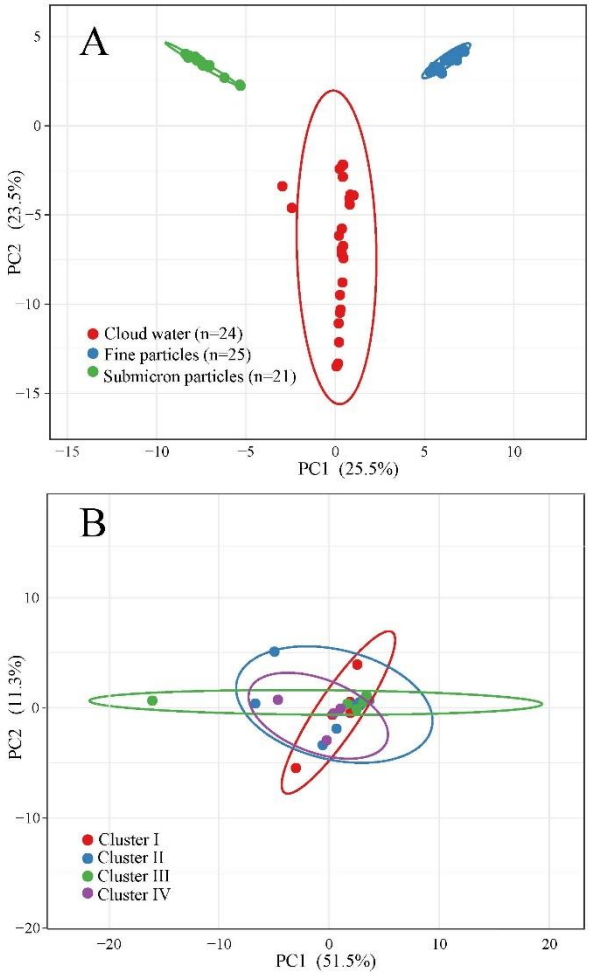

A
Cloud water (n=24)
Fine particles (n=25)
Submicron particles (n=21)
PC2 (23.5%)
PC1 (25.5%)
B
Cluster I
Cluster II
Cluster III
Cluster IV
PC2 (11.3%)
PC1 (51.5%)

Figure 2

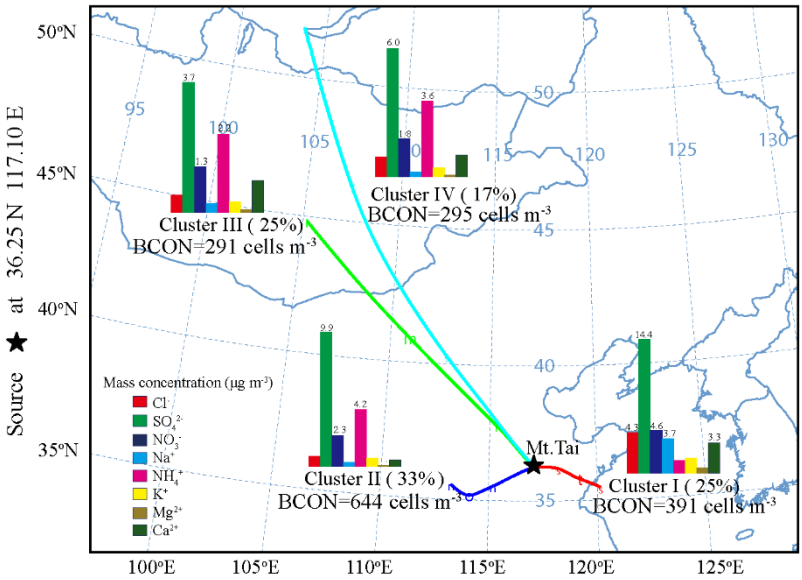

Source ★ at 36.25 N 117.10 E
50°N
45°N
40°N
35°N
100°E
105°E
110°E
115°E
120°E
125°E
Cluster III (25%)
BCON=291 cells m-3
Cluster IV (17%)
BCON=295 cells m-3
Cluster II (33%)
BCON=644 cells m-3
Cluster I (25%)
BCON=391 cells m-3
Mt.Tai
Mass concentration (μg m-3)
Cl-
SO4 2-
NO3-
Na+
NH4+
K+
Mg2+
Ca2+

Figure 3

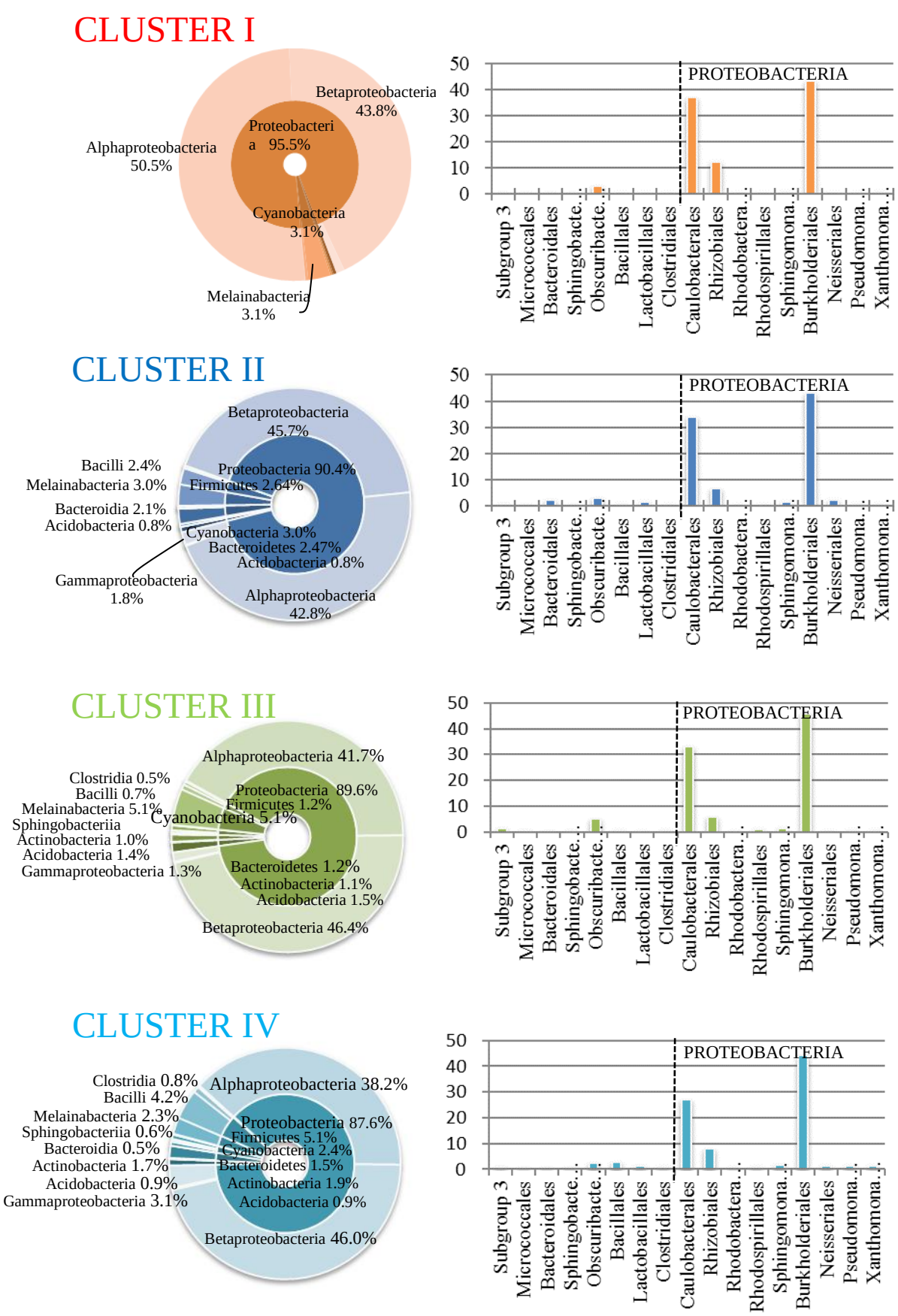
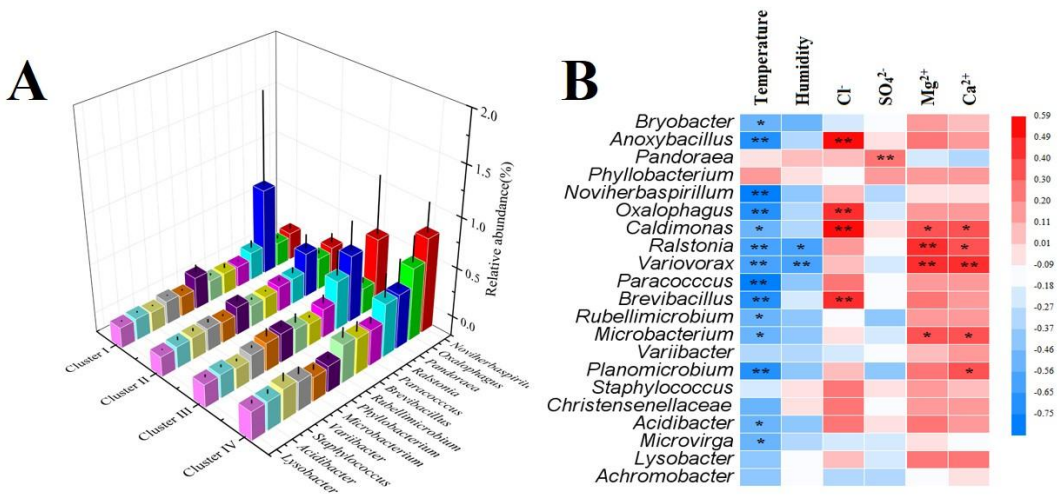

CLUSTER I
Betaproteobacteria 43.8%
Proteobacteria 95.5%
Alphaproteobacteria 50.5%
Cyanobacteria 3.1%
Melainabacteria 3.1%
PROTEOBACTERIA
Subgroup 3
Micrococcales
Bacteroidales
Sphingobacte...
Obscuribacte...
Bacillales
Lactobacillales
Clostridiales
Caulobacterales
Rhizobiales
Rhodobactera...
Rhodospirillales
Sphingomona...
Burkholderiales
Neisseriales
Pseudomona...
Xanthomona...
CLUSTER II
Betaproteobacteria 45.7%
Bacilli 2.4%
Melainabacteria 3.0%
Proteobacteria 90.4%
Firmicutes 2.64%
Bacteroidia 2.1%
Acidobacteria 0.8%
Cyanobacteria 3.0%
Bacteroidetes 2.47%
Acidobacteria 0.8%
Gammaproteobacteria 1.8%
Alphaproteobacteria 42.8%
CLUSTER III
Alphaproteobacteria 41.7%
Clostridia 0.5%
Bacilli 0.7%
Melainabacteria 5.1%
Sphingobacteriia
Actinobacteria 1.0%
Acidobacteria 1.4%
Gammaproteobacteria 1.3%
Proteobacteria 89.6%
Firmicutes 1.2%
Cyanobacteria 5.1%
Bacteroidetes 1.2%
Actinobacteria 1.1%
Acidobacteria 1.5%
Betaproteobacteria 46.4%
CLUSTER IV
Clostridia 0.8%
Bacilli 4.2%
Melainabacteria 2.3%
Sphingobacteriia 0.6%
Bacteroidia 0.5%
Actinobacteria 1.7%
Acidobacteria 0.9%
Gammaproteobacteria 3.1%
Alphaproteobacteria 38.2%
Proteobacteria 87.6%
Firmicutes 5.1%
Cyanobacteria 2.4%
Bacteroidetes 1.5%
Actinobacteria 1.9%
Acidobacteria 0.9%
Betaproteobacteria 46.0%

Figure 4


A
Relative abundance(%)
2.0
1.5
1.0
0.5
0.0
Cluster I
Cluster II
Cluster III
Cluster IV
Noviherbaspirillum
Oxalophagus
Pandoraea
Ralstonia
Paracocccus
Brevibacillus
Rubellimicrobium
Phyllobacterium
Microbacterium
Variibacter
Staphylococcus
Acidibacter
Lysobacter
B
Temperature
Humidity
Cl-
SO4 2-
Mg2+
Ca2+
Bryobacter
Anoxybacillus
Pandoraea
Phyllobacterium
Noviherbaspirillum
Oxalophagus
Caldimonas
Ralstonia
Variovorax
Paracocccus
Brevibacillus
Rubellimicrobium
Microbacterium
Variibacter
Planomicrobium
Staphylococcus
Christensenellaceae
Acidibacter
Microvirga
Lysobacter
Achromobacter
0.59
0.49
0.40
0.30
0.20
0.11
0.01
-0.09
-0.18
-0.27
-0.37
-0.46
-0.56
-0.65
-0.75

Figur 5