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# Effects of haze pollution on microbial community changes and correlation with chemical components in atmospheric particulate matter



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#### HIGHLIGHTS

## GRAPHICAL ABSTRACT

- · Soil and dust contributed most to the increase of the PM pollution.
- · Soil, water, plants and feces were the possible sources of the bacteria in PM.
- · Differences in bacterial community structure of clean and smoggy days were evident.
- · PM concentrations affected bacterial community more than PM sizes.
- The microbial community structure was dynamic due to different environmental factors.



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## ABSTRACT

In this study, particulate matter (PM) with aerodynamic diameters of  $\leq 2.5$  and  $\leq 10 \mu m$  (PM<sub>2.5</sub> and PM<sub>10</sub>, respectively), which was found at different concentrations in spring, was collected in Beijing. The chemical composition and bacterial community diversity of PM were determined, and the relationship between them was studied by 16S rRNA sequencing and mathematical statistics. Chemical composition analysis revealed greater relative percentages of total organic compounds (TOC) and secondary ions (NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and NH<sub>4</sub><sup>+</sup>). The concentrations of Ca<sup>2+</sup>,  $Na^+$ ,  $Mg^{2+}$ ,  $K^+$  and  $SO_4^{2-}$  increased in high-concentration PM, which was associated with the contribution of soil, dust and soot. Microbiological analysis revealed 1191 operational taxonomic units. Microbial community structure was stable at the phylum level. The most abundant phyla were Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Cyanobacteria. Community clustering analysis at the genus level showed that the difference in bacterial community structure between different PM concentrations (clean air vs. smog) was greater than that between different particle sizes. The dominant genera varied in different concentrations of PM. An unclassified genus of Cyanobacteria and Comamonadaceae were most abundant in low- and high-concentration PM, respectively. The microbial community structure was dynamic at the genus level due to different environmental factors. The dominant bacteria in high-concentration PM were widely distributed in soils, indicating that the soil contributed more to the increase in the PM. The individual microbes that were detected did not increase significantly as the PM concentration increased. The bacterial community structure was strongly correlated with  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ ,  $Mg^{2+}$ ,  $SO_4^{2-}$ and TOC in high-concentration PM and had a good correlation with NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, NH<sub>4</sub><sup>+</sup> and TIC in low-concentration PM. Soil and dust contributed to the increase in the concentration of the particles, and the relevant chemical components also produced differences in the bacterial community structure in different concentrations of PM.

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# 1. Introduction

Particulate matter (PM) has been identified as a human carcinogen (Gou et al., 2016) and is the fourth leading risk factor for public health. PM inhalation may lead to death (Hu et al., 2015; Wang and Wang, 2014) and can be deposited in different parts of the body (Cao et al., 2014; Yan et al., 2016). Particles with a submicron diameter can pass through the lungs or blood vessels (Xu et al., 2017). Exposure to a high concentration of haze, which is comprised of PM, contributes to an increase in morbidity and mortality caused by cardiovascular diseases, respiratory diseases and lung cancer (Cao et al., 2014; Gou et al., 2016; Xu et al., 2017). PM displays widely variant and complex compositions (Zhang and Su, 2016; Wang and Wang, 2014). These compositions include microorganisms, most of which adhere to the particles due to the inability of the microbes to use air components directly for reproduction and growth (Liao, 2013; Si, 2014). Microorganisms are an important route for the transmission of allergens and pathogens (Gao et al., 2017; Cao et al., 2014) and thus can be potentially harmful to humans (Ren et al., 2006; Cao et al., 2014).

To better understand the possible impacts of airborne microorganisms on public health, studies of microbial diversity and community structure have been conducted using traditional culture-dependent methods (Bertolini et al., 2013; Hu et al., 2015; Liao et al., 2013; Qian, 2012; Xu, 2013). The methods isolate and culture environmental samples to identify species, count and obtain a single strain of microorganisms according to morphological, physiological and biochemical characteristics (Liu et al., 2010). The early detection of airborne microorganisms was based on culture-dependent plate counting and morphological methods (Lighthart and Kim, 1989; Lindenann et al., 1982; Shaffer and Lighthart, 1997). The use of these methods laid the foundation for the studies of air microorganisms. Then, some researchers studied indoor and outdoor microbial contamination characteristics. For example, Fang et al. (2008, 2014) and Hu et al. (2015) studied the microbial aerosol concentration and particle size distribution. However, these methods underestimate microbial diversity (Deng et al., 2016; Ren et al., 2006) because only a small fraction of the microorganisms present will be cultivated (Deng et al., 2016). With the development of culture-independent techniques, modern molecular biology techniques analyze the nucleic acids directly extracted from the environmental samples. These methods use specific nucleic acid fragments as biomarkers to understand the composition and diversity of microorganisms in the environment. Specific target nucleic acid fragments are obtained by PCR and then identified by a variety of techniques to determine community composition and diversity, such as fingerprinting of community structure analysis, microarray and high-throughput sequencing. High-throughput sequencing technology, which can provide a comprehensive and detailed understanding of microbial diversity and community structure (Gandolfi et al., 2015; Ren et al., 2006; Smets et al., 2016), was used to study on the microbial ecology in various environments, such as soil (Hua et al., 2018; Geisen, 2016; Wang et al., 2017), water (Lu et al., 2016a, 2016b; Jiang et al., 2016; Schmeisser et al., 2003), sludge (Yoshizawa et al., 2014; Wang et al., 2016) and air (Gao et al., 2017; Cao et al., 2014; Xu et al., 2017).

In contrast to the extensive physical and chemical properties, little is known about microbes in PM (Yan et al., 2016). The majority of the existing studies focused on microbial diversity and the relationships between PM diameter, weather, location, time and atmospheric environment (Yoo et al., 2017). The microbial composition of PM<sub>1</sub> and PM<sub>10</sub> collected during the winter in the Chinese city of Urumqi was analyzed using 16S and 18S gene sequencing (Gou et al., 2016). The influence of different sampling points on relative bacterial abundance was greater than that of different particle sizes. Some studies investigated the relationships between microorganisms and environmental factors. The studies reported that the abundance and community structure of the air microorganisms were generally affected by temperature (Gao et al., 2014), relative humidity (Raisi et al., 2013), season (Bowers

et al., 2013; Bertolini et al., 2013; Bowers et al., 2012; Franzetti et al., 2011), geography (Bowers et al., 2011), and atmospheric pollutants (e.g., O<sub>3</sub>, NO<sub>2</sub>, SO<sub>2</sub>) (Xu et al., 2017). Since bacteria live in tight association with PM, the effects of particulate chemical components on airborne microorganisms were investigated in a few studies in recent years (Gandolfi et al., 2015; Gao et al., 2017). Gandolfi et al. (2015) compared the microbial air structure between the Italian cities of Milan and Venice using 16S rRNA and qPCR methods. In contrast to the major factors (environmental and meteorological conditions), the structure of bacterial communities was less affected by the chemical composition of PM. Allergens and pathogens were also detected in PM. Xu et al. (2017) examined the bacterial communities in submicron particles by high-throughput sequencing and quantitative polymerase chain reaction (qPCR) and some potential pathogens were detected. Cao et al. (2014) described the microbial compositions of PM with a diameter of 2.5  $\mu$ m (PM<sub>2.5</sub>) and 10  $\mu$ m (PM<sub>10</sub>) in Beijing using a metagenomic approach. The results suggested that bacteria were the most abundant microorganisms and that most of the inhalable microorganisms were from soils and were not pathogenic. The relative abundances of several detected microbial allergens and pathogens were positively correlated with the PM concentrations. A recent study found that the abundance of bacteria, as well as resistance genes in Beijing haze, was the highest among all researched types (Pal et al., 2016).

Some researchers found that the bacterial aerosol concentrations in clear days were significantly higher than those in haze days in summer and winter (Hu et al., 2015; Gao et al., 2014). However, Haas et al. (2013) investigated the concentration of particles and microorganisms in the center of Graz, Austria, in one year and found that bacterial aerosol concentrations were positively correlated with particle counts. Reports on the correlations between microbes and chemical components of PM are rare (Raisi et al., 2013; Alghamdi et al., 2014; Haas et al., 2013). Nonetheless, chemical constituents in PM are important in particulate origin, formation and growth mechanisms (Chang et al., 2015) and are closely associated with microbes as they are the survival environment of microorganisms. Therefore, high concentration of particulate matter and its adsorbed chemical components may inhibit or promote the growth of some microorganisms in the process of haze pollution, and thus affect the community structure. It is also possible that more harmful microorganisms, such as pathogenic bacteria and allergens, adsorb on the particles and threaten public health.

Herein, a method for the direct extraction of microbial DNA in PM under culture-independent conditions was explored. Bacterial 16S rRNA sequencing and ecology statistical analyses both in clean and somggy days were used to analyze the variability of bacterial diversity and community structure. Particulate chemical composition was also detected and used to evaluate whether the microbial community characteristics were related with it. The results may provide a reference for reducing particulate air pollution and protecting public health.

#### 2. Materials and methods

#### 2.1. Sample collection

PM<sub>2.5</sub> and PM<sub>10</sub> were sampled on the roof of the Department of the Environment (39°57′45.5″N, 116°21′51.3″E), Beijing Normal University in the Haidian District of Beijing located inside the third ring road of the city. The height of the sampling port was approximately 22 m and was approximately 300 m and 850 m from the nearest city traffic road and hospital, respectively. No obstructions and obvious sources of pollution were present. The samples were collected for approximately 24 h every day (9:00 am to 9:00 am the next day) from April 8 to 10 and May 5 to 6, 2016. The air was passed through two model TH-150C particulate matter samplers (Wuhan Tianhong Instruments Co., Ltd., China) with medium air flow (100 L/min) (one for PM<sub>2.5</sub> and another for PM<sub>10</sub>). In total, five PM<sub>2.5</sub> samples and five PM<sub>10</sub> samples were obtained. Before sampling, the quartz membranes ( $\emptyset$  90 mm, TISSUQUARTZ 2500QAT-

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