Meteorological factors had more impact on airborne bacterial communities than air pollutants

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HIGHLIGHTS

- The airborne bacteria concentrations and community structure showed strong seasonality.
- However, violent weather events broke seasonal trends of bacteria community.
- Within season, meteorological factors explained more variations in bacteria community structure than air pollution.

GRAPHICAL ABSTRACT
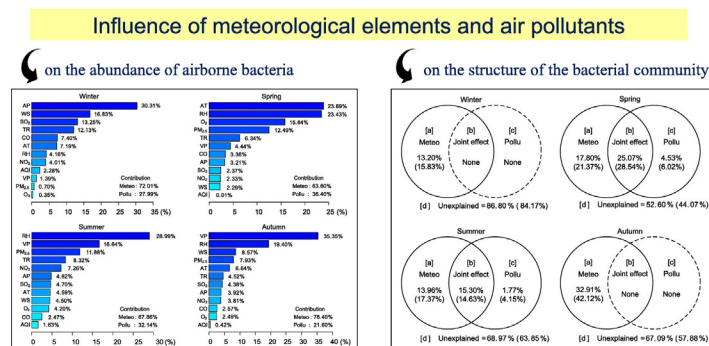
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ABSTRACT

Airborne bacteria have gained increasing attention because they affect ecological balance and pose potential risks on human health. Recently, some studies have focused on the abundance and composition of airborne bacteria under heavy, hazy polluted weather in China, but they reached different conclusions about the comparisons with non-polluted days. In this study, we tested the hypothesis that meteorological factors could have a higher impact on shaping airborne bacterial communities than air pollutants by systematically monitoring the communities for 1 year. Total suspended particles in Beijing were sampled for 20 consecutive days in each season of 2015. Bacterial abundance varied from 8.71×10^3 to 2.14×10^7 ribosomal operons per cubic meter according to the quantitative PCR analysis. There were relatively higher bacterial counts in spring and in autumn than in winter and summer. Airborne bacterial communities displayed a strong seasonality, according to the hierarchical cluster analysis. Only two exceptions overtook the seasonal trend, and both occurred in or after violent meteorological changes (sandstorm or rain). Aggregated boosted tree analysis performed on bacterial abundance showed that the dominant factors shaping bacterial communities were meteorological. They were air pressure in winter, air temperature and relative humidity in spring, RH in summer, and vapor pressure in autumn. Variation partition analysis on community structure showed that meteorological factors explained more variations than air pollutants. Therefore, both of the two models verified our hypothesis that the differences in airborne bacterial communities in polluted days or non-polluted days were mainly driven by the discrepancies of meteorological factors rather than by the presence of air pollutants.

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1. Introduction

Bacteria are ubiquitous on Earth. Despite low levels of moisture and nutrients, high solar radiation, and a large dispersal capability (Gandolfi et al., 2013), many species of bacteria survive in the atmosphere in large populations. The abundance of airborne bacteria ranges from 10^4 to 10^8 cells per m^3, depending on their habitats, such as agricultural fields, urban areas, forests, and mountains (Bowers et al., 2011, 2012; Cao et al., 2014). Airborne bacteria mainly come from natural sources, such as soils, plants, and water (Smets et al., 2016), and play prominent roles in ecological balance (Fang et al., 2007) and atmospheric processes (Gandolfi et al., 2013). In addition, some pathogens in the atmosphere may cause diseases, such as allergies, inflammation (Polymenakou, 2012), and wound infections (Whyte et al., 1982). Therefore, studying the dynamic characteristics of airborne bacteria and the factors that affect them is important. It can help researchers to positively identify environments that encourage more bacteria or pathogens, and allow people to take active control measures against microbial pollution and airborne diseases.

In recent years, the Chinese people have suffered from serious air pollution, especially frequent occurrences of heavy haze in winter and autumn (Cao et al., 2014; Li et al., 2015). Hazy days are characterized by high concentrations of fine particles (aerodynamic diameter <2.5 μm, $PM_{2.5}$) as well as low intensity of solar radiation. Since suspended particles could act as carriers of bacteria (Smets et al., 2016) and low solar radiation could reduce damages on bacteria (Tong and Lighthart, 1997a), some researchers have suggested that hazy environments might absorb large numbers of bacteria and benefit some species' survival that may increase health risks. However, investigations into airborne bacteria have not provided consistent results. For example, Gao et al. (2015a, 2015b) collected cultivable airborne bacteria in Beijing, and found a lower concentration during hazy days than in non-hazy days. In contrast, Li et al. (2015) used the same method and obtained opposite results in samples from Xi'an city. Wei et al. (2016) did not find significant differences in the composition of airborne bacteria between hazy and sunny days. In contrast, a study by Gao et al. (2017) suggested that atmospheric pollutants were the main factors in shaping bacterial community structure. Therefore, significant gaps remain in our knowledge about how, and to what extent, air pollutants affect airborne bacteria.

The above studies were conducted over a short timescale (Gao et al., 2017; Li et al., 2015) or focused mainly on the effects of air pollutants because they divided samples by air quality index (AQI) value (Wei et al., 2016) or $PM_{2.5}$ concentration (Gao et al., 2015a, 2015b; Li et al., 2015). We believe that long term systematic monitoring, which can cover more variations in airborne bacteria and environmental factors and compare their relative contributions to airborne bacteria with other possible driving factors, would improve our understanding of air pollutant effects. In this study, we hypothesized that meteorological factors play a more important role in shaping airborne bacterial communities than air pollutants do. We took meteorological factors into account for two reasons: i) the occurrence and degree of air pollution are closely related to meteorological conditions (Zhang et al., 2015); and ii) previous studies have shown that some meteorological factors have considerable effects on airborne bacteria (Jones and Harrison, 2004; Smets et al., 2016).

To test our hypothesis, we established a survey site in a fixed monitoring point in Beijing Urban Ecosystem Research Station, which monitored data on multiple meteorological factors, and monitored air pollutants that included fine particles ($PM_{2.5}$), ozone (O_3), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and carbon monoxide (CO). We not only focused on performing analysis on hazy days, but analyzed air pollutants throughout 2015. The size and diversity of the airborne bacterial communities were detected by quantitative PCR (qPCR) and high-throughput sequencing using bacterial 16S rRNA genes. To our knowledge, this is the first long-term, systematic monitoring of airborne bacteria in Beijing based on high-throughput sequencing methods and provides firsthand information about the airborne bacteria communities. We used multivariate statistical analysis to compare the relative contributions made by meteorological variables and air pollutants to airborne bacteria communities, and explained the divergence that was seen in our results and previous works (Gao et al., 2017; Li et al., 2015). We believe that this study will improve our understanding of airborne bacterial dynamics under various meteorological conditions and at different air pollution levels, and partly answer public doubts about the extent to which air pollutants influence airborne bacteria on polluted and non-polluted days.

2. Materials and methods

2.1. Particulate matter collection

The sampling site was located on the roof of a research building (40°0′31″N, 116°20′34″E, ~18 m above the ground) at the Research Center for Eco-Environmental Sciences (Chinese Academy of Sciences), in an area devoid of major pollution sources in the vicinity. Sampling was performed from the 5th to the 25th of January, April, July and October 2015; representing winter, spring, summer, and autumn. Totally suspended particles (TSPs) were extracted using a high-volume sampler (Type 2031, made by Qingdao Laoshan Applied Technology Research Institute, Qingdao, China) at an average flow rate of 1.05 m^3/min for 23 h (10:00 to 09:00 h on the next day). Approximately 1449 m^3 air flowed through square glass fiber filters (8×10 in.; Pall Life Sciences, Mumbai, India). Particulate matter with aerodynamic diameter ≤ 100 μm was captured by 99.98% typical aerosol retention. Before sampling, each filter was packaged by aluminum foil and baked in a muffle furnace at 500 °C overnight. The filter holder was disinfected with 75% ethanol and air-dried. Disposable aseptic gloves and sterile tweezers were used when exchanging filters. After wrapping with sterilized aluminum foil, the specimens were brought back to laboratory and stored at −80 °C until subsequent analyses were performed.

2.2. DNA extraction

One eighth of each filter was cut into strips and put into 50 ml aseptic centrifuge tubes on a clean bench. After adding 35 ml refrigerated sterile 1× PBS buffer, the tube was shaken at 4 °C for 20 min at 200 rpm, and then the suspension collected. The washing and shaking steps were repeated three times, generating approximately 105 ml elution for each sample. The elution was filtered using a 0.2 μm Supor 200 PES Membrane Filter (Pall, NY, USA), which was then cut up for DNA extraction using the PowerSoil DNA isolation kit (MOBIO, Carlsbad, CA, USA). To thoroughly lyse microbial cells and extract the DNA, the specimens were heated to 65 °C in PowerBead Tubes for 10 min, and then shaken at 6 m/s for 45 s (Fastprep-24, MP Biomedicals, Irvine, CA, USA). The next steps of the DNA extraction process were performed according to the standard MOBIO PowerSoil DNA isolation protocol. Genomic DNA quality and concentration were analyzed using a NanoDrop 1000 UV–Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Blank control samples were collected by placing a sterilized filter inside of the sampler without operation for 23 h, and processed in the same way as the experimental filters. DNA extraction from blank control samples resulted in DNA concentrations that were below the detection limit of our instruments. All the DNA samples were stored at −80 °C until further use.

2.3. qPCR

The abundance of airborne bacteria was estimated by quantifying the copy number of 16S rRNA genes. Universal bacterial primers and TaqMan probes that covered the positions 331 to 797 of the *Escherichia coli* coding region of the 16S rRNA gene (Nadkarni et al., 2002) were