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Spatial and seasonal variation of the airborne microbiome in a rapidly developing city of China

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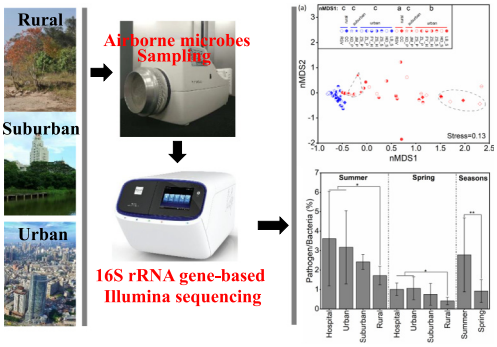
HIGHLIGHTS

- Firmicutes and Proteobacteria predominated in airborne bacterial communities.
- AM was higher diversity in summer compared with that in spring.
- Communities of AM and pathogens varied in sites with different urbanization levels.
- Relative abundance of potential human pathogens increased with urbanization levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Exposure to airborne microbes (AM) can affect the human microbiome and has various consequences for human health. Investigating the profiles of AM and the potential bacterial pathogens within, along with the factors influencing their community, is pivotal for understanding the impact of AM on human health. In this study, we collected AM during spring and summer from 11 sites with various levels of urbanization in the city of Xiamen, China. Bacterial community compositions of the AM were determined based on 16S rRNA gene amplicon sequencing. Firmicutes and Proteobacteria were the predominating phyla in the airborne bacterial communities, and a higher ($P < 0.05$) diversity of AM was found during the summer as compared to the spring. Significant differences in the community structure of the AM and the potential bacterial pathogens within airborne microbes were observed among the seasons and the sites with different levels of urbanization. Increases and/or decreases in the abundance of *Bacillus* and *Acinetobacter* could explain a major part of the variations in the AM community compositions. The proportion of potential bacterial pathogens during the summer was significantly higher ($P < 0.01$) than in the spring, and the relative abundance of several bacterial pathogens (i.e. *Burkholderia multivoran*, *Enterococcus faecium* and *Streptococcus thermophilus*) related to human diseases (39.8% of total pathogens on average) increased with increasing urbanization levels, suggesting that urbanization can increase the AM-associated human health risk.

1. Introduction

Air pollution is recognized as a global risk factor influencing human health. A recent report revealed a robust relationship between poor air

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quality and infant mortality in developing countries (Heft-Neal et al., 2018). In addition, reports have indicated a relationship between air quality, the airborne microbiome and human health. Bioaerosol particles harbor diverse microorganisms and play an important role in the exchange of microbes in air, water, soil, plants, animals and humans (Santl-Temkiv et al., 2018). Airborne microbes (AM) can affect human health by colonizing the skin, mucous membranes, digestive and respiratory tracts, and can subsequently cause a series of diseases such as infections, allergies (Ege et al., 2011), acute toxic effects (Xu et al., 2016), cardiovascular disease (Riggs et al., 2018), sterility infertility (Vizcaino et al., 2016) and even cancers (Walser et al., 2015).

The composition and biodiversity of AM, as well as the factors influencing the distribution of AM, have been investigated in previous studies (Hospodsky et al., 2015; Leung et al., 2018; Shamarina et al., 2017; Wilkins et al., 2016; Z. Xie et al., 2018). A significant higher abundance of AM can be detected during haze and foggy days as compared to non-haze days (Dong et al., 2016). Proteobacteria and Firmicutes has been established as the dominant phyla in AM communities, but a higher relative abundance of Actinobacteria was observed in New York (Robertson et al., 2013) and Milan (Bertolini et al., 2013). Wei et al. also demonstrated that the bacterial community compositions in air samples varied among 40 cities of China (Wei et al., 2015), where *Lactococcus* and *Pseudomonas* were the most abundant genera in Ji'nan (C.H. Xu et al., 2017) and Beijing (Wei et al., 2016), respectively. These variations could mainly be explained by environmental factors (i.e., temperature and the concentration of $PM_{2.5}$, PM_{10}, SO_2, O_3) (Lu et al., 2018), the sources of AM (i.e., soil, plant or water), seasonal variations and human activities (i.e., urbanization). Another study indicated that both the AM population and diversity as well as pathogenic bacteria within the AM were affected by air pollution (H Liu et al., 2018).

Contemporary environmental factors are significantly affected by human activities, among which urbanization is recognized as one of the most important forms. During urbanization, urban and residential areas including parks and impervious surfaces rapidly replace agricultural areas and forests. These shifts can lead to climate changes (i.e., temperature, CO_2 concentration, and wind speed) (Kalnay and Cai, 2003; J. Liu et al., 2018; Martinez-Zarzoso and Maruotti, 2011) in the terrestrial ecosystems, and can subsequently influence the composition and diversity of soil microbes. Ultimately, a shift in soil microbiome will influence the AM communities via dispersal of dust or aerosols. Reduction in plant species and populations, as caused by urbanization, will further influence the AM by changing the plant-related microbiota, which is an important source of AM. Laforest-Lapointe et al. have indicated that anthropogenic pressures, including urbanization, can modify leaf-associated microbe-host interactions, and change the plant microbiomes (Laforest-Lapointe et al., 2017). They further suggested that the feedback between urbanization pressures and the plant-related microbiota could shape urban microbiomes, including the airborne microbiota.

Urbanization is characterized with land use changes, demographic shifts and varying green space patterns, which may change the composition and diversity of AM. However, only a few studies have examined how urbanization affects the AM in a rapidly developing city. We hypothesized that the spatial-temporal distribution of AM, and in particular the potential bacterial pathogens within the AM, could be associated with urbanization level. In this study, AM samples from 11 sites of urban, suburban and rural regions in Xiamen, China were collected during spring and summer. The AM community was investigated by 16S rRNA gene amplicon sequencing, with the aim of characterizing the microbial communities and diversities. We especially aimed to detect the potential bacterial pathogens in the outdoor air and to explore how urbanization, seasons and environmental factors affect the microbial and pathogenic community compositions.

2. Methods and materials

2.1. Sampling sites and microbiota collection

A total of 11 sampling sites (Supplemental Fig. S1) with different levels of urbanization [characterized as urban, suburban or rural based on the population density, (Supplemental Fig. S1a), and based on land-use type (Supplemental Fig. S2)] were selected in Xiamen (24.48 N, 118.08 E), Fujian, China. The sampling sites included major streets, hospitals, parks and rural zones with fewer people, where some of the sites previously have been used for studying urbanization-induced changes of the soil microbiota (Wang et al., 2017). To collect the AM, a total of 6 m^3 air were filtrated (at a rate of 50 L min^{-1}) through a sterile 80 mm (diameter) gelatine filter (3 μm pore size, 12602-80-ALK, Sartorius stedim biotech, Supplemental Fig. S2) using a portable sampler ($AirPort_{MD8}$, Sartorius stedim biotech) during the daytime in the spring (March to May) and summer (July to August) of 2017. Samples were collected approximately 1.5 m above ground level, and there were no agricultural activities in rural sites when we were sampling airborne microbes. Triplicate samples from each site during each season were collected at the same time period during three days with similar weather condition. The filters with AM were placed into a pre-sterilized 50-mL tube and stored at −20 °C until DNA extraction. The concentration of gas pollutants, $PM_{2.5}$, $PM_{10,}$ and meteorological parameters were acquired from adjacent air monitoring sites, and are presented in Table S1.

2.2. DNA extraction

Genomic DNA from the AM was extracted using a bacterial genomic DNA kit (CW0552S, CWBIO, China) according to the provided protocol with some modifications. Briefly, the gelatin filter was dissolved in 20 mL sterile double distilled water (ddH_2O) at 37–40 °C, and cell pellets were subsequently obtained by centrifugation at 14,000 ×*g* for 10 min at 40 °C. A total of 360 μL of enzymatic lysis buffer (ELB, 20 nM Tris pH 8.0, 2 mM Na_2-EDTA, 1.2% TritonX-100 and 20 mg mL^{-1} lysozyme) was added to re-suspend and lyse the collected cells. DNA extractions were then conducted according to the manufacturer's instruction. Finally, 80 μL of sterile ddH_2O (pH = 8) was used to elute the purified DNA into a clean catch tube. The DNA was stored at −20 °C prior to target-gene amplification.

2.3. Amplification and sequencing

The V4-V5 region of the 16S rRNA gene was amplified using the primer set of 515F (5′-GTGCCAGCMGCCGCGG-3′) (Caporaso et al., 2012)/907R (5′-CCGTCAATTCMTTTRAGTTT-3′) (Lane et al., 1985). A 6-bp barcode was added at the 5′-end of 907R to identify the amplicons from different samples. The bacterial 16S rRNA gene amplification was performed in 50 μL reaction volumes containing 25 μL of premix Taq™ (Ex Taq™ version 2.0 plus dye, TaKaRa, Japan), 1 μL (10 nM) of each primer, 3 μL DNA and 20 μL sterile ddH_2O. The PCR thermal cycle was as follows: initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C 30 s, 58 °C 35 s and 72 °C 30 s, and a final extension step of 72 °C for 8 min. The PCR products with the correct size (Approx. 410 bp) were extracted from gel bands and purified using a universal DNA purification kit (DP214-3, Tiangen, China). Purified amplicons were quantified using a Qubit Fluoromoeter (version 3.0, Invitrogen), and equimolar concentrations of the samples were pooled for library constructions prior to high-throughput sequencing. Pooled samples were sent to BGI (Shenzhen, China) and were sequenced using a Illumina Hiseq 2500 platform.

2.4. Processing of Illumina sequencing data

Chimera and low-quality sequences (<300 bp in length, <25 in quality score, containing ambiguous characters and mismatch primer)

were removed from raw sequences using the Quantitative Insights Into Microbial Ecology pipeline (QIIME, version 1.9) (Caporaso et al., 2010). For analyses of the AM community composition and biodiversity, the remaining high-quality sequences were grouped into operational taxonomic units (OTUs) at a 97% nucleic acid similarity and taxonomically identified using BLAST against reference 16S rRNA gene sequences in the RDP database (Xu et al., 2014). Chloroplast and mitochondrial OTUs and OTUs with only one sequence (singletons) were removed in this study. All samples were rarefied to 32,720 sequences per sample for subsequent analyses. For determination of potential pathogens, the high-quality sequences were blasted against reference sequences from a constructed database reported by our previous study (Chen et al., 2016) with an E-value $< 1 \times 10^{-10}$ and a sequence identity threshold > 99%. All sequences have been deposited in the National Center for Biotechnology Information (NCBI) GenBank under SAR accession number of SRP144967.

2.5. Statistical analysis

Diversity indexes, including phylogenetic diversity (PD) _whole tree, Chao1, observed species, and Shannon-Wiener, were calculated using QIIME (version 1.9). Non-metric multidimensional scaling (nMDS) analysis, redundancy analysis (RDA) and constrained canonical analysis of principal coordinates (CAP) were performed using R with "Vegan" and "Mass" package (version 3.4.0) (Dixon, 2003) to determine environmental factors contributions to the AM community variations. Variations in the microbial community compositions between urban, suburban and rural regions were analyzed based on the nMDS1 values using SPSS software (version 20.0, SPSS Inc., Chicago, III, USA). All plots in this study were generated using OriginPro 2018.

3. Results

3.1. Microbial biodiversity and community structures

A total of 184,211 OTUs were detected across all sampling seasons and sites. To evaluate the bacterial biodiversity at the same sequencing depth, 32,720 sequences per sample were randomly selected to generate rarefaction curves. The alpha diversity of the AM was significantly higher ($P < 0.05$) during the summer than in the spring, as based on PD_whole tree (Fig. 1), Chao1, observed species and Shannon-Wiener (Supplemental Table S2). For the summer samples, the microbes collected from rural sites had the highest biodiversity followed by the samples from urban and suburban. However, for the spring samples, no significant variations in AM biodiversity were observed between the regions with different levels of urbanization.

Variations in the bacterial community composition, as visualized by nMDS (Fig. 2a), indicated a distinct pattern between summer and spring. The AM communities in the summer samples varied among sampling sites, but they exhibited an obvious separation between regions with different levels of urbanization. On the contrary, a significant region pattern was not observed in the spring samples (Fig. 2a).

3.2. Taxonomy and variation of bacterial taxa

The community structures of airborne bacteria in the collected samples are shown in Fig. 3 at the phylum (10 most abundant within each sample) and genus levels (relative abundance > 1.5% within each sample), respectively. At the phylum level (Fig. 3a), Firmicutes and Proteobacteria were the most dominant bacterial phyla, accounting for 21.87–92.86% and 4.28–31.82% of the total population, respectively. Firmicutes were significantly more abundant ($P < 0.01$) in the spring samples than in the summer samples. On the contrary, Proteobacteria ($P < 0.01$) and Acidobacteria ($P < 0.001$) were significantly more abundant in the summer samples as compared to the spring samples. At the

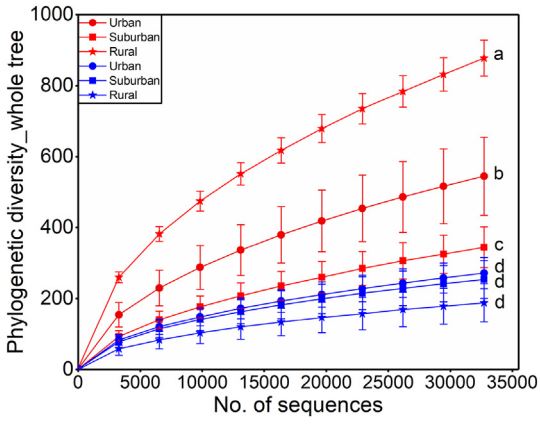

Fig. 1. Rarefaction curve based on phylogenetic diversity_whole tree (PD_whole tree) index. Red lines indicate samples from summer; Blue lines indicate samples from spring. Letters following each line indicate significant differences between samples from regions with different urbanization levels ($P < 0.05$). Error bar indicates sd (n > 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

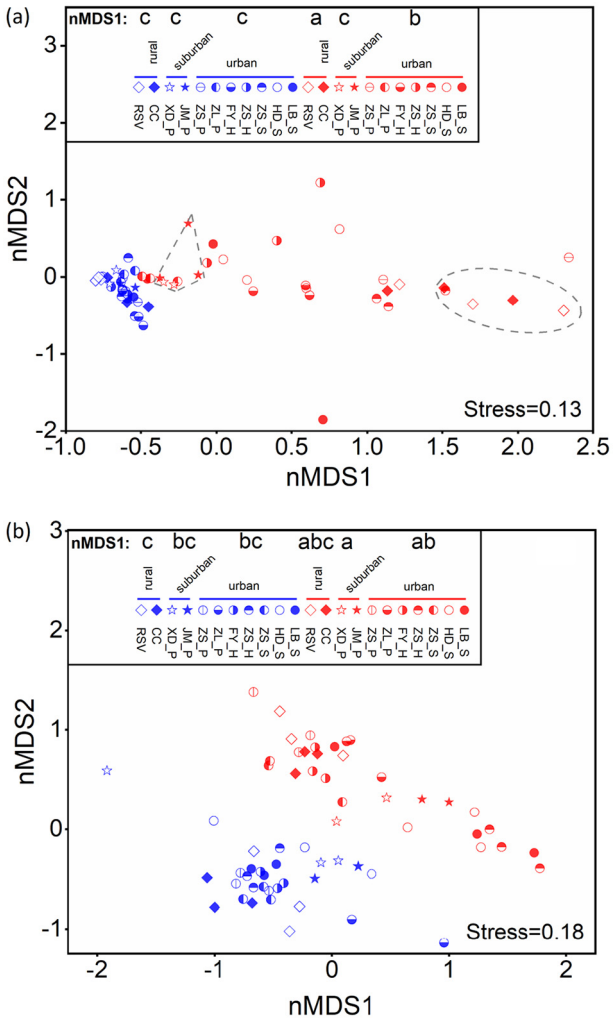

Fig. 2. Non-metric multidimensional scaling (nMDS) showing the patterns of airborne microbes (a) and potential bacterial pathogens (b) from regions with different urbanization levels during summer (red) and spring (blue). Letters indicate significant variations in communities among samples from urban, suburban and rural ($P < 0.05$) based on nMDS1 comparisons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

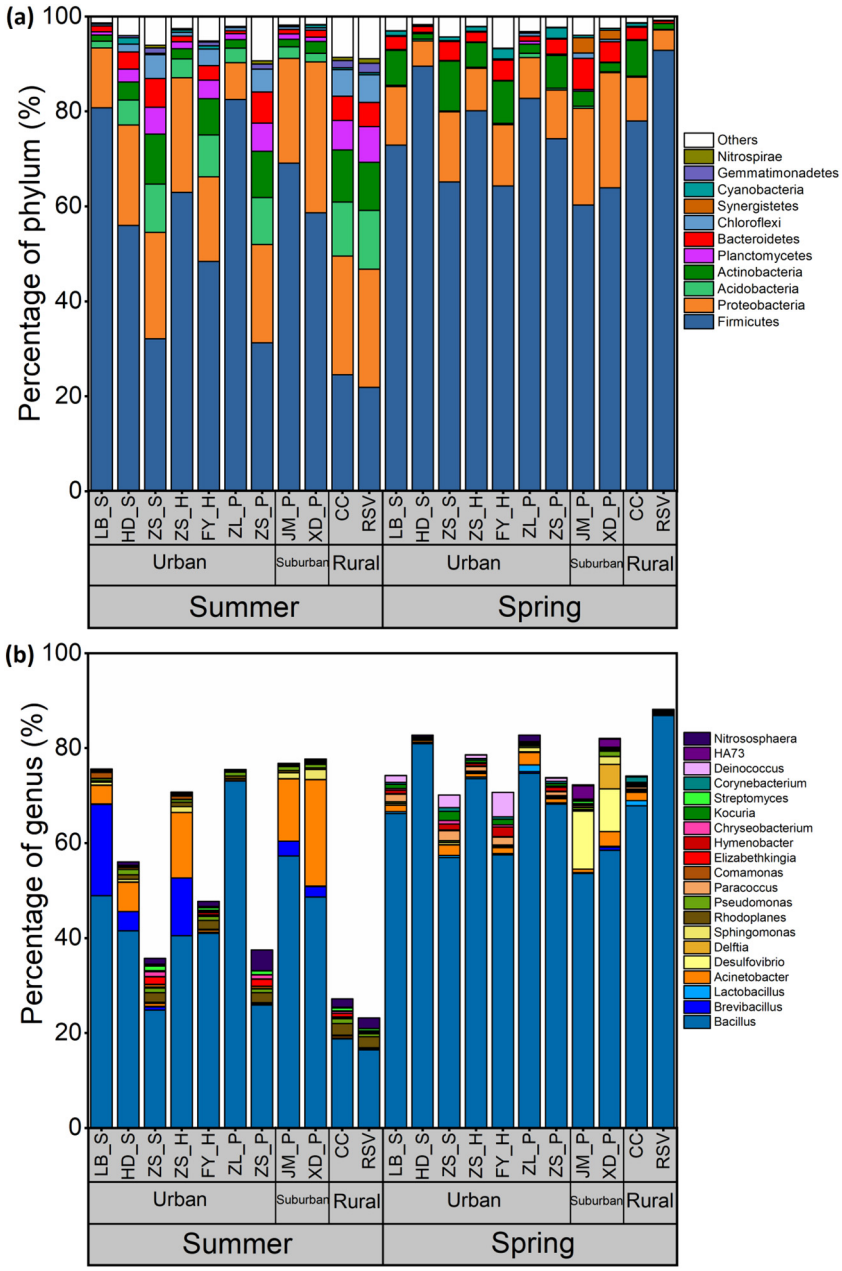

Fig. 3. Relative abundance of the most abundant phyla within each sample (a) and genera with relative abundance > 1.5% within each sample.

genus level (Fig. 3b), *Bacillus*, *Acinetobacter* and *Brevibacillus* were the most abundant genera in the air samples with relative abundances of 16.44–86.84% (average 53.71%), 0.21–22.40% (average 3.50%) and 0.006–19.24% (average 1.95%), respectively. The average relative abundances of the other genera were lower than 1%. The genera with significant variation among the measured samples were determined and are shown in Table 1. The relative abundance of *Bacillus* in the spring samples was significantly higher ($P < 0.05$) than in the summer samples, while, *Acinetobacter* was more abundant in summer samples from suburban and urban sites than in samples from other sites. In addition, the abundance of the archaea genus *Nitrososphaera*, was significantly higher ($P < 0.05$) in summer samples than in spring samples and varied between the regions in the summer samples. The *Nitrososphaera* genus exhibited a markedly temporal and spatial variation.

CAP analysis showed that season, temperature (TEM), wind speed (WS), solar radiation (SR), NO_x^-, NO_2^-, CO, SO_2, $PM_{2.5}$ and PM_{10} were key factors significantly ($P < 0.05$) related the AM communities and explained 20.9% (Bray-Curtis index) or 18.6% (Jaccard index) of the variations in the total bacterial community (Table 2). For the factors significantly affecting the total AM communities, $NO_x^- + NO_2^-$ explained 4.2% of the variation in AM communities, followed by TEM (3.5%), $PM_{10} + PM_{2.5}$ (3.1%), WS (2.8%) and season (2.5%). Different factors also significantly affected the AM community compositions during the different seasons, including gas pollutants, PM, meteorological parameters and sampling site (region). RDA revealed that region, PM_{10}, CO, O_3 and air quality index (AQI) significantly affected the AM community compositions in the summer samples (Supplemental Fig. S3a), while TEM, WS, NO_x^-, NO_2^-, O_3, CO and air pressure (AP) were detected as significant factors affecting the AM communities in the spring samples (Supplemental Fig. S3b).

3.3. Occurrence of potential bacterial pathogens

Approximately 1.95% of all sequences were identified as potential bacterial pathogens and were affiliated into 121 bacterial pathogenic

Table 1
Relative abundance (average ± sd) of genera with significant differences between regions with different urbanization levels, between summer and spring.

Genera	Summer			Spring		
	Urban	Suburban	Rural	Urban	Suburban	Rural
Bacillus	42.24 ± 16.18c	52.96 ± 6.14bc	17.62 ± 1.66d	68.29 ± 8.91ab	56.03 ± 3.47bc	77.35 ± 13.43a
Acinetobacter	3.64 ± 5.01b	17.75 ± 6.56a	0.33 ± 0.15b	1.35 ± 0.79b	1.92 ± 1.68b	1.03 ± 1.08b
Desulfovibrio	0.001 ± 0.003b	0.000 ± 0.000b	0.001 ± 0.000b	0.002 ± 0.002b	10.61 ± 2.23a	0.000 ± 0.000b
Nitrososphaera	1.12 ± 1.48ab	0.17 ± 0.00b	1.99 ± 0.33a	0.29 ± 0.48b	0.19 ± 0.05b	0.01 ± 0.01b
HA73	0.000 ± 0.001b	0.000 ± 0.000b	0.000 ± 0.000b	0.000 ± 0.000b	2.21 ± 0.78a	0.000 ± 0.000b
Sphingomonas	0.39 ± 0.44b	1.65 ± 0.59a	0.08 ± 0.01b	0.38 ± 0.26b	0.93 ± 1.01ab	0.24 ± 0.04b
Kocuria	0.12 ± 0.09b	0.08 ± 0.07b	0.06 ± 0.02b	0.74 ± 0.67a	0.10 ± 0.03b	0.20 ± 0.18b
Rhodoplanes	1.24 ± 0.74b	0.43 ± 0.09c	2.39 ± 0.04a	0.03 ± 0.01c	0.04 ± 0.02c	0.02 ± 0.02c
Paracoccus	0.08 ± 0.06b	0.08 ± 0.06b	0.07 ± 0.05b	1.05 ± 0.78a	0.26 ± 0.09b	0.25 ± 0.27b
Hymenobacter	0.02 ± 0.01b	0.03 ± 0.03b	0.01 ± 0.00b	0.80 ± 0.66a	0.10 ± 0.04b	0.16 ± 0.05b
Streptomyces	0.34 ± 0.38ab	0.04 ± 0.00b	0.55 ± 0.09a	0.02 ± 0.01b	0.34 ± 0.23ab	0.03 ± 0.03b
Corynebacterium	0.11 ± 0.08b	0.16 ± 0.16b	0.08 ± 0.08b	0.36 ± 0.24ab	0.14 ± 0.06b	0.61 ± 0.69a

Letters after each number indicate significant differences between the samples at $P < 0.05$ using a one-way ANOVA test with bootstrap = 1000.

species. *Acinetobacter oleivorans* (11.44%) and *Pseudomonas putida* (10.97%) were the predominant detected pathogenic species followed by *Staphylococcus saprophyticus* (8.73%), *Acinetobacter baumanii* (8.30%) and *Bacillus megaterium* (5.74%). The species with relative abundances > 1.5% are shown in Supplemental Fig. S4, in which most of the pathogenic species varied with the seasons. The communities of bacterial pathogens varied significantly ($P < 0.05$) with season and region, as based on nMDS1 comparisons (Fig. 2b). The ratio of pathogens to total bacteria in the summer samples (2.78%) was significantly higher ($P < 0.01$) than in the spring samples (0.92%), and a significantly higher ($P < 0.05$) pathogens to total bacteria ratio was observed in urban regions compared to rural areas. Samples collected from hospital areas (FY_H and ZS_H, Supplemental Table S1) had the highest pathogens to total bacteria ratio in the summer (3.62%) (Fig. 4). Several bacterial pathogenic species had significantly higher ($P < 0.05$) abundances in urban or/and suburban regions than in rural regions for both the summer (7 species, 35.51% of total pathogens on average, Fig. 5a, c) and the spring samples (25 species, 55.98% of total pathogens on average, Fig. 5b, c). Here, 39.8% of the total pathogens (i.e., *Acinetobacter baumanii*, *Staphylococcus saprophyticus*, *Burkholderia multivorans*, *Clostridium beijerinckii*, *Enterococcus faecium* and *Clostridium perfringens*) significantly increased with increasing levels of urbanization (Fig. 5c).

CAP analysis showed that season, region, AQI, AP, O_3, SR, $NO_x^- + NO_2^-$, PM_{10}, TEM, and WS were factors which significantly affected the pathogenic communities. These factors explained 47.3% of the variations in the pathogenic communities (Table 2), in which region explained 3.7%, followed by AP (3.3%), WS (2.7%), season (2.6%) and $NO_x^- + NO_2^-$ (2.3%). RDA analysis showed that pathogenic communities in the summer and spring samples were affected by different factors. The region was the most dominant factor affecting the distribution of pathogens in the summer and explained 4.78% of the variations (Supplemental Fig. S3c). Furthermore, AP, wind direction (WD), WS, SR, NO_x^- and NO_2^- were key factors related the pathogenic communities in the spring and explained 36.8% the community variations (Supplemental Fig. S3d).

Table 2
Constrained canonical analysis of principal coordinates (CAP) delineating the factors contributing to the variations of the bacterial and potential pathogenic communities.

Communities	Constrained	Bray-Curtis	Jaccard
	Factors	(%)	(%)
Bacterial communities	Season	2.5	2.3
	SO_2	1.1	1.2
	$NO_x^- + NO_2^-$	4.2	3.9
	CO	1.5	1.6
	$PM_{10} + PM_{2.5}$	3.1	3.3
	Temperature (TEM)	3.5	3.2
	Windspeed (WS)	2.8	2.7
	Solar radiation (SR)	1.4	1.4
	Unexplained	79.1	81.4
Pathogenic communities	Season	2.6	2.6
	Region	3.7	3.2
	Air quality index (AQI)	0.5	0.7
	Air pressure (AP)	3.3	2.8
	O_3	1.7	1.7
	Solar radiation (SR)	1.9	2.0
	$NO_x^- + NO_2^-$	2.3	2.5
	CO	0.7	1.0
	PM_{10}	0.6	0.9
	Temperature (TEM)	1.6	1.8
	Windspeed (WS)	2.7	2.4
	Unexplained	52.7	58.7

The factors listed in this table affected the communities of total AM and pathogens significantly ($P < 0.05$).

4. Discussion

In this study, we found that urbanization influenced the seasonal distribution of AM and potential bacterial pathogens within the AM. The ratio of pathogens to total bacteria increased with increasing urbanization levels, suggesting that urbanization may increase the risk of AM affecting human health.

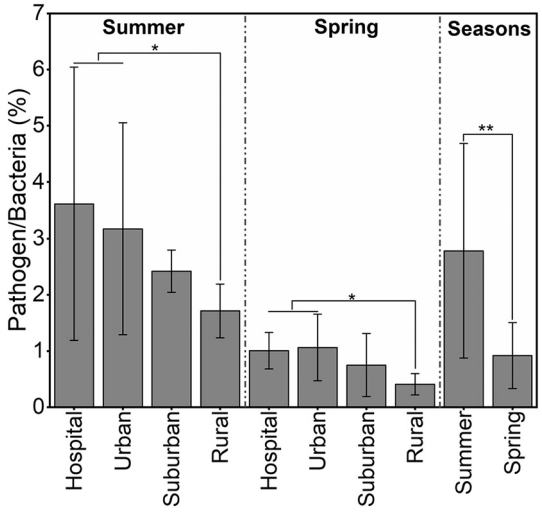

Fig. 4. Ratio of potential bacterial pathogen/total bacteria detected in the airborne microbial communities from regions with different levels of urbanization during summer and spring. * indicate significant differences between samples at $P < 0.05$; ** indicate significant differences between samples at $P < 0.01$ using a one-way ANOVA test with bootstrap = 1000. Error bar indicates sd (n > 3).

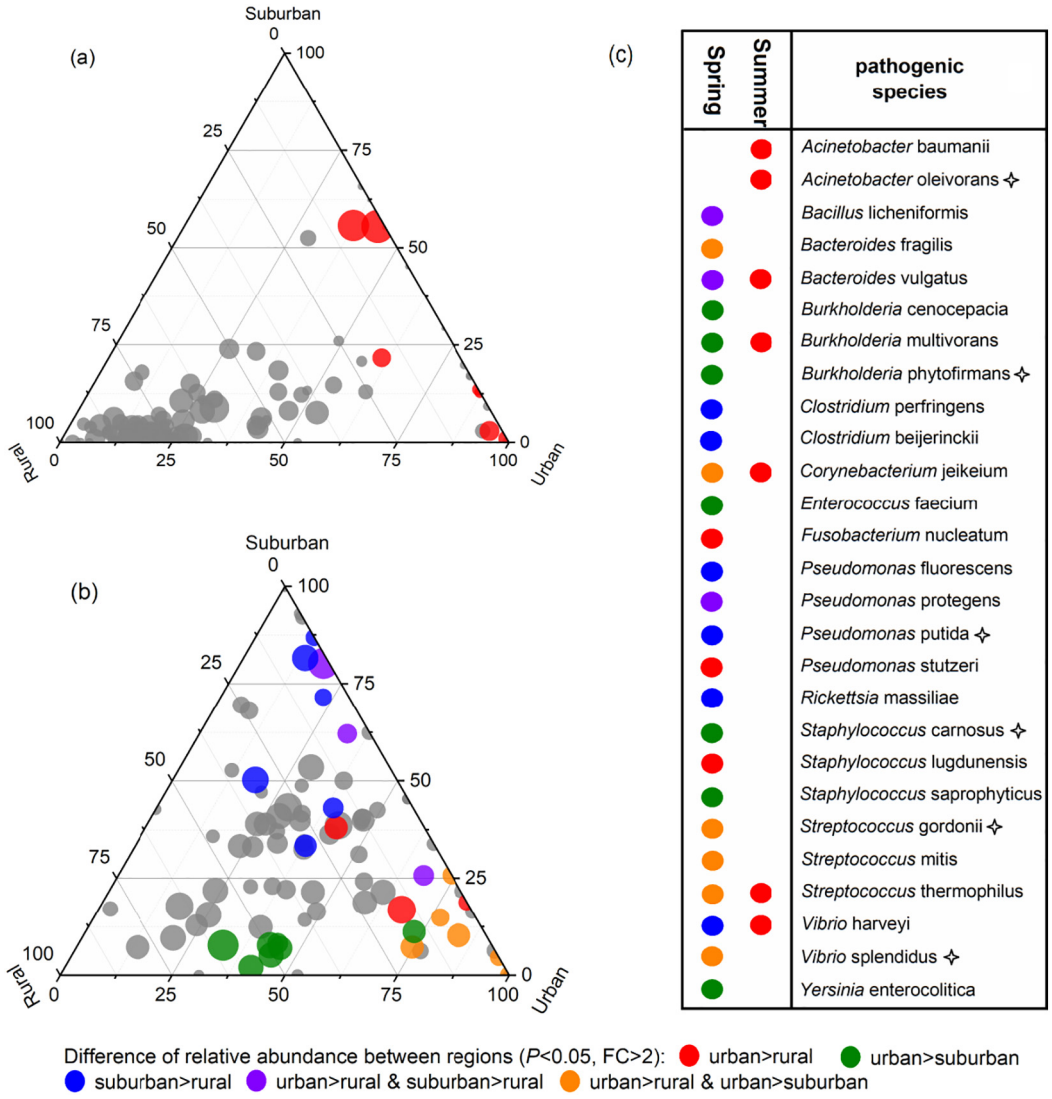

Fig. 5. Numbers of pathogenic species with significantly higher relative abundances in urban or suburban regions or both as compared to rural regions in both summer (a) and spring (b). Detailed information of the pathogenic species enriched in the urban or suburban regions or both (c). The size of the circles in plot (a) and (b) are proportional ✧ to the relative abundance of pathogenic species. Indicates pathogenic species with no risk to human health.

4.1. Factors affecting the distributions of AM

It was observed that AM communities showed a distinct seasonal pattern with a significantly higher diversity in the summer than in the spring. The shift of meteorological parameters between spring and summer may partially explain the seasonal variations in bacterial diversity. In general, summer is considered as a conducive season for microbial growth and reproduction (Quintero et al., 2010; Smets et al., 2016). Previous studies have also indicated that diversity and abundance of AM during the summer are significantly higher than during the winter due to higher temperatures (Bowers et al., 2013; Du et al., 2018; A.L. Xu et al., 2017). High wind speed can bring in exogenous microbes (Zhong et al., 2016), and can also strongly dilute ambient microbes in the air, thereby positively or negatively affecting the AM diversity. Moreover, the higher humidity and lower AP detected during the summer in Xiamen could partially explain the higher diversity of AM in the summer as compared to spring. It has been reported that high humidity (70–80%) is a sufficient condition for AM growth (Frohlich-Nowoisky et al., 2014; Leyronas and Nicot, 2013) and that increasing AP (Zhen et al., 2017) quickly can dilute AM concentrations.

In addition to the beneficial meteorological parameters during the summer, various plant species in rural areas may also partially account for a higher diversity of AM in the rural regions as compared to suburban and urban sites where there are fewer plant species. Previous studies (Jackson and Denney, 2011; Redford and Fierer, 2009; Thompson et al., 1993) have indicated that the higher abundance and diversity of plant-related microbes in the summer might be due to the blooming and withering of plants.

Since Xiamen is a coastal city, seawater-related microbes in the bioaerosols, which have been transported from the ocean to land, are important sources of AM. Thus, the seasonal variation of bacterial communities in seawater (Signori et al., 2018; Ward et al., 2017) may partially explain the AM diversity variations between spring and summer. Firmicutes and Proteobacteria were the top two phyla in both seasons, but their relative abundances significantly differed between the seasons. The seasonal shifts of dominant species have been reported previously in Mestre and Porto Marghera, where Burkholderiales and Actinomycetales were more abundant in colder seasons while Rhodobacterales was more abundant in warmer seasons (Gandolfi et al., 2015). In this study, the relative abundance of *Bacillus* and *Acinetobacter* displayed opposing patterns and partially contributed to

the seasonal variations in AM communities. High TEM and low AP during the summer in Xiamen accelerate the transportation of cold air from the ocean to land. The diluting effect of coastal AM, containing more Gram-negative bacteria (Fahlgren et al., 2010), may have led to an increase in *Acinetobacter* (Gram-negative bacteria) and a decrease in *Bacillus* (Gram-positive bacteria) which was observed in the summer samples, and it may also be responsible for the observed change in the AM community composition during the different seasons.

Spatial variation was also observed, with the lowest diversity observed at the suburban sites and the highest diversity observed at the rural sites during the summer. The AM communities during the summer varied with the different levels of urbanization. Bowers et al. have also indicated that the diversity and composition of AM in the near-surface atmosphere vary between urban and rural sites, and between two size fractions (Bowers et al., 2013). A possible reason for these variations is the higher concentration of gas pollutants in suburban and urban areas, caused by urbanization. Higher gas pollutants can provide considerable amounts of nutrients, enabling the survival and replication of microbes (Wei et al., 2017). Another possible reason for these spatial disparities is the relative contributions of the site-specific biological sources at the different sites. Human activities (J.W. Xie et al., 2018), seawater and limited vegetative species may be the main sources of AM at the urban and suburban sites of Xiamen. However, a diverse vegetation and soil might be the most important sources of AM at rural sites, resulting in the spatial variation of AM between regions with different urbanization levels.

4.2. Urbanization increases the risk of AM to human health

We further observed seasonal and spatial variations of potential pathogens within the AM in this study. Natural sources (i.e., soil, water, and plant materials), anthropogenic sources [i.e., hospital and wastewater treatment plant (WWTP) materials] (Zhu et al., 2017) and meteorological parameters are all suggested as the main drivers for these variations. The ratio of pathogens to total bacteria during the summer was significantly higher than in the spring, and urbanization significantly increased the relative abundance of potentially pathogenic species, especially in samples near hospital sites. Besides, several pathogens related to human disease (i.e., *Bacteroides vulgatus*, *Burkholderia multivorans*, *Enterococcus faecium*, *Streptococcus thermophiles*, and *Vibrio harveyi*) significantly increased with increasing urbanization levels, indicating that urbanization can increase the AM-associated human health risk.

Though natural source-related pathogens may increase due to beneficial conditions (Chen et al., 2018; Scevkova et al., 2016; Sonia et al., 2006), we speculate that the numerous potential pathogens from anthropogenic sources (i.e., human activity, hospital, and WWTP) may be the main players resulting in the increased ratio of pathogens during the summer in urban and suburban regions. Increasing human populations, as a result of urbanization, can lead to higher abundances of pathogens and infection rates due to human activities (Bradley and Altizer, 2007; Miller et al., 2002). Feces, industrial wastewater and domestic sewage with abundant pathogens (Fathi et al., 2017; Kulkarni et al., 2018; Li et al., 2015) are transferred into WWTPs, where the diversity and abundance of pathogens subsequently increase in effluents, activated sludge, biofilms, anaerobically digested sludge and in particular influents. Li et al. revealed by using a high-throughput metagenomic approach that the relative abundance of pathogens (bacterial pathogens/total bacteria) in sewage treatment plants (STP) ranged from 0.11% to 23.2% with the highest ratio found in the influent (Li et al., 2015). Furthermore, pathogens carried by patients can lead to a high abundance of pathogens in hospitals (Shimose et al., 2018). Pathogens in WWTPS and hospitals can be transferred into their surroundings via bioaerosol particles and can then be transmitted into ambient air, especially during the summer (Gao et al., 2018; Karra and Katsivela, 2007; Korzeniewska, 2011; Szyłak-Szydłowski et al., 2016). Studies have reported that higher diversities and abundances of bacterial pathogens can be detected in WWTPs and hospital surroundings as compared to adjacent background sites (Montagna et al., 2016; Offermann et al., 2016; Tong et al., 2017). In addition to bioaerosol particles with a high abundance bacterial pathogens transferred from WWTPs and hospitals, application of sewage sludge as fertilizers (Chen et al., 2016; Pillai et al., 1996) as well as reclaimed water (Piña et al., 2018; Wang et al., 2014) may also partially explain the increased abundance of bacterial pathogens in the urban and suburban sites.

Overall, seasonal variability of the total AM and bacterial pathogens were observed in this study. A higher diversity of AM and a higher ratio of pathogens to total bacteria were detected during the summer as compared to the spring. Urbanization exerted a season-dependent effect on the AM and bacterial pathogen profiles. The higher ratio of pathogens to total bacteria and the significant enrichment of pathogenic species at the sites with higher levels of urbanizing imply that urbanization may increase the AM-associated human health risk.

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References

Bertolini, V., Gandolfi, I., Ambrosini, R., Bestetti, G., Innocente, E., Rampazzo, G., et al., 2013. Temporal variability and effect of environmental variables on airborne bacterial communities in an urban area of Northern Italy. Appl. Microbiol. Biotechnol. 97, 6561–6570.

Bowers, R.M., Clements, N., Emerson, J.B., Wiedinmyer, C., Hannigan, M.P., Fierer, N., 2013. Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. Environ. Sci. Technol. 47, 12097–12106.

Bradley, C.A., Altizer, S., 2007. Urbanization and the ecology of wildlife diseases. Trends Ecol. Evol. 22, 95–102.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624.

Chen, Q.L., An, X.L., Li, H., Su, J.Q., Ma, Y.B., Zhu, Y.G., 2016. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environ. Int. 92-93, 1–10.

Chen, W., Hambleton, S., Seifert, K.A., Carisse, O., Diarra, M.S., Peters, R.D., et al., 2018. Assessing performance of spore samplers in monitoring Aeromycobiota and fungal plant pathogen diversity in Canada. Appl. Environ. Microbiol. 84.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930.

Dong, L.J., Qi, J.H., Shao, C.C., Zhong, X., Gao, D.M., Cao, W.W., et al., 2016. Concentration and size distribution of total airborne microbes in hazy and foggy weather. Sci. Total Environ. 541, 1011–1018.

Du, P.R., Du, R., Ren, W.S., Lu, Z.D., Fu, P.Q., 2018. Seasonal variation characteristic of inhalable microbial communities in PM 2.5 in Beijing city, China. Sci. Total Environ. 610, 308–315.

Ege, M.J., Mayer, M., Normand, A.C., Genuneit, J., Cookson, W.O.C.M., Braun-Fahrlander, C., et al., 2011. Exposure to environmental microorganisms and childhood asthma. N. Engl. J. Med. 364, 701–709.

Fahlgren, C., Hagstrom, A., Nilsson, D., Zweifel, U.L., 2010. Annual variations in the diversity, viability, and origin of airborne bacteria. Appl. Environ. Microbiol. 76, 3015–3025.

Fathi, S., Hajizadeh, Y., Nikaeen, M., Gorbani, M., 2017. Assessment of microbial aerosol emissions in an urban wastewater treatment plant operated with activated sludge process. Aerobiologia 33, 507–515.

Frohlich-Nowoisky, J., Nespoli, C.R., Pickersgill, D.A., Galand, P.E., Muller-Germann, I., Nunes, T., et al., 2014. Diversity and seasonal dynamics of airborne archaea. Biogeosciences 11, 6067–6079.

Gandolfi, I., Bertolini, V., Bestetti, G., Ambrosini, R., Innocente, E., Rampazzo, G., et al., 2015. Spatio-temporal variability of airborne bacterial communities and their correlation with particulate matter chemical composition across two urban areas. Appl. Microbiol. Biotechnol. 99, 4867–4877.

Gao, X.-L., Shao, M.-F., Wang, Q., Wang, L.-T., Fang, W.-Y., Ouyang, F., et al., 2018. Airborne microbial communities in the atmospheric environment of urban hospitals in China. J. Hazard. Mater. 349, 10–17.
Heft-Neal, S., Burney, J., Bendavid, E., Burke, M., 2018. Robust relationship between air quality and infant mortality in Africa. Nature 559, 254.
Hospodsky, D., Yamamoto, N., Nazaroff, W.W., Miller, D., Gorthala, S., Peccia, J., 2015. Characterizing airborne fungal and bacterial concentrations and emission rates in six occupied children's classrooms. Indoor Air 25, 641–652.
Jackson, C.R., Denney, W.C., 2011. Annual and seasonal variation in the Phyllosphere bacterial community associated with leaves of the southern Magnolia (Magnolia grandiflora). Microb. Ecol. 61, 113–122.
Kalnay, E., Cai, M., 2003. Impact of urbanization and land-use change on climate. Nature 423, 528.
Karra, S., Katsivela, E., 2007. Microorganisms in bioaerosol emissions from wastewater treatment plants during summer at a Mediterranean site. Water Res. 41, 1355–1365.
Korzeniewska, E., 2011. Emission of bacteria and fungi in the air from wastewater treatment plants - a review. Front. Biosci. 3, 393–407.
Kulkarni, P., Olson, N.D., Paulson, J.N., Pop, M., Maddox, C., Claye, E., et al., 2018. Conventional wastewater treatment and reuse site practices modify bacterial community structure but do not eliminate some opportunistic pathogens in reclaimed water. Sci. Total Environ. 639, 1126–1137.
Laforest-Lapointe, I., Messier, C., Kembel, S.W., 2017. Tree leaf bacterial community structure and diversity differ along a gradient of urban intensity. Msystems 2.
Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., Pace, N.R., 1985. Rapid-determination of 16s ribosomal-Rna sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. U. S. A. 82, 6955–6959.
Leung, M.H.Y., Tong, X., Tong, J.C.K., Lee, P.K.H., 2018. Airborne bacterial assemblage in a zero carbon building: a case study. Indoor Air 28, 40–50.
Leyronas, C., Nicot, P.C., 2013. Monitoring viable airborne inoculum of Botrytis cinerea in the South-East of France over 3 years: relation with climatic parameters and the origin of air masses. Aerobiologia 29, 291–299.
Li, B., Ju, F., Cai, L., Zhang, T., 2015. Profile and fate of bacterial pathogens in sewage treatment plants revealed by high-throughput metagenomic approach. Environ. Sci. Technol. 49, 10492–10502.
Liu, H., Zhang, X., Zhang, H., Yao, X.W., Zhou, M., Wang, J.Q., et al., 2018. Effect of air pollution on the total bacteria and pathogenic bacteria in different sizes of particulate matter. Environ. Pollut. 233, 483–493.
Liu, J., Gao, Z., Wang, L., Li, Y., Gao, C.Y., 2018. The impact of urbanization on wind speed and surface aerodynamic characteristics in Beijing during 1991–2011. Meteorog. Atmos. Phys. 130, 311–324.
Lu, R., Li, Y., Li, W., Xie, Z., Fan, C., Liu, P., et al., 2018. Bacterial community structure in atmospheric particulate matters of different sizes during the haze days in Xi'an, China. Sci. Total Environ. 637–638, 244–252.
Martinez-Zarzoso, I., Maruotti, A., 2011. The impact of urbanization on CO2 emissions: evidence from developing countries. Ecol. Econ. 70, 1344–1353.
Miller, M.A., Gardner, I.A., Kreuder, C., Paradies, D.M., Worcester, K.R., Jessup, D.A., et al., 2002. Coastal freshwater runoff is a risk factor for toxoplasma gondii infection of southern sea otters (Enhydra lutris nereis). Int. J. Parasitol. 32, 997–1006.
Montagna, M.T., Cristina, M.L., De Giglio, O., Spagnolo, A.M., Napoli, C., Cannova, L., et al., 2016. Serological and molecular identification of Legionella spp. isolated from water and surrounding air samples in Italian healthcare facilities. Environ. Res. 146, 47–50.
Offermann, F.J., Eagan, A., Offermann, A.C., Subhash, S.S., Miller, S.L., Radonovich, L.J., 2016. Potential airborne pathogen transmission in a hospital with and without surge control ventilation system modifications. Build. Environ. 106, 175–180.
Pillai, S.D., Widmer, K.W., Dowd, S.E., Ricke, S.C., 1996. Occurrence of airborne bacteria and pathogen indicators during land application of sewage sludge. Appl. Environ. Microbiol. 62, 296–299.
Piña, B., Bayona, J.M., Christou, A., Fatta-Kassinos, D., Guillon, E., Lambropoulou, D., et al., 2018. On the contribution of reclaimed wastewater irrigation to the potential exposure of humans to antibiotics, antibiotic resistant bacteria and antibiotic resistance genes – NEREUS COST action ES1403 position paper. J. Environ. Chem. Eng. https://doi.org/10.1016/j.jece.2018.01.011.
Quintero, E., Rivera-Mariani, F., Bolanos-Rosero, B., 2010. Analysis of environmental factors and their effects on fungal spores in the atmosphere of a tropical urban area (San Juan, Puerto Rico). Aerobiologia 26, 113–124.
Redford, A.J., Fierer, N., 2009. Bacterial succession on the leaf surface: a novel system for studying successional dynamics. Microb. Ecol. 58, 189–198.
Riggs, D.W., Yeager, R.A., Bhatnagar, A., 2018. Defining the human envirome: an omics approach for assessing the environmental risk of cardiovascular disease. Circ. Res. 122, 1259–1275.
Robertson, C.E., Baumgartner, L.K., Harris, J.K., Peterson, K.L., Stevens, M.J., Frank, D.N., et al., 2013. Culture-independent analysis of aerosol microbiology in a metropolitan subway system. Appl. Environ. Microbiol. 79, 3485–3493.
Santl-Temkiv, T., Gosewinkel, U., Starnawski, P., Lever, M., Finster, K., 2018. Aeolian dispersal of bacteria in southwest Greenland: their sources, abundance, diversity and physiological states. FEMS Microbiol. Ecol. 94.
Scevkova, J., Dusicka, J., Micieta, K., Somorcik, J., 2016. The effects of recent changes in air temperature on trends in airborne Alternaria, Epicoccum and Stemphylium spore seasons in Bratislava (Slovakia). Aerobiologia 32, 69–81.
Shamarina, D., Stoyantcheva, I., Mason, C.E., Bibby, K., Elhaik, E., 2017. Communicating the promise, risks, and ethics of large-scale, open space microbiome and metagenome research. Microbiome 5.
Shimose, L.A., Masuda, E., Caban, A.B., Munoz-Price, L.S., 2018. Air contamination in the hospital environment. In: Bearman, G., Munoz-Price, S., Morgan, D.J., Murthy, R.K. (Eds.), Infection Prevention: New Perspectives and Controversies. Springer International Publishing, Cham, pp. 163–172.
Signori, C.N., Pellizari, V.H., Enrich-Prast, A., Sievert, S.M., 2018. Spatiotemporal dynamics of marine bacterial and archaeal communities in surface waters off the northern Antarctic peninsula. Deep-Sea Res. II Top. Stud. Oceanogr. 149, 150–160.
Smets, W., Moretti, S., Denys, S., Lebeer, S., 2016. Airborne bacteria in the atmosphere: presence, purpose, and potential. Atmos. Environ. 139, 214–221.
Sonia, A., Andrew, D., Parviez, H., Peter, H., Mercedes, P., Pejman, R., 2006. Seasonality and the dynamics of infectious diseases. Ecol. Lett. 9, 467–484.
Szyłak-Szydłowski, M., Kulig, A., Miaśkiewicz-Pęska, E., 2016. Seasonal changes in the concentrations of airborne bacteria emitted from a large wastewater treatment plant. Int. Biodeterior. Biodegrad. 115, 11–16.
Thompson, I.P., Bailey, M.J., Fenlon, J.S., Fermor, T.R., Lilley, A.K., Lynch, J.M., et al., 1993. Quantitative and qualitative seasonal-changes in the microbial community from the Phyllosphere of sugar-beet (Beta-Vulgaris). Plant Soil 150, 177–191.
Tong, X., Xu, H., Zou, L., Cai, M., Xu, X., Zhao, Z., et al., 2017. High diversity of airborne fungi in the hospital environment as revealed by meta-sequencing-based microbiome analysis. Sci. Rep. 7, 39606.
Vizcaino, M.A.C., Gonzalez-Comadran, M., Jacquemin, B., 2016. Outdoor air pollution and human infertility: a systematic review. Fertil. Steril. 106, 897.
Walser, S.M., Gerstner, D.G., Brenner, B., Bunger, J., Eikmann, T., Janssen, B., et al., 2015. Evaluation of exposure-response relationships for health effects of microbial bioaerosols - a systematic review. Int. J. Hyg. Environ. Health 218, 577–589.
Wang, F.H., Qiao, M., Su, J.Q., Chen, Z., Zhou, X., Zhu, Y.G., 2014. High throughput profiling of antibiotic resistance genes in Urban Park soils with reclaimed water irrigation. Environ. Sci. Technol. 48, 9079–9085.
Wang, H.T., Marshall, C.W., Cheng, M.Y., Xu, H.J., Li, H., Yang, X.R., et al., 2017. Changes in land use driven by urbanization impact nitrogen cycling and the microbial community composition in soils. Sci. Rep. 7.
Ward, C.S., Yung, C.-M., Davis, K.M., Blinebry, S.K., Williams, T.C., Johnson, Z.I., et al., 2017. Annual community patterns are driven by seasonal switching between closely related marine bacteria. ISME J. 11, 1412.
Wei, M., Yu, Z.S., Zhang, H.X., 2015. Molecular characterization of microbial communities in bioaerosols of a coal mine by 454 pyrosequencing and real-time PCR. J. Environ. Sci. 30, 241–251.
Wei, K., Zou, Z.L., Zheng, Y.H., Li, J., Shen, F.X., Wu, C.Y., et al., 2016. Ambient bioaerosol particle dynamics observed during haze and sunny days in Beijing. Sci. Total Environ. 550, 751–759.
Wei, M., Xu, C.H., Chen, J.M., Zhu, C., Li, J.R., Lv, G.L., 2017. Characteristics of bacterial community in cloud water at Mt Tai: similarity and disparity under polluted and non-polluted cloud episodes. Atmos. Chem. Phys. 17, 5253–5270.
Wilkins, D., Leung, M.H.Y., Lee, P.K.H., 2016. Indoor air bacterial communities in Hong Kong households assemble independently of occupant skin microbiomes. Environ. Microbiol. 18, 1754–1763.
Xie, J.W., Jin, L., Luo, X.S., Zhao, Z., Li, X.D., 2018. Seasonal disparities in airborne bacteria and associated antibiotic resistance genes in PM2.5 between urban and rural sites. Environ. Sci. Technol. Lett. 5, 74–79.
Xie, Z., Li, Y., Lu, R., Li, W., Fan, C., Liu, P., et al., 2018. Characteristics of total airborne microbes at various air quality levels. J. Aerosol Sci. 116, 57–65.
Xu, H.J., Wang, X.H., Li, H., Yao, H.Y., Su, J.Q., Zhu, Y.G., 2014. Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. Environ. Sci. Technol. 48, 9391–9399.
Xu, C., Kan, H.D., Fan, Y.N., Chen, R.J., Liu, J.H., Li, Y.F., et al., 2016. Acute effects of air pollution on enteritis admissions in Xi'an, China. J. Toxicol. Environ. Health, Part A 79, 1183–1189.
Xu, A.L., Song, Z.W., Lang, X.L., Chen, X., Xia, Y., 2017. Seasonal variability in bacterial and fungal diversity and community composition of the near-surface atmosphere in coastal megacity. Aerobiologia 33, 555–575.
Xu, C.H., Wei, M.H., Chen, J.M., Wang, X.F., Zhu, C., Li, J.R., et al., 2017. Bacterial characterization in ambient submicron particles during severe haze episodes at Ji'nan, China. Sci. Total Environ. 580, 188–196.
Zhen, Q., Deng, Y., Wang, Y.Q., Wang, X.K., Zhang, H.X., Sun, X., et al., 2017. Meteorological factors had more impact on airborne bacterial communities than air pollutants. Sci. Total Environ. 601, 703–712.
Zhong, X., Qi, J.H., Li, H.T., Dong, L.J., Gao, D.M., 2016. Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region. Atmos. Environ. 140, 506–513.
Zhu, Y.G., Gillings, M., Simonet, P., Stekel, D., Banwart, S., Penuelas, J., 2017. Microbial mass movements. Science 357, 1099–1100.