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Urban greenness and plant species are key factors in shaping air microbiomes and reducing airborne pathogens

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ABSTRACT

Urban green space has been implicated in shaping airborne microbes, but there is an only rudimentary understanding of the key factors of urban green space affecting the composition and structures of airborne microbes. Here, we selected 40 urban sites based on stratified random sampling design and investigated the effects of multiple factors including landscapes, plant, soil, and anthropogenic factors on airborne microbial communities, especially bacterial and fungal pathogens. Bacterial and fungal communities in the control area with lower greenness were significantly ($P < 0.05$) different from those in other areas with a gradient of green space. The relative abundance of bacterial and fungal pathogens significantly ($P < 0.05$) decreased with increasing greenness. Other than soil thickness, soil type, slope position, and population density, plant species considerably contributed to the shift in the composition and abundance of potential bacterial and fungal pathogens. A significantly ($P < 0.05$) reduced abundance of bacterial and fungal pathogens was observed in areas with >30% masson pine. Together, these results provide insights into the importance of green space for providing health benefits for city dwellers by reducing pathogens in air, as well as providing support for the inclusion of plant species in the management of urban green space to reduce exposure risk of airborne pathogens.

1. Introduction

Urban green space provides numerous ecosystem services that benefit humans, including climate regulation, nutrient cycling, the supply of recreational areas, etc. (Hunter et al., 2019). There is strong evidence for human health benefits of urban green space, such as improved mental health and cognitive function, reduced cardiovascular morbidity and mortality, the prevalence of type 2 diabetes, and improved pregnancy outcomes (WHO, 2017). The positive effects could be partially explained by air quality improvement by decreasing air pollutant levels, reducing carbon dioxide (Orwell et al., 2004), and release of oxygen and aromatic compounds from plants and root-associated microbes (Pegas et al., 2012). In addition to positive effects, urban green space could be a potential source of antibiotic resistance genes that pose potential threaten to human health (Yan et al., 2019). Despite that the health effects of urban green space are well-known, the underlining mechanisms are yet to be fully understood.

Exposure to airborne microbes in green space has been increasingly implicated in the link between urban green space and human health (Robinson et al., 2020). Airborne microbes, including bacteria, fungi and viruses, are abundant in the air, of which some lineages are human pathogens or allergens that potentially threaten public health. It has been indicated that potential bacterial pathogen reduced in air collected from rural areas with high greenness (Li et al., 2019). Children living in urban areas have a higher risk of asthma and allergic rhinitis than those living in rural areas (Al-Qerem et al., 2016), and increasing air pollution and allergic exposure are identified as major risk factors associated with the increase in asthma (Brunekreef et al., 2009). Besides, frequent

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contact with environmental diverse microbes may reduce the chronic disease burdens associated with compromised immune (Flies et al., 2017; WHO, 2017). Exposure to environmental microbes influenced the human skin microbiota and increased anti-inflammatory cytokines (Hanski et al., 2012).

Airborne microbes are inevitably affected by various factors including particulate matter properties (Franzetti et al., 2011; Li et al., 2019), meteorological parameters (Rao et al., 2020), the sources of microbes (Berg et al., 2014; Zhai et al., 2018), and anthropogenic activities (Enloe et al., 2015; Zhang et al., 2020), and varied with sampling sites (Bowers et al., 2012; Yassin and Almouqatea, 2010) and time (Hu et al., 2020; Xie et al., 2018). However, research efforts have mainly focused on the diversity and community composition of bacteria and potential bacterial pathogens, for example, higher proportion of potential bacterial pathogens in air was detected in urban sites (Li et al., 2019). Only a few studies reported airborne fungal community and diversity (Lymperopoulou et al., 2016). Nevertheless, fungi potentially influence human health as some fungi are major pathogens and allergens for human beings. Distinct differences in the fungal community structures and compositions had been detected between coarse and fine particulate matter (Frohlichnowoisky et al., 2009). About 50% of detected fungi in household dust collected from Beijing were potential pathogens (Ding et al., 2020).

Of the factors affecting airborne microbes, plants were suggested to play a pivotal role in shaping airborne microbial communities. Previous studies have reported the importance of plants to airborne microbes including fungi (Su et al., 2002) and bacteria (Li et al., 2019). The greenness in a 50 m buffer zone explained 15% of the variation of the airborne bacterial communities between parks and parking lots (Mhuireach et al., 2016). Many typical and often dominant plant-associated bacteria could be detected in the air microbiome (Berg et al., 2014), and up to 50% of airborne bacteria in downwind air samples were contributed by local plants (Lymperopoulou et al., 2016). Ding et al (2020) demonstrated that plant was a source of dust-related microbes and significantly ($P < 0.05$) affected the bacterial communities in household dust. In addition to greenness, other plant-associated factors (e.g. plant age and plant species), soil factors (e.g. soil type and soil thickness), and landscapes (e.g. slope position and slope aspect) are important constituents in one green space. Plant species and ages significantly affected the phyllosphere microbial communities (Yang et al., 2001), which underwent frequent exchange with airborne microbes (Zhou et al., 2021), suggesting a potential role of phyllosphere microbiome in affecting the airborne microbial communities and potential pathogens. And Rottstock et al have demonstrated that higher plant diversity promoted higher diversity of fungal pathogens (Rottstock et al., 2014). On another hand, soil is suggested as the important source of microbes in air (Grady et al., 2019; Zarraonaindia et al., 2015). However, the effects of plant species, plant age, soil factors and landscapes on airborne microbes are yet to be explored.

In this study, airborne microbe samples were collected from 40 sites in Xiamen, China, in 2018. These sites were selected through a stratified random sampling design and were grouped into four functional zones (forest, street, residential quarter, and control area) based on multiple factors including plant factors, landscape features, soil factors, population density, and building coverage. Bacterial and fungal communities were characterized through amplicon sequencing of bacterial 16S rRNA gene and fungal internal transcribed spacer (ITS2), respectively. The aim of this study is to depict the effects of green space features on airborne microbes, especially on potential microbial pathogens, which could provide scientific basis for optimizing the design and management of urban green space towards benefiting human health. To achieve this, we compared the microbial community structures and potential microbial pathogens among different functional zones and to determine the factors influencing the profiles of microbial communities and pathogens. We then evaluated the contributions of green space composition and structure, including plant factors (e.g. plant species, plant age), soil factors (e.g. soil type) and landscape features (e.g. slope position and elevation), in shaping airborne bacteria and fungi, especially potential pathogens.

2. Materials and methods

2.1. Preliminary selection of sampling sites

Sampling site selection is critical in examining the underlying effects of local land use/land cover on communities of airborne microbes. In the present study, we used a stratified random sampling design approach to assure the representativeness of sampling (Catherine et al., 2008). Firstly, the strata were built by adopting the urban functional zones as spatial units, which provide a functional delineation, for which, forest characteristics, soil and topographical properties were available over the whole studied area. Three urban functional zones, including a forest zone and two other zones adjacent to an urban forest but with strong human activities, for example, street and residential quarter (RQ) zones, were selected to build the strata. An area apart from an urban forest with fewer trees was taken as a control area, where normalized difference vegetation index (NDVI) is significantly lower ($P < 0.05$) than those in other functional zones including forest, street and RQ (Table S1). A total of 50 candidate sites were selected on the map by this approach. After field examination, sites locating in the military areas or unreachable were excluded, resulting in 40 sampling sites that includes 12, 11, 12, and 5 for forest, street, RQ, and control area, respectively (Fig. S1a).

2.2. Characterization of urban functional zones

For each site, NDVI) in 50 m zone was obtained from www.gscloud.cn and population density was collected by the Xiamen Municipal Health and Family Planning Commission in 2012 (Table S1). Three plant factors (plant species, plant age, and forest category in the forest patch), four landscape features (slope declivity, slope position, slope aspect, and elevation), and two soil factors (soil type and soil thickness) in 50 m buffer zone (Table S1) were obtained from Forest Management Planning Inventory (FMPI, version 2018). The detailed methods for the digitization of each factor were described in the supplemental information. The functional zones including forest, street, RQ, and control area were distinctly separated based on above- mentioned factors and building coverage collected from OpenStreetMap data (https://www.openstreetmap.org/#map=12/24.4864/118.1202) using a discriminant analysis (Fig. S1b), which indicates the differences of green space and anthropogenic activity among functional zones.

2.3. Airborne microbe collection and DNA extraction

Airborne microbes were collected by filtering 6 m^3 air into a sterile gelatine filter (12602-80-ALK, Sartorius stedim biotech) with a rate of 50 L min^{-1} using a portable sampler ($Airport_{MD8}$, Sartorius stedim biotech). Triplicate samples for each site were collected from September to November (once a month) in 2018. All samples (n = 120) were collected at daytime with sunshine, and approximately 1.5 m above ground level at all sites. Filters with airborne microbes were stored at −20 °C until DNA extraction, which was performed according to the protocol described in the previous study (Li et al., 2019). Purified genomic DNA was eluted into 80 μL of sterile double distilled water (ddH_2O), and stored at −20 °C until amplification of bacterial 16S rRNA genes and fungal ITS2 region.

2.4. Target-gene amplification, sequencing, and data analysis

To explore the compositions of bacterial and fungal communities, bacterial 16S rRNA genes and fungal ITS2 region were amplified using primer sets of 338F/806R (Fadrosh et al., 2014) and fITS7/ITS4 (Karlsson et al., 2014), respectively. PCR amplification was performed in

a 25 μL mixture containing 12.5 μL of Phusion® Hot Start Flex 2 × Master Mix (Thermo Scientific), 0.1 μM of forward primer, 0.1 μM of reverse primer, 3.0 uL DNA as a template, and sterile ddH_2O. The PCR thermal cycle for bacterial 16S rRNA genes was performed as follows: initial denaturation at 98 °C for 30 s, followed by 29 cycles of 98 °C 10 s, 54 °C 30 s, and 72 °C 45 s, and a final extension at 72 °C for 10 min. For the fungal ITS2 region, PCR thermal cycle was processed as described in a previous study (Zhao et al., 2020). Amplicons were gel purified using a universal DNA purification kit (DP214-3, Tiangen, China). The concentration of purified amplicons was detected using a Qubit Fluorometer (version 3.0, Invitrogen), and subsequently, the equimolar of amplicons were pooled and sent to LC-BIO Bio-tech Ltd (Hangzhou, China) for library constructions and sequencing using an Illumina Miseq PE300 platform.

High-quality sequences were obtained after removal of low-quality sequences and chimera and were grouped into operational taxonomic units (OTUs) at a 97% nucleic acid similarity. For bacterial and fungal taxon identification, representative sequences for each OTUs were blasted against reference sequences in RDP (version 11.5, for bacteria and fungi), GenBank NT (for bacteria) and Unite (version 7.2, for fungi) databases using BLAST with confidence level = 0.8, identify threshold ≧ 90%, query coverage≧80% and e-value ≦10^{-5}. To excavate potential bacterial pathogens, bacterial 16S rRNA gene sequences were blasted against pathogenic sequences in Pathogen_16sPIP database using 16Spip software with identify threshold ≧ 99%, 10^{-20}≦ e-value ≦10^{-10} (Miao et al., 2017). Additionally, the directory of pathogenic fungi infecting human beings issued by the Ministry of Health of the People's Republic of China was used to explore potential fungal pathogens (Barberan et al., 2015; Du et al., 2018; Hernandez and Martinez, 2018). All sequences collected in this study have been deposited in the National Center for Biotechnology Information (NCBI) GenBank under SRA accession number of PRJNA592228 for bacterial 16S rRNA genes, and PRJNA592229 for fungal ITS2 genetic regions.

2.5. Statistical analysis

Principal coordinate analysis (PCoA) based on Bray Curtis distance and adonis test of the variations among microbial communities were calculated using R with "Vegan" and "Mass" packages (version 3.4.0). Correlation test, redundancy analysis (RDA), and partial redundancy analysis (pRDA) were performed using R with "psych", "Hmisc", "vegan" and "Mass" packages (version 3.4.0) to determine the contributions of landscapes, plant and soil factors in green space, and population density to microbial community compositions. One-way ANOVA was used to determine significant differences among samples from different sampling areas if necessary. All plots in this study were generated using OriginPro 2018.

3. Results

3.1. Composition of bacterial and fungal communities

Bacterial communities in four functional zones were dominated by Proteobacteria (29.7%–39.7%), Firmicutes (18.4%–31.0%) and Actinobacteria (14.3%–27.6%) (Fig. 1a), in which Actinobacteria was significantly ($P < 0.05$) higher in control area compared to the others, while Firmicutes exhibited a contrasting trend. At the class level of fungal communities, Dothideomycetes (39.59%–44.90%), Sordariomycetes (4.77%–8.18%) within Ascomycota and Agaricomycetes (17.75%–34.93%) within Basidiomycetes predominated in airborne microbes (Fig. 1b), in which the relative abundance of Sordariomycetes was the highest in control area but was the lowest ($P < 0.05$) in the forest area.

Significant differences in patterns of bacterial communities (adonis $P = 0.001$) were mainly driven by the bacterial communities in the control area (Fig. S2a and S2b), and we did not observe significant division (adonis $P = 0.094$) in bacterial profiles among the forest, street and residential quarter zones (Fig. S2b). Similar profiles of fungal communities were observed between street and residential quarter zone (adonis $P = 0.394$, Fig. S3d), and the variations in fungal communities among functional zones (adonis $P = 0.003$, Fig. S3a) were attributed to the significant different fungal communities in forest (adonis $P = 0.039$, Fig. S3b and S3d) and control areas (adonis $P = 0.004$, Fig. S3c and S3d).

No factors, including landscapes, plant and soil factors, and population density, detected in this study (Table S1) were observed to significantly affect the communities of total bacteria and fungi based on RDA analysis. For analyzing the relative contribution of these factors to the variations in bacterial and fungal community structures, we then reconstructed bacterial and fungal sub-communities including variant genus (fold change ≧ 2 in relative abundances among functional zones) significantly ($P < 0.05$) related to factors detected in this study (Fig. 2a and c). Soil thickness, plant factors including plant species, the ratio of masson pine, NDVI, and population density significantly ($P < 0.05$) affected the bacterial sub-communities, explaining 23.41% of the variations, in which soil thickness, plant factors, and population density explained 10.31%, 7.05%, 2.16% of the variations, respectively (Fig. 2b). While the fungal sub-communities were significantly ($P < 0.05$) influenced by slop position, plant factors (e.g. plant age, NDVI, plant species, the ratio of Masson pine and *Casuarina* equisetifolia) and population density (Fig. 2d), explaining 35% of the variations.

3.2. Potential bacterial pathogens and the associated factors

A total of 41 potential bacterial pathogenic species were detected in airborne microbial samples, with relative abundances ranging from 3.03% to 6.77%. The highest abundance of bacterial pathogens was observed in the street area, while the forest area harbored the lowest abundance (Fig. 3a). Significant variations in bacterial pathogen

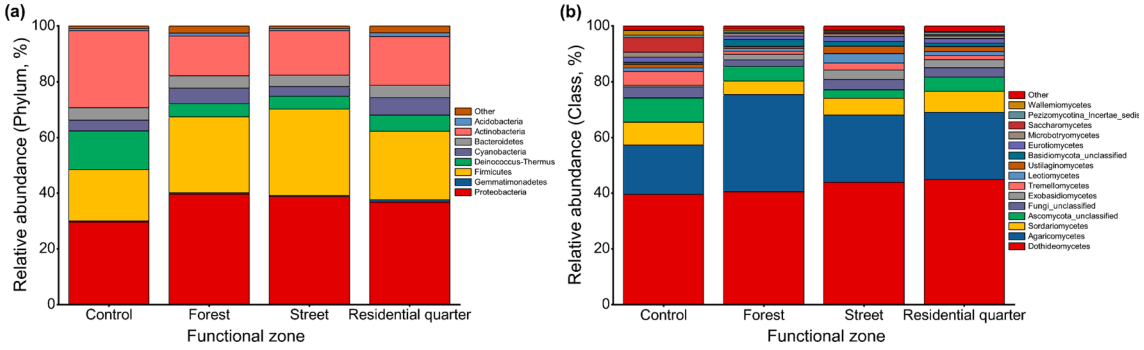

Fig. 1. Structures of bacterial (a) and fungal (b) communities in four functional zones.

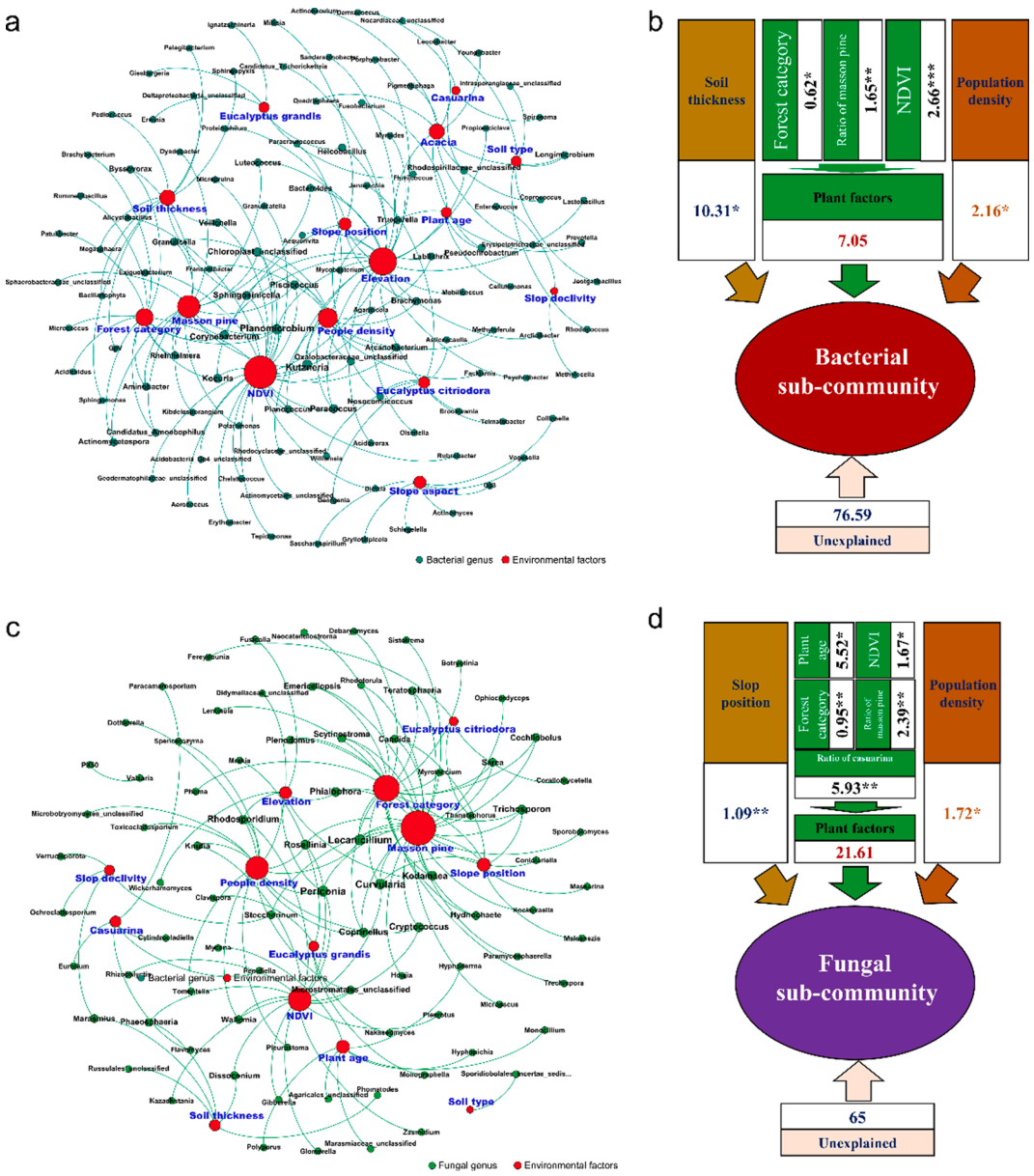

Fig. 2. Co-occurrence network between varying bacteria (a) and factors of green space, and contributions of green space and human activity to variation in patterns of bacterial sub-community (b); Co-occurrence network between varying fungi (c) and factors of green space, and contributions of green space and human activity to variation in patterns of fungal sub-community (d). The factor of green space includes soil factors, plant factors and landscape in this graph. Bacterial and fungal sub-community is the community of varying bacteria and fungi significantly related to the factors of green space, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

communities were detected among functional zones (adonis $P = 0.002$, Fig. S4a), which were mainly explained by the distinct pathogen communities in forest (adonis $P = 0.002$, Fig. S4b and S4d) and control areas (adonis $P = 0.041$, Fig. S4c and S4d). There was no significant difference in patterns of bacterial pathogen communities between street and residential quarter areas (adonis $P = 0.274$, Fig. S4d). *Acinetobacter baumannii* and *Pseudomonas aeruginosa* dominated in bacterial pathogen communities (Fig. 3e). There was a significantly positive (spearman R = 0.502, $P = 0.001$) relationship between bacterial pathogen abundances and population density (Fig. 3b), while, NDVIs (Fig. 3c) and ratio of masson pine (Fig. 3d) in 50 m buffer zone significantly and negatively (spearman R = −0.37, $P = 0.017$ and R = −0.467, $P = 0.004$, respectively) correlated with the relative abundance of bacterial pathogens in air.

We detected six bacterial pathogens with significantly varying relative abundance among functional zones (fold change ≥ 2, $P < 0.05$) which accounted for 8.2%–65.0% of total bacterial pathogens and were closely affiliated to *A. baumannii*, *Streptococcus oralis*, *Aeromonas punctate*, *Aeromonas hydrophila*, *Streptococcus sanguinis* and *Streptococcus equinus* (Fig. 3e). Four of these pathogens were significantly ($P < 0.05$) enriched in control, street and residential quarter areas compared with those in forest area (Fig. 3f). For example, *A. baumannii* with an average of 0.19% in forest area was 6.8-, 7.8- and 13.9-fold enriched in the residential quarter, street, and control areas, respectively (Fig. 3f).

These varying bacterial pathogens were significantly affected by plant factor (NDVI and ratio of masson pine) and population density, explaining 12.21% of the variations (Fig. 4a). Both *A. baumannii* and *S. oralis* were positively correlated with population density (Fig. 4b) but negatively correlated with NDVI (Fig. 4c) and the ratio of masson pine in 50 m buffer zone (Fig. 4d). The relative abundances of *A. baumannii*, *S. oralis*, and total bacterial pathogens were significantly ($P = 0.029$, 0.007, and 0.003, respectively) lower in sites with > 30% masson pine

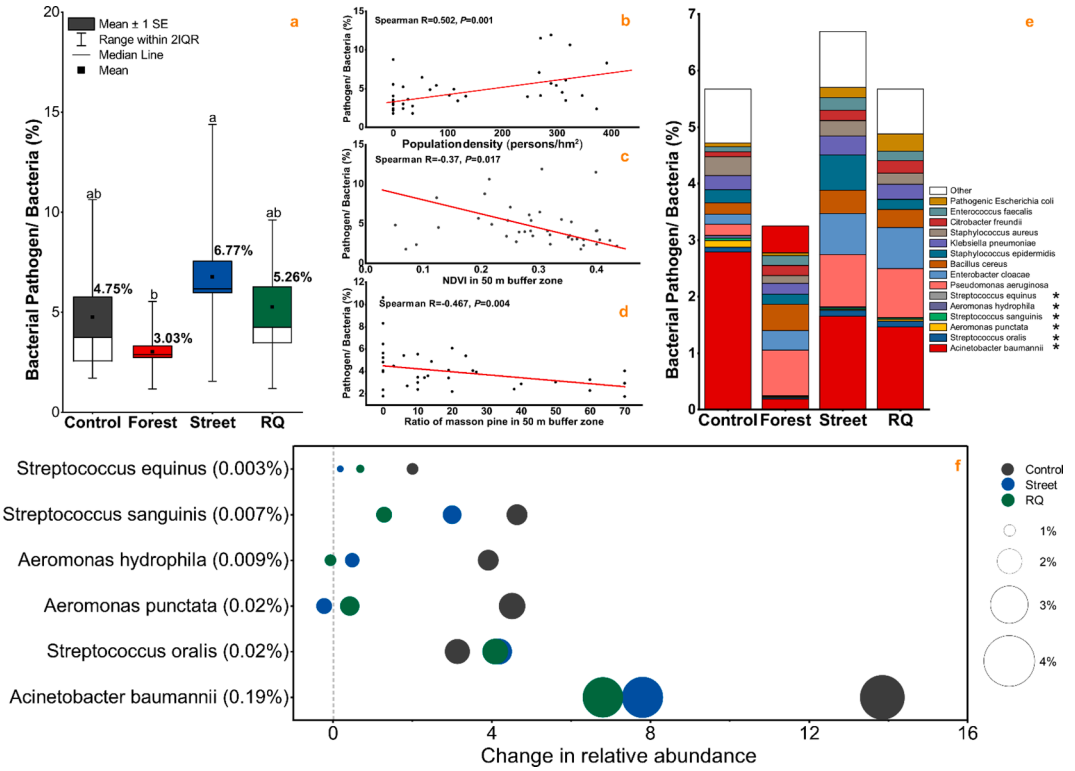

Fig. 3. Relative abundance (a) and structures (e) of bacterial pathogens, and relationship between total bacterial pathogens and population density (b), NDVI (c), and ratio of masson pine (d) in 50 m buffer zone; Net proportional changes in relative abundance of the varying bacterial pathogens in control, street and RQ areas compared with those in forest area (f). Y axis shows the relative abundance of the targeted pathogens in air collected from forest areas; Sizes of circles shows converted relative abundance of targeted pathogens using formula of $\log_{10}^{(\text{real relative abundance}*1000)}$; NDVI: normalized difference vegetation index; RQ: residential quarter; * in plot e indicates that these bacterial pathogens are significant ($P < 0.05$) difference among four functional zones.

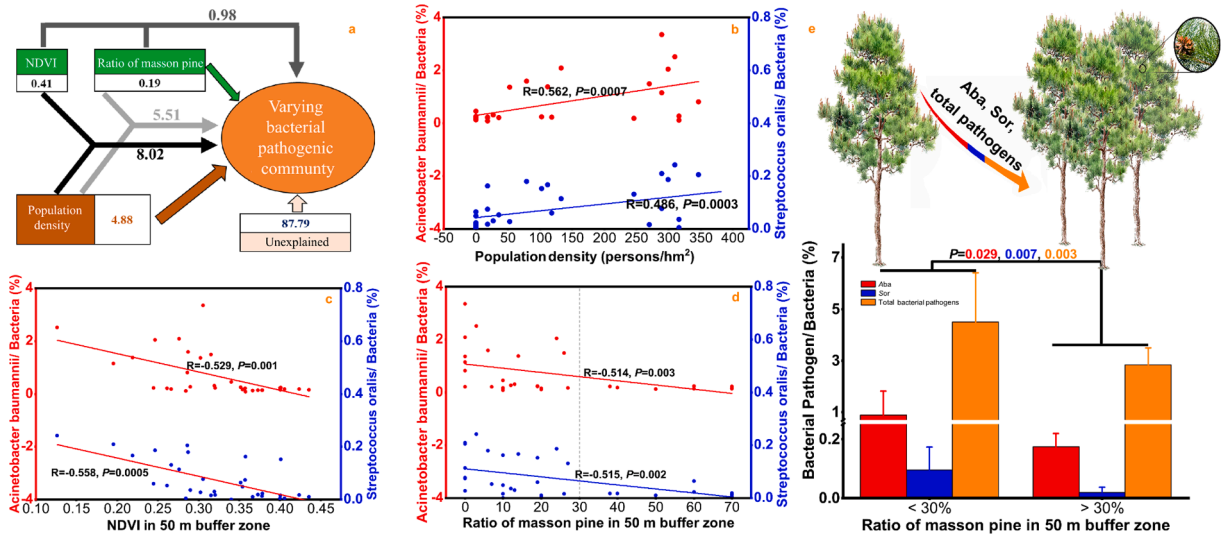

Fig. 4. Contributions of green space and human activity to variation in patterns of varying bacterial pathogens (a); Linear correlation between *Aba*, *Sor* and population density (b), between *Aba*, *Sor* and NDVI in 50 m buffer zone (c), and between *Aba*, *Sor* and ratio of masson pine in 50 m buffer zone (d); The relative abundance of total bacterial pathogens, *Aba* and *Sor* in areas with >30% and <30% of *Masson* pine (e). *Aba*: *Acinetobacter baumannii* and Sor: *Streptococcus oralis*; error bars represent the standard deviations of several air samples collected from the same functional zone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in 50 m buffer zone than those in sites with the ratio of masson pine < 30% (Fig. 4d, e).

3.3. Potential fungal pathogens and the associated factors

A total of 0.57%–1.31% of fungal sequences were identified as potential fungal pathogens affiliated to 17 species, with the highest abundance detected in the control area and the lowest abundance in the

forest area (Fig. 5a). Similar to bacterial pathogens, the relative abundances of fungal pathogens in forest areas were significantly ($P < 0.05$) lower than those in other functional zones (Fig. 5a). *Alternaria alternate*, *Candida tropicalis*, *Candida parapsilosis*, and *Fusarium solani* predominated and changed significantly ($P < 0.05$) among functional zones (Fig. 5d). In addition to these dominant species, *Penicillium digitatum*, *Candida glabrata*, *Clavuspora lusitaniae*, and *Aspergillus fumigatus* significantly increased in control, street and residential quarter areas compared with those in forest area (Fig. 5e). Compared with the forest area, *A. fumigatus*, *P. digitatum* and *C. parapsilosis* were 13.8-, 18.4- and 25.47-fold enriched in the control area (Fig. 5e). The relative abundances of fungal pathogens significantly and negatively correlated to elevation (Fig. 5b, $R = -0.365$, $P = 0.035$) and NDVI in 50 m buffer zone (Fig. 5c, $R = -0.336$, $P = 0.049$). Besides, soil type significantly ($P = 0.047$) influenced the relative abundances of airborne *A. alternate* (Fig. 6a). Differed from *A. alternate*, *C. parapsilosis* and *P. digitatum* increased with population density (Fig. 6b), but were significantly and negatively ($R = -0.485$, $P = 0.002$ and $R = -0.412$, $P = 0.009$) correlated with NDVI in 50 m buffer zone (Fig. 6c). Noteworthily, *C. parapsilosis* was significantly ($R = -0.492$, $P = 0.003$) related to ratio of masson pine (Fig. 6d), and greatly ($P = 0.031$) reduced in the sites with > 30% of masson pine in 50 m buffer zone (Fig. 6e).

4. Discussion

Human health benefit via regulating airborne microbes is a relatively unaccounted service provided by urban green space. Environmental microbiomes have profound impacts on human health, but the factors of urban green space affecting airborne microbes are not fully explored because of the complex compositions and structures in green space. This study provided a comprehensive comparison of airborne bacterial and fungal communities among various urban functional zones with different land use/cover types and a gradient of greenness. Multiple features covering plant, soil, landscape, and population aspects of urban green space were integrated to depict critical factors influencing airborne microbes, with particular focusing on potential pathogens. To the best of our knowledge, this is the first comprehensive report investigating the effects of plant species and greenness in urban green space on communities of airborne bacteria and fungi, especially the pathogens. Plant, soil and landscape feathers of urban green space all play important roles in airborne microbiomes. NDVI and ratio of masson pine in 50 m buffer zone were negatively correlated with potential bacterial and fungal pathogens in air, indicating positive effects of green space such as greenness and masson pine on human health. We also cannot ignore the effects of other components (e.g. soil type and elevation) in green space, and human activity (e.g. population density), on airborne pathogens.

The compositions of airborne microbes including bacteria and fungi varied with compositions and structures of green spaces, and population density in different urban functional zones. Proteobacteria, Firmicutes and Actinobacteria dominated in bacterial communities, which has been well documented in previous studies (Bertolini et al., 2013; Bowers et al., 2013; Li et al., 2019). *Dothideomycetes* and *Agaricomycetes* were the dominant fungi in airborne microbial communities, which is consistent with those observed in outdoor air samples collected in Mainz, Germany (Frohlichnowoisky et al., 2009) and Seoul, South Korea (Woo et al., 2018). Nevertheless, bacterial and fungal community structures significantly differed within four functional zones in this study, and soil thickness, plant species, the ratio of masson pine, NDVI, and population density were identified as important factors influencing the communities of varying bacteria significantly related to the factors of green space detected in this study. Soil, plant, water, and human beings are the major sources of airborne microbes, thus the factors influencing soil-, plant- and human-associated microbiomes may consequently affect airborne microbial communities. Previous studies have reported the effects of greenness (Mhuireach et al., 2016), human

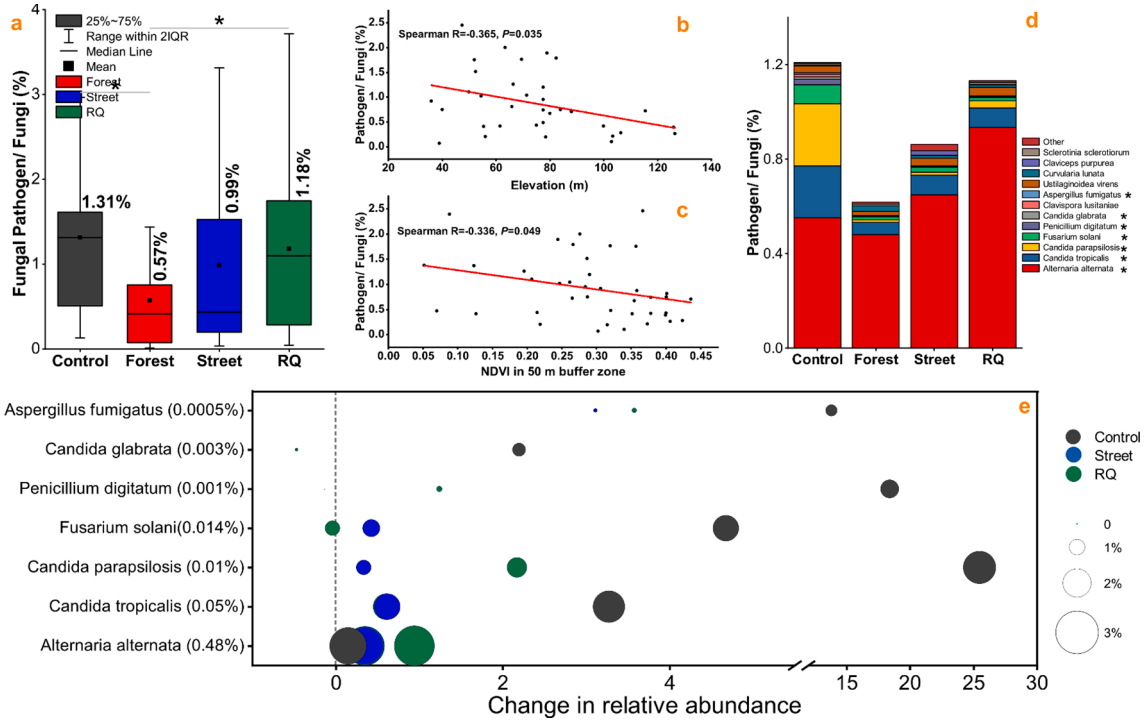

Fig. 5. Relative abundance (a) and structures (d) of fungal pathogens, and relationship between total fungal pathogens and elevation (b), and NDVI (c) in 50 m buffer zone; Net proportional changes in relative abundance of the varying fungal pathogens in control, street and RQ areas compared with those in forest area (f). Y axis shows the relative abundance of the targeted pathogens in air collected from forest areas; Sizes of circles shows converted relative abundance of targeted pathogens using formula of $\log_{10}^{(\text{real relative abundance}*1000)}$; NDVI: normalized difference vegetation index; RQ: residential quarter; * in plot e indicates that these bacterial pathogens are significant ($P < 0.05$) difference among four functional zones.

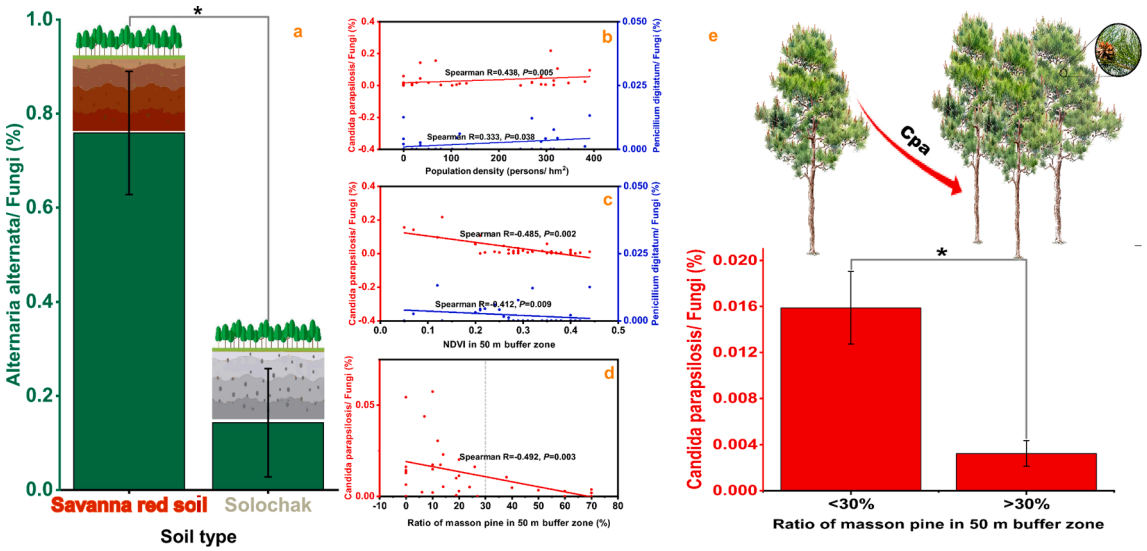

Fig. 6. Relative abundance of Alternaria alternate in air collected from sites with different soil type; Linear correlation between *Cpa*, *Pdi* and population density (b), between *Cpa*, *Pdi* and NDVI in 50 m buffer zone (c), and between *Cpa* and ratio of masson pine in 50 m buffer zone (d); The relative abundance of *Cpa* in areas with >30% and <30% of masson pine (e); *Cpa*: *Candida parapsilos* and *Pdi*: *Penicillium digitatum*; The error bars represent the standard deviations of several air samples collected from the same functional zone; * $P < 0.05$.

activity (Hu et al., 2020), air pollutants, and meteorological parameters (Zhai et al., 2018; Zhen et al., 2017) on airborne microbes. Here, population density explained only 2.16% and 1.72% of variations in subcommunities of bacteria and fungi with significantly changed abundance, respectively, which is significantly ($P < 0.01$) lower than those explained by plant factors (7.05% and 21.61%, respectively) (Fig. 2b and d). These results suggested that plant factors (e.g. NDVI, plant age, and plant species) of green space play a more important role in shaping airborne bacterial and fungal community structures compared to population density. Lymperopoulou et al (2016) indicated that up to 50% of air bacteria in downwind sites were presumably of local plant origin, and fungi from plants could influence the fungal composition of nearby air.

The proportion of potential bacterial pathogens (3.03%–6.77%) (Fig. 3a) is comparable to those detected in household dust (Ding et al., 2020) and air (Chakrawarti et al., 2020; Li et al., 2019), but higher than those detected in $PM_{2.5}$ collected from Zhejiang university (10 m above ground), with only 1.4%–1.9% of bacteria identified as potential pathogens (Hu et al., 2020). The relative abundance of potential bacterial pathogens positively correlated with population density, but negatively correlated with greenness (NDVI) and the ratio of masson pine, suggesting that human activity would increase the exposure health risk of airborne microbes, while increased greenness and masson pine could develop a positive influence on human health(Zhu et al., 2017).

In the forest area, the abundance of potential bacterial pathogens was significantly lower than those in other zones. Furthermore, *Acinetobacter baumannii* was significantly higher in control, street, and RQ areas compared with that in forest areas, and significantly ($P = 0.017$) correlated with greenness, plant species ($P = 0.004$), and population density ($P = 0.001$). *Acinetobacter* is prevalent in human-associated microbiota (Bik, 2009), especially in skin bacterial community (Gao et al., 2007), and *A. baumannii* has been identified as a critical bacterial pathogen cause hospital- and community-acquired infections. Several lineages affiliated with *Streptococcus* and *Aeromonas* were also significantly ($P < 0.05$) enriched in functional zones with high population density and low greenness (e.g. street, RQ, and control areas). These results indicate that the composition and structure of green space and human activity would affect the distribution of potential airborne bacterial pathogens.

Potential fungal pathogens occupied a smaller proportion of the total fungal community (0.57%–1.31%) (Fig. 5a) than those of potential bacterial pathogens. *Alternaria alterinate* was the most prevalent fungal pathogen detected in this study and was significantly higher in control, street, and RQ areas in comparison with that in the forest area. In this study, airborne microbial samples were collected from September to November with an average temperature of about 23 °C, which is beneficial to the dissemination of *A. alterinate* spores (Kustrzeba-Wojcicka et al., 2014). Besides, the increase of temperature caused by human activities (urban heat island effect) and the cooling effect of urban green space with trees (Shashua-Bar and Hoffman, 2000) might also contribute to the variation of *A. alterinate* among different functional zones. A recent global survey has indicated that warmer temperature increased the relative and total abundance of *Alternaria* in soil (Delgado-Baquerizo et al., 2020). Because of the surface-air aerosolization, factors influencing soil-borne fungal pathogens (e.g. *A. alterinate*) (Nguyen et al., 2016) would consequently affect airborne fungal pathogens, which may partially explain the detected significant correlation between soil type and fungal pathogen communities in this study.

The relative abundance of fungal pathogens in air detected in this study are lower than those in household dust collected in Beijing, where 0.78%–95.6% (median = 61%) of fungal sequences were identified as potential fungal pathogens (Ding et al., 2020), suggesting the human activity is a key factor influencing the abundance of airborne fungal pathogens, which could be supported by the significantly positive correlation between the dominant *C. parasilosis* and *P. digitatum* and population density (Fig. 6b). In addition, the low abundance of fungal pathogens in this study could be partially explained by the greenness and ratio of masson pine, which is negatively related to *C. parasilosis* and *P. digitatum* (Fig. 6c), and *C. parasilosis* (Fig. 6d), respectively.

One important finding of this study is that plant species were identified as an important factor influencing the community of both bacterial and fungal pathogens in the air. The proposed mechanisms underlying the effects of plants on airborne microbes include adsorbing atmospheric particles containing microbes, releasing negative oxygen ions (Jiang et al., 2020), and secreting volatile organic compounds (VOCs) influencing airborne microbial activities. Antimicrobial effects against various human pathogens have been demonstrated for various plant-associated volatile organic compounds (VOCs) (Dorman and Deans, 2000; Filipowicz et al., 2003), which vary greatly among different plant species. In this study, the relative abundances of total bacterial

pathogens, particularly *A. baumannii, S. oralis,* and *C. parapsilosis*, were negatively correlated with the ration of Masson pine in the urban functional zone. Pathogens in sites with >30% masson pine were significantly lower than those in sites with <30% Masson pine, suggesting the potential of masson pine in controlling airborne pathogens. This might be explained by the higher concentrations and diversities of organic compounds in pine (Arshadi et al., 2013; Bertaud and Holmbom, 2004; Vainio-Kaila, 2017). Volatile fraction and aqueous extracts from pine root, twigs, and needles have been reported to have antimicrobial activity (Feng et al., 2010; Koukos et al., 2000), and commonly used in traditional Chinese medicine and food processing.

In conclusion, we demonstrated the role of urban green space in shaping the airborne microbial community and controlling airborne pathogens. Plant species composition of urban green space plays an important role in reducing the relative abundance of bacterial and fungal pathogens. Our results provide evidence for explaining how the composition and structure of green space influence airborne pathogens, and suggested that modifying the plant species of urban green space might be a feasible approach to manipulate air microbiome towards health-promoting microbial exposure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

Hu Li, Zhi-Feng Wu, Yin Ren and Jian-Qiang Su designed this experiment; Hu Li and Zhi-Feng Wu performed the experiment; Hu Li analyzed the data and prepared the figures; Hu Li and Zhi-Feng Wu wrote the paper; Xiao-Ru Yang, Xin-Li An, Yin Ren and Jian-Qiang Su reviewed and commented on this manuscript. All authors read and approved the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106539.

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