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Original Research

Neighborhood garden's age shapes phyllosphere microbiota associated with respiratory diseases in cold seasons

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ABSTRACT

Neighborhood gardens serve as sensitive sites for human microbial encounters, with phyllosphere microbes directly impacting our respiratory health. Yet, our understanding remains limited on how factors like season, garden age, and land use shape the risk of respiratory diseases (RDs) tied to these garden microbes. Here we examined the microbial communities within the phyllosphere of 72 neighborhood gardens across Shanghai, spanning different seasons (warm and cold), garden ages (old and young), and locales (urban and rural). We found a reduced microbial diversity during the cold season, except for Gammaproteobacteria which exhibited an inverse trend. While land use influenced the microbial composition, urban and rural gardens had strikingly similar microbial profiles. Alarmingly, young gardens in the cold season hosted a substantial proportion of RDs-associated species, pointing towards increased respiratory inflammation risks. In essence, while newer gardens during colder periods show a decline in microbial diversity, they have an increased presence of RDs-associated microbes, potentially escalating respiratory disease prevalence. This underscores the pivotal role the garden age plays in enhancing both urban microbial diversity and respiratory health.

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1. Introduction

Multiple environmental exposures at the neighborhood level, including heavy metals, traffic, and air pollution, have been shown to influence human respiratory health [1–3]. Neighborhood gardens, serving as significant habitats for both indoor and outdoor microorganisms, constitute the primary origin of human exposure to microbes [4–6]. Phyllosphere microorganisms play critical roles in multiple ecological functions, including plant growth and fitness, and can also affect human respiratory health [7–9]. However, the health associations of phyllosphere microbial communities (PMCs) in neighborhood gardens and factors that may affect these microbial communities are largely uncharacterized.

Seasonal factors (wind, temperature, solar radiation, and rain) can affect the diversity and composition of microbial communities [10–12]. Hot and humid summer usually leads to fungal blooms, while cool, dry autumn and winter favor bacterial growth [13]. Related to asthma prevalence, the proportion of *Streptococcus* increases in the winter air, and the relative abundance of *Pseudomonas* increases in spring [14–16]. Whether the distribution of respiratory diseases (RDs) associated microbiota varies in different seasons remains to be explored.

Previous studies have shown that urbanization is associated with an increased burden of several respiratory diseases [17–19]. However, it is still largely unclear how urbanization (e.g., the

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garden age) affects PMCs, especially the human health-associated microbiota. As PMCs can be influenced by pollutants [9,20] and human activities [21], it is reasonable to presume that PMCs differ in neighborhood gardens between urban and rural areas. Different land-use types selectively affect microbial enrichment [22–26]. Urban areas tend to have a higher proportion of grey infrastructure, while rural areas are characterized by a greater abundance of green-blue landscapes. This is crucial since microbial diversity of organic soil promotes [27] while grey infrastructure and high-traffic areas were found to suppress naïve T-cell production [2]. Increased levels of greenery in the surrounding neighborhood and proximity to green-blue infrastructure both have the potential to facilitate the enrichment of microbiota associated with beneficial microbes (*Roseburia*, etc.) [28,29]. Furthermore, they exhibit a negative association with wheezing and allergy morbidity [30,31].

Urbanization has led to the coexistence of old and young neighborhood gardens. Soil microbial diversity tends to increase over time in urban parks, and the microbiota responds to vegetation type and park age [32–36]. Since the coupling between plants and soil tends to be more stable with time, the driving force of plants on soil properties and biological communities strengthens [37,38]. Consequently, this could potentially alter the diversity and composition of the PMCs. Besides, previous studies have found that the accumulation of microorganisms on building materials and the products of their degradation can also affect human respiratory health [39]. The human body's immune system is gradually developed and perfected with age [40]. This process is not only affected by the composition of the microorganisms in the body [41] but also interfered with and regulated by environmental microbiota [42,43]. Therefore, exploring the variation trend of PMCs with the increase of garden age, especially RDs-associated microbiota, is necessary.

Here, we analyzed microbial communities, using Illumina Miseq, residing in the leaf samples of *Cinnamomum camphora* in 72 neighborhood gardens in Shanghai, China. These gardens encompass different locations (urban and rural) and garden ages (old and young). The objectives of this study are to explore (i) how significant the seasonal variation in PMCs, especially the RDs-associated microbiota, and (ii) whether these microbes respond to the urban-rural gradient in neighborhood gardens with varying ages. In the current study, we formulated three key hypotheses. (i) Season affects the diversity and composition of PMCs in the neighborhood gardens. This is based on earlier results indicating that lower vegetation diversity and decreased litter content can reduce microbial habitats during the cold season [44]. (ii) PMCs differ between urban and rural areas, with more harmful microbiota enriched in urban areas, especially those related to respiratory inflammations. This conjecture is underpinned by observations showcasing the enrichment of pathogen-containing genera (e.g., *Streptococcus* and *Alternaria*) within built areas [45], coupled with observations that underscore the connection between yard vegetation and gut microbiota [46]. Furthermore, the green-blue space covers large areas in the surrounding rural gardens, which is characterized by health-supporting microbiota according to the "Biodiversity hypothesis" [47,48]. (iii) RDs-associated microbiota enriches in young neighborhood gardens. This inference is based on two parallel phenomena: formaldehyde and other airborne chemicals usually concentrated in the vicinity of new buildings, which are known to lead to the enrichment of RDs-associated harmful genera [49]. Additionally, such conditions are generally known to have a high prevalence in highly developed countries. Collectively, the three hypotheses are based on the general assumption that biodiversity peaks in summer and rural environments [50].

2. Materials and methods

2.1. Site description and sampling

We selected 72 neighborhood gardens in Shanghai, China (30.78° N, 121.53° E), including 42 urban neighborhood gardens and 30 rural neighborhood gardens (Fig. S1a, Table S1). These neighborhood gardens were divided into young (1–10 years) and old (22–85 years) categories. Every neighborhood was equipped with a garden, with an area that fluctuated from 800 to 5000 m^2. Sampling was conducted in late August and late December 2021, and all leaf samples were collected in three days to minimize temporal variation. The average temperature was 28.4 ± 4.4 °C in late August and 10.1 ± 3.3 °C in late December, referred to as the "warm season" and "cold season", respectively. We obtained data on the volume ratio and greening ratio of gardens from property managers. Besides, we also calculated the proportion of land-use types surrounding gardens. Combining the results of Shanghai's first-class land classification and remote sensing images at 30 m resolution (Fig. S1b), the proportion of each land-use type within the 1 km radius buffer zone was counted in ArcGIS software, with the sample site as the center of the circle, and divided into seven land-use types. We classified the land-use types into two categories based on their proportion: grey infrastructure (residential area, road, business district, and unutilized land) and green-blue infrastructure (vegetation, farmland, and water) (Fig. S1c).

We selected the camphor tree (*Cinnamomum camphora*) as the host tree species for its evergreen broad-leaved nature and widespread cultivation in Shanghai. We selected two mature and healthy camphor trees in each garden close to the residential building (within a 5–25 m distance). The tree age did not differ between old and young gardens ($P = 0.170$), nor between urban and rural gardens ($P = 0.095$). We used pruning shears to remove four branches 3–5 m above the ground from the south of each tree. The branches were intercepted before touching the ground. Twenty pieces of undamaged leaves were immediately and carefully removed from the branch. Then half of the leaves were mixed in a 50-ml tube to obtain a sample for microbial DNA extraction, and the remaining leaves were divided into two 20-ml tubes, which were used to measure leaf pH and dust retention, respectively. To avoid cross-contamination, ethanol (70.0%) was used to sterilize sampling tools (gloves, shears, and scissors). All tubes were placed into an icebox (4 °C), transported to the laboratory, and then handled within the same day.

2.2. Phyllosphere environmental detection

We took five leaves and punched three small discs on each leaf using a hole punch that had a diameter of 12 mm. This resulted in 15 discs, which were then placed into a test tube. To facilitate measurement, 10 ml of deionized water was added, and the system was evacuated with a vacuum pump for 2 h. The pH of the leaves was subsequently measured using the pH meter. Then we measured the dust retention per unit leaf [51]. All leaves in the tube were soaked in distilled water for 3 h, the attachments on each leaf were removed, the leaves were taken out, and the mixed cellulose ester microporous (with a pore size of 0.45 μm) filter membrane was used for suction filtration. Upon completion of the filtration, the filter membrane was dried again (60 °C, drying for 24 h to a constant temperature) and weighed by a balance. The weight of leaf particles was calculated as the difference between the initial weight (A_1) and the weight after drying (A_2), denoted as A_1-A_2. Then we used the laser leaf area meter (CI-203, USA) (resolution 0.01 square millimeter, accuracy ± 2%) to measure the total area of the dried

leaves (S). With this data, the calculation formula of leaf dust retention per unit area (LDR, g m^{-2}) was applied: $LDR = (A_1 - A_2)/S$.

2.3. DNA extraction, amplification, and sequencing

Phyllosphere microbial DNA was extracted from the leaf surface after arrival at the laboratory as described [52]. Total DNA was extracted using the Fast DNA SPIN extraction kits (MP Biomedicals, Santa Ana, CA, USA), according to the manufacturer's instructions. The extracted DNA was stored at −80 °C. PCR amplification of the bacterial 16S rRNA genes V4 region was performed using the forward primer 515 F 5′- GTGCCAGCMGCCGCGGTAA-3′ and the reverse primer 806R 5′-GGACTACHVGGGTWTCTAAT-3′ [53,54]. For fungi, the internal transcribed spacer (ITS) genes were amplified using the forward primer ITS7 5′ -GTGARTCATCGAATCTTTG-3′ and the reverse primer ITS4 5′ -TCCTCCGCTTATTGATATGC-3′ [55,56]. All procedures included negative controls to check for potential contamination. Sequencing was performed using the Illumina MiSeq platform (Illumina, USA). The paired files (.fastq) were available in the Sequence Read Archive at National Center for Biotechnology Information with BioProject number PRJNA875601 for bacteria and PRJNA875603 for fungi in the warm season, PRJNA876035 for bacteria and PRJNA876041 for fungi in the cold season.

2.4. Bioinformatics

Paired-end sequence data (.fastq) were processed using Mothur v1.44.3 according to the operating procedure [57]. Both bacterial and fungal sequences were aligned into contigs, and quality was controlled. Any sequence with an ambiguous base, more than one mismatch with the primers, longer than 8 bp for bacterial homopolymers and 13 bp for fungal homopolymers, and any sequence without a minimum overlap of 50 bp were removed [58]. The remaining sequences were aligned against the Greengene database [59] for bacteria and the UNITE-curated International Nucleotide Sequence Database reference database [60] for fungi. Non-target sequences (mitochondria, chloroplast, and archaea) were removed. Sequences were clustered to operational taxonomic units (OTUs) with 97% similarity, using nearest-neighbor (single linkage) joins to conservatively assign sequences to OTUs. The final OTU datasets contained 5811 bacterial OTUs and 2258 fungal OTUs in the warm season, 4089 bacterial OTUs, and 3299 fungal OTUs in the cold season. We estimated richness and diversity indices for bacterial and fungal communities in mothur. Observed OTU richness (S_{obs}), Shannon diversity ($H = -\sum P_i \times \ln P_i$), and Shannon's evenness ($E = H/\ln S_{obs}$), with P_i being the proportion of species i in the total community [61].

2.5. Identification of respiratory diseases - associated microbiota

We screened all bacterial and fungal OTUs according to the lists of potentially pathogenic genera (opportunistic and facultative) documented by Latham Taylor [62] and Xin-Li An [63]. We conducted the identification through five steps. (i) Genera with an average relative abundance larger than 0.6% were selected as a keyword list, resulting in a selection of 130 genera out of 639 genera for bacteria and 115 genera out of 445 genera for fungi in the warm season. Similarly, in the cold season, 128 genera were selected out of 629 genera for bacteria, while 120 genera out of 492 genera for fungi. (ii) Two sets of article searches were conducted using Web of Science in its Core Collection. We used the below algorithm for bacteria: TITLE (respiratory diseases) and TITLE (bacteri*). Given the existence of studies explicitly describing the taxonomy of fungal pathogens, our algorithm for fungi was: TOPIC (respiratory diseases) and (fung*). The search timespan covered all years. (iii) We adopted the Lost Pearls (https://rook iethx.shinyapps.io/LostPearls) [9] to select the RDs-associated genera. This tool required two input files: the target wordlist and the papers in PDF format to be searched. Each word in the wordlist was individually queried within each paper. When a match was found, the tool displayed the word, the sentence containing the word, and the respective paper. We used the list obtained in step (i) as the input wordlist, leading to 70 bacterial genera in 180 papers and 30 fungal genera in 21 files. (iv) We narrowed them into 14 bacterial and 13 fungal genera. Meanwhile, we defined the RDs-associated genera whose relative abundance peaked within the garden age categories as PRD genera. (v) To classify the RDs-associated microbiota at the species level, we assigned the representative sequences of each OTU in BLASTn sequence similarity search (E-value = 10^{-5}, qcover ≥90%, and identity ≥97%) (https://blast.ncbi.nlm.nih.gov) as described by Xin-Li An [63]and Hu Li [64].

2.6. Statistical analyses

All statistical analyses were performed in R v4.1.2 (R core team, 2019). The variations of bacterial and fungal diversity indices, the relative abundances of main taxonomic groups (phyla/classes/genera) to sampling seasons, urban-rural gradient, and garden age were tested using generalized linear mixed models (GLMM) with the lmer function in the lme4 package in R [65]. Since we selected the sampling gardens from both the east and west sides of the Huangpu River, the location of the sampling gardens was added as a random term to the models. Non-metric multidimensional scaling (NMDS) analyses were performed using the vegan package for bacterial and fungal datasets. The Bray–Curtis coefficient was used as the dissimilarity measure. The proportion of land-use types was correlated with the community structure as the vector fitting procedure in the same analyses. Linear discriminant analysis effect size (LEfSe) analyses were performed on the Galaxy website (http://huttenhower.sph.harvard.edu/galaxy/, v1.0) [66]. Based on the OTU dataset of microbes, microbial molecular ecological network analysis was performed to evaluate the differences in network complexity and stability in the warm and cold seasons on Molecular Ecological Network Analyses Pipeline (MENAP) (http://ieg2.ou.edu/MENA/) [67,68]. The cross-kingdom bacteria-fungi co-occurrence network and the correlations between major phyla were visualized on Gephi 0.9.2 and Cytoscape 3.9.1, respectively. The neutral community model (NCM) was constructed in R [69]. In this model, Nm was calculated to estimate the assembly mechanisms of microbial communities. In addition, the contribution of stochastic bacterial and fungal community assembly was assessed by normalized stochasticity ration (NST) in R with the NST package [70].

3. Results

3.1. The characteristics of neighborhood gardens and phyllosphere environment

The internal environment of the neighborhood gardens exhibited no variations between urban and rural gardens ($P = 0.244$) or according to garden age ($P = 0.269$). The average volume and greening ratios were 1.76 and 36.7% in urban and 1.69 and 36.8% in rural gardens. However, the surrounding environment of neighborhood gardens was significantly different between urban and rural areas. Urban gardens were surrounded by more grey infrastructure, up to 70.8%, while rural gardens contained more green-blue infrastructure, up to 58.0%, within a 1 km radius buffer zone. Regarding the phyllosphere environment, there were no

differences in leaf pH between urban and rural gardens, with a value of 4.2 ± 0.3, indicating that the effects of leaf pH on PMCs could be excluded. Furthermore, the dust retention per unit leaf area was similar between urban and rural areas, which were 0.205 ± 0.004 g m^{-2}. Thus, these key leaf characteristics hardly influenced the phyllosphere microbial community.

3.2. Diversity of phyllosphere bacterial and fungal communities

The diversity of PMCs was mainly affected by the season and garden age. The results showed that all bacterial diversity estimators (OTU richness, Shannon diversity, and Shannon's Evenness) were higher in the warm season, while the fungal diversity followed an opposite trend, higher in the cold season. Besides, both the bacterial and fungal communities were more diverse in old gardens (Fig. 1, Tables S2 and S3). The microbial diversity indices were similar between urban and rural gardens. However, the diversity of PMCs responded to land-use types. We found that the fungal communities were more sensitive to land-use types than bacterial communities (Fig. S2). Their diversity and evenness were negatively correlated with the percentage of farmland while positively correlated with the grey infrastructure (GI), especially residential areas and roads. Moreover, bacterial diversity and evenness positively correlated with the proportion of business districts (Fig. S2).

3.3. Compositional structure of phyllosphere bacterial and fungal communities

Both bacterial ($R^2 = 0.547$, $P < 0.001$) and fungal ($R^2 = 0.094$, $P < 0.001$) community compositions differed between warm and cold seasons, and their dissimilarities of them were lower in the cold season (Fig. S3). The proportion of business district and vegetation influenced the composition of bacteria (Fig. 2a), whereas the proportion of farmland affected the fungal community (Fig. 2d). Bacterial community composition had differences between old and young gardens in both warm ($R^2 = 0.042$, $P = 0.001$) and cold seasons ($R^2 = 0.031$, $P = 0.033$). The proportion of vegetation and residential area played a key role in the warm season (Fig. 2b). In comparison, the business district proportion was affected most in the cold season (Fig. 2c). Similar to the bacterial communities, there were significant differences in fungal communities between old and young gardens in both warm ($R^2 = 0.082$, $P < 0.001$) (Fig. 2e) and cold seasons ($R^2 = 0.058$, $P < 0.001$) (Fig. 2f). Surprisingly, there were no differences between urban and rural gardens in both bacterial and fungal communities. However, the proportion of surrounding land-use types can affect the microbial composition (Fig. S4).

Bacterial communities in the warm season were dominated by Proteobacteria (63.8%), followed by Cyanobacteria (11.8%) and Actinobacteria (5.5%). Among the major bacterial phyla, the relative abundances of Bacteroidetes and Firmicutes were higher in the warm season than in the cold season, while Proteobacteria displayed an inverse trend (Fig. S5, Table S2). As the most abundant phylum, Proteobacteria included three health-associated classes (Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria). The results showed a marked reduction in the relative abundance of Gammaproteobacteria in the warm season. Fungal communities were dominated by Ascomycota (71.5%), followed by Basidiomycota (10.7%) and Zygomycota (0.12%), and the most abundant genus was *Mycosphaerella* (23.3%) (Fig. S6). Moreover, the relative abundance of Ascomycota was higher in the cold season than in the warm season, and those in the young gardens were higher than in the old ones (Fig. S5, Table S3).

We also identified the characteristics genera by LEfSe analysis to explore the specific response of PMCs to seasons and garden ages. We observed that bacterial communities had more characteristic genera in the warm season than in the cold season, which was mainly from classes Gammaproteobacteria (*Acinetobacter*, *Enterobacter*, *Stenotrophomonas*), Alphaproteobacteria (*Sphingomonas*), and Saprospirae (*Sediminibacterium*) (Fig. 3a). Genera enriched in the old gardens were mainly from classes Synechococcophycideae (*Leptolyngbya*), while Gammaproteobacteria (*Halomonas*) in the young ones (Fig. 3b). In contrast, the fungal communities had more characteristic genera in the cold season, which were mainly from classes Dothideomycetes (*Alternaria*, *Aureobasidium*, *Pyrenochaetopsis*) and Sordariomycetes (*Verticillium*, *Colletotrichum*, *Cytospora*) (Fig. 3c). The young gardens had more fungal characteristic genera than the old ones, which were mainly from classes Dothideomycetes (*Mycosphaerella*, *Edenia*, *Elsinore*), Sordariomycetes (*Colletotrichum*, *Libertella*), and Eurotiomycetes (*Paecilomyces*) (Fig. 3d).

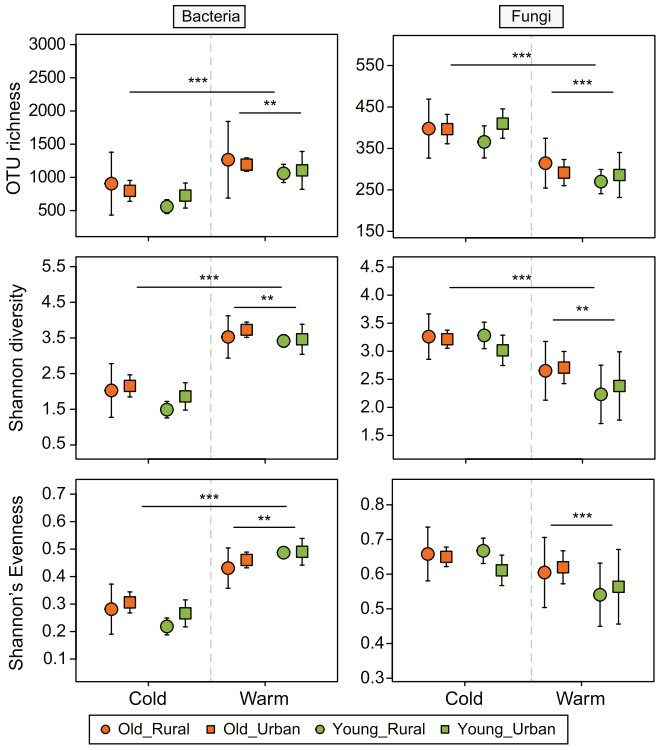

Fig. 1. Differences in OTU richness, Shannon diversity, and Shannon's Evenness between cold and warm seasons in old and young neighborhood gardens. Three alpha diversity indices for bacteria (left panels) and fungi (right panels). Bars represent standard errors. The upper asterisks represent the significance. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. For statistical results, see Tables S2 and S3.

3.4. Topological properties of seasonal microbial networks

To reveal the potential microbial interactions within different seasons, we constructed the following networks (Fig. 4). The microbial co-occurrence patterns varied greatly between warm and cold seasons, as indicated by multiple topological properties of the networks. For bacterial networks, the total number of nodes was 156 and 131, and the total number of links was 476 and 652, respectively. Notably, the average clustering coefficient and average degree of the network were higher in the cold season, indicating heightened complexity within the bacterial community during this period. There were more bacterial keystone taxa in the cold season,

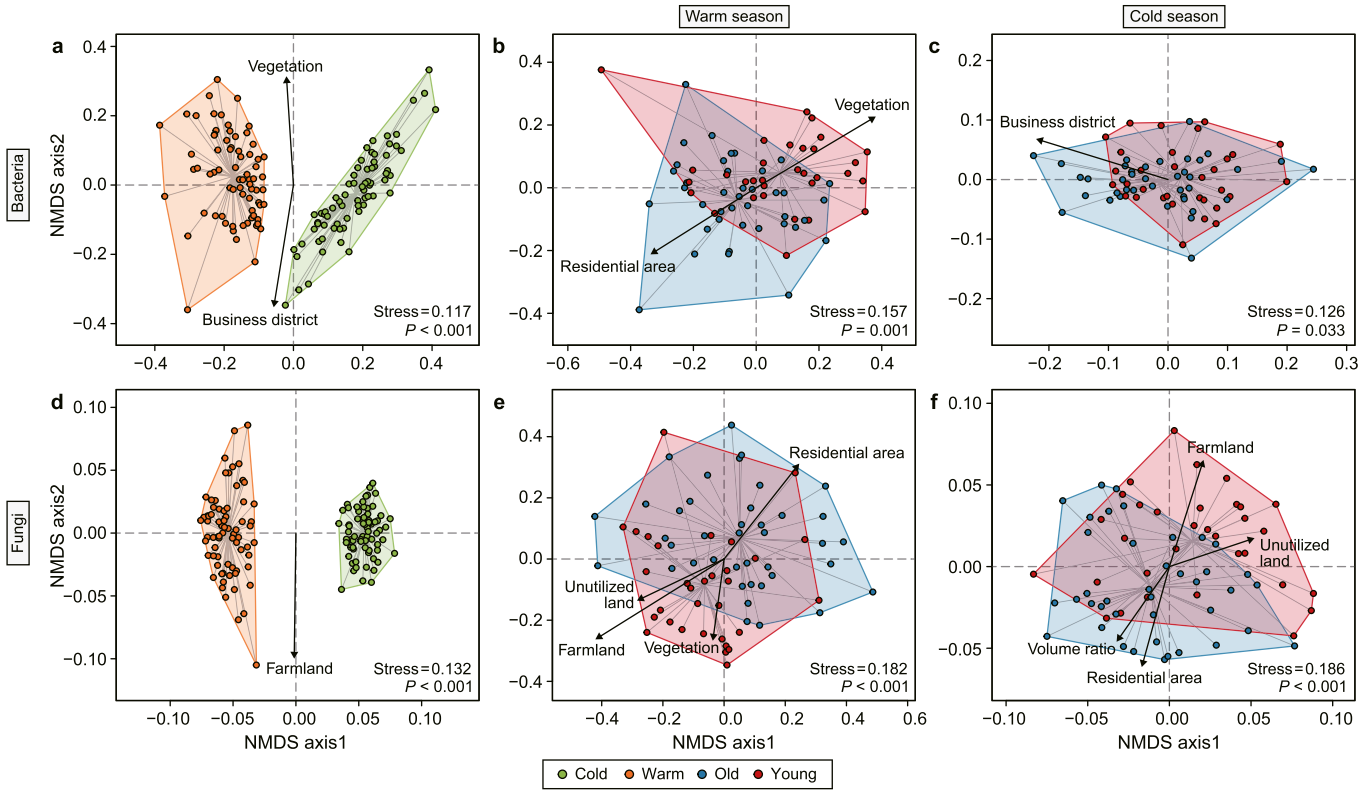

Fig. 2. NMDS ordination shows a significantly different phyllosphere microbial composition between the cold and warm seasons in the old and young neighborhood gardens using Bray–Curtis as the dissimilarity metric. The proportion of land-use types associated with PMCs is shown as arrows. **a**, **d**, the bacterial (**a**) and fungal (**d**) communities respond to season. **b**, **e**, The bacterial (**b**) and fungal (**e**) communities respond to garden age in the warm season. **c**, **f**, The bacterial (**c**) and fungal (**f**) communities respond to garden age in the cold season.

characterized by Proteobacteria, Actinobacteria, and Acidobacteria. Moreover, there were more positive correlations between bacterial phyla in the cold season compared with the warm season (Fig. S7). Regarding fungal networks, the total number of nodes was 91 and 119, and the total number of links was 309 and 195, respectively, while the average clustering coefficient and average degree of the network were higher in the warm season, representing that the fungal network was more complex in the warm season. We also found that the average clustering coefficient of the bacteria-fungi co-occurrence network in the old gardens was higher than that in the young gardens in both warm and cold seasons. Furthermore, a general trend of negative correlations was observed among phyla. We also found that the stochastic process played a more important role than the deterministic process for bacteria (Fig. S8c). However, the fungal communities showed the opposite trend (Fig. S8f).

3.5. Identification of respiratory diseases - associated microbiota

Notably, for bacteria and fungi, the total relative abundances of pathogenic microbial genera in the cold season were higher than those in the warm season (Fig. S9). We further identified the RDs-associated microbial genera from the potentially pathogenic genera and compared their relative abundances based on seasons and garden ages. Fourteen RDs-associated bacterial genera were identified in the warm and cold seasons, respectively (Fig. S10). In the warm season, the relative abundances of RDs-associated bacterial genera in different gardens fluctuated from 0.002% to 8.9%. While in the cold season, the relative abundances ranged from 0.02% to 33.1%. Among all RDs-associated bacterial genera, 56.0% (abundance percentage, including eight genera out of 14) had higher relative abundances in the cold season, including *Pseudomonas* (Fig. S10). Surprisingly, in the cold season, 95.9% (including six genera out of 14) of RDs-associated bacterial genera peaking in the range of 5–10 years (garden age) will be referred as PRD bacterial genera, and their proportion showed a decreasing trend with the extension of time, only 10.0% during the 10–25 years range (Fig. 5a). We further detected 13 RDs-associated fungal genera in warm and cold seasons, respectively (Fig. S10). The relative abundances of RDs-associated fungal genera at different gardens showed the same trend as the bacteria, with a higher percentage in the cold season. Among all RDs-associated fungal genera, 61.6% (including one genus out of 13) peaked in the range of 0–5 years in the cold season (Fig. 5b). Moreover, the proportion of land-use types around gardens affected the relative abundances of RDs-associated microbial genera in the warm season (Fig. 5c). While in the cold season, the enrichment effect of RDs-associated microbial genera in young gardens was mainly owing to the significant differences across garden age categories (Fig. 5d). Furthermore, RDs-associated bacterial and fungal species were identified as shown in Tables S4 and S5, including 20 bacterial species and 20 fungal species.

4. Discussion

4.1. Season affected the diversity and composition of PMCs

In terms of the response of PMCs to seasons, our results support the first hypothesis. The richness and diversity of bacteria were significantly higher in the warm season. This phenomenon is likely attributed to the capacity of the warm season to bolster bacterial

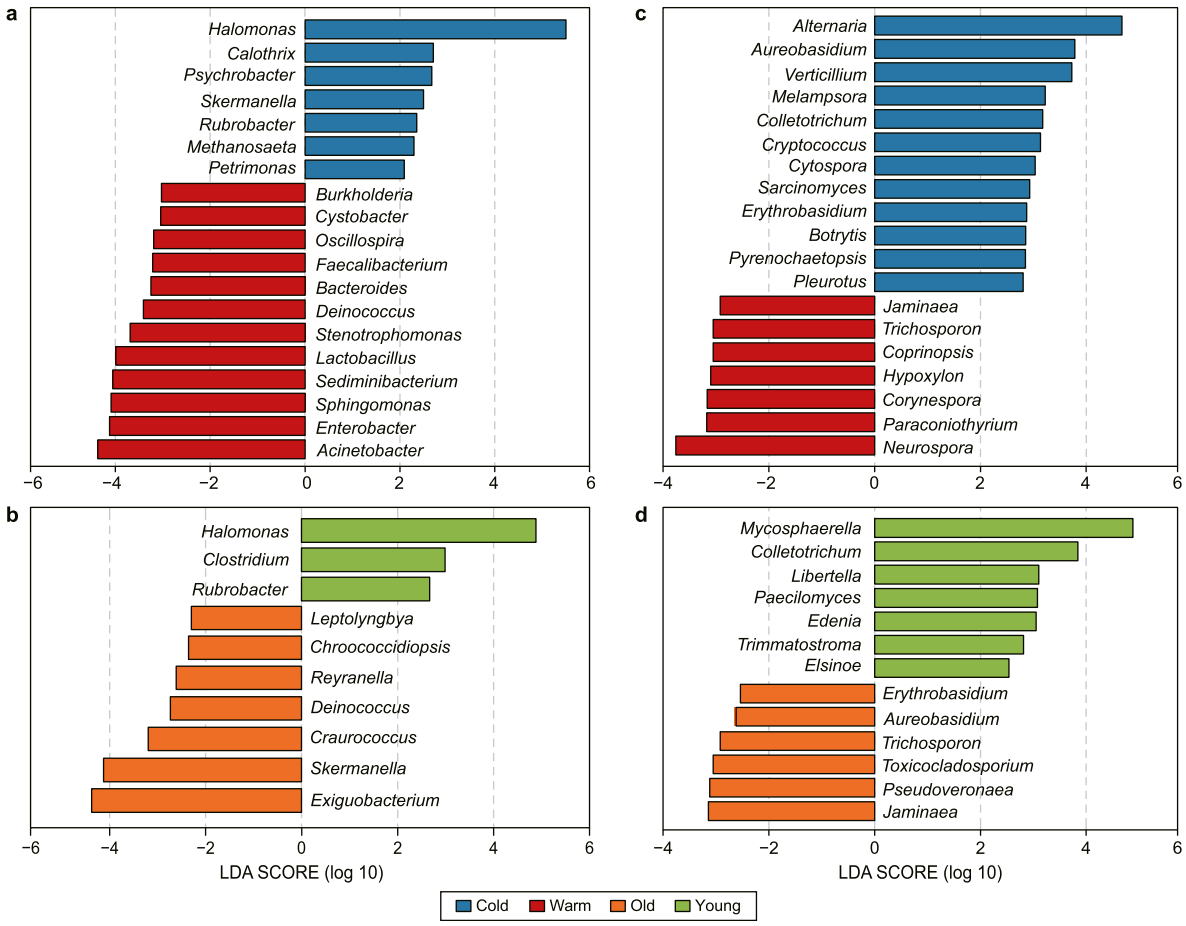

Fig. 3. LEfSe analysis for characteristic microbial genera between warm and cold seasons in the old and young neighborhood gardens. Only microbial genera with an LDA score >2 are shown. **a**–**b**, Characteristic bacterial genera in different seasons (**a**) and garden categories (**b**). **c**–**d**, Characteristic fungal genera in different seasons (**c**) and garden categories (**d**).

alpha diversity within the soil and the air, attributable to augmented vegetation diversity [50,71,72]. However, some bacterial characteristic genera showed an increasing trend during the cold season, most belonging to the class Gammaproteobacteria. Gammaproteobacteria are closely related to human health. Heightened diversity of Gammaproteobacteria on the skin has been demonstrated to promote regulatory T cells and immunomodulatory cytokines [73]. At the same time, the major phyla of phyllosphere bacteria established positive correlations to enhance the stability of the network to resist the cold climate [74]. The fungal network was mainly affected by the interaction process between vegetation and soil nutrients [75], so the network becomes more complex and stable in the warm season [76]. In addition, the fungal communities had higher total nodes in the cold than in the warm season due to decreased vegetation [77].

Seasons can also affect the composition of potentially pathogenic microbes, especially RDs-associated microbiota. The percentage of RDs-associated fungal genera in the cold season reached levels twice as high as that in the warm season, which is also similar to the feature of indoor airborne microbiota [78]. A previous study revealed that lungs are susceptible to biofilm infections, especially *Pseudomonas aeruginosa* [79], which belongs to the most abundant RDs-associated bacterial genus in our study, and its relative abundance was significantly higher in the cold season. Meanwhile, the most abundant RDs-associated fungal genus *Alternaria* also showed the same trend, and it is also closely related to human respiratory infections, such as chronic rhinosinusitis [45]. Generally, our findings may pave the way for environmental studies that explain the high incidence of respiratory diseases in the cold season [80].

4.2. The urban-rural gradient did not affect the PMCs

We found that urbanization did not affect PMCs in the neighborhood gardens, which does not support our second hypothesis. Compared with the traditional building materials (e.g., wood, clay) used in rural areas, most of the present-day rural neighborhood gardens comprise the same modern standard materials (cement, plates, etc.) as urban ones [81]. Microbial community composition may often be affected by building materials [82], possibly resulting in no differences in the PMCs. The composition of PMCs in neighborhood gardens was indeed affected by the surrounding land-use types. Interestingly, the proportion of business districts influenced the bacterial composition, while the fungal composition was affected by the proportion of vegetation and farmland, which likely promoted microbial diversity [83] in accordance with the "Biodiversity Hypothesis" by Ilkka Hanski [47].

4.3. The garden age affected the enrichment of RDs-associated microbiota

Another key finding of our current study is that the PMCs were shaped by the garden age, especially the RDs-associated

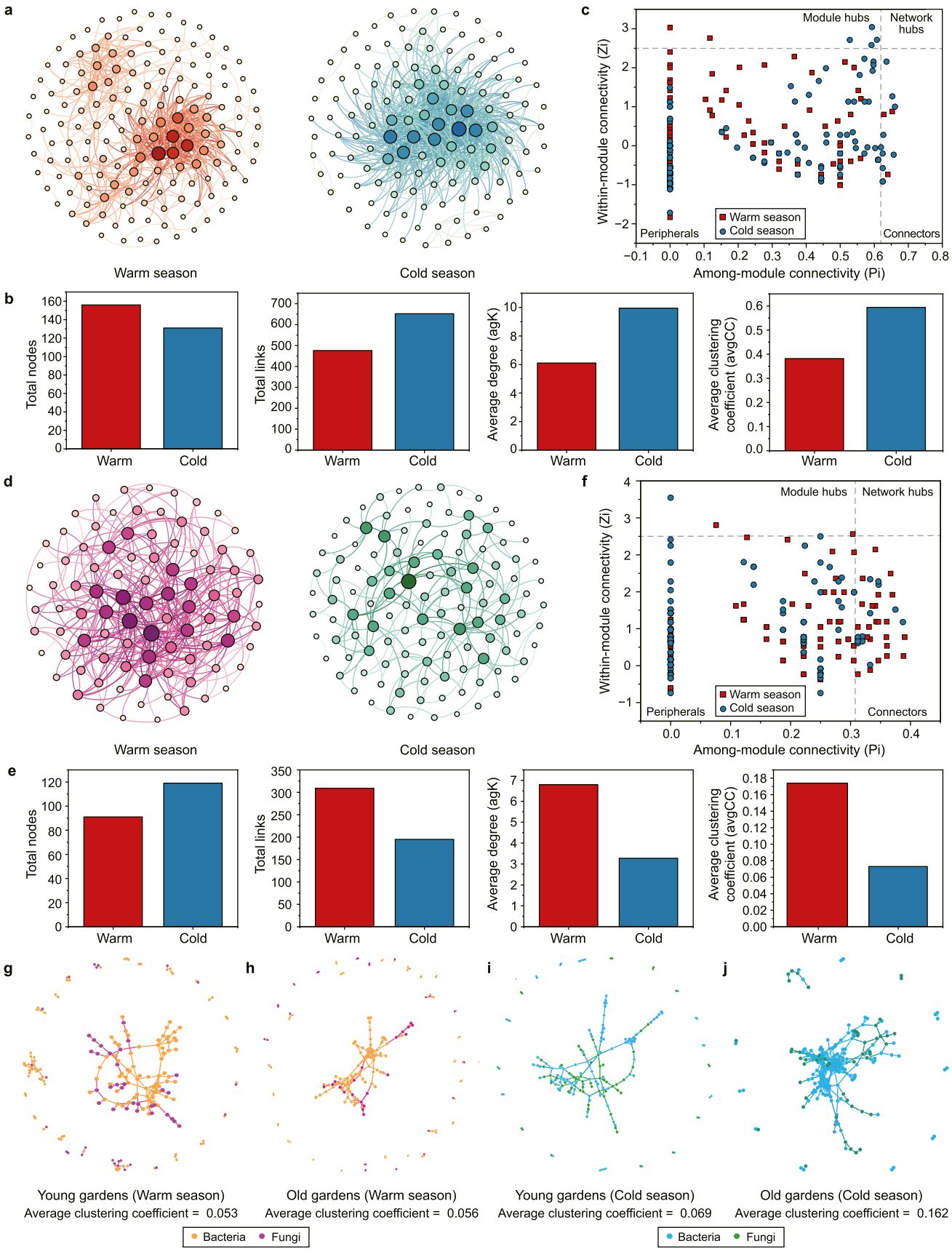

Fig. 4. Co-occurrence networks of PMCs. **a**, Visualization of bacterial networks in warm and cold seasons. **b**, Topological properties of the bacterial networks. **c**, Identification of bacterial keystone taxa. **d**, Visualization of fungal networks in the warm and cold seasons. **e**, Topological properties of the fungal networks. **f**, Identification of fungal keystone taxa. **g**–**h**, Cross-kingdom bacteria-fungi co-occurrence networks are visualized between young (**g**) and old (**h**) gardens in the warm season. **i**–**j**, Cross-kingdom bacteria-fungi co-occurrence networks are visualized between young (**i**) and old (**j**) gardens in the cold season.

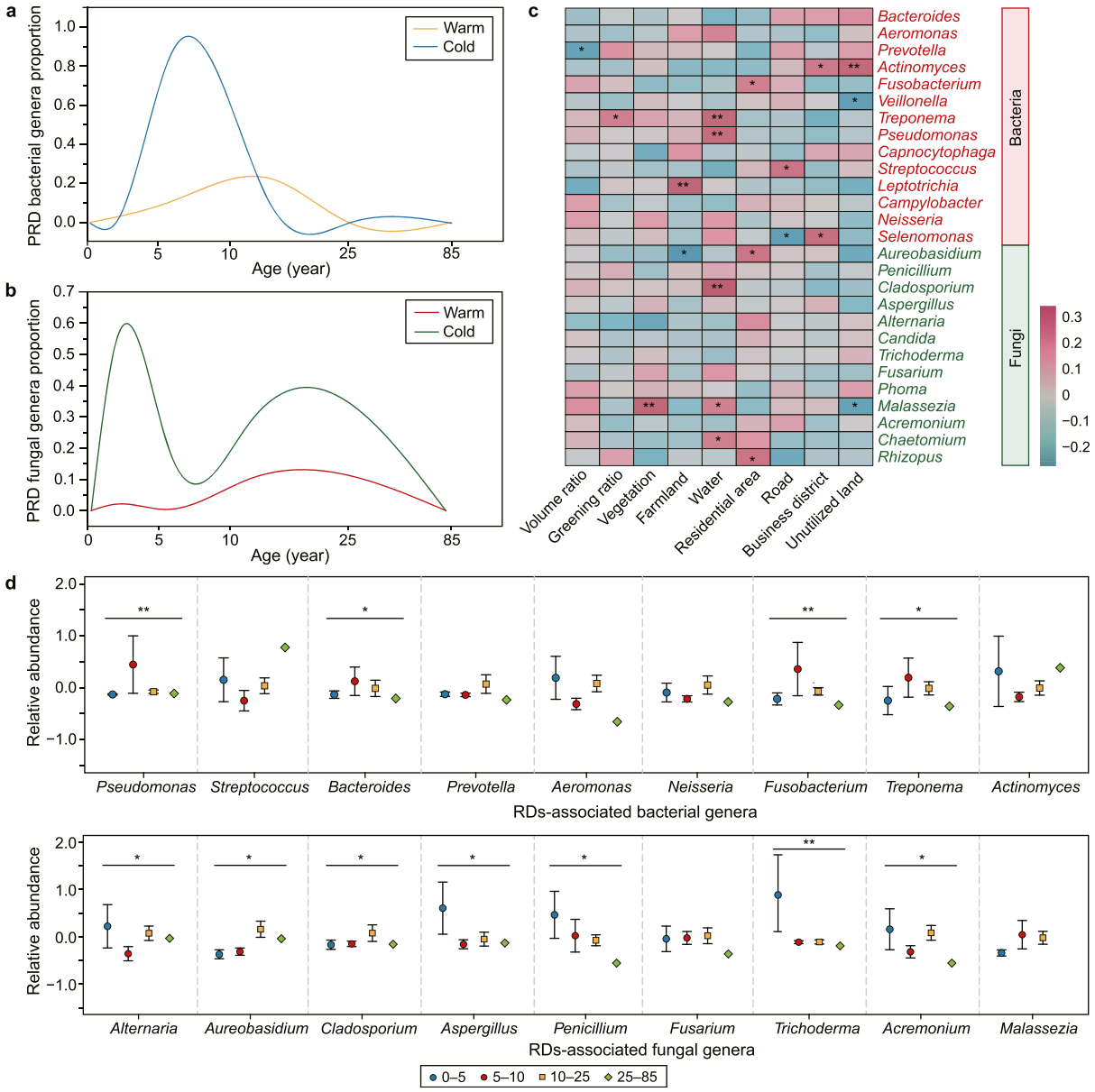

Fig. 5. The temporal variation and associated drivers of the RDs-associated microbial genera. Since each RDs-associated genus may contain one or more species, we use genera to represent the characteristics of the abundant taxa along the garden age gradient. The specific species and their abundance ratio are shown in Tables S4 and S5 **a**–**b**, Moving averages of the PRD bacterial (**a**) and fungal (**b**) genera proportion along the garden age in the cold season. **c**, Correlation analysis heatmap of RDs-associated microbial genera and environmental factors in the warm season, and the asterisks represent the significance. **d**, Relative abundances of the major RDs-associated bacterial and fungal genera across garden age categories in the cold season, and the asterisks represent the significance. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

microbiota, which fits our third hypothesis. We found that RDs-associated genera enriched in the young gardens in the cold season and were not affected by the urban-rural gradient. This may be due to standard building practices of both gardens and buildings. Urban soils tend to be produced commercially [84] and plants originate in commercial nurseries. The accumulation of building materials (gypsum board, mortar, etc.) in the early stage of neighborhood construction, since their surfaces are usually porous and rough, can provide a favorable breeding and growth environment for microbes under humid conditions. Building materials, e.g., plywood, laminate, paint, or glues, can gradually release volatile organic compounds (VOCs), including formaldehyde, toluene, benzene, etc. [85]. These VOCs can still be detected years after the completion of the construction [86]. Formaldehyde can inhibit the growth of PMCs by weakening their respiratory activity [87], while toluene can lead to the enrichment of its degrading bacteria (*Pseudomonas*) [88].

5. Conclusions

Our study indicates that PMCs in the neighborhood gardens responded to seasonal variation and garden age. The diversity of bacterial and fungal communities demonstrated an inverse correlation with seasons. In the cold season, PMCs were homogenized and contained more potentially pathogenic genera, particularly RDs-associated genera. Surprisingly, the RDs-associated species

significantly enriched in young neighborhood gardens. Collectively, our results highlight the essential impact of garden age on human health-associated microbiota. We also emphasize the need to reduce disturbance (e.g., constructions) and preserve old gardens in urban green space management. These findings extended our understanding of the park age effect from promoting ecosystem services [89] to reducing RDs-associated microbiota.

Credit authorship contribution statement

Chang Zhao: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Software, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing. **Xinxin Liu:** Methodology, Resources. **Haoxin Tan:** Investigation, Data Curation. **Shan Yin:** Project Administration, Resources. **Lantian Su:** Investigation, Formal Analysis, Software. **Baoming Du:** Investigation, Data Curation. **Muhammad Khalid:** Investigation, Data Curation. **Aki Sinkkonen:** Conceptualization, Writing - Review & Editing. **Nan Hui:** Conceptualization, Funding Acquisition, Investigation, Project Administration, Resources, Software, Supervision, Writing - Review & Editing.

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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Appendix A. Supplementary data

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