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# Urbanization influences the indoor transfer of airborne antibiotic resistance genes, which has a seasonally dependent pattern

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ABSTRACT

Over the last few years, the cumulative use of antibiotics in healthcare institutions, as well as the rearing of livestock and poultry, has resulted in the accumulation of antibiotic resistance genes (ARGs). This presents a substantial danger to human health worldwide. The characteristics of airborne ARGs, especially those transferred from outdoors to indoors, remains largely unexplored in neighborhoods, even though a majority of human population spends most of their time there. We investigated airborne ARGs and mobile genetic element (MGE, *IntI1*), plant communities, and airborne microbiota transferred indoors, as well as respiratory disease (RD) prevalence using a combination of metabarcode sequencing, real-time quantitative PCR and questionnaires in 72 neighborhoods in Shanghai. We hypothesized that (i) urbanization regulates ARGs abundance, (ii) the urbanization effect on ARGs varies seasonally, and (iii) land use types are associated with ARGs abundance. Supporting these hypotheses, during the warm season, the abundance of ARGs in *peri*-urban areas was higher than in urban areas. The abundance of ARGs was also affected by the surrounding land use and plant communities: an increase in the proportion of gray infrastructure (e.g., residential area) around neighborhoods can lead to an increase in some ARGs (*mecA*, *qnrA*, *ermB* and *mexD*). Additionally, there were variations observed in the relationship between ARGs and bacterial genera in different seasons. Specifically, *Stenotrophomonas* and *Campylobacter* were positively correlated with *vanA* during warm seasons, whereas *Pseudomonas*, *Bacteroides*, *Treponema* and *Stenotrophomonas* positively correlated with *tetX* in the cold season. Interstingly, a noteworthy positive correlation was observed between the abundance of *vanA* and the occurrence of both rhinitis and rhinoconjunctivitis. Taken together, our study underlines the importance of urbanization and season in controlling the indoor transfer of airborne ARGs. Furthermore, we also highlight the augmentation of green–blue infrastructure in urban environments has the potential to mitigate an excess of ARGs.

## 1. Introduction

The presence of antibiotic resistance poses a major worldwide concern for the health of both humans and animals (Ahmad et al., 2023; Tiedje et al., 2019). Numerous investigations have been carried out on the transmission of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in land-based ecosystems (Li et al., 2023; Symochko et al., 2023) as well as aquatic environments (Bonetta et al., 2023; Jia et al., 2022). However, small airborne particles can be inhaled, potentially impacting human respiratory health (Passi et al., 2021;

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Wang et al., 2021). Close correlation exists between respiratory health and indoor airborne microorganisms (Kumar et al., 2022; Walser et al., 2017), with the majority originating from the outdoor surroundings (Adams et al., 2015). However, the ARGs carried by airborne microbes and transferred indoors along with the factors influencing them remain poorly understood.

Land uses commonly differ in their microbial communities (Docherty et al., 2018) resulting in differences in airborne ARB from those environments. Proteobacteria are common in farms, whereas Firmicutes dominate in urban areas (Bai et al., 2022). Moreover, the ARGs found in rural regions primarily originate from animal husbandry (Swarthout et al., 2022). Conversely, the airborne ARGs detected in urban public settings primarily emanate from health care facilities (Zhou et al., 2023). Nevertheless, the impact of urbanization on airborne ARGs that transferred indoors in neighborhoods has not yet to be investigated comprehensively. Geographical variations in antibiotic emissions have been documented in prior investigations, utilizing a nationwide survey conducted in China. These disparities are presumed to stem from variances in human activities and socioeconomic factors (e.g., local GDP) (Guo et al., 2020; He et al., 2020). Shanghai, exemplifying economically developed region in China, exhibits a notable differences in land-use between its urban and *peri*-urban areas, coinciding with a gradual rise in the prevalence of respiratory diseases (RDs) (Zhang et al., 2015). Addtionally, investigating ARG variabilitytransferred indoors in both urban and *peri*-urban areas becomes crucial sinse it can establish a basis for enhancing the effectiveness of disease treatment.

Seasonal variation in precipitation leads to differences in ARG diffusion, and can also affect the propagation of airborne ARGs by influencing particulate matter concentration (Wang et al., 2022), particularly via airborne fine particles ($PM_{2.5}$)(Xie et al., 2018). A prior investigation revealed that climate variations in different regions have the potential to control the dynamics of ARGs in both public parks and pristine soil ecosystems (Khalid et al., 2023). In relation to the occurrence of respiratory illnesses, there is an elevation in the levels of *Streptococcus* increases during the winter season in the atmosphere, while there is a surge in the relative abundance of *Pseudomonas* during the spring time (Kennis et al., 2022; Psoter et al., 2013). Neverless, the correlation between respiratory diseases and the seasonal fluctuations of airborne ARGs remains ambiguous, especially concerning the airborne ARGs that are transmitted within indoor environments. Moreover, the spread of bacterial resistance is not exclusively associated with ARGs but also with class 1 integrons (Subirats et al., 2018), where the integron-integrase gene (*intI1*) plays a pivotal role as a fundamental component, facilitating the horizontal transfer of ARGs.

The transfer of ARGs in soil can be influenced by plant communities, as demonstrated by Xiao et al. (2023). Previous research conducted by Liu et al., 2021a has revealed that a healthy rhizosphere can support microbes with a higher reproductive capacity and a more complex microbial interaction network. This, in turn, contributes to plant growth promotion and reduces the levels of antibiotics present in soil. In aquatic ecosystems, certain bionic plants that utilize specialized chemical materials have been shown to facilitate the removal of ARGs, as highlighted by Zhou et al. (2022). Nevertheless, the understanding of the influence exerted by plant communities on the indoor transfer of airborne ARGs remains limited.

To explore the composition and abundance of different airborne ARGs that transferred indoors, as well as the influencing factors, we analyzed plant communities, and collected airborne materials on the corridor windows of residential buildings in 72 neighborhoods in late August and December 2021 in Shanghai. Following collection, we employed metabarcode sequenced airborne microbiota and conducted quantitative real-time PCR to detect ARGs. In our previous study, Khalid et al. (2023) found a remarkable disparity in ARG levels between urban green spaces and natural forests. Furthermore, we discovered that urban green spaces served as the primary contributor to airborne ARGs that transferred indoors. In the current study, we hypothesized that i) degree of urbanization can influence the abundance of ARGs transferred indoors. This is because complex anthropogenic activities can lead to more resistance sources (Lien et al., 2016). Further, we hypothesized that ii) the abundances of ARGs and MGE are higher in the warm season, likely attributable to the greater ventilation and resultant increase in airborne biomass transferred indoors (Wang et al., 2023b). Finally, we hypothesized that iii) the effect of urbanization on airborne ARGs that transferred indoors is greater than that of season. This is because the differences in land use types and corresponding anthropogenic activities can affect the airborne ARGs more than natural factors (Xiang et al., 2018).

## 2. Materials and methods

### 2.1. Site description and land-use type dataset

The present investigation was conducted in a total of 42 urban neighborhoods and 30 *peri*-urban neighborhoods situated in Shanghai, an expanding metropolis (Fig. S1a, Table S1). These neighborhoods were categorized based on their age: young (0 ~ 10 years) and old (22 ~ 85 years) (Zhao et al., 2024). All the 72 neighborhoods were located in residential areas, and neither industrial activity nor pollution presented in the vicinity of them. Typically, Shanghai encounters a subtropical monsoon climate, distinguished by an average temperature of 28.4 ± 4.4 °C in the latter part of August and 10.1 ± 3.3 °C in late December, denoted as the “warm season” and “cold season”, respectively. Furthermore, we approximated the $PM_{2.5}$ concentrations in the neighborhoods suring both the warm and cold seasons (https://www.aqistudy.cn/historydata/monthdata) (https://www.86 pm25.com/city/shanghai). Moreover, we gathered information concerning the proportion of neighborhood volume and the ratio of green space from property managers. Utilizing the primary land classification framework in Shanghai and remote sensing image with a resolution of 30 m, we computed the ratio of land-use types within a 1 km buffer zone and categorized them into seven distict categories, including vegetation, farmland, water, residential area, road, business district and unutilized land (Fig. S1b–c). Urban areas exhibit a higher proportion of grey infrastructure (residential area, road, business district, and unutilized land), while *peri*-urban areas possess a greater abundance of green–blue infrastructure (vegetation, farmland, and water). Additionally, we compiled data on the geographical location of all animal husbandry (e.g., chicken farms, pig farms) in Shanghai.

### 2.2. Airborne microbial collection

Air was sampled at each of the 72 neighborhoods using an airborne microbial collection device (Fig. S2). To minimize variation caused by vertical height, we installed the sampling device on the stairwell window ~ 3.0 m above ground (Fig. 1). The device’s inlet pipe was located outside the building while the filter element and outlet pipe were positioned inside. Since the battery of the airborne microbial collection device was designed to last for approximately 7 days, we retrieved the devices one week after we installed them. Then the samplers were transported to the laboratory. On the day of device removal, the filter membranes were removed from the devices and dispensed into labeled sterilized 50 mL centrifuge tubes under sterile conditions. The filter membrane samples were cut into smaller pieces and homogenized using a grinder (No: JXFSTPRP-48) after the addition of liquid nitrogen. The centrifuge tubes were filled with 20 mL of standard buffer (Tris-HCL, pH = 8.0), containing 0.1 M EDTA. After shaking for 3 min, the filter membranes were eliminated via filtration. The supernatant was removed following centrifugation for 3 min (6000 r/min) and the precipitate was then preserved at −80 °C until the extraction of the entire DNA occurred.

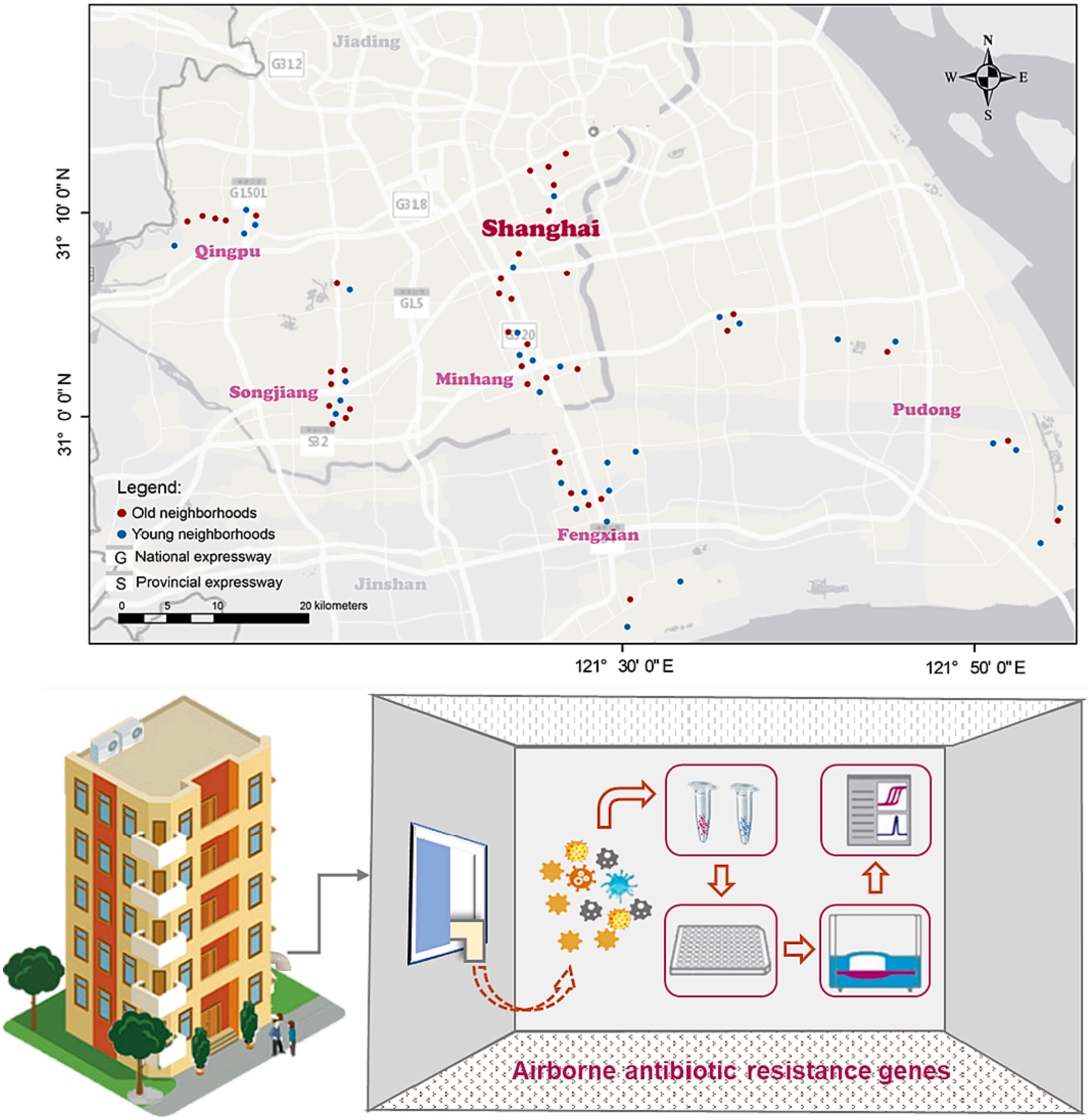

**Fig. 1.** Sampling design and the ARGs assay process.

### 2.3. *Microbial DNA extraction and sequence analysis*

According to the manufacture's guidelines, the OMEGA E.Z.N.A. Soil DNA Kit (D5625-01) was utilized for the extraction of total DNA. The automation of DNA extraction was performed on a Biomek i7 workstation (Beckman Coulter, USA), and subsequently, the extracted DNA was stored at −80 °C in preparation for polymerase chain reaction (PCR) amplification. The assessment of DNA quality was carried out using a 1 % agarose gel, while the determination of DNA concentration and purity was accomplished by employing a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). In order to conduct bacterial sequence analyses, amplification of the V4 region of the 16S rRNA gene was carried out using the following primers: forward primer 515F 5′- GTGCCAGCMGCCGCGGTAA-3′ and the reverse primer 806R 5′-GGACTACHVGGGTWTCTAAT-3′ (Caporaso et al., 2012; Suzuki and Giovannoni, 1996). As part of all processes, negative controls were incorporated to ensure that filter extracts and potential contamination were not present. In each PCR, a positive control (*Cupriavidus necator* JMP134, DSM 4058) was incorporated, as referenced in the studies by Roslund et al. (2022) and Singer et al. (2016) to maintain the integrity of the analysis. The sequencing process was executed on the Illumina MiSeq platform (Illumina, USA). The paired files (.fastq) are available in the Sequence Read Archive at National Center for Biotechnology Information with PRJNA932577 for bacteria.

Following the standard operating protocol, amplicon analysis was performed with QIIME2 (Bolyen et al., 2019). Paired-end sequences were demultiplexed and non-biological sequences (barcodes and primers) were removed. The DADA2 pipeline (Callahan et al., 2019) was utilized to denoise sequences into amplicon sequence variants (ASVs) with a threshold of 100 %. The Silva (v138) and Unite (v9) databases were employed for the annotation of bacterial taxonomy. We removed non-target sequences (mitochondria, chloroplast, archaea, metazoan, plantae, and Chlorophyta). In order to mitigate PCR and sequencing artifacts, ASVs of low abundance were eliminated from bacterial dataset if they had less than 10 sequences across all samples. We rarefied the dataset to 39072 sequences per sample using the 'rrarefy' function in the 'vegan' package.. The final ASV datasets contained 37136 bacterial ASVs.

### 2.4. Real-time quantitative PCR (qPCR) of target genes

Eleven target ARG subtypes (*aadA1*, *blaTEM*, *fox5*, *mecA*, *mexD*, *qnrA*, *ermB*, *sul2*, *tetX*, *vanA*, *sat4*), 16S rRNA and mobile genetic element (*intI1*) were quantitatively identified using a RealTime PCR System (QuantStudio 3, Thermofisher). The target gene-carrying plasmids were extracted from TA clones and using the QIAprep SpinMiniprep Kit (QIAGEN, Germany). Using the Nanodrop ND-2000 (Thermo Fisher Scientific, Wilmington, MA), the concentrations of standard plasmids (ng/mL) were ascertained, which facilitated the computation of gene copy concentrations (copies/mL). The reactions were conducted in a final 20 μL volume of reaction mixture, containing 10 μL of 2*SYBR Green qPCR Premix (Sharebio, Shanghai, China), 0.4 μL of forward and reverse primers for each gene (as specified in Table S2), 1 μL of extracted DNA, and 8.2 μL of nuclease-free water. The qPCR program consisted of an initial denaturation step at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 55–65 °C, and extension at 72 °C. A final melt curve stage was included with a temperature ramp from 55 °C to 95 °C, as described by (Srathongneam et al., 2024). The standard curves for each target gene displayed $R^2$ values above 0.99, demonstrating a strong correlation between the cycle threshold (Ct) values and the template DNA concentrations. Every qPCR reaction was conducted three times, in conjunction with standard curves and a negative control. In the negative control, nuclease-free water was used in place of the template genomic DNA. The relative abundances of ARGs or MGE were determined by caiculating the gene copy number /16S rRNA copy, facilitating comparisons of ARG abundances between urban and *peri*-urban areas.

### 2.5. Plant community and respiratory disease prevalence investigation

With the airborne microbial sampling device as the midpoint, a square quadrant was delimited by extending 20 m from the left and right, and 25 m vertically forward. In this quadrant (1000 $m^2$), we estimated the richness, diversity and abundance of three plant functional groups (Tree, Shrub and Grass).

To determine the prevalence of respiratory diseases, we conducted a questionnaire survey by recruiting 30 ± 5 volunteers in each of the old neighborhoods. Due to the short residency of residents in the young neighborhoods, we did not investigated the prevalence of dieases in the young ones. The volunteer selection criteria included: 1) age between 40 and 80, 2) neighborhood resident for more than 10 years, 3) the equal number of men and women. The survey of asthma, wheeze, shortness of breath, rhinitis, and rhinoconjunctivitis were included in the questionnaires as described in Fu et al. (2021). When analyzing the questionnaire data, we excluded volunteers who claimed non-respiratory diseases (mainly hypertension, diabetes, etc.). This resulted in 1026 questionnaires in the final dataset. The collection of health data was conducted with the approval of the Ethics Committee at Shanghai Jiao Tong University (B20230221I) in Shanghai, China. All participants provided their written consent, and the records were kept at Shanghai Jiao Tong University (Zhao et al., 2024).

### 2.6. Statistical analyses

Using the vegan package (R core team, 2019) in R version 4.3.1, we conducted statistical analyses. Dissimilarities were calculated using Bray-Curtis to analyze the composition of airborne ARGs. For result visualization, the 'metaMDS' function was employed to carry out nonmetric multidimensional scaling (NMDS). Shifts in the abundance of airborne ARGs and MGE were tested between warm and cold seasons using *t*-test. Correlations of environmental factors (e.g., land use types and plant communities) and the abundance of airborne ARGs were analyzed using the Spearman test. In ArcGIS, we drew the distribution map of the sample neighborhoods according to the abundance gradient of ARGs, and made the kernel density map of the animal husbandry distribution by using the latitude and longitude coordinates. Molecular Ecological Network Analysis Pipeline (MENAP) datasets were used for co-occurrence network analysis of airborne bacteria and ARGs (https://ieg2.ou.edu/MENA/) (Deng et al., 2012). The mantel test was performed between the abundances of potentially pathogenic bacterial genera and ARGs to explore their correlations (Sunagawa et al., 2015). Furthermore, the R basic function 'lm' was utilized to execute the linear regression of these bacteria and ARGs. The visualization of the scatter plot was accomplished using the ggplot2 package (Su et al., 2021). We used path analyses to explore the direct and indirect relationships among urbanization, season, land-use types, plant communities and the abundance of airborne ARGs and MGE that transferred indoors (Eisenhauer et al., 2015; Francini et al., 2021). Path analyses were conducted using the lavaan package (Rosseel, 2012) in R. The 'MLM' method, which incorporates maximum likelihood estimation with robust standard errors and a Satorra-Bentler scaled test statistic, was used to guarantee robust estimation. To account for exogenous categorical variables like urbanization and season, they were converted into dummy variables. Additionally, variation partitioning analyses (VPAs), as suggested by Yang et al. (2022), enabled the quantification of the relative contributions of airborne ARGs and MGE to the prevalence of respiratory diseases.

## 3. Results

### 3.1. Antibiotic resistance genes are affected by urbanization

We analyzed the distribution of ARGs in urban and *peri*-urban areas between warm and cold seasons. The ARG composition differed between urban and *peri*-urban neighborhoods, but only in the warm season ($R^2 = 0.025$, $P = 0.036$) (Fig. 2a), which was influenced by the proportion of farmland and unutilized land (Fig. 2a). The ARGs overall were more abundant in the *peri*-urban areas than in the urban areas, although distribution of ARGs differed. For example, *qnrA* was more abundant in the *peri*-urban areas, whereas *mexD* abundance did not differ between urban and *peri*-urban areas (Fig. 2b). In contrast to warm season, there were no significant differences in the ARG distributions between urban and *peri*-urban areas in the cold season ($R^2 = 0.007$, $P = 0.891$) (Fig. 2c). In the cold season, *mexD* and *mecA* were mainly distributed in urban areas, while *fox5* was more concentrated in *peri*-urban areas (Fig. 2d). In addition, we observed no significant difference in $PM_{2.5}$ concentrations between urban and *peri*-urban neighborhoods. Intriguingly, 81.5 % of the *peri*-urban neighborhoods were within 20 km of the maximum point of the animal husbandry kernel density map (Fig. 2e). In addition, the distances from the maximum point of the animal husbandry kernel density map were significantly lower in the *peri*-urban areas than urban areas (Fig. 2f).

### 3.2. Antibiotic resistance genes are affected by season

The total abundance of ARGs in the warm season was higher than in the cold season (Fig. 3a), and their distribution differed significantly ($R^2 = 0.044$, $P < 0.001$) between the warm and cold season (Fig. 3b). Among all ARGs and MGE, the abundance of *qnrA* was highest (37.5 %), followed by *mexD* (21.2 %) and *fox5* (14.3 %) in the warm season, while *intI1* had the highest abundance (28.6 %), followed by *mexD* (23.2 %) and *mecA* (15.6 %) in the cold season. Most ARGs, including *blaTEM*, *fox5*, *mexD*, *qnrA*, were more abundant in the warm season than in the cold season, with the exception of *tetX*. Moreover, the abundance of MGE (*intI1*) in the cold season was significantly higher than that in the warm season (Fig. 3c). In terms of $PM_{2.5}$, the concentration in the cold season ($43.0 \pm 0.5\ \mu g \cdot m^{-3}$) was significantly higher than that in the warm season ($19.0 \pm 0.4\ \mu g \cdot m^{-3}$).

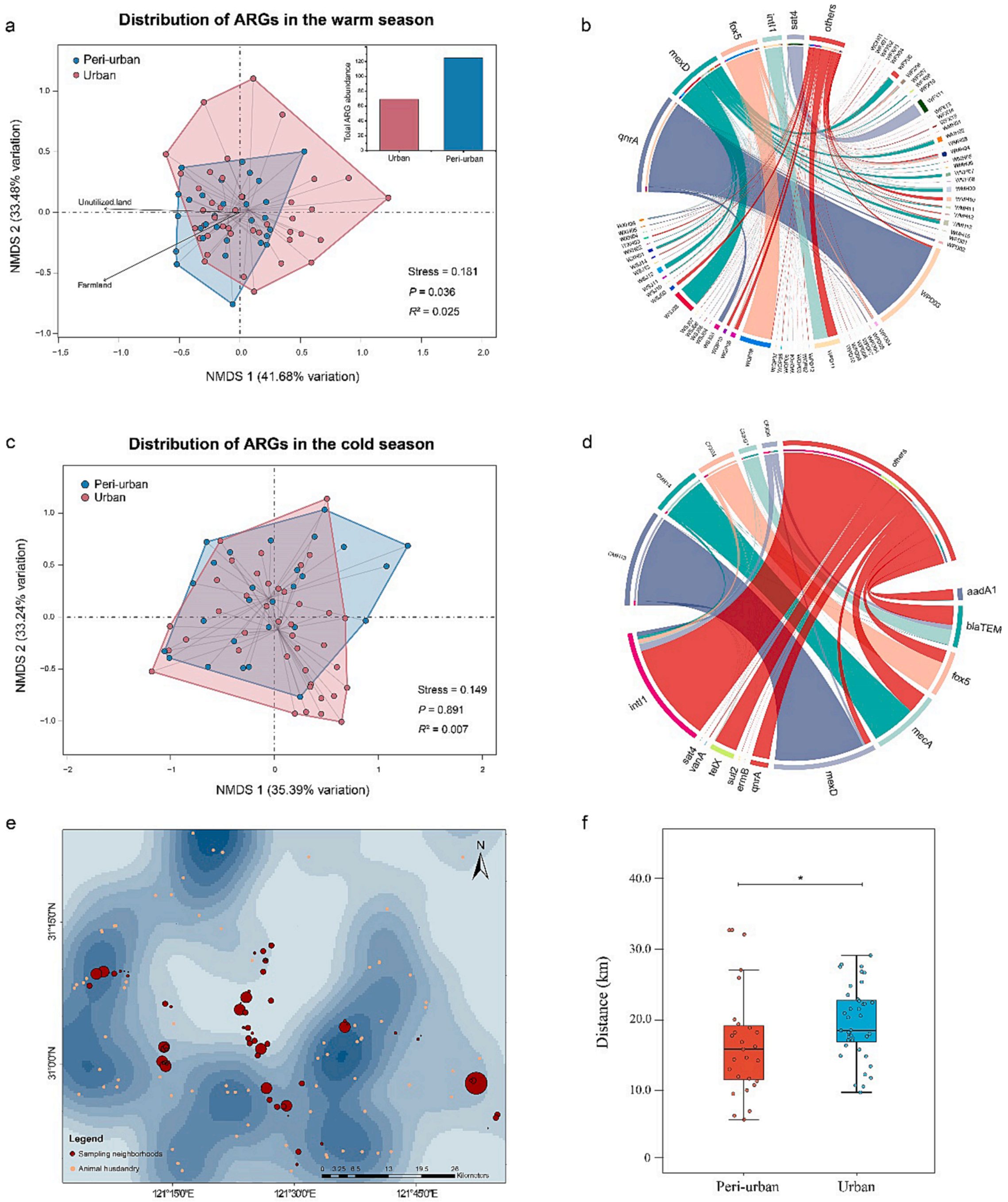

**Fig. 2.** Composition of ARGs and correlation of environmental factors and ARG abundance in the warm (a) and cold (c) seasons. During the warm season, the NMDS ordination reveals a marked difference in ARG composition between urban and *peri*-urban areas, with Bray-Curtis serving as the dissimilarity metric. Arrows are used to represent environmental factors that have a strong correlation with the two axes. Distribution characteristics of ARGs and MGE at different neighborhoods in the warm (b) and cold (d) seasons. Distribution kernel density map of animal husbandry around the sample neighborhoods. The red dots represent the sampling neighborhoods, and their sizes represent the abundance of ARGs in the warm season, and the orange dots represent animal husbandry (e).Urban and *peri*-urban distribution characteristics of minimum distance values from sampling neighborhoods to maximum points in animal husbandry kernel density map. The upper asterisks represent the significance (f). $^{*}P < 0.05$.

### *3.3. The effects of urbanization and season on airborne ARGs transferred indoors*

Our path analysis found that land use types surrounding the urban and *peri*-urban neighborhoods and plant communities inside the neighborhood gardens influenced the abundances of airborne ARGs and MGE. Season was also a key factor (Fig. 4). Urban areas had a greater proportion of residential areas, which contributed to the enrichment of *mecA*, *qnrA*, *ermB* and *mexD*. In contrast, the proportion of vegetation around the neighborhoods decreased with urbanization, which was associated with a decrease in *tetX* and an increase in *mecA* and *qnrA*. We also found that the reduction of all green–blue infrastructure (including vegetation, farmland and water) around neighborhoods due to urbanization contributed to an increase in *mecA*. Moreover, the decrease in grass diversity caused by urbanization can also promote the increase of *blaTEM*. Interestingly, despite the proportion of roads and unutilized land in urban and *peri*-urban areas showing no significant difference, both can still lead to an increase in some ARGs, including *blaTEM*, *ermB*,

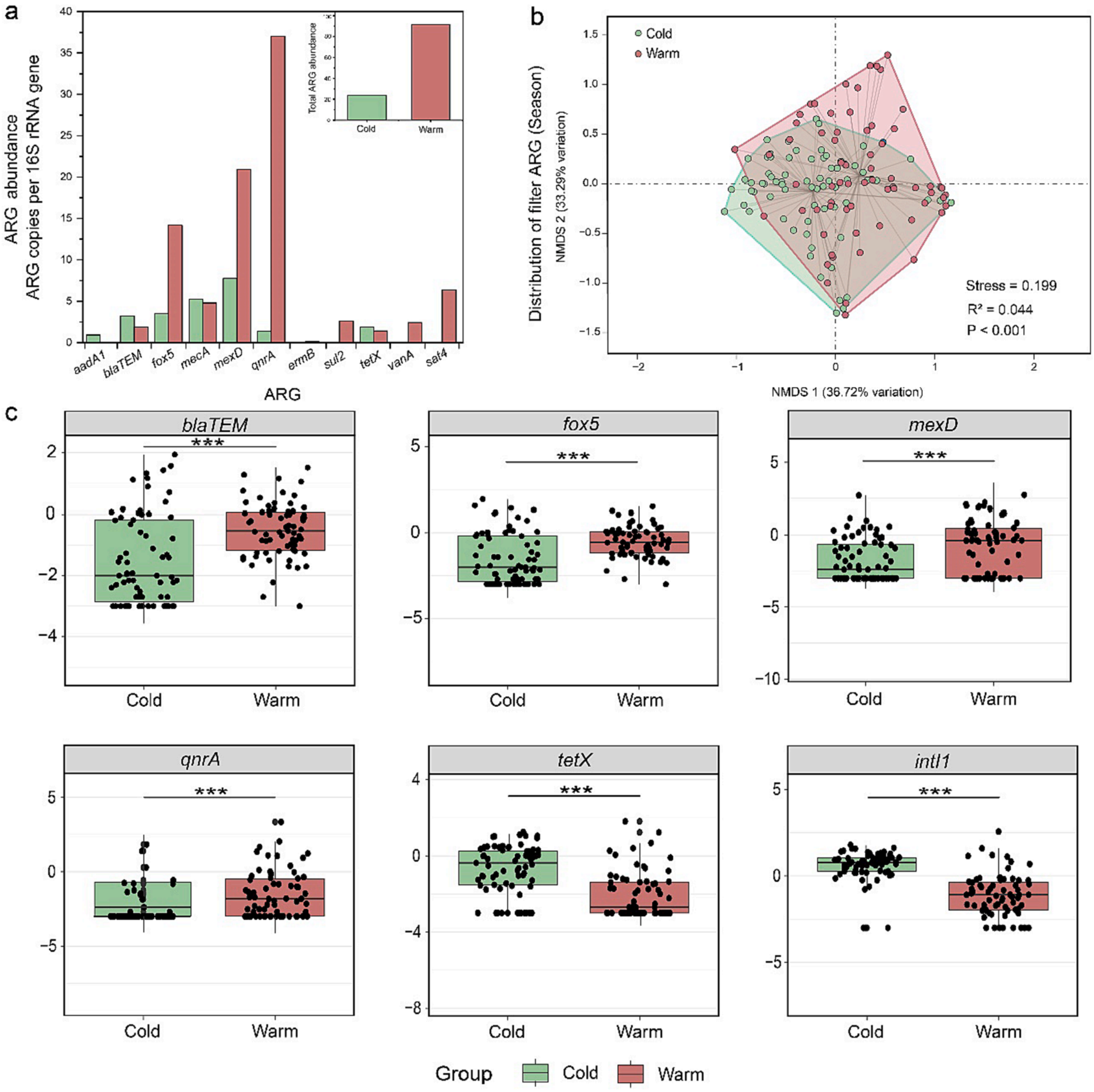

**Fig. 3.** Effect of seasonal variations on ARGs abundance and distribution. Total ARG abundance (ARG copies per 16S rRNA gene) between warm and cold seasons (a). Using Bray-Curtis as the dissimilarity metric, NMDS ordination illustrates a notable variance in ARG composition between the cold and warm seasons (b). Box plots show the composition of ARG average abundance between the cold and warm seasons (c). The upper asterisks represent the significance. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

*mecA*, and *mexD*. Unlike the indirect effect of urbanization, seasonal variations can directly cause changes in ARGs and MGE, where *sul2* increased with higher temperature, while *intI1* and *tetX* showed the opposite trend. It is noteworthy that the plant diversity increased in the warm season, which can result in the increase of *blaTEM*. In terms of the total effect, most ARGs had a stronger response to season than urbanization, and most of them were positively correlated with seasonal variations (Fig. 4b).

### *3.4. Co-occurrence among ARGs, MGE and microbial taxa*

During the warm season, we observed that the constructed networks comprised a total of 204 nodes, representing different subtypes of ARGs, MGE, and bacterial genera. The edges connecting these nodes amounted to 381, resulting in an average degree of 3.735 (Fig. 5a). By examining the modularity class, we noticed a clear division of the entire network into eight distinct modules. Notably, nodes within the same module exhibited greater interactions amongst themselves compared to interactions with nodes from other modules. Among these modules, Module I emerged as the largest with 61 modes, followed by modules II and III, which consisted of 26 and 18 nodes, respectively. We also identified specific associations between potentially pathogenic genera and ARGs in certain modules. For instance, *Actinomyces* showed a positive correlation with *aadA1* and *ermB* in module I. Moving on to the cold season, the networks presented a slightly different configuration. They consisted of 181 nodes, each representing a subtype of ARGs, MGE, or bacterial genera. The edges connecting these nodes amounted to 472, yielding an average degree of 5.215 (Fig. 5b). Similar to the warm season, the entire network exhibited a clear separation into eight major mudules based on the modularity class. Notably, Module I maintained its position as the largest module, accommodating 56 nodes. Modules II and III followed closely, comprising 48 and 39 nodes respectively. Among the different modules, we found a positive correlation between *Fusobacterium* and *aadA1*, *sat4* and *ermB* in module II.

In addition, we found that the association between the relative abundance of ARGs and bacterial genera varied between warm and cold seasons. The result showed that *vanA* showed the strongest correlation with airborne bacteria in the warm season (Fig. 5c) and was significantly positively correlated with *Stenotrophomonas* ($R^2 = 0.51$, $P = 0.00$) and *Campylobacter* ($R^2 = 0.02$, $P = 0.04$) (Fig. 5c, Fig. S3a–b). Notably, *tetX*

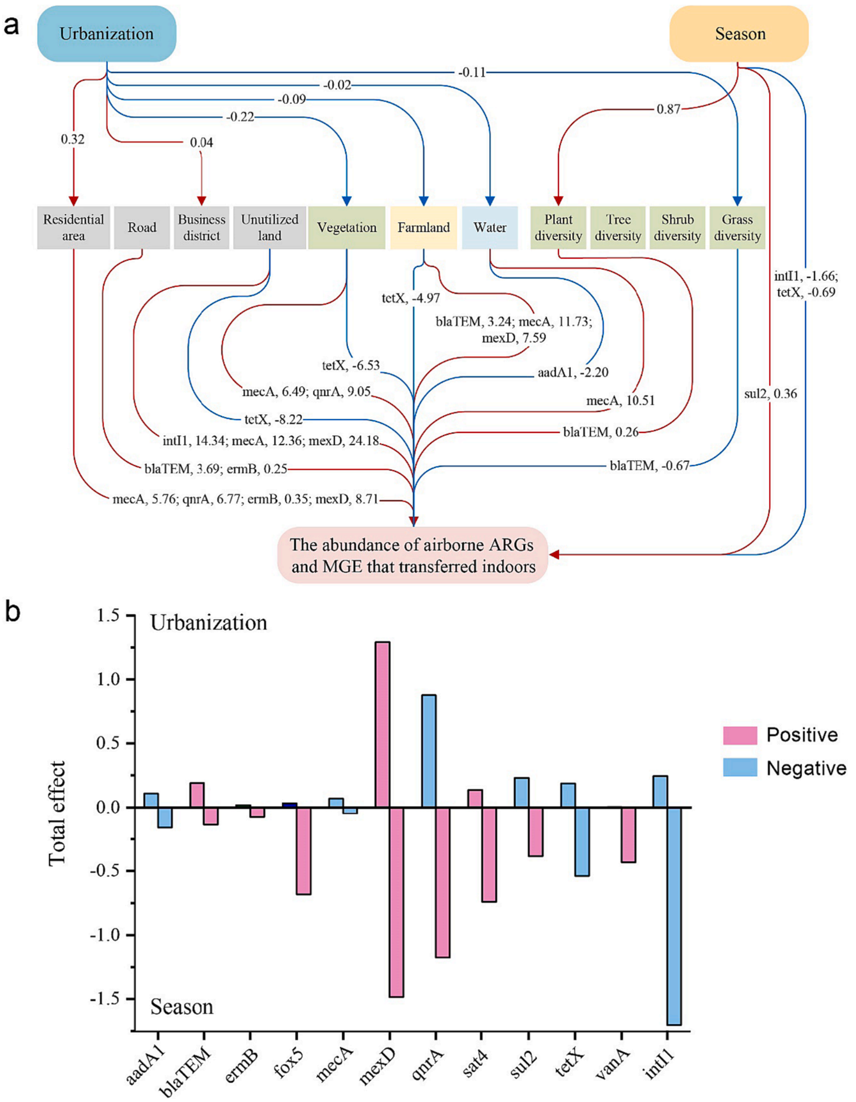

**Fig. 4.** Associations of urbanization (i.e., urban vs. *peri*-urban) and season on the abundance of airborne ARGs that transferred indoors. Schematic representation of the path analysis for airborne ARGs that transferred indoors. Exogenous (explanatory) variables are urbanization (urban and *peri*-urban) and season (warm and cold). Endogenous variables are land-use types (including residential area, road, business district, unutilized land, vegetation, farmland and water), plant communities (including plant diversity, tree diversity, shrub diversity and grass diversity) and the abundance of airborne ARGs and MGE that transferred indoors. Airborne ARGs and MGE included in the analysis are *aadA1*, *blaTEM*, *fox5*, *mecA*, *mexD*, *qnrA*, *ermB*, *sul2*, *tetX*, *vanA*, *sat4* and *intI1*. Red arrows indicate variables with significant positive correlations, while blue arrows represent those with significant negative correlations. For airborne ARGs and MGE that have a significant correlation, they are denoted above their corresponding paths (a). Differential analysis of the total effects of urbanization and season on different kinds of ARGs (b).

showed the strongest correlation with airborne bacteria in the cold season (Fig. 5d). The abundance of *tetX* was positively correlated with *Pseudomonas* ($R^2 = 0.31$, $P = 0.00$), *Bacteroides* ($R^2 = 0.53$, $P = 0.00$), *Treponema* ($R^2 = 0.15$, $P = 0.001$) and *Stenotrophomonas* ($R^2 = 0.71$, $P = 0.00$) (Fig. S3c–f).

### 3.5. Correlation between ARGs, MGE and respiratory diseases prevalence

As the abundance of ARGs was greater during the warm season compared to the cold season, we proceeded to investigate their association with the prevalence of respiratory diseases. The RDA analysis showed that ARGs and MGE explained 10.1 % of the total variation in respiratory diseases prevalence (Fig. S4). Among them, *vanA* contributed most to the prevalence of respiratory diseases (16.1 %), followed by *qnrA* (1.5 %) and *blaTEM* (1.1 %). Meanwhile, we also found that the combined effect of *vanA* and *tetX* was also higher, contributing up to 6.8 % to respiratory morbidity, followed by the combination of *vanA* and *blaTEM* (6.7 %), *vanA* and *fox5* (6.5 %). Furthermore, our analysis revealed a significantly higher prevalence of respiratory diseases in urban areas when compared to *peri*-urban regions, particularly for conditions such as wheezing, rhinitis, and rhinoconjunctivitis (Fig. 6a). The prevalence of wheezing was significantly positively correlated with the abundance of *qnrA*, while the prevalence of both rhinitis and rhinoconjunctivitis was positively correlated with the abundance of *vanA* (Fig. 6b).

## 4. Discussion

### 4.1. The distribution difference of ARGs between urban and peri-urban areas

Antibiotic usage is a key factor in the emergence and spread of ARGs (Sun et al., 2023). Urban areas have a higher population density and a greater number of hospitals than rural areas, which plausibly can lead to an increase in the variety and abundance of antibiotics (Kormos et al., 2022; Pires et al., 2023; Zhou et al., 2021). Surprisingly, our data

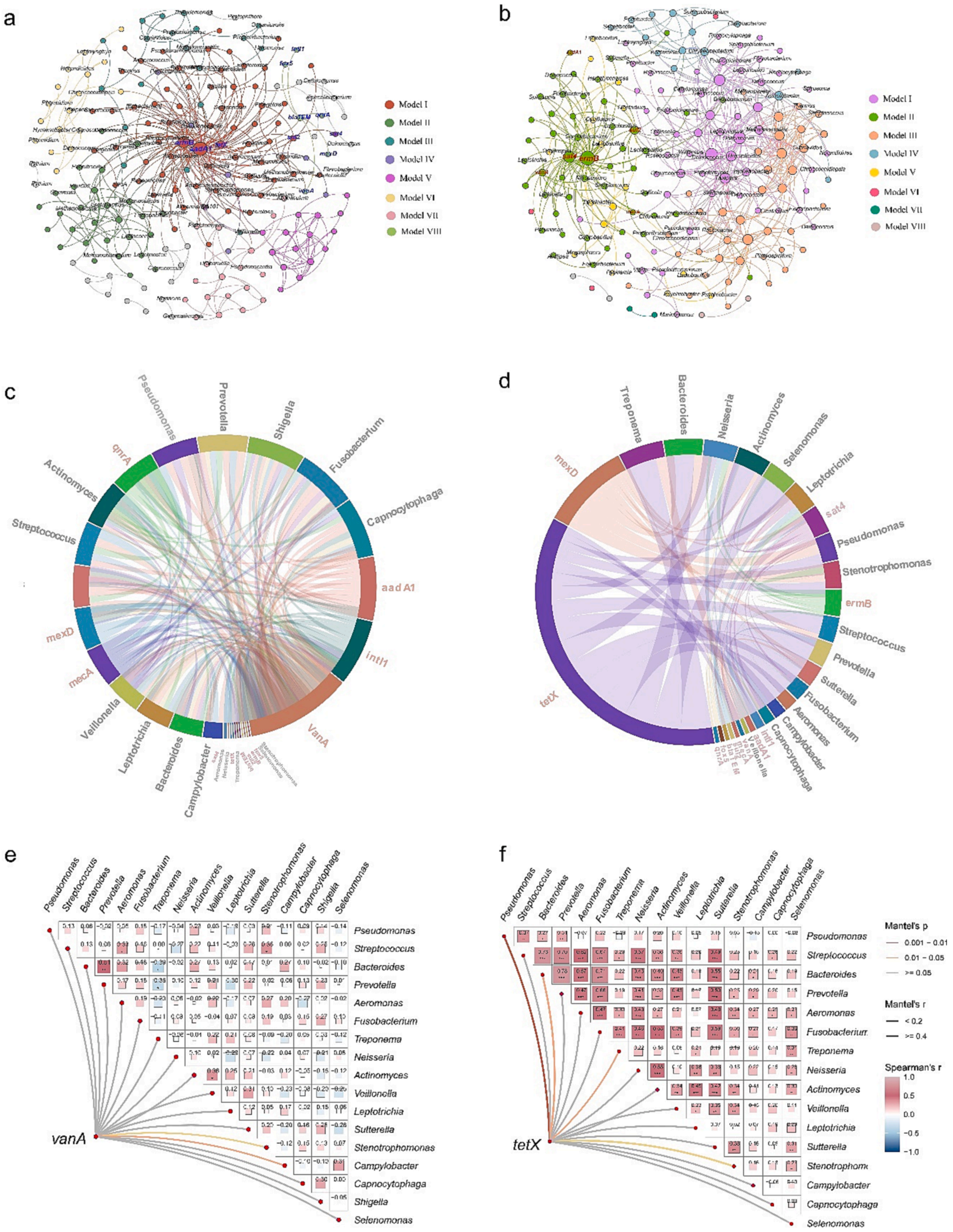

**Fig. 5.** Correlation between ARGs, MGE and airborne bacterial genera. Visualization of co-occurrence networks in the warm season (a). Visualization of co-occurrence networks in the cold season (b). The correlation between ARGs, MGE, and airborne bacterial genera during the warm season is depicted in (c). Similarly, their relationship during the cold season is illustrated in (d). Mantel test between the abundance of *vanA* and airborne bacterial genera in the warm (e) and cold (f) seasons, respectively.

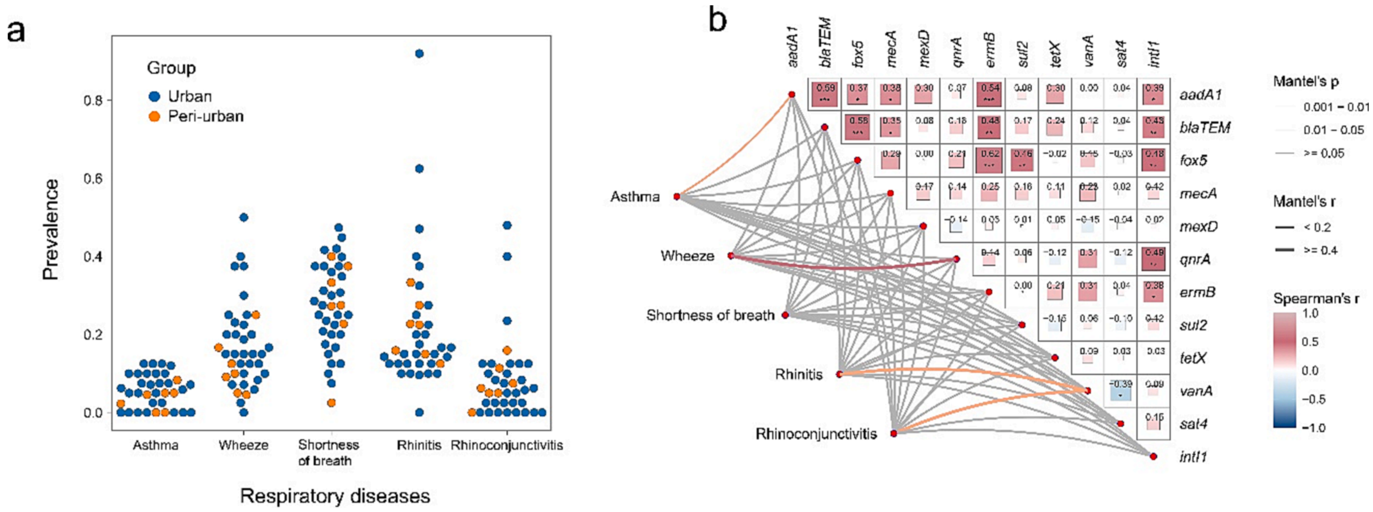

**Fig. 6.** Correlation between ARGs, MGE and respiratory diseases prevalence in the warm season. Prevalence of respiratory diseases (asthma, wheeze, shortness of breath, rhinitis and rhinoconjunctivitis) in the urban and *peri*-urban areas (a). Mantel test between the prevalence of respiratory disease and the abundance of ARGs and MGE (b).

indicate that the abundance of ARGs transferred indoors was higher in *peri*-urban neighborhoods than in urban ones, an observation mainly attributable to the proportion of farmland and adjacent animal husbandry. Peri-urban neighborhoods are closer to the animal husbandry, whose use of antibiotics may increase ARG abundance. In parallel, previous studies showed that built urban infrastructure was poor in microbiota (Li et al., 2022), while *peri*-urban areas may contain more diverse bacterial communities and thus host more diverse ARGs. In addition, more environmental pollution in the *peri*-urban areas can also lead to the enrichment of ARGs (Gao et al., 2023; Zhang et al., 2019). According to Liu et al., (2021b), the type of land use significantly influences the composition of ARGs. The direct association of *blaTEM* abundance with lesser grass diversity (mainly Poaceae) in urban neighborhood gardens observed here may be due to the extensive use of pesticides, e.g., nematicide avermectin (Zhao et al., 2023). For instance, avermectin application has been shown to substantially elevate the abundances of ARGs carried by opportunistic pathogens (Zhao et al., 2020). Meanwhile, proximity to animal husbandry can also contribute to a rise in airborne ARGs, a consequence of antibiotic usage (Sazykin et al., 2021; Xu et al., 2022). In addition, neighborhood gardens in both urban and *peri*-urban areas were relatively surrounded by dense vegetation, so we observed no difference in $PM_{2.5}$ concentrations between urban and *peri*-urban areas.

### 4.2. The abundances of ARGs and MGE responded to seasonal variations

Our findings clearly indicated a notable difference in ARG abundance between the warm and cold seasons, aligning with our initial hypothesis. Previous research conducted on farms (Song et al., 2022) and campuses (Hu et al., 2023) has consistently shown that ARG abundance in indoor air tends to be higher during winter compared to summer. These studies have attributed the increase in ARGs during winter to factors such as reduced ventilation in living spaces and prolonged confinement of animals in smaller areas, which create conditions favorable for bacterial exchange and enrichment. However, contrary to these findings, our study revealed that for most ARGs, their abundances were higher during the warm season compared to the cold season. The rise in ARGs during the warm season can be attributed to the elevated temperatures, which promote bacterial survival and cohabitation (Fig. 5a). Scientific literature has confirmed that higher temperature are linked to the proliferation of airborne bacteria and ARGs (Sequino et al., 2024). Our results suggest that the elevated ARGs are likely attribute to the lush urban plant communities in the warm season. Consequently, regulating ARGs through vegetation manipulation can be considered by urban green space managers or ecotechnology developers.. Interestingly, the abundance of MGE such as *intI1* exhibited an opposite pattern. This observation can be attributed to the fact that cold temperature environments facilitate enhanced horizontal gene transfer (HGT) processes mediated by *intI1* (Meng et al., 2023). Additionally, the concentration of $PM_{2.5}$ particles also play a role in determining the abundance of ARGs. Previous studies have indicated a positive correlation between the concentration of $PM_{2.5}$ and the abundance of ARGs in it (Wang et al., 2023a; Xie et al., 2019). Consequently, during the cold season with low wind speed, a significant number of airborne ARGs adhere to outdoor $PM_{2.5}$ particles, resulting in a decrease in the abundance of airborne ARGs transferred indoors.

### 4.3. Args and bacterial genera are co-spread in the air that transferred indoors

In the warm season, our network analysis revealed a co-occurrence between *aadA1*, *ermB* and *Actinomyces*. Similarly, in the cold season, there was a co-occurrence observed between *sat4* and *Fusobacterium*. Both *Actinomyces* (Chen et al., 2022) and *Fusobacterium* (Rattanaburi et al., 2022) can be associated with respiratory diseases. Based on our network analysis, it was revealed that both pathogens and common bacteria have the potential to co-disperse alongside numerous ARGs and MGE. Concerning the link between ARGs and bacterial groups, it was noted that *vanA* had the highest prevalence among ARGs in the warm season, while *tetX* was notably prominent during the cold season. Specifically, there was a considerable and affirmative correlation found between the high prevalence of *vanA* and the abundance of *Stenotrophomonas* as well as *Campylobacter*. *Stenotrophomonas* spp. can cause cystic fibrosis diseases, while *Campylobacter* spp. can cause chronic bronchitis and chronic obstructive pulmonary disease (Wang et al., 2015). In the cold season, the abundance of *tetX* positively correlated with *Pseudomonas* (Welp and Bomberger, 2020), *Bacteroides* (Sárvári et al., 2018), *Treponema* (Wang et al., 2017) and *Stenotrophomonas* (Maly et al., 2023), all of which were related to respiratory tract diseases.

### 4.4. Different contributions of ARGs and MGE to respiratory disease

We conducted an investigation on the potential impact of ARGs and MGE abundance on the occurrence of respiratory diseases. Our analysis of the single marginal effect (Fig. S4) revealed a strong correlation between the prevalence of respiratory diseases and the abundance of *vanA*.

Additionally, the enrichment of *vanA* was positively associated with the development of rhinitis and rhinoconjunctivitis. Previous research has emphasized the global risk that methicillin-resistant *Staphylococcus aureus* (MRSA) infections pose to public health. It is worth noting that vancomycin remains a crucial treatment option for MRSA infections (Cong et al., 2020). However, *Staphylococcus aureus* with complete resistance to vancomycin (e.g., *vanA*) has already emerged for years (Lindsay, 2010), which can lead to the occurrence of allergic rhinitis (Guzman-Avilan et al., 2022). In addition to rhinitis and rhinoconjunctivitis, wheeze also has higher prevalence, and positively correlated with the abundance of *qnrA*. Previous studies have shown that *Klebsiella pneumoniae* with quinolone resistance (e.g., *qnrA*) can cause chronic respiratory disease (e.g., wheeze) (Alvi et al., 2018). In terms of combined effects, the joint action of *vanA* and *tetX* showed the highest correlation with respiratory diseases. However, no significant contribution of *intI1* to the prevalence of respiratory diseases was found as either marginal or combined effect, suggesting that the prevalence of respiratory diseases was likely unlinked to the horizontal transfer of ARGs.

## 5. Conclusions

Our study explored the composition and distribution of airborne ARGs and MGE that spread into indoor environments. We found that 1) the effect of urbanization on ARGs was seasonally dependent and mainly affected by land use types, plant communities and animal husbandry. Particularly, a lesser proportion of green–blue infrastructure around neighborhoods in urban areas could lead to an increase in major ARGs such as *qnrA* and *mecA*. Interestingly, the abundance of *blaTEM* significantly and negatively correlated with the grass diversity in the gardens. 2) Seasonal variations affect the total abundance of ARGs and MGE. Higher temperatures contribute to a greater correlation between airborne bacteria and ARGs, thus increasing the total abundance of ARGs. 3) Some pathogenic bacterial genera are potential hosts of ARGs (e.g., *Stenotrophomonas* and *Campylobacter* positively correlated with *vanA* in the warm season). 4) ARGs can directly correlated with the prevalence of respiratory diseases. The abundance of *VanA* was significantly positively associated with the prevalence of rhinitis and rhinoconjunctivitis, whereas *qnrA* was significantly positively associated with wheeze. In summary, our research offers a novel approach for monitoring the transfer of ARGs and their potential hosts into indoor spaces, while also providing new measure for urban respiratory disease prevention.

## CRediT authorship contribution statement

**Chang Zhao:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xinxin Liu:** Resources, Methodology. **Haoxin Tan:** Investigation, Data curation. **Yucheng Bian:** Investigation, Data curation. **Muhammad Khalid:** Software, Investigation, Formal analysis. **Aki Sinkkonen:** Writing – review & editing, Conceptualization. **Ari Jumpponen:** Writing – review & editing, Conceptualization. **Saeed ur Rahman:** Software, Investigation, Formal analysis. **Baoming Du:** Investigation, Data curation. **Nan Hui:** Writing – review & editing, Supervision, Software, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

## 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.

## Data availability

I have shared the link to my data at the manuscript

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.108545.

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