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Vertical distribution of antibiotic resistance genes in an urban green facade

Shu-Yi-Dan Zhou^{a,b}, Qi Zhang^c, Roy Neilson^d, Madeline Giles^d, Hu Li^{a,b}, Xiao-Ru Yang^{a,b}, Jian-Qiang Su^{a,b}, Yong-Guan Zhu^{a,b,*}

^a Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen 361021, China

^b University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China

^c College of Environment, Zhejiang University of Technology, Hangzhou 310032, China

^d Ecological Sciences, The James Hutton Institute, Dundee, DD2 5DA, Scotland, UK

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ABSTRACT

The phyllosphere is considered a key site for the transfer of both naturally and anthropogenically selected antimicrobial resistance genes (ARGs) to humans. Consequently, the development of green building systems may pose an, as yet, unexplored pathway for ARGs and pathogens to transfer from the environment to outdoor plants. We collected leaves from plants climbing up buildings at 1, 2, 4 and 15 m above ground level and collected associated dust samples from adjacent windowsills to determine the diversity and relative abundance of microbiota and ARGs. Overall, a total of 143 ARGs from 11 major classes and 18 mobile genetic elements (MGEs) were detected. The relative abundance of ARGs within the phyllosphere decreased with increasing height above ground level. Fast expectation–maximization microbial source tracking (FEAST) suggested that the contribution of soil and aerosols to the phyllosphere microbiome was limited. A culture-dependent method to isolate bacteria from plant tissues identified a total of 91 genera from root, stem, and leaf samples as well as endophytes isolated from leaves. Of those bacteria, 20 isolates representing 9 genera were known human pathogenic members to humans. Shared bacterial from culture-dependent and culture-independent methods suggest microorganisms may move from soil to plant, potentially through an endophytic mechanism and thus, there is a clear potential for movement of ARGs and human pathogens from the outdoor environment.

1. Introduction

A diversity of microorganisms including bacteria, fungi, archaea and nematodes inhabit the above-ground portion of plants, known as the phyllosphere (Buonicontrò et al., 2018; Jariwala et al., 2017; Lindow and Brandl, 2003). Once seedling emergence begins, microorganisms colonize the phyllosphere from a variety of environments (Lemanceau et al., 2017) including bioaerosols originating from soil, irrigation water, plants and animals feces (Bulgarelli et al., 2013). Rainfall and air circulation can facilitate the movement of microorganisms to the phyllosphere (Allen et al., 2010; Zhou et al., 2021). Furthermore, internal transmission of microorganisms within plants (endophytes) through the vascular system can also shape the structure of phyllosphere microbiota (Whipps et al., 2008; Wulff et al., 2003).

There is emerging evidence that the phyllosphere antibiotic resistance can affect human health not only through the consumption of plant based agricultural produce (Martí et al., 2013), but also via public urban

green infrastructures (Yan et al., 2020). The phyllosphere can harbor antibiotic resistant bacteria (ARB) which can spread antimicrobial resistance genes (ARGs) through horizontal gene transfer via mobile genetic elements (MGE) (Dharmartha et al., 2019). There is a juxtaposition regarding urban green infrastructures (e.g. public parks, green facades and green roofs), between the benefits such as mitigating city heat island effects (Bowler et al., 2010; Ottele et al., 2011), facilitating human wellbeing (Gascon et al., 2015; Tzoulas et al., 2007) and increasing urban biodiversity (Bowler et al., 2010; Selway et al., 2020), and potential disbenefits such as being potential carriers of ARGs (via phyllosphere) that negatively impact human health (Yan et al., 2019). Furthermore, bioaerosols containing airborne ARGs occur not only in typical pollution sites like waste water treatment plants (Gaviria-Figueroa et al., 2019), but also in central urban areas (Yang et al., 2018). Compared to soil and waterborne based ARGs, airborne ARGs can more directly affect human health through inhalation (Xie et al., 2018) and direct interaction with the human skin microbiome (Solberg, 2000). It is,

* Corresponding author at: Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen 361021, China.

E-mail address: ygzhu@iue.ac.cn (Y.-G. Zhu).

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therefore, important to investigate the potential transfer of ARGs from green facades, used for building cooling and decoration (Koehler, 2008), to residential space.

Green facades are an important component of urban green space (Koehler, 2008), and typically comprise of climbing plants growing in soil at the base of a building or from preinstalled pots suspended on walls (Perez et al., 2011). To date, research on green facades has focused on energy savings, reducing carbon emissions and evaporative cooling (Besir and Cuce, 2018). However, to our knowledge no study has investigated their role in potential dissemination of ARGs and microbes despite their proximity to human residential space. ARGs from both soil and the atmosphere can impact the bacterial resistome found on the phyllosphere (Bringel and Couee, 2015; Chen et al., 2017) and as such climbing plants may impact the indoor environment and consequently human health. Previous studies have found that the soil–plant system could account for the transmission of ARGs between soil and vegetable products via microorganisms (Zhang et al., 2019; Zhu et al., 2016). Thus, the potential exists for vertical distribution of ARGs and phyllosphere microbiota via climbing plants.

Direct comparison of transmission mechanisms between vegetable and climbing plants are not possible because the profiles of ARGs in climbing plants have the potential to change over a greater range of heights. Aerosol-associated bacteria that originate from soil, soil fauna, nutrient application and human activities can affect phyllosphere microbiology (Bulgarelli et al., 2013; Jumpponen and Jones, 2010) but in climbing plants these effects will also interact with the physical distance from the soil in determining phyllosphere communities. Therefore, we hypothesize that the abundance of ARGs associated with green facades may decrease as vertical height increases. Furthermore, endophytic microorganisms, transported within plants, are likely to play a role in phyllosphere community formation (Bodenhausen et al., 2013), providing an interconnection between the soil, atmosphere and phyllosphere.

This study therefore aims to, (1) explore the vertical distribution of ARGs in the phyllosphere of climbing plants used as green facades, in terms of both composition and abundance; (2) assess the source of microbes to the phyllosphere at different heights above ground; and (3) understand the association between microbiota and ARG resistome compositions and how this varies in relation to height above ground.

2. Material and methods

2.1. Sample collection

Leaves from the climbing plant (*Parthenocissus tricuspidata*), a common plant species associated with public facilities and buildings, were collected from each of three neighboring buildings located in Xiamen city, Fujian, China (24° 36' N, 118° 03' E). The climbing plants, planted in soil, grew from the bottom of each building. A total of 4 heights (1, 2, 4 and 15 m above ground) were sampled. At each height, 4 leaves and associated windowsill dust samples were taken, with each sample analyzed separately. Windowsill samples were collected at a distance of approximately 20 cm from the phyllosphere samples. Collected leaves were chosen randomly and the stem cut with a tree trimmer. Dust samples from windowsills were taken as being representative of deposited aerosols and animal feces and samples were collected by wiping the windowsill with 4 sterile cotton swabs per sample which were then stored in a sterile 2.0 ml centrifuge tube until processing. To minimize sampling aerosols produced within buildings, windowsills which were associated with frequently closed windows were chosen for sampling. Rhizosphere soil and bulk soil samples were collected from the base of the studied plants. Bulk soil was collected from the top 10 cm of the soil profile, 5 m distant from buildings. To reduce the impact of rainfall on samples and samples sites, at least 7 days without any rainfall was required before sample collection. The physicochemical attributes of bulk and rhizosphere soil are listed in Table S1.

2.2. DNA extraction from phyllosphere, windowsill, rhizosphere soil and soil samples

DNA extraction from *P. tricuspidata* samples followed that described previously (Zhu et al., 2016) but with a few modifications as follows: 10 g fresh leaf samples were weighed into 250 ml conical flasks and 100 ml of 0.01 M sterile phosphate-buffered saline used to wash microbial communities from the leaf surface. Leaf samples were then sonicated for 10 mins and shaken for 1 h at 180 rpm and 30 °C. The saline solution was filtered through a 0.22 µm cellulose membrane capturing the phyllosphere microbiota. DNA was extracted from both the membranes and cotton swabs used to collect the windowsill dust samples. Membranes and swabs were cut into pieces by using sterile scissors. DNA was extracted using a Fast DNA Spin Kit for Soil (MP Biomedicals, CA) in accordance with the manufacturer's instructions. Microbial DNA was extracted from both bulk and rhizosphere soil (0.5g) samples also using a Fast DNA Spin Kit for Soil. Quality of extracted DNA was assessed using a spectrophotometer (NanoDrop ND-1000, Thermo Scientific, Waltham, MA) and DNA samples were stored at –20 °C prior to analysis.

2.3. High-throughput quantitative PCR for ARGs analysis

Analysis of ARGs using high-throughput quantitative PCR (HT-qPCR) followed methods described previously (Zhou et al., 2019) but with an additional new primer set (Table S2) (Yang et al., 2020). Three technical replicates were applied to each primer set and the threshold cycle (CT) was set to 31 and defined as the upper detection limit for a successful amplification (Chen et al., 2017). Only if all three technical replicates were positive, was the amplification considered successful. The formula for calculating the relative copy numbers of each ARG was as follows: relative copy number = $10^{(31-CT)/(10/3)}$, where CT represents the threshold cycle (Ouyang et al., 2015).

2.4. Microbial community composition

The V4-V5 hypervariable region of the bacterial and archaea 16S rRNA gene, was amplified using the primer set: 515F: GTGCCAGCMGCCGCGG and 907R: CCGTCAATCMTTTRAGTTT (Turner et al., 1999). The ITS region of fungi was amplified using ITS1F: CTTGGTCATTTAGAGGAAGTAA and ITS2R: GTCGCTTCTTCATC-GATGC (Prieto-Recio et al., 2012). PCR was conducted by Majorbio (Shanghai, China), with the following reagents; for 16S rRNA: 1 × 20 µl PCR reaction contained 4 µl 5 × FastPfu Buffer, 2 µl 2.5 mM dNTPs, 0.8 µl Forward Primer (5 µM), 0.8 µl Reverse Primer (5 µM), 0.4 µl FastPfu Polymerase, 0.2 µl BSA, 10 ng Template DNA and ddH₂O to make a final volume of 20 µl. For ITS; amplification: 1 × 20 µl PCR reaction contained 2 µl 10 × Buffer, 2 µl 2.5 mM dNTPs, 0.8 µl Forward Primer (5 µM), 0.8 µl Reverse Primer (5 µM), 0.2 µl rTaq Polymerase, 0.2 µl BSA, 10 ng Template DNA and ddH₂O to make a final volume of 20 µl. PCR conditions were: 3 mins at 95 °C, followed by 27 cycles (for 16S rRNA) or 37 cycles (for ITS) of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s. Thereafter, 10 mins at 72 °C and then 10 °C until halted by the user. All samples were run on an Illumina MiSeq PE300 sequencing platform (V3, Illumina, San Diego USA) by Majorbio (Shanghai, China).

Quantitative Insights Into Microbial Ecology (QIIME) (version 1.9.1) was used for, quality control of sequences (removing low quality sequences and chimeras), assigning taxonomy and identifying operational taxonomic units (OTUs). Single OTU sequences were discarded. Similarity for OTUs was set at 97% and determined by Usearch (version 7.0) (Edgar, 2010). OTUs were clustered using Uparse (version 7.0.1) (Edgar, 2013). The Greengenes data base (version 13.5), was used for assigning the taxonomic identity using a Ribosomal Database Project classifier. Fungal ITS sequences were compared with the UNITE database (version 8.1, <http://unite.ut.ee/>) (Nilsson et al., 2019). Analysis of alpha diversity was performed using Mothur (version 1.30.2) (Schloss et al., 2009). A constructed data base identified known human pathogenic

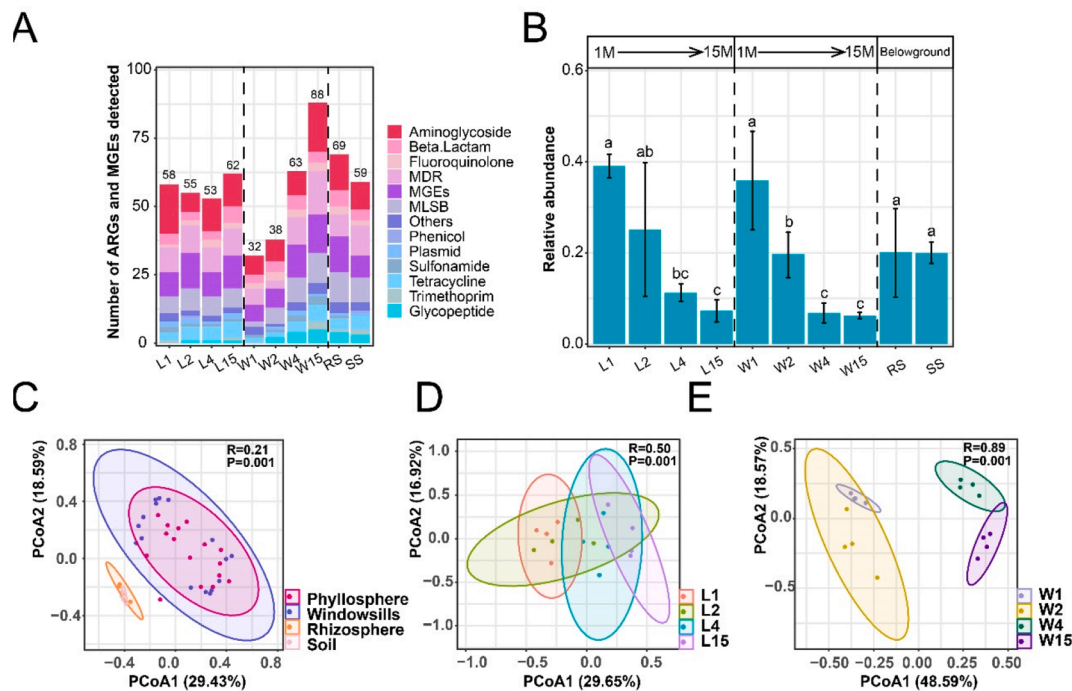


Fig. 1. (A) Detected number of ARGs and MGEs in phyllosphere (L), windowsill dust (W), rhizosphere soil (RS) and bulk soil samples (SS). L1, L2, L4 and L15 represent phyllosphere samples at 1, 2, 4 and 15 m. W1, W2, W4 and W15 represent windowsill dust samples at 1, 2, 4 and 15 m. ARGs were classified into 11 types (Aminoglycoside, Beta lactam, Fluoroquinolone, MDR, MLSB, Phenicol, Sulfonamide, Tetracycline, Trimethoprim, Glycopeptide and others.) (B) The relative abundance of ARGs and MGEs in all samples at different sampled heights. Different letters indicate significant differences at $P < 0.05$ level (ANOVA). Principal Coordinate Analysis (PCoA), based on Bray-Curtis dissimilarity metrics, was conducted to evaluate the resistome pattern in phyllosphere, windowsill dust, rhizosphere soil, bulk soil (C), at each height of phyllosphere (D) and windowsill dust samples (E) respectively.

bacteria (Chen et al., 2016). DNA sequences generated in this study are accessible in the NCBI Sequence Read Archive (SRA) under accession numbers PRJNA601894 and PRJNA601285 for bacteria and fungi, respectively

2.5. Culture dependent method

In parallel to the culture independent process for 16S rRNA amplicon sequencing, a subset of phyllosphere samples were assessed using culture dependent methods following the protocol reported previously (Zhang et al., 2019). A total of 48 randomly selected root ($n = 16$), stem ($n = 16$) and leaf ($n = 16$) samples from climbing plants were collected. Disinfection of root, stem and leaf surfaces and verification of disinfection followed (Schulz et al., 1993). Tissue samples were initially dipped in ethanol (75% v/v) for 1 min, then dipped in sodium hypochlorite solution (2% w/v available chlorine) for 2 min, followed by a final 30 s wash in ethanol. Bacteria from root, stem, and leaf endophytes and leaf surface samples were cultured in a 96-well microtiter plate using 1:10 (V/V) tryptic soy broth and incubated for 21 days at room temperature. Bacteria from each sample were isolated and DNA extracted using the Fast DNA Spin Kit as above. Extracted DNA was sequenced (Illumina HiSeq) using the bacterial 16S rRNA, region V4-V5, as previously described.

2.6. Statistical analyses

Microsoft EXCEL 2016 was used to calculate the sum, standard errors and means of the ARG HT-qPCR data. ANOVA using ARG data was conducted in SPSS. False discovery rate (FDR) adjustment was conducted in "R" with the package "fdrtool" (version 1.2.15) (Strimmer, 2008). Principal Coordinate Analysis (PCoA), Procrustes and Mantel tests based on the Bray-Curtis distances were conducted in the R statistical environment (version 3.6.1) using the R package "vegan" (Dixon,

2003; Oksanen et al., 2019) and considered significant at $P < 0.05$, while plotting was done using the "ggplot2" package (Wickham, 2010). LDA Effect Size (LEfSe) was conducted in the online tools "OmicStudio" (<http://www.omicstudio.cn/tool/>) (Segata et al., 2011). Venn diagrams were created online (<https://bioinfogp.cnb.csic.es/tools/venny/>). Fast expectation-maximization microbial source tracking (FEAST) was used for identifying the source of ARGs and bacterial communities and was conducted with the R package "FEAST" (Shenhav et al., 2019) and visualized as a pie chart. Bar charts and pie charts were drawn using the "ggplot2" package. Network analysis was calculated in "R" with package "Hmisc" (Yadav and Roychoudhury, 2018) and visualized in Gephi (0.9.2).

Structural equation models (SEM) were constructed in SPSS and visualized using AMOS graphic v21 (IBM) to evaluate the casual relationships between height, bacterial diversity, bacterial abundance, fungal diversity and ARGs. An *a priori* conceptual model was developed with the following assumptions: (1) height directly impacts the ARGs in phyllosphere; (2) both bacterial diversity and abundance directly affects the composition of ARGs; (3) height indirectly affects the composition of ARGs through directly impacting bacterial diversity and abundance. Multiple standards for goodness of fit were applied to evaluate the fitness of our model: chi-square (generally $P > 0.05$), high goodness of fit index (GFI, generally $GFI > 0.9$) and the root mean square error of approximation (RMSEA, value generally near 0) (Schermelleh-Engel et al., 2003).

3. Results

3.1. Profiles of antibiotic resistance genes with height

Overall, a total of 143 ARGs and 18 MGEs were detected from samples. ARGs detected from leaves, windowsill, rhizosphere soil and bulk soil samples were classified into 11 categories (Aminoglycoside,

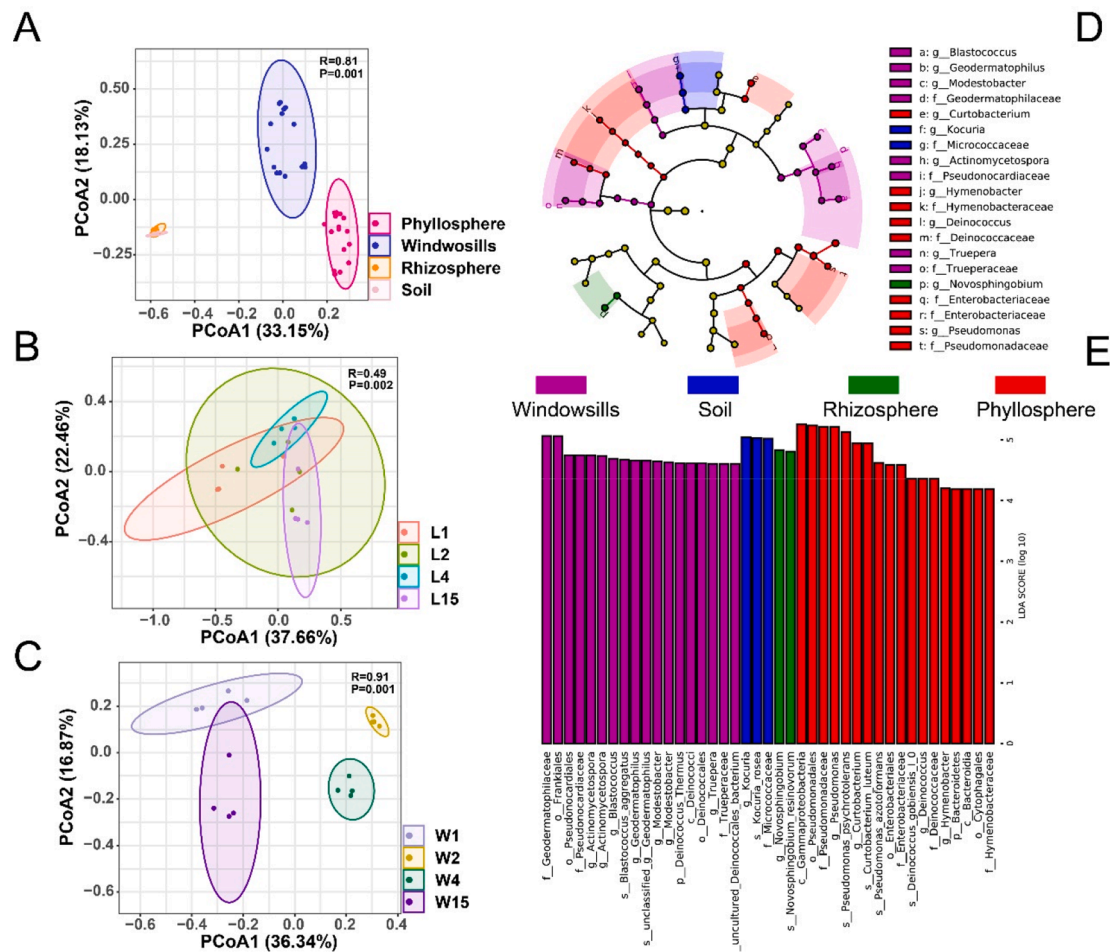


Fig. 2. Principal Coordinate Analysis (PCoA), based on Bray-Curtis dissimilarity metrics, was conducted to evaluate the bacterial communities in phyllosphere, windowsill dust, rhizosphere soil, bulk soil (C), at each height of phyllosphere (D) and windowsill dust samples (E) respectively. L1, L2, L4 and L15 represent phyllosphere samples at 1, 2, 4 and 15 m. W1, W2, W4 and W15 represent windowsill dust samples at 1, 2, 4 and 15 m. (D) (E) The LefSe analysis highlights the biomarkers in the different sampled habitats ($\alpha > 0.01$, LDA score > 3.0).

Beta Lactam, Fluoroquinolone, Multidrug resistance (MDR), Macrolide-lincosamide-streptogramin B resistance (MLS_B), Phenicol, Sulfonamide, Tetracycline, Trimethoprim, Glycopeptide and others). The average number of ARGs and MGEs detected ranged from 53 to 62 and 32 to 88 from leaves and windowsills, respectively (Fig. 1A). The detected number of ARGs from windowsill dust samples increased with height above ground ($P < 0.05$). An average of 69 and 59 resistome genes were detected in rhizosphere and bulk soil samples respectively (Fig. 1A). The relative abundances of ARGs in both windowsill and phyllosphere samples were greatest at heights sampled closest to ground level (Fig. 1B). The relative abundance of ARGs from 1 m phyllosphere samples was significantly greater ($P = 0.001$) than phyllosphere samples at 4 m and 15 m. For windowsill samples, those at 1 m were significantly ($P = 0.007$) more abundant than all other sampled heights. Samples at 2 m were also more abundant ($P = 0.017$) than those at either 4 m or 15 m. The composition of ARGs from bulk and rhizosphere soil samples clearly separated from all leaf and windowsill samples ($R = 0.21$, $P = 0.001$) (Fig. 1C). The composition of phyllosphere ARGs changed as height increased ($R = 0.50$, $P = 0.001$) (Fig. 1D). The composition of windowsill ARGs at 1 m were similar to those at 2 m, but significantly differed from the ARGs composition at 4 m and 15 m ($R = 0.89$, $P = 0.001$) (Fig. 1E). There were 29 shared, and 20, 30, 6 and 2 unique ARGs and MGEs from leaves, windowsills, rhizosphere soil and soil samples, respectively (Fig. S1).

3.2. Microbial community profiles from each habitat

A total of 2 288 678 high quality bacterial sequences, ranging from 26 083 to 73 940 sequences per sample, were obtained from phyllosphere, soil and windowsill samples. Bulk and rhizosphere soil had the greatest alpha diversity of all the sampled habitats (Shannon diversity index, Fig. S2A). Phyllosphere bacterial diversity was significantly lower than in other habitats ($P < 0.001$). Principal Coordinate Analysis (PCoA) using Bray Curtis distances ($R = 0.47$, $P = 0.001$) showed that the composition of bacterial communities at phylum level from phyllosphere and windowsills separated along PCoA 1, which explained 36.8% of the variation (Fig. S2B). In contrast, soil and rhizosphere samples did not separate along this axis (Fig. S2B).

At genus level, PCoA 1 accounted for 33.1% of the total variation and separated soil, windowsill and phyllosphere samples whereas, windowsill samples separated from both soil and phyllosphere samples along PCoA 2 (18.1% variation) ($R = 0.81$, $P = 0.001$) (Fig. 2A). At genus level, the composition of phyllosphere microbial community changed as height increased ($R = 0.49$, $P = 0.002$) (Fig. 2B). The bacterial composition of windowsill dust samples at 1 m were distinct from those at 2 m and 4 m along PCoA1, which explained 36.3% of the total variation ($R = 0.91$, $P = 0.001$) (Fig. 2C).

LefSe analysis identified bacteria that could be potential biomarkers for each sampled habitat (Fig. 2D and E). For those OTUs with a relative abundance $> 1\%$ samples at the resolution of phylum, class, order, family and genera differed by sample (Fig. 2D and E).

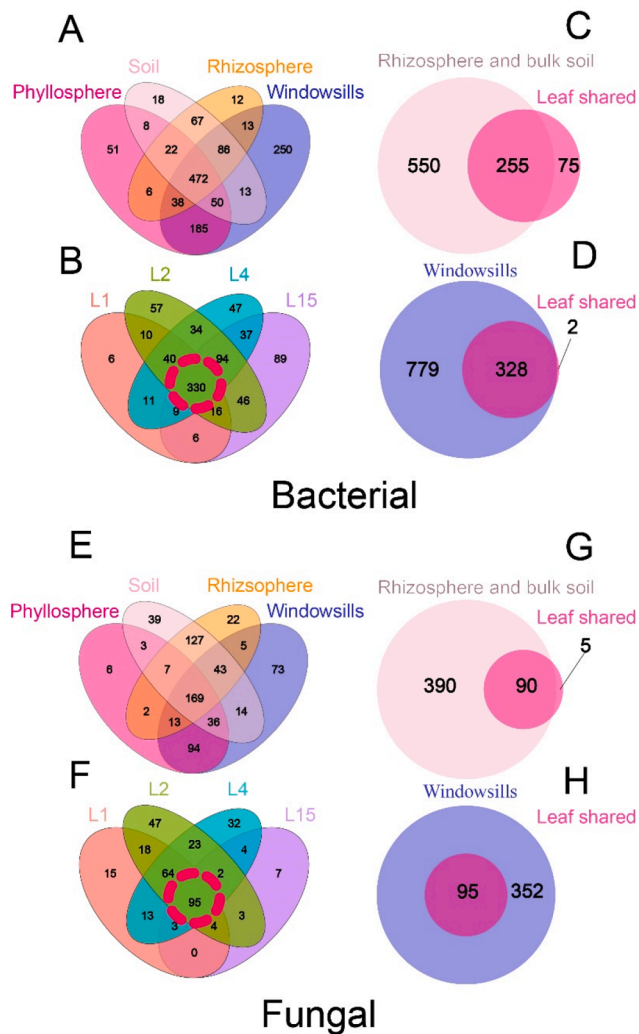


Fig. 3. (A) Venn diagram at bacterial genus level, for the four sampled habitats each represented by a different color. (B) Venn diagram at bacterial genus level, the red circle indicated the overlap of different heights of phyllosphere samples. (C) and (D) Comparing the overlap of different heights of phyllosphere samples with soil (bulk and rhizosphere soil), windowsills samples respectively. (E) Venn diagram at fungal genus level, for the four sampled habitats each represented by a different color. (F) Venn diagram at bacterial genus level, the red circle indicated the overlap of different heights of phyllosphere samples. (G) and (H) Comparing the overlap of different heights of phyllosphere samples with soil (bulk and rhizosphere soil), windowsill dust samples respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

A total of 91 bacterial genera were cultured from root endophyte, stem tissues, leaf endophyte and leaf surface samples (Table S3), 20 of which were identified as known human pathogens (Fig. S3). Four *Bacillus* genera were shared among three habitats. *Burkholderia* sp., *Pseudomonas putida*, *Salmonella enterica* and *Staphylococcus saprophyticus* were only detected on the leaf surface. Both *Klebsiella pneumoniae* and *Enterobacter cloacae* were shared among sampled leaf tissues.

A total of 2 496 136 fungal sequences were obtained, which consisted of phyllosphere (1 024 615), bulk soil (520 000) and windowsill (951 521). Fungal diversity in bulk and rhizosphere soil samples was greater compared to those from other sampled habitats (Fig. S4A; $P < 0.001$). PCoA showed fungal communities to have a similar significant separation between habitats as bacterial communities (Fig. S4B, $R = 0.54$, $P = 0.001$). The most abundant fungal genera present in all samples were: *Cladosporium*, *Toxicocladosporium*, *Phaeophleospora*,

Penicillium, *Naganishia*, *Hortaea*, *Aspergillus*, and *Colletotrichum* (Fig. S4C).

3.3. Identifying phyllosphere microbial sources

There were 18, 12, 51 and 250 unique bacterial genera in bulk and rhizosphere soil, phyllosphere and windowsill samples, respectively with 472 microbial shared genera between all samples (Fig. 3A). Also, there were a total of 330 genera shared among leaf samples sampled at each height (Fig. 3B). 255 of these genera were shared with bulk and rhizosphere soil, whereas 328 genera were shared with windowsill dust samples (Fig. 3C and D).

A total 169 fungal genera were shared among bulk and rhizosphere soil, phyllosphere and windowsill samples (Fig. 3E). 212 fungal genera were detected from leaf samples, however, only 95 genera were shared between all sample heights (Fig. 3F). Ninety of these genera were shared with bulk and rhizosphere soil, whereas 95 were shared with windowsill dust samples (Fig. 3G and H).

OTUs from 16S rRNA gene and ITS region sequencing were used for source tracking. FEAST evaluated the potential origin of bacterial and fungal communities at each sampled height (Fig. 4). At 1 m, 33.2%, 22.3% and 6.3% of the bacterial communities from phyllosphere samples originated from the phyllosphere at 2, 4 and 15 m, respectively (Fig. 4A). The fungal communities associated with phyllospheres at 1 m consisted of fungi from phyllosphere samples collected at 2 (76.0%), 4 (3.3%) and 15 m (8.3%) (Fig. 4B). Whereas, phyllosphere communities at 1 (20.1%), 4 (27.0%) and 15 m (21.3%) and windowsill dust (11.9%) accounted for the phyllosphere bacterial communities at 2 m (Fig. 4C). Phyllosphere fungal communities at 1, 4 and 15 m (82.1%) and windowsill dust (9.6%) were the main sources of fungi at 2 m (Fig. 4D). At 4 m height, the main sources of bacteria or fungi to the phyllosphere came from the other sampled heights with >70% of the bacterial or fungal communities associated with leaves from other heights (1, 2 and 15 m) (Fig. 4E, F). At 15 m, bacterial communities of the phyllosphere (1–4 m height) dominated (57.3%), whereas the contribution from windowsill dust was limited (3.4%). Similarly, for fungal communities at 15 m, phyllosphere samples at 2 and 4 m were the main (78.2%) sources of the fungal community with an additional contribution (17.7%) from windowsill samples.

3.4. Correlations between microbial communities and ARGs

Structural equation modelling identified the direct and indirect impact of height, bacterial abundance, bacterial diversity and fungal diversity on the composition of the antibiotic resistome. The model explained 68% of the antibiotic abundance. The height of sample sites above ground indirectly impacted the antibiotic resistome by significantly influencing the bacterial abundance ($\lambda = -0.75$, $P < 0.001$). Both bacterial abundance ($\lambda = 0.63$, $P < 0.05$) and diversity ($\lambda = -0.30$, $P < 0.05$), respectively, significantly and directly affected the abundance of ARGs (Fig. 5A). Height above ground, bacterial abundance and bacterial diversity had the greatest effects on the abundance of ARGs (Fig. 5B).

For bacterial communities, both the shared OTUs and ARGs among soil (bulk and rhizosphere soil) and leaves at each height had a significant Mantel correlation ($P < 0.01$). An equivalent correlation was not found among leaves and windowsills. Soil-leaves at all sampled heights and windowsill-leaves at 15 m had strong and significant correlations (Fig. S5).

After FDR adjustment, a network analysis identified a correlation between the bacterial microbiome and resistome, in which 13 genera were significantly correlated with 21 ARGs (Spearman's $\rho > 0.7$, P -value < 0.01) (Fig. 6A). For example, the genus *Curtobacterium* was significantly correlated with *tnp_6*, *erm (35)*, *aadA (16)*, *aac3_Via* and *qacH_351*, which belonged to MGES, MLSB, Aminoglycoside and multi-drug resistance, respectively. The relative abundance of the 13 genera varied with sample type and height above ground (Fig. 6B), and 8 of the

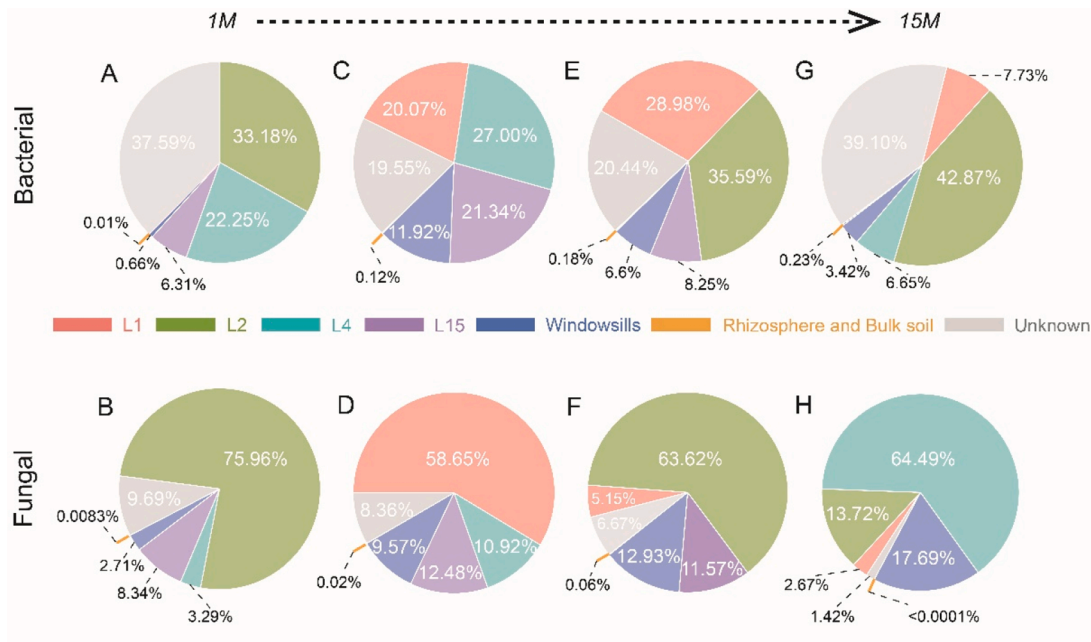


Fig. 4. Fast expectation–maximization microbial source tracking (FEAST). (A) to (H) highlight the bacterial and fungal sources of phyllosphere samples at 1, 2, 4 and 15 m above ground. L1, L2, L4 and L15 represent samples at 1, 2, 4 and 15 m.

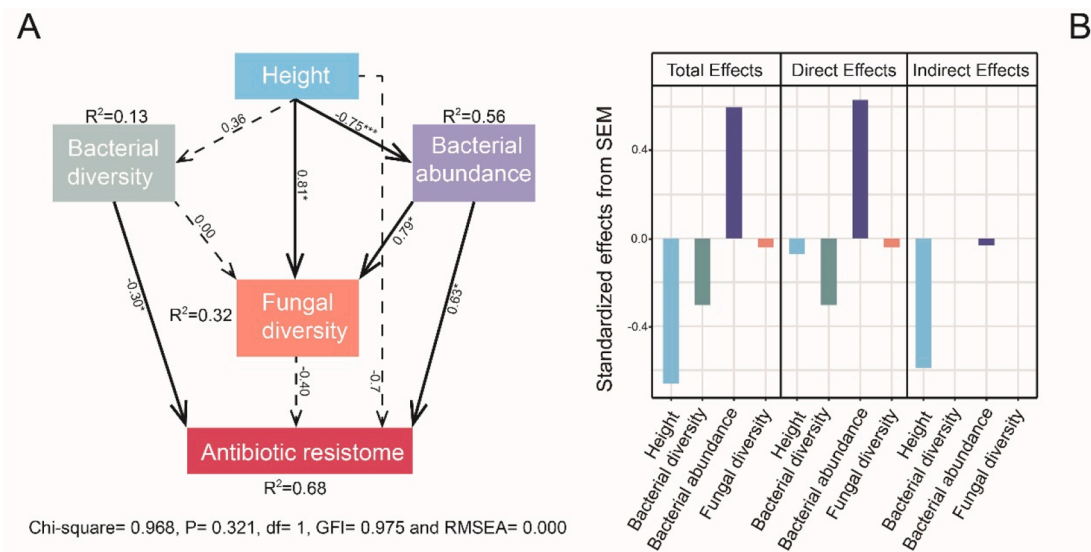


Fig. 5. (A) and (B) Structural equation models (SEM) indicate the direct and indirect impact of height, bacterial abundance, bacterial diversity and fungal diversity on the composition of the antibiotic resistome, respectively. Path coefficients are shown with arrows. The dotted and solid lines indicate the effects on the target (* $P \leq 0.05$, ** ≤ 0.01 , *** $P \leq 0.001$). R^2 indicates the proportion of variance explained. The parameters of the current model were: Chi-square = 0.968, $P = 0.321$, $df = 1$, GFI = 0.975 and RMSEA = 0.000.

genera, *Pseudomonas*, *Curtobacterium*, *Massilia*, *Sphingomonas*, *Methylobacterium*, *fEnterobacteriaceae*, *Microbacterium*, *Paracoccus* were cultured in the current study.

4. Discussion

4.1. Bacterial and fungal communities

In this study, FEAST analysis suggested that both bacterial and fungal communities may originate from phyllospheres located at the different sample heights, which may be a driver of the shared phyllosphere OTUs. Bacterial and fungal communities in both soil and windowsill dust made limited contribution to the phyllosphere microbiome, respectively.

Potentially one mechanism driving the shared bacteria or fungi between the phyllospheres located at different heights, is the transfer of endophytic microorganisms (Beattie and Lindow, 1999; Bodenhausen et al., 2013; Jumpponen and Jones, 2009). For example, the fungal genus *Cladosporium* and the bacterial genera *Pseudomonas* and *Curtobacterium* which dominated the phyllosphere in this study are commonly isolated as endophytes (Strobel et al., 2004; Teasdale et al., 2018; Zinniel et al., 2002). Additionally, *Pseudomonas*, *Curtobacterium*, *Sphingomonas* and *Microbacterium* were isolated from plant tissue samples, suggesting a potential pathway within the plant. Notably, these genera were also found in bulk soil samples though at low relative abundances, suggesting the bulk soil may act as a baseline reservoir for plant microbes. Notwithstanding this potential transportation of endophytes,

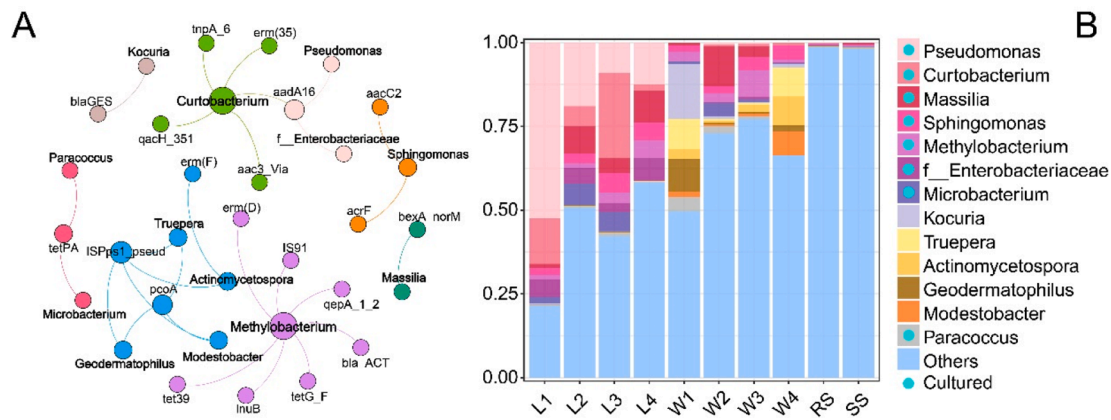


Fig. 6. (A) Network analysis showing the co-occurrence pattern between bacterial genera and ARGs (Spearman's correlation coefficient $R^2 > 0.7$, $P < 0.01$). (B) The relative abundance of the possible host of ARGs. Blue dots denote genera that were also identified from culture-dependent samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Pseudomonas and *Spingomonas* can also exist in aerosol samples, suggesting that air movement (Fahlgren et al., 2010) could also be a contributory mechanism for the spread of these genera.

Windowsill dust samples were considered as representative of airborne and animal fecal microbial communities in this study. Using this assumption, FEAST analysis suggested that airborne bacteria and fungi contributed members to the phyllosphere community which is consistent with previous studies (Courtois, 1990; Vokou et al., 2012). Although the phyllosphere can recruit microbial taxa from the external environment (e.g. air), not all microorganisms transferred to the phyllosphere will survive and form part of the community as selection can cause the disappearance of uncompetitive species (Whipps et al., 2008). The remaining sources were considered as “unknown”, which could be attributed to the inheritance of microbiome as plant microbes can be obtained from seeds, and differentiate into various microbial communities under selection pressure (e.g. nutrient level, water and UV light) (Abdelfattah et al., 2021). Nevertheless, the source tracking results were based on the statistic calculation from 16S rRNA and ITS gene sequence with low taxonomic resolution, which may not be sufficient to fully explain the contribution of sources.

4.2. Phyllosphere resistome profiles

To our knowledge this is the first study that demonstrates that urban green facades are potential reservoirs of ARGs and MGEs and that the profile of ARG communities differs with height above ground. The number of different ARG types remained consistent between phyllospheres at all heights, however the abundance of ARGs decreased with height, potentially driven by the proximity of lower leaves to the soil. The impact of soil-associated aerosols on ARGs may reduce as increasing heights. This concurs with previous studies carried out in agricultural systems where the proximity of leaves to the soil was found to be important for ARG transfer (Zhu et al., 2020). Moreover, as height of the green facade increased, the direct impact of human activities (e.g. irrigation and fertilization) on ARG abundance is likely to have diminished (Zhang et al., 2019). Similar to Chen et al. (2018) and based on SEM analysis, bacterial diversity and abundance had significant effects on the abundance of ARGs in phyllosphere. The bacterial abundance decreased as the height increase, indicating that the effect of soil-associate aerosols on phyllosphere microbiota gradually diminish. And it ultimately resulting in microbial differentiation between lower and higher location then further affect ARGs profile.

4.3. Source of the resistome within the phyllosphere

The association between bacterial communities and ARG profiles

suggests that as bacteria colonize the phyllosphere they could concurrently facilitate the spread of ARGs (Zhu et al., 2017; Chen et al., 2019). While atmospheric deposition will likely play a role in determining shared ARGs between the phyllosphere and windowsills, overlap in ARG types between the phyllosphere and soil suggests that some ARGs may have originated from the soil rather windowsills, and thus suggest an interconnection between the ARG reservoir in soil and the plant phyllosphere. The shared bacterial OTUs among the phyllosphere, rhizosphere soil and bulk soil were significantly correlated ($P < 0.01$, Mantel test) with the shared resistome. This further suggests the potential distribution of microbial organisms harboring ARGs within the soil and leaf system, even at different heights of climbing plants. In addition, animal feces (Lin et al., 2020) may also be a potential source of pathogens and ARGs in the phyllosphere though no further evaluation was conducted. Furthermore, invertebrate movement such as insects has potential to transfer resistome and pathogenic microorganisms from the environment to phyllosphere (Heaton and Jones, 2008; Power, 1990).

While buildings with green facades are believed to improve both wellbeing and indoor air quality (Meadow et al., 2014), we have demonstrated that a green facade may inadvertently introduce a possible pathway for ARGs carried by bioaerosols and soils. The corollary is that many beneficial microbes could also enter the indoor environment through this pathway (Mahnert et al., 2015) though this was not tested in this study. Although we have found known pathogenic members among the isolated bacteria from plant tissue, this does not detract from urban green spaces having a positive impact on human health. This is especially true in a modern society where people tend to spend more time indoors, significantly reducing their exposure to natural microorganisms (Spalt et al., 2016). Such exposure to a diversity of natural microorganism has several beneficial effects on human health, including skin health and demonstrable benefits to the respiratory system (Kim and Kim, 2019; Schuijs et al., 2015). Therefore, further studies should focus on how to better monitor the movement of microbes from green facades to indoor environments, and on how to manipulate healthy microbiomes in a “soil–plant–indoor environment” continuum.

CRediT authorship contribution statement

Shu-Yi-Dan Zhou: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft, Writing - review & editing. **Qi Zhang:** Data curation, Formal analysis, Investigation, Writing - review & editing. **Roy Neilson:** Funding acquisition, Writing - review & editing. **Madeline Giles:** Funding acquisition, Writing - review & editing. **Hu Li:** Methodology, Writing - review & editing. **Xiao-Ru Yang:** Funding acquisition, Resources, Writing - review & editing. **Jian-Qiang Su:** Resources,

Methodology, Writing - review & editing. **Yong-Guan Zhu:** Conceptualization, Funding acquisition, Resources, Project administration, 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 material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2021.106502>.

References

- Abdelfattah, A., Wisniewski, M., Schena, L., Tack, A.J.M., 2021. Experimental evidence of microbial inheritance in plants and transmission routes from seed to phyllosphere and root. *Environ. Microbiol.* 2021.
- Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 8, 251–259.
- Beattie, G.A., Lindow, S.E., 1999. Bacterial colonization of leaves: A spectrum of strategies. *Phytopathology* 89, 353–359.
- Besir, A.B., Cuce, E., 2018. Green roofs and facades: A comprehensive review. *Renew. Sustain. Energy Rev.* 82, 915–939.
- Bodenhausen, N., Horton, M.W., Bergelson, J., 2013. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS ONE* 8, 9.
- Bowler, D.E., Buyung-Ali, L., Knight, T.M., Pullin, A.S., 2010. Urban greening to cool towns and cities: A systematic review of the empirical evidence. *Landscape Urban Plann.* 97, 147–155.
- Bringel, F., Couee, I., 2015. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Front. Microbiol.* 6.
- Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L., Schulze-Lefert, P., 2013. Structure and functions of the bacterial microbiota of plants. In: Merchant S.S. (Ed.), *Annual Review of Plant Biology*, vol. 64.
- Buonincontro, D.S., Roberts, D.M., Goncalves Oliveira, C.M., Blok, V.C., Neilson, R., De Lima Oliveira, R.D.A., 2018. A Rapid Diagnostic for Detection of Aphelenchoides besseyi and A. fujianensis Based on Real-Time PCR. *Plant Disease* 102, 519–526.
- Chen, Q.-L., Cui, H.-L., Su, J.-Q., Penuelas, J., Zhu, Y.-G., 2019. Antibiotic resistomes in plant microbiomes. *Trends Plant Sci.* 24, 530–541.
- Chen, Q., An, X., Li, H., Su, J., Ma, Y., Zhu, Y.-G., 2016. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. *Environ. Int.* 92–93, 1–10.
- Chen, Q.L., An, X.L., Zhu, Y.G., Su, J.Q., Gillings, M.R., Ye, Z.L., Cui, L., 2017. Application of struvite alters the antibiotic resistome in soil, rhizosphere, and phyllosphere. *Environ. Sci. Technol.* 51, 8149–8157.
- Chen, Q.L., Fan, X.T., Zhu, D., An, X.L., Su, J.Q., Cui, L., 2018. Effect of biochar amendment on the alleviation of antibiotic resistance in soil and phyllosphere of *Brassica chinensis* L. *Soil Biol. Biochem.* 119, 74–82.
- Courtois, H., 1990. The mycoflora in the phyllosphere and in the rhizosphere of spruces (*Picea-abies* karst) and its significance to interpret forest decline symptoms. *Angewandte Botanik* 64, 381–392.
- Dharmarha, V., Guron, G., Boyer, R.R., Niemira, B.A., Pruden, A., Strawn, L.K., Ponder, M.A., 2019. Gamma irradiation influences the survival and regrowth of antibiotic-resistant bacteria and antibiotic-resistance genes on romaine lettuce. *Front. Microbiol.* 10, 16.
- Dixon, P., 2003. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* 14, 927–930.
- Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461.
- Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996–998.
- Fahlgren, C., Hagstrom, A., Nilsson, D., Zweifel, U.L., 2010. Annual variations in the diversity, viability, and origin of airborne bacteria. *Appl. Environ. Microbiol.* 76, 3015–3025.
- Gascon, M., Triguero-Mas, M., Martinez, D., Davdand, P., Forns, J., Plasencia, A., Nieuwenhuijsen, M.J., 2015. Mental health benefits of long-term exposure to residential green and blue spaces: A systematic review. *Int. J. Environ. Res. Public Health* 12, 4354–4379.
- Gaviria-Figueroa, A., Preisner, E.C., Hoque, S., Feigley, C.E., Norman, R.S., 2019. Emission and dispersal of antibiotic resistance genes through bioaerosols generated during the treatment of municipal sewage. *Sci. Total Environ.* 686, 402–412.
- Heaton, J.C., Jones, K., 2008. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. *J. Appl. Microbiol.* 104, 613–626.
- Jariwala, S., Redding, L., Hewitt, D., 2017. The severely under-recognized public health risk of strongyloidiasis in North American cities-A One Health approach. *Zoonoses Public Health* 64, 579–588.
- Jumpponen, A., Jones, K.L., 2009. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytol.* 184, 438–448.
- Jumpponen, A., Jones, K.L., 2010. Seasonally dynamic fungal communities in the *Quercus macrocarpa* phyllosphere differ between urban and non-urban environments. *New Phytol.* 186, 496–513.
- Kim, J.E., Kim, H.S., 2019. Microbiome of the skin and gut in atopic dermatitis (AD): Understanding the pathophysiology and finding novel management strategies. *J. Clinical Med.* 8.
- Koehler, M., 2008. Green facades-a view back and some visions. *Urban Ecosyst.* 11, 423–436.
- Lemanceau, P., Barret, M., Mazurier, S., Mondy, S., Pivato, B., Fort, T., Vacher, C., 2017. Plant communication with associated microbiota in the spermosphere, rhizosphere and phyllosphere. In: Becard, G. (Ed.), *How Plants Communicate with Their Biotic Environment*. Academic Press Ltd-Elsevier Science Ltd, London.
- Lin, Y., Dong, X., Sun, R., Wu, J., Tian, L., Rao, D., Zhang, L., Yang, K., 2020. Migratory birds-one major source of environmental antibiotic resistance around Qinghai Lake, China. *Sci. Total Environ.* 739.
- Lindow, S.E., Brandl, M.T., 2003. Microbiology of the phyllosphere. *Appl. Environ. Microbiol.* 69, 1875–1883.
- Mahnert, A., Moissl-Eichinger, C., Berg, G., 2015. Microbiome interplay: plants alter microbial abundance and diversity within the built environment. *Front. Microbiol.* 6, 11.
- Marti, R., Scott, A., Tien, Y.C., Murray, R., Sabourin, L., Zhang, Y., Topp, E., 2013. Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. *Appl. Environ. Microbiol.* 79, 5701–5709.
- Meadow, J.F., Altrichter, A.E., Kembel, S.W., Kline, J., Mhuireach, G., Moriyama, M., Northcutt, D., O'Connor, T.K., Womack, A.M., Brown, G.Z., Green, J.L., Bohannon, B.J.M., 2014. Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air* 24, 41–48.
- Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glockner, F.O., Tedersoo, L., Saar, I., Koljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* 47, D259–D264.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. *vegan: Community ecology package*, 2.5-6 ed, pp. Ordination methods, diversity analysis and other functions for community and vegetation ecologists.
- Ottele, M., Perini, K., Fraaij, A.L.A., Haas, E.M., Raiteri, R., 2011. Comparative life cycle analysis for green facades and living wall systems. *Energy Build* 43, 3419–3429.
- Ouyang, W.Y., Huang, F.Y., Zhao, Y., Li, H., Su, J.Q., 2015. Increased levels of antibiotic resistance in urban stream of Jiulongjiang River, China. *Appl. Microbiol. Biotechnol.* 99, 5697–5707.
- Perez, G., Rincon, L., Vila, A., Gonzalez, J.M., Cabeza, L.F., 2011. Green vertical systems for buildings as passive systems for energy savings. *Appl. Energy* 88, 4854–4859.
- Power, A.G., 1990. Cropping systems, insect movement, and the spread of insect-transmitted diseases in crops. *Ecol. Studies* 78, 47–69.
- Prieto-Recio, C., Romeralo, C., Bezos, D., Martin-Garcia, J., Martinez-Alvarez, P., Botella, L., Diez, J.J., 2012. First report of *Heterobasidion annosum* on *Pinus pinaster* in Spain. *Plant Dis* 96, 770–770.
- Schermelleh-Engel, K., Moosbrugger, H., Müller, H., 2003. Evaluating the fit of structural equation models: Tests of significance and descriptive goodness-of-fit measures. *MPR-online* 8, 23–74.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541.
- Schuijs, M.J., Willart, M.A., Vergote, K., Gras, D., Deswarte, K., Ege, M.J., Madeira, F.B., Beyaert, R., van Loo, G., Bracher, F., von Mutius, E., Chanez, P., Lambrecht, B.N., Hammad, H., 2015. ALLERGY Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. *Science* 349, 1106–1110.
- Schulz, B., Wanke, U., Draeger, S., Aust, H.J., 1993. Endophytes from herbaceous plants and shrubs - effectiveness of surface sterilization methods. *Mycol. Res.* 97, 1447–1450.
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. *Genome Biol.* 12, 18.
- Selway, C.A., Mills, J.G., Weinstein, P., Skelly, C., Yadav, S., Lowe, A., Breed, M.F., Weyrich, L.S., 2020. Transfer of environmental microbes to the skin and respiratory tract of humans after urban green space exposure. *Environ. Int.* 145, 106084–106084.

- Shenhav, L., Thompson, M., Joseph, T.A., Briscoe, L., Furman, O., Bogumil, D., Mizrahi, I., Pe'er, I., Halperin, E., 2019. FEAST: fast expectation-maximization for microbial source tracking. *Nat. Methods* 16, 627–632.
- Solberg, C.O., 2000. Spread of *Staphylococcus aureus* in hospitals: Causes and prevention. *Scandinavian J. Infect. Dis.* 32, 587–595.
- Spalt, E.W., Curl, C.L., Allen, R.W., Cohen, M., Adar, S.D., Stukovsky, K.H., Avol, E., Castro-Diehl, C., Nunn, C., Mancera-Cuevas, K., Kaufman, J.D., 2016. Time-location patterns of a diverse population of older adults: the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). *J. Exposure Sci. Environ. Epidemiol.* 26, 349–355.
- Strimmer, K., 2008. fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. *Bioinformatics* 24, 1461–1462.
- Strobel, G., Daisy, B., Castillo, U., Harper, J., 2004. Natural products from endophytic microorganisms. *J. Natural Prod.* 67, 257–268.
- Teasdale, S.E., Caradus, J.R., Johnson, L.J., 2018. Fungal endophyte diversity from tropical forage grass *Brachiaria*. *Plant Ecol. Divers.* 11, 611–624.
- Turner, S., Pryer, K.M., Miao, V.P.W., Palmer, J.D., 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J. Eukaryot. Microbiol.* 46, 327–338.
- Tzoulas, K., Korpela, K., Venn, S., Yli-Pelkonen, V., Kazmierczak, A., Niemela, J., James, P., 2007. Promoting ecosystem and human health in urban areas using Green Infrastructure: A literature review. *Landscape Urban Plann.* 81, 167–178.
- Vokou, D., Vareli, K., Zarali, E., Karamanoli, K., Constantinidou, H.I.A., Monokrousos, N., Halley, J.M., Sainis, I., 2012. Exploring biodiversity in the bacterial community of the Mediterranean phyllosphere and its relationship with airborne bacteria. *Microb. Ecol.* 64, 714–724.
- Whipps, J.M., Hand, P., Pink, D., Bending, G.D., 2008. Phyllosphere microbiology with special reference to diversity and plant genotype. *J. Appl. Microbiol.* 105, 1744–1755.
- Wickham, H., 2010. A Layered grammar of graphics. *J. Comput. Graphical Stat.* 19, 3–28.
- Wulff, E.G., van Vuurde, J.W.L., Hockenhull, J., 2003. The ability of the biological control agent *Bacillus subtilis*, strain BB, to colonize vegetable brassicas endophytically following seed inoculation. *Plant Soil* 255, 463–474.
- Xie, J.W., Jin, L., Luo, X.S., Zhao, Z., Li, X.D., 2018. Seasonal disparities in airborne bacteria and associated antibiotic resistance genes in PM2.5 between urban and rural sites. *Environ. Sci. Technol. Lett.* 5, 74–79.
- Yadav, M.L., Roychoudhury, B., 2018. Handling missing values: A study of popular imputation packages in R. *Knowl.-Based Syst.* 160, 104–118.
- Yan, Z.-Z., Chen, Q.-L., Zhang, Y.-J., He, J.-Z., Hu, H.-W., 2019. Antibiotic resistance in urban green spaces mirrors the pattern of industrial distribution. *Environ. Int.* 132, 105106.
- Yan, Z.Z., Chen, Q.L., Zhang, Y.J., He, J.Z., Hu, H.W., 2020. Industrial development as a key factor explaining variances in soil and grass phyllosphere microbiomes in urban green spaces. *Environ. Pollut.* 261, 10.
- Yang, K., Chen, Q.-L., Chen, M.-L., Li, H.-Z., Liao, H., Pu, Q., Zhu, Y.-G., Cui, L., 2020. Temporal dynamics of antibiotic resistome in the plastsphere during microbial colonization. *Environ. Sci. Technol.* 54, 11322–11332.
- Yang, Y., Zhou, R.J., Chen, B.W., Zhang, T., Hu, L.G., Zou, S.C., 2018. Characterization of airborne antibiotic resistance genes from typical bioaerosol emission sources in the urban environment using metagenomic approach. *Chemosphere* 213, 463–471.
- Zhang, J.Y., Liu, Y.X., Zhang, N., Hu, B., Jin, T., Xu, H.R., Qin, Y., Yan, P.X., Zhang, X.N., Guo, X.X., Hui, J., Cao, S.Y., Wang, X., Wang, C., Wang, H., Qu, B.Y., Fan, G.Y., Yuan, L.X., Garrido-Oter, R., Chu, C.C., Bai, Y., 2019a. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nat. Biotechnol.* 37, 676–684.
- Zhang, Y.-J., Hu, H.-W., Chen, Q.-L., Singh, B.K., Yan, H., Chen, D., He, J.-Z., 2019b. Transfer of antibiotic resistance from manure-amended soils to vegetable microbiomes. *Environ. Int.* 130, 104912.
- Zhou, S.-Y.-D., Zhu, D., Giles, M., Yang, X.-R., Daniell, T., Neilson, R., Zhu, Y.-G., 2019. Phyllosphere of staple crops under pig manure fertilization, a reservoir of antibiotic resistance genes. *Environ. Pollut.* 252, 227–235.
- Zhou, S.-Y.-D., Li, H., Giles, M., Neilson, R., Yang, X.-R., Su, J.-Q., 2021. Microbial flow within an air-phyllosphere-soil continuum. *Front. Microbiol.* 11.
- Zhu, B., Chen, Q., Chen, S., Zhu, Y.G., 2016. Does organically produced lettuce harbor higher abundance of antibiotic resistance genes than conventionally produced? *Environ. Int.* 98, 152–159.
- Zhu, D., Ding, J., Yin, Y., Ke, X., O'Connor, P., Zhu, Y.G., 2020. Effects of earthworms on the microbiomes and antibiotic resistomes of detritus fauna and phyllospheres. *Environ. Sci. Technol.* 54, 6000–6008.
- Zhu, Y.G., Gillings, M., Simonet, P., Stekel, D., Banwart, S., Penuelas, J., 2017. Microbial mass movements. *Science* 357, 1099–1100.
- Zinniel, D.K., Lambrecht, P., Harris, N.B., Feng, Z., Kuczumarski, D., Higley, P., Ishimaru, C.A., Arunakumari, A., Barletta, R.G., Vidaver, A.K., 2002. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl. Environ. Microbiol.* 68, 2198–2208.