Seasonal Disparities in Airborne Bacteria and Associated Antibiotic Resistance Genes in PM2.5 between Urban and Rural Sites

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ABSTRACT

The atmosphere represents an unappreciated compartment for the environmental dissemination of antibiotic resistance genes (ARGs), particularly via airborne *fi*ne particles ($PM_{2.5}$), with strong implications for the inhalational exposure of the general population. We examined the seasonal variations in airborne bacteria and several ARGs in $PM_{2.5}$ across an industrial− urban−rural transect in a megacity of China over an annual cycle. Seasonality was most apparent at the rural site with a remarkable wintertime reduction in the total level of bacteria and an enrichment of certain ARGs in winter but dilution in spring. This contrasted with the relative consistency across seasons at urban and industrial sites. The statistical correlation between ARGs and the mobile genetic element (MGE), *int*I1, weakened from rural to urban and industrial sites, which hints at the diluting role of *int*I1 in horizontal gene transfers across the land use gradient. Di*ff*ering mechanisms may regulate site-speci*fi*c population exposure to transferable ARGs, and the identi*fi*cation of additional MGEs is warranted. Compared to drinking water and the accidental ingestion of agricultural soil, airborne $PM_{2.5}$ contributes to a similar extent to the human daily intake of certain ARGs and *int*I1. Collectively, this study highlights the importance of $PM_{2.5}$ in the dissemination of, and pathways of human exposure to, common environmental ARGs.

INTRODUCTION

Antimicrobial resistance has been listed by the United Nations Environment Programme as one of the six global emerging environmental issues, [1] and there is widespread interest in elucidating its origin for informed risk management.[2,3] These efforts have led to a growing awareness of the distribution and dissemination of antibiotic resistance genes (ARGs) in natural environments (e.g., surface water, sediment, and soil [4−7]) and across engineered systems (e.g., wastewater

treatment plants and drinking water networks [8−14]). Among the environmental compartments for the dissemination of ARGs, the atmosphere, particularly via fine particulate matter ($PM_{2.5}$), is the least appreciated compared to its terrestrial counterparts, leaving the environmental loop of ARG flows unclosed. As a critical atmospheric component, airborne $PM_{2.5}$ influences air quality, regional climates, and human health. In particular, $PM_{2.5}$ can penetrate deeply into the alveolar region of the human lung. Disproportionate to the physico chemicaln characterization of $PM_{2.5}$, the understanding of its biological components is still in its infancy. [15−18] Evidence has emerged to distinguish airborne microbial communities [19] and associated antibiotic resistomes [20] from those in terrestrial and marine systems. Moreover, ambient air is subject to virtually no treatment upon inhalation, in contrast to processed food and water resources upon their ingestion. All of these factors would mean that airborne $PM_{2.5}$ is a unique pathway for the environmental dissemination of ARGs and for human exposure to these biological contaminants. The growing body of literature on airborne ARGs mainly focuses either on coarser particles (e.g., total suspended particulate and PM_{10} [21−23]) or on typical sources (e.g., clinical settings, dairy farms, and wastewater treatment plants [24,25]), with little consideration of ambient $PM_{2.5}$, which has greater implications for the exposure of the general population. [26,27] The highly dynamic nature of the atmospheric environment requires a spatiotemporally resolved characterization of airborne bacteria and ARGs. Therefore, we dedicated this study to investigating the distribution of total bacteria (16S rRNA gene), three representative ARGs (*erm*B, *tet*W, and *qnr*S), and a mobile genetic element (integron class 1, *intI*1) over an annual cycle along an industrial−urban−rural transect in Nanjing, China. Using a real-time quantitative polymerase chain reaction (qPCR), we elucidated the abundance, composition, and transferability of $PM_{2.5}$-associated ARGs as they evolved with seasonal cycles and land use gradients. We further assessed the relative importance

of $PM_{2.5}$ in human exposure pathways by estimating the daily intake of ARGs via the inhalation of $PM_{2.5}$ in comparison with that from drinking water and the accidental ingestion of agricultural soil. With the above quantitative information, the central aim of the study was to identify the contribution of ambient $PM_{2.5}$ to the dissemination of ARGs in the environment and eventually to human exposure.

MATERIALS AND METHODS

$PM_{2.5}$ Sampling

$PM_{2.5}$ samples were synchronously collected at industrial (37 m above ground), urban (17 m above ground), and rural (1.5 m above ground) sites (Figure S1 and Table S1) by high-volume (1000 L min^{-1}) samplers (TH- 1000C II, Wuhan Tianhong Instruments Co., Ltd.), using quartz microfiber filters (QMA, 203 mm × 254 mm, Whatman 1851-65; prebaked at 400 °C for 4 h). One 24 h sample (from 8:00 a.m. to 8:00 a.m.) was collected every 7−10 days at the industrial (n = 46) and urban (n = 48) sites and nearly every month at the rural site (n = 18) from March 2016 to May 2017. A filter was placed in an air sampler accessory box at each site throughout the sampling campaign to serve as field blanks. The seasonal variations in $PM_{2.5}$ concentrations at each site are summarized in Figure S2.

DNA Extraction and Real-Time Quantitative Polymerase Chain Reaction (qPCR) Detection

A quarter of each filter sample from industrial and urban sites was used for DNA extraction, while half of each sample was needed for the rural site because of the smaller amounts of DNA that were extracted there. A blank filter was treated simultaneously using the same operation that was used for the samples. After the filter was cut into pieces (one-eighth segments), each portion of

the filter sample was extracted with sterilized 1× phosphate-buffered saline following the procedures used in a previous study. [28] The extracts of each sample from the same month were combined and filtered through a 0.2 μm PES membrane disc filter (47 mm, Pall). All of the tools used in the pretreatment process were sterilized.

DNA was extracted from the disc filters (cut into small pieces) using the FastDNA SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's instructions, except using Agencourt AMPure XP beads (Beckman Coulter) in the last step for purification.[28] Several selected ARGs (*erm*B, *tet*W, and *qnr*S) and MGEs (*int*I1), as well as the 16S rRNA gene, were quantified on a StepOnePlus Real-Time PCR System (Applied Biosystems). Detailed information about the primer sets, thermocycling protocols, standard constructions, and quality control procedures can be found in Section S1 of the Supporting Information and Table S2.

Chemical Analysis

Trace metals were analyzed by inductively coupled plasma-mass spectrometry (Agilent model 7700) after acid digestion.[29] Major water-soluble inorganic ions (Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, Na^+, K^+, and NH_4^+) were analyzed by ion chromatography (Dionex). [30] Organic carbon and elemental carbon were analyzed using a carbon analyzer (model 2001, Desert Research Institute) [31] The seasonal average chemical compositions are summarized in Figure S3.

RESULTS AND DISCUSSION

Seasonal Contrast in Atmospheric Bacterial Loadings between Urban and Rural Sites

A general seasonal showed a distinct seasonal pattern in which the levels dramatically declined in winter while recovering in the following spring [one-way analysis of variance (ANOVA); $p < 0.05$ (Figure 1)]. The seasonal disparities were likely a result of the different

123 contributions of site-specific biological sources. Natural origins (e.g., soil and plant materials)
124 and anthropogenic sources (e.g., wastewater treatment plants, composting facilities, and livestock
125 farms) have already been identified as potential sources of ambient airborne microbes.
126 [18,32,33] We reasoned that airborne bacteria at the urban and industrial sites may largely
127 originate from seasonally independent sources, for example, from fugitive dust from paved roads
128 and human daily activities. Vegetation-related bacteria might dominate in rural areas, resulting
129 in the total bacteria loading changing with the blooming (spring and summer) and withering
130 (winter) of plants. It should be noted that, as the sampling height differed between the sites,
131 vertical gradients, particularly of bacteria resuspended from soil and vegetation, may exist as a
132 modifying factor of our site comparisons.
133 The total amount of airborne bacteria and their community structures could be regulated by
134 multiple meteorological conditions, including humidity and precipitation during their transfer
135 from sources to the atmosphere [34] (like the higher emission flux of soil-related bacteria in
136 autumn caused by the low level of humidity, which is then lower in winter because the soil is
137 frozen and covered by snow), and reshaped under different environmental factors, including
138 solar radiation, as selective pressures among seasons. [34−36] In addition, higher pollution
139 levels in winter (Figures S2 and S3) with frequent haze episodes in urban and industrial sites are
140 likely to provide considerable amounts of nutrients, such as soluble inorganic ions and low-
141 molecular weight organic acids, for the survival and replication of bacteria, [37,38] which could
142 partially explain the higher concentrations of bacteria in the atmosphere during this season at
143 urban and industrial sites compared to those in rural areas. The airborne microbial loadings
144 detected in other locations using staining or the qPCR technique are summarized in Figure S4,
145 where comparable bacterial loadings in outdoor $PM_{2.5}$ between eastern China (this study) and

146 northeastern America [39] could be found. These results indicate that there is some consistency
147 in bacterial loadings within regions and possibly even across continents in large-scale urban
148 areas, without significant disturbances from sources or long-range transports, an issue that needs
149 to be further investigated in the future. Unlike those in $PM_{2.5}$, airborne bacteria attached to larger
150 particles, including inhalable particulate matter (PM_{10}) and TSP, were unsurprisingly present at
151 either comparable or higher concentrations in the atmosphere but with great spatial variations.
152 This finding could be attributed to various factors, ranging from different sampling seasons to
153 diverse capture and extraction efficiencies of particle-attached bacteria, as well as the influence
154 of different dominant biological sources in different studies and regions.
155
156 **Differential Seasonal Enrichment of Atmospheric**
157 ARGs between Urban and Rural Sites. The absolute abundance and the relative abundance of
158 ARGs in this study, with the exception of those of *qnr*S, are comparable to, or several orders of
159 magnitude lower than, those reported previously (Figure S5 and S6). This is understandable
160 because most existing studies of airborne ARG were conducted at sites in the vicinity of ARG
161 sources. Nevertheless, urban ambient $PM_{2.5}$ has broader implications for the dissemination and
162 exchange of ARGs among the large urban populations that are affected by them.
163 Echoing the temporal evolution of total bacteria, the seasonality of associated *tet*W and *erm*B
164 was most pronounced at the rural site, in contrast to the relative seasonal consistency at the urban
165 and industrial sites, the exception being *qnr*S, with no notable trend due to frequent nondetection
166 in the samples (Figure 2). The increasing level of enrichment of ARGs from spring to winter and
167 their decline in the following spring at the rural site suggested the seasonal cycling of these
168 genetic elements at the less impacted site. Similar characterizations at background sites are

desirable for understanding the natural baseline of ARGs. In more densely populated urban and industrial areas with greater anthropogenic activities (Table S1), human-derived ARB from domestic activities [40] or outdoor fugitive dust may consistently contribute to airborne resistomes across seasons, resulting in the relatively small seasonal fluctuations in ARGs at these two sites. To quantify the direct human contribution, multiple lines of evidence from specific anthropogenic markers (e.g., *Hmt*, a human mitochondrial gene target) and the metagenomic profiling of host bacteria would be required to test the hypotheses described above.

The class 1 integron located on mobile genetic elements (MGEs) is often related to the dissemination of ARGs subject to anthropogenic impacts. [41] In our study, the relative abundance (normalized to the 16S rRNA gene) of ARGs, with the exception of *qnr*S (mostly below LOQ), strongly positively correlated with that of *int*I1 at the rural site (Figure 3 and Figure S7). Interestingly, the association weakened at the urban site and diminished at the industrial site. This finding is somewhat contradictory to the general belief that terrestrial *int*I1 of clinical origin is a proxy for elevated levels of anthropogenic pollution. [41] However, the nature of the airborne *int*I1 is yet to be determined with a broad coverage of MGEs and antibiotic resistance mechanisms. The current statistical results supported our hypothesis that different mechanisms in the propagation of airborne ARGs across land use gradients may exist in the environment. Horizontal gene transfer (HGT) by integrons may be mainly responsible for the dissemination of ARGs at the rural site, as opposed to the joint effects of multiple transfer mechanisms at urban and industrial sites, where the dominant role of the integron in HGT could possibly be substituted. The disparate mechanisms of ARG dissemination across land use gradients with specific local sources may have implications for site-specific exposure scenarios among populations upon inhalation and exchange with lung micro- biomes, even when integrons are present in quantities

192 similar to those in other studies (Figures S5 and S8).

193 The limited set of genes targeted in this study revealed spatially explicit signatures of biological
194 components between rural and urban areas (Figure S9), although the total amount of bacteria
195 accounted for <0.01% of the $PM_{2.5}$ by mass (assuming one copy of the 16S rRNA gene per cell
196 and 1 pg per cell). This observation suggests the predominance of local sources in shaping the
197 pro*fi*les of airborne bacteria and associated ARGs. Further evidence of this process is required
198 through an analysis of the bacterial community and multiple resistance mechanisms. By contrast,
199 the chemical components that were identi*fi*ed as the major contributors of mass to $PM_{2.5}$ [>70%
200 (Figure S3)] displayed relative spatial homogeneity across the industrial−urban−rural transect
201 (Figure S9), reflecting the regional influence predominantly on the chemical characteristics of
202 $PM_{2.5}$. [29] The chemical−microbial differentiation in the spatial distribution highlighted the
203 signi*fi*cance of the biological aspects in $PM_{2.5}$. Regional- and continental-scale investigations
204 should be conducted so that a complete picture of the situation can be obtained.

205

206 **Relevance of Inhalation to the Human Intake of Environmentally Disseminated ARGs**

207 To evaluate the relevance of airborne bacteria and ARGs in $PM_{2.5}$ to human exposure, we
208 estimated the daily intake (DI) of the studied genes via the inhalation of $PM_{2.5}$ and drinking water
209 and the accidental ingestion of agricultural soil from Nanjing or adjacent cities according to
210 eqs S1−S3 (Section S3 of the Supporting Information). Data on the concentrations of the targeted
211 genes in drinking water and agricultural soil were sought from the literature, [12,42,43] assuming
212 equal DNA extraction efficiencies across various environmental media.

213 It is interesting to note that the three exposure pathways contribute similar daily intakes of
214 macrolide resistance genes and MGEs in the studied region, although total bacteria and

tetracycline resistance genes via ingestion of drinking water or agricultural soil went beyond those via inhalation by at least 2 orders of magnitude. Given that Chinese people normally drink boiled water, these genes would likely be subject to substantial thermal degradation, suggesting that the inhalation of ambient $PM_{2.5}$ may outcompete the consumption of drinking water in the human intake of ARGs. Overall, the comparative analysis in this study highlights an emerging research need globally to ascertain the relative contribution of urban $PM_{2.5}$ as an exposure pathway to the human uptake of environmentally disseminated ARGs (Figure 4).

Environmental Implications

Our study demonstrated the critical role of ambient $PM_{2.5}$, a vector for ARGs that can be environmentally disseminated for consequent human exposure. Considering the high deposition efficiency of $PM_{2.5}$ in the respiratory tract, a number of research questions would arise, including whether and how airborne bacteria would, upon inhalation, interact with lung microbiomes and exchange potentially hazardous genetic elements such as ARGs. The seasonal dynamics of ARGs specific to functional categorizations of land use, in contrast with the relatively homogeneous chemical compositions across spatiotemporal scales (Figure S3), has signi*fi*cant implications for site-specific population exposure to $PM_{2.5}$-associated biological components. Using the study presented here, regional or continental comparisons are warranted to fully uncover the mechanisms regulating geo- graphical patterns in the abundance, transferability, and exposure of common environmental ARGs.

In addition, investigations of airborne ARGs in this study ranged from quanti*fi*cation of genetic levels to estimations of exposure intake by inhalation. However, the major hosts of ARGs in airborne microbial communities and their roles in driving the dissemination of ARGs are still

unknown but are of great significance if a complete picture of antibiotic resistance transfer is to be obtained, as well as of more types of ARGs and MGEs. Metagenomics-based host tracking may be conducted if the biomass available in $PM_{2.5}$ suffices for such an analysis to be conducted, [44,45] which is also conducive to the source apportionment of ARGs through a comparison of their host profiles with those of potential sources. [32,46] Moreover, assessments of the intercompartmental gene flow and of human exposure to these biological contaminants (e.g., biosolid−soil−plant continuum [47]) are desirable beyond the focus on ARGs in a confined environmental medium. To this end, internationally consistent efforts are needed to address the uncertainty of DNA extraction efficiencies among extraction methods and different sample types. It would then be possible to accurately report the absolute abundance of ARGs in environmental media, which is required to assess intercompartmentas mechanisms and human exposure.

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REFERENCES

(1) United Nations Environment Programme. Frontiers 2017: Emerging issues of environmental concern, 2017. https://wedocs. unep.org/handle/20.500.11822/22255.

(2) Pruden, A.; Larsson, D. J.; Amećquita, A.; Collignon, P.; Brandt, K. K.; Graham, D. W.; Lazorchak, J. M.; Suzuki, S.; Silley, P.; Snape, J. R.; Topp, E.; Zhang, T.; Zhu, Y.-G. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. *Environ. Health Persp.* 2013, *121*, 878−885.

(3) Vikesland, P. J.; Pruden, A.; Alvarez, P. J.; Aga, D.; Bürgmann, H.; Li, X.-d.; Manaia, C. M.; Nambi, I.; Wigginton, K.; Zhang, T.; Zhu, Y.-G. Toward a comprehensive strategy to mitigate dissemination of environmental sources of antibiotic resistance. *Environ. Sci. Technol.* 2017, *51*, 13061−13069.

(4) Zhu, Y.-G.; Zhao, Y.; Li, B.; Huang, C.-L.; Zhang, S.-Y.; Yu, S.; Chen, Y.-S.; Zhang, T.; Gillings, M. R.; Su, J.-Q. Continental-scale pollution of estuaries with antibiotic resistance genes. *Nat. Microbiol.* 2017, *2*, 16270.

(5) Luo, Y.; Mao, D.; Rysz, M.; Zhou, Q.; Zhang, H.; Xu, L.; Alvarez, P. J. J. Trends in antibiotic resistance genes occurrence in the Haihe River, China. *Environ. Sci. Technol.* 2010, *44*, 7220−7225.

(6) Xiong, W.; Sun, Y.; Zhang, T.; Ding, X.; Li, Y.; Wang, M.; Zeng, Z. Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. *Microb. Ecol.* 2015, *70*, 425−432.

(7) Chen, B.W.; Yuan, K.; Chen, X.M.; Yang, Y.; Zhang, T.; Wang, Y.; Luan, T.G.; Zou, S.C.; Li, X.D. Metagenomic analysis revealing antibiotic resistance genes (ARGs) and their genetic compartments in the tibetan environment. *Environ. Sci. Technol.* 2016, *50*, 6670−6679.

(8) Jia, S.; Shi, P.; Hu, Q.; Li, B.; Zhang, T.; Zhang, X.-X. Bacterial community shift drives antibiotic resistance promotion during drinking water chlorination. *Environ. Sci. Technol.* 2015, *49*, 12271−12279.

(9) Zhang, X.-X.; Zhang, T. Occurrence, abundance, and diversity of tetracycline resistance genes in 15 sewage treatment plants across China and other global locations. *Environ. Sci. Technol.* 2011, *45*, 2598−2604.

(10) Yang, Y.; Li, B.; Ju, F.; Zhang, T. Exploring variation of antibiotic resistance genes in activated sludge over a four-year period through a metagenomic approach. *Environ. Sci. Technol.* 2013, *47*, 10197−10205.

(11) Wang, F.-H.; Qiao, M.; Su, J.-Q.; Chen, Z.; Zhou, X.; Zhu, Y.-G. High throughput profiling of antibiotic resistance genes in urban park soils with reclaimed water irrigation. *Environ. Sci. Technol.* 2014, *48*, 9079−9085.

(12) Xie, W.-Y.; McGrath, S. P.; Su, J.-Q.; Hirsch, P. R.; Clark, I. M.; Shen, Q.; Zhu, Y.-G.; Zhao, F.-J. Long-term impact of field applications of sewage sludge on soil antibiotic resistome.

Environ. Sci. Technol. 2016, *50*, 12602−12611.

(13) Munck, C.; Albertsen, M.; Telke, A.; Ellabaan, M.; Nielsen, P. H.; Sommer, M. O. Limited dissemination of the wastewater treatment plant core resistome. *Nat. Commun.* 2015, *6*, 8452.

(14) Walsh, T. R.; Weeks, J.; Livermore, D. M.; Toleman, M. A. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: An environmental point prevalence study. *Lancet Infect. Dis.* 2011, *11*, 355−362.

(15) Fröhlich-Nowoisky, J.; Kampf, C. J.; Weber, B.; Huffman, J. A.; Pöhlker, C.; Andreae, M. O.; Lang-Yona, N.; Burrows, S. M.; Gunthe, S. S.; Elbert, W.; Su, H.; Hoor, P.; Thines, E.; Hoffmann, T.; Despreś, V. R.; Pöschl, U. Bioaerosols in the Earth system: Climate, health, and ecosystem interactions. *Atmos. Res.* 2016, *182*, 346−376.

(16) Jin, L.; Luo, X.S.; Fu, P.Q.; Li, X.D. Airborne particulate matter pollution in urban China: A chemical mixture perspective from sources to impacts. *Natl. Sci. Rev.* 2017, *4*, 593−610.

(17) Behzad, H.; Gojobori, T.; Mineta, K. Challenges and opportunities of airborne metagenomics. *Genome Biol. Evol.* 2015, *7*, 1216−1226.

(18) Smets, W.; Moretti, S.; Denys, S.; Lebeer, S. Airborne bacteria in the atmosphere: Presence, purpose, and potential. *Atmos. Environ.* 2016, *139*, 214−221.

(19) Cao, C.; Jiang, W.; Wang, B.; Fang, J.; Lang, J.; Tian, G.; Jiang, J.; Zhu, T. F. Inhalable microorganisms in Beijing's $PM_{2.5}$ and PM_{10} pollutants during a severe smog event. *Environ. Sci. Technol.* 2014, *48*, 1499−1507.

(20) Pal, C.; Bengtsson-Palme, J.; Kristiansson, E.; Larsson, D. J. The structure and diversity of human, animal and environmental resistomes. *Microbiome* 2016, *4*, 54.

(21) Ling, A. L.; Pace, N. R.; Hernandez, M. T.; LaPara, T. M. Tetracycline resistance and class 1 integron genes associated with indoor and outdoor aerosols. *Environ. Sci. Technol.* 2013, *47*, 4046− 4052.

(22) Gat, D.; Mazar, Y.; Cytryn, E.; Rudich, Y. Origin-dependent variations in the atmospheric microbiome community in Eastern Mediterranean dust storms. *Environ. Sci. Technol.* 2017, *51*, 6709− 6718.

(23) Mazar, Y.; Cytryn, E.; Erel, Y.; Rudich, Y. Effect of dust storms on the atmospheric microbiome in the Eastern Mediterranean. *Environ. Sci. Technol.* 2016, *50*, 4194−4202.

(24) Li, J.; Zhou, L.; Zhang, X.; Xu, C.; Dong, L.; Yao, M. Bioaerosol emissions and detection of airborne antibiotic resistance genes from a wastewater treatment plant. *Atmos. Environ.* 2016, *124*, 404−412.

(25) McEachran, A. D.; Blackwell, B. R.; Hanson, J. D.; Wooten, K. J.; Mayer, G. D.; Cox, S.

B.; Smith, P. N. Antibiotics, bacteria, and antibiotic resistance genes: Aerial transport from cattle feed yards via particulate matter. *Environ. Health Perspect.* 2015, *123*, 337−343.

(26) Gao, M.; Jia, R.; Qiu, T.; Han, M.; Wang, X. Size-related bacterial diversity and tetracycline resistance gene abundance in the air of concentrated poultry feeding operations. *Environ. Pollut.* 2017, *220*, 1342−1348.

(27) Schaeffer, J.; Reynolds, S. J.; Magzamen, S.; VanDyke, A.; Gottel, N.; Gilbert, J.; Owens, S.; Hampton-Marcell, J. T.; Volckens, J. Size, composition, and source profiles of inhalable bioaerosols from Colorado dairies. *Environ. Sci. Technol.* 2017, *51*, 6430−6440.

(28) Jiang, W.; Liang, P.; Wang, B.; Fang, J.; Lang, J.; Tian, G.; Jiang, J.; Zhu, T. F. Optimized DNA extraction and metagenomic sequencing of airborne microbial communities. *Nat. Protoc.* 2015, *10*, 768−779.

(29) Ming, L. L.; Jin, L.; Li, J.; Fu, P. Q.; Yang, W.; Liu, D.; Zhang, G.; Wang, Z. F.; Li, X. D. $PM_{2.5}$ in the Yangtze River Delta, China: Chemical compositions, seasonal variations, and regional pollution events. *Environ. Pollut.* 2017, *223*, 200−212.

(30) Zhang, T.; Cao, J.; Tie, X.; Shen, Z.; Liu, S.; Ding, H.; Han, Y.; Wang, G.; Ho, K.; Qiang, J.; Li, W. Water-soluble ions in atmospheric aerosols measured in Xi'an, China: seasonal variations and sources. *Atmos. Res.* 2011, *102*, 110−119.

(31) Cao, J.; Lee, S.; Ho, K.; Zhang, X.; Zou, S.; Fung, K.; Chow, J. C.; Watson, J. G. Characteristics of carbonaceous aerosol in Pearl River Delta Region, China during 2001 winter period. *Atmos. Environ.* 2003, *37*, 1451−1460.

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

(33) Yue, S.; Ren, H.; Fan, S.; Wei, L.; Zhao, J.; Bao, M.; Hou, S.; Zhan, J.; Zhao, W.; Ren, L.; Kang, M.; Li, L.; Zhang, Y.; Sun, Y.; Wang, Z.; Fu, P. High abundance of fluorescent biological aerosol particles in winter in Beijing, China. *ACS Earth Space Chem.* 2017, *1*, 493−502.

(34) Jones, A. M.; Harrison, R. M. The effects of meteorological factors on atmospheric bioaerosol concentrations - a review. *Sci. Total Environ.* 2004, *326*, 151−180.

(35) Zhen, Q.; Deng, Y.; Wang, Y.; Wang, X.; Zhang, H.; Sun, X.; Ouyang, Z. Meteorological factors had more impact on airborne bacterial communities than air pollutants. *Sci. Total Environ.* 2017, *601−602*, 703−712.

(36) Tong, Y.; Lighthart, B. Solar radiation is shown to select for pigmented outdoor atmosphere. *Photochem. Photobiol.* 1997, *65*, 103− 106.

(37) Wei, M.; Xu, C.; Chen, J.; Zhu, C.; Li, J.; Lv, G. Characteristics of bacterial community in

cloud water at Mt Tai: similarity and disparity under polluted and non-polluted cloud episodes. *Atmos. Chem. Phys.* 2017, *17*, 5253−5270.

(38) Cheng, C.; Wang, G.; Zhou, B.; Meng, J.; Li, J.; Cao, J.; Xiao, S. Comparison of dicarboxylic acids and related compounds in aerosol samples collected in Xi'an, China during haze and clean periods. *Atmos. Environ.* 2013, *81*, 443−449.

(39) Hospodsky, D.; Qian, J.; Nazaroff, W. W.; Yamamoto, N.; Bibby, K.; Rismani-Yazdi, H.; Peccia, J. Human occupancy as a source of indoor airborne bacteria. *PLoS One* 2012, *7*, No. e34867.

(40) Hartmann, E. M.; Hickey, R.; Hsu, T.; Betancourt Román, C.M.; Chen, J.; Schwager, R.; Kline, J.; Brown, G.; Halden, R. U.; Huttenhower, C.; Green, J. L. Antimicrobial chemicals are associated with elevated antibiotic resistance genes in the indoor dust microbiome. *Environ. Sci. Technol.* 2016, *50*, 9807−9815.

(41) Gillings, M. R.; Gaze, W. H.; Pruden, A.; Smalla, K.; Tiedje, J. M.; Zhu, Y.-G. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J.* 2015, *9*, 1269−1279.

(42) Peng, S.; Wang, Y.; Zhou, B.; Lin, X. Long-term application of fresh and composted manure increase tetracycline resistance in the arable soil of eastern China. *Sci. Total Environ.* 2015, *506−507*, 279− 286.

(43) Shi, P.; Jia, S.; Zhang, X.-X.; Zhang, T.; Cheng, S.; Li, A. Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. *Water Res.* 2013, *47*, 111−120.

(44) Ma, L.; Li, B.; Jiang, X.-T.; Wang, Y.-L.; Xia, Y.; Li, A.-D.; Zhang, T. Catalogue of antibiotic resistome and host-tracking in drinking water deciphered by a large scale survey. *Microbiome* 2017, *5*, 154.

(45) Ma, L.; Xia, Y.; Li, B.; Yang, Y.; Li, L.-G.; Tiedje, J. M.; Zhang, T. Metagenomic assembly reveals hosts of antibiotic resistance genes and the shared resistome in pig, chicken, and human feces. *Environ. Sci. Technol.* 2016, *50*, 420−427.

(46) Bowers, R. M.; McLetchie, S.; Knight, R.; Fierer, N. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *ISME J.* 2011, *5*, 601−612.

(47) Chen, Q.-L.; An, X.-L.; Zhu, Y.-G.; Su, J.-Q.; Gillings, M. R.; Ye, Z.-L.; Cui, L. Application of struvite alters the antibiotic resistome in soil, rhizosphere, and phyllosphere. *Environ. Sci. Technol.* 2017, *51*, 8149−8157.

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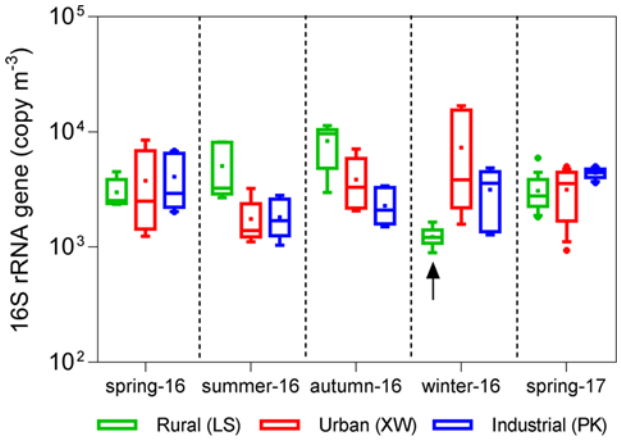


Figure 1. Seasonal variability in the $PM_{2.5}$-associated 16S rRNA gene indicative of total bacterial loading at the rural, urban, and industrial sites. The box plot presents the median, quartile, and 10% and 90% percentiles, with the dot symbol inside representing the seasonal mean value. The black arrow points out the most significant change across the seasonal cycle and land use gradient, i.e., wintertime reduction in bacterial load at the rural site (two-way ANOVA; $p < 0.05$).

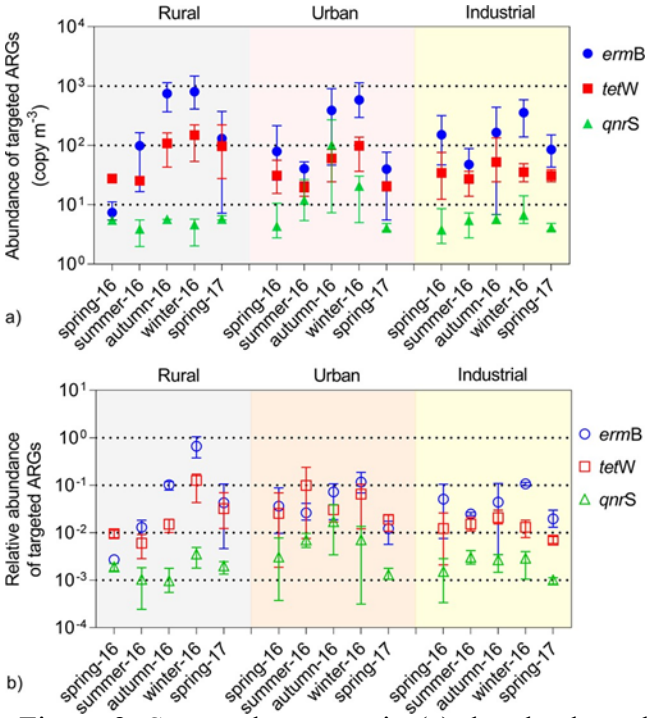
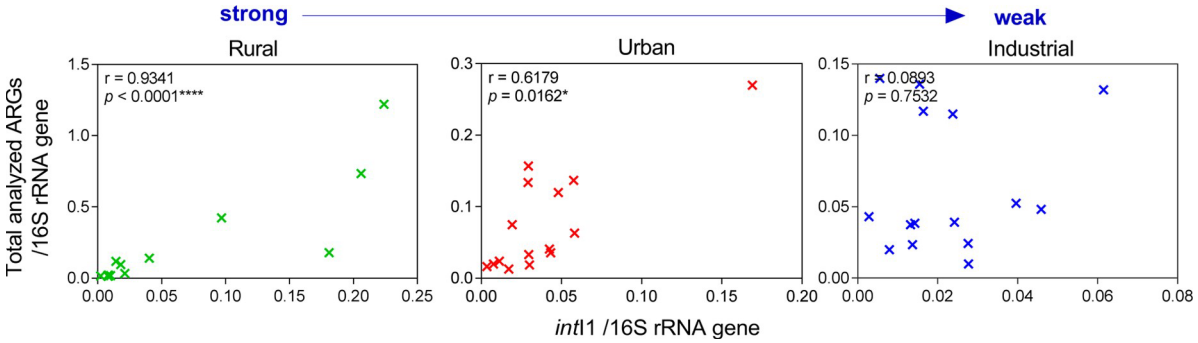


Figure 2. Seasonal patterns in (a) the absolute abundance of targeted ARGs normalized to air volume and (b) the relative abundance of targeted ARGs normalized to the 16S rRNA gene. Note that *qnr*S was not detected in most samples and that for the purpose of plotting *fi*gures the detection limit was assigned to those samples in which it was not detected.



459

460 Figure 3. Weakening statistical correlation between ARGs and *intI1* from the rural to urban
461 and industrial sites.

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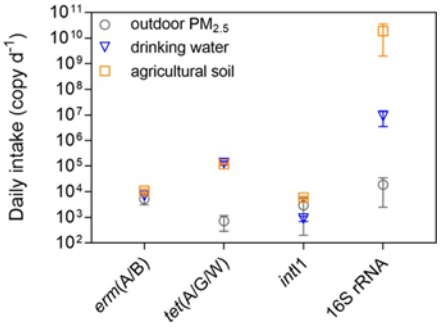

463

464 Figure 4. Comparison of the human daily intake of ARGs and 16S rRNA genes between inhalation
465 (gray circles for $PM_{2.5}$) and ingestion (blue triangles for drinking water and orange squares for
466 agricultural soil). The calculation was based on eqs S1−S3. The data on concentrations of targeted
467 genes in outdoor PM2.5 from urban Nanjing were generated by this study. Those in drinking water
468 from urban Nanjing were from ref 43. The soil concentrations of targeted genes from adjacent
469 cities in eastern China (data unavailable in urban Nanjing) were from refs 12 and 42.