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## SEASONAL VARIATION OF INDOOR BACTERIAL AEROSOLS IN NATURALLY VENTILATED URBAN CLASSROOMS WITH HIGH OUTDOOR PARTICULATE MATTER CONCENTRATIONS

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### INTRODUCTION

Both high particulate matter (PM) exposure and acute respiratory infections disproportionately affect children in less developed countries (Mathers et al., 2000), who are more likely to live in naturally ventilated buildings (McNeil and Letschert, 2007). This study aimed to describe the concentrations and intake fractions of particulate matter and bacteria in naturally ventilated classrooms with seasonal changes in operation, and the ecology of their indoor airborne bacteria.

### METHODS

Two preschool classrooms (sites A and B) in Lanzhou, a city located in northwest China, with high outdoor PM concentrations, were sampled indoors and outdoors in parallel. Separate sampling was conducted while the classrooms were occupied and vacant, in both summer and winter. Rooms were naturally ventilated by opening and closing windows. Particle mass concentrations were measured with 0.4-20  $\mu\text{m}$  size-resolved particle mass impactors fitted with polycarbonate filters and particle number concentrations were determined using optical particle counters (OPCs) measuring 0.3-10  $\mu\text{m}$ , both concurrently deployed indoors and outdoors. Carbon dioxide ( $\text{CO}_2$ ) was measured indoors with a Licor model LI-820 gas analyzer, and researchers continuously recorded occupancy levels from direct observation. Air exchange rates (AER) were estimated by a mass-balance method utilizing occupant-generated  $\text{CO}_2$ , tracer gas decay during the beginning of vacant periods, and outdoor (baseline)  $\text{CO}_2$  concentrations. Emission rates and source apportionment of indoor particulate matter and bacteria were calculated by a material balance method utilizing time-averaged measured indoor and outdoor concentrations along with estimates for the influence of ventilation and particle deposition. Intake fractions adjusted for size-resolved deposition rates were calculated based on methods from Nazaroff (2008).

To compare bacterial communities in indoor and outdoor air to two potential sources of indoor bioaerosols suggested by previous studies – resuspended floor dust and material shed from human occupants (Hospodsky et al, 2012) – floor dust was swept and collected after each day of sampling, and hand wash samples were obtained from adult occupants each

season. DNA was extracted from impactor filters, floor dust, and hand wash samples. Bacterial DNA concentrations were assessed by quantitative polymerase chain reaction (qPCR) targeting 16S rRNA marker genes relative to a *B. atrophaeus* standard. Amplified bacterial DNA was sequenced on a Roche 454 GS FLX platform. Sequence classification and diversity was analyzed with QIIME, with open-reference OTU picking (Caporaso et al., 2010).

## RESULTS AND DISCUSSION

**Ventilation, occupancy, emission rates and exposure estimates (Table 1):** Outdoor PM concentrations determined from impactor mass measurements were higher in winter than summer across all stages (outdoor means summer:  $130 \pm 50 \mu\text{g m}^{-3}$ , winter:  $340 \pm 110 \mu\text{g m}^{-3}$ ). Seasonal AER and CO<sub>2</sub> concentrations reflected the practice of closing classroom windows during winter and opening windows in the summer. In the winter, we observed six-fold reductions in AER compared to those measured in the summer (mean summer occupied 5.6, winter occupied 1.0 h<sup>-1</sup>). Indoor CO<sub>2</sub> concentrations also varied significantly between seasons ( $p < 0.05$ ), with indoor accumulation of CO<sub>2</sub> exceeding 2,500 ppm at both sites during winter.

Table 1. Ventilation data and estimates of % indoor aerosols attributable to indoor sources

		Air Exchange Rates (h <sup>-1</sup> ) (mean ± SD)		Indoor CO <sub>2</sub> occupied ppm (mean ±SD)	Persons during occupancy (mean)	Indoor emission rates (per person h)		% of indoor aerosols estimated from indoor sources	
		Occupied	Unoccupied			PM (mg)	Bacteria (10 <sup>6</sup> GCN)	PM	Bacteria
Summer	A	5.4 ± 1.3	1.3 ± 1.1	479 ± 65	8	31	169	73	57
	B	6.4 ± 0.6	0.4 ± 0.1	532 ± 56	19	18	46	23	81
Winter	A	1.1 ± 0.8	0.1 ± 0.04	1622 ± 859	25	20	79	86	97
	B	0.9 ± 0.1	0.1 ± 0.03	1825 ± 626	13	40	64	89	100

Despite significant seasonal differences in AER and outdoor PM concentrations, PM indoor/outdoor (I/O) ratios did not vary significantly between winter and summer. Mean I/O ratios across all sizes based on OPC data were 1.5 and 2.5 in summer at sites A and B respectively, and 2.5 and 1.6 in winter. Higher winter I/O ratios at site A compared to site B were likely due to elevated indoor particle number concentrations associated with atypically high indoor occupancy at site A in winter (25 mean occupants). The lowest I/O ratios were calculated for particle numbers in the 1-2.5 μm diameter size bin during winter (mean I/O 1.1), consistent with penetration factor estimates of > 0.9 for fine particulate matter of that size range (Nazaroff, 2004) and low indoor particle generation rates associated with occupancy for these smaller particles.

Indoor emission rates for PM and bacteria did not differ significantly by season. Mean indoor source contributions were 1.2 to 3.0 times higher in winter for both particulate matter and bacterial aerosols, but these differences were not statistically significant ( $p > 0.1$ ). Size-resolved intake fractions (iF) of particles in the size range 0.4-9 μm were 3 to 15 times higher in winter than summer at both sites, averaging 39,000 and 6,000 ppm respectively.

**Bacterial diversity:** Comparing alpha diversity within each type of sample, floor dust was most diverse, followed by ambient air, and occupants' hands, as measured by unique operational taxonomic units (OTUs) identified at the 97% identity level. When normalized for 200 sequences per sample, estimated numbers of observed species for dust, air, and hand samples were 150±5, 117±11, and 40±8 respectively. At the same rarefaction depth, in the absence of occupants, the richness of bacteria from outdoor air samples was greater during

winter (127±5 OTUs) than summer (113±10). When occupied, the richness of summer and winter air were not different relative to OTU estimation uncertainties. Differences in diversity between air, dust, and hand samples (beta diversity) were larger than differences between seasons, sites, occupancy states, or indoors versus outdoors. In both seasons, microbial communities from indoor and outdoor ambient air were more similar to communities from floor dust than the hands samples when compared using unweighted UniFrac distances.

Across all samples, three phyla — *Proteobacteria*, *Actinobacteria*, and *Firmicutes* — accounted for more than 90% of OTUs, while four taxa classified as human-associated from Hospodsky et al. (2012) accounted for 1-28% of OTUs. In both summer and winter, indoor air samples had higher percentages of human-associated taxa than parallel outdoor samples. This difference was statistically significant in winter ( $p < 0.05$ ). On average, floor dust samples had higher percentages of human-associated genera (17%) than indoor ambient air samples (11%). Both occupied and unoccupied dust had more human-associated taxa than concurrent air samples. At site A, summer dust samples from occupied sampling contained almost twice as many human-associated taxa compared to dust from unoccupied sampling (22% vs 12%).

## CONCLUSIONS

Both naturally ventilated sites investigated altered building operation (closed windows) in response to seasonal changes in temperature and air pollution. This shift in operational mode was associated with higher winter indoor levels of bioeffluent CO<sub>2</sub>, higher indoor PM concentrations, higher total and human-associated bacterial concentrations, and higher intake fractions of indoor airborne bacteria and particulate matter. Enrichment of human-associated bacteria in floor dust is consistent with the hypothesis that resuspension of floor dust contributes substantially to indoor bioaerosols. These results are also consistent with the inference that building operation — in particular, ventilation mode — can affect intake fraction of emissions (both bacterial and abiotic) with indoor sources.

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