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Characterizing airborne fungal and bacterial concentrations and emission rates in six occupied children's classrooms

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Abstract

Baseline information on size-resolved bacterial, fungal, and particulate matter (PM) indoor air concentrations and emission rates is presented for six school classrooms sampled in four countries. Human occupancy resulted in significantly elevated airborne bacterial (81 times on average), fungal (15 times), and PM mass (9 times) concentrations as compared to vacant conditions. Occupied indoor/outdoor (I/O) ratios consistently exceeded vacant I/O ratios. Regarding size distributions, average room-occupied bacterial, fungal, and PM geometric mean particle sizes were similar to one another while geometric means estimated for bacteria, fungi, and PM mass during vacant sampling were consistently lower than when occupied. Occupancy also resulted in elevated indoor bacterial-to-PM mass-based and number-based ratios above corresponding outdoor levels. Mean emission rates due to human occupancy were 14 million cells person^{-1} h^{-1} for bacteria, 14 million spore equivalents person^{-1} h^{-1} for fungi, and 22 mg person^{-1} h^{-1} for PM mass. Across all locations, indoor emissions contributed 83±27% (bacteria), 66±19% (fungi), and 83±24% (PM mass) of the average indoor air concentrations during occupied times.

Practical Implications

An extensive dataset of bacterial and fungal size-distributed indoor air concentrations and emission rates is presented. Analysis of these data contributes to an understanding of how indoor bacterial and fungal aerosols are influenced by human occupancy. This work extends beyond prior culture and DNA-based microbiome studies in buildings to include quantitative relationships between size-resolved bacterial and fungal concentrations in indoor air and building parameters such as occupancy, ventilation, and outdoor conditions. The work indicates that occupancy-associated emissions (e.g., via resuspension and shedding) contribute more to both bacterial and fungal indoor air concentrations than do outdoor sources for the occupied classrooms investigated in this study.

Introduction

Inhaling indoor air is an important route of human exposure to bacteria and fungi. Through resuspension and direct shedding, humans significantly influence the microbial composition and concentrations in indoor air (Fox et al. , 2005; Fox et al., 2008; Hospodsky et al., 2012; Mackintosh et al., 1978; Noble et al., 1976; Qian and Ferro, 2008; Qian et al., 2008; Qian et al., 2012). Nevertheless, baseline quantitative information on bacterial and fungal emissions resulting from human occupancy is limited. Understanding the dominant sources that shape indoor air microbiomes and improving microbial exposure models requires broader knowledge of bacterial and fungal emission rates associated with human occupants. It is also important to develop an accounting of size-resolved bacterial and fungal concentrations that are determined independently of the well known biases of culture-based methods (Amann et al., 1995).

This paper presents baseline characterizations of airborne particle, bacterial, and fungal concentrations and emission rates sampled from one important category of indoor environments: school classrooms. We studied a sample of six children's classrooms, respectively located in the United States (1), Germany (1), Denmark (2), and China (2). Size-resolved indoor and outdoor air samples were collected separately during times of occupancy and vacancy. Quantitative polymerase chain reaction (qPCR) analysis, targeting universal fungal 18S rDNA and bacterial 16S rDNA gene sequences, was used to determine microbial concentrations. A material balance approach using aerosol concentration data and measured classroom air exchange rates (AER) was applied to estimate size-resolved emission rates associated with human occupancy. Finally, to investigate the relationship between total particulate matter and biological material in air, ratios of bacteria and fungi to total particulate matter were determined on a mass and particle number basis. By combining size-resolved biological aerosol concentrations with building variables and material-balance models, the results provide important information to guide

quantitative approaches for investigating the sources of and human exposures to bacteria and fungi suspended in indoor air.

Materials and methods

Test environments

Sampling was conducted in six school classrooms selected for their geographic diversity. The schools were located in five cities, in four countries, from three continents. The choice of location was made to produce concentration and emission data from diverse locations. Table 1 provides physical descriptions and Table S1 lists outdoor environmental characteristics at each building site. School classrooms were chosen because of their consistent occupancy pattern and because of the high proportion of time spent there by children. All rooms had hard tile flooring. Five of the six classrooms (in the EU and China) relied on natural ventilation via windows only. One classroom, in Salinas (California), was mechanically ventilated by a wall-embedded fan that supplied 100% outdoor air into the classroom.

Room characterization included time-resolved measurements of indoor and outdoor temperature and relative humidity, time-resolved measurements of indoor CO_2 concentrations, and direct observation of time-resolved occupancy level. Indoor and outdoor temperatures were monitored during periods of occupancy using the Met One HHPC-6 optical particle counter's (OPC) built-in temperature and relative humidity sensors (HACH Ultra Analytics Inc., Loveland, CO). Indoor air carbon dioxide concentrations were tracked using a LI-COR 820 CO_2 gas analyzer (LI-COR, Lincoln, NE). The CO_2 measurements were then utilized to assess air exchange rates. In this approach, a material balance on CO_2 concentration in the classroom was applied by assuming that the classroom was well mixed, accounting for exchange of CO_2 with outdoor air via ventilation, and considering human occupants as the only important indoor emission source (Mullen, 2010). This material balance is expressed in Equation (1):

$$dY/dt = E(t)/V + \lambda Y_{out}(t) - \lambda Y(t) \quad (1)$$

where Y represents the indoor CO_2 concentration (ppm), Y_{out} represents the outdoor CO_2 concentration (ppm), E represents the aggregate emission rate of CO_2 by the adult and children occupants ($cm^3\ min^{-1}$), V represents room volume (m^3), and λ represents the air exchange rate (min^{-1}). Average CO_2 emission rates reported by Smith (1988) were used for classroom environments: 390 $cm^3\ min^{-1}$ per adult and 228 $cm^3\ min^{-1}$ per child. Equation (1) can be rearranged and integrated over an appropriate time interval, t_i to t_f, for which the air-exchange rate is assumed to be constant, to yield Equation (2):

$$\lambda = \frac{\left[\int_{t_i}^{t_f} \frac{E(t)}{V} dt\right] - (Y(t_f) - Y(t_i))}{\int_{t_i}^{t_f} Y(t)dt - \int_{t_i}^{t_f} Y_{out}(t)dt} \quad (2)$$

In applying Equation (2), $E(t)$ is calculated by summing the emissions of adults and children: $E(t) = A(t)e_A(t) + C(t)e_C(t)$ where A and C represent the number of adult and child occupants respectively, and e_A and e_C are the corresponding per-person CO_2 emission rates.

Aerosol sampling

Airborne particle, bacterial, and fungal sampling was conducted indoors and outdoors using six-stage Andersen non-viable multistage impactors (NewStar Environment, Roswell, GA). Each sampler was operated at 28 liters per minute with a rotary vane vacuum pump during occupied and vacant periods. Indoor and outdoor impactors were connected through a manifold to a single vacuum pump, sited

outdoors to reduce noise associated with sampling. The samplers collected airborne particles within nominal size ranges of 0.4-1.1 μm, 1.1-2.1 μm, 2.1-3.3 μm, 3.3-4.7 μm, 4.7-9.0 μm, and >9.0 μm aerodynamic diameter (d_p). A size bin of 9.0-20 μm was utilized for the largest particles for estimating geometric means and geometric standard deviations of the bacterial, fungal, and PM aerosol concentrations. Prior to operation, impactors were cleaned using a 70% ethanol solution and sterilized under UV light for at least 30 minutes. All size-specific sampling stages were then loaded with 81 mm diameter Nuclepore filters with a pore diameter of 0.8 μm (Whatman, Piscataway, NJ). After sampling, filters were removed and stored in parafilm-sealed sterile petri dishes at -20 °C until analysis. Occupied sampling was conducted in the presence of students and teachers, typically from 9 AM to 3 PM. Sampling for vacant conditions was conducted during weekends or overnight. Occupancy data and sampling dates are reported in Table S2. To ensure that microbial aerosol levels would be above limits of detection, air samples were collected for three to four consecutive days for approximately 25 total hours of occupied sampling and about 60 total hours of unoccupied sampling using the same filter substrates. During each of the four sampling conditions (parallel indoor and outdoor sampling during both vacant and occupied periods), each location was further characterized for particle number concentrations. Optical particle counters measured particle number concentrations in six size ranges from 0.3-0.5 μm, 0.5-1 μm, 1-2.5 μm, 2.5-5 μm, 5-10 μm, and >10 μm.

Quantification of size-resolved particle mass, bacterial, and fungal concentrations

To determine particulate matter mass concentrations, impactor filters were weighed before and after sampling as described by Qian et al. (2012). Number concentrations of total fungi and bacteria were determined by 18S and 16S qPCR targeting universal fungal and bacterial genes. For this purpose, DNA was extracted from a one-quarter section of each impactor filter using previously described methods (Boreson et al., 2004). Procedures included enzymatic, chemical, and physical cell lysis and DNA

purification using a commercial spin column DNA extraction kit (Mobio Laboratories, Carlsbad, CA). Modifications to the cited method included doubling of added chemical and enzyme volumes, proteinase K incubation at 54 ºC instead of 37 ºC, omission of the freeze-thaw cycle, and omission of the ethanol precipitation step before purification with the column kit. The extraction was performed using the Mobio low elution spin columns, which allowed for elution with a smaller volume to concentrate DNA from low biomass samples. Elution used 25 microliters of 10 mM Tris buffer (pH = 8), prewarmed to 50 ºC.

Quantitative PCR was performed in triplicate using an ABI 7500 fast real-time PCR system (Applied Biosystems, Carlsbad, CA). For bacteria, universal bacterial primers and TaqMan® probes covered the 331 to 797 *E. coli* numbering region of the 16S rRNA-encoding gene (Nadkarni et al., 2002). For the universal fungal DNA quantification, a SYBR Green assay was used with 18S RNA-encoding gene primers reported by Zhou et al. (2000). Real-time PCR cycle conditions and details of the qPCR protocols are as previously described for bacteria (Qian et al., 2012) and fungi (Yamamoto et al., 2011). Standard curves of bacterial cell and fungal spore quantities vs. cycle threshold number were developed using known amounts of *Bacillus atrophaeus* (ATCC 49337) and *Aspergillus fumigatus* (ATCC 34506) genomic DNA, respectively. For bacteria, cycle threshold values were calibrated versus total bacterial genomes and accounted for the ten rRNA operon copies in *B. atrophaeus* and the average of four rRNA operon copies per genome for all bacteria (Lee et al., 2009). Thus, bacteria values represent an "average" bacterial cell and are reported as cells per volume. Fungal universal qPCR values are presented as spore equivalents (SE) of a standard *A. fumigatus* strain (Yamamoto et al., 2011). To test for PCR matrix inhibition, standard curves for *B. atrophaeus* and *A. fumigatus* were also produced after spiking the standard with aerosol filter extracts. No inhibitory effects were observed.

Biological content of aerosols and emission rates

On a size-resolved basis, the ratio of bacteria to particulate matter and the ratio of fungi to particulate matter were estimated. These estimates were made on both a mass and particle number basis. For the mass basis, bacterial and fungal concentrations were first converted to mass concentrations, and then divided by the total particle mass concentration and reported as mass fractions, either in parts per million (ppm, 10^{-6}) or percent (%, 10^{-2}) terms. Bacterial number concentrations were converted to mass by assuming the average mass of a bacterium to be 665 fg (Ilic et al., 2001). Fungal spores were converted to mass by using the *A. fumigatus* spore geometric diameter of 2.5 µm (Cole and Samson, 1984), and assuming a spherical shape and unit density, yielding an estimated spore mass of 8.2 pg. To determine the ratio of bacterial cell number or fungal spores equivalent number to total particle number, size-resolved quantitative PCR results were used for bacterial and fungal number concentrations and these values were divided by optical particle counter results for size-resolved particle concentrations. The size channels of the Andersen impactor and the OPC were made similar by grouping the 2.1-3.3 and 3.3-4.7 µm stages of the impactor to compare against the 2.5-5 µm channel of the OPC, and by grouping the 0.3-0.5 µm and 0.5-1.0 µm channels of the OPC to compare against the 0.4 to 1.1 µm stage of the impactor.

We also estimated the size-resolved bacterial, fungal, and total mass emission rates associated with occupancy. Per-person hourly emission rates were calculated using a steady-state or time-averaged material balance method that equates ventilation and emission sources to ventilation and deposition losses and solves for the emission term (Equation 3):

$$QC_{out} + NE = QC + kVC \qquad (3)$$

Here, C_{out} is the time-averaged outdoor air concentration of total particle mass (μg m^{-3}), bacterial (cells m^{-3}), or equivalent fungal spores (SE m^{-3}); C is the time-averaged corresponding indoor concentration during the occupied period; N is the time-averaged number of persons in the room during occupied sampling as recorded by on-site observations (persons); E is the per-person emission rate of particle mass (mg h^{-1} $person^{-1}$), bacteria (cells h^{-1} $person^{-1}$), or fungal spore equivalents (SE h^{-1} $person^{-1}$); Q is the volumetric ventilation rate (m^3 h^{-1}); V is the measured room volume (m^3); and k is the size-specific deposition-rate coefficient for total particles, bacterial cells, or fungal spore equivalents (h^{-1}) based on the deposition experiments of Thatcher et al. (2002) and as previously reported by Qian et al. (2012). To solve for the species- and size-resolved emission rates, E, the parameters Q and V were grouped into air exchange rates (Q/V), which were determined from analysis of the CO_2 data, as summarized above.

Statistical analysis

Comparisons between the averages of two sets of data were made by assuming a normal distribution and performing homoscedastic *t*-tests. Correlations among bacterial, fungal, and PM emission rates were performed using Pearson product-moment correlations and reporting the correlation coefficient and *p* values. Variation between concentration and emission data from different sites was captured by presenting data in box and whisker plots that display the median, quartiles, and ranges.

Results

Tables 2 and S2 report building occupancy and ventilation parameters for the six study sites. Small variations in building occupancy, temperature, and relative humidity were found. For all sites, the mean time-averaged occupancy level during occupied periods was 10.8 students and instructors and values at individual sites ranged from 8.0 to 12.5. Indoor temperatures ranged from 20 ± 2 °C to 30 ± 2 °C and the average indoor relative humidity was 35 ± 7%. The average absolute difference in relative humidity

between indoor and outdoor environments was 16 ± 8%. Significant variability existed in the air-exchange rates measured during occupancy (see Table 2), which ranged among the sites from 1.0 ± 0.3 h^{-1} to 7.4 ± 2.3 h^{-1}.

Aerosol and bioaerosol concentrations

Concentration results for airborne bacterial cells, fungal spore equivalents, and particulate matter (PM) mass include values for indoor air (occupied and vacant) and outdoor air (occupied and vacant). Size-resolved concentration profiles are presented in Figure 1. At all sites, occupancy resulted in a pronounced increase in airborne bacterial cell and fungal spore equivalent concentrations as well as PM concentration. Across all classrooms and for all particle sizes, the average ratios of occupied to vacant concentrations were 81 for bacteria, 15 for fungi, and 9 for PM mass. The median occupied indoor to outdoor (I/O) ratios were 27 times, 4 times, and 5 times greater than I/O ratios during vacant periods for bacterial cells, fungal spores, and PM mass respectively (Figure 2). Occupied-period I/O ratios were typically greater than unity for bacteria and PM mass, but not for fungi.

Tables S3 (indoor occupied), S4 (indoor vacant), and S5 (outdoor occupied) provide geometric mean (GM) and geometric standard deviation (GSD) size distribution data for the six sites. These values were estimated using a best-fit GM and GSD method, which has the advantage of not requiring knowledge of the upper size limit on the largest impactor bin (Yamamoto et al., 2014). The size distributions for bacterial and fungal concentrations were similar during occupancy. Across all sites, the average geometric mean particle diameters for the indoor occupied conditions were 5.5 μm for bacterial cells and 5.9 μm for fungal spores. In 11 out of 12 cases considered (i.e., for five of six sites for bacteria and for all six sites for fungi), geometric mean aerodynamic diameters for the indoor occupied aerosols were larger than for the indoor vacant conditions.

The measurement of size-distributed concentrations of bacteria, fungi, PM mass and particle number enables exploration of the relationships between microbiological and total particles. The quantified relationships demonstrate an enrichment of the bacterial mass and number fraction of particulate matter in indoor air compared to outdoor air. The results also show that fungi dominate over bacteria in contributing to the biological mass fraction of indoor particulate matter. Figure 3 shows the microbial to PM mass fractions for bacteria (top) and fungi (bottom). The changes in this fraction with aerodynamic diameter follow the profile commonly observed with bacterial and fungal concentrations (see Figure 1), with minima in the 0.4 to 1.1 μm stage and maxima in the 3.3-4.7 or 4.7-9 μm stages. The median bacterial mass fraction for all sizes combined is 300 ppm for indoor occupied and 20 ppm for outdoor conditions (sampled during occupancy), respectively. The comparable results are much larger for fungi, where the median indoor occupied mass fraction is 0.3% (i.e., 10× larger than the corresponding value for bacteria) and the median outdoor mass fraction is 1.4%.

Figure 4 shows size-resolved number ratios comparing bacteria to particles and fungal spores to particles. This ratio increases with increasing particle size in all cases. Median number ratios range from 10^{-7} in the smallest sizes to 10^{-1} in the largest size bins for both bacteria and fungi. For bacteria, the median number ratio for indoor occupied conditions (0.4) was higher than for the outdoor occupied case (0.2). These ratio differences between indoor occupied and outdoor conditions were the largest (by one to two orders of magnitude) for the three smallest size ranges. For fungi, the median ratio of fungal spore equivalents to total particle number was 0.5 for indoor occupied versus 0.8 for outdoor occupied cases, and was also higher than the corresponding bacterial number ratios.

Emission rates associated with occupancy

Table 3 summarizes total (summed over all sizes) emission rates for each of the six classrooms monitored. These emission rates average 14×10^6 cells person^{-1} h^{-1} for bacteria, 14×10^6 spore equivalents person^{-1} h^{-1} for fungi, and 22 mg person^{-1} h^{-1} for total PM mass. Results spanned more than one order of magnitude for bacteria and fungi amongst the different locations. No correlations across the six sites were observed between bacterial and PM emissions (r = 0.25, p = 0.63), bacterial and fungal emissions (r = -0.32, p = 0.53), or between fungal and PM emissions (r = 0.25, p = 0.63). Size-distributed emission rate profiles for bacteria, fungi, and PM mass for each site are presented in Figures 5 and 6, and show the statistical properties of particle size distributions for these emission rates. Peak emissions occur in the same size bin (4.7-9 μm diameter) for bacteria, fungi, and particulate matter. Quantitative comparisons among bacteria, fungi, and PM mass emissions were conducted by transforming number-based emission rates for bacteria and fungi into mass emission rates (Figure 7). The rank ordering in mass emission rates was total PM mass >> fungi > bacteria. Considering median values of occupancy-associated emission rates, fungi comprise ~ 0.2% and bacteria comprise ~ 0.02% of total PM mass.

Sources of bacteria, fungi, and particulate matter in indoor air: Occupant-associated emissions versus ventilation supply from outdoors

Figure 8 compares the primary indoor air source categories of bacteria, fungi, and PM mass, summed across all particle sizes. Source categories are divided broadly into those attributable to human occupancy ($N \times E$ in units of bacterial cells, fungal SE or PM mass h^{-1}), and those introduced from outdoor air in association with ventilation ($Q \times C_{out}$, in units of bacterial cells, fungal SE, or PM mass h^{-1}). For all three outcomes — airborne bacteria, airborne fungi, and airborne PM mass — indoor air levels were dominated by indoor emissions during times of occupancy. Considering all six locations, the average contributions of indoor emissions to the total source during occupancy were 83 ± 27% for bacteria, 63 ± 19% for fungi,

and 83 ± 24% for PM mass. In the two Chinese schools, where outdoor concentrations and air-exchange rates were both relatively high, the percent contribution from outdoor air was also correspondingly high (Figure 8).

Regarding the variation of source strength with particle size, the general trend in bacteria, fungi, and particulate matter mass was an increase in the relative strength of the occupancy-associated indoor emissions with increasing particle size. In the 18 cases considered, the fraction attributable to indoor sources increased with increasing particle size in all but one case. In this case, bacteria at the Lanzhou 1 school, the outdoor source dominated instead of indoor emissions (Figure S1).

DISCUSSION

This paper reports baseline data on size-resolved concentrations and occupancy-associated emission rates of bacteria, fungi, and particulate matter mass in six occupied school classrooms from four countries. These size-resolved concentration and emission results are useful for developing insight into how building design, operation, and occupancy influences exposures to airborne bacteria and fungi in indoor environments.

Concentrations

Human occupancy is a strong contributor to bacterial, fungal, and total particulate-matter mass concentrations in the indoor air of densely occupied spaces, such as classrooms. The airborne concentrations of bacteria, fungi and PM mass during occupancy were always higher than indoor air concentrations during vacant sampling periods. Bacteria, fungi and PM mass I/O ratios for the occupied

conditions were greater than the I/O ratios for the vacant cases, indicating that the elevated concentrations during occupancy could not be ascribed to variable outdoor conditions. Rather, these occupancy-associated increases are consistent with prior studies indicating that resuspension and human shedding are significant sources of airborne microorganisms and coarse-mode PM mass (Fox et al., 2003; Hospodsky et al., 2012; Meadow et al., 2014; Qian et al., 2014; Chen and Hildemann, 2009).

This study presents novel information on the size ranges in which bacteria and fungi may exist in indoor aerosols. As previously observed in a smaller data set (Qian et al., 2012), the geometric mean aerodynamic diameters of bacteria and fungi reported here are larger than those typically reported for isolated bacteria and fungi. It has previously been demonstrated that in aerosols, many organisms exist in aggregate with other cells, spores or attached to abiotic particulate matter (Wittmaack et al., 2005). Occupancy corresponded to an increase in the geometric mean diameter of bacterial, fungal, and PM sizes as compared with vacant conditions. This observation appears consistent with the large reported aerodynamic diameters of bacteria associated with shed skin (Fox et al., 2005), as well as prior observations of an increasing resuspension rate with increasing particle size (Thatcher and Layton, 1995).

To provide specific examples of the biological portions of commonly measured PM mass and particle number measurements, we also utilized the data collected in this study to develop bacteria:PM and fungi:PM relationships. Two novel observations emerge. First, the biological species to particle number ratio assessments suggest that a high proportion of airborne particles in sizes above 2.1-μm aerodynamic diameter had some bacterial or fungal content. We note that this observation is true for the computed average and our analysis cannot determine if these elevated fractions are distributed throughout all particles, or are a result of a subset of particles that contain multiple biological entities. Second, particulate matter mass and number concentrations in indoor air during occupancy are substantially

enriched in bacterial mass and number concentration compared to outdoor air. No equivalent enrichment was observed for fungi where mass and particle fractions were found to be slightly higher in outdoor air as compared to indoor air (Figures 3 and 4). This second observation supports a previously described view that many bacteria in indoor air are generated indoors, for example, from growth on human skin and direct shedding into the air, or from deposition onto floors and other surfaces from indoor sources with subsequent resuspension (Hospodsky et al., 2012). On the other hand, the primary origin of fungi in indoor air may be outdoor air, but occupancy amplifies the airborne concentrations by shedding and/or resuspending previously deposited material, e.g. from flooring and from clothing (McDonagh and Byrne, 2014). These inferences are supported by our observation that occupancy has a more pronounced effect on elevating bacterial concentrations than on increasing fungal concentrations.

Emissions

It has been reported that humans are a major source of bacteria in indoor air (Hospodsky et al., 2012; Meadow et al., 2014; Dunn et al., 2013; Adams et al., 2013) and that the fungi in indoor air are mainly derived from the outdoor environment (Adams et al., 2013). To a large extent, these prior reports are based on investigations that identified microbial community members and matched them with previously known human or environmental sources of that organism. Using mass balances, emission rates and other building-based characteristics, this same theme was investigated in the six school classrooms. An analysis of the indoor and outdoor source strengths determined that, in most cases in these classrooms, occupancy-associated indoor emissions are dominant for both airborne bacteria and fungi. Prior research on occupant-associated emissions of biological particles suggests that resuspension is more important than direct shedding (Hospodsky et al., 2012), that biological emission rates can be related to CO_2 concentration (Bhangar et al., 2014), and that resuspension rates are influenced by flooring types, particle

size, surface loading, human activity, and relative humidity (Ferro et al., 2004; Tian et al., 2014; Chen and Hildemann, 2009).

Limitations

The most important limitations in the present study derive from the small number of sites sampled combined with the diverse characteristics of those sites. While revealing some consistent trends, the scope of this investigation can only hint at the full richness of bioaerosol concentrations and emissions in classrooms and other densely occupied indoor environments. Other limitations exist in estimating emission rates and in calculating biological mass fractions and number ratios. Regarding the emission rate calculations, the assumption of a 20-µm upper aerodynamic diameter for the top impactor stage and the uncertainty in the deposition velocities of bioaerosol particles estimates have previously been discussed and are expected to cause only minor to moderate uncertainty in the emission rate results (Qian et al., 2012). With respect to the biological to PM mass and number ratios, there are inherent uncertainties in estimating the bacterial and fungal number and mass concentrations from qPCR data. The rRNA gene copy numbers in fungi are highly variable, even within a species (Herrera et al., 2009) and are not well cataloged for the full diversity of fungi. Fungal universal qPCR values presented here are spore equivalents of a standard *A. fumigatus* strain (Yamamoto et al., 2011). Thus, the variability in the 18S rRNA gene copy numbers might complicate estimation of fungal biomass. We used a geometric mean diameter for fungi of 2.5 µm for the mass calculation of a single spore, which likely underestimates the overall fungal mass (especially for dominant larger fungi including *Cladosporium, Alternaria* and *Epicoccum* spp. (Yamamoto et al., 2012)). The use of the measured particle size distributions would likely have resulted in an overestimation due to the potential for fungal spores to be attached to abiotic particles. We note that, although different approaches were used, our average outdoor fungal mass fraction was 1.4%, which compares favorably with prior reports of fungal mass being 1 to 7% of PM in

the atmosphere (Bauer et al., 2008). Given the less variable size of bacteria and the known distribution of 16S rDNA genes per cell, biological mass and number estimates are less uncertain for bacteria than they are for fungi. Other comparisons and conclusions made using these biological to PM ratios focus on comparisons between indoor and outdoor cases, and these results, thus normalized, will not be impacted to the same extent by potential errors in the absolute values presented in Figures 3 and 4. Finally, the authors acknowledge that losses associated with particle bounce in impactors are common. To reduce these impacts, we used Nuclepore filter substrates, which are deformable, and which have been shown to significantly decrease bounce effects relative to aluminum substrates (Chang et al., 1999). Reported variation of bounce effects with particle size in the 2 to 9 μm are typically less than 10%, suggesting that bounce was not a major factor influencing our reported GM and GSD aerodynamic diameters. Furthermore, we have found in our earlier work that bacterial concentrations were typically not detected on after filters, which provides evidence that bounce does not result in the loss of total microorganisms from our impactor.

CONCLUSIONS

This study provides new baseline data for characterizing the size-resolved concentrations and emissions of bacteria and fungi in occupied indoor environments. In the environments tested, human occupancy resulted in the significant elevation of concentrations of airborne bacteria and fungi. The similar particle size distributions of bacteria, fungi, and PM mass in indoor occupied air, and the enrichment of bacterial mass and bacterial particle fractions in occupied settings, reveal the important role of human shedding and/or resuspension in shaping the indoor air microbiome. While outdoor air may be the ultimate origin for most airborne fungal particles in buildings without moisture problems, in these heavily occupied classrooms, occupancy driven mechanisms were dominant sources of both bacteria and fungi for indoor air. Novel information on bacterial and fungal particle size distributions and the size-

resolved relationships between microbial and total mass of indoor and outdoor aerosols is presented and archived. Because aerodynamic diameter strongly influences the fate of and human exposure to biological aerosols, the size-resolved data of the type presented here are of fundamental value for developing quantitative insights regarding human exposure in indoor air.

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Tables

Table 1 Physical characteristics of monitored classrooms.

Site name[a]	Volume [m^3]	Floor area [m^2]	Occupant density [person/m^2]	Number of windows	Operable windows? Yes/ No	Mechanical ventilation
Copenhagen	187	62	0.20	6	Yes	No
Berlin	225	60	0.17	2	Yes	No
Aarhus	260	80	0.15	8	Yes	No
Salinas	160	61	0.18	1	No	Yes
Lanzhou 1	185	53	0.15	4	Yes	No
Lanzhou 2	171	48	0.24	3	Yes	No

[a] Lanzhou 1, China (preschool classroom 1)

Lanzhou 2, China (preschool classroom 2)

Table 2 Building ventilation and thermal environment characteristic during occupied sampling periods (average **±** standard deviation).

Site name	AER [h^{-1}]	T_{in} [ºC]	T_{out} [ºC]	RH_{in} [%]	RH_{out} [%]
Copenhagen	1.8±0.5	20±2	14±3	47±4	64±12
Berlin	4.6±2.5	27±1	20±3	33±3	59±10
Aarhus	1.1±0.3	30±2	18±2	25±4	50±10
Salinas	1.0±0.3	26±1	31±4	38±2	25±7
Lanzhou 1	5.1±1.0	27±1	35±9	36±2	26±9
Lanzhou 2	7.4±2.3	28±5	33±6	31±8	23±8

Table 3. Emission rates for bacteria, fungi, and PM mass.

Site name	Bacteria [10^6 cells/person-h]	Fungi [10^6 SE[a]/person-h]	PM [mg/person-h]
Copenhagen	1.0	3.0	18
Aarhus	25	3.2	33
Berlin	4.3	57	30
Salinas	0.8	4.7	8.1
Lanzhou 1	35	8.5	25
Lanzhou 2	19	9.2	18
Arithmetic mean ± std. dev.	**14 ± 14**	**14 ± 21**	**22 ± 9**

[a] SE denotes spore equivalent

Figure Legends

Fig 1 Size distributions of airborne bacterial cell concentrations ($\Delta N/\Delta \log d_p$, 1000 cells m^{-3}), spore equivalent fungal concentrations ($\Delta N/\Delta \log d_p$, 1000 SE m^{-3}) and PM mass concentrations ($\Delta M/\Delta \log d_p$, µg m^{-3}) collected in indoor air during occupied and vacant periods and in outdoor air. Solid lines represent indoor occupied conditions, dashed lines represent indoor vacant conditions, and dotted lines represent outdoor air (sampled during occupancy). The numerical values within each frame report the total concentrations for all sizes and carry units of cells/m^3 for bacteria, spore equivalents m^{-3} for fungi, and µg m^{-3} for PM. An upper limit of 20-µm aerodynamic diameter is assumed for the largest impactor stage.

Fig 2 Indoor-to-outdoor concentration ratios (I/O) for occupied (closed circles) and vacant (open circles) conditions for bacteria (top), fungi (middle) and particulate matter mass (bottom). Because concentrations were below detection levels, the vacant I/O ratio was not determined for Salinas bacteria. For Lanzhou 2, the bacterial I/O ratios are nearly equal and the symbols overlap.

Fig 3 Particulate matter mass fractions of bacteria (top) and fungi (bottom) for indoor and outdoor occupied periods. These size-resolved mass ratios were determined by converting cell or spore concentration into a mass concentration, and then dividing by the PM mass concentration. Each box and whisker summarizes values from the six sites. Boxes frame the interquartile range, the middle lines represent medians, and the ends of the whiskers indicate the full data range.

Fig 4 Particle number ratios of bacterial cells (top) or fungal spores equivalents (bottom) to total particles for indoor and outdoor occupied periods. Number ratios were determined by dividing time-averaged bacterial concentrations from impactor samples by average particle concentrations determined by OPC monitoring. Each box and whisker includes values from all six sites. Boxes frame the interquartile range, the middle lines represent medians, and ends of the whiskers indicate the full data range.

Fig 5 Per person emission rates of total airborne particulate matter, bacterial cells, and fungal spores in the six occupied classrooms studied.

Fig 6 Fraction of total per person emission rates represented by specific size ranges. Dashed lines on the plots report the interquartile range, separated by the median (solid line).

Fig 7 Mass-based per person emission rates for bacteria, fungi, and PM. Boxes on the plots report the interquartile range, with the median indicated by the intermediate line. Error bars represent the full data range.

Fig 8 Comparison of the relative contributions of outdoor sources (QC_{out}) versus occupancy-associated indoor emission sources (emission rates, E) for bacteria (cells h^{-1}), fungi (spore equivalents h^{-1}), and airborne particle mass (g h^{-1}). Data represent the sums across all particle sizes measured.