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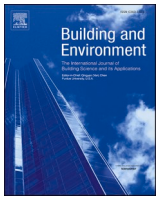

More diverse school microbiota may provide better protection against respiratory infections for school staff

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ABSTRACT

Our understanding of how exposure to school microbiota affects the respiratory health of staff and students in schools is limited. We examined the associations between exposure to school microbiota and respiratory and gastrointestinal infections. We performed an epidemiologic analysis of 1,529 school employees in the U.S. A questionnaire was administered to school staff to collect health information, and floor dust was vacuumed from 500 classrooms in 50 schools. Fungal internal transcribed spacer region and bacterial 16S amplicon sequencing were performed with extracted genomic DNA using Illumina Mi-Seq platform. The resulting DNA sequences were clustered into operational taxonomic units (OTUs). Staff were assigned the school-building-specific floor average number of bacterial or fungal OTUs from the same floor as their exposure. We used logistic regression models to estimate adjusted odds ratios of reported respiratory and gastrointestinal infections in the last 12 months. Exposure to the highest quartile in number of OTUs (Q4, highest richness) of the bacterial phyla Firmicutes or Actinobacteria was associated with 28–61 % lower odds of upper or lower respiratory infections compared to the lower three quartiles (Q123). Higher Firmicutes diversity was more strongly associated with upper respiratory infections, while greater Actinobacteria diversity showed a stronger association with lower respiratory infections. Fungal diversity was not associated with any type of infection, and neither bacterial nor fungal diversity was associated with gastrointestinal infections. Our study suggests that exposure to a highly diverse bacterial microbiota in school environments may play an important role in protecting school staff against respiratory infections.

1. Introduction

Indoor microbial exposure is an important contributing factor to occupants' health. The classroom is an indoor space where teachers and students spend the majority of their time while in school [1–3]. In the United States, there were approximately 3.8 million teachers for the 2020–2021 school year [4] and more than 49.6 million K-12 students were enrolled in Fall 2022 [5]. In schools, upper and lower respiratory infections frequently occur among teachers and students through the transmission of respiratory pathogens [6,7].

Humans constantly inhale airborne environmental microbes encountered both indoors and outdoors, and it has been hypothesized that the interaction between environmental and endogenous microbiomes in the upper and lower respiratory tracts influences occupants' susceptibility to infection from inhaled pathogens [8,9]. A 2018 review article [10] suggested that susceptibility to respiratory tract infections with airborne viruses or bacteria may be influenced by changes in the nasal bacterial microbiota that can occur due to environmental exposures as well as natural aging. The balanced microbial composition colonizing the human respiratory tract that helps resist infection may also be strengthened or disturbed by constant exposure to certain environmental microbiota [8,11]. This implies that environmental microbes in air we breathe may play an important role in maintaining our respiratory health. In addition, inadvertent ingestion of indoor dust containing diverse microbes may also affect the gut microbiota, potentially influencing gastrointestinal infections [12,13].

In indoor environments (e.g., classrooms) without combustion, cooking, or smoking, abiotic and biotic resuspension is the primary

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source of airborne particulates [14]. It has been estimated that resuspended dust from indoor surfaces can account for up to 60 % of the airborne indoor dust, and resuspended biological particles from the floor and other surfaces are a primary source of inhalation exposure to microbes in densely occupied indoor spaces [2,15,16]. Despite the importance of school indoor environments for student and teacher health, the microbiological composition of schools is relatively less studied than that in residential indoor environments. Similarly, epidemiologic studies of the effects of environmental microbiota on infections in school staff have not been reported in the literature. In this study, we examine these knowledge gaps and the associations between microbial richness in classroom floor dust and reported respiratory and gastrointestinal infections as part of a large epidemiologic study of public school workers in Philadelphia, Pennsylvania (PA) in the United States.

2. Methods

2.1. Epidemiologic study

We conducted a cross-sectional epidemiologic study of employees in 50 public elementary schools in Philadelphia, PA during 2015 – 2016 [17,18]. From a preliminary study that assessed water damage within the school district, we selected 50 schools with more than 350 students each, representing a spectrum from no to substantial moisture damage, for our study. During the summer of 2015, a total of 3006 staff members from these schools were invited to participate in a secure web-based health questionnaire survey. Information pertaining to informed consent was included on the first page of the questionnaire, and invitees were informed that completing the questionnaire would constitute consenting to participate in the study. The study was reviewed and approved by the National Institute for Occupational Safety and Health (NIOSH) Institutional Review Board (Protocol ID: 13-DRDS-02XP).

2.2. Environmental study

In the summer of 2015, we collected one composite floor dust sample from each of the 500 classrooms (hard floorings: >97 % of classrooms), with ten classrooms per school. These ten classrooms were carefully selected from various floors within each school, considering both spatial distribution and the number of study participants on each floor. To ensure better representation, more classrooms were selected from the floor with a higher number of participants. The details for the environmental sampling procedure were reported previously [17,18]. Briefly, we measured and marked the floor-wall junction around the entire perimeter of each room, covering a total of 12 square feet. We then vacuumed the area for eight minutes using a precleaned crevice tool attached to a L'il Hummer backpack vacuum sampler (100 ft^3/min, 1.5 horsepower, ProTeam Inc., Boise, ID, USA) equipped with a polyethylene filter sock (Midwest Filtration Company, Fairfield, OH, USA). The collected dust was sieved with a mesh (pore size: 250 μm), homogenized, and then partitioned into aliquots for analysis. Generally, all schools cleaned classrooms after school by vacuuming the floors. On the sampling day, we requested custodians to hold off on vacuuming or mopping the floors until we had completed our sample collection to ensure we maximized our dust collection. Unfortunately, one classroom had been vacuumed and mopped with water just before our sampling, and floor dust could not be collected from that classroom. Therefore, a total of 499 classroom floor dust samples were analyzed for bacterial and fungal DNA. The majority of the sampled classrooms relied on natural ventilation for air circulation by opening the windows. We used the National Institute for Occupational Safety and Health (NIOSH) Dampness and Mold Assessment Tool to collect dampness and mold-related information for the classrooms (https://www.cdc.gov/niosh/docs/2019–114/) and calculated average room dampness/mold scores.

2.3. Genomic DNA extraction

We extracted genomic DNA (gDNA) from 499 samples and 30 reagent blanks (negative controls) using the Roche High Pure PCR Template Preparation Kit (Roche Applied Sciences, Penzberg, Germany) as previously described [17–19]. Briefly, 5 mg of dust was suspended in the kit's tissue lysis buffer (250 μL), which was added to a 2 mL reinforced tube containing 300 mg of glass beads (212−300 μm, Sigma-Aldrich, St. Louis, MO) and homogenized with a bead mill homogenizer at a rate of 4.5 m/s for 30 s. Then, the tubes were centrifuged for two cycles at 20, 000 × g for 1 min to collect the lysis supernatants. The supernatants were placed in sterile 1.5 mL microcentrifuge tubes with 20 μL of Cel-Lytic B Cell Lysis reagent (Sigma-Aldrich) that was incubated at 37 °C for 15 min. We added the kit's binding buffer (200 μL) and proteinase K solution (40 μL) into the tubes that were incubated at 70 °C for 10 min. The samples were washed and eluted (Roche Applied Sciences, Penzberg, Germany), and 20 μL aliquots were stored at -80 °C until analysis.

2.4. Bacterial 16S and Fungal ITS Region Amplification, Sequencing, and Taxonomic Identification

Extracted gDNA samples were shipped to RTL Genomics (Lubbuck, TX) for Illumina Mi-Seq sequencing of the bacterial 16S rRNA genes and the fungal internal transcribed spacer 1 (ITS1) region. Bacterial DNAs were amplified using the primer pair (357wF: CCTACGGGNGGCWGCAG; and 806R: GGACTACHVGGGTWTCTAAT) and sequenced [20]. Fungal DNAs were amplified using the primer pair (ITS1F: CTTGGTCATTTAGAGGAAGTAA; and ITS2aR: GCTGCGTTCTTCATCGATGC) and sequenced as previously described [20]. Sequences with failed reads, low-quality tags and/or less than half the expected amplicon length were removed. Using the PEAR Illumina paired end read merger, paired sequences were merged [21]. Using an RTL Genomics internal trimming algorithm, the sequences were trimmed prior to clustering into operational taxonomic units (OTUs) using a 96 % similarity threshold with a USEARCH clustering algorithm [20,22,23]. Using the UPARSE OTU selection algorithm [23], OTUs were selected, and using the UCHIME chimera detection software chimeras were checked [24]. Bacterial and fungal taxa of representative OTU sequences were identified using a USEARCH global search algorithm [25] by comparing them to a database maintained by RTL Genomics of high-quality sequences derived from the National Center for Biotechnology Information database.

2.5. Statistical analyses

The health outcome variables for respiratory and gastrointestinal infections in our statistical models included self-reported influenza-like illness; lower respiratory infections such as pneumonia or acute bronchitis; upper respiratory infections involving the nose, sinuses, throat, ears, or the common cold; and gastrointestinal symptoms like a sudden onset of nausea, vomiting, or diarrhea lasting one or more days-all within the last 12 months. Influenza-like illness was defined as an episode of fever and cough that came on rapidly and lasted one or more days, potentially accompanied by fatigue, muscle aches, or sore throat. As not all the 1529 questionnaire participants had a floor dust sample from their own room, we assigned an average of the environmental measurements taken from classrooms on the same floor in each school building to those who spent most of their time on that floor for their exposure assessment. The number of samples per floor in each school ranged from one to nine, with a median of three. Out of the initial 1529 participants who completed the questionnaire, 116 were excluded from analysis because they either didn't provide school names, their school names weren't listed among the selected 50 schools with environmental samples, or their school floors lacked environmental measurements. Therefore, a total of 1413 participants were assigned exposure values.

The number of bacterial and fungal OTUs representing microbial

richness (the total number of different types of microorganisms), a measure of alpha diversity (diversity within a single community or sample) [26,27], was our exposure of interest. To identify the bacterial or fungal subgroups associated with infections, we also created richness variables for Gram-positive and negative bacteria, bacteria in the phyla Actinobacteria and Firmicutes, and yeasts for fungi by counting the number of OTUs for each subgroup classification. Categorical variables were created using the quartile (four-level variables) in the number of OTUs or the lowest three quartiles versus the highest quartile in the number of OTUs (binary variables: Q123 versus Q4). We also performed multivariable logistic regression models using richness (number of OTUs) as a continuous variable.

To examine the difference in average age or proportion of characteristics of the participants between teachers and non-teaching staff, a two-sample *t*-test for age and a two-sample test for equality of proportion were used [28,29]. An unadjusted two-sample test for equality of proportions of respiratory and non-respiratory infections was also performed between teachers and non-teaching staff, and low and high richness (Q123 versus Q4) of total fungi, yeast, total bacteria, Actinobacteria, and Firmicutes [28,29]. Unadjusted natural cubic spline curves (degrees of freedom=3) of the probability of respiratory infections against bacterial richness were examined. To examine adjusted associations between infections and bacterial or fungal diversity, we used multivariable logistic regression models. The models were adjusted for age, sex, race, ethnicity, current smoking, being a teacher, current asthma, hay fever, classroom dampness/mold score, and home mold odour in the last 12 months that could be independent risk factors or confounders. Due to the numerous infection variables and the many subclass variables of bacteria and fungi, which required extensive analyses, the statistical analysis began with a focus on the richness of Gram-positive or Gram-negative bacteria. Gram-positive bacteria consist of Actinobacteria and Firmicutes, while Gram-negative bacteria include all other phyla. Because the richness of Gram-positive bacteria was significantly associated with most respiratory infections, we conducted further analysis to determine which specific phylum within Gram-positive bacteria was driving the negative associations. However, since Gram-negative bacteria were not significantly associated with respiratory infections in the multivariable models, except for acute bronchitis, we did not pursue further analysis for its subclass or phylum. Generalized additive models adjusted for the covariates above were used to test for the non-linear exposure-response relationships with penalized cubic spline regression with 30 knots [30]. All statistical analyses were performed with R version 4.3.0 [31], and statistical significance was set at α=0.05 and marginal significance at=0.1.

3. Results

3.1. Demographics and distribution of richness

The overall participation rate was 50.9 %, with 1529 respondents; however, response rates for certain individual questions were lower due to some participants opting not to answer specific questions on the survey (Table 1). Of these respondents, 82.1 % were teachers, and the average age was 44.5 years (standard deviation=11.2) with teachers being 6-years younger on average than non-teachers (P-value < 0.01). The majority of the participants were female (85 %), white (70 %), non-Hispanic (95.7 %), and non-smokers (93.7 %). While 16 % of the respondents reported current asthma, more than half (56 %) reported physician-diagnosed hay fever. Eleven percent of participants reported mold odor in their homes in the last 12 months. Compared to non-teaching staff, the teacher participant group was comprised of significantly more white individuals, had fewer Hispanic individuals, and fewer current smokers (P-values < 0.04, Table 1).

The 75th percentile of the assigned floor average number of OTUs for total bacteria among the questionnaire participants was 460 (median=408) while the 75th percentile for total fungi was 181 (median=160) (Figure S1). The 75th percentile of the OTUs for Gram-positive bacteria (Actinobacteria and Firmicutes) was 230, which was similar to that for Gram-negative bacteria (229). Within Gram-positive bacteria, the 75th percentile of the assigned OTUs in Actinobacteria was higher (122) than that in Firmicutes (108). On average, yeast OTUs accounted for 15.6 % of total fungal OTUs (Table 2 and Figure S1). The 75th percentiles of the assigned OTUs were slightly lower than those in the original 499 samples (Table S1).

3.2. Unadjusted prevalence of infections

Prevalences of acute bronchitis, pneumonia, and influenza-like illnesses in the last 12 months were 18 %, 4.3 %, and 53 %, respectively (Table 3). Teachers reported more influenza-like illnesses than non-teachers (54 % versus 45 %, P=0.01). Of the upper respiratory infections, the common cold was the most prevalent (64 %), followed by sinus infection (55 %), nasal infection (52 %), throat infection (49 %), and ear infection (25 %). All upper respiratory infections were more prevalent in teachers than non-teachers (P-values<0.001), while the prevalence of ear infection was similar in two groups. Prevalences of gastrointestinal infections were generally lower than those of respiratory infections and also significantly (P-values<0.02) higher in teachers versus non-teachers. The most prevalent gastrointestinal infection reported was diarrhea (35 %) while vomiting was the lowest at 17 %.

In unadjusted analyses, prevalences of all infections in the highest richness quartile (Q4) for total bacteria were lower than those of the

Table 1
Characteristics of the study population by teachers and non-teaching staff.

Characteristics	Overall, N = 1501[1]	Teacher N = 1232[1]	Non-teacher N = 269[1]	Difference[2]	95 % CI[2, 3]	P-value[2]
Age: Mean ± SD, year	44.5 ± 11.2	43.4 ± 11.2	49.4 ± 9.9	-6.0	-7.5, -4.6	<0.001
Sex (female)	1230/1439 (85 %)	1016/1185 (86 %)	214/254 (84 %)	1.5 %	-3.7 %, 6.6 %	0.6
Race (white)	1027/1470 (70 %)	882/1211 (73 %)	145/259 (56 %)	17 %	10 %, 24 %	<0.001
Hispanic/Latino	59/1361 (4.3 %)	39/1127 (3.5 %)	20/234 (8.5 %)	-5.1 %	-9.1 %, -1.1 %	<0.001
Current smoker	85/1350 (6.3 %)	63/1122 (5.6 %)	22/228 (9.6 %)	-4.0 %	-8.4 %, 0.29 %	0.033
Current asthma	211/1345 (16 %)	173/1116 (16 %)	38/229 (17 %)	-1.1 %	-6.6 %, 4.4 %	0.8
Physician-diagnosed hay fever	767/1360 (56 %)	637/1129 (56 %)	130/231 (56 %)	0.14 %	-7.0 %, 7.3 %	>0.9
Mold odor at home in the last 12 months	149/1351 (11 %)	127/1122 (11 %)	22/229 (9.6 %)	1.7 %	-2.8 %, 6.2 %	0.5
Assigned floor average dampness score	1.3 (0.7)	1.3 (0.7)	1.4 (0.9)	-0.08	-0.2, 0.05	0.2

Mean ± SD for the age variable;
[1] n/N (%) for the rest of the binary variables; 28 people who did not answer the question on job title were removed from the analyses. Thus, the total number of observations for this analysis is 1501, instead of 1529. For individual questions, the denominators for percentage calculations are different, depending on the number of participants who did not answer a particular question (percent range of unanswered question: 1.8 % (job title)–13 % (age);
[2] Welch Two Sample *t*-test for the age variable and two sample test for equality of proportions for the rest of binary variables
[3] CI = Confidence Interval.

Table 2
Summary of the assigned floor average operational taxonomic units (OTUs) for health survey participants, categorized by bacterial and fungal groups.

Bacterial and Fungal Groups	Statistics, N = 1413[1]
Total bacteria	
Median (75th percentile)	408 (460)
Range	240–659
Gram positive bacteria	
Median (75th percentile)	205 (230)
Range	107–366
Actinobacteria	
Median (75th percentile)	110 (122)
Range	56–181
Firmicutes	
Median (75th percentile)	98 (108)
Range	35–185
Gram negative bacteria	
Median (75th percentile)	206 (229)
Range	116–334
Total fungi	
Median (75th percentile)	160 (181)
Range	63–277
Yeasts	
Median (75th percentile)	25 (30)
Range	6–49

[1] N: due to incomplete information on schools and floors, 116 participants in the health questionnaire survey could not be assigned floor average OTUs. Thus, the total number of observations in this table is 1413, instead of 1529.

combined lower three quartiles (Q123) (Table S2); however, the difference in prevalence was only significant (P=0.04) for diarrhea (-6.5 %); marginally significant (0.05< P-values <0.1) for the acute bronchitis, influenza-like illness, common cold, sinus infection, and nausea (range of difference: -4.6 % - -5.9 %); but not significant for the rest of the infections (-0.06 % - -5.2 %). The unadjusted prevalences of infections in Q4 and Q123 were reassessed by examining these prevalences in relation to the diversity within the phyla Actinobacteria and Firmicutes. The observed prevalence differences between Q4 and Q123 for OTUs within the phylum Actinobacteria were increased compared to those associated with total bacterial richness. Specifically, for influenza-like illness, this difference grew from -5.7 % to -8.4 %, representing a 47.4 % decrease in association; for common colds, from -5.4 % to -7.9 %, a 46.3 % decrease; for nasal infections, from -5.2 % to -8.4 %, a 61.5 % decrease; for sinus infections, from -5.7 % to -7.5 %, a 31.6 % decrease; and for throat infections, from -2.4 % to -5.3 %, an increase by a factor of 2.1 times. These heightened differences achieved statistical significance at an alpha level of 0.05, as indicated in Tables 4 and S1. In contrast, the magnitude of the negative associations between Actinobacteria and gastrointestinal infections diminished and became statistically insignificant when compared to those observed with total bacteria (Tables 4 and S1). Furthermore, we observed a consistent pattern in the shift of negative associations with infections between total bacteria and the phylum Firmicutes—the latter exhibited stronger negative associations with upper respiratory infections (Tables 5 and S1). On the other hand, prevalences of ear and nose infections in Q4 of total fungi were lower [difference: 6.1 % for ear infection (P=0.03) and 5.6 % for nasal infection (P=0.09)] than Q123 while prevalence of vomiting in Q4 was marginally (P=0.06) higher than that of Q123 (Table S3). Richness of yeast as a sub-group of total fungi did not show any significant difference in prevalences for any infections between Q4 and Q123 (Table S4).

3.3. Adjusted associations of infections with bacterial or fungal richness

Multivariable logistic regression models using richness as a continuous variable indicated that influenza-like illness, pneumonia, acute bronchitis, and sinus infections were linearly and negatively associated with the Actinobacteria (P-values<0.05) (data not shown). The common cold, and nose infections were linearly and negatively associated with the phylum Firmicutes (P-values<0.05) (data not shown). Unadjusted exposure-response relationships between probability of these infections and Actinobacteria or Firmicutes richness using the cubic spline function with three degrees of freedom are presented in Figure S2. In adjusted (multivariable) generalized additive models, the tests of non-linearity in the exposure-response relationships did not indicate significant non-linear associations, except for sinus infection with Firmicutes (P=0.025).

Multiple logistic regression models analyzing respiratory infections, with a binary variable comparing Q4 versus Q123 in terms of total bacterial richness, indicated decreased odds of infection in the group with the highest diversity (Tables 6 and S4). However, only the odds ratios for acute bronchitis, common cold, and nasal infections (with a range of odds ratios from 0.62 to 0.72) reached statistical significance (P-values < 0.05). However, analyses from models utilizing sub-taxonomic groups of bacteria revealed that the decreased odds associated with high diversity in the total bacterial community were primarily driven by the diversity found within either the Actinobacteria or Firmicutes phyla. The odds of all respiratory infections, except for ear infections, were significantly and inversely associated with richness in the Actinobacteria or Firmicutes phyla. Moreover, the odds ratios for the highest quartile of richness (Q4) within these phyla were similar to, or even lower than, those observed for total bacterial richness (Table 6). The odds of lower respiratory infections were significantly reduced-by

Table 3
Prevalence of respiratory and gastrointestinal infections reported in the past 12 months.

Type of Infection	Overall, N = 1501[1]	Teacher N = 1232[1]	Non-teacher N = 269[1]	Difference[2]	95 % CI[2,3]	P-value[2]
Acute bronchitis	247/1347 (18 %)	214/1123 (19 %)	33/224 (15 %)	4.3 %	-1.1 %, 9.8 %	0.2
Pneumonia	59/1358 (4.3 %)	50/1130 (4.4 %)	9/228 (3.9 %)	0.48 %	-2.6 %, 3.5 %	0.9
Influenza-like illness	719/1366 (53 %)	615/1135 (54 %)	104/231 (45 %)	9.2 %	1.9 %, 16 %	0.014
Common cold	871/1357 (64 %)	752/1127 (67 %)	119/230 (52 %)	15 %	7.7 %, 22 %	<0.001
Nose infection	712/1363 (52 %)	614/1131 (54 %)	98/232 (42 %)	12 %	4.8 %, 19 %	0.001
Sinus infection	748/1360 (55 %)	645/1130 (57 %)	103/230 (45 %)	12 %	5.0 %, 20 %	<0.001
Throat infection	670/1355 (49 %)	593/1128 (53 %)	77/227 (34 %)	19 %	12 %, 26 %	<0.001
Ear infection	334/1356 (25 %)	287/1126 (25 %)	47/230 (20 %)	5.1 %	-1.0 %, 11 %	0.12
Vomiting	230/1356 (17 %)	205/1126 (18 %)	25/230 (11 %)	7.3 %	2.5 %, 12 %	0.009
Diarrhea	481/1362 (35 %)	416/1132 (37 %)	65/230 (28 %)	8.5 %	1.8 %, 15 %	0.017
Nausea	442/1360 (32 %)	384/1129 (34 %)	58/231 (25 %)	8.9 %	2.4 %, 15 %	0.011

[1] n/N (%) for the rest of the binary variables; 28 people who did not answer the question on job title were removed from the analyses. Thus, the total number of observations for this analysis is 1501. For individual questions, the denominators for percentage calculations are different, depending on the number of participants who did not answer a particular question (percent range of unanswered question: 1.8 % (job title)–13 % (age);

[2] Two sample test for equality of proportions;

[3] CI = Confidence Interval. The denominators for percentage calculations are different, depending on the number of participants who did not answer a particular question (percent range of unanswered question: 8.8 % (Influenza-like illness)–10 % (Acute bronchitis).

Table 4
Unadjusted prevalence of respiratory and gastrointestinal infections by exposure group based on Actinobacteria richness (fourth quartile, Q4, versus reference group, the lower three quartiles, Q123 in the number of OTUs).

Type of Infection	Actinobacteria Q4, N = 353[1]	Q123, N = 1060[1]	Difference[2]	95 % CI[2, 3]	P-value[2]
Acute bronchitis	49/329 (15 %)	188/945 (20 %)	-5.0 %	-9.8 %, -0.18 %	0.054
Pneumonia	10/326 (3.1 %)	47/959 (4.9 %)	-1.8 %	-4.4 %, 0.69 %	0.2
Influenza-like illness	153/328 (47 %)	530/963 (55 %)	-8.4 %	-15 %, -1.9 %	0.010
Common cold	193/328 (59 %)	636/953 (67 %)	-7.9 %	-14 %, -1.6 %	0.012
Nose infection	152/329 (46 %)	524/959 (55 %)	-8.4 %	-15 %, -2.0 %	0.010
Sinus infection	162/326 (50 %)	548/958 (57 %)	-7.5 %	-14 %, -1.0 %	0.022
Throat infection	149/323 (46 %)	492/957 (51 %)	-5.3 %	-12 %, 1.2 %	0.11
Ear infection	70/328 (21 %)	246/953 (26 %)	-4.5 %	-9.9 %, 0.97 %	0.12
Vomiting	50/327 (15 %)	168/957 (18 %)	-2.3 %	-7.1 %, 2.5 %	0.4
Diarrhea	114/328 (35 %)	345/959 (36 %)	-1.2 %	-7.4 %, 5.0 %	0.7
Nausea	96/330 (29 %)	325/956 (34 %)	-4.9 %	-11 %, 1.0 %	0.12

[1] n/N (%) for the rest of the binary variables; 116 people who did not assigned exposure were excluded in the analyses. Thus, the total number of observations for this analysis is 1413;
[2] Two sample test for equality of proportions;
[3] CI = Confidence Interval.

Table 5
Unadjusted prevalence of respiratory and gastrointestinal infection reported by exposure group based on Firmicutes richness (fourth quartile versus reference group, the lower three quartiles in the number of OTUs).

Type of Infection	Firmicutes Q4, N = 353[1]	Q123, N = 1060[1]	Difference[2]	95 % CI[2, 3]	P-value[2]
Acute bronchitis	54/322 (17 %)	183/952 (19 %)	-2.5 %	-7.4 %, 2.5 %	0.4
Pneumonia	15/324 (4.6 %)	42/961 (4.4 %)	0.26 %	-2.6 %, 3.1 %	>0.9
Influenza-like illness	152/327 (46 %)	531/964 (55 %)	-8.6 %	-15 %, -2.1 %	0.009
Common cold	197/326 (60 %)	632/955 (66 %)	-5.7 %	-12 %, 0.55 %	0.071
Nose infection	154/328 (47 %)	522/960 (54 %)	-7.4 %	-14 %, -0.97 %	0.024
Sinus infection	159/325 (49 %)	551/959 (57 %)	-8.5 %	-15 %, -2.1 %	0.009
Throat infection	145/326 (44 %)	496/954 (52 %)	-7.5 %	-14 %, -1.1 %	0.023
Ear infection	74/326 (23 %)	242/955 (25 %)	-2.6 %	-8.2 %, 2.9 %	0.4
Vomiting	52/326 (16 %)	166/958 (17 %)	-1.4 %	-6.2 %, 3.5 %	0.6
Diarrhea	104/327 (32 %)	355/960 (37 %)	-5.2 %	-11 %, 0.93 %	0.11
Nausea	102/328 (31 %)	319/958 (33 %)	-2.2 %	-8.2 %, 3.8 %	0.5

[1] n/N (%) for the rest of the binary variables; 116 people who did not assigned exposure were excluded in the analyses. Thus, the total number of observations for this analysis is 1413;
[2] Two sample test for equality of proportions
[3] CI = Confidence Interval.

Table 6
Adjusted odds ratio (OR) and 95 % confidence interval (CI) of bacterial richness in floor dust (fourth quartile versus reference group, the lower three quartiles in the number of OTUs) for respiratory infections in the past 12 months.

Type of Infections	Type of bacteria	Adjusted OR (95 % CI)	P-value
Acute bronchitis			
	Total bacteria	0.62 (0.40–0.92)	0.02
	Gram-positive[1]	0.61 (0.40–0.91)	0.02
	Actinobacteria	0.56 (0.37–0.84)	0.01
	Firmicutes	0.83 (0.56–1.21)	0.34
	Gram-negative	0.54 (0.35–0.82)	<0.01
Pneumonia			
	Total bacteria	0.62 (0.27–1.28)	0.22
	Gram-positive[1]	0.83 (0.39–1.66)	0.62
	Actinobacteria	0.39 (0.14–0.88)	0.04
	Firmicutes	1.13 (0.56–2.17)	0.73
	Gram-negative	0.64 (0.28–1.32)	0.25
Influenza-like illness			
	Total bacteria	0.76 (0.57–1.02)	0.07
	Gram-positive[1]	0.72 (0.53–0.97)	0.03
	Actinobacteria	0.66 (0.49–0.89)	0.01
	Firmicutes	0.70 (0.52–0.94)	0.02
	Gram-negative	0.78 (0.58–1.04)	0.09
Common cold			
	Total bacteria	0.71 (0.52–0.97)	0.03
	Gram-positive[1]	0.67 (0.49–0.92)	0.01
	Actinobacteria	0.72 (0.52–0.98)	0.04
	Firmicutes	0.70 (0.51–0.97)	0.03
	Gram-negative	0.82 (0.60–1.12)	0.21
Nose infection			
	Total bacteria	0.72 (0.53–0.97)	0.03
	Gram-positive[1]	0.69 (0.51–0.95)	0.02
	Actinobacteria	0.72 (0.53–0.98)	0.03
	Firmicutes	0.68 (0.50–0.92)	0.01
	Gram-negative	0.79 (0.58–1.07)	0.12
Sinus infection			
	Total bacteria	0.75 (0.55–1.03)	0.07
	Gram-positive[1]	0.73 (0.53–1.00)	0.05
	Actinobacteria	0.76 (0.56–1.04)	0.09
	Firmicutes	0.67 (0.49–0.92)	0.01
	Gram-negative	0.83 (0.61–1.13)	0.23
Throat infection			
	Total bacteria	0.82 (0.60–1.11)	0.19
	Gram-positive[1]	0.86 (0.63–1.17)	0.33
	Actinobacteria	0.82 (0.61–1.11)	0.20
	Firmicutes	0.68 (0.50–0.91)	0.01
	Gram-negative	0.86 (0.64–1.16)	0.32
Ear infection			
	Total bacteria	0.92 (0.65–1.30)	0.65
	Gram-positive[1]	0.92 (0.65–1.31)	0.65
	Actinobacteria	0.86 (0.60–1.22)	0.41
	Firmicutes	0.73 (0.51–1.04)	0.09
	Gram-negative	1.03 (0.74–1.45)	0.87

The logistic regression models were adjusted for age, sex, race, ethnicity, current smoker, being a teacher, current asthma, hay fever, and home mold odor.
[1] Gram-positive bacteria included two phyla- Actinobacteria and Firmicutes.

44 % for acute bronchitis, 61 % for pneumonia, and 34 % for influenza-like illness-in association with the highest quartile of richness in Actinobacteria (Q4 versus Q123). Furthermore, significant associations were found between increased richness in both Actinobacteria and Firmicutes and decreased odds of influenza-like illness, common cold, and nasal infection. However, all upper respiratory infections-with the exception of ear infections-showed a tendency towards stronger negative associations with increased richness in Firmicutes (average protection of 32 %) compared to Actinobacteria (25 %). Only acute bronchitis was negatively associated (OR=0.54) with richness in Gram-negative bacteria. The adjusted exposure-response relationships using the quartile of Actinobacteria or Firmicutes richness for those significant associations indicated that the odds of the infections in the fourth quartile were always significantly lower than those in the first quartile (Fig. 1).

None of the gastrointestinal infections were significantly associated

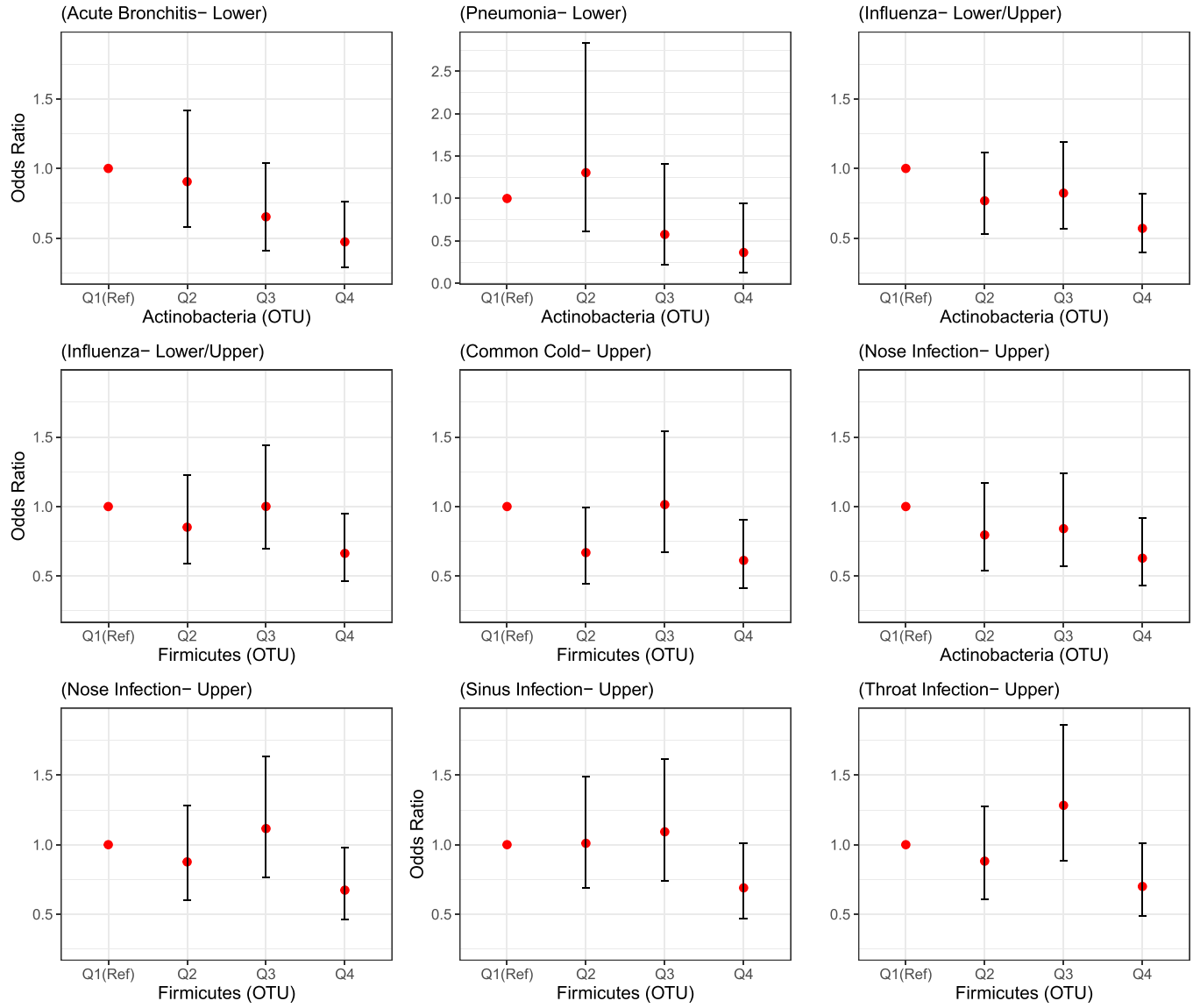

Fig. 1. Exposure-response relationships: adjusted odds ratio (OR) and 95 % confidence interval (CI) of quartiles in bacterial richness for lower and upper respiratory infections that were significantly associated with Actinobacteria or Firmicutes richness both in the linear and binary (Q4 versus Q123) logistic regressions. The logistic regression models were adjusted for age, sex, race, ethnicity, current smoker, being a teacher, current asthma, hay fever, home mold odor, and floor average dampness scores. Red dot: adjusted OR; Whiskers of OR: 95 % CI.

with increased richness of total bacteria, Actinobacteria, or Firmicutes in adjusted logistic regression models (Table S5). Furthermore, the richness of total fungi or yeasts were not significantly associated with any infections in adjusted regression models (data not shown). Individuals with physician-diagnosed hay fever or asthma were generally found to report a greater number of respiratory infections, with the notable exception of pneumonia in hay fever cases (Tables S6 and S7). For illustration, Table S8 presents the odds ratios for acute bronchitis associated with each variable included in the multivariable model. The full results of the model indicated that being older, female, being a teacher, having physician-diagnosed hay fever, and current asthma were significantly or marginally associated with the increased odds of acute bronchitis among the covariates.

4. Discussion

Our epidemiologic study of teaching and non-teaching staff from 50 schools has revealed the following: 1) exposure to higher richness of total bacteria in floor dust was associated with a 28–38 % decrease in the odds of reporting acute bronchitis, the common cold, and nasal infection compared to lower diversity; 2) within the total bacterial population, exposure to higher richness in the phylum Actinobacteria was associated with a 44–61 % decrease in the odds of lower respiratory infections (acute bronchitis and pneumonia) and a 34 % decrease in the odds of influenza-like illness compared to lower diversity, while exposure to Firmicutes was not associated with those infections; 3) within the total bacterial population, exposure to higher diversity in the phylum Firmicutes was associated with a 32 % decrease (on average) in the odds of upper respiratory infections (common cold, nasal, sinus, and throat infections), which represents a 7 % stronger association than that observed for the phylum Actinobacteria (25 %); 4) exposure to higher diversity in Gram-negative bacteria was associated with a 46 % decrease in the odds of acute bronchitis compared to lower diversity; and 5) similar negative associations of diversity in fungi or yeasts with respiratory infections were not observed. The exposure-response relationships for these negative associations appeared to be linear. None of the bacterial or fungal richness was associated with gastrointestinal infections in the multivariable regression models. Our study findings demonstrate that

exposure to a rich indoor microbiota, particularly within the bacterial phyla Actinobacteria and Firmicutes, may play an important role in the respiratory infections of school occupants.

4.1. Respiratory infections and bacterial diversity

There is limited information on the effects of exposure to diverse bacterial and fungal populations on respiratory infections. Furthermore, we are not aware of any epidemiologic studies that have examined associations between indoor microbial diversity and respiratory infections among school staff. A study of university students (n=357) living in dormitories in China reported that richness of the class Actinobacteria in settled dust was negatively associated with respiratory infections, indicating potential protective effects, whereas no such association was found with total bacteria [32]. In this study, respiratory infections were defined by the presence of symptoms such as the common cold, influenza, nasal congestion, and coughing reported within the last three months. Fu et al.'s school study involving 14- to 15-year-old students (n=308) in Malaysia documented that a higher richness of total bacteria was associated with a 22 % decrease in the odds of respiratory infections (OR=0.78, P-value=0.09) [33]. In this study, the absolute abundance (the actual number of microbial organisms) of bacterial and fungal taxa was calculated based on qPCR results. They found that the absolute abundance of certain bacterial genera within the phyla Actinobacteria, Proteobacteria, Deinococcota, Cyanobacteria, and Bacteroidetes in floor and surface dust from 21 classrooms was negatively associated (ORs=0.13–0.62, P-values<0.01) with reported respiratory infections. Similar to the findings of our study, Fu et al. did not observe any significant associations between total fungal richness or major fungal classes and respiratory infections. However, they did report significant protective effects associated with the absolute abundance of four fungal genera– *Devriesia, Endocarpon, Sarcinomyces*, and unclassified *Herpotrichiellaceae*–with odds ratios ranging from 0.10 to 0.25 (P-values≤0.001) for respiratory infections [33]. However, these fungal genera were not found in our elementary school study [17]. The findings from these two studies, which involved over 300 middle school and university students respectively, are consistent with our study findings from adults working in schools. In our study, the regression models were adjusted for demographic characteristics–age, sex, race, ethnicity, and smoking status–as well as additional covariates including asthma, hay fever, home mold odor, and classroom dampness scores that could be independently associated with respiratory infections. Altogether, our epidemiologic research suggests that exposure to higher diversity in indoor bacterial microbiota may offer protective effects against viral or bacterial respiratory infections among adults in the school environment.

The nares of adults are primarily colonized by diverse bacterial genera that mainly belong to the phyla Actinobacteria and Firmicutes, which are Gram-positive bacteria [9,34]. Bessesen et al. reported that adults colonized with methicillin-resistant *Staphylococcus aureus* (MRSA) in their nares, who are at an increased risk of staph infection, had microbial diversity in the nose that was 2.2 times lower than that of non-colonized healthy subjects [35]. They also demonstrated through in vitro experiments that competition among certain bacterial species, such as *Kocuria palustris* (Actinobacteria), *Lactobacillus gasseri* (Firmicutes), and *Streptococcus mitis* (Firmicutes), inhibits MRSA colonization in the nose, thereby providing a protective effect against staph infection. In the study by De Lastours et al., which included 338 U.S. adults, those infected with the influenza virus (n=35) exhibited significantly increased and imbalanced colonization of *Streptococcus pneumoniae* and *Staphylococcus aureus* in their nasal passages and throat [36]. In contrast to pathological conditions, microbiota in respiratory tracts of healthy adults is characterized by high diversity and low abundance [9]. These commensal bacteria inhabiting the nasal passages can impact the local bacterial community and influence the host immune response [8,10,37]. The healthy human nasopharynx is colonized by Gram-positive bacteria, such as *Staphylococcus, Streptococcus, Bifidobacterium, Dolosigranulum*, and *Corynebacterium*, as well as Gram-negative anaerobic bacteria, such as *Veillonella, Prevotella, Leptotrichia* and *Fusobacterium* [9,38]. However, an imbalance in the microbial composition of the nasopharynx could allow a particular pathogen to outgrow or modify responses to unexpected microbes in the lower airways [9,38–40]. Lee et al. reported that higher bacterial diversity in nasal passages and throat was associated with lower susceptibility to influenza virus infection [41]. Bacterial diversity generally increases as it moves from the anterior nose to the nasopharynx and to the oropharynx [38]. Those forementioned studies indicate that microbial diversity, not dominated by a single species, but balanced among resident species in respiratory tracts, appears crucial to resist colonization of pathogens [8,9]. Our epidemiologic study findings suggest that exposure to a more diverse array of environmental bacteria, particularly from the phyla Actinobacteria and Firmicutes, may have contributed to maintaining or strengthening a resilient microbiome in the respiratory tracts. This might have led to increased resistance against colonization by inhaled pathogenic microbes [8,9], ultimately providing a protective effect against respiratory infections.

4.2. Respiratory infections and fungal diversity

Roles of fungi in the respiratory microbiota and their contribution to health are not well understood [38,42,43]. Despite the paucity of studies, it appears that the respiratory tracts of healthy people are also inhabited by diverse fungi, commonly including *Cladosporium, Eurotium, Penicillium, Aspergillus, Candida*, and *Pneumocystis* [43]. In patients with cystic fibrosis or chronic obstructive pulmonary disease, lower lung function was associated with decreased fungal diversity that might have resulted from outgrowth of a single species or loss of rare species [43,44]. Additionally, cohort studies of U.S. children documented that increased diversity in the genus *Cryptococcus* may provide a protective effect on development of asthma in low-income Latino children [45] but that the fungal genus *Volutella* had an adverse effect on asthma severity in children with severe asthma [46]. However, it appears that no published studies have investigated the effects of fungal diversity on both respiratory and non-respiratory infections. In our study of adult school workers, we did not observe any adverse or protective effects of fungal diversity on respiratory and gastrointestinal infections. Nonetheless, additional epidemiologic studies are warranted to examine the impact of fungal diversity on respiratory infections in adult populations.

4.3. Microbiota in floor dust

From birth, inhaled microbes continuously interact with the microbiota of the respiratory tracts [8,9,47]. A study in France examined the associations between micro- and mycobiota in house dust and sputum from 22 adult patients with severe asthma (average age: 59.1). The results showed a higher proportion of shared fungal taxa between the sputum and house dust in patients experiencing acute exacerbations compared to those without exacerbations [48]. This study supports the notion that indoor environmental microbiota can be transferred into the respiratory tracts through inhalation. It is also well acknowledged that in densely occupied spaces, such as school classrooms, the resuspension of biological particles from indoor surfaces-including floors-due to occupants' activities, could be a primary source of airborne bacteria inhaled by those present [2,16]. Dust collected from the edges of classroom floors could better represent the accumulation of settled but once-airborne particles over the past year, as it is likely to escape regular vacuuming and mopping. Therefore, these floor dust samples may serve as a better proxy for long-term inhalation exposure, given that personal air sampling of bacteria or fungi has limitations in representing the temporal and spatial variation of airborne microbial concentrations [49–52]. Consequently, microbiomes in floor dust could significantly influence occupants' respiratory microbiome via resuspension and subsequent inhalation.

In our previous publication on bacterial microbiota in classroom

floor dust in the same schools, we reported that Proteobacteria was the richest (the most diverse) phylum, followed by the phyla Firmicutes and Actinobacteria [18]. However, Firmicutes was the most abundant phylum, with four genera-*Lactobacillus, Streptococcus, Clostridium*, and *Enterococcus*-ranking among the top 10 most abundant genera. As a single class within the identified phyla, Actinobacteria exhibited the greatest diversity of bacteria detected in this study [18]. In addition, we found that bacterial genera associated with humans were more diverse than those originating from outdoor environments, while genera linked to the outdoors were more abundant in these schools compared to human-associated bacteria. Our environmental analysis also revealed that fungal richness was less than half that of bacterial richness, although the quantity of fungal DNA sequences exceeded that of bacteria [17]. The top five richest fungal genera included *Aspergillus, Penicillium, Candida, Collectotrichum*, and *Alternaria* species. We identified DNA sequences derived from 724 fungal genera, which included 62 yeast genera-36 from the phylum Ascomycota and 26 from Basidiomycota-representing about 20 % of the total number of fungal DNA sequences [17]. In each classroom, we identified on average 17 yeast genera, indicating that yeasts are also prevalent fungi in these school environments. This environmental study demonstrated highly diverse bacteria and fungi in classroom floor dust.

4.4. Strengths and limitations of the study

The strength of our study lies in the relatively large number of participants-over 1500-from 50 schools in a major U.S. city, which afforded sufficient statistical power for the analyses. By assigning school building-specific floor averages as exposures for most questionnaire participants, we substantially increased the number of subjects included in the epidemiologic analyses compared to using individual classroom-based exposure assessments. This group-based exposure assignment resulted in less attenuated associations between exposure and health (data not shown), similar to the results previously reported from another large office building study by our group [53]. One limitation of our study is that we were unable to analyze the associations between the concentrations of individual microbial taxa and infections, as amplicon DNA sequencing yields only relative abundance information for each taxon. However, existing literature suggests that diversity in the respiratory tract-characterized by a lack of dominance by any single species-plays a more crucial role in protecting against respiratory diseases [8,9,43,44]. This supports our analytical strategy of using the diversity index to examine our hypothesis, rather than focusing on the concentrations of individual taxa [8,9]. Another potential limitation is the possibility of inaccurate taxonomic assignment, especially at lower taxonomic levels, which may arise from biases inherent in various stages of sample analysis. These stages include DNA extraction, PCR amplification, primer selection, and bioinformatics analysis involved in high-throughput sequencing. DNA amplicon sequencing does not indicate the viability of the identified microbes, leaving us uncertain about the proportion of viable organisms. This uncertainty represents a limitation if it is indeed only viable microbes that interact with the respiratory tract microbiome of the workers. Nonetheless, the use of amplicon sequencing and floor-average assignment for exposure categorization, as mentioned above, likely resulted in nondifferential misclassification of dichotomous exposure to diverse viable bacteria in floor dust. This would generally bias the associations towards the null [54]. Lastly, the infections reported over the past 12 months in our health questionnaire survey may have been subject to recall bias. Participants, unaware of their exposure status, might under- or over-report infections, which could lead to an attenuation of the actual associations. Nevertheless, our epidemiological study revealed significant negative associations between bacterial diversity and the odds of various respiratory infections, suggesting that the observed effects might have been even more pronounced in the absence of such misclassification or recall bias.

5. Conclusions

Our study's finding indicates that exposure to highly diverse indoor microbiota may confer protective effects against respiratory infections, suggesting that the inhalation of diverse microbial communities in indoor environments could play a significant role in promoting respiratory health. Exposure to high diversity in Actinobacteria may provide stronger protection against lower respiratory infections than Firmicutes, while diversity within the phylum Firmicutes may offer greater protection for upper respiratory infections compared to Actinobacteria. These findings imply that instead of traditional cleaning methods that kill most bacteria, a newly proposed probiotic cleaning-which introduces beneficial microbes that can outcompete harmful pathogens-may be a good practice to promote a healthier indoor microbial environment [55]. Therefore, probiotic intervention studies are needed to assess the efficacy of any probiotic cleaning method in prevention of respiratory tract infection before implementation.

In this adult population, we did not observe any protective effect of exposure to fungal or yeast richness on respiratory and gastrointestinal infections. As demonstrated in our previous publication on the bacterial microbiome of floor dust in these schools [18], exposure to a variety of human-associated bacteria, along with a rich presence of natural environmental bacteria such as Actinobacteria and Firmicutes, may contribute to a healthy and typical indoor microbial community. This could potentially have a positive impact on the respiratory health of the building's occupants. Nonetheless, further research to identify beneficial bacterial subgroups within the phyla Actinobacteria and Firmicutes-or variations within those subgroups that may confer protective effects against respiratory infections-would be valuable. This information may help us engineer healthier indoor microbiomes, promoting occupant health and offering better protection against infections.

Research data for this article

Due to the sensitive nature of the questions asked in this study, survey respondents were assured raw data would remain confidential and would not be shared.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

CRediT authorship contribution statement

Ju-Hyeong Park: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Angela R. Lemons:** Writing – review & editing, Resources, Methodology, Data curation. **Tara L. Croston:** Writing – review & editing, Resources, Methodology, Data curation. **Jerry Roseman:** Writing – review & editing, Validation, Investigation. **Brett J. Green:** Writing – review & editing, Resources, Funding acquisition. **Jean M. Cox-Ganser:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.buildenv.2025.112657.

Data availability

The data that has been used is confidential.

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