

Bacterial community assemblages in classroom floor dust of 50 public schools in a large city: characterization using 16s rRNA sequences and associations with environmental factors

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Abstract

Characterizing indoor microbial communities using molecular methods provides insight into bacterial assemblages present in environments that can influence occupants' health. We conducted an environmental assessment as part of an epidemiologic study of 50 elementary schools in a large city in the northeastern United States. We vacuumed dust from the edges of the floor in 500 classrooms accounting for 499 processed dust aliquots for 16S Illumina MiSeq sequencing to characterize bacterial assemblages. DNA sequences were organized into operational taxonomic units (OTUs) and identified using a database derived from the National Center for Biotechnology Information. Bacterial diversity and ecological analyses were performed at the genus level. We identified 29 phyla, 57 classes, 148 orders, 320 families, 1,193 genera, and 2,045 species in 3,073 OTUs. The number of genera per school ranged from 470 to 705. The phylum *Proteobacteria* was richest, followed by *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Cyanobacteria* while *Firmicutes* was most abundant. The most abundant order included *Lactobacillales*, *Spirulinales*, *Clostridiales*, *Bacteroidales*, *Pseudomonadales*, and *Micrococcales*. *Halospirulina* was the most abundant genus (the only genus within the order Spirulinales), followed by *Lactobacillus*, *Streptococcus*, *Sphingomonas*, *Clostridium*, and *Pseudomonas*. Gram-negative bacteria were more abundant and richer (relative abundance=0.53;1,632 OTUs) than gram-positive bacteria (0.47; 1,441). The Bray-Curtis dissimilarity index ranged from 0.22 to 0.63, with a median of 0.40. Effects of school location, degree of water damage, building condition, number of students, air temperature and humidity, floor material, and classroom's floor level on the bacterial richness or community composition were statistically significant but subtle. Our study indicates that classroom floor dust had a characteristic bacterial community represented by more gram-negative bacteria, in comparison to typical house dust that is represented by more gram-positive bacteria. Health implications of exposure to the microbiomes in classroom floor dust may be different from those in homes for school staff and students.

Background

Microbes are ubiquitous in the environment and there are at least ten times more microbes than cells in the human body [1]. The human microbiome constantly interacts with the microbiomes of the surrounding environments. Studies on the human microbiome have shown that most microbes native to our bodies are not pathogens but essential for functional human homeostasis [2, 3]. Still, the scientific community incompletely understands how environmental microbiomes interact with the human microbiome or affect human health. To understand this relationship, microbes in the environment and in the human body need to be fully characterized, which requires better analytical tools than traditional culture methods. The recent development of high-throughput sequencing technology has provided a powerful tool to analyze all genetic material in samples, and has led to discovery of microbial taxa that had never been identified before using the traditional methods [4, 5].

Over the past decade, there has been much interest in how microbiomes in the built environments affect respiratory illnesses such as asthma. A few environmental microbiome studies involving children suggested that exposure to a richer bacterial microbiome in house dust might have a protective effect on

the development of asthma, atopy, or wheezing [6–9]. These studies also documented certain bacterial taxa associated with the protective effects such as the order *Actinomycetales*, the families *Prevotellaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, the genera *Clostridium*, *Facklamia*, *Acinetobacter*, *Lactobacillus*, *Jeotgalicoccus*, *Corynebacterium*, and *Neisseria*, and the species *Staphylococcus sciuri*. In contrast, a pilot randomized controlled trial of 25 children reported that an inverse Simpson index used to measure classroom bacterial diversity was significantly associated with increased odds of asthma symptoms, whereas home bacterial diversity was not [10]. Another study reported an adverse effect of home bacterial richness (the number of different bacterial taxa) on severity of asthma in children [11]. Altogether, available peer-reviewed studies suggest that the effects of environmental bacteria on human health are complex; not only in bacterial richness but community composition, and presence and abundance of specific taxa may also play important roles.

The environmental microbiome is one of the core components in human exposures in the built environments that significantly contribute to occupants' health, and thus better understanding of its role in health and environments is crucial [12]. The committee of the 2017 National Academies' report on "Microbiomes of the Built Environment" emphasized the need for fundamental research on surface microbiome sources in indoor environments [2]. In the United States, on average, schoolteachers spend more than 50 h/week (> 2,600 hours per year) indoors whereas elementary school students spend 6.64 h/school day for approximately 180 days per year (about 1,195 hours per year) indoors [13, 14]. This demonstrates that school indoor environments contribute to environmental exposures more than any other locations for teachers and students except the home. Although there are a number of studies published about indoor microbiomes, most of them focused on residential environments or university lecture rooms [15–17]. Only a small number of studies using molecular methods have characterized classroom microbiomes in primary schools [10, 18]. In our study, we characterized the bacterial microbiome in floor dust of classrooms in 50 elementary schools in a large U.S. city. We also examined how school and classroom environmental factors influenced bacterial richness and community composition in floor dust collected from 500 classrooms.

Methods

Environmental study

An environmental assessment was conducted in June 2015 as part of a cross-sectional epidemiologic study to examine associations of microbial exposures with health in school staff. We selected 50 elementary schools in Philadelphia, PA to collect floor dust samples from ten selected classrooms in each school, for a total of 500 samples. The samples were collected from the floor near the edges of the room, at the junction of the floor and walls, where dust accumulation was observed. We measured and marked areas at the floor-wall junction around the full perimeter of each room (a total of 12 ft²). We vacuumed for eight-minutes the area with a precleaned crevice tool on a L'il Hummer backpack vacuum sampler (100 ft³/min, 1.5 horsepower, ProTeam Inc., Boise, ID, USA) equipped with a polyethylene filter sock (Midwest Filtration Company, Fairfield, OH, USA). After the collected dust was sieved with a mesh (pore

size: 250 μm), it was homogenized by rotating on a 360-degree rotary arm shaker at 65 r.p.m for 2 h and then partitioned into aliquots. After the sampling was completed, the relative humidity (RH) and temperature in classroom air were measured and recorded. Information on the average number of students for each sampled classroom was obtained from classroom teachers.

We collected dampness and mold information from all accessible rooms in the schools by visual assessment and evaluated mold odor using the Dampness and Mold Assessment Tool developed by the National Institute for Occupational Safety and Health (<https://www.cdc.gov/niosh/docs/2019-114/>). The average dampness and mold score was calculated using scores on water damage-related factors for all room components in each classroom. Information on the facility condition index [FCI=(cost of assessed deficiencies)/(replacement value)] for each school was obtained from a publicly available facility condition assessment report at <https://www.philasd.org/capitalprograms/wp-content/uploads/sites/18/2017/06/2015-FCA-Final-Report-1.pdf>.

Genomic DNA Extraction

Genomic DNA (gDNA) was extracted from 499 of 500 floor dust samples (one sample had no dust collected) and reagent blanks (n=30) as negative controls using the Roche High Pure PCR Template Preparation Kit (Roche Applied Sciences, Penzberg, Germany) as previously described [4, 19]. Five mg of dust was suspended in 250 μl of the kit's tissue lysis buffer and added to a 2-mL reinforced tube containing 300 mg of 212-300 μm glass beads (Sigma-Aldrich, St. Louis, MO). The tubes were then processed in a bead mill homogenizer at a rate of 4.5 m/s for 30 seconds. After two cycles of centrifugation at 20,000 $\times g$ for 1 minute, the lysis supernatant was placed in a sterile 1.5-mL microcentrifuge tube with 20 μl CellLytic B Cell Lysis reagent (Sigma) and incubated at 37°C for 15 minutes. Roche binding buffer (200 μl) and proteinase K solution (40 μl) were then added followed by a 10-minute incubation at 70°C. The samples were then washed and eluted as recommended by the manufacturer (Roche). Aliquots (20 μl) were stored at -80°C until shipment to the vendor for analysis.

Bacterial 16S amplification, sequencing and taxonomic identification

Extracted gDNA samples were submitted to RTL Genomics (Lubbock, TX) for Illumina Mi-Seq sequencing of the bacterial 16S rRNA genes. The samples were amplified using the 357wF (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTWTCTAAT) primer pair and sequenced as previously described [20]. The resulting sequences were quality checked to remove sequences with failed reads and low quality tags, and sequences that were less than half the expected amplicon length. Paired sequences were merged using the PEAR Illumina paired-end read merger, trimmed using a RTL internal trimming algorithm, and clustered into operational taxonomic units (OTUs) using a 96% similarity threshold using a USEARCH clustering algorithm [21, 22]. OTUs were selected using the UPARSE OTU selection algorithm [23] and chimeras were checked using the UCHIME chimera detection software [24]. For taxonomic identification, representative OTU sequences were compared to a database maintained by RTL Genomics of high-quality sequences derived from the National Center for Biotechnology Information database using a USEARCH global search algorithm [25].

Statistical analysis:

Taxonomy data from the sequencing results were analyzed with R using statistical packages *vegan*, *tidyverse*, *gridExtra*, *Hmisc*, *ggplot2*, *Mass*, and *broom* [26]. School and classroom-based Shannon-Weaver diversity (zero or positive number) and Bray-Curtis dissimilarity (constrained between 0 and 1) indices were calculated at the genus level due to 690 unidentifiable OTUs at the species level. The Bray-Curtis dissimilarity index is a taxonomy-based metric that allows the detection of small changes in community composition among the levels within a variable [27]. The Pielou's evenness index (constrained between 0 and 1) was also calculated. Hierarchical cluster analysis using Ward minimum variance method (minimizing within-cluster variance) was performed to cluster 50 schools using the Bray-Curtis index [28]. To compare the water damage scores among the clusters, we performed multiple comparisons using the Tukey honestly significant difference (HSD) test [29]. Since the Shannon-Weaver index and number of OTUs are influenced by sample size (i.e., groups with more samples show a higher diversity), rarefied genus accumulation curves were created to compare richness among the levels within each environmental factor. Although this method does not provide any statistical inference or compositional information, it estimates the number of genera in each level of the environmental variable using permutational sampling of the same number of DNA sequences in all levels [30]. Pearson correlation coefficients were calculated among Shannon-Weaver index and other environmental variables.

We used analysis of similarity (ANOSIM) to compare the mean ranks of between- and within-group (level) Bray-Curtis indices of the environmental variable (lower index has a lower rank value) [31]. R statistic was calculated by $[4(B-W)/N(N-1)]$, where B and W are the averages of the between-group and within-group ranks, respectively, and N is the number of samples. Permutational multivariate analysis of variance (PERMANOVA) modeling was performed to examine the adjusted relationships between community dissimilarity and environmental variables in full space without ordination [26]. The environmental variables included area of school, floor level, floor material, and quartiles of FCI scores, average water damage scores, number of students, air temperature, and air RH. Because ANOSIM and PERMANOVA are sensitive to unequal dispersion among the groups for unbalanced design, we examined homogeneity of dispersion among the levels in each environmental variable. The homogeneity of dispersion test indicated that the groups had similar dispersion for the categorical variables tested in the study (p-values > 0.05), except for RH (p-value=0.04) [31]

We used nonmetric multidimensional scaling (NMDS) to graphically present high dimensional data into a low dimensional space (k=3 in our analysis) in a way that the dissimilarity information between groups is reserved [32]. The NMDS is the most robust method that can handle any non-linear responses such as genus abundance [33]. Stress value (S, a statistic of goodness of fit) for the model was calculated [the smaller S value is the more favorable (< 0.2 as a rule of thumb)] [34]. We considered $P \leq 0.05$ as statistically significant and $P \leq 0.10$ as marginally significant.

Results

Bacterial richness, abundance, and diversity

We identified 3,073 unique OTUs from a total of 7.63 million sequences in the floor dust samples from the 499 classrooms, including 29 phyla, 57 classes, 148 orders, 320 families, 1,193 genera, and 2,045 species. Of the total 3,073 OTUs, 1,028 were not identifiable to the class or lower level. Among the top five richest phyla, the *Proteobacteria* had the largest number of OTUs (922 identified OTUs at the class level), followed by *Firmicutes* (770), *Actinobacteria* (669), *Bacteroidetes* (414), and *Cyanobacteria* (66) (Figure 1a). At the class level, *Actinobacteria* (the phylum *Actinobacteria*, 605 identified OTUs at the order level) was richest, followed by *Bacilli* (*Firmicutes*, 460), *Gammaproteobacteria* (*Proteobacteria*, 380), *Alphaproteobacteria* (*Proteobacteria*, 232), and *Clostridia* (*Firmicutes*, 218).

However, the rank order of the top five richest phyla identified was not concordant with that of the top five most abundant phyla. The phylum *Firmicutes* was most abundant (relative abundance: 0.29), followed by *Proteobacteria* (0.28), *Actinobacteria* (0.18), *Cyanobacteria* (0.14), and *Bacteroidetes* (0.09) (Supplemental Figure 1). The order *Lactobacillales* was most abundant (relative abundance: 0.14 in the phylum *Firmicutes*), followed by *Spirulinales* (0.11; *Cyanobacteria*), *Clostridiales* (0.07; *Firmicutes*), *Bacteroidales* (0.07; *Bacteroidetes*), *Pseudomonadales* (0.06; *Proteobacteria*), and *Micrococcales* (0.06; *Actinobacteria*). *Halospirulina* was the most abundant genus (the only genus within the order *Spirulinales*, relative abundance=0.11), followed by *Lactobacillus* (0.07), *Streptococcus* (0.03), *Sphingomonas* (0.03), *Clostridium* (0.03), and *Pseudomonas* (0.03) (Figure 1b). Of the most abundant top ten genera, only three were also in the richest top ten genera that included *Lactobacillus* (57 species identified), *Corynebacterium* (45), *Bacillus* (45), *Paenibacillus* (31), *Mycobacterium* (28), *Bacteroides* (25), *Pseudomonas* (24), *Provotella* (23), *Nocardiodes* (23), and *Flavobacterium* (23). We identified 15 *Staphylococcus* species with 0.018 in relative abundance including unidentified species and five *Propionibacterium* species with small relative abundance (<0.001). Gram-negative bacteria were more abundant (relative abundance=0.54) and richer (1,632 OTUs, 53%) than gram-positive bacteria [0.46; 1,441 OTUs (47%), respectively] in the bacterial community of these schools.

The number of bacterial genera identified in the 50 schools ranged from 470 to 705 (median=577) and the number ranged from 44 to 397 (246) for the 499 classrooms (Figure 2). The Shannon-Weaver diversity index ranged from 3.61 to 4.72 (4.14) for the schools and from 0.62 to 4.75 (3.64) for the classrooms. The Pielou's evenness index ranged from 0.57 to 0.72 (0.65) for the schools while the index ranged from 0.13 to 0.80 (0.67) for the classrooms. The Bray-Curtis index (1,225 unique pairs of schools) ranged from 0.23 to 0.63 (0.41), indicating that one-half of paired schools were at least 40% dissimilar in their genus composition. For the classrooms, the Bray-Curtis index (more than 124,000 pairs) ranged from 0.08 to 0.99 (0.66).

Relative abundance of dominant genera and hierarchical clustering of schools

We examined relative abundance of the top ten genera within each school. In 32 of 50 schools (64%), cumulative relative abundance of the top ten genera was 0.4 or higher (Figure 3). The cumulative relative abundance of the genera *Halospirulina* and *Lactobacillus* was higher than any other genus for all

schools, except for school number 34 where *Enterococcus* was more abundant than the summation of the two. The genus *Pseudomonas* was most abundant as a single genus in schools 46 and 49. The most abundant top ten genera in all classrooms included *Halospirulina* (relative abundance: 0.12), followed by *Lactobacillus* (0.07), *Streptococcus* (0.03), *Sphingomonas* (0.03), *Clostridium* (0.03), *Pseudomonas* (0.03), *Acinetobacter* (0.02), *Enterococcus* (0.02), *Corynebacterium* (0.02), and *Methylobacterium* (0.02) (Supplemental Figure 2).

Figure 3 presents four clusters by hierarchical clustering dendrogram of 50 schools and each of them showed characteristic genus composition. The cluster A included schools with *Halospirulina* at a medium level in relative abundance (~0.1 within the cluster) and *Bacillus* (0.075) along with low abundance of *Lactobacillus* (<0.02) (Supplemental Figure 3). The cluster B included 18 schools with the highest within-school relative abundance of *Halospirulina* (~0.2). The cluster C was composed of schools with lower relative abundance (0.06) of *Halospirulina* compared to other clusters along with medium abundance of *Lactobacillus* (~0.07) and higher abundances of *Sphingomonas* and *Pseudomonas* than other clusters. The cluster D consisted of schools with higher abundance of *Lactobacillus* (0.12) than those in other clusters. In the clusters A and C, the cumulative relative abundance of the top 10 genera was generally lower than the clusters B and D. When average water damage scores were compared among the clusters, cluster A had a significantly lower score than cluster D. Cluster D had the highest mean score of all the clusters (score of the cluster D > C > B > A). Multiple comparison adjusted with Tukey HSD showed that all pairwise comparisons were significantly different, except that two pairs of clusters (A and B, and C and D) were not different and that the clusters A and C were marginally different (Supplemental Figure 4).

Association of richness and community composition with school/classroom characteristics

Distributions of continuous environmental variables and their correlations with the Shannon-Weaver index are presented in Figure 4. Shannon-Weaver index was not associated with the average water damage score and the FCI score; however, it was positively but weakly correlated with the number of students in classroom (correlation coefficient=0.09, p-value=0.06). It was also weakly and negatively associated with air RH (-0.12, 0.01) and temperature (-0.12, <0.01). The FCI scores were moderately correlated with air RH (-0.35, p<0.001) and air temperature (0.45, p<0.001).

We created a rarefied genus accumulation curve for each level of categorical environmental variable (Figure 5). The steepest slope of the initial accumulation curve and the highest plateau for the schools in the southwestern area within the city indicated the highest proportion of relatively abundant genera and the highest richness, respectively. Interestingly, all three schools with the highest proportion of relatively abundant genera (Figure 5) were categorized in cluster C (Figure 2), which was also revealed by the steeper slope of the initial rarefaction curves than any other clusters (Supplemental Figure 5). All three schools showing the lowest proportion of relatively abundant genera were in cluster D and located in the northeastern area of the city. The continuous increase of rarefaction curves for clusters B and D indicated the presence of many rare genera (Supplemental Figure 5). The schools in need of the least repair (Q1, the first quartile of the school FCI scores) had the higher proportion of relatively abundant genera and

richer than any other FCI groups. The number of students in the classroom did not influence bacterial richness. School groups by quartile of classroom average water damage score showed slight differences in richness (similar height of plateau), but the most damaged schools (Q4) had a higher proportion of abundant genera compared to other groups. Schools with the lowest temperature had the richest and higher proportion of abundant genera while the effect of RH on richness was minimal.

ANOSIM (Figure 6) results indicated that the effects of the categorical variables on community composition were small (R values < 0.03) but significant, except for the type of floor materials. The classrooms in schools in need of least repair (Q1 of FCI score) were more dissimilar in composition than those in need of more repair (the group needing most repair was least dissimilar). The physical condition of the building (FCI score) affected dissimilarity the most among the environmental variables. Genus dissimilarity did not differ by classroom floor material type; however, it differed by floor levels of the classrooms ($R=0.02$, p -value < 0.01), with the first floors generally being the most dissimilar. There was a tendency that dissimilarity increased as water damage score or RH increased; whereas those with highest temperature in air were least dissimilar. The number of students had a marginal effect with classrooms with most students (Q4) being least dissimilar. All of the full and reduced multivariate models (PERMANOVA) adjusted for other environmental variables indicated that all of the environmental factors significantly affected dissimilarity in genus composition of paired classrooms although the effect was small (Table 1), which was consistent with the results of the univariate ANOSIM analyses. Mean water damage score of school classrooms in southwestern or northern region of the city were significantly higher than those in northeastern or southeastern region (Supplemental Figure 6). Because of this correlation, we constructed reduced PERMANOVA models without school area or water damage that yielded results similar to those of the full model. The PERMANOVA models also indicated that the effects of the physical condition of the building, area of the school, and classroom air temperature were greater than other environmental variables.

The first two of the three dimensions in unconstrained NMDS is presented in Figure 7. Stress values (0.1) indicate three-dimensional ordination is fair. Abundant phyla such as Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria, and Bacteroidetes were all clustered together in the majority of the schools. Schools in the northeastern area of the city had a distinct community composition compared to those in other areas. Schools in need of the least repair (Q1) had a unique genus composition, especially compared with those in Q2 and Q3. Schools with the least water damage (Q1) also had a unique composition compared with others, especially Q2 and Q3. The classrooms with the most students (Q4) had a different composition from those in Q1 and Q2. However, quartile groups of RH, and temperature did not show characteristic composition in NMDS.

Discussion

Bacterial community in the school classrooms

In floor dust from 499 classrooms in 50 elementary schools in a large U.S. city, we found that the *Proteobacteria* was the richest phylum although the *Firmicutes* was most abundant. Our top three phyla in relative abundance (*Firmicutes*, *Proteobacteria*, and *Actinobacteria*) were consistent with those of a longitudinal study of outdoor microbiomes in the atmosphere near surface in two Colorado cities [35]. In our study, human skin-associated bacterial genera (*Lactobacillus*, *Streptococcus*, *Corynebacterium*, and *Acinetobacter*) and human and animal feces-associated genera (*Enterococci*) [27, 36, 37] had rich species (155 in total) but were not predominantly abundant. However, we abundantly found *Halospirulina* and *Pseudomonas*, possibly carried on occupants' shoes into the classrooms as a soil component, and *Sphingomonas* and some *Acinetobacter* spp., possibly introduced from outdoor air after being released from plant leaf surfaces. These outdoor bacteria were not richer in species but more abundant than human-associated bacteria in our schools, which was similar to other study findings [35, 38]. The genus *Clostridium*, the 5th most abundant genus in our study, is ubiquitous in environments such as soils, sediments of a body of water and rivers, sewage, and human and animal intestinal tracks [39]. *Methylobacterium*, the 10th most abundant genus in our study, is also ubiquitous in nature and one of the common outdoor airborne bacteria, but generally found as part of a transient flora or as accidental contaminants [40]. In summary, human-associated genera in the top ten were more diverse (155 species) but less abundant (relative abundance=0.16) than outdoor environment-associated genera that were much less diverse (54 species) but more abundant (0.23) in our study. This finding may indicate that classroom environments had more proliferation of non-human-associated bacteria than those associated with human, although human occupants were one of the main sources for classroom microbiome. Our findings also emphasize that richness and abundance information need to be simultaneously considered in interpreting indoor microbiomes because they may have different health implications.

Grice et al., reported that 99% of human skin microbiome was represented by four phyla: *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* [41]. Of these, 62% were placed in three genera: *Corynebacteria* (the phylum *Actinobacteria*), *Propionibacteria* (*Actinobacteria*), and *Staphylococci* (*Firmicutes*). A Finnish study of four urban homes estimated that 16 to 41% of sequences (relative abundance) identified in floor dust might have originated from occupant's skin and another 6 to 40% likely from non-skin body parts of humans [17]. On the other hand, a U.S. study of indoor air in occupied university classrooms found that three human-associated main bacterial genera (*Corynebacterium*, *Staphylococcus*, and *Acinetobacter*) only accounted for 7.8% of observed bacterial sequences on average [42]. They also reported that 88% of the most common indoor species were from outdoors, emphasizing the impact of outdoor microbial communities on the microbiome within classroom environments. A most recent pilot randomized controlled trial found significant differences in alpha diversity and microbial community structure between homes and classrooms in the U.S. [10]. Along with these reports, our finding of diverse but relatively low abundance of human-associated bacteria in classroom floor dust indicated that there might be a characteristic difference in bacterial community and abundance between surface dust in residential buildings and floor dust in school classrooms.

We found that gram-negative bacteria were more abundant and richer than gram-positive bacteria in our schools. A study of four homes in Finland [17] reported a predominance of gram-positive bacteria (59% of species-level OTUs and 79% in relative abundance) resembling human skin microbiomes in house floor dust while we found a lower proportion (47% and 46%, respectively) in classroom floor dust. From a study of two nursing homes in Finland, Rintala et al. also reported dominantly abundant gram-positive bacteria in dust samples with the major phylum Firmicutes [43]. Hanson et al. also found predominantly abundant gram-positive bacteria (the major phylum Firmicutes, followed by Actinobacteria) in 12 surface dust samples from home bedrooms/living rooms and one classroom surface dust in the U.S. [44]. A literature review also summarized that the bacterial community in house dust is dominated by gram-positive bacteria [45]. Gram-negative bacteria generally require higher water activity for growth than gram-positive bacteria and fungi, and outdoor air is a rich source for gram-negative bacteria such as the phyla Proteobacteria and Bacteroidetes [45, 46]. Our findings indicated that the classroom environments in our study might have been frequently damp, and that outdoor sources might have played a more important role in shaping the microbiome in classroom floor dust than human sources, in contrast to residential dust.

In our study, we unexpectedly found that *Halospirulina* spp. were the most abundant in many schools, especially in the 18 schools categorized into the cluster B by hierarchical dendrogram. *Cyanobacteria* are photosynthetic prokaryotes that comprise approximately 165 genera and 1,500 species and produce cyanotoxins in some species [47]. Their habitats cover a wide range of environments including water with low or high salt concentration, terrestrial, and subaerial. Moreover, they have remarkable survivability in extreme temperatures (hot springs, Arctic and Antarctic lakes, snow, and ice), and in dried ponds with high saline concentrations [48-50]. An office building study in Kuala Lumpur, Malaysia identified *Cyanobacteria* in indoor air using the culture method that were likely tracked in from outdoor soils by humans or aerosolized from the soil of the indoor potted plants [38]. However, *Halospirulina* species were not cultured from their study. There are few published studies on the genus *Halospirulina*, and we are not aware of any literature that document the presence of this particular genus in indoor environments. Overall, *Cyanobacteria* are usually abundant in outdoor air of hot and humid tropical regions, favor high water content for growth, and have exceptional survivability in extreme conditions [48, 51]. We postulate that after introduction from outdoors into the buildings, *Halospirulina* might have proliferated in damp conditions of their microenvironments during the wet/dry cycles from recurring water damage. In addition, their extraordinary survivability in any extreme conditions of the microenvironments might have resulted in their abundance in these schools.

Effect of environments on richness, abundance, and community composition

In the genus accumulation curves with the rarefaction method, the classrooms in schools in the southwestern area of the city where there was a higher degree of water damage, or in the school buildings requiring the least repair (lower FCI score), presented a more steeply rising initial slope and a higher plateau than others. These features of rarefaction curves indicated a high proportion of relatively abundant genera and increased bacterial richness within the group [52]. Our finding of the highest

richness in bacterial genera in the classrooms with the most water damage compared with other quartile groups was consistent with the finding from a study of 198 homes in the southern New England region of the U.S. They reported an association between water leaks and increased bacterial richness in living room surface dust [15]. Classrooms with low air RH (Q1) had the fewest abundant genera and many rare genera compared to others with higher air RH; yet classrooms with the lowest air temperature (Q1) showed the highest proportion of abundant genera and the highest richness. Low RH in indoor air may decrease the available water for bacterial growth on the substrates or in microenvironments, which might have prevented proliferation of microbes and resulted in the fewest abundant genera [45]. The classrooms in schools requiring less repair tended to have higher RH in indoor air (Figure 4). Taken together, our study may indicate that location of school affects bacterial richness, and that high degree of water damage, low air temperature, and high humidity also increases the bacterial richness in floor dust in school classrooms.

Bacterial composition and abundance in built environments might depend on ventilation type, local climate, type of indoor fomites, season, and geographical area [43, 53, 54]. A study of living room floor dust from 286 homes in Germany [55] found from PERMANOVA models that the only significant environmental factor affecting bacterial community composition was natural ventilation in winter (but not in summer). From the ANOSIM, we found that the effects of most environmental factors on the bacterial community composition were subtle ($R \leq 0.03$) but statistically significant. PERMANOVA models adjusted for other variables confirmed that these subtle effects ($R^2 \leq 0.02$, smaller than those reported by other studies) of all environmental variables on the community composition were also significant. A New England home study in the U.S. found similar subtle ($R \leq 0.06$ in ANOSIM) but significant effects of home type (single vs. multifamily), home location (urban vs. suburban), and presence of pets on community composition [15]. Our finding of the effect of water damage on community composition is also consistent with that in a Finnish microbiome study of water-damaged homes with and without renovation [56]. The authors reported that bacterial genus composition in floor and other surface dust of water-damaged homes had a significant but only a small difference ($R < 0.1$ in ANOSIM) compared with that in dry homes. From ANOSIM, we observed the effect of the number of students on genus composition, showing a higher similarity in community composition in the classrooms with a higher number of students. Dannemiller et al. found that higher occupancy was associated with lower compositional variation (more similar) in living room surface dust, which is also in line with our observation [15]. Additionally, we found that classrooms on the first floor showed higher dissimilarity within the group compared to those on other floors, possibly indicating a greater effect of rich outdoor bacteria tracked into first floor classrooms than onto other floors. Although we did not observe statistical significance in univariate ANOSIM with only 13 classrooms with carpeted floor, PERMANOVA models adjusted for other environmental factors resulted in a significant effect of floor material on composition. Perhaps, the classrooms with carpeted floors might have a more dissimilar composition compared to those with smooth floors as shown in Figure 6. In aggregate, these findings implicate that individual environmental factors might marginally influence bacterial community composition in classroom floor

dust without a dominant single factor. This could indicate resilience of the indoor microbiome once it is established, unless there is a dramatic change in the environment such as water intrusion.

A strength of our study is that we had a large sample size (from 499 classrooms, 7.6 million sequences) from 50 schools, which allowed us to reliably examine bacterial diversity and community composition of classroom floor dust and determine effect of environmental factors within the study area. Yet, our cross-sectional study was conducted in summertime only, a limitation that may influence generalizing the results. The indoor microbiome is influenced by various outdoor sources that can vary by season [35, 43, 55]. However, because we collected cumulative dust that was likely to be settled or tracked in over extended periods of time (multiple seasons), the microbiomes in our dust might not be substantially affected by seasonal changes. Primer biases and factors such as gene copy number could result in differential amplification and identification of certain taxa [57]. We also recognize that extraction bias might have potentially influenced our identification [19].

Conclusions

From our cross-sectional study of 50 elementary schools, we found that outdoor bacterial sources and indoor environmental conditions might have played a dominant role in shaping classroom microbiome in floor dust, while human occupants were also an important source. In addition, school or classroom environmental factors significantly affected bacterial richness and community composition although their effects were subtle, indicating the relative stability of indoor microbiomes to environmental stress once established. Our findings demonstrate that microbiomes in school classrooms might be different from those in homes, which suggests that the health implication of exposure to microbiomes in schools could be different from that in residential environments. Thus, epidemiologic and clinical studies are warranted to better understand the effect of school or classroom microbiomes on health in school staff and students. The characteristics of bacterial microbiomes we found in this study will guide our future epidemiologic analysis of school-teachers' health related to microbial exposures.

List Of Abbreviations

OTU (Operational Taxonomic Unit); RH (Relative Humidity); FCI (Facility Condition Index); Tukey HSD (Tukey Honestly Significant Difference) test; ANOSIM (Analysis of Similarity); PERMANOVA (Permutational Multivariate Analysis of Variance); NMDS (Nonmetric Multidimensional Scaling)

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material:

The datasets generated during and/or analyzed during the current study are available via the Freedom of Information Act from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

JHP and JMC conceptualized and designed the study. JHP, JMC, and JR conducted the study and collected data. ARL performed sample extraction of gDNA and the sequencing data processing. JHP drafted the manuscript and performed statistical analyses. JHP, ARL, and BJG worked on data interpretation. All authors read, edited, and approved the final manuscript.

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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.

References

1. Cundell AM, Microbial Ecology of the Human Skin. *Microb Ecol.* 2018;76(1):113-120. doi:10.1007/s00248-016-0789-6.
2. The National Academies of Sciences Engineering and Medicine US, *Microbiomes of the Built Environment: A Research Agenda for Indoor Microbiology, Human Health, and Buildings.* Washington, D.C.: The National Academies Press;2017.

3. The National Academies of Sciences Engineering and Medicine US, The human microbiome, diet, and health: Workshop summary. Washington D.C.: The National Academies Press;2013.
4. Green BJ, Lemons AR, Park Y, Cox-Ganser JM, and Park J-H, Assessment of fungal diversity in a water-damaged office building. *J Occup Environ Hyg.* 2017;14(4):285-293. doi:10.1080/15459624.2016.1252044.
5. Peccia J and Hernandez M, Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. *Atmos Environ* (1994). 2006;40(21):3941-3961. doi:10.1016/j.atmosenv.2006.02.029.
6. Birzele LT, Depner M, Ege MJ, Engel M, Kublik S, Bernau C, et al., Environmental and mucosal microbiota and their role in childhood asthma. *Allergy.* 2017;72(1):109-119. doi:10.1111/all.13002.
7. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, et al., Exposure to environmental microorganisms and childhood asthma. *N Engl J Med.* 2011;364(8):701-9. doi:10.1056/NEJMoa1007302.
8. Karvonen AM, Kirjavainen PV, Taubel M, Jayaprakash B, Adams RI, Sordillo JE, et al., Indoor bacterial microbiota and development of asthma by 10.5 years of age. *J Allergy Clin Immunol.* 2019;144(5):1402-1410. doi:10.1016/j.jaci.2019.07.035.
9. Lynch SV, Wood RA, Boushey H, Bacharier LB, Bloomberg GR, Kattan M, et al., Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children. *J Allergy Clin Immunol.* 2014;134(3):593-601 e12. doi:10.1016/j.jaci.2014.04.018.
10. Lai PS, Kolde R, Franzosa EA, Gaffin JM, Baxi SN, Sheehan WJ, et al., The classroom microbiome and asthma morbidity in children attending 3 inner-city schools. *J Allergy Clin Immunol.* 2018;141(6):2311-2313. doi:10.1016/j.jaci.2018.02.022.
11. Dannemiller KC, Gent JF, Leaderer BP, and Peccia J, Indoor microbial communities: Influence on asthma severity in atopic and nonatopic children. *J Allergy Clin Immunol.* 2016;138(1):76-83 e1. doi:10.1016/j.jaci.2015.11.027.
12. Gilbert JA and Stephens B, Microbiology of the built environment. *Nat Rev Microbiol.* 2018;16(11):661-670. doi:10.1038/s41579-018-0065-5.
13. Goldring R, Gray L, and Bitterman A, Characteristics of Public and Private Elementary and Secondary School Teachers in the United States: Results From the 2011-12 Schools and Staffing Survey. US Department of Education.: Washington, DC. 2013.
14. National Center for Education Statistics US. 2007-2008 Schools and Staffing Survey. 2009 [cited 2020 June 23]; Available from: https://nces.ed.gov/surveys/sass/tables/sass0708_035_s1s.asp.
15. Dannemiller KC, Gent JF, Leaderer BP, and Peccia J, Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children. *Indoor Air.* 2016;26(2):179-92. doi:10.1111/ina.12205.
16. Hospodsky D, Qian J, Nazaroff WW, Yamamoto N, Bibby K, Rismani-Yazdi H, et al., Human occupancy as a source of indoor airborne bacteria. *PLoS One.* 2012;7(4):e34867. doi:10.1371/journal.pone.0034867.

17. Taubel M, Rintala H, Pitkaranta M, Paulin L, Laitinen S, Pekkanen J, et al., The occupant as a source of house dust bacteria. *J Allergy Clin Immunol.* 2009;124(4):834-40 e47. doi:10.1016/j.jaci.2009.07.045.
18. Hospodsky D, Yamamoto N, Nazaroff WW, Miller D, Gorthala S, and Peccia J, Characterizing airborne fungal and bacterial concentrations and emission rates in six occupied children's classrooms. *Indoor Air.* 2015;25(6):641-52. doi:10.1111/ina.12172.
19. Rittenour WR, Park JH, Cox-Ganser JM, Beezhold DH, and Green BJ, Comparison of DNA extraction methodologies used for assessing fungal diversity via ITS sequencing. *J Environ Monit.* 2012;14(3):766-74. doi:10.1039/c2em10779a.
20. Lemons AR, Hogan MB, Gault RA, Holland K, Sobek E, Olsen-Wilson KA, et al., Microbial rRNA sequencing analysis of evaporative cooler indoor environments located in the Great Basin Desert region of the United States. *Environ Sci Process Impacts.* 2017;19(2):101-110. doi:10.1039/c6em00413j.
21. Edgar RC, Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 2010;26(19):2460-1. doi:10.1093/bioinformatics/btq461.
22. Zhang J, Kobert K, Flouri T, and Stamatakis A, PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics.* 2014;30(5):614-20. doi:10.1093/bioinformatics/btt593.
23. Edgar RC, UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods.* 2013;10(10):996-8. doi:10.1038/nmeth.2604.
24. McCabe KM, Khan G, Zhang YH, Mason EO, and McCabe ER, Amplification of bacterial DNA using highly conserved sequences: automated analysis and potential for molecular triage of sepsis. *Pediatrics.* 1995;95(2):165-9.
25. Bokulich NA, Rideout JR, Kopylova E, Bolyen E, Patnode J, Ellett Z, et al., A standardized, extensible framework for optimizing classification improves marker-gene taxonomic assignments. *PeerJ.* 2015;3:e1502(3:e1502). doi:10.7287/peerj.preprints.934v2.
26. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, et al., *vegan: Community Ecology Package.* 2019.
27. Adams RI, Bateman AC, Bik HM, and Meadow JF, Microbiota of the indoor environment: a meta-analysis. *Microbiome.* 2015;3:49. doi:10.1186/s40168-015-0108-3.
28. Ward JH, Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association.* 1963;58(301):236-244. doi:10.1080/01621459.1963.10500845.
29. Tukey JW, Comparing individual means in the analysis of variance. *Biometrics.* 1949;5(2):99-114.
30. Kim BR, Shin J, Guevarra R, Lee JH, Kim DW, Seol KH, et al., Deciphering Diversity Indices for a Better Understanding of Microbial Communities. *J Microbiol Biotechnol.* 2017;27(12):2089-2093. doi:10.4014/jmb.1709.09027.
31. Anderson MJ and Walsh DC, PERMANOVA, ANOSIM, and the Mantel test in the face of heterogenous dispersions: What null hypothesis are you testing? *Ecological Monographs.* 2013;83(4):557-574.

32. Jakaitiene A, Sangiovanni M, Guarracino MR, and Pardalos PM, Multidimensional scaling for genomic data, in *Advances in stochastic and deterministic global optimization*, Pardalos PM, Zhigljavsky A, and Zilinskas J, Editors. 2016, Springer International Publishing: Cham, Switzerland. p. 129-139.
33. Minchin PR, An evaluation of the relative robustness of techniques for ecological ordination, in *Theory and models in vegetation science*, Prentice JC and Van Der Maarel E, Editors. 1987, Springer: Cham, Switzerland. p. 89-107.
34. Kruskal JB, Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*. 1964;29:1-27. doi:10.1007/BF02289565.
35. Bowers RM, Clements N, Emerson JB, Wiedinmyer C, Hannigan MP, and Fierer N, Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. *Environ Sci Technol*. 2013;47(21):12097-106. doi:10.1021/es402970s.
36. Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, and Harwood VJ, Enterococci in the environment. *Microbiol Mol Biol Rev*. 2012;76(4):685-706. doi:10.1128/MMBR.00023-12.
37. Meadow JF, Altrichter AE, Kembel SW, Moriyama M, O'connor TK, Womack AM, et al., Bacterial communities on classroom surfaces vary with human contact. *Microbiome*. 2014;2(1):7. doi:10.1186/2049-2618-2-7.
38. Chu W-L, Tneh S-Y, and Ambu S, A survey of airborne algae and cyanobacteria within the indoor environment of an office building in Kuala Lumpur, Malaysia. *Grana*. 2013;52(3):207-220. doi:10.1080/00173134.2013.789925.
39. Hatheway C, Bacterial sources of Clostridial neurotoxins, in *Botulinum neurotoxin and tetanus toxins*, Simpson LL, Editor. 1989, Academic Press Inc: San Diego, California. p. 3-25.
40. Green PN, *Bergey's manual of systematics of archaea and bacteria: Methylobacterium*. John Wiley & Sons, Inc., in association with Bergey's Manual Trust. 2005.
41. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al., Topographical and temporal diversity of the human skin microbiome. *Science*. 2009;324(5931):1190-2. doi:10.1126/science.1171700.
42. Meadow JF, Altrichter AE, Kembel SW, Kline J, Mhuireach G, Moriyama M, et al., Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air*. 2014;24(1):41-8. doi:10.1111/ina.12047.
43. Rintala H, Pitkaranta M, Toivola M, Paulin L, and Nevalainen A, Diversity and seasonal dynamics of bacterial community in indoor environment. *BMC Microbiol*. 2008;8:56. doi:10.1186/1471-2180-8-56.
44. Hanson B, Zhou Y, Bautista EJ, Urch B, Speck M, Silverman F, et al., Characterization of the bacterial and fungal microbiome in indoor dust and outdoor air samples: a pilot study. *Environ Sci Process Impacts*. 2016;18(6):713-24. doi:10.1039/c5em00639b.
45. Rintala H, Pitkaranta M, and Taubel M, Microbial communities associated with house dust. *Adv Appl Microbiol*. 2012;78:75-120. doi:10.1016/B978-0-12-394805-2.00004-X.

46. Hungaro HM, Pena WE, Silva NB, Carvalho RV, Alvarenga VO, and Sant'ana AS, Food microbiology, in Encyclopedia of agriculture and food systems, Van Alfen NK, Editor. 2014, Elsevier: San Diego, California. p. 213-231.
47. Bernstein JA, Ghosh D, Levin LS, Zheng S, Carmichael W, Lummus Z, et al., Cyanobacteria: an unrecognized ubiquitous sensitizing allergen? *Allergy Asthma Proc.* 2011;32(2):106-10. doi:10.2500/aap.2011.32.3434.
48. Moreira C, Ramos V, Azevedo J, and Vasconcelos V, Methods to detect cyanobacteria and their toxins in the environment. *Appl Microbiol Biotechnol.* 2014;98(19):8073-82. doi:10.1007/s00253-014-5951-9.
49. Organization WH, Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. London, UK: St Edmundsbury Press;1999.
50. Whitton BA, Ecology of Cyanobacteria II: Their diversity in space and time. NY, New York: Springer;2012.
51. Sharma NK, Rai AK, and Singh S, Meteorological factors affecting the diversity of airborne algae in an urban atmosphere. *Ecography.* 2006;29:766-772. doi:10.1111/j.2006.0906-7590.04554.x|.
52. Thompson GG and Withers PC, Effect of species richness and relative abundance on the shape of the species accumulation curve. *Austral Ecology.* 2003;28:355-360. doi:10.1046/j.1442-9993.2003.01294.x|.
53. Hewitt KM, Gerba CP, Maxwell SL, and Kelley ST, Office space bacterial abundance and diversity in three metropolitan areas. *PLoS One.* 2012;7(5):e37849. doi:10.1371/journal.pone.0037849.
54. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, et al., Architectural design influences the diversity and structure of the built environment microbiome. *ISME J.* 2012;6(8):1469-79. doi:10.1038/ismej.2011.211.
55. Weigl F, Tischer C, Probst AJ, Heinrich J, Markevych I, Jochner S, et al., Fungal and Bacterial Communities in Indoor Dust Follow Different Environmental Determinants. *PLoS One.* 2016;11(4):e0154131. doi:10.1371/journal.pone.0154131.
56. Jayaprakash B, Adams RI, Kirjavainen P, Karvonen A, Vepsäläinen A, Valkonen M, et al., Indoor microbiota in severely moisture damaged homes and the impact of interventions. *Microbiome.* 2017;5(1):138. doi:10.1186/s40168-017-0356-5.
57. Farrelly V, Rainey FA, and Stackebrandt E, Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. *Appl Environ Microbiol.* 1995;61(7):2798-801.

Tables

Table 1

Adjusted effect of environmental factors on dissimilarity in genus composition of paired classrooms.

Environmental Variable	Mean (SD)*	Full model (all categorical)**		Reduced model 1 (all categorical)**		Reduced model 2 (all categorical)**	
		R ²	P-value	R ²	P-value	R ²	P-value
Water damage score	0.17 (0.13)	0.01	< 0.001	-	-	0.01	< 0.001
Relative humidity	50.4 (9.9)	0.01	0.04	0.01	0.03	0.01	0.02
Temperature	81.1 (5.8)	0.01	< 0.001	0.01	< 0.001	0.02	< 0.001
Number of students	21.1 (9.3)	0.01	0.02	0.01	0.01	0.01	0.01
FCI score [†]	38.8 (17.9)	0.02	< 0.001	0.02	< 0.001	0.02	< 0.001
Area	-	0.02	< 0.001	0.02	< 0.001	-	-
Floor material type	-	0.01	< 0.01	0.01	< 0.01	0.01	< 0.01
Story in a building	-	0.01	< 0.001	0.01	< 0.001	0.01	< 0.001
Model residuals	-	0.89		0.90		0.91	
The table presents results of permutational multivariate analysis of variance (PERMANOVA).							
* Mean and standard deviation (SD) for the numerical variables.							
** All environmental variables in these models were categorical. Area: north (n = 159), north east (90), south east (90), south west (160); Type of floor material: carpet (13), tile (208), wood (223), others (55); Floor: 1st (165), 2nd (171), 3rd or higher (143), ground or lower (20). Continuous variable was categorized into four levels based on quartile to create a categorical variable.							
† FCI: facility condition assessment index.							

Figures

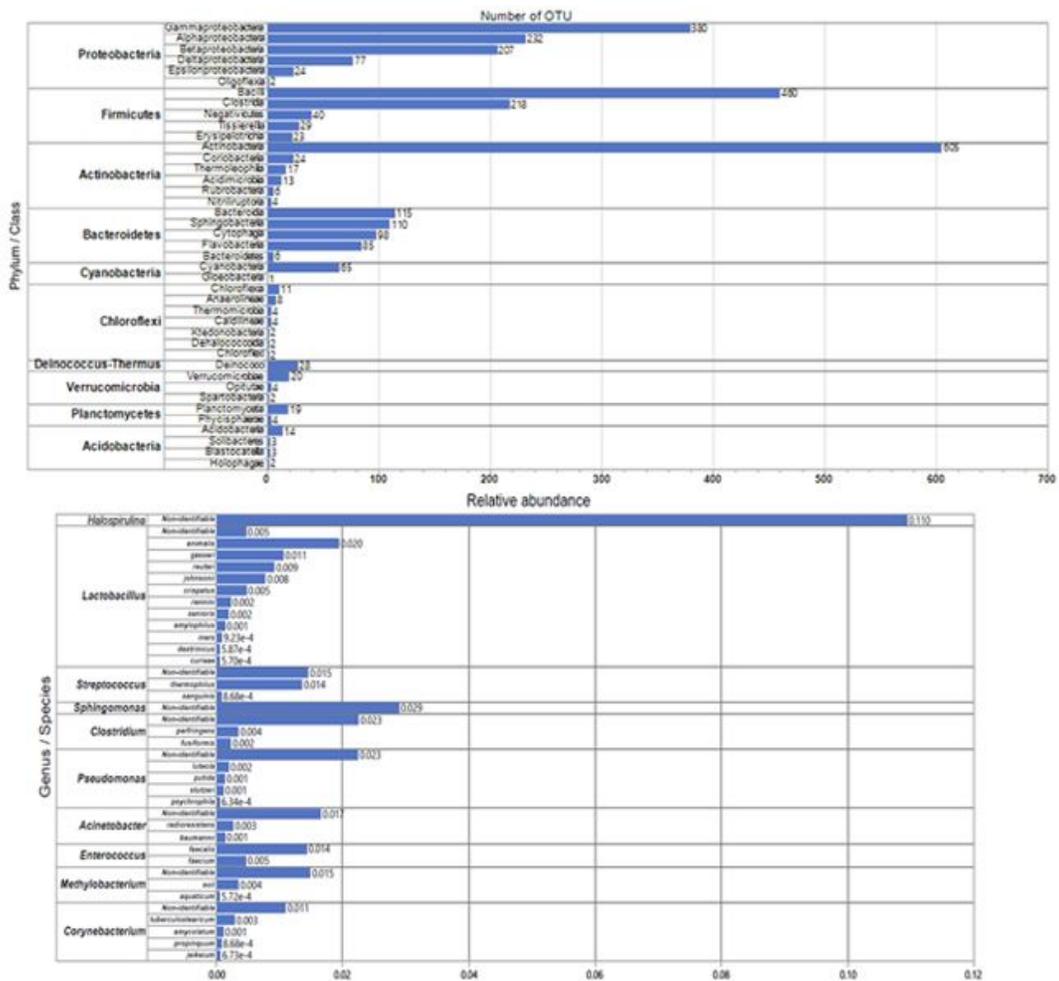


Figure 1

1a. Number of operational taxonomic units for each class of bacteria within the top 10 phyla. The bars are displayed by the descending order of the total number of OTUs in phylum and then in the class within each phylum. b. Relative abundance of species within the top 10 most abundant genera. Because of too many OTUs, species that were smaller than 0.0005 in relative abundance are not presented. For Halospirulina, no species were identifiable; and for Sphingomonas, relative abundances of all four identified species were smaller than 0.0005 (thus, not in the figure). The bars are displayed by the descending order of relative abundance in genus and then in identified species within each genus.

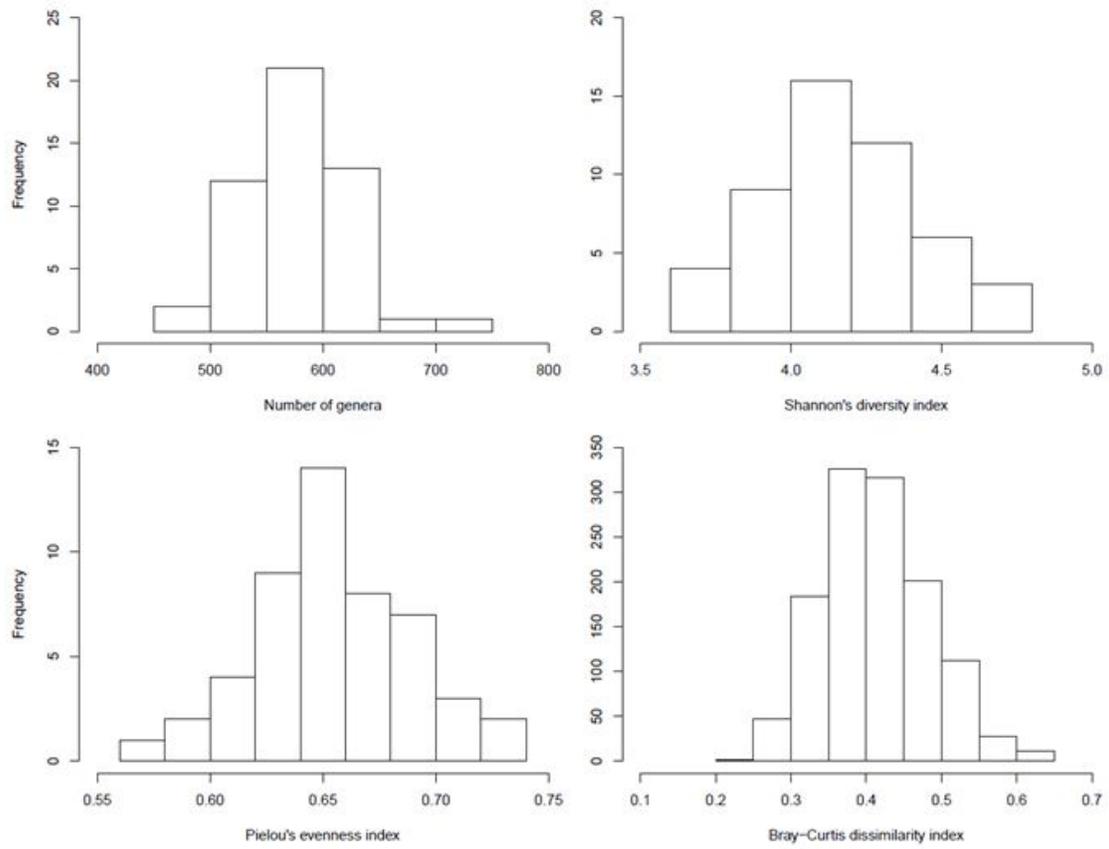


Figure 2

Bacterial richness, diversity, evenness, and dissimilarity indices in 50 schools.

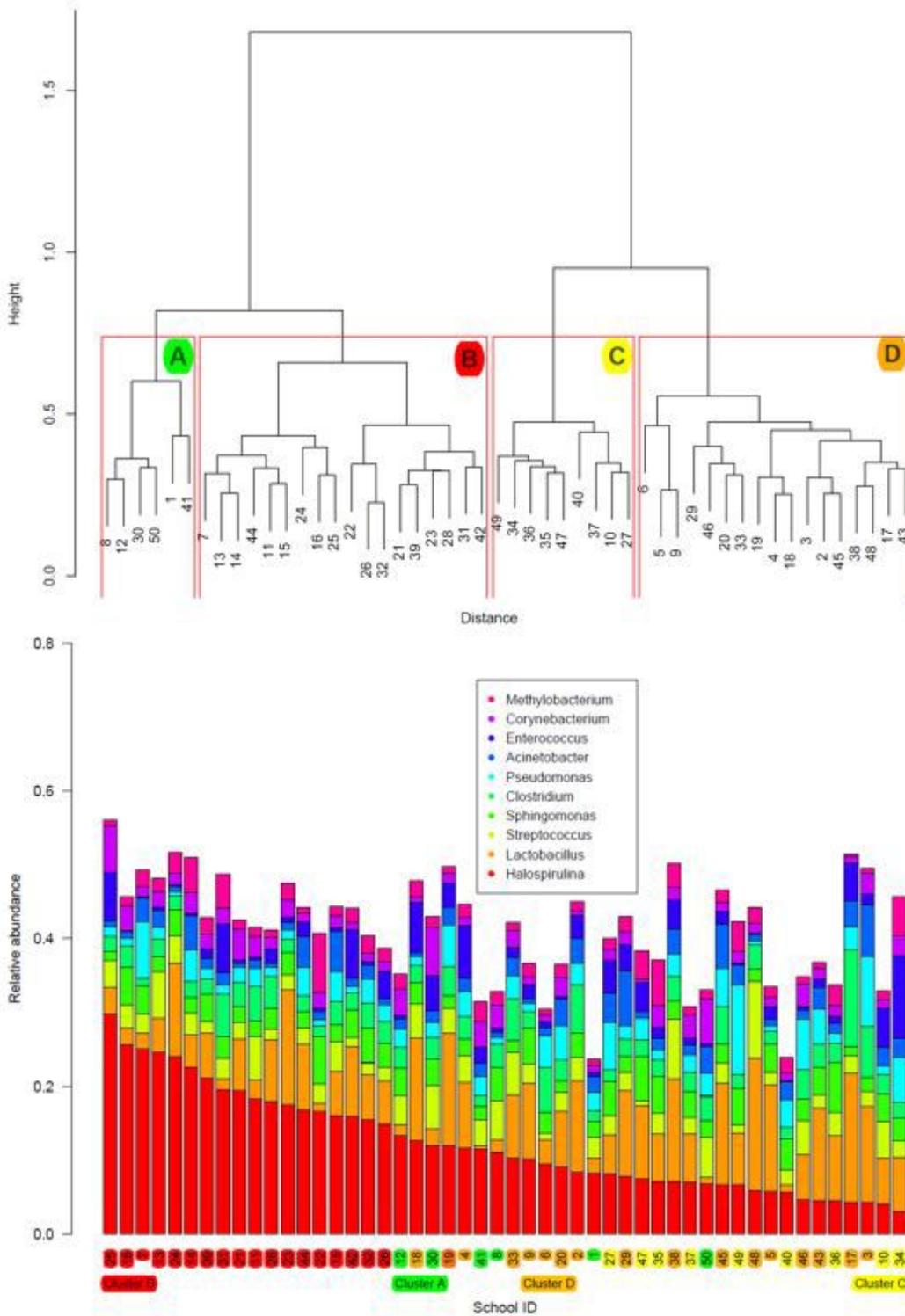


Figure 3

Hierarchical clustering dendrogram of 50 schools and relative abundance of the top ten bacterial genera. Hierarchical clustering dendrogram of 50 schools with four clusters (upper) and relative abundance of the top ten bacterial genera within each school (lower). Numbers highlighted with green color in the lower panel are schools in cluster A, red in cluster B, yellow in cluster C, and orange in cluster D.

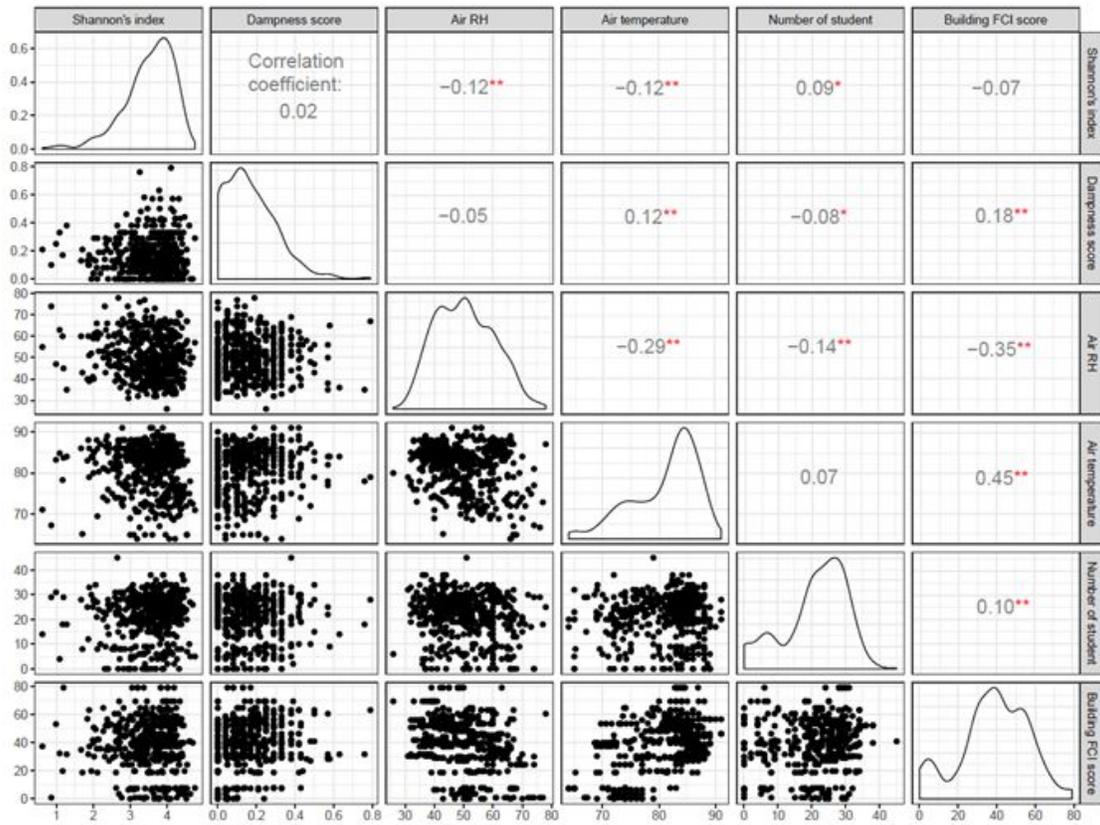


Figure 4

Correlations among bacterial diversity index and environmental parameters. FCI: facility condition assessment index; p-values: ** P-value ≤ 0.05 , * $0.05 < \text{P-value} \leq 0.1$.

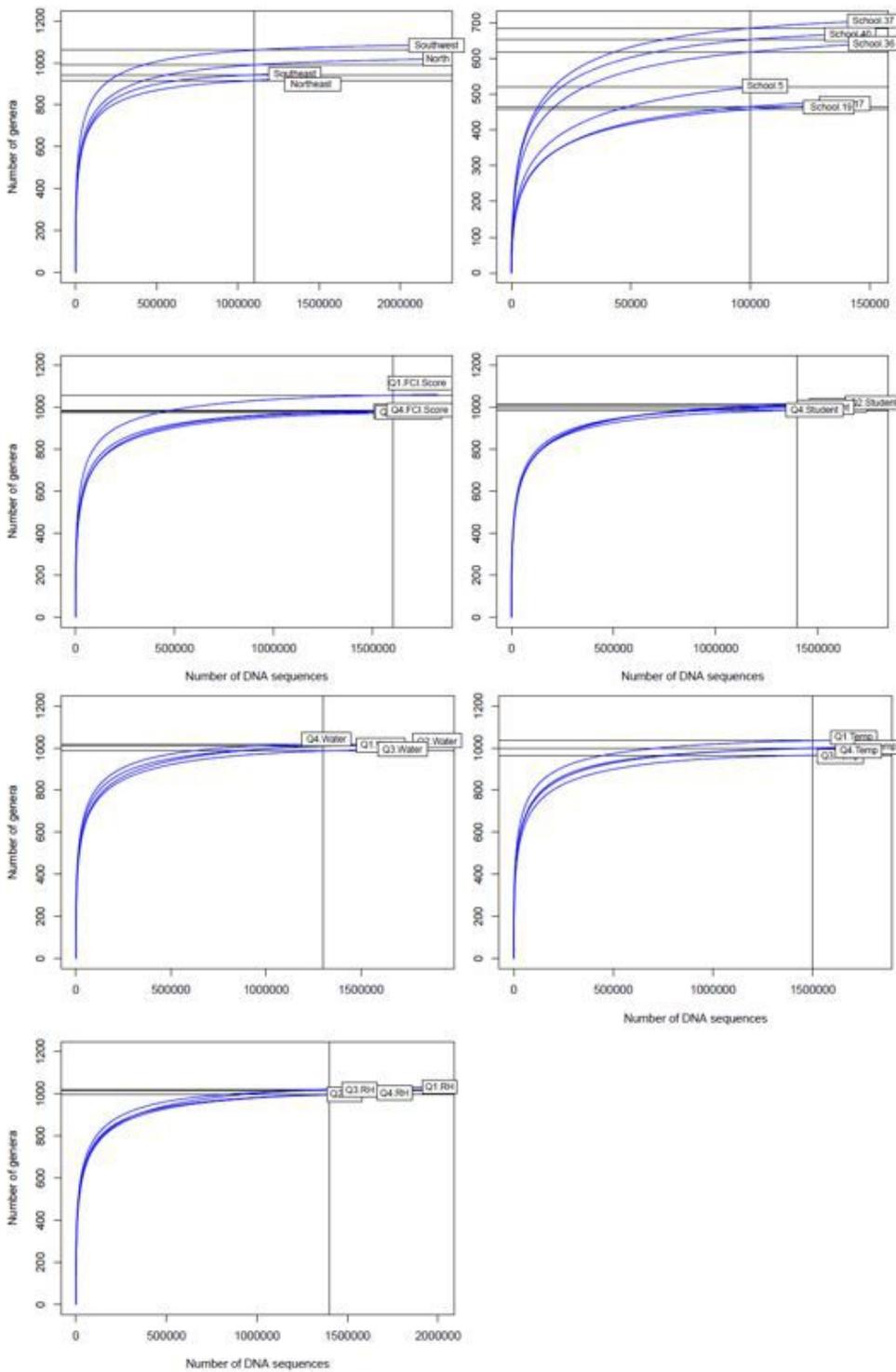


Figure 5

Each panel presents rarefied genus accumulation curves by the level of each environmental variable. Vertical line: the number of sampled sequences to compare richness; horizontal line: estimated number of genera by rarefaction with the same number of sampled DNA sequences.

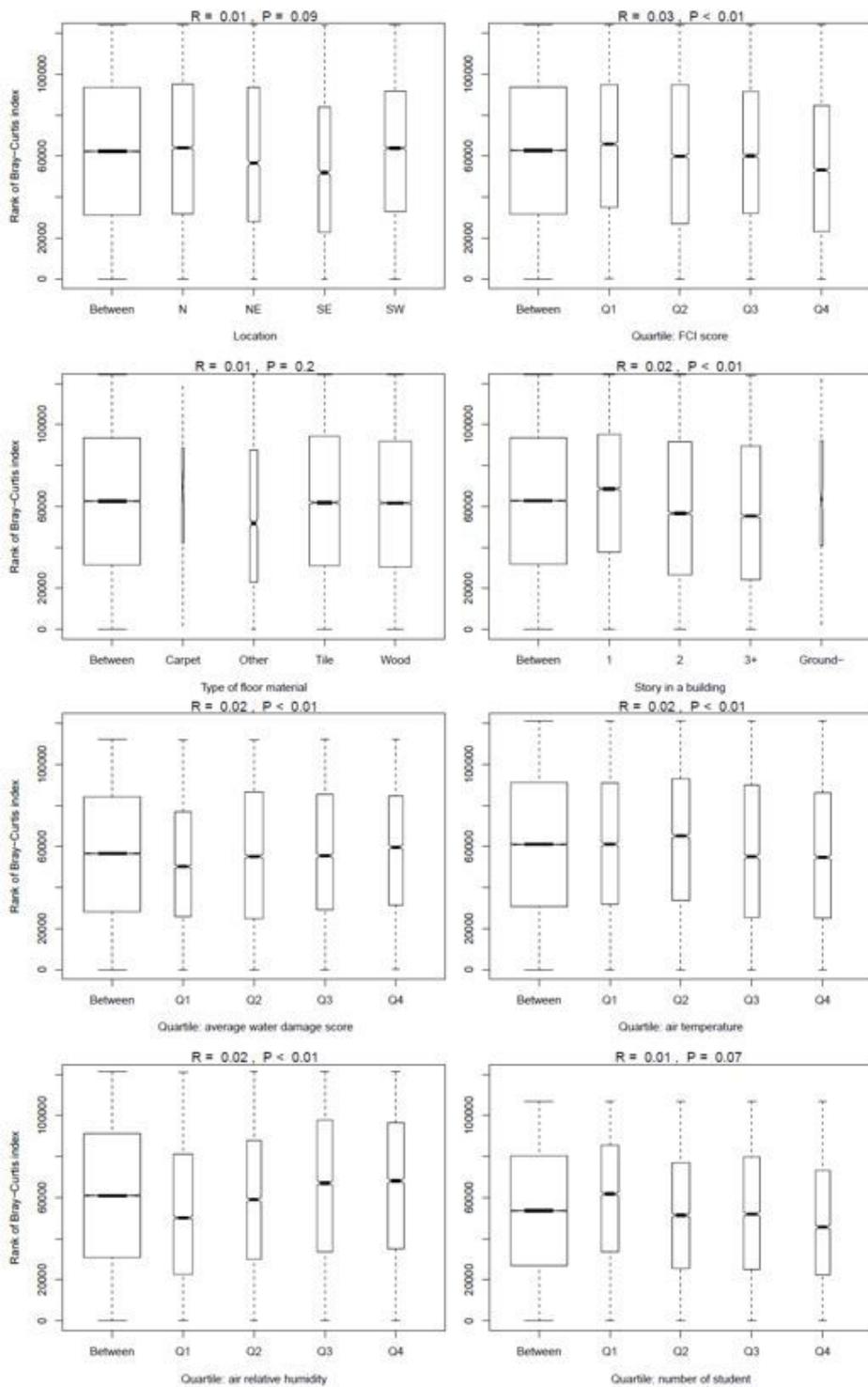


Figure 6

Analysis of similarity (ANOSIM) by the group or level of categorical environmental variables. ANOSIM was performed using rank of Bray-Curtis dissimilarity index over 499 samples.

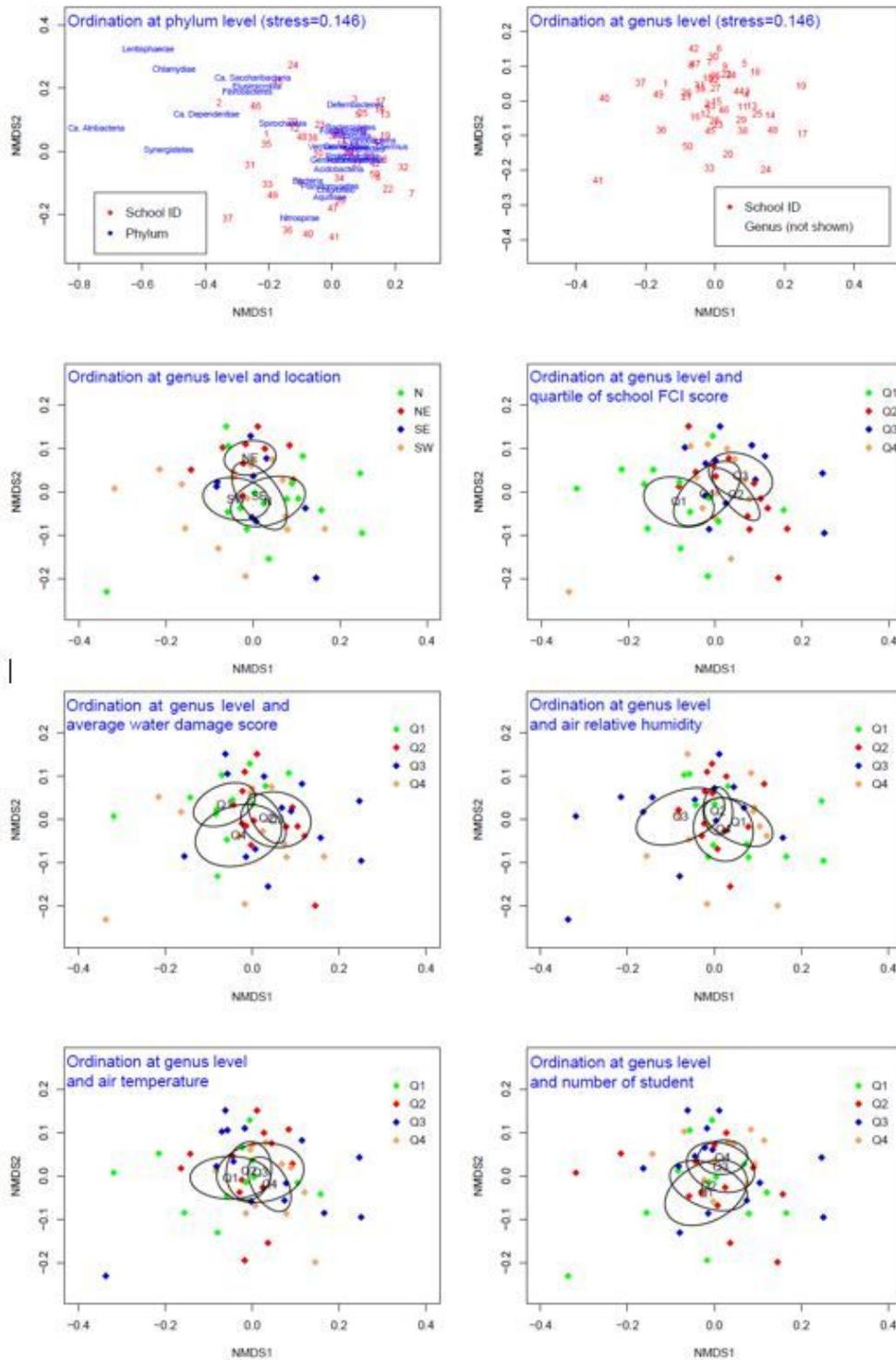


Figure 7

Unconstrained nonmetric multidimensional scaling (NMDS) of phylum and genus for 50 schools with environmental variable. NMDS of phylum (the first panel) and genus (the rest of the panels). Ellipses were constructed with a 95% confidence interval using standard error in chi-square distribution with two degrees of freedom.

Supplementary Files

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