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Mycobiota and the Contribution of Yeasts in Floor Dust of 50 Elementary Schools Characterized with Sequencing Internal Transcribed Spacer Region of Ribosomal DNA

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c01703.

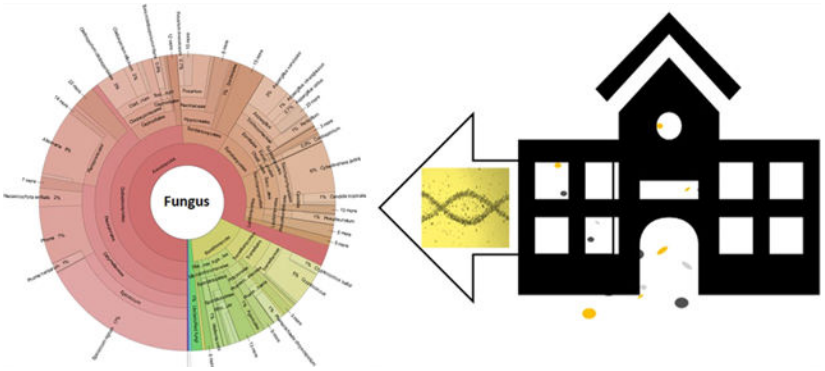
Relative abundance of the top 20 most abundant orders, cumulative relative abundance of the top 15 fungal genera for each school, cumulative relative abundance of the top 15 fungal species for each school, distance–decay relationship among all paired schools using Bray–Curtis dissimilarity indices and the negative exponential function at the species level, the top 30 most abundant fungal species in the quartile of schools based on school locations, ANOSIM at the species level for the water-damage score, air temperature, RH, and number of students in a classroom, the top 30 most abundant fungal species in the quartile of schools based on average temperature, the top 30 most abundant fungal species in the quartile of schools based on average RH of sampled classrooms, ANOSIM at the species level for the type of floor material, the top 30 most abundant fungal species in the quartile of schools based on average temperature, and yeasts or yeast-like fungal genera identified in our study by the descending order of relative abundance as percentage (PDF)

The authors declare no competing financial interest.

Abstract

The assemblage of fungi including unicellular yeasts in schools is understudied. We conducted an environmental study to characterize fungal communities in classroom floor dust. We collected 500 samples from 50 elementary schools in Philadelphia, PA, and evaluated room dampness/mold conditions. Genomic DNA from dust was extracted for internal transcribed spacer 1 Illumina MiSeq sequencing to identify operational taxonomic units (OTUs) organized from DNA sequences. Differential abundance analyses were performed to examine significant differences in abundance among groups. We identified 724 genera from 1490 OTUs. The genus *Epicoccum* was not diverse but the most abundant (relative abundance = 18.9%). Fungi were less diverse but most dissimilar in composition in the most water-damaged classrooms compared to the least water-damaged, indicating differential effects of individual classroom water-damage on fungal compositions. We identified 62 yeast genera, representing 19.6% of DNA sequences. *Cyberlindnera* was the most abundant (6.1%), followed by *Cryptococcus*, *Aureobasidium*, *Rhodotorula*, and *Candida*. The average relative abundance of yeasts tended to increase with increasing dampness and mold score and was significantly (p-value = 0.048) higher in the most water-damaged classrooms (22.4%) than the least water-damaged classrooms (18.2%). Our study suggests the need for further research on the potential health effects associated with exposures to yeasts in schools.

Graphical Abstract



Keywords

schools; yeasts; fungal microbiota; dampness and mold; classrooms

INTRODUCTION

Fungi are ubiquitous in both outdoor and indoor environments. It has been well acknowledged that exposure to fungi in damp indoor environments is negatively associated with various health outcomes,[1,2] although exposure to higher fungal richness early in life has been also reported to have protective effects against asthma development in young children.[3] To better understand these health associations in epidemiologic studies, complete characterization of indoor fungi is crucial. High-throughput sequencing methods for determining microbial diversity have made it possible to understand and characterize

the assemblage of fungal communities within indoor environments more fully. These DNA sequencing methods have identified some indoor fungi that were previously overlooked or not reported, such as the *Ustilaginomycetes* class belonging to the phylum *Basidiomycota*, indicating that these fungi may be prevalent and abundant indoors.[4–6] Overlooked fungal species may emerge as important sources of indoor fungal exposure. To date, large-scale studies characterizing fungal communities in elementary schools using high-throughput DNA sequencing have been limited.[7,8] Further characterization of these school environments is necessary to determine the broad diversity of fungi present that may contribute to occupant exposure and health.

Yeasts are largely unicellular fungi, with about 1500 described species, and ubiquitous in natural (soil, water, and plants) and built environments (foods and wet indoor niches such as kitchens and bathrooms).[9,10] Yeasts normally compose 10% or less of the environmental microbiota in indoor air,[11] but the proportion can increase up to 20% in damp environments.[12] These numbers may be underrepresented as yeasts are not easily cultured or identified using a traditional culture method.[13] This challenge may explain why they have received relatively little attention compared to other fungal taxa and why the health effects of indoor exposure to yeasts other than infection are less known. Depending on yeast taxa or diversity to which people are exposed indoors, the health effects may be protective or detrimental,[3,14] underscoring the need for better characterization of indoor yeasts and more epidemiologic studies.

Fungi in indoor floor dust can grow under damp conditions, and spores and fungal fragments in floor dust can be resuspended into air, which could significantly increase occupants' exposures.[15,16] Characterizing the fungal community composition in floor dust may help enhance our understanding of indoor fungal exposure and its health impacts on room occupants. In our study, we investigated fungal ecology in floor dust collected from 500 classrooms in 50 elementary schools in Philadelphia, PA, using the high-throughput DNA sequencing of the fungal internal transcribed spacer (ITS) region. We further examined the effects of environmental factors on the fungal community composition, as well as the significance of yeasts in school classrooms.

MATERIALS AND METHODS

Environmental Study.

We conducted an environmental assessment in June 2015 as part of a cross-sectional epidemiologic study of staff in 50 public schools in Philadelphia, PA. Schools were selected to represent a wide range of water-damage (from no to substantial damage) evaluated in a preliminary study. We collected one composite dust sample from the floor (hard floorings: >97% of classrooms) near the edges of the room, at the junction of the floor and walls (a total of 12 ft^2 around the full perimeter of each room) in 500 classrooms (one dust sample/classroom and 10 classrooms/school) using a Li'l Hummer backpack vacuum sampler (100 ft^3/min, 1.5 horsepower, ProTeam Inc., Boise, ID, USA). The details for the sample collection were reported previously.[17] The collected dust was sieved with a mesh (pore size: 250 μm), homogenized, and then partitioned into aliquots for analyses. A total of 499 samples were analyzed for fungal DNA (no dust was retrieved from one classroom

sample). The relative humidity (RH) and temperature in classroom air were measured at the end of the sampling, and information on the average number of students for each sampled classroom was also collected from the homeroom teacher. We used the NIOSH (National Institute for Occupational Safety and Health) Dampness and Mold Assessment Tool to collect dampness-related information for the classrooms (https://www.cdc.gov/niosh/docs/2019-114/). The calculated average dampness/mold scores for each classroom were used for data analyses.

Genomic DNA Extraction.

Genomic DNA (gDNA) was extracted 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.[5,17,18] Briefly, dust (5 mg) was suspended in the kit's tissue lysis buffer (250 μL) and added to a 2 mL reinforced tube containing 300 mg of glass beads (212–300 μm, Sigma-Aldrich, St. Louis, MO). The reinforced tubes were placed in a bead mill homogenizer and processed at a rate of 4.5 m/s for 30 s. The tubes were then centrifuged for two cycles at 20,000× *g* for 1 min, and the lysis supernatants were placed in sterile 1.5 mL microcentrifuge tubes containing 20 μL of CelLytic B Cell Lysis reagent (Sigma-Aldrich) and incubated at 37 °C for 15 min. The kit's binding buffer (200 μL) and proteinase K solution (40 μL) were then added and the tubes were incubated at 70 °C for 10 min. The samples were then washed and eluted as recommended by the manufacturer (Roche Applied Sciences, Penzberg, Germany). Aliquots (20 μL) were stored at −80 °C until they were shipped for analysis.

Fungal ITS Region Amplification, Sequencing, and Taxonomic Identification.

Extracted gDNA samples were submitted to RTL Genomics (Lubbuck, TX) for Illumina MiSeq sequencing of the fungal internal transcribed spacer 1 (ITS1) region. The samples were amplified using the ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2aR (GCTGCGTTCTTCATCGATGC) primer pair and sequenced as previously described.[19] The PEAR Illumina paired end read merger was used to merge paired sequences.[20] An RTL Genomics internal trimming algorithm was used to trim the sequences prior to clustering into operational taxonomic units (OTUs) using a 96% similarity threshold with a USEARCH clustering algorithm.[21] OTUs were selected using the UPARSE OTU selection algorithm[22] and chimeras were checked using the UCHIME chimera detection software.[23] The sequences were then quality-checked to remove sequences with failed reads, low-quality tags, and amplicon lengths less than half the expected length of 250–300 base pairs. Representative OTU sequences were queried against a database of high-quality sequences derived from the National Center for Biotechnology Information maintained by RTL Genomics using a USEARCH global search algorithm to determine taxonomic identifications.[24]

Statistical Analysis.

We analyzed taxonomy data with R (version 4.0.3)[25] and statistical packages vegan, phyloseq, and ANCOM-BC (analysis of compositions with bias correction).[26–28] We calculated the Shannon–Weaver diversity index for each school or classroom and Bray–Curtis dissimilarity between two classrooms or schools for all possible paired classrooms

(N = 124,251: the number of combinations of 499 classrooms taken two at a time without repetitions) or paired schools (N = 1225) at the species level. Pearson correlation coefficients were calculated among the Shannon–Weaver index, along with other environmental variables. To compare richness among the quartiles of school- or classroom-level average dampness/mold scores, we used rarefied species accumulation curves normalized by the same number of DNA sequences.[29] We used relative abundance in each sample or group for further data analysis. Relative abundance for individual taxa was calculated as overall relative abundance by dividing the sum of DNA sequences for each taxon across all 499 samples or all samples within each school or group (location or quartile) by the total number of DNA sequences. Analysis of similarity (ANOSIM) was performed, and R statistic was calculated to compare the mean ranks of between- and within-group Bray–Curtis dissimilarity of the environmental variable.[30] The homogeneity of the dispersion (equal variance) test among the groups in each school-average-based environmental variable indicated similar dispersion among the levels of the categorical variables tested (p-values > 0.05) at the species level, except for temperature (p-value = 0.02); however, it was homogeneous (p-value = 0.5) at the genus level for temperature. Therefore, ANOSIM for the quartile of the school average temperature was performed at the genus level identification. We graphically presented high dimensional data into a low dimensional space (k = 3 in our analysis) using nonmetric multidimensional scaling (NMDS) with ellipses showing the 95% confidence interval (CI).[17,31] We calculated stress value (S, a statistic of goodness of fit) for the NMDS model.[32] A distance–decay model was fitted to the Bray–Curtis dissimilarity indices using the negative exponential function to examine differences in dissimilarity by distance between the pairwise schools.[33] Using ANCOM-BC, differential abundance analyses of species among levels in categorical variables were performed, which excluded the species with zero abundance in more than 90% of samples. Then, the average abundances in individual levels were compared and p-values were adjusted with Holm–Bonferroni for multiple comparison.[26] We considered $p \leq 0.05$ as statistically significant.

RESULTS

Characteristics of Fungal Diversity.

We collected a total of 11.8 million DNA sequences, which clustered into 1490 OTUs, including 308 unidentifiable OTUs at class or lower taxonomy levels. The identified OTUs were placed in 5 phyla, 25 classes, 89 orders, 248 families, 724 genera, and 1182 species. The number of OTUs for each classroom ranged from 13 to 325 with a median of 163. The total number of OTUs (richness) in the phylum *Ascomycota* was much higher (1007 OTUs) than the phylum *Basidiomycota* (463 OTUs). At the class level, *Agaricomycetes* in the phylum *Basidiomycota* had the richest taxa (354 OTUs) of all (Figure 1). However, the class *Dothideomycetes* was the most abundant (overall relative abundance [RA] = 52%) and *Agaricomycetes* was placed at the sixth most abundant (5.6%) class. At the order level, *Pleosporales* (in the class *Dothideomycetes*) was considerably more abundant (RA = 42.3%) than others (Figure S1). The top three most abundant filamentous fungal genera (*Epicoccum*, RA = 18.9%; *Alternaria*, 8.3%; and *Phoma*, 8.1%) were all placed in *Pleosporales* (Figures 2 and S1). However, other abundant genera *Cladosporium* (RA = 7.1%), *Aspergillus*

(6.8%), and *Fusarium* (4.0%) belonged to the different orders *Capnodiales*, *Eurotiales*, and *Hypocreales*, respectively.

The top five richest genera included *Aspergillus* (26 taxonomically allocated species), *Penicillium* (19), *Candida* (19), *Collectotrichum* (14), and *Alternaria* (13). Of these five richest genera, only two (*Alternaria* and *Aspergillus*) were included in the top five most abundant genera (Figure 2). *Aspergillus* and *Cladosporium* were found in 100% of samples, *Epicoccum* in 99.8%, and *Cyberlindnera* (yeast) in 99.0%. At the species level, *Epicoccum nigrum* was most prevalent (99.8% of samples), followed by *Cladosporium cladosporioides* (99.6%), and *Cyberlindnera jadinii* (99.0%). Although *Epicoccum* and *Phoma* had only three allocated species for each, they were the ones among the most abundant (Figures 2 and S2). The top 15 fungal species represented, on average, 66% of DNA sequences in individual schools (range: 43.5–81.0%) and the top 15 genera 74.4% (60.7–84.3) (Figure S2). *Epicoccum nigrum* was the most abundant (RA = 27.5%) species of all. Four *Aspergillus* species (*A. versicolor*, *A. niveoglaucus*, *A. ustus*, and *A. penicillioides*) were included in the most abundant top 30 while any *Penicillium* species were not (Figures 3 and S3). *Fusarium* has a total of 11 taxonomically allocated species and *Fusarium graminearum* was found in 48.1% of classrooms but all 50 schools. Of the five taxonomically allocated *Stachybotrys* species (*S. chartarum*, *S. echinatus*, *S. elegans*, *S. microsporus*, and *S. nephrosporus*), *S. chartarum* was the most abundant and found in 144 classrooms (28.9%) and 48 schools (96%) but with low relative abundance (overall relative abundance = 0.07%).

Bray–Curtis dissimilarity indices for all paired schools at the species level ranged from 0.23 to 0.81 with a median of 0.50, while those for all paired classrooms ranged from 0.12 to 1.00 with a median of 0.74. In general, diversity indices and environmental factors (dampness score, temperature, RH, and number of students) were weakly or not correlated to one another (Figure 4). RH was weakly and negatively correlated with the number of students in classroom ($r = -0.14$, $p = 0.003$) and Shannon's index at the genus level ($r = -0.09$, $p = 0.057$), but not at the species level.

Fungal Community Composition.

The distance–decay model indicated that dissimilarity in community composition at the species level increased with the distance between the paired schools (slope = 0.45, $p = 0.001$) and NMDS analysis showed unique fungal composition by school locations (Figures 5 and S4, S5). ANOSIM showed that a group of schools or classrooms with highest average air temperature (fourth quartile: 84.8–88.4 °F) had the least dissimilar and those with lowest average air temperature (first quartile: 64.7–76.6 °F) had the most dissimilar species composition ($R = 0.035$ at the classroom level or 0.040 at the school level, p-values < 0.001). ANOSIM for the quartile of the school location showed homogeneous dispersion at the species level. On the other hand, the quartile of school average air temperature showed heterogeneous dispersion at the species level but homogeneous at the genus level. ANOSIM analysis is based on the assumption of homogeneous dispersion among groups. The classrooms with lowest RH (first quartile: 26.0–43.0%) also showed the least dissimilar composition (Figures S6 and S7). Carpeted floorings had the most dissimilar species composition compared to other hard floorings (Figure S8). The differential abundance

analyses indicated that average abundances of *E. nigrum*, *A. ustus*, *A. unguis*, *Phanerochaete chrysosporium*, and *Exidia glandulosa* were significantly (adjusted *p*-values < 0.05) higher in schools with higher temperature (third and fourth quartiles) than schools with the lowest temperature in the first quartile (Figure S9). A rarefied species accumulation curve by the quartile of the dampness/mold score indicated that richness was the lowest in classrooms with the most water-damage, but no clear trend in richness by the level of water-damage was observed (Figure 6A). ANOSIM showed that the fungal community within the group of classrooms with the most water-damage (fourth quartile) was the most dissimilar among classrooms compared to other groups, but the effect of water-damage on composition was small ($R < 001$, *p*-value = 0.08) (Figure S6). Species composition among the quartiles of dampness/mold scores was similar, except that the second (red color) and third (blue) quartiles were distinctly different in composition (Figure 6B). Differential abundance analyses indicated that abundances of *A. versicolor*, *Toxicocladosporium irritans*, *Clavispora lusitaniae*, and *Thielavia hyalocarpa* in the most water-damaged schools were significantly (adjusted *p*-values < 0.021) higher than those in the least damaged schools. However, the effects of these environmental factors on compositional dissimilarity were small (range of *R* values = 0.006–0.040) (Figures 5 and S6). The number of students per classroom was not associated with fungal species composition in floor dust (ANOSIM *p*-value > 0.1).

Contribution of Yeasts to Fungal Community.

Of the total 724 fungal genera identified, we found 62 yeasts or yeast-like genera (Table S1) that collectively represented 19.6% of the total number of fungal DNA sequences. On average, 17 yeast genera were identified in each classroom (range: 2–33 genera/classroom). Of the 62 genera, 36 belonged to the phylum *Ascomycota* and 26 to *Basidiomycota*. Eight of the top 30 most abundant fungal species were yeasts (denoted with a red asterisk above the bar in Figure 3), the top three of which (*Cyberlindnera jadinii*, *Candida tropicalis*, and *Cryptococcus saitoi*) collectively represented 12.3% of total DNA sequences. Of the 13 fungal genera that were isolated in more than 90% of classrooms, five were yeasts—*Cyberlindnera*, *Cryptococcus*, *Aureobasidium*, *Rhodotorula*, and *Candida*. *Cyberlindnera* was not only the most prevalent but also the most abundant yeast genus (RA = 6.1%), followed by *Cryptococcus* (5.2%), *Candida* (1.5%), and *Rhodotorula* (1.1%). The black yeasts (*Aureobasidium* and *Exophiala*) and *Coniochaeta* were also prevalent (97.8, 84.6, and 55.7%, respectively), although their relative abundances were less than 1%. *Malassezia restricta* was the only species in this genus and isolated from only 34 of the 499 classrooms (6.8%) with small relative abundance (RA = 1.5×10^{-3}%).

The average relative abundance of yeasts tended to increase with increasing dampness score quartiles (first quartile = 18.2%; second = 20.3%; third = 21.3%; and fourth = 22.4%). Yeast abundance was significantly ($p = 0.048$) higher in classrooms in the highest quartile than in the lowest one; however, the significance was lost when the Holm–Bonferroni adjustment for multiple comparisons was applied. The abundance of the yeast species *Clavispora lusitaniae* was significantly (adjusted *p*-value < 0.001) higher in the most water-damaged schools than in the least damaged ones.

DISCUSSION

Characteristics of Fungal Community Composition in Schools.

We found from our study of 500 classrooms in 50 elementary schools that the number of fungal DNA sequences in floor dust was substantially higher than bacteria (11.8 vs 7.6 million), although the number of OTUs was slightly less than half of that of bacteria (1490 versus 3073 OTUs).[17] Our finding of the highest richness of the class *Agaricomycetes* in the phylum *Basidiomycota* is different from Amend et al.'s finding of the class *Dothideomycetes* in the phylum *Ascomycota* being the richest class in settled dusts in homes, offices, shops, and a church in six continents.[34] Yet, *Agaricomycetes* only accounted for 5.6% of DNA sequences in contrast to the class *Dothideomycetes* that represented 51.9% in our study, which is similar to the finding from a study of elementary schools in Finland and the Netherlands.[8] The abundance of the class *Dothideomycetes* in the school classroom samples is likely attributed to xerotolerance and the production of melanized fungal spores that help them to survive longer under unfavorable conditions.[34,35] *Pleosporales* is the largest order, representing 25% of species in *Dothideomycetes*,[36] and *Alternaria*, the second most abundant genera in our study, is one of the most commonly identified anamorphic fungi in the family *Pleosporaceae*.[36]

Epicoccum is a saprophytic fungus and considered slightly xerophilic requiring minimum water activity 0.86–0.90 but can also grow under wet conditions.[37] *Epicoccum* species produce numerous airborne conidia, similar to *Aspergillus*, *Alternaria*, *Penicillium*, or *Cladosporium* species that have been commonly cultured in indoor air.[38] Adams et al., also found *Epicoccum* as one of the most abundant genera in Finnish and Dutch elementary schools similar to our study.[8] *Alternaria* is a phylloplane fungus and its conidia can easily become airborne. If the outdoor-originated genera *Epicoccum* and *Alternaria* were introduced indoors through air, their relative abundance in floor dust may be associated with their quick settlement on indoor surfaces owing to the large spore size during no occupancy[7] and growth in the floor dust under high RH conditions.[15] They could also be tracked in by occupants (shoes or cloths) from outdoors. *Phoma* species, which is the third most abundant in these schools, produce numerous conidia from fruiting bodies and can grow at a high level of water activity similar to *Epicoccum*.[38] *Fusarium graminearum* and *Stachybotrys chartarum* were also prevalent in these schools, but their relative abundances were low (not included in the top 30 abundant fungi) and not associated with dampness scores.

Many of the fungi in the top 15 most abundant genera (Figure 2) in our study, such as *Epicoccum*, *Alternaria*, *Cladosporium*, *Aspergillus*, *Fusarium*, and *Penicillium*, have been associated with allergic rhinitis.[14,39] *Epicoccum nigrum* has been associated with allergic fungal sinusitis in world populations[39] and hypersensitivity pneumonitis in two children in a mold-contaminated home.[40] Dannemiller et al., reported adverse effects of exposure to *Epicoccum* genus or *Epicoccum nigrum* on asthma severity in atopic children.[14] *Cladosporium cladosporioides*, producing a known allergen,[39] was the third most abundant species in our school samples; however, *Cladosporium herbarum*, which produces 60 known allergens,[39] was not highly abundant. Interestingly, the commonly cultured fungal species in indoor environments, such as *Penicillium* species and *Aspergillus fumigatus* that produce

many known allergens,[39] were not included in the most abundant top 30 species identified in our study (Figure 3). Although our current study does not report any health data, the associations of exposures to these fungi with respiratory diseases and symptoms are being examined in ongoing epidemiologic analyses.

Effects of Environmental Conditions on Fungal Diversity and Community Composition.

The species accumulation curve may indicate that floor dust in classrooms with the most water-damage had the lowest richness in fungal species, but there was no clear trend in richness by the level of water-damage. On the other hand, Dannemiller et al. found higher diversity in house dust collected from homes with damp walls in the United States.[3] A Finnish study examining the effects of remediation of water-damaged homes on the microbiome showed significant reduction in fungal richness after renovation.[41] Conversely, another Finish study investigating the mycobiome in settled dust from two pairs of office buildings in two locations (one reference and one water-damaged building in each location) reported inconsistent results in fungal diversity in water-damaged buildings. Higher diversity in a water-damaged building was found in one location but lower diversity in another, compared to the reference building matched by age, construction type, usage, condition, and ventilation type.[13] In addition, a study of public housing in the United States showed that fungal diversity in settled dust was lower but compositions were more unique in water-damaged compared to nondamaged homes.[42] A chamber study of the microbiome of impregnated carpet dust demonstrated that fungal richness decreased as equilibrium RH increased.[15] Our finding of the highest dissimilarity within the group of the most water-damaged classrooms than other groups indicated that water-damage in individual classrooms within the group may have differentially affected fungal community depending on the type or pattern of the damage and the conditions of classrooms. This differential effect might have resulted in a unique fungal composition despite low fungal diversity.

Amend et al. found that fungal compositional similarity was most strongly influenced by geography.[34] Our distance–decay model also indicated that dissimilarity in community composition at the species level increased with the distance between the paired schools, demonstrating compositional difference by distance even within the same geographical region. We found that classrooms with the highest air temperature in summer had the lowest fungal dissimilarity. The school average air temperature of the fourth quartile during the sampling campaign ranged from 84.8 to 88.4 °F (29.3–31.3 °C), which was much higher than that recommended by ASHRAE (72.0–80.5 °F during summer).[43] Considering that the optimal growth temperature for most indoor fungi is approximately 77 °F (25 °C),[44] such high temperatures during the summer might have restricted the growth of a majority of fungi in floor dust, except for minor thermophiles (being able to grow in 86 °F [30 °C] or higher), resulting in low dissimilarity.[44] However, the effects of these environmental factors on compositional dissimilarity of fungi were subtle as we also found from our bacterial community study in the same schools.[17] Taken all together, fungal composition in floor dust of school classrooms may not be determined by a single factor, but by the complex collective effects of types and patterns of water-damage, building materials, school location, and other environmental conditions such as temperature and humidity.

Contribution of Yeasts to School Fungal Community.

From our study, we found that (1) a high proportion of fungal DNA sequences (RA = 19.6%) were yeasts; (2) on average, 17 yeast genera in each classroom were identified; and (3) the relative abundance of yeasts increased by the level of water-damage. The study of elementary schools in Finland and the Netherlands mentioned earlier reported that the abundant yeast taxa in settled dust differed between countries—i.e., the genera *Mrakia* and *Apiotrichum* were the most abundant in the Netherlands while the genera *Cyberlindnera* and *Phaeococcomyces* were in Finland.[8] A study of two daycare centers in Oslo, Norway, reported that the order *Saccharomycetales* (yeasts) was one of the most abundant ascomycetes.[45] A study of two water-damaged and two paired nondamaged buildings reported 24% of yeast-like fungal species in settled dust.[13] The presence of black yeast-like fungi, such as *Aureobasidium pullulans* and *Exophiala dermatitidis*, were frequently associated with water-related household appliances.[9] Dannemiller et al. also reported positive association of the presence of the yeast species *Cryptococcus uzbekistanensis*, *Cryptococcus albidus*, and *Coniosporium apollinis* with mold/moisture indicators such as water-damage, visible mold, or leaks.[3] These indicate that yeasts that have been easily overlooked are likely an important fungal group deserving of more attention in indoor microbiome or epidemiologic studies, especially in environments that might have been frequently influenced by water incursion.

Studies indicated that the yeast *Malassezia* among the fungal genera and the species *M. restricta* (of their 11 species identified) predominated in most skin sites of healthy adults.[46–48] *Candida* (*C. tropicalis*, *C. parapsilosis*, and *C. orthopsilosis*) and *Cryptococcus* species (*C. flavus*, *C. dimennae*, and *C. diffluens*) were also occasionally identified from adult human skin.[46] An environmental microbiome study reported that yeasts *Cryptococcus*, *Malassezia*, *Saccharomyces*, *Candida*, *Rhodotorula*, *Mrakia*, and *Cystofilobasidium* were among the most abundant top 20 fungal genera in settled dust collected from four office buildings in Finland.[13] The most prevalent yeast genera found in settled dust from various buildings in 72 locations across six continents included *Aureobasidium*, *Cryptococcus*, and *Filobasidium*.[34] However, other environmental studies did not observe predominance of *Malassezia* or *Cryptococcus* sp. in residential surface dust in California, United States, and air in apartments in Moscow, Russia.[49,50] In our study, we identified only one *Malassezia* species (*M. restricta*) with low abundance (1.5×10^{-3}%) and prevalence (6.8%). Rather, we found 62 diverse yeast taxa with the most prevalent (more than 90%) genera *Cyberlindnera*, *Cryptococcus*, *Aureobasidium*, and *Candida*. In our floor dust, *Filobasidium* and various *Trichosporon* species accounted for only 0.61% of DNA sequences, although they were prevalent (>55%). *Candida*, *Rhodotorula*, and *Naganishia* can survive harsh environments, such as cleaning agents, and high temperature, salt concentration, and pH.[38,51] High prevalence and relative abundance of black yeasts such as *Exophiala* sp. and *Aureobasidium pullulans* are also likely associated with the production of melanin that protects them from environmental stress, such as ultraviolet light.[51] Although whether or not the origin of some dominant yeasts in our study was from human skin was unclear, our findings may indicate that yeast ecology in classroom floor dust was likely influenced by survival characteristics of individual yeasts and classroom water-damage. Additional studies of yeasts in school

environments using sequencing-based methodologies would provide a better understanding of yeast ecology and its role in occupants' health.

Cyberlindnera jadinii is a teleomorph parental species for *Candida utilis*.[52] It is currently placed in the order *Saccharomycetales* (ascomycetous yeast) but has historically had alternative taxonomic classifications.[53] In our study, species belonging to the genus *Candida* were found in 25% of the classrooms with generally low relative abundance (0.10%). *Candida tropicalis*,[50] the second most abundant (2%) single yeast species in our study, can become pathogenic in wounds or immunocompromised people.[46,54] The genus *Cryptococcus*, a basidiomycetous yeast, has 19 known species including two pathogenic species, *C. neoformans* and *C. gattii*, that can cause meningitis.[55] *Cryptococcus* has been associated with trees, humans, bird excreta, and soil,[46,55] and was one of the six most common fungi found in residential surface dust.[49] We found *Cryptococcus* species in almost all classrooms (492; 98.6%) with a total relative abundance of 5.2%, the second highest among yeasts. *Candida* and *Cryptococcus* species were commonly found in classroom floor dust in this study and our ongoing epidemiologic analyses may inform their health impact, if any, among school staff.

A cohort study of atopic children in the United States found mixed results—some *Cryptococcus* species (*C. nyarrowii*, *C. skinneri*, and *C. podzolicus*) in house dust adversely affected severe asthma and other species (*C. paraflavus* and *C. heimaeyensis*) beneficially affected mild asthma.[14] In our study, these specific *Cryptococcus* species were not identified. The same research group also found that no specific fungal taxon but the decreased diversity in the genus *Cryptococcus* had an adverse effect on asthma development in a low income, Latino birth cohort study in the United States.[3] In our study, we identified four *Cryptococcus* species (*C. gattii*, *C. neoformans*, *C. saitoi*, and *C. tephrensis*) of which *C. saitoi* showed the highest prevalence (87.2%) and relative abundance (1.6%). A U.S. study of homes reported *C. saitoi* has been associated with low asthma prevalence in children.[56] Exposure to some yeasts in the first year of life had a protective effect on the development of asthma at age 13 and on fungal sensitization.[57] However, exposure to yeasts in the genera *Rhodotorula* and *Sporobolomyces* in a damp building increased the odds of developing asthma in adult occupants.[58] In the current study, the genus *Rhodotorula* (RA = 1.1%) was isolated from 94% of the classrooms and *Sporobolomyces* (0.2%) from 68.3%. Taken together, health effects following exposures in schools to the yeast genera *Candida*, *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces*, or total yeasts may differ by composition or diversity of exposed species as well as occupant age (teachers versus students). Thus, our ongoing epidemiologic analyses of the potentially diverse effects of yeast exposure in schools on teachers' health would be of interest.

One of the strengths of our study is the large number of samples collected from 500 classrooms in 50 schools that made our results more reliable. However, there are some limitations in our study as well. We did not collect potentially important information such as presence of indoor plants and pets, type of ventilation, or surrounding outdoor environmental conditions that could have also affected fungal diversities and community compositions in the studied classrooms. As known, DNA sequencing does not differentiate live and dead fungal cells, which provides no direct information of potential pathogenicity

of the identified fungi.[59] Other weaknesses of the high-throughput sequencing method include potential biases involved in the different stages of sample analysis (e.g., DNA extraction, PCR amplification, primer selection, and bioinformatics analysis), which might have resulted in inaccurate sequencing or taxonomic assignment, especially at the species level.[6,60,61]

In summary, we found that the number of fungal OTUs was much lower than bacterial OTUs in school classrooms. In addition, the phylum *Ascomycota* was richer and more abundant than *Basidiomycota* in the classrooms. Although the genus *Penicillium* was highly diverse in our study, it was not relatively abundant. *Epicoccum* and *Alternaria* species, likely originating from outdoors, were prevalent and the most abundant in schools. Both release potent allergens, implicating the allergic potential of classroom floor dust. We also found that classrooms with the most water-damage had the lowest richness of fungi in floor dust, compared to those with less water-damage, while most water-damaged classrooms had the most varied composition. This indicated that the water-damage in individual classrooms in this high water-damage group may have differentially affected fungal compositions among the classrooms. Yeasts identified with high richness, abundance, and prevalence in our study increased in relative abundance with water-damage, but the dominant human skin yeast genus, *Malassezia*, was less prevalent in these environments. Our findings highlighted that yeasts are a significant part of indoor fungal ecology, especially in damp environments. An abundance of yeasts in floor dust may implicate dampness and mold conditions in schools, which also suggests that more research is needed on the health impact of yeast exposure in classrooms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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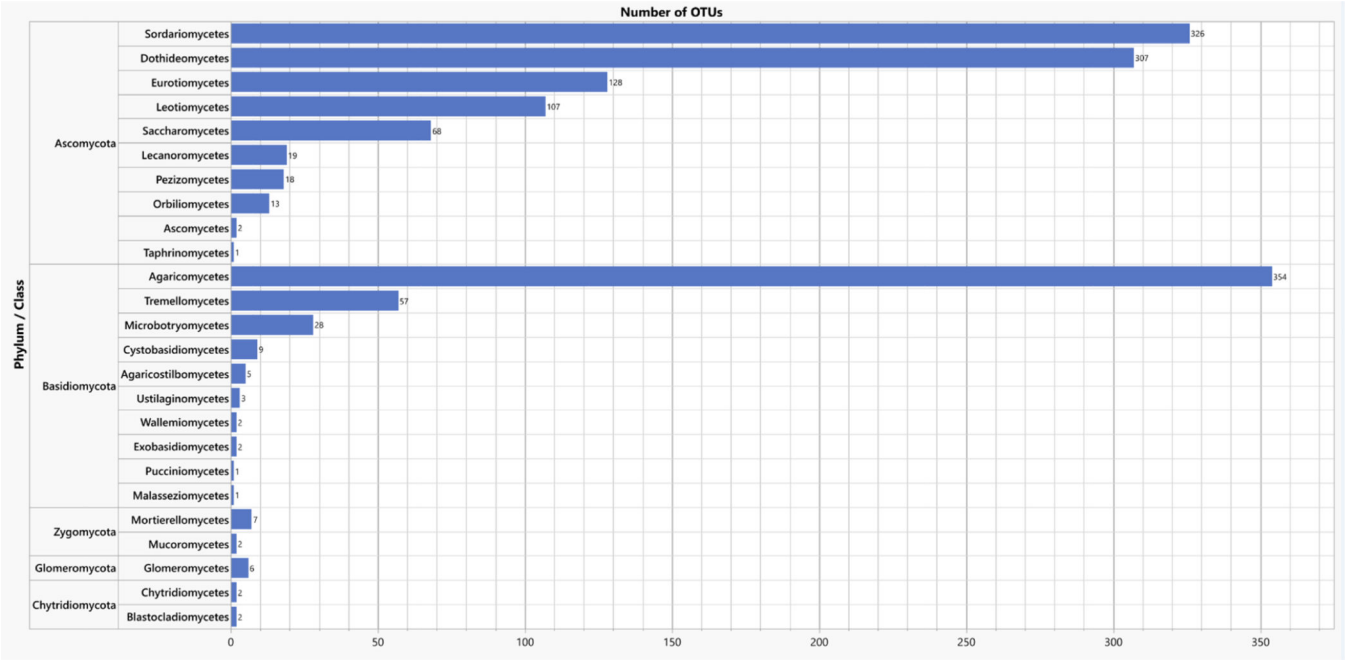

Figure 1.
Number of OTUs for each of the 25 classes identified in classroom floor dust.

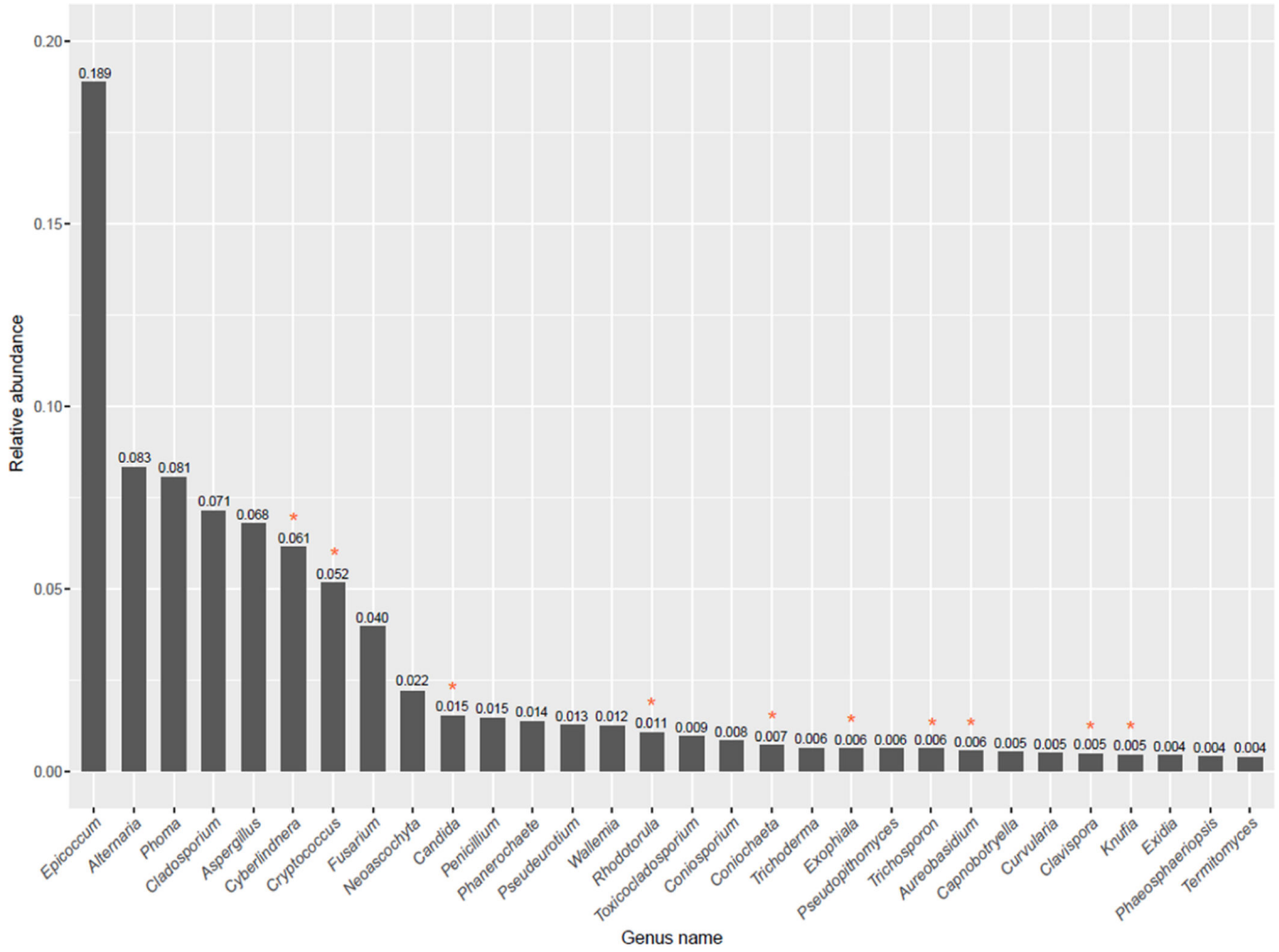

Figure 2.

Top 30 most abundant fungal genera. Yeast or yeast-like fungi are denoted with red asterisks and relative abundance on the *y*-axis is the overall relative abundance of all 499 samples.

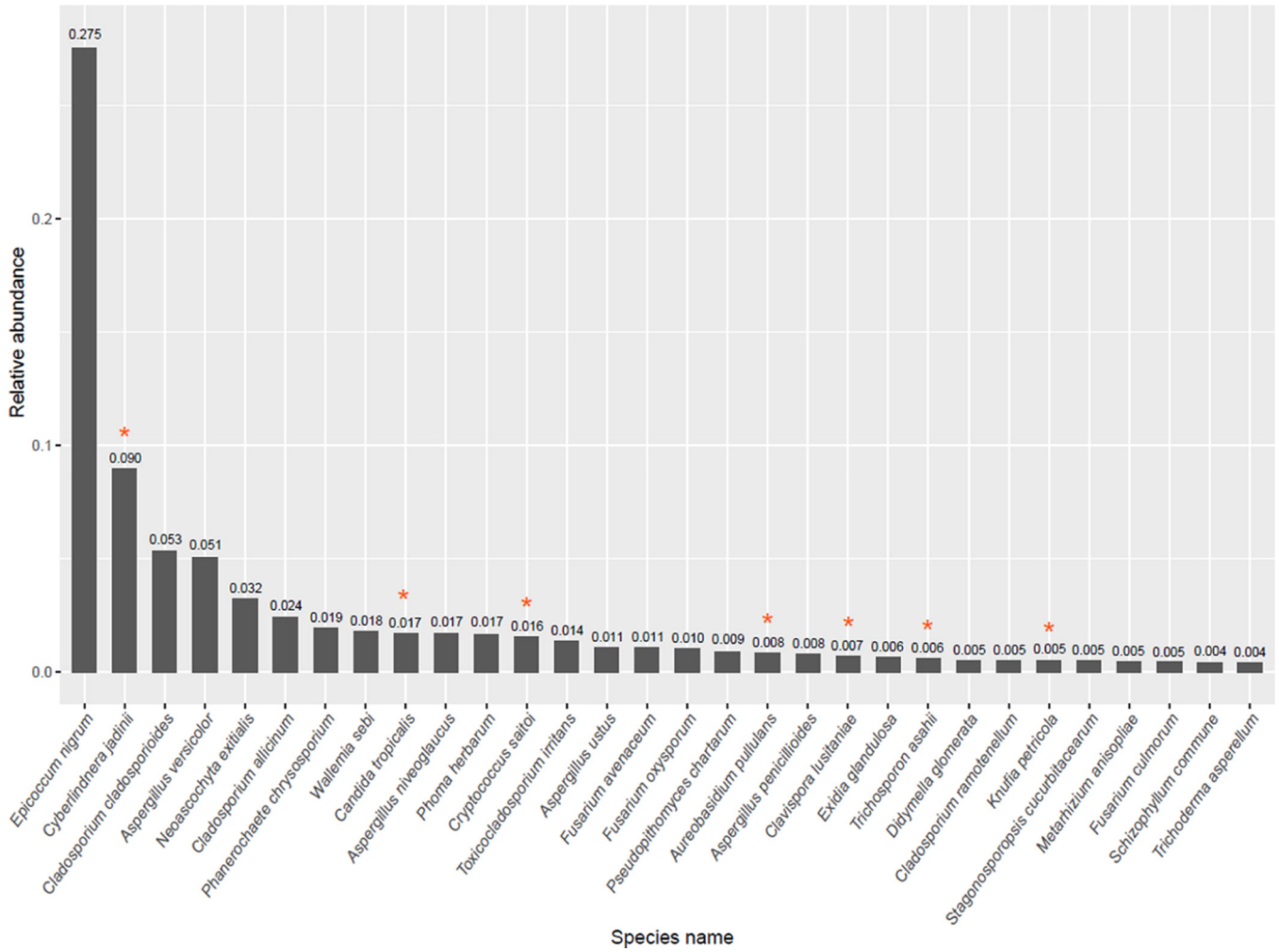

Figure 3.

Top 30 most abundant fungal species. Yeasts or yeast-like fungi are denoted with red asterisks and relative abundance on the *y*-axis is the overall relative abundance of all 499 samples.

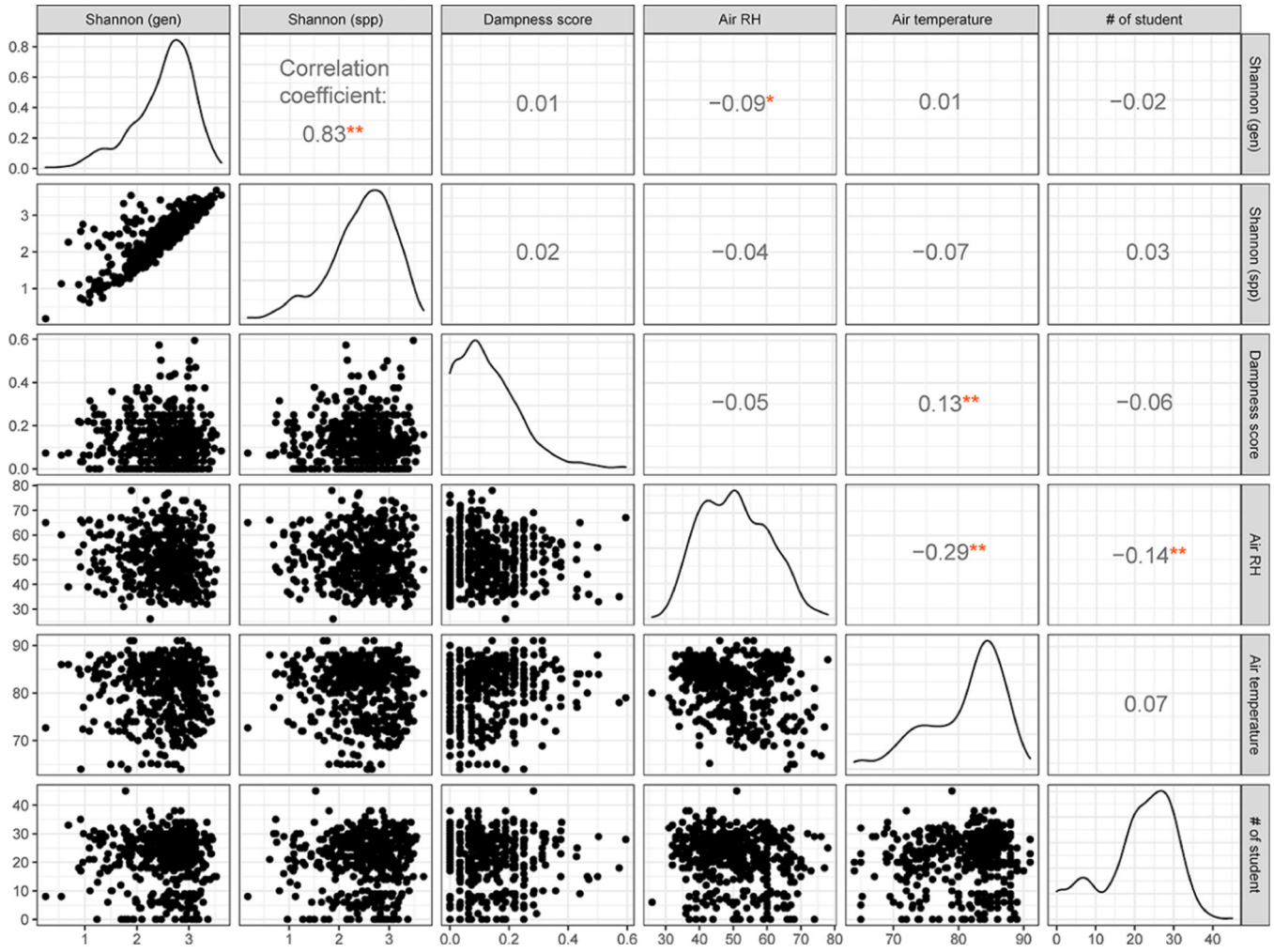

Figure 4.

Correlations among Shannon–Weaver diversity indices and environmental variables. * 0.05 < *p*-value < 0.1; ** *p*-value < 0.05.

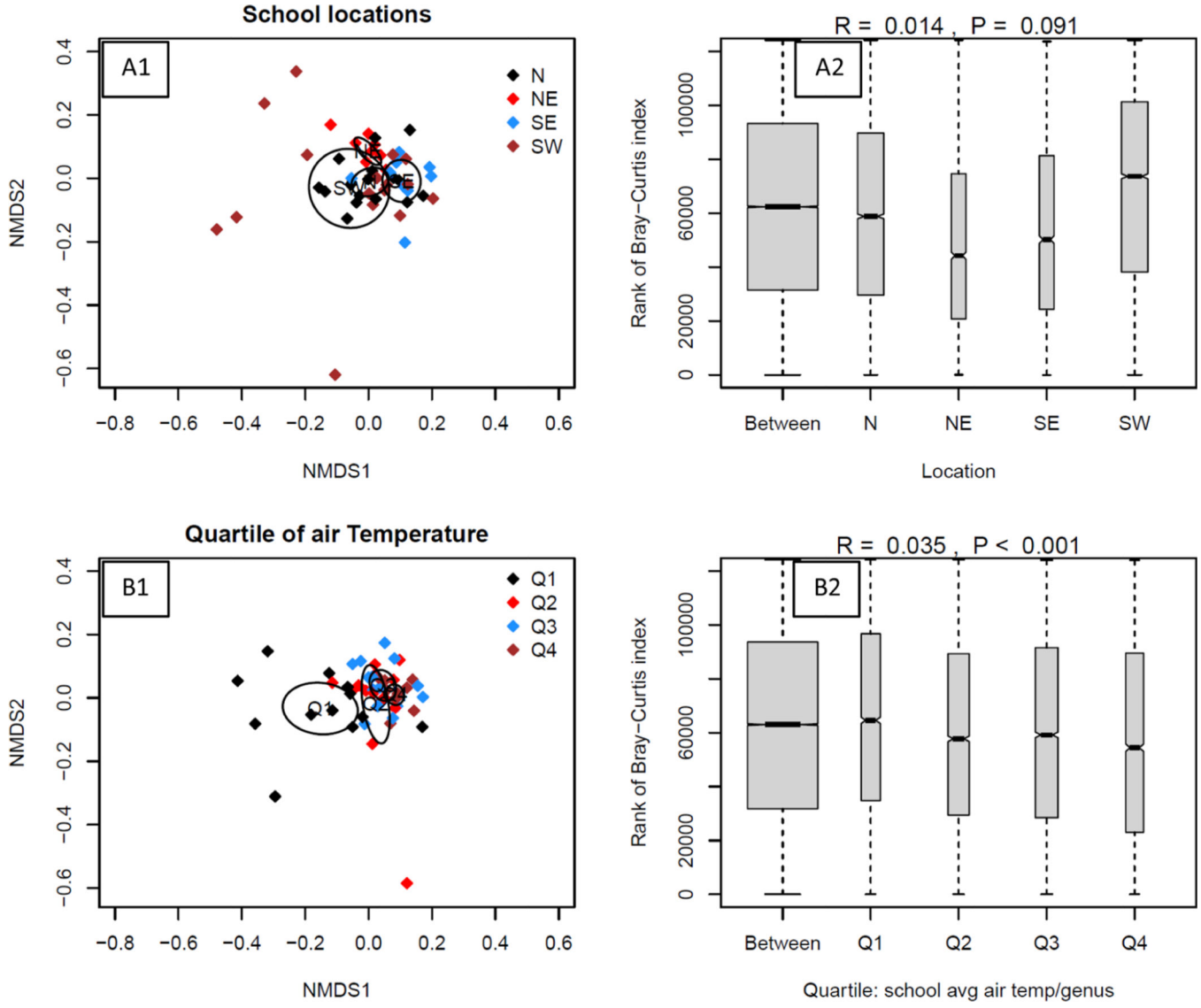

Figure 5.

NMDS plots with 95% CI ellipse in plots A1 and B1 and ANOSIM at the species level for the categorical variable of the school location (N: northern area; NE: northeastern area; SE: southeastern area; and SW: southwestern area within the city) and the genus level for average school temperature (minimum = 64.70, first quartile = 76.58; median = 81.07; third quartile: 84.80; maximum 88.40 °F) in plots A2 and B2, respectively. The rank of the Bray–Curtis index is the rank in ascending order of the dissimilarity index scores for all paired classroschools or classrooms with highest average air temperatureoms (N = 124,251).

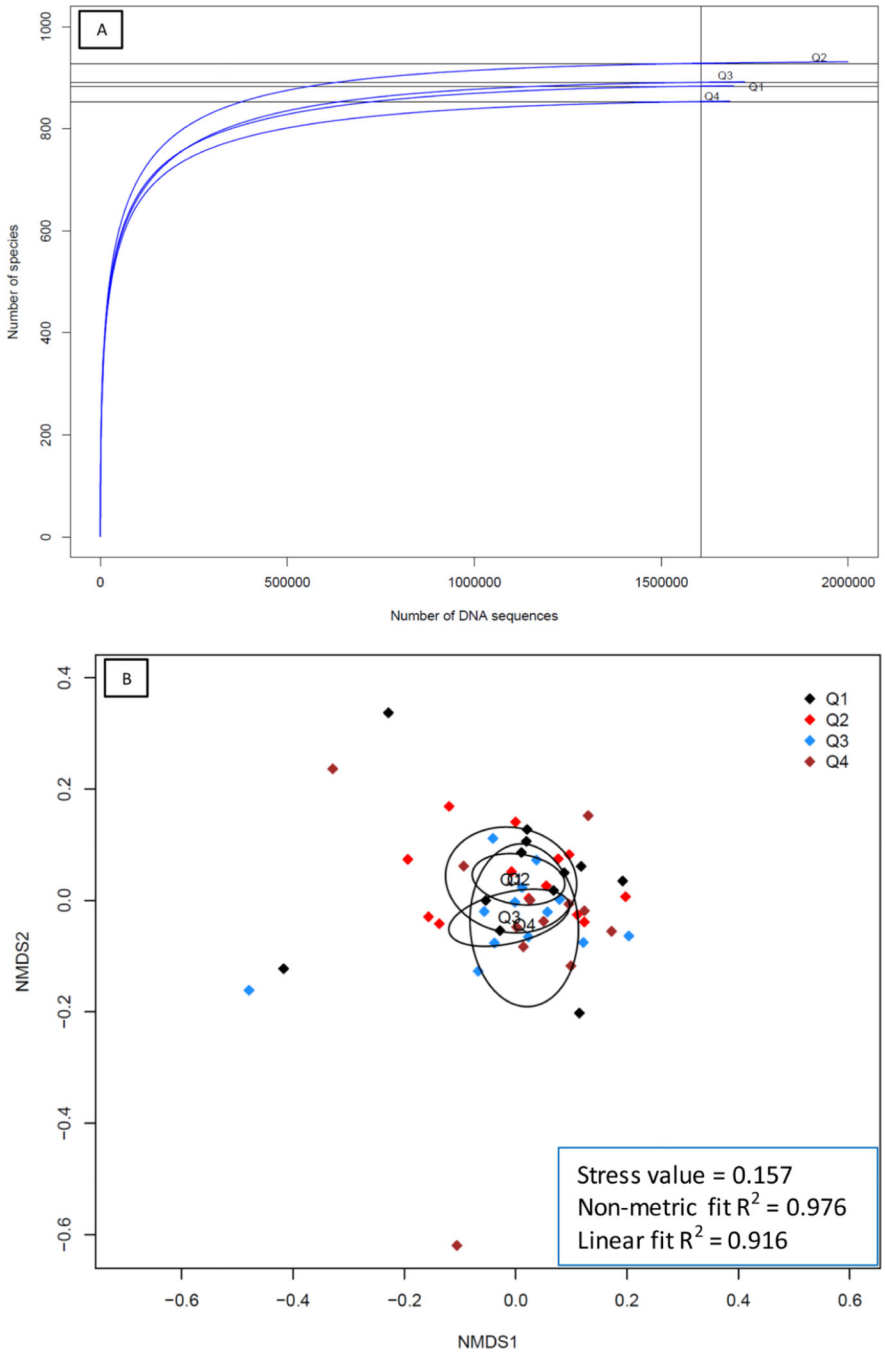

Figure 6.
Species rarefaction curve (A) and NMDS plot (B) by quartiles of the water-damage score.