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Associations of observed home dampness and mold with the fungal and bacterial dust microbiomes†

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The objective of this analysis was to examine and compare quantitative metrics of observed dampness and mold, including visible mold and moisture damage, and fungal and bacterial microbiomes. In-home visits were conducted at age 7 for children enrolled in the Cincinnati Childhood Allergy and Air Pollution Study. Trained study staff evaluated the primary residence and measured total areas of visible moisture and mold damage in the home. Floor dust was collected and archived. Archived dust samples collected from each home (n = 178) were extracted and analyzed using bacterial (16S rRNA gene) and fungal (internal transcribed spacer region) sequencing. Fungi were also divided into moisture requirement categories of xerophiles, mesophiles, and hydrophiles. Data analyses used Spearman's correlation, Kruskal–Wallis, Permanova, DESeq, and negative binomial regression models. Comparing high moisture or mold damage to no damage, five fungal species and two bacterial species had higher concentrations (absolute abundance) and six fungal species and three bacterial species had lower concentrations. Hydrophilic and mesophilic fungi showed significant dose-related increases with increasing moisture damage and mold damage, respectively. When comparing alpha or beta diversity of fungi and bacteria across mold and moisture damage levels, no significant associations or differences were found. Mold and moisture damage did not affect diversity of fungal and bacterial microbiomes. Instead, both kinds of damage were associated with changes in species composition of both bacterial and fungal microbiomes, indicating that fungal and bacterial communities in the home might be influenced by one another as well as by mold or moisture in the home.

Environmental significance

Various methods have been used to characterize the microbial exposure in homes and workplaces. Quantitative measures of observed mold and moisture damage have been explored as a way to quantify the exposure to microorganisms and/or their products. Amplicon-based sequencing performed on indoor dust samples has recently been used to characterize microbial diversity. Few studies have investigated both fungal and bacterial microbiomes in relation to observed mold and moisture damage. In the current study, mold and moisture damage were found to be associated with changes in the species composition of both bacterial and fungal microbiomes, indicating that the fungal and bacterial communities in the home might be influenced by one another as well as by mold or moisture in the home.

1. Introduction

It is estimated that the United States population spends 87% of their time indoors.[1] Therefore, there is potential for significant microbial exposure from the indoor environment. According to the World Health Organization, 10–50% of indoor environments in Europe, North America, Australia, India, and Japan are affected by increased dampness that could lead to increased microbial growth.[2] Various methods have been used to characterize the microbial exposure in homes and workplaces, including those that arise from dampness.

Quantitative measures of observed mold and moisture damage have been explored as a way to quantify the exposure to microorganisms and/or their products so that specific health-relevant guidelines could be set. We previously determined that the highest categories of measured areas of moisture and mold damage had significant associations with early childhood wheeze; however, at lower damage levels, the available data were too sparse to support specific health-relevant thresholds.[3]

Fungi in floor dust can be used as a surrogate for historical indoor inhalation exposure and has the added benefit of being

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less influenced by short-term variability in airborne concentrations from indoor activities and ventilation.[4] Floor dust has been evaluated for fungal species *via* culture methods,[5,6] species-specific quantitative polymerase chain reaction (qPCR),[7,8] and amplicon-based sequencing.[9,10] Amplicon-based sequencing performed on indoor dust samples has recently been used to characterize microbial diversity in dwellings, allowing an improved understanding of the indoor microbiome.[11,12]

Evaluating the microbial composition of homes and workplaces may provide insight as to the healthfulness of the environment. Healthy indoor microbiomes should promote health and minimize harmful exposures.[13] Many types of potentially pathogenic microorganisms have been identified in dust at various workplaces, including bacteria, such as *Bacillus*, *Actinomyces*, *Corynebacterium*, *Prevotella*, *Clostridium*, and *Rickettsia*, and fungi, such as *Alternaria*, *Cladosporium*, *Penicillium*, and *Aspergillus*.[14] When looking at the microbiome as a whole, an increased risk of asthma development has been associated with decreased fungal and bacterial diversity.[15,16] While some studies have explored the fungal or bacterial microbiome indoors, few studies have investigated both fungal and bacterial microbiomes in relation to observed mold and moisture damage.[17–19] Therefore, further research has been needed into how the microbiome is associated with the results of traditionally measured moisture or mold damage exposure in homes and workplaces.

In the current study, amplicon-based sequencing of the fungal and bacterial microbiome is compared with previously measured mold and moisture damage. Analyses included both untargeted statistical analyses to identify any microbial species associated with mold or water damage, as well as hypothesis-based analyses investigating whether the differing moisture requirements for growth of different fungal species influenced their associations with mold or water damage.

2. Methods

2.1 Moisture damage and mold damage

In-home visits were conducted in 2010 at age seven for children enrolled in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) (Fig. 1). Eligibility for the study required that at least one parent was atopic, which was defined as having allergic symptoms and a positive reaction on a skin prick test to at least 1 of 15 common aeroallergens.[20] Trained study staff evaluated the primary residence and measured the total areas of moisture damage and of visible mold in the home. Mold damage (m^2) was defined as the largest measured single surface area with mold, or mold and water damage, in any room in a home (*i.e.*, visible mold was required). Moisture damage (m^2) was defined as the maximum damaged surface from either water, mold, or both on a single surface in any room in a home.[3] Floor dust samples from CCAAPS homes were collected, archived, and stored in a freezer at −20 °C in 2010. Collection method has been previously described.[21] Mold damage was analyzed as a continuous variable and also in three categories: high ≥ 0.19 m^2, low < 0.19 m^2, and no mold damage. Similarly, moisture damage was analyzed both as a continuous variable and categorically: high ≥ 0.29 m^2, low < 0.29 m^2, and no moisture damage. These categories are based on previously published work.[3]

2.2 Universal qPCR and microbiome for fungi and bacteria

In the current study, DNA was extracted from the archived floor dust samples using the MOBIO PowerLyzer® PowerSoil® DNA isolation kit according to the manufacturer's instructions (MOBIO, Carlsbad, California) modified by adding additional glass beads.[22] Ultra-high-throughput microbial community analysis (Illumina MiSeq) was performed by the Research and Testing Laboratory in Lubbock, TX. The internal transcribed spacer (ITS) region of fungal ribosomal DNA was amplified by PCR with ITS1F/ITS2aR primers. For bacteria, extracted DNA was amplified with 16S ribosomal DNA (rDNA) 515F and 806R primers that target the V4 region.

Total DNA concentration was measured using qPCR with universal fungal primers 5.8F1/5.8R1 that target the ITS region[23] and universal bacterial primers that target the 16S rRNA gene[24] (Step One Plus, Applied Biosystems, Life Technologies, Carlsbad, CA). The assay and cycling conditions have been previously described.[25] The cycle threshold determinations were automatically performed by the instrument using default parameters. Samples were tested in triplicate with R^2 > 0.9 and efficiency > 90%. Extracted DNA from a solution of *Aspergillus fumigatus* or *Bacillus atrophaeus* with a known concentration of spores was used as the standard. A set of PCR reaction mixtures

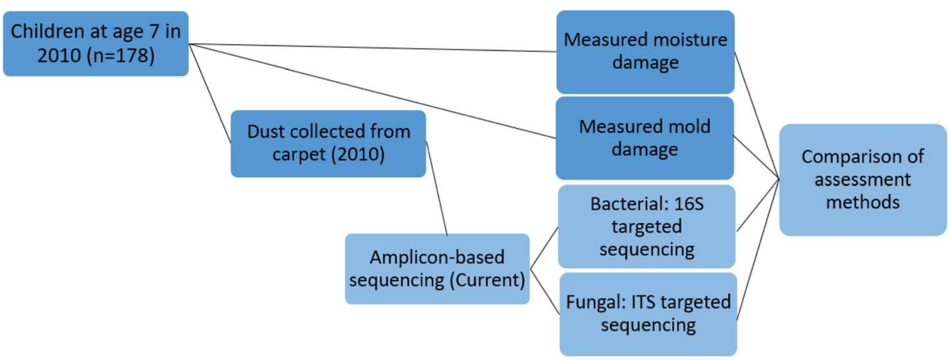

Fig. 1 Flow diagram of assessment methods.

were spiked with a known concentration of DNA to test for inhibition.[9]

The DADA2 Pipeline in R Statistical Analysis Software was used to remove chimeras and develop the amplicon sequence variant (ASV) table and to assign taxonomy using Silva version 132 and UNITE database.[26,27] Analysis of the sequencing reads, including refined phylogenic, abundance, and diversity analyses was conducted at the University of Cincinnati.

2.3 Data analysis

Downstream analyses were conducted using the R package, phyloseq.[28] *P*-Values less than 0.05 were considered significant for all tests. Adjusted *p*-values that accounted for multiple testing were utilized for PERMANOVA and DESeq analyses. We examined four aspects of the microbiome data and how they were linked with measured mold and moisture damage: alpha richness, beta diversity, indicator taxa, and fungi with known moisture requirements. Those species identified by name were verified in NCBI to corroborate the phylogenetic approach.

2.3.1 Alpha diversity. Alpha diversity measures calculated for each sample, based on rarefied data, included Shannon's diversity, the number of observed ASVs (observed), and Faith's phylogenetic diversity (PD). Spearman correlation coefficients were determined for associations between fungal and bacterial alpha diversity measures (Shannon, observed, and PD) and continuously measured variables of mold and moisture damage. A Kruskal–Wallis test was performed to evaluate the differences in median alpha diversity measures between the three categories of mold and moisture.

2.3.2 Beta diversity. Beta diversity analysis was performed with relative abundance data. Multivariate analysis of variance using distance matrices was utilized with PERMANOVA (Adonis) and Bray–Curtis to assess differences in beta diversity of the relative abundance of fungal and bacterial species, between the mold damage categories (high, low and no) and between the moisture damage categories (high, low, and no). Heatmaps were generated by selecting only species that were present in at least 20% or more of the samples. The non-metric multi-dimensional scaling (NMDS) of fungal and bacterial relative abundance was plotted between the three mold damage and the three moisture damage categories.

2.3.3 Indicator taxa. Differential abundance testing with DESeq was used to determine which fungal and bacterial species differed between categories of mold and moisture damage. We accounted for differences in sequencing depth by multiplying the relative abundance values by the qPCR (fungal or bacterial total DNA per gram of dust) values for each sample to calculate absolute abundance, a term based on previous literature.[29,30] Traditional DESeq is performed with raw counts and is normalized by estimating size factors within the first step. We used a modified DESeq in which we eliminated the first step of estimating size factors, since our data was already normalized. Species were filtered by requiring their presence in 20% or more of samples, plus a species log 2 fold change greater than 2 or less than −2, with an adjusted *p*-value < 0.05.

2.3.4 Fungi with known moisture requirements. Fungal species were categorized into hydrophilic ($a_w \geq 0.90$), mesophilic ($0.80 \leq a_w < 0.90$), and xerophilic ($a_w < 0.80$) based on moisture requirements found in the literature.[10] Spearman correlation coefficients were also determined for comparisons between the sum of the absolute concentrations of fungal species within each of the three water requirement categories (hydrophilic, mesophilic, and xerophilic) and continuously measured variables of mold and moisture damage.

For mesophiles, negative binomial regression models were utilized to analyze the absolute abundance differences of fungi across the three levels of mold and moisture damage categories.[10,31] For xerophiles and hydrophiles, each with large proportions of zero counts, zero-inflated negative binomial regression models were used for these analyses. We note that in zero-inflated negative binomial regression models, excess zeros are modeled independently of the counts.

3. Results

3.1 Overview of sequencing

For fungi, an average of 7354 (range 186–26 276) sequences per sample was identified with a total of 3855 amplicon sequence

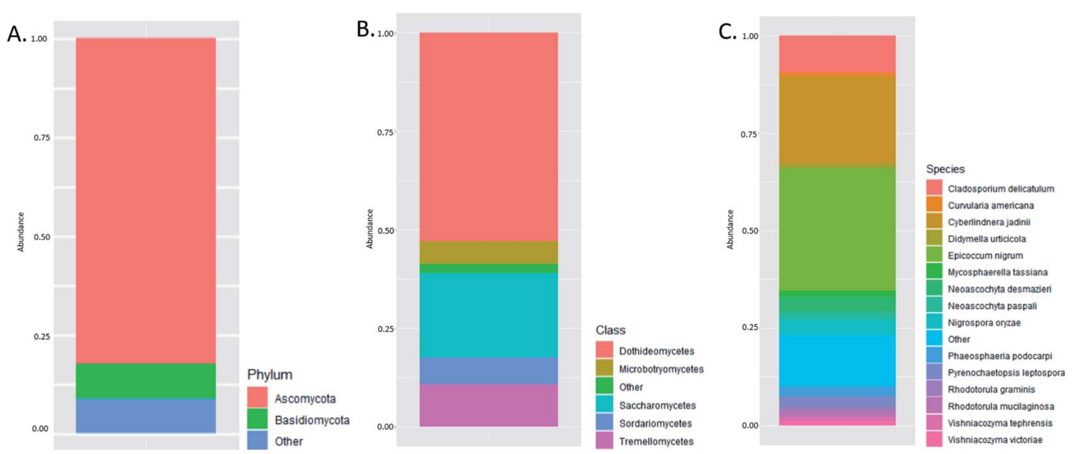

Fig. 2 Relative abundance of the most abundant fungal microbiome (A) 2 phyla, (B) 5 classes, and (C)15 species.

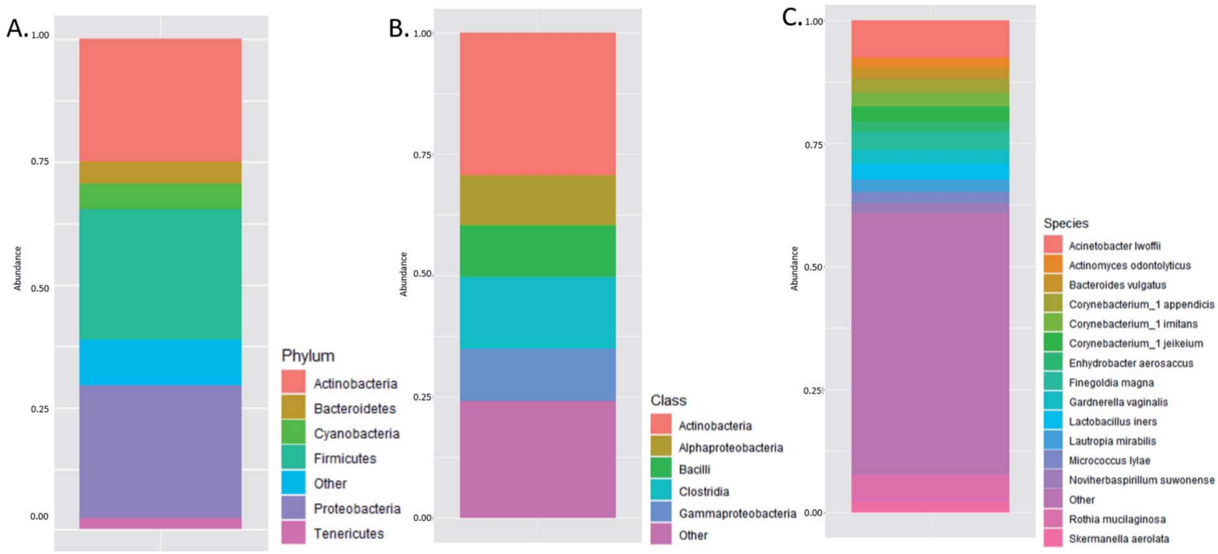

Fig. 3 Relative abundance of the most abundant bacterial microbiome (A) 6 phyla, (B) 5 classes, and (C)15 species.

variants (ASVs). For bacteria, an average of 28 234 (range 6033–53 036) sequences per sample was identified with a total of 11 150 ASVs. Rarefaction curves for fungal analyses are presented in the ESI (Fig. S1†). For fungal samples, 1000 reads was selected as the threshold for rarefaction, as this maintained 1942 taxa and excluded only 6 samples. For bacterial analyses, the minimum read count was 6,033, which was used as the number of reads to rarefy the data for alpha diversity analyses. The predominant fungal and bacterial phyla, classes, and species, based on relative abundance, can be seen in Fig. 2 and 3, respectively. *Epicoccum nigrum* was the most abundant fungal species of the mycobiome, accounting for 17% of the fungal microbiome. *Acinetobacter lwoffii* was the most abundant bacterial species accounting for 1% of the bacterial microbiome. At the genus level, with 11% relative abundance of the bacterial microbiome consisting of *Corynebacterium*, followed by *Staphylococcus* (9%), *Streptococcus* (6%), *Acinetobacter* (4%), *Massilia* (2%), *Pseudomonas* (2%), and *Lactococcus* (1%) (data not shown). Species identified in the indicator taxa analysis and fungi with known moisture requirements were verified on the NCBI database and closely-related species (>98% identity match and >98% query cover) to the target sequences are identified in Table S1.†

3.2 Alpha diversity

We did not find any correlations between the alpha diversity measures (Shannon, PD, and observed) of fungal and bacterial microbiomes and visibly measured mold and moisture damage (Table 1). Furthermore, there were no significant differences between categories of mold damage (high/low/no) or moisture damage (high/low/no) and the bacterial or fungal alpha diversity measures using Kruskal–Wallis (Table 2).

3.3 Beta diversity

Multivariate analysis of variance demonstrated no significant differences in fungal or bacterial species between the categories of mold or moisture damage (Table 3). For the heat maps, 61 fungal species and 103 bacterial species remained after trimming so that species that were not observed in at least 20% of

Table 1 Correlation of fungal and bacterial alpha diversity to continuous variables of mold and moisture damage with Spearman correlation coefficient

	Spearman correlation coefficient, ρ (p-value)	
Diversity measure	Mold damage	Moisture damage
Fungal Shannon	0.069 ($p = 0.38$)	0.099 ($p = 0.20$)
Fungal PD	0.019 ($p = 0.80$)	0.057 ($p = 0.46$)
Fungal observed	0.042 ($p = 0.59$)	0.071 ($p = 0.36$)
Bacterial Shannon	0.15 ($p = 0.047$)	−0.049 ($p = 0.53$)
Bacterial PD	0.058 ($p = 0.45$)	−0.079 ($p = 0.31$)
Bacterial observed	0.044 ($p = 0.57$)	−0.092 ($p = 0.23$)

Table 2 *P*-Values for differences between fungal and bacterial alpha diversity across mold and moisture damage categories with Kruskal–Wallis test, *p*-values[a]

	Kruskal–Wallis, p-value	
Diversity measure	Mold damage (high/low/no)	Moisture damage (high/low/no)
Fungal Shannon	0.58	0.33
Fungal PD	0.40	0.46
Fungal observed	0.25	0.27
Bacterial Shannon	0.10	0.74
Bacterial PD	0.83	0.29
Bacterial observed	0.84	0.34

[a] Mold damage categorical (high $\geq$ 0.19 m^2); moisture damage categorical (high $\geq$ 0.29 m^2).

Table 3 Permanova (ADONIS) dissimilarity matrices using Bray's dissimilarity between fungal and bacterial species across mold and moisture categories

	Permanova (ADONIS), R^2 (p-value)	
Relative abundance	Mold damage (high/low/no)	Moisture damage (high/low/no)
Fungal species	0.01 ($p = 0.29$)	0.02 ($p = 0.13$)
Bacterial species	0.01 ($p = 0.54$)	0.01 ($p = 0.48$)

the samples were excluded.[32] A heat map displaying the fungal and bacterial species with all categorical data, including mold high, low, and no categories, and moisture damage high, low, and no categories, can be seen in the ESI (Fig. S2 and S3†). Heat maps did not reveal any major clustering between any categorical data. The non-metric multi-dimensional scaling (NMDS) of fungal and bacterial species within the categories of mold damage and moisture damage, presented in Fig. S4 and S5,† showed no discernable clustering patterns.

3.4 Indicator taxa

Table 4 shows the DESeq results on the differential absolute abundance (referred to as concentration) of fungal and bacterial genera and species between no and high categories of mold and moisture. Three species of fungi (*Toxicocladosporium irritans*, *Plectosphaerella oratosquillae*, and *Phaeosphaeria podocarpi*) and two species of bacteria (*Alkanindiges illinoisensis* and *Dialister invisus*) had higher concentrations in high mold damage compared to no mold damage. Four fungal species (*Septoriella phragmitis*, *Exophiala xenobiotica*, *Candida tropicalis*, and *Vishniacozyma carnescens*) and four bacterial species (*Staphylococcus aureus*, *Coprococcus 2 eutactus*, *Corynebacterium matruchotii*, and *Akkermansia muciniphila*) had lower concentrations in high mold damage compared to no mold damage. In high moisture damage compared to no moisture damage, four species of fungi (*T. irritans*, *P. oratosquillae*, *R. mucilaginosa* and *Candida parapsilosis*), as well as two bacterial species (*C. eutactus* and *A. illinoisensis*) had higher concentrations. Three species in high moisture damage compared to damage had lower concentrations (fungal: *Filobasidium oeirense* and *Hannaella phetchabunensis*; bacterial: *S. aureus*).

Only a few species differed across the categories of both kinds of damage (high mold and moisture damage categories compared to none). The fungal species, *T. irritans* and *P. oratosquillae*, and the bacterial species *Alkanindiges illinoisensis* had higher concentrations in both types of high damage categories, and the bacteria species of *S. aureus* had lower

Table 4 Differential of absolute abundance (DESeq) between no and high mold and moisture damage (log 2 fold change, all data has adjusted p-values < 0.05). Red: negative log-fold change; blue: positive log-fold change

Kingdom	Mold	Moisture	Species
Fungus		4.31	*Candida parapsilosis*
	2.42		*Phaeosphaeria podocarpi*
	2.69	2.98	*Plectosphaerella oratosquillae*
		2.55	*Rhodotorula mucilaginosa*
	5.52	6.11	*Toxicocladosporium irritans*
	−3.69		*Candida tropicalis*
	−2.06		*Exophiala xenobiotica*
		−2.04	*Filobasidium oeirense*
		−2.56	*Hannaella phetchabunensis*
	−2.25		*Septoriella phragmitis*
	−2.42		*Vishniacozyma carnescens*
Bacteria	2.36	2.03	*Alkanindiges illinoisensis*
	2.05		*Dialister invisus*
	−2.07	2.53	*Coprococcus 2 eutactus*
	−2.30		*Akkermansia muciniphila*
	−2.90		*Corynebacterium matruchotii*
	−2.96	−2.10	*Staphylococcus aureus*

concentrations for both types of high damage categories. *C. eutactus*, however, showed inconsistent associations, decreasing in the high mold category but increasing in the high moisture damage category.

The complete DESeq results on the differential absolute abundance of fungal and bacterial genera and species among the three categories of mold and moisture damage are presented in Tables S2 and S3.† For fungi, DESeq analysis identified 25 species that differed between categorical values of mold damage (no/low/high) and moisture damage (no/low/high) (Table S2†). For fungi, the DESeq analysis demonstrated that with increasing moisture and/or mold damage, 13 fungal species had higher concentrations, six species had lower concentrations, and five species concentrations were neither consistently higher nor lower. For bacteria, DESeq analysis identified 14 species (9 Gram-positive and 5 Gram-negative) that differed between categorical values of mold damage (no/low/high) and moisture damage (no/low/high; Table S3†). In general, the DESeq analysis demonstrated that, with increasing moisture and/or mold damage, the absolute concentrations of seven bacterial species were higher, and the concentrations of two species were lower.

3.5 Fungi with known moisture requirements

From the 3855 ASVs, 759 fungal species were identified in our samples, but only 25 had known moisture requirements and could be categorized as hydrophiles, mesophiles, or xerophiles (Table 5). Based on the relative abundance, the xerophilic species consisted of 0.08% of the total fungal microbiome and the hydrophilic species consisted of 0.5%, with a majority being *Rhodotorula mucilaginosa*. The mesophilic species consisted of 23% of the mycobiome, with *E. nigrum* as the most dominant species (17%), followed by *Cladosporium delicatulum* (5%).

Spearman correlation coefficients for hydrophiles, mesophiles and xerophiles with mold damage were 0.08, 0.11, and 0.07, respectively, and with moisture damage, 0.10, 0.06, and 0.11, respectively, with *p*-values ranging from 0.15 to 0.42.

Based on the negative binomial regression model, mesophiles were 2.42 and 1.95 times greater in the high and low mold damage categories, respectively, than in the no mold damage category (both $p = 0.01$). Mesophiles showed no significant association with high and low moisture damage categories compared to no moisture damage (estimated relative change [ERC] = 1.29 and 1.21, respectively). Based on the zero-inflated negative binomial regression model, hydrophiles were 6.13 and 5.00 times greater in the high and low moisture damage categories, respectively, than in the no moisture damage category ($p < 0.01$ and $p = 0.01$, respectively). Hydrophiles did not show any significant association with high and low mold damage categories compared to no mold damage (ERC = 0.34 and 3.39, respectively). The zero-inflated negative binomial regression model for xerophiles showed no significant relationships with high and low mold damage (ERC = 0.94 and 0.55, respectively) or high and low moisture damage (ERC = 0.75 and 0.62) compared to no damage. The models of the mesophiles, hydrophiles, and xerophiles appear to have a dose–response relationship with increasing mold or moisture damage categories with the exception of hydrophiles and mold damage categories. The zero-inflation components of the models did not demonstrate statistically significant relationships of the occurrence of excess zeros with the different damage categories of no, low and high for mold or moisture damage, for either xerophiles or hydrophiles (Table 6).

4. Discussion

This study utilized amplicon-based sequencing of the fungal and bacterial microbiome and compared these results with previously measured mold and moisture damage. Mold and moisture damage were found to be associated with changes in bacterial and fungal species of the indoor microbiome, but not in the overall composition as measured by alpha or beta diversity. High moisture or mold damage was significantly associated with higher or lower concentrations of specific

Table 5 Xerophiles, mesophiles and hydrophiles present in our samples based on established water requirements, and relative abundance percentage in the mycobiome[a]

Xerophiles	% of mycobiome	Mesophiles	% of mycobiome	Hydrophiles	% of mycobiome
Wallemia sebi	2.75×10^{-3}	*Penicillium aurantiogriseum*	7.28×10^{-4}	*Pichia membranifaciens*	1.34×10^{-3}
Aspergillus ruber	3.62×10^{-3}	*Penicillium glabrum*	9.06×10^{-4}	*Rhodotorula mucilaginosa*	4.56×10^{-1}
Aspergillus penicillioides	0.00×10^{0}	*Cladosporium halotolerans*	3.17×10^{-2}	*Stachybotrys chartarum*	8.80×10^{-3}
Aspergillus sydowii	7.35×10^{-2}	*Cladosporium sphaerospermum*	3.70×10^{-2}	*Mucor racemosus*	1.97×10^{-3}
Penicillium brevicompactum	5.21×10^{-4}	*Debaryomyces hansenii*	9.38×10^{-3}	*Sistotrema brinkmannii*	2.63×10^{-3}
Hortaea werneckii	5.47×10^{-4}	*Wickerhamomyces anomalus*	3.26×10^{-3}		
		Cladosporium dominicanum	1.45×10^{-3}		
		Cladosporium ramotenellum	1.34×10^{-1}		
		Epicoccum nigrum	1.74×10^{1}		
		Fusarium sporotrichioides	4.69×10^{-2}		
		Cladosporium delicatulum	5.11×10^{0}		
		Aureobasidium pullulans	3.68×10^{-1}		
		Fusarium solani	3.26×10^{-2}		
		Saccharomyces cerevisiae	8.41×10^{-3}		

[a] Xerophiles $a_w < 0.80$; mesophiles $0.80 \leq a_w < 0.90$; hydrophiles $a_w \geq 0.90$ (Adams *et al.*, 2020).[10]

Table 6 Estimated relative change in absolute abundance from a zero-inflated negative binomial regression models for xerophiles and hydrophiles and negative binomial regression model for mesophiles,[a] with *p*-values of the models coefficients

		Mold damage				Moisture damage			
		Count modeling		Zero modeling		Count modeling		Zero modeling	
Zero-inflated negative binomial	Mold or moisture damage comparison	ERC	*p*-Value	ERC	*p*-Value	ERC	*p*-Value	ERC	*p*-Value
Xerophiles	High	0.94	0.93	0.35	0.10	0.75	0.68	0.47	0.15
	Low	0.55	0.42	1.38	0.51	0.62	0.49	0.93	0.87
	No	1.00	—	1.00	—	1.00	—	1.00	—
Hydrophile	High	0.34	0.13	1.34×10^{-4}	0.93	6.13	**<0.01**[b]	1.19	0.87
	Low	3.39	0.08	2.26	0.15	5.00	**0.01**[b]	2.29	0.45
	No	1.00	—	1.00	—	1.00	—	1.00	—
Mesophiles	High	2.42	**0.01**[b]	—	—	1.29	0.40	—	—
	Low	1.95	**0.01**[b]	—	—	1.21	0.44	—	—
	No	1.00	—	—	—	1.00	—	—	—

[a] ERC: estimated relative change (ERC) in the absolute abundance is the exponential of the negative binomial model estimate; mold damage categorical (high ≥ 0.19 m^2; 0 > low > 0.19 m^2; no = 0 m^2); moisture damage categorical (high ≥ 0.29 m^2; 0 > low > 0.29 m^2; no = 0 m^2); only negative binomial regressions were used with mesophiles. [b] *p*-Value < 0.05.

fungal and bacterial species. In addition, the concentration of hydrophilic fungi was significantly greater with the presence of moisture damage, and the concentration of mesophilic fungi was significantly greater with the presence of mold damage, with a dose–response pattern in both instances. Fungal and bacterial measures of alpha diversity (Shannon, number of observed ASVs and phylogenetic diversity indices) yielded no significant correlations with continuously measured mold and moisture damage, as well as no significant differences between the three categories of mold or moisture damage. Fungal and bacterial measures of beta diversity utilizing Permanova with Bray–Curtis similarly yielded no significant differences between the three categories of mold or moisture damage.

In high mold damage homes compared to no mold damage, we found three fungal and two bacterial species with higher concentrations and four fungal and four bacterial species with lower concentrations. There is limited data regarding the effects of mold or moisture damage on indoor fungal and bacterial microbiomes. Some studies have found no specific bacterial or fungal species associated with mold damage in homes.[9] Adams *et al.*[10] found associations between water damage status and indicator fungal taxa in vacuum dust samples, including *R. mucilaginosa*, and in door trim swabs, including *T. irritans*. Both of those species had significantly higher concentrations with increasing moisture damage in our study. Fungal species *P. oratosquillae*, *C. parapsilosis*, and *P. podocarpi* also had higher concentrations with moisture and/or mold damage; Dannemiller *et al.*[9] has reported positive associations between *Plectosphaerella* spp. and bacterial and human cell concentrations, *Phaeosphaeria* spp. and bacterial, fungal, and human cell concentrations, and *C. parapsilosis* and bacterial and fungal concentrations. *C. parapsilosis* and bacterial genus *Alkanindiges* were also more common in multi-family homes when compared to single-family homes.[9] In our study, both high mold and moisture damage categories were associated with higher concentrations of three species, *A. illinoisensis*, *T. irritans*, and *P. oratosquillae*. In addition, *Acremonium* spp. has been shown to be very common on damp building materials.[33] In our study, *A. fusidioides* had a higher concentration (3.1 log fold change) with increasing mold damage from low to high mold damage. Several bacterial and fungal species had lower concentrations with increasing mold or moisture damage. These species potentially could thrive at lower water content levels but were outcompeted at higher water content levels. For example, *S. aureus* has an a_w value as low as 0.82 (15% w/v NaCl),[34] and our data demonstrated greater concentrations with no mold and moisture damage, and significantly lower concentrations with both high moisture and mold damage. A study by Jayaprakash in 2017 found a similar trend where *Staphylococcus* had significant increases in the relative abundance after moisture damage renovations.[19] A majority of bacterial species evaluated for growth of microorganisms in food, however, have a minimum inhibitory a_w value of 0.90.[35] Emerson *et al.*[36] evaluated the abundant genera in flooded homes and found the most abundant bacterial families to be Pseudomonadaceae, Enterobacteriaceae, and Moraxellaceae which was dominated by *Acinetobacter johnsonii* and *Acinetobacter rhizosphaerae*. As our study was a categorical species comparison, *Alkanindiges illinoisensis* was the only species found within these families that had a significantly greater abundance in high mold and moisture damage homes. Overall, this study found 24 fungal species and 14 bacterial species with significant differences between categories of mold damage and moisture damage. At higher levels of mold and moisture damage, some bacterial and fungal species had higher concentrations and some lower concentrations. This raises the question of how the indoor species composition of the bacterial and fungal microbiomes may interact with each other.

Hydrophilic fungi demonstrated a dose-related increase across categories of no, low, and high moisture damage, with *R. mucilaginosa* as the most prevalent hydrophile. Adams *et al.*[10] found similar results from vacuum dust samples, where the relative abundance of hydrophilic fungi were significantly higher in homes with more visible mold areas, with a dose-related increase across categories of no, low, and high mold damage. *R. mucilaginosa* is a yeast and likely an opportunistic human pathogen.[37] We have previously demonstrated that children residing in homes with high moisture damage were significantly more likely to have wheezing at age 3 and persistent wheeze through age 7 (adjusted odds ratio [aOR] = 2.2; 95% confidence interval [CI] = 1.0, 4.3 and aOR = 3.2; CI = 1.3, 7.5, respectively).[3] When comparing the indicator species and those with water requirement values, *R. mucilaginosa* was present on both lists and was among the most abundant 15 species overall. *R. mucilaginosa* concentration was higher with higher moisture and mold damage conditions. This agrees with findings by Adams *et al.*,[10] who also saw significantly higher concentrations of *R. mucilaginosa* in vacuum samples within water-damaged homes. Mesophilic fungi concentrations were greater in the highest category of mold damage, with *E. nigrum* as the most prevalent mesophile. *E. nigrum* and *C. delicatulum* were two of the most abundant fungal species within this study, accounting for 17% and 5% of the fungal microbiome, respectively. Both are mesophilic fungi with known moisture requirements. *E. nigrum*, considered to be mostly an outdoor saprophyte, is commonly found indoors, and has been shown to cause respiratory allergy disorders in humans.[8,38–41] We have also shown previously that children in homes with high mold damage were significantly more likely to have wheezing at age 3 and a marginally significant increase in persistent wheeze through age 7 (aOR = 3.5; CI = 1.5, 8.2, and aOR = 2.8; CI = 0.9, 8.6, respectively).[3]

Dust in various typical indoor environments and workplaces has been shown to contain many types of potentially pathogenic microorganisms, including fungi, such as *Alternaria*, *Cladosporium*, *Penicillium*, and *Aspergillus*.[14,42] Our most abundant fungal genera included *Epicoccum*, *Alternaria*, *Cyberlindnera*, *Pseudopithomyces*, and *Cladosporium*. As for potentially pathogenic bacteria, typical dust in indoor environments also includes *Bacillus*, *Actinomyces*, *Corynebacterium*, *Prevotella*, *Clostridium*, *Staphylococcus*, *Lactococcus*, and *Rickettsia* bacteria.[14,42] Of those genera, *Staphylococcus*, *Corynebacterium*, *Streptococcus*, and *Lactococcus* were among the most abundant genera in our study.

We did not see different trends among Gram-positive *vs.* Gram-negative bacteria in the differential abundance of species across levels of water damage or mold, whereas other studies have found that water damage or visible mold/mildew in the home was associated with a 20–66% increase in the levels of Gram-negative bacteria (measured by endotoxin and midchain 3-hydroxy fatty acids).[43] In addition, we did not see different trends for the fungal phyla Ascomycota *vs.* Basidiomycota in the species differential abundance across damage levels, whereas other studies have reported an increase in Basidiomycota : Ascomycota ratios when evaluating the degradation of fungi in soil litter over time.[44] Within our study, generally species were differentially abundant, either increasing or decreasing, independently, and not based on the Gram-stain category or fungal phylum. Cross-detection of closely-related species was possible with a few of the specific species identified, however similar to Adams *et al.*,[10] when comparing multiple databases, the overall analysis and findings did not differ.

Our observations of alpha diversity measures were consistent with Adams *et al.*,[10] who found that the overall taxonomic richness of fungi (observed, Shannon diversity, and inverse Simpson index) did not differ between homes with and without visible mold damage nor for any building damage indicators. Dannemiller *et al.*[9] evaluated the effect of housing characteristics on the fungal and bacterial microbiomes, and found mold but not bacteria to be significantly associated with the beta diversity of fungi. By utilizing NMDS for fungal and bacterial species between mold or moisture damage categories, there were no observations of visual clustering when utilizing NMDS and no significant differences seen when utilizing Adonis with Bray–Curtis.

A possible limitation of this study is that the samples were archived for eight years. When DNA in blood samples was evaluated for long-term storage, Bulla *et al.*[45] found that DNA yield, but not integrity, may be impacted. No studies to our knowledge, however, have evaluated long-term storage of DNA yield in dust samples. Another possible limitation is the use of *Aspergillus fumigatus* or *Bacillus atrophaeus* for universal qPCR calibration due to the varying number of genome copies across species of bacteria and fungi.[46,47] In addition, the use of universal primers may introduce certain biases, or differences in qPCR efficiency, depending on the primers used, however, the universal primers offer an estimation of the fungal load and provide a method to standardize the relative abundance. This approach of adjusting high-throughput sequencing has been utilized and discussed previously.[9,10,48–51] While floor dust has been used as a surrogate for inhalation exposure, airborne exposure will differ from dust exposure. In addition, it is possible the microenvironment of the floor dust may not be completely identical to other types of dust in the home. However, in our previous study performed in 2017, the species composition in wipe dust samples collected from above floor surfaces were found to be strongly correlated to vacuumed floor dust ($r = 0.67$, $p < 0.05$). Furthermore, electrostatic settling cloths left for one month were found to have a moderate correlation with vacuum dust ($r = 0.53$, $p = 0.06$).[8] *Penicillium* species are commonly found to be part of the indoor microbiome,[36,52,53] however, in this study *Penicillium* was not found to be among the most abundant or significantly different between mold or moisture damage categories. It is possible there was an error in extraction or amplification, however, it also is possible these samples lacked abundance in floor dust as in Coombs *et al.*,[54] who used the same methods as the current study. They found no *Penicillium* among the most abundant in floor dust samples but did find this genus to be among the most abundant genera in bed dust and air samples. Lastly, we acknowledge that housing and seasonal factors are known to influence the microbial composition of the home,[55,56] however, due to a lack of data availability and an attempt to capture overall trends, these factors were not evaluated in this study.

This study helps to further describe the complex issues of the indoor microbiome and how it is related to mold and moisture damage in the home. Mold and moisture damage were found to be associated with changes in the species composition of both bacterial and fungal microbiomes. Specific fungal and bacterial species had significantly higher or lower concentrations with increasing moisture or mold damage. The impacted species were not found to belong consistently to a specific water requirement or Gram-staining category. This study demonstrates that increasing levels of mold and moisture damage could have significant effects on the microbiome, but further evaluation is necessary in order to gain a more comprehensive picture.

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Conflicts of interest

The authors declare no conflicts of interest.

References

1 N. E. Klepeis, W. C. Nelson, W. R. Ott, *et al.*, The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants, *J. Exposure Sci. Environ. Epidemiol.*, 2001, **11**, 231.

2 WHO, *World Health Organization guidelines for indoor air quality: dampness and mould*, WHO Regional Office Europe, 2009.

3 J. Cox, P. Ryan, J. Burkle, *et al.*, Quantitative and semiquantitative estimates of mold exposure in infancy and childhood respiratory health, *Environmental Epidemiology*, 2020, **4**, e101.

4 H. Würtz, T. Sigsgaard, O. Valbjørn, *et al.*, The dustfall collector–a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB), *Indoor Air*, 2005, **15**, 33–40.

5 H. W. Meyer, H. Würtz, P. Suadicani, *et al.*, Molds in floor dust and building-related symptoms in adolescent school children, *Indoor Air*, 2004, **14**, 65–72.

6 S. Cho, J. H. Park, K. Kreiss, *et al.*, Levels of microbial agents in floor dust during remediation of a water-damaged office building, *Indoor Air*, 2011, **21**, 417–426.

7 S. Vesper, J. M. Cox-Ganser, L. Wymer, *et al.*, Quantification of mold contamination in multi-level buildings using the Environmental Relative Moldiness Index, *J. Occup. Environ. Hyg.*, 2018, **15**(1), 38–43.

8 J. Cox, R. Indugula, S. Vesper, *et al.*, Comparison of indoor air sampling and dust collection methods for fungal exposure assessment using quantitative PCR, *Environ. Sci.: Processes Impacts*, 2017, **19**, 1312–1319.

9 K. C. Dannemiller, J. F. Gent, B. P. Leaderer, *et al.*, Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children, *Indoor Air*, 2016, **26**, 179–192.

10 R. I. Adams, I. Sylvain, M. P. Spilak, *et al.*, Identifying a fungal signature of moisture damage in buildings: targeted and untargeted approaches with mycobiome data, *Appl. Environ. Microbiol.*, 2020, **86**, e01047-20.

11 S. Rocchi, B. Valot, G. Reboux and L. Millon, DNA metabarcoding to assess indoor fungal communities: Electrostatic dust collectors and Illumina sequencing, *J. Microbiol. Methods*, 2017, **139**, 107–112.

12 A. Barberán, J. Ladau, J. W. Leff, K. S. Pollard, H. L. Menninger, R. R. Dunn and N. Fierer, Continental-scale distributions of dust-associated bacteria and fungi, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 5756–5761.

13 K. C. Dannemiller, Moving towards a robust definition for a “Healthy” indoor microbiome, *mSystems*, 2019, **4**, e00074-19.

14 B. Gutarowska, J. Szulc, A. Nowak, *et al.*, Dust at various workplaces—Microbiological and toxicological threats, *Int. J. Environ. Res. Public Health*, 2018, **15**, 877.

15 K. C. Dannemiller, M. J. Mendell, J. M. Macher, *et al.*, Next-generation DNA sequencing reveals that low fungal diversity in house dust is associated with childhood asthma development, *Indoor air*, 2014, **24**, 236–247.

16 M. J. Ege, M. Mayer, K. Schwaiger, *et al.*, Environmental bacteria and childhood asthma, *Allergy*, 2012, **67**, 1565–1571.

17 E. M. Kettleson, A. Adhikari, S. Vesper, *et al.*, Key determinants of the fungal and bacterial microbiomes in homes, *Environ. Res.*, 2015, **138**, 130–135.

18 F. Weikl, C. Tischer, A. J. Probst, *et al.*, Fungal and bacterial communities in indoor dust follow different environmental determinants, *PLoS One*, 2016, **11**, e0154131.

19 B. Jayaprakash, R. I. Adams, P. Kirjavainen, *et al.*, Indoor microbiota in severely moisture damaged homes and the impact of interventions, *Microbiome*, 2017, **5**, 138.

20 P. H. Ryan, G. LeMasters, J. Biagini, *et al.*, Is it traffic type, volume, or distance? Wheezing in infants living near truck and bus traffic, *J. Allergy Clin. Immunol.*, 2005, **116**, 279–284.

21 T. Reponen, U. Singh, C. Schaffer, *et al.*, Visually observed mold and moldy odor versus quantitatively measured microbial exposure in homes, *Sci. Total Environ.*, 2010, **408**, 5565–5574.

22 N. Yamamoto, K. Bibby, J. Qian, *et al.*, Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air, *ISME J.*, 2012, **6**, 1801.

23 R. Haugland and S. Vesper, United States Patent: Method of identifying and quantifying specific fungi and bacteria, *US Pat.*, 6387652B1, 2002.

24 M. A. Nadkarni, F. E. Martin, N. A. Jacques, *et al.*, Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set, *Microbiology*, 2002, **148**, 257–266.

25 J. Cox, K. Isiugo, P. Ryan, *et al.*, Effectiveness of a portable air cleaner in removing aerosol particles in homes close to highways, *Indoor Air*, 2018, **28**, 818–827.

26 B. J. Callahan, P. J. McMurdie, M. J. Rosen, *et al.*, DADA2: high-resolution sample inference from Illumina amplicon data, *Nat. Methods*, 2016, **13**, 581–583.
27 B. Callahan, *Silva taxonomic training data formatted for DADA2 (Silva version 132)*, Zenodo, 2018.
28 S. McMurdie PJHolmes, phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data, *PLoS One*, 2013, **8**, e61217.
29 C. Jian, P. Luukkonen, H. Yki-Jarvinen, *et al.*, Quantitative PCR provides a simple and accessible method for quantitative microbiome profiling, *bioRxiv*, 2018, 478685.
30 K. C. Dannemiller, N. Lang-Yona, N. Yamamoto, *et al.*, Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations, *Atmos. Environ.*, 2014, **84**, 113–121.
31 Y. Xia, J. Sun and D.-G. Chen, *Statistical analysis of microbiome data with R*, Springer, 2018.
32 C. Niemeier-Walsh, P. H. Ryan, J. Meller, *et al.*, Exposure to Traffic-Related Air Pollution is Associated with Greater Bacterial Diversity in the Lower Respiratory Tract of Children, *bioRxiv*, 2020, 1–40, DOI: 10.1101/2020.12.09.417501.
33 B. Andersen, J. C. Frisvad, I. Søndergaard, *et al.*, Associations between fungal species and water-damaged building materials, *Appl. Environ. Microbiol.*, 2011, **77**, 4180–4188.
34 W. Grant, Life at low water activity, *Philos. Trans. R. Soc., B*, 2004, **359**, 1249–1267.
35 M. Brewer, Traditional preservatives–sodium chloride, *Encyclopaedia of Food Microbiology*, ed. R. K. Robinson, C. A. Blatt and P. D. Patel, vol. 3, 1999.
36 J. B. Emerson, P. B. Keady, T. E. Brewer, *et al.*, Impacts of flood damage on airborne bacteria and fungi in homes after the 2013 Colorado Front Range flood, *Environ. Sci. Technol.*, 2015, **49**, 2675–2684.
37 L. Irinyi, C. Serena, D. Garcia-Hermoso, *et al.*, International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database—the quality controlled standard tool for routine identification of human and animal pathogenic fungi, *Med. Mycol.*, 2015, **53**, 313–337.
38 C. W. Mims and E. A. Richardson, Ultrastructure of sporodochium and conidium development in the anamorphic fungus Epicoccum nigrum, *Can. J. Bot.*, 2005, **83**, 1354–1363.
39 N. Yamamoto, D. Schmechel, B. T. Chen, *et al.*, Comparison of quantitative airborne fungi measurements by active and passive sampling methods, *J. Aerosol Sci.*, 2011, **42**, 499–507.
40 N. Yamamoto, W. Nazaroff and J. Peccia, Assessing the aerodynamic diameters of taxon-specific fungal bioaerosols by quantitative PCR and next-generation DNA sequencing, *J. Aerosol Sci.*, 2014, **78**, 1–10.
41 J. Noble, S. Crow, D. Ahearn, *et al.*, Allergic fungal sinusitis in the southeastern USA: involvement of a new agent Epicoccum nigrum Ehrenb. ex Schlecht. 1824, *J. Med. Vet. Mycol.*, 1997, **35**, 405–409.
42 R. I. Adams, S. Bhangar, K. C. Dannemiller, *et al.*, Ten questions concerning the microbiomes of buildings, *Build. Environ.*, 2016, **109**, 224–234.
43 J. E. Sordillo, U. K. Alwis, E. Hoffman, *et al.*, Home characteristics as predictors of bacterial and fungal microbial biomarkers in house dust, *Environ. Health Perspect.*, 2011, **119**, 189–195.
44 E. E. Kuramae, R. H. Hillekens, Md Hollander, *et al.*, Structural and functional variation in soil fungal communities associated with litter bags containing maize leaf, *FEMS Microbiol. Ecol.*, 2013, **84**, 519–531.
45 A. Bulla, B. De Witt, W. Ammerlaan, *et al.*, Blood DNA yield but not integrity or methylation is impacted after long-term storage, *Biopreserv. Biobanking*, 2016, **14**, 29–38.
46 P. Větrovský TBaldrian, The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses, *PLoS One*, 2013, **8**, e57923.
47 L. A. Lofgren, J. K. Uehling, S. Branco, *et al.*, Genome-based estimates of fungal rDNA copy number variation across phylogenetic scales and ecological lifestyles, *Mol. Ecol.*, 2019, **28**, 721–730.
48 K. C. Dannemiller, N. Lang-Yona, N. Yamamoto, *et al.*, Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations, *Atmos. Environ.*, 2014, **84**, 113–121.
49 X. Zhang, Z. Zhao, T. Nordquist, *et al.*, A longitudinal study of sick building syndrome among pupils in relation to microbial components in dust in schools in China, *Sci. Total Environ.*, 2011, **409**, 5253–5259.
50 M. Bomberg, J. Mäkinen, M. Salo, *et al.*, High diversity in iron cycling microbial communities in acidic, iron-rich water of the Pyhäsalmi mine, Finland, *Geofluids*, 2019, 7401304, DOI: 10.1155/2019/7401304.
51 X. Fu, D. Norbäck, Y. Li, *et al.*, Associations between indoor microbiome exposure and allergic and non-allergic rhinitis for junior high school students in Terengganu, Malaysia, *Authorea Preprints*, October 15 2020, DOI: 10.22541/au.160279753.37398847/v1.
52 S. R. Haines, J. A. Siegel and K. C. Dannemiller, Modeling microbial growth in carpet dust exposed to diurnal variations in relative humidity using the "Time-of-Wetness" framework, *Indoor air*, 2020, **30**, 978–992.
53 B. Hegarty, A. Pan, U. Haverinen-Shaughnessy, *et al.*, DNA Sequence-Based Approach for Classifying the Mold Status of Buildings, *Environ. Sci. Technol.*, 2020, **54**, 15968–15975.
54 K. Coombs, D. Taft, D. V. Ward, *et al.*, Variability of indoor fungal microbiome of green and non-green low-income homes in Cincinnati, Ohio, *Sci. Total Environ.*, 2018, **610**, 212–218.
55 M. Frankel, M. Timm, E. Hansen, *et al.*, Comparison of sampling methods for the assessment of indoor microbial exposure, *Indoor Air*, 2012, **22**, 405–414.
56 A. M. Madsen, S. Moslehi-Jenabian, M. Z. Islam, *et al.*, Concentrations of Staphylococcus species in indoor air as associated with other bacteria, season, relative humidity, air change rate, and S. aureus-positive occupants, *Environ. Res.*, 2018, **160**, 282–291.

