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Aerosol fluorescence, airborne hexosaminidase, and quantitative genomics distinguish reductions in airborne fungal loads following major school renovations

Marina Nieto-Caballero[1] | Odessa M. Gomez[1] | Richard Shaughnessy[2] | Mark Hernandez[1]

[1]Civil, Environmental and Architectural Engineering Department, Environmental Engineering Program, University of Colorado, Boulder, Colorado, USA

[2]Chemical Engineering Department, Indoor Air Program, University of Tulsa, Tulsa, Oklahoma, USA

Correspondence
Mark Hernandez, Civil, Environmental and Architectural Engineering Department, Environmental Engineering Program, University of Colorado, Boulder, CO, USA.
Email: mark.hernandez@colorado.edu

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Abstract

Fluorescent aerosol cytometry (FAC) was compared to concurrent recovery of airborne β-N-acetylhexosaminidase (NAHA) and quantitative polymerase chain reaction (qPCR) for the respective ability of these methods to detect significant changes in airborne fungal loads in response to building renovations. Composite, site-randomized indoor aerosol samples for airborne fungi measurements were acquired from more than 70 occupied classrooms in 26 different public schools in the Colorado Rocky Mountain Front Range region of the United States. As judged by ANOVA and Pearson's correlation test, statistically significant associations were observed between real-time FAC and airborne NAHA levels, which detected significant reductions in airborne fungal loads immediately following building rehabilitations. With lower confidence, a statistically significant association was also resolved between fluorescing aerosols, NAHA levels, and the recovery of fungal 18S rRNA gene copies by qPCR from simultaneous, collocated aerosol samples. Quantitative differences encountered between the recovery of common genomic markers for airborne fungi and that of optical and biochemical methods are attributed to the variance in 18S rRNA target gene copies that different fungal species can host.

KEYWORDS
airborne fungi, PBAP, public schools, quantitative polymerase chain reaction, ultraviolet laser/light-induced fluorescence, β-N-Acetylhexosaminidase

1 | INTRODUCTION

Indoor exposure to airborne pollen, fungi, bacteria, their spores, and their fragmented parts has been associated with a variety of negative health effects, including infectious, allergenic, toxigenic, and hypersensitivity diseases.[1-4]

Concerning the different materials that contribute to airborne particle loads, bioaerosols remain the least studied,[5] even though they are now recognized as ubiquitous in both indoor and outdoor environments.[6-8] While the occurrence of select gases and particulate matter mass (PM_x) is the subject of well-established ambient air guidelines in the Americas, parts of Asia, Europe, and Australia, exposures to airborne microorganisms indoors remain unregulated in much of the world. This is in large part because of the cost, effort, and uncertainties associated with characterizing airborne microbial biomass in time frames relevant to indoor air ventilation rates and human exposures. The indoor microbial exposure assessments, which are still used in industrial hygiene practice, remain largely based on conventional culturing and/or phenotypic manual direct microscopy methods.

Studies of building occupation patterns in developed regions suggest that urban dwellers spend as much as 90% of their life indoors[9,10]—where the indoor air quality (IAQ) of the built environment delineates respiratory exposure.[11-13] In this context, (sub) urban children are considered the most susceptible populations to indoor air pollutants, given that a significant amount of their time is spent breathing recirculated school air in the classroom. School-associated childhood aerosol exposure is second only to their residences[14]; hence, characterizing childhood educational environments is a significant societal concern.[15-18] As judged by patterns in indoor carbon dioxide concentrations, previous public school building studies have shown a preponderance of relatively low classroom (CR) air change rates—a condition that is also associated with relatively high particulate matter levels[17,19,20] as well as elevated humidity and/or dampness indoors.[21]

In turn, respiratory discomfort, including asthma or asthma-like symptoms in children, has been reportedly associated with elevated humidity in moisture damaged buildings,[22-24] as has the increased occurrence of surface-associated and airborne fungi.[25-27] While water-damaged building materials in these environments can be subject to optical inspection (eg, trained examiners and infrared cameras) and real-time moisture assessment (eg, relative humidity (RH) and resistance meters), no such methods are available to characterize airborne microbes with the same degree of economy and/or confidence in short timeframes.

The last decade has witnessed the development and application of mechanized optical, biochemical, and genomic methods that have been applied to help characterize bioaerosol loads in different indoor environments—particularly in response to buildings impacted by extensive water damage.

Exemplars applying genomic bioaerosol characterization methods include the aftermath of flooding events in the eastern Rocky Mountain Front Range, Colorado, USA.[28-30] Here, long-term passive aerosol samples were analyzed for their content of bacterial and fungal DNA from flooded indoor environments. While semi-quantitative, these genomic-based results showed that airborne microbial communities can be significantly affected by water inundations, and can persist after indoor environment conditions return to normality and/or after professional construction efforts have been executed to remove water damage.[28]

Genomic methods have also been used to characterize seasonal indoor bioaerosol exposures in two schools,[31] where PM and bacterial bioaerosols were compared during winter and summer. The authors used quantitative polymerase chain reaction (qPCR), targeting the bacterial 16S rRNA marker genes, in addition to amplicon sequencing of the same region.

Examples also exist that report pilot application of biochemical and optical methods to characterize bioaerosol occurrence, mostly in high- and low-density buildings[32-34]; however, rarely have these emerging bioaerosol assessment technologies been adapted to assess airborne particulate matter in educational environments.[33]

While classic mass spectrometry (MS) has been applied to characterize some airborne endo- and mycotoxins indoors, these MS-based analytical chemistry methods require tedious sampling protocols and extensive preparation[35-37] nor can their results be extrapolated to estimate airborne microbial biomass *in situ*. An emerging biochemical assay that quantifies β-N-acetylhexosaminidase (NAHA) enzymes appears to be a robust biomarker that can provide an estimate of airborne fungal biomass.[38-40] Quantitation of this enzyme, which is found in most filamentous fungi and their spores, has been leveraged for recent indoor environment surveys[32,41]; however, NAHA observations have yet to be reported as part of a quantitative bioaerosol exposure assessment practice in educational environments.

Real-time optical technologies for airborne microorganism detection, using ultraviolet laser/light-induced fluorescence (UV-LIF), were originally developed in a military context to detect biological warfare agents.[42-45] This technology has been translated into a mobile cytometry platform that has since been applied to environmentally controlled chamber studies, as well as a plethora of indoor and outdoor environments. Fluorescent aerosol cytometry (FAC) has also been used in schools to broadly characterize total fluorescent particle concentrations, associated with biological aerosols.[46] Laboratory studies have identified specific fluorescence properties, that in conjunction with optical diameters, can distinguish between different bioaerosol types (*ie*, fungi, pollen, and bacteria).[47]

While residential studies have leveraged genomic tools for bioaerosol exposure assessments, few reports compile associations of systematic school renovation on bioaerosol exposure or respiratory symptom prevalence in children.[25] Further, no studies have concurrently compared, or otherwise leveraged, observations of indoor bioaerosol levels across biochemical, optical, and genomic platforms in a practical capacity.

In response, the goals of this study were as follows: (1) to adapt cytometric (FAC) and hexosaminidase (NAHA) as complimentary quantitative biomarkers for near-real-time assessment of airborne indoor fungal loads; (2) to benchmark these emerging biomarkers against each other and genomic-based aerosol surveillance using widely accepted qPCR methods; and (3) to demonstrate the application of these methods for resolving the effects of common building rehabilitation practices on airborne fungal loads under realistic conditions.

Here, we report a seasonal survey of airborne fungal concentrations in the occupied classrooms of public schools immediately before and following major building renovations. A demonstration of a non-invasive use of three independent characterization methods is presented—where optical, biochemical, and genomic fungal biomarkers were concurrently collected for periods that were equivalent to the characteristic time of an average (volumetric) air change in more than 70 classrooms in 26 different school sites. Results suggest a statistically significant correlation between airborne fungal loads estimated by FAC, NAHA quantitation, and quantitative PCR. The convergence of these fundamentally independent methods supports a high degree of confidence in their analytical sensitivity to distinguish changes in bioaerosol loads in response to renovations under realistic occupation conditions.

2 | METHODS

Concurrent, collocated air samples were collected in 71 occupied K-12 public school classrooms according to the following experimental design and analytical methods.

2.1 | School characterization and metadata collection

Air samples and metadata were collected during two different sampling campaigns, corresponding to pre- and post-renovation conditions in public school buildings in the Colorado Front Range Area (United States). During the spring semester of 2017 (February to May), bioaerosols were collected from 32 randomly chosen classrooms in eleven different schools; while two years later (February to May 2019), 41 randomly chosen classrooms distributed across fifteen different schools were observed during an otherwise identical sampling campaign following major building renovations. These major architectural renovations included, but were not limited to the following: roofing, (dry) wall, window, carpet and flooring replacements; acoustic textile installations; and foundation upgrades. At least three random classrooms were selected and characterized in each school, where carbon dioxide (CO_2) levels, temperature (T), relative humidity (RH), room configuration, and occupancy data were systematically recorded, archived, and reported by Haverinen-Shaughnessy and co-workers.[48] Additionally, concurrent outdoor environmental measurements and air samples were taken immediately outside the schools during these same time periods, making a total of 97 sampling sites, including both indoor and outdoor samples.

Concurrent collection of airborne samples for aerosol cytometry, fungal enzyme (β-N-acetylhexosaminidase (NAHA)), and genetic material for quantitative polymerase chain reaction was performed with three independent bioaerosol samplers, collocated and concurrently operated at each sampling site, both indoors and out.

Metadata from each of the classrooms were also collected, which included ventilation type, architectural textiles, occupant levels, occupation times, plant and/or animal presence, and visual evidence of water damage (Figure 1).

2.2 | Carbon dioxide and classroom air change rate estimates

Calibrated electronic CO_2 sensors (Netatmo, France) were placed in the classrooms and were set to continuously record CO_2 concentrations, temperature, and RH levels on 5-minute intervals for the spring semesters of 2017 and 2019. The CO_2 concentration profiles were analyzed for each classroom, and ventilation rates were calculated as follows. Effective classroom air change rates were estimated using analysis of CO_2 concentration decay following class dismissals according to widely accepted methods[49-52] and international

FIGURE 1 An example of visual evidence for mold growth due to water damage from water pipe leakage on a classroom ceiling. This was commonly observed at multiple schools

standards (ISO12569:2017).[53] The CO_2 concentration decay was modeled assuming well-mixed conditions in the classrooms (Equations 1 and 2), where $C(t)$ is the gas concentration at time t, C_{ex} is the outdoor concentration, C_o is the initial concentration at time zero, Q is the internal-external flow rate, V is the classroom volume, and n is the air change rate per hour (ACH)[52] as adapted for educational environments.[54]

$$C(t) = C_{ex} + (C_o - C_{ex})\, e^{-\frac{Q}{V}t} \quad (1)$$

$$n = \frac{Q}{V} = \frac{1}{t}\ln\left(\frac{C_o - C_{ex}}{C(t) - C_{ex}}\right) \quad (2)$$

2.3 | Genomic fungal characterization with qPCR

Samples for genomic analyses were collected with a Spot Sampler (Aerosol Devices, Fort Collins, CO), modified for bioaerosol condensation capture directly into liquid genomic preservatives.[55] Samples for genomic analyses were collected at a flow rate of 1.5 L/min for 30 minutes with temperatures set to 5, 45, 10, and 25°C for the conditioner, initiator, moderator, and nozzle regions of the sampler, respectively.

Samples were transferred to *ZR BashingBead Lysis Tubes* (0.1 and 0.5 mm) (Zymo Research, Irvine, CA, USA) with a total volume of 1 ml DNA/RNA shield for storage until further DNA extraction following preparation with ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research, Irvine, CA, USA) protocols into an elution volume of 50 μl ultrapure molecular grade water.

Quantitative polymerase chain reaction (qPCR) has been widely used to quantify the number of copies of different target genes in environmental samples since 2003,[56] including water,[57] soil,[58] and air[59] samples. In this study, a 132 base pair (bp) target region in the fungal 18S rRNA marker gene was targeted,[60] following the DNA extraction and purification of the bioaerosol samples collected with

a condensation-based Spot Sampler. These samples were used to quantify fungal occurrence with qPCR using universal primer sets reported by Radwan et al[60] with dye modifications, shown in Table 1, used to perform the analysis with a QuantStudio3 platform (Applied Biosystems, Foster City, CA, USA).

Estimates of fungal 18S rRNA gene copy numbers recovered from environmental samples were obtained by comparing the amplicons generated from the collected bioaerosols to a standard target DNA sequence reproduced from the genome of *Stachybotrys* spp. (gBlocks Gene Fragments, Integrated DNA Technologies, Coralville, USA). The primers for the standard correspond to the 18S cDNA sequence circumscribing all known *Stachybotrys* species. The standard's DNA sequence, its GenBank reference, and the qPCR standard curve (CT values) are provided in the Appendix S1.

Triplicates of seven 10-fold dilutions were used as standards for each qPCR analysis (10^7 to 1 copies/μl). Each reaction was performed in triplicate with a final concentration of 1X iTaq Universal Probes Supermix (Bio-Rad, Hercules, CA), 0.3 μM forward primer, 0.3 μM reverse primer, 0.1 μM TaqMan probe, 3.5 mM $MgCl_2$ (includes $MgCl_2$ contained in the master mix), and 1 μl of DNA template in a total volume of 20 μl. Real-time PCRs were performed in standard chemistry mode with an initial step to activate the polymerase at 95°C for 5 minutes, and a cycle that was repeated 40 times with a first stage of DNA denaturization at 95°C for 15 s and a second stage of annealing/extension and plate reading at 60°C for one minute. As judged by the standard curve, the amplification efficiency using this qPCR protocol was 87%.

TABLE 1 Sequences for primer set and TaqMan probe for qPCR analysis with QuantStudio3

	Sequence 5'→3'
Fungi forward primer	GCYYGAATAYATTAGCATGGAATAAYRDA AYAVGA
Fungi reverse primer	CCAAGAATTKCACCTCTGACARYWSAAT ACTGA
Fungi TaqMan MGBNFQ Probe	5'-FAM-TTGTTGGYTTSYARGACCVHCGTA ATGATTAATAGGGAYRGTCG-3'

TABLE 2 Fluorescence properties of particles excited with two different lasers at wavelengths of 280 and 370 nm, and detection of fluorescent particles at two different detection ranges (*ie*, 310 to 400 nm and 420 to 650 nm)

Fluorescence Channel	Excitation (nm)	Detection range (nm)
Channel A	280	310–400
Channel B	280	420–650
Channel C	370	420–650

2.4 | Optical Particle Characterization

Real-time optical particle analysis was conducted with a portable dual wavelength fluorescent aerosol cytometer (InstaScope, Boulder, CO, USA) using the manufacturer's fluorescence and size calibration settings. Optical particle data were collected at an air sample flow rate of 0.835 L/min over 15-minute increments. Particles were classified according to the respective fluorescence signal and aerodynamic particle size, in order to discern mold-like particles from the bulk in the classroom air as follows.

The fluorescence excitation and emission ranges for each of the three channels are shown in Table 2. Based on their optical response, measured particles were classified into seven different fluorescent-type identities (type A, B, C, AB, AC, BC, and ABC). Particle classification depended on the measured optical size and the fluorescence channel(s), in which the detected fluorescence intensity of each particle was above the instrument noise threshold of at least 3 standard deviations above the intrinsic instrument fluorescence "noise."[61] Optical particle data from the environmental surveys were sorted and classified based on the results obtained from a previous study, which cataloged the fluorescence spectra of airborne spores generated from pure cultures of common indoor fungi using this technology.[47] In this study, fungal (mold-like) particles were defined as fluorescent particles above the noise threshold, with optical diameters between 2 and 10 μm, and that were fluorescent in channel A, AB, and ABC, including a correction factor for the three fluorescent types of 0.8, 0.15, and 0.05, respectively. These correction factors were calculated based on the study by Hernandez et al,[47] in which 80% of the fungal particles aerosolized in an environmental chamber showed fluorescence signal in channel A, 15% of the fungal particles were fluorescent in channels AB, and 5% of the fungal particles were fluorescent in channels ABC. The code used for fungal aerosol assignment to raw fluorescent and size data generated by the FACs used in this study is publicly available on GitHub (https://github.com/marinanieto/InstaScope).

2.5 | β-N-acetylhexosaminidase (NAHA) characterization

Air samples were collected using ultraclean 0.8 μm pore MCE filter cassettes at a flow rate of 15 L/min for 20 minutes. Filter extracts were fluorometrically analyzed for their β-N-acetylhexosaminidase (NAHA) content in the laboratory 4 h after collection using the methods previously described by the manufacturer (Mycometer-Air, Tampa, FL, USA), adapted from the accepted Danish standard DS3033.[39]

This method, generically referred to here as "mycometry," consists of a β-N-acetylhexosaminidase enzyme-substrate biochemical reaction that results in a 4-methyl-umbelliferyl fluorophore that is then quantified at an excitation of 365 nm. The NAHA activity results here were normalized by *Stachybotrys* spp. spore NAHA enzymatic activity and reported as spore equivalents per liter of air (#/L), as described in the Mycometer manufacturer's protocol and U.S. EPA Environmental Technology Verification Report.[62]

The average air change in the classrooms monitored was approximately 3 air changes per hour (ACH) and was not significantly different in the classrooms monitored before or after renovations (2.85 ± 1.45 ACH). Regardless of the collection platform, all bioaerosol collected during this study was under occupied conditions, with samplers centrally located in the students' (seated) breathing zone, through a minimum characteristic time defined by the average air change (20 minutes). These sampling campaigns were conducted well into the normal school day, after several air changes had occurred when it was reasonable to assume that the CO_2 and PM levels had reached near and steady-state; thus, the respective bioaerosol samples recovered were likely representative of that in classroom air during normal occupation periods. In all cases, the bioaerosol samples recovered were above the detection limit of the respective analytical method (*ie*, optical fluorescence, biochemical (NAHA), or genomic).

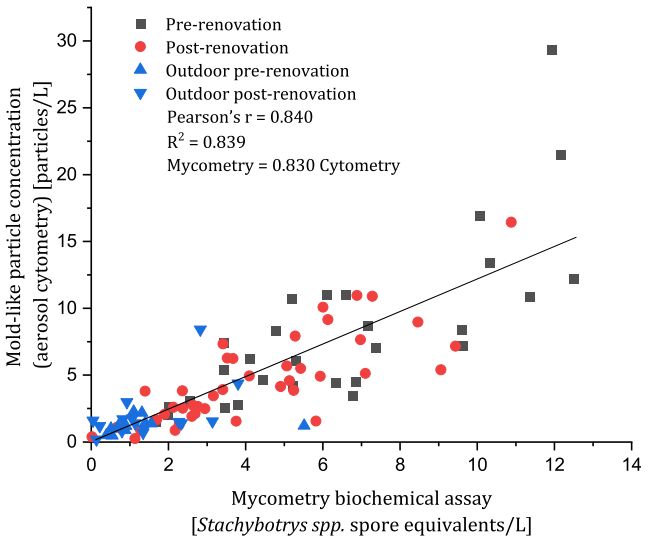

FIGURE 2 Correlation of mold-like particles measured in occupied K-12 classrooms with two independent methods, including fluorescent cytometry data discerning between mold-like particles from the bulk PM (*y*-axis) and β-N-acetylhexosaminidase biochemical assay or mycometry (*x*-axis). Fungal airborne observations from pre-renovation (■), post-renovation (■), and from immediately outdoors (■). Linear regression is shown with a black line

3 | RESULTS

3.1 | Correlation between aerosol cytometry and mycometry

Airborne fungi concentrations were measured with two independent methods, which included optical measurements with a real-time fluorescent aerosol cytometer (FAC) and a biochemical assay that measures fungal enzyme concentrations (NAHA enzyme activity) for the pre-renovation and post-renovation sampling campaigns in 2017 and 2019, respectively. Detailed results on the mold-like aerosol concentrations (aerosol cytometry) and mycometry measurements obtained from the classrooms can be found in Table S1.

To test for potential associations between aerosol cytometry and mycometry, the respective Pearson correlation coefficient (*r*) was calculated. Results from both indoor and outdoor observations are included in this study. A statistically significant positive linear correlation was found between the two independent aerosol sampling methods (Pearson's $r = 0.840$ with a p-value < 0.000), as presented in Figure 2.

Additionally, a linear regression analysis was performed comparing results obtained with the optical and biochemical methods. Consistently higher mold-like particle counts were obtained with FAC when compared to NAHA recovery. FAC consistently reports 1.211 ± 0.005 times more fungi-like particles than mycometry (Mycometry = 0.826 (aerosol cytometry)), with an *R*-squared of 0.839.

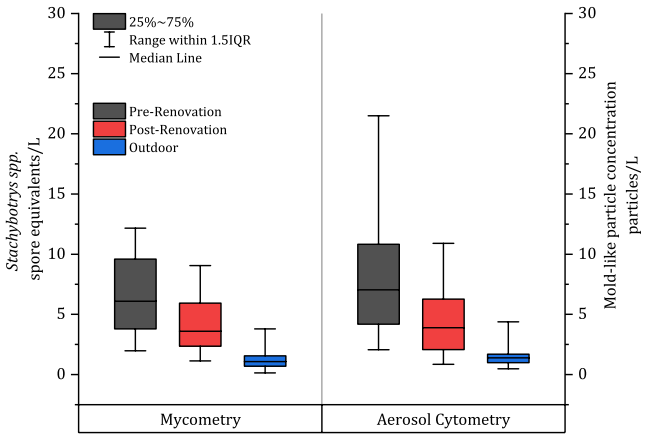

FIGURE 3 Airborne fungal concentrations as judged by mycometry (left) and aerosol cytometry (right). Pre-renovation (■), post-renovation (■), and outdoor (■) concentrations are presented here. Building renovations and/or ventilation system improvements were associated with reductions in airborne fungal loads as judged with both methods. Outdoor fungi concentrations were significantly lower than the fungi concentrations indoors

3.2 | Fungal reductions after building and ventilation system renovations in schools

Besides obtaining a significant positive linear correlation between optical and biochemical (NAHA) results, statistically significant reductions of fungal loads were observed with both methods when assessing airborne fungal concentrations in classroom air before and after school building and/or ventilation system renovations. This was tested with analysis of variance (ANOVA) for mycometry and aerosol cytometry pre- and post-renovations, shown in Figure 3, where boxes represent quartiles 1 and 3 from the observations, whiskers represent the ranges with 1.5 interquartile range (IQR), the transparent squares within the boxes show the means—outliers are marked with asterisks. Both methods report significant reductions with the same p-value of 0.003. Independently, these results suggest that building and/or ventilation system renovations played a

significant role in airborne fungal load concentration reductions in occupied classrooms with 95% confidence. Outdoor measurements, shown in Figure 3 in blue, had significantly lower fungal loads than indoors, as judged by both aerosol cytometry and mycometry methods. Outdoor observations are commonly reported below indoor airborne fungal concentrations,[63] acknowledging that short-term variations in outdoor bioaerosol levels can be highly variable, based on locale and meteorological conditions.[64]

3.3 | Correlation between aerosol cytometry, mycometry, and qPCR

In addition to the two independent methods used during the 2017 sampling campaign (*ie*, FAC and mycometry), Figure 4A, a condensation-based particle sampler (Spot Sampler) was incorporated during the 2019 campaign, Figure 4B. Samples from mycometry and aerosol cytometry were assessed for correlations with 18S rRNA gene copies, measured with fungal-specific primers, as described in the methods section.

A significant correlation was found between airborne fungal loads as determined by mycometry and the 18S rRNA gene copies recovered from the condensate of the Spot Sampler. A positive linear correlation was observed, with lower degree of confidence than FAC correlations, based on Pearson's *r* coefficient of 0.734 and a p-value lower than 10^{-4}, shown in Figure 5A.

Following building renovations, a significant correlation was also observed between mold-like particle concentrations reported from aerosol cytometry and 18S rRNA gene copies in the classroom air, Figure 5B. This correlation showed a slightly lower degree of confidence, Pearson's r coefficient of 0.695, when compared to mycometry.

Even though positive linear correlations were observed between data obtained from quantitative PCR and mycometry or FAC, they were significantly lower than the correlations observed between mycometry and FAC, shown in Figure 2, with size and fluorescence gating for mold-like particles. This is likely due to the fact that many fungal spores often possess more than one copy of the 18S rRNA gene in their genome, and different fungal species can have markedly different number of gene copies in their spores or hyphae.[65]

4 | CONCLUSIONS

The monitoring of IAQ in K-12 schools is important, as children spend a significant part of their days breathing school indoor air, which is known to be influenced by building factors and ventilation system conditions. School-age children constitute one of the more susceptible populations toward developing respiratory conditions, in response to occupation of water-damaged buildings. The Environmental Protection Agency (EPA)[66] reports that moisture problems in schools are usually caused by roof and plumbing leaks, extreme weather events, and excess of humidity (*ie*, poor performing of HVAC systems) in the classrooms.

Evaluating airborne fungal presence indoors in school air is not a common practice, as methods for assessing fungi in the air in real-time are not well established. Rigorous industrial hygiene assessments are costly; thus, a systematic evaluation of airborne mold in school buildings is relegated to subjective visual inspections that can bypass potential impacts on indoor air quality.

The COVID-19 pandemic has highlighted the importance of real-time IAQ monitoring (eg, with report or portable CO_2, PM, and FAC monitors) to confirm indoor ventilation performance. Poorly designed and under-performing HVAC systems are associated with increasing bioaerosol accumulation during a typical indoor school day. Using portable unintrusive CO_2 monitors, air change rates in this study were found to be significantly lower than ANSI/ASHRAE guidelines (*ie*, 4 to 6 ACH),[67] with an average of 2.8 ACH. Based on the ventilation results alone, these particular schools could likely benefit from improved building operations.

In this study, airborne fungal loads were assessed in a total of 71 classrooms in 26 schools with two independent sampling methods (*ie*, mycometry and aerosol cytometry), which were found to have a significant positive linear correlation. These results suggest that aerosol fluorescence cytometry could potentially be used as a rapid and reliable tool to periodically screen airborne fungal concentrations indoors in real time.

As judged by mycometry and aerosol cytometry, significant reduction in airborne fungal loads was observed after school renovations, suggesting that these rehabilitation efforts played a significant role in IAQ improvement in these schools, as it directly relates to airborne fungi concentrations. These measurements were taken during two sampling campaigns separated by two years and were both performed

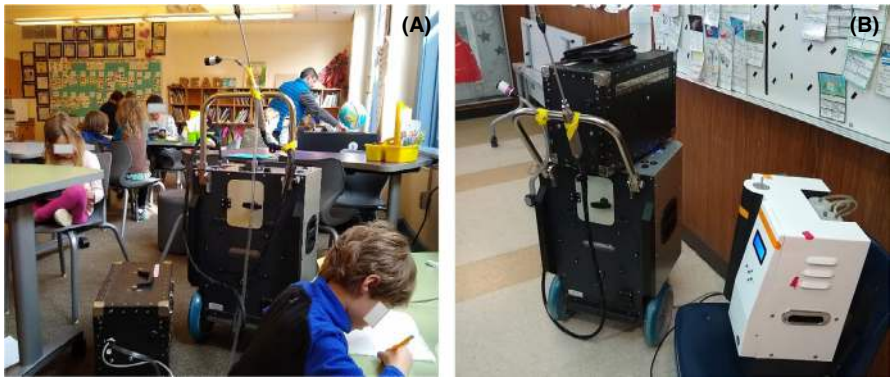

FIGURE 4 (A) Bioaerosol sampling in 2017. Aerosol cytometry and mycometry were set to sample in parallel during class periods. (B) Bioaerosol sampling in 2019. Aerosol cytometry, mycometry, and condensation-based sampler (Spot Sampler) for genetic material assessment were set to sample side-by-side during class periods

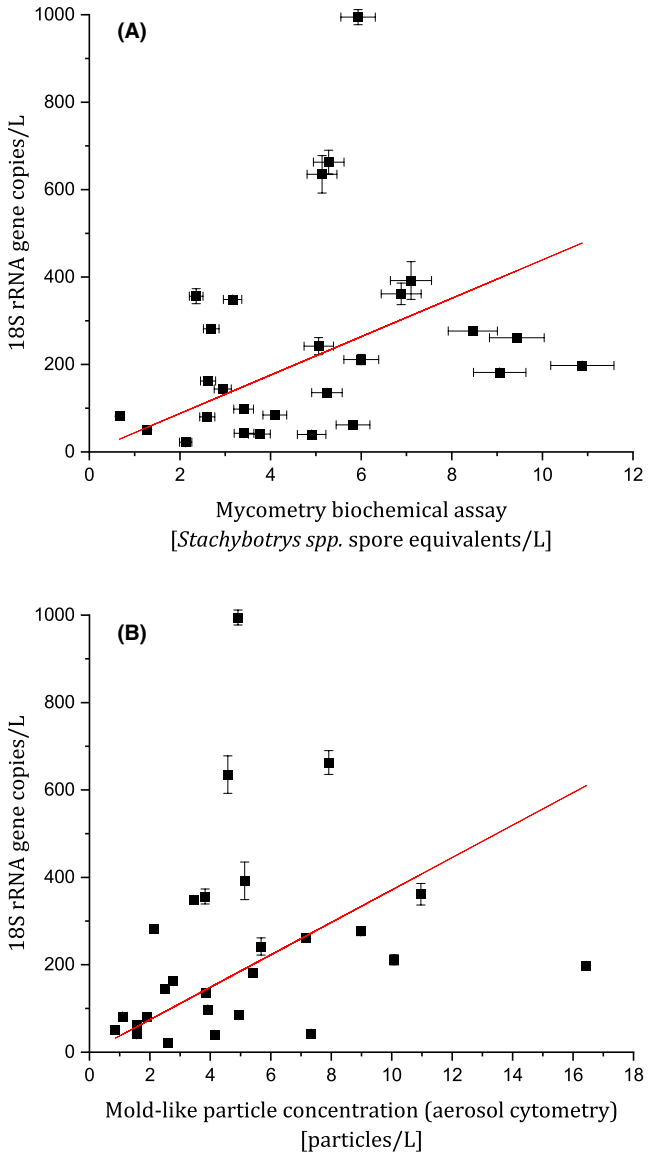

FIGURE 5 Quantitative PCR analysis of the 18S rRNA gene region, specific to fungi, and normalized by *Stachybotrys* spp. 18S gene copies (■). These recovered from the air of post-renovation classrooms show a positive linear correlation (red lines) with the following biomarkers: (A) mycometry biochemical assay for airborne fungal NAHA concentration; and, (B) fluorescent aerosol cytometry

during spring; thus, the seasonality factor was normalized. However, differences in indoor airborne fungal concentrations fluctuate with season, as ventilation practices are usually modified with respect to atmospheric conditions (eg, mechanical ventilation such as opening windows in warm days or more recirculation of air during winter).

Like all field studies, this demonstration had the following caveats. Samples for genomic characterization were exclusively collected for the classrooms in schools in which renovations already occurred, implying that the use of qPCR with fungal primers was not included in fungal reduction assessment in indoor air in this study, as condensation particle capture equipment was not available at that time. Moreover, qPCR correlations for airborne fungal load estimations indoors were found to have lower confidence with respect to mycometry and aerosol cytometry; hence, the use of mycometry or aerosol cytometry for this purpose appears more accurate with respect to airborne fungal concentrations. This result is likely associated with multiple copies of the 18S rRNA gene in the different fungal genomes (*ie*, copy number polymorphism). Not only can fungi carry multiple copies of this gene target, but additionally they can vary widely in gene copy number across different taxa.[65]

This work presents the application of complimentary assays for the characterization of airborne fungi. These analyses are fundamentally independent of each other—optical, biochemical, and genomic. When applied as a suite, they provide a rapid non-invasive format for more robust characterizations of indoor atmospheres, regardless of occupancy.

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AUTHOR CONTRIBUTIONS

Marina Nieto-Caballero: Conceptualization (lead), data Curation, formal analysis, investigation, methodology, project administration, software, supervision, validation, visualization, writing—original draft preparation. **Odessa M. Gomez:** Conceptualization (supporting), investigation, supervision, writing—review and editing. **Richard Shaughnessy:** Resources. **Mark Hernandez:** Conceptualization (lead), supervision, funding acquisition, visualization, resources (lead), writing—review and editing.

PEER REVIEW

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SUPPORTING INFORMATION

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