UC Berkeley
UC Berkeley Electronic Theses and Dissertations

Title
The Impact of Water-Damage on Microbial Communities in North American Public Housing

Permalink
https://escholarship.org/uc/item/3sm0q3z2

Author
Sylvain, Iman Angelique

Publication Date
2018

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

The Impact of Water-Damage on Microbial Communities in North American Public Housing

By

Iman Sylvain

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Microbiology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor John Taylor, Chair
Professor Thomas Bruns
Professor Rachel Morello-Frosch

Fall 2018

Abstract

The Impact of Water-Damage on Microbial Communities in North American Public Housing

by

Iman Sylvain

Doctor of Philosophy in Microbiology

University of California, Berkeley

Professor John Taylor, Chair

Water intrusion can result from hurricanes and flood damage, condensation, and elevated humidity indoors. Increased water availability and moisture indoors allows for the proliferation of fungi and other microorganisms that are able to grow on most building materials. Damp, moldy housing has been associated with a number of negative health outcomes, principally respiratory disease, allergy, asthma, anxiety and depression. For the promotion of public health, there has been a long history of studying the microorganisms that inhabit the built environment. Nonetheless, there are currently no federal standards set by the Environmental Protection Agency distinguishing what is 'safe' or 'abnormal' microbial exposure indoors. In part this is because until very recently, the main tools used to identify microbes have relied on culture-based methods, microscopy, and immunochemical techniques. We now know that the vast majority of microorganisms cannot be cultured in the laboratory, and we have made epic strides in fungal and bacterial taxonomy by using molecular tools for phylogenetic identification.

My dissertation research used high-throughput DNA sequencing technologies to characterize the microbiomes of water-damaged public and private housing in Richmond, CA, and New York City, NY. We collected house dust from over 90 homes and profiled bacterial and fungal communities using multiple sampling methods. We surveyed one public housing project in Richmond that was condemned by the local Housing Authority, yet remained occupied by seniors, disabled persons, and low-income residents for years after being deemed uninhabitable. We also surveyed over 40 buildings in Brooklyn and Manhattan, NYC, in the notoriously underserved New York City Housing Authority (NYCHA) buildings and privately-owned apartments in the same neighborhoods. The NYC buildings endured Hurricane Sandy in 2012 and the public housing projects were woefully neglected in the storm's wake. A year later, NYCHA had yet to address major structural damage caused by the flooding and many residents fell ill. A class action lawsuit was successfully filed against the Housing Authority for violating the Americans with Disabilities Act (which provides protections to persons with asthma and breathing disorders) for failing to abate spreading mold contamination in NYCHA buildings and exacerbating the deterioration of residents' health.

By working collaboratively with building scientists, non-profit organizations, resident activists, and attorneys, we were able to deduce a microbiological signature of building negligence and the impact of long-term water-damage on residential buildings. We conducted amplicon sequencing and quantitative-PCR on dust collected from the outdoors, in kitchens, bathrooms, bedrooms, and living rooms. We found that there are distinct microbial communities in water-damaged homes compared to homes with no damage, and different microbial communities in public housing units compared to private housing.

In Richmond, we evaluated fungal communities in units with visible mold and compared these to fungi in units with no visible mold and the outdoors. We learned that fungal communities in units with visible mold are less diverse than communities in units with no visible mold and outdoors. Fungal communities in units with visible mold have increased abundance of Eurotiomycetes, Saccharomycetes, and Wallemiomycetes. Samples collected from actively growing mold on walls and surfaces were dominated by two *Cladosporium* species. We also saw that fungi growing on walls became airborne and could be detected in our passive samplers that collected settled dust over the course of four weeks.

In NYC, we similarly saw that units with water-damage were characterized by lower bacterial and fungal diversity, lower fungal biomass, and compositionally distinct microbial communities. Microbial communities in water-damaged units had a significant reduction in outdoor microbes and increased abundance of Clostridia, Coriobacteriia, Agaricomycetes, and Pezizomycetes fungi. These classes include taxa that are known opportunistic pathogens and could trigger an allergic response. In addition, we observed evidence of microbial dysbiosis in public housing units. Compared to private housing, microbial communities in public housing had reduced alpha- and beta-diversity, and increased abundance of Bacteroida, Erysiphelotrichia, Negativicutes, and Saccharomycetes; budding yeasts and taxa found in association with the human gut. Beyond this, we evaluated the utility of various sampling methods, including swabbing surfaces and door trims, vacuuming floors, collecting passively settled dust, and using electrostatically charged wipes, to collect biological material. We provide recommendations to the field of indoor air microbiology for sampling methodology, and advocate for the use of simple, empty, sterile petri dishes to collect settled dust. This allows for accumulation of airborne taxa over the course of one month, both indoors and outdoors, accounting for fluctuations in season, diurnal dynamics, and resident behaviors.

Housing has long been deemed a major social determinant of health. Living in substandard housing increases risk of injury, exposure to environmental toxins like lead, asbestos, outdoor pollution, mold, and allergens. Public housing residents have the worst documented health outcomes of any population in the United States. Nearly half of all public housing in this country is occupied by African Americans, yet this population only accounts for 12% the national census. The concentration of People of Color in substandard and unhealthy housing today is the result of a long history of housing segregation and racial exclusion from the federal subsidies that led white Americans to become home-owners and build wealth. Afterwards, neoliberal policies focused on diminishing social welfare dramatically defunded New Deal-style public housing and began demolishing thousands of public housing complexes. This ultimately led to the current sociopolitical catastrophe and chronic disrepair of North American public housing stock. A

consequence of this malignant building management is an asthma epidemic, to which low-income Children of Color living in urban public housing suffer disproportionately.

Characterizing the microbiota of low-income housing is a critical step towards addressing disparate exposure to indoor air pollution caused by microorganisms. Elevated moisture indoors, which leads to the growth of toxic mold, is preventable and can be remediated. But to fix a problem we must first know it exists. In the case of a microbiological problem, the culprits are invisible to the naked eye due to their microscopic size, so it is imperative to use tools that are rapid, efficient, and informative for adequate detection. Here is where environmental justice and activism meets basic microbiology and microbial ecology. Here is where a DNA sequence is fuel for proletariat resistance. Here is where science becomes a tool of empowerment, self-advocacy, and for healthy affordable housing. For years the residents of low-income housing have complained they can't breathe in their homes. By finally putting names on the fungi and bacteria that comprise the mold on their walls and circulate in the air, we now have a cleared picture of why.

Dedication

This dissertation is dedicated to the spirits of African slaves, whose labor was stripped from their bodies under the constant threat of the whip, abhorrent physical and sexual violence, and deep psychological trauma. Those who watched their kin sold at auction and endured a systemic and legal system of dehumanization – all in order to build this country's wealth. This dissertation is dedicated to the descendants of African slaves who are still subjugated in this country, fed the same slop for food, and housed in similar conditions under an air of modernity. This dissertation is dedicated to those resilient residents in public housing, who everyday affirm their lives have worth and meaning. Those Black, Brown, poor, old, immigrant, indigenous, disabled, and queer folk, who took to the streets or the courts simply to remind blood-thirsty capitalists that they exist and deserve to live in health-promoting conditions. This is dedicated to all those mothers and fathers and sisters and brothers and aunties and uncles and grandmas and grandpas and nonbinary folx and chosen families who had to watch their babies suffer from asthma attacks in the middle of the night, knowing that an ambulance won't make it to the hood in time. This is for those who believe that living in poverty should not be a death sentence.

This is dedicated to my elders – the Sylvain/Bullock/Greenwood/Phifer family - for giving me strength to navigate academic bureaucracy, near-constant microaggression, and the psychic erasure of Students of Color at this campus that ironically prides itself on diversity and liberalism. Thank you, Momma, for giving me the courage to use my brain and my words as my primarily tools of resistance. This is dedicated to my friends who held me and fed me and consoled me every time I wanted to quit, and who encouraged me to have a full life outside of the university, blessed with art and activism, travel, and love: Akiko, Hana, Charmaine, Quynh-Anh, Angus, Divya, Ina, Amber, Heather, Natalia, Juliana, and Jonny, in particular, I could not have finished this project without. And finally, this is dedicated to my descendants – may you never have your breath taken away by the constructions of white supremacy.

Table of Contents

Chapter 1: A Different Suite: The Assemblage of Distinct Fungal Communities in Water-Damaged Units of a Poorly-Maintained Public Housing Building pg. 1

Chapter 2: Evidence of Disparate Microbial Communities Associated with Water-Damaged Units in New York City Public Housing Residences Using High-throughput Sequencing pg. 20

Chapter 3: A Comparison of Sampling Methods Used in High-throughput Sequencing Analysis for Microbiome Studies of the Built Environment pg. 43

Chapter 4: Hope's Children: The Dismantling of North American Public Housing Caused Childhood Asthma Disparity pg. 55

References pg. 63

Acknowledgments

I would like to thank my advisor John Taylor for many valuable lessons about the culture, structure, tempo, and politics of science. I would like to thank Rachel Adams for hands-on training in the laboratory. I would like to thank Pierre Gladieux and Sara Branco and for being my first mentors in the Lab. I would like to thank my Lab Brother Chris Hann-Soden – Congratulations on your graduation! I would like to thank Saru Jayaraman, Carolyn Finney, Rachel Morello-Frosch, and Laura Nader, for being my necessary women faculty members outside of my department who helped me to make sense of the university. I would like to thank Sydney Glassman for showing me finishing is possible. I would like to thank Thomas Bruns and Else Vellinga for regularly reminding me about the joy of mycology. Thank you Cheng Gao, Cat Adams, and additional members of the Taylor/Bruns lab complex for their intellectual and social contributions. Additionally, this project would not be possible with the financial contributions of Paula Olsiewski and the Sloan Foundation. Constance Gary and the residents of Hacienda, and Lisa Cowan of the Red Hook Initiative.

A Different Suite: The Assemblage of Distinct Fungal Communities in Water-Damaged Units of a Poorly-Maintained Public Housing Building

Iman A Sylvain, Rachel I Adams, and John W Taylor

ABSTRACT

Water-damaged housing has been associated with a number of negative health outcomes, principally respiratory disease and asthma. Much of what we know about fungi associated with water-damaged buildings has come from culture-based and immunochemical methods. Few studies have used high-throughput sequencing technologies to assess the impact of water-damage on microbial communities in residential buildings. In this study we used amplicon sequencing and quantitative-PCR to evaluate fungal communities on surfaces and in airborne dust in multiple units of a condemned public housing project located in the San Francisco Bay Area.

We recruited 21 households to participate in this study and characterized their apartments as either a unit with visible mold or no visible mold. We sampled airborne fungi from dust settled over a month-long time period from the outdoors, in units with no visible mold, and units with visible mold. In units with visible mold we additionally sampled the visible fungal colonies from bathrooms, kitchens, bedrooms, and living rooms.

We found that fungal biomass in settled dust was greater outdoors compared to indoors, but there was no significant difference of fungal biomass in units with visible mold and no visible mold. Interestingly, we found that fungal diversity was reduced in units with visible mold compared to units with no visible mold and the outdoors. Units with visible mold harbored fungal communities distinct from units with no visible mold and the outdoors. Units with visible mold had a greater abundance of taxa within the classes Eurotiomycetes, Saccharomycetes, and Wallemiomycetes. Colonies of fungi collected from units with visible mold were dominated by two *Cladosporium* species, *C. sphaerospermum* and *C. halotolerans*. This study demonstrates that high-throughput sequencing of fungi indoors can be a useful strategy for distinguishing microbial exposures in water-damaged homes with visible and nonvisible mold growth and may provide a microbial means for identifying water-damaged housing.

INTRODUCTION

It is well established that humans spend most of their time in their homes; the estimate is 70% for residents of the United States (1). It is equally well established that housing is a major social determinant of health (2). Damp, moldy housing is linked with a number of negative health outcomes, such as respiratory infections, asthma, allergy, and compromised mental health (3). Given that ethnic minorities and low-income populations disproportionately occupy inadequate and unhealthy housing, there is resultant public health disparity (4). Acknowledging these factors, the World Health Organization recognizes access to a healthy indoor environment as a basic human right (5), and some public health practitioners view the desegregation of North American housing as an environmental justice priority (6).

The health of homes can be compromised by water intrusion through leaks (in roofs, windows, or plumbing), floods, or condensation, because increased moisture promotes the proliferation of microbes on indoor surfaces (7). There has long been an effort to identify microbial, and specifically fungal, signatures of water-damage in buildings, and to pinpoint the potential cause of ill-health effects associated with damp housing conditions (8). These efforts have largely relied on assessment of microbial communities through cultivation and immunological techniques. Based on this work, many of the taxa that proliferate on water-damaged building materials have been identified (9). Some of these taxa are capable of producing mycotoxins (10), which creates an additional concern for public health. While some studies have found that concentrations of cultivable fungi or fungal cell wall components are higher in damaged houses compared to dry homes (11), this pattern has not always been observed (12).

Reliance on cultivation as part of the process to identify microbes is now known to be inadequate following the discovery that the vast majority of microorganisms are unculturable under laboratory conditions (13). Instead, cultivation-free approaches provide a promising new approach to grant insight into the microbiology of the built environment (14), including water-damaged buildings. By utilizing DNA sequencing platforms such as Illumina MiSeq and 454 Pyrosequencing for samples of house dust, much has been learned about the diverse bacteria and fungi that co-occur in our dwellings (15). It is now known that indoor microbial communities are structured by patterns of geography and climate (16), season (17), building design (18), ventilation systems (19), and occupants (20). Fungal taxa found in house dust from healthy buildings have been shown largely to be a stochastic subset of outdoor fungi, presumably trafficked inside via open doors, windows, and on residents (21), and their presence is principally determined by the geographic location of the home (22).

Advances in sampling indoor air have also facilitated our greater understanding of the airborne mycobiome. Indoor samples typically fall into one of two types, either surface sampling using swabs, wipes, or tape, to recover microbes from suspected colonies, or vacuum sampling of either air or dust settled on floors and shelving (8). Swab and tape sampling is useful for identifying colonies of visible fungi; air sampling provides a sample of airborne microbes at a defined time, typically a few minutes; and settled dust provides a sample integrated over a long, but undefined, period (23). The adoption of a new approach to sampling airborne microbes by gravity settlement over a defined period of at least one-week (24) can account for diurnal variation in airborne microbes and variation in occupant behavior. Importantly, this approach avoids the expense of vacuum pumps and trained technicians, allowing for far greater replication and implementation by residents themselves.

Researchers are just beginning to apply high-throughput sequencing techniques to characterize fungal communities in water-damaged housing. Recently Jayaprakash et al. (25) applied amplicon sequencing to severely moisture-damaged residences undergoing renovation, complimented with quantitative-PCR (qPCR) and chemical-analytical approaches. Renovation of residences damaged by moisture resulted in a decrease in overall fungal richness and had a small but significant effect on fungal community composition. Prior to this, Emerson et al. (26) conducted amplicon sequencing and qPCR on house dust from passive dust collectors and HVAC filters in flooded and non-flooded homes six months after a historic weather event in Boulder, Colorado. They found

significant differences in fungal community composition between flooded and non-flooded homes, with flooded homes hosting three times greater fungal biomass, and experiencing dominance by *Penicillium* taxa.

We have heeded the call to further study the relationship between the water-damaged built environment, microbial communities, and human occupants (27). We have previously examined the processes that govern fungal community assemblage in healthy housing by sampling settled airborne dust in newly-constructed university housing in the San Francisco Bay Area (21). Here we used the same sampling and identification approaches to examine fungal assemblages in poorly maintained, water-damaged residences in the Bay Area. We sampled from a public housing project that had been condemned by the local Housing Authority due to chronic disrepair. The building had long-term water-damage including a leaking roof that had produced stalactites from dissolved concrete. The resulting water intrusion supported visible fungal colonies in units, and the building was additionally plagued with pest infestations. With this data, we asked whether broad differences in fungal community structure could be detected in units with visible mold when compared to units with no visible mold or the outdoors.

MATERIALS AND METHODS

Sampling. Fungi in air and on surfaces were sampled at a 6-story, 150-unit, concrete public housing project in the San Francisco Bay Area that was built in 1966. The building remained occupied with residents despite having been declared uninhabitable in 2014. Federal reports had documented the roof leaks that over two decades of disrepair led to the formation of stalactites, as well as pest infestations, sewage problems, asbestos, and exposed electrical wire.

At a Residents Council meeting, 21 households spanning the 6 stories in the building and inhabiting units with varied layouts volunteered to participate in this study. At the initial sampling visit, experienced mycologists surveyed the apartment and categorized the units (individual households within the building) as having either 'visible mold' growth or 'no visible mold'. Visible mold was present in kitchens, bathrooms, livings, and bedrooms, and appeared as green, black, or pinkish-orange growths and discoloration on walls; the extent of the visible mold was not recorded. We categorized 11 units as having 'visible mold' and 10 units as having 'no visible mold'. We noted the floor level of the unit and room where samples were collected for each sample.

Airborne house dust was collected from each unit using settled dust collectors (open, empty, sterile 10 cm diameter petri dishes) (24), that were left open for four weeks in kitchens, bathrooms, living rooms, and bedrooms (FIGURE 1a). Indoor settled dust samples were paired with outdoor samples (FIGURE 1b). Outdoor samples were obtained from collectors suspended from railings or placed on top of light fixtures adjacent to the units and protected from precipitation by an overhang. In all units, settled dust collectors were placed in living rooms, bathrooms, and the outdoor walkway. In units with visible mold, additional settled dust collectors were also placed to sample all rooms with visible mold. In total, 68 settled dust samples were analyzed (18 outdoor samples, 22 indoors samples from units with no visible mold, and 28 indoor samples from units with visible mold). In units with visible mold, fungal colonies were sampled directly (FIGURE 1c) with dry Floq Swabs (Copan Diagnostics). These 24 surface samples were collected and frozen until processed. The

study was approved by the University of California Committee for the Protection of Human Subjects under protocol 2014-08-6589.

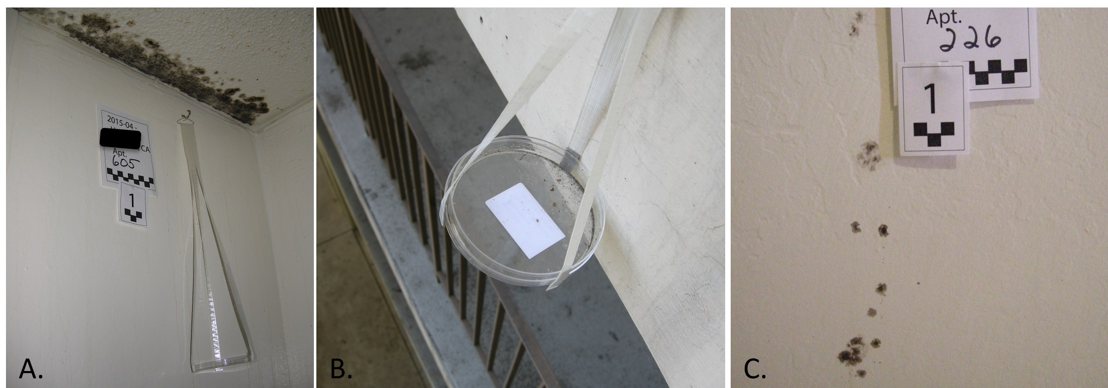


FIGURE 1. *Photographs of Sampling Methods. Indoor (A) and outdoor (B) settled dust collectors. These empty, sterile petri dishes collect airborne fungi by settlement of dust over the course of four weeks. Patches of visible mold or obvious discoloration on walls were swabbed for surface samples (C).*

DNA extraction and library preparation. Fungal genomic DNA (gDNA) was extracted from settled dust samples using a phenol:choloform-isoamyl alcohol extraction protocol, followed by MoBio PowerSoil Kit, as previously described (21). Surface samples (collected from units with visible mold) were processed following the PowerSoil Kit without modification. As controls, we also processed gDNA from unexposed swabs and petri dishes in order to determine potential contamination or sequencing errors in downstream analyses. Additionally, we included a Mock Community sample composed of gDNA from 18 known taxa, including *Rhodotorula*, *Cladosporium*, *Phoma*, *Candida* in high relative concentrations, *Penicillium* and *Cryptococcus* in intermediate relative concentrations, and *Neurospora*, *Chaetomium*, *Tetrasphaeria*, *Beauveria*, *Leptosphaerulina*, *Pestalotiopsis*, and *Exophiala*, in low relative concentrations.

Fungal community composition was determined by constructing amplicon libraries from gDNA isolated from settled dust samples and swabbed colonies. Briefly, PCR primers for the ribosomal DNA internal transcribed spacer (ITS) regions, ITS1 and ITS2 (28) were adapted for Illumina MiSeq 250 paired-end sequencing with V2 chemistry, following methods previously described (29). Quality of PCR amplicons was assessed by gel electrophoresis, prior to further cleaning with magnetic beads, and quantification using a Quant-iT dsDNA Assay Kit. Equimolar concentrations of PCR product from 92 samples were pooled into a single Illumina lane. Library sequencing was conducted at the Vincent J. Coates Genomic Sequencing Laboratory in the California Institute for Quantitative Biosciences (QB3) at the University of California, Berkeley.

Quantities of airborne fungi in settled dust samplers could be compared because the same time and area of collections were used for all samples. The relative amount of fungal DNA in each settled dust sample was determined using quantitative-PCR (qPCR) with the Bio-Rad CFX96 Touch

Real-Time PCR Detection System. qPCR employed ITS primers (30) and SYBR Green. The quantification standard consisted of in-house ITS plasmids that had been constructed from *Aspergillus fumigatus*. Fungal biomass for airborne fungi was estimated by dividing the qPCR fungal gene copy number by the petri dish surface area (56.5cm^2 for a 10cm plate).

Sequence processing. Using Cutadapt (31) adapter sequences were removed with no quality filtering, but with a minimum read length of 75bp. Further processing into "amplicon sequence variants" (ASVs) was implemented in the DADA2 library (32) in the R environment with some additional software. First, forward and reverse reads were filtered (truncQ=2, and maxEE=2 for forward and maxEE=5 for reverse reads). Then paired forward and reverse reads were identified using Fastq-pair (https://github.com/linsalrob/EdwardsLab/) and paired using Pear (33). Returning to DADA2, sequences with N's were removed, dereplicated, and then sequence variants inferred. Chimeric sequences were removed, and taxonomy assigned against the UNITE database (34).

The two negative controls were clean, containing low total number of reads and low abundance of particular taxa that were abundant in biological samples, indicating likely "sample bleed" (35). Two low-abundance taxa were identified as contaminants using the Decontam package (36) in R, and these were removed. The mock community contained DNA from 18 taxa, and 28 ASVs were identified. Four of the ten taxa put in low abundance were not recovered, while the 8 taxa input at medium and high concentration were identified. This workflow provided 8,970 ASVs with resolved fungal taxonomic identification, which were used in further analysis. Raw sequences are available through NCBI (SRP144641).

Statistical analysis. We compared fungal biomass, richness, evenness, and community composition in dust samples from units with visible mold, units with no visible mold, and the outdoors. Statistical analysis of ASVs and quantitative-PCR data was conducted principally in QIIME (37) and R (38) using the Vegan (39), BiodiversityR (40), Phyloseq (41), ggplot2 (42), and Codaseq (43) packages. Gloor et al. (43) argue that microbiome datasets generated by high-throughput sequencing are compositional in nature because the number of DNA sequence reads is limited by the capacity of the sequencing machinery. Thus we analyzed this dataset compositionally by first filtering with CodaSeq (min.reads = 5000, min.occurrence = 0.001, min.prop = 0), then conducting a center log-ratio transformation (clr), instead of using standard counts and rarefying.

We used a one-way ANOVA to analyze qPCR data from settled dust samples and tested for differences in mean fungal biomass across units with no visible mold, units with visible mold, and the outdoors. A Wilcoxon test was used to test for differences in biomass indoors and outdoors, and between units with and without visible mold. Spearman Correlation was used to test whether fungal biomass was significantly different on various floors of the building. ANOVA was used to test whether biomass differed between rooms indoors.

To assess alpha diversity amongst fungal communities in units with or without visible mold as well as outdoors, richness was measured as Chao1 and evenness was measured as Shannon Diversity. Significant differences in means of Chao1 and Shannon between the outdoors, units with visible mold, and units with no visible mold were tested using ANOVA. Spearman Correlation was used to test whether fungal richness was significantly different on various floors

of the building, and Kruskal-Wallis was used to test whether richness differed between rooms indoors.

Beta diversity was assessed with Aitchison distance (Euclidian distance between samples) and variance-based compositional principal component (PCA) plots. Two samples were removed as outliers after plotting due to low read counts. Significant differences of fungal community composition in settled dust from units with visible mold, units with no visible mold, and the outdoors was tested using ADONIS (PERMANOVA). We conducted a multivariate homogeneity of groups dispersion test to examine among-community similarity in outdoor samples, units with visible mold, and units with no visible mold, using pairwise permutation tests with Tukey's HSD. ADONIS was used to test whether fungal composition was significantly different on various floors of the building and rooms indoors.

A Kruskal-Wallis test of median abundance was used to determine whether the abundance of fungal classes were significantly different in outdoor samples, units with no visible mold, and units with visible mold. Finally, for units with visible mold, Kruskal-Wallis testing was used to determine whether the abundance of classes was significantly different across sampling methods (settled dust and surface swabs).

RESULTS

In our study, we addressed the following questions: a) Does fungal biomass, diversity, or community composition, differ between units with visible mold, units with no visible mold, and the outdoors? b) What taxa dominate units with visible mold? C) Do fungi forming visible colonies on surfaces become airborne and contribute to the indoor air microbiome?

Fungal Biomass. We found that mean fungal biomass was marginally significant (ANOVA; $p=0.055$) across indoor units with visible mold, indoor units with no visible mold, and the outdoors (FIGURE 2). This trend was largely a response to the difference of biomass indoors and outdoors. Biomass outdoors was three times greater than indoors (Wilcoxon; $p<0.001$). In units with visible mold and units with no visible mold, no significant difference in biomass was detected (Wilcoxon, $p=0.87$).

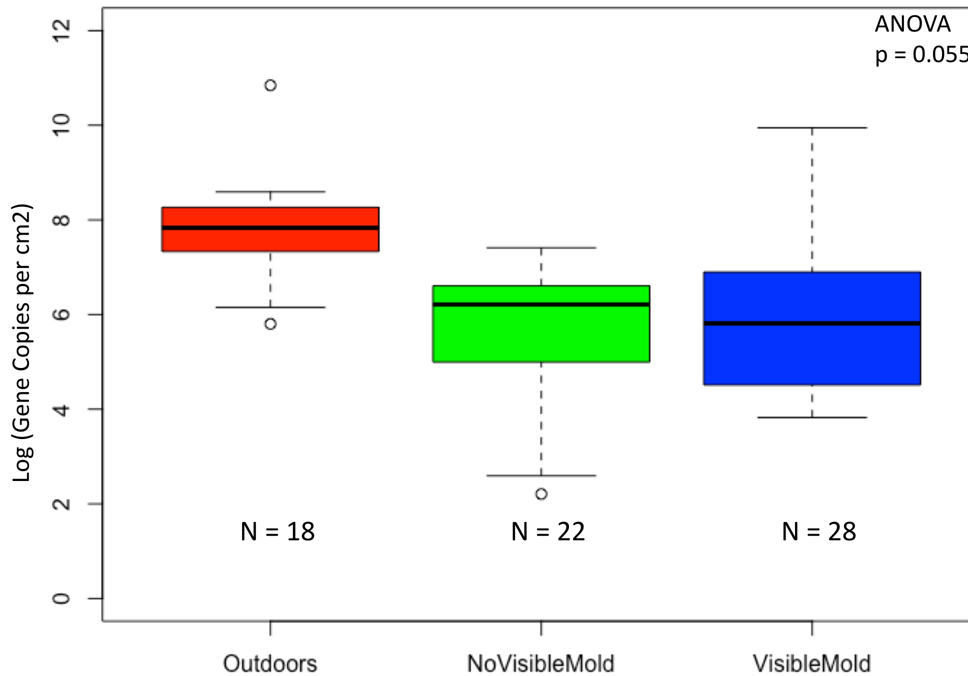


FIGURE 2. *Fungal Biomass. Comparison of mean fungal biomass in settled dust from outdoor air (red), indoor air in units with no visible mold (green), and indoor air in units with visible mold (blue). ANOVA was used to compare all three environments. Fungal gene copy number was divided by petri dish surface area as a proxy for biomass per cm² per month. Biomass is log-transformed for visual clarity. Fungal biomass was marginally significantly different between the outdoors, units with no visible mold, and units with visible mold (p=0.055).*

Fungal Diversity. We found significant differences in fungal richness (ANOVA; p<0.001) in settled dust from units with visible mold, units with no visible mold, and the outdoors (FIGURE 3a). Outdoor samples had the greatest richness, followed by units with no visible mold, and then units with visible mold. Fungal communities in units with visible mold were significantly less even (ANOVA, p=0.019) than units with no visible mold and the outdoors (FIGURE 3b).

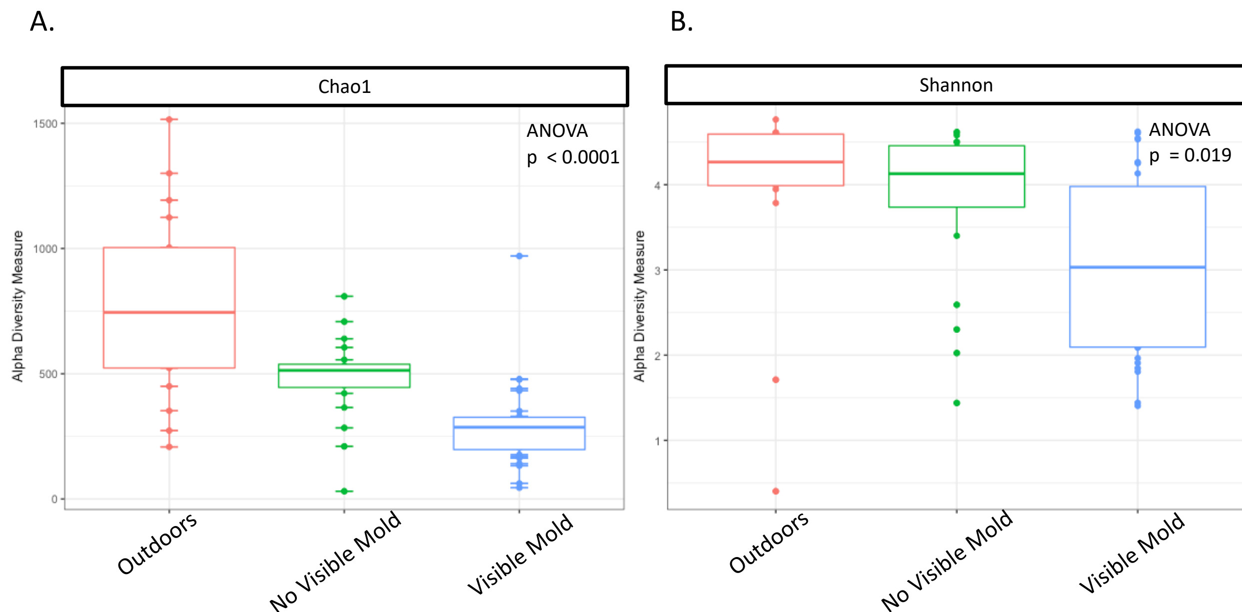


FIGURE 3. *Fungal Diversity. Community richness is measured as Chao1 (A) and community evenness is measured with Shannon Diversity Index (B) in settled dust from outdoor air (red), indoor air in units with no visible mold (green), and indoor air in units with visible mold (blue). There are significant differences in community richness (p<0.0001) and evenness (p=0.019) between these environments. Units with visible mold are less rich and less even than units with no visible mold and the outdoors.*

Fungal Communities. We found distinct fungal communities outdoors, in units without visible mold, and in units with visible mold (ADONIS; p<0.001; R^2 = 0.11). Outdoor samples are compositionally distinct from indoor samples, and within indoor samples there are distinct fungal communities in units with visible mold and units with no visible mold (FIGURE 4). Units with visible mold had the least dispersion, while the outdoor samples showed the greatest dispersion, or greatest dissimilarity to each other (SUPPLEMENTAL FIGURE 1; Tukey HSD; p=0.02).

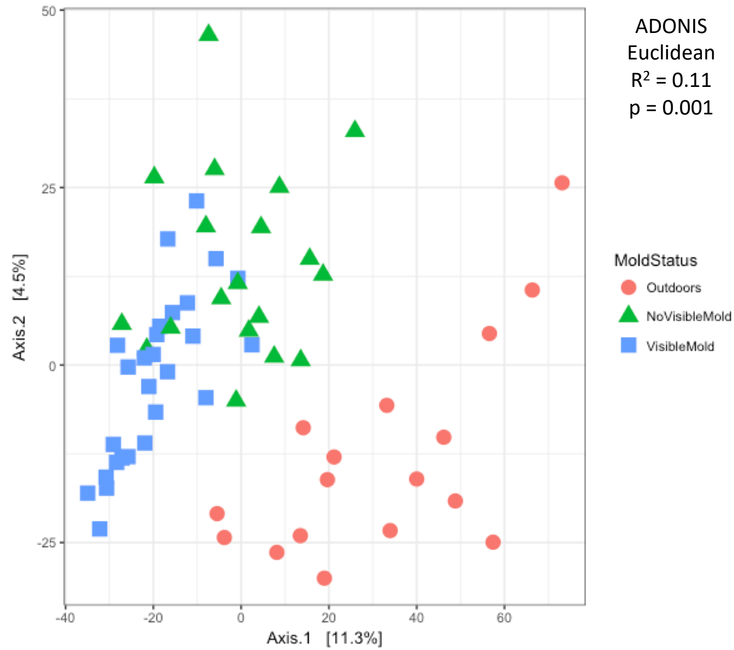
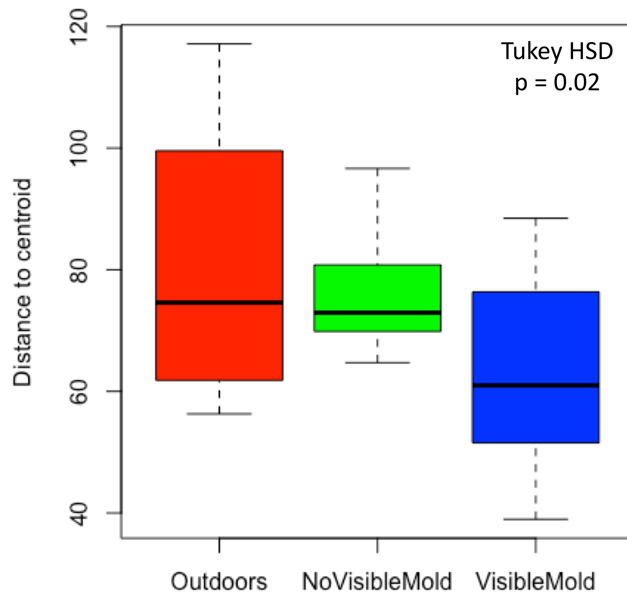


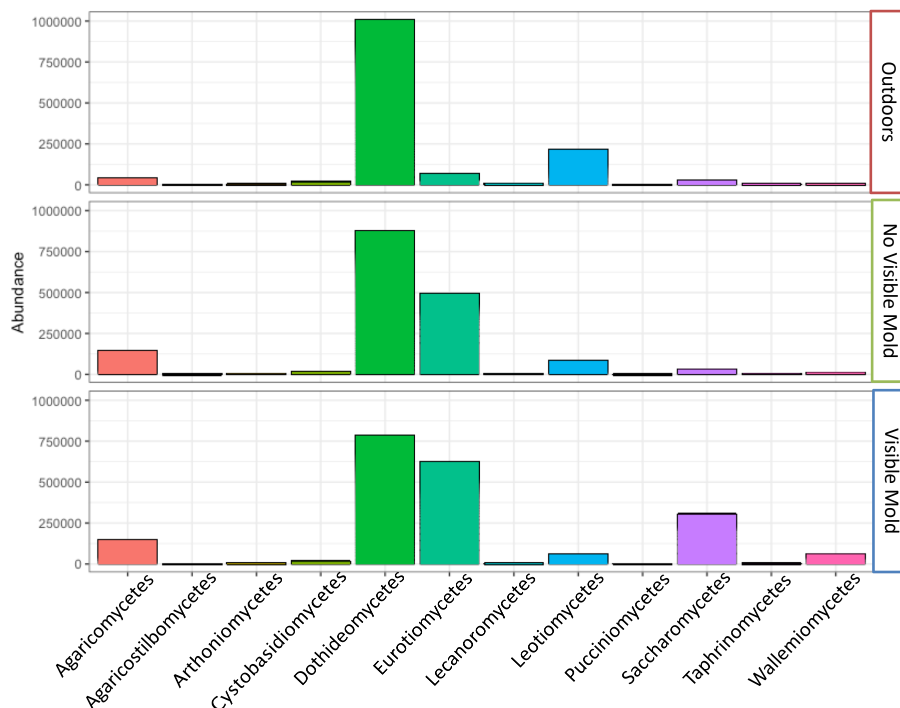
FIGURE 4. *PCA of Samples. Variance-based principal components analysis plot showing the dissimilarity of communities in settled dust from outdoor air (red), units with no visible mold (green), and units with visible mold (blue). Outdoor communities are distinct from indoor samples, and the presence of mold indoors distinguishes the composition of indoors samples. ADONIS test shows that the compositional distance between these three environments is significant ($p=0.001$; $R^2=0.11$).*



SUPPLEMENTAL FIGURE 1. *Among-Community Beta-diversity Across Environments. Comparison of homogeneity of variance of communities in settled dust sampled from outdoor air (red), indoor air of units with no visible mold (green), and indoor air of units with visible mold (blue). Significantly less dissimilarity (p=0.02) is seen among communities sampled from units with visible mold compared to those sampled in units without visible mold or the outdoors (Tukey's HSD test).*

Fungal Community Structure by Rooms and Floors. We detected no significant differences in biomass across different floors in the building (Correlation; p=0.13), or in different rooms indoors (Kruskal-Wallis; p=0.67). We detected no significant difference in fungal richness across floors (Correlation; p = 0.67), or in different rooms (ANOVA; p =0.3). We likewise detected no significant differences in fungal community composition across floors (ADONIS; p=0.26), or in different rooms indoors (ADONIS; p=0.311).

Fungal Taxa in Settled Dust. Twelve fungal classes were shown to have significantly different (Kruskal-Wallis; p<0.05) abundances across outdoor samples, units with no visible mold, and units with visible mold (SUPPLEMENTAL FIGURE 2). Agaricomycetes, Agaricostilbomycetes, Arthoniomycetes, Cystobasidiomycetes, Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes, Pucciniomycetes, Saccharomycetes, Taphrinomycetes, and Wallemiomycetes showed differentially abundant across these three environments. The abundance was significantly greater outdoors in all but four classes. Agaricomycetes, Eurotiomycetes, Saccharomycetes and Wallemiomycetes, had greater abundance indoors compared to the outdoors. Of these, Eurotiomycetes, Saccharomycetes, and Wallemiomycetes, were more abundant in units with visible mold compared to units with no visible mold (FIGURE 5).



SUPPLEMENTAL FIGURE 2. *Abundance of Fungal Classes Across Environments. Sequence abundance of fungal classes found in settled dust from outdoor air (top panel), units with no visible mold (middle), and units with visible mold (bottom). These twelve classes have significantly different abundance across the environments, as determined by Kruskal-Wallis test (p<0.05). Eight classes are more abundant outdoors, but Agaricomycetes, Eurotiomycetes, Saccharomycetes, and Wallemiomycetes are more abundant indoors.*

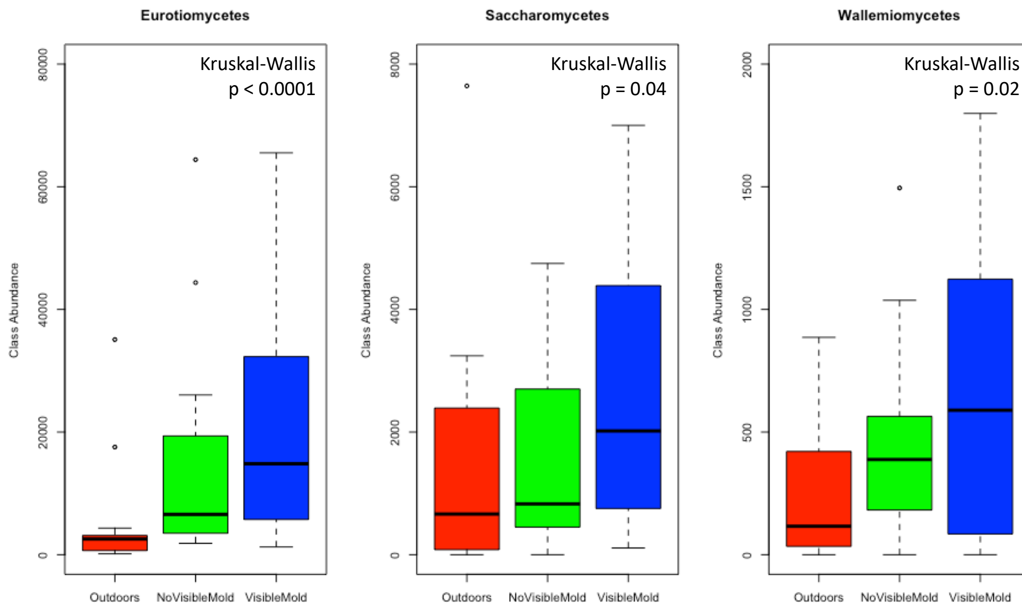


FIGURE 5. *Increased Abundance of Three Classes in Units with Visible Mold. A Kruskal-Wallis test of median abundance determined that three classes, Eurotiomycetes, Saccharomycetes, and Wallemiomycetes, were significantly more abundant in units with visible mold compared to units with no visible mold and the outdoors (p<0.05).*

Visible, Surface Communities Compared to Airborne Settled Dust. In units with visible mold, swabs were used to collect surface samples. Surface samples were dominated by one major taxon, identified as *Cladosporium sphaerospermum* (FIGURE 6). Other Dothidiomycetes and Eurotiomycetes were major constituents of surface samples, with *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Cyberlindnera*, *Cystobasidium*, *Didymella*, *Paraphoma*, *Penicillium*, *Pyrenochaeta*, and *Stachybotrys* species comprising the top 15 most abundant taxa in surface samples.

In units with visible mold we also compared the single most abundant taxon in each unit as determined by sampling either with surface swabs or settled dust collectors (SUPPLEMENTAL TABLE 1). For 9 out of 11 units with visible mold, there is discordance between sampling methods for determining which taxon is the most abundant. Surface samples and settled dust collectors detect a different assortment of fungi in units with visible mold. 15 out of 22 classes detected in units with visible mold were found to have significant differential abundance (Kruskal-

Wallis; $p<0.05$) between sampling methods (FIGURE 7). Settled dust samples detect a wider array of fungal classes than surface samples, and greater abundance of taxa within shared classes. Cystobasidiomycetes and Dothideomycetes were the only classes found to be more abundant in surface samples.

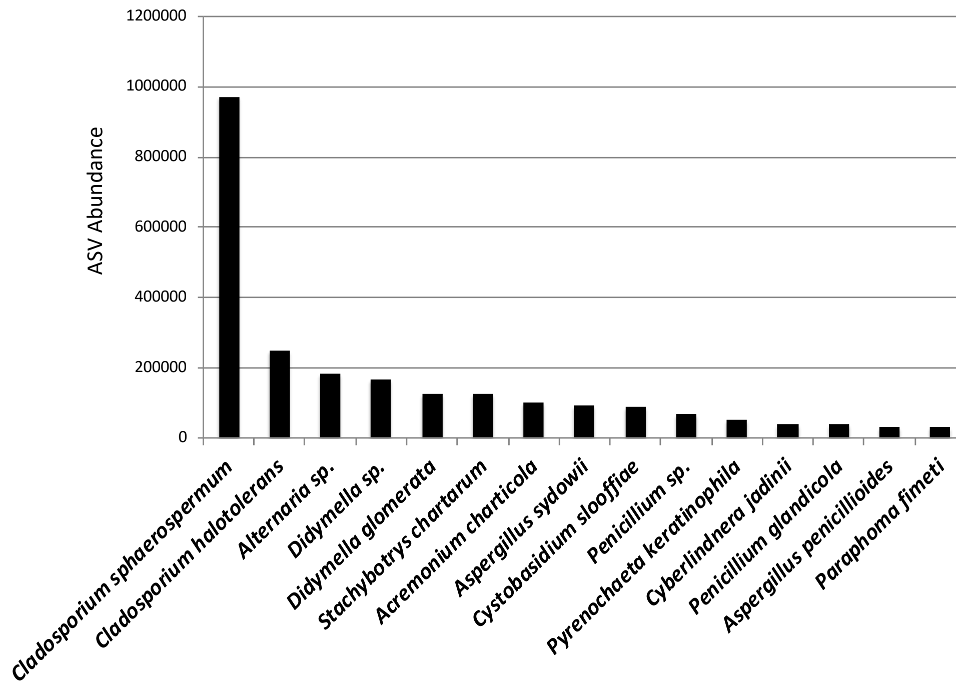


FIGURE 6. *Most Abundant Taxa in Surface Samples. The top 15 most abundant taxa recovered in surface samples.* Cladosporium *spp. comprise the majority of reads from surface samples. Other Dothideomycetes and Eurotiomycetes are major constituents of colonies of visible mold in water-damaged units.*

Unit	Most Dominant Taxon in Settled Dust	Most Dominant Taxon on Surfaces
A	* *Aspergillus proliferans* | Eurotiomycetes	*Cladosporium sphaerospermum* | Dothideomycetes
B	* *Mycosphaerella tassiana* | Dothideomycetes	*Aspergillus sydowii* | Eurotiomycetes
C	* *Cladosporium delicatulum* | Dothideomycetes	* *Mycosphaerella tassiana* | Dothideomycetes
D	*Clavispora lusitaniae* | Saccharomycetes	* *Cryptococcus uniguttulatus* (syn. *C. neoformans*) | Tremellomycetes
E	* *Blumeria graminis* | Leotiomycetes	*Cladosporium sphaerospermum* | Dothideomycetes
F	*Aspergillus sydowii* | Eurotiomycetes	*Cyberlindnera jadinii* (syn. *Pichia jadinii*) | Saccharomycetes
G	*Aspergillus sydowii* | Eurotiomycetes	*Aspergillus sydowii* | Eurotiomycetes
H	* *Mycosphaerella tassiana* | Dothideomycetes	*Aspergillus sydowii* | Eurotiomycetes
I	*Penicillium sp.* | Eurotiomycetes	*Didymella sp.* | Dothideomycetes
J	* *Acremonium charticola* | Sordariomycetes	* *Cladosporium halotolerans* | Dothideomycetes
K	*Alternaria sp.* | Dothideomycetes	*Alternaria sp.* | Dothideomycetes

* Denote taxa that have previously not been reported in indoor environments

SUPPLEMENTAL TABLE 1. *Predominant Taxon in Each Unit. Table showing which ASV was identified as the most abundant taxon in each unit with visible mold, either by surface samples or settled dust collectors. There is discordance in what taxon predominates each unit depending on sampling method used to survey the community. Stars denote taxa that have not previously been reported from the indoor environment or water-damaged buildings.*

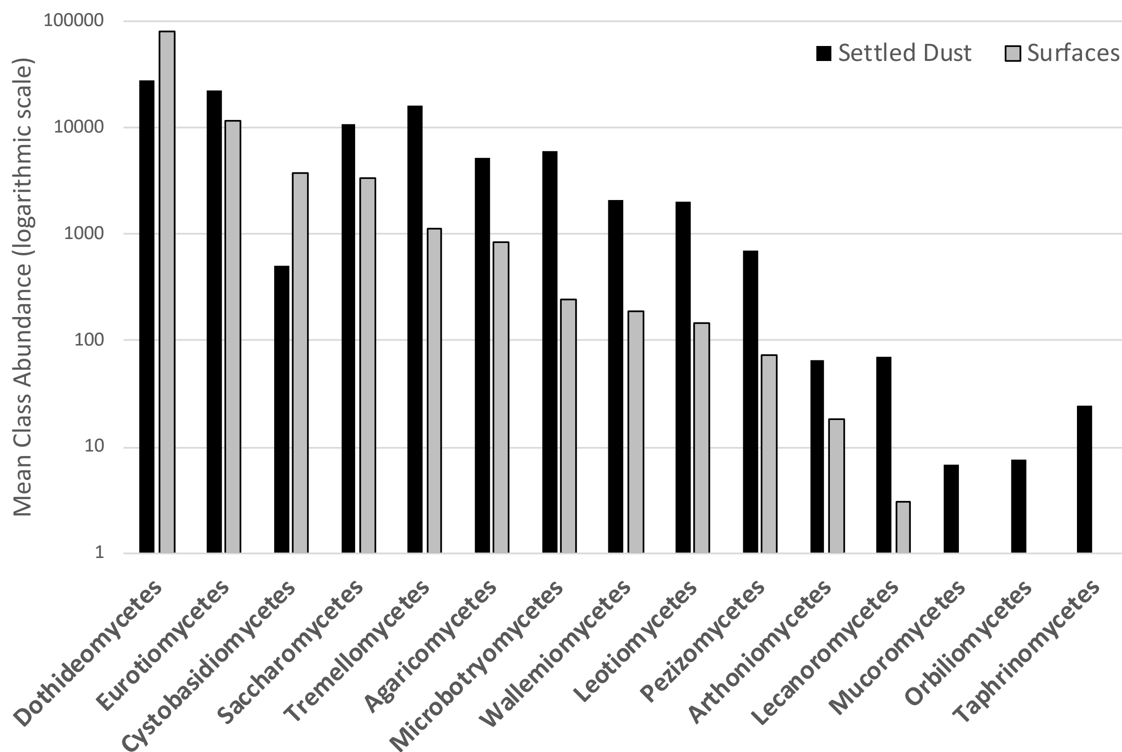
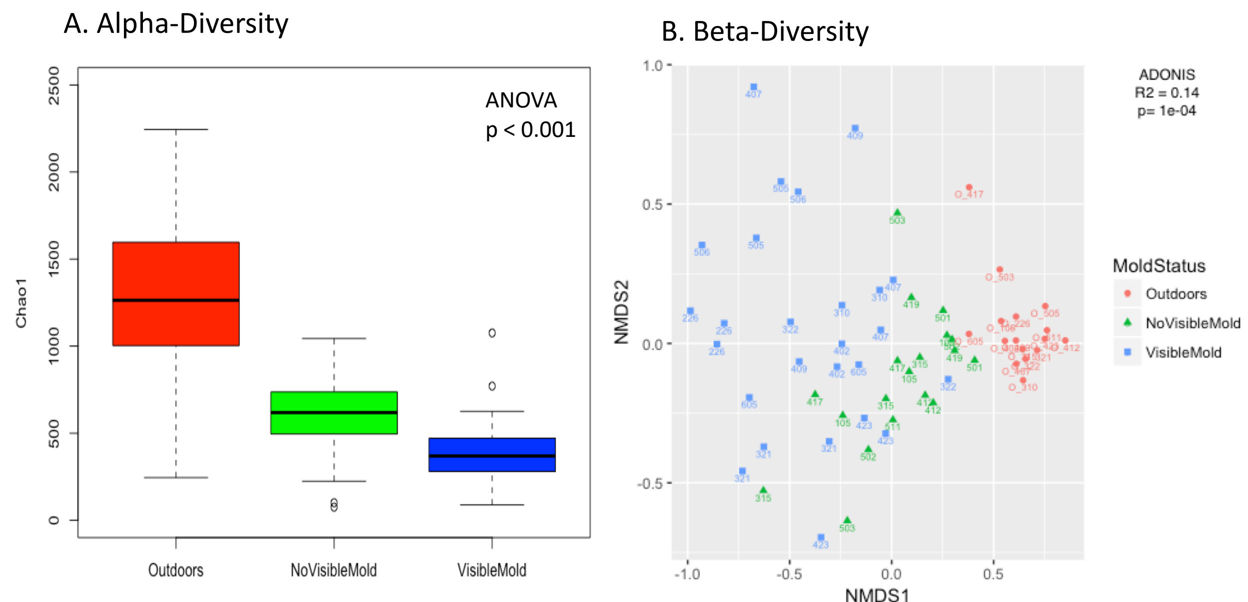


FIGURE 7. Abundance of Fungal Classes with Differential Abundance Across Sampling Methods. 15 classes were found to have significant differential abundance (p<0.05) when sampled using either surface swabs or settled dust collectors in units with visible mold. Settled dust collectors detect greater abundance of fungal classes compared to surface samples in all but two class, Cystobasidiomycetes and Dothideomycetes.

Comparison of statistical analytical methods. To enable comparison of our treatment of sequence data as compositional with the traditional treatment of such data as abundance counts (as has been done in almost all publications on fungi in indoor air), we also analyzed our data in the traditional manner. Results of both approaches arrive at similar conclusions about the biology of fungi in the built environment. As seen with compositional analysis, count analysis provided an ANOVA of OTU richness that was significantly different ($p<0.001$) between outdoor samples, units with no visible mold, and units with visible mold (SUPPLEMENTAL FIGURE 3a). With count analysis, an NMDS and ADONIS test showed that the composition of units with visible mold were distinct from units with no visible mold and outdoors samples ($R^2=0.14$; $p<0.0001$; $R^2=0.14$; SUPPLEMENTAL FIGURE 3b). Count analysis found that samples from units with no visible mold were compositionally more similar to outdoor samples, and samples from units with visible mold were distinct from each other and not tightly clustered.



SUPPLEMENTAL FIGURE 3. Alpha- and beta-diversity of fungal communities in samples when analyzed by traditional count-based methods. (A) Alpha-diversity showing significant differences in OTU richness among all sample types, outdoor air (red), indoor air of units with no visible mold (green), and indoor air of units with visible mold (blue). (B) Beta-diversity showing significant differences in community composition among environments. Comparison of these results with those from analyses that treat sequence data as compositional (Figures 3 and 4) show similar trends in richness and community composition.

DISCUSSION

The features that combine to make our study unique in the field of indoor air microbiology include: (1) simultaneous collection indoors and outdoors of fungi passively settling on sterile surfaces over a defined period long enough to account for daily and weekly variation in fungal abundance and occupant behavior. (2) Sampling 21 units within one water-damaged building in residences both with and without visible mold colonies. (3) Culture independent characterization of fungal communities by high-throughput DNA sequencing. (4) Analytical treatment of microbial DNA sequence reads as a compositional dataset rather than the standard statistical treatment of rarefied read counts.

By sequencing settled dust from units with visible mold, units with no visible mold, and outdoor air, we queried the impact of water-damage on fungal biomass, richness, and community composition indoors. By sequencing indoor surfaces with visible mold growth, we asked which taxa dominate units with visible mold, and whether these taxa become airborne. With these data we found distinct fungal communities associated with moldy housing. Compared to units with no visible mold and the outdoors, units with visible mold had reduced community richness, reduced community evenness, and a demonstrably different suite of taxa, principally within the Dothideomycetes and Eurotiomycetes. The abundances of Eurotiomcyetes, Saccharomycetes, and Wallemiomyctes were significantly greater in units with visible mold compared to units with no visible mold and the outdoors. Fungal community structure (biomass, richness, and composition) did not differ significantly across floors of the building or between rooms indoors.

A prior study of fungal communities inside healthy homes of the San Francisco Bay Area found that movement of fungi from the outdoors was sufficient to explain fungal assemblages indoors (21). In our study of a poorly-maintained building in the same region, we saw evidence that the presence of excess water in units, judged by visible mold, allowed for the proliferation of a few indoor taxa and the development of fungal communities that are distinct from those found in units without visible mold or the outdoors.

Comparison of Units With and Without Visible Mold

Quantitative Analysis Did Not Allow for the Detection of Differences Between Units. In this study we found no significant difference of fungal biomass in units with visible mold and units with no visible mold. Fungal biomass was predictably greater outdoors compared to indoors, but qPCR analysis alone was not sufficient to distinguish differences among fungal communities in units with and without visible mold. There is a long history of using quantitative-PCR to ascertain the concentrations of mold indoors (i.e. (44), (45)), such that Mold-Specific Quantitative PCR informed the development of the Environmental Relative Moldiness Index (ERMI), a scoring system used to predict whether dust in buildings can indicate water-damage (46).

In comparison to culture-based methods, qPCR is more sensitive, accurate, and better able to detect different microbial concentrations in house dust of moisture-damaged and undamaged homes (47). Early studies using qPCR suggested that high concentrations of particular fungi indoors (some *Aspergillus*, *Eurotium*, *Chaetomium*, *Paecilomyces*, *Penicillium*, *Scopulariopsis*, *Stachybotrys*, *Trichoderma*, and *Wallemia*) could be indicator species used to detect water-damage in homes (44). In this study we used universal fungal primers to quantify the relative concentrations of all

fungi indoors and outdoors, rather than specific taxa, and overall fungal biomass indoors was not significantly different in units either with or without visible mold. Rather, in our study, community composition was more indicative of differences between units than total fungal load. Alpha- and beta-diversity measures allowed for greater detection of differences attributable to water-damage than quantification of fungal gene copies.

Fungal Communities in Units with Visible Mold Are Less Diverse than Units with No Visible Mold and Outdoor Air. In our study, units with visible mold had lower fungal richness (number of fungal taxa) than units with no visible mold or outdoor air. In addition, fungal communities in units with visible mold were less even, suggesting a dominance of a few taxa within these units. An assessment of fungal diversity in a water-damaged office building likewise found reduced fungal richness in sequences collected from lower floors that had incurred worse water-damage (48). But in a study evaluating which housing characteristics impact microbial communities indoors, Kettleson et al. (49) found homes with higher ERMI and high humidity housed more fungal taxa than homes with low ERMI scores and lower humidity. Using clone libraries, Pitkaranta et al. (12), observed elevated fungal diversity was associated with water-damage in office buildings. Dannemiller et al. (50) found no significant difference in fungal richness in homes with and without visible mold, but did correlate the presence of water leaks with increased fungal richness. Later Dannemiller et al. (51) showed that in homes with no visible mold, increased moisture led to significantly greater fungal richness, but in homes with visible mold an increase in moisture did not increase fungal richness.

Taken together, these publications suggest that the elevated presence of water indoors may increase fungal richness until a threshold is reached where visible mold becomes present and begins to dominate the community. At that point, actively growing mold may lower observed fungal diversity in water-damaged homes because the airborne spores of a few dominant taxa comprise the majority of sequence data. Such a trend was demonstrated in Adams et al. (52) when a unique signal from abundantly sporulating Basidiomycota fruiting bodies distorted the perception of species richness in mycology classrooms, which appeared to have lower overall richness compared to other classrooms.

Fungal Communities from Units with Visible Mold are Distinct from Units with No Visible Mold and Outdoor Air. Detecting differences in fungal community composition within homes impacted by water-damage remains challenging, even with the advent of high-throughput sequencing, possibly because there is currently no consistent measurement used to characterize moisture in buildings (53). In our study we used the appearance of visible mold as the main determinant of water-damage in the units, and we were able to detect clear compositional differences in units with and without visible mold. We found that fungal communities in units with visible mold are dissimilar to outdoor air communities and to units with no visible mold. Adams et al. (21), surveyed the microbiota of healthy homes in the Bay Area and found that outdoor air fungi dominate the patterning of indoor air, and no taxa were indicators of the indoor environment. Here we observed the presence or absence of visible mold indoors as a major factor distinguishing the microbial communities within a building. Emerson et al. (26) surveyed homes that had experienced water-damage directly attributable to a historic flooding event and found significant differences in fungal community composition between flooded and non-flooded homes in Colorado. Jayaprakash et al. (25) surveyed buildings with less obvious causes of water-damage

and did not see fungal community structure differentiate as a response to water intrusion in severely-damaged homes in Finland.

Units with Visible Mold are Dominated by Fungal Taxa Previously Associated with Water-Damaged Buildings. Units with visible mold were dominated by Dothideomycetes in the genera *Alternaria*, *Cladosporium*, *Didymella*, and *Mycosphaerella,* which presumably migrated indoors from the outdoors (21), where their abundance was much greater. The migration of these fungi could also be by air currents or by occupants and their pets (54). In addition, it is possible that the fungi entered the units on contaminated fruits or vegetables. Three fungal classes, Eurotiomceyetes, Saccharomycetes, and Wallemiomceyetes, showed significantly greater abundance in units with visible mold compared to units with no visible mold and the outdoors, suggesting an indoor source for these taxa in water-damaged units. Agaricomycetes also had greater abundance indoors compared to the outdoors, but their abundance was highest in units with no visible mold. A clone-library study of fungi in dust collected from water-damaged and renovated buildings likewise discovered increased fungal diversity in the Agaricomyetes and Dothideomcyetes fungi was associated with water-damage (12).

Many of the taxa that we found to dominate units with visible mold in surface samples have previously been associated with the indoor environment, and in particular water-damaged buildings. The most abundant taxon recovered from colonies of visible mold growth on surfaces was *Cladosporium sphaerospermum*. Other predominant taxa that we recovered in our sequencing of units with visible mold included species in the genera *Acremonium*, *Alternaria*, *Aspergillus, Penicillium*, *Stachybotrys*, and *Wallemia*. In a qPCR study of Finnish homes, rising concentrations of *Cladosporium sphaerospermum* and *Wallemia sebi* in house dust were associated with increasing severity of moisture damage (47). Where we have detected fungal species not previously associated with the indoor environment, the genera or classes that harbor these species have been reported from water-damaged building materials (e.g. in (8), (9), (44)). We identified *Acremonium charticola, Aspergillus proliferans, Blumeria graminis, Cladosporium delicatulum, Cladosporium halotolerans, Cryptococcus uniguttulatus*, and *Mycosphaerella tassiana,* as dominant taxa in units with visible mold that had not previously been reported indoors.

Comparison of Sampling Methods

Airborne Fungi and Surface Communities are Distinct in Units with Visible Mold. In units with visible mold we collected swabs from actively growing colonies on surfaces, as well as airborne settled dust over the course of one month. The two collection methods provided different profiles of the mycobiota within homes. The dominant taxon recovered within each unit varied between collection methods. Only in two out of eleven units were the same dominant taxon identified using both collection methods. Surface samples were largely dominated by *Cladosporium* spp., while settled dust samples recovered more phylogenetic diversity. Settled dust collectors were able to recover significantly greater abundance of a number of fungal classes: Agaricomycetes, Arthoniomycetes, Lecanoromycetes, Leotiomcyetes, Microbtroymycetes, Pezizomycetes, Saccharomceyes, Tremellomycetes, and Wallemiomycetes, to name a few. These taxa appear to become airborne, and over the course of one month are able to deposit in settled dust collectors. Interestingly, Basidiomycete yeasts in the Cystobasidiomycetes were recovered in greater abundance from surface samples compared to settled dust; possibly suggesting they are less likely to produce airborne spores or be inhaled by residents.

The utilization of varied collection methods provided for a composite view of fungal communities indoors. Collection by swabbing surfaces selects for live fungi that may be actively sporulating in homes at the moment of sampling. These colonies may also be very large in size and have pigmented spores, making them visually detectable. In contrast, settled dust samples are a collection of both live and dead airborne fungi that accumulate in the home over time. Settled dust samples also collect a greater abundance of fungi because they are set out for weeks at a time and can acquire fungal material from undetected colonies as well. The settled dust collection captures longer-term dynamics in the fungal community composition, while the surface sample is a snapshot of predominant taxa in the unit at a particular moment in time. Temporal, ecological, and presumable physiological differences are detected by different sampling methods, and both proved valuable to characterize fungi associated with water-damaged buildings.

Limitations of This Study. We collected 68 settled dust samples from outdoor air, units with no visible mold, and units with visible mold, within a chronically water-damaged 150-unit building in the San Francisco Bay Area. Although some units had visibly greater water-damage than others (demonstrated by actively growing mold on surfaces), it is possible that all units in the building experienced some level of water-damage due to long-term structural issues, and thus we may actually have compared mildly and severely water-damaged units. A more comprehensive assessment of building water intrusion, including the measurement of relative humidity, temperature, and the surface area of mold, might have facilitated a more precise categorization of the level of water-damage in each unit. It would have been ideal to have also surveyed a building of similar design and age with no prior history of water-damage in close proximity to our building. This survey would allow for the comparison of microbial communities within a water-damaged building and a healthy building within the same timescale, season, and weather regime. Additionally, surface samples were only collected from units with visible mold where we swabbed actively growing colonies. Though we presumed surface samples would be dominated by one taxon, sequence data recovered additional ASVs in surface samples at lower abundance. These additional taxa are constituents of the surface community that were not necessarily visible. It would be good to standardize surface collections in each unit and have background knowledge of which ASVs are found on surfaces in each unit regardless of the presence of visible mold.

CONCLUSIONS

This study is the first to analyze the microbial inhabitants of a condemned building using high-throughput sequencing methods. This is also one of only a handful of studies to use culture-independent techniques to explore the impact of water-damage on microbial communities in buildings. The distinction between outdoor microbial communities, units with no visible mold, and units with visible mold, shows that insufficient building maintenance can drastically shift the assemblage of fungi indoors.

In this study we showed that sampling replicated units in one poorly maintained structure can reveal differences among the airborne mycobiome seen outdoors and indoors, as well as in units with and without visible fungal colonies. Furthermore, sampling fungal spores, yeast cells, and hyphae, by gravity settling over a time period long enough to account for daily variation in airborne fungi and weekly variation in occupant behavior characterized airborne fungal communities that

correlated with the presence of visible fungal colonies. Biomass of the settled fungi, however, did not correlate with the presence or absence of visible fungi in units. In units with visible mold, the airborne fungal communities were less diverse and dominated by a few major taxa.

With the onset of high-throughput sequencing, it is no longer "impractical to measure all the molds in a home" collected in dust, as was suggested by Vesper et al. (46). We look forward to comparing our study with others of poorly maintained buildings that include replication in the form of many units in one building, sampling of airborne fungi over a defined period, and fungal identification by high-throughput sequencing. Through these comparisons we hope to arrive at an economical and accurate means to detect progress toward the WHO mandate of healthy housing as a basic human right.

Evidence of Disparate Microbial Communities Associated with Water-Damaged Units in New York City Public Housing Residences using High-throughput Sequencing

Iman A Sylvain, John W Taylor, Michal P Spilak, Michael S Waring, Rachel I Adams

ABSTRACT

Housing is a primary social determinant of public health. Microbial exposure indoors can result in a number of negative health outcomes, including injury, respiratory disease, asthma, depression and anxiety. Asthma morbidity is disproportionately experienced by low-income People of Color living in public housing. The New York City Housing Authority (NYCHA) is the largest public housing authority in the United States. NYCHA is woefully underfunded and the 2,000+ buildings managed by the housing authority suffer from major structural damage and disrepair. In the wake of Hurricane Sandy NYCHA failed to adequately address water-damage caused by the storm, exacerbating previously poor living conditions. Mold contamination became a serious health concern for NYCHA residents who eventually sought legal restitution. Working collaboratively with community organizations, resident advocates, attorneys, and building scientists, we assessed microbial communities within NYCHA buildings and neighboring privately-owned apartments to determine whether New York City had a mold problem, or specifically NYCHA buildings were microbially disordered.

Three years after Hurricane Sandy flooded buildings in New York City in October 2012, we characterized microbial communities from 70 households in public and private housing complexes across Brooklyn and Manhattan. Comparison of airborne dust samples from the outdoors and inside units with or without water-damage showed that, compared to outdoors and undamaged units, water-damaged units had reduced fungal biomass, reduced bacterial and fungal richness, distinct bacterial and fungal communities, and lower within-group dissimilarity (beta-dispersion). Water-damaged units had significantly lower abundance of many outdoor taxa and increased abundance of two bacterial classes: Clostridia and Coriobacteriia, and two fungal classes, Agaricomycetes, and Pezizomycetes. Additionally, public housing showed different microbial community composition from private housing and the outdoors. Microbial communities in public housing were characterized by lower diversity and increased abundance of three bacterial classes, Bacteroida, Erysipelotrichia, Negativicutes, and one fungal class, Saccharomycetes.

The shift in microbial communities in water-damaged units and public housing projects may be indicative of environmental filtering indoors. Building materials compromised by water intrusion and long-term neglect may begin to select for a distinct community of microbes dominated by a comparatively small number of taxa with potential health consequence. This study demonstrates that high-throughput sequencing can be leveraged to investigate the microbial impact of natural and sociopolitical disasters on the built environment.

INTRODUCTION

There is a long history of research investigating the microorganisms that cohabit the indoor environments humans have constructed. The recent adoption of high-throughput sequencing

(HTS) of bacterial and fungal rRNA has dramatically changed the way we view the microbiology of the built environment (55). With the advent of HTS, we have identified previously unculturable organisms and come to learn about factors and conditions that pattern how outdoor microbes enter and persist in the built environment (56). HTS has shown that indoor microbiomes are governed by several key factors, including geography (57), climate (16), heating and ventilation systems (58), building design (19), moisture (59), occupancy by humans (54) and pets (49). Specifically, fungi and bacteria found on residential surfaces in healthy buildings have been shown to be mainly a filtered sample of outdoor origin (60) with little indoor growth, augmented by some human-associated bacteria (61).

Damp, moldy housing has been associated with a number of negative health effects mediated by microbial exposure, principally respiratory symptoms, allergies, asthma, and perturbations of the immunological system (5). As such, many studies have investigated the effect of water-damage on microbial communities within the built environment. Culture-based and immunochemical studies using microscopy and detection of endotoxin or glucans have elucidated numerous taxa that are indicators of water-damage (62), (63), (64), (11), (65). With bacteria, the Actinobacteria, *Micropolyspora, Streptomyces,* and *Thermoactinomyces* have been found in association with water-damaged buildings (8). With fungi, culture-based studies have reported *Aspergillus, Aureobasidium, Eurotium, Chaetomium, Cladosporium, Penicillium, Stachybotrys, Trichoderma,* and *Wallemia* as fungal indicators of water-damage indoors (46). Many of these fungi are able to produce secondary metabolites that include mycotoxins, fostering a debate over the level of concern to public health (66), (67). The potential of indoor fungi to cause allergies and asthma is less controversial, but a full understanding of this area awaits improved fungal sampling (68). While fungi have long been demonstrated to grow at elevated levels of water availability (a_w) indoors (53), Hegarty et al. recently confirmed using metatranscriptomics that fungi in house dust are metabolically active at a range of 0.5-1.0 a_w, and genes related to allergenicity, mycotoxin production, and pathogenicity are upregulated as water availability increases (69).

There is a well-documented association between housing quality and public health (70). Housing is a prime social determinant of health, and poor housing has been linked with a wide range of negative conditions, including respiratory infection, asthma, lead poisoning, injuries, and adverse effects on mental health (3), (71). The ways in which housing conditions - which are shaped by social forces like racial segregation (72) and poverty (73) - affect exposure to intoxicants and translate social adversity into individual illness has also been deeply explored (2), (6). The inadequate housing often occupied by marginalized communities is believed to be the underlying cause of ethnic/racial public health disparity in the United States (74). Asthma, in particular, is a disease triggered by environmental allergens often found in association with substandard housing where there is increased exposure to cockroaches, mice, fungi, and proximity to highways and other sources of outdoor pollutants (75). Asthma morbidity and mortality disproportionately impacts low-income, urban, Communities of Color (76), (77). This impact has led many public health practitioners and environmental justice advocates to press for improved housing conditions as major interventions for asthma morbidity, (78), (79), and specifically for the remediation of mold in public housing residences (80), (81,82), (83).

The New York City Housing Authority (NYCHA) is the largest public housing authority in the country, housing 7% of NYC residents in 175,636 units and 2,418 buildings (84), while wrestling

with the deterioration that comes from severe debt (85). When Hurricane Sandy struck NYC on October 29, 2012, NYCHA was unable to provide timely and adequate disaster responses to the estimated 80,000 NYCHA residents who lost electricity, heat, and essential services (86). In a survey conducted in the aftermath of the disaster, 56% of surveyed residents noted negative health effects associated with the presence of visible mold (86).

Political advocacy coordinated by community organizations, Community Health Workers, and a legal team from the Natural Resources Defense Council and the National Center for Law and Economic Justice, resulted in the filing of a class action lawsuit against NYCHA. The suit was successful in employing the Americans with Disabilities Act by alleging that NYCHA violated the civil rights of residents with asthma and breathing disabilities by failing to eradicate leaks, excessive moisture, mold, and ventilation issues (83). After one year it was clear that NYCHA was out of compliance with the order and overwhelmed by the vast requirements for capital improvements. NYCHA has since launched the Mold Busters program that will attempt to address key structural issues like "roofs that have not been replaced in 20 years, facades that are no longer sealing the building from rainwater, antiquated ventilation systems that do not work properly, and decaying in-wall piping."

The microbiomes of water-damaged building are just beginning to be studied using HTS, such that a clear understanding of how water intrusion structures microbial biomass, richness, and community composition indoors has yet to be established. One of the first studies of water-damaged buildings by HTS occurred after a major flooding event in Boulder, CO. Here, researchers found differences in fungal and bacterial community composition between flooded and non-flooded homes and documented increased abundance of *Penicillium*, Psuedomonadaceae, and Enterobacteriaceae in flooded homes (26). In another study, changes in the microbiota associated with severely water-damaged buildings before and after damage renovations were observed as reductions in bacterial and fungal richness, specifically Actinomycetales, and subtle effects on bacterial and fungal community structure (25). More recently, we surveyed one multi-unit, public housing building in Richmond, CA, with chronic water-damage and found distinct fungal communities in units with visible mold compared to units with no visible mold and the outdoors {Sylvain:wz}. In the Richmond, CA study, units with visible mold had lower fungal richness and increased abundance of three fungal classes.

In the New York City study reported here, we used high-throughput sequencing to examine microbial communities associated with water-damaged units in NYC public housing projects. We sampled bacteria and fungi from indoor and outdoor air in the summer and winter in Brooklyn and Manhattan, conducted building assessments, recorded a number of environmental measures that included temperature, relative humidity, CO_2, and air exchange rate, and tracked occupant behaviors over the course one month. We sampled indoors and outdoors from NYCHA public housing and privately-owned apartments in the same boroughs. With this data we queried whether water-damage results in greater microbial biomass indoors, if water-damaged units have greater microbial richness than non-damaged units, if there are shifts in microbial community structure attributable to water-damage, and whether there are obvious distinctions between microbial communities associated with public and private housing? By conducting amplicon sequencing and quantitative-PCR on rRNA of bacteria and fungi that settled in passive dust samplers, we were

able to detect differences in microbial community composition associated with indoor water-damage and public versus private housing.

MATERIALS AND METHODS

Study Design and Sampling Methods. In the winter of 2015, three years after the October 2012 damage caused by Hurricane Sandy, we partnered with local community organizations to recruit volunteers to participate in this study of airborne bacteria and fungi in New York City public housing. We successfully recruited 60 households for the first season, and an additional 10 households the following season. Participation involved an initial inspection of the unit by our team of building specialists, the structure and maintenance of the building, an evaluation of the presence of mold and vermin, and the installation of HOBO Data Loggers (Onset Computer Corp.) to continually record environmental measurements related to thermal comfort. On the day of airborne sampler and data logger installation, an interview with residents was also conducted, where basic questions regarding cleaning and cooking habits, health, and socioeconomic status was recorded. Residents were asked to maintain a diary over the course of the study to track their indoor behavior.

In total, we surveyed 70 units in 43 buildings in Brooklyn and Manhattan over the course of two seasons. We sampled in five major NYCHA housing complexes, as well as in privately-owned buildings in the same boroughs. We surveyed units that we deemed water-damaged due to the presence of cracking paint, water-leakage, visible mold, and self-reports by occupants. Wherever possible we sampled non-damaged units in the same building, TABLE 1. This study was approved by the University of California Committee for the Protection of Human Subjects under protocol 2014-08-6589.

Airborne Dust Samples	BACTERIA (123 Total) n =	FUNGI (100 Total) n =
Indoor	101	79
Outdoor	22	21
Winter	65	53
Summer	58	47
Water Damaged	50	43
Not Water Damaged	51	36
Public Housing	79	61
Private Housing	22	18
Brooklyn	77	65
Manhattan	46	35
Units	65	61
Buildings	41	40
Housing Complexes	8	8

TABLE 1. *Summary of samples collected in this study. A total of 123 bacterial and 100 fungal samples were analyzed. These passively settled dust samples were sourced from multiple locations,*

indoors and outdoors, in different neighborhoods, from different housing types, and multiple housing complexes. The units were either water-damaged or not damaged and collected over two seasons in New York City. Water-damage was determined by the presence of cracking paint, water-leakage, visible mold, and self-reports by residents.

Either by visual inspection, resident interviews, or from occupant diaries, we recorded whether cigarettes were smoked in the unit, if residents developed health concerns (e.g., asthma or sinus issues), if a musty smell could be detected in the unit, if there was water leakage, visible mold, mice, or roaches in the home. We recorded the unit number, floor of the building, whether the building was operated by NYCHA or a private landlord, and if a NYCHA building, the name of housing complex. For each collection, we recorded the borough (Brooklyn or Manhattan) and season (summer or winter). We recorded the age of the building, number of floors, and the total area of the surveyed home. We recorded the total number of occupants, including adults, children, and pets. In addition, we tracked outdoor and indoor temperature (°C), relative humidity (RH %), air exchange rate (h^{-1}), and CO^2 concentration (ppm) indoors. (Building assessment results reported elsewhere, see (87)).

To pair the building measurements with microbiological data, in each household within a building (hereafter, unit) we sampled passively settled airborne dust over one month by deploying dust collectors – open, empty, sterile, 10-cm diameter petri dishes with no growth media, as previously described (88) in living rooms or bedrooms. Where possible and in compliance with NYCHA rental policies, we also deployed passive airborne dust collectors outdoors that had been modified to prevent the entry of precipitation. Outdoor samplers were placed on top of air-conditioner units, on windows, or on balconies.

DNA Extraction and Amplicon Library Preparation. Recovered samplers and control samplers (sterile Petri dishes that had not been deployed) were treated identically in all laboratory manipulations. For each sample, bacterial and fungal genomic DNA (gDNA) was extracted using a phenol:choloform-isoamyl alcohol extraction protocol with the Qiagen DNeasy PowerSoil HTP 96 Kits, as previously described (21), (88). Briefly, for indoor and outdoor Passive Airborne samples, a sterile cotton swab dipped in PCR-grade double-distilled water was used to remove all of the dust on the plate and placed in a Miller Phosphate-Miller SDS buffer. The quality of gDNA was checked with gel electrophoresis before library preparation.

PCR was conducted using primers for the DNA regions coding for 16S ribosomal RNA in bacteria and ITS in fungi and adapted for Illumina MiSeq 250 paired-end sequencing with V2 chemistry, following methods previously described (29). Quality of PCR amplicons was assessed by gel electrophoresis, prior to further cleaning with magnetic beads and concentration with the SequalPrep Normalization Plate. Final PCR-product was quantified using a Quant-iT dsDNA Assay Kit, and equimolar concentrations of samples were pooled into six lanes for Illumina Amplicon Sequencing.

Quantitative-PCR (qPCR) was conducted to determine microbial biomass in airborne dust samples. Plasmids were constructed in-house for the 27F/518R in bacteria using *Escherichia coli* and FF2/FR1 region for fungi with *Aspergillus fumigatus*. qPCR analysis was performed on a CFX96 Touch Real-Time Detection System using SYBR Green. Microbial biomass for airborne

bacteria and fungi was estimated by dividing the qPCR Starting Quantity mean by the Petri dish surface area (56.5cm^2) to create a metric of Gene Copies per cm^2. Library sequencing was conducted at the Vincent J. Coates Genomic Sequencing Laboratory in the California Institute for Quantitative Biosciences (QB3) at the University of California, Berkeley.

Sequence Processing. Sequences were processed differently for bacteria and fungi, resulting in the construction of amplicon sequence variants (ASVs) for bacteria and operational taxonomic units (OTUs) for fungi. For bacteria, using Cutadapt (31), adapter sequences were removed with no quality filtering, but with a minimum read length of 75bp. Further processing into ASVs was implemented in the DADA2 library (32) in the R environment with some additional software. First, forward and reverse reads were filtered (truncQ=2, and maxEE=2 for forward and maxEE=5 for reverse reads). Then paired forward and reverse reads were identified using Fastq-pair (https://github.com/linsalrob/EdwardsLab/) and paired using Pear (33). Returning to DADA2, sequences with N's were removed, dereplicated, and then sequence variants inferred. Chimeric sequences were removed, and taxonomy assigned against the GreenGenes database (89).

For fungi, forward and reverse read pairs were processed with Cutadapt (31) and Trimmomatic (90) to remove the linker and adapter sequence. Usearch (91) was then used to merge pairs, quality-filter by length and score, and then check for chimeras. The resulting sequences were then clustered into non-chimeric OTUs at 97% identity, and OTU representative sequences were compared to the QIIME database (37) using Blastn in order to assign fungal taxonomy. Raw sequences will be submitted to NCBI's Sequence Read Archive upon publication.

For bacteria and fungi we used the Decontam package (36) in R to remove contaminant taxa that were prevalent in the Negative Controls or Blank samples. This step resulted in the removal of 76 bacterial taxa, and 201 taxa for fungi, representing a loss of less than 1% ASVs or OTUs from the dataset. We removed 'Eukaryotic' and 'unidentified' domains from the bacteria dataset, and 'unidentified' or 'incertae sedis' phyla for fungi. We then used CodaSeq (43) to filter the dataset to a minimum of 6,000 reads per sample and performed a center-log-ratio (CLR) transformation, constructing a compositional dataset. The final dataset used for analysis included 123 bacterial samples comprising 16,382 taxa and 100 fungal samples comprising 11,293 taxa.

Statistics Analysis. Statistical analysis of ASVs, OTUs, and quantitative-PCR data was conducted principally in R (38) using the packages Vegan (39), BiodiversityR (92), Phyloseq (41), ggplot2 (42), and Codaseq (43). We recorded a host of categorical and numerical variables associated with each sample to explore the integration of robust building science measurements with microbiological assays. Categorical variables included status of water-damage, season, borough, location indoors or outdoors, housing type, housing complex, and presence of visible mold. Numerical variable included environmental measures related to thermal comfort like temperature, relative humidity, CO^2 concentration, occupancy, and unit area.

For both bacteria and fungi, a Kruskal-Wallis test was used to test for significant differences in mean biomass across categorical variables. A Pearson's Correlation was used to test for correlations amongst biomass and numerical variables. Wilcoxon test was used to test for differences in biomass for indoor samples only. Alpha-diversity was measured as Observed Species Richness in filtered but not CLR-transformed data. Significant differences in mean

observed richness across groups was tested using ANOVA for categorical variables and Pearson's Correlation for numerical variables. Tukey's Honest Significant Difference (HSD) was used to independently test for pairwise differences in richness between the outdoors and water-damaged units, water-damaged units and units with no damage, and outdoors to units with no damage. Tukey's HSD was also used to test for pairwise differences in richness between housing types and the outdoors.

To evaluate differences in beta-diversity, a distance matrix of taxa shared between samples was generated using Aitchinson Distance (Euclidean distance of CLR-transformed data). PERMANOVA, executed as ADONIS in R, was then used to test for compositional differences in the distances between different groups (categorical data), and a Mantel test was used to correlate distances between numerical data with beta-diversity. Community composition was then visualized with a Principal Components Analysis plot using Phyloseq. A permutation test of multivariate homogeneity of group dispersions (Betadisper) and Tukey's HSD was then conducted using Vegan.

Taxonomic explorations of the data were initially conducted using Krona (93) to look at proportional abundance of taxa across groups. Then taxa were conglomerated to the levels of phylum or class and a Kruskal-Wallis test was used to look for significant differences in abundance across groups. To look at a finer scale, ANCOM (94) was used to test for differences in the abundance of genera across groups.

RESULTS

Microbial Communities Associated with Water-damaged Housing.

Microbial Biomass Responses to Water-damage. We found that mean bacterial biomass was not significantly different across airborne dust samples collected from the outdoors, units with no water-damage, or water-damaged units (Kruskal-Wallis; p=0.119), FIGURE 1a. We did detect significant differences in fungal biomass between samples from the outdoors, units with no water-damage, and units with water-damage (Kruskal-Wallis; p=0.006), FIGURE 1b. We could show that the major differences were between fungal biomass indoors and outdoors by restricting analysis of fungal biomass to indoor samples and finding that there was no significant difference between units with or without water-damage (Wilcoxon; p=0.67).

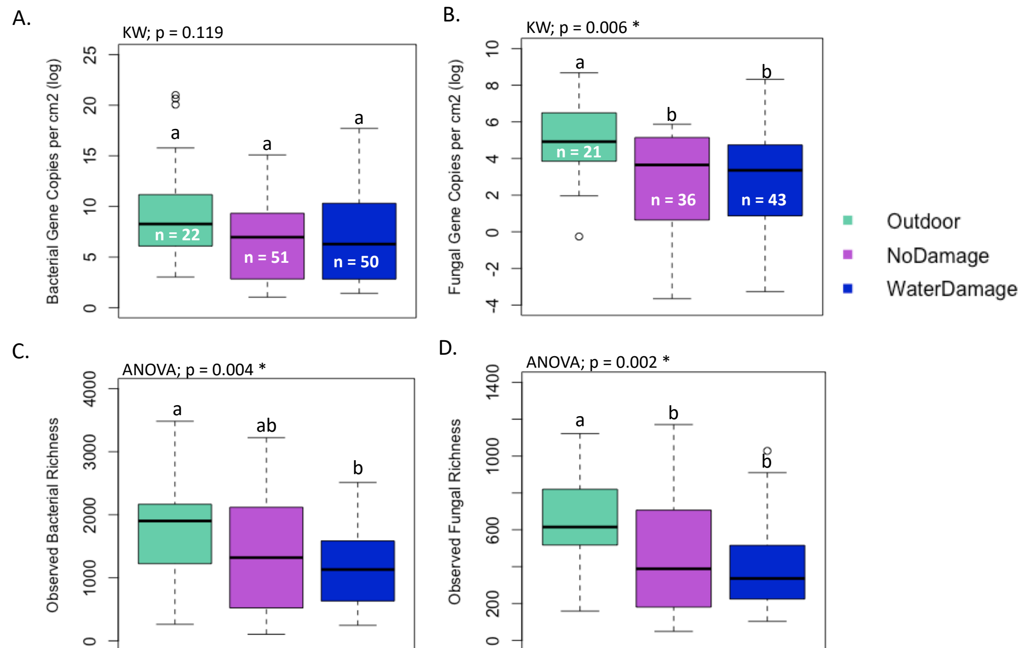


FIGURE 1. *Biomass and alpha-diversity associated with water-damage. Differences in bacterial (A) and fungal (B) biomass in outdoor dust samples, indoors samples from units with no water-damage, and water-damaged units, was tested using Kruskal-Wallis (KW). Bacterial (C) and fungal (D) alpha-diversity, measured as Observed Species Richness, was compared using ANOVA. Letters above boxplots denote group differentiation. Shared letters denote no significant difference between groups.*

Microbial Richness Responses to Water-damage. Alpha diversity, measured as Observed Species Richness, differed significantly between samples from the outdoors, units with no water-damage, and water-damaged units for both bacteria (ANOVA; p=0.004), FIGURE 1c., and fungi (ANOVA; p=0.002), FIGURE 1d. Observed richness was greatest outdoors, reduced in units with no water-damage, and lowest in water-damaged units. Pairwise comparisons of Observed Species Richness using Tukey's HSD showed that for bacteria, water-damaged units had significantly lower richness than the outdoors (p=0.003), but water-damaged units were not significantly different from undamaged units (p=0.31), and richness in units with no water-damage was not significantly different from the outdoors (p=0.07). For fungi, pairwise comparisons showed water-damaged units had significantly lower richness than the outdoors (p=0.001), as did non-damaged units (p=0.012), but richness in water-damaged units and non-damaged units was not significantly different (p=0.75).

Microbial Community Composition Responses to Water-damage. We detected distinct microbial communities in samples from the outdoors, units with no water-damage, and water-damaged units, FIGURE 2. Outdoor samples were compositionally different from indoor samples, accounting for the majority of the explained variability in PC1 (Bacteria: PERMANOVA; p=0.001, R^2=0.078), (Fungi: PERMANOVA; p=0.001, R^2=0.060). Nearly all samples from water-damaged units formed tight clusters far removed from outdoor samples. Some samples from units

with no visible mold resembled the outdoors and some samples from units with no water-damage overlaid samples from water-damaged units, reflecting similar bacterial and fungal composition in these samples and raising the possibility of imperfections in our assessment of water-damage. The overall trend shows that samples from units with no water-damage are similar to outdoor communities, and outdoor communities host significantly different microbial communities than water-damaged units. This pattern was most striking when the data were parsed by season. In the winter, outdoor samples became more dissimilar to samples from water-damaged units, and samples from water-damaged units clustered even tighter than was seen in summer.

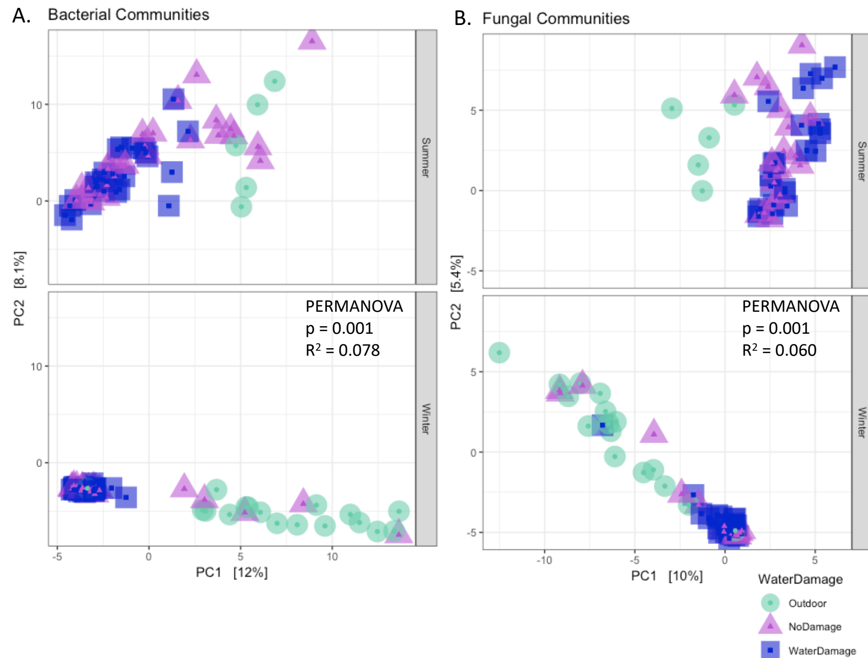


FIGURE 2. *Microbial Community Composition for bacteria (A) and fungi (B) associated with water-damage. Differences in beta-diversity were tested using PERMANOVA and visualized using a Principal Components Analysis (PCA). The samples are separated by season in the PCA.*

Beta-dispersion (distance to the centroid of microbial communities) also differed significantly between samples from outdoors, units with no water-damage, and water-damaged units, FIGURE 3. Sample to sample variation within groups was greatest for outdoor samples, reduced in indoor units with no water-damage, and the least in samples from water-damaged units for bacteria (Tukey's HSD; p=0.004) and fungi (Tukey's HSD; p=0.022). Pairwise comparisons of bacterial communities showed that samples from nondamaged units were not more dispersed than outdoor samples (T-test; p=0.129), unlike samples from water-damaged units that were significantly less dispersed than outdoor samples (T-test; p=0.001). Indoors, samples from water-damaged units were significantly less dispersed than non-damaged units (T-test; p= 0.039). For fungi, samples from water-damaged units were significantly less dispersed than outdoor samples (T-test; p= 0.002,) and indoor samples from units with no water-damage were significantly less dispersed than outdoor samples (T-test; p=0.019), but indoors, samples from water-damaged units were not significantly less dispersed than samples from units with no water-damage (T-test; p= 0.68). This

trend shows that for bacteria, non-damaged units were comparable to outdoor communities, but water-damaged units were very different from the outdoors. Also, indoors, units with water-damage and no damage had significantly different beta-dispersion. For fungi, indoor communities had reduced beta-dispersion compared to the outdoors, both for units with and without water-damage, but dispersion between indoor units was not significantly different, regardless of damage status.

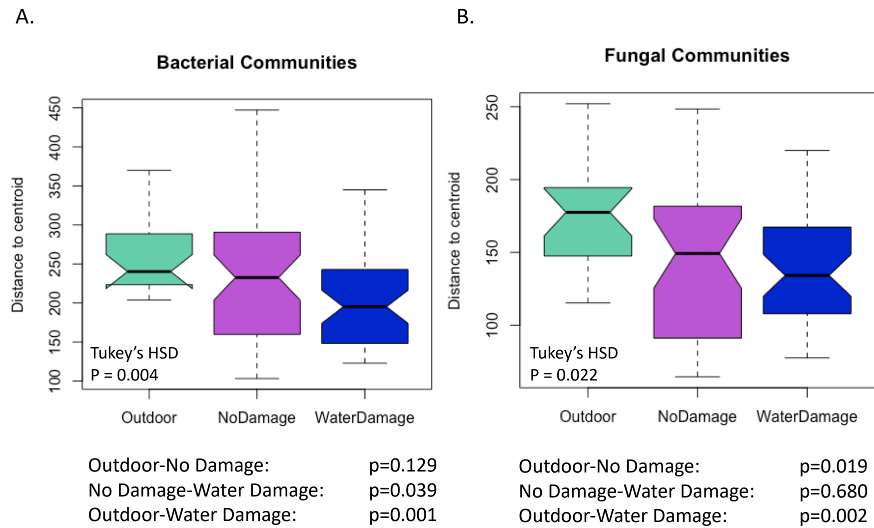


FIGURE 3. *Beta-dispersion (average distance to the centroid) of bacterial (A) and fungal (B) communities associated with water-damage was tested with Tukey's HSD. Pairwise comparisons of distance to the centroid with permutation T-test are listed below.*

Microbial Taxa Associated with Water-Damaged Units. The bacterial and fungal taxonomic diversity of airborne dust in water-damaged units is diverse, albeit less diverse than from units with no water-damage or the outdoors, FIGURE 4, SUPPLEMENTAL FIGURE 1. We discovered four microbial classes that were significantly more abundant in samples from water-damaged units, FIGURE 5. For bacteria, the classes Clostridia (Kruskal-Wallis; p=0.006) and Coriobacteriia (Kruskal-Wallis; p=0.02) were found to be significantly more abundant in samples from water-damaged units compared to units with no water-damage and the outdoors, FIGURE 5a. For fungi, we found significantly greater abundance of the classes Agaricomycetes (Kruskal-Wallis; p<0.0001) and Pezizomycetes (Kruskal-Wallis; p=0.002) in samples from units with water-damage compared to units with no water-damage and the outdoors, FIGURE 5b.

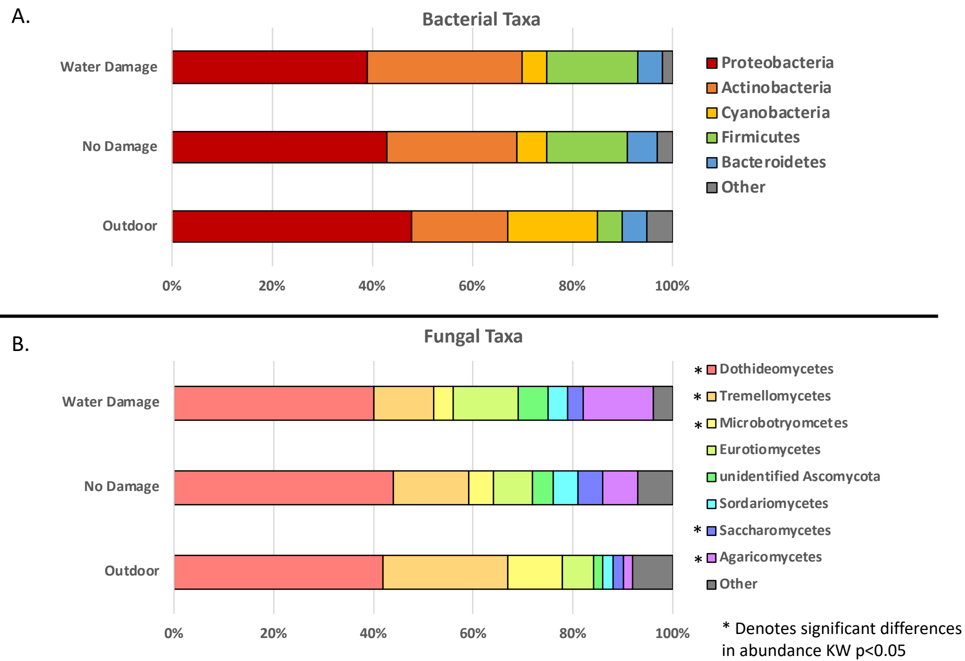
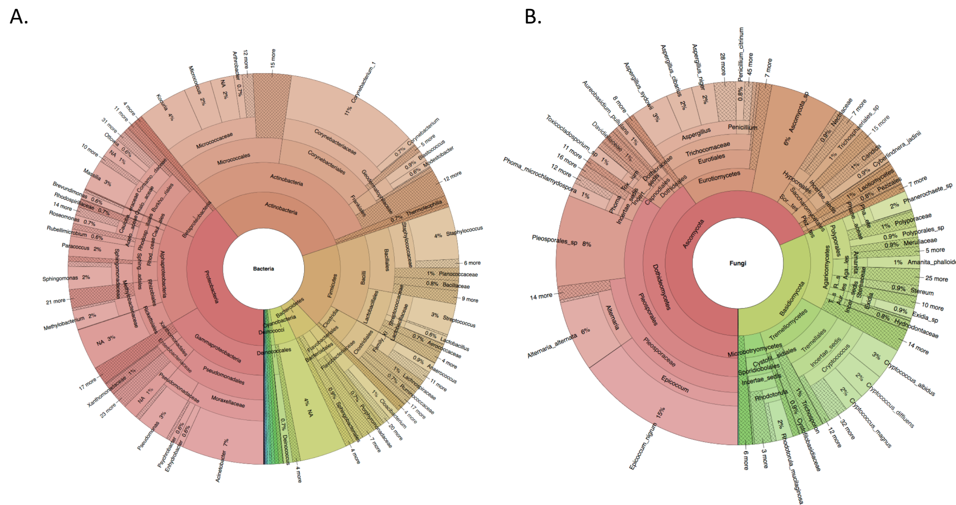


FIGURE 4. *Major taxa recovered with airborne dust for bacteria (A) and fungi (B) from outdoor samples, units with no water-damage, and water-damaged units. Bacteria phyla and fungal classes are listed. Mean abundance across groups was tested with Kruskal-Wallis. "*" denotes significant differences in mean abundance of taxa.*



SUPPLEMENTAL FIGURE 1. *The aeromicrobiota of water-damaged housing units in New York City. Proportional representation of bacterial (A) and fungal (B) taxa collected from airborne samples in water-damaged homes.*

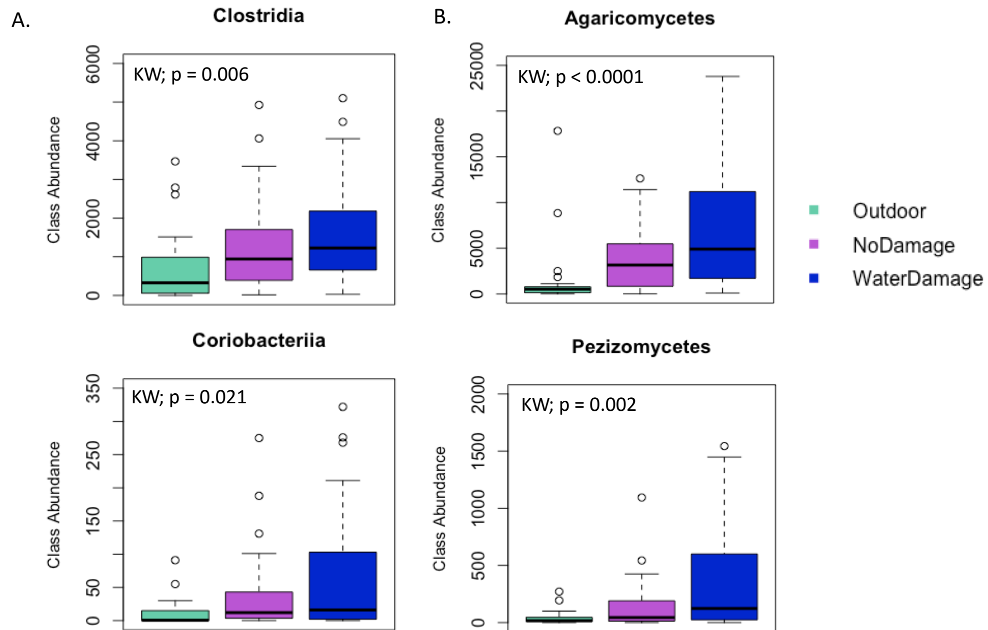
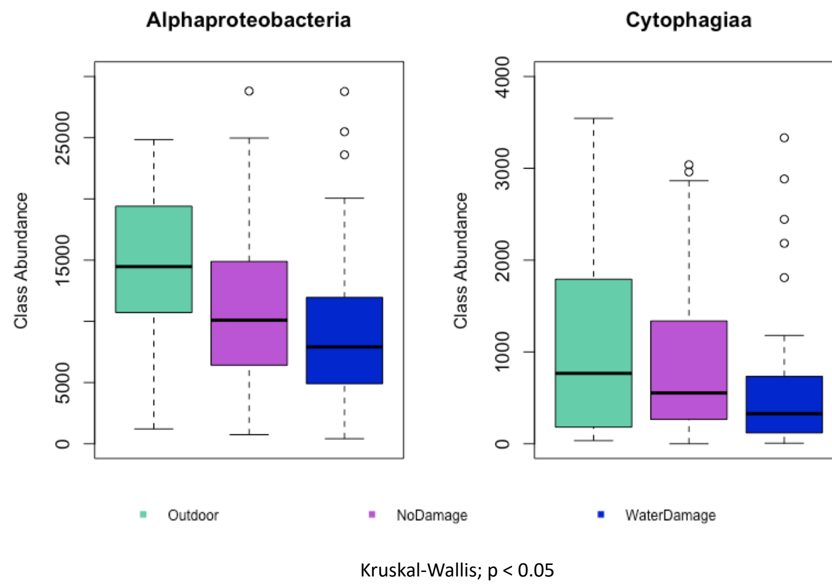
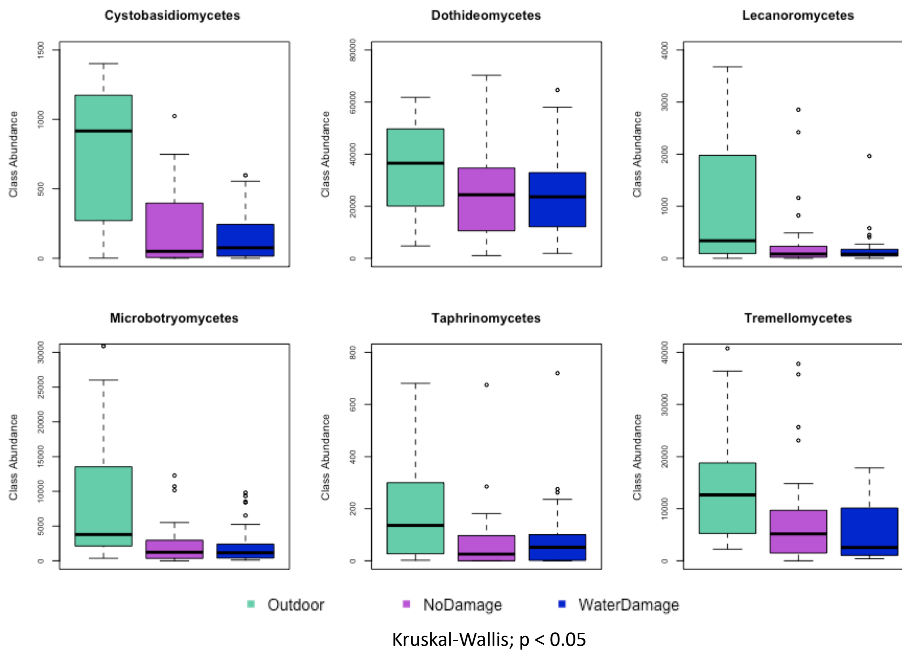


FIGURE 5. *Bacterial (A) and fungal (B) classes with greater taxonomic abundance in samples from units with water-damage compared to units with no damage and the outdoors. Abundance of microbial classes was tested using Kruskal-Wallis.*

Significant reductions in the abundance (Kruskal-Wallis; $p<0.05$) of the bacterial classes Alphaproteobacteria and Cytophagiaa were also observed in samples from water-damaged units in comparisons to the other two types of samples, outdoors and undamaged units indoors SUPPLEMENTAL FIGURE 2. Six fungal classes showed significantly less abundance in water-damaged units compared to the other two sample types, these included Cystobasidiomycetes, Dothideomycetes, Lecanoromycetes, Microbotryomycetes, Taphrinomycetes, and Tremellomycetes SUPPLEMENTAL FIGURE 3. Using ANCOM we found that two bacterial genera and eight fungal genera had significantly different abundances across the three environments (Kruskal-Wallis; $p<0.05$). These genera included the bacterial genera *Cryobacterium* and *Ottawia,* which were reduced in water-damage units compared to the other two sample types. For fungi, the genera *Curvularia, Eyrsiphe, Ganoderma,* and *Setosphaeria* were reduced in water-damaged units compared to the other two sample types, while the genera *Cystofilobasidium, Mrakia, Paecilomyces,* and *Pyronema* showed significant increases in abundance.



SUPPLEMENTAL FIGURE 2. *Bacterial taxa with significantly different abundance outdoors compared to indoors in units with no water-damage and units with water-damage. Mean abundance of classes was tested using Kruskal-Wallis.*



SUPPLEMENTAL FIGURE 3. *Fungal taxa with significantly different abundance outdoors compared to indoors in units with no water-damage and units with water-damage. Mean abundance of classes was tested using Kruskal-Wallis.*

Microbial Communities Associated with Public Housing.
Our sampling regime of airborne dust allowed for even sampling of water-damaged and non-damaged homes, and independently, private and public housing. Though the sample sizes are smaller for the evaluation of differences across housing types, a Chi-Square Contingency Test showed that there is no significant association between housing type and water-damage in our dataset (χ^2: p=0.40). Observed frequencies of water-damage within each housing type are as expected statistically, thus we were able to analyze housing type independently from water-damage.

Microbial Biomass and Richness in Public Housing. Bacterial biomass did not differ significantly between outdoor samples, samples taken inside private housing, or samples from inside public housing (Kruskal-Wallis; p=0.079), FIGURE 6a. Fungal biomass did differ significantly between outdoors, private housing, and public housing (Kruskal-Wallis; p=0.006), FIGURE 6b. When only indoor samples were analyzed, fungal biomass did not differ significantly between samples from public housing and private housing (Wilcoxon; p=0.695), indicating the major differences are between indoor and outdoor samples. Bacterial richness, FIGURE 6c, and fungal richness, FIGURE 6d, differed significantly between outdoor samples, samples from private housing, and samples from public housing. Outdoor samples were the most species rich, followed by private housing, and then public housing (ANOVA; Bacteria: p=0.004, Fungi: p<0.0001). Pairwise comparisons with Tukey's HSD showed that public housing units are significantly less rich than outdoor communities in both bacteria (p=0.004) and fungi (p=0.0004). Richness in private housing was not significantly different from the outdoors for bacteria (p=0.27) or fungi (p=0.23). Indoors, bacterial (p=0.36) and fungal richness (p=0.18) do not differ significantly between public and private housing units.

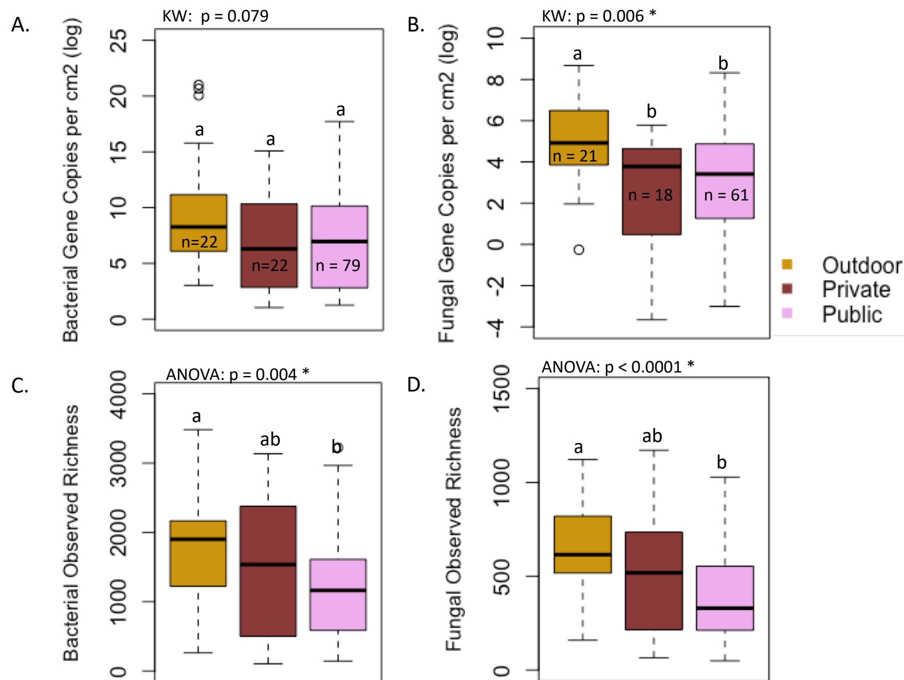


FIGURE 6. Biomass and alpha-diversity associated with public and private housing. Bacterial (A) and fungal (B) biomass between outdoor dust samples, indoor samples from private housing and public housing was tested using Kruskal-Wallis (KW). Bacterial (C) and fungal (D) alpha-diversity, measured as Observed Species Richness, of communities from outdoors, private housing, and public housing was compared using ANOVA. Letters above boxplots denote group differentiation. Shared letters denote no significant difference between groups.

Microbial Community Composition in Public Housing. The compositions of bacterial and fungal communities were distinct between the outdoors, samples from private housing, and samples from public housing. Significant differences in beta-diversity were detected for bacteria and fungi across the housing types (PERMANOVA; Bacteria: p=0.001, R^2=0.033, Fungi: p=0.001, R^2=0.062). When analyzing beta-dispersion, FIGURE 7, we found that the distance to the centroid was greatest in outdoor samples, followed by samples from private housing, and least in samples from public housing for bacteria (Tukey's HSD; p=0.002) and fungi (Tukey's HSD; p=0.005). For bacteria, pairwise comparisons of beta-dispersion showed that the distance to the centroid was not significantly different between outdoor samples and private housing (T-test; p =0.443), but the difference between the outdoors and public housing was significant (T-test; p=0.004), as was the difference between public and private housing (T-test; p=0.040). For fungi, the average distance to the centroid was not significantly different between outdoor samples and indoor samples from private housing (T-test; p=0.133), or between indoor samples from public and private housing (T-test; p=0.140). However, when comparing outdoor samples to indoor samples from public housing, the distance to the centroid was found to be significantly different (T-test; p=0.003).

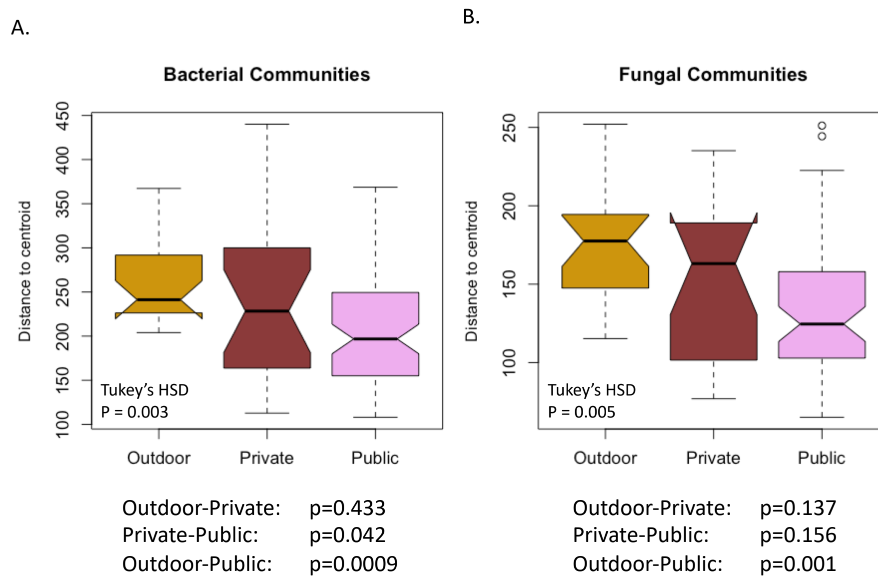


FIGURE 7. Beta-dispersion (average distance to the centroid) of bacterial (A) and fungal (B) communities associated with housing type was tested using Tukey's HSD. Pairwise comparisons of distance to the centroid with a T-test showed are shown below.

Microbial Taxa Associated with Public Housing. Public housing projects had significantly greater indoor abundance of four bacterial classes and two fungal classes compared to samples from indoor private housing and the outdoors, FIGURE 8. Bacteroidia (Kruskal-Wallis, p=0.014), Clostridia (Kruskal-Wallis, p=0.0003), Erysipelotrichia (Kruskal-Wallis, p=0.0005), Negativicutes (Kruskal-Wallis, p=0.02), Pezizomycetes (Kruskal-Wallis, p=0.002), and Saccharomycetes (Kruskal-Wallis, p=0.0002) were more abundant in public housing. Samples from indoor air in public housing also had a significant reduction in the abundance of Alphaproteobacteria (Kruskal-Wallis, p=0.008), and the fungal classes, Cystobasidiomycetes (Kruskal-Wallis, p=0.00002), Dothideomycetes (Kruskal-Wallis, p=0.02), Lecanoromycetes (Kruskal-Wallis, p=0.002), Microbotryomycetes (Kruskal-Wallis, p=0.0006), Taphrinomycetes (Kruskal-Wallis, p=0.007), and Tremellomycetes (Kruskal-Wallis, p=0.002) compared to private housing and the outdoors.

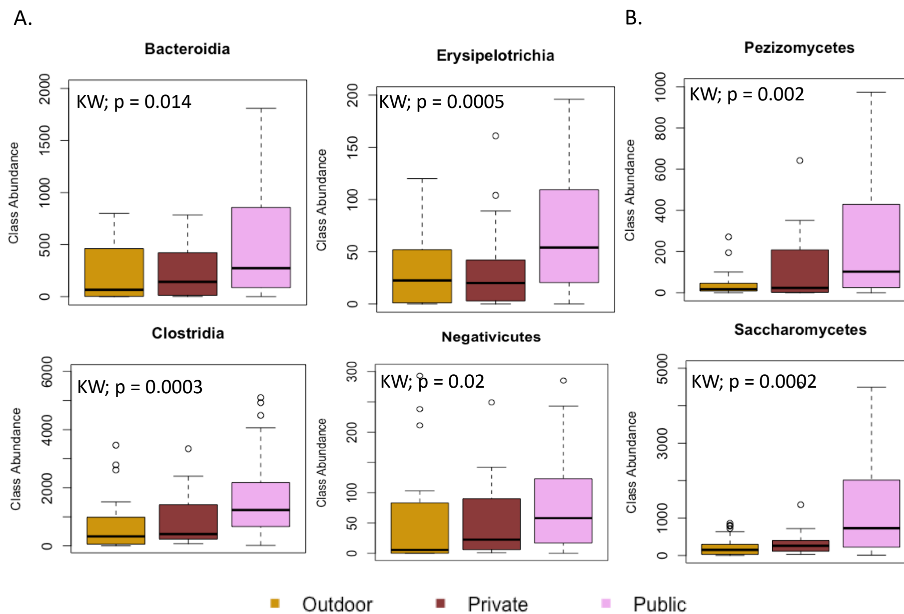


FIGURE 8. Bacterial (A) and fungal (B) taxa with significantly greater abundance in public housing compared to private housing and the outdoors. Mean abundance of fungal classes was tested with Kruskal-Wallis.

Additional Factors Influencing Microbial Communities.

As our dataset included records of many environmental variables and building measurements, we observed additional factors that influenced microbial communities. Principally, these included whether the sample was collected indoors or outdoors, in summer or winter, whether the unit had visible mold, and which housing complex the sample was from, TABLE 2. Overall, indoor samples had lower biomass (Kruskal-Wallis; Bacteria: p=0.049, Fungi: p=0.002), richness (ANOVA; Bacteria: p=0.003, Fungi: p=0.0001), and were distinct from the outdoors (PERMANOVA; Bacteria: p=0.001, R^2=0.067, Fungi: p=0.001, R^2=0.050). Summer samples had greater biomass (Kruskal-Wallis; Bacteria: p<0.00001, Fungi: p p<0.00001) and richness (ANOVA; Bacteria:

p<0.00001, Fungi: p<0.00001) than winter samples, and community composition varied by season (PERMANOVA; Bacteria: p=0.001, R^2=0.057, Fungi: p=0.001, R^2=0.066).

BACTERIA	Biomass	α-Diversity	β −Diversity		β −Dispersion
	p-value	p-value	p-value	r2	p-value
Housing Complex	/	/	0.001	0.090	0.006
Housing Type	/	0.004	0.001	0.033	0.002
Water Damage	/	0.004	0.001	0.078	0.004
Indoor/Outdoor	0.049	0.003	0.001	0.067	0.008
Season	2.E-16	5.E-07	0.001	0.057	0.001
Visible Mold	/	0.002	0.001	0.049	0.004

FUNGI	Biomass	α-Diversity	β −Diversity		β −Dispersion
	p-value	p-value	p-value	r2	p-value
Housing Complex	/	/	0.036	0.083	0.033
Housing Type	0.006	5.E-04	0.001	0.062	0.005
Water Damage	0.006	0.002	0.001	0.060	0.022
Indoor/Outdoor	0.002	5.E-04	0.001	0.050	0.004
Season	8.E-09	0.007	0.001	0.066	0.030
Visible Mold	0.002	2.E-04	0.003	0.035	0.003

TABLE 2. Additional factors influencing bacterial and fungal biomass, alpha-diversity, and beta-diversity in airborne microbial communities. "/" denotes analysis that was conducted but not statistically significant.

Units with visible mold had less bacterial richness (ANOVA; p=0.002), fungal richness (ANOVA; p=0.0002), and less fungal biomass (Kruskal-Wallis; p=0.002) than units with no visible mold and the outdoors. Community composition in units with visible mold was distinct from units with no visible mold and the outdoors (PERMANOVA; Bacteria: p=0.001, R^2=0.049, Fungi: p=0.003, R^2=0.035). And microbial communities from the same housing complexes were more compositionally similar to each other than samples from different complexes (PERMANOVA; Bacteria: p=0.001, R^2=0.090, Fungi: p=0.036, R^2=0.083). Beyond this, we observed the detection of a musty smell indoors, leaking pipes, the presence of mice, the development of health issues in the residents, smoking indoors, the size of the unit, temperature and relative humidity indoors also significantly influenced bacterial and fungal communities indoors, SUPPLEMENTAL TABLE 1 & SUPPLEMENTAL TABLE 2.

BACTERIA INDOORS	Biomass p-value	α-Diversity p-value	β −Diversity p-value	β −Diversity r2	β −Dispersion p-value
Cigarettes	0.573	0.728	0.124	/	/
Developed Health Issues	0.830	0.586	**0.001**	0.032	**0.001**
Musty Smell	0.900	0.888	**0.001**	0.024	**0.002**
Water Leakage	0.612	0.749	**0.005**	0.023	**0.002**
Mice	0.304	0.222	**0.009**	0.022	**0.045**
Unit	0.621	0.854	0.712	/	/
Building	0.685	0.483	0.143	/	/
Borough	0.789	0.663	0.108	/	/
Floor Level	0.697	0.903	0.758	/	/
Age	0.572	0.924	0.173	/	/
Roaches	0.765	0.440	0.185	/	/
Pets	0.650	0.870	0.334	/	/
Household Income	0.428	0.328	0.171	/	/
Adults	0.552	0.637	0.074	/	/
Kids	0.539	0.675	0.715	/	/
Total Occupants	0.732	0.946	0.082	/	/
Occupant Density	0.954	0.311	0.068	/	/
Total Area	**0.030**	0.451	0.598	/	/
Outdoor Temp	**0.023**	0.304	0.054	/	/
Winter Temp	0.134	**0.002**	0.356	/	/
Winter AER	0.400	0.653	0.685	/	/
Winter CO2	0.980	0.880	0.700	/	/
Winter RH	**0.035**	**4.E-04**	0.291	/	/
Summer RH	0.189	0.628	0.964	/	/
Summer Temp	0.051	**0.037**	0.137	/	/

SUPPLEMENTAL TABLE 1. Additional environmental variables inlfuencing bacterial community structure in airborne samples. Significant p-values are bolded. / denotes analysis that was conducted but not statistically significant.

FUNGI INDOORS	Biomass p-value	α-Diversity p-value	β −Diversity p-value	β −Diversity r2	β −Dispersion p-value
Cigarettes	0.536	**0.037**	**0.003**	0.024	0.052
Developed Health Issues	0.233	1.000	**0.001**	0.030	0.225
Musty Smell	0.754	0.522	**0.005**	0.026	0.238
Water Leakage	0.942	0.174	**0.001**	0.034	0.290
Mice	0.246	0.113	0.546	/	/
Unit	0.717	0.426	0.937	/	/
Building	0.853	0.494	0.816	/	/
Borough	0.796	0.705	0.400	/	/
Floor Level	0.946	0.490	0.733	/	/
Age	0.441	0.766	0.132	/	/
Roaches	0.645	0.740	0.370	/	/
Pets	0.803	0.493	0.474	/	/
Household Income	0.051	0.132	0.077	/	/
Adults	0.560	0.656	0.295	/	/
Kids	0.691	0.656	0.864	/	/
Total Occupants	0.509	0.701	0.274	/	/
Occupant Density	0.250	0.193	0.186	/	/
Total Area	0.735	0.692	0.448	/	/
Outdoor Temp	0.211	0.197	0.228	/	/
Winter Temp	0.099	0.248	0.306	/	/
Winter AER	0.217	0.261	0.564	/	/
Winter CO2	0.889	0.598	0.846	/	/
Winter RH	0.065	0.136	0.884	/	/
Summer Temp	0.365	**0.016**	0.181	/	/
Summer RH	0.539	0.161	0.980	/	/

SUPPLEMENTAL TABLE 2. Additional environmental variables influencing fungal community structure in airborne samples. Significant p-values are bolded. / denotes analysis that was conducted but not statistically significant.

DISCUSSION

The data reported here demonstrates that high-throughput sequencing can be used to detect broad compositional differences in homes that have experienced water-damage and have yet to be remediated owing to intent or neglect. In New York City this lack of remediation occurred after Hurricane Sandy when NYCHA failed to enact a robust plan to address water intrusion in public housing units. Today, bacterial and fungal communities in previously water-damaged units have a distinct signature of biomass, richness, community structure, and assortment of taxa. These disparate microbial signatures have resulted in microbiome dysbiosis in public housing units, which may contribute to the failing health of many low-income residents.

Fungal Biomass is Reduced in Units with Water-damage. Our comparison of microbial biomass in units with and without water-damage and the outdoors, found that fungi, but not bacteria, showed significant differences in biomass across these environments, due to fungal biomass being lower indoors compared to outdoors. Our result is in contrast to other studies that have reported increased fungal concentrations in water-damaged buildings and homes with mold (95). The difference may be due to the use, in these studies, of the Environmental Relative Moldiness Index, which infers biomass from qPCR analysis of 36 fungal taxa (46), rather than the entire microbial community. Perhaps fungi in the ERM panel show increased biomass, whereas other fungi do not. A broader concern about the use of bacterial or fungal biomass to identify water-damaged units is the inability to know if the rDNA amplified in microbiome studies is recovered from living or dead microorganisms (96).

Bacterial and Fungal Richness is Reduced in Units with Water-damage. We measured alpha-diversity for bacteria and fungi in outdoor airborne dust and indoor house dust sampled from units with water-damage and no water-damage. We found that there was less microbial richness in water-damaged units compared to the outdoors. Pairwise comparisons showed that in units with no water-damage, bacterial richness is comparable indoors and outdoors, but in units with water-damage there is significantly lower bacteria diversity than outdoors. Fungal richness was lower indoors compared to the outdoors, for both water-damaged and non-damaged units. In the literature there does not appear to be a clear understanding of how alpha-diversity shifts as moisture is increased indoors. Our results are corroborated by Green et al., who used Sanger sequencing to assess fungal diversity in a water-damaged office building and found lower fungal richness on bottom floors than top floors, presumably owing to the greater water-damage lower in the building (48). In contrast, using clone libraries and mold-specific qPCR to evaluate the impact of remediation on fungi in moisture damaged buildings, Pitkäranta et al. found elevated fungal diversity associated with water-damage before remediation (12). Similarly, Dannemiller found that higher moisture was associated with increased fungal diversity in house dust until visible mold was present, at which point diversity was reduced (50). An explanation for these results comes from the observation that overabundance of a few taxa can reduce the ability of HTS to detect rare taxa, leading to a decrease in total richness, as was shown for classrooms exposed to abundant fungal spores, which had lower richness than classrooms lacking this exposure (52).

The evidence of reduced microbial richness in water-damaged units compared to non-damaged units and the outdoors could be indicative of microbiological dysbiosis, which could become a public health threat. In a birth cohort study of low-income Latinx families, lower fungal diversity, particularly of *Cryptococcus*, was significantly associated with increased risk of asthma development (50). Furthermore, it has been demonstrated that children who grow up on farms are exposed to greater microbial diversity, and this is protective against the development of asthma and atopy (97). Thus, low microbial diversity in water-damaged housing may expose New York City residents to a greater risk of asthma development.

Different Bacterial and Fungal Communities Accumulate in Water-damaged Units. We measured beta-diversity for bacterial and fungal communities sampled from outdoor air and compared these to indoor communities sampled from units with and without water-damage. Microbial communities in the three environments were distinct, and indoor samples from units with no water-damage were more compositionally similar to the outdoors than units with recorded water-damage. This result provides a promising avenue for evaluating levels of mold contamination indoors. By comparing study homes with presumed damage to the outdoors, or a dust profile from a "microbially healthy home," we may be able to establish a metric of disrepair using HTS. As the outdoor air is more mixed and has greater taxonomic diversity and abundance than the indoor environment, we can observe how far microbial communities have diverged from the outdoor source as a response to environmental conditions like water intrusion and building negligence.

Looking within the microbial communities, we can correlate the presence of particular taxa with water-damage. For example, water-damaged units had greater abundance of two bacterial classes, Clostridia and Coriobacteriia, and two fungal classes, Agaricomycetes, and Pezizomycetes, when compared to units with no damage or the outdoors. Clostridia are anaerobic spore-forming bacilli within the phylum Firmicutes that are ubiquitous in soil and also typical constituents of the skin or intestinal microflora of humans and animals. Although most Clostridia are benign, a number are opportunistic human pathogens, causing a variety of diseases including gas gangrene, tetanus, botulism, pseudomembranous colitis, and food poisoning (98). Coriobacteriia are gram-positive anaerobic bacteria within the phylum Actinobacteria, and one of the most prevalent bacterial classes found in the distal gut of human adults. Its biology has largely been elucidated by next-generation sequencing technologies (99) and implicated in a range of human diseases.

Agaricomycetes include many charismatic mushroom-forming fungi within the phylum Basidiomycota, whose roles in nature vary from edible to medicinal to toxic species, as well as symbionts, decomposers, and pathogens (100). Pezizomycetes are apothecial (cup) fungi within the Ascomycota. They are saprobic, mycorrhizal, or parasitic on plants, and include choice edibles like truffles and morels (101). These fungi have previously been reported as abundant on wet and dry surfaces in residences (60), and in association water-damaged building materials (12), (48). In our previous study using HTS to profile a condemned public housing project in the San Francisco Bay Area, we found that units with visible mold had significantly greater abundance of Eurotiomycetes, Saccharomycetes, and Wallemiomycetes, characteristic molds and yeasts. In both studies, water-damaged units and units with visible mold were characterized by a significant

reduction in the abundance of many outdoor taxa, which presumably contributed to their signal of lower alpha-diversity.

Similar to our results, Emerson et al. conducted HTS and found distinct bacterial and fungal communities in flooded and non-flooded homes in Boulder, CO, 2-3 months after a historic flooding event using PERMANOVA. Flooded homes had significantly greater abundance of Moraxellaceae, Pseudomonadaceae, and Enterobacteriaceae bacteria, and Eurotiales fungi (26). Jayaprakash et al. likewise surveyed severely moisture damaged homes before and after renovation using HTS and found subtle effects of renovation on bacterial and fungal community structure using ANOSIM. In their study, renovated homes had reduced abundance of Actinomycetales bacteria and a number of taxa within the Agaricomycetes, Dothideomycetes, Microbotryomycetes, Sordariomycetes, Taprinomycetes, and Tremellomycetes (25).

Microbial Dysbiosis is Evident in Public Housing. We detected different microbial communities in public and private housing in New York City. Compared to the outdoors, public housing had lower bacterial and fungal richness and fungal biomass. Microbial communities in public housing were also compositionally distinct from communities in private housing and the outdoors. Pairwise comparisons of bacterial and fungal beta-dispersion showed that private housing was not significantly different from the outdoors, but public housing was significantly dissimilar to the outdoors. Public housing projects hosted significantly greater abundance of four bacteria classes, Bacterodia, Clostridia, Erysipelotrichia, Negativicutes, and two fungal classes, Pezizomycetes, and Saccharomycetes. All of these bacteria are known members of the gut microbiome, capable of causing disease associated with gut dysbiosis, and Saccharomycetes comprises a number of opportunistic pathogens including *Candida* and *Cryptoccocus*.

The distinct microbial communities accumulating in NYCHA building leads to differential microbial exposure in public housing and private housing in New York City. These disordered microbial communities may contribute to the disproportionately high rates of asthma documented in public housing residents. Northridge et al. conducted a parent-questionnaire of 4,853 children attending 26 NYC elementary schools and found that even when adjusting for individual risk factors like ethnicity/race and socioeconomic status, children living in public housing had significantly higher odds of asthma than children living in private housing; 22% of children living in public housing had been diagnosed with asthma or chest wheeze (102). Previously, Corburn et al. mapped neighborhood environment factors in New York City and found hotspots of asthma hospitalization were located in neighborhoods with the highest public housing units (103). Furthermore, nearly half of all asthma hospitalizations reported between 1997-2000 were localized in four asthma hotspots, although these neighborhoods only accounted for 14% of the city population. Our study of airborne microbial communities was conducted in two of these asthma hotspots in Harlem and Brooklyn.

Our study adds to a growing number of investigations of disparate environmental factors within public housing that could negatively impact the health of residents (104), (81). The microbial communities inside NYCHA buildings are demonstrably different from the microbes found in neighboring privately-owned buildings and the outdoors. This is indicative of an indoor environment that either selects for a different suite of taxa or building design/maintenance with poor ventilation not capable of adequately mixing indoor and outdoor air. Deterministic processes

of community composition by environmental filtering, rather than stochasticity, may be enlisted to explain the differences in microbial communities found in public housing units. Though extensively documented in macroorganisms, the role of deterministic processes in shaping communities for microorganisms has received less attention. Cao et al. conducted amplicon sequencing of bacteria and archaea in grassland ecosystems and noted that deterministic environmental filtering processes were a greater contributor to soil community beta-diversity patterns than dispersal limitation (105). Kilvin et al. likewise found that environmental filtering by abiotic factors affected fungal regional community assembly in soils more than dispersal limitation (106).

The Importance of Sampling Indoor and Outdoor Microbial Communities Over Extended Periods. To our knowledge, this study is the first to compare microbial communities in multiple water-damaged buildings to outdoor microbial communities using HTS with high replication. The comparison proved invaluable to determining the differentiation of microbial communities in units with water-damage and no damage, and the distinction between microbial communities in public and private housing. Pairwise comparisons to the outdoors consistently showed that microbial communities in water-damaged units had less biomass, less diversity, and different taxonomic composition. Pairwise comparisons to the outdoors showed that public housing units had less biomass, were less diverse, and had less dispersion than the outdoors, which was not the case for private housing units. This suggests that differences between indoor microbial communities can be subtle, and possibly undetectable, without comparison to source populations like the outdoor community. Future microbiome studies should be sure to simultaneously sample outdoor air as a 'control' sample while exploring shifts in microbial communities that may be attributable to various environmental factors.

Another way to establish controls beyond simultaneously sampling the indoor and outdoor environment is with longitudinal studies. In addition to sampling outdoors, we also sampled airborne dust over the course one month in two different seasons in New York. We saw that season was a major determinant of community structure for bacteria and fungi. Summer samples had greater biomass and richness compared to winter. Seasonal variation has been demonstrated in indoor microbiome studies previously (107), (21). A study of the many environmental factors that shape indoor microbial communities reported that time of the year was the most decisive parameter structuring bacterial and fungal communities (108). Furthermore, we collected airborne dust by sampling passive dust for multiple weeks at a time. Most studies of the indoor microbiome sample for a period 1-10 minutes by vacuuming or swabs, which does not account for changes in microbial communities due to season, climate, or diurnal fluctuations in occupant behavior. By collecting settled dust over the course of one month, we were able to ascertain that the taxa recovered in this study have the potential to be inhaled by residents, which is a major concern for the allergen- or mycotoxin-producing taxa detected here.

Long-term Microbial Impacts of Hurricane Sandy. The experience of Hurricane Sandy might have long-term impacts on the microbial communities inside water-damaged units that have yet to receive sufficient remediation from NYCHA. A metagenomic profile of New York City subway stations showed that the microbiological impacts of Hurricane Sandy were long-standing (109). One train station was fully submerged during the storm and remained closed for years. When

surveyed, 10 unique bacterial taxa were detected only in the flooded station; these taxa appeared to be associated with cold marine environments and fish.

Microbiological studies in the City of New Orleans after Hurricane Katrina affirm that natural disaster and collapsed infrastructure can have withstanding effects on the microbial ecology of a city long after the storm. Rao et al. sampled airborne microorganisms from the outdoors, and homes with mild and moderate/heavy water-damage following Hurricane Katrina and Rita in New Orleans, LA, and found high concentrations of molds, glucans, and endotoxin indoors, two months after the waters receded (11). Later, Bloom et al. detected verrucarol and sterigmatocystin, mycotoxins produced by *Stachybotrys* and *Aspergillus* respectively, in dust samples from mold-contaminated homes five months post-Katrina (65).

CONCLUSIONS

We used high-throughput sequencing technologies to examine microbial communities in airborne dust associated with water-damaged and undamaged units of New York City Housing Authority buildings and neighboring privately-owned homes over two seasons post-Hurricane Sandy. Our study is the first to use HTS to study the assembly of microorganisms in water-damaged residences with high replication within and across buildings, including low-income and market-rate housing, over extended sampling periods, and to correlate the microbiology with extensive building science measurements. By comparing the biomass, alpha-diversity, beta-diversity, and abundance of bacteria and fungi in homes to the outdoor community, we were able to detect distinct microbial communities associated with water intrusion and housing type. Increased water availability and moisture levels resultant from unaddressed hurricane damage appears to have curated a distinct microbial assemblage within water-damaged units in NYCHA. As the hurricane occurred in 2012, this is now indicative of a chronic disturbance indoors that has structured a narrow assortment of microbes, composed of opportunistic pathogens, allergen-producing taxa, and a community of low diversity that may trigger asthma and respiratory disease. This shows that the budgetary priorities and housing policies enacted by the US Department of Housing and Urban Development (which subsidizes low-income housing) and NYCHA not only impact the lives of residents living within neglected public housing projects, but also the microorganisms within the buildings.

A Comparison of Sampling Methods Used in High-throughput Sequencing Analysis for Microbiome Studies of the Built Environment

Iman A Sylvain, John W Taylor, Michal P Spilak, Michael S Waring, Rachel I Adams

ABSTRACT

Microbiome studies have used high-throughput sequencing technologies to elucidate the factors that pattern indoor microbial communities. We now know that the built environment is vastly more diverse and complex than previously thought. The methods used to sample indoor environments range from the collection of settled dust, to active air sampling, to the swabbing of surfaces. Depending on the method used in analysis, the concentration and composition of bacteria and fungi detected in samples may differ. As people spend the majority of their lives indoors, it is important that we glean accurate profiles of the microorganisms cohabiting our spaces. Here we compared five different sampling methods used to assess indoor microbial communities in 70 homes in New York City. In each home we deployed a passive airborne dust collector, paired with a charged airborne dust collector, we vacuumed the floor in a high traffic area, swabbed surfaces with visible mold or obvious discoloration, and swabbed door trims. We found that the sampling methods recover very different communities in terms of microbial biomass, richness, community composition, and taxon abundance. As each sampling method has its own set of challenges and benefits, serious consideration must be given when establishing a protocol for the assessment of indoor microbes. For a high-efficiency method that is capable of capturing diurnal and seasonal changes in the indoor environment, we recommend the use of passive airborne dust collectors.

INTRODUCTION

Technological advancements in the characterization of microbial communities, both bacterial microbiomes and fungal mycobiomes, has been facilitated by high throughput sequencing (HTS), and matured to the point that best practices are now discussed for the aspects that are common to all such studies, e.g., taxon identification and analysis of community composition (110), (96). Where the studies differ is in sampling because protocols must be tailored to the environment in question. Here, we compare five methods of sampling airborne and settled bacteria and fungi in the built environment and discuss the relative merits and demerits of each approach.

Recognizing that Americans spend nearly 90% of their time indoors, the built environment is increasingly being viewed as a critical site for public health (1). In efforts to abate unhealthy conditions in homes, offices, schools, and hospitals, many studies have attempted to assess microbial concentration and composition indoors. These attempts have led to the development of varied sampling methods and analytical tools. Until the 2000s, detection of fungi indoors primarily relied on microscopic examination and identification of fungi directly, or after cultivation from material collected by adhesive tape, swabs, and vacuuming of floor dust or microbes in air (55). Samples were then analyzed for allergens, mycotoxins, cell wall components, or taxonomic identification and semi-quantitative measures from spores (111). Of these methods, the only one that sampled fungi over a defined period and that allowed for comparison of fungi indoors and outdoors was vacuum-assisted filtration of air, but the period sampled was typically shorter than

30 minutes. Knowing that there is a diurnal pattern of fungal spore release, and that the built environment is used differently on work days and the weekend, this period was too short to adequately sample airborne microbes.

The traditional, microscopic approach to fungal identification has been replaced by a molecular approach following the application of polymerase chain reaction (PCR) and direct sequencing of the internal transcriber spacer (ITS) to phylogenetically identify fungi (28). Among the most thoughtful applications of PCR to indoor air are those of the Environmental Protection Agency. Water intrusion alters the built environment by enabling the growth of fungi on many substrates, including paint, caulk, wood, and the paper covering wall board (112). Using quantitative-PCR, Vesper et al. were able to generate a list of 26 fungal taxa that are indicators of fungal growth on water-damaged building material, and an additional 10 taxa that are common indoor inhabitants (95). Results of these analyses led to the development of the Environmental Relative Moldiness Index (ERMI) which was then used to calculate potential health risks for building occupants (46) and linked epidemiological studies with microbial exposure in water-damaged buildings (64), (113).

The introduction of HST to research on the built environment has allowed researchers to study thousands of microbes in samples rather than the fewer than 50 recognized by ERMI. It is now established that traditional culture and microscopy-based approaches vastly underestimate the types and quantity of microbes in environmental samples. There has been a dramatic improvement in our understanding of the factors that pattern bacterial (61) and fungal communities within the built environment (55). The abundance, composition, and diversity of microbial communities indoors are structured by interactions between outdoor air, occupants, ventilation strategies, moisture levels, and building materials (15). While a number of studies have compared the utility of surveying indoor microbiota using molecular methods and culture-based techniques (47), (114), (12), generally reporting the improved ability of molecular tools to recover a greater number of taxa, few studies have compared sampling methods used in high-throughput sequencing studies (115), (26), (116).

Here we compared five sampling methods (FIGURE 1) with a total of 751 samples to survey the aeromicrobiota and surfaces of 70 homes with and without water-damage in New York City using quantitative-PCR and amplicon sequencing of ITS and 16S. Three of the methods sample microbes that have settled from air or grown over a period defined by the last round of cleaning or maintenance, a date that typically is unknown. These included vacuum sampling of microbes settled on floors (vacuumed floor dust), swab sampling of microbes settled on the top of door trim (swabbed door trim), and swab sampling of microbes settled on or growing on walls with or without visible mold (swabbed surfaces). Two of the methods sample microbes that have settled from the air onto a sterile surface over a period defined by the researcher and differ from one another by the absence (passively settled dust) or placement of a negatively charged Teftex wipe (electrostatically settled dust) on the sterile surface. We found that these methods recovered vastly different bacterial and fungal communities with significant differences in biomass, diversity, composition, beta-dispersion, and taxon abundance.

	Floor Dust	Passive Airborne	Door trim	Surface Sample	Charged Airborne
Field Materials	Vacuum	Dust Collector	Swab	Swab	Electrostatic Dust Collector
Surface Area	1-m2	56.5-cm2	not standardized	not standardized	39.1-cm2
Accumulation Time	Unknown	1 month	Unknown	Unknown	1 month
Extraction Difficulty	Medium	Medium	Low	Low	High

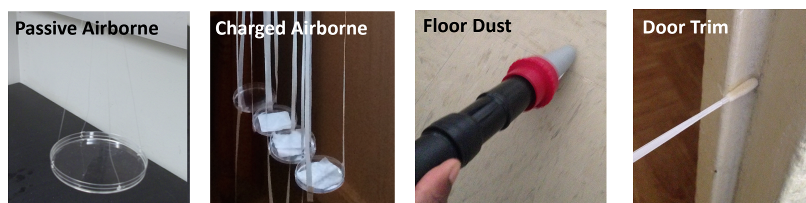


FIGURE 1. Comparison of sampling methods used to collect house dust. Sample types vary in the materials that are required in the field to make the collection, the amount of time the samplers are able to accumulate dust, their surface area, and the ease of utility in downstream processing of gDNA.

MATERIALS AND METHODS

Sampling Collection. Specimens using each of the five sampling methods were recovered as follows. Vacuumed floor dust samples were obtained using a Dustream Collector DU_ST-1 (Indoor Biotechnologie) and a portable vacuum, covering 1 square meter of flooring in a heavily trafficked area. Swabbed door trim samples were obtained using a dry Floq (Copan Diagnostics) to collect dust from the upper surface of the trim framing the interior of the front door. Swabbed surface samples were obtained using a Floq swab on walls showing discoloration or apparent fungal colonies. Passively settled dust was allowed to accumulate over one month in suspended Petri dish bottoms having a sterile surface area of 56.5cm^2. Electrostatically settled dust was allowed to accumulate over one month on a sterile Teftex pad with a surface area of 39.1 cm^2 that had been placed in the bottom plate of a sterile, suspended Petri dish.

DNA Library Preparation and Sequence Processing. Transfer of microbes to buffer for DNA extraction (21) depended on the sample type. For vacuumed floor dust, 200mg were weighted and transferred into a 1.5ml Eppendorf tube with 800 μl Miller SDS-Miller Phosphate Buffer. Swabs from door trim or walls were designed to easily break from the stick and become enclosed in 2ml tube which was then filled with Miller buffer when ready to be processed. Passively settled dust was swabbed from the Petri dish and the swab treated as above. Electrostatically settled dust required first sonicating the Teftex wipe for 30minutes in 10ml of PBS buffer, followed by filtering and concentrating the sample to 400μl. Downstream genomic DNA extraction and sequence

processing methods proceeded exactly as previously described (88). With the inclusion of field and laboratory controls, we generated a total of 751 samples. After processing, filtering, and center-log-transformation (43), we arrived at a final dataset of 420 bacteria samples comprising 16,382 Amplicon Sequence Variants (ASVs), and 425 fungal samples comprising 11,293 Operational Taxonomic Units (OTUs).

Statistical Analysis. Analysis was principally conducted in R (38) using the vegan (39) and phyloseq (41) packages, as previously described (117). Briefly, ANOVA was used to test for differences in Observed Species Richness across all sample types. Tukey's HSD was used to test for pairwise differences in richness across sampling methods. A Kruskal-Wallis test was used to test for significant differences in mean bacterial and fungal biomass in sample types with standardized surface area, which included Vacuumed floor dust, Passively settled dust, and Electrostatically settled dust samples. PERMANOVA and Principal Components Analysis were used to test and visualize differences in Aitchinson distance between microbial communities detected by different sampling methods. A permutation test of multivariate homogeneity of group dispersions (Beta-dispersion) was used to conduct pairwise comparisons between sample group distances. Kruskal-Wallis Tests were used to detect significant differences in taxon mean abundance.

RESULTS

Alpha Diversity. Mean Observed Species Richness varied markedly across sampling methods, ranging from the average detection of merely 67 OTUs for fungi in airborne collectors augmented with electrostatically charged, Teftex wipes (hereafter, charged airborne collectors), to over ten times greater richness in floor dust samples FIGURE 2. For both fungi and bacteria, there was a significant difference in the mean observed richness detected across sampling methods (ANOVA; $p<0.00001$). Pairwise comparisons using Tukey's HSD showed that bacterial richness varied significantly ($p<0.05$) among all methods with the exception of the two swab samples (Door trim vs Surface Samples, $p=0.15$). Likewise, for fungi, richness varied significantly ($p<0.05$) for all methods except the two swab methods (Door trim vs Surface Samples, $p=0.07$) and the comparisons between Surface Samples and Charged airborne samplers ($p=0.07$). For both bacteria and fungi, floor dust detected the greatest richness, followed by the passive airborne collectors, then swabs. Charged airborne collectors detected the least number of fungal taxa. The challenges of recovering microbial DNA from Teftex wipes caused us to limit our processing to winter samples and our analysis to fungi. When only winter samples were analyzed, Charged airborne samples still showed the least richness.

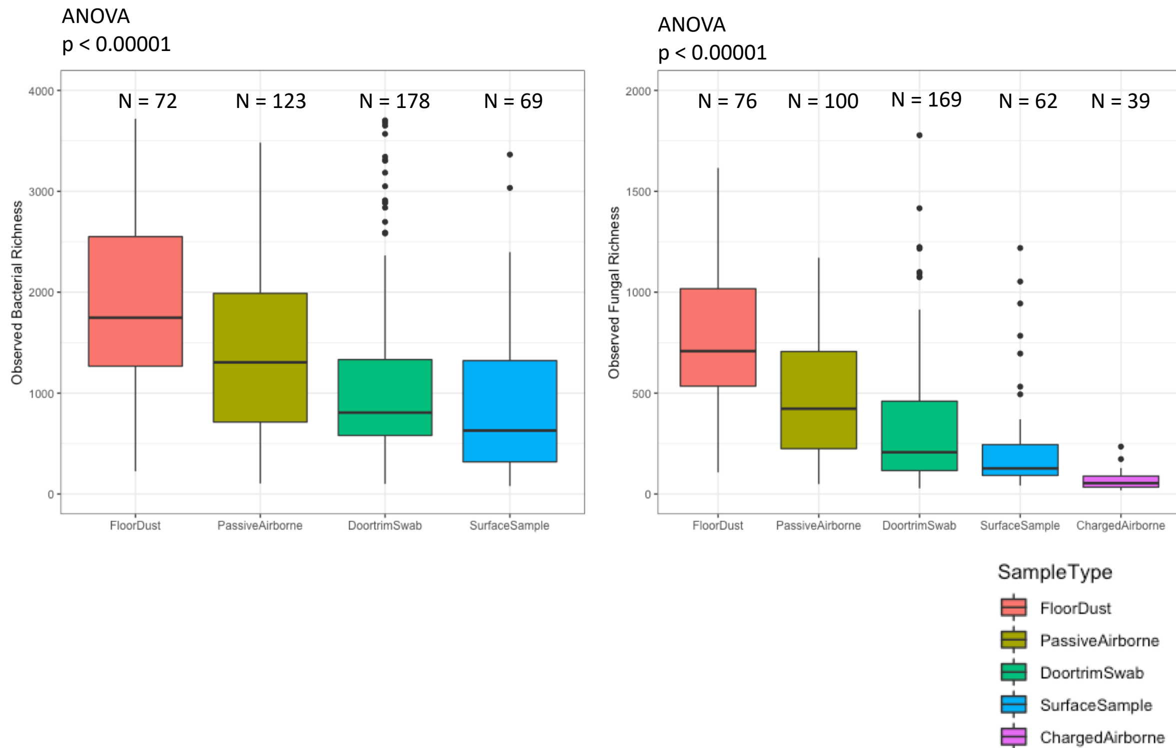


FIGURE 2. Alpha diversity, measured as Observed Species Richness, varied significantly across sampling type and was tested using ANOVA.

Biomass. For samples with standardized surface area, we conducted quantitative-PCR to determine the biomass accumulated with each sampling method. This analysis included Floor Dust samples and Passive Airborne samples for bacteria and fungi, and additionally Charged Airborne samples for fungi, FIGURE 3. Floor Dust accumulated significantly greater bacterial and fungal biomass than Passive Dust Collectors, and both Floor Dust and Passive Airborne samplers accumulated significantly greater fungal biomass than Charged Airborne collectors (Kruskal-Wallis; $p < 0.0001$).

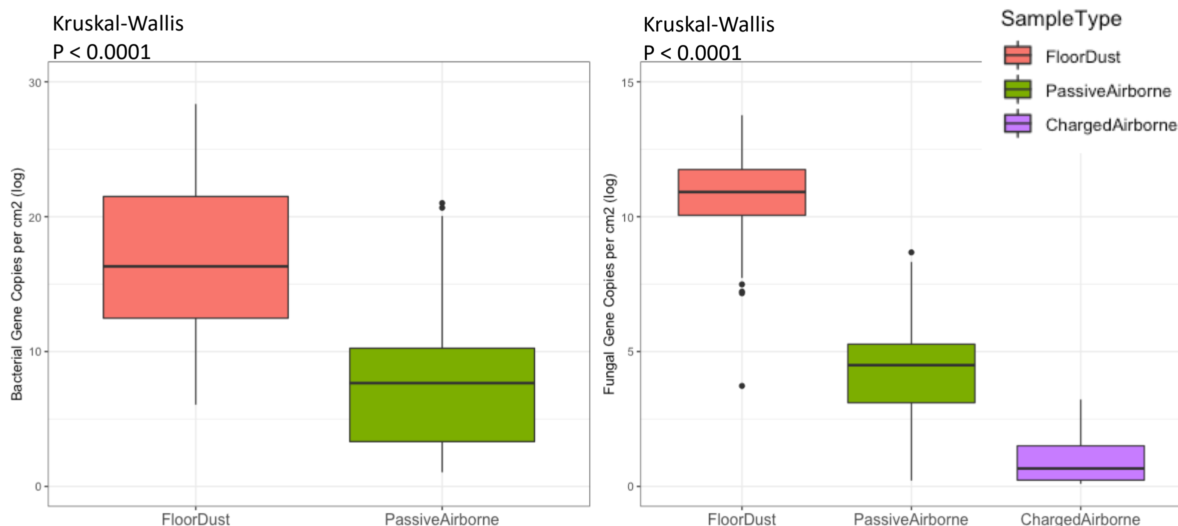


FIGURE 3. Microbial biomass, measured as gene copies per cm^2, varied significantly (Krustal-Wallis test) across sampling methods with standardized surface area. Visualized by log-scale.

Community Composition. Sampling method had a strong effect on determination of microbial community composition. As seen in FIGURE 4, Principal Components Analysis showed clustering of communities by sample type (PERMANOVA; Bacteria: p=0.001, R^2=0.04, Fungi: p=0.001, R^2=0.055). We considered it a surprise that the effect of sampling type could be detected by principal components analysis despite the large variation in samples taken from housing in New York City that included differences in location, season, ownership (public or private), and degree of water-damage. The first two principal components explained 10.5% of the variance for bacteria and 11.9% for fungi. The two sample types that captured the greatest difference among samples were passive airborne collectors and vacuumed floor dust. Passive Dust samples were also the only sampling method deployed outdoors. When outdoor samples were included in the analysis, SUPPLEMENTAL FIGURE 1, the trend remained robust and microbial composition differed significantly (p<0.05) across sampling methods. Sampling types also have significantly different mean distances to the centroid (beta-dispersion) in communities (Bacteria: p=0.001; Fungi: p=0.001), FIGURE 5. Sample-to-sample variation within groups was greatest for Floor Dust samples, then Passive Airborne, Door trim, and Surface Samples for bacteria and fungi. The least dispersion was observed in Charged Airborne samples for fungal communities.

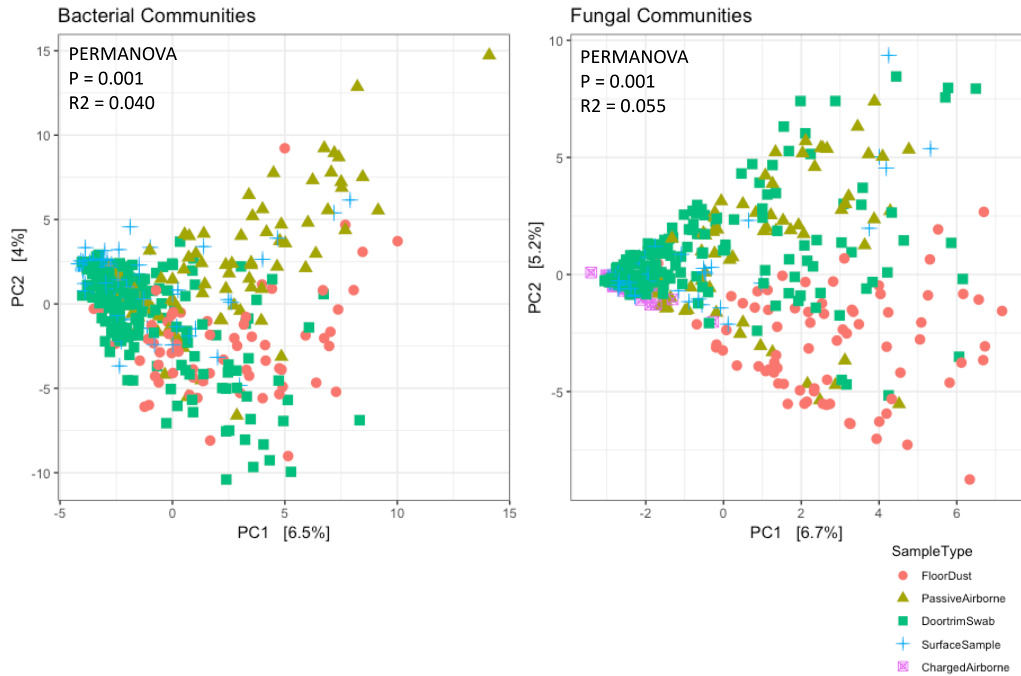
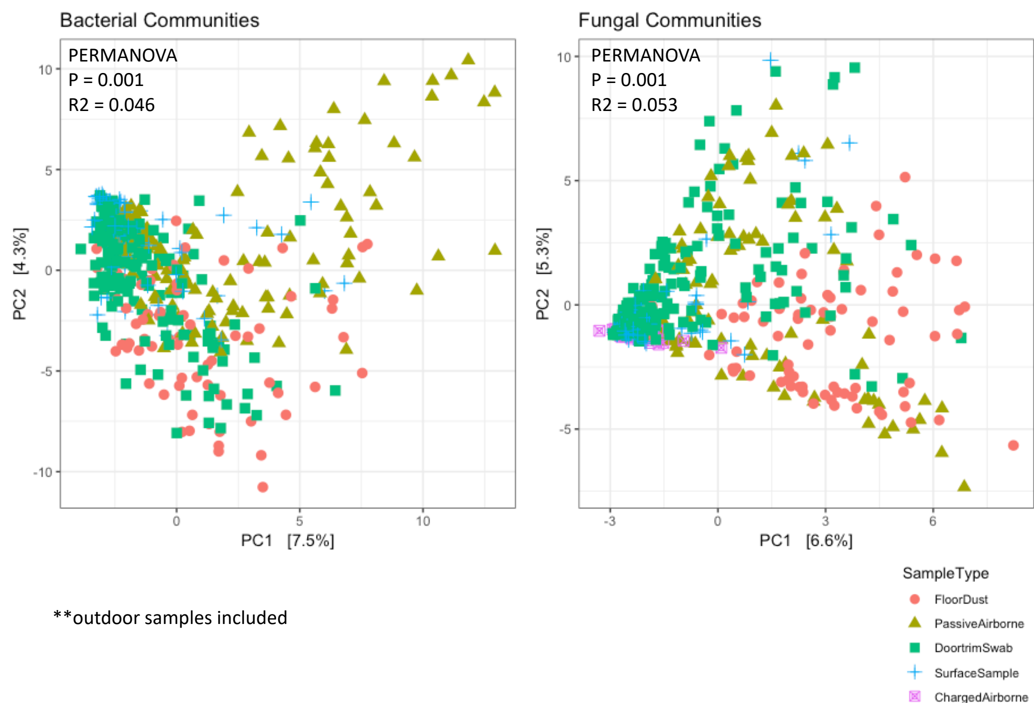


FIGURE 4. Microbial Community Composition, visualized with a Principal Components Analysis and tested with PERMANOVA, showed significant differences in beta-diversity across sampling methods. Microbial communities clustered by sample type.



SUPPLEMENTAL FIGURE 1. Microbial community composition including outdoor samples visualized with a Principal Components Analysis and tested with PERMANOVA. Results remain robust and show distinction between microbial communities in different sampling methods.

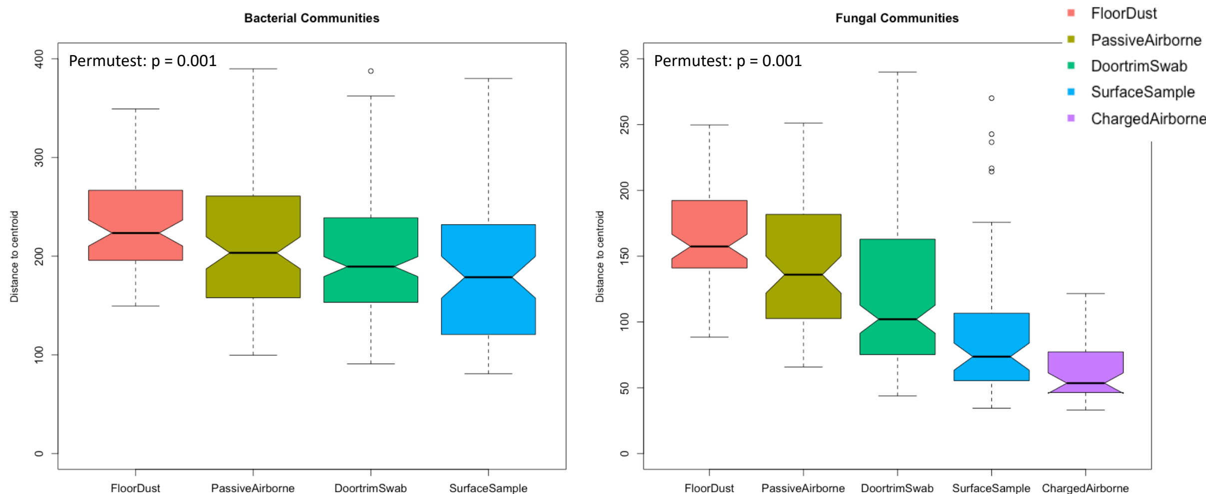


FIGURE 5. Beta-dispersion (distance to the centroid) of microbial communities, tested with permutation of homogeneity of groups, is significantly across sample types.

Taxa Associated with Sampling Methods. The abundance of microbial taxa recovered across sampling methods differed significantly, FIGURE 6. The proportional representation of Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria recovered from Floor Dust, Passive Airborne dust, Door trim Swabs, and Surface Samples showed shifts in dominant members of the community. Floor Dust detected the greatest abundance of Cyanobacteria (Kruskal-Wallis: p=6.57e-05) and Bacteroidetes (Kruskal-Wallis; p=0.02). Passive Dust detected the greatest abundance of Actinobacteria (Kruskal-Wallis; p=4.5e-08). Door trims had the greatest abundance of Firmicutes (Kruskal-Wallis; p=0.008), and Surface Samples detected the greatest abundance of Proteobacteria (Kruskal-Wallis; p=8.79e-07). For fungi, communities found by each collection method principally comprised Ascomycota and Basidiomycota. Charged Airborne samples had a greater expansion in the Basidiomycota, with half of the taxa being classified in the phylum, compared to ranges of 29-39% in other sampling methods. Within fungal classes, Charged Airborne samples recovered the greatest proportional abundance of Tremellomycetes (Kruskal-Wallis: p=1.37e-11) and Pezizomycetes marginally (Kruskal-Wallis: p=0.09). Floor Dust detected the greatest proportion of Saccharomycetes (Kruskal-Wallis: p=2,28e-08), Sordariomycetes (Kruskal-Wallis: p=4.07e-09), and Microbotryomycetes (Kruskal-Wallis: p=2.39e-05). Door trim samples detected the greatest abundance of Agaricomycetes (Kruskal-Wallis: p=1.5e-14). Surface Samples detected the greatest abundance of Dothideomycetes (Kruskal-Wallis: p=6.73e-11).

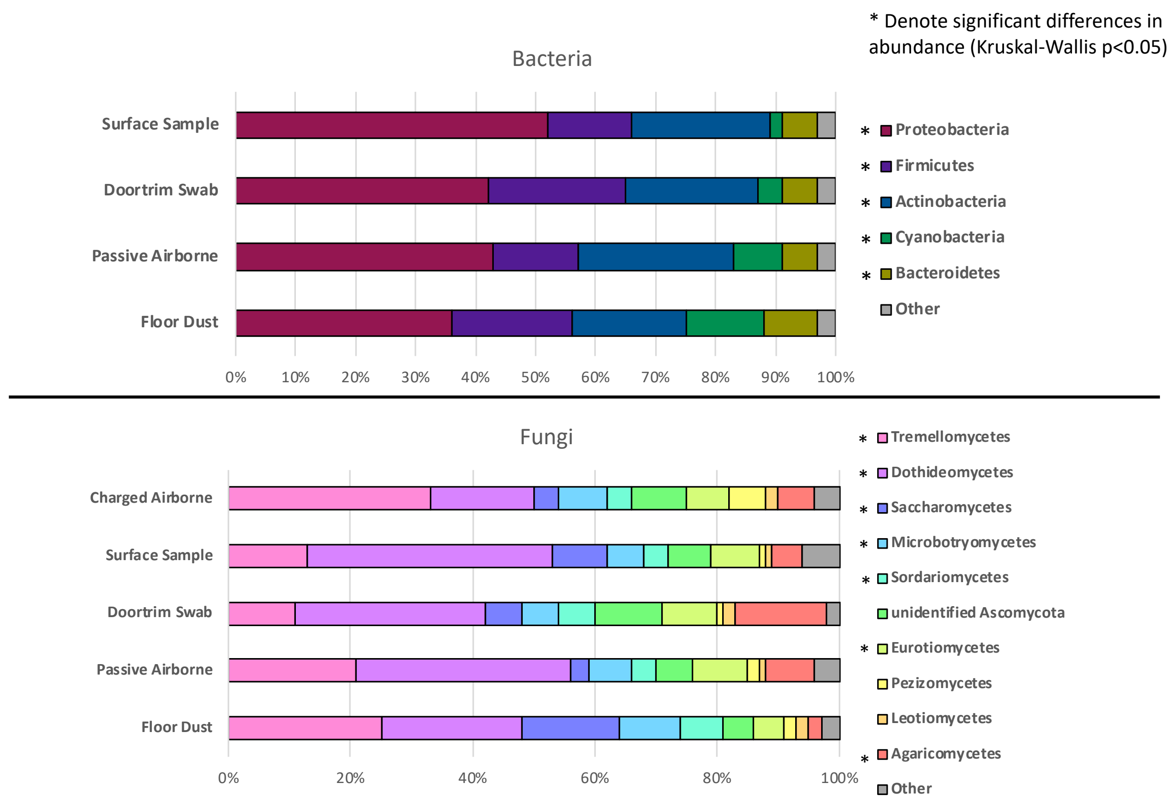


FIGURE 6. Differential abundance of taxa associated with different sampling methods. Kruskal-Wallis was used to test mean abundance of sequence reads in bacterial Phyla and fungal Classes.

DISCUSSION

Microbes that have settled on surfaces are often collected in studies that seek to correlate human exposure to various indoor contaminants. House dust is thought to provide an integrated long-term sample of previously airborne particles, but because microbial assemblages in dust readily shift due to deposition, resuspension, and removal of particles, different dust samples may also have different sources of microbes (23). As indoor microbiome studies develop, researchers should be aware that the choice of sampling methodology could result in very different profiles of microbial communities. Here we compared the recovery of indoor microbiota using HST with five methods that used vacuuming of floor dust, swabbing of door trim or walls, passive settling, and electrostatic settling of airborne dust over one month. We conducted amplicon sequencing and quantitative-PCR on 420 bacterial and 425 fungal samples collected from 70 homes in New York City over two seasons. Overall, we detected 16,382 bacteria ASVs and 11,293 fungal OTUs. We found that these various sampling methods detect significantly different microbial biomass, quantities of taxa, and composition of communities.

We found dust vacuumed from 1 meter squared of flooring recovered the greatest biomass and richness of any sampling method tested. The large biomass recovered by vacuuming floor dust may reflect the large surface area and potentially long period of accumulation, the latter a function

of the housekeeping habits of the occupants (23). The high richness may also be due to augmentation of settled airborne dust with soil tracked indoors by human occupants and their pets. Passively settled dust samples provided the next greatest microbial biomass and richness. Door trim Swabs and Surfaces Samples followed in terms of microbial community richness but did not allow for comparisons of biomass because neither their surface area nor the period of accumulation were standardized. Charged airborne samples had the lowest recorded biomass and richness of any sample type. In contrast to our results, the ERMI approach with qPCR of 36 taxa was used to compare five sampling methods (air sampling, two EDCs, floor vacuuming, and swabbing), finding no significant differences in the calculated ERMI scores of any sampling method, but observing significant differences in the total fungal biomass of air samples compared to dust samples and significant differences in the abundances of specific taxa (118).

The composition of microbial communities was also significantly different in each of the sampling methods. Analysis with PERMANOVA and a permutation tests of the Distance to Centroid showed that the microbial communities detected within each sampling method are distinct. Hoisington et al. sampled a large retail space using multiple sampling methods, including HVAC filters and bioaerosol samplers over the course of two weeks. They found that while bacterial communities sampled using the same method appeared to be compositionally similar to each other, this was not the case for fungi, whose communities clustered by time of collection (119). Hospodsky et al. compared bacteria populations sampled from floor dust and airborne particles in a classroom setting and found that biomass and diversity were highest in floor dust samples and observed distinct communities associated with different sampling types (116). In the present study, we detected significant differences in community composition for both bacteria and fungi, with collection method being a major driver of differences between samples.

Different sampling methods favored different classes of bacteria and fungi. Of all the sampling methods tested, Floor Dust had significantly greater abundance of the bacterial phyla Bacteroidetes and Cyanobacteria and fungal classes Microbotryomycetes and Saccharomycetes. Bacteroidetes and Saccharomycetes are found in a numerous of environments, but also include species of opportunistic human pathogens, some of which have been previously found indoors in homes of asthmatic children (18), (120). Microbotryomycetes comprises molds that have also been associated with the indoor environment (60), (8). Cyanobacteria are characteristically outdoor bacteria and were presumably trafficked indoors on shoes or paws (17). Swabs of Door trim had the greatest abundance of bacterial Firmicutes and fungal Agaricomycetes, while swabs of surfaces had the greatest abundance of Proteobacteria and fungal Dothideomycetes. Proteobacteria and Firmicutes are diverse bacterial taxa often found the human and animal guts, which have been demonstrated as dominant bacterial phyla associated with human occupancy indoors (54). Agaricomycetes, home to mushrooms and wood decay fungi, are abundant spore producers and it is logical that their spores would accumulate on door trim. Dothideomycetes are known to grow on building materials and it is also logical that their species would be recovered from visible colonies.

These five sampling methods allowed us to gain greater spatial and temporal understanding of microbiological dynamics indoors. The Passive and Charged airborne samples had a standard surface area and were open to collect falling dust for a period of four weeks, which captured daily changes in microbial abundance and weekly changes in occupant behavior. Although the surface

area of the vacuumed floor dust sample was standardized, the amount of time for floor dust to accumulate was unknown and likely determined by the housekeeping habits of the occupants. The door trim samples were presumed to sample the longest period, because it is unlikely that any resident dusted the tops of their doors, raising the potential for dust above the door to have been accumulating for years. The surface samples of visible mold colonies sample a short moment in time when a fungus growing indoors produces spores that are melanized or otherwise pigmented such that they can be observed.

Charged Airborne dust samples were only analyzed for fungi in one season due to the difficulty in streamlining the DNA extraction protocol. This process was tedious and had low-efficiency, resulting in the lowest recovery of any sampling method. Adams et al. compared the similarity of bacterial communities and quantities using passive dust collectors and charged electrostatic dust collectors with fibrous material. In this study although different samplers varied in their collection, retention, and extraction efficiency, microbial composition was similar across sampling methods. The authors suggested passive dust collectors constructed from empty petri dishes were sufficient to study microbial communities aerosolized in house dust (115). We concur.

In our study, Passive Airborne samplers were simply constructed with standard Petri dishes (a low-cost material), and easily deployed inside homes and the outdoors. Our study sites included water-damaged housing units in NYC housing. Setting up Passive Airborne samplers outdoors allowed for insightful comparisons between water-damaged and non-damaged units (reported elsewhere; (117)), which could not be gleaned by analyzing indoor samples only. Furthermore, by collecting Passive Airborne dust over the course of one month, we know that the communities detected with this method became aerosolized and had the potential to be regularly inhaled by occupants, possibly leading to acute infection (15). Emerson compared microbial communities using HTS with passive samplers and HVAC samples in a study of water-damage homes in Boulder, CO. They also reported significant differences in community composition between the two types of samplers, regardless of flooding conditions (26). Their use of passive samplers over defined periods to evaluate microbial communities allowed them to discern the impact of renovation after a historic flood event.

CONCLUSIONS

Few studies have directly compared the structure of bacterial and fungal communities in house dust using multiple sampling methods and high-throughput sequencing technologies with high replication. In this study we compared biomass, richness, community composition, and taxon abundance of bacteria and fungi collected in 70 homes using Floor Dust, Passive Airborne dust, Door trim Swabs, Surface Swabs, and Charged Airborne dust. We found that sampling methods strongly influenced our depiction of indoor microbiomes, resulting in significantly different measurements of biomass, alpha-diversity, and beta-diversity. Furthermore, certain methods had an affinity for different taxa. Researchers focused on querying the relative abundances of particular microbes should be aware that their choice of sampling regimen might influence their observation.

The sampling methods also vary in terms of their surface area, accumulation time, portability, cumbersomeness in the field, and difficulty in downstream processing of genomic DNA. The intent of the research should determine which sampling method best suits the study. If the aspiration of

the study is to determine which bioaerosols residents presently contend with, we recommend the use of Passive Airborne Dust collectors. Passive Airborne samplers accumulate dust over a standard surface area for long-term yet specific time courses and can be processed with relative ease. Passive Airborne samplers are easily portable into the field, can be set up both indoors and outdoors, and are able to be deployed with discretion in the home so as not the disturb the occupants. If the intention of the study is to determine any potential contaminants that have been in the home in the past, Floor Dust samples may be useful. Floor Dust samples collect a great deal of biomass that could also be used for compendium analysis of cockroach, mite, or mouse allergens, or additional household chemicals that may be eliciting a health response. If the research focus is identification of fungi actively sporulating in homes, amplicon sequence of swab samples may suffice.

The Dismantling of North American Public Housing Caused Childhood Asthma Disparity

Iman A Sylvain and Rachel Morello-Frosch

ABSTRACT

Asthma is a respiratory disease that disproportionately impacts low-income communities of color living in urban environments, particularly in federally-assisted housing. Since 2001 there has been a noted racial/ethnic disparity in childhood asthma that is arguably attributable to the dismantling of North American public housing. Asthma is mediated by allergic responses to environmental triggers that are commonly found in segregated substandard housing. Racist, classist, neoliberal policies of the Clinton Administration led to the creation of the social welfare reform program HOPE VI (Housing Opportunities for People Everywhere), whose hallmark attribute was the demolition of over 260,000 public housing units. Under the auspice of ending concentrated poverty, HOPE VI heralded a shift of the Department of Housing and Urban Development towards subsidizing vouchers for rentals in the private housing market, rather than secured housing in government-owned buildings for those in need. This program forcibly relocated thousands of Black families and has led to the deteriorating health of HOPE VI communities. Those families remaining in the vestiges of public housing projects have been malignantly neglected and are currently experiencing an asthma epidemic resultant from poor building maintenance at the hands of their government slumlord. Asthma interventions that are not focused on reforming public housing policy, ending poverty, or informed by the epidemiological impacts of racial housing segregation will be ever hindered. In order to end the asthma epidemic in America we must reinvest in public housing infrastructure and ensure that affordable housing is an arena where low-income residents can choose to live in good health and dignity.

THE CHILDHOOD ASTHMA EPIDEMIC: A RESPIRATORY SIEGE ON THE BLACK URBAN POOR

Today approximately 7 million children aged 0-17 in the United States have asthma (121). The prevalence of childhood asthma in the United States doubled from 1980-1995 and continually increased until 2010 (122). Significant disparity in asthma prevalence has been noted since 2001. Asthma affects all races, ethnicities, sexes, age groups, and regions of the country, but poor inner-city minorities experience disproportionate rates of asthma morbidity and mortality (76). Many environmental justice advocates view asthma as a “politicized illness experience” (123) because the burden of asthma falls disproportionately on Black and Puerto Rican low-income, urban children (124).

Asthma disparity for Black children is particularly alarming. Black children are twice as likely to be hospitalized, more than twice as likely to have an emergency department visit, and four times more likely to die from asthma than white children (121). Socioeconomic characteristics at the individual, household, and community level also affect a child’s risk of asthma (125). 12.2% of children living below the federal poverty level have asthma. Asthma is now the leading cause of school absence among children in impoverished urban neighborhoods (126). Evidence has shown that while socioeconomic status plays an important role in predicting asthma, it has different

effects depending on race and ethnicity (127). Childhood asthma prevalence in low-income minority neighborhoods reaches 23%, exceeding the national average four times (76).

Urban residence has been suggested as an explanation for the high prevalence of asthma in poor minorities, and living in public housing has been identified as a particular risk factor for asthma (2). Either by "design or evolution" public housing developments are primarily located in urban poor minority neighborhoods (126). A study conducted in New York City found that even after adjusting for individual risk factors like ethnicity/race and low socioeconomic status, children living in public housing have higher odds of asthma than children living in any type of private housing (102). Childhood asthma prevalence in NYC public housing was nearly 22%. These rates were concordant with studies from Boston that showed public housing residents are more likely to have asthma than other city residents; asthma rates in Boston public housing reached 19% (128).

Asthma is a complex disease characterized by enhanced bronchial activity and reversible airway construction, oftentimes mediated by an allergic response to biological agents like dust mites, cockroaches, and fungi (51), and a range of other socio-environmental factors. Elevated asthma rates among poor, minority, inner-city residents has been linked to the low quality housing they typically occupy which exposes them to greater asthma triggers more commonly found in substandard housing (77). In New York City racial/ethnic disparity in asthma is thought to be due to more Blacks and Latinx living in public housing units suffering from structural and maintenance deficiencies like interior/exterior leaks, chipping paint, broken plaster, holes in the floor/walls, and pest infestations. Inadequate housing occupied by economically disadvantaged minority inner-city families leads to greater exposure to a number of asthma triggers such as dust mites, pet dander, cockroaches, mice feces, and mold (77).

THE IMPACT OF SUBSTANDARD HOUSING ON HEALTH

It is well established that housing is an important determinant of health, and substandard housing is a major threat to wellbeing (3). Since the 19th century physicians and public health officials have worked to improve ventilation, sanitation, structural stability, and reduce crowding in homes in order to reduce death and illness amongst the working poor concentrated in urban slums near factories (129). Today our understanding of the connections between housing and health have expanded beyond the last century's concern with sanitation, infectious disease, and safety, and onto a number of agents associated with morbidity and mortality, like radon, asbestos, lead, pests and pet allergens, chemicals in wall coatings, furnishing, and consumer products, outdoor toxicants, pesticides, secondhand smoke, combustion by products, and mold (6). Poor housing conditions are associated with a myriad of negative health outcomes, including respiratory infections, asthma, lead poisoning, injuries, and compromised mental health (3).

Damp, moldy housing has been linked to anxiety and depression, as well as associated with asthma and other chronic respiratory symptoms by providing environment for mites, roaches, and viruses (3). Common sources of moisture inside homes are from condensation, structural membrane leaks, damp foundations and crawl spaces, leaky pipes, inadequate ventilation and daily activities like bathing and cooking (129). Inadequate ventilation and moisture management in housing contributes to asthma, mold-related illness, and carbon monoxide poisoning (74). Old, dirty carpeting is also an important reservoir for dust, allergens, and toxic chemicals (3). Exposure to

household allergens like tobacco smoke, cockroaches, mold, dust mites, mice, and pets can cause airway hyperresponsiveness, wheezing, and increase the likelihood of developing asthma (130).

Populations living in inadequate housing are more likely to have environmental diseases and injuries (74). Economically disadvantaged neighborhoods have poorer housing conditions, increased crowding, and higher rates of housing code violations that can lead to indoor allergen exposure (130). Neighborhoods with low socioeconomic status also have elevated rates of intentional injury, poor birth outcomes, cardiovascular disease, HIV, gonorrhea, and tuberculosis, in addition to poor air quality due to their proximity to major sources of vehicle exhaust emissions (3).

Indoor concentrations of multiple pollutants have been shown to be elevated in low socioeconomic status households due to indoor and outdoor sources, physical housing structures, and residential activity patterns (6). Low-income people are more than twice as likely to live in homes with severe physical problems compared to the general populations (3). Disparity in asthma morbidity is arguably caused by disproportionate exposure to indoor environmental asthma triggers associated with living in substandard housing.

RACIAL HOUSING SEGREGATION CAUSES PUBLIC HEALTH DISPARITIES

The Center for Disease Control reported that in 2009 Blacks had the highest odds of living in inadequate and unhealthy housing compared to any other racial/ethnic group (4). African Americans are disparately exposed to pests and mold, lead paint hazards, and fires. Racial residential segregation is believed to be a fundamental cause of racial disparities in health (131). 7.5% of Blacks and 6.3% of Latinx live in substandard housing, compared to only 2.8% of Whites (74). Residential segregation may account for the well-established disparities between Blacks and whites in adverse birth outcomes, health behaviors, mortality, and chronic diseases such as asthma, diabetes, and hypertension (74). The etiology of asthma in particular, strongly implicates the role of racial residential segregation and disparate access to quality housing on child health.

Black communities are more segregated than any other ethnic/racial group in the United States, and no immigrant group has ever lived under the level of segregation that exists for African Americans (131). The experience of residential segregation has meaningful impacts on the health of Black Americans irrespective of their socioeconomic status. There is evidence that Black neighborhoods have significantly poorer healthcare facilities staffed by less competent physicians (lacking board certification), higher exposure to toxic environmental pollutants, and less access to fresh produce in supermarkets or recreational facilities, compared to white neighborhoods (72). African Americans tend to live in hyper-segregated areas that are concentrated with low-income housing projects and are markedly different from white areas (127).

There is a long history of segregation and Anti-Black racism demonstrated in federal housing policies, which has threatened the health and wellbeing of Black people. The creation of the Federal Housing Administration (FHA) and Veterans Administration (VA) fundamentally changed the American housing market in 1934 and made homeownership an affordable reality for millions of Americans for the first time. The FHA and VA financed nearly half of the nation's suburban housing between 1950-1960 (132). By defining communities of color as contaminants

that would erode national property values and rewarding neighborhoods that "protected" property using racially restrictive convents mortgage and outright violence, the federal government prescribed unambiguously racist and discriminatory policies that reserved mortgage insurance exclusively for Whites. As a result, merely 2% of the FHA mortgage insurance was made available to non-white homeowners, and of the 3.9 million mortgage loans guaranteed by the VA, less than 30,000 went to Black veterans (132).

Communities of color were intentionally excluded from the opportunity to own a home, build wealth, or cohabitate in white neighborhoods, a legacy that remains today. Despite the illegality of institutionally exclusionary policies, racial residential segregation between whites and Blacks has remained high and strikingly stable in both Northern and Southern cities since 1940 (131). Segregation scores (dissimilarity index values) of large metropolitan cities like Detroit, Milwaukee, Chicago, and Cleveland are alarmingly close to the scores of South African cities during apartheid (125).

The historical and ongoing exclusion of Black people from affordable housing opportunities has manifested in the overrepresentation of African Americans in federal public housing. Today African Americans make up 12% of American households, but occupy 45% of public housing (133). These public housing projects tend to be clustered in census tracts with high poverty rates and resounding racial segregation. A study by the National Low Income Housing Coalition found that Black public housing residents have an average annual income of less than $20,000 and are four times more likely to live in high poverty neighborhoods compared to their white counterparts (133). The dense concentration of poor African Americans in public housing may ironically be the result of the deliberate destruction of North American public housing.

CLINTON ERA POLICIES DISMANTLED PUBLIC HOUSING

The towering high-rise public housing projects painting the skyline of metropolitan cities like New York City, Chicago, and Detroit, are increasingly becoming images of the past. Public housing assistance today is characterized by low-rise mixed-income neighborhoods of townhouses and apartments (134), and section 8 vouchers to subsidize rent in the private housing market. Today, where once stood public housing projects, there are luxury condominiums, shopping and convention centers, air force bases, medical colleges, and corporate headquarters (135). Since 1992 the U.S. Department of Housing and Urban Development (HUD) has spent over $6 billion to "redevelop" dilapidated public housing, principally through demolition, and has actively displaced thousands of people, mostly African Americans (136).

A herald of the Clinton Administration was social welfare reform, which ushered in neoliberal strategies to reduce the role of government in providing housing for the poor, and effectively dismantled the New Deal model of publicly-owned and operated housing which had been in place for 50 years (137). Growing concerns about the concentration of poverty, crime, and drug use in public housing projects resulted in the National Commission on Severely Distressed Public Housing in 1992, which estimated that there were 86,000 public housing units in distress (representing only 6% of the total public housing stock). This report became the cornerstone of HUD's Housing Opportunities for People Everywhere (HOPE VI) program, which as of 2010 has

demolished nearly 260,000 public housing units (138) in the name of neighborhood revitalization and the belief that tenant mobility creates greater access to health and economic opportunities.

The Obama Administration's premiere public housing initiatives, the Rental Assistance Demonstration (RAD) program and Choice Neighborhoods are continuations of Clinton's HOPE VI, and rely heavily on private dollars and section 8 vouchers to relocate public housing tenants and rebuild decrepit housing and surrounding neighborhoods slated for gentrification (136). The key distinction between Choice Neighborhoods Initiative/RAD and HOPE VI is the right-of-return policy for displaced tenants that guarantee previous low-income residents the opportunity to live in the redeveloped mixed-income units (137). It is estimated that only 14-25% of original residents returned to HOPE VI redeveloped mixed-income sites, which were typically smaller than the units they replaced, had very few public housing units available, and enforced stricter tenant screening criteria (138) (e.g. work requirements, criminal background checks, and drug tests) (139).

The belief that forcibly removing public housing tenants and granting section 8 vouchers would dissolve concentrated areas of poverty proved to be unfounded. Most HOPE VI displacees moved a median distance of 2.9 miles nationally, and into other neighborhoods of high poverty where landlords were willing to accept section 8 vouchers or other public housing existed (138). Race was clearly a driving factor for HOPE VI project demolition, as nearly all of the projects scheduled for demolition were majority non-white, and half of the demolished projects were comprised of 95% or greater African American households. Thus, the majority of households directly displaced by HOPE VI public housing demolition across the country are African American (139).

THE DETERIORATING HEALTH OF HOPE VI RESIDENTS

HOPE VI strategies to redevelop blighted public housing communities by demolishing buildings and replacing them with low-density developments inhabited by socioeconomically diverse residents was predicated on neoliberal philosophies of mobility, which sought to rollback the powers of the state in favor of market processes that promote economic efficiency (140). Imbroscio describes the 'mobility paradigm' inherent in urban housing policy that presumes economic opportunity for the poor can only be reached through a physical move towards opportunity, such a spatial move from the inner city to the suburbs, rather than through economic investments in the places where poor already live (140). The mobility paradigm is central to the preservation vs. mobility debate that queries whether federal housing policy should focus on preserving existing public housing that is located in low-income predominantly Black neighborhoods, or helping the residents to move to higher income and less racially segregated neighborhoods (141).

HOPE VI sought to promote self-sufficiency in public housing residents, which was measured by their ability to gain employment and get off welfare, while simultaneously addressing the deteriorating physical conditions of public housing developments, rampant drug dealing, and violent crime. Ultimately this plan forcibly displaced resident populations with high rates of unemployment, welfare receipts, teen pregnancy, and low levels of education and literary (142). Long-term tracking studies of HOPE VI residents found that while some relocates were able to use their rental vouchers to move to less economically distressed neighborhoods, there has been virtually no change in racial segregation. Nearly all members of the HOPE VI Panel Study moved into predominantly Black neighborhoods; a large portion moved into other traditional public

housing developments, and some became homeless or imprisoned (143). Of those who used the voucher in the private market, 45% reported having trouble paying their utility bills and purchasing food.

Families deemed ‘hard-to-house’ were inhibited from utilizing HOPE VI vouchers on the private housing market or gaining reentry to redeveloped mixed-income developments. ‘Hard-to-house’ families were comprised of households with elderly people, grandparents that cared for children, physically or mentally disabled persons, large families that required four or more bedrooms, or members that had a history of arrest, substance abuse, unemployment, or no high school diploma (139). These hard-to-house families were forced to remain in traditional public housing, which rapidly deteriorated as vacancies increased. 38% of HOPE VI Panel Study participants either remained in their original developments or moved to other traditional housing projects (143).

Keene and Geornimus (2001) proposed that involuntary relocation in the wake of HOPE VI is contributing to the weathering (biosocial process underlying early health decline and excess mortality) and poor health of urban, predominantly African American, public housing residents. HOPE VI displacees experienced the erosion of geographically rooted social ties that buffered against structural disadvantage and now contend with a multitude of stressors associated with crime, violence, lack of resources, and psychic stress from ghettoization in their new racially segregated, high-poverty neighborhoods (144). As residential stability has been identified as one of the most important predictors of community health (76). HUD’s HOPE VI, Choice Neighborhoods, and RAD programs that enforce residential mobility are implicated in the deterioration of health status for thousands of low-income Americans.

Despite the rhetoric which suggested that demolishing high-rise public housing would provide new health and economic opportunities for residents, longitudinal studies of HOPE VI families has found little to no health improvements in adults 4-5 years after relocation (142). In fact, the HOPE VI Panel Study found increased mortality rates for HOPE VI voucher users, who reported being in poor health prior to involuntary relocation, and later experienced serious physical and emotional distress. Evidence shows that public housing residents have the worst health of any population in the United States (142). The demolition and forced relocation of public housing communities did not rectify this trend. Ruel et al.’s study of health status in public housing communities slated for demolition under HOPE VI found that public housing functions more as a safety net than a cause of poor health, as the majority of public housing residents had poor health prior to entry to their Atlanta projects and looked to such affordable housing options as a last resort (145).

Scientist evaluated outdoor air quality surrounding three public housing high-rises authorized for demolition under HOPE VI that were located within 100m of other occupied high-rise buildings, schools, and community buildings (146). The study showed that the mere act of demolishing public housing projects exposes public housing residents to high concentrations of particulate matter air pollution, which may adversely affect the respiratory heath of nearby residents. Another study of residents living in four Chicago public housing projects sought to identify predictors of asthma-related hospitalizations in adults and noted increased asthma morbidity and wheezing associated with inadequate building maintenance pending building demolition under HOPE VI (80). The HOPE VI Panel Study found high rates of a number of chronic debilitating conditions, such as arthritis, obesity, depression, diabetes, hypertension, stroke, and asthma (143). Not only

has HOPE VI failed to improve the physical health conditions of public housing residents, it has led to the deteriorated health status of impacted communities.

CONCLUSIONS

The Clinton Administration viewed public housing as one of the biggest and most visible failures of American social welfare policy, citing public housing as constantly underfunded and poorly maintained, and creating hazardous conditions that put residents at risk for injury and disease (143). The solution to underfunding is not defunding. Rather than abolishing public housing, the government should have made significant reinvestment in our public housing stock. At its core, the debate of whether to preserve public housing rather than demolish projects and provide vouchers to subsidize rent costs in the private market was a question of race in America, and it rooted housing policy in the belief that it is problematic for too many low-income folks or people of color to live near one another (141). This focus prompted spatial dispersion of poor people as a solution to concentrated poverty rather than rectifying the fundamental causes of housing inequity in America, notably the long history of discrimination in mortgage lending and racial residential segregation (132).

Today Black women disproportionately occupy federal public housing (138), and their children have alarmingly high rates of asthma due to inadequate housing (102). Public housing should be clean, safe, energy efficient, and rich with programs and services to provide residents the choice of remaining in the government safety net, or transitioning to the private market (142). New York City is one of the last cities to maintain high-rise housing projects in America (134), and every year the loss of ~10,000 units to disrepairs threatens the total demise of public housing nationwide (136).

Clinton Era social welfare reform demonstrated rampant neglect for the health of our nation's poor, palpable disregard for the lives of public housing residents, and further cemented segregation. After a decade and a half of neoliberal policies that sought to shift the responsibility of providing affordable housing away from the federal government and to the private market, those who remain in the vestiges of America's public housing have been notably neglected and deprioritized by the government. These gingerly veiled racist and classist housing policies have negatively impacted the health of ethnic minorities in America, and may be an underlying factor contributing to the ethnic/racial disparity in the respiratory disease asthma.

Under the Obama Administration there is a coordinated interagency federal action plan to reduce racial and ethnic disparity in childhood asthma (121). Despite the involvement of the Department of Housing and Urban Development in this initiative, and its stated mission to "address the multifactorial nature of asthma disparities through holistic, coordinated, community-wide intervention," the action plan only mentions federally assisted housing once. Though the plan acknowledges that asthma disproportionately affects minority children with family incomes below the poverty line, the proposal does not include an effort to improve the physical, structural, or environmental elements of public housing where impacted children live. The HUD Healthy Homes Initiative likewise concedes exposure to household allergens may contribute to the disproportionate burden of asthma among African American children in poor households (147),

but has yet to propose an initiative to address the respiratory problems HUD created by demolishing thousands of public housing developments.

The rise in asthma disparity may have been preventable if the government chose to invest healthy, affordable housing for our nation's most vulnerable populations. Asthma is an epidemic disproportionately affecting low-income urban Black communities (121) largely attributable to living in substandard housing (3). Genuine effort to desegregate cities might have reduced the disparate exposure to environmental allergens and toxicants now associated with racially segregated poor quality housing (74). Those who disproportionately suffer from asthma (inner-city, low-income, Black youth, living in poorly maintained public housing) are the children of HOPE VI legacies. Ending disparity in American childhood asthma will require housing reparations for economically disadvantaged Black families, genuine reinvestment in public housing infrastructure, and a national plan to make affordable housing safe and healthy for all.

References

1. Klepeis N, Nelson W, Orr W, Robinson J, Tsang A, Switzer P, et al. The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants. Journal of Exposure Analysis and Environmental Epidemiology. 2001 Jun 27;11:231–52.

2. Rauh V, Landrigan P, Claudio L. Housing and Health. Annals of the New York Academy of Sciences. 2008;:1–14.

3. Krieger J, Higgins D. Housing and Health: Time Again for Public Health Action. Research and Practice. 2002;:1–11.

4. Center for Disease Control. CDC Health Disparities and Inequalities Report — United States, 2011. 2011 Jan 14;:1–116.

5. World Health Organization. WHO Guidelines for Indoor Air Quality: Dampness and Mould. 2009;:1–248.

6. Adamkiewicz G, Zota A, Fabia P, Chahine T, Julien R, Spenger J, et al. Moving Environmental Justice Indoors: Understanding Structural Influences on Residential Exposure Patterns in Low-Income Communities. Research and Practice. 2011;:1–9.

7. Health NYCDO, Hygiene M. Guidelines on Assessment and Remediation of Fungi in Indoor Environments. 2008;:1–25.

8. Flannigan B, Samson RA, Miller DJ. Microorganisms in Home and Indoor Work Environments. 2nd ed. 2011. 511 p.

9. Andersen B, Frisvad JC, Søndergaard I, Rasmussen IS, Larsen LS. Associations between Fungal Species and Water-Damaged Building Materials. Appl Environ Microbiol. Sixth edition. 2011 Jun 7;77(12):4180–8.

10. Nielsen KF, Frisvad JC. Mycotoxins on building materials. In: Fundamentals of mold growth in indoor environments and strategies for healthy living. Wageningen: Wageningen Academic Publishers; 2011. pp. 245–75.

11. Rao CY, Riggs MA, Chew GL, Muilenberg ML, Thorne PS, Van Sickle D, et al. Characterization of Airborne Molds, Endotoxins, and Glucans in Homes in New Orleans after Hurricanes Katrina and Rita. Appl Environ Microbiol. 2007 Feb 26;73(5):1630–4.

12. Pitkäranta M, Meklin T, Hyvärinen A, Nevalainen A, Paulin L, Auvinen P, et al. Molecular profiling of fungal communities in moisture damaged buildings before and after remediation - a comparison of culture-dependent and culture-independent methods. BMC Microbiol. 2011;11(1):235–16.

13. Amann RI, Ludwig W, Schleifer K-H. Phylogenetic Identification and In Situ Detection of Individual Microbial Cells without Cultivation. Microbiological Reviews. 1995 Mar 1;59(1):1–33.

14. Stephens B. What Have We Learned about the Microbiomes of Indoor Environments? Gibbons SM, editor. mSystems. 2016 Jul 26;1(4):202–9.

15. Adams RI, Bhangar S, Dannemiller KC, Eisen JA, Fierer N, Gilbert JA, et al. Ten questions concerning the microbiomes of buildings. Building and Environment. Elsevier Ltd; 2016 Nov 15;109(C):224–34.

16. Amend AS, Seifert KA, Samson R, Bruns TD. Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. Proc Natl Acad Sci USA. 2010;107(31):13748–53.

17. Adams RI, Miletto M, Lindow SE, Taylor JW, Bruns TD. Airborne Bacterial Communities in Residences: Similarities and Differences with Fungi. Moreau CS, editor. PLoS ONE. 2014 Mar 6;9(3):e91283–7.

18. Dannemiller KC, Gent JF, Leaderer BP, Peccia J. Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children. Indoor Air. 2015 Apr 17;26(2):179–92.

19. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, et al. Architectural design influences the diversity and structure of the built environment microbiome. The ISME Journal. Nature Publishing Group; 2012 Jan 26;6(8):1469–79.

20. Meadow JF, Altrichter AE, Bateman AC, Stenson J, Brown GZ, Green JL, et al. Humans differ in their personal microbial cloud. PeerJ. 2015;3(5):e1258–22.

21. Adams RI, Miletto M, Taylor JW, Bruns TD. Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. The ISME Journal. 2013 Feb 21;7(7):1262–73.

22. Barberán A, Dunn RR, Reich BJ, Pacifici K, Laber EB, Menninger HL, et al. The ecology of microscopic life in household dust. Proc R Soc B. 2015 Aug 26;282(1814):20151139–9.

23. Rintala H, Pitkäranta M, Täubel M. Microbial Communities Associated with House Dust. In: Advances in Applied Microbiology. Elsevier; 2012. pp. 75–120. (Advances in Applied Microbiology; vol. 78).

24. Adams RI, Tian Y, Taylor JW, Bruns TD, Hyvärinen A, Täubel M. Passive dust collectors for assessing airborne microbial material. Microbiome. Microbiome; 2015;3(46):1–11.

25. Jayaprakash B, Adams RI, Kirjavainen P, Karvonen A, Vepsäläinen A, Valkonen M, et al. Indoor microbiota in severely moisture damaged homes and the impact of interventions. Microbiome. Microbiome; 2017;:1–17.

26. Emerson JB, Keady PB, Brewer TE, Clements N, Morgan EE, Awerbuch J, et al. Impacts of Flood Damage on Airborne Bacteria and Fungi in Homes after the 2013 Colorado Front Range Flood. Environ Sci Technol. 2015 Feb 10;49(5):2675–84.

27. Committee on Microbiomes of the Built Environment: From Research to Application, Board on Life Sciences, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, Health and Medicine Division, Board on Infrastructure and the Constructed Environment, et al. Microbiomes of the Built Environment. Washington, D.C.: National Academies Press; 2017. 317 p.

28. White TJ, Bruns T, Lee S, Taylor J. Amplification And Direct Sequencing Of Fungal Ribosomal Rna Genes For Phylogenetics. In: PCR Protocols: a Guide to Methods and Applications. Academic Press, Inc; 1990. pp. 315–22.

29. Adams RI, Lymperopoulou DS, Misztal PK, De Cassia Pessotti R, Behie SW, Tian Y, et al. Microbes and associated soluble and volatile chemicals on periodically wet household surfaces. Microbiome. Microbiome; 2017 Sep;:1–16.

30. Zhou G, Whong W-Z, Ong T, Chen B. Development of a fungus-specific PCR assay for detecting low-level fungi in an indoor environment. Molecular and Cellular Probes. 2000;14(6):339–48.

31. Martin M. Cutadapt Removes Adapter Sequences from High-throughput Sequencing Reads. EMBnetjournal. 2011 May 11;:1–3.

32. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016 May 23;13(7):581–3.

33. Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics. 2014 Feb 25;30(5):614–20.

34. The New Phytologist Tansley Medal. 2010 Mar 18;:1–35.

35. Carlsen T, Aas AB, Lindner D, Vralstad T, Schumacher T, Kauserud H. Don't make a mista(g)ke: is tag switching an overlooked source of error in amplicon pyrosequencing studies? Fungal Ecology. Elsevier Ltd; 2012 Dec 1;5(6):747–9.

36. Davis NM, Proctor D, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. 2017 Nov 17;:1–38.

37. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high- throughput community sequencing data. Nature Publishing Group. Nature Publishing Group; 2010 May 1;7(5):335–6.

38. Ihaka R, Gentleman R. R: A Language for Data Analysis and Graphics. Journal of Computational and Graphical Statistics. 1996 Sep;5(3):299–314.

39. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Package "vegan." 2018 Oct 25;:1–297.

40. Tree diversity analysis. 2005 Nov 8;:1–7.

41. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. Watson M, editor. PLoS ONE. Public Library of Science; 2013 Apr 22;8(4):e61217–11.

42. Wickham H. ggplot2. 2009. 211 p.

43. Gloor GB, Wu JR, Pawlowsky-Glahn V, Egozcue JJ. It's all relative: analyzing microbiome data as compositions. Annals of Epidemiology. Elsevier Inc; 2016 May 1;26(5):322–9.

44. Vesper SJ, Wymer LJ, Meklin T, Varma M, Stott R, Richardson M, et al. Comparison of populations of mould species in homes in the UK and USA using mould-specific quantitative PCR. Letters in Applied Microbiology. 2005 Oct;41(4):367–73.

45. Meklin T, Reponen T, McKinstry C, Cho S-H, Grinshpun S, Nevalainen A, et al. Comparison of mold concentrations quantified by MSQPCR in indoor and outdoor air sampled simultaneously. Science Total Environment. 2007 Aug 15;:1–6.

46. Vesper S, McKinstry C, Haugland R, Wymer L, Bradham K, Ashley P, et al. Development of an Environmental Relative Moldiness Index for US Homes. Journal of Occupational and Environmental Medicine. 2007 Aug;49(8):829–33.

47. Lignell U, Meklin T, Rintala H, Hyvärinen A, Vepsäläinen A, Pekkanen J, et al. Evaluation of quantitative PCR and culture methods for detection of house dust fungi and streptomycetes in relation to moisture damage of the house. Letters in Applied Microbiology. 2008 Oct;47(4):303–8.

48. Green BJ, Lemons AR, Park Y, Cox-Ganser JM, Park J-H. Assessment of fungal diversity in a water-damaged office building. Journal of Occupational and Environmental Hygiene. Taylor & Francis; 2017;14(4):285–93.

49. Kettleson EM, Adhikari A, Vesper S, Coombs K, Indugula R, Reponen T. Key determinants of the fungal and bacterial microbiomes in homes. Environmental Research. Elsevier; 2015 Apr 1;138(C):130–5.

50. Dannemiller KC, Mendell MJ, Macher JM, Kumagai K, Bradman A, Holland N, et al. Next-generation DNA sequencing reveals that low fungal diversity in house dust is associated with childhood asthma development. Indoor Air. 2014;24(3):236–47.

51. Dannemiller KC, Gent JF, Leaderer BP, Peccia J. Indoor microbial communities: Influence on asthma severity in atopic and nonatopic children. The Journal of Allergy and Clinical Immunology. Elsevier Ltd; 2016 Mar 22;:1–9.

52. Adams RI, Amend AS, Taylor JW, Bruns TD. A Unique Signal Distorts the Perception of Species Richness and Composition in High-Throughput Sequencing Surveys of Microbial Communities: a Case Study of Fungi in Indoor Dust. Microb Ecol. 2013 Jul 24;66(4):735–41.

53. Dedesko S, Siegel JA. Moisture parameters and fungal communities associated with gypsum drywall in buildings. Microbiome. Microbiome; 2015 Dec 2;:1–15.

54. Adams RI, Bhangar S, Pasut W, Arens EA, Taylor JW, Lindow SE, et al. Chamber Bioaerosol Study: Outdoor Air and Human Occupants as Sources of Indoor Airborne Microbes. Zhang Y, editor. PLoS ONE. 2015 May 29;10(5):e0128022–18.

55. Gilbert JA, Stephens B. Microbiology of the built environment. Nature Reviews Microbiology. Springer US; 2018 Nov;:1–10.

56. Leung MHY, Lee PKH. The roles of the outdoors and occupants in contributing to a potential pan-microbiome of the built environment: a review. Microbiome. Microbiome; 2016 Apr 25;:1–15.

57. Grantham NS, Reich BJ, Pacifici K, Laber EB, Menninger HL, Henley JB, et al. Fungi Identify the Geographic Origin of Dust Samples. Rokas A, editor. PLoS ONE. 2015 Apr 13;10(4):e0122605–13.

58. Meadow JF, Altrichter AE, Kembel SW, Kline J, Mhuireach G, Moriyama M, et al. Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. Indoor Air. 2nd ed. 2013 May 24;24(1):41–8.

59. Dannemiller KC, Weschler CJ, Peccia J. Fungal and bacterial growth in floor dust at elevated relative humidity levels. Indoor Air. 2017;27(2):354–63.

60. Adams RI, Miletto M, Taylor JW, Bruns TD. The Diversity and Distribution of Fungi on Residential Surfaces. PLoS ONE. 2013 Oct 15;:1–9.

61. Fujiyoshi S, Tanaka D, Maruyama F. Transmission of Airborne Bacteria across Built Environments and Its Measurement Standards: A Review. Front Microbiol. 2017 Nov 29;8:224–17.

62. Park JH, Cox-Ganser JM, White SK, Laney AS, Caulfield SM, Turner WA, et al. Bacteria in a water-damaged building: associations of actinomycetes and non-tuberculous mycobacteria with respiratory health in occupants. Indoor Air. 2015;27(1):24–33.

63. Park J-H, Cox-Ganser JM, Kreiss K, White SK, Rao CY. Hydrophilic Fungi and Ergosterol Associated with Respiratory Illness in a Water-Damaged Building. Environmental Health Perspectives. 2008 Jan;116(1):45–50.

64. Chew GL, Wilson J, Rabito FA, Grimsley F, Iqbal S, Reponen T, et al. Mold and Endotoxin Levels in the Aftermath of Hurricane Katrina: A Pilot Project of Homes in New Orleans Undergoing Renovation. Environmental Health Perspectives. 2006 Dec;114(12):1883–9.

65. Bloom E, Grimsley LF, Pehrson C, Lewis J, Larsson L. Molds and mycotoxins in dust from water-damaged homes in New Orleans after hurricane Katrina. Indoor Air. 2009 Apr;19(2):153–8.

66. Nevalainen A, Täubel M, Hyvärinen A. Indoor fungi: companions and contaminants. Indoor Air. 2015 Jan 20;25(2):125–56.

67. Kelman BJ, Robbins CA, Swenson LJ, Hardin BD. Risk from Inhaled Mycotoxins in Indoor Office and Residential Environments. Int J Toxicol. 2004;23(1):3–10.

68. Kanchongkittiphon W, Mendell MJ, Gaffin JM, Wang G, Phipatanakul W. Indoor Environmental Exposures and Exacerbation of Asthma: An Update to the 2000 Review by the Institute of Medicine. Environmental Health Perspectives. 2015 Jan;123(1):6–20.

69. Hegarty B, Dannemiller KC, Peccia J. Gene expression of indoor fungal communities under damp building conditions: Implications for human health. Indoor Air. 2017;28(4):548–58.

70. Shaw M. Housing and Public Health. Annu Rev Public Health. 2004 Apr;25(1):397–418.

71. Bonnefoy X. Inadeqate housing and health: an overview. International Journal of Environment and Pollution. 2007;30:1–19.

72. Landrine H, Corral I. Separate and Unequal: Residential Segregation and Black Health Disparities. Ethnicity and Disease. 2009;:1–6.

73. Morello-Frosch R, Lopez R. The riskscape and the color line: Examining the role of segregation in environmental health disparities. Environmental Research. 2006 Oct;102(2):181–96.

74. Jacobs DE. Environmental Health Disparities in Housing. Research and Practice. 2011;:1–9.

75. Krieger J, Jacobs DE, Ashley PJ, Baeder A, Chew GL, Dearborn D, et al. Housing Interventions and Control of Asthma-Related Indoor Biologic Agents. Journal of Public Health Management and Practice. 2010;16:S11–S20.

76. Hill TD, Graham LM, Divgi V. Racial Disparities in Pediatric Asthma: A Review of the Literature. Curr Allergy Asthma Rep. 2011;11(1):85–90.

77. Rosenbaum E. Racial/Ethnic Differences in Asthma Prevalence: The Role of Housing and Neighborhood Environments*. Journal of Health and Social Behavior. 2008;:1–15.

78. Wang C, Abou El-Nour MM, Bennett GW. Survey of Pest Infestation, Asthma, and Allergy in Low-income Housing. Journal of Community Health. 2007 Aug 22;33(1):31–9.

79. Jacobs DE, Kelly T, Sobolewski J. Linking Public Health, Housing, and Indoor Environmental Policy: Successes and Challenges at Local and Federal Agencies in the United States. Environmental Health Perspectives. 2007 Jun;115(6):976–82.

80. Lambertino A, Turyk ME, Curtis L, Persky VW. Asthma Morbidity in Adult Chicago Public Housing Residents. Journal of Asthma. 2009 Jul 2;46(2):202–6.

81. Hynes HP, BRUGGE D, Watts J, Lally J. Public Health and the Physical Environment in Boston Public Housing: A Community-based Survey and Action Agenda. Planning Practice Research. 2000;15:31–49.

82. Brugge D, Vallarino J, Ascolillo L, Osgood ND, Steinbach S, Spengler J. Comparison of multiple environmental factors for asthmatic children in public housing. Indoor Air. 2003 Apr 23;:1–11.

83. López R, Chantarat T, Bozack A, Lopez A, Weiss L. Reducing Childhood Asthma Triggers in Public Housing: Implementation and Outcomes from an East Harlem Community Health Worker Program. Environmental Justice. 2015 Oct;8(5):185–91.

84. Authority NYCH. Nycha 2018 Fact Sheet. 2018 Sep 13;:1–4.

85. Bach V, Waters T. Strengthening New York City's Public Housing. Community Service Society. 2014 Jul 9;:1–32.

86. Rebuilding AFAJ. Weathering The Storm. 2014 May 5;:1–48.

87. Spilak M, Waring MS, Adams RI, Sylvain I. Use of indoor air parameters as indicators of water damage in buildings: Results from a field study of Public and Private housing in New York City. 2016 Feb pp. 1–7.

88. Sylvain I, Adams RI, Taylor JW. A Different Suite: The Assemblage of Distinct Fungal Communities in Water-Damaged Units of a Poorly-Maintained Public Housing Building. *in revision*. :1–100.

89. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. Appl Environ Microbiol. 2006 Jul 4;72(7):5069–72.

90. Bolger A, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.

91. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26(19):2460–1.

92. Kindt R. Package "BiodiversityR" version 2.7-2. 2016 Aug 29;:1–138.

93. Ondov BD, Bergman NH, Phillippy AM. Interactive metagenomic visualization in a Web browser. BMC Bioinformatics. BioMed Central Ltd; 2011 Sep 30;12(1):385.

94. Mandal S, Van Treuren W, White RA, Eggesbø M, Knight R, Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. Microbial Ecology in Health & Disease. 2015 May 29;26(0):1–8.

95. Vesper SJ, McKinstry C, Yang C, Haugland RA, Kercsmar CM, Yike I, et al. Specific Molds Associated With Asthma in Water-Damaged Homes. Journal of Occupational and Environmental Medicine. 2006 Aug;48(8):852–8.

96. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, et al. Best practices for analysing microbiomes. Nature Reviews Microbiology. Springer US; 2018;:1–13.

97. Ege M, Mayer M, Normand A-C, Genuneit J, Cookson WOCM, Braun-Fahrlander C, et al. Exposure to Environmental Microorganisms and Childhood Asthma. The New England Journal of Medicine. 2011 Feb 24;364(8):701–9.

98. Wells CL, Wilkins TD. Clostridia: Sporeforming Anaerobic Bacilli. In: Medical Microbiology. 4 ed. 1996. pp. 1–25.

99. Bisanz JE, Soto-Perez P, Lam KN, Bess EN, Haiser HJ, Allen-Vercoe E, et al. Illuminating the microbiome's dark matter: a functional genomic toolkit for the study of human gut Actinobacteria. bioRxiv. Cold Spring Harbor Laboratory; 2018 Apr 30;:1–15.

100. de Mattos-Shipley KMJ, Ford KL, Alberti F, Banks AM, Bailey AM, Foster GD. The good, the bad and the tasty: The many roles of mushrooms. Studies in Mycology. ELSEVIER B.V; 2016 Nov 11;85:125–57.

101. Ekanayaka AH, Hyde KD, Jones EBG, Zhao Q. Taxonomy and phylogeny of operculate discomycetes: Pezizomycetes. Fungal Diversity. Springer Netherlands; 2018 May 25;90(1):161–243.

102. Northridge J, Ramirez OF, Stingone JA, Claudio L. The Role of Housing Type and Housing Quality in Urban Children with Asthma. J Urban Health. 2010 Jan 9;87(2):211–24.

103. Corburn J, Osleeb J, Porter M. Urban asthma and the neighbourhood environment in New York City. Health Place. 2006;12:167–79.

104. Chew GL, Carlton EJ, Kass D, Hernandez M, Clarke B, Tiven J, et al. Determinants of cockroach and mouse exposure and associations with asthma in families and elderly individuals living in New York City public housing. Annals of Allergy, Asthma & Immunology. American College of Allergy, Asthma & Immunology; 2006 Oct 1;97(4):502–13.

105. Cao P, Wang J-T, Hu H-W, Zheng Y-M, Ge Y, Shen J-P, et al. Environmental Filtering Process Has More Important Roles than Dispersal Limitation in Shaping Large-Scale Prokaryotic Beta Diversity Patterns of Grassland Soils. Microb Ecol. 2016 Apr 12;72(1):221–30.

106. Kivlin SN, Winston GC, Goulden ML, Treseder KK. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecology. 2014 Dec;12:14–25.

107. Korpelainen H, Pietiläinen M. Diversity of indoor fungi as revealed by DNA metabarcoding. Gulick P, editor. Genome. 2017 Jan;60(1):55–64.

108. Weikl F, Tischer C, Probst AJ, Heinrich J, Markevych I, Jochner S, et al. Fungal and Bacterial Communities in Indoor Dust Follow Different Environmental Determinants. Moreau CS, editor. PLoS ONE. 2016 Apr 21;11(4):e0154131–16.

109. Afshinnekoo E, Meydan C, Chowdhury S, Jaroudi D, Boyer C, Bernstein N, et al. Geospatial Resolution of Human and Bacterial Diversity with City-Scale Metagenomics. Cell Systems. The Authors; 2015 Jul 29;1(1):72–87.

110. Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nature Reviews Microbiology. Springer US; 2018;:1–15.

111. Méheust D, Le Cann P, Reboux G, Millon L, Gangneux J-P. Indoor fungal contamination: Health risks and measurement methods in hospitals, homes and workplaces. Critical Reviews in Microbiology. 2014;40(3):248–60.

112. EPA US. Mold Remediation in Schools and Commercial Buildings. 2008 Sep;:1–56.

113. Vesper S, Cox-Ganser JM, Wymer L, Park J-H. Quantification of mold contamination in multi- level buildings using the Environmental Relative Moldiness Index. Journal of Occupational and Environmental Hygiene. Taylor & Francis; 2018 Jun 20;15(1):38–43.

114. Park JH, Sulyok M, Lemons AR, Green BJ, Cox-Ganser JM. Characterization of fungi in office dust: Comparing results of microbial secondary metabolites, fungal internal transcribed spacer region sequencing, viable culture and other microbial indices. Indoor Air. 2018 Jun 7;28(5):708–20.

115. Adams RI, Tian Y, Taylor JW, Bruns TD, Hyvärinen A, Täubel M. Passive dust collectors for assessing airborne microbial material. Microbiome. Microbiome; 2015;3(46):1–11.

116. Hospodsky D, Qian J, Nazaroff WW, Yamamoto N, Bibby K, Rismani-Yazdi H, et al. Human Occupancy as a Source of Indoor Airborne Bacteria. Wold LE, editor. PLoS ONE. Public Library of Science; 2012;7(4):e34867–10.

117. Sylvain I, Taylor JW, Spilak M, Waring MS, Adams RI. Evidence of Disparate Microbial Communities Associated with Water-Damaged Units in New York City Public Housing Residences using High-throughput Sequencing. *in preparation*. pp. 1–40.

118. Cox J, Indugula R, Vesper S, Zhu Z, Jandarov R, Reponen T. Comparison of indoor air sampling and dust collection methods for fungal exposure assessment using quantitative PCR. Environmental Science: Processes & Impacts. Royal Society of Chemistry; 2017 Oct 13;19:1312–9.

119. Hoisington AJ, Maestre JP, King MD, Siegel JA, Kinney KA. Impact of sampler selection on the characterization of the indoor microbiome via high-throughput sequencing. Building and Environment. Elsevier Ltd; 2014 Oct 1;80(C):274–82.

120. Ciaccio CE, Barnes C, Kennedy K, Chan M, Portnoy J, Rosenwasser L. Home dust microbiota is disordered in homes of low-income asthmatic children. Journal of Asthma. 2015 Sep 12;52(9):873–80.

121. EPA. President's Task Force on Environmental Health Risks and Safety Risks to Children. 2012;:1–24.

122. Akinbami LJ, Simon AE, Rossen LM. Changing Trends in Asthma Prevalence Among Children. Pediatrics. 2016 May 12;137(1):e20152354–9.

123. Brown P, Mayer B, Zavestoski S, Luebke T, Mandelbaum J, McCormick S. The health politics of asthma: environmental justice and collective illness experience in the United States. Social Science & Medicine. 2003 Aug;57(3):453–64.

124. Asthma and Allergy Foundation of America. Ethnic Disparities in the Burden and Treatment of Asthma. 2005;:1–60.

125. Williams DR, Sternthal M, Wright RJ. Social Determinants: Taking the Social Context of Asthma Seriously. Pediatrics. 2016 Mar;123(Supplement 3):S174–84.

126. Peters JL, Levy JI, Rogers CA, Burge HA, Spengler JD. Determinants of Allergen Concentrations in Apartments of Asthmatic Children Living in Public Housing. J Urban Health. 2007 Jan 10;84(2):185–97.

127. Thakur N, Oh SS, Nguyen EA, Martin M, Roth LA, Galanter J, et al. Socioeconomic Status and Childhood Asthma in Urban Minority Youths. The GALA II and SAGE II Studies. Am J Respir Crit Care Med. 2013 Nov 15;188(10):1202–9.

128. Digenis-Bury EC, Brooks DR, Chen L, Ostrem M, Horsburgh R. Use of a Population-Based Survey to Describe the Health of Boston Public Housing Residents. Research and Practice. 2008 Jan;:85–91.

129. Weitzman M, Baten A, Rosenthal DG, Hoshino R, Tohn E, Jacobs DE. Housing and Child Health. Current Problems in Pediatric and Adolescent Health Care. Elsevier; 2013 Sep 1;43(8):187–224.

130. Camacho-Rivera M, Kawachi I, Bennett GG, Subramanian SV. Associations of Neighborhood Concentrated Poverty, Neighborhood Racial/Ethnic Composition, and Indoor Allergen Exposures: a Cross-Sectional Analysis of Los Angeles Households, 2006–2008. J Urban Health. 2014 Apr 26;91(4):661–76.

131. Williams DR, Collins C. Racial Residential Segregation: A Fundamental Cause of Racial Disparities in Health. Public Health Reports. 2001;116:1–13.

132. Woods LL II, Shaw-Ridley M, Woods CA. Can Health Equity Coexist With Housing Inequalities? A Contemporary Issue in Historical Context. Health Promotion Practice. 2014 Mar 31;15(4):476–82.

133. National Low Income Housing Coalition. Housing Spotlight. Vol. 2. 2012 Nov pp. 1–6.

134. Heathcott J. The Strange Career of Public Housing. Journal of the American Planning Association. 2012 Sep;78(4):360–75.

135. Hanlon J. Beyond HOPE VI: Demolition/Disposition and the uncertain future of public housing in the U.S. J Hous and the Built Environ. 2nd ed. 2012 Jan 10;27(3):373–88.

136. Blumgart J. The Slow Death of Public Housing. Planning. 2015;:1–9.

137. Goetz EG. The Transformation of Public Housing Policy, 1985–2011. Journal of the American Planning Association. 2012 Sep;78(4):452–63.

138. Goetz EG. New Deal Ruins. Cornell University Press; 2013. 257 p.

139. Popkin SJ, Cunningham MK, Burt M. Public housing transformation and the hard-to-house. Housing Policy Debate. 2005 Jan;16(1):1–24.

140. Imbroscio D. Beyond Mobility: The Limits Of Liberal Urban Policy. Journal of Urban Affairs. 2011. pp. 1–20.

141. Crowley S, Pelletiere D. Affordable Housing Dilemma: The Preservation Vs. Mobility Debate. National Low Income Housing Coalition. 2016 May 1;:1–48.

142. Manjarrez CA, Popkin SJ, Guernsey E. Poor Health: Adding Insult to Injury for HOPE VI Families. Vol. 5, Ubran Institute. 2007 Jun pp. 1–10.

143. Popkin SJ, Levy DK, Buron L. Has Hope Vi Transformed Residents' Lives? New Evidence From The Hope Vi Panel Study. Housing Studies. 2nd ed. 2009 Jul;24(4):477–502.

144. Keene DE, Geronimus AT. “Weathering” HOPE VI: The Importance of Evaluating the Population Health Impact of Public Housing Demolition and Displacement. J Urban Health. 2011 May 24;88(3):417–35.

145. Ruel E, Oakley D, Wilson GE, Maddox R. Is Public Housing the Cause of Poor Health or a Safety Net for the Unhealthy Poor? J Urban Health. 2010 Jun 29;87(5):827–38.

146. Dorevitch S, Demirtas H, Perksy VW, Erdal S, Conroy L, Schoonover T, et al. Demolition of High-Rise Public Housing Increases Particulate Matter Air Pollution in Communities of High-Risk Asthmatics. Journal of the Air & Waste Management Association. 2012 Feb 27;56(7):1022–32.

147. Ashley PJ. HUD's Healthy Homes Program: Progress and Future Directions. Journal of Environmental Health. 2015 Sep;:1–5.