

RESEARCH ARTICLE

The short-term effect of residential home energy retrofits on indoor air quality and microbial exposure: A case-control study

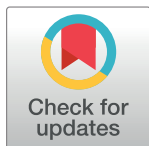
Mytien Nguyen¹^{2a}, Eric C. Holmes²^{ab}, Largus T. Angenent^{1,2,3}^{*}

1 Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, United States of America, **2** Department of GeoSciences, University of Tübingen, Tübingen, Germany, **3** Max Planck Institute for Developmental Biology, Tübingen, Germany

^{2a} Current address: Yale University School of Medicine, New Haven, CT, United States of America

^{2b} Current address: Stanford University, Stanford, CA, United States of America

* l.angenent@uni-tuebingen.de



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Abstract

Weatherization of residential homes is a widespread procedure to retrofit older homes to improve the energy efficiency by reducing building leakage. Several studies have evaluated the effect of weatherization on indoor pollutants, such as formaldehyde, radon, and indoor particulates, but few studies have evaluated the effect of weatherization on indoor microbial exposure. Here, we monitored indoor pollutants and bacterial communities during reductions in building leakage for weatherized single-family residential homes in New York State and compared the data to non-weatherized homes. Nine weatherized and eleven non-weatherized single-family homes in Tompkins County, New York were sampled twice: before and after the weatherization procedures for case homes, and at least 3 months apart for control homes that were not weatherized. We found that weatherization efforts led to a significant increase in radon levels, a shift in indoor microbial community, and a warmer and less humid indoor environment. In addition, we found that changes in indoor airborne bacterial load after weatherization were more sensitive to shifts in season, whereas indoor radon levels were more sensitive to ventilation rates.

Introduction

Twenty-two percent of the total U.S. energy consumption is used in residential buildings, mainly to heat and cool homes [1]. Since the energy crisis of the 1970s, national policy discussions have been held on energy efficiency and energy conservation [2]. This led to the establishment of building energy codes such as the 1975 ASHRAE Energy Code and the Energy Policy and Conservation Act (EPCA) [3]. Included in the EPCA was the creation of the Weatherization Assistance Program (WAP) by the Department of Energy [4]. Weatherization is the practice of protecting a building from environmental fluctuations to reduce energy consumption in the building by air sealing and insulation of the home (only air sealing reduces the air leakage of a building). WAP provides financial assistance to low-income households in single-

family dwellings to weatherize their home [4, 5]. Besides improving the household energy efficiency, weatherization also provides an additional cost-saving incentive by lowering the heating expenses and by improving the thermal comfort in the home. Since the implementation of WAP in 1976, more than 7 million homes have been weatherized [6].

According to the National Human Activity Pattern Survey (NHAPS), United States residents spend 80% of their time indoors [7]. During the COVID-19 pandemic, this percentage has risen dramatically after the U.S. entered a national lockdown [8, 9]. Furthermore, research has shown that COVID-19 remains active in indoor air for up to 3 hours [10]. Therefore, the indoor environment has been increasingly recognized for its relevance to human health and well-being [11]. Prior studies on the effects of residential ventilation on indoor exposure has led to a continued concern about the impact of weatherization on human health [12–18]. The World Health Organization (WHO) established a set of 12 housing inadequacies that had sufficient evidence for estimating disease burden [19]. These 12 housing inadequacies include indoor temperature, home energy efficiency, radon exposure, and humidity in dwellings. One of the main outcomes of weatherization is air leakage control, or the sealing of cracks and bypasses in the building envelope, to reduce energy loss *via* air leakage [5]. As a result, indoor-sourced pollutants, such as radon, formaldehyde, and biological pollutants, can accumulate in indoor air [20]. Logue et al. identified 31 chemical pollutants and 9 priority chemical pollutants that are important in considerations of indoor residential health. Radon is among the 31 pollutants identified, though, it is not a priority pollutant. The 9 priority chemical pollutants includes particulate matter of 2.5 μm and smaller in aerodynamic diameter, which particle mass in one cubic meter of air is known as $\text{PM}_{2.5}$ [21]. $\text{PM}_{2.5}$ in the home can originate from outdoor air, while $\text{PM}_{2.5}$ also can be generated inside the home by, for example, combustion [22–24]. The contribution of outdoor $\text{PM}_{2.5}$ to indoor concentrations can range from 23% to 70% [23, 25, 26]. Epidemiological studies have also found PM_{10} to be a relevant indicator for health effects [27]. Both $\text{PM}_{2.5}$ and PM_{10} have been associated with decreases in lung function and exacerbations of respiratory diseases [28].

Current research shows varying impact of weatherization on indoor pollutants and respiratory health. Radon is a radioactive gas that is the leading cause of lung cancer among non-smokers [29]. Studies have found that weatherization leads to an 0.44 pCi L^{-1} increase in radon concentration [30] or did not significantly affect radon concentration when controlled for environmental parameters [31]. On the contrary, a World Health Organization review concluded that household energy improvements in Europe lead to improved respiratory health [32].

A more recent consideration of indoor health is the microbial composition of indoor air, which is referred to here as the indoor microbiome, and which can persist in air or settle on surfaces and carpets. The residential indoor microbiome has become a growing consideration in public health because of its proposed role in shaping the human immune development in allergic diseases [33, 34]. The biodiversity hypothesis states that a reduced human contact with microbiota in early life will lead to inadequate immune development [35]. Studies of the indoor microbiome have shown that ventilation and occupancy rates are important drivers of its microbial composition. Skin-associated bacteria have been commonly found in the indoor microbiome [36, 37]. In addition, bacterial community diversity and richness were found to be lower in naturally ventilated homes compared to mechanically ventilated homes. Specifically, Miletto and Lindow found that airborne bacterial load increased with increasing frequency of natural ventilation, occupancy, and indoor activity in residential homes [38].

Although one of the main aims of weatherization is to decrease the natural ventilation rate in residences, the direct effect of weatherization on the indoor bacterial community composition is yet to be studied. This study aims to gain some insight into the effect of weatherization

on the residential indoor environment and human health in a small-scale case-control study. We examined the indoor air quality and bacterial community of single-family residential homes before and after weatherization procedures. We compared these weatherized case homes to similar single-family control residential homes that did not undergo weatherization.

Methods

Study design

This study was conducted in Tompkins County, NY, which is an area of approximately 100,000 inhabitants located in the International Energy Conservation Code (IECC) Climate Zone 5A (cold and moist). Twenty single families with residential homes that were located in Tompkins County, NY were recruited for this study on a voluntary basis. The Institutional Review Board at Cornell University's Office of Research Integrity and Assurance specifically approved this study. All study homes were chosen to represent these characteristics; the house: (1) was occupied by one family; (2) was a smoke-free environment; (3) included a basement; (4) was constructed before 1960; (5) was occupied continuously for 6-months prior to enrollment in the study, as well as throughout the study period; and (6) was not located near a waste management plant. All home visits and sampling were conducted between November 2014 and May 2016 (S1 Table). Each home was sampled twice. Detailed methodology can be found in S1 File.

Each sampling campaign spanned between 3 and 4 continuous days. Equipment and samplers were installed on the first day. Upon arrival to the study home, we conducted a survey of the house with the homeowner to identify ideal locations for equipment placement and to note air quality concerns and water damage. In the meantime, the homeowners were instructed in detail about the sampling setup and protocol. Homeowners were also given a questionnaire package that included: building survey; occupant health and activities survey for each occupant; and an hourly occupancy log sheet for each floor of the house (S2 File). After initial set up, all equipment and samplers were left on for 3–4 days. After 3–4 days, equipment and samplers were turned off and removed from the house. During this visit, we also collected household samples of dust and floor surfaces, and measured building dimensions. This was replicated for the second sampling campaign.

Building leakage

We measured the building leakage *via* the blower-door test (Retrotec, Everson, WA) in cubic meter *per* h at 50-pascal (CMH₅₀). We calculated the air change rate at 50-pascal (ACH₅₀) by normalizing the CMH₅₀ by the building volume [39]:

$$ACH_{50} = \frac{CMH_{50}}{V_{house}} \quad (1)$$

ACH₅₀ = Air changes *per* h at 50 pascal (h⁻¹)

CMH₅₀ = Building leakage (m³h⁻¹)

V_{house} = House volume (m³)

During each sampling campaign, the air change rate was also measured *via* tracer gas (CO₂) decay to estimate the natural ventilation rates (ACH_{CO2}). The indoor and outdoor CO₂ levels were simultaneously measured with a Li-COR CO₂ Monitor (Li-COR Biosciences, Lincoln, NE) and an SBA-5 CO₂ Gas Analyzer (PP Systems, Amesbury, MA), and recorded with data loggers (HOBO 4-Channel Analog Data Logger, Onset Computer Corporation, Bourne, MA). After sampling, we calculated the air change rate *per* h as an average of the decay rates

during the 3–4 days sampling period, using the following equation from Laussmann and Helm [40]:

$$\lambda = \frac{\ln C_f - \ln C_i}{t_f - t_i} \quad (2)$$

λ = ACH_{CO₂} = air changes *per* h (h⁻¹)
 C_i = Initial CO₂ concentration (ppm)
 C_f = Final CO₂ concentration (ppm)
 t_i = Initial time (h)
 t_f = Final time (h)

Recording environmental conditions and radon concentrations

The indoor temperature and the relative humidity for 11 locations inside the house were recorded every min with temperature- and relative-humidity-loggers (HOBO UX100-003 Temperature-Relative Humidity data logger, Onset Computer Corporation). Both the basement and living area radon levels were measured simultaneously during each sampling campaign. The basement radon levels were measured with the Short Term Liquid Scintillation Kit (AccuStar, Medway, MA) to obtain a time-average. The living room radon levels, on the other hand, were measured hourly with the RadStar RS800 Continuous radon monitor (AccuStar).

Air, surface, and dust samples collection

The indoor and outdoor air samples were collected through impaction onto 0.2- μ m pore sized polycarbonate membrane filters (Nucleopore Track-Etched Polycarbonate Membrane, GE Healthcare Biosciences, Pittsburgh, PA) at 60-70-lpm in a 4-stage Andersen cascade impactor (Tisch Environment Inc., Cleves, OH), with size bins (μ m): <2.1; 2.1–4.7; 4.7–9.0; and >9.0. All 90-mm diameter membranes were cut to 81-mm diameter in a laminar flow hood with flame- and ethanol-sterilized scissors to fit the Andersen impactor. We included one field blank for each set of air samples. The indoor air sampler was located in the living room area and at least 1-m elevated from the floor surface. Most indoor air samplers were elevated to eye-level at approximately 1.5-m from the floor. The outdoor air sampler was placed between 0.6-m to 2-m from the building exterior and elevated between 0.5-m to 2-m from the ground. After sampling, we weighed all filters before and after sampling using a Mettler Toledo Microbalance (Columbus, OH) with a resolution of 0.01 mg. Floor-surface samples were collected using sterile wipes. We collected carpet-dust samples using a 3-stage sampling cassette (Zefon International Inc., Ocala, FL), which had been in a sterile, DNA-free laminar flow hood with a sterile 37-mm, 0.4- μ m-pore size polycarbonate membrane filter (Steriltech Corporation, Kent, WA). All air, dust, and floor surface samples were stored at -20°C *prior* to further processing.

Sample processing and DNA extraction

We conducted the sample processing and DNA extraction in a laminar-flow hood to prevent laboratory contamination. The surface samples from wipes were eluted using a method described in Yamamoto et al. [41]. In addition, the wipes were submerged in 100 mL of auto-claved- and UV-sterilized phosphate buffer saline (PBS) solution with 0.1% Tween-80 and then shaken at 250 rpm for 6 h. The eluted liquid was filtered through a 0.2- μ m pore size, gridded Supor® membrane filter funnel (Pall Corporation, Port Washington, NY). One quarter of each air and wipes filter and approximately 100 mg of coarse dust from the sampling cassette were added directly to DNA extraction tubes.

The DNA extraction method for all samples was described previously [41]. Briefly, samples were bead-beaten for 30 s to elute biomass from filters and then cell walls were broken down with lysozyme (15 mg mL⁻¹) at 37°C for 30 min, and proteinase K (0.4 mg mL⁻¹) in SDS at 56°C for 60 min. DNA was further extracted with a bead-beating procedure at room temperature for 3 min and then purified in an equal volume of a solution that consisted of phenol:chloroform:isoamyl alcohol (25:24:1 ratio). After these steps, we completed the DNA concentration and purification with the MO BIO PowerSoil solutions C2 to C6 (MO BIO Laboratories Inc., Carlsbad, CA).

Enumeration of total airborne bacterial load

Indoor airborne bacterial load was quantified using a *B. subtilis* standard in a quantitative polymerase chain reaction (qPCR) targeting the V3-V4 region of the universal 16S rRNA gene, as previously described by Nadkarni et al. [42] on an ABI 7300 real-time thermocycler system (Applied Biosystems) using the following: probe (5' - /56-FAM/CGTATTACC/ZEN/GCGGCTGCTGGCAC/3IABkFQ/-3'), forward primer (334F: 5' -TCCTACGGGAGG CAGCAGT-3'), and reverse primer (806R: 5' -GGACTACCAGGGTATCTAATCCTGTT-3').

PCR and sequencing

To analyze the bacterial community of air, dust, and surface samples, the V4 variable region of the 16S rRNA gene was amplified and sequenced. DNA was amplified using the Earth Microbiome Project protocol (www.earthmicrobiome.org), using 192 Golay-barcoded 806 reverse primers (R: 5' -GGACTACHVGGGTWTCTAAT-3') and the 515 forward primer (F: 5' -GTG CCAGCMGCCGCGGTAA-3') [43]. The amplicons were purified using magnetic beads with the MagBind RxnPure (Omega BioTek, Norcross, GA). All samples were quantified with the Quant-iT™ PicoGreen® dsDNA Assay (Promega, Eugene, OR) with the lambda standard (Invitrogen, Carlsbad, CA), and pooled into three separate libraries at 4-ng amplicon dsDNA *per* sample. Libraries were sequenced with the 2 x 250-bp paired end Illumina MiSeq platform at the Cornell Genomics Facility (Cornell University Institute of Biotechnology, Ithaca, NY).

Bacterial community analysis

Raw sequences were quality filtered and demultiplexed in QIITA and QIIME v1.9.0 [44]. Sequences were clustered into operational taxonomic units (OTUs) based on 97% similarity using the sortmerna method [45]. Recent discussions focusing on the best method for analysis of marker gene sequences have focused on OTUs *versus* exact sequence variances (ESV). Although there is a certain robustness associated with ESVs, statistical inferences were indistinguishable between ESV and OTU analyses for ecological samples [46]. Given this similarity, we opted to analyze our sequences with OTUs. The OTUs were aligned against the Greengenes database v13.8 [47] using PyNAST [48]. Further analyses were performed with MacQIIME (www.wernerlab.org). Rare taxa (singletons) were removed from the bacterial community analysis. The diversity of the bacterial community within each sample was calculated using the Shannon index [49], which calculates both the richness and evenness of a community.

Statistical analysis

The bacteria concentration in air was log-normalized to account for skewness in bacteria counts *prior* to correlation tests. We tested group comparisons using student's paired and unpaired *t*-tests. In addition, group comparisons of dichotomous variables were tested using the Cochran-Mantel-Haenszel test. *P*-values less than 0.05 were considered significant.

Correlations between samples was determined by calculating the partial Pearson r to control for covariates using the function *pcor.test* in R. ANOVA was utilized to determine significant environmental parameters in multiple linear regressions.

Results

The sizes of the 9-case and 11-control single-family homes in this study varied from a 272-m³ house volume to a 1518-m³ house volume, with 1–3 floors (Table 1). We did not find a significant difference in building volume and numbers of floors between case and control homes (Table 1). The maximum carpet coverage of floors for all homes was only 25%. Within this relatively small coverage, the case homes had a significantly larger carpet coverage compared to control homes (Table 1). Both case and control houses had an assortment of different heating systems, including hot-air circulation, wood stove, hot-water heating, and electric heating (Table 1). Most basements were unfinished, with the exception of 2 case homes and 3 control homes. Indoor plants were present in 6 out of 9 case homes, while they were present in all control homes. Three case homes and 1 control home were occupied by people who were diagnosed with asthma, while 6 case homes and 7 control homes had occupants with environmental allergies (Table 1). In addition, in 7 case homes and 7 control homes, occupants reported having respiratory symptoms when inside the home, including: sinusitis, intermittent sneezing, and sore-dry throat (Table 1). However, no significant differences in basement type, indoor plants, asthma, allergies, and respiratory symptoms were found between case and control homes (Table 1).

Air-sealing effectiveness of weatherization

Weatherization efforts for the case homes were performed by a local company that had been contracted by the city, and included a combination of air sealing to reduce natural ventilation, as well as attic or basement insulation to improve thermal comfort. For the 9 case homes, improved air sealing after weatherization resulted in an average total decrease in building leakage of 1690 m³ h⁻¹ (± 887 m³ h⁻¹) (Table 1), corresponding to an average decrease of 21.8% ($\pm 9.68\%$) from the pre-retrofit building leakage measurement (ordered from low to high in Fig 1A). When these building leakage values from blower-door tests were corrected for house volume, the magnitude of impact changed, with an average decrease in ACH₅₀ of 0.7 h⁻¹ (Fig 1A). For the combined data points, the difference in natural ventilation rate for the 9 case homes between the sampling events was significantly more negative than the control homes (Fig 1B), showing again a measured effect of weatherization. The natural ventilation rate for each individual case home either decreased or remained the same after weatherization, with an average decrease in ACH_{CO2} of 0.7 h⁻¹ (Fig 1B). Natural ventilation for the control homes fluctuated within an air change of ± 1 h⁻¹, with an average air change of 0.2 h⁻¹ for all data points during the two sampling events, but did not differ significantly (Fig 1B). The reduction in natural ventilation rates in case homes correlated with an increase in indoor-outdoor temperature ratio (S2 Table). Indeed, we found that weatherization resulted in a significant increase in indoor-outdoor temperature ratio, as well as a significant decrease in indoor-outdoor relative humidity ratio (Fig 1C and 1D). These results showed that weatherization reduced the leakiness and natural ventilation rates of older homes in Tompkins County, NY, leading to improved shielding from the outdoor environment.

Effect of weatherization on occupant health and indoor exposure

Occupant-reported surveys of health symptoms indicated no changes in homebound respiratory symptoms between sampling periods for both case and control homes. These symptoms

Table 1. Characteristics of study homes and weatherization.

Building Volume (m ³)	# Floors ¹	Pre-Weatherization Building Leakage (m ³ h ⁻¹)	Δ Building Leakage (m ³ h ⁻¹)	Est. % Carpet ²	Heating	Basement	Household size (# occupants # children)	Occupant Density (person / 10 ² -m ²)	Indoor Plants	Asthma (# occupants)	Allergies (# occupants)	Respiratory Symptoms ²	Δ Respiratory Symptoms	Δ Thermal Comfort
448	2	4460	-1400	5	Hot air	Unfinished	3 (1)	2.04	No	0	0	2	No	Yes
777	2	5862	-850	25	Hot air	Unfinished	3 (1)	1.18	No	0	2	2	No	Yes
1336	2	17364	-1682	15	Hot air	Unfinished	2 (0)	0.46	Yes	1	1	1	No	Yes
352	1	3364	-595	15	Wood	Finished ³	4 (1)	3.46	Yes	0	2	2	No	No
285	2	10704	-3058	10	Hot air	Unfinished	3 (1)	3.21	No	2	2	2	No	Yes
272	2	13592	-3058	5	Hot water	Unfinished	3 (0)	3.36	Yes	0	1	2	No	Yes
1518	1	3367	-987	15	Electric	Finished	2 (0)	0.40	Yes	1	2	3	No	No
509	3	7985	-1954	5	Wood	Unfinished	3 (1)	1.80	Yes	0	0	0	No	No
553	2	8835	-1631	15	Hot air	Unfinished	1 (0)	0.55	Yes	0	0	0	No	No
378	2	4018	--	5	Wood	Unfinished	2 (0)	1.61	Yes	0	2	3	No	No
527	3	7747	--	10	Hot water	Unfinished	5 (2)	2.89	Yes	0	2	0	No	No
513	2	9531	--	5	Hot air	Unfinished	5 (2)	2.97	Yes	0	1	1	No	No
464	1	3041	--	5	Hot air	Finished	6 (2)	3.94	Yes	0	1	2	No	No
618	2	3933	--	5	Wood	Unfinished	3 (1)	1.48	Yes	0	0	1	No	No
499	2	3169	--	5	Hot air	Finished ¹	4 (1)	2.44	Yes	0	1	1	No	No
715	2	10534	--	5	Hot water	Unfinished	5 (2)	2.13	Yes	1	0	2	No	No
570	1	4749	--	5	Hot water	Finished	2 (0)	1.07	Yes	0	2	2	No	No
523	2	14136	--	1	Wood	Unfinished	3 (1)	1.75	Yes	0	0	0	No	No
778	2	3203	--	5	Wood	Unfinished	1 (0)	0.39	Yes	0	0	0	No	No
412	2	5649	--	20	Hot air	Unfinished	2 (0)	1.48	Yes	0	1	3	No	No
Avg. Δ means ±145.00	0.22 ±0.24	1498±1927	--	5.14 ±2.66	--	--	0.62±0.61 (0.36±0.34)	0.05±0.50	--	0.25±0.24	0.60±0.36	0.21±0.48	--	--
p-value ⁴	0.38	0.29	--	0.04	--	0.64	0.32 (0.30)	0.91	0.15	0.16	0.61	0.70	0.65	0.02

¹ Excluding the basement

² Percent of total floor area that are covered by rugs and carpets

³ Partially finished basement

⁴ Comparison between case and control homes

Δ: defined as value at second sampling event minus value at first sampling event (building leakage) or different answer at questionnaire (respiratory symptoms and heating comfort).

Case homes are shaded in grey, and control homes are in white.

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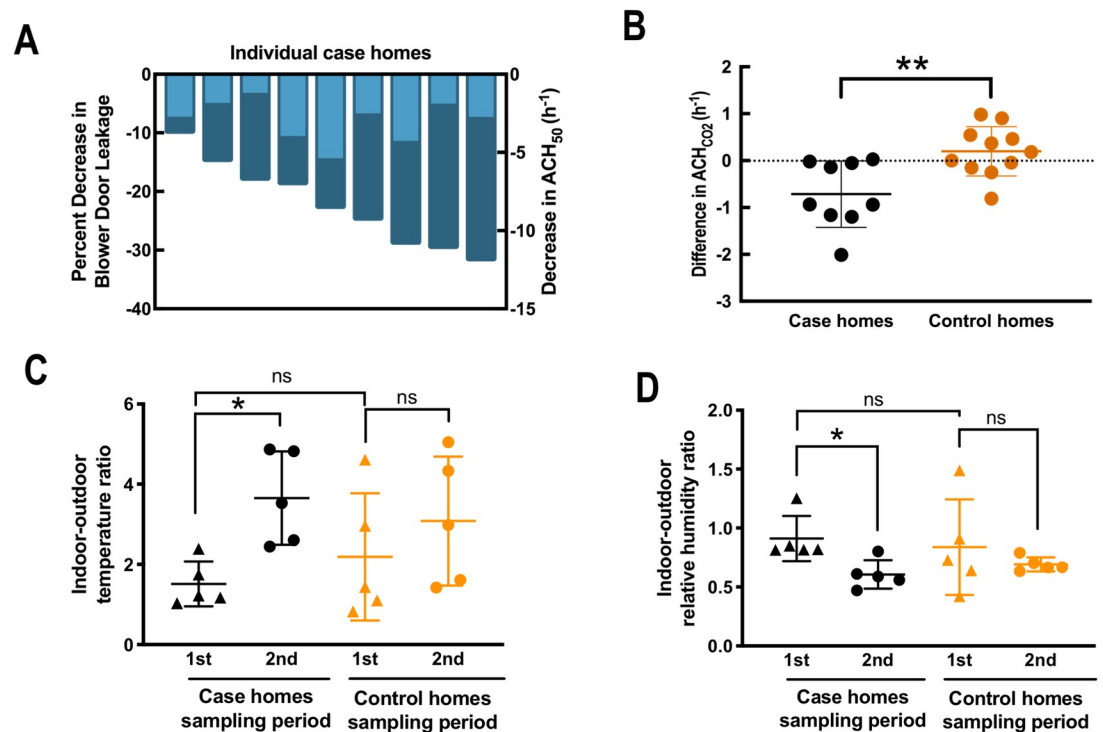


Fig 1. Building leakage, temperature, and relative humidity. A) Decrease in building leakage (dark blue; left y-axis) and decrease in air changes *per h* at 50 pascal depressurizations (ACH_{50}) (light blue; right y-axis) for each case home are shown as negative numbers. Both were measured by the blower-door test. Greater decreases of building leakage were used as the order to visualize the data. The decrease in building leakage is presented as a percent of pre-weatherization building leakage in each case home. B) Decreases in air change *per h* under normal pressure conditions as measured by carbon dioxide tracer gas decay are shown as negative numbers. Black indicates case homes, and orange indicates control homes. A more negative change indicates a greater decrease in ventilation rate between the first and second sampling for case and control homes. C) Average indoor-outdoor ratio for temperature during the first and second sampling periods for case and control homes. D) Average indoor-outdoor ratio for relative humidity during the first and second sampling periods for case and control homes. *: $p < 0.05$; **: $p < 0.01$.

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included sneezing, sore and dry throat, coughing, and exacerbations of pre-existing respiratory diseases (Table 1). Interviews with occupants of only the case homes indicated a significant increase in thermal comfort (Table 1) and noticeably lower heating requirements (data not shown). We did not observe significant differences in basement and living-area radon levels between case and control homes for the first sampling period when the data was averaged (Fig 2A and 2C). Weatherization increased the *basement* radon levels for the case homes, while no significant change in the average basement radon level in control homes was observed between the first and second sampling event (Fig 2A). No significant difference for any of the comparisons in average *living-area* radon levels was observed (Fig 2C). We then re-analyzed the same radon level data by calculating the difference in radon levels between the first and second sampling periods for each individual home (Fig 2B and 2D). Again, we found that the average differences in *basement* radon levels in case homes were significantly greater than those in control homes (Fig 2B). The average difference in radon levels between the first and second sampling periods in the living area was also significantly greater in case homes compared to control homes (Fig 2D). Thus, we observed that radon levels increased during weatherization of these older homes in Tompkins County, NY.

We collected indoor particulates and filtered them into four size bins based on particulate diameter: particulate matter greater than 9.0- μm in diameter (PM_{9+}); particulate matter

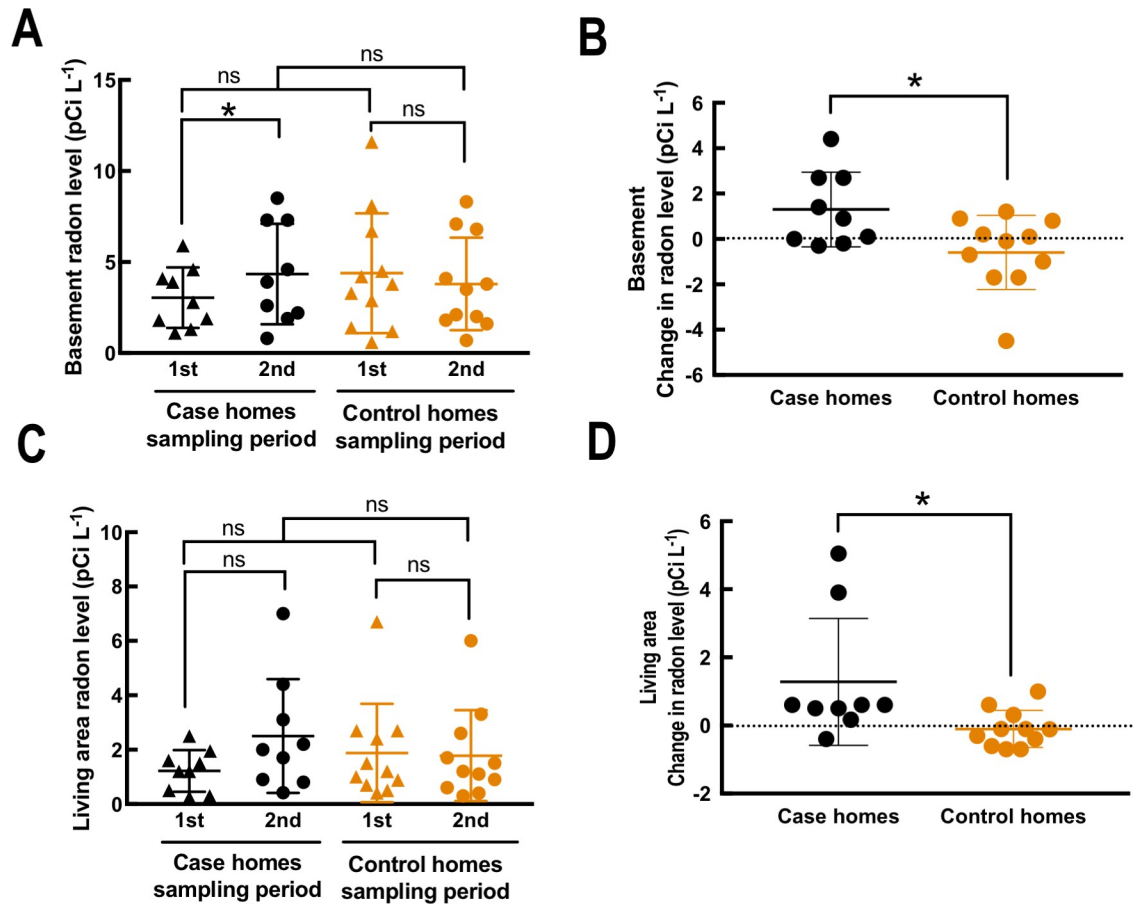


Fig 2. Radon levels in residential homes. Radon levels (A,C) and differences in radon levels (B,D) in the first floor living area (A,B) and basement (C,D) between sampling periods. A,C) The average radon level during each sampling period is shown for the living room area (A) and the basement (C), with black points corresponding to case homes and orange points corresponding to control homes. Triangles represent the first sampling period, and circles represent the second sampling period. Significance between sampling periods were determined using the paired *t*-test, while significance between the 1st sampling periods of case and control homes were determined using the unpaired *t*-test. B,D) The differences in radon levels is colored by house, with black for case homes, and orange for control homes. A positive value indicates that radon level increased in the second sampling period compared to the first sampling period.

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between 4.7- μm and 9.0- μm in diameter ($\text{PM}_{4.7-9}$); particulate matter between 2.1- μm and 4.7- μm in diameter ($\text{PM}_{2.1-4.7}$); and particulate matter between 0.22- μm and 2.1- μm in diameter ($\text{PM}_{2.1}$). The size-separation sampling was performed twice for each home as part of the experimental design. We measured the mass of the sample for each size bin, resulting in consistent average particulate matter mass concentrations below $5 \mu\text{g per m}^3$ per size bin with only a few outliers (S1 Fig). For three out of four size bins, a small increase in average particulate matter mass concentration was found for the case homes between the pre-weatherization and the post-weatherization event, but this was not significant (S1 Fig). No trend or significant difference were observed for the control homes. Based on the same measurements, the indoor-outdoor (IO) ratios of each size bin were computed for each home and sampling event by dividing the indoor particulate matter mass concentration by the outdoor particulate matter mass concentration (S2 Fig). When these IO ratios were then averaged per each home category and sampling period, the inside mass concentration was always higher than the outside mass concentration for all size bins (above the dotted line [1] in S2 Fig). For case homes, the average

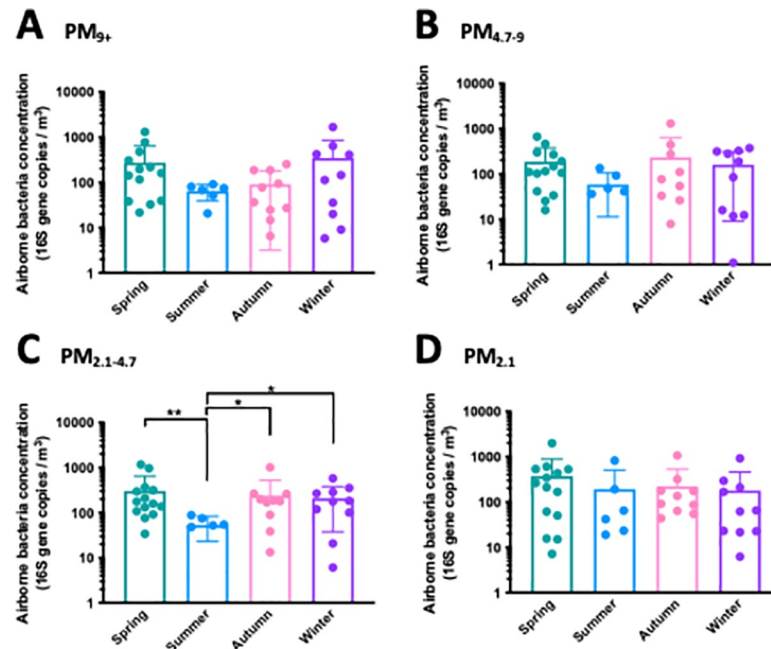


Fig 3. Airborne bacteria concentration in indoor air. Concentration of bacteria in indoor air across all homes, in 16S gene copies *per m*³. All air samples from both sampling periods and all size bins were consolidated and categorized by season based on date of sample collection. Bars are colored based on season, with the following grouping: Spring: green, *n* = 14; Summer: blue, *n* = 6; Autumn: pink, *n* = 10; Winter: purple, *n* = 10. The unpaired *t*-test was used to determine significance between seasons. Significant differences are shown. *: *p* < 0.05, **: *p* < 0.01.

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IO ratios showed small increases in IO ratio with weatherization for all size bins. However, these trends were again not significant (S2 Fig). For control homes, no trend or significant changes in IO ratios were found for all size bins (S2 Fig).

We also measured the average airborne bacteria concentration in each size bin with qPCR, which was approximately 200 16S rRNA (16S) gene copies *per m*³ of air and did not change significantly between sampling periods for both case and control homes (S3 Fig). In addition, when we consolidated all air samples from both case and control homes, we found that seasons are a major determinant of the airborne bacterial concentration. Overall, airborne bacteria concentrations in the home were lower in the summer season and differed throughout the season, however, this was only significant for PM_{2.1-4.7} and not for the other size bins (Fig 3). Within each season, there was no significant difference between the particulate matter size bins (S4 Fig).

Assessment of bacterial community diversity

We also sequenced the bacterial 16S rRNA genes from extracted genomic DNA from: (1) the size-separation air sampler (indoor and outdoor); (2) the dust samples; and (3) the floor-surface samples. The Shannon index (alpha diversity) was chosen for its robustness in calculating both the evenness (the distribution of species within a community) and richness (the number of unique species in a community). A community with many different species that are evenly distributed will have a high Shannon index. We calculated the difference in outdoor and indoor alpha diversity by subtracting the Shannon index from the outside air sample with the Shannon index from the inside air sample of the individual home for the corresponding size bin (S5A Fig). A negative value represents a higher diversity indoors compared to outdoors.

We observed a trend with smaller particulates having a higher diversity indoors vs. outdoors, resulting that $PM_{2.1}$ was characterized with the greatest indoor-outdoor difference for both case and control homes in both sampling events (S5A Fig). No significant difference in indoor vs. outdoor diversity was observed between case and control homes ($p = 0.117$ [PM_9], 0.404 [$PM_{4.7-9}$], 0.725 [$PM_{2.1-4.7}$], 0.271 [$PM_{2.1}$]) (S5A Fig).

Similarly, we compared the Shannon index from the indoor air for each home between the first and second sampling event (second minus first), indicating that a negative value would show a higher diversity after weatherization for the case homes. However, there was no significant difference in each particulate-matter size bin (S5B Fig). We then also performed the same comparison for dust and floor-surface samples, but again we did not find significant differences (even though, the carpeted areas within the living area of case homes looked like they were less diverse in bacterial composition ($p = 0.09$) compared to the control homes) (S6 Fig). A multiple linear regression analysis with the Shannon indices from indoor samples (air, dust, and floor) and environmental factors, such as occupancy, ventilation rate, temperature, relative humidity, and season only found that ACH_{50} was found to be significantly correlated with the Shannon index of $PM_{4.7-9}$ ($p = 0.003$).

Relationship between air, dust, and floor-surface bacterial communities

The relationships between air, dust, and floor-surface bacterial communities were calculated using the weighted UniFrac metric (beta diversity), which determines a distance of dissimilarity based on differences in phylogeny and abundance between samples. An overview of all air, dust, and floor-surface bacterial communities from each sample shows that dust and floor-surface areas samples were more similar to each other than to air sample (S7 Fig), although a considerable overlap between sample types was observed. To relate samples more quantitatively, the UniFrac distances from the first sampling period were subtracted from the UniFrac distances from the second sampling period (2nd sampling event minus 1st sampling event) for each home. Differences in UniFrac distance between corresponding pairs of indoor and outdoor air samples showed a significant positive value in case homes between sampling periods (Fig 4). This was not observed in control homes (S8 Fig), which indicates that for case homes after the second sampling event, the indoor air bacterial community diverged more from the outdoor air community (the communities were more dissimilar during the second sampling event than the first sampling event). In addition, through the same calculation method we also compared: indoor air and living area carpet-dust samples; and indoor air and living-area floor-surface samples. No significant differences were observed, though, for these comparisons in both case and control homes (Fig 4; S8 Fig).

Drivers of indoor air microbiome composition

To determine environmental factors that drive the indoor airborne microbiome composition, we consolidated all indoor air samples from case and control homes. Next, constrained ordination was utilized to correlate microbiome composition to environmental factors *via* multiple linear regression. We maintained the samples separated by the particulate size bins, and therefore we would include factors that not only drive compositional changes in the airborne bacteria community between sampling periods, but also factors that can potentially drive compositional difference between particle size bins, such as evaporation and condensation. However, we did not observe other factors than the environmental factors to drive the microbial composition. We included these changes in environmental factors in the ordination: indoor-outdoor temperature; indoor-outdoor relative humidity; occupancy rate; occupant density; percent the house is occupied; ventilation rate; average wind speed; and average rain-

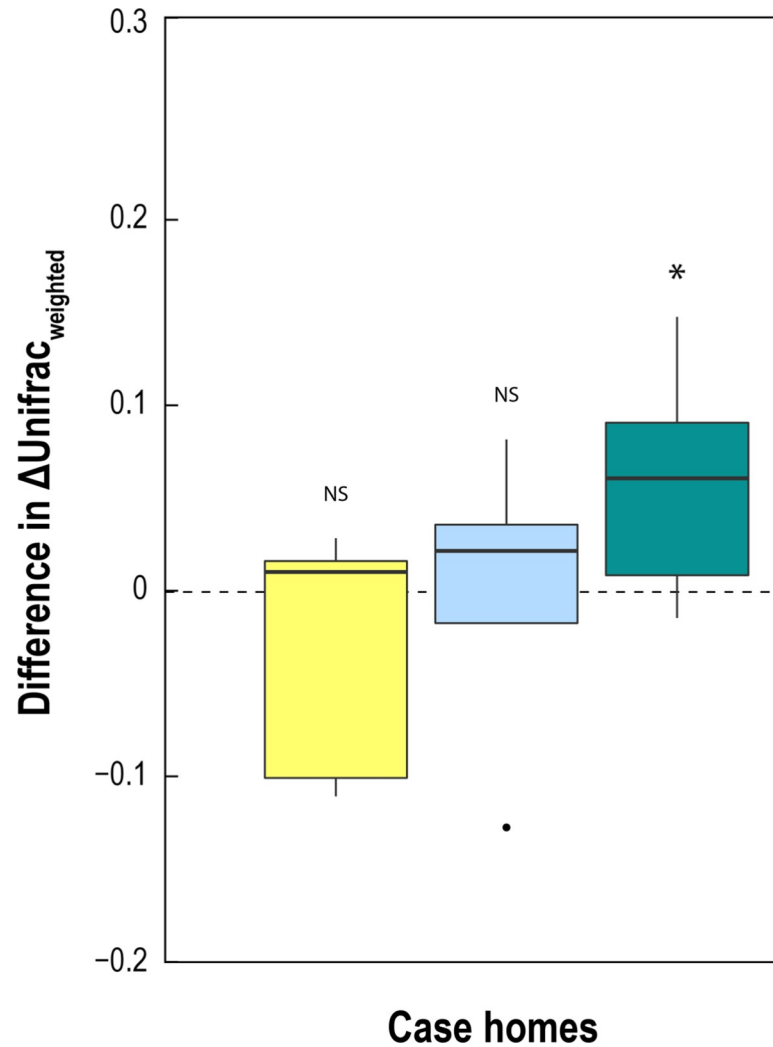


Fig 4. Similarities between air, dust, and floor-surface bacterial communities in case homes. Indoor airborne bacteria communities in each air particle size bins were compared to their corresponding outdoor airborne bacteria communities (turquoise). The same indoor air samples were also compared to living room floor surface (yellow) and living room carpet dust (blue) communities. Positive values indicate that two communities are more dissimilar in the second sampling period compared to the first sampling period. Negative values indicate that the two communities became more similar.

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snow. Of these, four environmental conditions were found to significantly drive the changes in the indoor airborne bacteria composition (in the order of importance): (1) occupant density; (2) ACH_{50} ; (3) indoor temperature; and (4) indoor relative humidity (Fig 5).

Quality control for microbial community analysis

We compared each sample type and their corresponding processing blank, *prior* to the above analyses of bacterial community in air, carpet dust, and floor-surface areas. The bacterial communities of samples for one home, processing blanks, and the hand microbiome of a researcher are summarized in S9 Fig at the phylum level. The bacterial communities of air, carpet dust, and floor-surface samples differ from their corresponding processing blanks, as well as the hand microbiome of the researcher. Even though the DNA concentrations of, for

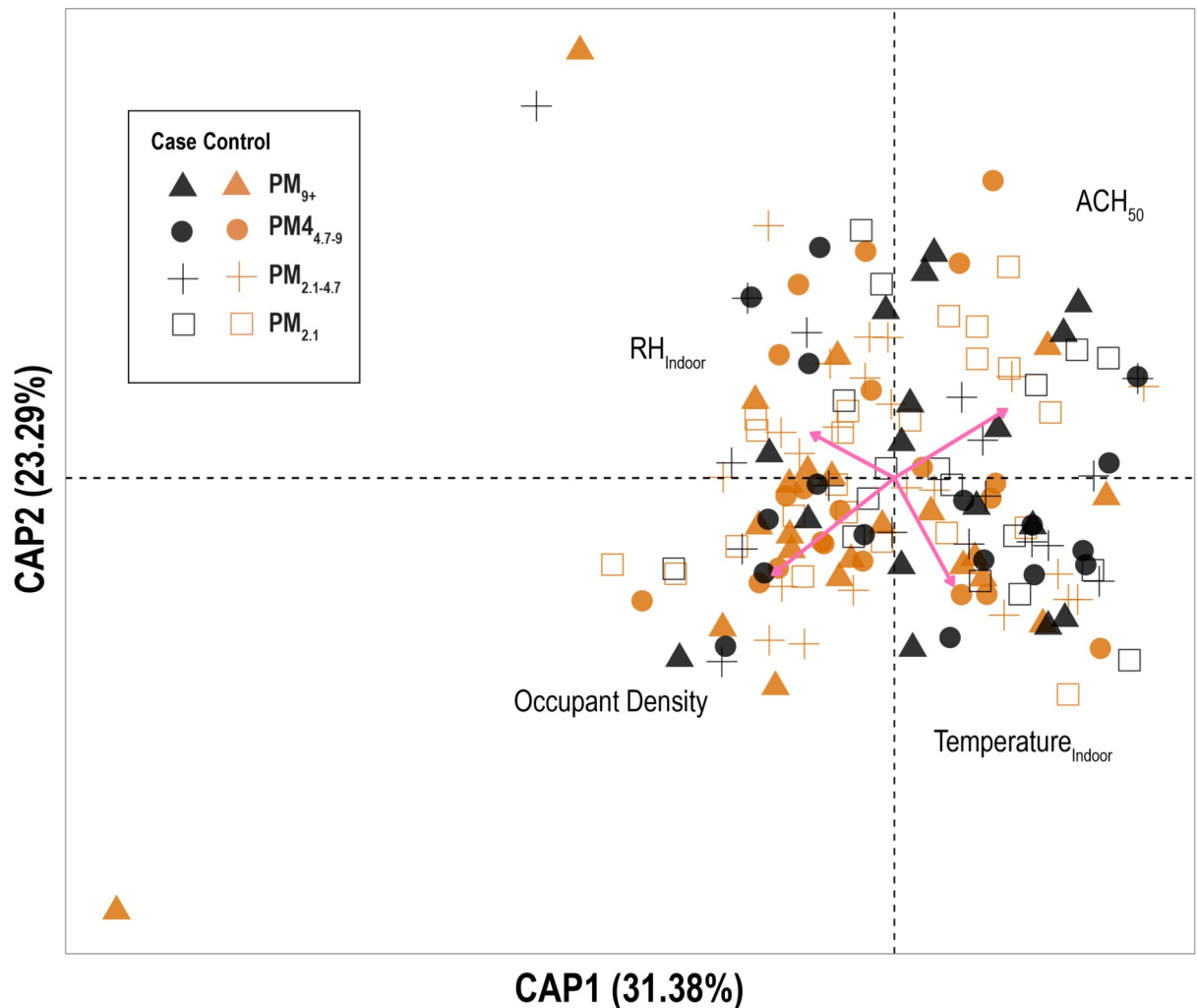


Fig 5. Constrained ordination analysis of indoor air microbiome. The weighted UniFrac metric that was calculated from bacterial composition and abundance was used to present dissimilarity between samples. Pairwise distances between air samples of both case and control homes were constrained against all collected environmental parameters to elucidate significant predictors of changes in indoor airborne bacteria composition. Each sample is colored by house, with black for case homes and orange for control homes. Samples are also shaped by air particle size bin.

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example, air samples were low, we have characterized different communities from environmental samples compared to blanks, with the phylum Verrucomicrobia (p -value < 0.0001) as a strong identifier of an indoor home sample.

Discussion

Weatherization led to an increase in indoor exposures with no significant impact on health

This study evaluated the impact of weatherizing of older single-family homes in climate region 5A by comparing changes in case homes to baseline changes in control homes that did not undergo weatherization between sampling periods. Weatherization led to a reduction in building leakage by an average of 22 percent (Fig 1). This is similar to previous evaluations of weatherization effectiveness across the United States [5]. After weatherization, a significant

number of families reported being thermally more comfortable, which was reflected quantitatively by an increase in indoor-outdoor temperature ratio and a decrease in indoor-outdoor relative humidity ratio after weatherization (Fig 1). A part of these results could be explained by the rebound effect, where home energy consumption is actually increased. Previous studies had found that although there is a rebound effect observed for approximately 27% of homes after weatherization [50], homeowners in weatherized home, on average, set their thermostat lower compared to non-weatherized homes [51]. Despite reporting greater thermal comfort, there was no noticeable change in respiratory symptoms or exacerbation of pre-existing symptoms among residents after weatherization. Our work agrees with larger studies [5, 52, 53], but our result is only representative of health effects after a relatively short period of several months.

Other studies have identified indoor pollutants that are an indicator for potential long-term health effects. Logue et al. [21] identified multiple compounds that are important in indoor respiratory health, including radon and $PM_{2.5}$. In a study of weatherized homes in North Carolina, Doll et al. [54] found that the radon level was weakly correlated with a decreased natural ventilation rate. We found that both basement and living area radon levels were significantly increased in case homes after weatherization when compared to baseline fluctuations in control homes. In the living area, where occupants spend the majority of their time, we observed minimal weatherization effect on radon levels in most case homes. For two case homes, however, we observed an exception because the radon levels exceeded the EPA action level of 4.0 pCi L^{-1} after weatherization (Fig 2). These two homes had different building leakage decreases of 14% and 30%, which indicates that living area radon concentration was not strictly correlated with weatherization effectiveness. Therefore, it is necessary for homeowners to measure the radon levels in a home after weatherization, regardless of the extent of the retrofit performed because each building envelope results in different indoor air quality outcomes after weatherization.

In multiple epidemiological studies, airborne particulate matter concentrations have been implicated as an important determinant of human health [55]. A previous study found that particulates with a relatively small particulate matter size (PM_3) were weakly correlated with a decrease in natural ventilation after weatherization [54]. However, we did not observe this finding. For our study, the average IO mass ratio in both case and control homes were greater than one, indicating significant indoor contribution to occupant exposure (S2 Fig), which would have masked the contribution of outdoor air to the indoor air mass concentration. Therefore, the change in outside particulates played a smaller role on the change in inside particulates due to weatherization. Instead, we found a significant correlation between changes in indoor mass concentrations and changes in occupancy rate. However, we did not record the occupant activity and the duration of these activities. Therefore, besides observing a significant correlation between indoor residential particulate matter concentration and occupancy rate, we did not have the information to observe a correlation between environmental conditions and air change rate. The contribution factor by different indoor sources, such as combustion, heating, and occupant activities, has been shown to vary with the nature of the activity [56–59]. In addition, the effect size of certain activities may be different for varying particulate matter size bins [59].

Indoor microbial community shifts are minimally affected by weatherization

Airborne bacteria concentration is an important consideration in public health [25, 60–62]. In respiratory health, both the concentration of inhaled microbes and its size distribution play

considerable roles in health outcomes [63]. Previous studies have shown that indoor concentrations of bacteria range from 10^1 to 10^3 colony forming units *per m*³ of air with conflicting size distributions [63–65]. Our average bacteria concentration of 200 16S gene copies *per m*³ air is within the range found in previous research [64, 65]. Unlike previous studies, we found that airborne bacteria concentration was relatively uniform throughout all size bins (S3 Fig). However, this may be due to the variation in sample collection methods used between studies. Regardless, we did not observe changes in bacterial loads due to weatherization (S3 Fig).

Previous studies had also implicated temperature, relative humidity, ventilation rate, and occupancy as determinants of bacterial load in the residential environment [37, 63, 66–68]. We found that the bacterial load within the homes did not change with weatherization, and mainly correlated with occupancy rate for all homes, with some other correlations between bacterial load and temperature and relative humidity. We observed that there is a seasonal effect on the airborne bacterial concentration (Fig 3). In the summer, there appears to be a lower bacterial load in the air compared to other seasons. This trend is most clearly seen in PM_{2.1–4.7} (Fig 3C). This seasonal effect on bacteria concentration indoors was previously described by Frankel et al. [66], where season was found to be a more significant driver of indoor bacteria concentration than ventilation.

Characterization of the indoor bacterial community shows that the diversity of airborne bacteria community did not change with weatherization (S6 Fig). Previous studies have found conflicting results on the effect of ventilation on bacterial community richness and diversity [36, 69, 70]. Here, we observed that weatherization and the corresponding reduced ventilation rate does not significantly change the diversity of the airborne bacterial community 6-months after weatherization. We also found that the indoor air microbiome became less similar to the outdoor air microbiome after weatherization (Fig 4). The indoor air microbiota is driven mainly by indoor occupants and outdoor air. The occupant identity within each home were constant before and after weatherization (no one moved in or out), and the outdoor source remained constant. Therefore, we were not surprised that a small reduction in ventilation due to weatherization did not significantly shift the indoor microbiota. However, understanding what drives the airborne bacterial community could aid us in shaping our residential bioaerosols dynamics. We aggregated air samples from all particulate matter size bins and homes to determine the environmental factors that drive the compositional changes between airborne communities. Though season was a significant driver of indoor bacterial exposure, it did not significantly impact the indoor microbial community (Fig 5). We found that indoor relative humidity, temperature, occupant density, and the airtightness are the main drivers of community differences between air samples. In two preliminary studies, we also identified relative humidity and ventilation rates as major determinants of bacterial communities in residential homes (S1 Fig). These determinants of bacterial community composition were also found by others in previous studies [38, 71].

Our study has a number of limitations. This study has a small sample size of 9 case homes and 11 control homes. This study also took place in climate region 5A (cold and moist) with four distinctive seasons. New York State is known to have higher than national average levels of radon, and the effect of weatherization in different climate regions will most likely differ. The findings from this study can be applied most accurately other regions with four distinctive seasons. Future studies in different regions with a larger sample size would provide additional insights into the findings presented. In addition, we collected health data by surveying occupants, which can be subjected to recall bias, and did not evaluate for rebound effect after weatherization. Lastly, samples were collected within 6 months post-weatherization, which only capture the short-term effects of weatherization. Continual monitoring of weatherized homes over a longer period of time and detailed evaluation of occupant behaviors for rebound

effect will enable us to evaluate both short and long-term effects of weatherization. This is critical for evaluations of human health as many health effects of poor indoor air quality are gradual.

In conclusion, we demonstrated that weatherization of older homes reduced the building leakage by about 22%, and led to an increase in basement and living room radon, an increase in indoor-outdoor temperature ratio, and a decrease in indoor-outdoor relative humidity. The resulting reduction in ventilation rates, however, did not in itself lead to changes in particulate matter concentration, airborne bacteria load, or bacteria community composition. Even though total airborne bacterial load was not significantly correlated with ventilation rates, occupancy rates, and indoor environmental conditions, the microbial composition of indoor air was governed by these factors. The occupants did not report any related health concerns for the case homes after weatherization, while the thermal comfort did increase. In light of the national lockdown during COVID-19, this is good news for the Weatherization Assistance Program for the Department of Energy in the US. However, weatherization may indirectly result in negative health outcomes. We found that indoor-outdoor humidity ratio decreased with weatherization, indicating that there was a decrease in indoor ambient humidity. Research has shown that low ambient humidity (below 50%) impairs host innate immune response against viruses, such as SARS-CoV-2, and increase host susceptibility to inflammatory-mediated diseases [72]. Although the indoor relative humidity was never below 50%, residents should be vigilant about monitoring and maintaining normal humidity indoors after weatherization efforts. Another parameter of concern that could cause long-term health effects was radon levels, which increased in the basements and living area for some of our case homes, and which increased to levels that were above the EPA action level of 4.0 pCi L^{-1} . These increases in radon level did not correspond to the most effective weatherization retrofits. This observation suggests that individual homes exhibit unique sets of characteristics that govern how the radon level shifts with weatherization. Due to the non-predictive behavior of radon levels we, therefore, recommend performing a radon test after weatherization to find whether mitigation would be necessary in locales where radon has posed a problem. Overall, we demonstrated that while weatherization improved thermal comfort with no change in microbial exposure, the effect on the indoor pollutant radon was variable. We recommend that energy efficient programs promote and budget for strategies to assess individual homes for indoor air quality after weatherization.

Supporting information

S1 Fig. Sampling strategy and particulate matter mass concentration of case and control homes during each sampling period. Sampling strategy (A) for case and control homes. Mass concentration of particulate matter size bins (B: PM_{9+} ; C: $\text{PM}_{4.7-9}$; D: $\text{PM}_{2.1-4.7}$; E: $\text{PM}_{2.1}$) across all homes and sampling periods. Points are colored based on house type, with case homes as black points and control homes as orange points. Triangles represent the first sampling period, and circles represent the second sampling period. Significance test *via* the paired *t*-test indicated no significant difference between sampling periods in both case and control homes.

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S2 Fig. Particulate matter mass concentration Indoor-Outdoor (IO) ratio. Indoor-outdoor concentration ratio of particulate matter size bins (A: PM_{9+} ; B: $\text{PM}_{4.7-9}$; C: $\text{PM}_{2.1-4.7}$; D: $\text{PM}_{2.1}$) across all homes and sampling periods. IO ratio is defined as the indoor particulate matter mass concentration over the outdoor mass concentration. Black points represent case homes and orange points represent control homes. Triangles represent the first sampling

period, and circles represent the second sampling period. A dashed line represents a 1:1 ratio. Significance test *via* the paired *t*-test indicated no significant difference between sampling periods in both case and control homes.

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S3 Fig. Airborne bacterial concentration in case and control homes during each sampling period. Airborne bacterial concentration in four particulate matter size bins (A: PM₉₊; B: PM_{4.7-9}; C: PM_{2.1-4.7}; D: PM_{2.1}) across all homes and sampling periods. Black points represent case homes and orange points represent control homes. Empty bars represent the first sampling period, and filled bars represent the second sampling period. Significance test *via* the paired *t*-test indicated no significant difference between sampling periods in both case and control homes.

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S4 Fig. Airborne bacteria concentration in indoor air. Concentration of bacteria in indoor air across all homes, in 16S gene copies *per m*³. All air samples from both sampling periods were consolidated and categorized by size bins for each season. Bars are colored based on size bin, with the following grouping: PM₉₊: green; PM_{4.7-9}: blue; PM_{2.1-4.7}: pink; PM_{2.1}: purple. The unpaired *t*-test was used to determine significance between seasons. No significant were found between groups.

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S5 Fig. Alpha diversity of indoor and outdoor airborne bacterial communities. Community diversity is reported as a change in the Shannon index for airborne bacteria samples in both the case (black) and control (orange) homes. A) Outdoor-indoor alpha diversity comparison of airborne bacterial communities, separated into particle size bins. Change in Shannon Index was calculated as outdoor air minus indoor air alpha diversity for each home. Therefore, negative values indicate greater diversity indoors compared to outdoors. No significance was found between case and control homes. B) Differences in alpha diversity of indoor air bacterial community, calculated by subtracting the first sampling alpha diversity from the alpha diversity of the second sampling. There is no significance between case and control homes across all air particle size bins.

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S6 Fig. Alpha diversity of surface and carpet dust samples. Bacterial community diversity is reported as a change in the Shannon Index for airborne bacterial samples in both the case (black) and control (orange) homes. Differences in alpha diversity of surface and dust bacterial communities, calculated by subtracting the first sampling alpha diversity from the alpha diversity of the second sampling.

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S7 Fig. Beta diversity analysis of all air, dust, and floor-surface, and samples. Principal coordinates analysis with the weighted UniFrac dissimilarity metric of all air, dust, and surface samples. Only the first two axes are shown. Dissimilarity distances are calculated from the differences in taxonomic composition and abundance between samples. Each point represents one sample, colored by sample source: air (turquoise), carpet dust (yellow), and floor surface (blue).

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S8 Fig. Differences in similarities between air, dust, and floor-surface bacterial communities of control homes. Indoor airborne bacterial communities in each air particles size bins were compared to their corresponding outdoor airborne bacterial communities (turquoise).

The same indoor air samples were also compared to living room floor surface (yellow) and living room carpet dust (blue) communities. Positive values indicate that two communities are more dissimilar in the second sampling period compared to the first sampling period. Negative values indicate that the two communities became more similar. NS: no significant.

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S9 Fig. Taxonomic summary of representative samples and processing blanks. 16S rRNA gene sequences summarized at the phylum level of home samples (air, carpet dust, and floor surface) and their corresponding processing blanks. Processing blanks are defined as sampling media (*i.e.* sterile filters for air samples) that were processed in a similar manner during the sampling process through PCR amplification. Therefore, processing blanks captures both field and lab contaminants. The hand microbiome of one researcher is also included.

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S10 Fig. Constraint ordination of two preliminary residential homes studies. Data from two preliminary analyses of airborne bacterial communities in residential homes. **A)** A small study of two homes. Each home was sampled multiple times throughout the year, with samples representing every season (Summer, Spring, Autumn, and Winter). Bacterial community was analyzed from PM₁₀, or particulate matter with diameters lower than 10- μ m. The results from constraining bacterial community differences against environmental parameters and building characteristics are shown. RH is relative humidity (%); AER is air change rate (h⁻¹). **B)** Bacterial community analysis of a small group of 4 homes: 2 case and 2 control homes. Air samples were also constrained against environmental parameters and building characteristics.

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S1 Table. Sampling timeline of case and control homes.

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S2 Table. Partial Pearson correlation coefficients r between ventilation rate, occupancy, and environmental conditions.

(PDF)

S1 File. Methods.

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S2 File. Building/occupant survey.

(PDF)

S1 Graphical abstract.

(TIFF)

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Author Contributions

Conceptualization: Largus T. Angenent.

Data curation: Mytien Nguyen.

Formal analysis: Mytien Nguyen.

Funding acquisition: Largus T. Angenent.

Investigation: Mytien Nguyen, Eric C. Holmes.

Methodology: Mytien Nguyen, Eric C. Holmes, Largus T. Angenent.

Project administration: Largus T. Angenent.

Supervision: Largus T. Angenent.

Validation: Mytien Nguyen.

Visualization: Mytien Nguyen.

Writing – original draft: Mytien Nguyen.

Writing – review & editing: Largus T. Angenent.

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