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Modeling microbial growth in carpet dust exposed to diurnal variations in relative humidity using the "Time-of-Wetness" framework

Sarah R. Haines[1,2,3] | Jeffrey A. Siegel[4] | Karen C. Dannemiller[2,3,5]

[1]Environmental Science Graduate Program, Ohio State University, Columbus, Ohio

[2]Department of Civil, Environmental & Geodetic Engineering, College of Engineering, Ohio State University, Columbus, Ohio

[3]Division of Environmental Health Sciences, College of Public Health, Ohio State University, Columbus, Ohio

[4]Department of Civil and Mineral Engineering, University of Toronto, Toronto, ON, Canada

[5]Sustainability Institute, Ohio State University, Columbus, Ohio

Correspondence
Karen C. Dannemiller, Department of Civil, Environmental & Geodetic Engineering, Environmental Health Sciences, Ohio State University, 470 Hitchcock Hall, 2070 Neil Ave, Columbus, OH 43210.
Email: Dannemiller.70@osu.edu

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Abstract

Resuspension of microbes in floor dust and subsequent inhalation by human occupants is an important source of human microbial exposure. Microbes in carpet dust grow at elevated levels of relative humidity, but rates of this growth are not well established, especially under changing conditions. The goal of this study was to model fungal growth in carpet dust based on indoor diurnal variations in relative humidity utilizing the time-of-wetness framework. A chamber study was conducted on carpet and dust collected from 19 homes in Ohio, USA and exposed to varying moisture conditions of 50%, 85%, and 100% relative humidity. Fungal growth followed the two activation regime model, while bacterial growth could not be evaluated using the framework. Collection site was a stronger driver of species composition (P = 0.001, R^2 = 0.461) than moisture conditions (P = 0.001, R^2 = 0.021). Maximum moisture condition was associated with species composition within some individual sites (P = 0.001-0.02, R^2 = 0.1-0.33). *Aspergillus*, *Penicillium*, *and Wallemia* were common fungal genera found among samples at elevated moisture conditions. These findings can inform future studies of associations between dampness/mold in homes and health outcomes and allow for prediction of microbial growth in the indoor environment.

KEYWORDS

carpet, exposure, fungi, house dust, moisture, mold

1 | INTRODUCTION

Exposure of dampness and mold in homes to individuals with asthma costs $22.4 billion each year in the United States alone.[1] Many of these costs arise from exacerbations of existing asthma, such as exposure to a trigger in the residential environment.[2] Additionally, exposure to inhalant allergens, such as from fungi, is responsible for up to 44% of physician-diagnosed asthma cases.[3] Some fungal species contain known allergens, including species in the genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium*, *Wallemia*, and others.[4-6] Most of our exposure to these indoor microbial communities is through the resuspension of floor dust,[7-9] and dust exposure may occur through inhalation, ingestion, and dermal exposure routes.[10] When humans are present in an indoor environment, depending on the size of the particles, the amount of particulate matter in the air increases by 3-68 times, with exposure to fungal genomes increasing by 1.5-5.2 times and bacterial genomes increasing by 12-2700 times.[7] House dust is an environmental measurement that is commonly used as

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an indicator of human exposure as microbial communities in floor dust are often correlated with the microbial composition of indoor air samples.[11,12]

Carpet can act as a reservoir for microbes in house dust[13] and constitutes about half of the flooring market in the United States.[14] Resuspension of dust from carpet is also generally higher than from solid flooring materials, which may increase human exposure.[13] Microbes grow in house dust at increased relative humidity conditions,[15] with fungi able to grow at lower relative humidity levels than bacteria.[16] Increased equilibrium relative humidity (ERH) is associated with increased microbial growth rates,[16] and moisture availability also influences fungal morphology in carpet under these conditions.[17] Fungal growth typically occurs above about 80% ERH.[18] Metabolic activity also increases at elevated relative humidity levels[15,19] and can result in degradation of chemicals in the dust such as phthalate esters.[20]

Moisture is the critical limiting factor for fungal growth in the indoor environment.[21-23] Moisture can include the presence of small amounts of liquid water or water vapor in the air. The United States Environmental Protection Agency (US EPA) recommends that relative humidity should be maintained between 30%-50% and definitively below 60% to inhibit mold growth in the home.[24]

There are many different ways to describe available water, which is a challenge when investigating indoor moisture. Relative humidity (RH) is the partial pressure of water vapor relative to the saturation pressure and is not strongly linked to microbial growth under most normal conditions in a home unless it is high enough (>80% RH) that condensation or a substantial adsorbed water layer is present on surfaces. Water activity (a_w) is a term that is widely used in microbiology[25] to describe available moisture and is the partial vapor pressure of water in a substance relative to the water vapor pressure in air at equilibrium (and thus is not measurable under non-equilibrium conditions). Under equilibrium conditions, a_w and RH are equal (although a_w is expressed fractionally and RH in %). However, building materials are rarely at equilibrium and it is not possible to measure a_w in the field. Equilibrium relative humidity, ERH, is the relative humidity in a sealed headspace above a material, and may represent a measurable representation of relative humidity at the surface and therefore available water in dynamic indoor systems.[21]

The relative humidity does not remain at a constant level indoors and instead fluctuates due to seasonal or daily temperature variations, ventilation, sources, such as showers, moisture adsorption or desorption from surrounding rooms, and the absolute humidity of the outdoor air. The American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) Standard 160 "Design Criteria for Moisture Control in Building" contains a methodology to calculate indoor humidity by balancing moisture production and removal rates through ventilation or dehumidification while ignoring the impacts of moisture storage of hygroscopic materials and the impact of humidity on the moisture production rate.[26-28]

The models of moisture in air often do not translate to moisture in building materials. In drywall, various models exist to predict microbial growth that occurs under variations in relative humidity in a typical home. The time-of-wetness (TOW) framework is an approximation for microbial growth in drywall.[22,29] This framework uses the fraction of time that the equilibrium relative humidity is at or above an 80% threshold to predict the relative growth rate of the fungus on the surface (Figure 1). Even when the initial saturation of the drywall only lasted 10 minutes, the surface remained above the critical growth threshold for over 6 hours.[29] Within the TOW framework, there are four different fungal growth models: (a) the standard model, (b) the activation limited growth model, (c) the deactivation limited growth model, and (d) the two activation regime model. The standard model was derived from steady-state data that was extrapolated to growth predictions under transient

Practical Implications

- Exposure to mold in housing costs society billions of dollars every year and can originate from fungal growth in carpet dust when relative humidity in the air is elevated.
- Utilization of the time-of-wetness framework allowed for improved prediction of human exposure at both the individual and population level.
- The data presented here indicates that site of sampling is a stronger driver of microbial communities in dust than moisture effects, which has implications for detection of undesirable microbial growth in buildings.
- This work allows for accurate modeling of fungal growth in carpet dust upon exposure to elevated relative humidity, and also emphasizes the difficulty of detecting the presence of indoor mold growth using available methods.

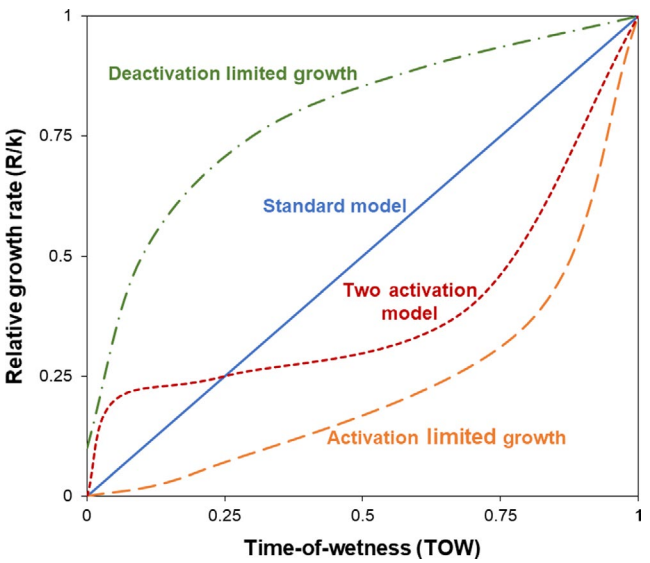

FIGURE 1 Theoretical standard TOW models. R/k is the relative growth rate and TOW is the fraction of total time spent above 80% equilibrium relative humidity

conditions. In this model, it is assumed that the organisms respond quickly to changes in relative humidity and temperature. The activation limited growth model occurs when deactivation (active to passive biomass) is a faster process than activation (passive to active biomass), and there is a lag time in initiation of microbial growth from the beginning of the wet period. Because of this, the relative growth rate is lower than expected based on the standard model. Deactivation limited growth refers to the model in which activation is a faster process than deactivation, and there is a lag in suspension of microbial growth at the beginning of the dry period. Similarly, the relative growth rate is higher than expected based on the standard model. Finally, the two activation regime model was created by modifying the activation limited growth model accounting for the time necessary for organisms to adjust to wet period and the quick activation of the passive biomass for short wet periods.[29]

The TOW framework utilizes an 80% critical growth threshold to model fungal growth in drywall. However, it is unknown whether this framework can be utilized when modeling microbial growth in carpet dust, which has important implications for human health. Through the utilization of this framework, we may better predict microbial growth in carpet dust to influence how we manage, construct, and design buildings.

The goal of this project is to model the fungal growth rate in carpet dust at variable relative humidity conditions using the TOW framework. We simulated varying relative humidity conditions in laboratory chambers containing carpet and dust collected from homes in Ohio, USA. We measured total fungal concentration over time and calculated the relative growth rate. We assessed the fit with the four models of the TOW framework to determine which was most applicable to fungal growth in carpet dust in homes, which has important implications for mold-related exposures.

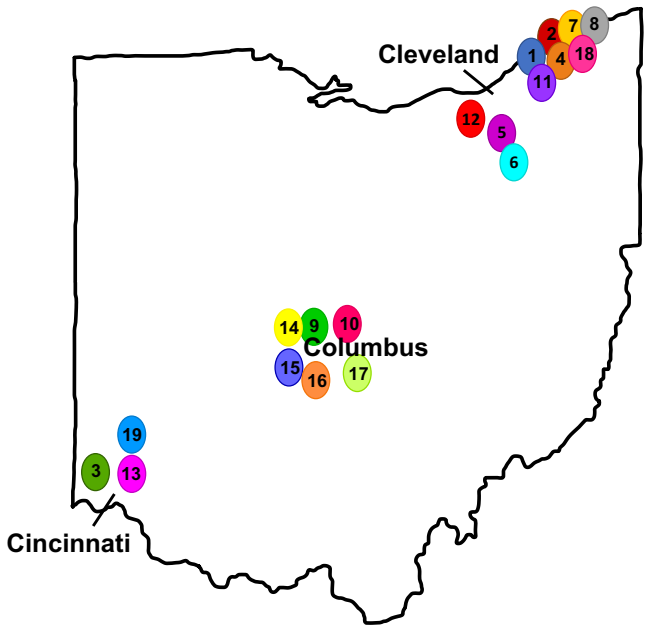

FIGURE 2 Map of Ohio showing each of the 19 locations for carpet and dust collection

2 | MATERIALS AND METHODS

Samples of carpet and corresponding dust were collected from 19 different homes across Ohio, USA, from May 2016 to August 2018 (Figure 2) using a previously described protocol.[30] A 240 cm × 160 cm section of carpet was removed from a high traffic area such as the living room of each home and cut into twelve 20 cm × 160 cm strips. Previously sterilized aluminum foil (baked at 500°C for 4 hours) was placed on top of each strip of carpet, and the carpet was rolled such that the bottom of the carpet did not come in contact with the top. This roll was then sealed in a polyethylene zip top bag.

Matching dust was collected using either the home vacuum or a cleaned Eureka Mighty Mite handheld vacuum (Eureka®) from the remaining carpet in the home. If the home vacuum cleaner did not contain a vacuum bag, the dust was removed from the canister and placed in a zip top bag. After returning to the laboratory, the collected dust was hand-sieved to 300 µm to remove larger debris and was stored at 25°C for up to 2 weeks prior to incubations. Triplicate samples of 100 mg of collected dust from each home were stored at −20°C to be later used in analysis as "Original Dust." The carpet was cut into 10 cm × 10 cm squares, wrapped in baked aluminum foil, and stored at room temperature until future use.

All participants included in the study were asked to complete a survey containing information about the age of the carpet, the frequency of vacuuming, the number of occupants (adults and children), and number of pets (dogs and cats) (Table S1). Participants were also asked if there was a history of water damage or visible mold growth in the home. If participants answered in the affirmative, then samples were not collected. Carpet fiber material was determined through burn testing. This project was approved by the Ohio State University Behavioral Institutional Review Board under study number 2016B0132.

2.1 | Chamber study

For the incubation experiments, carpet coupons embedded with dust were placed in relative humidity-controlled chambers. The baseline ERH was 50%, and the ERH was elevated to either 85% or 100% for 0, 6, 12, 18, or 24 hours per day over periods of 2 weeks to simulate variations in relative humidity in a home (Figure 3). Salt solutions with a a_w of 0.50 and 0.85 were used to maintain the relative humidity in the glass incubation chambers using sodium chloride (NaCl) and magnesium chloride ($MgCl_2$). The 0.50 a_w solution consisted of 100 mL of distilled water and 42.84 g of $MgCl_2$, while the 0.85 a_w solution consisted of 100 mL of distilled water and 23.37 g of NaCl. For the 1.00 a_w solution, only distilled water was used. The a_w of these solutions was tested for accuracy using an AquaLab™ Dew Point Water Activity Meter (Decagon 125 Devices) with a margin of error of +/−0.003 and adjusted as needed.

Each 10 cm × 10 cm carpet square was then evenly covered with 250 mg of sieved dust from the corresponding home, excluding the 1 cm of carpet closest to the edge to avoid edge effects. The dust was embedded using a modified American Society for Testing and

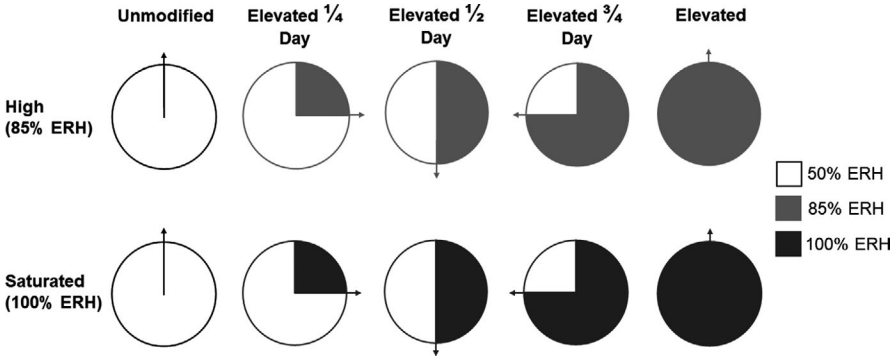

FIGURE 3 ERH Condition Pictographs: Each circle above represents the daily humidity cycle in an incubation chamber.

Materials (ASTM) method F608-13 in which a 12 cm long, 1440 g steel pipe covered in baked aluminum foil was rolled over the carpet squares 30 times.[30] Three embedded carpet squares from the same site were placed in an autoclaved 3.8 L glass jar along with 100 mL of the appropriate salt solution. One carpet square each was removed on day 5, 10, or 14. An Onset® HOBO® Data logger (Onset Computer Corporation) was sterilized with ethanol and used to record the ERH conditions within the jar. The jar was sealed using parafilm to retain moisture, prevent ambient contamination, and allow the release of CO_2. An example of Onset® HOBO® Data logger results compared to theoretical variations can be found in the Supplemental Information Figures S1 and S2. These glass incubation chambers were placed in an incubator set at 25°C for 14 days, and the salt solutions were changed as needed to create the required variations in ERH.

After incubation, the dust was collected using a 19 mm × 90 mm cellulose Whatman thimble inserted into a Eureka Mighty Mite using a cylindrical adapter.[30] The dust was vacuumed in a circular motion moving around the carpet square, followed by a linear up and down motion for ~3 minutes. The filter was then placed in a zip top bag in a −20°C freezer until further analysis.

2.2 | Particle size analysis

Analysis of the different particle sizes within each sample of collected dust was conducted using a Mastersizer S, particle size analyzer (Malvern Instruments). For this analysis, the dust was added to distilled water in the sample dispersion unit and stirred at 1500 rpm. The dust was added to the solution until the obscuration value (the reduction in light intensity due to the addition of dust) was approximately 30%. Once the obscuration was met, the particle size was measured. This analysis was conducted on dust from sites 2-19, as there was insufficient dust from site 1.

2.3 | DNA extractions and QPCR

2.3.1 | DNA extraction

To begin the analysis, 50 mg of dust was aliquoted from each collected dust sample and placed in a 2.0 mL screw top vial. Aliquots of original, non-incubated, collected dust were also extracted to determine a baseline fungal and bacterial concentration. The DNA extractions were performed using the QIAGEN DNeasy PowerSoil Microbial Kit (Qiagen) and modified with the alterations to the bead beating in which 0.3 g of 100 µm glass beads, 0.1 g of 500 µm glass bead, and 1 g of PowerBeads are used, in addition to a reduction in the final elution volume to 50 µL.[31] During each extraction, a sample of only beads and solution was used as a negative control. DNA extracts were then stored at −20°C.

2.3.2 | qPCR

Quantitative polymerase chain reaction (qPCR) was performed using a QuantStudio™ 6 Flex System (Applied Biosystems™) with samples prepared on a 96-well (0.2 mL) plate to determine the quantity of spores and cells in the dust samples. DNA extracts were initially diluted to concentrations of 10× and 100× using Tris-EDTA (10 mmol/L Tris, 1 mmol/L EDTA, pH 8). The quantity of the 10× and 100× dilutions determined from qPCR was then compared to check for PCR inhibition due to contaminants in dust. All samples were run in triplicate. Each plate also included two wells without sample solution added as no template controls.

Universal assays targeting the whole kingdom were used for the bacterial and fungal quantification of the samples. For bacteria, TaqMan® master mix (Applied Biosystems™) was used to target the 16S rRNA gene, with forward primer 5′-TCCTACGGGAGGCAGCAGT-3′, reverse primer 5′GGACTACCAGGGTATCT AATCCTGTT-3′ and probe (6-FAM)-5′-CGTATTACCGCGGCTGCTGGCAC-3′-(BHQ).[7,32,33] For fungi, SYBR® Green (Applied Biosystems™) was used with forward primer, FF2, 5′-GGTTCTATTTTGTTGGT TTCTA-3′ and reverse primer, FR1, 5′-CTCTCAATCTGTCAATCCTTATT-3′.[32] To quantify the number of spores or cells in a sample, standards were created and run with samples during qPCR *Bacillus atrophaeus* (ATCC® 49337™) was used for bacterial standards, and *Aspergillus fumigatus* (ATCC® MYA-4609™) was used for fungal standards. For bacteria, a hemocytometer was utilized to count cells and a total cell quantity was then determined from this count and diluted to 10^1-10^6 genome copies per µL of standard. For fungus, *A fumigatus* was grown on potato dextrose agar for 14 days, after which

conidia were collected using a nylon-flocked swab and suspended into a PBS-T solution. Again, the concentration in solution was measured with a hemocytometer prior to creation of qPCR standards by DNA extraction. To measure intact DNA of living and dead organisms, the initial hold stage was set at 50°C for 2 minutes and then 95°C for 15 minutes with a PCR stage of 40 cycles of denaturation at 95°C for 15 seconds and annealing at 60°C for 1 minute.

The quantity determined from qPCR was expressed as "spore equivalents (SE) per mg of dust" for fungi and "cells per mg of dust" for bacteria. We specify "spore equivalents" of DNA for fungi to quantify the value and also recognize that DNA originates from both spores and other fungal fragments and that gene copy numbers can vary between microorganisms and fungal fragments. For bacteria, we expressed values from qPCR as average bacterial cells. We accounted for the average 7.75 rRNA operon copies in the *B atrophaeus* genome and the average 4.7 rRNA operon copies/genome in all bacteria for our bacterial samples.[34,35] The quantity of spore equivalents or bacterial cells determined from qPCR is multiplied by 50 μL (the amount of solution at the end of the DNA extraction) and by the dilution factor of either 10× or 100×. This is then divided by the amount of original dust used in the extraction of that sample, which was approximately 50 mg.

2.4 | DNA sequencing

Samples were sequenced using an Illumina MiSeq™ with bacterial 16S 515F (5′-GTGCCAGCMGCCGCGGTAA) and 806R (5′-GGACTACHVHHHTWTCTAAT) sequencing primers[36,37] and fungal ITS1F (CTTGGTCATTTAGAGGAAGTAA)[38] and ITS2aR (GCTGCGTTCTT CATCGATGC)[39] ribosomal DNA primers[40] with 2 × 300 bp chemistry. Only samples set at elevated moisture conditions continuously (50% 24 hours, 85% 24 hours, or 100% 24 hours), original dust samples, and sites 1-3 were included in the sequenced with the bacterial primers. Samples from sites 1-3 were sequenced at the Molecular and Cellular Imaging Center at the Ohio State University, Wooster Campus, and samples from sites 4-19 were sequenced at RTL Genomics in Lubbock, TX. These sequence data have been submitted to the European Nucleotide Archive under accession number PRJEB37053.

The bioinformatic pipeline Quantitative Insights Into Microbial Ecology (QIIME) version 1.9 was utilized to analyze the retrieved raw FASTQ sequencing data.[41] First, primers and spacers were trimmed and the paired ends were joined using the SeqPrep method.[42] These files were then quality trimmed to a Phred score of 20 with 3 low-quality base cells allowed before truncating. Beta diversity was determined through QIIME using the Bray-Curtis dissimilarity statistic.[43] Principle coordinate analysis (PCoA) was conducted from a beta diversity analysis[44] using Bray-Curtis.

For bacteria, we utilized weighted and unweighted UniFrac.[45] The weighted and unweighted UniFrac analysis accounts for both phylogenetic relatedness of operational taxonomic units (OTUs) and abundance, while Bray-Curtis only accounts for abundance. PCoA figures were created for all the bacterial data to show the dissimilarity/similarities between moisture conditions.

Fungal taxonomy was identified using the Basic Local Alignment Search Tool (BLAST) version 2.2.28+,[46] the User-friendly Nordic Internal Transcribed spacer Ectomycorrhiza (UNITE) database[47] and Fungal High-throughput Taxonomic Identification tool for use with Next-Generation Sequencing (FHiTINGS) version 1.4.[48] One note about the UNITE 2016 database is the reclassification of certain species and the creation of space-holder for these reclassified species. Species *Pleosporales fam* and *Tremellales fam* are likely placeholders for taxa that has been reclassified, and the sequences were not recognized during taxonomical reshuffling. Bacterial taxonomic identification was determined using QIIME summarize_taxa.py and the Greengenes database version 13_5.[49] Using the qPCR quantity results, we were able to calculate absolute abundance of the species from the sequencing data.

2.5 | Statistics

Statistical Analysis System (SAS), version 9.4 (SAS Institute, Inc), was used to compare microbial diversity and taxonomy to different characteristics such as moisture condition and incubation time. To calculate the adjusted *P*-value, the false discovery rate (FDR) was used to determine statistical significance ($P < 0.05$) of varying parameters as they relate to species composition.[50] We calculated the false discovery rate (FDR) instead of the positive false discovery rate (PFDR),[50] as we were using a sample size of 48 species which is considerably smaller than the datasets normally used with a PFDR analysis.[51] A 95% confidence ellipse was created in SAS and later graphed in Microsoft Excel using the PCoA data to compare the elevated moisture conditions to each other and determine the true mean of each dataset. The 95% confidence ellipse is determined using an algorithm that states that if you were to replicate your sampling distribution 100 times, then 95% of the time the ellipses that were created would contain the same underlying mean. A smaller ellipse around the data indicates less variance in the dataset. The adonis function in QIIME was used to determine statistical significance of different groupings from the Bray-Curtis and UniFrac distance matrix. The adonis function was performed on each set of fungal data from each site to determine site-specific characteristics.

When conducting a statistical analysis to the relationship of particle size to species, the PROC MULTTEST FDR Test Mean function on SAS was utilized to observe where the highest percentage of particles was found (by volume %) and compared peak size particles ≥40 μm to <40 μm (Table S2).

To determine species composition, an inverse hyperbolic sine (IHS) transformation was used to normalize absolute abundance values of each species. The IHS transformation performs a similar analysis to that of a logarithmic transformation but allows for the use of values <1 and 0.[16,52,53] Species that were not found in 80% of the samples were then removed from the analysis to reduce multiple

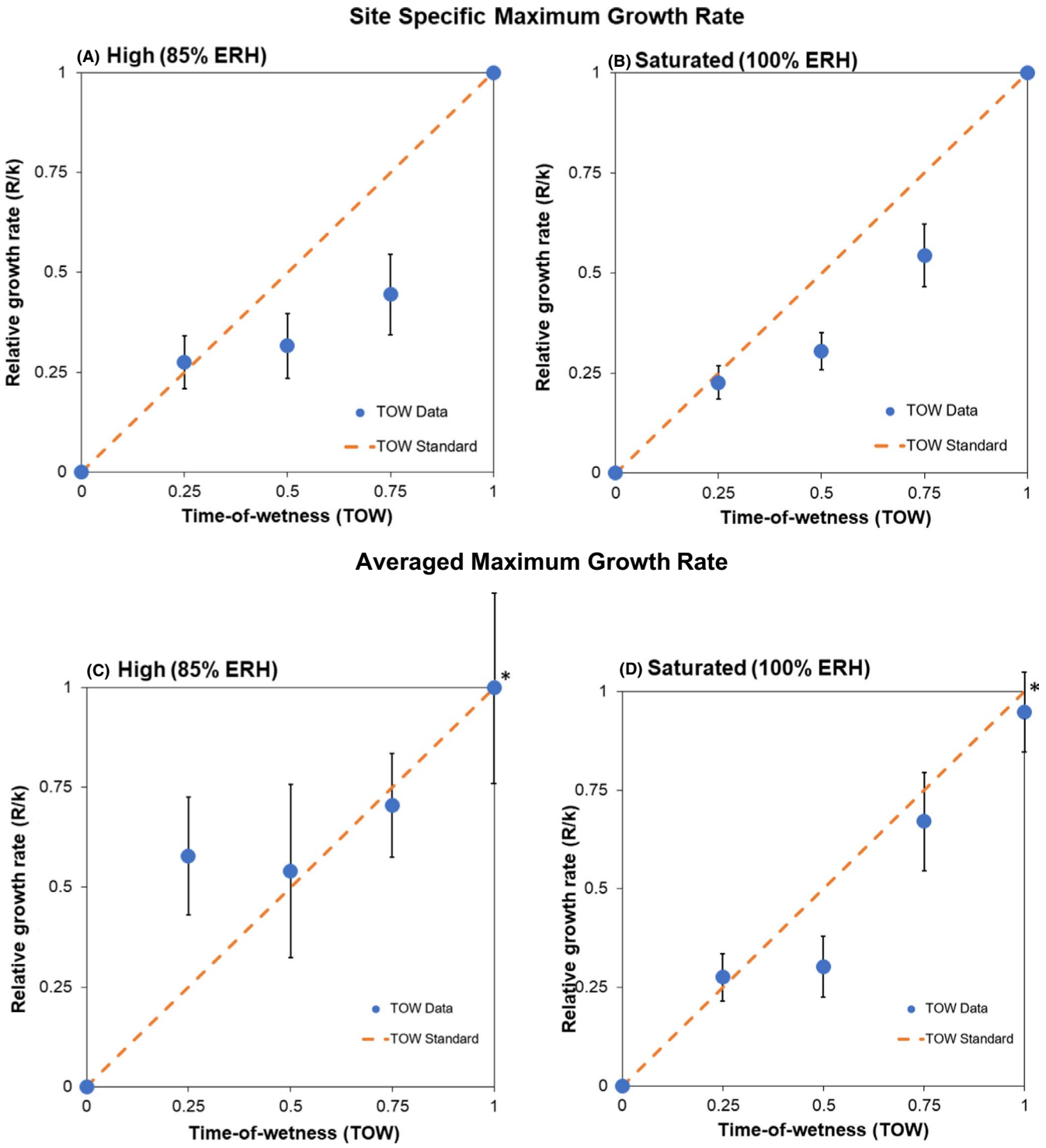

FIGURE 4 Evaluation of the TOW framework for fungi at High (85% ERH) (A) and Saturated (100% ERH) (B) for all 19 sites. The IHS mean of the exponential growth constant (0.330 for High and 0.121 for Saturated conditions) was calculated for each site, averaged, and used in place of the relative growth rate to recalculate the TOW model for both High (85% ERH) (C) and Saturated (100% ERH) (D). The *x*-axis represents the fraction of time spent above 80% relative humidity, while the *y*-axis is the relative growth rate. The error bars represent standard error. For both 85% TOW and 100%, the data most closely follow the two activation model. *For easier visualization, the y-axis has a maximum value of 1 although the error bars exceed this limit. For the High (85% ERH), the error bar reached a value of 1.24 (C), and for the Saturated (100% ERH), the error bar reached a value of 1.1 (D)

comparison concerns and to allow us to select for species potentially associated with higher moisture conditions. The IHS mean of the growth rate of fungal species was calculated for the Saturated and High moisture conditions as well as for certain abundant fungal species to determine an average growth rate from all the sites. The IHS mean is similar to a geometric mean, but allows for the use

of negative and 0 values.[54] The IHS mean is determined by taking the IHS of each value, averaging these values and transforming back using the hyperbolic sine.

The Spearman rank correlation coefficient was calculated for both the High and Saturated TOW models.[55] The Spearman rank correlation coefficient determines the strength and direction in the relationship between the data with a value of 1 having the strongest positive correlation. A two-tailed *t* test was calculated to determine the significance of the exponential growth rates under the Saturated (100% ERH) condition compared to the High (85% ERH) condition.

2.6 | Time-of-Wetness Model development

We applied the theoretical standard TOW framework[29] to our fungal concentration results from each sample to determine the growth rate (*k*) and effective growth rates (*R*) for each incubation condition and also to calculate the relative growth rate (*R/k*). The value *k* is the growth rate under steady-state conditions (at continuously high ERH), while *R* is the effective growth rate when samples are above the critical moisture level required for growth for only a subset of the time. The relative growth rate can then be determined from the two values as *R/k*. Equation 1 below can be used to calculate the relative growth rate during wet and dry periods where *A* = active biomass growth, *P = passive biomass growth*, *μ = rate* from *P->A*, *k = growth rate*, and *t = time*.

$$A(\Delta t) = A(0)\, e^{(k\Delta t)} + P(0)\, \frac{\mu}{\mu + k} \left[e^{(k\Delta t)} - e^{(-\mu\Delta t)} \right]. \quad (1)$$

Exponential growth was then assumed as the TOW increased. When exponential growth is assumed, the effective growth rate, *R*, can be determined using Equation 2.

$$F(nT) = F(0) \exp(RnT). \quad (2)$$

In this case, *F = A + P*, *nT* = total time after n cycles.[29] The maximum growth rate occurs when *R = k* as this is the highest ERH condition for a period of 24 hours. To solve for *k*, the fungal concentration was measured over time at the continuously High (85%) or Saturated (100%) relative humidity conditions (Figure S3). The exponential equation of the best fit line was determined, and the value of *k* was recorded (Equation 2). The *R* values were then calculated using the same method from samples exposed to variations in ERH. *R/k* was calculated at each TOW period, with the TOW periods corresponding to either a High or Saturated condition, 0.25 = Elevated ¼ Day, 0.50 = Elevated ½ Day, 0.75 = Elevated ¾ Day, and 1 = Elevated. The Unmodified condition refers to the samples that were exposed to 50% ERH for the total incubation period.

The *R/k* values for all sites were then averaged to get one *R/k* value for each moisture condition. If the calculated *R/k* for an individual site was found to be negative, a zero was used instead, as there was no true growth rate for that condition, such as at constant 50% ERH. The resulting graph was used to evaluate the fit to the standard, deactivation limited growth, activation limited growth, and two activation models.

3 | RESULTS

3.1 | Time-of-Wetness framework

The fungal concentrations ranged from 1.05×10^2 spore equivalents/mg dust to 1.92×10^{10} spore equivalents/mg dust. The bacterial concentrations ranged from 4.37×10^4 cells/mg dust to 2.33×10^{10} cells/mg dust. The ERH conditions within the jars generally exceeded the 80% critical growth threshold under High or Saturated conditions, and were below the 80% threshold at Unmodified ERH conditions. However, the ERH was often higher or lower than the intended value (Figures S1 and S2). This may be due to the hygroscopic nature of the carpet and other materials in the jar.

The fungal growth rate increased as the time spent above the critical growth threshold increased. This was consistent whether the maximum growth rate *k* was used from each site (Figure 4A,B) or calculated as the IHS mean exponential growth constant from all sites (Table S3) (Figure 4C,D). Comparing the site average growth values to the TOW for the High (85% ERH) and Saturated (100% ERH) conditions yielded a value of a Spearman rank correlation coefficient of 1.0. Using all individual values from the 19 sites results in Spearman's rank correlation coefficients of 0.44 for the High (85% ERH) model and 0.78 for the Saturated (100% ERH) model. On average, the exponential growth rates were higher under Saturated (100% ERH) compared to High (85% ERH) conditions (Table S3, two-tailed *t* test $P < .0001$). A representation of the data used to calculate the averaged TOW models in Figure 4 can be found in the Figure S4.

The data most closely conformed to the two activation regimes model when compared to the standard model, deactivation limited growth model, or activation limited growth model. This may indicate that activation of the passive biomass occurs quickly, and as the wet period continues, the system switches from a high to low activation rate.[29] However, no data were collected with TOW between 0 and 0.25 to confirm this observation.

A TOW model was also constructed for bacterial growth (Figure S5), but over half (55%) of the data did not follow an exponential best fit line, resulting in excessive zeros and ones to be added to the final TOW value calculation. This caused the relative growth rate for each TOW point (0.25, 0.50, and 0.75) to equal 0.50. A model cannot be determined from these data.

3.2 | Taxonomic identification

Sequencing results yielded 4 042 067 total quality bacterial reads and 21 559 346 quality fungal reads. After removing species not

present in at least 80% of samples, there were 48 fungal species and 27 bacterial species included in the analysis. The most common fungal species (found in >99% of all samples) were *Epicoccum nigrum*, *Byssochlamys spectabilis*, and *Cladosporium delicatulum*, and the most common bacterial species (found in >99% of all samples) were *Microbispora rosea* and *Bacillus flexus*. The most common fungal genera (found in >99% of all samples) were *Aspergillus*, *Alternaria*, *Cladosporium*, *Epicoccum*, *Wallemia*, and *Byssochlamys* , while the most common fungal orders (found in >99% of all samples) were determined as *Eurotiales*, *Capnodiales*, *Pleosporales*, *Helotiales*, *Incertae sedis*, *Tremellales*, *Wallemiales*, and *Hypocreales*.

3.3 | Fungal communities

PCoA was used to evaluate changes in fungal communities incubated under different conditions (Figure 5). Sites are displayed in different colors, and the darker shades correspond to samples with higher moisture conditions. Samples visually separated by site to higher degree (P = 0.001, R^2 = 0.461) than by maximum moisture exposure level (P = 0.001, R^2 = 0.021), which indicates that site was a stronger driver of microbial community rather than moisture condition.

To determine statistical significance of the x and y axes of the PCoA (Figure 5), a linear regression was performed using different variables. Site was determined to be a main driver of species composition along PC1, as each site has its own fungal community (P < 0.001) (Figure 5). Within the individual sites, moisture and the amount of time spent at certain moisture conditions were the most important factors indicating species composition (Table S4, Figure S6).

As an additional analysis, we considered only the samples incubated at a constant ERH for the duration of the experiment (Figure 6). Certain individual sites were found to have statistically different microbial communities concerning the maximum moisture conditions at 100% ERH and 85% ERH compared to 50% ERH (Table S5). Through adonis, maximum moisture was determined to be statistically significant (P = 0.001, R^2 = 0.14). In regard to the 95% confidence ellipse analysis, these groupings were not different based on ERH level, although a subset of samples incubated at 85% and 100% ERH did fall outside the 95% confidence ellipses for the original dust and 50% ERH.

3.4 | Fungal taxonomy

We compared the species composition to these moisture conditions and time to determine how these factors influence microbial composition. *Penicillium chrysogenum* (P = 0.001), *Aspergillus sydowii* (P = 0.001), *Cladosporium sphaerospermum* (P = 0.002), *Wallemia mellicola* (P = 0.01), *Aspergillus penicillioides* (P = 0.03), and *Cladosporium halotolerans* (P = 0.03) were more associated with the Saturated (100% ERH) Elevated condition than the Unmodified (50% ERH) condition (Table 1). *Tremellales fam* (P = 0.001), *Monographella cucumerina* (P = 0.001), and *Pleosporales fam* (P = 0.007) were some of the species

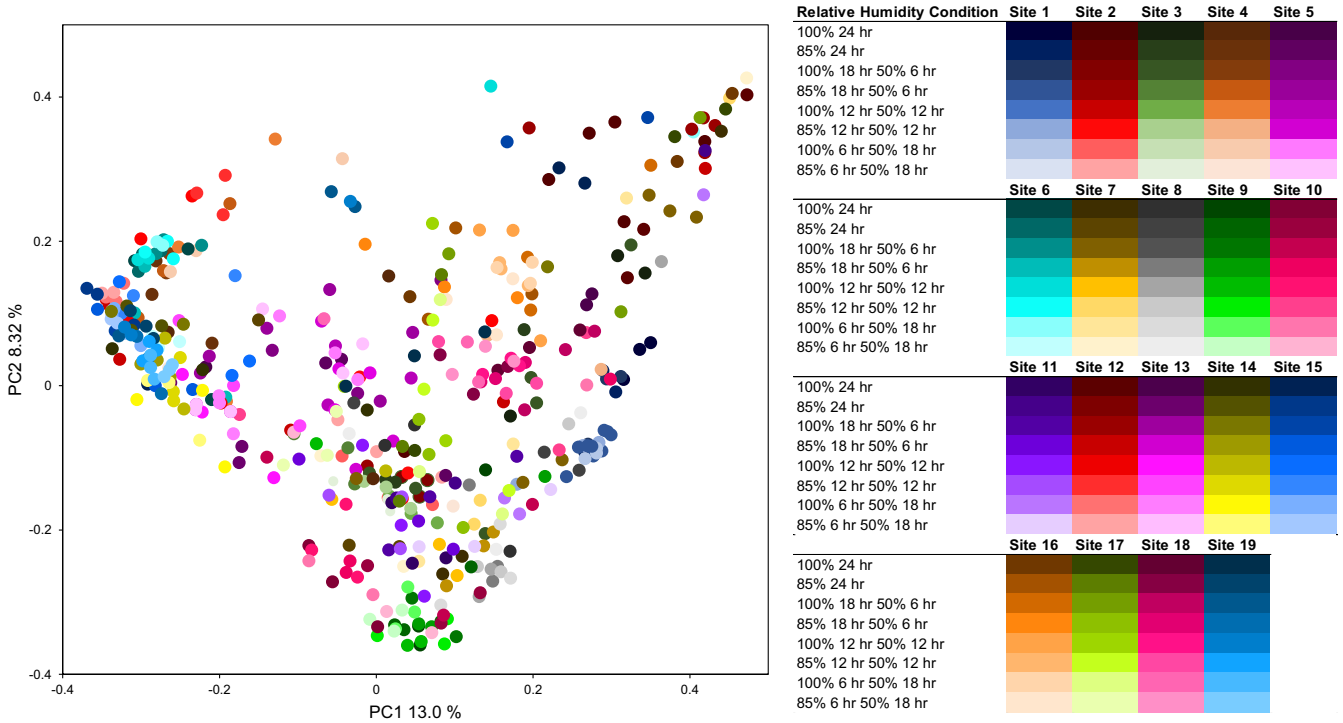

FIGURE 5 Principle coordinate analysis plot (PCoA) in which samples with similar microbial communities are clustered together. The colors represent an individual site with the color gradient representing moisture condition. Darker colors indicated a higher moisture condition

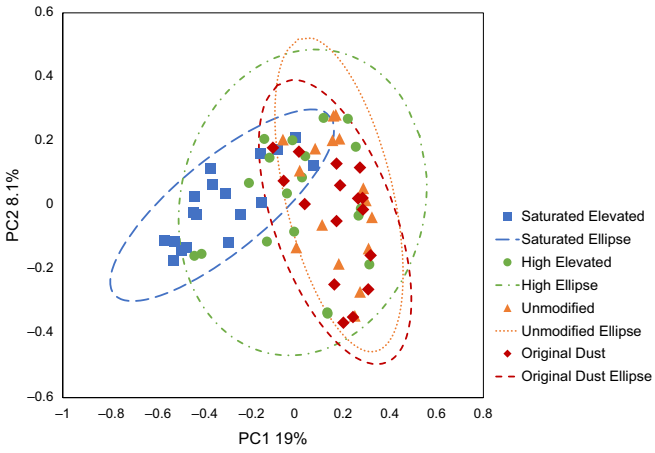

FIGURE 6 Principle coordinate analysis (PCoA) of and the original dust sample data and the three Elevated conditions [Saturated (100% ERH), High (85% ERH), and Unmodified (50% ERH), incubated for 14 days] . A 95% confidence ellipse was added for each condition. The 95% confidence ellipse represents the confidence region the true mean of the dataset

more statistically associated with Unmodified moisture condition than the Saturated Elevated condition (Table 1). When comparing the Saturated Elevated condition to the High (85% ERH) Elevated condition, no species were statistically associated with 85% ERH, and *Aspergillus austroafricanus* (P = 0.0002), *Cladosporium sphaerospermum* (P = 0.009), *Aspergillus sydowii* (P = 0.03), and *Penicillium chrysogenum* (P = 0.04) were associated with the Saturated Elevated condition (Table 2). Comparing all of the sample conditions set at Saturated (100% ERH) and High (85% ERH) conditions, *Aspergillus austroafricanus* (P = 0.03) and *Aspergillus sydowii* (P = 0.04) were statistically associated with the Saturated condition (Table 3). Finally, *Aspergillus austroafricanus* (P = 0.003), *Aspergillus sydowii* (P = 0.01), *Cladosporium sphaerospermum* (P = 0.009), and *Penicillium chrysogenum* (P = 0.03) were more associated with the Saturated Elevated samples set at 24 hours consistently for 14 days compared to the original dust samples (Table 4). The number of days and the time spent at a maximum moisture conditions did not have a statistically significant impact on species composition. The genera *Aspergillus*, *Penicillium*, and *Wallemia* were statistically associated with Saturated Elevated moisture condition (Table S6).

Variations in relative humidity did impact certain species composition. Samples incubated at the Saturated Elevated condition compared to samples incubated at the Saturated Elevated ¼ condition did lead to statistical differences in species composition. *Aspergillus austroafricanus* (P = 0.0001), *Aspergillus sydowii* (P = 0.003, *Penicillium chrysogenum* (P = .02), and *Cladosporium sphaerospermum* (P = 0.02) were all associated with the Saturated Elevated condition, while *Didymella nigricans* (P = 0.02), *Knufia perforans* (P = 0.02), *Tremellales fam* (P = 0.02), and *Monographella cucumerina* (P = 0.04) were associated with the Saturated Elevated ¼ condition (Table S7). Comparing the High Elevated to High Elevated ¼ conditions as well as Saturated Elevated did not result in any significant species. Also, the relationship between Saturated Elevated ¼ and Saturated Elevated ¾ as well as High Elevated ¼ and High Elevated ¾ did not reveal statistically significant species.

TABLE 1 Samples at Saturated (100% ERH) elevated condition and Unmodified (50% ERH) Elevated condition were compared to determine species statistically associated with each moisture condition. We evaluated species that were found in ≥80% of all samples

Fungal species	Unadjusted *P*-value	Adjusted FDR *P*-value
More abundant at saturated elevated		
Penicillium chrysogenum	<.0001	0.001
Aspergillus sydowii	0.0002	0.002
Cladosporium sphaerospermum	0.0003	0.002
Wallemia mellicola	0.004	0.01
Aspergillus penicillioides	0.02	0.04
Cladosporium halotolerans	0.02	0.04
More abundant at unmodified elevated		
Tremellales fam	<.0001	0.001
Monographella cucumerina	0.0002	0.002
Pleosporales fam	0.001	0.007
Epicoccum brasiliense	0.001	0.007
Didymella nigricans	0.001	0.007
Knufia perforans	0.002	0.01
Filobasidium magnum	0.003	0.01
Cryptococcus victoriae	0.003	0.01
Rhodotorula graminis	0.003	0.01
Holtermanniella takashimae	0.004	0.01
Physciella chloantha	0.004	0.01
Cystofilobasidium macerans	0.005	0.01
Vishniacozyma carnescens	0.008	0.02
Articulospora proliferata	0.01	0.03
Paramyrothecium roridum	0.01	0.04
Gibberella baccata	0.02	0.04
Phaeosphaeria podocarpi	0.02	0.04
Pyrenochaetopsis leptospora	0.02	0.05
Pyrenochaetopsis pratorum	0.03	0.05

3.5 | Fungal growth rate

The growth rate of certain fungal species over the 14 days of incubations was estimated for High and Saturated Elevated conditions. The linear growth rate and the exponential growth constants were determined for *Aspergillus sydowii*, *Aspergillus versicolor*, *Cladosporium sphaerospermum*, *Cryptococcus victoriae* (synonym *Vishniacozyma victoriae*), *Epicoccum nigrum*, *Penicillium chrysogenum*, and *Wallemia mellicola* (Table S8 and S9). Growth rates differed between species and between time of growth, for example from day 5 to day 10 and from day 10 to day 14. The overall fungal linear growth rate and exponential growth

TABLE 2 Samples set at Saturated (100% ERH) Elevated condition and High (85% ERH) Elevated condition were compared to determine species statistically associated with each moisture condition

Fungal species	Unadjusted *P*-value	Adjusted FDR *P*-value
More abundant at saturated elevated		
Aspergillus austroafricanus	<.0001	0.0002
Cladosporium sphaerospermum	0.0004	0.01
Aspergillus sydowii	0.002	0.03
Penicillium chrysogenum	0.004	0.05
More abundant at unmodified elevated		
–	–	–

Note: No species were associated with 85% Max ERH condition when compared to samples at 100% Max ERH condition.

rate for High Elevated and Saturated Elevated conditions for each individual home was also calculated (Tables S10 and S3). The Saturated Elevated condition had an IHS mean linear growth rate of 3.26×10^5 spore equivalents/mg dust/day from day 5 to day 14, and samples experiencing a High Elevated condition had an IHS mean linear growth rate of 1.34×10^1 spore equivalents/mg dust/day from day 5 to day 14.

3.6 | Bacterial communities

Bacterial beta diversity was compared among samples using both unweighted and weighted Unifrac distance (Figure 7). This analysis includes day 14 samples at either a Saturated Elevated, High Elevated, or Unmodified Elevated condition as well as original non-incubated dust samples from each site. The PCoA was used to evaluate differences in samples due to moisture condition. Using SAS version 9.4, both the weighted and unweighted PCoAs (Figure 7A,B) were analyzed and PC1 was determined to be statistically associated with site ($P < 0.0001$), and PC2 was statistically associated with maximum moisture condition ($P < 0.0001$). Using the adonis statistical test, the clustering of the different samples was revealed to be associated with the maximum moisture condition of either 0%, 50%, 85%, or 100% ERH (unweighted Unifrac $P = 0.001$, $R^2 = 0.068$ and weighted Unifrac $P = 0.001$, $R^2 = 0.17$). However, overlap was still observed in the 95% confidence interval ellipses at the different ERH levels. A subset of the samples at 100% ERH was located outside the other ellipses.

TABLE 3 All samples set at either a Saturated (100% ERH) or High (85% ERH) condition for 6, 12, 18, and 24 hours were compared to determine species statistically associated with each moisture condition

Fungal species	Unadjusted *P*-value	Adjusted FDR *P*-value
More abundant at saturated condition		
Aspergillus austroafricanus	0.0008	0.04
Aspergillus sydowii	0.002	0.04
More abundant at High condition		
–	–	–

Note: No species were more statistically associated with the High (85% ERH) moisture condition.

3.7 | Bacterial taxonomy

The bacterial species and the moisture conditions were compared in SAS using the same FDR analysis that was used in the fungal analysis. *Bacillus firmus* ($P = .0005$), *Bacillus flexus* ($P = .0008$), *Brachybacterium conglomeratum* ($P < .0001$), *Kocuria rhizophila* ($P = .004$), and *Microbispora rosea* ($P = .0002$) were more associated with samples incubated at Saturated (100% ERH) Elevated than samples incubated at Unmodified (50% ERH) Elevated over 2 weeks (Table 5). *Acinetobacter johnsonii* ($P = .01$) and *Pseudomonas stutzeri* ($P = .0069$) were statistically associated with samples incubated at the Unmodified Elevated condition than samples set at the Saturated Elevated condition (Table 5). The Saturated (100% ERH) Elevated condition samples were also compared to the original dust samples from each site (Table 6), and *Acinetobacter lwoffii* ($P = .002$), *Acinetobacter johnsonii* ($P = .005$), and *Pseudomonas stutzeri* ($P = .002$) were all statistically associated with the original dust samples. When comparing samples at High (85% ERH) elevated to the Saturated, Unmodified, and original dust samples, no species were statistically associated with this condition.

Virgibacillus, *Bacillus*, *Brachybacterium*, and *Staphylococcus* were among the genera more associated with the Saturated (100% ERH) Elevated moisture condition when compared to samples at High condition, Unmodified condition, and original dust (Table S11). These 16 genera were the most abundant and found in every sample analyzed: *Actinomycetospora*, *Agrobacterium*, *Bacillus*, *Corynebacterium*, *Devosia*, *Erwinia*, *Flavobacterium*, *Lysinibacillus*, *Microbispora*, *Modestobacter*, *Paracoccus*, *Pseudomonas*, *Pseudonocardia*, *Rhodoplanes*, *Sphingomonas*, *and Staphylococcus* (found in > 99% of all samples).

TABLE 4 The Saturated (100% ERH) Elevated samples were compared to the original dust samples to determine statistically associated species

Fungal species	Unadjusted *P*-value	Adjusted FDR *P*-value
Aspergillus austroafricanus	<.0001	0.004
Aspergillus sydowii	0.0005	0.01
Cladosporium sphaerospermum	0.002	0.03
Penicillium chrysogenum	0.003	0.03
Original dust		
–	–	–

Note: No species were more statistically associated with the original dust samples.

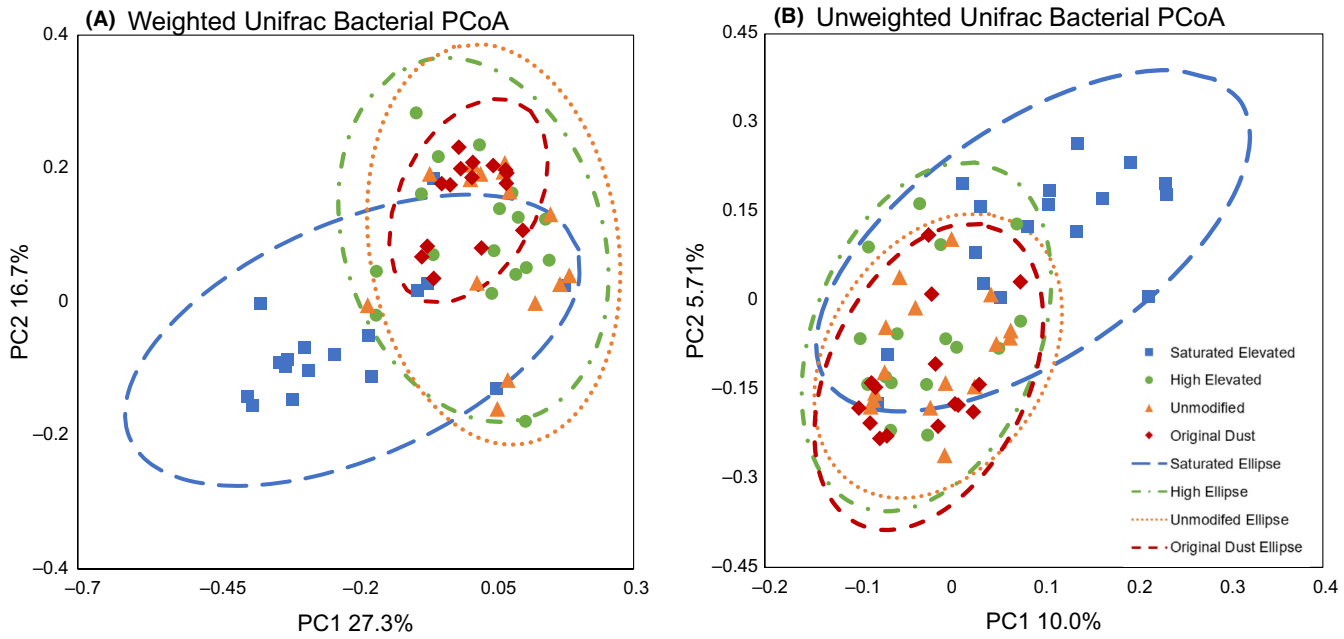

FIGURE 7 Weighted and Unweighted Unifrac for bacterial PCoA analysis showing day 14 samples from each of the 19 sites at a constant elevated moisture condition of either Saturated, High, or Unmodified. A 95% confidence ellipse was added for each condition to show comparison

TABLE 5 List of bacterial species more associated either the Saturated (100% ERH) Elevated condition or the Unmodified (50% ERH) Elevated condition when samples are compared

Bacterial Species	Unadjusted *P*-value	Adjusted FDR *P*-value
Saturated elevated		
Bacillus flexus	<.0001	<.0001
Brachybacterium conglomeratum	<.0001	0.0004
Microbispora rosea	0.0002	0.0018
Bacillus firmus	0.0005	0.0035
Kocuria rhizophila	0.0039	0.0209
Unmodified elevated		
Pseudomonas stutzeri	0.0069	0.0308
Acinetobacter johnsonii	0.0112	0.0432

3.8 | Physical analysis

The particle size range of the sieved dust was approximately 10-100 µm (Figure S7).

TABLE 6 List of species that were more associated with a condition of Saturated (100% ERH) Elevated and the original dust when samples were compared

Bacterial species	Unadjusted *P*-value	Adjusted FDR *P*-value
Saturated elevated		
Bacillus flexus	0.0025	0.0229
Original dust		
Acinetobacter lwoffii	0.0016	0.0229
Pseudomonas stutzeri	0.0017	0.0229
Acinetobacter johnsonii	0.0053	0.0358

Wallemia mellicola (P = .0009), *Aspergillus penicillioides* (P = .001), and *Penicillium chrysogenum* (P = .03) were all positively associated with a peak particle size ≥40 µm, while *Nigrospora oryzae* (P = .002) and *Didymella nigricans* (P = .007) were statistically significantly associated with peak particle size <40 µm.

4 | DISCUSSION

Overall, we demonstrated that the TOW framework can be applied to model fungal growth in house dust, particularly when using the two activation model. The results of this study have important implications for the development of quantitative indicators to measure mold growth in homes. Site of origin was a stronger driver of final microbial communities than moisture conditions. Thus, species composition may not be sufficient to indicate fungal growth indoors because the moisture signature may be masked by site differences.

Each home had a unique microbial signature, and there is high variability of species composition between sites. This may partially explain why efforts have not yet revealed a group of fungal species consistently associated with dampness in a home.[56,57] In a study testing the applicability of one metric designed to quantify mold in US homes, it was determined that the metric was not practical for use in differing climates and regions and must account for local fungal groups and specific moisture damage characteristics in homes.[58] The microbial communities in a home also naturally change over time, such as by season and with different usage patterns.[30,59-61] This highlights the difficulties of developing a metric of mold growth in homes. Currently, subjective measures such as visual inspection and detection of moldy odor continue to have the strongest associations with health effects from dampness in a home when compared to quantitative metrics.[62]

Some fungal species were determined to be more associated with the Saturated (100% ERH) moisture condition when compared to the Unmodified (50% ERH) moisture condition. Of these, *A penicillioides*, *C sphaerospermum*, *P chrysogenum*, and *W mellicola* were found to be present in every home, while *A sydowii* was not present in site 10 and *C halotolerans* was not found in site 18. These species are commonly found in the indoor environment particularly in water-damaged buildings or those with increased moisture.[63-69] However, several of these species have also been identified in other surveys of homes without explicit moisture damage.[30,53,70-72] *C halotolerans* has also been found to be more resistant to changes in a_w when compared to other fungal species.[69] The concentration of these species was higher at the 100% moisture condition, but they were also present in samples incubated at the Unmodified (50% ERH) moisture condition in every home (with the exception of *A sydowii* in site 10). It is likely that using these species as a marker for moisture in the indoor environment would be difficult and would need to be associated with increases in concentration.

Certain species may be better adapted to relative humidity cycling in the indoor environment (Table S7). Xerophilic fungi, species adept to grow at or below a a_w of 0.85, may be more inclined to grow under these variations in relative humidity.[73] Xerophilic fungi such as *Aspergillus penicillioides*, *P chrysogenum*, *Penicillium brevicompactum*, and *W mellicola* may proliferate in house dust with low moisture content.[74] Few studies, however, have been conducted measuring microbial growth on building materials while varying the relative humidity conditions. In one particular study by Johansson (2013), the fluctuations of relative humidity and temperature were measured in an attic space to determine mold growth on different building materials. They found that when the relative humidity did exceed their critical moisture levels determined for these different materials for a long period of time, there was increased growth.[75] One study conducted by Park (1982) identified the impact of drying and rewetting on fungal growth on cellophane membranes by observing fungal growth on culture plates where the colony diameter was regularly measured. *Cladosporium cladosporioides* was able to restart growth after 1 hour of a wet period, while certain *Penicillium* species needed an additional 50 hours of a wet period to restart growth after incubating for 2 weeks at a dry period.[76] Park (1982) determined that phylloplane fungi, or fungi that commonly grow on the surface of leaves, are more adapted to cycling conditions. Adan (1994) found that the fungal species *P chrysogenum* would grow on gypsum drywall if the duration of the wet periods was at least half of the total duration studied, and in our study, we found that it was also less prevalent under Elevated ¼ conditions compared to Elevated. Pasanen (2000) determined that allowing for a drying stage at 50% relative humidity did not decrease the viability of spores in materials such as gypsum board, particle board, and wood, while fast drying decreases the viability of fungal spores. However, *Penicillium* species seem to be adapted to tolerate stress from variations in humidity.[77]

Results from our study are consistent with findings in previous studies of microbes and microbial growth in house dust. *W mellicola* (formerly *Wallemia sebi*) is commonly found in the built environment, and exposure to this species can cause allergic sensitization.[68] Moisture-associated species *P chrysogenum* was found to be statistically associated with the Saturated (100% ERH) moisture condition, and other studies found this species to be the most common fungal species in water-damaged buildings.[67] The bacterial genera *Bacillus*, *Brachybacterium*, *Staphylococcus*, *Kocuria*, and *Virgibacillus* were found to be associated with Saturated (100% ERH) maximum moisture condition which is consistent with findings in a previous study.[16] Overall, the total fungal and bacterial concentrations of the original dust samples were fairly consistent with findings from previous studies, but the concentrations post-incubation often exceeded these initial concentrations (Table S12).[30]

4.1 | Limitations

This work utilized laboratory-based experiments that do not wholly represent the indoor environment and factors that may impact changes in relative humidity and temperature. Indoor conditions will not accurately mirror those in our laboratory chambers, but the findings from this study reiterate the importance of maintaining relative humidity and provide insights for creating moisture standards indoors.

Samples of dust and carpet were collected from 19 homes in the state of Ohio. Future work using dust and carpet from other areas of the United States and the world would be helpful in determining differences in microbial communities. Collecting samples from both arid and humid climates would also allow for comparison in how the dust and communities in the dust react to changes in relative humidity conditions. In this study when utilizing the TOW framework, active biomass was assumed causing the model to shift to an exponential growth model. This assumption was made as passive biomass cannot be determined through the current means.

Standard limitations of DNA sequencing and analysis methods also apply to this study. Universal qPCR and sequencing primers are prone to error due to copy number variation and amplification bias. Additionally, the estimated growth rates of different species within the samples are prone to compounding errors and should be viewed as estimates only.

4.2 | Future work and implications

The results of this study allow for the prediction of microbial growth in indoor dust under more realistic, varying relative humidity conditions. This capability can provide important insights into the impacts of harmful mold growth in buildings and unintended health outcomes. For instance, a previous study by Fabian et al[78] evaluated how indoor environmental conditions impact asthmatic children and modeled building interventions to determine how these interventions may reduce harmful exposure to asthma triggers. They found that integrated pest management and fixing kitchen exhaust fans caused a 7%-12% reduction in asthma events, while increasing some weatherization efforts on a home led to a 20% increase in the seriousness of an asthma event and a 67% increase in damp homes.[78] However, they used a model for mold exposure from 1999 that was based on an initial "mold index" scale.[79] The TOW framework could be used to evaluate similar home interventions to limit human microbial exposure. Future studies may account for this microbial growth under realistic changes in relative humidity when making recommendations to limit exposure to fungal allergens and asthma triggers. Our results also highlight the importance of identifying improved methods to measure microbial growth in homes for better associations with health outcomes. Improved models of microbial growth can lead to more accurate results and ultimately influence policy decisions.

Future work is needed to determine the mechanism of how microbes in dust uptake water. We currently do not understand dust moisture content or how much water is available to the microbes. The TOW framework will be useful to combine with models of water uptake of dust to predict health outcomes, including asthma and asthma exacerbation, due to increased moisture and mold growth.

This work is also timely, as wet weather events are expected to increase within the next 60 years.[80] These events may cause increased flooding and higher humidity in the indoor environment. Additionally, the increased use of air conditioning increases the risk of condensate leakage, as well as cool indoor surfaces with high ERH. The results presented here can help to predict which indoor surfaces are at increased risk of mold growth.

5 | CONCLUSION

Moisture is generally the limiting factor for microbial growth in the indoor environment. This is also true in carpet dust, which is an important source of human exposure. This study demonstrated that microbial growth in carpet dust can be sustained in the indoor environment even with short 6-hour bursts of elevated relative humidity in a room. The microbial growth can be predicted using the TOW framework with the two activation regime model. The two activation regime model had the closest fit to our data. These results emphasize the importance of controlling moisture indoors and that even short periods of time at elevated relative humidity levels have the potential to support fungal growth.

The results have important implications for building maintenance to protect human health. Special consideration should be applied when selecting building materials for periodically damp areas, such as bathrooms, and wall-to-wall carpeting and other dust reservoirs should be avoided in these areas. Prediction of microbial growth using this model can influence both building design and policy simulations of moldy homes to provide more accurate results and inform future decisions. Ultimately, these results indicate the importance of maintaining proper relative humidity levels within the home and being cognizant of areas where moisture levels may become elevated, even temporarily.

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AUTHOR CONTRIBUTION

Sarah R. Haines: Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Writing-original draft (lead); Writing-review & editing (equal). **Jeffrey A. Siegel:** Conceptualization (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Resources (equal); Supervision (equal); Writing-review & editing (equal). **Karen C. Dannemiller:** Conceptualization (lead); Data curation (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Supervision (equal); Writing-review & editing (equal).

ORCID

Sarah R. Haines https://orcid.org/0000-0003-3791-6947
Jeffrey A. Siegel https://orcid.org/0000-0001-5904-169X
Karen C. Dannemiller https://orcid.org/0000-0003-4754-6804

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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