Modeling microbial growth in carpet dust under diurnal variations in relative humidity

Thesis

Presented in Partial Fulfillment of Requirements to graduate with Research Distinction in Environmental Engineering at The Ohio State University

By

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2017

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Abstract

People spend 90% of their time indoors where resuspension of dust from the floor represents a major source of human microbial exposure. Microbial communities in floor dust can grow under elevated equilibrium relative humidity (ERH) and contribute to these exposures. However, it is not yet known how diurnal variations in relative humidity will affect this growth. The goal of this work is to demonstrate that fungal growth in house dust can be modeled using the "time-of-wetness" (TOW) concept from growth on drywall, and that as the TOW of the dust increases so will the relative growth rate of fungi and bacteria. Carpet was collected from different homes, cut into 10 cm x 10 cm carpet squares, embedded with dust from the same home and placed inside incubation chambers with controlled ERH for two weeks. ERH was varied from 50% to either 85% or 100% for a period of 0, 6, 12, 18, or 24 hours per day over the two week period. Data loggers were placed in a subset chambers to record ERH and temperature. The embedded carpet was hygroscopic as indicated by the hysteresis behavior of the relative humidity in the chambers. Quantitative polymerase chain reaction (qPCR) performed on the dust DNA extractions revealed that the TOW for the 85% and 100% samples relates to the standard TOW curve. These measurements demonstrated that the relative growth rate fit the TOW model within the determined Pearson Correlation Coefficient of 0.974. This model will be refined as the study continues. Ultimately, this data can be used to accurately model fungal growth in housing based on moisture and can be utilized in future public health, policy, and epidemiological models.

Acknowledgements

I would like to thank my mother and father for always encouraging me to follow my dreams, my sister for showing up with cups of coffee after a long day in the lab and Jack for late nights helping me edit my research papers and supporting me through this new adventure.

Also I would like to thank Dr. Karen Dannemiller for taking me on as one of her first undergraduate research assistants and always believing in my potential to succeed. To Professor Bielicki who has supported this research from the beginning. To Ashleigh, Sam, Quentin and Ningling for never hesitating to help with various parts of my research, be it cutting carpet or changing samples at 9 pm. For that I am truly grateful.

Finally thank you to the OSU Undergraduate Research Office, the Research Scholar Award and the College of Engineering undergraduate research project for funding my last year of research as well as the Alfred P Sloan Foundation for grant number G-2016-7262 which will also support me in completing this research as a graduate student.

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Introduction

People spend 90% of their time indoors, where the air may contain chemicals and microbes that are harmful to human health. One of the leading causes of hospital visits among children is asthma, which affects 1 in 13 people in the United States (Merrill, 2002). Prolonged exposure to microbes suspended in the air as well as water dampness in homes contributes to asthma exacerbations. These microbes enter homes through tracked in soil, outdoor air and human shedding. Once present in the home microbes may be resuspended from carpets into the air, which is an important source of human exposure (Qian, 2012). When relative humidity levels are elevated the growth of microbes in floor dust increases at an exponential rate (Dannemiller, 2016). There is limited knowledge on if these microbes, once in the home, can grow in carpets and increase the total number of microbes. We lack a quantitative understanding of this growth and do not have the ability to model it, which would be beneficial for future epidemiological studies (Fabian, 2014).

Application of the time-of-wetness (TOW) concept in drywall was developed by researcher Olaf Adan in 1994(Adan, 2011). This model shows that as the TOW of gypsum drywall increases so does the relative growth rate of fungi in the drywall. The TOW model is based off the fraction of time it takes for the relative humidity increase above the 80% threshold. When the relative humidity in an insulated room is elevated the

interior drywall can become saturated due to condensation. Though this saturation may last for 10 minutes the overall effects may last for over 6 hours with the relative humidity maintaining above 80% therefore increasing microbial growth (Adan, 2011).

A study conducted by Dr. Karen Dannemiller looked at microbes growing in carpet dust above constant 50% relative humidity levels (Dannemiller, 2016). This study maintained relative humidity at constant levels for a two week period of time. However it did not account for diurnal variations in relative humidity. The home environment does not stay at constant ERH conditions indefinitely and instead varies throughout the day. The goal of this study is to model microbial growth in carpet dust under diurnal variations within buildings to determine how this may impact human microbial exposure.

Materials and Methods

Collection. Beginning in May 2016, we collected carpet with no known mold growth from 6 homes throughout Ohio using a previous protocol (Dannemiller, 2016) (Figure 1). The carpet was removed from high traffic areas of the home such as the living room or family room. 240cm x 160 cm sections were removed and cut into twelve 20 cm x 160 cm strips labeled A through H. Aluminum foil previously baked to 500 °C for 4 hours was placed on top of each strip which was then rolled, ensuring that the bottom of the carpet did not touch the top and sealed in a polyethylene zip top bag.

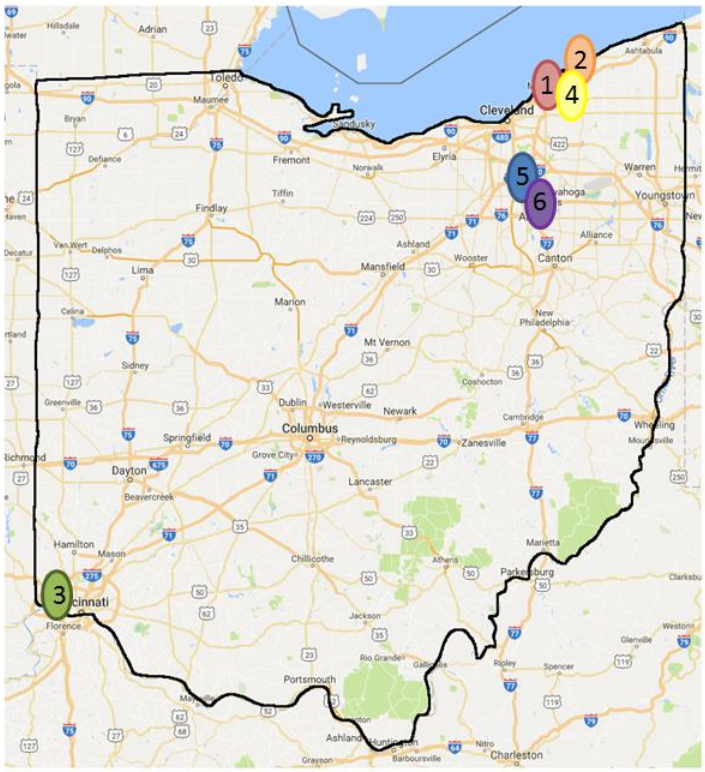

Figure 1. Carpet site locations across the state of Ohio.

The remaining carpet was vacuumed and dust was collected either using the in home vacuum or a Eureka Mighty Mite. This collected dust was then passed through a 300 um sieve to obtain fine dust particles and stored at room temperature for later use. Once back in the laboratory, the carpet was cut into 10 cm X 10 cm squares and wrapped in aluminum foil for later use.

Chamber Study. Carpet coupons were placed in temperature-controlled chambers where relative humidity was varied from 50% to either 85% or 100% for 0, 6, 12, 18, or 24 hours to mimic diurnal variations in buildings. Relative humidity in the chambers was controlled with salt solutions. The salt solutions were created based on the assumption that water activity equals the relative humidity of air denoted as a ratio. Water activity is measured when a solution is at equilibrium with the air above it and no transfer of water vapor occurs. It is calculated by using the equation $a_w = \frac{RH}{100\%}$ ranging between values of 0 to 1, with a value of 1 referring to pure water and values less than 1 referring to aqueous solutions (Adan, 2011). Salt solutions at 50%, 85% and 100% ERH were created using NaCl and $MgCl_2$. 100 mL of distilled water (DI) and 42.84 g of $MgCl_2$ were used to make the 50% solution while 100 mL of DI and 23.37 g of NaCl were used to make the 85% solution. The two salt solutions were utilized because NaCl cannot be used to make a salt solution above 75% water activity or it will become too saturated (Barbosa-Canovas, 2008). These solutions were then tested for accuracy using an AquaLab Dew Point Water Activity Meter (Decagon 125 Devices, Pullman, WA, USA) and adjusted if needed. Each 10 cm X 10 cm carpet square was then sprinkled with 250 mg of dust from the same home, excluding the 1 cm closest to the edges to avoid edge effects. The dust was embedded using ASTM method F608-13 modified by using a 12 cm long, 1440 g

steel pipe (Dannemiller, 2016). Three embedded squares from the same site labeled as day 5, day 10 and day 14 were placed in autoclaved glass incubation jars along with 100 mL of the correct ERH solution and an Onset ® HOBO® Data logger (Onset Computer Corporation 470 MacArthur Blvd. Bourne, MA 02532) that recorded the ERH conditions continuously every minute.

The incubation jars were placed in the incubator set at 25 °C for two weeks. To simulate diurnal variations the ERH was changed from 50%, 85% or 100% every 18/6 hours (in one set) or every 12 hours (in the second set)(Figure 2).

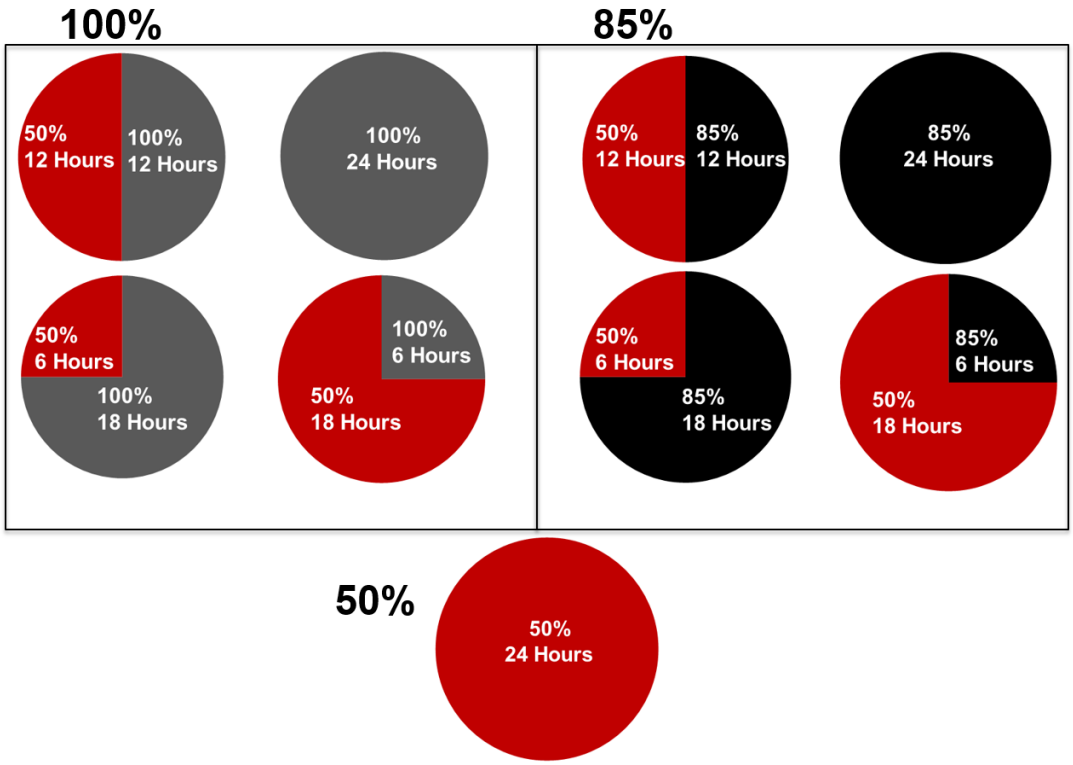

Figure 2. Each circle above represents an incubation chamber, with the variation of time and humidity condition over 1 day. The ERH condition was changed in each chamber over each time period given above. For example, at 9am the 12 hour 100% ERH samples were changed to 50% ERH and then at 9pm were changed back to 100% ERH. These variations were continued for 14 days and completed for each site.

On day 5, day 10 and day 14 one square from each chamber was removed, weighed and dust was collected using 19 mm x 90 mm cellulose Whatman thimble inserted into a Eureka Mighty Mite using a cylindrical adapter (Dannemiller, 2016). The carpet was vacuumed in a linear motion moving up and down the carpet square and then in a circular

motion for around 3 minutes. The dust was then stored at -20°C until further analysis.

Analysis. To begin analysis dust was removed from the Whatman thimbles and weighed. Aliquots of 50 mg of the weighed dust were then placed into 2.0mL screw top vials for extraction. DNA from the dust was extracted using a modified protocol for the MoBio PowerSoil DNA extraction kit (MoBio, Carlsbad, CA, USA) with modification (Yamamoto, 2012). Once extracted the DNA extract was diluted to a concentration of 10x using Tris-EDTA (TE) to prevent PCR inhibition. To test for inhibition the samples were spiked with a known concentration of DNA from 106 *Bacillus atropheaus* cells or 10^8 *Aspergillus fumigatus* spores depending on which was being tested and then compared to the expected value.

Samples from site 1-3 experienced both fungal and bacterial inhibition with the 18 and 6 hour 10x dilution samples. The samples were diluted to 100x and qPCR was run again to check for inhibition. The Site 1-3 samples were run at 100x dilutions and at this concentration did not experience inhibition. Quantatative polymerase chain reaction (qPCR) was then completed for these samples at 100x concentrations, which was then accounted for in later calculations. Site 4-6 samples showed little inhibition at 10x, though the quantity of the amount of microbes in the samples was much less than the Site 1 - 3 samples. Site 4 - 6 samples were subsequently run through qPCR at the 10x

dilutions and the quantities of spore equivalent/mg dust were calculated and recorded. Once qPCR was completed the samples were ready to be sent to sequencing.

76 samples diluted to 10x with molecular water, including 3 blank samples were sent to be sequenced at the Molecular and Cellular Imaging Center at the Ohio State University Wooster Campus. Bacterial 16S and fungal ITS ribosomal DNA primers were used to sequence the samples using 2x300 bp chemistry on the Illumina Mi Seq platform. Once sequencing data was received it was analyzed using QIIME (Caporaso, 2010).

Upon receiving the data adapters and identifiers were already removed, and primers and spacers were trimmed. Paired end reads were joined using the SeqPrep method. Reads were quality trimmed to a Phred score of 20, reads with any N were bases were removed, and the maximum number of low quality base calls allowed before truncating a read was 3. For alpha and beta diversity analysis we trimmed the reads in all samples for bacterial samples at 4172 reads per sample and fungal at 8580 reads per sample. The Bray-Curtis method was utilized to verify the dissimilarity between each site for fungi. For bacteria we considered both weighted and unweighted Unifrac (Lozupone, 2005).

Results

Data loggers. Information from the data loggers was compiled using HOBO software and exported to spreadsheets, listing the time, temperature (C°) and ERH. With this information we determined how long the ERH was above 60%, 65%, 70%, 75%, 80% and 85% ERH for each of the different conditions. A MATLAB code was created to determine how many times the ERH was above this certain level according to the data loggers.

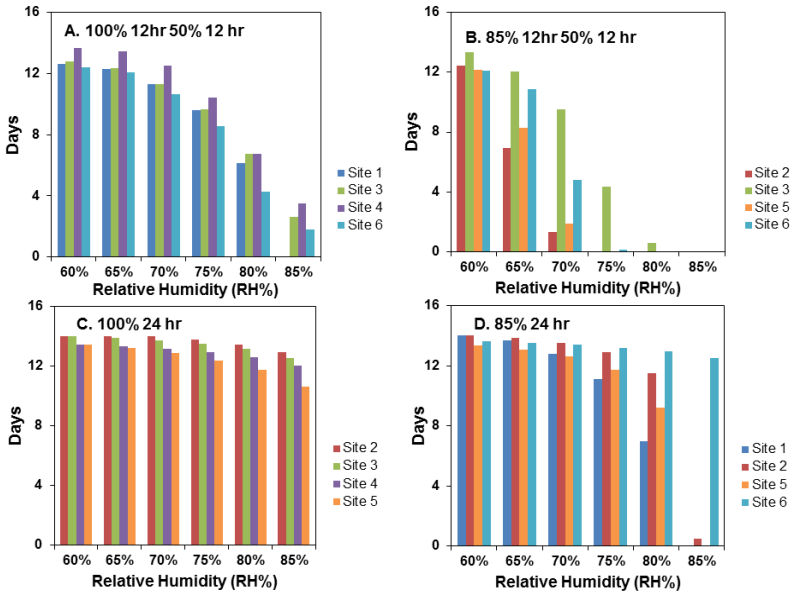

Figure 3. Total number of days that relative humidity was elevated above potential growth thresholds from 12/24 hour conditions.

When the ERH was changed from 100% to 50% or 85% to 50% for a 12 hour cycle the ERH in the chamber remained at 65% or above for over half the time (7 days total time out of 14 days). From these results it was concluded that the carpet can be considered hygroscopic, in that it retains the moisture for a longer period of time than the air. If the carpet was not hygroscopic we would have noticed the ERH dropping to 50% for about the half of the time and then rising to 85% or 100% for the other half of the time. Figure 4 exemplifies this hygroscopic nature showing that at 100% the ERH would experience a rapid decrease when the parafilm was removed and the ERH jars were switched to 50% but would remain around 80% ERH until the jars were switched back.

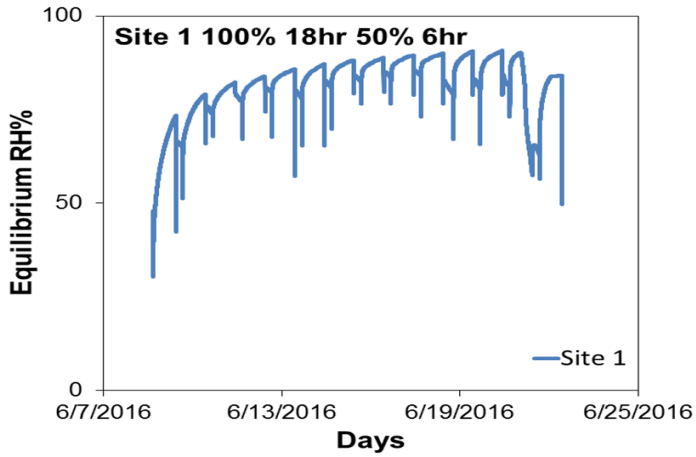

Figure 4. Example of ERH data taken from the HOBO data loggers after 2 weeks.

TOW. We calculated the growth rate (*k*) and effective growth rates (*R*) from each incubation jar and site to determine the TOW at both 85% ERH and 100%. The value *k* is considered the growth rate under steady state conditions. *R* is calculated as the effective growth rate when exponential growth is assumed. 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* and *k*=growth rate.

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

Because of the nature of the experiment with elevated moisture conditions, all growth was considered active. This forces $\mu = 0$, thus canceling the second half of the equation leaving Equation 2.

$$A(\Delta t) = A(0)e^{(k\Delta t)} \qquad \textbf{Equation 2}$$

To solve for *k*, the exponential growth curves were determined by plotting each site's 24 hour sample quantities taken from qPCR by the number of days of incubation. The *R* values were then calculated assuming exponential growth and Equation 3 where nT= total time after n cycles (Adan, 2011).

$$F(nT) = F(0)exp(RnT) \qquad \textbf{Equation 3}$$

The exponential growth curve equation was determined by plotting quantity and days of incubation, but this time for every other incubation combination. This information was then used to calculate R/k values at 0, 0.25, 0.50 and 1.

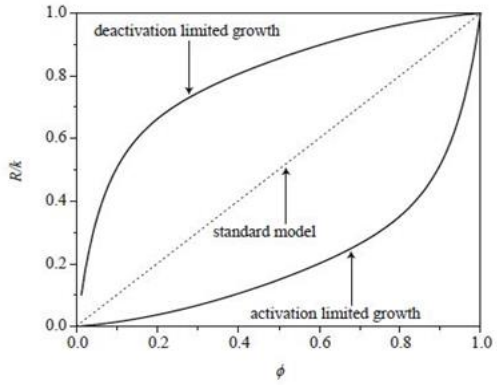

Figure 5. Typical time-of-wetness versus the relative growth rate curves, showing the activated limited growth curve and the deactivation limited growth curve. Reproduced from (Adan, 2011).

Figure 5 represents the standard TOW model established by Olaf Adan using drywall. The standard model represents ideal conditions in which the *R/k* value = TOW. Activation limited growth occurs when there is a lag in fungal growth after ERH conditions exceed the threshold and deactivation limited growth curve occurs when there is a lag in growth stopping after ERH decreases below the critical growth threshold (Adan, 2011).

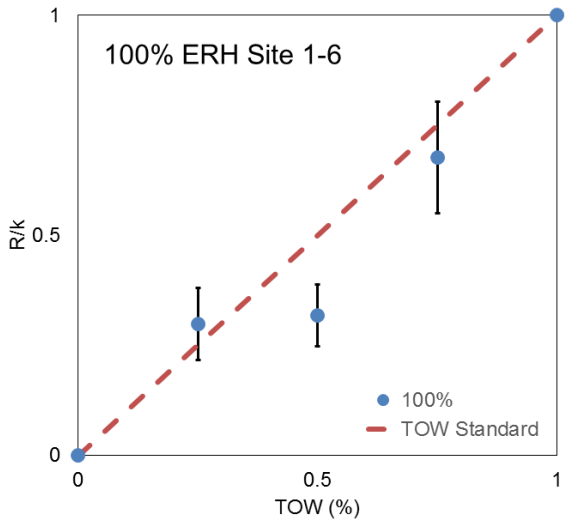

Figure 6. TOW compared to R/k, averaging sites 1 through 6 at the 100% conditions.

Figure 6 shows the 100% ERH data follows the TOW standard model. These points were determined by averaging the R/k values from each site to get a representative point. Looking at the 85% ERH data from each site it was determined that only data from site 1 – 3 would accurately represent the model. Further analysis must be done on sites 4 -6 prior to inclusion in the model. The TOW plot from sites 1 – 3 at 85% ERH can be seen below in Figure 7.

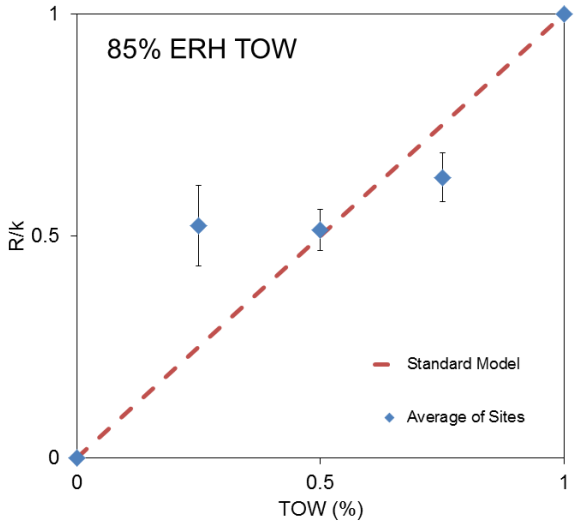

Figure 7. TOW compared to R/k at 85% ERH using data from Sites 1 – 3 against the standard model.

There was a strong correlation between the R/k and the TOW as determined by the Pearson correlation coefficient. The Pearson correlation coefficient is a measure of the linear dependence between two sets of variables. When the coefficient is close to 1 this means that the data sets are correlated on each other (Mukaka, 2012). Using the data in Figure 7 the coefficient was calculated as 0.897. The coefficient using the data in Figure 6 was determined to be 0.974 which shows a strong correlation between data.

DNA Sequencing. Data from sequencing results were separated into bacterial and fungal results, with 1,223,318 total bacterial reads and 2,394,479 total fungal reads. The graphs below were completed using the principal coordinate analysis (PCoA) data taken from the QIIME core diversity analyses. PCoA takes all of the given data and the variability within the data and clusters similar samples together (Ramette, 2007). Because of this the axes of the PCoA within the different plots in Figure 8 and 9 do not relate to each other but instead represent the similarities or dissimilarities of the data set. The axes were therefore assumed to correspond to either site, moisture condition or length of ERH incubation.

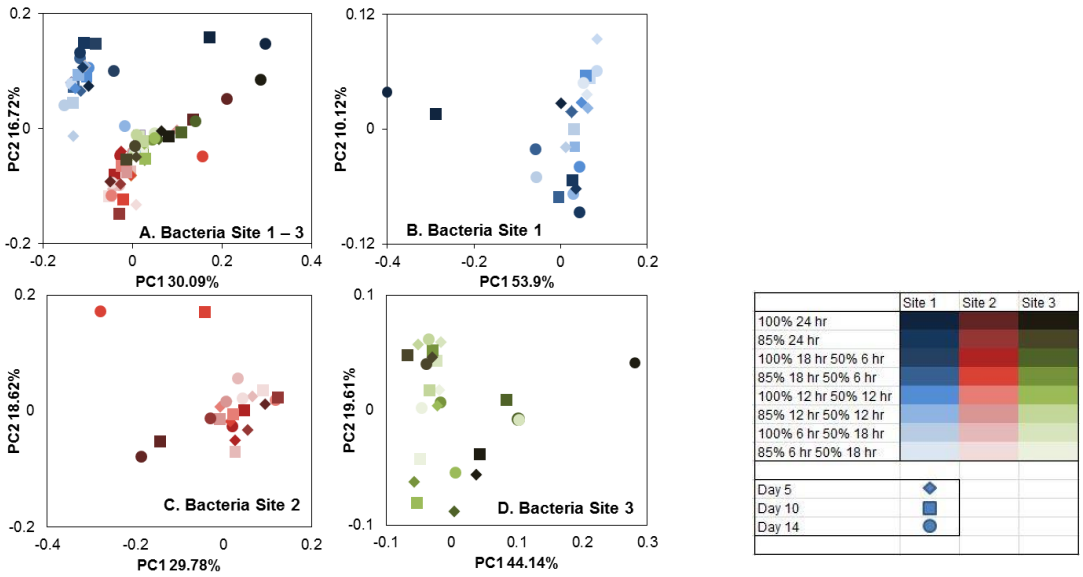

Figure 8. Bacterial principal coordinate plots.

In bacteria, site and moisture conditions lead to differences in the communities (Figure 8A). In Figure 8A the distinct separation between sites can be seen along PC1 as well as the color gradient representing the moisture condition lessening moving down PC2 and therefore accounting for less growth. After separation by site, Figures 8B, 8C, and 8D demonstrate that moisture conditions lead to changes in the bacterial communities. The darker markers (representing higher moisture) separate from the lighter markers (representing lower moisture conditions). Figure 8B and 8C separate the most with little change occurring at day 5, as the values are seen clustering together and then separating at 10 and 14 days. Samples held at 100% RH 24 hours a day for 14 days showed the most separation from the rest of the data.

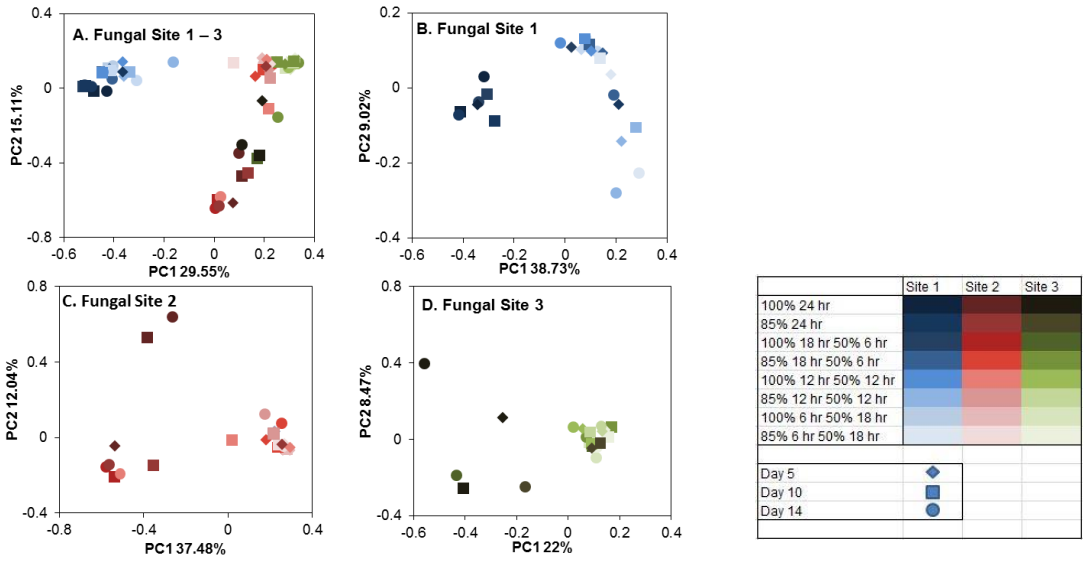

Figure 9. Fungal principal coordinate plots using QIIME core diversity analyses.

The fungal analyses from QIIME showed more variation among days and ERH condition compared to the bacterial data. Five days of incubation, unless kept at 100% ERH for 24 hours, did not produce changed in the microbial communities (the points did not separate). This is most likely due to the lag time in the activation growth curve as discussed previously. From Figure 9B, which depicts site 1 fungal data, a large amount of separation occurred from the 100% 24hr, 85% 24hr and 100% 18hr values. There also tended to be more separation from the 100% 12hr than the 85% 18 hr. 6 hours of incubation at 100% ERH or 85% ERH then switched to 50% ERH for 18 hours was normally not enough time for a large separation between days meaning a limited amount

of growth occurred after 2 weeks at this level.

Discussion

Fungal growth in carpet dust follows the TOW trend as determined with growth in drywall. When the ERH is above the 80% threshold growth occurs at an exponential rate. It is also noted that 5 days at varying relative humidity is not enough time for fungal spores to begin their growth cycle, following the activated limited growth model. The carpet maintains moisture even when ERH is changed from a high level to a lower level. When the ERH in the chamber was decreased the actual decrease was gradual with ERH staying above the 80% for over half the time.

Fungal Growth. As the carpet dust is exposed to diurnal ERH conditions for a two week period fungal growth occurs at an exponential rate. This growth is more likely to follow an activation limited growth model than a deactivation limited growth model as seen by the lag in growth occurring over the first few days followed by exponential growth up to day 14 (Figure 6). When carpet is wetted fungal concentration is known to increase exponentially from day 1 to day 7 and then level off between day 7 to day 30 (Ong, 2014). The growth in our study may be slower because the carpet will contain less moisture than if water was applied directly.

Microbial and Fungal Communities. Bacterial sequencing showed the greatest variance among the data as site differentiation while fungal sequencing varied around both the site and ERH differences. This is consistent with previous studies showing that bacteria are influence by occupants while fungal communities are largely influenced by outdoor air and moisture (Meadow, 2015, Dunn, 2013, Hospodsky, 2012, Adams, 2013, Täubel, 2009). Fungal and bacterial communities are associated with specific site or home characteristics that can cause variation in communities. This study also demonstrated this pattern but established an association with the TOW model, showing that as ERH conditions were gradually increased from 50%, 85% and 100% throughout a two week period growth occurred at around day 10 and onwards.

Implications of work. Resuspension of floor dust in the home is a major source of human microbial exposure (Qian, 2012). The high levels of microbial and fungal spores in carpet dust under diurnal variations may be inhaled easily through resuspension. These communities are also known to be associated with certain health outcomes (Dannemiller, 2015). This may increase the chance of asthma development in homes with similar conditions. It is important to monitor RH levels in homes containing carpet in much of the living spaces. The EPA recommends maintaining indoor relative humidity between 30-50% and definitely below 60% (Mold Course Chapter 2, 2017). Our work

demonstrates the importance of maintaining proper relative humidity in buildings to prevent unwanted microbial growth and exposure. RH monitors can be purchased from local hardware stores to determine any fluctuations in RH in the home, but this may not be helpful if the ERH of the carpet is at a higher RH than the rest of the house because of its hygroscopic nature. One way to inactivate fungal and microbial growth is through use of steam treatment, but this unfortunately does not remove the spores from the surface (Ong, 2014).

Limitations. Only 6 homes were sampled across Ohio, USA in this study, with most homes located in Northeast Ohio and one in the Cincinnati, Ohio area, as can be seen in Figure 1. To achieve a more representative sample 14 more homes are planned to be sampled throughout the course of 2017-2018 totaling at 20 sites. It is also hoped that microscopy will be utilized to further analyze the results of this project. This research will be continued through my graduate studies at The Ohio State University.

Conclusion

The information given throughout this paper will be useful in future epidemiological studies looking into fungal growth in carpets and homes. Carpet dust does follow the TOW trend similar to gypsum drywall in which under elevated relative humidity conditions growth occurs at an exponential rate. Maintaining proper ERH in homes

would be the first step in monitoring growth in carpets. It may be interesting to determine the growth rate of bathroom carpets and how constant moisture from the shower may contribute to this growth. This study demonstrates that increased relative humidity in homes could increase human exposure to microbes.

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