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Variability of Indoor Fungal Microbiome of Green and Non-Green Low-Income Homes in Cincinnati, Ohio

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Abstract

"Green" housing is designed to use low-impact materials, increase energy efficiency and improve occupant health. However, little is known about the indoor mycobiome of green homes. The current study is a subset of a multicenter study that aims to investigate the indoor environment of green homes and the respiratory health of asthmatic children. In the current study, the mycobiome in air, bed dust and floor dust was compared between green (study site) and non-green (control site), low-income homes in Cincinnati, Ohio. The samples were collected at baseline (within four months following renovation), and 12 months after the baseline at the study site. Parallel sample collection was conducted in non-green control homes. Air samples were collected by PM2.5 samplers over 5-days. Bed and floor dust samples were vacuumed after the air sampling was completed. The DNA sample extracts were analyzed using ITS amplicon sequencing. Analysis indicated that there was no clear trend in the fungal communities between green and non-green homes. Instead, fungal community differences were greatest between sample types - air, bed, and floor. Microbial communities also changed substantially between sampling intervals in both green

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and non-green homes for all sample types, potentially indicating that there was very little stability in the mycobiomes. Research gaps remain regarding how indoor mycobiome fluctuates over time. Longer follow-up period might elucidate the effect of green renovation on microbial load in buildings.

Keywords

mycobiome; occupancy; air sampling; dust sampling; sequencing

1. INTRODUCTION

The built environment microbiome, coupled with the extensive amount of time spent by individuals indoors, has been known to influence human health (Kanchongkittiphon et al., 2015; Mendell et al., 2011). Exposure to fungi has been linked to a range of detrimental health effects (Douwes et al., 2003) including asthma (Jaakkola et al., 2010; Reponen et al., 2011). However, protective effects of fungi have also been reported. Exposure to increased levels of mold-derived components early in life was found to protect children from allergic diseases and allergic sensitization (Iossifova et al., 2007). Due to these links to human health, it is imperative to better understand the complex microbial habitat of the indoor built environment, especially if immunocompromised or mold-sensitized individuals are present.

With the “green” building movement, more and more homes are opting to be energy efficient. “Green” housing is designed to use low-impact materials, increase energy efficiency and improve occupant health (Kibert, 2016). Previous studies have shown that green and non-green materials support microbial growth similarly (Mensah-Attipoe et al., 2015) (Coombs et al., submitted for publication). However, trends in energy efficiency, having led to “tighter” buildings with reduced ventilation could potentially result in increased humidity and lead to altered microbial load (Fabian et al., 2014; Macher et al., 2016).

High-throughput DNA sequencing has recently been used for obtaining a culture-independent and comprehensive picture of the microbial dimension of a variety of ecosystems (Konya and Scott, 2014). Microbial diversity has also been assessed in a variety of indoor environments, ranging from homes and offices to healthcare facilities and transportation environments, as previously reviewed (Ramos and Stephens, 2014). The majority of studies examining residences have focused on the bacterial diversity in the indoor environment (Dunn et al., 2013; Flores et al., 2013; Kelley et al., 2004). One prior study (Kembel et al., 2014) characterized bacterial biomes in dust samples collected in a “green” university building. The few studies of fungal taxa within homes have mostly investigated swabbed surfaces, vacuumed floor dust or indoor air using either gravity settled air samples or portable air samplers (Adams et al., 2013a; Adams et al., 2013b; Dannemiller et al., 2014; Kettleson et al., 2015; Rittenour et al., 2014; Yooseph et al., 2013). No previous studies, however, have compared fungal communities in air, bed dust and floor dust. Furthermore, very limited data are available on the effect of “green” building practices on indoor fungal load. Lower levels of ergosterol (an estimate of fungal biomass) were found

after a year of residency in green-renovated homes compared to levels measured in the old home before moving out (Takaro et al., 2011).

This study is a subset of a multicenter study designed by the Centers for Disease Control and Prevention (CDC) and the Department of Housing and Urban Development (HUD). The goal of the multicenter study is to investigate the relationship between the indoor environment of green homes and the respiratory health of children with asthma living in low-income homes. Previously, we reported that no difference was found in the levels of PM2.5, black carbon, sulfur, ultrafine particles, total volatile organic carbons or formaldehyde between green and non-green homes (Coombs et al., 2016). Here we characterize and compare the mycobiomes (fungal microbiomes) of indoor air, bed dust and floor dust between green and non-green homes in Cincinnati, Ohio. The goal of the study was to determine if green renovation altered richness and diversity of the indoor mycobiome.

2. MATERIALS AND METHODS

2.1. Study Design

The study included 52 low-income homes (26 green-renovated apartments, and 26 non-green control apartments) (Figure 1). Green-renovated apartments were drawn from a low-income, 800 apartment complex in Cincinnati. All of the green homes were renovated from previously non-green units. The characteristics of the study homes have been reported previously (Coombs et al., 2016). Briefly, green features that were expected to affect the humidity and thereby, the microbial load included energy efficient windows and doors, whole house insulation, energy efficient central heating/cooling system, and bathroom fans. The first post-renovation (baseline) samples from green-renovated homes were collected within four months of renovation, and another set of samples was collected 12 months later. Parallel sampling, matched by the season, was conducted in non-green homes; 6 non-green homes were located in the same community as the green-renovated homes and 20 were located at the control site about 6 miles from the green-renovated homes. Sampling at a control home was matched with the study home by season. Both apartment complexes receive federal assistance to allow them to provide subsidized housing to low-income families (U.S. Housing Act of 1937). Homes were considered for inclusion if a child who lived in the home was age 7–12 years and the caregiver reported the child had a diagnosis of asthma and current symptoms in past six months.

Temperature and relative humidity were measured using a HOBO® data logger (Onset Computer Corporation, Bourne, MA) and were continuously recorded every five minutes throughout the five-day air sampling duration. The data were downloaded, and a five-day average was used in the data analysis. The mean relative humidity (± standard deviation) was 40.5±13.5% in green homes and 41.8±10.7% in non-green homes. The difference was not significant (t-test: $p=0.323$). The respective values for temperature were 24.5±1.9°C and 24.6±1.8°C ($p=0.399$). A checklist was used to record data on home characteristics, such as signs of visible mold. Residents in control homes reported visible mold and moldy smell more often than residents in green homes at the baseline (Table S1). Home characteristics have been presented previously in more detail (Coombs et al., 2016).

2.2. Sample collection and handling

Air samples were collected over a period of 5 days in child's bedroom. In some cases, the child slept mainly in the primary caregiver's bedroom, so air samples were collected there instead. Air samples were collected onto 37 mm diameter, 2.0 μm pore size Polytetrafluroethylene (PFTE) membrane filters using single-stage PM2.5 Personal Modular Impactors (SKC, Inc., Eighty Four, PA) connected to AirChek 2000 pumps (Model 200–2002; SKC, Inc., Eighty Four, PA). The pumps were calibrated to a flow rate of 3 (± 10%) L/min before the start of sampling and checked immediately after sampling with a BIOS DryCal DC-2 flow meter (SKC, Inc., Eighty Four, PA). Post sampling, the filter samples were stored in sterile containers at −20°C until analyzed.

Dust samples were vacuumed from the living room floor and the child's bed or, alternatively, the primary caregiver's bed on the fifth air sampling day as described earlier (Adhikari et al., 2014). The dust was sieved (355 μm mesh sieve), and the resulting fine dust was stored at −20°C before analyses (Adhikari et al., 2014). For bed samples, the mattress and pillows associated with the upper half of the beds were vacuumed for three minutes. Post-sampling, the filters containing the dust were sealed in sterile plastic tubes and stored at −20°C until analyzed. The bed samples were not sieved because it was homogeneous throughout the surface of a bed and made of small fine particles that would typically go through a sieve.

The number of air and bed samples included in the analysis was not the same as the floor samples because 12% of the air samples failed (either the filter was damaged, or the flow rate decreased >10%) and 57% of the beds did not contain sufficient amounts of dust for analysis. Participant attrition contributed to lower sample numbers at 12-months.

2.3. Fungal Analysis using Illumina MiSeq

DNA was extracted by using the PowerMax Soil DNA Isolation Kit (MO BIO) from 20 mg of dust collected from the floor, 20 mg of dust collected from bed surfaces and the entire filter containing air samples, as described previously (Yamamoto et al., 2012). DNA extracts were sent to the Research and Testing Laboratory (Lubbock, TX, USA) for Illumina MiSeq sequencing. All samples were analyzed in one sequencing run.

The ITS1 region from the DNA sample extracts was amplified for sequencing using a forward and reverse fusion primer. The forward primer included the (5'-3') Illumina i5 adapter (AATGATACGGCGACC-ACCGAGATCTACAC), an 8–10bp barcode, a primer pad, and the ITS1F primer (CTTGGTCATTTAGAGGAAGTAA). The reverse fusion primer included the (5'-3') Illumina i7 adapter (CAAGCAGAAGACGGCATACGAGAT), an 8–10bp barcode, a primer pad, and the unlabeled ITS2 primer (GCTGCGTTCTTCATCGATGC). Primer pads were designed to ensure the primer pad/primer combination had a melting temperature of 63–66°C according to methods described previously (Kozich et al., 2013). Twenty-five μl reactions were prepared using Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), plus 1 μl of each 5 μM primer, and 1 μl of the template. ABI Veriti thermocyclers (Applied Biosystems, Carlsbad, California) were used to cycle the reactions, with the following thermal profile: 95°C for 5 min, then 35 cycles of 94°C for 30 sec, 54°C for 40 sec, 72°C for 1 min, followed by one

cycle of 72°C for 10 min and 4°C hold. Internal negative controls were run together with the samples. Negative control samples did not amplify.

The PCR products were visualized with an eGel Imager (Life Technologies, Grand Island, New York). Resulting amplicons were then pooled at equimolar concentration, and size selected using Agencourt AMPure XP (BeckmanCoulter, Indianapolis, Indiana). The size selected pool was quantified by using the Qubit 2.0 fluorometer (Life Technologies) and loaded onto 2×300 flow cell at 10 pM in an Illumina MiSeq (Illumina, Inc. San Diego, California).

2.4. Identification of Taxa and Statistical Analysis

Forward and reverse reads in FASTQ format were merged using the PEAR illumina paired-end read merger (Zhang et al., 2014) and the resulting sequences converted to FASTA format. Prefix dereplication was performed using USEARCH (Edgar et al., 2011). Sequences with less than 70% identity to alpha release of the UNITE/QIIME 12_11 ITS reference dataset (http://qiime.org/home_static/dataFiles.html), approximately 0.48% of the sequences, were removed from further analysis. De novo OTU clustering and generation of an OTU table was performed using the UPARSE pipeline (Edgar 2013) at 97% threshold identity. Taxonomic assignment of OTUs was performed using QIIME 1.8.0 and the reference dataset (Abarenkov et al., 2010; Caporaso et al., 2010). OTUs were filtered from the de novo OTU table, removing taxa below a minimum fractional count of 0.01% (e.g. --min_count_fraction 0.0001).

Alpha and beta diversity analysis were performed with QIIME and with the vegan package in R. For beta diversity (Bray-Curtis dissimilarity), single rarefaction was performed at a depth of 3000 sequences per sample. The data and sample clustering and visualization were performed by principal component analysis (PCA) and hierarchical clustering. Alpha diversity was characterized using Shannon and Chao 1 measures. First, rarefaction plots were generated using the maximum depth of 3000 sequences per sample. After exporting PCA plots from QIIME and the non-rarefied OTU table, it was noted that no additional samples would be lost using a rarefaction depth of 10000 reads. Therefore, for statistical testing of differences in alpha diversity, single rarefaction was performed at a depth of 10000 sequences per sample using the vegan package in R (Oksanen et al., 2015). The Kruskal-Wallis test was used to compare alpha diversity between baseline and 12-month samples and between green and non-green housing. Additional comparisons were conducted using the Kruskal-Wallis test between the green and non-green housing and between baseline and 12 months for each particular sample type (air, bed, and floor). Each sample was included in six comparisons:

1. a comparison between all green and all non-green samples;
2. a comparison between all baseline samples and all 12-month samples;
3. a set of comparisons separated by particular sample type (air, bed or floor) to compare green and non-green samples;
4. a set of comparisons separated by particular sample type (air, bed or floor) to compare baseline and 12-month samples;

5. a set of comparisons broken down by sample type AND time point (for example baseline air samples) comparing green and non-green;
6. a set of comparisons by particular sample type, particular renovation status (for example green bed samples) for baseline vs. 12-month.

A Bonferroni correction was applied to a p-value of 0.05 resulting in a significance level set at p=0.008.

To explore differential abundance in the taxa between the housing environments, the linear discriminant analysis effect size estimator (LEfSe) software was used (Segata et al., 2011). In order to further validate and visualize patterns observed in data, we used hierarchical clustering (bi-clustering) of samples and OTUs. In order to reduce the noise, the OTUs had to be present in at least 25% of the samples and classified to the genus level to be included in the analysis. OTU counts were normalized to express them in relative terms as the fraction of the total number of counts per sample. For generating the heat map, OTUs with unknown genus were discarded. Both, Euclidean and Pearson distance measures were used generating similar results

Finally, the Jaccard distance was calculated to assess how much the composition of each sample type in each home changed from baseline to 12-months in green versus non-green homes.

3. RESULTS

Post quality trimming, there were over 2.3 million fungal reads from air samples (approximately 1.25 million from green samples and 1.1 million from non-green samples), over 1.2 million fungal sequences from bed dust samples (521,826 from green samples and 726,690 from non-green samples) and over 7.7 million sequences from floor samples (approximately 4 million from green samples and 3.7 million from non-green samples).

Principal component analysis (PCA) and hierarchical clustering were performed in order to assess clustering (and potential separation) of samples of different housing type, e.g., green vs. non-green houses, in terms of beta diversity. While there was no separation between green and non-green units (Figure 2A) or between baseline and 12-month samples (Figure 2B), there was clear clustering based on sample type (Figure 2C). Similar results were obtained using hierarchical clustering (Figure S2). Samples collected from the air were most dissimilar from those collected from the bed and floor and formed a distinct cluster. Samples from the bed and floor also clustered distinctly although samples from these two groups overlapped in the PCA plot as well as in the heat map generated using hierarchical clustering. Based on the separation visible in the PCA, we sought to confirm if difference in beta diversity between the three sample types was significantly different. As there were significant differences in dispersion (betadisper, vegan package of R; $p<0.001$) we were unable to use PERMANOVA to test if the sample types differed significantly. We instead used MRPP (mrpp, vegan package of R) to test for differences in beta diversity between air, bed, and floor samples based on the Bray-Curtis distance matrix used to generate the PCA plots in QIIME. This resulted in a chance corrected within-group agreement of 0.084 and p-

value of 0.001 indicating that the beta diversity was significantly different among the three sample types.

Furthermore, Shannon's diversity index showed samples collected from air to have the lowest diversity in taxa followed by floor and bed samples (Figure 3A). Similarly, the Chao 1 richness index showed samples collected from air to have the least amount of richness in taxa followed by bed and floor samples (Figure 3B). There was no difference in diversity or richness between green and non-green units or between the baseline and 12-month samples (Figure S1).

We performed further analysis of the significance of alpha diversity measures between sample groups as described in the methods section. Overall, there was no specific trend detected in the richness and diversity of taxa between green and non-green floor and air samples (Table S2). However, it was evident from the mean Shannon's diversity values that the fungal communities exhibited moderately high levels of diversity. Values for the Shannon index typically range from 0 to 5 (Adams et al., 2013b; Margalef, 1972); our values ranged from 2.8 to 4.2 (between 15 and 69 estimated species) with most values being greater than 3 (estimated species >20). While no statistically significant difference in alpha diversity as measured by Chao 1 (richness) or Shannon (diversity) were detected using the Kruskal-Wallis test after adjustment for multiple comparisons, some comparisons had uncorrected p-values less than 0.05 as shown in Table S2.

Table 1 represents a summary of results from the analysis of differences between samples using Jaccard distance measure separately on data from all three sample types, determining if there was any change in fungal communities from baseline to 12-month. No significant difference was detected in the Jaccard distance values between green and non-green homes in air or floor samples. However, a significant difference was observed in Jaccard distance values between bed samples collected from green and non-green homes, where the non-green units had a greater degree of difference between baseline and 12-month samples than the green units. Furthermore, the mean Jaccard distance value comparing baseline to 12 months post-renovation in each of our three sample types ranged from 0.74 to 0.88 across the different sample types. Values close to 1 indicate that both green and non-green communities changed substantially from the baseline time point to 12 months. This shows that there was substantial variation in the mycobiomes of both green and non-green communities from the baseline time point to the 12-month time point, although this change did not follow clear patterns by renovation status or time point as seen by the lack of clustering in the PCA plots discussed above.

Table 2 shows the ten most abundant fungal genera by sample type (air, bed and floor dust). Two yeasts were abundant in all sample types: *Candida* and *Rhodotorula*. The following taxa were found among the ten most abundant in two of the three sample types: *Clavispora* in air and floor dust, *Penicillium* in air and bed dust, and *Cryptococcus, Eurotium*, and *Fusarium* in bed and floor dust. Additionally, the following taxa were among the 10 most common in one of the three sample types: *Memnoniella, Schizophyllum, Aspergillus*, *Collectrotrichum, Ischnoderma and Oudemansiella* in air samples; *Alternaria, Phoma Debaryomyces*, and

Capnobotryella in bed samples; and *Rhizoctonia*, *Plectrosphaerella*, and *Galactomyces* in floor samples.

LEfSe analysis showed a significant enrichment of several fungal taxa in samples collected in green-renovated homes compared to non-renovated and in 12-month samples compared to baseline samples (Table S3). Most of the enriched genera/species were unique to an individual sample type. For example, *Trametopsis cervina*, wood rotting fungus, was more frequently present in air samples collected in green than in non-green homes at baseline and *Aspergillus unguis* was more often present in floor samples collected from green than in non-green homes at 12 months. Four taxa were enriched in several sample types. *Rhodotorula glutinis* yeast was present more frequently in air samples collected in green than in non-green both at baseline and at 12-months. *Penicillium sinulosum* was more frequent in air samples collected in green than in non-green at baseline and in air samples collected in non-green buildings at 12 months than at baseline. Wood rotting *Sistotrema* had a higher relative abundance at 12-months than at baseline both in green and non-green homes. Another wood rotting fungus *Polyporous squamosus* was present more frequently at 12 months than at baseline in both air and dust samples.

4. DISCUSSION

Fungal diversity or richness did not have any consitent trends between green and non-green homes. This also supports our previous findings on the similarity in non-biological indoor contaminants between these two building types (Coombs et al., 2016).

The PCA results showed clear separation of fungal communities in air, bed and floor samples. This confirms previous findings from culture-based studies that have concluded that the taxa and concentrations of fungi in house dust samples poorly correlate with corresponding results in indoor air samples (Chew et al., 2003; Hyvärinen et al., 2006; Miller et al., 1988; Park et al., 2000; Ren et al., 1999). Our results are also supported by the findings from previous studies (Rittenour et al., 2014); (Adams et al., 2015); (Hoisington et al., 2014), which compared the microbiomes of air samples and floor dust indoors. To the best of our knowledge, no prior studies compared bed samples to air and floor samples using next generation sequencing. Bed dust is the most homogenous of dust samples in a home. It is less prone to things like tracking in dust on your shoes or harsh cleaning agents, which can either interfere with the microbial agent itself or appear as a contaminant during the laboratory analysis to measure that agent. Furthermore, bed dust served as our internal control as it is not expected to be clearly changed during a housing renovation as could happen to floor dust.

High Shannon diversity indices showed that the fungal communities in all sample types had high levels of diversity. This finding has been previously shown in Cincinnati, using next generation sequencing (Kettleson et al., 2015). Shannon diversity and Chao 1 richness showed that air samples had the lowest diversity and richness, whereas bed samples had the highest diversity and richness. The richness of fungi in floor dust being higher than samples collected from the indoor air have been shown in other studies using next generation sequencing (Adams et al., 2015). A study (Augustyniuk-Kram and Dmowska, 2013) using

conventional techniques showed that the richness of fungi in floor dust was higher than in bed dust samples.

Air samples had higher richness of taxa at 12-months compared to baseline. The Jaccard distance analyses compared the similarity of samples of the same type from the same house between baseline and twelve months, while the PCA analysis looked for clustering by sample type, renovation status, and time across all samples. The highest Jaccard distance values were observed for air samples indicating that fungal composition changed more in air samples than the bed or floor samples between baseline and 12-months. The composition of indoor mycobiome is known to be strongly dependent on the fungal community in the surrounding outdoor environment which can vary even within short distances (<500 m) (Adams et al., 2013a; Adams et al., 2013b). Although the baseline and 12-month samples were collected during the same season, there could be variation in the outdoor fungal communities between subsequent years that are reflected in the 5-day air samples. Dust samples represent longer term average than air samples (Casas et al., 2016) and therefore, are not expected to follow the variation in the outdoor mycobiome as readily as air samples.

Additionally, bed samples had a higher richness of taxa in non-green than green homes. The higher richness of 12-month bed samples from non-green homes primarily contributed to this difference. Also, Jaccard distance values showed that fungal composition changed in non-green bed samples more than in green bed samples between baseline and 12-months. The comparable or greater level of difference in the non-green homes was unexpected, as the families in the non-green homes had been living in their units for several years presumably providing the opportunity for the mycobiome to equilibrate. Overall, the Jaccard distance values were high, indicating that the fungal communities changed considerably between the baseline and 12-months. To the best of our knowledge, this study is the first to show such temporal change in mycobiomes within housing communities, using next generation sequencing. Large variation of the bacterial communities in indoor air within one year has recently been reported (Emerson et al., 2017) supporting the conclusion that air sampling in multiple time points is needed to characterize microbial communities in indoor air.

Many of the fungal taxa that we found (*Alternaria, Aspergillus*, *Cryptococcus, Eurotium, Fusarium, Penicillium*, *Rhodotorula)* have been frequently reported in studies that used conventional culture or microscopy methods as well as in studies that employed next generation sequencing (Adams et al., 2015; Flannigan et al., 2016; Kettleson et al., 2015; Rittenour et al., 2014). *Candida* was the most abundant genus in bed samples indicating the dominance of human associated fungi (Adams et al., 2013b). *Cryptococcus*, abundant in both bed and floor samples, has been shown to be inversely associated with asthma risk in children (Dannemiller et al., 2014). Surprisingly, the most abundant fungus in air samples was *Memnoniella*, followed by *Penicillium, Schizophyllum*, and *Aspergillus*. Phylogenetically, *Memnoniella* is closely related to *Stachybotrys* and is often reported under *Stachybotrys* genus (Haugland et al., 2001). In culture-based studies, *Stachybotrys* is usually less abundant, whereas *Penicillium* and *Aspergillus* dominate air samples (Flappan et al., 1999). Furthermore, *Stachybotrys* spores are not expected to be collected in the PM2.5 samples due to their large size. However, *Memnoniella* has important features that facilitate the aerosolization its conidia. The conidia grow in dry chains and are relatively small

(physical size: 3–6 × 3–5 μm), whereas *Stachybotrys* conidia grow in slimy masses and are larger (9×4 μm) (Lombard et al., 2016). Some of the taxa among the 10 most common genera in the current study have not been previously reported in indoor environments: plant pathogens *Collectrotrichum*, and *Rhizoctonia*, yeasts *Debaryomyces* and *Galactomyces*, as well as mushrooms *Ischnoderma* and *Oudemansiella*. Most of the taxa identified in the LEfSe analysis were enriched in a specific sample type, except four that were enriched in several sample types: *Penicillium sinulosum, Polyporous squamosus, Rhodotorula glutinis, and Sistotrema*. The relevance of these taxa to human health is currently not known.

5. STUDY LIMITATIONS

A larger sample size would allow us to model the taxa in relation to potential confounding covariates. Additionally, since we only analyzed samples from two time-points (baseline and 12-month) over the course of a year, more frequent sampling may better resolve the temporal changes. Further, since we used PM2.5 samplers to collect the air samples, bioaerosols >PM2.5 could not be collected or analyzed. However, we did this consistently across homes and throughout repeated sampling, so our results have internal validity. Previous culture-based studies have shown that the largest number of fungal spores in indoor air is within the size range of 2.1–3.3 μm (Reponen et al., 1994). However, recent studies that used next generation sequencing for size-fractionated air samples collected in classrooms (Qian et al., 2012; Yamamoto et al., 2014) reported larger aerodynamic diameters than those previously found in culture-based studies.

Furthermore, there are inherent biases in using next-generation DNA sequencing technology to explore fungal communities. The primers used for amplifying the ITS region can bias the results towards specific taxa, the primers used here tend to skew the results towards basidiomycetes (Bellemain et al., 2010) but are useful at discriminating against plants (Lindahl et al., 2013). This means that the samples may contain higher levels of ascomycetes or other groups than measured here. In addition, DNA extraction technique affects OTU recovery (Tedersoo et al., 2010). Work with soil mycobiomes demonstrates that the nature of bias introduced by DNA extraction protocol will depend on the starting material (Young et al., 2015), but we are unaware of any studies that directly compare the PowerMax kit used in this study to other methods used with dust samples. As such, we are uncertain of which taxa this extraction method may be biased towards. The use of the same extraction protocol and sequencing protocol for all samples should result in samples that can successfully be compared across groups (Lindahl et al., 2013).

6. CONCLUSIONS

To our knowledge, this is the first study to use next generation sequencing to determine the differences in the mycobiomes of air, bed and floor dust within the green and non-green homes. The research revealed three main findings. First, there was no clear trend in the fungal communities between green and non-green homes. Notable differences between green and non-green homes were seen only in bed samples at 12-months. Second, air samples had the lower diversity and richness compared to bed and floor samples. Third, microbial communities changed considerably within one year in both green and non-green

homes for all sample types, as demonstrated by the high Jaccard distances. These results indicate that the mycobiomes in all three sample types (air, bed, and floor) were not stable over the course of a year, for both green and non-green communities. The taxa differences found in the current study between sample types support the use of multiple sample types, depending on the goal of the study. A longer follow-up period and more frequent sampling might elucidate any differences between the two housing types.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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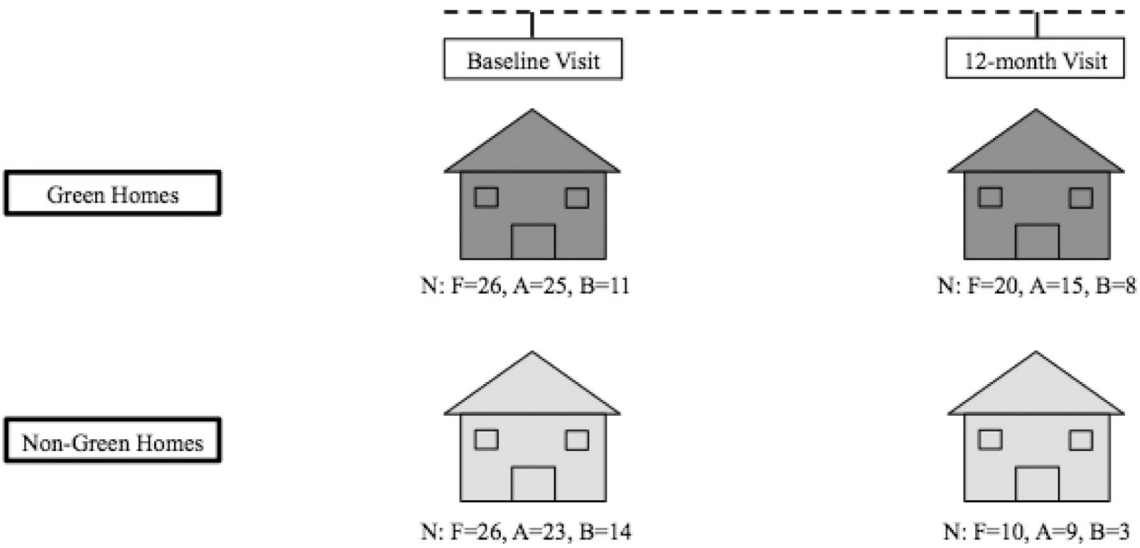

Figure 1.

The Cincinnati Green Housing Mycobiome study design. Homes were assessed at baseline (within four months post-renovation) and 12 months after baseline. The number of samples included in the analysis is indicated as Air (A), Bed (B), and Floor (F).

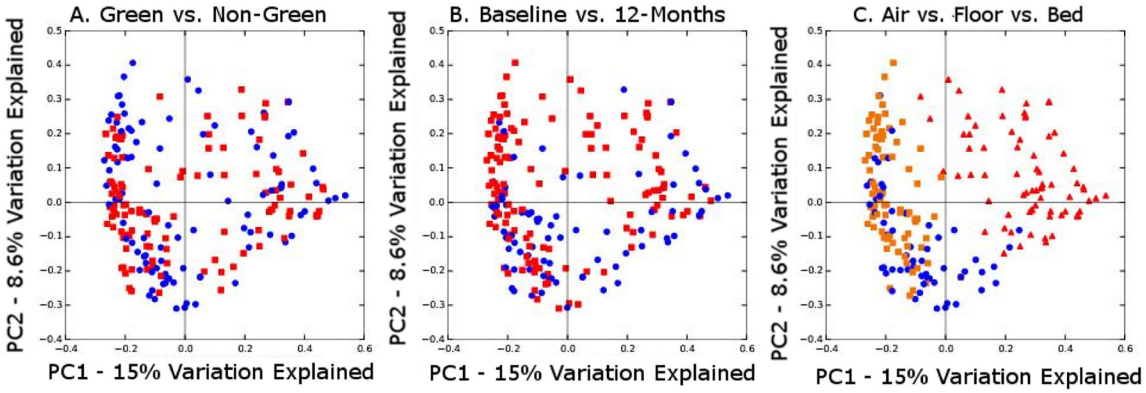

Figure 2.

Two-dimensional PCA plot based on the Bray-Curtis dissimilarity metric. Percentage of the diversity distribution is explained by the axes on the plot. (A) Samples associated green (red square) and non-green (blue circle) units are shown as single points. (B) Same PCA plot, this time showing baseline (red square) and 12-month (blue circle) samples. (C) Same PCA plot, showing the three sample types: the floor (orange square), air (red triangle) and bed (blue circle).

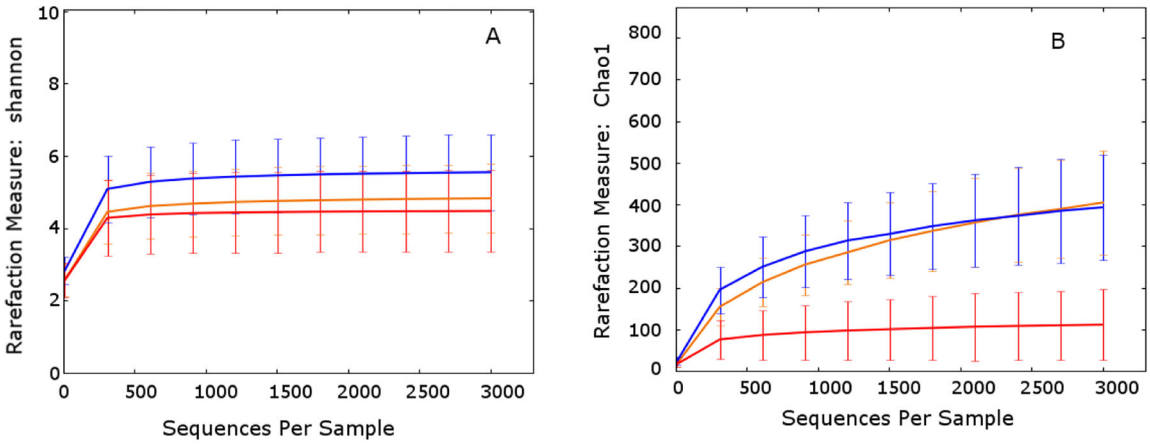

Figure 3.

Rarefaction curves depicting within-sample (α) Shannon diversity (A) and Chao 1 richness (B) based on sample type: the air (red line), floor (orange line), and bed (blue line). The error bars present standard deviations.

Table 1.

Summary of Jaccard distance analysis comparing air, bed and floor dust samples collected at baseline and 12-months post-renovation from green and non-green homes.

Sample Type	Renovation Status	Mean Jaccard Value[1]	p-Value
Air	Green	0.88	0.76
	Non-Green	0.87	
Bed	Green	0.72	0.04
	Non-Green	0.83	
Floor	Green	0.77	0.63
	Non-Green	0.74	

[1]Jaccard distances range from 0 to 1; higher values indicate more different fungal composition between baseline and 12-months.

Table 2.

Ten most abundant identified genera, by sample type with average percent relative abundance (RA)

Air	RA	Bed	RA	Floor	RA
Memnoniella	8.9%	*Candida*	3.5%	*Rhodotorula*	1.9%
Penicillium	2.0%	*Eurotium*	1.6%	*Fusarium*	1.9%
Schizophyllum	1.9%	*Rhodotorula*	1.4%	*Candida*	1.4%
Aspergillus	1.7%	*Fusarium*	1.4%	*Cryptococcus*	0.6%
Rhodotorula	1.1%	*Alternaria*	1.2%	*Rhizoctonia*	0.6%
Clavispora	1.1%	*Phoma*	0.8%	*Clavispora*	0.6%
Colletrotrichum	0.9%	*Cryptococcus*	0.5%	*Myrothecium*	0.4%
Ischnoderma	0.8%	*Penicillium*	0.4%	*Plectosphaerella*	0.2%
Candida	0.5%	*Debaryomyces*	0.4%	*Eurotium*	0.2%
Oudemansiella	0.4%	*Capnobotryella*	0.4%	*Galactomyces*	0.2%