

## Evaluation of HVAC filters as a sampling mechanism for indoor microbial communities

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### ABSTRACT

HVAC filters are in place for extended periods of time and can serve as integrated air samplers. This paper presents a comparison of bacterial and fungal concentrations and communities in HVAC filter dust and other sampling locations in occupied residences and in the unoccupied UTest House. A DNA-based, culture-independent approach was utilized to characterize the microbial communities. Microbial concentrations and communities in HVAC filter dust samples were not statistically different from those in high surface dust samples in occupied homes. Despite the general similarity in the communities, Proteobacteria were present in greater proportion in HVAC filter dust samples than in surface dust samples suggesting the air origin of this phylum. Gram-positive bacteria were present in greater proportion in occupied residences than in an unoccupied test house, confirming the potential association of this group with occupants. HVAC filter microbial communities were not different from those present in a composited month-long indoor air sample providing preliminary evidence that filters could be a viable option for long-term investigation of airborne biological contaminants.

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### 1. Introduction

The presence of microorganisms indoors has been related to several health and discomfort outcomes including respiratory diseases, odors, and occupant dissatisfaction (Gyntelberg et al., 1994; Verhoeff and Burge, 1997). Some researchers have associated indoor microbial concentrations with asthma symptoms (Park et al., 2006; Ross et al., 2000; Smedje et al., 1997). However, the association between culturable fungal levels in air or dust samples and health problems has been inconsistent (Nelson et al., 1995; Peat et al., 1998; Verhoeff and Burge, 1997). This discrepancy may be attributable to the fact that bioaerosol samples are typically short-term in nature and provide only a snapshot of microbial contaminant levels in air at a particular time and place. Even when collected from the same location, airborne bacterial samples have significant temporal variability (Fierer et al., 2008), highlighting the need to develop an integrative methodology to assess indoor biological contaminants. Floor dust may provide an integrated sample of

contaminant levels but these samples are influenced by material tracked-in from the outside and may be skewed towards larger particle-bound contaminants (Lewis et al., 1999).

The majority of previous indoor biological studies have relied on an assessment of culturable microorganisms that represent only a small fraction of the total microorganisms present indoors (Toivola et al., 2002). In recent years, several studies have applied culture-independent, DNA-based approaches to better characterize the diverse bacterial and fungal communities present in indoor environments (Kelley et al., 2004; Pakarinen et al., 2008; Pitkäranta et al., 2008; Rintala et al., 2008; Täubel et al., 2009; Tringe et al., 2008). The application of molecular biology tools to indoor environmental investigations should reveal a much greater fraction of the microbial community present than culturable methods, a finding recently confirmed by Pitkäranta et al. (2008). Vesper et al. (2007) reported an association between asthma symptoms and the Relative Moldiness Index (RMI), an index based on molecular biology tools, confirming that these techniques may provide a better characterization of health effects from microorganisms.

A potential alternative to the use of settled dust and air samples for microbial evaluation is the use of heating, ventilation, and air conditioning (HVAC) filters for indoor environment investigations. Collecting samples of HVAC dust may improve our understanding of indoor occupant exposure by providing an integrated measure of pollutant concentrations associated with indoor particles. Greater

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than 70% of the residential buildings in the United States have a central forced-air HVAC system (U.S. Bureau of Census, 2005), almost all with a built-in filtration system. These filters essentially serve as passive, long-term samplers that can be collected with minimal effort and analyzed for a broad range of indoor contaminants. Recently, Stanley et al. (2008) utilized filters in two large public buildings as bioaerosol sampling devices to determine the culturable bacteria concentrations for selected species present in air. While most of the molecular-based studies described above focused solely on settled dust, Tringe et al. (2008) investigated the bacterial communities present on the dust that collected on two HVAC filters in two large shopping centers in Singapore. They reported that the two HVAC filters (air samples) have more in common to each other than with environmental (outdoor soil and water) samples collected nearby. They also found greater similarity between the bacterial communities in filter samples and indoor floor dust compared to outdoor ground-level dust suggesting that the filter community originates from an indoor niche. The purpose of the current study is to explore the microbial concentrations and communities on filters and compare them to indoor settled dust and air communities as a first step towards using HVAC filter dust as an integrated measure of microbial levels in residences. This paper focuses on bacterial and fungal culturable concentrations and DNA-based analysis of microbial communities present in HVAC filter dust and other indoor sampling locations in occupied residences and in an unoccupied full-scale test house (U-Test House). The investigation was divided into two phases: 1) Investigation of culturable microbial concentrations in settled and HVAC filter dust in eight occupied residences in Austin, Texas; 2) Study of microbial communities, using a culture-independent approach in four sites as well as in the full-scale U-Test House where more detailed sampling was performed.

## 2. Methods

### 2.1. Phase 1: culturable microorganisms

A sample of convenience of eight residential buildings located in Austin, Texas was selected for this part of the investigation. More detailed information regarding the sites and the occupants is reported in Noris et al. (2009). All of the buildings had central air conditioning that recirculated indoor air, as is typical of residences in the Southern U.S.

### 2.2. Sample collection

Floor dust, high surface dust and HVAC filter dust samples were collected 2–3 times over a six-month period from each residence during the cooling season (summer and fall). The filters were classified according to the minimum efficiency reporting value (MERV) as determined by ASHRAE Standard 52.2 (ASHRAE, 2007) and reported by the filter manufacturers. The sample included seven low-efficiency (MERV <5), six mid-efficiency (MERV 5–8) and three high-efficiency (MERV 9–14) filters. A composite sample of living room and main bedroom floor dust was acquired from each building using a Dynamite Plus, Dirt Devil vacuum equipped with an Indoor Biotechnologies Duststream Collector. Approximately 1 m<sup>2</sup> of floor area was sampled for 2 min each, avoiding tracked-in dust areas. A composite high surface (horizontal surfaces >1 m above the floor) dust sample was collected from elevated surfaces such as door frames, shelves, and tops of furniture, using the same vacuum technique utilized for floor samples. We collected three settled dust samples from sites 1, 2, 3, 4 and 7 and two samples from the remaining three sites. Two HVAC filters were collected from each site approximately three months apart, while

the settled dust samples were collected approximately four weeks apart from each other. In the same five buildings where three floor and high surface dust samples were collected, air samples were also collected from a height of 1 m–1.5 m above floor level. An impinger (Biosampler, SKC Inc., Eighty Four, PA) was connected to a vacuum pump operating at a constant volumetric flow rate of 12.5 L min<sup>-1</sup> for a period of 1 h. The microorganisms were captured in a phosphate buffer solution (PBS) consisting of 8 g L<sup>-1</sup> NaCl, 0.2 g L<sup>-1</sup> KCl, 1.44 g L<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub>, and 0.24 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>. The bioaerosol samples were plated immediately, while the dust samples were stored in a 4 °C environmental chamber maintained at approximately 70% RH until the analyses were performed.

### 2.2.1. Sample analysis

The enumeration of culturable bacteria and fungi present in the bioaerosol samples, settled dust, and HVAC dust samples were completed using the standard spread plate method 9215C (APHA, 1998). For the settled dust and the HVAC dust samples, the microorganisms present in the dust were transferred into PBS by sonication and vortexing for 10 min each. An aliquot of PBS was plated on R<sub>2</sub>A agar containing 0.04% cycloheximide and incubated at 30 °C for bacterial enumeration or on Sabouraud dextrose agar (SDA) plates containing 0.01% chloramphenicol and incubated at room temperature (approximately 23 °C) for fungal quantification. To estimate the spore-forming fraction of the population, an aliquot of each sample was pasteurized for 15 min at 75 °C, following the procedure developed by Barbeau et al. (1997), and then plated as described above. The Wilcoxon Rank–Sum Test, which does not assume any specific distribution of the data, was applied to compare and identify dissimilarities between the different data groups. A significance level of 0.1 was assumed owing to the small sample size and the conservative nature of this statistical test.

### 2.3. Phase 2: microbial communities

In the second phase of the investigation, the analysis was expanded to a culture-independent, DNA-based approach potentially capable of more fully characterizing the microbial communities present indoors. This phase was conducted in a subset of four of the above residential sites as well in an unoccupied, 120 m<sup>2</sup> manufactured home (U-Test House) where the fan of the HVAC system was operated continuously during the investigation. The lack of occupants in the U-Test House reduced localized particle and microbial emissions and represented an idealized site for evaluating HVAC filters as a sampling mechanism for indoor microbial communities. At the beginning of this phase, clean high-efficiency (MERV >11) HVAC filters were installed at all the test sites. Several high surfaces at each site were also cleaned at filter installation and the homeowners were instructed not to clean the designated surfaces.

### 2.3.1. Sample collection

Filters and high surface samples were collected during the cooling season, two months after the installation of the filter in the occupied residences (October–November) and one month after installation in the U-Test House (October). At the time of filter removal, a composite sample of high surface dust from the previously cleaned surfaces was collected using the vacuum mechanism described above in Phase 1. During the month-long investigation in the U-Test House, indoor and outdoor bioaerosol samples were collected five days per week using the same impinger method described in Phase 1. Indoor bioaerosol samples were acquired from a central location in the building, while outdoor samples were collected at least 3 m from any doors or windows. The DNA extracted from each daily bioaerosol sample in the U-Test House was

pooled together by combining equal volume aliquots of the DNA extractions from the 20 daily samples into a composite, integrated sample.

### 2.3.2. Sample analysis

Approximately 50 mg of each high surface dust sample was immersed into 50 ml of PBS, sonicated and vortexed for 10 min each to transfer the microorganisms to the liquid phase. Subsequently, the liquid solution was filtered first through a Whatman #41 (Whatman Inc., Piscataway, NJ) to remove large particles and then through a 0.2  $\mu\text{m}$  GTP Membrane Filter (Millipore, Billerica, MA) to separate the microbes from the particles. The filters were stored at  $-80^\circ\text{C}$  until analysis. To sample the dust from each HVAC filter, nine 2.54 cm square pieces of filter material distributed in each quadrant were cut from the filter. Subsequently, they were immersed into 50 ml of PBS, sonicated, vortexed and filtered as described above for the surface dust samples. For the bioaerosol samples, the PBS containing the microorganisms was directly filtered through a 0.2  $\mu\text{m}$  filter.

The DNA from the microorganisms captured on the 0.2  $\mu\text{m}$  filters was extracted using the Power Soil DNA (MoBio Laboratories, Carlsbad, CA) kit per manufacturer's specifications except for the following modifications. 100  $\mu\text{l}$  of lysozyme (3 mg ml $^{-1}$ ) and 300  $\mu\text{l}$  of a phenol–chloroform–isoamylalcohol (24:24:1) solution were added at the initial step in addition to the normal reagents. Also, the MP FastPrep-24 (QBiogene) was used instead of the vortexing step. DNA samples were then PCR amplified using bacterial-specific primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GCYTACCTGTACGACTT-3') or fungal-specific primers ITS1F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Each 50  $\mu\text{l}$  PCR reaction contained 1 $\times$  PCR buffer, 1.6 mM MgCl $_2$ , 0.2 mM each dNTP, 0.2  $\mu\text{M}$  each primer, 2 U of *Taq* polymerase, and 2  $\mu\text{l}$  of DNA. The PCR amplification conditions consisted of 10 min at 94  $^\circ\text{C}$ , followed by 35 cycles of 60 s at 94  $^\circ\text{C}$ , 60 s at 55  $^\circ\text{C}$ , and 60 s at 72  $^\circ\text{C}$  and a final extension of 7 min at 72  $^\circ\text{C}$ . For each sample investigated, we performed triplicate PCR reactions to reduce amplification biases; the amplicons were pooled prior to cloning. After confirming the amplicon length on agarose gel, the amplicons were purified using the QIAquick Gel Extraction kit (QIAGEN, Valencia, CA). Negative controls were performed and never showed amplification. Amplicons were then cloned using the TOPO TA cloning kit for sequencing (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions and subsequently sequenced in one direction with an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). The nonredundant sequences were deposited at the GenBank database with accession number GU595461–GU596375 for the bacterial clones and GU721174–GU722092 for the fungal clones.

Sequences were aligned against the GenBank database utilizing the BLAST algorithm (Altschul et al., 1990) using 97% sequence similarity as the criterion to determine the similarity to known microorganisms. The web-based tool FastGroupII (Yu et al., 2006) was used to estimate the number of Operational Taxonomic Units (OTUs), Chao1 richness estimator and the Shannon–Wiener diversity index. The sequences from bacteria and fungi were then aligned separately using MEGA (Tamura et al., 2007). The phylogenetic trees, containing the archeal sequence *Haloferax Volcanii* as an outgroup, were created in the CIPRESS portal (<http://www.phylo.org/portal>) using the RAxML algorithm (Stamatakis et al., 2005). Finally, the microbial communities present in the different samples were compared using the Weighted UniFrac algorithm (Lozupone et al., 2006, 2007). This method utilizes the phylogenetic distances between the microorganisms to compare microbial communities in two or more environments (de Evgrafov et al., 2010; Fierer et al., 2008; Lauber et al., 2008; Rintala et al., 2008).

A significance level of 0.1 was assumed due to the limited number of sites and the exploratory nature of the investigation.

## 3. Results and discussion

### 3.1. Phase 1: culturable microorganisms

Fig. 1 shows the mean bacterial and fungal culturable concentrations for each of the sampling locations investigated in the residences. Multiple samples at the same site are given equal weighting; there are 21 samples each of floor and high surface dust, 16 HVAC filter dust samples and five air samples shown in the figure.

The culturable concentrations for both fungi and bacteria in Fig. 1 were generally consistent with the published literature. Indoor concentrations for bacteria and fungi vary considerably with reported values ranging from 10 $^2$  to 10 $^4$  CFU m $^{-3}$  for indoor air and from 10 $^5$  to 10 $^7$  CFU g $^{-1}$  for settled dust (Andersson et al., 1999; Bouillard et al., 2005; Dales et al., 1997; Koch et al., 2000; Ren et al., 1999; Ross et al., 2000). For all of the sampling locations, the observed viable bacterial concentrations were higher than fungal concentrations and the estimated spore concentrations were approximately two orders of magnitude lower than total concentrations. Total bacteria concentrations ranged from 10 $^4$  to 10 $^7$  CFU g $^{-1}$ , with a greater median concentration found on the floor, followed by high surfaces, and HVAC filter samples with median concentrations of  $1.9 \times 10^7$ ,  $4.4 \times 10^6$  and  $1.1 \times 10^6$  CFU g $^{-1}$ , respectively. This would suggest that larger particles or clusters of bacterial cells that are more likely to settle may have greater bacterial concentrations than small particles that remain suspended in air and are captured on the filter. Another possible explanation could be that the survival/growth conditions and nutrient availability on surfaces may be more favorable than on the filters. Fungal concentrations in the dust samples ranged from 10 $^3$  to 10 $^7$  CFU g $^{-1}$  with reasonably consistent distributions across the dust sampling locations. In the air samples, there was a greater fungal median concentration than bacterial and greater variability. Several studies have shown that indoor air fungal concentrations have elevated temporal and spatial variability (Hyvärinen et al., 2001; Koch et al., 2000) and thus the short sampling time may have affected the results. Stanley et al. (2008) calculated low indoor air culturable concentrations for selected bacterial species, often below 4 CFU m $^{-3}$ , based on HVAC filter concentrations. The results in the current study diverge from those perhaps due to differences in quantification techniques and the fact that residential HVAC

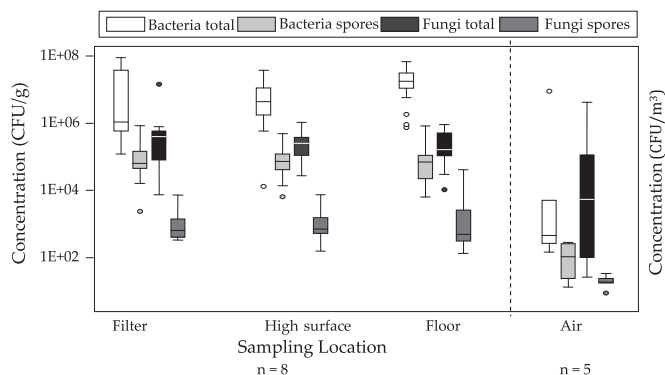


Fig. 1. Culturable microbial concentrations by sampling location. Air samples have dimensions of CFU m $^{-3}$  and all others have dimensions of CFU g $^{-1}$ , with  $n$  = number of residences. The lowest end of the box represents the 25th percentile, the top represents the 75th percentile, and the horizontal bar inside the box indicates the median of the distributions. Single points outside the box are the outliers.

systems generally supply 100% recirculated indoor air and operated only when the thermostat calls for conditioning, typically less than 25% of the time (Stephens et al., 2010; Thornburg et al., 2004).

Fig. 2 summarizes the mean culturable concentrations of bacteria and fungi in the floor dust, high surface dust and HVAC filter dust at each of the eight sites investigated. The concentration of bacteria was fairly consistent within one order of magnitude across most sites except for Sites 1, 2 and 3. At Site 3, HVAC filter and high surface dust concentrations were quite similar but the floor dust samples had much greater concentrations and may have been influenced by tracked-in particles from outside. The difference between the HVAC filter and the high surface dust samples at Sites 1 and 2 may be due to the reduced efficiency of the filters collected from these sites, specifically one low- and one mid-efficiency filter for Site 1 and two low-efficiency filters for Site 2. The reduced efficiency of these filters makes them less representative sampling devices because they capture fewer particles and increases the probability of observing differing levels on the filters than on the floor or high surface. The difference in microbial concentrations on the filters and those found in surface and floor dust at these three sites may also be attributable to the cycling of the HVAC system (Noris et al., 2009), suggesting that HVAC filters in residential buildings where the HVAC system is operated sporadically may be less representative of indoor contaminant levels. Nevertheless, despite some site-specific differences, the Wilcoxon sign-rank test reveals that the culturable microbial concentrations encountered at different dust sampling locations for all the sites were not statistically different.

From Figs. 1 and 2 it is clear that both bacteria and fungi are able to populate and survive in the dust present indoors. Importantly for this work, in a humid and warm environment like central Texas during the cooling season, microorganisms appeared to survive and colonized the dust on HVAC filters with concentrations similar to those found in the dust that settles inside the residences, suggesting that these filters may be a promising location for collecting samples for indoor assessments. While the culturable concentrations were comparable, the composition of the microbial communities may differ with sampling location because of specific environmental conditions that may favor some species over others. This aspect, as well as the influence of occupants on the composition of indoor microbial communities, was addressed in Phase 2.

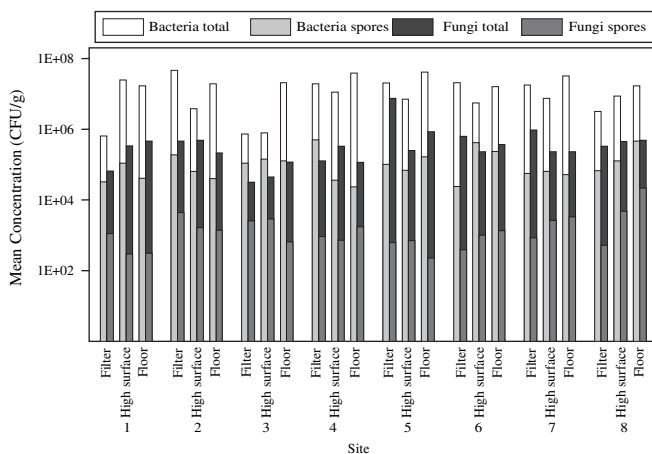


Fig. 2. Mean culturable microbial concentrations in dust samples by location within the buildings.

### 3.2. Phase 2: microbial communities

Culture-based techniques have limited utility for microbial community investigations because they detect only the fraction (sometimes a very small fraction) of the total number of species present that are capable of forming colonies on a specific agar plate. Similarly, even DNA-based approaches to community analysis may introduce biases during the DNA extraction, amplification, cloning and/or sequencing steps. Nevertheless, molecular methods are an attractive alternative because they can detect a greater fraction of the community present and typically reveal a more diverse community than culture-based techniques. Thus, in Phase 2, the study was expanded to a DNA-based analysis of the microbial communities present in a subset of four residential sites and in a 120 m<sup>2</sup> one-story, mostly unoccupied full-scale UTest House. The identification of microbial species common to HVAC filters, as well as the association between occupants and microbial species, was also investigated. After eliminating all potentially chimeric or poor quality sequences, we obtained a total of 915 bacterial clones and 919 fungal clones, corresponding to 248 and 295 OTUs, respectively. The bacterial clones had an overall Chao1 value of 426 and a Shannon–Wiener index of 4.58, while fungal clones had values of 508 and 4.62, respectively. These values indicate a microbial representation similar to that observed in other indoor studies (Pitkäranta et al., 2008; Rintala et al., 2008; Täubel et al., 2009) confirming the diverse bacterial and fungal communities present in indoor environments.

Fig. 3 summarizes the bacterial composition at the phylum level for all the samples analyzed. The most common phyla encountered were gram-negative Proteobacteria, and gram-positive Actinobacteria and Firmicutes, a finding in agreement with recent DNA-based studies by Rintala et al. (2008) and Täubel et al. (2009). These three phyla represent 96% of the clones encountered on the residential filters and 90% of the clones found in the high surface residential samples in the current study. For all the residential sites investigated, Proteobacteria were present in greater proportion in the filter dust samples than in the high surface samples, with mean values of 65% and 39% respectively. Tringe et al. (2008) utilized a DNA-based technique similar to the current study and also observed an elevated proportion of Proteobacteria on HVAC filters in two commercial buildings. These results contrast with culture-based results reported by Stanley et al. (2008) who observed that the gram-positive *Bacillus* (of the Firmicutes phylum) was the most commonly identified group in a culture-based study of HVAC filter bacterial communities. The prevalence of gram-positive bacteria in the Stanley et al. (2008) study may be due to a bias of culturing techniques that favor gram-positive bacteria or due to differences in the types of building sampled (e.g., large shopping mall in the Stanley study versus individual residences in the current study). The results from culture-independent studies described herein and by others suggest that Proteobacteria represent a significant fraction of the indoor air bacterial community and that this phylum may better tolerate the environmental conditions encountered in air (Brodie et al., 2007; Fierer et al., 2008) and on HVAC filters. One explanation could be that they possess a greater fraction of key genes involved with resistance to desiccation and oxidative damage, as suggested by Tringe et al. (2008). While Proteobacteria (mainly *Ralstonia*, *Pantoea* and *Enterobacter* spp.) dominated the filter dust samples, an opposite trend was observed for Actinobacteria, with the mean percentage in the high surface samples more than four times higher than that found on the filters, 26% versus 6%. In the high surface sample of Site 6 there was a dominance of Actinobacteria and an absence of Firmicutes. This site had the highest number of occupants of all the sites investigated (four occupants) and was the only one where children were present.

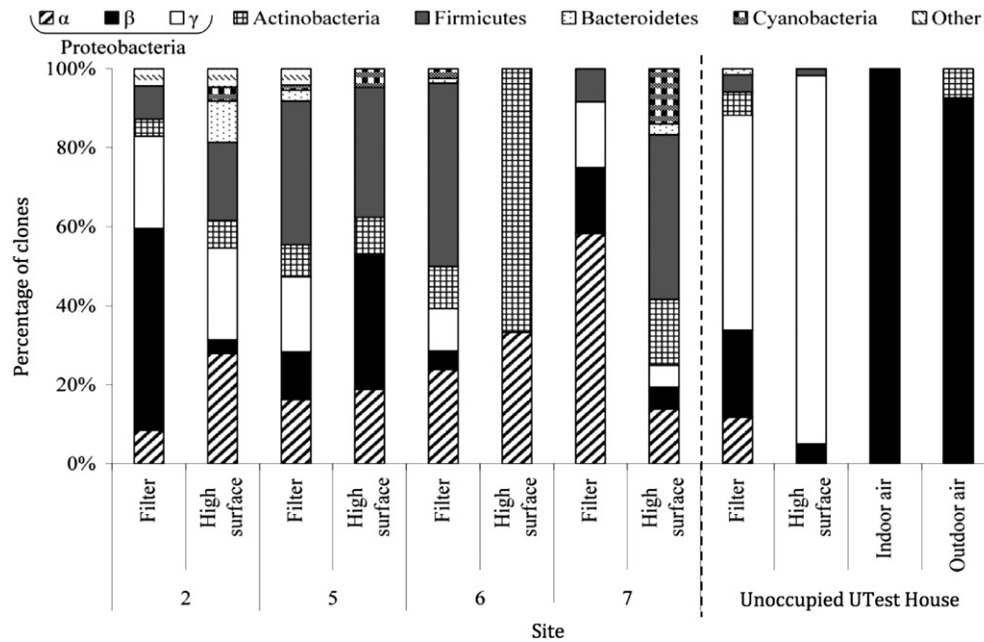


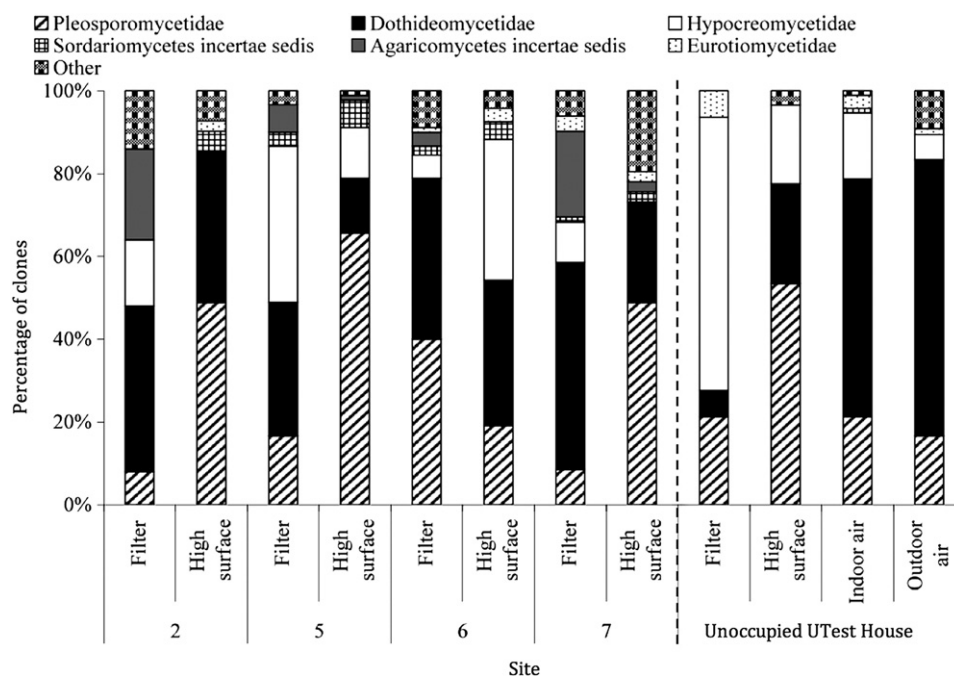
Fig. 3. Bacterial composition at the phylum level for the sequence libraries obtained from the samples collected from the four residences (Site 2, 5, 6, and 7) and the UTest House using 97% similarity criterion. The y-axis represents the phylogenetic distribution of rRNA sequences obtained for each sample.

Therefore, a higher proportion of Actinobacteria in this higher occupancy residence may suggest the human origin of this phylum (Rintala et al., 2008).

Comparison of the clone libraries generated from the dust samples in occupied residences to those in the unoccupied UTest House indicates that a much greater proportion of gram-positive bacteria, mainly Firmicutes and Actinobacteria, were present in the residences versus in the UTest House, with mean values of 41% and 6% respectively. This increased proportion of gram-positive bacteria in occupied buildings supports the speculation that many gram-positive bacteria found indoors may be attributable to human sources (Horak et al., 1996; Pakarinen et al., 2008; Rintala et al., 2008; Täubel et al., 2009). Rintala et al. (2008) examined the bacterial communities in surface dust in two occupied buildings across seasons. They observed higher variation in microbial composition between buildings than between seasons suggesting the development of site-specific bacterial communities, and that building users may be responsible for the presence of the dominant bacterial groups. Täubel et al. (2009) made a similar suggestion after examining the bacterial communities in mattress dust, floor dust and skin surface samples of occupants in four residences. In the current study, we observed a greater proportion of gram-negative bacteria, primarily Proteobacteria, in the dust samples collected in the unoccupied UTest House (mean value of 93%) versus those collected in the residences (52%), corroborating the supposition that gram-negative bacteria, and specifically Proteobacteria, may be of outdoor (i.e., environmental) origin. In the UTest House, we observed a dominance of Proteobacteria in all the samples analyzed, including the indoor and outdoor air samples. Fierer et al. (2008) reported elevated temporal variability in outdoor samples with dominance, across the five sampling days, of Proteobacteria and Bacteroidetes. This latter phylum was rarely observed in the current study and may be more typical of colder climates (Miteva et al., 2004; Yi et al., 2005). An elevated presence of Proteobacteria in outdoor air communities was also reported by Brodie et al. (2007) for the same geographic area of this study, confirming that this may be the most abundant phylum in ambient air samples.

Fig. 4 shows the fungal composition at the subclass level for all the clone libraries acquired. The majority of the sequences belonged to the phylum Ascomycota, with a much smaller fraction assigned to the Basidiomycota phylum. The majority of the fungal clones encountered in the samples analyzed belong to the *Dothideomycetes* (*Pleosporomycetidae*, *Dothideomycetidae* subclasses), *Sordariomycetes* (*Hypocreomycetidae*, *Sordariomycetes incertae sedis*) or *Agaricomycetes* (*Agaricomycetes incertae sedis*) class. Specifically, the *Dothideomycetes* class seems to be dominant, with *Cladosporium* and *Alternaria* spp. being the most abundant representatives. Pitkäranta et al. (2008) also observed an abundance of the *Dothideomycetes* class in indoor dust from two nursing homes in Finland even though the most common phylum was Basidiomycota. However, they also observed an increase in *Dothideomycetes*, and therefore in Ascomycota, during the summer months, which represent a more similar climate to that encountered in central Texas in summer and fall. Amend et al. (2010) also reported that *Dothideomycetes* was the dominant class when investigating fungal communities in indoor dust samples collected in buildings from different continents; their findings agree with the results of the current study. In the *Sordariomycetes* class, the genus *Fusarium* was the most commonly detected, which is consistent with results of other studies (O'Brien et al., 2005; Pitkäranta et al., 2008) that also used a molecular-based approach. Some culture-based studies reported elevated concentrations of *Penicillium* and *Aspergillus* spp. in indoor and outdoor communities (Koch et al., 2000; Ren et al., 1999). However, in the current study, we observed a limited proportion of the class corresponding to these genera, Eurotiomycetes, a result similar to that reported by Pitkäranta et al. (2008). This discrepancy could be due to a specific bias of the culturing methods that favor these species.

When comparing the fungal composition within a given site, we observed that for all the sites except Site 6, the proportion of *Dothideomycetes* was greater in high surface dust (a mean of 76%) than in filter dust samples (a mean of 59%). An opposite finding was observed for *Agaricomycetes* that were present in filter dust samples in much greater proportion than in high surface samples for all the residential sites, with mean values of 16% and 1%,



**Fig. 4.** Fungal composition at the subclass level for the sequence libraries obtained from all the samples analyzed using 97% similarity as criterion. The y-axis represents the phylogenetic distribution of rRNA sequences obtained for each sample.

respectively. *Sordariomycetes* were present in a greater proportion in filters than in the high surface dust samples, suggesting the air origin of this group. This is especially true for the UTest House filter, where this class seems to proliferate constituting 66% of the fungal clones obtained. The proportion of *Sordariomycetes* increased from a mean value of 19%–42% among all the dust samples in the residences and those in the UTest House. This class has been observed to dominate in outdoor air samples (Fierer et al., 2008) confirming the potential environmental origin of this class in the UTest House. However, the fraction of this class was not particularly high in indoor air, but seems to proliferate in the UTest House filter, therefore other factors may be important. Both indoor and outdoor air samples for the UTest House site were dominated by Ascomycetes, as also reported by Fierer et al. (2008) for outdoor air, supporting the hypothesis that indoor fungal communities strongly depend on outdoor fungi microbiota (Pitkäranta et al., 2008).

Some of the clones encountered in the HVAC filter samples in the occupied residences have high similarity to species that are reported to be potential opportunistic pathogens (Taylor et al., 2001). The bacterial species include *Pantoea agglomerans*, *Ralstonia pickettii*, *Enterobacter hormaechei*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*, as well as *Bacillus cereus*, *Bacillus pumilus*, and *Bacillus thuringiensis* among others. Fungal potential pathogens include *Alternaria alternata* and *Alternaria tenuissima*, *Fusarium proliferatum* and *F. oxysporum*, *Penicillium expansum*, *Cladosporium cladosporioides* and *Cladosporium axysporum*. The presence of these opportunistic pathogens on HVAC filters confirms the potential application of filters as samplers for detecting harmful microorganisms. However, additional analyses would be required to determine if these microbes were actually in a viable state and at an airborne concentration that could lead to infection.

To evaluate the potential use of HVAC filters as a sampling mechanism for indoor microbial communities, the similarity between microbial communities in different indoor sampling locations was evaluated using the UniFrac significance metric (Lozupone et al., 2006). This parameter compares the phylogenetic distance between the sequences encountered in a pair of samples. Table 1

presents the UniFrac values for the comparisons between the HVAC filter and high surface dust samples in the residential sites. From the *p*-values, it appears that both the bacterial and fungal communities in the filter and high surface dust samples within each residence investigated were not statistically different. Thus, the UniFrac results suggest that in a given residence, the microbial community present in high surface dust was not different from that present in HVAC filter dust and high-efficiency filters may be suitable samplers for assessing the composition of indoor microbial communities. This result relies on a statistical comparison of phylogenetic distances between two communities (the UniFrac analysis) while the compositional findings described above (Figs. 3 and 4) were based on a macroscopic division of the microbial community into different phyla or subclasses. The UniFrac and compositional analyses effectively complement each other since the UniFrac analysis determines if the communities in a given sample pair are statistically different on a phylogenetic level while the compositional analysis is useful for comparing the predominant microorganisms present with those identified in different environments or in different studies.

While the UniFrac comparisons indicate that the microbial communities in HVAC filter dust and high surface dust were not different in a given residence, Site 7 had the lowest *p*-values of all the residences for both the bacterial and fungal community comparison, with a bacteria *p*-value of 0.10, the threshold of significance. One possible explanation is the location of the HVAC filter in this residence. The filter was located at the return grille in the hallway, away from some of the rooms where the high surface

**Table 1**  
UniFrac significance (*p*-value) for the filter to high surface comparison in the four residential sites.

	Site			
	2	5	6	7
Bacteria	0.41	0.35	0.48	0.10
Fungi	0.30	0.65	0.55	0.16

dust sample was collected (living room and two of the four bedrooms) potentially resulting in differences in the particles collected on the filter versus those being deposited on indoor surfaces. The fungal and bacterial concentrations determined using culturing techniques (Fig. 2) were similar for the filter and high surface dusts at Site 7, while greater variations in the microbial concentrations between filter and high surface dusts were observed at other sites (i.e., bacteria for Site 2, fungi for Site 5) that had non-different microbial communities (Table 1). This lack of correlation between culture-based and culture-independent results highlights the fact that similarity in culturable concentrations is not necessarily associated with similarities in the microbial communities. When we monitored the HVAC system usage for 2–3 day-long monitoring periods during Phase 1 (Noris et al., 2009), Site 7 had the highest cooling duty cycle (34% compared to a median of 18% for the other sites), suggesting that other factors other than the HVAC system usage could be important. More intuitively, for the other two sites where a high cooling duty cycle was found, Site 5 (32%) and 6 (29%), the *p*-values were well above the threshold. However, caution is suggested in interpreting these results because of lack of direct monitoring of HVAC operation during the measurements described here.

Table 2 shows the UniFrac significance values for the samples collected in the unoccupied full-scale UTest House. The *p*-values for the microbial community comparisons between the filter and high surface dust samples (second column) were lower than those determined for the residences (Table 1) suggesting that in the unoccupied UTest House, the microbial communities in these two sampling locations are more distinct from each other. In contrast, human-associated microorganisms seem to dominate the communities in occupied residences (Rintala et al., 2008; Täubel et al., 2009) suggesting that occupied residences have a more homogeneous microbial distribution at the different sampling locations. This could be due to the fact that in residences, occupants generate particles through their activities and introduce microorganisms that could deposit onto surfaces or be captured by the filter leading to a more homogeneous distribution of microorganisms in the indoor environment. In contrast, the fungal communities in the HVAC filter and high surface dust samples in the UTest House were statistically different, while bacteria were not. The difference in the fungal communities observed in filter and high surface dust in the UTest House could be attributable to the increased proportion of Sordariomycetes in filter dust (Fierer et al., 2008).

The results from the month-long investigation in the unoccupied UTest House indicates that the filter and the composite indoor air sample (third column) were not statistically different supporting the findings of Tringe et al. (2008) that suggested that HVAC filter dust can be used as an integrated measure of airborne microbial communities, even though some specific differences in the community may occur. A comparison of the OTUs identified in the integrated indoor air sample to that encountered in the HVAC filter sample in the UTest House indicated that approximately 92% of the species (OTUs) encountered in the integrated indoor air sample were also encountered in the HVAC filter sample. HVAC filters are in place for extended periods of time, therefore, during their usage, a great volume of air is filtered through them (Stanley

et al., 2008) and only the microorganisms that were, at some point in time, airborne have the opportunity to be captured on the filter. In this study, the airborne microbial communities were derived from the analysis of a composite sample of 20 daily 1-h bioaerosol samples and thus represent a more integrated measurement than typical bioaerosol samples. Short-term air samples are reported to have a great temporal variability (Brodie et al., 2007; Fierer et al., 2008) and by compositing daily collections we tried to overcome this limitation so as to be able to compare the indoor air samples to the dust that collected on the surfaces and on the filter over the same period.

The values reported in the fourth column of Table 2 indicate that both bacterial and fungal communities in high surface dust and indoor air were statistically different in the unoccupied UTest House. For such a building, high surface dust samples may not be representative of airborne microbial communities possibly because surface dust samples may be influenced by the microorganisms attached to the particles that are more likely to deposit on surfaces rather than stay in the air (i.e., larger particles). Single bacterial and fungal cells range from 0.5  $\mu\text{m}$  to 50  $\mu\text{m}$ , with fungal spores generally larger than bacterial spores (Li and Li, 1996; Terzieva et al., 1996). Also, both bacteria and fungi are often associated with other particles, which alter their effective size. The size of these biological particles influences their fate and the probability of being detected in the different sampling locations, since larger particles are likely to settle while smaller particles may stay suspended in air longer and have more opportunities to be captured on HVAC filters. In the unoccupied UTest House, differences in microbial communities at the different sampling locations may have been more evident due to the limited human occupancy. However, in the residences the presence of occupants tended to homogenize the communities present (Table 1).

The significantly different bacterial community present in the high surface dust and indoor air in the idealized UTest House seems to be largely attributable to the different composition of Proteobacteria in each sample location. Air samples were dominated by  $\beta$ -Proteobacteria which constituted greater than 93% of the clone libraries for the indoor and outdoor air samples. In contrast, dust samples in the unoccupied UTest House were dominated by  $\gamma$ -Proteobacteria which constituted 54% of the bacterial clones encountered on the filter dust and 93% of those found on high surface dust sample (Fig. 3). For fungi in the unoccupied house, the difference between high surface and air may reside in the composition of the Dothideomycetes class. The high surface sample was composed of 53% of the subclass Pleosporomycetidae and 24% of the subclass Dothideomycetidae. For indoor air the proportion for these two subclasses was inverted, with the former accounting for 21% and the latter 57% of the sequences (Fig. 4). Finally, the microbial communities in indoor and outdoor air were not statistically different in the unoccupied UTest House (last column of Table 2) where the HVAC system was continuously operated and there were limited presence of occupants. Most importantly for this investigation, the findings reported in Table 2 suggest that high-efficiency HVAC filters located in HVAC systems operating a great fraction of time can be used as a surrogate for long-term air samples. This would suggest that HVAC filters could be used as an

**Table 2**  
UniFrac significance (*p*-value) for the samples collected in the UTest House. The bold values indicate statistically different comparisons.

	Filter versus high surface	Filter versus indoor air	High surface versus indoor air	Indoor versus outdoor air
Bacteria	0.13	0.14	<b>0.01</b>	0.12
Fungi	<b>0.01</b>	0.24	<b>0.03</b>	0.19

alternative to extensive periodic air sample collections and yield statistically similar information.

#### 4. Conclusions

This study evaluated the use of HVAC filters as long-term air samplers for indoor biological contamination. Microbial concentrations and communities on HVAC filter dust samples were not statistically different from high surface dust samples in occupied residences. Proteobacteria were present in greater proportion in HVAC filter dust samples than in high surface dust samples and they were present in greater proportion in the unoccupied UTest House than in residences suggesting the outdoor air origin of this phylum. Gram-positive bacteria were present in greater proportion in occupied residences than in the unoccupied UTest House, confirming the potential association of this group with occupants. HVAC filter microbial communities were not statistically different from a composite indoor air sample in a mostly unoccupied UTest House. The results indicate that HVAC filters may be a viable option for investigating indoor biological contaminants and could be used as a surrogate for long-term air samples, as suggested by other researchers. The current study represents an exploratory investigation of the potential use of HVAC filters as a sampling mechanism for indoor microbial communities. The results are promising and suggest that a more comprehensive investigation of this technique is warranted.

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