

ORIGINAL ARTICLE

The reestablishment of microbial communities after surface cleaning in schools

S.E. Kwan¹, R.J. Shaughnessy², B. Hegarty¹, U. Haverinen-Shaughnessy² and J. Peccia¹ ¹ Department of Chemical and Environmental Engineering, Yale University, New Haven, CT 06520-8286, USA² Department of Chemical Engineering, Indoor Air Program, The University of Tulsa, Tulsa, OK 74101-9700, USA**Keywords**

allergens, cleaning, human microbiome, indoor microbiome, schools.

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Abstract**Aims:** The goal of this study was to quantify the indoor microbiome dynamics of bacterial and fungal communities on school desk surfaces during a cleaning intervention.**Methods and Results:** Quantitative PCR and DNA sequenced-based approaches were employed to describe microbial community dynamics on ten desk surfaces, spread across three schools, located in the Northeast region of the United States. Six samples were taken from each desk, one precleaning, and five postcleaning at 30 min, 1, 3, 7 and 21 days. Cleaning of the desks physically removed *c.* 50% of bacteria, fungi, and human cells and a full recovery of the surface microbial concentrations occurred within 2–5 days. This recovery period is much shorter than the schools' once per semester cleaning schedule. The dominant source of bacteria and fungi on desks at all time points came from the human microbiome (skin, oral cavity, and gut). More than 50% fungi on desks were members of genera that contain known allergens.**Conclusions:** Microbial communities on these school desks are primarily generated and maintained from the deposition of human-associated bacteria and fungi. Current school surface cleaning protocols and cycles may be ineffective at reducing student exposure to fungal allergens and microbes of human origin.**Significance and Impact of Study:** Multiple students often share desks in schools. Results on the removal and reestablishment of microbial communities on these surfaces are critical for setting cleaning schedules and practices that effectively interrupt exposure to surface-associated pathogens and allergens.**Introduction**

Bacteria and fungi are a central component of the indoor microbiome. Common sources include microbes shed from the skin, saliva or secretions from humans and pets, microbes deposited from indoor air or outdoor air that entered via infiltration or ventilation, and microbial growth on surfaces where elevated water activities exist (Nielsen *et al.* 2004; Adams *et al.* 2013; Prussin and Marr 2015). Human exposure to these microbes can occur from direct contact with surfaces or through resuspension from surfaces and subsequent inhalation (Boor *et al.* 2013). It is well established that exposure to specific bacteria and fungi in the built environment results in

infectious and noninfectious disease, and there is growing evidence that microbial exposure in early life may have beneficial health effects (Arrieta *et al.* 2015; Lynch 2016; Peccia and Kwan 2016). Factors that modify the surface concentrations of microbes are expected to be significant for modulating human health.

Regular and effective surface cleaning is an important modifying factor for reducing human exposure to microbes in buildings—especially in hospitals and schools where occupant density is high and individuals may be immunocompromised. Prior studies in this area have focused on specific agents or microbial indicators to demonstrate that improved cleaning interventions can reduce surface-associated pathogen viability and

concentration (Gibson *et al.* 1999; Donskey 2013), lower exposure to asthma triggers, reduce instances of upper respiratory symptoms (Wälinder *et al.* 1999; Krieger *et al.* 2002), significantly decrease microbial indicators on school surfaces (Shaughnessy *et al.* 2013), and lessen adverse student health outcomes (Haverinen-Shaughnessy *et al.* 2015).

Recent focus has been placed on cataloging microbial communities on building surfaces (Flores *et al.* 2011, 2013; Dunn *et al.* 2013; Hsu *et al.* 2016). The DNA-based study of surfaces enables the detection of a diverse set of microbes (e.g. bacterial pathogens, fungal allergens, or beneficial classes of microbes) and community profiles offer the ability to infer the origins (human or environmental) of microbes and hence the potential for disease transmission. While these prior surface ecology studies have led to a better understanding of the sources, diversity, and potential health implications of microbial exposures indoors, the impact of cleaning on both the physical reduction and the reestablishment of bacterial and fungal surface communities remains uncharacterized. Such information is critical for setting cleaning schedules and practices that effectively interrupt exposure to surface-associated organisms.

This study quantified the dynamics of bacterial and fungal communities on school surfaces during a cleaning intervention. Experiments were conducted in three schools located in the Northeast region of the U.S. Quantitative PCR and DNA sequenced-based approaches were utilized to stratify bacterial and fungal community removal and reestablishment of microbial community dynamics by taxa and origin.

Materials and methods

Sample collection

Three Connecticut schools, grades 7 to 12, were recruited from the same school district in the spring of 2015. A total of ten classrooms were selected across the three schools. Classrooms were carefully selected to ensure no chemicals (other than cleaning chemicals), food, or animals were present in the room at any time. Desk across the 3 schools were of the same and age and type (purchased 2007, Mel-sur tops). Schools, and classrooms remained in sessions and occupied during the 21 days sampling campaign. Sampled classrooms occupancies ranged from 18 to 25 people throughout the 21 days sampling period. See Table S1 for full classroom details. The most highly used desk was selected for sampling from each classroom. Current cleaning schedules call for all desks to be cleaned at the end of every semester (i.e. twice per year). Desk sampling occurred during the middle of the semester, at least

2 months after the most prior desk cleaning. Each desk was sampled for baseline microbial concentrations, then cleaned by the study's field sampler by wetting the surface with a cleaning agent (glass cleaner, all-purpose cleaner or anti-bacterial cleaner) and wiping the surface in its entirety with a fresh paper towel, in accordance with the school district's protocols, see Table S1. Once cleaned, five subsequent samples were collected over 21 days to track the reestablishment of microbial communities (Table 1). No cleaning events took place over this time period. Environmental characteristics recorded included the specific type of cleaning product used, room occupancy, observations of excess moisture or water damage in each classroom, room air temperature and relative humidity, and architectural details including room volume, flooring type, building age, and methods of heating, cooling and ventilating (Table S1).

For sampling, surface-associated microbes were collected from 2.5 cm × 2.5 cm high touch-point areas of each desk (one sample area for each sample time slot) using sterile cotton swabs moistened with autoclaved and filtered 0.15 M NaCl, 9×10^{-4} M Tween 20[®] solution as previously described (Fierer *et al.* 2008). Surface sampling over time requires that spatially distinct samples be collected for each surface time point. Preliminary work in our lab was conducted to determine the spatial variability in microbial concentrations on a desk surface, and to compare the magnitude of this variability with sample concentration differences at the multiple time points after cleaning. Eight adjacent locations on a desk were simultaneously sampled and total bacteria enumerated by qPCR (Fig. S1). The uncertainty introduced due to spatial variance (COV = 42%) was less than the concentration differences introduced by time (COV = 131%), suggesting that our sampling approaches could track temporal changes in concentrations. We also tested the cell removal efficiency of moistened cotton tip swabs. A sterile surface, comparable in material to the school desks, was spiked with a known concentration of bacteria and fungi and left to dry. The bacteria and fungi were then removed with a moistened cotton tip swab, as described above, and re-sampled 5 times. These tests were performed in triplicate. Figure S2

Table 1 Desk sampling summary. Samples were collected on each of the 10 desks over 6 time points, 1 precleaning (t_1) and 5 postcleaning timepoints (t_2 : directly postcleaning, t_3 : 1 day, t_4 : 3 days, t_5 : 7 days, t_6 : 21 days postcleaning)

School	Number of classrooms/ desks sampled	Sample time points (t_1 – t_6)
School B	3 classrooms/1 desk per room	t_1 , t_2 , t_3 , t_4 , t_5 , t_6
School O	3 classrooms/1 desk per room	t_1 , t_2 , t_3 , t_4 , t_5 , t_6
School H	4 classrooms/1 desk per room	t_1 , t_2 , t_3 , t_4 , t_5 , t_6

indicates that *c.* 65% of spiked bacteria and fungi were removed from a desk surface using our swabbing technique, with significantly diminishing removals if additional swabs are used. One cotton-tipped swab was therefore deemed sufficient for biomass sampling.

DNA extraction and quantitative PCR

After sampling, swab tips were dropped into 2 ml screw top tubes and DNA extracted using a DNeasy PowerSoil Kit (Qiagen Inc., Germantown, MD, USA), modified to improve cell lysis via bead beating (Yamamoto *et al.* 2012). Material from desk surfaces was quantified via qPCR and included analysis of total bacteria, total fungi, and total human cells. Universal bacterial primers and TaqMan[®] probes were used for total bacterial concentration analysis targeting the 331 to 797 *E. coli* numbering region of the 16S rDNA encoding gene with forward primer 5'-TCCTACGGGAGGCAGCAGT-3', reverse primer 5'-GGA CTACCAGGGTATCTAATCCTGTT-3', and TaqMan[®] probe, (6-FAM)-5'-CGTATTACCGCGGCTGC TGGCAC-3'-(BHQ1) (Nadkarni *et al.* 2002). The SYBR Green method was used for universal fungal and human cell detection with the fungal primers FF1 (5'-GGTTCTATT TTGTTGGTTTCTA-3') and FR2 (5'-CTCTCAATCTGTC AATCCTTATT-3') targeting the 18S rDNA gene (Zhou *et al.* 2000) and the human assay intra-Yb8 primers 5'-CGA GGCGGGTGGATCATGAGGT-3 and 5'-TCTGTGCGCCCA GGCCGGACT-3' targeting *Alu* elements (Walker *et al.* 2003; Dannemiller *et al.* 2014b). All qPCR measurements were conducted in triplicate, and amplification was performed on an ABI 7500 fast real-time PCR system (Applied Biosystems, Carlsbad, CA, USA). Standard curves of serial dilutions of whole-cell concentration vs. cycle threshold number were generated, using *Bacillus atrophaeus* (ATCC 49337) for bacteria reported as cells (Qian *et al.* 2012), using *Aspergillus fumigatus* (ATCC 34506) for fungi reported as *A. fumigatus* spore equivalents (SE) (Qian *et al.* 2012), and using human embryonic kidney cells (HEK 293) for human cells, reported as cells (Dannemiller *et al.* 2016). Standard curve procedures and thermocycling conditions were described previously (Hospodsky *et al.* 2012; Qian *et al.* 2012; Yamamoto *et al.* 2012; Dannemiller *et al.* 2014b). PCR inhibition was tested by spiking all sample extracts with known target DNA concentrations of *B. atrophaeus*. No inhibition was observed.

DNA sequencing and analysis

The Bacterial V1/V2 region of 16S rRNA was sequenced using Hyb8F/Hyb338R primers (Campbell *et al.* 2012). Fungal taxonomic libraries, were prepared by targeting the internal transcribed spacer (ITS) region that is

circumscribed by ITS-1F and ITS2 primers (White *et al.* 1990; Gardes and Bruns 1993); (Dannemiller *et al.* 2014b). Library preparation, sequencing, and de-multiplexing of bacterial and fungal sequences were performed at the University of Texas Genomic Sequencing and Analysis Facility (UT GSAF, Austin, TX, USA) on an Illumina MiSeq platform to generate paired-end reads of 250 length bases in each direction. Sequences were archived in the European Nucleotide Archive (accession numbers PRJEB23735 and PRJEB23736 for bacteria and fungi respectively).

Bacterial data read analysis was conducted using the bioinformatics analysis toolkit QIIME, ver. 1.9 (Caporaso *et al.* 2010). Sequences were overlapped, paired-end reads were merged, quality trimmed, clustered at 97% similarity, aligned using PyNAST against the GreenGenes 13.8 open reference sequence collection database, and phylogenetic assignments were made using the Ribosomal Database Project classifier in QIIME. Bacterial taxa are referred to as operational taxonomic units (OTU's) due to the clustering. Bacterial rarefaction curves and richness were assessed based on the number of observed OTUs in 1084 sequences per sample. Bacterial β diversity and principal coordinate analyses were performed using both weighted and unweighted UniFrac approaches (Lozupone and Knight 2005).

For diversity analysis of fungal data, QIIME, ver. 1.9 (Caporaso *et al.* 2010) was used for overlapping paired-end read merging, quality trimming, and clustering at 97% similarity (Dannemiller *et al.* 2014b). For fungal α -diversity and rarefaction analysis, the number of observed species per sample was assessed based on the number of observed OTUs in 1134 sequences per sample. Fungal taxonomic identification was assigned by FHiTINGS, ver. 1.4 (Dannemiller *et al.* 2013) and the UNITE fungal database (Nilsson *et al.* 2009), as described previously (Dannemiller *et al.* 2014b). For β diversity and principal coordinate analysis (PCoA), Morisita Horn (Horn, 1966) (nonphylogenetic) distance was calculated from all quality-trimmed reads.

Relative abundances for each identification were calculated from the QIIME and FHiTINGS annotation output files. Absolute bacterial and fungal concentrations per m² were calculated by multiplying relative abundance values with total bacterial cell and total fungal SE quantities per m², as determined by qPCR with universal bacterial and fungal primers (Dannemiller *et al.* 2014a).

Statistics

A two-tailed, independent-sample *t*-test was used to compare differences in the cumulative efficiency of a swab to remove bacteria and fungi from a preseeded surface, the

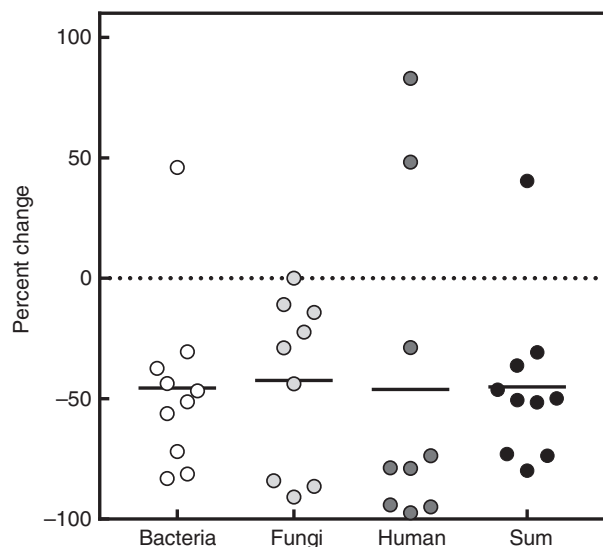


Figure 1 Per cent change immediately after surface cleaning for bacteria (cells per m²), fungi (SE per m²), human (cells per m²), and qPCR-sum-total (qPCR per m²). Horizontal bar denotes sample mean.

concentration of total fungi with human-associated fungi on pre and postcleaned desks, and the number of bacterial and fungal observed OTUs from each desk pre and 1 and 21 days postcleaning. A multi-line t-test with Bonferroni–Dunn corrections compared the temporal variations pre and 1 and 21 days postcleaning for the top 20 bacterial and fungal taxa. An analysis of similarity (ANOSIM, available through QIIME) was used to test for differences

between precleaning and postcleaning microbial communities for bacteria (weighted and unweighted UniFrac) and fungi (Morisita Horn). The recovery rate of microbes, after a cleaning event, was calculated using a linear regression of the first three postcleaning sample time points (30 min, 1 and 3 days postcleaning) plotted against qPCR concentrations of bacterial cells per m², fungal SE per m², human cells per m², and the qPCR sum-total qPCR per m².

Results

Microbial community reestablishment rate

Cleaning resulted in a significant reduction in microbial and cellular concentrations. These concentrations then steadily increased, beyond the initial concentration, over the 21 days sampling period. Paired average microbial and cellular concentration reductions ($\pm$ SD) due to cleaning were $46 \pm 37\%$, $42 \pm 36\%$, $46 \pm 67\%$, and $45 \pm 34\%$ for bacteria, fungi, human cells, and qPCR sum-total, respectively (Fig. 1). Quantitative PCR (qPCR) sum-total represents the addition of bacterial and human cells with fungal spore equivalents (SE). While variability was observed in cleaning reduction, the average reductions were statistically different from zero for bacteria ($P = 0.001$), fungi ($P = 0.003$), and qPCR sum-total ($P = 0.0006$), and nearly significant for human cells ($P = 0.06$).

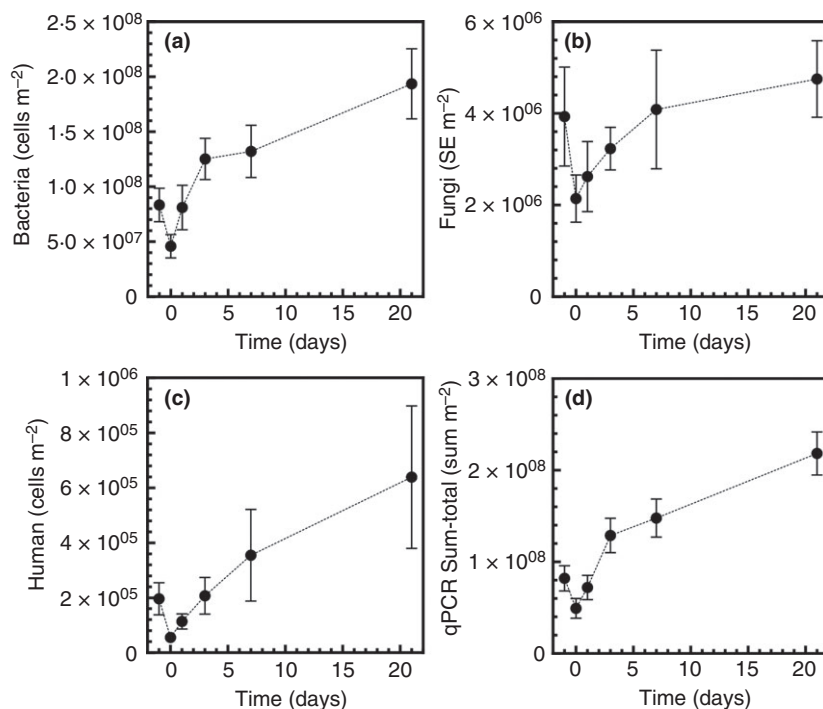


Figure 2 Desk microbial concentrations pre and postcleaning. Zero time denotes the time of cleaning and qPCR Sum-total represents the summation of total bacteria, total fungi, and human cells. Error bars represent one standard error.

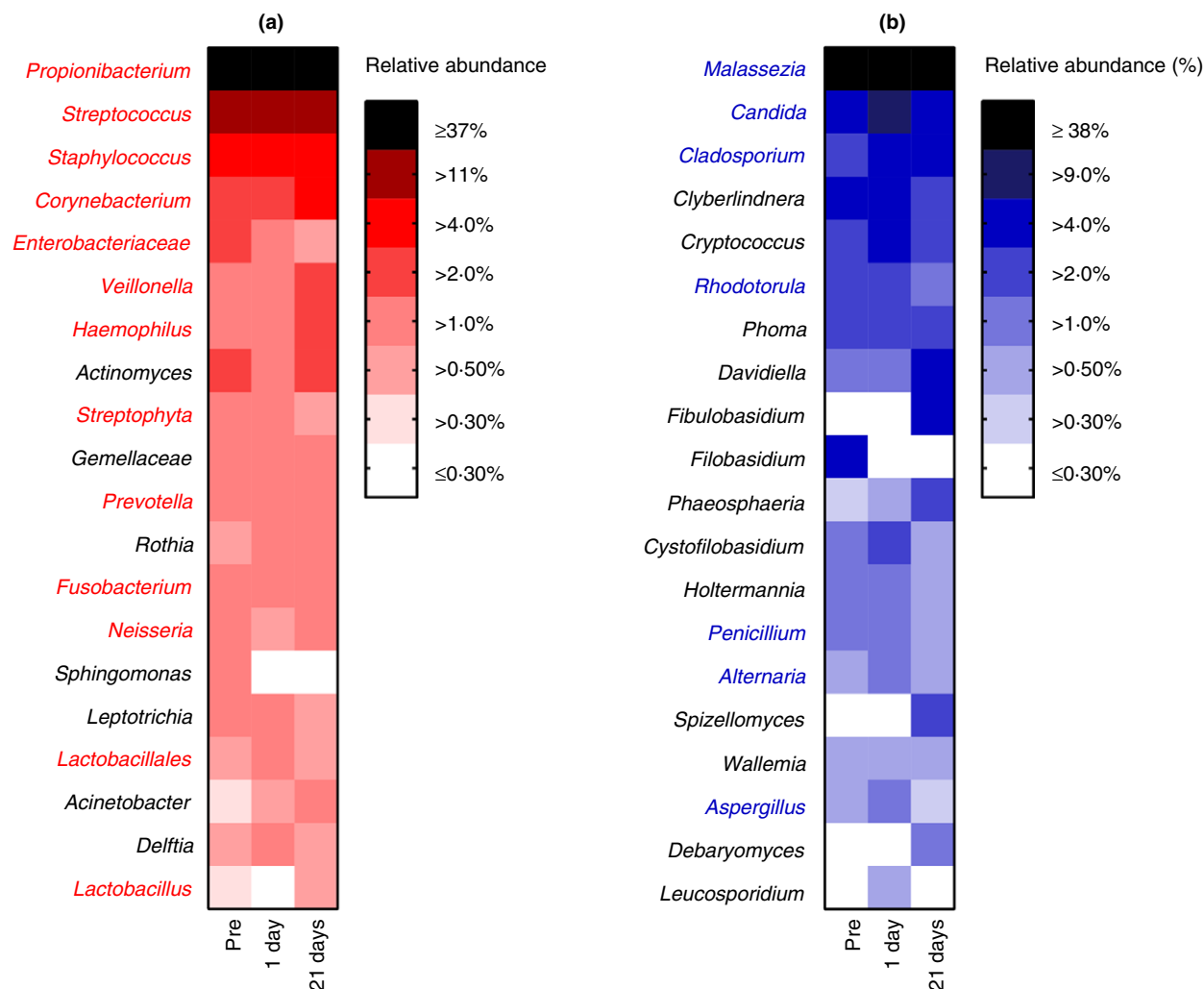


Figure 3 Relative abundance of the 20 most abundant (a) bacterial genera in red and (b) fungal genera in blue for school desk surface samples taken prior to a cleaning event (pre) and 1 and 21 days postcleaning. Taxa displayed represents (a) $83.1 \pm 0.06\%$ of all bacterial sequences and (b) $81.7 \pm 11.5\%$ of all fungal sequences. Bacterial taxa names listed in red are all human associated and fungal taxa names listed in blue represent genera with known fungal allergens. [Colour figure can be viewed at wileyonlinelibrary.com]

The biological communities on the surface after cleaning were reestablished at average recovery rates of $1.1 \times 10^6 \pm 0.1 \times 10^6$ cells per m^2 -hr ($\pm$ SD) for bacteria (Fig. 2a), $15\,000 \pm 1\,700$ SE per m^2 -hr for fungi (Fig. 2b), $2\,100 \pm 120$ cells per m^2 -hr for human cells (Fig. 2c), and $1.1 \times 10^6 \pm 0.06 \times 10^6$ sum per m^2 -hr for qPCR sum total (Fig. 2d). Microbial and cellular surface concentrations rapidly returned to precleaning quantities after 2.0 ± 2.1 days ($\pm$ SD) for bacteria, 3.6 ± 3.0 days for fungi, and 4.9 ± 7.2 days for human cells. By number, bacteria dominated surface microbial concentrations at each of the six sampling time points accounting for 95% of the qPCR sum-total concentration precleaning, 95% directly postcleaning, 97% 1 day, 97% 3 days, 97% 7 days, and 97% 21 days postcleaning. No

significant difference in removal or reestablishment rates of surface associated microbial communities were observed as a function of the three different cleaning agents used ($P \geq 0.8$ for paired average qPCR sum-total concentration reduction due to cleaning; $P \geq 0.4$ for after cleaning qPCR sum-total reestablishment rate).

Microbial origin

After quality trimming, 56,123 bacteria and 213,804 fungal sequences were retained for analysis from 30 samples. Among all samples, a total of 3,418 different bacterial OTUs, 316 bacterial genera, 2,115 fungal species and 395 fungal genera were identified. Rarefaction analysis demonstrated a minor impact of surface cleaning on bacterial and

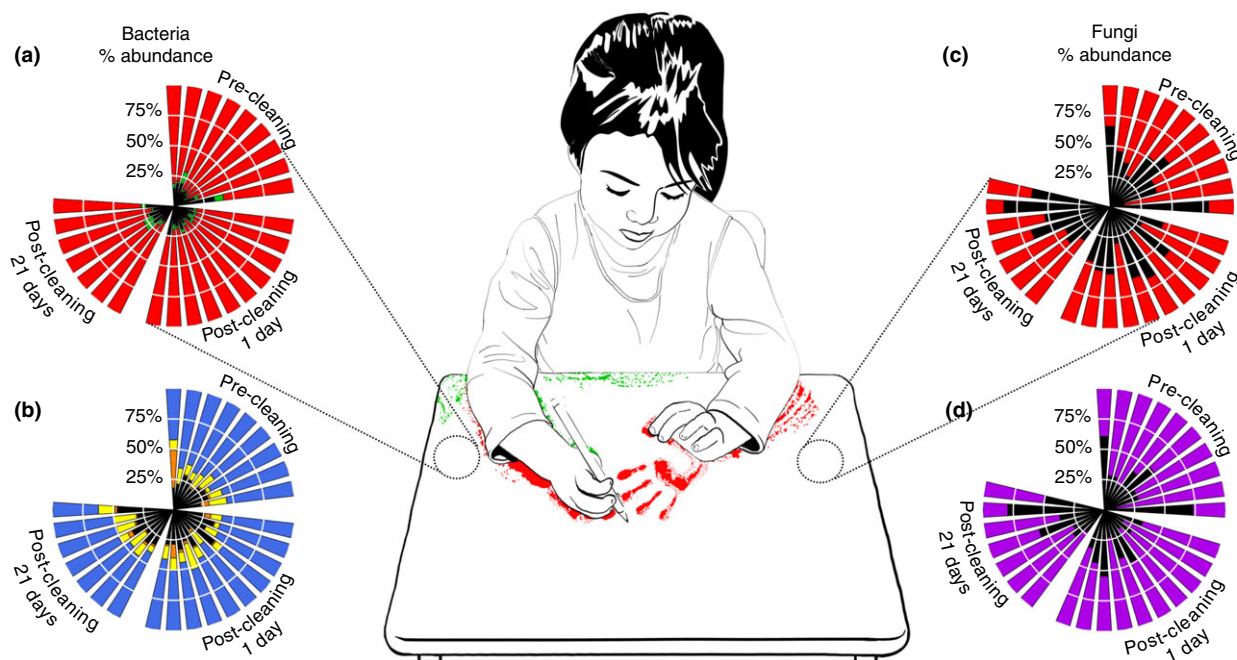


Figure 4 Relative abundance of bacterial and fungal genera found on desk surfaces pre and postcleaning categorized as (a) bacteria that is human associated (red), environmentally associated (green), and other bacteria (black); (b) bacteria that is skin associated (blue), oral cavity associated (yellow), gut/faecal associated (orange), and other human associated bacteria (black); (c) fungi that is human associated (red), and other fungi (black); and (d) fungal genera containing allergens (purple), and other fungi (black). (■) Human associated; (■) Environmentally associated; (■) Other; (■) Skin associated; (■) Oral associated; (■) Gut/Faecal associated, and (■) Genera that contain allergens. [Colour figure can be viewed at wileyonlinelibrary.com]

fungal communities (Fig. S3a,b). Out of 1084 randomly chosen sequences, mean ($\pm$ SD) bacterial richness was 128 ± 33 sequences for precleaning, 127 ± 14 OTUs sequences 1 day postcleaning, and 143 ± 21 OTUs sequences 21 days postcleaning (Table S2). Mean fungal richness from 1,134 random sequences per sample was 72 ± 13 OTUs precleaning, 73 ± 10 for 1 day postcleaning, and 105 ± 13 OTUs 21 days postcleaning (Table S3). Bacterial richness was not impacted by pre or postcleaning time (pre vs. 1 day, $P = 0.9$, pre vs. 21 days, $P = 0.3$, and 1 day vs. 21 days, $P = 0.09$). Fungal richness increased over time, with day 21 greater than precleaning ($P < 0.01$) and greater than 1 day postcleaning ($P < 0.01$).

Only minor changes in temporal patterns for bacteria ecology were observed (Fig. 3a and Fig. S6). Both weighted and unweighted UniFrac analysis demonstrated a lack of broad changes in bacterial community structure or relative abundances on desks pre versus postcleaning (ANOSIM $R = 0.2$, $P = 0.02$ and $R = 0.2$ $P = 0.03$ for unweighted and weighted UniFrac distance, respectively) (Fig. S4). The most dominant bacterial genera among all samples were known human microflora including: *Propionibacterium* (44.2–37.2%), *Streptococcus* (13.5–11.4%), *Staphylococcus* (6.8–4.4%), *Corynebacterium* (6.4–2.4%), *Enterobacteriaceae* (3.2–0.7%), *Veillonella* (2.4–1.8%), and *Haemophilus*

(2.24–1.24%). The top 20 bacterial taxa showed little change in per cent abundance between precleaning and 1 and 21 days after a cleaning event from their precleaning per cent abundance (multiple line t -test with Bonferroni–Dunn corrections, $P_{\text{adjusted}} > 0.4$ for all samples) (Fig. 3a). Overall, bacteria from human origin accounted for $79 \pm 9\%$, $80 \pm 3\%$, and $77 \pm 5\%$, precleaning and 1 and 21 days postcleaning. Among all sampling times, the majority of human bacteria were skin-associated ($44 \pm 7.5\%$), with $8.6 \pm 3.2\%$ oral microbiota, and $2.7 \pm 5.0\%$ gut microbiota (Fig. 4a,b), see Table S4 for a list of human-associated bacteria.

For fungi, Morisita Horn distance analysis demonstrated that there was little difference in fungal community structure on desks postcleaning and precleaning (ANOSIM $R = -0.01$, $P = 0.5$) (Fig. S5). Fungal genera were on average greater than 40% human-associated including *Malassezia* (40.4–45.3%) and *Candida* (4.15–10.8%). Taxa belonging to the genera *Cladosporium* (2.54–7.57%), *Cyberlindnera* (2.06–8.64%), *Cryptococcus* (2.76–4.58%), and *Rhodotorula* (1.95–4.27%) were also highly enriched. The top 20 fungal taxa and 1 and 21 days after a cleaning event showed little change from their precleaning per cent abundance (multiple line t -test with Bonferroni–Dunn corrections, $P_{\text{adjusted}} > 0.99$ for all

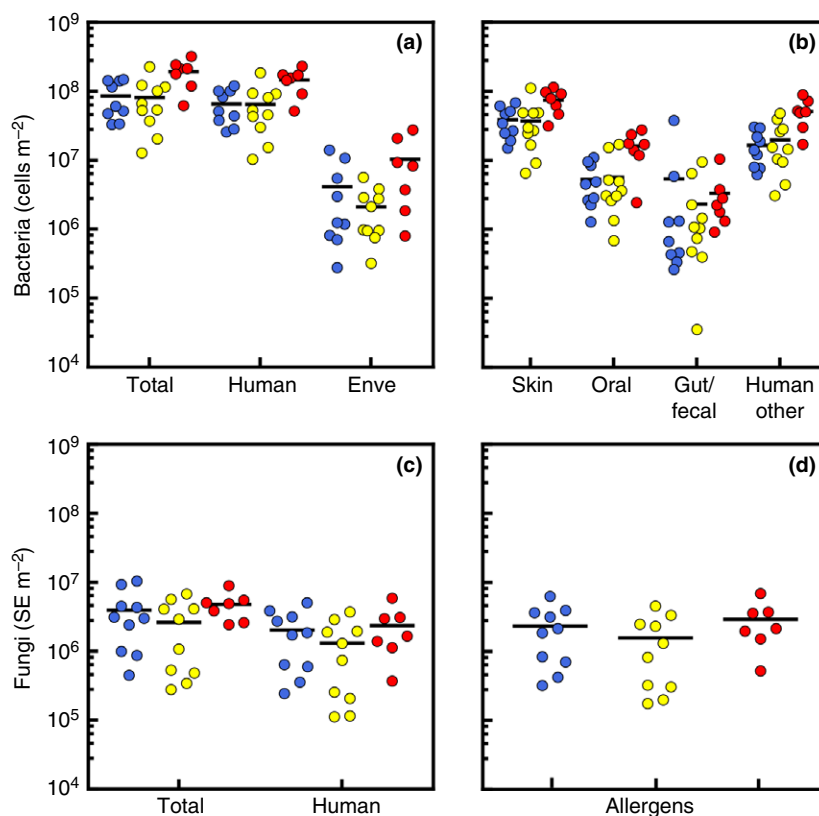


Figure 5 Absolute concentrations of bacteria and fungi found on desk surface samples pre and postcleaning for (a) total, human associated, and environmentally associated bacteria; (b) Pre and postcleaning absolute concentrations of human associated bacterial concentrations grouped into skin, oral, gut, and other human origin categories; (c) total and human associated fungi; and (d) Pre and postcleaning absolute concentrations of genera containing known allergenic fungi. Horizontal bar denotes sample mean. (●) Precleaning; (●) 1 day postcleaning; and (●) 21 days postcleaning. [Colour figure can be viewed at wileyonlinelibrary.com]

samples, except *Cladosporium* $P_{\text{adjusted pre vs. 1d}} = 0.01$ and *Davidella* $P_{\text{adjusted pre vs. 1d}} = 0.005$ and $P_{\text{adjusted 1d vs. 21d}} = 0.007$) (Fig. 3b and Fig. S7). Human-associated fungi accounted for $54 \pm 19\%$, $53 \pm 14\%$, and $31 \pm 26\%$, precleaning and 1 and 21 days postcleaning. Fungi that were members of genera with known allergens accounted for $63 \pm 19\%$, $66 \pm 14\%$, and $39 \pm 32\%$, precleaning and 1 and 21 days postcleaning (Fig. 4c,d). See Table S6 for a list of genera found in these desk samples that contain known allergenic species.

Dynamics of fungal allergens and human-associated

Fig. 5a–d plots the absolute concentrations of bacteria and fungi on desks and is separated by pre and post (1 and 21 days) cleaning. Microbial concentrations are parsed into relevant groups including environmental, skin, oral, and gut/faecal bacteria and fungal allergens (Tables S4–S6). The concentrations of these specific microbes at 1 day postcleaning were not different than precleaning concentrations ($P = 0.9$ for skin microbiota, $P = 0.8$ for oral

microbiota; $P = 0.04$ for gut/faecal microbiota; and $P = 0.3$ for genera containing fungal allergens).

Discussion

The results of this study highlight two important concepts for understanding how cleaning impacts the concentration and ecology of microbes on surfaces. First, c. 50% of bacteria and fungi were physically removed from desks during the cleaning intervention and the recovery of microbial concentrations was rapid compared to the surfaces cleaning schedule. Second, the ecology of school desks was strongly influenced by the human microbiome, namely human skin and oral microbiota, included fungal allergens, and did not significantly change in community profile throughout cleaning and community reestablishment.

The impacts of surface cleaning on microbial culturability are well established through disinfection literature (Bloomfield *et al.* 2006; Donskey 2013) and have been indirectly addressed in studies focused on biological contamination and the microbial ecology of surfaces. One

cleaning study saw a 90% reduction in biological contamination measurements (based on ATP) between pre- and postcleaned surfaces in schools, in which surfaces were sprayed with a disinfection spray and then wiped down with a microfiber cloth (Shaughnessy *et al.* 2013). Similar studies conducted on hospital bedrails and overbed tables saw average reductions of 39, 51, and 99% of bacterial after disinfection cleaning (Attaway HH *et al.* 2012; Bogusz *et al.* 2013; Stewart *et al.* 2014). Another concluded that although cleaning through disinfection was effective in reducing microbial contamination, the effects were short-lived; most surfaces became re-contaminated in 1.5 to 3 h (Scott *et al.* 1984; Josephson *et al.* 1997). Comparative studies on hospital bedrails and overbed tables similarly showed the relatively short-lived effect of cleaning through disinfection with surface recontamination reaching approximate precleaning levels from 3 to 48 h after cleaning (Attaway HH *et al.* 2012; Bogusz *et al.* 2013; Stewart *et al.* 2014). This study focused on the physical removal of microbiota from desk surfaces since most school cleaning practices may not provide the proper cleaners and contact time for disinfection and some features of microbial communities such as fungal allergens are not responsive to disinfectants. Here fungal allergens, in particular, were reduced by 42% due to current school cleaning practices, but recovery was rapid. Prior studies that focused on cat, dog, dust mite, and cockroach allergens observed a 60–70% reduction in allergens in rooms cleaned four times a day compared to those only cleaned once a day (Tranter 2005). The rate of microbial reestablishment on these surfaces was a unique contribution of this study. In this case, we assumed reestablishment was due to shedding and deposition of microbial aerosols, rather than microbial replication due to the lack of moisture on desk surfaces. Communities were reestablished within 2–5 days of cleaning. Thus, the cleaning interval would need to be less than this period to result in a significantly lower exposure to children. These intervals are important, even when considering a cleaning protocol that provides complete physical removal and disinfection. Relationships between the reduction in allergens found on school desks and increased cleaning frequency have been observed (Smedje and Norbäck 2001). Other frequently touched surfaces such as keyboards, lockers, pencil sharpeners, doorknobs, and paper towel dispensers in schools have been shown to harbour bacterial communities, and children occupying uncleaned classrooms as opposed to those cleaned daily with disinfectant wipes are more likely to be absent, due to illness (Bright *et al.* 2010).

The microbial ecology revealed a strong influence of human microbiota ($79 \pm 6.2\%$) on desks. These communities were not only skin-associated ($44 \pm 7.5\%$) but

contained significant proportions of microbes from oral cavities ($8.6 \pm 3.2\%$) and faecal/gut microbiota ($2.7 \pm 5.0\%$) and suggest modes of disease transmission from desks as a fomite. This is of special significance in schools where desks are inhabited by a variety of students throughout the day. Temporal variation in microbial ecology pre- versus postcleaning were small ($P_{\text{adjusted}} > 0.4$ for all bacteria genera and $P_{\text{adjusted}} > 0.99$ for most fungal genera), suggesting a consistent human source of microbes on the desk surfaces.

In conclusion, the desk cleaning protocols observed in this study were ineffective at the full physical removal of surface microbes from desks, only *c.* 50% physical removal of bacteria, fungi, and human cells was observed. Recovery of these microbes occurred in 2–5 days, far more rapidly than current, once per semester desk cleaning intervals. No significant microbial shift was seen in the desk microbes after cleaning or during the postcleaning recovery time indicating that there was one dominant, human source. These results were confirmed by the high abundance of bacteria and fungi from human skin, the oral cavity and the gut. These data demonstrate how desks can be an important surface for mediating the transmission of disease in schools and the limitations of standard cleaning schedules.

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Conflict of Interest

No conflict of interest declared.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Adjacent area test for desk areas 1 to 8 (a), mean qPCR bacterial Qty for adjacent desk areas 1 to 8 (b).

Figure S2 Surface cumulative per cent removal of bacteria (a) and fungi (b) for swabs 1 through 5.

Figure S3 Rarefaction curves for (a) bacteria and (b) fungi. (a, b) Lines are displayed in blue for precleaning, yellow for 1 day postcleaning, and red for 21 days postcleaning.

Figure S4 Principal coordinate analysis plots for bacterial communities from desk swab samples taken pre and postcleaning for (a) unweighted UniFrac distance, (b) Weighted UniFrac distant.

Figure S5 Principal coordinate analysis plots for fungal communities from desk swab samples taken pre and postcleaning based off of a Morisita Horn distance analysis.

Figure S6 Data represent the 20 bacterial genera with the highest relative abundances found on school desks precleaning and 1 and 21 days postcleaning. Coloured figure annotations indicate cleaning time line: blue, yellow, and red represent precleaning, 1 and 21 days postcleaning timepoints, respectively. Taxonomic names highlighted in red represent human associated bacteria. * indicates genera is found abundantly in the environment and on humans.

Figure S7 Data represent the 20 fungal genera with the highest relative abundances found on school desks precleaning and 1 and 21 days postcleaning. Coloured figure annotations indicate cleaning time line: blue, yellow, and red represent precleaning, 1 and 21 days postcleaning timepoints respectively. Taxonomic names highlighted in blue represent genera that contain known allergenic fungi.

Table S1 Desk, classroom, occupant, and environmental characteristic details.

Table S2 Number of observed Bacterial OTUs from desk surface samples taken before a cleaning event and 1 and 21 days post the cleaning event.

Table S3 Number of observed fungal OTUs from desk surface samples taken before a cleaning event and 1 and 21 days post the cleaning event.

Table S4 Characterization of the bacterial microbiome populating different body sites.

Table S5 Environmentally associated bacteria.

Table S6 Genera identified in this study that contain allergenic species.

Supplementary Information

The Redevelopment of Microbial Communities after Surface Cleaning in Schools

Journal of Applied Microbiology

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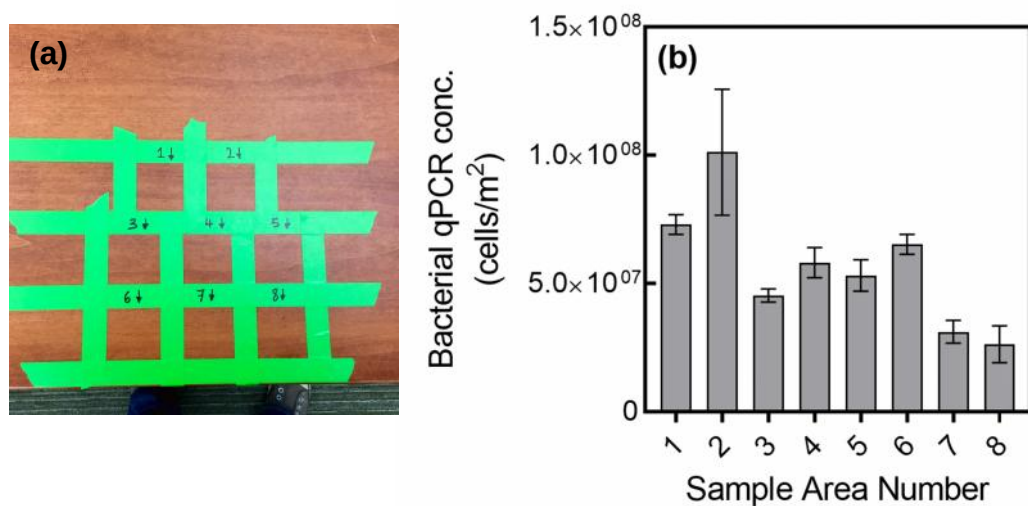


Figure S1. Adjacent area test for desk areas 1 to 8 (a), mean qPCR bacterial Qty for adjacent desk areas 1 to 8 (b). Error bars represent standard error, n=3 for each area, n=24 for average.

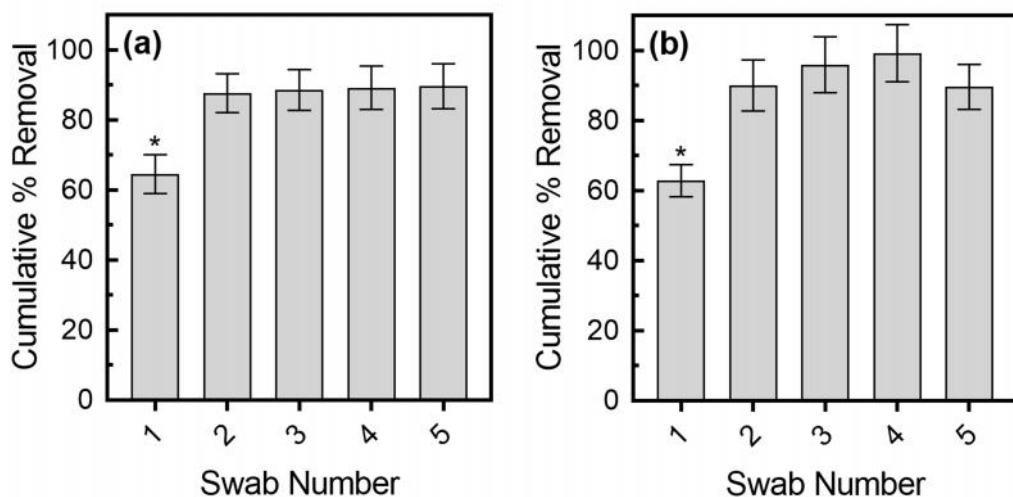


Figure S2. Surface cumulative percent removal of bacteria (a) and fungi (b) for swabs 1 through 5. Significance difference of swab 1 mean from other means was tested using an unpaired, two-tail t-test (a) $0.0405 \leq p \leq 0.0421$ and (b) $0.0175 \leq p \leq 0.0341$.

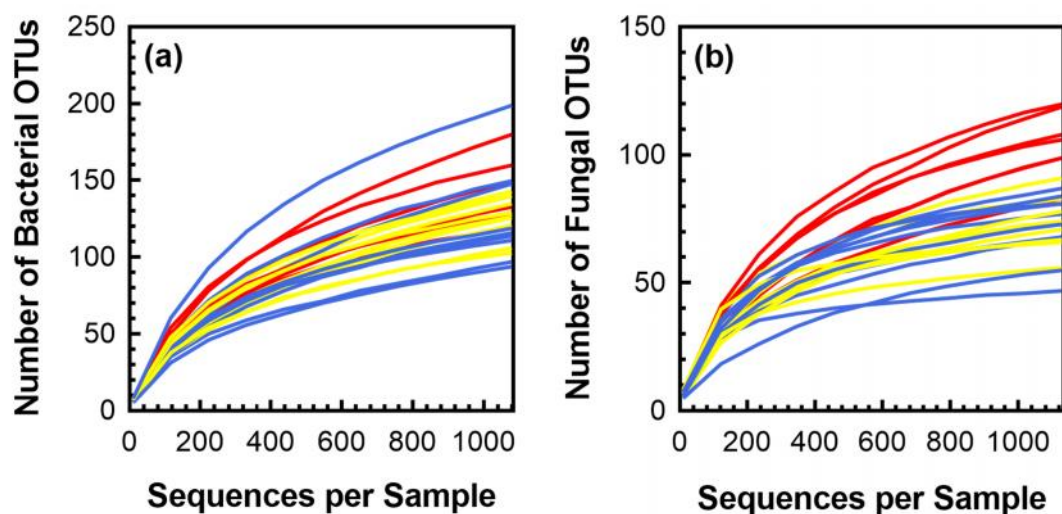


Figure S3. Rarefaction curves for (a) bacteria and (b) fungi. (a, b) Lines are displayed in blue for pre-cleaning, yellow for 1 day post-cleaning, and red for 21 days post-cleaning. Bacteria was normalized to 1084 sequences per sample and fungi was normalized to 1134 sequences per sample prior to analysis.

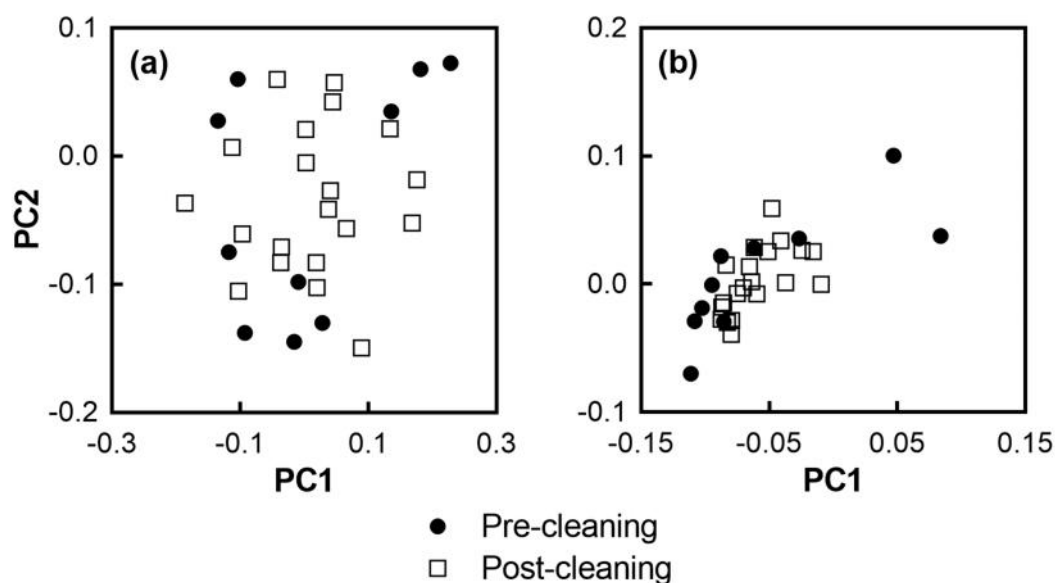


Figure S4. Principal coordinate analysis plots for bacterial communities from desk swab samples taken pre- and post-cleaning for (a) unweighted UniFrac distance, (b) Weighted UniFrac distant. ANOSIM testing showed the bacterial communities of desk prior to and after a cleaning event had differences, but grouped poorly ($p=0.02$, $R=0.2$), ($p=0.03$, $R=0.2$) for unweighted and weighted UniFrac distances, respectively.

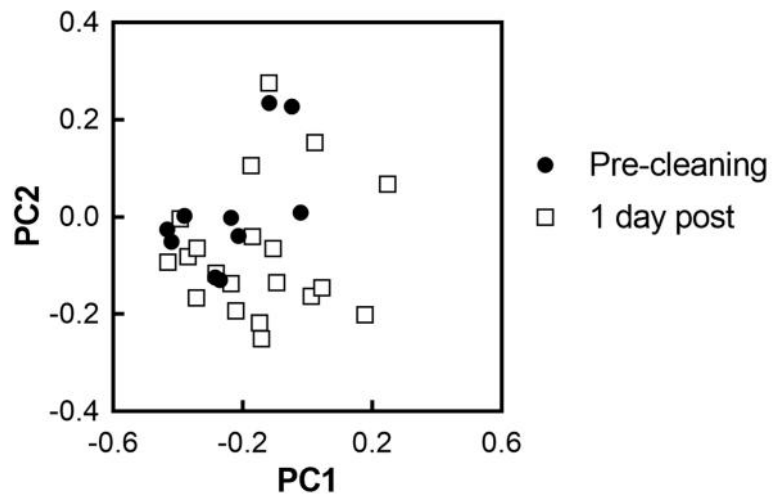


Figure S5. Principal coordinate analysis plots for fungal communities from desk swab samples taken pre- and post-cleaning based off of a Morisita Horn distance analysis. ANOSIM testing showed the fungal communities of desk prior to and after a cleaning event had no significant difference ($p=0.5$, $R=-0.1$).

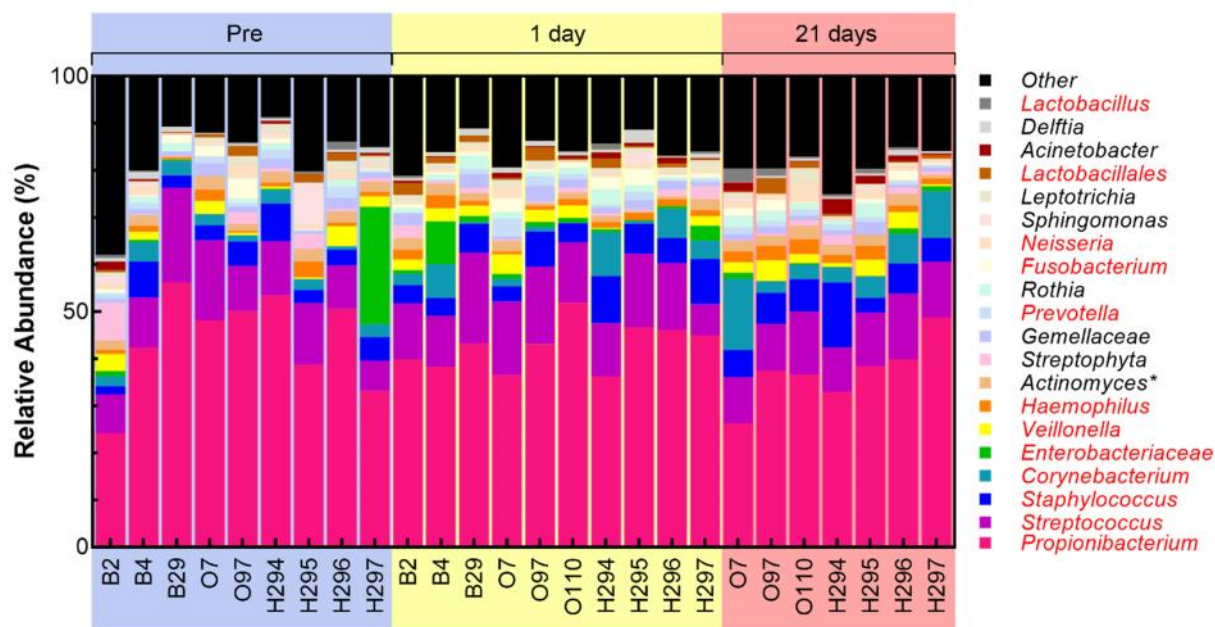


Figure S6. Data represent the 20 bacterial genera with the highest relative abundances found on school desks pre-cleaning and 1 day and 21 days post-cleaning. Colored figure annotations indicate cleaning time line: blue, yellow, and red represent pre-cleaning, 1 day, and 21 days post-cleaning timepoints, respectively. Taxonomic names highlighted in red represent human associated bacteria. * indicates genera is found abundantly in the environment and on humans.

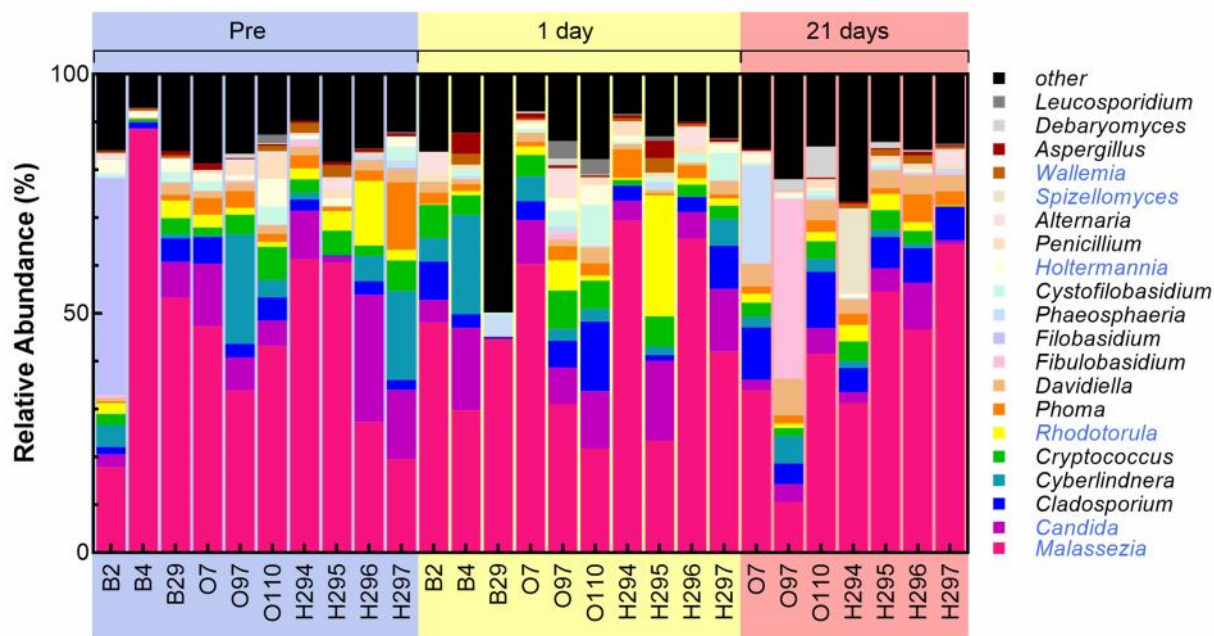


Figure S7. Data represent the 20 fungal genera with the highest relative abundances found on school desks pre-cleaning and 1 day and 21 days post-cleaning. Colored figure annotations indicate cleaning time line: blue, yellow, and red represent pre-cleaning, 1 day, and 21 days post-cleaning timepoints, respectively. Taxonomic names highlighted in blue represent genera that contain known allergenic fungi.

Table S1. Desk, classroom, occupant, and environmental characteristic details.

Desk	Cleaner used*	Room Occupancy	Grade Level	Classroom Type	Excess Moisture?	Building Age	Room Air Temp (°C)	Room RH (%)	Room Volume (m ³)	Desk Material	Flooring Type	Method for heating/cooling
B2	Glass	23-25	7	Spanish	No	Renovated 2007	22.02 ±0.68	57.61 ±10.07	153	Melsur	Vinyl	Central school HVAC
B4	Glass	23-25	7	Math	No	Renovated 2007	22.02 ±0.68	57.61 ±10.07	153	Melsur	Vinyl	Central school HVAC
B29	Glass	23-25	8	Math	No	Renovated 2007	21.83 ±1.04	61.31 ±9.46	153	Melsur	Vinyl	Central school HVAC
O7	All purpose	18-24	8	Spanish	No	Renovated 2007	21.40 ±0.74	65.46 ±10.25	159	Melsur	Vinyl	Central school HVAC
O97	All purpose	18-24	7	Spanish	No	Renovated 2007	21.60 ±0.67	64.61 ±9.70	157	Melsur	Vinyl	Central school HVAC
O110	All purpose	18-24	7	English	No	Renovated 2007	21.60 ±0.67	64.61 ±9.70	165	Melsur	Vinyl	Central school HVAC
H294	Anti-Bacteria	25	9-12	Math	No	Built 2007	21.65 ±1.39	56.33 ±5.50	190	Melsur	Vinyl	Central school HVAC
H295	Anti-Bacteria	25	9-12	Math	No	Built 2007	21.65 ±1.39	56.33 ±5.50	190	Melsur	Vinyl	Central school HVAC
H296	Anti-Bacteria	25	9-12	Math	No	Built 2007	21.01 ±1.26	57.43 ±6.65	190	Melsur	Vinyl	Central school HVAC
H297	Anti-Bacteria	25	9-12	Math	No	Built 2007	21.01 ±1.26	57.43 ±6.64	190	Melsur	Vinyl	Central school HVAC

* Desks were sprayed down with cleaner then wiped off with a clean paper towel. Glass cleaner - Hillyard Arsenal, Green Select Glass Cleaner, concentrated cleaner for all glass surfaces, 27. All purpose - Hillyard, Arsenal Suprox Multi Purpose #33, Dilution Control Concentrate, and Anti-Bacterial - Hillyard Arsenal Re-Juv-Nal® One-Step Disinfectant germicidal detergent and deodorant, 16, active ingredients: Didecyl dimethyl ammonium chloride (0.04%).

Table S2. Number of observed Bacterial OTUs from desk surface samples taken before a cleaning event and 1 day and 21 days post the cleaning event from 1084 sequences randomly subsampled for each library.

Location	Desk number	Pre-cleaning	1 day post cleaning	21 days post cleaning
Middle School B	B2	199	140	-
	B4	150	135	-
	B29	94	106	-
Middle School O	O7	97	143	160
	O97	116	128	119
	O110	n.a.	121	149
High School	H294	114	129	180
	H295	111	103	129
	H296	119	126	129
	H297	148	140	133

*OTUs are based on 97% similarity. Abbreviations and symbol: n.a., samples not available due to small number of sequences produced (fewer than 1084 reads); - samples not included as qPCR data indicated desks were cleaned before sampling.

Table S3. Number of observed fungal OTUs from desk surface samples taken before a cleaning event and 1 day and 21 days post the cleaning event from 1134 sequences randomly subsampled for each library.

Location	Desk number	Pre-cleaning	1 day post cleaning	21 days post cleaning
Middle School B	B2	87	n.a.	-
	B4	55	56	-
	B29	84	66	-
Middle School O	O7	47	74	99
	O97	68	83	83
	O110	77	71	108
High School	H294	73	74	119
	H295	81	67	106
	H296	68	78	120
	H297	81	91	99

*OTUs are based on 97% similarity. Abbreviations and symbol: n.a., samples not available due to small number of sequences produced (fewer than 1084 reads); - samples not included as qPCR data indicated desks were cleaned before sampling.

Table S4. Characterization of the bacterial microbiome populating different body sites. Bacteria that were associated with more than one body site were placed the category in which it is found in the highest percent abundance according to González et al. (González, Vázquez-Baeza and Knight, 2014).

Skin	Human Site		
	Oral	Gut/Fecal	Human Other
<i>Anaerococcus</i>	<i>Haemophilus</i>	<i>Bacteroidales</i>	<i>Alloiococcus</i>
<i>Fusobacterium</i>	<i>Neisseria</i>	<i>Bacteroides</i>	<i>Atopobium</i>
<i>Propionibacteriaceae</i>	<i>Neisseriaceae</i>	<i>Clostridiaceae</i>	<i>Bifidobacteriaceae</i>
<i>Propionibacterium</i>	<i>Paraprevotellaceae</i>	<i>Clostridiales</i>	<i>Bifidobacterium</i>
<i>Streptophyta</i>	<i>Pasteurellaceae</i>	<i>Erysipelotrichaceae</i>	<i>Corynebacterium</i>
<i>Tissierellaceae</i>	<i>Porphyromonas</i>	<i>Firmicutes</i>	<i>Lactobacillales</i>
	<i>Prevotella</i>	<i>Lachnospiraceae</i>	<i>Lactobacillus</i>
	<i>Veillonella</i>	<i>Parabacteroides</i>	<i>Leptotrichiaceae</i>
	<i>Veillonellaceae</i>	<i>Ruminococcaceae</i>	<i>Moraxella</i>
		<i>Ruminococcus</i>	<i>Staphylococcus</i>
			<i>Streptococcus</i>

Table S5. Environmentally associated bacteria.

Bacteria	References
<i>Achromobacter</i>	(Garrity et al., 2005; Vaneechoutte et al., 2011; Huys, 2014)
<i>Acinetobacter</i>	(Ulrike, 2008; Huys, 2014)
<i>Aeromonadaceae</i>	(Huys, 2014)
<i>Brevundimonas</i>	(Chi et al., 2004)
<i>Chryseobacterium</i>	(P R Hsueh et al., 1996; Po Ren Hsueh et al., 1996; Vaneechoutte et al., 2011)
<i>Delftia</i>	(Bilgin et al., 2015)
<i>Pseudomonas</i>	(Walker et al., 2004; Huys, 2014)
<i>Serratia</i>	(Huys, 2014)
<i>Sphingobium</i>	(Yabuuchi et al., 2002; Murakami, Otsuka and Senoo, 2010)
<i>Sphingomonas</i>	(Yabuuchi et al., 2002; Murakami, Otsuka and Senoo, 2010)

Table S6. Genera identified in this study that contain allergenic species (Simon-Nobbe *et al.*, 2008; Yamamoto *et al.*, 2012).

Genera

Alternaria
Aspergillus
Candida
Cladosporium
Curvularia
Embellisia
Fusarium
Penicillium
Saccharomyces
Stachybotrys
Thermomyces
Trichophyton
Malassezia
Rhodotorula

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