



What Have We Learned about the Microbiomes of Indoor Environments?

Webinar and Discussion for EPA, September 22, 2016

Brent Stephens, PhD

Associate Professor

Civil, Architectural and Environmental Engineering



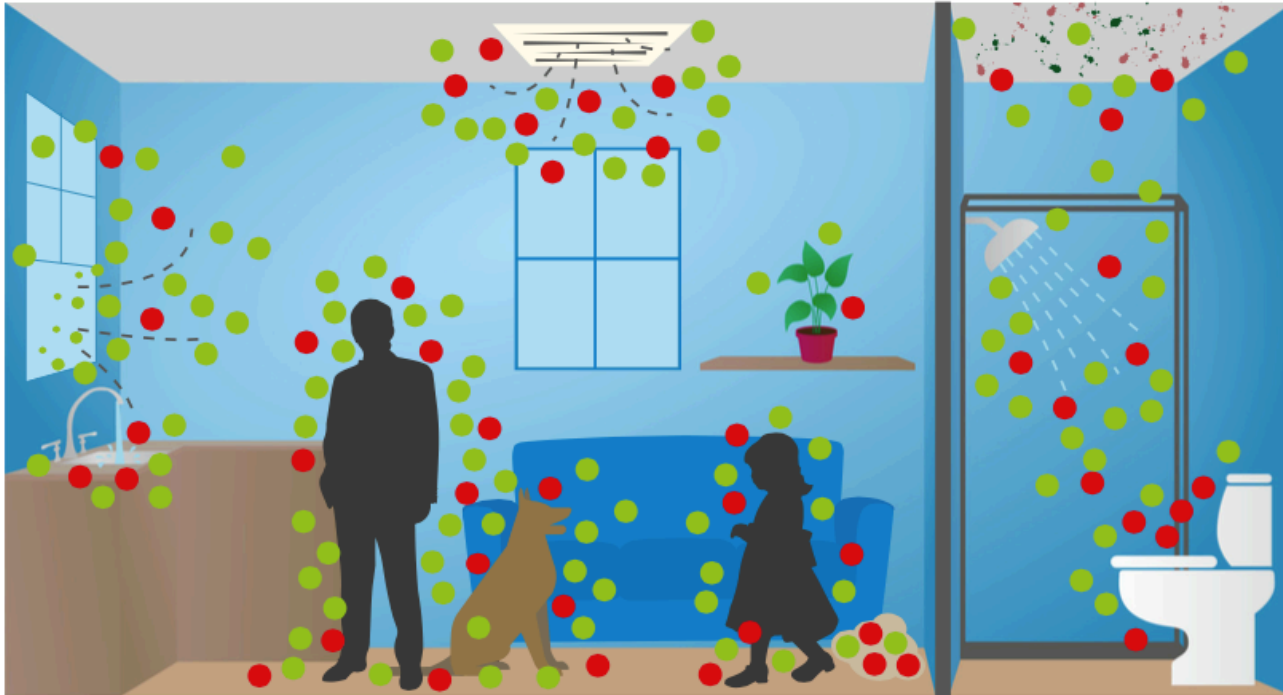
The Built Environment Research Group

advancing energy, environmental, and sustainability
research within the built environment
at Illinois Institute of Technology



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Introduction and motivation



Prussin and Marr **2015** *Microbiome* 3:78

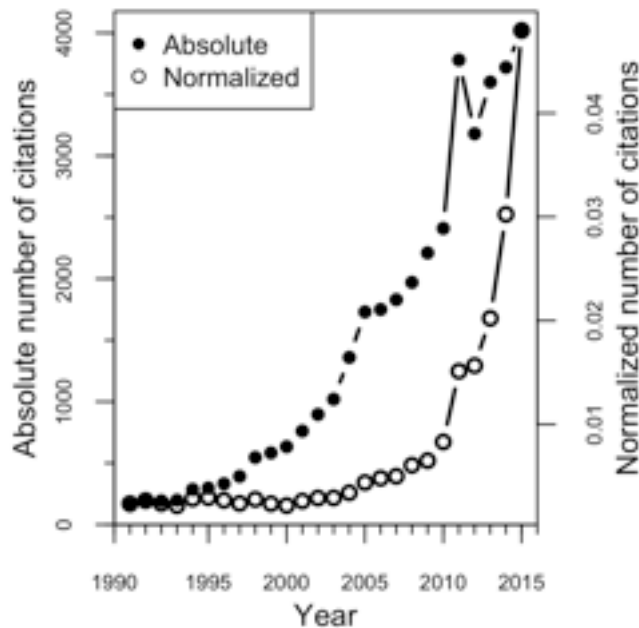
Studying the role of the built environment in exposing humans to specific microbes (e.g., pathogens or allergens) and the role of microbes responsible for the deterioration of building materials has a very rich history

Adams et al. Ten questions concerning the microbiomes of buildings. Accepted to *Building and Environment*

Introduction and motivation

There has been a dramatic increase in the use of high-throughput molecular techniques to analyze microbial communities in indoor environments in the last ~10 years

Google Scholar citations by the keywords: *microbiology* OR *microbiome* OR *bioaerosol* AND *indoor*



Adams et al. Ten Questions concerning the microbiomes of buildings. Accepted to *Building and Environment*

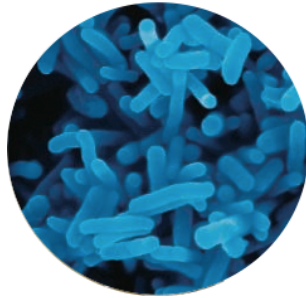
In a recent presentation to the National Academies of Sciences' committee on 'Microbiomes of the Built Environment: From Research to Application', I reviewed recent studies on the microbiology of the built environment (MoBE) and organized their findings into 12 major categories.

I also proposed that:

1. We have added new layers of complexity to our rich existing knowledge from a long history of applying culture-based methods.
2. The practical implications of this added complexity remain somewhat elusive.

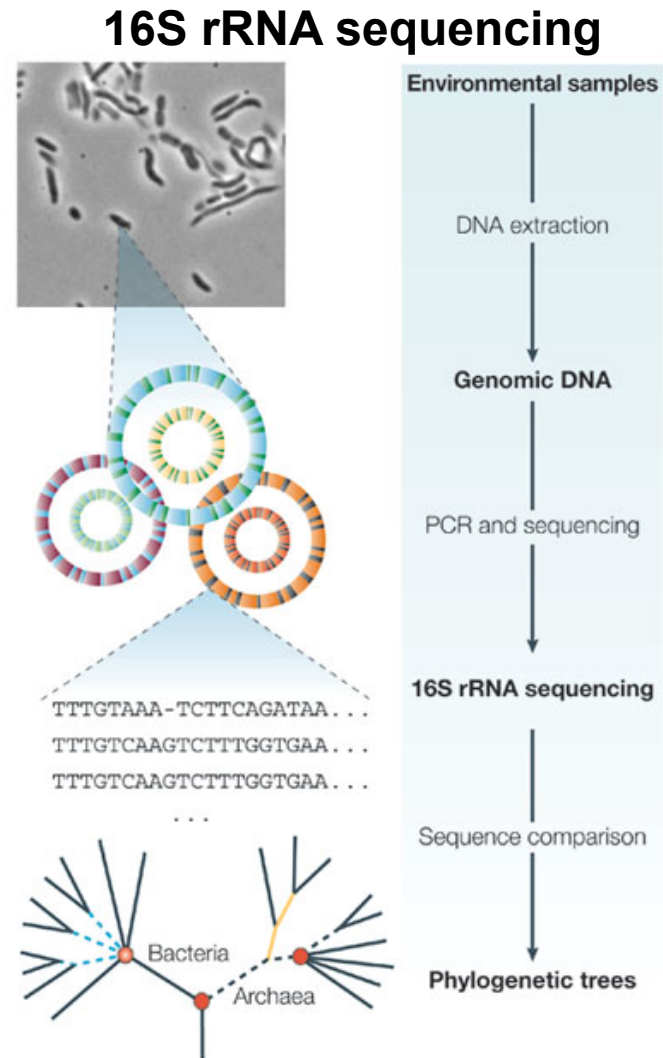
**What have we learned about the
built environment microbiome?**

1. **Culture-independent methods** reveal vastly greater microbial diversity compared to culture-based methods



“The ability to sequence DNA samples from the environment has allowed scientists to **detect far more than the 1% of microbes that can be cultured** in the laboratory. It has also revealed how they vary from place to place.”

Whitfield, J. **2005** *Science* 310:960-961



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Nature Reviews | Genetics

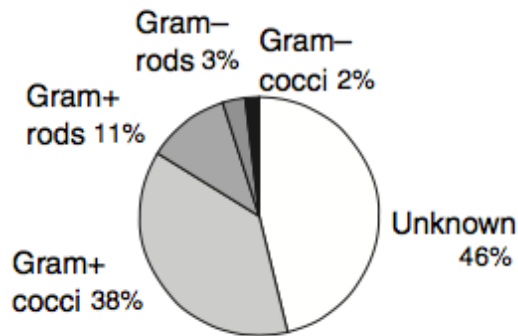
Tringe, S., Rubin, E. **2005** *Nature Rev Gen* 6:805-814

1. Culture-independent methods reveal vastly greater microbial diversity compared to culture-based methods

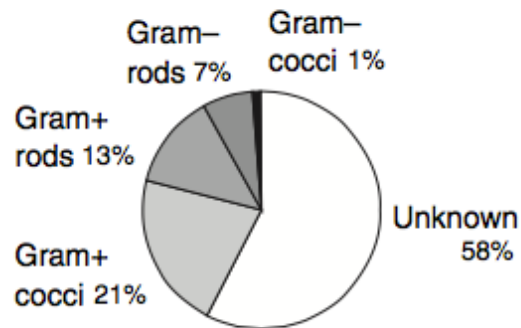
Culture-based methods

BASE study (100 offices)

Indoor air (102 CFU/m³)



Outdoor air (179 CFU/m³)



Chicago residences (20 homes)

Spatial Variation of Viable Bacteria

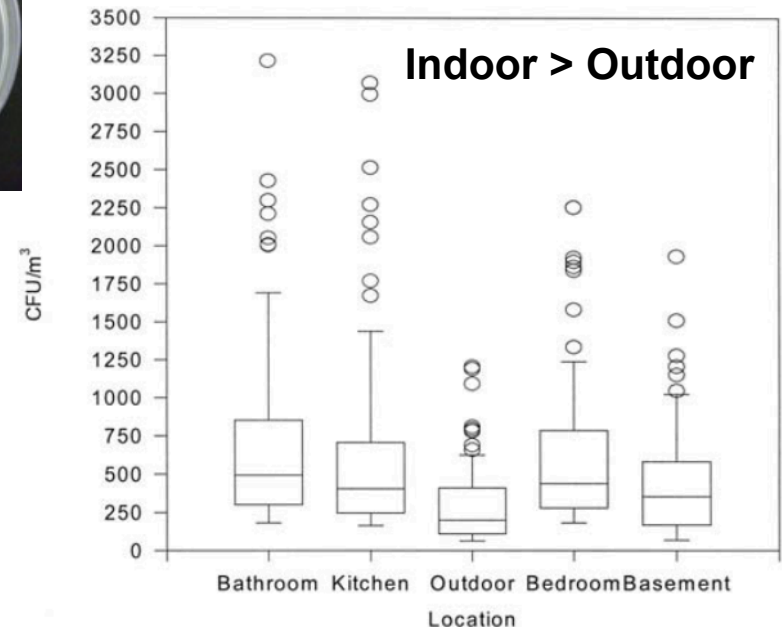
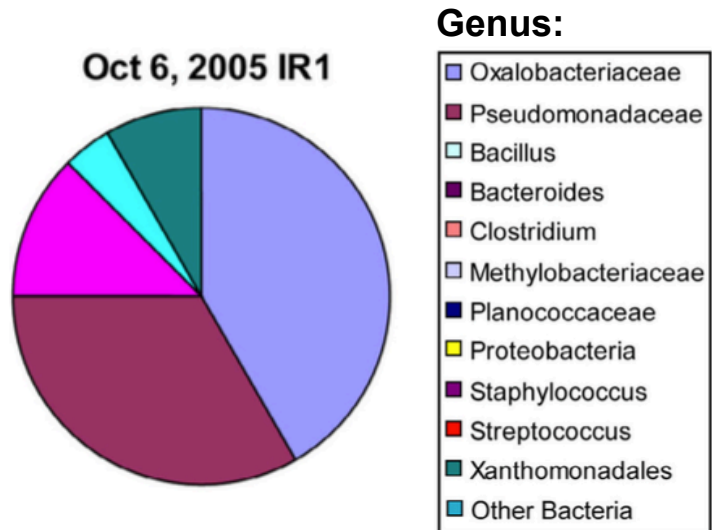


Fig. 1 Composition of culturable bacteria in indoor and outdoor air in 100 large office buildings

Gram-positive bacteria and *Staphylococcus* sp. levels as a percentage of total culturable bacteria

	Bedroom (%)	Bathroom (%)	Kitchen (%)	Basement (%)	Outdoors (%)
Gram-positive bacteria	58	62	75	68	50
<i>Staphylococcus</i>	37	26	30	16	10

1. Culture-independent methods reveal vastly greater microbial diversity compared to culture-based methods

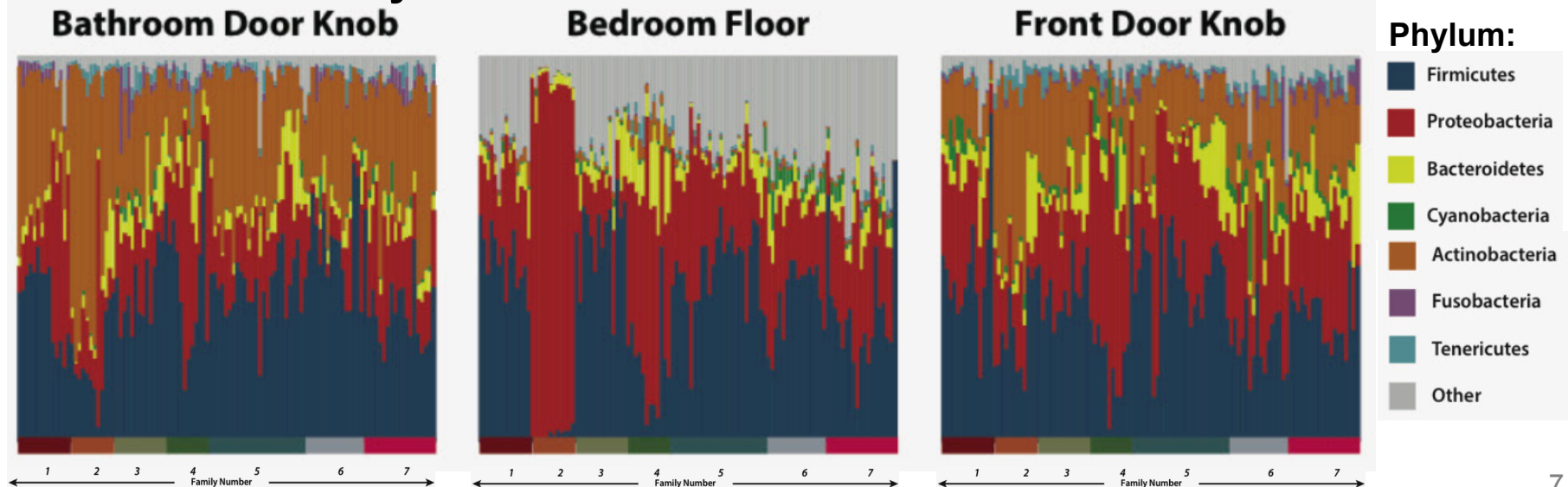


Bacterial diversity in a child-care facility

“Culture-independent methods based on 16S rRNA gene sequencing revealed **an entirely new dimension of microbial diversity**, including an estimated 190 bacterial species from 15 bacterial divisions”

Lee et al. 2007 *BMC Microbiology* 7:27

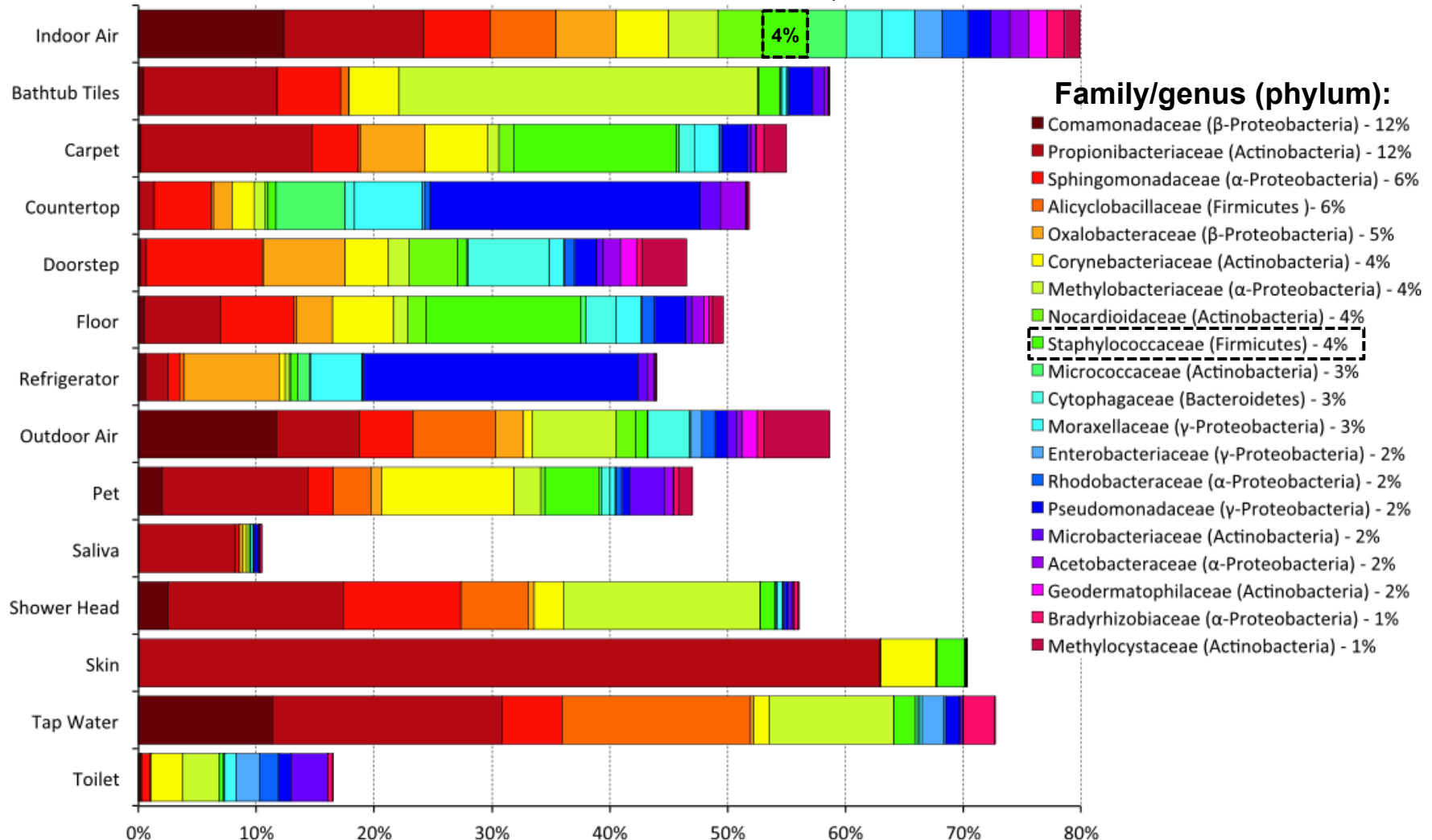
Bacterial diversity in 7 residences



Lax et al. 2014 *Science* 345(6200):1048-1052

1. Culture-independent methods reveal vastly greater microbial diversity compared to culture-based methods

29 homes in San Francisco, CA

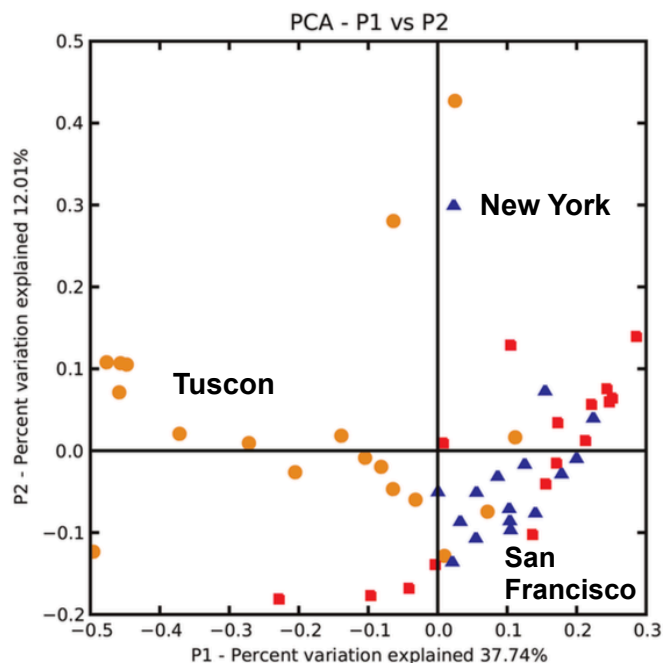


2. Indoor spaces often harbor **unique** microbial communities

Bacterial communities on office surfaces in 3 U.S. cities

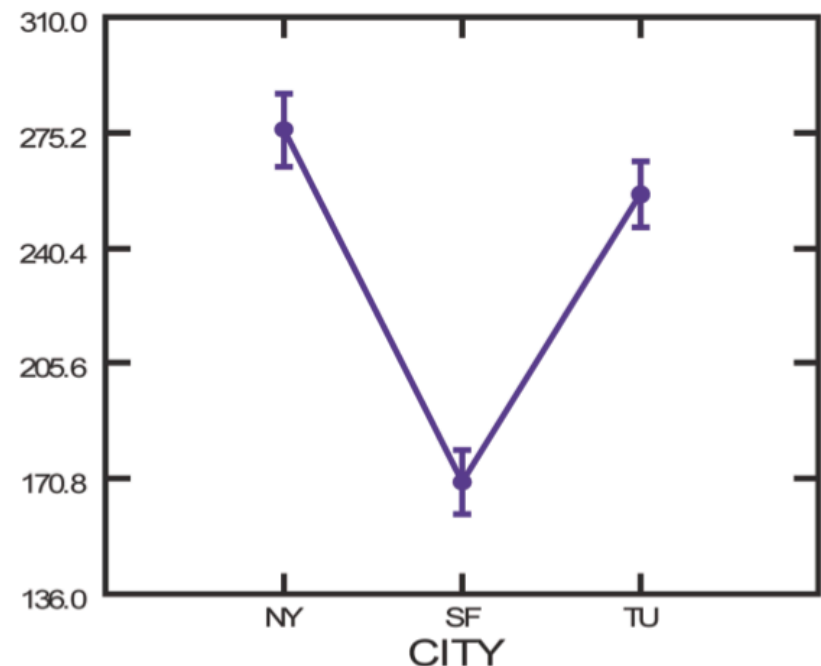
Bacterial diversity

“Bacterial community **diversity** of the Tucson samples was **clearly distinguishable** from that of New York and San Francisco, which were indistinguishable”

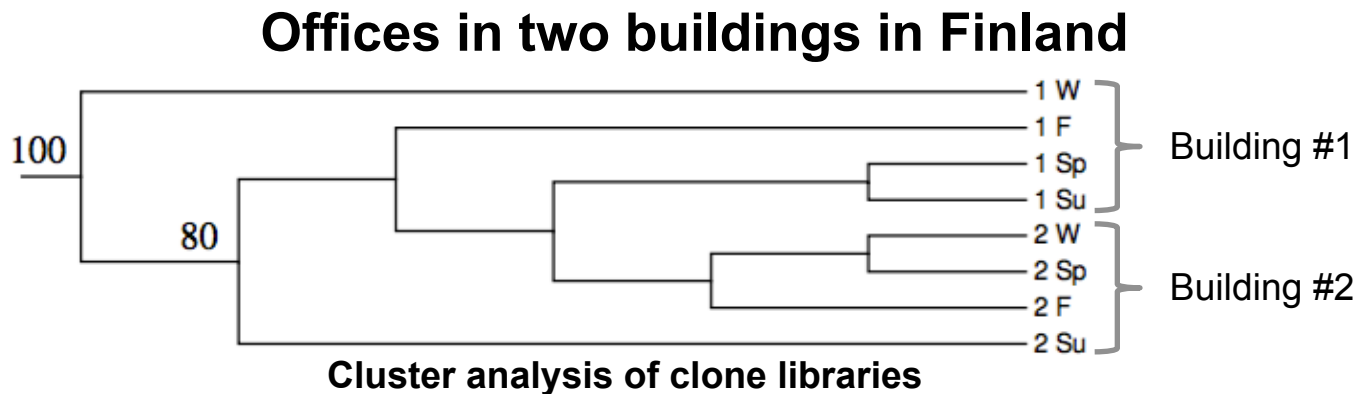


Bacterial abundance

“Multiplex pyrosequencing identified more than **500 bacterial genera from 20 different bacterial divisions**. The most abundant of these genera tended to be common inhabitants of human skin, nasal, oral or intestinal cavities.”



2. Indoor spaces often harbor **unique** microbial communities



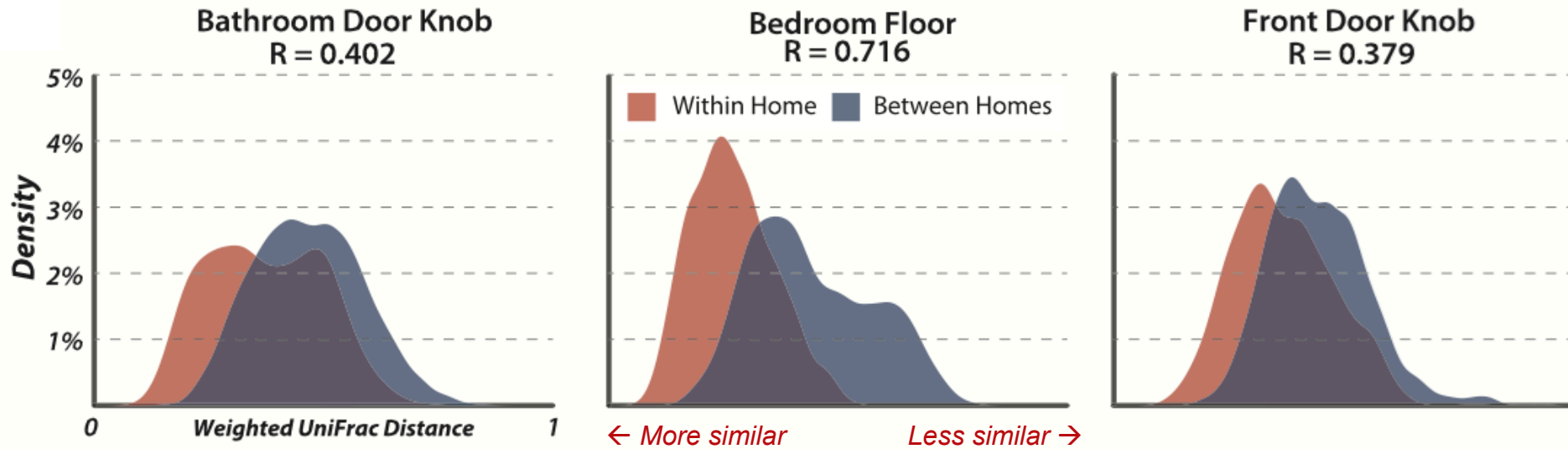
“The composition and dynamics of **indoor dust bacterial flora** were investigated in two buildings over a period of one year”

“Bacterial flora of the two buildings differed during all seasons except spring, but differences between seasons within one building were not that clear, indicating that **differences between the buildings were greater than the differences between seasons**”

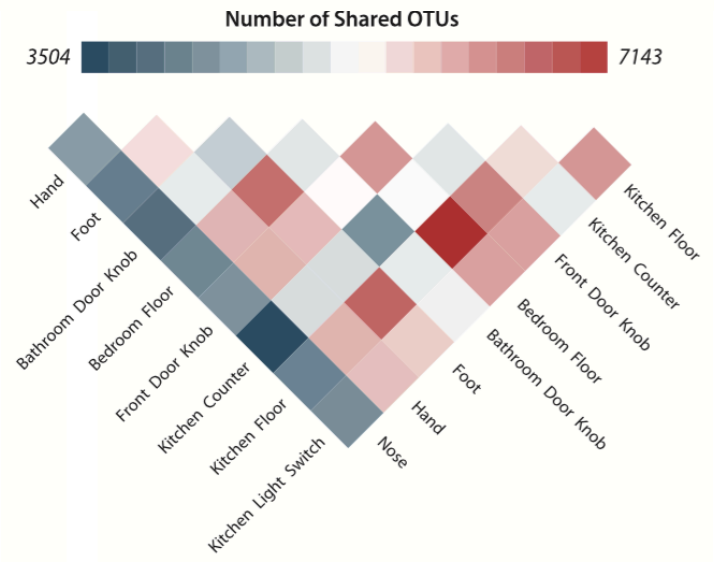
“...the bacterial flora of indoor dust is complex and dominated by Gram-positive species. The dominant phylotypes most probably **originated from users** of the building”

2. Indoor spaces often harbor **unique** microbial communities

7 families and their homes over 6 weeks
(including 3 families that moved)



- Microbial communities **differed substantially** among homes
- Microbiota in each home were **identifiable by family**
- Floors resemble feet and other floors
- Kitchen counters do not resemble noses

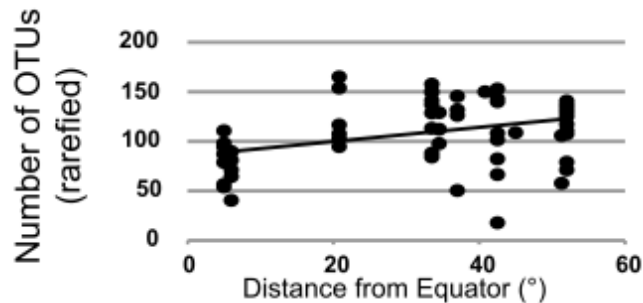


3. Indoor **fungal** communities are largely driven by **outdoor** fungal communities (in non-damp buildings)

Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics

Anthony S. Amend^{a,1}, Keith A. Seifert^b, Robert Samson^c, and Thomas D. Bruns^a

“Contrary to common ecological patterns, we show that **fungal diversity** is significantly **higher in temperate zones** than in the tropics, with distance from the equator being the best predictor of phylogenetic community similarity”



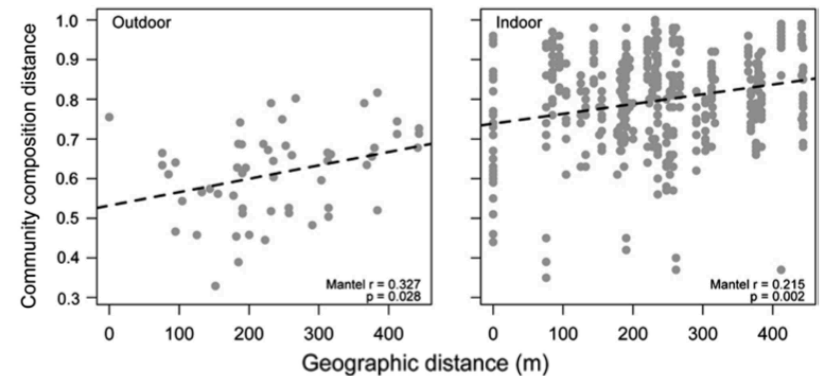
“Remarkably, building function has **no significant effect** on indoor fungal composition, despite stark contrasts between architecture and materials of some buildings in close proximity”

Amend et al. **2010** *PNAS* 107(31):13748

Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances

Rachel I Adams, Marzia Miletto, John W Taylor and Thomas D Bruns
Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

“Fungal assemblages indoors were diverse and **strongly determined by dispersal from outdoors**, and no fungal taxa were found as indicators of indoor air”



“More **fungal biomass** was detected **outdoors** than indoors”

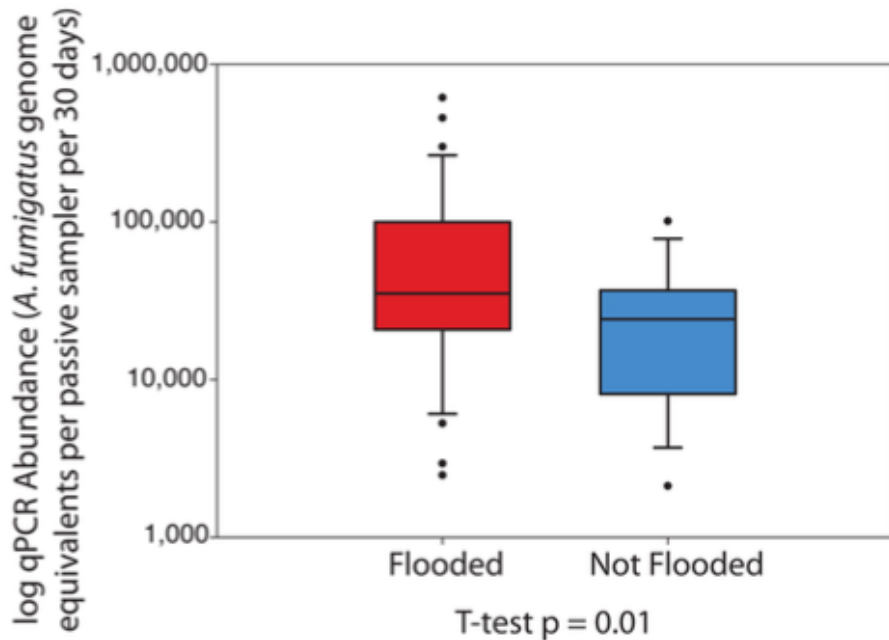
“Room and occupant behavior had **no detectable effect** on the fungi found in indoor air”

Adams et al. **2013** *ISME J* 1:1-12

4. Indoor **fungal** communities in **damp buildings** are often distinct from those in non-damp buildings

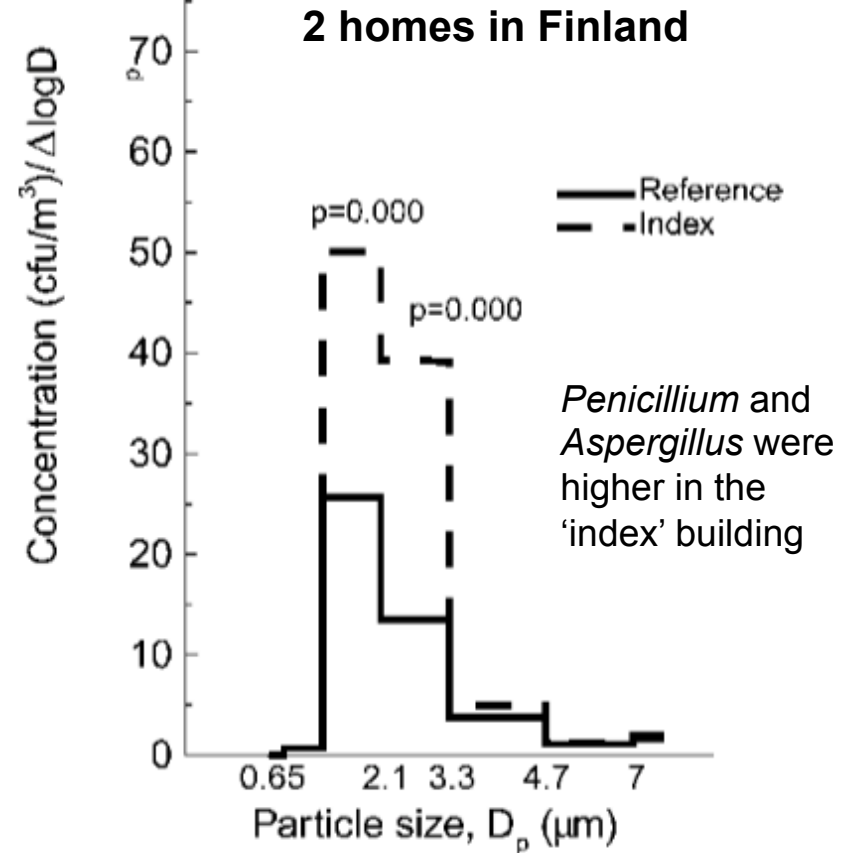
36 flood-damaged and 14 non-flooded homes in Boulder, CO

B. Fungal abundances



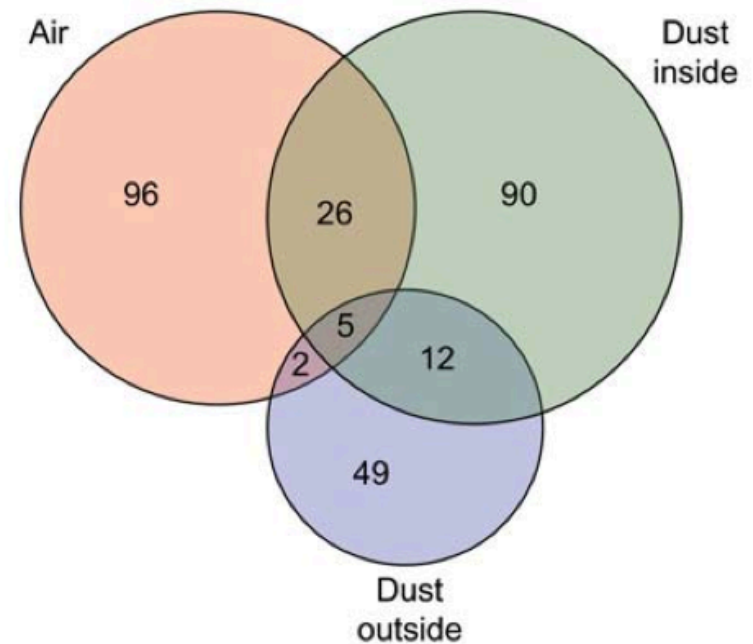
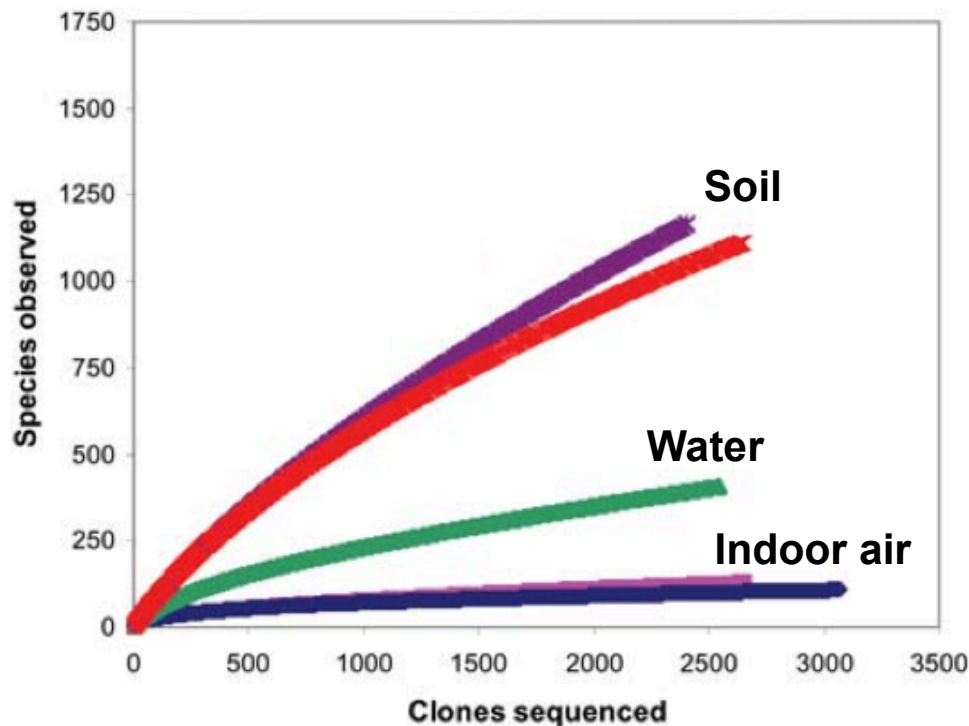
- Fungal abundances were estimated to be 3x higher in flooded homes
- Penicillium* were the most abundant taxa

Size distribution of viable fungi
Two residences



5. Indoor bacteria **often originate from indoor sources**

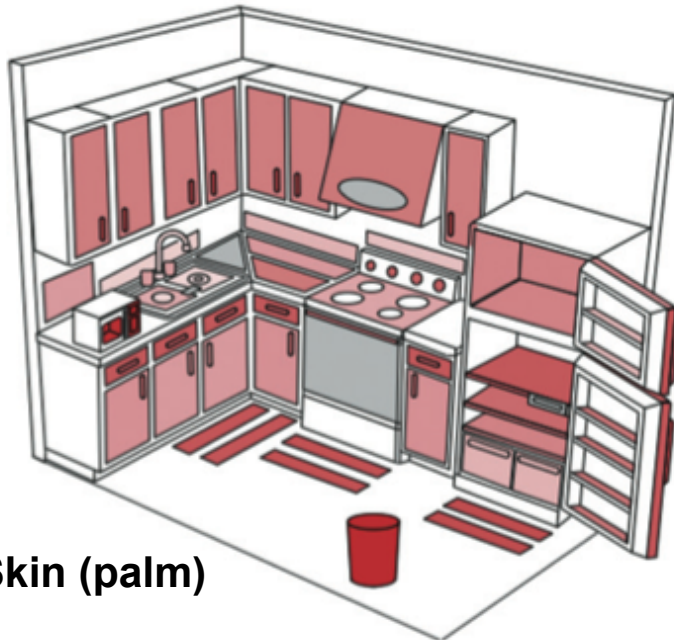
“Comparison of air samples with each other and nearby environments suggested that the **indoor air microbes** are not random transients from surrounding outdoor environments, but rather **originate from indoor niches**.”



6. Source tracking techniques demonstrate that **humans and pets often dominate** bacterial communities on indoor surfaces

Diversity, distribution and sources of bacteria in residential kitchens

“**Human skin** was the **primary source** of **bacteria** across all kitchen surfaces, with contributions from food and faucet water dominating in a few specific locations”



Skin (palm)

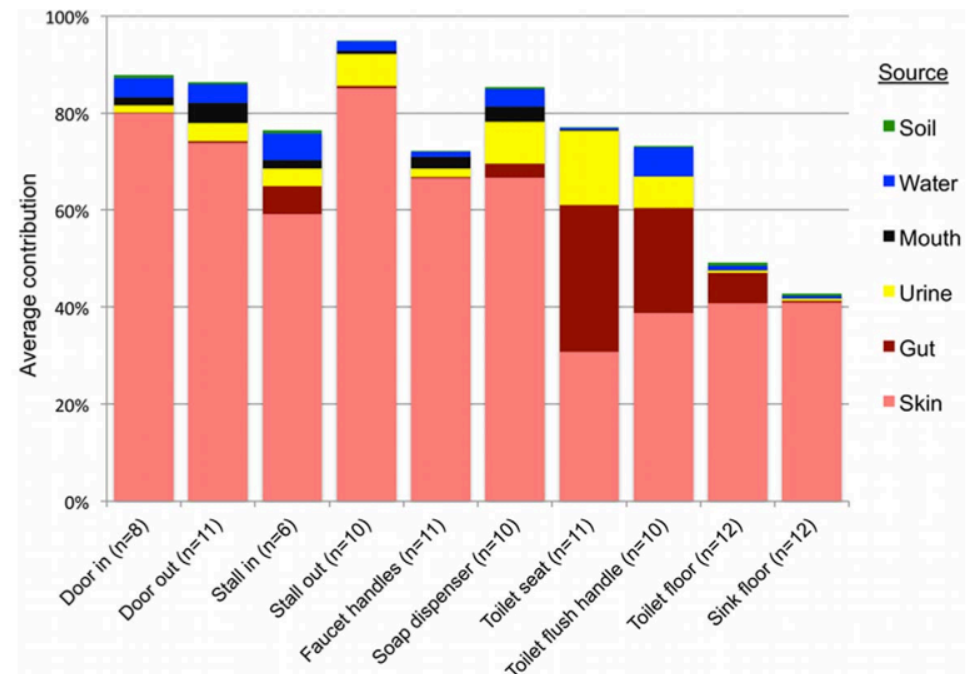
Flores et al. **2013** *Environ Microbio* 15:588-596

Microbial Biogeography of Public Restroom Surfaces

Gilberto E. Flores¹, Scott T. Bates¹, Dan Knights², Christian L. Lauber¹, Jesse Stombaugh³, Rob Knight^{3,4}, Noah Fierer^{1,5*}

“**Human-associated microbes** are commonly found on restroom surfaces”

“Bacterial pathogens could readily be transmitted between individuals by the touching of surfaces”



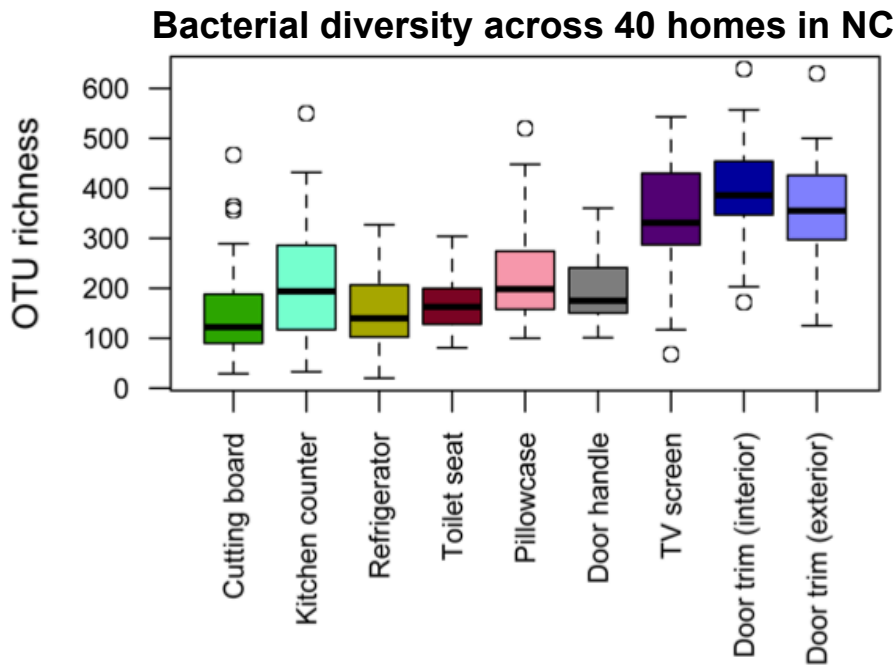
Flores et al. **2011** *PLoS ONE* 6(11):e28132

6. Source tracking techniques demonstrate that **humans and pets often dominate** bacterial communities on indoor surfaces

Home Life: Factors Structuring the Bacterial Diversity Found within and between Homes

Robert R. Dunn^{1,3}, Noah Fierer^{2,3*}, Jessica B. Henley^{2,3}, Jonathan W. Leff^{2,3}, Holly L. Menninger^{1,3}

- Specific locations were distinct
- Presence of **dogs** → greater diversity
- Correlations between I and O communities

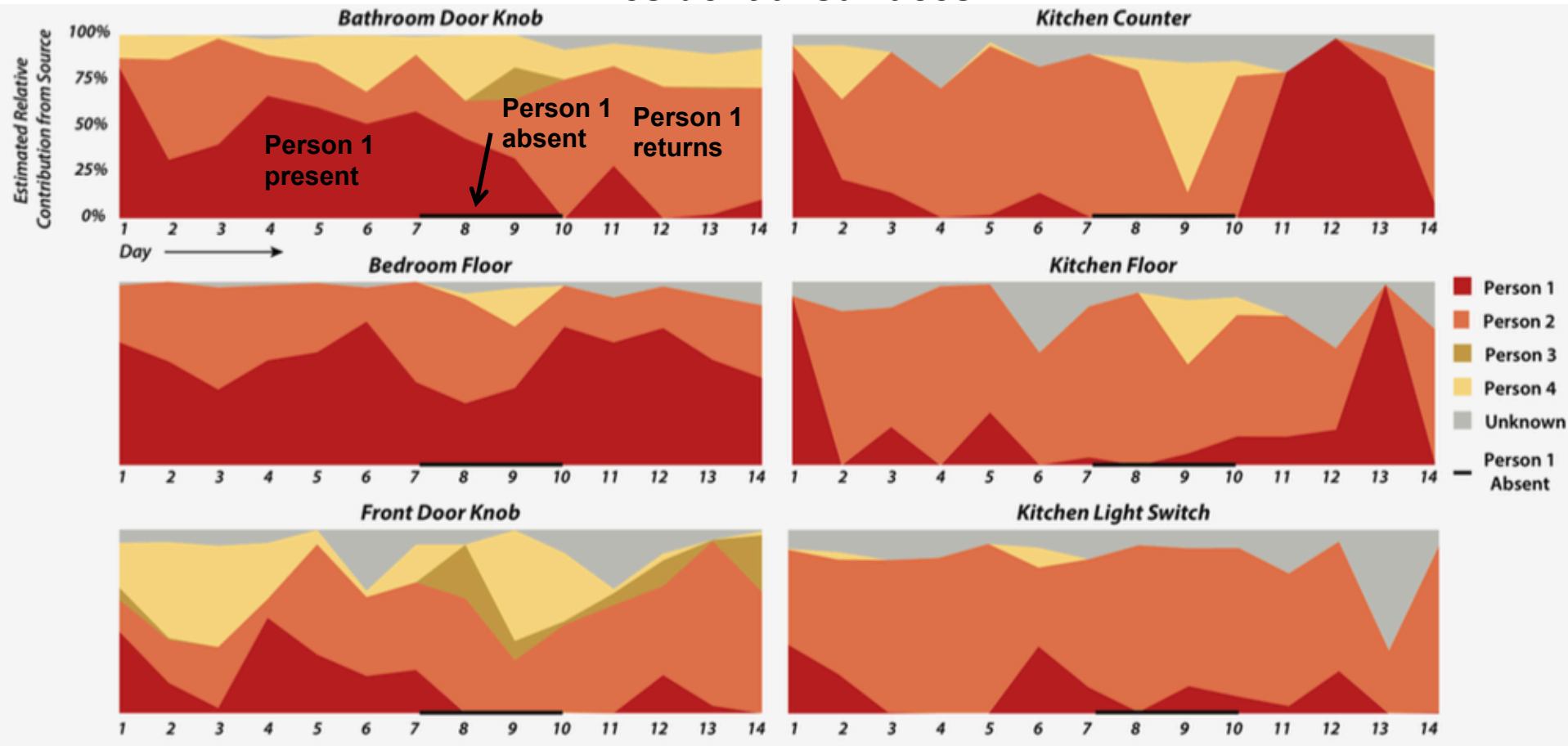


	Human skin	Human oral cavity	Human stool	Leaves	Soil
Cutting board	0.6	0.7	0.1	1.2	0.6
Kitchen counter	2.5	2.4	0.4	1.8	1.2
Refrigerator	2.0	1.3	0.2	1.1	0.7
Toilet seat	17.2	7.9	5.7	0.1	0.3
Pillowcase	9.3	24.2	1.3	0.6	0.7
Door handle	5.7	9.0	0.7	1.2	2.5
Television	7.9	6.4	1.9	1.7	3.3
Door trim (interior)	4.8	2.3	1.7	3.3	4.8
Door trim (exterior)	0.7	0.2	0.2	3.7	7.0

Figure 4. Source tracking analysis showing relative proportion of bacteria at each sampling site associated with given sources.

6. Source tracking techniques demonstrate that **humans and pets often dominate** bacterial communities on indoor surfaces

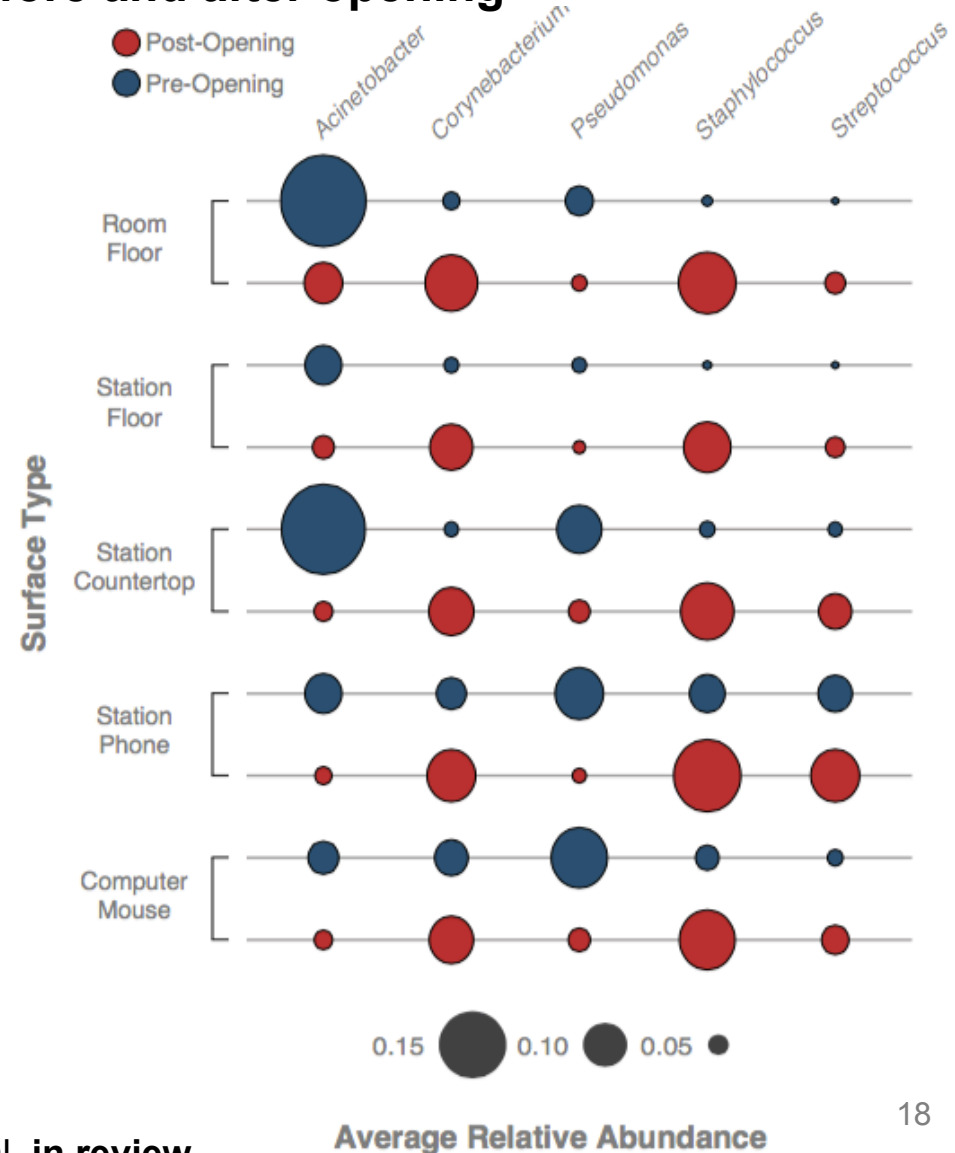
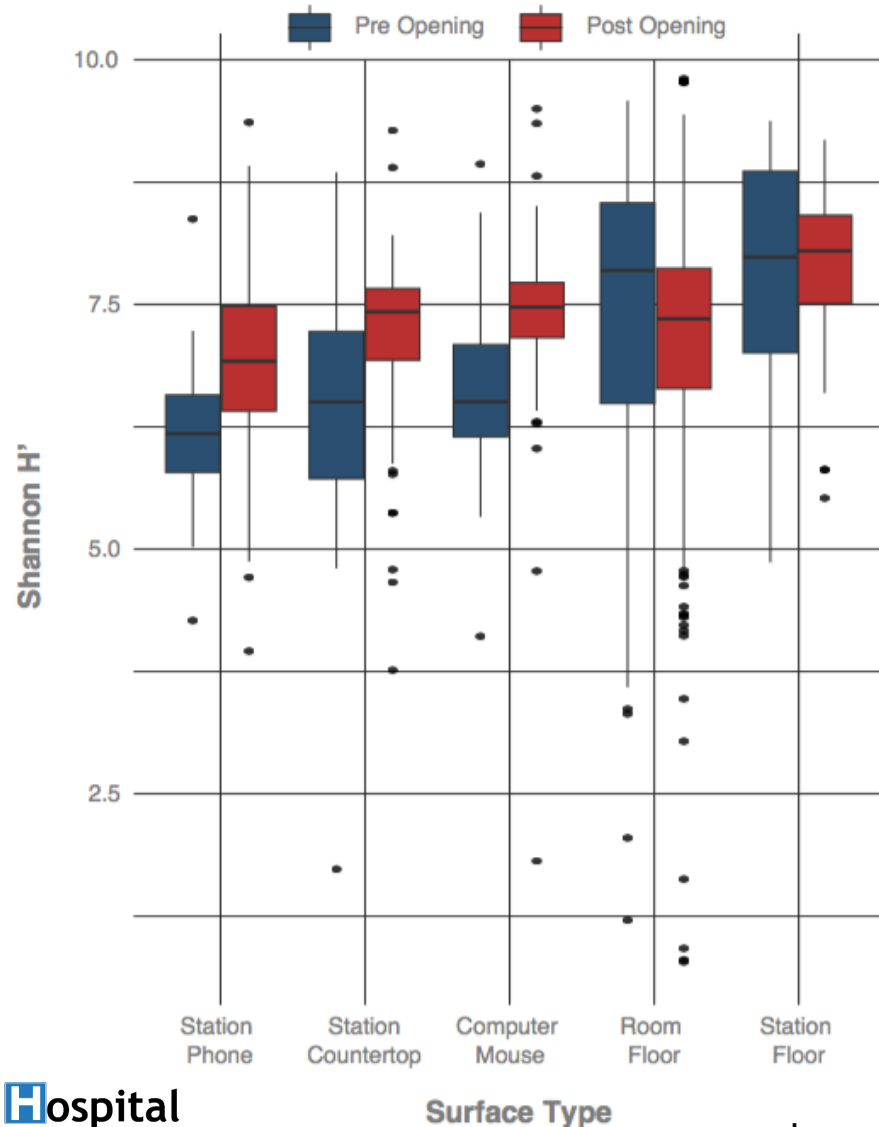
Residential surfaces



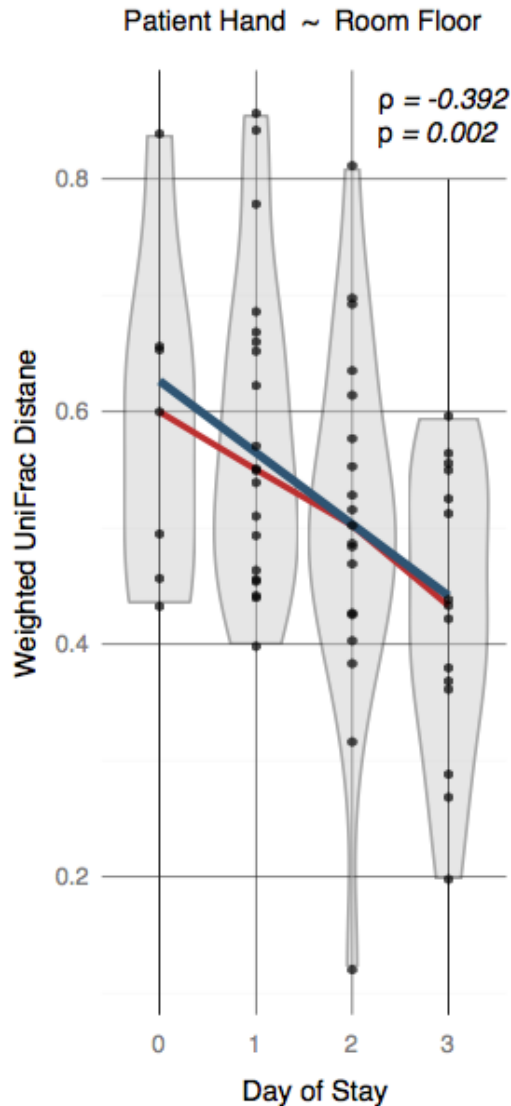
Also, after a move to a **new house**, the microbial community in the new house **rapidly converges** on the microbial community of the occupants former house

6. Source tracking techniques demonstrate that **humans and pets often dominate** bacterial communities on indoor surfaces

Hospital surfaces before and after opening

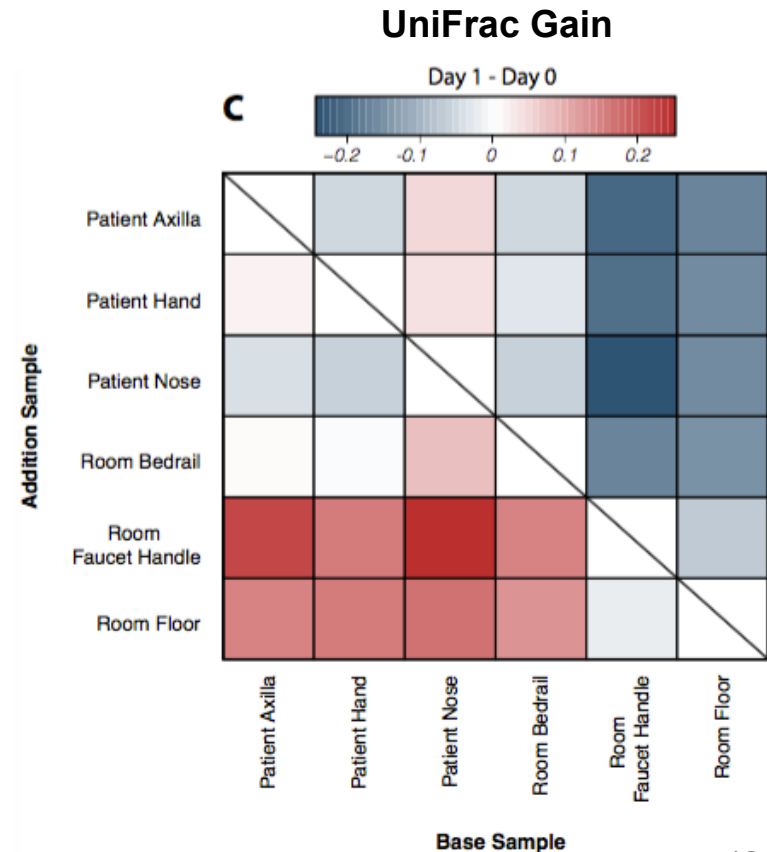


7. Occupants and surfaces interact in **both directions**



“...taxa shared with the skin of the current patient are more abundant on room surfaces **after** the patient has spent a night in the room, while taxa shared with room surfaces are more abundant on patient skin when a patient **first enters** the room”

“This asymmetry may suggest that **patients initially pick up room-associated taxa that predate their stay**, but that their own microbial signatures begin to influence the room with time.”



8. Humans are also major sources of bacteria to indoor air

Human Occupancy as a Source of Indoor Airborne Bacteria

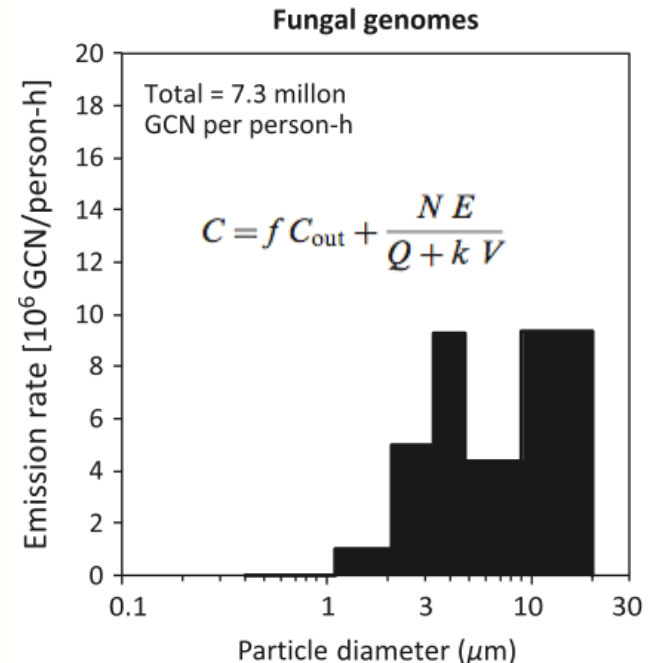
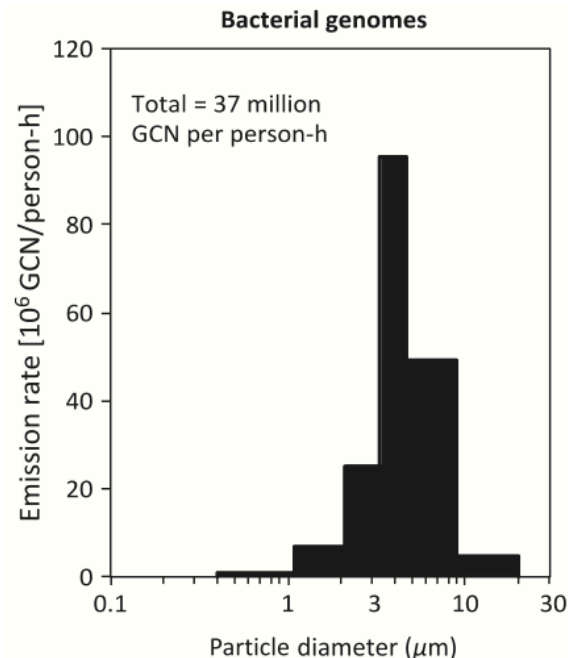
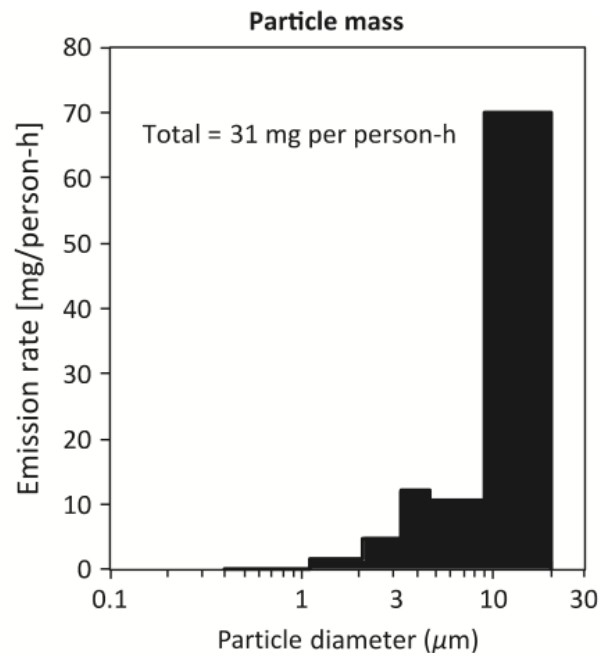
Denina Hospodsky¹, Jing Qian^{1na}, William W. Nazaroff², Naomichi Yamamoto^{1,3}, Kyle Bibby¹, Hamid Rismani-Yazdi^{1nb}, Jordan Peccia^{1*}

Hospodsky et al. **2012** *PLoS ONE* 7(4):e34867

“Occupancy increased the total aerosol mass and bacterial genome concentration in indoor air... with an increase of nearly two orders of magnitude in airborne bacterial genome concentration in PM₁₀”

Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom

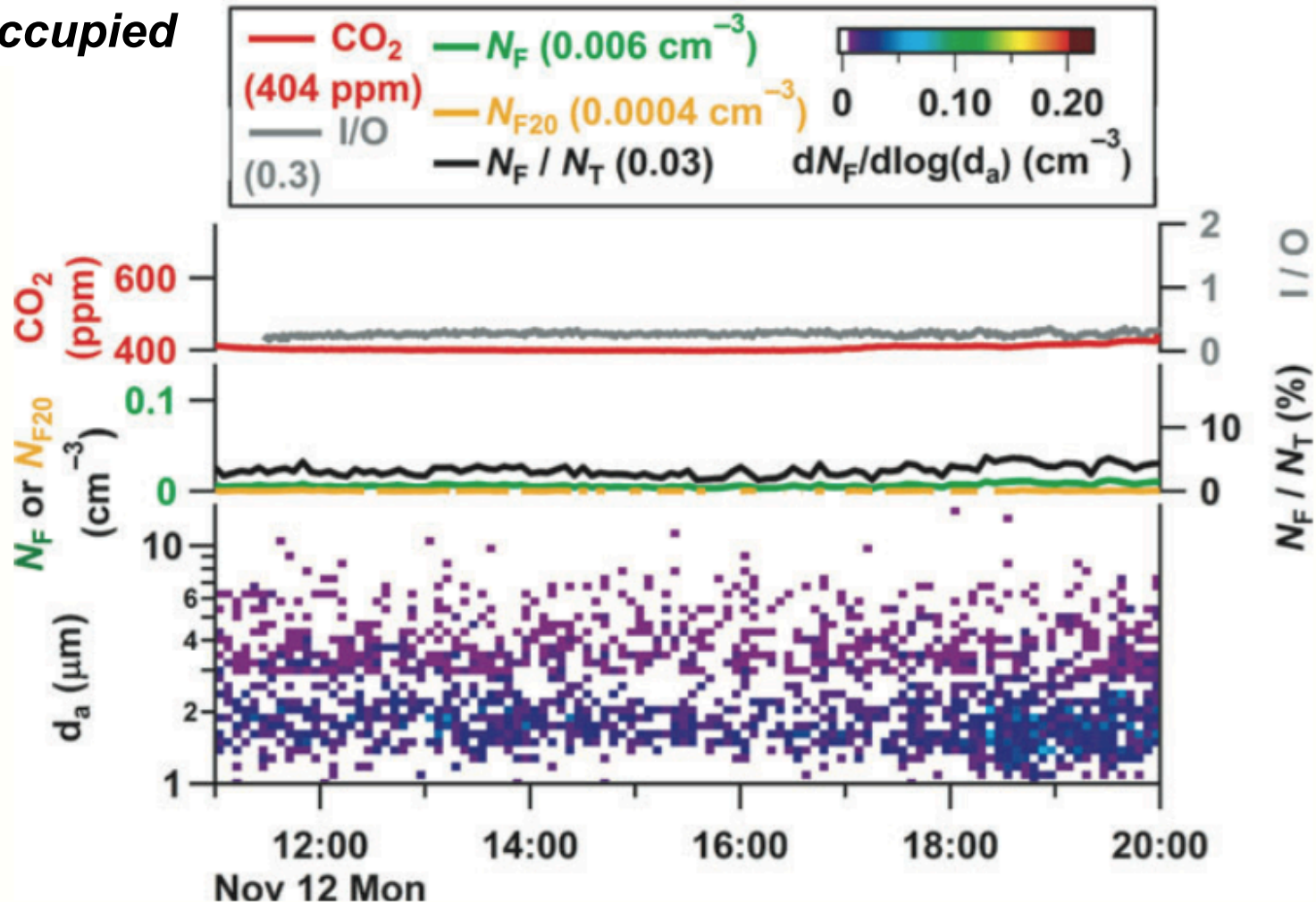
Qian et al. **2012** *Indoor Air* 22:339-351



8. Humans are also major sources of bacteria to indoor air

Classroom fluorescent bioaerosol study

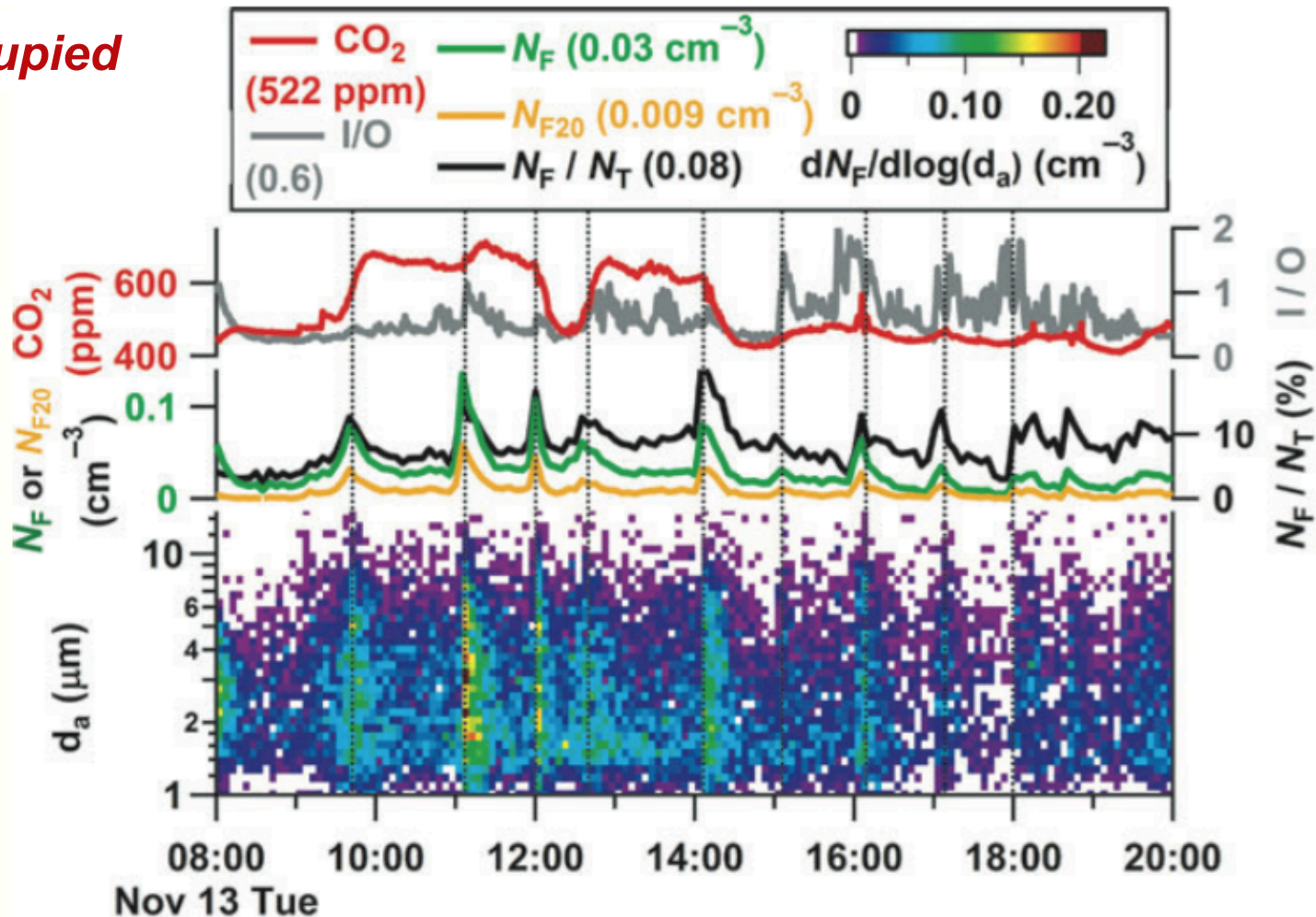
Unoccupied



8. Humans are also major sources of bacteria to indoor air

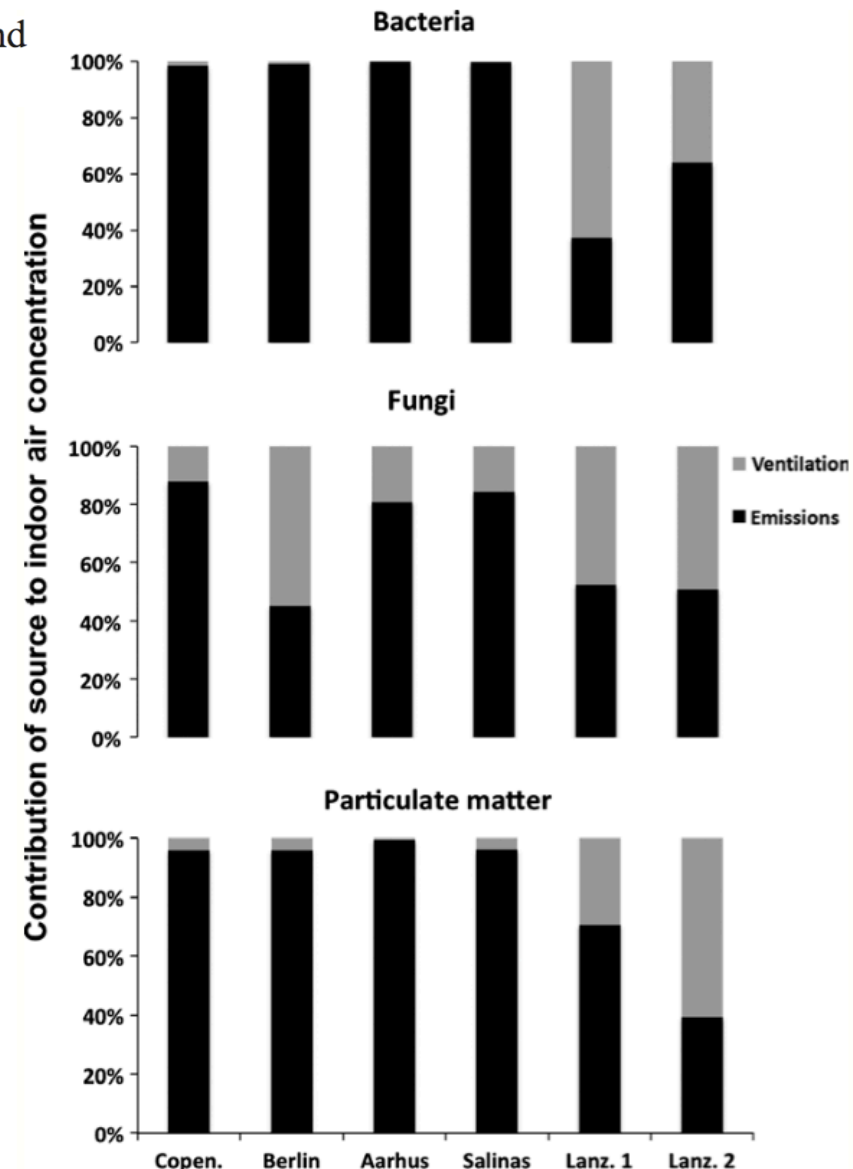
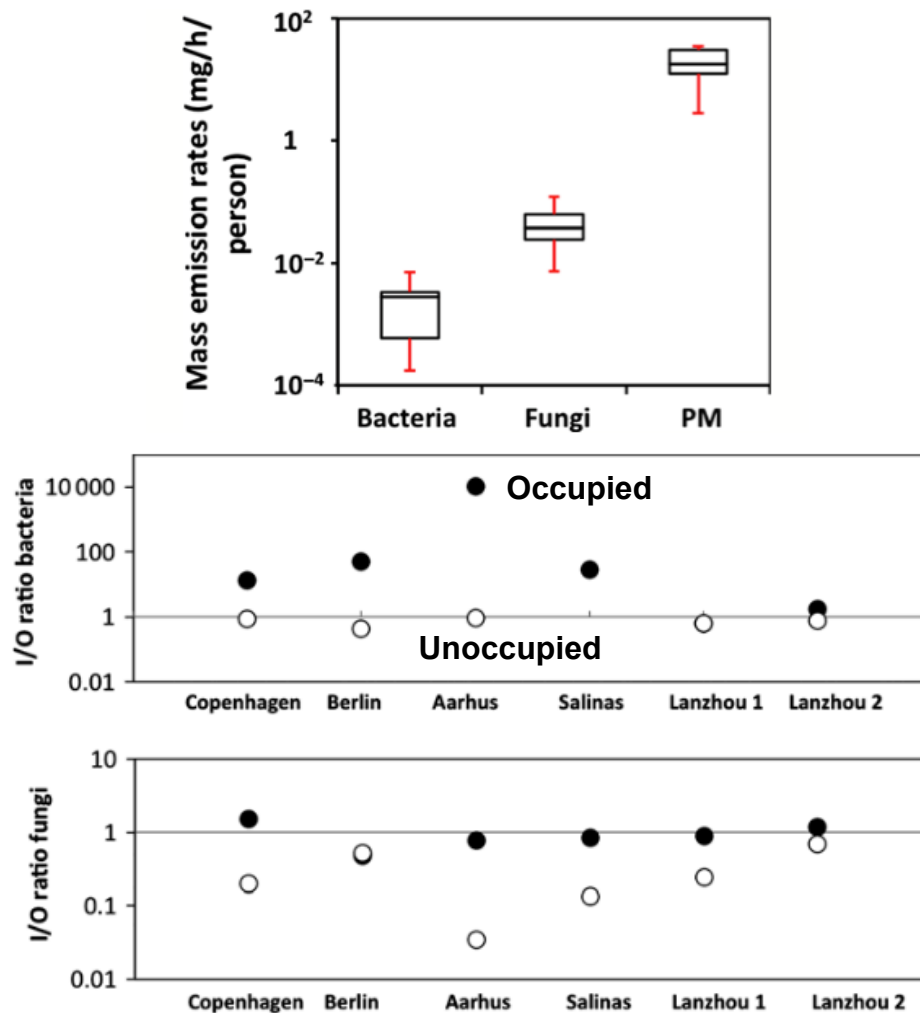
Classroom fluorescent bioaerosol study

Occupied



8. Humans are also major sources of bacteria to indoor air

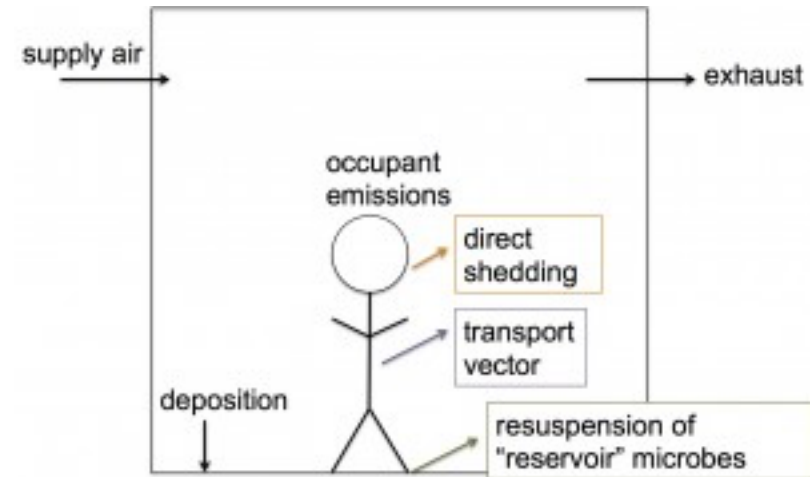
Characterizing airborne fungal and bacterial concentrations and emission rates in six occupied children's classrooms



9. Controlled studies can elucidate the **mechanisms** of human microbial emissions

Mechanisms of human emissions:

1. Direct shedding
2. Resuspension of settled particles
3. Direct surface contact



Courtesy of Rachel Adams

9. Controlled studies can elucidate the **mechanisms** of human microbial emissions

Mechanisms of human emissions:

1. Direct shedding
2. Resuspension of settled particles
3. Direct surface contact

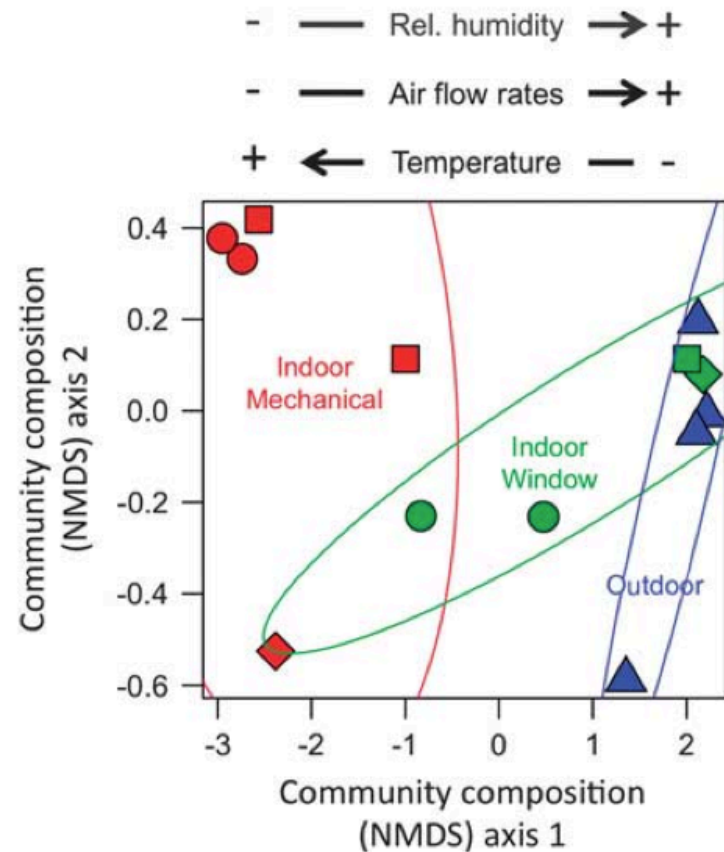
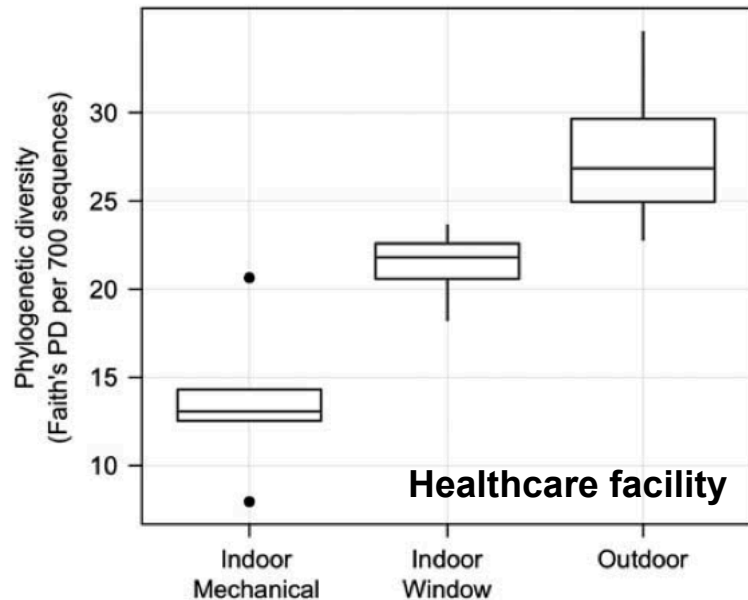


- Seated, simulated office work conditions: **$\sim 10^6$ particles per hour per person**
 - Fluorescence (UV-APS)
- **Walking increased this 5-6x**
 - Mostly attributable to resuspension
 - And some additional direct shedding (arm movements)
- During both walking and sitting, **more than 65%** of the emissions originated from the floor
 - **Resuspension was dominant**
- Dominant particle size: $\sim 3-5 \mu\text{m}$

10. Building **design and operation** can influence indoor microbial communities

Architectural design influences the diversity and structure of the built environment microbiome

Steven W Kembel¹, Evan Jones¹, Jeff Kline^{1,2}, Dale Northcutt^{1,2}, Jason Stenson^{1,2}, Ann M Womack¹, Brendan JM Bohannan¹, G Z Brown^{1,2} and Jessica L Green^{1,3}

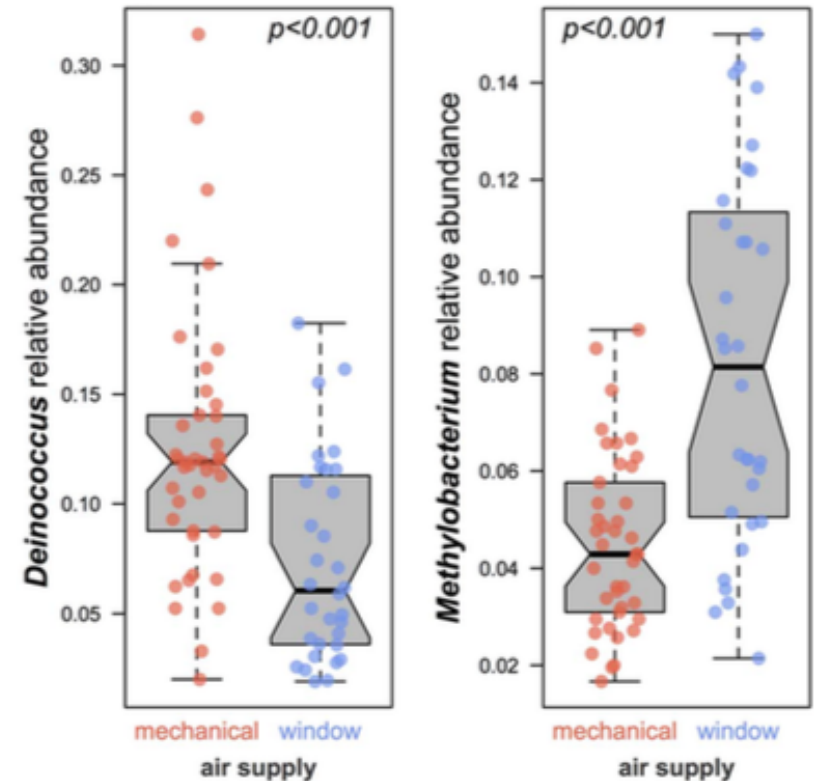
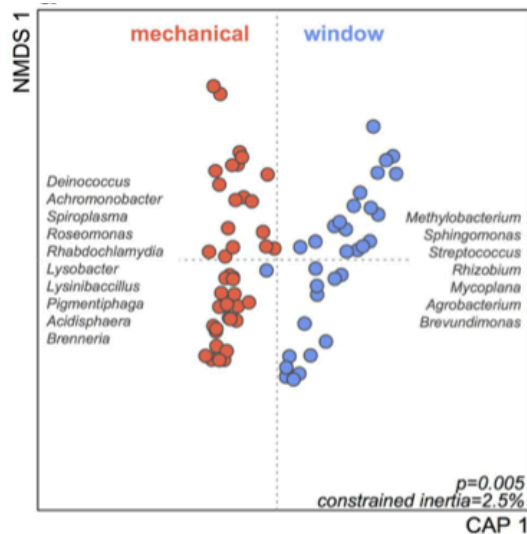
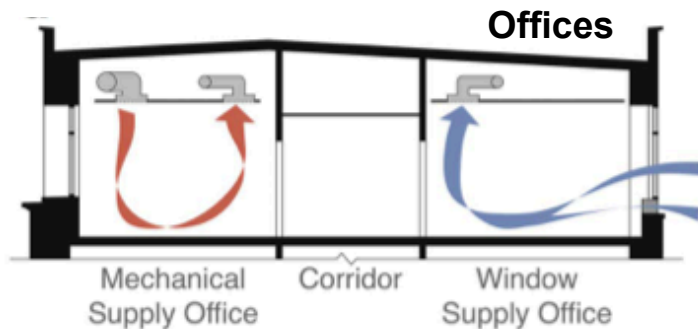


- Bacterial diversity: IA < OA
- Rooms w/ HVAC were less diverse than open window rooms
- **Source of ventilation air** and **T/RH** correlated w/ composition of IA bacteria

10. Building **design and operation** can influence indoor microbial communities

Architectural Design Drives the Biogeography of Indoor Bacterial Communities

Steven W. Kembel^{1,2,3,9}, James F. Meadow^{2,3,9}, Timothy K. O'Connor^{2,3,4}, Gwynne Mhuireach^{2,5}, Dale Northcutt^{2,5}, Jeff Kline^{2,5}, Maxwell Moriyama^{2,5}, G. Z. Brown^{2,5,6}, Brendan J. M. Bohannon^{2,3}, Jessica L. Green^{2,3,7}



“Within offices, the **source of ventilation air** had the greatest effect on bacterial community structure”

- Even if absolute abundance remains similar

10. Building **design and operation** can influence indoor microbial communities

Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children

“Increased microbial **richness** was associated with the presence of pets, **water leaks**, longer AC use, suburban (vs. urban) homes, and dust composition measures”

“The most significant differences in community composition were observed for **AC use and occupancy** (people, children, and pets) characteristics”

“Occupant density measures were associated with **beneficial bacterial taxa**, including *Lactobacillus johnsonii*”

Table 1 Richness analysis of housing characteristics for fungi and bacteria. Associations with $P < 0.05$ are in bold

Category	<i>n</i> (yes)	Mean number of fungal OTUs			Mean number of bacterial OTUs		
		Yes	No	<i>P</i> -value	Yes	No	<i>P</i> -value
More than 5 people in home	49	95.7	91.8	0.59	735	735	0.91
More than 3 children in home	87	96.6	91.8	0.54	701	743	0.26
Urban home (vs. Suburban)	112	90.1	96.2	0.33	707	772	0.031
Single family (vs. Multifamily)	94	92.8	92.6	0.98	755	717	0.22
Mold	85	94.0	91.8	0.73	738	728	0.75
Water leaks	80	102	86.6	0.017	729	740	0.73
AC use (yes or no)	178	92.6	94.4	0.86	737	712	0.63
AC use (more than 2 months) ^a	105	86.6	102.4	0.021	730	757	0.40
Pets	85	101	86.2	0.015	772	704	0.024

‘Yes’ and ‘no’ refer to the category, that is, for pets, ‘yes’ homes had pets and ‘no’ homes did not have pets.

^aExcludes homes with no AC use.

10. Building **design and operation** can influence indoor microbial communities

RESEARCH ARTICLE

Fungal and Bacterial Communities in Indoor Dust Follow Different Environmental Determinants

Fabian Weigl^{1*}, Christina Tischer^{2,3}, Alexander J. Probst⁴, Joachim Heinrich^{2,5}, Iana Markevych^{2,6}, Susanne Jochner⁷, Karin Pritsch¹

Fungal communities in house dust (286 homes) impacted by:

- Surrounding greenness
- Outdoor PM concentrations
- Age of building
- Window opening behavior

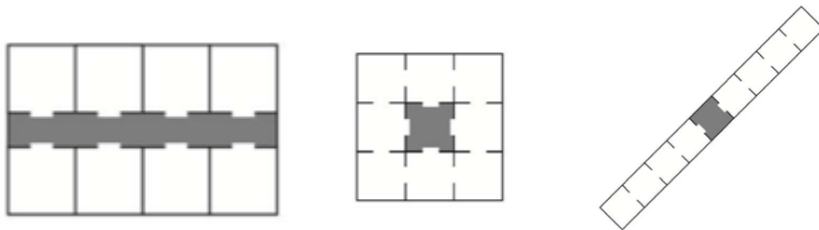
Table 1. Significance of associations between environmental determinants and microbial community variation (based on Bray—Curtis dissimilarities).

Environmental characteristics	Fungi		Bacteria	
	<i>P</i> ^a	δ ^b	<i>P</i> ^c	δ ^d
Indoor characteristics				
N° of rooms within the flat	0.75	0.87	0.71	0.79
N° of occupants in the flat	0.85	0.79	0.36	0.28
Dampness	0.69	0.29	0.16	0.39
Mould at home	0.04	0.03	0.09	0.13
Water leakage	0.81	0.85	0.57	0.62
Tightness of the windows ^e	0.03	0.04	0.36	0.36
Ventilation living room through windows—summer	0.27	0.24	0.71	0.93
Ventilation living room through windows—winter	0.67	0.64	0.05	0.05
Heating within the home	0.03	0.02	0.36	0.41
Renovation measures last 12 months	0.44	0.61	0.65	0.65
Pets	0.27	0.28	0.62	0.75
Type of living room floor	< 0.001	< 0.001	0.08	0.02
Smoking of tobacco in the flat	0.42	0.41	0.71	0.78
Outdoor characteristics				
Age of the building	0.01	0.01	0.28	0.31
Position of the home	0.49	0.67	0.1	0.05
Building density of the neighbourhood	0.52	0.59	0.39	0.44
Traffic jams in rush hour	0.83	0.83	0.29	0.24
Facility with noticeable air pollution within 50 and 100 m	0.58	0.72	0.45	0.40
Facility with noticeable air pollution within 50 m	0.33	0.28	0.85	0.94
Surrounding greenness (500 m buffer)	0.72	0.63	0.84	0.94
Surrounding greenness (100 m buffer)	0.05	0.006	0.19	0.22
Surrounding greenness (30 m buffer)	0.06	0.01	0.33	0.30
Urban index	0.02	0.01	0.51	0.60
NO ₂	0.23	0.06	0.63	0.75
NO _x	0.06	0.03	0.37	0.41
PM _{2.5}	0.004	0.005	0.51	0.44
PM ₁₀	0.54	0.32	0.82	0.70
PM _{coarse}	0.04	0.008	0.41	0.46
PM _{2.5} absorbance	0.07	0.06	0.37	0.42

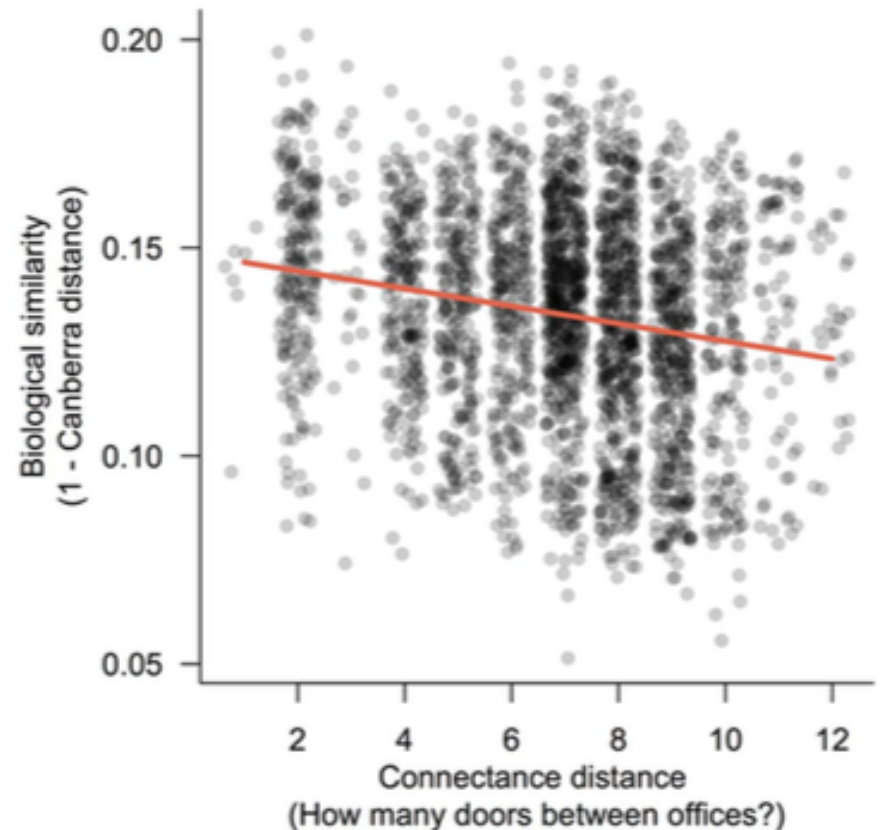
10. Building **design and operation** can influence indoor microbial communities

Architectural Design Drives the Biogeography of Indoor Bacterial Communities

Steven W. Kembel^{1,2,3,9}, James F. Meadow^{2,3,9}, Timothy K. O'Connor^{2,3,4}, Gwynne Mhuireach^{2,5}, Dale Northcutt^{2,5}, Jeff Kline^{2,5}, Maxwell Moriyama^{2,5}, G. Z. Brown^{2,5,6}, Brendan J. M. Bohannon^{2,3}, Jessica L. Green^{2,3,7}



“Spaces with high human occupant diversity and a high degree of **connectedness** to other spaces via ventilation or human movement contained a distinct set of bacterial taxa when compared to spaces with low occupant diversity and low connectedness”

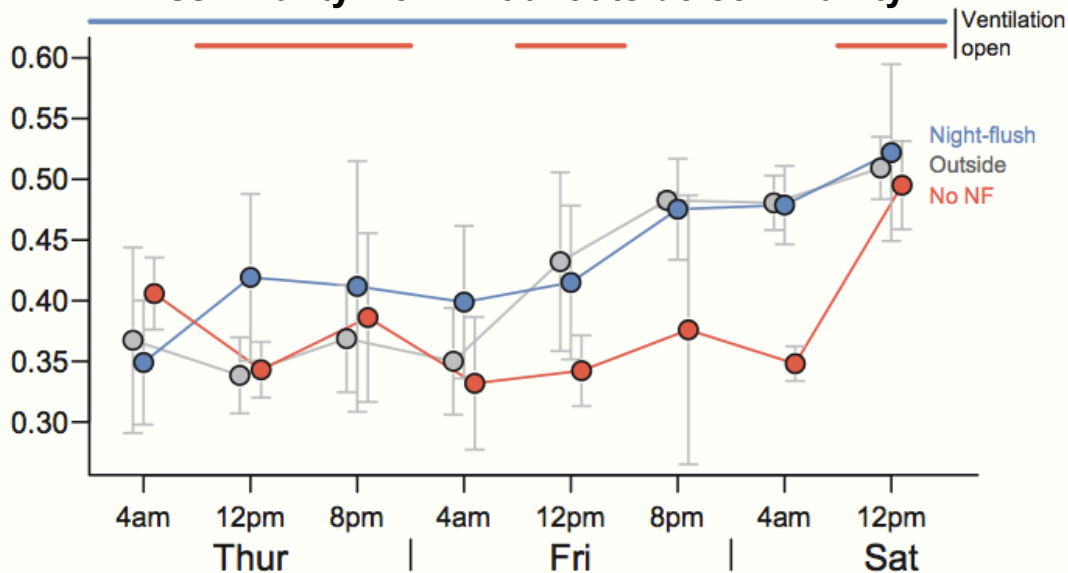


10. Building **design and operation** can influence indoor microbial communities

Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source

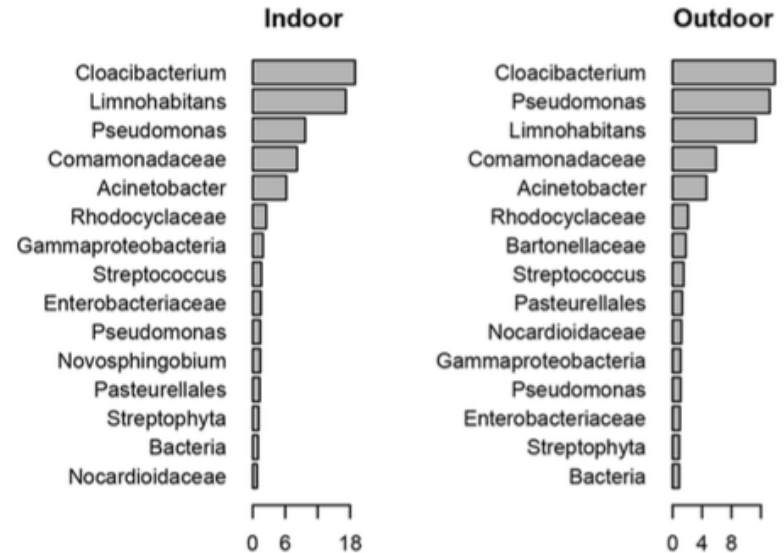
- Indoor air communities **closely tracked OA**
- Human-associated bacterial genera were more than 2x as abundant in IA vs. OA
- Ventilation** had a demonstrated effect on indoor airborne bacterial community composition (following a time lag)

Dissimilarity from initial outside community



Meadow et al. **2013** *Indoor Air* 24(1):41-48

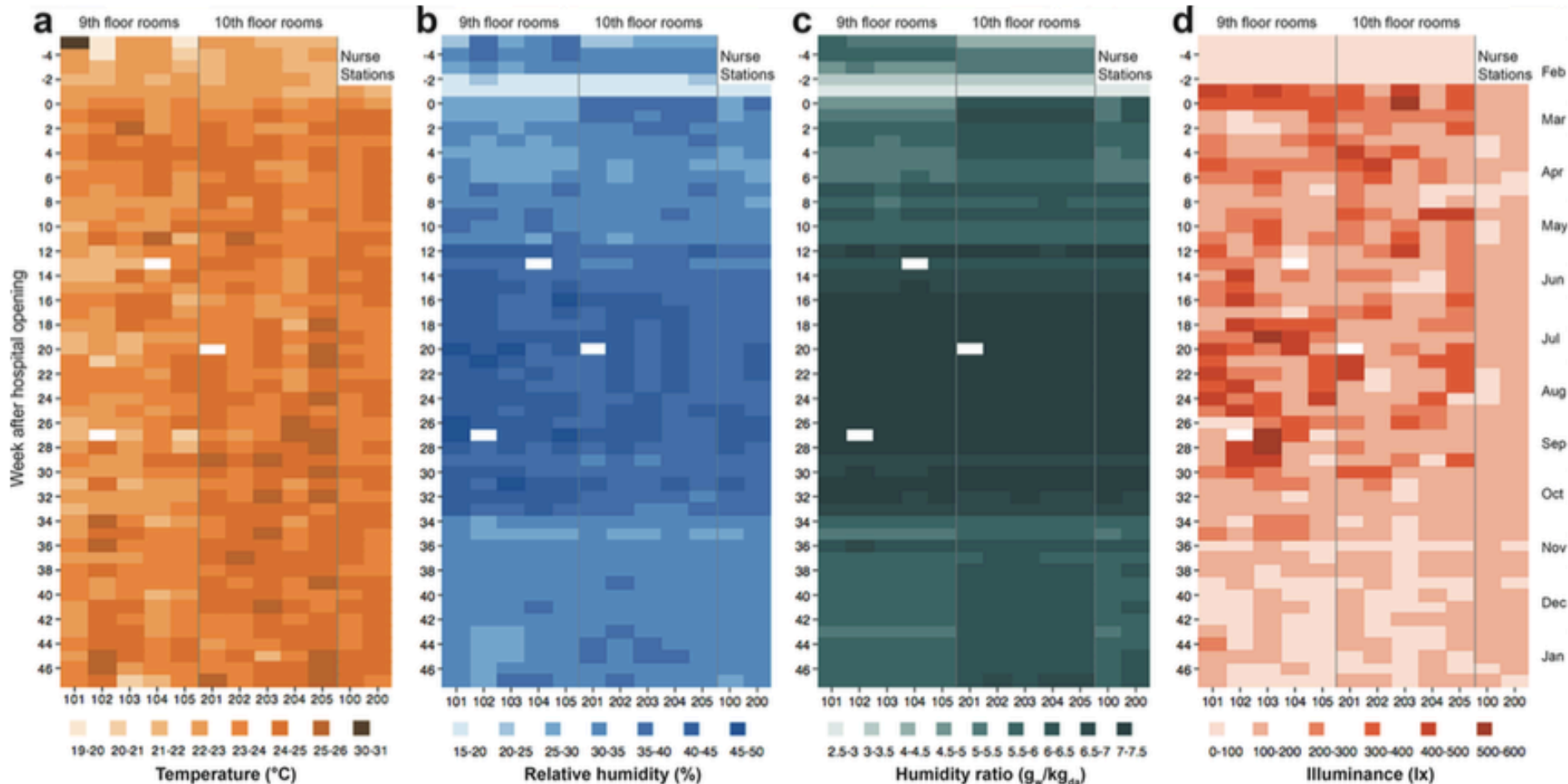
Another chamber study (bacteria):



- In a mechanically ventilated office setup (~3 ACH and MERV 7 filtration), indoor microbial composition **mostly tracked that of outdoor** composition
 - The number of occupants and their activity had a smaller influence on indoor bioaerosol composition than expected

11. Building **environmental conditions** often have a **small influence** on indoor microbial communities

Long-term environmental conditions in a new hospital

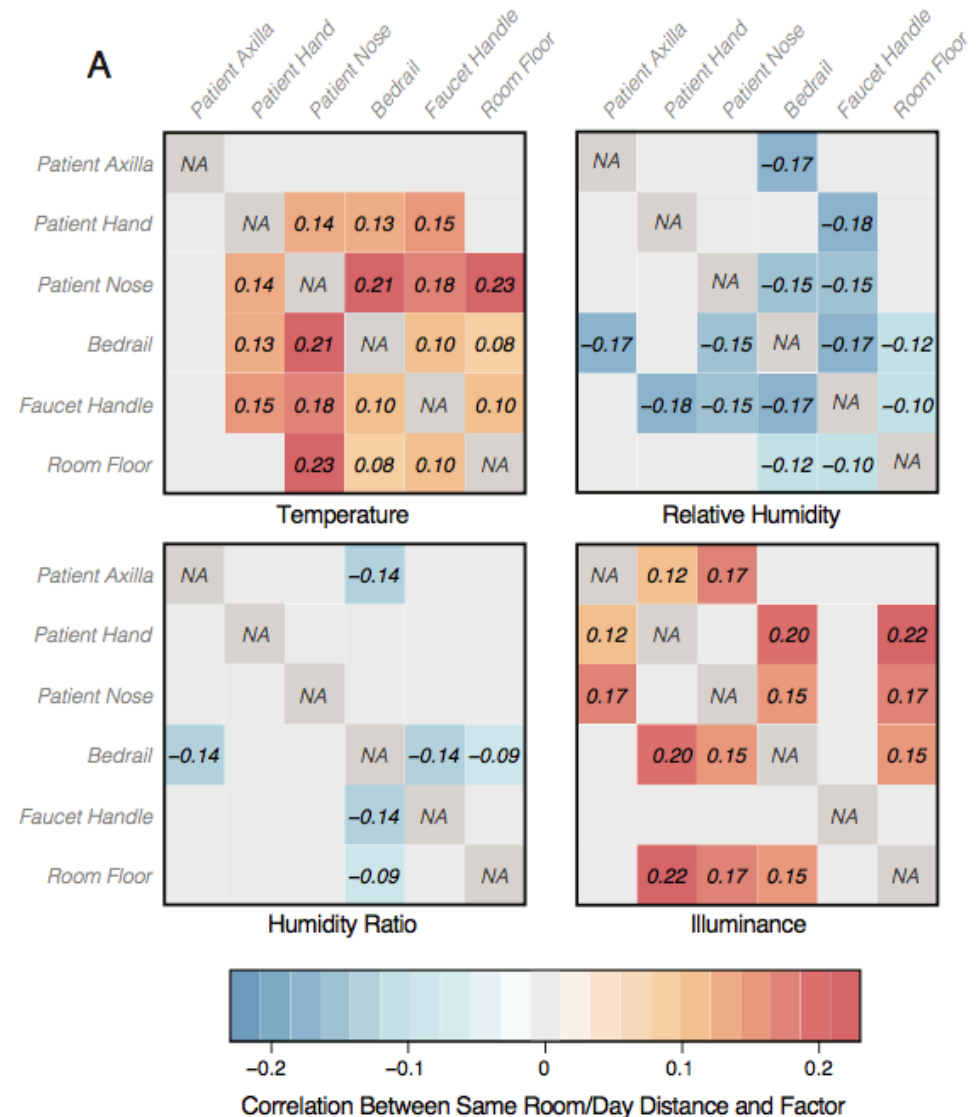


Ramos et al. **2015** *PLOS ONE* 10(3):e0118207

11. Building **environmental conditions** often have a **small influence** on indoor microbial communities

Bacterial community similarity and long-term environmental conditions in a new hospital

“...**higher temperatures and higher illuminance** were consistently associated with **greater microbial dissimilarity** between patient and surface microbial communities, while **higher relative humidity and humidity ratio** were consistently correlated with **greater microbial similarity**”



11. Building **environmental conditions** often have a **small influence** on indoor microbial communities

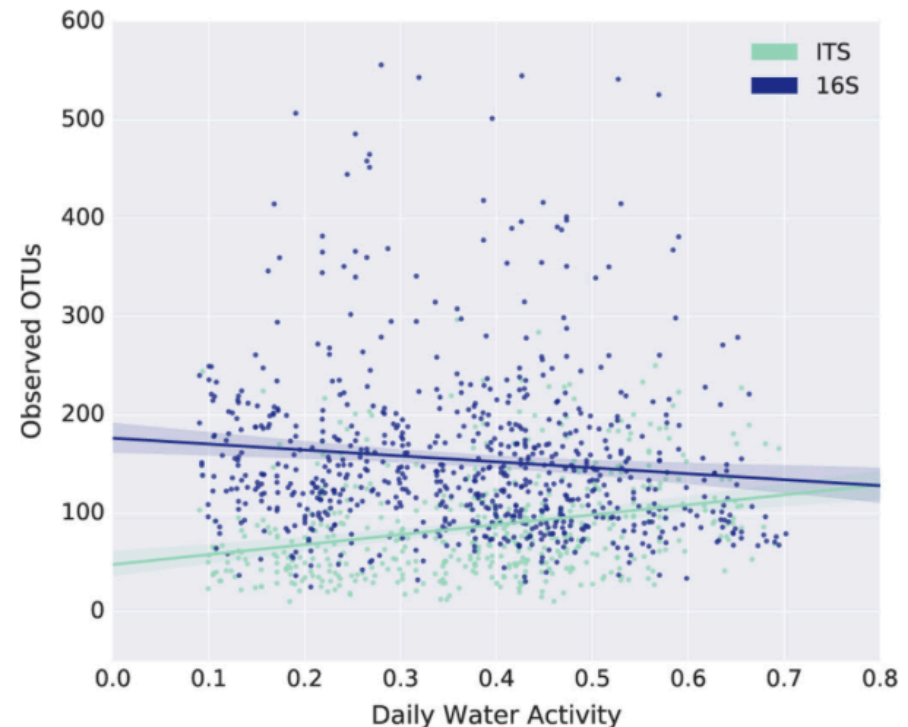
The Built Environment Is a Microbial Wasteland

Gibbons 2016 *mSystems*



Geography and Location Are the Primary Drivers of Office Microbiome Composition

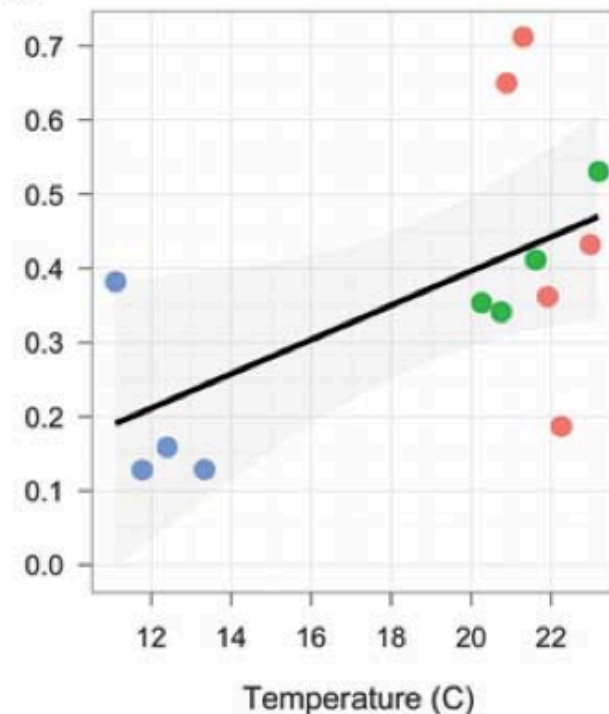
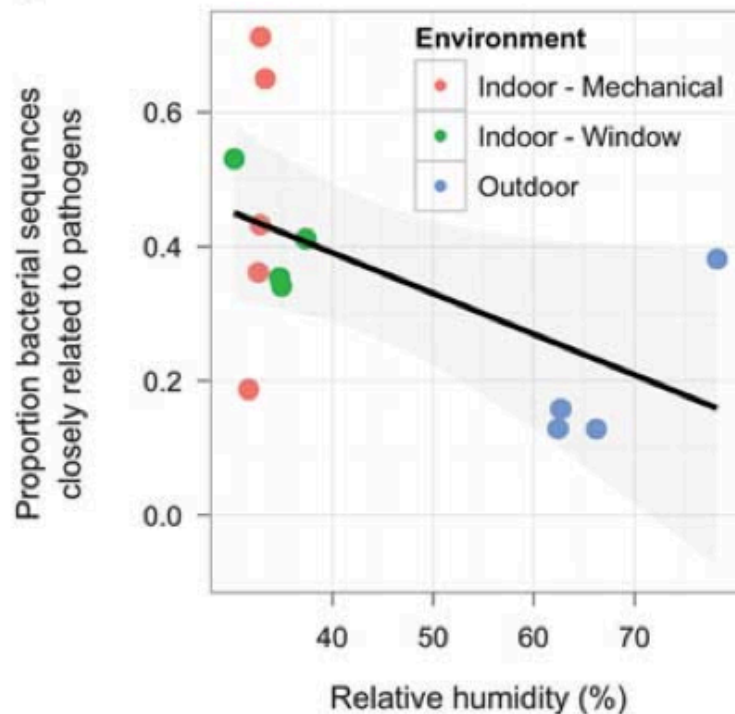
Chase et al. 2016 *mSystems* 1(2):e00022-16



11. Building **environmental conditions** often have a **small influence** on indoor microbial communities

Correlations of bacterial findings with environmental conditions in a hospital room

***Factors are correlated**



Kembel et al. **2012** *ISME J* 6:1469-1479

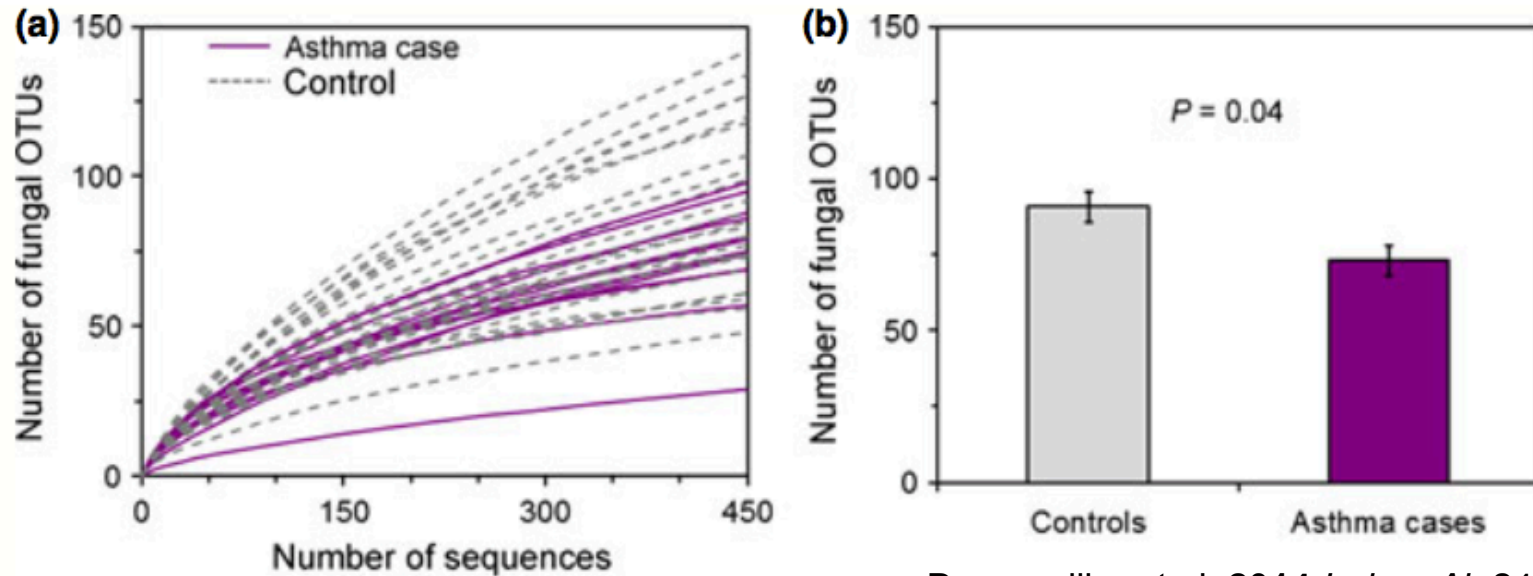
Correlations of bacterial findings with homes characteristics

“Bacterial richness tended to be higher in those four (of 11) units that reported at least occasional humidifier use”

Adams et al. **2014** *PLoS ONE* 9(3):e91283

12. Exposures to microbial diversity (i.e., the ‘right’ number of the ‘right’ kinds of microbes) can be **beneficial** for health

Next-generation DNA sequencing reveals that low fungal diversity in house dust is associated with childhood asthma development



Dannemiller et al. **2014** *Indoor Air* 24(3):236-247

Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children

“...children with the highest exposure to specific allergens and bacteria during their first year were least likely to have recurrent wheeze and allergic sensitization”

Lynch et al **2014** *J Allergy Clin Immunol* 134:593-601

Summary of (some of) what we have learned

1. Culture-independent methods reveal vastly greater microbial diversity compared to culture-based methods
2. Indoor spaces often harbor unique microbial communities
3. Indoor fungal communities are largely driven by outdoor fungal communities (in non-damp buildings)
4. Indoor fungal communities in damp buildings are often distinct from those in non-damp buildings
5. Indoor bacteria often originate from indoor sources
6. Source tracking techniques demonstrate that humans and pets often dominate bacterial communities on indoor surfaces
7. Occupants and surfaces interact in both directions
8. Humans are also major sources of bacteria to indoor air
9. Controlled studies can elucidate the mechanisms of human microbial emissions
10. Building design and operation can influence indoor microbial communities
11. Building environmental conditions often have a small influence on indoor microbial communities (but sometimes larger)
12. Exposures to microbial diversity (i.e., the 'right' number of the 'right' kinds of microbes) can be beneficial for health

Practically, what have we learned?

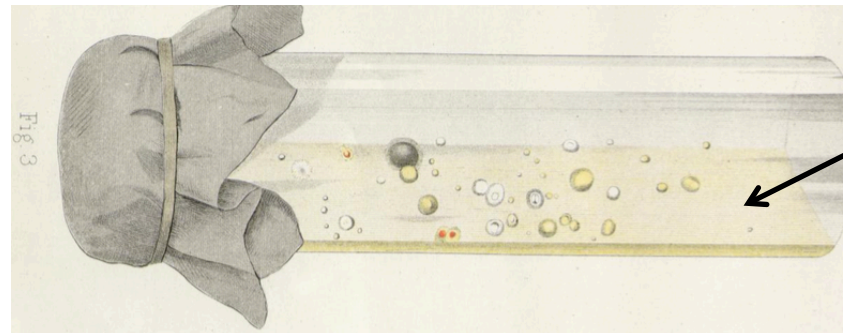
IV. *The Carbonic Acid, Organic Matter, and Micro-organisms in Air, more especially of Dwellings and Schools.*

By Professor THOS. CARNELLEY, D.Sc., and J. S. HALDANE, M.A., M.B., University College; and A. M. ANDERSON, M.D., Medical Officer of Health, Dundee.

Communicated by Sir HENRY E. ROSCOE, F.R.S.

Received June 10,—Read June 10, 1886.

5. An investigation into the sources of the organic matter and micro-organisms of air inside buildings, and the circumstances affecting the number of micro-organisms; also of the relative number of bacteria and moulds in both outside and inside air.



Meat jelly

Adding to the evidence (from 1887)

Always compare indoor air to outdoor air

In order to draw conclusions from an examination of air inside buildings, it is of course necessary to know the state of the outside air. As regards each of the constituents estimated, considerable variations were found at different times and places.

Occupant density drives bacterial counts (but has no impact on fungi)

Cubic space per person.	No. of houses.	Temperature.	Carbonic Acid.	Organic matter.	Total micro-organisms.	Bacteria.	Moulds.
Cubic feet.							
100– 180	14	55	11·5	15·1	80	78	1·8
180– 260	18	54	10·7	15·1	49	47	1·5
260– 340	6	53	10·3	11·8	32	31	0·7
340– 500	4	57	9·2	8·4	42	40	2·1
500–1000	6	54	8·6	5·6	6	6	0
1000–2500	8	53	6·7	3·9	9·1	8·5	0·7
2500–4000	4	57	7·9	5·0	13·1	12·8	0·4

The explanation of the ratio $\frac{\text{Bacteria}}{\text{Moulds}}$ increasing with the vitiation of the air is that moulds come mostly from the outside air. When the air in a room becomes vitiated the bacteria increase largely, while the number of moulds is affected to a relatively much less extent, if at all.

Adding to the evidence (from 1887)

Resuspension is a key source of indoor microbes

It has been shown by HESSE (*loc. cit.*) that when a room is left quiet the micro-organisms settle out in a few hours, so that the air becomes comparatively free (cf. TYNDALL's experiments on sterilisation of air by subsidence). Hence it is clear that a certain amount of physical disturbance in a room is a condition necessary to the presence of micro-organisms in the air. It might naturally be supposed that the effects of physical disturbance would tend to obscure all other factors affecting the number of micro-organisms present in air. It is, therefore, necessary to consider first what, other things being equal, are the limits of the influence of ordinary physical disturbances on the number of micro-organisms.

be expected that a large number of bacteria would be given off from bed clothes when shaken.*

2. The skin and clothes of the persons present in a room at the time of an observation also occur naturally as a probable source of infection of air. That this source, however, is of much less importance than might be supposed may, we think, be shown from our observations.

Adding to the evidence (from 1887)

The “pig pen effect” is very real

B. *Cleanliness of rooms and persons habitually present in them.*—In order to show the influence of differences as regards cleanliness, we have classified the houses and schools as shown in the following Table. This Table requires no comment. It shows most conclusively the enormous influence of differences as regards cleanliness on the number of micro-organisms.

		No. of cases.	Average space per person.	Average carbonic acid.	Average organic matter.	Average micro-organisms.
One-roomed houses	{ Clean	1	295	8·0	13·1	18
	{ Dirty	7	200	9·9	18·1	41
	{ Dirtier	13	221	10·7	13·5	49
	{ Very dirty	6	220	11·0	15·1	93
Two-roomed houses	{ Very clean	2	273	12·2	10·8	10
	{ Clean	4	264	9·3	7·7	22
	{ Dirty	7	233	9·4	11·2	69

Adding to the evidence (from 1887)

Source (and rate) of ventilation air delivery impacts indoor microbes

	Mechanically ventilated.	Naturally ventilated.
Cubic space per person	1	1·0
Temperature in excess of outside air .	1	0·66
Carbonic acid " " .	1	1·7
Organic matter " " .	1	7·0
Micro-organisms " " .	1	9·2
Bacteria " " .	1	9·4
Moulds " " .	1	2·0

**Note: Naturally ventilated spaces had lower ventilation rates than mechanically ventilated spaces*

The all-important argument for mechanical ventilation is that it maintains a certain standard of purity, and, unless some simpler method which will maintain a similar standard can be devised, its adoption in crowded schools seems to be very much required.

Adding to the evidence (from 1887)

Indoor microbes and built environment factors are weakly correlated

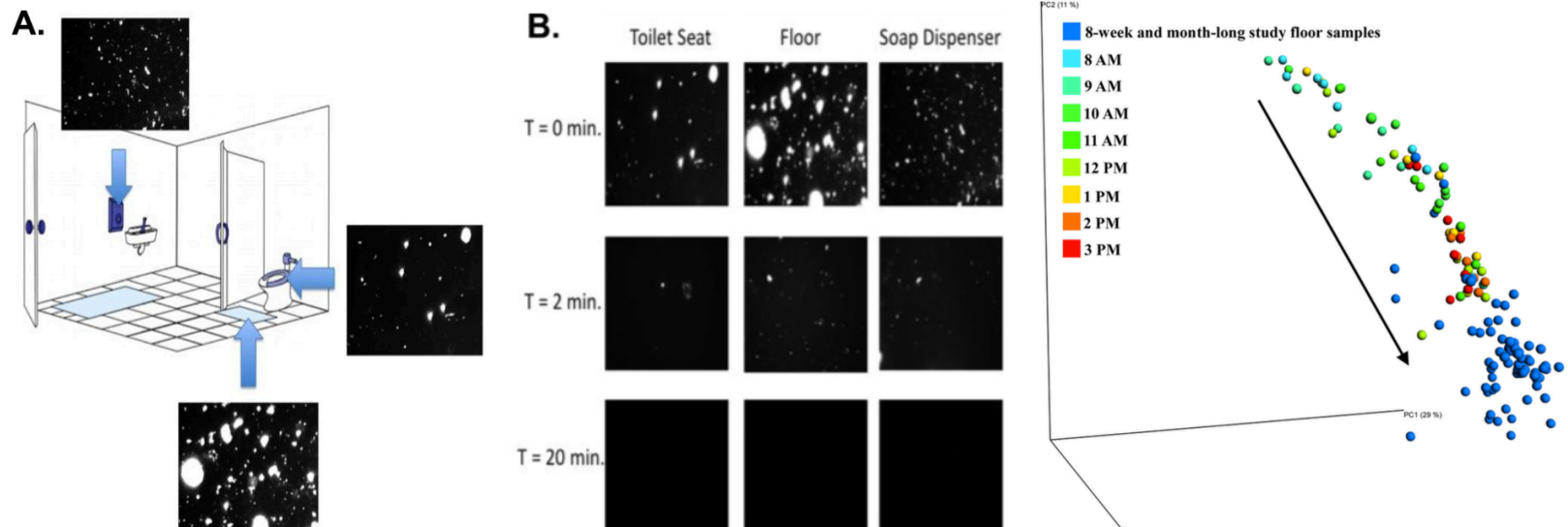
No constant relation between the quantities of carbonic acid, organic matter, and micro-organisms can be detected in individual cases (see PARKES, p. 147; also DE CHAUMONT, 'Roy. Soc. Proc.,' vol. 23, p. 188). Sometimes we find a high organic matter accompanied by a low carbonic acid, whilst under other circumstances the reverse may be the case. A determination of carbonic acid alone is therefore never a sufficient indication of the purity or otherwise of a given sample of air. Nevertheless, by taking the average of a considerable number of observations, we find that there is a *general* relationship, so that a high carbonic acid is, as a rule, accompanied by a high organic matter, and *vice versa*, though this is by no means always the case. There appears, however, to be no definite connexion between the number of micro-organisms and the amount of carbonic acid (see page 93).

From 1887 to 2016 and beyond

“The **combination of culture and culture-independent methods** provided powerful means for determining both viability and diversity of bacteria in child-care facilities.”

“Although our study identified a remarkable array of microbial diversity present in a single daycare, it also revealed just **how little we comprehend the true extent of microbial diversity** in daycare centers or other indoor environments.”

Lee et al. **2007** *BMC Microbiology* 7:27

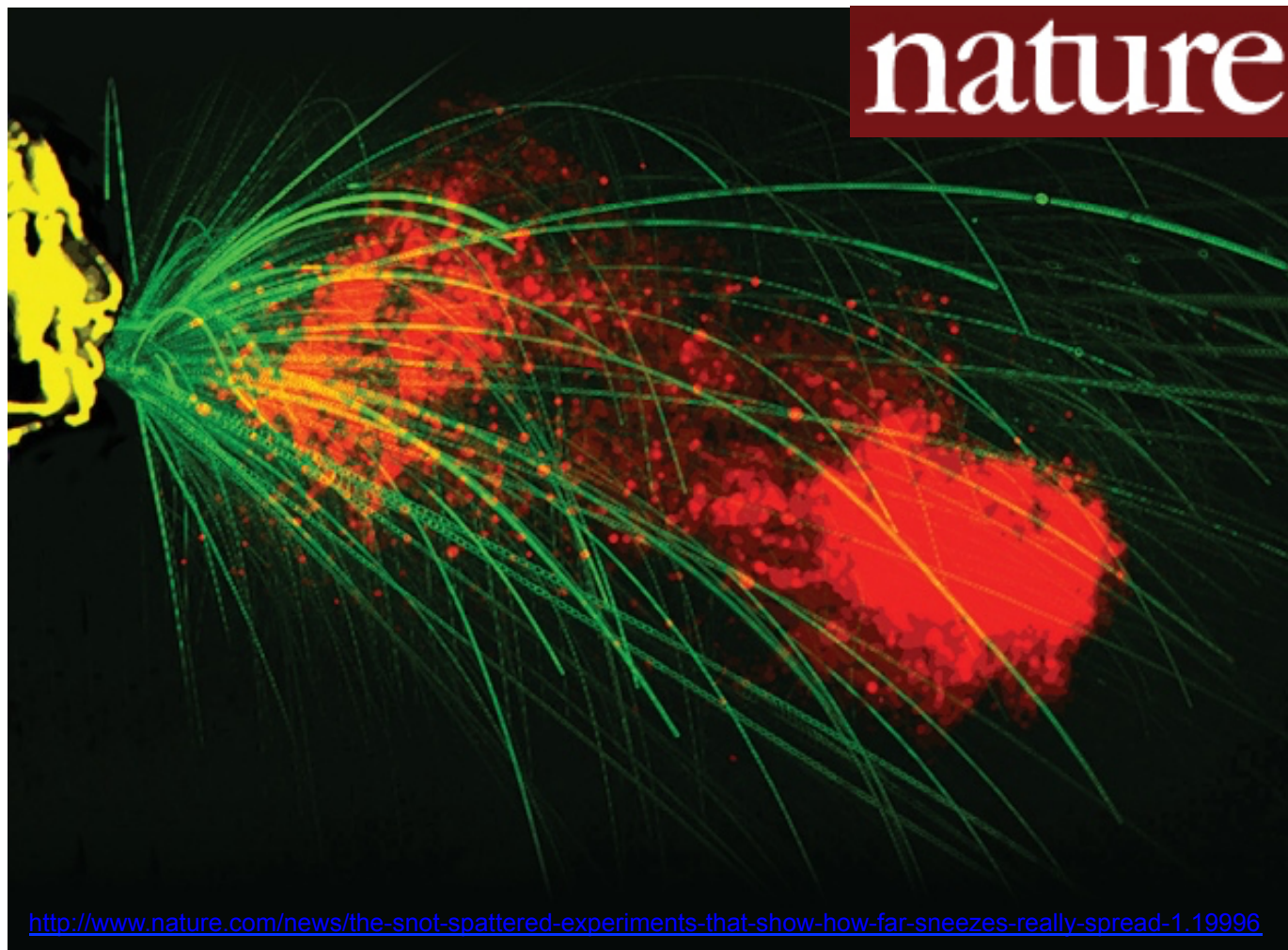


Gibbons et al. **2015** *Applied and Environ Microbio* 81:765-773

Where are we going?

1. We continue to build upon existing knowledge by adding *complexity* (physically, ecologically, statistically, etc.) to our existing knowledge base
2. We are beginning to think differently about microbes in indoor environments and health
 - But we are somewhat stuck between two distinctly different positions

Example: The complexity of sneezes (2016)



“A sneeze captured on high-speed video. After a sneeze, large droplets of saliva and mucus (green) shoot out of the mouth, but fall relatively quickly. A turbulent cloud carries smaller droplets (red) and allows them to drift for up to 8 metres.”

The complexity of sneezes (2016)



Bourouiba and Bush (2014)

Rapid evaporation of droplets after sneezing, by *Mythbusters* (2010)



Infection of Air*

Bacteriologic and Epidemiologic Factors

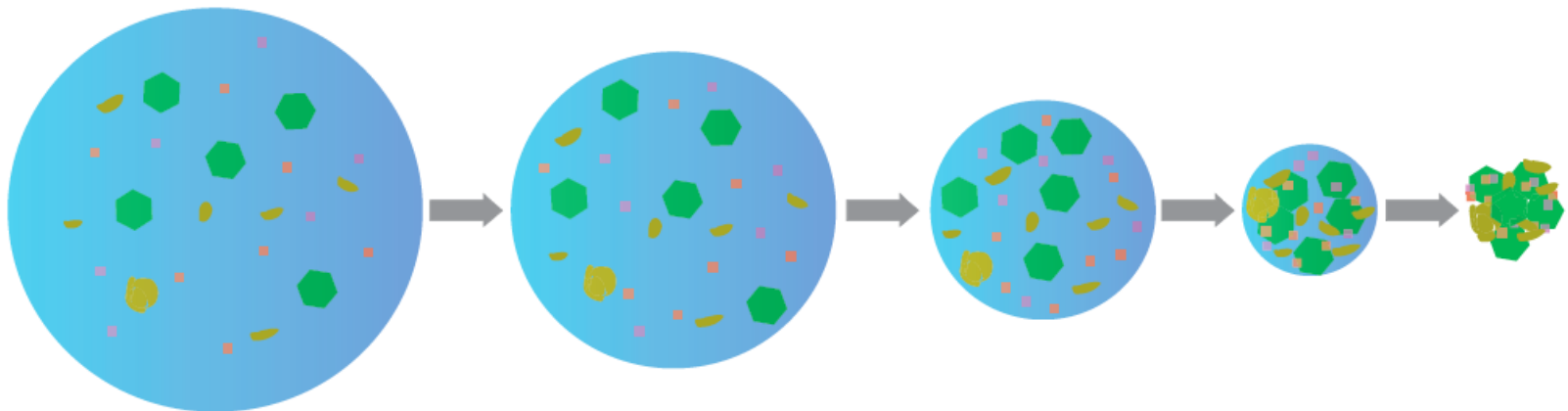
W. F. WELLS, M. W. WELLS, AND STUART MUDD, M.D.



FIGURE II—Droplet nuclei (contrasted with Flüge droplets) photographed by the Tyndall effect. Upper left, sneezing; upper right, pronouncing the letter "p"; lower left and right, pronouncing the letter "t." (Taken from F. Weyrauch and J. Rzymkowski. Photographien zur Tröpfcheninfektion. *Ztschr. f. Hyg. u. Infektionskr.*, 120:444, 1938.)

The complexity of sneezes (2008)

- When a person coughs, sneezes, speaks, or even breaths:
 - Particles of liquid water, proteins, salts, and other matter are expelled
 - These are called **droplets**
 - These particles may contain smaller infectious organisms
 - Droplets rapidly deposit to surfaces and/or decrease in size as the surrounding liquid evaporates
 - **Droplet nuclei** remain after evaporation
 - Typically 40-50% smaller diameter (d_p) than original droplets
 - Still contain infectious organisms



Evidence of airborne rhinovirus transmission (1987)

My favorite experiment...

Aerosol Transmission of Rhinovirus Colds

Elliot C. Dick, Lance C. Jennings, Kathy A. Mink,
Catherine D. Wartgow, and Stanley L. Inhorn

Rhinovirus infections may spread by aerosol, direct contact, or indirect contact involving environmental objects. We examined aerosol and indirect contact in transmission of rhinovirus type 16 colds between laboratory-infected men (donors) and susceptible men (recipients) who played cards together for 12 hr. In three experiments the infection rate of restrained recipients (10 [56%] of 18), who could not touch their faces and could only have been infected by aerosols, and that of unrestrained recipients (12 [67%] of 18), who could have been infected by aerosol, by direct contact, or by indirect fomite contact, was not significantly different ($\chi^2 = 0.468$, $P = .494$). In a fourth experiment, transmission via fomites heavily used for 12 hr by eight donors was the only possible route of spread, and no transmissions occurred among 12 recipients ($P < .001$). These results suggest that contrary to current opinion, rhinovirus transmission occurs chiefly by the aerosol route.

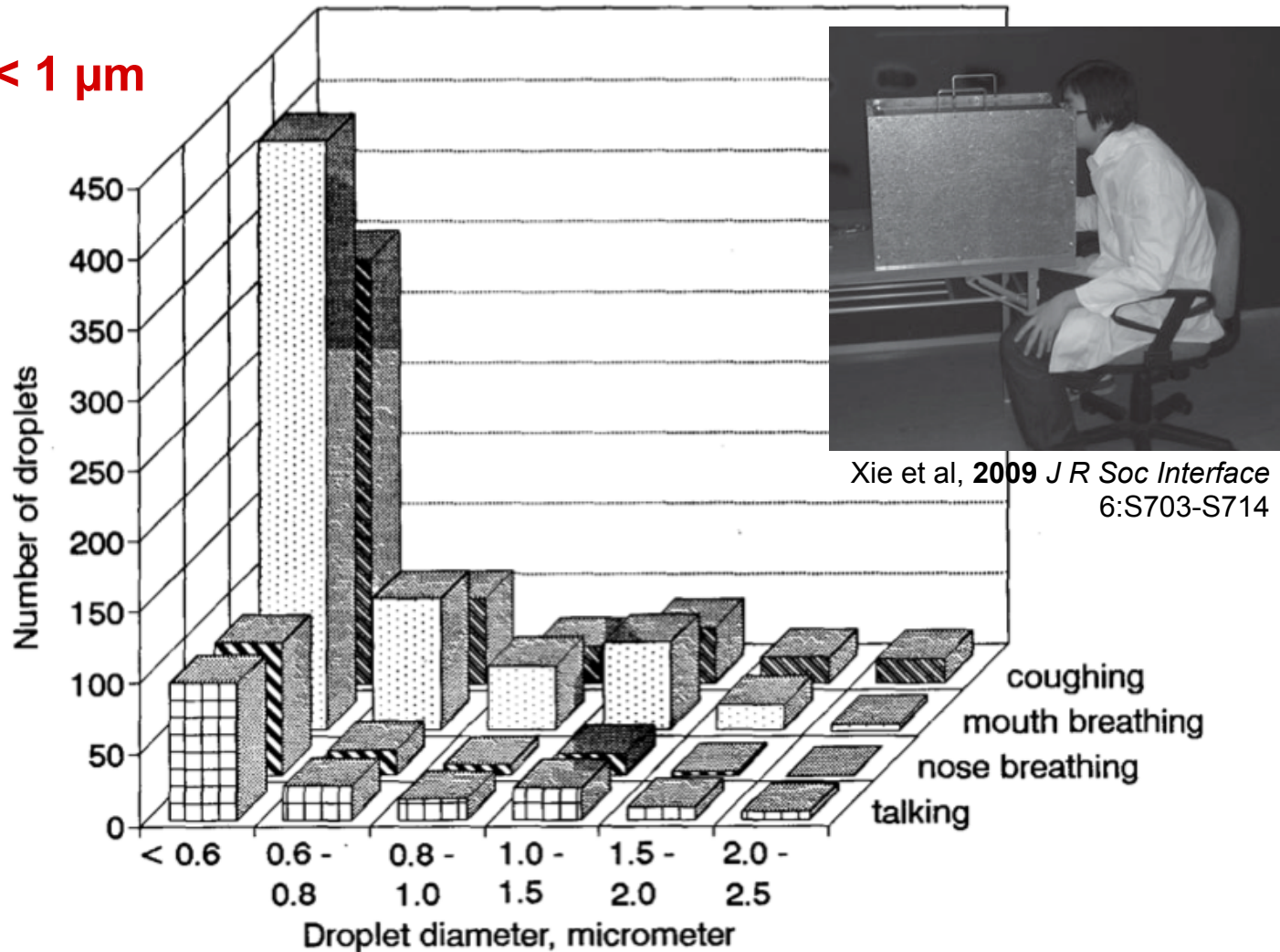
ments. Twenty-seven to 34 men >18 years of age were inoculated intranasally with 560–2,400 TCID₅₀ of safety-tested RV16 [5] by pipette and spray on two successive days. On the third day, eight men with the most severe colds (donors) played stud and draw poker with 12 antibody-free (no neutralization of virus by the undilute [1:1] serum specimen against a 20–25 TCID₅₀ challenge) men (recipients) between hours of 8 a.m. and 11 p.m. The ending hour was

Evidence of airborne rhinovirus transmission (1987)

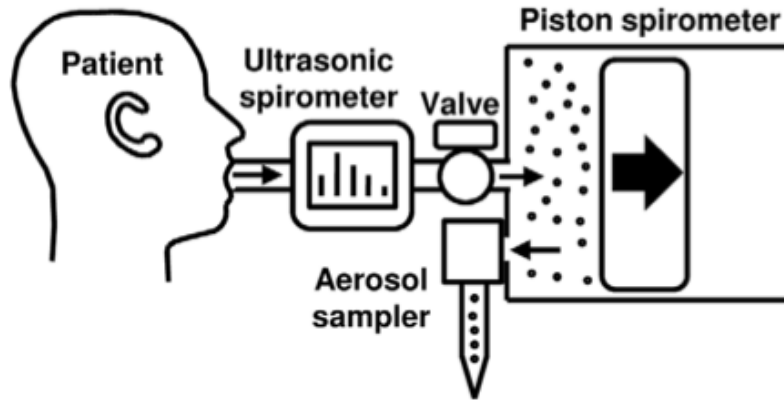


Particle emissions from coughing subjects (1997)

Nearly all
particles < 1 μm



Viral RNA contained in size-resolved aerosol samples (2010)



qPCR reveals influenza viral RNA size distribution in human coughs:

- 42% < 1 μm
- 23% 1-4 μm
- 35% > 4 μm

Table 1. Influenza viral RNA detected in the NIOSH two-stage aerosol sampler.

Aerosol particle size range (aerodynamic diameter)	Median # of viral copies per cough	% of viral RNA contained in particles in this size range	% of subjects whose cough aerosol contained viral RNA-laden particles in this size range
>4 μm	6.3 (SD 9.0)	35%	90%
1 to 4 μm	3.3 (SD 6.9)	23%	81%
<1 μm	3.7 (SD 23.7)	42%	75%
All particles	15.8 (SD 29.3)	100%	100%

Although ~90% of emitted particles (number concentrations) are < 1 μm

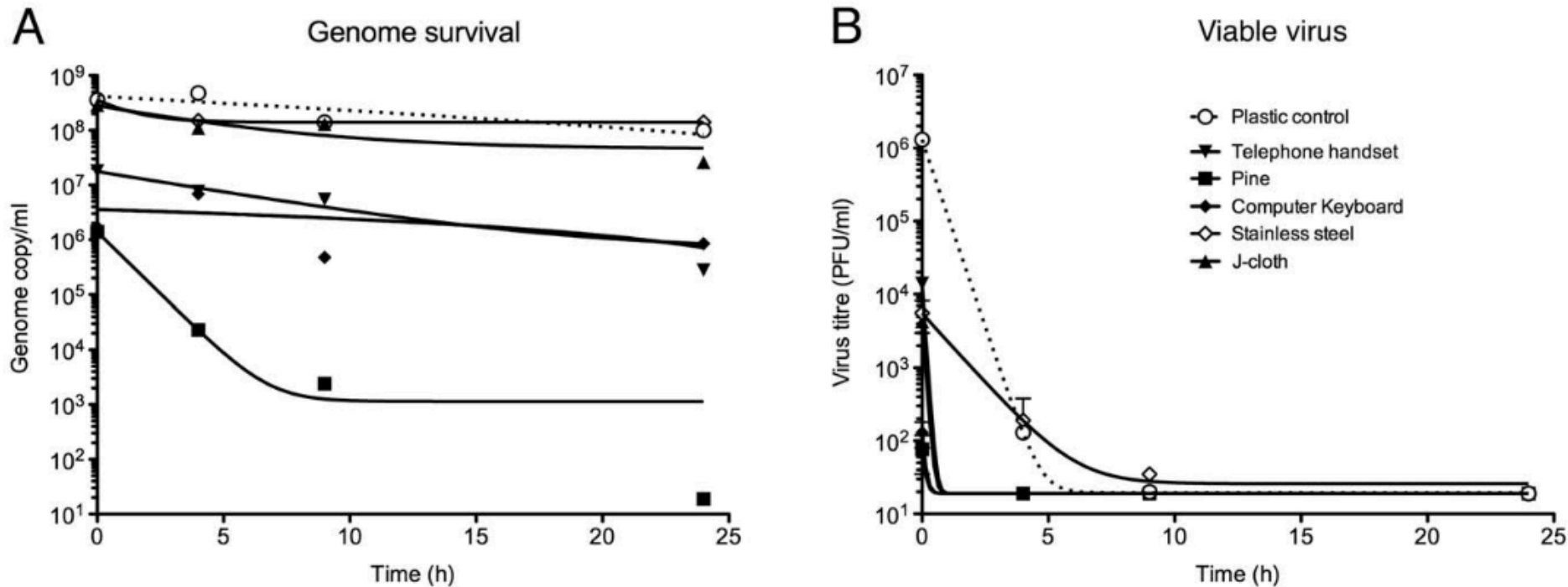
- Only ~40% of viral RNA is contained in that fraction

Others have detected airborne influenza viruses in real environments: Lindsley et al., 2010 *Clin Infect Dis*

50:693-698; Yang et al., 2011 *J R Soc Interface* 8:1176-1184

Influenza virus survival on surfaces (2011)

The usefulness of **combining biological methods**:



Conclusions/Significance: The genome of either virus could be detected on most surfaces 24 h after application with relatively little drop in copy number, with the exception of unsealed wood surfaces. In contrast, virus viability dropped much more rapidly. Live virus was recovered from most surfaces tested four hours after application and from some non-porous materials after nine hours, but had fallen below the level of detection from all surfaces at 24 h. We conclude that influenza A transmission via fomites is possible but unlikely to occur for long periods after surface contamination (unless re-inoculation occurs). In situations involving a high probability of influenza transmission, our data suggest a hierarchy of priorities for surface decontamination in the multi-surface environments of home and hospitals.

The complexity of sneezes (2016)

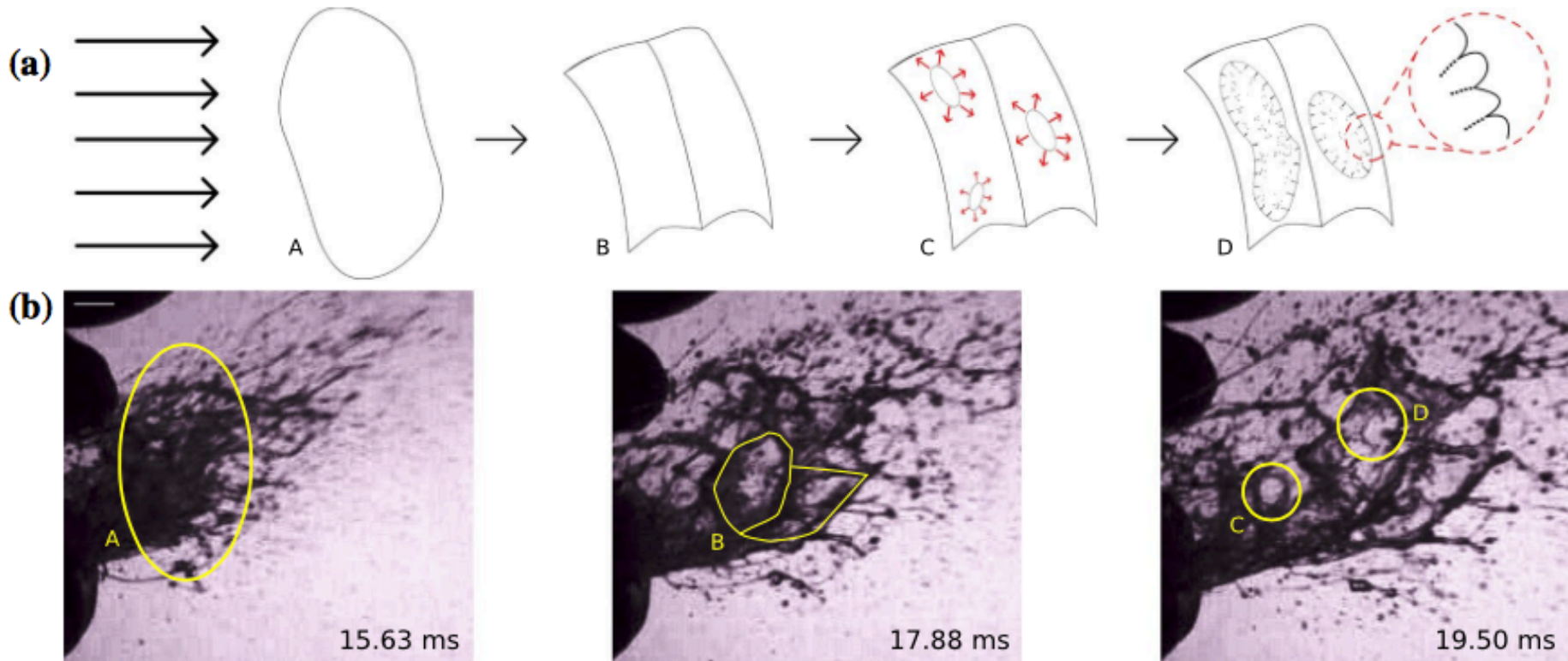


Bourouiba and Bush (2014)

The complexity of sneezes (2016)

“High-speed imaging elucidates the physical mechanisms of droplet formation at the exit of the mouth during sneezing”

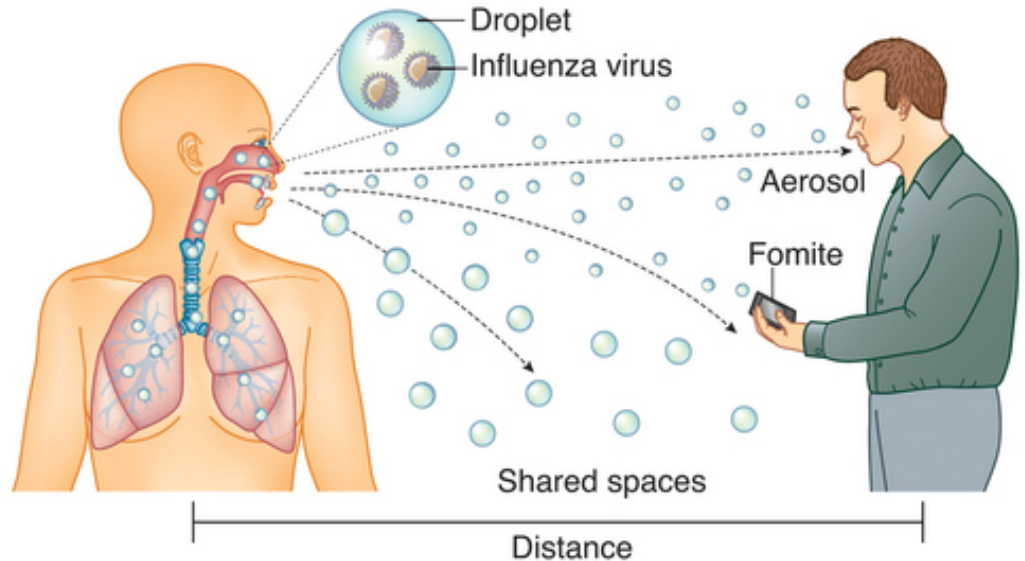
“The breakup of the fluid into droplets continues to occur outside of the respiratory tract”



“Such breakup involves a complex cascade of events from sheets, to tag bursts, to ligaments, which finally break into droplets”

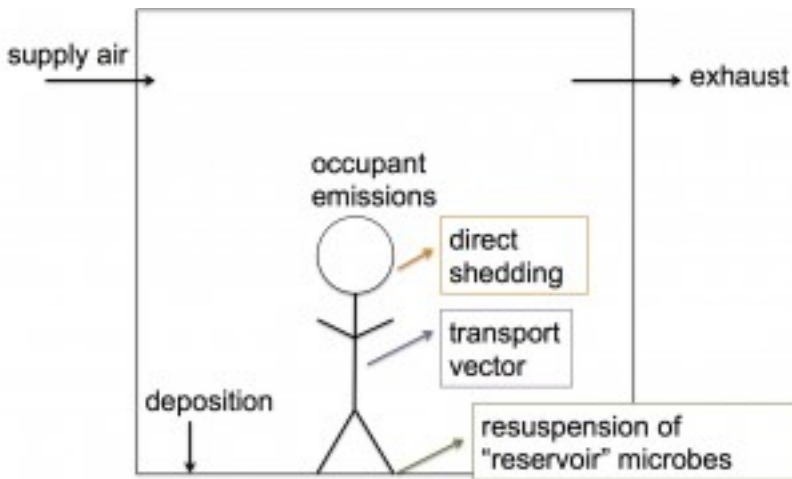
Microbial transmission: from pathogens to protection

A long history of **infectious disease and asthma/allergy** research has primed us to consider the **negative health impacts** of microbes in the built environment



Lakdawala and Subbarao 2012 *Nature Medicine* 18:1468-1470

But on the opposite end of the spectrum, some measures of microbial diversity/abundance have been associated with **positive health benefits**



Courtesy of Rachel Adams

How will we change the way we design and operate buildings?

New Sherwin-Williams paint kills infection-causing bacteria

Nathan Bomey, USA TODAY 7:07 a.m. EDT October 28, 2015



Can bioinformed design promote healthy indoor ecosystems?

Jessica L. Green
University of Oregon

?



Antibiotic

Probiotic

The “wrong” microbes

The “right” microbes

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- NAS study committee members and EPA staff
 - Especially Laura Kolb



email: brent@iit.edu
web: www.built-envi.com
twitter: @built_envi