

The indoor microbiome, asthma and allergies

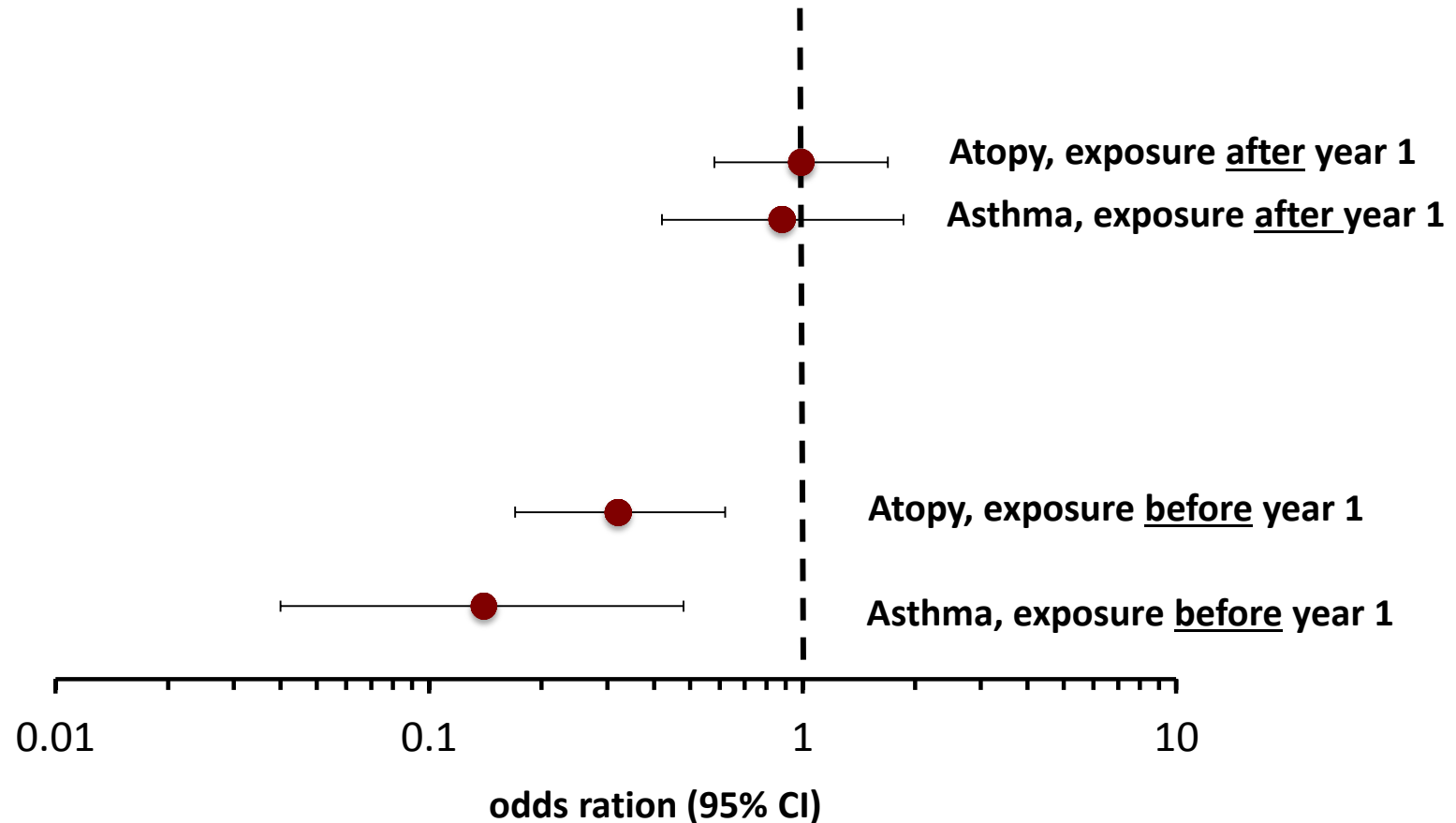
**Jordan Peccia
Associate Professor
Environmental Engineering
Yale University, U.S.A.**

The incidence of asthma and allergies:

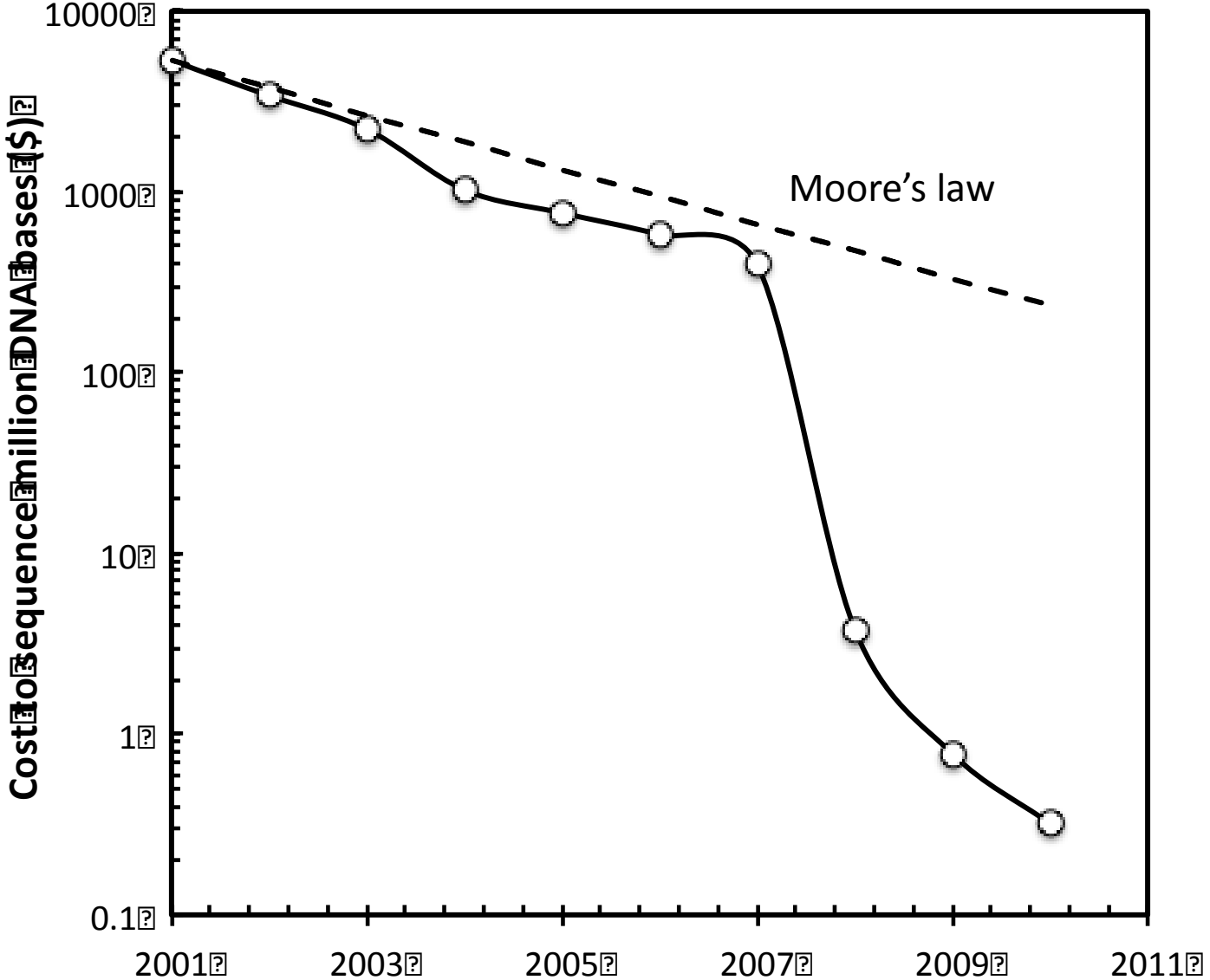
- **One in five children have respiratory allergies;**
- **One in ten children have asthma and rates have doubled since 1980;**
- **50% of Americans have some form of allergen sensitization. This number is only 7% among the Amish community in Indiana.**(Holbreich, JACI 2012)
- **In China, asthma prevalence is six times greater in urban children versus rural children.** (Ma et al., 2009 Pediatric Pulomology)



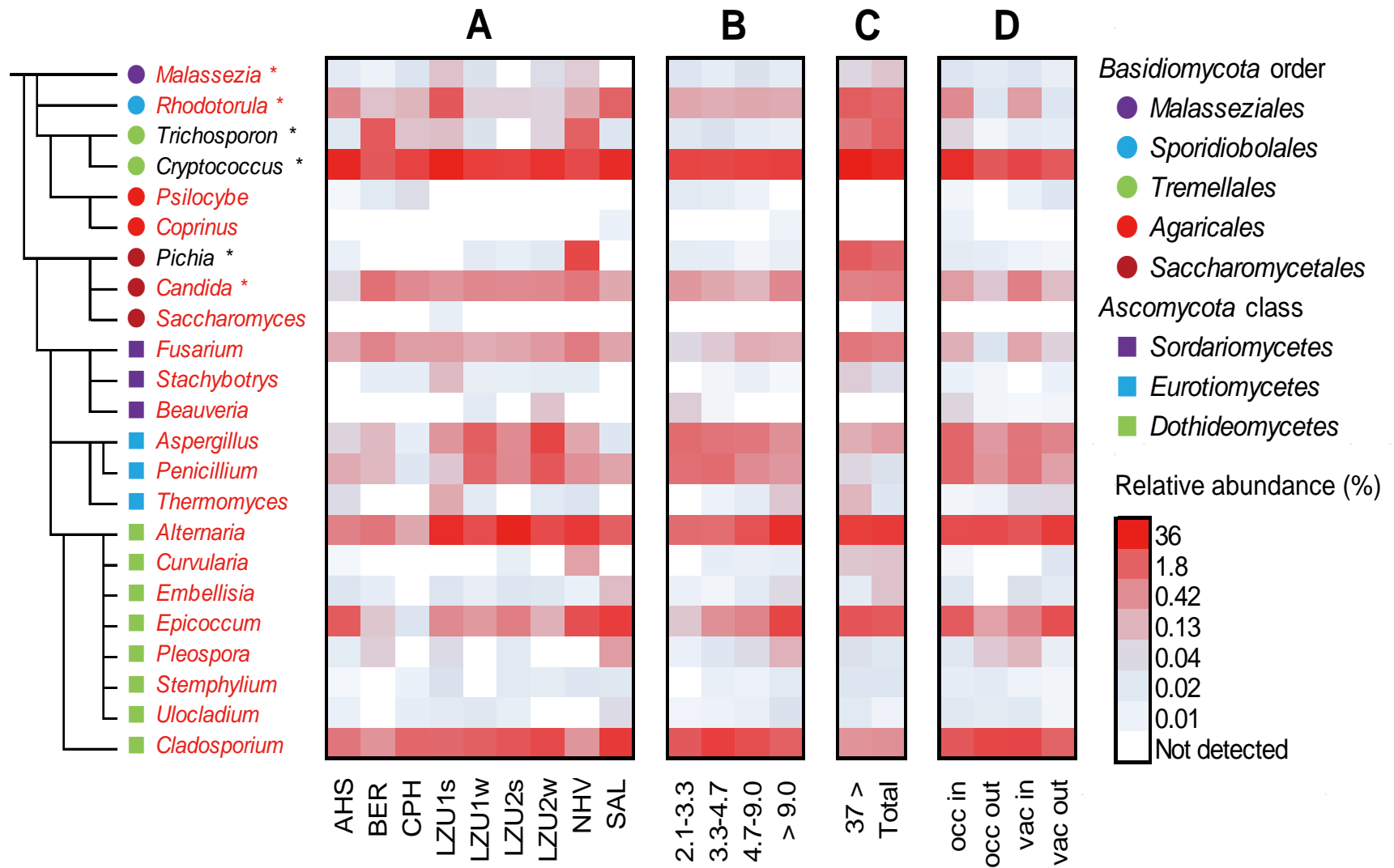
Living on a farm and drinking unpasturized milk



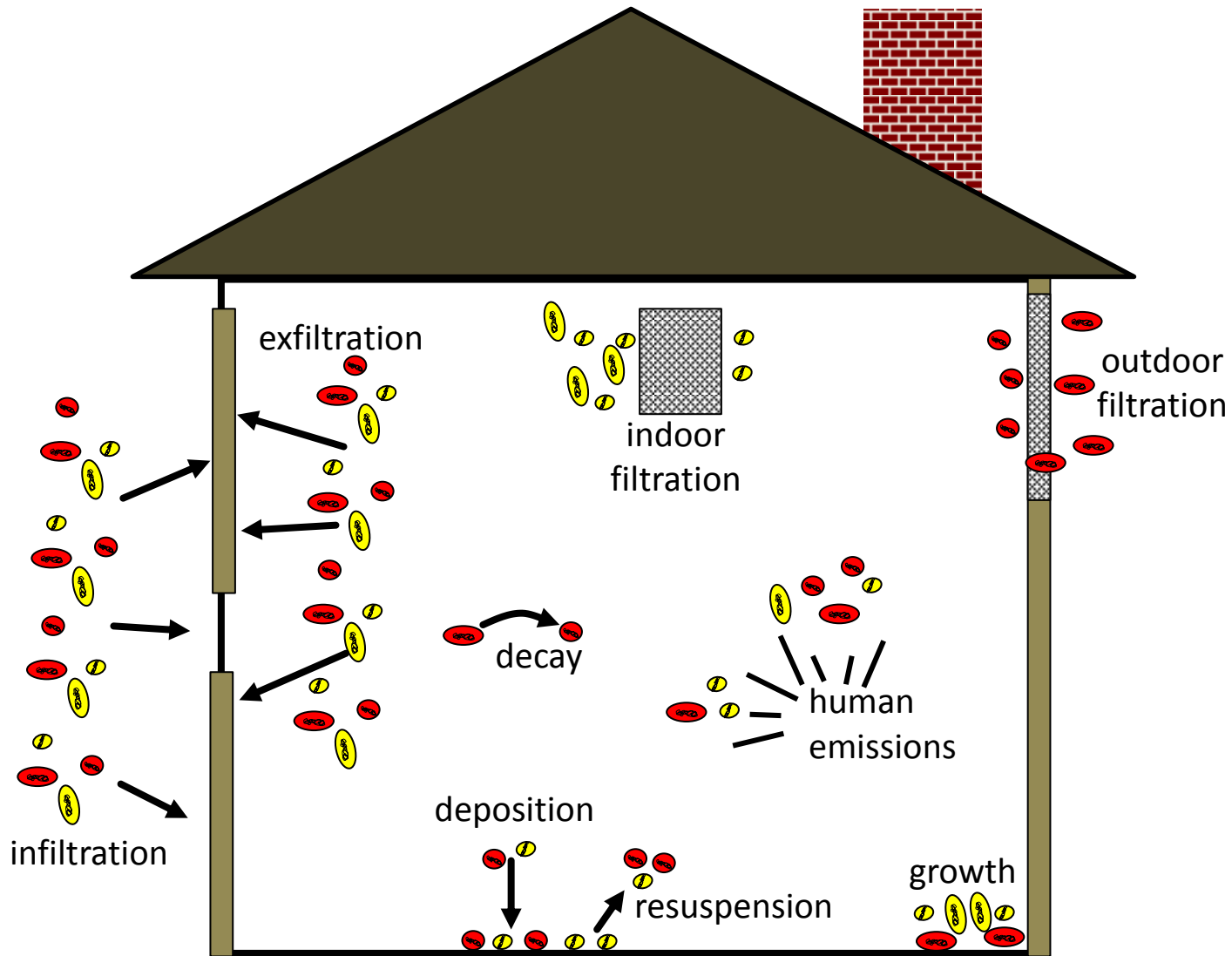
Cost of DNA Sequencing:



Build catalogs of microbes on surfaces and in air



Particle dynamics and the indoor microbiome





**Can next-generation DNA sequencing methods improve upon
asthma-microbe associations?**

Fungal ecology in epidemiology studies



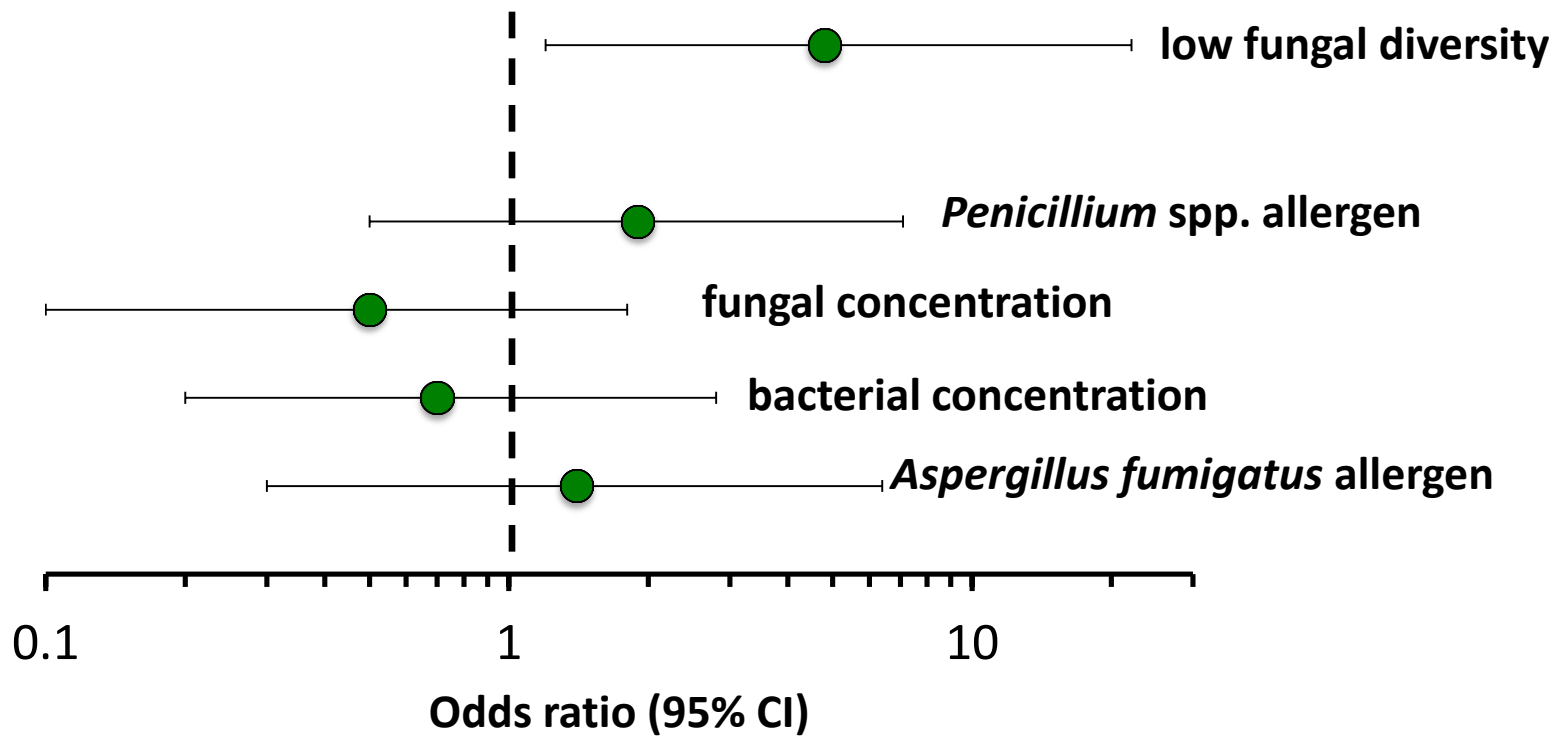
Karen C. Dannemiller, Mark J. Mendell, Janet M. Macher, Kazukiyo Kumagai, Jordan Peccia

- Longitudinal birth cohort study
- Enrolled 601 pregnant women living in the Salinas Valley and followed them (292) through age 12.
- Sequenced fungal populations in house dust of 13 asthmatic children and 28 control cases. House dust was collected and home moisture conditions were cataloged when children were 12 months.



Asa Bradman, Nina Holland, Kim Harley, Brenda Eskenazi

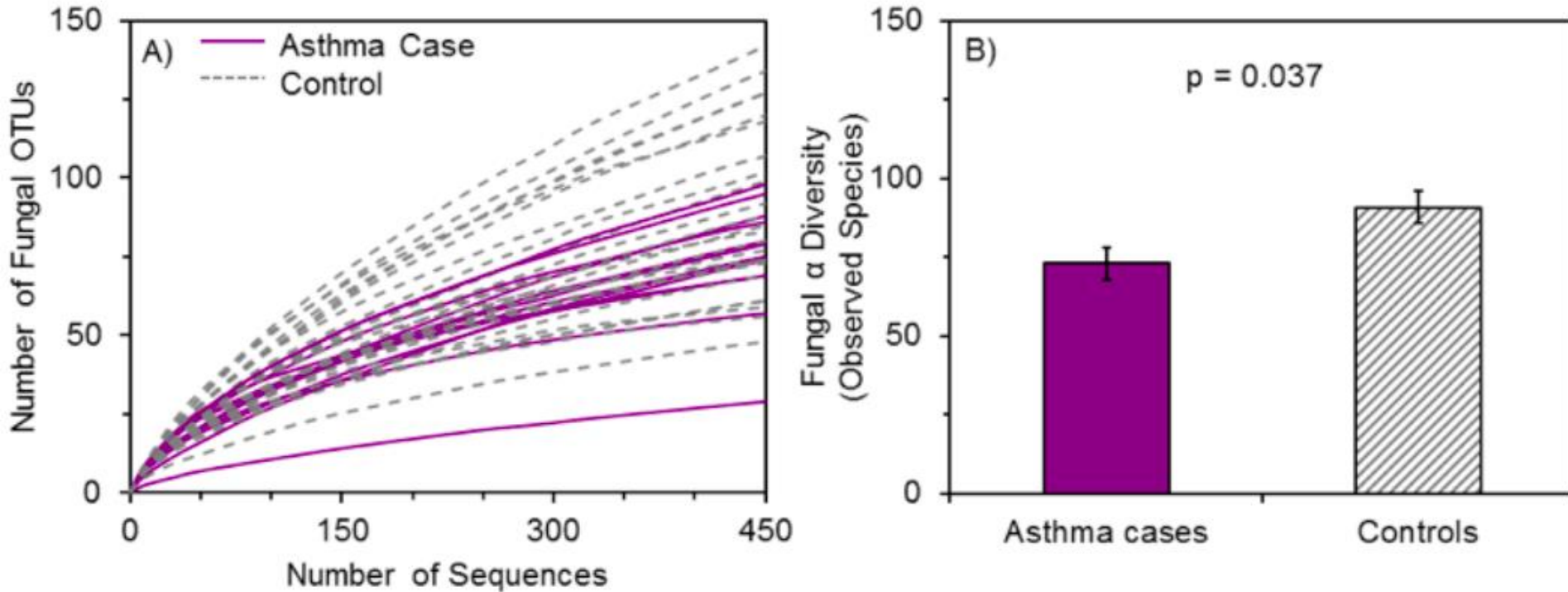
Asthma development was associated with...



Dannemiller et al., Indoor Air (2012)

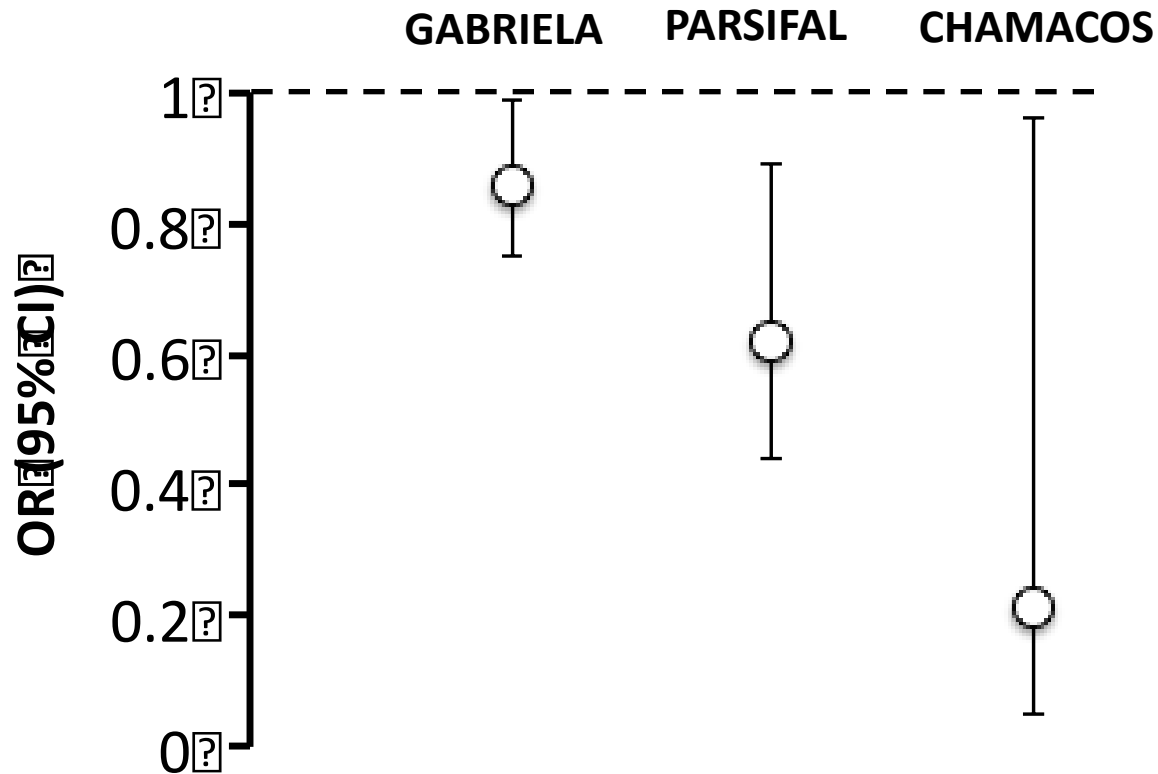
DOI:101111 /ina.12072

Fungal diversity is associated with the development of asthma in children

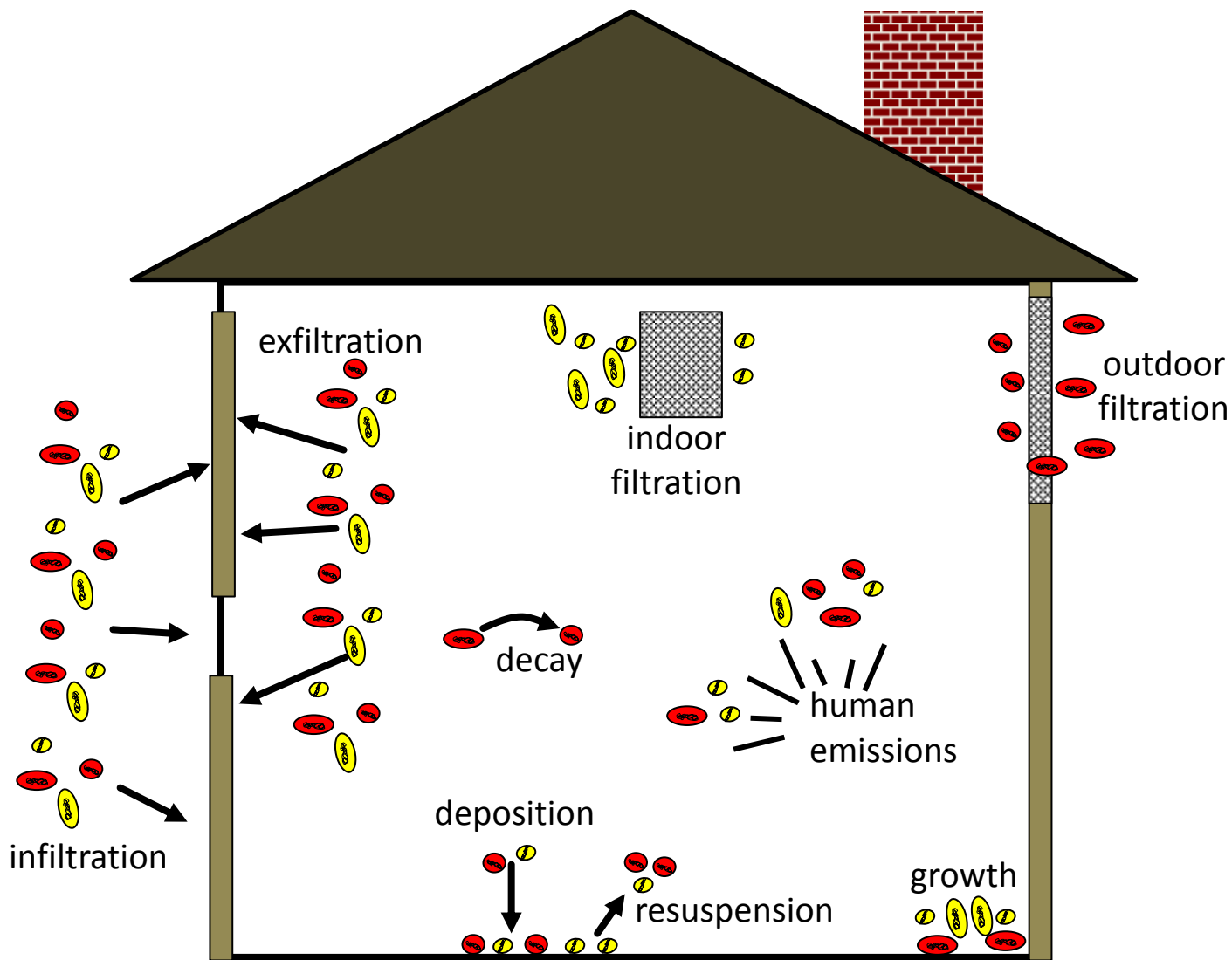


unadjusted Odds Ratio (OR) 4.80, 95% CI is 1.04 to 22.1

Microbial diversity (richness) is protective against asthma development



Can we design buildings to promote a health indoor microbiology

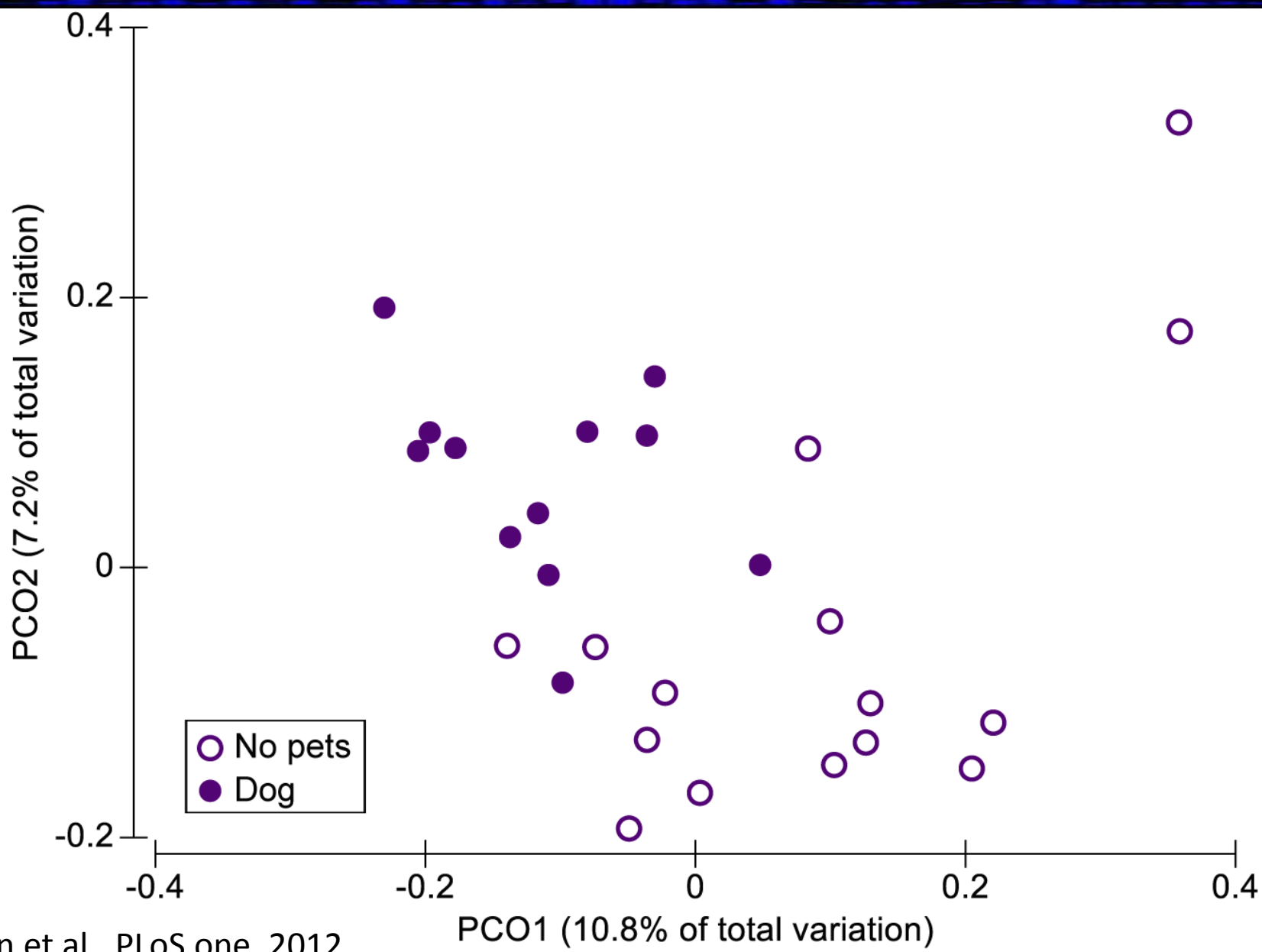


**Can we design buildings to promote
a health indoor microbiology?**

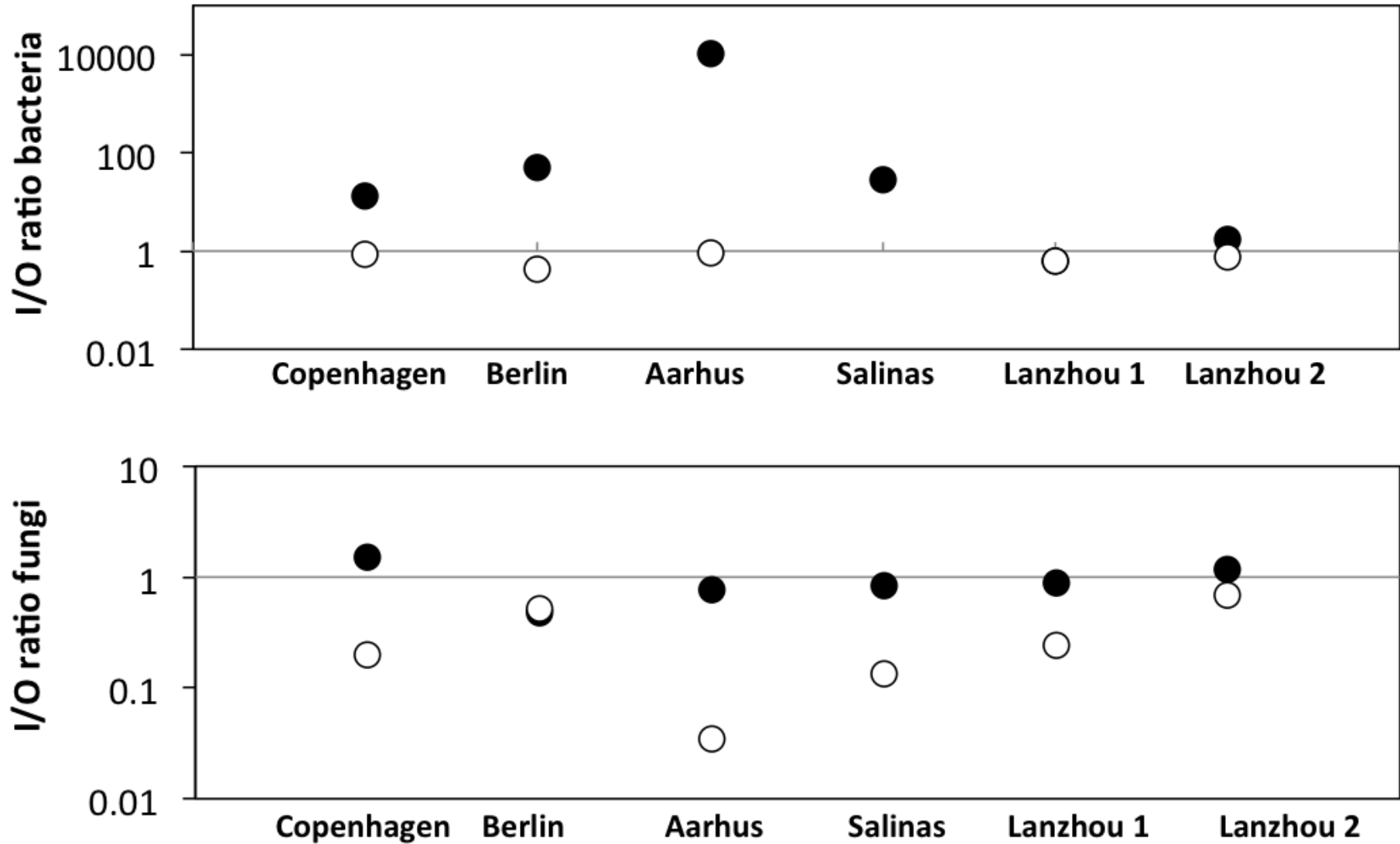
CT and MA 200 home study –richness

Category	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	95.7	91.8	0.59	735	735	0.91
More than 3 children in home	96.6	91.8	0.54	701	743	0.26
Urban home (vs. Suburban)	90.1	96.2	0.33	707	772	0.031
Single family (vs. Multifamily)	92.8	92.6	0.98	755	717	0.22
Mold	94.0	91.8	0.73	738	728	0.75
Water leaks	102	86.6	0.017	729	740	0.73
AC use (yes or no)	92.6	94.4	0.86	737	712	0.63
AC use (more than 2 months)	86.6	102.4	0.021	730	757	0.40
Pets	101	86.2	0.015	772	704	0.024

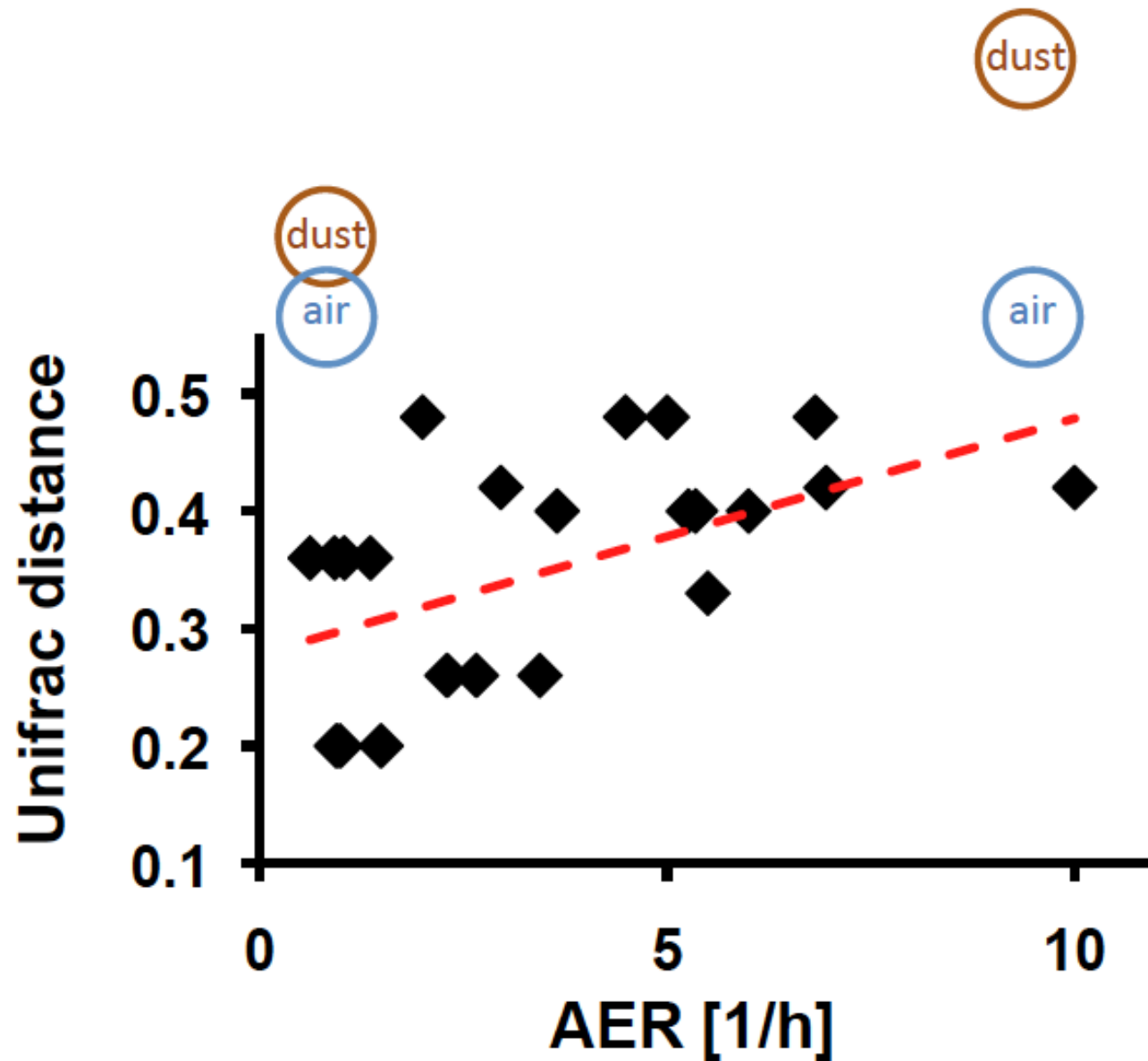
Dogs change the microbiome of your home



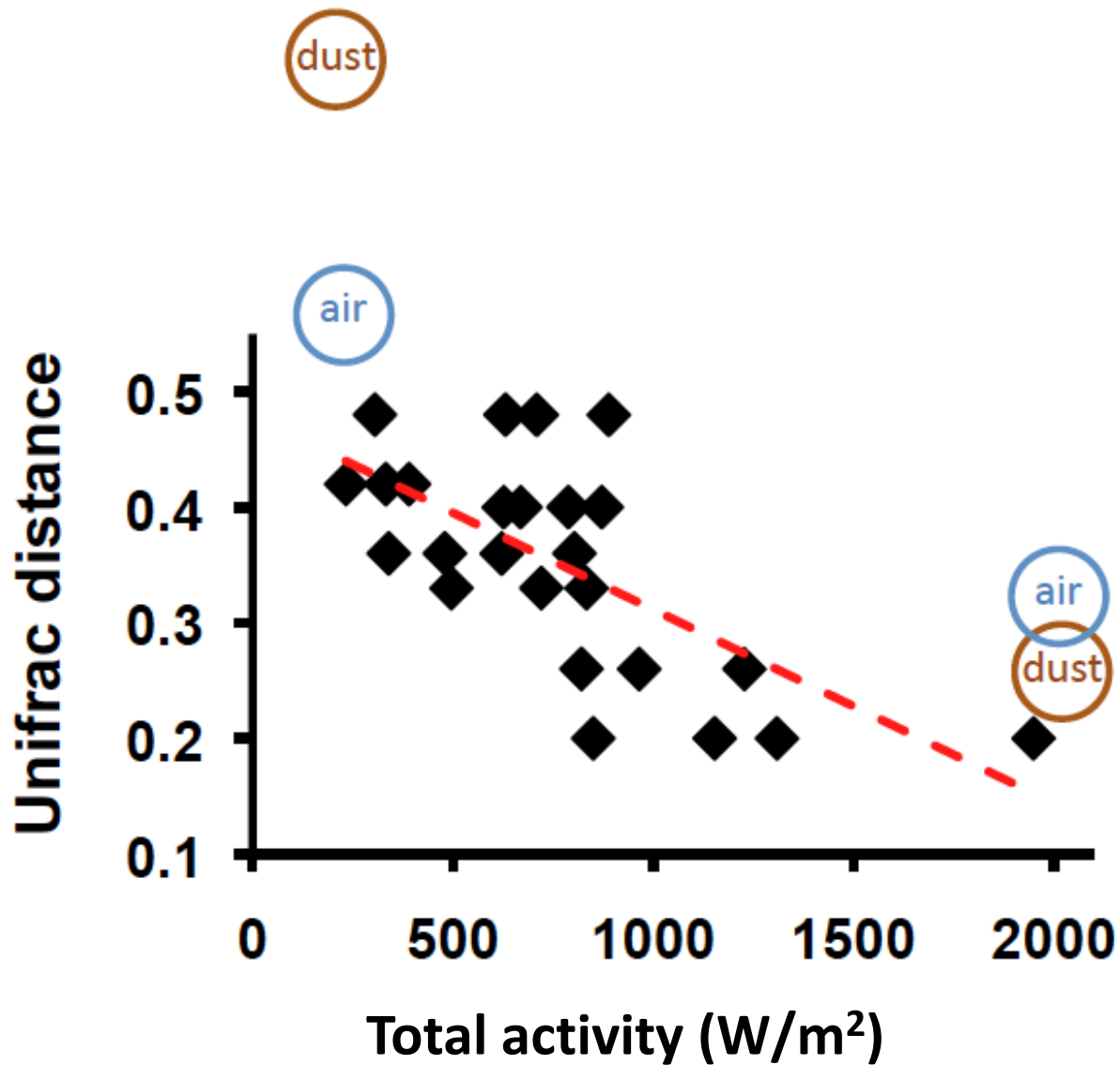
indoor outdoor ratio-occupancy



Ventilation correlation

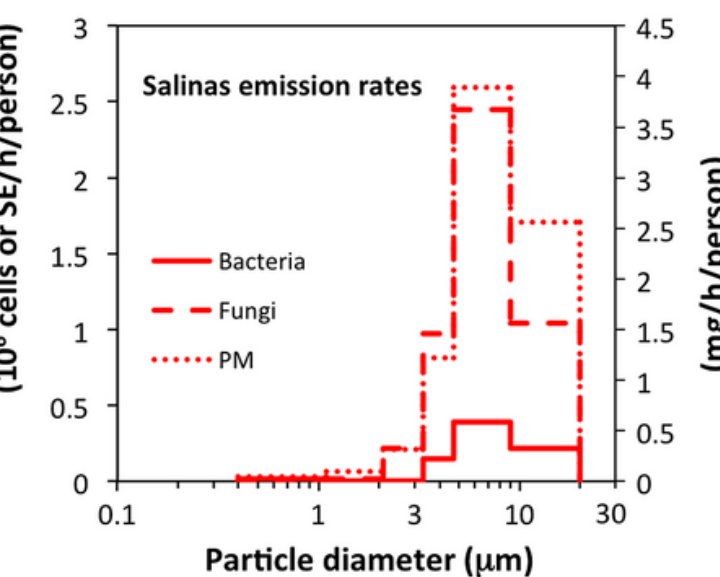
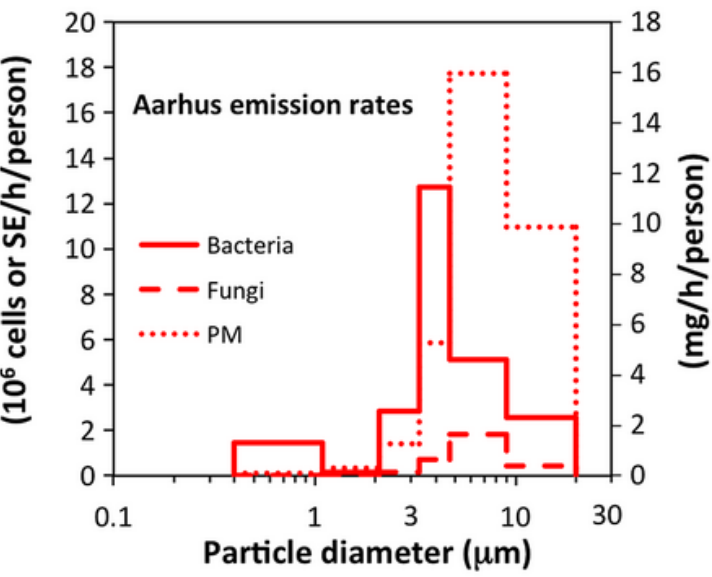
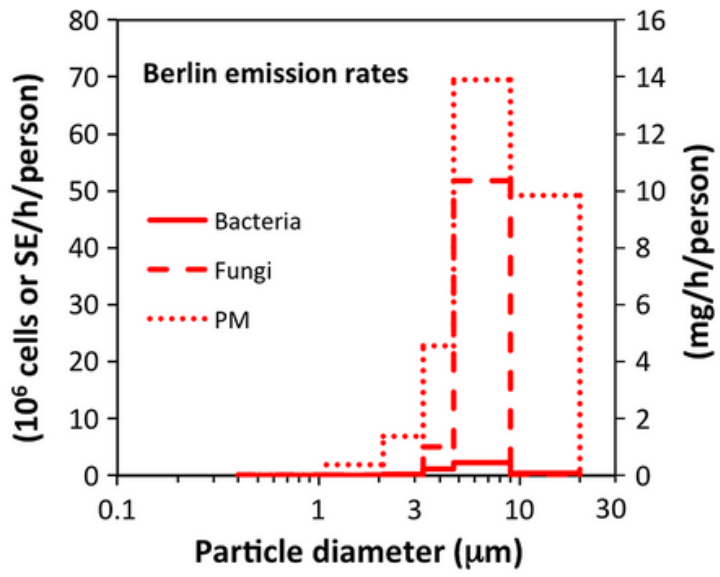
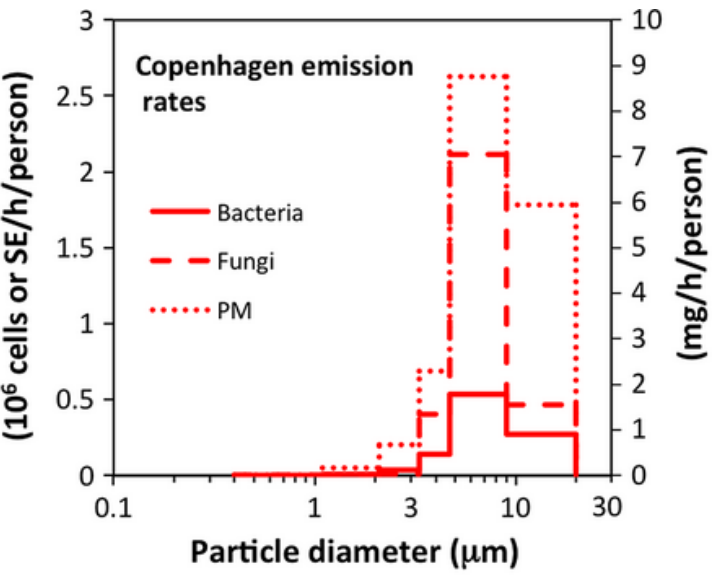


Activity correlation



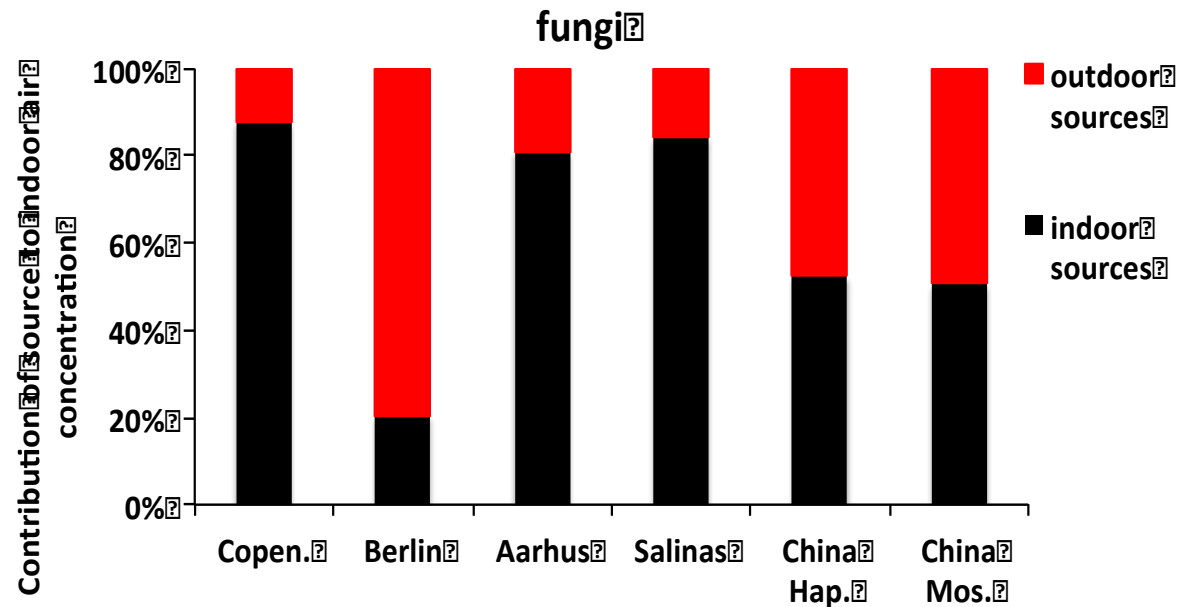
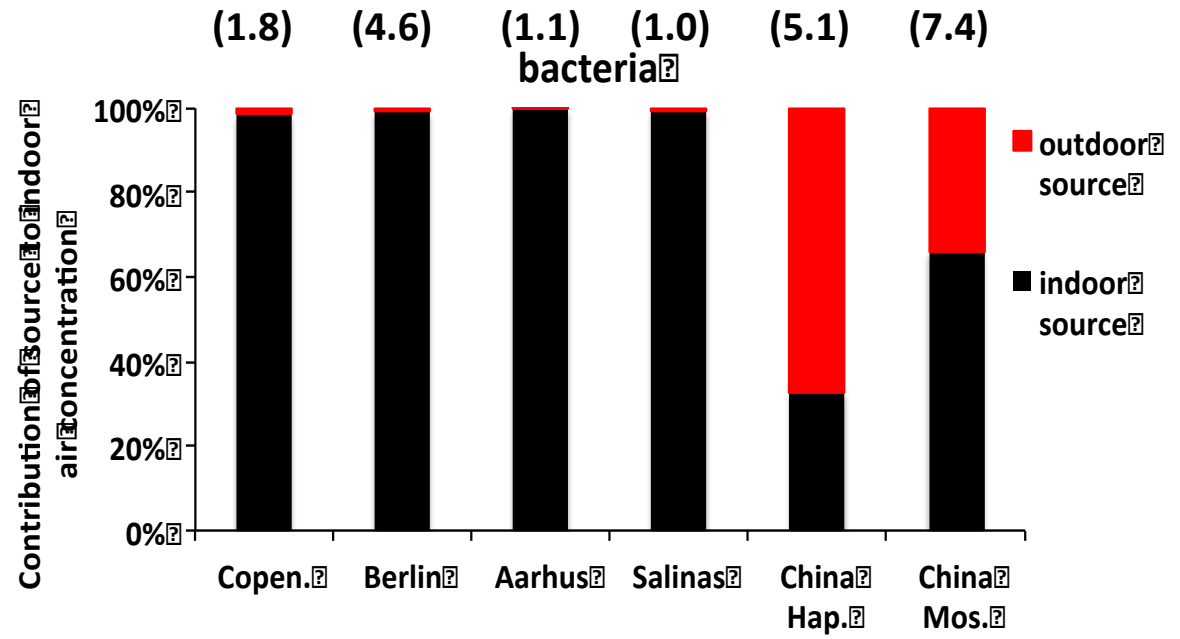
Mechanistic approaches: emissions

$$QC_{out} + NE = QC + kVC$$

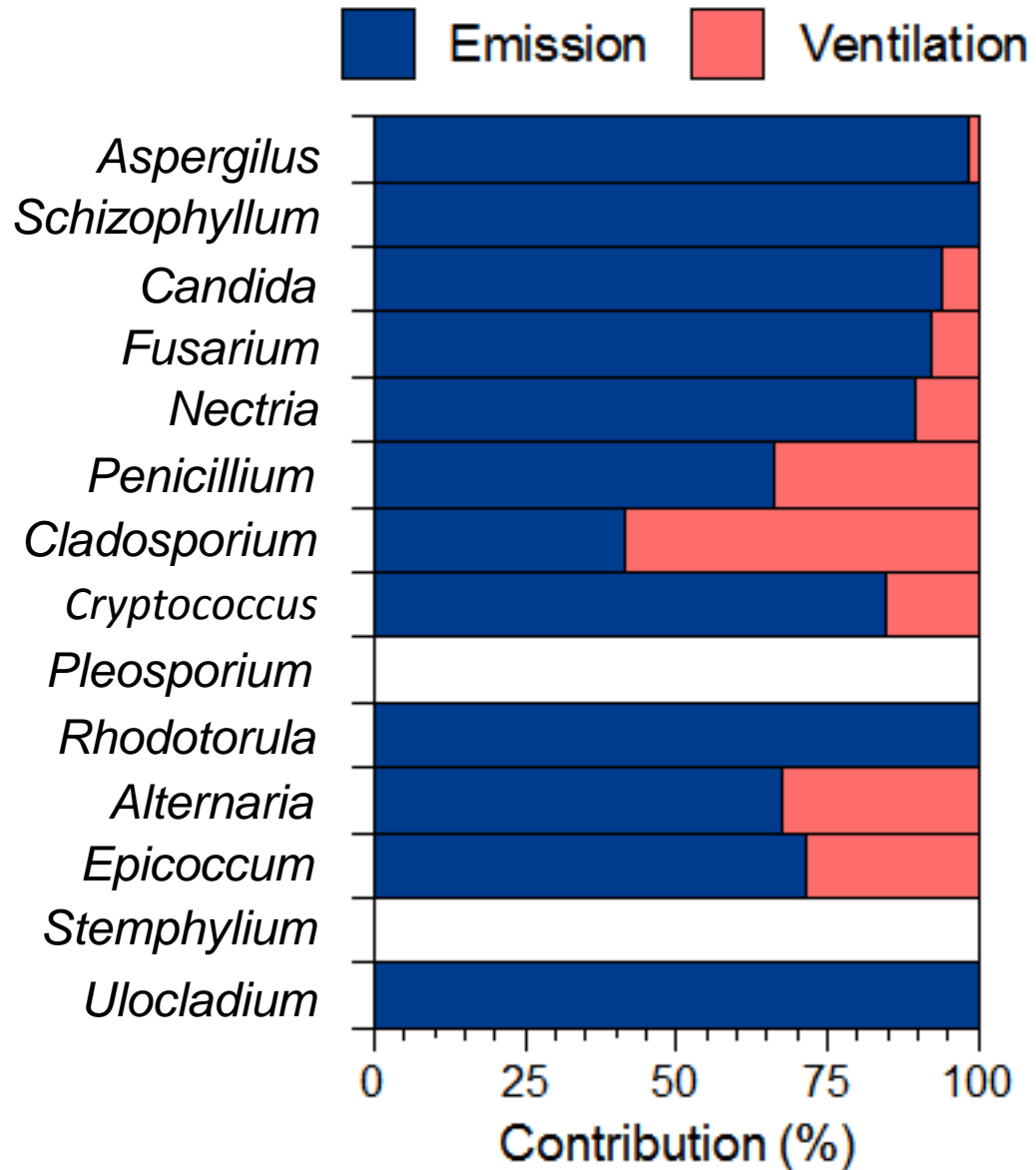


Hospodsky et al., (2014), Indoor Air

Impacts of ventilation?



Apportionment of sources of fungi in buildings



How close are we to being able to model and predict exposures?



Infiltration, exfiltration,
mechanical ventilation,
filtration (*AER*, *outdoor concentrations*,
penetration and filtration efficiency)

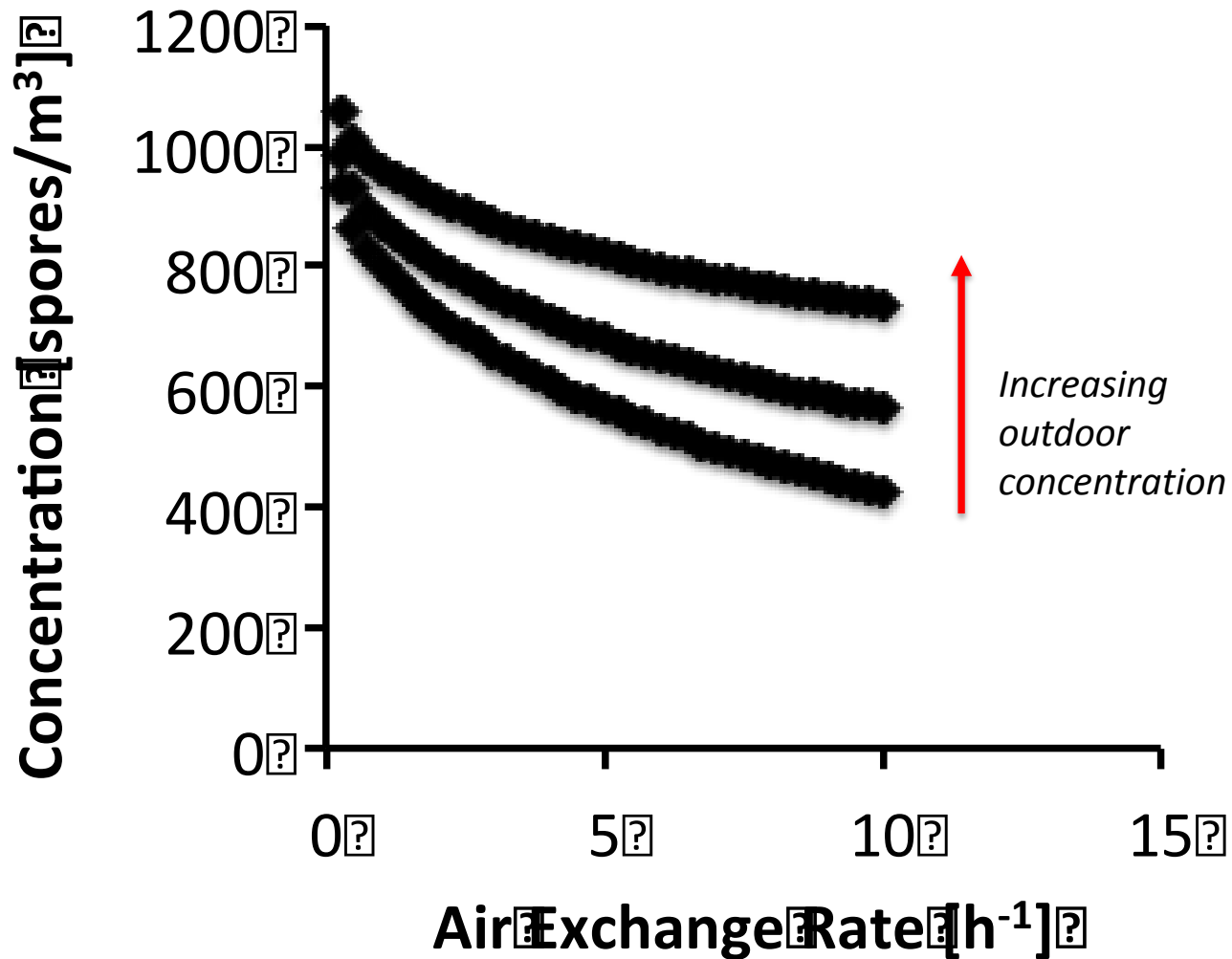


Resuspension
(*occupancy, metabolic activity, shedding rates, surface materials, surface concentrations, resuspension factor*)

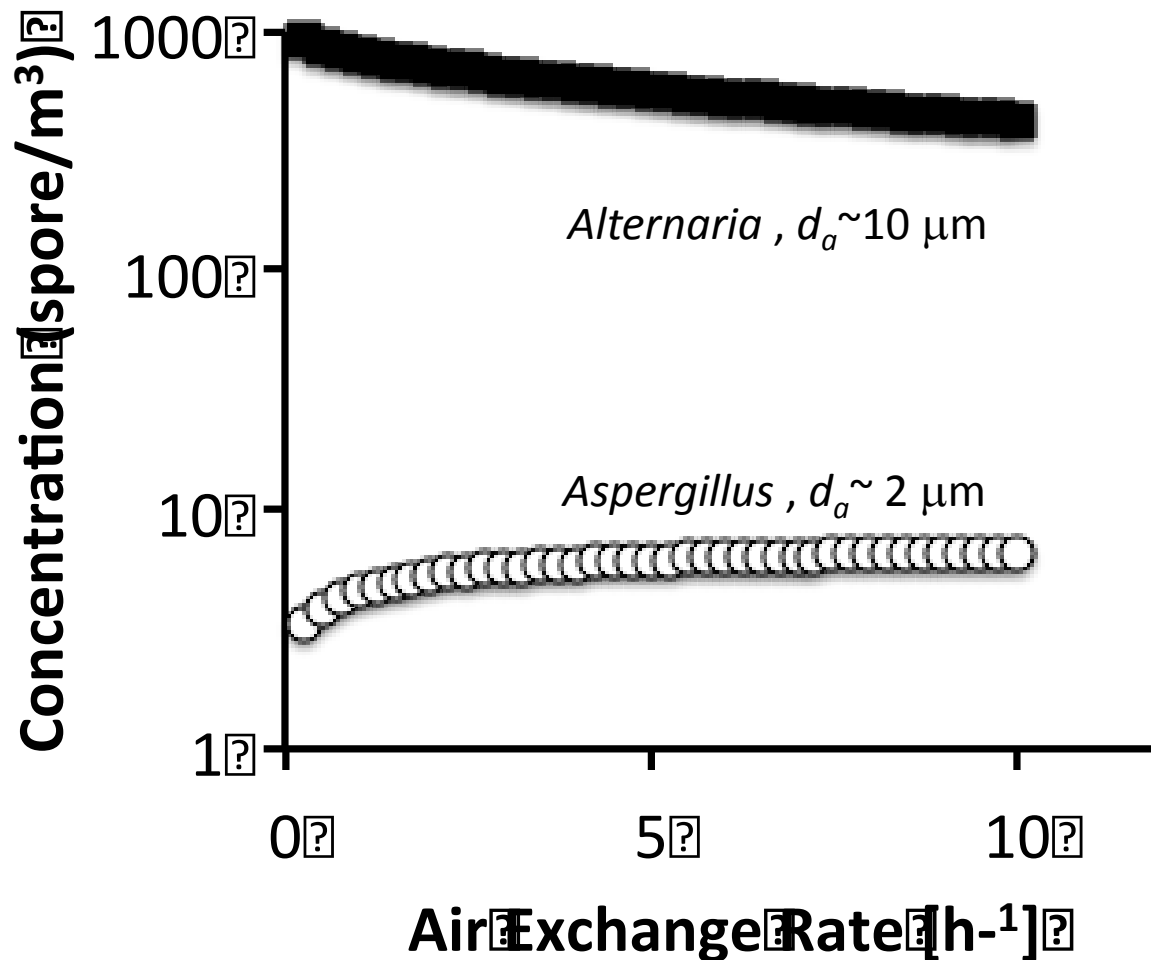


Deposition (***rate coefficient***)

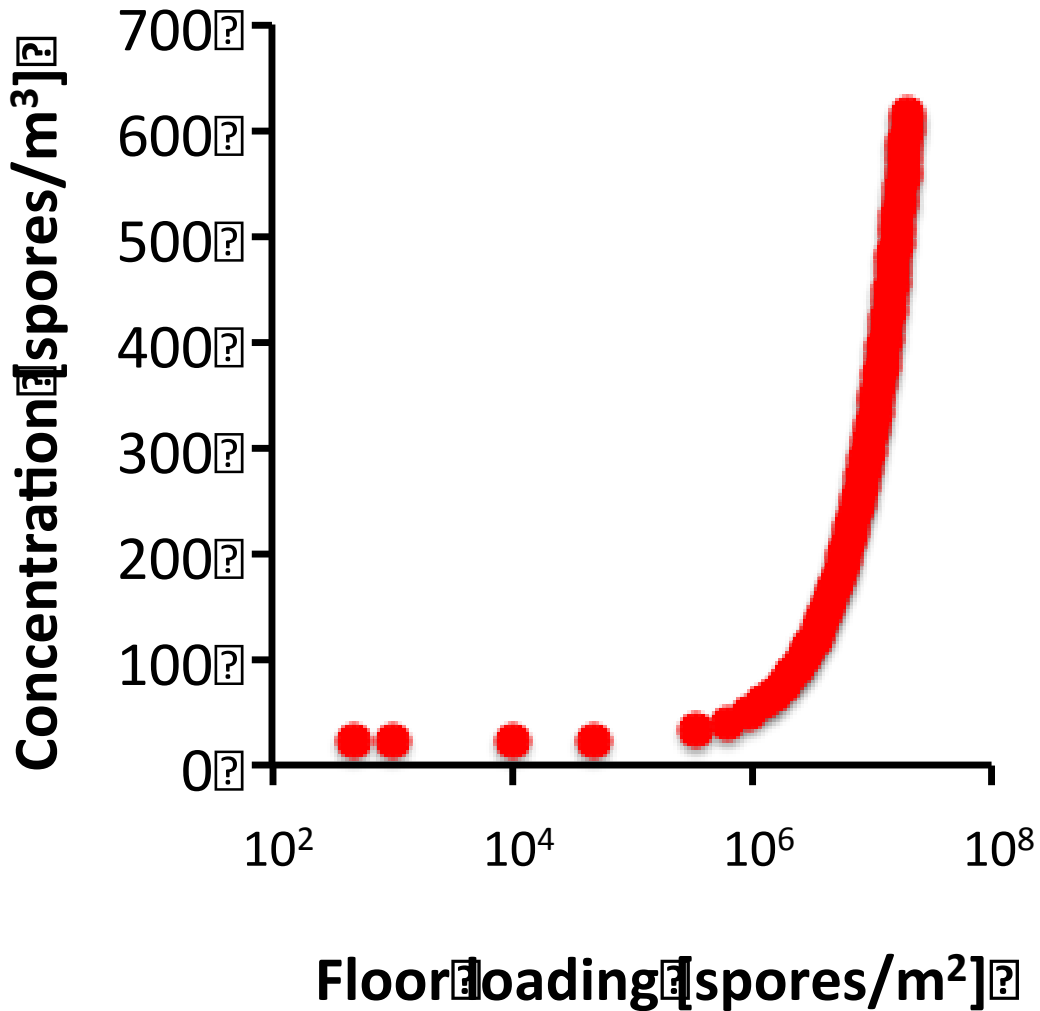
Impact of outdoor air concentration on indoor air concentration



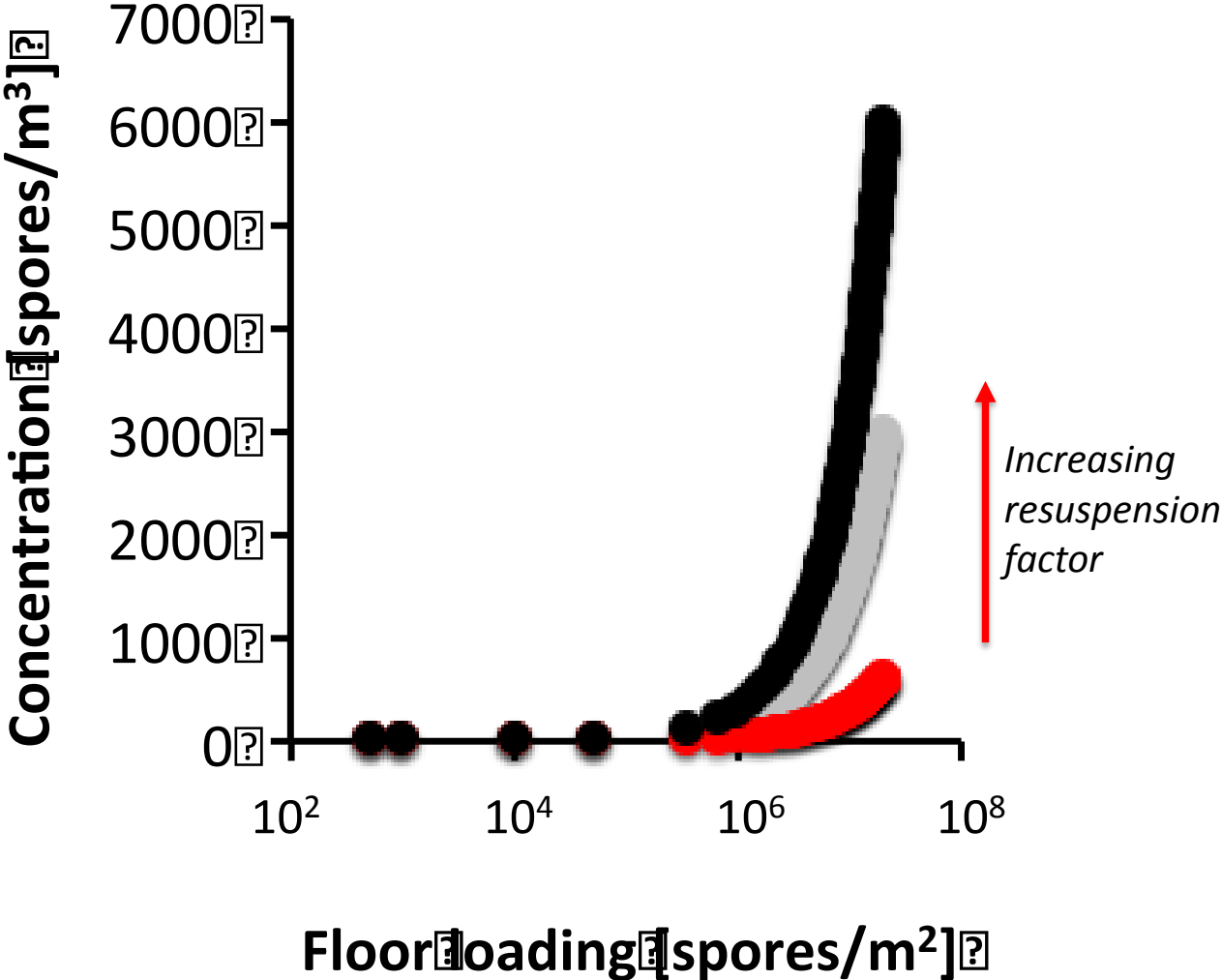
Different paths for different microbes



Floor loading can strongly influence indoor air exposure



Flooring material impacts indoor air exposures



Conclusions

- **Asthma development associated with low fungal diversity (richness)**

- **Once health-relevant microbial targets are met, rational, mechanistic approaches can be used as a basis to design buildings that encourage or control exposures**

For more Information contact:

Jordan.Peccia@yale.edu



Peccia Lab:

Karen Dannemiller

Naomichi Yamamoto

Denina Hospodski

Jing Qian

Collaborators:

William Nazaroff, UC Berkeley

Mark Mendell, CDPH

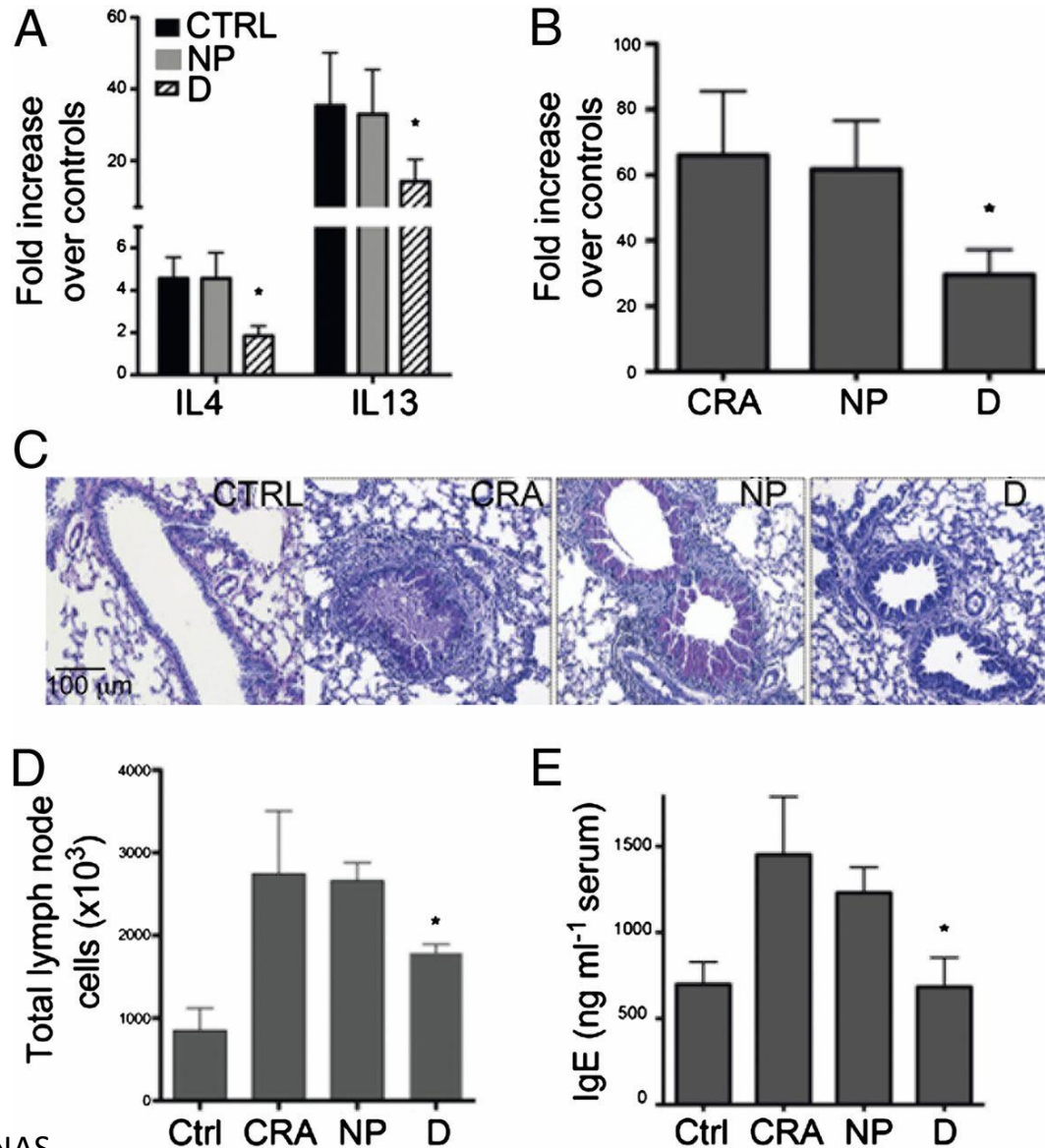
Janet Macher, CDHP

Kazukiyo Kumagai, CDPH

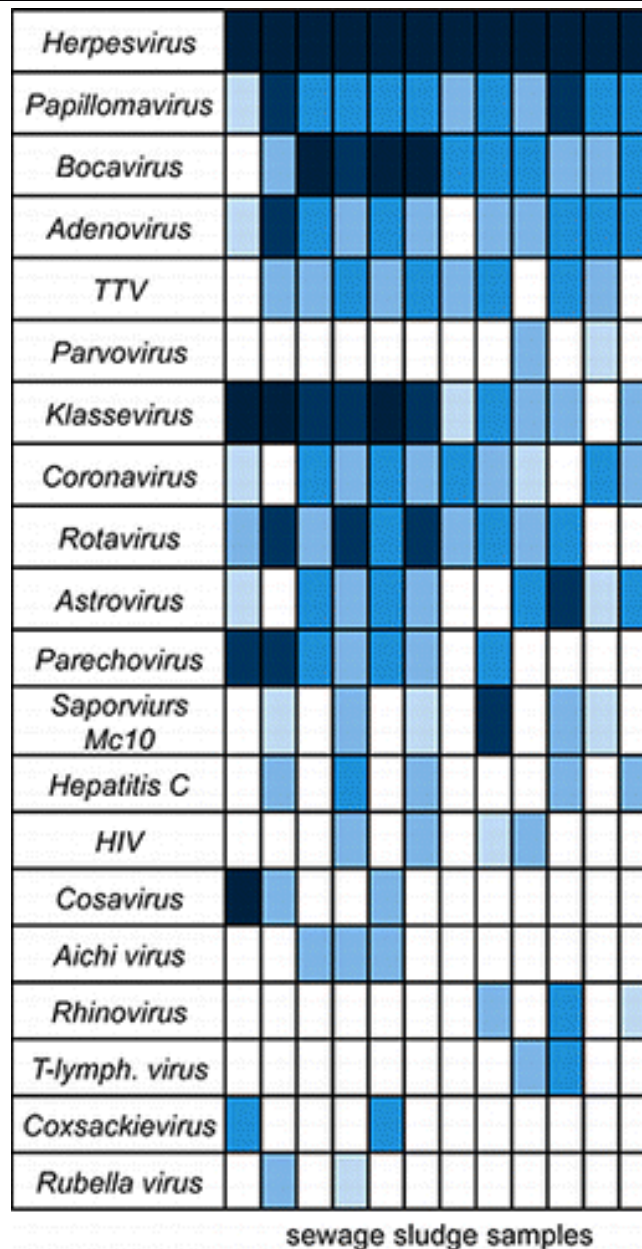
CHAMACOS cohort, UC Berkeley

Leaderer group, Yale Public Health

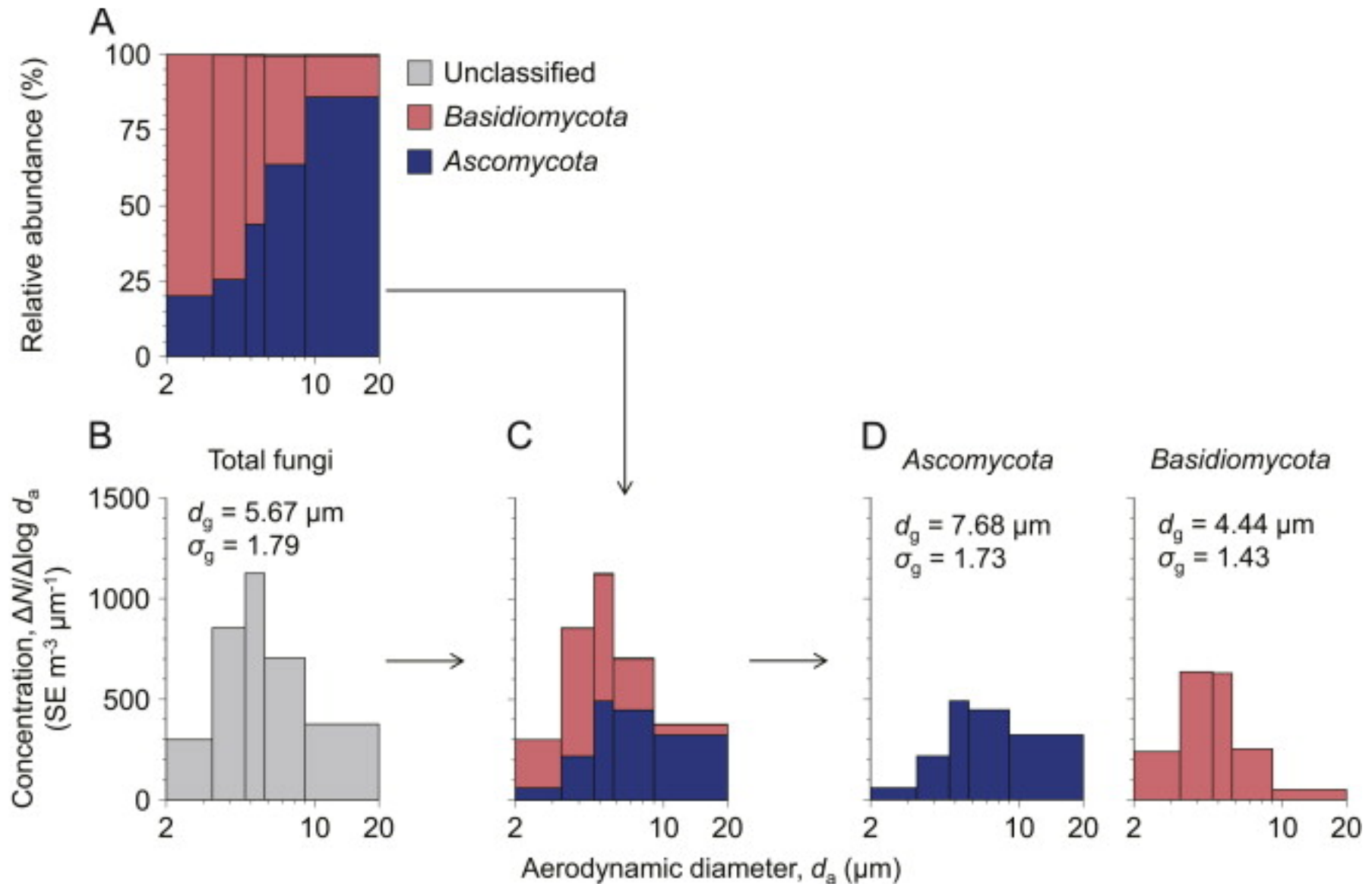
Exposure to dust from homes with dogs attenuates the development of allergen-induced airways



Metagenomics to get to bacteria and viral pathogens and functional genes



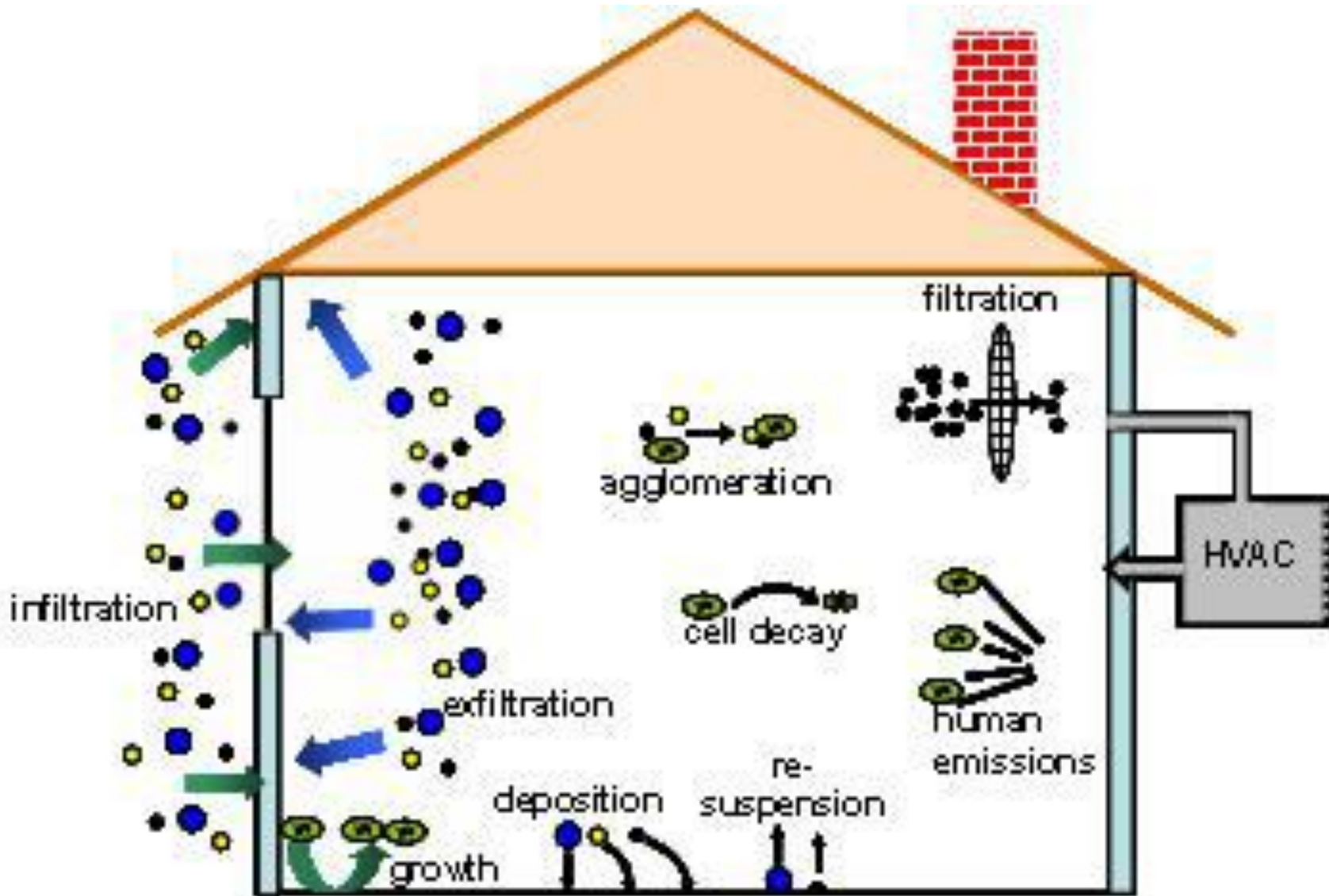
Taxa-specific aerodynamic diameter



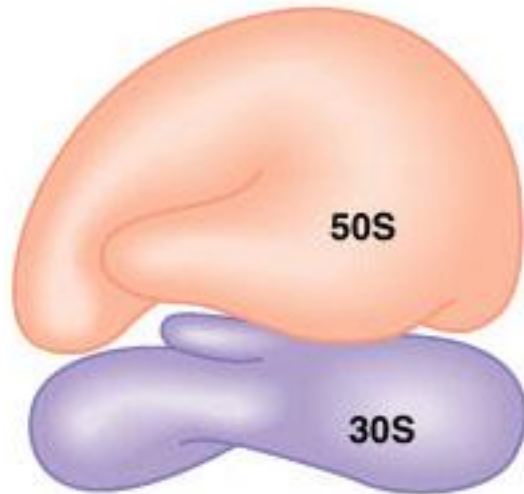
Future Directions

- **Air conditioners and microbial emissions**
- **Growth and gene function of microbes on building materials**
- **Improving frameworks for classifying mold in homes**
- **Ventilation and microbes in schools**

Particle dynamics and the indoor microbiome



Structure for Ribosomal RNA:

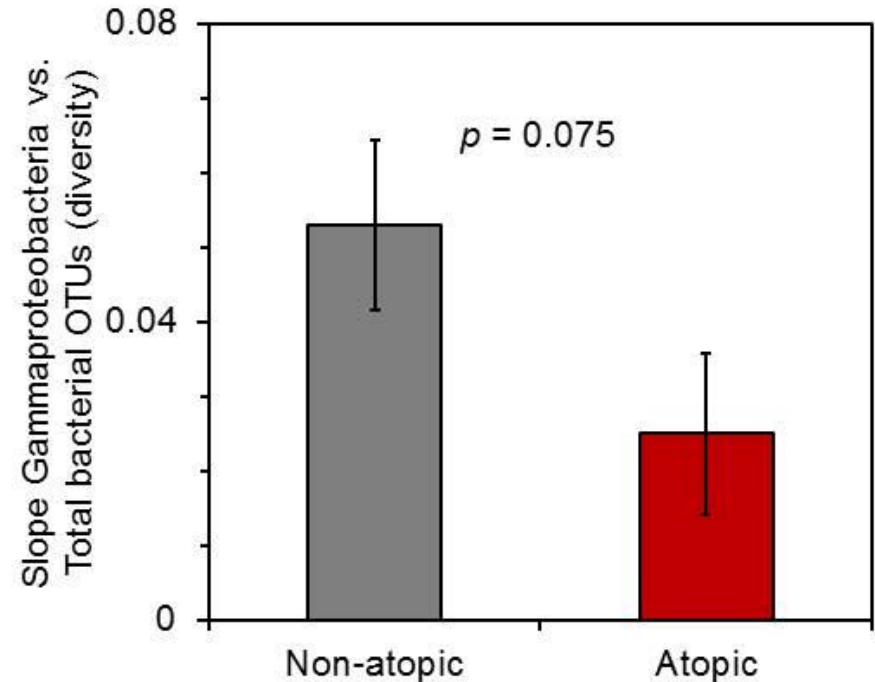
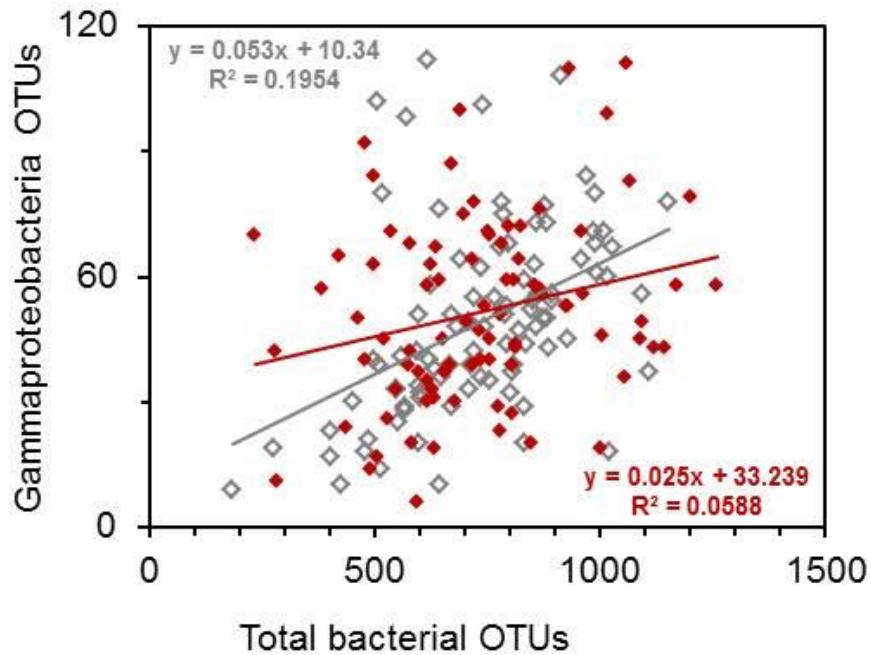


	<u>Eukaryotes</u>	<u>Bacteria</u>
Total	80S size	70S size
LSU	60S	50S
SSU	40S	30S
LSU rRNA	5.8S, 28S	5S, 23S
SSU rRNA	18S	16S

transcribed intragenic spacer regions (important for fungi)



Gamma Proteobacteria diversity and allergic status



“Compared with healthy individuals, atopic individuals had lower environmental biodiversity in the surroundings of their homes and significantly lower diversity of γ -Proteobacteria on their skin.” Hanske, et al. PNAS 2012

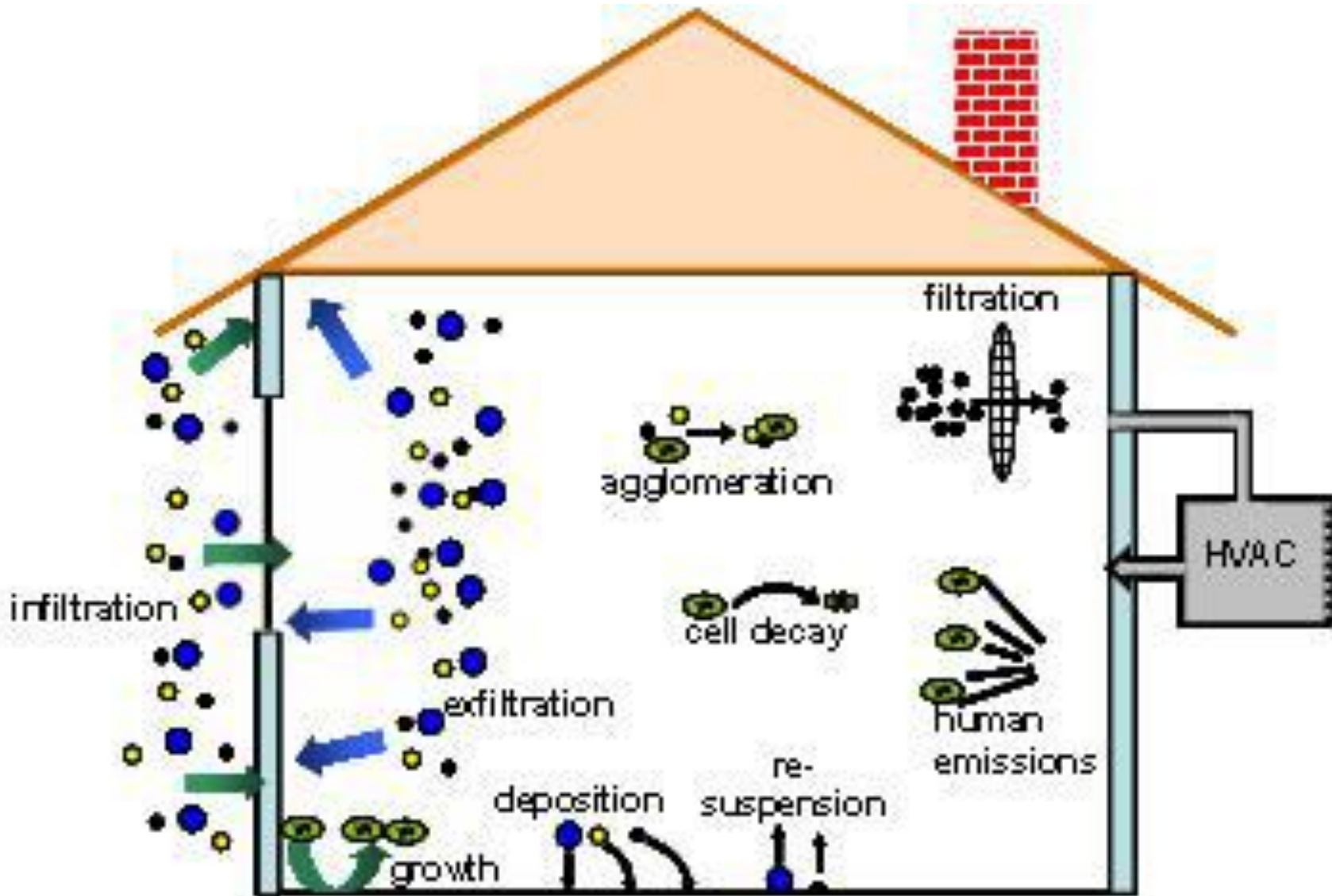
Different features of the microbial communities were associated with SEVERITY based on asthma sub-type

	All children OR (95% CI)	Atopic children OR (95% CI)	Non-atopic children OR (95% CI)
<i>Fungi</i>			
Low fungal richness	1.29 (0.70-2.38)	1.05 (0.45-2.46)	1.61 (0.66-3.93)
Allergenic species	2.53 (1.28-5.00)	2.71 (0.99-7.39)	2.38 (0.94-6.01)
Allergenic genera	1.79 (0.92-3.45)	2.55 (0.97-6.67)	1.27 (0.51-3.18)
Fungal concentration	2.02 (1.14-3.56)	1.69 (0.77-3.75)	2.40 (1.06-5.44)
(1–3)- β -D-glucan	0.55 (0.24-1.26)	0.47 (0.13-1.68)	0.60 (0.20-1.83)

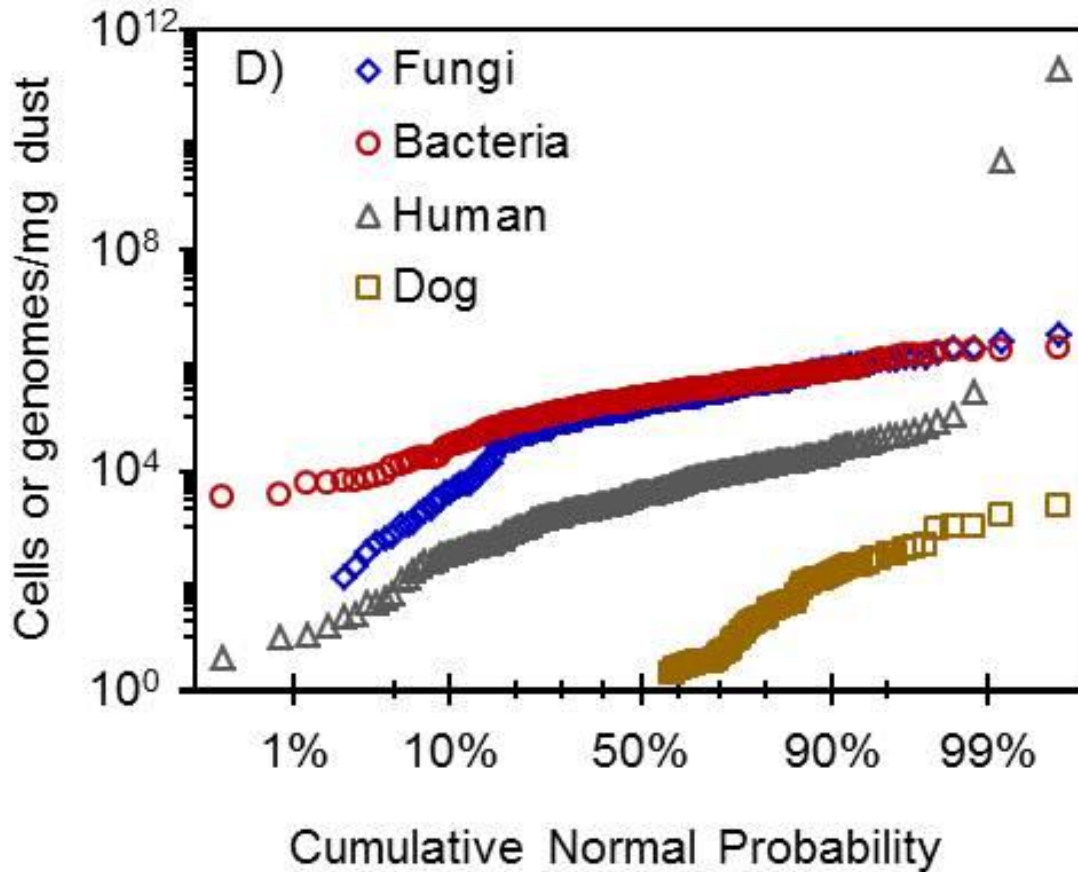
Taxa associated with allergic asthma

Taxa associated with severe asthma in allergic children		
Taxon	<i>p</i> - value	<i>q</i> - value
Species		
<i>Volutella colletotrichoides</i>	0.0003	0.12
<i>Thelebolus microsporus</i>	0.002	0.41
<i>Cryptococcus nyarrowii</i>	0.007	0.66
<i>Trichosporon porosum</i>	0.01	0.72
<i>Candida galli</i>	0.02	0.72
<i>Cryptococcus skinneri</i>	0.02	0.72
<i>Scoliciosporum umbrinum</i>	0.040	0.72
<i>Mycocalicium victoriae</i>	0.042	0.72
<i>Cryptococcus podzolicus</i>	0.042	0.72
<i>Teratosphaeria ohnowa</i>	0.042	0.72
<i>Lambertella tubulosa</i>	0.046	0.72
<i>Pseudaegerita viridis</i>	0.047	0.72
<i>Rhizocarpon petraeum</i>	0.047	0.72
<i>Leptosphaerulina americana</i>	0.047	0.72
<i>Epicoccum nigrum</i>	0.047	0.72
Genus		
<i>Volutella</i>	0.0001	0.044
<i>Thelebolus</i>	0.0161	0.66
<i>Lambertella</i>	0.0258	0.66
<i>Cochliobolus</i>	0.0367	0.66
<i>Cordyceps</i>	0.0474	0.66
<i>Phaeoconiella</i>	0.0474	0.66
<i>Phialocephala</i>	0.0474	0.66
<i>Rhizocarpon</i>	0.0474	0.66
<i>Epicoccum</i>	0.0474	0.66

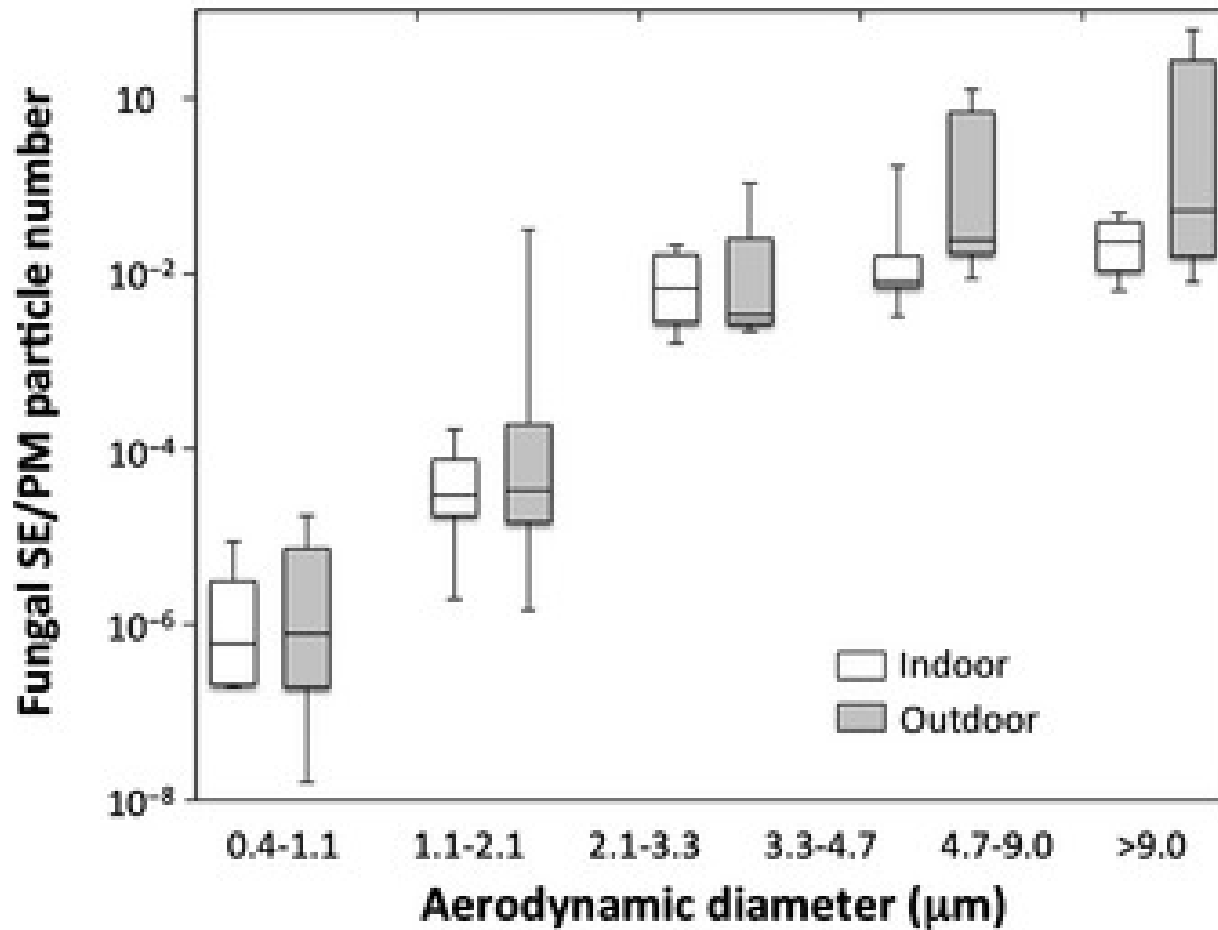
Design



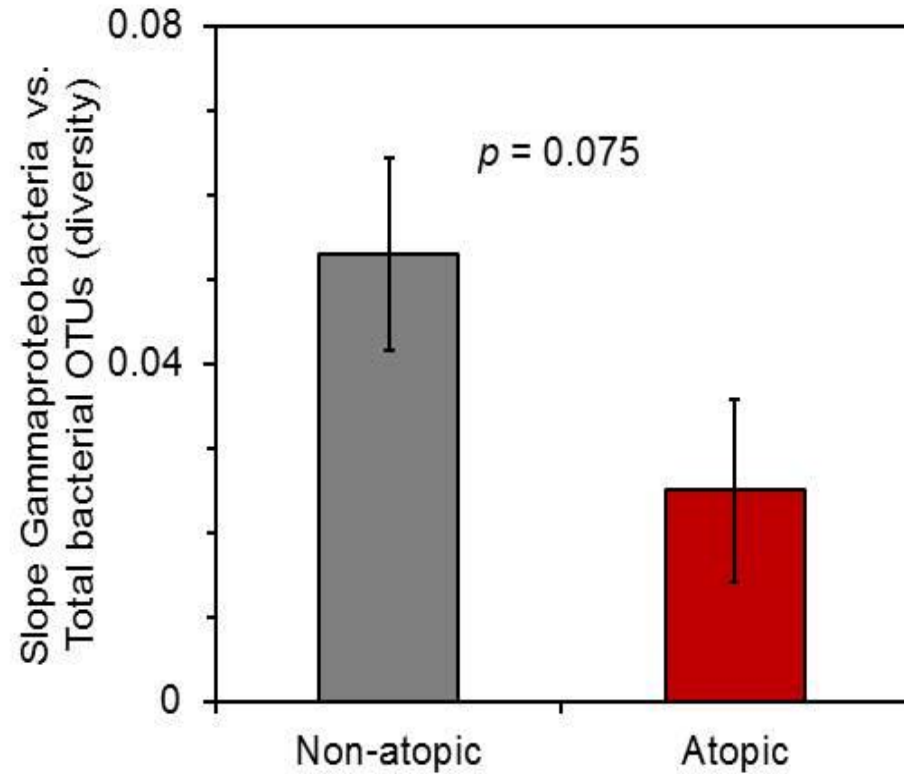
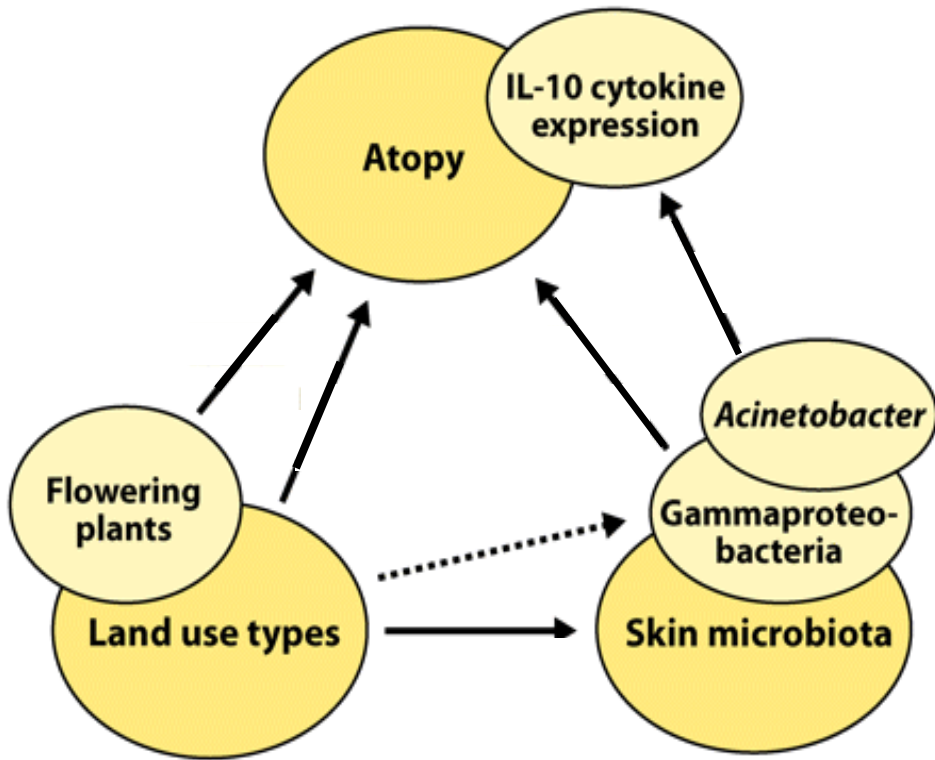
Housing factors that influence diversity



How close are we to modeling?



Allergy and the environment



200 Home study



with Brian Leaderer, Janneane Gent
Yale School of Public Health

STAR EXIT INTERVIEW

3/17/08

STUDY #:

MONITORING PERIOD 5 MP

RA: _____ EXTRA

DATE OF HOME INTERVIEW: MONTH DAY YEAR
EHIDMN EHIDDY EHDYR

DATE OF LAST SUCCESSFUL INTERVIEW: MONTH DAY YEAR
ELCDMN ELCCDY ELCDYR

DATE OF EXIT INTERVIEW: MONTH DAY YEAR
EXDMN EXDDY EXDYR

Time Interview Began: EXSTMH EXSTMM EXSAP

CODER'S INITIALS _____

Time Interview Ended: EXETMH EXETMM EXEP

NUMBER OF ATTEMPTS TO REACH RESPONDENT EXNT

RA: Was the Respondent named on the assignment card the person who completed this interview?
(IF NO - MAKE CHANGES TO ASSIGNMENT CARD AND DESCRIBE CHANGE IN COMMENTS) ERAR

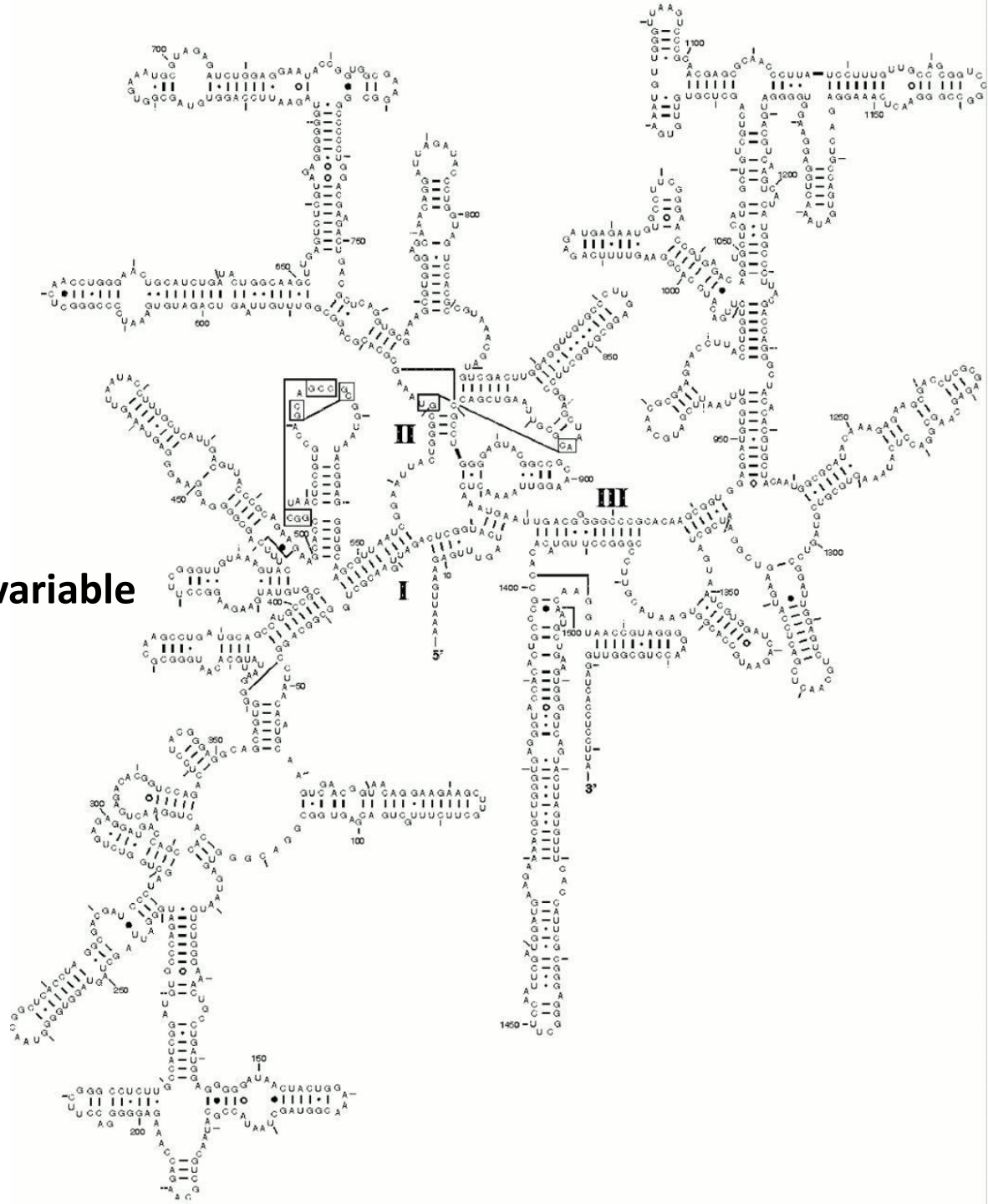
(FOR ALL QUESTIONS)
 No Response
 Don't Know DM

CENTER FOR PERINATAL, PEDIATRIC AND ENVIRONMENTAL EPIDEMIOLOGY
YALE UNIVERSITY SCHOOL OF MEDICINE
ONE CHURCH STREET, 6TH FLOOR, NEW HAVEN, CT 06510
Phone: 203-764-9375 or 1-800-41-INFANT (1-800-414-6326); Fax: 203-764-9378

- 50 allergic asthma, severe
- 50 allergic asthma, non-severe
- 50 non-allergic asthma, severe
- 50 non-allergic asthma, non-severe

Describing microbial communities

- ~1500 nucleotides long
- a structural portion of the ribosome
- present in all organisms
- evolved slowly and includes conserved, variable and hypervariable

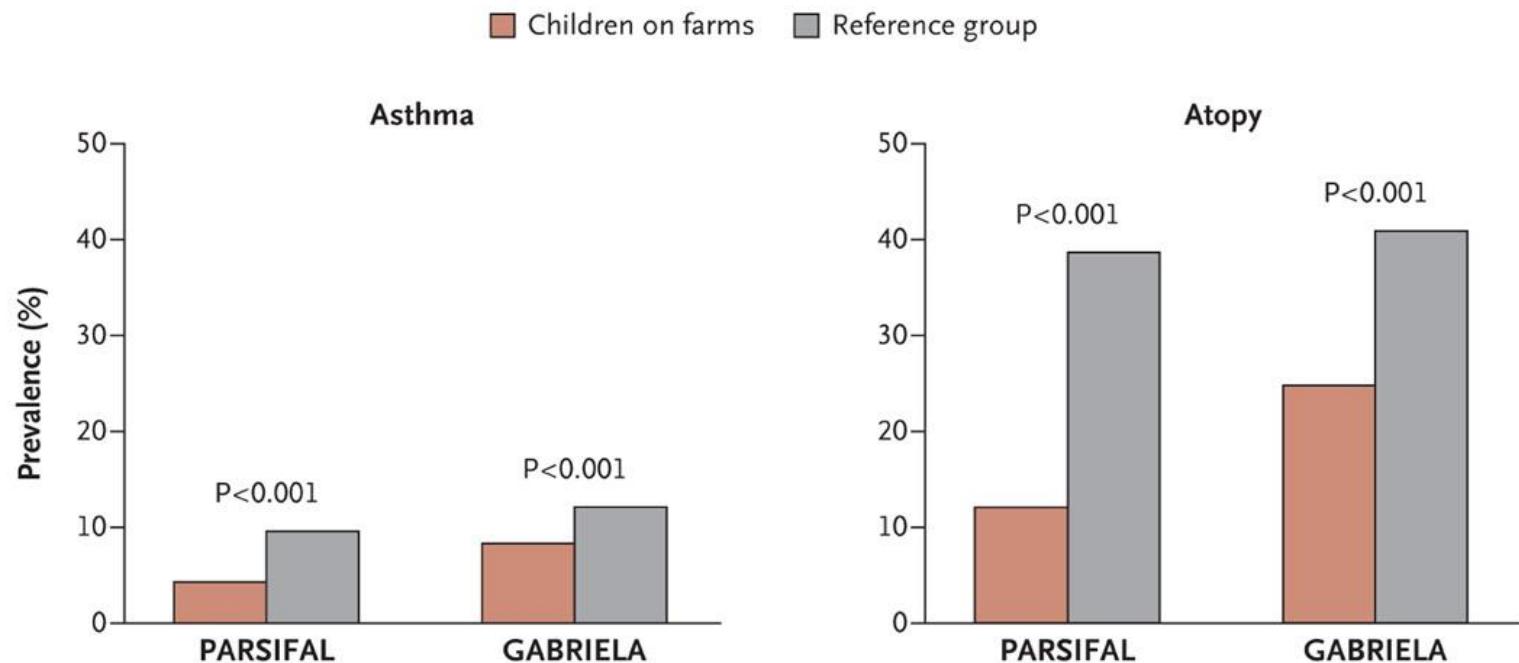


Asthma, allergy and the environment

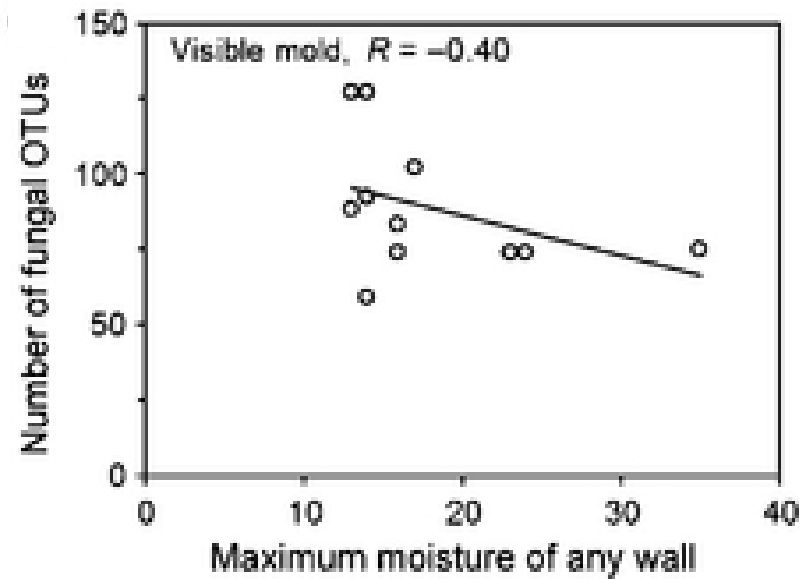
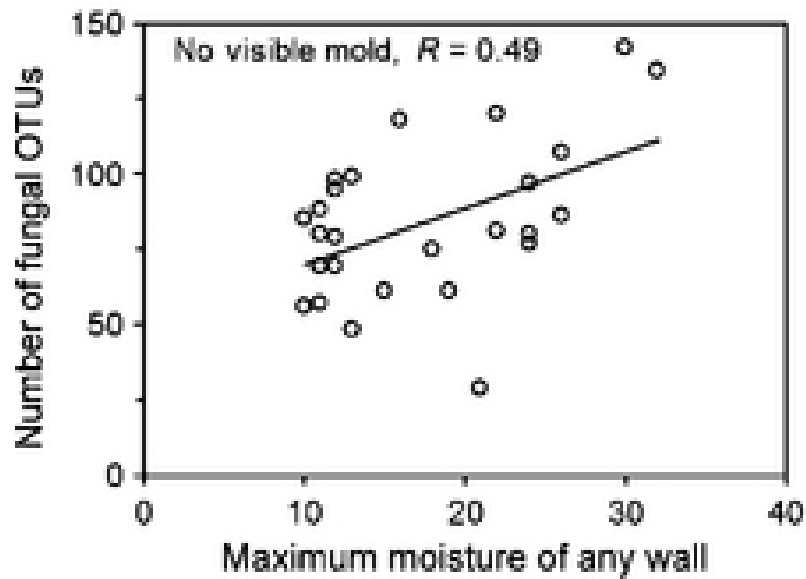
- Microbial empirical associations between allergy and asthma development and the environment are moving in a “more defined” direction:

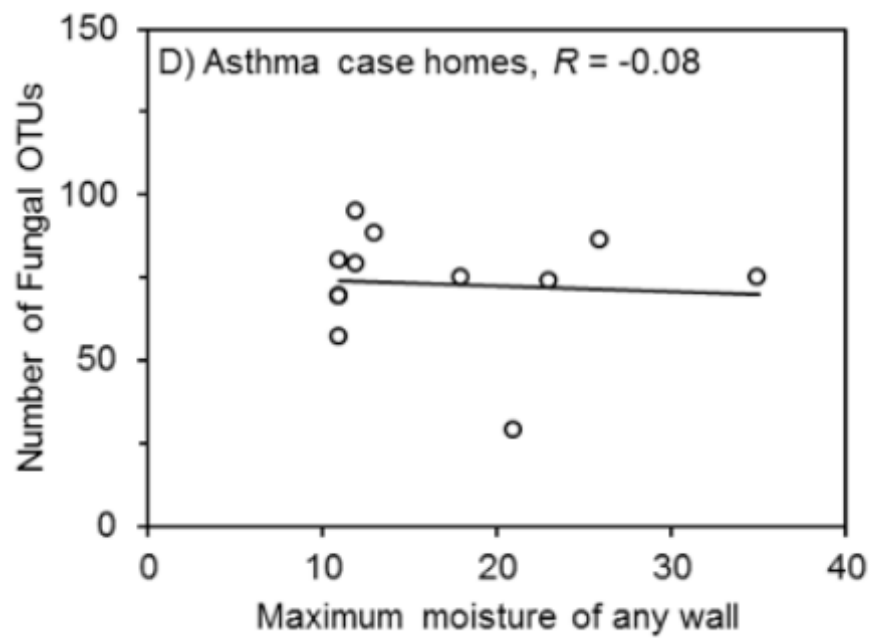
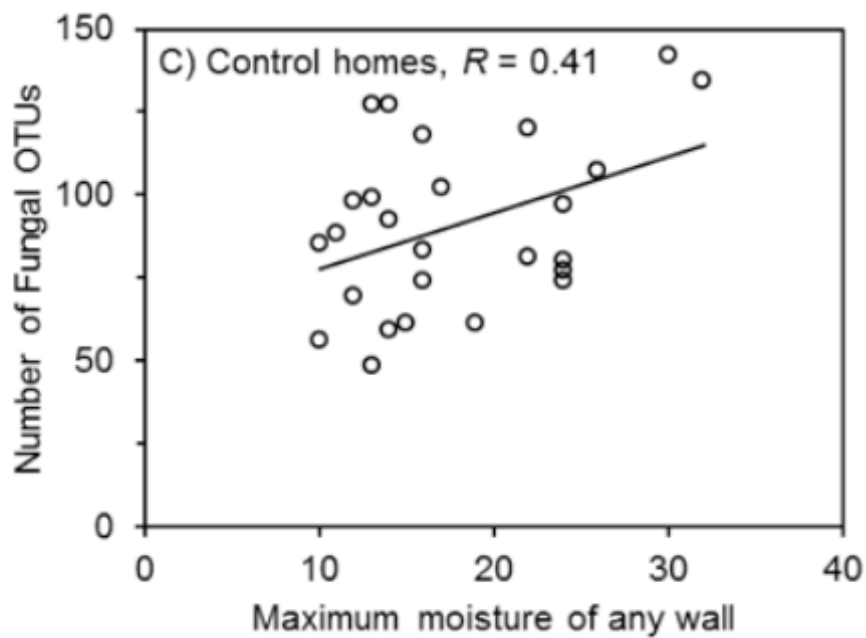
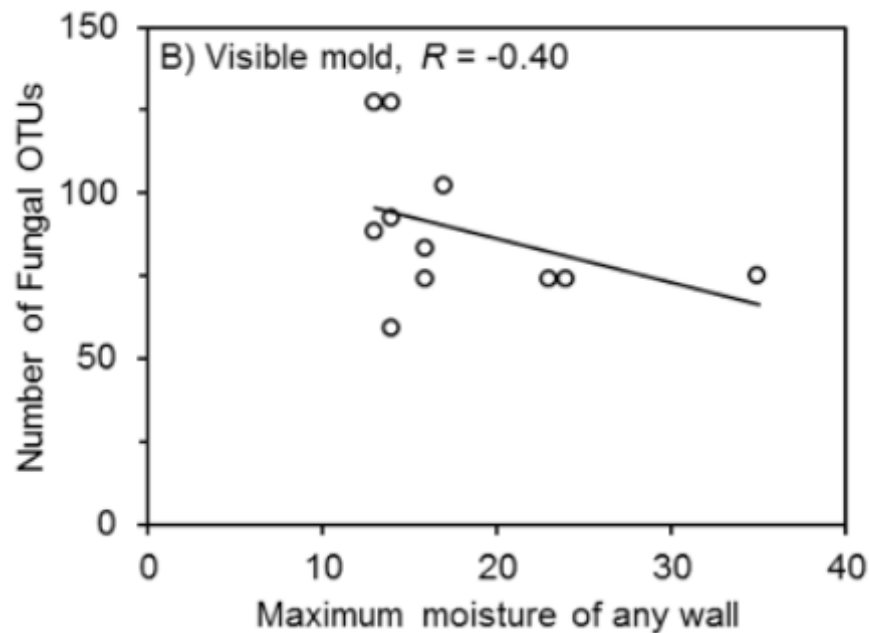
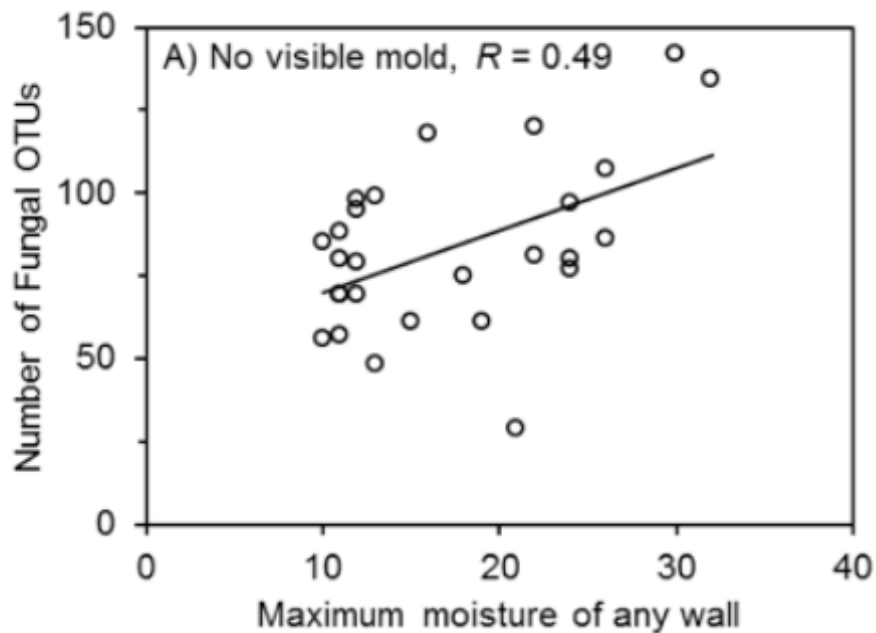
Living on a farm
Owning animals (pets)
Living in a rural environment
Drinking unpasturized milk

> protective



Further observations on moisture and diversity





Evolution

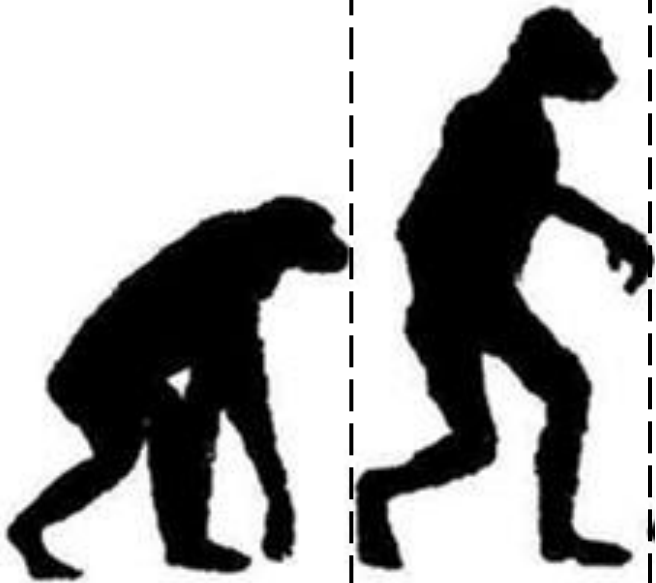
Culturing



Evolution

Culturing

**DNA
methods**

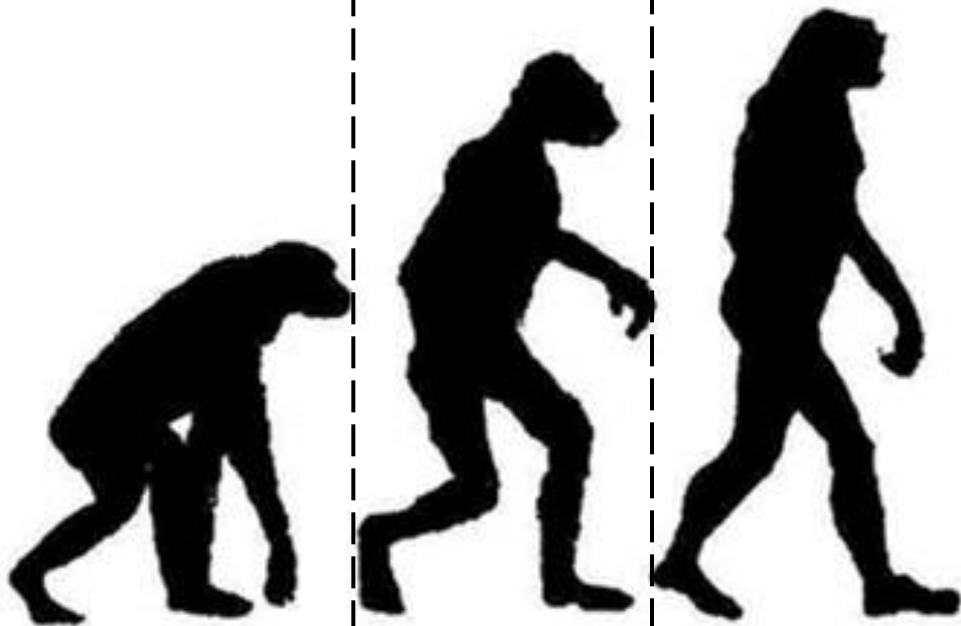


Evolution

Culturing

**DNA
methods**

**Build
catalogs**



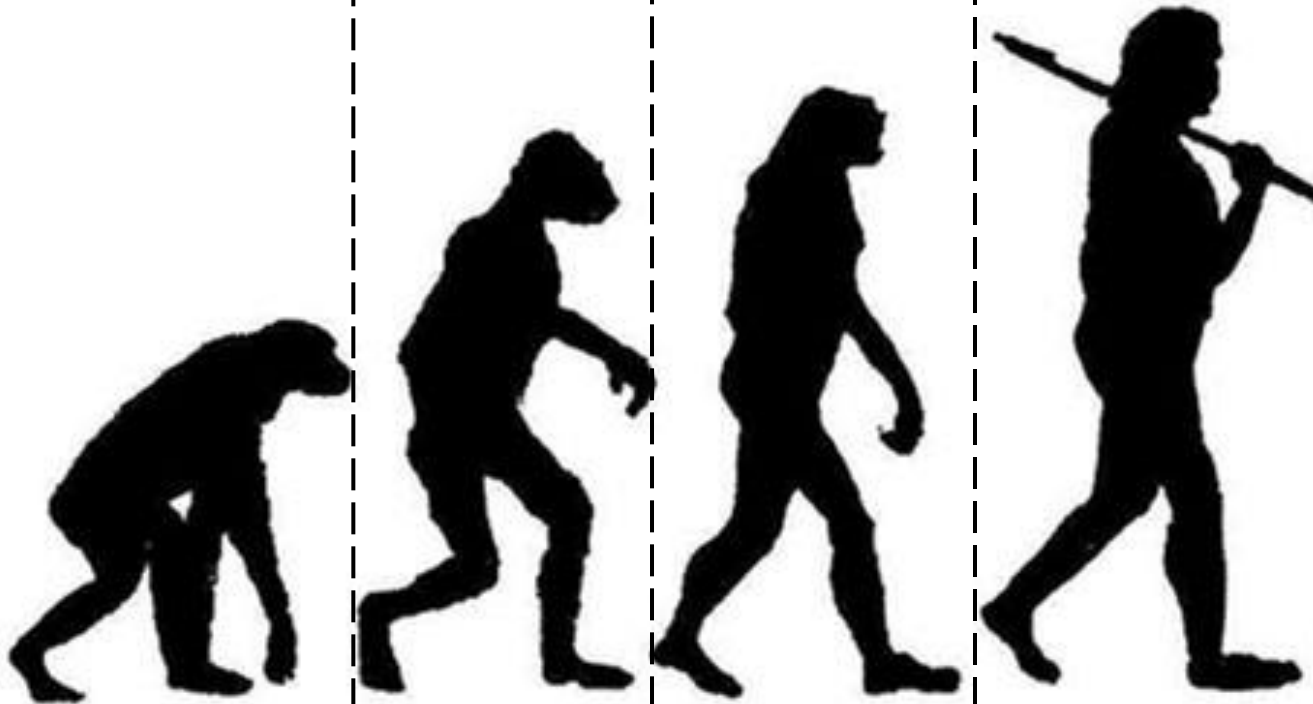
Evolution

Culturing

**DNA
methods**

**Build
catalogs**

**Empirical
association
between
microbes
and
buildings**



Evolution

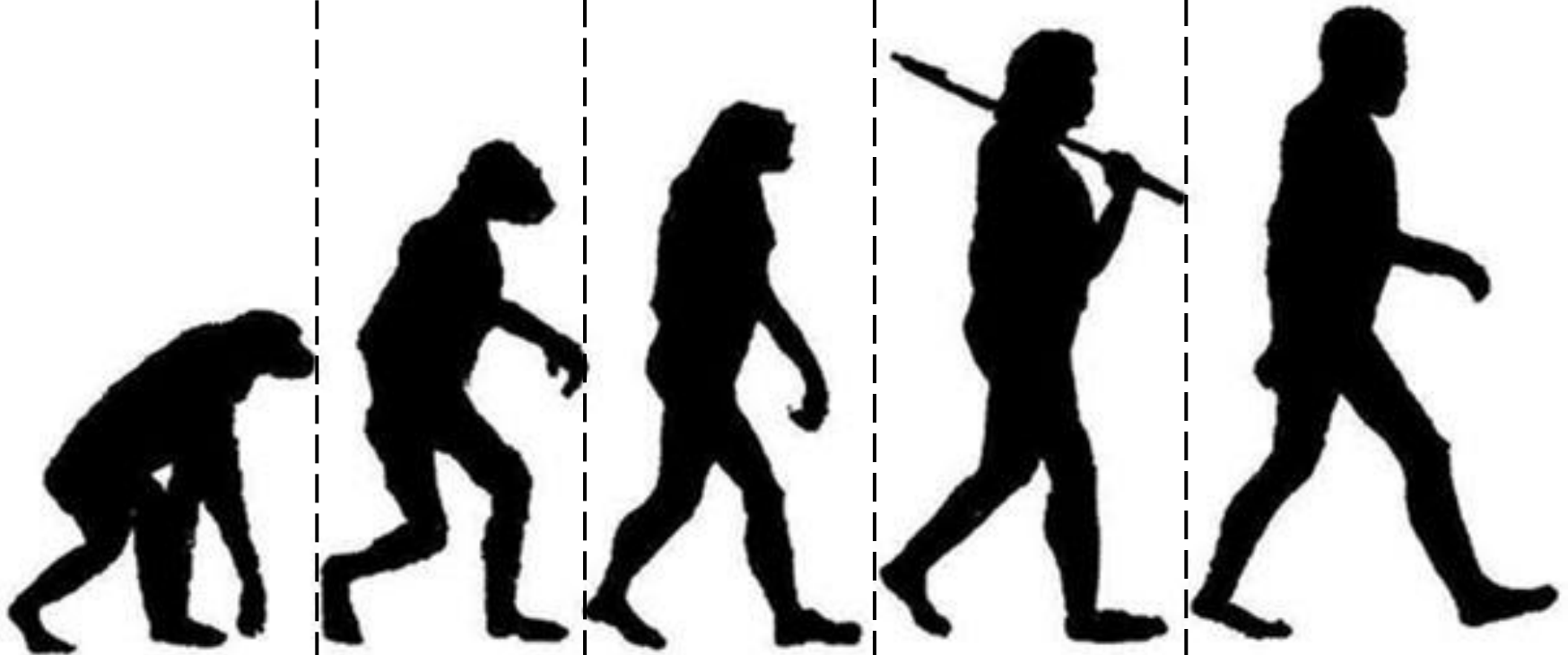
Culturing

**DNA
methods**

**Build
catalogs**

**Empirical
association
between
microbes
and
buildings**

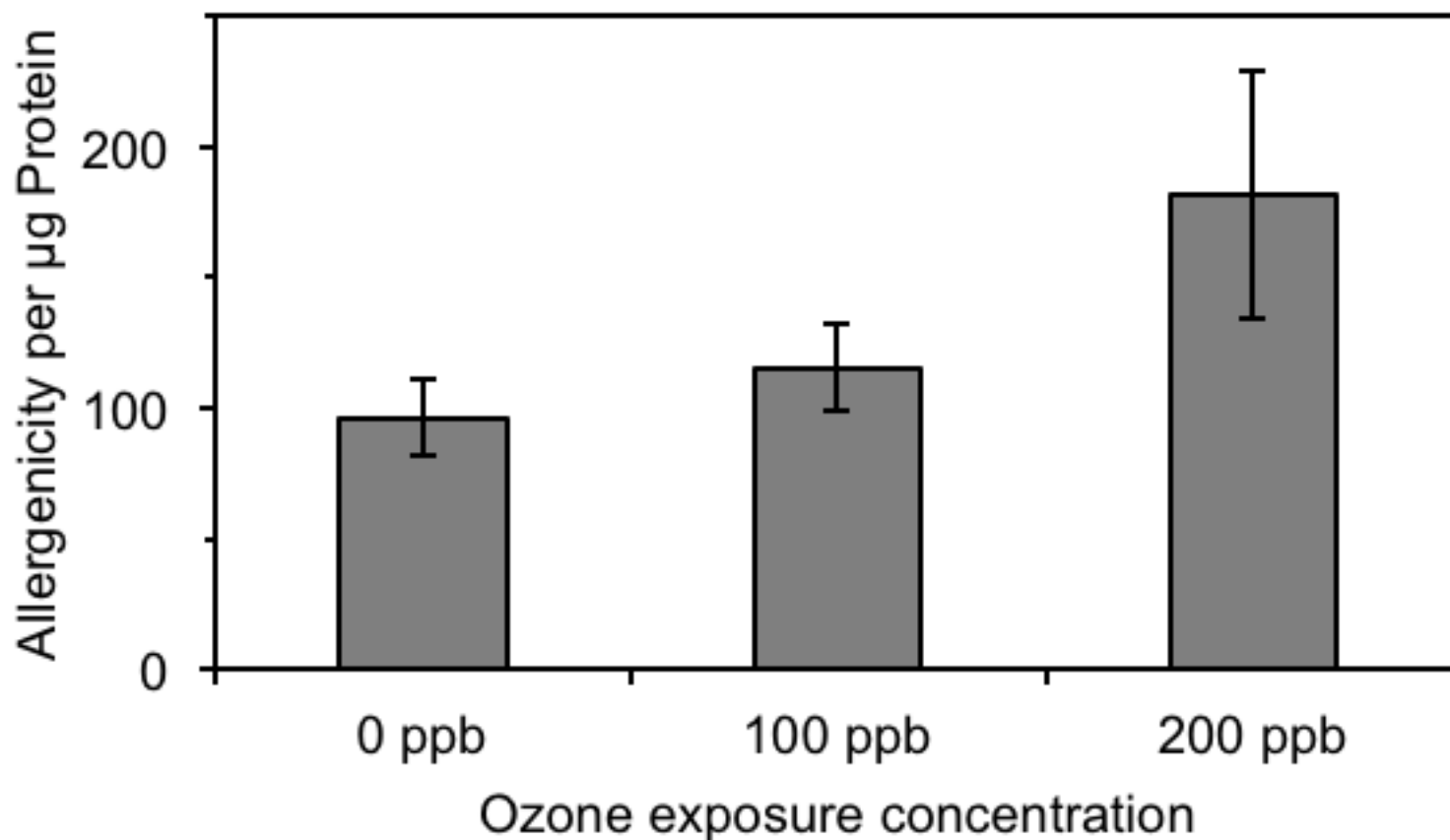
**Mechanistic
understanding
(design)**



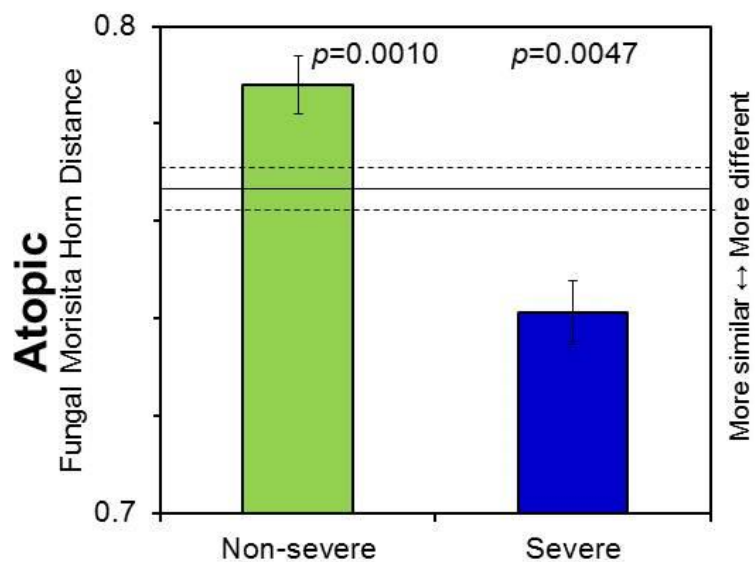
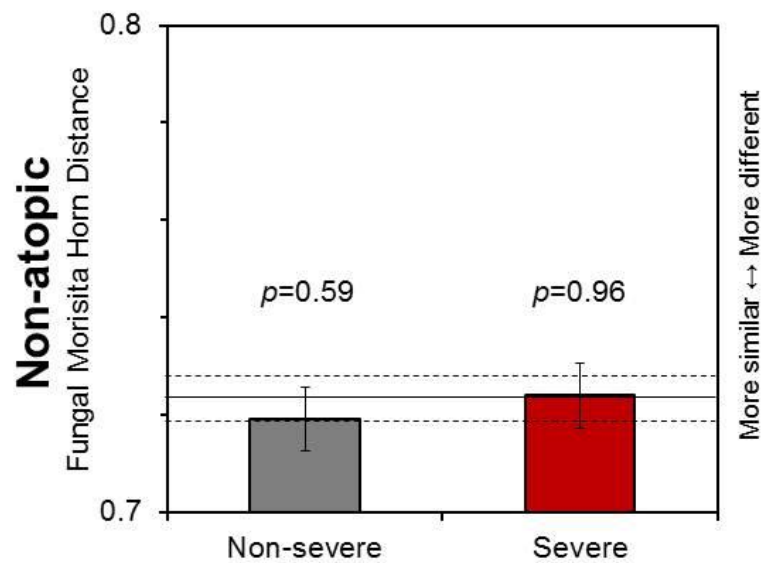
Proteomic analysis of *A. fumigatus* allergens, effect of O₃

Allergen	Accession	Log ₂ fold Change	ANOVA
Asp f 8	AFUA_2g10100	0.91	0.0079
Asp f 9	AFUA_5g13300	0.68	0.0138
Asp f 11	AFUA_5g04170	-0.29	0.0173
Asp f 13	AFUA_2g12630	0.63	0.0332
Asp f 18	AFUA_7g04930	-0.50	0.0432
Asp f 10	AFUA_2g03720	4.66	0.0519
Asp f 23	AFUA_2g11850	0.45	0.0877
Asp f 3	AFUA_6g02280	0.75	0.131
Alt a 4 homologue	AFUA_2g06150	-0.15	0.193
Asp f 22	AFUA_6g06770	-0.17	0.530
Alt a 10 homologue	AFUA_7g01000	0.23	0.585
Alt a 10 homologue	AFUA_4g08600	0.01	0.927

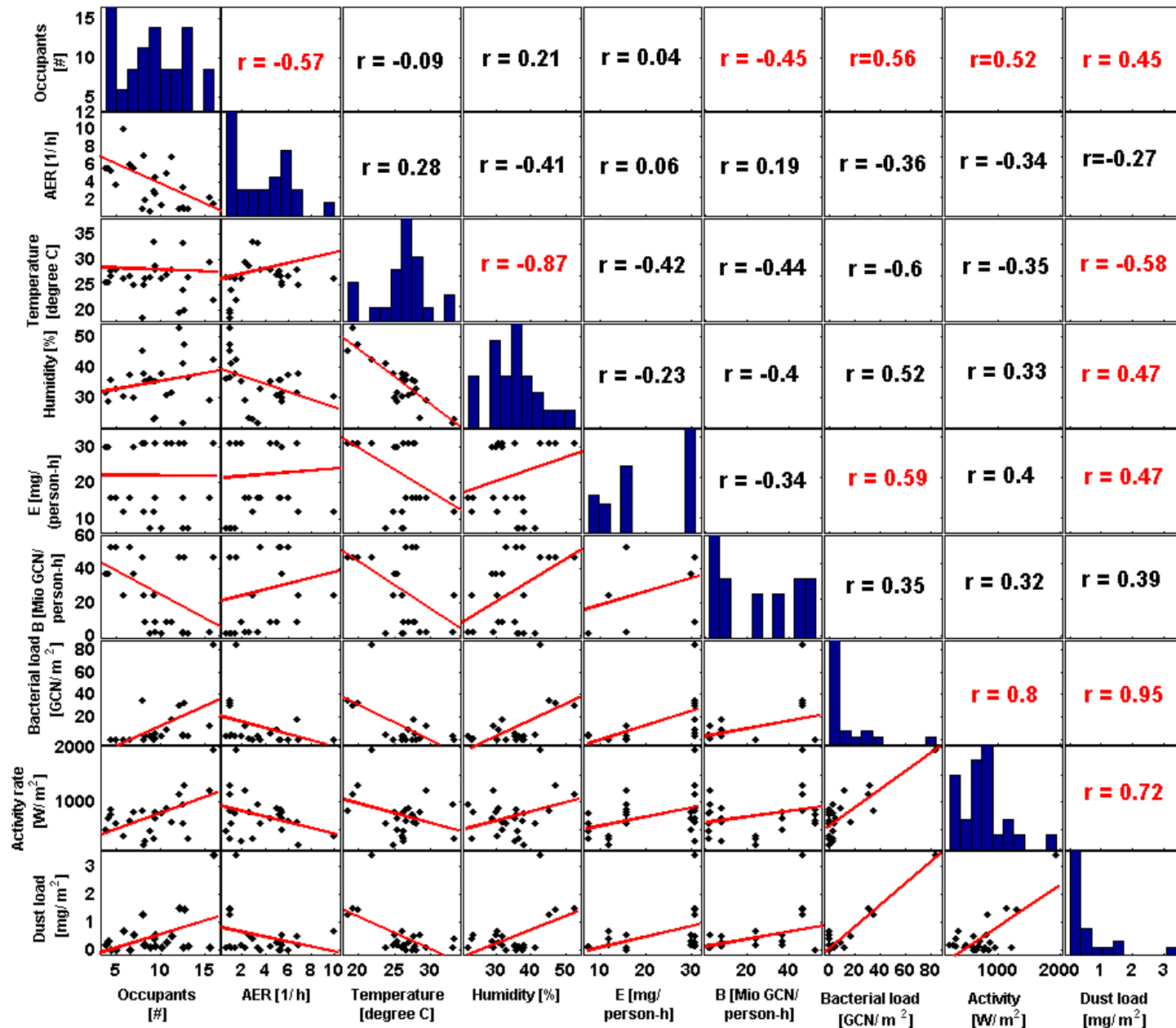
Effects of O₃ on *A. fumigatus* allergenicity (IgE binding)



Fungal community structure seems to matter for allergic asthma



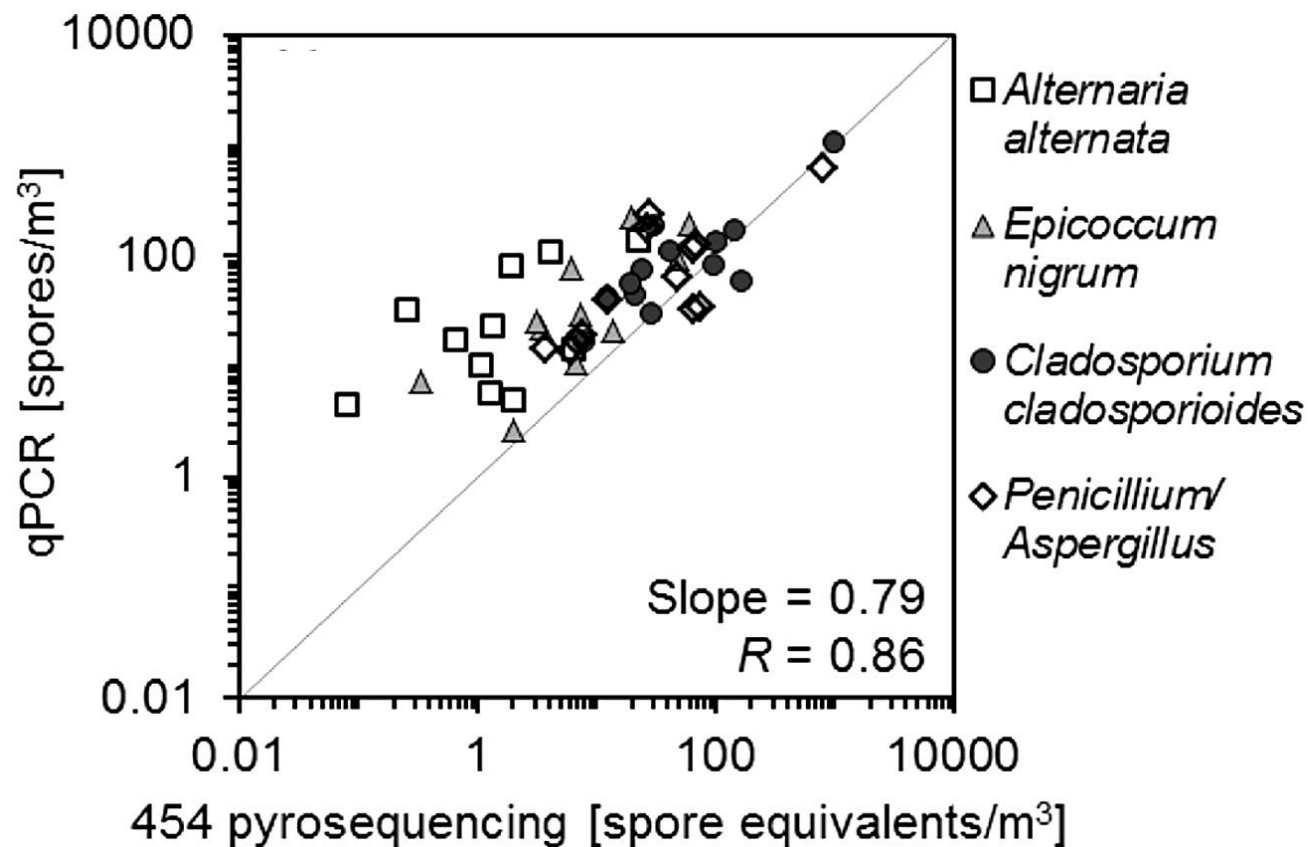
Statistics: Look for Associations



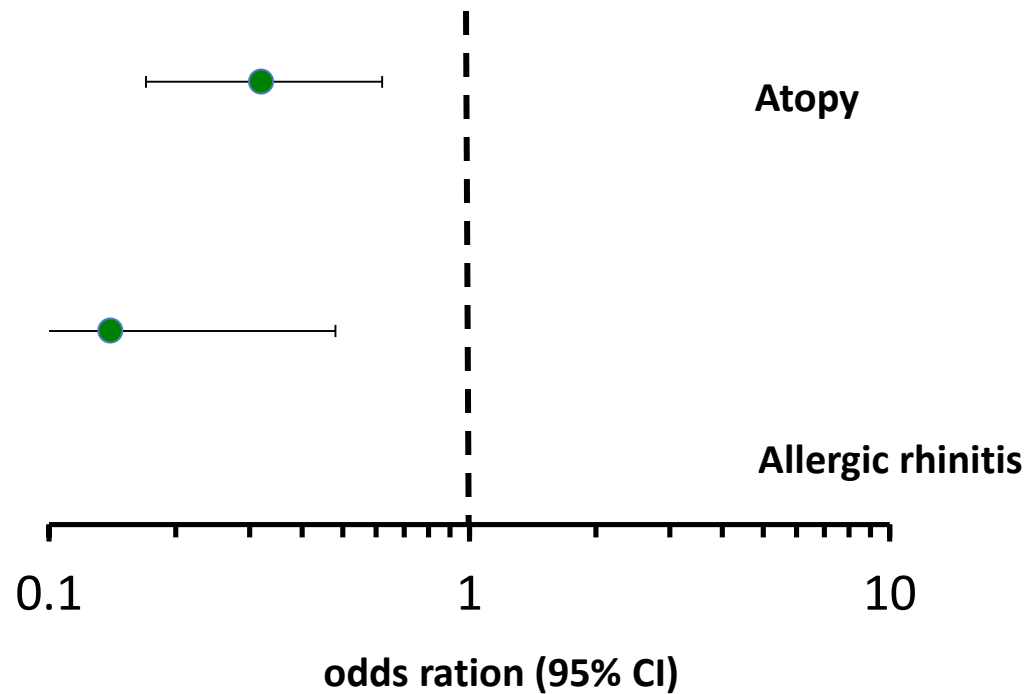
(Hospodsky, Nazaroff, Peccia unpublished data)

A push for absolute quantitativeness

Absolute concentration taxa = qPCR total fungi X relative abundance taxa



cesarian section and allergies



When we adjust for confounders, the low diversity/asthma association is stronger

Low Fungal Diversity and Asthma Development

Potential Confounder

n (%)

OR

95% CI

<i>none</i>	—	4.80	1.04	22.10
<i>Rainy Season</i>	22 (58%)	6.96	1.27	38.23
<i>Pets</i>	5 (13%)	4.74	1.03	21.88
<i>Other children</i>	19 (50%)	4.83	1.04	22.29
<i>Below poverty line</i>	28 (74%)	5.54	1.11	27.59
<i>Moisture (>17)</i>	15 (39%)	4.79	1.04	22.07
<i>Moisture (>21)</i>	12 (32%)	4.68	1.01	21.65
<i>Moisture (>24)</i>	5 (13%)	6.43	1.15	35.80
<i>Visible mold</i>	11 (29%)	4.76	1.03	21.95
<i>Two or more moisture indicators</i>	12 (29%)	5.61	1.12	28.10

Adjusted logistic regression models for association between asthma development and low fungal diversity.

Revolution/Evolution—DNA Sequencing to Identify Indoor Microorganisms

**Jordan Peccia
Environmental Engineering
Yale University**

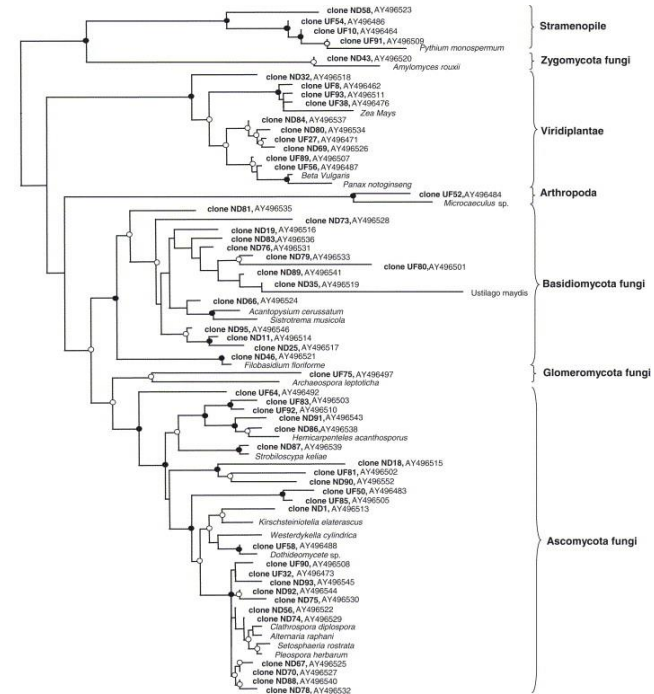
Revolution

■ New Haven and Yale has nurtured great leaders



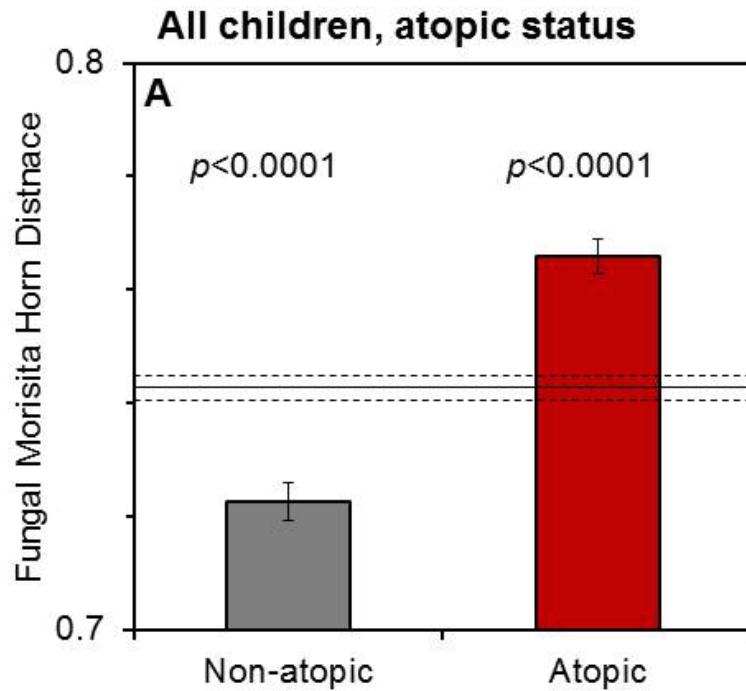
■ New Haven and Yale invented the frisbees, bicycles, pizza, helicopters, cemeteries, hamburgers, American football, can openers, vulcanized rubber, lollipops, the dictionary

■ Yale researchers published the first phylogenetic library of an air sample (Boreson et al, 2004 *Atmos. Environ.*) *,**

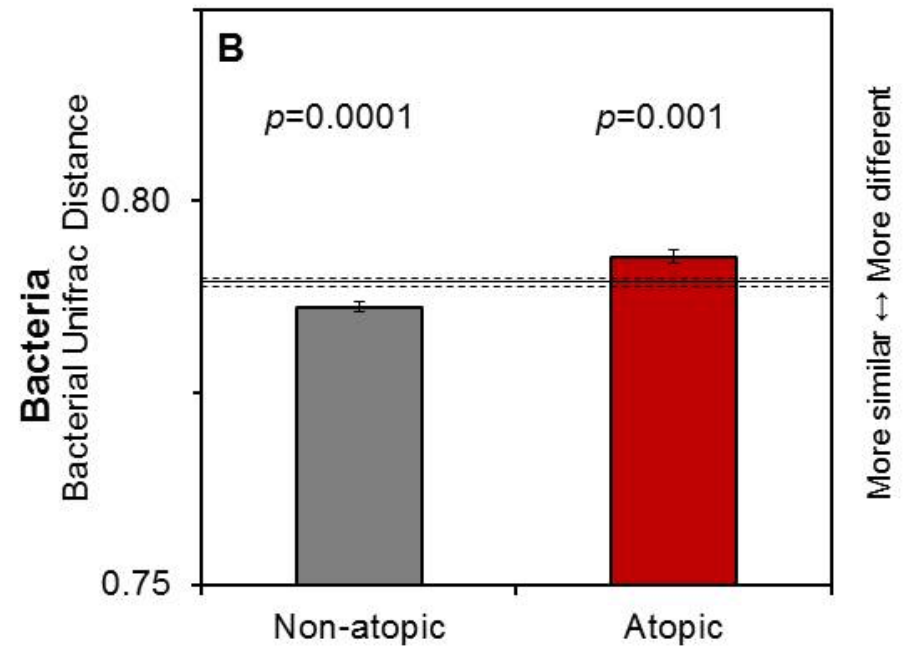


* Ok, the second. First was in 1996 by A. Wakefield, where she cloned air samples of *Pneumocystis carini i (jirovecii)*, using species specific primers. Wakefield, *J Clin Microbiol*, (1996). ** at the time of publication, the corresponding author was employed by Arizona State University, and had never heard of New Haven, CT

Fungal community structure seems to matter for allergic asthma



More similar ↔ More different



More similar ↔ More different

Asthma

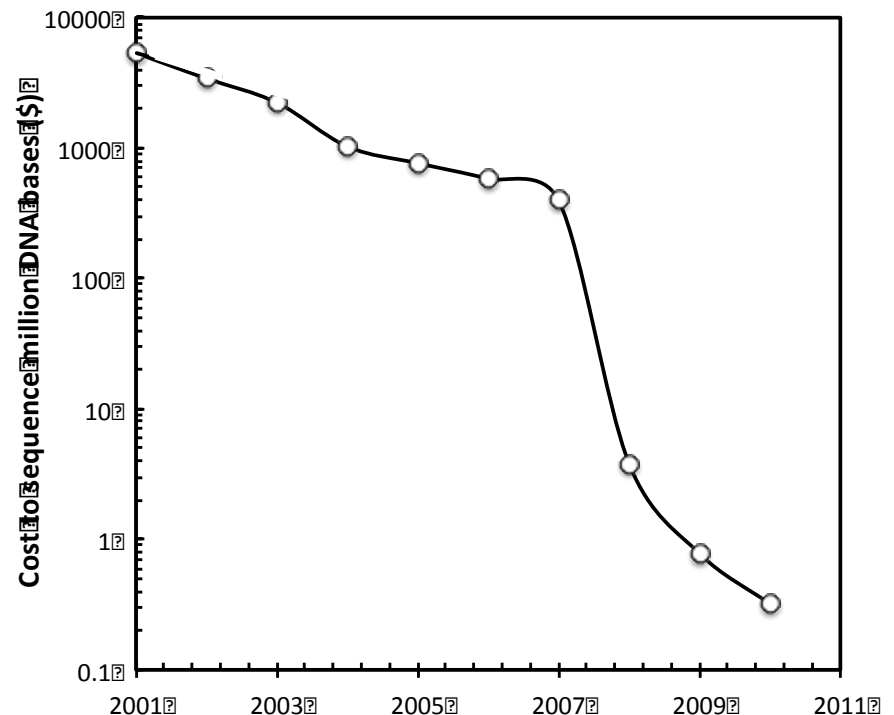
- Multi-factorial disease. Hygiene hypothesis suggests that exposure to microbes (infectious or not) in early childhood helps to modulate the immune system and allow for its proper development.
- Atopic or allergic asthma refers to a predisposition to developing type 1 hypersensitivity reactions

Papers on fungi in urban air don't cause revolutions:

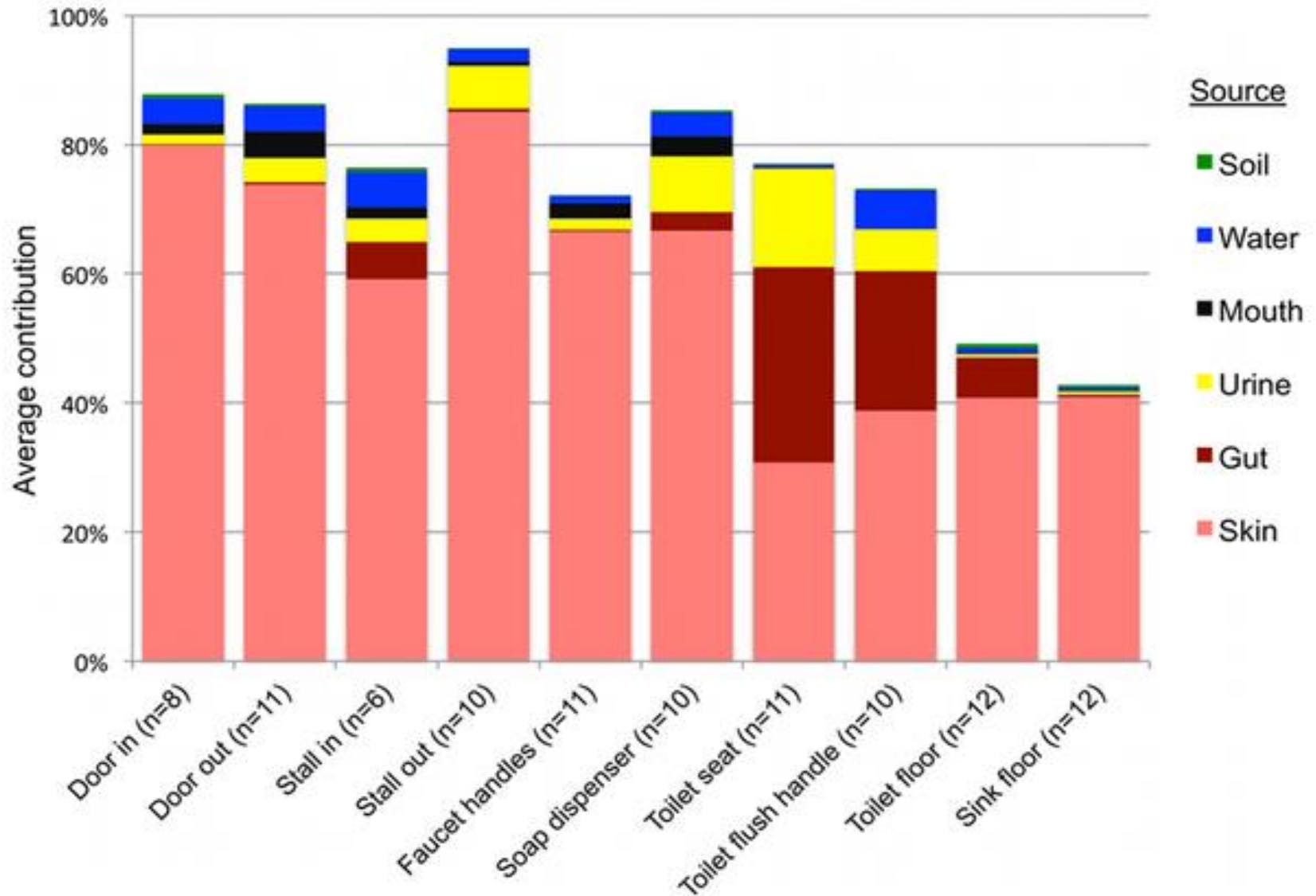
- September 11, 2001. Anthrax in the mail, renewed interest in bioterrorism.
- Alfred P. Sloan Foundation became interesting the “background” indoor microbiome, and forced world leading microbial ecologist to work with engineers/building scientists.

Sanger sequencing and cloning was slow and costly

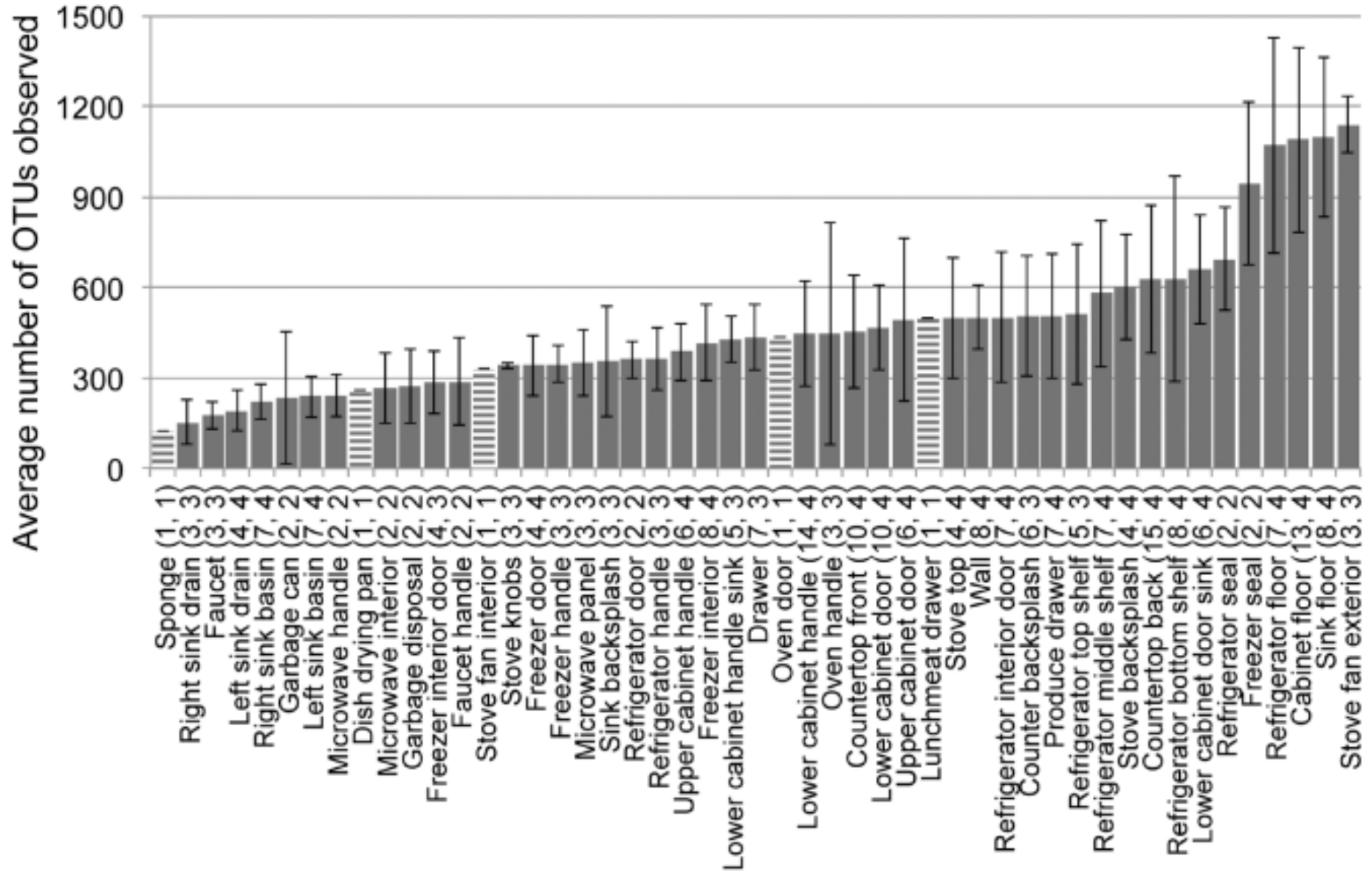
Next generation sequencing: low cost per sequence and rapid, cloning not required



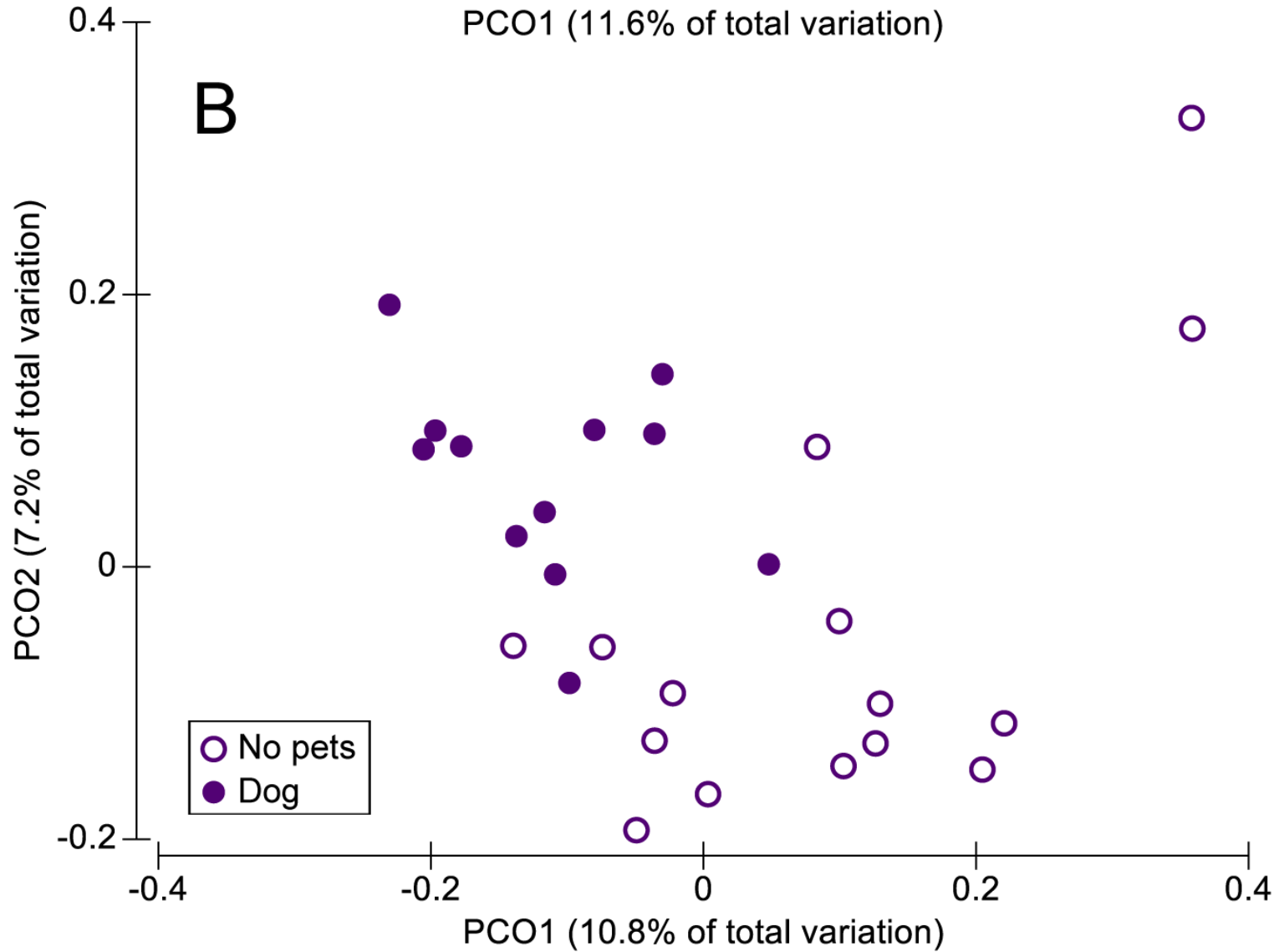
Track sources of bacteria on toilet seats



Estimate bacterial diversity (richness) of surfaces in residential kitchens



Determine quantitative comparisons of different environments (dogs versus no pet)



Evolution

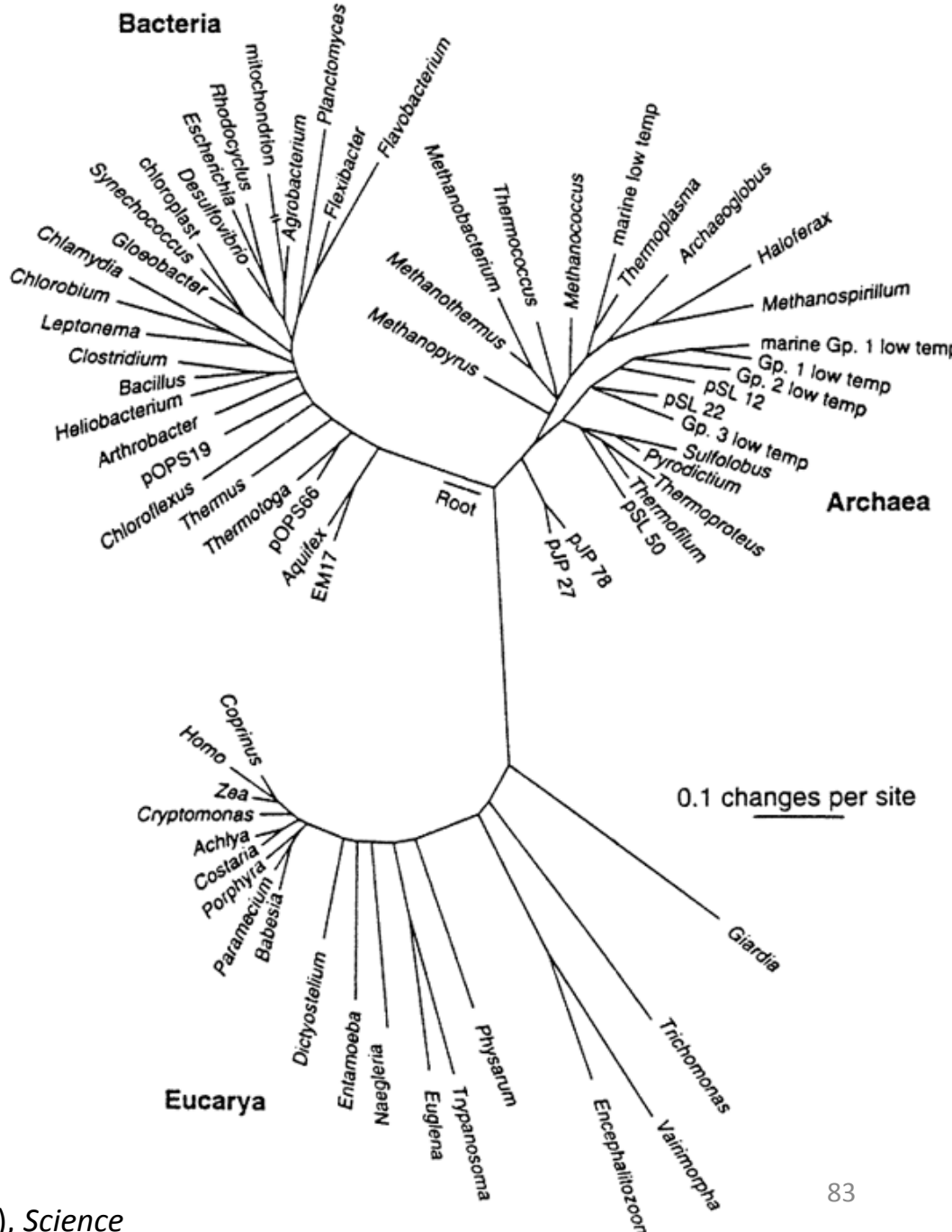
Continuing to evolve

- **Limited standardization of sequence processing pipelines;**
- **Incorporation of building microbiome databases in new research studies;**
- **Improving data analysis for unraveling building-microbe relationships;**
- **Lack of absolute quantitiveness in DNA-based methods;**
- **Incorporate metagenomes and transcriptomics.**

SSU rRNA Alignment Forms the Tree of Life and a Basis for Identification

■ rRNA-based Taxonomy:

- Domain
- Phylum
- Class
- Order
- Family
- Genus
- Species



A move toward mechanisms

EMPIRICAL



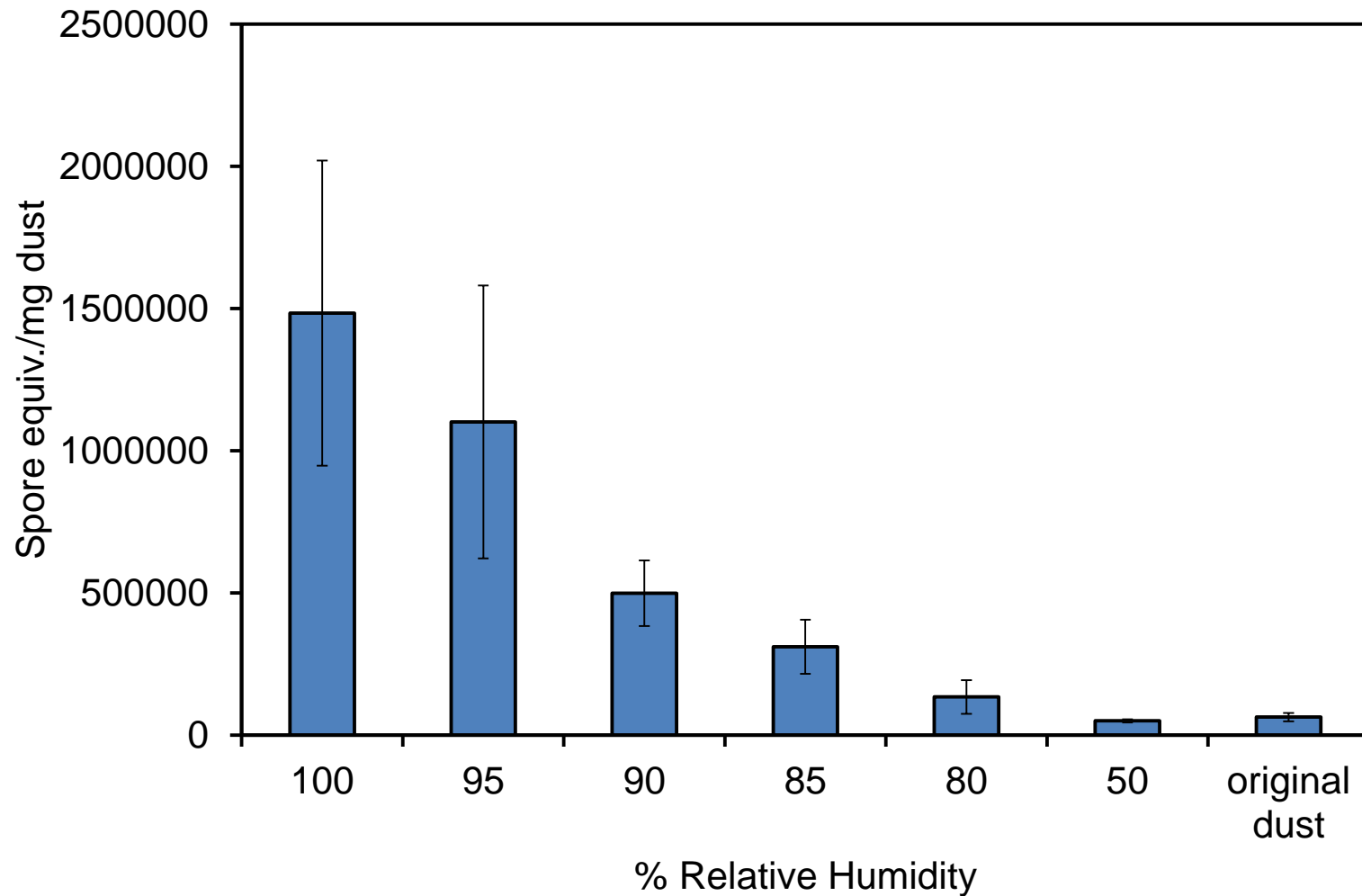
- Large number of environments;
- Low sample density;
- Cursory building variables (RH, light, occupancy);
- Statistical frameworks.

MECHANISTIC



- Fewer buildings with spatial, temporal, and size fractionated sampling;
- DNA sequencing, metagenomics, transcriptomics, culturing, network analysis and other “big data” methods;
- Intense study of buildings (ventilation, energy consumption, human activity);
- Links to fundamentals in physics, chemistry, engineering and medicine (Particle dynamics, mass balances).

Transcriptomics? They are not all dead!



For more Information contact:

Jordan.Peccia@yale.edu



House dust fungal diversity is important for Asthma Development

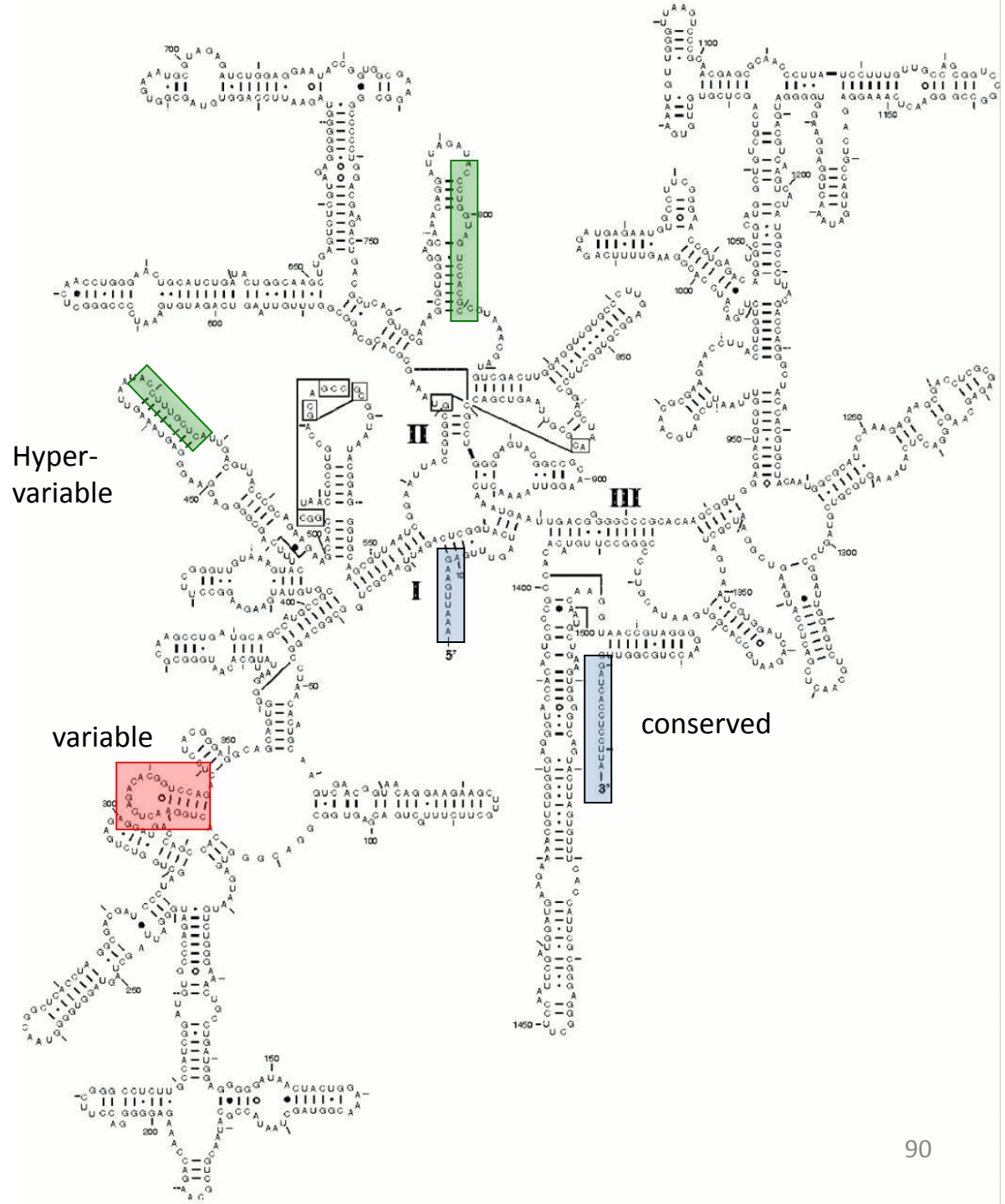
What if you already have asthma.....?

Asthma Conclusions – next gen. sequencing tools useful

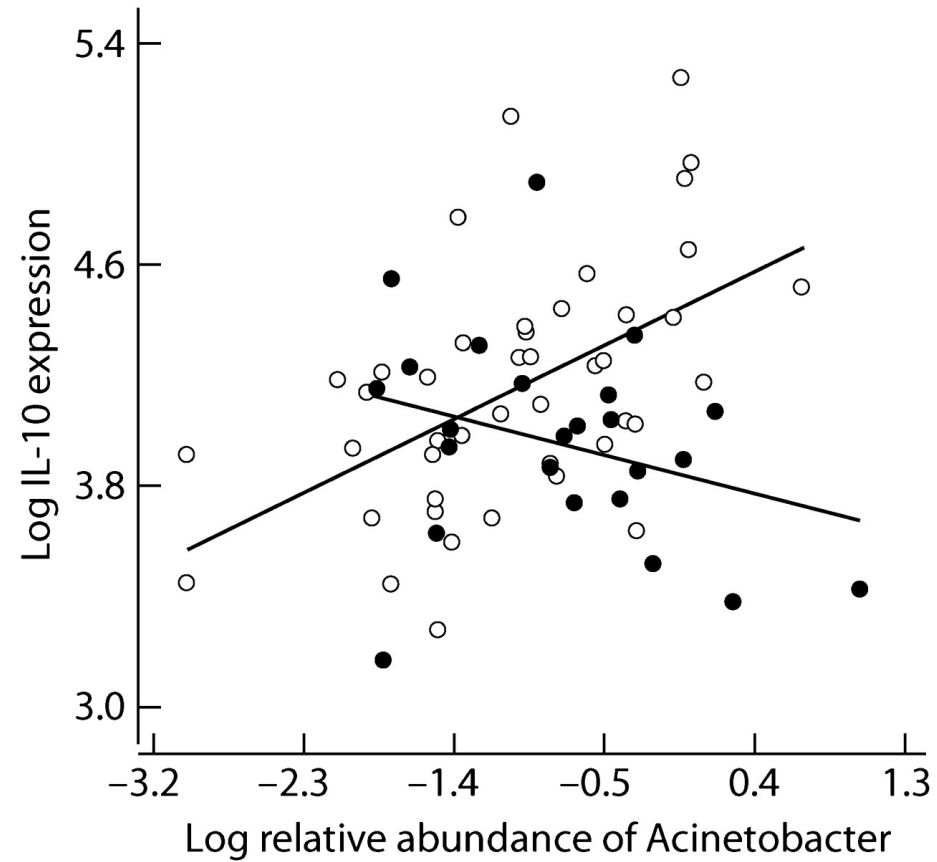
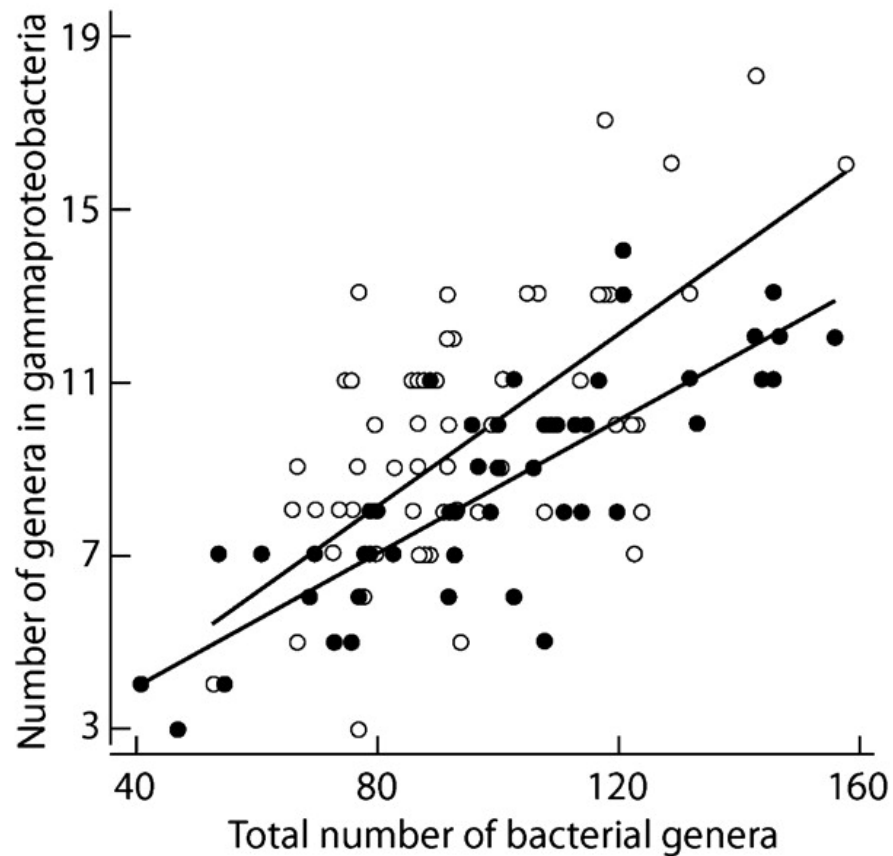
- **Associations between fungal diversity (richness) and asthma DEVELOPMENT**
- **Non-allergic asthma severity associated with total fungi, but not specific taxa in the fungal community**
- **Allergic asthma severity associated with allergens, and specific taxa in the fungal community.**
- **Molecular tools can play an important role in asthma epidemiology by providing diversity (richness) analyses, concentrations of specific allergens, and opening up the fungal populations to consider the broad range of taxa that are present**

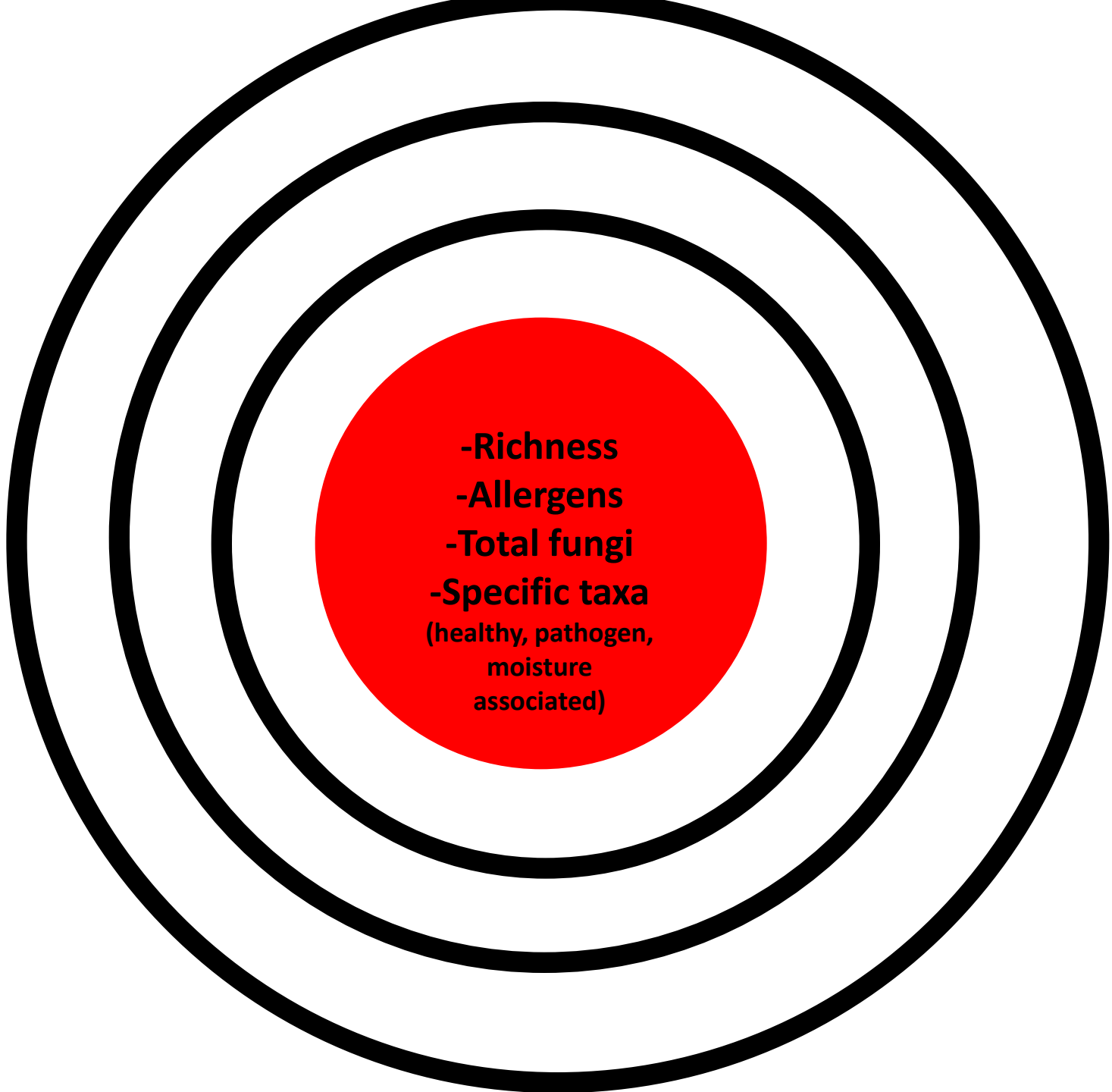


Jordan Peccia
Environmental Engineering
Yale University



Allergy, diversity, and *Acinetobacter*





-Richness
-Allergens
-Total fungi
-Specific taxa
(healthy, pathogen,
moisture
associated)

Associating targets with building characteristic

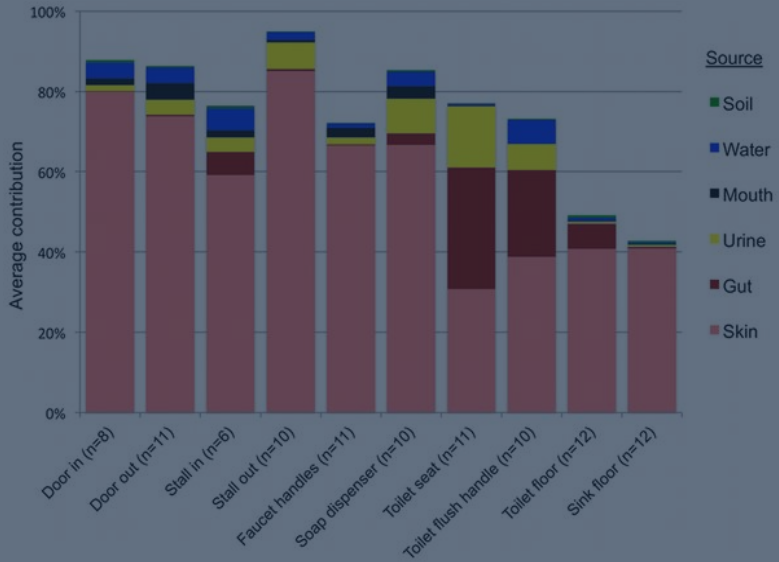
Category	<i>n</i> (yes)	Mean number of fungal OTUs			Mean number of bacterial OTUs		
		Yes	No	<i>P</i> -value	Yes	No	<i>P</i> -value
More than 5 people in home	49	95.7	91.8	0.59	735	735	0.91
More than 3 children in home	87	96.6	91.8	0.54	701	743	0.26
Urban home (vs. Suburban)	112	90.1	96.2	0.33	707	772	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

**Imagine the applications for
industry?**

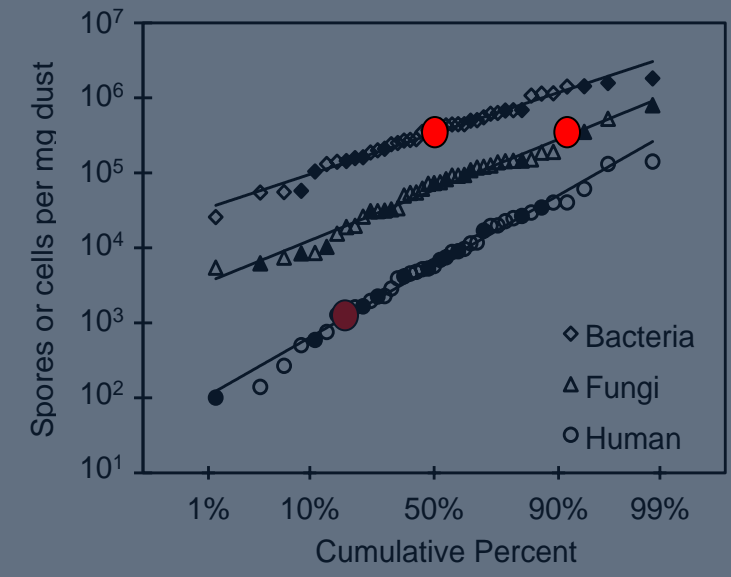
What you get:

- **A list of fungi including relative abundances. Typically 2,000 to 10,000 identifications per sample;**
- **Species level identifications: list of allergens, list of pathogens;**
- **Quantitative comparisons from one location or time-point to another;**
- **Diversity—How many different species of fungi are present;**
- **Ability to track the sources of fungi.**

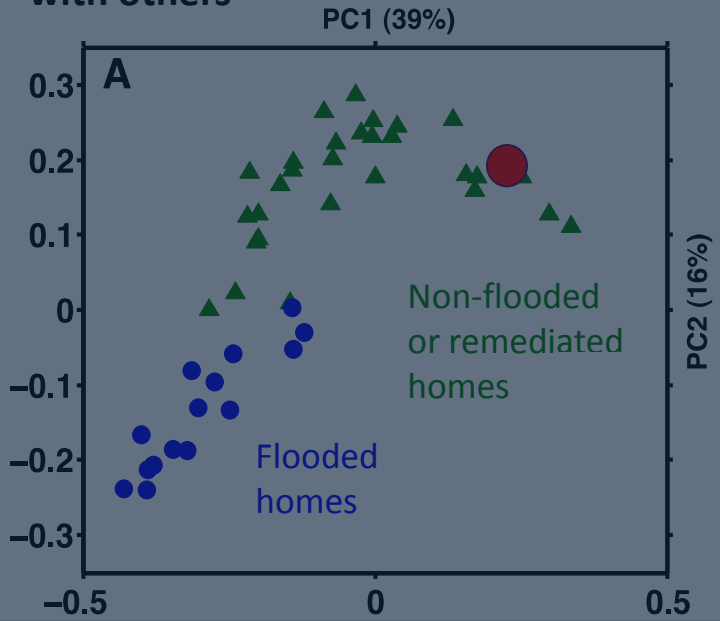
Raw data: lists of species present and abundances, Lists of allergens, and a source tracker



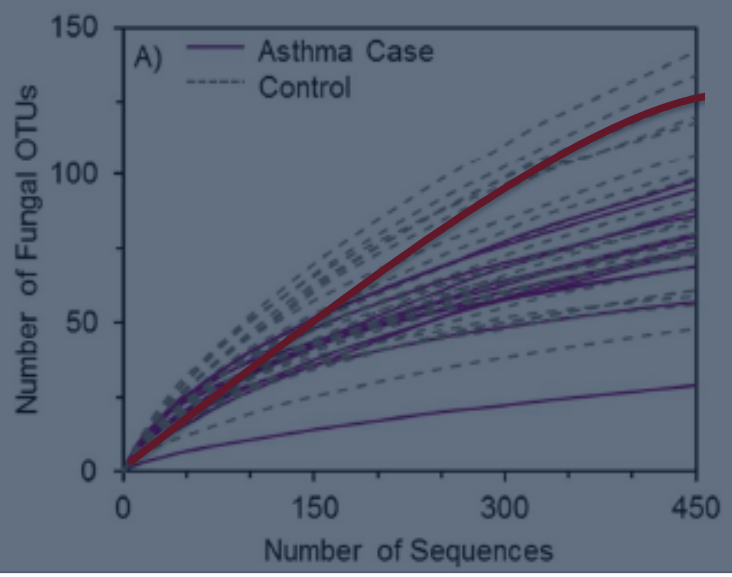
Comparisons: how much fungi, bacteria and human DNA is in your home



Comparisons: How does your home compare with others



Health metrics: Do communities correspond with disease states



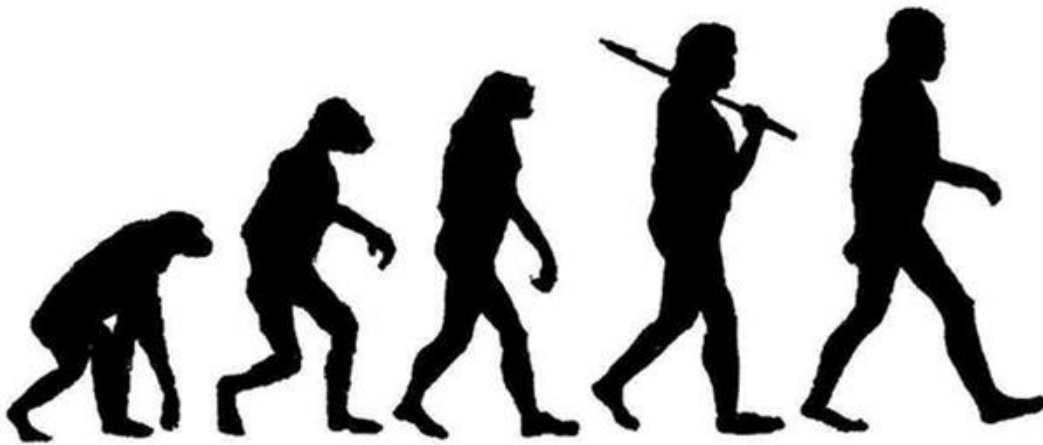
- **What are the targets?**

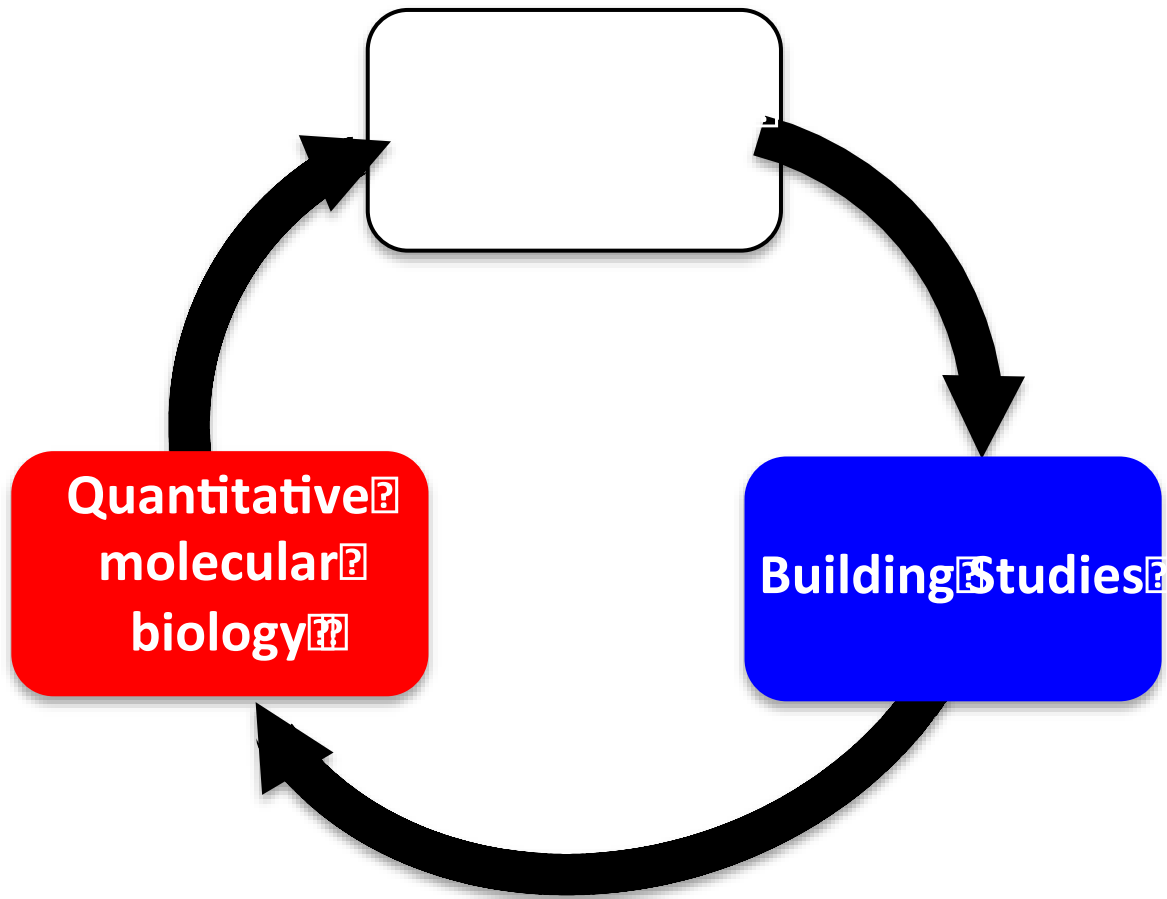
- **How do we integrate these methods and this knowledge with the building industry**
 - mold remediation and assessment
 - Building design;
 - Building maintenance

- **What do we know about how buildings impact the target presence and abundance?**

Research

Practice





We are not alone in these types of observations

The NEW ENGLAND
JOURNAL *of* MEDICINE

ESTABLISHED IN 1812

FEBRUARY 24, 2011

VOL. 364 NO. 8

Exposure to Environmental Microorganisms and Childhood Asthma

Markus J. Ege, M.D., Melanie Mayer, Ph.D., Anne-Cécile Normand, Ph.D., Jon Genuneit, M.D.,
William O.C.M. Cookson, M.D., D.Phil., Charlotte Braun-Fahrlander, M.D., Dick Heederik, Ph.D.,
Renaud Piarroux, M.D., Ph.D., and Erika von Mutius, M.D., for the GABRIELA Transregio 22 Study Group

- Living on a farm is associated with a lower prevalence of asthma
- Diversity of bacterial and fungal exposure (mostly by culturing) is inversely related with the asthma risk

Objectives

- Determine the impact of building ventilation on airborne and surface microbial communities.
- Estimate the impact of cleaning on surface and floor dust microbial communities.

Approach

qPCR
total
bacteria

Air
exchange
rate

*DNA sequencing
16S, ITS*

Occupancy
level

Indoor air,
outdoor air

Emission
rates

qPCR
total
fungi

Surface
samples

ATP

*RH,
Temp*

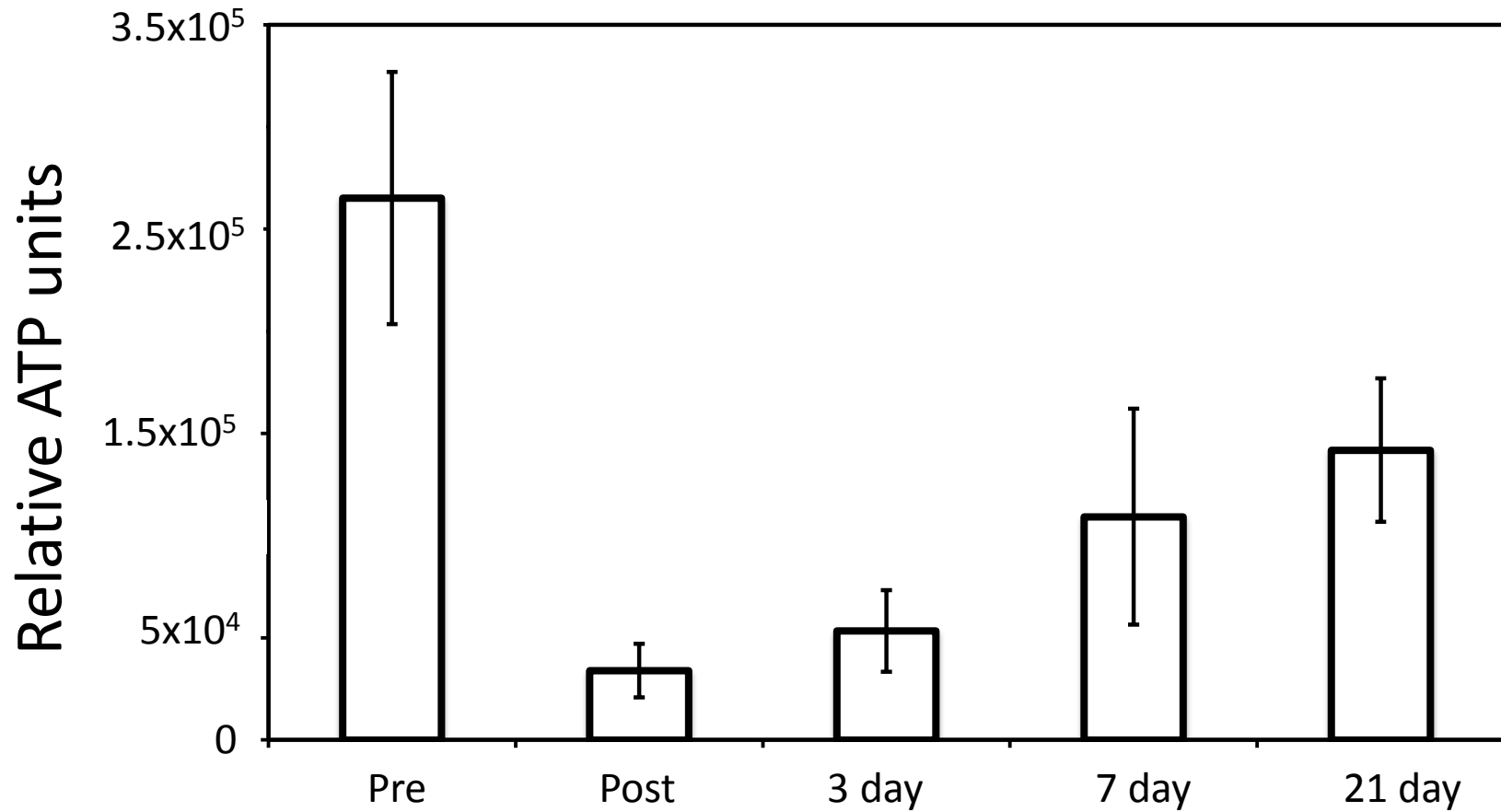
Cleaning
regimes

*Concentrations
of specific taxa
and allergens*

Cleaning approach (26 treatment and 26 control homes)

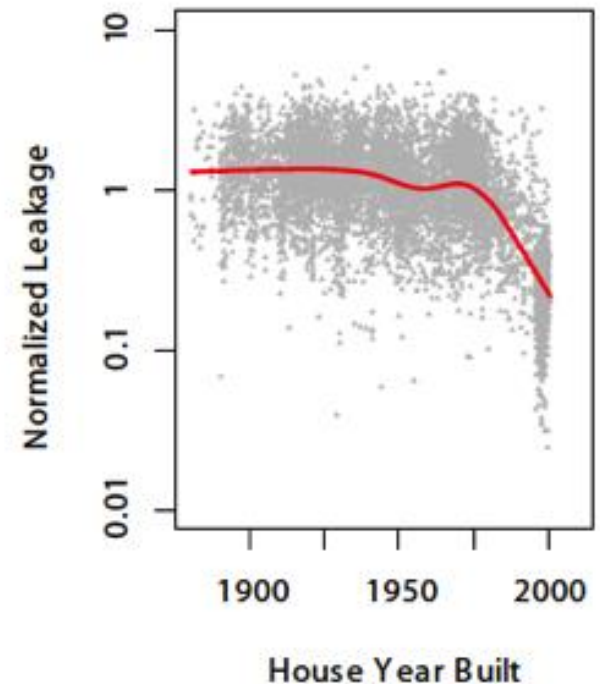
- **Pre cleaning, t=0**
 - **Post cleaning, t=6 months**
 - **Post cleaning, t=12 months**
-
- **Microbial community development over time (door knobs, desks, floors)**

ATP on desk surfaces pre and post cleaning



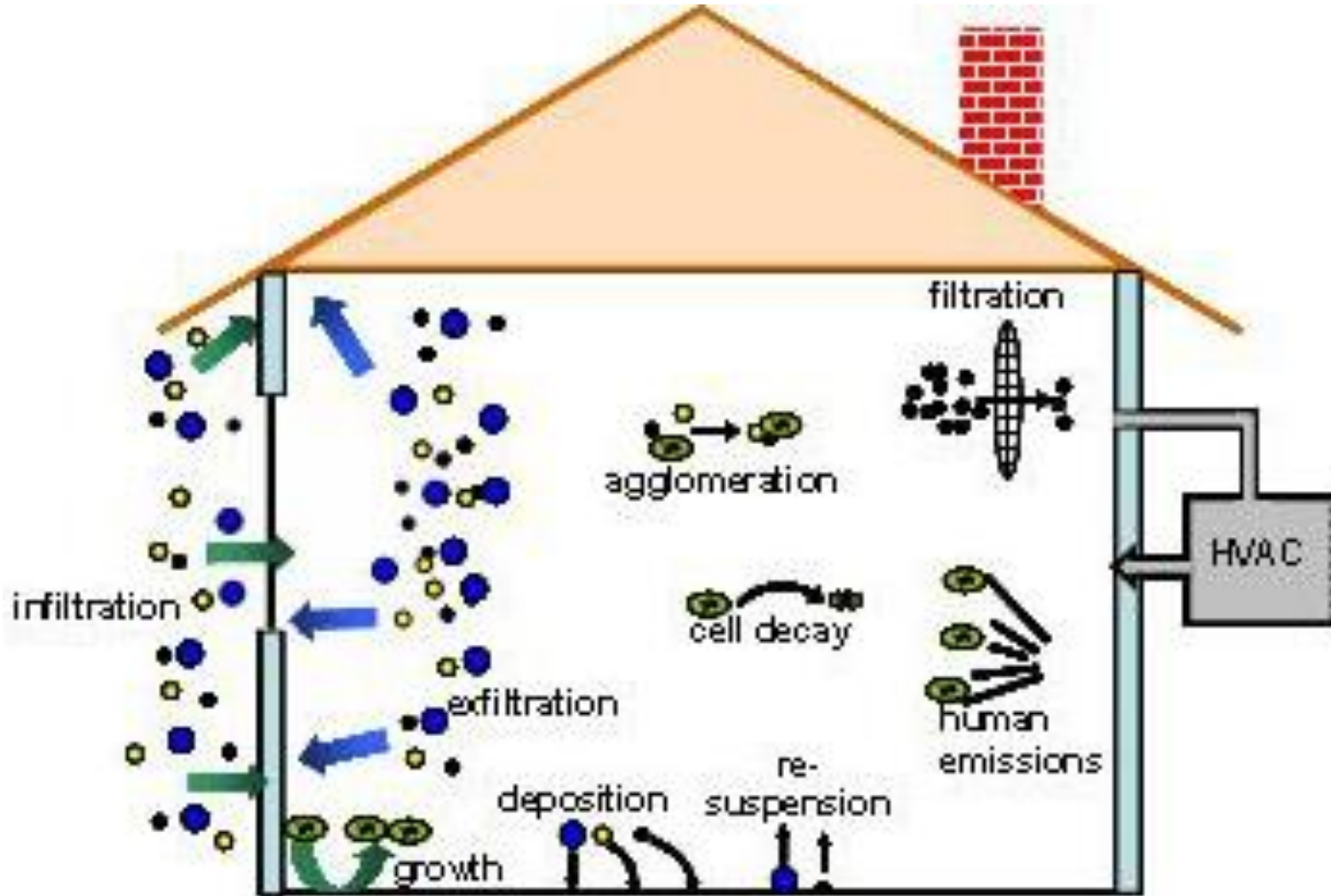
Ventilation Approach

- ***Statistical and deterministic*** microbial analyses (26 homes, 2 seasons, perfluorocarbon tracer)



(Chan, Price et al. 2003,
Chan, Nazaroff et al. 2005).

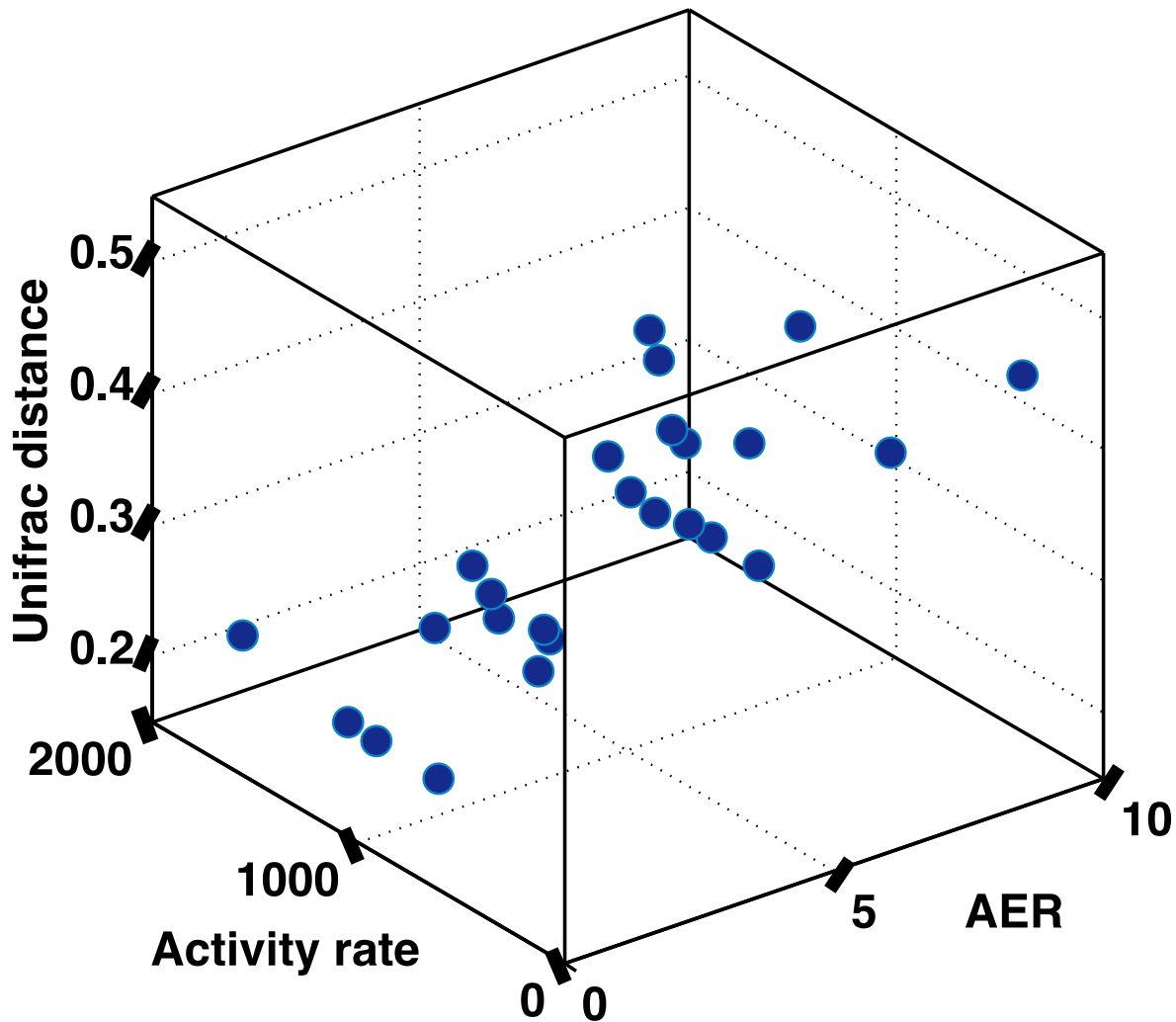
Ventilation sources + indoor emissions = deposition + ventilation losses



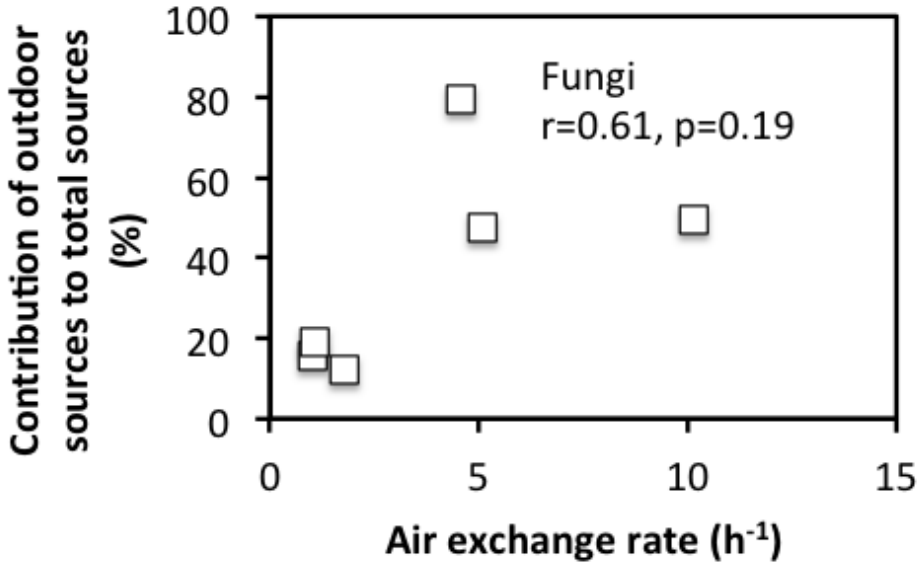
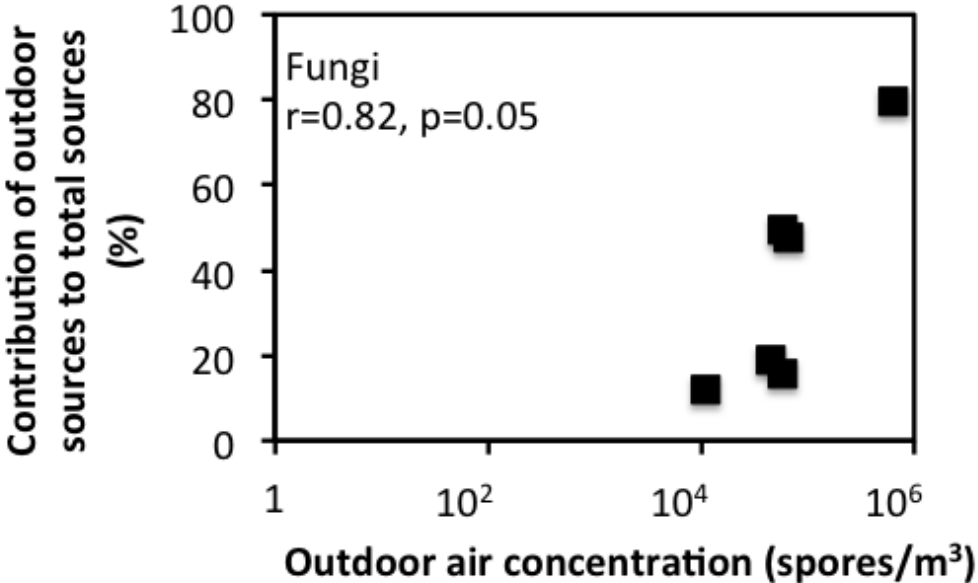
Tribal home cleaning and ventilation study

- We are focusing on tribal homes;
- Exploring to important factors on air and surface dust microbes: ventilation and cleaning;
- Applying both statistical and number balance data analysis approaches.

Asthma, allergy and the environment



Do we expect ventilation to be important



Sometimes, even if I stand in the middle of the room, no one acknowledges me.



The need for baseline data to enable design

**Jordan Peccia
Environmental Engineering
Yale University**

Rational design

- **By continuing to produce data on taxa abundance, taxa size, and developing rate coefficients, we can produce models that PREDICT exposure and guide RATIONALE design.**

