Derived ecological niches of indoor microbes are crucial for asthma symptoms in university dormitories

Xi Fu ^{1,3#}, Yanling Li ^{1,2#}, Yi Meng ^{1,2}, Qianqian Yuan ^{1,2}, Zefei Zhang ⁵, Dan Norbäck ⁴, Yiqun Deng ^{1,2*}, Xin Zhang ^{5*}, Yu Sun ^{1,2*}

¹Guangdong Provincial Key Laboratory of Protein Function and Regulation in Agricultural Organisms, College of Life Sciences, South China Agricultural University, Guangzhou, Guangdong, 510642, PR China.

²Key Laboratory of Zoonosis of Ministry of Agriculture and Rural Affairs, South China Agricultural University, Guangzhou, Guangdong, 510642, PR China.

³School of Public Health, Sun Yat-sen University, Guangzhou, PR China.

⁴Occupational and Environmental Medicine, Dept. of Medical Science, University Hospital, Uppsala University, 75237 Uppsala, Sweden.

⁵Institute of Environmental Science, Shanxi University, Taiyuan, PR China.

These authors contributed equally to this work.

* To whom corresponds should be addressed. Email: sunyu@scau.edu.cn, xinzhang0051@sxu.edu.cn, yqdeng@scau.edu.cn.

Competing interests

The authors declare that they have no competing interests.

Funding

We thank for funding support from Guangdong Province (2018KTSCX021), Natural Science Foundation of China (81861138005), Key Research and Development (R&D) Projects of Shanxi Province (201803D31021), Shanxi One Hundred Excellent Experts Project (nr 9) and South China Agricultural University.

Abstract

31

32 Increasing evidences from home environment indicate that microbiome community is
33 associated with asthma. However, indoor microbiome composition can be highly diverse and
34 dynamic, and thus current studies fail to produce consistent association. Chinese university
35 dormitories are special high-density dwellings with a standard built environment and personal
36 characteristics for occupants, which can be used to disentangle the complex interactions between
37 microbes, environmental characteristics and asthma.

38 Settled air dust and floor dust was collected from 87 dormitory rooms in Shanxi University.
39 Bacterial community was characterized by 16S rRNA amplicon sequencing. Students (n = 357)
40 were surveyed for asthma symptoms.

41 Asthma symptoms were not associated with the overall bacterial richness, but associated with
42 different phylogenetic classes. Taxa richness and abundance in Clostridia and Bacteroidia were
43 positively associated with asthma ($p < 0.05$), and these taxa were mainly derived from human gut.
44 Taxa richness ($p < 0.1$) and abundance ($p < 0.05$) in Alphaproteobacteria and Actinobacteria were
45 protectively associated with asthma, and these taxa were mainly derived from outdoor
46 environment. Building age, floor and curtain cleaning frequency shaped the overall bacterial
47 community of air dust ($p < 0.05$). Frequent curtain cleaning increased the relative abundance of 10
48 protective genera ($p < 0.05$), and old buildings had mix effects to protective genera ($p < 0.05$).

49 Our data shows that taxa from different phylogenetic classes and ecological niches have
50 different health effects, indicating the importance of incorporating evolutionary and ecological
51 concepts in revealing general patterns in the microbiome asthma association analysis.

52

53 Introduction

54

55 With the progress of urbanization and industrialization in many developed and developing
56 countries, the prevalence of metabolic and immune diseases has been remarkably increasing in the
57 past few decades, including obesity, diabetes, inflammatory bowel disease, allergies and asthma [1,
58 2]. Recent culture-independent microbiome studies suggest that the increasing prevalence could be
59 due to loss of microbial diversity in human body [3]. Specifically, the development of asthma is
60 suggested to be associated with reduction and variation of microbiome composition in the outdoor
61 and indoor environment, as well as in the human respiratory and gastrointestinal tract [4-6].
62 However, it is not easy to identify a consistent pattern between microbial exposure and asthma;
63 many studies produced different or even conflicting results. For example, several studies show that
64 high microbial diversity exposure at home is protective against asthma, especially in the farm
65 environment [7, 8]. But there are several studies indicating that higher microbial diversity does not
66 relate to asthma [9, 10], or even be a risk factor to impair respiratory health [11, 12]. Besides the
67 conflicting results in microbial diversity, the studies surveying the association between relative or
68 absolute abundance of microbial taxa and asthma also produce heterogeneous results. For example,
69 one child cohort study in northeastern United States find only one protective taxa for asthma,
70 whereas another child cohort study in several cities of United States find 373 bacterial taxa are
71 associated with childhood asthma [10, 11].

72 The first challenge of microbiome analysis is to deal with the complex multidimensional
73 dataset, which contains abundant variations for thousands of taxa. In addition, indoor microbiome
74 composition is affected by many indoor and outdoor environment characteristics, including
75 latitude, climate and humidity, soil and plant, traffic pollution, building design and ventilation,
76 number of occupants and pets, indoor surface material and plumbing systems, settled dust
77 resuspension and so on [13-15]. Microbial community can vary across geographic distance even
78 between rooms in the same building [16-19], and the variation can be temporally changed, as
79 indicated in longitudinal studies in home, hospital and kindergarten rooms [20-22]. The scale of
80 variation could be astonishing even among the same sample type and ecological niche. For
81 example, individual human gut and hand microbiome can be 80%-90% different from one another
82 [23, 24]. Similarly, people inhabit in different locations or different rooms in the same building or
83 different time can be exposed to distinct microbial compositions [18]. Thus, to conduct a clear
84 health association study, either the number of microbiome sampling is large enough to account for
85 the variations, or applying a solid study design to reduce the microbiome variation. Another
86 problem for asthma association analysis is that many personal characteristics are associated with
87 asthma, such as parental asthma, tobacco smoking, obesity, respiratory infection, pollen allergy,
88 age, gender, education level and lifestyle [25], and it is necessary to adjust these variables in the
89 statistical model. However, studies adjusted different set of confounding factors can introduce
90 biases, leading to incomparable conclusions. Overadjustment biases and unnecessary adjustment

have also been reported in epidemiologic studies [26]. Overall, the microbiome asthma association study is still in the early exploratory stage, and thus it is optimal to design study with minimal confounding effects to reduce analysis variation.

Chinese university dormitory is a special high-density dwelling. Each room is shared by ~4-8 occupants and connected by a long corridor. Rooms within a building share same or similar outdoor characteristics, such as greenness and soil type and traffic pollution, as well as similar indoor characteristics, such as room size and design, window orientation, ventilation system, indoor decoration and furniture. No kitchen is in the dormitory building, and thus no particulate matters emitted from cooking activity. Thus, many factors leading to drastic microbiome variation in home environment are standardized in the dormitory environment. In addition, many confounding variables associated with asthma are also standardized. For example, dormitory occupants have similar age, education level and life style, including eating in the same student canteen, similar sleeping and wake up time and mandatory physical training classes in the university. The participants of this dormitory study are mainly from northern china, which are shown to have low genetic diversity from a previous large-scale genomic survey of China [27].

Few studies have been conducted in Chinese dormitories, and these studies focused on health effect of environmental characteristics, including visible dampness and mold, room ventilation and crowdedness [28, 29]. In this study, we conducted the first microbiome asthma association study in Chinese university dormitories. The analysis aims to disentangle the association between microbial diversity/abundance, environmental characteristics and asthma. Previous hypothesis and observation were also tested in the dormitory dataset.

113 Materials and Methods

114

115 Study population

116 A total of 87 rooms in 10 dormitory buildings were randomly selected in Shanxi University,
117 Taiyuan, China. Photos for building, corridor and dormitory rooms were taken (Figure S1). Settled
118 airborne dust and floor dust were collected for each room in November and December, 2013.
119 Environmental characteristics were recorded. Self-reported questionnaires on asthma related
120 symptoms and personal information were sent to students living in these rooms, and 357
121 participants responded (response rate 97.3%). The study was approved by University Ethical
122 Committee, and all participants gave their informed consent.

123

124 Assessment of health and environmental data

125 Questions about current asthma was obtained from the European Community Respiratory
126 Health Study (ECRHS). The asthma related questions included wheeze, breathlessness during
127 wheeze, feeling of chest tightness, shortness of breath during rest, shortness of breath during
128 exercise, woken by attack of shortness of breath [30]. The prevalence of ever had asthma, attack of
129 asthma, and current asthma medication use was very low and not included in the study. Recall
130 time for all these questions were 12 months. The number of asthma symptoms for each participant
131 was counted and categorized as 0, 1, 2, ≥ 3 . Questions about current smoking and parental
132 asthma/allergy were also included.

133

134 Dust sampling, DNA extraction and sequencing

135 Detailed information for dust sampling, DNA extraction and sequencing protocol can refer to
136 a previous publication of microbiome analysis in the same dormitory rooms [31]. Settled airborne
137 and floor dust were collected by petri-dish and vacuum cleaner from 87 randomly selected rooms.
138 A few samples failed to amplify enough DNA for sequencing, and thus in total 83 floor dust and
139 86 air dust samples were sequenced by Illumina MiSeq platform with MiSeq Reagent Kit v3.
140 DNA extraction and multiplexed high-throughput sequencing were conducted by Personalbio
141 (www.personalbio.cn). Sequencing data was deposited in Qiita with study ID 12841
142 (<https://qiita.ucsd.edu/study/description/12841>).

143

144 Bioinformatics and association analysis

145 Microbiome data were mainly analyzed by Quantitative Insights Into Microbial Ecology
146 (QIIME, v1.8.0) [32] and R. All samples were rarefied to even depth of 11,000 reads. Beta
147 diversity analysis were conducted by weighted UniFrac distance metrics [33], and visualized by
148 non-metric multidimensional scaling (NMDS) analysis [34].

149 Bivariate analysis between environmental characteristics and diversity was performed using
150 Kruskal-Wallis test. Environmental characteristics with p values lower than 0.1 were further input

151 in the multivariate analysis by forward stepwise approach in IBM SPSS Statistics (v21.0).
152 Permutational bivariate and multivariate analyses of variance (Adonis) [35] between
153 environmental characteristics and microbiome data were conducted by the vegan package in R.
154 Associations between bacterial richness/abundance and asthma symptom were examined by
155 hierarchical ordinal regression in STATA (v15.0), adjusted for variables including gender, smoking,
156 parental asthma. Dormitory building was second hierarchy. For each sample, the overall richness
157 of bacteria and richness within the dominant phyla and classes were estimated as number of
158 observed taxonomic units (OTUs) within each phylogenetic lineage. Associations between
159 bacterial abundance and asthma score were analyzed at the phylum, class and genus level. Parallel
160 lines test was conducted to examine the model fitness. If $p < 0.01$ in the parallel lines test, we
161 further conducted nominal regression. Microbes with significant associations in nominal
162 regression model were kept as associated microbes. The associations between environmental
163 characteristics and protective and risk microbes were examined by linear regression in STATA
164 (v15.0).
165

166 Results

167

168 In total, 357 participants were surveyed with health data from 87 randomly selected
169 dormitory rooms. All rooms had natural ventilation by windows and doors. The size of the
170 dormitory rooms ranged from 12 to 23 m² with 4 to 8 occupants in each room, and the average
171 living space was 3.1 m² per person (interquartile range IQR, 2.3-3.5 m²). 69.3% of the participants
172 were girls. The mean age of the participants was 21 years (IQR 20-23 years). The prevalence of
173 smoking, parental asthma and pollen allergy was 4.8%, 1.4% and 5.6%, respectively. Attack of
174 shortness of breath during rest and exercise were common (10.4% and 31.7%; Table 1). The
175 prevalence of feeling of chest tight and waken up by attack of shortness of breath were 6.4% and
176 2.8%. Only 1.1% of participants reported breathlessness during wheeze (Table 1).

177

178 Association between microbial diversity/abundance and asthma symptoms

179

180 Detailed microbiome data analysis in these dormitories has been reported in a previous
181 publication [31]. Overall, the microbial composition of airborne and floor dust were drastically
182 different, as shown in the NMDS (Figure 1) and Adonis analysis ($R^2 = 0.65$, $p < 0.001$). Except
183 two floor samples in building No. 9 that were more similar to the composition of airborne dust, the
184 air and floor dust samples clustered separately along NMDS1. Thus, we conducted association
185 analysis for air and floor dust separately.

186 The overall bacterial richness was not associated with asthma symptoms for both floor and
187 air dust ($p > 0.2$). In air dust, richness in Alphaproteobacteria and Actinobacteria were marginally
188 negatively associated with asthma ($p = 0.07$ and 0.08), and richness in Clostridia and Bacteroidia
189 were positively associated with asthma ($p = 0.02$ and 0.03 ; Table 1). In floor dust, richness in
190 Proteobacteria had a slight trend toward negatively associated with asthma ($p = 0.14$), and richness
191 in Clostridia had a trend toward positively associated with asthma ($p = 0.12$; Table S1).

192 We further calculated the association between relative abundance of each bacterial phylum,
193 class and genus and asthma symptoms (Table S2 and S3). In air dust, we found 5 genera of
194 Alphaproteobacteria and 8 genera of Actinobacteria were protectively associated with asthma ($p <$
195 0.05 ; Table 3), consistent with the richness analysis. Similarly, 4 genera in Clostridia were mainly
196 positively associated with asthma, and *Eubacterium* and *Peptococcus* were also detected in floor
197 dust. Two genera in Bacteroidia were positively associated with asthma. Other classes showed
198 mixed associations with asthma. For example, in the class of Betaproteobacteria, two genera were
199 positively associated and two general were negatively associated with asthma. In floor dust, six
200 genera in the class of Clostridia were positive association with asthma (Table 4), also consistent
201 with the richness analysis.

202 An interesting phenomenon is that taxa from different ecological niches were differently
203 associated with asthma. Alphaproteobacteria and Actinobacteria were mainly derived from

outdoor environment, and were presented in low abundance in human derived niches (0.001% and 0.92% in gut, data from Human Microbiome Project [36, 37]), and the protective genera were not detected in human gut. Clostridia and Bacteroidia were dominant classes in human gut. Bacteroidia accounted for 74.0% and Clostridia accounted for 19.4% of human gut microbiota [36, 37]. The risk genera in the indoor environment, including *Prevotella* (3.7%), *Eubacterium* (4.8%), *Ruminococcus* (1.5%), *Blautia* (0.92%), *Faecalibacterium* (3.9%), *Subdoligranulum* (4.3%), *Collinsella* (0.2%) and *Bifidobacterium* (0.63%), were present in high abundance in human gut (abundance values in parenthesis).

A recent indoor microbiome study reported that the sum relative abundance of intercorrelated genera showed much stronger association to asthma than any single genus [38]. Thus, the protective effect can be more accurately assessed by microbial clusters. We conducted factor analysis for the protective and risk microbes in air dust and found a similar pattern (Table S4 and S5). The protective microbes were mainly clustered into 2 factors (Table S4). The first factor included 5 genera of Actinobacteria and 4 genera of Proteobacteria, and the second factor included 3 genera of Actinobacteria and 1 genera of Alphaproteobacteria. Both factors were strongly associated with asthma protection ($p = 0.005$ and 0.001).

Association between environmental factors and microbial diversity/abundance

We first conducted the association analysis between environmental characteristics and the overall microbial composition and diversity. Three environmental characteristics were removed in the analysis as they were positively correlated with other characteristics (Table S6). Visible dampness and mold were detected in few rooms, and were also excluded. The bivariate analysis were shown in Table S7 and S8. Selected environmental characteristics ($p < 0.1$) were further calculated in the multivariate model (Table 5). Building age, curtain cleaning frequency and floor cleaning frequency were associated with bacteria composition in air dust. Building age, sex of occupants and wall surface type were associated with bacterial composition variation in floor dust ($p < 0.05$). These characteristics can explain 2.7% - 4.5% of total variation. East/west window orientation increased bacterial richness in air dust, while plants in room increased bacterial richness in floor dust ($p < 0.05$).

We further conducted association analysis between environmental characteristics and the protective and risk microbes for asthma (Table S9 and S10), and significant associations for air dust samples were summarized in Table 6. Old buildings had higher abundance of several protective bacteria, including *Caulobacter* and 2 unidentified Bacteroidetes, and lower abundance of several protective bacteria, *Microbacterium*, *Tessaracoccus* and an uncultured Bradyrhizobiaceae. Frequent curtain cleaning increased the abundance of 10 protective genera. Other environmental characteristics only affected one asthma-associated microbes.

Discussion

Here we presented the first microbiome asthma association study in Chinese university dormitories. Similar pattern were observed between the two sampling strategies at higher taxonomic level. For example, bacterial richness and abundance in Clostridia were positively associated with asthma, and Alphaproteobacteria were mainly protectively associated with asthma. But the results were more heterogeneous at lower taxonomic level. We identified 23 protective and 15 risk genera in air dust and 1 protective and 9 risk genera in floor dust, but only 3 risk genera were identified in both dataset. The difference may be partly due to the significant composition variation between air and floor dust, which has been thoroughly analyzed and discussed in a previous paper [31]. One of the major compositional differences was that floor dust had high proportion of *Pseudomonas* (75.1%). The dominant taxon can swamp low frequency organisms in the sequencing and rarefaction analysis, leading to biased microbial richness estimation [39]. Similarly, we found significant lower number of OTUs [31] and asthma-associated taxa in floor dust compared with air dust in dormitories. Thus, we argued that the microbes identified from air dust is more comprehensively represent the associated microbes in this indoor environment, and in the following discussion, we mainly compared the result from air dust with other studies.

In the past few years, the “hygiene hypothesis” and the later expanded version “diversity hypothesis” have drawn a lot of attention and discussion [40]. The hypotheses suggest that the increasing prevalence of metabolic and immune diseases including asthma is related to loss of biodiversity, especially microbial diversity, in human gut and outdoor/indoor environment [1]. The mechanism is suggested to relate to inadequate stimulation and maturation of immunoregulatory circuits in low microbial exposure environment. The theory has gained supports from human gut [1], but heterogeneous results were produced for indoor microbiome studies. A cross-sectional study reported that children lived in farm environment were exposed to greater variety of environmental microorganisms, providing protective effects for asthma and atopy [7], and a few subsequent cohort studies also supported the claim [38, 41]. However, there were several studies failed to identify the pattern [9-11, 42]. Indeed, the number of studies does not support the “diversity hypothesis” is higher than studies support it in the indoor microbiome area. In this study, we found asthma was not associated with the overall bacterial richness, but was associated with specific classes, such as Clostridia, Bacteroidia, Alphaproteobacteria and Actinobacteria. The results need to be further verified in other studies. If the conclusion holds, then the “diversity hypothesis” can be modified as that high exposure in specific classes or lineages, rather than the whole microorganisms, is associated with respiratory health in the indoor environment.

In this study, we found Alphaproteobacteria and Actinobacteria were mainly protective for asthma. Alphaproteobacteria is an extraordinarily diverse and ancient group of bacteria, and its members are widely distributed in various ecological niches, such as soil, water, air and even low-nutrient environments as deep oceanic sediments and glacial ice [43, 44]. Two birth cohort

studies showed that Alphaproteobacteria was present in higher abundance in farm homes compared to urban homes, providing protective effect for asthma development [9, 38]. Similarly, Actinobacteria is also a diverse class of bacteria that is widely distributed in terrestrial and aquatic ecosystems [45, 46]. The protective association between Actinobacteria and asthma has been reported in one indoor study [38], and also in a sputum study of nonasthmatic subjects compared with asthmatic subjects [47].

Taxa diversity and abundance in Clostridia were risk factors for asthma in dormitories. Previous studies showed conflicting results between Clostridia and asthma. Studies in gut microbiome reported that colonization of *Clostridium difficile* in youth children was associated with eczema, wheeze, asthma and atopy [48, 49]. Our unpublished study in middle school classrooms of Johor Bahru, Malaysia also reported *Robinsoniella* from Clostridia is a risk factor for asthma. But one case-control study for adults suggested that *Clostridium* cluster XI was protectively associated with asthma prevalence [50]. In addition, *Clostridium butyricum* was suggested to be used as a potential therapeutic microbe for asthma treatment [51]. The discrepancy suggests that Clostridia may have complex effects for asthma. It is possible that the taxa from this class mainly pose risk effects, but exposure with certain genera may be involved in immune training and thus provide protective effects.

An interesting result from this dormitory study is that the protective and risk taxa were mainly derived from different ecological niches. Protective taxa in Alphaproteobacteria and Actinobacteria were mainly from outdoor environment, whereas the risk taxa in Clostridia and Bacteroidia are mainly derived from human gut. Other risk genera, such as *Bifidobacteria* from Actinobacteria and *Collinsella* from Coriobacteriia, also followed the rule and were highly abundant in human gut. Human sheds and releases large amount of microbes to the indoor environment every day. Previous studies reported that students in university classroom emitted ~ 36 million bacterial and 7.3 million fungal genomes per person per hour [52], and the microbiome in a new house can be rapidly changed by new occupants in a few days [21]. A review article summarized that human-derived microbes accounted for 4-40% of the total bacterial load in various indoor environments [53]. Thus, human-derived microbes account for large proportion of indoor microbiome and can further influence the human respiratory health, as shown in this study. The conceptual advances can promote practical advances in the indoor environment. Practical solutions, such as increasing air and microbial exchange between indoor and outdoor environment and frequently cleaning or use less textile fabrics, can promote the respiratory health for indoor occupants.

A strength of this study is that we choose Chinese university dormitory rooms, a standard built environment with similar outdoor and indoor characteristics and relatively low microbiome variation, to disentangle the association between microbial exposure and asthma. The personal characteristics of dormitory occupants were also standardized with similar age, education level and life style. As many potential confounding factors were controlled, consistent results between

microbial diversity and abundance analyses were produced, and the protective and risk taxa showed clear phylogenetic pattern with distinct derived ecological niches. A limitation of this study is that due to the small amount of dust collected by petri-dish collector, we cannot conducted the third-generation sequencing with higher taxonomical resolution. Also, the cross-sectional study design limits the possibility to draw conclusions on causal effects.

In conclusion, we present here the first microbiome asthma association study in university dormitories, and identified a list of protective and risk microbes derived from different ecological niches. The study demonstrate the importance of incorporating ecological and evolutionary concepts in disentangle the microbes associated with respiratory health. This study can promote current understanding of microbial exposure and asthma and future practice to maintain healthy indoor microbiome.

Acknowledgement

We thank Personalbio (www.personalbio.cn) for assistant in sequencing and bioinformatics analysis.

References

1. Bello MGD, Knight R, Gilbert JA, Blaser MJ: **Preserving microbial diversity**. *Science* 2018, **362**(6410):33-34.
2. Eder W, Ege MJ, von Mutius E: **The asthma epidemic**. *The New England journal of medicine* 2006, **355**(21):2226-2235.
3. Clemente JC, Pehrsson EC, Blaser MJ, Sandhu K, Gao Z, Wang B, Magris M, Hidalgo G, Contreras M, Noya-Alarcon O *et al*: **The microbiome of uncontacted Amerindians**. *Science advances* 2015, **1**(3).
4. Eder W, Mutius E: **Hygiene hypothesis and endotoxin: What is the evidence?** *Current opinion in allergy and clinical immunology* 2004, **4**:113-117.
5. Man WH, de Steenhuijsen Piter WAA, Bogaert D: **The microbiota of the respiratory tract: gatekeeper to respiratory health**. *Nature Reviews Microbiology* 2017, **15**:259.
6. Sbihi H, Boutin RC, Cutler C, Suen M, Finlay BB, Turvey SE: **Thinking bigger: How early-life environmental exposures shape the gut microbiome and influence the development of asthma and allergic disease**. 2019, **74**(11):2103-2115.
7. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, Heederik D, Piarroux R, von Mutius E, Group GTS: **Exposure to environmental microorganisms and childhood asthma**. *The New England journal of medicine* 2011, **364**(8):701-709.
8. Dannemiller KC, Lang-Yona N, Yamamoto N, Rudich Y, Peccia J: **Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations**. *Atmospheric Environment* 2014, **84**:113-121.

- 357 9. Kirjavainen PV, Karvonen AM, Adams RI, Täubel M, Roponen M, Tuoresmäki P, Loss
358 G, Jayaprakash B, Depner M, Ege MJ *et al.* **Farm-like indoor microbiota in non-farm**
359 **homes protects children from asthma development.** *Nature medicine* 2019,
360 **25(7):1089-1095.**
- 361 10. O'Connor GT, Lynch SV, Bloomberg GR, Kattan M, Wood RA, Gergen PJ, Jaffee KF,
362 Calatroni A, Bacharier LB, Beigelman A *et al.* **Early-life home environment and risk of**
363 **asthma among inner-city children.** *Journal of Allergy and Clinical Immunology* 2018,
364 **141(4):1468-1475.**
- 365 11. Dannemiller KC, Gent JF, Leaderer BP, Peccia J: **Indoor microbial communities:**
366 **Influence on asthma severity in atopic and nonatopic children.** *Journal of Allergy and*
367 *Clinical Immunology* 2016, **138(1):76-83.e71.**
- 368 12. Pitkäranta M, Meklin T, Hyvärinen A, Nevalainen A, Paulin L, Auvinen P, Lignell U,
369 Rintala H: **Molecular profiling of fungal communities in moisture damaged buildings**
370 **before and after remediation--a comparison of culture-dependent and**
371 **culture-independent methods.** *BMC microbiology* 2011, **11:235-235.**
- 372 13. Leung MH, Lee PK: **The roles of the outdoors and occupants in contributing to a**
373 **potential pan-microbiome of the built environment: a review.** *Microbiome* 2016,
374 **4(1):21.**
- 375 14. Prussin AJ, 2nd, Marr LC: **Sources of airborne microorganisms in the built**
376 **environment.** *Microbiome* 2015, **3:78.**
- 377 15. Yamamoto N, Hospodsky D, Dannemiller KC, Nazaroff WW, Peccia J: **Indoor**
378 **Emissions as a Primary Source of Airborne Allergenic Fungal Particles in Classrooms.**
379 *Environmental Science & Technology* 2015, **49(8):5098-5106.**
- 380 16. Amend AS, Seifert KA, Samson R, Bruns TD: **Indoor fungal composition is**
381 **geographically patterned and more diverse in temperate zones than in the tropics.**
382 *Proc Natl Acad Sci U S A* 2010, **107(31):13748-13753.**
- 383 17. Barberán A, Ladau J, Leff JW, Pollard KS, Menninger HL, Dunn RR, Fierer N:
384 **Continental-scale distributions of dust-associated bacteria and fungi.** *Proceedings of*
385 *the National Academy of Sciences of the United States of America* 2015,
386 **112(18):5756-5761.**
- 387 18. Adams RI, Miletto M, Taylor JW, Bruns TD: **Dispersal in microbes: fungi in indoor air**
388 **are dominated by outdoor air and show dispersal limitation at short distances.** *The*
389 *ISME journal* 2013, **7:1262.**
- 390 19. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, Bohannan BJ,
391 Brown GZ, Green JL: **Architectural design influences the diversity and structure of the**
392 **built environment microbiome.** *The ISME journal* 2012, **6(8):1469-1479.**
- 393 20. Lax S, Sangwan N, Smith D, Larsen P, Handley KM, Richardson M, Guyton K,
394 Krezalek M, Shogan BD, Defazio J *et al.* **Bacterial colonization and succession in a**
395 **newly opened hospital.** *Science translational medicine* 2017, **9(391).**
- 396 21. Lax S, Smith DP, Hampton-Marcell J, Owens SM, Handley KM, Scott NM, Gibbons

397 SM, Larsen P, Shogan BD, Weiss S *et al.* **Longitudinal analysis of microbial interaction**
398 **between humans and the indoor environment.** *Science* 2014, **345**(6200):1048-1052.

399 22. Nygaard AB, Charnock C: **Longitudinal development of the dust microbiome in a**
400 **newly opened Norwegian kindergarten.** *Microbiome* 2018, **6**(1):159.

401 23. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML,
402 Jones WJ, Roe BA, Affourtit JP *et al.* **A core gut microbiome in obese and lean twins.**
403 *Nature* 2009, **457**(7228):480-484.

404 24. Fierer N, Hamady M, Lauber CL, Knight R: **The influence of sex, handedness, and**
405 **washing on the diversity of hand surface bacteria.** *Proc Natl Acad Sci U S A* 2008,
406 **105**(46):17994-17999.

407 25. Castro-Rodriguez JA, Forno E, Rodriguez-Martinez CE, Celedon JC: **Risk and**
408 **Protective Factors for Childhood Asthma: What Is the Evidence?** *The journal of allergy*
409 *and clinical immunology In practice* 2016, **4**(6):1111-1122.

410 26. Schisterman EF, Cole SR, Platt RW: **Overadjustment bias and unnecessary**
411 **adjustment in epidemiologic studies.** *Epidemiology* 2009, **20**(4):488-495.

412 27. Liu S, Huang S, Chen F, Zhao L, Yuan Y, Francis SS, Fang L, Li Z, Lin L, Liu R *et al.*
413 **Genomic Analyses from Non-invasive Prenatal Testing Reveal Genetic Associations,**
414 **Patterns of Viral Infections, and Chinese Population History.** *Cell* 2018,
415 **175**(2):347-359.e314.

416 28. Sun Y, Zhang Y, Bao L, Fan Z, Sundell J: **Ventilation and dampness in dorms and**
417 **their associations with allergy among college students in China: a case-control study.**
418 *Indoor air* 2011, **21**(4):277-283.

419 29. Sun Y, Zhang Y, Sundell J, Fan Z, Bao L: **Dampness in dorm rooms and its**
420 **associations with allergy and airways infections among college students in China: a**
421 **cross-sectional study.** *Indoor air* 2009, **19**(4):348-356.

422 30. Pekkanen J, Sunyer J, Anto JM, Burney P: **Operational definitions of asthma in studies**
423 **on its aetiology.** *The European respiratory journal* 2005, **26**(1):28-35.

424 31. Sun Y, Li Y, Yuan Q, Zhang Z, Deng Y, Norbäck D, Zhang X, Fu X: **Distinct**
425 **microbiome composition of floor and air dust distort richness estimation in university**
426 **dormitories.** *BioRxiv* 2019, **2019/855684**.

427 32. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK,
428 Fierer N, Pena AG, Goodrich JK, Gordon JI *et al.* **QIIME allows analysis of**
429 **high-throughput community sequencing data.** *Nature methods* 2010, **7**(5):335-336.

430 33. Lozupone C, Knight R: **UniFrac: a new phylogenetic method for comparing microbial**
431 **communities.** *Applied and environmental microbiology* 2005, **71**(12):8228-8235.

432 34. Ramette A: **Multivariate analyses in microbial ecology.** *FEMS microbiology ecology*
433 2007, **62**(2):142-160.

434 35. McArdle BH, Anderson MJ: **Fitting multivariate models to community data: A comment**
435 **on distance-based redundancy analysis.** *Ecology (Washington D C)* 2001,
436 **82**(1):290-297.

- 437 36. Lloyd-Price J, Mahurkar A, Rahnavard G, Crabtree J, Orvis J, Hall AB, Brady A,
438 Creasy HH, McCracken C, Giglio MG *et al.* **Strains, functions and dynamics in the**
439 **expanded Human Microbiome Project.** *Nature* 2017, **550**(7674):61-66.
- 440 37. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI: **The**
441 **human microbiome project.** *Nature* 2007, **449**(7164):804-810.
- 442 38. Karvonen AM, Kirjavainen PV, Täubel M, Jayaprakash B, Adams RI, Sordillo JE, Gold
443 DR, Hyvärinen A, Remes S, von Mutius E *et al.* **Indoor bacterial microbiota and**
444 **development of asthma by 10.5 years of age.** *Journal of Allergy and Clinical*
445 *Immunology* 2019.
- 446 39. Adams RI, Amend AS, Taylor JW, Bruns TD: **A unique signal distorts the perception of**
447 **species richness and composition in high-throughput sequencing surveys of microbial**
448 **communities: a case study of fungi in indoor dust.** *Microbial ecology* 2013,
449 **66**(4):735-741.
- 450 40. von Hertzen L, Hanski I, Haahtela T: **Natural immunity.** *EMBO reports* 2011,
451 **12**(11):1089-1093.
- 452 41. Dannemiller KC, Mendell MJ, Macher JM, Kumagai K, Bradman A, Holland N, Harley
453 K, Eskenazi B, Peccia J: **Next-generation DNA sequencing reveals that low fungal**
454 **diversity in house dust is associated with childhood asthma development.** *Indoor air*
455 2014, **24**(3):236-247.
- 456 42. Huang YJ, Nelson CE, Brodie EL, DeSantis TZ, Baek MS, Liu J, Woyke T, Allgaier M,
457 Bristow J, Wiener-Kronish JP *et al.* **Airway microbiota and bronchial**
458 **hyperresponsiveness in patients with suboptimally controlled asthma.** *Journal of*
459 *Allergy and Clinical Immunology* 2011, **127**(2):372-381.e373.
- 460 43. Fierer N: **Embracing the unknown: disentangling the complexities of the soil**
461 **microbiome.** *Nature Reviews Microbiology* 2017, **15**:579.
- 462 44. Morris RM, Rappé MS, Connon SA, Vergin KL, Siebold WA, Carlson CA, Giovannoni
463 SJ: **SAR11 clade dominates ocean surface bacterioplankton communities.** *Nature*
464 2002, **420**(6917):806-810.
- 465 45. Ghai R, Rodriguez-Valera F, McMahon KD, Toyama D, Rinke R, Cristina Souza de
466 Oliveira T, Wagner Garcia J, Pellon de Miranda F, Henrique-Silva F: **Metagenomics of**
467 **the water column in the pristine upper course of the Amazon river.** *PloS one* 2011,
468 **6**(8):e23785-e23785.
- 469 46. Embley TM, Stackebrandt E: **The molecular phylogeny and systematics of the**
470 **actinomycetes.** *Annual review of microbiology* 1994, **48**:257-289.
- 471 47. Marri PR, Stern DA, Wright AL, Billheimer D, Martinez FD: **Asthma-associated**
472 **differences in microbial composition of induced sputum.** *The Journal of allergy and*
473 *clinical immunology* 2013, **131**(2):346-352.e523.
- 474 48. van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof
475 M, Reijmerink NE, Dompeling E, van den Brandt PA, Ferreira I *et al.* **Mode and place**
476 **of delivery, gastrointestinal microbiota, and their influence on asthma and atopy.**

477 *Journal of Allergy and Clinical Immunology* 2011, **128**(5):948-955.e943.

478 49. Penders J, Thijs C, van den Brandt PA, Kummeling I, Snijders B, Stelma F, Adams H,
479 van Ree R, Stobberingh EE: **Gut microbiota composition and development of atopic**
480 **manifestations in infancy: the KOALA Birth Cohort Study.** *Gut* 2007, **56**(5):661-667.

481 50. Pekkanen J, Valkonen M, Täubel M, Tischer C, Leppänen H, M. Kärkkäinen P, Rintala
482 H, Zock J-P, Casas L, Probst-Hensch N *et al*: **Indoor bacteria and asthma in adults: a**
483 **multicentre case-control study within ECRHS II**, vol. 51; 2018.

484 51. Liao H-Y, Tao L, Zhao J, Qin J, Zeng G-C, Cai S-W, Li Y, Zhang J, Chen H-G:
485 **Clostridium butyricum in combination with specific immunotherapy converts**
486 **antigen-specific B cells to regulatory B cells in asthmatic patients.** *Scientific reports*
487 2016, **6**:20481-20481.

488 52. Qian J, Hospodsky D, Yamamoto N, Nazaroff WW, Peccia J: **Size-resolved emission**
489 **rates of airborne bacteria and fungi in an occupied classroom.** *Indoor air* 2012,
490 **22**(4):339-351.

491 53. Adams RI, Bhargava S, Dannemiller KC, Eisen JA, Fierer N, Gilbert JA, Green JL, Marr
492 LC, Miller SL, Siegel JA *et al*. **Ten questions concerning the microbiomes of buildings.**
493 *Build Environ* 2016, **109**:224-234.
494
495
496
497

509 calculated by 2-level hierarchic ordinal regression models adjusted for gender, smoking and
 510 parental asthma. Besides the five major phyla, other phyla had relatively small number of
 511 observed OTUs and thus merged as “Others”.

Phylum	Class	OR (95% CI)	p value
Proteobacteria		0.9(0.72-1.12)	0.35
	Alphaproteobacteria	0.75(0.55-1.02)	0.07
	Betaproteobacteria	0.97(0.8-1.17)	0.73
	Gammaproteobacteria	0.84(0.43-1.62)	0.60
Firmicutes		1.06(0.84-1.35)	0.62
	Bacilli	0.97(0.74-1.28)	0.82
	Clostridia	1.3(1.04-1.62) *	0.02
Actinobacteria		0.8(0.59-1.09)	0.16
	Actinobacteria	0.76(0.55-1.04)	0.08
Bacteroidetes		1.12(1.01-1.26) *	0.04
	Bacteroidia	1.17(1.02-1.34) *	0.03
Deinococcus-Thermus		0.91(0.8-1.05)	0.19
Others		1.19(0.95-1.5)	0.13
Total OTUs		0.97(0.86-1.1)	0.64

512 OR calculated for 100 OTU increase in Proteobacteria and Total OTUs; 50 OTU increase in
 513 Firmicutes, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria,
 514 Bacilli; 25 OTU increase in Others; 10 OTU increase in Deinococcus-Thermus and Clostridia; 5
 515 OTU increase in Bacteroidetes and Bacteroidia.

516 * p < 0.05.

517

Table 3. Association between bacterial abundance in air dust and asthma symptoms among student (N = 357) in dormitories. Odds Ratio (OR) and 95% confident interval (CI) were calculated by 2-level hierarchic ordinal regression models adjusted for gender, smoking and parental asthma. Significant positive association is coded with red color and significant negative association is coded with green color.

phylum	OR (95% CI)	p-value	class	OR (95% CI)	p-value	genus	OR (95% CI)	p-value
Proteobacteria	0.24(0.04-1.39)	0.111	Alphaproteobacteria	0.01(0-1.39)	0.067	<i>Altererythrobacter</i>	0.45(0.21-0.95)	0.036
						<i>Aminobacter</i>	0.01(0-0.56)	0.026
						<i>Dongia</i>	0.06(0-0.82)	0.035
						<i>Paracoccus</i>	0.06(0.01-0.45)	0.006
						uncultured_Bradyrhizobiaceae	0.23(0.05-0.99)	0.048
			Betaproteobacteria	0.42(0.03-5.39)	0.503	<i>Hydrogenophaga</i>	35.61(3.98-318.59)	0.001
						<i>Noviherbaspirillum</i>	0.34(0.12-0.99)	0.048
						uncultured_bacterium	4.8(1.08-21.28)	0.039
			Gammaproteobacteria	0.36(0.13-0.98)	0.045	<i>Variovorax</i>	0.32(0.11-0.88)	0.028
Firmicutes	3.94(0.66-23.47)	0.132	Bacilli	3.1(0.53-18.25)	0.21	<i>Enhydrobacter</i>	0(0-0.13)	0.002
			Clostridia	18.33(0.8-418.21)	0.068	<i>Facklamia</i>	0.05(0.01-0.44)	0.007
						<i>Eubacterium</i>	1.36(1.08-1.71)	0.009
						<i>Peptococcus</i>	4.11(1.14-14.89)	0.031
						Ruminococcaceae_UCG-005	0.57(0.34-0.97)	0.037
						Ruminococcaceae_UCG-013	5.81(1.45-23.23)	0.013
						uncultured_Halanaerobiales	2.06(1.1-3.86)	0.023
			Erysipelotrichia	0.95(0.65-1.41)	0.803	<i>Faecalibaculum</i>	0.07(0.01-0.64)	0.019
			Negativicutes	1.29(0.97-1.73)	0.084	<i>Dialister</i>	1.11(1.02-1.2)	0.013

Actinobacteria	1.01(0.7-1.44)	0.976	Acidimicrobiia	0.61(0.42-0.88)	0.008	uncultured_Actinobacterium	0.6(0.4-0.92)	0.02
			Actinobacteria	0.96(0.67-1.37)	0.81	<i>Aeromicrobium</i>	0.04(0-0.7)	0.027
						<i>Bifidobacterium</i>	1.17(1.03-1.32)	0.016
						<i>Dietzia</i>	0.09(0.01-0.93)	0.044
						<i>Kytococcus</i>	0.36(0.15-0.87)	0.023
						<i>Microbacterium</i>	0.68(0.49-0.94)	0.018
						<i>Modestobacter</i>	0.1(0.02-0.5)	0.005
						<i>Okibacterium</i>	0.03(0-0.64)	0.025
						<i>Tessaracoccus</i>	0.12(0.02-0.71)	0.02
						unidentified_Micrococcaceae	0.51(0.27-0.97)	0.039
			Coriobacteriia	1.65(1.19-2.31)	0.003	<i>Collinsella</i>	2.17(1.3-3.62)	0.003
Bacteroidetes	2.37(1.22-4.6)	0.011	Bacteroidia	2.36(1.22-4.57)	0.011	<i>Enorma</i>	1.21(1.01-1.45)	0.037
						Prevotella_7	1.28(1.04-1.58)	0.021
			Cytophagia	1.25(0.55-2.85)	0.597	Prevotella_9	1.1(1.02-1.18)	0.012
						<i>Cytophaga</i>	3.39(1.03-11.2)	0.045
						uncultured_Lentimicrobiaceae	20.86(3.35-130.04)	0.001
Armatimonadetes	0.78(0.51-1.18)	0.235	Sphingobacteriia	0.98(0.64-1.5)	0.924	uncultured_Bacteroidetes	0.02(0-0.69)	0.031
						unidentified_Sphingobacteriales	0.19(0.04-0.78)	0.021
Chloroflexi	1.28(0.34-4.86)	0.72	Chthonomonadetes	0.05(0.01-0.23)	<0.001	<i>Chthonomonas</i>	0.05(0.01-0.23)	<0.001
			Thermomicrobia	1.16(0.85-1.6)	0.349	unidentified_Thermomicrobia	3.03(1.15-8.03)	0.025

OR calculated for 0.001 increase for taxa with abundance < 0.001; 0.01 increase for taxa with abundance 0.001-0.01; 0.1 increase for taxa with abundance 0.01 – 0.1.

Table 4. Association between bacterial abundance in floor dust and asthma symptoms among student (N = 357) in dormitories. Odds Ratio (OR) and 95% confident interval (CI) were calculated by 2-level hierarchic logistic regression models adjusted for gender, smoking and parental asthma. Significant positive association is coded with red color and significant negative association is coded with green color (* p < 0.05, ** p < 0.01).

phylum	OR (95% CI)	p-value	class	OR (95% CI)	p-value	genus	OR (95% CI)	p-value
Proteobacteria	0.77(0.15-4.07)	0.763	Alphaproteobacteria	0.55(0.21-1.45)	0.225	<i>Nitrobacter</i>	0.25(0.09-0.73)	0.011
			Betaproteobacteria	0.51(0.27-0.99)	0.048			
Firmicutes	1.1(0.91-1.33)	0.325	Bacilli	1(0.8-1.25)	0.994	<i>Geobacillus</i>	1.39(1.12-1.72)	0.003
			Clostridia	1.13(1.04-1.22)	0.004	<i>Eubacterium</i>	1.99(1.12-3.53)	0.018
						<i>Ruminococcus</i>	1.19(1.06-1.33)	0.004
						<i>Blautia</i>	1.22(1.07-1.38)	0.002
						<i>Faecalibacterium</i>	1.27(1.04-1.55)	0.022
						<i>Peptoclostridium</i>	1.07(1.02-1.12)	0.004
						<i>Subdoligranulum</i>	1.09(1.03-1.15)	0.004
Actinobacteria	1.43(0.69-2.98)	0.334	Erysipelotrichia	1.09(1.03-1.15)	0.003			
			Negativicutes	1.4(1.11-1.76)	0.004	<i>Megamonas</i>	1.04(1.01-1.06)	0.004
			Coriobacteriia	1.11(1.03-1.19)	0.003	<i>Collinsella</i>	1.2(1.06-1.35)	0.004

OR calculated for 0.001 increase for taxa with abundance < 0.001; 0.01 increase for taxa with abundance 0.001-0.01; 0.1 increase for taxa with abundance 0.01 – 0.1.

Tables 5. Multivariate analysis between environmental characteristics and bacterial richness (α -diversity) and community variation (β -diversity). The α -diversity was calculated by forward stepwise linear multiple regression, and β -diversity was calculated by forward stepwise Adonis multivariate analysis on 10,000 permutations.

Air dust			Floor dust		
	observed OTU (α -diversity)	community variation (β -diversity)	observed OTU (α -diversity)	community variation (β -diversity)	
	beta (95% CI)	R2	beta (95% CI)	R2	
Environmental characteristics					
Building age a		0.035 **		0.035 *	
Sex of occupants b				0.044 **	
Floor level c					
Floor surface type d					
Floor cleaning method e					
Floor cleaning frequency f		0.027 *			
Wall surface type g				0.045 **	
Window orientation h	152.18 (38.63, 265.72) **				
Curtain cleaning frequency i		0.037 *			
Plant in room j			40.50 (3.47, 77.53) *		

*** p < 0.001, ** p < 0.01, * p < 0.05

^a Building age: new (0), old (1); ^b Sex of occupants: male (0), female (1); ^c Floor level: ground (1), top (2), middle (3); ^d Floor surface type: concrete (1), ceramic (2), stone (3), wood (4); ^e Floor cleaning method: dry (1), wet (2); ^f Floor cleaning frequency: < 3 times/week (1), > 3 times/week (2); ^g Wall surface type: paint (1), wall paper (2); ^h Window orientation: south/north (0), east/west (1); ⁱ Curtain cleaning frequency: 2 times/year (0), 4 times/years (1); ^j Plant in room: no (0), yes (1).

Table 6. Association between environmental characteristics and risk/protective genera in air dust. Beta coefficient was calculated by linear regression models and only significant beta are shown (p < 0.05). Genera identified to be protective in this study are coded with green color, and genera identified to be risk are coded with green color.

		Building age	Sex of occupants	Floor surface type	Floor cleaning method	Floor cleaning frequency	Wall surface type	Window orientation	Curtain cleaning frequency
Proteobacteria	<i>Caulobacter</i>	0.13							

	Noviherbaspirillum					0.11		-0.64	
	Methyloversatilis								0.99
	uncultured_Bradyrhizobiaceae	-0.11							0.22
	Variovorax						-0.11		0.5
Firmicutes	Facklamia				0.03				
	Eisenbergiella								0.2
	Peptococcus	-0.1							
Actinobacteria	Microbacterium	-0.43							1.63
	Unidentified_Actinobacteria								0.61
	Kytococcus								1.04
	Tessaracoccus	-0.08							
	Okibacterium								0.09
	Dietzia								0.33
	Unidentified_Acidimicrobiia								1.06
Bacteroidetes	Prevotella_7		-0.5						
	Cytophaga			-0.03					
	uncultured_Bacteroidetes	0.03							
	Unidentified_Sphingobacteriia	0.08							
Parcubacteria	Candidatus_Sonnebornia_yantaiensis			0.07					

544 OR calculated for 0.001 increase for taxa with abundance < 0.001; 0.01 increase for taxa with abundance 0.001-0.01; 0.1 increase for taxa with abundance 0.01 – 0.1.

1 Supplementary Figures

2

3

4

5
6 Figure S1. Photos for dormitory building, corridor and rooms.
7