Fungi and bacteria in the beds of rural and urban infants correlate with later risk of atopic diseases

Lehtimäki, Jenni; Gupta, Shashank; Hjelmsø, Mathis; Shah, Shiraz; Thorsen, Jonathan; Rasmussen, Morten Arendt; Soverini, Matteo; Li, Xuanji; Russel, Jakob; Trivedi, Urvish; Brix, Susanne; Bønnelykke, Klaus; Chawes, Bo Lund; Bisgaard, Hans; Sørensen, Søren J.; Stokholm, Jakob

Published in:
Clinical and Experimental Allergy

DOI:
10.1111/cea.14414

Publication date:
2023

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
**Original Article**

**Fungi and bacteria in the beds of rural and urban infants correlate with later risk of atopic diseases**

**Jenni Lehtimäki**1,2 | Shashank Gupta3 | Mathis Hjelmsø2 | Shiraz Shah2 | Jonathan Thorsen2,4 | Morten Arendt Rasmussen2,5 | Matteo Soverini2 | Xuanji Li3 | Jakob Russel3 | Urvish Trivedi3 | Susanne Brix6 | Klaus Bønnelykke2 | Bo Lund Chawes2 | Hans Bisgaard2,† | Søren J. Sørensen3 | Jakob Stokholm2,5

1Finnish Environment Institute, Helsinki, Finland
2COPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Gentofte, Denmark
3Section of Microbiology, Department of Biology, University of Copenhagen, Universitetsparken 15, Copenhagen, Denmark
4Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
5Section of Microbiology and Fermentation, Department of Food Science, University of Copenhagen, Frederiksberg C, Denmark
6Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark

**Correspondence**
Jakob Stokholm, COPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Gentofte, Denmark.
Email: stokholm@copsac.com

Søren J. Sørensen, Section of Microbiology, Department of Biology, University of Copenhagen, Universitetsparken 15, Copenhagen 2100, Denmark.
Email: sjs@bio.ku.dk

**Funding information**
The Lundbeck Foundation, Grant/Award Number: R180-2014-3356, R93-A8499 and R16-A1694; The Danish Ministry of Health, Grant/Award Number: 903516; the Danish Council for Strategic Research, Grant/Award Number: 0603-00280B; The Capital Region Research Foundation

**Abstract**

**Introduction:** Rural children have a lower risk of asthma and atopic diseases than urban children. However, whether indoor microbiota in non-farming rural homes provides protection is unclear.

**Methods:** Here, we examine if microbes in the beds of rural and urban infants are associated with later development of atopic diseases. We studied fungi and bacteria in the beds of 6-month-old infants (n = 514) in association with the risk of asthma, allergic rhinitis, eczema and aeroallergen sensitization at 6 years of age in the prospective COPSAC2010 cohort.

**Results:** Both fungal and bacterial diversity were lower in the beds of children, who later developed allergic rhinitis (−0.22 [−0.43,−0.01], p_adj = .04 and −.24 [−0.42,−0.05], p_adj = .01 respectively) and lower bacterial richness was discovered in beds of children developing asthma (−41.34 [−76.95,−5.73], p_adj = .02) or allergic rhinitis (−45.65 [−81.19,−10.10], p_adj = .01). Interestingly, higher fungal diversity and richness were discovered in the beds of children developing eczema (0.23 [0.02,0.43], p_adj = .03 and 29.21 [1.59,56.83], p_adj = .04 respectively). We defined a limited set of fungal and bacterial genera that predicted rural/urban environment. Some rural-associated bacterial genera such as Romboutsia and Bacillus and fungal genera Spegazzinia and Physcia were also associated with reduced risk of diseases, including eczema. These fungal and bacterial fingerprints predicting the living environment were associated with...
INTRODUCTION

In high-income countries, humans are extensively exposed to the microbes of indoor environments as they spend on average 90% of their time indoors. The vast exposure to indoor microbes has been proposed to influence human health, possibly by shaping the human microbiota composition or through immune activation. The indoor microbiota can be affected by numerous factors, such as pet ownership, household size, type of house, ventilation and geography, many of which have been associated with risk of atopic diseases in children. Importantly, the composition of indoor microbiota early in life has been associated to the later development of asthma, eczema or aeroallergen sensitization, whereas findings in adults are more contrasting, suggesting the importance of early microbial exposures.

Dust microbiota in the homes of farmers may protect their children from asthma and atopic diseases. Mice exposed to farm dust had reduced lung inflammation compared to a control group, supporting a causal protective effect of the farm microbiota. Nevertheless, in high-income countries, farming is becoming an increasingly rare profession, even in rural areas. Hence, reported rural–urban differences in the prevalence of asthma and atopic diseases cannot be fully explained by the ‘farm effect’. For example, increasing greenness of the living environment has been associated with decreases in the prevalence of aeroallergen sensitization not just among children in general, but also in asthma and allergic rhinitis, but not eczema, with rural compositions being protective. The bed dust bacteria mediated 27% of the protective association of a rural living environment for allergic rhinitis (p = .04).

Conclusions: Bed dust microbes can be differentially associated with airway- and skin-related diseases. The differing bed dust microbiota between rural and urban infants may influence their later risk of asthma and allergic rhinitis.

KEYWORDS
childhood, dust microbiome, indoor microbiome, non-communicable diseases, residential environment, urbanization

GRAPHICAL ABSTRACT
Living environment-associated bacteria and fungi in the beds of 6 months old infants were studied in association with their risk of developing asthma and atopic diseases at 6 years of age. Bacteria that associated with rural environment were also associated with reduced risk of asthma and allergic rhinitis, while rural fungi was associated with reduced risk of allergic rhinitis. Finally, rural bacteria partially mediated the protective association with rural environment in allergic rhinitis.

Key Messages
- Asthma and allergic rhinitis associated with different microbial exposures during infancy than eczema.
- Microbial taxa that associated with rural environment had protective association with asthma and allergic rhinitis.
- Microbes in infants’ beds could provide a modifiable factor for future translational approaches.
children living on farms. Additionally, recent studies have shown that in non-farming rural homes, the indoor microbiota is more diverse and composed of different taxa than in urban homes. Therefore, indoor microbial exposure in non-farming rural homes may partly explain rural and urban differences in the prevalence of asthma and atopic diseases. As far as we know, only one, recent study addressed this question. However, they combined rural and urban populations from different countries and found cohort-specific associations.

A developing child is susceptible to environmental exposures within a limited time window early in life, where the immune system is trained via microbe-mediated regulatory signals that are crucial for maintaining a healthy host-microbiota mutualism. As young children spend a lot of time in their beds, we hypothesized that early exposure to specific taxa in bed dust can influence their later health. Importantly, we hypothesized that taxa originating from surrounding living environment (rural/urban) can mediate lasting health impacts. We tested these hypotheses in the prospective COPSAC mother–child cohort with deep clinical phenotyping, where we have previously discovered major differences in the prevalence of asthma and atopic diseases between rural and urban children.

2 | METHODS

2.1 | Data collection

Bed dust samples (n = 580) for the characterization of microbiota were collected from 6-month old children in the COPSAC mother–child cohort (n = 700). Children from the cohort have been followed prospectively since birth, and extensive data have been collected during scheduled and acute care visits at the COPSAC clinic. Dedicated study physicians were solely responsible for the diagnosis and treatment of all respiratory, allergy and skin-related symptoms. An objective definition of the living environment (rural or urban) was based on the CORINE Land Cover database as previously described.

The parents collected bed dust samples from the child’s bed. They attached an external filter [DUSTREAM® Collector, Indoor Biotechnologies (n = 50) or Dust Collecting Device from ALK-Abello (n = 464)] to their vacuum cleaner and vacuumed throughout the sheets and pillow for 5 min as instructed. They were instructed not to change sheets prior to vacuuming. Filters were frozen in the home for 3 days to kill house dust mites and then shipped by mail to the research unit where they were afterwards kept at −20°C until DNA extraction.

2.2 | Diagnosis of diseases

**Asthma** at age 6 years: Diagnosis was made prospectively throughout childhood based on an exhaustive quantitative symptom algorithm as previously described.

**Allergic rhinitis** at age 6 years was diagnosed based on allergic sensitization and clinical interviews.

**Eczema** at age 6 years: Diagnosis was made prospectively throughout childhood based on the criteria of Hanifin and Rajka.

**Aeroallergen sensitization** at age 6 years was determined from serum IgE measurements and/or skin prick test to 10 aeroallergens as described. All sequencing data are deposited at the Sequence Read Archive (PRJNA605085).

2.4 | Bioinformatics and quality control

Reads were analysed by QIIME2 (qiime2-2018.11) pipeline through DADA2 to infer the amplicon sequence variants (ASVs) present and their abundances across the samples. Taxonomy was assigned using a pre-trained sklearn-based taxonomy classifier specific for the V3-V4 16S region (Silva, release-132, 99% ASV) and for ITS sequencing, UNITE database (dynamic-2017-12-01) were used. Samples with exceptionally low or high read depth were excluded. Lower cut-off was 6000 for fungal samples (n excluded = 5) and 4000 for bacterial samples (n = 3) and higher cut-off for bacterial samples was 60,000 sequences (n = 6). The samples were rarefied to the lowest sequencing depth after cut-off (bacterial ASVs to 6774 sequences and fungal ASVs to 9942 sequences) to avoid bias due to sampling depth. See Appendix S1 for details.

2.5 | Statistical analysis

From the 580 children who had bed dust samples collected, we excluded children (n = 53) who had their dust samples collected after 1 year of age as well as children living on farms (n = 14), leaving us 514 children for the downstream analysis. Figure S1 shows the distribution of data collection time points in the final data sets.

The R-package phyloseq was used for handling the microbiota and metadata. Differences in the observed richness and Shannon diversity according to disease status were tested with adjusted linear models. The community structure of microbiota and metadata. Differences in the observed richness and Shannon diversity according to disease status were tested with adjusted linear models. The community structure of microbiota and metadata. Differences in the observed richness and Shannon diversity according to disease status were tested with adjusted linear models. The community structure of microbiota and metadata. Differences in the observed richness and Shannon diversity according to disease status were tested with adjusted linear models. The community structure of microbiota and metadata. Differences in the observed richness and Shannon diversity according to disease status were tested with adjusted linear models.
Suitable method for testing differential abundance was determined with DA Test R package. Differences in relative abundances at genus level between children with and without diseases were analysed using zero-inflated log-normal model provided in metagenomeseq R package (function fitFeatureModel) as DA Test suggested it to be the most robust method for our data. Corrections for multiple testing (significance threshold was $q < .05$) were done within families of tests, for example, separately for Figures 1A–D or 2A–D.

Sparse partial least squares (sPLS) models with log-transformed relative abundances of bacterial and fungal genera, separately, were used to predict the rural and urban groups (mixOmics and caret R-packages). The optimum model was selected based on the root mean squared error (RMSE) statistics, from repeated 10-fold cross-validation to avoid overfitting. In other words, as we have previously reported a significant effect of the living environment (rural/urban) on the risk of developing the studied diseases and on the bed dust microbiota, we decided to define a limited set of fungal and bacterial genera, separately, which were the best predictors of the living environment. Based on each sample’s resemblance to the composition of these models, a score was extracted for each child, that is, a fungal living environment score and a bacterial living environment score. Higher predicted values indicated a more urban composition, while lower values indicated more rural composition in the bed dust. These scores were subsequently tested against diseases with linear models. Finally, we used a model-based causal mediation analysis (mediation R-package) utilizing bootstrapping approximation with 1000 simulations for disentangling the effect of living environment and fungal or bacterial scores in the development of diseases. Marginal significance indicates $p$-values above significance level ($≤ .05$) but $< .1$. All analyses were conducted in R version 4.3.0.43

3 | RESULTS

We discovered in total 18,997 fungal amplicon sequencing variants (ASVs) and 43,233 bacterial ASVs from the beds of 6-month-old infants. The median observed richness was 243 (range: 6–505) and 274 (55–756) for the fungal and bacterial samples respectively. The median Shannon diversity was 3.71 (ranging between 1.05 and 5.02) in the fungal samples and 3.94 (1.13–5.49) in bacterial samples. The infants’ age at the time of dust sampling (Figure S1) was associated with fungal (PERMANOVA, $F = 1.99, R^2 = .004, p = .002$) and bacterial ($F = 6.05, R^2 = .012, p < .001$) community structure, that indicates the number, types and relative abundances of the different taxa within a sample in relation to other samples’ compositions.

3.1 | Higher richness associated with reduced risk of asthma and allergic rhinitis

From the 514 studied children, 7.7% (38) had asthma, 7.0% (35) had allergic rhinitis, 7.7% (38) had eczema ongoing at age 6 years and 23.5% (87) were sensitized to aeroallergens. Fungal Shannon diversity was lower in the beds of infants who had allergic rhinitis at 6 years of age (adjusted linear models, Estimate $= −0.22, 95\% CI [−0.43,−0.01], p = .04$). Opposite directionality was discovered for eczema, that is, a higher fungal diversity in the infant beds was associated with an increased risk of having eczema at 6 years of age ($0.23, [0.02,0.43], p = .03$). Bacterial Shannon diversity was lower in infants having allergic rhinitis at 6 years of age ($−0.24, [−0.42,−0.05], p = .01$). No differences in fungal or bacterial diversity were discovered in children with or without aeroallergen sensitization at age of 6 years (Table 1).

The fungal richness was also higher in the beds of infants, who had eczema at 6 years of age (29.21, [1.59,56.83], $p = .04$). The bacterial richness was lower in the beds on infants who had asthma or allergic rhinitis at 6 years of age ($−41.34, [−76.95,−5.73], p = .02$ and $−45.65, [−81.19,−10.10], p = .01$ respectively). No differences in richness were discovered in children with or without aeroallergen sensitization at 6 years of age (Table 1).

Only 1% of children had both asthma and allergic rhinitis, 0.6% had both asthma and eczema, while 1.4% of children had both allergic rhinitis and eczema. Children, who had both asthma and allergic rhinitis had both very low fungal and bacterial richness and diversity (all $p < .004$). Interestingly, children who had either asthma or rhinitis in co-morbidity with eczema had higher bacterial richness than children with only asthma or rhinitis. Marginal associations indicated that children with only eczema had higher fungal richness and diversity than children with co-morbidity with of either asthma or rhinitis and eczema (Table S1).

The significance levels and conclusions did not change when children growing on farms were included in the analysis.

The fungal community structure (Bray–Curtis dissimilarity) was associated with asthma and allergic rhinitis at 6 years of age (adjusted PERMANOVA: $F = 1.63, R^2 = .003, p = .03$ and $F = 2.02, R^2 = .004, p < .01$ respectively, Table 2). We found no significant associations between these diseases and bacterial community structure. However, marginally significant association were found for asthma, eczema and aeroallergen sensitization (Table 2).

3.2 | Some of the most common genera in the beds of infants were associated to the later risk of developing diseases

From the 100 most abundant fungal and bacterial genera, we discovered both taxa with positive and negative association with later diseases in differential abundance testing, (Figures 1 and 2). These fungal and bacterial genera tended to partly overlap in asthma, allergic rhinitis and aeroallergen sensitization, while different genera associated with eczema. For example, bacterial genus Neisseria was found in higher abundances in both asthma and allergic rhinitis. Genera Lawsonella and Prevotella were higher in allergic rhinitis and aeroallergen sensitization, while Paracoccus and Bacillus were lower in these diseases. From fungal genera, Spegazzinia was negatively associated with asthma and allergic rhinitis and taxa from family Xylariaceae negatively associated with allergic rhinitis and aeroallergen sensitization.
TABLE 1 The fungal and bacterial alpha diversity in the beds of infants in association to asthma, allergic rhinitis, eczema and aeroallergen sensitization at 6 years of age.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Disorder</th>
<th>Disease (yes)</th>
<th>Disease (no)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Shannon</td>
<td>Fungi</td>
<td>Asthma</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhinitis</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eczema</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sensitization</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Asthma</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhinitis</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eczema</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sensitization</td>
<td>86</td>
</tr>
</tbody>
</table>

Note: Adjusted linear models on association between alpha diversity (Shannon diversity and observed richness) of fungal and bacterial community and diseases. Bold values mark significant p-values. Models were adjusted for sampling age and season, dust filter type, sequencing run, pet ownership, number of older siblings and rural/urban classification.

TABLE 2 The fungal and bacterial community structure in the beds of infants in association to asthma, allergic rhinitis and eczema at 6 years of age.

<table>
<thead>
<tr>
<th>Diagnosis at 6 years of age</th>
<th>Fungi</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>F</td>
</tr>
<tr>
<td>Asthma</td>
<td>467</td>
<td>1.631</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>469</td>
<td>2.02</td>
</tr>
<tr>
<td>Eczema</td>
<td>467</td>
<td>1.75</td>
</tr>
<tr>
<td>Sensitization</td>
<td>348</td>
<td>1.398</td>
</tr>
</tbody>
</table>

Note: Significance was tested with PERMANOVA. Models were adjusted for sampling age and season, dust filter type, sequencing run, pet ownership, number of older siblings and rural/urban classification. F indicates effect sizes, that is, the magnitude of the differences between groups. R² indicates the proportion of variation that was explained by the variable of interest. Bold value indicates significant p-values.

However, very few of these associations remained significant after correction for false discovery rate. These included following associations: Bacterial taxa from *Family Saccharimonadales* was positively associated with asthma. Bacterial genus *Prevotella* and fungal genus *Daldinia* were positively associated with allergic rhinitis.

3.3 Bed dust microbiota can mediate the protective effect of rural environment in allergic rhinitis

The limited set of fungal and bacterial taxa that predicted living environment, that is, the *fungal and bacterial living environment scores* (Figure 3A,B), included some taxa that were also differentially abundant between healthy and diseased children (Figures 1 and 2). Especially, taxa that had protective association with diseases were associated with rural environment in the sPLS analysis. For example, bacterial genus *Rombeutia* that associated rural living environment community was negatively associated with eczema, while genus *Bacillus* that associated with rural living environment was negatively associated with allergic rhinitis and aeroallergen sensitization. Fungal taxa from *Family Sporidiobolaceae* that associated with rural living environment was negatively associated with eczema, while genus *Spegazzinia* that associated with rural living environment was negatively associated with allergic rhinitis and asthma. Moreover, genus *Physcia* that associated with rural living environment was negatively...
associated with asthma. Yet, less coherently, higher abundance of genus *Aspilicia* was a risk factor for eczema but was associated to rural living environment.

A higher bacterial living environment score (i.e. more urban composition) was associated with the development of later asthma (linear model, Estimate = 0.07, 95% CI [0.00,0.13], p = .04; Figure 3E), while both higher fungal and bacterial living environment scores were associated with the later development of allergic rhinitis (0.08, [0.00,0.15], p = .04 and .09, [0.02,0.15], p = .01 respectively; Figure 3G,H). No association existed between the scores and eczema or aeroallergen sensitization.

Finally, the bacterial living environment score significantly mediated the protective effect of the rural environment in the development of allergic rhinitis (27% was mediated by bacterial score, p = .04), while a marginally significant association was discovered for asthma (complete mediation, p = .06; Table 3). No mediation effect was observed for the fungal living environment score or eczema and aeroallergen sensitization.

**FIGURE 1** The top bacterial genera in infant beds, which differ in relative abundance between children with or without asthma, rhinitis, eczema or allergic sensitization. Relative abundances of top 100 bacterial genera were tested with metagenomeseq feature model and significant differences were plotted. *q*-values indicate *p*-values corrected for false discovery rate.

**FIGURE 2** The top fungal genera in infant beds, which differ in relative abundance between children with or without asthma, rhinitis, eczema or allergic sensitization. Relative abundances of top 100 fungal genera were tested with metagenomeseq feature model and significant differences were plotted. *q*-values indicate *p*-values corrected for false discovery rate.
**DISCUSSION**

Our results showed that bed dust microbes early in life were associated with the development of asthma and atopic diseases. Interestingly, the associations between richness, diversity and community structure of the bed dust microbiota differed among diseases. Higher diversity and richness were associated with lower risk of asthma and allergic rhinitis while they were associated with a higher risk of eczema by age 6 years. Moreover, some disease-associated taxa were shared between asthma, allergic rhinitis and aeroallergen sensitization, while different taxa were found to be associated with eczema. Based on our previous research, we
TABLE 3 Results from a causal mediation analysis testing if bed dust microbes can mediate the effect of living environment on health.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Mediator</th>
<th>Outcome</th>
<th>n</th>
<th>Effect</th>
<th>Estimate</th>
<th>[95% CI]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Living environment (Rural/urban)</td>
<td>Bacterial living environment score</td>
<td>Asthma</td>
<td>466</td>
<td>ACME</td>
<td>0.017</td>
<td>[-0.00,0.03]</td>
<td>.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ADE</td>
<td>[-0.06,0.04]</td>
<td>.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proportion (ACME/Total)</td>
<td>1.407</td>
<td>[-8.60,10.4]</td>
<td>.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>0.012</td>
<td>[-0.03,0.05]</td>
<td>.59</td>
</tr>
<tr>
<td></td>
<td>Fungal living environment score</td>
<td>Rhinitis</td>
<td>468</td>
<td>ACME</td>
<td>0.015</td>
<td>[0.00,0.03]</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ADE</td>
<td>[-0.01,0.09]</td>
<td>.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proportion (ACME/Total)</td>
<td>0.272</td>
<td>[-0.01,1.44]</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>0.061</td>
<td>[0.00,0.10]</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ACME</td>
<td>0.003</td>
<td>[-0.02,0.03]</td>
<td>.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asthma</td>
<td>468</td>
<td>ADE</td>
<td>[-0.05,0.06]</td>
<td>.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proportion (ACME/Total)</td>
<td>0.339</td>
<td>[-5.33,5.87]</td>
<td>.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>0.009</td>
<td>[-0.04,0.06]</td>
<td>.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ACME</td>
<td>0.013</td>
<td>[-0.01,0.04]</td>
<td>.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhinitis</td>
<td>470</td>
<td>ADE</td>
<td>[-0.01,0.09]</td>
<td>.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proportion (ACME/Total)</td>
<td>0.251</td>
<td>[-0.34,1.18]</td>
<td>.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>0.051</td>
<td>[0.00,0.10]</td>
<td>.03</td>
</tr>
</tbody>
</table>

Note: Causal mediation analysis tests whether the mediation effect estimated by logistic regression is significant. Average Causal Mediated Effect (ACME) indicates the effect of mediator, that is, the fungal or bacterial living environment score. Average Direct Effect (ADE) indicates the direct effect of predictor, that is, living environment (rural/urban) and the proportion of mediation (ACME/Total) indicates the proportion of the fungal or bacterial living environment score explained by the total effect of the living environment on asthma and rhinitis. The total effect indicates the influence of the predictor on outcome (without mediator). Bootstrapping approximation with 1000 simulations was used for confidence intervals. Note that even though the name of the analysis contains ‘causal’, these data are observational. Bold value indicates significant p-values.

Further hypothesized that rural and urban microbes in the infants’ beds can affect the later risk of asthma and atopic diseases. We discovered that living in a rural environment may provide a source of taxa associated to lower risk of asthma and allergic rhinitis. For example, many of the rural environment-associated taxa were negatively associated with diseases, including eczema. Importantly, we found that taxa associated to rural environment can mediate the protective effect in allergic rhinitis. This suggests that exposure to environment-specific microbes instead of other rural/urban associated factors can be important for the development of allergic rhinitis. Throughout the study, associations with bed dust microbiota were most robust with allergic rhinitis, though the end points are overlapping and might to some extent resemble part of the same children. Moreover, associations with bed dust microbiota were less coherent with eczema than with other outcomes.

The strengths of our study include longitudinal data enabling a focus on early life effects on later health, identical definition of diseases in the cohort and utilization of multiple layers of data including land cover, microbiome and health outcomes. Our study is limited by the observational setup, which does not enable confirmation of causality. Even though several significant differences were discovered, children with and without disease also overlapped in several measures such as richness, diversity and taxa abundances. Moreover, proportion of the entire 6-month dust community structure that was explained by whether or not the child developed atopic disease at age 6 years was rather small. Our study is probably unable to cover all the complexities in associations between early life living environments, microbial exposures and later health. For example, our previous study indicated that the window of opportunity, that is, the sensitive time period for microbial exposures, might differ between diseases. Moreover, even though we had only a single sample per child, the age at collection time varied between individuals. This might cause noise in the data and limit statistical power.

Our findings suggest that early life microbial exposures may have distinct effects in airway and skin-related diseases. The difference can be due to differing aetiology of these diseases or the route of entry of the microbes, that is, inhaled or through skin contact. The diagnosis of one atopic disorder increases the probability of developing or having another atopic disorder. However, in our data, the co-morbidities between asthma, rhinitis or eczema were rare. In previous research, asthma and allergic rhinitis frequently coexist, which is not surprising as they comprise lower and upper airway diseases respectively. Eczema (atopic dermatitis) has been associated with numerous co-morbidities, many of which present skin-related infections and disorders. However, eczema is also likely to coexist with asthma. Despite limited power to evaluate co-morbidities, out data suggest that co-morbidity between asthma and eczema is associated with very high richness and diversity of dust microbiota while co-morbidity of asthma and rhinitis is associated with very low richness and diversity. Thus, it is possible that early life microbial exposures can be either too
limited or too abundant and perfect balance exists somewhere between these extremes. For example, in a recent Finnish study that combined several cohorts, greenness around the home did not associate with eczema risk at age 2 years, but coniferous and mixed forests associated with increased risk of eczema.47 However, living in a rural environment during the first year of life was associated with lower risk of eczema in our cohort.27 In other studies, indoor microbial exposures tend to associate with lower risk of eczema but inconsistency between studies exist.48-50 Yet, previous studies have consistently shown that increased exposure to indoor microbes is associated with lower risk of asthma9,12,15,17,18,51 and allergic rhinitis.25

Our findings propose that a rural environment can generally provide beneficial microbial exposures early in life, supporting later health. Previous research shows that a farming lifestyle supports health-promoting indoor microbiomes.15,18 The protective effect of both a farming lifestyle and a general rural environment may have a shared basis, such as extensive microbial exposure. However, key taxa tend to differ between our study and studies on the farm effect. Farming lifestyle increases cattle-related microbes indoors,15 which can form a distinct indoor microbiota in farming homes. Moreover, sampling location (e.g. bed vs. floor) may influence on taxa discovered. Additionally, the difference in indoor microbiota structure between farming homes and other (rural) homes is more contrasting than between rural and urban homes in this Danish cohort. We can consider two potential explanations for this. Microbes originating from the child can have profound effects on bed dust microbiota, lessening the contrast between rural and urban areas. Another option is a gradient of decreasing exposures to beneficial microbes from farms to other rural homes and finally to urban areas.52

5 | CONCLUSIONS

We discovered that microbes associated with rural environment in the infants’ beds are associated with lower risk of asthma and allergic rhinitis. In allergic rhinitis, rural microbes are partly mediating the protective association of rural environment. Microbes in beds were also associated with the later risk of eczema, but rural environment may not provide protective exposures against eczema. This is probably because different aspects in microbial exposures are associated with the development of airway- and skin-associated diseases.

AUTHOR CONTRIBUTIONS

All co-authors have contributed substantially to the analyses and interpretation of the data, and have provided important intellectual input. Jenni Lehtimäki wrote the first draft of the article, performed the data analysis and initial interpretation, and prepared figures and tables. Shashank Gupta performed the laboratory analysis and bioinformatics. All authors have agreed that the accuracy and integrity of any part of the work has been appropriately investigated and resolved and all have approved the final version of the article. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication. No honorarium, grant or other form of payment was given to anyone to produce the article.

ACKNOWLEDGEMENTS

This study would not be possible without the time and efforts that all children and parents in the COPSAC cohort have contributed to science advancement. We express our deepest gratitude to them. We also thank numerous members of the COPSAC research team who have participated in data collection and processing.

FUNDING INFORMATION

The Lundbeck Foundation (Grant Nos. R16-A1694, R93-A8499 and R180-2014-3356), The Danish Ministry of Health (Grant No. 903516), the Danish Council for Strategic Research (Grant No. 0603-00280B) and The Capital Region Research Foundation.

CONFLICT OF INTEREST STATEMENT

All authors declare no potential, perceived or real conflict of interest regarding the content of this article. The funding agencies did not have any role in design and conduct of the study; collection, management and interpretation of the data; or preparation, review or approval of the article. No pharmaceutical company was involved in this study.

DATA AVAILABILITY STATEMENT

The data set analysed during this study are available in the Sequence Read Archive (SRA) repository under project id PRJNA605085 for bed dust samples (both bacterial and fungal raw sequencing reads). All other data are available from the corresponding author.

ETHICS STATEMENT AND CONSENT TO PARTICIPATE

This study was approved by the local ethics committee (H-B-2008-093), and the Danish Data Protection Agency (2015-41-3696). Both parents gave verbal and written informed consent before enrolment.

CONSENT FOR PUBLICATION

Not applicable.

ORCID

Jakob Stokholm https://orcid.org/0000-0003-4989-9769

REFERENCES


SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.