HHS Public Access

Author manuscript

Pediatr Res. Author manuscript; available in PMC 2021 December 17.

Published in final edited form as:

Pediatr Res. 2021 December ; 90(6): 1153–1160. doi:10.1038/s41390-021-01400-1.

Altered Microbiomes in Thirdhand Smoke-Exposed Children and their Home Environments

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Abstract

Introduction.—Tobacco smoke contains numerous toxic chemicals that accumulate in indoor environments creating thirdhand smoke (THS). We investigated if THS-polluted homes differed in children's human and built environment microbiomes as compared to THS-free homes.

Methods.—Participants were N=19 THS exposed children and N=10 unexposed children (≤ 5 years) and their parents. Environmental and biological samples were analyzed for THS pollutants and exposure. Swab samples were collected from the built environment (floor, table, armrest, bedframe) and child (finger, nose, mouth, and ear canal) and 16S ribosomal RNA genes were analyzed for bacterial taxa using high-throughput DNA sequencing.

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Authorship contributions

Each author has met the *Pediatric Research* authorship requirements by making substantial contributions to

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3) analysis and interpretation of data: Matt, Kelley, Liu, Quintana, Hoh, Dodder

4) drafting the article or revising it critically for important intellectual content: Matt, Kelley, Liu, Quintana, Hoh, Dodder, Mahabee-Gittens

5) Final approval of the version to be published: Matt, Kelley, Quintana, Mahabee-Gittens

Declaration of Interests

The authors declare no competing interests.

Category of Study

Clinical population study of children exposed to thirdhand smoke

Consent Statement

The study was approved by the San Diego State University Institutional Review Board. A research assistant verbally reviewed the consent form, and participants signed the consent form.

Results.—Phylogenetic α-diversity was significantly higher for the built environmental microbiomes in THS-polluted homes compared to THS-free homes ($p<0.014$). Log2 fold comparison found differences between THS-polluted and THS-free homes for specific genera at built environment (e.g., *Acinetobacter, Bradyrhizobium, Corynebacterium, Gemella, Neisseria, Staphylococcus, Streptococcus, Veillonella)* and in samples from children s (esp. *Corynebacterium, Gemella, Lautropia, Neisseria, Rothia, Staphylococcus, and Veillonella).*

Conclusion.—When exposed to THS, indoor and children microbiomes are altered in an environment-specific manner. Changes are similar to those reported in previous studies for smokers and secondhand smoke-exposed persons. THS-induced changes in child and environmental microbiome may play a role in clinical outcomes in children.

INTRODUCTION

Some of the most prominent chemical constituents of tobacco smoke (e.g., nicotine, polycyclic aromatic hydrocarbons) have antifungal and antibacterial properties, and there is a growing body of research demonstrating that active smoking and exposure to secondhand smoke alters the human microbiome (MB).(1–3) These tobacco-smoke induced alterations of the mouth, nose, ear, and gut MB are believed to interfere with the normal functioning of the immune system and are suspected to have other harmful systemic impacts in humans (e.g., immune system).(4–11) Tobacco smoke may have an even broader impact on the microbial ecologies coexisting with humans and their built-environments (BE), however, because tobacco smoke leaves behind a persistent chemical residue on surfaces, in settled house dust, and in materials and objects. Known as thirdhand smoke (THS), these pollutants are found at high concentrations on surfaces and in dust of indoor environments long after the last known or suspected tobacco use.(12, 13) THS pollutants also inhabit the same physical space (e.g., skin, mouth, surfaces of materials) and exist on similar physical scales as microorganisms.(14–17)

A recent study of 220 low-income multiunit housing units of nonsmokers showed that THS residue was found in all homes regardless of the current smoking status of the residents and the presence of smoking bans.(15) Moreover, 10% of nonsmoker units showed surface nicotine concentrations at levels associated with homes of active smokers in previous studies.(15) In indoor environments where tobacco has been smoked frequently over long periods of time, considerable reservoirs of THS pollutants can accumulate in carpets, furniture, upholstery, building materials, and other objects. From these reservoirs, new THS pollutants may be re-emitted or transferred via physical contact leading to the involuntary and unsuspected exposure of residents.(12, 13) Tobacco residue has been found in private homes, hotels, used cars, neonatal intensive care units, and on the hands of children and adults moving into homes formerly occupied by smokers, and living in homes of smokers who quit smoking.(18–23) Because of higher frequency of hand-to-mouth behaviors, higher amounts of dust ingestion, and the ongoing development of the immune system and major organs, young children are at particular risk of exposure to pollutants in dust and on surfaces and are most sensitive to their adverse health effects.(16, 24–27) This study investigated if: (1) homes of smokers with indoor home smoking bans were polluted with higher levels of THS in reservoirs accessible to young children (i.e., dust, surfaces), (2) children in homes

of smokers with indoor smoking bans had higher levels of THS on their hands and in their bodies, (3) homes of smokers differed in their BE microbiomes (e.g., bedframe, pillow, table) from homes of nonsmokers, and (4) human MB (e.g., mouth, ear, nose, finger) of children in THS-polluted homes differ from those of THS-free homes.

METHODS

Research Design

We applied a quasi-experimental design that compared multiple samples of the indoor BE and child MB in homes polluted with THS (i.e., THS-exposed group, TEG) to homes free of tobacco smoke pollutants (i.e., no exposure group, NEG). To be eligible for TEG, residences had to have at least one adult who smoked at least 20 cigarettes per week, lived with a child ≤5 years of age, did not smoke in the presence of the child, and had not smoked inside the home during the past week. To be eligible for NEG, all residents in the home and primary caregivers of the child had to be nonsmokers, no smoking may have taken place inside the home over the past 6 months, there was a strictly enforced smoking ban inside the home, and the child was not exposed to tobacco smoke outside the home in the past month. Since parent-reported smoking status may not accurately reflect the levels of THS in a home or a child's exposure, we measured THS levels in dust, in surfaces, and on the hands and in the urine of children living in the homes. As described in the Supplemental Material, we created validated NEG (vNEG) and TEG (vTEG) groups based on the measured levels of nicotine in dust, on surface, and on hands and based on urinary cotinine. The comparisons of MB samples are based on these validated THS groups.

Participants

After approval by the San Diego State University Institutional Review Board, interested participants were screened by telephone to determine if they qualify and to explain study procedures. At the home visit, a research assistant verbally reviewed the consent form, and participants signed the consent form. Participants received $50 for completing an interview and allowing the collection of environmental and biological samples. Tables S1 and S2 in the online supplemental material provide demographic data and describe tobacco product use in NEG and TEG homes. The participating children in NEG and TEG had a median age of 3.7 and 3.9 years, and 50% and 58% were identified as White, respectively. None of the residents of NEG homes were current users of any tobacco products, and all homes had strict smoking bans. In TEG, all homes had at least one current user of combustible cigarettes, six homes also had users of electronic cigarettes, two of smokeless tobacco, and two of hookah. None of the TEG homes reported indoor use of conventional cigarettes, electronic cigarettes, or hookah inside the home during the past month.

Measures

Pairs of research assistants visited participants' homes to conduct in-person interviews and collect environmental and biological samples.

Personal Interviews—Interviews were conducted with the eligible adult participant who self-reported smoking history in the apartment, personal and other residents' use of

conventional cigarettes, electronic cigarettes, hookah, and smokeless tobacco in the home. The adult participant also reported about the exposure of the child to any of these tobacco products at home and away from home; sociodemographic characteristics of the participant and child and home characteristics.

Surface Nicotine—Prescreened cotton rounds (100% cotton facial wipes) were wetted with 1.5 mL of 1% ascorbic acid and wiped over a 100 cm^2 area - typically a wooden door unlikely to be frequently cleaned. Field blanks were collected in all homes, and a random sample of 20% was analyzed. The wipe sample preparation and nicotine analysis methods were previously published.(28) Surface nicotine levels were reported as micrograms of nicotine per square meter of surface ($\mu g/m^2$; loading), and the limit of detection (LOD) was approximately 0.019 μg nicotine/m^2. The estimated LOD was defined based on an instrumental signal/noise ratio of 5.(29) We compared the dining room table, bedframe, and arm rest MBs based on groups with low (i.e., THS-free) and high (i.e., THS-polluted) surface nicotine levels.

Dust nicotine—Dust samples were collected from a 1 m^2 area (or from a larger area if needed to collect approximately 1 cm of dust in collection bottle) with a High-Volume-Small Surface-Sampler (HVS4, CS3 Inc., Venice, FL) into methanol-washed amber bottles. The dust sample preparation and nicotine analysis methods were previously published (30, 31). The dust nicotine LOD was 2.6 ng/g dust, or 0.020 ng/m^2 to 12 ng/m^2 (due to variability in the area vacuumed and collected dust mass). We compared the living room floor, pillowcase, and bed sheet MBs based on groups with low (i.e., THS-free) and high (i.e., THS-polluted) dust nicotine levels.

Hand Nicotine—A wipe sample of the child's dominant hand was taken by wiping the palm and volar aspect of all fingers.(30, 31) Hand wipes were prepared and analyzed as described above for surface wipe samples. Hand nicotine levels were reported in nanograms of nicotine per hand wipe (ng/wipe), and the LOD was approximately 0.19 ng nicotine/wipe. We compared the hand MB between children based on groups with low (i.e., THS-free) and high (i.e., THS-polluted) hand nicotine levels.

Urine cotinine—A urine sample was collected from each child. Samples were frozen at −20°C until analysis for cotinine concentration by LC-MS/MS, using previously published sample preparation and LC/MS/MS methods.(32) The cotinine LOD was 0.033 ng/mL. We compared the ear, nose, and mouth MBs based on groups with low (i.e., THS-free) and high (i.e., THS-polluted) urinary cotinine levels.

Microbiome sampling—Biological samples from children and from indoor home surfaces were collected using sterile rayon tipped swabs (P25–806WR, Puritan Medical Products). From each child, we sampled a finger, nose, ear, and mouth (cheek). The child was instructed to not eat or drink for at least thirty minutes prior to the mouth sampling. To collect finger samples, we gently swabbed around and under the child's index finger of their non-dominant hand. To collect nasal samples, we inserted the swab one centimeter into the anterior nares and directed it up into the tip of the nose and gently rotated three times and repeated this for the other nostril with the same swab. The outer ear canal (dominant

hand side) was sampled by placing the swab into the outer ear and gently rotating it. Mouth samples were collected by swabbing the area between the cheek and gum for approximately 10 seconds.

Environmental samples from the indoor BE were collected from surfaces frequently touched by the children; specific locations were chosen in consultation with the parent. In each home, we sampled the child's pillowcase, bedframe, bottom bed sheet, dining room table, an armrest from a living room sofa or chair, and the living room floor. To standardize sampling among surfaces and homes, we taped a 10cm × 10cm square cardboard template to each surface and swabbed the complete area within the frame. Field blanks (i.e., negative controls) of mock swabs were collected and analyzed from each home. After swabbing, the swab tip was quickly snapped off at the swab tip and placed in 500μL of sterile-filtered Phosphate Buffered Saline (1X PBS, pH 7.4, Fisher BioReagents) as transport medium within a 1.5mL Eppendorf tube soon after the sampling procedure. The 10 samples plus one control field blank from each home were sealed in a plastic freezer bag and transported on ice to San Diego State University where they were stored at −80°C until processing. DNA isolation, PCR, and 16S rRNA sequencing methods are presented in detail in the Supplemental Material.

Statistical Analyses

To control for non-normal distributions and heterogeneous error variances, we subjected all response variables to logarithmic transformation, and we report minima, maxima, and quartiles of distribution and geometric means and their 95% confidence intervals. Permutation multivariate analysis of variance (PERMANOVA) tests were used to test for differences in β-diversity between THS-polluted and THS-fee homes. For log2 fold-change analyses comparing the abundance of ASVs between THS-polluted and THS-free environments, the False Discovery Rate (FDR) was protected at 5%.

The Sample-Location-by-THS-Status design was analyzed using linear mixed-effects models where homes were the random factor, the sample location (finger, nose, ear, mouth, pillow case, bed frame, bed sheet, table, arm rest, floor) was the fixed within-subject factor, and the validated THS status (vNEG vs vTEG) was the fixed between-subjects factor. The Type I error rate was set at 5% (two-tailed). In the absence of interaction effects, we tested for main effects of location and THS status. To explore the robustness of these models, we repeated these mixed linear models with different indices of microbial diversity (Faith, Chao1, Shannon, Sequence Count) as well as validated THS groups based on dust, surfaces, and hand nicotine, and urinary cotinine. R statistical software (version 3.6.3) and Stata (version 16) were employed for analyses.(33, 34)

RESULTS

Thirdhand Smoke Pollution and Exposure

Figure 1 shows surface, dust, and hand nicotine and urinary cotinine levels in THS-free and THS-polluted homes based on the cut-offs applied to the measured levels of nicotine on surface, in dust, and on hands and cotinine in urine. All THS-free homes (vNEG) showed

levels in the lower range of nicotine and cotinine found in previous studies of THS pollution and exposure.(18, 20, 22, 35) The geometric mean and median levels in vTEG were 55–200 times higher than in vNEG, establishing strong and statistically significant contrasts between the THS-free and THS-polluted homes ($p<0.001$). Dust loadings in the reported and validated THS-free and THS-polluted homes showed significantly higher dustiness than THS free homes ($p=0.29$ and $p=0.0034$, respectively). Dust nicotine, surface nicotine, hand nicotine, and urine cotinine showed strong linear association with each other ranging from $r=0.59$ (cotinine -surface nicotine; $N=29$; $p<0.001$) to 0.87 (cotinine – hand nicotine; $N=29$; $p<0.001$).

Bacterial Diversity in THS-Free and THS-Polluted Homes

Linear mixed-effects model analyses of all diversity indices showed statistically significant Sample Type-by-THS Status interaction effects. Figure 2 shows the geometric means and 95% confidence intervals for the Faith PD index comparing the sample types for THS-free and THS-polluted homes based on nicotine in dust, on surface, on hands and urinary cotinine. Further investigations of the significant Sample Type-by-THS status interactions revealed that α-diversity was consistently higher on floors, arm rests, and tables in THS-polluted homes compared to THS-free homes. Higher α-diversity was also observed for ear samples of children in homes with THS-polluted dust and surfaces. Figure S2 in the online supplemental material shows similar α-diversity patterns based on Chao1, Shannon, and sequence count indices.

Permutation ANOVA tests of β-diversity found no clear separation visually or statistically between MB samples from THS-polluted and THS-free environments in any individual MB habitat (Figure S3 and Table S4 in the online supplement). We did detect a significant effect of THS when we combined all the BE samples ($R^2=0.021$; FDR-adjusted $p=0.012$) but not with the combined human MB samples ($R^2=0.012$; FDR-adjusted $p>0.50$). We did find noticeable differences in β-diversity among environments expected to be different (e.g., finger, mouth and floor; Figure S3 l. in the online supplement).

Cell Counts in THS-Free and THS-Polluted Homes

Linear mixed-effects model analyses of cell count data from finger, mouth, bed frame, and floor samples revealed no THS status-by-Sample Type interaction but a significant main effect of Sample Type ($p<0.0001$). Mouth and floor samples had higher cell counts than finger and bed frame samples, and THS-free homes had overall higher cell counts ($p=0.0528$). Figure S4 in the online supplement provides further information about the cell counts.

Comparison of Species-Specific Abundances in THS-free and THS-polluted Homes

An examination of relative abundances via a log2 fold-change analysis of bacterial species between vTEG and vNEG homes identified numerous ASVs with differential abundances in every home environment studied. In the ear samples (see Figure 3), the majority of the differentially abundant ASVs had a higher relative abundance in THS-exposed children, with ASVs from the genera *Staphylococcus*, *Neisseria* and *Corynebacterium* being the most differentiated. *Bradyrhizobium* and a species of *Staphylococus* were the exceptions,

showing higher abundance in THS-free children. A similar pattern held for the armrest samples (see Figure 4); i.e., the majority of the differentiated ASVs were relatively more abundant in the THS-exposed homes. *Bradyrhizobium, Labrys, Prevotella, and a species of Sphingobacterium* showed higher abundance in THS-free homes.

Figures S5–S12 in the online supplement show the log2 fold-change results for the rest of the environments. Supplemental Table S4 summarizes the ASV patterns for all 10 environments, indicating with "+" higher abundances in vTEG homes and with "–" higher abundances in vNEG homes. Overall, there was no common abundance pattern across the four human and the six environmental MBs or across the 43 ASVs. That is, there was no evidence that THS exposure universally suppresses or enhances bacterial growth. There was, however, evidence for potential environment specific patterns.

Supplemental Table S4 shows that among the human MBs, the nose MB exhibited a similar patter to that discussed above for the ear (see Figure 3); i.e., there was a higher abundance across ASVs in THS-polluted compared to THS-free homes. In contrast, mouth and finger showed the opposite effect. Notable, there were 17 ASVs in the mouth MB with significantly higher abundance in children living in THS-free homes, compared to eight ASVs that were more abundant in THS polluted homes. Eight of the 17 ASVs with higher abundance in THS-free homes were associated with *Rothia* and *Lautropia*.

Among the environmental MBs, bed sheets and floors exhibited a similar pattern to that shown above for arm rests (also see Figure 4); i.e., THS-polluted homes were associated with higher abundance for the vast majority of differentiating ASVs. The opposite effect could be observed for the pillow case and table MB. Notably, the table MB had 23 ASVs that were more abundant and only one that was less abundant in THS-free homes. Seven of the 23 ASVs were associated with *Streptococcus* and three each with *Corynebacterium* and *Granulicatella*.

Across the 43 ASVs that significantly distinguished between THS-free and THS-polluted homes, 21 show evidence of suppression as well as enhancement in THS-polluted homes. Notable exceptions were *Gemella* and *Porphyromonas*, both showing consistently higher abundance in THS-polluted than THS-free homes. For *Gemella*, this pattern held for ear, nose, bedframe, armrest and bed sheet MBs. For *Porphyromonas*, this pattern held for ear, pillow case, and bed sheet MBs.

Discussion

This is the first study to examine the impact of THS pollution on the built environment MB and the human MB of children living in THS-polluted homes. In comparison to THS-free homes of nonsmokers' homes with smoking bans, our findings confirmed previous studies that homes of smokers with indoor smoking bans are polluted with THS toxicants as indicated by elevated levels of nicotine on home surfaces and in settled house dust.(30, 31, 35) Children living in THS-polluted homes are exposed to THS as indicated by elevated levels of nicotine on their hands and cotinine in their urine. We found significantly higher phylogenetic α-diversity at multiple sampling

locations of the BE in THS-polluted homes compared to THS-free homes but no differences for human MB. We found no differences in β-diversity for BE or human MB. However, we did find differences between THS-polluted and THS-free homes for specific genera, including the following: *Corynebacterium, Staphylococcus, Neisseria, Gemella, Veillonella, Acinetobacter, Bradyrhizobium, Micrococcus, Streptococcus, Moraxella, Labrys, Porphyromonas, Prevotella, Granulicatella, Rothia, and Lautropia.*

THS and Microbial Diversity

We observed the strongest association between THS-pollution and microbial diversity with measurements of α-diversity on BE surfaces. The floor, table, and arm rests of THS-polluted homes all had significantly higher bacterial biodiversity than the same environments in THS-free homes. These differences were replicated, distinguishing THS-polluted from THS-free environments based on nicotine in dust, on surfaces, on children's hands, and cotinine in children's urine. In contrast, none of the human MB samples showed a difference in α-diversity between THS-polluted and THS-free samples. These results held regardless of the α-diversity metric used. Bacterial cell-counting via epifluorescence microscopy found clear and expected differences in mean bacterial abundance among environments (mouth > floor > finger > bedframe) but no difference between THS-polluted and THS-free homes within the four environments tested. This supports the conclusion that the observed differences in α-diversity (esp. on the floor) were not a consequence of greater bacterial abundance in THS-polluted environments (e.g., the floor). Moreover, the cell count data contradicted the notion that THS has general overall bactericidal properties.

The observed differences in α-diversity between built and human samples are likely the result of the physical, chemical, and biological properties of these environments. Dry surfaces in the BE have been described as a "microbial wasteland"; i.e., they tend to contain a high diversity driven by passive settling of microbes originating from multiple different environments (mainly skin and soils but also water, pets and feces depending on the setting).(36) Human MB environments, with the exception of the skin, tend to be highly metabolically active and experience rapid turnover, and have a highly selective range of diversity that is much lower and less variable than dry BE surfaces.(37) Skin surfaces tend to be drier and more diverse than other human MB environments. The passive accumulation of THS chemicals on dry surfaces puts them in direct and persistent contact with the microbes at the same spatial scale. Comparatively, THS chemicals in a human MB environment, such as the mouth or nose, are metabolized relatively rapidly. Some human MB environments are also likely to be washed or cleaned more often (e.g., hand washing, bathing, tooth brushing) than BE environments (e.g., floors, arm rests). This may partly explain why we observed a THS-effect on community-wide diversity metrics, especially α-diversity, in BE but not in human MB environments. The differential effects of THS on β-diversity among BE may be attributable to not only physical and chemical properties of the settings, but also how often surfaces are cleaned. For example, table surfaces are likely cleaned on a more regular basis than floor surfaces.

Log2 fold-change analyses identified numerous putative bacterial species (ASVs) whose relative abundances differed significantly between THS-polluted and THS-free homes in BE

and human MB environments. Interestingly, while the impact of THS relative abundance ratios on the more commonly present genera was apparent in every MB, the direction of the effect was MB location-specific. For example, *Staphylococcus and Corynebacterium* species in the ear and armrest had higher relative abundances in THS-polluted than in THS-free samples, but precisely the opposite pattern was observed for mouth, pillow case, and table samples for *Staphylococcus* and for finger and table samples for *Corynebacterium*. Species of *Corynebacterium*, which were consistently higher in THS-polluted homes, are readily isolated from tobacco water-pipes and also from the human oral cavity of cigarette smokers.(38–40) One study identified *Gemella* in higher abundance in the respiratory tract of smokers than non-smokers.(41) ASVs belonging to two other mostly non-pathogenic genera, *Veillonella* and *Bradyrhizobium*, were also differentially abundant in THS-polluted and THS-free environment. *Veillonella* is known as an oral and gut commensal and has been shown to be more abundant in the mouths of non-smokers, but the effect of smoking on species within this genus appears to be species dependent.(42, 43)

While our results strongly suggest THS-pollution has a significant impact on bacteria abundances and overall biodiversity, it is curious that the effect is so environment-specific that species within the same genus could have opposite responses among MB samples within the same households and in the same children. We suggest four possible factors to explain this environment-specific effect: (1) the mixture of THS constituents present at specific sampling locations, (2) the amount of time the microbial communities are exposed to and interact with THS at specific sampling locations, (3) the overall metabolic activity of the microbes at specific sampling locations, and (4) child activity level and interaction with the environment. In the BE samples, the length of time that bacteria are exposed to THS chemicals is likely affected by the amount of disturbance and the physical nature of the surface. Floors and tables are both non-porous two-dimensional surfaces, but most people clean table surfaces much more often than floors. Bedsheets, pillowcases and armrests, on the other hand, are porous highly structured three-dimensional environments where microbial organisms and THS constituents in particulate and gas-phases co-exist and interact. Such structures provide larger surface areas for microbial ecologies as well as the adsorption of THS pollutants. In terms of human environments, direct exposure of the MB to THS chemicals could vary considerably. Young children explore their environments with their hands and mouth, sampling and collecting THS pollutants as well as microbial organism.(16, 18, 22, 31, 35) Because of absorptive properties of the skin, we suspect that nicotine may persist much longer on fingers and in the ear canal than in the mouth where it is rapidly absorbed into the bloodstream. The metabolic activity of the finger MB, a dry and salty low-growth environment, and the ear canal is much lower than rapid turnover of the mouth (saliva).(37) BE environments are also considered ‘microbial deserts’ and we expect that the effects of THS could be considerably different depending on whether the microbes were in a dormant (spore-like) or vegetative growth state.

Limitations and Future Directions

The results of this study demonstrate that THS not only exposes children to toxic THS pollutants but may also alter human and BE microbial diversity of preschool age children. The effect of THS pollution appears to be MB specific and differential with respect to

bacterial species. Given the relatively small number of households in this study, many more households should be included in future field studies to determine the replicability of these results and to provide greater statistical power. Based on this pilot study, future studies should continue to measure THS pollution and exposure specific to MB samples and include methods such as quantitative PCR targeting specific bacterial species or genera (e.g., *Staphylococcus*, *Corynebacterium*, *Gemella*, *Neisseria*, *Veillonella*) to confirm the deep sequencing and relative abundance results. Laboratory experiments in controlled chambers and animal studies could also be used to directly test the effects of nicotine and other tobacco smoke compounds on microbial communities and the potential importance of material type, environmental conditions, disturbance (e.g., cleaning), behavior, and the impact of the THS-induced changes in the human MB on disease pathways and outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

The authors express their appreciation to Joy M. Zakarian for her invaluable contributions as project coordinator for this study.

Financial Support

The work that provided the basis for this publication was supported by the California Tobacco Related Disease Research Program (#26IP-0047; Principal Investigator: G.E. Matt) and the National Institute of Environmental Health Studies (NIH Grant Number R01ES030743; MPI: E.M. Mahabee-Gittens, G.E. Matt).

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Impact statement

- Despite smoking bans, children can be exposed to tobacco smoke residue (i.e., thirdhand smoke) that lingers on surfaces and in settled house dust.
- Thirdhand smoke exposure is associated with changes in the microbiomes of the home environment and of the children living in these homes.
- Thirdhand smoke is associated with increased phylogenetic diversity of the home environment and changes in the abundances of several genera of the child microbiome known to be affected by active smoking and secondhand smoke (e.g., *Corynebacterium, Staphylococcus, Streptococcus*).
- Thirdhand smoke exposure by itself may induce alterations in the microbiome that play a role in childhood pathologies.

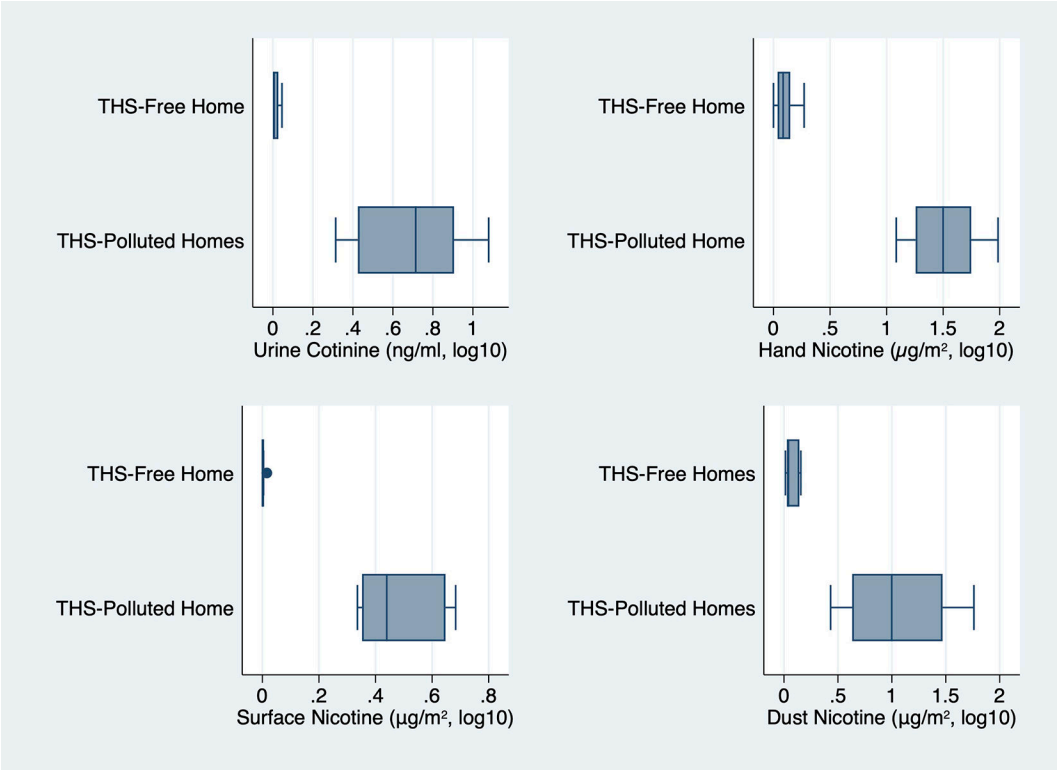

Figure 1.

THS pollution and exposure in validated THS-free and THS-polluted homes based on cut-offs for urinary cotinine, hand nicotine, surface nicotine, and dust nicotine.

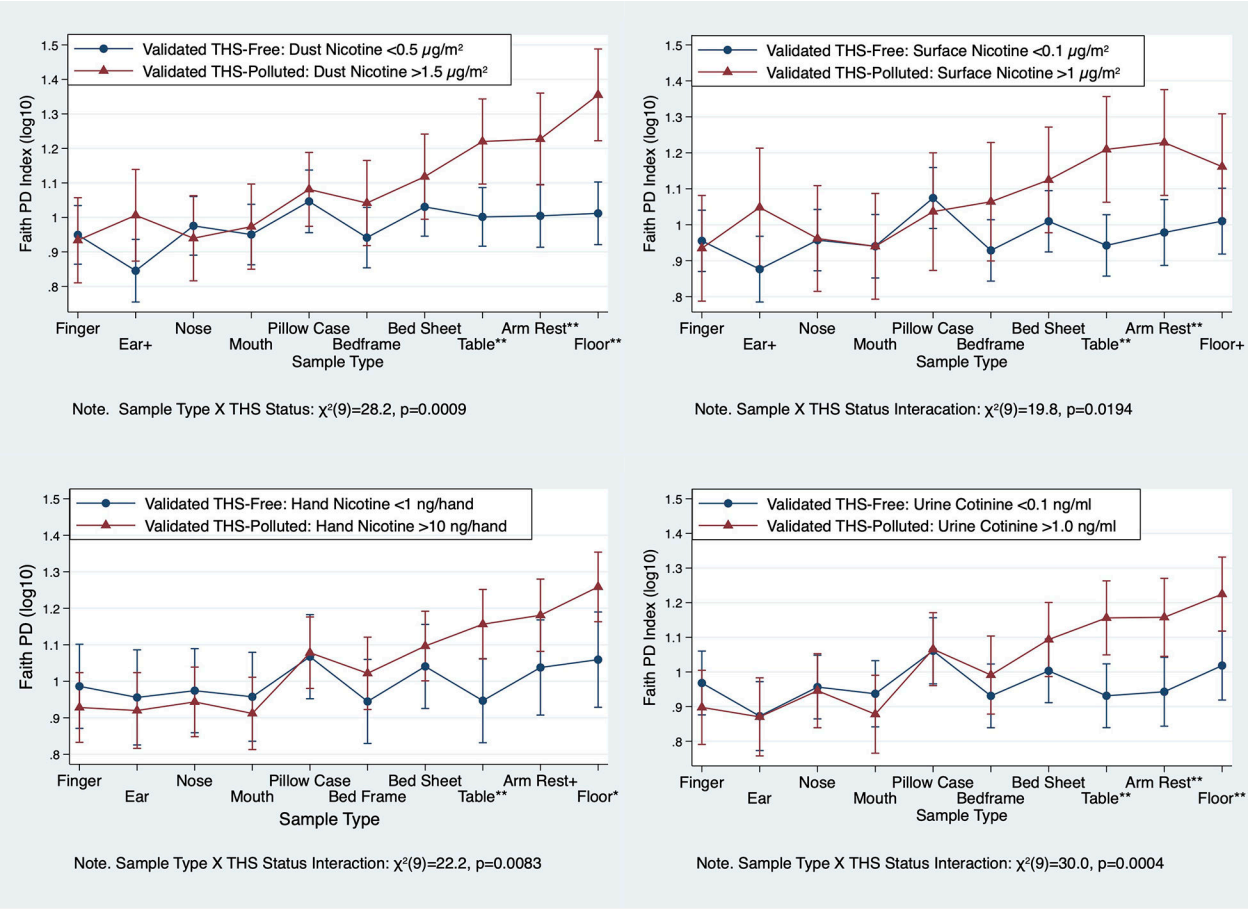

Figure 2.

Faith PD α-diversity (GeoMean and 95% Confidence Interval) in human and environmental samples taken from validated THS-free (blue, vNEG) and THS-polluted (red, vTEG) homes based on dust nicotine, surface nicotine, hand nicotine, and urinary cotinine cut-offs.

Note. +: p<0.10; **: p<0.01

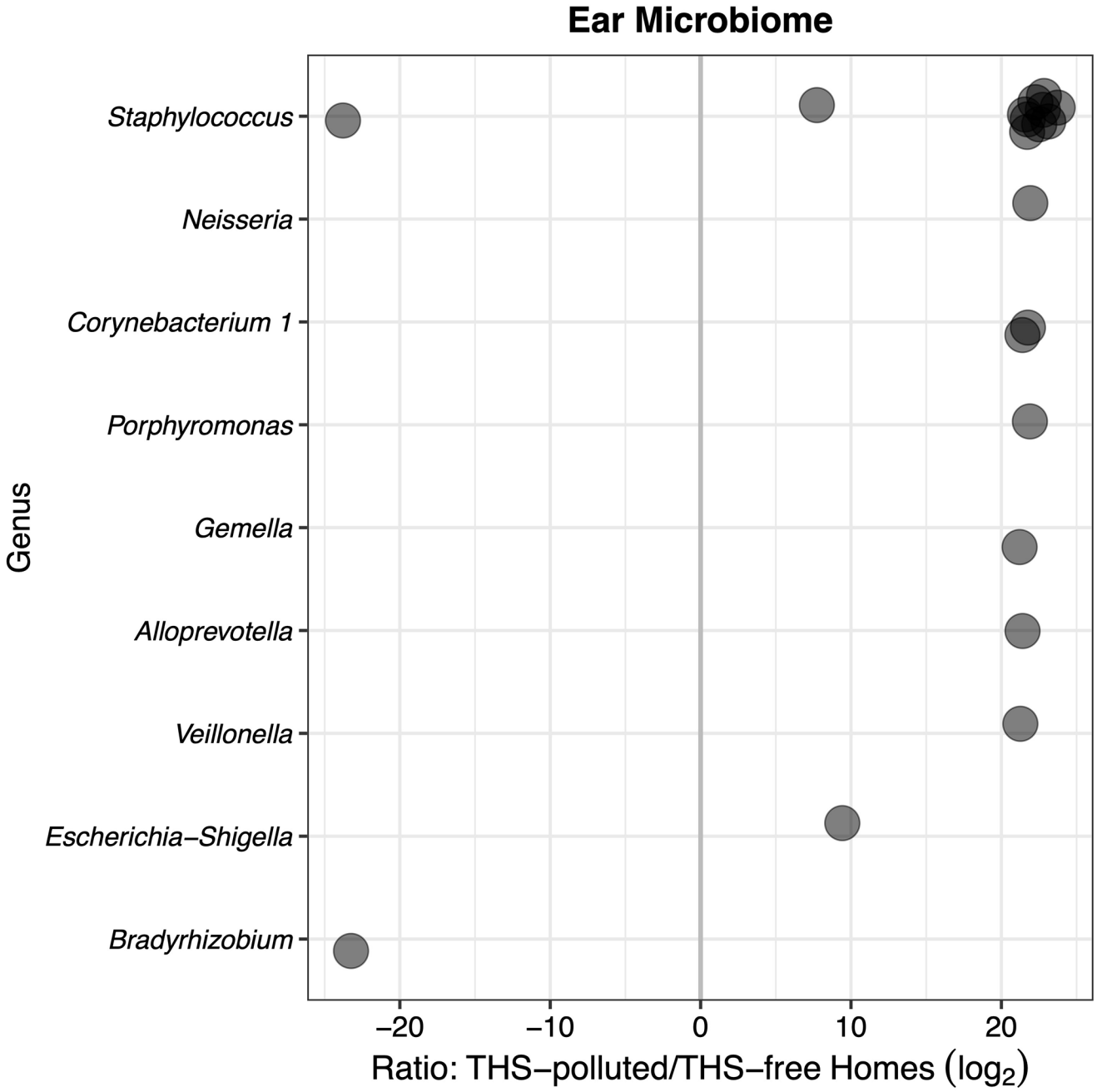

Figure 3.

Results of the log2 fold analysis based on the ASVs identified in the 16S sequencing libraries from ear samples. Each dot indicates a specific ASV taxonomically identified from the genus indicated on the Y-axis. Positive values on the X-axis indicate the ASV is significantly more abundant (FDR-adjusted $p<0.05$) in THS-polluted homes (vTEG; N= 7 samples) than in THS-free (vNEG; N=9 samples) homes, whereas negatives values indicate the opposite. Only ASVs with log2 fold-change values >|5| are shown.

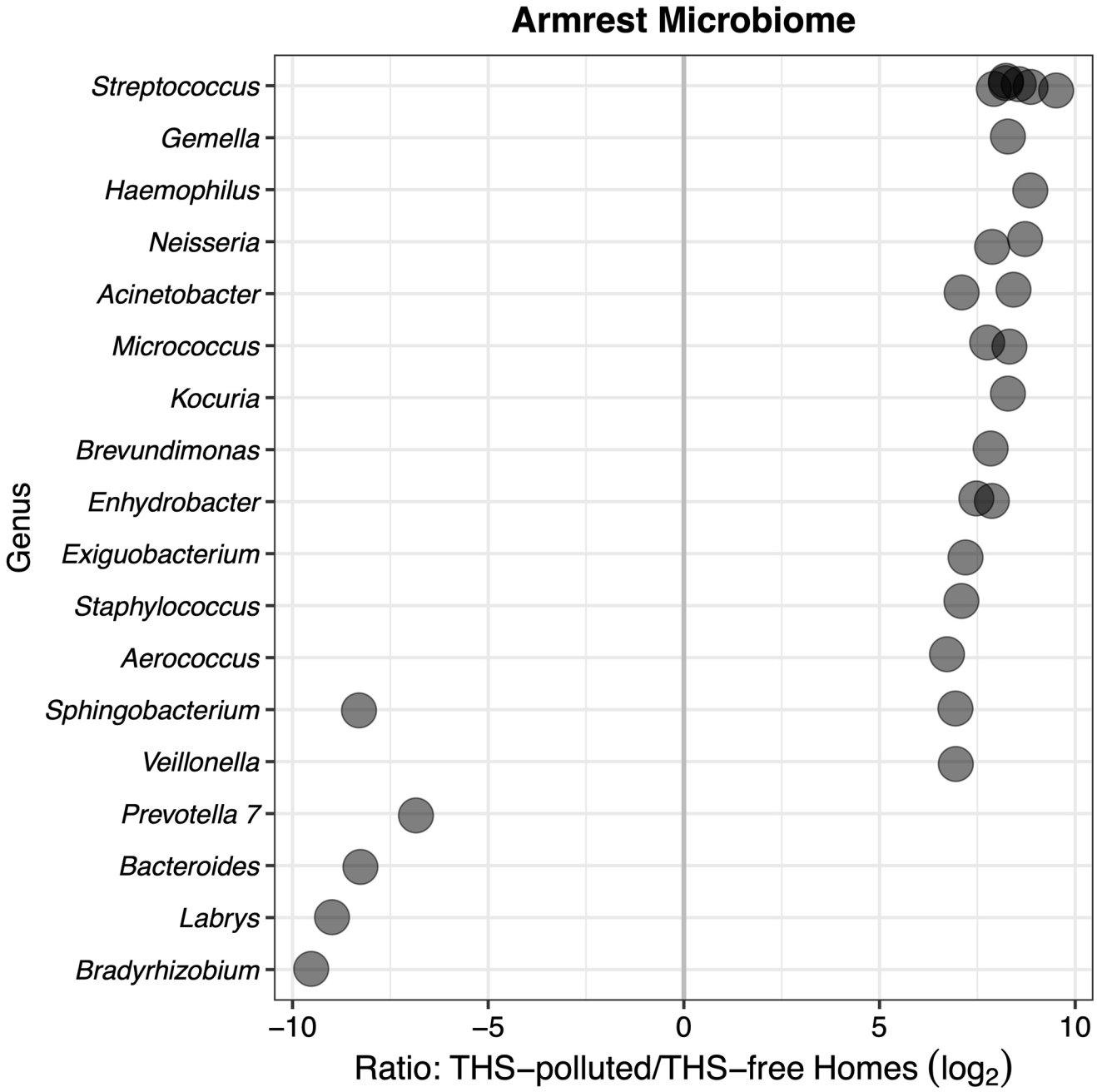

Figure 4.

Results of the log2 fold analysis based on the ASVs identified in the 16S sequencing libraries from armrest samples. Each dot indicates a specific ASV taxonomically identified from the genus indicated on the Y-axis. Positive values on the X-axis indicate the ASV is significantly more abundant (FDR-adjusted $p<0.05$) in THS-polluted homes (vTEG; N= 4 samples) than in THS-free (vNEG; N=8 samples homes, whereas negative values indicate the opposite. Only ASVs with log2 fold-change values >|5| are shown.