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Article

Effect of Household Air Pollution on the Gut Microbiome and Virome of Adult Women Living in Uganda

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ABSTRACT: **BACKGROUND:** Emerging observational studies suggest that air pollution can influence the gut microbiome. However, this association is often highly confounded by factors, such as diet and poverty. The gut virome may influence respiratory health independent of the gut microbiome. We recently demonstrated in a randomized waitlist-controlled trial (ClinicalTrials.gov NCT03351504) that a clean lighting intervention reduced the level of personal exposure to air pollution among adult women in rural Uganda. **OBJECTIVES:** To determine the effect of a solar lighting intervention on changes to the gut microbiome and virome and secondarily to determine the association between these changes on lung health. **METHODS:** Between 2018 and 2019, we collected stool samples and assessed respiratory symptoms and spirometry from 80 adult women living in rural Uganda at baseline and 12 and 18 months postrandomization. The intervention group received a solar lighting system after randomization, while the waitlist-controlled group received one at 12 months. Deep metagenomics sequencing of stool was performed and profiled for nonviral and viral taxonomic composition. The primary analysis focused on pre- vs postintervention changes due to power considerations, adjusting for potential confounding by age, diet, antibiotic use, and season. A sensitivity analysis was conducted using intention-to-treat principles. When comparing pre- vs postintervention periods, we used sparse partial least-squares models to identify nonviral and viral signatures of reduced air pollution exposure. Mixed effects models were used to evaluate changes in health outcomes as well as associations between microbial signatures of reduced air pollution exposure and health. **RESULTS:** The average age was 39.2 years. The solar lighting intervention led to larger changes in viral compared to nonviral microbial community structure and differential abundance of bacteria, eukaryotes, and viruses. Provision of solar lighting systems was associated with a reduction in the presence of respiratory symptoms from 57.1% to 36.1% ($p = 0.002$), while there was no impact on lung function. Microbiome and virome signatures had AUCs of 0.74 and 0.76, respectively, in predicting pre- vs postintervention stool samples. Microbiome signatures were associated with a lower risk of respiratory symptoms (OR = 0.68 (0.49 − 0.94), $p = 0.020$). **CONCLUSION:** Among adult women living in rural Uganda, both nonviral and viral components of the gut microbial community changed after a clean lighting intervention. Microbiome signatures reflective of lower air pollution exposures were associated with improved respiratory symptoms. These observations suggest that air pollution may influence lung health through the gut-lung axis, warranting further exploration in future intervention studies.

INTRODUCTION

Approximately 2.4 billion people worldwide are exposed to household air pollution (HAP) resulting in an estimated 3.2 million premature deaths annually, with a disproportionate impact on women and children living in resource-limited settings.[1] HAP has been identified as a common cause of acute and chronic respiratory diseases in adults and is a leading cause of pneumonia in children. The mechanism of harm has been postulated to revolve around impaired host immunity and persistent inflammation. The literature on the effect of air pollution exposure on the microbiome is sparse and comprises either animal studies or published human studies that are largely observational in design[2−8] and performed in high or middle income settings. Associations between fine particulate matter ($PM_{2.5}$) exposure and changes in the human microbiota have been described, though these studies have largely focused on airway microbial communities. Air pollution exposure may also influence the gut microbiome.[8−11] A substantial portion of particles inhaled into the upper respiratory tract are ingested.[8] Even ingested nonmicrobial components of air pollution[12,13] have been shown to alter the gut microbiome in animal studies. This suggests that air pollution exposure can alter the gut microbiome by serving as either a source of microbes, nutrients for microbes, or more indirectly by disrupting host mucosal barrier function or altering host immune function.[14] Human gut phage populations have been shown to influence human immune regulation, transfer DNA to bacteria thus altering functionality such as antimicrobial resistance, and can lead to bacterial cell lysis thus regulating human microbial commun-

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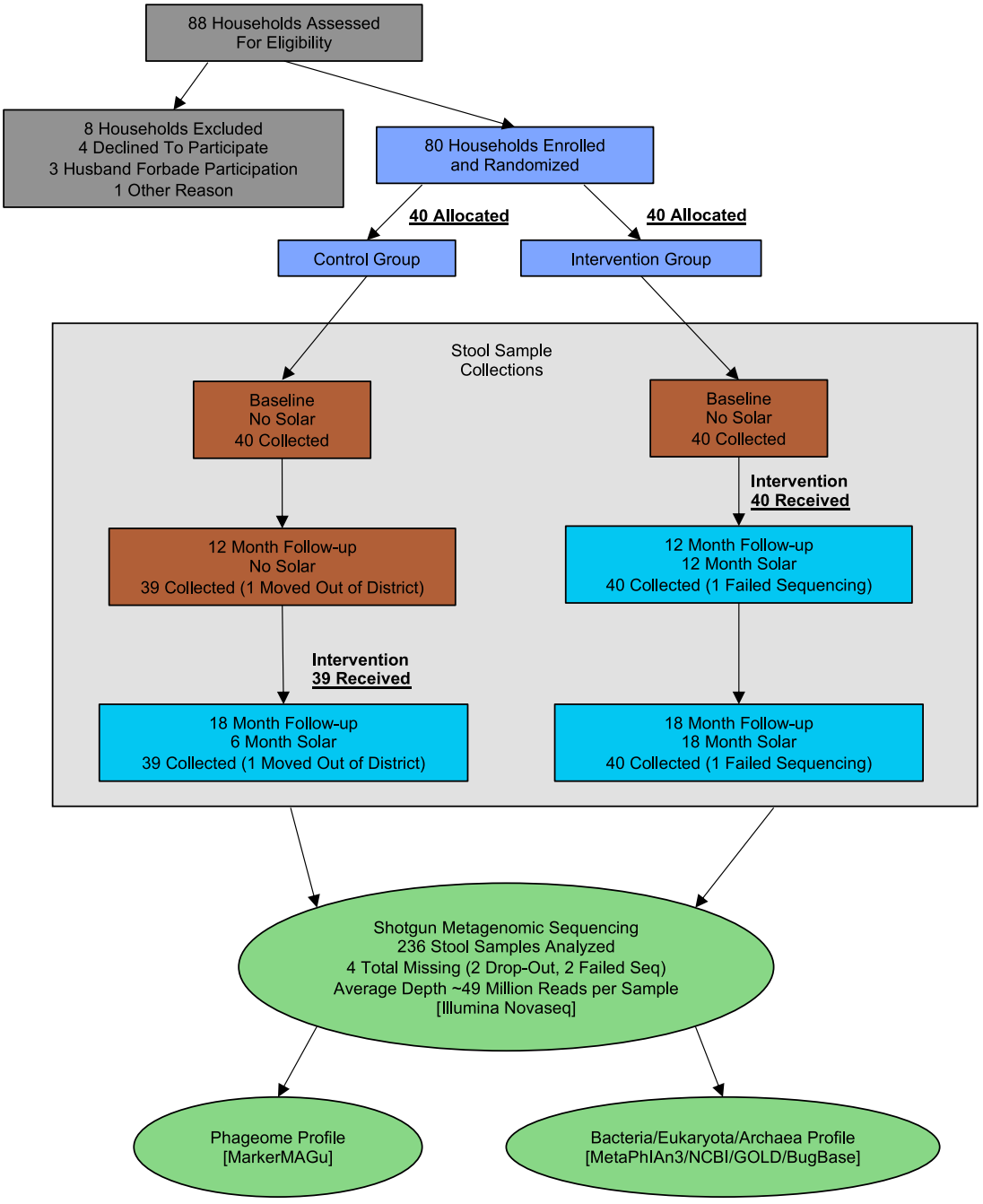

Figure 1. CONSORT Flow diagram. Flowchart overview shows the experimental process from enrollment of participants to final shotgun metagenomic sequencing profiles. Of 88 households assessed for participation, 80 were enrolled in the study and randomized in a 1:1 ratio into control and intervention groups of 40 households each. The intervention group received the solar lighting system immediately after baseline assessment and randomization, while the control group received it 12 months after randomization. An additional follow-up was conducted after an additional 6 months (18 months total elapsed). Collections shaded in orange are preintervention, and those shaded in blue are postintervention. Surveys and assessments were conducted, and stool samples were collected for all participants at each of these time points. The resulting 236 stool samples all underwent shotgun sequencing and were profiled for microbial taxonomy at the species level and predicted bacteria host of viruses at the genus level.

ities.[15,16] How air pollution exposure might shape the human virome is unknown.

The gut-lung axis is important for lung health.[17] Germ-free mice are highly susceptible to lung infection due to the inability to generate an appropriate immune response as well as the absence of a resident microbiota for colonization resistance; the ability to defend against pulmonary infection is restored with gut microbiota transplantation.[18] Animal and human studies have identified strong links between gut microbiota and other lung diseases such as asthma[19−22] and chronic obstructive pulmonary disease.[23] Furthermore, viral components of the gut microbial community have largely been overlooked, but recent studies show that gut commensal viruses (particularly phages, viruses that infect human-associated bacterial communities) predict future risk of lung disease such as asthma independent of the gut bacteriome.[24]

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While a person often has little control over air pollution exposure in resource-limited settings, the human microbiome has been shown to be modifiable and may be a viable target of intervention to improve health even in low and middle income countries.[25] Thus, it is critical that more definitive human studies demonstrate how air pollution exposure may influence the gut microbiome and virome.

An often-overlooked source of HAP is kerosene-based lighting. Simple open-wick kerosene lamps are widely used in some resource-limited settings as a household lighting source and produce fine particulate matter ($PM_{2.5}$) concentrations up to eight times the World Health Organization-recommended limits.[26] We previously demonstrated in a randomized controlled trial (ClinicalTrials.gov NCT03351504) among adult women living in rural Uganda that provision of an indoor solar lighting system completely displaced fuel-based lighting in 92% of participants.[27] Uptake of the solar lighting system was high with daily use averaging 8.23 h.[28] In intent-to-treat analyses, the intervention led to 36.1 $\mu g/m^3$ (95% CI −70.3 to −2.0) in $PM_{2.5}$ exposure and 10.8 $\mu g/m^3$ (95% CI −17.6 to −4.1) to black carbon, corresponding to a reduction from baseline of 37% and 91%.[27] This exposure reduction was observed despite no attempts to change the use of biomass fuel for cooking.

Here, we test the hypothesis that this clean lighting intervention changes the gut microbiome and virome and, in secondary analysis, determine the association between gut microbial changes and respiratory health.

METHODS

Study Design and Population

An overview of the study design and methodology is outlined in Figure 1 CONSORT Flow Diagram. Between 2018 and 2019, we conducted a one-year parallel group, randomized, waitlist-controlled trial of indoor solar lighting systems in Nyakabare parish, located in a rural region of southwest Uganda. The use of biomass fuel for cooking and kerosene lamps for lighting is common in the region. The local economy is largely based on subsistence agriculture, animal husbandry, and small-scale enterprise and trading. Few households have access to potable water through in-home taps, and both food and water insecurity are prevalent.[29,30]

Inclusion criteria included adult women living in Nyakabare Parish with no history of chronic lung disease. Exclusion criteria included active tuberculosis in any family member. Participants were randomly selected from a census conducted in the parish. Of 88 contacted, 80 consented to participate and were randomized (1:1) to intervention or waitlist-control (Figure 1). A waitlist-controlled design means that the control participants received the same solar-lighting intervention after 12 months, preserving an initial control comparison period while ensuring eventual access for all participants. This approach was taken in response to local community and institutional review board concerns regarding the equitable treatment of the control group. Home and research clinic visits took place at baseline, 12 months, and 18 months postrandomization to collect standardized surveys, perform health measurements, and obtain stool specimens. The intervention group received solar lighting immediately after baseline, while the waitlist-control group received solar lighting after their 12-month visit.

Written informed consent was obtained from the study participants. This study was approved by the Mbarara University of Science and Technology (Protocol #02/11−16) and the Partners Human Research Committee (Protocol 2017P000306/PHS). We obtained clearance to conduct the study from the Ugandan National Council of Science and Technology (Protocol #PS 42) and the Ugandan President's office. The trial was registered with Clinical-Trials.gov (NCT03351504) prior to commencement of the study. The primary outcome of the trial was personal exposure to fine particulate matter ($PM_{2.5}$) and black carbon. Change to the stool microbiome was a prespecified secondary outcome.

Randomization, Masking, and Study Intervention

Randomization was based on a random number generator and was stratified by participants' pre-existing primary lighting source (national electrical grid or battery-powered devices, solar lamps or systems, candles, hurricane kerosene lamps, and open wick kerosene lamps). The intervention involved receipt of an indoor solar lighting system at the time of randomization (intervention group) or after 12 months postrandomization (waitlist-controlled group). Blinding of participants and field staff was not possible due to the nature of the study intervention.

The study intervention was an indoor solar lighting system costing approximately $150 U.S. dollars, obtained from Allmar Solar Systems, a local vendor based in the Mbarara township. These lighting systems were deployed in the intervention households after baseline surveys between February and April 2018 and in the control households between 12 to 14 months after baseline between June and July 2019.

Personal Exposures to Air Pollution

Measurement of personal exposure to $PM_{2.5}$ and black carbon was assessed at baseline and 12 months as previously described.[27] Briefly, at each study visit, participants wore custom-built samplers composed of a compact multistage cascade impactor with a with a 2.5 μm cutpoint for 48 h where particles smaller than 2.5 μm to be collected onto a preweighed 37 mm, 2.0 μm pore size Teflon filter (Pall Life Sciences; Teflo).[31] Filters underwent gravimetric measurement for fine particulate concentrations and for black carbon (BC) concentrations by measuring filter blackness using a smoke stain reflectometer (model EEL M43D, Diffusion Systems Ltd., London, United Kingdom). Field blanks were used to account for potential bias in filter weight due to sampling methods.

Administration of Surveys for Demographics, Diet, Medication Use, and Respiratory Symptoms

During study visits at baseline, 12 months, and 18 months after randomization, trained research assistants administered surveys in Runyankole, the local language. The surveys administered questions eliciting demographic characteristics, household assets, cooking and lighting practices, diet[32] using a food frequency questionnaire adapted to the local context, medication use (including antimicrobials), as well as a modified version of the American Thoracic Society Respiratory Disease questionnaire[33] to assess respiratory symptoms. Exposure to household air pollution can cause different respiratory symptoms, with the most commonly reported symptoms being chronic cough, wheeze, and shortness of breath.[34−36] These symptoms are also independent predictors of mortality[37,38] even after accounting for lung function.[39] For this reason, our primary symptom outcome was a composite outcome of these three symptoms, although we also report each component outcome as well. These component outcomes were (1) chronic cough defined as cough lasting at least 3 continuous months during the year; (2) wheeze occurring with or without a cold; and (3) dyspnea when hurrying on level ground or walking up a slight hill.

Lung Function Testing

During study visits at baseline, 12 months, and 18 months after randomization, forced expiratory maneuvers (up to seven trials to produce three acceptable curves) according to American Thoracic Society guidelines were performed under the direction of a trained technician on the Easy On-PC (nDD) spirometer without bronchodilator administration. The highest values for forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were used given that they met criteria for acceptability and reproducibility.

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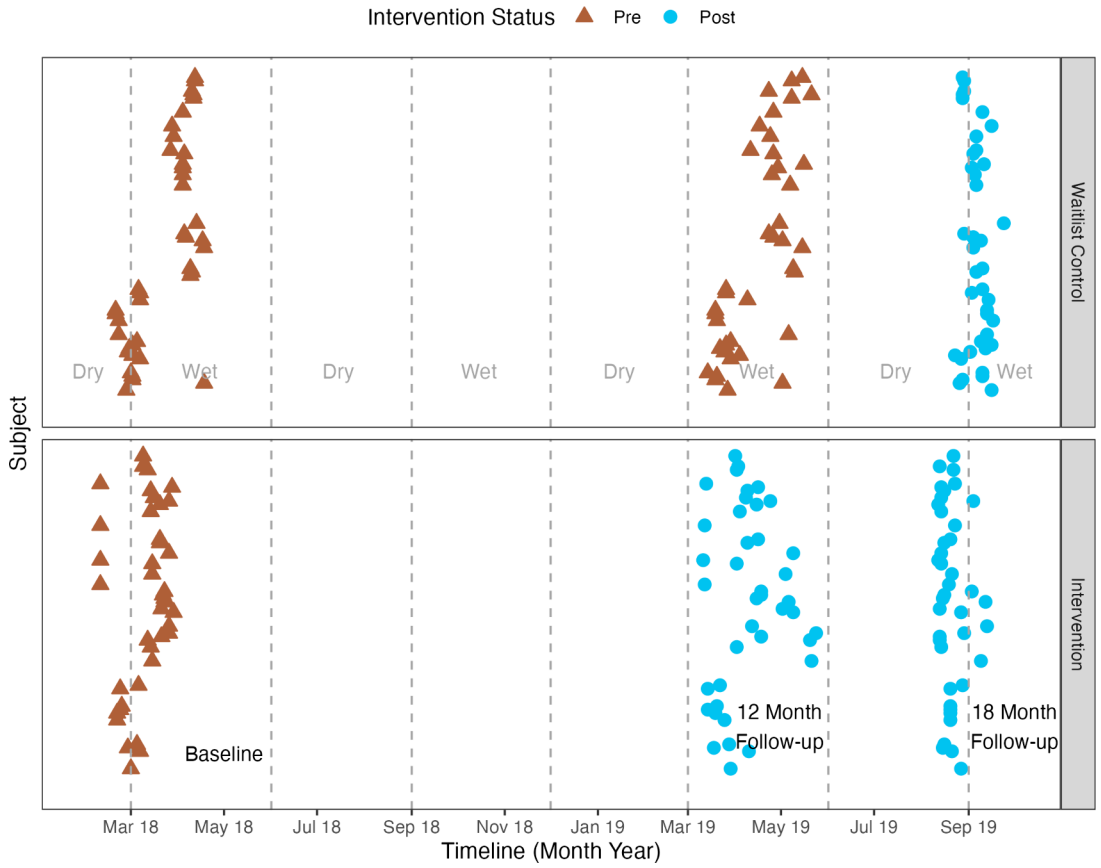

Figure 2. Timeline plot of study procedures, stratified by intervention vs waitlist control group. Randomization into intervention vs waitlist control groups occurred after 80 participants were enrolled. The intervention group received solar lighting systems after they completed the baseline assessment, while the control group received solar lighting systems after they had completed their 12 month follow-up study activities. Triangles and circles indicate the timing of research clinic visits for collection of stool samples, assessment of respiratory symptoms, and lung function testing for each research participant. Colors and shapes indicate whether study procedures occurred before the participant received a solar lighting system (brown and triangle) or after (blue and circle). Season annotated as "wet" (March to May and September to November) vs "dry" (June to August, December to February).

Stool Sample Collection, Nucleic Acid Extraction, Metagenomic Sequencing

During study visits at baseline, 12 months, and 18 months after randomization, at each research study visit at Mbarara Regional Referral Hospital, morning stool samples were collected from participants using disposable stool collection containers (Fisher Scientific) and frozen within one h of collection at −80 °C.

At the time of nucleic acid extraction, 20 million cells of *Imtechella halotolerans*, an environmental halophile not found in human biological samples,[40,41] were spiked into 0.25 g of stool for absolute abundance estimation. Samples, reagent-only negative controls and mock community positive controls (Zymo)[41] were extracted with a validated magnetic bead-based protocol using the Maxwell HT 96 gDNA Blood Isolation System (Promega) on a KingFisher Flex (ThermoFisher) instrument as previously described.[42] Extracted nucleic acids were quantified using the PicoGreen dsDNA Assay Kit (Invitrogen), and library prep was performed using the Nextera XT DNA Library Preparation Kit (Illumina) and sequenced on the NovaSeq 6000 platform to generate 2 × 150 base pair reads.

Bioinformatics Processing

Raw data files in binary base call (BCL) format were converted into FASTQ files and demultiplexed based on the dual-index barcodes using the Illumina bcl2fastq software. FASTQ files were processed using bioBakery3[43] version v3.0.0-alpha.6 07−10−2020. Demultiplexed raw fastq sequences were processed using KneadData,[44] including the removal of human host "contaminant" sequences, removal of low-complexity and repetitive sequences, removal of adapter and low-quality bases with Trimmomatic,[45] and contaminant checks with bowtie2.[46] Taxonomic profiling of the sequenced microbial samples at the species level was performed by using MetaPhlAn3. Taxonomy was further annotated with data from NCBI,[47] JGI GOLD,[48] and BacDive.[49] Taxonomic profiling of the virus samples with the predicted bacteria host at the genus level was performed using Marker-MAGu[50] database. To filter out potentially spurious features due to sequencing or classification errors, we performed prevalence filtering, i.e., excluding taxa identified in less than 10% of samples (≥25 samples).[51] We also performed variance filtering at the first quartile; that is, features with zero or low variance in the sample are removed because they are unlikely to contribute to the overall variation. We did not perform abundance filtering; that is, rare taxa were retained if they were present in at least 5% of sequenced samples.

Statistical Analyses

A timeline plot of study procedures including stool sample collection is detailed in Figure 2.

Due to the requirements for higher power for the high dimensional outcomes of the gut microbiome and virome, the primary approach focused on a prepost analysis with the intention-to-treat analysis as a sensitivity analysis. Adequate power is essential in microbiome research to detect meaningful effects, particularly given the high variability and complexity of the microbial communities. The decision to focus on a prepost analysis effectively doubles the sample size for comparison. In the prepost analysis, samples collected from all three time periods (baseline, 12 month, 18 months) are used for analysis and allow us to assess the effect of the intervention in all 80

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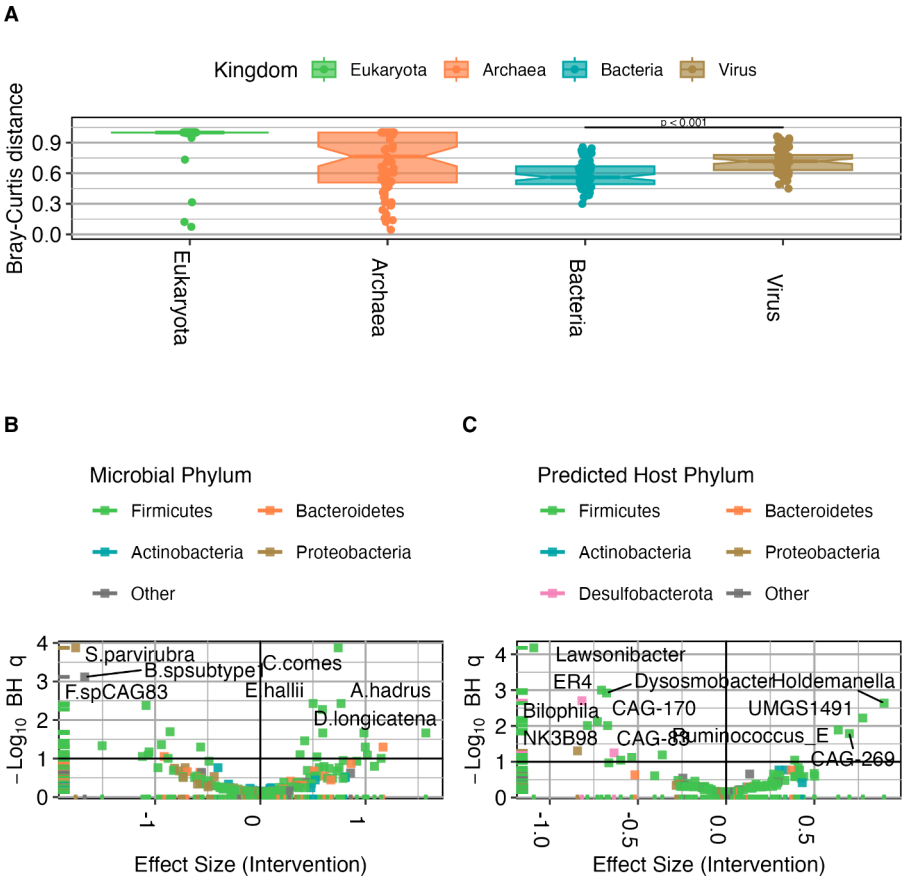

Figure 3. Change in gut microbial community structure after the intervention depicted using Bray−Curtis distance from preintervention sample, stratified by microbial kingdom and volcano plots showing the effect of solar lighting intervention on differential abundance of nonviral and viral gut microbes. The Bray−Curtis distance (**A**) was calculated between baseline (preintervention) and 18 months follow-up (postintervention) for all participants, separately for each kingdom. Nonzero values indicate a change. There was a change in microbial community structure for all microbial kingdoms, although the change was greater for viruses than bacteria; comparison evaluated using pairwise Wilcoxon rank sum test with Benjamini & Hochberg correction. Viruses in the volcano plot (**C**) depicted as predicted bacteria host of each virus to facilitate biological interpretation, as most viruses are poorly annotated. Each point in volcano plot corresponds to a microbial species (**B**) and predicted bacteria host of viruses at the genus level (**C**). X axis maps log-2 fold change estimate of solar lighting intervention on the relative abundance of each microbe (**B**) and bacteria host (**C**) as calculated by mixed effects models using lme4. The points above the Y = 1 line are differentially abundant after multiple hypothesis correction (q-value <0.1). Colors correspond to the phylum each microbe (nonvirus) and predicted bacteria host (virus) belongs to.

participants, as in this design each participant serves as their own control. In contrast, in the intention-to-treat analysis, only the baseline and 12 month follow-up stool samples are used in the analysis, and the effect of the intervention is compared between the 40 participants randomized to the intervention group and the 40 participants randomized to the waitlist control group, accounting for temporal trends and individual variability. In microbiome studies, there are known large temporal fluctuations in the gut microbiome even within the same individual that may exceed between-individual differences;[52,53] thus, longitudinal studies recommended by most guidelines are over cross-sectional comparisons.[54] Thus, both baseline and 12 month stool samples were used in the intention-to-treat analysis, as described in detail below.

Alpha diversity calculations were performed to estimate the Reciprocal Simpson indices and Shannon indices. The phyloseq package (with dependencies on the vegan package) was used to calculate the Bray−Curtis distances between the baseline and 18 months for each kingdom (Figure 3A) to evaluate global shifts in microbial community structure before and after the intervention. The Wilcoxon rank sum test was used to test the Bray−Curtis differences between bacteria and viruses. Relative abundance estimates for microbial species (see Supplemental Figure 1, per-sample summary of microbial sequencing profile of gut microbiomes) and relative abundance estimates for viruses with a taxonomy of predicted bacteria host at the genus level (see Supplemental Figure 2, per-sample summary of predicted bacteria host sequencing profile of gut viromes in relative abundance) were used as the profile outcomes. In our primary outcome analyses, a prepost analysis was performed, comparing the outcomes before and after the intervention as the control group also received a solar lighting system at 12 months postrandomization, with stool samples collected at 18 months. The intention-to-treat analysis was conducted as the sensitivity analysis instead using samples under the randomized controlled design only (samples collected at baseline and 12 months). We used mixed effects regression models where solar lighting intervention status was a fixed effect, a random intercept term for subject was incorporated to account for repeated measures within each participant, and a random intercept term for village was incorporated to account for the clustering effect. Age, time-varying diet (here modeled as the first principal component of the food frequency questionnaire), time-varying antibiotic use in the prior three months, and time-varying season (here modeled as a binary variable, where March to May and September to November are defined as the wet season and the others as the dry season) were included as potential confounders for prepost analysis only. Two separate linear mixed effect models for prepost analysis and ITT analysis were formulated with linear models for the microbiome and lung function outcomes and logistic models for symptom outcomes:

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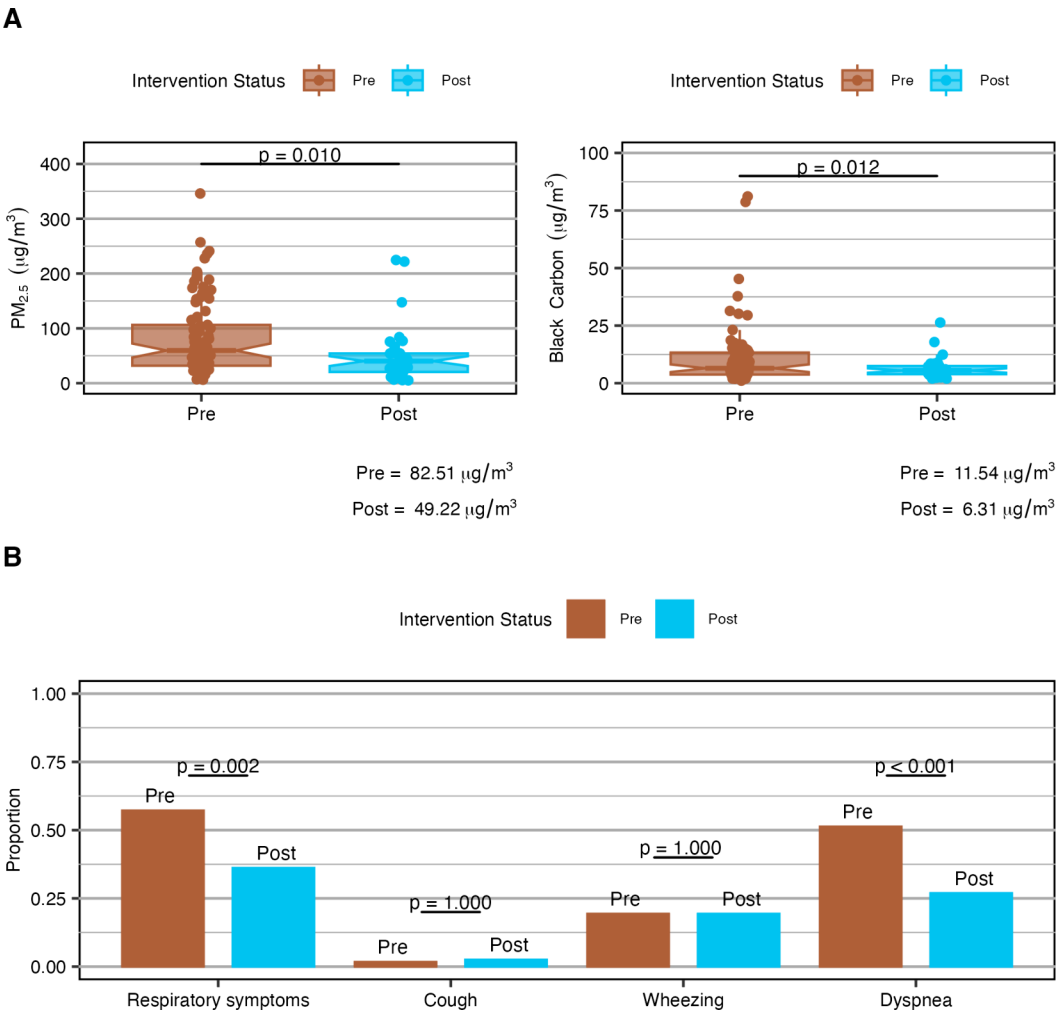

Figure 4. Personal exposure to fine particulate matter and black carbon before vs after the clean lighting intervention and a composite score for respiratory symptoms. Distribution of fine particulate ($PM_{2.5}$) and black carbon concentration measurements before and after solar lighting intervention (A), in micrograms per cubic meter of air. Measurements were taken using personal air quality monitors with gravimetric collection worn by participants over a period of 72 h at each time point. Personal exposure to $PM_{2.5}$ and black carbon was not measured at 18 months. There were 39 observations missing in $PM_{2.5}$ and 42 observations missing in black carbon due to equipment malfunction. Model estimates in below plots are mixed effects linear model predictions for each pollutant before (baseline) and after solar lighting intervention, controlling for participant and village as random effects. Both pollutant concentrations showed statistically significant decreases postintervention. The bar plot of the lung symptom category (B) is derived as a composite score marked as reported if a subject has experienced any of these symptoms from cough, wheezing, or dyspnea. Each X axis mark corresponds to one of these conditions, while the Y axis indicates the proportion of participants reporting that symptom. P-values are from Pearson's chi-squared tests for each condition.

Prepost Analysis.

$$\log it(P(Y_{ijk} = 1)) = \beta_0 + \beta_1 PostIntervention_{ij} + \beta_2 Age_j + \beta_3 Diet_{ij} + \beta_4 AntibioticUse_{ij} + \beta_5 Season_{ij} + V_j + U_k$$

.

Where Y_{ijk} is the binary **respiratory** symptom for the i-th measurements in the j-th subject within the k-th village, β_0 is the fixed intercept, β_1 is the fixed effect of the intervention for the i-th measurements in the j-th subject, V_j is the random effect for the j-th subject, and U_k is the random effect for the k-th village. Adjusting for confounding variables, age, diet, antibiotic use, and season. β_2 is the fixed effect of age in the j-th subject, β_3, β_4, and β_5 are the fixed effect of other confounders for the i-th measurements in the j-th subject.

ITT Analysis.

$$\log it(P(Z_{mnp} = 1)) = \alpha_0 + \alpha_1 Intervention_n \times Post_{mn} + W_n + S_p$$

.

Where Z_{mnp} is the binary **respiratory** symptom for the m-th measurements in the n-th subject within the p-th village, α_0 is the fixed intercept, α_1 is the fixed effect of the interaction between the intervention assignment and postintervention measurement, W is the random effect for the n-th subject, and S_p is the random effect for the p-th village.

This analysis was implemented using the lme4[55] package for differential abundance with a centered log-ratio transformation for the compositional microbial clades (Figure 3B and Figure 3C for prepost analysis, and Supplemental Figure 3A and Supplemental Figure 3B for ITT analysis). The Benjamini-Hochberg multiple hypothesis correction was also applied for differential abundance analysis. Additional taxonomic information for metagenome-assembled genomes was manually obtained from the Genome Taxonomic Database (GTDB)[56] to facilitate biological interpretations. Secondary analyses estimated associations between solar lighting intervention to $PM_{2.5}$ and black carbon concentrations, which were obtained only at baseline and 12 months post intervention (Figure 4A and Figure 4B).

For feature selection to identify microbiome and virome signatures of reduced air pollution exposure, we trained the models for pre- vs

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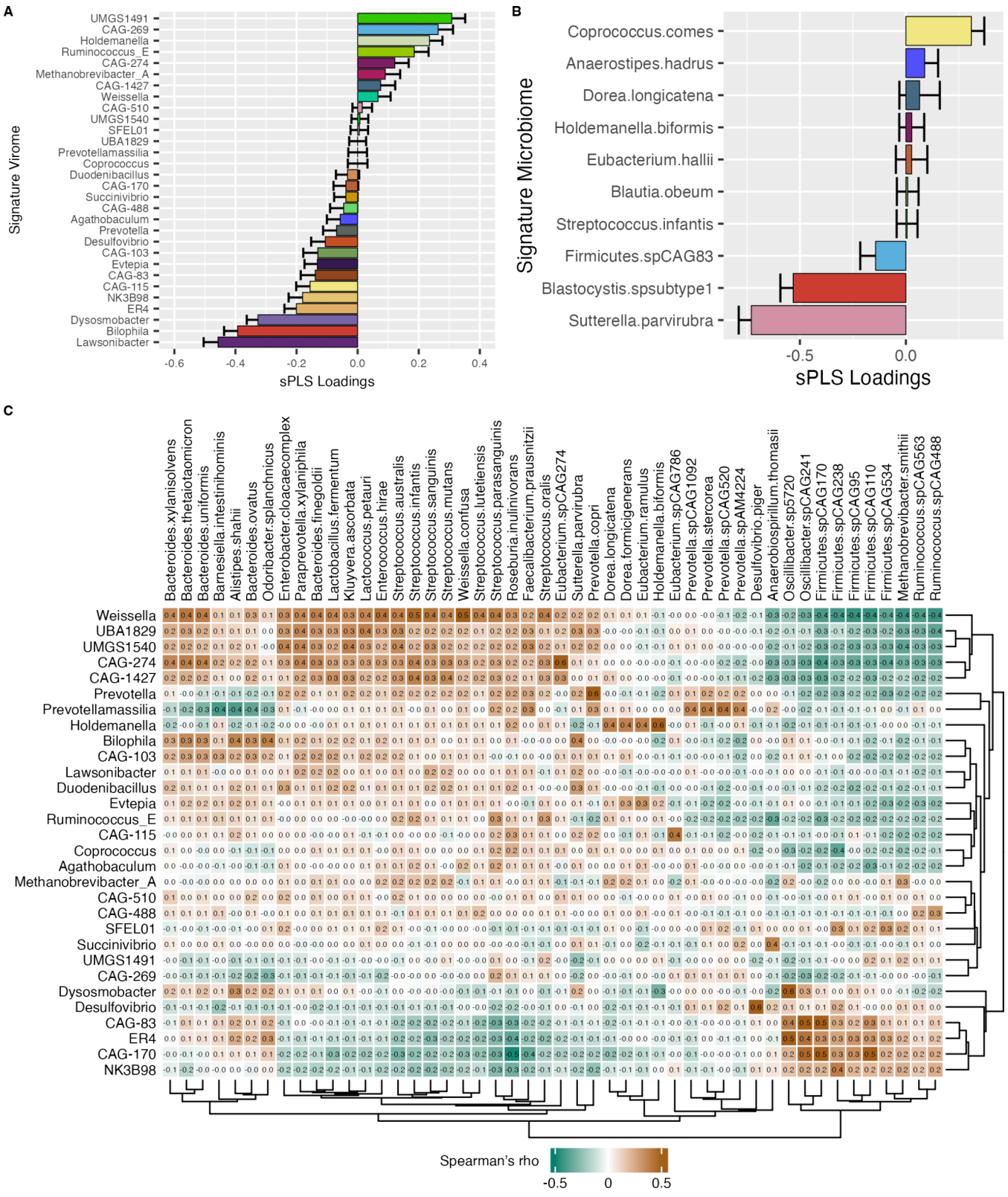

Figure 5. Virome and microbiome signatures of air pollution exposure with correlation between individual nonviral and viral microbes to demonstrate relationship between the gut microbiome and virome communities. Viruses annotated with predicted microbial host to facilitate biological interpretation. Loading from sparse partial least-squares regression (sPLS) model predicted by the solar lighting intervention using 10 repeats of 10-fold CV for the cross-validation for (**A**) virome signature with 30 viral clades and (**B**) microbiome signature with 10 nonviral microbial. (**C**) Spearman correlation heatmap with viruses selected by the sPLS model for the virome score (here depicted as predicted bacteria host of viruses in the rows) and nonviral microbes selected in the columns if at least one of their absolute correlations to the selected bacteria host is 0.35. Hierarchical clustering was performed using the averaging method for both rows and columns.

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Table 1. Characteristics of Study Participants[a]

	Overall cohort	Waitlist control	Intervention	p-value
n	80	40	40	
Age, years	39.16 (10.05)	36.83 (6.61)	41.50 (12.24)	0.037
Education (%)				0.754
None	11 (13.8)	4 (10.0)	7 (17.5)	
Primary 1−2	22 (27.5)	12 (30.0)	10 (25.0)	
Primary 3−6	28 (35.0)	15 (37.5)	13 (32.5)	
Primary 7	19 (23.8)	9 (22.5)	10 (25.0)	
Marital status (%)				0.602
Married	71 (88.8)	35 (87.5)	36 (90.0)	
Cohabiting	8 (10.0)	4 (10.0)	4 (10.0)	
Separated/divorced	1 (1.2)	1 (2.5)	0 (0.0)	
Land ownership (%)	78 (97.5)	39 (97.5)	39 (97.5)	1.000
Owns a radio (%)	62 (77.5)	31 (77.5)	31 (77.5)	1.000
Owns a motorcycle (%)	10 (12.5)	6 (15.0)	4 (10.0)	0.735
Owns a car (%)	1 (1.2)	0 (0.0)	1 (2.5)	1.000
Wealth quintile	2.99 (1.34)	2.90 (1.24)	3.08 (1.46)	0.562
Hours spent indoors daily	16.12 (2.92)	15.81 (3.56)	16.42 (2.11)	0.352
Self-reported hours of light use daily (mean (SD))	4.75 (3.10)	4.35 (2.81)	5.15 (3.36)	0.251
Primary lighting source (%)				0.995
Candles	2 (2.5)	1 (2.5)	1 (2.5)	
Kerosene (open wick) lamp	24 (30.0)	12 (30.0)	12 (30.0)	
Kerosene (hurricane) lamp	9 (11.2)	4 (10.0)	5 (12.5)	
Flashlight	5 (6.2)	3 (7.5)	2 (5.0)	
Solar panel powered bulbs	27 (33.8)	14 (35.0)	13 (32.5)	
Electrical bulbs (national grid)	13 (16.2)	6 (15.0)	7 (17.5)	
Primary cook in house (%)	78 (97.5)	40 (100.0)	38 (95.0)	0.474
Hours spent cooking daily	4.08 (1.57)	3.98 (1.49)	4.18 (1.65)	0.565
Cooking fuel type (%)				0.500
Charcoal	3 (3.8)	1 (2.5)	2 (5.0)	
Firewood	76 (95.0)	39 (97.5)	37 (92.5)	
LPG/Natural gas	1 (1.2)	0 (0.0)	1 (2.5)	
Trash burning (%)	40 (50.0)	18 (45.0)	22 (55.0)	0.502
Respiratory symptoms (%)	50 (62.5)	27 (67.5)	23 (57.5)	0.488
Cough	1 (1.2)	0 (0.0)	1 (2.5)	1.000
Wheeze	18 (22.5)	11 (27.5)	7 (17.5)	0.422
Dyspnea	45 (56.2)	24 (60.0)	21 (52.5)	0.652
FEV_1, liters	2.38 (0.40)	2.38 (0.40)	2.38 (0.40)	1.000
% predicted FEV_1	87.91 (13.41)	86.66 (13.19)	89.16 (13.67)	0.409
FVC, liters	2.98 (0.46)	2.98 (0.44)	2.98 (0.49)	0.971
% predicted FVC	92.44 (12.80)	91.61 (11.85)	93.26 (13.78)	0.567

[a]Note only Ugandan women were recruited for this study. Values presented as mean (standard deviation) for continuous variables and number (%) for categorical variables. Two-tailed p-value based on Pearson chi-square test for categorical variables and one-way ANOVA test for continuous variables. Abbreviations: LPG = liquefied petroleum gas. FEV_1 = forced expiratory volume in the first second, a measure of lung function. FVC = forced vital capacity, a measure of lung function.

postsolar lighting intervention with a number of variables ranging from 10 to 30 to determine the optimal number of features that provide better performance. This training was performed using the sparse partial least-squares (sPLS) method with 10 repeated 10-fold cross validation using the caret[57] and mixOmics[58] R packages. The model with 30 virus features was selected as it has a higher area under curve (AUC) (Figure 5A). The virome scores were derived from the prediction of the best trained model. The microbiome scores were acquired through a similar approach, and 10 microbes were selected (Figure 5B). A heatmap was created using the ComplexHeatmap[59] package with the average method for the hierarchical clustering for both rows and columns (Figure 5C). The rows were the selected predicted bacteria host of the virome score, and the columns (microbial species) were filtered by at least one of the absolute correlations with the selected bacteria host was higher than 0.35. We calculated Spearman's rho correlations between microbiome score and the virome score using the cor() function and tested the Spearman's correlation test using the cor.test() function. The mixed effect models using the lme4 package were then conducted.

To assess the relationship of the clean lighting intervention, gut microbiome and virome scores, and respiratory symptoms, we first used pre- vs postsolar lighting intervention as the outcome and the time-varying scores as the predictor for both microbiome score and virome score, individually. Subsequently, the respiratory symptom was treated as the longitudinal outcome with the time-varying microbiome score and the time-varying virome score treated separately as fixed effects. In the mixed effect models, age, time-varying diet, time-varying antibiotic use in the previous three months, and time-varying season were included as potential confounding variables, and subjects and villages were adjusted as random effects. Potential confounders were chosen based on prior biological knowledge, with age, diet, and antibiotic use potentially confounding the relationship between air pollution exposure and gut microbial communities, while season was included given the prepost approach used in the primary analysis.

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There was no missingness in clinical covariates, including respiratory symptoms, age, intervention status, diet, antibiotic use, and season. Four stool microbiome samples were missing due to either failed sequencing (two stool samples) or study dropout (one participant moved to another district, see Figure 1). There were 39 observations missing in $PM_{2.5}$ and 42 observations missing in black carbon due to equipment malfunction. The mechanism of missingness was considered to be missing at random; thus, a complete case analysis was performed using mixed effects models with a random intercept for study participant and village. Two-sided p-values <0.05 were considered significant. All analyses were performed using R version 4.3.2.

RESULTS

Cohort Characteristics

80 women were recruited for this study (CONSORT diagram, Figure 1). Baseline characteristics have been presented previously[27] (Table 1). Table 2 showed the differences for health outcomes between pre- and postintervention status. The average age was 39.2 years, with 76.3% receiving a primary level of education or less. Participants spent the majority of their time indoors (15.8 and 16.4 h a day for control and intervention groups, respectively). There were no differences in the prevalence of respiratory symptoms (composed of cough, wheeze, and dyspnea) at baseline between the control and intervention groups and no difference in baseline lung function.

Table 2. Health Outcomes Stratified by Pre- and Postintervention Status[a]

	Preintervention	Postintervention	p-value
Observations (n)	119	119	
Respiratory symptom (%)	68 (57.1)	43 (36.1)	0.002
Cough	2 (1.7)	3 (2.5)	1.000
Wheeze	23 (19.3)	23 (19.3)	1.000
Dyspnea	61 (51.3)	32 (26.9)	<0.001
FEV_1, liters	2.37 (0.39)	2.32 (0.38)	0.295
FVC, liters	2.97 (0.45)	2.91 (0.44)	0.272
% predicted FEV_1	87.04 (12.99)	85.67 (12.25)	0.403
% predicted FVC	91.66 (12.27)	89.98 (11.68)	0.280

[a]This was a repeated measures study with three time points. Symptoms assessed at baseline (pre-intervention for all participants), 12 months (post-intervention for intervention group, pre-intervention for waitlist control group), and 18 months (post-intervention for all participants). Values presented as mean (standard deviation) for continuous variables and number (%) for categorical variables. Two-tailed p-value based on Pearson chi-square test for categorical variables and one-way ANOVA test for continuous variables. Abbreviations: FEV_1 is Forced Expiratory Volume in 1 Second. FVC is Forced Vital Capacity. % predicted calculated based on Global Lung Function Initiative 2022 equations.

Gut Metagenomic Profiles

Stool was sequenced to an average read depth (±standard deviation) of 49.1 ± 14.5 million reads. No singletons or doubletons were observed in any of the sequencing profiles. All negative (reagent-only) controls failed library prep, indicating absence of contamination. Four stool profiles were missing, two due to failed library prep, and two follow-up stool samples were not collected since a participant dropped out (Figure 1, CONSORT flow diagram). The per-sample taxonomic composition (both nonviral and viral relative abundance) are depicted in Supplemental Figure 1 and Supplemental Figure 2 (**Per-sample summary of microbial sequencing profile of gut microbiomes** and **Per-sample summary of predicted bacteria host sequencing profile of gut viromes in relative abundance**).

Effects of Clean Lighting Intervention on Microbial Communities

When evaluating changes in alpha diversity of the nonviral microbial community (for brevity hereafter referred to as the microbiome while viral communities are referred to as the virome), there was no statistically significant change, with a reciprocal Simpson index change from 13.22 to 14.72 (p = 0.05) and the exponential Shannon index from 3.16 to 3.22 (p = 0.203) after the intervention. In contrast, for the virome, the reciprocal Simpson index decreased from 32.70 species to 28.58 (p = 0.036) and the exponential Shannon index of viruses decreased from 3.94 to 3.81 (p = 0.059) after the intervention. There was a greater change in microbial community structure for viruses as compared to bacteria (median Bray–Curtis difference 0.72 vs 0.56; p < 0.001, Wilcoxon rank-sum test). The difference in Horn-Morisita distance between baseline and postintervention for viruses versus bacteria was also significant (median 0.75 vs 0.56, p < 0.001, Wilcoxon rank-sum test). Figure 3A illustrates the difference in Bray–Curtis distance between baseline and postintervention stool samples for each kingdom (stratified by eukaryota, archaea, bacteria, and viruses); no change in overall microbial community structure would result in a Bray–Curtis difference of zero.

Differentially Abundant Nonviral and Viral Gut Microbiota

The 10 most abundant microbial species in the observed stool communities were *Prevotella copri* (12.15% of average stool community), *Faecalibacterium prausnitzii* (6.14%), *Bifidobacterium adolescentis* (3.95%), *Collinsella aerofaciens* (3.65%), *Eubacterium rectale* (3.41%), *Ruminococcus bromii* (3.01%), *Escherichia coli* (2.54%), *Prevotella sp.* CAG:279 (2.36%), *Methanobrevibacter smithii* (2.25%, an archaeon), and *Phascolarctobacterium succinatutens* (2.22%). Identified viral species all belonged to the *Caudoviricetes* class, that is, double-stranded DNA tailed phages whose hosts are bacteria and archaea. Figure 3B and Figure 3C depict volcano plots demonstrating the effect of the clean lighting intervention on the differential abundance of microbial (B) and viral (C) clades. Of the nonviral clades, 12 decreased postintervention and belong to the Firmicutes, Proteobacteria, Bacteroidetes, and Bigyra (an eukaryote) phylum. The remaining 14 microbes increased postintervention and are largely from the Firmicutes and Bacteroidetes phylum. For viruses, here depicted using predicted bacterial host genus to facilitate biological interpretation, 12 decreased postintervention, with predicted hosts from the Firmicutes, Desulfobacterota, and Proteobacteria phyla. The remaining 5 increased postintervention and all predicted viral hosts belong to the Firmicutes phylum. The differentially abundant microbes and virally predicted microbial hosts were different. Supplemental Table 1 and Supplemental Table 2 (**Taxonomy and differential abundance of prevalent microbes** and **Taxonomy and differential abundance of prevalent predicted bacteria host of viruses at the genus level**) include the complete list of these differentially abundant microbial species and viral predicted microbial hosts for the effects of the clean lighting intervention on the gut microbiome.

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Microbial Signatures of Reduced Air Pollution Exposure

When comparing pre- to postintervention personal exposure to air pollution (Figure 4A), the provision of solar lighting systems reduced personal exposure to $PM_{2.5}$ from an average of 82.51 $\mu g/m^3$ to 49.22 $\mu g/m^3$ (95% CI (24.47, 74.34), p = 0.010) and reduced black carbon exposure from 11.54 $\mu g/m^3$ to 6.31 $\mu g/m^3$ (95% CI (2.33, 10.32), p = 0.012).

To determine whether microbial signatures of air pollution exposure were associated with health, we created microbiome and separately virome scores using a sparse partial least-squares model to predict reduced air pollution exposure. The model selected 30 viruses and 10 microbes for the virome and microbiome scores, with model AUCs of 0.758 and 0.739, respectively, in predicting whether stool samples were obtained before or after the air pollution intervention (Figure 5A and Figure 5B). Among the 30 selected predicted bacterial hosts comprising the virome score, *Dysosmobacter*, ER4 (a metagenome assembled genome for *Oscillibacter*), *Lawsinobacter*, and *Biolphila* were the predicted virus host genera that provided the largest loadings in the negative direction, while *Holdemanella*, CAG-269 and UMGS1491 (both metagenomes assembled genomes for phages infecting *Clostridium* species) contributed to the positive direction (Figure 5A). Among the 10 selected bacteria and eukaryote species contributing to the microbiome score, *Sutterella parvirubra* and *Blastocystis sp* subtype 1 contributed the most to the negative direction of the loadings. *Coprococcus comes* had the largest positive contribution to the loadings (Figure 5B).

Intervention Effect on Respiratory Symptoms

Frequency of any reported respiratory symptoms (a composite of cough, wheezing, and/or dyspnea based on the American Thoracic Society Respiratory Disease questionnaire[33]) decreased postintervention from 57.1% to 36.1% (p = 0.002) and was largely driven by a reduction in dyspnea frequency from 51.3% to 26.9% (p < 0.001) (Figure 4B). There were no statistically significant associations between the intervention and lung function measured by prebronchodilator spirometry (Supplemental Table 3 and Supplemental Table 4).

Microbial Signatures of Reduced Air Pollution Exposure and Respiratory Symptoms

A higher microbiome signature score (higher score reflects lower air pollution exposure in the postintervention period) was associated with a lower odds of having respiratory symptoms (OR 0.68 (0.49 − 0.94), p = 0.020) in mixed effects models adjusting for age, diet, antibiotic use, and season. The virome score did not reach statistical significance (OR 0.74 (0.53 − 1.03), p = 0.076). Overall virome and microbiome scores were modestly correlated (Spearman's rho = 0.45, p < 0.001). To demonstrate the relationship between individual microbial and viral clades, given the known effect of phages on bacterial and archaeal communities, their correlation is depicted in Figure 5C; only microbes with at least one correlation greater than 0.35 among the 30 selected viruses (depicted as predicted bacterial hosts) of the viruses are shown.

To better understand how gut microbiome vs virome signatures of reduced air pollution exposure influences respiratory symptoms, we binarized the microbiome score and virome score at a z-score of zero and further classified them into 4 categories based on low vs high microbiome and virome scores (Supplemental Figure 4). The proportion of respiratory symptoms was lower when the microbiome score or virome score was high in the group. However, the proportion of respiratory symptoms was lowest in the group with high microbiome score and low virome score rather than the group with both high microbiome and virome scores suggesting greater importance in the microbiome signature.

Sensitivity Analysis: Intention-to-Treat Analysis (ITT)

The clinical trial was powered for an intention-to-treat analysis of the primary clinical trial outcome of change in personal exposure to air pollution rather than for high dimensional outcomes such as microbiome sequencing data sets, which require larger sample sizes. Thus, our primary analysis focused on pre- vs postintervention effects as the control group also received the solar lighting intervention at 12 months postrandomization, and we collected stool samples at 18 months postrandomization. Thus, we had 80 participants who had stool samples collected both before and after the intervention, rather than 40 participants if an intent-to-treat analysis only was performed. To verify that our pre- vs postanalysis reflected the effect size of an intention-to-treat analysis, in sensitivity analysis, we performed an intention-to-treat analysis using samples under the randomized controlled design only (stool samples collected at baseline and 12 months only). In mixed effects models accounting for repeated measures in participants and clustering effect in villages, the solar lighting intervention reduced respiratory symptoms (OR 0.41 (0.18−0.94), p = 0.034). The effect size was similar to our primary prepost analysis, with an odds ratio 0.41 (0.21−0.78), p = 0.007 (Supplemental Table 4, **Comparison of prepost vs intention-to-treat analysis on clinical outcomes**). When evaluating the effect of the solar lighting intervention on differential abundance, as expected, there were fewer statistically significant microbes due to the need to adjust for multiple testing of a high number of features, with seven members of the Firmicutes phylum and one from the Actinobacteria phylum for the microbiome analysis and ten differentially abundant predicted bacteria hosts from the Firmicutes and Desulfobacterota phyla. In evaluating the rank order of differentially abundant microbes for the ITT vs prepost analysis, the Spearman's correlations of q-values between sensitivity analysis and primary analysis were significant for both differentially abundant nonviral and viral analyses (Spearman's rho 0.422 with p < 0.001 for microbiome, 0.595 with p < 0.001 for virome). The volcano plots are depicted using raw p value on the y axis for both microbiome and virome (see Supplemental Figure 3, **Effect of solar lighting intervention on differential abundance of nonviral and viral gut microbes, ITT analysis**). These results suggest that the ITT analysis shows similar effect sizes to the prepost analysis but had less power to detect differentially abundant microbes.

DISCUSSION

A solar lighting intervention changed the gut microbiome and virome of women living in rural Uganda. Gut microbial signatures of reduced air pollution exposure were associated with improved respiratory symptoms but no change in lung function. These findings support the hypothesis that, in humans, air pollution exposure can influence the gut microbiome and virome. A candidate mechanism by which air pollution might impact lung health is through changes in gut microbial communities. A future therapeutic approach to countering the adverse health effects of air pollution might include targeting beneficial members of the gut microbiome.

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Individuals in resource-limited settings often lack the ability to reduce their personal exposure to air pollution, while microbiome-targeted therapies are within individual control.

While there are some studies evaluating the effect of air pollution on the respiratory microbiome,[60−63] fewer have examined associations with the gut microbiome, none have evaluated the gut virome, and none have examined the gut microbial changes and influence on pulmonary outcomes. Available studies, largely observational in nature and performed in high or middle income studies, have focused on outdoor air pollution exposure and changes in the gut microbiome. A longitudinal observational study of 76 older-age healthy adults in China who had personal air pollution monitoring and stool collected for 16S rRNA gene sequencing showed associations between $PM_{2.5}$ and differential abundance of several bacterial taxa; these taxa appeared to mediate the association between air pollution exposure and insulin resistance.[3] While the investigators collected stool after a prescribed uniform diet for 5 days prior to stool collection, confounders were not included in their mediation analysis, nor did they account for the compositional nature of their generated microbiome data in mediation analyses. Several other observational studies have examined the association between traffic-related air pollutants (TRAP) and gut microbiome composition,[5,8,25] assessed mostly with amplicon sequencing, although one study used metagenomics sequencing. These have shown associations between TRAP and reduced alpha diversity, changes to overall microbial community composition based on beta diversity, and a differential effect based on the type of pollutant assessed (e.g., $PM_{2.5}$, ozone, nitrogen dioxide). Given the large number of potential confounders (both measured and unmeasured) in microbiome studies,[64] intervention studies are particularly useful for strengthening the evidence base. To our knowledge, no other published gut microbiome study in humans has been conducted in the context of an effective intervention to reduce air pollution exposure. Therefore, this study provides further support that one way air pollution exposure may influence lung health through changes in the gut microbiome.

In our study, when examining gut microbiome signatures of reduced air pollution exposure, *Sutterella parvirubra*, *Coprococcus comes*, and *Anaerostipes hadrus* were the three most influential nonviral microbes that contributed to the signature. *Sutterella parvirubra* decreased postintervention and is known as a gut commensal with proinflammatory properties and is associated with inflammatory bowel disease.[65] *Coprococcus comes* and *Anaerostipes hadrus* are two gut commensal butyrate-producing bacteria that increased in relative abundance postintervention and have been associated with anti-inflammatory properties.[66−68] None of these microbes have been studied specifically in the context of lung disease and could be considered in future gut-lung axis mechanistic studies to determine how their effect on systemic inflammation or the production of circulating metabolites might influence pulmonary outcomes in the context of air pollution.

This study has several strengths. Intervention studies are less prone to residual confounding compared with observational studies. We used metagenomic rather than amplicon sequencing, allowing assessment of bacterial, archaeal, fungal, and viral (predominantly phage) communities and allowing for finer taxonomic resolution. Our average sequencing depth was 49 million reads per sample, sufficiently deep to characterize complex fecal microbial communities.[69] We address the paucity of high quality microbiome data emerging from resource-limited settings.[25] The major limitation of this study is the modest sample size; thus, the primary statistical comparison focused on a pre- vs postintervention effect to leverage the additional sample size gained after the control group received a solar lighting system, with an intention to treat analysis presented as a sensitivity analysis. This substudy was designed as a proof-of-concept study, with the main trial powered to detect a reduction in personal exposure to air pollution as the primary outcome.[27] Beyond issues of power, the modest sample size may affect the generalizability of these findings. A prepost analysis cannot account for other temporal changes that may have occurred outside of the study intervention, although we did adjust for season, diet, and antibiotic use in our models, with the intention-to-treat analysis presented as a sensitivity analysis. The time frame of the entire study was short, at 18 months, and during that period, there were no major changes in local infrastructure and governance with no major infectious disease outbreaks. While the gut microbiome was registered as an outcome on ClinicalTrials.gov as a secondary outcome prior to the start of the study, the statistical analysis plan for this secondary outcome was not prespecified prior to trial initiation, although it was preregistered in Open Science Foundation. Due to budgetary and logistic limitations, personal exposure to air pollution was not measured at 18-month follow-up, limiting our ability to define microbiota changes to specific air pollutants. While we are not able to describe exposure-response relationships between measured personal $PM_{2.5}$ or black carbon exposure and changes to the gut microbiome and virome, we have shown that the solar lighting intervention leads to sustained decreases in personal exposure to personal $PM_{2.5}$ and black carbon,[27] demonstrating the validity of our modeling approach. Though the reduction in air pollution exposure is more modest than that described for recent clean cooking trials using liquified petroleum gas (LPG) stoves,[70] clean lighting may be an easier-to-sustain intervention as it is a one-time financial investment for the solar lighting device, whereas LPG as a fuel remains too expensive to sustain for many households in the long term without significant long-term government and private investment.[71,72] It was not possible to blind the participants to the intervention; thus, it is possible that, in an unblinded study, participants may be inclined to say positive things as a gesture of gratitude to the study team. However, the composite respiratory symptom outcome, derived from the validated American Thoracic Society Respiratory Disease questionnaire, is composed of three questions pertaining to cough, wheezing, and dyspnea. The lack of change in cough and wheeze but significant improvement in dyspnea suggests that participants did not uniformly report better outcomes across all symptoms. This suggests that the finding of improved dyspnea was less likely to be driven by the influence of an unblinded intervention. While the intervention improved dyspnea, it did not change lung function. Dyspnea has been shown to be an independent predictor of all-cause mortality in community dwelling adults;[37,38] this is true even after controlling for lung function.[39] Future intervention studies in household air pollution can address these limitations, including larger sample sizes powered for intention-to-treat analyses, longer duration of follow-up, exposure reduction during critical windows of exposure in early life, detailed characterization of potential

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confounding variables, and additional investigation of causal pathways.

While not intuitive, air pollution exposure has the potential to alter the gut microbiome through the ingestion of inhaled particles. A chamber study using radiolabeled aerosols showed that 20% of inhaled fine particles are immediately ingested.[73] Beyond the microbial components of air pollution serving as a source of microbes to the gastrointestinal tract,[74] nonmicrobial components of air pollution such as $PM_{2.5}$ have been shown in animal studies to alter the gut microbiome through the induction of inflammatory changes.[13,75] Our study provides evidence that air pollution alters the gut microbiome and that the gut microbiome signatures were associated with improved respiratory symptoms. Further mechanistic work is needed to better define the underlying mechanisms that may serve as the basis for microbial-based therapeutics.

This study adds to the nascent literature on air pollution exposure and changes in the gut microbiome and virome. Future therapeutic interventions to ameliorate the adverse effects of air pollution exposure on lung health may consider targeting the gut microbiome, an approach that has been used successfully in several clinical trials in resource-limited settings focused on neonatal sepsis and growth stunting.[25] More broadly, the infant microbiome is seeded by the maternal microbiome at birth, with the vertical transmission of beneficial microbes affected by environmental factors such as the route of delivery.[76] Some studies suggest that route of birth delivery (vaginal vs caesarean section) has persistent effects on the gut microbiome through adulthood.[33] Many household air pollution intervention studies focused on improved child health recruit pregnant women[77] to address this critical window of development in the prenatal and early neonatal period. It is possible that a mechanism by which household air pollution interventions may impact lung health[78] is through changes to the maternal microbiome that are then transmitted to their children, though this requires further study.

In summary, a clean lighting intervention previously shown to decrease personal exposure to household air pollution also decreased respiratory symptoms and changed the gut microbiome and virome of adult women living in rural Uganda. Microbiome signatures of reduced air pollution exposures were associated with improved respiratory symptoms. Future randomized controlled trials focused on ameliorating the harmful effects of household air pollution on health should consider the role of the gut microbiome as a potential target of intervention.

■ ASSOCIATED CONTENT

Data Availability Statement

Metagenomics sequencing files for stool samples are available on the NCBI Sequence Read Archive at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA976942. This analysis has been registered on the Open Science Foundation (OSF): https://osf.io/6ax8n/ where the R code for the main analyses is deposited.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/ehp.6c00064.

Descriptions of Excel Tables S1 and S2; Tables S3 and S4 in full; Figures S1−S4 (PDF)
Excel Tables S1 and S2 (XLSX)
Source code (ZIP)

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○CYH and EN contributed equally and should be considered cofirst authors.

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Notes

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