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Article

Performance of PUF-Disk Passive Air Samplers for Quantitative and Compositional Assessment of Airborne Bacteria

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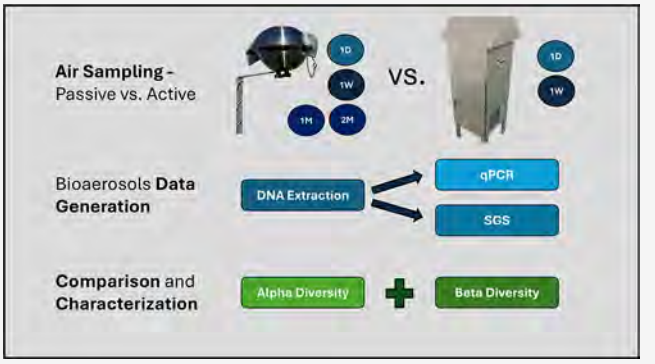
ABSTRACT: We present the first comprehensive comparison of polyurethane foam disk passive air samplers (PUF−PASs) and active high-volume air samplers (Hi-Vol) for bioaerosol monitoring in side-by-side deployments. Using qPCR, 16S rRNA gene sequencing, and multivariate analysis, we demonstrated that the PUF−PASs detected higher bacterial biomass, as evidenced by significantly elevated gene copy numbers and estimated bacterial cells per m^3 of air volume, but exhibited lower diversity compared to Hi-Vol samplers. Hi-Vol samplers recovered a more taxonomically diverse community, including transient and rare taxa, during sampling periods of 1 day and 1 week. Hi-Vol detected genera not detected in long-term PUF−PAS sampling, while PUF−PAS detected species not observed in short-term Hi-Vol. PUF−PAS samples were enriched with environmental and spore-forming persistent genera. Hi-Vol samples were enriched with opportunistic and human-associated episodic spikes in a range of bacterial species. PCoA analysis confirmed a substantial divergence in bacterial community structure by sampler type and duration. Temporal analysis results showed a progressive shift in bacterial community composition with increasing sampling duration in PUF−PAS. The findings highlight the complementary benefits of both sampler types: active air sampling for capturing short-lived human-associated bioaerosols and taxonomic richness, while passive air samplers favor biomass accumulation and chronic exposure profiling, enabling exposure assessment and ecological surveillance.



KEYWORDS: *passive air sampler, hi-volume air sample, bacteria, qPCR, PUF disk, sampling duration*

INTRODUCTION

Airborne bacteria are ubiquitous in urban environments, and several airborne bacterial communities influence the atmospheric process, ecosystem dynamics and human health. For example, airborne bacteria such as *Legionella* spp. and *Mycobacterium* spp. are known to cause human diseases via aerosolization of the microbes. Inhalation exposure to some airborne bacteria, when dispersed through the atmosphere, can cause infection, allergies, inflammation, and respiratory diseases.[1] Airborne bacteria such as *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Streptomyces*, and *Xanthomonas* can also cause plant diseases. While airborne bacteria are widely reported in academic literature due to their pathogenicity in relation to human health, some bacteria have positive effects, such as bioremediation. For instance, several airborne microorganisms, such as *Pseudomonas* and *Mycobacterium*[2−4] have the capability to metabolize organic compounds such as benzo[a]pyrene and pyrene. Further, studies have indicated that exposure to some microbes or bacteria can benefit health, protecting children from allergies and asthma by helping to develop a healthy immune system.[5,6]

Airborne bacteria are emitted in both indoor and outdoor environments. They can originate from natural sources (wildfire, volcanic eruptions), traffic emissions, aerosolized soil and dust resuspension, molds, hospital operating rooms and contaminated water. The human health impacts caused by airborne bacteria have led to various sampling techniques being designed to characterize them. Active air samplers are commonly used for short sampling periods, but these require electricity to operate, are costly and need frequent maintenance and calibration.[7−9] A number of studies have described the advantages and disadvantages of passive and active air samplers.[10,11]

Passive air samplers, including Petri dishes, agar settle plates, dust fall collectors, Rutgers Electrostatic Passive Samplers (REPSs), and Personal Aeroallergen Samplers (PAASs), and active air samplers such as high-volume air samplers and multistage size impactors, have been widely used to measure airborne bacteria.[8,10,12−14]

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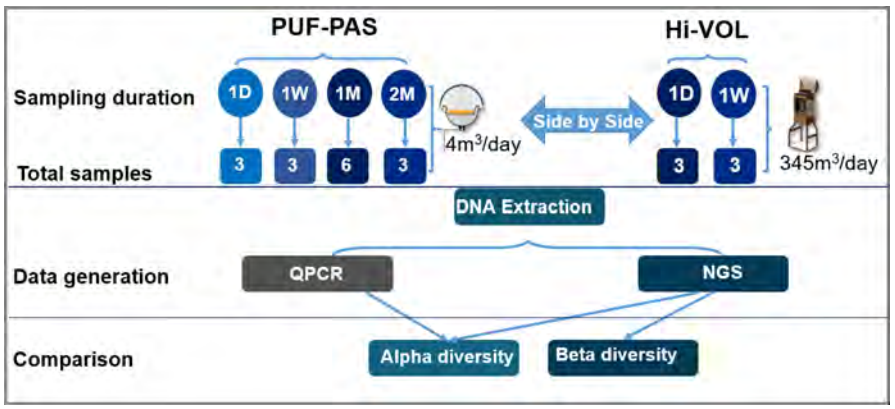

Figure 1. General workflow of the study design showing codeployed (side-by-side) PUF−PAS and Hi-Vol active samplers at the same site, with samples collected over 1 day and 1 week (both Hi-Vol and PUF−PAS), and extended to 1 and 2 months for PUF−PAS. Data were generated using quantitative polymerase chain reaction (qPCR) and second-generation sequencing (SGS).

A recent study demonstrated that PUF−PASs can be used over days, weeks and months, especially in low-resource settings. A previous study provided a proof-of-concept exercise for the characterization of airborne bacteria and fungi using high-throughput DNA sequencing in the Canadian atmosphere.[7] Their findings suggested that updates on performance and development of a methodology for characterization of airborne microorganisms collected by the PUF−PAS system were required to provide quantitative information to more effectively investigate sources and potential exposure.[7] Briefly, a PUF−PAS is a diffusion-based passive air sampler that functions as an integrative sampler, by collecting gas- and particle-phase compounds with a size fraction of 1−5 μm from outdoor air, including those that can be inhaled.[15] In PUF−PASs, gas phase and semivolatile compounds are transferred across an air-side boundary layer to the PUF disk and retained by absorption or adsorption with the PUF matrix.[16,17] The detailed mechanism of PUF−PAS operation has been described in previous studies.[16]

Here, we provide a follow-up to a previous study to evaluate the airborne bacterial composition and diversity collected using PUF−PASs and analyzed using second-generation sequencing. For the successful assessment of the PUF-Disk sampler and development of the methodology for characterization of airborne microorganisms, PUF−PASs were codeployed in a side-by-side comparison alongside conventional high-volume samplers in Toronto at the Downsview site mixed source. We conducted sampling and quantitative PCR (qPCR)-based measurements to compare the sampling performance of the PUF−PAS with that of a reference colocated active Hi-Vol air sampler using sampling durations ranging from 24 h to 2 months. Ultimately, this study used the PUF−PAS to generate volumetric air concentrations for bioaerosols, which are essential for equivalent sampling flow rates[18] and logistically more feasible and cost-efficient.[7] This study will contribute to the longer-term study of airborne microorganisms and the health risks they pose across Canadian cities and globally.

MATERIALS AND METHODS

Sampler Deployment

Sampling campaigns were conducted by colocating a PUF−PAS (14 cm diameter × 1.35 cm thick, surface area 365 cm², mass 4.40 g, volume 207 cm^3, PUF−PAS, Tisch Environmental, TE-200-PAS, Cleves, USA) and a PS-1 high-volume sampler, (Tisch Environmental, TE-Hi-Vol TSP, Cleves, USA) at the Downsview site in Toronto from January to March 2023 (Figure S1 of the Supporting Information (SI)). All colocated passive and active air sampling experiments were conducted at 1 Day and 1 Week (both Hi-Vol and PUF), extended to 1 and 2 months for PUF−PAS, and were run for the same sampling durations at the same site (Downsview), characterized as light traffic, industry, park and residential. The Hi-Vol sampler could not obtain samples for 1 month and 2 months consecutively due to its high flow rate and logistic difficulties. The Downsview monitoring site used in this study was selected based on an existing network of air sampling sites operated by the National Air Pollution Surveillance (NAPS) network, Government of Canada. The passive samplers were mounted approximately 2 m above the ground on a fence (Figure S1). As part of the PUF disk performance exercise, active high-volume air samplers that collected total gas- and particle-phase compounds were codeployed (side-by-side) at the same site. Additional workflow information about the PUF disk sampler and Hi-Vol active air sampler and deployment is provided in Figure 1. A total of 21 samples (PUF, n = 15) and Hi-Vol (n = 6), including blank samples, were collected, which were triplicated (24 h, 1 week and 2 months) and six samples were collected at 1 month. We also collected four blank samples (2 for PUF-Disk and 2 for Hi-Vol active samplers).

The Hi-Vol samples were collected at a 240 L/min flow rate equivalent to ∼ 345m^3/day. All Hi-Vol filters (47 mm) glass fiber filters were baked overnight in the muffle furnace at a constant 400 °C temperature from 4 pm to 8 am before deployment to remove organic contaminants. Immediately after field sampling, filters were folded twice, wrapped in aluminum foil, placed inside sterilized plastic bags, sealed in polyethylene and kept refrigerated at 4 °C

The PS-1 Hi-Vol sampler was operated in total suspended particulate (TSP) mode, which does not include a size-selective inlet and therefore does not have a defined aerodynamic cutoff point (d_{50}). The effective d50 for TSP samplers such as this is typically 10s of um up to 100um, depending on operational parameters and meteorology during sampling.

All samples were stored in a fridge at 4 °C immediately after field sampling. All PUF disks were housed horizontally inside precleaned stainless steel chambers and were kept refrigerated until the campaign concluded in March, after which they were stored in precleaned, solvent-rinsed glass jars following the protocols of the Global Atmospheric Passive Sampling GAPS Network[19] and immediately shipped to the laboratory for DNA extraction.[7,8,20,21] PUF−PASs collect gas and particle species in ambient air, including those that can be inhaled (up to about 5 μm and with a similar particle size distribution as the PS-1 high-volume sampler used in this study.[15] All PUF-disk and Hi-Vol active air samples were packed in a sealed box and shipped from Canada to Molecular Research LP (MR DNA, Texas, USA) for DNA sequencing.

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DNA Extraction, Sequencing, and Real-Time qPCR

Genomic DNA from PUF−PAS and Hi-Vol filter samples was extracted using the DNeasy PowerMax Soil Kit (Qiagen) following the manufacturer's instructions. Details of extractions are provided in our previous study.[7] To illustrate DNA extraction aerosolization issues, all 4 blank samples collected were extracted along with the samples. Briefly, one-half pieces of the PUF disk and full filter (QFF, 47 mm) samples, including a blank sample, were extracted for DNA in the same way. Briefly, four field blanks were included for quality assurance and quality control (QA/QC). For each blank, the sampling media were taken to the field site, inserted into the sampler, and briefly exposed to ambient air for a few seconds without operating the pump. The blanks were then removed and processed through the entire DNA extraction and qPCR workflow to evaluate background contamination arising from handling, transport and field procedures. Bacterial quantification via extracted DNA was determined by qPCR using Bacteria2F and Bacteria2R primers.[7,22,23] qPCR was performed using a hydrolysis-probe (Taqman) assay rather than the amplicon-based primer set commonly used for sequencing for the V4 region, such as 515*F*/806R.[7,22] In this study, total bacterial 16r RNA gene abundance was quantified using the following primer-probe set: Bacteria 2F (5′-CCATGAAGTCGGAATCGCTAG-3′), Bacteria 2R: (5′- GCTTGACGGGCGGTGT-3′) and Bacteria 2 Probe: (5′-/56-FAM-TACAAGGCC/ZEN/CGGGAACGTATTCACCG/3LAIBkFQ/-3′).[24−26] Three extractions were performed for each PUF−PAS by dividing them into three equal parts and adding them to a PowerMax Bead Tube to undergo cell lysis. Each filter underwent one extraction. The purified DNA was eluted from the MB Maxi Spin Column in 5 mL of solution C6 and stored at −20 °C until PCR amplification. One hundred μL of extracted DNA from each PUF replicate, totaling 300 μL, were pooled prior to PCR. DNA quantity and quality were determined using a NanoDrop2000 (Thermo Scientific).

One μL of the template DNA was used to perform the qPCR reaction using 2X Universal TaqMan PCR Master Mix (Applied Biosystems) in QuantStudio3 Real-Time PCR System (Applied Biosystems). Each sample was run in duplicate. The PCR reaction was performed with an initial holding stage of 50°C for 2 min and 95°C for 10 min. The cycling stage consisted of 40 cycles of 95°C for 15 s, followed by 60°C for 1 min. Genomic DNA from *E. coli* was used as a standard. Quality assurance and control (QA/QC) procedures were incorporated throughout the workflow. In this study, no-template controls (NTCs) were included in each qPCR run and consistently yielded no detectable amplification. DNA extraction blanks were submitted with the sample batch and were processed and sequenced in parallel, allowing assessment of background signal and potential contamination. For the generation of a standard curve, an *E. coli*(*E. coli*) K-12 strain was used as the reference material. qPCR amplicons were not sequenced, as they were used solely for quantitative assessment, following standard library preparation protocols. Routine QA/QC were applied at all stages, including DNA quality control, library preparation, and postsequencing data quality assessment. Amplicon sequencing was performed at Molecular Research Laboratories (MR DNA, Shallowater, TX, USA) on the Illumina NovaSeq 6000 platform using a paired-end 2 × 250 bp chemistry, targeting about 20,000 reads per sample. Briefly, the V4 region of the bacteria's 16S rRNA gene was amplified using a primer set (515*F*/806R) selected for this study, with PCR conditions following MR DNAs standardized protocol, including read joining, length and quality filtering, dereplication, denoising and chimera removal, which were taxonomically classified using BLAST against a curated NCBI database (Dowd et al., 2008) and detailed methods are described in bioinformatic section PCR products were visualized on agarose gels to confirm successful amplification and were normalized based on band intensity and DNA concentration. Amplicons were indexed with dual barcodes, pooled in equimolar concentration, and purified using Ampure XP beads. Purified pooled libraries were preprepared following Illumina guidelines and sequenced on the NovaSeq 6000 (Dowd et al., 2008).

Bioinformatic Analysis

DNA amplicon samples were analyzed through high-throughput sequencing on the Illumina MiSeq platform, following the manufacturer's protocols at Molecular Research LP (MRDNA, Shallowater TX, USA). The raw reads were processed with cutadapt v4.2[27] to remove the primer and adaptor sequences. Amplicon sequence variants (ASVs) were then inferred with DADA2 v1.26[28] by truncating the reads to a uniform length (truncLen) of 185 or 130 and removing reads exceeding 2 or 5 expected errors (maxEE), for forward and reverse reads, respectively. The ASVs were inferred with the "pseudo-pooling" option enabled to increase the sensitivity in detecting rare variants. The forward and reverse read pairs were merged and suspected chimeric sequences (specifically, two-parent chimeras) were removed using the removeBimeraDenovo function. Taxonomic classification of the ASV representative sequences was done with the RDP classifier implemented in DADA2, using the SILVA v138.1 reference database.[29] While DADA2 allows for species level assignment with the assign Species or add Species functions by exact matching between the ASV sequences and the reference database, we decided not to assign species level taxonomy due to the limitation of the relatively short V4 amplicon sequences.[30] Non-targeted reads such as those classified as mitochondrial and chloroplast in origin as well as suspected contaminant ASVs were removed from further analysis. Suspected contaminant ASVs were identified and removed via the R decontam package v. 2.12.[31] A suspected contaminant is called if identified by either the frequency or prevalence methods based on the community profiles (from amplicon sequencing) and gene copy number quantification (from qPCR) of the blank sample controls and the actual samples. The statistical tests in assigning contaminant ASVs were conducted with a more-aggressive-than-default probability threshold of 0.35.

Microbial ecological analyses were primarily conducted using the R package phyloseq v1.41.[32] Data visualization was performed using ggplot2 v4.0.1.[33] Differential abundance analysis was conducted using the R package ANCOM-BC v 2.8.1,[34] which performed Analysis of Compositions of Microbiomes with Bias Correction at the genus level, comparing between communities from the two sampler types.

Data Processing and Analysis

Our previous methods have described the data analysis procedure.[7] Briefly, to estimate the bacteria load, qPCR targeting the 16S rRNA gene was used to estimate the bacterial load, and the volume of air normalized the bacterial load. Microbial diversity and richness were analyzed using Shannon and Chao 1 indices. The 16s rRNA sequencing used Illumina MiSeq to assess the taxonomic composition at the phylum, class and genus level. Principal Coordination Analysis (PCoA) based on Bray−Curtis dissimilarities and the PERMANOVA test[35] on weighted UniFrac distance matrices was carried out using the R package vegan v.2.5.4[36] to investigate variance between the bacterial communities partitioned by sampler type and sampling durations. ANCOM-BC[34] was used to identify different abundant taxa between the PUF−PAS and Hi-Vol active samplers.

Our previous study[7] detailed the procedure for blank samples of PUF−PAS disks and filters from Hi-Vol analysis. Briefly, we compared bacterial 16S rRNA gene copy numbers to assess potential background contamination and validation of sample integrity. The results of the Wilcoxon test ($p = < 0.001$) showed that the 16S rRNA gene copy numbers in actual air samples were significantly higher than in the blank control, showing high differences between the two groups, confirming that bacterial signals were distinct from laboratory or field contamination and supporting the validity of downstream quantitative analyses without background contamination or noise from blank samples, validating the qPCR data set.

RESULTS AND DISCUSSION

Alpha Diversity and Bacterial Load

Bacterial genera richness (Chao 1) and diversity (Shannon) indices varied between sampler types and sampling durations

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(Figure 2A). During the short sampling durations (1D, 1W), the Hi-Vol showed higher richness than the PUF−PAS. The

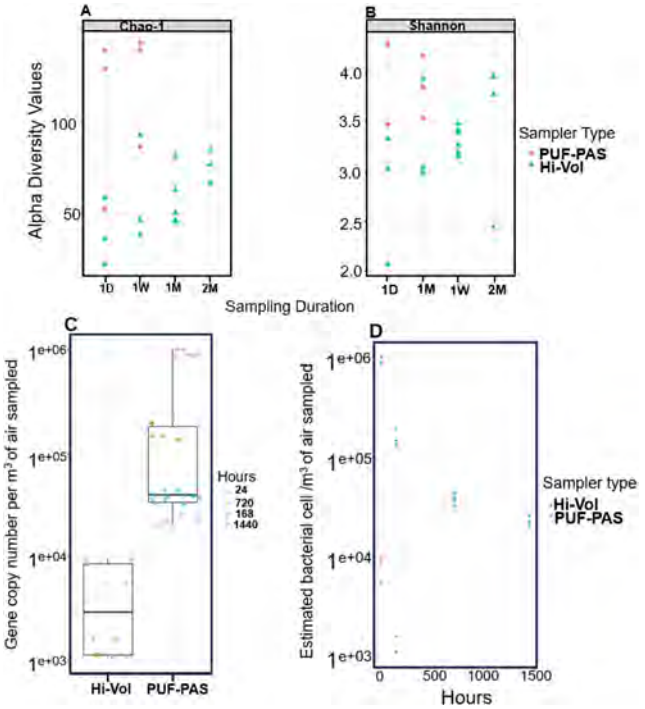

Figure 2. Average alpha diversity and biomass metrics of bacteria across sampler type (Hi-Vol and PUF−PAS) and sampling durations (1 Day (1D), 1Week (1W), 1Month (1M)). The Hi-Vol sampler showed higher taxonomic richness and diversity (A-B), whereas PUF−PAS samples yielded higher gene copy numbers and bacterial load per unit of air volume: (A) Alpha diversity indices (Chao1) and Shannon(B) indices; (C) Gene copy numbers (qPCR) per μL DNA extract by air volume; and (D) Estimated bacterial cells per normalized cubic meter of air volume sampled over increasing durations (the regression line represents the banded area (95% confidence interval).

Chao 1 richness declined for both Hi-Vol and PUF−PAS over time, especially for PUF−PAS between 1 M and 2M, compared to 1W and 1D for Hi-Vol. With long sampling durations (1M, 2M), the richness captured by the PUF−PAS was comparable to that of Hi-Vol samples over shorter sampling durations, suggesting a detection limit issue for PUF−PASs for shorter deployment periods; i.e., insufficient air for detecting some taxa. However, a slight decline in genera richness was also observed for the PUF−PAS over 2 months, which may also suggest potential microbial degradation or saturation of the PUF-disk capacity.

Shannon diversity index values were also consistently higher for Hi-Vol samples at short sampling durations (1D, 1W) than for PUF−PAS samples (Figure 2B), suggesting that a short sampling duration reflects real taxa with greater microbial evenness and richness in Hi-Vol active samples. At a short duration of 1D and 1W, PUF−PAS showed a lower Shannon diversity value, but this value increased with an extended sampling period of 1 M and 2M. These findings suggest that PUF−PAS is better at maintaining recoverable DNA from microbial populations and can improve diversity capture compared to its short sampling duration. Our previous proof-of-concept study[7] showed that PUF−PASs can recover airborne microbial DNA over long deployments and may capture low-abundance or episodic taxa missed in short sampling durations using the Hi-Vol active sampler compared to high-flow rate and mechanical stress of active samplers, which can damage cells or degrade DNA. These results agreed with a study in France that showed greater bacterial richness and evenness in a passive sampler than in an active air sampler over long sampling periods.[10]

To further justify these findings, the results of normalized volumes comparing both samples showed that there was less DNA in Hi-Vol than PUF−PAS, and the 1D sampling duration had the highest amplifiable 16S rRNA gene (Figure 2C), which reflects the number of bacteria (alive or dead) with DNA that was still intact. We have demonstrated that DNA quantity does not necessarily reflect higher richness and diversity: a high DNA concentration or gene copy number yields more bacterial biomass (high qPCR counts and higher estimated bacterial cells per unit of air volume). A decline in bacterial loads over time was observed with PUF−PAS, with a stable plateau after prolonged deployment of approximately 700 h (Figure 2D).

PUF−PAS showed a steady increase in load and diversity over long sampling durations. Bacterial concentrations (cells/m^3) varied with sampler type and sampling durations. The qPCR and normalized volume results indicated that both Hi-Vol and PUF−PAS (Figure 2D) showed consistency over short sampling durations of 1D and 1W; however, PUF−PAS results exhibited high variability, which may reflect the variability in bacterial concentrations in air over time.

A similar trend was also found for several observed amplicon sequence variants (ASVs) per m^3 of air. Figure S2 in the Supporting Information (SI) shows that PUF−PAS accumulated more biomass but with lower per-volume richness, whereas Hi-Vol captured higher ASV diversity over 1D and 1W. These findings showed that DNA yields reflected quantity, while the diverse metric reflected community structure.

Overall, these results suggest that PUF−PAS and Hi-Vol should not be directly compared when monitoring bioaerosols without assessing the trade-off between temporal resolution and cumulative diversity in capturing airborne microbial populations. They have potential advantages in detecting short and long-term in capturing airborne microbial populations and can be selected based on specific monitoring objectives.[14] Our findings can support the aerobiology research for designing sampling strategies for ecological surveillance and health risk assessment in city and remote environments.[12]

Airborne Bacterial Population and Temporal Analysis

Proteobacteria were the most prevalent airborne bacteria across all sampler types and durations (Figure 3A), and these phyla are commonly detected in high relative abundance in aerosol science using different types of active and passive air samplers.[7,10−13,18] However, we observed a shift in the phyla over longer sampling periods. Over extended sampling periods (1 M and 2M), the PUF−PAS samples accumulated a more diverse and balanced microbial population, with high levels of phyla from environmental origins, such as Bacteroidetes.

Figure 2A also showed that PUF−PAS samples had increased evenness and diversity with extended sampling periods of 1 M and 2M. In contrast, Hi-Vol samples showed low richness and dominance at the phylum level, supporting current evidence that over an extended sampling period, PUF−PASs accumulate a broad snapshot of airborne bacterial diversity at the phylum level. These findings showed that Hi-Vol samplers detected a more dynamic and taxonomically

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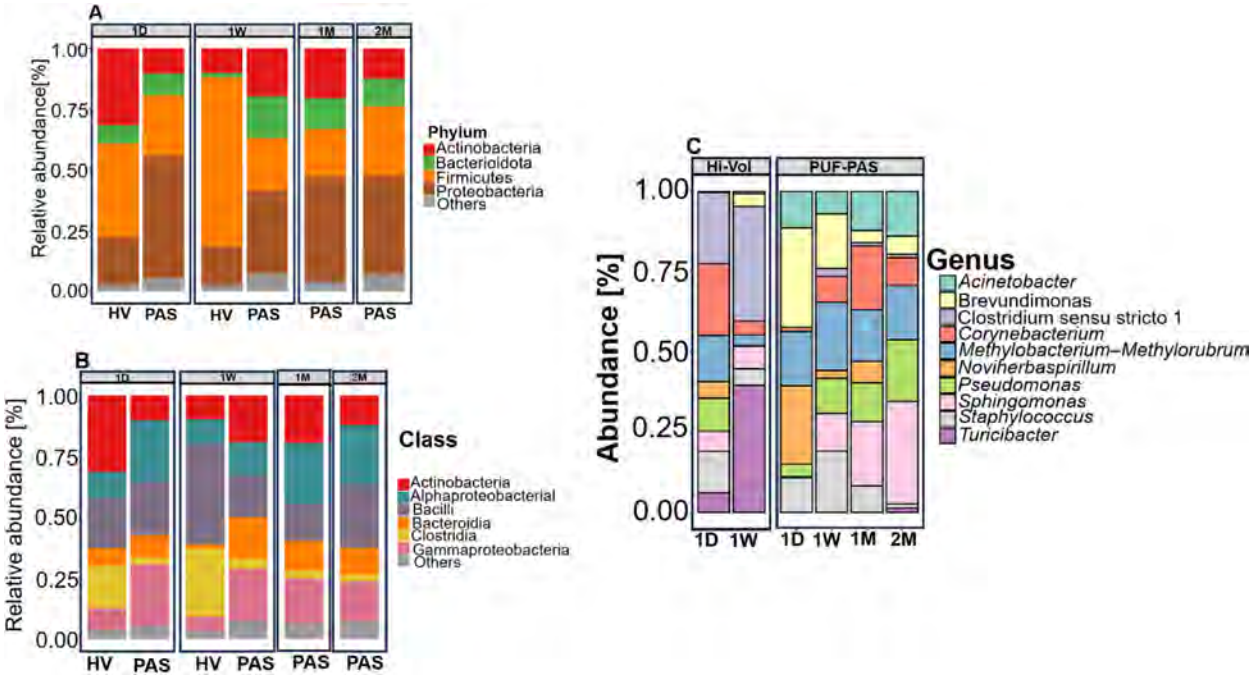

Figure 3. Relative abundance of different bacterial populations showing taxonomic profiles of phyla (A), classes (B), and genera detected in high-volume air samplers (HV) (C) and polyurethane foam disk passive air samplers (PUF−PAS) with different sampling durations (1 Day (1D), 1Week (1W), 1Month (1M) and 2 Months (2M)) showing that the Hi-Vol sampler captured diverse and transient taxa, while persistent bacterial groups dominated PUF−PAS samples. Figure 3C only shows the top 10 most abundant genera.

varied bacterial profile. PUF−PAS samples showed more stable, less variable profiles, especially over 1- and 2-month extended sampling periods. These findings revealed that sampling duration and sampler type influence the observed microbial profile and may be useful complementary methods. Our findings were consistent with a study in Europe that found that both active and passive types identified similar dominant phyla.[10]

The Hi-Vol sampler detected more taxonomic variability between 1D and 1W, including Gammaproteobacteria and Bacilli at the class level (Figure 3B). For example, Clostridia and Bacilli had a strong representation at 1 W, and a more balanced community composition was observed at 1 W, supporting our previous finding of high Shannon diversity. The consistent and stable dominant classes of Actinobacteria, Alphaproteobacteria and Gammaproteobacteria were observed in PUF−PAS samples during short and long sampling durations (Figure 3B). A lower proportion of Clostridia in PUF−PAS samples indicated less transient variability. In contrast, little change between 1 and 2 M in PUF−PAS reflected stability in microbial composition over extended sampling periods (Figure 3B). A temporary shift in microbial composition was observed, showing a successional change with PUF−PAS. In comparison, Hi-Vol was optimal for acute exposure monitoring due to its focused snapshot of dominant bacteria over a short sampling period. For example, the relative abundance of Bacilli declined with an extended sampling period. At the same time, Alphaproteobacteria increased from 1 W to 2M, suggesting that long deployment durations enable or favor a broad range of taxa to adapt, leading to temporal succession or time-integrated accumulation of diverse airborne taxa on the sampler over long deployment periods rather than biological adaptation.[7,37] These observed class shifts with increasing sampling duration in PUF−PAS samples provide evidence that sampling times influence dominant taxa and microbial diversity. These findings support the earlier conclusion that the PUF−PAS is optimal for time-integrated samples over weeks/months and favors the detection of persistent and resilient microbial taxa while under-representing transient or rare bacterial communities. Figure 3C shows that the composition of airborne bacterial communities at the genus level differed markedly between Hi-Vol and PUF−PAS samples across different sampling durations. The high variability of genera detected at 1D and 1 W showed the temporal sensitivity. For example, a balanced mix of genera such as *Corynebacterium*, *Staphylococcus*, *Methylobacterium*, *Romboutsia*, and *Clostridium* was found in 1D samples in HI-Vol samplers while in 1W samples, *Turicibacter*, *Thermoactinomyces*, and *Terrisporobacter* dominated, suggesting the influence of local environment factors. These genera (*Thermoactinomyces* and *Terrisporobacter*) are rarely detected in atmospheric environments, supporting the higher richness and diversity observed in Hi-Vol samplers. Nevertheless, stable core microbiomes such as *Acinetobacter*, *Brevundimonas*, *Corynebacterium*, *Sphingomonas*, *Pseudomonas*, and *Staphylococcus were detected across* 1D to 2 M samples in PUF−PAS (Figure 3D). Interestingly, a greater temporal shift was observed in PUF−PAS than in Hi-Vol samples. Within all sampling durations, *Methylobacterium* and *Methylorubrum* were always present, while *Hymenobacter* appeared more prominent at 2 M (Figure 3D), suggesting a desiccation of airborne-resistant bacterial taxa. Some genera detected in this study (Figure 3) are known to include pathogenic species.

These results reveal the capacity of PUF−PASs for integrating an extensive range of airborne bacteria, especially those with natural, soil and vegetation origins. Hi-Vol and PUF−PASs capture different yet complementary aspects of the airborne microbiome. When the study goal is to offer a short-

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term snapshot focused on the airborne microbial profile, especially for human health risk assessment, Hi-Vol is optimal and essential for understanding small-scale ecological dynamics; however, when the goal is to assess environmental exposure, a PUF−PAS is optimal for accumulating a broader and more balanced community with both low- and high-abundance environmental bacteria, critical for cumulative exposure tracking. When designing an air microbiome study, PUF−PAS is the preferred choice because of the observed temporal shift in microbial composition from short to long sampling durations.

The significant variability observed in Hi-Vol supports our finding that Hi-Vol is optimal for detecting taxonomic richness and community fluctuation. In contrast, moderate and broad signals were found for genera such as *Pseudomonas* and *Geodermatophilus* (Table S1); these dominant genera can withstand desiccation, UV exposure and other atmospheric stresses due to spore formation that allows them to remain detectable in ambient air over extended sampling periods. The overall findings observed in this study (Table S1) confirmed that Hi-Vol detected transient spikes in community composition, detecting episodic microbial events associated with rain, traffic and local emissions, while PUF−PAS samples reflected stable and time-integrated bacteria. The lack of a strong peak in PUF−PAS samples confirmed its tendency to underestimate rare taxa and favor the dominant group over extended periods. Due to the high volume of air collected by Hi-Vol samplers, this sampler captures more low-abundance taxa, while PUF−PAS tends to accumulate dominant taxa while underrepresenting rare groups.

Over two months of long-term sampling, we observed unique microbial fingerprints originating from soil, water, and plant-associated genera. These bacteria were not observed in the short-duration samples from either Hi-Vol or PUF−PAS, demonstrating the value of including longer time-integrated passive sampling, suggesting that the PUF−PAS exhibited temporal microbial succession, and diversity increases tended to accumulate over time. Our recent work in the same region indicates that geographic variation accounts for more variation in bioaerosol sources than short-term seasonal or meteorological fluctuations.[7]

These results were also confirmed by PCoA analysis of samplers and durations when all samples were combined (Figure 4). Hi-Vol samples showed a tight clustering toward the left side for 1D and 1W and low variability within the group, suggesting that bacterial communities were similar across short-term samples and that Hi-Vol is likely to detect a distinct, consistent subset of the bacterial community. PUF−PAS samples were widely spread toward the right side, especially for 1 and 2 M samples. This observed variability in community composition across long sampling periods indicated the power of PUF−PASs for collecting time-integrated and compositionally distinct microbial populations, suggesting that sampler type has a substantial influence on the detection of taxa composition and diversity, supporting our previous observations that both duration and sampler type influence bacterial composition. We observed a temporal gradient within PUF−PAS samples, and this was demonstrated by a clear separation by duration, where samples at 1 and 2 M were compositionally distinct from 1D and 1W, reflecting community shifts over time and accumulation of slow-deposition taxa. Due to the nature of PUF−PAS, we observed variation within 1D, 1W, and 1M, reflecting its sensitivity to

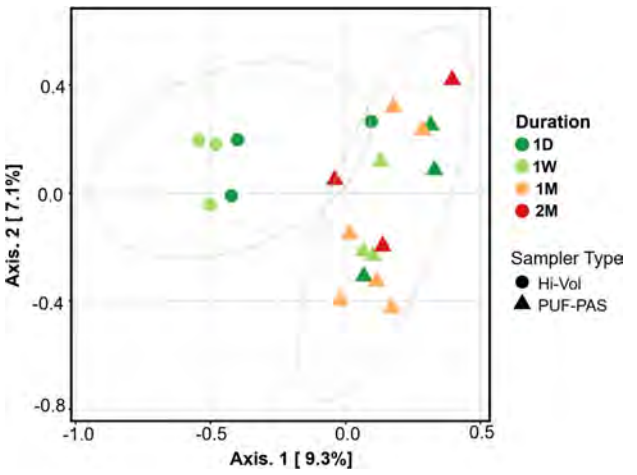

Figure 4. Principal coordinates analysis (PCoA) of the to Hellinger distances of bacterial communities colored by sampling duration and shaped by sampler type (B), where Hi-Vol samples cluster close to each other (short-term events), while PUF−PAS samples disperse along a time-integrated gradient.

short-term fluctuations compared with the pumped active Hi-Vol. These differences do not suggest that microbial growth on the sampler, as PUF−PAS is not designed to support proliferation, but rather indicate saturation over time instead of biological activity. Overall, PUF−PASs appear to be more sensitive to change with sampling duration, while Hi-Vol provides a snapshot and does not vary much within a short-duration sampling period.

Differential Abundance

We applied the log-linear model of the Analysis of Composition of Microbiomes with Bias Correction (ANCOM-BC) to compare the bacterial communities detected by Hi-Vol and PUF−PAS. The ANCOM-BC can differentiate between taxa and adjust for compositional bias. The W-statistic indicates the effect size, showing how often a community is detected as differentially abundant in pairwise comparisons. A positive value showed higher abundance in Hi-Vol samplers, and a negative W indicated higher abundance in PUF−PAS samples. In parallel, we used the natural log-transformed ratio, which reports log fold change (LFC), showing taxa abundance in Hi-Vol relative to PUF−PAS samples: a positive LFC value indicated enrichment in Hi-Vol samples and a negative value indicated enrichment in PUF−PAS samples. Our results (Figure 5 and Table S2) also revealed differences in the microbial composition between sampler types. For example, genera indicating human influence and urban environments, such as *Acinetobacter*, *Bacteroides*, *Ralstonia*, and *Deinococcus*, were more abundant in Hi-Vol. These genera are primarily detected in urban aerosols. They are likely to be detected more effectively using Hi-Vol samplers due to the high air intake during a short sampling period. In contrast, spore-forming, aerobic and environmental taxa were enriched in PUF−PAS samples, including *Turicibacter*, *Clostridium*, and *Thermoactinomyces*, suggesting their enrichment in long-term sampling durations reflects cumulative settling and resistance to desiccation and environmental exposure. At the same time, LFC quantified the magnitude and direction of abundant bacterial genera. For instance, *Acinetobacter* (W = 3.94. LCF = 5.20) was substantially more abundant in Hi-Vol samples, while *Thermoactinomyces* (W-

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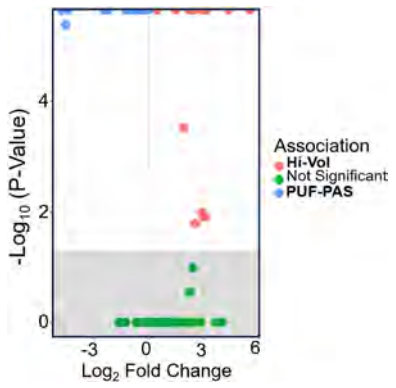

Figure 5. A volcano plot comparing the differential abundance of bacterial taxa between Hi-Vol and PUF−PAS samplers. Taxa enriched in Hi-Vol samples appear on the right (red), while taxa enriched in PUF−PAS samples appear on the left (blue). Green points indicate taxa with no statistically significant difference between samplers.

60.6, LFC = −5.07) was substantially enriched in PUF−PAS samples. These findings confirm our previous observation that PUF−PAS and Hi-Vol complement each other, and we believe that understanding these differences is significant for designing future bioaerosol monitoring campaigns, especially for studies comparing microbial diversity across multiple environments or evaluating exposure risk. However, a previous study indicated that a passive method was preferable to an active one for assessing bacterial and fungal air contamination in operating theaters because such systems are noiseless, low-cost, electricity-free, and require no maintenance.[13] The proper selection of sampling duration and frequency is essential for successful bioaerosol sampling campaigns.[11] The volcano plot (Figure 5, Table S2) shows that only a small subset of taxa exhibited significant sampler-specific enrichment, with most taxa showing no strong differential association between Hi-Vol (*Acinetobacter, Bacteroides, Alcaligenes, Blastomonas, Bradyrhizobium*) and PUF−PAS (*Turicibacter, Clostridium, Thermoactionomyces, Saccharopolyspora, Romboutsia*). This indicates that neither sampler systematically biases the community structure, and that the two methods are primarily complementary. Hi-Vol provides greater sensitivity for low-abundance taxa because of its large volume, while PUF−PAS offers time-integrated collection that captures dominant and episodic taxa. Overall, the results support the use of both samplers as complementary tools for analyzing airborne microbial diversity.

CONCLUSIONS

This is the first study to quantitatively compare Hi-Vol and PUF−PAS air samplers across time scales and link them to taxonomic shifts and qPCR-based load estimates. Hi-Vol and PUF−PAS samplers offer distinct and complementary perspectives on airborne microbial composition and diversity, and the results inform best practices for bioaerosol monitoring using different sampler types and durations, highlighting fundamental trade-offs in bioaerosol sampling strategies and methods.[18,38]

Hi-Vol consistently detected more diverse microbial populations, but over long sampling periods, PUF−PAS samples yielded a significantly greater amount of bacterial biomass. Overall, the results indicated that biomass yield and microbial diversity are decoupled, and that Hi-Vol and PUF−PAS are complementary approaches for assessing the aerobiome. When normalized by air volume, Hi-Vol was more efficient per cubic meter of air. PUF−PAS samples tended to underestimate concentrations during short-term deployment, but showed increasing richness and taxonomic diversity over time, suggesting cumulative microbial accumulation. To capture transient microbial signatures, Hi-Vol offers the high resolution necessary for evaluating exposure in a dynamic urban environment, despite the operational costs, high maintenance requirements and daily logistical support that limit wide-scale, long-term deployment.[18] The PUF−PAS is suitable for long-term monitoring, is cost-effective, easy to use, and can be used in both short and long-term sampling. Our findings align with previous work showing that active samplers detect transient airborne microbiota,[39,40] while passive methods yield cumulative community profiles.[41]

When the air volume was normalized for PUF−PAS and Hi-Vol, the 1D samples had the highest amplifiable levels of the 16s RNA gene, reflecting the number of live or dead bacteria with intact DNA. Hi-Vol was better at detecting short-lived taxa. However, PUF−PAS was better for retaining the detectable DNA of microbial populations, confirming that these differences are not driven by sampling volume alone but reflect fundamental distinctions in microbial capture efficiency. Previous work has shown that the Hi-Vol sampler and PUF−PAS samples capture nearly the same PM size and distribution.[15]

The results support the development of duration-specific performance models and suggest the complementary use of both active and passive sampler strategies depending on the research goal. For example, combining short and long sampling durations for both Hi-Vol and PUF−PASs reinforced the importance of detecting potentially pathogenic airborne bacteria for integrating air microbiome surveillance with public health assessment, revealing the need for strategic sampler choice based on surveillance goal.[12] Although the identification and discussion of pathogenic bacteria was outside the scope of this study, our screening of potential genera detected in the PUF−PAS and Hi-Vol samplers provides important information about the need for strategic sampler choice selection to assess the potential health risk of bioaerosols. In our study, several potentially pathogenic genera were detected with both sampler types and all durations. During sampling periods of 1D and 1W, some bacteria, such as *Staphylococcus* and *Enterococcus*, were detected in both Hi-Vol and PUF−PAS, although Hi-Vol showed higher relative abundance. In contrast, genera such as *Corynebacterium* and *Lawsonella* (opportunistic pathogens) were detected more in longer-duration PUF−PAS sampling.

Our study provides key strategies for using active and passive air samplers in airborne microbial surveillance.

Our research offers essential sampling methodologies for using PUF-passive and Hi-Vol active air samplers in bioaerosol monitoring. Sampling duration significantly affects microbial diversity and community structure. These changes may reflect actual temporal variation and sampling strategies. Additional studies have shown that sampling duration influences the effectiveness of bioaerosol samplers.[42,43] These results highlight the importance of considering sampling time when designing passive monitoring programs.

STUDY LIMITATIONS

The study was constrained by the minimal sample size per group and the use of a single sampling site. Because of the

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elevated flow rate of Hi-Vol, we could not obtain samples for 1 month and 2 months. Lower-volume active sampling could be explored for longer-term comparison with PUF−PAS sampling. Sample storage parameters such as storage, duration and temperature should also be assessed for impact on environmental DNA (eDNA) viability/detection. Integrating PUF−PAS sampling with molecular techniques has significant potential for extensive and cost-effective air quality assessment.

Although we examined taxa that include potentially pathogenic members, the 16S rRNA V4 region provides limited taxonomic resolution and does not reliably enable species-level identification. Consequently, genus-level assignment should be interpreted with caution, and we have deliberately avoided attributing specific pathogenic traits to species or strains. Some genera detected are known to contain pathogenic species, but the V4 region alone cannot distinguish pathogenic potential, which remains speculative. Future work with larger sample sizes and more comprehensive sequencing approaches could incorporate particle-size-resolved bioaerosol sampling or direct measurement using flow cytometry to further address the influence of particle size on sampling and quantification of airborne microorganisms.

■ ASSOCIATED CONTENT

Data Availability Statement

The sequencing reads were deposited in the EMBL-EBI European Nucleotide Archive (ENA) under study accession number: **PRJEB95104**.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestair.5c00336.

Additional materials include site map, an estimated bacterial cell concentration per m^3, the top bacteria genera detected in each sampler and the differential abundance of bacteria between the Hi-Vol active air sampler and PUF passive air sampler (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Stetzenbach, L. D. Airborne Infectious Microorganisms. *Encycl. Microbiol.* **2009**, 175−182.

(2) Ghosal, D.; Ghosh, S.; Dutta, T. K.; Ahn, Y. Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *Front. Microbiol.* **2016**, *7*, 7.

(3) Nzila, A.; Musa, M. M. Current Status of and Future Perspectives in Bacterial Degradation of Benzo[a]Pyrene. *Int. J. Environ. Res. Public Health* **2021**, *18* (1), 262.

(4) Lladó, S.; Jiménez, N.; Viñas, M.; Solanas, A. M. Microbial Populations Related to PAH Biodegradation in an Aged Biostimulated Creosote-Contaminated Soil. *Biodegradation* **2009**, *20* (5), 593−601.

(5) Kim, K.-H.; Kabir, E.; Jahan, S. A. Airborne Bioaerosols and Their Impact on Human Health. *J. Environ. Sci.* **2018**, *67*, 23−35.

(6) Srikanth, P.; Sudharsanam, S.; Steinberg, R. Bio-Aerosols in Indoor Environment: Composition, Health Effects and Analysis. *Indian J. Med. Microbiol.* **2008**, *26* (4), 302−312.

(7) Kalisa, E.; Saini, A.; Lee, K.; Mastin, J.; Schuster, J. K.; Harner, T. Capturing the Aerobiome: Application of Polyurethane Foam Disk Passive Samplers for Bioaerosol Monitoring. *ACS EST Air* **2024**, *1* (5), 414−425.

(8) Kalisa, E.; Kuuire, V.; Adams, M. A Preliminary Investigation Comparing High-Volume and Low-Volume Air Samplers for Measurement of PAHs, NPAHs and Airborne Bacterial Communities in Atmospheric Particulate Matter. *Environ. Sci.: Atmos.* **2022**, *2* (5), 1120−1131.

(9) Kalisa, E.; Archer, S.; Nagato, E.; Bizuru, E.; Lee, K.; Tang, N.; Pointing, S.; Hayakawa, K.; Lacap-Bugler, D. Chemical and Biological Components of Urban Aerosols in Africa: Current Status and Knowledge Gaps. *Int. J. Environ. Res. Public Health* **2019**, *16* (6), 941.

(10) Dillon, K. P.; Tignat-Perrier, R.; Joly, M.; Grogan, S. N. C. M.; Larose, C.; Amato, P.; Mainelis, G. Comparison of Airborne Bacterial Populations Determined by Passive and Active Air Sampling at Puy de Dôme, France. *Aerosol Air Qual. Res.* **2023**, *23*, 220403.

(11) Mainelis, G. Bioaerosol Sampling: Classical Approaches, Advances, and Perspectives. *Aerosol Sci. Technol.* **2020**, *54* (5), 496−519.

(12) Balyan, P.; Ghosh, C.; Das, S.; Banerjee, B. D. Comparison of Efficiency of Active and Passive Methods of Bioaerosols' Estimation. In *Indoor Environmental Quality*, Sharma, A.; Goyal, R.; Mittal, R., Eds.; Springer: Singapore, 2020; pp. 85−94. DOI:

(13) Karigoudar, R.; Wavare, S.; Kakhandki, L.; Bagali, S.; Kumar, I. Comparison of Active and Passive Methods of Air Sampling to Evaluate the Microbial Contamination of Air in Operation Theaters. *J. Pure Appl. Microbiol.* **2020**, *14*, 2691−2697.

(14) Yamamoto, N.; Schmechel, D.; Chen, B. T.; Lindsley, W. G.; Peccia, J. Comparison of Quantitative Airborne Fungi Measurements by Active and Passive Sampling Methods. *J. Aerosol Sci.* **2011**, *42* (8), 499−507.

(15) Markovic, M. Z.; Prokop, S.; Staebler, R. M.; Liggio, J.; Harner, T. Evaluation of the Particle Infiltration Efficiency of Three Passive Samplers and the PS-1 Active Air Sampler. *Atmos. Environ.* **2015**, *112*, 289−293.

(16) Herkert, N. J.; Hornbuckle, K. C. Effects of Room Airflow on Accurate Determination of PUF-PAS Sampling Rates in the Indoor Environment. *Environ. Sci.: Process Impacts* **2018**, *20* (5), 757−766.
(17) Harner, T.; Mitrovic, M.; Ahrens, L.; Schuster, J. Characterization of PUF Disk Passive Air Samplers for New Priority Chemicals: A Review. *Organohalogen Compd.* **2014**, *76*, 442−445.
(18) Manibusan, S.; Mainelis, G. Passive Bioaerosol Samplers: A Complementary Tool for Bioaerosol Research. A Review. *J. Aerosol Sci.* **2022**, *163*, 105992.
(19) Saini, A.; Harner, T.; Chinnadhurai, S.; Schuster, J. K.; Yates, A.; Sweetman, A.; Aristizabal-Zuluaga, B. H.; Jiménez, B.; Manzano, C. A.; Gaga, E. O.; Stevenson, G.; Falandysz, J.; Ma, J.; Miglioranza, K. S. B.; Kannan, K.; Tominaga, M.; Jariyasopit, N.; Rojas, N. Y.; Amador-Muñoz, O.; Sinha, R.; Alani, R.; Suresh, R.; Nishino, T.; Shoeib, T. GAPS-Megacities: A New Global Platform for Investigating Persistent Organic Pollutants and Chemicals of Emerging Concern in Urban Air. *Environ. Pollut.* **2020**, *267*, 115416.
(20) Kalisa, E.; Nagato, E.; Bizuru, E.; Lee, K.; Tang, N.; Pointing, S.; Hayakawa, K.; Archer, S.; Lacap-Bugler, D. Pollution Characteristics and Risk Assessment of Ambient PM2. 5-Bound PAHs and NPAHs in Typical Japanese and New Zealand Cities and Rural Sites. *Atmos. Pollut. Res.* **2019**, *10* (5), 1396−1403.
(21) Kalisa, E.; Nagato, E. G.; Bizuru, E.; Lee, K. C.; Tang, N.; Pointing, S. B.; Hayakawa, K.; Archer, S. D.; Lacap-Bugler, D. C. Characterization and Risk Assessment of Atmospheric $PM_{2.5}$ and PM_{10} Particulate-Bound PAHs and NPAHs in Rwanda, Central-East Africa. *Environ. Sci. Technol.* **2018**, *52* (21), 12179−12187.
(22) Thijs, S.; Op De Beeck, M.; Beckers, B.; Truyens, S.; Stevens, V.; Van Hamme, J. D.; Weyens, N.; Vangronsveld, J. Comparative Evaluation of Four Bacteria-Specific Primer Pairs for 16S rRNA Gene Surveys. *Front. Microbiol.* **2017**, *8*, 494.
(23) Zemb, O.; Achard, C. S.; Hamelin, J.; De Almeida, M.; Gabinaud, B.; Cauquil, L.; Verschuren, L. M. G.; Godon, J. Absolute Quantitation of Microbes Using 16S rRNA Gene Metabarcoding: A Rapid Normalization of Relative Abundances by Quantitative PCR Targeting a 16S rRNA Gene Spike-in Standard. *MicrobiologyOpen* **2020**, *9* (3), No. e977.
(24) Suzuki, M. T.; Taylor, L. T.; DeLong, E. F. Quantitative Analysis of Small-Subunit rRNA Genes in Mixed Microbial Populations via 5′-Nuclease Assays. *Appl. Environ. Microbiol.* **2000**, *66* (11), 4605−4614.
(25) Nadkarni, M. A.; Martin, F. E.; Jacques, N. A.; Hunter, N. Determination of Bacterial Load by Real-Time PCR Using a Broad-Range (Universal) Probe and Primers Set. *Microbiology* **2002**, *148* (1), 257−266.
(26) Hospodsky, D.; Yamamoto, N.; Peccia, J. A. Precision, and Method Detection Limits of Quantitative PCR for Airborne Bacteria and Fungi. *Appl. Environ. Microbiol.* **2010**, *76* (21), 7004−7012.
(27) Martin, M. Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads. *EMBnet. J.* **2011**, *17* (1), 10−12.
(28) Callahan, B. J.; McMurdie, P. J.; Rosen, M. J.; Han, A. W.; Johnson, A. J. A.; Holmes, S. P. DADA2: High-Resolution Sample Inference from Illumina Amplicon Data. *Nat. Methods* **2016**, *13* (7), 581−583.
(29) Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F. O. The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Res.* **2012**, *41* (D1), D590−D596.
(30) Abellan-Schneyder, I.; Matchado, M. S.; Reitmeier, S.; Sommer, A.; Sewald, Z.; Baumbach, J.; List, M.; Neuhaus, K. P. Pipelines, Parameters: Issues in 16S rRNA Gene Sequencing. *mSphere* **2021**, *6* (1), 10−1128.
(31) Davis, N. M.; Proctor, D. M.; Holmes, S. P.; Relman, D. A.; Callahan, B. J. Simple Statistical Identification and Removal of Contaminant Sequences in Marker-Gene and Metagenomics Data. *Microbiome* **2018**, *6* (1), 226.
(32) McMurdie, P. J.; Holmes, S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* **2013**, *8* (4), No. e61217.
(33) Wickham, H. *Ggplot2: elegant Graphics for Data Analysis*; Springer: New York, 2009.
(34) Lin, H.; Peddada, S. D. Analysis of Compositions of Microbiomes with Bias Correction. *Nat. Commun.* **2020**, *11* (1), 3514.
(35) Anderson, M. J.; Ellingsen, K. E.; McArdle, B. H. Multivariate Dispersion as a Measure of Beta Diversity. *Ecol. Lett.* **2006**, *9* (6), 683−693.
(36) Oksanen, J.; Blanchet, F. G.; Kindt, R.; Legendre, P.; Minchin, P. R.; O'Hara, B.; Simpson, G. L.; Solymos, P.; Stevens, H.; Wagner, H. H.*vegan" Title Community Ecology PackageCommunity Ecol. Package R Package Version*MASS2016
(37) Harner, T.; Saini, A.; Shahpoury, P.; Eng, A.; Schuster, J.; Kalisa, E.; Mastin, J. Cross-Cutting Research and Future Directions under the GAPS Networks. *Environ. Sci. Adv.* **2024**, *3* (6), 798−807.
(38) Durand, K. T. H.; Muilenberg, M. L.; Burge, H. A.; Seixas, N. S. Effect of Sampling Time on the Culturability of Airborne Fungi and Bacteria Sampled by Filtration. *Ann. Occup. Hyg.* **2002**, *46* (1), 113−118.
(39) Gandolfi, I.; Bertolini, V.; Ambrosini, R.; Bestetti, G.; Franzetti, A. Unravelling the Bacterial Diversity in the Atmosphere. *Appl. Microbiol. Biotechnol.* **2013**, *97* (11), 4727−4736.
(40) Yooseph, S.; Andrews-Pfannkoch, C.; Tenney, A.; McQuaid, J.; Williamson, S.; Thiagarajan, M.; Brami, D.; Zeigler-Allen, L.; Hoffman, J.; Goll, J. B.; Fadrosh, D.; Glass, J.; Adams, M. D.; Friedman, R.; Venter, J. C. A Metagenomic Framework for the Study of Airborne Microbial Communities. *PLoS One* **2013**, *8* (12), No. e81862.
(41) Mahnert, A.; Vaishampayan, P.; Probst, A. J.; Auerbach, A.; Moissl-Eichinger, C.; Venkateswaran, K.; Berg, G. Cleanroom Maintenance Significantly Reduces Abundance but Not Diversity of Indoor Microbiomes. *PLoS One* **2015**, *10* (8), No. e0134848.
(42) Haig, C. W.; Mackay, W. G.; Walker, J. T.; Williams, C. Bioaerosol Sampling: Sampling Mechanisms, Bioefficiency and Field Studies. *J. Hosp. Infect.* **2016**, *93* (3), 242−255.
(43) Mescioglu, E.; Paytan, A.; Mitchell, B. W.; Griffin, D. W. Efficiency of Bioaerosol Samplers: A Comparison Study. *Aerobiologia* **2021**, *37* (3), 447−459.

https://doi.org/10.1021/acsestair.5c00336
ACS EST Air 2026, 3, 710−718