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Capturing the Aerobiome: Application of Polyurethane Foam Disk Passive Samplers for Bioaerosol Monitoring

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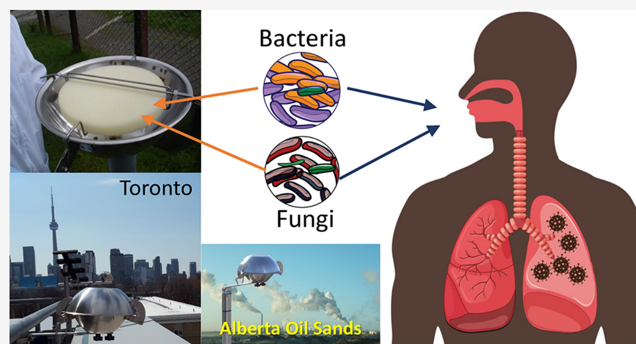
ABSTRACT: Bioaerosols are ubiquitous and play a significant role in global climate and human health due to inhalation exposure. Passive air sampling of bioaerosols, as a complementary method to active sampling using pumps, is increasingly valued due to its simplicity, electricity-free operations, and cost-effectiveness in providing time-integrated samples over weeks/months. In this study, polyurethane foam disk passive air samplers (PUF-PAS), passive dry deposition air samplers (PAS-DDs), and active high-volume (Hi-Vol) air samplers were deployed in Toronto and the Athabasca oil sands region (OSR) in the first stages of a proof-of-concept exercise for bioaerosols. Airborne bacterial and fungal communities were characterized using MiSeq DNA sequencing. All sampler types were shown to successfully collect bioaerosols. The dominant bacterial and fungal phyla observed by all samplers were qualitatively similar. Species richness and community structure of the airborne bacterial and fungal communities varied with sites and seasons. Principal coordination analysis indicated that bacterial and fungal communities differed between Toronto and OSR. Further work is required to calibrate and characterize the uptake of PUF-PAS and PAS-DD for bioaerosols to derive quantitative information on their abundance to better assess sources and potential exposure risks.



KEYWORDS: *bioaerosol, bacteria, fungi, polyurethane foam disk (PUF disk), passive air samplers*

INTRODUCTION

Bioaerosols are ubiquitous in the ambient air and significantly affect the global climate, urban visibility, and human health. They represent 15 to 25% of particulate matter (PM) by mass.[1−3] Bioaerosols include viruses, bacteria, and fungi and range in size from 0.003 to 30 μm in diameter.[4−7] The sources of bioaerosols influence their physical properties (size, surface area, and density).[2,8] The atmosphere is considered a habitat for bioaerosols originating from the soil, water/seawater, oil sands, vegetation, animals, and humans. Bioaerosols contain different species, some of which have adverse health effects on humans.[2] For example, some bacterial genera (*Acinetobacter, Bacillus, Staphylococcus, Corynebacterium*) and fungi (*Cladosporium, Aspergillus, Penicillium, Alternaria*) are known as human pathogens and have been associated with respiratory tract allergies.[2,4,6,9−12]

Bioaerosols vary substantially according to time, location, seasons, and climate, resulting in spatial and temporal variations in characteristics, concentration, and toxicity.[1−4] Studies have indicated that the diversity and concentration of bioaerosols in the atmosphere depends on different environmental factors (wind speed and wind direction, temperature, and humidity) and physical properties of aerosols (size, density, and shape)[5] and land use type.[6] Temperature and relative air humidity (RH) were reported to influence the concentrations of airborne microorganisms,[7,8] and a positive correlation was found between RH and bacteria and fungi.[9] Furthermore, the variation of airborne microorganisms is influenced by the origins of air currents in downwind locations.[2] Sandstorms were observed to increase the concentrations of cultural and total microbes in the bioaerosols in the Qingdao coastal region.[10] Many species of airborne bacteria were observed at high altitudes (800 m), and those species vary by the direction and interaction of free tropospheric winds.[11] The concentration of both indoor and outdoor airborne microorganisms varies between seasonal shifts in atmospheric conditions and location.[12] The mean bacterial abundance was lower in the winter than in other seasons,[13] whereas levels of airborne microorganisms were higher during the day than at night.[14]

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The Public Health Agency of Canada established a national safety and security program to protect the health and safety of the public against risks posed by bioaerosol pathogenic bacteria (*Mycobacterium spp*, *Pseudomonas spp*, etc.) and fungi (*Aspergillus spp*, *Cryptococcus spp*), classified as Risk Group 2 Human Pathogens.[13] Recent studies also showed that bioaerosols are an essential mode of transmission of COVID-19 and, thus, a public health concern.[14−16] A recent report by the United Nations Environment Programme also highlighted bioaerosol monitoring as an essential component of "*One Health*" integrated surveillance for antimicrobial resistance to complement traditional public health surveillance systems.[17] Another recent study conducted in the UK indicated that air pollution could be used to gather vital genetic data to track changes in the environment and biodiversity at a continental scale using environmental DNA.[18] However, there are also potential positive benefits of bioaerosol exposure. For example, recent studies indicated that exposure to aerobiomes can support healthy human immune function and reduce blood pressure.[19,20]

In response to concerns about the toxicity and health effects associated with inhaling microorganisms, advances have been made in recent decades in the sampling and characterization of bioaerosols in ambient air. Air sampling on filters is an essential tool in the collection of bioaerosols.[1,2,6,21,22] DNA extraction of filters from high-volume (Hi-Vol) air samples (active sampling) followed by downstream analysis using high-throughput DNA sequencing is the favored method to characterize bioaerosols.[23,24] The advantage of this method is that it collects enough air to perform several consecutive analyses: various chemical and biological components can be performed offline on the same sample. However, sampling using active samplers is costly and often represents a "grab sample" over hours or days, i.e., it is typically not integrated over extended periods (e.g., weeks/months). Numerous consecutive Hi-Vol samples would need to be collected to characterize ambient air over more extended periods, which would further add to the cost and complexity of the study. Active samplers require maintenance, electricity, can be noisy, and are not designed for continuous, unattended sampling over several days. It requires frequent replacement of collection media and skilled personnel to perform calibrations.[25,26]

Technological developments have shown the utility of passive sampling as a complementary approach to active sampling methods (using pumped air and filtration) for bioaerosol sampling.[27] Passive samplers such as Petri dishes, agar settle plates, dust fall collectors, and Personal Aeroallergen Samplers are most commonly used for bioaerosol sampling in ambient and indoor environments.[28,29] These tools are inexpensive, simple, easy to operate, and can continuously sample for days, weeks, and months. Several studies have compared passive samplers to active sampling for bioaerosol monitoring,[25,26,30] and extensive reviews have been published.[29] Correlation between passive and active samplers was observed in microbial diversity and composition.[31] In addition, Angel et al.[14] indicated that passive samplers are more useful for investigating personal exposure to airborne respiratory viruses than active samplers. These passive samplers, such as polydimethylsiloxane, are less intrusive and can provide a time-integrated sample for the early detection of potential cases and the direction of the specific infection site control protocols that forestall community transmission.[14]

Studies using passive air samplers in bioaerosol monitoring have used culture-dependent methods.[32] However, this method has many limitations; only viable and culturable microorganisms can be identified. In contrast, culture-independent methods (such as high-throughput DNA sequencing) have been used with active bioaerosol samplers in Hong Kong,[33] Colorado, U.S.A.[22] and New Haven, U.S.A.[34] Culture-independent methods reflect the diversity of viable microbes and can be applied to any biological sample containing nucleic acid.[35,36]

Polyurethane foam (PUF) disk passive air samplers (PUF-PASs) and passive dry deposition air samplers (PAS-DDs),[37] have mainly been used to assess organic pollutants, trace metals, and pesticides in ambient air.[38−40] These samplers have been used in many areas, such as remote areas to address air monitoring needs and where active air sampler installation was not feasible. These samplers have also been used in cities such as Toronto, international monitoring programs such as the Global Atmospheric Passive Sampling (GAPS) and GAPS-Megacities Networks and the Oil Sands Monitoring (OSM) program in Alberta, Canada.[40,41]

Studies are yet to comprehensively assess PUF disk samplers for bioaerosol collection. The present study used next-generation DNA sequencing to evaluate the diversity and microbial composition (bacteria, fungi) of samples collected by PUF-PASs and PAS-DDs from archived air samples. These samples represent different land-use types in the Athabasca oil sands region (OSR), where open pit mining activities contribute significant amounts of soil dust to ambient air. PUF-PAS samples from a variety of site types in Toronto were also included and a filter-based Hi-Vol active air sampler was included at one site in Toronto, as a reference.

Our study aimed to begin a proof-of-concept that PUF-disk passive samplers are a simple and cost-effective approach to characterize long-term bioaerosol diversity and compositional trends in bioaerosols at high spatial resolution. Understanding the sources and their associated microbial compositions can provide insight into exposure and possible mitigation strategies to control the effects of toxic chemical compounds and potential microbial allergens and pathogens on human health.

■ MATERIALS AND METHODS

Samplers and Sampling Sites. In this study, PUF-PASs were deployed in four and three sites in the Athabasca oil sands region (OSR) and Toronto, respectively, which were subject to different emission sources. PAS-DDs were co-deployed at 2 of the four sites in OSR. Schematic descriptions of PUF−PAS and PAS-DD are shown in the Figure S1, and details of sampling sites are given in Table S1 of the Supporting Information (SI). The OSR is in the Northeast of Alberta, and fugitive dust from mining and oil sands transportation processes significantly contributes to air pollutants in the OSR's atmosphere.[42] The sites in OSR were selected based on an existing network of air sampling sites operated by the Wood Buffalo Environmental Association (WBEA) in OSR and sites in Toronto were part of existing air pollution monitoring sites operated by the ATOUSA (Assessing Toxicity of Organics in Urban Sectors for Air) program.[41]

The sites in OSR included a community site (AMS6) in the town of Fort McMurray (population of 68000), two of the enhanced deposition sites (AMS13 and AMS11), which are within 3 and 5 km, respectively, of open-pit mining operations,

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and a background forest site (JP104), which is further away from open-pit mining operations. The areas in Toronto ranged from a traffic site adjacent to the 16-lane Highway 401 (MOE) to urban/traffic land-use in Downtown Toronto (WB-Wallberg Building), a residential site (NY-North York) and an Urban background site (DV-Downsview) characterized as heavy traffic, light traffic, industry, park and residential. The historical weather data (temperature and RH) across all sampling sites in OSR and Toronto were retrieved from the Government of Canada and the WBEA weather stations. The mean temperature across all sampling periods were between −4.0°C and 14.0°C in Toronto and −13.0°C and 19.0°C in OSR while the mean RH ranged from 69 to 88% in Toronto and 63 to 80% in the OSR (Table S1 of the SI).

Previous studies have described PUF-PAS and PAS-DD devices[37] including sample preparation information.[40] Briefly, a PAS-DD comprises two parallel flat plates (cover plate and open plate) and collects the larger depositing particles as well as ambient particles and gases (Figure S1 of the SI). PUF-PAS collects gas and particle species (up to about 5 μm) in ambient air, including those that can be inhaled and with similar particle size distribution as the PS-1 Hi-Vol sampler used in this study,[43] while for the PAS-DD, the particle sampling range is extended beyond 10 μm to include larger particles that deposit from the air to terrestrial surfaces.[44] Both the PUF-PAS and PAS-DD samplers are useful and applied to studies of chemical pollution in ambient air (PUF-PAS) and its dry deposition to surfaces (PAS-DD). The porous nature of the PUF disk substrate ensures that deposited particles are retained and not lost during sample deployment.[37] The PUF disk passive air samplers (PUF-PAS) exhibit an average air sampling rate of ∼4 m^3/day.[45,46]

Sample Collection. PUF disks (14 cm diameter × 1.35 cm thick, surface area 365 cm^2, mass 4.40 g, volume 207 cm^3) were exposed for consecutive 2-month periods in the OSR and Toronto sites, approximately 2 m or higher above the ground. PUF disks were housed horizontally inside precleaned stainless steel chambers (PUF-PAS, Tisch Environmental, TE-200-PAS) and between the parallel plates of the PAS-DD (Tisch Environmental, TE-PAS-DD) without any enclosure. PUF-PAS and PAS-DDs samplers were deployed in OSR in 2017, and PUF-PAS samplers were deployed in Toronto in 2019, spanning all four seasons. Table S1 of the SI details each site characteristic, season, and sample−start and end time for the deployment and total volume collected. Field blanks (n = 2) were also collected along with the samples in the OSR and Toronto, by exposing PUF disks to air for 1 min.[47] As part of the PUF disk reference, Hi-Vol active air samplers were also deployed in Toronto at one site (Downsview), and samples (n = 2) were collected for 24 h (from 10:00 am to 10:00 am of the following day) on 47 mm filters. A Hi-Vol blank (n = 1) filter was collected the same way as PUF disk blank samples by exposing them to air and installing them in the sampling device for 1 min[47] without switching on the air pump. All Hi-Vol filters used in this study were preheated for 240 min before deployment to remove organics contaminants.[21] All samples were stored in the fridge at 4 °C immediately after field sampling. All PUF samples were stored in precleaned, solvent-rinsed glass jars. Filter samplers were wrapped in aluminum foil, stored in a sterilized sealed plastic bag.[48] All PUF disks and Hi-Vol samples were packed in boxes and shipped immediately to Molecular Research LP at MR DNA (Shallowater, TX, U.S.A.) for sequencing following FEDEX shipping sample protocols.

Analysis of Microbial Community Structures. DNA from both PUF disk and glass fiber filters (GFF) were extracted using the same method. Genomic DNA was isolated from all samples using the DNeasy PowerMax Soil Kit (Qiagen) following the manufacturer's instructions and adapting the soil method for the analysis of PUF disk segments. In this study, PUF disk samples were divided into six equal parts and each segment was extracted separately. All six extracts were then combined and added to the PowerMax Bead Tube to undergo cell lysis. One extraction per Hi-Vol filter sample was also performed. The purified DNA was eluted from a Maxi Spin Column in 5 mL of solution C6 and stored at -20°C until Polymerase Chain Reaction (PCR) amplification. The GFF filter samples were extracted following the protocol for the PUF disk samples. All PUF and filter blanks were treated the same way as actual samples. Figure S2 of the SI summarizes a schematic description of DNA extraction from PUF-disks and Hi-Vol filter samples.

For all PUF disk and filter samples, the prokaryotic 16S and fungal ITS PCR conditions were treated similarly. Before PCR, the PUF-DNA extraction replicates were pooled, and 20 μL of each replicate was pipetted into a new tube. DNA concentration was quantified using a NanoDrop2000 (Thermo Scientific). Polymerase chain reaction (PCR) was used to amplify DNA using primer pairs targeting the V3−V4 regions (∼300 base pair (bp)) of the bacterial 16S rRNA gene (PCR1 forward and PCR1 reverse), and the internal transcribed spacer region (∼400 bp) of fungal 18S and 5.8S RNA genes (ITS1 forward and ITS2 reverse).[49] One μL of extracted DNA, or pooled DNA, was used as a template for PCR using Dual-Indexed (UDI) primers 515F-(GTGYCAGCMGCCGCGG-TAA) and 806R(GGACTACNVGGGTWTCTAAT)[50] for bacteria. For fungal PCR conditions, 1 μL of extracted DNA, or pooled DNA, was used as a template for PCR using barcoded primers ITS1F (CTTGGTCATTTAGAGGAAGT-AA)[51] and ITS2R (GCTGCGTTCTTCATCGATGC).[52] The first thermal cycling was performed using the thermocycler, with the following conditions: 95 °C for 5 min, then 25 cycles of 95 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. A second PCR reaction to add i5 and i7 adapters was performed. One μL from the first PCR reaction was used as a template. The second thermal cycling conditions were performed using the following conditions: 95 °C for 5 min, then five cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, 72 °C for 5 min, and followed by a final step of 4 °C − ∞. The thermal cycling conditions for fungal analysis were performed under the following conditions: 95 °C for 5 min, then 30 cycles of 95 °C for 30 s, 53 °C for 40 s, 72 °C for 1 min, 72 °C for 10 min and the final elongation stage of 4 °C − ∞. After amplification, PCR products were checked in 2% agarose gel to determine the amplification's success and the bands' relative intensity. Samples were multiplexed using unique dual indices and were pooled together (e.g., 100 samples) in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then, the pooled and purified PCR product was used to prepare an Illumina DNA library. Sequencing was performed at MR DNA (Shallowater, TX, U.S.A.) using an Illumina MiSeq, following the manufacturer's guidelines.

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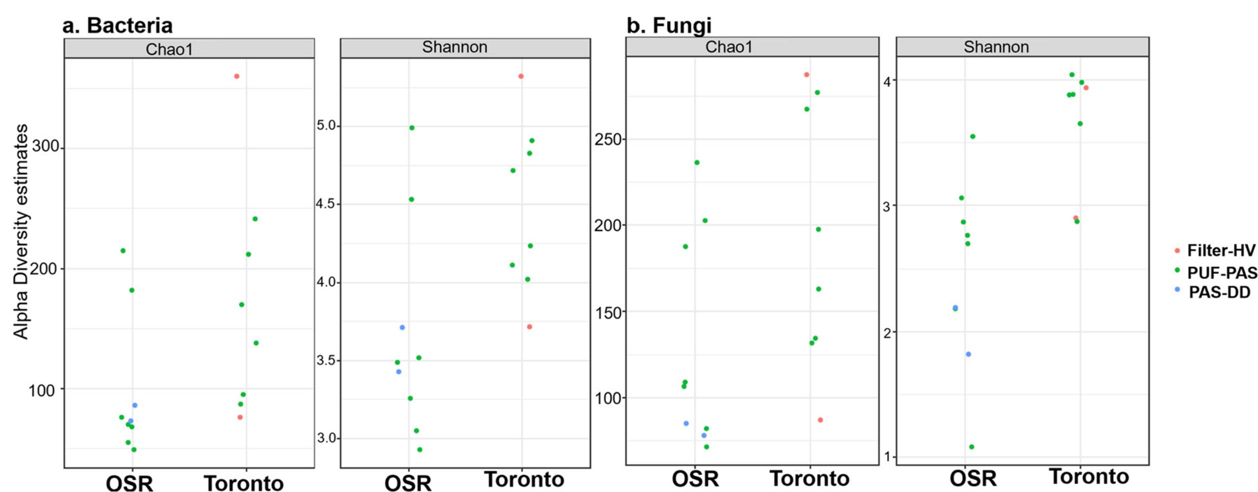

Figure 1. Average alpha diversity of bacteria (a) and fungi (b) estimated by Chao1 (richness) and Shannon (diversity) from different sampler types: PUF-PAS, PAS-DD, and filter-Hi-Vol active sampler for all sites combined in the Oil Sands Region (OSR) and Toronto. There is difference in duration of the samples, i.e., 24 h Hi-Vol vs 2-month PUF passives; Toronto passive samples (n = 6, PUF-PAS) and oil sands region (OSR) samples (n = 7, PUF-PAS and n = 2, PAS-DD) represent sites with very different land use (see Table S1 for details).

Blank samples of PUF disks and filters were used as the negative sampling controls for each sampler type. These blank samples were analyzed for potential artifacts that may represent the background noise found in both blank and actual samples, and amplicon sequences variants (ASVs) found in blank samples were removed from downstream analysis, leaving the organisms from the actual samples. The R Package Deficiency Adenosine Deaminase 2 (DADA2) was used to process the sequence data for PCR[53] Cutadapt V2.8 removed the primer sequences.[54] The reads were quality-filtered by removing reads with N symbols or removing all the reads that exceeded the highest anticipated errors (>5 and >2 for reverse and forward reads, respectively).[49] The dereplicated or unique reads were denoised using a trained error model to acquire unique sequences that were used for inferring the ASVs. The Ribosomal Database Project (RDP) classifier performed in DADA2 was used to identify the representative ASV sequences.[49] The taxonomic compositions were acquired from the SILVAnr v132 and UNITE 0.4.02.2020 databases.[49] The alpha diversity analysis estimated by Chao1 and Shannon indices[55] representing species richness and diversity were assessed using the phyloseq package.[56] The sequencing reads were deposited online in the EMBL-EBI European Nucleotide Archive with Accession Number PRJEB71037.

■ RESULTS AND DISCUSSION

Considerations for Comparability. At the onset of the study, we expected differences to be observed for bacterial and fungal richness in diversity across the two study regions, OSR and Toronto, as well as some degree of heterogeneity within the sites from each region. In addition, we expected to see differences associated with the air sampling method and time and duration of sample deployment. This range of factors that may impact bioaerosol measurements in air are explained further below and need to be considered as limitations when interpreting the results. A more detailed characterization of these factors is beyond the scope of the current paper and will be the topic of future work on sampler calibration, uptake and characterization and related factors for bioaerosols levels in air measured by different approaches. Key factors to consider when interpreting the current results include:

i. Differences in Particle Sizes Captured by PUF-PAS, PAS-DD, and Hi-Vol Samplers. Although the PUF-PAS and PAS-DD samplers use the same PUF disk substrate for collecting particles, the more open parallel plate design of the PAS-DD sampler housing will result in a broader range of deposited bioaerosols, which includes coarse particles. Whereas the PUF-PAS sampler collects particles in the range of ultrafine particles to about PM_5 (i.e., <5 μm), the PAS-DD sampler collects larger settling particles carried by air currents.[44] Air sampling rates may also differ between PUF-PAS and PAS-DD since the PUF-PAS has a greater surface area available for deposition,[37] while the PAS-DD is more exposed to higher wind speed (direct air flow) due to its open design, which may result in episodes of higher sampling rates. These differences in particle size distribution and sampling rates for the two samplers can lead to differences in bioaerosol content for co-deployed samples. Both PUF-PAS and PAS-DD samplers operate by diffusive transfer/deposition rather than active air flow; therefore, the breakthrough of particles is less likely. Any deposited particles will be retained by the porous PUF substrate.[37]

In the case of the Hi-Vol sampler, sampling rates are measured directly and, therefore, are more quantitative. The size range of sampled particles is dependent upon the particle size inlet of the sampler housing (~10 μm in the current study) and the particle-size cut-off of the filter used (0.1 μm in the current study), although this lower cut-off will vary over time with the degree of particle loading onto the filter. This means that particles captured by the Hi-Vol sampler will be in the range of about 0.1 to 10 μm, which is quite different compared to particles captured by PUF-PAS and PAS-DD, which includes particles smaller than 0.1 μm.

ii. Difference in Sample Duration. Bioaerosols at a given location may exhibit high temporal and spatial variability within consecutive hours, between days and weeks.[57] This presents challenges when comparing results from Hi-Vol samplers, which are typically 24-hour "grab samples", from time-integrated sampling using PUF-PAS and PAS-DD, which are typically 2−3 months in duration. This has implications for comparing results from Toronto in the current study, which includes both High-Vol and passive samples.

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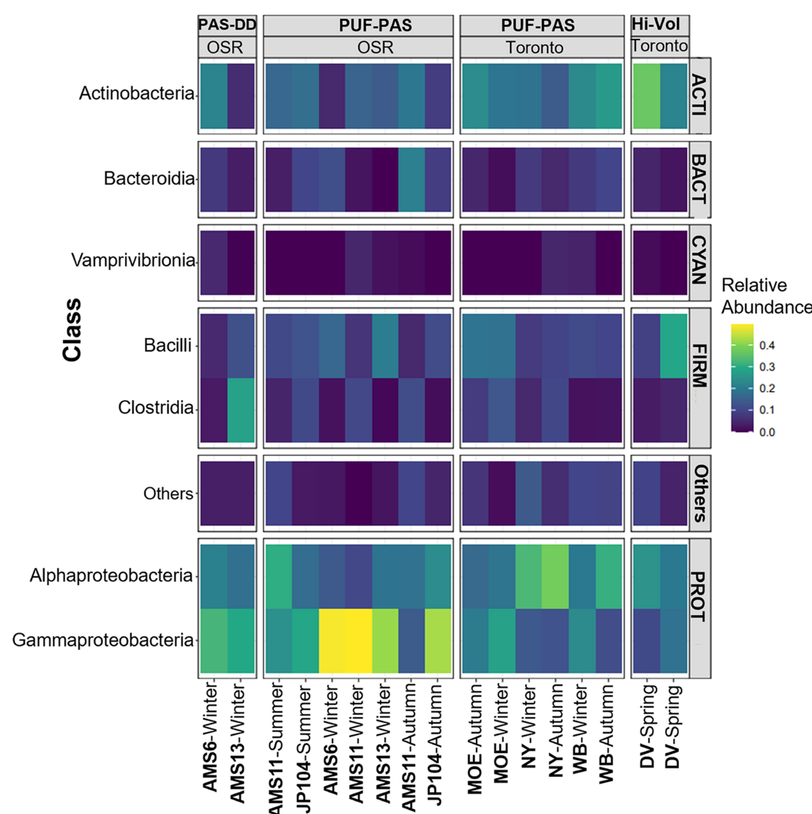

Figure 2. Heatmap diagram showing the distribution of bacteria relative abundance at class level (ordered by phylum, ACT = *Actinobacteria*, BACT = *Bacteroidota*, CYAN = *Cyanobacteria*, PROT = *Proteobacteria*)) from three sampler types (PUF-PAS, PAS-DD, and Hi-Vol (DV)), as detected in air samples during fours seasons from the Athabasca Oil Sands Region and Toronto samples (see Table S1 for details).

iii. Differences among Seasons. Bioaerosols originate from terrestrial surfaces (soil, plants, water bodies, animals, people) and, therefore, temperature, humidity, wind speed, and direction, and other climate and meteorological factors impact their emission, transport and deposition from the air.[58−62] These are all important factors governing variability in bacterial and fungal richness in ambient air.

iv. Differences in Land Use and Point Sources. Land use and the presence of point sources in the vicinity of air sampling sites may have a significant impact on measured bioaerosols and may also introduce temporal variability in bioaerosols due to temporal variability in emissions (e.g., agricultural practices may result in suspension of bioaerosols; traffic-associated emissions; episodic wildfires, industrial practices, and mining such as open-pit mining in the OSR). These topics associated with source attribution of bioaerosols are considered here, but any detailed evaluation of sources is beyond the scope of the current study design and is a topic of future work.

Species Richness and Community Diversity. To better validate the quality of the blanks, we performed a principal coordination analysis (PCoA) of blank vs biological samples and compared their 16s rRNA gene copies number. Blank samples of PUF disks and filters were used as the negative sampling controls for each sampler type. The results (Figure S3 of the SI) showed a significant distinction between blank and actual samples ($P = 0.00016$) and a clear distinction was observed between control (blank) and samples.

Bacterial and fungal species richness and diversity (alpha diversity metrics, i.e. Chao and Shannon, respectively)[63] exhibited variations between land-use and sampler types. Table S2 of the SI shows the Chao 1 and Shannon values for each individual site data. The airborne bacterial species diversity and richness levels were elevated at Toronto sites in comparison to the OSR. The bacterial and fungal species diversity and richness were elevated in Hi-Vol samplers compared to PUF-disk samplers (Figure 1). As discussed in the section above, the observed differences in bacteria and fungal diversity metrics among PUF−PAS, PAS-DD and Hi-Vol active samplers are due to many factors (e.g., time and duration of sampling, land use, point sources, and sampler characteristics) and cannot be resolved with our current understanding and characterization of the samplers. Other studies have also shown bacterial and fungal species heterogeneity and richness between co-located passive and active samplers.[16,64,65] Bioaerosols range in size as follows: viruses (0.01−0.3 μm), bacteria (0.1−10 μm), fungal and fern spores (1−30 μm), and plant pollen (5−100 μm) matter.[15−19] Studies have shown that microbial diversity increases with aerosol size.[20,21] Recent studies have compared airborne bacteria collected by passive and active air samplers and showed differences in diversity and microbial community composition detected by both samplers, suggesting the influence of differences in particle sampling across particles size ranges for the two sampler types.[22]

In this study, the alpha diversity metrics (Chao and Shannon) of the PUF-PAS and PAS-DD can be compared for two sites in OSR (town of Fort McMurray (AMS6) and at the open pit mining (AMS13), with both sites having co-located PUF-PAS and PAS-DD with the same sampling duration and season. The results showed that PAS-DD exhibited higher levels of bacteria richness and diversity than PUF-PAS at both sites. Conversely, the fungal richness and

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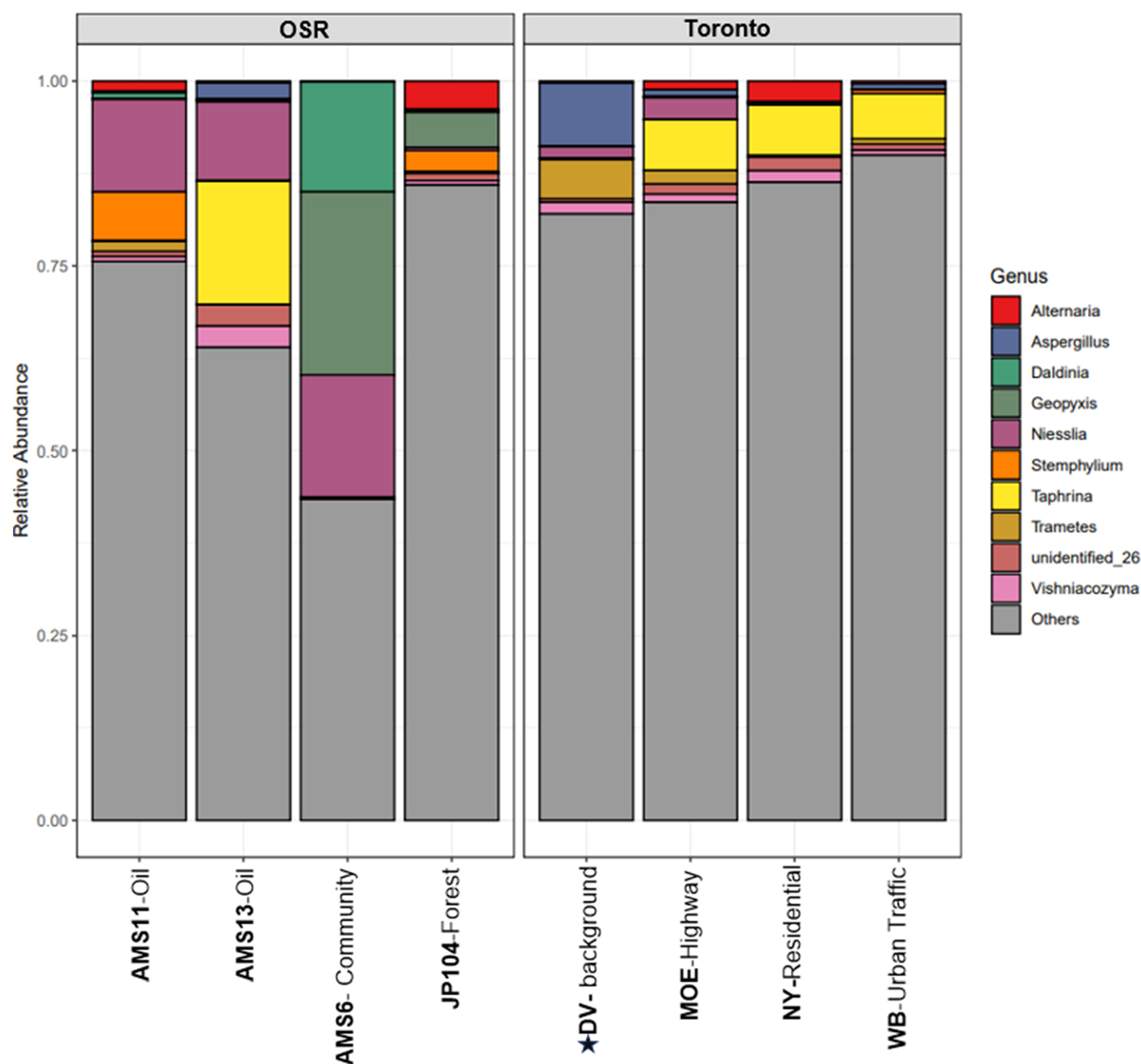

Figure 3. Relative abundance of different fungal genera detected at OSR site (AMS11, AMS13, AMS6, JP104) sites for (PUF-PAS and PAS-DD) combined samplers and Toronto sites (MOE, WB, NY, DV) for (PUF-PAS) combined samplers and Hi-Vol (DV site indicated by asterisk). See Table S1 for details.

diversity were higher in the PUF-PAS than in PAS-DD at AMS13 (Table S2 of the SI). However, the result observed from the *t*-test showed no statistically significant differences in PUF-PAS and PAS-DD for species richness (p value = 0.1144) or diversity level (p value = 0.0712) for bacteria. Similar findings were also observed for fungi for species richness (p value = 0.3514) and diversity (p value = 0.4818). These results suggest that the difference in sampling rates and particle sizes had little effect on bacteria and fungal richness and diversity when PUF-PAS and PAS-DD were co-deployed side-by-side. Further, few studies have studied the effect of sampling time on bioaerosol collected using passive samples. Available studies suggested the range of suitable sampling time should be considered or determined for existing and newly developed samplers.[23] The most recent study found that passive air samplers effectively capture airborne microorganisms while maintaining bioaerosol for over 21 days of continuous sampling.[23]

Airborne Microbial Community Composition. The taxonomic assignment revealed that the most abundant airborne bacteria across all PUF-disk and Hi-Vol filter samples were qualitatively similar. However, the relative abundances of specific taxa were site-, sampler type-, and region-dependent (Figure 2). The bacterial communities were dominated by *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidota*, with *Proteobacteria* (Figure 2) being the most dominant in both PUF-disk type passive air samplers and Hi-Vol active air samplers. This has similarly been identified in other ambient air samples in France,[25,66] China,[4,67] Germany,[68] over the Great Barrier Reef (Australia),[69] Greece,[70] and Rwanda,[21] in which similar next-generation sequencing methods were also used. The airborne bacterial communities sampled in this study with PUF disks and Hi-Vol active air samplers contained similar phyla. This was consistent with other studies showing that passive and active air samplers produce comparable results for the same major bacterial taxa in the atmosphere.[25,71] In this study, *proteobacteria* dominated the PUF-PAS, while *Firmicutes* and *Actinobacteria* were dominant in the PAS-DD and Hi-Vol active samplers, respectively. Previous studies have shown that using different air sampler types can potentially result in

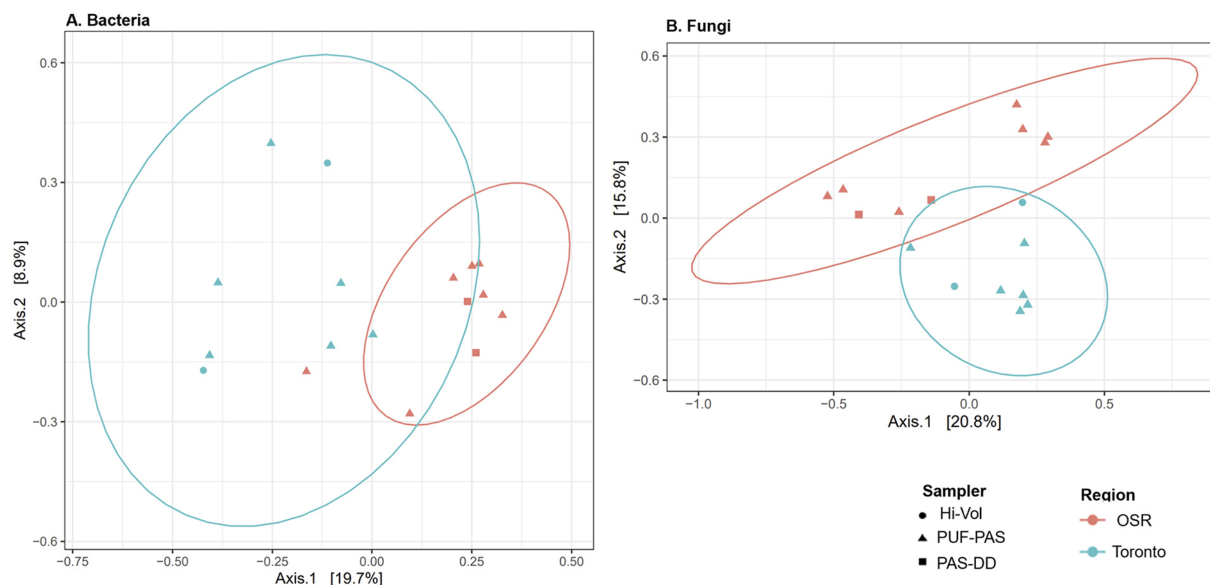

Figure 4. Principal coordinates analysis (PCoA) of the Bray-Curtis dissimilarities of bacterial (A) and fungal (B) communities (relative abundances) by region samples collected in the Oil Sands Region (OSR) and Toronto sites.

variation in the detection of airborne microorganisms despite being co-located.[24,65] Despite similarities in airborne bacterial community structure at the phylum level, there are noteworthy distinctions between the dominant classes of *proteobacteria*, with *Gammaproteobacteria* being the most prevalent class in all phyla of bacteria in OSR (Figure 2). This finding supported an earlier report showing that *Alpha*- and *Gammaproteobacteria* were the most abundant *proteobacteria* identified in the Gulf of Mexico (oil spill area).[72,73] Several studies have reported *Gammaproteobacteria* as the most abundant class in more heavily polluted oiled samples and deepwater oil plumes.[74] *Gammaproteobacteria* exhibited similar profiles in the two sites closest to oil sands open pit mining (AMS11 and AMS13) and at the town of Fort McMurray (AMS6), whereas the background forest site (JP104) exhibit abundance profiles of *Clostridia* and *Bacteroides*. For sites in Toronto, *Bacilli*, *Alphaproteobacteria*, and *actinobacteria* were the dominant classes of bacteria. These results suggest that airborne microorganisms are widely distributed in the atmosphere and could vary according to different atmospheric environments and land-use types (Table S1).[75]

At the genus level, the most abundant bacterial and fungal genera across all PUF-disk and Hi-Vol filters are shown in Figure S4 and Figure 3, which exhibited different relative abundances. Figure S5 shows that the relative abundances of bacteria genera were qualitatively similar between OSR and Toronto samples and within sites in the OSR and Toronto. In contrast, fungal genera are much more heterogeneous (Figures 3 and S6). For example, for bacteria, *Pseudomonas*, *alistipes*, *Brundimonas*, *Sphingomonas*, *Lactobacillus*, *Methylobacterium*, and *Acinetobacter* were the most abundant airborne bacteria genera in the OSR and Toronto. The bacterial genera (*Pseudomonas*) were observed in high abundance at OSR sites, similar to those previously observed in other direct 16S rRNA gene sequencing studies of Alberta oil sands tailings ponds.[76,77] However, bacterial genera differed across various sampling sites and areas, as expected. Studies have indicated that *Pseudomonas* is enriched in burning samples,[78] which may have originated from forest fires that often occur in Alberta and affect the ambient level of air pollution.[79]

The predominant fungal classes detected in this study were found in the phyla *Ascomycota* (*Dothideomycetes* and *Sordariomycetes*) and *Basidiomycota* (*Agaricomycetes*) (Figure S4 of the SI), which have been shown to dominate ambient air samples in several previous studies.[4,60,80,81] The fungal class detected in this study exhibited different abundance profiles at each site in the OSR in contrast to Toronto samples that showed similar profiles. For example, the two sites closest to oil sands open pit mining (AMS11 and AMS13) exhibited different profiles with dominant *Malasseziomycetes* and *Eurotiomycetes*, respectively. The class of *Dothideomycetes* dominated the background forest site (JP104). In contrast, the community site (AMS6) in the town of Fort McMurray has a profile dominated by *Sordariomycetes*. The primary sources of these dominant classes of *Dothideomycetes*, *Sordariomycetes*, and *Agaricomycetes* have all been identified in soil, humus, and plants.[36,80,82] *Agaricomycetes*, which are the dominant class of *Basidiomycota* in Toronto for this study, is known as mushroom-forming fungi,[83] commonly found in soil[84] and on leaf surfaces of plants.[85] *Basidiomycota* is typically too large to aerosolize in warm climates,[86] suggesting that the conditions during the collection of the Toronto samplers were on average warmer compared to the timing of OSR sample collection (see Table S1 of the SI).

In contrast to bacteria, the fungal community in the OSR sites exhibit unique abundance profiles; the two sites closest to oil sands open pit mining (AMS11 and AMS13) have the highest relative abundance of *Niesslia*, *Taphrina*, and *Stemphylium*, whereas the background forest site (JP104) was dominated by *Alternaria* and the community site (AMS6) in the town of Fort McMurray has a profile dominated by *Daldinia* and *Geopyxis* (Figure 3). Toronto sites exhibited a similar profile to each other; for instance, three of the four sites in Toronto with influence from traffic emissions sources were dominated by *Taphrina*, whereas the fourth site, DV (Hi-Vol samplers only), exhibits a unique profile dominated by *Aspergillus* and *Trametes*. However, as discussed previously,

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differences at the DV sites could be associated with several factors, including shorter duration of sample (24 h vs 2 months), different time/year of sampling and differences in particle size distribution collected by Hi-Vol compared to PUF-PAS.

Previous studies showed that soil ecosystems can change the microbial composition and community structure of the microbes.[78] In this study, *Taphrina* was most dominant at the traffic site in Toronto and the AMS13, suggesting the influence of traffic emissions sources. AMS13 is closest to mining activities and could also be influenced by emissions from large trucks and other equipment on the site. *Niesslia* was the dominant genera found at the OSR site closest to oil sands open pit mining sites, suggesting the influence of mining activity. *Alternaria* and *Aspergillus*, pathogenic fungi detected in previous culture-independent studies, were detected in higher abundance at Toronto sites and are major fungal allergens known to cause invasive infections and aspergillosis of the lung.[80,87−89]

Comparing Variability in Microbial Composition between Samplers. This study compared microbial communities detected by the consecutive Hi-Vol samplers and the co-located PUF-PAS and PAS-DD. The active Hi-Vol samples (HV-1 and HV-2) and PUF-PAS and PAS-DD at AMS6 (AMS6-1 and AMS6-2) and AMS13 (AMS13-1 and AMS13-2) samples show significant differences for some classes of bacteria and fungi (Table S1, Figures 2 and S7); these were two consecutive and co-located samples, and this result may reflect the dynamic nature of the airborne microorganism and its dependence on meteorology. The two Hi-Vol samples from Toronto do not map next to each other in Figure 4, suggesting that consecutive samples may reflect the dynamic nature of the airborne microorganism over time for a given location. Mhuireach et al. (2016) showed that land-use types could affect variation in airborne bacteria communities between parks and parking lots, even when these are near each other. The bacteria and fungal communities detected in co-located PUF-PAS and PAS-DD showed different patterns and relative abundance, which may reflect differences in the time of sampling but also differences in the particle size range captured by PUF-PAS and PAS-DD. In agreement, previous studies have compared and characterized the performance of the co-located PUF-PAS and PAS-DD for organic compounds analysis in air samples.[90] They found that PAS-DD captured more and larger particle-associated target chemicals compared to PUF-PAS due to its open design with a cover plate where the PUF disk is protected from precipitation and photolytic degradation from direct sunlight.[90] Although the co-located PUF-PAS and PAS-DD samplers use the same PUF disk substrate for collecting particles, differences observed in detecting bacteria and fungi could be related to differences in advected air masses carrying bioaerosols or in meteorological conditions (wind speed, temperature, humidity), particle size collected, sampling rate, and surface conditions (e.g., soil moisture), which could impact local emissions of bioaerosols. These results suggest that bioaerosol among samplers should be compared carefully, especially when samples were collected at different sites and during different periods.

Community Structure (Bacteria and Fungi). To explore bacteria and fungal community structures by land- use type and seasons, PCoA was performed. The figure shows bacteria results (Figure 4A) and fungal results (Figure 4B) clustered by sampler types and locations. The bacterial and fungal communities showed apparent clustering (distinct bacterial and fungal communities) that differentiated OSR versus Toronto air samples (Figure 4). In agreement, Bowers analyzed bioaerosols in the U.S. and reported that land use is an essential factor affecting airborne bacterial communities.[22,91] In China, Cao et al.[1] indicated that samples collected at different locations exhibited different dominant bacteria and fungal genera, suggesting that geographic variation can enhance variation in bioaerosol sources more than seasonal and meteorological conditions. The OSR sites were located ∼4000 km from the Toronto sites and are characterized by petroleum reserves in the form of bitumen. In contrast, traffic and mixed sources generally influenced the Toronto sites, including traffic, light industry, parkland, and residential areas.

Microbial community compositions at the genus level showed seasonal variation with more pronounced fungi than bacteria. A clear separation by season (ignoring differences attributed to sampling location) in fungi was observed in winter, autumn, summer, and spring samples (Figure S8), indicating a different seasonal distribution of the fungal community structures. However, the results for bacteria across seasons were not clustered, indicating a similar seasonal distribution of the bacterial community structures in spring, summer, and autumn, consistent with previous studies.[75] In this study, winter samples showed high species richness and diversity compared to other seasons. Studies have suggested that a cold climate is the most favorable season for fungi, suggesting that they sporulate more in winter than in other seasons.[60,80] Global studies have suggested that fungi are significantly associated with seasonal changes more so than bacteria,[92] highlighting that bacteria exist in small-size fractions, enabling them to attach to fine particles easily.[93] As a result, bacteria can undergo long-range transport from one region to another, especially during strong winds, whereas temperature and precipitation have been indicated to explain the main variations in global fungal diversity.[94] Both temperature and rain increase the metabolic rates of organism multiplication[95] and could play different roles in altering the structure of microbial communities present in the air.

Limitations of This Study and Future Directions. This screening study was the initial stage of a proof-of-concept for the application of PUF-disk type passive air samplers for tracking bacteria and fungi in air, with application to selected samples and sites from OSR and Toronto. Although the results presented here are positive for the sensitivity of the method for characterizing diversity and richness in bacterial and fungal populations, sources of variability have been highlighted and discussed. These sources of variability include *inter alia* the mode of sample collection, differences in sampling rate, differences in particle-size range collection, meteorological factors, and the dynamic nature of bioaerosols from day-to-day and across seasons. It is also important to recognize that differences exist between the passive samplers used here and other passive sampling approaches for bioaerosols. As interest in bioaerosols continues to grow, it will be necessary to compare performance of the most widely used bioaerosol sampler types and to assess sampler-dependent biases and artefacts in the collections of bioaerosols. Related to this point, certain bacteria community's genera, such as Acinetobacter, Pseudomonas, Methylobacterium and Micrococcus, have been found to degrade chemical PAH compounds especially those with lower molecular weights (2 to 4 rings) as their sole carbon

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source.[24] Interactions between chemicals and bioaerosols are expected to occur and may in fact change the chemical or bioaerosol composition over longer time periods and perhaps also during sample storage. It is proposed that future work include calibration studies with PUF-PAS and PAS-DD samplers in order to characterize and quantify their uptake profiles, and better address the additional questions raised here. This will contribute to the potential application of these simple samplers for tracking bioaerosols and assessing their changes over time, which may indicate biodiversity loss that may be associated with environmental pressures, including chemical pollution and climate change.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestair.3c00107.

Details regarding the active and passive air samplers, information on the locations and dates of all samples collected, and a schematic of the DNA extraction and analysis method. Tabulated and graphic results are provided for bacteria and fungi diversity, richness, and relative abundance, as well as results from principal coordination analysis (PDF)

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