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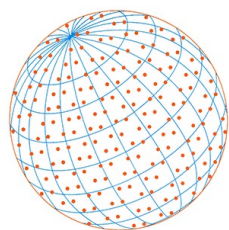
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Comparison of Airborne Bacterial Populations Determined by Passive and Active Air Sampling at puy de Dôme, France

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ABSTRACT

Bioaerosols have impacts on atmospheric processes, as well as ecosystem and human health. Common bioaerosol collection methods include impaction, liquid impingement, filtration, and electrostatic precipitation. These methods are used by active samplers that require an air mover and power, but this requirement can also represent a major constraint in field studies. Alternatively, passive samplers do not require power and can operate for long times. In this study, the Rutgers Electrostatic Passive Sampler (REPS), which captures particles by electrostatic attraction and gravitational settling, was deployed at the summit of puy de Dôme (1465 m a.s.l., France) alongside an active PM₁₀ sampler (~1000 L min⁻¹) collecting aerosols on a quartz fiber filter. The diversity of the airborne bacteria captured by both samplers across six weekly sampling periods was examined by 16S rRNA gene amplicon sequencing. The dominant phyla observed by both samplers were similar and included Firmicutes, Proteobacteria, and Actinobacteriota. Overall, 12 to 63% of the total bacterial richness at the genus level was shared between the two samplers, depending upon a paired sample, i.e., sampling week. The PM₁₀ sampler and REPS detected the same dominant genera, including *Lysinibacillus* and *Sphingomonas*, although their relative abundances for each paired sampler varied. The observed bacterial richness and diversity, as estimated through Shannon's and Simpson's indexes, were significantly greater in REPS samples compared to the PM₁₀ samples. The results suggest that REPS could be used for simple and convenient sampling of bioaerosols, especially in remote areas and other locations with limited power access.

Keywords: Passive sampler, Bioaerosols, Diversity

1 INTRODUCTION

Bioaerosols are particles of biological origin suspended in the air. In general, airborne proteins, toxins, viruses, fungi, pollen, and bacteria, as well as fragments thereof, are within this category (Frölich-Nowoisky *et al.*, 2016). These particles can be free-floating (e.g., a single bacterium in the air) or part of a larger particle agglomerate (e.g., a virion in a respiratory particle or bacterium attached to a soil particle). Bioaerosols have been demonstrated to have impacts on Earth processes (e.g., cloud and ice formation, chemical reactions) as well as human health (Frölich-Nowoisky *et al.*, 2016). In the atmosphere, they can act as nuclei for the formation of water droplets and ice crystals, affecting the hydrological cycle (Möhler *et al.*, 2007). In public health, infectious bioaerosols,

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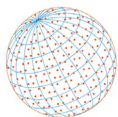
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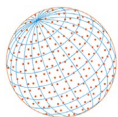


containing agents such as *Mycobacterium tuberculosis* and SARS-CoV-2, can be transmitted through the air and infect humans (Mbareche *et al.*, 2019). Furthermore, agriculture is heavily impacted by aerosolized plant pathogens. For instance, the causative agent of wheat stem rust is dispersed through the air and can infect crops in distant locations (Meyer *et al.*, 2017). As a result, the collection and identification of the constituent microbial populations of the air/atmosphere are essential to numerous disciplines.

To identify airborne microbial populations, bioaerosol samplers are commonly employed, which fall into two possible categories: active or passive samplers. Active samplers use an internal or external air mover, requiring power, to sample a known volume of air, allowing the determination of bioaerosol concentrations. Passive samplers do not require any external power or an air mover and rely on gravity, diffusion, and/or electrostatic forces to collect airborne particles, including bioaerosols. Passive sampling devices based on these collection principles include spore traps (Durham, 1946; Serrano-Silva and Calderón-Ezquerro, 2018), dust collectors (Cox *et al.*, 2017), agar settling plates (Andon, 2006), polydimethylsiloxane pads (Angel *et al.*, 2022), and specially-arranged polarized ferroelectric polymer films (Therkorn *et al.*, 2017a). In particular, electrostatic collection combined with gravitational particle deposition can allow for greater collection efficiency of bioaerosols compared to gravitational settling alone (Therkorn *et al.*, 2017b). For a comprehensive review of passive samplers for bioaerosols, refer to Manibusan and Mainelis (2022).

The different air sampling methods affect the types of analyses that can be conducted on the collected biological material (Mainelis, 2020). Until recently many bioaerosols studies, especially those using passive samplers, have been limited to culture-based methods (Gandolfi *et al.*, 2013). However, the use of only culture-based methods will not provide information on viable but not culturable (VBNC) or non-viable microbes, which includes the vast majority of airborne cells (e.g., Vařtilingom *et al.*, 2012). Additionally, common active bioaerosol samplers are known to impact the viability and physiological status of microbes (Zhen *et al.*, 2013, 2018), which can create more biases in culture-based studies. However, even the microbes detected via culture-independent methods (e.g., marker-gene-surveys) can be influenced by sampler selection (Fahlgren *et al.*, 2011; Hoisington *et al.*, 2014; Mbareche *et al.*, 2018; Serrano-Silva and Calderón-Ezquerro, 2018; Bøifot *et al.*, 2020). In marker-gene survey studies, different samplers often agree about the presence of the more abundant phylotypes, but not the rarer phylotypes (Fahlgren *et al.*, 2011). This might be explained by air samplers that use disparate sampling principles, so more studies comparing airborne biodiversity with multiple air samplers are needed.

Few studies have been conducted concurrently with both active and passive samplers to examine and compare bioaerosol diversity. Additionally, most of the bioaerosol studies using marker-gene surveys (typically targeting the 16S rRNA gene) have been conducted with active samplers. Only a select few utilized passive samplers (Adams *et al.*, 2015; Mhuireach *et al.*, 2016; Maestre *et al.*, 2018; Serrano-Silva and Calderón-Ezquerro, 2018). Since passive samplers offer certain advantages over active sampling methods, such as simplicity of operation and long sampling times, more studies using passive samplers for marker-gene surveys are needed. One recently developed passive sampler is the Rutgers Electrostatic Passive Sampler (REPS). It uses electrostatic deposition caused by a poled polarized ferroelectric film as well as gravitational settling to collect bioaerosol particles (Therkorn *et al.*, 2017a, 2017b). It has been shown to effectively collect bioaerosols up to 21 days in the outdoor environment (Grogan and Mainelis, 2022). Furthermore, REPS was deployed to sample air in Greece and detected 793 bacterial operational taxonomic units (OTUs) by sequencing part of the 16S rRNA gene (Metaxatos *et al.*, 2022). However, REPS's ability to determine airborne bacterial diversity has not been compared to other samplers. Thus, the goal of this study was to compare the ability of REPS to detail the diversity of airborne bacteria with that of an established active filter-based sampler. Specifically, REPS and an active high flow rate PM₁₀ sampler were deployed simultaneously at the summit of puy de Dôme (1465 m a.s.l.), France (see Tignat-Perrier *et al.* (2020) for results of the PM₁₀ filter-based study), where 6 weekly samples were collected by both samplers and the bacterial diversity in samples was analyzed and compared.



2 METHODS

2.1 Samplers and Sample Collection

REPS and an active PM₁₀ sampler were located at the meteorological station at the summit of puy de Dôme in the Auvergne region of central France (1465 m a.s.l., 45.772°N, 2.9655°E). The station is operated by the Observatoire de Physique de Clermont-Ferrand (OPGC) and is part of the Cézeaux-Aulnat-Opme-Puy de Dôme (CO-PDD) platform for atmospheric measurements (Baray *et al.*, 2020). Relative humidity, temperature, and wind speed were logged every five minutes during the sampling periods. It was also observed that clouds traveled over the location during sampling. The cloud droplet sizes were assessed during Weeks 2, 3, and 5, with the described instrumentation at the station (Baray *et al.*, 2020). The percentage of time of the total sampling period when the cloud droplets were greater than 10 µm was calculated, because the active sampler collects PM₁₀ particles and REPS does not separate particles by size. The calculated percentage could potentially have helped to explain results.

The filter-based PM₁₀ samples were collected on a weekly basis for nearly a year, and the obtained results are presented elsewhere (Tignat-Perrier *et al.*, 2020). The PM₁₀ samples were collected on a quartz fiber 5.9" diameter round filter inside a high-volume air sampler equipped with a PM₁₀ size-selective inlet (Dommergue *et al.*, 2019; Tignat-Perrier *et al.*, 2019, 2020). The collection and preparation of the filter samples were previously described in (Tignat-Perrier *et al.*, 2020) and sampling methods for the filters were outlined in (Dommergue *et al.*, 2019). The quartz-fiber filters were heated to 500°C for 8 hours. The transportation blanks (filters brought to sampling site, but not mounted inside the sampler) and field blanks (mounted inside the sampler, but no air sampled) were used to assess contamination. Filters sampled over 8,000 m³ of air (approximately 44–66 m³ hr⁻¹; Table 1) over each 6–7-day period.

During select weeks of the previous study, described in Table 1, the Rutgers Electrostatic Passive Sampler (REPS) was co-deployed with the PM₁₀ sampler. Weeks 1 through 6 were May 24–30, April 5–12, March 29–April 5, May 10–17, March 22–29, and May 17–24, 2017, respectively. Comparison of bacteria observed by the two samplers during the co-located sampling periods is the focus on this paper. During the assembly of REPS, its holder and bases were cleaned, disinfected, and irradiated with UV light for 15 minutes. The ferroelectric films were disinfected with 70% (v/v) ethanol and rinsed with sterile water (Therkorn *et al.*, 2017a; Metaxatos *et al.*, 2022). The samplers were assembled at Rutgers based upon previous designs (Therkorn *et al.*, 2017a; Metaxatos *et al.*, 2022) and shipped in sterile 50 mL conical tubes to Clermont-Ferrand, France for sampling at puy de Dôme. At the sampling site, REPS were placed in a small protective cage within five meters of the PM₁₀ sampler. The top of the cage was covered by plastic to prevent rain from directly hitting the sampler. The handling base of REPS was disinfected with 70% (v/v) ethanol prior to installation of the sampler. One REPS was kept in the cage for one weekly sampling period.

2.2 Sample Processing

Three punches (38 mm diameter) of each PM₁₀ filter were used for DNA extraction with the DNeasy PowerWater kit. PM₁₀ samples were extracted as part of Tignat-Perrier *et al.* (2020) (Tignat-Perrier *et al.*, 2020). Collected cells were lysed at 65°C for one hour, the lysate was vortexed for 10 minutes at the maximum speed and then centrifuged in a syringe for 4 minutes at 1000 × *g* (Dommergue *et al.*, 2019; Tignat-Perrier *et al.*, 2020). The biological material was eluted from REPS by placing the sampler in 30 mL of ultrapure water (0.2 µm filtered) in a tube, vortexed for 2 minutes, and placed in an ultrasonic bath for 10 minutes. Samples were aliquoted for flow cytometry (450 µL) and cultivation (100 µL in duplicate or triplicate). The remainder of the sample was filtered through MoBio 0.22 µm Water Filters (MoBio 14880-50-WF) and stored at –80°C. Three blank controls of REPS that were not used for sampling but otherwise handled in the same way were processed as above. Eluate from REPS was extracted following the same protocols as the PM₁₀ samples.

2.3 Cultivation and Flow Cytometry

100 µL aliquots of the REPS elution liquid from Weeks 1, 4, and 6 were plated on R2A agar.

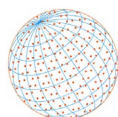
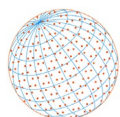


Table 1. Meteorological and Sampling Details. The collection times occurred in 2017 and are in UTC. The data were collected at 5-minute intervals during the described sampling period. The percentage of the time of the entire sampling period in which relative humidity was 100% and the cloud droplet radius was greater than 10 μm were calculated. Cloud droplet radius was not measured during Weeks 1, 4, and 6.

Week	Collection Period	Flow rate (L min^{-1})	Total air sampled (m^3)	Average \pm Standard Deviation Range			Percentage of Sampling Time	
				Temperature ($^{\circ}\text{C}$)	Relative Humidity (%)	Wind Speed (m s^{-1})	Relative Humidity = 100%	Cloud Droplet > 10 μm
1	Start: 8:40 May 24 End: 10:05 May 30	949	8282	15.5 \pm 3.4 8.6–23.6	70.0 \pm 20.2 21.9–99.9	5.0 \pm 3.1 0.5–17.7	0	n/a
2	Start: 8:30 April 5 End: 13:20 April 12	1006	10438	6.4 \pm 4.7 –1.1–16.9	69.0 \pm 23.9 13.3–100	5.6 \pm 3.5 0.5–15.7	18.5	37.8
3	Start: 9:15 March 29 End: 8:15 April 5	999	10015	5.2 \pm 3.7 –0.4–15.4	77.3 \pm 23.0 24.8–100	5.0 \pm 3.5 0.7–15.9	32.3	23.1
4	Start: 8:40 May 10 End: 13:50 May 17	978	10154	9.2 \pm 3.5 4.4–20.5	76.6 \pm 18.8 23.6–100	6.3 \pm 3.4 0.6–16.2	17.8	n/a
5	Start: 13:30 March 22 End: 9:15 March 29	940	9478	3.1 \pm 3.3 –4.3–12.3	79.4 \pm 17.2 35.3–100	7.2 \pm 4.1 0.2–19.1	19.3	15.1
6	Start: 13:50 May 17 End: 8:20 May 24	987	9664	7.9 \pm 5.1 –0.4–19.4	84.0 \pm 20.4 19.5–100	7.6 \pm 4.3 0.3–24.1	29.1	n/a

n/a = not applicable.



After incubation, the number of bacterial colony-forming units (CFU) were counted and converted to CFU mL⁻¹. For all six sampling periods, the number of cells collected by REPS was assessed with flow cytometry following methods from [Amato *et al.* \(2017\)](#). Briefly, 225 µL of sample was mixed with 25 µL of 5% glutaraldehyde (Sigma-Aldrich G7651). These were stored at 4°C until analysis. Prior to analysis, 1 vol of Tris-EDTA buffer (pH 8.0, 40 mM Tris-base, 1 mM EDTA) and 5 µL of SYBRGreen I (Molecular Probes Inc., Eugene, OR) were added and incubated in the dark for five minutes. Cells were counted with a FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and the numbers were extrapolated to the total number of cells collected during the sampling period.

2.4 DNA Extraction, PCR, and Sequencing

For all REPS and PM₁₀ samples, DNA was extracted with the DNeasy PowerWater kit (Qiagen) following its associated protocol. The V3-V4 region of the 16S rRNA gene was amplified from the extracted DNA following protocols described in [Tignat-Perrier *et al.* \(2019\)](#). Briefly, the primers 341f and 805r (with Illumina adapters) were used with Platinum Taq Polymerase (ThermoFisher Scientific) to generate ~510 bp amplicons. The thermal cycling conditions were 95°C for 3 mins, then 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension step at 72°C for 5 mins. Library preparation was performed using standard Illumina library preparation protocols and sequenced by paired-end MiSeq (2 × 250).

2.5 Bioinformatics and Data Analysis

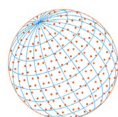
Adapter sequences were removed by Illumina software following sequencing. The sequences of the PM₁₀ samples were available from a previous study ([Tignat-Perrier *et al.*, 2020](#)), but were re-analyzed alongside the REPS sequences obtained here. The fastq files of both REPS and PM₁₀ filter samples were imported into QIIME2 (v2021.4) ([Bolyen *et al.*, 2019](#)) and primers were removed with cutadapt ([Martin, 2011](#)). The sequences were trimmed and denoised with the DADA2 ([Callahan *et al.*, 2016](#)) plugin. Chimeras were detected by the consensus method and the sequences were truncated to 220 bp. The reads were merged to yield amplicon sequence variants (ASVs). The sequences of the transportation and field blanks for the PM₁₀ filters were removed from all samples during the denoising step with DADA2. After denoising all samples, ASVs present in REPS blanks were manually removed from all samples. The filtered ASVs from all the samples were grouped into 97% operational taxonomic units (OTUs) with the vsearch ([Rognes *et al.*, 2016](#)) plugin in QIIME2. A naïve Bayes scikit learn classifier ([Pedregosa *et al.*, 2011](#); [Bokulich *et al.*, 2018](#)), trained on 341f and 805r primers using extracted reference full-length operon sequences of the SILVA v138 database ([Quast *et al.*, 2013](#)), was used for classification. Sequences classified as chloroplasts or mitochondria were filtered out with QIIME2.

The final filtered sequences were imported into R with qiime2R ([Bisanz, 2018](#)). The observed OTUs, Shannon's diversity index, Simpson's diversity index, and the weighted UniFrac distances were assessed with the phyloseq package ([McMurdie and Holmes, 2013](#)) (version 1.38.0). A principal coordinates analysis of weighted UniFrac was done with phyloseq as well. The OTUs were collapsed at the genus level with phyloseq and the genera shared between the co-located samplers each week was assessed with VennDiagram ([Chen and Boutros, 2011](#)) (version 1.7.1). A paired one-tailed t-test was performed ($\alpha = 0.05$) using stats (version 4.1.2) package ([R Core Team, 2021](#)) in R comparing the diversity metrics of samples collected with REPS and the filter from the same sampling period. The phyloseq objects were converted into DESeq2 objects and used for determining the differential abundance of OTUs based upon samplers. Using DESeq2, the counts for OTUs were modeled based on a negative binomial generalized distribution. The estimated fold change of the OTUs in REPS vs. PM₁₀ samples were compared by Wald test and the p-values were adjusted with the Benjamini-Hochberg method ($\alpha = 0.01$). This assessed if OTUs were more abundant in REPS or PM₁₀ samples.

3 RESULTS

3.1 Sampling Conditions

The sampling periods and meteorological conditions are shown in [Table 1](#). The REPS and PM₁₀



sampling occurred over six collection periods, referred to as Weeks (Table 1). The air volume sampled for PM₁₀ in that period ranged from approximately 8282 to 10438 m³ (Table 1) (Tignat-Perrier *et al.*, 2020). The average temperature across all collection periods was between 3.1 and 15.5°C. The temperatures reached below 0°C during Weeks 2, 3, 5, and 6. The wind speed (measured every 5 mins) ranged between 0.2 and 24.1 m/s across all collection periods. The relative humidity during the sampling periods ranged from 21.9% to 100%. A relative humidity of 100% was observed for 0 to 29.1% of the time during individual weekly sampling periods. The relative humidity of 100% could have been indicative of a cloud. The cloud droplet sizes were greater than 10 µm for 37.8, 23.1, and 15.1% of the entire sampling period in Weeks 2, 3, and 5 (Table 1). Due to its nature, the PM₁₀ sampler would not have collected particles greater than 10 µm, including cloud droplets, while REPS could have collected these particles due to gravitational settling and impaction. Thus, during these fractions of Weeks 2, 3, and 5 (e.g., 37.8, 23.1, and 15.5%), the size distribution of particles collected by the two samplers could have been different.

3.2 Biomass Collected and Biodiversity Metrics

For all REPS samples, the number of cells collected was enumerated by flow cytometry. The average number of cells collected with REPS ranged from 90,720 cells to 1,256,730 cells per sample across all the collection time periods (Table S1). There were cells detected in the blank samples from REPS, but their number was orders of magnitude lower compared to samples and no culturable bacteria were detected (Table S1). For Weeks 1, 4, and 6, the samples collected with REPS were cultured on R2A agar. On average, there were 900, 1200, and 2400 total CFU collected with REPS for those weeks, respectively (Table S1).

After cultivation and flow cytometry, the remainder of the eluate from REPS was used for DNA extraction and 16S rRNA gene amplicon sequencing. The number of raw reads for REPS ranged from 42,344 to 162,586 and the number of raw reads for PM₁₀ samples ranged from 44,945 to 67,953 reads (Table S1). There were no reads from the PM₁₀ blank samples after quality control. The REPS blanks had 735–7,981 reads (Table S1), but these were manually removed from all samples. After denoising and initial quality control, REPS samples had 1,319 to 12,144 reads and the PM₁₀ samples had 77 to 8,930 reads (Table S1). When sampling bioaerosols, there are always issues with contaminants and low biomass (Behzad *et al.*, 2015). Since there were ASVs associated with REPS negative controls that passed quality control, ASVs derived from controls were manually removed. Because REPS blanks were handled the same way as actual REPS samplers, except exposing them to air for sampling, the ASVs in blank REPS likely resulted during the process of assembling REPS, transportation, and/or from the DNA extraction/sequencing procedures.

The number of observed OTUs ranged from 90 to 480 for REPS and from 10 to 276 for the PM₁₀ samples (Fig. 1) across the sampling periods. Overall, 1439 unique OTUs were detected across all the sampling periods for both samplers. The Shannon index values ranged from 1.6 to 4.5 for PM₁₀ filter samples and from 4.0 to 5.2 for REPS samples. Finally, the Simpson index values ranged from 0.68 to 0.97 for PM₁₀ and from 0.97 to 0.99 for REPS samples. In terms of α -diversity metrics, the observed OTUs, Shannon's index, and Simpson's index were significantly greater for samples collected by REPS compared to the PM₁₀ sampler ($p = 0.018$, $p = 0.0036$, $p = 0.038$ respectively). The weighted Unifrac metric was used to compare the samplers and weeks to each other through principal coordinates analysis (PCoA) (Fig. S1). The analysis demonstrated that the sampler type and sampling period did not fully explain the variation amongst the bacterial populations collected (Fig. S1).

3.3 Detected Biodiversity

For the paired weekly REPS and PM₁₀ filter samples, Firmicutes and Proteobacteria were the most abundant phyla, along with Actinobacteriota (Fig. 2). In Weeks 1, 2, 3, and 6, Firmicutes were detected in higher relative abundance in PM₁₀ filter samples compared to REPS samples. However, Proteobacteria were detected in higher relative abundance in REPS compared to PM₁₀ filter samples in Weeks 2, 3, and 6 (Fig. 2).

The 63 bacterial genera detected in this study varied in relative abundance from 0 to 68.8% (Table S2). The top 10 most relatively abundant genera across all the samples (PM₁₀ filter and REPS) were *Bacillus*, *Clostridioides*, *Desulfovibrio*, *Jatrophihabitans*, *Lysinibacillus*, *Microbacterium*,

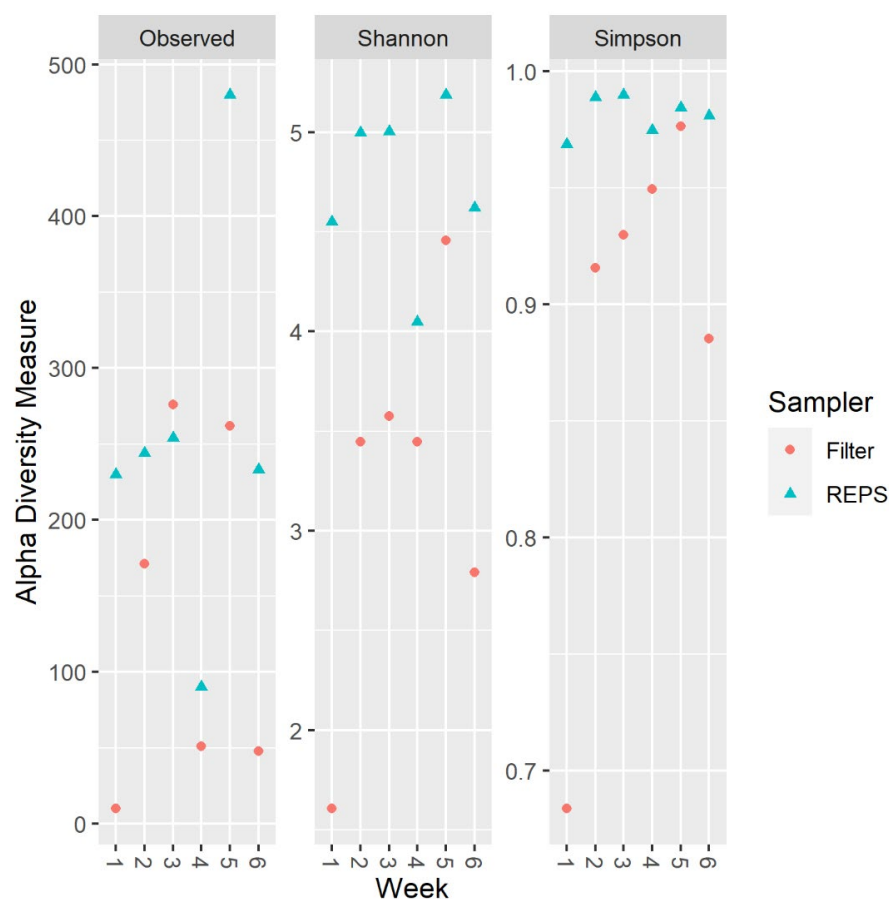
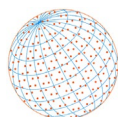


Fig. 1. Alpha diversity metrics for REPS and filter sampling. The alpha diversity metrics were the observed OTUs, Chao1 index, Shannon diversity index, and the Simpson's index. Each week of sampling is presented (Table 1) with the corresponding sample from REPS or filter.

Pandoraea, *Sphingomonas*, *Streptomyces*, and *Turicibacter*. *Lysinibacillus*, a member of Firmicutes, was much more abundant for the PM₁₀ filter samples in Weeks 2 and 3 (Fig. 3, Table S2). *Turicibacter* showed relative abundance of less than 1.3% in REPS samples, and was not detected in Week 4 by REPS, while it was present in larger proportions in all six PM₁₀ filter samples. On the other hand, *Streptomyces* was not detected by the PM₁₀ filter sampler in Weeks 1 and 4 but was detected with REPS across all sampling periods and ranged from 1.2 to 8.2% in relative abundance. *Clostridioides* was detected in 10 samples and not detected by REPS during Weeks 2 and PM₁₀ during Week 1. *Jatrophihabitans* was detected by REPS across all weeks but was not detected in the PM₁₀ samples during Weeks 1 and 4 (Fig. 3, Table S2).

Overall, the top 10 genera had different relative distributions between the samplers. When comparing the presence/absence of all the annotated genera, there were some genera that were shared by the paired samplers and others that were detected either by REPS or by the PM₁₀ filter (Fig. 4, Table S2). However, in Week 1, all four genera detected by the PM₁₀ sampler were also detected by REPS, while REPS detected 26 genera in low abundance (Fig. 4, Table S2). Most of the genera that were not shared between the samplers across all sampling periods were rare taxa representing < 1% in relative abundance of the total bacterial population detected.

When comparing the bacterial populations collected by REPS and PM₁₀ sampler, there were differences just based upon composition (Figs. 3 and 4). Thus, the fold-difference in the abundance of the OTUs when detected by REPS vs. PM₁₀ sampler was compared (Fig. 5). This was done to assess if an OTU was more likely to be detected by REPS or the PM₁₀ sampler. No OTUs were significantly more represented ($p > 0.01$) in the PM₁₀ samples compared to REPS samples. However, ten OTUs were significantly more present ($p < 0.01$) in REPS samples compared to the PM₁₀ samples. Five of these OTUs were not annotated at the genus level. The remaining OTUs were associated

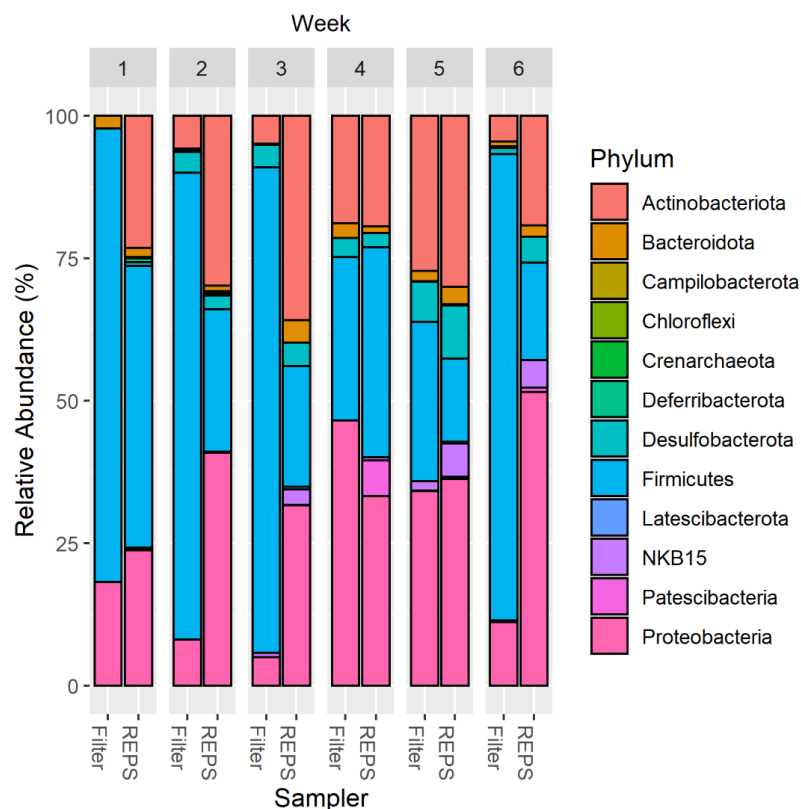
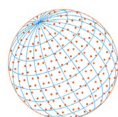


Fig. 2. Relative abundance of detected phyla with REPS and filter sampling.

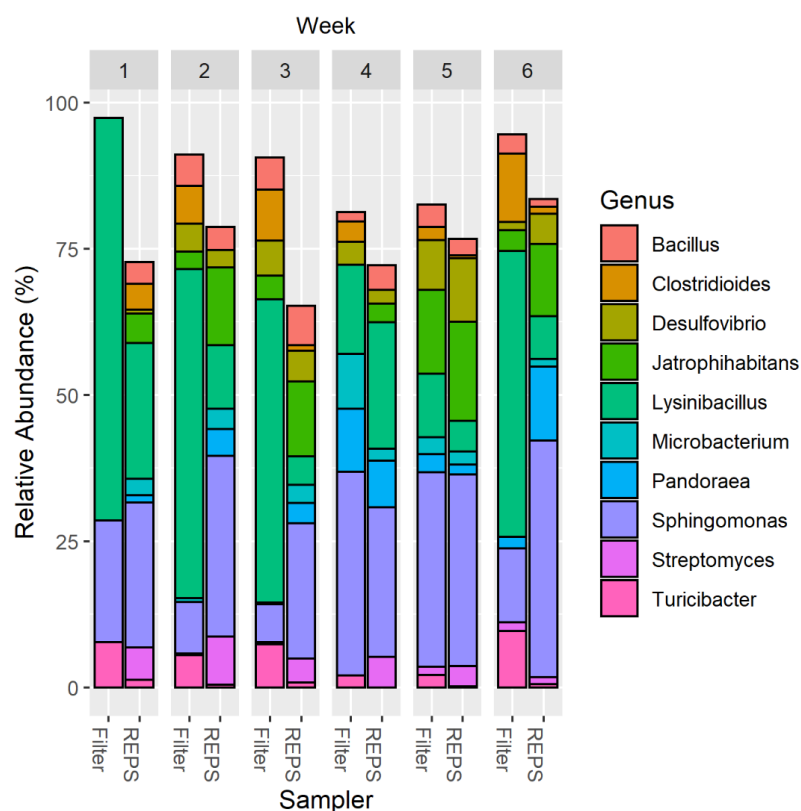


Fig. 3. Relative abundance of top 10 genera detected across REPS and filter sampling. All genera were ranked in their relative abundance and the top 10 were selected. Their distribution was presented across all the samples.

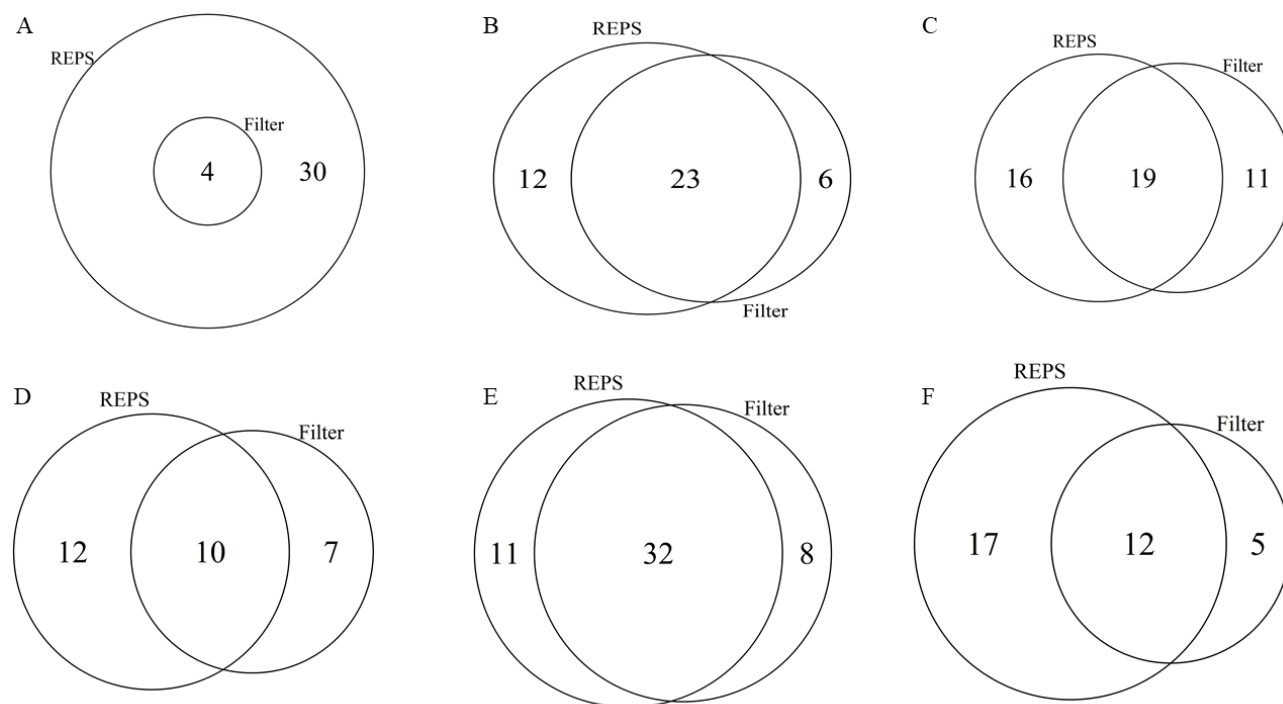
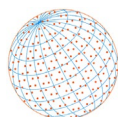


Fig. 4. Comparison of shared and distinct genera by REPS and filter (PM₁₀) sampling for each week. (A) Week 1, (B) Week 2, (C) Week 3, (D) Week 4, (E) Week 5, and (F) Week 6.

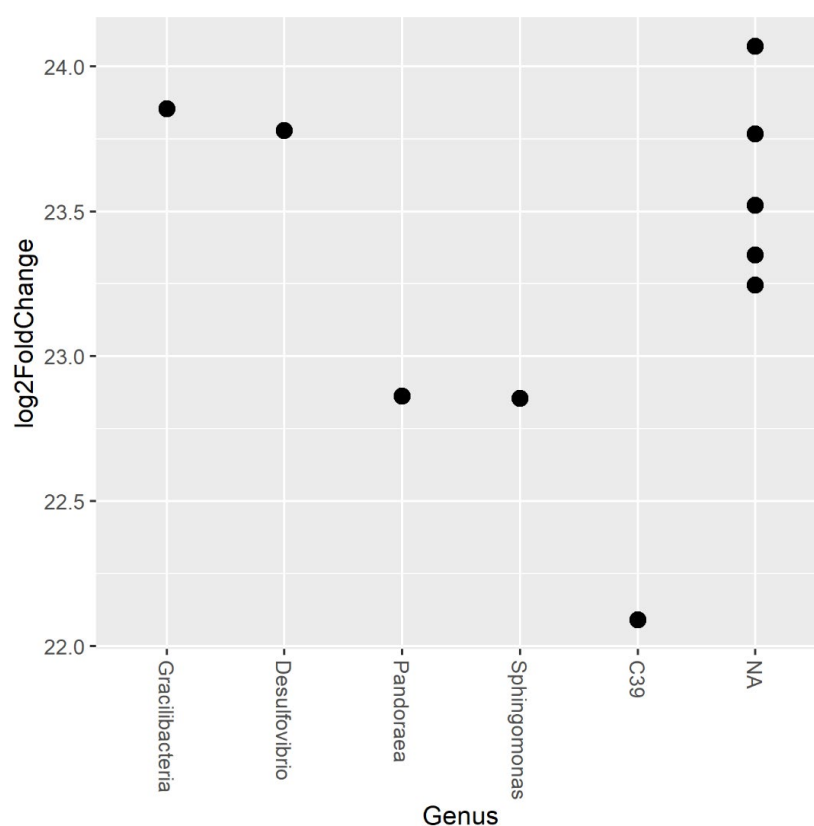
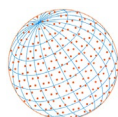


Fig. 5. Differential abundance of OTUs detected with REPS and filter. Ten OTUs were significantly differentially abundant ($p < 0.01$) across all weeks between the samplers. Their assignment at the genus level was presented versus the log-2 fold change of the OTUs. NA were OTUs that were not affiliated with a taxon at the genus level.



with the genera C39 (family *Rhodocyclaceae*), *Desulfovibrio*, *Gracilibacteria*, *Pandoraea*, and *Sphingomonas*. These OTUs were only present in one or two of the samples collected with REPS. The absence of these OTUs in other weekly REPS samples likely resulted in the significant differential abundance when compared to the PM₁₀ samples overall. There was about a 22 to 24 log-2-based fold difference in the abundances of these 10 OTUs out of a total of 1439 OTUs detected. At the genus level, no difference was observed in the abundance of taxa shared by REPS and PM₁₀.

4 DISCUSSION

4.1 Microbial Populations Detected

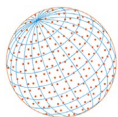
Samples in this study were collected between late March and late May. A year-long study, in which the same PM₁₀ sampler was used, found that during spring many of the bacterial bioaerosols at puy de Dôme were derived from the phyllosphere (i.e., leaf surface) (Tignat-Perrier *et al.*, 2020). The area around the mountain is mostly covered in vegetation or used for agriculture. Furthermore, phyllosphere bacteria are typical members of the atmospheric microbiome (Lindemann *et al.*, 1982; Bowers *et al.*, 2011). Thus, these airborne bacteria were expected in this location.

Sphingomonas, a typical member of phyllosphere and often present in atmospheric assemblages (Mancinelli and Shulls, 1978; Péguilhan *et al.*, 2021), was one of the most abundant genera detected in our study (Fig. 3). The other main bacterial genera detected was *Lysinibacillus*, which is often found in soil (Miwa *et al.*, 2009). Interestingly, strains of this genus are able to nucleate ice, which has implications for precipitation initiation (Failor *et al.*, 2017). Other detected genera in the samples described here such as *Jatrophihabitans* (Kim *et al.*, 2015), *Bacillus* (Martin and Travers, 1989), *Microbacterium* (Lee *et al.*, 2014), *Pandoraea* (Coenye *et al.*, 2000), and *Streptomyces* (Bontemps *et al.*, 2013) are common members of soil/rhizosphere. The detected *Desulfovibrio*, *Clostridioides*, and *Turicibacter* in this study could be derived from livestock (Liu *et al.*, 2016) that frequent the areas near the sampling site. *Streptomyces* has been detected in atmospheric precipitation (Sarmiento-Vizcaino *et al.*, 2018) and is frequently recovered from clouds at the sampling site of this study (Amato *et al.*, 2005, 2017). A typical atmospheric bacterial genus, *Pseudomonas* (Fahlgren *et al.*, 2010), was detected, but not amongst the most relatively abundant bacteria (Table S2). Other genera were detected, but were typically present < 1% in relative abundance across most sampling periods (Table S2). The bacteria detected at the sampling location would reflect local sources and the history of the traveling air masses.

4.2 Comparison of Diversity Determined by the Two Samplers

Overall, the taxa detected by REPS and the PM₁₀ sampler were similar. However, the relative abundances of certain taxa were sampler-dependent. This is consistent with multiple other studies showing that the use of different samplers can lead to a difference in the detection of bacteria (Hoisington *et al.*, 2014; Mbareche *et al.*, 2018; Zhen *et al.*, 2018). Despite the different nature of the two samplers used, i.e., a small passive sampler and a large-volume active filter sampler, only 10 OTUs out of 1439 OTUs detected were significantly more present in REPS samples as compared to the PM₁₀ samples (Fig. 5). It is important to note that there is no standard technique or methodology for sampling bioaerosols for diversity analysis; thus, while sampler-based differences could be expected, it is important to estimate the extent of such difference and their potential causes. The genera present in low relative abundances were driving the main differences between REPS and the filter from the PM₁₀ sampler (Fig. 4, Table S2). A lack of agreement of rarer taxa between different bioaerosol samplers has been found in previous studies (Mbareche *et al.*, 2018; Bøifot *et al.*, 2020), and this was an expected result given the spatial and/or temporal heterogeneity inherent in scarcity.

The α -diversity metrics were significantly greater in the samples collected by REPS compared to the samples collected by the PM₁₀ filter sampler. There could be several reasons for the observed differences. First, there is usually heterogeneity in the bacteria that are detected between co-located samplers. Previous studies have found differences in diversity metrics amongst co-located active samplers and between active and passive samplers operated at the same time (Mhuireach *et al.*, 2016; Mbareche *et al.*, 2018; Bøifot *et al.*, 2020).

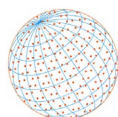


Second, the mechanisms utilized by the samplers may account for the differences observed. For example, active filter-based Button samplers (SKC Inc., Eighty Four, PA) collected samples with higher Shannon's index values than samples collected by passive settling plates in the urban environment (Mhuireach *et al.*, 2016). However, the reverse result was observed in our study: passive REPS yielded higher diversity than the active filter sampler. One of the potential reasons is the collection mechanisms of REPS. REPS uses gravitational settling and an electrostatic field created by permanently polarized PVDF film, which collects greater biomass compared to solely gravity-based passive samplers (Therkorn *et al.*, 2017b). In addition to the sampling principle, the diversity observed may have been affected by the nature of active versus passive sampling. The air being pulled through the filter continuously for 6–7 days would create desiccative conditions and could negatively impact the bacteria collected, especially with the high sampling flow rate used (Zhen *et al.*, 2018). The cells collected by REPS would still be exposed to desiccative conditions while on the sampler's stationary surface, but the desiccation would not be as intense as in the filter because there is no active air flow. It is well-known that desiccation, amongst other variables, would affect the viability of collected microbes (Cox and Wathes, 1995), but this was not the focus of the current study. This desiccation occurs in all long-term sampling studies using filters to profile bacterial aerosol diversity (Smith *et al.*, 2012; Bowers *et al.*, 2013; Wehking *et al.*, 2018; Mescioglou *et al.*, 2019; Tignat-Perrier *et al.*, 2020). It has been shown that during air sampling, including by filters, cells can become damaged and release DNA (Zhen *et al.*, 2013). Since extracellular DNA was not characterized here, some phylotypes collected by the PM₁₀ filter might not have been detected. Because REPS used less harsh sampling mechanisms, it may have been conducive for the detection of rarer taxa.

Additionally, the observed differences in diversity metrics and detected bacterial taxa might be due to potentially different particle sizes captured by the two samplers. While there is no aerodynamic cut-off size for REPS, the active sampler was equipped with a PM₁₀ size-selector, i.e., particles larger than 10 µm were not collected. If there were bioaerosol particles larger than 10 µm, the presence and abundances of phylotypes associated primarily with larger particles might not agree between samplers. For instance, differing distributions of microbes in PM₁₀ and PM_{2.5} have been noted (Bowers *et al.*, 2013; Fan *et al.*, 2019). Furthermore, there were cloud events with droplets larger than 10 µm during the sampling period. Obviously, the PM₁₀ sampler would not have collected cloud droplets > 10 µm. During Weeks 2, 3, and 5 cloud droplets > 10 µm were present 15.1 to 37.8% of the total sampling time (Table 1). Even though the PM₁₀ sampler did not collect these particles, REPS could have collected them, especially by gravitational settling. These unequal sampling efficiencies for particles > 10 µm may have led to the significant difference in observed diversity metrics. Even though the bacterial populations were more even within samples collected by the PM₁₀ sampler, the higher Shannon's diversity and observed OTU values indicated that REPS detailed a richer population of bacteria. This would suggest that there were diverse bacteria associated with particles larger than 10 µm. Even though the liquid water content of clouds was not measured in Weeks 4 and 6, the relative humidity was 100% for 17.8 and 29.1% of the sampling period, respectively. During those periods, cloud droplets > 10 µm were likely present, as in the other Weeks. Furthermore, the cloud droplets would have reached REPS. Even if some biomass was removed from REPS as a result of wash-off, the impact was minimal because the diversity data obtained by REPS is very similar to the active sampler data. Also, REPS was previously found to determine increasing quantities of culturable bacteria as the sampling progressed across a 21-day sampling period, despite varying meteorological conditions (Grogan and Mainelis, 2022). Regardless of the differences and varying conditions highlighted here, our co-located active and passive samplers agreed on the major airborne bacterial taxa, which demonstrates that passive sampling can be used to effectively detail airborne bacterial diversity.

4.3 Limitations, Perspectives, and Conclusions

This study found that the bacterial assemblages sampled using REPS and an active PM₁₀ sampler were very similar, demonstrating that a simple passive sampler yields similar results to more common active bioaerosol sampling methods. Since REPS is a small passive sampler that does not require an air mover or a power source, it can be easily deployed for long durations. Its use could be especially beneficial for sampling air in remote areas that do not have access to power



required for active samplers. In addition, the device is cost-effective. Although the amount of shared OTUs varied between weekly paired samples, the differences could be due to the principles of active and selective (PM₁₀) vs. passive sampling. However, these differences were mostly for low abundance taxa, which are inherently more variable in space and time than dominant taxa. Furthermore, as there is no standard for bioaerosol sampling for diversity analysis, differences among different samplers are expected. Determining the exact reasons for the presence/absence of certain OTUs with the different sampling methods should be investigated further. Multiple sample replicates of an expanded study would be needed to have a more robust statistical analysis. Additionally, comparisons of REPS to other sampling methods, including liquid-based samplers, would provide further insight into how the air sampling principle can affect observed microbial diversity, as has been noted for other samplers (Mbareche *et al.*, 2018), and the physical collection of biomass. Although we determined the culturable and total number of bacteria collected by REPS, this was not done with the active sampler, so no comparisons can be made between the two methods. Overall, this study shows that REPS is an effective passive bioaerosol sampler, requiring no pumps or power source, as it yields similar bacterial diversity results compared to a more commonly used active sampler.

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ADDITIONAL INFORMATION

Data Availability

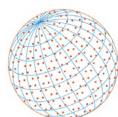
The filter sample fastq files analyzed in this study were generated from a previous study, which were deposited in ftp://ftpadn.ec-lyon.fr/Tignat-Perrier_2020-Stoten_Puy-de-Dome_amplicon_INHALE/. The REPS sequences generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under accession No. PRJNA929526

Supplementary Material

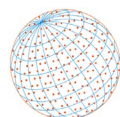
Supplementary material for this article can be found in the online version at <https://doi.org/10.4209/aaqr.220403>

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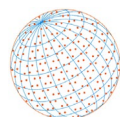
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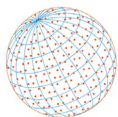
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