Efficiency of bioaerosol samplers: a comparison study

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Abstract Bioaerosols, including bacteria and fungi, are ubiquitous and have been shown to impact various organisms as well as biogeochemical cycles and human health. However, sample collection poses a challenge for aeromicrobiologists and can determine the success of a study. Establishing a standard collection procedure for bioaerosol sampling could help advance the field. We tested the efficiency (number of organisms collected and DNA yield per unit time) of three sampling devices: a membrane filtration device, a liquid impinger, and a portable electrostatic precipitator bioaerosol collector. We compared the efficiency of these three devices for both culture-dependent studies, by enumerating colony forming units (CFUs), and culture-independent studies, by extracting and quantifying total DNA. Our results show that the electrostatic precipitator collected microorganisms significantly more efficiently than the membrane filtration and liquid impingement in both types of studies over the same time interval. This is due to the high flow rate of the device. This work is important and timely because aeromicrobiology is currently restricted by long sampling times and risk of evaporation, desiccation, or freezing during sample, which increases with sampling times. Fieldwork convenience and portability of instruments are an additional challenge for sampling. Using a sampler that can overcome these technical hurdles can accelerate the advancement of the field, and the use of a lightweight, battery-powered, inexpensive, and portable bioaerosol collection device could address these limitations.

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1 Introduction

Microorganisms can become aerosolized, transported and deposited by wind, and $\sim$ 1–20% of these airborne microorganisms remain viable after deposition (Smith 2013; Posfai et al. 2003; Prospero et al. 2005; Deguillaume et al. 2008; Womack et al. 2010; Polymenakou 2012). These airborne microbes, referred to as bioaerosols, can transmit diseases to

new environments (Eames et al. 2009; Li et al. 2007; Roy et al. 2004; Yu et al. 2004), impacting humans, animals, and plants (Shinn et al. 2000; Hayes et al. 2001; Garrison et al. 2003; Weir-Bush et al. 2004; Griffin and Kellogg 2004; Griffin et al. 2016). Recent studies have also demonstrated that airborne microbes deposited into the ocean can contribute to increases in marine bacterial production (Rahav et al. 2016) and N_2 fixation rates (Rahav et al. 2016; 2018), impacting nutrient cycles and possibly the biological carbon pump. Although the importance of studying airborne microbes is clear, environmental aeromicrobiology (the abundance and diversity of airborne microbes in open spaces) is still a relatively unexplored field. However, recent advances in molecular biology, specifically the availability of affordable and rapid genetic sequencing, have advanced the field (Behzad et al. 2015). One of the current limitations of the field is the lack of consensus on optimal sampling methods that provide good sensitivity and specificity for various types of analyses.

Many environmental aerobiology studies that have addressed microbes (bacteria and fungi) or viruses have utilized different collection protocols, devices and analytical assays (Gandolfi et al. 2013; Behzad et al. 2015), and this lack of standardization interferes with the ability to compare data between studies (Gandolfi et al. 2013; Behzad et al. 2015). This is especially true for studies that require quantification techniques such as quantitative polymerase chain reaction (qPCR), epifluorescence microscopy and flow cytometry (Gandolfi et al. 2013), but also applies to qualitative bioaerosol microbial diversity studies (Gandolfi et al. 2013). Until recently, most diversity studies on airborne microbes have used culture-based methods (Griffin et al. 2003; 2007; Prospero et al. 2005). These methods rely on high collection rates, which ensure that the sampling period is short enough to that cells will not be desiccated and will remain viable. Although culture-based studies have been key in advancing aeromicrobiology, only 1–10% of total bacteria and fungi are culturable in the laboratory (Amann et al. 1995), and therefore, these methods shed light only on a small portion of the airborne microbial communities. Recently, there has been a shift to using next-generation sequencing for assessing airborne microbial diversity (Metzker, 2009; Rahav et al. 2016; Mazar et al. 2016; Gat et al. 2017; Mayol et al. 2017; and many more), which provides a more complete representation of the microbial communities (Sharpton 2014) and has the potential to shed unprecedented light on bioaerosol diversity (Peccia et al. 2010). However, application of metagenomic sequencing techniques relies on high DNA yields of sufficient quality, which can be challenging due to the low biomass in most outdoor aerosol samples (between 10^4 and 10^6 microbes m^{-3}) (Lighthart et al. 1997). Therefore, aerosol samples require the collection of large volumes of air in relatively short periods.

Membrane filtration-based devices (MF) and liquid impingement (LI) devices are the most commonly used instruments by aeromicrobiologists (Fahlgren et al. 2011; Fields et al. 1974; Jensen et al. 1992; Kesavan et al. 2010; Griffin et al. 2001; Buttner et al. 1997), who study the microbial community of the air in many environments including indoors, mountains, the ocean and even the lower atmosphere using small unmanned aircraft systems. For example, Chen and Li (2005) used a MF sampler to test *Mycobacterium tuberculosis* levels in an indoor healthcare facility to develop a detection method using quantitative polymerase chain reaction (qPCR). In contrast, Angenent et al. (2005) used LI to detect and identify microorganisms in a hospital therapy pool. Tanaka et al. (2019) and Smith (2013) both used MF-based instruments on mountains to determine high altitude airborne microbial communities. Griffin et al. (2010) used both MF and LI devices at the Mount Bachelor Observatory in Bend, Oregon, and compared the CFUs in samples collected by each instrument. In another high-elevation setting, Bowers et al. (2012) used MF to attain bacterial counts to study bacterial community shifts throughout the seasons. While investigating the annual variability of airborne microbes on the coast of the Baltic Sea, Fahlgren et al. (2010) used MF to quantify CFUs. Similarly, aeromicrobiologists who collect samples over the ocean typically install instruments on the upper deck of research vessels, and the samplers that are currently used include MF-based devices (Griffin et al. 2007; Xia et al. 2014; Mescioglu et al. 2019), impingers (Cho and Hwang 2011) and, less commonly, cyclonic collectors (Mayol et al. 2017). Studies using both conventional (Kellogg et al. 2004; Prospero et al. 2005) and molecular methods (Rahav et al. 2016) investigating airborne microbes during dust events have typically used MF systems. Cyclone-based collectors have also been developed for short-term sampling of aerosols to monitor environmental and occupational bioaerosol

exposure (Tolchinsky et al. 2011). More recently, researchers have used remote-controlled small unmanned aircraft systems (sUAS) to collect airborne microorganisms from the lower atmosphere (Jimenez-Sanchez et al. 2018).

MF collection devices work by pumping air through a membrane filter composed of a chosen material and pore size. MF devices are low-cost, easy to build and operate, and are used widely in aerosol chemistry (Aparicio-Gonzáles et al. 2012) and aeromicrobiology research (Prospero et al. 2005; Brodie et al. 2007; Griffin et al. 2007; Bowers et al. 2011; Jiang et al. 2015; and many more). MF systems used for aeromicrobiology are set to have airflow rates between 10 and 30 l min^{-1} to limit cell stress caused by impaction (Fahlgren et al. 2011). Some of the disadvantages of using MF devices include loss of cell viability with increased collection time due to desiccation (Griffin et al. 2010). It is convenient to use filters in culture-based studies by directly placing the filters with the samples, facing up, onto agar plates. The filters then act as a wick and bring the nutrients up to microorganisms collected onto the filter, allowing viable microbes to develop colonies on the filter. However, it is challenging to use filters in culture-independent studies because it is necessary, yet not trivial, to remove microorganisms from the filter before downstream processing to prevent the inhibitory materials of the filter from reducing assay efficiency (Despres et al. 2007).

LI devices work by pumping air through an inlet into liquid collection medium and can have multiple compartments that separate particles based on size fractions. LI devices have a higher airflow rate than the MF systems, which reduces collection time and has a lower likelihood of cell desiccation since the organisms are kept in liquid during sampling. It is also possible to use the sampled liquid in multiple assays by easily dividing the collected material after homogenization (Griffin et al. 2010). Since cells are already in liquid, the medium can be centrifuged to concentrate cells to a smaller volume and used directly in nucleotide extraction kits. However, LI devices are less convenient to use in the field since they are heavy, need to be autoclaved after each use, and are not recommended for long sampling periods because of evaporation (Grinshpun et al. 1996) or for sampling in high latitudes due to freezing of the liquid medium.

There are also volumetric air sampling devices, like the Burkard sampler or one designed by Pastuszka et al. (2013), that impact aerosols directly onto agar plates instead of onto filters. These samplers have similar flow rates (10–30 l min^{-1}) to MF devices (Pastuszka et al. 2013), but likely increase cell stress and viability loss due to direct contact with the agar (Stewart et al. 1995). Furthermore, these devices are used less commonly than MF-based devices in studies reporting on the total airborne population, but seem to work well for fungal spore collection (Ho et al. 2004; Wu et al. 2004).

A less commonly used sampler is an electrostatic precipitator (EP), which uses a high voltage electric charge to attract airborne particles to a grounded surface. Studies have used a variety of EP collection devices to collect airborne microbes (Grinshpun et al. 1996; Mainelis et al. 1999; 2002a; 2002b; Hogan et al. 2004; Dybwad et al. 2014; Mbareche et al. 2018). Recently, an EP sampler was developed by the United States Department of Agriculture (USDA) that is small, lightweight (0.9 kg), inexpensive, portable and battery-powered (Gast et al. 2003). The battery lifetime of the device is ~ 9 h using standard 9 V batteries (500 mAh) and ~ 21 h using 1200 mAh batteries, and the unit can be adapted to run using a 12 V source or an AC adapter. The USDA EP has a relatively high air flow rate (100 l min^{-1}) and can be used to collect airborne microbes directly onto agar media plates (Gast et al. 2004). The USDA EP can be used in the field during consecutive sampling runs because the device can be disinfected by spraying and the used agar plate can be switched out with a new premade sterile agar plate at the beginning of each run. This specific EP has been used for the detection of the pathogen *Salmonella enteritidis* in poultry house environments alongside an impaction device and a passive exposure collector (Gast et al. 2003). The EP was the most reliable of the devices tested in the *S. enteritidis* detection study (Gast et al. 2003). At present, however, the EP is designed to work only with agar plates, which works for culturing but is not the best "substrate" for genetic material (DNA/RNA) extraction. Thus, the designer of the EP has suggested collecting samples onto a bare metal plate for culture-independent studies and washing off microbes with a PSB solution for downstream processing. This EP has not yet been tested in a culture-independent study where DNA yield is quantified.

Aerobiology studies would be advanced by use of an aerosol sampling instrument that can provide a

solution to the evaporation, desiccation, freezing and fieldwork convenience problems, such as power source, size and weight of collector, and disinfection between runs, and that can be used for both culture-dependent and culture-independent studies. The success of any instrument hinges on the efficiency of the collection (the number of organisms collected per unit time) and the representativeness of the collected assemblage. Here we compare EP, LI and MF devices operating simultaneously in St. Petersburg, Florida, during normal atmospheric conditions to evaluate how they compare in the efficiency of collection for culture-dependent and culture-independent studies over the same time of collection.

2 Methods

2.1 Samplers and the experimental setup

A multi-stage LI (Burkard Manufacturing Co Ltd, UK) with three particle size fractions ($> 10 \mu M$, $10–4 \mu M$ and $< 4 \mu M$) was used in the experiment. A MF system that was assembled in-house (110 V vacuum pump, Fisher Scientific, PVC two-place-manifold, and housing) was used with pre-sterilized filter housings containing 47-mm-diameter, 0.2-μM-pore-size cellulose acetate filter membranes to collect samples (Fisher Scientific, Atlanta, GA). The EP used was manufactured by the United States Department of Agriculture (Gast et al. 2003) with a reported hypothetical flow rate of 100.05 l min^{-1} (Gast et al. 2003), which was used in our calculations. The flow rates of the MF and LI samplers were measured before each sampling event and were 11.491 l min^{-1} and 0.9352 l min^{-1}, respectively.

The LI, MF and EP samplers were tested outdoors at ground level during the daytime at the U.S. Geological Survey in St. Petersburg, Florida. The samplers were set next to one another, and metadata, including start and end time, temperature, humidity and flow rates were collected for each run (Table 1). A particle counter (IQAir Particle Scan Pro) was set up alongside the samplers to assess levels of particulate matter in the air during the sampling runs. The particle counter was used to report six size fraction ranges ($\geq 0.3 \mu M$, $\geq 0.5 \mu M$, $\geq 0.7 \mu M$, $\geq 1.0 \mu M$, $\geq 2.0 \mu M$ and $\geq 5.0 \mu M$). The duration of sample collection for the culture-dependent and culture-independent

experiments was approximately 1 to 2 h and 2 h, respectively. A total of 5 samples were collected for each of the culture-dependent and culture-independent study sample sets over five days, and we did not include replicates within the same run because only a single device for each system was used.

2.2 Culture-dependent experiments

Tryptic soy agar (TSA) media was used to culture the microorganisms. Samples were collected directly onto agar plates with the EP. For the MF samples, filters were removed from the plastic holder and placed onto an agar plate facing up using sterile forceps. The LI was autoclaved between runs and prepared with 7 mL of sterile 1X phosphate saline buffer (PSB) in each of the three compartments. After the run was completed, liquid from the impinger was pipetted into 15-mL tubes and centrifuged to a pellet at $5900 \times g$ for 20 min. The liquid above the pellet was pipetted off until 1 mL remained. The pellet and remaining liquid were then mixed thoroughly by vortexing, and 200 μL was spread onto an agar plate (in triplicates). All the agar plates were incubated at 36° C, and CFUs were enumerated manually after ~ 36 h. For the LI, the averages of the triplicate CFU values were used in the analysis.

2.3 Culture-independent experiments

2.3.1 Membrane filters

The membrane filters were kept in a -20 degrees freezer following collection and until processing (between 3 and 7 days). The filters were placed into autoclaved 47-mm filter holders and backflushed using 15 mL of sterile 1X PSB to remove collected microbes from the filter. The liquid was pelleted at $5900 \times g$ for 20 min, and excess liquid was pipetted off until 1 mL remained. The samples were vortexed, and 200 μL was used to extract DNA.

2.3.2 Liquid Impinger

The PSB solution containing bioaerosols was pipetted from each compartment into separate sterile tubes. The solution was reduced in volume by evaporation to ~ 5 mL. PSB solution (0–2 mL) was added to each sample to a final volume of 7 mL. The PSB

Table 1 Study type, run ID, sampler type (MF = membrane filtration, LI = liquid impinger, EP = electrostatic precipitator and EP_A = electrostatic precipitator with agar plate), start time, humidity, temperature, end time, run duration, flow rate and volume of air (m^3) pumped for the experiments

Study type	Run ID	Sampler	Date	Start time	Humidity	Temp (F)	End time	Run duration (h)	Run time (min)	Flow rate (l min^{-1})	Volume of air (m^3)
Culture-dependent	A	MF	4-May-18	12:51	56.7	83.7	14:52	2:01	121	11.49	1.39
	A	LI	4-May-18	12:46	56.7	83.7	14:52	2:06	126	9.35	1.18
	A	EP	4-May-18	12:47	56.7	83.7	14:54	2:07	127	100.05	12.71
	D	MF	8-May-18	8:12	62.3	74.1	9:13	1:01	61	11.49	0.70
	D	LI	8-May-18	8:13	62.3	74.1	9:13	1:00	60	9.35	0.56
	D	EP	8-May-18	8:14	62.3	74.1	9:13	0:59	59	100.05	5.90
	J	MF	11-May-18	8:05	72	75.1	9:05	1:00	60	11.49	0.69
	J	LI	11-May-18	8:06	72	75.1	9:06	1:00	60	9.35	0.56
	J	EP	11-May-18	8:06	72	75.1	9:06	1:00	60	100.05	6.00
	L	MF	11-May-18	14:55	42.7	89.9	15:55	1:00	60	11.49	0.69
	L	LI	11-May-18	14:55	42.7	89.9	15:55	1:00	60	9.35	0.56
	L	EP	11-May-18	14:55	42.7	89.9	15:55	1:00	60	100.05	6.00
	V	MF	23-May-18	12:42	83	80.6	13:53	1:11	71	11.49	0.82
	V	LI	23-May-18	12:42	83	80.6	13:53	1:11	71	9.35	0.66
	V	EP	23-May-18	12:42	83	80.6	13:53	1:11	71	100.05	7.10
Culture-independent	C	MF	7-May-18	11:51	54.1	82.8	14:00	2:09	129	11.49	1.48
	C	LI	7-May-18	11:53	54.1	82.8	14:00	2:07	127	9.35	1.19
	C	EP	7-May-18	11:54	54.1	82.8	14:00	2:06	126	100.05	12.61
	E	MF	8-May-18	11:15	55.2	80.2	13:15	2:00	120	11.49	1.37
	E	LI	8-May-18	11:17	55.2	80.2	13:15	1:58	118	9.35	1.10
	E	EP	8-May-18	11:20	55.2	80.2	13:15	1:55	115	100.05	11.51
	I	MF	10-May-18	11:09	57.9	80.3	13:13	2:04	124	11.49	1.42
	I	LI	10-May-18	11:10	57.9	80.3	13:13	2:03	123	9.35	1.15
	I	EP	10-May-18	11:11	57.9	80.3	13:13	2:02	122	100.05	12.21
	K	MF	11-May-18	10:58	61.3	81.5	13:00	2:02	122	11.49	1.40
	K	LI	11-May-18	10:59	61.3	81.5	13:00	2:01	121	9.35	1.13
	K	EP	11-May-18	10:59	61.3	81.5	13:00	2:01	121	100.05	12.11
	M	MF	14-May-18	8:05	78	73	10:10	2:05	125	11.49	1.44
	M	LI	14-May-18	8:00	78	73	10:10	2:10	130	9.35	1.22
	M	EP	14-May-18	8:10	78	73	10:10	2:00	120	100.05	12.01
	P	EP_A	17-May-18	10:37	74	78.7	12:40	2:03	123	100.05	12.31
	Q	EP_A	18-May-18	11:38	66.5	81.7	13:42	2:04	124	100.05	12.41
	R	EP_A	18-May-18	13:45	62.6	84.4	15:47	2:02	122	100.05	12.21
	S	EP_A	21-May-18	8:48	80.4	74.9	10:52	2:04	124	100.05	12.41
	T	EP_A	21-May-18	10:54	65	88.6	12:57	2:03	123	100.05	12.31
	U	EP_A	21-May-18	13:02	76.1	74.2	15:03	2:01	121	100.05	12.11

solution with bioaerosols was then pelleted down at 5900 × g for 20 min, and excess liquid was pipetted off until 1 mL remained. The remaining 1 mL was vortexed to homogenize, and 200 μL was used for DNA extraction.

2.3.3 Electrostatic precipitator

The EP was used in two ways. Samples we refer to as EP in the culture-independent study sample set, were collected onto a sterile metal plates without agar, rinsed with 10 mL of PSB after collection, centrifuged to pellet (5900 × g for 20 min), and decanted to 1 mL. The pellet was vortexed with the remaining 1 mL solution, and 200 μL was used to extract DNA. For samples we refer to as EP_A, we collected material onto a TSA agar plate and transferred the material into two DNA extraction tubes, which were later combined, using swabs.

DNA was extracted from all the samples using the Qiagen DNeasy PowerSoil Kit following the manufacturer's protocol until the last step, where instead of using the elution buffer, Qiagen AE was used to elute DNA. DNA was quantified using a Qubit Fluorometer with the Qubit dsDSNA HS Assay Kit and reported in ng μL^{-1}.

2.4 Statistical analysis

All statistical tests were carried out using R. We did not process the data beforehand, except for normalizing the results to the volume of air pumped. It was not necessary to control for additionally measured co-factors because (1) there was no clear relationship between the measured co-factors and the DNA yield (even after log transformation of data) and (2) while humidity and temperature had some effect on CFUs, the effects of the instrumentation were much stronger. Furthermore, the experimental design controlled for these co-factors because each sampler was run at the same time and location alongside the other samples, and therefore, they were subjected to the same range in temperature and humidity. CFU and DNA yield data were both nonparametric; thus, Kruskal–Wallis test was used to test differences between groups. Spearman's test was used to test for correlation between two variables.

3 Results

3.1 Particle counts

The particle concentrations in the air varied throughout the study. The average abundances for the six size fractions and the total particle counts are reported for culture experiments and nucleotide experiments in Supplementary Figs. 1 and 2, respectively. Most of the particles during the experiments were in the ≥ 0.3 μM size range, and therefore , the number of total particles is primarily influenced by particles ≥ 0.3 μM. The total number of particles ranged from 2.3×10^4 to 6.83×10^4 per liter of air (median = 4.04×10^4) during collection of samples for the culture-dependent experiment. Run L and A had the highest number of particles in all size fractions for the culture-dependent experiments. Total particles ranged between 2.35×10^4 and 7.40×10^4 per liter of air (median = 3.42×10^4) during collection for the culture-independent experiments, with the highest number of particles in run C and U. Run U had the highest number of particles in all size fractions, except for particles ≥ 0.3 μM, which were highest in number during run C.

3.2 Culture-dependent experiments comparison

The number of total colony forming units (CFUs) for samples collected with the LI ranged from 2 to 104 colonies (median = 8) (Table 2). The number of bacterial and fungal colonies were also counted separately (Table 2), and when the total number of CFUs was high (84 and 104), the bacteria made up 69% and 84% of the total CFUs, respectively. CFUs per m^{-3} of air for samples collected with the LI ranged from 3 to 186 (median = 12) (Table 2.)

CFUs in samples collected with the MF ranged from 1 to 80 CFUs (median = 5) (Table 2). The number of bacterial colonies ranged from 0 to 55, and there is no data on the fraction of bacteria and fungi in sample L_MF due to the similar appearance of many of the colonies (Table 2). CFUs per m^{-3} of air for samples collected with the MF ranged from 1 to 116 (median = 7) (Table 2).

The EP samples had the largest number of total CFUs grown, ranging from 22 to 929 CFUs (median = 77) (Table 2, Fig. 1). Sample A_EP and L_EP had nine times more CFUs than the samples with the highest number of CFUs from the other samplers (LI sample L_L with 104 CFUs). For the samples with very high counts, it was not possible to accurately differentiate between the bacterial and fungal colonies because the colonies appeared homogenous. CFUs per m^{-3} of air for the EP samples ranged from 3 to 160 (median = 13) (Table 2).

Table 2 Run ID, plate ID, total CFUs, bacterial CFUs, fungal CFUs, total CFUs m^{-3} air, bacterial CFUs m^{-3} air, fungal CFUs m^{-3} air and aerosol collection method

| Run ID	Sampler	Plate ID	Total CFUs	Bacterial CFUs	Fungal CFUs	Total CFUs m^{-3} air	Bacterial CFUs m^{-3} air	Fungal CFUs m^{-3} air
A	MF	A_MF	64	55	9	46.03	3.96	6.47
A	EP	A_EP	929	U	U	73.11	0.00	0.00
A	LI	A_LI	58	40	18	49.22	3.39	15.30
D	MF	D_MF	5	1	4	7.13	0.14	5.71
D	EP	D_EP	77	63	14	13.04	1.07	2.37
D	LI	D_LI	2	0	1	3.56	0.06	2.38
J	MF	J_MF	1	0	1	1.45	0.00	1.45
J	EP	J_EP	40	23	17	6.66	0.38	2.83
J	LI	J_LI	2	0	2	3.56	0.06	2.97
L	MF	L_MF	80	U	U	116.03	0.00	0.00
L	EP	L_EP	956	952	4	159.25	15.86	0.67
L	LI	L_LI	104	87	17	185.34	15.50	30.90
V	MF	V_MF	3	1	2	3.68	0.12	2.45
V	EP	V_EP	22	14	8	3.10	0.20	1.13
V	LI	V_LI	8	1	7	12.05	0.20	10.00

MF Membrane filtration; *LI* liquid impinge; *EP* electrostatic precipitator with no agar

3.3 CFUs relation to particle counts

There was a larger total number of CFUs during experiments that corresponded with the highest particle counts (Fig. 1). There was a significant positive correlation between the total CFUs collected by the LI and particle counts in the $\geq 0.5\ \mu M$, $\geq 0.7\ \mu M$ and $\geq 1.0\ \mu M$ size fractions (Spearman's

Fig. 1 Number of colony forming units (CFUs) collected by the three intruments compared and the total particles in the air during sampling. Each bar represents CFUs in one sample (y-axis on the left), and the colors correspond to which instrument was used to collect the sample. The y-axis on the right corresponds to total particle count during sampling
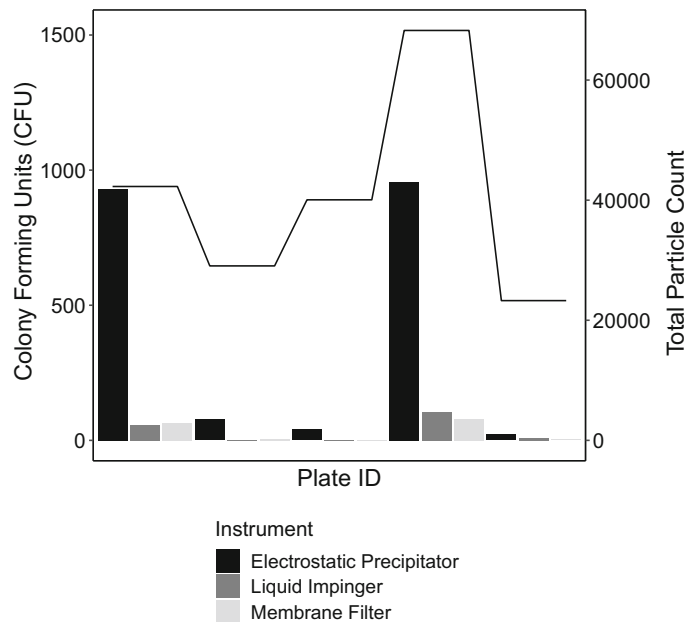
correlation: rho = 0.97 p = 0.0048). CFUs per m^{-3} of air collected with the LI were also correlated to particle counts in the $\geq$ 0.5 μM and $\geq$ 0.7 μM size fractions (Spearman's correlation: rho = 1.00, p = 0.01667). Total CFUs and CFUs per m^{-3} of air collected with the MF were significantly correlated to particle counts in the $\geq$ 2.0 μM size fractions (Spearman's correlation: rho = 1.00, p = 0.01667). The EP also had a larger total number of CFUs and CFUs per m^{-3} of air when total particle counts and particles in the $\geq$ 2.0 μM size fraction were higher, but the correlation was not significant (Spearman's correlation: rho = 0.80, p = 0.080).

3.4 Culture-independent experiments comparison

The DNA concentrations of samples collected using each of the instruments are listed in Table 3 and illustrated in Fig. 2. Since the LI has three compartments, the highest concentration from the three was used for the analysis. DNA was not detectable (< 0.50 ng/mL) by the Qubit dsDSNA HS Assay Kit in 6 out of 28 sample (4 = EP with metal plate, 1 = LI, and 1 = MF). The DNA yield was significantly different between the LI (median = 0.02 μg/mL), MF (median = 0.021 μg/mL), EP with a metal plate (median = 0.05 ng/mL) and EP with an agar plate (median = 0.1355 μg/mL) (Kruskal–Wallis test: H = 13.73, df = 3, p = 0.003296). The EP with an agar plate yielded the highest concentration of DNA, significantly outperforming the EP with a metal plate (p = 0.027), the MF (p = 0.026) and the LI (p = 0.026) (Fig. 2) (Pairwise Mann–Whitney U test). Similarly, when nucleotide concentrations were normalized to the volume of air pumped, there was a significant difference between samplers (Kruskal–Wallis test: H = 8.25, df = 3, p = 0.041), but the difference was only significant between EP with a metal plate and EP with an agar plate (0.04) (Supplementary Fig. 3). The nucleotide concentrations of samples did not significantly correlate to particle counts regardless of the collection instrument used. However, the highest concentrations of DNA throughout the experiment

Table 3 Run ID, sampler method and DNA yield (ug/mL)

Run ID	Sampling method	None	< 0.1 (ug/mL)	$\geq$ = 0.1 (ug/mL)
C	Membrane filter		0.0355	
C	Liquid impinger	Not detectable		
C	Electrostatic precipitator with no agar	Not detectable		
E	Membrane filter		0.0214	
E	Liquid impinger			0.11
E	Electrostatic precipitator with no agar	Not detectable		
I	Membrane filter		0.0205	
I	Liquid impinger		0.02	
I	Electrostatic precipitator with no agar	Not detectable		
K	Membrane filter	Not detectable		
K	Liquid impinger		0.024	
K	Electrostatic precipitator with no agar		0.028	
M	Membrane filter		0.014	
M	Liquid impinger		0.023	
M	Electrostatic precipitator with no agar	Not detectable		
P	Electrostatic precipitator with no agar		0.0877	
Q	Electrostatic precipitator with agar			0.2
R	Electrostatic precipitator with agar			0.121
S	Electrostatic precipitator with agar			0.11
T	Electrostatic precipitator with agar			0.15
U	Electrostatic precipitator with agar			0.244

Fig. 2 DNA concentrations (y-axis) in samples collected, grouped by sampler type (x-axis). EP = electrostatic precipitator with metal plate, EP_A = electrostatic precipitator with agar plate, LI = liquid impinger, and MF = membrane filtration device
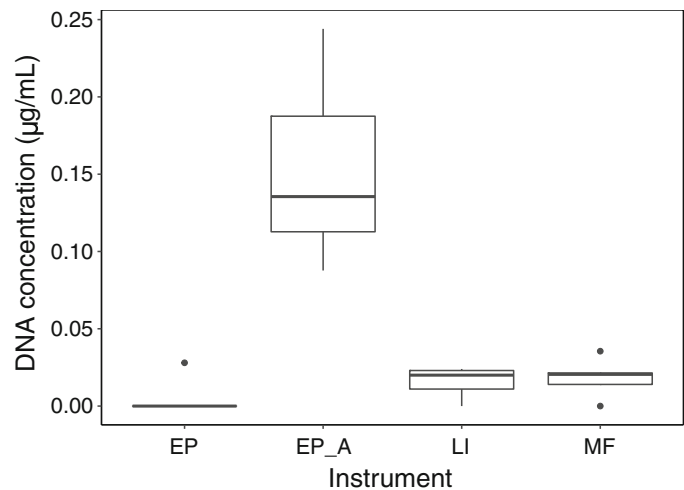
was collected during the run with the highest number of particles in the ≥ 0.5 μM, ≥ 0.7 μM, ≥ 1.0 μM, ≥ 2.0 μM and ≥ 5.0 μM size fractions (Run U).

4 Discussion

Our results show that sampling for the same length of time resulted in a larger total number of CFU's in samples collected by the USDA EP than the LI and MF devices, indicating that the USDA EP was more efficient for culture-dependent methods (Fig. 1, Table 2). CFUs per m^{-3} of air were not significantly different between the instruments, and during two runs (L and V), the EP collected fewer CFUs per m^{-3} than the MF and LI (Supplementary Fig. 4). These results indicate the high flow rate of the EP results in an increase in total microbes collected and, therefore, in the CFUs recovered. Although the LI collected more CFUs per m^{-3} of air during two sampling events, the sampling duration would have to be increased by $\sim$ 2–20-fold to ultimately collect the same absolute number of CFU's as the EP (Table 2). These results indicate that all three sampling devices collect similar numbers of culturable organisms per volume of air, but because the EP has a higher airflow rate, more organisms are retrieved per unit time. This is an important quality because a sampler that can collect more airborne organisms over a shorter time will potentially allow the detection of rare pathogens that otherwise would be missed, and samples can be processed before quality degradation. Moreover, increasing sampling time may not be an option for

studies at certain field sites. While the total number of airborne microbes collected and cultured does not correlate to real-life health impacts, the increased chance to detect rare pathogens is relevant as early detection is important to curtail the spread of contagious disease. Ultimately, if the flow rates of MF and LI can be increased without compromising the viability of airborne microbes, the difference in efficiency between the instruments may not be significant. We suggest that additional tests with higher flow rates for these devices be carried out.

The USDA EP used with an agar plate yielded the highest concentrations of DNA (Fig. 2, Table 3) in our experiment, indicating that it is more efficient and effective than the LI and MF devices Fig. 2, Table 3). While the EP with a metal plate, LI and MF devices almost always yielded between undetectable and < 0.1 μg/mL of DNA (5 out of 6 and 6 out of 6 runs, respectively), the EP with an agar plate consistently (5 out of 6 sampling events) yielded > 0.1 μg/mL of DNA (Table 3, Fig. 2). Similar to the CFU results, the concentration of DNA per m^{-3} of air was not significantly different between the instruments (Supplementary Fig. 3), indicating that the high flow rate of the EP was key in its outperformance of other samplers (more air pumped hence more microbes collected). It would be interesting to determine if the higher DNA yield corresponds to the detection of rare organisms and if there is a difference in the presence of rare organisms between the tested instruments.

It is interesting and important to note the difference between DNA yield when using a metal plate and an agar plate with the EP sampler. One explanation could

be that some microbes grew on the agar plates during the collection time. However, although we used TSA agar in our EP collection device, the plates were processed immediately after sampling and fast enough to prevent substantial growth that could account for the observed differences. There were no visible CFUs on the agar at the end of sampling. Since only $\sim 1\%$ of microorganisms are culturable in the laboratory in optimal conditions, we can rule out that growth could have led to the high DNA yield we observed. For future studies, it may be best to use agar without nutrients for culture-independent studies. Alternatively, we hypothesize that the adhesive nature of the agar is effective in trapping particles with associated microorganisms and preventing them from desiccating, whereas the metal plate does not have the same effect and in fact, particles may bounce off the plate. Additionally, we hypothesize that washing the metal plate with PSB did not recover as many microorganisms as swabbing the agar plates to obtain the DNA. It would be interesting to use liquid (similar to the LI system) instead of agar for a more direct comparison of the effect of trapping or bouncing of the different collection alternatives.

Bioaerosols are found in indoor (Tringe et al. 2008; Kembel et al. 2012; Rintala et al. 2008; Adams et al. 2014; Dunn et al. 2013) and outdoor environments (Kellogg and Griffin, 2006; Griffin et al. 2007; Katra et al. 2014; Rahav et al. 2016; Gat et al. 2017; Mayol et al. 2017) and may impact both human health (Kellogg et al. 2004; Sultan et al. 2005; Brodie et al. 2007; Oh et al. 2014; An et al. 2014) and natural ecosystems (Sharoni et al. 2015; Rahav et al. 2016, 2018). From a public health perspective, it is especially important to monitor and manage the air quality of environments with high bioaerosol exposure (Bragoszewska 2019; Bragoszewska 2020; Gamero et al. 2018) or areas that are frequented by populations vulnerable to bioaerosols (Shinn et al. 2015). Adverse health effects associated with bioaerosol exposure is reportedly higher for workers in certain working environments including waste-sorting plants (Bragoszewska 2019; Bragoszewska 2020) and landfills (Gamero et al. 2018). Bragoszewska (2020) and Gamero et al. (2018) both reported high concentrations of Aspergillus genera in a waste-sorting plant and in a landfill, respectively. Another population that is vulnerable to bioaerosols are children, and Shin et al. (2015) reported a diverse array of human associated airborne bacteria in childcare facilities in Seoul, Korea. Despite the importance of monitoring and managing bioaerosols, particularly given of future decreases in air quality and increasing desertification, there are no standardized methods of studying bioaerosols (Behzad et al. 2015). This makes conducting new aeromicrobiology studies difficult due to issues related to replicating, interpreting and comparing existing studies (Behzad et al. 2015). Because the biomass of airborne organisms in aerosol samples is low, one of the most challenging aspects of aeromicrobiology studies is sample collection and establishing an efficient (i.e., reduction in time and complexity of operation) and effective (i.e., obtaining an accurate and representative assessment of organisms in the air) collection instrument would help advance the field.

Although previous studies have compared different collection instruments in parallel, this is the first to compare the EP recently manufactured by the USDA (Gast et al. 2003) and two most commonly used collection devices (LI and MF). We found higher yield using the USDA EP with agar plates for both culture-based (quantifying CFUs; Fig. 1, Table 1,2) and culture-independent (quantifying DNA concentrations; Fig. 2, Table 1,3) methods. The main driver for the increase in yield is the higher flow rates and effective capture efficiency generated by strong electrostatic attraction of the EP compared to the LI or FM samplers. The EP is also relatively lightweight, battery-powered, inexpensive and portable. However, if other devices can achieve higher airflow rates without compromising trapping efficiency, they may be as effective since the number of airborne microbes detected when normalizing to the volume of air pumped is similar for all instruments tested here. One of the limitations of this study was not including a sampling instrument with an airflow rate similar to the USDA EP, and future comparison studies may consider including other commonly used bioaerosol sampling devices with similar airflow rates. Future studies should also increase sampling size and include replicates if access to multiple devices of the same kind is possible. Finally, it would be of great interest to test the relationship between sampling instrumentation choice and resulting microbial community structure. In the case that instruments capture different parts of the microbial community, different sampling methods could be used in parallel to gain a more

complete picture of the airborne microbial community structure.

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