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Temporal Variability of the Bioaerosol Background at a Subway Station: Concentration Level, Size Distribution, and Diversity of Airborne Bacteria

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Naturally occurring bioaerosol environments may present a challenge to biological detection-identification-monitoring (BIODIM) systems aiming at rapid and reliable warning of bioterrorism incidents. One way to improve the operational performance of BIODIM systems is to increase our understanding of relevant bioaerosol backgrounds. Subway stations are enclosed public environments which may be regarded as potential bioterrorism targets. This study provides novel information concerning the temporal variability of the concentration level, size distribution, and diversity of airborne bacteria in a Norwegian subway station. Three different air samplers were used during a 72-h sampling campaign in February 2011. The results suggested that the airborne bacterial environment was stable between days and seasons, while the intraday variability was found to be substantial, although often following a consistent diurnal pattern. The bacterial levels ranged from not detected to 10^3 CFU m^{-3} and generally showed increased levels during the daytime compared to the nighttime levels, as well as during rush hours compared to non-rush hours. The airborne bacterial levels showed rapid temporal variation (up to 270-fold) on some occasions, both consistent and inconsistent with the diurnal profile. Airborne bacterium-containing particles were distributed between different sizes for particles of >1.1 μm, although ~50% were between 1.1 and 3.3 μm. Anthropogenic activities (mainly passengers) were demonstrated as major sources of airborne bacteria and predominantly contributed 1.1- to 3.3-μm bacterium-containing particles. Our findings contribute to the development of realistic testing and evaluation schemes for BIODIM equipment by providing information that may be used to simulate operational bioaerosol backgrounds during controlled aerosol chamber-based challenge tests with biological threat agents.

Subway transportation systems are found in at least 133 cities worldwide, transporting about 200 million passengers daily (www.uitp.org). Subway stations are typically confined and crowded underground public environments that may be regarded as potential bioterrorism targets.

A major challenge with bioterrorism incidents involving aerosolized biological threat agents is that exposure to even lethal doses will in most cases not induce any immediate symptoms. Thus, in the absence of on-site biological detection-identification-monitoring (BIODIM) capabilities, the public will most likely remain unaware of such potential health hazards until symptomatic individuals seek medical assistance up to several days later. Such a time frame can result in exposed individuals traveling worldwide before an alarm is raised, thus complicating postincident response efforts and potentially leading to the unnecessary loss of human life.

An urgent need for early-warning BIODIM systems capable of operating in complex environmental backgrounds has been expressed by both military and civilian authorities, exemplified by the Joint Biological Point Detection System (JBPDS) program of the U.S. Department of Defense (www.defense.gov) and the BioWatch Generation-3 program of the U.S. Department of Homeland Security (www.dhs.gov). Early-warning BIODIM systems may allow for timelier implementation of effective countermeasures (e.g., containment, evacuation, and prophylactic treatment with medical countermeasures) which could contribute to reduce the consequences of bioterrorism incidents.

In operational environments, the natural aerosol background will challenge BIODIM systems with a dynamic and complex mixture of both biological and nonbiological airborne particulate matter that could interfere with the system's performance. The natural occurrence of similar but nonpathogenic environmental relatives of biological threat agents may lead to false-positive or -negative detection responses by the BIODIM system. Currently, few if any available systems have been able to meet all of the user requirements regarding rapid, robust, reliable, cost-effective, sensitive, and specific surveillance of biological threat agents in different operational environments (2).

Obtaining more-detailed information about various bioaerosol backgrounds may contribute to provide an overview of bioaerosol background-related conditions that will be encountered by BIODIM systems. Such information may assist in the development and testing and evaluation (T&E) of BIODIM equipment by allowing more-realistic operational conditions to be taken into account.

Airborne bacteria have been surveyed at subway stations in several countries around the world, including the United States (3, 4), Japan (5), South Korea (6–8), China (9), Russia (10), Egypt (11), the United Kingdom (12), Hungary (13, 14), Iran (15), and Norway (16). However, certain characteristics of the airborne bac-

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terial environments encountered at subway stations have not previously been addressed in detail, including the size distribution of airborne bacterium-containing particles and the temporal variability of the concentration level, size distribution, and diversity of airborne bacteria.

The current work investigated airborne bacteria at the Nationaltheatret subway station in Oslo, Norway, aiming at providing detailed information concerning the temporal variability of airborne cultivable bacteria during a 72-h continuous-sampling campaign in the winter season of 2011. Three air samplers were used to obtain data sets with different properties, including (i) particle size-resolved bacterial concentration levels and diversity data using an Andersen six-stage cascade impactor, (ii) concentration levels with a high temporal resolution using a MAS-100 high-volume impactor, and (iii) concentration levels and diversity data using a SASS 3100 high-volume electret filter sampler, in accordance with a sampling scheme that was used during a previous study at the same station in the spring, summer, and fall seasons of 2010 (16). The airborne bacterial diversity was investigated by analyzing representative bacterial isolates using the Biotyper 3.0 MALDI-TOF MS microbial identification system. The sampling campaign also included meteorological and total particle measurements using a VXT520 weather station and an APS3321 aerodynamic particle sizer, respectively.

MATERIALS AND METHODS

Study location. The study was conducted at the Nationaltheatret subway station in Oslo (ca. 600,000 inhabitants), Norway. The subway transportation network in Oslo has six bidirectional lines which all pass through the Nationaltheatret station, transporting over 70 million people annually. The underground station consists of a single double-tracked tunnel hall housing both the eastbound and westbound routes. On average, one train departs every minute during the operating hours of the station. No heating, ventilation, and air conditioning (HVAC) or passenger screening door (PSD) systems are installed at the station. A previous survey of the airborne bacterial environment at the same station (May to September 2010) has been reported (16).

The sampling campaign was initiated on 14 February 2011 at 09:00 and ended on 17 February 2011 at 09:00 after 72 h of continuous sampling activities at the station. The air sampling and monitoring equipment were colocated in the middle of the westbound concourse about 4 m from the train tracks. Throughout the campaign, the subway lines were running, with (i) increasing train frequency from 05:10 (first train) to 06:00, (ii) constant train frequency from 06:00 to 22:00, and (iii) decreasing train frequency from 22:00 to 01:15 (last train). The station was nonoperative and closed to the public from 01:15 to 05:10. On some occasions during this period, maintenance personnel were working at the station and diesel-powered maintenance trains were operating in the adjacent tunnel network.

Bioaerosol collection. Air samples were collected during the sampling campaign using three different instruments, as follows: (i) an Andersen six-stage cascade impactor (28.3 liters of air per minute [lpm], TE-10-800; Tisch Environmental, Cleves, OH), (ii) a MAS-100 high-volume single-stage impactor (100 lpm; Merck, Billerica, MA), and (iii) an SASS 3100 high-volume electret filter sampler (300 lpm; Research International, Monroe, WA). The Andersen sampler was used to obtain particle size-resolved airborne cultivable-bacterial concentration levels by sampling for 20 min at the start of every h. The Andersen sampler separates aerosols based on their aerodynamic diameter by cascade impaction onto cultivation plates in six size-resolved stages, as follows: stage 1, >7.1 μm; stage 2, 4.7 to 7.1 μm; stage 3, 3.3 to 4.7 μm; stage 4, 2.1 to 3.3 μm; stage 5, 1.1 to 2.1 μm; and stage 6, 0.65 to 1.1 μm. The airflow through the Andersen sampler was monitored using a mass flow meter (TopTrak 826; Sierra Instruments, Monterey, CA). The MAS-100 was used to obtain airborne cultivable bacterial concentration levels with a higher temporal resolution than the Andersen sampler by sampling for 2.5 min at the start of every 10-min period. The MAS-100 was scheduled to continue sampling until 17 February at 09:00, but due to a battery problem, the sampling had to be discontinued on 16 February at 11:00. The SASS 3100 obtained airborne cultivable bacterial concentration levels by sampling for 2 h per sample, generating three different sample types, as follows: (i) nighttime samples (03:00 to 05:00) in the station, (ii) daytime samples (07:00 to 09:00) in the station, and (iii) daytime samples (07:00 to 09:00) at a square adjacent to the station's entrance (outdoor reference location). Two SASS 3100 samplers were used to allow simultaneous air sampling in the station and at the outdoor reference location.

The air samplers were mounted on tripods with inlet heights of about 1.5 m. The samplers were disinfected with ethanol (70%) between samples to avoid cross-contamination. Field blanks were generated by mounting cultivation plates (Andersen and MAS-100) or filters (SASS 3100) without sampling air and subjecting them to the same analyses as the air samples. The Andersen and MAS-100 impactors were operated with Reasoner's 2a (R2A) (Oxoid, Cambridge, United Kingdom) plates supplemented with 100 μg ml^{-1} cycloheximide (Sigma-Aldrich, St. Louis, MO) (R2Ac) to avoid fungal growth. Air samples collected on SASS 3100 filters were extracted into liquid using an extraction buffer (phosphate-buffered saline with 0.05% Triton X-100 [pH 7.4]) and the SASS 3010 extractor instrument (Research International) according to the manufacturer's instructions. The filter extracts (100 μl) were diluted as needed with extraction buffer and plated as triplicates on R2Ac plates. A similar procedure, but including a heat shock (75°C for 20 min), was also performed on the SASS 3100 filter extracts to exclusively enumerate aerobic bacterial spores. The incubation of all cultivation plates was performed at 30°C for 48 h before the colonies were enumerated. The results obtained from SASS 3100 samples were expressed as an average of the triplicate cultivation plates, while the results from the Andersen and MAS-100 were corrected using the standard positive-hole correction method (17). The airborne bacterial levels were expressed as CFU per cubic meter of air (CFU m^{-3}). The limit of detection (LOD) was 6 CFU m^{-3} for the SASS 3100 cultivation assay, corresponding to the observation of at least one CFU on each of the triplicate cultivation plates. The LODs for the Andersen and MAS-100 were 2 and 4 CFU m^{-3}, respectively.

Total particle and meteorological data collection. The total particle concentration level and size distribution at the station were monitored with an APS 3321 aerodynamic particle sizer (TSI, Shoreview, MN). The APS 3321 was positioned on a table with an inlet height of about 1.3 m. The data were logged at 10-s intervals in 51 channels for particles with an aerodynamic diameter between 0.5 and 20 μm and reported as particles per cubic meter of air (particles m^{-3}). Meteorological parameters, including temperature, humidity, wind speed, and wind direction, were monitored using a VXT520 weather station (Vaisala, Helsinki, Finland) mounted on a tripod at a height of about 1.5 m. The number of people on the station's westbound concourse was regularly counted, averaged over a 30-min period, and reported as 0, 1 to 10, 10 to 50, 50 to 100, or >100 passengers. The outdoor meteorological conditions during the sampling campaign were retrieved from the Norwegian Meteorological Institute (www.yr.no).

Bacterial isolation. Representative selections of morphologically distinct bacterial colonies were isolated from 17 Andersen and 9 SASS 3100 samples. The triplicate primary cultivation plates from each SASS 3100 sample were considered together as one sample when colonies were selected. For each Andersen sample, the six size-resolved primary cultivation plates were considered separately during colony selection. Five daily samples were included for the Andersen sampler, two rush hour samples (08:00 and 16:00), two non-rush hour samples (12:00 and 21:00), and one nighttime sample (04:00). Two additional single samples were also included, (i) a morning sample on 14 February (09:00), since the sampling campaign was initiated after the peak morning rush on the first day, and

(ii) a nighttime sample on 16 February (03:00), when the airborne cultivable bacterial level was temporarily increased compared to the general nighttime level.

The selected colonies were transferred from the primary cultivation plates to fresh R2A plates (secondary cultivation plates) and incubated at 30°C for 48 h. This process was repeated twice or until pure isolates were observed by subculturing from the secondary cultivation plates. The final bacterial isolates were stored at −80°C in brain heart infusion broth (Oxoid) supplemented with 18% glycerol (Merck).

MALDI-TOF MS. The bacterial isolates were classified using the Biotyper 3.0 microbial identification system (Bruker Daltonics, Bremen, Germany) coupled with a MicroFlex LT matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) instrument (Bruker Daltonics), in accordance with the manufacturer's standard direct transfer method. The bacterial isolates were recovered from frozen stocks and cultivated on Trypticase soy agar (TSA) plates (Oxoid) at 30°C for 24 to 48 h before single colonies were transferred onto an MSP 96 ground-steel target (Bruker Daltonics) as triplicates. The α-cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker Daltonics) was prepared in accordance with the manufacturer's recommendations and overlaid on each target spot (1 μl). The Biotyper 3.0 was run in automatic classification mode, and the reference database used was the Bruker Taxonomy database (version 3.3.1.0, containing 4,613 library entries), coupled with the security-relevant add-on database (Bruker Daltonics). The Biotyper 3.0 reports classification score values (SV) of between 0 and 3, which are generally interpreted to suggest (i) probable species identification if the SV is ≥2.3, (ii) secure genus identification and probable species identification if the SV is ≥2.0, (iii) probable genus identification if the SV is ≥1.7, and (iv) no reliable identification if the SV is <1.7. Bacterial isolates that failed to generate an SV of ≥2.0 during the first analysis round were subsequently cultured and analyzed again.

Statistical analysis. The results were subjected to statistical analyses using SigmaPlot version 12.3 (Systat Software, Inc., San Jose, CA). Normality testing was done with the Shapiro-Wilk test, and depending on whether the normality and equal variance criteria were fulfilled or not, significance testing was performed with the Student *t* test or the Mann-Whitney rank-sum test, respectively. Consistencies regarding the temporal variation both within and between the bacterial and particle concentration level data sets were investigated using the Pearson product-moment correlation coefficient. The Pearson chi-square test was used for the bacterial diversity (categorical) data sets. The significance level was set at a P value of <0.05 for all statistical tests.

RESULTS

Airborne cultivable bacterial concentration level. During the sampling campaign (14 February at 09:00 to 17 February at 09:00), 72 and 300 air samples were collected at the subway station by the Andersen six-stage cascade impactor and the MAS-100 high-volume single-stage impactor, respectively. The average bacterial levels reported by the Andersen and MAS-100 were 377 ± 227 CFU m^{-3} (mean ± standard deviation) and 378 ± 288 CFU m^{-3}, respectively. Both samplers showed significant correlation (r = 0.86) concerning the temporal variation of the airborne bacterial level during the campaign and revealed several consistent diurnal trends, i.e., (i) the daytime level was higher than the nighttime level, (ii) during the daytime, the morning (~07:00 to 10:00) and afternoon (~15:00 to 18:00) rush hours showed higher levels than the noon (~10:00 to 15:00) and evening (~18:00 to 00:00) non-rush periods, and (iii) following the afternoon rush hours, the bacterial level typically decayed throughout the evening and night, with a diurnal minimum just before the train activity recommenced in the morning (Fig. 1).

During the nighttime period, when the bacterial levels were typically low and decaying, two atypical events occurred, and both samplers reported (i) a temporary strong increase in the bacterial level on 16 February between 02:00 and 04:00 and (ii) a similar but modest increase on 15 February at about 01:00 (Fig. 1). When these two atypical events were considered together, the Andersen sampler reported a significantly higher (4-fold) bacterial level (395 ± 213 CFU m^{-3}) during the events than during the same periods on nonevent days (98 ± 38 CFU m^{-3}). Similar results were observed with the MAS-100, which reported a significantly higher (7.2-fold) bacterial level during the events (819 ± 225 CFU m^{-3}) than during nonevent periods (113 ± 100 CFU m^{-3}).

Despite the nighttime atypical events and for both samplers, significant correlations were observed when the individual diurnal periods were compared to each other (Andersen, r = 0.61 to 0.85, and MAS-100, r = 0.46 to 0.71). Based on the day-to-day diurnal consistencies, the Andersen and MAS-100 results from individual sampling days were each merged into a single diurnal period averaged hourly (Table 1). The two nighttime atypical events were not included in the averaged data.

To compare the daytime and nighttime bacterial levels at the station, the following time period definitions were used: daytime, between 05:20 and 00:00, and nighttime, between 00:00 and 05:20. The nighttime-to-daytime boundary was based on the arrival of the first morning trains between 05:10 and 05:20, with the subsequent rapid increase in anthropogenic activities (i.e., trains and passengers). The daytime-to-nighttime boundary was chosen because the passenger counts (Fig. 1) and train frequency decreased rapidly after midnight. The Andersen results showed that the daytime bacterial level (452 ± 198 CFU m^{-3}) was significantly higher (4.2-fold) than the nighttime level (107 ± 68 CFU m^{-3}). Similarly, the daytime level was significantly higher (2.9-fold) than the unfiltered (i.e., including atypical events) nighttime level (154 ± 151 CFU m^{-3}). The MAS-100 results showed that the daytime level (443 ± 275 CFU m^{-3}) was significantly higher (4.3-fold) than the nighttime level (103 ± 102 CFU m^{-3}) and also significantly higher (3.3-fold) than the unfiltered nighttime level (137 ± 187 CFU m^{-3}).

A total of nine air samples were collected with the SASS 3100 high-volume electret filter sampler during the sampling campaign, corresponding to three daily samples, as follows: (i) nighttime in the station (03:00 to 05:00), (ii) daytime at the station (07:00 to 09:00), and (ii) daytime in the outdoor reference location (07:00 to 09:00). The average bacterial levels reported by the SASS 3100 were 493 ± 153, 25 ± 22, and 41 ± 17 CFU m^{-3}, for daytime and nighttime in the station and the daytime outdoor reference, respectively (Table 1). The daytime level in the station was significantly higher (19.4-fold) than the nighttime level and also significantly higher (11.9-fold) than the daytime outdoor level. The bacterial levels reported by the SASS 3100 in the station were not significantly different from those obtained with the Andersen and MAS-100 impactors when these were averaged using only the corresponding sampling periods. However, the nighttime bacterial level reported by the SASS 3100 was, although not significantly different, 1.3-fold and 3.8-fold lower than those reported by the Andersen and MAS-100, respectively.

The spore-specific cultivation analysis performed on the SASS 3100 samples consistently reported airborne bacterial spore levels of less than the LOD (5.5 CFU m^{-3}), except during daytime sampling in the station (7 ± 5 CFU m^{-3}). Still, the daytime level of cultivable bacterial spores corresponded to less than 2% of the total cultivable bacterial level at the station.

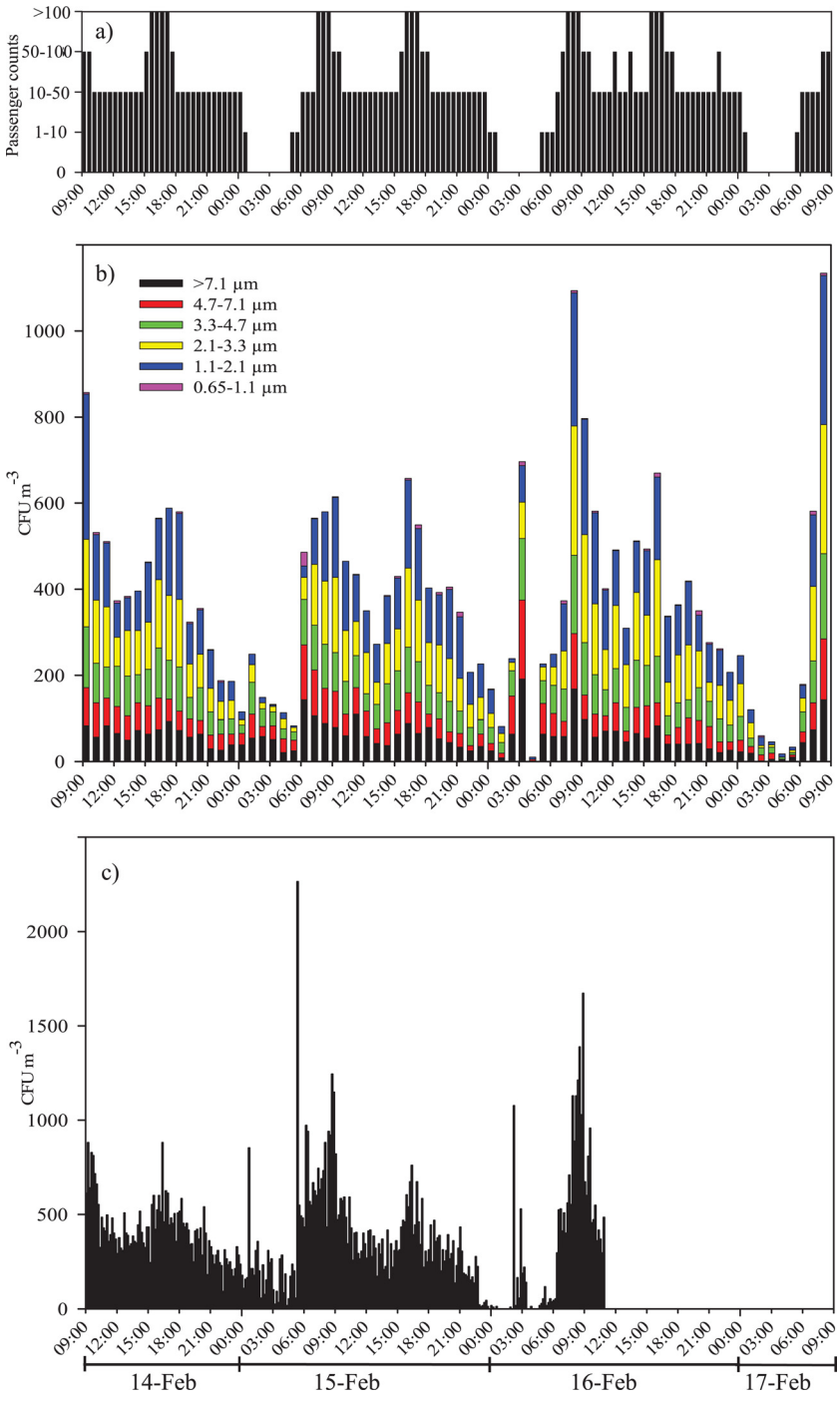

FIG 1 Airborne cultivable bacterial concentration levels and passenger counts during the 72-h sampling campaign at the subway station. (a) Numbers of passengers on the westbound concourse (30-min average). (b) Particle size-resolved airborne bacterial levels based on data from the Andersen six-stage cascade impactor. (c) High-temporal-resolution airborne bacterial levels based on data from the MAS-100 impactor. MAS-100 sampling was discontinued on 16 February at 11:00 due to a power supply failure.

Airborne cultivable-bacterium-containing particle size distribution. The average particle size-resolved bacterial levels reported by the Andersen six-stage cascade impactor were as follows: stage 1 (>7.1 μm), 58 ± 36 CFU m^{-3}; stage 2 (4.7 to 7.1 μm), 50 ± 33 CFU m^{-3}; stage 3 (3.3 to 4.7 μm), 69 ± 38 CFU m^{-3}; stage 4 (2.1 to 3.3 μm), 93 ± 67 CFU m^{-3}; stage 5 (1.1 to 2.1 μm), 105 ± 78 CFU m^{-3}; and stage 6 (0.65 to 1.1 μm), 3 ± 4 CFU m^{-3}. These results showed that the various particle sizes contributed to 17, 15, 19, 22, 26, and ≤1% of the total level, respectively (Fig. 1).

The bacterial levels reported within each particle size stage correlated significantly with the total level (r = 0.33 to 0.95). By

TABLE 1 Concentration levels and particle size distribution of airborne cultivable bacteria and total particles in the subway station[a]

Time period (hh:mm)	Bacterial level (mean CFU m^{-3} ± SD) for indicated sampler and location				Bacterial level (mean % contribution to total bacterial level ± SD) in airborne particle size (μm) stage:						Total particle level (mean particles m^{-3} ± SD)	Particle size distribution (0.5–20 μm) (mean ± SD)[c]	
	In station:		SASS 3100[b]		1 (>7.1)	2 (4.7–7.1)	3 (3.3–4.7)	4 (2.1–3.3)	5 (1.1–2.1)	6 (0.65–1.1)		CMAD (μm)	GSD
	Andersen	MAS-100	In station	Outdoors									
00:00–01:00	176 ± 54	79 ± 81			19 ± 11	15 ± 6	20 ± 2	20 ± 8	25 ± 7	<1	$3.2 \times 10^7 \pm 1.8 \times 10^7$	0.72 ± 0.04	1.56 ± 0.11
01:00–02:00	101 ± 19	111 ± 127			16 ± 5	16 ± 4	25 ± 7	24 ± 6	18 ± 6	<1	$4.0 \times 10^7 \pm 2.6 \times 10^7$	0.70 ± 0.05	1.48 ± 0.12
02:00–03:00	104 ± 44	152 ± 104			23 ± 15	25 ± 9	26 ± 1	9 ± 1	15 ± 13	2 ± 3	$4.0 \times 10^7 \pm 2.3 \times 10^7$	0.78 ± 0.10	1.62 ± 0.22
03:00–04:00	89 ± 43	114 ± 100	25 ± 22		26 ± 11	27 ± 3	25 ± 4	11 ± 1	10 ± 5	1 ± 1	$3.4 \times 10^7 \pm 2.7 \times 10^7$	0.72 ± 0.03	1.45 ± 0.10
04:00–05:00	47 ± 47	67 ± 80			25 ± 6	25 ± 10	23 ± 10	13 ± 4	13 ± 8	<1	$2.1 \times 10^7 \pm 5.8 \times 10^6$	0.70 ± 0.02	1.39 ± 0.07
05:00–06:00	114 ± 82	362 ± 605			30 ± 1	26 ± 7	21 ± 4	14 ± 1	9 ± 8	<1	$2.4 \times 10^7 \pm 8.3 \times 10^6$	0.76 ± 0.05	1.63 ± 0.17
06:00–07:00	304 ± 131	499 ± 276			26 ± 3	20 ± 5	25 ± 2	15 ± 3	11 ± 5	3 ± 3	$3.6 \times 10^7 \pm 1.0 \times 10^7$	0.86 ± 0.08	1.73 ± 0.04
07:00–08:00	506 ± 95	656 ± 171	493 ± 153	41 ± 17	16 ± 2	14 ± 4	18 ± 1	26 ± 3	26 ± 5	1 ± 1	$3.4 \times 10^7 \pm 8.7 \times 10^6$	0.81 ± 0.06	1.67 ± 0.04
08:00–09:00	936 ± 252	1073 ± 294			14 ± 1	13 ± 1	17 ± 1	26 ± 1	29 ± 1	<1	$3.2 \times 10^7 \pm 6.4 \times 10^6$	0.75 ± 0.03	1.61 ± 0.06
09:00–10:00	756 ± 103	666 ± 149			12 ± 1	10 ± 3	15 ± 1	28 ± 3	34 ± 4	<1	$3.7 \times 10^7 \pm 6.3 \times 10^6$	0.80 ± 0.06	1.64 ± 0.04
10:00–11:00	526 ± 48	435 ± 104			11 ± 1	12 ± 2	16 ± 1	27 ± 1	33 ± 3	<1	$3.7 \times 10^7 \pm 5.2 \times 10^6$	0.79 ± 0.05	1.66 ± 0.04
11:00–12:00	449 ± 46	375 ± 67			20 ± 4	12 ± 2	15 ± 1	23 ± 4	29 ± 4	<1	$3.8 \times 10^7 \pm 7.3 \times 10^6$	0.78 ± 0.04	1.64 ± 0.04
12:00–13:00	405 ± 62	357 ± 68			16 ± 1	16 ± 2	18 ± 6	25 ± 5	25 ± 3	<1	$4.3 \times 10^7 \pm 7.1 \times 10^6$	0.82 ± 0.05	1.66 ± 0.05
13:00–14:00	322 ± 46	313 ± 70			14 ± 1	12 ± 3	21 ± 3	26 ± 5	27 ± 5	<1	$4.3 \times 10^7 \pm 1.2 \times 10^7$	0.81 ± 0.06	1.65 ± 0.05
14:00–15:00	431 ± 58	356 ± 94			14 ± 4	14 ± 2	20 ± 3	27 ± 3	25 ± 2	<1	$4.1 \times 10^7 \pm 7.7 \times 10^6$	0.79 ± 0.05	1.62 ± 0.06
15:00–16:00	462 ± 26	446 ± 111			13 ± 2	14 ± 1	20 ± 1	23 ± 1	29 ± 1	<1	$4.1 \times 10^7 \pm 7.3 \times 10^6$	0.79 ± 0.06	1.63 ± 0.07
16:00–17:00	631 ± 46	597 ± 113			13 ± 1	10 ± 2	18 ± 2	30 ± 3	28 ± 3	<1	$4.3 \times 10^7 \pm 1.0 \times 10^7$	0.76 ± 0.05	1.59 ± 0.07
17:00–18:00	492 ± 110	408 ± 108			13 ± 2	9 ± 3	15 ± 2	25 ± 1	37 ± 6	<1	$4.5 \times 10^7 \pm 8.4 \times 10^6$	0.78 ± 0.06	1.63 ± 0.07
18:00–19:00	449 ± 94	421 ± 86			14 ± 4	9 ± 1	17 ± 1	27 ± 2	33 ± 1	<1	$4.4 \times 10^7 \pm 9.1 \times 10^6$	0.78 ± 0.07	1.62 ± 0.08
19:00–20:00	378 ± 40	332 ± 67			14 ± 3	13 ± 1	14 ± 2	28 ± 3	31 ± 3	1 ± 1	$4.5 \times 10^7 \pm 8.8 \times 10^6$	0.79 ± 0.09	1.62 ± 0.08
20:00–21:00	370 ± 25	316 ± 107			14 ± 3	10 ± 4	20 ± 2	24 ± 1	31 ± 7	2 ± 1	$4.4 \times 10^7 \pm 1.0 \times 10^7$	0.80 ± 0.11	1.62 ± 0.09
21:00–22:00	294 ± 38	265 ± 71			11 ± 11	13 ± 4	19 ± 3	20 ± 3	36 ± 4	2 ± 1	$4.3 \times 10^7 \pm 1.4 \times 10^7$	0.79 ± 0.11	1.61 ± 0.10
22:00–23:00	218 ± 31	196 ± 81			11 ± 2	12 ± 6	20 ± 1	26 ± 3	30 ± 5	1 ± 1	$3.9 \times 10^7 \pm 7.7 \times 10^6$	0.75 ± 0.06	1.58 ± 0.11
23:00–00:00	206 ± 17	130 ± 116			16 ± 3	12 ± 2	18 ± 2	24 ± 2	30 ± 4	<1	$3.3 \times 10^7 \pm 1.0 \times 10^7$	0.73 ± 0.04	1.58 ± 0.10

[a] Data from the 72-h sampling campaign are presented as 1-h averages over the diurnal period, unless otherwise indicated.
[b] Two-hour averages: the SASS 3100 sampled for 2 h during the nighttime (03:00 to 05:00) and daytime (07:00 to 09:00).
[c] CMAD, count median aerodynamic diameter; GSD, geometric standard deviation.

comparing the different stages to each other, significant correlations were also observed between stages ($r = 0.38$ to 0.94), except when stage 4 or 5 was compared to stage 6 ($r = 0.17$ and $r = 0.20$, respectively). The general trend was that the correlation strength was inversely related to the distance between the stages (i.e., neighboring stages correlated better than distant stages) (Fig. 1). The particle size-resolved bacterial levels also showed significant correlation ($r = 0.63$ to 0.89) between sampling days (Fig. 1), except for stage 2 on 14 February compared to 16 February and for stage 6 on all sampling days. The weak correlation observed between 14 February and 16 February for stage 2 could be explained by a higher variability in this stage on 16 February (Fig. 1). The weak correlations observed between all sampling days for stage 6 could be explained by frequent observations of bacterial levels that were ≤LOD, with sporadic increases on some occasions (Fig. 1). Based on the observed general temporal consistency concerning the various particle sizes' contributions to the total level, the results from individual sampling days were merged into a single diurnal period averaged hourly (Table 1).

Compared between sampling days, the size distribution of airborne bacterium-containing particles showed no significant differences, and similar results were obtained when different daytime periods (rush hours versus non-rush hours) were compared to each other (see Fig. S1 in the supplemental material). Taken together, these results suggested that the size distribution of airborne bacterium-containing particles at the station showed limited day-to-day variation and was also conserved between different daytime periods. The largest temporal variability concerning the size distribution of airborne bacterium-containing particles was consistently observed during two diurnal periods, (i) the daytime-to-nighttime transition period and (ii) the nighttime-to-daytime transition period (Table 1).

When daytime and nighttime periods were compared to each other, significant differences were revealed regarding the size distribution of airborne bacterium-containing particles in the station (Fig. 2). Bacterium-containing particles of between 1.1 and 3.3 μm corresponded to a significantly larger fraction during the daytime (56% ± 4% [mean ± standard deviation]) than at night (30% ± 8%), while a significantly greater fraction of bacterium-containing particles of >3.3 μm was observed at night (70% ± 7%) than during the day (44% ± 3%) (Fig. 2). By taking into account the general trend showing low and decaying bacterial levels at night (Fig. 1), the results suggested that the nighttime shift in size distribution was caused by a reduction in 1.1- to 3.3-μm bacterium-containing particles rather than an increase in >3.3-μm bacterium-containing particles. Taken together, these results suggested that the contributing sources for 1.1- to 3.3-μm bacterium-containing particles were predominantly present in the station only during daytime.

When the size distribution of bacterium-containing particles observed during nighttime atypical events (16 February from 02:00 to 04:00 and 15 February at 01:00) was compared to those observed during the temporally closest daytime periods showing corresponding total bacterial levels (15 February at 23:00, 16 February at 16:00, and 14 February at 21:00, respectively), bacterium-containing particles of >3.3 μm were found to be present as a significantly larger fraction during the nighttime atypical events (79% ± 4%) than during the corresponding daytime periods (44% ± 3%) (Fig. 2). The opposite result was observed for 1.1- to 3.3-μm bacterium-containing particles, which showed a significantly smaller fraction during the nighttime atypical events (21% ± 3%) than during the corresponding daytime periods (56% ± 4%) (Fig. 2). Taken together, these results suggested that the nighttime atypical events were caused by sources that generated a larger fraction of bacterium-containing particles of >3.3 μm and that these sources probably differed from the predomi-

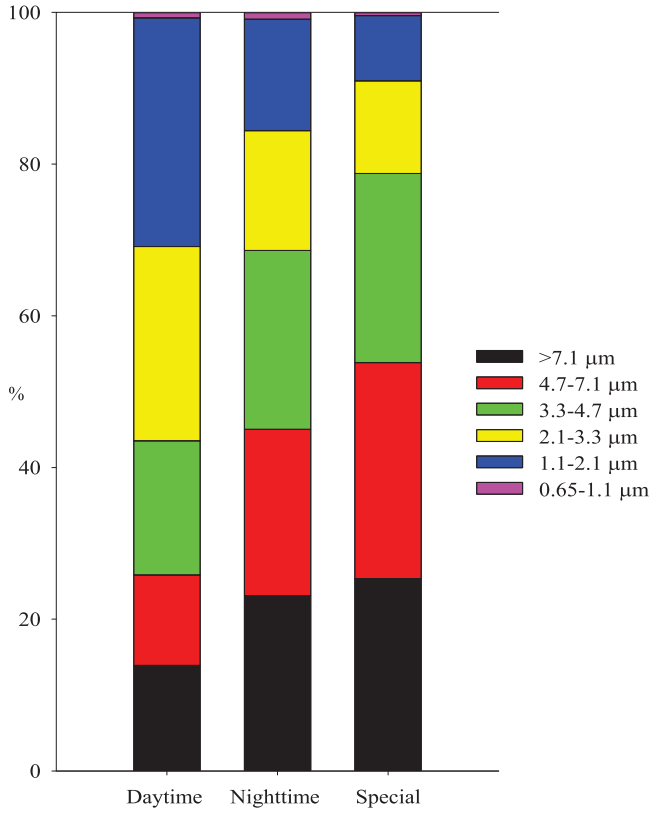

FIG 2 Size distribution of airborne cultivable-bacterium-containing particles at the subway station. Size-resolved airborne cultivable bacterial levels were obtained with the Andersen six-stage cascade impactor, expressed as percentages of the total level and categorized into three groups: daytime, nighttime, and atypical (special) nighttime events (16 February from 02:00 to 04:00 and 15 February at 01:00).

nant daytime sources, which generated a larger fraction of bacterium-containing particles between 1.1 and 3.3 μm.

Airborne cultivable bacterial diversity. The airborne bacterial diversity in the station was investigated by analyzing representative bacterial isolates using the Biotyper 3.0 MALDI-TOF MS system. A total of 1,832 bacterial isolates were recovered from the primary cultivation plates of 17 Andersen and 9 SASS 3100 samples and analyzed using the Biotyper 3.0 standard direct transfer method. The results showed that 1,293 isolates (71% of the total) were successfully classified at the species or genus level. Of the total number of isolates classified, 1,141 and 152 isolates were derived from the Andersen and SASS 3100 samples, respectively. The isolates recovered from the Andersen impactor consisted of 17% Gram-negative bacteria, while only 4% Gram negatives were recovered from the SASS 3100 electret filter sampler.

When considering the total bacterial diversity from both samplers, 39 different bacterial genera belonging to the following four bacterial phyla were observed at the station: *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteriodetes*/*Chlorobi* (Table 2). The predominant bacterial genera were *Micrococcus*, *Rhodococcus*, *Arthrobacter*, *Moraxella*, *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Kocuria*, and *Dermacoccus*, corresponding to the following bacterial species: *Micrococcus luteus*, *Rhodococcus fascians*, *Arthrobacter* spp. (e.g., *Arthrobacter parietis*), *Moraxella osloensis*, *Staphylococcus* spp. (e.g., *Staphylococcus epidermis*), *Bacillus* spp. (e.g., *Bacillus megaterium*), *Pseudomonas* spp. (e.g., *Pseudomonas stutzeri*), *Kocuria* spp. (e.g., *Kocuria rosea*), and *Dermacoccus nishinomiyaensis* (Table 2).

Of the total number of SASS 3100-derived isolates classified, 60% were pigmented (92/152), showing yellow, orange, or pink colony pigmentation, and the predominant species were *M. luteus*, *R. fascians*, and *Rosemonas mucosa*/*K. rosea*, respectively. Of the total number of Andersen-derived isolates classified, 44% were pigmented (507/1,141), showing yellow, orange, or pink colony pigmentation, and the predominant species were *M. luteus*/*A. parietis*/*Kocuria marina*/*Kocuria rhizophila*, *R. fascians*/*Bacillus atrophaeus*, and *R. mucosa*/*K. rosea*, respectively.

The Andersen-derived bacterial diversity showed only limited day-to-day variation (see Fig. S2 in the supplemental material). Similarly, the Andersen-derived data for various daytime periods (morning rush hour, 08:00; noon non-rush period, 12:00; afternoon rush hour, 16:00; and evening non-rush period, 21:00) also corresponded to each other (see Fig. S2). These results suggested that the airborne bacterial diversity at the station was conserved between sampling days, as well as between different daytime periods. The Andersen-derived diversity results were therefore categorized into three groups, (i) daytime, (ii) nighttime, and (iii) nighttime atypical event (16 February at 03:00). Similarly, the SASS 3100-derived diversity results were also categorized into three groups, but the daytime outdoor reference location group replaced the nighttime station atypical event group. The results obtained with both samplers showed that the daytime bacterial diversity at the station differed significantly from the nighttime diversity (Fig. 3).

The Andersen-derived daytime diversity was dominated by *Micrococcus* (37%), followed by *Rhodococcus* (14%), *Arthrobacter* (11%), *Moraxella* (9%), *Staphylococcus* (6%), and *Dermacoccus* (5%), while the nighttime diversity was dominated by *Rhodococcus* (49%), followed by *Arthrobacter* (20%) and *Micrococcus* (8%) (Fig. 3). The diversity observed during the atypical event on 16 February at 03:00 was similar to the diversity of nonevent nighttime samples, being dominated by *Rhodococcus* (45%) and *Arthrobacter* (27%) (Fig. 3). However, no *Micrococcus* isolates were recovered during the nighttime atypical event, while *Pseudomonas* isolates (16%) were abundantly recovered. Interestingly, *Pseudomonas* was not observed during nonevent nighttime periods and was only at low abundance during daytime periods (3%).

The SASS 3100-derived daytime diversity at the station was dominated by *Micrococcus* (37%), followed by *Rhodococcus* (17%), *Staphylococcus* (14%), *Bacillus* (12%), and *Dermacoccus* (6%), while the nighttime diversity was dominated by *Rhodococcus* (46%), followed by *Staphylococcus* (17%), *Arthrobacter* (13%), and *Bacillus* (13%) (Fig. 3). The relative abundances of *Micrococcus*, *Rhodococcus*, and *Dermacoccus* observed with the SASS 3100 were consistent with the Andersen-derived results. However, *Bacillus* and *Staphylococcus* were observed more frequently with the SASS 3100 than with the Andersen impactor, while *Arthrobacter* (1%) and *Moraxella* (1%) were less frequently observed with the SASS 3100 (Fig. 3).

When the SASS 3100-derived daytime diversity in the station was compared to the daytime outdoor diversity, a higher abundance of *Micrococcus* (58%) and lower abundances of *Rhodococcus* (8%), *Staphylococcus* (8%), and *Bacillus* (8%) were observed at the outdoor location (Fig. 3). Additionally, several minor differences were observed for bacterial genera that were generally observed at

TABLE 2 Airborne cultivable bacterial diversity in the subway station

Genus[a]	Species	Occurrence using indicated sampler in:		
		Current study[b]		Previous study, SASS 3100[c]
		Andersen	SASS 3100	
Acinetobacter	*A. johnsonii*, *A. lwoffii*, *A. pittii*, *A. nosocomialis*, *A. schindleri*, *A. towneri*	X	X	
Aerococcus		X		
Agrococcus	*A. jenensis*	X		
Agromyces			X	
Arthrobacter	*A. parietis*, *A. scleromae*, *A. polychromogenes*, *A. oxydans*, *A. sulfonivorans*, *A. chlorophenolicus*, *A. castelli*, *A. crystallopoietes*	X	X	X
Bacillus	*B. megaterium*, *B. atrophaeus*, *B. simplex*, *B. flexus*, *B. pumilus*, *B. cereus sensu lato* group species	X	X	X
Brevibacillus	*B. choshinensis*	X		X
Brevibacterium		X		
Brevundimonas	*B. nasdae*, *B. vesicularis*, *B. diminuta*	X		X
Chryseobacterium		X		
Corynebacterium	*C. flavescens*	X		X
Curtobacterium	*C. flaccumfaciens*		X	X
Dermacoccus	*D. nishinomiyaensis*	X	X	X
Dietzia		X		X
Enterococcus	*E. gallinarum*	X		
Kocuria	*K. rosea*, *K. palustris*, *K. rhizophila*, *K. marina*, *K. polaris*	X	X	X
Lysinibacillus	*L. fusiformis*, *L. sphaericus*	X	X	X
Macrococcus		X		
Massilia	*M. timonae*	X	X	
Microbacterium	*M. phyllosphaerae*, *M. lacticum*	X		X
Micrococcus	*M. luteus*	X	X	X
Moraxella	*M. osloensis*	X	X	X
Ochrobactrum	*O. intermedium*	X		
Paenibacillus	*P. pasadenensis*, *P. amylolyticus*	X	X	X
Paracoccus	*P. yeei*	X		X
Pseudoclavibacter	*P. helvolus*	X		
Pseudomonas	*P. stutzeri*, *P. xanthomarina*, *P. gessardii*, *P. brenneri*, *P. libanensis*	X	X	X
Psychrobacillus		X		
Rhizobium	*R. rubi*	X		
Rhodococcus	*R. fascians*	X	X	X
Roseomonas	*R. mucosa*	X	X	X
Rothia	*R. amarae*	X		X
Sphingobacterium	*S. multivorum*	X		
Sphingobium		X		
Sphingomonas	*S. aerolata*	X		X
Staphylococcus	*S. aureus*, *S. hominis*, *S. capitis*, *S. haemolyticus*, *S. epidermis*, *S. saprophyticus*, *S. warneri*, *S. equorum*	X	X	X
Stenotrophomonas	*S. maltophilia*	X		
Streptococcus	*S. salivarius*	X		
Streptomyces	*S. badius*, *S. griseus*	X	X	X
Total		37	17	22

[a] The most-abundant bacterial genera in the current study are highlighted in boldface.
[b] Results were obtained using the Biotyper 3.0 MALDI-TOF MS system.
[c] Dybwad et al. (16); results were obtained using the Biotyper 2.0 MALDI-TOF MS system and partial 16S rRNA gene sequencing. Only genera corresponding to the current study are presented.

low frequencies. At the outdoor location, *Dermacoccus* (8%), *Roseomonas* (4%), and *Lysinibacillus* (4%) were more abundant than they were in the station, while *Acinetobacter* (1%), *Arthrobacter* (1%), *Massilia* (1%), *Moraxella* (1%), *Paenibacillus* (1%), *Pseudomonas* (2%), *Streptomyces* (2%), and *Agromyces* (1%) were only observed in the station (Fig. 3).

In an attempt to investigate particle size-dependent diversity differences, the 1,141 successfully classified Andersen-derived isolates were also categorized with respect to particle size based on the size-resolved stages of the Andersen sampler. The analysis was done without temporal considerations due to the limited number of isolates obtained during the nighttime periods. The size distribution observed for the isolates classified were as follows: stage 1 (>7.1 μm), 20% (228/1,141); stage 2 (4.7 to 7.1 μm), 18% (208/

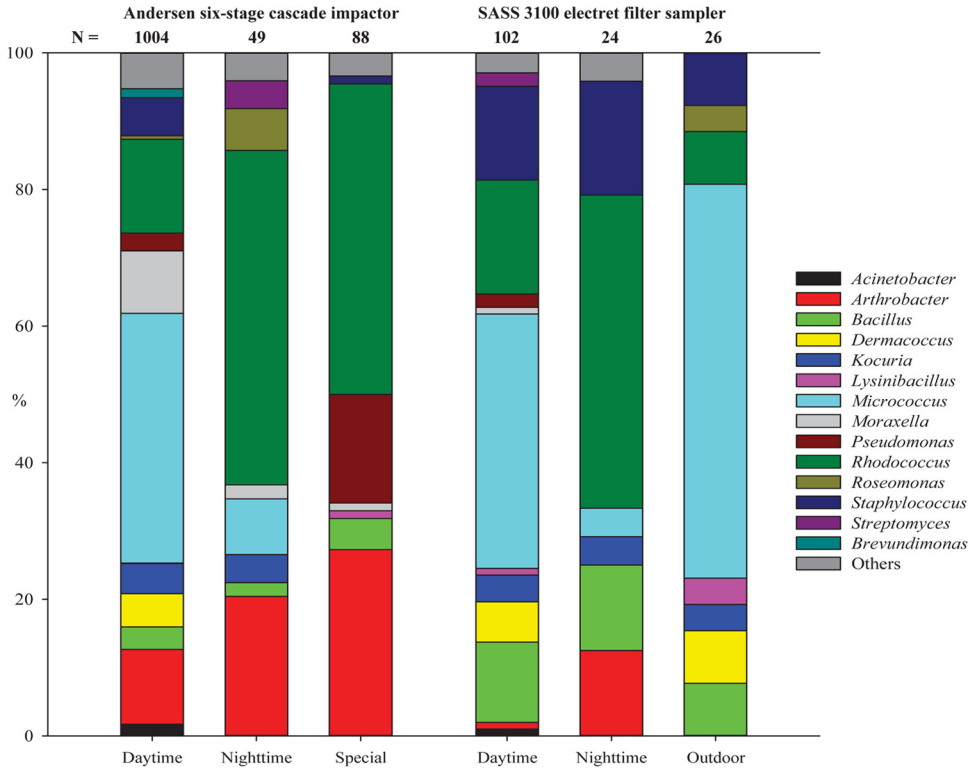

FIG 3 Airborne cultivable bacterial diversity in the subway station. The airborne bacterial diversity was based on Biotyper 3.0 MALDI-TOF MS analysis of representative bacterial isolates from 17 Andersen six-stage cascade impactor samples and 9 SASS 3100 electret filter samples. Andersen-derived results were categorized into three groups, daytime, nighttime, and nighttime atypical (special) events (16 February at 03:00) at the station. SASS 3100-derived results were categorized into three groups: daytime and nighttime in the station and daytime at the outdoor reference location. The number of bacterial isolates (N) classified within each category is also presented.

1,141); stage 3 (3.3 to 4.7 μm), 19% (218/1,141); stage 4 (2.1 to 3.3 μm), 19% (216/1,141); stage 5 (1.1 to 2.1 μm), 22% (253/1,141); and stage 6 (0.65 to 1.1 μm), 2% (18/1,141). The results showed that a very limited number of isolates were recovered from the 0.65- to 1.1-μm size range. However, by taking into account only major differences, the relative abundance of *Micrococcus* was low in stage 6 compared to its abundance in the other stages, while *Kocuria*, *Acinetobacter*, *Moraxella*, and *Pseudomonas* isolates were not observed in stage 6 (Fig. 4). Additionally, the relative abundance of *Arthrobacter*, *Bacillus*, *Rhodococcus*, *Dermacoccus*, *Brevundimonas*, and *Microbacterium* seemed to be higher in stage 6 than in the other stages, although for *Brevundimonas* (5%) and *Microbacterium* (5%), the abundance estimates were based on the observation of a single isolate (Fig. 4). When stage 6 was not taken into account, the observed bacterial diversity was similar for all other stages (Fig. 4). The only exception was an increased relative abundance of *Micrococcus* in the stages corresponding to particles of between 1.1 and 3.3 μm (stages 4 and 5) compared to particles of >3.3 μm (stages 1 to 3) (Fig. 4). These results suggested that the airborne cultivable bacterial diversity at the station was fairly conserved for bacterium-containing particles of different sizes greater than 1.1 μm (Fig. 4).

Total particle concentration level and size distribution. The average total particle level (mean ± standard deviation) at the station was $3.8 \times 10^7 \pm 1.4 \times 10^7$ particles m^{-3} (0.5 to 20 μm). The average count median aerodynamic diameter (CMAD) and geometric standard deviation (GSD) (mean ± standard deviation) were 0.77 ± 0.07 μm and 1.60 ± 0.11, respectively. The total particle levels were generally stable during the daytime periods, except for some sporadic short-duration events with strongly increased particle loads (Fig. 5). The daytime particle levels did not show a consistent temporal pattern that corresponded to the daytime bacterial levels (Fig. 5). The total particle levels generally decayed during the late evening period and continued to decay throughout the night, consistent with the general trend observed for the bacterial levels during the same period. The results suggested that the underlying baseline particle level was lower at night than in daytime but that the observed nighttime particle level variability was higher than the daytime variability, showing multiple short- and long-duration events with increased particle loads that even exceeded the daytime maximum levels (Fig. 5). The nighttime events showing increased particle loads commonly coincided with periods of increased maintenance activities at the station or in the adjacent tunnel network. Based on the observed diurnal consistencies, the total particle levels from the individual sampling days were merged into a single diurnal period averaged hourly (Table 1).

The average daytime total particle level was $3.9 \times 10^7 \pm 1.0 \times 10^7$ particles m^{-3}, while the average nighttime level was $3.3 \times 10^7 \pm 2.2 \times 10^7$ particles m^{-3}. The average daytime CMAD and GSD were 0.79 ± 0.07 μm and 1.63 ± 0.08, respectively, while the average nighttime CMAD and GSD were 0.72 ± 0.06 μm and 1.50 ± 0.16, respectively. No significant differences were observed with respect to total particle levels or particle size distributions

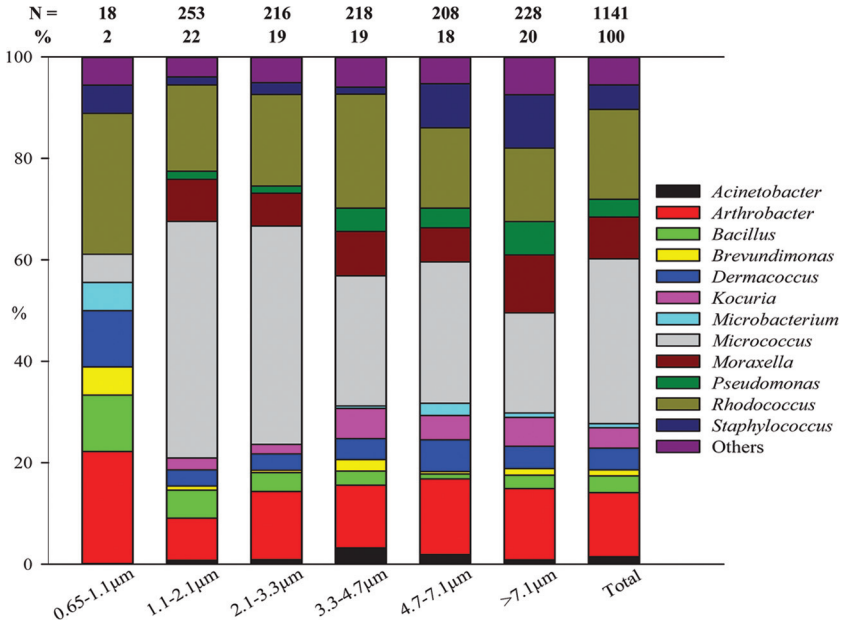

FIG 4 Particle size-resolved airborne cultivable bacterial diversity at the subway station. The particle size-resolved bacterial diversity data are based on Biotyper 3.0 MALDI-TOF MS analysis of representative bacterial isolates from 17 Andersen six-stage cascade impactor samples. The number of bacterial isolates (N) classified within each size bin and in total are presented.

when the daytime and nighttime periods were compared to each other (Table 1). Since the total particle levels did not show a temporal pattern that could be correlated with the bacterial levels, an attempt was made to investigate whether a high abundance of small particles could be masking an underlying correlation. Three different particle size ranges were extracted from the measured range (0.5 to 20 μm), as follows: (i) particles of ≥0.5 μm (i.e., total), (ii) particles of ≥1 μm, and (iii) particles of ≥3.3 μm. However, even after excluding particles of <1 μm and <3.3 μm, the correlation between the temporal variation profile of the total particle levels and the bacterial levels did not improve (Fig. 5).

Meteorological conditions. The average meteorological conditions (mean ± standard deviation) at the station were as follows: (i) temperature, 7.5 ± 0.4°C; (ii) relative humidity, 63 ± 8.4%; (iii) wind speed, 0.4 ± 0.2 m s^{-1}; and (iv) wind direction, 173 ± 69° with 0° west (i.e., wind from east toward west). The temperatures at the station did not show consistent diurnal or day-to-day trends, except that rapid fluctuations seemed to occur more frequently during the day than at night (Fig. 6). The humidity levels showed several consistent diurnal trends, including (i) lower levels at night than during the day, (ii) rapidly fluctuating but stable or increasing average daytime levels, and (iii) decaying levels during the late evening and nighttime periods, showing less fluctuation than in the daytime period (Fig. 6). Both the daytime and nighttime humidity levels showed day-to-day variation in terms of an overall increase throughout the sampling campaign, with the largest change observed for the nighttime levels (Fig. 6). The wind speed levels followed a trend similar to that of the humidity levels, i.e., (i) lower levels at night than during the day, (ii) rapid fluctuations during daytime, and (iii) rapid decay during the early nighttime period. The rapid drop in wind speed coincided with the daily shutdown of train operations, suggesting that train-induced piston effects were the main driving force (Fig. 6). During the nighttime, when no trains were running, the air movement was almost exclusively from east toward west (Fig. 6). During the daytime, the air movement fluctuated rapidly between all directions, although the dominant direction was the same as during night (Fig. 6). These results supported the hypothesis that train-induced piston effects were the main driving force behind the speed, as well as the direction, of air movements at the station but also suggested that a stable air movement from east toward west was present independent of the train activity. The average outdoor meteorological conditions were representative of typical winter conditions in the region and remained stable throughout the sampling campaign, i.e., (i) temperature of −7.4 ± 1.6°C, (ii) relative humidity of 79.0 ± 4.8%, (iii) wind speed of 4.1 ± 0.6 m s^{-1}, wind direction 36 ± 10° with 0° north (i.e., wind from the north-northeast), (iv) cloud cover of ≥90%, and (v) snow depth of ~0.5 m.

DISCUSSION

The current study characterized airborne bacteria during a 72-h continuous sampling campaign at a Norwegian subway station. The results obtained contribute novel information concerning the airborne bacterial community encountered in a subway station environment, including previously unaddressed properties like the size distribution of airborne bacterium-containing particles and the temporal variability of airborne bacteria with respect to their concentration level, size distribution, and diversity on several different timescales. Additionally, the results contributed to confirm and extrapolate results from previous efforts to characterize airborne bacteria at subway stations (5–9, 11, 12, 15, 16). The diurnal and day-to-day variation was specifically addressed in the current work (winter 2011), while seasonal variability assessments were made possible by including results obtained during our previous study (16) at the same station (spring, summer, and fall of 2010).

The results reported here may assist in the development of BIODIM equipment and enable improved T&E schemes involv-

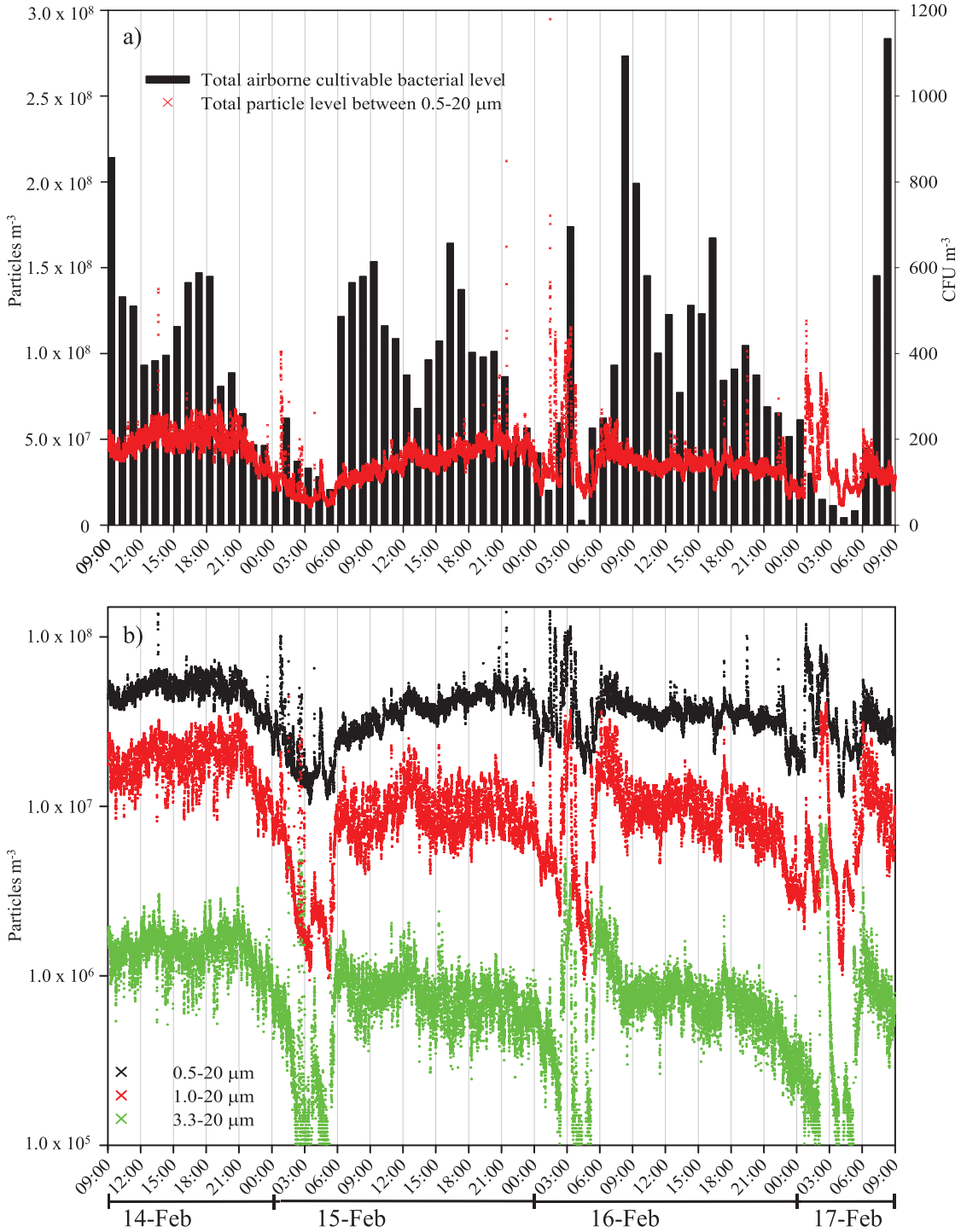

FIG 5 Total particle concentration levels during the 72-h sampling campaign at the subway station. (a) Levels of total particles between 0.5 and 20 μm obtained using the APS 3321 aerodynamic particle sizer (left *y* axis) and airborne cultivable bacterial levels obtained using the Andersen six-stage cascade impactor (right *y* axis). (b) Total levels of particles in various size ranges (indicated by the key), obtained using the APS 3321.

ing simulated operational conditions, including realistic bioaerosol backgrounds, during controlled aerosol chamber-based challenge tests with biological threat agents. Furthermore, the knowledge generated may also be of great interest to the public health, occupational health, and microbial ecology communities.

Airborne bacterial levels. Previous characterization efforts at subway stations have reported airborne cultivable bacterial levels ranging from not detected to 10^4 CFU m^{-3} (5–9, 11, 12, 15, 16). In the current work, the airborne cultivable bacterial levels, based on sampling with three air samplers possessing different properties

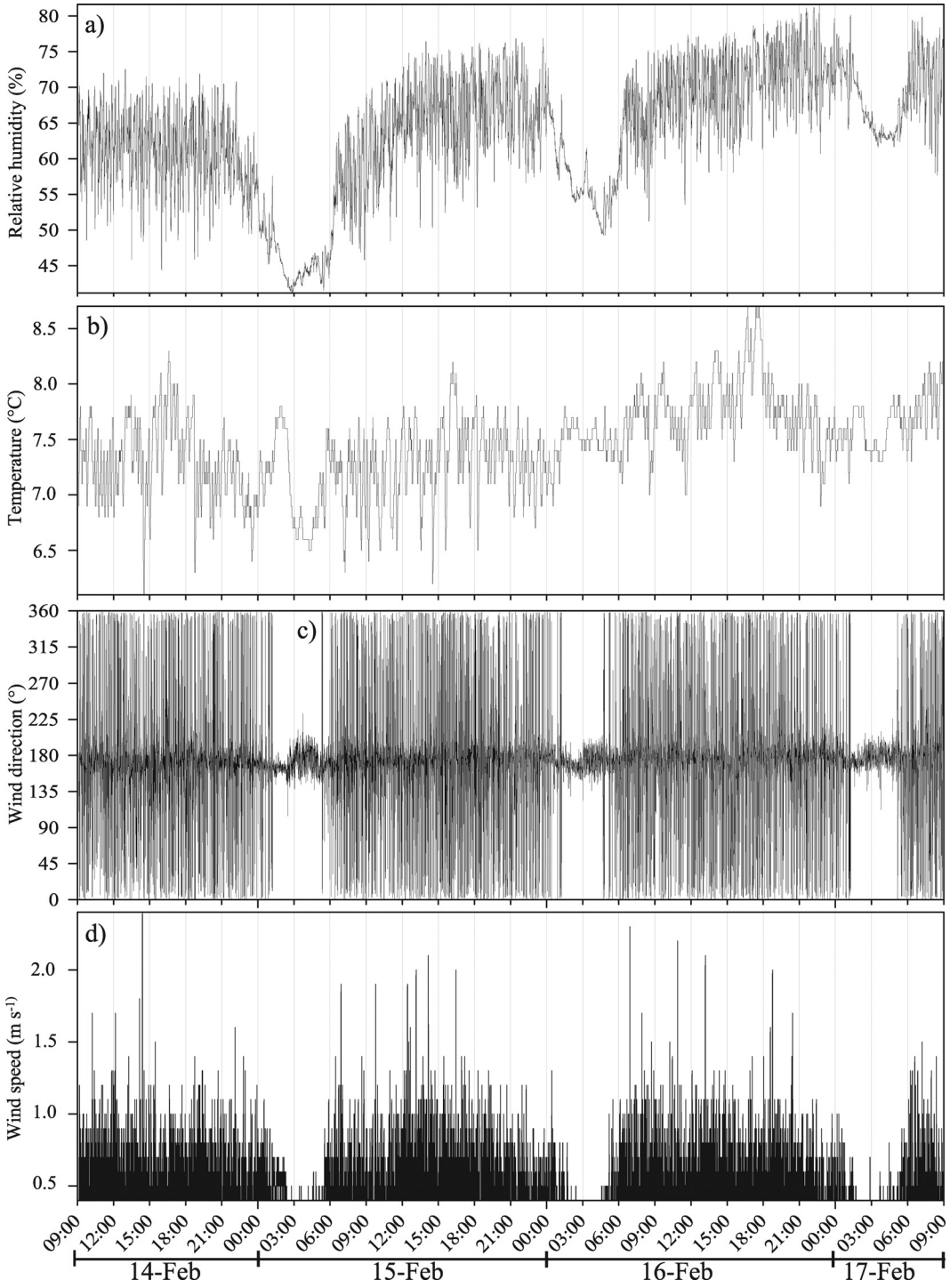

FIG 6 Meteorological conditions during the 72-h sampling campaign at the subway station. The condition measured is indicated on the *y* axis of each panel. In panel c, 180° corresponds to wind directly from east to west.

(Andersen, MAS-100, and SASS 3100), ranged from not detected to 10^3 CFU m^{-3}.

A consistent diurnal pattern regarding the levels of airborne cultivable bacteria was observed at the station, as follows: (i) the daytime levels were higher than the nighttime levels, (ii) the diurnal maximum levels were generally observed during the morning and afternoon rush hours, which both showed increased levels compared to the noon and evening non-rush periods, and (iii) following the afternoon rush hours, the bacterial levels generally decayed throughout the evening and nighttime periods, with diurnal minimum levels just before the train activity recommenced in the morning.

To the authors' knowledge, the diurnal variability of airborne bacteria in a subway station environment has not previously been addressed in detail. However, Birenzvige et al. (3) characterized the diurnal variation of the aerosol background at the Pentagon subway station (Washington, DC) and also attempted to address the diurnal variation of airborne bacteria. Unfortunately, airborne bacterial concentration levels could not be established due to an erratic air sampler, although some diurnal trends were proposed (3). A comparison between the diurnal concentration profile of airborne bacteria observed in the current work and the trends proposed by Birenzvige et al. (3) suggested that the profiles at both stations were highly similar. The observed diurnal concentration profiles of airborne bacteria were also found to correspond to the diurnal PM10 (airborne particulate matter of <10 μm) mass concentration profile reported by Salma et al. (18) from a subway station in Budapest, Hungary.

On two separate occasions during the current sampling campaign, atypical nighttime events deviating from the general nighttime trend occurred. The atypical events showed temporarily increased bacterial levels, which commonly exceeded daytime levels except for the rush hour maxima. The atypical events seemed to coincide with periods of increased maintenance activities at the station or in the adjacent tunnel network.

The largest temporal variability of the airborne bacterial levels was consistently observed during two distinct diurnal periods at the subway station, i.e., (i) the nighttime-to-daytime transition period, when the diurnal minimum levels at the end of the night were followed by a rapid increase in anthropogenic activities (i.e., passengers and trains) and the diurnal maximum levels during the peak morning rush hours, and (ii) the two nighttime atypical events, which showed increased bacterial levels that deviated from the general nighttime trend of low and decaying levels. During the nighttime-to-daytime transition period, the largest observed differences in the bacterial levels of adjacent samples were 20- and 40-fold, based on hourly (Andersen) and 10-min (MAS-100) sampling frequencies, respectively. Similarly, during the nighttime atypical events, the largest differences between adjacent samples were 66- and 270-fold, based on the Andersen and MAS-100 results, respectively. These results demonstrated that the airborne bacterial levels in subway stations may be associated with significant temporal variation over a short period of time and that such changes occur as a consequence of both predictable (e.g., nighttime-to-daytime transition periods) and unpredictable (e.g., atypical nighttime events) events.

The average daytime levels of cultivable airborne bacteria found at the station were 452 ± 198 (Andersen), 443 ± 275 (MAS-100), and 493 ± 153 (SASS 3100) CFU m^{-3}. In addition to being highly consistent when compared, these results were also in close agreement with the daytime level (396 ± 93 CFU m^{-3}) reported by the SASS 3100 during the previous study (16) at the same station. The average nighttime bacterial levels found in the current study were 107 ± 68 (Andersen), 103 ± 102 (MAS-100), and 25 ± 22 (SASS 3100) CFU m^{-3}. The average nighttime bacterial level (21 ± 13 CFU m^{-3}) reported by the SASS 3100 during the previous study (16) corresponded better to the SASS 3100 results in the current work than to the Andersen and MAS-100 results. This could be explained by the fact that the SASS 3100 collected a single 2-h nighttime sample during a period commonly corresponding to the diurnal minimum bacterial levels, while the Andersen and MAS-100 sampled throughout the night at an hourly and a 10-min sampling frequency, respectively. In summary, these results suggested that the airborne bacterial levels at the station appeared to be similar during both studies, with a consistent daytime-to-nighttime difference.

Considering the fact that the current study was performed during the winter season (February 2011), while the previous study (16) was performed during the spring, summer, and fall seasons (May to September 2010), the combined results suggested that the airborne bacterial levels at the station were conserved on a seasonal time scale. Additionally, the current results, which show a consistent diurnal pattern on three consecutive days, in combination with the previous results (16), which show consistent daytime and nighttime levels over a period of several months, suggested that the airborne bacterial levels at the station were also conserved on a day-to-day time scale.

Based on the fact that the station was nonoperational and closed to the public during the night, it could be suggested that anthropogenic activities, such as passengers and trains, were the major sources of airborne bacteria at the station. The increased bacterial levels observed during the morning and afternoon rush hours compared to the levels in the noon and evening periods corresponded to increased passenger numbers, while the train frequency remained constant. This result demonstrated more specifically that passengers were a major source of airborne bacteria at the station.

Airborne bacterium-containing particle size distribution. The size distribution of airborne particles containing cultivable bacteria has, to the authors' knowledge, not previously been studied in a subway environment. Lee et al. (8) described the use of an Andersen six-stage cascade impactor in various public environments, including a subway station, but did not report size-resolved results from the station. The size distribution of bacterium-containing particles can have an impact on the regional deposition of airborne bacteria in the human respiratory tract upon inhalation and, therefore, may be of importance for health hazard assessments (19). Furthermore, information concerning the size distribution of bacterium-containing particles in various operational environments is also of interest to the BIODIM community (2).

The current work revealed that airborne bacterium-containing particles of between 0.65 and 1.1 μm contributed on average less than 1% of such particles at the station, while particles of between 1.1 and 2.1, 2.1 and 3.3, 3.3 and 4.7, 4.7 and 7.1, and >7 μm contributed 26, 22, 19, 15, and 17% of the total level, respectively. The averaged results suggested that bacterium-containing particles of >1.1 μm were relatively evenly distributed between different particle sizes, although the maximum level was observed for particles of between 1.1 and 2.1 μm and ~50% of the particles were between 1.1 and 3.3 μm.

The size distribution of airborne bacterium-containing particles was found to be conserved between sampling days, as well as between different daytime periods (rush hours versus non-rush hours). The largest temporal variation in the size distribution of airborne bacterium-containing particles was consistently observed during two distinct diurnal periods at the station, i.e., (i) the daytime-to-nighttime transition period and (ii) the nighttime-to-daytime transition period.

Both the relative and absolute amounts of 1.1- to 3.3-μm bacterium-containing particles were found to be higher during the daytime than at night, suggesting that the contributing sources for

such particles were primarily present in the station during the daytime. Since the major difference between daytime and nighttime periods was an almost complete absence of passengers and trains during the latter, it is reasonable to suggest that anthropogenic activities were the predominant sources of 1.1- to 3.3-μm bacterium-containing particles at the station.

The relative amount of airborne bacterium-containing particles of >3.3 μm observed during the nighttime atypical events was found to be much higher than the amounts observed during the temporally closest daytime periods that showed comparable bacterial levels, while the reverse was observed for 1.1- to 3.3-μm particles. Interestingly, these observations suggested that the nighttime atypical events were caused by different sources than the daytime sources, which generated a larger relative amount of 1.1- to 3.3-μm airborne bacterium-containing particles.

Airborne bacterial diversity. To our knowledge, no previous study has investigated the temporal variability of the airborne bacterial diversity at a subway station. The airborne cultivable bacterial diversity at subway stations has been shown to consist largely of the bacterial genera *Micrococcus*, *Staphylococcus*, and *Bacillus*, but several other genera, such as *Pseudomonas*, *Corynebacterium*, *Paracoccus*, *Kocuria*, *Aerococcus*, *Moraxella*, and *Enterococcus*, have also been observed, although generally less consistently (3, 5, 7, 11, 15).

The previous study (16) identified 37 bacterial genera in the station air and revealed that the airborne bacterial diversity appeared to be comparable with the diversity found in other stations (7). In the current work, 39 bacterial genera were observed in the subway air, and the most frequently observed were *Micrococcus*, *Rhodococcus*, *Arthrobacter*, *Moraxella*, *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Kocuria*, and *Dermacoccus*. Of the total number of observed genera, 56% (22/39) were also encountered during the previous study (16). If only the most frequently observed genera in the current work were taken into account, a complete qualitative consistency was observed between the two studies concerning the diversity of airborne cultivable bacteria.

The results obtained for the temporal variability of the airborne bacterial diversity in the current study, in combination with our previous results (16), suggested that the diversity was conserved (i) between seasons, at least qualitatively, (ii) between days, and (iii) between different daytime periods, such as rush hours and non-rush hours.

The airborne bacterial diversities obtained with the Andersen impactor and the SASS 3100 electret filter sampler were generally similar, although some differences were observed, i.e., (i) the Andersen-derived diversity corresponded to 17% Gram-negative bacteria, while only 4% was observed with the SASS 3100, (ii) the Andersen-derived diversity showed a higher relative abundance of *Arthrobacter* and *Moraxella* than the SASS 3100, while the SASS 3100 showed a higher relative abundance of *Staphylococcus* and *Bacillus*, and (iii) the Andersen-derived diversity corresponded to 44% pigmented bacteria, while 60% was observed with the SASS 3100.

The results from both samplers suggested that the daytime diversity was strongly dominated by *Micrococcus* (*M. luteus*), while the nighttime diversity was strongly dominated by *Rhodococcus* (*R. fascians*) and had a very low relative abundance of *Micrococcus* compared to the daytime period. Several other bacterial genera appeared to have higher relative abundances in the station during the daytime than at night, including *Moraxella*, *Dermacoccus*, *Pseudomonas*, *Brevundimonas*, and *Acinetobacter*, while *Arthrobacter* and *Roseomonas* appeared to have higher relative abundances during the night. Compared to the previous study (16), the relative abundance of *Rhodococcus* (*R. fascians*) reported by both samplers was much higher in the current work.

An interesting observation was made regarding the airborne bacterial diversity during an atypical nighttime event (16 February at 03:00) that showed strongly increased bacterial levels at night. While the atypical event diversity generally resembled the nighttime diversity with respect to the dominance of *Rhodococcus* and *Arthrobacter*, a high relative abundance of *Pseudomonas* that deviated from both the typical daytime and nighttime abundances was observed. Additionally, no *Micrococcus* was observed during the atypical event. Taken together, these observations suggested that the nighttime atypical event sources differed from the predominant daytime sources and that the atypical event sources appeared to aerosolize a larger relative fraction of *Pseudomonas*.

When the daytime bacterial diversity in the station was compared to that of the equivalent outdoor reference location, a higher relative abundance of *Micrococcus* and lower relative abundances of *Rhodococcus*, *Staphylococcus*, and *Bacillus* were observed outdoors. The results concerning the difference between the station diversity and the outdoor bacterial diversity did not correspond to those obtained in our previous study (16), which showed a higher relative abundance of *Bacillus* and a lower relative abundance of *Micrococcus* at the outdoor location than in the station. The reasons behind these discrepancies are not known, although the composition of outdoor airborne bacterial communities has been shown to display seasonal variation (20).

The airborne cultivable bacterial diversity in the station generally showed a conserved diversity profile for bacterium-containing particles of different sizes >1.1 μm. Interestingly, the largest deviation from the general trend was observed for *Micrococcus*, which showed an increased relative abundance in airborne bacterium-containing particles of between 1.1 and 3.3 μm compared to its abundance in particles of >3.3 μm. Compared to the diversity observed for airborne bacterium-containing particles of >1.1 μm, particles of between 0.65 and 1.1 μm appeared to have very low relative abundances of *Micrococcus*, *Moraxella*, and *Pseudomonas*.

Total particle level and size distribution. The total particle levels and size distributions observed in this study were comparable to those reported previously at the same station (16), as well as to those reported by Birenzvige et al. (3). However, at the same time, several consistent differences were identified concerning the diurnal profiles and short-term temporal variability of the total particle levels. While the previous study (16) revealed a significant difference between the daytime and nighttime particle levels, the current work showed no significant difference between particle levels categorized into daytime and nighttime periods. The current results nevertheless revealed that the underlying baseline particle level was lower at night than during the day but that the particle level variability was higher at night, with multiple short- and long-duration events showing increased particle loads that exceeded the daytime maximum levels. The nighttime events commonly coincided with periods of increased maintenance activities at the station or in the adjacent tunnels, which suggested that a causal link existed. However, a direct correlation between specific maintenance activities and the nighttime events was not identified.

A low temporal correlation was observed between the total particle levels and the airborne bacterial levels at the station during the

current work, even when particles of <1 and <3.3 μm were excluded from the total particle size range (0.5 to 20 μm). However, some general consistencies were observed between the total particle levels and the airborne bacterial levels, i.e., (i) the baseline total particle levels and the airborne bacterial levels were both higher during the day than at night and (ii) the baseline total particle levels and airborne bacterial levels both showed a generally decaying trend during the late evening period that continued throughout the night.

Implication and future directions. This work represents an important step forward in obtaining a more-complete understanding of the bioaerosol backgrounds that will be encountered by BIODIM systems at subway stations. The knowledge generated helps to define a range of relevant bioaerosol background-related conditions that may be used to improve the development and T&E of BIODIM equipment by allowing realistic operational conditions to be taken into account. A set of representative and well-defined bioaerosol backgrounds may be developed and standardized for use in aerosol test chambers, thus simulating realistic operational conditions during controlled aerosol chamber-based challenge tests with biological threat agents.

This study specifically addressed airborne bacteria, although other types of airborne microorganisms (e.g., fungi and viruses), their products (e.g., toxins), and other types of nonmicrobial bioaerosols (e.g., pollen) may also correspond to relevant components of the bioaerosol background with respect to the development and T&E of BIODIM systems.

Since the current work provided novel information concerning the size distribution of airborne bacterium-containing particles and the temporal variability of airborne bacteria in a subway environment, very limited data were available for interstudy comparisons. To confirm and extend the results obtained in the current work, it is also of interest to address the spatiotemporal variability of airborne bacteria by performing similar studies in different subway stations.

Cultivation has traditionally been the method of choice when studying airborne bacteria, although cultivation-independent techniques are now becoming more widespread, also within aerobiological research (4). Cultivation-independent analyses (e.g., quantitative PCR, next-generation sequencing, and microarrays) are able to elucidate not only cultivable bacteria but also viable but not cultivable (VBNC) and dead bacterial fractions. The cultivable fractions of environmental bacteria have in some environments been shown to correspond to <1% of the total number (21), clearly suggesting that the use of cultivation-independent techniques may extend our understanding of the bioaerosol background. Currently, few reports exist on the use of cultivation-independent analyses to study airborne bacteria in subway stations (3, 4). We have recently performed a cultivation-independent diversity study of airborne bacteria using 16S rRNA gene-based PhyloChip microarrays at the same Norwegian subway station (unpublished data), which may contribute interesting information regarding differences between the cultivation-dependent and -independent airborne bacterial diversities.

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