Science of the Total Environment 957 (2024) 177539

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Characterization of bioaerosols associated with commuter transport micro-environments using high throughput sequencing

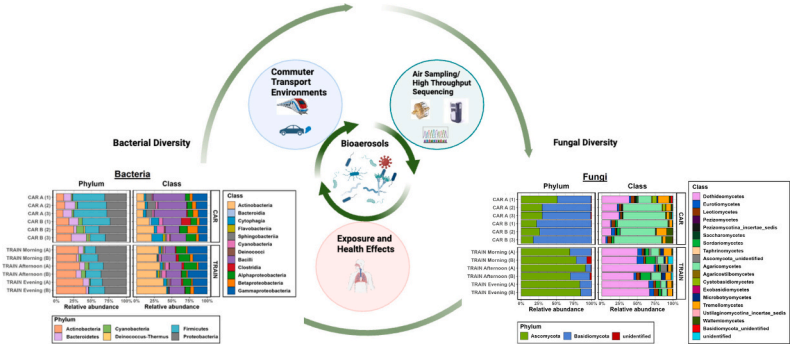
N. Grydaki, I. Colbeck, C. Whitby *

School of Life Sciences, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, Essex, UK

HIGHLIGHTS

- Currently there is a lack of data on the microbial fraction of airborne particles that commuters are exposed to.
- High throughput sequencing was used to characterize the microbiome of bioaerosols inside trains and automobiles in the UK.
- Distinct bacterial and fungal communities were found in the air with both modes of transport, especially with fungi.
- Airborne microbial taxa of human health concern were found with both modes of transport.
- Choice of commuter transport has a potentially significant impact on the aerosol microbiome that passengers are exposed to.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Philip Hopke

Keywords:
Bioaerosols
Bacteria
Fungi
Bacterial 16S rRNA gene
Fungal ITS gene
High throughput sequencing

ABSTRACT

Air quality inside commuter transport is an important public health issue. However, there is currently limited information on commuter exposure to the microbial fraction of airborne particles (i.e. bioaerosols) in different types of transport. Here we investigated the abundance and diversity of bioaerosols in public trains and private automobiles in the UK using molecular approaches. Overall, bacterial 16S rRNA gene abundances were significantly greater with the train (between 3.07×10^5 and 8.97×10^5 copies/m^3) compared to the car (between 4.21×10^4 and 4.78×10^5 copies/m^3) (p-value 0.019 $<$ 0.05), with no significant differences found with train journeys throughout the day (p-value $>$0.05). In terms of microbial composition, significant differences were found between the two modes of transport, for both bacterial and fungal communities. Specifically, bacteria were dominated by Proteobacteria (trains: 37 %; cars: 30 %), Firmicutes (trains: 20 %; cars: 36 %), Actinobacteria (trains: 34 %; cars: 16 %) and Bacteroidetes (trains: 6.1 %; cars: 13 %). Within the fungi, Ascomycota were predominant in the train (80 %), while the car was dominated by Basidiomycota (70 %), which may be due to the time of year sampled. Additionally, a core bacterial and fungal microbiome, including human commensals and outdoor-originating micro-organisms, alongside several taxa of human health concern were found in the air of both modes of transport. This study provides an important insight into the aerosol microbiome in transport micro-environments, which is crucial for the evaluation of commuter exposure to potential health risks.

* Corresponding author at: School of Life Sciences, University of Essex, Colchester CO4 3SQ, UK.
E-mail address: cwhitby@essex.ac.uk (C. Whitby).

https://doi.org/10.1016/j.scitotenv.2024.177539
Received 9 June 2024; Received in revised form 24 October 2024; Accepted 11 November 2024
Available online 23 November 2024
0048-9697/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Studies have shown that commuting may make a significant contribution to the daily exposure to air pollutants (DeSouza et al., 2021; Fernández-Iriarte et al., 2021; Kumar et al., 2021). Moreover, the degree of exposure is highly affected by the mode of transport (Yang et al., 2021; Rivas et al., 2017a; Cepeda et al., 2017). Air quality and exposure assessment during commuting has mainly focused on particulate matter (e.g. Wang et al., 2021a; Rivas et al., 2017b). Yet, the microbial fraction of particles (i.e. bioaerosols) in public transport is a public health concern (e.g. in the transmission of SARS-CoV-2) (Gartland et al., 2022; Wang et al., 2021b; Tirachini and Cats, 2020). Among the various different public transport modes, the metro system has perhaps been the most studied in terms of airborne microorganisms, particularly on subway station platforms, rather than the interior of carriages (Grydaki et al., 2021; Leung et al., 2021; Passi et al., 2021). In contrast, despite the typically longer journey time and hence greater exposure time, overground trains have received much less attention (García-Mozo et al., 2020; Wang et al., 2010).

In the UK in 2018, 68 % of workers travelled to work by car and 10 % by rail, although this varied by region, with only 27 % commuting by car into London (Department of Transport, 2019). Overall, in 2018 the average time taken up by commuting into work by any means was 29 min, ranging from up to 59 min for travelling by rail (Department of Transport, 2019). Across the UK, air quality within trains has been the subject of recent research by the Rail Safety and Standards Board (RSSB, 2021). It was concluded that the main PM_{10} sources in the carriages were the exhaust, passenger movement and the incoming mixed ambient air (RSSB, 2021).

Exposure of commuters in London to air pollution, and in particular to particulate matter, has been the subject of a number of studies (Rivas et al., 2017a; Kaur et al., 2007; Adams et al., 2001). Regarding bioaerosols, Patel et al. (2018) analyzed dust samples from 17 train stations, of which 9 were in London. Across all stations, the median bacterial count value was 756,000 CFU per cm^2. In another study, Green et al. (2021) found no traces of SARS-CoV-2 RNA in air samples from three London Underground stations, an underground train carriage and a bus.

In-vehicle exposure is also associated with elevated levels of several pollutants (Bista et al., 2022; Campagnolo et al., 2019; Cepeda et al., 2017). However, despite the fact that the inside of an automobile is considered to be an ideal environment for transmission of biological agents due to its confined space, information on bioaerosol exposure in car interiors is currently limited. Studies on in-cabin air quality have measured the bacterial and fungal CFU levels in passenger cars by employing culture-dependent methods and found that the most dominant fungal genera in automobiles are similar to the ones found in public transport (i.e. *Cladosporium, Penicillium, Aspergillus* and *Alternaria)* (Prakash et al., 2014; Wang et al., 2013; Jo and Lee, 2008; Lee and Jo, 2005). Buitrago et al. (2021) measured total airborne bacteria and fungi in three different types of car as well as buses and trains in Lisbon and found that cars had lower fungal and bacterial loads than public transport, based on cultivation and microscopic methods. Moreover, the airborne microbiota was highly influenced by the ventilation mode and the occupancy of the vehicles (Buitrago et al., 2021).

Currently, there is limited information on commuter exposure to bioaerosols in the UK. The overall aim of this study was to investigate the abundance and diversity of airborne microorganisms in the transport environment using culture-independent methods. We postulated that the choice of commuter transport mode had a significant impact on the aerosol microbiome that passengers were exposed to, and commuters using public transport, rather than private automobiles, would be exposed to different microbial taxa. To address this, a comparative investigation was conducted in two different types of microenvironments (i.e. train and private vehicle), covering similar commuter transport routes.

2. Materials and methods

2.1. Commuter transport sampling sites

Each travel scenario was examined under a fixed route between Colchester (south-eastern UK) and London. Colchester is located about 80 km northeast of London and is connected to the capital through a major road and a railway line (Fig. 1). Both routes are heavily used daily by people commuting, as well as for leisure purposes. Sampling was conducted between September 2016 and March 2017, with the train sampling during September 2016 and car sampling between February and March 2017. A total of six journeys were carried out for each transport mode.

Train sampling was carried out in trains running along the electrified rail line connecting Colchester to London Stratford, which is one stop before the line terminus (London Liverpool Street). Stratford is a major multilevel interchange station serving various overground and underground lines linking Stratford to other stations in and around central London. In 2016–17 Stratford was ranked as the 6th busiest station in terms of passengers' number and station usage in Great Britain (UK Government, 2017). Each sampling period consisted of a two-way trip and included the time spent inside train carriages as well as the time spent waiting and walking on the (outdoor) station platform for the outward and return journey. The total duration of each sampling trip depended on the number of stops, as well as the waiting time between the arrival at the destination station and the departure of the return train, and ranged from 110 to 159 min. In order to get a better representation of exposure, sampling was carried out in different time periods (i.e. during the morning, afternoon and evening). Journey duration and ventilation conditions are shown on Table 1.

Car sampling was carried out with two different petrol-fuelled vehicles, both mid-sized hatchbacks (car A and B). The number of regular passengers in car A varied between 1 and 2 adults, while car B was typically used by 1–4 people, including 2 toddlers. For the purposes of the study, the automobiles were occupied with two passengers (the driver and the researcher responsible for the sample collection). All round trips were carried out during afternoon hours, with the duration of each one-way journey ranging from 83 to 106 min. The majority of the route was via the A12, a dual carriageway used by approximately 65,000 vehicles per year (Road Traffic Statistics, 2017). During the journey, the vehicle windows were kept closed, with the air conditioning on in car A and the fan on and recirculation air off in car B (Table 2).

Temperature, relative humidity and carbon dioxide concentration were recorded every minute using a Rotronic CP11 indoor air quality meter (Table S1, Fig. S1). For the train, the median temperature ranged from 23.3 °C (morning) to 27.1 °C (evening) and the median relative humidity varied between 46 % (afternoon) to 71 % (morning). For the car journeys, the median temperature was higher in car A (18.7–20.8 °C), due to the use of the air conditioning system, compared to car B (16.4–18.6 °C). The relative humidity median levels were lower in car A (31–39 %) than in car B (43–59 %). CO_2 levels varied significantly among the journeys (Kruskal Wallis, p-value <0.05), with the lowest median concentration (478 ppm) observed during evening train journeys when the windows were kept open. Median values for the morning and afternoon journeys were 669 ppm and 792 ppm, respectively. Between the two automobiles, car A, that had the A/C system switched on during all three sampling trips, exhibited a lower median concentration (697 ppm) compared to car B (781 ppm).

2.2. Air sampling

Air samples were collected using Advantec polypropylene filter holders (Cole-Parmer, UK) loaded with 47-mm nuclepore polycarbonate filters (0.4 μm-pore size, Whatman, UK). Filters were sterilised by autoclaving and filter holders were washed with 10 % (v/v) bleach, rinsed with ultrapure water (Milli-Q, Millipore) and autoclaved at

121 °C for 15 mins. Each sampler was sealed with sterilised aluminium foil and stored in a sealed sterile bag until ready for sampling. A portable battery-operated Leland Legacy Pump (SKC, UK) set at 8 l min^{-1} was used for sampling while commuting by train. Sampling in the cars was performed using a Linear Diaphragm LD50 DE pump (Charles Austen, UK), operated at the same flow rate used for the train sampling. Filter holders were connected to the pumps using Tygon tubing (R-3603, Sigma-Aldrich, UK). When sampling the trains, all sampling equipment was carried in a backpack that was specially reinforced with sound-proofing foam and the inlets were positioned on the outside of the bag and at the breathing height (~1.5 m) (Fig. S2). When sampling in the automobiles, sampling inlets were placed below the driver's seat head-rest facing towards the back seats (Fig. S3).

2.3. Bacterial and fungal analysis

DNA was extracted from the samples using methods described previously (Grydaki et al., 2021). Briefly, filters were inserted into 5 ml screw-cap tubes containing 0.1-mm zirconium/silica beads and cells were disrupted by bead-beating in sodium dodecyl sulfate (SDS)-based extraction buffer and incubation at 75 °C, followed by phenol-chloroform purification and isopropanol precipitation, with addition of co-precipitant glycogen, and final elution of pelleted DNA in 35 μl sterile water. Negative controls (3 field blanks and 3 no-template extraction controls) were included in all of the molecular analysis steps to check for contamination.

qPCR and amplicon sequencing of the 16S rRNA gene was performed using the primers 341F/805R (Herlemann et al., 2011) and approaches previously described (Grydaki et al., 2021; Ferguson et al., 2021, 2019; Pankhurst et al., 2012). The qPCR standard curve was constructed using *E. coli* genomic DNA (qPCR efficiency = 97.2 %, slope = 3.39, $R^2 = 0.99$, y-intercept = 37.43, Ct values of the non-template controls >36) and results were expressed as the number of gene copies per cubic meter of air. Primers ITS1-F (Gardes and Bruns, 1993) and ITS2 (White et al., 1990) were used for amplicon sequencing of the ITS1 region. All libraries were prepared using Illumina Nextera XT Index kit, pooled with an internal control 20 % PhiX and loaded to a v3-chemistry 600 cycle-kit reagent cartridge (Illumina, Inc.). Sequencing (2×300 bp) was performed using an Illumina MiSeq platform and bioinformatics analysis of sequence libraries was performed as previously described (Grydaki et al., 2021), except Operational Taxonomic Units (OTUs) that appeared in blank controls were subtracted from the data using filter_otus_from_otu_table.py script within QIIME 1.9.1 (Caporaso et al., 2010). The sequencing data obtained from the Illumina MiSeq have been submitted to NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1079228.

A total of 625,162 bacterial and 629,002 fungal sequencing reads were generated for all samples ($n = 12$) and negative controls ($n = 6$). After quality trimming and chimera filtering, a total of 312,155 16S rRNA gene and 335,359 ITS region sequences clustered into 10,060 bacterial and 2970 fungal OTUs, respectively. After removal of unassigned reads, non-bacterial/non-fungal sequences and subtraction of OTUs that were determined to be present in the controls from the samples, 203,668 16S rRNA gene reads (median/sample = 11,040 ± 10,251 read counts), representing 8394 bacterial OTUs, and 332,307 ITS reads (median/sample = 27,374 ± 4462 read counts), corresponding to 2413 fungal OTUs, were retained. The numbers of sequences per samples were normalised based on the number of sequences obtained from the smallest library (3837 sequences for bacteria and 20,066 for fungi). The total numbers of remaining sequences were 46,044 for bacteria and 240,792 for fungi. Abundance-based filtering carried out to remove OTUs with 5 or fewer counts further reduced the numbers to 19,618 bacterial and 224,321 fungal sequences, represented by 139 bacterial and 347 fungal OTUs.

2.4. Statistical analysis

For the purposes of analysis, samples obtained during commuting by train in the morning ($n = 2$), in the afternoon ($n = 2$) and in the evening ($n = 2$), as well as samples obtained in car A ($n = 3$) and car B ($n = 3$), were grouped in order to give an average representation of the microbial abundance and diversity. Shapiro-Wilk test was used for normality testing of bacterial 16S rRNA gene abundance as well as carbon dioxide concentration data. Group means were compared using Student's *t*-test

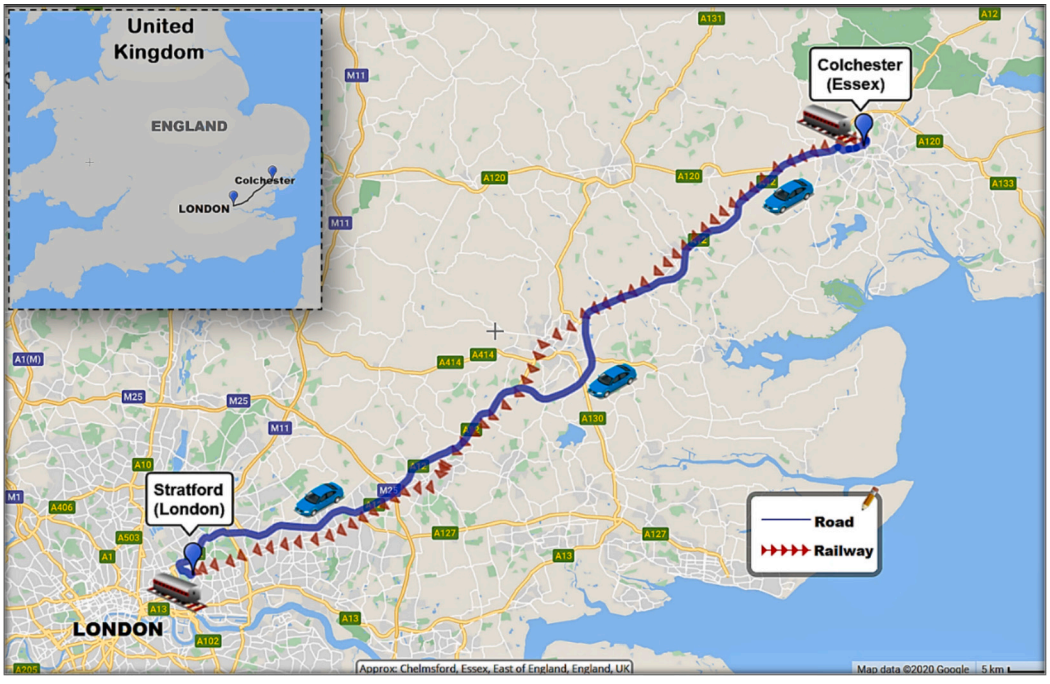


Fig. 1. Location of origin and destination points of the study route. The zoomed map shows the railway line (red line) used for train sampling and the road transport route (blue line) used for car sampling.
Map was created using Scribble Maps based on data imported from Google Maps (UK).

and one-way analysis of variance (ANOVA) on normally distributed variables, whereas Kruskal-Wallis test was used to compare group medians when non-parametric tests were necessary. Correlations between bacterial abundance, microbial richness and carbon dioxide concentration were assessed using Spearman's rank coefficient analysis.

Beta diversity analysis was carried out using the abundance-based Bray-Curtis and incidence-based binary Jaccard distance metrics of community dissimilarity. The metrics were computed using beta_diversity_through_plots.py and the resulting distance matrices were visualized with Principal Coordinates Analyses (PCoA) 3D-plots, generated by EMPeror software (Vázquez-Baeza et al., 2013) within QIIME 1.9.1. The statistical significance of differences in microbial composition among groups of samples was determined using permutation-based multivariate analysis of variance (PERMANOVA) (Anderson, 2001) with 9999 permutations, performed with compare_categories.py. The taxonomic distribution of OTUs across samples was determined using QIIME script summarize_taxa.py and visualized with bar plots and heat maps generated in R statistical computing environment (R Core Team, 2014). The number of core OTUs, defined as the OTUs that are present in all samples, was determined using compute_core_microbiome.py within QIIME. Venn diagram analysis was performed using *jvenn* (Bardou et al., 2014).

3. Results

3.1. Total bacterial quantification

Overall, airborne bacterial 16S rRNA gene abundances varied significantly among journeys (one-way ANOVA, p-value = 0.005). When comparing the two types of transport mode, bacterial 16S rRNA gene abundances were significantly greater with the train (between 3.07×10^5 and 8.97×10^5 copies/m^3) compared to the car (between 4.21×10^4 and 4.78×10^5 copies/m^3) (independent samples t-test, p-value = 0.019 < 0.05) (Fig. 2, left). No significant differences were found with train journeys throughout the day, with between 4.03×10^5 and 6.57×10^5 copies/m^3 (morning), 3.07×10^5 and 5.52×10^5 copies/m^3 (afternoon) and 6.61×10^5 and 8.97×10^5 copies/m^3 (evening), or between journeys by train and car A (Post hoc Tukey's Honest Significant Difference test, p-value >0.05). However, 16S rRNA gene abundances in trains were found to be significantly higher compared to car B, during morning (p-value = 0.03) and evening (p-value = 0.003) journeys (Fig. 2, right). In addition, Spearman's rank coefficient analysis demonstrated that there is a significant negative association ($\rho = -0.64$, p-value <0.05) between 16S rRNA gene abundance and carbon dioxide levels.

3.2. Diversity of airborne bacteria and fungi

In general, PCoA analysis of bacteria and fungi using the abundance-based Bray-Curtis metric (Fig. 3, top) revealed three distinct clusters delineated by mode of transport (i.e. train, car A and car B). Significant differences were found between the train and car for both bacteria (PERMANOVA, pseudo-$F_{1,10} = 2.90$, $R^2 = 0.22$, p-value = 0.002) and fungi (pseudo-$F_{1,10} = 8.58$, $R^2 = 0.46$, p-value = 0.002), but not between cars A and B (p-value >0.05 for both bacteria and fungi). Journeys with car A appeared to be more similar, compared to the ones with car B, only in terms of bacterial composition. However, results based on Jaccard's distance (Fig. 3, bottom) demonstrated that the transport mode had a weaker overall effect on bacterial diversity (pseudo-$F_{1,10} = 1.82$, $R^2 = 0.15$, p-value = 0.007), with car A being more similar to the train. In contrast, fungal diversity was highly distinct with the train, car A and B. PERMANOVA revealed significant differences between the two transport modes (pseudo-$F_{1,10} = 3.18$, $R^2 = 0.24$, p-value = 0.002), with the effect size larger compared to the one on bacteria, in agreement with results obtained based on the Bray-Curtis metric.

Within the bacterial communities, at the phylum and class level, bioaerosols in both transport modes were dominated by Proteobacteria (train 37 %, car 30 %, on average); notably *Gammaproteobacteria* (train 22 %, car 14 %), Firmicutes (train 20 %, car 36 %); particularly *Bacilli* (train 18 %, car 33 %), Actinobacteria (train 34 %, car 16 %); notably *Actinobacteria* (train 37 %, car 16 %), and Bacteroidetes (train 6.1 %, car 13 %). Cyanobacteria (train 3.1 %, car 4.4 %) and Deinococcus-Thermus (train 0.32 %, car 0.76 %) were also identified but in low relative abundance (Fig. 4, top).

At the genus level, the most abundant bacteria detected in the train were human skin commensals, such as *Corynebacterium* (11.2 %), *Staphylococcus* (10.5 %), *Acinetobacter* (7.3 %) and *Kocuria* (4.5 %) (Fig. 5, left). *Corynebacterium* (10.9 %) were also dominant in car B. *Streptococcus and Gemella*, that are part of the oral microbiome, were present in higher relative abundance in car B (8.5 % and 4.3 %, respectively), compared to car A (1.37 % and 0.34 %) and the train (2.45 % and 0.64 %). Indeed, some of the most frequently encountered bacterial genera, including *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Acinetobacter*, *Kocuria*, *Pseudomonas*, *Gemella*, *Pantoea* (detected at high abundance in morning-A train: 9.7 % and evening-B train: 6 %) and *Streptomyces* (mainly detected during evening-A: 4.9 % and B: 13.8 % commutes by train), comprise several opportunistic pathogenic species.

Pseudomonas spp., that are ubiquitous in the environment, were highly abundant in the train (8.1 %) and car A (11.2 %). Interestingly, car A exhibited a high relative abundance of *Carnobacterium* (24.5 %) and some genera commonly found in soil, water and plants, such as *Planomicrobium* (8.0 %), *Pedobacter* (9.3 %) and *Massilia* (4.9 %), that were in much lower mean relative abundances in car B (0.7 %) and the train (0.4 %). *Hymenobacter* (5.8 %) and *Cyanobacteria GpI* (6.6 %) were more abundant in car B. Other non-human associated bacterial taxa that were detected in varying abundances in the air of all transport environments included *Blastococcus*, *Brachybacterium* and *Arthrobacter*.

Analysis of OTU richness showed that the numbers of different bacterial OTUs identified were similar for the train (104–118) and car A

Table 2
Sampling duration per each two-way car journey and ventilation conditions.

Sampling trip	Date	Time inside car (total sampling duration)	Car ventilation
Car A (1)	11/2/17	193 min	A/C ON (22 °C)[a]
Car A (2)	19/2/17	192 min	A/C ON (21 °C)[a]
Car A (3)	11/3/17	190 min	A/C ON (20.5 °C)[a]
Car B (1)	20/3/17	184 min	Car ventilation ON
Car B (2)	22/3/17	173 min	Car ventilation ON
Car B (3)	23/3/17	195 min	Car ventilation ON

[a] A/C temperature set by the passengers.

Table 1
Sampling duration per each two-way train journey, number of stops and ventilation conditions.

Sampling trip	Date	Time in train (min)	Time on platform (min)	Outward journey stops	Outward journey ventilation	Return journey stops	Return journey ventilation	Total sampling duration (min)
Afternoon (A)	12/9/16	100	34	4	A/C ON	1	A/C ON	134
Evening (A)	12/9/16	111	48	6	Windows open	4	A/C ON	159
Evening (B)	13/9/16	121	9	5	Windows open	6	Windows open	130
Morning (A)	15/9/16	106	23	4	–	6	A/C ON	129
Morning (B)	16/9/16	111	20	5	–	6	A/C ON	131
Afternoon (B)	16/9/16	103	7	4	A/C ON	4	A/C ON	110

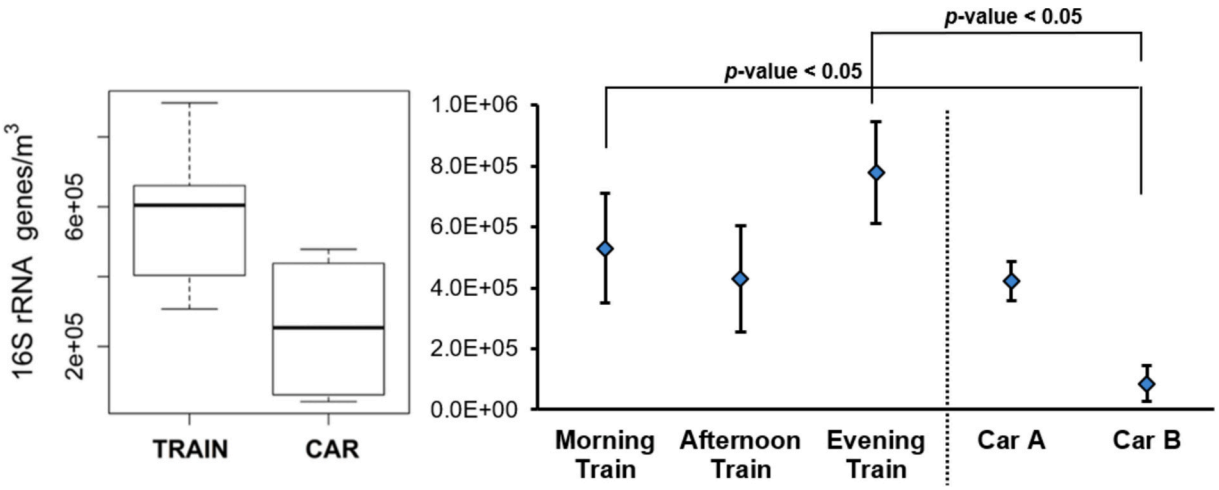


Fig. 2. Abundance of bacterial 16S rRNA gene copies per cubic meter of air for the total of aerosol samples collected during commuting by train and by car (left), as determined by qPCR. Bacterial 16S rRNA gene copies/m^3 of air presented per time-zone of train journey (morning, afternoon and evening, $n = 2$) and type of car (A and B, $n = 3$) (right). Error bars represent standard deviation of the mean. Tukey's HSD pair-wise comparisons were used to denote statistical significance (p-value <0.05) between types of journeys.

(98–114), but were lower for car B (52–74) (Table S2), with significant positive correlation found between bacterial OTU richness and 16S rRNA gene abundance ($\rho = 0.84$, p-value <0.001), as shown by Spearman's rank test. In total, 53 bacterial OTUs were found to overlap between the 85 identified core OTUs from both automobiles and the 72 core OTUs from the train (Fig. S4). From those, 48 OTUs were shared between the train and car A, while 15 OTUs were present in both the train and car B. However, only a set of 11 OTUs was found to be common between the two vehicles, from which 10 OTUs (*Corynebacterium* spp.; 2 OTUs, *Streptococcus* spp., *Staphylococcus* spp.; 2 OTUs, *Blastococcus* spp., *Brachybacterium* spp., *Hymenobacter* spp., *Arthrobacter* spp. and *Carnobacterium* spp.) were also present across all the train samples. The genera representing the unique core bacterial OTUs detected in each transport environment are shown in Table 3.

Within the fungal communities, at the phylum level, Ascomycota and Basidiomycota were dominant, with distinct proportions found for each mode of transport. Specifically, on average, 70 % of OTUs obtained for the car were Basidiomycota, with the remaining reads assigned to Ascomycota. In contrast, Ascomycota were predominant (80 %) in the train (Fig. 4, bottom).

At the class level, the majority of Basidiomycota in cars was represented by *Agaricomycetes* (53 %), followed by *Tremellomycetes* (8 %) and *Wallemiomycetes* (4.4 %). Wood-degrading *Agaricomycetes Trametes* (car A 13 %, car B 19 %), *Bjerkandera* (car A 14 %, car B 15 %) and *Daedaleopsis* (car A 4.3 %, car B 4 %) were the most abundantly detected basidiomycetes in the car environment (Fig. 5, right). *Wallemia* (*Wallemiomycetes)*, a common indoor mold, was found most notably in car B (6.38 %), whereas the skin-associated yeast *Cryptococcus* (*Tremellomycetes*), comprising species with pathogenic potentials, was more enriched in car A (3.61 %). The majority of Basidiomycota in the trains was also represented by *Agaricomycetes* (8 %), followed by *Tremellomycetes* (6.61 %). The human skin basidiomycetous yeasts of *Malassezia*, *Rhodotorula* and *Trichosporon* were also detected in the majority of journeys by both train and car, but only at very low fractions (< 0.5 %).

Within the Ascomycota, mainly dominated by *Dothideomycetes* (train 58 %, car 19 %), *Cladosporium* spp. was the most abundant genus in both the train (26 %) and car A (19 %) (Fig. 5, right). Other *Dothideomycetes* that were more abundant in the train, compared to the car, were *Mycosphaerella* (13 %), *Alternaria* (6 %) and *Aureobasidium* (5.1 %), followed by *Eurotiomycetes Penicillium* (4.7 %). The widespread genus *Aspergillus* (*Eurotiomycetes*) (train 0.45 %, car A 0.12 % and car B 1.27 %) and skin-associated yeast *Candida* (< 0.5 %), both associated with well-known opportunistic pathogens, were only detected at low proportions.

Overall, the numbers of different fungal OTUs were higher compared to the ones observed for bacteria, across all the samples (i.e. between 116 and 193 for train, 120–161 for car A and 88–111 for car B). The number of common fungal OTUs between the automobiles and the train core datasets was 23, from which 22 were shared between car A and the train, while only 7 taxa were present in both car B and the train (Fig. S5). The OTUs shared between the two study cars were 19, whereas only 6 OTUs (*Cladosporium* spp.; 2 OTUs, *Alternaria* spp., *Trametes* spp., *Mycosphaerella* spp. and *Sporobolomyces* spp.) were found in all three fungal core datasets. The genera representing the unique core fungal OTUs found in each transport environment are shown in Table 4.

4. Discussion

Bioaerosols inside public and private modes of transport are an important health issue. Currently there is limited information on the airborne bacterial and fungal microorganisms in commuter trains and cars. Our study examined the aerosol microbiome in car and train environments, covering similar commuter routes from London to Colchester (UK), using high throughput sequencing approaches.

Results based on qPCR showed that airborne bacterial 16S rRNA gene abundances were significantly greater with the train compared to the car, which might be associated with the higher throughput of passengers using rail services compared to cars. However, the negative correlation found between the 16S rRNA gene levels and the measured CO_2 meant that the highest bacterial concentrations were found in the absence of elevated CO_2, which might suggest that indoor levels of bacterial aerosols in the transport environments are also strongly affected by sources other than the occupants. Indeed, the highest 16S rRNA gene levels were observed during the examined evening journeys by train, when the windows were open, indicating that outdoor particles introduced indoors with ventilation air play a major role in the increase of biological particles. Wang et al. (2010) also found a negative correlation between CO_2 and total (viable) bacterial concentrations inside trains in Taiwan. However, in contrast, Lee et al. (2024) found that bacteria were positively influenced by CO_2 levels.

In both rail and road transport environments, bacterial aerosols were dominated by Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes, as it has been consistently observed in other types of indoor environments (e.g., Wilkins et al., 2016; Gaüzère et al., 2014a; Kembel et al., 2012). Airborne bacteria identified in the train carriages were represented by similar levels of Gram-positive and Gram-negative

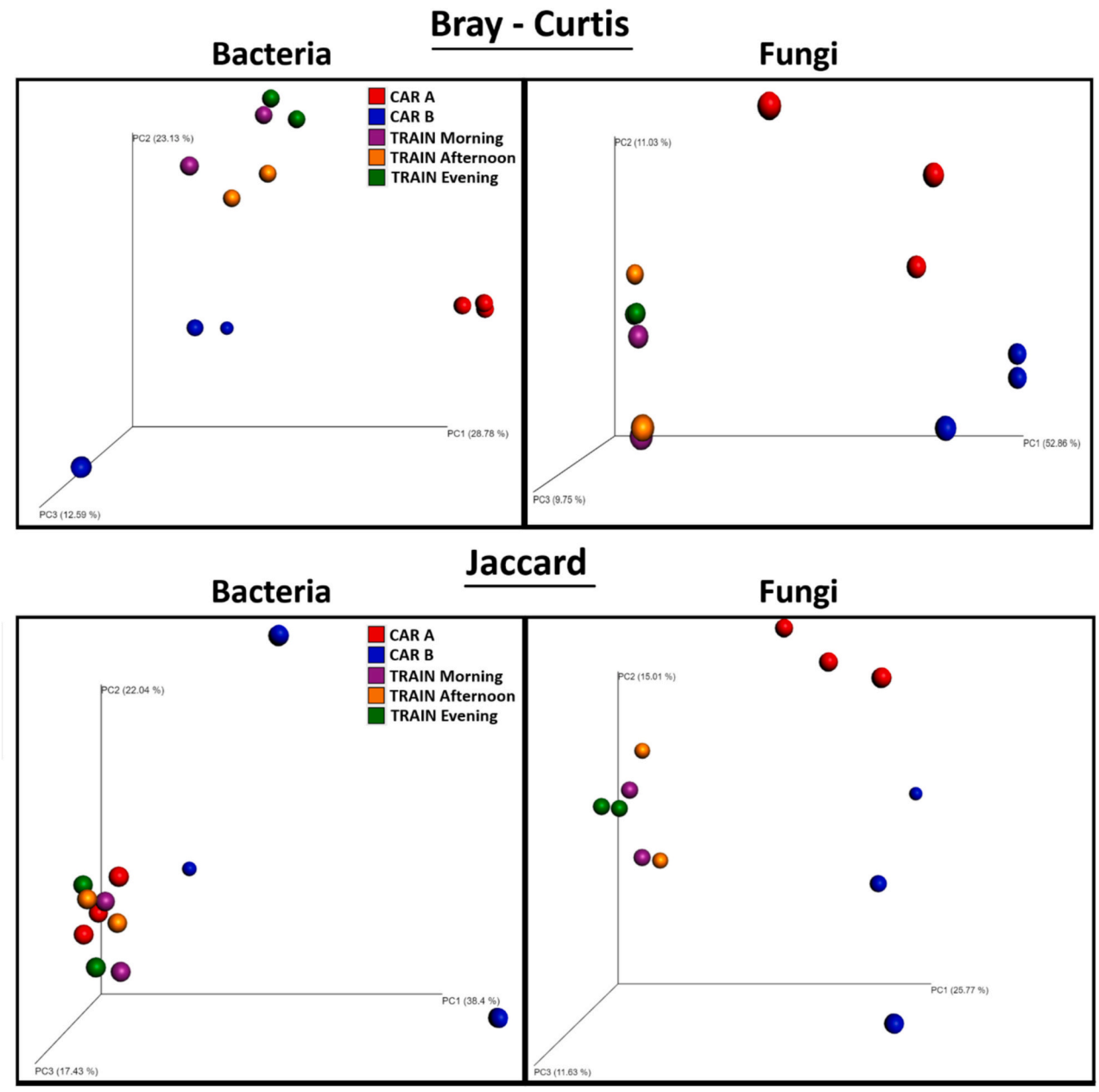


Fig. 3. Principal coordinate analysis 3D-plots of bacterial (left) and fungal (right) beta diversity based on Bray-Curtis dissimilarity (top) and Jaccard distance matrix (bottom).

microorganisms; whereby Gram-positive Actinobacteria and Firmicutes *Bacilli* (e.g. *Staphylococcus*, *Streptococcus*) accounted for ~52 %, in total, and Gram-negative Proteobacteria, Bacteroidetes and Cyanobacteria accounted for ~46 % of the bacterial 16S rRNA gene sequences. Wang et al. (2010) found that Gram-positive and Gram-negative bacteria comprised 70.5 % and 20.5 % of the total bacteria, respectively, in train bioaerosols based on cultivation methods. However, culture-dependent bioaerosol studies may bias towards the over-representation of Gram-positive taxa (Rendon et al., 2017; Fang et al., 2014). Gołofit-Szymczak et al. (2019) found that the most prevalent viable airborne bacteria in car cabins were Gram-positive. Yet, the predominance of *Bacilli* in the air of the examined automobiles in the present study was in line with the findings of Stephenson et al. (2014) and Li et al. (2013), which was based on culture-independent analysis of samples collected from interior surfaces and HVAC filters, respectively, inside vehicle cabins.

Overall, despite the fact that the two types of transport microenvironments in our study were examined during different times of the year, results showed that the air in both modes shared a common bacterial signature, mainly comprised from human-related (*Corynebacterium*, *Streptococcus*, *Staphylococcus*) and outdoor environment-associated taxa (*Blastococcus*, *Brachybacterium*, *Hymenobacter*, *Arthrobacter*, *Carnobacterium*). The great contribution of bacteria originating from the occupants to the aerosol microbiome encountered in the indoor environment has been well-established (Luongo et al., 2017; Adams et al., 2015; Hospodsky et al., 2012). In addition, it has been reported that the predominance of human occupancy-associated microbiota in indoor spaces might result in a rather homogenised aerosol bacterial composition over time (Prussin II et al., 2016; Adams et al., 2014; Gaüzère et al., 2014b), which might not reflect the seasonal dynamics observed in outdoor air (Núñez et al., 2021; Bowers et al., 2013). The noteworthy difference in the proportions of human microbiota between the two examined cars in our study might be related to the regular usage of car B by more passengers (3–4) compared to car A (1–2 people). Moreover, differences were also exhibited among train journeys

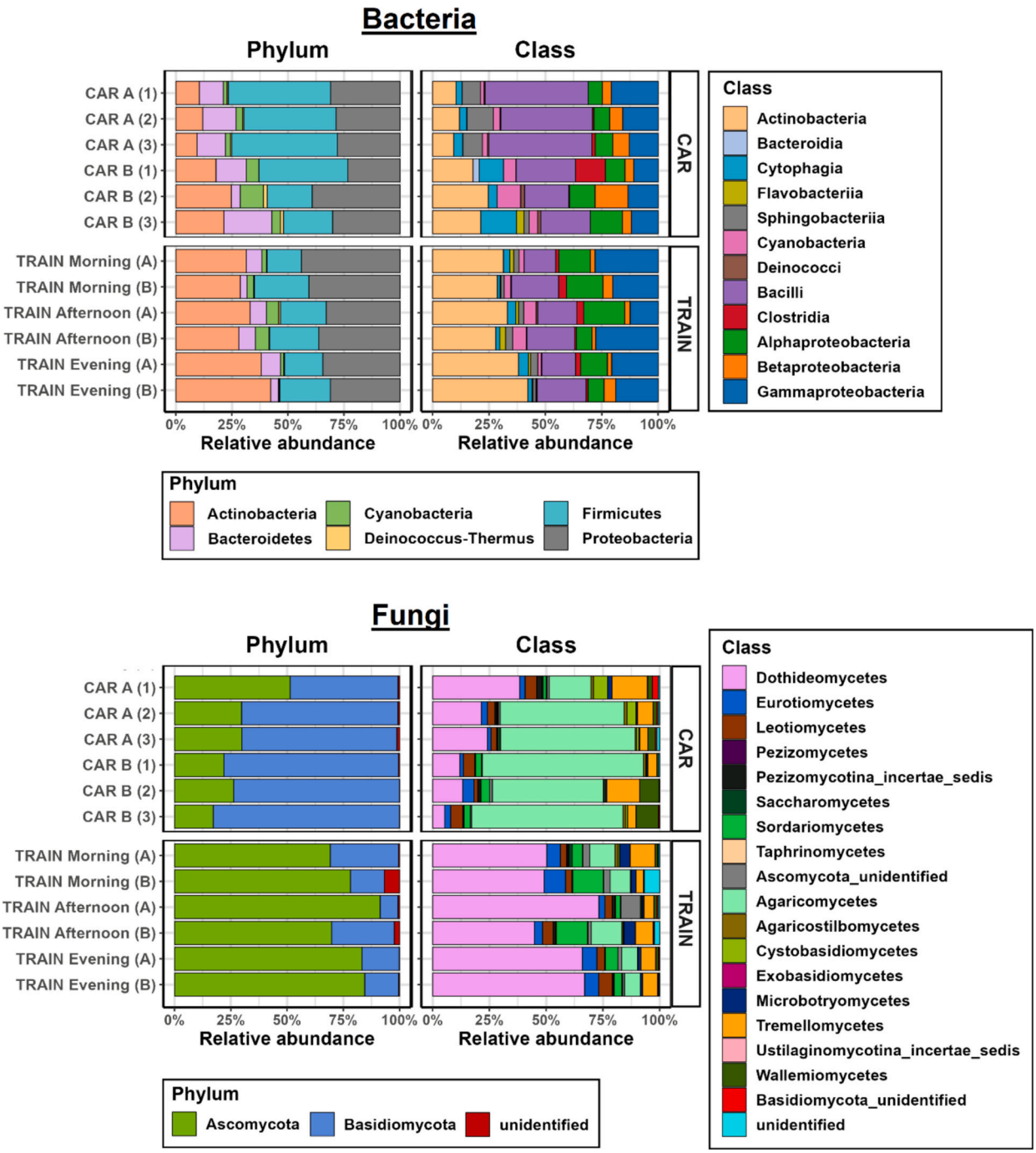


Fig. 4. Relative abundance of bacterial (top) and fungal (bottom) OTUs at the phylum and class level per each sample.

throughout the day, e.g. *Corynebacterium* spp. proportion was higher during morning and afternoon commutes, most likely attributed to the ridership levels. At the same time, evening journeys exhibited higher fractions of outdoor-originating taxa, such as *Arthrobacter* or *Rhodococcus*, which might be linked to the natural ventilation occurring through open windows at the specific trains. In addition, passengers can also be potential passive transport vectors of microbes from outdoors and therefore, introduction of outdoor particles is not only affected by the ventilation system.

Staphylococcus spp., affiliated with *Staphylococcus aureus* and *Methicillin-Resistant Staphylococcus aureus* (MRSA) - causative agents of invasive infections, which were more enriched in the air of the trains compared to the cars, have been commonly detected in public buses, both on surfaces (e.g. Conceição et al., 2013; Simoes et al., 2011) and in the air (e.g. Nowakowicz-Dębek et al., 2017; Onat et al., 2017). Moreover, *Staphylococcus* spp. have also been previously identified in the air of car cabins (Gołofit-Szymczak et al., 2019), as well as in dust samples collected from automobile air conditioning filters (Li et al., 2013) revealing another important source and dispersal mechanism for the specific genus. Various other genera that were found on the automobile A/C filters by Li et al. (2013), such as *Kocuria*, *Arthrobacter*, *Massilia*, *Pseudomonas*, *Pantoea* and *Acinetobacter*, were also detected in the air of the investigated cars in the present work. Early studies have pointed out the microbial colonisation capability in automobile A/C systems (Simmons et al., 1999; Kumar et al., 1990) and have demonstrated that the in-vehicle microbial air quality is mainly associated with the state of

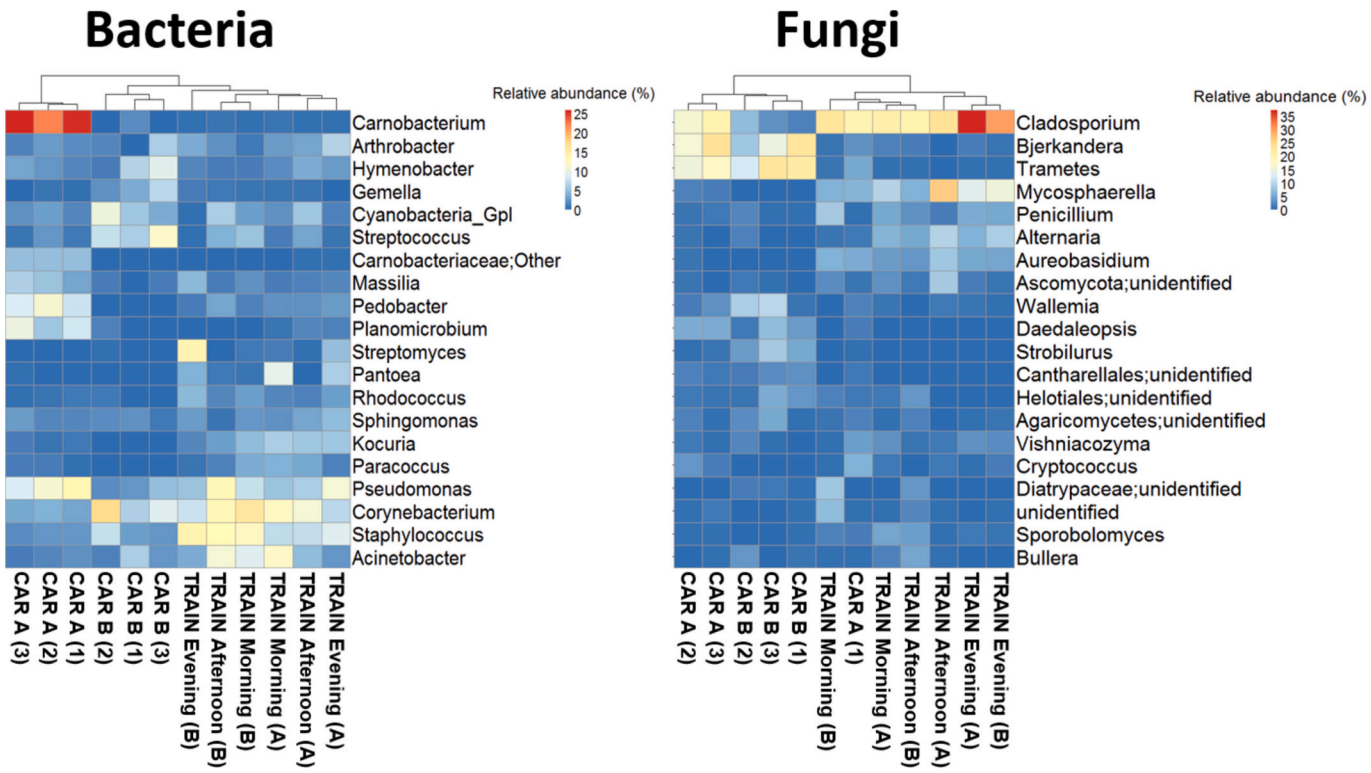


Fig. 5. Heatmap displaying the relative abundance of the 20 most dominant bacterial (left) and fungal (right) genera across the samples.

Table 3
Genus of unique core bacterial OTUs found only in the train or the car environment. The number in brackets denotes the occurrence of the OTU.

Bacterial OTUs		
Train	Automobile	
	Car A	**Car B**
Pedobacter	*Skermanella*	*Gemella*
Pseudomonas	*Acetobacteraceae* (unidentified) (2)	*Haemophilus*
Acinetobacter (3)	*Algoriphagus*	*Neisseria*
Corynebacterium (4)	*Carnobacteriaceae* (unidentified)	**Car A & Car B**
Finegoldia	*Virgibacillus*	*Nocardioides*
Empedobacter	*GpI* (unidentified) (2)	
Romboutsia	*Streptococcus*	
Lactobacillus	*Psychrobacter* (2)	
Brevibacterium	*Mycobacterium*	
Streptomyces	*Planomicrobium* (2)	
Brachybacterium	*Sanguibacter*	
Blastococcus	*Carnobacterium*	
Pantoea	*Pseudomonas* (2)	
Moraxellaceae (unidentified)	*Microbacteriaceae* (unidentified)	
	Sphingomonas (2)	
	Neisseria	
	Massilia (2)	
	Acinetobacter	
	Polaromonas	
	Thermoactinomyces	
	Arthrobacter	

the A/C filter (Gołofit-Szymczak et al., 2019; Vonberg et al., 2010). In addition, the pronounced high abundance of *Carnobacterium*, a psychrophilic genus, in car A (A/C switched on during all journeys), along with the unique presence of smaller proportions of *Psychrobacter* and *Thermoactinomyces*, which have all been previously detected on HVAC systems (Li et al., 2013), might provide further evidence that the A/C system consists a major source of bioaerosols in the automobile cabin environment. Moreover, the role of the air conditioning system as a source of microbial aerosols should be investigated in public transit modes, too.

Currently, there is a lack of sequence information on the aerosol mycobiome in transportation environments, especially the interior of trains and cars. Our study found that Ascomycota (particularly *Dothideomycetes*) were dominant in the train, while Basidiomycota (mainly *Agaricomycetes*) dominated in the car. Previous cultivation-based studies of indoor air tended to recover Ascomycota, while the Basidiomycota were largely missed (Simon-Nobbe et al., 2008). *Dothideomycetes* are saprotrophic molds that are frequently found indoors, while *Agaricomycetes* are mainly outdoor mushroom-forming and plant-decaying fungi. The prevalence of the two classes within their divisions has also been reported in previous indoor and outdoor bioaerosol studies (e.g., Shin et al., 2015; Hoisington et al., 2014; Yamamoto et al., 2012).

The higher prevalence of molds inside train carriages, compared to cars, might be associated with the higher number of passengers using rail services, as many fungi enter indoor environments via humans (e.g. on footwear). However, seasonality may influence the fungal bioaerosol microbiome, given that vegetation is one of the main sources of fungi, and the highest concentrations of fungi in the air are typically found during summer and autumn (Frankel et al., 2012; Yamamoto et al., 2012; Kaarakainen et al., 2008). Although absolute concentrations for fungi were not determined in the present work, our sampling took place in autumn and winter, so one might expect higher levels in the summer. Fernández-Iriarte et al. (2021) observed a decrease in the relative abundance of various taxonomic genera from the summer to the winter in buses. As samples in our study were collected during different periods of the year for the two examined types of transport, it is possible that some of the differences observed in the relative abundances of fungal communities between the train and automobile journeys could be attributed to season.

Cladosporium spp. (e.g. *Cladosporium cladosporioides*), which was the most abundant fungal genus found in both trains and car A, is generally prevalent in both outdoor (Kaarakainen et al., 2008) and indoor (Peimbert and Alcaraz, 2023) air, including transport environments.

Table 4
Genus of unique core fungal OTUs encountered only in the train or the car environment. The number in brackets denotes the occurrence of the OTU.

Fungal OTUs		
Train	Automobile	
	Car A	**Car B**
Articulospora	*Buckleyzyma*	*Hyphodontia*
Hypocreaceae (unidentified)	*Saccharomyces*	*Vestigium*
Neodevriesia	*Ascomycota* (unidentified) (2)	*Aspergillus*
Phlebia	*Ramularia*	*Helotiales* (unidentified) (2)
Plectosphaerella	*Extremus*	*Steccherinum*
Dissoconium	*Articulospora*	*Agaricomycetes* (unidentified)
Debaryomyces	*Flammulina*	*Skeletocutis*
Phaeosphaeria	*Cladosporium*	*Diatrypaceae* (unidentified)
Basidiomycota (unidentified)	*Agaricales* (unidentified)	*Dioszegia*
Pyrenochaetopsis	*Itersonilia* (2)	*Zymoseptoria*
Cordycipitaceae (unidentified)	*Chalastospora*	*Entylomatales* (unidentified)
Cladosporium	*Symmetrospora* (2)	**Car A & Car B**
Cordyceps	*Nectriaceae* (unidentified)	*Resinicium*
Sarocladium	*Cantharellales* (unidentified) (3)	*Aspergillus*
Ascomycota (unidentified)	*Postia* (2)	*Itersonilia*
Penicillium	*Sporobolomyces*	*Phlebiella*
Monographella	*Chaetothyriales* (unidentified)	*Cantharellales* (unidentified)
Bullera	*Kondoa* (2)	*Daedaleopsis*
Unidentified	*Tetrachaetum*	*Strobilurus*
	Unidentified (3)	*Bjerkandera*
	Cryptococcus	*Piptoporus*
	Polyporales (unidentified)	*Wallemia* (2)
	Guehomyces	*Agaricomycetes* (unidentified)
		Stereum

Cladosporium, along with *Penicillium*, *Altermaria, Aureobasidium* and *Aspergillus*, have been found in a variety of culture-based surveys investigating public transport vehicles, such as buses (Nowakowicz-Dębek et al., 2017; Prakash et al., 2014) and underground trains (Hernández-Castillo et al., 2014; Hoseini et al., 2013), as well as private vehicles (Wang et al., 2013; Jo and Lee, 2008; Lee and Jo, 2005). Interestingly, the particular taxa have also been found to colonise A/C filters and vents in automobiles (Li et al., 2013; Jo and Lee, 2008), indicating that air conditioning systems can be a significant source of these microorganisms in the indoor air of vehicles, in accordance with results previously discussed.

However, as it has been well-documented, the origin of fungi found indoors is primarily located outdoors in various natural sources, mainly associated with plant material and rotting wood. Apart from the aforementioned taxa, the vast majority of airborne fungi, detected across both modes of transport, were related to vegetation, either as plant-pathogens, epiphytes, endophytes, or just wood-decomposers (*Bjerkandera*, *Trametes*, *Mycospaerella*, *Daedaleopsis*, *Strobilurus*, *Vishniacozyma*, *Bullera*, *Sporobolomyces*, *Diatrypaceae*, *Cantharellales* and *Helotiales*), suggesting that the particular taxa are indicative of the outdoor sources that represent the local surrounding environment (Adams et al., 2013; Amend et al., 2010). García-Mozo et al. (2020), also, found that trains may act as a reservoir for outdoor biological particles. Our results indicate that the outdoor sources are important contributors to the airborne fungal diversity in transport vehicles.

In our study, the examination of a variety of public transport journeys by train throughout the day (morning, afternoon, evening) allowed us to investigate the overall exposure of commuters. Although compositional variation is expected, as different train carriages were sampled as well as various environmental factors contributing to the shaping of the indoor air microbiome in the trains, such as number of passengers, outdoor air composition and ventilation conditions (Meadow et al., 2014), our results identified the presence of a common airborne microbiome in all train journeys regardless of the time of day. Interestingly, bacterial and fungal communities from car A were more similar to those found in the train carriages, rather than in car B. Even though the same route with the same number of passengers was examined for both automobiles, each vehicle is a unique microenvironment with its own distinct characteristics (e.g. ventilation settings) and the recovered microbial taxa may be influenced by its usage and regular passengers. Meadow et al. (2015) showed that occupants emit a unique identifiable microbial cloud in the surrounding environment and therefore private vehicles may carry a distinct microbial signature linked to the passengers using them.

Of particular human health concern was the identification of several bacterial and fungal taxa that are affiliated with opportunistic pathogenic or allergenic species found in various relative abundances in both types of transport. In addition to the several common human skin/oral commensals (e.g. *Corynebacterium*, *Staphylococcus*, *Streptococcus* among others) identified, widely distributed taxa such as *Pseudomonas*, as well as *Pantoea* and *Streptomyces* that were mainly detected in the trains, are also associated with potential pathogenic strains. Moreover, there were also bacterial OTUs detected uniquely in each transport environment that are associated with species responsible for various infectious human diseases, such as *Finegoldia* (Goto et al., 2008) in trains, *Thermoactinomycetes* (Selman et al., 2017) in car A, *Haemophilus* and *Neisseria* (Stephens and Farley, 1990) in car B. Known fungal allergens such as *Wallemia* and *Aspergillus* spp. were more abundant in the cars, while *Cladosporium*, *Alternaria*, *Penicillium* and *Aureobasidium* were more predominant in the trains. Fungal taxa associated with respiratory infections were also found (e.g. *Cryptococcus*, *Sporobolomyces*, *Bjerkandera*).

5. Limitations of the study

Despite the small sample size used in the present study, our investigation demonstrated the importance of characterizing the microbiome of aerosols in the indoor environments of trains and cars in order to better assess commuter exposure and human health risk. Although the two types of transport micro-environments were investigated during different periods of the year, which may have affected the results obtained in the current work, our findings represent an important step towards advancing our knowledge of the composition of the airborne microbiome that commuters are exposed to. Nevertheless, a more extended collection of samples would be required in order to provide a robust evaluation of possible links between the in-transit aerosol microbiome variability and the environmental parameters. Furthermore, it has to be highlighted that indoor bioaerosols are a mixture of

particles originating from both the various indoor sources and the outdoor air transferred to the inside via air exchange, which is again dependent on the various sources located outdoors, and, therefore, when studying the indoor aerosol microbiome both indoor and outdoor bioaerosols should be taken into account and both indoor and outdoor conditions should be considered. Since our study did not include a source-tracking analysis, we recommend that future studies implement outdoor air sampling, as well as collection of potential source samples (e.g. surfaces) from both the interior of the transport cabins and the outdoor surrounding environments, in order to accurately determine the origin of the observed airborne taxa.

6. Conclusions

Studies characterizing the microbial aerosols in transportation environments are crucial in order to evaluate their role in human health and exposure during commuting (Colbeck and Whitby, 2019). This study examined the aerosol microbiome in train and car transport microenvironments, covering similar commuter routes, using high throughput sequencing. While a core bacterial and fungal microbiome, including human commensals and outdoor-originating micro-organisms, was found between the two transport modes, with more common taxa between the train and car A, distinct bacterial and fungal communities were also found. Interestingly, distinct fungal communities were found associated with the different modes of transport, with an abundance of Basidiomycota associated with the car environment compared to the train where Ascomycota were predominant, which may be related to the time of year sampled. Of particular human health concern was the recovery of microbial genera affiliated with potentially pathogenic or allergenic species found in both the train and car, suggesting that in all transport environments ventilation should be optimised to reduce passenger's exposure to potentially harmful bioaerosols. Our findings show that choice of transport mode has a significant impact on the bioaerosol microbiome that passengers are exposed to.

CRediT authorship contribution statement

N. Grydaki: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **I. Colbeck:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **C. Whitby:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no competing interests to declare.

Acknowledgements

The study was part of the Marie Skłodowska-Curie project "Human EXposure to Aerosol COntaminants in Modern Microenvironments" (HEXACOMM), supported by the European Union 7th Framework Programme (FP7/2007-2013) under Grant agreement no. 315760, with additional support from NERC (NE/M010813/1). We would like to thank our HEXACOMM collaborators from Institute of Environmental Assessment and Water Research (IDÆA-CSIC), Barcelona, Spain, for providing the portable Leland Legacy Pump (SKC Ltd., UK) used for air sampling in trains.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177539.

Data availability

Data will be made available on request.

References

Adams, H.S., Nieuwenhuijsen, M.J., Colvile, R.N., McMullen, M.A.S., Khandelwal, P., 2001. Fine particle (PM2.5) personal exposure levels in transport microenvironments, London, UK. Sci. Total Environ. 279 (1–3), 29–44.
Adams, R.I., Miletto, M., Taylor, J.W., Bruns, T.D., 2013. Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. ISME J. 7 (7), 1262–1273.
Adams, R.I., Miletto, M., Lindow, S.E., Taylor, J.W., Bruns, T.D., 2014. Airborne bacterial communities in residences: similarities and differences with fungi. PloS One 9 (3), e91283.
Adams, R.I., Bhangar, S., Pasut, W., Arens, E.A., Taylor, J.W., Lindow, S.E., Nazaroff, W. W., Bruns, T.D., 2015. Chamber bioaerosol study: outdoor air and human occupants as sources of indoor airborne microbes. PloS One 10 (5), e0128022.
Amend, A.S., Seifert, K.A., Samson, R., Bruns, T.D., 2010. Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. Proc. Natl. Acad. Sci. 107 (31), 13748–13753.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26 (1), 32–46.
Bardou, P., Mariette, J., Escudié, F., Djemiel, C., Klopp, C., 2014. *jvenn*: an interactive Venn diagram viewer. BMC Bioinformatics 15 (1), 293.
Bista, S., Dureau, C., Chaix, B., 2022. Personal exposure to concentrations and inhalation of black carbon according to transport mode use: the MobiliSense sensor-based study. Environ. Int. 158, 106990.
Bowers, R.M., Clements, N., Emerson, J.B., Wiedinmyer, C., Hannigan, M.P., Fierer, N., 2013. Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. Environ. Sci. Technol. 47 (21), 12097–12106.
Buitrago, N.D., Savdie, J., Almeida, S.M., Verde, S.C., 2021. Factors affecting the exposure to physicochemical and microbiological pollutants in vehicle cabins while commuting in Lisbon. Environ. Pollut. 270, 116062.
Campagnolo, D., Cattaneo, A., Corbella, L., Borghi, F., Del Buono, L., Rovelli, S., Spinazzé, A., Cavallo, D.M., 2019. In-vehicle airborne fine and ultra-fine particulate matter exposure: the impact of leading vehicle emissions. Environ. Int. 123, 407–416.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Gonzalez Pena, A., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336.
Cepeda, M., Schoufour, J., Freak-Poli, R., Koolhaas, C.M., Dhana, K., Bramer, W.M., Franco, O.H., 2017. Levels of ambient air pollution according to mode of transport: a systematic review. Lancet Public Health 2 (1), e23–e34.
Colbeck, I., Whitby, C., 2019. In: Harrison, R.M., Hester, R.E. (Eds.), Biological Particles in the Indoor Environment. Issues in Environmental Science and Technology No. 48 Indoor Air Pollution. Royal Society of Chemistry.
Conceição, T., Diamantino, F., Coelho, C., de Lencastre, H., Aires-de-Sousa, M., 2013. Contamination of public buses with MRSA in Lisbon, Portugal: a possible transmission route of major MRSA clones within the community. PloS One 8 (11), e77812.
Department of Transport, 2019. Transport Statistics Great Britain 2019. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/870647/tsgb-2019.pdf.
DeSouza, P., Lu, R., Kinney, P., Zheng, S., 2021. Exposures to multiple air pollutants while commuting: evidence from Zhengzhou. China. Atmospheric Environment 247, 118168.
Fang, Z., Gong, C., Ouyang, Z., Liu, P., Sun, L., Wang, X., 2014. Charac- teristic and concentration distribution of culturable airborne bacteria in residential environments in Beijing. China. Aerosol Air Qual Res 14 (3), 943–953.
Ferguson, R.M.W., Garcia-Alcega, S., Coulon, F., Dumbrell, A.J., Whitby, C., Colbeck, I., 2019. Bioaerosol biomonitoring: sampling optimisation for molecular microbial ecology. Mol. Ecol. Resour. 19, 672–690.
Ferguson, R.M.W., Neath, C.E.E., Nasir, Z.A., Garcia-Alcega, S., Tyrrel, S., Coulon, F., Dumbrell, A.J., Colbeck, I., Whitby, C., 2021. Size fractionation of bioaerosol emissions from green-waste composting. Environ. Int. 147, 106327.
Fernández-Iriarte, A., Duchaine, C., Degois, J., et al., 2021. Bioaerosols in public and tourist buses. Aerobiologia 37, 525–541.
Frankel, M., Bekö, G., Timm, M., Gustavsen, S., Hansen, E.W., Madsen, A.M., 2012. Seasonal variations of indoor microbial exposures and their relation to temperature, relative humidity, and air exchange rate. Appl. Environ. Microbiol. 78 (23), 8289–8297.
García-Mozo, H., López-Orozco, R., Canalejo, C., Oteros, J., 2020. Indoor biological particles in a train: comparative analysis with outdoor atmosphere. Aerobiologia 36 (3), 481–492.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2 (2), 113–118.
Gartland, N., Fishwick, D., Coleman, A., Davies, K., Hartwig, A., Johnson, S., Van Tongeren, M., 2022. Transmission and control of SARS-CoV-2 on ground public transport: a rapid review of the literature up to May 2021. J. Transp. Health 26, 101356.

Gaüzère, C., Godon, J.J., Blanquart, H., Ferreira, S., Moularat, S., Robine, E., Moletta-Denat, M., 2014a. 'Core species' in three sources of indoor air belonging to the human micro-environment to the exclusion of outdoor air. Sci. Total Environ. 485, 508–517.
Gaüzère, C., Moletta-Denat, M., Blanquart, H., Ferreira, S., Moularat, S., Godon, J.J., Robine, E., 2014b. Stability of airborne microbes in the Louvre Museum over time. Indoor Air 24 (1), 29–40.
Gołofit-Szymczak, M., Stobnicka-Kupiec, A., Górny, R.L., 2019. Impact of air-conditioning system disinfection on microbial contamination of passenger cars. Air Qual. Atmos. Health 12, 1127–1135.
Goto, T., Yamashita, A., Hirakawa, H., Matsutani, M., Todo, K., Ohshima, K., Toh, H., Miyamoto, K., Kuhara, S., Hattori, M., Shimizu, T., 2008. Complete genome sequence of Finegoldia magna, an anaerobic opportunistic pathogen. DNA Res. 15 (1), 39–47.
Green, D., Zhou, J., Desouza, C., 2021. Transport for London SARS-CoV-2 RNA sampling study, Environmental Research Group, Imperial College London. https://content.tfl.gov.uk/covid-sampling-paper-phase-1.pdf.
Grydaki, N., Colbeck, I., Mendes, L., Eleftheriadis, K., Whitby, C., 2021. Bioaerosols in the Athens Metro: Metagenetic insights into the PM_{10} microbiome in a naturally ventilated subway station. Environ. Int. 146, 106186.
Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5 (10), 1571.
Hernández-Castillo, O., Mugica-Álvarez, V., Castañeda-Briones, M.T., Murcia, J.M., García-Franco, F., Briseño, Y.F., 2014. Aerobiological study in the Mexico City subway system. Aerobiologia 30 (4), 357–367.
Hoisington, A.J., Maestre, J.P., King, M.D., Siegel, J.A., Kinney, K.A., 2014. Impact of sampler selection on the characterization of the indoor microbiome via high-throughput sequencing. Build. Environ. 80, 274–282.
Hoseini, M., Jabbari, H., Naddafi, K., Nabizadeh, R., Rahbar, M., Yunesian, M., Jaafari, J., 2013. Concentration and distribution characteristics of airborne fungi in indoor and outdoor air of Tehran subway stations. Aerobiologia 29 (3), 355–363.
Hospodsky, D., Qian, J., Nazaroff, W.W., Yamamoto, N., Bibby, K., Rismani-Yazdi, H., Peccia, J., 2012. Human occupancy as a source of indoor airborne bacteria. PloS One 7 (4), e34867.
Jo, W.K., Lee, J.H., 2008. Airborne fungal and bacterial levels associated with the use of automobile air conditioners or heaters, room air conditioners, and humidifiers. Arch. Environ. Occup. Health 63 (3), 101–107.
Kaarakainen, P., Meklin, T., Rintala, H., Hyvärinen, A., Kärkkäinen, P., Vepsäläinen, A., Hirvonen, M.R., Nevalainen, A., 2008. Seasonal variation in airborne microbial concentrations and diversity at landfill, urban and rural sites. CLEAN–Soil, Air. Water 36 (7), 556–563.
Kaur, S., Nieuwenhuijsen, M.J., Colvile, R.N., 2007. Fine particulate matter and carbon monoxide exposure concentrations in urban street transport microenvironments. Atmos. Environ. 41 (23), 4781–4810.
Kembel, S.W., Jones, E., Kline, J., Northcutt, D., Stenson, J., Womack, A.M., Bohannan, B.J., Brown, G.Z., Green, J.L., 2012. Architectural design influences the diversity and structure of the built environment microbiome. ISME J. 6 (8), 1469.
Kumar, P., Lopez, M., Fan, W., Cambre, K., Elston, R.C., 1990. Mold contamination of automobile air conditioner systems. Ann. Allergy 64 (2), 174–177.
Kumar, P., Hama, S., Abbass, R.A., Nogueira, T., Brand, V.S., Abhijith, K.V., de Fatima Andrade, M., Asfaw, A., Aziz, K.H., Cao, S.J., El-Gendy, A., 2021. Potential health risks due to in-car aerosol exposure across ten global cities. Environ. Int. 155, 106688.
Lee, B.G., Kim, Y.J., Shim, J.E., Lee, H., Yeo, M.K., 2024. Estimation of microbial inhalation exposure and prediction of microbial concentrations in rail transportation facilities during the COVID-19 pandemic. Aerosol Sci. Tech. 58 (3), 309–322.
Lee, J.H., Jo, W.K., 2005. Exposure to airborne fungi and bacteria while commuting in passenger cars and public buses. Atmos. Environ. 39 (38), 7342–7350.
Leung, M.H.Y., Tong, X., Bøifot, K.O., Bezdan, D., Butler, D.J., Danko, D.C., Gohli, J., Green, D.C., Hernandez, M.T., Kelly, F.J., Levy, S., 2021. Characterization of the public transit air microbiome and resistome reveals geographical specificity. Microbiome 9 (1), 1–19.
Li, J., Li, M., Shen, F., Zou, Z., Yao, M., Wu, C.Y., 2013. Characterization of biological aerosol exposure risks from automobile air conditioning system. Environ. Sci. Technol. 47 (18), 10660–10666.
Luongo, J.C., Barberán, A., Hacker-Cary, R., Morgan, E.E., Miller, S.L., Fierer, N., 2017. Microbial analyses of airborne dust collected from dormitory rooms predict the sex of occupants. Indoor Air 27 (2), 338–344.
Meadow, J.F., Altrichter, A.E., Kembel, S.W., Kline, J., Mhuireach, G., Moriyama, M., Northcutt, D., O'Connor, T.K., Womack, A.M., Brown, G.Z., Green, J.L., 2014. Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. Indoor Air 24 (1), 41–48.
Meadow, J.F., Altrichter, A.E., Bateman, A.C., Stenson, J., Brown, G.Z., Green, J.L., Bohannan, B.J., 2015. Humans differ in their personal microbial cloud. PeerJ 3, e1258.
Nowakowicz-Dębek, B., Pawlak, H., Wlazło, Ł., Maksym, P., Kapica, J., Chmielowiec-Korzeniowska, A., Trawińska, B., 2017. Evaluating bioaerosol exposure among bus drivers in the public transport sector. J. Occup. Environ. Hyg. 14 (11), D169–D172.
Núñez, A., García, A.M., Moreno, D.A., Guantes, R., 2021. Seasonal changes dominate long-term variability of the urban air microbiome across space and time. Environ. Int. 150, 106423.
Onat, B., Alver Şahin, Ü., Sivri, N., 2017. The relationship between particle and culturable airborne bacteria concentrations in public transportation. Indoor Built. Environ. 26 (10), 1420–1428.
Pankhurst, L., Whitby, C., Pawlett, M., Larcombe, L., McKew, B.A., Deacon, L., Morgan, S., Villa, R., Drew, G., Tyrrel, S., Pollard, S., Coulon, F., 2012. Temporal and spatial changes of the microbial bioaerosol communities in green-waste composting. FEMS Microbiol. Ecol. 79, 229–239.
Passi, A., Nagendra, S.S., Maiya, M.P., 2021. Assessment of exposure to airborne aerosol and bio-aerosol particles and their deposition in the respiratory tract of subway metro passengers and workers. Atmos. Pollut. Res. 12 (11), 101218.
Patel, K.V., Bailey, C.L., Harding, A.H., Biggin, M., Crook, B., 2018. Back- ground levels of micro-organisms in the busy urban environment of transport hubs. J. Appl. Microbiol. 125 (5), 1541–1551.
Peimbert, M., Alcaraz, L.D., 2023. Where environmental microbiome meets its host: Subway and passenger microbiome relationships. Mol. Ecol. 32 (10), 2602–2618.
Prakash, N.U., Bhuvaneswari, S., Kumar, M.R., Lankesh, S., Rupesh, K., 2014. A study on the prevalence of indoor mycoflora in air-conditioned buses. British Microbiology Research Journal 4 (3), 282–292.
Prussin II, A.J., Vikram, A., Bibby, K.J., Marr, L.C., 2016. Seasonal dynamics of the airborne bacterial community and selected viruses in a children's daycare center. PloS One 11 (3), e0151004.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rendon, R.V.C., Garcia, B.C.B., Vital, P.G., 2017. Assessment of airborne bacteria in selected occupational environments in Quezon City, Philippines. Arch. Environ. Occup. Health 72 (3), 178–183.
Rivas, I., Kumar, P., Hagen-Zanker, A., 2017a. Exposure to air pollutants during commuting in London: are there inequalities among different socio-economic groups. Environ. Int. 101, 143–157.
Rivas, I., Kumar, P., Hagen-Zanker, A., de Fatima Andrade, M., Slovic, A.D., Pritchard, J. P., Geurs, K.T., 2017b. Determinants of black carbon, particle mass and number concentrations in London transport microenvironments. Atmos. Environ. 161, 247–262.
Road Traffic Statistics, 2017. UK Road Traffic Statistics -A12 Brentwood. https://roadtrafficstats.uk/traffic-statistics-essex-a12-16196#.Yvpvc3bMJPY.
RSSB, 2021. CLEAR: Analysis of Air Quality on Board Trains T1188. Board, Rail Safety and Standards.
Selman, M., Buendía-Roldán, I., Navarro, C., Gaxiola, M., 2017. Hyper- sensitivity pneumonitis. In: Pulmonary Hypertension and Interstitial Lung Disease. Springer, Cham, pp. 145–164.
Shin, S.K., Kim, J., Ha, S.M., Oh, H.S., Chun, J., Sohn, J., Yi, H., 2015. Metagenomic insights into the bioaerosols in the indoor and outdoor environments of childcare facilities. PloS One 10 (5), e0126960.
Simmons, R.B., Rose, L.J., Crow, S.A., Ahearn, D.G., 1999. The occurrence and persistence of mixed biofilms in automobile air conditioning systems. Curr. Microbiol. 39 (3), 141–145.
Simoes, R.R., Aires-de-Sousa, M., Conceiçao, T., Antunes, F., da Costa, P.M., de Lencastre, H., 2011. High prevalence of EMRSA-15 in Portuguese public buses: a worrisome finding. PloS One 6 (3), e17630.
Simon-Nobbe, B., Denk, U., Pöll, V., Rid, R., Breitenbach, M., 2008. The spectrum of fungal allergy. Int. Arch. Allergy Immunol. 145 (1), 58–86.
Stephens, D.S., Farley, M.M., 1990. Pathogenic events during infection of the human nasopharynx with Neisseria meningitidis and Haemophilus influenzae. Rev. Infect. Dis. 13 (1), 22–33.
Stephenson, R.E., Gutierrez, D., Peters, C., Nichols, M., Boles, B.R., 2014. Elucidation of bacteria found in car interiors and strategies to reduce the presence of potential pathogens. Biofouling 30 (3), 337–346.
Tirachini, A., Cats, O., 2020. COVID-19 and public transportation: current assessment, prospects, and research needs. J. Public Transp. 22 (1), 1.
UK Government, 2017. Estimates of station usage: 2016 to 2017. https://www.gov.uk/government/statistics/estimates-of-station-usage-2016-to-2017.
Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A., Knight, R., 2013. EMPeror: a tool for visualizing high-throughput microbial community data. Gigascience 2 (1), 16.
Vonberg, R.P., Gastmeier, P., Kenneweg, B., Holdack-Janssen, H., Sohr, D., Chaberny, I. F., 2010. The microbiological quality of air improves when using air conditioning systems in cars. BMC Infect. Dis. 10 (1), 146.
Wang, C., Xu, J., Fu, S.C., Chan, K.C., Chao, C.Y., 2021b. Respiratory bioaerosol deposition from a cough and recovery of viable viruses on nearby seats in a cabin environment. Indoor Air 31 (6), 1913–1925.
Wang, C.Y., Lim, B.S., Wang, Y.H., Huang, Y.C.T., 2021a. Identification of high personal PM2. 5 exposure during real time commuting in the Taipei metropolitan area. Atmosphere 12 (3), 396.
Wang, Y.F., Wang, C.H., Hsu, K.L., 2010. Size and seasonal distributions of airborne bioaerosols in commuting trains. Atmos. Environ. 44 (35), 4331–4338.
Wang, Y.F., Tsai, C.H., Huang, Y.T., Chao, H.R., Tsou, T.C., Kuo, Y.M., Wang, L.C., Chen, S.H., 2013. Size distribution of airborne fungi in vehicles under various driving conditions. Arch. Environ. Occup. Health 68 (2), 95–100.
White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J.L., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A Guide to Methods and Applications 18 (1), 315–322.
Wilkins, D., Leung, M.H., Lee, P.K., 2016. Indoor air bacterial communities in Hong Kong households assemble independently of occupant skin microbiomes. Environ. Microbiol. 18 (6), 1754–1763.
Yamamoto, N., Bibby, K., Qian, J., Hospodsky, D., Rismani-Yazdi, H., Nazaroff, W.W., Peccia, J., 2012. Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. ISME J. 6 (10), 1801–1811.
Yang, Z., He, Z., Zhang, K., Zeng, L., de Nazelle, A., 2021. Investigation into Beijing commuters' exposure to ultrafine particles in four transportation modes: bus, car, bicycle and subway. Atmos. Environ. 266, 118734.