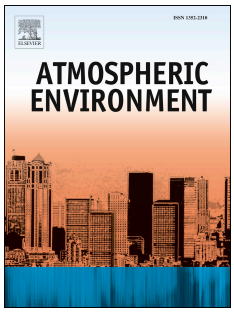

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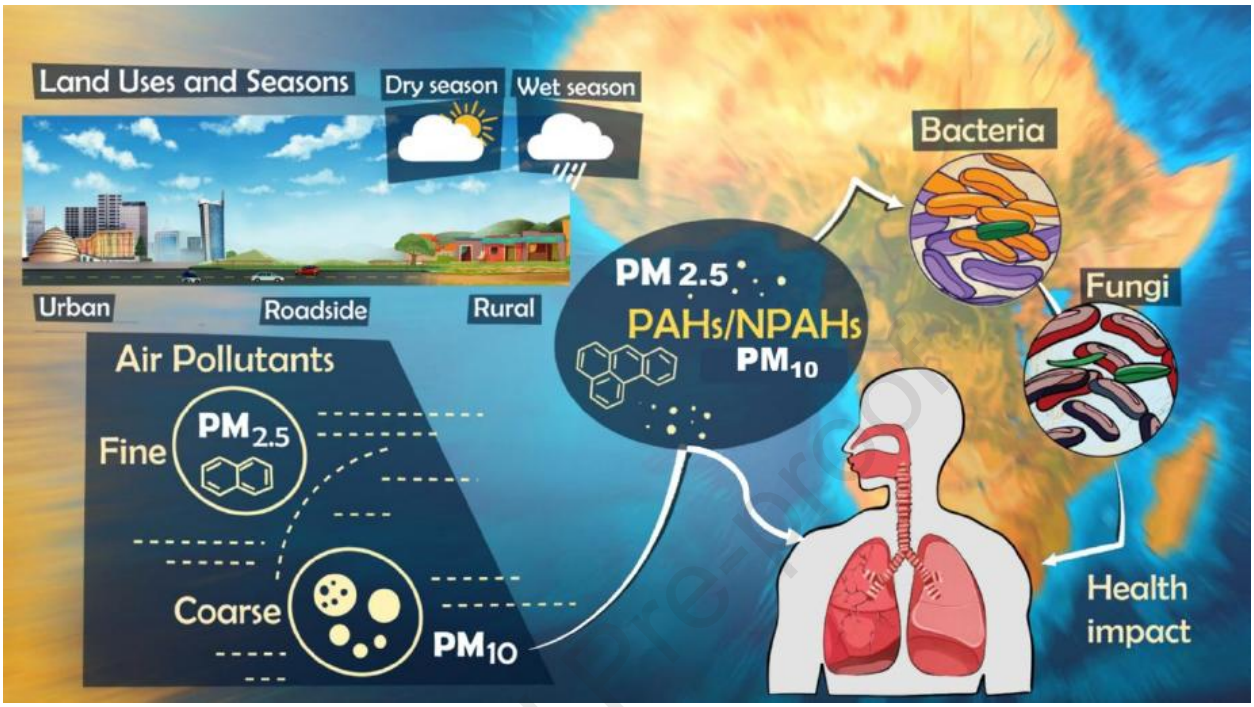
Graphical Abstract



Variability of Airborne Microbial Communities and Associations with Organic Pollutants in African Air Particulate Matter Across Land-Use Types

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Abstract

Exposure to particulate matter (PM) is a major global health concern, yet the potential relationships between its chemical and microbial components remains poorly understood, particularly in rapidly urbanizing, understudied settings. This study presents an integrated assessment of polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs (NPAHs), bacteria, and fungi in both fine ($PM_{2.5}$) and coarse (PM_{10}) aerosols across urban, roadside, and rural sites in sub-Saharan Africa, with a focus on Rwanda across dry and wet seasons. Microbial analysis revealed that the richness and community structure of the airborne bacterial and fungal communities varied with land-use type, linked with PAH/NPAH abundance, PM size fraction, and season. Spearman correlation coefficient confirmed that bacterial communities were more strongly associated with PAH and NPAH compounds, whereas fungal communities were shaped primarily by environmental factors. One bacterial genus, *Sphingobium*, exhibited evidence of selective enrichment within the PAH rich $PM_{2.5}$ size fraction, highlighting the potential for direct interaction between the biological and chemical compositions in air. We provide a critical baseline for African cities where air quality data are scarce. Current air quality standards, which prioritize chemical thresholds, overlook the biological burden carried by PM.

Keywords: *Particulate matter, airborne bacteria, airborne fungi, polycyclic aromatic hydrocarbons, Chemical-microbial interaction, Rwanda, Sub-Saharan Africa*

1. Introduction

Atmospheric particulate matter (PM) is a complex mixture of organic and inorganic material, and is the largest environmental cause of premature human mortality worldwide, particularly in Africa (Bauer et al., 2019; Bililign et al., 2024; Kalisa et al., 2019a; World Health Organization, 2016). PM_{10} (particulate matter, <10 μm) and $PM_{2.5}$ (particulate matter, <2.5 μm) are commonly the focus of air quality monitoring in Africa because of their known effects on the respiratory system (Petkova et al., 2013). Bound to PM, and increasing the health risks, are a broad variety of chemicals, including heavy metals, nitrates, sulphates, minerals, polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs (NPAHs), and microbial components such as viruses, bacteria, and fungi, some of which are associated with allergenic and pathogenic potential to humans (Després et al., 2012; Haas et al., 2013; Kalisa et al., 2019a; Morakinyo et al., 2016; Šantl-Temkiv et al., 2022; Vermani et al., 2010; Zhai et al., 2018). Although biological particles in the atmosphere account for 25% of the PM (Jaenicke, 2005), most research focuses on its chemical components (Yoo et al., 2017). When multiple pollutants have been investigated in the same study, the health effects are greater than the sum of the cumulative effects of each variable, indicating the need for transdisciplinary understandings of air pollution (Adhikari et al., 2006; Morakinyo et al., 2016; Rosselli et al., 2015). These studies also demonstrated that pollutants affect microbial communities, and that these communities vary by region and size fraction.

Few studies have characterized both the chemical and biological composition of PM (Gandolfi et al., 2015; Jalava et al., 2015; Runlan et al., 2019; Sánchez de La Campa et al., 2013; Sun et al., 2018; Yan et al., 2018), and Limited studies have reported a correlation between bioaerosol communities and chemical composition (Murakami et al., 2010; Petroselli et al., 2021; Shi et al., 2022). The objectives of this study were to address this knowledge gap by to: (1) characterizing

airborne bacterial and fungal communities by aerosol size fractions and from three locations (rural, urban background and urban roadside) in Rwanda using high-throughput DNA sequencing, and (2) examining these communities association with PAH and NPAH levels.

2. Materials and Methods

2.1 Sampling of Atmospheric Particulate Matter

A high-volume air sampler (SIBATA, Electric Company Limited, HVS-RW-1000F, Japan), equipped with $PM_{2.5}$ and PM_{10} fractionating inlets at an average flow rate of $1m^3$/min, was used to collect PM_{10} and $PM_{2.5}$ samples over a 24-hour period. Samples were collected on Whatman glass microfibre filters (GFF, 8″ x 10″) from three land-use types to capture a range of air pollution exposure scenarios in Rwanda. The first was an urban background (UB) site on the rooftop of the five-storey Muhabura Building at the College of Science and Technology. The UB site (1.9616° S, 30.0640° E) was chosen for its distance from immediate emissions sources (major roads, factories) and represents a city-wide background air quality condition in Kigali. The nearest main road and residential area are located ~3km away. The site is in a green environment with relatively few direct pollution hotspots. The urban background site reflects typical Rwandan urban conditions, including widespread domestic wood burning, a healthy residential area and a green environment.

The urban roadside (UR) site was located on the rooftop of the Rwanda Ministry of Environment Nyarugenge Pension Plaza building, near a high-traffic corridor. The UR site (1.9519° S, 30.0739° E) was adjacent to a main road and experienced high volumes of private vehicles, buses and motor-taxi traffic. The UR site was located within 10m of the primary corridors, and surrounding mixed-use/commercial land use with high traffic density (>10,000 vehicles/motorcycle per day).

The rural site was situated on the rooftop of the Rwanda Energy Group (REG) station in Musanze. The rural site (1.5367° S, 29.68253° E) was 102 km north of Kigali City, distant from high densities of industrial and traffic emissions sources, and served as a rural reference point for microbial and chemical. These sites were chosen to represent typical urban, urban roadside, and rural conditions in sub-Saharan Africa, considering differences in population density, emission sources (such as biomass burning and traffic), and environmental settings. Rooftop placement (10-15 m above ground level) ensured security and access to electricity, prevented vandalism, and minimized direct interference from road dust and local dust disturbances. Kigali City was chosen because it is the largest city in Rwanda, with high levels of economic development and industry compared to other urban settings. Rwanda is a landlocked country situated at a high altitude, which creates a stable tropical climate, characterized by reduced temperature variation and slower wind speeds than many other African countries. Consequently, emissions throughout the year are relatively constant.

All samples were collected at the UB site (wet season: 01 April to 21 April; dry season: 15 June to 30 June) and at the UR site (wet season: 15 May to 31 May; dry season: 01 June to 14 June). Due to logistical constraints, samples were collected only during the wet season (23 April to 14 May) at the rural site. **Table S1** provides details of the study sites and characteristics and Figure 1 shows the location of sampling site within map of Rwanda and Africa In Rwanda, the long wet season usually occurs from March to May, characterized by sustained heavy rainfall, while the long dry season from June to August features lower precipitation and cooler temperatures. These seasons reflect the primary climatic cycles in the region and are commonly used in environmental

and public health research to distinguish between wet and dry periods (DeWitt et al., 2019; Kalisa et al., 2025, 2023; Kalisa and Adams, 2022; Subramanian et al., 2020).

A total of 88 $PM_{2.5}$ and PM_{10} samples were collected from three land-use types (urban, rural and roadside) in Rwanda.

Meteorological parameters (relative humidity and temperature) were obtained from the Rwanda Meteorological Agency, located ~ 2-5 km from the nearest urban and rural sampling sites.

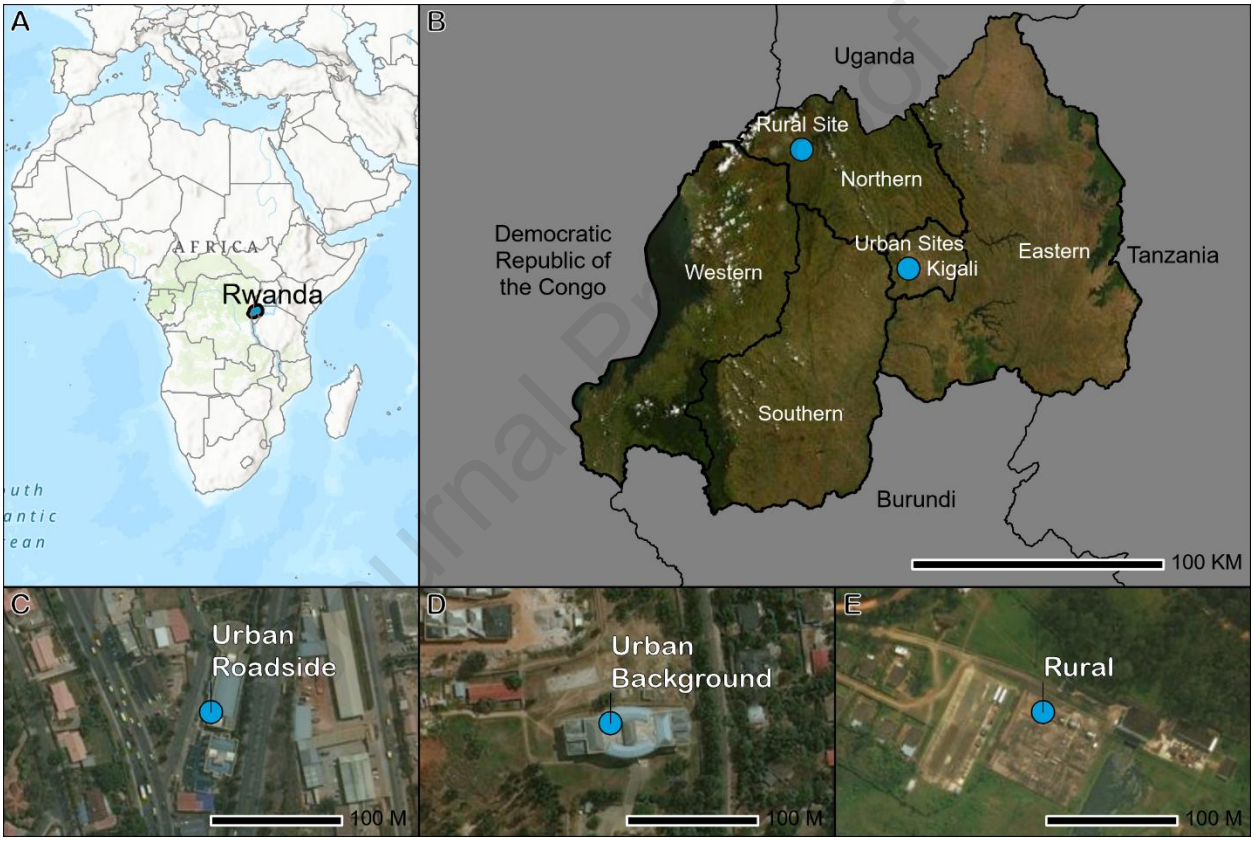

Figure 1. A – Rwanda location within Africa. B – Provinces of Rwanda including locations for rural site and urban sites. C – Urban Roadside site. D – Urban Background site. E – Rural site. Sources: ESRI, DigitalGlobal, GeoEYE, Earthstar Geographics, CNES/Airbus DS, USDA, AeroGRID, and IGN. Map projection: EPSG:3857.

2.2 Gravimetrical analysis of PM

PM_{10} and $PM_{2.5}$ concentrations were calculated gravimetrically using methods described in our previous study (Kalisa et al., 2018c). Briefly, the PM_{10} and $PM_{2.5}$ filters were weighed prior to

sampling using an electronic microbalance (KERN, Balingen, Germany, readability 0.1µg). The filter holders were sterilized with 70% ethanol before each sampling. After sampling, filters were transported from the site to the laboratory packed in dry ice. Gravimetric analysis was carried out for each PM sample in an environment-controlled room by dividing the net change in weight of filters before and after sampling by the total volume of air (1440 m^3) for 24-hour sampling. All filters were then stored at −20°C until further analysis. Each PM filter was cut into two equal parts using sterilized scissors: one part was analyzed for 16 PAH and 8 NPAH compounds, and the other part was analyzed for biological components (airborne bacteria and fungi).

2.3 Biological: Extraction of DNA and sequencing

DNA was extracted directly from the PM filter samples. A portion (49.2 cm^2) from each half of the filter was cut into small pieces and placed in a Nucleospin bead tube (2ml, Machinery–Nagel, Germany) filled with ceramic beads (1.4 mm, Qiagen, Germany). Cetyl trimethylammonium bromide (CTAB) was used to extract genomic DNA following the protocol described previously (Archer et al., 2015; Kalisa et al., 2022, 2024). The genomic DNA from PM samples was quantified in ng/µL using Qubit (Fluorometer, Invitrogen, USA). Polymerase chain reaction (PCR) was used to amplify DNA using primer pairs targeting the V3-V4 hypervariable regions of the bacterial 16S rRNA gene (PCR1 forward and PCR1 reverse), and the internal transcribed spacer region of fungal 18S and 5.8S RNA genes (ITS1 forward and ITS2 reverse), as detailed in our previous studies (Kalisa et al., 2024, 2022).These gene regions provide an extensive estimate of the taxonomic compositions of microorganisms in communities (Delgado-Baquerizo et al., 2018; Schoch et al., 2012; Thompson et al., 2017). AMPure XP beads (California, USA) were used to purify the PCR product and a Nextera XT index kit was used to index the amplicons (Archer et

158 al., 2019). The amplicon libraries were analysed using the Illumina MiSeq platform following the
159 manufacturer's protocol (Illumina, CA, USA).

160
161 *2.4 Sequence data analysis*
162
163 Amplicon Sequence Variants (ASVs) were determined for the 16S region using the R package
164 DaDa2 (Callahan et al., 2016). The taxonomy of the ASVs was assigned using the built-in RDP
165 naïve Bayesian classifier in DADA2 with the SILVA nr v132 database (Quast et al., 2012).
166 USEARCH version 9.0.2132 (Edgar, 2010) was used to process the ITS amplicon sequence data
167 to generate operational taxonomic units (OTUs). Fungal OTUs based on the ITS sequences were
168 clustered at the 97% similarity threshold following the workflow described in Archer et al. (2019).
169 Alpha diversity was estimated using Shannon and Chao1 indices. The PERMANOVA test
170 (Anderson et al., 2006) on weighted UniFrac distance matrices was carried out using the R package
171 vegan v.2.5.4 (Oksanen et al., 2016) to investigate variance between the microbial communities
172 partitioned by land use types, PM size fraction (PM_{10} and $PM_{2.5}$), and seasons. Spatial and temporal
173 factors in airborne microbial communities were visualized using Principal Coordinate Analysis
174 (PCoA). The sequencing reads were deposited in the EMBL-EBI, European Nucleotide Archive
175 (ENA) with the study access number PRJEB33617.

176
177 *2.5 Chemical: PAHs and NPAHs analysis*
178
179 PAH and NPAH compounds were extracted from $PM_{2.5}$ and PM_{10} and analyzed following our
180 previous protocol (Kalisa et al., 2018a). Briefly, a 16 PAH mixture (Sigma-Aldrich, USA) and an
181 8 NPAH mixture (Chiron, Trodheim, Norway) were used as standards. The names and
182 abbreviations of these PAHs are detailed in Table S1. One portion (49.2 cm^2) from half of each of
183 the PM_{10} and $PM_{2.5}$ sample filters was divided into small portions and placed in a conical flask.

Five deuterated PAHs – naphthalene-d_8 (Nap-d_8), acenaphthylene-d_{10}, (Ace-d_{10}), phenanthrene-d_{10} (Phe-d_{10}), chrysene-d_{12} (Chr-d_{12}), perylene-d_{12} (Pyr-d_{12}), and an NPAH surrogate (2-fluoro-7-nitrofluorene (FNF)) – were added as internal standards. The PM_{10} and $PM_{2.5}$ filter samples were sonically extracted with benzene-ethanol and the extract was cleaned with sodium hydroxide, sulfuric acid, and ultrapure water. Detailed extraction steps have been previously reported (Hayakawa et al., 2018; Kalisa et al., 2019b, 2018a). About 100 µL of the extract were analyzed using high-performance liquid chromatographic systems (HPLC, Kyoto, Japan) with a fluorescence and chemiluminescence detector for NPAH analysis. The HPLC system has been previously detailed (Hayakawa, 2018).

2.6 Correlation analysis

In this study, total PM mass, total PAHs, temperature, and relative humidity were considered environmental variables that could potentially influence aerosol characteristics, while individual PAHs and NPAHs were treated as pollutant species examined for their correlations with bacterial and fungal community composition. The correlations between environmental factors (PM_{10}, $PM_{2.5}$, total PAHs, total NPAHs, temperature (T^oC) and relative humidity (RH)), airborne bacterial and fungal abundances and communities' diversity in relation to PAH and NPAH compounds were investigated using Spearman′s rank correlation coefficients. Pairwise Pearson correlations were calculated across all composite samples, using pairwise deletion of missing values. The resulting symmetric matrix was visualized as a heat map. Rows and columns were jointly clustered by complete-linkage hierarchical clustering on Euclidean distances of the correlation profiles. Global model, axis-specific and term-specific significances were assessed with permutation ANOVA. Skew-positive pollutant concentrations were log-transformed as ln(1 + x) to accommodate zeros

and approximate normality. All quantitative variables were then centred and scaled ($\mu = 0$, $\sigma = 1$) so that regression coefficients were on comparable metrics.

3. Results and Discussion

3.1 Patterns of Occurrence for Particulate-bound PAHs and NPAHs

Mean concentrations of the collected PM_{10} and $PM_{2.5}$ showed seasonal variation, with lower levels during the wet season than the dry season (Table S2). The highest average dry season concentration at each site was measured at the UR location (216.7 ± 46.3 μg/m^3 and 188.0 ± 41.3 μg/m^3, respectively), in contrast to the UB site (143.0 ± 21.4 μg/m^3 and 124.3 ± 18.5 μg/m^3, respectively). The highest PM concentration occurs during the dry season, primarily due to the increased resuspension of dust from unpaved roads, biomass burning, and agricultural activities (Kalisa et al., 2025, 2018b).

The mean concentrations of ΣPAHs and ΣNPAHs were both higher in the dry season than in the wet season (Table S2). The means of PM_{10}-bound ΣPAHs and ΣNPAHs at the UR site were 60.7 ± 19.4 ng/m^3 and 1155.0 ± 540 pg/m^3, respectively, representing the highest mean values for total PAH and NPAH concentrations. The lowest mean concentration of PM was recorded at the rural site (53.6 ± 17.1 and 45.0 ± 15.6 μg/m^3, respectively) during the wet season (Table S2) across locations. However, the ΣPAH concentrations at the rural site (37.5 ± 19.5 ng/m^3) were higher than those at the urban background site (23.07 ± 8.0 ng/m^3) during the wet season (Table S2). The average air temperature (°C) and relative humidity (%) during the 3-month sampling period indicated stable conditions with little variability between dry and wet seasons, or between land-use types, as demonstrated by a recent study in Rwanda (Kalisa et al., 2022). High PAHs at rural sites are due to wood and charcoal burning for heating and cooking, while at urban sites, traffic

diesel emissions are the primary contributor to air pollution (Kalisa et al., 2025, 2018b; Kalisa and Adams, 2022; Subramanian et al., 2020). The PAH and NPAH levels measured in Rwanda are higher than those reported in America, Asia and Europe (Bai et al., 2024; Hayakawa and Aoki, 2025; Kim et al., 2013).

3.2 Overview of Microbial Diversity and Distribution in Aerosols

Bacterial species richness (Chao 1) and diversity (Shannon) varied with land-use type, PM size, and season (Table S3). The observed variation in bacterial richness and diversity across land-use types, PM size fraction and season aligned with the findings of Archer et al. (2023), who reported that strong environmental factors, macroclimatic conditions, and dominance of local sources shaped airborne bacterial diversity (Archer et al., 2023).

In the present study, the number of bacterial species detected in the $PM_{2.5}$ samples across all sites was higher than in PM_{10}. Conversely, the fungal taxa richness was slightly greater in the PM_{10} samples than in $PM_{2.5}$, peaking in the rural samples during the wet season. The species richness remained consistent across samples from different land-use types, PM sizes, and seasons. Airborne bacterial and fungal alpha diversity levels in Rwanda were higher than those reported from high-altitude terrestrial areas in Japan (Maki et al., 2015), China (Xu et al., 2019) and Germany (Fröhlich-Nowoisky et al., 2009). During both seasons, species richness and Shannon evenness were highest at the UR site, followed by the UB and then the rural site, suggesting an urban influence on bacterial populations. However, for fungi the rural site, close to forest and agricultural activities, displayed the highest species richness and community diversity. This aligns with findings in the USA (Bowers et al., 2011; Yamamoto et al., 2012).

3.3 Airborne Microbial community composition

3.3.1 Bacteria in $PM_{2.5}$ and PM_{10}

We found spatial heterogeneity in urban and rural microbiomes, with the most abundant airborne bacteria varying significantly with site and PM size (**Fig. 2 A&B**). Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes were the dominant bacterial phyla for all sites and in both PM size fractions. Roadside samples were characterized by a marked reduction in microbial diversity, with a high dominance of Gammaproteobacteria (15-20%). Bacilli were notably higher in PM_{10} than in $PM_{2.5}$ and were dominant at urban sites, while Actinobacteria were the dominant bacteria at the rural site (15-20% of the total community). Airborne microbial communities sampled in Rwanda were similar to those identified in Australia, China and the USA (Archer et al., 2020; Bowers et al., 2011; Bowers et al., 2013; Cao et al., 2014a; Gao et al., 2017; Lee et al., 2010). A global study has shown that bacteria are recruited to the atmosphere from both local and distant soils across different biomes (Archer et al., 2023). Actinobacteria, a taxa primarily derived from soil, dominated rural sites (20%) (Barka et al., 2016; Jose et al., 2021) likely caused by agricultural activities and more labile surfaces than urban environments that were dominated by Bacilli, Bacteroidetes and Proteobacteria frequently associated with human activities (Hospodsky et al., 2012).

At the genus level, bacterial community structures also varied between PM_{10} and $PM_{2.5}$ samples with land use types (**Fig. 2 C&D**). In $PM_{2.5}$, Sphingobium and Sphingomonas were consistently detected at all sites (**Fig. 2 C**), suggesting that these two genera are abundant and likely to be less influenced by local land use (Murakami et al., 2010; Spring et al., 2021). PM_{10} samples exhibited greater bacterial diversity than $PM_{2.5}$. Some genera were only observed in PM_{10} samples, not in $PM_{2.5}$, confirming that size fraction may drive variation in bacterial community composition.

We detected various known PAH degrading bacterial genera in the PM_{10} fraction (*Psedomonas*, *Micrococcus*, and *Sphingobium)*, and a substantial proportion of the $PM_{2.5}$ bacterial community was *Sphingobium* which are well known for their ability to degrade PAHs and NPAHs ;(Ghosal et al., 2016a; Zhang et al., 2022).

The high proportion of *Sphingobium* , which are hydrophobic biofilm formers, in $PM_{2.5}$ likely reflects two main processes: their ability to adhere to smaller, combustion-derived particles which serve as microhabitats for hydrocarbon-degrading bacteria (Li et al., 2023); and the ability for greater selective pressures and activity in the atmosphere due to the longer atmospheric residence time and a more stable microenvironment for this taxa in a highly polluted environment. In the PM_{10} size fraction, *Pseudomonas* and *Micrococcus* species are metabolically versatile and capable of utilizing a broad range of hydrocarbons (, Wei et al., 2021,). but may not be as well adapted to PAH degradation as *Sphingobium*. They are also stress resistant taxa frequently identified in coarse atmospheric samples (Bowers et al., 2013; Bowers et al., 2011) so may represent the natural background abundance in the shorter atmospheric residents found in $PM_{10,}$ while being outcompeted in $PM_{2.5}$.

Overall, these results indicate strong environmental structuring by PAHs with distinct land-use-specific distributions, suggesting that the bacterial community can be shaped by urbanization and land-use type, and that particle size fraction can influence their composition and significantly shape the structure and diversity of airborne bacterial communities. The dominance of *Sphingobium* in $PM_{2.5}$ may not only signify its adaptive potential but also indicate that it is a good bioindicator of airborne combustion-derived pollution.

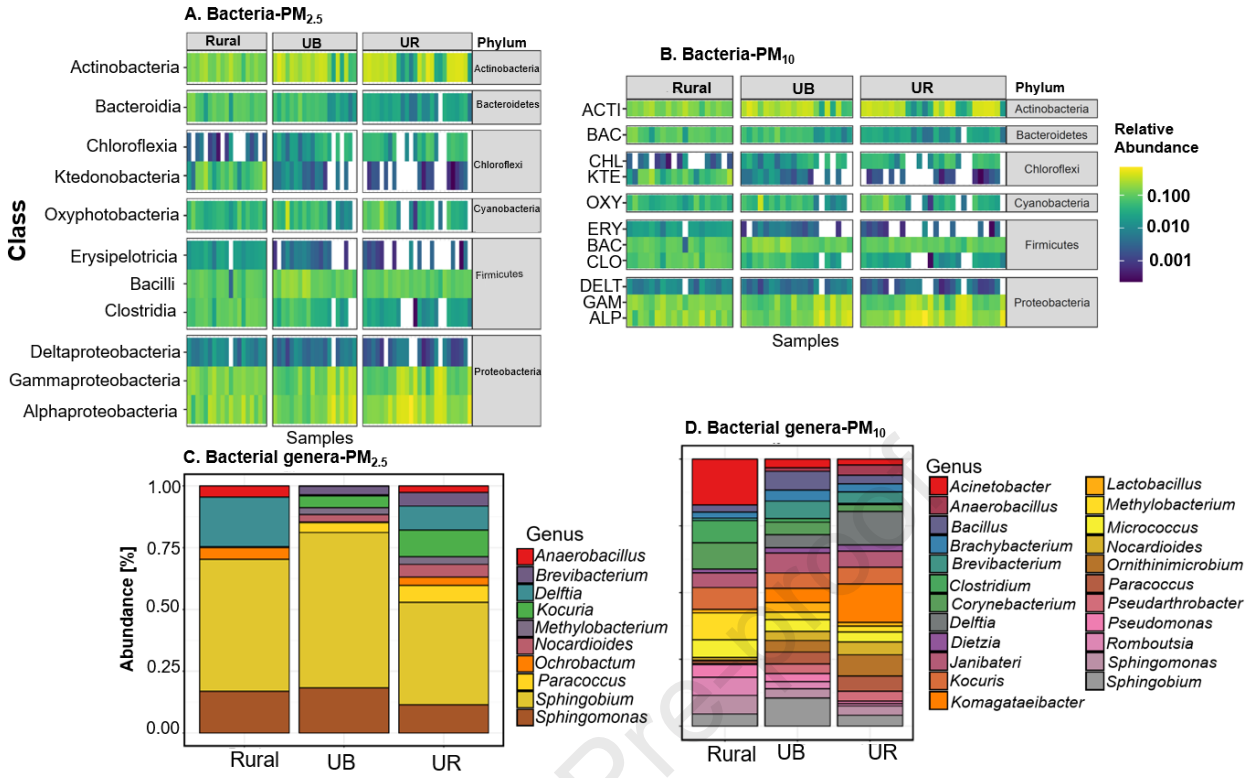

Figure 2. Relative abundance of the most abundant bacterial ASVs at class level with mean relative abundance in three land-use types (rural, urban background (UB) and urban roadside (UR) sites) for $PM_{2.5}$ (A) and PM_{10} (B) samples. Relative abundances of the most abundant bacterial genera for $PM_{2.5}$ samples (C) and PM_{10} samples (D);Phyla abbreviation : Actinobacteria (ACTI), Bacteroidia (BAC), Chloroflexia (CHL), Ktedonobacteria (KTE), Oxyphotobacteria (OXY), Erysipelotricia (ERY), Bacilli (BAC), Clostridia (CLO), Deltaproteobacteria (DELT), Gammaproteobacteria (GAM) and Alphaproteobacteria (ALP)

3.3.2 Fungi in $PM_{2.5}$ and PM_{10}

The abundances of dominant fungal OTUs varied with PM size and site (**Fig. 3 A&B**). Ascomycota was the dominant phylum in both $PM_{2.5}$ and PM_{10} across all sites, especially in PM_{10} at the rural site, likely due to environmental spore sources and agricultural plant debris. Dothideomycetes was the most abundant class (30-35%) in all samples in $PM_{2.5}$ and PM_{10}, being higher in $PM_{2.5}$ and at UB and UR in PM_{10} than the rural site, implying a link to plant degeneration, pollution tolerance, or urban vegetation. In contrast, Agaricomycetes and Tremellomycetes were

detected mainly at rural sites and showed higher proportions in PM_{10} than $PM_{2.5}$, which was consistent with a study of fungi in aerosols along an urbanization gradient in Hong Kong (Woo et al., 2013). Agaricomycetes are commonly found in tropical soil (Wardle and Lindahl, 2014) and on leaf surfaces (Kembel and Mueller, 2014). Compared to bacteria, urbanization has a weaker effect on fungal composition, suggesting greater ecological resilience of dominant airborne fungal communities in African rural settings, as has been recently reported in rural Eastern China (Zhao et al., 2025).

At the genus level, the fungal community varied by PM size and sampling sites (**Fig. 3 C&D**). There were insufficient sequence reads generated from the $PM_{2.5}$ rural sample to be included in downstream analyses. *Phoma,* and *Davidiella* were the dominant fungal genera, accounting for >50% of the $PM_{2.5}$ fungal community, both being more abundant in the UR site . Intrestingly, the most abundant genus in PM_{10} varied between the Rural site (*Itersonillia)* and the Urban sites (*Davidiella).*

In contrast to the bacterial communities, only one PAH degrading taxa, *Alternaria,* was repeatedly detected in the $PM_{2.5}$ fraction (Després et al., 2012; Fröhlich-Nowoisky et al., 2016).*Alternaria* is a ubiquitous, prolific, small-spored ascomycete explaining its detection in the $PM_{2.5}$ fraction. Given the short transit time, living microscopic fungi would not have had time to gain a competitive advantage via PAH degradation are they rely on using extracellular ligninolytic enzymes or intracellular monooxygenases that take time to act. This may also be due to the fact that many taxa with PAH degrading ability do not produce spores, or live in substrate matrices that do not provide opportunity for aerosolization to occur (Haritash and Kaushik, 2009; Pointing, 2001).In contrast, the larger mass of PAHs identified in $PM_{2.5}$ would is likely enriched in soot and

condensed PAHs from combustion (Li et al., 2023) providing a selective advantage for bacteria that are small, hydrophobic, and able to attach to soot surfaces (Ghosal et al., 2016a).

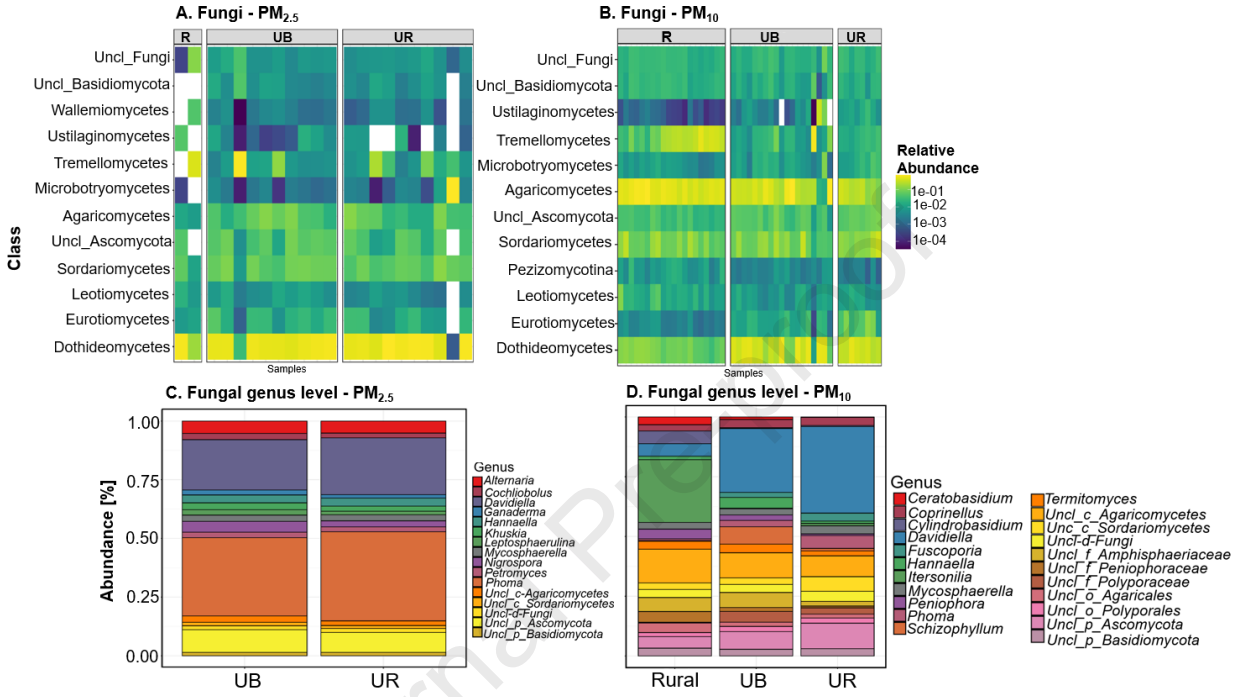

Figure 3. Heatmap diagram showing distribution of Operational Taxonomic Unit (OTUs)- Internal Transcribed Space (ITS) relative abundance at class level (ordered by phylum) from three land-use types, as detected in $PM_{2.5}$ (A) and PM_{10} (B) samples from the rural, urban background (UB) and urban roadside (UR) sites. Relative abundances of the fungal genera for PM_{10} samples (C) and $PM_{2.5}$ (D) from three land-use types: rural, urban background (UB) and urban roadside (UR).

3.4 Community Structure and Seasonal Variation of Atmospheric Bacteria and Fungi

PCoA was used to elucidate the differences in the bacterial communities from PM_{10} and $PM_{2.5}$ by land use type **(Fig. 4 A&B)**. Bacterial communities clustered according to land-use type for both PM size (PERMANOVA PM_{10} ($p < 0.001$) and $PM_{2.5}$ ($p < 0.001$). PCoA of 16S rRNA of $PM_{2.5}$ data showed that Axis 1 and 2 accounted for 44.8% of the total variation. Variance was high and bacterial communities overlapped between Rural, UB and UR sites. This suggests that $PM_{2.5}$ bacterial communities are not distinctly structured by land use type. On the other hand, PM_{10}

samples showed significant clustering between Rural and Urban sites suggesting that bacterial communities in fine particles can travel long distances due to particle physics, while coarse particles are shaped by localized environmental conditions such as dust, soil, vegetation, and human activities, consistent with the findings of Precha et al. (2025).

PCoA was used to elucidate the differences in the microbial communities in PM_{10} and $PM_{2.5}$ by land-use type **(Fig. 4 C&D)**. Bray–Curtis dissimilarities showed distinct clustering of fungal communities across rural, UB, and UR sites in both PM_{10} (PERMANOVA, $p < 0.001$) and $PM_{2.5}$ ($p < 0.071$), respectively. The UB and UR samples clustered together, and these overlaps were observed for both PM size fractions, highlighting that the urban environment homogenizes airborne fungal assemblages due to diminished greenery, and shared anthropogenic sources such as traffic, dust, and buildings. The increased overlap between sites in PM_{10}, especially between UB and UR, suggests that coarse particles may act as carriers of fungal spores from various sources, reducing site-specific profiles. These findings indicate that fine and coarse particles differ in their capacity to preserve the land-use profile in fungal community structure.

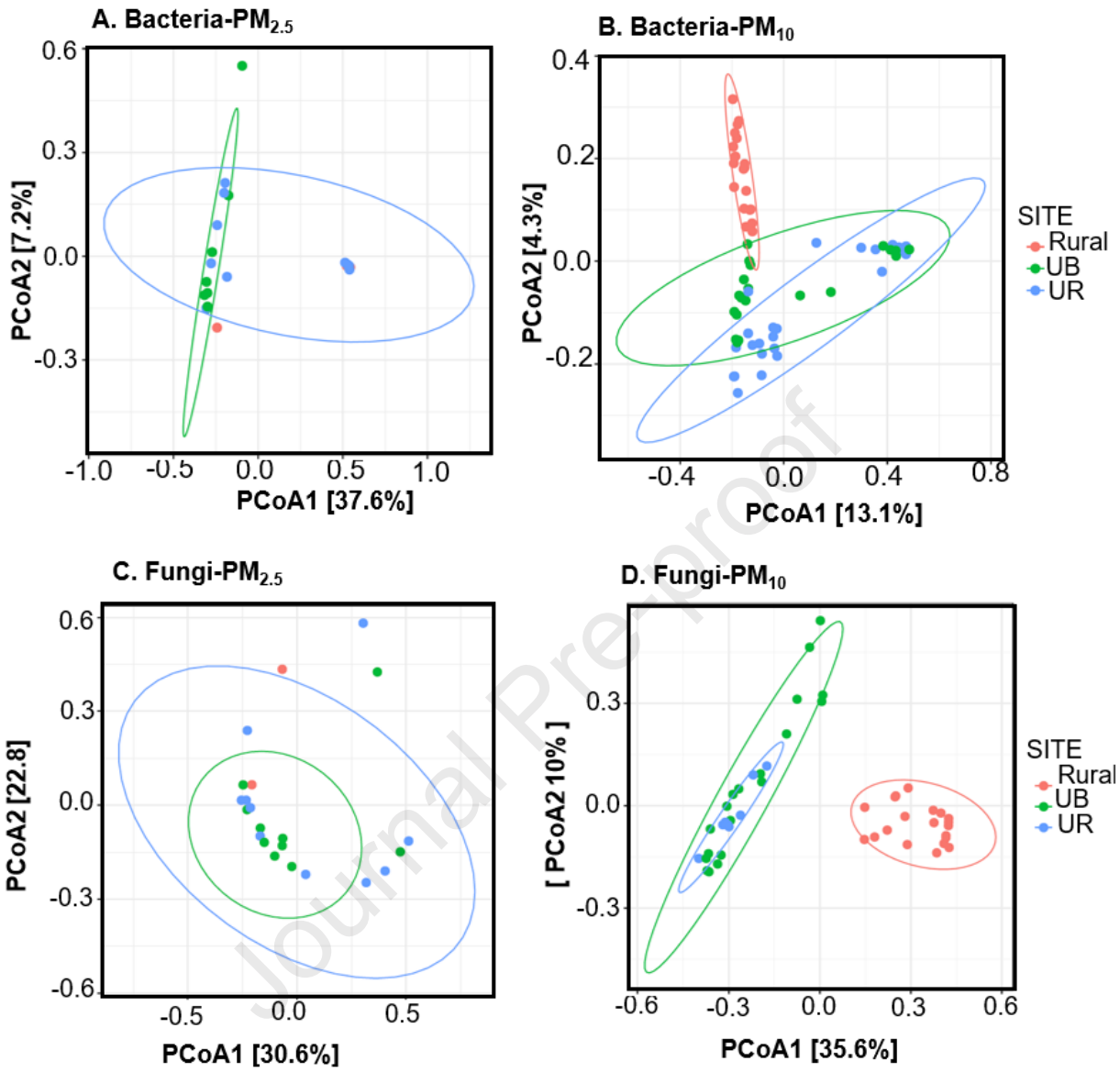

Figure. 4. Principal coordinates analysis (PCoA) of the Bray-Curtis dissimilarities of bacterial communities (relative abundances) from PM_{10} samples (A) and $PM_{2.5}$ samples (B) and fungal communities from PM_{10} samples (c) and $PM_{2.5}$ samples (d) from three land-use types in Rwanda: rural, urban background (UB) and urban roadside (UR). (A) Bacteria in $PM_{2.5}$, where the first two axes explain 37.6% (PCoA1) and 7.3% (PCoA2) of the total variance; (B) Bacteria in PM_{10}, explaining 13.1 % (PCoA1) and 4.3 % (PCoA2); (C) Fungi in $PM_{2.5}$ explaining 30.6 % (PCoA1) and 22.8 % (PCoA2); and Fungi in PM_{10}, explaining 35.6 % (PCoA1) and 10.0% (PCoA2) of the total variance. Points represent individual samples, and ellipses indicate group dispersion by site.

The fungal taxa richness was slightly greater in the PM_{10} samples than in the $PM_{2.5}$ samples and was highest in the rural samples (**Table S3**). Seasonal variation influenced airborne bacterial communities between dry and wet seasons for $PM_{2.5}$. Compared to bacteria, fungal communities

showed weaker seasonal patterns **(Figure S1).** Some clustering between dry and wet season samples was visible, with slightly broader separation in the dry season for $PM_{2.5}$. Bacterial PM_{10} exhibited the most seasonal variation, while fungal PM_{10} samples from the wet season showed variation among themselves, indicating that the season is more variable for fungi. However, there was more variation between samples in the dry season for bacteria in PM_{10} (Jiang et al., 2022). Overall, fine particles captured more season-specific bacterial shifts than fungal shifts. During the dry season, higher dust resuspension and reduced atmospheric moisture likely favored soil- and dust-associated bacteria, while the wet season may have promoted the proliferation of water- or plant-associated bacteria (Zuo et al., 2024). For the fungal community, seasonal differences were less pronounced than for bacteria, with partial separation in $PM_{2.5}$. These patterns indicate that airborne fungal communities are relatively stable across seasons (Wang et al., 2021), especially for PM_{10}. The results indicate that bacteria are more sensitive to seasonal environmental changes than fungi and that $PM_{2.5}$ better reflects seasonal microbial dynamics than PM_{10}.

3.5 Association between PAHs, NPAHs, Bacteria, Fungi and Environmental Factors

The Spearman correlation coefficient was used to investigate the association between airborne bacterial diversity and environmental factors including PAHs, NPAHs, temperature and relative abundance. This revealed significant associations between several PAH/NPAH compounds and specific bacterial taxa (Fig. S2), indicating that variation in pollutant levels and meteorological conditions are associated with the distribution and clustering of microbial communities (Archer et al., 2023; Petroselli et al., 2021; Shi et al., 2022; Woo et al., 2013).

The observed links between PAHs/NPAHs and airborne microbial communities reflect both shared emission sources (vehicular and biomass combustion) and environmental factors related to

meteorology and particle size. These results support the idea that PM connects chemical and microbial processes in the atmosphere (Markowicz et al, 2018; Shi et al, 2022).

Among bacteria, genera such as *Sphingobium, Paracoccus,* and *Acinetobacter* showed strong positive correlations with high-molecular-weight PAHs and nitrated PAHs. The concentrations of PAH and NPAH compounds were significantly negatively correlated with airborne bacterial genera, including *Micrococcus* (Ghosal et al., 2016b; Vetrova et al., 2023). Environmental parameters, including PM_{10} total mass and RH, showed both positive and negative correlations with pollutant concentrations and bacterial variables. In contrast, multiple fungal taxa exhibited significant negative correlations with these same PAHs and NPAHs (**Fig. S2**), confirming that fungal community structure is climate-sensitive and influenced by moisture conditions, making fungi important indicators of both chemical and climatic conditions in polluted air. The inverse relationship between RH and certain PAHs/NPAHs suggests that high humidity favours the removal or transformation of these pollutants in the atmosphere. Lower humidity conditions may enhance pollutant persistence and concentration during the dry season.

4. Conclusion, Limitations of the study and Future Perspectives

This study presents a preliminary investigation into the characterization of chemical pollutants and airborne microbial populations in $PM_{2.5}$ and PM_{10} across urban, roadside, and rural environments in Sub-Saharan Africa. Microbial communities varied between environments based on land use type (rural, urban background and urban roadside). Our findings show that variation in local sources has a greater effect than meteorological factors and seasonal variations. By comparing these sites, our study highlights how different land-use types contribute to variations in bacterial and fungal components.

We found that PAHs and NPAHs were associated with airborne bacterial populations, particularly *Sphingobium*, while airborne fungi were more strongly linked to climatic factors. This variation could be related to particle physical/chemical microenvironments or biological form during atmospheric transport. These findings highlight the need for localized, context-sensitive risk assessments rather than one-size-fits-all regulatory approaches. PM is not simply a passive vector, but a dynamic matrix where chemical and biological interactions occur, potentially creating chemical-microbial synergies, with public health implications where co-inhalation of pollutants and viable microbes may amplify the risks of respiratory and systemic illness.

Collectively, our study provides baseline African evidence that the chemical (PAHs, NPAHs) and biological (bacterial, fungal) fractions of PM are not independent but covary with land-use type and particle size ($PM_{2.5}$ and PM_{10}). Multivariate analyses demonstrated that source regimes and abiotic conditions jointly structure aerosols. Organic pollutants can act as environmental filters, selecting for tolerant taxa, while microbial communities trace combustion sources. We have demonstrated that African landscapes harbour unique and pollutant-microbiome associations that remain underrecognized in global air quality frameworks. When such associations are observed, they most likely reflect different underlying drivers rather than direct effects. Our findings provide a foundation for policy-relevant monitoring of co-exposure mixtures in rapidly urbanizing African environments.

While this study can serve as a baseline for further research in Africa, its limited duration (three months), limits our conclusions where future research should employ larger sample sizes and longer sampling periods to improve robustness and temporal resolution. While co-exposure to organic pollutants and microbes is a valid and important concept in health research, we did not

include a formal risk assessment framework. Pathogenicity was not the primary focus, and no strain-level or viability data were generated and could not be with amplicon sequence data. Future research should include exposure assessment, microbial viability testing, and epidemiological data along with metagenomics, metatranscriptomics, or functional gene assays, so that inferences can be made about degradative pathways, pollutant tolerance and health risks. The associations observed between chemical and microbial taxa are correlative and cannot be interpreted as causal interactions. Pollutants and microbial communities may co-vary in response to common emission sources (traffic, biomass burning) in different land use types or environmental drivers (temperature, relative humidity), rather than directly influencing each other.

Non-traditional pollutants (PAHs, NPAHs, bacteria, and fungi) are rarely monitored or considered in air pollution campaigns in Africa, despite their significant health impacts. Future research should include other chemical components of aerosols, such as toxic metals, sulphates, nitrates, mineral dust, and black carbon, and assess the physical properties of aerosols and factors such as wind speed and wind direction to find correlations with microbial communities in the atmosphere. Our results show that organic pollutants and airborne microbial communities vary together across different land-use types and particle sizes.

Future epidemiological studies should integrate long-term air quality and health data across multiple countries to assess the synergistic effects of chemical and microbial pollutants on respiratory and other health outcomes. Microbial indicators, such as virulence potential, biodegradation capacity, and antibiotic resistance gene profiles, could be integrated into monitoring frameworks. Future efforts could also expand chemical profiling and explore the influence of urban infrastructure and microclimate on aerosol dynamics.

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Author Contributions

EK: Conceptualization, Data curation, Methodology, Investigation, Funding acquisition, Formal analysis, Validation, Writing- original draft, Writing-review & Editing and Visualization.

SDA, MA, AS, GH, GU, KH, SP, DLB, EK: Conceptualization, Methodology, Writing-review & Editing.

JB, KL, TL, SDA: Methodology, Visualization, Writing, Review, and Editing

Conflicts of Interest

We declare that the authors have no known competing financial interests that could have influenced the results presented in this study.

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Highlights:

- We characterize atmospheric PAHs, NPAHs, bacteria, and fungi in PM from Africa
- PM shows co-variation between PAHs/NPAHs and microbial composition.
- Each land-use type revealed distinct pollutant-microbiome signatures
- Bacteria responded to PAHs/NPAHs, while airborne fungi were driven by climate factors.
- Bioaerosol surveillance should be incorporated into air quality monitoring

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: