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Aerobiological study of bacterial and fungal community composition in the atmosphere of Mexico City throughout an annual cycle☆

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ABSTRACT

The atmosphere as a temporary habitat for airborne microbial communities is a valuable topic to explore, and it is through aerobiological studies that the diversity of biological particles and their release, emission, transport, deposition, and impact are assessed. Specific microorganisms are involved in meteorological processes, and phytosanitary and public health concerns. Airborne microbial composition is related to factors such as geographic region and weather conditions.

In this study a metagenomic approach was used to determine the composition of bacterial and fungal communities in the air of two different land-use areas (urban area and semi-rural area), during dry and rainy seasons in Mexico City. Air sampling was carried out with a Hirst-type spore trap, collecting the samples simultaneously in both study areas. Forty-two bioaerosol samples were collected, and the DNA obtained was sequenced using Next-Generation Sequencing. The results indicated that the bacterial communities were represented mainly by the phyla Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes, Cyanobacteria, and the fungal communities by the phyla Ascomycota followed by Basidiomycota. The evident changes in microbial composition were related more to seasonality than to locality, since both UA and SRA showed a high degree of urbanization, despite some differences in land use. Continuous monitoring of atmospheric bioaerosols is essential to determine the influence of meteorological factors on the composition of the aerial microbiota.

1. Introduction

The atmosphere as a temporary habitat for airborne microbial communities is a valuable topic to explore, and it is through aerobiological studies that the diversity of airborne biological particles and their release, emission, transport, deposition, and impact are assessed (Edmonds and Benninghof, 1973; Isard and Gage, 2001). Primary biological particles, or bioaerosols, are a subset of atmospheric particles that are released directly from the biosphere into the atmosphere. They are made up of living and dead organisms such as bacteria, fungi, viruses, bacterial and fungal spores, microbial fragments, pollen, etc. (Fröhlich-Nowoisky et al., 2016; Romano et al., 2019). Since bioaerosols are ubiquitous in the atmosphere, it plays a fundamental role in their transport and dispersal around the planet (Yoo et al., 2017; Després et al., 2012).

Various studies have focused on airborne microbial communities, showing their relevance in different areas such as human health, environmental quality, and atmospheric processes (for example, ice/cloud nucleation properties) (Šantl-Temkiv et al., 2015; Fang et al., 2018; Kim et al., 2018; Xie et al., 2018). Although the atmosphere is considered a hostile environment for the survival of microorganisms, studies have revealed diverse and metabolically active microbial communities (Womack et al., 2010; Gandolfi et al., 2013).

The composition of airborne microbial communities is affected by meteorological conditions associated with seasonal shifts (Li et al., 2019). Due to the dynamic nature of the atmosphere, microorganisms can be transported and dispersed, impacting the environment and human health (Romano et al., 2019).

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The study of microbial communities in the air has been underestimated, and until a few years ago, it was restricted to those populations that can be easily cultivated and isolated with biological culture-dependent techniques. More recently, the collection of DNA from airborne samples and new methods such as Next-Generation Sequencing (NGS) have expanded the potential to explore the diversity and total composition of the microbial communities found in different environmental conditions. Some studies have correlated the temporal variability of bacterial and fungal communities with meteorological conditions and the chemical composition of the particles (Maron et al., 2006; Cao et al., 2014; Gandolfi et al., 2013; Aziz et al., 2018).

Although outdoor air quality in Mexico has been widely studied, mainly focusing on chemical pollutants, only a few studies have assessed the biological quality of the air through the evaluation of atmospheric bioaerosols (Rosas et al., 1986, 1989; Santos-Burgoa et al., 1994; García-Mena et al., 2016; Serrano-Silva and Calderón-Ezquerro, 2018; Calderón-Ezquerro et al., 2020). Bioaerosols not only have a profound impact on public health, as a source of pathogens and allergenic components, but also on agricultural production, atmospheric processes, the distribution of clouds, global precipitation, and biodeterioration (Yao, 2018; Di Carlo et al., 2017; Fröhlich-Nowoisky et al., 2016).

Mexico City is one of the most populated cities in the world and one of the most badly-affected by air pollution. This situation has serious and widespread effects due to the enormous amounts of pollutants emitted into the environment, mainly into the atmosphere (gases and airborne inorganic/organic particles), which can be transported over long distances, affecting both public health and ecosystems (Baklanov et al., 2016).

Mexico City is a heterogeneous region with urban and semi-rural areas. Coyoacán is an urban area located in the southeast of the Valley of Mexico with a total area of 54.4 km^2, which corresponds to 3.6% of the city's territory. It has an average altitude of 2256 m.a.s.l and a population density of 11404.70 inhabitants/km^2. Coyoacán has three ecological reserves: Reserva Ecológica del Pedregal de San Angel; Viveros de Coyoacán, and Ciudad Universitaria in the Universidad Nacional Autónoma de México (UNAM) (SNIM, 2010). Xochimilco is a semi-rural area located southeast of Mexico City with a total area of 118 km^2, which corresponds to 7.91% of the city's territory. It has an average altitude of 2275 m.a.s.l and a population density of 3400 inhabitants/km^2. This area has 12517.8 ha, of which 20% is urban land and 80% is represented by conservation areas. A lacustrine landscape characterizes the conservation area with a network of canals on the edge of Xochimilco's residual lake (Salles, 1992; SNIM, 2010). There is currently severe concern regarding pollution, such as salinization, sinking, and floods generated by the expansion of the population and the discharge of black water, grey water, garbage, and agrochemical products dumped directly into the canals (Rosas et al., 1984; Peralta, 2011; Embarcadero-Jiménez et al., 2016; SECITI, 2017; Milenio, 2015).

In this context, the aims of the present study were: 1) to thoroughly characterize the biodiversity of airborne bacterial and fungal communities collected in an urban and semi-rural area of Mexico City; 2) to explore the impact of dry and rainy seasons on the structure of the bacterial and fungal community of each evaluated area.

2. Materials and methods

2.1. Bioaerosol sampling

Air sampling was carried out weekly through an annual cycle: this comprised the dry season, divided into cold-dry (from November 4, 2015, to March 3, 2016) and hot-dry (from March 4 to May 2, 2016), and the rainy season (from May 3 to September 2, 2016). Two locations with different types of land-use were selected. The UA, located in Coyoacán (19° 19′ 35″ N, 99° 10′ 34″ W), had mean temperatures of 14.8, 20, and 18 °C and accumulated precipitation of 10.00, 13.40, and 234.20 mm during the cold-dry, hot-dry, and rainy seasons, respectively (data obtained from the RUOA [Red Universitaria de Observatorios Atmosféricos] network). The SRA, located in Xochimilco (19° 15′ 45″ N, 99° 05' 33" W), had mean temperatures of 14.8, 18.4, and 17.7 °C and accumulated precipitation of 10.20, 12.60, and 219.00 mm during the cold-dry, hot-dry, and rainy seasons, respectively (Table 1). A total of 42 bioaerosol samples were collected during this study (n = 21 samples per studied area; seven samples in each season, hot-dry, cold-dry, and rainy).

The meteorological parameters were selected to characterize each area and determine differences between the dry season and the rainy season. For this reason, we use daily averages of temperature, relative humidity, and accumulated precipitation. Records of temperature, precipitation, relative humidity, and solar radiation in the UA (Coyoacán) were also obtained from the RUOA network. In the SRA (Xochimilco) a Davis Vantage Pro2 Weather Station (Davis Instruments™) was installed to obtain this data.

A Hirst-type Spore Trap (HST) was used to collect the bioaerosol samples at both locations. The HST incorporates a rotating drum that moves clockwise, the air sample is sucked in by a vacuum pump (10 L/min airflow), and the bioaerosols are deposited by impaction on cellophane tape (Melinex®, DuPont, USA). Briefly: the tape was attached to the surface of the drum and covered with a thin layer of a mixture of petroleum jelly and hexane (1:5) (Calderon-Ezquerro et al., 2015). The drum was prepared in a sterile cabinet with ultraviolet light to avoid contamination of the collection surfaces. The drum was thoroughly cleaned with benzalkonium chloride (0.1%) and then irradiated with ultraviolet light, together with the tape (Melinex tape), the mixture of petroleum jelly with hexane, the scissors, and two dissector forceps used to manipulate the tape. The bioaerosol collection tape was fixed to the drum, and the petroleum jelly was spread evenly with a sterile brush in a very thin layer. The drum was transported within a sterile container provided by the manufacturer until installed in the HST. Before the drum was installed, the HST was cleaned, verifying that the inlet hole was free of dirt, and sprayed with benzalkonium chloride (0.1%) and it was operated for 10 min, verifying that the

Table 1
Average values of the meteorological variables recorded in semi-rural and urban areas during this study.

Climatic season	Urban area (Coyoacán)				Semi-rural area (Xochimilco)			
	Temp (°C)	Accumulated precipitation (mm)	Relative humidity (%)	Solar radiation (W/m2)	Temp (°C)	Accumulated precipitation (mm)	Relative humidity (%)	Solar radiation (W/m2)
Cold-dry	14.84	10.00	50.59	174.16	14.83	10.20	53.11	165.23
Hot-dry	20.00	13.40	41.04	221.62	18.44	12.60	53.84	219.28
Rainy	18.18	234.20	67.30	198.91	17.72	219.00	75.48	193.76

intake orifice was free of dirt (Serrano-Silva and Calderón-Ezquerro, 2018). The air samples were collected simultaneously in the two areas.

2.2. Extraction, purification, and measurement of DNA

The samples collected on the Melinex tape were processed in a light laminar flow hood that was previously sterilized with UV and benzalkonium chloride (0.1%). DNA extraction was carried out as described in Serrano-Silva and Calderón-Ezquerro (2018). The DNA was eluted in 10 μL of DNase-free water (QIAGEN) and stored at −80 °C until subsequent analysis. DNA concentration and purity were verified in a Qubit 4 Fluorometer using the Qubit dsDNA HS Assay Kit (Thermo Scientific).

The Melinex tapes from the HST were processed for DNA extraction and PCR amplification of the 16S and the Internal Transcribed Spacer (ITS) regions of the ribosomal RNA. For the negative controls a clean Melinex tape (no impacted bioaerosols) was used. DNA extraction and PCR were as described in Serrano-Silva and Calderón-Ezquerro (2018). No PCR products were obtained from the negative controls.

2.3. DNA sequencing

Bacterial 16S and fungal ITS regions were amplified by polymerase chain reaction (PCR), following the workflow for preparing gene amplicons for the Illumina MiSeq System. The variable regions, V3 and V4, of the bacterial 16S rRNA gene were targeted in PCR using 12.5 ng DNA with primers 341F/805R (Herlemann et al., 2011) at an alignment temperature of 55 °C. For the fungal ITS2 region, the Herlemann et al. (2011) protocol was followed, but using the primers ITS3_KY02 (Toju et al., 2012)/ITS4 (White et al., 1990) and an alignment temperature of 52 °C.

2.4. Data analysis

Reads with Phred scores lower than Q30 in the outer sections were discarded using Trimmomatic PE (Bolger et al., 2014). Qiime v1.9.1 (Caporaso et al., 2010) metagenomic pipeline was used to assign 16S and ITS sequences to taxonomic units for bacteria and fungi, respectively. Assignment of Operational Taxonomic Units (OTUs) was conducted via the open reference approach with USEARCH v6.1 (Edgar, 2010), clustering at 99% identity for bacteria and fungi. Representative phylogenetic OTUs (phylotypes) were assigned using the UCLUST consensus taxonomy assigner. The Greengenes v13.8 Database was used for bacteria and a combined dataset of UNITE v7.0 plus Findley for fungi. Chimeric sequences were removed, given <80% similarity with the reference database, either Greengenes or UNITE + Findley. The data obtained from the libraries for each season were grouped and treated as a single sample to be compared with library data from the other seasons.

Microbial diversity was determined by calculating the Shannon diversity index (H), the Simpson Index (D), and the abundance-based estimator of species richness Chao1, based on the OTU matrix generated by QIIME. Rarefaction curves were generated through QIIME scripts and rendered in an R Core Team package. Pie charts showing abundance were plotted using Krona Tools v2.7 (Ondov et al., 2011). Three-dimensional abundance bar plots were generated with the lattice Extra R package. Principal Component Analysis was performed for bacteria and fungi and was grouped by season and location with R package vegan.

Table 2
Descriptive characteristics of the metagenomic analysis (16S and ITS).

Area	UA	SRA	UA	SRA
Characteristic	16S (Bacteria)		ITS (Fungi)	
Post-QC sequences	730,231.00		6, 515. 064	
Total OTUs assigned	6047		7561	
Total sequences assigned	564, 967		4, 885	
Unique OTUs	789	849	1013	1013
Core microbiome - OTUs	2135	3277	2258	2019
Total Known Genera	2659	2659	5141	5141
Unique Known genera	397	397	627	627
Core microbiome - Genera	1121	1600	1597	1401

UA= Urban area; SRA=Semi-rural area.

2.5. Nucleotide sequence accession numbers

The metagenomic data from this study were submitted to the NCBI Sequence Read Archive (SRA) under the bioproject accession number: (PRJNA668864).

3. Results

3.1. Microbial diversity of bioaerosol samples

From the 42 samples, 730231.00 16S and 6515.064 ITS reads of high quality were obtained using deep sequencing (Illumina MiSeq). A total of 564967 16S sequences were assigned to bacteria, of which 2659 genera were identified in the atmosphere of Mexico City during this study. Of the 4,885,054 total sequences assigned to fungi, 5141 were identified to genus level (Table 2). According to the rarefaction curves and diversity indices, bacterial and fungal diversity was adequately characterized during the study (Fig. 1, Fig. 2).

3.2. Bacterial community composition

A Principal Component Analysis (PCA) plot of the observed

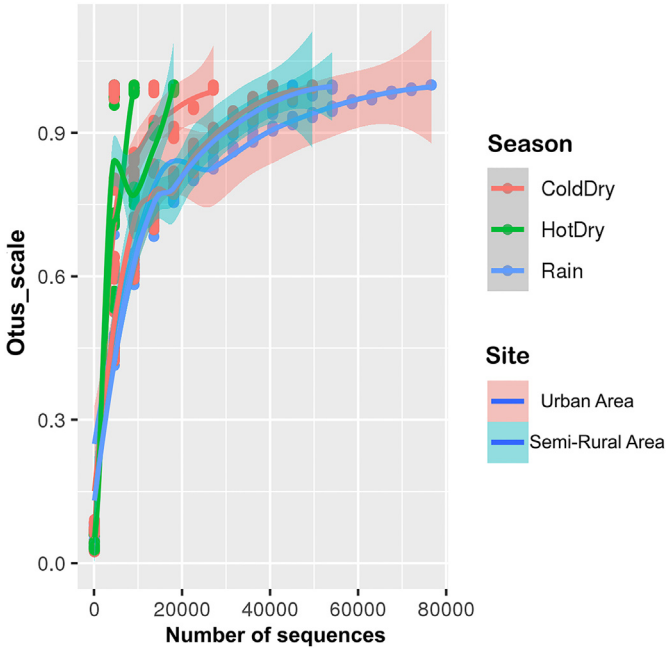

Fig. 1. Rarefaction curve of observed taxonomic units (OTU) of bacterial taxa throughout an annual cycle (dry and rainy season) in an urban area (UA) and a semi-rural area (SRA) in Mexico.

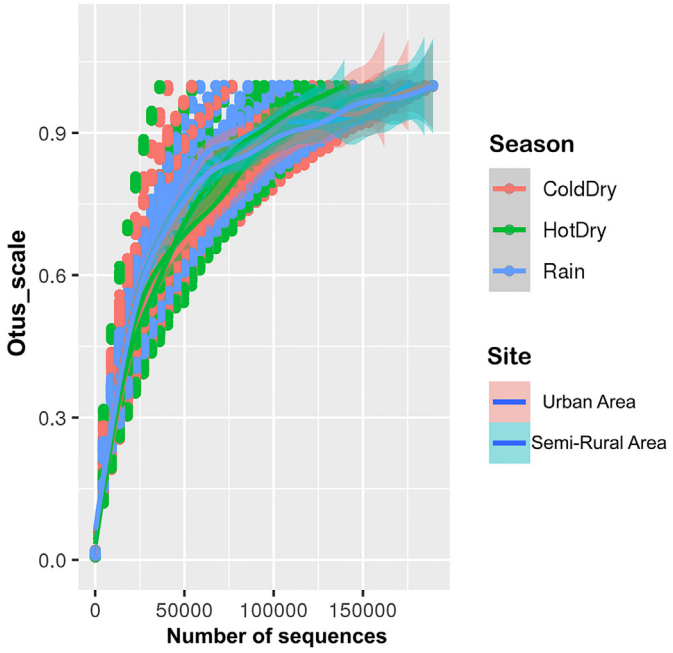

Fig. 2. Rarefaction curve of observed taxonomic units (OTU) of fungal taxa throughout an annual cycle (dry and rainy season) in an urban area (UA) and a semi-rural area (SRA) in Mexico.

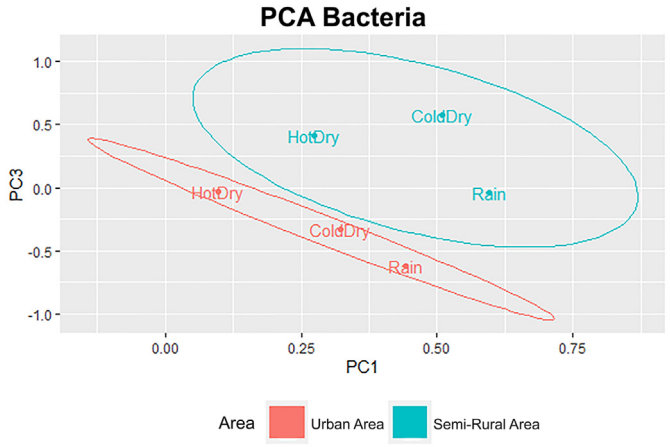

Fig. 3. Principal Component Analysis (PCA) plot of season and area of airborne bacteria; blue = semi-rural area (SRA) and red = urban area (UA). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

airborne bacterial taxonomic units (OTUs) recorded during an annual cycle showed two groups corresponding to samples from UA and SRA during the cold-dry, hot-dry, and rainy seasons. The clusters were separated into defined groups of bacteria for each station (Fig. 3). Table 3 presents the analysis performed by area and station for the assigned OTUs of bacteria. The results showed a greater richness in the SRA during the dry season (cold and hot) but the values in the rainy season were similar for both areas; the diversity indices showed similar values, independent of the area and season (Table 3).

3.3. Annual bacterial community composition

The phylum Actinobacteria showed an annual relative abundance of 41% in the UA and 42% in the SRA, followed by Proteobacteria, Firmicutes, Bacteroidetes, Cyanobacteria, and Chloroflexi (Fig. 4A and B, Supplementary File 1).

Thirteen bacterial genera that were identified (>1%) occurred only in the UA (Fig. 4C, Supplementary File 1); 17 bacterial genera (>1%) were only found in the semi-rural samples (Fig. 4D, Supplementary File 1).

3.4. Seasonal bacterial community composition

Analysis of the samples collected during the dry and rainy seasons in the UA and the SRA showed nine bacterial phyla with a relative abundance >1% (Table 4) (Fig. 5A and B; Supplementary Files 2 and Supplementary Files 3).

3.4.1. Dry season

The bacterial community composition showed that Actinobacteria dominated during the dry season, followed by Proteobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria (Table 4; Fig. 5A and B; Supplementary File 2).

The genera with the highest relative abundances were *Microbispora*, *Kocuria, Paracoccus, Corynebacterium*, some genera of the Nocardioidaceae (*Friedmanniella*, *Propionicimonas*, *Aeromicrobium*, and *Nocardioides*), some genera of the Geodermatophillaceae (*Modestobacter* and *Geodermatophillus*), some genera of the Intrasporangiaceae (*Arsenicicoccus*, *Phycicoccus*, and *Janibacter*), some genera of the Acetobacteraceae (*Roseomonas*, *Bacillus*), some genera of the Staphylococcaceae (*Staphylococcus*, *Jeotgalicoccus*, and *Salinicoccus*) (Table 5; Supplementary File 2).

3.4.2. Rainy season

During the rainy season, the relative abundance of Actinobacteria decreased slightly, resulting in codominance with Proteobacteria. Firmicutes, Chloroflexi, and Cyanobacteria were also present. The phylum Bacteroidetes showed an increase in occurrence during the rainy season (Table 3, Fig. 5A and B; Supplementary File 2). The genera with the highest relative abundances were *Microbispora*, *Kocuria*, *Paracoccus*, *Corynebacterium*, *Pseudomonas*, *Sphingomonas*, *Rheinheimera*, *Bacillus*, *Streptococcus*, genera of the Oxalobacteraceae (such as *Janthinobacterium*), and genera of the Enterobacteraceae.

With regard to the composition of bacterial communities by area, the UA showed greater richness in terms of the core microbiome (3277 OTUs) compared to the SRA (2135 OTUs) (Table 2,

Table 3
Bacterial diversity indices by area and season.

Area	SRA			UA		
Index	Cold_Dry season	Hot_Dry season	Rainy season	Cold_Dry season	Hot_Dry season	Rainy season
Chao1 (16S)*	909.92 ± 147.7	1096.44 ± 147.7	869.37 ± 245.4	762.69.±87.3	784.29 ± 216.4	853.56 ± 265.8
Total OTUs	1956	2015	1996	2061	2025	2153
H (16S)*	7.79 ± 0.1505	7.8 ± 0.1093	7.71 ± 0.2459	7.73 ± 0.1245	7.7 ± 0.1625	7.68 ± 0.2545
D (16S)*	0.99 ± 0.0016	0.99 ± 0.0012	0.99 ± 0.0021	0.99 ± 0.0014	0.99 ± 0.0015	0.99 ± 0.0022

UA= Urban area; SRA= Semi-rural area; H= Shannon diversity index; D = Simpson Index (D), and Chao1 = Abundance-based estimator of species richness; *Mean values.

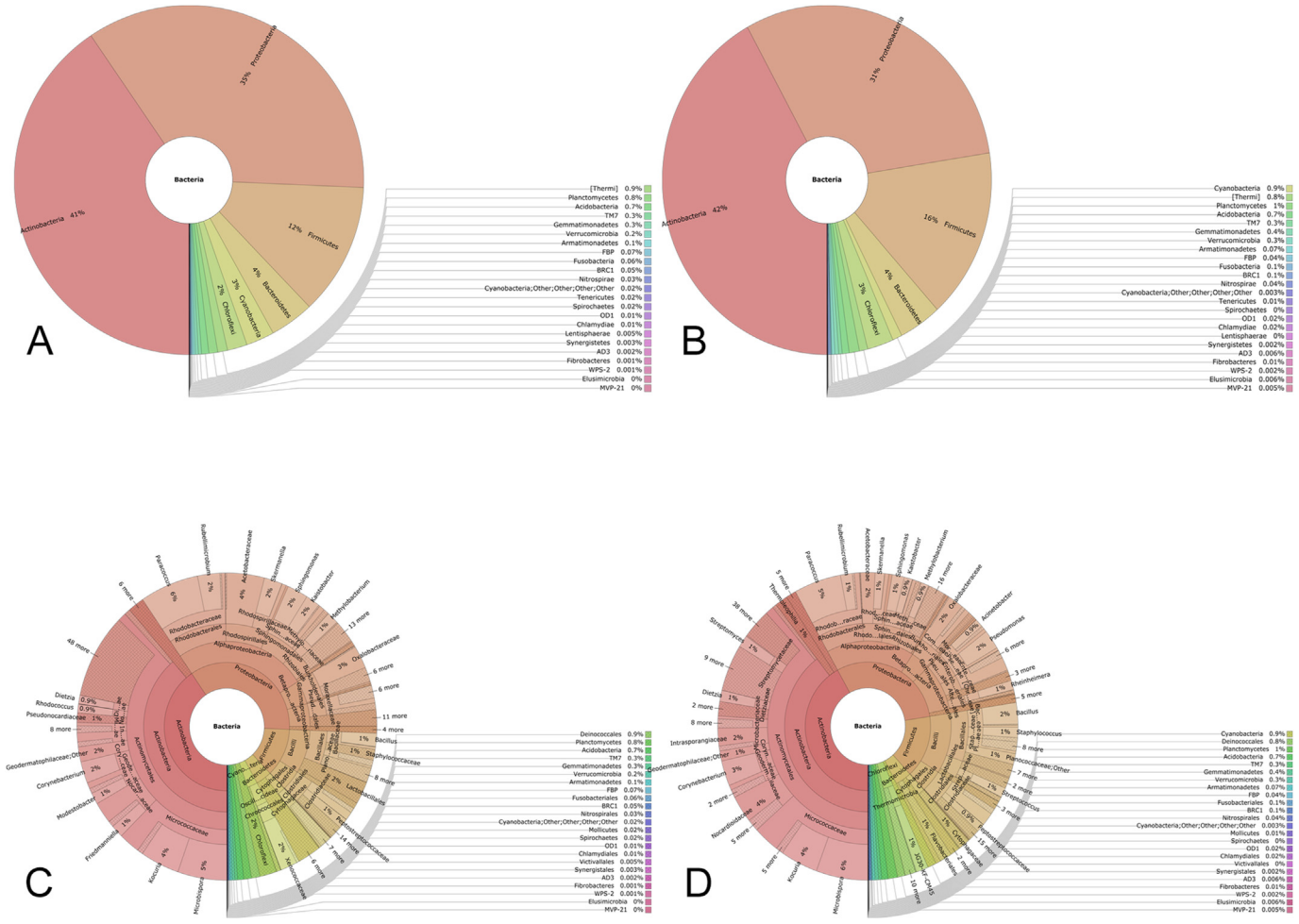

Fig. 4. Taxonomic distribution of airborne bacteria in an urban area (UA) and a semi-rural area (SRA) in Mexico City. A) Bacterial communities at phylum level in the urban area; B) Bacterial communities at phylum level in the semi-rural area; C) Bacterial communities at genus level in the urban area; D) Bacterial communities at genus level in the semi-rural area.

Table 4
Relative abundance of the main bacterial phyla by area and season.

Area	UA		SRA	
Phyla	% Dry season	% Rainy season	% Dry season	% Rainy season
Actinobacteria	41	39	47	36
Proteobacteria	32	38	25	37
Firmicutes	14	10	18	13
Bacterioidetes	3	5	3	6
Cyanobacteria	3	2	1	0.8
Chloroflexi	2	2	3	2
Therni	1	1	0.9	1
Planctomycetes	0.7	0.9	1	1
Acidobacteria	0.7	0.6	0.7	___

UA= Urban area; SRA=Semi-rural area.

Fig. 4C and D, Supplementary File 1). Cyanobacteria showed greater relative abundance in the UA, while Firmicutes showed greater relative abundance in the SRA. Further, genera such as *Pseudomonas*, *Sphingomonas*, *Rheinheimera*, and *Bacillus* were present in the SRA with high relative abundances.

This study demonstrated shifts in the bacterial community composition related to seasonality and subtle shifts related to location (either urban or semi-rural).

3.5. Fungal community composition

Principal Component Analysis (PCA) based on the OTUs showed that the fungal communities were grouped into two clusters, differentiating the seasonality of the samples. Fungal communities collected during the rainy season in both areas of the study were different from those in the dry seasons (Fig. 6).

The OTUs assigned to fungi in both locations and seasons of the year are presented in Table 6. Regarding fungal diversity, the results

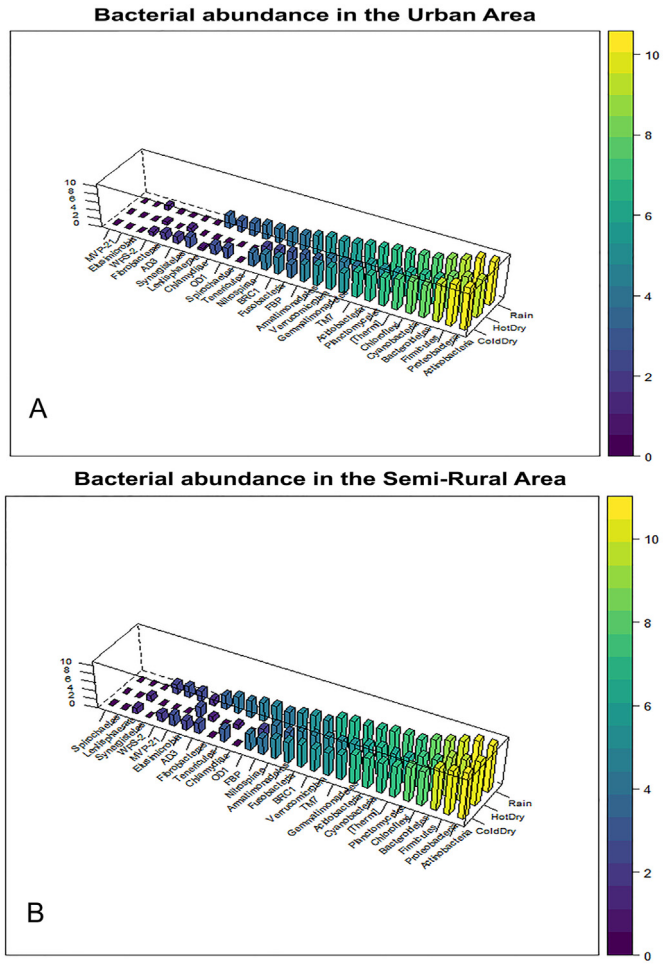

Fig. 5. Seasonal variation in the main bacterial phyla in the atmosphere of A) an urban area (UA) and B) a semi-rural area (SRA) in Mexico.

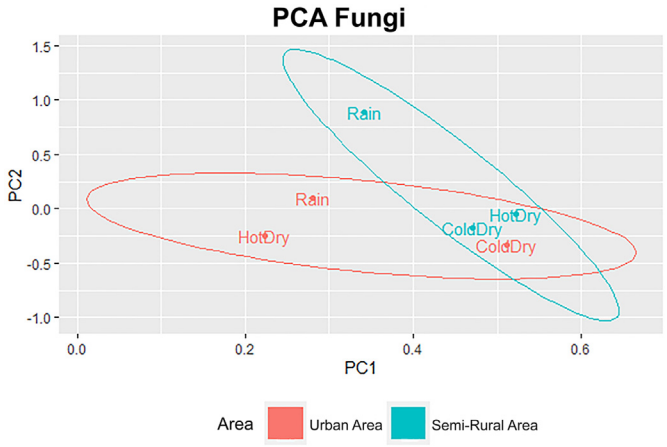

Fig. 6. Principal Component Analysis (PCA) plot of season and area of airborne fungi; blue = semi-rural area (SRA) and red = urban area (UA). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

showed seasonal shifts with higher richness and diversity during the rainy season in both areas, and there were higher values for richness and diversity in the UA (Table 7).

3.6. Annual fungal community composition

The annual analysis identified the presence of the phyla Ascomycota (UA, 81% and SRA, 80%) and Basidiomycota (UA, 10% and SRA, 11%). *Cladosporium* predominated in both areas with high values (>40%), being the most abundant genus throughout the year. The genera of fungi >1% are shown in Fig. 7A–D and Supplementary File 4. In both the UA and SRA 8% were assigned to "Unclassified Fungi; " 4% in the UA were assigned as "Unidentified Plantae."

Table 5
Relative abundance of the main bacterial taxa by area and season.

Area	UA		SRA	
Genera	%Dry season	%Rainy season	%Dry season	%Rainy season
Microbispora	5	5	7	4
Paracoccus	5	8	6	4
Kocuria	3	5	4	4
Nocardiodaceae:	<1	<1	5	3
Friedmanniella Propionicimonas				
Aeromicrobium Nocardioides				
Corynebacterium	2	2	3	3
Rubellimicrobium	2	2	1	1
Acetobacteraceae	4	4	2	1
Roseomonas				
Roseococcus				
Skermanella	2	2	1	1
Rhizobiales	<1	<1	3	<1
Sphingomonas	2	2	1	2
Kaistobacter	1	2	1	<1
Bacillus	2	1	2	2
Staphyloccocaceae	1	1	2	0.9
Staphylococcus				
Jeotgalicoccus				
Salinicoccus				
Streptococcus	<1	<1	<1	2
Other < 1				

UA= Urban area; SRA= Semi-rural area.

Table 6
Relative abundance of the main fungal phyla by area and season.

Area	UA		SRA	
Phyla	% Dry season	% Rainy season	% Dry season	% Rainy season
Ascomycetes	84	70	86	67
Basidiomycetes	6	21	6	23

UA= Urban area; SRA= Semi-rural area.

Table 7
Fungal diversity indices by area and season.

Area	SRA			UA		
Index	Cold_Dry season	Hot_Dry season	Rainy season	Cold_Dry season	Hot_Dry season	Rainy season
Chao1 (ITS)*	3751.52 ± 1017.4521	3920.28 ± 932.5782	4222.4 ± 1235.0579	3403.69 ± 785.4993	3172.44 ± 906.1936	4602.45 ± 865.74
Total OTUs	16,352	13,871	15,791	15,819	17,998	17,274
H (ITS)*	4.96 ± 1.6325	4.84 ± 1.4161	6.22 ± 2.0432	5.18 ± 1.5823	4.66 ± 1.2916	6.68 ± 1.8376
D (ITS)*	0.76 ± 0.1466	0.74 ± 0.153	0.84 ± 0.1268	0.82 ± 0.1231	0.85 ± 0.0692	0.87 ± 0.1081

UA= Urban area; SRA= Semi-rural area; H= Shannon diversity Index; D = Simpson Index (D), and Chao1 = Abundance-based estimator of species richness; *Mean values.

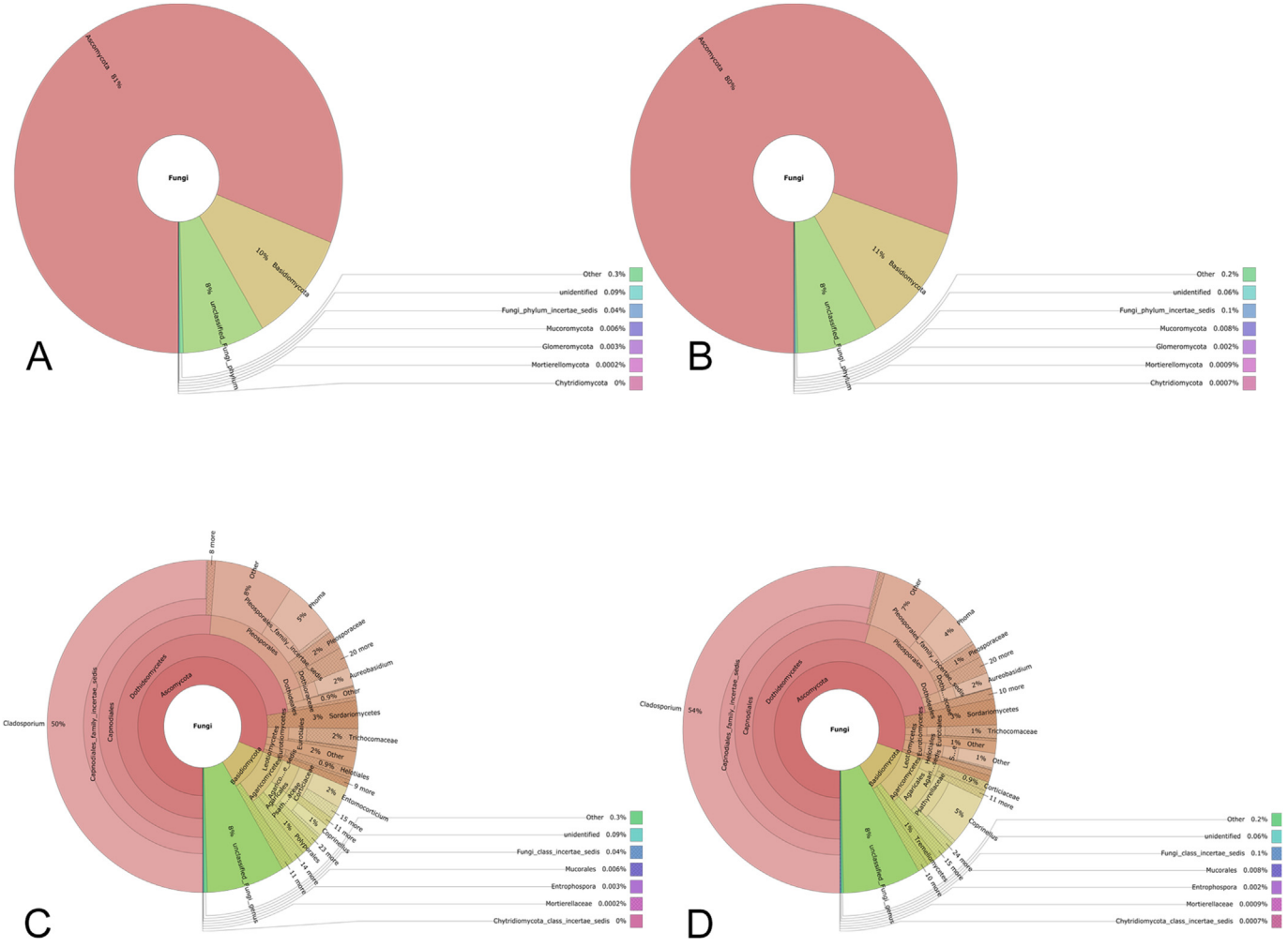

Fig. 7. Taxonomic distribution of airborne fungi in an urban area (UA) and a semi-rural area (SRA) in Mexico City. A) Fungal phyla in the UA; B) Fungal phyla in the SRA; C) Genera of fungi in the UA; D) Genera of fungi in the SRA.

3.7. Seasonal fungal community composition

3.7.1. Dry season

The fungal community composition did not differ in terms of area during the dry season. Relative abundance showed similar values in both areas. Ascomycota dominated with 84% in the UA and 86% in the SRA; Basidiomycota was identified with a relative abundance of 6% for both areas (Table 7).

Table 8
Relative abundance of the main fungal genera by area and season.

Area	UA		SRA	
Genus	% Dry season	% Rainy season	% Dry season	% Rainy season
Cladosporium	58.00	40.00	59.00	42.00
Phoma	5.00	5.00	5.00	3.00
Aureobasidium	2.00	1.00	2.00	0.80
Bipolaris	0.50	0.30	0.40	0.20
Penicillium	0.60	0.70	0.60	0.50
Eurotium	0.60	0.80	0.70	0.30
Cryptococcus	0.30	0.20	0.40	0.20
Entomocorticium	0.30	5.00	0.10	1.00
Heterobasidion	0.20	0.20	0.03	0.01
Nigrospora	0.30	0.30	0.30	0.20
Coprinellus	2.00	3.00	2.00	11.00
Hyphodontia	0.20	2.00	0.03	0.20
Leptosphaerulina	0.20	0.30	0.20	0.10
Periconia	0.08	0.50	0.40	0.60
Leptosphaeria	0.30	0.90	0.30	1.00
Aspergillus	0.10	0.10	0.09	0.06
Fusarium	0.20	0.20	0.20	0.30
Neurospora	0.10	0.20	0.20	0.10
Eutypella	0.20	0.20	0.20	0.20
Chaetomium	0.10	0.03	0.10	0.80
Alternaria	0.30	0.30	0.30	0.30

UA= Urban area; SRA= Semi-rural area.

Cladosporium showed the highest relative abundance in both study areas. The main genera found (>1%) were *Phoma*, *Aureobasidium*, and *Coprinellus* (Table 8; Fig. 8A and B; Supplementary Files 5 and Supplementary Files 6).

3.8. Rainy season

During the rainy season the phylum Ascomycota decreased in abundance, while the Basidiomycota showed a considerable increase. Also, slight shifts between areas were evident. *Cladosporium* showed lower relative abundance than in the dry season. The main genera found (>1%) were *Phoma*, *Aureobasidium*, *Entomocorticium*, *Coprinellus*, *Hyphodontia*, and *Leptosphaeria* (Table 8; Fig. 8A and B; Supplementary Files 5 and 6).

Regarding the composition of fungal communities by area, the UA showed greater richness in terms of the core microbiome (2258 OTUs) compared to the SRA (2019 OTUs) (Table 2, Fig. 4C and D, Supplementary File 1). *Cladosporium* was found to be the most abundant genus, in both the UA and the SRA.

This study demonstrated shifts in the fungal community composition related to seasonality and subtle shifts related to location (either urban or semi-rural).

4. Discussion

The atmosphere is a hostile habitat due to variations in temperature, pH, ultraviolet radiation, resources, and water availability (Womack et al., 2010; Aguilera et al., 2018). Despite such harsh conditions the atmosphere harbours abundant and variable microbial communities (Genitsaris et al., 2017). Some culture-dependent studies have shown that airborne bacteria and fungi are metabolically active and play active roles in atmospheric events (Behzad et al., 2015). It is not yet clear if the atmosphere is merely a medium for the dispersion of bioaerosols or if a complex of ecological communities exists (Morris et al., 2011). The diversity and distribution of airborne communities are still being studied due to the complexity of their behaviour (Chan and Yao, 2008; Womack et al., 2010; Aguilera et al., 2018). New technologies, such as NGS, have provided more detailed analyses of the abundance and diversity of atmospheric bioaerosols and have the advantage of not requiring culture-dependent methods.

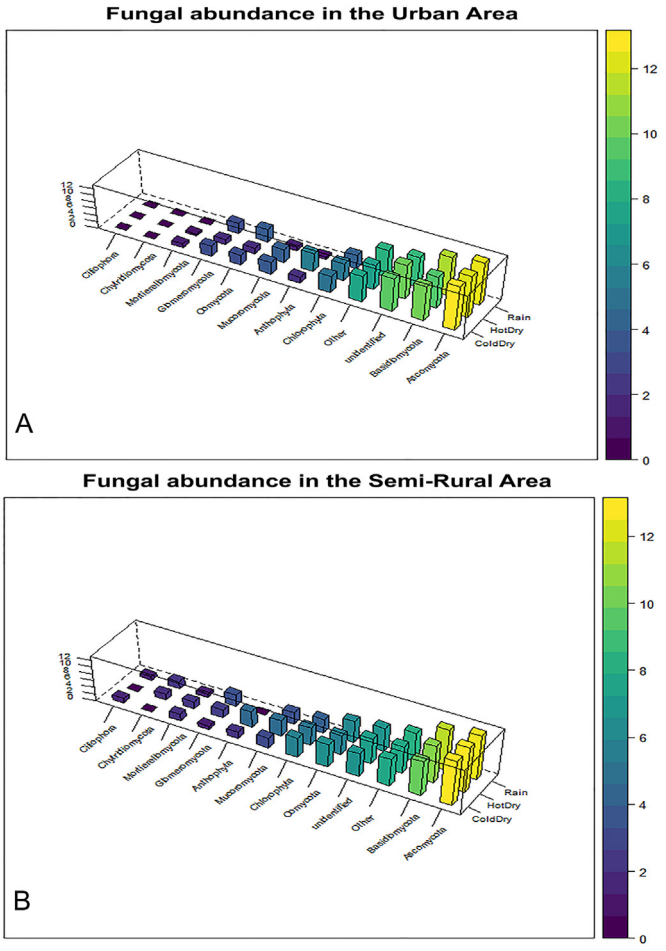

Fig. 8. Seasonal variation in the main fungal genera in the atmosphere of A) an urban area (UA); B) a semi-rural area (SRA) in Mexico.

4.1. Airborne bacteria

Airborne samples were collected from an UA and an SRA during an annual cycle in this study. The composition of the airborne bacterial and fungal communities identified in this study agrees with previous studies that describe the most common microbial community in the air of urban outdoor environments as belonging to the phyla Firmicutes, Proteobacteria, Bacteroides, Actinobacteria, Cyanobacteria, Ascomycota and Basidiomycota (Bowers et al., 2011; Oh et al., 2014; Barberán et al., 2015; García-Mena et al., 2016; Prussin et al., 2016; (Núñez et al., 2017); Genitsaris et al., 2017; Serrano-Silva and Calderón-Ezquerro, 2018; Mhuireach, 2019; Chen et al., 2020). Numerous human pathogens have been reported within these phyla, being responsible for severe health problems, such as asthma, respiratory infections, skin and wound infections, acne, and allergic reactions (Frank et al., 2007).

Factors such as meteorological conditions, land-use type, seasonality, and anthropogenic activities can alter the microbial community composition. It has been shown that urban and rural areas can present different bacterial communities depending on the environmental sources of each area (Després et al., 2007; Bowers et al., 2011; Dallimer et al., 2011; Barberán et al., 2015; Mhuireach et al., 2019). In studies carried out traditional cultivation techniques, higher numbers of bacterial CFU were registered in UAs than in rural areas in the same geographical regions (Rosas et al., 1993; Després et al., 2007, 2012; Bowers et al., 2011; Barberán et al., 2015).

In this study the composition of the bacterial communities showed an increase in richness in the SRA during the dry season, while in the rainy season richness values were similar for the two areas. With reference to fungi, however, the results indicated seasonal changes with an increase in the richness and diversity indices during the rainy season in both areas. It is essential to take into account that the SRA of Xochimilco, although it has agricultural areas and water canals (chinampas), presents a high degree of urbanization, similar to that of the UA of Coyoacán. Related to this, similar meteorological conditions were recorded for each season in the two areas, therefore, in this study, the factor that seems to mark the differences in richness and diversity of both bacteria and fungi was the seasonal pattern. The dry season induced the development of some groups of bacteria and fungi, while the precipitation and humidity in the environment during the rainy season promoted other phyla, which were not common in the dry season.

Regarding the airborne bacterial phyla identified in this study, Actinobacteria dominated during the dry season, decreasing slightly in the rainy season; this finding is similar to previous studies in Mexico City (Serrano-Silva and Calderon-Ezquerro, 2018), and the results reported by Cha et al. (2016), where they identified an increase in Actinobacteria and Acidobacteria during a dust event in Seoul, South Korea. Actinobacteria has been described as a phylum resistant to desiccation conditions, which favours its permanence in hostile environments (Anandan et al., 2016; Calderón-Ezquerro et al., 2020). Furthermore, among the Actinobacteria recorded in this study, genera such as *Corynebacterium* and *Mycobacterium* were identified. Within these genera, causative agents of many human and animal infections such as diphtheria (*Corynebacterium diphtheriae*) and tuberculosis (*Mycobacterium tuberculosis*) may be found. Likewise, *Corynebacterium* have been reported to produce antagonistic effects through phytohormones, polysaccharides, and toxins causing several plant diseases (Anandan et al., 2016).

Proteobacteria were one of the most abundant bacterial phyla, and similar studies report this as a ubiquitous phylum in indoor and outdoor environments (Yooseph et al., 2013; Ruiz-Gil et al., 2020). Also, in agreement with our results, it has been shown that Proteobacteria was more abundant in the rainy season and UA (García-Mena et al., 2016; Serrano-Silva and Calderón-Ezquerro, 2018). The vast majority of Proteobacteria are pathogenic species. Recent studies suggest they play a role in lung diseases, such as asthma and chronic obstructive pulmonary disease, conditions that present varying degrees of inflammation, which is characteristic of the diseases related to this Phylum (Rizzati et al., 2017).

Firmicutes increased in relative abundance in the dry season, although no marked differences were observed between the study areas for each season. Firmicutes are commonly associated with human skin microbiota (Prussin et al., 2016) and have been found on plant surfaces (Lymperopoulou et al., 2016). At genus level *Bacillus* and *Clostridium* were found in our study. Although most of the species belonging to these genera are saprophytic organisms, prevalent in soils, water, air, and various plants, there are relevant human-pathogen species as *Bacillus anthracis*, *Clostridium botulinum*, and *C. tetani*.

Cyanobacteria were recorded throughout the year in both areas (with relative abundances $\geq$3%), although they increased in the UA (Coyoacán) during the dry season. This bacterial phylum was found in high proportions in previous studies conducted in Mexico City (Roy-Ocotla and Carrera, 1993; Serrano-Silva and Calderón-Ezquerro, 2018). The Cyanobacteria concentration in the air is mainly influenced by temperature and humidity (Sharma et al., 2007; Brewer and Fierer, 2018). Further, Cyanobacteria have been reported to represent a considerable share of atmospheric bioaerosols, predominating in tropical regions (Sharma et al., 2007; Genitsaris et al., 2011; Sahu and Tangutur, 2015). The most abundant Cyanobacteria found in our study were the Xenococcacea. This family was found during the dry season and was absent in the rainy season, likely because it was removed from the atmosphere by heavy rains (precipitation 220–234 mm). Members of the Cyanobacteria have been reported to be able to survive under high radiation, extreme temperatures, osmotic stress, and extreme pH values. (Magana-Arachchi and Wanigatunge, 2013).

There are several species of human pathogens within the phylum Cyanobacteria, so it would be relevant for public health to carry out more studies to evaluate their identity at species level, and their concentration and viability in the air through the annual cycle.

Many of the bacteria collected in this study have been reported to be resistant to extreme environmental conditions such as desiccation (*Bacillus*), radiation (*Deinococcus*), and high temperatures (some species of *Microbispora* and *Rubellimicrobium*) (Roszak et al., 1987; La Duc et al., 2007). We found several genera of human pathogens; relevant phytopathogens include *Kocuria rhizophila*, various *Staphylococcus* spp., *Acinetobacter* spp., *Psychrobacter sanguinis*, *Mycobacterium arupense*, *Rhodococcus fascians*, *Enterococcus cecorum*, *Pseudomonas viridiflava*, and *Erwinia* sp.

4.1.1. Airborne fungi

Airborne fungi were found in the atmosphere throughout the annual cycle. The main phyla were Ascomycetes and Basidiomycetes. The analysis showed high diversity in both the SRA and the UA, but lower species richness compared to bacteria; the dominant species included *Cladosporium*, *Phoma*, *Alternaria*, and *Aureobasidium* (frequently found in airborne samples worldwide) (Patel et al., 2018; Ortega Rosas et al., 2019).

According to the Shannon index, the fungal communities were more diverse in the UA than in the SRA.

The fungal diversity in the UA may be related to the great diversity of native and exotic species (flora, fungi, and lichens) found in the ecological reserve (REPSA, 2019) located on the University

campus (Coyoacan).

Ascomycota showed a higher relative abundance throughout the annual cycle, followed by Basidiomycota. This finding coincides with previous studies that demonstrated fungal community composition was related more to seasonal changes than location (Nicolaisen et al., 2017). Furthermore, common microbial phyla, such as the Ascomycota and Basidiomycota, have been shown to contain numerous pathogens (Chen et al., 2020).

Regarding the taxonomic characterization of airborne fungi, the results indicated that, throughout the year, *Cladosporium* was the fungal genus with the highest annual relative abundance in the SRA (54.2%) and the UA (48.5%). This result agrees with several studies that have shown this fungus to be the main component of the air mycobiota in several regions of the world and a predominant fungus over other types of spores (Rodríguez-Rajo et al., 2005; Patel et al., 2018), regardless of collection methods, whether culture-dependent (Shelton et al., 2002; Awad, 2005; Fang et al., 2005), by spore impaction (Calderón et al., 1997; Kasprzyk and Worek, 2006; Oliveira et al., 2010; Ortega Rosas et al., 2019), or by metagenomic exploration (Barberán et al., 2015; Nicolaisen et al., 2017). The presence of *Cladosporium* in both areas (UA and SRA) can be attributed to geographical location, climatic conditions, human activity, and vegetation type (Lacey, 1981; Pasanen, 1992; Awad, 2005).

Alternaria, *Aureobasidium*, and *Phoma* were collected from the air in low proportions at both monitored locations. *Alternaria* is frequently found on plants, so it is prevalent and widespread in rural areas (Mitakakis et al., 2001); *Aureobasidium* is a common airborne fungus that has been identified in forested and UA (Irga and Torpy, 2016); *Phoma* is commonly found in aquatic systems and soil. These three genera have the potential to be pathogenic in plants, humans, and non-human animals. In humans these fungi act as opportunistic pathogens when individuals are immunocompromised, as in organ transplants, chemotherapy, or the use of immunosuppressive agents, causing phaeohyphomycosis.

Penicillium and *Aspergillus* were also identified in this study. It has been shown that the infection of *Aspergillus fumigatus* occurs by inhalation of airborne spores. The fungus enters the respiratory tract and alters its morphology (from yeast to hyphae). In immunocompromised patients can infect the lungs, cause pneumonia, and in severe cases, disseminate to other organs (Delgado-Rizo et al., 2017). Moreover, the presence of spores of these fungi in the air is associated with allergic rhinitis and allergic asthma (Kurup, 2000).

Seasonal analysis of the fungal communities in this study showed the occurrence of important Basidiomycetes in agricultural and forest environments. Our results are consistent with studies that determined that the seasonality of basidiomycetes are developed mainly in the rainy season in areas with abundant vegetation (Sundari et al., 2018).

Within the Basidiomycetes we found *Entomocorticium*, a mutualist of the southern pine beetle, *Coprinellus*, a saprophytic fungus associated with disturbed soils, such as those found in roads, parking lots, gardens, and construction sites, and rotten wood in humid environments, and *Hyphodontia*, a fungus associated with *Abies religiosa* and *Pinus* spp. These genera were present in both areas, mainly during the rainy season when the conditions stimulate the development, growth, and maturation of the spores.

Within the Basidiomycetes, *Cryptococcus* is not commonly reported to be a genus that occurs in the air. It should be noted that *Cryptococcus neoformans* and *C. gatii* are relevant pathogens for human health. Although these species are generally considered to be opportunistic pathogens, some strains have shown a remarkable ability to manipulate the human immune response in order to facilitate establishment and spread, even in immunocompetent people (May et al., 2016).

The use of NGS to study bioaerosols contributes to the enrichment of databases, facilitating more robust identification of emerging airborne human and plant pathogens. We consider the continuous and permanent evaluation of the microbiota of the atmosphere to be necessary, since bacterial and fungal communities vary in space and time. Likewise, it is important to evaluate the direct relationship between bioaerosols and meteorological factors (temperature, relative humidity, daylight hours, precipitation, direction, and magnitude of the wind, among others) that allow its development, permanence, viability, and transport to be determined, both seasonally and day by day. The above will allow us to deepen our knowledge of the bioaerosols responsible for damage to human health and ecosystems and the risk factors associated with exposure to pathogens. This will also result in the development of adequate prevention and control measures for the benefit of the human population and the environment.

5. Conclusions

- Potential human and plant pathogen genera were identified as forming part of the airborne microbiota of the UA and the SRA.
- The composition of the fungal and bacterial communities was consistent with previous aerobiological studies, even with different identification techniques.
- The microbial composition was similar between locations, likely because of a high degree of urbanization in both areas, but with evident seasonal changes in the microbial community.
- Initially, the study areas were selected because they represent different land uses, urban and semi-rural, so we hypothesized that there would be differences between the compositions of the respective microbial communities. However, the similarities in the microbial communities and in the meteorological conditions in the study areas allowed us to determine that urban growth has permeated the SRA (Xochimilco). In this context, community composition was more related to seasonal changes than location.
- It is essential to carry out continuous monitoring of atmospheric bioaerosols to determine the direct influence of each meteorological factors over the airborne microbiota composition.

Author statement

Calderon-Ezquerro MC: Conceptualization, Investigation, writing and edition, and methodology. Serrano-Silva N: Reference review, technical methodology contribution. Brunner-Mendoza C: Supervision, Investigation, writing and edition.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.116858.

Compliance with ethical standards

This paper does not contain any studies with human participants or animals performed by any of the authors.

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