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The Differential Vertical Distribution of the Airborne Biological Particles Reveals an Atmospheric Reservoir of Microbial Pathogens and Aeroallergens

Andrés Núñez[1,2] · Diego A. Moreno[1,3]

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Abstract

The most abundant biological particles present in the air are bacteria, fungal propagules and pollen grains. Many of them are proved allergens or even responsible for airborne infectious diseases, which supports the increase of studies in recent years on their composition, diversity, and factors involved in their variability. However, most studies in urban areas are conducted close to ground level and a factor such as height is rarely taken into account. Thus, the information about how the composition of biological particles changes with this variable is scarce. Here, we examined the differential distribution of bacteria, fungi, and plants at four altitudes (up to ~ 250 m) in a metropolitan area using high-throughput DNA sequencing. Most taxa were present at all levels (common taxa). However, a transitional layer between 80 and 150 m seemed to affect the scattering of these bioaerosols. Taxa not present at all altitudes (non-common) showed an upward tendency of diversity for bacteria and plants with height, while the opposite trend was observed for fungi. Certain patterns were observed for fungi and specific plant genera, while bacterial taxa showed a more arbitrary distribution and no patterns were found. We detected a wide variety of aeroallergens and potential pathogens at all heights, which summed a substantial portion of the total abundance for fungi and plants. We also identified potential connections between the biological particles based on their abundances across the vertical section.

Keywords Urban airborne biodiversity · Next-generation sequencing · Height · Bacteria · Fungi · Pollen

Introduction

Human population in urban environments is daily exposed to an enormous variety of airborne biological particles that include microorganisms such as viruses, bacteria, archaea, fungi, and parts of higher organisms like pollen [1]. Some of them play a relevant role in human health causing allergic symptoms (mainly by fungal spores and pollen) [2] and airborne infectious diseases such as influenza, legionaries' disease, and tuberculosis [3–5]. The origin of these particles is very diverse (soil, plant surface, water sources, other organisms, etc.) [1], and their presence, abundance, and dispersion are highly influenced by meteorological factors [6–8], resulting in seasonal and daily variations [9–13].

The presence of biological entities has been described in the upper atmosphere (up to 77 km), with no clear limit beyond which life is not detected [14, 15]. Previous aerobiological studies addressing the study of distribution of fungal spores and pollen grains with height have revealed the complexity of the matter. For instance, a differential pattern was initially proposed for pollen depending on whether it is from trees or herbaceous sources, suggesting that pollen grains from the former are capable of reaching higher altitudes so total counts increase with height, while the opposite occurs with the latter [16, 17]. Further studies have shown that this is

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✉ Diego A. Moreno
diego.moreno@upm.es; diego.moreno@uclm.es

[1] Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (ETSII-UPM), c/ José Gutiérrez Abascal 2, E-28006 Madrid, Spain

[2] Department of Genetics and Microbiology, Facultad de Biología, Universidad de Murcia, E-30100 Murcia, Spain

[3] Facultad de Farmacia, Universidad de Castilla-La Mancha (FF-UCLM), Avda. Dr. José María Sánchez Ibáñez s/n, E-02008 Albacete, Spain

only partly true and under the influence of the pollen type studied and meteorological conditions [18–20]. Similarly, Khattab and Levetin [21] found that the fungal basidiospores counts were higher at ground level (e.g., *Penicillium/Aspergillus* type) while ascospores concentration increased with the height (e.g., *Alternaria* spp.), although this difference was not confirmed by further studies [22]. A recent study conducted by Damialis et al. at rural landscapes [23], which identified pollen and spores at different heights up to 2000 m, demonstrated different dispersion models depending on the taxa. Therefore, further studies to unravel both total diversity and patterns of distribution with height are still required.

In metropolitan areas, the knowledge on composition of the airborne biological particles and how they are dispersed in the urban atmosphere is particularly relevant to evaluate the real exposure to pathogenic organisms and aeroallergens [24–26]. However, most surveys are conducted within the maximum exposure area for human (< 55 m above ground level), so the real reservoir of microscopic biodiversity above this height is still poorly understood. Furthermore, the majority of studies have been conducted by microscopy or culture approaches, so the total diversity is usually underestimated.

In addition, microbial biodiversity can be associated to larger particles as $PM_{2.5}$ and PM_{10} in metropolitan areas [27–31]. In fact, an increase in the prevalence of allergic diseases by a synergistic effect of these air pollutants and pollen grains has been described [32, 33]. Likewise, pollen grains can develop a rich microbiome that influences their allergenicity [34–36], so other interactions between microscopic organisms present in the air may remain unnoticed even though they are of a particular importance from both ecological and health points of views.

Next-generation DNA sequencing (NGS) has become recently a promising technology in this field and has been successfully applied to airborne bacteria, fungi and plant pollen [37–40], solving additionally the bias of the culture-based analyses. Here, we examined the patterns of distribution by height of the microbial (bacteria and fungi) and plant diversity present in the urban atmosphere using NGS technology. We characterized the fundamental taxa and identified the exposure to allergens and potential pathogens in this environment. Furthermore, we also correlated the presence and abundance between the different organisms, identifying putative new interspecific relationships present in the original source or during transport and whose concomitant exposure may result in significant relevance for human health.

Materials and Methods

Location and Sampling Methodology

Sampling was carried out in "Torre CEPSA," the second tallest tower in Spain, sited in Madrid (40.475677° N, 3.687686° W). The building is located in an urban location, surrounded by high traffic streets, highways and a few gardens, and close to a train station and two hospitals.

Airborne particles samples were taken using a DUO SAS Super 360 (VWR), with an airflow rate of 180 L/min. The sampler was customized in production to overcome the time/volume limitation of the standard equipment. This device uses two Petri dishes simultaneously (biological replicates) that were covered with sterile petroleum jelly (Vaseline, *Interapothek*) in a biosafety cabinet using sterilized materials and kept closed until they were placed into the sampler. The heads of the sampler were autoclaved the day of the sampling, and soaked in ethanol 96% and dried out with sterilized paper after each sampling. On Thursday 9th March 2017, a total of 8 samples (4 paired samples or duplicates) were taken at four different heights in the following order: ground level (~ 647 m above sea level), 80 m, 150 m, and ~ 250 m, corresponding with the accesses to the exterior of the tower (external corridors) and the rooftop, both areas used by the staff only. "Torre CEPSA" is a glass windowed building, which is frequently cleaned, and the sampler was placed at a minimum distance of 1 m from the closest wall. Each sample is a 1-h collection (~ 10.8 m^3). The meteorological data are shown in Supplementary Information ESM1 as a description of the meteorological condition during the sampling time. An additional sample keeping the device off was taking as a negative control.

DNA Extraction and High-Throughput Sequencing

Petri dishes were kept at 4 °C after collection until the DNA extraction was performed the following day. Working in a biosafety cabinet, the petroleum jelly of each dish containing the particle collected was recovered using a sterilized razor and put into an extraction tube of DNeasy PowerSoil Kit (Qiagen). DNA was extracted and purified following the manufacturer's instructions. DNA libraries and high-throughput sequencing using Illumina® MiSeq platform (2 × 300 bp reads) were performed at "Parque Científico de Madrid" (Madrid, Spain). Amplicon libraries were obtained using a different set of universal primers for each group of the biological entity studied (Bacteria: Bakt_341 (F): 5′-CCTACGGGNGGCWGCAG-3′; Bakt_805 (R): 5′-GACTACHVGGGTATCTAATCC-3′ [41], for partial amplification of the V3–V4 hypervariable regions of the gene 16S rRNA; Fungi: ITS86 (F): 5′-GTGAATCATCGAATCTTTGAA-3′ [42]; ITS-4 (R): 5′-TCCTCCGCTTATTGATATGC-3′ [43], for the region 5.8S–ITS2; Plants: ITS-p3 (F): 5′-YGACTCTCGGCAACGGATA-3′ [44]; ITS-4 (R): 5′-TCCTCCGCTTATTGATATGC-3′ [43], for the region 5.8S–ITS2). DNA amplification was null for the negative control under the same conditions, but it was included in the sequencing batch to discard contaminations and sequencing issues.

Raw Sequence Data obtained in this study are available in the National Center for Biotechnology Information (NCBI), Sequence Read Archive (SRA), under the Accession number SRP140605 (Bioproject PRJNA450512).

Sequence Assembly, Preprocessing, and Normalization

Bioinformatic processing of the next-generation sequencing (NGS) data was performed as described in Núñez et al. [38]. A first check of the total number of sequences per sample, sequence quality and length distribution was performed with FastQC software (version 0.11.3, Babraham Bioinformatics Group, Babraham Institute, UK [www.bioinformatics.babraham.ac.uk/projects/fastqc/]). Paired-ends sequences were assembled with PANDAseq ([45]; version 2.8, https://github.com/neufeld/pandaseq/wiki/PANDAseq-Assembler), filtering by Q-score quality (default 0.6), trimming the primer sequences and removing the sequences exceeding the length of the amplicon (bacteria: min 400 bp, max 500; plants: min 320, max 550). For fungal ITS library, as the sequencing protocol exceeded the length of the amplicon, we employed "read_fastq" from Biopieces (version 2.0, http://maasha.github.io/biopieces/) to remove the primer sequence followed by "fastq-join" ([46]; https://github.com/brwnj/fastq-join) to pair the reads. General processing of the sequences was carried out in Qiime suite environment [47]; version 1.9.1, http://qiime.org). Chimeras were subtracted using USEARCH v8.1 (https://drive5.com/usearch/) with default values. OTUs clustering and taxonomic assignments were performed with the default algorithm of Qiime (pick_open_reference_otus.py), using UCLUST for picking OTUs

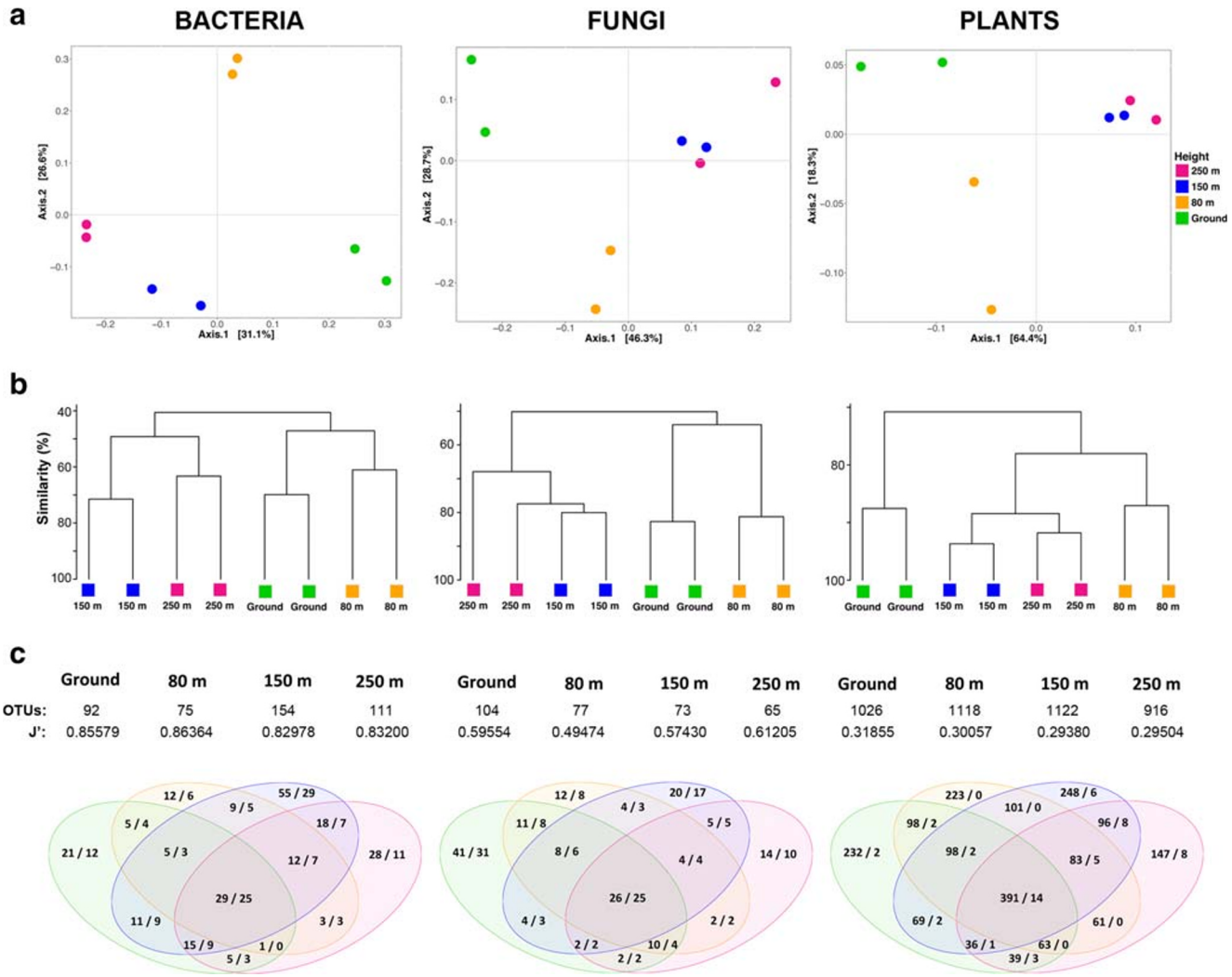
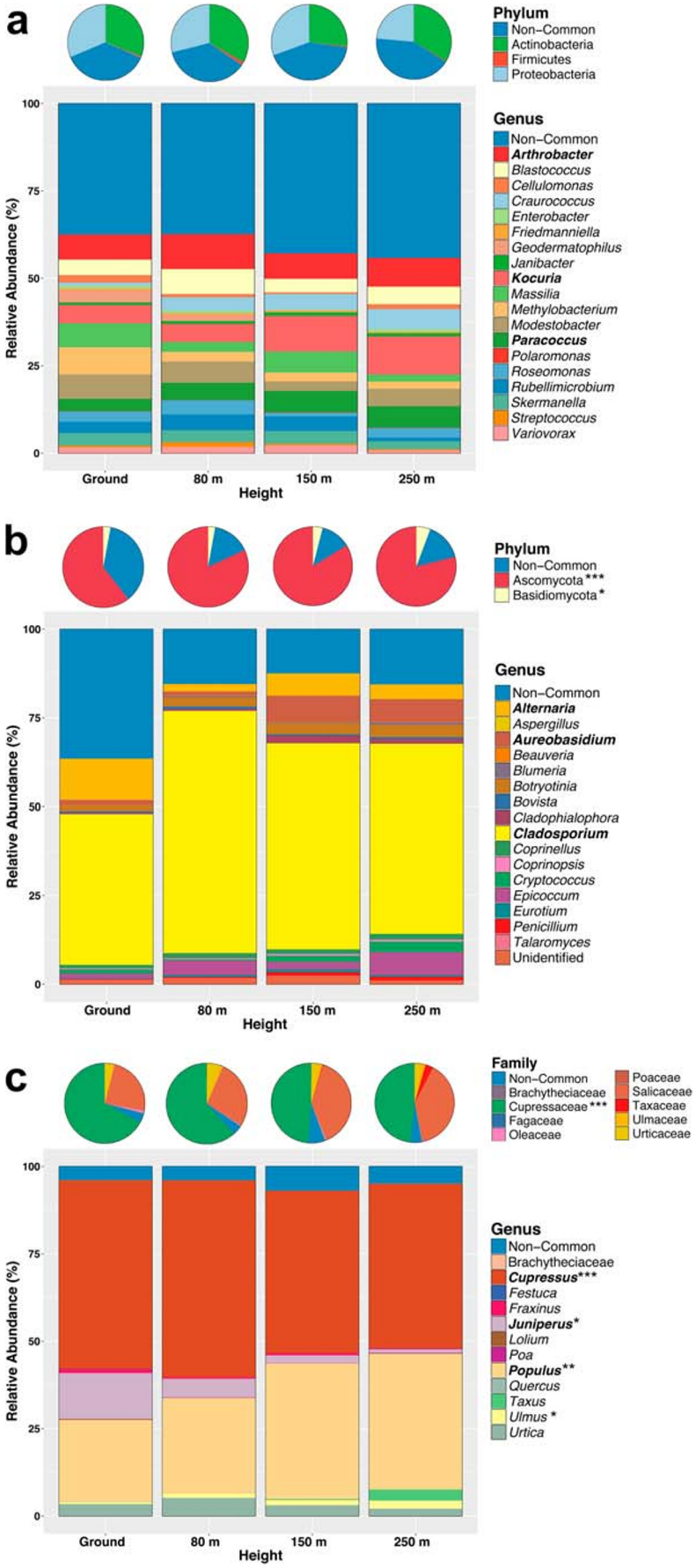


Fig. 1 Differential distribution of the biological airborne particles in height. **a** Principal coordinate Analyses (PCoA) showing the similarity of the biological composition between samples for the different communities. **b** Cluster analyses based on Bray-Curtis indices. **c** Richness (Observed OTUs), Evenness (*J'*) coefficients, and Venn diagrams showing the OTUs/phylotype distribution for the different heights


a
Phylum
Non−Common
Actinobacteria
Firmicutes
Proteobacteria
Genus
Non−Common
Arthrobacter
Blastococcus
Cellulomonas
Craurococcus
Enterobacter
Friedmanniella
Geodermatophilus
Janibacter
Kocuria
Massilia
Methylobacterium
Modestobacter
Paracoccus
Polaromonas
Roseomonas
Rubellimicrobium
Skermanella
Streptococcus
Variovorax
Relative Abundance (%)
100
75
50
25
0
Ground
80 m
150 m
250 m
Height
b
Phylum
Non−Common
Ascomycota ***
Basidiomycota *
Genus
Non−Common
Alternaria
Aspergillus
Aureobasidium
Beauveria
Blumeria
Botryotinia
Bovista
Cladophialophora
Cladosporium
Coprinellus
Coprinopsis
Cryptococcus
Epicoccum
Eurotium
Penicillium
Talaromyces
Unidentified
Relative Abundance (%)
Ground
80 m
150 m
250 m
Height
c
Family
Non−Common
Brachytheciaceae
Cupressaceae ***
Fagaceae
Oleaceae
Poaceae
Salicaceae
Taxaceae
Ulmaceae
Urticaceae
Genus
Non−Common
Brachytheciaceae
Cupressus***
Festuca
Fraxinus
Juniperus*
Lolium
Poa
Populus**
Quercus
Taxus
Ulmus *
Urtica
Relative Abundance (%)
Ground
80 m
150 m
250 m
Height

◀ **Fig. 2** Characterization of the common atmospheric biodiversity (present at all heights). Bar plots of the taxa associated to the common OTUs (core) for each community: bacteria (**a**), fungi (**b**), and pollen (**c**). The most abundant genera are highlighted in bold. Statistical differences of the abundance distribution (Wilcoxon signed-rank test) are indicated based on the level of significance: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

[48], which were clustered with 97% similarity cutoff. Taxonomic assignments were performed applying UCLUST algorithm (minimum consensus 0.51, similarity > 0.9, max accepts 3) with SILVA database for bacteria ([49]; release_132), and UNITE ([50]; version 7.0, https://unite.ut.ee/) for fungi. A customized database was created for plants assignment as described previously in Núñez et al. [38].

The OTUs assigned to chloroplast and mitochondria in 16S analysis were filtered out. When the OTUs found in a library were assigned or identified to a different organism of that analyzed in the library (for instance, OTUs assigned to Plantae in Fungi library), and those that did not reach at least the taxonomic rank of Order were removed. As a part of the denoising process performed in R software environment ([51]; version V3.4.2, https://www.r-project.org/), we estimated a base threshold (detection limit) to consider a given OTU as detected. Firstly, we identified all the OTUs with a mix of zero and non-zero values in all the samples, and for each of these OTUs we took the smallest non-zero count value. The median of all these values was considered as our technical detection limit. Then, we filtered out the OTUs below this value (1 count for pollen, 5 for fungi, and 4 for bacteria). Finally, only the OTUs present in both samples of the same duplicate were taken into consideration. The differences in counts were then normalized with "metagenomeSeq" package [52] and subsequent analyses were performed using the package "phyloseq" ([53]; version 1.20.0). See Supplementary Material ESM2 for additional information and ESM4–6 for complete final records. The package "Tax4fun" [54] was used to explore the functional diversity of the bacterial communities.

Statistical Analyses

The statistical analyses were performed with different packages in R environment. Permutational multivariate analyses of variance (PERMANOVA) associated to PCoAs were performed with "vegan" package using the Bray-Curtis distance matrices and 999 permutations ("adonis" function). Evenness index (J') described by Pielou [55] and other diversity indexes (ESM3) were calculated subtracting the detection limit from all the counts, so the OTUs with one count above the detection limit are considered as singletons, and below as absent. Wilcoxon signed-rank test (paired test, two-sided, and confidence level at 0.95) was applied to evaluate the significance of the abundance distribution of the main taxa. Only those genera with > 3 observations (OTUs) were assessed. Spearman's rank correlations between organisms (two-sided, confidence level at 0.95, and p values adjusted using Benjamini-Hochberg correction) was performed using the matrix with the sum of relative abundance compiled by each genus from the core communities present in each sample, so a total of 8 observations per taxa were used in the test.

Results and Discussion

Differential Distribution of the Biological Airborne Particles in Height

As shown in Fig. 1a, the different biological particles showed a similar distribution, with differences fundamentally based on the height (PERMANOVA R2 values 0.730, 0.790, and 0.825 for bacteria, fungi, and plants, respectively, with associated p values < 0.01), indicating different composition between the levels. Similar ordinations were obtained when phylogenetic information based on Unifrac distance matrices was included, although fewer differences between samples were found (Supplementary Information ESM3). A particular tendency to cluster in two heights was observed in both cases (Fig. 1b and ESM3), suggesting the existence of a transitional layer between 80 and 150 m around which the biological entities showed a different community composition.

After merging the data of the replicates from each altitude, the OTUs distribution showed different patterns (Fig. 1c). In contrast to other studies performed at higher altitudes in non-urban areas [15, 56, 57], bacterial richness (number of OTUs) displayed an upward tendency with height, increasing above 80 m. This fact would explain the fast changes described in short periods by other authors [9, 58, 59], simply because the potential source is just a few meters above. On the contrary, the highest value for fungi was found at ground level, with a remarked decrease over this elevation. Other diversity indexes (Chao1, Shannon, Fisher) showed similar results (Supplementary Information ESM3). These results would suggest that bacteria are more easily dispersed in the atmosphere than fungal propagules, which tend to remain at lower heights. Size, shape, and the ease to get attached to other particles may explain these tendencies.

In the case of airborne plant DNA (mostly from pollen grains although the contribution of microscopic plant debris cannot be discarded), richness was very steady across the four heights.

Fundamental Biodiversity Present in a Vertical Section

The common OTUs (those found at all the heights) for bacteria represented 57–63% of the total abundance. This core was

formed by 12 different families and 19 genera (Fig. 2a), which belonged to the main phyla Actinobacteria (25–30%), Proteobacteria (25–30%), and Firmicutes (< 2%). *Kocuria*, *Arthrobacter*, and *Paracoccus* were the most abundant genera in all the samples, in accordance with the bacterial biodiversity described previously for this season and area [37]. Although the relative abundance of some taxa varied with the height, no pattern associated with this variable was found at any taxonomic rank examined ($p > 0.05$ for Wilcoxon tests). Additionally, the relative abundance of the non-common OTUs (those not present at all heights) is the greatest of the three communities (bacteria, fungi, or plant) analyzed (35–42% of the total abundance for each height).

The core of fungal diversity (Fig. 2b) was formed mainly by DNA sequences corresponding to the phylum Ascomycota (> 60% of each sample) and a minority to Basidiomycota (< 6%). Among the 13 different families and 16 genera that were identified, Davidiellaceae and the genus *Cladosporium*/*Davidiella* were clearly predominant (> 40% of the relative abundance in each height), followed by *Alternaria* (3–12%) and *Aureobasidium* (1–9%). Although no significant variation in the abundance of the constitutive genera was found between heights, the abundance of Ascomycota was significantly lower at ground level ($p < 0.001$), while the opposite tendency was observed for Basidiomycota ($p < 0.05$).

In the case of the plant diversity (Fig. 2c), the 391 OTUs in common compiled between 93 and 96% of the total abundance, which would explain the high similarity between the samples' composition (Fig. 1b, right panel). The plant core was formed by 8 families of vascular plants and 1 bryophyte, with a total of 11 different genera, and a high predominance of *Cupressus* spp. (Fig. 2c). Both the presence and the relative abundance of pollen detected by NGS are supported by the pollen calendar of the region and previous studies [37, 60]. The pollen of *Cupressus* spp. and *Juniperus* spp. would tend to accumulate in the area next to the base (ground and 80 m, $p < 0.001$ and $p < 0.05$, respectively, and family Cupressaceae $p < 0.001$) while the pollen of *Populus* spp. and *Ulmus* spp. (Ulmaceae) would follow the opposite tendency and be more abundant at 250 m ($p < 0.05$, respectively). We also evaluated if these differential patterns would be related to the size of the pollen grains. The plant genera were classified into "Small" (10–25 μm), "Medium" (26–51 μm), and "Large" (51–100 μm) according to the average size of their pollen grains (information extracted from http://www-saps.plantsci.cam.ac.uk/pollen/; https://pollenatlas.net/atlas/pollen-profiles; https://www.paldat.org; and technical documents from the aerobiological network of the region "Red Palinocam," http://www.comunidad.madrid/servicios/salud/polen). However, no significant correlation of this feature with height was found ($p > 0.05$ for the Kruskal-Wallis test, Supplementary Information ESM3). In fact, the plant genera with small pollen grains seemed to accumulate at the lower altitudes. A plausible explanation comes from the fact that many pollen grains are not completely spherical and present morphologies and some additional structures to help in their aerodynamics, so the size cannot be considered as a unique variable to explain the patterns observed across the vertical section.

Recently, Damialis et al. [23] found an increase of the diversity and concentration of fungal spores and many pollen types with height up to 2000 m. The number of OTUs and evenness index variations determined in our work suggested that the pollen diversity do not significantly change up to 250 m in the metropolitan areas, although we agree that particular differences exist for specific taxa.

Distribution of the Divergence Taxa Between Heights

A total of 69 bacterial genera, which mostly belonged to the phyla Actinobacteria and Proteobacteria (Fig. 3a), were differently distributed in the vertical section. Jointly, they gathered 25–31% of the total relative abundance in each height with no remarked difference between levels. Thirty-six genera were detected only in one height (19 at 150 m) but their contribution to the total abundance was marginal (< 6% of each sample jointly). In regard to fungi, 21 out of 53 genera were exclusively found at ground level (Fig. 3b), which mostly belonged to genera of the Phylum Basidiomycota (> 23% corresponded to *Strobilurus* spp.). For plants, the contribution of the non-common genera (32) represented a minor fraction (0.5–3.4% of the total abundance of each height sample), with a tendency to find more diversity when height increases (Fig. 3c). Unlike previously proposed [16, 17], we did not observe any correlation with the type of plant (herbaceous or tree) and height, finding DNA from several herbaceous species above the ground level (Fig. 2c) and at least 14 genera of this type were only detected over 150 m (Fig. 3c).

Pathogens and Allergenic Particles

Our results showed that urban population is widely exposed to fungal and pollen allergens at any height. Most of the diversity found in this work has been previously reported to have consequences in human health, acting as allergens or opportunistic pathogens [26, 61, 62]. Although a taxonomic resolution to species level would be required to be confident about the pathogenicity of the microbial genera we found, several genera are fundamentally associated to health issues. For instance, *Enterobacter* or *Streptococcus* were detected as part of the biodiversity core, and were found at all the heights (Table 1). Other genera related with the human microbiome included *Acinetobacter*, *Corynebacterium*, *Cutibacterium* (former *Propionibacterium*), *Klebsiella* and *Lactobacillus*, gathering ≤ 5% from each sample.

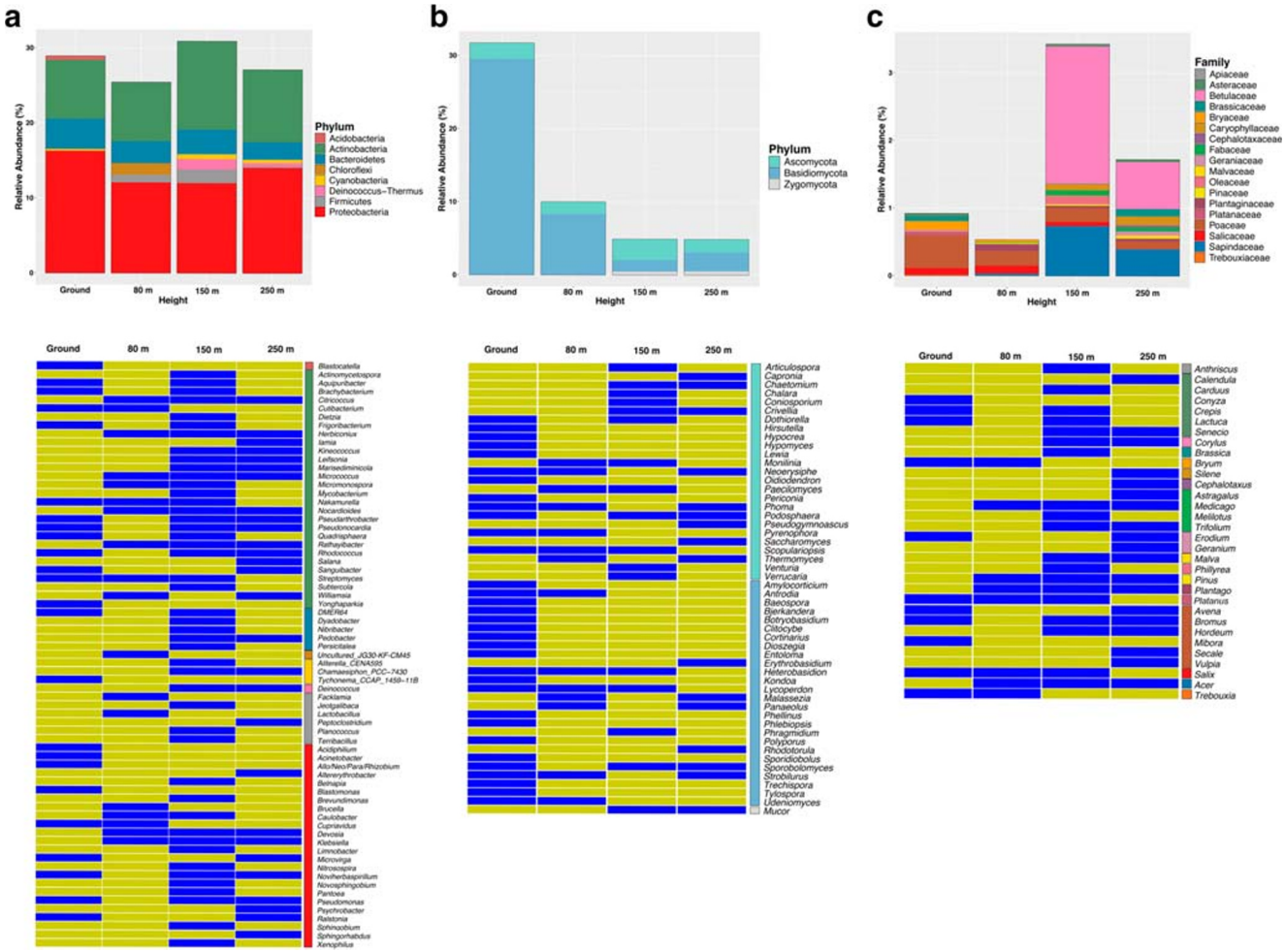


Fig. 3 Detailed description of the non-common taxa by height. Bar plots (relative abundance) and heatmap (presence/absence) representations of bacteria (**a**), fungi (**b**), and pollen (**c**)

On the other hand, a total of 16 fungal genera with allergenic/pathogenic representatives were present in the atmosphere, with *Cladosporium* spp. dominating the relative abundance at all the heights, but relevant genera such as *Alternaria*, *Aspergillus*, *Epicoccum*, and *Penicillium* were also present. In addition, most pollen grains have an allergenic potential, especially the diversity found at this season. This was dominated by representatives from Cupressaceae (*Cupressus* spp. and *Juniperus* spp.) and Salicaceae (*Populus* spp.), so that > 99% of the abundance belonged to genera that may cause allergy symptoms [26].

Excepting fungi, which showed a lower abundance of pathogens/allergens at ground level, the exposure to relevant entities did not show important differences between the heights regarding diversity or abundance.

Potential Interaction Between Organisms

Microorganisms are hardly found isolated in an ecosystem. One interesting attempt is to elucidate the potential connections between the different entities by using their abundances as a quantitative variable. Therefore, we performed Spearman's rank correlations between the relative abundance of each organism of the core communities and the other taxa at genus level. As shown in Fig. 4, positive strong correlations ($rho > 0.7$) were resolved into two scenarios of connections, with no crossover interactions between them, which would suggest either a different source location or total independency. Some interactions have been previously confirmed by other methods. For instance, the presence of *Aspergillus* spp. in the leaf surface of *Ulmus* sp. (Fig. 4) was described by Levetin and Dorsey [63]. Also the most abundant bacteria, *Kocuria* (Actinobacteria), *Paracoccus* (Alphaproteobacteria), and the fungus *Aureobasidium* showed all positive correlations with the trees *Populus*, *Ulmus* and *Taxus*, associations described in former studies [63–66], coinciding with our results. However, in our study, most of these correlations failed to past the statistical significance test ($p > 0.05$), likely because of the shortage of observations (see Supplementary Material Dataset ESM7 and ESM8 for complete data).

Table 1 Potential allergenic/pathogenic genera and their relative abundance distribution by height

Organism	Genus	Relative abundance (%)			
		Ground	80 m	150 m	250 m
Bacteria	*Acinetobacter*	0.608	0.000	0.000	0.000
	Enterobacter	0.721	0.684	0.273	0.680
	Geodermatophilus	4.869	1.841	0.284	0.006
	Klebsiella	0.000	0.168	0.067	0.120
	Micromonospora	0.000	0.202	0.324	0.000
	Mycobacterium	0.000	0.000	0.191	0.000
	Pseudomonas	2.410	0.000	0.275	0.168
	Roseomonas	3.034	6.928	2.135	5.891
	Streptococcus	0.983	1.329	0.632	1.313
	Total	12.625	11.152	4.181	8.178
Fungi	*Alternaria*	12.498	2.983	6.759	4.639
	Aspergillus	0.084	0.231	0.547	0.978
	Aureobasidium	1.381	1.108	7.351	9.095
	Botryotinia	1.426	2.496	3.085	3.448
	Chaetomium	0.000	0.000	0.452	0.298
	Cladosporium	42.580	68.218	57.966	53.586
	Cryptococcus	2.000	1.044	4.140	3.491
	Epicoccum	1.710	4.094	2.135	8.264
	Eurotium	0.171	0.511	0.772	0.450
	Mucor	0.000	0.000	0.470	0.506
	Oidiodendron	0.036	0.000	0.000	0.000
	Paecilomyces	0.000	0.107	0.077	0.000
	Penicillium	0.400	0.515	1.703	1.702
	Phoma	0.029	0.043	0.000	0.029
	Scopulariopsis	0.040	0.247	0.312	0.000
	Talaromyces	0.065	0.143	0.183	0.038
	Total	62.420	81.740	85.952	86.524
Plants	*Acer*	0.000	0.030	0.738	0.395
	Corylus	0.000	0.000	2.028	0.694
	Cupressus	56.787	59.288	48.932	49.398
	Fraxinus	1.200	0.463	1.116	0.468
	Juniperus	13.161	5.396	2.296	1.041
	Poaceae[a]	0.868	0.374	0.316	0.468
	Pinus	0.000	0.019	0.022	0.010
	Platanus	0.054	0.001	0.001	0.000
	Populus	23.424	27.570	39.021	39.256
	Quercus	0.002	0.004	0.003	0.003
	Salix	0.099	0.112	0.059	0.000
	Taxus	0.063	0.061	0.295	3.185
	Ulmus	0.583	1.310	1.559	2.422
	Urtica	3.434	5.197	3.226	2.119
	Total	99.324	99.606	99.442	99.450

[a] Family Graminaceae/Poaceae includes the following detected genera: *Avena*, *Bromus*, *Festuca*, *Hordeum*, *Lolium*, *Mibora*, *Poa*, *Secale*, and *Vulpia*

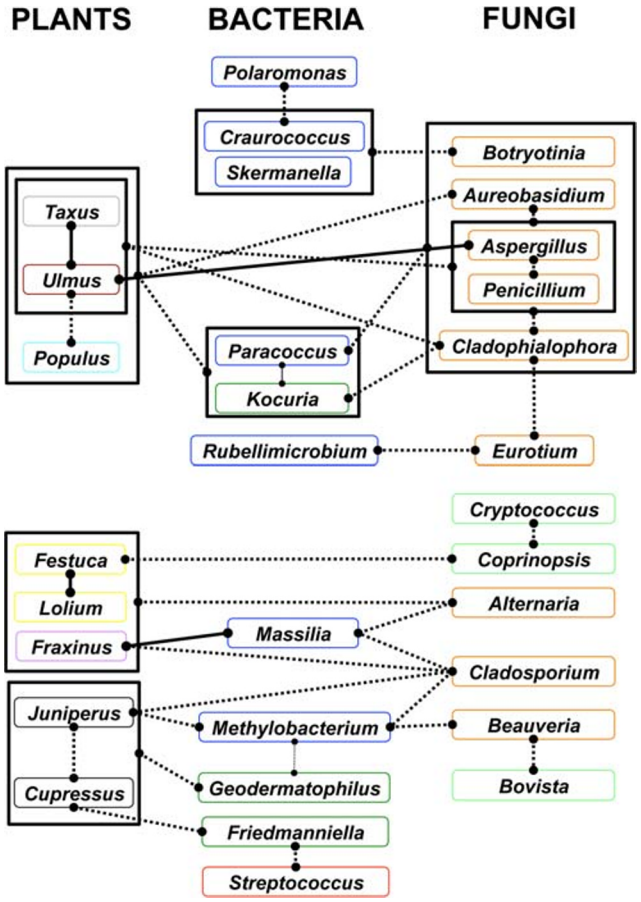


Fig. 4 Representation of the correlation between taxa. Color frames indicate taxonomic groups. Bacteria: (green box) Actinobacteria; (red box) Firmicutes; (blue box) Proteobacteria. Fungi: (orange box) Ascomycota; (green box) Basidiomycota. Plants: (black box) Cupressaceae; (purple box) Oleaceae; (yellow box) Poaceae; (turquoise box) Salicaceae; (gray box) Taxaceae; (brown box) Ulmaceae. Relations connected by dotted lines indicate p values > 0.05 while continuous lines indicate p values < 0.05. Only those with two or more correlations with a *rho* value > 0.7 are shown (see Supplementary Material EMS7 and EMS8 for complete results)

Some recent publications have reported that the combination of two or more allergenic particles like pollen grains with fungal spores or endotoxins can exacerbate the immune response [34, 35, 67]. Accordingly, we observed a positive correlation between grass pollen (*Festuca* spp. and *Lolium* spp., Poaceae) and the ash tree (*Fraxinus* spp., Oleaceae) with the also allergenic fungi *Alternaria* spp. These associations presented low statistical reliability ($p > 0.05$), but they point out a relationship to be confirmed in further studies due to its interest in allergy. On the other hand, the correlations between plant taxa would suggest a similar aerodynamics of the pollen grains or a close source location instead of real interspecific interactions of the individuals.

We also explored the functional diversity of the bacterial communities found in the air (Supplementary Information ESM3). No significant differences were observed between heights. The carbohydrate and amino acid metabolism

pathways and membrane transport were the main functional pathways at any level. Similar results were reported by Wei et al. [68] for airborne bacterial communities.

Conclusions and Final Remarks

Our work examined the concomitant presence of the main airborne biological particles present in an urban environment (bacteria, fungi, and plants) at different heights with DNA-based accuracy. Our results showed that the urban atmosphere is very rich in microscopic biodiversity, which is particularly steady in height for pollen but more fluctuating for fungi and bacteria. The divergences between the different heights analyzed are mainly due to minor representatives, excepting for bacteria, whose non-common taxa may exceed the 40% of the total diversity of one height. We also observed some trends on the distribution of the biological entities, with an increase of different taxa for bacteria and pollen at higher altitudes and the opposite tendency for fungi. Nonetheless, some additional variables besides height may influence the results, so this preliminary study must be confirmed with more extensive works. For instance, a comparative analysis with a building-independent sample to evaluate the contribution from the buildings surfaces would be interesting. Such samples could be collected employing unmanned aerial vehicles (drones) or helium balloons, although law restrictions to use these devices in metropolitan areas can make it difficult to conduct. Although the potential emissions from building walls are inherent to this type of study, to measure their relative contribution to the total diversity is something to consider, even when our study was carried out in a glass windowed building (frequently cleaned) and samples were taken at 1 m from the walls. The influence of the meteorological factors should be also taken into account. Our collection was conducted in a short period of time (≈ 5 h), which would show a minimal effect caused by these variables. However, some previous studies reported even intra-diurnal changes that may alter the patterns of abundance and distribution of the biological particles [69–71], although such patterns are still imprecise. Lighthart and Shaffer [69] reported several changes along a day time, with peaks during morning. Fang and colleagues [70] found the opposite pattern, and recently Gusareva et al. [71] reported changes day a night. Moreover, architecture and building design in the city may create main wind corridors or, contrary, obstructing barriers affecting to the exposure not only to the exposure of $PM_{2.5}$ as suggested by Zhang and colleagues [72] but also to biological particles.

Our results also showed that urban population is continuously exposed to a significant amount of aeroallergens and potentially harmful microorganisms, which would support a continuous monitoring. The correlations of the abundances between the organisms (co-occurrence) were hampered by the limited number of observations. Nonetheless, some of the associations found in our study have been previously described, and more thorough studies as those conducted by Manijaran et al. [73], Fan et al. [74], or Gusareva et al. [71] may set the spot on new bilateral relationships, which is particularly interesting from an ecological point of view as well as public health.

Finally, although this work shows a particular picture in time and place of the urban atmosphere, the observations are interesting enough to conduct further studies about the biological entities present at different scales in the cities in addition to the chemical pollution.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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