Environment International 150 (2021) 106423

Contents lists available at ScienceDirect

Environment International

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

Seasonal changes dominate long-term variability of the urban air microbiome across space and time

Andrés Núñez [a,b], Ana M. García [a], Diego A. Moreno [a,c,*], Raúl Guantes [d,e,*]

[a] Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (ETSII-UPM), Madrid, Spain
[b] Department of Genetics and Microbiology, Facultad de Biología, Universidad de Murcia, Murcia, Spain
[c] Facultad de Farmacia, Universidad de Castilla-La Mancha (FF-UCLM), Albacete, Spain
[d] Department of Condensed Matter Physics and Material Science Institute 'Nicolás Cabrera', Science Faculty, Universidad Autónoma de Madrid, Madrid, Spain
[e] Institute for Condensed Matter Physics (IFIMAC), Science Faculty, Universidad Autónoma de Madrid, Madrid, Spain

ARTICLE INFO

Handling editor: Frederic Coulon

Keywords:
Bioaerosol monitoring
Airborne bacteria and fungi
Metagenomics
Environmental factors
Urban air

ABSTRACT

Compared to soil or aquatic ecosystems, the atmosphere is still an underexplored environment for microbial diversity. In this study, we surveyed the composition, variability and sources of microbes (bacteria and fungi) in the near surface atmosphere of a highly populated area, spanning ~ 4,000 Km^2 around the city center of Madrid (Spain), in different seasonal periods along two years. We found a core of abundant bacterial genera robust across space and time, most of soil origin, while fungi were more sensitive to environmental conditions. Microbial communities showed clear seasonal patterns driven by variability of environmental factors, mainly temperature and accumulated rain, while local sources played a minor role. We also identified taxa in both groups characteristic of seasonal periods, but not of specific sampling sites or plant coverage. The present study suggests that the near surface atmosphere of urban environments contains an ecosystem stable across relatively large spatial and temporal scales, with a rather homogenous composition, modulated by climatic variations. As such, it contributes to our understanding of the long-term changes associated to the human exposome in the air of highly populated areas.

1. Introduction

The composition and dynamics of the microbial diversity present in the atmosphere is still under intensive research and discovery. Bacteria and fungi propagules constitute a significant fraction of this aerobiota, which are aerosolized from different terrestrial and aquatic ecosystems (Fröhlich-Nowoisky et al., 2016). Although the atmospheric conditions may not favor their survival, meteorological factors like rainfall or wind currents are key factors affecting their abundance and prevalence in the air (Burrows et al., 2009; Smets et al., 2016). Moreover, air masses can carry these particles across trans-continental distances before being precipitated (Cáliz et al., 2018; Griffin et al., 2017; Maki et al., 2019; Mayol et al., 2017). During their presence in the atmosphere they play an ecological role by acting like ice nuclei, activating cloud formation and triggering the bioprecipitation (Morris et al., 2014), although a minor part is also withdrawn by dry deposition (Jones et al., 2008). Once deposited, microbial interactions start in the new environment and contribute to the biogeochemical cycles (Falkowski et al., 2008). Some times they can also exert a negative effect by disseminating plant and animal diseases throughout both natural and livestock (Bradford et al., 2013; Fisher et al., 2012). Furthermore, because of their ubiquity, their adaptable metabolism and the large volume of biomass that they represent as a whole, monitoring microorganisms may be crucial in a climate change scenario (Cavicchioli et al., 2019; Smith et al., 2019).

Among all environments, the characterization of these microorganisms in metropolitan areas is attracting much attention because such particles may have harmful consequences on human health. As part of the inhalable fraction, some may trigger allergic reactions, cause pulmonary diseases or aggravate respiratory pathologies (Murray et al., 2018). However, the dynamics and composition of the microbial aerosols within the cities is still unclear due to several factors. Firstly, different land-use can provide diverse sources of microorganisms,

* Corresponding authors at: Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (ETSII-UPM), Madrid, Spain (D.A. Moreno). Department of Condensed Matter Physics and Material Science Institute 'Nicolás Cabrera', Science Faculty, Universidad Autónoma de Madrid, Madrid, Spain (R. Guantes).
E-mail addresses: diego.moreno@upm.es (D.A. Moreno), raul.guantes@uam.es (R. Guantes).

https://doi.org/10.1016/j.envint.2021.106423
Received 1 December 2020; Received in revised form 25 January 2021; Accepted 25 January 2021
Available online 9 February 2021

setting up differences in abundance and diversity between urban locations depending on the degree of urbanization or the particularities of surrounding areas (Bowers et al., 2011a; Mhuireach et al., 2016; Newbound et al., 2010; Stewart et al., 2020). Thus, urban parks provide soil and plant-related niches, in addition to fauna-related microbes, while ponds and fountains are sources of aquatic microbial life (Després et al., 2012). In addition, it is known that environmental changes drive important variations in the airborne communities both at short-term (Fierer et al., 2008; Gusareva et al., 2019; Yan et al., 2018) and associated to seasonal variability (Bowers et al., 2012; Cáliz et al., 2018; Fan et al., 2019; Innocente et al., 2017; Núñez et al., 2019; Tignat-Perrier et al., 2020). Especially relevant are the latter. Since many of the microbial organisms are commensals or saprophytes, they are linked to the life cycle of higher organisms to grow and multiply, which are usually synchronized with seasonal changes, e.g. plant growth and decay. Lastly, atmospheric transport and mixing of air masses as well as extreme atmospheric events (dust intrusions, pollution hazes or storms) may add biological variability across time and space (DeLeon-Rodriguez et al., 2013; Gat et al., 2017; Mazar et al., 2016; Yan et al., 2016; Yoo et al., 2018). Altogether, the potential influence of these many environmental factors may hinder the characterization of the aerobiota in urban environments. Although urbanization has been proposed to homogenize airborne microbiota (Barberán et al., 2015; Docherty et al., 2018) the influence of different environmental factors on prokaryotic and eukaryotic diversity in the urban atmosphere is not properly understood yet.

Previous works on the effect of seasonality in the air microbiome of urban environments have been conducted in one or a few points in the same city (Bowers et al., 2013; Genitsaris et al., 2017; Hiraoka et al., 2017; Innocente et al., 2017; Lee et al., 2017; Stewart et al., 2020). On the other hand, amplicon-based surveys of largely sampled urban areas have been usually restricted to short time periods (Docherty et al., 2018; Li et al., 2019; Mhuireach et al., 2016; Mhuireach et al., 2019). Here, we used targeted amplicon sequencing to simultaneously survey bacterial and fungal communities at 11 different sites scattered throughout a large metropolitan region in Madrid (Spain), across different seasonal periods for two years. The sampling locations are representative of urban scenarios with different degrees of urbanization and population density. Combined with meteorological and air pollution data, the present work provides a comprehensive analysis of the dominant and season specific bacterial and fungal taxa present in the near surface atmosphere of a wide urban area, evaluating the relative contribution of spatial and long-term temporal characteristics and assessing the influence of different environmental and pollution factors.

2. Materials and methods

2.1. Sampling sites characteristics

Eleven sites scattered throughout the Community of Madrid (Spain) (Fig. 1a), were sampled, covering an area of ~ 4,000 Km^2 around the city center and being representative of different urbanization levels. The Community of Madrid is located in the center of the Iberian Peninsula, ca. 320 Km away from any coastal place and 678 m above mean sea level. Total population is estimated in 6,6 million people differently distributed throughout the territory, with over 3 million living in the city center.

Around 16 million journeys are made each day by the population, 43% related to daily commuting (official data from Madrid Regional Transport Consortium, 2018, https://www.crtm.es/). Aerial pictures of the region (Google Earth version 7.3.3; https://www.google.com/earth/download/ge/) were used to examine the percentage of green areas, parks and non-urbanized lots in 1 Km around the sampling point and, accordingly, the sites were classified into "Green" (>7% non-urbanized area: G1-G3), "Parks" (between 3 and 7%, P1-P3) or "Built" (<3%, B1-B5). Thus, G sites (mostly found in the north of the region) are mainly residential areas characterized by large wild zones, clearing areas and large green zones. Sampling points in P sites (eastern regions) are set in urban environments but surrounded by short buildings and some urban parks. B sites (center and southern region) are placed in highly built areas, with scarce or small green areas and busy streets around. General district demography associated to the sampling sites showed that G and P places are less populated (median: 2500 ± 1937 and 2563 ± 2558 inhabitants/km^2, respectively), compared to B sites (median: 4359 ± 2181 inhabitants/km^2) (official data 2018, http://www.madrid.org/iestadis/).

2.2. Sampling methodology and DNA extraction and quantification

Samples at the 11 sites were collected simultaneously using volumetric spore traps (Burkard Manufacturing Co., England, UK), placed rooftop at a height of 15–21 m. Each sample covers a 7-days collection period, corresponding to ~ 100 m^3 of air sampled (at the typical rate of the spore traps, which is 10 L/min). Sampling procedures, DNA extraction (DNeasy PowerSoil Kit, Qiagen®) and quantitation (Quant-iT PicoGreen double-stranded DNA (dsDNA) assay kit (Invitrogen, Molecular Probes) were performed as described previously in (Núñez et al., 2017), obtaining a range of DNA concentration of 0.10–48.90 pg/μl across the samples (median = 4.66 pg/μl; mean = 3.03 pg/μl). A synchronous sample collection was conducted each season along a period of two years (henceforth year A and B), starting Summer 2015 and finishing Spring 2017. A total of 87 samples were collected (1 sample was missing because of a device failure during collection in site B2, Fall.A).

2.3. High-throughput DNA sequencing

High-throughput sequencing analyses were performed using the purified DNA from each sample in a targeted amplicon sequencing (TAS) approach. Hypervariable regions V3-V4 of the 16S rRNA gene of bacteria and region 5.8S – ITS2 of fungi were amplified using the following universal primers attached to adaptors and multiplex identifier sequences: Bakt_341 (F): 5′- CCTACGGGNGGCWG- CAG −3′; Bakt_805 (R): 5′- GACTACHVGGGTATCTAATCC −3′ (Herlemann et al., 2011) for bacteria; and ITS86 (F): 5′- GTGAATCATCGAATCTTTGAA-3′ (Turenne et al., 1999); ITS-4 (R): 5′-TCCTCCGCTTATTGATATGC −3′ (White et al., 1990) for fungi. Purified-amplicon libraries were sequenced in Illumina® MiSeq platform (2 × 300 bp reads) at Madrid Scientific Park (Madrid, Spain), with a minimum sequencing depth of 100,000 reads/amplicon. 1 sample of the 16S library (G3 Winter.B) was discarded because DNA amplification failed, so a total of 86 samples were analyzed for bacteria. DNA from a negative control (sample obtained with the same procedure applied in sample collection but keeping the device off) was also included in the sequencing protocol to discard sample contamination.

2.4. Sequence preprocessing

PANDAseq v2.8 (Masella et al., 2012) was used for assembling paired-ends sequences of the 16S DNA library, filtering by Q-score quality (0.6), trimming the primers sequences and excluding the sequences exceeding the length of the amplicon (min: 400 bp, max: 500 bp). For fungal sequences, the average length of the amplicon (<300 bp) would lead to complete overlap between the reads, so we first employed "read_fastq" from Biopieces v2.0 (http://maasha.github.io/biopieces/) to remove the primer sequence at the end of the amplicon, followed by "fastq-join" (Aronesty, 2013) (https://github.com/brwnj/fastq-join) to pair the sequences. Global processing of the sequences was conducted in Qiime suite environment (Caporaso et al., 2010) (version 1.9.1, http://qiime.org). Chimeras were excluded using USEARCH v8.1 (https://drive5.com/usearch/) in default mode. OTUs clustering and taxonomic assignments were performed with the default algorithm of Qiime (pick_open_reference_otus.py), using UCLUST as method for picking

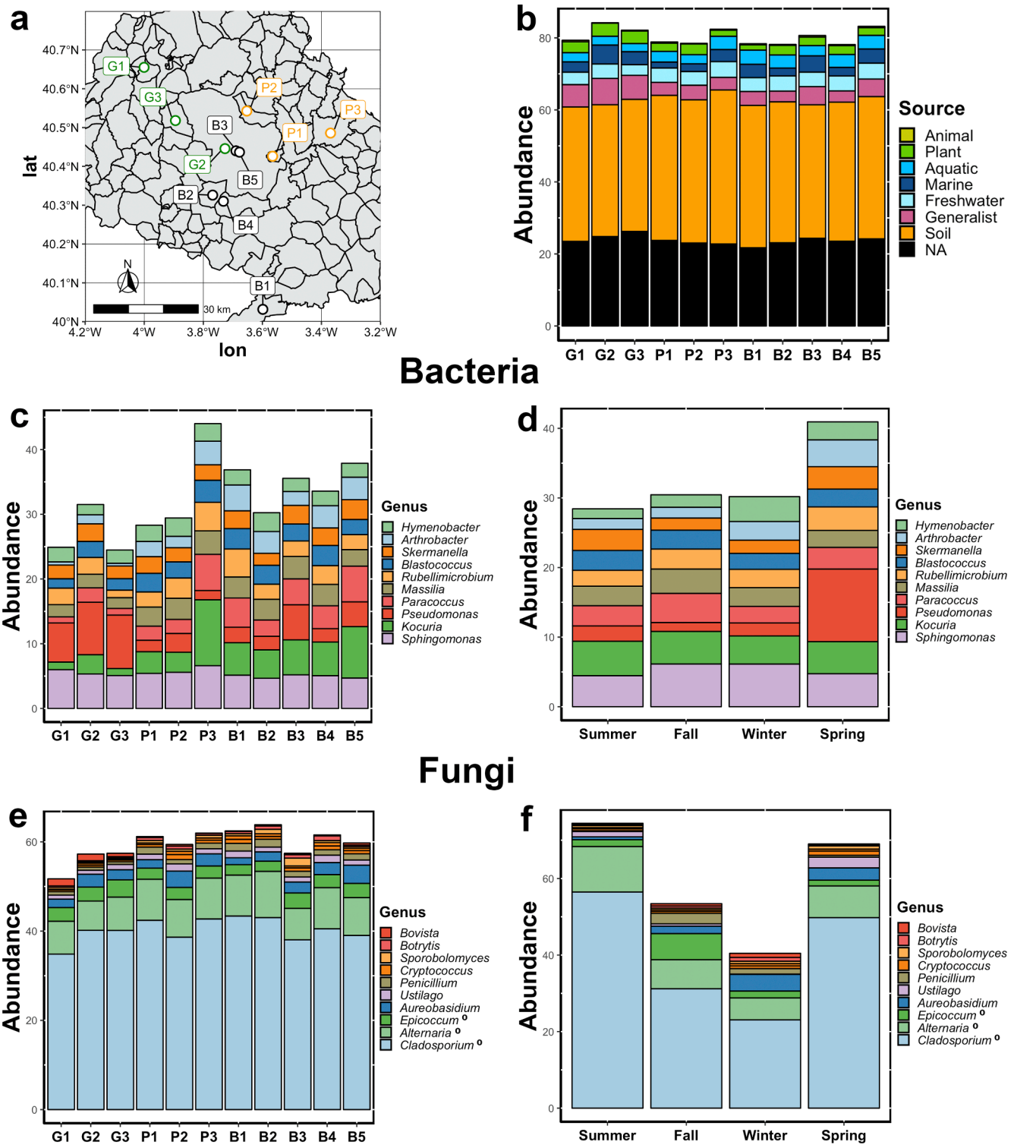

Fig. 1. Sampling points and overview of the airborne microbial composition. Geographical location of the sampling sites (a). Contribution of the different predicted sources of bacterial taxa to the relative abundance across each sampling site (b). Relative abundances of the 10 most abundant bacterial genera by sampling location (c) or season (d). Relative abundances of the Top 10 fungal genera by sampling location (e) or season (f).

OTUs (Edgar, 2010) at 97% similarity cutoff. Silva database (release_132) for bacteria (Quast et al., 2013), and UNITE v7.0 (Kõljalg et al., 2013) for fungi were used for the taxonomic assignments. OTUs assigned to chloroplast, mitochondria, "Unassigned" or that did not reach a defined taxonomic rank at *Phylum* level were filtered out. For bacteria, we conducted an additional manual revision to search for potential contaminations from insect or human-skin microbiota during manipulation, as in (Núñez et al., 2019), identifying a total of 9 OTUs that were removed for further analyses.

2.5. Filtering and normalization

Since spurious OTUs with very low counts may appear due to PCR and sequencing errors (Quince et al., 2011; Weiss et al., 2017), we

estimated a 'noise floor' following the statistical procedure outlined in (Núñez et al., 2019), which resulted in one and two counts for bacteria and fungi, respectively. As a pre-analysis step, we thus removed singletons (OTUs present with an abundance of one count in one sample and zero in the rest) for bacteria, as well as singletons and doubletons for fungi. This procedure eliminated 16,845 OTUs in bacterial samples (30% of the original table) and 3,582 in fungal samples (26%). However, they only represented between 0.3 and 1.5% (bacteria) or 0.2–0.29% (fungi) of the samples relative abundances.

To establish the core of bacterial and fungal genera (taxa, at the *Genus* level, present in at least 95% of all samples) we considered a more restrictive criterion of 'presence' taking into account experimental variability with duplicate and simultaneously running spore traps, as described in (Núñez et al., 2019). This sets a threshold of 0.032% in relative abundance for an OTU to be reliably observed in a given sample, which we used as a criterion for presence/absence.

In all calculations comparing relative abundances (as those shown in Fig. 1 and Figures S3, S4, S9) we corrected for biases due to differences in size between samples using cumulative sum scaling normalization (Paulson et al., 2013), as implemented in the "metagenomeSeq" package.

2.6. Environmental and pathogen annotations

We collected the top 150 OTUs of every sample (covering at least 70% of relative abundance per sample, giving a total of ~ 2,000 OTUs) as representatives of the airborne bacterial community. The predicted sources (Fig. 1b and Figure S1) were assigned using the Seqenv pipeline (Sinclair et al., 2016). Briefly, DNA sequences corresponding to the OTUs are aligned against the NCBI database. The taxonomic information for each sequence is extracted and associated with Environmental Ontology (ENVO) annotations. The annotations of the top 5 matches

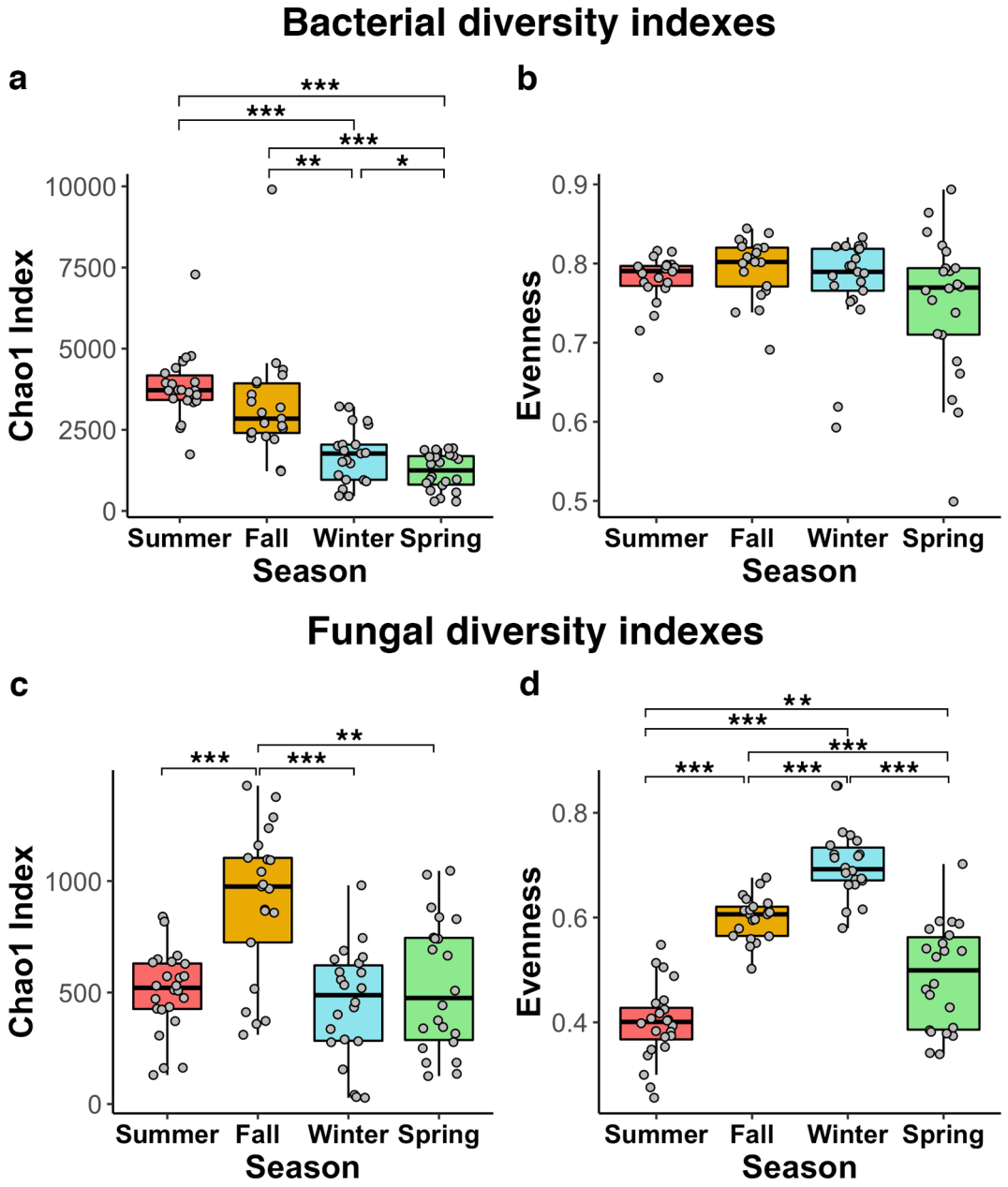

Fig. 2. Alpha-diversity estimators change across seasons. Chao1 index (species richness) and Pielou's index (relative evenness) for airborne bacteria (a and b, respectively) and fungi (c and d, respectively). Welch's-tests were performed to determine statistical differences between seasons and asterisks represent their significance: ***: $P < 0.001$; **: $0.001 < P < 0.01$; *: $0.01 < P < 0.05$.

(>97% similarity) for each sequence were taken and assigned to 7 different habitats: Animal-related (which includes gut- and skin related microbiota), Plant (phyllosphere and rhizosphere-related bacteria), Water (including Freshwater, Marine or Aquatic when the annotation was not specific enough to discern the type of aquatic environment) and Soil (including sediment, sand, sludge, desert, and related expressions). The most frequent habitat of those 5 annotations was selected as the predicted habitat for each sequence submitted, or the term "Generalist" was used when a tie occurred, which is a relatively common situation for bacteria (many of which are usually isolated, for instance, from plant surfaces as well as soil samples). For those sequences without environmental assignation but defined to species level, a manual assignment of the ecological habitat was set based on the related literature. A total of 654 (~33%) bacterial OTUs could not be assigned to any habitat (NA), mainly sequences from culture-independent studies and incomplete taxonomic description (rank Family or higher).

The same pipeline was used to assign most likely habitats to fungal taxa, including a Lichen category (Figure S1). The top 150 OTUs per sample (giving a total of ~ 2,600 OTUs) covered > 90% of the relative abundance in each sample. However, around 54% of fungal OTUs could not be associated to a defined habitat using this procedure.

The ecological guilds for fungal OTUs were assigned using the FUNGuild pipeline (Nguyen et al., 2016) for the whole set of fungal taxa. We gathered the results in the eleven different categories shown in Figure S2, including each taxon into one or several of these categories according to their potential ecological guild. Around 18% of fungal OTUs could not be assigned to any guild.

For the identification of pathogenic bacteria and fungi (Figure S9), we compiled a list of potential human pathogens from references (Abd Aziz et al., 2018; Fan et al., 2019; Kowalski and Bahnfleth, 1998; Liu et al., 2018).

2.7. Richness and evenness estimates

Indexes for estimation of alpha-diversity were calculated based on Hill numbers (effective number of species) (Chao et al., 2014) as implemented in the package 'iNEXT' (Hsieh et al., 2016). For richness (total number of species) we give the asymptotic estimate (Chao1 index), and for evenness (similarity in species relative abundance in a sample) we use Pielou's evenness index (Jost, 2010). Pielou's index varies between 0 and 1, with larger values representing more even distributions in abundance among species. This index is calculated from the asymptotic values of the Hill numbers q = 0 (Chao1 richness) and q = 1 (Shannon diversity, SD) as $\ln(SD)/\ln(Chao1)$.

2.8. Mantel tests

A matrix of spatial distances (in Km) between the different sampling locations was obtained from the latitude and longitude coordinates of each sampling site, using the function 'distm' from package 'geosphere' with geodesic distance. The Mantel test was calculated with function 'mantel' in 'vegan' R package, using Spearman rank correlation and permutation tests (1,000 permutations) for significance.

2.9. Beta-diversity

The Morisita-Horn distance was used as it is an abundance-based measure of similarity dominated by the most abundant species, resistant to under-sampling (rare species have little effect) (Chao et al., 2006). Since composition in our samples is dominated by few relatively abundant and pervasive genera, this distance allows a better visualization of spatiotemporal influences on the similarity of our microbial communities. Principal Coordinate Analysis (PCoA) was applied after rarefaction to minimum sampling depth and Hellinger standardization. Taxa abundances were grouped at the genus level. Contributions to principal coordinates axes were corrected for negative eigenvalues using 'cailliez' method. PERMANOVA tests were performed with 'adonis' function in 'vegan' package, checking first for homogeneity of group variances ('permutest.betadisper' function in 'vegan').

For Fig. 3a,b and Fig. 4, the samples in each location belonging to the same seasonal period were combined (summing up abundances of common OTUs in the two different years). The most abundant genera were correlated to dimensions in principal coordinates space using 'envfit' function in 'vegan' (Fig. 3a,b).

2.10. Indicator species

Species indicators of a given group of samples (e.g. a seasonal period) are characterized by an index (*IndVal*) between 0 and 1, which is the product of two components (Dufrêne and Legendre, 1997): specificity, or abundance of the species in the group relative to its total abundance, and fidelity, or relative frequency of occurrence of the species in samples belonging to the group. *IndVal* indices for all bacterial and fungal genera were obtained with the R package labdsv. Significance was calculated by 10,000 randomizations of groups, followed by Benjamini-Hochberg correction for multiple testing. Only species with *IndVal* values > 0.4/0.5 (for bacteria and fungi respectively) and $P < 0.01$ are shown in Fig. 3.

2.11. Environmental characteristics and data of Madrid area

The Community of Madrid is located in the center of the Iberian Peninsula, flanked by the mountain chain "Sistema Central" (with 2,000 m high peaks) to the north and the plateau "Meseta Central" of the Peninsula to the south. The weather in the region shows features of both semiarid and Mediterranean climates. Winters are mildly cold and not very rainy, while summers are dry and hot.

According to the data provided by the State Agency of Meteorology in Spain (AEMET, "Agencia Estatal de Meteorología", http://aemet.es), normal temperature values in urban areas ranged from 6.0 to 25.6 °C, with an average value across the year of ~ 15 °C (Figure S10). The lowest temperatures are found in Winter, with an average value of 6.5 °C, although normal minimum temperatures range 0.0–2.6 °C, and normal maximum temperatures vary between 10.4 and 12.5 °C. On the other hand, July and August are the warmest months, with mean temperatures over 25 °C, maximum values around 32.5 °C and minimum ones in the range 15.4–18.0 °C.

The climate is slightly dry, with average relative humidity values that fluctuate between 36% (Summer) and 77% (Winter), and a mean relative humidity of 56–58% throughout the year. In correspondence with a Mediterranean weather, the rainy seasons are Fall-early Winter and Spring. The number of days with precipitation over 1 mm is always below 60, compiling ~ 410 mm of total annual precipitation with peaks in October/November and April/May (40–60 mm), and finding the lowest values in July/August (~10 mm). The region of Madrid accumulates annually ~ 100 clear days, with peaks of solar radiation in July.

The average wind speed varies between 2 and 3 m/s throughout the year (Figure S11), being Winter and Spring the seasons with the highest values. NE seems the dominant one for most of the year, mainly coming from central Europe and eastern Mediterranean regions (Gregale). There are significant contributions from W winds in Winter (Ponente, humid current from the Atlantic Ocean), and SSE in Fall (likely originated in dry conditions from the North Africa deserts).

In accordance with this global description of the climate in the region, sampling times coincide with periods that represent typical characteristics of each season (red lines in Figure S10).

With respect to the atmospheric pollution of Madrid area, there is an Air Quality Network with a total of 24 air quality stations distributed across the region and classified in 3 urban areas and 3 rural areas (http://gestiona.madrid.org). All these stations take hourly measures of the main atmospheric pollutants in Madrid area affecting human health (NO_2, O_3, particulate matter < PM_{10}) and some of them also measure

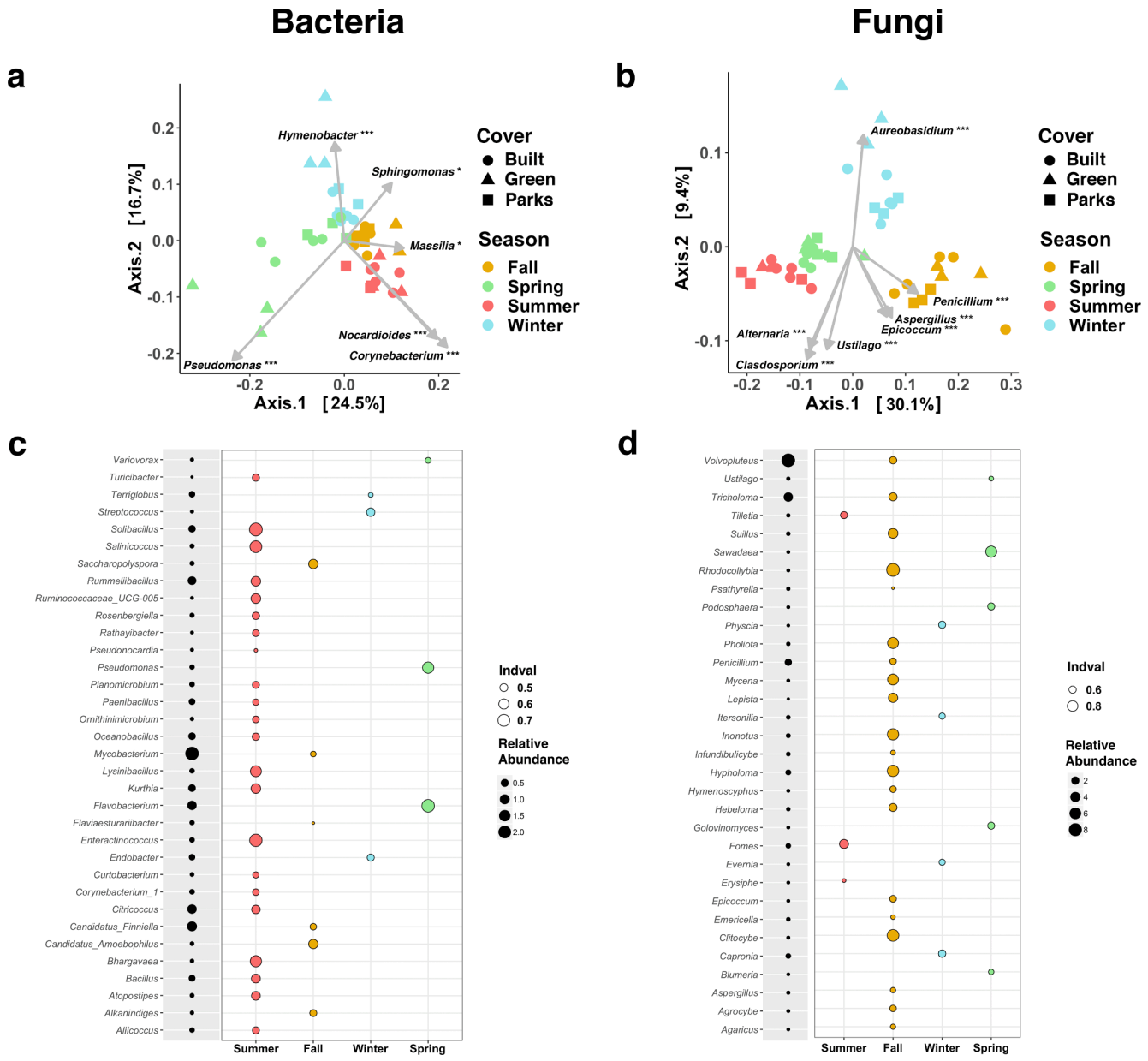

Fig. 3. Seasonal gradients in microbial communities and indicator taxa. Principal Coordinates Analysis of samples grouped by seasonal period for bacteria (a) and fungi (b) using Morishita-Horn distance (*Methods*). The most abundant taxa (at the *genus* level) of each community were correlated to the ordinations, and statistically significant correlations are shown as arrows within the ordination plots. Arrow lengths are proportional to the correlation, and point towards the direction of most rapid change of the explanatory taxa. Asterisks represent significance: ***: $P < 0.001$; **: $0.001 < P < 0.01$; *: $0.01 < P < 0.05$. Bacterial (c) and fungal (d) genera indicator of different seasonal periods. Only genera with an indicator value (*IndVal*) ≥ 0.4 (for bacteria) or ≥ 0.5 (for fungi) are shown, together with their relative abundances in all samples.

levels of other pollutants such as SO_2, CO, benzene, $PM_{2.5}$ or NO. In addition, Madrid central district has its own air quality network with another 24 stations scattered across the city center, providing hourly measures of some contaminants (https://www.madrid.es/portal/site/munimadrid).

Among all the pollutants, NO_2 is the most problematic in the region, especially in the central district with daily heavy traffic conditions (around 80% of this pollutant is originated by road traffic). NO_2 mean values are thus higher in densely urbanized areas compared to locations with parks or vegetation (Figure S12). An annual mean of 40 $\mu g/m^3$ is considered as a risk threshold for human health and this limit is overpassed in downtown sampling sites B3 and B5. The concentration of tropospheric O_3 is over the limits (daily mean of 120 $\mu g/m^3$) only in certain days of Summer, when high temperatures and lack of wind coincides. The values in less urbanized areas tend to be higher than in urban environments (Figure S12), although monthly average concentrations are clearly under the risk limit and daily values never exceeded it during the sampling periods. Concerning particulate matter (PM), its presence in the tropospheric air is due both to human activity (traffic, industrial processes, coal heaters) as well as to natural sources (such as calimas, or dust storms originated in the Sahara Desert). The risk limit of PM_{10} concentration for human health (daily mean of 50 $\mu g/m^3$) was only surpassed during Fall.B and Winter.B periods in four of the eleven locations surveyed. Possible causes were a sporadic biomass combustion event registered in Fall.B and a calima from North Africa affecting Spain central region in Winter.B (Table S3).

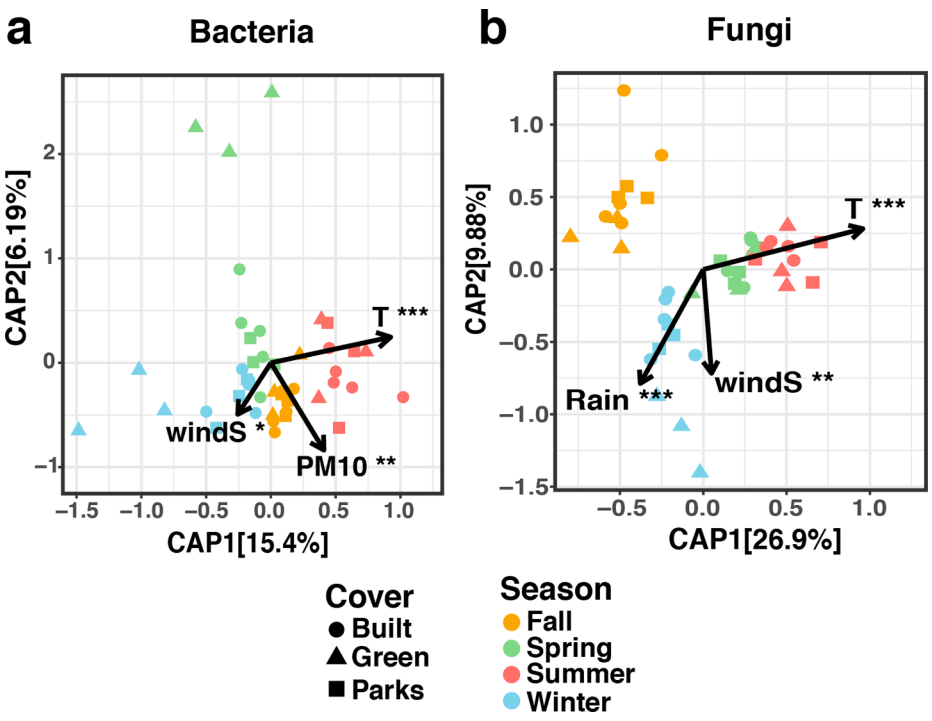

Fig. 4. Environmental factors explain main trends in seasonal changes. Constrained ordinations of samples grouped by seasonal period with environmental factors (*Methods*). Asterisks represent significance of the environmental variables under permutation tests (1,000 permutations): *** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$. The represented variables explain ~ 27% of the sample variance for bacteria and ~ 41% for fungi (adjusted partial variances), with temperature the most explanatory variable for both communities. The ordinations shown correspond to correlation biplots: angles between samples and arrows reflect their correlations. Other statistical information from dbRDA is shown in Table S4.

SO_2 concentrations are always very low in the city compared to the risk limit (daily mean 125 μg/m^3, and hourly mean 350 μg/m^3, see also Figure S12), and it is not considered a significant problem for human health in Madrid.

All meteorological and pollution data used for factor analyses and constrained dbRDA were obtained from above mentioned governmental open access resources (Agencia Estatal de Meteorología, AEMET, http://www.aemet.es/; Air Quality Network of Comunidad de Madrid http://gestiona.madrid.org and Air Quality Network of Madrid central district https://www.madrid.es/portal/site/munimadrid), assigning each sampling site to the nearest meteorological and air quality stations. We collected daily averaged data of temperature, relative humidity, wind speed, and PM_{10}, NO_2 and ozone levels, as well as total amount of precipitation and solar radiation during the sampling periods. These were the environmental factors with available data in all surveyed locations during the sampling periods. The environmental matrix (the set of environmental factors assigned to each sample) contained weekly averages of these environmental factors for each sampling week, with the exception of rain (total amount).

2.12. Analysis of environmental variables

2.12.1 Factor analysis

Exploratory factor analysis was performed using the R package FactoMineR (Lê et al., 2008). Principal component analyses (PCA) were done on the environmental matrix with standardized data,

2.12.2 dbRDA

PCoA ordinations of taxa grouped at *Genus* level, using Hellinger standardization after rarefaction and Morishita-Horn distance, were constrained to the environmental variables using function capscale in vegan package. Variance explained by the different variables was corrected as in (Peres-Neto et al., 2006) (adjusted R^2). Biases due to linear dependencies between explanatory (environmental) variables were assessed calculating variance inflation factors (vif) with function 'vif.cca' in 'vegan'. In addition, we explored environmental variables significantly associated to community variation applying model selection with function 'ordiR2step' in 'vegan'. After these pre-analysis steps, we retained for final analyses variables with values of vif < 3, keeping only unbiased factors with more plausible ecological meaning and stronger associations. In this way, we excluded ozone, solar radiation and relative humidity from further analysis based on strong correlations with temperature, and weaker explanatory power than temperature as assessed by model selection. For constrained dbRDA ordinations in Fig. 4, we only show the variables that were significantly associated to community variation (permutations tests using 'anova.cca' function for 'terms').

3. Results

3.1. Composition and sources of microorganisms

Using spore traps (Núñez et al., 2017), we collected air samples along four seasonal periods during two years. We sampled simultaneously in 11 sites scattered across a wide area within the territorial demarcation of the Community of Madrid, Fig. 1a. The sampling sites include three locations within Madrid city center (G2, B3 and B5) as well as different urban and *peri*-urban scenarios belonging to medium to large population size towns surrounding the central area (*Methods*). A total of 87 samples (7-days period each) were subjected to targeted amplicon DNA sequencing to monitor the bacterial and fungal diversity gathered in every sampling site (*Methods* and Table S1).

We first analyzed the composition and possible sources of the taxa present in our samples. Most of the bacterial taxa were of soil origin (~70% of the total number of taxa with identified origin, Figure S1a), according to the most frequent matches of Environmental Ontology (ENVO) terms (*Methods*), followed by those related to aquatic environments (~14%). These potential sources agree with other studies of airborne bacterial composition in urban and rural environments (Barberán et al., 2015; Bowers et al., 2013; Bowers et al., 2011a; Bowers et al., 2011b; Cáliz et al., 2018; Hiraoka et al., 2017). In contrast to some of these studies, however, we found a quite low proportion of bacteria (~3%) directly associated to plants, likely because of the different approach used to assign the predicted source (*Methods*). The

contribution of different habitats to bacterial relative abundance was rather homogeneous across sampling locations, Fig. 1b. This agrees with other works on airborne communities that found no significant differences between frequency of potential sources among rural and urban areas (Barberán et al., 2015).

Fungal communities were also dominated by taxa of soil origin (~60% of total number of taxa with assigned source, Figure S1b) and to a less extent by fungi frequently associated to plants (~20%). Fungal taxa were also classified into different ecological guilds (*Methods* and Figure S2). Many of the identified taxa (~46%) were saprotrophs, as it is the case for many fungi found in soil. Especially frequent in this group were taxa related to wood decay (Figure S2, 'Wood saprotroph'), with ubiquitous presence of several species of *Peniophoraceae*. Many of the fungal taxa were also potential plant and animal pathogens, with similar frequency distributions across sampling sites, Figure S1.

To provide a comprehensive characterization of bacterial and fungal diversity, we first gathered the abundance of all OTUs belonging to the same genus. This resulted in 1,086 identified bacterial genera (distributed across 27 different phyla) and 570 identified fungal genera (5 phyla). We then investigated the existence of steady airborne communities across space and time, looking for taxa (at the genus level) present in at least 95% of all samples (*Methods*). For bacteria, we found a common core of 26 genera, Table S2. Remarkably, these few common genera constituted a sizable fraction of all the samples (between 31% and 72% of individual sample abundance, ~50% of total prokaryotic abundance). The prokaryotic core is dominated by members of *Actinobacteria* and *Proteobacteria*, which are the most common phyla found in soil(Delgado-Baquerizo et al., 2018), such as *Sphingomonas*, *Kocuria*, *Pseudomonas* and *Paracoccus* Fig. 1c-d and Figure S4a-b. These genera can be also found within the most abundant taxa in other studies on very different urban areas (Li et al., 2019; Polymenakou et al., 2020; Serrano-Silva and Calderón-Ezquerro, 2018; Yan et al., 2018).

The dominant genera were distributed in similar proportions across sites (Fig. 1c) and seasonal periods (Fig. 1d) with the exception of *Pseudomonas*, whose presence is remarkably higher in Spring at the less urbanized sampling locations (G1-G3). This increase is however observed only during the first spring period ($P < 0.05$, Welch's test), Figure S3b, pointing out to characteristic environmental conditions favoring the outbreak of some *Pseudomonas* species. In particular, the accumulated precipitation during the two weeks previous to this sampling period was much higher in these locations. Cloud formation can be triggered by ice nucleation activity proteins present in several species of *Pseudomonas*. Thus, these bacteria can be deposited on the earth surface and increase their local abundance rapidly (Failor et al., 2017). In addition, many *Pseudomonas* species are saprophytes and plant pathogens, which would favor their rise in sites with abundant plant covering.

The fungal community was dominated by *Ascomycota,* and to a much less extent by *Basidiomycota* (Figure S4c-d). We identified only 4 core genera (*Cladosporium, Alternaria, Epicoccum* and *Eurotium*, all assigned to *Ascomycota*) that made up a noticeable but very variable fraction of the eukaryotic community across all the samples (between ~ 4% and 86% of individual sample abundance, and ~ 54% of total fungal abundance). This inter-sample variability of the core taxa suggests that fungal airborne communities are more sensitive to local sources or environmental factors than prokaryotic communities. A possible explanation is that most of the prevalent fungal taxa found in our samples are soil and plant saprotrophs feeding from debris of dead plants, whose presence may be largely influenced by the climatic season and the availability of nearby sources. As for bacteria, we collected the 10 most abundant genera across all samples and calculated their distribution by sampling sites, Fig. 1e, or seasonal period, Fig. 1f. Apart from the above mentioned core genera, other fungal genera such as *Penicillium* or *Sporobolomyces* are highly prevalent (>90% of the samples). While different sites show a rather homogeneous distribution of the main fungal taxa, seasonal periods display significant differences, with a smaller contribution of these genera in Fall/Winter samples compared to the Spring/Summer periods (Welch's test, $P < 0.001$).

3.2. Seasonal features modulate microbial diversity

The microbiome in the near surface atmosphere can be influenced by changes both from nearby sources and from environmental factors (Bowers et al., 2011b; Fierer et al., 2008; Fröhlich-Nowoisky et al., 2016; Jones and Harrison, 2004; Mhuireach et al., 2019; Tanaka et al., 2019). As a first characterization of diversity across space and time, we estimated two alpha-diversity indicators for each sample: number of taxa (richness, Chao 1 index (Gotelli and Chao, 2013)) and similarity in species relative abundance (evenness, Pielou's index (Jost, 2010)) (*Methods*). We then grouped these indicators by samples belonging to the same location or seasonal period.

For each location, richness exhibited a large variability depending on the sampling period, Figure S5, which prevents to detect significant differences among sites. In contrast, gathering samples by seasonal period revealed different trends among seasons. For bacteria, Spring/Winter periods are characterized by significantly lower richness than Fall/Summer samples, Fig. 2a. Evenness estimates are relatively high in all seasonal periods (Fig. 2b), consistent with the presence of a core of highly abundant taxa varying across seasons. Summer samples differed from each other less than those collected in other seasons, in agreement with other works in urban areas (Bertolini et al., 2013), which hints to a strong effect of temperature on bacterial community composition. Seasonal variations in the number and abundance of airborne bacteria have been observed in previous studies, with a larger abundance during Summer periods in many of them (Be et al., 2015; Bertolini et al., 2013; Bowers et al., 2012; Bowers et al., 2011b; Genitsaris et al., 2017). Other studies, however, reported a higher bacterial diversity in different seasons (Cáliz et al., 2018; Du et al., 2018; Lee et al., 2017) pointing to an influence of multiple environmental factors combined with the climatic characteristics of the region.

Fungal communities show a significant increase in richness during Fall, Fig. 2c. In contrast to bacteria, all seasonal periods exhibited marked differences in evenness, Fig. 2d, with the more dissimilar communities corresponding to the Summer periods. This agrees with the results of previous surveys in the Iberian Peninsula using microscopy techniques that showed an increase in abundance and types of fungal spores during Summer and, especially, Fall seasons (Díez-Herrero et al., 2006; Oliveira et al., 2009; Sánchez-Reyes et al., 2016).

Analyzing seasonal diversity in different years, similar general trends are observed (Figure S6). However, some inter-annual variability is also apparent. In bacteria, richness is significantly different between the Spring periods of both years, Figure S6a (likely associated to a dust event from North Africa, Table S3). Fungal communities show significant inter-annual differences in richness in Fall, Winter and Spring samples (Figure S6c).

We next studied spatial and temporal variation in species composition between samples (beta-diversity). We first investigated the possibility of spatial correlation in our data (if closer locations contain more similar microbial communities) using Mantel tests separately for each sampling period (*Methods*). No significant correlations were found among beta-diversity and site geographical distances for the sampling periods analyzed. In addition, we used Mantel correlograms and distance-based Moran eigenvalue maps to check that no significant spatial structure is present in the microbial communities sampled. Then, we applied principal coordinate analysis (PCoA), as described in *Methods*, to visualize gradients in our samples. When taxa were grouped by seasonal period, we observed a clear separation by season in both bacterial and fungal samples, Fig. 3a,b [$R = 0.5$ and 0.8 for bacteria and fungi, respectively, $P < 0.001$, permutational analysis of variance (PERMANOVA)]. In contrast, grouping by sampling location did not show a significant influence (using PERMANOVA tests). Seasonal patterns were still distinguishable and significant irrespectively of the year (Figure S7), albeit with smaller contributions to total variance ($R = 0.29$

and 0.32 for bacteria and fungi, respectively, $P < 0.001$, PERMANOVA).

To find which taxa are mainly responsible for the observed seasonal gradients, we fitted the most abundant genera to the ordinations. Taxa with most significant correlations with sample ordinations are shown as correlation arrows in Fig. 3a, b. In analogy with the results for alpha-diversity (richness and evenness), the Winter and Spring samples were significantly different from the Fall/Summer samples in the composition of bacterial communities. The differential abundance of pervasive genera accounted for the main gradients, such as the increase in abundance of *Pseudomonas* during Spring. The gradient along the second main component in Winter samples is due in part to the increased presence of *Hymenobacter*, whose species are known to be well adapted to extreme temperature and desiccation conditions. Summer samples are especially enriched in some genera of *Actinobacteria*, such as *Nocardioides* and *Corynebacterium*, whose environmental species are frequently found in soil environments and usually resistant to the high irradiation, temperature and dryness characteristic of this season.

Fungal taxa show even stronger seasonal trends, where species belonging to the most abundant genera (*Cladosporium/Davidiella, Alternaria*, *Aureobasidium* and *Penicillium*) fluctuate in abundance following seasonal environmental changes, Fig. 3b. In addition to seasonal gradients, the fungal communities show a clear separation by year of sampling, Figure S7 [$R = 0.35$, $P < 0.001$, PERMANOVA], suggesting a higher sensitivity to particular environmental conditions. A consistent difference between both years was observed in the total amount of rain during Fall and Winter sampling periods, being significantly larger during the second year in most locations (Figure S13). This environmental factor is indeed strongly correlated to the separation of both years along the two main coordinates in PCoA (Figure S7).

3.3. Indicator taxa

Despite main gradients in ordinations are due to seasonal variations of most abundant taxa (corresponding to core genera in bacterial samples), we also investigated the presence of taxa characteristic of specific seasons, using indicator species values (*Methods*). Several microbial genera were identified as indicators for each season (Fig. 3c,d), while there were no indicators for type of site (G, P or B) or specific locations ($P > 0.1$ with Benjamini-Hochberg correction for multiple testing). Summer presented the highest number of indicators for bacteria, and Fall for fungi, in correspondence with the seasons showing the highest species richness for each community. With the exception of *Rosenbergiella* and *Succinivibrio*, Gram-positive actinobacteria were predominant indicators of the Summer season, likely due to their resistance to dry conditions. The abundance of *Streptococcus,* also Gram-positive bacteria related to the human microbiome, increases notably in Winter. In addition, two genera with acidic soil-related members, *Terriglobus* and *Endobacter*, were identified as characteristic of the Winter season.

Several fungi of the phylum *Basidiomycota* were associated with Summer season such as *Tilletia* spp., a pathogen of several species of grasses, and *Fomes* spp., a wood-decay fungi. The *Ascomycota Erysiphe* spp., obligate parasite of leaves and fruits, was also characteristic of the warm season. Most of the genera identified as indicator for the Spring period belong to the order *Erysiphales*: *Blumeria*, *Golovinomyces, Podosphaera* and *Sawadaea,* that cause the powdery mildew on plants and trees during the growing season favored by humidity and moderate temperatures.

In contrast to the bacterial community, which did not show specific genera indicative of the year of sampling, up to 20 fungal genera appeared as indicator taxa of the sampling year with *indval* values > 0.5 (Figure S8). They were mostly plant leaves colonizers and pathogens. Of note, some abundant genera, Fig. 1e-f, with potentially harmful representatives for humans like *Aureobasidium*, *Cryptococcus* or *Epicoccum* were selected as fungal markers for the first year of sampling, in accordance with their predominant presence during this year (99%, 89% and 93% respectively). Some habitants of angiosperms surfaces, such as *Botrytis* spp. and *Stemphylium* spp. were almost exclusively present during the second sampling year.

3.4. Human pathogens and aeroallergens

Pathogenic microorganisms are frequently found in air microbiome studies (Abd Aziz et al., 2018; Fan et al., 2019; Kowalski and Bahnfleth, 1998; Liu et al., 2018). In our survey, we found a small fraction of potentially pathogenic bacteria (average 12% *per* sample, Figure S9). Some of the most abundant genera with pathogenic taxa (*Pseudomonas, Geodermatophilus, Staphylococcus, Roseomonas, Acinetobacter* and *Clostridium*), are also included in the bacterial core (prevalence > 95%), while other abundant pathogenic genera such as *Streptococcus* and *Bacillus* are also highly prevalent (>90% of the samples). The most abundant *Streptococcus* species found in our samples, *Streptococcus gallolyticus*, is an opportunistic pathogen causing septicemia and endocarditis, and also associated to colorectal cancer (Pasquereau-Kotula et al., 2018). Likewise, *Acinetobacter baumannii* and *Acinetobacter lwoffii,* two documented human pathogens found in health care units (the former being listed by the WHO as a critical antibiotic resistant microorganism), are the dominant species of this genus in our survey.

Some of the relatively abundant pathogens identified show a clear seasonal influence, as it is the case with *Thermoactinomyces vulgaris*, associated to pneumonia and peaking in Fall. Especially abundant in Fall are also *Serratia plymuthica* and *Serratia marcescens*, both causing opportunistic infections. With lower abundance, we identified DNA of *Pseudomonas aeruginosa* and *Pseudomonas pseudoalcaligenes,* cause of widespread infections in hospitals, with almost exclusive presence in Spring and Winter, respectively.

Occasionally, potential enteropathogens like *Campylobacter jejuni*, *Enterobacter cloacae* or *Escherichia coli* were also found, but their presence was detected in very few samples and with low abundance. This is also the case with representatives of the genus *Legionella*, responsible for the Legionaries' disease and Pontiac fever (Sánchez-Parra et al., 2019).

Regarding fungal taxa, because of their life style, around a third of the total sequences was associated to plant or animal pathogens, with the core genera *Cladosporium*, *Alternaria*, and *Epicoccum* among the most relevant for human health as cause of different allergy symptoms. The most abundant allergenic species identified across samples were *Cladosporium herbarum* (*Davidiella tassiana*), *Epicoccum nigrum*, *Aureobasidium pullulans*, *Alternaria tenuissima* and *Alternaria alternata.* These allergens showed an almost exclusive presence only in one of the sampling years (year A for the first four species, and year B for *Alternaria alternata*). Other fungal pathogens showed also high inter-annual variability, as it is the case of some *Penicillium* spp. (*Penicillium digitatum*, *P. expansum*, *P. chrysogenum*) causing keratitis and mycosis, only detected during the first sampling year, and *Fusarium proliferatum* detected only during the second year in Fall samples. Other prevalent pathogenic taxa showed a marked seasonal variability, such as *Aspergillus niger* and *Aspergillus fumigatus*, causing pulmonary infections, which were especially abundant during Fall. As with bacteria, some pathogens were detected very occasionally in our samples, as it is the case of *Cryptococcus neoformans* and *Cryptococcus albidus.*

3.5. Influence of environmental variables on airborne microbial communities

The seasonal patterns apparent in the community composition of airborne microorganisms are likely driven by long-term changes in environmental factors. We collected a common set of meteorological and pollution data taken from meteorological and air quality stations close to the different sampling sites (Section 2.11 in *Methods*). These data included daily values of air temperature, amount of rain, relative humidity, solar radiation and wind speed, as well as daily levels of particulate matter ($<PM_{10}$), NO_2 and O_3. Values of environmental

variables across the different sampling periods are summarized in Figure S13, showing clear seasonal trends but with some differences among years, particularly in the amount of rain and PM_{10} during Fall and Winter periods.

We first investigated the structure of the environmental variables using factor analysis (*Methods*). Samples were clearly grouped by seasonal period in the principal components of the environmental matrix (Figure S14a), with temperature, relative humidity, solar radiation and ozone levels explaining the scatter of samples along the first dimension, while values on the second component were mainly influenced by the amount of rain and PM_{10} levels. The third dimension, which also contributed noticeably to the amount of explained variance, was almost exclusively determined by wind speed. The environmental variables were not all independent. Regarding pollutants, we observed a strong positive correlation of ozone levels with temperature and solar radiation, and a negative correlation between ozone and NO_2, Figure S14b. These correlations are well documented and likely due to the chemistry of ozone production and destruction (Coates et al., 2016). With respect to meteorological variables, temperature and relative humidity showed also a significant negative correlation (Figure S14b), as expected from the climatic conditions of Madrid, with a mixture of cold semiarid and Mediterranean characteristics. In addition, and due to atmospheric transmittance, solar radiation is highly correlated with air temperature and relative humidity.

To assess the influence of different environmental variables on seasonal changes in community composition, we regressed the species matrix on the environmental factors using distance-based redundancy analysis (db-RDA) (Borcard et al., 2018; Legendre and Anderson, 1999). Explanatory variables were selected based on possible collinearities among factors and significant associations by model selection (Section 2.12.2 in *Methods*).

Seasonal trends in bacterial diversity are most significantly explained by the temperature gradient (Fig. 4a), followed by amount of PM_{10} and average wind speed. While total rain fallen during the sampling periods was not selected as one of the main explanatory variables, it may show an influence on the composition of the Winter communities in the 'green' locations, where especially G1 and G3 sites registered an elevated amount of precipitation during this season.

Temperature was also the dominant environmental factor explaining seasonal variations in fungal communities. Unlike bacteria, these communities seem to be also especially sensitive to rain levels (Fig. 4b), while wind speed explains the trend of Winter samples, in a similar way as in bacteria. Of note, the main representative fungal genus in our samples, *Cladosporium*, has been found to be positively influenced by temperature (Katial et al., 1997; Oliveira et al., 2009; Peternel et al., 2004), in agreement with its more abundant presence in Summer and Spring samples (Fig. 1f).

Regarding chemical contaminants, NO_2 levels were not significantly associated to changes in composition of bacterial or fungal communities.

Solar radiation, ozone levels and relative humidity were ruled out from final analyses due to their strong correlations with temperature. In addition, relative humidity showed either no significant (for bacteria) or weak (for fungi) associations with community variation as assessed by model selection. Solar radiation and ozone levels were statistically associated to compositional variations, but their impact on the concentration of airborne microorganisms is less clear than that of temperature. On one hand, there is no evidence for a direct influence of ozone levels on bacteria or fungi at the concentrations present in the near surface atmosphere of Madrid (maximum of $\sim 100\ \mu g/m^3$ in some Summer samples, Figure S13) (Sousa et al., 2008; Ueda et al., 2016). On the other hand, although solar radiation can influence the viability of bacteria in the air, its effect on bioaerosol concentrations is difficult to assess independently of temperature and relative humidity. Several studies in fungi pointed that spore release could be favored by increasing solar radiation incident on leave or soil surfaces, which acts by reducing surface moisture and promoting release (Jones and Harrison, 2004). Solar radiation can also have also an impact on short-term variability of airborne bacteria, such as in diurnal cycles (Lighthart, 1997). A recent extensive study of diel variability of microorganisms (bacteria and fungi) in the air (Gusareva et al., 2019) showed that temperature had the strongest effect on diurnal cycles.

4. Discussion

The presence of a common abundant core of bacterial genera modulated by environmental factors resembles findings in very different ecological niches, such as the soil (Delgado-Baquerizo et al., 2018), marine (Fuhrman et al., 2015) and gut microbiomes (Falony et al., 2016; Zhernakova et al., 2016). The bacterial core found in the near atmosphere of Madrid metropolitan area conforms a rich microbiome, stable across different spatial and temporal changes. Many of these core genera have been identified in other culture dependent and sequencing surveys in the air of different cities (Table S5), suggesting that urban environments constitute an ecosystem with many similarities around the globe. *Sphingomonas*, *Corynebacterium*, *Nocardioides*, *Clostridium*, *Kocuria* and *Paracoccus* are usually among the most abundant genera across studies, with changes in relative abundances that could be caused by the different biases in sampling methods and the specific features of the urban areas surveyed. While the vast majority of the bacterial taxa found in our samples are of soil origin, as expected in the near surface urban air, other long time series of airborne diversity, as in high elevations (Cáliz et al., 2018; DeLeon-Rodriguez et al., 2013), tropical (Gusareva et al., 2019) or rural environments (Bowers et al., 2013) show a greater diversity of habitat.

The fungal community was less diverse and dominated by a few genera of *Ascomycota*, corresponding to taxa commonly found in soil that dominate across different ecosystems and geographies (Egidi et al., 2019; Tedersoo et al., 2014). These genera are also ubiquitous in the atmosphere, and have been found in places with different environmental conditions and urbanization levels (Barberán et al., 2015; Grinn-Gofron and Bosiacka, 2015; Oliveira et al., 2009; Tanaka et al., 2019; Woo et al., 2018), Table S6. The likely reasons for the global prevalence of these taxa are their wind dispersal abilities, but also their flexible trophic capabilities and the higher potential for resource utilization (Egidi et al., 2019), as it is the case with members of *Alternaria*, which are potential opportunistic plant pathogens, or *Cladosporium*, also common inhabitants of organic debris.

Evaluating the proportion of variance in beta-diversity explained by location, land coverage, seasonal period and year of sampling, we clearly found a large contribution of seasonal factors driving variations in community composition across relatively large spatiotemporal scales. These seasonal shifts of airborne microbes have been observed in other studies with different sampling methodologies and environments (Bowers et al., 2013; Bowers et al., 2012; Cáliz et al., 2018; Franzetti et al., 2011; Hiraoka et al., 2017; Innocente et al., 2017; Lee et al., 2017; Tignat-Perrier et al., 2020; Zhong et al., 2016). Seasonal variations are mainly manifested as changes in the abundance of pervasive and most representative taxa, some of which are especially adapted to particular climatic conditions. This is the case of the bacterial genus *Hymenobacter*, whose species are found in cold ecosystems and show a strong signal in Winter. In addition, we found some taxa unequivocally associated to particular seasonal periods, especially to Summer for bacteria and Fall for fungi. In contrast to seasonal indicators, we did not find taxa significantly associated to sampling sites or land coverage. This may seem unexpected, since other studies have found that local sources and plant cover may play important roles (Bowers et al., 2011a; Mhuireach et al., 2016; Mhuireach et al., 2019; Stewart et al., 2020). We notice that our samples represent the accumulated microbial diversity along a week, which could hinder the clear identification of local representative taxa as well as of short-term variability in community composition (Bertolini et al., 2013; Fierer et al., 2008; Gusareva et al., 2019). On the other hand, our study supports the idea that the atmospheric

microbiome is quickly homogenized and redistributed among relatively distant areas.

In addition to seasonal changes, we also found a noticeable inter-annual variability among airborne fungi, with some prevalent taxa preferentially present in one of the sampling years. This could be partly attributable to inter-annual differences in meteorological conditions, particularly precipitation levels (Shi et al., 2020), and hints to a greater sensitivity of fungi to climatic drivers (Tedersoo et al., 2014; Vetrovsky et al., 2019).

The seasonal patterns in airborne community composition can be explained in part by seasonal variations in environmental factors. Temperature had a strong effect on both fungal and bacterial diversity, followed by average precipitation levels for fungal communities. Global studies of soil fungal communities have shown that temperature and precipitation explain main variations in worldwide fungal diversity (Bahram et al., 2018; Tedersoo et al., 2014; Vetrovsky et al., 2019; Zhou et al., 2016), with stronger contribution than soil features. Thus, it is reasonable to expect seasonal variations of these two factors to be tightly linked to seasonal changes in the composition of airborne fungi. These variables can influence in different ways fungal bioaerosols: temperature can directly accelerate metabolic rates favoring organism multiplication (Brown et al., 2004; Zhou et al., 2016) and also contribute to physical detachment of fungi from soil and plant surfaces (Jones and Harrison, 2004). Likewise, precipitation can play different roles both altering the structure of the soil and plant communities (Shi et al., 2020), the main source of airborne fungi, as well as influencing their dispersion by promoting production of conidia or spore release (Jones and Harrison, 2004).

As for airborne bacteria, several works have reported an increase in total bacterial numbers during warm seasons (Genitsaris et al., 2017; Harrison et al., 2005) consistent with the effect of air temperature on growth rates (Harrison et al., 2005; Zhou et al., 2016). This could explain the high richness and low inter-sample variability found during the summer periods. In contrast to fungi, total particulate matter (PM_{10}) is found to be significantly associated to seasonal changes in bacteria, and likely influences both Summer and Fall communities. Due to their smaller sizes, bacteria can easily attach to fine inorganic particles and be transported jointly. In fact, previous studies have found correlations between total particulate matter and the amount and diversity of airborne bacteria (Du et al., 2018; Hara and Zhang, 2012).

5. Conclusions

In summary, our study provides evidence that the urban air microbiome is dominated by a few cosmopolitan taxa frequently found in soil, with a more homogenous composition than the airborne microbiome of rural or pristine environments, or at high altitudes. This urban community is likely assembled by emission and dispersal from nearby sources, and homogenized by typical transport processes in the boundary layer of the atmosphere. While particularities of the local sources, such as plant coverage or differences in human activity, can have an impact on short-term and spatial variability, we find that most of long-term variability is associated to seasonal and climatic changes. The present work thus contributes to our knowledge of the human exposome in metropolitan areas and to the environmental drivers responsible for its variation.

6. Availability of data and material

The datasets generated and/or analysed during the current study are available in the National Center for Biotechnology Information (NCBI), Sequence Read Archive (SRA), under the accession number PRJNA664957.

Funding

This work was funded in part by the Community of Madrid (Spain) under the Programs AIRBIOTA-CM (S2013/MAE-2874) and AIRTEC-CM (S2018/EMT4329). The funding agency played no role in the design of the study and collection, analysis, interpretation of data or in writing the manuscript.

Author contributions

A.M.G, D.A.M. and R.G. conceived the study; A.N., A.M.G. and D.A.M designed experiments; A.N. collected and processed air samples; A.N. and R.G. analyzed data; A.N. and R.G. wrote the manuscript, with input from all the co-authors.

CRediT authorship contribution statement

Andrés Núñez: Investigation, Methodology, Data curation, Formal analysis, Writing - original draft. **Ana M. García:** Conceptualization, Methodology, Funding acquisition, Writing - review & editing. **Diego A. Moreno:** Conceptualization, Supervision, Methodology, Project administration, Funding acquisition, Writing - review & editing. **Raúl Guantes:** Conceptualization, Supervision, Methodology, Formal analysis, Funding acquisition, Writing - original draft.

Declaration of Competing Interest

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.

Acknowledgements

We thank Dr. A.M. Guitiérrez-Bustillo and the palynological network of Madrid PALINOCAM for their collaboration during the collection of samples for this study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106423.

References

Abd Aziz, A., Lee, K., Park, B., Park, H., Park, K., Choi, I.-G., et al., 2018. Comparative study of the airborne microbial communities and their functional composition in fine particulate matter (PM2.5) under non-extreme and extreme PM2.5 conditions. Atmos. Environ. 194, 82–92. https://doi.org/10.1016/j.atmosenv.2018.09.027.

Aronesty, E., 2013. Comparison of Sequencing Utility Programs. Open Bioinform. J. 7, 1–8. https://doi.org/10.2174/1875036201307010001.

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., et al., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233–237. https://doi.org/10.1038/s41586-018-0386-6.

Barberán, A., Ladau, J., Leff, J.W., Pollard, K.S., Menninger, H.L., Dunn, R.R., et al., 2015. Continental-scale distributions of dust-associated bacteria and fungi. Proc. Natl. Acad. Sci. USA 112, 5756–5761. https://doi.org/10.1073/pnas.1420815112.

Be, N.A., Thissen, J.B., Fofanov, V.Y., Allen, J.E., Rojas, M., Golovko, G., et al., 2015. Metagenomic Analysis of the Airborne Environment in Urban Spaces. Microb. Ecol. 69, 346–355. https://doi.org/10.1007/s00248-014-0517-z.

Bertolini, V., Gandolfi, I., Ambrosini, R., Bestetti, G., Innocente, E., Rampazzo, G., et al., 2013. Temporal variability and effect of environmental variables on airborne bacterial communities in an urban area of Northern Italy. Appl. Microbiol. Biotechnol. 97, 6561–6570. https://doi.org/10.1007/s00253-012-4450-0.

Borcard, D., Gillet, F., Legendre, P., 2018. Numerical ecology with R, 2nd Edition. Springer, Switzerland.

Bowers, R.M., Clements, N., Emerson, J.B., Wiedinmyer, C., Hannigan, M.P., Fierer, N., 2013. Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. Environ. Sci. Technol. 47, 12097–12106. https://doi.org/10.1021/es402970s.

Bowers, R.M., McCubbin, I.B., Hallar, A.G., Fierer, N., 2012. Seasonal variability in airborne bacterial communities at a high-elevation site. Atmos. Environ. 50, 41–49. https://doi.org/10.1016/j.atmosenv.2012.01.005.

Bowers, R.M., McLetchie, S., Knight, R., Fierer, N., 2011a. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. ISME J. 5, 601–612.
Bowers, R.M., Sullivan, A.P., Costello, E.K., Collett Jr., J.L., Knight, R., Fierer, N., 2011b. Sources of bacteria in outdoor air across cities in the midwestern United States. Appl. Environ. Microbiol. 77, 6350–6356.
Bradford, S.A., Morales, V.L., Zhang, W., Harvey, R.W., Packman, A.I., Mohanram, A., et al., 2013. Transport and Fate of Microbial Pathogens in Agricultural Settings. Crit. Rev. Environ. Sci. Technol. 43, 775–893. https://doi.org/10.1080/10643389.2012.710449.
Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789. https://doi.org/10.1890/03-9000.
Burrows, S.M., Elbert, W., Lawrence, M.G., Pöschl, U., 2009. Bacteria in the global atmosphere – Part 1: Review and synthesis of literature data for different ecosystems. Atmos. Chem. Phys. 9, 9263–9280. https://doi.org/10.5194/acp-9-9263-2009.
Cáliz, J., Triado-Margarit, X., Camarero, L., Casamayor, E.O., 2018. A long-term survey unveils strong seasonal patterns in the airborne microbiome coupled to general and regional atmospheric circulations. Proc. Natl. Acad. Sci. USA 115, 12229–12234. https://doi.org/10.1073/pnas.1812826115.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303.
Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., et al., 2019. Scientists' warning to humanity: microorganisms and climate change. Nat. Rev. Microbiol. 17, 569–586. https://doi.org/10.1038/s41579-019-0222-5.
Chao, A., Chazdon, R.L., Colwell, R.K., Shen, T.J., 2006. Abundance-based similarity indices and their estimation when there are unseen species in samples. Biometrics 62, 361–371. https://doi.org/10.1111/j.1541-0420.2005.00489.x.
Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., et al., 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecol. Monogr. 84, 45–67. https://doi.org/10.1890/13-0133.1.
Coates, J., Mar, K.A., Ojha, N., Butler, T.M., 2016. The influence of temperature on ozone production under varying NOx conditions – a modelling study. Atmos. Chem. Phys. 16, 11601–11615. https://doi.org/10.5194/acp-16-11601-2016.
DeLeon-Rodriguez, N., Lathem, T.L., Rodriguez, R.L., Barazesh, J.M., Anderson, B.E., Beyersdorf, A.J., et al., 2013. Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. Proc. Natl. Acad. Sci. USA 110, 2575–2580. https://doi.org/10.1073/pnas.1212089110.
Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-Gonzalez, A., Eldridge, D. J., Bardgett, R.D., et al., 2018. A global atlas of the dominant bacteria found in soil. Science 359, 320–325. https://doi.org/10.1126/science.aap9516.
Després, V.R., Huffman, J.A., Burrows, S.M., Hoose, C., Safatov, A.S., Buryak, G., et al., 2012. Primary biological aerosol particles in the atmosphere: a review. Tellus B: Chem. Phys. Meteorol. 64, 15598. https://doi.org/10.3402/tellusb.v64i0.15598.
Díez-Herrero, A., Ruiz, S.S., Bustillo, M.G., Morales, P.C., 2006. Study of airborne fungal spores in Madrid. Spain. Aerobiologia 22, 133–140. https://doi.org/10.1007/s10453-006-9025-z.
Docherty, K.M., Pearce, D.S., Lemmer, K.M., Hale, R.L., 2018. Distributing regionally, distinguishing locally: examining the underlying effects of local land use on airborne bacterial biodiversity. Environ. Microbiol. 20, 3529–3542. https://doi.org/10.1111/1462-2920.14307.
Du, P., Du, R., Ren, W., Lu, Z., Zhang, Y., Fu, P., 2018. Variations of bacteria and fungi in PM2.5 in Beijing, China. Atmos. Environ. 172, 55–64. https://doi.org/10.1016/j.atmosenv.2017.10.048.
Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67, 345–366. https://doi.org/10.1890/0012-9615(1997).
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.
Egidi, E., Delgado-Baquerizo, M., Plett, J.M., Wang, J., Eldridge, D.J., Bardgett, R.D., et al., 2019. A few Ascomycota taxa dominate soil fungal communities worldwide. Nat. Commun. 10, 2369. https://doi.org/10.1038/s41467-019-10373-z.
Failor, K.C., Schmale, D.G., Vinatzer, B.A., Monteil, C.L., 2017. Ice nucleation active bacteria in precipitation are genetically diverse and nucleate ice by employing different mechanisms. ISME J. 11, 2740–2753. https://doi.org/10.1038/ismej.2017.124.
Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The microbial engines that drive Earth's biogeochemical cycles. Science 320, 1034–1039. https://doi.org/10.1126/science.1153213.
Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., et al., 2016. Population-level analysis of gut microbiome variation. Science 352, 560–564. https://doi.org/10.1126/science.aad3503.
Fan, C., Li, Y., Liu, P., Mu, F., Xie, Z., Lu, R., et al., 2019. Characteristics of airborne opportunistic pathogenic bacteria during autumn and winter in Xi'an, China. Sci. Total Environ. 672, 834–845. https://doi.org/10.1016/j.scitotenv.2019.03.412.
Fierer, N., Liu, Z., Rodriguez-Hernandez, M., Knight, R., Henn, M., Hernandez, M.T., 2008. Short-term temporal variability in airborne bacterial and fungal populations. Appl. Environ. Microbiol. 74, 200–207. https://doi.org/10.1128/AEM.01467-07.
Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., et al., 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484, 186–194. https://doi.org/10.1038/nature10947.
Franzetti, A., Gandolfi, I., Gaspari, E., Ambrosini, R., Bestetti, G., 2011. Seasonal variability of bacteria in fine and coarse urban air particulate matter. Appl. Microbiol. Biotechnol. 90, 745–753. https://doi.org/10.1007/s00253-010-3048-7.
Fröhlich-Nowoisky, J., Kampf, C.J., Weber, B., Huffman, J.A., Pöhlker, C., Andreae, M. O., et al., 2016. Bioaerosols in the Earth system: Climate, health, and ecosystem interactions. Atmos. Res. 182, 346–376. https://doi.org/10.1016/j.atmosres.2016.07.018.
Fuhrman, J.A., Cram, J.A., Needham, D.M., 2015. Marine microbial community dynamics and their ecological interpretation. Nat. Rev. Microbiol. 13, 133–146. https://doi.org/10.1038/nrmicro3417.
Gat, D., Mazar, Y., Cytryn, E., Rudich, Y., 2017. Origin-Dependent Variations in the Atmospheric Microbiome Community in Eastern Mediterranean Dust Storms. Environ. Sci. Technol. 51, 6709–6718. https://doi.org/10.1021/acs.est.7b00362.
Genitsaris, S., Stefanidou, N., Katsiapi, M., Kormas, K.A., Sommer, U., Moustaka-Gouni, M., 2017. Variability of airborne bacteria in an urban Mediterranean area (Thessaloniki, Greece). Atmos. Environ. 157, 101–110. https://doi.org/10.1016/j.atmosenv.2017.03.018.
Gotelli, N.J., Chao, A., 2013. Measuring and Estimating Species Richness, Species Diversity, and Biotic Similarity from Sampling Data. In: Levin, S.A. (Ed.), Encyclopedia of Biodiversity (Second Edition). Academic Press, Waltham, pp. 195–211.
Griffin, D.W., Gonzalez-Martin, C., Hoose, C., Smith, D.J., 2017. Global-Scale Atmospheric Dispersion of Microorganisms. Microbiol. Aerosols 155–194. https://doi.org/10.1002/9781119132318.ch2c.
Grinn-Gofron, A., Bosiacka, B., 2015. Effects of meteorological factors on the composition of selected fungal spores in the air. Aerobiologia (Bologna) 31, 63–72. https://doi.org/10.1007/s10453-014-9347-1.
Gusareva, E.S., Acerbi, E., Lau, K.J.X., Luhung, I., Premkrishnan, B.N.V., Kolundzija, S., et al., 2019. Microbial communities in the tropical air ecosystem follow a precise diel cycle. Proc. Natl. Acad. Sci. USA 116, 23299–23308. https://doi.org/10.1073/pnas.1908493116.
Hara, K., Zhang, D., 2012. Bacterial abundance and viability in long-range transported dust. Atmos. Environ. 47, 20–25. https://doi.org/10.1016/j.atmosenv.2011.11.050.
Harrison, R.M., Jones, A.M., Biggins, P.D.E., Pomeroy, N., Cox, C.S., Kidd, S.P., et al., 2005. Climate factors influencing bacterial count in background air samples. Int. J. Biometeorol. 49, 167–178. https://doi.org/10.1007/s00484-004-0225-3.
Herlemann, D.P., Labrenz, M., Jurgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5, 1571–1579. https://doi.org/10.1038/ismej.2011.41.
Hiraoka, S., Miyahara, M., Fujii, K., Machiyama, A., Iwasaki, W., 2017. Seasonal Analysis of Microbial Communities in Precipitation in the Greater Tokyo Area, Japan. Front. Microbiol. 8, 1506. https://doi.org/10.3389/fmicb.2017.01506.
Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods Ecol. Evol. 7, 1451–1456. https://doi.org/10.1111/2041-210X.12613.
Innocente, E., Squizzato, S., Visin, F., Facca, C., Rampazzo, G., Bertolini, V., et al., 2017. Influence of seasonality, air mass origin and particulate matter chemical composition on airborne bacterial community structure in the Po Valley, Italy. Sci. Total Environ. 593–594, 677–687. https://doi.org/10.1016/j.scitotenv.2017.03.199.
Jones, A.M., Harrison, R.M., 2004. The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. Sci. Total Environ. 326, 151–180. https://doi.org/10.1016/j.scitotenv.2003.11.021.
Jones, S.E., Newton, R.J., McMahon, K.D., 2008. Potential for atmospheric deposition of bacteria to influence bacterioplankton communities. FEMS Microbiol. Ecol. 64, 388–394. https://doi.org/10.1111/j.1574-6941.2008.00476.x.
Jost, L., 2010. The Relation between Evenness and Diversity. Diversity 2, 207. https://doi.org/10.3390/d2020207.
Katial, R.K., Zhang, Y., Jones, R.H., Dyer, P.D., 1997. Atmospheric mold spore counts in relation to meteorological parameters. Int. J. Biometeorol. 41, 17–22. https://doi.org/10.1007/s004840050048.
Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F., Bahram, M., et al., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277. https://doi.org/10.1111/mec.12481.
Kowalski, W.J., Bahnfleth, W., 1998. Airborne respiratory diseases and mechanical systems for control of microbes. Heating, Piping, and Air Conditioning.
Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. 25, 18.
Lee, J.Y., Park, E.H., Lee, S., Ko, G., Honda, Y., Hashizume, M., et al., 2017. Airborne Bacterial Communities in Three East Asian Cities of China, South Korea, and Japan. Sci. Rep. 7, 5545. https://doi.org/10.1038/s41598-017-05862-4.
Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69, 1–24. https://doi.org/10.1890/0012-9615(1999).
Li, H., Zhou, X.-Y., Yang, X.-R., Zhu, Y.-G., Hong, Y.-W., Su, J.-Q., 2019. Spatial and seasonal variation of the airborne microbiome in a rapidly developing city of China. Sci. Total Environ. 665, 61–68. https://doi.org/10.1016/j.scitotenv.2019.01.367.
Lighthart, B., 1997. The ecology of bacteria in the alfresco atmosphere. FEMS Microbiol. Ecol. 23, 263–274. https://doi.org/10.1111/j.1574-6941.1997.tb00408.x.
Liu, H., Zhang, X., Zhang, H., Yao, X., Zhou, M., Wang, J., et al., 2018. Effect of air pollution on the total bacteria and pathogenic bacteria in different sizes of particulate matter. Environ. Pollut. 233, 483–493. https://doi.org/10.1016/j.envpol.2017.10.070.
Maki, T., Lee, K.C., Kawai, K., Onishi, K., Hong, C.S., Kurosaki, Y., et al., 2019. Aeolian Dispersal of Bacteria Associated With Desert Dust and Anthropogenic Particles Over Continental and Oceanic Surfaces. J. Geophys. Res.: Atmos. 124, 5579–5588. https://doi.org/10.1029/2018JD029597.

Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G., Neufeld, J.D., 2012. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinf. 13, 31. https://doi.org/10.1186/1471-2105-13-31.

Mayol, E., Arrieta, J.M., Jimenez, M.A., Martinez-Asensio, A., Garcias-Bonet, N., Dachs, J., et al., 2017. Long-range transport of airborne microbes over the global tropical and subtropical ocean. Nat. Commun. 8, 201. https://doi.org/10.1038/s41467-017-00110-9.

Mazar, Y., Cytryn, E., Erel, Y., Rudich, Y., 2016. Effect of Dust Storms on the Atmospheric Microbiome in the Eastern Mediterranean. Environ. Sci. Technol. 50, 4194–4202. https://doi.org/10.1021/acs.est.5b06348.

Mhuireach, G., Johnson, B.R., Altrichter, A.E., Ladau, J., Meadow, J.F., Pollard, K.S., et al., 2016. Urban greenness influences airborne bacterial community composition. Sci. Total Environ. 571, 680–687. https://doi.org/10.1016/j.scitotenv.2016.07.037.

Mhuireach, G.Á., Betancourt-Román, C.M., Green, J.L., Johnson, B.R., 2019. Spatiotemporal Controls on the Urban Aerobiome. Front. Ecol. Evol. 7 https://doi.org/10.3389/fevo.2019.00043.

Morris, C.E., Conen, F., Alex Huffman, J., Phillips, V., Pöschl, U., Sands, D.C., 2014. Bioprecipitation: a feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. Glob. Change Biol. 20, 341–351. https://doi.org/10.1111/gcb.12447.

Murray, K.A., Olivero, J., Roche, B., Tiedt, S., Guégan, J.-F., 2018. Pathogeography: leveraging the biogeography of human infectious diseases for global health management. Ecography 41, 1411–1427. https://doi.org/10.1111/ecog.03625.

Newbound, M., McCarthy, M.A., Lebel, T., 2010. Fungi and the urban environment: A review. Landscape Urban Plann. 96, 138–145. https://doi.org/10.1016/j.landurbplan.2010.04.005.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., et al., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006.

Núñez, A., Amo de Paz, G., Ferencova, Z., Rastrojo, A., Guantes, R., Garcia, A.M., et al., 2017. Validation of the Hirst-Type Spore Trap for Simultaneous Monitoring of Prokaryotic and Eukaryotic Biodiversities in Urban Air Samples by Next-Generation Sequencing. Appl. Environ. Microbiol. 83 https://doi.org/10.1128/AEM.00472-17.

Núñez, A., Amo de Paz, G., Rastrojo, A., Ferencova, Z., Gutiérrez-Bustillo, A.M., Alcamí, A., et al., 2019. Temporal patterns of variability for prokaryotic and eukaryotic diversity in the urban air of Madrid (Spain). Atmos. Environ. 217, 116972 https://doi.org/10.1016/j.atmosenv.2019.116972.

Oliveira, M., Ribeiro, H., Delgado, J.L., Abreu, I., 2009. The effects of meteorological factors on airborne fungal spore concentration in two areas differing in urbanisation level. Int. J. Biometeorol. 53, 61–73. https://doi.org/10.1007/s00484-008-0191-2.

Pasquereau-Kotula, E., Martins, M., Aymeric, L., Dramsi, S., 2018. Significance of Streptococcus gallolyticus subsp. gallolyticus Association With Colorectal Cancer. Front. Microbiol. 9 https://doi.org/10.3389/fmicb.2018.00614.

Paulson, J.N., Stine, O.C., Bravo, H.C., Pop, M., 2013. Differential abundance analysis for microbial marker-gene surveys. Nat. Methods 10, 1200–1202. https://doi.org/10.1038/nmeth.2658.

Peres-Neto, P.R., Legendre, P., Dray, S., Borcard, D., 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology 87, 2614–2625. https://doi.org/10.1890/0012-9658(2006).

Peternel, R., Culig, J., Hrga, I., 2004. Atmospheric concentrations of Cladosporium spp. and Alternaria spp. spores in Zagreb (Croatia) and effects of some meteorological factors. Ann. Agric. Environ. Med. 11, 303–307.

Polymenakou, P.N., Mandalakis, M., Macheras, M., Oulas, A., Kristoffersen, J.B., Christakis, C.A., et al., 2020. High genetic diversity and variability of microbial communities in near-surface atmosphere of Crete island. Greece. Aerobiologia 36, 341–353. https://doi.org/10.1007/s10453-020-09636-w.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl. Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

Quince, C., Lanzen, A., Davenport, R.J., Turnbaugh, P.J., 2011. Removing noise from pyrosequenced amplicons. BMC Bioinf. 12, 38. https://doi.org/10.1186/1471-2105-12-38.

Sánchez-Parra, B., Núñez, A., Moreno, D.A., 2019. Preventing legionellosis outbreaks by a quick detection of airborne Legionella pneumophila. Environ. Res. 171, 546–549. https://doi.org/10.1016/j.envres.2019.01.032.

Sánchez-Reyes, E., de la Cruz, D.R., Sánchez, J.S., 2016. First fungal spore calendar of the middle-west of the Iberian Peninsula. Aerobiologia 32, 529–539. https://doi.org/10.1007/s10453-016-9430-x.

Serrano-Silva, N., Calderón-Ezquerro, M.C., 2018. Metagenomic survey of bacterial diversity in the atmosphere of Mexico City using different sampling methods. Environ. Pollut. 235, 20–29. https://doi.org/10.1016/j.envpol.2017.12.035.

Shi, Y., Zhang, K., Li, Q., Liu, X., He, J.-S., Chu, H., 2020. Interannual climate variability and altered precipitation influence the soil microbial community structure in a Tibetan Plateau grassland. Sci. Total Environ. 714, 136794 https://doi.org/10.1016/j.scitotenv.2020.136794.

Sinclair, L., Ijaz, U.Z., Jensen, L.J., Coolen, M.J.L., Gubry-Rangin, C., Chronakova, A., et al., 2016. Seqenv: linking sequences to environments through text mining. PeerJ 4, e2690. https://doi.org/10.7717/peerj.2690.

Smets, W., Moretti, S., Denys, S., Lebeer, S., 2016. Airborne bacteria in the atmosphere: Presence, purpose, and potential. Atmos. Environ. 139, 214–221. https://doi.org/10.1016/j.atmosenv.2016.05.038.

Smith, T.P., Thomas, T.J.H., Garcia-Carreras, B., Sal, S., Yvon-Durocher, G., Bell, T., et al., 2019. Community-level respiration of prokaryotic microbes may rise with global warming. Nat. Commun. 10, 5124. https://doi.org/10.1038/s41467-019-13109-1.

Sousa, S.I.V., Martins, F.G., Pereira, M.C., Alvim-Ferraz, M.C.M., Ribeiro, H., Oliveira, M., et al., 2008. Influence of atmospheric ozone, PM10 and meteorological factors on the concentration of airborne pollen and fungal spores. Atmos. Environ. 42, 7452–7464. https://doi.org/10.1016/j.atmosenv.2008.06.004.

Stewart, J.D., Shakya, K.M., Bilinski, T., Wilson, J.W., Ravi, S., Choi, C.S., 2020. Variation of near surface atmosphere microbial communities at an urban and a suburban site in Philadelphia, PA, USA. Sci. Total Environ. 724, 138353 https://doi.org/10.1016/j.scitotenv.2020.138353.

Tanaka, D., Sato, K., Goto, M., Fujiyoshi, S., Maruyama, F., Takato, S., et al., 2019. Airborne Microbial Communities at High-Altitude and Suburban Sites in Toyama, Japan Suggest a New Perspective for Bioprospecting. Front. Bioeng. Biotechnol. 7 https://doi.org/10.3389/fbioe.2019.00012.

Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N.S., Wijesundera, R., et al., 2014. Fungal biogeography. Global diversity and geography of soil fungi. Science 346, 1256688. https://doi.org/10.1126/science.1256688.

Tignat-Perrier, R., Dommergue, A., Thollot, A., Magand, O., Amato, P., Joly, M., et al., 2020. Seasonal shift in airborne microbial communities. Sci. Total Environ. 716, 137129 https://doi.org/10.1038/s41598-019-51073-4.

Turenne, C.Y., Sanche, S.E., Hoban, D.J., Karlowsky, J.A., Kabani, A.M., 1999. Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. J. Clin. Microbiol. 37, 1846–1851. https://doi.org/10.1128/JCM.37.6.1846-1851.1999.

Ueda, Y., Frindte, K., Knief, C., Ashrafuzzaman, M., Frei, M., 2016. Effects of Elevated Tropospheric Ozone Concentration on the Bacterial Community in the Phyllosphere and Rhizoplane of Rice. PLoS ONE 11, e0163178. https://doi.org/10.1371/journal.pone.0163178.

Vetrovsky, T., Kohout, P., Kopecky, M., Machac, A., Man, M., Bahnmann, B.D., et al., 2019. A meta-analysis of global fungal distribution reveals climate-driven patterns. Nat. Commun. 10, 5142. https://doi.org/10.1038/s41467-019-13164-8.

Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., et al., 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5, 27. https://doi.org/10.1186/s40168-017-0237-y.

White, T., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Shinsky, J., White, T. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp. 315–322.

Woo, C., An, C., Xu, S., Yi, S.M., Yamamoto, N., 2018. Taxonomic diversity of fungi deposited from the atmosphere. ISME J. 12, 2051–2060. https://doi.org/10.1038/s41396-018-0160-7.

Yan, D., Zhang, T., Su, J., Zhao, L.-L., Wang, H., Fang, X.-M., et al., 2018. Structural Variation in the Bacterial Community Associated with Airborne Particulate Matter in Beijing, China, during Hazy and Nonhazy Days. Appl. Environ. Microbiol. 84, e00004–e18. https://doi.org/10.1128/AEM.00004-18.

Yan, D., Zhang, T., Su, J., Zhao, L.L., Wang, H., Fang, X.M., et al., 2016. Diversity and Composition of Airborne Fungal Community Associated with Particulate Matters in Beijing during Haze and Non-haze Days. Front. Microbiol. 7, 487. https://doi.org/10.3389/fmicb.2016.00487.

Yoo, K., Yoo, H., Lee, J.M., Shukla, S.K., Park, J., 2018. Classification and Regression Tree Approach for Prediction of Potential Hazards of Urban Airborne Bacteria during Asian Dust Events. Sci. Rep. 8, 11823. https://doi.org/10.1038/s41598-018-29796-7.

Zhernakova, A., Kurilshikov, A., Bonder, M.J., Tigchelaar, E.F., Schirmer, M., Vatanen, T., et al., 2016. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 352, 565–569. https://doi.org/10.1126/science.aad3369.

Zhong, X., Qi, J., Li, H., Dong, L., Gao, D., 2016. Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region. Atmos. Environ. 140, 506–513. https://doi.org/10.1016/j.atmosenv.2016.06.034.

Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D., et al., 2016. Temperature mediates continental-scale diversity of microbes in forest soils. Nat. Commun. 7, 12083. https://doi.org/10.1038/ncomms12083.