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Effect of the passive natural ventilation on the bioaerosol in a small room

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ABSTRACT

Natural passive ventilation (windows opening) is frequently used in many houses and old buildings to renovate the air, remove unpleasant odors and dust, and reduce the physicochemical pollutants indoor. However, little is known about the effect on biological particles such as pollen grains and fungal spores (both allergenic) or bacteria (potentially infectious and pathogenic). In the present research, the bioaerosols composition in a small room naturally ventilated was analyzed by high-throughput DNA sequencing. Pollen grains were the most abundant particles outdoors while microbial phyla *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Ascomycota* were predominant indoors. The main divergences in bioaerosols between indoor and outdoor environments were caused by the different abundance of the biological particles rather than the different taxa composition. Keeping the window open for 2 h did not change significantly the microbial community present indoors, although there was a tendency to mix the components of both environments. The abundance of human-related and potentially harmful microorganisms was higher indoors and was not remarkably affected by natural ventilation. In our study, natural ventilation through window opening had a poor effect on removing these microorganisms from the atmosphere indoor, suggesting that additional mechanisms such as air filtering systems would be required in order to improve the air quality of these environments from a microbiological point of view.

1. Introduction

Biological particles such as pollen, fungal spores, bacteria and viruses are emitted from natural (soil, water, plant surfaces, animal depositions) and artificial sources (fountains, wastewater treatment plants, dumps) in metropolitan areas [1–3]. These bioaerosols are present in the air that we daily breathe and, although most of them are innocuous, a significant part has the capability of triggering negative effects on human health. For instance, pollen grains from several species usually found in parks and gardens cause allergy, affecting thousands of people worldwide [4,5]. Most fungal spores are also considered strong aeroallergens, with the most known representatives belonging to the genera *Alternaria*, *Cladosporium* or *Aspergillus* because of their high abundance and prevalence in the atmosphere [6,7]. Moreover, maladies such as tuberculosis or legionellosis are airborne bacterial diseases, and jointly with infectious fungi like *Histoplasma capsulatum* (histoplasmosis), *Cryptococcus neoformans* (cryptococcosis) or *Aspergillus* spp. (aspergillosis, aspergilloma and allergy) are a real threat, especially for immunocompromise patients [8,9].

Recent events caused by SARS-Covid'19 has highlighted how potentially dangerous airborne microbes can be and how less we know yet about their dynamics and transport in the atmosphere. Unlike physical and chemical air pollutants, bioaerosols are not daily monitored in the cities, being pollen concentration the most frequent exception [10]. Microbial bioaerosols, e.g., fungi and bacteria, are more difficult to monitor because of the lack of standardized protocols for the measurements, their high diversity, variability and the complexity of the analyses [11,12]. Moreover, bioaerosols outdoor are affected by meteorological factors, seasons, nearby sources and can be even deposited after long-transport from other continents [13–15]. Therefore, the real exposure to these particles remains unclear.

Similar scarce knowledge exists about the exposure to biological particles inside the buildings. It has been estimated that current human population in the cities spend >80% indoors, considering the time at the workplace and home [16,17], which has promoted the interest in the air pollutants in these environments. Thus, several works have described the potential effect of the exposure to fungi and bacteria inside public buildings and health centers, paying particular attention to children as the most vulnerable citizens. These studies remark the potential negative consequences of these particles to trigger and exacerbate allergic

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sensitization, asthma and respiratory diseases [18–21]. Even so, the concentration of biological particles is not submitted to any regulation, except particular places such as operation or clean rooms. Only general guidance exists, as the one proposed by the European Commission, which considers a highly polluted environment when the microbial load is > 500 CFU/m^3 for non-industrial premises, and >1000 or 2500 CFU/m^3 for fungi and bacteria, respectively, in case of houses [22]. Moreover, most studies are limited to culturable microorganisms and focused on only one type (fungi or bacteria), simplifying the real diversity present indoors.

The main reason for this lack of consensus is that there are several factors affecting the concentrations of microorganisms in the air indoor: the composition and concentration outdoors, the grade of occupancy and activity in the room, and even certain seasonality have been observed, which can be associated to changes in the sources and in people patterns [23–27]. In addition, the type of ventilation is also an important parameter to take into account. Most workplaces are usually equipped with a mechanical ventilation system, but many houses and large buildings like academic premises frequently resort, total or partially, to natural ventilation through windows, especially during the warm seasons. Moreover, this approach has been proposed as a crucial strategy to diminish SARS-Cov-2 infection in closed environments [28]. However, although several previous works have surveyed the bioaerosols outdoor and indoor in naturally ventilated buildings [24, 29–31], a comparison to evaluate how the window opening affect these particles indoor has not being addressed properly yet. Here, we evaluate how indoor microbial communities are affected by natural ventilation. For this purpose, we characterized the bioaerosols composition in a small isolated room with high-throughput DNA sequencing accuracy under two different situations: the window closed and open. We also showed special interest in the taxa with relevance for human health and analyzed their Indoor/Outdoor ratios in order to clarify the effect of this strategy to reduce biological pollution inside the buildings.

2. Materials and Methods

2.1. Sampling methodology and location

Air samples were taken in “Escuela Técnica Superior de Ingenieros Industriales”, an academic building of “Universidad Politécnica de Madrid” founded in 1845. The edification is located in an urban area of the city of Madrid with high traffic avenues around and next to a small urban park (Spain, 40.439881°N 3.689409°W, 705 m AMSL). The sampling indoors was conducted in a small room rarely used (only for the storage of academic documents) of 2.70 × 2.90 × 3.60 m (w x l x h) located in the fourth floor of the building (17.5 m AGL), with only one window of 0.70 × 1.0 m (w x h) and one door, which was kept always closed. The room was isolated and it was not in used during the sampling period. No cleaning treatments were conducted in the room before the sampling except for the transfer of the stored materials one week earlier. The sampling outdoor was conducted at 3 m over the ground, at the same location where the air quality station is placed and the meteorological parameters were measured during the sampling time (−70 m from the indoor location) (Table S1).

The collection period ranged from 10th to February 25, 2020, collecting a total of 22 air samples: 11 outdoors and 11 indoors. To evaluate the effect of passive ventilation, the samples indoors taken on dates 10th – 14th, 24th and 25th were collected with the window closed. Those from 18th – 21st were taken with the window open during the collection time and, afterward, the window was closed the rest of the day (Table S1).

Two air samplers DUO SAS Super 360 (VWR) were used to collect the samples. This is an impactor-type device with two heads for Petri dishes that run simultaneously during the collection (replicates), with an airflow rate of 180 L/min. The heads of the samplers were cleaned and autoclaved each day after sampling. Empty sterile Petri dishes were covered with pharmaceutical petroleum jelly (Vaseline, Interapothek, Spain), which was used as adhesive surface to collect the airborne particles. They were prepared in a biosafety cabinet using sterilized materials and kept closed at 4 °C until the sampling time. 2 h samples (−21.6 m^3 of air per Petri dish) were collected with each device, which were running synchronically (indoor and outdoor). Collection was conducted from 8:30 a.m. to 10:30 a.m. each day during the sampling period.

2.2. DNA extraction and sequencing

After collection, the Petri dishes were kept at 4 °C until DNA extraction was conducted later in the same day. Using a sterilized razor, the petroleum jelly with the particles collected was retrieved and put into an extraction tube of DNeasy Powersoil Kit (Qiagen). DNA was extracted and purified following the manufacture’s guideline. As DNA concentration was expected to be low and also to reduce the variability in biological particles between replicates, the buffers containing the DNA of each replicate were merged and spined through the same purification column of the kit so we can obtain a more representative sample. DNA concentration (quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit; Invitrogen, MolecularProbes®) ranged from 23 pg/m^3 of air to 3600 pg/m^3, with the samples taken indoors always yielding lower values (mean: 50 ± 30 pg/m^3; median: 35 pg/m^3) compared to samples outdoors (mean: 974 ± 998 pg/m^3; median: 628 pg/m^3) (Table S1).

DNA samples were submitted to high-throughput amplicon sequencing at the Genomics service of “Parque Científico de Madrid” (Madrid, Spain), using Illumina® Mi-Seq platform (2 × 300 reads). Amplicon libraries were obtained using the following universal primers sets: Bakt_341 (F): 5′- CCTACGGGNGGCWGCAG −3′; Bakt_805 (R): 5′-GACTACHVGGGTATCTAATCC -3′ [32] for partial amplification of the hypervariable regions V3–V4 of the gene 16S rRNA of bacteria; and ITS86 (F): 5′- GTGAATCA TCGAATCTTTGAA-3′ [33], ITS-4 (R): 5′-TCCTCCGCTTATTGATATGC -3′ [34], for the region 5.8S – ITS2 of fungi. The negative control (the same sampling practice but with the air sampler turn-off, followed by the same DNA extraction procedure) resulted in no DNA amplification following the same protocol, as did the sample from 14th Feb taken indoor, so it was discarded.

2.3. Sequence assembly, filtering and normalization

Raw DNA sequence data was processed (demultiplexed, trimmed, paired-reads merged and chimera filtered) using the default parameters of DADA2 pipeline (v1.17.5) [35] in R environment [36]. The Amplicon Sequence Variants (ASVs) obtained were submitted to taxonomy assignment using SILVA [37] (release 138) and UNITE [38](version 8.2) databases for bacteria and fungi, respectively. The sequences assigned to the order “chloroplast” were annotated as “Plantae” at phylum and genus ranks for figures and analyses purposes. In the case of fungal sequences, since the primer set also amplified, in less extent, DNA from plants, those ASVs with only kingdom rank assigned were curated with PLANiTS database [39] (release 29-03-2020) and reassigned to “Plants” when confirmed. Additionally, those ASVs retrieving “Unassigned” from this database were submitted to the online version of BLAST (National Center for Biotechnology Information, NCBI) and assigned correspondingly to plants or fungi. Similar to the “chloroplast” sequences, these were annotated as “Plantae” at phylum and genus ranks.

Normalization of the data was performed with the R package “metagenomeSeq” [40] to compensate the bias due to differences in sequencing depth, especially when the plant DNA sequences were removed.

2.4. Data analyses and statistics

Global analyses were performed in R environment using the packages “phyloseq” [41] (version 1.34.0) and “vegan” (version 2.5–6).

Principal Coordinates Analyses (PCoA) were conducted using Bray-Curtis dissimilarity matrices and the analysis of similarity (ANOSIM) was set to 999 permutations. To evaluate the statistical differences between the abundances of particular taxonomical groups, the corresponding ASVs of the groups (phyla or genera) were selected and their accumulative abundances were used to pairwise comparisons by Welch's test. Alpha-diversity indexes, Chao1 (richness) and Shannon (diversity), were calculated after conducting a rarefaction set to the sample with the lowest number of reads in the matrix. The IndVal indices (species indicator) were obtained with the R package "labdsv".

3. Results

3.1. Global characterization

A total of 11 air samples were taken simultaneously across the sampling period in a pairwise approach (indoor-outdoor) and organized as follows: 11 outdoors ("Outdoor") and 11 indoors ("Indoor", although one was discarded during processing, see Materials and Methods). In order to analyze whether the passive ventilation has any effect on the microbial communities composition, the latter were collected with the window closed (6, "Indoor WinC") or keeping the window open (4, "Indoor WinO"). The DNA extracted from the samples was submitted to high-throughput sequencing to characterize airborne bacteria and fungi, retrieving 7541 and 2071 ASVs, respectively (see Materials and Methods for details).

As shown in Fig. 1, bioaerosols composition in the samples indoor was easily differentiated from those taken outdoors in both bacterial and fungal analyses (ANOSIM $R_1 = 0.725$ and 0.766, respectively). Among all possible clustering, the differentiation of 3 groups ("Indoor WinC", "Indoor WinO" and "Outdoor") retrieved the highest R-value for bacteria and fungi ($R_3 = 0.863$ and 0.831, respectively). This result suggests an alteration of the indoor airborne biological components when the window was open, but still differentiable from those samples taken outside (R2, cluster with two groups: "Indoor WinC" and "Indoor WinO"-"Outdoor").

The main differential component in both analyses was the contribution of plant DNA, presumably from pollen grains and detected indirectly by the set of the universal primers employed during the high-throughput sequencing protocol (see Materials and Methods). Those sequences were identified in our samples as chloroplast DNA (which contains copies of the 16S rRNA gene) in the bacterial analysis, and genomic plant DNA in the fungal analysis. Thus, the samples taken outside were dominated by DNA from pollen grains over bacteria and fungi, while indoor environment was enriched with DNA from microbial entities.

Notably, the relative abundances of this plant DNA outdoors showed significant differences with the samples taken indoors even when the window was kept open (Fig. 2). The most abundant microbial phyla (those with a relative abundance >1%) were *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Bacteroidota* and *Cyanobacteria* for bacteria; and *Ascomycota* and *Basidiomycota* for fungi. The abundance of these bacterial phyla showed statistically significant differences between the three types of samples (Welch's test $p < 0.05$), which suggests that keeping the window open has indeed an effect by altering the airborne bacterial community indoors (Fig. 2a): *Firmicutes* and *Proteobacteria* tended to decrease their abundances indoor while *Bacteroidota* followed the opposite trend. On the contrary, no differences were found in the fungal community regarding the state of the window, open or closed, although the contribution of the phyla *Ascomycota* and *Basidiomycota* were notably different compared to samples Outdoor due to the large contribution of plant DNA (Fig. 2b). It is worth mentioning that the abundance of these bacterial and fungal taxa did not show statistically significant differences in the samples Outdoor across the periods when the window was open or closed (Welch's test $p > 0.05$), despite of the changes in the meteorological variables. The values shown in Fig. 2 are a good representation for the abundances of these groups in these samples.

3.2. Shared taxa and diversity indexes

Next, we compared the taxa present in each scenario ("Indoor WinO", "Indoor WinC" or "Outdoor"). A common procedure in DNA based studies of bioaerosols is to filter out those sequences assigned to organisms other than those under analysis. Therefore, here on, the sequences assigned to chloroplasts or plants were removed from the analyses to focus on the microbial component, and the raw data was proportionally arranged to make feasible the comparison with other previous works (see Materials and Methods). A total of 1034 and 656 different ASVs were identified for bacteria and fungi, respectively. Those numbers corresponded to 787 and 555 defined genera to each type of microorganism. The genera present in the three types of samples

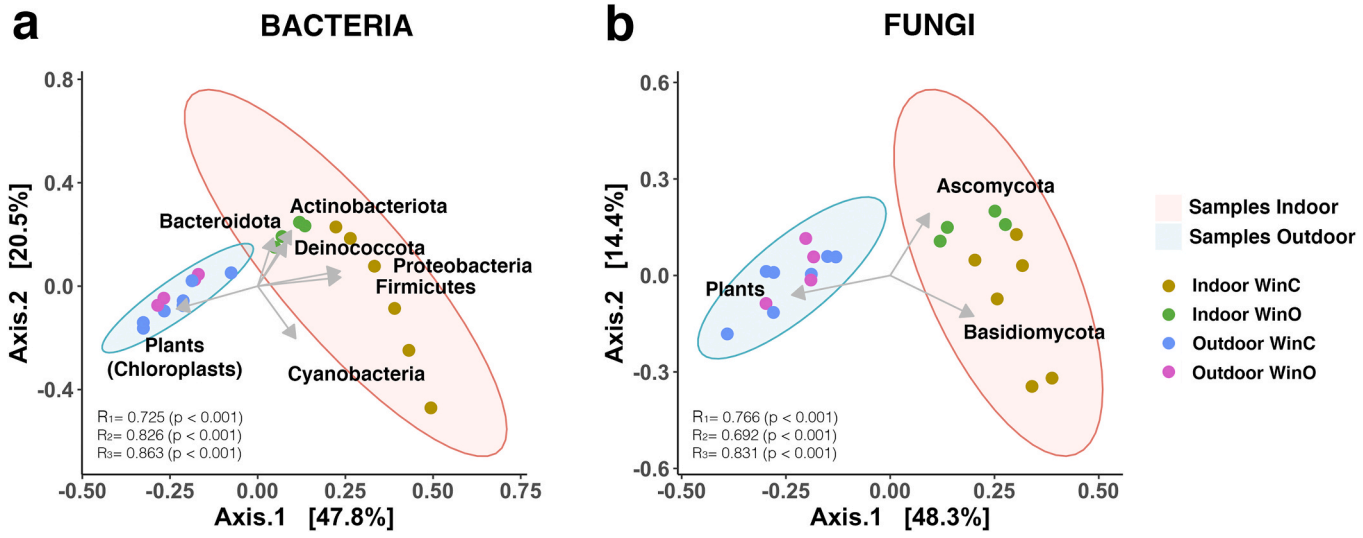

Fig. 1. The bioaerosols composition differs between samples taken indoor and outdoor. Principal Coordinates Analysis (PCoA) of the samples for bacterial (a) and fungal (b) analyses based on Bray-Curtis dissimilarity matrices. The most abundant phyla were correlated to the ordinations as explanatory variables. Those with statistically significant correlations ($p \leq 0.001$ for bacteria and $p \leq 0.01$ for fungi) were plotted as arrows, whose lengths are proportional to the value of the correlation. The direction of the arrows indicates the samples with higher abundance for that particular taxon.

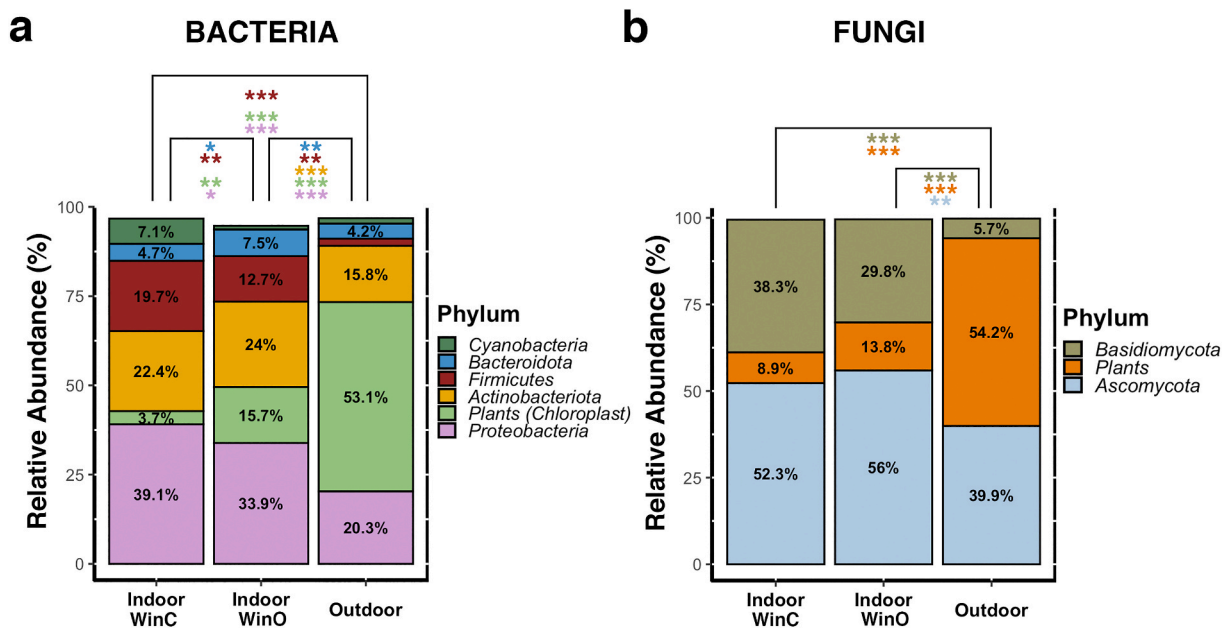

Fig. 2. Global description of the bioaerosols. Relative abundances of the most abundant phyla (>2%) of bacteria (a) and fungi (b) by type of sample (Indoor WinC, Indoor WinO or Outdoor). Welch's tests were performed to determine statistical differences in the abundances of the phyla between the types of samples in a pairwise comparison. Asterisks represent their significance: ***: $p < 0.001$; **: $0.001 < p < 0.01$; *: $0.01 < p < 0.05$; blank: $p > 0.05$. The colors of the asterisks are correlated with the phyla showed in the legend. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

gathered >88% of the relative abundance present in all the samples, with a higher number for bacteria (321, ~41%) in comparison to fungi (145, ~26%) (Fig. 3). Several genera were detected only in one situation, especially outdoors (147 and 136). However, considering the genera found exclusively indoors (WinO + WinC), the numbers were only slightly higher in this environment (155 and 181 for bacteria and fungi, respectively). Either way, those genera found exclusively in one type of scenario represented a small fraction of each sample in terms of accumulative abundance (<4.3% and 4.8% for bacteria and fungi, respectively, outdoor), with similar proportions to those taxa found only indoors (<2.9% and 3.3%). These data suggest that the microbial core of both environments, indoor and outdoor, is very similar and most of the taxa are shared, representing the highest fraction of the bioaerosols (Fig. 3).

Richness and diversity indexes showed results in agreement with this idea (Fig. S1). Bacterial communities were similar in richness and no significant differences were found in diversity (Shannon index, Welch's test $p > 0.05$ for pairwise comparisons). However, a tendency to higher diversity was observed when the ambient air is involved (Figs. S1a–b). This is supported by a bacterial community dominated by a few taxa with high abundance in the samples indoor WinC, mostly the genera *Bradyrhizobium* and *Paenibacillus*, in addition to *Sphingomonas*, *Kocuria* and *Paracoccus*, which also appeared with high abundance in the samples Indoor WinO and Outdoor (Fig. S2a).

The number of fungal species was lower indoors, with a trend to increase when the window was kept open (Fig. S1c), likely because of the external contribution of species. The opposite trend in diversity is observed compared to bacterial communities: the indoor environment tended to be more diverse compared to samples from outdoors (Fig. S1d), likely because the latter were dominated by *Cladosporium* spp. and its teleomorph *Mycosphaerella* spp., which accumulated ca. 50% of the relative abundance of these samples (Fig. S2b).

3.3. Human microbiome species and taxa indicators

Previous works have established that areas indoors are enriched in

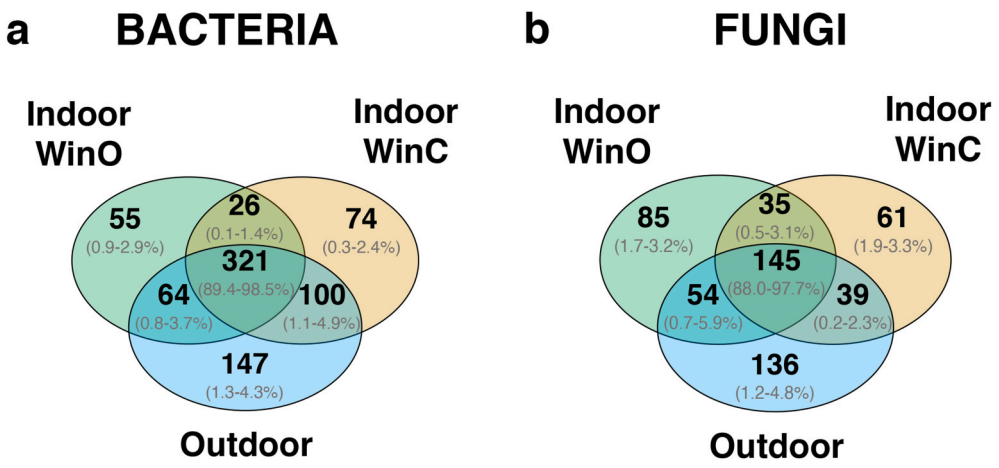

Fig. 3. Venn diagrams showing the relations of the genera between the sets of samples. The number of genera is shown in bold, with the range of relative abundance that those taxa gather in the individual samples in grey between parentheses.

microorganisms related to human microbiome [42,43]. In accordance, our analyses identified a larger number of bacterial genera related to human in samples Indoor (WinC and WinO) compared to Outdoors (Fig. 4a). These genera gathered ~12-10% of the total relative abundance of the samples Indoor, while their abundance was <3% in the samples Outdoor. The most abundant group corresponded with the most generalist bacteria, found in the oral cavity as well as in the respiratory tract and skin (e.g., *Staphylococcus*, *Streptococcus* or *Corynebacterium*), followed by buccal bacteria (e.g., *Micrococcus*, *Lactobacillus*) and skin-related (e.g., *Brachybacterium*, *Enhydrobacter*) (Table S2). In contrast, the most abundant human-related bacteria in the samples Outdoor were associated to the respiratory tract and represented by different genera, such as *Pseudomonas* or *Sphingobacterium*. Although only a few fungi are considered as human-associated microbiome, there are several genera frequently isolated from the human body. Thus, the most abundant in all the sample types were those found in the buccal cavity and respiratory tract (*Penicillium*, *Cladosporium*), followed by the genera exclusively identified in the oral cavity (*Aureobasidium*, *Alternaria* or *Cryptococcus*). Unlike the bacteria analysis, no significant differences in the abundances were found between samples indoors and outdoors for fungi (Fig. 4b).

The analysis of species indicators identified genera from indoor or outdoor areas in a reasonable way (Fig. S3). Environmental bacteria *Blastococcus* and the cyanobacteria *Nostoc PCC-73102* were associated to samples taken outdoors, while *Haemophilus* and *Cutibacterium* (human-related bacteria) were assigned to Indoor samples. Also, *Bradyrhizobium* and *Paenibacillus* were selected as indicators. These two genera have been described previously in ambient air of urban and rural environments in a diversity of studies [44–46]. However, in our survey, they were dominant in the samples Indoor WinC, while their abundances were diluted in the samples with ambient air present (WinO and Outdoor, Fig. S2).

Among the fungi, plant pathogens and saprophytes as *Mycosphaerella* and *Thermomyces* were selected as species indicators for samples Outdoor alongside the aeroallergen *Alternaria*, all belonging to the phylum *Ascomycota*. Contrariwise, the group of *Basidiomycota* was predominantly preferred as indicator of the air indoors. For instance, *Filobasidium*, a teleomorph of *Cryptococcus* found in indoor environments [47,48], and the human-related fungi *Malassezia* were identified.

3.4. Pathogenic genera and indoor/outdoor ratios

Several potentially pathogenic microbes can be present in both environments, indoors and outdoors, as stated by several authors [25,26,29]. As shown in Fig. 5a, the air indoors, independently on the state of the window, carried significantly more potentially harmful bacteria than the air outdoors. The most abundant genera correlated with some of the previously identified as human-related (*Pseudomonas*, *Staphylococcus*, *Acinetobacter* or *Corynebacterium*), but also pathogenic genera as *Roseomonas*, *Mycobacterium*, *Legionella* or *Geodermatophilus* were present. In regards to fungi, most genera were allergenic (*Cladosporium*, *Penicillium*, *Alternaria*, *Aureobasidium*) and the ventilation from the window has little impact in their abundance (Indoor WinC vs Indoor WinO), finding no significant differences between the three scenarios ("Indoor WinC", "Indoor WinO" and "Outdoors") (Fig. 5b).

Therefore, when the ratio of abundances indoors/outdoors (I/O) for these potentially harmful genera are calculated (Fig. S4), the air outdoor seemed cleaner for the majority of these pathogens than the air indoor (considering >2.00 as a threshold), excepting for the bacterium *Geodermatophilus* and the fungi *Phoma* and *Alternaria*, which showed a greater abundance (ratio <0.5) when the ambient air is involved (samples Indoor WinO and Outdoor).

4. Discussion

The concern about air quality inside the buildings is increasing as people spend more and more time indoors. Closed environments tend to have a different composition of gases and particles because of their predisposition to accumulation and the specific activities occurring inside. Nonetheless, outdoor air and pollutants can still penetrate into the building by infiltration (joints, openings, leakages) and natural ventilation (windows and doors), which, in some cases, can contribute to deteriorate the interior atmosphere. Many studies have analyzed the dynamics of physicochemical compounds inside the buildings, showing that air pollutants indoor in natural ventilated buildings are frequently correlated with the concentrations outdoor of $PM_{2.5}$, PM_{10} and CO_2 in urban environments [49–51], and even certain seasonality exists because of the change of patterns from summer to winter (windows opening, AC, etc.) [52,53]. However, there is a shortage of knowledge on bioaerosols indoor and their response to natural passive ventilation, which is evaluated in this work.

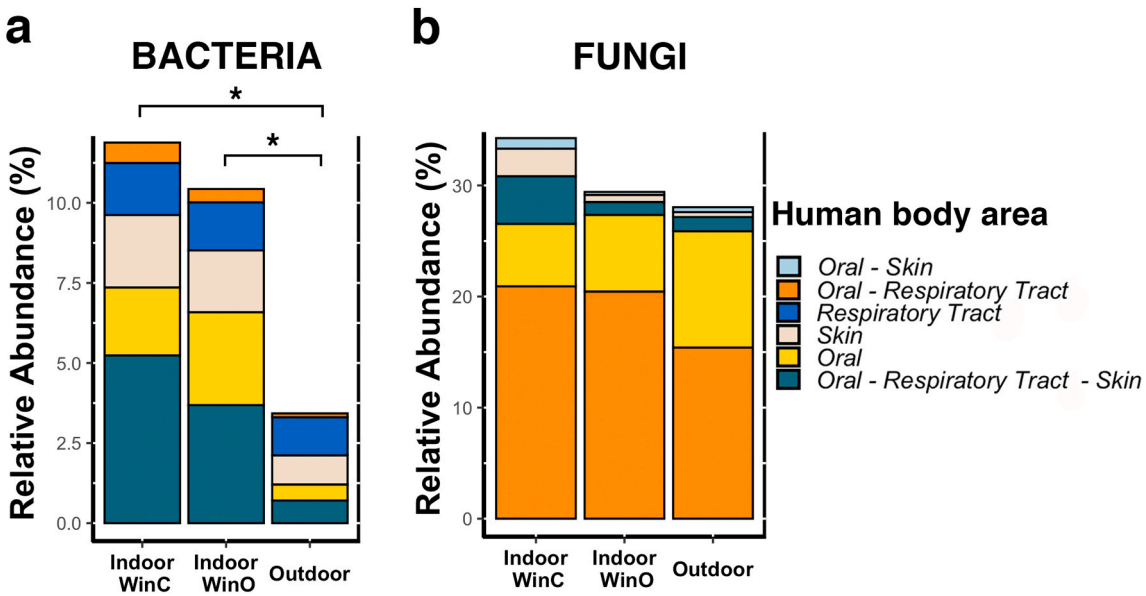

Fig. 4. Contribution of the microbial genera associated to human microbiome. The relative abundances of the bacterial (a) and fungal (b) genera are plotted according with the location on the human body.

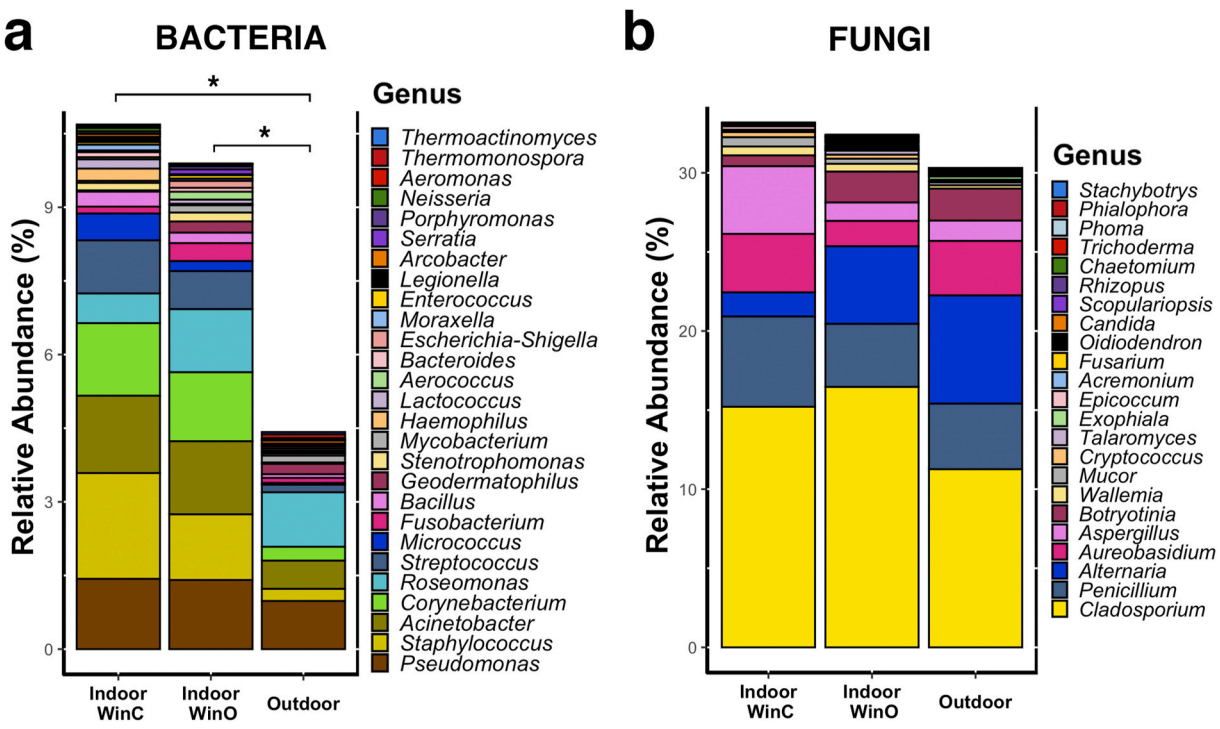

Fig. 5. Relative abundances of the potentially harmful microbes. Bacterial (a) and fungal (b) genera with potentially pathogenic representatives were selected and their contribution to the total abundance of the microbial community was plotted by type of sample.

Our results employing DNA sequencing for the characterization of the bioaerosols showed that the main divergences between indoor and outdoor environments are caused by the different abundance of the biological particles rather than the different taxa composition (Figs. 1 and 2). We found that pollen grains were the most abundant particles outdoors compared to the abundances inside the building by the time the study was conducted. Several works have confirmed that pollen grain concentrations are frequently higher outdoors compared to indoors [54,55], effect that can be exacerbated in our case because of the urban park nearby. Even a negative gradient from the window to inner places in the same room have been described [56], which may be related to the large size of the pollen grains (usually >20 μm).

We observed that the microbial phyla *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Ascomycota* were dominant indoors (Fig. 2), whose representatives have been also detected in high abundance in previous works [27,29,42]. Most of the microbial taxa are present in both environments (indoor and outdoor) and the relative abundance gathered by the common taxa represents a majority (>88%, Fig. 3), which is in agreement with recent DNA-based [27,43,57] and culture-based studies [24,29,31]), suggesting a general tendency in these environments. Across all the samples, the most abundant bacterial genera were *Sphingomonas*, *Kocuria* and *Paracoccus*, which coincides with those previously described outdoors in the area [14,58] and worldwide [59,60], and supports the importance of the air outdoor as a main source of microorganisms indoors. Environmental fungus *Mycosphaerella/Cladosporium* dominated both indoor and outdoor air, and based on their saprophytic lifestyle, it is expected that air outdoor to be the origin, as previous works reported their high abundance outdoor as well [14,58].

When the window was kept open for 2 h, the microbial community present indoor did not change severely. The analysis at phylum level showed a more noticeable effect on the airborne bacteria over the fungal community (Fig. 2). The average size of these particles (bacteria <3 μm; fungi <20 μm) could influence on their distribution, being the transport of bacteria favored comparing to fungal propagules, idea that is also support by the scarce exchange of bigger particles like pollen from outdoor to indoor (Plants, Fig. 2). However, richness and diversity indexes showed bigger changes for fungi, which could be explained by the entrance of new taxa inside the building, whereas, in the case of bacteria, the window opening would alter mainly the abundance of the taxa already present indoor.

Although some taxa were found only in some type of sample (WinC, WinO or Outdoor; Fig. 3), the low relative abundance compiled by them (≤4.8%) and the fact that we detected some unique taxa in WinO when the potential sources are either indoor or outdoor air could be explained as a bias associated to the sampling strategy of the study (limited time instead of continuous sampling for 24 h) in addition to the high variability that bioaerosols outdoor can show caused by environmental and meteorological factors [12,61].

As described by previous works [43,62], we did observe a significant difference in the contribution of the human-related bacteria, with higher abundances indoors (Fig. 4). A recent study conducted by Zhou and colleagues [27] did not show such difference, maybe influenced by the ventilation type sampled (mechanically ventilated buildings). In our study, the natural ventilation has little impact on this group of bacteria, whose composition and abundance remains very similar to those found with the windows closed, confirming the indoor air as a major source. Correspondingly, a higher abundance of potential pathogens was found indoors (Fig. 5), likely because the most abundant coincides with human-associated microbiota. Again, the influence of the window opening was minor for this distinct set (pathogens/aeroallergens) in both microbial groups bacteria and fungi. Consequently, these results highlight that natural ventilation through a window has a poor effect on removing these microorganisms from the atmosphere indoor in this case-of-study, indicating that additional mechanisms such as air filtering systems would be required. This conclusion is in agreement with a recent work conducted by Kwan et al. [63], which pointed out that the differences in the air exchange rates in naturally ventilated houses did not alter significantly the microbial concentration indoor.

New public buildings and workplaces have usually built-in HVAC (heating, ventilation and air conditioning) systems. These mechanisms have shown to reduce successfully the microbial load indoors in densely

populated buildings such as educational premises [64,65] or sensitive buildings like hospitals [66,67]. HVAC are especially useful to reduce fungal propagules compared to the concentrations outdoors, but bacterial ratio I/O tended to >1 regardless of the type of ventilation [64,68,69]. Our approach cannot determine total concentrations of the microorganisms. However, we found ratios I/O > 2 for the relative abundance of several microbial pathogenic genera, suggesting that the air outdoor carries less potentially harmful microorganisms or they are more diluted (Fig. S4). Similarly, previous studies have described I/O ratios >1.5 for bacteria (indicating a higher load indoors) and also for particular pathogens like *Staphylococcus* spp. or *Streptococcus* spp. in non-mechanically ventilated buildings [25,26,29,70]. On the contrary, the same studies showed global ratios I/O for fungi between 0.5 and 1, which implies similar concentrations between both environments. In accordance, our results did not show significant differences for potentially pathogenic fungi as a group (Figs. 4 and 5) but showed some differences in the ratios for specific genera. For instance, we found I/O > 2 for *Aspergillus*, *Cladosporium*, *Cryptococcus*, *Epicoccum* or *Penicillium*, which are well-known aeroallergens and potentially infectious. 10 out of 26 potentially harmful bacterial genera (~38%) reduced their relative abundance when the ambient air accesses to the room indoor for 2 h (samples WinO), while only 4 out of 17 (~23%) did for fungi. Consequently, additional mechanisms must be implemented to reduce dangerous microbes in indoor environments, a fact with special importance to fight against the current pandemic virus SARS-Cov-2. Finally, some points must be taken into account for the interpretation of our results. First, the room was not in use during the sampling periods, which makes it ideal for analyzing the exchange of the particles without any other perturbation. However, this particularity implies that the extrapolation to indoor environments where human activities are involved should be taken with caution because, not only new additional sources of microorganisms can be added but also the air turbulences may contribute to the re-suspension and dispersion of the particles. Second, the window was the only opening in the room. Hence, the exchange rate could be improved by opening other windows or doors in the floor to create air currents that increases the exchange rate and accelerates the mix of air masses between indoor and outdoor. New studies making these comparisons should be addressed in the future. In addition, the samples indoor were taken using only one sampler (collecting 2 replicates that were merged in one). Although the room was very small, we cannot assure that other points in the room would show some divergences. However, the sampler was located in the center of the room, right in front of the window, so it would be expected that other spots in the room beyond that point would be even less affected by the window opening.

On the other hand, additional data such as the ventilation rate or the gradient of temperature between indoor and outdoor, among other parameters, would provide a more thorough understanding of our results. In addition, meteorological factors like wind speed and direction may have an impact in the exchange of bioaerosols when the window is open, variables that we could not evaluate formally because of the limited number of samples. Nonetheless, our study is based on the comparison of paired-samples from several independent observations (one each day) and the results were very consistent (Fig. S5), despite the possible changes on these and other parameters. The effect of mechanical instruments like fans indoors, which are very popular in many countries during warm seasons, would be interesting to analyze in order to evaluate the efficiency to reduce the potentially harmful microbial load in interior rooms.

5. Conclusions

Our results suggest that outdoor air is the main source of bioaerosols for an isolated room indoor, although human-related bacteria and potentially harmful taxa accumulate in higher abundance in the latter. Natural ventilation through window opening has a low impact on the microbial composition and abundance and it should be supplemented with additional mechanisms to improve the exchange rate of bioaerosols inside the buildings.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.buildenv.2021.108438.

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