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Effects of seasonality and air pollution on the nasal microbiota in healthy Italian adults

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ABSTRACT

Air pollution is a major environmental risk factor for respiratory health, yet its interaction with seasonality in shaping the upper airway microbiota remains poorly understood. We conducted a longitudinal repeated-measures study to investigate whether seasonality modulates the effects of indoor and outdoor air pollution on the nasal microbiota of healthy adults. Twenty-six participants were sampled weekly for three weeks in winter and three weeks in summer . Microbial composition was characterized using 16S rRNA gene sequencing (124 samples) and whole-genome shotgun sequencing (141 samples). Weekly exposure to indoor total suspended particles (TSP) and outdoor pollutants (particulate matter, black carbon, benzene, and carbon monoxide) was assessed using environmental monitoring data. The nasal microbiota was stable within seasons but differed significantly between seasons, with winter enrichment of *Moraxella* species, particularly among women with children. Across seasons, higher pollutant levels were negatively associated with relative abundance of commensal taxa, particularly *Corynebacterium* species. In addition, this study identified significant season-pollutant interactions. For example, in summer, commensal bacteria (e.g., *Staphylococcus epidermidis* and *Cutibacterium granulosum*) were found to be negatively associated with particulate matter exposure. Among host factors, sex explained the largest proportion of variance in microbial diversity, while household characteristics contributed additional compositional variability. These findings indicate that the respiratory microbiome varies across seasons and is associated with air pollution, suggesting that both seasonality and environmental exposures can contribute to differences in respiratory microbial communities.

1. Introduction

Air pollution is one of the leading environmental risk factors for respiratory morbidity and mortality worldwide. In addition to its direct inflammatory and toxic effects, increasing evidence suggests that airborne pollutants may alter the ecological balance of the upper respiratory tract microbiota, a microbial community that plays a key role in maintaining airway homeostasis. The respiratory microbiota, has gained attention for its potential role in respiratory conditions including asthma, allergies, and viral infections (Kumpitsch et al., 2019; Hou et al., 2022; Man et al., 2017). However, the microbiome of the respiratory tract remains less well characterized compared to the microbiome of other sites (Man et al., 2017).

Given its direct exposure to the environment, characterizing the microbial community of the airways and its interactions with environmental factors is key to understanding how external influences shape its

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complexity. While there are studies on the seasonal variation of the gut microbiota (Davenport et al., 2014; Hisada et al., 2015; Smits et al., 2017; Zhang et al., 2014; Castonguay-Paradis et al., 2025; Kortekangas et al., 2020; Tani et al., 2023), very few studies look at the respiratory microbiota across seasons (Odendaal et al., 2024; Cai et al., 2023). Conversely, a larger number of studies have investigated associations between exposure to air pollutants and the respiratory microbiota (Arca-Lafuente et al., 2025; Qin et al., 2019; Solazzo et al., 2025; Mariani et al., 2018; Vieceli et al., 2023; Xue et al., 2020). For example, recent studies suggest that exposure to Particulate Matter (PM) can lead to a decrease of microbial diversity and an increase in the relative abundance of *Moraxella* and other opportunistic pathogens (Qin et al., 2019; Mariani et al., 2018). However, these studies did not consider the combined effects of seasonality and air pollution on the respiratory microbiome.

Host-related factors also contribute to shaping the respiratory microbiota. Age and sex have been associated with differences in microbial diversity and composition in the upper airways, and environmental and lifestyle exposures further influence community structure. Although the respiratory microbiome becomes more stable in adulthood, inter-individual variability remains substantial and may reflect both biological and environmental determinants (Odendaal et al., 2024; Biesbroek et al., 2014).

Taken together, these findings suggest that variation in the respiratory microbiota of healthy individuals is driven by the interplay of seasonality, air quality, and host characteristics. However, few studies have systematically evaluated the combined effect of these factors on the airway microbiota. In addition, no prior study has simultaneously examined seasonality, air pollution, and the nasal microbiome in a repeated-measures design. Moreover, most studies in this field have relied on 16S ribosomal RNA sequencing, a technique with limited resolution for analyzing microbial communities. We therefore conducted a longitudinal repeated-measures study to determine whether seasonal context modifies pollution–microbiota associations in healthy adults, integrating high-resolution microbial profiling with detailed assessment of indoor and outdoor air pollutant exposures.

2. Methods

2.1. Recruitment

Healthy volunteers employed by the University of Milan and the University of Insubria, Como, participated in a six-week study, which included three weeks in winter (November–March) and three weeks in summer (May–July). Flyers were used to inform potential participants about the study, and enrollment was based on volunteer availability. In addition, participants enrolled in the winter session were also asked to confirm their availability to participate again approximately five-six months later, during the summer session. Eligibility criteria for enrollment required participants to be aged 20–65 years, with no known infectious diseases, and not under medication or antibiotic treatment during the week of enrollment and the three weeks prior. Each participant completed a questionnaire to provide relevant information, including household and work environment information, smoking habits, participation in sports, and other weekly activities. The study received ethical approval from the Ethics Committee of the University of Milan (approval number 24/22) and was conducted in accordance with the principles of the Helsinki Declaration. Written informed consent was obtained from all participants.

2.2. Air pollution data collection

Indoor Total Suspended Particles (TSP) concentrations were measured weekly in 13 offices across 4 buildings (two at the University of Milan and two at the University of Insubria, Como) over a period of 6 weeks total. TSP sampling was conducted using a low-flow SKC air sampling pump attached to a filter cassette containing a 37-mm, 0.8-μm mixed cellulose esters (MCE) membrane filter. A detailed description of the collection and measurement procedures for TSP samples has been reported in a previous publication (Solazzo et al., 2025). In addition to the collection of indoor TSP samples, we also included the outdoor concentrations of five main air pollutants, i.e., Benzene, Black Carbon (BC), Carbon Monoxide (CO), and Particulate Matter (PM_{10} and $PM_{2.5}$), obtained from the Italian Regional Agency for Environmental Protection (ARPA) database. The selection of these pollutants was based on their biological importance and effects on the respiratory microbiota considering previous studies and the availability of their concentrations in the ARPA database (Solazzo et al., 2025; Mariani et al., 2018; Boniardi et al., 2025).

Air pollution data were obtained from the ARPA Lombardy monitoring network (https://www.arpalombardia.it/temi-ambientali/aria/rete-di-rilevamento/). Data quality was ensured through standardized protocols, routine instrument calibration, and validation by the Regional Metrological Office. Participants were georeferenced by residential address and linked to the nearest station. Hourly or daily pollutant data were used; for benzene and CO, 24-hour average weekly concentrations were derived from hourly values. For the other pollutants, for which only daily averages were available, weekly mean concentrations were determined.

2.3. Nasal swab collection, DNA extraction and sequencing

At the end of each monitoring week, swabs from the anterior nares were collected from each participant using FLOQSwabs (Copan, Italy). The swab was inserted into one nostril and slowly rotated 4–5 times while pressing gently against the nasal walls. The same swab was then used to sample the other nostril. To monitor contamination, clean swabs were opened and swirled in the room as negative controls during the collection process. All samples, including controls, were immediately stored at −80 °C after collection. DNA was extracted from the biological samples using the QIAamp UCP Pathogen Mini Kit spin column protocol (Qiagen, Hilden, Germany). Negative controls were included during the extraction process. Following DNA extraction, the DNA concentration was quantified using the Qubit dsDNA High Sensitivity Assay Kit (Invitrogen). An aliquot of the DNA extracted from anterior nares swabs was shipped to Personal Genomics Srl (Verona, Italy) for 16S rRNA gene sequencing. Library preparation for sequencing included amplification of the V3-V4 region using primers Pro314F and Pro805R. The library quality and quantity were assessed using the LabChip DNA High Sensitivity Kit (Perkin Elmer, Waltham, MA, USA) and the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) to generate 300 bp paired-end reads. The remaining DNA extracted from the anterior nares swabs was used for whole-genome shotgun sequencing on the Illumina NovaSeq 6000 S2 platform, as described in our previous paper (Solazzo et al., 2025).

2.4. Bioinformatics and statistical analyses

Bioinformatics. The nasal microbiome was analyzed using both 16S rRNA and WGS data. The 16S rRNA data were used to determine microbial diversity and relative abundance at the genus level, while WGS data were used to analyze species level taxonomy. The paired-end sequencing data (150 bp read length) from the WGS were demultiplexed and trimmed using Bcl2fast (v 2.20) and fastp (v 0.20.1), respectively. Taxonomic classification was performed using Kraken (v 2.1.2) and Bracken (v 2.5). Further details are provided in (Solazzo et al., 2025). The sequences generated from the 16S rRNA sequencing were imported and denoised using QIIME 2 v. 2022 DADA2 pipeline (Bolyen et al., 2019). Taxonomy was assigned using a pre-trained Naïve Bayes classifier (Silva database, release 138, 99%). The QIIME outputs were imported in R using the *qiime2R* package (v. 0.99.6) to perform the

diversity and taxonomic analyses. The following R packages were used to analyze the microbiota: phyloseq (1.46), microbiome (1.24), microViz (0.12), pctax (0.1.3), microeco (1.12).

We used the 16S rRNA data to analyze both alpha and beta diversity. Alpha (α) diversity was estimated using the Shannon Index. The difference in alpha diversity between the groups was tested using the Wilcoxon test. Beta (ß) diversity was estimated with a Bray-Curtis distance matrix, and the difference in beta diversity was calculated using the permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. The redundancy dimension analysis was performed using the pctax R package (v. 0.1.3). Bacterial abundance was centered log ratio (CLR) transformed before running the analysis. The differences in bacterial relative abundance were analyzed with the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) package (v. 1.6.4). The ANCOM-BC was performed using the default settings, and the mean difference (W) was considered significant when a p-value < 0.05 and FDR < 0.10 were reached. For the host factors, the ANCOM model included sex, age, BMI, smoking habits, allergies, owning furry pets, having a child, and active in sports. Monitoring time was included in the model to account for potential bias, and the season was used included as fixed effect.

Statistical analysis. The relationship between air pollution exposure and nasal microbiota was first assessed by testing correlations between each pollutant concentration and the CLR-transformed abundance of the top 15 bacterial species using a linear mixed-effects model with a random intercept for subject. Subsequently, season-pollutant interaction were tested using linear mixed-effects models fitted separately for each pollutant-taxon combination. CLR-transformed bacterial relative abundance was the dependent variable. Pollutant exposure, season, and their interaction term were included as fixed effects, along with indoor TSP, smoking habits, having a child, and allergies. A random intercept for subject was included in all models to account for within-subject correlation due to repeated measurements. Marginal season-specific estimates and their 95% confidence intervals were derived for each pollutant–taxon combination. Denominator degrees of freedom were estimated using the Kenward-Roger approximation and vary across models due to differences in the number of available observations per pollutant–taxon combination. Of the 156 planned observations (26 participants × 6 weekly visits), 141 were available for WGS analysis and 124 for 16S rRNA analysis. Missing exposure measurements occurred in 9 out of 26 participants, accounting for 15 observations (10.6% of the 141 WGS observations); no participant had >3 missing exposure measurements out of 6 visits. No missing exposure measurements were present in the 16S rRNA dataset. Missing data were handled implicitly by the mixed-effects models under a missing-at-random assumption. To account for multiple comparisons in the interaction analysis, FDR-adjusted p-values were computed using the Benjamini–Hochberg procedure, applied separately for each pollutant across all bacterial species tested. All statistical analyses were si graperformed with R software v 4.2.1 and SAS software v 9.4.

3. Results

3.1. Population description

This study includes 26 healthy volunteers employed by the University of Milan (N = 17) and the University of Como (N = 9), (Table 1). Participants were monitored in the winter season (November 2021 to March 2022) and the summer season (May to July 2022) for a total of 6 weekly samplings resulting in 124 samples for 16S analysis and 141 samples for WGS analysis (Supplemental Fig. 1). Each participant completed a weekly questionnaire to provide information about their activities during the monitoring weeks, including days spent in the office, use of face masks at work, smoking habits, use of antibiotics, and other relevant factors. Most subjects (~ 62%) were in the healthy weight category (BMI range: 18.5–24.9), with the rest in the overweight range (BMI range: 25–29.9). Additionally, most of the participants were non-smokers (73%), and none of them shared indoor spaces with smokers. About half of the participants declared doing regular physical activity, although a decline was observed during the summer season (Table 1). None of the participants used antibiotics during the sample collection period or the weeks before the sampling. However, about 30% reported a mild allergic reaction to pollen, pets, dust, or mold. Most participants shared living quarters with other adults, and a small proportion had children (~20%) with age equal or lower than 10 yrs old, and/or pets (~30%). When comparing personal habits between the two seasons (e.g., physical activity, use of antibiotics, days in the office), no significant differences were observed, except for face mask usage in the office. Face mask use was significantly higher in the winter compared to the summer (Fisher's test, OR = 20.2, p-value = 0.01). This difference is a consequence of COVID-19 risk management protocols in public spaces and workplaces that were different between the two seasons. Although face

Table 1
Demographics, lifestyle, household, and work environment information on participants.

Characteristics	(N = 26)	
***Sex,* n (%)**		
Female	17.0 (65%)	
Male	9.0 (35%)	
***Age*, mean (SD)**	38.9 (8.7)	
***BMI*, mean (SD)**	24.3 (3.2)	
***Smoking*, n (%)**		
Yes	7.0 (27%)	
No	19.0 (73%)	
Allergies*[1], *n (%)		
Yes	10.0 (38%)	
No	16.0 (62%)	
Residential area, n (%)		
City	12.0 (46.2%)	
Small town	13.0 (50%)	
Rural area	1.0 (3.8%)	
***N of people in the household,* n (%)**		
0	3.0 (12%)	
1	9.0 (35%)	
2	4.0 (15%)	
3	10.0 (38%)	
***Number of people in the office*, n (%)**		
2 or higher	24.0 (92%)	
1	2.0 (8%)	
***Have children,* n (%)**		
Yes	7.0 (27%)	
No	19.0 (73%)	
***Have pets,* n (%)**		
Yes	10.0 (38%)	
No	16.0 (62%)	
***Office ventilation system,* n (%)**		
Operable windows	16.0 (62%)	
Force mechanical HVAC[2] system or hybrid	10.0 (38%)	
Differences observed between the two seasons	**Winter**	**Summer**
***Physical activity*, n (%)**		
Yes	14.0 (54%)	9.0 (35%)
No	12.0 (46%)	17.0 (65%)
Recent vaccination, n (%)		
Yes	2.0 (8%)	0.0
No	24.0 (92%)	26.0 (100%)
***Days in the office per week,* mean (SD)**	4.4 (0.8)	4.2 (0.8)
***Use of mask in the office*[3], n (%)**		
Yes	17.0 (65%)	7.0 (27%)
No	9.0 (35%)	19.0 (73%)
***Office sanitation,* n (%)**		
Yes	2.0 (8%)	0.0
No	24.0 (92%)	26.0 (100%)

Note.
[1] Food allergies not included.
[2] Heating, ventilation, and air condition system.
[3] This variable was significant different between the two seasons (p-value = 0.01).

mask use differed significantly between seasons, it was not included as an additional covariate in the microbiome analysis because preliminary analyses in our cohort did not provide evidence of a significant association between mask use and respiratory microbiome (data not shown). Some other minor, but not significant, differences among the seasons include office sanitation and recent vaccination. Office sanitation was performed in two offices during the winter season, like the use of face masks, as a part of COVID-19 risk management. The difference in vaccination is due to the winter campaign beginning at the end of the seasonal flu vaccination period (Table 1).

3.2. *The nasal microbiota of healthy subjects' changes across seasons*

We characterized the nasal microbiota in healthy volunteers to determine variations across seasons. The samples were analyzed by 16S rRNA sequencing and WGS. A detailed description of the sample collection and sequencing is reported in Supplemental Fig. 1. After quality filtering, we retained 16S rRNA data of 124 samples (median sampling depth = 13,241 reads, range: 4106 – 57,834 reads) and metagenomic data of 141 samples (median sampling depth = 3506,075 reads, range: 2180,154 – 94,975,134) for taxonomic assignment and other downstream analyses. The 16S rRNA data were used to determine microbial diversity changes across the seasons and the differences in genus relative abundance, while the metagenomic data were used to study changes at the species level. Before analyzing seasonal differences, we examined whether there were variations in the nasal microbiota across the three weeks within the same season. No significant differences were found in alpha diversity (within-sample diversity) or beta diversity (between-sample diversity) across weeks. Conversely, significant variations between seasons were observed. Alpha diversity was higher in summer compared to winter (Wilcoxon test, p-value = 0.001), with approximately 70% of the summer samples displaying higher diversity than their corresponding winter samples (Fig. 1A). In addition, seasonality accounted for about 5% of the variance in beta diversity (PERMANOVA, p-value = 0.001). In both seasons, we identified 10 dominant bacterial genera across all samples, including common airway-associated taxa such as *Corynebacterium, Dolosigranulum, Moraxella, Streptococcus*, and *Staphylococcus* (Fig. 1B). Most samples (85%) had

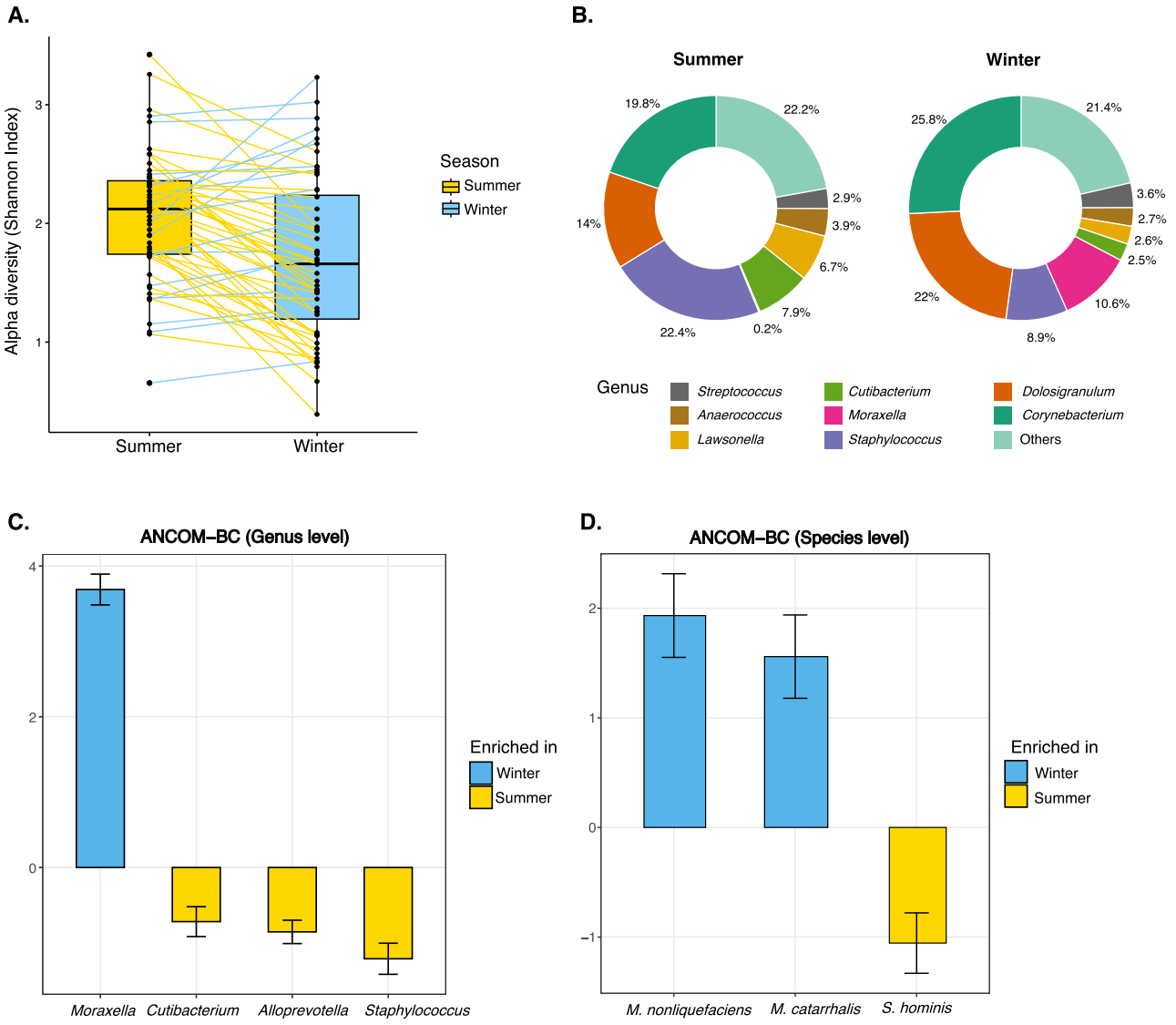

Fig. 1. Seasonal variation in nasal microbiota of healthy adults. A. Alpha diversity comparisons using the 16S rRNA data between microbiota of healthy subjects in summer (yellow boxplot) and winter (blue boxplot). The line connects the alpha diversity values of the same subjects between the two sampling times. When the alpha diversity is higher in summer, the line is yellow, when the alpha diversity is higher in winter, the line is blue. The alpha diversity was estimated using the Shannon Index. B. Each donut plot represents the relative abundance of the top genera identified in both seasons. C-D. ANCOM-BC analysis on nasal microbiota of healthy individuals. C. Analysis performed at the genus level using 16S rRNA sequencing data. D. Analysis performed at the species level using WGS data.

microbiota dominated by Gram-positive bacteria. Significant correlations among Gram-positive bacteria were identified (Fig. 2). We observed that the relative abundance of *Dolosigranulum* has a negative correlation with the relative abundance of other common Gram-positive commensals, including *Cutibacterium* (rho = −0.55), *Staphylococcus* (rho = −0.50), and *Streptococcus* (rho = −0.35). Conversely, *Staphylococcus* was positively correlated with *Streptococcus* (rho = 0.40) and *Cutibacterium* (rho = 0.46). At the species levels, we were still able to observe these correlations (Fig. 2).

In addition to differences in microbial diversity, we observed significant variations in bacterial relative abundance between seasons. At the genus level, the relative abundance of *Moraxella* was higher in the winter, while it was lower for *Staphylococcus, Alloprevotella,* and *Cutibacterium* (Fig. 1C). At the species level, the relative abundance of two species of *Moraxella* (*M. catarrhalis, M. nonliquefaciens*) was higher in the winter and one species of *Staphylococcus* (*S. hominis*) was higher in the summer (Fig. 1D). These findings suggest that while the nasal microbiota remains stable over a period of weeks, it is significantly influenced by seasonal changes

3.3. Seasonality influences the effects of air pollution exposure on commensal bacteria

We investigated whether, beyond seasonal variations, exposure to air

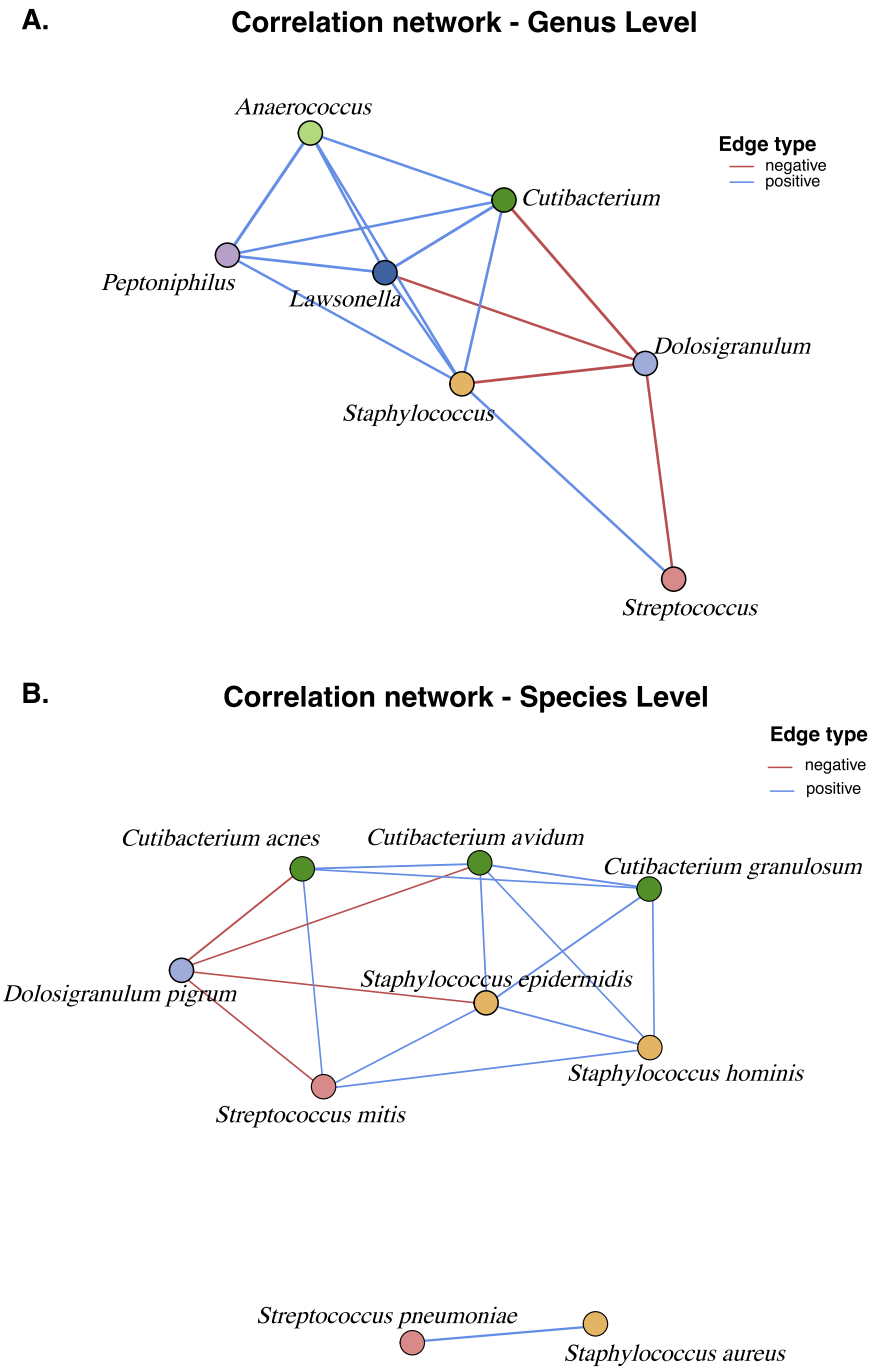

Fig. 2. Taxa correlation networks in the nasal microbiota of healthy subjects. The networks were generated using MetaNet. Each node represents a different taxon. The red edges indicate negative correlations between genera, the blue edges indicate positive correlations. A. Correlation network created from the 16S rRNA sequencing data (genus level). B. Subnetwork created with the WGS data (species level) focusing on the genera found already significant in the genera network.

pollution affects the nasal microbiota of healthy individuals. For this, we assessed the combined effects of season and air pollution on the respiratory microbiota. We analyzed five outdoor air pollutants, including gaseous pollutants (e.g., Benzene, CO) and airborne particles (e.g., PM_{10}, $PM_{2.5}$, BC). Additionally, we also considered indoor TSP exposure measured inside the offices. Overall, outdoor air pollution concentrations were higher in winter than in summer, whereas the weekly measurements of indoor TSP did not show significant variations between seasons (Table 2). As expected, positive correlations were identified among air pollutants in winter (Fig. 3A) and summer (Fig. 3B), with a particularly strong correlation between PM_{10} and $PM_{2.5}$ concentrations. When we analyzed the effects of air pollution on the microbiota, alpha diversity was negatively correlated with all pollutants, but the correlation was statistically significant only for BC (Pearson, rho = −0.2, p = 0.04). In contrast, beta diversity was significantly associated with all pollutants and explained about 2–3% of the diversity (Supplemental Table 1). After analyzing the effects on microbial diversity, we investigated the effects on the top 15 taxa. We observed significant negative associations between Gram-positive commensals and air pollution. Specifically, there was a negative association between two *Corynebacteria* species (e.g., *C. accolens, C. striatum*) and outdoor exposure to Benzene (*C. accolens*, beta = −0.29, p-value = 0.02; *C. striatum*, beta = −0.22, p-value = 0.02), $PM_{2.5}$ (*C. accolens*, beta = −0.02, p-value = 0.005; *C. striatum*, beta = −0.01, p-value = 0.005), PM_{10} (*C. accolens*, beta = −0.01, p-value = 0.02; *C. striatum*, beta = −0.01, p-value = 0.02), and BC (*C. accolens*, beta = −0.16, p-value = 0.007; *C. striatum*, beta = −0.13, p-value = 0.005). In the interaction analysis, we observed significant interactions between seasonality and air pollution, at the species level. After FDR correction (Benjamini-Hochberg, applied separately for each pollutant across all 20 species tested), the interaction between benzene exposure and season for *Dolosigranulum pigrum* was statistically significant (p = 0.001, p-FDR = 0.020): in summer, benzene exposure was positively associated with the relative abundances of this species, while no significant association was observed in winter. Additional nominally significant interactions, between PM_{10} and *Staphylococcus epidermidis* (p = 0.042), $PM_{2.5}$ and *Cutibacterium granulosum* (p = 0.027), did not survive FDR correction (p-FDR = 0.06–0.28) and should therefore be interpreted as exploratory associations (Fig. 4– Supplemental Table 2). At the genus level, in the summer air pollution was negatively associated with the relative abundance of *Staphylococcus*, and positively associated with the relative abundance of *Dolosigranulum*, although these associations did not reach significance (Supplemental Table 3). While our findings confirm the negative correlations already observed in previous studies between commensal bacteria and these pollutants, our analyses indicate how seasonality influences the effects of air pollutants on the respiratory microbiota.

Table 2
Air pollutant concentration in winter and summer.

Type of exposure	Concentrations, Median (Q1-Q3)	Winter	Summer	p-value
Indoor	***TSP exposure [μg/m^3]***	33.7 (25.1 – 40.6)	26.0 (20.7 – 36.7)	0.5
Outdoor	***PM$_{10}$ exposure [μg/m^3]***	38.9 (30.4 – 46.3)	23.9 (17.9 – 26.2)	< 0.001
	PM$_{2.5}$ *exposure [μg m^3]*	29.2 (24.2 – 37.1)	14.0 (11.4 – 16.3)	< 0.001
	Black Carbon [μg/m^3]	2.3 (2.1 – 3.1)	0.8 (0.7 – 0.9)	< 0.001
	Benzene [μg/m^3]	1.1 (1.0 – 1.8)	0.5 (0.5 – 0.8)	< 0.001
	Carbon monoxide (CO) [mg/m^3]	0.6 (0.5 – 0.8)	0.3 (0.3 – 0.4)	< 0.001

3.4. Host factors and their impact on the nasal microbiota of healthy adults

After analyzing the effects of external factors (i.e., seasonality and air pollution) we investigated the influence of host factors (i.e., sex, age, BMI) and behaviors (e.g., smoking habits, owning a furry pet, having a child) on the nasal microbiota of healthy adults. Among the host factors, sex had the most significant impact on the nasal microbiota. Males exhibited higher alpha diversity (Wilcoxon test, p-value < 0.001) than females in both seasons (Supplemental Fig. 2). Beta diversity also differed significantly between males and females (PERMANOVA, R^2 = 0.06, p-value = 0.001). This difference seems driven by enrichment of Gram-positive bacteria in males, as also observed from the ANCOM-BC analysis of WSG data (Supplemental Fig. 3). In contrast, age had a limited impact on the nasal microbiota as it did not significantly correlate with microbial diversity, and only a few associations with bacterial relative abundance were observed, such as *Corynebacterium propinquum* (W = 3.47, p-value adj = 0.05), *Corynebacterium pseudodiphtheriticum* (W = 3.46, p-value adj = 0.03), and *Prevotella buccalis* (W = 3.53, p-value adj = 0.03). BMI had a positive correlation with alpha diversity (rho = 0.35, p-value < 0.001), and appeared to influence beta diversity (PERMANOVA, R^2 = 0.04, p-value = 0.001). However, no significant associations were identified between BMI and bacterial relative abundance at either the genus or species levels. When considering other host factors (smoking habits, physical activity, allergies, owning a furry pet, and having a child), physical activity was the only factor that did not show any significant correlations with microbial diversity. All the other variables explain part of the microbial diversity observed across the samples (Fig. 5A and B). Smoking and allergies are associated with increased alpha diversity (Wilcoxon, p-value < 0.05), while people who have kids and/or own furry pets tend to have lower alpha diversity (Fig. 5A and B). In addition, allergies had a negative impact on the relative abundance of several common commensals of the human airways suggesting an increase in relative abundance of other bacteria (Fig. 6). Finally, in subjects with children, we observed an enrichment in *Moraxella nonliquefaciens* (ANCOM-BC, W = 4.8), and *Moraxella catarrhalis* (ANCOM-BC, W = 4.0). This suggests a potential transmission from children to adults. However, this enrichment was observed only in women and not in men with children. The significant increase in *Moraxella* relative abundance was mostly in the winter (from <5% to >50%) and resulted in significantly lower microbial diversity in the winter. In contrast, during the summer, we did not find notable differences between people with children and other individuals. Taken together, these analyses indicate that sex, pet ownership, presence of children, and mild allergies are key determinants of microbial diversity and composition in healthy adults.

4. Discussion

Air pollution represents a major environmental determinant of respiratory health, yet its impact on the upper airway microbiota remains incompletely understood. In this exploratory observational longitudinal study, we demonstrate that seasonality influences pollution–microbiota associations in healthy adults. Across the two seasons, while individual characteristics and habits remained stable, significant changes were observed in nasal microbial diversity and composition. In over 70% of participants, microbial diversity increased in the summer, driven by a higher abundance of Gram-positive commensals. In contrast, winter samples showed enrichment of Gram-negative species, particularly *Moraxella catarrhalis* and *M. nonliquefaciens*, which dominated the microbiota and reduced overall diversity. This antagonistic relationship between *Moraxella* species and Gram-positive commensals in the upper airways has been documented in previous studies. The Gram-positive bacteria usually found negatively associated with *Moraxella* include *Corynebacterium* and *Dolosigranulum*. These two bacteria have been negatively associated with *Moraxella* in several studies, with one

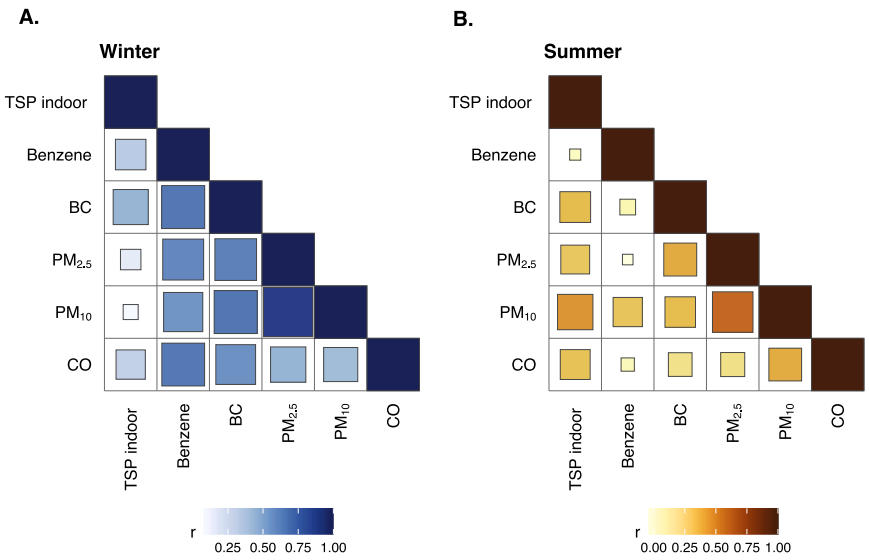

Fig. 3. Correlations between air pollutant concentrations in winter (A) and summer (B). Correlations were calculated using the Pearson correlation test, with color intensity indicating the Pearson correlation coefficient (rho, r). Darker colors represent higher r values.

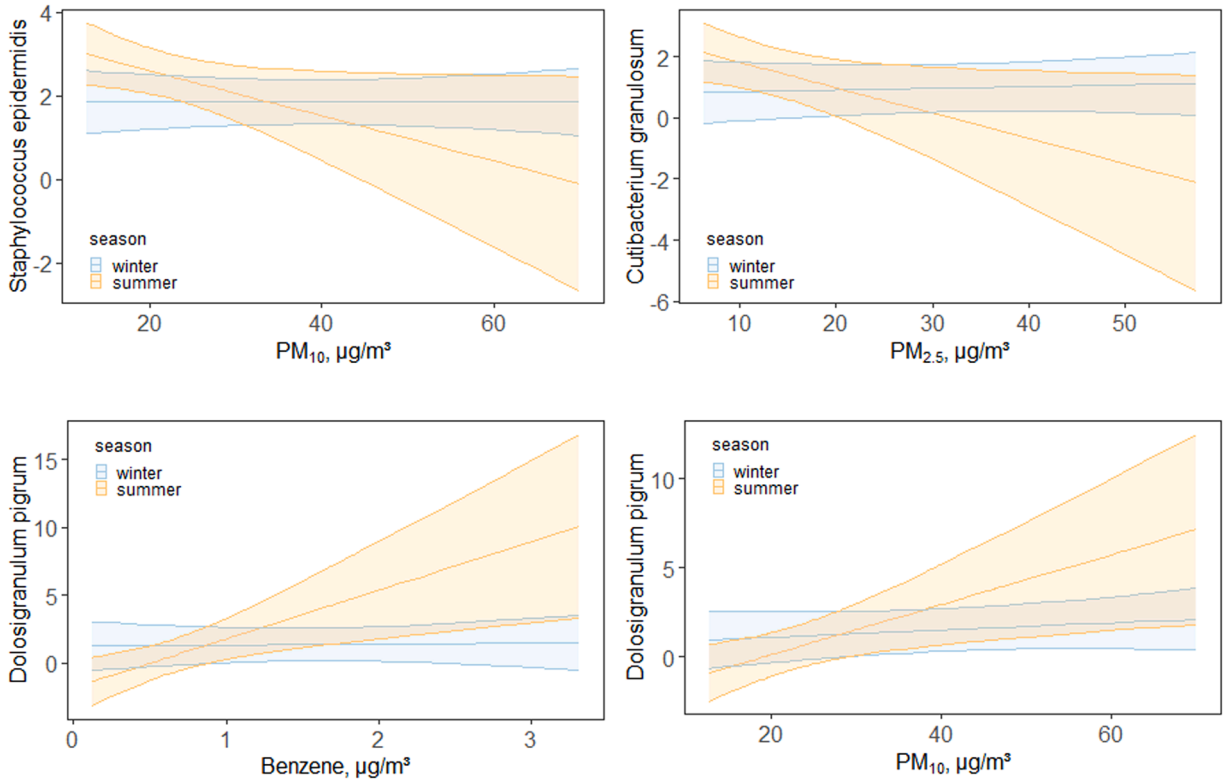

Fig. 4. Association between weekly exposure to air pollutants and CLR-transformed bacterial abundance, stratified by season. Marginal means and 95% confidence intervals were derived from linear mixed-effects models including an interaction term between air pollutant levels and season. All models were adjusted for indoor TSP exposure, having a child, smoking habits, and allergy status. Random intercepts were included for each subject to account for repeated measures. Lines represent predicted values by season; shaded areas indicate 95% confidence intervals.

experimental study showing that *Corynebacterium* can inhibit the growth of *Moraxella*. However, the mechanism behind this growth inhibition is still unclear (Lappan and Peacock, 2019). To date, only one study has investigated seasonal variations in the respiratory microbiome of healthy adults. Consistent with our findings, it reported reduced microbial diversity in winter compared to summer, along with an increased relative abundance of *Moraxella* during the winter season (Odendaal et al., 2024). Some similar studies were performed in children with asthma (Boniardi et al., 2025; McCauley et al., 2022). Also in children, during the cold seasons Gram-negative bacteria like *Moraxella* were found to be enriched and to correlate with several respiratory viruses (e.g., Rhinovirus and RSV), which are typically less prevalent in the summer season (Monto, 2002; Dissanayake et al., 2022; Jeannoël et al., 2019).

We included in our analysis indoor exposure to TSP and outdoor exposure to PM_{10}, $PM_{2.5}$, BC, Benzene and CO. Both indoor and outdoor

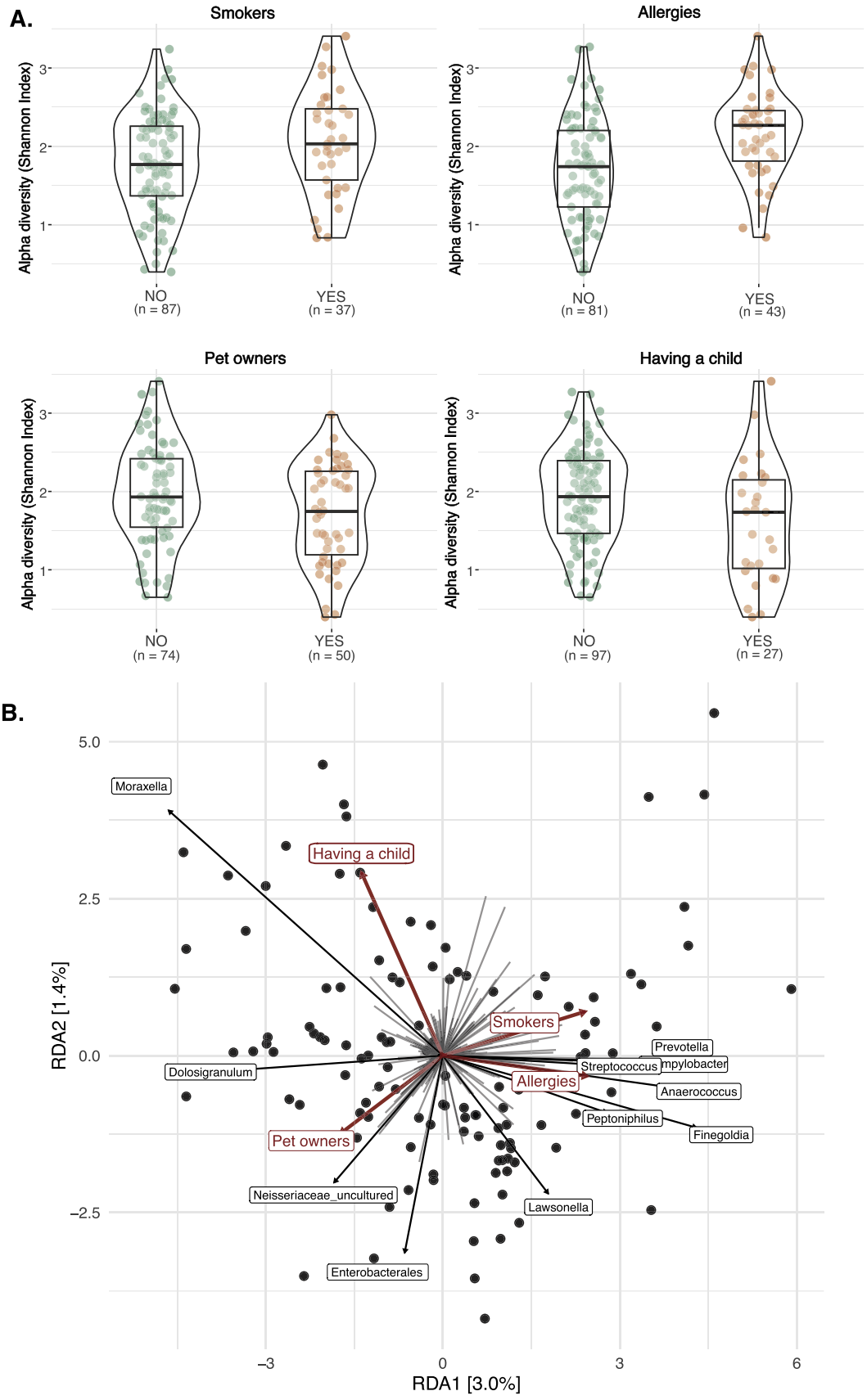

Fig. 5. (A) Differences in microbial alpha diversity, measured using the Shannon Index, show that smokers and individuals with mild allergies have higher alpha diversity compared to non-smokers and those without allergies (Wilcoxon, p-value < 0.05). Conversely, having a child or owning a pet is correlated with lower alpha diversity, but this difference was not significant. (B) Redundancy analysis plot shows in red the variables that significantly explain part of the beta diversity variance observed across the samples. The black arrows indicate the bacterial genera that contribute to this variability.

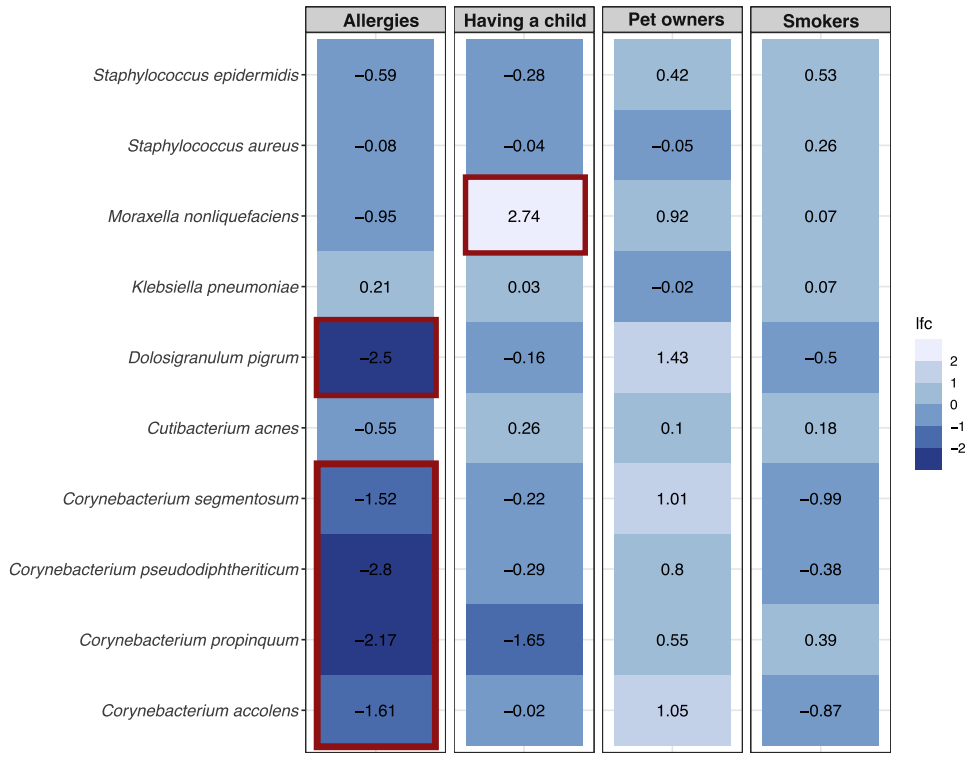

Fig. 6. Heatmap showing the results from the ANCOM-BC analysis. The y axis lists the 10 main bacterial species identified in the nasal microbiota; the x axis lists the variables tested in the analysis. The intensity of the blue color indicates the log-fold change value estimated by the ANCOM-BC analysis. The red boxes highlight the significant results (p-value < 0.05, FDR < 0.05) that also passed the sensitivity test.

exposures were associated with microbial diversity and composition. As previously observed (Arca-Lafuente et al., 2025), exposure to air pollution negatively correlates with the relative abundance of *Actinomycetota*, such as *Corynebacterium*. The presence of certain species of this genus (e.g., *C. accolens. C. segmentosum*) in the respiratory microbiome is known to play a protective role against respiratory conditions and it has been found negatively associated with air pollution in other studies (Arca-Lafuente et al., 2025; Lappan and Peacock, 2019). However, we show how seasonality influences this relationship in healthy adults. Specifically, we found significant interactions between air pollution exposure and the summer season. This difference may reflect seasonal variations in exposure patterns or environmental conditions, including meteorological factors such as temperature and humidity, which co-vary with season and may influence both pollutant concentrations and mucosal immune responses. In winter, significant pollution–microbiota interactions were not observed. This finding coincides with the marked enrichment of Moraxella species characteristic of the winter season, which substantially alters the overall microbial community structure compared to summer. The directional trends observed in winter, while not statistically significant, may represent exploratory signals worthy of investigation in larger studies with greater statistical power. After FDR correction, the only statistically robust interaction was observed for *Dolosigranulum pigrum* and benzene exposure: in summer, benzene was positively associated with the relative abundance of this species ($p = 0.001$, p-FDR $= 0.020$), while no significant association was observed in winter. Additional exploratory associations — including negative associations of Staphylococcus epidermidis and *Cutibacterium granulosum* with particulate matter, and a positive association of *Dolosigranulum pigrum* with PM10, in summer — were nominally significant but did not survive FDR correction and warrant confirmation in larger studies.

This positive association of *Dolosigranulum* could be due to a decrease in the relative abundance of *Staphylococcus* and *Cutibacterium*, since these genera tend to be antagonistic in the upper airways (Yu et al., 2025), as also suggested by our network analysis. However, our study is observational, and functional studies are needed to assess whether air pollution causes a decrease in commensal bacteria. Overall, these significant interactions between seasonality and air pollution highlight the importance of including seasonality when studying the effects of outdoor air pollution on the respiratory microbiome.

We also examined the influence of host factors on the nasal microbiome in healthy adults. We observed that, in both seasons, females tend to have a microbiota with lower diversity compared to males. Additionally, males exhibited a higher relative abundance of several genera, including those less represented in the respiratory microbiota. This was also observed in a previous study on the microbiota of the nasopharynx where the authors found a higher abundance of *Finegoldia, Peptoniphilus*, and *Anaerococcus* in males 15 years and older (Odendaal et al., 2024). Interestingly, in our study as well as previously observed (Solazzo et al., 2022), the increase in *Moraxella* during the winter season is mostly seen in females who have a young child. This suggests a potential household transmission between mothers and children, but to date no studies on direct transmission of *Moraxella* have been performed. Previous studies on respiratory microbiome transmission suggested that both commensals and pathogens might be transmitted between co-habitants, especially those in close contact (Ren et al., 2025). The hypothesis of a potential household transmission of *Moraxella* needs to be confirmed by mechanisticstudies, and the sex-specific pattern warrants further investigation to clarify whether behavioral or biological mechanisms are involved. In our analysis, we also found that even if the age range of our subjects was relatively wide—from 26 to 65 yrs old– age was not a factor associated with the nasal microbiome. This suggests that even if over the lifetime the nasal microbiome changes, the differences observed in adults are driven by other factors. Among these, we found that owning a furry pet influenced the nasal microbiota, as previously described by other studies (Lederer and Endres, 2024). In our study smoking habits seemed to have a slight effect on the nasal microbiota, while other studies found a greater impact of tobacco smoking habits on individuals. This might be a consequence of our cohort composition as only a small

percentage of the subjects involved are smokers and all declared no daily exposure to passive smoke.

Overall, our study suggests that seasonality, air pollution, and host factors collectively shape the nasal microbiota of healthy individuals. These findings support the concept that environmental exposures interact with airway microbial ecology in a context-dependent manner.

We acknowledge some limitations of the present study. First, the relatively small sample size (n = 26) may limit statistical power and the generalizability of the findings. Nevertheless, the repeated within-subject sampling across two seasons strengthens the robustness of temporal comparisons. Second, the proportion of variance in beta diversity explained by air pollution was modest (approximately 2–3%), reflecting the multifactorial nature of microbiome composition, where environmental and host-related factors simultaneously contribute to community structure. Moreover, meteorological variables — including temperature, relative humidity, wind speed, and rainfall — are plausible modulators of both pollutant dispersion and host–microbiome interactions, and could partly underlie the seasonal differences in pollution–microbiota associations observed in our study. Sensitivity analyses including these variables did not identify independent meteorological effects in our dataset, likely due to their strong collinearity with the season term and the limited sample size; however, future larger studies should model meteorological exposures explicitly to disentangle their contribution from that of season per se. In addition, the indoor TSP was assessed only in the office settings, future studies should consider the combined effect of both household and office indoor TSP exposure. Outdoor pollutant concentrations were assigned based on the nearest ARPA Lombardy monitoring station to each participant's residential address, without accounting for commuting routes. Although participants completed weekly questionnaires capturing workplace attendance and relevant lifestyle factors, personal inhalation exposure may not be fully captured by these area-level and office-level proxy measures. This represents an additional source of potential exposure misclassification that should be addressed in future studies through personal monitoring devices or time-activity models. Participants were healthy adults residing in urban areas of Northern Italy, which may limit extrapolation to populations with different environmental exposures, climatic conditions, or health status. Finally, genetic information was not available for this study. Genetic factors may influence susceptibility to allergic conditions and host–microbiome interactions and could therefore contribute to part of the inter-individual variability observed in the nasal microbiota. However, the primary aim of this study was not to investigate the genetic determinants of allergic disease, and allergy-related analyses were exploratory in nature. Future studies integrating genomic, environmental, and microbiome data in larger and geographically diverse populations are needed to validate and extend these observations and to better understand the complex interplay between host genetic factors, environmental exposures, and respiratory microbiome dynamics.

5. Conclusion

This exploratory study demonstrates that seasonality influences the association between air pollution exposure and the nasal microbiota in healthy adults. Beyond direct pollutant–microbiota relationships, our findings indicate that environmental context plays a critical role in shaping airway microbial ecology. Host-related factors, including sex and household characteristics, further contribute to inter-individual variability. These results underscore the importance of considering sampling season and host-related factors when evaluating the impact of air pollution on the respiratory microbiome.

Ethics approval

This study was approved by the Ethics Committee of the University of Milan (approval number 24/22), in agreement with the principles of the Helsinki Declaration.

CRediT authorship contribution statement

Giulia Solazzo: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sabrina Rovelli:** Writing – original draft, Investigation, Conceptualization. **Simona Iodice:** Formal analysis, Data curation. **Andrea Spinazzé:** Writing – review & editing, Resources. **Domenico Maria Cavallo:** Writing – review & editing, Resources. **Valentina Bollati:** Writing – review & editing, Supervision. **Elodie Ghedin:** Writing – review & editing, Resources. **Luca Ferrari:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Conceptualization.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.heha.2026.100189.

Data availability

Sequencing data from this study are available in the Sequence Read Archive (SRA) under the following accession number: PRJNA1129090 and PRJNA1013427.

References

Arca-Lafuente, S., Nuñez-Corcuera, B., Ramis, R., Karakitsios, S., Sarigiannis, D., García, Dos, Santos, S., et al., 2025. Effects of urban airborne particulate matter exposure on the human upper respiratory tract microbiome: a systematic review. Respir. Res. 26, 118. https://doi.org/10.1186/s12931-025-03179-9.

Biesbroek, G., Tsivtsivadze, E., Sanders, E.A.M., Montijn, R., Veenhoven, R.H., Keijser, B. J.F., et al., 2014. Early respiratory microbiota composition determines bacterial succession patterns and respiratory health in children. Am. J. Respir. Crit. Care Med. 190, 1283–1292. https://doi.org/10.1164/rccm.201407-1240OC.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., GA, Al-Ghalith, et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. Nat. Publish. Gr. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Boniardi, L., Solazzo, G., Favero, C., Campo, L., Ferrari, L., Fustinoni, S., 2025. Short-term personal exposure to multiple air pollutants affects nasal microbiota in school-age children. Sci. Tot. Environ. 981, 179588. https://doi.org/10.1016/j.scitotenv.2025.179588.

Cai, S., Gao, J., Liu, X., Yang, J., Feng, D., Li, G., et al., 2023. Seasonal dynamics of the upper Respiratory tract microbiome in chronic obstructive pulmonary disease. Int. J. Chron. Obstruct. Pulmon. Dis. 18, 1267–1276. https://doi.org/10.2147/COPD.S403198.

Castonguay-Paradis, S., Demers-Potvin, É., Rochefort, G., Lacroix, S., Perron, J., Martin, C., et al., 2025. Seasonal variations in circulating endocannabinoidome mediators and gut microbiota composition in humans. Gut Microb. 17, 2476563. https://doi.org/10.1080/19490976.2025.2476563.

Davenport, E.R., Mizrahi-Man, O., Michelini, K., Barreiro, L.B., Ober, C., Gilad, Y., 2014. Seasonal variation in human gut microbiome composition. PLoS One 9, e90731. https://doi.org/10.1371/journal.pone.0090731.

Dissanayake, E., Brockman-Schneider, R.A., Stubbendieck, R.M., Helling, B.A., Zhang, Z., Bochkov, Y.A., et al., 2022. Rhinovirus increases Moraxella catarrhalis adhesion to the respiratory epithelium. Front. Cell Infect. Microbiol. 12, 1060748. https://doi.org/10.3389/fcimb.2022.1060748.

Hisada, T., Endoh, K., Kuriki, K., 2015. Inter- and intra-individual variations in seasonal and daily stabilities of the human gut microbiota in Japanese. Arch. Microbiol. 197, 919–934. https://doi.org/10.1007/s00203-015-1125-0.

Hou, K., Wu, Z.-X., Chen, X.-Y., Wang, J.-Q., Zhang, D., Xiao, C., et al., 2022. Microbiota in health and diseases. Sig transduct target ther. Nat. Publish. Gr. 7, 1–28. https://doi.org/10.1038/s41392-022-00974-4.

Jeannoël, M., Lina, G., Rasigade, J.P., Lina, B., Morfin, F., Casalegno, J.S, 2019. Microorganisms associated with respiratory syncytial virus pneumonia in the adult population. Eur. J. Clin. Microbiol. Infect. Dis. 38, 157–160. https://doi.org/10.1007/s10096-018-3407-3.

Kortekangas, E., Kamng'ona, A.W., Fan, Y.-M., Cheung, Y.B., Ashorn, U., Matchado, A., et al., 2020. Environmental exposures and child and maternal gut microbiota in rural Malawi. Paediatr. Perinat. Epidemiol. 34, 161–170. https://doi.org/10.1111/ppe.12623.

Kumpitsch, C., Koskinen, K., Schöpf, V., Moissl-Eichinger, C., 2019. The microbiome of the upper respiratory tract in health and disease. BMC Biol. 17, 87. https://doi.org/10.1186/s12915-019-0703-z.

Lappan, R., Peacock, C.S., 2019. Corynebacterium and dolosigranulum: future probiotic candidates for upper respiratory tract infections. Microbiol. Aust. 40, 172–177. https://doi.org/10.1071/MA19051. CSIRO PUBLISHING.

Lederer, A.-K., Endres, K., 2024. The upper airway microbiota: how host and environment shape this ecological niche. Sig transduct target ther. Nat. Publish. Gr. 9, 1–3. https://doi.org/10.1038/s41392-024-01996-w.

Man, W.H., de Steenhuijsen Piters, W.A.A., Bogaert, D., 2017. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat. Rev. Microbiol. 15, 259–270. https://doi.org/10.1038/nrmicro.2017.14.

Mariani, J., Favero, C., Spinazzè, A., Cavallo, D.M., Carugno, M., Motta, V., et al., 2018. Short-term particulate matter exposure influences nasal microbiota in a population of healthy subjects. Environ. Res. 162, 119–126. https://doi.org/10.1016/j.envres.2017.12.016.

McCauley, K.E., Flynn, K., Calatroni, A., DiMassa, V., LaMere, B., Fadrosh, D.W., et al., 2022. Seasonal airway microbiome and transcriptome interactions promote childhood asthma exacerbations. J. Allerg. Clin. Immunol. 150, 204–213. https://doi.org/10.1016/j.jaci.2022.01.020.

Monto, A.S., 2002. The seasonality of rhinovirus infections and its implications for clinical recognition. Clin. Ther. 24, 1987–1997. https://doi.org/10.1016/S0149-2918(02)80093-5.

Odendaal, M.-L., de Steenhuijsen Piters, W.A.A., Franz, E., Chu, M.L.J.N., Groot, J.A., van Logchem, E.M., et al., 2024. Host and environmental factors shape upper airway microbiota and respiratory health across the human lifespan. Cell 187, 4571–4585. e15. https://doi.org/10.1016/j.cell.2024.07.008 e15.

Qin, T., Zhang, F., Zhou, H., Ren, H., Du, Y., Liang, S., et al., 2019. High-level PM2.5/PM10 exposure is associated with alterations in the Human pharyngeal microbiota composition. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.00054 [Internet]. Frontiers[cited 2025 Feb 12].

Ren, L., Yang, J., Xiao, Y., Guo, L., Rao, J., Wu, C., et al., 2025. Transmission of the human respiratory microbiome and antibiotic resistance genes in healthy populations. Microbiome 13, 115. https://doi.org/10.1186/s40168-025-02107-9.

Smits, S.A., Leach, J., Sonnenburg, E.D., Gonzalez, C.G., Lichtman, J.S., Reid, G., et al., 2017. Seasonal cycling in the gut microbiome of the Hadza Hunter-Gatherers of Tanzania. Science 357, 802–806. https://doi.org/10.1126/science.aan4834.

Solazzo, G., Iodice, S., Mariani, J., Persico, N., Bollati, V., Ferrari, L., et al., 2022. Upper Respiratory microbiome in pregnant women: characterization and influence of parity. Microorganisms 10, 2189. https://doi.org/10.3390/microorganisms10112189.

Solazzo, G., Rovelli, S., Iodice, S., Chung, M., Frimpong, M., Bollati, V., et al., 2025. The microbiome of Total suspended particles and its influence on the respiratory microbiome of healthy office workers. Ecotoxicol. Environ. Saf. 291, 117874. https://doi.org/10.1016/j.ecoenv.2025.117874.

Tani, M., Shinzaki, S., Asakura, A., Tashiro, T., Amano, T., Otake-Kasamoto, Y., et al., 2023. Seasonal variations in gut microbiota and disease course in patients with inflammatory bowel disease. PLoS One 18, e0283880. https://doi.org/10.1371/journal.pone.0283880.

Vieceli, T., Tejada, S., Martinez-Reviejo, R., Pumarola, T., Schrenzel, J., Waterer, G.W., et al., 2023. Impact of air pollution on respiratory microbiome: a narrative review. Intens. Crit. Care Nurs. 74, 103336. https://doi.org/10.1016/j.iccn.2022.103336.

Xue, Y., Chu, J., Li, Y., Kong, X., 2020. The influence of air pollution on respiratory microbiome: a link to respiratory disease. Toxicol. Lett. 334, 14–20. https://doi.org/10.1016/j.toxlet.2020.09.007.

Yu, K., Tenaglia, V., Chua, E.G., Haines, R., Bahal, G., Nicol, M.P., et al., 2025. Interactions between bacteria in the human nasopharynx: a scoping review. Lancet Microb. 6, 101062. https://doi.org/10.1016/j.lanmic.2024.101062.

Zhang, J., Guo, Z., Lim, A.A.Q., Zheng, Y., Koh, E.Y., Ho, D., et al., 2014. Mongolians core gut microbiota and its correlation with seasonal dietary changes. Sci. Rep. 4, 5001. https://doi.org/10.1038/srep05001.