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Short-term passive greenspace exposures have little effect on nasal microbiomes: A cross-over exposure study of a Māori cohort

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ABSTRACT

Indigenous health interventions have emerged in New Zealand aimed at increasing people's interactions with and exposure to macro and microbial diversity. Urban greenspaces provide opportunities for people to gain such exposures. However, the dynamics and pathways of microbial transfer from natural environments onto a person remain poorly understood. Here, we analysed bacterial 16S rRNA amplicons in air samples ($n = 7$) and pre- and post-exposure nasal samples ($n = 238$) from 35 participants who had 30-min exposures in an outdoor park. The participants were organised into two groups: over eight days each group had two outdoor park exposures and two indoor office exposures, with a cross-over study design and washout days between exposure days. We investigated the effects of participant group, location (outdoor park vs. indoor office), and exposures (pre vs. post) on the nasal bacterial community composition and three key suspected health-associated bacterial indicators (alpha diversity, generic diversity of Gammaproteobacteria, and read abundances of butyrate-producing bacteria). The participants had distinct nasal bacterial communities, but these communities did not display notable shifts in composition following exposures. The community composition and key health bacterial indicators were stable throughout the trial period, with no clear or consistent effects of group, location, or exposure. We conclude that 30-min exposure periods to urban greenspaces are unlikely to create notable changes in the nasal microbiome of visitors, which contrasts with previous research. Our results suggest that longer exposures or activities that involves closer interaction with microbial rich ecological components (e.g., soil) are required for greenspace exposures to result in noteworthy changes in the nasal microbiome.

1. Introduction

Disconnection from natural environments is a characteristic of urban lifestyles and one which is associated with poorer health outcomes (Sibthorpe and Brymer, 2020; Robinson et al., 2024). For Indigenous Peoples, whose identity, culture, and health are intertwined with the natural environment (Warbrick et al., 2016; Durie, 2004), the disconnection from ancestral lands and natural environments generally, is particularly concerning. Warbrick et al. (2023) recently proposed that the relationship between environmental microbiomes and health has important implications for the health of Indigenous Peoples, despite Indigenous people rarely being represented in studies of the microbiome. With the majority of people now living in cities (United Nations, 2018), urban greenspaces and their accompanying aerobiomes are key points of exposure to natural environmental microbiomes (Robinson et al., 2023).

Bacterial colonisation of the human body occurs during and after birth, with post-birth bacterial communities primarily shaped by people's environments (Rothschild et al., 2018). Pathways of exposure to environmental bacteria include ingested and inhaled substances, either directly or indirectly (e.g., via hand-to-face transfer). Air is a well-understood transmission medium for microbiota, which triggers health conditions such as allergies and infectious disease (Kim et al., 2018). However, the transmission pathway of health-supporting airborne bacteria (e.g., bacteria that have been associated with protection from allergies (Haahtela, 2019);) has received much less attention (Robinson et al., 2023). Airborne bacterial communities (aerobiomes) of built indoor environments are highly variable due to a wide range of possible conditions (Ghosh et al., 2015). Outdoor environments are also rich aerobiome reservoirs (Robinson et al., 2021). Because airborne

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dispersal of microbiota is a key pathway of bacterial exposure and transfer, air transfer dynamics (i.e., quantities of specific taxa transferred to humans during a specific timeframe) can be studied via sampling nasal bacterial communities (Robinson et al., 2023). Nasal microbiome changes may reflect the characteristics of aerobiomes of recent exposure, suggesting that the study of outdoor aerobiomes can provide critical insights into human microbiome assemblages (Selway et al., 2020). However, few studies have examined how nasal microbiomes change after exposure to outdoor air.

Greenspace aerobiomes originate from leaf surfaces and soil, with modulating effects from vegetation complexity and height above the ground (i.e., vertical stratification (Robinson et al., 2021);), air pollution (Franchitti et al., 2022), and wind-carried airshed influences (Robinson et al., 2023). In urban settings, land cover has a strong influence on the composition of aerobiomes. For example, the aerobiomes of parks have different community compositions than adjoining parking lots (Mhuireach et al., 2019). Among greenspaces, amenity grassland aerobiomes have different compositions to remnant native vegetation aerobiomes and possess consistent alpha diversity at heights up to 2 m (Robinson et al., 2021). Thus, urban amenity grasslands should have distinct aerobiomes compared to indoor offices and provide useful locations to study the transfer of aerobiomes into the airways of people. Yet, the use of amenity grassland aerobiomes in bacterial transfer studies is limited.

Several aerobiome characteristics and taxonomic groups have been linked with human health. Salutogenic functions of bacteria include maintenance of the mucosal barriers (Ashida et al., 2012), immune signalling (Hosseinkhani et al., 2021), vitamin production (LeBlanc et al., 2013), and synthesis of short-chain fatty acids such as butyrate (Brame et al., 2021). Administration of probiotics via oral and nasal routes has been linked with the alleviation of allergic diseases (Jamalkandi et al., 2021). Furthermore, the Biodiversity Hypothesis describes how exposure to a greater amount of microbial diversity in the natural environment may be required to promote innate immune training and immunoregulation (Haahtela, 2019). In a complex network of interactions, exposure to bacterial diversity can modulate immune responses and reduce pro-inflammatory and allergenic antibodies and cytokines (Haahtela, 2019). Thus, exposure to higher alpha diversity of bacteria within outdoor aerobiomes with a low level of pathogenic taxa could potentially support human health (Spragge et al., 2023).

The diversity of Gammaproteobacterial genera on the skin has been associated with increased plasma transforming growth factor beta 1 (TGF-β1) levels, decreased interleukin-17 (pro-inflammatory cytokines), and increased relative abundance of regulatory T-cells (Roslund et al., 2020). Increased TGF-β1 and decreased interleukin-17 are associated with an anti-inflammatory molecular profile, and regulatory T-cells are critical for immunotolerance, including tolerance of commensal taxa (Roslund et al., 2020). Butyrate-producing bacteria are key members of the human and animal gut with numerous health benefits, and after birth they are primarily supplied by the environment with nutritional support via ingestion of fibre (Brame et al., 2021). Certain outdoor environments are reservoirs of butyrate producers that could disperse into the aerobiome and transfer onto people visiting those environments (Brame et al., 2022). Thus, aerobiome Gammaproteobacterial diversity and butyrate-producing bacterial read abundances could provide indicators of human health-associated benefits of aerobiome exposure.

Here we studied the changes in 16S rRNA amplicons in pre- and post-exposure nasal microbiome samples from 35 Māori (Indigenous New Zealand) participants, divided into groups (A and B), who spent two repeated 30-min exposure periods in each of two locations: an indoor office and an outdoor park (amenity grassland). We utilised a cross-over study design to control for effects of group and day, with two exposure days in one location (Days 1 and 3), followed by a two-day washout period, then two further exposure days in the other location (Days 6 and 8). To understand the influences of exposures on the nasal microbiomes, we examined the effects of location, individual, group, single exposures, and repeated exposures on (Sibthorpe and Brymer, 2020) the nasal bacterial alpha diversity, (Robinson et al., 2024) nasal bacterial community composition, and (Warbrick et al., 2016) specific bacterial taxonomic groups with known health associations (Gammproteobacterial diversity and butyrate-producing bacterial abundances).

2. Materials and methods

2.1. Experimental design

We utilised a crossover trial design (Fig. 1A). We recruited 35 participants into the trial, which took place March 15–22, 2023. The participants were divided into two groups: group A ($n = 18$) and group B ($n = 17$). On days 1 and 3, group An underwent outdoor exposures and group B underwent indoor exposures, with crossover for exposure days 6 and 8. Exposure days were on March 15 and 17, then on March 20 and 22, allowing for a single washout day (i.e., a day with no assigned exposures) between testing days and two washout days before the crossover.

The outdoor treatment group met at the Te Arawa Whānau Ora office in Rotorua, New Zealand, at approximately 8:30am. Te Arawa Whānau Ora is an Indigenous community health organisation, and all participants in this study were employees of the organisation and identify as Māori. Their noses were swabbed pre-exposure (hereafter referred to as "Pre", see description below), and they then went for a walk to Kuirau Park, approximately 600 m from the office, for 30 min (Fig. 1B). Upon their return, before entering the office, they were re-tested with a second nasal swab (hereafter referred to as "Post").

The indoor treatment group met at the same office at the same time and day as the outdoor treatment group. Their noses were swabbed using the same methods. However, during the 30-min exposure period, they remained in the office.

2.2. Nasal swabbing

All nasal swab samples were obtained by inserting a sterile nylon-flocked swab tip (FLOQSwabs Lot, 2011490, Copan Flock Technologies, Bescia, Italy) into the anterior nares and rotating in a circular motion for 3–5 s per naris, then repeated in the opposite naris using the same swab. The swab tip was then immediately snipped into a sterile 15 mL falcon tube, sealed with the lid, wrapped with parafilm, and placed in a −20 °C freezer in the office.

2.3. Air sampling

Air samples were obtained in Kuirau Park along the same walking path where participants walked during their outdoor period and at a central indoor location in the Te Arawa Whānau Ora office. At Kuirau Park, air samples were collected at a single air station over an approximately 8-h period during each exposure day, following the method described in Mhuireach (Mhuireach et al., 2016). The aerobiome sampling stations were set up on site between 08:00 and 08:30 h and collected between 15:00 and 15:30 h. Kuirau Park is a highly managed mature amenity grassland with no active changes in greenspace coverage or vegetation, regularly tended lawns that dominate the park, and active tree maintenance (e.g., pruning, watering). At the Te Arawa Whānau Ora office site, air samples were collected at a single air station following the same procedures, times, and days. On one day, March 17, the weather was rainy and the air station assembly using protective umbrellas was vandalised, thus an outdoor air sample was not obtained for that day.

The outdoor park air sampling station was made of plastic boxes and achieved a height of 1.2 m. Sampling at this height should be representative of aerobiome exposure potential for children and adults alike, and is within the 2 m height range of similar alpha diversity as measured elsewhere in amenity grassland aerobiomes (Robinson et al., 2021). The indoor office sampling station was a single plastic box placed on a table,

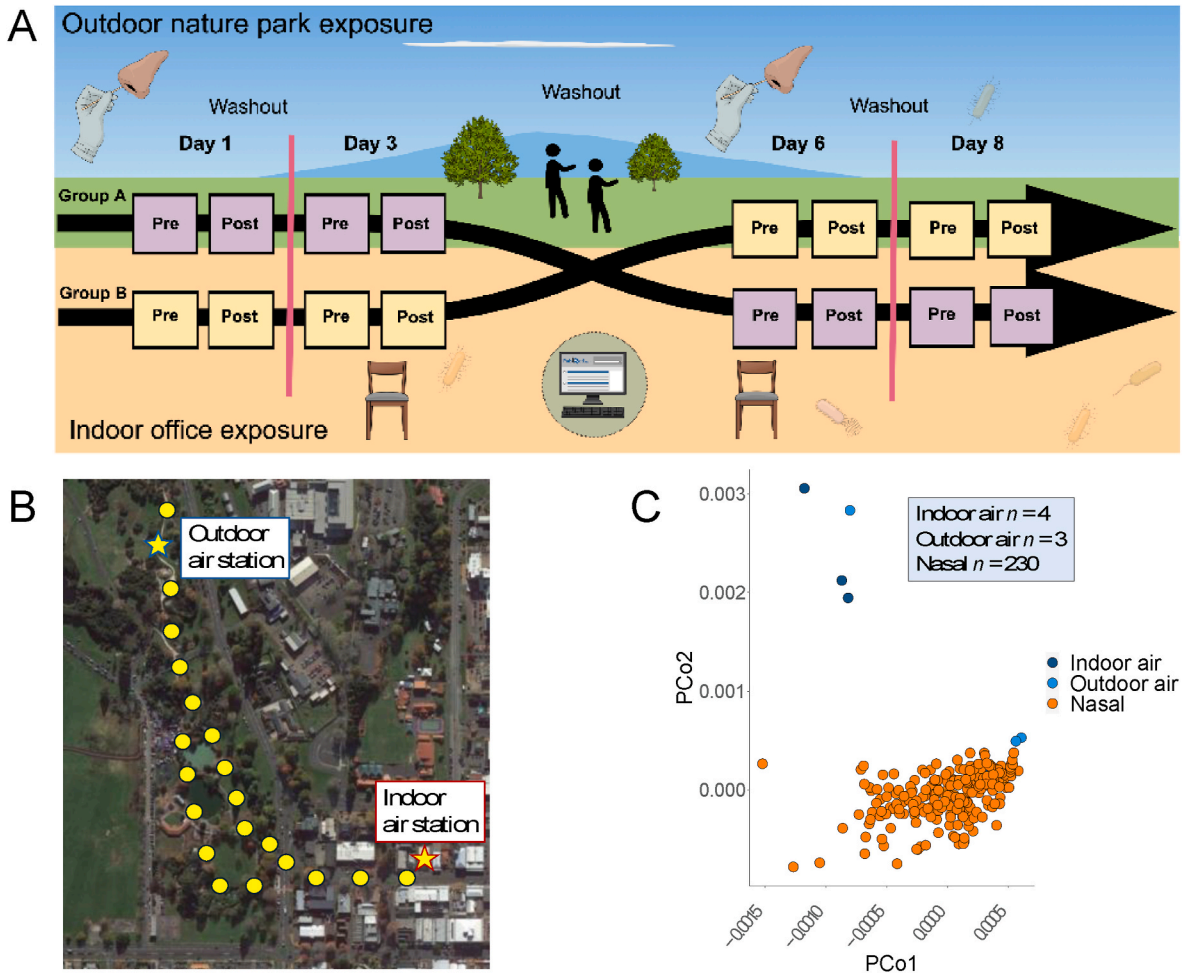

Fig. 1. (A) Overview of the cross-over experimental design. (B) Walking path map of the outdoor treatment group in Rotorua, New Zealand. Map generated with Google maps. Yellow dots denote the walking path. (C) Principal coordinates analysis based on centred-log ratio compositional abundance data displaying variation in community composition by sample type (Adonis PERMANOVA: $F = 5.515$, $R^2 = 0.023$, $p = 0.001$).

achieving a height of approximately 1.5 m. On the top of each station, we opened and placed three sterile clear plastic Petri dish bases and lids, which provided six collection surfaces per site. This method of passive aerobiome sampling has been shown to be as effective as active sampling methods (Mhuireach et al., 2016). On two days, a field control was generated by holding open an additional Petri dish for 30 s at the equipment box. Immediately after the air sampling activity, each Petri dish was sealed, labelled, and placed in the office freezer at −20 °C until DNA extraction (described below).

2.4. DNA extraction, PCR, sequencing

Within one week of obtaining all samples, DNA extractions and quantifications were performed in a PC2 laboratory at Auckland University of Technology, Auckland, New Zealand. To transport samples from Rotorua to Auckland, samples were removed from the office freezer, placed onto ice in a sealed insulated container, and transported by vehicle to the lab. Upon arrival at the lab, they were immediately placed into a −20 °C freezer.

The petri dishes for each site were opened and swabbed with sterile nylon-flocked swab tips (FLOQSwabs) inside a laminar flow cabinet. One swab and 40 μL of added sterile phosphate-buffered saline was used for swabbing all six surfaces, except for surfaces that showed visual signs of damage or contamination, for approximately 4 min total using a consistent pattern of swabbing. The tips were cut directly into 15 mL sterile falcon tubes. We obtained an extraction blank control for each extraction batch using the same process as samples but without a swab tip.

We used the QIAamp DNA Mini Kit (QIAGEN) for all samples and followed the manufacturer's instructions with two modifications to increase final concentration: the incubation step was extended from 10 min to 15 min, and the final elution buffer volume was reduced from 80 μL to 60 μL. The extraction concentrations were then quantified using a Qubit High Sensitivity dsDNA assay (ThermoFisher Scientific). Once DNA concentrations were verified, PCR amplification of the bacterial 16S rRNA V3–V4 regions was performed in the lab at Auckland University of Technology using Kappa HiFi Taq mix with 341F-805R primers (Kapa Biosystems) via PCR on an Eppendorf Vapo.Protect Mastercycler Pro thermocycler. The first PCR round included 38 amplification cycles. Plate clean-up was performed via AMPure XP reagent. To normalise clean PCR products to 1 ng/uL, samples below 1 ng/μL were concentrated using the Eppendorf Concentration and using the following conditions: D-AQ, 30 C, 18 min. Second round PCR used the Nextera XT Index Kit to index samples, with eight cycles of amplification. Samples were then pooled, cleaned with AMPure XP reagent, and quantified using Qubit High Sensitivity. The Bioanalyzer 2100 expert High Sensitivity DNA assay was performed to check library quality and molarity, and libraries were pooled for equal molarity. Upon completion of library preparation, sequencing of amplicon sequence variants was completed on the Illumina Miseq V3 using the Illumina MiSeq Reagent Kit v3 (600 cycle). Four PCR negative blanks were generated during the library preparation steps for quality control.

2.5. Bioinformatics

From the 16S rRNA raw sequence data, amplicon sequence variants (ASVs) were trimmed and filtered using an established Qiime2 pipeline (version 2023.5), with forward reads truncated at 260 bp and reverse reads truncated at 198 bp. Taxonomy was assigned using the onboard Naïve Bayes taxonomic classifier and Silva database v 138.1. Sequences were then cleaned using scripts utilising the R *phyloseq* package (version 1.42.0 (McMurdie and Holmes, 2013);) by removing the following sequences: those assigned to mitochondria and chloroplasts, taxa that did not occur in at least two samples, and ASVs with total sums <20 reads. Sequences that were likely of contamination origin were identified and removed using the R *decontam* package (version 1.18.0 (Davis et al., 2018);) using the function "isNotContaminant" suited for low biomass samples.

2.6. Statistical analysis

All statistical analyses were performed using R (version 4.2.3 (R Core Team, 2023);). To maintain consistency with prior aerobiome studies, statistical significance was set at alpha = 0.05. To compare nasal bacterial alpha diversity between samples, Hill numbers were examined using R *hillR* package (Li, 2018), which integrates sample size and coverage. We set the q parameter for Hill numbers at 0.80 for reduced sensitivity to relative abundances compared with Shannon index.

To prepare for beta diversity tests, the read abundance data were evaluated using R *zCompositions* package (version 1.4.0.1 (Palarea-Albaladejo and Martín-Fernández, 2015);), zeros were imputed using the R *scImpute* package (version 0.0.9 (Li and Li, 2018);), and eight low total read abundance samples were discarded to reduce data sparsity. The resultant read abundances were then transformed with centred-log ratio using the R *compositions* package (verion 2.0.6 (Van den Boogaart and Tolosana-Delgado, 2008);), followed by ordination with principal coordinates analysis using R *ecodist* package (version 2.0.9 (Goslee and Urban, 2007);), based on Aitchison distances obtained with the R *vegan* package (version 2.6.4 (Oksanen et al., 2022);); statistics were generated using PERMANOVA (Adonis) tests via the R *vegan* package. To determine the homogeneity of community composition between participants, distance-to-centroid analyses were performed using the R *vegan* package. Maps were created using the R *ggmap* package (version 3.0.2 (Kahle and Wickham, 2013);). To determine the differential abundance of bacterial taxa and specific taxonomic groups between samples, the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) method was performed on untransformed amplicon data with the *ancombc2* function in the R *ANCOMBC* package (version 2.0.3 (Lin and Peddada, 2020);). Participant was set as a random effect (rand_formula) for mixed effects modelling. The p-value adjustment was set as "fdr", and prv_cut and lib_cut were set at "0". The ANCOMBC algorithm has been shown to minimise bias due to sampling fractions and reduces false discovery rates. We downloaded a comprehensive list of pathogens from Bartlett, Padfield (Bartlett et al., 2022)) to examine pathogenic read abundances in the samples. To determine the effects of repeated exposures, time-series analyses were performed using repeated-ANOVAS with R *rstatix* package (version 0.7.2 (Kassambara, 2023);). R *ggplot2* package (version 3.4.2 (Wickham, 2016);) was used for data visualisations.

3. Results

3.1. Aerobiomes were different from nasal microbiomes

Aerobiomes had a higher alpha diversity (hill number = 82.3 ± 64.4 SD, $n = 7$) than nasal microbiomes (hill number = 19.5 ± 10.6 SD, $n = 238$; W = 1378, $p = 0.003$). Aerobiome location had no effect on alpha diversity between outdoor (hill number = 60.4 ± 70.3 SD, $n = 3$) and indoor (hill number = 98.8 ± 64.6 SD, $n = 4$) samples (t = 0.740, df = 4.2, $p = 0.50$). Overall, aerobiomes and nasal microbiomes had different community compositions (Adonis PERMANOVA: F = 5.515, $R^2 = 0.023$, $p = 0.001$; Fig. 1C), and outdoor aerobiomes were compositionally similar to indoor aerobiomes (Adonis PERMANOVA: F = 1.268, $R^2 = 0.20$, $p = 0.17$).

3.2. Exposure effect on composition, diversity and differential ASV abundances

The 30-min outdoor exposures did not change the nasal bacterial community composition for either group A (Adonis PERMANOVA: F = 0.686 $R^2 = 0.013$, $p = 0.99$) or group B (Adonis PERMANOVA: F = 0.726, $R^2 = 0.013$, $p = 0.98$) (Fig. 2A). The 30-min indoor exposures also did not change the community composition for either group A (Adonis PERMANOVA: F = 0.809, R2 = 0.014, $p = 0.89$) or group B (Adonis PERMANOVA: F = 0.675, $R^2 = 0.012$, $p = 0.99$) (Fig. 2D).

There was no effect of group on changes in nasal bacterial alpha diversity after 30-min exposures among both outdoor exposures (Wilcox: W = 433, p = 0.83) and indoor exposures (Wilcox: W = 358, p = 0.18), even though the groups visited the locations on separate days. When the two groups were combined, there was no effect on the alpha diversity by either the outdoor exposures (W = 1388, $p = 0.11$; Fig. 2B) or the indoor exposures (W = 1818, $p = 0.93$; Fig. 2E), although the treatment location effect (i.e., indoor vs outdoor) was significant (Wilcox: W = 2169, $p = 0.02$).

For group A, the 30-min outdoor exposure had no effect on the read abundance of any genus on day 1. However, on day 3, the outdoor treatment resulted in a significant decrease in the genera *Escherichia-Shigella* (ANCOMBC: log fold change (lfc) = −1.91, adjusted-p (q) < 0.001) and *Pseudomonas* (ANCOMBC: lfc = −1.72, $q < 0.001$) (Fig. 2C). For group B, on day 6, the outdoor treatment resulted in five taxa with significantly decreased read abundances: *Rheinheimera* (ANCOMBC: lfc = −3.37, $q < 0.001$), *Massilia* (ANCOMBC: lfc = −3.22, $q < 0.001$), *Acinetobacter* (ANCOMBC: lfc = −3.16, $q < 0.001$), *Flavobacterium* (ANCOMBC: lfc = −3.16, $q < 0.001$), and family Comomonadaceae (ANCOMBC: lfc = −2.10, $q = 0.004$; Fig. 2F). The outdoor treatment had no effect on any genus on day 8 for group B (all data are in Table S1).

3.3. Exposure effects on health-associated bacterial groups

30-min exposures had different effects in groups A and B on the number of Gammaproteobacteria genera (t-test: t = −2.111, df = 115.12, $p = 0.036$), so we examined the two groups separately. Indoor exposure significantly decreased the Gammaproteobacteria diversity in group A (t-test: t = −2.221, df = 56.61, $p = 0.03$) but had no effect in group B (Wilcox: W = 358, $p = 0.91$). Outdoor exposure had no effect on Gammaproteobacteria diversity for group A (t-test: t = −1.015, df = 49.3, $p = 0.32$) but weakly decreased Gammaproteobacteria diversity for group B (t-test: t = −1.905, df = 54.99, $p = 0.062$).

There was no effect of group on changes in nasal butyrate-producing bacterial read abundances after 30-min exposures among both outdoor exposures (Wilcox: W = 329, $p = 0.24$) and indoor exposures (Wilcox: W = 396.5, $p = 0.43$). With Groups A and B combined, we observed no effect of treatment location on butyrate producer read abundances (Wilcox: W = 1940, $p = 0.28$).

3.4. Aerobiome-associated taxa in nasal microbiomes

We identified 1098 bacterial taxa in the outdoor aerobiome samples and then constrained nasal microbiome analyses with only these taxa. 30-minute exposures had no effect on the percentage of aerobiome taxa in nasal samples in either outdoor (t-test: t = 0.331, df = 114.77, $p = 0.74$; Fig. 3A) or indoor treatments (W = 1740, $p = 1$; Fig. 3D). 30-minute outdoor exposures had no effect on the community composition of aerobiome taxa in nasal samples in either group A (Adonis PERMANOVA: F = 0.761, $R^2 = 0.014$, $p = 0.96$; Fig. 3B) or group B (Adonis

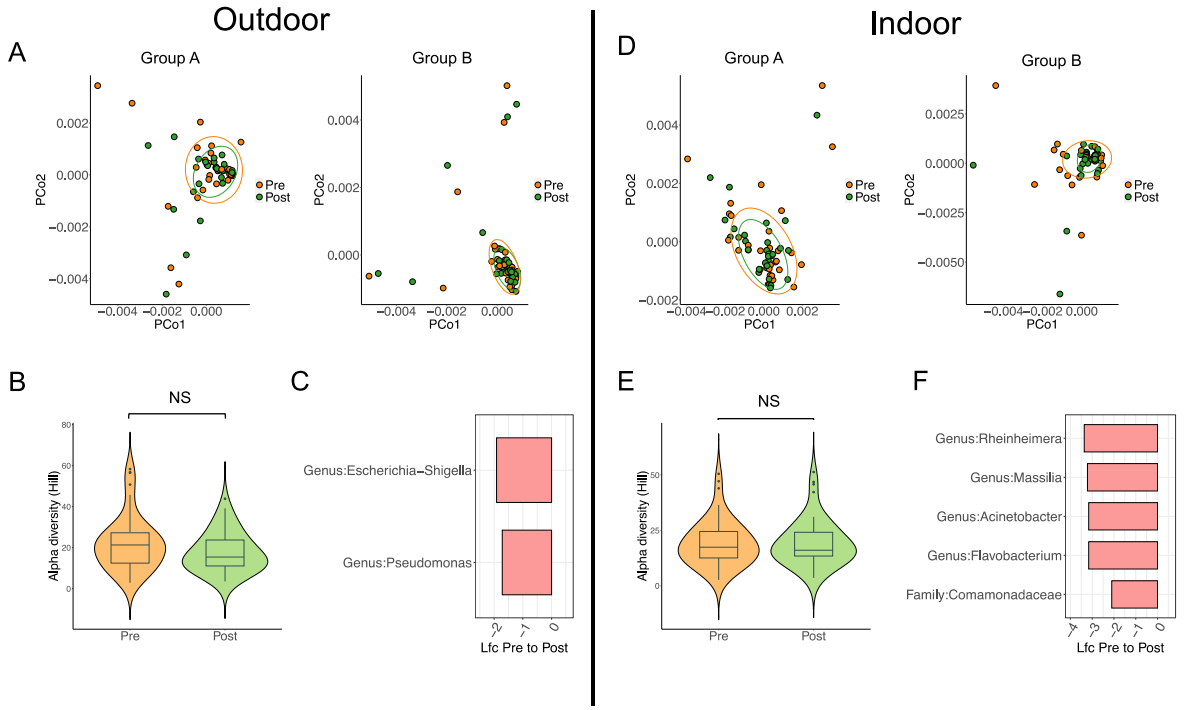

Fig. 2. (A) Principal coordinates analysis based on centred-log ratio compositional abundance data displaying variation in community composition before (Pre) and after (Post) outdoor exposure for groups A and B. (B) Boxplots of changes in alpha diversity from before (Pre) and after (Post) outdoor exposure. The y-axis shows the alpha diversity based on Hill numbers. Boxes show the median and interquartile range, while whiskers extend to the remaining range of data. (C) Significantly differentially abundant genera in nasal microbiomes after outdoor exposure. The x axis shows the log fold change from before pre-exposure to post-exposure. Red bars indicate a decrease in log fold change. (D) Principal coordinates analysis based on centred-log ratio compositional abundance data displaying variation in community composition before (Pre) and after (Post) indoor exposure for groups A and B. (E) Boxplots of changes in alpha diversity from before (Pre) and after (Post) indoor exposure. The y-axis shows the alpha diversity based on Hill numbers. Boxes show the median and interquartile range, while whiskers extend to the remaining range of data. (F) Significantly differentially abundant genera in nasal microbiomes after indoor exposure. The x axis shows the log fold change from pre-exposure to post-exposure. Red bars indicate a decrease in log fold change.

PERMANOVA: F = 0.861, R^2 = 0.015, p = 0.77; Fig. 3C), and 30-min indoor exposures had no effect on the community composition of aerobiome taxa in nasal samples taxa in either group A (Adonis PERMANOVA: F = 0.862, R^2 = 0.015, p = 0.80; Fig. 3E) or group B (Adonis PERMANOVA: F = 0.704, R^2 = 0.013, p = 0.99; Fig. 3F).

3.5. Time-series effects on nasal microbiome characteristics

Participant had a strong effect on nasal bacterial communities from Day 1 to Day 8 (Adonis PERMANOVA: F = 3.667, R^2 = 0.382, p = 0.001; Fig. S1). However, time had no effect on post-exposure group nasal bacterial community composition (Fig. 4A–D). Group homogeneity (beta dispersion) also did not change from Day 1 to Day 8 (ANOVA: F = 1.147, p = 0.29).

Time had no effect on alpha diversity for group A (repeated measures ANOVA: ges = 0.084, p = 0.30) or group B (repeated measures ANOVA: ges = 0.192, p = 0.16; Fig. 5A). Group B showed a time effect on Gammaproteobacteria diversity, with significantly reduced diversity from Day 1 post to Day 8 post (repeated measures ANOVA: ges = 0.344, p = 0.002; Fig. 5B), but showed no effect on Group A (repeated measures ANOVA: ges = 0.082, p = 0.31). Time had no effect on the sum of relative abundances of butyrate-producing bacteria for group A (repeated measures ANOVA: ges = 0.119, p = 0.14) or B (repeated measures ANOVA: ges = 0.2, p = 0.24), although time had a weak effect on increasing read abundances of butyrate-producing bacteria from Day 1 post to Day 8 post in group B (repeated measures ANOVA: ges = 0.167, p = 0.058); Fig. 5C).

4. Discussion

We ran a short-term greenspace cross-over exposure trial of a Māori cohort and showed that this exposure had little effect on nasal microbiomes. This low responsiveness of the nasal microbiome was following repeated 30-min passive exposures to an outdoor nature park. Location, participant, and time had weak or no effect on the nasal microbiome alpha diversity, community composition, aerobiome taxa present in nasal samples, and health-associated bacterial groups. Overall, our results contrast with an earlier study that reported changes in nasal microbiomes after greenspace exposure (Selway et al., 2020). We suggest that nasal microbiomes are relatively stable over short periods of passive greenspace exposure, and 30 min of this passive exposure (i.e., walking in greenspaces) does not result in notable and/or consistent changes in the nasal bacterial communities of participants. Our work raises important questions about the types of activities and duration of exposure to greenspaces required to result in meaningful changes to the nasal microbiome.

4.1. Aerobiomes had higher alpha diversity than nasal microbiomes

We found that overall aerobiomes had higher alpha diversity than nasal microbiomes. This is consistent with the findings from Selway et al. (2020), where outdoor air samples had higher alpha diversities than nasal samples. To our knowledge, no previous studies have compared the aerobiome alpha diversity of indoor office and urban greenspace environments. Our findings showed no difference between office and amenity park aerobiome alpha diversity; however, we had only seven air samples (three outdoor and four indoor), which likely limited our power to detect an effect. In addition, we utilised the passive

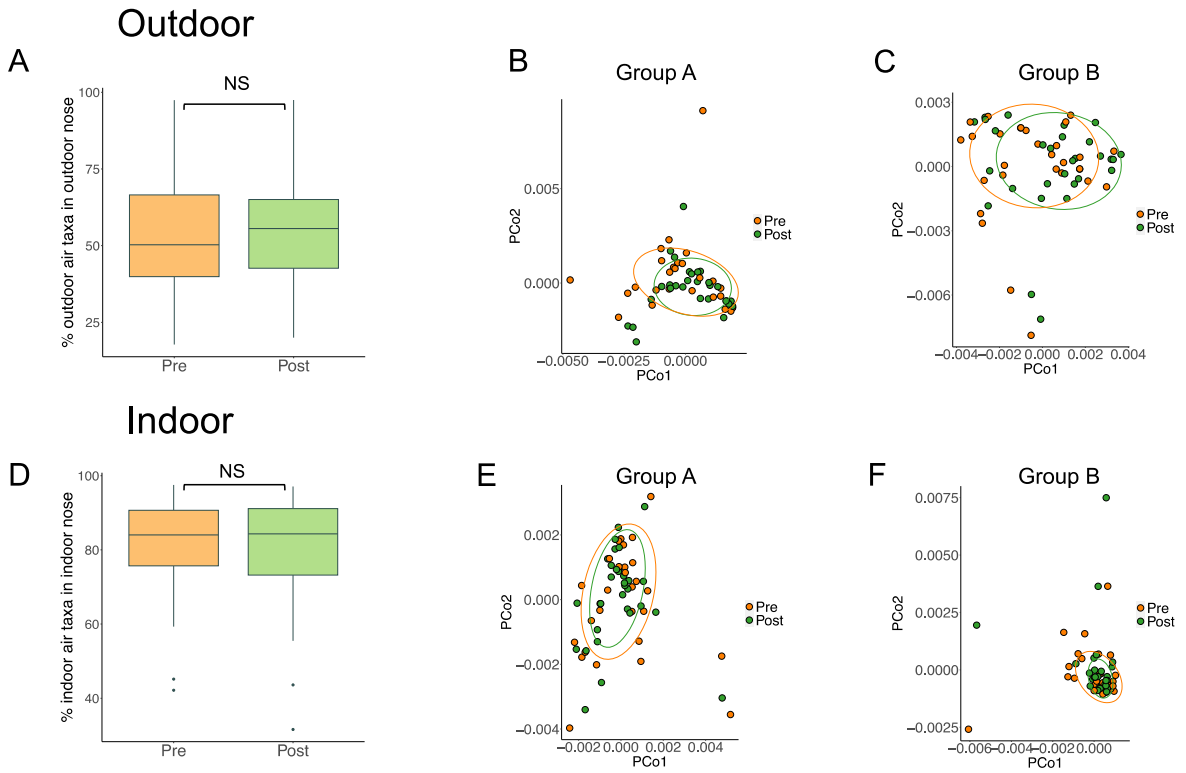

Fig. 3. (A) Boxplots of the percentage of outdoor air taxa that were found in the nose (y-axis) before (Pre) and after (Post) outdoor exposure. Boxes show the median and interquartile range, while whiskers extend to the remaining range of data. (B–C) Principal coordinates analysis based on centred-log ratio compositional abundance data of only aerobiome-associated taxa found in the nose, displaying variation in community composition before (Pre) and after (Post) outdoor exposure for groups A (panel B) and B (panel C). (D) Boxplots of the percentage of outdoor air taxa that were found in the nose (y-axis) before (Pre) and after (Post) outdoor exposure. Boxes show the median and interquartile range, while whiskers extend to the remaining range of data. (E–F) Principal coordinates analysis based on centred-log ratio compositional abundance data of only aerobiome-associated taxa found in the nose, displaying variation in community composition before (Pre) and after (Post) outdoor exposure for groups A (panel E) and B (panel F).

settlement method for aerobiome sampling based on Mhuireach et al. (2016), who found comparable results between passive and active aerobiome sampling methods. However, additional studies comparing these two methods could better inform future aerobiome study design. Recent studies have placed value on urban greenspaces and natural outdoor locations as environmental reservoirs of immunoregulatory biodiversity for urban residents (Robinson et al., 2021; Roslund et al., 2020). However, future direct comparisons of indoor and outdoor aerobiomes across a range of built environments and outdoor settings are needed to establish the conditions that may drive potential health-promoting exposure effects.

4.2. Short greenspace exposures had little effect on nasal microbiomes

We found no clear effects of the 30-min exposures on nasal microbiome alpha diversity or community composition. Even when filtering the microbial taxa to just particular health-associated bacterial groups (i.e., Gammaproteobacteria, butyrate-producing bacteria), the only notable effects were a reduction in generic diversity of Gammaproteobacteria and an increase in butyrate-producing bacterial read abundances in group B across the trial period. Roslund et al. (2020) recently found that generic diversity of Gammaproteobacteria on the skin of children associated with shifts in blood plasma markers TGF-β1 and interleukin-17 toward an anti-inflammatory profile. Our observed reduction in generic diversity of Gammaproteobacteria and an increase in butyrate-producing bacterial read abundances may be part of normal temporal bacterial variability (Vandeputte et al., 2021) or could have been driven by an unmeasured factor. However, since so few studies have generated data directly comparable to ours, the capacity to compare our findings with other studies is limited.

4.3. Exposure times

Our trial ran for eight days, with four 30-min exposure events across these days. We found only minimal changes in nasal microbiome characteristics after each exposure. Our 30-min exposure length was intended to represent a typical nature exposure of, for example, going for a walk in a park during a lunch break or walking a pet. Similar human exposure trials are limited, but some provide noteworthy discussion. In a study with two or three participants spending time in urban greenspaces, Selway et al. (2020) found skin and nasal microbiome changes, but participants performed activities that encouraged more direct interaction with soils and/or vegetation and utilised ca. 1.5 h exposure periods. Roslund et al. (2020) added biodiverse forest floor and sod into daycare centres, then found changes in the skin and gut microbiomes of participant children (3–5 years old) over 28 days with approximately 1.5 h daily exposure periods. Lai et al. (2017) examined the exposure impacts of academic mouse researchers working in the dirty cage wash area on nasal and skin microbiomes. Their exposure period was a single 8-h shift, and they found no significant change in the nasal microbiome between pre- and post-shift samples. Studies assessing the effects of land cover surrounding a person's home on their skin microbiome are able to integrate much longer exposure periods to show effects on residents' microbiomes. For example Hanski et al. (2012) assessed the influence of living near biodiversity and found notable effects on the bacterial classes in the skin. Thus, longer and/or repeated exposure periods plus more direct exposure (e.g., handling soils) may be required to elicit changes in nasal and skin microbiomes. Future urban greenspace research should

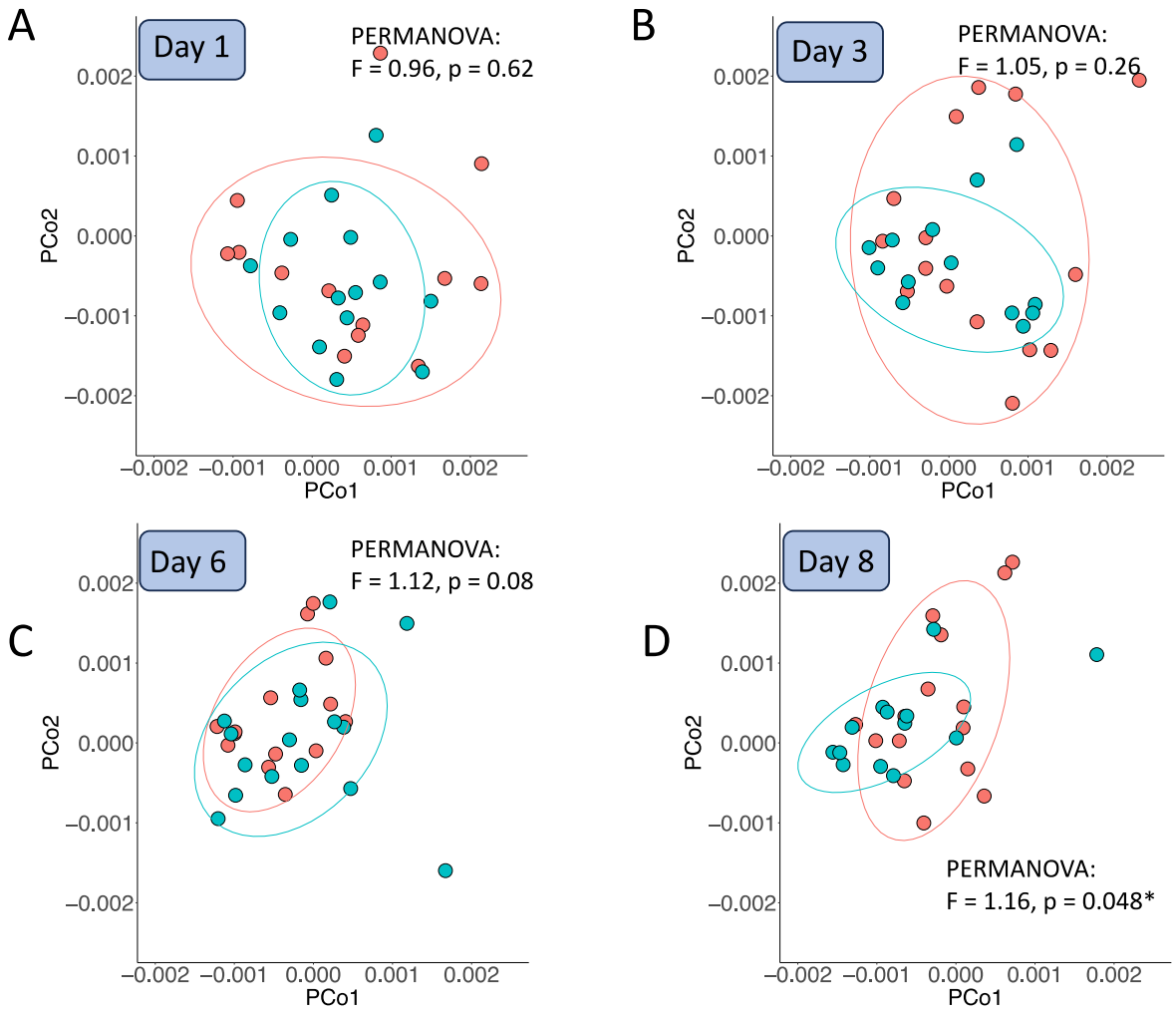

Fig. 4. Display of time-series effects on post-exposure community composition between group A and group B, using principal coordinates analysis based on centred-log ratio compositional abundance data for days 1 (panel A), 3 (panel B), 6 (panel C), and 8 (panel D). Red points and ellipses are Group A. Blue points and ellipses are Group B. Outliers were removed on Days 1, 3, and 8. * indicates significance at $p < 0.05$.

further examine the effect of different activities (e.g., passive walking as in our study, direct handling of microbially-rich ecosystem components such as soil), durations (e.g., short 30-min periods as in our study, longer and/or repeated short exposures) as well as adjacency and ecological quality of greenspaces on causing changes to human nasal microbiomes.

4.4. Individual participant nasal microbiome stability

We showed a relatively stable participant nasal microbiome over our study period, with strong between-subject effects found on all days. This finding corroborates with human gut microbiome studies, that are generally stable over time (Goodrich et al., 2014). Costello et al. (2012) described how the host shapes the microbiota through environmental selection processes. We show that the composition of an individual's nasal microbiome appeared to change over the eight-day period, but not in ways that could be explained by our environmental exposure treatments. Our groups rotated through the same two sites, with similar exposures to the associated aerobiomes. The stability of between-subject microbiome diversity provides additional evidence that more direct, longer, and/or more frequent exposure is necessary for environmental exposures to overcome other host selection pressures to modulate an individual's nasal microbiome.

5. Conclusions

Spending time in urban greenspaces can provide a person with exposure to outdoor aerobiomes that may have health-beneficial properties, such as by providing exposure to high bacterial diversity (Robinson et al., 2021; Roslund et al., 2020). Our study utilised pre- and post-exposure bacterial data to identify changes in the nasal microbiome following 30-min walks in an outdoor urban park. We observed stability of the alpha diversity, community composition, and abundances of specific health-associated bacterial groups across exposure periods and across the trial period. Between-subject differences in nasal microbiomes were maintained during the trial period, although some evidence indicated a reduction in the diversity of Gammaproteobacteria and an increase in butyrate producing taxa. Our results suggest that 30 min of passive exposure to greenspaces provides insufficient aerobiome exposure to results in changes in nasal bacterial diversity and communities. Indigenous initiatives, which are driven by Indigenous knowledge and emphasise cultural connection as a motivator, could benefit from the expanding collection of microbiome data to better understand the complex (and holistic) relationship between health and the environment. Our study demonstrates the need for future human exposure trials investigating urban greenspace health benefits to examine the types of activity and duration of exposure.

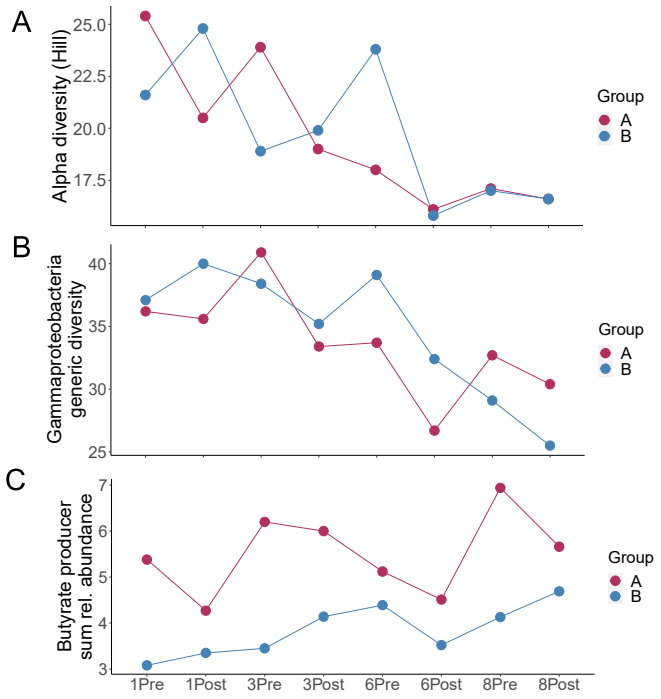

Fig. 5. Line plots showing pre-versus post-exposure measures of human health-associated bacterial characteristics in nasal samples of groups A and B across the trial period: (A) alpha diversity (Hill numbers), (B) Gammaproteobacterial generic diversity, and (C) sums of relative abundances of butyrate-producing bacteria.

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Human ethics approval

Ethical approval for this study was obtained on Dec 7, 2021 from AUTEC – Auckland University of Technology Ethics Committee (Application 21/414). All procedures were performed in compliance with relevant laws and institutional guidelines. Informed consent for experimentation was obtained by all human subjects.

Data uploads

All study data and custom R code is available at Figshare at the following doi: 10.6084/m9.figshare.24993471.

CRediT authorship contribution statement

Joel E. Brame: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Isaac Warbrick:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Deborah Heke:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Craig Liddicoat:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Martin F. Breed:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, 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.

Data availability

All study data and custom R code are available at Figshare at the following doi: 10.6084/m9.figshare.24993471

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118814.

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