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Transfer of environmental microbes to the skin and respiratory tract of humans after urban green space exposure

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ABSTRACT

Background: In industrialized countries, non-communicable diseases have been increasing in prevalence since the middle of the 20th century. While the causal mechanisms remain poorly understood, increased population density, pollution, sedentary behavior, smoking, changes in diet, and limited outdoor exposure have all been proposed as significant contributors. Several hypotheses (*e.g.* Hygiene, Old Friends, and Biodiversity Hypotheses) also suggest that limited environmental microbial exposures may underpin part of this rise in non-communicable diseases. In response, the Microbiome Rewilding Hypothesis proposes that adequate environmental microbial exposures could be achieved by restoring urban green spaces and could potentially decrease the prevalence of non-communicable diseases. However, the microbial interactions between humans and their surrounding environment and the passaging of microbes between both entities remains poorly understood, especially within an urban context.

Results: Here, we survey human skin (n = 90 swabs) and nasal (n = 90 swabs) microbiota of three subjects that were exposed to air (n = 15), soil (n = 15), and leaves (n = 15) from different urban green space environments in three different cities across different continents (Adelaide, Australia; Bournemouth, United Kingdom; New Delhi, India). Using 16S ribosomal RNA metabarcoding, we examined baseline controls (pre-exposure) of both skin (n = 16) and nasal (n = 16) swabs and tracked microbiota transfer from the environment to the human body after exposure events. Microbial richness and phylogenetic diversity increased after urban green space exposure in skin and nasal samples collected in two of the three locations. The microbial composition of skin samples also became more similar to soil microbiota after exposure, while nasal samples became more similar to air samples. Nasal samples were more variable between sites and individuals than skin samples.

Conclusions: We show that exposure to urban green spaces can increase skin and nasal microbial diversity and alter human microbiota composition. Our study improves our understanding of human-environmental microbial interactions and suggests that increased exposure to diverse outdoor environments may increase the microbial diversity, which could lead to positive health outcomes for non-communicable diseases.

1. Introduction

Over the past 70 years, industrialized countries have experienced an increased prevalence of non-communicable diseases (NCDs), such as cancers, diabetes, asthma, allergies, and cardiovascular, mental and autoimmune diseases (Bach, 2002; Boutayeb and Boutayeb, 2005; Okada et al., 2010). This increase was initially observed in industrialized countries (Wagner and Brath, 2012); however, NCDs are

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also rapidly increasing in industrializing countries (Okada et al., 2010). Many of these diseases are linked to immune system dysfunction, where the body attacks itself (autoimmune disease) or overreacts to harmless substances (allergy; *e.g.* pollen or dust mites). For example, reduced exposure to these harmless substances during immune development leads to a higher chance of developing asthma (Lynch et al., 2014). Increased NCD rates have been linked to a wide range of factors, including genetics, environmental and lifestyle factors (diet, sedentary behavior and smoking), and more recently documented, microbial communities on and in the human body (microbiota) (Rook, 2012).

Industrialized societies experiencing this higher prevalence of NCDs have undergone an excelled rate of environmental and sociological change in the recent past (*e.g.* The Great Acceleration) (Graham and White, 2016; Steffen et al., 2015). Since the 1950s, urbanization has become one of the biggest drivers of industrialization (Steffen et al., 2015). Urbanization is associated with a decrease in outdoor exposure, fewer green space environments, and reduced environmental microbial diversity (Graham and White, 2016; McMichael, 2013). For instance, there was a 14–55% decline in green cover of single-family homes across 20 cities in Los Angeles County from 2000 to 2009 (Lee et al., 2017). This decrease of urban vegetation, as well as the increased access to medicine (*e.g.* antibiotics), are both hypothesized to reduce microbial diversity within humans (Rook, 2012; Graham and White, 2016).

The Hygiene (Strachan, 1989), Old Friends (Rook et al., 2003), and Biodiversity (von Hertzen et al., 2011) Hypotheses each suggest mechanisms that may explain why an urbanized lifestyle is associated with decreased human microbial diversity. The Hygiene Hypothesis suggests that limiting early childhood exposure to microbes, such as infectious agents, symbiotic microorganisms, and parasites, increases their susceptibility to various allergens (Strachan, 1989). The Old Friends Hypothesis also highlights the impacts of decreased microbial exposure during immune development but suggests that modern exposures contain less microbial diversity than those that occurred throughout human history (Rook et al., 2003). The Biodiversity Hypothesis further expands on the Hygiene and Old Friends Hypotheses and suggests that reduced exposure to microbiota from natural environments may be the cause (von Hertzen et al., 2011). Each of these hypotheses infers causal relationships between reduced microbial exposure and increased incidences of NCDs, despite few studies directly examining the causal links and mechanisms between decreased environmental microbiota exposure and human disease.

The Microbiome Rewilding Hypothesis (Mills et al., 2017) provides a potential solution to reduce NCD incidence. This hypothesis posits that exposure to remnant and revegetated ecosystems may restore the microbial diversity in human habitats and improve current industrialized microbial states. In turn, increased microbial diversity could help reduce NCD prevalence. Natural plant communities (*e.g.* remnant vegetation) tend to be more microbially variable than human-impacted environments (*e.g.* lawns) (Mhuireach et al., 2016, 2019), and therefore, could serve as a source of diverse microbiota to colonize the human body and rewild human microbiomes.

Several studies have suggested that microbiota transfer from the environment to humans. For example, there is more bacterial diversity on the skin of individuals living near agricultural and forest environments compared to urban environments (Hanski et al., 2012). Murine models have further supported this observation; farm dust containing microorganisms can protect the epithelium in the lungs to minimize allergies, perhaps through transfer of protective microorganisms (Schuijs et al., 2015). Further, a study has suggested that spending at least 120 min outdoors over a week does improve health and well-being (White et al., 2019) and may help decrease the NCD epidemic in industrialized countries. If the Microbiome Rewilding Hypothesis is true, exposure to microbially diverse outdoor environments could be a solution to decreasing NCD rates in urban centers. Indeed, it has already been shown in a randomized controlled mouse trial that high-biodiversity soils potentially supply the gut with anxiety-reducing microbes (*e.g.* butyrate-producing bacteria) (Liddicoat et al., 2020).

Despite these hypotheses and observations, specific studies tracking how the human microbiome is affected when visiting urban green spaces have not yet been conducted. In this paper, we monitor changes in human-associated microbial diversity and composition after exposure to urban green spaces on both the skin and nasal passage of volunteers in Adelaide, Australia; Bournemouth, United Kingdom; and New Delhi, India. In Adelaide, we characterized environmental samples from this urban environment to track the direct transfer of specific environmental microbiota to the human subjects. Overall, this study aims to better understand the relationship between humans and their surrounding microbial environment and provide evidence to better understand the Hygiene, Old Friends, and Biodiversity Hypotheses.

2. Methods

2.1. Sampling locations

Samples were collected in urban green spaces of Adelaide, Australia (environmental samples: September 2016; human samples: October-November 2016), Bournemouth, England (human samples: October 2016), and New Delhi, India (human samples: October 2016). Locations were logged with GPS (Table S1). Five different types of urban green space were sampled three times in each city (n = 15 locations per city) to represent different vegetation types and to capture the city's green space heterogeneity (Table S1). The vegetation types varied between cities due to biome differences but were largely analogous. Adelaide's vegetation types were vacant lots (covering in annual weeds), lawns (*e.g.* sports fields), parklands (*i.e.* annual understory with tree canopy), revegetated open woodlands, and remnant open woodlands. Bournemouth's vegetation types consisted of bare ground, lawns, parklands, young regrowth forests (revegetated), and old regrowth forests (remnant). New Delhi's vegetation types were bare ground, lawns, parklands, invaded shrublands (*i.e.* predominantly invasive shrub species), and savannah woodlands (*i.e.* native grassland with sparse tree cover).

2.2. Sample collection

2.2.1. Soils

Soil was collected from the 0–10 cm soil horizon in Adelaide. Within each green space, approximately 100 g of soil was collected from each of nine points using sterilized (flamed) trowels and homogenized in a sterile bag. From the homogenized soil, a 50 g sample was collected in a sterile falcon tube and frozen at −20 °C at the research facility on the evening of collection.

2.2.2. Air

In Adelaide, an Airchek Sampler 224-PCXR7 (SKC Inc.) was run for 1 h to collect air samples. The sampler ran at 2 $L.min^{-1}$ and was calibrated using a rotameter before and after sampling to determine average flow rate. Samples were collected on 1 μm pore-size, 25 mm diameter glass fiber filters (SKC Inc.). The filter cartridge was sterilized before each filter was inserted. After collection, filters were placed into 2 mL sterile microcentrifuge tubes with sterilized forceps. A field control was taken at each site by loading a filter into a sterilized filter cartridge without running the sampler. Air filters were frozen at −20 °C at the research facility until DNA extraction.

2.2.3. Leaves

Leaf samples were collected in Adelaide while wearing sterile gloves from vegetation strata (mixed species) in two sterile Falcon tubes per site and frozen −20 °C at the research facility until washing. Care was taken to collect healthy, undamaged leaves. Samples were standardized for leaf surface area, and surface microbes were washed into 15 mL of

TE buffer from which DNA was extracted.

2.2.4. Skin and nasal swabs

In each city, two of the three subjects directly interacted with elements of urban green space by collecting air, soil, and leaf samples. These interactions, *i.e.* digging in the dirt and brushing against vegetation, were an attempt to mimic behaviors of children interacting with the environment. Subject 1 visited urban green spaces in all three countries, whereas subject 2 visited urban green spaces in Australia, and subject 3 visited UK and India. For subjects that traveled internationally, samples were taken at least three days after landing.

Under ethics approval (H-2016–235; University of Adelaide Human Research Ethics Committee), participants swabbed themselves (skin and nasal) in the morning before leaving their accommodation and then after spending over 1 h in Adelaide green spaces (October-November 2016; n = 4 days) or after ~15 min in Bournemouth and New Delhi green spaces (October 2016; n = 2 days each). Given time constraints, multiple sites were visited on the same day (Table S1). Two drops of sterile saline solution were applied to the inside (volar) left wrist of each participant who then rubbed a swab (Sterile Catch-All™ Sample Collection Swab; Epicentre Biotechnologies) on the area for 30 sec. Participants also swirled a swab in their nasal cavity no more than 1 cm up the nose. Swab tips were snipped into sterile 2 mL microcentrifuge tubes. Swab field controls (sterile swab that followed the same process as sampling, without touching the skin or nasal cavity) to capture background signals from outdoor swab collection were also taken in each green space. At each location, the skin field control involved applying 2 drops of saline to a swab and then enclosing the tip in a 2 mL microcentrifuge tube, and the nasal control was a dry swab placed into separate tube.

2.3. DNA extraction

To allow for a direct comparison, skin and nasal samples were prepared identically to the environmental samples. Bacterial DNA was extracted using the DNeasy Powersoil kit (QIAGEN), following manufacturer's instructions. Extraction blank controls (*i.e.* extractions without any biological sample; EBCs) were performed in parallel to monitor DNA present within the laboratory and reagents.

2.4. 16S ribosomal RNA amplification and sequencing preparation

Using Illumina primers (515F: 5'-AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3'; reverse-barcoded 806R: 5'-CAAGCAGAAGACGGCATACGAGATnnnnnnnnnnnnAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2011, 2012), the V4 region of the 16S rRNA gene was targeted and amplified. Each individual polymerase chain reaction (PCR; 25 μL) contained 18.05 μL DNA-free water, 2.5 μL 10× High Fidelity PCR Buffer (Invitrogen), 1.0 μL $MgSO_4$ (50 mM), 0.2 μL dNTPs (25 mM) (Invitrogen), 0.25 μL Platinum® Taq DNA Polymerase High Fidelity (Invitrogen), 1.0 μL of each V4 primer (10 μM), and 1.0 μL of DNA extract. Cycling conditions were as follows: 95 °C for 6 min; 38 cycles of 95 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 90 sec; and a final extension at 60 °C for 10 min. A no-template amplification control (NTC) was included during each PCR group to monitor laboratory and reagent contamination. Additionally, all extracts and NTCs were amplified in triplicate to minimize PCR bias (Goodrich et al., 2014). Once amplified, PCR products were pooled, and the presence of a 16S V4 gene product was verified by gel electrophoresis. Amplifiable products from EBCs and NTCs were prepared for sequencing alongside the samples to characterize laboratory and reagent contamination.

16S rRNA libraries were quantified using a Qubit dsDNA HS assay kit (Invitrogen) and pooled in equimolar concentrations into groups of ~30 samples. Pooled libraries were then cleaned using AxyPrep Mag PCR Clean-up Kit (Axygen Scientific). The pooled, cleaned libraries were then quantified using a broad sensitivity D1000 Screentape on an Agilent 2200 Tapestation. Quantitative PCR (qPCR; KAPA Illumina Primer mix) was carried out to accurately determine the concentration of this final library (LightCycler 96 System, Roche Life Science), before diluting to a 2 nM concentration for sequencing. Libraries were sequenced on the Illumina Miseq using 2 × 150 bp kit with and custom sequencing primers, as previously described (Caporaso et al., 2011, 2012).

2.5. Pre-processing and high-quality sequence selection

Using QIIME2 (v 2019.7) (Bolyen et al., 2019), all sequencing runs were demultiplexed by the unique barcode fused to each individual sample. Using only the forward sequences, sequences were filtered based on the quality scores (minimum 4) and presence of ambiguous base calls (Bokulich et al., 2013). Following this, sequences were denoised using Deblur (Amir et al., 2017), with sequences trimmed to 150 bp. Amplicon sequence variant (ASV) tables and representative sequences from each sequencing run were then merged to allow for comparative analysis. Any ASV that was observed less than 10 times in the dataset and ASVs observed in one sample only were removed. The remaining representative ASVs were assigned taxonomy based on the SILVA database (v 132) (Quast et al., 2013). Next, a SEPP insertion tree was created for phylogenetic diversity analyses (Janssen et al., 2018). Decontam (Davis et al., 2018) was then used to identify contaminants found from EBCs and NTCs at a 0.5 prevalence, and these contaminants were removed. Finally, singletons and any sample with less than 500 sequences was also removed.

2.6. Diversity, statistical and taxonomic analyses

Next, alpha- and beta diversity were calculated at a rarefied depth of 1,000 sequences. For alpha diversity, both the species richness (observed species) and phylogenetic diversity (Faith's PD) (Faith, 1992) were calculated, and a Wilcoxon test (Wilcoxon, 1945) was applied to calculate the significant differences in sample groups. Unweighted UniFrac (Lozupone and Knight, 2005) was used to examine the phylogenetic beta diversity. PERMANOVA (Anderson, 2001) was implemented as a pairwise comparison to compare the UniFrac distances between sample groups. To analyze taxa at the genus level, ASVs were summarized into their taxonomic classification. Presence and absence of taxa between the post-exposure samples and pre-exposure and environmental samples were calculated. To further characterize taxa shared between sample groups, a core microbiome analysis (80% present in all samples) was performed both before and after the removal of pre-exposure taxa from post-exposure samples.

3. Results

3.1. Sequencing analysis and quality control

This study included human skin (n = 16 pre-exposure; n = 90 post-exposure) and nasal (n = 16 pre-exposure; n = 90 post-exposure) swabs, environmental samples (*i.e.* air filters, leaves, and soils; n = 45), environmental background controls (field controls; swab or air filter opened from packaging in the field but no sample was taken; n = 52), and laboratory controls (EBCs and NTCs; n = 51). As these samples range in biomass, we first examined the microbial signal in each sample type for artefacts. First, we removed singletons and low abundance taxa by excising any amplicon sequence variant (ASV) represented by less than 10 sequences and any ASV only observed in one sample over the dataset. Next, we detected 65 contaminant ASVs found in laboratory controls using Decontam (Davis et al., 2018) (Table S2) and removed these ASVs from the analysis. Once laboratory contaminant sequences were removed, EBC and NTC samples were then removed for downstream analysis. We then removed samples with less than 500

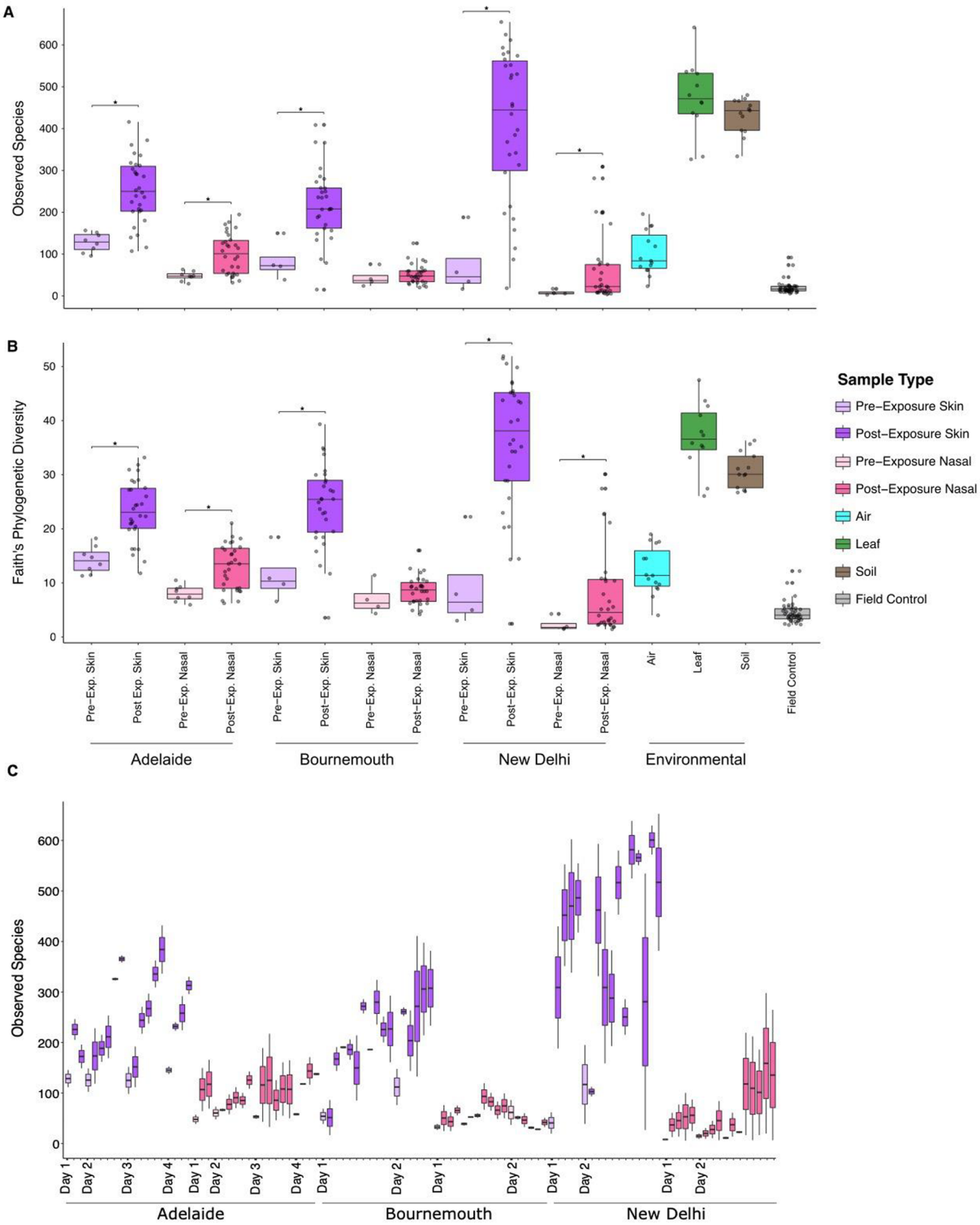

Fig. 1. Skin and nasal diversity increased after exposure to urban green space environments. Alpha diversity (*i.e.* within sample diversity) was calculated at a rarefied depth of 1,000 sequences for each sample, before binning into the respective sample type and location group. Two metrics were measured: A) Observed species and B) Faith's phylogenetic diversity. Statistical significance between pre- and post-exposure samples are denoted with * ($q < 0.05$). C) Alpha diversity was also calculated for each site visited throughout the day for both skin and nasal samples (displayed in order of sites visited). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

sequences (12 samples; Table S3). Post-filtering, 14,300,289 sequences (mean = 48,149 sequences; range = 529–791,282 sequences; see Table S4 for individual sample types) and 23,845 ASVs (see Table S4 for individual sample types) were retained from 297 samples.

3.2. Increased microbial diversity after green space exposure in Adelaide

First, we focused our analysis on a single city—Adelaide, Australia—to explore changes in skin and nasal microbiota after urban

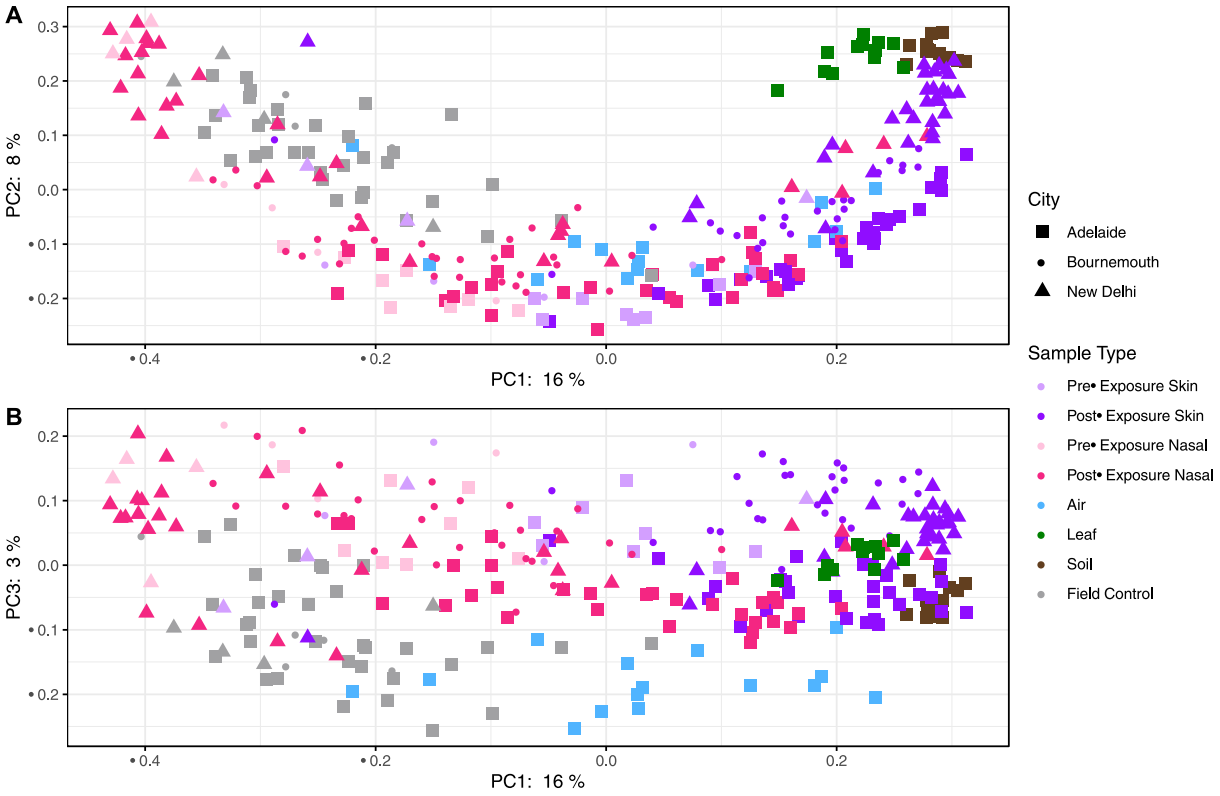

Fig. 2. Skin and nasal samples became more similar to environmental samples after urban green space exposure. PCoA plots were generated to compare compositional differences using the unweighted UniFrac metric. The first three principle component axes are shown as (A) PC1 vs. PC2 and (B) PC1 vs. PC3. Colors represent the sample type, and shapes represent the location. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

green space exposure. We assessed the diversity (microbial richness (observed species; OS) and phylogenetic (Faith's PD)) in skin and nasal samples after ~1.5 h of exposure to 15 different urban green space sites over four days. We observed a significant increase in skin microbiota diversity after urban green space exposure (Fig. 1; pre vs. post; Wilcoxon; OS: q = 0.0002; PD: q = 0.0001). Similarly, we observed an increase in nasal microbiota diversity after urban green space exposure (Fig. 1; OS: q = 0.0035; PD: q = 0.0022). In most cases, we noted an increase in diversity throughout the day, but after showering, the skin and nasal microbiota 'reset' to pre-exposure diversity levels as seen from the previous day (Fig. 1C). Overall, these results suggests that skin and nasal microbiota diversity increases after urban green space exposure.

Next, we evaluated the microbial composition changes (beta diversity) after exposure to urban green space environments in Adelaide. We found that the skin and nasal composition significantly shifted after urban Adelaide green space exposure (Fig. 2; pre vs. post; PERMANOVA; skin: pseudo-F = 3.351, q = 0.001; nasal: pseudo-F = 2.887, q = 0.001), and that microbiota in skin and nasal samples became more similar to the environmental samples after this exposure. More specifically, the skin samples showed closer approximation to soil samples after urban green space exposure (Fig. 2; pre vs. soil: pseudo-F = 10.62, q = 0.001; post vs. soil: pseudo-F = 10.77, q = 0.001). Further, the microbial composition of the nasal samples became more similar to the air samples (Fig. 2; PERMANOVA; pre vs. air: pseudo-F = 4.040, q = 0.001; post vs. air: pseudo-F = 2.687, q = 0.001) compared with the pre-exposure nasal samples. Overall, these results suggest that environmental microbes can colonize the human body during urban green space exposure over the timeframe observed.

We then investigated the origin or source of microbes in skin and nasal swabs after exposure to Adelaide urban green spaces. Before green space exposure, skin samples were consistently dominated (> 3% relative abundance) by *Micrococcus* (11.6% relative abundance), *Staphylococcus* (10.9%), *Tetrasphera* (10.3%), *Corynebacterium* (7.5%), *Paracoccus* (6.9%), *Acinetobacter* (6.7%), *Brevundimonas* (3.8%), and *Cutibacterium* (3.5%) (Fig. 3), and these genera decreased in relative abundance post-exposure. Rare taxa (less than 3% relative abundance; combined relative abundance = 45.8%) and *Sphingomonas* (5.2%) increased in abundance post-exposure (Fig. 3). We observed similar trends in the nose. *Staphylococcus* (57.6%), *Corynebacterium* (20.8%), *Lawsonella* (6.6%), and *Anaerococcus* (3.9%) taxa were the dominant genera in the nose before urban green space exposure (Fig. 3). After urban green space exposure, *Staphylococcus* and *Lawsonella* decreased in abundance, while *Corynebacterium* and *Anaerococcus* remained similar (Fig. 3). Similar to the skin, rare ASVs increased by 7.5% post-exposure in the nose (Fig. 3). Altogether, this suggests that increases in diversity and shifts in microbial abundances are linked to an increase in rare taxa and a decrease in common, human-associated taxa after green space exposure at this city.

3.3. Introduced microorganisms originate from the air, plants, and soil

We next identified the relative proportions of ASVs shared between human and environmental samples after green space exposure in Adelaide. In the skin, 18% of ASVs in post-exposure samples were also found in pre-exposure skin samples, while more ASVs were shared with environmental samples: 23% with air, 30% with leaf, and 40% shared with soil samples (Fig. 4). In the nose, only 14% of post-exposure nasal ASVs were shared with pre-exposure nasal samples, while there were more ASVs shared with environmental samples: 28% shared with air, 30% with leaf, and 37% with soil samples (Fig. 4). This highlights the presence of environmental ASVs in both skin and nasal samples post-exposure. Following this, we completed a core microbiome analysis to further explore the taxa consistently found on the human body after

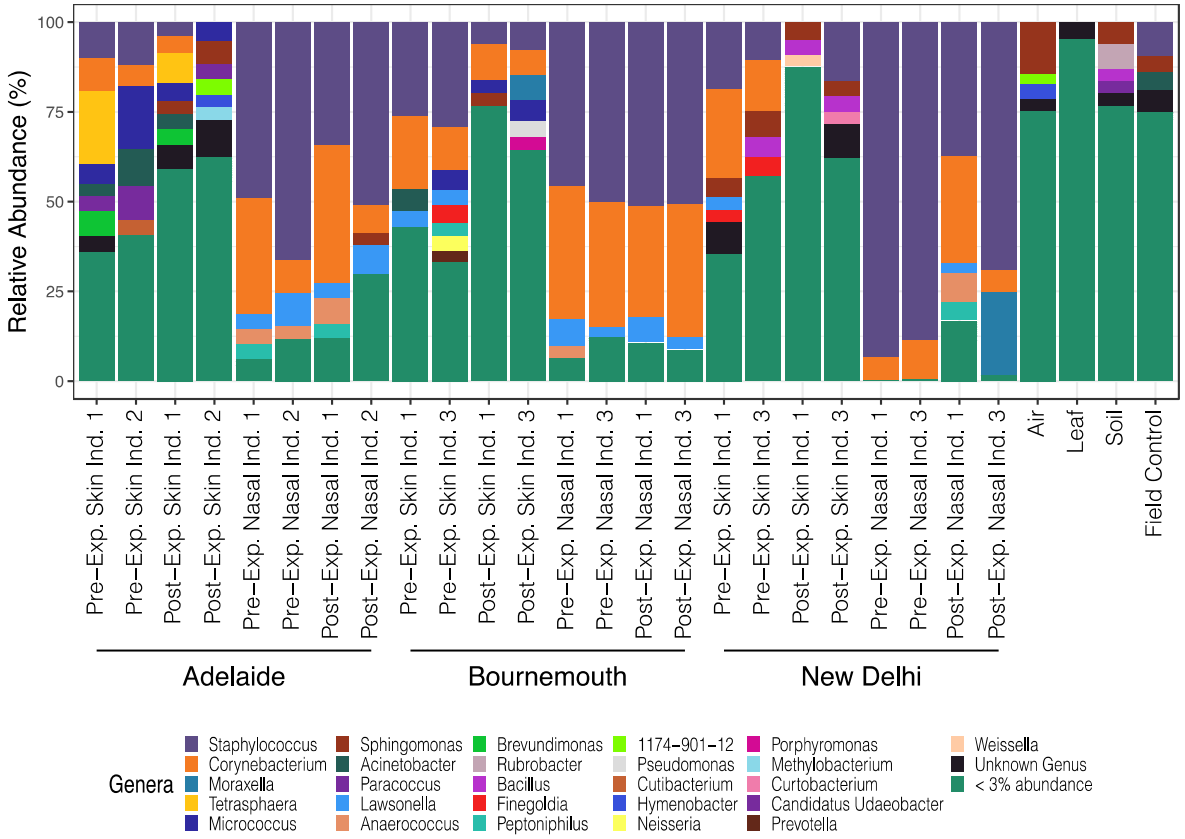

Fig. 3. Urban green space exposure is associated with an increase in low abundance taxa. The proportion of bacterial genera are shown in pre- and post-exposure skin and nasal samples, as well as environmental samples. Samples were grouped according to sample type, location, and subject. Genera with < 3% relative abundance were collapsed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

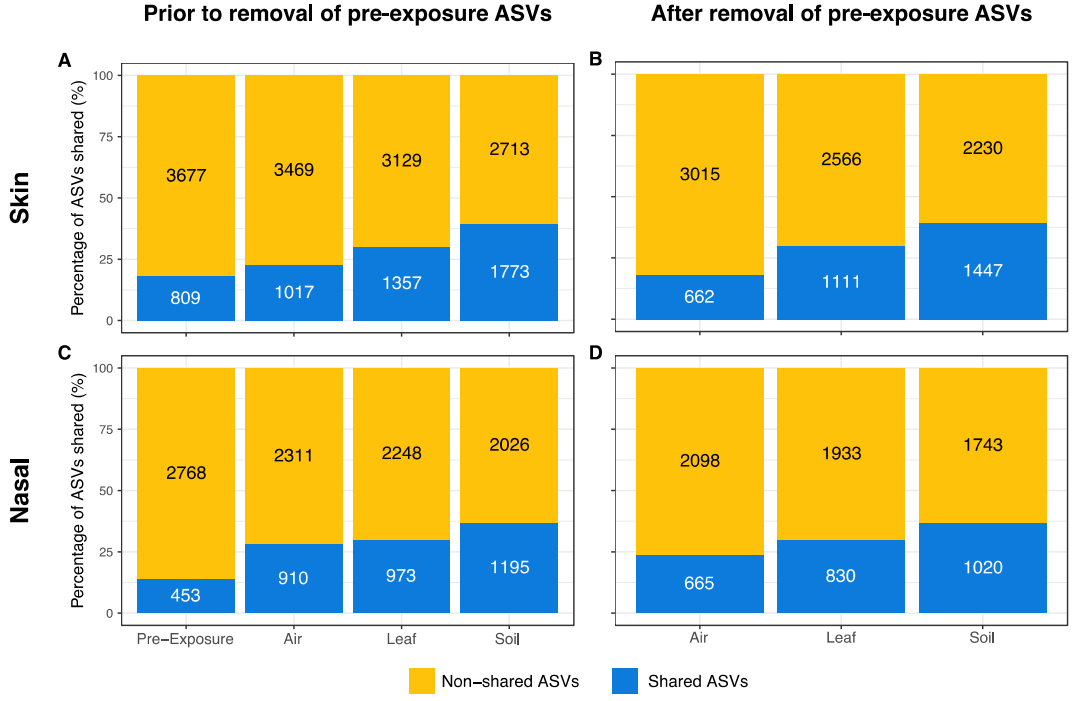

Fig. 4. Environmental ASVs are shared with skin and nasal samples after urban green space exposure. The presence or absence of ASVs from post-exposure samples and pre-exposure and environmental samples was calculated. This observation was calculated both before the removal of pre-exposure ASVs for skin (A) and nasal (C) samples, as well as after the removal of pre-exposure ASVs for skin (B) and nasal (D) samples. The percentage of non-shared ASVs were colored yellow, while the percentage of shared ASVs were blue. The number of shared or non-shared ASVs were noted in the bars for each sample type. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

urban green space exposure (*i.e.* present in 80% of samples). While 40 and 16 core ASVs were shared between the pre- and post-exposure skin and nasal samples, respectively, there were far fewer ASVs consistently shared between the environmental and post-exposure samples. For example, 13 air, 3 leaf, and 3 soil ASVs were shared with post-exposure skin samples, while 9 air, 1 leaf, and 3 soil ASVs were shared with post-exposure nasal samples (Table S5). When examining the core of the biological samples with all environmental samples, only a *Sphingomonas*, *Blastococcus*, and *Solirubrobacter* ASV were shared in 80% of all post-exposure skin and environmental samples, while a *Sphingomonas*, *Cutibacterium*, *Blastococcus,* and *Massilia* ASV were identified in 80% of all post-exposure nasal and environmental samples (Table S5). Overall, this suggests that microbes shared between the environment and the body are variable across environmental sites and the two individuals, but suggests that some environmental organisms can be identified in post-exposure skin and nasal swabs.

As the goal of this paper is to track newly introduced environmental species and not environmental species that may have been present on the skin and in the nose before the study, we excluded any ASV that was present in both pre- and post-exposure samples and then investigated the remaining post-exposure ASVs with environmental ASVs. In post-exposure skin samples, 18% of post-exposure skin ASVs were shared with air, 30% with leaf, and 39% with soil samples (Fig. 4). In post-exposure nasal samples, 24% of ASVs were shared with air, 30% with leaf, and 37% with soil samples (Fig. 4). The proportion of shared ASVs were very similar to before the removal of pre-exposure taxa, which indicates that very few pre-exposure microbes were shared with the environment, and this further confirms that these ASVs were transferred from the environment to the human body.

We next examined the core ASVs between post-exposure and environmental samples to investigate if the same ASVs were introduced onto the skin from the environment. No specific ASVs were present in the core microbiome between post-exposure and environmental samples, suggesting that the transfer of individuals species is highly variable across individuals and sites. While ASVs may be different, it is plausible that certain genera or types of species are preferentially transferred. Therefore, we performed a core microbiome analysis at the genus taxonomic level to provide a general description of the ASVs transferred from the environment. In the post-exposure skin samples, 34 genera were shared with at least one environmental type (Table 1). For example, *Sphingomonas*, *Nocardioides*, and *Solirubrobacter* genera, as well as unknown genera belonging to Sphingomonadaceae, Burkholderiaceae (family), and Solirubrobacterales (order), were shared in all of the environmental and post-exposure skin samples (Table 1). In the post-exposure nasal samples, 22 genera were shared with at least one environmental type (Table 1). *Sphingomonas, Nocardioides*, and Burkholderiaceae genera were observed in all three environmental sources and the post-exposure nasal samples (Table 1). As these genera were shared in multiple environmental types and humans, it may indicate their ubiquitous presence across the study sites.

3.4. Skin diversity increased with outdoor exposure in different locations

To investigate whether increased human-associated microbial diversity after urban green space exposure occurs in vastly different outdoor environments, skin and nasal samples from two individuals were collected during 15-minute, urban green space exposures in Bournemouth, England and New Delhi, India. In the skin samples, both microbial richness and phylogenetic diversity significantly increased after exposure to green space environments in Bournemouth (Fig. 1; pre vs. post; Wilcoxon; OS: $q = 0.0075$; PD: $q = 0.013$) and New Delhi (Fig. 1; OS: $q = 0.0016$; PD: $q = 0.0023$). In the nose, microbial richness and phylogenetic diversity significantly increased in nasal samples collected after urban green space exposure in New Delhi (Fig. 1; OS: $q = 0.034$; PD: $q = 0.031$); however, this was not the case in Bournemouth (Fig. 1; OS: $q = 0.27$; PD: $q = 0.15$). Similar to Adelaide, in most cases we saw an increase in microbial richness over the day for both skin and nasal samples in Bournemouth and New Delhi (Fig. 1C). Overall, these findings support an increase in microbial diversity of the skin, but not necessarily the nasal cavity, after green space exposure in different green space locations around the world.

Next, we examined the microbial composition of samples collected before and after green space exposure in Bournemouth and New Delhi. Exposure to urban green spaces resulted in a significant change in microbial composition in skin and nasal samples from Bournemouth (Fig. 2; pre vs. post; PERMANOVA; skin: pseudo-$F = 2.354$, $q = 0.002$; nasal: pseudo-$F = 1.722$, $q = 0.004$). In New Delhi, skin samples collected post-exposure were also significantly different in composition compared to pre-exposure skin samples (Fig. 2; pseudo-$F = 3.449$, $q = 0.001$). However, nasal samples showed no significant compositional changes after green space exposure (Fig. 2; pseudo-$F = 1.747$, $q = 0.063$). These results indicate that skin microbiota composition significantly changes after this type of green space exposure, but nasal microbiota compositional changes were not always observed during green space exposures, possibly due to the shorter exposure time frame.

Lastly, we examined changes in relative abundance of bacterial genera after green space exposure in Bournemouth and New Delhi (Fig. 3). On the skin, exposure to green spaces in both Bournemouth and New Delhi resulted in a decrease in abundance of *Staphylococcus* (20.7% decrease in Bournemouth; 5.4% decrease in New Delhi), *Corynebacterium* (7.7% decrease in Bournemouth; 17.9% decrease in New Delhi), *Finegoldia* (1.5% decrease in Bournemouth; 4.1% decrease in New Delhi), *Lawsonella* (2.8% decrease in Bournemouth), *Acinetobacter* (2.4% decrease in Bournemouth), and *Sphingomonas* (1.6% decrease in New Delhi) (Fig. 3). The genera that increased after green space exposure in Bournemouth were *Micrococcus* (1.8% increase) and *Moraxella* (3.4% increase), while only *Bacillus* (1.4% increase) increased in New Delhi (Fig. 3). Rare taxa also increased in both Bournemouth (26.1% increase) and New Delhi (30.6% increase) (Fig. 3). Similar to Australia, Bournemouth nasal samples were dominated by *Staphylococcus* (47.8% relative abundance), *Corynebacterium* (36.0%), and *Lawsonella* (5.2%) before green space exposure (Fig. 3). However, the relative abundance of these genera did not vary by more than 3% post-exposure, and these genera remained dominant post-exposure in Bournemouth. In contrast, the abundances in post-exposure nasal samples from New Delhi were more variable between individuals and were distinct from pre-exposure nasal samples. Again, *Staphylococcus* (90.7% relative abundance) and *Corynebacterium* (8.9%) were the most abundant taxa pre-exposure; however, *Staphylococcus* (53.1% relative abundance) decreased after green space exposure, and *Corynebacterium* (17.9%) and low abundance taxa (12.3%) increased post-exposure (Fig. 3). Interestingly, both individuals were affected by different taxa post-exposure in New Delhi. For example, *Moraxella* increased to 23.1% relative abundance after green space exposure in one subject, yet *Anaerococcus* (7.9%) and *Peptoniphilus* (5.1%) increased in abundance in the other subject (Fig. 3). While compositional and abundance-based differences were dependent on the city and individual, *Staphylococcus* consistently decreased, and low abundance taxa consistently increased in post-exposure skin and nasal samples in all three cities.

4. Discussion

There is a need to improve our understanding of the interactions between environmental and human microbiota, especially if this interaction is to be used to reduce the prevalence of NCDs in urban human populations. In this study, we monitored the bacterial diversity, composition, and relative abundance of microbiota in the skin and nasal passages from three individuals before and after exposure to several urban green spaces in three cities. The diversity and composition of skin microbiota consistently increased after exposure to urban green space environments. The response of nasal microbiota was unique to specific green spaces and individuals studied. Overall, we show that microbiota

Table 1
Core genera are shared between post-exposure samples and environmental samples after removing of pre-exposure ASVs.

ASV Genus	Skin			Nasal		
	Air	Leaf	Soil	Air	Leaf	Soil
Sphingomonas	■	■	■	■	■	■
Hymenobacter	■	■		■	■	
Nocardioides	■	■	■	■	■	■
Methylobacterium	■					
Solirubrobacter	■	■	■		■	■
Unknown Genus (Family 67-14)	■	■	■		■	■
Unknown Genus (Family Sphingomonadaceae)	■	■	■			■
Unknown Genus (Family Burkholderiaceae)	■	■	■	■	■	■
Unknown Genus (Family Chitinophagaceae)		■	■		■	■
Chthoniobacter		■	■			
Unknown Genus (Family Acetobacteraceae)		■				
Conexibacter		■	■		■	■
Candidatus Udaeobacter		■	■			
Bryobacter		■	■			
Unknown Genus (Family Solirubrobacteraceae)		■				
Novosphingobium		■	■		■	■
Gemmatimonas		■	■			
Unknown Genus (Family Beijerinckiaceae)		■	■			■
Mycobacterium		■	■		■	■
Streptomyces		■	■			
Pedobacter		■	■		■	■
Unknown Genus (Family Caulobacteraceae)		■	■			
Jatrophihabitans		■				
Unknown Genus (Family Isosphaeraceae)		■	■			
Rubrobacter			■			■
Microvirga			■			■
Flavisolibacter			■			■
RB41 (Family Pyrinomonadaceae)			■		■	■
Bacillus			■			
Segetibacter			■			■
Marmoricola			■			
Actinoplanes			■			■
Geodermatophilus			■			
Unknown Genus (Family Longimicrobiaceae)			■			
Devosia					■	■
Pseudomonas					■	

A core microbiome analysis (present in at least 80% of samples) was performed for post-exposure samples and environmental samples at the genus taxonomic level. Shaded boxes denote shared genera between post-exposure skin (light grey) and nasal (dark grey) samples and environmental samples.

can transfer from the environment onto the human body, for at least the period observed here. Demonstrating such a colonization pathway lays a foundation to further examine how human microbiota shift during interactions with the natural environment.

4.1. Environmental microbiota transfer onto people

We identified increases in skin and nasal microbial diversity and composition after green space exposure in three different cities. Before exposure to green spaces, we observed that the skin and nasal samples were dominated by typical skin microbiota, including *Staphylococcus* and *Corynebacterium* (Grice et al., 2009; Oh et al., 2014). However, after exposure to urban green spaces, we saw an increase in environmental microbial taxa. For instance, *Sphingomonas*, *Blastococcus* (skin and nose), *Solirubrobacter* (skin), and *Massilia* (nasal)—all common soil microbes—were consistently observed on the individuals in this study after urban green space exposure. *Cutibacterium*, which was shared between the air and post-exposure nasal samples, is normally found in high abundance on the skin (Gannesen et al., 2019), and may possibly be cross-contamination from the individual who collected the air samples. After removing the ASVs found in pre-exposure samples, we also found *Nocardioides* (skin and nose), *Burkholderiaceae* (skin and nose), Solirubrobacterales (skin), and Sphingomonadaceae (skin) taxa transferred from the environment to the human body after green space exposure. Of these aforementioned taxa, both *Sphingomonas* and *Massilia* have also been shown to colonize the human body. For example, some species of *Sphingomonas* have been associated with nosocomial infections (Ryan and Adley, 2010) and others have been observed as a human skin commensal (Cosseau et al., 2016). While most known species of *Massilia* are also found in the air (Weon et al., 2008), freshwater (Gallego et al., 2006), and plant roots (Ofek et al., 2012); *Massilia* has also been detected in blood, cerebrospinal fluid, and bone of immunocompromised patients (Lindquist et al., 2003) and from the eye of a patient suffering with endophthalmitis (Kämpfer et al., 2012). While we found taxa transferred from the environment to both individuals, it is unknown how long these microbes remained present on the human body. Indeed, Bateman (Bateman, 2017) showed that most soil microbes transferred onto an individual were lost after 2 h, but rare taxa still remained present on the skin for longer than 24 h, including after washing (Bateman, 2017). Further research is also needed to explore how exposure to these and similar microbes from different environments (*e.g.* indoor environments) may impact skin and nasal microbiota or how much human use of certain green spaces may also influence microbial transfers. With longer tracking across more individuals and

explorations of different environment types, further research can investigate how these microbes do or do not colonize the human body and whether or not that colonization has health benefits or risks in healthy individuals.

Surprisingly, the change in diversity and composition before and after urban green space exposure was generally similar in the three different cities, despite numerous factors in timing (*e.g.* seasonality), diet, and exposures (*e.g.* temperature, humidity, pollution) differing between the three cities. Several studies have explored human microbiota changes when traveling (Youmans et al., 2015; Vangay et al., 2018; Voorhies et al., 2019), and these studies have implications to our results. For example, microbiota are impacted by circadian rhythms (Deaver et al., 2018), which were likely to be altered in the individuals studied here as they traveled internationally to these locations over a three-week period. We also only sampled our participants in the morning; future studies should examine the maintenance of these microbes throughout the day and collect control samples from these same individuals when they are not exposed to green spaces at different times of day. Further studies have also shown that diseases acquired during traveling, such as travelers' diarrhea, which affects up to 60% of individuals traveling to industrializing countries (Greenwood et al., 2008), can alter gut microflora composition, but not diversity, after the illness (Youmans et al., 2015). Altered diets and immunological responses to microbes in these locations may also have influenced our results. Another study showed that International Space Station astronauts had significant changes to skin and gut microbiota both during and after space flight, suggesting that the exposure to a single indoor environment can influence microbial composition and increase diversity (Voorhies et al., 2019). While space capsules are very different from urban environments, our participants were on long-haul flights during this study traveling from country to country. Lastly, Thai immigrants had altered gut microbial composition, decreased microbial diversity, and an increase in obesity rates after shifting their environment and moving to the United States (Vangay et al., 2018). Nevertheless, these studies support the idea that exposure to environments with different levels of diversity can alter the relative abundance of existing skin taxa, such as *Staphylococcus*, *Streptococcus*, and *Corynebacterium* (Voorhies et al., 2019). However, longitudinal investigations into microbial changes associated with different environmental contexts (*e.g.* indoor, non-green space, and different urban environments), as well as their concomitant functional changes, are needed to understand the role of environmental exposure in shaping the human microbiome, especially in the context of health. Further studies are also needed to examine if environmental exposure influences the microbiota in the mouth, urogenital tract, and other body sites.

4.2. Microbial diversity and health

Associations between decreased environmental or human diversity and disease have been noted in the skin and respiratory tract. For example, lower biodiversity on the skin is associated with atopic dermatitis (Bjerre et al., 2017; Kim and Kim, 2019), and exposure to farm dust—with a higher microbial load compared to house dust—has been shown to protect individuals against respiratory disease, such as asthma (Schuijs et al., 2015). These studies continue to support the idea that either having increased exposure to microbes or being colonized with more diverse microbes may have positive health benefits. In our study, we observed a significant increase in microbial diversity on the skin and in the nasal cavity of individuals that were exposed to diverse urban green spaces, indicating that exposure to these environments may be able to assist in providing a protective effect against immune-mediated diseases. Downstream studies looking at the health outcomes of individuals with regular outdoor exposure are needed to determine if these increases can be applied to successfully prevent or treat human disease.

While a substantial number of studies suggest that increased microbial biodiversity may generally be good for human health, the timing of this exposure to biodiverse microbial communities may also be important. For instance, increased biodiversity exposure is likely critical during immune development, as intense immune training and development occurs during the first three months following birth (Lloyd and Saglani, 2019; Knight et al., 2014) through to the age of three (Dzidic et al., 2018; Gensollen et al., 2016; Yatsunenko et al., 2012). During birth, increased infant microbial diversity has been associated with a vaginal birth and breast-feeding of the infant, compared to caesarean section births and formula feeding (Dominguez-Bello et al., 2019; Dzidic et al., 2018). However, there are many additional factors that contribute to the development of a child's microbiota, including the environment, diet, and medication (Munyaka et al., 2014). Understanding how these environmental factors alter the human microbiota during this critical window is especially important in the context of limiting downstream health issues. There is a growing body of literature suggesting that several diseases and disorders can be reduced by exposing an infant to particular 'allergens' early in immune development (von Hertzen et al., 2011; Burbank et al., 2017). Alternatively, there are negatively associated factors, such as antibiotics and reduced outdoor exposure, that may disturb a naturally developing microbiota and underpin disease development later in life (*e.g.* obesity) (Dominguez-Bello et al., 2019). While our study was conducted on adults, similar studies examining outdoor exposure in children should be conducted to see if an increase in their microbial biodiversity reduces downstream development of NCDs. Health consequences of outdoor exposure in school age children would also be a fruitful avenue to pursue in future research.

In most industrialized countries, individuals now spend an average of 146 h inside each week (~87%) (Spalt et al., 2016). Children also spend less time in outdoor activities than any other point in our history, especially with an increase in the availability of technology; for example, children spent ~30 min less in outdoor play between 1975 and 2015 (Mullan, 2019). In response to this, several studies have identified that limiting indoor exposure—by spending time outside—can improve health. For example, White et al. (2019) revealed that spending at least two hours outdoors each week was associated with self-reported good health and wellbeing (White et al., 2019). While numerous factors may contribute to this improvement in health, such as vitamin D production and exercise, the interaction with a microbially diverse environment could also make a long-term contribution to health. Recently, researchers have identified that some microorganisms in biodiverse soil (*e.g. Mycobacterium vaccae* and *Kineothrix alysoides*) can improve mood and limit anxiety and depression in mouse models (Reber et al., 2016; Liddicoat et al., 2020), suggesting that exposure to biodiverse outdoor microbiota may also contribute to improved human health. Our study indicates that a pathway for colonization of these environmental microbiota can occur through exposure to biodiverse environments (Mills et al., 2019; Aerts et al., 2018), which can happen in addition to eating from home or community-planted vegetable gardens (Tresch et al., 2019; Robinson et al., 2018; Mills et al., 2019), incorporating indoor plants into an office space or at home (Mahnert et al., 2015), planting roof-top gardens on apartment buildings (Robinson et al., 2018), or living with a dog that spends time outdoors.

5. Conclusions

Decreases in environmental microbial diversity and increases in NCDs have been observed over the last 70 years in both industrialized and industrializing countries. Exposure to biodiverse urban green spaces may improve health by increasing microbial diversity and shaping the composition of human microbiota after exposure. This study suggests that short-term (*e.g.* 15–30 min) exposure can introduce environmental microbes to the skin, and in some cases, to the nose. Further research is needed to examine if these microbiota alterations linked to green space exposure have positive health benefits, especially

in children, across different locations, and within other body sites.

Ethics approval and consent to participate

Ethics approval for this project was obtained from the University of Adelaide Human Research Ethics Committee (H-2016–235). Approvals included informed consent from participants and education to safely and reproducibly collect swabs.

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Author contributions

LSW, MFB, JGM, PW, CS, SY, and AL conceptualized and designed the study; JGM, MFB, CS, and SY collected samples; CAS and JGM performed experiments; CAS performed bioinformatics analysis; CAS and LSW provided interpretation of the data; CAS wrote the manuscript; all authors contributed to editing the manuscript.

CRediT authorship contribution statement

Caitlin A. Selway: Data curation, Investigation, Formal analysis, Visualization, Validation, Writing - original draft, Writing - review & editing. **Jacob G. Mills:** Conceptualization, Methodology, Data curation, Investigation, Writing - review & editing. **Philip Weinstein:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Chris Skelly:** Conceptualization, Methodology, Writing - review & editing. **Sudesh Yadav:** Conceptualization, Methodology, Writing - review & editing. **Andrew Lowe:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Martin F. Breed:** Conceptualization, Methodology, Resources, Writing - review & editing, Funding acquisition, Supervision. **Laura S. Weyrich:** Conceptualization, Methodology, Resources, Writing - review & editing, Funding acquisition, Supervision.

Data availability statement

The datasets analyzed in this article are available in the QIITA repository with Study ID 13064 (https://qiita.ucsd.edu/study/description/13064). Scripts for QIIME2 and R analysis are available on Figshare (https://adelaide.figshare.com/articles/Microbial_Transfer_To_Humans_Selway2020_txt/12016443).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106084.

References

Aerts, R., Honnay, O., Van Nieuwenhuyse, A., 2018. Biodiversity and human health: mechanisms and evidence of the positive health effects of diversity in nature and green spaces. Br. Med. Bull. 127, 5–22. https://doi.org/10.1093/bmb/ldy021.

Amir, A., McDonald, D., Navas-Molina, J.A., Kopylova, E., Morton, J.T., Xu, Z.Z., et al., 2017. Deblur rapidly resolves single-nucleotide community sequence patterns. MSystems 2, e00191–e216. https://doi.org/10.1128/mSystems.00191-16.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral. Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp. x.

Bach, J.-F., 2002. The effect of infections on susceptibility to autoimmune and allergic diseases. N. Engl. J. Med. 347, 911–920. https://doi.org/10.1056/NEJMra020100.

Bateman, A., 2017. The dynamics of microbial transfer and persistence on human skin.

Bjerre, R.D., Bandier, J., Skov, L., Engstrand, L., Johansen, J.D., 2017. The role of the skin microbiome in atopic dermatitis: a systematic review. Br. J. Dermatol. 177, 1272–1278. https://doi.org/10.1111/bjd.15390.

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., et al., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59. https://doi.org/10.1038/nmeth.2276.

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

Boutayeb, A., Boutayeb, S., 2005. The burden of non communicable diseases in developing countries. Int. J. Equity Health 4, 2. https://doi.org/10.1186/1475-9276-4-2.

Burbank, A.J., Sood, A.K., Kesic, M.J., Peden, D.B., Hernandez, M.L., 2017. Environmental determinants of allergy and asthma in early life. J. Allergy Clin. Immunol. 140, 1–12. https://doi.org/10.1016/j.jaci.2017.05.010.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. 108, 4516–4522. https://doi.org/10.1073/pnas.1000080107.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Cosseau, C., Romano-Bertrand, S., Duplan, H., Lucas, O., Ingrassia, I., Pigasse, C., et al., 2016. Proteobacteria from the human skin microbiota: species-level diversity and hypotheses. One Health 2, 33–41. https://doi.org/10.1016/j.onehlt.2016.02.002.

Davis, N.M., Proctor, D.M., Holmes, S.P., Relman, D.A., Callahan, B.J., 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6, 226. https://doi.org/10.1186/s40168-018-0605-2.

Deaver, J.A., Eum, S.Y., Toborek, M., 2018. Circadian disruption changes gut microbiome taxa and functional gene composition. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00737.

Dominguez-Bello, M.G., Godoy-Vitorino, F., Knight, R., Blaser, M.J., 2019. Role of the microbiome in human development. Gut 68, 1108–1114. https://doi.org/10.1136/gutjnl-2018-317503.

Dzidic, M., Boix-Amorós, A., Selma-Royo, M., Mira, A., Collado, M.C., 2018. Gut microbiota and mucosal immunity in the neonate. Med. Sci. 6, 56. https://doi.org/10.3390/medsci6030056.

Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. Biol. Conserv. 61, 1–10. https://doi.org/10.1016/0006-3207(92)91201-3.

Gallego, V., Sánchez-Porro, C., García, M.T., Ventosa, A., 2006. Massilia aurea sp. nov., isolated from drinking water. Int. J. Syst. Evol. Microbiol. 56, 2449–2453. https://doi.org/10.1099/ijs.0.64389-0.

Gannesen, A.V., Zdorovenko, E.L., Botchkova, E.A., Hardouin, J., Massier, S., Kopitsyn, D.S., et al., 2019. Composition of the biofilm matrix of cutibacterium acnes acneic strain RT5. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.01284.

Gensollen, T., Iyer, S.S., Kasper, D.L., Blumberg, R.S., 2016. How colonization by microbiota in early life shapes the immune system. Science 352, 539–544. https://doi.org/10.1126/science.aad9378.

Goodrich, J.K., Di Rienzi, S.C., Poole, A.C., Koren, O., Walters, W.A., Caporaso, J.G., et al., 2014. Conducting a microbiome study. Cell 158, 250–262. https://doi.org/10.1016/j.cell.2014.06.037.

Graham, H., White, P.C.L., 2016. Social determinants and lifestyles: integrating environmental and public health perspectives. Public Health 141, 270–278. https://doi.org/10.1016/j.puhe.2016.09.019.

Greenwood, Z., Black, J., Weld, L., O'Brien, D., Leder, K., Von Sonnenburg, F., et al., 2008. Gastrointestinal infection among international travelers globally. J. Travel Med. 15, 221–228. https://doi.org/10.1111/j.1708-8305.2008.00203.x.

Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J., Young, A.C., et al., 2009. Topographical and temporal diversity of the human skin microbiome. Science 324, 1190–1192. https://doi.org/10.1126/science.1171700.

Hanski, I., von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., et al.,

2012. Environmental biodiversity, human microbiota, and allergy are interrelated. Proc. Natl. Acad. Sci. 109, 8334–8339. https://doi.org/10.1073/pnas.1205624109.
Janssen, S., McDonald, D., Gonzalez, A., Navas-Molina, J.A., Jiang, L., Xu, Z.Z., et al., 2018. Phylogenetic placement of exact amplicon sequences improves associations with clinical information. MSystems 3. https://doi.org/10.1128/mSystems.00021-18.
Kämpfer, P., Lodders, N., Martin, K., Falsen, E., 2012. Massilia oculi sp. nov., isolated from a human clinical specimen. Int. J. Syst. Evol. Microbiol. 62, 364–369. https://doi.org/10.1099/ijs.0.032441-0.
Kim, J.E., Kim, H.S., 2019. Microbiome of the skin and gut in atopic dermatitis (AD): understanding the pathophysiology and finding novel management strategies. J. Clin. Med. 8, 444. https://doi.org/10.3390/jcm8040444.
Knight, J.M., Davidson, L.A., Herman, D., Martin, C.R., Goldsby, J.S., Ivanov, I.V., et al., 2014. Non-invasive analysis of intestinal development in preterm and term infants using RNA-Sequencing. Sci. Rep. 4, 5453. https://doi.org/10.1038/srep05453.
Lee, S.J., Longcore, T., Rich, C., Wilson, J.P., 2017. Increased home size and hardscape decreases urban forest cover in Los Angeles County's single-family residential neighborhoods. Urban For Urban Green 24, 222–235. https://doi.org/10.1016/j.ufug.2017.03.004.
Liddicoat, C., Sydnor, H., Cando-Dumancela, C., Dresken, R., Liu, J., Gellie, N.J.C., et al., 2020. Naturally-diverse airborne environmental microbial exposures modulate the gut microbiome and may provide anxiolytic benefits in mice. Sci. Total Environ. 701, 134684. https://doi.org/10.1016/j.scitotenv.2019.134684.
Lindquist, D., Murrill, D., Burran, W.P., Winans, G., Janda, J.M., Probert, W., 2003. Characteristics of massilia timonae and massilia timonae-like isolates from human patients, with an emended description of the species. J. Clin. Microbiol. 41, 192–196. https://doi.org/10.1128/JCM.41.1.192-196.2003.
Lloyd, C.M., Saglani, S., 2019. Opening the window of immune opportunity: treating childhood asthma. Trends Immunol. 40, 786–798. https://doi.org/10.1016/j.it.2019.07.004.
Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005.
Lynch, S.V., Wood, R.A., Boushey, H., Bacharier, L.B., Bloomberg, G.R., Kattan, M., et al., 2014. Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children. J. Allergy Clin. Immunol. 134 (593–601), e12. https://doi.org/10.1016/j.jaci.2014.04.018.
Mahnert, A., Moissl-Eichinger, C., Berg, G., 2015. Microbiome interplay: plants alter microbial abundance and diversity within the built environment. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.00887.
McMichael, A.J., 2013. Globalization, climate change, and human health. N. Engl. J. Med. 368, 1335–1343. https://doi.org/10.1056/NEJMra1109341.
Mhuireach, G.Á., Betancourt-Román, C.M., Green, J.L., Johnson, B.R., 2019. Spatiotemporal controls on the urban aerobiome. Front. Ecol. Evol. 7. https://doi.org/10.3389/fevo.2019.00043.
Mhuireach, G., Johnson, B.R., Altrichter, A.E., Ladau, J., Meadow, J.F., Pollard, K.S., et al., 2016. Urban greenness influences airborne bacterial community composition. Sci. Total Environ. 571, 680–687. https://doi.org/10.1016/j.scitotenv.2016.07.037.
Mills, J.G., Weinstein, P., Gellie, N.J.C., Weyrich, L.S., Lowe, A.J., Breed, M.F., 2017. Urban habitat restoration provides a human health benefit through microbiome rewilding: the Microbiome Rewilding Hypothesis. Restor. Ecol. 25, 866–872. https://doi.org/10.1111/rec.12610.
Mills, J.G., Brookes, J.D., Gellie, N.J.C., Liddicoat, C., Lowe, A.J., Sydnor, H.R., et al., 2019. Relating urban biodiversity to human health with the 'holobiont' concept. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.00550.
Mullan, K., 2019. A child's day: trends in time use in the UK from 1975 to 2015. Br. J. Sociol. 70, 997–1024. https://doi.org/10.1111/1468-4446.12369.
Munyaka, P.M., Khafipour, E., Ghia, J.-E., 2014. External influence of early childhood establishment of gut microbiota and subsequent health implications. Front. Pediatr. 2. https://doi.org/10.3389/fped.2014.00109.
Ofek, M., Hadar, Y., Minz, D., 2012. Ecology of root colonizing massilia (oxalobacteraceae). PLoS ONE 7, e40117. https://doi.org/10.1371/journal.pone.0040117.
Oh, J., Byrd, A.L., Deming, C., Conlan, S., NISC Comparative Sequencing Program, Barnabas, B., et al., 2014. Biogeography and individuality shape function in the human skin metagenome. Nature 514, 59–64. Doi: 10.1038/nature13786.
Okada, H., Kuhn, C., Feillet, H., Bach, J.-F., 2010. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin. Exp. Immunol. 160, 1–9. https://doi.org/10.1111/j.1365-2249.2010.04139.x.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.
Reber, S.O., Siebler, P.H., Donner, N.C., Morton, J.T., Smith, D.G., Kopelman, J.M., et al., 2016. Immunization with a heat-killed preparation of the environmental bacterium Mycobacterium vaccae promotes stress resilience in mice. Proc. Natl. Acad. Sci. 113, E3130–E3139. https://doi.org/10.1073/pnas.1600324113.
Robinson, J.M., Mills, J.G., Breed, M.F., 2018. Walking ecosystems in microbiome-inspired green infrastructure: an ecological perspective on enhancing personal and planetary health. Challenges 9, 40. https://doi.org/10.3390/challe9020040.
Rook, G.A.W., 2012. Hygiene hypothesis and autoimmune diseases. Clin. Rev. Allergy Immunol. 42, 5–15. https://doi.org/10.1007/s12016-011-8285-8.
Rook, G.A.W., Martinelli, R., Brunet, L.R., 2003. Innate immune responses to mycobacteria and the downregulation of atopic responses. Curr. Opin. Allergy Clin. Immunol. 3, 337–342. https://doi.org/10.1097/01.all.0000092602.76804.ad.
Ryan, M.P., Adley, C.C., 2010. Sphingomonas paucimobilis: a persistent Gram-negative nosocomial infectious organism. J. Hosp. Infect. 75, 153–157. https://doi.org/10.1016/j.jhin.2010.03.007.
Schuijs, M.J., Willart, M.A., Vergote, K., Gras, D., Deswarte, K., Ege, M.J., et al., 2015. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. Science 349, 1106–1110. https://doi.org/10.1126/science.aac6623.
Spalt, E.W., Curl, C.L., Allen, R.W., Cohen, M., Adar, S.D., Stukovsky, K.H., et al., 2016. Time-location patterns of a diverse population of older adults: the multi-ethnic study of atherosclerosis and air pollution (MESA Air). J. Expo. Sci. Environ. Epidemiol. 26, 349–355. https://doi.org/10.1038/jes.2015.29.
Steffen, W., Broadgate, W., Deutsch, L., Gaffney, O., Ludwig, C., 2015. The trajectory of the anthropocene: the great acceleration. Anthr. Rev. 2, 81–98. https://doi.org/10.1177/2053019614564785.
Strachan, D.P., 1989. Hay fever, hygiene, and household size. BMJ 299, 1259–1260. https://doi.org/10.1136/bmj.299.6710.1259.
Tresch, S., Frey, D., Bayon, R.-C.L., Mäder, P., Stehle, B., Fliessbach, A., et al., 2019. Direct and indirect effects of urban gardening on aboveground and belowground diversity influencing soil multifunctionality. Sci. Rep. 9, 1–13. https://doi.org/10.1038/s41598-019-46024-y.
Vangay, P., Johnson, A.J., Ward, T.L., Al-Ghalith, G.A., Shields-Cutler, R.R., Hillmann, B.M., et al., 2018. U.S. immigration westernizes the human gut microbiome. Cell 175 (962–972), e10. https://doi.org/10.1016/j.cell.2018.10.029.
von Hertzen, L., Hanski, I., Haahtela, T., 2011. Natural immunity. EMBO Rep. 12, 1089–1093. https://doi.org/10.1038/embor.2011.195.
Voorhies, A.A., Mark Ott, C., Mehta, S., Pierson, D.L., Crucian, B.E., Feiveson, A., et al., 2019. Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-46303-8.
Wagner, K.-H., Brath, H., 2012. A global view on the development of non communicable diseases. Prev. Med. 54, S38–S41. https://doi.org/10.1016/j.ypmed.2011.11.012.
Weon, H.-Y., Kim, B.-Y., Son, J.-A., Jang, H.B., Hong, S.K., Go, S.-J., et al., 2008. Massilia aerilata sp. nov., isolated from an air sample. Int. J. Syst. Evol. Microbiol. 58, 1422–1425. https://doi.org/10.1099/ijs.0.65419-0.
White, M.P., Alcock, I., Grellier, J., Wheeler, B.W., Hartig, T., Warber, S.L., et al., 2019. Spending at least 120 minutes a week in nature is associated with good health and wellbeing. Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-019-44097-3.
Wilcoxon, F., 1945. Individual comparisons by ranking methods. Biom. Bull. 1, 80–83. https://doi.org/10.2307/3001968.
Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., et al., 2012. Human gut microbiome viewed across age and geography. Nature 486, 222–227. https://doi.org/10.1038/nature11053.
Youmans, B.P., Ajami, N.J., Jiang, Z.-D., Campbell, F., Wadsworth, W.D., Petrosino, J.F., et al., 2015. Characterization of the human gut microbiome during travelers' diarrhea. Gut Microbes 6, 110–119. https://doi.org/10.1080/19490976.2015.1019693.