Environment International 157 (2021) 106811

Contents lists available at ScienceDirect

Environment International

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

# Long-term biodiversity intervention shapes health-associated commensal microbiota among urban day-care children

Marja I. Roslund [a], Riikka Puhakka [a], Noora Nurminen [b], Sami Oikarinen [b], Nathan Siter [c], Mira Grönroos [a], Ondřej Cinek [d], Lenka Kramná [d], Ari Jumpponen [e], Olli H. Laitinen [b], Juho Rajaniemi [c], Heikki Hyöty [b], Aki Sinkkonen [f,*], the ADELE research group [1]

[a] Ecosystems and Environment Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Niemenkatu 73, FI-15140 Lahti, Finland
[b] Faculty of Medicine and Health Technology, Tampere University, Arvo Ylpön katu 34, FI-33520 Tampere, Finland
[c] Faculty of Built Environment, Tampere University, Korkeakoulunkatu 5, FI-33720 Tampere, Finland
[d] Department of Pediatrics, Second Faculty of Medicine, Charles University, V Úvalu 84, Praha 5, 150 06 Prague, Czech Republic
[e] Division of Biology, Kansas State University, Manhattan KS66506, KS, United States of America
[f] Natural Resources Institute Finland, Turku, Finland

ARTICLE INFO

Handling editor: Yong-Guan Zhu

ABSTRACT

*Background:* In modern urban environments children have a high incidence of inflammatory disorders, including allergies, asthma, and type 1 diabetes. The underlying cause of these disorders, according to the biodiversity hypothesis, is an imbalance in immune regulation caused by a weak interaction with environmental microbes. In this 2-year study, we analyzed bacterial community shifts in the soil surface in day-care centers and commensal bacteria inhabiting the mouth, skin, and gut of children. We compared two different day-care environments: standard urban day-care centers and intervention day-care centers. Yards in the latter were amended with biodiverse forest floor vegetation and sod at the beginning of the study.

*Results:* Intervention caused a long-standing increase in the relative abundance of nonpathogenic environmental mycobacteria in the surface soils. Treatment-specific shifts became evident in the community composition of Gammaproteobacteria, Negativicutes, and Bacilli, which jointly accounted for almost 40 and 50% of the taxa on the intervention day-care children's skin and in saliva, respectively. In the year-one skin swabs, richness of Alpha-, Beta-, and Gammaproteobacteria was higher, and the relative abundance of potentially pathogenic bacteria, including *Haemophilus parainfluenzae*, *Streptococcus* sp., and *Veillonella* sp., was lower among children in intervention day-care centers compared with children in standard day-care centers. In the gut, the relative abundance of *Clostridium sensu stricto* decreased, particularly among the intervention children.

*Conclusions:* This study shows that a 2-year biodiversity intervention shapes human commensal microbiota, including taxa that have been associated with immune regulation. Results indicate that intervention enriched commensal microbiota and suppressed the potentially pathogenic bacteria on the skin. We recommend future studies that expand intervention strategies to immune response and eventually the incidence of immune-mediated diseases.

## 1. Introduction

Today, the vast majority of children in the world live in urban areas with limited access to natural environments (United Nations, 2018). Coincidently, an increasing number of children living in urban areas are also suffering from immune-mediated diseases, including asthma (Ege et al., 2012), type 1 diabetes (Kondrashova et al., 2005), atopy, and allergies (Hanski et al., 2012). Urban children, in comparison with rural children, have distinct commensal microbiota, which is hypothesized to be a cause of the high immune-mediated disease incidence among children living in an urban environment (Hanski et al., 2012; Kondrashova et al., 2013; Lehtimäki et al., 2017). According to the altered

* Corresponding author.
*E-mail address:* aki.sinkkonen@luke.fi (A. Sinkkonen).
[1] The ADELE research group.

https://doi.org/10.1016/j.envint.2021.106811
Received 4 June 2021; Received in revised form 17 July 2021; Accepted 30 July 2021
Available online 14 August 2021

environmental microbiota hypothesis, both biodiversity loss (Haahtela, 2019) and urban pollution alter environmental and commensal microbiota, potentially leading to an imbalance in human microbiota (Parajuli et al., 2017; Roslund et al., 2019, 2018; Vari et al., 2021). Several studies suggest that an imbalance in human microbiota may eventually lead to increased incidence of noncommunicable immune-mediated diseases (Cekanaviciute et al., 2017; Ege et al., 2012; Hanski et al., 2012; Santoru et al., 2017; Stein et al., 2016).

An imbalance in human microbiota (a.k.a dysbiosis), is characterized by overgrowth of some bacterial species, while the relative abundance of other bacteria decreases. The dysbiosis in commensal microbiota inhabiting the skin, mouth, and gut may be an important factor determining the transition from health to disease (Karkman et al., 2017; Lynch and Pedersen, 2016; Said et al., 2014; Stokholm et al., 2018). Dysbiosis on the skin is associated with dermatologic disorders, including atopic eczema (Fyhrquist et al., 2014; Nakatsuji and Gallo, 2019), acne vulgaris, and rosacea (Murillo and Raoult, 2013). Many studies have reported an increased abundance of *Staphylococcus aureus* on skin among atopic compared with healthy individuals (Byrd et al., 2017; Kobayashi et al., 2015; Nakatsuji et al., 2017; Nakatsuji and Gallo, 2019). In addition, lower gammaproteobacterial diversity on the skin is associated with atopy, whereas *Acinetobacter* species induce anti-inflammatory responses on skin (Fyhrquist et al., 2014; Hanski et al., 2012). Childhood salivary bacteria in the mouth may contribute to priming the microbiota in the gut (Wade, 2013). In addition to dysbiosis in the oral cavity, salivary bacteria are also connected to imbalances elsewhere in the human body (Wade, 2013), such as inflammatory bowel disease (Docktor et al., 2012; Said et al., 2014). Gut microbiota shapes the priming of the immune system in early childhood; thus, perturbations in the gut microbiome may trigger inflammatory disorders later in life (Gensollen et al., 2016; Stokholm et al., 2018). Dysbiosis in the gut has been linked to immune-mediated diseases, such as type 1 diabetes (Brown et al., 2011), rheumatoid arthritis (Chen et al., 2016), asthma (Stokholm et al., 2018), and inflammatory bowel disease (Morgan et al., 2012). The production of butyrate is crucial in maintaining gut health and most butyrate-producing bacteria belong to the families Ruminococcaceae and Lachnospiraceae (Geirnaert et al., 2017).

Attempts to restore dysbiotic microbiota have often been unsuccessful. Although various treatments are available to remedy gut dysbiosis, for example probiotics, prebiotics, and fecal or vaginal microbe transplantation, these are not effective for most immune system disorders, such as asthma and allergy prevention (Mennini et al., 2017). Probiotics may even delay the recovery of gut microbiota after antibiotic perturbation (Severyn and Bhatt, 2018; Suez et al., 2018). A potential explanation for the delay and ineffectiveness is that a few strains cannot mimic the influence of diverse natural bacterial communities; these are typically slow-growing and cannot be cultivated in laboratory conditions (Kauppi et al., 2012; Parajuli et al., 2020; Primm et al., 2004; Yu et al., 2015). In contrast, exposure to diverse environmental microbes via multiple routes including skin and mucosal tissues may trigger a wider spectrum of innate immune responses and lead to the induction of adaptive immune responses (Kawai and Akira, 2010).

Our previous studies have introduced novel strategies to modulate human commensal microbiota by exposing people to natural biodiversity. These strategies are currently based on short-term skin contact with materials containing high microbial biodiversity, including rubbing hands with organic materials (Grönroos et al., 2019; Hui et al., 2019a; Nurminen et al., 2018) and exposure via fabric packets (Grönroos et al., 2019). Until this study, the longest trial was our 28-day intervention where urban environmental biodiversity was enriched in order to examine the effects of biodiversity on commensal microbiota and immune regulation of urban day-care children (Roslund et al., 2020). Children participating in the intervention, which included the enrichment of day-care center playground with microbially diverse vegetation and organic soil, had more diverse commensal microbiota than children in the control group, whose day-care yards were not modified. In that study, the increase in gammaproteobacterial diversity on skin was associated with enhanced immune regulation (Roslund et al., 2020). However, the stability of the alterations in bacterial communities associated with biodiversity intervention in the long-term still remains unexplored.

In the current long-term study, we follow the shifts in commensal microbiota of urban day-care children for 1–2 years. The commensal microbiota shifts were studied among 89 children residing in two different day-care environments: (1) intervention yards amended with biodiversity elements and (2) standard urban control yards with no amendments. We analyzed children's skin, salivary, and gut microbiota before the intervention, after a 28-day intervention period, and after 1 and 2 years of intervention. We also analyzed how stable the environmental microbiota was at the intervention day-care centers for 2 years. We hypothesized that long-term shifts in commensal microbiota vary between the intervention and standard day-care children, and that these shifts reflect the composition of environmental microbiota at the intervention yards.

## 2. Methods

### 2.1. Study subjects and experimental setup

Study subjects were healthy urban day-care children born in Finland and aged between 3 and 5 years at the beginning of the study. Exclusion criteria included immune deficiency, immunosuppressive medication, a medical condition affecting the immune response, and a cancer diagnosis. Standardized questionnaires were used to record the use of antibiotics, probiotics, medication, and other background information.

Altogether, 89 children from 13 day-care centers participated in the study (Table 1). Day-care centers were located in three cities in southern Finland: Espoo (n = 6), Tampere (n = 2), and Lahti (n = 5). All the day-care yards included similar playing eguipments, i.e. a sandbox, a playground, a swing, and a jungle gym. Seven of the day-cares were non-modified standard urban day-care centers, referred to as "standard day-care centers" hereafter, which served as controls. These standard day-care centers, had approximately 500 $m^2$ yards with limited access to natural biodiversity. Six day-care centers were modified with forest floor (100 $m^2$) and sod (200 $m^2$) as described in Roslund et al. (2020) and

**Table 1**
**Counts of the study subjects in intervention and standard day-care centers.** Individuals using probiotics and/or antibiotics were excluded from the gut microbial statistical analyses. Human data were analyzed with intention-to-treat (ITT) (all study subjects) and per-protocol (PP) (complete cases) populations. The PP population was used to analyze changes between time points. Two-year gut bacterial shifts were not analyzed due to the limited number of study subjects.

| | Intervention | Standard | Total |
|---|---|---|---|
| Number of day-care centers | 6 | 7 | 13 |
| Children in total | 61 | 28 | 89 |
| Boys | 31 | 17 | 48 |
| Girls | 30 | 11 | 41 |
| Excluded from gut analyses: | | | |
| Probiotics users | 7 | 2 | 9 |
| Antibiotics users | 4 | 2 | 6 |
| All study subjects | **Intention-to-treat** | | |
| Saliva | 20 | 12 | 32 |
| Skin | 61 | 28 | 89 |
| Gut | 49 | 25 | 74 |
| Year-one study subjects | **Per-protocol** | | |
| Saliva | 10 | 6 | 12 |
| Skin | 27 | 15 | 42 |
| Gut | 17 | 6 | 23 |
| Year-two study subjects | **Per-protocol** | | |
| Saliva | 7 | 0 | 7 |
| Skin | 8 | 0 | 8 |
| Gut | 3 | 0 | 3 |

Puhakka et al. (2019). Day-care centers in Lahti and Tampere were modified in May 2016 and day-care centers in Espoo in May 2017. Children also received peat blocks for playing and planting boxes for growing vegetables and flowers at the day-care centers. Originally, the intervention lasted for 28 days from May to June (Roslund et al., 2020). Personnel in the day-care centers ensured that children touched the green materials during the original 28-day intervention period. After that, the green materials remained in the yards, but our research group neither encouraged nor discouraged day-care personnel to organize prearranged exposure to the biodiverse materials. Nevertheless, in Finland, children usually play in the yards daily in the morning and afternoon for approximately 2 to 4 h altogether. Children were served three meals daily (breakfast, lunch, and afternoon snack) in the participating day-care centers, with the same daily menu in each city, as advised by the Finnish Food Authority National Nutrition Council (https://www.ruokavirasto.fi/en/themes/healthy-diet/national-nutrition-council/). Because diet and other lifestyle factors may affect human microbiota (Graham-Rowe, 2011; Parajuli et al., 2020; Saarenpää et al., 2021), information about the consumption of berries and vegetables, time spent outdoors or in nature, contact with animals, sick days, and number of siblings were recorded in a survey on day 28.

### 2.2. Sample collection

To investigate the microbial shifts associated with the biodiversity intervention, we collected skin, saliva, and stool samples from 89 day-care children residing in either intervention day cares or in non-modified standard urban day-care centers (Table 1). We also collected surface soil samples from day-care yards. Samples were collected at baseline (day 0), day 28, and year one (day 365) from the intervention and standard groups. Since many children changed day-care center, started school, or did not provide signed informed consent for the 2-year follow-up, year-two (day-730) samples were acquired only from eight children, who all belonged to the intervention group. Soil samples were also collected at year-two from intervention yards.

For skin bacterial community analyses, we collected swab samples (a sterile cotton-wool stick wetted in 0.1% Tween® 20 in 0.15 M NaCl) from the back of each child's dominant hand (2 × 2 cm area, about 10 s wiping). Skin swabs were immediately put in dry ice and stored at − 70 °C until further processing. Skin samples were analyzed also in two groups, divided according to the year when the study started (2016 or 2017). Among children who started the study in 2016, skin samples were collected in the afternoon after outdoor play at the yards, whereas among children who started the study in 2017 skin samples were collected before children went outdoors in the morning or afternoon. As children were required to wash their hands with soap for 0 to 2 h before assistants took skin swabs, and since handwashing and soap simplify the skin bacterial community (Fierer et al., 2008; Hui et al., 2019a), skin bacterial community analyses were divided into two groups.

For salivary bacterial community analyses, study subjects were asked to refrain from eating and drinking before saliva samples were taken in the morning immediately after waking up. Saliva samples were collected using sterile cotton swabs. The children held three cotton swabs in their mouth for approximately 40 s. Parents/guardians collected the saliva and stool samples in sterile plastic tubes and they were stored in their home freezers (−18 to − 20 °C) for 1 to 2 days until the researchers collected them and stored them at − 70 °C.

Soil samples were collected from the intervention and standard day-care yards from five locations: from the sandbox and playground, and next to the swing, jungle gym, and main door. Within each location, five 2 g subsamples were randomly drawn and combined to generate one sample per each location. The uppermost surface soil (depth < 1 cm) was collected since the children are in daily contact with the soil surface.

### 2.3. Microbial analyses

Bacterial communities were analyzed using Illumina MiSeq 16S rRNA gene metabarcoding with read length 2 × 300 bp using a v3 sequencing reagent kit. Samples for MiSeq sequencing were prepared as in Roslund et al. (2019). Soil, stool, and saliva bacterial samples were extracted with a PowerSoil® DNA Isolation Kit (Qiagen, Hilden, Germany) and skin swabs with a Fast DNA spin kit for soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's standard protocol. The V4 region within the 16S rRNA gene was amplified in PCR in triplicate using 505F and 806R primers (Caporaso et al., 2012). Negative and positive controls were included during the sampling process as recommended by Hornung et al. (2019). Control field blank swabs were taken in the same surroundings and same manner as the skin samples by holding the sterile swab in the air for 10 s. Negative controls were included during the sampling process and at all further steps (DNA extraction, PCR, and sequencing controls) and a positive control (*Cupriavidus necator* JMP134, DSM 4058) was included in each PCR to ensure the quality of the analysis. Bacterial sequence data were deposited into the Sequence Read Archive (BioProject PRJNA531814).

Raw sequence data were processed using Mothur (version 1.42.3) (Schloss et al., 2009) as in Roslund et al. (2019), except that OptiClust was used to assign sequence data to OTUs (Westcott and Schloss, 2017). Bacterial sequences were aligned against a SILVA reference (version 123) and operational taxonomic units (OTUs) were classified using the Mothur version of a Bayesian classifier with RDP training set version 16. Low abundance OTUs (number of observations in the data set ≤ 10) were removed from the raw sequence data. The level of contamination was determined by comparing the biological samples with the controls (sampling blanks, positive controls, DNA extraction, PCR, and sequencing of negative controls) as recommended by Eisenhofer et al. (2019) and Hornung et al. (2019). To minimize the influence of contaminant DNA, OTUs observed in negative controls were removed from sequence data taking into account possible index hopping (if the OTU had 1000% or more sequence reads more on average in samples than in negative controls, the OTU was not removed). Removed taxa found in negative controls were *Nesterenkonia*, *Halomonas*, *Caldalkalibacillus*, *Undibacterium*, Comamonadaceae unclassified, Bacillaceae 1 unclassified, Bacillales unclassified, and Xanthomonadaceae unclassified. The 16S ribosomal RNA (16S rRNA) sequencing generated a mean read count of 82 814 ± 62 571 for skin samples, 29 050 ± 7456 for saliva samples, 20 365 ± 10 696 for stool samples, and 63 996 ± 47 119 for soil samples. Due to variation in sequence read counts and to keep as many samples as possible in the downstream analyses, skin samples were rarefied to 735, saliva samples to 8568, stool samples to 3156, and soil samples to 11 426 sequences. All the sequences were also rarefied to 1050 sequences to allow analyses including all sample types. Good's coverage index (average ± standard deviation: saliva 1.00 ± 0.00, soil 0.97 ± 0.02, stool 0.99 ± 0.00, and skin 0.82 ± 0.14) was used to determine OTU coverage adequacy for diversity and community composition analyses. OTUs of interest were further identified to species level with microbial nucleotide BLAST (version 2.11.0 + ) (Zhang et al., 2000). Potential human pathogens were identified as in Hui et al. (2019a), and a list of bacterial genera was used that includes potentially pathogenic species (opportunistic + facultative) as published in Appendix A in Taylor et al. (2001).

### 2.4. Statistics

All the statistical tests were done with R v3.6.1 (R core team, 2018) and the *vegan* (Oksanen et al., 2019) and *lme4* (Bates et al., 2015) packages. Bacterial community composition was analyzed between children in intervention versus standard day-care centers with PERMANOVA (function *adonis* in the *vegan* package) (Anderson, 2017) with the Bray–Curtis metric. Two independent PERMANOVA analyses were done: one within time points and another between time points.

PERMANOVA was done at OTU, genus, order, family, class, and phylum level with abundance and presence/absence (standardization method "pa" with *decostand* function) data sets. The abundance and presence/absence data sets are also called as weighted and unweighted distances, respectively. In addition, PERMANOVA was done within specific phyla and class if the relative abundance was over 1% (skin, saliva, and stool samples) or 0.2% (soil samples); the abundance and diversity of bacteria in particularly organic soils are extremely high (Orgiazzi et al., 2016; Sinkkonen et al., 2013; Yu et al., 2015, 2014). Multivariate homogeneity of group dispersions (PERMDISP, function *betadisper* in the *vegan* package) was used to examine whether the significant differences in PERMANOVA are caused by different within-group variation (dispersion) instead of different centroid positions. Principal coordinate analysis with the Bray–Curtis distance was used to visualize the difference in bacterial community composition (*cmdscale* function). The Shannon and Simpson diversity indices were determined using the function *diversity* and rarefied species richness using the function *rarefy* in the *vegan* package.

Linear mixed models (LMMs) (function *lmer* in the *lme4* package) were constructed to analyze temporal shifts in bacterial variables, taking into account clustering of participants and repeated measures. In LMMs, bacterial richness, diversity, or relative abundance was used as the dependent variable, treatment (intervention vs standard), age, gender, and time point as a repeated measures factor (fixed factor), and individual participants nested within the cluster (day-care center) as a grouping variable (random factor). The differences in the bacterial variables between day-care children were determined using the *t*-test or, in the case of non-normally distributed data, the Wilcoxon signed-rank test. The LMMs, t-tests, and Wilcoxon signed-rank tests were done for bacterial taxa that have a relative abundance of at least 0.1%. Analysis of variance (ANOVA) was constructed to test bacterial richness and Shannon diversity differences in intervention materials. Pairwise comparison was done with Tukey's honestly significant difference (HSD) test.

Statistical analyses were done for intention-to-treat (ITT) and per-protocol (PP) populations (complete cases i.e., study subjects who provided all day-0, day-28, and year-one samples, and in the case of 2-year period analyses also a year-two sample). ITT includes all participants regardless of whether they provided samples at all time points. The PP population was used when changes between two time points were analyzed to investigate whether conclusions are sensitive to assumptions regarding the pattern of missing data. To estimate how much children within different day-care centers resemble each other, the intraclass correlation coefficients (ICCs) (function *icc* in the *ICC* package) were calculated as in Vandeputte et al. (2017). ICCs were calculated for OTU counts using a two-way analysis of variance (ANOVA) model defining each day-care center as a distinct class. Reliability was based on absolute agreement (Kim, 2013).

To conceptualize the false discovery rate all the statistical tests were carried out with the Benjamini–Hochberg correction (referred to as the Q value in the results) (Benjamini and Hochberg, 1995). All statistical tests were considered significant at the $Q < 0.05$ level.

## 3. Results

### 3.1. Characterization of soil, skin, saliva, and stool bacterial communities

After quality control, soil samples had 7885, skin samples 3075, saliva samples 677, and gut samples 1225 OTUs. Skin and soil had 1843 OTUs in common, while skin and gut had 242 and skin and saliva 203 OTUs in common (Table S1 A). Three OTUs were distinguishable in all sample types. According to the BLAST results two of these OTUs were *Streptococcus salivarius* (query cover = 100%, E value = $2 \times 10^{-130}$, identity = 100%), and *Haemophilus parainfluenzae* (query cover = 100%, E value = $4 \times 10^{-131}$, identity = 100%). The third OTU, *Veillonella montpelliernsis* (query cover = 100%, E value = $2 \times 10^{-48}$, identity = 81%) had relatively low sequence similarity for a conspecific call. These OTUs are human commensal bacterial species; they were found rarely in mineral soil samples but not in intervention materials (Table S1 B). Bacterial communities in saliva samples formed a visually tight cluster adjacent to skin bacterial communities (Fig. S1). In a joint analysis that included all sample types and both day-care groups, time was a significant factor shaping environmental and commensal bacterial communities in the intervention treatment but not in the standard treatment (Table S1 C).

On day 28, the consumption of berries and vegetables, time spent outdoors or in nature, contact with animals, sick days, and number of siblings were similar among day-care groups ($P > 0.06$).

### 3.2. Exploration of shifts in soil microbiota upon intervention

#### 3.2.1. Bacterial community composition in intervention and standard yards

Permutational multivariate analysis of variance (PERMANOVA) showed that the intervention shifted the community composition of surface soil bacteria from OTU to phylum level, and the community composition of the main phyla and classes differed between baseline and day 28 ($Q = 0.001$, Table S2 A) and between baseline and year-one samples ($Q < 0.01$, Table S2 B). Bacterial community composition shifted also between day-28 and year-one samples ($Q < 0.002$, Table S2 C), and the coefficients of determination from baseline to year one were generally smaller compared with day 28 (Table S2). When comparing the shifts between the year-one and year-two samples, the bacterial community composition was more stable, i.e., there were no shifts in the community composition at the OTU level or within major phyla and classes (Table S2 D).

In PERMANOVA, bacterial communities differed between the intervention and standard yards before (Table S3 A) and after (Table S3 B) the intervention period, and the coefficient of determination ($R^2$) increased by 50% from 0.06 to 0.09. Pairwise PERMANOVA revealed that, in particular, one recently renovated standard day-care center consisting mostly of asphalt and rubber matting differed from the other day-care centers on day 0 (Table S4 A).

There were shifts in the bacterial communities at the standard day-care yards during year one ($Q < 0.05$, Table S3 C), indicating normal regional and temporal variation in environmental microbiota (Fierer, 2017; Soininen et al., 2021). When both day-care groups were plotted together in a principal coordinate analysis, the overall and gammaproteobacterial communities in the intervention yards shifted toward those in intervention materials over time, while no visually evident shift was observable in standard day-care yard soils (Fig. 1 A and B). OTU counts did not vary between day-care centers on day 0 or within day-care groups in day-28 or year-one samples (intraclass correlation coefficient, $ICC < 0$; Table S5).

#### 3.2.2. Soil taxonomic differences between day-care groups

Intervention materials on day 1 contained a high diversity and richness of bacteria, particularly in sod (Table 2); most of the genera belonged to the classes Actinobacteria (10%), Sphingobacteriia (17%), and Alpha- (16%), Beta- (9%), and Gammaproteobacteria (8%) (Table S6).

Within the first 28 days, the intervention shifted the relative abundances of 60 soil bacterial genera (Table S7 A). Compared with the baseline (day 0), 34 of these 60 genera still differed after 1 year, and 17 genera after 2 years (Table S7). Importantly, ten genera met the following four requirements: 1) the relative abundance shifted within 28 days at the intervention yards (Table S7 A); 2) no or an opposite shift occurred at the standard yards (Table S7 C); 3) after 1 year, the relative abundance of these genera still differed between intervention and standard yards (Table S8 A); 4) the relative abundance stayed at the altered level for 2 years (Table S7 B). In detail, an increase at the intervention yards meeting these requirements occurred in *Mycobacterium*, *Mucilaginibacter*, and unclassified genera within

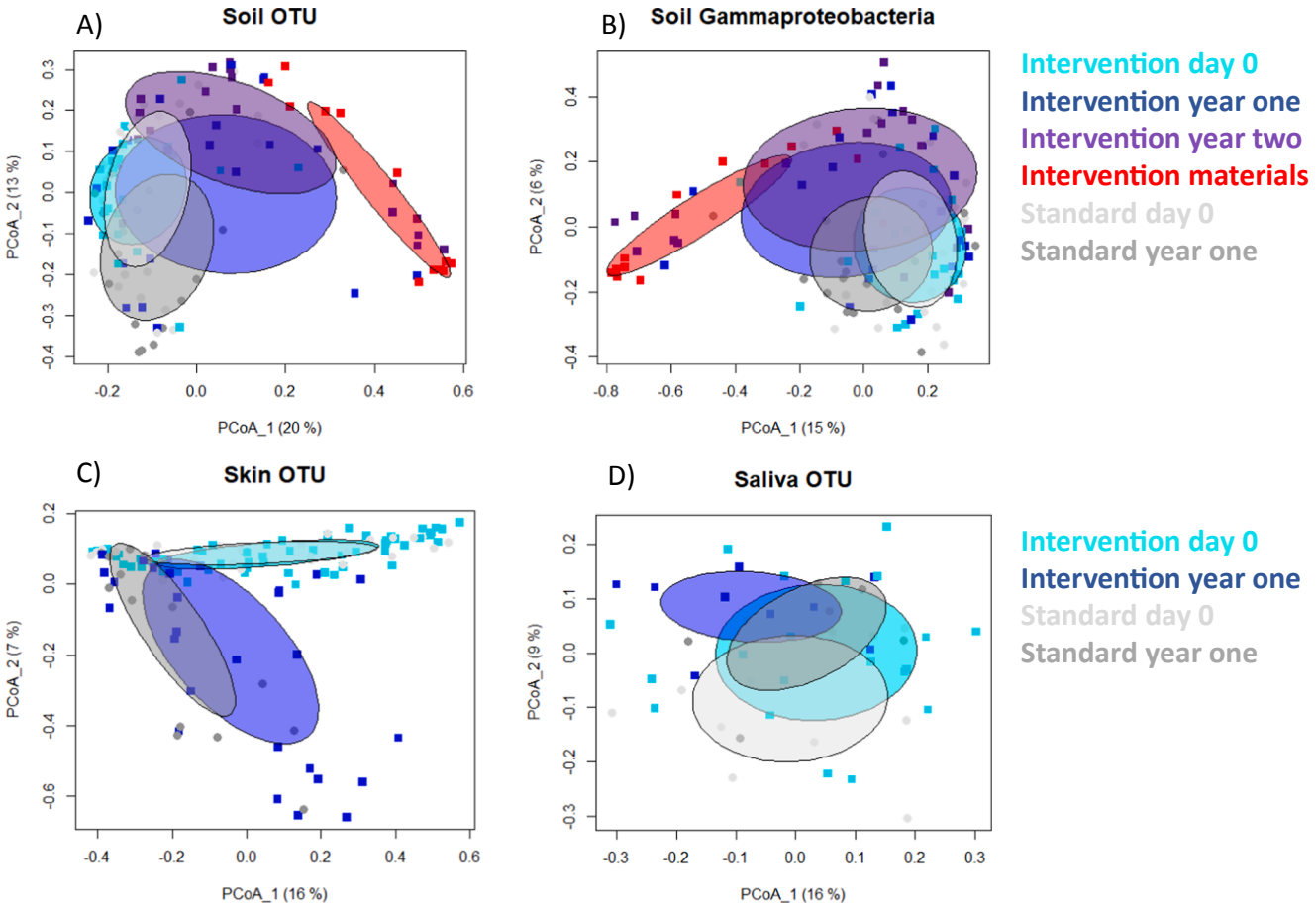


**Fig. 1.** Principal coordinate analysis (PCoA) representing shifts in bacterial communities between intervention and standard treatments. A) Soil bacterial OTU and B) gammaproteobacterial shifts, C) skin, and D) saliva bacterial OTU shifts with abundance data. Significance was determined by PERMANOVA. In short, the bacterial communities in the intervention yards shifted toward those in intervention materials (P and Q = 0.001) (A, B), and after year one the (C) skin (P = 0.02 and Q = 0.04) and (D) salivary bacterial community differed between children in the intervention versus standard day-cares (P = 0.01, Q = 0.02; Table 3). Statistics are shown in Tables S2 (within treatment) and S3 for soil (between treatments) (A, B), and Table 3 for skin and saliva (C and D).

Gammaproteobacteria and Granulicella, and a decrease occurred in *Novosphingobium*, *Roseomonas*, *Flavisolibacter*, *Segetibacter*, *Beijerinckia*, and an unclassified genus within *Burkholderiales* (Table S7 and S8).

The diversity (Shannon index) of Gammaproteobacteria and Sphingobacteriia was higher and that of Deinococci lower at the intervention yards compared with the standard day-care yards after year one (Table S8 B). These differences were not observed at the baseline (day 0) (Table S9). In addition, the intervention was associated with an increase in acidobacterial Gp1 Shannon diversity and richness, whereas these indices decreased at the standard yards ($R^2 > 0.09$, $P < 0.001$, Q = 0.002, Table S10).

### 3.3. Salivary microbiota

#### 3.3.1. Bacterial community composition shifts in saliva

At the baseline, the community composition of bacteria at the OTU level did not differ between day-care groups; however, there was a difference in the Flavobacteriia community ($R^2 = 0.16$, $P = 0.001$, Q = 0.008; Table S11). On day 28, gammaproteobacterial community compositions differed in saliva ($R^2 = 0.11$, $P = 0.002$, Q = 0.02; Table S11 E). After year one, the community composition of salivary bacteria differed at the OTU and class levels between the intervention and standard day-care groups; in particular we observed differences within the classes Gammaproteobacteria, Negativicutes, and Fusobacteriia (Table 3 A). In addition, among intervention children the community composition of the class Bacilli shifted between day-28 and year-one samples ($R^2 = 0.26$, $P = 0.002$, Q = 0.02; Table S12 A) and this shift remained in year-two samples (Table S12 B and C).

#### 3.3.2. Salivary taxonomic differences between day-care groups

Among the intervention children, the relative abundance of Firmicutes, particularly Lactobacillales ($R^2 = 0.51$, P and Q < 0.001), increased whereas that of Fusobacteriia decreased ($R^2 = 0.43$, $P < 0.001$, and Q = 0.001) during year one (Table S13 A). Also gender was associated with the salivary bacterial community. Girls had more *Prevotella* (Bacteroidia) ($R^2 = 0.23$, $P < 0.001$, Q = 0.008) and *Veillonella* (Negativicutes) ($R^2 = 0.31$, $P = 0.001$, Q = 0.009) and a higher diversity of Firmicutes ($R^2 = 0.25$, $P = 0.002$, Q = 0.01) compared with boys (Table S14).

The richness of phylum Proteobacteria was higher in day-28 than year-one samples among intervention children (Table S13 A and B). Therefore, proteobacterial richness in saliva was higher among children in standard day-care centers compared with children in intervention day-care centers in year one (Table S15 C). Shifts in salivary bacterial diversity were rare and ephemeral. Within the ITT population, the alpha diversity of Proteobacteria in saliva increased slightly among intervention children during the 28-day intervention period (Table S16 A), and it was higher than that of children in standard day-care centers on day 28 (Table S15 E). However, the alpha diversity of Proteobacteria had returned to the baseline level in the year-one samples among intervention children (Table S13).

**Table 2**
A) Shannon diversity and B) richness of the intervention materials. Data are presented as mean ± standard deviation (number of samples = 3). Differences were analyzed with the analysis of variance (ANOVA) and pairwise comparisons were done with Tukey's honestly significant difference (HSD) tests. OTU = operational taxonomic unit.

| A) Shannon diversity | Forest floor | Peat bar | Planting box soil | Sod | ANOVA *P* value |
|---|---|---|---|---|---|
| Total bacteria | 5.63 ± 0.20* | 5.09 ± 0.88** | 5.19 ± 0.21** | 6.87 ± 0.31 | < 0.001 |
| Actinobacteria | 3.93 ± 0.33** | 3.21 ± 0.51** | 3.14 ± 0.15** | 4.51 ± 0.18 | < 0.001 |
| Sphingobacteriia | 2.90 ± 0.22* | 2.35 ± 0.72** | 3.11 ± 0.13* | 3.95 ± 0.37 | < 0.001 |
| Alphaproteobacteria | 3.93 ± 0.33 | 3.68 ± 0.56 | 3.52 ± 0.16* | 4.29 ± 0.22 | 0.01 |
| Betaproteobacteria | 2.00 ± 0.22** | 1.50 ± 0.53**,a | 2.77 ± 0.14 | 3.39 ± 0.44 | < 0.001 |
| Gammaproteobacteria | 2.00 ± 0.22** | 2.43 ± 0.59*,a | 1.27 ± 0.24** | 3.30 ± 0.22 | < 0.001 |
| **B) Richness** | **Forest floor** | **Peat bar** | **Planting box soil** | **Sod** | **ANOVA *P* value** |
| Observed OTU richness | 2121 ± 184* | 2177 ± 1764* | 2354 ± 464* | 4630 ± 1448 | 0.007 |
| Actinobacteria | 89 ± 10** | 132 ± 53* | 97 ± 16** | 202 ± 30 | < 0.001 |
| Sphingobacteriia | 80 ± 10* | 52 ± 40** | 95 ± 14 | 138 ± 31 | < 0.001 |
| Alphaproteobacteria | 179 ± 15 | 177 ± 77 | 156 ± 14 | 197 ± 40 | 0.519 |
| Betaproteobacteria | 33 ± 4** | 32 ± 22** | 49 ± 6** | 81 ± 15 | < 0.001 |
| Gammaproteobacteria | 48 ± 4* | 37 ± 23* | 40 ± 5* | 77 ± 15 | < 0.001 |

* Tukey's HSD $P < 0.05$.
** $P < 0.001$ compared with sod.
[a] Tukey's HSD $P < 0.001$ compared with planting box soil.

### 3.4. Skin microbiota

#### 3.4.1. Bacterial community composition shifts on the skin

The skin bacterial community differed between children in the intervention versus standard day-care centers at all taxonomic levels in year-one samples, when all children regardless of the starting year were analyzed jointly (Table 4 B). Within-group variation at the genus level in abundance data was higher among intervention children compared with children in standard day-cares in year-one samples (Table S17 C). Shifts in skin bacterial communities of the intervention children were similar to those in saliva: on skin, the class Bacilli community shifted during year one (Table S18 B) and this shift remained in year-two samples (Table S18). Other class-level shifts and differences between day-care groups in year one included gammaproteobacterial and Negativicutes communities (Table S18).

When the data were analyzed according to the starting year, these differences between children in the intervention and standard day cares were observed only among children who participated in the study in 2016, i.e., whose skin samples were taken after the children spent time outdoors at the day-care yards during the sampling day (Table S18 L). These community composition differences between intervention and standard day-care groups did not exist at the baseline (Table S18 J) or after the 28-day intervention period (Table S18 K).

Among intervention children who started the study in 2017, i.e., whose skin swabs were taken before spending time at day-care yards, shifts were found in the community composition of classes Bacilli and Gammaproteobacteria (Table S18 B), and these shifts were not observed among the children in the standard day-care (Table S18 I). Among these intervention children, the total bacterial, Gammaproteobacteria, and Bacilli community composition shifted during the 28-day intervention period (Table S18 A), whereas there were no shifts among children in standard day-care centers (Table S18 G). Among intervention children who participated in the study in 2016, the shifts between day 0 and day 28 were observed within phyla Proteobacteria, Actinobacteria, Bacteroidetes, and Acidobacteria (Table S18 A), whereas no shifts were observed among children in standard day-care centers (Table S18 G) (note the results are similar with the ITT and PP populations).

#### 3.4.2. Skin taxonomic differences between day-care groups

The relative abundance of four opportunistic pathogens became lower among intervention children compared with children in standard day-care centers in the year-one samples. These were *Gemella* sp. and *Streptococcus* sp. within class Bacilli, *Veillonella* sp. within class Negativicutes, and *H. parainfluenzae* (Otu000003) within class Gammaproteobacteria (Table S19 C). In addition, the total relative abundance of genera containing potential human pathogens (acquired from Taylor et al., 2001) was lower among intervention children compared with children in standard day-care centers in the year-one samples (*t*-test: $P = 0.002$, $Q = 0.013$; Table S19 C). The differences were observed with the PP population and the trend was similar among all children regardless of the study starting year and with ITT populations (Table S19).

Gender was a significant factor affecting taxonomic composition of Clostridiales but only among children who started the study in 2017, i.e., whose skin swabs were taken before spending time at day-care yards (Table S20). Specifically, relative abundances of *Anaerococcus*, *Peptoniphilus*, and *Ezakiella* were greater for girls than boys (Table S20).

#### 3.4.3. Species richness and diversity on the skin

The richness of Alpha-, Beta-, and Gammaproteobacteria was higher among children in the intervention day-care centers compared with children in the standard day-care centers in year-one samples when all children were in the model (Fig. 2; Table S19 C). This difference was not observed at the baseline or on day 28 (Table S19 A and B).

Among children who started the study in 2016, evenness based on the Shannon diversity index decreased less among children in the intervention compared with those in the standard day-care centers ($R^2 = 0.64$, $P = 0.002$, $Q = 0.014$; Table S21 A) during the first year. Otherwise, the two day-care groups did not differ in their diversity. Total bacterial alpha diversity decreased in both the standard ($R^2 = 0.71$, P and $Q < 0.001$, Table S21 C) and intervention day-care groups during year one ($R^2 = 0.37$, P and $Q < 0.001$, Table S21 B).

### 3.5. Shifts in gut bacterial community

Gut microbial communities shifted in the ITT population within both day-care groups during the study period (Table S22). While several shifts were minor and seemed haphazard within the first year of intervention, the relative abundance of *Clostridium sensu stricto* decreased by more than a third in 28 days among the intervention children ($R^2 = 0.07$, $P = 0.001$, $Q = 0.01$; Table S22 A and Fig. 3). In addition, the relative abundance was less than a half of the baseline value in the year-one samples of the intervention group ($R^2 = 0.07$, $P = 0.008$, $Q = 0.3$; Table S22 A), while in the standard group the relative abundance of *C. sensu stricto* remained constant throughout year one ($R^2 = 0.01$, $P > 0.5$, $Q > 0.9$; Table S22 A).

Additional relative abundance shifts in the ITT population included a decrease in genus *Romboutsia* among the intervention children within 28 days ($R^2 = 0.03$, $P < 0.001$, $Q < 0.01$; Table S22 A). *Romboutsia* abundance remained low in the year-one samples among the children in the intervention day-care group, whereas among the children in the standard day-care group the relative abundance of *Romboutsia* remained at the original, high level in the year-one samples (Table S22 A).

**Table 3**
**Permutational multivariate analysis of variance results for salivary and skin bacterial communities.** A) Salivary and B) skin bacterial communities differed between the intervention (skin samples n = 27, salivary samples n = 9), and standard day-care children (skin samples n = 15, salivary samples n = 6) in year-one samples. Analyses included the study subjects that gave both day-0 and year-one samples. Statistics are reported as F statistics, coefficient of determination ($R^2$), probability *P* value, and Benjamini–Hochberg adjusted Q value. OTU = operational taxonomic unit.

| **A) Salivary bacteria** | **Abundance data** | | | | **Presence/absence data** | | | |
|---|---|---|---|---|---|---|---|---|
| | F value | $R^2$ | *P* value | Q value | F value | $R^2$ | *P* value | Q value |
| OTU level | 1.514 | 0.104 | 0.112 | 0.168 | 2.043 | 0.136 | **0.006** | **0.021** |
| Genus level | 1.961 | 0.131 | **0.029** | 0.108 | 1.708 | 0.116 | 0.098 | 0.110 |
| Family level | 1.807 | 0.122 | **0.036** | 0.108 | 2.086 | 0.138 | 0.077 | 0.110 |
| Order level | 1.524 | 0.105 | 0.142 | 0.168 | 2.306 | 0.151 | **0.033** | 0.066 |
| Class level | 1.500 | 0.103 | 0.168 | 0.168 | 3.533 | 0.214 | **0.007** | **0.021** |
| Class Bacilli | 0.612 | 0.045 | 0.662 | 0.662 | 0.882 | 0.064 | 0.589 | 0.673 |
| Class Betaproteobacteria | 1.231 | 0.087 | 0.287 | 0.385 | 0.521 | 0.039 | 0.675 | 0.675 |
| Class Gammaproteobacteria | 2.807 | 0.178 | 0.069 | 0.382 | 3.790 | 0.226 | **0.001** | **0.008** |
| Class Bacteroidia | 1.089 | 0.077 | 0.337 | 0.385 | 1.645 | 0.112 | 0.153 | 0.245 |
| Class Clostridia | 1.113 | 0.079 | 0.330 | 0.385 | 0.922 | 0.066 | 0.529 | 0.673 |
| Class Negativicutes | 2.002 | 0.133 | 0.153 | 0.382 | 3.914 | 0.231 | **0.004** | **0.016** |
| Class Fusobacteriia | 1.548 | 0.106 | 0.125 | 0.382 | 3.914 | 0.231 | **0.007** | **0.019** |
| Class Flavobacteriia | 1.456 | 0.101 | 0.191 | 0.382 | 3.887 | 0.23 | **0.035** | 0.070 |
| **B) Skin bacteria** | **Abundance data** | | | | **Presence/absence data** | | | |
| | F value | $R^2$ | *P* value | Q value | F value | $R^2$ | *P* value | Q value |
| OTU level | 1.900 | 0.041 | 0.067 | 0.067 | 1.595 | 0.035 | **0.021** | **0.042** |
| Genus level | 2.473 | 0.053 | **0.039** | **0.047** | 1.955 | 0.043 | **0.020** | **0.042** |
| Family level | 3.121 | 0.066 | **0.024** | **0.047** | 2.342 | 0.051 | **0.014** | **0.042** |
| Order level | 3.326 | 0.070 | **0.028** | **0.047** | 2.212 | 0.048 | **0.040** | 0.060 |
| Class level | 3.291 | 0.070 | **0.034** | **0.047** | 2.157 | 0.047 | 0.068 | 0.082 |
| Class Bacilli | 3.039 | 0.065 | **0.018** | **0.046** | 0.929 | 0.021 | 0.520 | 0.607 |
| Class Betaproteobacteria | 0.939 | 0.021 | 0.372 | 0.372 | 0.279 | 0.006 | 0.910 | 0.910 |
| Class Gammaproteobacteria | 2.877 | 0.061 | **0.007** | **0.042** | 2.341 | 0.051 | **0.015** | 0.105 |
| Class Alphaproteobacteria | 1.417 | 0.031 | **0.049** | 0.074 | 1.470 | 0.032 | **0.050** | 0.121 |
| Class Bacteroidia | 1.486 | 0.033 | 0.136 | 0.163 | 2.016 | 0.044 | 0.052 | 0.121 |
| Class Negativicutes | 2.994 | 0.064 | **0.023** | **0.046** | 2.071 | 0.045 | 0.133 | 0.186 |

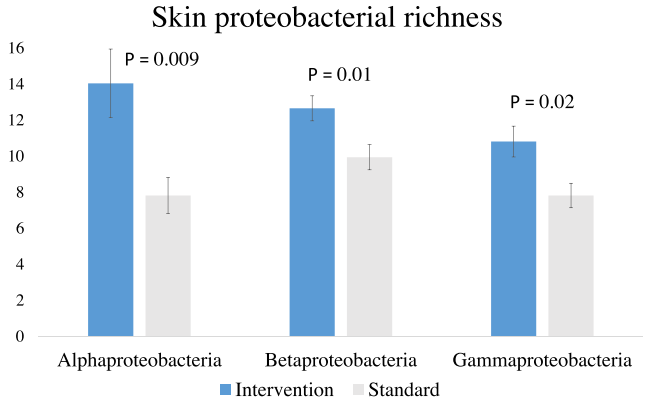


**Fig. 2.** Skin alpha-, beta- and gammaproteobacterial richness in intervention and standard groups at year one. Richness is shown for the PP population as mean ± standard error. Statistics are shown in Table S19.

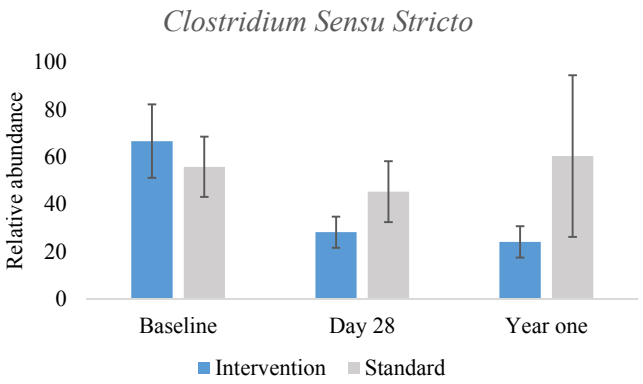


**Fig. 3.** Relative abundance of *Clostridium sensu stricto* in the intervention and standard groups. Abundances were rarefied to 3156 sequences and relative abundance is shown for the ITT population as mean ± standard error. Statistics are shown in Table S22.

However, when the PP group was analyzed, the baseline values for *Romboutsia* in the intervention group were about half of those in the standard group and in year-one samples (Table S22 A). There were no additional genus-level abundance shifts in the ITT populations.

Three shifts that occurred only in the PP population and not in the ITT population were observed within the order Clostridiales. Among the intervention children, the relative abundance of *Ruminococcus* 2 (Lachnospiraceae), *Dorea* (Lachnospiraceae), and an unclassified genus within Clostridiales increased, while among the children in standard day-care centers these abundances decreased during the 28-day intervention period ($P < 0.002$, $Q < 0.03$; Table S23 A). However, the baseline relative abundances of these three taxa were also higher in the standard than in the intervention group (Table S23).

The community composition of gut bacteria did not differ between intervention and standard day-care children on day 28, but we observed several differences in the day-0 and year-one samples (Table S24). Gender was not a significant factor in the context of gut bacterial community.

## 4. Discussion

The current biodiversity intervention is the first one that follows the changes in commensal microbiota over the time periods of 1 and 2 years. The study demonstrates how the intervention altered environmental microbiota, which in turn was linked to alterations in commensal microbiota (See graphical abstract).

### 4.1. Soil microbiota

Introduction of intervention materials resulted in significant shifts in bacterial taxonomies. The microbial shifts remained for 2 years even

though children trampled the sod and forest floor vegetation. Bacterial shifts at the day-care yards were particularly observed within phyla Actinobacteria, Bacteroidetes, and Acidobacteria Gp1, and classes Alpha- and Gammaproteobacteria. The relative abundance of bacteria typically found in boreal forests increased at the intervention day-care yards (Pankratov et al., 2007; Pankratov and Dedysh, 2010; Sun et al., 2014). Importantly, shifts in the soil surface that remained for 2 years included a higher abundance of genus *Mycobacterium*. Environmental mycobacteria, such as *M. vaccae*, have been proposed to have anti-inflammatory properties and improve mood and learning, while limiting anxiety and depression (Fonken et al., 2018; Frank et al., 2018; Matthews and Jenks, 2013; Reber et al., 2016). Humans, like their ancestors, have been exposed to nonpathogenic mycobacteria throughout their evolution (Rook et al., 2004), but according to our study mycobacterial abundance is low at typical urban day-care yards. The innate immune system recognizes environmental mycobacteria as harmless and adjuvant for regulatory T cells and anti-inflammatory interleukin 10, i.e., mycobacteria prime our immune system to fight against pathogens (Frank et al., 2018; Rook et al., 2004; Smith, 2017). Therefore, in the context of knowledge of environmental mycobacteria, the increase in the mycobacterial abundance at the intervention day-care yards may be beneficial from the viewpoint of the immune regulation of children.

In particular, boreal forest floor included mycobacteria (see also Iivanainen et al., 1997; Niva et al., 2006), but boreal forest floor is unfortunately of limited supply, and it cannot be used in temperate and warmer regions. Therefore, there is a need for novel, microbiologically diverse landscaping materials that include a high diversity and richness of anti-inflammatory health-associated bacteria (Nurminen et al., 2018; Puhakka et al., 2021, 2019a,b). In our previous study, we manufactured and tested biodiverse mineral soil materials and observed a shift in microbial communities and an increase in bacterial diversity on skin (Hui et al., 2019a). Similar materials could be produced for children's playgrounds and other urban green spaces to facilitate daily contacts with diverse environmental microbiota for children who have no daily access to agricultural or forest areas. If these man-made biodiverse materials are implemented in playgrounds and day-care yards, the changed microbial environment might alleviate the development of the immune system and decrease the risk of several immune-mediated diseases in urbanized societies.

### 4.2. Skin and saliva microbiota

The fact that between-treatment differences in skin and saliva microbiota in the year-one samples were evident, particularly within the class Gammaproteobacteria, fits nicely with the higher gammaproteobacterial diversity in the intervention compared with standard yards and supports the view that differences between intervention and standard day-care children are linked to the biodiversity intervention per se. In addition, since the relative abundance of common skin commensals and potential pathogens *H. parainfluenzae* (Gammaproteobacteria), *Streptococcus* sp. (Bacilli), and *Veillonella* sp. (Negativicutes) became lower within year one among the intervention compared with standard day-care group, factors modulating skin bacterial community were plausibly treatment-specific. Importantly, the current study found evidence that microbial changes on skin are associated with changes in the gastrointestinal tract; the community composition of classes Bacilli, Gammaproteobacteria, and Negativicutes shifted both on skin and in saliva in a treatment-specific way in the intervention and standard groups. In general, the current findings indicate that the microbiological changes reported in our recent short-term day-care intervention (Roslund et al., 2020) are likely to persist over a long time period after the establishment of green yards.

An interesting aspect in our study is that biodiversity intervention might have prevented the season- or age-dependent loss (Hui et al., 2019b; Lehtimäki et al., 2017) of skin alpha-, beta-, and gammaproteobacterial richness since these were lower among children in standard day-care centers in year one. As higher gammaproteobacterial diversity on the skin and in the soil surface has previously been associated with proper immune modulation (Roslund et al., 2020), these findings are in line with the "old friends" and "biodiversity" hypotheses (Hanski et al., 2012; Rook et al., 2004) that frequent contact with rich environmental microbiota help to reduce the risk of immune-mediated, noncommunicable diseases. Indeed, in the context of children's health, gammaproteobacterial diversity on skin has previously been associated with a decreased risk of atopy and allergies (Fyhrquist et al., 2014; Hanski et al., 2012). In addition, since the relative abundance of *H. parainfluenzae*, *Streptococcus* sp., and *Veillonella* sp. became lower within year one among the intervention compared with standard day-care group, and since these were the most common OTUs found in all sample types, including gut and soil microbiota, our results indicate that biodiversity intervention may affect taxa not distinguished in previous research that tested the old friends and biodiversity hypotheses. While *H. parainfluenzae* and *Streptococcus* have frequently been associated with human infections (Delorme et al., 2015; Nwaohiri et al., 2009; O'Neil et al., 2016), *Veillonella* is rarely reported to cause infection (Rovery et al., 2005; Sillanpää et al., 2017). *Streptococcus* and *Veillonella* have been more abundant on the skin and in the airways when the environment lacks natural biodiversity (Voorhies et al., 2019), and the high abundance has been associated with childhood asthma (Ahluwalia et al., 2020; Lehtimäki et al., 2021) and atopic skin (Chng et al., 2016). Interestingly, the same bacterial taxa, including *Haemophilus*, *Streptococcus*, *Veillonella*, and *Gemella*, that were observed to be lower among intervention children's skin compared with children in standard day cares may contribute to imbalance in salivary microbiota and to inflammatory bowel disease (Said et al., 2014). It is important to note that we did not observe these potential pathogens in the intervention materials, and they were only rarely found in mineral soil samples taken from the yards, indicating that they belong to commensal microbiota that children transferred to yards. The reasons why they were not observed in year-one and year-two forest floor samples include high resistance of forest floor microbiota to commensal invaders, and the fact that boreal forest floor is a different habitat compared with the human body. This raises the interesting question of whether biodiversity intervention can be utilized to delay or prevent the spread of pathogens and inflammatory disorders (Haahtela, 2019).

Interestingly, we found over two times more bacterial OTUs in skin swabs compared to stool samples. The reason might be that while stool contains several dominating bacterial species that are capable to live in acidic gut environment (Huttenhower et al., 2012), skin microbiota is highly influenced by the diverse environment and human behavior (Haahtela et al., 2021; Hanski et al., 2012; Lehtimäki et al., 2017; Saarenpää et al., 2021). In other words, skin – as the first frontier of the human body – is exposed to the huge variety of environmental microbes and may thus encounter numerous bacterial species. Several shifts in skin microbiota were not observed in day-care centers located in the city of Espoo where the study started in 2017, a year later than in Lahti and Tampere. One explanation for these differences is that samples were taken at different times of the day. In 2016, most skin swabs were taken after the children spent time outdoors at the day-care yards, whereas in 2017, the swabs were taken before the children spent time outdoors. Another explanation is that the weather conditions differed between years and affected the children's clothing. May 2016 was dry and sunny, the temperature was 3 to 5 °C higher than usual (daily mean temperature 10 to 21 °C), whereas May 2017 was rainy and the temperature was 1 to 3 °C lower than usual (daily mean temperature 2 to 18 °C) (Finnish Meteorological Institute: http://www.ilmastokatsaus.fi). In 2016, children often wore T-shirts, while in 2017 they mostly wore shirts with long sleeves, raincoats, and gloves. Possibly, diverse environmental microbiota reached arms and hands easier in 2016 when children wore coats and gloves less frequently. Alternative explanations include age: as most of our study children were 4 or 5 years old at the beginning of the study,

we were not able to compare different age groups within years. However, the physiology and structure of the skin changes with age (Waller and Maibach, 2005), possibly shaping skin bacterial communities. In addition, behavior is typically related to age; younger children play in close contact with soil, lick mucky hands, and ingest the soil more than the older children, which might affect the commensal bacterial communities. In any case, gammaproteobacterial diversity on skin declined only among children in the standard day-care centers, which indicates that biodiversity intervention might prevent a weather- or age-related decline in anti-inflammatory bacteria on skin.

### 4.3. Gender and microbiota

As salivary *Prevotella* is abundant in patients with certain immune-mediated diseases (Larsen, 2017), the finding that girls had more salivary *Prevotella* than boys indicates that gender-specific differences in oral bacteria may be related to health outcomes. Similarly, *Veillonella* was more abundant in the salivary microbiome of girls compared with boys, but as the association between *Veillonella* and caries is species-specific (Djais et al., 2019), we cannot state whether the difference is of medical interest. On the skin, girls had more *Peptoniphilus*, which is associated with diabetic skin and tissue infection (Dowd et al., 2008; Walter et al., 2014); however, *Peptoniphilus* and the other genera observed in greater abundance on the skin of the girls are part of the human vaginal microbiota and have only occasionally been observed on the skin (Brown et al., 2014; Diop et al., 2017; Lagier et al., 2015; Sharma et al., 2014). Gender-specific differences in the commensal microbiome may be related to diet, host physiology, and other living habits that differ between girls and boys (Ma and Li, 2019; Merkiel-Pawłowska and Chalcarz, 2017). Gender- and environment-related shifts might also be interdependent since boys and girls may choose different areas to play (Lucas and Dyment, 2010). Importantly, they both prefer green areas to play if available (Lucas and Dyment, 2010). Indeed, we previously studied how biodiversity intervention affected children's well-being, play, and environmental relationships (Puhakka et al., 2019b). Based on that study, biodiverse yards motivated children's play, diversified their activities, and increased their physical activity level. As biodiverse green areas promote well-being (Puhakka et al., 2019b) and health (Flandroy et al., 2018), and as microbial changes in our intervention group were previously associated with health benefits (Roslund et al., 2020), we recommend that green spaces are accessible for everyone.

### 4.4. Shifts in the gut

We are cautious in drawing conclusions based on gut results, since the number of participants that provided year-one samples was very small in the standard day-care group, and some of the between-group differences and shifts were observed in either the ITT or PP population but not in both. Keeping this in mind, the decrease found in the relative abundance of *C. sensu stricto* among intervention children is consistent with our previous study where this taxon was decreased with an increasing number of shrub species in home yards (Parajuli et al., 2020). Other shifts in gut bacteria were observed within family Lachnospiraceae, which has previously been associated with health promotion (Candela et al., 2014; Lynch et al., 2014). These included changes in the diversity, richness, or relative abundance of Lachnospiraceae, *Ruminococcus* 2, *Dorea*, and *Blautia* among children in the intervention and standard day-care centers. These bacteria include important butyrate producers, and they have been associated with protection against obesity (Cho et al., 2012), inflammatory bowel disease (Chumpitazi et al., 2016), antibiotic resistant *Clostridium difficile* infection (Petrof et al., 2013), and even colorectal cancer (Candela et al., 2014). However, certain Lachnospiraceae strains have been linked to onset of type 1 diabetes (Giongo et al., 2011; Kameyama and Itoh, 2014), while the higher abundance of Lachnospiraceae family has been observed in healthy subjects compared to type 1 diabetes cases (Giongo et al., 2011; Roesch et al., 2009). There were baseline differences between children in the intervention and standard day-care centers within these taxa, and therefore we cannot state that these shifts are associated with biodiversity intervention. In addition, intervention materials did not include the above-mentioned genera known for butyrate production, indicating that other factors, such as the entire microbial community (e.g., bacteria, fungi, archaea, protozoa, invertebrates, and viruses) bound to soil and plant particles might modulate the gut bacterial community. Also, of course, factors like health, living habits, diet, and genetic differences between individuals shape commensal microbiota (Huttenhower et al., 2012; Lozupone et al., 2012).

We also checked for differences in gut bacterial functional orthologs between the two day-care groups but did not find any (methodology in Roslund et al., (2019)). This further strengthens the conclusion that the effects of intervention on the gut microbiota were minor in this study compared with our earlier studies (Nurminen et al., 2018; Roslund et al., 2020). In the earlier studies, changes in the gut microbiota were observed after well-instructed direct contact with soil-based environmental biodiversity, while in the current study, day-care personnel were not asked to actively guide the children to play with intervention materials after the 28-day study period. Nevertheless, the current intervention trial and earlier studies using animal models (Ottman et al., 2019; Zhou et al., 2016), and our previous observational and intervention studies with humans (Nurminen et al., 2018; Parajuli et al., 2020; Roslund et al., 2020), indicate that being in contact with biodiversity components in everyday life may shape the bacterial flora in the gastrointestinal tract.

### 4.5. Factors needing attention

During the long trial, we had to adjust two details while the trial was ongoing. In our previous 28-day intervention trial the nurses at the day-care centers guided the children to be in close contact with the green materials (Roslund et al., 2020). In contrast, in the current long-term follow-up study we did not ask for the systematic continuation of this guidance. We observed a decline in the total bacterial diversities on skin over time, and we cannot distinguish whether the decline was caused by normal age-dependent variation or whether it was caused by the lack of guidance. The second detail was related to the moment of sampling skin microbiota. In 2016, children gave skin bacterial swabs in the afternoon after playing at the yards, while in 2017 children gave the swabs after washing hands with soap and before going outdoors. While this adjustment possibly reduced random variation between swabs, it might have reduced variation between treatments. An interesting observation is related to the variation within day-care versus between day-care centers. While in most cases the ICCs were remarkably low, indicating that there was no significant variation in bacterial communities caused by the day-care center, soil bacterial communities differed between day-care centers before the intervention period. This is not surprising since the coverage by asphalt, concrete, rubber safety matting, various mineral soil materials, and natural rock varied between day-care yards; it is well-known that soil heterogeneity, patchiness, and structure modulates the variation in soil microbiota. Another occasion when intraclass coefficients indicated a day-care center effect was year-one skin swabs in standard day-care centers. These differences can be considered as normal variation caused by an urban, non-green environment. Importantly, our modeling approach to include individual participants nested within the cluster, i.e., day-care center, in multilevel modeling is the standard way to take potential cluster effects into account (Huang, 2016; Moen et al., 2016).

The current intervention was designed to change the overall microbial environment of children at day care yards, including altered airborn and phyllosphere microbiome and interlinkages between these and soil microbiota. Due to practical limitations, surface soil samples were used as proxies of the exposomic shift. Regardless of the methodology used in

the current study, there is no rason why future studies should not investigate how biodiversity interventions shape airborne and phyllosphere microbiome, and how these are connected to the human commensal microbiome. In addition, because the study season may affect the exposure to diverse environmental microbiota, i.e. exposure is diminished in winter (Hui et al., 2019b; Nurminen et al., 2021), future intervention trials should pay attention to seasonal variation in the context of human commensal microbiota.

One of the core questions in environmental microbiological studies is whether the changes observed are a haphazard consequence of the joint analysis of rich taxa. It is obvious that intervention shifted the soil bacterial communities at the intervention yards, since the relative abundance of forest floor bacterial taxa increased in the intervention yards and the bacterial community shifted toward bacterial communities in intervention materials (see Fig. 1). If the changes were random, taxa associated with a forest or an agricultural environment should not be overrepresented. In the current study, the situation was quite the opposite (see Table S7), i.e., typical forest soil microbiota such as *Mycobacterium*, *Mucilaginibacter*, and gammaproteobacterial communities were enriched in the intervention yards only. Further, the changes observed on the skin and in saliva were previously distinguished in comparisons of urban versus rural populations and in earlier intervention or comparative trials (Hui et al., 2019a; Kirjavainen et al., 2019; Roslund et al., 2020; Ruokolainen et al., 2017). Further evidence of non-random between-group variation comes from the comparison of boys versus girls. Skin bacterial community differences between girls and boys were observed only among children whose skin swabs were taken before spending time at day-care yards, indicating that playing in a microbial-rich environment reduced the relative abundance of human originated microbiota on the skin. For these reasons, we conclude that shifts in microbiota observed in the current study were likely caused by the intervention *per se*.

## 5. Conclusions

This long-term intervention study shows that changes in environmental and commensal microbiota are prolonged. Taken together the findings of this study and our previous studies (Grönroos et al., 2019; Hui et al., 2019a; Nurminen et al., 2018; Parajuli et al., 2020; Roslund et al., 2020, 2019, 2018) encourage the implementation of biodiverse nature-based solutions in the management and planning of urban environments. Conceptually, our findings indicate that there is a possibility to enhance commensal microbiota and reduce the relative abundance of potential pathogens in urban environments using biodiversity interventions. This could eventually provide prophylactic alternatives against inflammatory disorders.

## 6. Declarations

**Ethics approval and consent to participate:** Ethical approval for the study was obtained from the ethical committee of the local hospital district (Tampereen yliopistollisen sairaalan erityisvastuualueen alueellinen eettinen toimikunta, Pirkanmaa, Finland). Approvals to conduct the study were obtained from the cities of Espoo, Tampere, and Lahti. All participants received oral and written information about the study and parents/guardians of the children provided a written informed consent that was in accordance with the Declaration of Helsinki.

**Availability of data and materials:** Raw sequencing data has been deposited to the Sequence Read Archive (SRA) under BioProject PRJNA531814. The sensitive data that support the findings of this study are available from University of Helsinki but restrictions defined in General Data Protection Regulation (EU 2016/679, 2016) and Finnish Data Protection Act 1050/2018 (Finnish Ministry of Justice, 2018) apply to the availability of these data, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission from the ethical committee of the local hospital district (Tampereen yliopistollisen sairaalan erityisvastuualue, Pirkanmaa, Finland).

**Funding:** The funding was provided by Business Finland (grant numbers 40333/14 and 6766/31/2017) and the Helsinki Metropolitan Region Urban Research Program (Finland).

**Author contributions:** A.S., H.H., O.H.L., R.P., J.R., and M.I.R. designed the study. R.P., M.G., M.I.R., A.S., N.N., J.R., N.S., and O.H.L. implemented the study. M.I.R., N.N., L.K., O.C., A.J., and S.O. generated the data. M.I.R. and A.S. analyzed the data. M.I.R. and A.S. wrote the first draft of the manuscript. R.P., O.C., N.N., and A.J. provided critical editing of the manuscript. A.S., H.H., and J.R. obtained funding and were the principal investigators of the project. The authors read and approved the final manuscript.

## Declaration of Competing Interest

A.S., H.H., O.H.L., M.G., N.N., and S.O. have been named as inventors in a patent application "immunomodulatory compositions" submitted by University of Helsinki (patent application number 20165932 at Finnish Patent and Registration Office). M.G., M.I.R. and A.S. have been named as inventors in a patent application "Immunomodulatory gardening and landscaping material" submitted by University of Helsinki (patent application number 175196 at Finnish Patent and Registration Office). None of the inventors have received royalties from the patent application.

## Acknowledgement

The authors wish to acknowledge the children and their parents/guardians, the day-care personnel, and the cities of Espoo, Lahti, and Tampere for enabling the study. The authors also acknowledge the CSC – IT Center for Science, Finland, for computational resources, the ADELE team members and AlmaLab for their support, and three reviewers for critical comments.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106811.

## References

Ahluwalia, T.S., Eliasen, A.U., Sevelsted, A., Pedersen, C.T., Stokholm, J., Chawes, B., Bork-jensen, J., Grarup, N., Pedersen, O., Hansen, T., Linneberg, A., Sharma, A., Weiss, S.T., Evans, M.D., Jackson, D.J., Gern, J.E., Jr, R.F.L., Ober, C., Pedersen, A. G., Bisgaard, H., Bønnelykke, K., 2020. FUT2–ABO epistasis increases the risk of early childhood asthma and Streptococcus pneumoniae respiratory illnesses. Nat. Commun. https://doi.org/10.1038/s41467-020-19814-6.

Anderson, M.J., 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef Stat. Ref. Online 1–15. https://doi.org/10.1002/9781118445112.stat07841.

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67. https://doi.org/10.18637/jss.v067.i01.

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57 (1), 289–300.

Brown, C.T., Davis-Richardson, A.G., Giongo, A., Gano, K.A., Crabb, D.B., Mukherjee, N., Casella, G., Drew, J.C., Ilonen, J., Knip, M., Hyöty, H., Veijola, R., Simell, T., Simell, O., Neu, J., Wasserfall, C.H., Schatz, D., Atkinson, M.A., Triplett, E.W., Roop, R.M., 2011. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLoS One 6 (10), e25792. https://doi.org/10.1371/journal.pone.0025792.

Brown, K., Church, D., Lynch, T., Gregson, D., 2014. Bloodstream infections due to Peptoniphilus spp.: Report of 15 cases. Clin. Microbiol. Infect. 20 (11), O857–O860. https://doi.org/10.1111/1469-0691.12657.

Byrd, A.L., Deming, C., Cassidy, S.K.B., Harrison, O.J., Ng, W.-I., Conlan, S., Belkaid, Y., Segre, J.A., Kong, H.H., 2017. Staphylococcus aureus and staphylococcus epidermidis strain diversity underlying pediatric atopic dermatitis. Sci. Transl. Med 9 (397), eaal4651. https://doi.org/10.1126/scitranslmed.aal4651.

Candela, M., Turroni, S., Biagi, E., Carbonero, F., Rampelli, S., Fiorentini, C., Brigidi, P., 2014. Inflammation and colorectal cancer, when microbiota-host mutualism breaks. World J. Gastroenterol. 20, 908–922. https://doi.org/10.3748/wjg.v20.i4.908.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6 (8), 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Cekanaviciute, E., Yoo, B.B., Runia, T.F., Debelius, J.W., Singh, S., Nelson, C.A., Kanner, R., Bencosme, Y., Lee, Y.K., Hauser, S.L., Crabtree-Hartman, E., Sand, I.K., Gacias, M., Zhu, Y., Casaccia, P., Cree, B.A.C., Knight, R., Mazmanian, S.K., Baranzini, S.E., 2017. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. Proc. Natl. Acad. Sci. U. S. A. 114 (40), 10713–10718. https://doi.org/10.1073/pnas.1711235114.

Chen, J., Wright, K., Davis, J.M., Jeraldo, P., Marietta, E.V., Murray, J., Nelson, H., Matteson, E.L., Taneja, V., 2016. An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. Genome Med. 8, 1–14. https://doi.org/10.1186/s13073-016-0299-7.

Chng, K.R., Tay, A.S.L., Li, C., Ng, A.H.Q., Wang, J., Suri, B.K., Matta, S.A., McGovern, N., Janela, B., Wong, X.F.C.C., Sio, Y.Y., Au, B.V., Wilm, A., De Sessions, P.F., Lim, T.C., Tang, M.B.Y., Ginhoux, F., Connolly, J.E., Lane, E.B., Chew, F.T., Common, J.E.A., Nagarajan, N., 2016. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. Nat. Microbiol. 1, 1–10. https://doi.org/10.1038/nmicrobiol.2016.106.

Cho, I., Yamanishi, S., Cox, L., Methé, B.A., Zavadil, J., Li, K., Gao, Z., Mahana, D., Raju, K., Teitler, I., Li, H., Alekseyenko, A.V., Blaser, M.J., 2012. Antibiotics in early life alter the murine colonic microbiome and adiposity. 488 (7413), 621–626. https://doi.org/10.1038/nature11400.

Chumpitazi, B.P., Cope, J.L., Hollister, E.B., Tsai, C.M., Mcmeans, A.R., Luna, R.A., Versalovic, J., Shulman, R.J., 2016. Randomised Clinical Trial: Gut Microbiome Biomarkers are Associated with Clinical Response to a Low FODMAP Diet in Children with Irritable Bowel Syndrome 42, 418–427. https://doi.org/10.1111/apt.13286.Randomised.

Delorme, C., Abraham, A.L., Renault, P., Guédon, E., 2015. Genomics of Streptococcus salivarius, a major human commensal. Infect. Genet. Evol. 33, 381–392. https://doi.org/10.1016/j.meegid.2014.10.001.

Diop, K., Raoult, D., Bretelle, F., Fenollar, F., 2017. "Ezakiella massiliensis" sp. nov., a new bacterial species isolated from human female genital tract. New Microbes New Infect. 15, 16–17. https://doi.org/10.1016/j.nmni.2016.09.011.

Djais, A.A., Theodorea, C.F., Mashima, I., Otomo, M., Saitoh, M., Nakazawa, F., 2019. Identification and phylogenetic analysis of oral veillonella species isolated from the saliva of japanese children. F1000Research 8, 1–19. https://doi.org/10.12688/f1000research.18506.4.

Docktor, M.J., Paster, B.J., Abramowicz, S., Ingram, J., Wang, Y.E., Correll, M., Jiang, H., Cotton, S.L., Kokaras, A.S., Bousvaros, A., 2012. Alterations in diversity of the oral microbiome in pediatric inflammatory bowel disease. Inflamm. Bowel Dis. 18 (5), 935–942. https://doi.org/10.1002/ibd.21874.

Dowd, S.E., Wolcott, R.D., Sun, Y., McKeehan, T., Smith, E., Rhoads, D., Egles, C., 2008. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). PLoS One 3 (10), e3326. https://doi.org/10.1371/journal.pone.000332610.1371/journal.pone.0003326.g00110.1371/journal.pone.0003326.t001.

Ege, M.J., Mayer, M., Schwaiger, K., Mattes, J., Pershagen, G., Van Hage, M., Scheynius, A., Bauer, J., Von Mutius, E., 2012. Environmental bacteria and childhood asthma. Allergy Eur. J. Allergy Clin. Immunol. 67, 1565–1571. https://doi.org/10.1111/all.12028.

Eisenhofer, R., Minich, J.J., Marotz, C., Cooper, A., Knight, R., Weyrich, L.S., 2019. Contamination in Low Microbial Biomass Microbiome Studies: Issues and Recommendations. Trends Microbiol. 27 (2), 105–117. https://doi.org/10.1016/j.tim.2018.11.003.

EU 2016/679, 2016. The General Data Protection Regulation (GDPR) is a regulation in EU law on data protection and privacy in the European Union (EU) and the European Economic Area (EEA). Regulation of the European Parliament.

Fierer, N., 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15 (10), 579–590. https://doi.org/10.1038/nrmicro.2017.87.

Fierer, N., Hamady, M., Lauber, C.L., Knight, R., 2008. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc. Natl. Acad. Sci. U. S. A. 105 (46), 17994–17999. https://doi.org/10.1073/pnas.0807920105.

Finnish Ministry of Justice, 2018. Data protection act 1050/2018.

Flandroy, L., Poutahidis, T., Berg, G., Clarke, G., Dao, M.C., Decaestecker, E., Furman, E., Haahtela, T., Massart, S., Plovier, H., Sanz, Y., Rook, G., 2018. The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems. Sci. Total Environ. 627, 1018–1038. https://doi.org/10.1016/j.scitotenv.2018.01.288.

Fonken, L.K., Frank, M.G., D'Angelo, H.M., Heinze, J.D., Watkins, L.R., Lowry, C.A., Maier, S.F., 2018. Mycobacterium vaccae immunization protects aged rats from surgery-elicited neuroinflammation and cognitive dysfunction. Neurobiol. Aging 71, 105–114. https://doi.org/10.1016/j.neurobiolaging.2018.07.012.

Frank, M.G., Fonken, L.K., Dolzani, S.D., Annis, J.L., Siebler, P.H., Schmidt, D., Watkins, L.R., Maier, S.F., Lowry, C.A., 2018. Immunization with Mycobacterium vaccae induces an anti-inflammatory milieu in the CNS: Attenuation of stress-induced microglial priming, alarmins and anxiety-like behavior. Brain. Behav. Immun. 73, 352–363. https://doi.org/10.1016/j.bbi.2018.05.020.

Fyhrquist, N., Ruokolainen, L., Suomalainen, A., Lehtimäki, S., Veckman, V., Vendelin, J., Karisola, P., Lehto, M., Savinko, T., Jarva, H., Kosunen, T.U., Corander, J., Auvinen, P., Paulin, L., von Hertzen, L., Laatikainen, T., Mäkelä, M., Haahtela, T., Greco, D., Hanski, I., Alenius, H., 2014. Acinetobacter species in the skin microbiota protect against allergic sensitization and inflammation. J. Allergy Clin. Immunol. 134 (6), 1301–1309.e11. https://doi.org/10.1016/j.jaci.2014.07.059.

Geirnaert, A., Calatayud, M., Grootaert, C., Laukens, D., Devriese, S., Smagghe, G., De Vos, M., Boon, N., Van De Wiele, T., 2017. Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. Sci. Rep. 7, 1–14. https://doi.org/10.1038/s41598-017-11734-8.

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 (6285), 539–544. https://doi.org/10.1126/science:aad9378.

Giongo, A., Gano, K.A., Crabb, D.B., Mukherjee, N., Novelo, L.L., Casella, G., Drew, J.C., Ilonen, J., Knip, M., Hyöty, H., Veijola, R., Simell, T., Simell, O., Neu, J., Wasserfall, C.H., Schatz, D., Atkinson, M.A., Triplett, E.W., 2011. Toward defining the autoimmune microbiome for type 1 diabetes. ISME J. 5 (1), 82–91. https://doi.org/10.1038/ismej.2010.92.

Graham-Rowe, D., 2011. Lifestyle: When allergies go west. Nature 479 (7374), S2–S4. https://doi.org/10.1038/479S2a.

Grönroos, M., Parajuli, A., Laitinen, O.H., Roslund, M.I., Vari, H.K., Hyöty, H., Puhakka, R., Sinkkonen, A., 2019. Short-term direct contact with soil and plant materials leads to an immediate increase in diversity of skin microbiota. MicrobiologyOpen 8 (3), e00645. https://doi.org/10.1002/mbo3.2019.8.issue-310.1002/mbo3.645.

Haahtela, T., 2019. A biodiversity hypothesis. Allergy Eur. J. Allergy Clin. Immunol. 1445–1456 https://doi.org/10.1111/all.13763.

Haahtela, T., Alenius, H., Lehtimäki, J., Sinkkonen, A., Fyhrquist, N., Hyöty, H., Ruokolainen, L., Alenius, H., Mäkelä, M., 2021. Immunological resilience and biodiversity for prevention of allergic diseases and asthma. Allergy 1–14. https://doi.org/10.1111/all.14895.

Hanski, I., von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., Karisola, P., Auvinen, P., Paulin, L., Makela, M.J., Vartiainen, E., Kosunen, T.U., Alenius, H., Haahtela, T., 2012. Environmental biodiversity, human microbiota, and allergy are interrelated. Proc. Natl. Acad. Sci. 109 (21), 8334–8339. https://doi.org/10.1073/pnas.1205624109.

Hornung, B.V.H., Zwittink, R.D., Kuijper, E.J., 2019. Issues and current standards of controls in microbiome research. FEMS Microbiol. Ecol. 95, 1–7. https://doi.org/10.1093/femsec/fiz045.

Huang, F.L., 2016. Alternatives to Multilevel Modeling for the Analysis of Clustered Data. J. Exp. Educ. 84 (1), 175–196. https://doi.org/10.1080/00220973.2014.952397.

Hui, N., Grönroos, M., Roslund, M.I., Parajuli, A., Vari, H.K., Soininen, L., Laitinen, O.H., Sinkkonen, A., 2019a. Diverse Environmental Microbiota as a Tool to Augment Biodiversity in Urban Landscaping Materials. Front. Microbiol. 10, 1–10. https://doi.org/10.3389/fmicb.2019.00536.

Hui, N., Parajuli, A., Puhakka, R., Grönroos, M., Roslund, M.I., Vari, H.K., Selonen, V.A.O., Yan, G., Siter, N., Nurminen, N., Oikarinen, S., Laitinen, O.H., Rajaniemi, J., Hyöty, H., Sinkkonen, A., 2019b. Temporal variation in indoor transfer of dirt-associated environmental bacteria in agricultural and urban areas. Environ. Int. 132, 105069. https://doi.org/10.1016/j.envint.2019.105069.

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J.H., Chinwalla, A.T., Creasy, H.H., Earl, A.M., Fitzgerald, M.G., Fulton, R.S., Giglio, M.G., Hallsworth-Pepin, K., Lobos, E.A., Madupu, R., Magrini, V., Martin, J.C., Mitreva, M., Muzny, D. M., Sodergren, E.J., Versalovic, J., Wollam, A.M., Worley, K.C., Wortman, J.R., Young, S.K., Zeng, Q., Aagaard, K.M., Abolude, O.O., Allen-Vercoe, E., Alm, E.J., Alvarado, L., Andersen, G.L., Anderson, S., Appelbaum, E., Arachchi, H.M., Armitage, G., Arze, C.A., Ayvaz, T., Baker, C.C., Begg, L., Belachew, T., Bhonagiri, V., Bihan, M., Blaser, M.J., Bloom, T., Bonazzi, V., Paul Brooks, J., Buck, G.A., Buhay, C. J., Busam, D.A., Campbell, J.L., Canon, S.R., Cantarel, B.L., Chain, P.S.G., Chen, I.M. A., Chen, L., Chhibba, S., Chu, K., Ciulla, D.M., Clemente, J.C., Clifton, S.W., Conlan, S., Crabtree, J., Cutting, M.A., Davidovics, N.J., Davis, C.C., Desantis, T.Z., Deal, C., Delehaunty, K.D., Dewhirst, F.E., Deych, E., Ding, Y., Dooling, D.J., Dugan, S.P., Michael Dunne, W., Scott Durkin, A., Edgar, R.C., Erlich, R.L., Farmer, C.N., Farrell, R.M., Faust, K., Feldgarden, M., Felix, V.M., Fisher, S., Fodor, A.A., Forney, L.J., Foster, L., Di Francesco, V., Friedman, J., Friedrich, D.C., Fronick, C.C., Fulton, L.L., Gao, H., Garcia, N., Giannoukos, G., Giblin, C., Giovanni, M.Y., Goldberg, J.M., Goll, J., Gonzalez, A., Griggs, A., Gujja, S., Kinder Haake, S., Haas, B.J., Hamilton, H.A., Harris, E.L., Hepburn, T.A., Herter, B., Hoffmann, D.E., Holder, M.E., Howarth, C., Huang, K.H., Huse, S.M., Izard, J., Jansson, J.K., Jiang, H., Jordan, C., Joshi, V., Katancik, J.A., Keitel, W.A., Kelley, S. T., Kells, C., King, N.B., Knights, D., Kong, H.H., Koren, O., Koren, S., Kota, K.C., Kovar, C.L., Kyrpides, N.C., La Rosa, P.S., Lee, S.L., Lemon, K.P., Lennon, N., Lewis, C.M., Lewis, L., Ley, R.E., Li, K., Liolios, K., Liu, B., Liu, Y., Lo, C.C., Lozupone, C.A., Dwayne Lunsford, R., Madden, T., Mahurkar, A.A., Mannon, P.J., Mardis, E.R., Markowitz, V.M., Mavromatis, K., McCorrison, J.M., McDonald, D., McEwen, J., McGuire, A.L., McInnes, P., Mehta, T., Mihindukulasuriya, K.A., Miller, J.R., Minx, P.J., Newsham, I., Nusbaum, C., Oglaughlin, M., Orvis, J., Pagani, I., Palaniappan, K., Patel, S.M., Pearson, M., Peterson, J., Podar, M., Pohl, C., Pollard, K.S., Pop, M., Priest, M.E., Proctor, L.M., Qin, X., Raes, J., Ravel, J., Reid, J. G., Rho, M., Rhodes, R., Riehle, K.P., Rivera, M.C., Rodriguez-Mueller, B., Rogers, Y. H., Ross, M.C., Russ, C., Sanka, R.K., Sankar, P., Fah Sathirapongsasuti, J., Schloss, J. A., Schloss, P.D., Schmidt, T.M., Scholz, M., Schriml, L., Schubert, A.M., Segata, N., Segre, J.A., Shannon, W.D., Sharp, R.R., Sharpton, T.J., Shenoy, N., Sheth, N.U., Simone, G.A., Singh, I., Smillie, C.S., Sobel, J.D., Sommer, D.D., Spicer, P., Sutton, G. G., Sykes, S.M., Tabbaa, D.G., Thiagarajan, M., Tomlinson, C.M., Torralba, M., Treangen, T.J., Truty, R.M., Vishnivetskaya, T.A., Walker, J., Wang, L., Wang, Z., Ward, D.V., Warren, W., Watson, M.A., Wellington, C., Wetterstrand, K.A., White, J. R., Wilczek-Boney, K., Wu, Y., Wylie, K.M., Wylie, T., Yandava, C., Ye, L., Ye, Y., Yooseph, S., Youmans, B.P., Zhang, L., Zhou, Y., Zhu, Y., Zoloth, L., Zucker, J.D.,

Birren, B.W., Gibbs, R.A., Highlander, S.K., Methé, B.A., Nelson, K.E., Petrosino, J.F., Weinstock, G.M., Wilson, R.K., White, O., 2012. Structure, function and diversity of the healthy human microbiome. Nature 486, 207–214. https://doi.org/10.1038/nature11234.

Iivanainen, E.K., Martikainen, P.J., Räisänen, M.L., Katila, M.L., 1997. Mycobacteria in boreal coniferous forest soils. FEMS Microbiol. Ecol. 23, 325–332. https://doi.org/10.1016/S0168-6496(97)00040-8.

Kameyama, K., Itoh, K., 2014. Intestinal Colonization by a Lachnospiraceae Bacterium Contributes to the Development of Diabetes in Obese Mice. Microbes Environ. 29, 427–430. https://doi.org/10.1264/jsme2.ME14054.

Karkman, A., Lehtimäki, J., Ruokolainen, L., 2017. The ecology of human microbiota: dynamics and diversity in health and disease. Ann. N. Y. Acad. Sci. 1399, 78–92. https://doi.org/10.1111/nyas.13326.

Kauppi, S., Romantschuk, M., Strömmer, R., Sinkkonen, A., 2012. Natural attenuation is enhanced in previously contaminated and coniferous forest soils. Environ. Sci. Pollut. Res. 19 (1), 53–63. https://doi.org/10.1007/s11356-011-0528-y.

Kawai, T., Akira, S., 2010. review The role of pattern-recognition receptors in innate immunity : update on Toll-like receptors. Nat. Immunol. 11, 373–384. https://doi.org/10.1038/ni.1863.

Kim, H.-Y., 2013. Statistical notes for clinical researchers: Evaluation of measurement error 1: using intraclass correlation coefficients. Restor. Dent. Endod. 38 (2), 98. https://doi.org/10.5395/rde.2013.38.2.98.

Kirjavainen, P. V., Karvonen, A.M., Adams, R.I., Täubel, M., Roponen, M., Tuoresmäki, P., Loss, G., Jayaprakash, B., Depner, M., Ege, M.J., Renz, H., Pfefferle, P.I., Schaub, B., Lauener, R., Hyvärinen, A., Knight, R., Heederik, D.J.J., von Mutius, E., Pekkanen, J., 2019. Farm-like indoor microbiota in non-farm homes protects children from asthma development. Nat. Med. https://doi.org/10.1038/s41591-019-0469-4.

Kobayashi, T., Glatz, M., Horiuchi, K., Kawasaki, H., Akiyama, H., Kaplan, D.H., Kong, H. H., Amagai, M., Nagao, K., 2015. Dysbiosis and Staphyloccus aureus Colonization Drives Inflammation in Atopic Dermatitis. Immunity 42, 756–766. https://doi.org/10.1016/j.immuni.2015.03.014.

Kondrashova, A., Reunanen, A., Romanov, A., Karvonen, A., Viskari, H., Vesikari, T., Ilonen, J., Knip, M., Hyöty, H., 2005. A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. Ann. Med. 37 (1), 67–72. https://doi.org/10.1080/07853890410018952.

Kondrashova, A., Seiskari, T., Ilonen, J., Knip, M., Hyöty, H., 2013. The “Hygiene hypothesis” and the sharp gradient in the incidence of autoimmune and allergic diseases between Russian Karelia and Finland. Apmis 121 (6), 478–493. https://doi.org/10.1111/apm.2013.121.issue-610.1111/apm.12023.

Lagier, J.C., El Karkouri, K., Nguyen, T.T., Armougom, F., Raoult, D., Fournier, P.E., 2015. Non-contiguous finished genome sequence and description of Anaerococcus senegalensis sp. nov. Stand. Genomic Sci. 6, 116–125. https://doi.org/10.4056/sigs.2415480.

Larsen, J.M., 2017. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology 151 (4), 363–374. https://doi.org/10.1111/imm.2017.151.issue-410.1111/imm.12760.

Lehtimäki, J., Karkman, A., Laatikainen, T., Paalanen, L., Von Hertzen, L., Haahtela, T., Hanski, I., Ruokolainen, L., 2017. Patterns in the skin microbiota differ in children and teenagers between rural and urban environments. Sci. Rep. 7, 1–11. https://doi.org/10.1038/srep45651.

Lehtimäki, J., Thorsen, J., Rasmussen, M.A., Hjelmsø, M., Shah, S., Mortensen, M.S., Trivedi, U., Vestergaard, G., Bønnelykke, K., Chawes, B.L., Brix, S., Sørensen, S.J., Bisgaard, H., Stokholm, J., 2021. URBANIZED MICROBIOTA IN INFANTS, IMMUNE CONSTITUTION AND LATER RISK OF ATOPIC DISEASES. J. Allergy Clin. Immunol. 148 (1), 234–243. https://doi.org/10.1016/j.jaci.2020.12.621.

Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., Knight, R., 2012. Diversity, stability and resilience of the human gut microbiota. Nature 489 (7415), 220–230. https://doi.org/10.1038/nature11550.

Lucas, A.J., Dyment, J.E., 2010. Where do children choose to play on the school ground ? The influence of green design 4279. https://doi.org/10.1080/03004270903130812.

Lynch, S.V., Pedersen, O., 2016. The Human Intestinal Microbiome in Health and Disease. N. Engl. J. Med. 375 (24), 2369–2379. https://doi.org/10.1056/NEJMra1600266.

Lynch, S.V., Wood, R.A., Boushey, H., Bacharier, L.B., Bloomberg, G.R., Kattan, M., O’Connor, G.T., Sandel, M.T., Calatroni, A., Matsui, E., Johnson, C.C., Lynn, H., Visness, C.M., Jaffee, K.F., Gergen, P.J., Gold, D.R., Wright, R.J., Fujimura, K., Rauch, M., Busse, W.W., Gern, J.E., 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.

Ma, Z., Li, W., 2019. How and why men and women differ in their microbiomes: medical ecology and network analyses of the microgenderome. Adv. Sci. 6 (23), 1902054. https://doi.org/10.1002/advs.v6.2310.1002/advs.201902054.

Matthews, D.M., Jenks, S.M., 2013. Ingestion of Mycobacterium vaccae decreases anxiety-related behavior and improves learning in mice. Behav. Processes 96, 27–35. https://doi.org/10.1016/j.beproc.2013.02.007.

Mennini, M., Dahdah, L., Artesani, M.C., Fiocchi, A., Martelli, A., 2017. Probiotics in Asthma and Allergy Prevention. Front. Pediatr. 5, 1–5. https://doi.org/10.3389/fped.2017.00165.

Merkiel-Pawłowska, S., Chalcarz, W., 2017. Gender differences and typical nutrition concerns of the diets of preschool children - the results of the first stage of an intervention study. BMC Pediatr. 17, 1–11. https://doi.org/10.1186/s12887-017-0962-1.

Moen, E.L., Fricano-Kugler, C.J., Luikart, B.W., O’Malley, A.J., Harezlak, J., 2016. Analyzing clustered data: Why and how to account for multiple observations nested within a study participant? PLoS One 11 (1), e0146721. https://doi.org/10.1371/journal.pone.0146721.

Morgan, X.C., Tickle, T.L., Sokol, H., Gevers, D., Devaney, K.L., Ward, D.V., Reyes, J.A., Shah, S.A., LeLeiko, N., Snapper, S.B., Bousvaros, A., Korzenik, J., Sands, B.E., Xavier, R.J., Huttenhower, C., 2012. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 13 (9), R79. https://doi.org/10.1186/gb-2012-13-9-r79.

Murillo, N., Raoult, D., 2013. Skin microbiota: overview and role in the skin diseases acne vulgaris and rosacea. Future Microbiol. 8 (2), 209–222. https://doi.org/10.2217/fmb.12.141.

Nakatsuji, T., Chen, T.H., Narala, S., Chun, K.A., Two, A.M., Yun, T., Shafiq, F., Kotol, P. F., Bouslimani, A., Melnik, A.V., Latif, H., Kim, J.N., Lockhart, A., Artis, K., David, G., Taylor, P., Streib, J., Dorrestein, P.C., Grier, A., Gill, S.R., Zengler, K., Hata, T.R., Leung, D.Y.M., Gallo, R.L., 2017. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci. Transl. Med. 9, 1–12. https://doi.org/10.1126/scitranslmed.aah4680.

Nakatsuji, T., Gallo, R.L., 2019. The role of the skin microbiome in atopic dermatitis. Ann. Allergy, Asthma Immunol. 122 (3), 263–269. https://doi.org/10.1016/j.anai.2018.12.003.

Niva, M., Hernesmaa, A., Haahtela, K., Salkinoja-Salonen, M., Sivonen, K., Haukka, K., 2006. Actinobacterial communities of boreal forest soil and lake water are rich in mycobacteria. Boreal Environ. Res. 11, 45–53.

Nurminen, N., Cerrone, D., Lehtonen, J., Parajuli, A., Roslund, M., Lönnrot, M., Ilonen, J., Toppari, J., Veijola, R., Knip, M., Rajaniemi, J., Laitinen, O.H., Sinkkonen, A., Hyöty, H., 2021. Land Cover of Early Life Environment Modulates the Risk of Type 1 Diabetes. Diabetes Care May; dc201. https://doi.org/https://doi.org/10.2337/dc20-1719.

Nurminen, N., Lin, J., Grönroos, M., Puhakka, R., Kramna, L., Vari, H.K., Viskari, H., Oikarinen, S., Roslund, M., Parajuli, A., Tyni, I., Cinek, O., Laitinen, O., Hyöty, H., Sinkkonen, A., 2018. Nature-derived microbiota exposure as a novel immunomodulatory approach. Future Microbiol. 13 (7), 737–744. https://doi.org/10.2217/fmb-2017-0286.

Nwaohiri, N., Urban, C., Gluck, J., Ahluwalia, M., Wehbeh, W., 2009. Tricuspid valve endocarditis caused by Haemophilus parainfluenzae: a case report and review of the literature. Diagn. Microbiol. Infect. Dis. 64 (2), 216–219. https://doi.org/10.1016/j.diagmicrobio.2009.02.015.

O’Neil, C.R., Wilson, E., Missaghi, B., 2016. Bone and Joint Infections due to Haemophilus parainfluenzae: Case Report and Review of the Literature. Can. J. Infect. Dis. Med. Microbiol. 2016, 1–6. https://doi.org/10.1155/2016/4503025.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.M., Wagner, H., 2019. Package “vegan”: Community ecology package. https://doi.org/ISBN 0-387-95457-0.

Orgiazzi, A., Bardgett, D.R., Barrios, E., Valerie, B.-P., Briones, M., Chotte, J.-L., Deyn, G. B., Eggleton, P., Fierer, N., Fraser, T.D., Hedlund, K., Simon, J., Johnson, N., Jones, A., Kandeler, E., Kaneko, N., Lavelle, P., Lemanceau, P., Miko, L., Wall, D., 2016. Global Soil Biodiversity Atlas. European Comission.

Ottman, N., Ruokolainen, L., Suomalainen, A., Sinkko, H., Karisola, P., Lehtimäki, J., Lehto, M., Hanski, I., Alenius, H., Fyhrquist, N., 2019. Soil exposure modifies the gut microbiota and supports immune tolerance in a mouse model. J. Allergy Clin. Immunol. 143 (3), 1198–1206.e12. https://doi.org/10.1016/j.jaci.2018.06.024.

Pankratov, T.A., Dedysh, S.N., 2010. Granulicella paludicola gen. nov., sp. nov., Granulicella pectinivorans sp. nov., Granulicella aggregans sp. nov. and Granulicella rosea sp. nov., acidophilic, polymer- degrading acidobacteria from Sphagnum peat bogs. Int. J. Syst. Evol. Microbiol. 60, 1–24.

Pankratov, T.A., Tindall, B.J., Liesack, W., Dedysh, S.N., 2007. Mucilaginibacter paludis gen. nov., sp. nov. and Mucilaginibacter gracilis sp. nov., pectin-, xylan- and laminarin-degrading members of the family Sphingobacteriaceae from acidic Sphagnum peat bog. Int. J. Syst. Evol. Microbiol. 57, 2349–2354. https://doi.org/10.1099/ijs.0.65100-0.

Parajuli, A., Grönroos, M., Kauppi, S., Płociniczak, T., Roslund, M.I., Galitskaya, P., Laitinen, O.H., Hyöty, H., Jumpponen, A., Strömmer, R., Romantschuk, M., Hui, N., Sinkkonen, A., 2017. The abundance of health-associated bacteria is altered in PAH polluted soils - Implications for health in urban areas? PLoS One 12 (11), e0187852. https://doi.org/10.1371/journal.pone.0187852.

Parajuli, A., Hui, N., Puhakka, R., Oikarinen, S., Grönroos, M., Selonen, V.A.O., Siter, N., Kramna, L., Roslund, M.I., Vari, H.K., Nurminen, N., Honkanen, H., Hintikka, J., Sarkkinen, H., Romantschuk, M., Kauppi, M., Valve, R., Cinek, O., Laitinen, O.H., Rajaniemi, J., Hyöty, H., Sinkkonen, A., 2020. Yard vegetation is associated with gut microbiota composition. Sci. Total Environ. 713, 136707. https://doi.org/10.1016/j.scitotenv.2020.136707.

Petrof, E.O., Gloor, G.B., Vanner, S.J., Weese, S.J., Carter, D., Daigneault, M.C., Brown, E. M., Schroeter, K., Allen-vercoe, E., 2013. Stool substitute transplant therapy for the eradication of Clostridium difficile infection : ‘ RePOOPulating ’ the gut 1–12.

Primm, T.P., Lucero, C.A., Falkinham, J.O., 2004. Health Impacts of Environmental Mycobacteria. Clin. Microbiol. Rev. 17 (1), 98–106. https://doi.org/10.1128/CMR.17.1.98-106.2004.

Puhakka, R., Haskins, A.H., Jauho, M., Grönroos, M., Sinkkonen, A., 2021. Factors Affecting Young Adults’ Willingness to Try Novel Health-Enhancing Nature-Based Products. J. Int. Consum. Mark. 1–18. https://doi.org/10.1080/08961530.2021.1873887.

Puhakka, R., Ollila, S., Valve, R., Sinkkonen, A., 2019a. Consumer Trust in a Health-Enhancing Innovation – Comparisons between Finland, Germany, and the United Kingdom. J. Int. Consum. Mark. 31 (2), 162–176. https://doi.org/10.1080/08961530.2018.1498757.

Puhakka, R., Rantala, O., Roslund, M.I., Rajaniemi, J., Laitinen, O.H., Sinkkonen, A., 2019b. Greening of daycare yards with biodiverse materials affords well-being, play

and environmental relationships. Int. J. Environ. Res. Public Health 16, 1–16. https://doi.org/10.3390/ijerph16162948.
R core team, 2018. R: A Language and Environment for Statistical Computing.. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
Reber, S.O., Siebler, P.H., Donner, N.C., Morton, J.T., Smith, D.G., Kopelman, J.M., Lowe, K.R., Wheeler, K.J., Fox, J.H., Hassell, J.E., Greenwood, B.N., Jansch, C., Lechner, A., Schmidt, D., Uschold-Schmidt, N., Füchsl, A.M., Langgartner, D., Walker, F.R., Hale, M.W., Lopez Perez, G., Van Treuren, W., González, A., Halweg-Edwards, A.L., Fleshner, M., Raison, C.L., Rook, G.A., Peddada, S.D., Knight, R., Lowry, C.A., 2016. Immunization with a heat-killed preparation of the environmental bacterium Mycobacterium vaccae promotes stress resilience in mice. Proc. Natl. Acad. Sci. U. S. A. 113 (22), E3130–E3139. https://doi.org/10.1073/pnas.1600324113.
Roesch, L.F.W., Lorca, G.L., Casella, G., Giongo, A., Naranjo, A., Pionzio, A.M., Li, N., Mai, V., Wasserfall, C.H., Schatz, D., Atkinson, M.A., Neu, J., Triplett, E.W., 2009. Culture-independent identification of gut bacteria correlated with the onset of diabetes in a rat model. ISME J. 3 (5), 536–548. https://doi.org/10.1038/ismej.2009.5.
Rook, G.A.W., Adams, V., Hunt, J., Palmer, R., Martinelli, R., Brunet, L.R., 2004. Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. Springer Semin. Immunopathol. 25, 237–255. https://doi.org/10.1007/s00281-003-0148-9.
Roslund, M.I., Grönroos, M., Rantalainen, A.-L., Jumpponen, A., Romantschuk, M., Parajuli, A., Hyöty, H., Laitinen, O., Sinkkonen, A., 2018. Half-lives of PAHs and temporal microbiota changes in commonly used urban landscaping materials. PeerJ 6, e4508. https://doi.org/10.7717/peerj.4508.
Roslund, M.I., Puhakka, R., Grönroos, M., Nurminen, N., Oikarinen, S., Gazali, A.M., Cinek, O., Kramná, L., Siter, N., Vari, H.K., Soininen, L., Parajuli, A., Rajaniemi, J., Kinnunen, T., Laitinen, O.H., Hyöty, H., Sinkkonen, A., 2020. Biodiversity intervention enhances immune regulation and health-associated commensal microbiota among daycare children. Sci. Adv. 6 (42), eaba2578. https://doi.org/10.1126/sciadv.aba2578.
Roslund, M.I., Rantala, S., Oikarinen, S., Puhakka, R., Hui, N., Parajuli, A., Laitinen, O. H., Hyöty, H., Rantalainen, A.-L., Sinkkonen, A., Grönroos, M., Hui, N., Kalvo, R., Nurminen, N., Oikarinen, S., Parajuli, A., Puhakka, R., Rantala, S., Roslund, M.I., Soininen, L., Yan, G., Laitinen, O.H., Rajaniemi, J., Hyöty, H., Sinkkonen, A., 2019. Endocrine disruption and commensal bacteria alteration associated with gaseous and soil PAH contamination among daycare children. Environ. Int. 130, 104894. https://doi.org/10.1016/j.envint.2019.06.004.
Rovery, C., Etienne, A., Foucault, C., Berger, P., Brouqui, P., 2005. Veillonella montpelliérensis endocarditis. Emerg. Infect. Dis. 11, 1112–1114. https://doi.org/10.3201/eid1107.041361.
Ruokolainen, L., Paalanen, L., Karkman, A., Laatikainen, T., von Hertzen, L., Vlasoff, T., Markelova, O., Masyuk, V., Auvinen, P., Paulin, L., Alenius, H., Fyhrquist, N., Hanski, I., Mäkelä, M.J., Zilber, E., Jousilahti, P., Vartiainen, E., Haahtela, T., 2017. Significant disparities in allergy prevalence and microbiota between the young people in Finnish and Russian Karelia. Clin. Exp. Allergy 47, 665–674. https://doi.org/10.1111/cea.12895.
Saarenpää, M., Roslund, M.I., Puhakka, R., Grönroos, M., Parajuli, A., Hui, N., Nurminen, N., Laitinen, O.H., Hyöty, H., Cinek, O., Sinkkonen, A., 2021. Do Rural Second Homes Shape Commensal Microbiota of Urban Dwellers ? A Pilot Study among Urban Elderly in Finland. Int. J. Environ. Res. Public Health 18, 3742. https://doi.org/10.3390/ijerph18073742.
Said, H.S., Suda, W., Nakagome, S., Chinen, H., Oshima, K., Kim, S., Kimura, R., Iraha, A., Ishida, H., Fujita, J., Mano, S., Morita, H., Dohi, T., Oota, H., Hattori, M., 2014. Dysbiosis of salivary microbiota in inflammatory bowel disease and its association with oral immunological biomarkers. DNA Res. 21, 15–25. https://doi.org/10.1093/dnares/dst037.
Santoru, M.L., Piras, C., Murgia, A., Palmas, V., Camboni, T., Liggi, S., Ibba, I., Lai, M.A., Orrù, S., Blois, S., Loizedda, A.L., Griffin, J.L., Usai, P., Caboni, P., Atzori, L., Manzin, A., 2017. Cross sectional evaluation of the gut-microbiome metabolome axis in an Italian cohort of IBD patients. Sci. Rep. 7 (1) https://doi.org/10.1038/s41598-017-10034-5.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Horn, D.J. Van, Weber, C.F., 2009. Introducing mothur : Open-Source. Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.
Severyn, C.J., Bhatt, A.S., 2018. In Translation With Probiotics, Resistance Is Not Always Futile. Cell Host Microbe 24 (3), 334–336. https://doi.org/10.1016/j.chom.2018.08.014.
Sharma, H., Tal, R., Clark, N., Segars, J.H., 2014. Microbiota and pelvic inflammatory disease. Semin. Reprod. Med. 32 (01), 043–049. https://doi.org/10.1055/s-0000007210.1055/s-003-2623510.1055/s-0033-1361822.
Sillanpää, S., Kramna, L., Oikarinen, S., Sipilä, M., Rautiainen, M., Aittoniemi, J., Laranne, J., Hyöty, H., Cinek, O., Fey, P.D., 2017. Next-Generation Sequencing Combined with Specific PCR Assays To Determine the Bacterial 16S rRNA Gene Profiles of Middle Ear Fluid Collected from Children with Acute Otitis. Media. 2 (2) https://doi.org/10.1128/mSphere.00006-17.
Sinkkonen, A., Kauppi, S., Simpanen, S., Rantalainen, A.-L., Strömmer, R., Romantschuk, M., 2013. Layer of organic pine forest soil on top of chlorophenol-contaminated mineral soil enhances contaminant degradation. Environ. Sci. Pollut. Res. 20 (3), 1737–1745. https://doi.org/10.1007/s11356-012-1047-1.
Smith, D., 2017. Discovery and Characterization of an Anti-Inflammatory Lipid Derived from Mycobacterium vaccae. University of Colorado. Chemistry & Biochemistry Graduate Theses & Dissertations. 232.
Soininen, L., Grönroos, M., Roslund, M.I., Sinkkonen, A., 2021. Long-term storage affects resource availability and occurrence of bacterial taxa linked to pollutant degradation and human health in landscaping materials. Urban For. Urban Green. 60 (May 2021): 127065. https://doi.org/10.1016/j.ufug.2021.127065.
Stein, M.M., Hrusch, C.L., Gozdz, J., Igartua, C., Pivniouk, V., Murray, S.E., Ledford, J.G., Marques dos Santos, M., Anderson, R.L., Metwali, N., Neilson, J.W., Maier, R.M., Gilbert, J.A., Holbreich, M., Thorne, P.S., Martinez, F.D., von Mutius, E., Vercelli, D., Ober, C., Sperling, A.I., 2016. Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children. N. Engl. J. Med. 375 (5), 411–421. https://doi.org/10.1056/NEJMoa1508749.
Stokholm, J., Blaser, M.J., Thorsen, J., Rasmussen, M.A., Waage, J., Vinding, R.K., Schoos, A.M.M., Kunøe, A., Fink, N.R., Chawes, B.L., Bønnelykke, K., Brejnrod, A.D., Mortensen, M.S., Al-Soud, W.A., Sørensen, S.J., Bisgaard, H., 2018. Maturation of the gut microbiome and risk of asthma in childhood. Nat. Commun. 9, 1–10. https://doi.org/10.1038/s41467-017-02573-2.
Suez, J., Zmora, N., Zilberman-Schapira, G., Mor, U., Dori-Bachash, M., Bashiardes, S., Zur, M., Regev-Lehavi, D., Ben-Zeev Brik, R., Federici, S., Horn, M., Cohen, Y., Moor, A.E., Zeevi, D., Korem, T., Kotler, E., Harmelin, A., Itzkovitz, S., Maharshak, N., Shibolet, O., Pevsner-Fischer, M., Shapiro, H., Sharon, I., Halpern, Z., Segal, E., Elinav, E., 2018. Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. Cell 174 (6), 1406–1423. e16. https://doi.org/10.1016/j.cell.2018.08.047.
Sun, H., Terhonen, E., Koskinen, K., Paulin, L., Kasanen, R., Asiegbu, F.O., 2014. Bacterial diversity and community structure along different peat soils in boreal forest. Appl. Soil Ecol. 74, 37–45. https://doi.org/10.1016/j.apsoil.2013.09.010.
Taylor, L.H., Latham, S.M., Woolhouse, M.E.J., 2001. Risk factors for human disease emergence. Philos. Trans. R. Soc. B Biol. Sci. 356, 983–989. https://doi.org/10.1098/rstb.2001.0888.
United Nations, 2018. World Urbanization Prospects: The 2018 Revision. UNDESA 1–2.
Vandeputte, D., Kathagen, G., D'hoe, K., Vieira-Silva, S., Valles-Colomer, M., Sabino, J., Wang, J., Tito, R.Y., De Commer, L., Darzi, Y., Vermeire, S., Falony, G., Raes, J., 2017. Quantitative microbiome profiling links gut community variation to microbial load. Nature 551 (7681), 507–511. https://doi.org/10.1038/nature24460.
Vari, H.K., Roslund, M.I., Oikarinen, S., Nurminen, N., Puhakka, R., Parajuli, A., Grönroos, M., Siter, N., Laitinen, O.H., Hyöty, H., Rajaniemi, J., Rantalainen, A.-L., Sinkkonen, A., 2021. Associations between land cover categories, gaseous PAH levels in ambient air and endocrine signaling predicted from gut bacterial metagenome of the elderly. Chemosphere 265, 128965. https://doi.org/10.1016/j.chemosphere.2020.128965.
Voorhies, A.A., Mark Ott, C., Mehta, S., Pierson, D.L., Crucian, B.E., Feiveson, A., Oubre, C.M., Torralba, M., Moncera, K., Zhang, Y., Zurek, E., Lorenzi, H.A., 2019. Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. Sci. Rep. 9, 1–17. https://doi.org/10.1038/s41598-019-46303-8.
Wade, W.G., 2013. The oral microbiome in health and disease. Pharmacol. Res. 69 (1), 137–143. https://doi.org/10.1016/j.phrs.2012.11.006.
Waller, J.M., Maibach, H.I., 2005. Age and skin structure and function, a quantitative approach (I): blood flow, pH, thickness, and ultrasound echogenicity 221–235.
Walter, G., Vernier, M., Pinelli, P.O., Million, M., Coulange, M., Seng, P., Stein, A., 2014. Bone and joint infections due to anaerobic bacteria: An analysis of 61 cases and review of the literature. Eur. J. Clin. Microbiol. Infect. Dis. 33, 1355–1364. https://doi.org/10.1007/s10096-014-2073-3.
Westcott, S.L., Schloss, P.D., 2017. OptiClust, an improved method for assigning amplicon-based sequence data to operational taxonomic units. mSphere 2 (2). https://doi.org/10.1128/mSphereDirect.00073-17.
Yu, D., Kurola, J.M., Lähde, K., Kymäläinen, M., Sinkkonen, A., Romantschuk, M., 2014. Biogas production and methanogenic archaeal community in mesophilic and thermophilic anaerobic co-digestion processes. J. Environ. Manage. 143, 54–60. https://doi.org/10.1016/j.jenvman.2014.04.025.
Yu, D., Sinkkonen, A., Hui, N., Kurola, J.M., Kukkonen, S., Parikka, P., Vestberg, M., Romantschuk, M., 2015. Molecular profile of microbiota of Finnish commercial compost suppressive against Pythium disease on cucumber plants. Appl. Soil Ecol. 92, 47–53. https://doi.org/10.1016/j.apsoil.2015.03.005.
Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A Greedy Algorithm for Aligning DNA Sequences. J. Comput. Biol. 7 (1-2), 203–214. https://doi.org/10.1089/10665270050081478.
Zhou, D., Zhang, H., Bai, Z., Zhang, A., Bai, F., Luo, X., Hou, Y., Ding, X., Sun, B., Sun, X., Ma, N., Wang, C., Dai, X., Lu, Z., 2016. Exposure to soil, house dust and decaying plants increases gut microbial diversity and decreases serum immunoglobulin E levels in BALB/c mice. Environ. Microbiol. 18 (5), 1326–1337. https://doi.org/10.1111/1462-2920.12895.