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Full length article

Urban indoor gardening enhances immune regulation and diversifies skin microbiota — A placebo-controlled double-blinded intervention study

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A B S T R A C T

According to the hygiene and biodiversity hypotheses, frequent exposure to environmental microbiota, especially through soil contact, diversifies commensal microbiota, enhances immune modulation, and ultimately lowers the risk of immune-mediated diseases. Here we test the underlying assumption of the hygiene and biodiversity hypotheses by instructing volunteers to grow edible plants indoors during the winter season when natural exposure to environmental microbiota is low. The one-month randomized, placebo-controlled double-blind trial consisted of two treatments: participants received either microbially diverse growing medium or visually similar but microbially poor growing medium. Skin microbiota and a panel of seven immune markers were analyzed in the beginning of the trial and after one month. The diversity of five bacterial phyla (Bacteroidetes, Planctomycetes, Proteobacteria, Cyanobacteria, and Verrucomicrobia) and one class (Bacteroidia) increased on the skin of participants in the intervention group while no changes were observed in the placebo group. The number of nodes and edges in the co-occurrence networks of the skin bacteria increased on average three times more in the intervention group than in the placebo group. The plasma levels of the immunomodulatory cytokine interleukin 10 (IL-10) increased in the intervention group when compared with the placebo group. A similar trend was observed in the interleukin 17A (IL-17A) levels and in the IL-10:IL-17A ratios. Participants in both groups reported high satisfaction and adherence to the trial. The current study provides evidence in support of the core assumption of the hygiene and biodiversity hypotheses of immune-mediated diseases. Indoor urban gardening offers a meaningful and convenient approach for increasing year-round exposure to environmental microbiota, paving the way for other prophylactic practices that might help prevent immune-mediated diseases.

1. Introduction

Autoimmune and immune-mediated diseases (e.g., asthma and allergies) are becoming more and more prevalent in developed countries (Lerner et al., 2016; To et al., 2012). Exposure to diverse environmental microbiota, especially through soil, is important for the natural development and functioning of the immune system and, ultimately, for human health (Ege et al., 2012; Fyhrquist et al., 2014; Noverr & Huffnagle, 2005; Ottman et al., 2019; Rook, 2009; Stein et al., 2016; Stiemsma et al., 2015; Valkonen et al., 2015; von Hertzen & Haahtela, 2006). According to the hygiene hypothesis and its variant, the biodiversity hypothesis, this is linked to biodiversity loss and reduced microbial exposure (Aerts et al., 2018; Civitello et al., 2015; Haahtela, 2019; Haahtela et al., 2021; Hanski et al., 2012; Lehtimäki et al., 2017; Rohr et al., 2020; Rook, 2009; Ruokolainen et al., 2017; von Hertzen et al., 2011; von Hertzen & Haahtela, 2006). Beneficial microbial exposure has decreased among urban dwellers (Gupta et al., 2020; Parajuli et al., 2018; Shan et al., 2020), and people living in urban areas have distinct microbiota compositions compared to their rural counterparts (Hanski et al., 2012; Lehtimäki et al., 2017, 2018; Ruokolainen et al., 2015, 2020).

Compelling evidence suggests that home surroundings play a key

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role in determining microbiota-related health outcomes. Forest and agricultural land cover during the first year of life is inversely associated with the risk of atopic sensitization and type 1 diabetes (Hanski et al., 2012; Nurminen et al., 2021; Ruokolainen et al., 2015). Biodiverse and green living areas correlate with respiratory health (Donovan et al., 2018; Liddicoat et al., 2018). Diverse yard vegetation and outdoor nature-related activities have been linked to health-related changes in the gut microbiota composition (Parajuli et al., 2020; Sobko et al., 2020). Living in rural or farm-like conditions is directly associated with diverse commensal microbiota and a well-functioning immune system (Ege et al., 2012; Kirjavainen et al., 2019; Stein et al., 2016; von Mutius & Radon, 2008). Rural second homes and active contacts with urban green space have been linked to differences in commensal microbial communities (Saarenpää et al., 2021; Selway et al., 2020), and the number of visits to urban green space is associated with reduced need for asthma and psychotropic medication (Turunen et al., 2023). In line with comparative studies on disease incidence, biodiversity intervention trials (i.e., introduction of microbiologically diverse vegetation and soil to everyday living environment) have been shown to enrich commensal microbiota and enhance immune modulation among urban citizens, at least temporarily, but as notified by several authors, many of the studies have been of pilot character (Hui et al., 2019a; Roslund et al., 2020, 2021, 2022, 2023; Tischer et al., 2022).

Even though built urban space is known to reduce transfer of health-associated microbiota indoors (Hui et al., 2019b; Parajuli et al., 2018; Zhao et al., 2024), only a handful of studies have concentrated on ways to modify indoor exposure to rich environmental microbiota in densely built areas. A single study suggested that indoor green walls enrich skin microbiota and may enhance immunomodulation (Soininen et al., 2022), and a few intervention trials indicated that short-term contact with microbiologically rich soil products supports diverse skin microbiota (Grönroos et al., 2019; Nurminen et al., 2018; see also Shaffer & Lozupone, 2018). Gardening has successfully been used as a form of therapy for centuries, and recently it has been scientifically proven to provide substantial health benefits ranging from alleviated depression and stress to elevated cognitive functions (Soga et al., 2016). While some studies have looked into whether urban gardening affects the human microbiota (Gascon et al., 2020; Mhuireach et al., 2023), and a single study has even recorded plasma IL-6 levels among breast cancer survivors to understand the effect of home gardening on inflammation (Bail et al., 2018), no study has, to our knowledge, investigated the impact of indoor gardening on commensal microbiota and immunomodulation simultaneously in the context of the hygiene and biodiversity hypotheses of immune-mediated diseases (see Rook & Lowry, 2022).

Here we present the results of a test of the core idea on which the hygiene and biodiversity hypotheses were built on, i.e., whether exposure to soil-based microbiota causes changes in commensal microbiota and enhances immune system modulation. For this, we provided volunteers with growing medium and crop species for indoor gardening. To understand the role of soil microbiota exposure in the shifts of immune marker levels in plasma, we built a randomized double-blind trial. Half of the volunteers received microbially rich growing medium and the other half visually similar but microbially poor peat-based growing medium. As far as we know, this is the first randomized double-blind clinical trial examining the effects of indoor gardening on both the microbiota and immune system in parallel. The detailed hypotheses were that i) urban indoor gardening with microbially rich growing medium (intervention group) causes changes in the skin microbial communities while gardening with peat-based growing medium (placebo group) causes no or minuscule changes; ii) the co-occurrence networks of the skin bacteria are more complex in the intervention than in the placebo group after but not before the intervention; and that iii) immune modulation is enhanced in the intervention but not in the placebo group.

2. Materials and methods

2.1. Participants

Participants of this trial were recruited from the cities of Lahti (population > 120 000) and Hyvinkää (population > 45 000) in Finland. The trial followed the recommendations of the Finnish Advisory Board on Research Integrity and was approved by the ethics committee of the Pirkanmaa Hospital District (approval code: R19077, date: 19.8.2019). Written informed consent in accordance with the Declaration of Helsinki was signed by all participants. Exclusion criteria included age under 18 years, severe doctor-diagnosed immune deficiency (e.g., HIV, antibody deficiency), immunosuppressive medication, immune-mediated disease (e.g., colitis ulcerosa, Crohn's disease, rheumatoid arthritis), leukopenia, tetanus antibody deficiency, diabetes, mental or memory disorder, cancer diagnosis, rash or eczema, daily smoking, owning indoor pets, and living on an operating farm.

Participants were placed either in an intervention or placebo group through a stratified randomization (variables: age, reported gender). All participants were asked to fill out questionnaires assessing their living conditions and lifestyle both during their everyday life and the trial (e.g., medication and food supplements, diet and changes in it, outdoor activities and hobbies, contact with soil). Adherence to the trial, negative and positive experiences related to the trial, and hand-washing and hand-sanitizing habits were also recorded. All participants using antibiotics during or six months before the trial or anti-inflammatory drugs during the trial were excluded from the analyses. Samples from thirteen placebo and fifteen intervention group participants were used in the analyses (Table 1).

Table 1
Demographic characteristics of the study participants.

			Placebo group	Intervention group
No of participants			13	15
Gender	Female		10	11
	Male		3	4
Age	<35		3	4
	35–60		6	6
	>60		4	5
	Average		47	46
	Range		29–72	29–70
Type of residence	Apartment		5	6
	Rowhouse		4	3
	Detached house		4	6
Gardening during growing season	Outdoor vegetable garden		5	3
	Outdoor flower bed		2	2
	Indoor vegetables		2	1
	Daily		1	0
	Weekly		5	6
	Monthly		1	0
	Rarely		2	6
	Never		4	3
Outdoor recreation	Walking	Daily	8	5
		Weekly	3	6
		Monthly	0	4
		Rarely	2	0
		Never	0	0
	Cycling	Daily	3	4
		Weekly	3	3
		Monthly	2	5
		Rarely	5	1
		Never	0	2
	Other (e.g., berry picking, swimming, hiking, fishing)	Daily	5	3
		Weekly	3	6
		Monthly	3	4
		Rarely	2	2
		Never	0	0
Diet	Omnivorous		13	15
	Vegetarian/vegan		0	0
	Changes during trial		No: all	No: all

2.2. Planters and crop species

Participants were provided with a plastic planter (size 70x14x18 cm), spray bottle, desk lamp with a bulb (20 W 3000 K 2000 lm), seeds and plants, and growing medium (Fig. 1). The placebo group used commercial horticultural peat (Luonnonturve by Kekkilä, Finland) mixed with heat-expanded lightweight clay pebbles (Leca by Saint-Gobain, Finland) and inorganic fertilizer sticks (Substral by Transmeri, Finland). Horticultural peat was chosen because of its wide use and low bacterial diversity (1.3×10^8 16S rRNA sequences per gram soil; Roslund et al., 2022; Fig. 2). The intervention group used the same horticultural peat and clay pebble blend mixed with a microbiologically rich compost-based mixture (3.5×10^9 16S rRNA sequences per gram mixture; Hui et al., 2019a) developed and used in our previous studies (Hui et al., 2019a; Nurminen et al., 2018). This mixture contains sieved composted materials such as tree bark and mulch, dung, deciduous leaf litter, peat, agricultural sludge, and *Sphagnum* moss.

Both groups were provided with the same seven crop species: lettuce (*Lactuca sativa*), white mustard (*Sinapis alba*), radish (*Raphanus raphanistrum* subsp. *sativus*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), pea (*Pisum sativum*), and fava bean (*Vicia faba*). This set of species was chosen as some of them can be harvested shortly after sowing as microgreens (mustard) and sprouts (pea, fava bean), and some later as leaves (lettuce), taproots (radish), bulbs (garlic), and rhizomes (ginger).

Both groups were given the same instructions. First, a layer of clay pebbles was placed at the bottom of the planter to improve drainage. The planter was then filled either with the peat-based or compost-based growing medium. The seeds, rhizomes, and bulbs were planted in different sections of the planter according to the instructions. These first steps were completed barehanded. The planter was then placed close to a window and watered, and the lamp was set up nearby. As the trial was conducted during the winter season, in February and March, grow lights were needed. Daily tasks included both soil and dietary exposure: Monitoring the moisture level by inserting a bare finger deep into the growing medium, watering and misting with the spray bottle, harvesting, and resowing.

2.3. Skin and growing medium samples

Skin swab samples for bacterial analyses were collected at the beginning of the trial (0 mo) and at one month (1 mo) by a trained nurse. The back of the hand (2x2 cm) was swabbed with ten horizontal and ten vertical strokes with a cotton swab wetted with saline buffer (0.1 % Tween 20 in 0.15 M NaCl). The cotton tips were placed in separate sterile plastic tubes and stored at −80 °C until further processing. Growing medium samples were collected into separate zip lock bags with sterilized plastic spoons by a university researcher before the start of the trial, and they were stored at −80 °C until further processing.

Bacterial DNA was extracted from the skin samples with the Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) and from the growing medium samples with the PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany) as per the manufacturers' standard protocols. DNA concentrations were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The V4 region of the 16S rRNA gene was amplified with the 505F and 806R primers. Sterile water was used as a negative control during the DNA extraction and no-template control was used during the PCR. *Cupriavidus necator* JMP134 (DSM4058) was used as a positive control. All samples were sequenced at the Institute for Molecular Medicine Finland (FIMM, Helsinki, Finland) using the Illumina MiSeq platform (2x300 bp, V3 reagent kit).

2.4. Blood samples

Blood samples were used to analyze the levels of anti-inflammatory cytokines interleukin 10 (IL-10) and transforming growth factor beta (TGF-β1), which have been connected to a lower risk of several immune-mediated diseases (Burmeister & Marriott, 2018; Li et al., 2006; Opal & DePalo, 2000; Prud'homme and Piccirillo, 2000); proinflammatory cytokines interleukin 17A (IL-17A), interleukin 1 beta (IL-1β), and tumor necrosis factor (TNF-α), which have been associated with the risk of immune-mediated diseases (Dinarello, 2000; Honkanen et al., 2010; Kuwabara et al., 2017); and multifunctional cytokines interleukin 6 (IL-6) and interleukin 21 (IL-21), which have both anti-inflammatory and proinflammatory properties (Mehta et al., 2004; Rose-John, 2012).

The blood samples were collected at the beginning of the trial (0 mo) and at one month (1 mo) by a trained nurse. Venous blood was drawn into two Vacutainer CPT Mononuclear Cell Preparation tubes containing sodium citrate (BD Biosciences, NJ, USA). The tubes were centrifuged as per the manufacturer's instructions to separate the plasma. The plasma samples were stored at −80 °C until further processing. IL-17A, IL-10, IL-1β, IL-6, IL-21, and TNF-α concentrations were measured from the plasma samples using the Milliplex MAP High Sensitivity T Cell Panel kit (Merck KGaA, Darmstadt, Germany) with the Bio-Plex 200 system (Bio-Rad Laboratories, Hercules, CA, USA) and Bio-Plex Manager software (version 4.1, Bio-Rad Laboratories, Hercules, CA, USA). TGF-β1

Fig. 1. Gardening equipment provided for the participants consisted of a plastic planter, lamp, bulb, spray bottle, crop species, and growing medium.

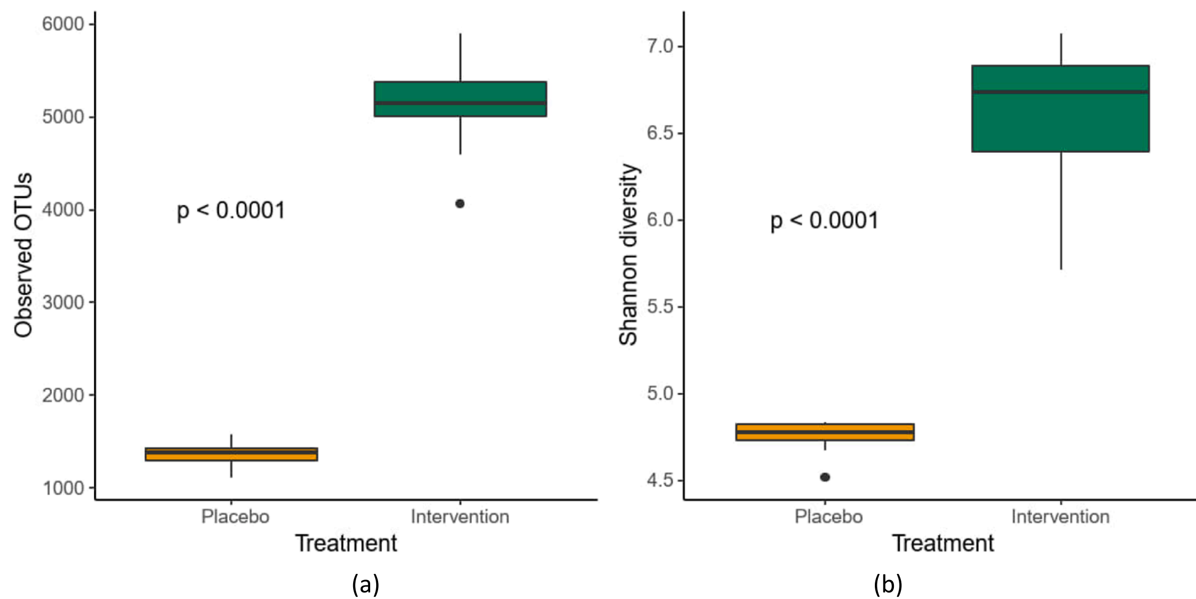

Fig. 2. Horticultural peat used by the placebo group had lower OTU richness (a) and Shannon diversity (b) than the compost-based mixture used by the intervention group.

concentrations were measured using ELISA (BioVendor, Czech Republic).

2.5. Bioinformatics

Paired-end sequence data (.fastq) from the skin and growing medium (i.e. soil) samples were processed using mothur (version 1.39.5, https://www.mothur.org, accessed on 27 July 2022; Schloss et al., 2009) following the protocols by Schloss & Westcott (2011) and Kozich et al. (2013) as described in our earlier studies (Nurminen et al., 2018; Parajuli et al., 2020). General quality of the sequencing data was good; per sequence quality scores for all samples were (Q score) > 30. The overall number of reads was 4 639 172 in skin samples and 3 449 602 in soil samples. There were 56 575 ± 25 692 and 49 280 ± 8 770 (mean ± SD) reads per skin and soil sample, respectively. The sequences were aligned using the mothur version of SILVA bacterial reference (version 132; Pruesse et al., 2007). Less abundant (≤10 sequences across all experimental units) operational taxonomic units (OTUs) were removed to avoid PCR or sequencing artifacts. Contaminant OTUs were removed as described by Roslund et al. (2021). Both the skin and soil samples were subsampled to the lowest sequence counts (5 907 and 33 805, respectively; 1000 iterations). Good's coverage index (rarefaction curve analysis) was 0.91 ± 0.06 for soil and for skin 0.96 ± 0.06 (mean ± SD).

2.6. Statistics

All statistical analyses and data visualizations were performed using the R statistical software environment (version 4.1.2, R Foundation, Vienna, Austria; R Core Team, 2020). Following packages were used: phyloseq (version 1.38.0; McMurdie & Holmes, 2013) for community composition analyses and diversity calculations, vegan (version 2.5–7; Oksanen et al., 2019) for diversity calculations, ggplot2 (version 3.3.5; Wickham, 2016) for visualizations, MKinfer (version 0.6; Kohl, 2020) for permutation tests, lme4 (version 1.1–29; Bates et al., 2015) and rsq (version 2.5; Zhang, 2018) for linear mixed-effects models (LMM), and cooccur (version 1.3; Griffith et al., 2016) and visNetwork (version 2.1.0; Thieurmel, 2021) for co-occurrence network analyses.

Statistical analyses of the skin and growing medium bacterial communities were conducted at different taxonomic levels (i.e., OTU, genus, family, order, class, phylum). At the OTU level, analyses were conducted within the most abundant phyla and classes (relative abundance > 0.025 % across all samples). The data were transformed to proportions by dividing the reads for each operational taxonomic unit (OTU) in a sample by the total number of reads in that sample (McKnight et al., 2019). The cytokine concentration data were $\log_{10}$ transformed to normalize the distributions. In the cytokine analyses, changes in the $\log_{10}$ cytokine concentrations were used. These were calculated by extracting the starting value (0 mo) from the one-month value (1 mo).

Bacterial Shannon and Simpson diversity indices, observed richness, and relative abundances were compared between the intervention and placebo group as well as between the time points using the Student's *t*-test or Mann–Whitney *U* test. The Student's *t*-test was used when the data were normally distributed based on the Shapiro–Wilk test, and the Mann–Whitney *U* test was used when the data were not normally distributed. Paired tests were used when different time points were compared within the groups. Changes in the cytokine levels were compared between the intervention and placebo group using a permutation *t*-test. LMMs were used to study the relationship between the bacterial and cytokine variables. Participants were used as a random factor in all models. Bacterial co-occurrence network analyses were conducted at different taxonomic levels (OTU, genus, family, order, class, phylum) using binary presence-absence data, and the number of nodes and edges was recorded. *p*-values were corrected using the Benjamini–Hochberg method (Benjamini & Hochberg, 1995).

3. Results

3.1. Anti-inflammatory cytokine IL-10 increased in the intervention group

Compared to the placebo group, $\log_{10}$ IL-10 levels increased in the intervention group during the trial (Fig. 3a, Table 2). A similar difference was observed in $\log_{10}$ IL-17A levels and $\log_{10}$ IL-10:IL-17A ratios (Fig. 3b–c, Table 2). No statistically significant differences were observed in $\log_{10}$ TNF-α, IL-21, IL-6, IL-1β or TGF-β1 levels (Fig. 3d–h, Table 2). After the Benjamini–Hochberg *p*-value correction, only differences in the $\log_{10}$ IL-10 levels remained significant (Fig. 3a, Table 2).

3.2. Skin bacterial diversity increased in the intervention group

The Shannon diversity of the phyla Proteobacteria, Bacteroidetes,

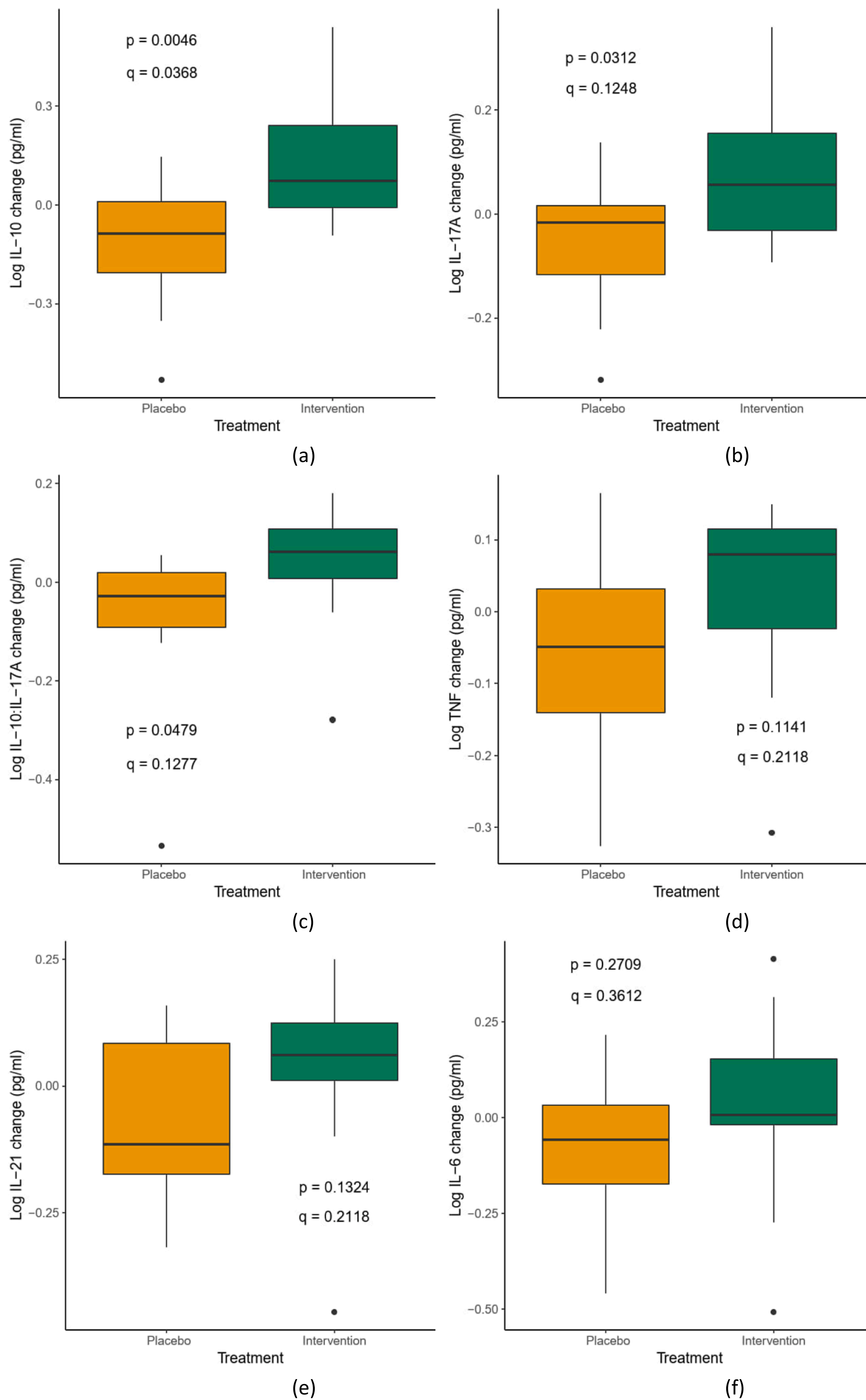

Fig. 3. Log_{10} IL-10 (a) and IL-17A (b) concentrations and log_{10} IL-10:IL-17A ratio (c) increased in the intervention group during the trial. No differences between the groups were observed in log_{10} TNF-α (d), IL-21 (e), IL-6 (f), IL-1β (g) or TGF-β1 (h) concentrations. Boxplots show medians (thicker line), upper and lower hinges (box), values 1.5 times the interquartile range (whiskers), and values outside hinges (data points, outliers).

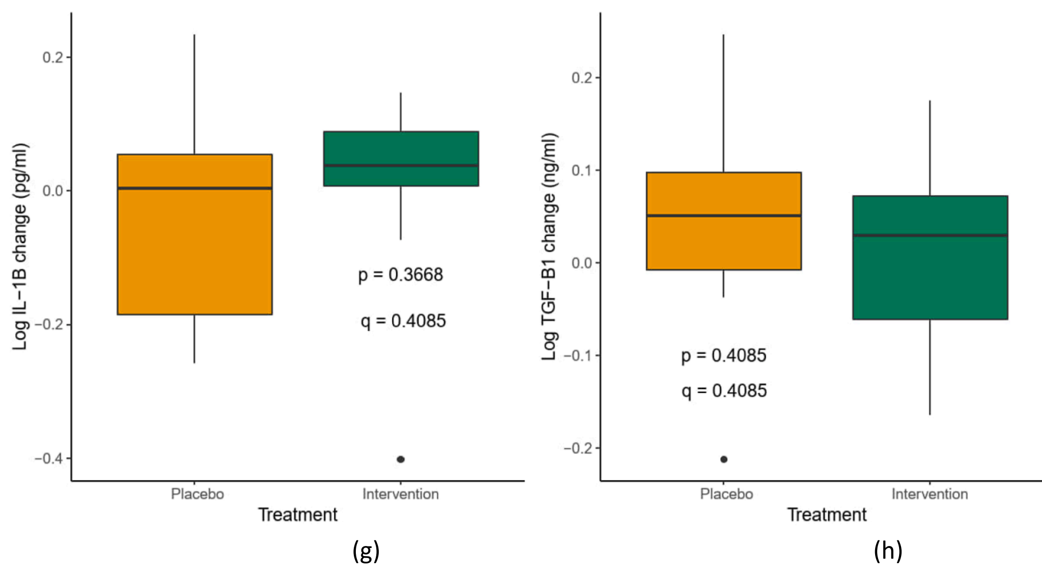

Fig. 3. (*continued*).

Table 2
Differences in $\log_{10}$ cytokine concentrations between the groups were tested with a permutation *t*-test (9999 permutations). *p*-values, q-values, and significance after the correction are given for all cytokines and for IL-10:IL-17A ratio.

Cytokine	*p*-value	q-value	Significant with FDR 0.1
IL-10	0.0046	0.0368	Yes
IL-17A	0.0312	0.1248	No
IL-10:IL-17A	0.0479	0.1277	No
TNF-α	0.1141	0.2118	No
IL-21	0.1324	0.2118	No
IL-6	0.2709	0.3612	No
IL-1β	0.3668	0.4085	No
TGF-β1	0.4085	0.4085	No

Planctomycetes, Cyanobacteria, and Verrucomicrobia and of the class Bacteroidia increased ($q < 0.10$) in the intervention group during the trial (Fig. 4, Table 3). No differences in the Shannon diversities between the time points were observed in the placebo group (Fig. 4, Table 3). There were no differences ($q > 0.10$) at order, family or genus levels, neither between time points nor between treatments. More than 60 % of the skin bacterial community belonged to the 15 most abundant genera, while uncultured genera covered less than one tenth of the skin bacterial community (Fig. 5). The most abundant genera were always *Staphylococcus*, *Streptococcus*, *Ralstonia*, and *Corynebacterium* (Fig. 5).

3.3. Nodes and edges increased in the co-occurrence networks

The topological parameters of the skin bacterial co-occurrence networks were compared between the intervention and placebo group (Table 4). The complexity of the networks grew in both groups as the number of nodes (individual taxa or OTUs) and edges (correlations) increased at most of the taxonomic levels. Although these increases were multiple times higher in the intervention group at most levels, they were most pronounced at the OTU and genus levels. At the genus level, the number of nodes and edges in the intervention group increased by 146 and 6236, respectively, and in the placebo group by 50 and 677, respectively (Table 4, Fig. 6). The proportion of positive correlations increased in both groups at most of the taxonomic levels. After the trial, the skin bacterial co-occurrence networks in the intervention group had more nodes and edges than the networks in the placebo group.

3.4. Adherence to the trial

Adherence to the trial was 100 % in both groups, and most participants cared for their crops on a daily basis. A few participants reported taking short breaks during the weekends (Table A.1), but these breaks did not take place right before sampling. Participants in both groups reported minimal soil contact unrelated to the trial (Table A.1), for example through their outdoor gardens or outdoor recreation, most probably due to the winter season.

4. Discussion

The results of this biodiversity intervention trial support the core assumption on which the hygiene and biodiversity hypotheses were built on. During the trial, skin bacterial diversity became higher and co-occurrence networks more complex in the intervention group than in the placebo group, and these differences were reflected in changes in the immune response. Since the diversity of five bacterial phyla and one bacterial class increased in the intervention but not in the placebo group during the exposure, the results support the first hypothesis that handling microbially rich growing medium enriches the skin microbiota. The second microbially oriented hypothesis was supported as the number of co-occurrence nodes and edges were higher in the intervention group than in the placebo group after the trial. As between-treatment differences in changes in the immunomodulatory cytokine levels were evident, the trial is also in accordance with the hypothesis that immune modulation was enhanced in the intervention but not in the placebo group.

The current study indicates that daily contact with microbially rich soil—instead of any soil—is vital for immune modulation. However, while the plasma levels of the anti-inflammatory IL-10 increased in the intervention group, the underlying trigger is unclear. Although shifts in IL-10 levels have previously been associated with Gammaproteobacteria on skin (Fyhrquist et al., 2014; Hanski et al., 2012; Roslund et al., 2020, 2022), the finding was not repeated in the current study (LMM: Gammaproteobacteria ~ IL-10, $p > 0.05$, data not shown). This may be related to the mode of exposure: while in previous studies participants have been exposed to the environmental microbiota mainly externally, in the current study the participants were exposed also via gastrointestinal tract while consuming the crops. This might cause shifts in the gut microbiota, and the potential gut microbiota differences might affect the immune response. Indeed, edible plants contain microbiota that are hypothetically linked to human immune response (Wicaksono et al.,

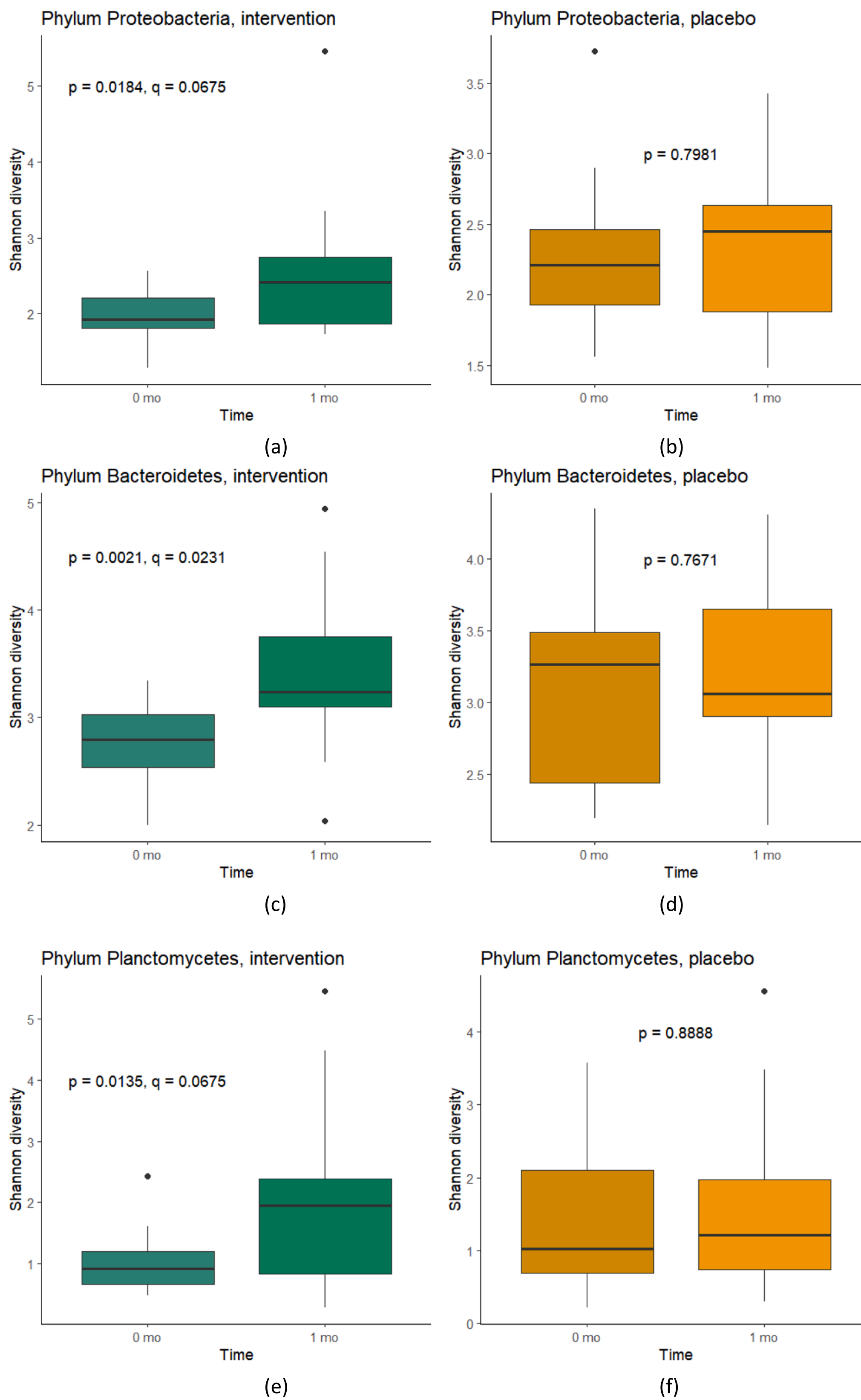

Fig. 4. Shannon diversity of the phyla Proteobacteria (a), Bacteroidetes (c), Planctomycetes (e), Cyanobacteria (g), Verrucomicrobia (i) and of the class Bacteroidia (k) increased in the intervention group during the trial while no changes were observed in the placebo group (b, d, f, h, j, l). Boxplots show medians (thicker line), upper and lower hinges (box), values 1.5 times the interquartile range (whiskers), and values outside hinges (data points, outliers).

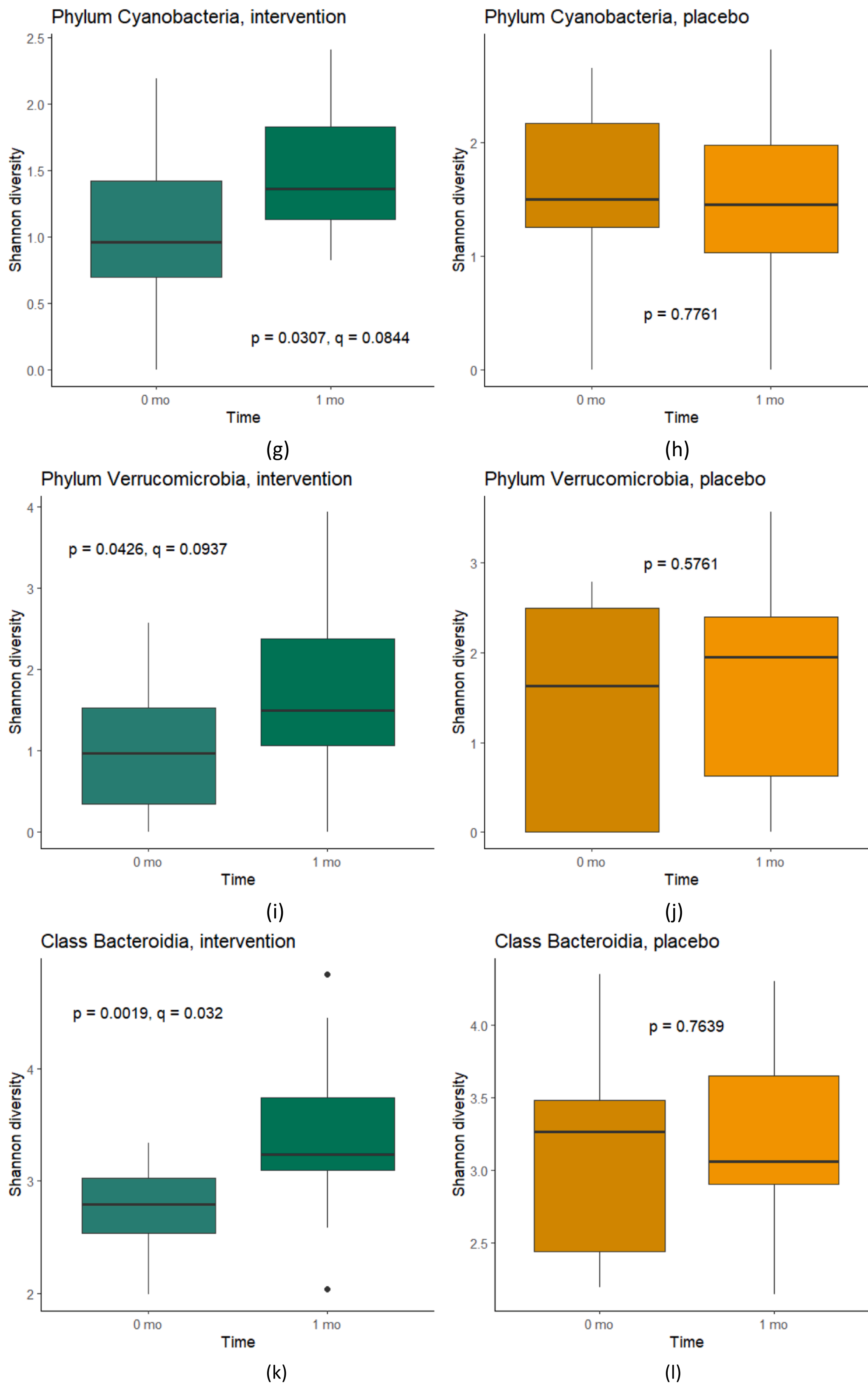

Fig. 4. (*continued*).

2022, 2023). To conclude, the lack of associations between specific skin bacterial taxa and immune markers does not nullify the importance of the shifts in immune markers as these might have been affected by changes in the gut microbiota.

Interestingly, changes in the skin bacterial diversity in the current study occurred mainly at the phylum level, while all the hub OTUs in the co-occurrence networks had a low relative abundance (Table A.2). The implications of this are twofold. First, the common protocol of searching for associations between immune responses and shifts in abundances or diversities at higher taxonomic levels hardly distinguishes the potential

Table 3
Shannon diversities at different time points (0 and 1 month) were compared within the intervention and placebo groups using paired tests. Shannon diversities (mean ± standard deviation), *p*-values, q-values (only intervention group), statistical significance, and test types are given for all tested phyla and classes. Statistical significance is marked with bold font.

Taxon	Treatment	Shannon diversity index (mean ± SD)		*p*-value	q-value	Significant with FDR 0.1	Test
		0 mo	1 mo				
Phylum Bacteroidetes	**Intervention**	**2.76 ± 0.35**	**3.44 ± 0.73**	**0.0021**	**0.0231**	**Yes**	***t*-test**
	Placebo	3.14 ± 0.66	3.22 ± 0.62	0.7671	–	No	*U* test
Class Bacteroidia	**Intervention**	**2.75 ± 0.35**	**3.42 ± 0.71**	**0.0019**	**0.0320**	**Yes**	***t*-test**
	Placebo	3.14 ± 0.66	3.22 ± 0.62	0.7639	–	No	*t*-test
Phylum Planctomycetes	**Intervention**	**1.02 ± 0.50**	**1.99 ± 1.40**	**0.0135**	**0.0675**	**Yes**	***U* test**
	Placebo	1.42 ± 0.94	1.56 ± 1.19	0.8888	–	No	*t*-test
Class Planctomycetacia	Intervention	1.08 ± 0.67	2.16 ± 1.31	0.0214	0.1712	No	*U* test
	Placebo	1.47 ± 1.13	1.91 ± 0.95	0.2790	–	No	*t*-test
Phylum Proteobacteria	**Intervention**	**1.99 ± 0.32**	**2.51 ± 0.91**	**0.0184**	**0.0675**	**Yes**	***U* test**
	Placebo	2.32 ± 0.54	2.38 ± 0.60	0.7981	–	No	*U* test
Class Alphaproteobacteria	Intervention	3.43 ± 0.45	3.40 ± 0.91	0.9201	0.9321	No	*t*-test
	Placebo	3.52 ± 0.48	3.51 ± 0.53	0.9746	–	No	*t*-test
Class Deltaproteobacteria	Intervention	2.16 ± 0.40	2.66 ± 0.98	0.2220	0.4364	No	*U* test
	Placebo	2.24 ± 0.80	2.36 ± 0.91	0.6477	–	No	*t*-test
Class Gammaproteobacteria	Intervention	1.69 ± 0.31	1.93 ± 0.68	0.2220	0.4364	No	*U* test
	Placebo	1.93 ± 0.34	1.96 ± 0.48	0.8511	–	No	*t*-test
Phylum Cyanobacteria	**Intervention**	**1.07 ± 0.53**	**1.50 ± 0.48**	**0.0307**	**0.0844**	**Yes**	***t*-test**
	Placebo	1.56 ± 0.68	1.46 ± 0.77	0.7761	–	No	*U* test
Class Oxyphotobacteria	Intervention	0.76 ± 0.62	1.00 ± 0.59	0.1091	0.4364	No	*t*-test
	Placebo	1.28 ± 0.70	1.23 ± 0.68	0.8742	–	No	*t*-test
Phylum Verrucomicrobia	**Intervention**	**0.99 ± 0.76**	**1.73 ± 1.04**	**0.0426**	**0.0937**	**Yes**	***t*-test**
	Placebo	1.40 ± 1.07	1.65 ± 1.15	0.5761	–	No	*t*-test
Class Verrucomicrobiae	Intervention	0.99 ± 0.76	1.73 ± 1.04	0.0426	0.2272	No	*t*-test
	Placebo	1.40 ± 1.07	1.65 ± 1.15	0.5761	–	No	*U* test
Phylum Acidobacteria	Intervention	2.26 ± 0.51	2.72 ± 0.84	0.1193	0.2187	No	*t*-test
	Placebo	2.47 ± 0.77	2.68 ± 0.74	0.4629	–	No	*t*-test
Class Acidobacteriia	Intervention	1.86 ± 0.50	2.21 ± 0.82	0.2455	0.4364	No	*t*-test
	Placebo	2.11 ± 0.84	2.34 ± 0.73	0.4761	–	No	*t*-test
Phylum Fusobacteria	Intervention	1.73 ± 0.48	1.59 ± 0.25	0.1641	0.2579	No	*U* test
	Placebo	1.85 ± 0.30	1.73 ± 0.35	0.4017	–	No	*t*-test
Class Fusobacteriia	Intervention	1.73 ± 0.48	1.59 ± 0.25	0.1641	0.4364	No	*U* test
	Placebo	1.85 ± 0.30	1.73 ± 0.35	0.4017	–	No	*U* test
Phylum Deinococcus-Thermus	Intervention	1.68 ± 0.42	1.50 ± 0.41	0.2799	0.3849	No	*t*-test
	Placebo	1.58 ± 0.67	1.61 ± 0.43	0.8888	–	No	*U* test
Class Deinococci	Intervention	1.68 ± 0.42	1.50 ± 0.41	0.2799	0.4478	No	*t*-test
	Placebo	1.58 ± 0.67	1.61 ± 0.43	0.8888	–	No	*U* test
Phylum Chloroflexi	Intervention	1.81 ± 0.95	2.05 ± 1.04	0.6062	0.7409	No	*t*-test
	Placebo	1.66 ± 0.94	1.78 ± 0.84	0.7297	–	No	*t*-test
Phylum Firmicutes	Intervention	1.85 ± 0.36	2.07 ± 1.13	0.7120	0.7832	No	*U* test
	Placebo	2.22 ± 0.40	2.12 ± 0.74	0.9443	–	No	*U* test
Class Negativicutes	Intervention	1.03 ± 0.34	1.20 ± 0.34	0.1883	0.4364	No	*t*-test
	Placebo	1.11 ± 0.27	1.30 ± 0.37	0.2318	–	No	*t*-test
Class Clostridia	Intervention	2.82 ± 0.72	3.15 ± 0.72	0.4432	0.5909	No	*U* test
	Placebo	3.04 ± 0.36	3.19 ± 0.47	0.4264	–	No	*t*-test
Class Bacilli	Intervention	1.37 ± 0.33	1.47 ± 0.91	0.9321	0.9321	No	*U* test
	Placebo	1.66 ± 0.23	1.57 ± 0.53	0.8339	–	No	*U* test
Phylum Actinobacteria	Intervention	2.47 ± 0.48	2.50 ± 0.61	0.8266	0.8266	No	*t*-test
	Placebo	2.68 ± 0.58	2.78 ± 0.66	0.5559	–	No	*t*-test
Class Acidimicrobiia	Intervention	1.11 ± 0.73	1.58 ± 1.17	0.3163	0.4601	No	*t*-test
	Placebo	0.97 ± 0.89	1.35 ± 0.96	0.2240	–	No	*U* test
Class Thermoleophilia	Intervention	1.92 ± 0.60	2.10 ± 1.03	0.5815	0.7157	No	*t*-test
	Placebo	1.98 ± 0.91	2.20 ± 0.79	0.5399	–	No	*t*-test
Class Actinobacteria	Intervention	2.43 ± 0.48	2.41 ± 0.51	0.8153	0.9318	No	*t*-test
	Placebo	2.62 ± 0.55	2.66 ± 0.56	0.8030	–	No	*t*-test

importance of rare taxa, simply because random variation caused by numerous confounding factors in the everyday living environment is likely multiple orders of magnitude higher among rare taxa than major phyla and classes. Second, since variation in phylogeny and niche specialization within classes and phyla can be high, it may not be optimal to use classes and phyla as indicators of health or immune response in studies using soil exposure. Nevertheless, the diversity of the phylum Proteobacteria increased in the intervention but not in the placebo group in the current study, and in an earlier study Proteobacteria have been identified as possible health indicators of commercially cultivated edible plant species (Köberl et al., 2017). Also, the phylum Bacteroidetes that increased in diversity in the intervention group has an important role in maintaining homeostasis and healthy gastrointestinal functions, especially mucosal immunity (Gibiino et al., 2018; Huttenhower et al., 2012; Troy & Kasper, 2010). Although we did not study if skin Bacteroidetes were transmitted into the gastrointestinal tract, skin exposure to soil with high microbial diversity is capable of modifying the gut microbiota (Nurminen et al., 2018). Our study did not separate whether skin Bacteroidetes diversity increased via soil contact or via plant contact in the intervention group, while no change occurred in the control group. Interestingly, some studies have found residential green spaces–vegetation patches–to be negatively associated with Bacteroidetes levels in the gut (Van Pee et al., 2023). In addition to Proteobacteria and Bacteroidetes, the Shannon diversity of the phylum Cyanobacteria increased in the intervention group. While not much is known of their role in health and disease, some studies have found them

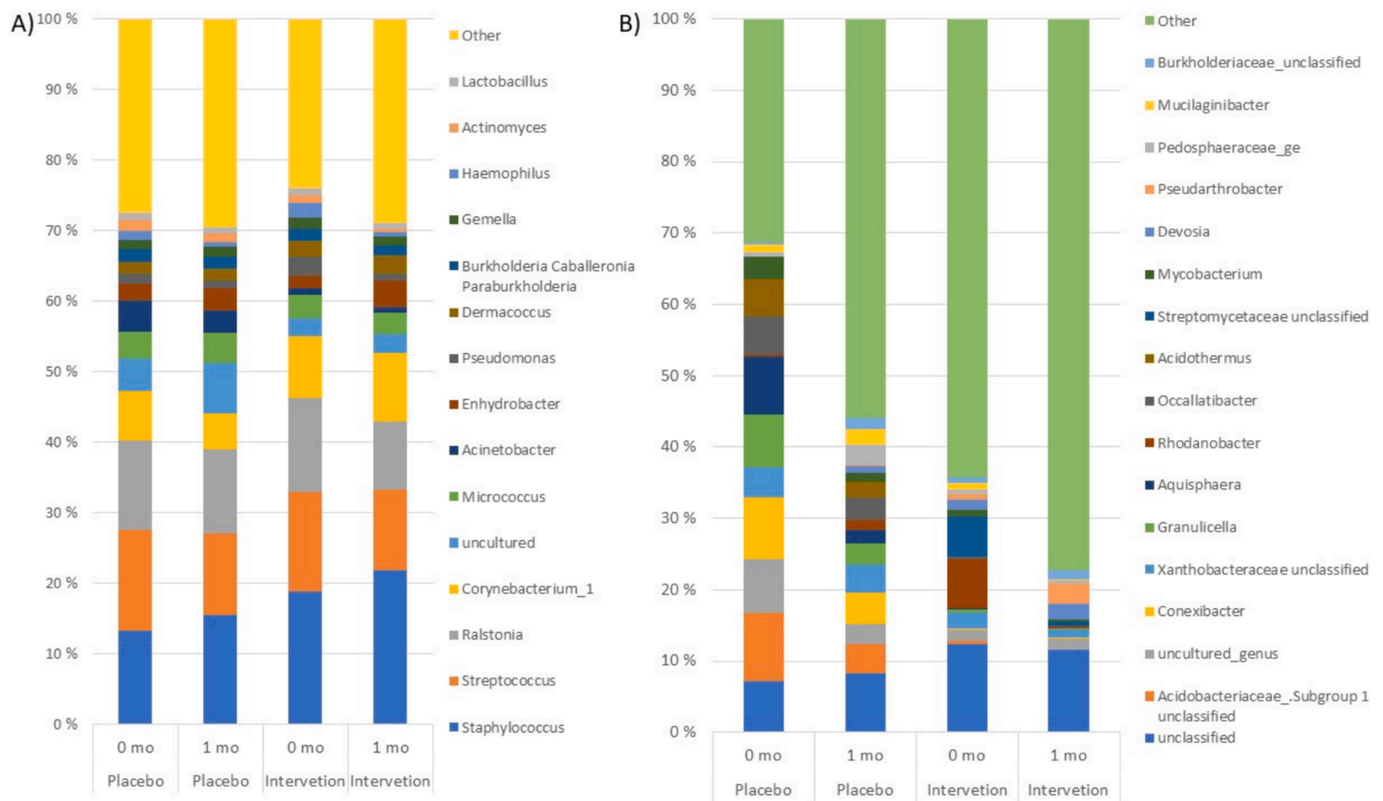

Fig. 5. The relative abundance of the most common bacterial genera (>1 %) A) on the skin and B) in the soil in the placebo and intervention treatment in the beginning (0 mo) and after one month (1 mo) of the intervention.

Table 4
Topological measures of the skin bacterial co-occurrence networks.

Taxonomic level	Treatment	Time point	Nodes	Node change	Edges	Edge change	Positive	Positive %	Negative	Negative %
OTU	Placebo	0 mo	1202	66	21171	−369	18071	85 %	3100	15 %
		1 mo	1268		20802		17720	85 %	3082	15 %
	Intervention	0 mo	980	502	9630	19365	6890	72 %	2740	28 %
		1 mo	1482		28995		23444	81 %	5551	19 %
Genus	Placebo	0 mo	427	50	3478	677	2983	86 %	495	14 %
		1 mo	477		4155		3828	92 %	327	8 %
	Intervention	0 mo	404	146	1882	6236	1335	71 %	547	29 %
		1 mo	550		8118		7858	97 %	260	3 %
Family	Placebo	0 mo	188	31	763	233	686	90 %	77	10 %
		1 mo	219		996		967	97 %	29	3 %
	Intervention	0 mo	182	60	437	890	325	74 %	112	26 %
		1 mo	242		1327		1286	97 %	41	3 %
Order	Placebo	0 mo	92	22	224	87	187	83 %	37	17 %
		1 mo	114		311		301	97 %	10	3 %
	Intervention	0 mo	76	41	95	237	72	76 %	23	24 %
		1 mo	117		332		313	94 %	19	6 %
Class	Placebo	0 mo	28	12	30	40	26	87 %	4	13 %
		1 mo	40		70		66	94 %	4	6 %
	Intervention	0 mo	31	21	23	58	16	70 %	7	30 %
		1 mo	52		81		78	96 %	3	4 %
Phylum	Placebo	0 mo	5	8	3	7	2	67 %	1	33 %
		1 mo	13		10		9	90 %	1	10 %
	Intervention	0 mo	6	8	3	9	3	100 %	0	0 %
		1 mo	14		12		11	92 %	1	8 %

to protect the skin against UV-induced damage and pigmentation (Fuentes-Tristan et al., 2019; Li et al., 2020).

Co-occurrence network analyses focusing on the microbe-microbe interactions on human skin have not been reported in any previous intervention trials testing the hygiene and biodiversity hypotheses. Despite their limited use, co-occurrence networks can be a convenient tool in revealing positive and negative interactions between microbial taxa. A positive correlation can imply that two species simply favor similar environmental conditions, or that they interact with each other and even co-operate, for example by producing metabolites that benefit one another (Das et al., 2018). In the same way, a negative correlation may indicate that the species require different environmental conditions, or that they are competing with each other. Since in our study both groups had daily soil contact, it is not surprising that the number of

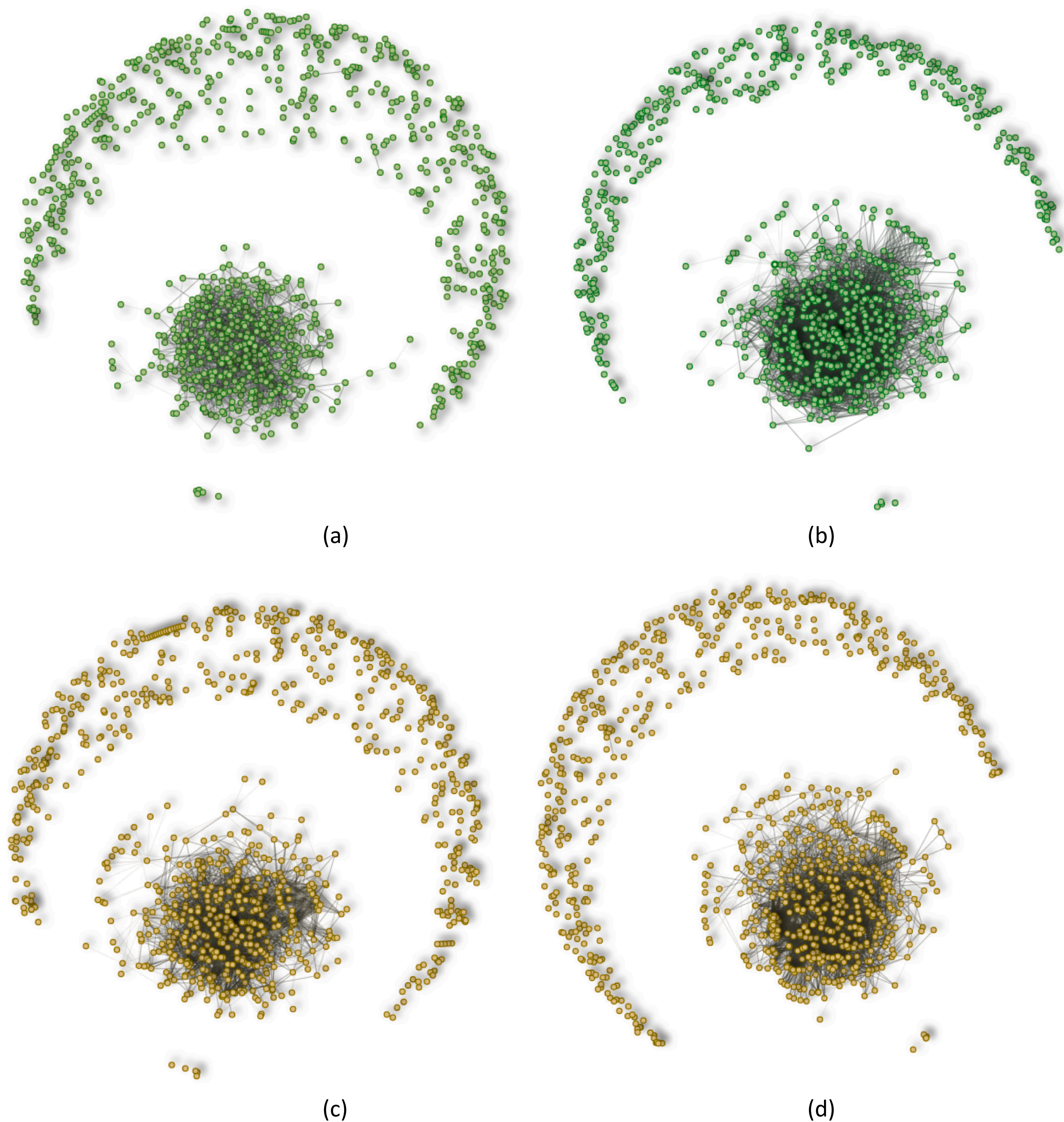

Fig. 6. Skin bacterial co-occurrence networks at the genus level: intervention group (green) before (a) and after the trial (b), placebo group (yellow) before (c) and after the trial (d).

nodes and edges increased in both groups at almost all taxonomic levels during the trial. As the increases were stronger in the intervention group, the results of the co-occurrence network analyses indicate that microbially diverse soil is superior to microbially poor soil in diversifying the skin microbiota.

The lack of clinical trials, especially those focusing on skin, that report co-occurrence network results makes forming any estimates on possible health outcomes hard. Comparing co-occurrence results between different types of clinical trials can be particularly challenging, not least due to the obscure nature of positive and negative correlations. Relvas et al. (2021) used co-occurrence network analysis to study the impact of oral health on the salivary microbiome, and found that the health-associated network was characterized by more interconnections between the nodes than the disease-associated. In the current study, the intervention group network contained considerably more interconnections than the placebo group network after the trial. Relvas et al. (2021) also found the health-associated network to be better balanced in terms of the proportion of positive correlations. In our study, the proportion of positive correlations increased in both groups, which is understandable as soil bacteria have been found to benefit from interspecific cooperation (Ren et al., 2015). The hub OTUs identified in our study had a low relative abundance (<1 %)—a finding that Relvas et al. (2021) also reported—which implies that even rare and less-abundant taxa can have an important role in the microbial communities of

human skin.

The more complex intervention group co-occurrence networks detected at one month are in accordance with the changes in the skin microbiota diversity. It is logical to assume that there are well-established microbial interaction networks in the intervention growing medium as it consists of multiple natural components with high microbial activity and diversity. As the participants interact with the growing medium, some parts of these complex interconnection networks are transferred onto the skin. The skin swab samples were taken from the back of the hand, not from fingers that the participants mostly used while tending to the crops, which indicates that the detected networks are not solely a result of soil residues being sampled. The nurse visited the participants at different times of the day, and while the participants were given instructions to perform the daily tasks prior to the visit, we, due to ethical reasons, could not monitor the exact time of performing the tasks or handwashing. Due to this limitation, we can only assume that some participants had soil contact and/or washed their hands immediately before sampling while others did not.

Another weakness of the current study is its short duration due to the COVID-19 pandemic. Skin and blood samples were not collected at three months of gardening as originally planned. Samples collected at multiple timepoints are needed to account for the high temporal variability observed in some individuals (Flores et al., 2014). As the follow-up samples are missing, it is unknown whether the changes in the skin microbiota would have persisted after the trial. Mhuireach et al. (2023) found that soil contact through gardening increases the number of bacterial taxa shared between soil and skin, but this effect largely disappears within 12 h. Hence, we assume that no long-lasting microbiota changes would have been detected in the midst of the COVID-19 pandemic. In general, gardening is often a hobby or even a profession for those involved in it, leading to recurring soil contact and microbial exposure, which—based on the current study—might lead to long-term shifts in the immune response (Roslund et al., 2020,2021; Tischer et al., 2022). In addition to being longer-lasting, future studies should be considerably larger and involve people from different geographic regions. The current study was heavily skewed towards the female gender, and the results might not be fully generalizable, particularly due to sex differences in immune responsiveness (Beenakker et al., 2019). An optimal study would also consider all possible routes of inoculation. The current study did not differentiate between the role of the consumed plants and aerobiome. Previous studies have found that indoor plants are able to shape the indoor microbial communities (Dockx et al., 2022; Mahnert et al., 2015) and potentially even enhance immune regulation (Soininen et al., 2022). Wider cytokine panels could be utilized to add to the knowledge of the impact of soil contact on immune response. Future studies should also consider using shotgun metagenomic sequencing instead of targeted 16S rRNA as it allows for a more in-depth analysis of the microbial assemblages by enabling the identification of not only bacteria but also fungi, viruses, and other microorganisms and their functional potential.

As our study was conducted during the winter season, it demonstrates how beneficial microbial exposure can be obtained year-round. Overall microbial exposure, especially among urbanites, has been found to be low in winter and high in spring and summer (Hui et al., 2019b; Mhuireach et al., 2021). This might also explain why gardening studies executed during the summer months have failed to detect microbial effects (Gascon et al., 2020). In addition to the season, our trial differs from previous gardening studies by taking place indoors and by being double-blinded and more rigorously controlled. In many gardening studies that focused on varying health outcomes the placebo or control group has consisted of wait-list participants not actively gardening (Bail et al., 2018; Davis et al., 2016; Demark-Wahnefried et al., 2018; Gascon et al., 2020), but in our study both the intervention and placebo group engaged in gardening using identical equipment, crop species, and instructions. This plausibly is a major advantage of the current study.

The majority of the participants in our study reported being satisfied with the trial and that they plan to continue gardening, which might lead to long-lasting immunomodulatory effects. For most, information related to the role of beneficial bacterial exposure in immune regulation was new, but almost everyone was eager to learn more. The findings of this trial show how urban indoor gardening offers a space- and cost-efficient approach with which to increase beneficial microbial exposure at all stages of life, for example in kindergartens, schools, offices, and nursing homes.

5. Conclusion

The current biodiversity intervention trial demonstrated for the first time that urban indoor gardening has the potential to diversify the microbiota on human skin and to increase anti-inflammatory cytokine levels in plasma. Our findings are in accordance with the hygiene and biodiversity hypotheses, which state that these shifts may ultimately lead to a lower risk or weaker symptoms of certain immune-mediated diseases, such as allergies. Furthermore, the experimental setting illustrates how beneficial microbial exposure can be obtained indoors and year-round through an activity that is both meaningful and satisfying.

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CRediT authorship contribution statement

Mika Saarenpää: Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Marja I. Roslund:** Conceptualization, Methodology, Validation, Writing – review & editing. **Noora Nurminen:** Conceptualization, Formal analysis, Methodology, Validation. **Riikka Puhakka:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. **Laura Kummola:** Formal analysis, Methodology, Validation, Writing – review & editing. **Olli H. Laitinen:** Funding acquisition, Methodology, Resources, Writing – review & editing. **Heikki Hyöty:** Methodology, Resources, Validation. **Aki Sinkkonen:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization, Data curation, Funding acquisition, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Aki Sinkkonen reports financial support was provided by The Strategic Research Council of Finland. Olli Laitinen reports financial support was provided by The Strategic Research Council of Finland. Heikki Hyöty reports financial support was provided by Horizon Europe. Aki Sinkkonen reports financial support was provided by Business Finland. Mika Saarenpää reports financial support was provided by Kone Foundation. Mika Saarenpää reports financial support was provided by Finnish Cultural Foundation. Aki Sinkkonen, Noora Nurminen, Olli Laitinen, Heikki Hyoty has patent #EP3551196 issued to EPO. Aki Sinkkonen, Noora Nurminen, Olli Laitinen, Heikki Hyoty has patent #US-11786564-B2 issued to USPTO. Aki Sinkkonen, Noora Nurminen, Olli Laitinen, Heikki Hyoty has patent #US-11318173-B2 issued to USPTO. Aki Sinkkonen, Marja Roslund has patent #EP3589300 issued to EPO.

Aki Sinkkonen, Olli Laitinen and Heikki Hyoty are board members of Uute Scientific Ltd that provides solutions for immune modulation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper].

Data availability

Permission to use personal data can be applied from the ethics committee of the Pirkanmaa Hospital District according to local laws. Sequence data is publicly available.

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Appendix

Table A1
Questionnaire data on additional soil contact and adherence to the trial.

Participant	Treatment	Additional soil contact during trial	Break from gardening during trial
P1	Placebo	No	No
P2	Placebo	No	No
P3	Placebo	No	No
P4	Placebo	No	No
P5	Placebo	No	No
P6	Placebo	Played with kids outside	One weekend trip
P7	Placebo	No	No
P8	Placebo	No	One day during a weekend
P9	Placebo	No	No
P10	Placebo	No	One day during a weekend
P11	Placebo	Installed a flower bed edging	No
P12	Placebo	Planted indoor herbs	A long weekend trip
P13	Placebo	Raked old leaves	No
I1	Intervention	No	No
I2	Intervention	No	No
I3	Intervention	No	No
I4	Intervention	No	No
I5	Intervention	No	No
I6	Intervention	Cut down a yard tree	No
I7	Intervention	Raked old leaves	No
I8	Intervention	No	No
I9	Intervention	Repotted houseplants	No
I10	Intervention	No	No
I11	Intervention	No	No
I12	Intervention	No	One day during a weekend
I13	Intervention	Played with kids outside	One weekend trip
I14	Intervention	No	No
I15	Intervention	No	No

Table A2
Three main skin bacterial hub OTUs in the intervention and placebo group were identified based on their degree (number of edges). The hub OTUs were unique for both groups and time points, and none of them were among the hundred most abundant OTUs (relative abundances varied between 0.0012 % and 0.0392 %).

Treatment	Time point	Hub OTU	Phylum	Class	Order	Family	Genus
Placebo	0 mo	1.	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia
		2.	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter
		3.	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Flavitalea
	1 mo	1.	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Unclassified
		2.	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Luteolibacter
		3.	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Polaromonas
Intervention	0 mo	1.	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Unclassified
		2.	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Filomicrobium
		3.	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Shinella
	1 mo	1.	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter
		2.	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella
		3.	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Uncultured

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