



Review

Interplay between natural environment, human microbiota and immune system: A scoping review of interventions and future perspectives towards allergy prevention



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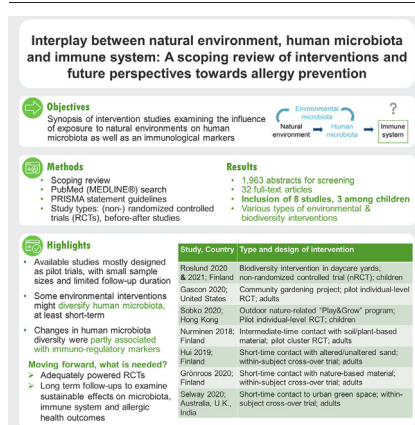
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HIGHLIGHTS

- Only few environmental intervention studies of mainly pilot character were identified.
- Environmental interventions appear to diversify human microbiota.
- Changes in microbiota diversity partly associate with immunoregulatory markers.
- Environmental interventions might be translated into allergy preventive approaches.
- Adequately powered studies with long-term follow up are needed.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Urbanization and biodiversity loss are linked to chronic disorders, in particular allergic diseases. The aim of this scoping review was to provide a synopsis of intervention studies specifically examining the influence of exposure to natural environments on human microbiota as well as immunological markers as suggested interlink between natural environment and the development of allergic diseases.

Methods: We searched PubMed (MEDLINE®) and all references cited in the included studies following the PRISMA statement guidelines. No restrictions regarding age and sex of study participants, language or publication date were made. The protocol was registered at OSF REGISTRIES (<https://osf.io/musgr>).

Abbreviations: ASV, Amplicon sequencing variant; EAACI, European Academy of Allergy and Clinical Immunology; HTS, High-throughput sequencing; IL-10, Interleukin-10; IL-17A, Interleukin-17A; KEGG, Kyoto Encyclopedia of Genes and Genomes; NGS, Next-generation sequencing; OTU, Operational taxonomic unit; PBMCs, Peripheral blood mononuclear cells; qPCR, Quantitative polymerase chain reaction; RCT, Randomized controlled trial; sASV, Species-level amplicon sequencing variant; sp., species; spp, species (plural); TGF-β1, Transforming growth factor β1; Treg, regulatory T cells.

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Results: After screening, eight intervention studies were included. The interventions reported were mainly of pilot character and various, ranging from nature-related educational programs, biodiversity interventions in day-cares to short-term contact with soil- and sand-preparations. Most of the studied interventions appeared to increase human microbiota richness and diversity in specific taxa groups in the short-time. Immunological markers were assessed in only two studies. In these, their associations with human microbiota richness were pre-dominantly reported. Conclusion: There is some evidence that the so-called biodiversity interventions have the potential to diversify human microbiota, at least over a short period. Adequately powered randomized controlled trials with long term follow-up are required to examine sustainable effects on microbiota and immune system.

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1. Introduction

The pre- and postnatal periods are particularly important for immunological training and development of regulatory mechanisms that prevent insufficiently controlled inflammatory responses and hyper-responsiveness associated with asthma and allergic diseases in humans (Kirjavainen et al., 2019a). It is well established that microbial exposures, including those from the developing resident microbiota on human body surfaces, can influence this process (Kirjavainen et al., 2019a; Renz and Skevaki, 2020). The notion that changes of early-life microbial exposures, human microbiota development in modern living environments and the increase in allergies are interrelated has been evolving since the introduction of the hygiene hypothesis three decades ago (Strachan, 1989; Kirjavainen and Gibson, 1999; Liu, 2015; Reynolds and Finlay, 2017; Gordon, 2011). More recently, the so-called biodiversity hypothesis emphasizes the role of reduced environmental biodiversity as the primary cause for the impoverished microbial exposures, consequent lack of promotion of homeostatic immunological development and ultimately the allergy epidemic (Haahela et al., 2021; Haahela, 2019; Hertzen et al., 2011; Mutius and Smits, 2020).

Based on observational studies, natural or native environments with enriched and specific microbial exposures like traditional farms (Casas et al., 2016; Mutius and Vercelli, 2010; Müller-Rompa et al., 2018; Stein

et al., 2016; Ober et al., 2017), and less consistently the extent of residential greenness (Lambert et al., 2017; Dadvand et al., 2019; Ruokolainen et al., 2015; Ferrante et al., 2020) may protect from allergies and asthma. While some of these prospective observational studies imply and experimental studies (Stein et al., 2016; Debarry et al., 2007; Schuijs et al., 2015) support a causal relationship between microbial exposures and the beneficial health effects associated with these environments, actual evidence of causality of the suggested biological mechanism remains to be shown in randomized interventions studies. A recent systematic review on nature exposure in relation to immune system functioning encompassing human, animal and in vitro studies observed some evidence of positive effects of nature exposure on immune system responses such as anti-inflammatory cell activity (Andersen et al., 2021). As compared to our research hypothesis, the authors suggested a direct immunoregulation through inhalation of airborne, volatile substances such as biogenic volatile organic compounds (BVOCs).

The role of human microbiota in health and disease is undisputed, and subsequently, human microbiota are a concrete and promising target for therapeutic approaches (El-Sayed et al., 2021a; El-Sayed et al., 2021b; El-Sayed et al., 2021c). It is also well established that the developing human commensal microbiota affects the maturation of immunological regulation, as best shown for intestinal microbiota (Renz and Skevaki, 2020; Su et al., 2021; Zimmermann et al., 2019). However, the claim that the protective

effects of health beneficial natural environments on immune response is mediated via an influence on the commensal human microbiota, is highly hypothetical at this point, despite some indication from observational studies (Kirjavainen et al., 2019b; Depner et al., 2020; Depner et al., 2017). At present, evidence is scarce on how the natural environment interacts with the human microbiome and by doing so possibly affects the (developing) immune response (Nielsen et al., 2020). A clear overview of the findings from published studies on this topic is necessary to identify knowledge gaps, plan future research efforts and develop health-promoting interventions. Therefore, the primary aim of this scoping review was to identify children and adult intervention trials on exposure to natural environments or isolated aspects thereof, such as yard vegetation or natural soil preparations, and describe their methods. Our second aim was to compare the effects of the environmental interventions in relation to the commensal microbiota and immunomodulation as one possible interlink between exposure and allergic immune response.

2. Results

Our search yielded 1963 references, of these, four were obtained by hand search, but not included in the synthesis as these studies did not meet the inclusion criteria (Nielsen et al., 2020; Chatenoud et al., 2020; Hakonen et al., 2018; Lehtimäki et al., 2018a). After title and abstract screening, 32 full text articles were assessed for eligibility, leading to the inclusion of eight studies. Details of the selection process

including reasons for exclusion are displayed in Fig. 1 (Moher et al., 2015).

2.1. Key characteristics of included studies

Of the eight included studies investigating the effect of exposure to various (aspects of) natural environments on human microbiota diversity and composition (Gascon et al., 2020; Grönroos et al., 2019; Hui et al., 2019; Nurminen et al., 2018; Roslund et al., 2020; Selway et al., 2020; Sobko et al., 2020), two studies additionally evaluated immunological effects (Nurminen et al., 2018; Roslund et al., 2020). Five studies were conducted in Finland (Grönroos et al., 2019; Hui et al., 2019; Nurminen et al., 2018; Roslund et al., 2020; Roslund et al., 2021), one study each in the U.S. (Gascon et al., 2020), Hong Kong (Sobko et al., 2020) and one multi-centre study in Australia, the U.K. and India (Selway et al., 2020) (Table 1). The size of the study populations ranged from two to 89 participants, with three investigations in preschool children and five among adult populations. All studies were conducted in an urban environmental setting or among urban residents. Two of the studies were designed as pilot RCTs (Gascon et al., 2020; Sobko et al., 2020), two as nRCT (cluster-non-randomized (Roslund et al., 2020; Roslund et al., 2021)), and one as pilot nRCT (individual-level between-subject (Nurminen et al., 2018)). Three studies can be considered as before-after-studies with pre- versus post-intervention comparisons within the same individuals (Grönroos et al., 2019; Hui et al., 2019; Selway et al., 2020).

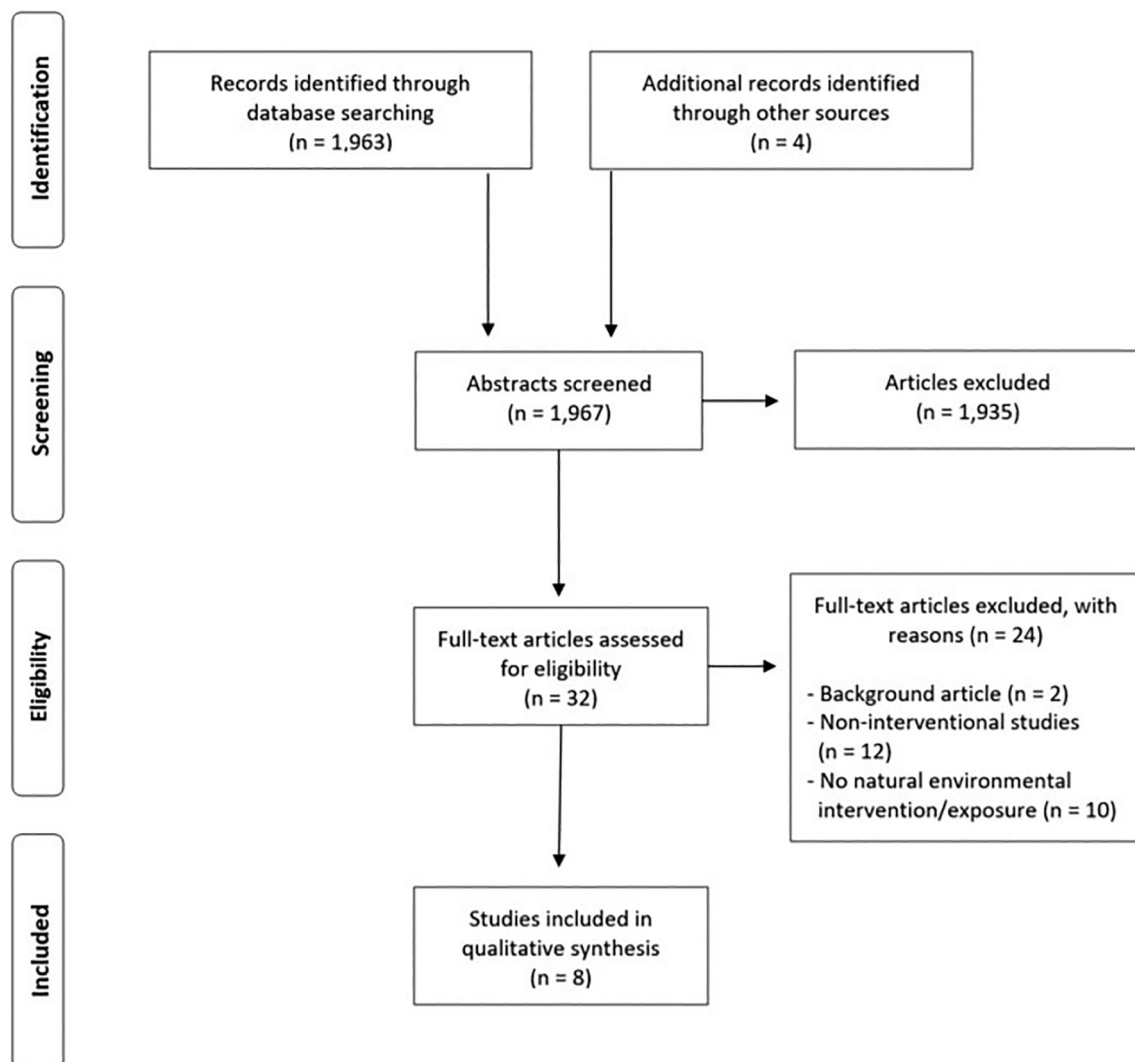


Fig. 1. PRISMA flow chart for literature search.

Table 1

Description of the study design along with the intervention/exposure concepts and measurements of the included studies.

Author (year and country)	Study design	Description of intervention/exposure	Pre-/Post-intervention measurements
Roslund et al. (2020, Finland), Lahti, Tampere	N = 75, children in day-cares (3-5y)	Biodiversity intervention in day-care yards	ENVIRONMENTAL microbiota in surface soil of day-cares
	Intervention - cases Modified day-cares N = 4, N = 36 children	Standard day-care with intervention were equipped with biodiversity material and elements (forest floor, sods, planters and peat blocks for climbing and digging) in day-care yards	HUMAN microbiota: skin swabs (back of the hand), faecal samples
	Controls - A Standard day-cares N = 3, N = 16 children	Duration intervention: 28 days	IMMUNE RESPONSE: blood samples (IL-17A, IL-10, TGF- β 1, CD4 + CD25 + CD127lowFOXP3 + Treg cells)
	Controls - B Nature-oriented day-cares N = 3, N = 23 children	Control groups: 1. Standard day-cares without intervention 2. Nature-oriented day-cares	SAMPLING TIME POINTS: - once at baseline - day 28 (immediately at the end of intervention)
Roslund et al. (2021, Finland), Lahti, Tampere, Espoo - follow-up of Roslund et al., 2020, but enriched with additional daycares and children	N = 89, children in day-cares (3-5y)	Biodiversity intervention in day-care yards	ENVIRONMENTAL microbiota in surface soil of day-cares
	Intervention – cases Modified day-cares N = 6, N = 61 children	The initial duration of the intervention with guided/encouraged contact with the biodiverse daycare materials was 28 days (see above). After that period, the material remained in the daycare yards, and children continued having contact with those materials, however, without guidance or specific encouragement.	HUMAN microbiota: skin swabs (back of the hand), faecal samples, salivary samples
	Controls – A Standard day-cares N = 7, N = 28 children		SAMPLING TIME POINTS: - once at baseline (32/89/74 individuals for saliva/skin/faecal sampling) - day 28 (during intervention) - 1 year follow-up (during intervention) 12/42/23 individuals for saliva/skin/faecal sampling) - 2 years follow-up (at the end of intervention) (7/8/3 individuals for saliva/skin/faecal sampling; no controls)
Nurminen et al. (2018, Finland)	Pilot Cluster-RCT (group-level between-clusters)	Intermediate-time contact with soil/plant-based material	HUMAN microbiota: skin swabs (volar surface of the forearm), faecal samples, blood samples
	N = 14 healthy adult volunteers (27-63y)	Exposure by rubbing hands with a soil- and plant-based immunomodulatory composition	IMMUNE response: Change of immune system markers in PBMCs cytokine mRNA expression: TGF- β , IL-10
	N = 7 cases, N = 7 controls	Duration intervention: 14 days, 3-times a day Control group: no exposure	Sampling time points - microbiota: - once at baseline - day 14 (immediately after exposure) - day 35 (only microbiota)
Gascon et al. (2020, U.S.)	Pilot individual-level RCT (between-subject)	Community gardening project	HUMAN microbiota (faeces, mouth, hand, forehead)
	N = 16, adults $\geq$ 18 years	Community garden project close to the home environment including receiving a garden plot, vegetable seeds and transplants to support food production and a gardening class	SAMPLING TIME POINTS: - once at baseline - every three weeks - last: immediately after intervention
	8 cases, 8 controls [Full info on 5 cases, 6 controls]	Duration intervention: 20 weeks, summer	
Sobko et al. (2020, Hong Kong)	Pilot individual-level RCT (between-subject)	Control group: on 1 year waiting list and no gardening activities Outdoor nature-related “Play&Grow” program on intestinal microbiome	HUMAN microbiota: faecal samples
	N = 27 cases N = 18 controls	Early environmental education programme for families, e.g. guided nature activities and “hands-on” experiences including related homework and a package of healthy lifestyle recommendations published by the Hong Kong government	SAMPLING TIME POINTS: - once at baseline - week 10 (immediately after intervention)
		Duration: One intervention ‘event’ per week for 10 weeks	
Grönroos et al. (2020, Finland)	Within-subject individual-level (randomisation unclear) cross-over trial	Control group: no program/activities except the package of healthy lifestyle recommendations Short-time contact with microbiologically diverse nature-based material	HUMAN microbiota: skin
	N = 2, healthy adults, multiple tests performed on the same individual	1st experiment: “Direct hand-exposure” testing of 16 different soil and plant-based materials	SAMPLING TIME POINTS: once before and once after (within minutes of intervention)
	2 experimental set-ups, multiple materials tested	2nd experiment: “Exposure via fabric packets” Three different types of fabric packets filled with dried, crushed, and sieved Sphagnum moss placed on the inner forearm for 3 h Short-time contact of altered and unaltered sand material	
Hui et al. (2019, Finland)	Within-subject individual-level (randomisation: fingers and persons were randomized) cross-over trial/experiment	Each N = 5 participants performing 3 experiments:	HUMAN microbiota: skin (finger)
	N = 5, healthy adults (30-50y), multiple tests performed on the same individual	1st experiment: sieved sand (1. unaltered and 2. enriched with freeze-dried organic material)	SAMPLING TIME POINTS: once before and once after (within minutes of intervention)

Table 1 (continued)

Author (year and country)	Study design	Description of intervention/exposure	Pre-/Post-intervention measurements
Selway et al. (2020, AUS, U.K., India)	Within-subject individual-level (randomisation unclear) cross-over trial/experiment	2nd experiment: safety sand used in children's playground (1. unaltered and 2. enriched with freeze-dried organic material)	HUMAN microbiota: skin, nasal swabs
		3rd experiment: sandbox sand (1. unaltered and 2. enriched with freeze-dried organic material)	
		Short-time but high intensity exposure to different urban green exposure	
		Five different types of urban green space were sampled three times in three different cities ($N = 15$ locations in Adelaide (~1.5 h exposure), Bournemouth and New Delhi, both 15 min exposure)	
	N = 3, adults, multiple tests among those 3 adults, carried out in 3 countries and in different urban green space environments	Samples (exposure) within each type of green space: 1. Air 2. Soil 3. Leaves	SAMPLING TIME POINTS: before (morning) and shortly after the green space contact
		Sampling and direct interaction with soil and leaves in the urban green spaces was the actual exposure scenario (mimicking children interacting with the environment)	

2.2. Description of the environmental exposure interventions

The included studies encompassed a broad range of different intervention concepts along with various sampling approaches and measurement time points. A brief description of the intervention trials including their design, population, sampling and other key characteristics is provided below and summarized in Table 1.

2.2.1. (Pilot) Non-randomized controlled trials

A Finnish non-randomized cluster trial examined a 28 days long biodiversity intervention in urban day-care centres with originally no or only little green spaces among 75 preschool children (three-five years) in Lahti and Tampere (Roslund et al., 2020). Within the intervention group ($n = 36$ children), four standard day-care yards were equipped with segments of forest floor, sod, planters for growing annuals and peat blocks for climbing and digging. In order to intensify contact with these materials, nurses of the day-care centres offered guided outdoor activities and encouraged children to engage with the material brought into the yard. The remaining standard day-care centre/yards (three day-cares, $n = 16$ children) served as controls with no nature guidance activities and no intervention materials in the yard. Further, children ($n = 23$) from three nature-oriented day-care centres served as “positive” controls with a suggested ideal microbial exposure already at baseline. Skin swab and blood samples were taken before and immediately after intervention (no specific point in time stated) by a trained study nurse, faecal samples were collected by the parents. Children with an age below three or over five years at the beginning of the study, the native country being other than Finland, immune deficiency, immunosuppressive medication, a condition affecting immune response, or cancer diagnosis, were excluded from the study. The use of antibiotics, probiotics, medication, and other background information was recorded using standardized questionnaires.

The results of a one year and two-year follow-up of this initial trial were presented in another, recent publication (Roslund et al., 2021). The results of the subsequent study are partly based on the first study, however, encompassing also children from a third city in Southern Finland (Espoo). In total, the 89 children from 13 day-care centres ($n =$ seven standard day-care yards and $n =$ six modified day-care yards) were followed-up until two years after the intervention took place. The study population was heavily reduced, however, at sampling time-point one year (12–42 individuals depending on the sample type) and especially after two years (three to eight individuals depending on the sampling type). The initial duration of the intervention with guided/encouraged contact with the biodiverse day-care yard materials was 28 days, as described above. After that period the material remained in the day-care yards and children continued having contact

with those materials, i.e. the intervention assumingly continued to take place, even without particular guidance and documentation.

A Finnish pilot (non-randomized cluster) trial, described the effects of short-time (two-weeks) contact to soil and plant-based materials on human microbiota and immunological markers (Nurminen et al., 2018). This study included 14 healthy volunteers aged 27–63 years (seven treated, seven non-treated) within an urban setting. The exposure materials were composed of various nature-derived composted Finnish soil and plant materials. Within the intervention group, participants were rubbing their hands with the soil and plant-based materials for 20 s twice a day for a two-week exposure period. After the exposure, study subjects washed their hands with tap water for five seconds, followed by drying with a towel. The controls were matched by similarities regarding sex, age, pet ownership and dwelling type. There was no specific exposure planned for the control group. All participants collected faecal and skin swab samples before (day 0), immediately after (day 14) and three weeks after the intervention period (day 35). Blood samples were collected by a study nurse at day 0 and day 14. Exclusion criteria were as follows: immune deficiencies, immunosuppressive medications, three or more infections within a year that required hospitalization, a condition affecting the immune response, memory disorder diagnosed by a medical doctor, acute depression or acute or earlier psychosis, cancer diagnosis within the last two years or on-going cancer treatment, type 1 or type 2 diabetes, rash or ulcers in hands, living outside city area, age under 18 or over 65, “incompetency” and daily smoking.

2.2.2. Pilot randomized controlled trials

A pilot randomized controlled trial (RCT) with 16 adults (eight each in intervention and control group) evaluated the influence of community gardening in the Denver metropolitan region (U.S.) on human microbiota composition over a 20-week study period (Gascon et al., 2020). The study participants had to live within short distance to the study garden. The participants of the intervention group were provided a community garden plot, vegetable seeds and transplants to support food production in addition to a gardening class and access to events associated with the community gardening project. Participants of the control group were assigned to the waiting list for the community gardening project and they further agreed to not perform any gardening activities during the study period. Human microbiota sample collection (faeces, oral swabs, skin swabs from hand and forehead) was performed every three weeks at six time points before, during and after intervention (latest: no specific point in time stated). Exclusion criteria were not reported for this study and 11 of the included study participants reported having chronic diseases.

A pilot RCT among 54 healthy preschool children in Hong Kong investigated the contact with nature and its impact on gut microbiota and

psychosocial behaviour in a 10-week intervention (Sobko et al., 2020). Families of two- to five-year old healthy children were recruited to participate in a “Play & Grow” programme where nature connection was enhanced by outdoor activities in public parks once a week for 10 consecutive weeks within Hong Kong. The families (respective children) were randomly assigned to the intervention ($n = 27$) and control group ($n = 18$). The intervention group was offered a weekly guided nature activity where the children had skin contact with materials found in nature in addition to related weekly homework activities such as growing plants. No activities were assigned for the control group. Caregivers of the intervention and control group completed questionnaires before and after the intervention trial. In addition, faecal samples of the children were collected prior and after the intervention (no specific point in time stated). Caregivers in the intervention and control group was recommended a package of healthy lifestyle recommendations published by the Hong Kong government. Children from non-local families, children who were on antibiotic therapy (two months prior to the start of the trial), and children with chronic diseases were excluded from the study.

2.2.3. Before-after studies

The influence of short-time contact with soil and moss on skin microbiota was examined in a Finnish pilot before-after study among two adult (no age specified) volunteers in two experiments (Grönroos et al., 2019). In the first study, two volunteers rubbed their hands with sixteen different soil, composted and plant-based materials for 20 s, with a maximum of two materials being tested on the same individual on the same day. After every exposure, hands were cleaned with soap in tap water and dried with a paper towel. Skin swab samples of the right hand were taken before exposure and immediately after washing and drying hands. In the second experiment, two volunteers were exposed to fabric packets filled with dried, crushed, and sieved Sphagnum moss, commonly known as peat moss (exposure not further specified; Sphagnum represents a genus of several hundred different moss species). These bags were placed on the inner forearms for three hours and 45 min. Skin swab samples were taken right before placing the packets and immediately after exposure period. Exclusion criteria were not reported for this study.

A Finnish (cross-over) before-after study evaluated the effect of short-time contact with microbe-enriched sand material on skin bacterial diversity (Hui et al., 2019). In this study among five healthy adults aged 30–50 years, three different sand materials, commonly used in playgrounds and other public spaces were divided into two parts: altered and unaltered. The altered sand materials were enriched with a microbial inoculant from forest and agricultural materials that resembles microbiota in organic soils, whereas the unaltered soils remained without further treatment. Skin swab samples from two fingers of each hand were collected by an assistant after cleaning hands with soap and tap water and drying, before testing the enriched and unaltered sand materials. After that, the participants rubbed one finger in the unenriched and the other finger in the same material enriched with the inoculant. The post-intervention set of skin swab samples from both fingers were collected five minutes after washing and drying the hands. All participants tested all materials in a random order but there was only one testing a day and a one-day break between the tests. Exclusion criteria were not reported for this study.

Lastly, a cross-over multi-centre study conducted in Australia, India and the U.K. evaluated the effect of contact with different types of exposures related to urban green space in relation to bacterial diversity on skin and in airways (Selway et al., 2020). Five different types of local urban green space (vacant lots, lawns, parklands, revegetated open woodlands/invaded shrub lands and remnant open woodlands/ savannah woodlands) were sampled three times in each city ($n = 15$ locations in Adelaide, Bourne-mouth and New Delhi). Three healthy adults (no age specified) sampled three types of potential exposures - air, leaves, soil - within each type of urban green space. Direct interaction with soil and leaves in addition to breathing the air in the urban green spaces was done to imitate exposure of children interacting with the environment. Skin swab samples and

nasal swab samples were taken before and immediately after exposure. Exclusion criteria were not reported for this study.

2.3. Environmental and human microbiota measures in the included studies

Environmental and human microbiota outcome measures were done from various sample types, including faeces, oral/nasal swabs, skin swabs, soil/sands, plant-based materials and air. All of these measurements aimed at characterizing the bacterial microbiota using 16S rRNA amplicon sequencing approach. While “microbiome” and “microbiota” were often used interchangeably in the examined studies, it was observations of bacterial communities, or microbiota that were reported in all cases. Thus, we use the more correct term microbiota in this scoping review. “Microbiota” refers to the assembly of microbes in a sample/environment, whereas “microbiome” includes also the “theatre of activity” of the microbiota, that is including their internal and external structural elements and metabolites (Berg et al., 2020). Amplicon sequencing approaches do neither target entire genomes nor structural elements and metabolites, but specific gene sequences – here the bacterial 16S rRNA genes – that allow the determination of the microbial composition, i.e. microbiota in a sample (Cox et al., 2013).

Three main groups of outcomes were reported from the bacterial microbiota analyses: i) outcomes related to α -diversity within samples, including observed and estimated (Chao1 estimator) taxa richness, i.e. number of different taxa, and various diversity indices that take into account not only the number of different taxa in a sample, but also their relative abundance distribution and in some cases their phylogenetic relatedness (e.g. Shannon diversity index, Simpson diversity index, Faith's phylogenetic diversity). Further, ii) outcomes related to β -diversity, that is between sample microbiota composition differences, largely presenting similarity/dissimilarity between groups of samples (for example pre versus post intervention) with respect to microbiota composition. And lastly iii) differences in the relative abundance of specific taxa on different taxonomic levels between groups of samples, from ASVs (amplicon sequencing variants) and OTUs (operational taxonomic units) as the smallest units of categorization, to species (e.g. *Staphylococcus aureus*) and genera (e.g. *Staphylococcus*) and to broader and more general taxonomic groups, like phyla (e.g. Firmicutes). In addition, two studies reported results from KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis, which typically uses PICRUST (phylogenetic investigation of communities by reconstruction of unobserved states) software (Langille et al., 2013). In essence, this is a computational approach that attempts to predict functionality within a microbial community based on 16S marker gene data and a database of reference genomes, i.e. based the presence of specific microbes in a sample without actual measurement of the respective genetic functions within the community. One major assumption using such an approach is that the detected microbes in a community are alive and metabolically active, which may to some extent or largely be true for human microbiota. This may, however, very well not be the case for environmental microbes deposited on a human surface that is being sampled, for instance for soil microbes remaining on skin or microbes from the air being deposited in the nasal cavity.

2.4. Interventions and human microbiota

The main results in relation to human microbiota and immune response markers before and after the intervention/exposure are displayed in Table 2a–2c.

The non-randomized controlled trial among 75 three to five year old children in Finnish day-care centres investigated the effect of enhanced biodiversity exposure by equipping standard day-care yards with material such as forest floor, sods, and planters on human microbiota (faecal samples and skin swabs) over a period of 28 days (Roslund et al., 2020). At baseline, α -diversity within Alphaproteobacteria determined from skin swabs was significantly higher in children from nature-oriented day-cares (positive controls) as compared to the standard day-cares before interventions. When comparing bacterial diversity in skin samples pre versus post

Table 2a

Outcome measurements and summary of results of the included (pilot) non-randomized controlled trials.

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
Roslund et al. (2020; Finland)	Biodiversity intervention in day-care yards (28 days)	ENVIRONMENTAL microbiota (surface soils) α-diversity Differences in diversity of bacterial microbiota on various taxa levels between standard and intervention day-cares, and in before- versus after intervention day-cares β-diversity Differences in community composition of Gammaproteobacteria before versus after intervention and between intervention yards vs. standard yards SKIN microbiota <i>Before versus after intervention</i> α-diversity Alphaproteobacteria diversity increased in intervention day-cares but not in standard day-cares β-diversity Not reported. <i>Between day-care type comparisons</i> α-diversity Higher diversity in a-Proteobacteria in children from nature-based compared to intervention daycares (before intervention) β-diversity Difference in bacterial community composition between nature-oriented and standard day-cares (before intervention) <i>After intervention comparison</i> α-diversity Children in intervention day-cares had more diverse skin Proteobacteria and Gammaproteobacteria communities compared to standard day-cares β-diversity Not reported. GUT microbiota <i>Before versus after intervention</i> α-diversity Intervention day-cares: increase in diversity of <i>Ruminococcaceae</i> β-diversity Not reported. Taxa Intervention day-cares: decrease in relative abundance of Clostridiales <i>Between day-care type comparisons</i> α-diversity Not reported. β-diversity Differences between standard and nature-oriented daycare children in <i>Ruminococcaceae</i> community composition before intervention,

Table 2a (continued)

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
Roslund et al. (2021), Lahti, Tampere, Espoo	Biodiversity intervention in day-care yards – ongoing (28 days guided intervention, continuous non-guided and documented intervention thereafter)	and in <i>Faecalibacterium</i> communities after the intervention. Similar communities of <i>Ruminococcaceae</i> at the beginning of the intervention but slightly different post intervention between standard day-care and intervention day-care children IMMUNOLOGICAL response SKIN microbiota IL-10:IL-17A ratio significantly increased among intervention children, but not in standard or nature daycares before and after intervention period All children: an increase in skin Gammaproteobacterial diversity was associated with a significant increase in TGF-β1 plasma concentration Intervention day-care children: an increase in skin Gammaproteobacterial diversity was associated with a significant decrease in IL-17A concentration and increase in the percentage of T-reg cell Nature-oriented day-care children: High Gammaproteobacterial diversity in skin microbiomes was associated with an increase in IL-10 plasma concentration Standard day-care children: low Gammaproteobacterial diversity in skin was associated with a decrease in TGF-β1 expression ENVIRONMENTAL microbiota α-diversity Differences in soil bacterial microbiota diversity (Shannon index) for Gammaproteobacteria, Sphingobacteriia and Deinococci between intervention and standard-daycares at year-one. β-diversity Differences in community composition of the main phyla and classes between before and day 28, before and year 1 and day 28 vs. year 1 follow-up. Bacterial communities in the intervention yards shifted towards those in intervention materials (before vs. day 28 and before vs. year 1) Taxa Shifts in relative abundance of 60 soil bacterial genera between day 0 and day-28, and of 34 and 17 bacterial genera after year-one and year-two compared to day 0. Differences in some of these shifts between intervention and standard-daycares were reported as well.follow-up. Bacterial communities in the intervention yards shifted towards those in intervention materials (before vs. day 28 and before vs. year 1) SALIVA microbiota

Table 2a (continued)

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
		<i>Before versus at the end of the intervention</i> α-diversity Higher Proteobacteria richness in children from standard day-cares as compared to children in intervention day-cares at year 1 follow-up versus baseline Intervention day-cares: Richness of phylum Proteobacteria was higher at day 28 as compared to year 1 follow-up. β-diversity Significant different community composition between intervention and standard day-cares at OTU and class levels (in particular Gammaproteobacteria, Negativicutes, and Fusibacteriia) at year 1 follow-up versus baseline Taxa Relative abundance of Lactobacillales increased, while Fusobacteriia decreased during year one in the intervention daycare population; differences in salivary microbiota reported by gender. SKIN microbiota <i>Before versus at the end of intervention</i> α-diversity Richness of Alpha-, Beta-, and Gammaproteobacteria was higher among children in intervention day-cares (all children together) as compared to standard day-cares in year 1 follow-up, but not before and not at day 28 versus baseline: Total bacterial α-diversity decreased during year one in both intervention and standard-daycares. β-diversity Intervention day-care children (starting 2017): shifts in total bacteria, Gammaproteobacteria and Bacilli community composition during the 28-day intervention (not visible in children from standard day-cares) versus baseline Intervention day-care children (starting 2016): shifts within phyla Proteobacteria, Actinobacteria, Bacteroidetes, and Acidobacteria before versus baseline (no shifts observed among children from standard day-cares) <i>Between day-care type comparisons</i> α-diversity Intervention day-care children (starting 2016): evenness based on the Shannon diversity index decreased less compared with those in the standard day-cares during the first year. Otherwise,

Table 2a (continued)

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
		no differences and overall bacterial α-diversity decreased during year-one in both day-care types. β-diversity Differences in the bacterial community composition at all taxonomic levels between children in the intervention versus standard day-cares at year 1 follow-up. Taxa Relative abundance of four taxa groups harboring potentially opportunistic pathogens (genera Gemella, Streptococcus, Veillonella. and class Bacilli) became lower among children from intervention day-cares as compared to standard day-cares at year 1 follow-up. Total relative abundance of genera containing potential human pathogens was lower among intervention children. Gender was a significant factor affecting taxonomic composition of Clostridiales. GUT microbiota <i>Between day-care type comparisons</i> α-diversity Not reported β-diversity No differences in the community composition of gut bacteria at day 28, but some differences at baseline and year 1 follow-up Taxa Intervention day-cares: decrease in relative abundance of <i>Clostridium sensu strictu</i> at day 28 and at year 1 follow-up Lower levels of <i>Romboutsia</i> at day 28 and at year 1 follow-up samples among children from intervention day-cares SKIN microbiota α-diversity no significant changes in diversity in before versus after exposure period GUT microbiota α-diversity Significant increase of bacterial diversity in faecal samples of intervention group in before vs. immediate after exposure period, in particular for the phylum Bacteroides (day 14). No change in before- versus three weeks after exposure (day 35) IMMUNE response TGF-β The level of TGF-β expression was significantly associated with the change of bacterial diversity in both the skin and faeces right after exposure period (day 14) IL-10 No significant association
Nurminen et al. (2018, Finland)	Intermediate-time contact with soil/plant-based material	
	Duration intervention: 3-times a day, 2 weeks	

Table 2b

Outcome measurements and summary of results of the included pilot randomized controlled trials.

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
Gascon et al. (2020, U.S.)	Community gardening project*	HUMAN microbiota (faeces, mouth, hand, forehead)
	Duration intervention: 20 weeks, summer	α-diversity No significant difference between intervention and control groups over time β-diversity No significant differences in the bacterial composition and community structure in samples between and within participants and between interventional groups over time GUT microbiota
Sobko et al. (2020, Hong Kong)	Outdoor nature-related "Play&Grow" program on intestinal microbiome	α-diversity No significant differences in the bacterial diversity between two time points before versus after intervention) in the intervention and control groups. Richness within Proteobacteria increased and within Bacteroidetes decreased significantly before versus after intervention in the intervention group
	Duration: One intervention per week for 10 weeks	β-diversity Difference in the bacterial composition of faecal samples in intervention and control groups before versus after exposure
		Taxa Increase abundance in <i>Roseburia</i> in the control but not intervention group before versus after intervention; increased abundance in <i>Blautia</i> in the intervention group
		Predicted microbiota functions (KEGG) More metabolic function pathways were modified in the intervention group compared to the control group

intervention, Alphaproteobacteria diversity but not richness was significantly increased for children in intervention day-cares, which was not case for control children in non-intervention, standard day-cares. After the biodiversity exposure period, children in intervention day-cares had more diverse skin Proteobacteria and Gammaproteobacteria communities as compared to control standard day-cares that did not undergo intervention. There were no significant findings reported for changes in β -diversity of skin microbiota. With respect to *faecal samples*, a significant increase in diversity but not richness of Ruminococcaceae was observed among children in intervention day-cares before versus after the intervention, while no results have been reported for the other day-care types. Statistically significant differences in the bacterial community composition (β -diversity) within specific taxonomic groups, here Ruminococcaceae and Faecalibacterium, have been observed between standard and nature-oriented day-care children. In the post- versus pre-intervention comparison, a significant decrease in the relative abundance of Clostridiales sequences was observed among children in intervention day-cares.

The follow-up study by Roslund and colleagues (Roslund et al., 2021) among initially 89 children (year one: 12–42 individuals depending on the sample type; year two: three to eight individuals) confirmed differences in day-care yard soil bacterial microbiota diversity (Shannon index of specific taxa classes), community and taxa composition also after one year versus baseline and further between standard and intervention day-cares at year one follow-up. The authors confirmed that soil microbiota composition was shifting towards the intervention material in the intervention daycares, predominantly in the first year of the follow-up. The *skin bacterial community* composition differed between intervention and standard-daycare children at all taxonomic levels in the year-one samples. Shifts in

Table 2c

Outcome measurements and summary of results of the included before-after studies.

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
Grönroos et al. (2020, Finland)	Short-time contact with microbiologically diverse nature-based material	SKIN microbiota
	1st experiment: "Direct hand-exposure"	1st experiment: α -diversity
	2nd experiment: "Exposure via fabric packets"	Shannon diversity index and taxa richness were significantly higher in skin swabs after exposure at all tested taxonomic levels tested, and within all phyla, except for the phylum Firmicutes
		β -diversity
		Bacterial community composition on skin (fingers) was significantly different after exposure, at all tested taxonomic levels and in all tested taxonomic groups
		Variability in bacterial composition significantly higher after compared to before exposure
		Taxa
		Relative abundance of Acidobacteria and Bacteroidetes, as well as other less abundant phyla in skin swabs increased (no info on statistical testing)
		Total bacterial abundance was significantly higher after exposure
		2nd experiment: α -diversity
		Bacterial richness and Shannon diversity index were higher on forearm skin in samples taken after the use of packets containing the <i>Sphagnum moss</i> except for packages using the fabric ST047DIA_2 (no info on statistical testing)
		β -diversity
		Bacterial community composition on skin after exposure was different from the samples taken before the experiment and more similar to the composition of the <i>Sphagnum moss</i> (no info on statistical testing)
Hui et al. (2019, Finland)	Short-time contact of altered and unaltered sand material	SKIN microbiota
	1st experiment: sieved sand (1. unaltered and 2. enriched with freeze-dried organic material)	α -diversity
	2nd experiment: safety sand used in children's playground (1. unaltered and 2. enriched with freeze-dried organic material)	Bacterial OTU richness, Simpson and Shannon indices were significantly higher on skin swabs after exposure to the inoculant-enriched sand compared to untreated sand
	3rd experiment: sandbox sand (1. unaltered and 2. enriched with freeze-dried organic material)	β -diversity
		The before skin swab samples, samples after exposure to non-enriched sands, and samples after exposure to microbial inoculant-enriched sands, had distinct skin bacterial communities
		Taxa
		Generally, relative abundances of Acidobacteria, Actinobacteria, and Proteobacteria were lower and the relative abundance of Firmicutes was higher in the skin swab samples after exposure to inoculant-enriched compared to the non-enriched sand samples.

Table 2c (continued)

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
Selway et al. (2020, AUS, U.K., India)	Short-time but high intensity exposure to different urban green exposure	Relative abundance of seven out of nine most dominant genera was higher after touching non-modified sands compared to enriched sands and it was higher for the remaining two most dominant genera after touching inoculant-enriched sand.
		Potentially pathogenic taxa Significant drop in relative abundance of genera possibly containing pathogenic taxa was greater after touching inoculated sands (40–50% rel. Abundance before versus 4% after) compared to non-inoculated sands (40–50% before versus 14% after) compared to before exposure.
		KEGG analysis Genes associated with <i>Staphylococcus</i> were associated with touching inoculant-enriched sand. No other associations with potential bacterial soil pathogens were found SKIN microbiota
	Samples (exposure) within each type of green space: 1. Air 2. Soil 3. Leaves	1. ADELAIDE (AUSTRALIA) α-diversity Species richness and phylogenetic diversity (Faith's PD) in skin: Significant increase after urban green space exposure β-diversity Bacterial composition significantly shifted after urban green space exposure and became more similar to the source (soil, leaves, air) after exposure. Skin samples post exposure more similar to soil Taxa Increases in diversity and shifts in microbial abundances are linked to an increase in rare taxa and a decrease in common, human-associated taxa after green space exposure in Adelaide (no statistical testing) Excluding ASVs (amplicon sequence variants) present in both before versus after exposure skin samples (18% of all ASVs) showed that after exposure skin samples shared 23%, 30% and 40% of ASVs with air, leaves and soil samples, respectively 2. BOURNEMOUTH (U.K.) a-diversity Species richness and diversity (Faith's PD) significantly increased after urban green space exposure in skin samples β-diversity Significant shifts in bacterial composition of samples after urban green space exposure Taxa After urban green space exposure, decrease in abundance of <i>Staphylococcus</i> , <i>Corynebacterium</i> , <i>Finegoldia</i> , <i>Lawsonella</i> ,

Table 2c (continued)

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
		<i>Acinetobacter</i> <i>Micrococcus</i> , <i>Moraxella</i> and rare taxa increase in abundance after exposure
		3. NEW DELHI (INDIA) a-diversity Species richness and diversity (Faith's PD) significantly increased after urban green space exposure β-diversity Significant shifts in bacterial composition Taxa After green space exposure, decrease in abundance of <i>Staphylococcus</i> , <i>Corynebacterium</i> , <i>Finegoldia</i> , <i>Lawsonella</i> , <i>Acinetobacter</i> <i>Bacillus</i> and rare taxa increase in abundance after exposure NASAL microbiota
		1. ADELAIDE (AUSTRALIA) α-diversity Species richness and phylogenetic diversity (Faith's PD): Significant increase after urban green space exposure β-diversity Bacterial composition significantly shifted after urban green space exposure and became more similar to the source (soil, leaves, air) after exposure. Nasal samples post exposure more similar to the air samples Taxa Increases in diversity and shifts in microbial abundances are linked to an increase in rare taxa and a decrease in common, human-associated taxa after green space exposure in Adelaide In post-exposure nasal samples, 24% of ASVs were shared with air, 30% with leaf, and 37% with soil samples 2. BOURNEMOUTH (U.K.) a-diversity Species richness and diversity (Faith's PD) were not significantly increased after urban green space exposure β-diversity Significant shifts in bacterial composition after urban green space exposure Taxa Relative abundance of genera did not vary by more than 3% after exposure 3. NEW DELHI (INDIA) a-diversity Species richness and diversity (Faith's PD) significantly increased after urban green space exposure β-diversity No significant shifts in bacterial composition of nasal samples after urban green space exposure Taxa

Table 2c (continued)

Author (year and country)	Type of intervention	Summary of results on human microbiota and immunological response
		Relative abundance of <i>Staphylococcus</i> decreased after green space exposure Corynebacterium and low abundance taxa increased in nasal samples after exposure

Bacilli, Gammaproteobacteria and Negativicutes were reported between day-28 and year-one reported for the intervention daycares. During the course of one year, total bacterial alpha-diversity on skin decreased in children of both standard and intervention day-care types. However, more specific richness within Alpha-, Beta-, and Gammaproteobacteria was higher among children from intervention day-cares compared to children from standard-daycares at year one. Moreover, the total relative abundance of genera containing typical human commensals and potential pathogens (e.g. *Gemella*, *Streptococcus*, and *Veillonella* genera and *Haemococcus influenza*) was lower among children in the intervention group. The authors reported a significantly different community composition in *saliva microbiota* between children of intervention and standard day-cares at year one follow-up in specific taxa classes (Gammaproteobacteria, Negativicutes, Fusobacteriia), and shifts in community composition between day-28 and year-one in the intervention population. On taxon level, relative abundance of Lactobacillales increased, while Fusobacteriia decreased during year one in the intervention daycare population, while also differences in salivary microbiota were also noted by gender. Differences in saliva α -diversity was inconclusive. Lastly, no results for alpha-diversity of *gut bacteria* at year one were reported and there were only slight differences in the community composition (beta-diversity). Decreases in relative abundance of *Clostridium sensu stricto* and *Romboutsia* at day-28 and year-one in intervention children were highlighted by the authors.

In a Finnish pilot trial among 14 healthy adults (Nurminen et al., 2018), intermediate-time contact with various soil- and plant-based material by rubbing hands over a period of two weeks was not associated with changes in bacterial microbiota diversity on skin. However, exposure was associated with an increase of overall bacterial α -diversity and in the phylum Bacteroides in faecal samples in the intervention but not the control group. However, statistically significant effects were only seen immediately after the intervention period (day 14), but not at a later sampling time-point thereafter (day 35).

As the remaining studies were pilot studies and of mainly explorative character, the main focus was on feasibility of the intervention and less on strictly testing hypotheses and estimating statistically robust effects. For instance, findings from a pilot trial did not suggest differences in bacterial α - and β -diversity in human microbiota samples (faeces, mouth, hand, and forehead) between the intervention and the control group after 20 weeks of a community gardening project among 16 adults in Denver (U.S.). In the same study, no differences were observed in human microbiota measurements over time and between pre-and post-intervention (baseline vs 20 weeks) for the interventional and control group, but it cannot be concluded whether this was due to lack of statistical power or absence of effect (Gascon et al., 2020).

The pilot trial among 54 pre-school children in Hong Kong (Sobko et al., 2020) did not observe a change in overall bacterial diversity in the faecal samples collected pre versus post intervention, in children participating a 10-week outdoor nature-related “Play&Grow” program. However, the authors reported an increase of richness within the phylum Proteobacteria and a decrease in richness within Bacteroidetes in the intervention but not in the control group post versus pre intervention. They reported further a difference in the β -diversity comparing post- versus pre-intervention assessments. Increases were observed in the relative abundance of the bacterial genus *Blautia* in the intervention group and of *Roseburia* in the control group. According to the KEGG analysis, more metabolic function pathways were modified within the intervention group as compared to the control group at a statistically significant level.

All three before-after studies evaluated the effect of short-time contact with nature based materials or surrogates of environmental exposure on human skin and nose microbiota. They all included healthy adults, but no study sample exceeded five participants. The first Finnish study on two adults tested the impact of microbiologically diverse nature-based material on human skin microbiota by direct hand exposure to 16 soil and plant-based materials and via fabric packets with dried Sphagnum moss laced on the inner forearm (Grönroos et al., 2019). For both experiments, an increase in richness, α -diversity and also changes in bacterial community composition directly after exposure have been observed. However, no information on statistical testing for the second experiment was provided (likely not feasible due to the small sample size).

Another small Finnish (within-subject cross-over, N = five subjects, three experiments per subject) study investigated the effects of short-time contact to microbial enriched and untreated sand materials on skin microbiota and revealed significant changes in richness, bacterial α - and β -diversity of skin samples in the course of the intervention (Hui et al., 2019). Increases in richness and diversity measures were significant after versus before touching enriched sands, and post-exposure bacterial diversity was higher in skin samples derived from enriched sands compared to non-enriched sands. Further, the relative abundance of specific bacterial phyla (Actinobacteria, Acidobacteria, Proteobacteria and Firmicutes) and the dominant bacterial genera differed significantly after exposure to inoculant-enriched compared to non-enriched sand materials, and this was also reflected in the KEGG functional prediction analysis. The authors also report a reduction in relative abundance of potentially pathogenic taxa on skin after touching both enriched and non-enriched sands, though it should be stated that the taxonomic resolution of the microbial analysis applied did not allow for a detection of specific bacterial pathogenic species.

Finally, a multi-centre study conducted in Australia, India and the U.K. among three adults investigated short-time but high intensity exposure to air, soil, and leaves in five different kinds of urban green space (in total 15 locations in three countries), thereby mimicking children interacting with the environment (Selway et al., 2020). Significant increase in α -diversity in both skin and nose (except from the U.K.) swab samples were observed for all study locations, a significant change in β -diversity was reported for Adelaide (Australia) and Bournemouth (U.K.) for skin and nasal samples, and only for skin samples in New Delhi (India). This study also reported taxa changes that can be generally summarized as indicating i) transfer of bacterial taxa from source to skin and nasal cavity post exposure; and ii) reduction in relative abundance of human source bacterial taxa at an increase of more rare bacterial taxa, the latter likely sources from the environment.

2.5. Interventions and human immune response markers

Two studies additionally investigated the effects of the interventions on immune response markers (Table 2a and b). In the biodiversity intervention study in day-cares (Roslund et al., 2020) only few significant associations with immunological markers were seen. The authors reported increase in plasma IL-10:IL-17A ratio for children in intervention day-cares during the trial period. In exploratory analyses, some of the variables of skin and gut microbiota that were reported to be increased by the intervention were shown to correlate positively with plasma levels of TGF- β 1 and relative abundance of Treg cells in blood and negatively with the plasma levels of IL-17A. For the Finnish study on exposure to soil and plant materials among 14 healthy adults (Nurminen et al., 2018), the level of TGF- β but not IL-10 expression in peripheral blood mononuclear cells was significantly associated with the increase in bacterial diversity in both skin and faecal samples immediately after exposure (day 14) but not at a later time point post-exposure (day 35).

3. Discussion

3.1. Summary of main findings

For our scoping review we found only a limited number of intervention studies that evaluated the impact of natural environmental exposure in

relation to human microbiota and immune response. The available investigations were largely designed as pilot studies with explorative character. They encompassed only small study populations, varied considerably in study design, type and duration of intervention as well as follow-up assessments. The majority of the studies reported that the exposure to natural environment increased taxa richness or diversity in human microbiota samples and pre-dominantly on skin, as this body site was sampled and assessed most frequent in the studies. Diversity changes within larger bacterial groups (phylum level) were reported, and in five out of eight studies increases in overall bacterial diversity in human microbiota samples were observed. A significant shift in microbial composition (beta-diversity) in human samples was observed upon intervention in four studies. These changes were mostly seen in samples taken immediately after the intervention took place and only one study reported also findings two years after the start of intervention. Immunological markers were assessed in only two studies and some direct effects were reported in one of them. Both studies reported that the intervention-associated changes in the human microbiota diversity were associated with increased levels of immunoregulatory markers but also these effects seemed short lived.

3.2. Comparison to previous work in the field

Although the early microbial colonization is suggested to be a key regulator of immune response development and exposures to microbe-rich environments are convincingly associated with relevant beneficial health effects, the contribution of the living environment to human microbiota composition is still poorly understood (Kirjavainen et al., 2019a). A study analyzing worldwide soil and gut microbiota in relation to richness, diversity and species identity revealed that Bacteroidetes and Firmicutes dominated human faecal samples whereas Proteobacteria and Verrucomicrobia were the prominent phyla in the soil samples (Tasnim et al., 2017). Interestingly, the most consistent changes in the studies we reviewed were related to Proteobacteria phyla and Bacteroides genus. Two studies in children (Roslund et al., 2020; Sobko et al., 2020) and one study in adult populations (Grönroos et al., 2019) observed an increase in Proteobacteria α -diversity and richness (Hui et al., 2019; Sobko et al., 2020) immediately after the trial in the intervention group. Whereas two studies found also an increased α -diversity and richness for Bacteroides (Hui et al., 2019; Nurminen et al., 2018), another study reported a decrease in α -diversity of Bacteroides (Sobko et al., 2020). Similar observations with respect to human microbiota diversity have been made in association studies. For instance, a study investigating the relationship between land cover and skin microbiota among six year old children observed that 20% of the variation in the relative abundance of Proteobacteria could be attributed to traditional environments including forest and agricultural land cover (Ruokolainen et al., 2015). Similarly, lower biodiversity around residential home has been associated with a significantly lower diversity of Gammaproteobacteria in skin samples of adolescents and also, a lower prevalence of allergic sensitization (Hanski et al., 2012). Moreover, a canine model studying a possible interrelation between environmental and host skin microbiota observed that forest and arable land were also associated with a greater amount of different Proteobacteria and further soil-related taxa (Lehtimäki et al., 2018b). In contrast, a study among 355 four months old infants in Canada observed that for children living in close proximity to natural environments, there was a significant inverse association with the Proteobacteria phylum and a significant positive association with the Actinobacteria phylum in faecal samples (Nielsen et al., 2020).

There is some evidence from the so called “farm studies” in Europe and the U.S. that farm exposure, typically associated with an enriched microbial environment, appears to modify immune profiles already at birth and is further associated with a lower prevalence of asthma and allergic diseases (Ober et al., 2017). For instance, Amish and Hutterite children have similar genetic ancestries but grow up under distinct environmental conditions. The living environment of Amish children who live in close contact to farm animals and family members and without any technological equipment was associated with an expression in innate immunity genes involved

in the response to microbial exposures as compared to Hutterite children living on highly-industrialized farms (Stein et al., 2016; Ober et al., 2017). Related to this, the gut microbiome maturation seems to play a decisive role in modifying allergic and asthma disease risk (Vercelli, 2021). In the Protection against Allergy study in Rural Environments (PASTURE) birth cohort, exposure related to farming was associated with the maturation of the gut microbiome until 12 months of age. Moreover, the estimated microbiome age (EMA) further mediated some of the protective farm effect on asthma at school age, and it was suggested that this would be mainly attributable to butyrate producing bacterial communities such as Roseburia and Coprococcus (Depner et al., 2020). Somewhat related, though not linking to human microbiota changes, forest bathing has been suggested to be associated with anti-inflammatory and anti-asthmatic effects for instance by inhaling natural volatile substances (Debarry et al., 2007). In this recent systematic review, forest bathing was linked to increased natural killer cell levels and cytotoxic activity in the majority of the respective studies (Andersen et al., 2021).

Although findings of previous studies are intriguing, further intervention studies are needed in particular to determine whether observed effects of exposure to natural environments on human microbiota and subsequent immune response markers are a result of transient “hitchhiking” of bacteria on human body sites, or the effect of an actual colonization in consequence of an intervention (Tasnim et al., 2017). Ultimately, given the current evidence, more accurate exposure characterization and especially longer observation periods are needed in order to characterize the suggested beneficial aspects of exposure to natural environments. Sufficient time of observation might facilitate the attribution of exposure to nature in relation to health benefits including immunological response or even allergic symptoms.

3.3. Critical appraisal of the included studies

The studies ultimately included in this scoping review comprised a wide range of interventional strategies that imitate the engagement with natural environmental exposure. In general, as six of the eight investigations have been initially designed as feasibility (pilot) studies, the consideration of methodological issues was not given priority by most of the included studies.

In general, sample sizes were very small, ranging from only two to 89 study subjects and therefore, the interpretation of the results need to take into account the lack of required sample size calculation in order to detect an effect of the intervention, the lack of information on statistical testing and possibly meaningfulness. In addition, not all studies provided sufficient information on which parameters have been tested beyond those for which results were presented (in particular for taxa) or how many tests have been performed. The problem of multiple testing has been only addressed by two studies (Gascon et al., 2020; Roslund et al., 2020). Moreover, two of the eight included studies seemed to be structured as cluster-randomisation (comparison on group-level) (Roslund et al., 2020; Roslund et al., 2021) and the remaining compared the effects of the intervention on individual-level. However, information on the exact approach and terminology was not explicitly stated. Further, there was no information in the methods whether multi-level analyses have been performed to model the correlation of data within the clusters.

As most of the studies performed the last measurements immediately after the intervention period, there is no information available for how long the observed effects might have continued beyond that. There were only two studies with additional post-intervention measurements (Nurminen et al., 2018; Roslund et al., 2021). Whereas one study failed to detect any significant effects two weeks after the end of the intervention period (Nurminen et al., 2018), the study on biodiversity interventions in standard day-care yards reported changes in α - and β -diversity on skin and in specific taxa groups which were visible also at year one of the ongoing intervention (Roslund et al., 2021). The method description in this publication suggests that the intervention never ended during the follow-up period, i.e. the biodiverse intervention materials were present in the daycare centers throughout

the study period, but there was no specific guidance to be in contact with those materials after day-28 of the study. This means there was no follow-up sampling conducted after the intervention ended, but several sampling campaigns were carried out throughout the intervention. Unfortunately, the sample size for the two-year follow-up was too small to draw meaningful conclusions. This also leads to considerations concerning the duration of the environmental contact that might be required to result in a short- or medium-term modification of the human microbiota, even possibly involving microbial colonization. While in the majority of studies interventions were very short-term, in the order of minutes and hours, three out of the eight studies described a longer-term interventional period of 10 (Sobko et al., 2020) weeks, 20 weeks (Gascon et al., 2020) and even two years (ongoing) (Roslund et al., 2021). The trial among 54 children with a 10-week intervention period observed significant changes in specific bacterial groups post versus pre-intervention in the intervention group. On the other hand, no changes in human microbiota samples were reported in the pilot trial in 16 adults after a 20 weeks intervention period, which, however, might be very likely due to the very small sample size. The long-term biodiversity intervention among 89 children seemed to be still ongoing, however, the reduced sample size after two years clearly aggravates a reasonable interpretation of the findings.

The methodological quality of an intervention study depends also on a detailed description of the study and a thorough reflection of possible confounding factors. In particular in the context of gut microbiota changes, current diet and a current antibiotic therapy should be recorded. Two out of four studies looking at gut microbiota considered current antibiotic treatment as a possible confounding factor (Roslund et al., 2020; Roslund et al., 2021) and one study as exclusion criteria (Sobko et al., 2020). Gascon et al. and Sobko et al. took into account current diet or a possible change of diet throughout the interventional period, however, this was not considered as a potential confounder in the analyses. Four studies did not report any exclusion criteria (Gascon et al., 2020; Grönroos et al., 2019; Hui et al., 2019; Selway et al., 2020).

3.4. Future perspectives including recommendations for future studies

The design and implementation of an environmental intervention study for primary or secondary allergy prevention among infants and young children are logistically and mechanistically challenging, in particular considering the young age group, the design of suitable and longitudinally arranged intervention concepts and issues of adherence. Consequently, the number of studies that report such attempts has been very limited. This is, however, a high-risk high-reward area in research and development of preventive strategies and therefore well-designed, focused and hypothesis driven studies with sufficient statistical power are worthwhile to pursue, specifically since indicative findings are not discouraging at the least.

Based on the lessons learned from the already conducted studies and existing literature in this study area, the following methodological issues should be taken into consideration in planning future environmental intervention studies in children as primary or secondary allergy prevention approach.

3.4.1. The window of opportunity – critical exposure periods

There is strong epidemiological evidence that the potentially protective effects of an increased microbial exposure occur in particular within the first years of life and/or already prenatally (Mutius and Vercelli, 2010; Mutius, 2016). In Europe, about 34% of children under age three and around 95% as of age four visited regularly day-care facilities in 2019 (European Commission, 2019). For the U.S. in the same year, the enrolment rate among three to four year-olds and as of age five was indicated with about 61% and 87%, respectively (National Center for Education Statistics, 2019). Day-care facilities may therefore represent a unique possibility to implement interventions already early in life within a protected framework and the possibility for a longer interventional or observational period (Lehtimäki et al., 2018a; Roslund et al., 2020). In this context, interventions such as guided nature activities (Sobko et al., 2020) or the

installation of (permanent) microbial diverse materials (Roslund et al., 2020) seems to be a promising and feasible approach considering the age-group and long-term exposure within several possible settings, e.g., day-cares or kindergarten.

3.4.2. Duration of intervention and follow-up periods

In order to achieve and maintain microbial colonization on human body sites or at least a chronic-like nearly daily exposure, which may be required to provoke the suggested beneficial allergy protective effects, a longer-term duration of environmental exposure is recommended (Lehtimäki et al., 2017; Sun et al., 2020). It is unclear whether a one-time rubbing of soil onto skin has any effect beyond a momentary, well conceivable increase in bacterial richness (numbers of human bacterial taxa + soil bacterial taxa > number of human bacterial taxa alone). Results from one bacterial microbiota measurement from one body site immediately before and one immediately after the intervention are inconclusive. In this context, repeated measurements of skin, faeces and blood samples over extended intervention and long-term post-intervention periods are crucial in order to evaluate temporal changes and persistence of human microbiota in relation to certain environmental exposure related to the intervention. It is further crucial to take into account factors to be suspected of affecting human microbiota and/or immune system responses including health status, diet, physical activity, antibiotic or other medication, residential surrounding green or naturally diverse area as well as time spending outdoors (Selway et al., 2020; Maher et al., 2020; Lozupone et al., 2012; Moitinho-Silva et al., 2021).

3.4.3. Significance on disease outcomes

While the studies included in the present review showed that the interventions may have some short-term impact on human microbiota and these changes may even correlate with some immunological markers, there is no indication that the associated changes are beneficial in allergy prevention or to health overall. Therefore, future trials should clearly focus on how a direct link to potential beneficial health effects could be obtained.

3.4.4. Study design

Further, a statistical sound and robust study design in the form of a randomized controlled trial is essential. For instance, further (pilot) interventional studies are advised to follow suitable checklists such as The Consolidated Standards of Reporting Trials (CONSORT) statement (Eldridge et al., 2016a; Schulz et al., 2010). This evidence-based, minimum set of recommendations for reporting randomized trials to improve transparency and quality of reporting by informing on required sample size, numbers of eligible participants, type and practicability of the interventions, response rate to questionnaires, retention and dropout rates as well as time needed to collect and analyse data (Eldridge et al., 2016b).

3.5. Strengths and limitations of the scoping review

To the best of our knowledge, this is the first scoping review on the effects of exposure to natural environment or isolated aspects of natural environments in relation to human microbiota as suggested interlink between environmental exposure and immune response. A strength of our approach was the comprehensive scope of the search strategy with few exclusion criteria in order to include a broad range of biodiversity interventions and outcomes. The results of our search confirmed the innovative aspect of this research area considering that all 8 included studies have been published within the past three years. Unfortunately, due to the novelty of this approach, most regions of the world are not covered. As of now, most of the studies were conducted in Europe (Finland). Another limitation that should be noted is related to the critical appraisal of the included studies. The included trials, many designed as pilot studies and with small sample sizes, were predominantly considered as having high risk of bias. Therefore, our findings must be interpreted with caution, but can certainly be used as a basis for future larger randomized trials with longer follow-up.

We identified only few intervention studies that were predominantly designed as pilot trials with rather explorative character. Based on the presented evidence, our results suggest that some environmental interventions might diversify human microbiota, at least temporarily. Whereas there were little direct immunological effects reported for the two available studies, intervention-associated changes in human microbiota diversity was associated with increased levels of some immunoregulatory markers. However, a direct link to beneficial health effects remains highly hypothetical at this stage. Carefully designed proof-of-concept studies are needed in order to evaluate on a suitable interventional concept along with a sufficiently long observational period which allows the examination of sustainable health effects. The identification of microbial signatures of human microbiota in response to natural environment interventions might help to determine strategies for the prevention of treatment of asthma and allergic outcomes in childhood and beyond.

In conclusion, the number of studies investigating the effect of exposure to natural environments on human microbiota as well as immunological markers is very limited. The studies published to-date are mostly of pilot character and limited in population size and follow-up duration, and the environmental intervention approaches chosen in these studies vary widely. There is, however, some evidence that those so-called biodiversity interventions have the potential to diversify human microbiota, at least over a short period of time. Adequately powered randomized controlled trials with long term follow-up are required to examine sustainable effects on microbiota and immune system.

4. Materials and methods

The current scoping review was performed according to the methodological framework as outlined by Arksey and O'Malley (Arksey and O'Malley, 2005) and the protocol for this review was registered at OSF REGISTRIES (<https://osf.io/musgr>) on 26.02.2021.

4.1. Literature search and study selection

Two authors (CT, MT) developed the initial search strategy and performed a systematic search in the medical database PubMed (MEDLINE®). The final search was conducted on the 18th of December 2020 and updated on the 3rd of September 2021 using search-terms related to natural environmental exposure and search terms related to human microbiome and/or immune system responses in combination with terms related to the study type or the mechanism of the suggested microbial transfer (Supplementary table 1). There were no restrictions with regard to age, gender of study participants, language or date of publication. Finally, references of all included articles were examined for further potential studies meeting the inclusion criteria.

4.2. Inclusion and exclusion criteria

Criteria for inclusion of a study were: a) peer-reviewed original research article; b) interventions examining the exposure to natural environment and/or specific factors representing aspects of contact with natural environments (e.g. forest, parks, natural green space or natural green land cover, yard vegetation, soil and/or plant materials); and c) outcomes including the human microbiome (skin, gastrointestinal, lung, airways) or immune system responses linked to asthma and allergic disorders (e.g. T-cell phenotypes, regulatory T-cells, plasma Transforming Growth Factor beta (TGF- β 1) levels, immunoregulatory cytokines). In order to evaluate whether an intervention is provoking any changes in the health outcomes described above, the included studies were further required to have outcome measurement(s) performed before and after the intervention, without requirements on the duration of the follow-up period. Not considered were studies with a cross-sectional study design (association/correlation studies), narrative reviews, editorials, as well as studies on animal populations. Furthermore, we excluded studies investigating oral route applications of diet variations, pro, pre- and synbiotics or any other dietary supplements,

exposure to (house dust mite) allergens, objectively and subjectively assessed moisture and/or mould, and fungal as well as bacterial components not associated with a suggested natural environmental exposure.

4.3. Study selection and data extraction

Citations of all studies that we identified were downloaded, de-duplicated and transferred to Rayyan, a web application that assists in the production of systematic reviews (Ouzzani et al., 2016). The authors (CT, UM, JT, PK, MT) screened the identified titles and abstracts in dyads and in blind mode. Any conflicts with respect to inclusion or exclusion were resolved jointly by the respective authors. The authors independently performed full text screening in dyads (blind mode) and three authors (CT, PK, MT) synthesized findings according to the guidelines as proposed in the PRISMA-ScR (Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews) checklist (Tricco et al., 2018). The following data were extracted from the included studies: author, year of publication, country of study, study type/design/method, study population (including sample size), age of the study population, description of intervention and/or exposure(s), and description of outcome(s). Disagreements were resolved by discussion (CT, UM, JT, PK, MT). As a formal assessment of the methodological quality of the included studies is beyond the scope of this scoping review, we limited our evaluation pointing out the major methodological issues.

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CRediT authorship contribution statement

CT: Conceptualization, methodology, screening, data extraction, writing—original draft preparation, writing—review and editing.

PK, MT: Conceptualization, screening, data extraction, writing—original draft preparation, writing—review and editing.

UM, JT, KW, TK, CA: Writing—review and editing.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.153422>.

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