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## Greenspace and human microbiota: A systematic review

Yi-Dan Zhang<sup>a,1</sup>, Gang-Long Zhou<sup>a,1</sup>, Lu Wang<sup>a</sup>, Matthew H.E.M. Browning<sup>b</sup>,  
Iana Markevych<sup>c</sup>, Joachim Heinrich<sup>d,e</sup>, Luke D. Knibbs<sup>f,g</sup>, Tianyu Zhao<sup>h,i</sup>, Yizhen Ding<sup>j</sup>,  
Shi Chen<sup>k</sup>, Kang-Kang Liu<sup>l</sup>, Payam Dadvand<sup>m,n</sup>, Guang-Hui Dong<sup>a</sup>, Bo-Yi Yang<sup>a,\*</sup>

<sup>a</sup> Joint International Research Laboratory of Environment and Health, Ministry of Education, Guangdong Provincial Engineering Technology Research Center of Environmental Pollution and Health Risk Assessment, Department of Occupational and Environmental Health, School of Public Health, Sun Yat-sen University, Guangzhou 510080, China

<sup>b</sup> Department of Park, Recreation, and Tourism Management, Clemson University, Clemson, SC 29634, USA

<sup>c</sup> Institute of Psychology, Jagiellonian University, Krakow, Poland

<sup>d</sup> Institute and Clinic for Occupational, Social and Environmental Medicine, LMU University Hospital Munich, Munich 80036, Germany

<sup>e</sup> Allergy and Lung Health Unit, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, VIC 3010, Australia

<sup>f</sup> School of Public Health, The University of Sydney, Camperdown, NSW 2006, Australia

<sup>g</sup> Public Health Research Analytics and Methods for Evidence, Public Health Unit, Sydney Local Health District, Camperdown, NSW 2025, Australia

<sup>h</sup> Institute and Clinic for Occupational, Social and Environmental Medicine, LMU University Hospital Munich, Comprehensive Pneumology Center (CPC) Munich, German Center for Lung Research (DZL), Munich 80036, Germany

<sup>i</sup> Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, 85764 Neuherberg, Germany

<sup>j</sup> Department of Landscape Architecture and Urban Planning, Texas A&M University, College Station, TX 77843, USA

<sup>k</sup> School of Public Health, Sun Yat-Sen University, Guangzhou 510080, China

<sup>l</sup> Department of Research Center for Medicine, the Eighth Affiliated Hospital, Sun Yat-Sen University, Shenzhen, China

<sup>m</sup> ISGlobal, Dr. Aiguader 88, 08003 Barcelona, Spain

<sup>n</sup> CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

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## ABSTRACT

**Background:** Potential effect of greenspace exposure on human microbiota have been explored by a number of observational and interventional studies, but the results remained mixed. We comprehensively synthesized these studies by performing a systematic review following Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

**Methods:** Comprehensive literature searches in three international databases (PubMed, Embase, and Web of Science) and three Chinese databases (China National Knowledge Infrastructure, Wanfang, and China Biology Medicine disc) were conducted from inception to November 1, 2023. Observational and interventional studies that evaluated associations between greenspace exposure and human microbiota at different anatomical sites were included. Studies were assessed using the National Toxicology Program's office of Health Assessment and Translation risk of bias tool and certainty of evidence was assessed using the Grading of Recommendations, Assessment, Development and Evaluation framework. Two authors independently performed study selection, data extraction, and risk of bias assessment, and evidence grading. Study results were synthesized descriptively.

**Results:** Twenty studies, including 11 observational studies and 9 interventional studies, were finally included into the systematic review. The microbiota of the included studies was from gut (n = 13), skin (n = 10), oral cavity (n = 5), nasal cavity (n = 5) and eyes (n = 1). The majority of studies reported the associations of greenspace exposure with increased diversity (e.g., richness and Shannon index) and/or altered overall composition of human gut (n = 12) and skin microbiota (n = 8), with increases in the relative abundance of probiotics (e.g., *Ruminococcaceae*) and decreases in the relative abundance of pathogens (e.g., *Streptococcus* and *Escherichia/Shigella*). Due to limited number of studies, evidence concerning greenspace and oral, nasal, and ocular microbiota were still inconclusive.

\* Corresponding author at: Joint International Research Laboratory of Environment and Health, Ministry of Education, Guangdong Provincial Engineering Technology Research Center of Environmental Pollution and Health Risk Assessment, Department of Occupational and Environmental Health, School of Public Health, Sun Yat-sen University, Guangzhou 510080, China.

E-mail address: [yangby23@mail.sysu.edu.cn](mailto:yangby23@mail.sysu.edu.cn) (B.-Y. Yang).

<sup>1</sup> These authors contributed equally to this work.

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**Conclusion:** The current evidence suggests that greenspace exposure may diversify gut and skin microbiota and alter their composition to healthier profiles. These findings would be helpful in uncovering the potential mechanisms underlying greenspace and human health and in promoting a healthier profile of human microbiota.

## 1. Introduction

### 1.1. Background

Greenspace, referring to land with natural vegetation (e.g., forest, shrubs and grassland), parks, and other nature-rich spaces (Taylor and Hochuli, 2017), provides a wide range of ecosystem services that could benefit humans, including pollutant remediation, carbon maintenance, and nutrient cycling, and improving health (Groffman et al., 2009). There is accumulating evidence concerning beneficial effects of greenspace on human health, such as promoting mental wellbeing, reducing morbidity and mortality, and improving pregnancy outcomes (Yang et al., 2021). However, the precise mechanisms underlying these effects remain unclear. Several hypotheses have been proposed, including reducing stress, mitigating environmental hazards (i.e., air pollutants, heat, and noise), encouraging physical activity, and improving social cohesion (Markevych et al., 2017; Marselle et al., 2021). Recent evidence also suggests that human microbiota might be involved in the pathways through which greenspace influences human health (Mills et al., 2017).

Human microbiota are dynamic ecosystems of commensal microbes that contribute to metabolic functioning, protect against pathogens, and train and develop the human immune system (Rackaityte and Lynch, 2020; Ahn and Hayes, 2021). Changes in human microbiota in different body sites (e.g., skin, respiratory tract, and digestive tract) can cause pathological processes and mechanisms that are involved in the development of diseases, such as allergy, inflammatory bowel disease and diabetes (Hou et al., 2022). While the key drivers for microbial diversity and community structure are still being investigated, the biodiversity hypothesis suggests that exposure to natural environments such as greenspaces may shape the development of the human microbiota (Haahtela, 2019). Specifically, the air, soil and plants in biodiverse greenspace harbor a variety of the microorganisms, and humans may interact with these microbes via direct or indirect contacts while in greenspace. In addition, greenspace can reduce air pollutant levels (Hirabayashi and Nowak, 2016) and subsequently alleviate human microbiota perturbation caused by air pollution (Mousavi et al., 2022). Furthermore, greenspace provides residents with a place to unwind from stress and conduct sports activities (Marselle et al., 2021; Foo, 2016), both of which can improve gut microbial health by modulating hormone levels and energy metabolism (Wegierska et al., 2022; Agirman and Hsiao, 2021).

Recently, an increasing number of studies have evaluated the association between greenspace exposure and human microbiota, but the results remain mixed. Some studies have reported that greenspace exposure was associated with increased human skin and gut microbial diversity (Nurminen et al., 2018; Grönroos et al., 2019; Roslund et al., 2020; Selway et al., 2020; Roslund et al., 2021; Wuet al., 2022), while others didn't find such associations (Gascon et al., 2020; Nielsen et al., 2020; Pearson et al., 2020). Moreover, the assessment of microbiota encompasses a spectrum of anatomical sites (e.g., gut, skin, nasal cavity), which further complicates the interpretations of these results. Systematic reviews are widely recognized for their ability to address limitations in primary studies such as small sample sizes, publication biases, and chance of biases, and to synthesize and critically appraise the existing evidence standardly and transparently. Thus, conducting a systematic review that comprehensively evaluates the association between greenspace exposure and human microbiota would provide a synthesis of the existing evidence to inform the existing knowledge gaps and to translate available evidence into interventions and policies for

improving public health.

### 1.2. Aims and objectives

We aimed to conducted systematic review to comprehensively synthesize the available observational and interventional evidence on the association between greenspace exposure and human microbiota. The research objective of this systematic review was to address the following Population, Exposure, Comparator, Outcome, and Study (PECOS) question: among the general population (Population), what is the effect of higher greenspace exposure (Exposure) vs. lower and no greenspace exposure (Comparison) on human microbial diversity and composition (Outcome), observed in epidemiology observational and interventional studies (Study design)? Specifically, the population refers to general population regardless of age, gender, and health status. Exposure refers to exposure to greenspace, including but not limited to parks, forests, urban green areas, and natural environments. Comparator is defined as comparing the microbiota in participants with relatively high versus relatively low greenspace exposure, or among participants with greenspace exposure versus those without greenspace exposure. Outcome includes microbial alpha-diversity and beta-diversity, represent the diversity of whole microbial community, and relative abundance of each taxon in the microbial community (Table 1).

## 2. Methods

### 2.1. Planning and scoping

In order to provide a scoping and framing of associations between greenspace exposure and human microbiota, databases including PubMed, Epistemonikos, and China National Knowledge Infrastructure (CNKI) were searched from establishment to Nov 1st, 2023 for previous reviews of this topic. International Prospective Register of Systematic Reviews (PROSPERO) and Open Science Framework Registry were also accessed on Nov 1st, 2023 to find any ongoing systematic reviews on this topic. Terms including “greenspace”, “greenness”, “natural environment”, “vegetation”, “forest”, “grassland”, or “park”, combined with “human microbiota”, “skin microbiota”, “gut microbiota”, or “bacteria” were searched (See detailed search strategies in Table S1).

Through a systematic search, we identified one scoping review and one systematic review that similarly focused on greenspace and human microbiota (Table S2). The scoping review conducted in 2021 focused solely on eight interventional studies published before September 2021 (Tischer et al., 2022). The systematic review concentrated on seven epidemiological studies pertaining to greenspace and human gut microbiota, up until October 2022 (Van Pee et al., 2023). These reviews, due to their restricted scopes, did not incorporate all available research

**Table 1**  
Population-Exposure-Comparator-Outcome-Study (PECOS) statement.

Category	Describe
Population	General population regardless of age, gender, and health status.
Exposure	Exposure to greenspace, including but not limited to parks, forests, urban green areas, and natural environments.
Comparator	Higher greenspace exposure versus lower greenspace exposure; with greenspace exposure versus without greenspace exposure
Outcome	Alpha diversity, beta-diversity of the whole community and relative abundance of each taxon of microbiota from any site of human body.
Study	Human epidemiological studies, including observational studies and interventional studies.

on greenspace and human microbiota. Furthermore, both lacked clear PECOS statements, risk of bias assessments, and certainty of evidence evaluations, which could potentially lead to subjective conclusions. Hence, an updated and more comprehensive review is needed. Firstly, a broader range of evidence must be considered to assess the association between greenspace exposure and human microbiota. This includes various study designs and microbiota from different body sites, along with new studies published after October 2022. Secondly, formal research evaluation or analysis of risk of bias and certainty of existing evidence should be conducted.

Our systematic review was conducted and reported following the standard protocols recommended by the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 (Table S3).

## 2.2. Search strategy and selection criteria

Two investigators (YDZ and GLZ) searched three international databases – PubMed, Embase, and Web of Science (Science Citation Index Expanded and Social Science Citation Index in Web of Science Core Collection), and three Chinese database – CNKI, Wanfang, and China Biology Medicine, for pertinent literature published prior to November 1, 2023. The search strategy was proposed by literature searchers (YDZ, GLZ) after extensive reading of systematic reviews and articles relevant to greenspace and human microbiota, and then refined with the help of information experts (IM, TZ) in this field (Table S4). To balance the sensitivity and specificity of our search strategy, the strategy consists of two domains: (1) different terms for greenspace; (2) different terms for human microbiota. Publications were limited to those with the full-texts available in English and Chinese. The papers included in the previous two reviews (Tischer et al., 2022; Van Pee et al., 2023) were used as benchmark papers for pilot search to test the comprehensiveness of the search strategy. All benchmark papers were captured by our search strategy. We also manually searched the reference lists of the previous two reviews and articles included in our current review.

Studies were eligible if they met the below criteria following PECOS criteria (Table 2): (i) Population: studies conducting among general population. (ii) Exposure: studies assessed greenspace exposure at individual-level with objective metrics [e.g., remote sensing-based indices of greenspace such as normalized difference vegetation index (NDVI) or the percentage of greenspace cover], subjective metrics that assessed by trained auditors using a consistent tool (e.g., self-reported proximity to greenspace and perception of greenspace) or specific interventions (e.g., walking in urban parks). (iii) Comparators: studies comparing human microbial diversity and/or composition between individuals exposed to different greenspace levels. (iv) Outcome: studies reporting human microbial diversity and composition. (v) Study: human epidemiological studies, including observational studies and interventional studies.

## 2.3. Study selection and data extraction

Identified records were imported into Endnote 20 (Clarivate Analytics, Philadelphia, PA, USA). One investigator (YDZ) removed 3708 duplicates using this software's automated tool. Then, two investigators (YDZ and GLZ) independently screened titles and abstracts for relevance. Lastly, two independent investigators (YDZ and GLZ) scanned the full texts of the remaining articles for eligibility. Any disagreements were addressed through discussion with a third investigator (BYY).

The following information was independently extracted from eligible studies by two investigators (YDZ and GLZ): authors, year of publication, study design, location (country/region), sample size, greenspace exposure assessment(s), the method to characterize microbiota, microbial outcomes, adjustments and main findings. Again, any disagreements in data extraction were resolved by discussions with a third investigator (BYY).

**Table 2**

Eligibility criteria informed by Population-Exposure-Comparator-Outcome-Study (PECOS).

Inclusion criteria	Exclusion criteria
Participants/Population: General populations across the lifespan.	Studies did not conduct in human
Exposure/Intervention: For observational studies, greenspace exposure was measured objectively by use of a satellite system, land cover maps, or an assessment by trained auditors using a consistent tool; For interventional studies, greenspace exposure interventions included conducting activities in greenspace (i.e., parks, gardens) and contacting greenspace features (i.e., vegetations and soils in greenspace), etc. Greenspace exposure was included as a separate variable within the analysis, and results were reported specifically for greenspace. Greenspace exposure was assessed at individual level.	Greenspace exposure not measured at individual level.
Comparators: People who expose to lower greenspace levels or not expose to greenspace.	/
Outcomes: Assessment of diversity and/or relative abundance of specific taxa of microbiota from any human body sites (e.g., skin, gut) . Using genomic sequencing technology.	The microbiota was detected by culture method.
Studies: Observational studies (i.e., cross-sectional studies; cohort studies; case-control studies) and interventional studies (i.e., RCT, non-RCT, Before-after interventional studies).	In vitro and in vivo experimental studies

Abbreviation: RCT: Randomized Controlled Trial.

## 2.4. Risk of bias assessment

Two investigators (YDZ and GLZ) independently assessed the risk of bias (RoB) for each study using the National Toxicology Program's Office of Health Assessment and Translation (OHAT) RoB tool (2015). Any disagreement was resolved by discussion with a third investigator (BYY). Based on the tool, the included studies were assessed with three critical criteria (exposure assessment, outcome assessment and confounding bias) and four non-critical criteria (selection bias, attrition/exclusion bias, selective reporting bias, and other source of bias). Each criterion in the OHAT tool was categorized as "low", "probably low", "probably high" or "high" risk (detailed RoB ratings are presented in Table S5). Based on the above rating, evidence from each of the included studies were ranked as "not serious", "serious", or "very serious" RoB (Matta et al., 2021), which were then used to support assessments of the RoB of the body of evidence and decision-making for certainty rating. Detailed protocols on the three-tier scale can be found in Table S5.

## 2.5. Evidence synthesis

Evidence synthesis was conducted using the Synthesis Without Meta-analysis (SWiM) reporting guideline (Campbell et al., 2020). Studies were first grouped by the studied microbiota in different body sites, such as skin and gut, and then grouped by the reported outcomes (i.e., alpha-diversity, beta-diversity, and the relative abundance of specific taxa). For each type of microbiome, differences in microbial alpha- and beta-diversity, relative abundance of bacterial or fungal taxa, and effect estimates of the associations of greenspace with diversity or abundance, along with their 95 % confidence intervals, were extracted and reported as the main results. We used a vote-counting approach supported by

tables to summarize the directions of the association between greenspace exposure and microbial alpha-diversity, and the relative abundance of specific taxa, as well as the significance of the difference in microbial beta-diversity between people with different greenspace exposure levels. We explored sources of heterogeneity qualitatively by comparing study designs, exposures assessed, outcome studied, and statistical methods used for studies included in the narrative analysis. Meta-analyses were planned if there were more than two sufficiently homogenous studies (i.e., similar study design, greenspace exposure measurement, microbial determination methods, and outcome metrics) available.

## 2.6. Rating certainty in the body of evidence

We assessed the certainty of the evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework adapted to environmental health reviews (Morgan et al., 2016). The initial GRADE certainty was based upon the following key features of the study design (high certainty for 4 features, moderate certainty for 3 features, low for 2 features, very low for 1 feature): exposure prior to outcome, individual outcome data, comparison group used, and controlled exposure. Evidence could then be downgraded on the basis of risk of bias concerns (i.e., more than a half of studies composing the body of evidence were assessed as having “serious RoB”), imprecision of effects (studies with high variability of effect estimates), inconsistency, indirectness, and publication bias. Finally, ratings could be upgraded if all plausible confounders or other biases increase certainty in the observed effect, if a large or very large effect was observed, or a dose–response gradient was observed (Table S6). The certainty assessment was conducted independently by two reviewers (YDZ and GLZ). An overall strength of evidence rating was assigned for each of the studied outcomes (i.e., alpha-diversity, beta-diversity, and relative abundance of specific taxa for microbiota from each body site) in the review.

## 3. Results

### 3.1. Literature retrieval and study characteristics

Our database search yielded 17419 articles. After removing 6070 duplicates, titles and abstracts of 11,349 articles were screened and 11,326 were excluded. After a full-text review of the remaining 23 articles, 20 were included (Fig. 1) (Asri et al., 2023; Bowyer et al., 2022; Brown et al., 2022; Gacesa et al., 2022; Gascon et al., 2020; Gisler et al., 2021; Grönroos et al., 2019; Hanski et al., 2012; Hui et al., 2019; Nielsen et al., 2020; Nurminen et al., 2018; Pearson et al., 2020; Pearson et al., 2019; Roslund et al., 2020; Roslund et al., 2021; Ruokolainen et al., 2015; Selway et al., 2020; Sobko et al., 2020; Wu et al., 2022; Zhang et al., 2023). After manual searches of the reference lists of the finally included 20 studies and two previous reviews (Tischer et al., 2022; Van Pee et al., 2023), we did not identify new eligible articles. The records of disagreements and corresponding final decisions during the process of study selection were shown in Table S7.

The characteristics of the studies were summarized in Table 3. These studies were conducted across 35 countries, including seven studies in Finland (Grönroos et al., 2019; Hanski et al., 2012; Hui et al., 2019; Nurminen et al., 2018; Roslund et al., 2020; Roslund et al., 2021; Ruokolainen et al., 2015), four in the United States of America (USA) (Brown et al., 2022; Gascon et al., 2020; Pearson et al., 2020; Pearson et al., 2019), two studies in Canada (Gisler et al., 2021; Nielsen et al., 2020), two studies in China (Asri et al., 2023; Wu et al., 2022), one study in the United Kingdom (UK) (Bowyer et al., 2022), one study in Netherland (Gacesa et al., 2022), one study conducted in UK, Australia, and India (Selway et al., 2020), and one study covering 34 countries (Zhang et al., 2023). Ten of these studies had a cross-sectional design (Asri et al., 2023; Bowyer et al., 2022; Gacesa et al., 2022; Hanski et al., 2012; Nielsen et al., 2020; Gascon et al., 2020; Pearson et al., 2020; Pearson et al., 2019; Ruokolainen et al., 2015; Wu et al., 2022; Zhang et al., 2023), one had a cohort design (Gisler et al., 2021). Nine had an interventional design, including two randomized controlled trials (Gascon et al., 2020; Sobko et al., 2020), five non-randomized controlled trials (Brown et al., 2022; Hui et al., 2019; Nielsen et al., 2020; Roslund et al., 2020; Roslund et al., 2021) and two before-after interventional studies (Grönroos et al., 2019; Selway

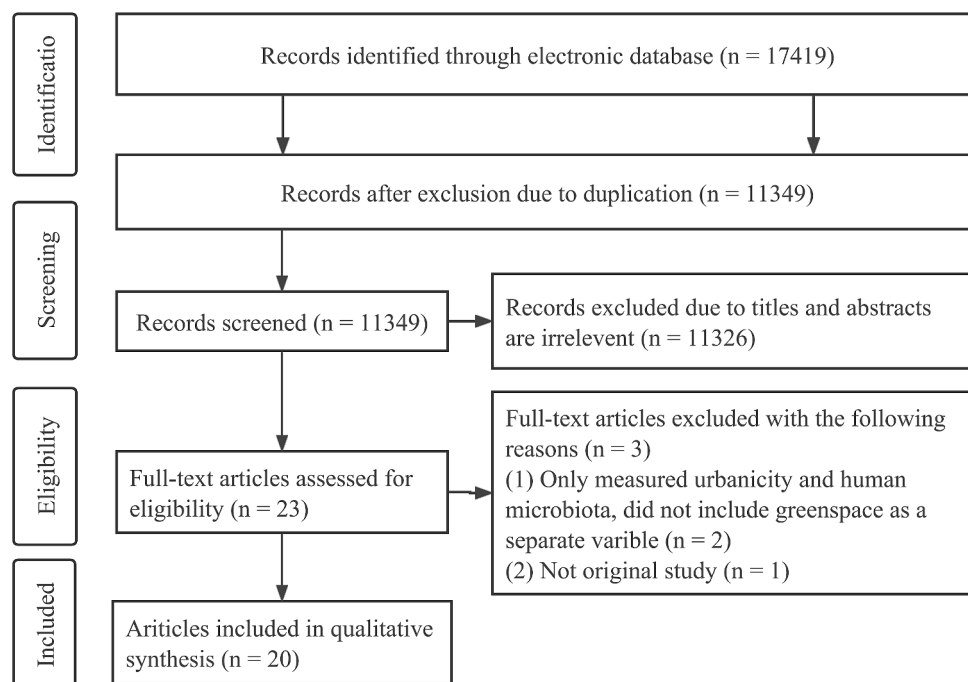


Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart for the study selection.

**Table 3**  
Characteristics of the included studies.

Author/year	Study characteristics			Greenspace exposure assessment			Determination of microbiota			Covariates
	Participants	Country	Design	Metrics	Radius	Source	Sample type	Technique	metrics	
I. Hanski; 2012	118 children aged 14 to 18	Finland	Cross-sectional study	Percentage of agricultural land and forest	3000 m	CORINE20-00 land cover database	Skin	16S rRNA; V1-V3 region	Principal component positively correlated with the diversity of protobacteria	/
L. Ruokolainen; 2015	118 children and adolescents aged 14 to 18	Finland and Estonia	Cross-sectional study	Percentage of agricultural land and forest	3000 m	CORINE2006 landcover database	Skin	16S rRNA; V1-V3 region	Relative abundance of taxa	/
A L. Pearson; 2019	48 dead adults with mean age 43	USA	Cross-sectional study	A binary variable that whether any green remediation (including city parks, trees and urban farms),	400 m	Public records	Mouth, nasal, rectum, eyes	16S rRNA; V4 region	Alpha-diversity: Taxon richness; Simpson's diversity; Simpson's richness; Beta-diversity: Bray-Curtis distances, Relative abundance of genera	Age, ethnicity, BMI, circumstance of death, summer death, outdoor death, and poverty, stratified by sex.
C. C. Nielsen; 2020	355 infants aged 4 months	Canada	Cross-sectional study	A binary variable that whether any natural environment around home.	500 m; 1000 m	uPLVI. map	Gut	16S rRNA; V4 region	Alpha-diversity: Chao1, Shannon and Simpson index; Beta-diversity: Bray-Curtis distances.	Infants' age, season at time of fecal sample collection, breastfeeding status, and birth mode-antibiotic use
A. L. Pearson; 2020	126 decedents	USA	Cross-sectional study	Mean and standard deviation of NDVI; percentage of trees and grassland	100 m	National Agriculture Imagery Program MODIS	Nasal, oral and gut	16S rRNA; V1-V3 region	Alpha-diversity The number of ASVs; Faith PD; Shannon index; evenness.	Sex, age, death type, season, ethnicity and BMI.
A. Gisler; 2021	47 infants	Canada	Cohort study	NDVI	250 m	MODIS	Nasal	16S rRNA; V3-V5 region	Alpha-diversity: Shannon index; Species richness. Relative abundance of genera	date of swab, siblings, gender, childcare at swab, age at swab, feeding type at swab, delivery mode, vaccination and parental smoking
Ruth. Bowyer; 2022	2443 adults with mean age of 60.3	UK	Cross-sectional study	Percentage of greenspace	800 m; 3000 m; 5000 m	25 m <sup>2</sup> Land Cover Map of Great Britain 2015	Gut	16S rRNA; V4 region	Beta-diversity: Bray-Curtis distance. Relative abundance of genera.	Family structure, library size, geographic region, BMI, healthy eating index, frailty index, age, antibiotic use in the previous month, highest educational attainment and the area-based Index of Multiple Deprivation
Kun-Peng Wu; 2022	1758 adults with mean age of 47.5	China	Cross-sectional study	NDVI, EVI	250 m; 500 m; 1000 m	MODIS	Gut	16S rRNA; V3-V4 region	Alpha-diversity: Shannon index; Chao1; ACE; The number of ASVs. Relative abundance of taxa.	Age, gender, residence, years of education, annual household income, BMI, antibiotic use, total physical activity, pure alcohol intake, smoking status, weekly nutrition consumption, and the season of investigation
R. Gacesa; 2022	8298 people aged 4 to 84	Netherlands	Cross-sectional study	NDVI	100 m	LANDSAT 5 satellite image	Gut	shotgun metagenomes	Relative abundance of species.	Age, sex, BMI, Bristol stool scale of the fecal sample and technical factors (DNA concentration, sequencing read depth, sequencing batch and sampling season).
Yi-Dan Zhang; 2023	360 palm samples, 255 forehead samples, 9219 gut samples and 899 oral samples	34 countries	Cross-sectional study	NDVI, EVI	250 m; 500 m; 1000 m	MODIS	Forehead, Palm, gut, oral	16S rRNA; V4 region	Alpha-diversity: The number of ASVs; Evenness, Shannon index. Beta-diversity: Bray-Curtis distances. Relative abundance of genera.	Age, sex, ethnicity, season of samples collection, population destiny.
Aji Kusumaning Asri; 2023	47 children	China	Cross-sectional study	NDVI	250 m; 500 m; 750 m; 1000 m	MODIS	Nasal	16S rRNA; V3-V4 region	Alpha-diversity: The number of species; Species diversity; Species richness. Relative abundance of genera (Dolosisgranulum,	Age, sex, childcare center, siblings, secondhand smoke, pet, fuel, cockroaches, cleaning frequency

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Table 3 (continued)

Author/year	Study characteristics			Greenspace exposure assessment			Determination of microbiota			Covariates
	Participants	Country	Design	Metrics	Radius	Source	Sample type	Technique	metrics	
N.Nurminen; 2018	14 healthy adults aged 27–63	Finland	Randomized controlled trial	Exposure by rubbing their hands with a soil- and plant-based materials	/	/	Skin gut	16S rRNA; V4 region	Corynebacterium, Staphylococcus, Moraxella, Haemophilus, and Streptococcus) Alpha-diversity: Shannon index. Relative abundance of taxonomy	Gender, age, pet ownership and dwelling type
M. Grönroos; 2019	two healthy adults	Finland	Before-after interventional study	(1) Direct hand exposure to eight composted soils. (2) Exposure via fabric packets-plant based materials ;	/	/	Skin	16S rRNA; V1-V3 region	Alpha-diversity: Shannon index; Richness. Beta-diversity: distances.	/
N. Hui; 2019	Five adults aged 30 to 50	Finland	Non-randomized controlled trial	Contact with microbial inoculant forest and agricultural materials	/	/	Skin	16S rRNA; V3-V4 region	Alpha-diversity: Shannon index; Simpson index; Richness; Evenness. Beta-diversity: Bray-Curtis distance. Relative abundance of taxa.	/
Mireia Gascon; 2020	16 healthy adults	USA	Non-randomized controlled trial	Gardening activity	/	/	Gut, oral, palm; forehead	16S rRNA; V4 region	Alpha-diversity: Shannon index. Beta-diversity: Bray-Curtis distance. Relative abundance of taxa.	/
C. A. Selway; 2020	90 skin samples and 90 nasal samples from 3 adults	Australia; UK; India	Before-after interventional study	Exposed to air, soil, and leaves in greenspace	/	/	Gut	16S rRNA; V3-V4 region	Alpha-diversity: observed species; Faith PD. Beta-diversity: Bray-Curtis distance. Relative abundance of taxa.	/
T. Sobko; 2020	54 children aged 7	China	Randomized controlled trial	Nature activity once per week, totally 10 weeks	/	/	Gut	16S rRNA; V3-V4 region	Alpha-diversity: Shannon index. Relative abundance of taxonomy	/
M. I. Roslund; 2020	75 children aged 3 to 5	Finland	Non-Randomized controlled trial	Increased forest vegetation in kindergartens	/	/	Skin, gut	16S rRNA; V4 region	Alpha-diversity: Shannon index and richness in total bacterial community, phylum, and class level (skin); Shannon index at order level (gut). Beta-diversity: Bray-Curtis Distance in genus Faecalibacterium (gut) and family Ru-minococcaceae (gut).	Gender

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Table 3 (continued)

Author/year	Study characteristics			Greenspace exposure assessment			Determination of microbiota			Covariates
	Participants	Country	Design	Metrics	Radius	Source	Sample type	Technique	metrics	
M. I. Roslund; 2021	89 children aged 3 to 5	Finland	Non-Randomized controlled trial	Increased forest vegetation in kindergartens	/	/	Oral, skin, gut	16S rRNA; V4 region	Relative abundance of taxa at family level (gut), Alpha-diversity: Shannon index, Beta-diversity: Bray-Curtis Distance.	Age, gender and time point
M. D. Brown; 2022	56 participants aged 5–18 years from 19 families	USA	Non-Randomized controlled trial	Within the gardening families, the primary gardener needed to garden at least 30 min per week in the gardening season (April to August).	/	/	Gut	16S rRNA; V4 region	Relative abundance of taxa Alpha-diversity: The number of features; Faith's PD; Beta-diversity: Unweighted-uniFrac, Bray-Curtis distance, Jaccard distance. Relative abundance of taxa.	Age, gender, residence, years of education, annual household income, BMI, antibiotic use, total physical activity, pure alcohol intake, smoking status, weekly nutrition consumption, and the season of investigation

Abbreviations: CORINE, Coordination of Information on the Environment; uPLVI, Urban Primary Land and Vegetation Inventory; PERMANOVA, Permutational Multivariate Analysis of Variance; NDVI, Normalized Difference Vegetation Index; sd, standard deviation; MODIS, Moderate-Resolution Imaging Spectroradiometer; ASVs, Amplicon Sequence Variants; Faith's PD, Faith's phylogenetic diversity; EVI, Enhanced Vegetation Index. /: not applicable.

et al., 2020). Sample size ranged from two (Grönroos et al., 2019) to 9219 (Zhang et al., 2023). Eight studies focused on infants and children (Asri et al., 2023; Gisler et al., 2021; Hanski et al., 2012; Nielsen et al., 2020; Roslund et al., 2020; Roslund et al., 2021; Ruokolainen et al., 2015; Sobko et al., 2020), one was conducted in a cohort with an older population (Bowyer et al., 2022), and the remaining was carried out in adults (Brown et al., 2022; Gacesa et al., 2022; Gascon et al., 2020; Grönroos et al., 2019; Hui et al., 2019; Nurminen et al., 2018; Pearson et al., 2020; Pearson et al., 2019; Selway et al., 2020; Wu et al., 2022; Zhang et al., 2023).

### 3.1.1. Greenspace exposure assessment

In the observational studies that explored the association between greenspace exposure and human microbiota, greenspace exposure was assessed using objective metrics, which are derived from satellite images or land maps combined with participants' residential locations. This kind of assessment ensures that greenspace exposure is allocated to each individual and minimizes the nondifferential measurement bias that could bias the effect estimates toward null. Specifically, among the 11 observational studies, six utilized the NDVI driven from sources including the Moderate Resolution Imaging Spectroradiometer with a 250-m resolution (Asri et al., 2023; Gisler et al., 2021; Wu et al., 2022; Zhang et al., 2023), Landsat remote sensing images with a 30 m resolution (Gacesa et al., 2022) and American National Agriculture Imagery Program with a 1 m resolution (Pearson et al., 2020). Four studies used the percentage of greenspace cover obtained from Coordination of Information on the Environment (CORINE) land cover database (Hanski et al., 2012; Ruokolainen et al., 2015) or land cover maps specific to the study regions (Bowyer et al., 2022; Pearson et al., 2020). Two studies employed binary variables indicating the presence of any greenspace around residential locations, sourced from either public record (Pearson et al., 2019) or the Urban Primary Land and Vegetation Inventory of the city of Edmonton, which had a resolution of 30 m (Nielsen et al., 2020). The buffer size of these objective metrics ranged from 100-m (Pearson et al., 2020) to 5000-m (Bowyer et al., 2022) radii around residential locations. Additionally, seven studies measured greenspace exposure before microbial sampling (Pearson et al., 2019; Nielsen et al., 2020; Pearson et al., 2020; Gisler et al., 2021; Gacesa et al., 2022; Zhang et al., 2023; Asri et al., 2023), while the remaining four studies measured greenspace exposure levels during the same period as microbial sampling, which weakens the causality of the observed associations (Hanski et al., 2012; Ruokolainen et al., 2015; Bowyer et al., 2022; Wu et al., 2022).

Regarding the nine interventional studies, three employed interventions that required participant to rub their hands with soil- and plant-based materials once with a duration of 20 s (Grönroos et al., 2019; Hui et al., 2019) or for 20 s per day over a two-week period (Nurminen et al., 2018). Four studies instructed participants to engage in activities in urban parks and neighborhood gardens from 3 h to several weeks (Brown et al., 2022; Gascon et al., 2020; Selway et al., 2020; Sobko et al., 2020). Two covered a portion of the gravel in kindergartens with vegetations for varying duration, ranging from 28 days to two years (Roslund et al., 2020; Roslund et al., 2021).

### 3.1.2. Outcome assessment

The 20 studies explored microbial communities in multiple human body sites, including the gut, skin, oral cavity, nasal cavity, and ocular region. The gut microbiota was the most frequently studied with, with 13 studies (Bowyer et al., 2022; Brown et al., 2022; Gacesa et al., 2022; Gascon et al., 2020; Nielsen et al., 2020; Nurminen et al., 2018; Pearson et al., 2020; Pearson et al., 2019; Roslund et al., 2020; Roslund et al., 2021; Sobko et al., 2020; Wu et al., 2022; Zhang et al., 2023), followed by the skin microbiota with ten studies (Gascon et al., 2020; Grönroos et al., 2019; Hanski et al., 2012; Hui et al., 2019; Nurminen et al., 2018; Roslund et al., 2020; Roslund et al., 2021; Ruokolainen et al., 2015; Selway et al., 2020; Zhang et al., 2023). Oral (Gascon et al., 2020;

**Table 4**  
Risk of bias for each study.

Study	Critical Criteria <sup>a</sup>			Non-critical Criteria <sup>a</sup>					Overall RoB rank <sup>b</sup>
	Exposure assessment	Outcome assessment	Confounding bias	Selection bias	Attrition/Exclusion bias	Selective Reporting bias	Conflict of interest	Other Sources of bias	
I. Hanski/2012	Low	Low	Probably High	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Serious
L. Ruokolainen/2015	Low	Low	Probably High	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Serious
A. L. Pearson/2019	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
C. C. Nielsen/2020	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	High	Not serious
A. L. Pearson/2020	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
A. Gisler/2019	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
Ruth C.E. Bowyer/2021	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
Kunpeng Wu/2022	Low	Low	Probably High	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Serious
R. Gacesa/2022	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
Yi-Dan Zhang/2023	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
A.K.Asir/2023	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
N.Nurminen/2018	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
Mireia Gascon/2019	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
T. Sobko/2019	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
M. Grönroos/2019	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	High	Not serious
N. Hui/2019	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	High	Not serious
C. A. Selway/2020	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	High	Not serious
M. I. Roslund/2020	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
M. I. Roslund/2021	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
M. D. Brown/2022	Low	Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Probably Low	Not serious
Risk of bias rating	Low		Probably low		Probably High		High		

<sup>a</sup>Risk of bias assessment was conducted for each study using the National Institutes of Environmental Health Sciences National Toxicology Program Office of Health Assessment and Translation (OHAT) tool (OHAT, 2015) and the University of California at San Francisco Navigation Guide (Lam et al., 2016; Woodruff and Sutton, 2014). Each of domain was evaluated as “low”, “probably low”, “probably high”, or “high” risk according to specific criteria.

<sup>b</sup>Studies were categorized into three overall RoB rating: “Not serious” RoB: study must be rated as “Low” or “Probably Low” RoB for critical criteria and have most non-critical criteria answered “Low” or “Probably Low”; “Serious” RoB: study meets criteria for neither “Not serious” RoB nor “Very serious” RoB; “Very serious” RoB: study must be rated as “High” or “Probably High” RoB for critical criteria and have most non-critical criteria answered “High” or “Probably High”. RoB, Risk of bias.

Pearson et al., 2020; Pearson et al., 2019; Roslund et al., 2021; Zhang et al., 2023) and nasal microbiota (Asri et al., 2023; Gascon et al., 2020; Gisler et al., 2021; Pearson et al., 2020; Pearson et al., 2019) were each reported in five studies, while ocular microbiota was examined in only one study (Pearson et al., 2019). Most of the studies used amplicon sequencing targeting the 16S rRNA gene (V1-V5 region) for microbiota determination. Only one study employed shotgun metagenomic sequencing (Gacesa et al., 2022). Pipelines using for bioinformatic analysis varied between studies. Eleven used Quantitative Insights Into Microbial Ecology (QIIME) or QIIME2 (Pearson et al., 2019; Nielsen et al., 2020; Pearson et al., 2020; Gisler et al., 2021; Bowyer et al., 2022; Zhang et al., 2023; Nurminen et al., 2018; Gascon et al., 2020; Selway et al., 2020; Sobko et al., 2020; Brown et al., 2022), six used Mothur (Hanski et al., 2012; Ruokolainen et al., 2015; Asri et al., 2023; Hui, 2019; Roslund; 2020; Roslund; 2021), and two used Usearch pipelines (Wu et al., 2022; Asri et al., 2023). All but one study (Gacesa et al., 2022) used Greengenes, SLIVA database or Ribosomal Database Project classifier as a reference for defining bacterial taxa (Table S8). The outcomes reported by these studies included three aspects: (i) microbial alpha-diversity, which represents the richness, evenness, and diversity of taxa and measured using various alpha-diversity indexes such as the number of operational taxonomic units (OTUs) or amplicon sequence variates (ASVs), Pielou’s evenness, Shannon index, and Faith’s phylogenetic diversity (PD). (ii) microbial beta-diversity, which reflects differences in microbial composition between samples, was measured using various distance metrics (e.g., Bray-Curtis distance and UniFrac distance). (iii) the relative abundance of specific taxa at different taxonomic levels between groups of samples, ranging from phyla to species.

### 3.2. Risk of bias assessment

The risk of bias assessment was summarized in Table 4 and visualized in Fig. S1. Three of the included studies were rated as having “serious” RoB (Table 4) (Hanski et al., 2012; Ruokolainen et al., 2015; Wu et al., 2022). These studies may have high or probably high confounding bias, either due to under-adjustment for any potential confounder (Hanski et al., 2012; Ruokolainen et al., 2015), or over-adjustment for possible mediators between greenspace exposure and human gut microbiota, such as physical activity (Wu et al., 2022). Additionally, four studies may have a probably high risk of bias from other source, stemming from inappropriate statistical approaches and limited sample size. One study conducted subgroup analyses but did not statistically compare the estimates from subgroups (Nielsen et al., 2020). The reliability of intervention effects in three studies may be compromised by their small sample sizes (e.g., two to five participants) (Grönroos et al., 2019; Hui et al., 2019; Selway et al., 2020). The detailed risk bias assessments of each study were presented in the supplementary materials.

### 3.3. Associations between greenspace and the human gut microbiota

Thirteen studies, including seven cross-sectional studies (Bowyer et al., 2022; Gacesa et al., 2022; Nielsen et al., 2020; Pearson et al., 2020; Pearson et al., 2019; Wu et al., 2022; Zhang et al., 2023) and six interventional studies (Brown et al., 2022; Gascon et al., 2020; Nurminen et al., 2018; Roslund et al., 2020; Roslund et al., 2021; Sobko et al., 2020) explored the association between greenspace and the human gut microbiota. In total, twelve of these studies reported that greenspace



**Table 5**  
Summary of the main findings of included studies.

Author/year	Alpha-diversity	Beta-diversity	Relative abundance of taxa
<i>Gut microbiota</i>			
A. L. Pearson; 2019	Green remediation-richness ( $\beta$ :1.99, 95 % CI, 1.61, 2.37)	No significant association were found between green remediation and gut microbial beta-diversity.	(+) Genera: Pedobacter, Agrobacterium, Comamonadaceae other, Pseudomonas, Lactobacillus, Luteimonas. (-) Genera: Xanthomonadaceae unspecified, Lactobacillaceae unspecified.
C. C. Nielsen; 2020	Natural environment-Shannon index (OR:0.63, 95 %CI:0.40–0.98) Natural environment-Simpson index (OR:0.63, 95 %CI:0.41–0.98).	Beta-diversity was statistically significant with exposure to a natural space among infants not breastfed and living with pets.	(+): Akkermansia; Enterobacteriales (only in all formula-fed infants) (-): Clostridiales (only in all formula-fed infants)
A. L. Pearson; 2020	NDVI sd - the number of ASVs ( $\beta$ :187.51, 95 % CI:-370.45- -4.57) NDVI sd - Faith PD ( $\beta$ :17.27, 95 %CI:-32.41- -2.14);	/	/
Ruth. Bowyer; 2022	/	Beta-diversity was associated with 3000 m greenspace percentage, (PERMANOVA test: $R^2 = 0.002$ , $P = 0.003$ ).	(+): Roseburia, Anaerostipes, Roseburia inulinivorans; Ruminococcaceae_UCG_014; Lachnospiraceae. (-): Escherichia/Shigella, Haemophilus
Kun-Peng Wu; 2022	NDVI <sub>250m</sub> - Shannon index: ( $\beta$ :0.108, 95 % CI:0.027–0.188); EVI <sub>250m</sub> - Shannon index: ( $\beta$ :0.102, 95 %CI:0.02–0.184), NDVI <sub>250m</sub> - ACE ( $\beta$ :0.153, 95 %CI:0.077–0.209); EVI <sub>250m</sub> - ACE ( $\beta$ :0.151, 95 %CI:0.074–0.228), NDVI <sub>250m</sub> - Chao1 ( $\beta$ :0.153, 95 %CI:0.076–0.229); EVI <sub>250m</sub> - Chao1 ( $\beta$ :0.149, 95 %CI:0.072–0.227); NDVI <sub>250m</sub> - the number of ASVs ( $\beta$ :0.154, 95 %CI:0.077–0.232); EVI <sub>250m</sub> - the number of ASVs ( $\beta$ :0.152, 95 % CI:0.073–0.23).	/	(+): Firmicutes (phylum), Clostridia (class), Subdivision3 (class), Ruminococcaceae (family), Eubacteriaceae (family), Clostridiales (order), Actinomycetales (order), Turicibacter (genus), Romboutsia (genus), Macrocooccus (genus), Lachnospiraceae (genus), Kurthia (genus), Klebsiella (genus), Gemmiger (genus), Alistipes (genus), Peptostreptococcaceae (genus). (-): Euryarchaeota (phylum), Methanobacteriaceae (family), Methanobacteria (class), Methanobacteriales (order), Methanobrevibacter (genus), Erysipelotrichaceae incertae sedis (genus).
R. Gacesa; 2022	/	/	(+) species: Bacteroides plebeius; Desulfovibrio piger, Mitsukella unclassified, (-) species: Bacteroides fragilis, Clostridium bolteae; Bilophila wadsworthia, Flavonifractor plautii, Anaerotruncus colihominis.
Yi-Dan Zhang; 2023	NDVI <sub>250m</sub> -the number of ASVs ( $\beta$ :7.63, 95 % CI:1.65–13.61); EVI <sub>250m</sub> -the number of ASVs ( $\beta$ :9.00, 95 %CI:1.23, 16.78)	Significant differences at the genus and ASV levels were observed between the two greenness groups (low vs. high) in gut microbiota (NDVI <sub>500m</sub> : PERMANOVA: $F = 4.0$ , $p = 0.001$ ).	(+) genera: Bifidobacterium, Haemophilus, Clostridium; (-) Genera: Bacteroides, Parabacteroides, Lachnospira, Holdemania, Blautia, Coprococcus, Eubacterium, Streptococcus, Odoribacter, Anaerococcus (+) Phyla: Bacteroides.
N.Nurminen; 2018	After exposure, interventional group had higher Shannon index compared with control groups, ( $p = 0.029$ ).	/	
Mireia Gascon; 2020	No significant difference was observed.	No significant difference was observed.	(+) Phylum: Verrucomicrobi
T. Sobko; 2020	After intervention, Chao1 richness of phyla Bacteroidetes significantly decreased ( $p = 0.02$ ), while that of Proteobacteria increased ( $p = 0.03$ ).	/	(+) Species: An unclassified species of Blautia (-) Species: An unknown species of Bacteroides, Parabacteroides distasonis, an unclassified Acidaminococcus, an unclassified Dialister, an unclassified Bilophila.
M. I. Roslund; 2020	Shannon index of family Ruminococcaceae increased after intervention.	Beta-diversity of family Ruminococcaceae (PERMANOVA; $F = 0.049$ ; $p = 0.027$ ) in gut microbiota was different between control and interventional group after intervention.	(-) Order: Clostridiales
M. I. Roslund; 2021	/	/	(-) Genus: Clostridium sensu stricto
M. D. Brown; 2022	Gardening families' fecal samples tended to have a higher Shannon index at peak garden season compared to the control group.	Beta diversity analysis including unweighted UniFrac ( $p = 0.07$ ), Bray Curtis ( $p = 0.02$ ) and Jaccard ( $p = 0.01$ ) distances varied between gardening families at peak season compared to control family distances at peak season	(+) Species: Bacteroides stercoris, Alistipes inops, Bacteroides ovatus. (+) Genus: Romboutsia, Terrisporobacter
<i>Skin microbiota</i>			
I. Hanski; 2012	The diversity of preteobacteria was higher on the skin of individuals living in an environment with more forest and agricultural land.	/	/
L. Ruokolainen; 2015	/	/	(+) Phylum: Proteobacteria.
Yi-Dan Zhang; 2023	NDVI <sub>500m</sub> -the number of ASVs: ( $\beta$ :262.49, 95 % CI:85.99–438.99); EVI <sub>500m</sub> -the number of ASVs: ( $\beta$ :483.48, 95 %CI:261.81–705.15)	Significant differences at the genus and ASV levels were observed between the two greenness groups (low vs. high) in the palm microbiota (NDVI <sub>500m</sub> : $F = 4.0$ , $p = 0.002$ ).	(+) Genera: Lactobacillus, Staphylococcus, Actinomycetospora, Brachy bacterium, Brevibacterium, Dietzia, Bifidobacterium, Oscillospira, Solibacter, Leuconostoc, Pseudoxanthomonas, Acetobacter, Vibrio, Salinisphaera.

(continued on next page)

Table 5 (continued)

Author/year	Alpha-diversity	Beta-diversity	Relative abundance of taxa
N.Nurminen; 2018	Shannon index of the skin microbiota increased clearly in two individuals but the overall change was not significant.	/	(-) Genera: Chryseomicrobium, Anaerococcus, Enhydrobacter, Rubellimicrobium, Lactococcus, Corynebacterium, Finegodia. (+) Phyla: Bacteroidetes, Chlorflexi and Actinobacteria (only in two individuals' skin microbiota).
M. Grönroos; 2019	Exposures increased Shannon index of skin microbiota (t = 3.39, p = 0.004) and the diversity of Acidobacteria (t = 4.51, p < 0.001), Actinobacteria (t = 8.09, p < 0.001), Bacteroidetes (t = 3.39, p < 0.004), Proteobacteria (t = 5.56, p < 0.001) and Alpha- (t = 5.6, p < 0.001), Beta- (t = 2.18, p = 0.045) and Gamma-proteobacteria (t = 4.7, p < 0.001).	Beta-diversity on hands' microbiota significantly changed (PERMANOVA; F = 8.68; p = 0.001).	/
N. Hui; 2019	After intervention, skin microbial Shannon index (p < 0.001), Simpson index (p < 0.001) and richness (p < 0.001) increased;	Microbial composition changed (PERMANOVA; R <sup>2</sup> = 0.536, P = 0.002).	(+) Genera (after contact with microbiota-enriched sand): Luteimonas, Planomicrobium, Chryseobacterium (-) Genera (after contact with microbiota-enriched sand): Acinetobacter, Burkholderia, Corynebacterium, Ralstonia, Rothia, Staphylococcus (+) Genera: Sphingomonas. (-) Genera: Micrococcus, Staphylococcus, Tetrasphera, Corynebacterium, Paracoccus, Acinetobacter, Bervundimonas, Cutibacterium.
C. A. Selway; 2020	Observed species and faith PD increased after greenspace exposure (Observed species: p = 0.0002; faith PD: p = 0.0001).	Beta-diversity in skin microbiota changed (PERMANOVA; F = 3.351; p = 0.001). Microbiota in skin samples became more similar to the environmental after exposure.	(-) Genus: Gemella sp., Streptococcus sp. within class Bacilli, Veillonella sp., H. parainfluenzae (Otu000003).
Mireia Gascon; 2020	No significant change was observed.	No significant change was observed.	/
M. I. Roslund; 2020	In the interventional group, Shannon index of Alphaproteobacterial, Proteobacterial and Gammaproteobacterial on skin increased after intervention.	/	/
M. I. Roslund; 2021	Shannon index increased (p = 0.014) in skin samples after one-year intervention.	Beta-diversity were changed in skin microbiota (PERMANOVA; F = 2.473; p = 0.039)	(-) Genus: Gemella sp., Streptococcus sp. within class Bacilli, Veillonella sp., H. parainfluenzae (Otu000003).
<i>Oral microbiota</i>			
A. L. Pearson; 2019	green remediation-richness (β: 1.03, 95 % CI, 0.26, 1.80)	Beta-diversity of oral microbiota were different between people with neighborhood green remediation high and low level (p = 0.005).	(+) Genera: Acidobacteria unspecified, Bacteroides, Faecalibacterium, Lactobacillus, Blautia, Veillonellaceae other
A. L. Pearson; 2020	No significant association was found between greenspace and oral microbial alpha-diversity.	/	/
Yi-Dan Zhang; 2023	No significant association was found between greenspace and oral microbial alpha-diversity.	No significant association were found between greenspace and oral microbial beta-diversity.	(+) Genus: Lautropia (-) Genera: Prevotella, Megasphaera
Mireia Gascon; 2020	No significant difference was observed.	No significant difference was observed.	/
M. I. Roslund; 2021	Shannon diversity of Proteobacteria in saliva increased slightly among intervention children during the 28-day intervention period, and it was higher than that of children in standard day-care centers on day 28, but returned to the baseline level after one-year intervention period.	Beta-diversity in oral samples were different between interventional group and control group at OTU level and within the classes Gammaproteobacteria, Negativicutes, and Fusobacteriia after one-year intervention	(+): Firmicutes (Phyla), Lactobacillaless (Order) (-): Fusobacteriia (Class)
<i>Nasal microbiota</i>			
A. L. Pearson; 2019	Green remediation-Shannon index (β = 1.84, 95 % CI, 1.61, 2.37)	No significant association were found between green remediation and nasal beta-diversity.	(+) Genus: Alloiococcus.
A. L. Pearson; 2020	Percent grassland –the number of ASVs (β: -0.91, 95 % CI, -1.58, -0.25) Percent grassland - Faith PD (β: -0.09, 95 % CI, -0.15, -0.02)	/	/
A. Gisler; 2021	No significant association was observed.	/	No significant association was observed.
Aji Kusumaning Asri; 2023	No significant results of greenness with nasal microbial alpha-diversity in asthmatic children.	/	(-) Genus: Streptococcus.
C. A. Selway; 2020	Observed species and faith PD increased after greenspace exposure. (Observed species: p = 0.0035; faith PD: p = 0.0022)	Beta-diversity of nasal microbiota changed (PERMANOVA; F = 2.887; p = 0.001) after intervention. Nasal microbiota became more similar to the environmental after exposure.	(+) Genera: rare taxa. (-) Genera: Staphylococcus, Lawsonella.
<i>Ocular microbiota</i>			
A. L. Pearson; 2019	No significant association was found between green remediation and ocular microbial alpha-diversity.	No significant association were found between green remediation and ocular microbial beta-diversity.	(+) Genera: Gemella, Gemellaceae other, Firmicutes other, Streptococcaceae unspecified, Lautropia. (-) Genus: Blautia.

Abbreviations: CORINE, Coordination of Information on the Environment; uPLVI, Urban Primary Land and Vegetation Inventory; PERMANOVA, Permutational Multivariate Analysis of Variance; NDVI, Normalized Difference Vegetation Index; sd, standard deviation; MODIS, Moderate-Resolution Imaging Spectroradiometer; ASVs, Amplicon Sequence Variants; Faith's PD, Faith's phylogenetic diversity; 95 %CI, 95 % Confidence interval; EVI, Enhanced Vegetation Index. /: not applicable. (+): The relative abundance of taxa increased with greenspace or increased after intervention. (-): The relative abundance of taxa decreased with greenspace or decreased after intervention.

exposure was associated with higher alpha-diversity ( $n = 7$ ) (Brown et al., 2022; Nurminen et al., 2018; Pearson et al., 2019; Roslund et al., 2020; Sobko et al., 2020; Wu et al., 2022; Zhang et al., 2023), altered overall compositions ( $n = 5$ ) (Bowyer et al., 2022; Brown et al., 2022; Nielsen et al., 2020; Roslund et al., 2020; Zhang et al., 2023), and/or changed relative abundances of taxa ( $n = 12$ ) in gut microbiota (Table 5) (Bowyer et al., 2022; Brown et al., 2022; Gascon et al., 2020; Nielsen et al., 2020; Nurminen et al., 2018; Pearson et al., 2020; Pearson et al., 2019; Roslund et al., 2020; Roslund et al., 2021; Sobko et al., 2020; Wu et al., 2022; Zhang et al., 2023).

### 3.3.1. Observational studies on greenspace and human gut microbiota

Five out of the seven cross-sectional studies detected associations between greenspace exposure and gut microbial alpha-diversity (Table 5) (Nielsen et al., 2020; Pearson et al., 2020; Pearson et al., 2019; Wu et al., 2022; Zhang et al., 2023). Among these, three reported significant positive associations (Pearson et al., 2019; Wu et al., 2022; Zhang et al., 2023), while two indicated negative associations (Nielsen et al., 2020; Pearson et al., 2020). In a study of 1758 Chinese adults, higher NDVI levels in 250 m, 500 m, and 1000 m radii around the home were positively associated with gut microbial alpha-diversity, measured by the Shannon index, *chao1*, ACE index and the number of ASVs (Wu et al., 2022). Similarly, a study across 34 countries covering 9219 individuals reported a positive association between NDVI and the number of ASVs in gut microbiota (Zhang et al., 2023). Another study in the USA also found a positive association between the neighborhood green remediation (i.e., park, tree and urban farm within a 400 radius of homes) and the richness of gut microbial communities in 14 women (Pearson et al., 2019). In contrast, a study among 126 American adults identified a negative association between the standard deviation of NDVI within a 100 m radius of homes and the number of ASVs, as well as Faith's phylogenetic diversity (Faith's PD) in gut microbiota (Pearson et al., 2020). Another study involving 355 Canadian infants found that greenspace within a 500 m radii around home was associated with lower Simpson index and Shannon index in the gut microbiota (Nielsen et al., 2020).

Four cross-sectional studies also explored the association between greenspace and gut microbial beta-diversity (Bowyer et al., 2022; Nielsen et al., 2020; Pearson et al., 2019; Zhang et al., 2023). Among them, a study of a UK cohort of twins (Bowyer et al., 2022), a study in 355 Canadian infants (Nurminen et al., 2018), and a study conducted across 34 countries (Zhang et al., 2023) all demonstrated a significant difference of gut microbial beta-diversity across varying levels of residential greenspace, indicating an altered overall composition with increased exposure to greenspace. However, a study in 48 American adults did not observe any significant differences in beta-diversity based on neighborhood greenspace (Pearson et al., 2019).

Five studies have reported associations between greenspace and relative abundances of specific taxa (Bowyer et al., 2022; Nielsen et al., 2020; Pearson et al., 2019; Wu et al., 2022; Zhang et al., 2023), encompassing 29 taxa that increased with greenspace exposure and 25 taxa that decreased with greenspace exposure (Table 5). However, there is a lack of consistency in the specific bacterial taxa associated with greenspace exposure across these diverse studies. The relative abundance of some beneficial genera or species showed an increase with greenspace exposure, including *Akkermanisia* (Nielsen et al., 2020), *Rosburia* (Bowyer et al., 2022), *Ruminococcaceae* UCG\_014 (Bowyer et al., 2022), *Lachnoclostridium* (Bowyer et al., 2022), *Lachnospiraceae*, *Gemmiger* (Wu et al., 2022), *Anerostipes* (Zhang et al., 2023), *Bifidobacterium* (Zhang et al., 2023), and the species *Bacteroides plebius*

(Gacesa et al., 2022). These microbes play a pivotal role in the production of short-chain fatty acids (SCFAs) like propionic acid and acetic acid, which are instrumental in maintaining intestinal barrier integrity, mucus production, and safeguarding against inflammation (Ahn and Hayes, 2021). Meanwhile, among taxa with decreased relative abundance in higher greenspace levels, several were found to be detrimental to human health, including the opportunistic pathogenic genera/species *Escherichia/Shigella* (Bowyer et al., 2022), *Streptococcus* (Zhang et al., 2023), *Clostridium bolteae* (Gacesa et al., 2022), as well as the genera/species related to intestinal inflammation such as *Holdemania* (Zhang et al., 2023) and *Bilophila wadsworthia* (Gacesa et al., 2022).

### 3.3.2. Interventional studies on greenspace and human gut microbiota

Five of the six interventional studies examined changes in gut microbial alpha-diversity following greenspace interventions (Table 5) (Brown et al., 2022; Gascon et al., 2020; Nurminen et al., 2018; Roslund et al., 2020; Sobko et al., 2020), and four of them found significant results (Brown et al., 2022; Nurminen et al., 2018; Roslund et al., 2020; Sobko et al., 2020). Among these, two reported significant increases in Shannon index in overall microbial communities after participants had direct contact with soil and plant-based materials (Nurminen et al., 2018), or engaged in four-months of gardening activities (Brown et al., 2022). In addition, two studies conducted in children measured alpha-diversity at the phyla and family levels. They found increases in Shannon indexes of the phyla *Bacteroidetes* and the family *Ruminococcaceae* in children's gut microbiota after playing in urban parks for 10 weeks (Sobko et al., 2020) and after adding vegetation in kindergartens for 28 days (Roslund et al., 2020), respectively. Beta-diversity was assessed by three studies (Brown et al., 2022; Gascon et al., 2020; Roslund et al., 2020). One study involving 56 adults found a significant difference in gut microbial beta-diversity between those who participated in gardening and those who did not (Brown et al., 2022). Another study with 75 Finnish children also reported that in comparison with the baseline, the gut microbial beta-diversity changed after conducting interventions which included the addition of vegetations in kindergartens (Roslund et al., 2020). The remaining study did not observe a significant difference (Gascon et al., 2020).

All six studies reported changes in specific relative abundances after interventions (Table 5). Three studies reported changes in relative abundance at phyla and order levels. Among them, two studies noted an increase in phyla *Bacteroides* and *Verrucomicrobi* in adults exposed to plant-based material and engaged in gardening activities, respectively (Nurminen et al., 2018; Sobko et al., 2020). In another study, the addition of vegetations to kindergartens for 28 days led to an increased relative abundance of order *Clustidium* in children's gut (Roslund et al., 2020). The remaining three studies delved into changes at the genus or species level. Notably, Roslud et al. observed a decrease in the abundance of the pathogenic bacterium *Clostridium sensu stricto* in the children's gut after adding vegetations to the kindergarten for one year (Roslund et al., 2021). After four months of gardening, participants exhibited an increase in the relative abundance of several genera and species associated with digestion and the maintenance of intestinal health, including *Bacteroides stercoris*, *Alistipes inops*, *Bacteroides ovatus*, *Romboutsia*, and *Terrisporobacter* (Brown et al., 2022). Sobko et al. also reported changes in the relative abundance of several species in the gut of children after ten weeks of playing in urban parks. However, the classification of most species failed, rendering the potential health effects of these change unknown (Sobko et al., 2020).

### 3.4. Association between greenspace and the human skin microbiota

Ten studies investigated the association between greenspace exposure and the human skin microbiota. Three of them were cross-sectional studies (Hanski et al., 2012; Ruokolainen et al., 2015; Zhang et al., 2023), while the remaining were interventional (Gascon et al., 2020; Grönroos et al., 2019; Hui et al., 2019; Nurminen et al., 2018; Roslund et al., 2020; Roslund et al., 2021; Selway et al., 2020). Eight of these studies reported that greenspace exposure was associated with higher alpha-diversity (in seven studies) (Grönroos et al., 2019; Hanski et al., 2012; Hui et al., 2019; Roslund et al., 2020; Roslund et al., 2021; Selway et al., 2020; Zhang et al., 2023), altered compositions (in five studies) (Grönroos et al., 2019; Hui et al., 2019; Roslund et al., 2021; Selway et al., 2020; Zhang et al., 2023), and/or changed relative abundances of taxa (in six studies) (Hui et al., 2019; Nurminen et al., 2018; Roslund et al., 2021; Ruokolainen et al., 2015; Selway et al., 2020; Zhang et al., 2023) in skin microbiota (Table 5).

#### 3.4.1. Observational studies on greenspace and human skin microbiota

Two cross-sectional studies found positive associations between higher percentages of forest and agricultural land cover within 3-km radii around home and diversities and relative abundance of *Proteobacteria* in children's skin (Table 5) (Hanski et al., 2012; Ruokolainen et al., 2015). Additionally, a recent study conducted among 34 countries observed positive associations between NDVI within 500-m radii around homes and the number of ASVs in human palm microbiota (Zhang et al., 2023). Microbial beta-diversity on the palm also significantly differed between people living with high-level and low-level of greenspace (Zhang et al., 2023). Higher abundances of several genera, including potential beneficial members like *Lactobacillus* and *Oscillospira*, were observed in people living with high level of greenspace. The abundances of several genera commonly found in the natural environment (i.e., *Actinomycetospira*, *Brachy bacterium*, and *Dietzia*) were also enriched in people with higher greenspace levels (Zhang et al., 2023).

#### 3.4.2. Interventional studies on greenspace and human skin microbiota

Regarding greenspace intervention and microbial alpha-diversity, five studies found that conducted activities in greenspace (Selway et al., 2020), increasing vegetations in kindergartens (Roslund et al., 2020; Roslund et al., 2021) or contacting soil- and plant-based materials could increase the alpha-diversity (Grönroos et al., 2019; Hui et al., 2019) (i.e., Shannon diversity, observed species and Faith's PD) of the overall bacteria in skin, while two studies did not find significant results (Nurminen et al., 2018; Gascon et al. 2019). Three studies also found that the Shannon indexes in *Alpha-proteobacteria*, *Beta-proteobacteria* and *Gamma-proteobacteria* increased after the interventions including adding kindergartens with forest floor and rubbing participants' hands with soil and plant-based materials (Grönroos et al., 2019; Roslund et al., 2020; Roslund et al., 2021). Additionally, contacting with greenspace features or conducting activities in greenspaces led to changes in overall microbial compositions (Grönroos et al., 2019; Hui et al., 2019; Roslund et al., 2021; Selway et al., 2020). For instance, compositions of skin microbiota were closer to that of the environment and contact materials after walking in urban parks and rubbing hands with plant-based materials (Grönroos et al., 2019; Selway et al., 2020).

Various microbial taxa changed depending on the greenspace type, materials participants contacted, and duration of exposure (Table 5). Specifically, on the palms of participants exposed to microbiota-enriched soil, the relative abundance of several genera containing opportunistic pathogens, such as *Acinetobacter*, *Burkholderia*, *Corynebacterium*, *Ralstonia*, *Rothia*, *Staphylococcus*, decreased (Hui et al., 2019). Roslund et al. found that adding forest vegetation in kindergartens led to a reduction in the relative abundance of opportunistic pathogens (including *Gemella* sp. and *Streptococcus* sp.) on the skin of children aged 3–5 (Roslund et al., 2021). Moreover, Selway et al. observed that walking in urban greenspaces increased the presence of natural sourced

genera (*Sphingomonas*, *Corynebacterium*, and *Fingoldia* etc.) on participants' hands (Selway et al., 2020).

### 3.5. Association between greenspace and human microbiota in other body sites

Several studies explored greenspace exposure and human microbiota at other body sites, including oral cavities, nasal cavities and eyes. Current findings on greenspace and microbiota on these body sites were still mixed (Table 5).

#### 3.5.1. Association between greenspace and human oral microbiota

Five studies, including three cross-sectional studies (Pearson et al., 2020; Pearson et al., 2019; Zhang et al., 2023) and two interventional studies (Gascon et al., 2020; Roslund et al., 2021) explored greenspace exposure and the human oral microbiota. A cross-sectional study involving 48 American adults noted that the presence of parks and trees within a 400-m radius of the home was associated with higher oral microbial richness (Pearson et al., 2019). Nevertheless, the remaining two cross-sectional studies did not observe significant associations between greenspace exposure and oral microbial alpha- and beta-diversity (Pearson et al., 2020; Zhang et al., 2023). Additionally, Zhang et al. found that individuals residing in areas with higher greenspace levels exhibited an enriched relative abundance of *Lautropia*, while the relative abundance of *Prevotella* and *Megasphaera* were lower in these individuals (Zhang et al., 2023).

Among the one interventional study conducted by Roslund et al. 2021, where a part of the gravel was replaced with vegetation in the kindergartens, the community composition of children's salivary microbiota differed at the OTU and class levels between the interventional and control groups. In addition, one year after intervention, the relative abundance of *Lactobacillales* increased while the relative abundance of *Fusobacteria* decreased (Roslund et al., 2021). The other interventional studies conducted by Gascon et al did not find any significant changes in participants' oral microbiota after engaging in gardening activities (Gascon et al., 2020).

#### 3.5.2. Association between greenspace and human nasal microbiota

Five studies, comprising three cross-sectional (Asri et al., 2023; Pearson et al., 2020; Pearson et al., 2019), one cohort (Gisler et al., 2021), and one interventional design (Selway et al., 2020), examined nasal microbiota. Among these, cross-sectional studies reported inconsistent findings. One involving 48 American adults found higher Simpson's diversity in nasal microbiota with the presence of parks and trees within a 400 m radius of homes (Pearson et al., 2019). In contrast, another study of 126 American adults showed a negative association between the percentage of grassland within a 100 m radius and nasal microbial alpha-diversity measured by Faith PD (Pearson et al., 2020). A study in 47 children did not find a significant association between NDVI within a 250-m radius around the home and any alpha-diversity index (Asri et al., 2023). The cohort study involving 47 Canadian infants also observed no significant association between NDVI within a 250-m radius around the home and nasal microbial diversity and composition (Gisler et al., 2021). In addition, an interventional study found that one-hour of walking in urban greenspaces could increase the alpha-diversity in nasal microbiota (Selway et al., 2020). Several commensal genera (i.e., *Staphylococcus* and *Corynebacterium*) decreased and several rare genera increased after this intervention (Selway et al., 2020).

#### 3.5.3. Association between greenspace and human ocular microbiota

Only one study in 48 American adults explored the association between green remediation around home and ocular microbiota (Pearson et al., 2019). They test the association between "having parks, trees, and urban farms" and richness, evenness and Simpson's diversity of ocular microbiota, and stratified by sex. However, no-significant association were observed either in females or males.

**Table 6**  
GRADE assessments of certainty of evidence for greenspace exposure and microbial alpha-diversity.

Outcome	Health effect <sup>a</sup>	Initial rating	Downgrade	Upgrade	Certainty in body of evidence
		Features: controlled exposure, exposure prior to outcome, individual outcome data, comparison used High: meet 4 features Moderate: meet 3 features Low: meet 2 features Very Low: meet 0–1 features	Possible Reasons: Inconsistency, imprecision, Risk of Bias concerns, indirectness, publication bias	Possible reasons: All plausible confounders or other biases increase certainty in the observed effect, large effect observed, dose–response gradient	Low, Very Low, Moderate, or High
Alpha-diversity (Gut microbiota) N = 10	7 of 10 studies support	High Reason: 5 studies met 4 features (Nurminen et al., 2018; Gascon et al., 2020; Sobko et al., 2020; Roslund et al., 2020; Brown et al., 2022), 4 studies met 3 features (Pearson et al., 2019; Pearson et al., 2020; Nielsen et al., 2020; Wu et al., 2022; Zhang et al., 2023), and 1 study met 2 features (Wu et al., 2022)	High -> Moderate Reason: imprecision	No	Moderate
Alpha-diversity (Skin microbiota) N = 9	7 of 9 studies support	High Reason: 5 studies met 4 features (Nurminen et al., 2018; Hui et al., 2019; Gascon et al., 2020; Roslund et al., 2020; Roslund et al., 2021), 3 studies met 3 features (Zhang et al., 2023; Grönroos et al., 2019; Selway et al., 2020), and 1 study met 2 features (Hanski et al., 2012).	High -> Moderate Reason: imprecision	No	Moderate
Alpha-diversity (Oral microbiota) N = 5	2 of 5 studies support	Moderate Reason: 2 studies met 4 features (Gascon et al., 2020; Roslund et al., 2021) and 3 studies met 3 features (Pearson et al., 2019; Pearson et al., 2020; Zhang et al., 2023).	Moderate -> Very Low Reason: Inconsistency and imprecision	No	Very Low
Alpha-diversity (Nasal microbiota) N = 5	2 of 5 studies support	Moderate Reason: 5 studies met 3 features (Pearson et al., 2019; Pearson et al., 2020; Gisler et al., 2021; Asri et al., 2023; Selway et al., 2020).	Moderate -> Very Low Reason: Inconsistency and imprecision	No	Very Low
Alpha-diversity (Ocular microbiota) N = 1	0 of 1 study support	Moderate Reason: 1 study met 3 features (Pearson et al., 2019).	Moderate -> Very Low Reason: Single study and small sample size	No	Very Low

<sup>a</sup>Health effect: the health effects of greenspace exposure refer to increased alpha diversity.

### 3.6. Certainty assessment of evidence on greenspace and human microbiota

#### 3.6.1. Certainty assessment of evidence on greenspace and gut microbiota

For greenspace and human gut microbial alpha-diversity, because 7 of 10 studies found significant positive associations between greenspace and gut microbial alpha-diversity (Nurminen et al., 2018; Sobko et al., 2020; Roslund et al., 2020; Brown et al., 2022; Pearson et al., 2019; Wu et al., 2022; Zhang et al., 2023), and 5 studies met the four main features in study design (Nurminen et al., 2018; Gascon et al., 2020; Sobko et al., 2020; Roslund et al., 2020; Brown et al., 2022) (Table S9), the initial certainty was rated as “high”. However, the evidence was downgraded to “moderate” due to imprecision (Table 6). Similarly, the evidence on greenspace and human gut beta-diversity was rated as “moderate” initially, but was downgraded for imprecision and finally had a “low” certainty for the body of evidence (Table 7). Regarding the relative abundance of specific taxa, the initially rating of evidence was “moderate”, but was downgraded to “very low” because of inconsistency in the observed taxa and imprecision (Table 8).

#### 3.6.2. Certainty assessment of evidence on greenspace and skin microbiota

The initial certainty for evidence on greenspace and skin microbial alpha-diversity was rated as “high” because the study design of more than a half of studies met the four features (Nurminen et al., 2018; Hui et al., 2019; Gascon et al., 2020; Roslund et al., 2020; Roslund et al., 2021), and downgraded to “moderate” due to imprecision (Table 6). Evidence on greenspace and skin microbial beta-diversity was rated as “moderate” certainty initially as half of studies met three features

(Zhang et al., 2023; Grönroos et al., 2019; Selway et al., 2020), and also downgraded to “low” certainty because of imprecision (Table 7). Relative abundance changes associated with greenspaces were found in all six studies (Nurminen et al., 2018; Hui et al., 2019; Roslund et al., 2021; Zhang et al., 2023; Selway et al., 2020; Ruokolainen et al., 2015), but the evidence was finally rated as “very low” due to study design, inconsistent observed taxa, and imprecise results (Table 8).

#### 3.6.3. Certainty assessment of evidence for greenspace and microbiota in other body sites

The GRADE certainty of the evidence on greenspace and human oral microbiota (including alpha-, beta-diversity and relative abundance of taxa) was initially rating as “moderate”, but all downgraded because of inconsistency and imprecision in results. In sum, the certainty of evidence was “very low” (Tables 6–8). The evidence on greenspace and human nasal microbiota (including alpha-diversity, beta-diversity, and relative abundance of taxa) initially rated as “moderate”. However, all these ratings were subsequently downgraded due to inconsistency and imprecision in results. Consequently, the overall certainty of the evidence was assessed as “very low” (Tables 6–8). In addition, the certainty of the evidence for greenspace and human ocular microbiota was very low as the single study on greenspace and human ocular microbiota (Pearson et al., 2019) was initially rated as “moderate” and downgraded due to the small sample size (n = 48) (Tables 6–8).



**Table 7**  
GRADE assessments of certainty of evidence for greenspace exposure and microbial beta-diversity.

Outcome	Health effect a	Initial rating	Downgrade	Upgrade	Certainty in body of evidence
		Features: controlled exposure, exposure prior to outcome, individual outcome data, comparison used High: meet 4 features Moderate: meet 3 features Low: meet 2 features Very Low: meet 0–1 features	Possible Reasons: Inconsistency, imprecision, Risk of Bias concerns, indirectness, publication bias	Possible reasons: All plausible confounders or other biases increase certainty in the observed effect, large effect observed, dose–response gradient	Low, Very Low, Moderate, or High
Beta-diversity (Gut microbiota) N = 7	5 of 7 studies support	Moderate Reason: 3 studies met 4 features (Gascon et al., 2020; Roslund et al., 2020; Brown et al., 2022), 2 studies met 3 features (Pearson et al., 2019; Nielsen et al., 2020; Zhang et al., 2023), and 1 study met 2 features (Bowyer et al., 2022).	Moderate -> Low Reason: imprecision	No	Low
Beta-diversity (Skin microbiota) N = 6	5 of 6 studies support	Moderate Reason: 3 study met 4 features (Hui et al., 2019; Gascon et al., 2020; Roslund et al., 2021) and 3 studies met 3 features (Zhang et al., 2023; Grönroos et al., 2019; Selway et al., 2020).	Moderate -> Low Reason: imprecision	No	Low
Beta-diversity (Oral microbiota) N = 4	2 of 4 studies support	Moderate Reason: 2 studies met 4 features (Gascon et al., 2020; Roslund et al., 2021) and 2 studies met 3 features (Pearson et al., 2019; Zhang et al., 2023).	Moderate -> Very Low Reason: Inconsistency and imprecision	No	Very Low
Beta-diversity (Nasal microbiota) N = 2	1 of 2 studies support	Moderate Reason: 2 studies met 3 features (Pearson et al., 2019; Selway et al., 2020).	Moderate -> Very Low Reason: Inconsistency and imprecision	No	Very Low
Beta-diversity (Ocular microbiota) N = 1	0 of 1 study support	Moderate Reason: 1 study met 3 features (Pearson et al., 2019).	Moderate -> Very Low Reason: Single study and small sample size	No	Very Low

<sup>a</sup>Health effect: the health effects of greenspace exposure refer to changed beta diversity.

## 4. Discussion

### 4.1. Main findings

Our systematic review of the available evidence on the association of the greenspace exposure with the human microbiota retrieved eleven observational studies and nine interventional studies. These studies were mostly conducted in Europe and North America and mainly investigated microbiota in gut and skin samples. Moderate to low certainty of evidence supported the associations of greenspace exposure with increased alpha-diversity and altered overall composition of human gut and skin microbiota, with increases in the relative abundance of probiotics and decreases in the relative abundance of pathogens. However, published studies have high heterogeneity in study designs, greenspace exposure assessments, and participants' characteristics. In addition, the number of studies was still small, and several studies had relatively high risk of bias.

### 4.2. Comparisons with prior reviews and interpretations

To our best knowledge, this is the most comprehensive systematic review on greenspace exposure and human microbiota to date. We are aware of one scoping review and one systematic review on this topic (Tischer et al., 2022). The scoping review summarized eight interventional studies up to 25 September 2021, with most reporting that short-term interventions increased human microbial richness and diversity in specific taxa groups. The previous systematic review, which focused on greenspace and human gut microbiota, included seven studies (Van Pee et al., 2023). The majority reported a positive association between greenspace and the gut microbial diversity, richness, and evenness. Furthermore, bacteria positively associated with greenspace are mainly beneficial for human health and bacteria negatively associated with

greenspace are linked to adverse health outcomes. Our review's findings generally align with the previous reviews. By comparison, we systematically reviewed more than twice the number of studies ( $n = 20$ ), encompassing all studies in the prior review (except for two studies that only focused on urbanicity and gut microbiota), as well as the most recent observational and interventional and studies on greenspace and human microbiota. Additionally, we also assessed the risk of bias in each study using OHAT tools. Consequently, the current review provides more comprehensive evidence on greenspace and human microbiota than the two prior reviews.

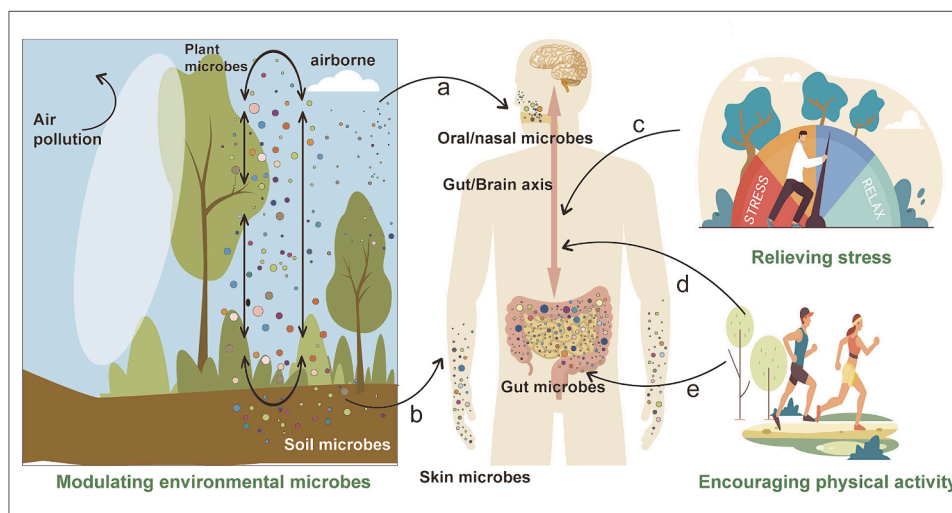
### 4.3. Potential mechanisms

The associations between greenspace exposure and human microbiota are consistent with several following mechanisms (Fig. 2). First, natural environments exhibit a higher microbial diversity compared to urban built environments (Kirjavainen et al., 2019). The root systems of plants release substantial amounts of organic compounds into the soil, nourishing microbes and thus diversifying the soil microbiota (Bonkowski et al., 2009). The phyllosphere also hosts a large number of microbes originating from soil and seeds (Zhou et al., 2020). During processes like plant evapotranspiration, rain splash and wind gusts, plant and soil microbes can become airborne. When people conduct activities in greenspaces, many microbes may adhere to the skin, be inhaled into the respiratory tract or be swallowed into the digestive tract, thus altering the human microbiota. Second, ambient particulate matter has been shown to carry pathogenic bacteria, and several air pollutants, such as  $\text{NO}_2$  can disrupt human skin and intestinal microbes (Mousavi et al., 2022). Since greenspace can help to reduce air pollutant levels (Markevych et al., 2017), the microbiota of people who live around greenspaces is less likely to be disturbed by air pollutants. In addition, living in proximity of greenspaces may lead to an improvement

**Table 8**  
GRADE assessments of certainty of evidence for greenspace exposure and relative abundance of taxa.

Outcome	Health effect a	Initial rating	Downgrade	Upgrade	Certainty in body of evidence
		Features: controlled exposure, exposure prior to outcome, individual outcome data, comparison used High: meet 4 features Moderate: meet 3 features Low: meet 2 features Very Low: meet 0–1 features	Possible Reasons: Inconsistency, imprecision, Risk of Bias concerns, indirectness, publication bias	Possible reasons: All plausible confounders or other biases increase certainty in the observed effect, large effect observed, dose–response gradient	Low, Very Low, Moderate, or High
RA of taxa (Gut microbiota) N = 12	12 of 12 studies support	Moderate Reason: 6 studies met 4 features (Nurminen et al., 2018; Gascon et al., 2020; Sobko et al., 2020; Roslund et al., 2020; Roslund et al., 2021; Brown et al., 2022) and 6 studies met 3 features (Pearson et al., 2019; Nielsen et al., 2020; Bowyer et al., 2022; Wu et al., 2022; Gacesa et al., 2022; Zhang et al., 2023).	Moderate -> Very Low Reason: inconsistency and imprecision	No	Very Low
RA of taxa (Skin microbiota) N = 6	6 of 6 studies support	Moderate Reason: 3 studies met 4 features (Nurminen et al., 2018; Hui et al., 2019; Roslund et al., 2021), 2 studies met 3 features (Zhang et al., 2023; Selway et al., 2020), and 1 study met 2 features (Ruokolainen et al., 2015).	Moderate -> Very Low Reason: Inconsistency and imprecision	No	Very Low
RA of taxa (Oral microbiota) N = 3	3 of 3 studies support	Moderate Reason: 1 study met 4 features (Roslund et al., 2021) and 2 studies met 3 features (Pearson et al., 2019; Zhang et al., 2023).	Moderate -> Very Low Reason: Inconsistency and imprecision	No	Very Low
RA of taxa (Nasal microbiota) N = 4	3 of 4 studies support	Moderate Reason: 4 studies met 3 features (Pearson et al., 2019; Gisler et al., 2021; Asri et al., 2023; Selway et al., 2020).	Moderate -> Very Low Reason: Inconsistency and imprecision	No	Very Low
RA of taxa (Ocular microbiota) N = 1	1 of 1 studies support	Moderate Reason: 1 study met 3 features (Pearson et al., 2019).	Moderate -> Very Low Reason: Single study and small sample size	No	Very Low

<sup>a</sup>Health effect: the health effects of greenspace exposure refer to altered relative abundance of specific taxa. Abbreviations: RA, relative abundance.



**Fig. 2.** Potential pathway by which greenspace enriches human microbiota. Greenspaces are able to change the human microbiota by modulating the environmental microbiota that humans may inhale/ingest (a) and contact (b), relieving mental stress (c) and encouraging physical (d; e). This figure was designed using assets from Freepik.com.

in lifestyles, such as engaging in regular physical activity, which has been reported to alter gut environment by modifying bile acids, reducing transit time, and regulating mucus production (Wegierska et al., 2022). These changes can promote the growth of health-related bacteria and decrease pathogens in the gut. Finally, greenspace exposure is associated

with the reduction of psychological stress, (Markevych et al., 2017) which can alter gut microbiota by activating the hypothalamic–pituitary–adrenal axis (Agirman and Hsiao, 2021).

#### 4.4. Limitations of systematic reviews and included studies

Our review faced a number of limitations. First, due to the limited number of studies and the heterogeneity in exposure assessment, we were not able to perform a *meta-analysis*. Second, we included only formally published studies due to the limited accessibility of grey literature. This approach may have prevented us from obtaining gray literatures and introduced publication bias. Third, we restricted our search to English and Chinese language articles, which may have inevitably led to the exclusion of relevant studies written in languages other than English and Chinese. Fourth, comparing measures of the microbiota across studies is challenging, as these measures depend on the characteristics of the population studied and the methodologies employed. Notably, considering beta-diversity is a measure subjective to the population within which it is derived and generally not analyzed using models that produce effect sizes and standard deviations, the results of beta-diversity is contextual and hard to synthesize. Fifth, although we concluded that evidence generally supported the positive associations between greenspace exposure and increased alpha-diversity in skin and gut microbiota, the links between microbial higher diversity and the health status of individuals are still uncertain. Thus, the further health interpretation of this conclusion might be limited.

In addition, the interpretation of our findings should be cautious given the limitations of studies included in this review. First, the inclusion of cross-sectional studies precludes us from inferring a causal relationship between greenspace and microbiota. Moreover, most of the existing interventional studies lacked a randomized controlled design. The absence of parallel control groups or randomized allocation to groups, along with limited sample sizes, may introduce selection and detection biases. Second, the majority of studies focused on changes after short-term greenspace exposure or a “snapshot” cross-sectional association between greenspace and microbiota. The one-time robbing of soil onto the skin or short walks in greenspace may result in only temporary changes in the microbiota, which could have limited health effects. Third, the assessment of greenspace exposure in the review study was mainly limited to the presence of greenspace. Such assessments overlook other potentially critical dimensions, such as quality, macro-biodiversity, vegetation type and physical access, that could affect micro-biodiversity value of greenspace (Ho et al., 2005; McCormack et al., 2010). Fourth, while all of the included studies measured the microbial diversity and composition, it remains unclear whether and to what extent these aspects could affect health. Fifth, we observed that several kinds of bias existed in current studies, which may influence the strength and direction of observed associations. Specifically, we identified confounder bias in three studies. Two studies did not adjust their analyses for any confounders, which could overestimate the strength of the association. One study adjusted for the factor that could be partially explain the casual link between greenspace exposure and human microbiota (i.e., physical activity), which may bias the effect estimates toward null. In addition, other sources of bias, including in appropriate statistical methodology and small sample size could also made the conclusion less solid. Sixth, the existing studies were predominantly conducted in Europe and North America, while most other regions not being adequately represented. The generalizability of the current evidence from these high-income countries may be limited to low- and middle-income countries due to contextual differences in climate, fauna and flora, culture, diet, and sociodemographic characteristics. Seventh, individuals’ age, gender, physiology, and living habits could lead to differences in commensal microbiota, potentially influencing the response to greenspace exposure. However, only three studies to date performed subgroup analysis according to participants’ characteristics (i.e., age, sex, climate zones, breastfeeding or pet ownership) (Pearson et al., 2019; Nielsen et al., 2020; Zhang et al., 2023).

#### 5. Concluding remarks and future perspectives

Overall, the available evidence generally indicated that greenspace exposure may diversify gut and skin microbiota and alter their composition to healthier profiles. However, drawing a robust conclusion requires further well-designed, longitudinal studies and interventional studies to enhance our understanding of the effect of greenspace exposure on human microbiota, as well as the downstream health effect. We suggest that further studies pay particular attention to the following aspects:

- (1) Conducting longitudinal studies and intervention studies with long-term and repeated measurements of greenspace exposure and microbiota.
- (2) Comprehensively assessment of greenspace exposure including aspects such as diversity and type of flora and fauna, use of greenspaces, and type of interactions with natural features when the participants are in greenspaces.
- (3) Implementing metagenomic sequencing method to provides information of functional pathways and metabolites.
- (4) Extending on human microbiota to additional body sites, such as respiratory tract.
- (5) Selecting covariates using methods based on modern confounding theory, such as directed acyclic graph, to avoid under- or over-adjustment.
- (6) Investigating the modifying effects of participants’ characteristics, such as age, sex/gender, pet ownership, diet, and health status on the association between greenspace exposure and microbiota.
- (7) Validating the role of enriched microbiota as a mechanism underlying the associations between greenspace exposure and human health.

#### 6. Contributors

Y-D Z and G-L Z designed the search strategy and selection criteria, screening, data extraction, quality assessment, and wrote the first draft of the paper. LW, MB, IM, JH, LK, TY Z, YZ D, SC, K-K L, PD, G-H D reviewed and edited the manuscript. B-Y Y conceptualized the study, provided overall supervision and reviewed and edited the manuscript. B-Y Y is the guarantor of this work.

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#### CRediT authorship contribution statement

**Yi-Dan Zhang:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Gang-Long Zhou:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Lu Wang:** Methodology, Software, Validation, Visualization. **Matthew H.E.M. Browning:** Methodology, Writing – review & editing. **Iana Markevych:** Methodology, Writing – review & editing. **Joachim Heinrich:** Writing – review & editing. **Luke D. Knibbs:** Writing – review & editing. **Tianyu Zhao:** Methodology, Writing – review & editing. **Yizhen Ding:** Writing – review & editing. **Shi Chen:** Writing – review & editing. **Kang-Kang Liu:** Writing – review & editing. **Payam Dadvand:** Writing – review & editing. **Guang-Hui Dong:** Writing – review & editing. **Bo-Yi Yang:** Conceptualization, Funding acquisition, Project administration, Validation, Writing – review & 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2024.108662>.

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