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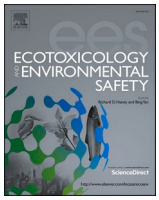

Intervention effects of greenspace exposure on human microbiota: A randomized controlled trial in Chinese young adults

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ABSTRACT

Enriching human microbiota has been proposed as a mechanism by which greenspace exposure improves human health. The existing evidence is scarce with few studies able to evaluate causality. We conducted a randomized controlled trial of 30 healthy undergraduate students to explore the intervention effects of greenspace on human gut and oral microbiota alpha-diversity, composition, differential genera and functional pathways. The study participants were divided into three groups, including outdoor greenspace (GS) group, outdoor non-greenspace (NGS) group, and indoor group, who visited a park, an open space without vegetation, and a classroom, respectively, for two hours per day over seven days. Differences in microbial alpha-diversity and composition across various groups were tested using Wilcoxon test and permutational multivariate analysis of variance, respectively. Linear discriminant analysis effect size analysis was performed to test differences in genera and functional pathways. Greenspace intervention significantly increased gut microbiota alpha-diversity, especially the observed Amplicon Sequence variant indexes and the Faith indexes (both $p < 0.05$). In addition, the intervention substantially changed the composition of gut microbiota, of which the relative abundances of potentially beneficial bacteria increased. Further, the greenspace intervention affected several functional pathways of gut microbiota, including "substance dependence", "specific types of cancer", and "viral infectious diseases". However, we did not find any significant effect of greenspace intervention on oral microbiota. Our results suggest that greenspace intervention diversifies the gut microbiota and alters its composition. These findings could help to reinforce the potential of increasing people's access to greenspace as a public health intervention.

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1. Introduction

Greenspaces, including parks, gardens, forests and greenbelts, are critical components of the human living environment in urban areas. With rapid industrialization and urbanization, large areas of natural environments including greenspace have been replaced with built-up land covers such as buildings and paved surfaces, challenging people's access to health-promoting natural landscapes (Hartig and Kahn, 2016). The potential effects of greenspace on human health have thus attracted worldwide attention, and large volumes of scientific evidence on such topic has been accumulated (Yang et al., 2021). The existing evidence generally supports that greenspace exposure could exert various benefits on human physical and psychological health and wellbeing (Xu et al., 2023; Yang et al., 2021). However, the exact mechanisms underlying such benefits are yet to be established, although there have been several hypothesized pathways, including mitigating urban-related environmental hazards such as air pollution, noise, and heat, encouraging physical activity, enhancing social cohesion, and recovering from stress (Markevych et al., 2017; Zhang et al., 2023). In recent years, enriching human microbiota diversity has emerged as a novel mechanism by which greenspace may affect human health, although the evidence for this pathway is still not well established (Zhang et al., 2024).

The human microbiota, including the gut, oral, and skin bacteria, play critical roles in regulating immune function (Clarke et al., 2010), contributing to metabolic health (Fan and Pedersen, 2021) and providing beneficial nutrients (e.g., vitamins (Kau et al., 2011) and short chain fatty acids (Topping and Clifton, 2001)). On the other hand, microbial dysbiosis (i.e., loss of diversity and imbalance in the composition) has been linked to various diseases including gastrointestinal inflammation (Cohen et al., 2019), cancer (Tong et al., 2021), and psychiatric disorders (Järbrink-Sehgal and Andreasson, 2020). The gut and oral microbiota are more stable than the skin and nasal microbiomes (Zhou et al., 2024). As the largest organ in the human body in terms of surface area (250–400 m^2) (Thursby and Juge, 2017), the gastrointestinal tract hosts a resident bacterial population which is particularly crucial for human life (Mizutani et al., 2020). The oral cavity, hosting the largest amount of aerobic bacteria, is the entrance of the digestive system that may affect gut microbiota and thus influence human health (Gasmi et al., 2021). Specifically, certain oral bacteria, such as Streptococcus and Veillonella species, have been found to translocate to the gut, particularly in conditions like periodontal disease or poor oral hygiene. This translocation can disrupt the balance of gut microbiota, leading to dysbiosis, which has been linked to various systemic health issues, including inflammatory bowel disease, cardiovascular disease, and metabolic disorders (Chen et al., 2019).

Exposure to greenspace may affect human microbiota in direct and indirect ways. Specifically, vegetation can regulate the microbiome of the rhizosphere and subsequently change the soil microbial diversity (Mills et al., 2017). Vegetation can also shape the air microbiome by secreting volatile organic compounds, releasing plant particles with microbes and influencing the microclimates (Pearson et al., 2020). Subsequently, microbes in the soil and air of greenspace can be directly contacted, ingested, and inhaled by humans, which ultimately affect their microbiota (Li et al., 2021). In addition, greenspace may indirectly affect human microbiota by reducing ambient air pollution (Feng et al., 2020; Sabedotti et al., 2023), encouraging physical activity (Mueller et al., 2021), and reducing psychological stress (Yang et al., 2021)—pathways that are reported to be closely related to the human microbiota (Hart et al., 2023; Liu et al., 2024; Molina-Torres et al., 2019). Therefore, it is biologically plausible to hypothesize that greenspace affects human gut microbiota.

Several epidemiological studies have examined the associations between greenspace exposure and human microbial diversity and composition (Zhang et al., 2024), yet the results are mixed. In addition, half of the existing studies were cross-sectional, limiting the ability to confirm causality between greenspace exposure and changes in microbiota. A few studies have adopted an intervention design (Brown et al., 2022; Gascon et al., 2020; Nurminen et al., 2018; Roslund et al., 2020, 2021; Selway et al., 2020; Sobko et al., 2020), but most of them have limitations in lacking randomization of participants (Roslund et al., 2020). Further, most of the prior studies were carried out in European and North American countries and the microbiome effects of greenspace in other areas, such as Asia, are particularly unclear. Such evidence would be of interest considering that the biological effects of greenspace can be context-specific and depend on its types, constructions, and vegetation species (Huang et al., 2023; Lambert et al., 2018). In addition, compared to the Western countries, Chinese cities are much more compact and dense (Sun et al., 2017). With high population density and less per capita greenspace, the study of greenspace and human health is more urgent in China, especially in the city of rapid economic development.

To address these research gaps, we conducted a randomized controlled trial (RCT) on young adults in southern China to discover and rigorously attribute the effects of greenspace exposure on human gut and oral microbial diversity and composition as well as their functional pathways.

2. Methods

2.1. Study design

This open-label and randomized controlled trial was carried out in Guangzhou city, Southern China, which undergoes rapid economic development. Guangzhou is a typical southern city with oceanic subtropical monsoon climate, characterized by warm and rainy weather, abundant light and heat, and long summer. We recruited 30 participants and randomly and evenly assigned them into three groups: outdoor greenspace (GS) group, outdoor non-greenspace (NGS) group, and indoor group. The participants were asked to stay in their assigned environment for two hours every day for 7 consecutive days. A questionnaire was employed to collect data on socio-demographic and lifestyles at baseline, and fecal and saliva samples were collected before and after intervention for microbiota test. The study was approved by Ethics Committee of Sun Yat-sen University.

2.2. Participants

Originally, we recruited 33 healthy undergraduate students from Sun Yat-Sen University as study participants. The intervention occurred in vegetation growing season in June, 2022. Due to the scheduling reasons, three students quitted our study. Finally, 30 participants were included, according to the following criteria: (1) aged 18 years or older; (2) having no communicable or non-communicable diseases; (3) not reporting recent consumption of prescribed or over-the-counter-medication or supplements, including antibiotics and probiotics; and (4) not reporting smoking cigarettes or drinking alcohol. Prior to the trial, the study aims and procedures were fully explained to all participants, both orally and in writing. Participation in the study was voluntary and the participants confirmed their willingness to be involved in the research through a written consent form.

2.3. Randomization and masking

At the time of randomization, eligible participants were randomly assigned in a 1:1:1 ratio to visit the GS, NGS and indoor groups. The randomization list was computer-generated using the EXCEL software (version 2019). Neither participants nor field assessors were masked to study group assignment because it was not possible so that this is an open-label study.

2.4. Procedure

Prior to the intervention, we employed a questionnaire to collect data on participants' demographics (e.g., age, gender, height, and weight) and socioeconomic information. Meanwhile, a fecal sample and saliva sample were collected from all participants at the research location and stored at −80 °C for detection. Then, the participants in three groups left for the exposure location from the same point by walking (Fig. 1). The distance from the university campus to both the urban park and the commercial streets was approximately 1 kilometer, with a travel time of less than 10 minutes for each destination. The participants in the GS group were exposed to greenspace in an urban park (i.e., Guangzhou Martyr Memorial Park) located in the center of Guangzhou city with a size about 0.26 square kilometers and adequate sky view (Fig. 1A). The park has an abundance of plant species including trees (e.g., *Ficus altissima*, *Araucaria heterophylla*, *Livistona chinensis*), shrubs (e.g., *Alpinia sanderae*, *Cinnamomum burmannii*, *Excoecaria cochinchinensis*), and grasses, and the canopy cover is as high as 70 % (Feng et al., 2009). Participants in the NGS group were exposed to an outdoor open space near commercial streets, without any vegetation, and surrounded by high building density (Fig. 1B). There was little greenspace on the way to the exposure location of NGS group. Participants in the indoor group were exposed in a classroom on the campus of the university, without indoor potted plants or window views of plants (Fig. 1C). Participants in the three groups were accompanied by researchers at all times and stayed in their corresponding environment for two hours (from 3:00 pm to 5:00 pm) per day, continuously lasting for seven days. During each two-hour intervention, participants were asked to walk slowly, stand, or sit in a state of peace and silence without strenuous exercise. Outside of each intervention, participants were asked to stay at their university dormitory for as much time as possible to avoid other environmental exposures. In addition, we requested all the participants to have a normal and balanced diet (i.e., avoid to have too much meats or vegetables), and to have meals in the university's canteen. After the intervention, fecal and saliva samples were recollected and stored at −80 °C until the detection (Fig. 2).

2.5. Fecal and saliva sample collection and microbe sequencing

The baseline fecal and saliva samples were collected one day before the intervention. Specifically, participants were given a fecal collection tube and requested to collect a fecal sample and bring their samples to the laboratory within six hours after collection. Saliva samples of participants were collected from the dorsum and ventral surfaces of the tongue and the mucosa of the alveolar ridges (pooled microbial sample) by a trained person at research location. DNA was extracted from fecal and saliva samples and the V3 and V4 hypervariable region of the bacterial 16S rRNA gene was amplified by PCR using universal primers and sequenced by the Illumina Miseq platforms (Ravi et al., 2018). The sequencing data were trimmed to 240 nt and clustered to distinct Amplicon Sequence Variants (ASVs) using the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline (Callahan et al., 2016). The ASV data of the fecal and saliva samples were rarified to 5000 and 20, 000 reads, respectively. We used observed ASVs, Pielou's evenness, Shannon index, and Faith index to estimate microbial alpha-diversity. We also employed abundance-based Unweighted-Unifrac matrices to quantitatively measure the compositional dissimilarity between different groups and visualized the dissimilarity using Principal co-ordinates analysis (PCoA) plots. Samples with similar microbial composition are closer on the PCoA plots. Taxonomic assignments were based on the Silva reference database (version 138), and abundances of taxa were calculated on the genus level. The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (Douglas et al., 2020) software and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000) (level 2) were used to infer the functional pathways and calculate their relative abundances. It is important to note that these functional predictions are estimations based on phylogenetic inference and not direct measurements. All calculations were performed in the Quantitative Insights Into Microbial Ecology 2 (QIIME2) software (2021.11 release) and R software (version 4.2.1.) with vegan R package.

2.6. Statistical analyses

Differences in the distribution of baseline characteristics were analyzed using one-way analysis of variance (ANOVA) tests for continuous variables and chi-square tests for categorical variables. We investigated within-group changes in alpha-diversity before and after the intervention using the paired samples Wilcoxon tests. We also compared differences in alpha-diversity between groups using the grouped Wilcoxon tests. Differences in beta-diversity before and after the intervention within the same group as well as the differences between groups were tested using the permutational multivariate analysis of variance (PERMANOVA) (function adonis in *vegan* R package) (Oksanen et al., 2020) based on PCoA analysis. Linear discriminant analysis effect size (LEfSe) was used to detect significant differential genera and functional pathways among different groups, as well as within each group before and after the intervention (Chang et al., 2022). Generally, genera with higher Linear Discriminant Analysis (LDA) values are regarded as more significant in distinguishing the microbial composition between distinct groups. If LDA values was > 2, then the relative abundance of the genus or the functional pathway was considered significantly differed between groups (Huang et al., 2020). Statistical significance was defined by two-sided $p < 0.05$, and analyses were carried in R software (version 4.2.1.).

3. Results

3.1. Characteristics of participants

The study sample consisted of 15 males and 15 females of a median age of 20 years old and a mean (SD) of BMI was 20.79 (2.84) kg/m^2 (Table 1). About half of the participants' parents had higher education levels (i.e., $\geq$ high school degree) and higher household income levels (i.e., $\geq$ 8000 Yuan [1118.5 USD] per month). There were no significant differences among groups by demographic, social-economic variables, indicating good comparison in baseline characteristics across the three groups. During the intervention, the GS group experienced lower air temperature levels but higher relative humidity levels than the NGS group and the indoor group, though the differences were not significant.

3.2. Effects of greenspace intervention on alpha-diversity of human microbiota

We observed that greenspace exposure significantly increased the diversity of human gut microbiota. Specifically, before the intervention, alpha-diversities of gut microbiota were similar across the three groups. The intervention increased the observed ASVs and Faith indices in the GS group, but significantly decreased the two indexes in the NGS group (Supplemental Figure 1). Consequently, after the intervention, the observed ASVs and Faith indices of gut microbiota were significantly higher in the GS group than those in the other two groups (Fig. 3) and the two indices were also significantly higher in the indoor group comparing to the NGS group (Supplemental Figure 4). On the other hand, Pielou's evenness and the Shannon index did not change significantly after the intervention. With regard to the oral microbiota, the intervention effects of greenspace on alpha-diversity were weak. All four alpha-diversity indexes did not change substantially before and after the intervention.

Fig. 1. Locations of exposure environments. The starting point (O) and the corresponding locations of three groups, including a park (A), an open space without any vegetation (B), and a classroom (C).

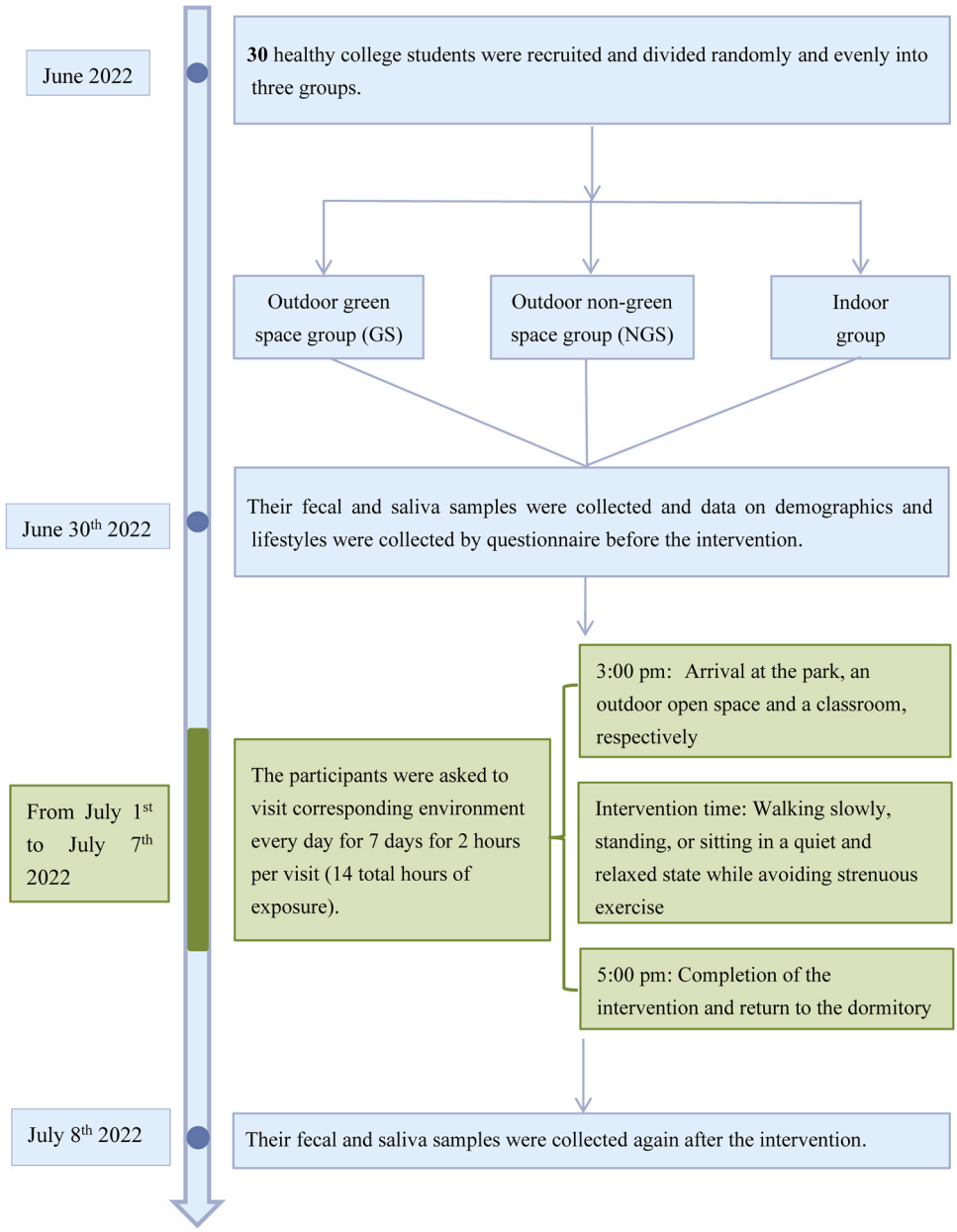

Fig. 2. Flow chart of study process.

3.3. *Effects of greenspace intervention on composition of human microbiota*

We observed that greenspace intervention influenced the composition of the human gut microbiota. Before the intervention, the composition of gut microbiota was different between the GS group and the NGS group (F=1.702, p = 0.01), but was similar between the GS group and the indoor group (Fig. 4, panels A–B). The intervention significantly changed the composition of the gut microbiota in all three groups (Supplemental Figure 2). Consequently, the magnitude of the discrepancy in gut microbiota composition between the GS and NGS group became greater after the intervention (F=6.473, p = 0.001), consistent with the result of the PCoA plot that the two groups were farther apart (Fig. 4). Also, the composition of the gut microbiota between the GS and the indoor groups differed after the intervention (ANOSIM, r = 0.139, p = 0.001). The composition of gut microbiota was significantly different between the NGS and the indoor groups both before and after the intervention (Supplemental Figure 4). Regarding the oral microbiota, the intervention had little effects on its composition (Fig. 4 and Supplemental Figure 2).

Looking at the intervention effects of greenspace on specific genera of microbiota, we observed that the intervention significantly enriched the beneficial genera in the gut microbiota (note: this does not absolutely mean the elimination or detection of new taxa) (Fig. 5). The health implications of specific genera are shown in Supplemental Table 1. For instance, in the GS group, the abundances of seven genera, including four (57 %) potential beneficial genera (e.g., *Lactobacillus*and and *Eubacterium_eligens_group*), significantly increased as a result of the intervention. In addition, the relative abundances of nine genera including five (55.6 %) potential beneficial genera (e.g., *Peptostreptococcus* and *Blautia*) and two genera including one (50 %) potential beneficial genera (i.e., *Allobaculum*) significantly increased in the NGS and the indoor groups, respectively, as a result of the intervention.

We further compared the relative abundances of specific genera between the GS and NGS groups and found that, before the intervention, there were only 12 genera that significantly differed between the groups

Table 1
Baseline characteristics.

Variable	Outdoor greenspace group (GS) (N = 10)	Outdoor non-greenspace group (NGS) (N = 10)	Indoor group (N = 10)	*P*
Age, median (IQR), year	20 (1)	20 (1)	21 (1)	0·07
Gender, No. (%)				1·00
Male	5 (50)	5 (50)	5 (50)	
Female	5 (50)	5 (50)	5 (50)	
Father or mother's higher education level, No. (%)				0·79
Less than high school	6 (60)	4 (40)	3 (30)	
High school	1 (10)	1 (10)	2 (20)	
Higher than high school	3 (30)	5 (50)	5 (50)	
Monthly-average household income, No. (%), yuan				0·40
< 4000	3 (30)	3 (30)	4 (40)	
4000–8000	0 (0)	3 (30)	2 (20)	
> 8000	7 (70)	4 (40)	4 (40)	
BMI, mean (SD), kg/m^2	20·98 ± 2·14	21·86 ± 3·67	19·53 ± 2·23	0·18
Air temperature, mean (SD),°C	31·27 ± 2·25	32·03 ± 2·07	31·69 ± 2·16	0·81
Relative humidity, mean (SD), %	66·9 ± 12·32	64·14 ± 13·69	65·37 ± 13·70	0·93

Note: BMI, body mass index; SD, standard deviation; IQR, interquartile range.

and 52 genera that differed between the groups after the intervention (Fig. 5). Additionally, after the intervention, 24 potentially beneficial microbial genera (e.g., *Prevotellaceae_UCG-001, Butyrivibrio* and *Lachnospiraceae XPB1014 group*), were enriched in the GS group, whereas only six beneficial microbial genera (e.g., *Akkermansia, Fusicatenibacter* and *Lachnospiraceae_UCG-004*) were enriched in the NGS group (Fig. 5). Similar patterns were observed when comparing the GS and indoor groups. For example, before the intervention, only eight genera showed significantly different abundances between the two groups, whereas this number reached 22 after the intervention. Of them, the relative abundances of 12 potentially beneficial genera significantly increased in the GS group (such as *Prevotellaceae_UCG-001* and *Sussiniclasticum*) and one in the indoor group (i.e., *Dialister*).

3.4. Effects of greenspace intervention on functional pathways of gut microbiota

We mapped functional pathways using KEGG analysis and found that after the intervention, comparing to those before the intervention, six (i.e., "cellular community of prokaryotes", "substance dependence", "immune system", "signal transduction", "cancer of specific types" and "forting, sorting and degradation") pathways were enriched in the GS groups (Supplementary Figure 3).

We also compared the relative abundances of pathways between the GS and NGS groups after the intervention. We found that five (i.e., "substance dependence", "virus infectious disease", "cardiovascular disease", "circulatory system" and "cancer of specific types") were enriched in the GS group, but only one (i.e., "carbohydrate metabolism") were enriched in the NGS group. When comparing the GS and indoor groups after the intervention, three pathways (i.e., "substance dependence", "virus infectious disease" and "cellular community of prokaryotes") were enriched in the GS group. None were significantly enriched in the indoor group.

4. Discussion

4.1. Key findings

This is the first RCT to evaluate the impact of greenspace exposure on human microbiota in China and one of few such studies worldwide. We observed that seven days of a greenspace intervention significantly increased the participants' gut microbiota alpha-diversity and changed gut composition, with potentially beneficial bacteria showing increased relative abundances. In addition, we observed that a greenspace intervention affects several functional pathways of gut microbiota, including those related to substance dependence, cancer, and viral infectious diseases.

4.2. Effects of greenspace intervention on alpha-diversity of gut microbiota

In line with our findings that a greenspace intervention increased the gut microbial alpha-diversity, a prior case-controlled cohort study that compared the gut microbiota of 10 American gardening families whose members gardened at least 30 minutes per week in the gardening season (April through August) with nine non-gardening families. The investigators observed that gardeners had greater observed ASV features and higher Faith's PD scores in gut microbiota than the non-gardeners (Brown et al., 2022). Similarly, Nurminen and colleagues carried out a controlled trial in which seven participants experienced a two-week long intervention that involved rubbing their hands with a soil-and plant--based material three times per day and seven other participants did not (Nurminen et al., 2018). The investigators observed that the intervention was associated with a significant increase in the Shannon index of the gut microbiota. However, in another pilot RCT, Gascon and colleagues observed that a greenspace intervention involving gardening activities did not significantly change the gut microbial alpha-diversity of the participants (Gascon et al., 2020).

Apart from intervention studies, there are also several cross-sectional studies that examined the association between greenspace exposure and gut microbial alpha-diversity. In our review of the literature through October 2022, we found five cross-sectional studies of greenspace exposure and gut alpha-diversity (Zhang et al., 2024). Of them, three observed positive associations, and two observed null associations between greenspace exposure and gut alpha-diversity (Zhang et al., 2024). These inconsistencies may be caused by the difference of climate, greenspace types, and study designs among other factors. Collectively, although the existing evidence concerning greenspace and gut microbial alpha-diversity is not entirely consistent, more than half of the prior studies, combined with our current findings, suggest that greenspace exposure may influence gut microbial richness.

4.3. Effects of greenspace intervention on composition of gut microbiota

We also observed that a greenspace intervention significantly changed the composition of the gut microbiota, and particularly increased the abundances of genera like *Alistipes*, *Lactobacillus*, *Sussiniclasticum*, and *Eubacterium_eligens_group*. These genera are documented to be beneficial to human health by producing active components including short chain fatty acid and vitamins (Eicher and Mohajeri, 2022; Vemuri et al., 2019). Consistent with our findings, Brown and colleagues observed that four months of gardening significantly changed the gut microbial composition of the gardeners, with *Alistipes inops, Bacteroides stercoris, Romboutsia, Bacteroides ovatus,* and *Terrisporobacter* showing significant enrichment (Brown et al., 2022). Results of another intervention trial involving 75 Finnish children indicated that adding more vegetation in kindergartens significantly changed the children's gut microbial composition and enriched the abundance of *Ruminococcaceae*, which is known to contain established or candidate probiotics (Roslund et al., 2020). A pre-post study also showed that approximately

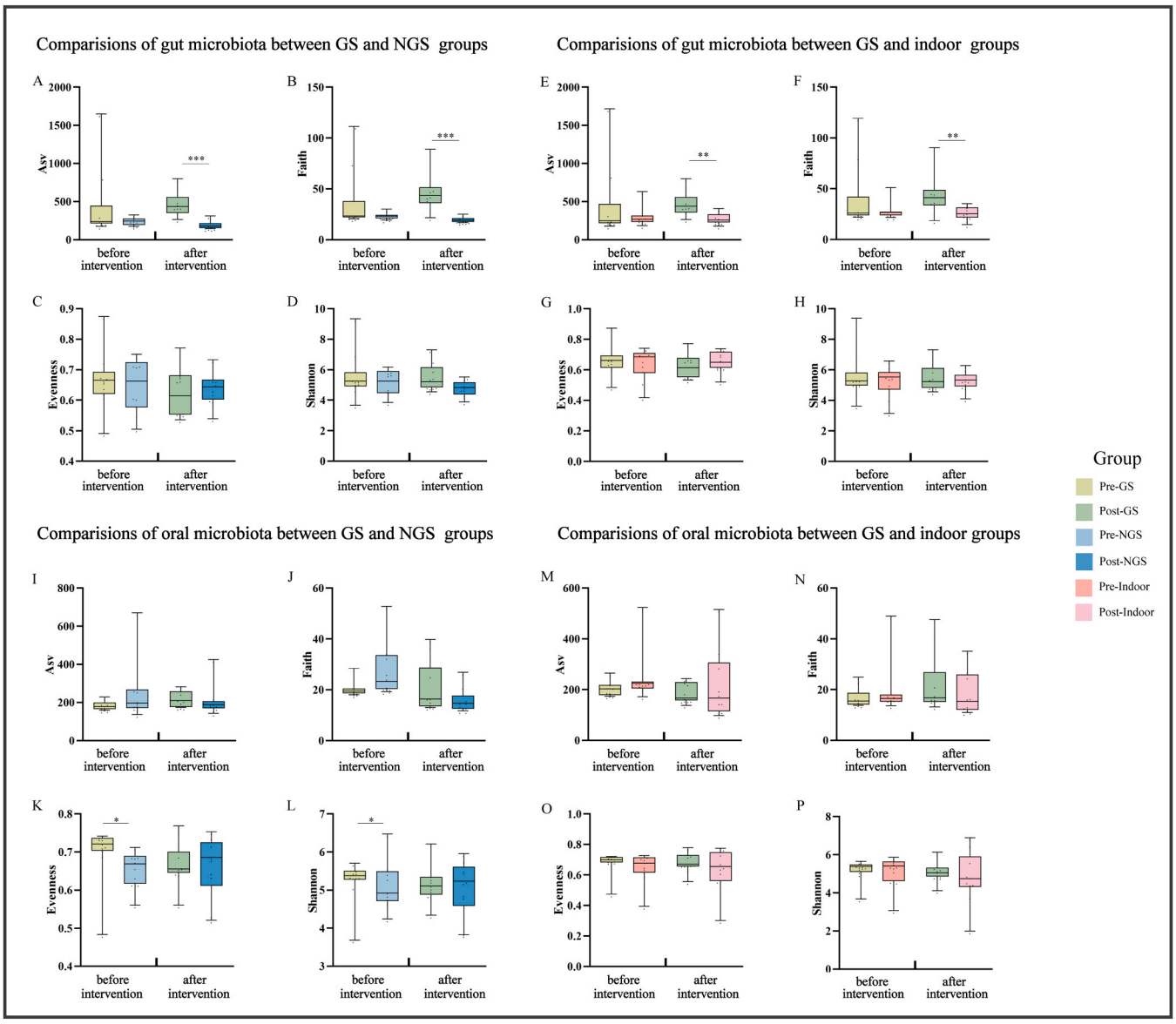

Fig. 3. Differences of alpha-diversity across groups. Comparisons of ASV, Faith index, Pielou's Evenness, and Shannon index of human gut microbiota between GS and NGS groups (A–D) as well as between GS and indoor groups (E–H) before and after interventions, respectively; comparisons of ASV, Faith index, Pielou's Evenness, and Shannon index of human oral microbiota between GS and NGS groups (I–L) as well as between GS and indoor groups (M–P) before and after interventions, respectively. See also Supplemental Figure 1. Note: Significance levels are indicated by asterisks: ***$p < 0.001$, **$p < 0.01$.

an hour of urban greenspace exposure resulted in adults' palm microbiota becoming more similar to the soil microbiota, and abundances of *Corynebacterium*, which correlate with opportunistic infections (Byeon et al., 2021), decreasing (Selway et al., 2020). Several cross-sectional studies have also explored the associations of greenspace exposure with gut microbial composition and the relative abundances of specific taxa (Bowyer et al., 2022; Nielsen et al., 2020; Wu et al., 2022; Zhang et al., 2023), and the results generally support our current findings that greenspace exposure is associated with altered overall composition of gut microbiota with an increased abundance of beneficial genera or species (e.g., *Akkermanisia*, *Rosburia*, *Ruminococcaceae_UCG-014*, *Lachnospiracea* and *Bifidobacterium*).

4.4. *Effects of greenspace intervention on functional pathways of gut microbiota*

Further, we observed that a greenspace intervention affected gut microbiota functions, especially those related to substance dependence and the development of cancers and viral infectious diseases. It is difficult to compare our findings with others given the novelty of this investigation. However, our findings are indirectly supported by epidemiological studies on greenspace exposure in relation to substance dependence, cancers, and infectious diseases (Li et al., 2023; Takashima et al., 2024; Wiley et al., 2022). Specifically, a cross-sectional study among 14,070 Canadian adolescents and young adults observed that greater greenspace exposure was associated with less drinking alcohol or tobacco use (Wiley et al., 2022). In addition, a review of 14 cohort studies concluded that greenspace exposure was associated with reduced incidence of prostate, lung, and breast cancers (Li et al., 2023). Similarly, a study carrying out in a community-based birth-cohort of 158 Australian children reported that higher greenness levels were associated with fewer viral and *M. catarrhalis* detections in the first 3-months after birth (Takashima et al., 2024). Thus, we posit that greenspace exposure may promote human health by modulating the abundance of functionally specific gut microbiota taxa that encode key metabolic pathways. Despite this, our findings concerning the effects of greenspace

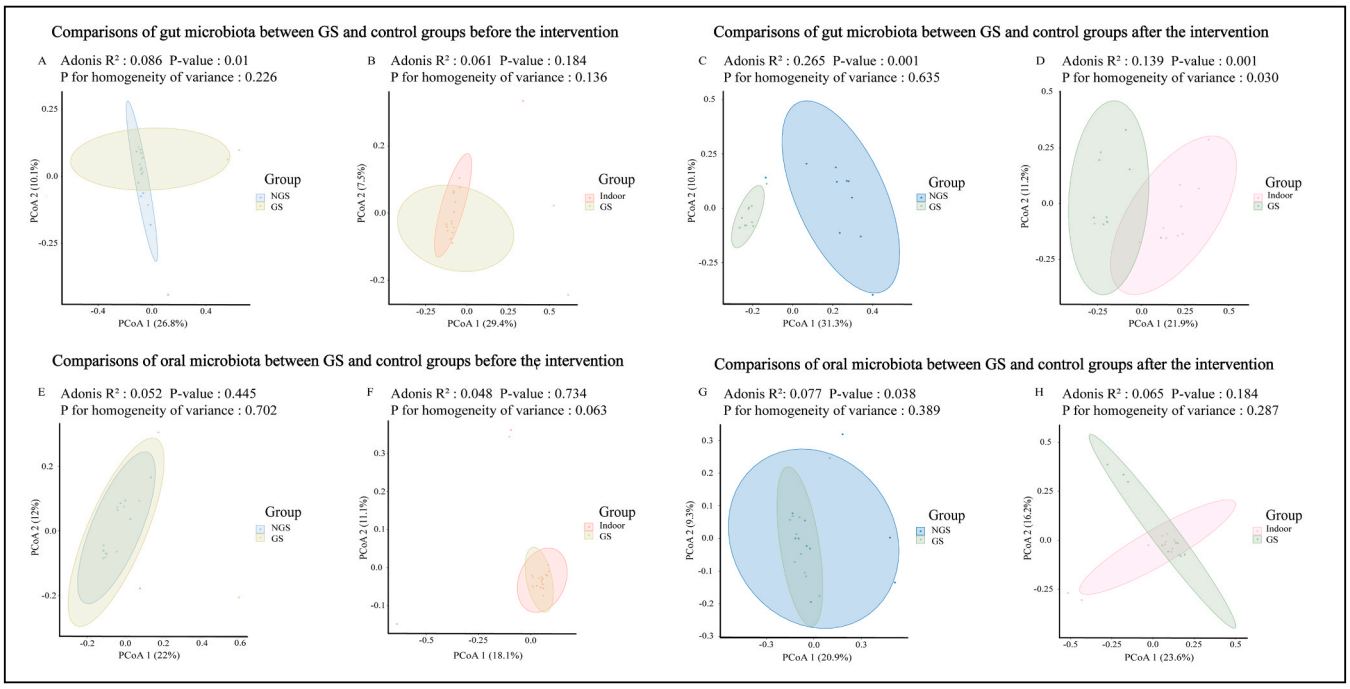

Fig. 4. Differences of beta-diversity across groups. Comparisons of beta-diversity of human gut microbiota between GS and NGS groups as well as between GS and indoor groups before the intervention (A and B) as well as after interventions (C and D); comparisons of beta-diversity of human oral microbiota between GS and NGS groups as well as between GS and indoor groups before the intervention (E and F) as well as after interventions (G and H). See also Supplemental Figure 2.

intervention on gut microbial functions and downstream health still need to be validated in future research.

4.5. Effects of greenspace intervention on oral microbiota

We also estimated the effects of greenspace intervention on oral microbiota, but did not detect significant effects. Our null findings for oral microbiota were supported by a few previous studies on greenspace and oral microbiota, such as an interventional study with 16 adults in the U.S. (Gascon et al., 2020) and two cross-sectional studies with 126 adults from the U.S. (Pearson et al., 2020) and 899 adults from 34 other countries (Zhang et al., 2023); these studies found that greenspace exposure was not significantly associated with the overall alpha-diversity in oral microbiota. Similarly, studies by Gascon (Gascon et al., 2020) and Zhang (Zhang et al., 2023) found no significant association between greenspace and microbial composition. One potential explanation for such null associations is that the oral microbial ecosystem may be relatively stable and that only the microbiome that adapt to a unique oral structure and ecosystem survive and reproduce (Mark et al., 2020; Wang et al., 2020). In addition, saliva is frequently swallowed, carrying environmental microbiota into the gastrointestinal tract, thus it is difficult for microbiota colonization in the mouth. Additionally, our study was conducted in the summer, so participants needed to drink water frequently, and resulting microbiota in the mouth may have been washed into the gastrointestinal tract.

4.6. Potential mechanisms

The exact mechanisms by which greenspace exposure affects human gut microbiota are unclear, but there are several hypothesized pathways. Specifically, greenspace can directly change the environmental microbiota, which further influences human gut microbiota. For example, vegetation can affect soil microbial diversity by regulating the microbiome of the rhizosphere and phyllosphere (Mills et al., 2017). Trees, grasses and flowers can also shape the air microbiome. Specifically, flowers release pollen and particulates that may carry microbes, and when the grass is cut or leaves fall from trees, microbes become airborne (Pearson et al., 2020). Exposure to greenspace thus may change gut microbiota by accidental (or intentional) contact, ingestion, or inhalation. Moreover, greenspace is associated with alleviating psychological stress, which can alter gut microbiota through activating the hypothalamic-pituitary-adrenal axis (Dinan Cryan, 2017; Yang et al., 2021).

4.7. Strengths and limitations

A major strength of this study is its RCT design, which enhances the causality of the evidence concerning greenspace exposure and human gut microbiota. Additionally, we set up two control groups, including the NGS group and the indoor group, which strengthened the robustness of our effect estimates. Thirdly, the participants were exposed to real-world so that we could directly examine the effects of greenspace on human microbiota. Fourthly, four different indices of alpha-diversity were used in our study, and we looked into microbiota composition of both gut and oral microbiota and predictive functional pathways of gut microbiota.

However, our study also has limitations. First, although we provided clear guidance and training to participants to maintain a healthy and balanced diet and have confidence in their compliance, the lack of dietary standardization and measures is a major limitation. Dietary variations are closely related to microbiota diversity and may have compromised the precision of our effect estimates. Future studies should incorporate standardized dietary monitoring to better isolate the effects of greenspace exposure. Second, the intervention duration was relatively short (14 hours total), which might not have been enough to make a significant change in microbiota and also prevented us from detecting potential longer-term effects of exposure on microbiota. Third, greenspace exposure during the non-intervention time might have biased our estimates. However, since all participants were students from the same university and resided in the same dormitory building, their living environment and daily routines were highly similar. Additionally, we conducted training sessions to emphasize the importance of staying in

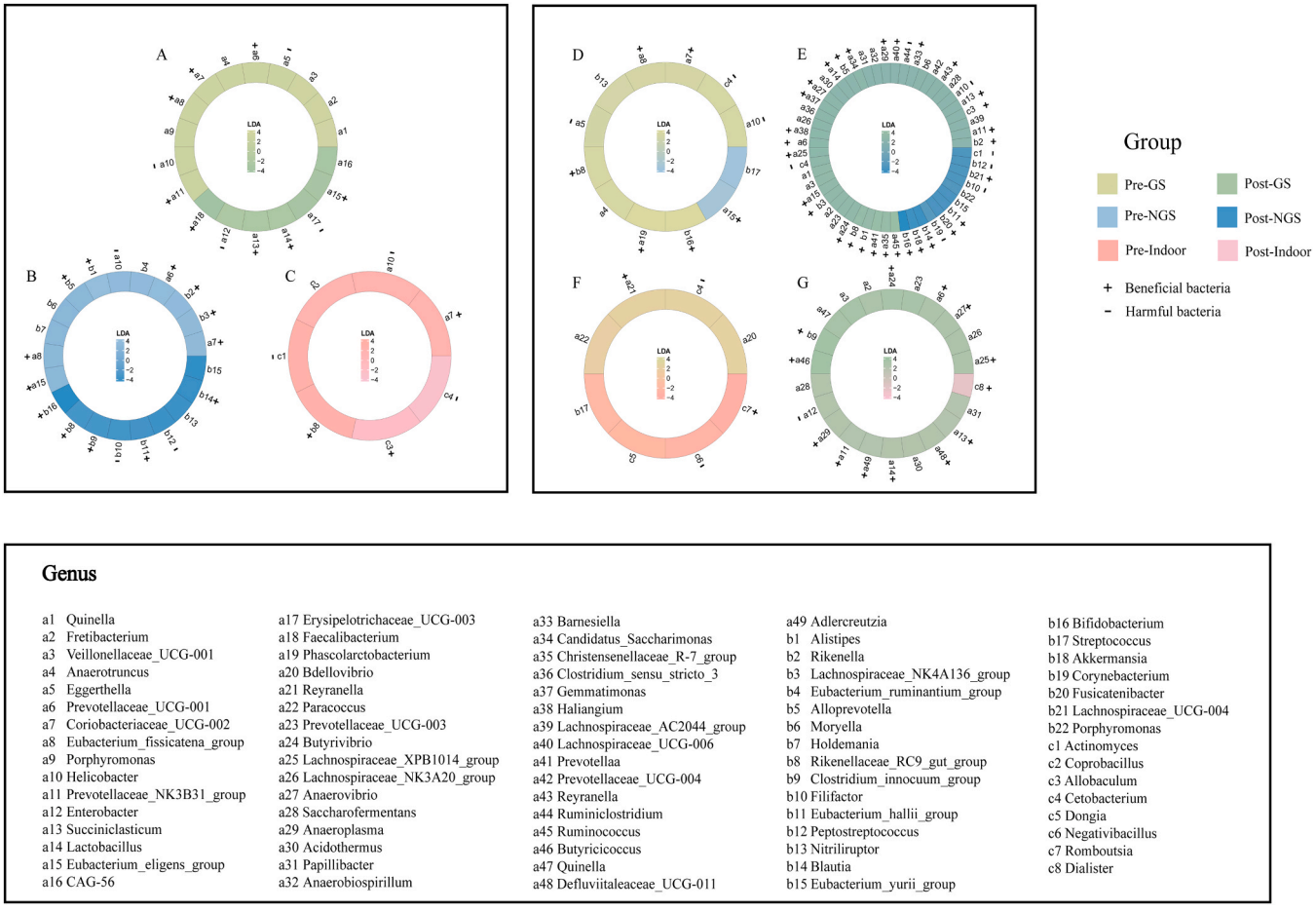

Fig. 5. Differences of relative abundances of specific genera across groups. Comparisons of relative abundances of specific genera of human gut microbiota within the same group before and after the intervention (A–C) and across different groups before and after interventions(D–G). Genera with $p < 0.01$ and LDA value > 2 was considered significant and are shown here. The '+ ' symbol indicates genera that have been previously reported as beneficial in the literature, while the '-' symbol indicates genera associated with potential harm.

their dormitories as much as possible during non-exposure times, and we believe they followed these protocols as instructed. Although we did not collect data on their activities during non-intervention time, we expect the confounding effects of incidental environmental exposures to be minimal. Fourth, we did not collect environmental microbiota samples, such as those from soil or air which could have offered additional mechanistic explanations for the observed changes in microbial diversity. Fifth, in the real world we could not control (or adjust) other co-exposures like air pollutants, which might have confounded our results. Last, our study was carried out in only one city and greenspace exposures were within a single park, which limits the generalization of our results to other locations and greenspaces with different types, constructions and species.

5. Conclusion

Our randomized control trial suggests that spending a couple of hours every day for one week in greenspace can diversify adult's human gut microbiota and alter its composition, enriching beneficial taxa (e.g., Prevotellaceae_UCG-001, Butyrivibrio) and modulating disease-relevant functional pathways. These findings indicates that short-term greenspace interventions may change gut microbial communities toward potential health-promoting states, offering a mechanistic foundation for epidemiological observations of greenspace-health associations. Still, future studies with larger sample sizes and longer intervention durations across diverse regions, populations, and locations are needed to validate our results.

CRediT authorship contribution statement

Wang Zhi: Writing – review & editing. **Zheng Zi-Han:** Writing – review & editing, Visualization. **Markevych Iana:** Writing – review & editing, Funding acquisition. **Zhao Tian-Yu:** Writing – review & editing. **Huang Ji-Lin:** Writing – review & editing. **Zhu Xiao-Qi:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis. **Chen Chen:** Writing – review & editing. **Li Jun-Yi:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yang Bo-Yi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Li Chuang:** Writing – review & editing, Methodology. **Dong Guang-Hui:** Writing – review & editing. **Jiang Jian-Cheng:** Writing – review & editing, Visualization, Validation, Software, Methodology. **Heinrich Joachim:** Writing – review & editing. **Browing Matthew H. E. M.:** Writing – review & editing. **Wang Lu:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dadvand Payam:** 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118183.

Data availability

Data will be made available on request.

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