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The role of early life factors and green living environment in the development of gut microbiota in infancy: Population-based cohort study

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ABSTRACT

Objective: Early life microbial exposure influences the composition of gut microbiota. We investigated how early life factors, and the green living environment around infants' homes, influence the development of gut microbiota during infancy by utilizing data from the Steps to Healthy Development follow-up study (the STEPS study).
Methods: The gut microbiota was analyzed at early (~3 months, n = 959), and late infancy (~13 months, n = 984) using 16S rRNA amplicon sequencing, and combined with residential green environment, measured as (1) Normalized Difference Vegetation Index, (2) Vegetation Cover Diversity, and (3) Naturalness Index within a 750 m radius. We compared gut microbiota diversity and composition between early and late infancy, identified significant individual and family level early life factors influencing gut microbiota, and determined the role of the residential green environment measures on gut microbiota development.
Results: Alpha diversity (*t*-test, $p < 0.001$) and beta diversity (PERMANOVA, $R^2 = 0.095$, $p < 0.001$) differed between early and late infancy. Birth mode was the strongest contributor to the gut microbiota community composition in early infancy (PERMANOVA, $R^2 = 0.005$, $p < 0.01$) and the presence of siblings in late infancy (PERMANOVA, $R^2 = 0.007$, $p < 0.01$). Residential green environment showed no association with community composition, whereas time spend outdoors did (PERMANOVA, $R^2 = 0.002$, $p < 0.05$). Measures of greenness displayed a statistically significant association with alpha diversity during early infancy, not during late infancy (glm, $p < 0.05$). In adjusted analysis, the associations remained only with the Naturalness Index, where higher human impact on living environment was associated with decreased species richness (glm, Observed richness, $p < 0.05$).
Conclusions: The role of the residential green environment to the infant gut microbiota is especially important in early infancy, however, other early life factors, such as birth mode and presence of sibling, had a more significant effect on the overall community composition.

1. Introduction

The process of microbial colonization and compositional development, alongside the immune and metabolic programming by the microbiota, are believed to have a lasting impact on individuals' health (Rautava et al., 2012). The initial colonization is generally believed to occur at birth when the first microbes from the environment and maternal birth cavity start to colonize the infant gut (Koenig et al., 2011; Thursby and Juge, 2017).

Early life factors, including gestational age at birth, birth mode, infant diet, presence of older siblings or household pets, and maternal and infant antibiotic use, have been widely documented to influence the

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establishment and composition of the gut microbiota during infancy (Bäckhed et al., 2015; Laursen et al., 2015; Tun et al., 2017; Uzan-Yulzari et al., 2021). While numerous studies have investigated the effects of individual factors, less is known about the collective influence and relative importance of different variables on gut microbiota development in infancy.

Recent research, such as the study by Jokela et al. (2023), has assessed the effects of a wide array of factors and their relative importance on infant gut microbiota profiles. This study identified technical variables alongside birth mode, defecation frequency, and parity/siblings as significant contributors to microbiota variation. Similarly, findings from Stewart et al. (2018) highlighted significant associations between breastfeeding, birth mode, and early infancy microbiota, while also underlining the impact of environmental factors such as geographical location and household exposures (e.g., siblings and furry pets) on microbiota composition from infancy to three years of age. Both studies underscored the significance of birth mode and the presence of siblings as key contributors to early infancy gut microbiota composition. Despite these and other findings, traditional gut microbiota determinants explain only a small portion of the variation in gut microbiota composition among infants. It remains unclear whether there are other important uncharacterized variables, including environmental factors such as exposure to residential green spaces, that might play a role in explaining variation in gut microbiota composition. (Schmidt et al., 2018; Van Pee et al., 2023).

Only a limited number of studies have been conducted to reliably assess the role of green environments in infant gut microbiota development. Previous research has primarily focused on adults (Bowyer et al., 2022; Parajuli et al., 2020; Pearson et al., 2020; Zhang et al., 2023) or has been limited to crude environmental measures such as urbanicity (Lehtimäki et al., 2021). There have been few intervention studies examining the association between natural environments e.g. biodiversity and gut microbiota, as well as immune regulation, or fecal serotonin in daycare children (Roslund et al., 2020; Sobko et al., 2020). To our knowledge, only one previous study has assessed the effects of natural environments on gut microbiota diversity and composition in infancy (Nielsen et al., 2020). The effects of residential green environments on gut microbiota diversity and composition vary between the studies, and in infants, Nielsen et al. (2020) found the green environments to be associated with reduced likelihood of having a high gut microbiota diversity. Moreover, to our knowledge, there are no previous studies assessing the role of green environments in the development of gut microbiota in relation to the other early life factors in infancy.

This study aims to extend the current research by (1) studying the maturation of gut microbiota in infancy, (2) identifying the significant early life factors influencing gut microbiota during both early (around 3 months) and late (around 13 months) infancy, (3) establishing the relative effects of residential green environment measures in the development of gut microbiota. Our focus was on understanding how the residential greenery including; greenness (Normalized Difference Vegetation Index, NDVI) (Rhew et al., 2011), vegetation cover diversity (Simpson's Diversity Index of Vegetation Cover, VCDI) (Ritsema van Eck and Koomen, 2008), and naturalness (NI) (Walz and Stein, 2014) as well as the time spent outdoors, influenced the gut microbiota diversity and composition in infancy by utilizing data from Steps to Healthy Development follow-up study (the STEPS study).

2. Material and methods

2.1. Study cohort

This study was based on data from children and parents participating in a longitudinal population-based follow-up study, Steps to Healthy Development of Children (the STEPS Study), which has previously been described in detail elsewhere (Lagström et al., 2013). Briefly, all Finnish- and Swedish-speaking mothers delivering a child between January 2008 and March 2010 in the Hospital District of Southwest Finland formed the source population (in total, 13,436 mothers and their 14,946 children). Of the cohort families, 1797 mothers and 1658 fathers with 1805 neonates volunteered as participants for the intensive follow-up group of the STEPS Study (Fig. 1). No selection criteria other than language (Finnish or Swedish speaking family) were applied to recruiting the families in the STEPS Study. The ethics committee of the Hospital District of Southwest Finland has approved the STEPS Study (2/2007). The parents gave their written informed consent for the study. The legal basis for processing personal data is public interest and scientific research (EU General Data Protection Regulation 2016/679 (GDPR), Article 6(1)(e) and Article 9(2)(j); Data Protection Act, Sections 4 and 6).

2.2. Sample collection and gut microbiota analyses

A total of 1823 fecal samples from 1033 full term infants, collected between 2008 and 2010, were included in the study (Fig. 1). Samples were collected both in early infancy (0.5–5 months; n = 892) with a mean age of 2.8 months, and late infancy (11–17 months; n = 931) with a mean age of 13.5 months. Parents collected the fecal samples at their homes into sterile collection tubes with no additives, following written instructions provided with sampling equipment. Parents were instructed to mark the date and time of the sample collection and either mail or bring the samples to the laboratory at the ambient temperature as soon as possible after the collection. In late infancy, parents were instructed to place the samples to + 4 °C after collection. On average, fecal samples were stored for two days before being stored at − 80 °C in the laboratory. Specifically, in early infancy, the mean time to storage was 2.0 days, with a maximum of 18 days, while in late infancy, the mean time was 1.8 days, with a maximum of 10 days. The DNA was extracted in 2020 from 30-100 mg of fecal material using a Qiagen DNeasy 96 PowerSoil Pro QIAcube HT kit according to the manufacturer's protocol. The DNA was extracted by Center of Evolutionary Applications, University of Turku. The composition of the gut microbiota was analyzed using 16S rRNA sequencing, targeting the V3-V4 regions. The length of amplified region was approximately 460 bp. The sequencing library was prepared following the Illumina 16S metagenomic sequencing library preparation protocol using primers Bakt_341F: CCTACGGGNGGCWGCAG and Bakt_805R: GACTACHVGGGTATCTAATCC (Illumina, 2013). The MiSeq v3 instrument (Illumina, San Diego, CA) was used for the sequencing. Both sequencing library preparation and sequencing was performed in Finnish Functional Genomics Centre Facility supported by University of Turku, Åbo Akademi University and Biocenter Finland. Both positive (ZymoBIOMICS Microbial Community DNA Standard D6305/D6306) and negative (water) control samples were added to the sequencing and used to assess the accuracy and reliability of the sequencing results.

2.3. Bioinformatics

The sequences were processed using the nfcore/ampliseq pipeline version 2.1.0, which encompasses sequencing quality control, trimming of reads, amplicon sequence variant (ASV) calling, phylogenetic placement, and taxonomic classification (Straub et al., 2020). Taxonomic assignments were achieved by aligning reads against the Silva 138.1 prokaryotic SSU taxonomic database (Quast et al., 2013). Read numbers were not equalized by rarefying as this procedure causes a significant and unnecessary loss of data (McMurdie and Holmes, 2014), but samples with less than 1,000 reads (early infancy n = 6 and late infancy n = 1) were discarded (Fig. 1). After pre-processing, we had a total of 45,948,753 reads from 1823 samples (on average 25,205 reads per sample, range 1,034 – 168,892 reads per sample). The reads accounted for 34,639 ASVs that represented 10 known bacterial phyla and 304 known genera.

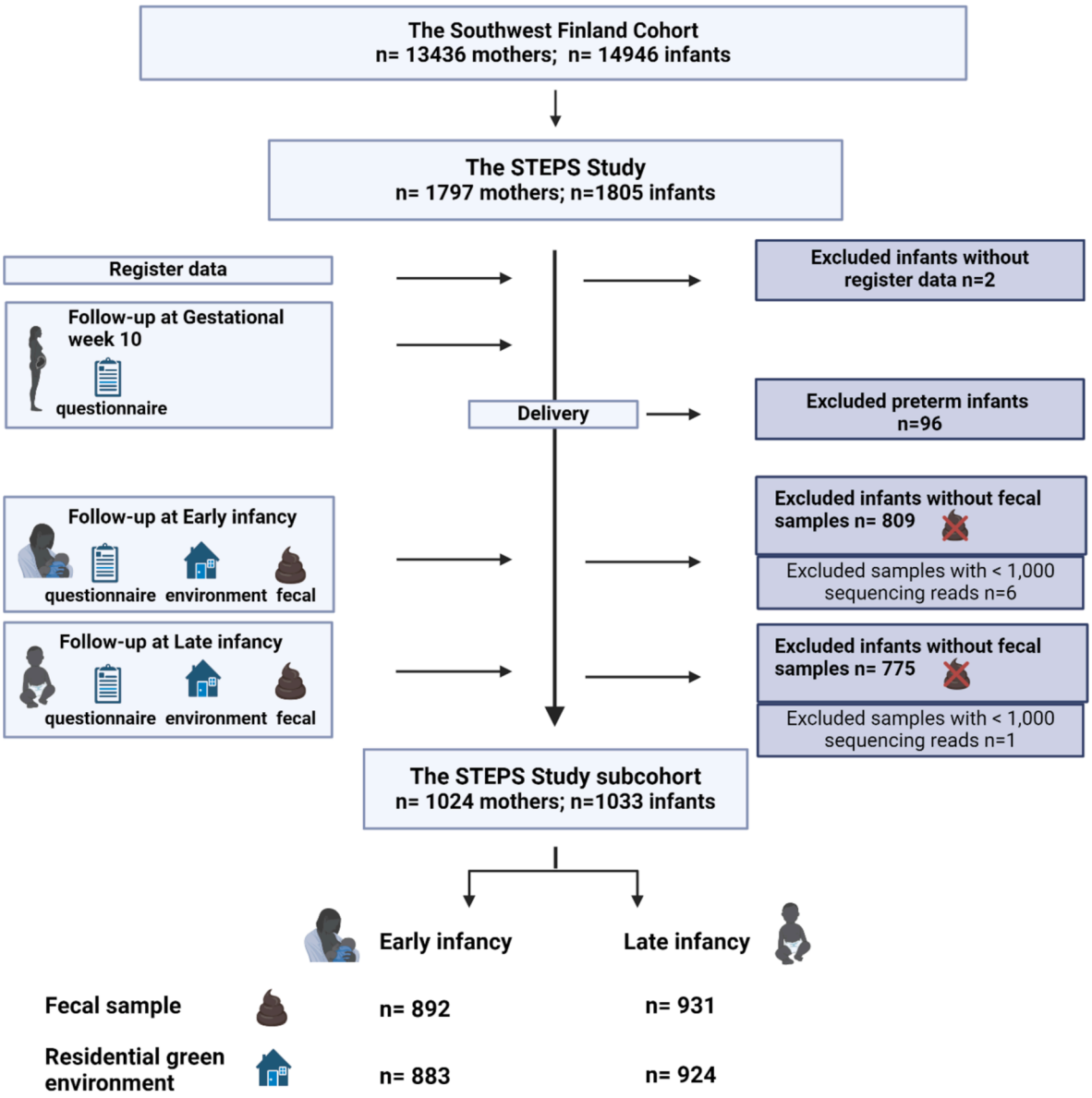

Fig. 1. Flow chart of the STEPS Study. This study focuses on the subcohort of the STEPS Study, comprising infants who have provided fecal samples. Fecal samples were collected at both early and late infancy, representing two distinct time points in the study. At the bottom of the figure, overlapping measures of the residential green environments for the subcohort are depicted, representing the environmental data collected in parallel with the fecal samples. Created with BioRender.com.

2.4. Early life factors

Pre- and perinatal characteristics of the infants and their mothers were extracted from the Medical Birth Register on parturients, deliveries, and births maintained by the Finnish Institute for Health and Welfare. These variables included: child birthweight, sex assigned at birth, and preterm birth (birth occurring before 37 weeks of gestation), maternal age, mode of delivery (vaginal or cesarean section), primiparity (no previous deliveries), maternal weight and height before pregnancy, gestational diabetes mellitus (GDM), and intrapartum antibiotic treatment as well as antibiotic therapy during the 7 days after birth.

Information about breastfeeding (BF) (and complementary feeding) was obtained via a self-administered follow-up diary. The records were collected in real-time. Breastfeeding status was categorized into three groups: exclusive BF (defined as an infant receiving no food other than a mother's breast milk, except for water, at the time of fecal sample collection), partial BF (infants who had been introduced to milk formulas or solid foods and continue breastfeeding at the time of fecal sample collection) and no BF (infants who were not receiving breast milk at the time of fecal sample collection). Infants with missing information on breastfeeding where categorized to unknown. Information about probiotic use before the fecal sampling was acquired from the same follow-up diary.

Self-administered questionnaires during gestational week 10 provided information on family net income and occupational social class at that time. Occupational social class was categorized based on the International Standard Classification of Occupations (ISCO) into two groups according to the occupational titles: "manual" workers (eg, farmers, construction workers, process/transportation workers, or others; ISCO classes 5–9) and "non-manual" workers (eg, as managers, specialists, experts, office workers; ISCO classes 1–4) (Statistics Finland, 2001). Information regarding the mother's and father's age and parental education, as well as living environment including the residential area (rural, urban, settlement area), was also acquired from the self-administered questionnaires during gestational week 10. Information

about the time that the child spent outdoors playing as well as attendance at day care was based on questionnaires at the child's age of 13 months. The outdoor time variable was collected retrospectively by asking parents to report in hours and minutes the time that the child spends outdoors a day. Information regarding exposure to household pets at the age of three months was obtained from the self-administered questionnaires at the child age of 24 months. The season of sampling was determined based on the information provided by the parents about the date and time of the fecal sample collection.

2.5. Residential green environment

Residential mobility data, based on a complete history of the residential addresses with latitude and longitude coordinates, were obtained from the Population Register Center for each mother and her child during the follow-up. Using open-source Geographical Information Systems (QGIS, https://www.qgis.org/en/site/), data on residential green environment were linked to the cohort participants' home addresses by the latitude and longitude coordinates at the time points the fecal samples were collected; the first time when the child was born (early infancy) and the second time when the child was one year old (late infancy).

The selected residential green environment variables measure the properties of the green environments surrounding the homes of the participants (750 m × 750 m grid size) and excluding the indoor environment and actual use of green spaces by the participants. The following residential green environment measures were used for they have been previously connected to health (Reyes-Riveros et al., 2021) as well as microbiota levels (Dockx et al., 2021): greenness (Normalized Difference Vegetation Index, NDVI) (Rhew et al., 2011), vegetation cover diversity (Simpson's Diversity Index of Vegetation Cover, VCDI) (Ritsema van Eck and Koomen, 2008), and Naturalness Index i.e. how much human impact and intervention there has been in the residential area (NI) (Walz and Stein, 2014). The variables of the residential green environments were derived from multispectral satellite images series, with a 30 m × 30 m of spatial resolution (NDVI; Landsat TM 5, National Aeronautics and Space Administration—NASA) and land cover data (other greenness variables; CORINE).

The green environment measures have been described in detail elsewhere (Lahdenperä et al., 2023) but briefly, we used Landsat TM images obtained over the summertime (June–August, greenest months in Finland), to minimize the seasonal variation of living vegetation and cloud cover and NDVI (Rhew et al., 2011) was calculated from selected images. The final NDVI map averaged data from 2008 to 2010, ensuring cloud-free coverage. NDVI measures vegetation, ranging from − 1 to 1 where values below zero represent water surfaces which were excluded, thus the values were constrained to 0–1 (Lahdenperä et al., 2023).

Second, we used calculated indicators related to the diversity and naturalness of the land cover from CORINE Land Cover data sets of the year 2012. Two indexes, VCDI (Ritsema van Eck and Koomen, 2008) and NI (Walz and Stein, 2014), were calculated from this information. VCDI focuses on vegetation classes (agriculture, broad-leave forest, coniferous forest, mixed forest, shrub/grassland and wetland), approaching 1 with increased vegetation class diversity and more equitable distribution among land use classes. NI measures human impact and degree of human interventions on ecological components, ranging from 1 to 7, where low values represent low human impact (≤ 3 = Natural), medium values moderate human impact (4–5 = Semi-Natural), and high values strong human impact (6–7 = Non-Natural) (Lahdenperä et al., 2023).

2.6. Statistical analysis

The statistical analyses were conducted using the R computing environment version 4.4.0 (R Core Team, 2024), employing a structured approach in three main parts: (1) Study the maturation of gut microbiota by characterizing the differences in the gut microbiota diversity and composition between early and late infancy. (2) Identify the significant early life factors influencing microbiota during both time points. (3) Determine the role of the residential green environment measures on gut microbiota development. The analysis encompassed core microbiota analysis techniques by analyzing alpha diversity for within-sample diversity and richness, beta diversity (community composition) for between-sample similarity, and differential abundance (DA) analysis. Given the descriptive approach taken in the beta diversity analysis, and to enhance the reliability of microbial community composition, rare ASVs were removed from the data if they appeared less than 1 time in at least 1 % of the samples. Additional sensitivity analyses were conducted to assess the impact of technical variables. All analyses were conducted with complete case analysis.

2.6.1. Gut microbiota diversity and composition in early and late infancy

Alpha diversity, represented by the Observed richness and Shannon diversity, were determined using the mia R package version 1.12.0 (Ernst et al., 2023). Shannon index was used to describe the bacterial diversity in a sample by counting the abundance and evenness of the ASVs present, while Observed richness estimates the bacterial richness in a sample based on the number of different ASVs present in a sample. The association between alpha diversity and age was tested using the Welch two sample *t*-test. Beta diversity was visualized with principal coordinates analysis (PCoA) for ASV-level data using Bray-Curtis distances and functions from ggplot2 version 3.5.1 (Wickham, 2016). The statistical significance was tested with permutational multivariate analysis of variance (PERMANOVA) with 999 permutations using the adonis2 function from the vegan package version 2.6–6.1 (Oksanen et al., 2022). The homogeneity of the group dispersions was assessed using the permdist function (Oksanen, et al., 2022). Differential abundance analysis of the microbiota was performed using the LinDA package version 0.2.0 (Zhou et al., 2022), which fits linear regression model, and the ALDEx2 package version 1.36.0 (Fernandes et al., 2014) which fits regression model on the centered log-ratio (clr) transformed data. The DA analysis was conducted at the genus level data, focusing on genera present in > 10 % of the samples. All p-values were adjusted for multiple comparisons using the Benjamin Hochberg (BH) procedure and only adjusted p-values < 0.05 were considered statistically significant. Only genera that were statistically significant in both LinDA and ALDEx2 analysis were discussed in the text.

2.6.2. Early life factors accounting for the variation in the gut microbiota community composition

Potential variables influencing gut microbiota were investigated based on existing literature (Bäckhed et al., 2015; Jokela et al., 2023; Lapidot et al., 2022; Laursen et al., 2015; Martin et al., 2016; Mortensen et al., 2018; Mueller et al., 2016; Rutayisire et al., 2016; Uzan-Yulzari et al., 2021). Given the possibility that some variables may have influenced the microbiota already before the studied time period, and that others may modify the microbiota for an unknown time frame, the time-dependent variables, such as the green environment measures, season of sampling, and breastfeeding status, were measured at the time of the sample collection, while other variables remained constant. The statistical significance of each variable on microbiota beta diversity during both early and late infancy was evaluated by employing separate PERMANOVA models for both time points with adjustments for multiple comparisons (BH). In total, 23 variables with known associations with gut microbiota development in infancy, including the residential green environment measures, and technical variables were included in the PERMANOVA analyses. LinDA and ALDEx2 analyses was used to determine statistically significant genera associated with each variable of interest similarly as described in chapter 2.6.1. The models were adjusted for covariates detected in previous PERMANOVA analyses. The effects of each variable of interest on the alpha diversity were evaluated using a non-parametric Wilcoxon test.

2.6.3. The role of the residential green environment in the gut microbiota development

Regression models were used to investigate relationships between green environment measures and alpha diversity (Shannon diversity and Observed richness) using the glm function in R. We tested both linear and quadratic relationship as well as crude and adjusted models using covariates detected in chapter 2.6.2. Effect estimates were presented as regression coefficients (b) along with their corresponding 95 % confidence intervals (CI). To meet the model assumptions Observed richness was log-transformed. LinDA and ALDEx2 analyses was used to determine statistically significant genera associated with each green environment measure as well as outdoor time similarly to chapter 2.6.1. Carrier analyses were conducted to further assess the relationship between outdoor time and specific bacterial genera. Odds ratios (ORs) for a carrier/ non-carrier of *Ruminococcus, Roseburia, Clostridioides,* and *Alistipes* in response to time spent outdoors, were calculated with logistic regression models adjusted for covariates detected in chapter 2.6.2.

3. Results

3.1. Participant characteristics

Of the infants included in the present study, 52 % were boys, 46 % had at least one older sibling in the home at birth, 11 % were delivered by cesarean section, and 10 % received antibiotics at a maternity hospital. More than 50 % of the infants lived in urban areas and Semi-Natural or Non-Natural areas. Most of the infants lived in areas of moderate or high vegetation cover (Table 1). More detailed information about the study population is in the supplementary 1.

3.2. Gut microbiota diversity and composition in early and late infancy

Infant gut microbiota community composition was strongly associated with age. Age explained 9 % of the variation in the gut microbiota between early infancy and late infancy (Fig. 2a, PERMANOVA, p-value = 0.001). The homogeneity of group dispersion significantly differed between early and late infancy and was higher in early infancy (beta-disper, p-value = 0.001). Early infancy was dominated by *Actinobacteriota* (54 %) and late infancy with *Firmicutes* (59 %). At the genus level, bacterial ASVs representing *Bifidobacterium* (50 %) were the most abundant in early infancy followed by *Bacteroides* (23 %) and *Clostridium sensu stricto 1* (8,4%). Transitioning to late infancy *Bacteroides* (22 %) took the place as the most abundant genus, followed by *Bifidobacterium* (14 %), *Blautia* (14 %), and *Faecalibacterium* (11 %). Notably, *Faecalibacterium* was nearly absent during early infancy (Fig. 2b, Supplementary 2). Infant age was also associated with gut microbiota alpha diversity. Both Shannon diversity (Fig. 2c, *t*-test, p-value < 0.001) and Observed richness (Supplementary Figure 1, *t*-test, p-value < 0.001) were statistically significantly lower in early infancy compared to late infancy.

3.3. Sources of variation in gut microbiota during early and late infancy

The gut microbiota community composition in both early and late infancy was significantly influenced by several factors (Fig. 3). The presence of siblings, birth mode, and maternal age exhibited statistically significant associations with variation in gut microbiota community composition in both early and late infancy (Fig. 3, separate PERMANOVA models, p-value < 0.05). From the technical variables, both sequencing library size and DNA concentration displayed statistically significant association with the community composition at both time points (Fig. 3, separate PERMANOVA models, p-value < 0.01). Additionally, factors such as family income, perinatal antibiotic exposure, breastfeeding status, outdoor time, and sequencing batch demonstrated significant associations with the gut microbiota during either early or late infancy (Fig. 3, separate PERMANOVA models, p-value < 0.05).

Table 1
Descriptive characteristics of the subcohort of the STEPS Study in relation to infant age (early infancy / late infancy). Sample sizes and percentages are given for categorical variables and means with standard deviations for continuous variables.

Characteristics	n (%) / Mean (SD) Early infancy n = 892	Late infancy n = 931
Infant characteristics		
Sex		
Boy	462 (52 %)	477 (51 %)
Girl	430 (48 %)	454 (49 %)
Birth mode		
Cesarean	100 (11 %)	105 (11 %)
Vaginal	792 (89 %)	826 (89 %)
Siblings		
Yes	408 (46 %)	432 (46 %)
No	484 (54 %)	499 (54 %)
Breastfeeding status		
Exclusive	373 (42 %)	0 (0 %)
Partial	243 (27 %)	104 (11 %)
No	68 (7.6 %)	596 (64 %)
Unknown	208 (23 %)	231 (25 %)
Pets (at 3 months)		
Yes	222 (25 %)	246 (26 %)
No	329 (37 %)	380 (41 %)
Unknown	341 (38 %)	305 (33 %)
Day care		
Yes	NA	191 (21 %)
No	NA	704 (79 %)
Family characteristics		
Maternal age (years)	31.21 (4.42)	31.29 (4.46)
Pre-pregnancy BMI (kg/m2)	24.30 (4.81)	24.34 (4.80)
Family monthly income		
> 3000 €	450 (51 %)	473 (52 %)
< 3000 €	426 (49 %)	443 (48 %)
Occupational social class		
Manual	163 (19 %)	169 (19 %)
Non-manual	673 (81 %)	705 (81 %)
Antibiotics		
Antibiotics at birth (infant)		
Yes	87 (10 %)	93 (10 %)
No	805 (90 %)	838 (90 %)
Antibiotics at birth (mother)		
Yes	105 (12 %)	106 (11 %)
No	787 (88 %)	825 (89 %)
Environmental characteristics		
Residential area		
Rural	157 (18 %)	162 (18 %)
Urban	508 (58 %)	536 (58 %)
Settlement area	211 (24 %)	220 (24 %)
Season of sampling		
Summer (March–Sep)	419 (49 %)	454 (51 %)
Winter (Oct–Feb)	436 (51 %)	430 (49 %)
Outdoor time (h)	NA	2.17 (1.35)
Naturalness (NI)	5.18 (0.86)	5.11 (0.85)
Greenness (NDVI)	0.56 (0.11)	0.59 (0.11)
Vegetation cover diversity (VCDI)	0.58 (0.15)	0.35 (0.14)

3.3.1. Early life factors accounting for the variation in the gut microbiota community composition

In early infancy, the birth mode (cesarean/vaginal) accounted for 0.5 % of the variation in gut microbiota community composition, gradually decreasing to 0.3 % in late infancy (Fig. 3). Birth mode was significantly associated with bacterial alpha diversity (Table 2, Wilcoxon test, p-value < 0.05) and specific bacterial genera (Table 2, LinDA and ALDEx2, adjusted p-value < 0.05). The presence of siblings accounted for a marked portion of the variation in gut microbiota community composition between infants, explaining 0.5 % of the variability in early infancy and increasing to 0.7 % in late infancy (Fig. 3). Siblings were associated with increased alpha diversity in both age groups (Table 2, Wilcoxon test, p-value < 0.001) and specific bacterial genera (Table 2, LinDA and ALDEx2, adjusted p-value < 0.05). Breastfeeding was statistically significantly associated with gut microbiota

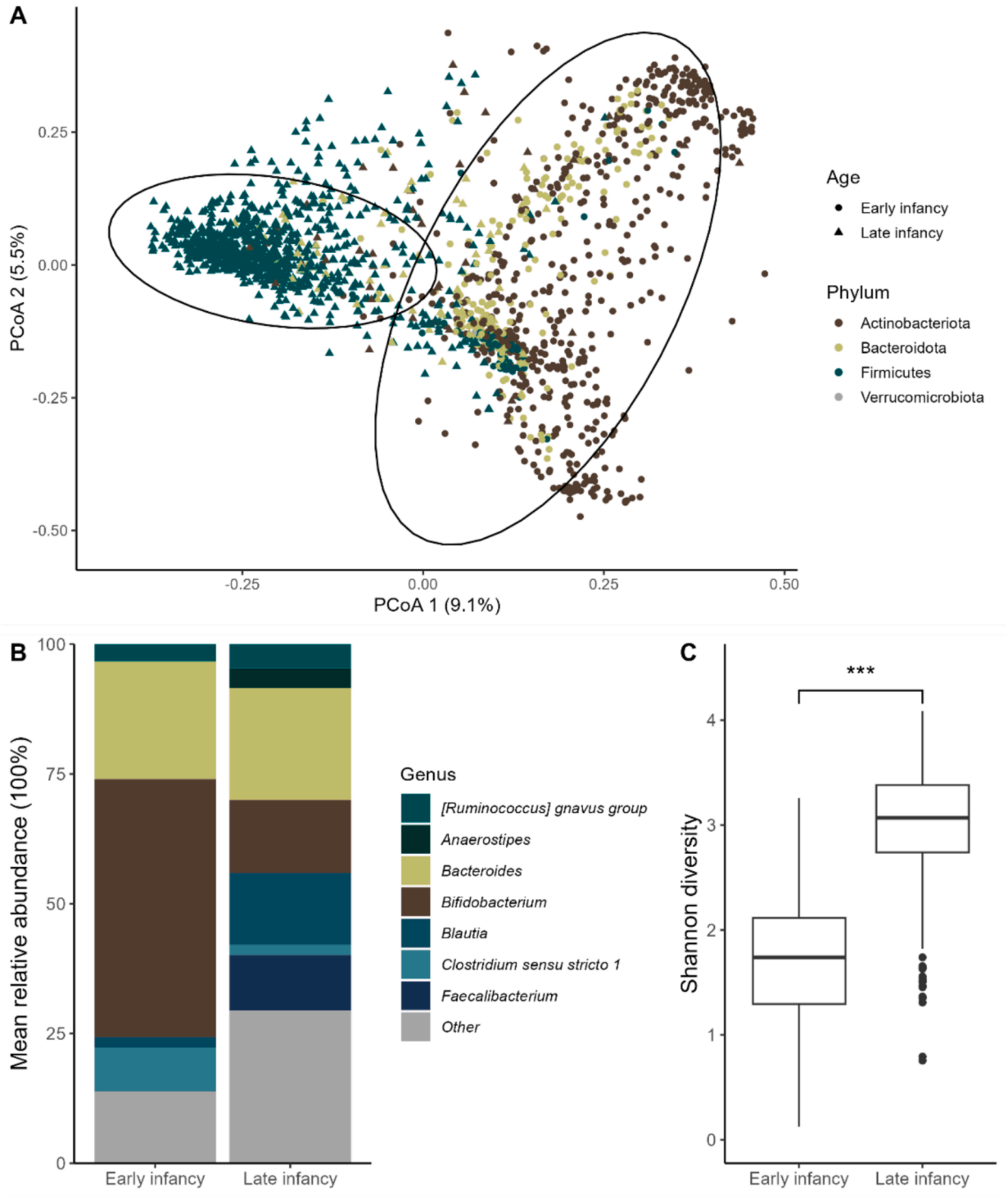

Fig. 2. Differences in the gut microbiota between early and late infancy. (a) Community composition at different time points visualized with PCoA for ASV-level data using Bray-Curtis distances. Colors represent the dominant Phyla in each sample and the ellipses, calculated using ggplot2, represent the early and late infancy time points. (b) Composition bar plot displaying the mean relative abundances of genus-level taxonomy during early and late infancy. (c) Differences in Shannon alpha diversity between early and late infancy. The statistical significance (*t*-test, p-value < 0.001) is indicated with stars.

community composition in late infancy, explaining 0.4 % of the variation, while in early infancy, although not statistically significantly, breastfeeding accounted for 0.5 % of the variation explained between the BF groups (Fig. 3).

Among early life factors related to the infant's family, namely maternal age and family income were associated with the gut microbiota community composition in early and late infancy, each explaining 0.4 % and 0.3 % of the variation respectively (Fig. 3). While the influence of family income diminished in late infancy, the impact of maternal age remained statistically significant (Fig. 3). Exposure to perinatal antibiotics was significantly associated with the gut microbiota in early infancy. The antibiotics administered to the mother explained more variation in the gut microbiota (0.4 %) compared to the variation explained by antibiotics received directly by the neonate (0.3 %)

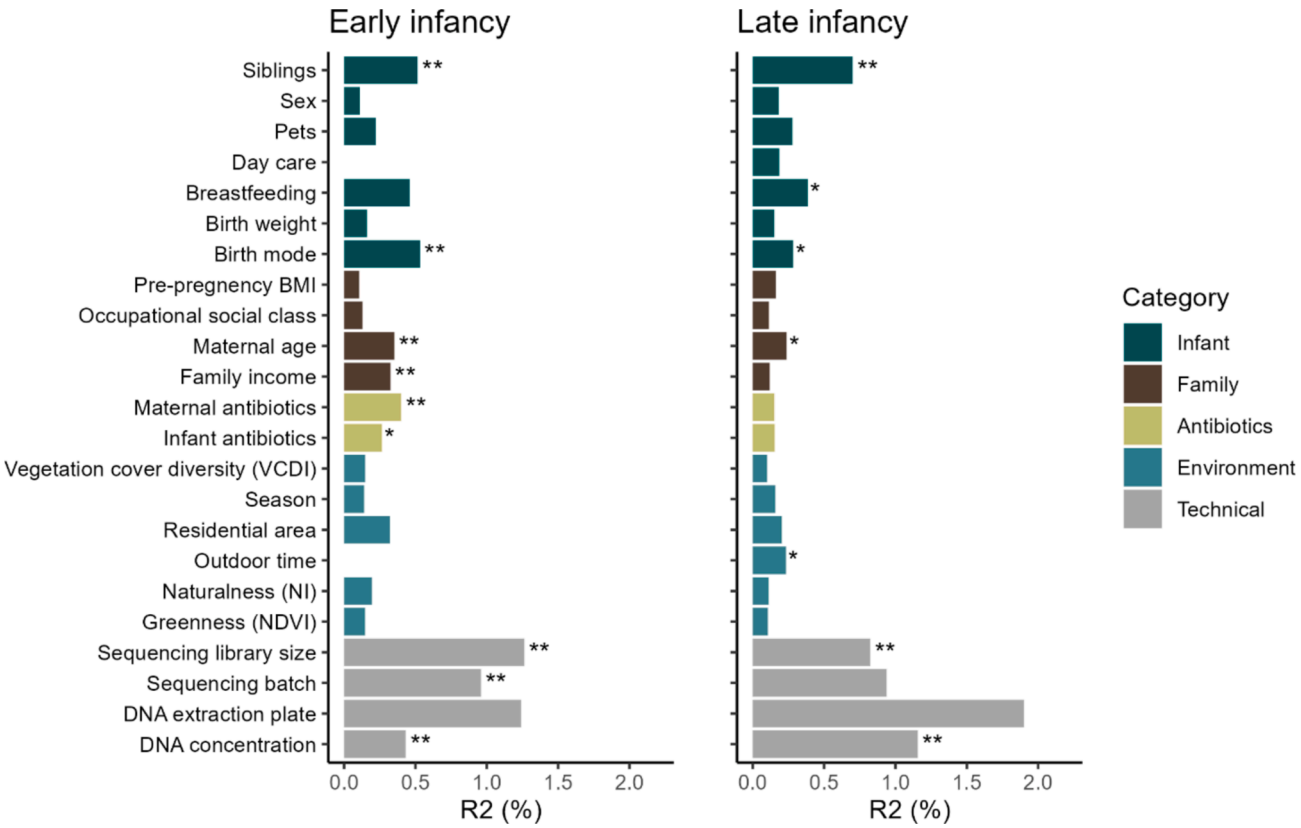

Fig. 3. Sources of variation in gut microbiota community composition during early and late infancy. Separate PERMANOVA models for early (n = 764) and late infancy (n = 742) were employed to evaluate the variation explained (R2 %) and the statistical significance of each variable. To address multiple comparisons, p-values were adjusted using BH method. Statistical significance is denoted as follows (** for p-value < 0.01, * for p-value < 0.05).

Table 2
The effects of early life factors on specific bacterial genera and gut microbiota alpha diversity.

	Early infancy Genera [a]	Shannon/ Observed [b]	Late infancy Genera [a]	Shannon/ Observed [b]
Birth mode (ref cesarean)	*Clostridium sensu stricto 1*↑	↓ **	*Bacteroides* ↓	↓ *
	Vellionella ↑	NS		NS
	Blautia ↑			
	Bacteroides↓			
Presence of siblings (ref yes)	*Eggerthella*↑	↑ **	*Faecalibacterium*↑	↑ ***
	Bifidobacterium↑	↑ *	*Subdoligranulum*↑	↑ ***
	Clostridium sensu stricto 1↓		*Fusicatenibacter*↑	
			Veillonella↓	
			Erysipelatoclostridium↓	
Breastfeeding (BF) status [c] (ref exclusive BF /partial BF)	*Staphylococcus*↑	↓ ***	*Veillonella*↑	NS
	Blautia ↓	↓ ***	*Bifidobactrerium*↑	NS
			Lachnoclostridium↓	
Maternal antibiotics (ref yes)	*Collinsella*↓	↓ *		NS
		NS		NS
Infant antibiotics (ref yes)	*Clostridium sensu stricto 1*↑	↓ **		↓ *
	Bacteroides↓	NS		↓ *

[a] Statistically significant genera (adjusted p-value < 0.05) in both adjusted LinDA and ALDEx2 models (early infancy: mode of delivery, breastfeeding status, mothers age, family income, presence of siblings, antibiotic treatment mother and infant; late infancy: mode of delivery, breastfeeding status, mothers age, presence of siblings and time spent outdoors). The arrows indicate whether the abundance of given genus increases [↑] or decreases [↓] for the reference group given in the brackets.

[b] Alpha diversity measured by Shannon index and Observed richness. The statistical significance was tested using a nonparametric Wilcoxon test (*** for p-value < 0.001, ** for p-value < 0.01, * for p-value < 0.05). The arrows indicate whether Shannon diversity/ Observed richness increase [↑] or decrease [↓] for the reference group given in the brackets. NS indicate a non-significant association

[c] Statistically significant genera (adjusted p-value < 0.05) in both adjusted LinDA and ALDEx2 models comparing exclusive BF with partial BF in early infancy and partial BF with no BF in late infancy.

(Fig. 3). The alpha diversity decreased in early infancy when antibiotics were administered to either the mother or the neonate and persisted into the late infancy when antibiotics were directly administered to the neonate (Table 2, Wilcoxon test, p-value < 0.05). The uneven beta-dispersion was observed between compared groups: siblings in early and late infancy, family income, DNA extraction plate, sequencing batch in early infancy, and day care attendance in late infancy.

3.3.2. The role of the residential green environment in the gut microbiota development

No significant associations between residential green environment measures and gut microbiota community composition were detected after adjusting p-values for multiple testing (Fig. 3). The greenness measures showed no association with specific bacterial genera using both adjusted LinDA and ALDEx2 models. However, measures of greenness displayed a statistically significant association with alpha diversity during early infancy, but not during late infancy, as shown in Table 3. During early infancy, higher levels of NDVI (Table 3, b = 0.038, 95 %CI = 0.004, 0.072) and lower NI (Table 3, b = -0.049, 95 %CI = -0.093, −0.005) was associated with increased Shannon diversity. In addition, VCDI demonstrated a significant quadratic relationship with the Shannon diversity (b2 = 0.041, 95 %CI = 0.002, 0.081), suggesting a non-linear association between VCDI and Shannon diversity. However, these associations were observed only in the crude models. Notably, the association between the NI and the Observed richness persisted even after adjusting for covariates (Table 3, b = -0.035, 95 %CI = -0.070, < −0.001)).

The amount of time spent outdoors was associated with both adjusted alpha (glm, Shannon diversity, b = 0.053, 95 %CI = 0.028,0.077) (glm, Observed richness, b = 0.053, 95 %CI = 0.033,0.072) and beta diversity (PERMANOVA, R^2(%) = 0.2, adjusted p-value = 0.03) in late infancy. The time spent outdoors was only recorded for the late infancy time point, where we observed a positive association between the time spent outdoors and the abundance of bacterial genera *Alistipes, Faecalibacterium, Roseburia,* and *Ruminococcus* (LinDA, adjusted p-value < 0.05). In addition, we found a negative association between outdoor time and the genus *Clostridioides* (LinDA, adjusted p-value < 0.05). While the adjusted ALDEx2 model yielded similar results, it was insufficient to achieve statistical significance. The associations between outdoor time and the four most significant genera *Alistipes, Faecalibacterium, Roseburia,* and *Clostridioides* were further analyzed using an adjusted logistic regression model to study the associations deeper (Supplementary 1, Table 2).

The strongest association with the time spent outdoors was found within the genus *Alistipes*, where a one-hour increase in outdoor time was associated with a nearly 30 % rise in the likelihood of being a carrier of this genus (Supplementary 1 Table 2, OR = 1.281, adjusted p-value < 0.001). Also, the negative association with the genus *Clostridioides* was notable and a one-hour increase in outdoor time decreased the probability of being a *Clostridioides* carrier by 25 % (Supplementary 1 Table 2, OR = 0.755, adjusted p-value = 0.001). A marked rise in *Alistipes* carriers with increased outdoor time is evident in Fig. 4, but in case of the genera *Faecalibacterium, Roseburia,* and *Clostridioides* the relationship was less pronounced. However, the associations remained statistically significant for both *Faecalibacterium* (Supplementary 1 Table 2, OR = 1.175, adjusted p-value = 0.017) and *Roseburia* (Supplementary 1 Table 2, OR = 1.175, adjusted p-value = 0.017).

To evaluate the impact of technical variables on our results, we performed additional sensitivity analyses. Initially, we examined the influence of technical variables on gut microbiota community composition between early and late infancy. Age remained a statistically significant determinant of community composition differences, despite a reduction in explained variance (PERMANOVA, R2(%) = 1.3, p-value = 0.001). Subsequently, we included technical variables as additional covariates in the remaining analyses to account for potential technical confounding. The variables added were statistically significantly associated with gut microbiota community composition in early (sequencing batch, sequencing library size, and DNA concentration) and late infancy (sequencing library size and DNA concentration) (Fig. 3, separate PERMANOVA models, p-value < 0.05). Sensitivity analyses was conducted to assess effects of early life factors on specific bacterial genera (Table 2), gut microbiota alpha diversity and residential environment (Table 3), as well as the association between outdoor time and presence of specific bacterial genera (Supplementary 1, Table 2). The results remained similar with minor changes detailed in the supplementary material 1 Tables 3, 4, 5. Notably, when the associations with gut microbiota alpha diversity and exposure to natural environments were adjusted with additional covariates, the statistically significant association between Naturalness index and Observed richness in early infancy diluted.

Table 3
Associations between being exposed to natural environments within 750 m x 750 m of the residential address and Observed richness and Shannon diversity [a] in infant gut microbiota at early infancy (n = 867) and late infancy (n = 838).

	log(Observed) richness		Shannon index	
Early infancy				
	Crude model b [c] (95 %CI)	Adjusted model [b] b (95 %CI)	Crude model b (95 %CI)	Adjusted model [b] b (95 %CI)
NI [d]	−0.039 (−0.073, −0.005)	−0.035 (−0.070, < −0.001)	−0.049 (−0.093, −0.005)	−0.038 (−0.083, 0.006)
NDVI	0.022 (−0.004, 0.049)	0.021 (−0.006, 0.048)	0.038 (0.004, 0.072)	0.034 (< −0.001, 0.069)
VCDI	0.011 (−0.009, 0.030)	0.012 (−0.008, 0.031)	−0.009 (−0.016, 0.034)	0.013 (−0.012, 0.03)
Late infancy				
NI	−0.005 (−0.035, 0.024)	0.007 (−0.024, 0.038)	−0.004 (−0.042, 0.033)	0.011 (−0.028, 0.050)
NDVI	−0.005 (−0.027, 0.017)	−0.016 (−0.039, 0.007)	0.006 (−0.022, 0.034)	−0.008 (−0.038, 0.021)
VCDI	−0.006 (−0.024, 0.012)	−0.007 (−0.025, 0.012)	< 0.001 (−0.023, 0.0228)	< −0.001 (−0.024, 0.0228)

[a] Higher Observed richness (species richness) values indicate a greater number of different microbial species; higher Shannon index values indicate higher microbial diversity, as measured by species richness and evenness.

[b] Regression models are adjusted for (early infancy): mode of delivery, breastfeeding status, mothers age, family income, presence of siblings, antibiotic treatment mother and infant (late infancy): mode of delivery, breastfeeding status, mothers age, presence of siblings, time spent outdoors.

[c] b > 0, = 0, and < 0 suggest increasing, no, and decreasing effects of greenness on human microbial diversity, respectively.

[d] The effect estimates were expressed as (b) and the corresponding 95% confidence interval (CI) per 1-unit increase in NI and 0.1-unit increase in NDVI and VCDI.

4. Discussion

Our main findings indicate that the development of gut microbiota in infancy is characterized by a shift in microbial dominance as well as community development towards diverse microbial populations. Birth mode correlated markedly with the gut microbiota community composition in early infancy and the presence of siblings in late infancy. Additionally, measurements of outdoor time associated with gut microbiota community composition and specific bacterial genera in late infancy. While residential greenness did not emerge as a significant factor in determining gut microbiota community composition during infancy, green environment measures were associated with gut microbiota species richness in early infancy.

Age emerged as a significant factor driving changes in gut microbiota composition, explaining 9 % of the variation between early and late infancy. The infant gut microbiota evolves from an initially diverse and heterogeneous composition to a simpler, and finally more complex adult-like configuration (Bäckhed et al., 2015). The initial colonizers are microbes from maternal as well as other environmental sources, and many of them are transiently present only in some infants, as they are poorly adapted or unsuited to colonize the infant's gastrointestinal tract (Wampach et al., 2017). Early colonizers are mainly attributed to genera

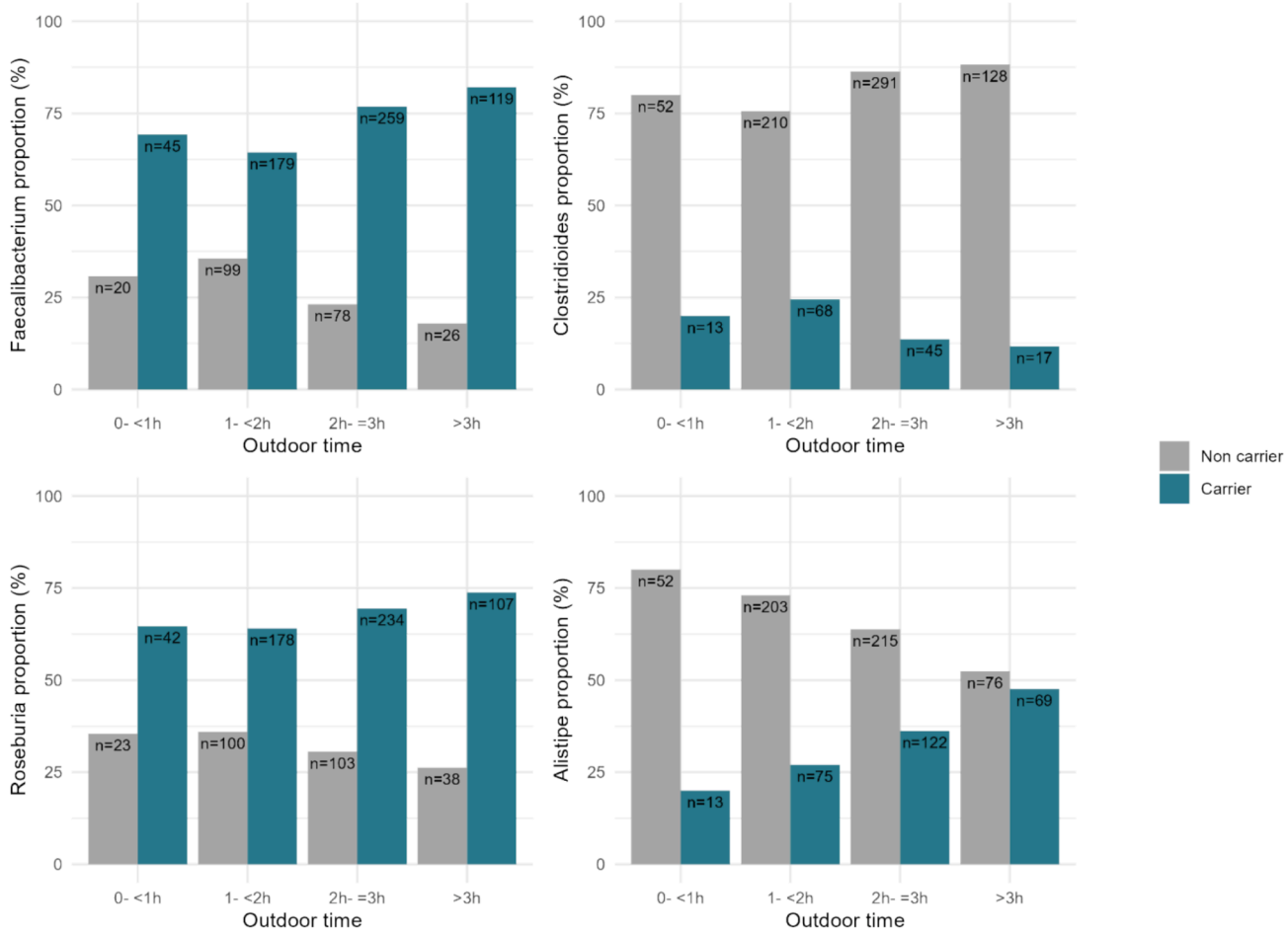

Fig. 4. The association between outdoor time and genera *Faecalibacterium, Clostridioides, Roseburia,* and *Alistipes* visualized in four outdoor time categories. The proportions of carrier/ non-carriers are presented in the y-axis.

described as saccharolytic facultative aerobic i.e., oxygen-tolerant sugar fermenting bacteria (Ferretti et al., 2018; Vieira-Silva et al., 2016). In our study we focused on the periods of simpler community composition, characterized by the high rates of breastfeeding, during early infancy (about 3 months), and the more complex adult-like configuration in late infancy (about 13 months). Because there were minor differences in the sample storage between early and late infancy, one must acknowledge the possible effects of short-term room temperature storage of the early infancy samples, while interpreting the results.

The transition from a simple bacterial community, towards a more diverse microbiota is not random but likely driven by factors such as the establishment of anaerobic conditions, cessation of breastfeeding, and microbial interactions (Bäckhed et al., 2015). The well-established association with the abundance of *Bifidobacterium* in infancy was also observed in our data, where the gut microbiota in early infancy was predominantly composed of this genus. Another abundant genus in early infancy was *Clostridium sensu stricto 1,* a genus that belongs to the pioneer butyrate producers in infant gut microbiota (Appert et al., 2020). The production of butyrate by bacteria is of key importance in the creation and maintenance of the anaerobic conditions that characterize healthy and more adult like gut microbiota (Appert et al., 2020). Toward late infancy, the prevalence of several genera within the phylum *Actinobacteriota* decreased, whereas the prevalence of genera within the phylum *Firmicutes* such as *Blautia* and *Faecalibacterium* increased. The rise in *Faecalibacterium*, a butyrate-producing bacterium sensitive to oxygen, likely results from the development of anaerobic conditions in the gut during late infancy (Miquel et al., 2014). In the case of *Blautia,* the increase in late infancy can be attributed to the cessation of breastfeeding (Galazzo et al., 2020), which also accounts for the decrease in *Bifidobacterium* during this period (Fehr et al., 2020). Genus *Bacteroides* maintained consistently high abundance throughout both early and late infancy. All of these genera are stable colonizers in the gut microbiota, as they comprise a substantial proportion of the overall microbial relative abundance, and for that reason, they work as good hallmarks of microbial maturation. It is still important to note, that gut microbes evolve and survive as a community, and not a single genus can be considered as a representation of microbial maturation.

Microbes exhibit various survival mechanisms, with some capable of independent survival while others rely on coexistence within a community of different microbes. This community development also serves as an indicator of microbial maturation. Our data revealed greater interindividual variation in community composition during early infancy compared to late infancy, with microbiota complexity increasing toward late infancy. This suggests that in late infancy, microbial communities have evolved to include a greater diversity of simultaneous microbial strains, likely shared among infants. This mirrors the characteristics of a healthy, more adult-like microbiota, where microbial populations collaborate to synthesize essential metabolites, such as amino acids, nucleotides, and vitamins (Bäckhed et al., 2015; Watson et al., 2023).

Among all the early life factors assessed, the most pronounced effects on gut microbiota community composition were observed with technical variables related to microbiota analysis, similarly as in the study by Jokela et al. (2023). By reporting these effects, we aimed to increase the transparency of our results and highlight the relative impacts of sample processing, sequencing, and other laboratory procedures. Including

technical variables in the analysis helps mitigate confounding but can also dilute biologically relevant associations by reducing statistical power. In our study, the relatively large sample size allowed us to control for technical variables in the sensitivity analyses without significantly affecting the biological interpretation of our results. On one hand, one of the main findings between gut microbiota alpha diversity in early infancy (Observed richness) and Naturalness Index (NI) diluted after adding additional variables to control the analysis. Future research should consider the balance between controlling for technical variability and maintaining biological relevance.

Surprisingly, our analysis revealed that breastfeeding status during early infancy did not exert a statistically significant influence on the gut microbiota community composition even though the variation explained were notable. However, this finding could be attributed to the fact that in early infancy the largest breastfeeding groups (exclusive BF and partial BF) are not distinguished by the cessation of breastfeeding, but rather the introduction to milk formulas or solid foods. Conversely, in late infancy, we observed a statistically significant impact of breastfeeding status on the gut microbiota community composition, where the two largest groups (partial BF and no BF) were distinguished by the cessation of breastfeeding. The breastfeeding status in late infancy was associated with the increased abundance of *Veillonella* and *Bifidobacterium* in breastfed infants. The increase in *Veillonella* likely stems from its ability to use lactate from breast milk as its main carbon source (Zhang and Huang, 2023). Additionally, previous research indicates that the increase in *Veillonella* may also be influenced by bacterial transfer through milk microbiota and extended breastfeeding duration (Fehr et al., 2020).

We observed an enrichment of *Clostridium sensu stricto 1* during early infancy among infants delivered via cesarean section and those exposed to antibiotics. We also observed a decrease in *Clostridium sensu stricto 1* among infants with siblings compared to those without siblings. These findings align with previous literature associating *Clostridium sensu stricto 1* with cesarean section delivery (Hill et al., 2017; Long et al., 2021), antibiotic exposure (Barnett et al., 2023; Endika et al., 2023), and absence of siblings (Jokela et al., 2023; Penders et al., 2013). Given the variation in microbial community composition between early and late infancy, we found that different bacterial genera were associated with early life factors at different time points. Specifically, the presence of siblings exhibited distinct effects on the microbiota composition during early and late infancy. The observed decrease in *Clostridium sensu stricto 1* among infants with siblings were not seen anymore in late infancy, where the relative abundance of this genus is smaller. Instead, during late infancy, the presence of siblings was associated with a decrease in *Veillonella*, along with an increase in *Faecalibacterium*. The presence of siblings was also associated with the increased gut microbiota alpha diversity in both time points, suggesting that it might contribute to the maturation of infant gut microbiota (Laursen et al., 2015; Stewart et al., 2018).

Conversely, the intrapartum antibiotic treatment and antibiotic therapy during the 7 days after birth, were associated with the decreased alpha diversity. In previous studies, this has been associated with delayed microbial maturation, along with risks of long-term health consequences for the infant (Zhang et al., 2021). The antibiotic treatment was also associated with the gut microbiota community composition in early infancy but not in late infancy, contributing to the knowledge of the duration of the effects of antibiotics in infant gut microbiota. The prevailing perception is, that antibiotic exposure in the first week of life, alters fecal microbiota development with deviations in the relative abundance of individual taxa until 1 to 2 year of age (Uzan-Yulzari et al., 2021; Van Daele et al., 2022).

The relative effect of the residential green environment on the gut microbiota community composition was small compared to some of the early life factors. Despite not being a statistically significant, the residential area (rural, urban, settlement area) appears to account for a greater degree of variation in gut microbiota community composition compared to green environment measures themselves: greenness (NDVI), naturalness (NI), or vegetation diversity (VCDI). Studies with urban–rural distinctions have received more attention in microbiota studies, diverging from the studies with green environment, by incorporating not only the physical surroundings but also the impact of social environment and lifestyle factors (Lehtimäki et al., 2021).

The influence of environmental greenness on gut microbiota is an emerging area of interest, and our study was among the first to explore this association in a population-based cohort of infants. This adds a new dimension to understanding how living environments contribute to microbiota development and subsequent health outcomes. In addition, we analyzed the effects separately in two distinct time points, being able to demonstrate the importance of early infancy exposures to green and natural environments. In early infancy, the gut microbiota alpha diversity was associated with all of the greenness measures (NI, NDVI, and VCDI). While the effects with Shannon diversity index were diluted after accounting for covariates, an association persisted between NI and the Observed richness, suggesting that infants residing in more natural areas exhibit a greater diversity of observed species in their gastrointestinal systems. The stronger association between residential green environment and alpha diversity during early infancy could be attributed to the infant gut's aerobic state during this period. During early infancy, the gut environment may still retain some oxygen, enabling oxygen-tolerant microbes from outdoor environments to colonize the gut microbiota more effectively than in late infancy. However, additional mechanistic studies are required to confirm these observations. On one hand, our findings are supported by our earlier study, which revealed a higher diversity of human milk oligosaccharides (HMOs) among mothers living in natural environments (Lahdenperä et al., 2023). Since HMOs act as substrates for specific microbes capable of metabolizing them, their diverse composition can enhance the richness of microbial species in infants' gastrointestinal systems, particularly during early infancy when breastfeeding is predominant. On the other hand, this raises a question whether the association between residential green environment and infant gut microbiota is mediated by HMOs.

In contrast to our findings, a previous study in four-month-old infants found that proximity to natural environments, measured by the Urban Primary Land and Vegetation Inventory (uPLVI), was associated with reduced microbial diversity (Shannon index), but no association was observed with microbial richness (Chao1 index) (Nielsen et al., 2020). Variations in environmental measures and statistical analyses utilized may account for the differences in outcomes. Although not statistically significant, we also observed that greener environments (measured by NDVI) and more diversely vegetated environments (measured by VCDI) were associated with reduced microbial diversity (Shannon index) and microbial richness (Observed richness) in adjusted models during late infancy. As the sole studies examining the effects of residential green environments on infant gut microbiota during this pivotal phase of microbiota and immune system maturation, additional research is essential to confirm and build upon these findings. Special attention should also be paid to the timing of the exposure and the direction of the effect.

In addition to assessing green environment measures, we investigated the impact of time spent outdoors on gut microbiota diversity and composition. Interestingly, the role of outdoor time in late infancy was notable, also with respect to other early life factors. The increased outdoor time was positively associated with the genera *Alistipes, Faecalibacterium,* and *Roseburia,* with the strongest association observed for the genus *Alistipes*. These genera are recognized for their butyrate-producing capacity (Brame et al., 2021; Vital et al., 2017). Butyrate, produced by the intestinal microbiota, plays a crucial role in maintaining host health by providing energy to the intestinal epithelium, modulating the immune system, and affecting various metabolic pathways throughout the body. Depletion in butyrate-producing taxa has been linked to several emerging noncommunicable diseases (Vital et al., 2017).

Outdoor environments provide conducive conditions for the growth

of butyrate-producing bacteria, which can be transferred into homes through various means such as airflow, pet activity, and clothing (Brame et al., 2021). For example, Parajuli et al. (2018) identified butyrate-producing bacteria *Roseburia* and *Faecalibacterium* in doormat debris while investigating the effects of urbanization on the transfer of environmental microbiota indoors. The research showed that increased urbanization led to decrease in the Firmicutes community, including *Roseburia* and *Faecalibacterium*, in doormat debris. However, this trend was less pronounced in households without pets, suggesting that pet ownership might influence *Firmicutes* abundance, which is typically low in surface of soils (Parajuli et al., 2018). In our study, we observed elevated levels of *Roseburia* and *Faecalibacterium* in infants spending more time outdoors. Yet, the precise contribution of outdoor exposure to this increase remains uncertain, particularly due to the anaerobic and non-spore forming nature of these genera.

We also noted a negative association between outdoor time and the presence of *Clostridioides*. The genus *Clostridioides* include more than 200 species, some of which are known for producing toxins that cause invasive infections (Ioannou et al., 2023). Our findings suggest that outdoor activities may potentially reduce the likelihood of carrying *Clostridioides* species in infancy.

The main strengths of this study include the longitudinal study design, access to the rich metadata in the STEPS study, and large sample size. Our study benefits from detailed information on early life factors collected from both infant and their families, as well as objectively determined accurate place of residence linked to specific measures on the surrounding green environment around the homes of the infants (750 m × 750 m grid sizes). The environmental measures were treated as a continuous variable and the gut microbiota analysis was conducted primarily on the ASV level, avoiding unnecessary categorization. The utilization of two measurement points for assessing gut microbiota composition, strict model adjustments, along with sensitivity analyses with technical variables, enhances the validity of our findings. Furthermore, the exclusion of preterm infants, population-based sample, and the homogeneity of the study population contribute to its generalizability.

However, certain limitations in the current study should be noted. Firstly, while we had comprehensive information on various early life factors, validated data on infant diet were lacking, and some variables had missing information. Additionally, some variables, such as outdoor time, were only acquired at one time point and were not collected in real time, potentially introducing recall bias. Furthermore, we lack information about the specific outdoor environments where the time was spent. Secondly, the predominantly green living environments in Finland, even in urban areas, may limit the variability of residential greenness compared to other populations, potentially influencing the strength of our results. Thirdly, the gut microbiota development was studied on cross-sectional data and no longitudinal analysis were conducted in this study. Finally, the current study was performed using 16S rRNA sequencing which offers comprehensive taxonomic profiling, but other methods, such as shotgun sequencing, could offer better resolution on a functional strain level.

5. Conclusions

Our study explored the impact of various early life factors and residential green living environments on infant gut microbiota composition and diversity. Through comprehensive analyses, we uncovered how distinct early life factors, including birth mode and presence of sibling, influenced gut microbiota community composition in both early and late infancy, while other factors, such as antibiotic exposure exerted a stronger influence on gut microbiota in early infancy and cessation of breastfeeding as well as outdoor time in late infancy. Moreover, our analysis revealed that associations between residential green environment and gut microbiota alpha diversity were more pronounced in early infancy than in late infancy, indicating a critical period of susceptibility to environmental influences on gut microbiota diversity. This study sheds light on the interplay between environmental factors and the early development of gut microbiota, a crucial aspect of infant health and development.

CRediT authorship contribution statement

Minka Ovaska: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Manu Tamminen:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Mirkka Lahdenperä:** Writing – review & editing, Supervision, Conceptualization. **Jussi Vahtera:** Writing – review & editing. **Samuli Rautava:** Writing – review & editing. **Carlos Gonzales-Inca:** Data curation. **Marja A. Heiskanen:** Writing – review & editing. **Hanna Lagström:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.109093.

Data availability

Data will be made available on request.

Due to Finnish federal legislation, the research data cannot be made available online, but data can potentially be shared with Material Transfer Agreement as part of research collaboration. Requests for collaboration can be sent to the Executive Committee of the STEPS Study, please contact principal investigator Hanna Lagström (hanlag@utu.fi). The 16S rRNA sequencing data generated and analyzed during the current study are not yet publicly available. We are in the process of submitting the data to National Center for Biotechnology Information (NCBI) Sequence Read Archive. For any immediate inquiries or requests for the data, please contact Hanna Lagström (hanlag@utu.fi).

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