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Yard vegetation is associated with gut microbiota composition

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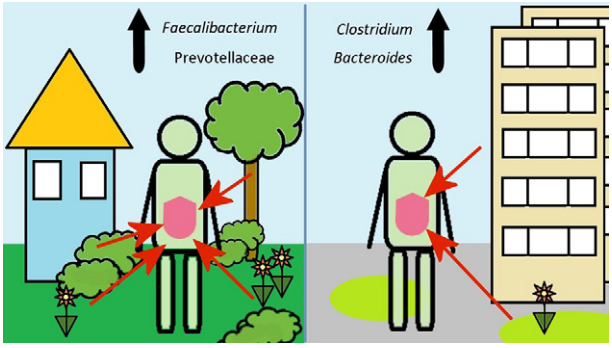
HIGHLIGHTS

- The influence of external environment on human gut microbiota remains elusive.
- We studied the effect of garden diversity and built area (anthroposphere) on gut bacteria (biosphere).
- Yard shrubs and built area correlated with abundance of many health-related bacterial taxa.
- Living environment, and changes therein, likely shape gut microbiota composition.

GRAPHICAL ABSTRACT



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ABSTRACT

Gut microbes play an essential role in the development and functioning of the human immune system. A disturbed gut microbiota composition is often associated with a number of health disorders including immune-mediated diseases. Differences in host characteristics such as ethnicity, living habit and diet have been used to explain differences in the gut microbiota composition in inter-continental comparison studies. As our previous studies imply that daily skin contact with organic gardening materials modify gut microflora, here we investigated the association between living environment and gut microbiota in a homogenous western population along an urban-rural gradient. We obtained stool samples from 48 native elderly Finns in province Häme in August and November 2015 and identified the bacterial phylotypes using 16S rRNA Illumina MiSeq sequencing. We assumed that yard vegetation and land cover classes surrounding homes explain the stool bacterial community in generalized linear mixed models. Diverse yard vegetation was associated with a reduced abundance of

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Living environment
Garden diversity
Built area coverage

Clostridium sensu stricto and an increased abundance of *Faecalibacterium* and Prevotellaceae. The abundance of *Bacteroides* was positively and strongly associated with the built environment. Exclusion of animal owners did not alter the main associations. These results suggest that diverse vegetation around homes is associated with health-related changes in gut microbiota composition. Manipulation of the garden diversity, possibly jointly with urban planning, is a promising candidate for future intervention studies that aim to maintain gut homeostasis.

1. Introduction

The role of microbial contacts in the development and functioning of the human immune system is well-recognized (Macpherson and Harris, 2004; Round and Mazmanian, 2009). A balanced composition of the human commensal microbiota, particularly the gut microflora, has been deemed crucial for the development of a healthy immune system. Accordingly, shifts in the composition of the human gut microbiota, referred to as "microbial dysbiosis" are associated with various immunological disorders (Frank et al., 2007, Sjögren et al., 2009, Sekirov et al., 2010). In stool samples of patients exhibiting dysbiosis, several bacterial genera including the butyrate producing *Prevotella*, *Faecalibacterium*, and *Bifidobacterium* are depleted and others, such as *Bacteroides*, *Ruminococcus* and *Dorea* are enriched compared to healthy controls (Rajilić–Stojanović et al., 2011; Lopetuso et al., 2013; Laursen et al., 2017).

In addition to the role of the commensal human microbiota, a number of studies have now demonstrated that microbial inputs from the environment are also important for the development of a normal immune system and protection from immune-mediated non-communicable diseases (Strachan, 1989; Bach, 2002). In addition, the recently proposed "biodiversity hypothesis" states that interaction with diverse and abundant environmental microbiota is important for the prevention of immune-mediated non-communicable diseases (Von Hertzen et al., 2011). This notion has been exemplified by a high prevalence of immune-mediated non-communicable diseases in the densely built urban environment, which is characterized by low environmental biodiversity and reduced probability of nature contacts (Chapin Iii et al., 2000; Haahtela et al., 2015). In line with this hypothesis, studies have demonstrated that the skin microbiota of children and adolescents are associated with the vegetation type and land cover, such as the proportion of built area in western urban society (Hanski et al., 2012; Ruokolainen et al., 2015; Lehtimäki et al., 2017).

There are indirect evidences suggesting that the composition of the gut microbiota is associated with the living environment. A number of studies have reported a higher abundance of gut *Bacteroides*, *Bifidobacterium*, *Blautia* and *Dorea* in populations from highly urbanized areas in USA and Europe compared to rural populations from Africa and South America who had enriched *Prevotella* and members belonging to Clostridiaceae family (Yatsunenko et al., 2012; Ou et al., 2013; Schnorr et al., 2014; Obregon-Tito et al., 2015; O'Keefe et al., 2015; Gomez et al., 2016). Even though, these studies compare the gut microbiota composition in terms of geographical diversity, the differences observed have been primarily attributed to host factors such as ethnicity, living habits including diet, access to modern health care and use of antibiotics, prebiotics and probiotics. Therefore, it is not known whether such differences can also be observed along an urban-rural gradient, and how environmental factors including yard vegetation and the proportion of built areas are associated with gut microbiota. Our recent studies show that direct contact with gardening materials induces shifts in commensal microbiota composition and the immune response (Nurminen et al., 2018; Grönroos et al., 2019; Hui et al., 2019). In addition, our another recent study revealed that yard greening enhances outdoor activities as well as a higher microbial diversity and biomass in the environment (Puhakka et al., 2019), and the ecological heterogeneity of yard vegetation has been distinguished important in maintaining several ecosystem services, which in turn have been associated with the incidence of at least some immune mediated diseases such as atopy (Hanski et al., 2012; Groffman et al., 2017; Torres-Camacho et al., 2017). It is therefore logical to think that living environment, particularly yard vegetation, influences human interaction with environmental microbes, which ultimately affect the gut microbiota and the immune system. This aspect, however, has not been explored before.

Another factor that is generally assumed to be important in shaping the human gut microbiota is an exposure to pets and other domestic animals. Exposure to dogs, cows and cats have been observed to reduce the incidences of several immune mediated diseases (Ownby et al., 2002; Gern et al., 2004) by increasing the indoor exposure to environmental microbiota (Fujimura et al., 2010). Animal ownership is known to increase gut microbial diversity in children (Azad et al., 2013) and even sleeping on animal fur such as sheep skin has been suggested to reduce the probability of atopy and asthma (Tischer et al., 2015). However, despite growing support for the role of pets and domestic animals in influencing the human microbiota, it is not known how animal ownership shapes potential associations between living environment and gut bacterial community within a western population.

To summarize the knowledge gap, it is not known whether health-associated differences in gut microbiota composition exist along an urban-rural gradient. More importantly, the roles of yard vegetation, built environment and pet ownership in shaping the gut microbiota remain elusive. Therefore, we investigated the stool microbiota of healthy aging Finns as a model population from a single geographic location and ethnic background. We recorded yard vegetation and land cover in the vicinity of their residences, and used these and animal ownership as proxies of environmental diversity to explain stool microbiota composition. We hypothesized that yard vegetation and urban living environment explain the variation in the relative abundance of various stool bacterial taxa. Additionally, we assumed that animal ownership is also important in shaping the gut microbiota composition.

2. Materials and methods

2.1. Study area and participants

Our study participants comprised of 48 elderly retired people (65–79 years) residing within the city of Lahti and rural municipalities in Päijät-Häme and two rural municipalities (Iitti and Pukkila) in the immediate vicinity of Päijät-Häme in Southern Finland (map in Supplementary Fig. S1), additional details in Parajuli et al. (2018). Participants were chosen from a large prospective study called GOAL (Good Aging in Lahti region; Fogelholm et al., 2006) from which participants were randomly selected. A half of the study participants were living in urban apartment houses in the city of Lahti and the remaining half in rural areas in detached houses outside densely populated communities (demographic information in Table 1). The average residence time (mean ± SD) of urban participants was 23 (±10, min = 4, max = 45) years and the average residence time of rural participants was 49 (±18, min = 9 and max = 71) years.

We excluded participants that had one or more non-communicable chronic diseases affecting the immune response, including diabetes, rheumatoid arthritis, chronic obstructive

Table 1
Characteristics of urban and rural participants of the study.

	Urban	Rural
Total participants	23	25
Gender		
Female	10	10
Male	13	15
Animal ownership		
Indoor/outdoor animals	3	12
No animals	20	13

pulmonary disease, celiac disease, psoriasis, dementia, multiple sclerosis, asthma with cortisone treatment, or cancer (active treatment during the last year or largely spread). We also excluded daily smokers, people taking immunosuppressive medication and cortisone pills. Participants who were treated with antibiotics within the last six months were excluded before statistical analysis. Participants owning indoor pets or outdoor domestic animals (cat, dog, cow, horse, chicken and pig) were later separated from the main dataset in subsequent statistical analyses to study the effect of animal-ownership on gut microbiota composition (details below).

For the potential effects of diet, study participants' food habits were recorded in earlier surveys conducted in 2002 and 2012 by the GOAL study. The surveys included 23 preassigned food types that were divided into six food categories. For each food category, the participants were asked whether they consumed the food type 1–2 days, 3–5 days, 6–7 days, or not at all (0 days) during the preceding week. We did not find differences between rural and urban participants' food habits (Supplementary Table S1). Data was similar in 2002 and 2012, and therefore only data from 2012 is shown.

2.2. Stool sample collection, DNA extraction, amplification and sequencing

A total of 90 stool samples were collected from 48 study participants repeatedly in August (45 samples) and November (45 samples; see Supplementary Fig. S2 for the flowchart describing sample collection and subsequent processes), i.e. the three participants who did not provide us stool samples in August were not the same as the three who failed to do so in November. Details of sample collection, DNA extraction, and amplicon sequencing are described in our previous study (Nurminen et al., 2018). Briefly, the participants took the stool samples themselves and stored them at −20 °C until collected by our study personnel a few days later. Samples were transferred in dry ice and stored at −80 °C until analyzed. DNA was extracted from 30 to 60 mg of frozen and unprocessed stool sample and bacterial community was assessed by the amplification of V4 region within the 16S rDNA on Illumina MiSeq equipment (Nurminen et al., 2018).

2.3. Bioinformatics

Paired end sequence data (.fastq) from the rRNA gene dataset of stool bacterial communities were processed using Mothur (version 1.39.5; Schloss et al., 2009) following the protocol by Schloss and Westcott (2011) and Kozich et al. (2013) and as described earlier (Nurminen et al., 2018). Sequences were aligned using the Mothur version of SILVA bacterial reference (version 132). Less abundant operational taxonomic units (OTUs) represented by 10 or fewer sequences across all experimental units were removed to avoid PCR or sequencing artifacts (Oliver et al., 2015). To control the varying number of sequences, each sample was subsampled to 4024 sequences. Observed OTU richness (Sobs), the complement of Simpson's diversity (1/D: $1/\sum pi2$), and Simpson's evenness (ED: $1/\sum pi2/S$), with pi representing frequency of each OTU within a sample, were calculated in Mothur.

2.4. Land cover class and garden diversity determination

The proportions of land cover types within 200 m radiuses from participants' homes' were estimated using the Pan-European CORINE Land Cover 2012 database. The percentages of four different land cover categories. i.e., built area (including hardscapes), open area (spaces with voluminous open nature), forest, and transitional area were characterized and three of them i.e. percentage of built area, forest and transitional area were included in statistical analyses. Open area correlated highly significantly with other variables.

Plant inventory was done between June and July in 2015. The number and type of vascular plant species in study participants' yards were recorded using a 0.1 ha sampling area that excluded roads, forests, fields and buildings. All vascular plant species were classified into 10 different morphological-taxonomic categories: shrubs, trees, tree seedlings (one year old), non-woody flowering plants (excluding monocots), pteridophytes (ferns), edible berry bushes (e.g. currants), fruit trees (e.g. apple, pear, cherries, and plums), non-woody edible plants, perennial plants, and monocots. Owing to a high correlation between some vegetation categories and an insufficient number of plant species (particularly in the urban region), the total number of plant species and the number of species in shrubs, trees, non-woody flowering plants and ferns were included in the analyses. In addition, we did a separate analysis to study the association between gut microbial community and the number of edible plant species in rural areas.

2.5. Statistical analyses

All statistical analyses were performed in R computing environment (version 3.3.3, R Development Core Team, 2017). Differences in bacterial community composition between rural and urban areas and between males and females were analyzed using permutational multivariate analysis of variance (PERMANOVA). Non-metric multidimensional scaling (NMDS) (Vegan package in R; Oksanen et al., 2013) was used to visualize the bacterial community compositions. Plant inventory and land-cover data were correlated with the community structure using permutation tests; the vector fitting procedure (the envfit function in Vegan) and the Bray-Curtis coefficient was used as the dissimilarity measure.

The association between stool bacterial diversity indices as well as relative abundances of bacterial taxa and land-cover types, sampling seasons, genders and yard vegetation were evaluated using generalized linear mixed model (GLMM) with the lmer function in the lme4 package in R. We used altogether three separate GLMM models that produced all the probability values presented in the results. The models were designed to evaluate the association of yard vegetation within 0.1 ha, land cover within 200 m and edible plants in the rural area (within 0.1 ha) separately with stool bacterial community to have a maximum of 5 continuous predictor variables in both models.

To study the association with yard vegetation, predictor variables included sampling area (rural or urban), and sampling season as dichotomous factors and their interaction, and number of plant species in each vegetation category as continuous variables. We compared the difference in diversity, richness and relative abundance of bacterial taxa between rural and urban sites in this model. We visualized the results by plotting the predicted values of the dependent variables from each model against the rural-urban classification (permanent residence in Lahti or outside Lahti) wherever relevant.

For the land cover data (a separate model), we removed rural-urban classification from the factors and used land cover types as continuous variables. The reason was that the sole rural-urban classification did not reduce but variation in land cover, and particularly the coverage of built area, reduced the transfer of environmental microbiota indoors in our earlier study (Parajuli et al., 2018). To

determine the season-wise variation in stool bacteria, we considered only those dependent variables that had significant associations with season in both models (where applicable) for a robust prediction. In case of the dataset about the association between gut microbial community and the number of edible plant species in rural yards, we removed rural-urban classification, added animal ownership as a dichotomous variable, and used berry bushes, non-woody edible plants and fruit trees as continuous variables. The reasons were that these vegetation types were abundant around rural houses but were present in only a few urban houses, and that almost 50% of rural participants owned an animal.

We performed all analyses for the whole dataset (90 samples) as well as for the dataset generated by excluding the study participants with animals (63 samples) separately in the first two models because of unequal distribution of animal owners between rural (12) and urban (3) participants. However, for the association between edible plants in the yard and stool bacterial taxa in rural area, we included animal ownership as a factor since a half of the participants in the rural area owned an animal.

In each case, we checked the model assumptions. We inspected the distribution of residuals and plotted fitted values against residuals. Response variables were transformed as log, square root or cubic root where necessary to ensure that model assumptions were met. For model selection, we removed non-significant terms, starting with the term with the highest *p*-value. The continuous variables, i.e. plant inventory and land cover were initially subject to model simplification until only terms with *p*-values <0.1 were left. If the interaction between factors remained non-significant (p-values >0.1), it was also removed. However, to remain true to our study design, the main effects (sampling month and rural-urban classification (in vegetation analyses)) were retained in the model irrespective of their significance. At finer taxonomic (family and genus) levels, we selected 8 and 12 most abundant taxa respectively that had no or very few zero abundances across samples to obviate the problems caused by zero inflation that is common with microbial sequence data. At all other taxonomic levels, we analyzed the taxa that were represented by >1% of the sequences.

To double-check the results, we analyzed each taxon separately so that samples in which the abundance was zero were removed case by case (Maurice et al., 2015) to confirm that zero abundances did not cause bias.

3. Results

3.1. Bacterial community characterization

We analyzed the 16S rRNA gene amplicon dataset from 90 stool samples collected repeatedly from 48 elderly volunteers (25 rural and 23 urban) in August and November 2015. We obtained 9239 OTUs that represented 9 known bacterial phyla. These bacterial communities belonged to 18 identified classes and 81 known genera. Firmicutes and Bacteroidetes were the two dominant phyla representing >90% of the total sequences (91% in urban and 92% in rural). Bacterial OTUs representing Firmicutes were the most abundant in rural stool samples with 48% of the total sequences, while Bacteroidetes was the most abundant in urban stool samples with more than half (51%) of the sequences. These were followed by Actinobacteria, Proteobacteria and Verrucomicrobia (Fig. 1). In rural stool samples, the abundance of Firmicutes ranged from 5 to 68% and that of Bacteroidetes was 16–93% (Supplementary Fig. S3a), while the range was 10–83% for Firmicutes and 13–71% for Bacteroidetes in urban stool samples (Supplementary Fig. S3b).

3.2. Bacterial community composition

We performed PERMANOVA to study the differences in the bacterial community composition between rural and urban areas and between males and females and used NMDS ordination to visualize the differences graphically. PERMANOVA and NMDS revealed that the community composition did not differ between male and female volunteers in August ($p = 0.06$; Fig. 2a) but differed in November ($R^2 = 0.08$, $p = 0.009$; Fig. 2b) in the whole dataset as well as when the animal owners were excluded (Supplementary Fig. S4). Four environmental vectors, percentage of built area ($R^2 = 0.14$, $p = 0.033$), and transitional area ($R^2 = 0.11$, $p = 0.042$), and the number of shrub species ($R^2 = 0.08$, $p = 0.012$) and berry bushes ($R^2 = 0.13$, $p = 0.023$) was associated with the stool bacterial community composition in November while the number of tree species ($R^2 = 0.12$, $p = 0.036$) was associated with the community composition in August samples (Fig. 2) in the whole dataset. In contrast, when the animal owners were excluded, only the number of shrub species ($R^2 = 0.20$, $p = 0.048$) was associated with the community composition in August and no environmental

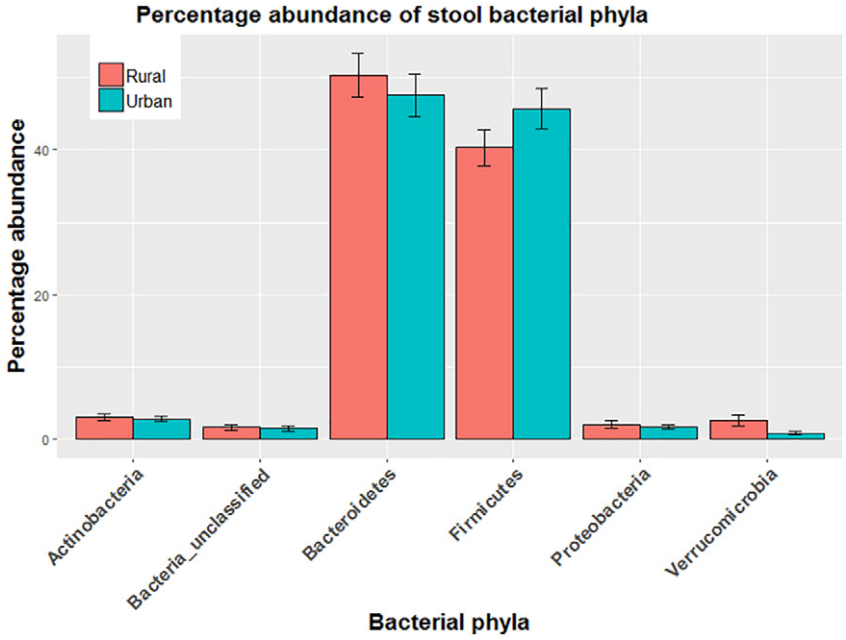

Fig. 1. Major bacterial phyla in rural and urban stool samples (values are expressed as mean ± SE and a cut off value of 1% abundance was used).

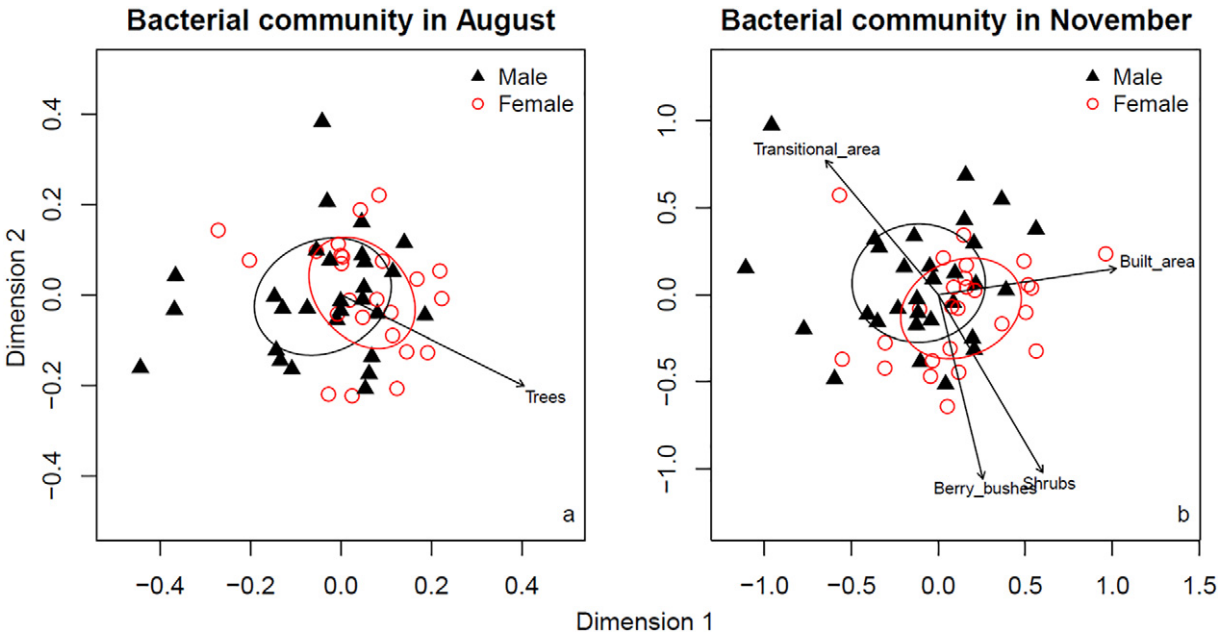

Fig. 2. NMDS ordination revealing difference in bacterial community composition in stool samples between males and females in August (a) and November (b) in the whole dataset. Statistically significant ($p < 0.05$) environmental variables are shown.

variables correlated with the community composition in November (Supplementary Fig. S4). In addition, we did not observe differences in the bacterial community composition between rural and urban stool samples in either datasets.

3.3. Association between vegetation and land cover type and stool microbial communities

3.3.1. Number of yard shrub species is an explanatory variable of gut bacterial community

We performed GLMM analysis to study the association between vegetation type as well as land cover surrounding the permanent residences and the gut microbiota of our study participants separately. GLMM of the model containing vegetation type as predictor variables revealed that the number of shrub species in the yard had the strongest association with stool bacterial community. The relative abundance of *Faecalibacterium* sp. ($p = 0.005$) and *Bifidobacterium* sp. ($p = 0.003$) increased while that of *Clostridium* sensu stricto ($p = 0.002$) decreased with an increase in the number of shrub species (Table 2). When the animal owners were removed from the data, the associations between the number of shrub species and *Faecalibacterium* sp. ($p = 0.006$) and *Clostridium* sensu stricto ($p = 0.006$) were retained (Supplementary Table S2).

Table 2
Statistical parameters revealing the association between relative abundance of stool bacterial taxa and number of yard shrub species from GLMM analysis.

Taxa	Estimate	Standard error	*p* value
Genus level			
Bifidobacterium[a]	0.0077	0.0026	0.003
Clostridium sensu stricto[a]	−0.0047	0.0016	0.002
Faecalibacterium[a]	0.0131	0.0047	0.005
Family level			
Bifidobacteriaceae[a]	0.0077	0.0026	0.003
Clostridiaceae_1[a]	−0.0047	0.0016	0.002
Prevotellaceae[a]	−0.0288	0.0109	0.008
Ruminococcaceae	0.0094	0.0033	0.004
Class level			
Bacteroidia	−0.0171	0.0064	0.007
Phylum level			
Bacteroidetes	−0.0179	0.0061	0.003
Firmicutes	0.0143	0.0054	0.008
(Firmicutes:Bacteroidetes)[b]	0.080	0.029	0.005

a = square root transformation, b = log transformation.

The same highly significant associations existed at the family level: the relative abundance of Ruminococcaceae (that includes *Faecalibacterium* sp.) increased and Clostridiaceae cluster I decreased when the number of yard shrub species increased ($p = 0.004$ and $p < 0.001$ with and without animal owners for Ruminoccaceae and $p = 0.002$ and 0.006 for Clostridiaceae, respectively, Table 2 and Supplementary Table S2). Additionally, the abundance of family Prevotellaceae declined with increase in the number of shrub species regardless of whether the animal owners were excluded or not ($p = 0.008$ with animals and $p = 0.014$ without animal owners, Table 2 and Supplementary Table S2). In contrast, the association between shrubs and Bifidobacteriaceae family and *Bifidobacterium* sp. became insignificant ($p > 0.05$) when animal owners were removed from the data. In addition, *Blautia* sp. ($p = 0.001$) increased and *Prevotella* sp. ($p = 0.009$) decreased as the number of yard shrub species increased when animal owners were excluded from the analysis (Supplementary Table S2).

At the phylum level, the relative abundance of Firmicutes increased when the number of yard shrub species increased ($p = 0.008$ with and $p = 0.004$ without animal owners), whereas Bacteroidetes ($p = 0.003$) declined, but only when the animal owners were included. The Firmicutes to Bacteroidetes ratio, however, exhibited positive association with yard shrub species with and without animal owners (Table 2 and Supplementary Table S2). At class level, the abundance of Bacteroidia ($p = 0.007$) declined with increase in yard shrub species but only when animal owners were included (Table 2).

As several garden shrubs are cultivated for edible fruits, we analyzed shrubs species bearing edible fruits separately. The relative abundance of stool *Parabacteroides* sp. increased ($p = 0.026$) and *Prevotella* sp. decreased with an increase in the number of shrubs species bearing edible fruit ($p = 0.005$; Supplementary Table S3).

3.3.2. Built environment is associated with gut bacteria composition

The strongest positive association between the percentage of built area and the relative abundance of stool bacteria was in case of *Parabacteroides* sp. ($p = 0.002$; Table 3). The relative abundance of *Bacteroides* sp. and family Bacteroidaceae increased with increasing

Table 3
Statistical parameters revealing the association between relative abundance of the stool bacterial families and genera with coverage of built area.

Taxa	Estimate	Standard error	p value
Genus level			
Bacteroides[a]	0.0020	0.0007	0.008
Parabacteroides[a]	0.0006	0.0002	0.002
Prevotella[a]	−0.0024	0.0011	0.028
Family level			
Bacteroidaceae[a]	0.0020	0.0007	0.008

a = square root transformed.

percentage of built area ($p = 0.008$; Table 3). When the animal owners were removed from the analysis, these associations became less significant (p = 0.009 and $p = 0.019$ for *Parabacteroides* and *Bacteroides*, respectively; Supplementary Table S4). The relative abundance of *Prevotella* sp. decreased with an increase in the percentage of built area regardless of the inclusion of the animal owners ($p = 0.028$ and $p = 0.014$, respectively; Table 3 and Supplementary Table S4).

We also used urban-rural division as one of the factors in GLMM but it did not turn out to be a significant factor affecting the relative abundances of stool bacterial taxa (Supplementary Fig. S5). Genus *Dorea* was the only taxon that was associated with rural-urban classification (higher in urban), regardless of animal ownership ($p = 0.010$ and p = 0.026 with and without animal owners; Supplementary Fig. S5). *Faecalibacterium* sp. was less abundant ($p = 0.027$) and *Parabacteroides* sp. ($p = 0.025$) and class Bacilli ($p = 0.015$) were more abundant among urban participants in the whole dataset. When the animal owners were excluded, Family Clostridiaceae 1 and genera *Clostridium* sensu stricto (p = 0.025) and *Prevotella* (p = 0.025) were less and genus *Blautia* (p = 0.008) was more abundant in urban stool samples, but the significances were not strong (Supplementary Fig. S5). Finally, we analyzed the importance of other land cover types and observed that they did not have particularly significant associations with stool bacterial taxa (Supplementary Table S5).

3.3.3. Non-woody flowering plants diversity is associated with the abundance of gut bacterial taxa

The number of non-woody flowering plant species had a direct association with the relative abundance of the family Prevotellaceae ($p < 0.001$ with animal owners and $p = 0.029$ without animal owners, Supplementary Table S6) but not the genus *Prevotella sp.* ($p > 0.05$). As an opposite, the relative abundances of Bacteroidaceae and *Bacteroides* sp. ($p = 0.005$) decreased as the number of non-woody flowering plants increased in the whole dataset. Likewise, the relative abundance of the class Bacteroidia ($p = 0.009$ and $p = 0.004$ with and without animal owners, respectively) and phylum Bacteroidetes ($p = 0.011$ and $p = 0.003$ with and without animal-owners) increased with an increase in the number of flowering plant species (Supplementary Table S6). In contrast to flowering plants and particularly shrubs, the total number of plant species and ferns had very few associations with stool microbiota (Supplementary Table S7).

We also analyzed the whole data by removing samples having zero abundance of the taxa being tested as a response variable. However, the results were hardly affected, suggesting that our observations were not biased by the nature of the bacterial sequence data (Supplementary Tables S8–S12).

4. Discussion

In this study, we showed that yard vegetation and land cover in the immediate vicinity of residences were important explanatory variables for stool microbiota composition along an urban-rural gradient while sampling time had no effect. Particularly, the diversity of yard shrub species demonstrated the most profound relationships and was associated with shifts in the relative abundance of two major gut phyla, i.e. Bacteroidetes (reduced) and Firmicutes (increased) as well as the Firmicutes to Bacteroidetes ratio (increased). Reduction in Firmicutes to Bacteroidetes ratio is typical for gut flora among the elderly (Mariat et al., 2009), probably an indication of unstable and deteriorating gut microbiota, and the reduction is also associated with an increased incidence of inflammatory bowel disease and allergic reactions (Clemente et al., 2012; Kim et al., 2018). The observed increase in this ratio with an increase in the number of yard shrub species, regardless of whether the animal-owners were included or not, suggests that abundant yard shrubs could promote gut microbiota homeostasis, particularly among the elderly.

This assumption is supported further by the associations observed at the family and genus level. Family Ruminococcaceae and genus *Faecalibacterium* became more abundant and Clostridiaceae cluster I and *Clostridium* sensu stricto less abundant with increasing number of yard shrub species, regardless of whether animal owners were included or not. Several members of Clostridiaceae and *Clostridium* sensu stricto are known to be pathogenic (Lakshminarayanan et al., 2013; Rajilić-Stojanović and de Vos, 2014) and have been seen as a marker of degrading gut microbiota among the elderly (Drago et al., 2012; Lopetuso et al., 2013), whereas, *Faecalibacterium* has been associated with a healthy gut (Rajilić–Stojanović et al., 2011; Rajilić-Stojanović and de Vos, 2014). Therefore, even though we cannot say for sure whether the shrubs were the sources of gut *Faecalibacterium*, there is a good reason to assume that yard shrub diversity is linked to gut homeostasis and can turn out to be an important revelation in understanding how living environment and particularly the green environment is associated with human health.

Because of the nature of our study design, we cannot state the very reason why yard shrubs had more associations with stool microbiota compared to other morphological vascular plant groups. However, shrubs are about the same height as humans, they need weeding, are cut regularly, and their microbiota in fallen leaves invade paths and patios in October and early November and therefore enhance the chances of human contacts. Among rural participants, the number of shrubs bearing edible fruit promoted *Parabacteroides,* known to cut down sugars (Nihira et al., 2013), but the association was not highly significant ($p > 0.02$), possibly due to a small sample size. As the applied value of any strong associations between yard vegetation and gut microbiota can be huge, we encourage further research on shrubs and their effect on gut microbiota by, for instance, utilizing metagenomics approaches for the assessment of microbial functions.

Number of non-woody flowering plant species was associated with the relative abundance of a number of stool bacteria including positive associations with the phylum Bacteroidetes and class Bacteroidia. Bacteroidia comprises two of the most abundant families in gut flora, i.e. Prevotellaceae, that became more abundant, and Bacteroidaceae (including only genus *Bacteroides* in this study) that declined with increasing number of non-woody flowering plant species. Since these two families and the *Bacteroides* genus have context-dependent association with health disorders (Wexler, 2007; Ley, 2016), the associations between flowering plants species and gut microbiota were not straightforward indicators of human health. Interestingly, however, Hanski et al. (2012) observed a negative correlation between the abundance of non-native flowering plants in garden and atopy in Finnish children and atopic children were found to host less Bacteroidetes and Bacteroidia in another study (Chen et al., 2016). Although the study populations differ, these interesting associations suggest that non-woody flowering plant species may have a role in gut homeostasis together with woody shrubs and will require further investigations.

We observed that the built area coverage was associated with an increased abundance of genera *Bacteroides* and *Parabacteroides* and a reduced abundance of genus *Prevotella*. Previously, *Parabacteroides* has been linked with a healthy gut microflora in the elderly and a high abundance of *Bacteroides* and *Dorea* have been associated with several

adverse health conditions and obesity (Lucke et al., 2006; Pozuelo et al., 2015; Del Chierico et al., 2017) but the effects of *Bacteroides* are assumed bidirectional (Wexler, 2007). This suggests that built environment does not necessarily cause solely negative changes in the stool of healthy elderly people. Interestingly, increase in *Bacteroides* and *Dorea* and decrease in *Prevotella* with increased coverage of built area correspond to cross-continental studies comparing urban and rural populations (Yatsunenko et al., 2012; Ou et al., 2013; Schnorr et al., 2014; Obregon-Tito et al., 2015; O'Keefe et al., 2015; Gomez et al., 2016). Therefore, it seems that living in highly rural areas and having a preindustrial life style is not needed for a healthy gut microbiota. As our study population comprised of a homogenous group of people, we can exclude factors such as ethnicity, socioeconomic status, high parasite load, access to modern health care, use of antibiotics and even diet (Supplementary Table S1). Our findings therefore underline the role of everyday nature contacts and transfer of environmental microbiota indoors (Parajuli et al., 2018; Hui et al., 2019) in the homeostasis of gut microbiota.

Even though animal ownership affected the bacterial composition between rural and urban participants, it did not alter the key finding in our study, i.e. it did not affect the associations found between shrubs and *Faecalibacterium* and *Clostridium* sensu stricto. Instead, all the associations between stool microbiota and *Bacteroides* and those found between land cover classes and stool microbiota depended on the inclusion of animal owners. As animal owners seemed to have less *Parabacteroides* and *Bacteroides* than non-animal owners, the increased relative abundance of these two taxa in built environment might be balanced by pets and domestic animals (Parajuli et al., 2018). This association that may even turn out to be a causal effect, however, is presumably dependent on nature contacts; if animals and their owners do not have access to surface soil or if the environment is polluted, surface soil microbiota is not transferred indoors or it is different compared to microflora found in pristine environments (Parajuli et al., 2017; Grönroos et al., 2019; Roslund et al., 2018; Hui et al., 2019) Further, as severe pollution changes not only environmental and human microbial but also plant communities (Sinkkonen et al., 2013; Hansi et al., 2014; Belz and Sinkkonen, 2016; Roslund et al., 2019) associations found in relatively unpolluted Southern Finland may not necessarily hold under severe air pollution e.g. in megalopolises. We encourage research that takes into account environmental factors such as land cover, traffic and variation in air and soil pollution. Intervention studies based on modification of yard vegetation are recommended to study the importance of immediate living environment in shaping gut microbiota and to develop easy-to-use management strategies that modify the load of environmental microbiota urban dwellers are exposed to (Puhakka et al., 2018; Hui et al., 2019).

5. Conclusion

Our findings suggest that yard vegetation, particularly shrubs and non-woody flowering plants, and the coverage of built area are associated with gut microbiota. As the relative abundance of *Faecalibacterium* was high and that of *Clostridium* sensu stricto was low with an increasing number of shrub species, we have a good reason to hypothesize that yard vegetation have cascading effects on health associated gut microflora in urbanized, developed societies. Living in areas with tdiverse plant communities seems to be negatively associated with dysbiotic shifts in gut microbiota.

Ethics approval and consent to participate

This study was carried out according to the recommendations of the "*Finnish Advisory Board on Research Integrity*" and ethical approval was obtained from the regional ethical committee. Written informed consents were obtained from all subjects in accordance with the *Declaration of Helsinki*.

Availability of data and materials

Raw sequences reads generated in the study are available in Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) with accession numbers SAMN08991885-SAMN08992045.

Disclosure statement

The authors report no conflict of interest.

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CRediT authorship contribution statement

Anirudra Parajuli: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Nan Hui:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draftWriting - review & editing. **Riikka Puhakka:** Validation, Investigation, Data curation, Writing - review & editing. **Sami Oikarinen:** Investigation, Resources, Data curation.**Mira Grönroos:**Validation, Investigation, Data curation, Writing - review & editing, Supervision. **Ville A.O. Selonen:** Investigation, Data curation. **Nathan Siter:** Validation, Investigation, Resources, Data curation. **Lenka Kramna:** Investigation, Resources, Data curation. **Marja I. Roslund:** Investigation, Data curation. **Heli K. Vari:** Investigation, Data curation. **Noora Nurminen:** Investigation, Data curation, Writing - review & editing. **Hanna Honkanen:** Investigation, Data curation. **Jukka Hintikka:** Validation, Resources, Data curation, Writing - review & editing. **Hannu Sarkkinen:** Validation, Resources, Data curation, Writing - review & editing. **Martin Romantschuk:** Validation, Resources, Writing - review & editing. **Markku Kauppi:** Validation, Resources, Data curation, Writing - review & editing. **Raisa Valve:** Validation, Resources, Data curation, Writing - review & editing. **Ondřej Cinek:** Validation, Resources, Data curation, Writing - review & editing. **Olli H. Laitinen:** Methodology, Validation, Writing - review & editing, Supervision. **Juho Rajaniemi:** Validation, Resources, Data curation, Writing - review & editing. **Heikki Hyöty:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision. **Aki Sinkkonen:** Funding acquisition, Supervision, Visualization, Writing - review & editing, Resources, Validation, Methodology, 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.scitotenv.2020.136707.

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