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Associations between land cover categories, gaseous PAH levels in ambient air and endocrine signaling predicted from gut bacterial metagenome of the elderly

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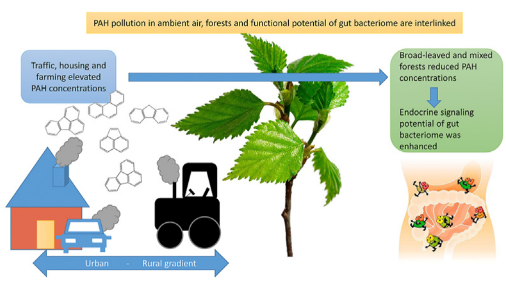
HIGHLIGHTS

- We studied consequences of ambient air PAH pollution along an urban-rural gradient.
- We found associations between land cover, gaseous PAHs and gut functional microbiome.
- PAH levels in air were high in active farms and low next to broad-leaved forests.
- Functional orthologs for endocrine signaling increased with broad-leaved forest cover.
- Air purification by forests may alleviate the endocrine disruption potential of PAHs.

GRAPHICAL ABSTRACT



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ABSTRACT

There is evidence that polycyclic aromatic hydrocarbons (PAHs) and human gut microbiota are associated with the modulation of endocrine signaling pathways. Independently, studies have found associations between air pollution, land cover and commensal microbiota. We are the first to estimate the interaction between land cover categories associated with air pollution or purification, PAH levels and endocrine signaling predicted from gut metagenome among urban and rural populations.

The study participants were elderly people (65–79 years); 30 lived in rural and 32 in urban areas. Semi-Permeable Membrane devices were utilized to measure air PAH concentrations as they simulate

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the process of bioconcentration in the fatty tissues. Land cover categories were estimated using CORINE database and geographic information system. Functional orthologues for peroxisome proliferator-activated receptor (PPAR) pathway in endocrine system were analyzed from gut bacterial metagenome with Kyoto Encyclopaedia of Genes and Genomes.

High coverage of broad-leaved and mixed forests around the homes were associated with decreased PAH levels in ambient air, while gut functional orthologues for PPAR pathway increased along with these forest types. The difference between urban and rural PAH concentrations was not notable. However, some rural measurements were higher than the urban average, which was due to the use of heavy equipment on active farms.

The provision of air purification by forests might be an important determining factor in the context of endocrine disruption potential of PAHs. Particularly broad-leaved forests around homes may reduce PAH levels in ambient air and balance pollution-induced disturbances within commensal gut microbiota.

1. Introduction

Polycyclic aromatic hydrocarbons or PAH compounds are ubiquitous pollutants in the environment as they are formed during incomplete combustion or pyrolysis of carbonaceous material. They are emitted to the atmosphere from many anthropogenic sources the most common being traffic, heating, and industrial processes (Boström et al., 2002). PAHs exposure generally occurs at low, chronic doses, and due to the lipophilicity of PAH-compounds, they are likely to attain high local concentrations in the exposed tissues such as lungs (Boström et al., 2002). Since PAH exposure causes a variety of health risks, ranging from carcinogenic and mutagenic changes to endocrine disruption, they have been classified as priority environmental pollutants by several authorities, including the European Environment Agency (EEA) and the United States Environmental Protection Agency (USEPA) (Boström et al., 2002; Kamal et al., 2015; Kim et al., 2013).

In urban areas, many studies have assessed traffic and vehicular exhaust to be the major contributor of PAH emissions (Afshar-Mohajer et al., 2016; Albuquerque et al., 2016; Parajuli et al., 2017; Viippola et al., 2016). In rural areas, burning of biomass is a regular part of agricultural practices, including burning of grassland and crop residues. A few studies have determined these practices have a major impact on local ambient PAH concentrations (Noth et al., 2011; Sevimoglu and Rogge, 2016). On the other hand, very few studies have commented on the role that the use of heavy agricultural or grain drying machinery plays in rural PAH concentrations, even though agriculture has been reported as a major source of particulate matter PAHs in Europe (Lelieveld et al., 2015). Therefore, there is need to examine potential associations between active farming and PAHs levels in ambient air.

As found in our recent study (Roslund et al., 2019) and further explored by Fouladi et al. (2020), air pollution may induce shifts in gut microbiota, which are associated with bacterial functional capacity and endocrine signaling. Many endocrine disrupting chemicals are in the gas phase, which is readily bioavailable (Annamalai and Namasivayam, 2015; Novák et al., 2009; Oziol et al., 2017). Human microbiota transforms bioavailable PAHs to toxic reactive metabolites (Claus et al., 2016; Sowada et al., 2017; Van de Wiele et al., 2005). However, many studies have focused solely on pollutants associated with air particles and health consequences (Amarillo et al., 2014; Bailey et al., 2020; Kamal et al., 2015; Lelieveld et al., 2015; Oliveira et al., 2019). The toxic effects of PAHs are strongly related to their capability to activate the aryl hydrocarbon receptor (AhR) (Vondráček et al., 2017). AhR interacts with peroxisome proliferator-activated receptor (PPAR) in the endocrine system that regulates metabolism, inflammation and insulin sensitivity (Dou et al., 2019; Wang et al., 2011). In addition, commensal gut bacteria modulate endocrine signaling pathways which drive the hormonally mediated processes, metabolism and inflammation (Kelly et al., 2004; Nepelska et al., 2017). While communication between the AhR and PPAR (Borland et al., 2014; Wang et al., 2011) and between human gut microbiota and PPAR (Kelly et al., 2004; Nepelska et al., 2017) is demonstrated, the potential of PAHs to disrupt the PPAR pathway via gut microbiota has been less characterized.

While, green spaces may sequestrate PAHs from ambient air and thus improve air quality in urban areas (De Nicola et al., 2017; Vieira et al., 2018), traffic, industry and domestic wood burning may worsen the air quality (Boström et al., 2002). Therefore, it is possible that land cover categories associated with air purification or pollution play a role in environmental health risks associated with endocrine signaling. Despite this, studies estimating the interaction between land cover categories and PAHs in the ambient air, and modulation of PPAR pathway via differences in gut metagenome are lacking.

The general purpose of this study was to examine potential associations between land cover categories associated with air pollution or purification, PAH levels in ambient air and PPAR pathway in endocrine system predicted from gut bacterial metagenome. The gaseous compounds of PAHs were measured around homes of 32 urban and 30 rural study subjects who donated stool samples and allowed outdoor passive samplers to collect PAHs from ambient air. We had four specific hypotheses. First, we hypothesized that urban and rural areas have different distribution of PAH levels in the ambient air. Second, we sought to estimate the effect of land cover categories consisting of typical sources of air pollution (i.e. industrial units, road and rail networks and detached houses with fireplaces) on PAH concentrations in ambient air. Third, we aimed to quantify the provision of air purification given by different green spaces, including coniferous, mixed and broad-leaved forests, and transitional woodland and shrub areas. Finally, we estimated the correlations between PPAR signaling predicted from gut metagenome, PAH levels in ambient air and land cover categories associated with PAH pollution or purification.

2. Materials and methods

2.1. Study area and participants

This study took place in the region of Päijät-Häme, southern Finland, and two of its neighboring municipalities, i.e. Iitti and Pukkila (Fig. 1). The study participants were 62 elderly people aged 65–79 years. Thirty participants lived in farmhouses (active or non-active) or in other detached houses in rural areas away from the populated centres (called the rural sites from here on after), and 32

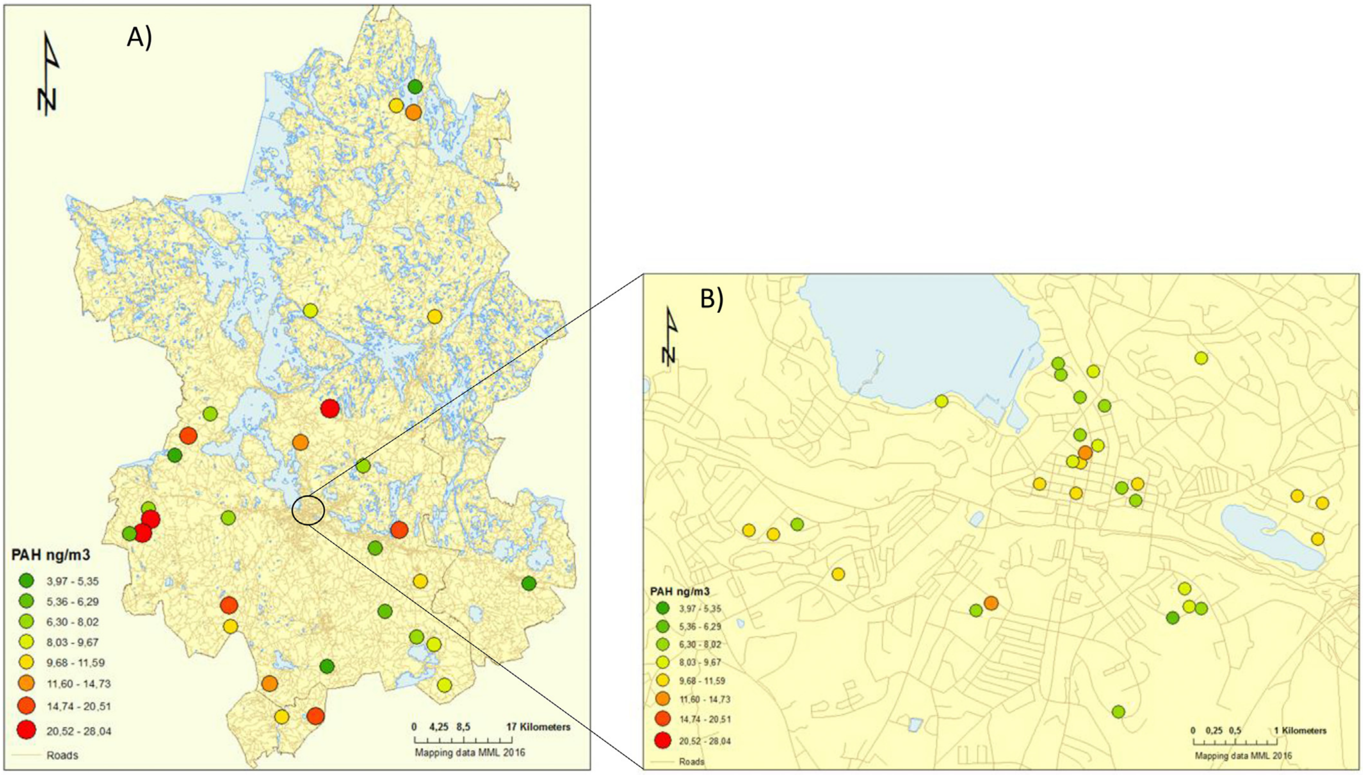

Fig. 1. Geographical distribution of **(A)** rural and **(B)** urban sampling sites of the current study.

participants resided in apartments in the urban area of the city of Lahti (referred to as the urban sites afterwards). The study participants were selected from a previous *GOAL (Good Aging in Lahti region*) –study that researched associations between the living environment and chronic diseases and functional disabilities among the elderly (Fogelholm et al., 2006). The characteristics of study participants and study sites, including the criteria for rural-urban classification are described in more detail in our previous studies (Hui et al., 2019b; Parajuli et al., 2018, 2020).

This study was carried out according to the recommendations of the "*Finnish Advisory Board on Research Integrity*" and ethical approval was obtained from the Ethics Committee of the Pirkanmaa Hospital District, Finland. Written informed consent was obtained from all subjects in accordance with the *Declaration of Helsinki*. The outdoor recreation habits and other background information was recorded using standardized questionnaires.

2.2. Air sampling

The samplers were installed at study sites during a 3-day period, from August 31 to September 2, 2015. The deployment time was 28 days and, subsequently, the samplers were collected for analysis between September 28 and 30, 2015. Out of the 62 sampler units installed, one sampler unit was not recovered and thus, 61 samples were analyzed.

The passive sampling device (PSD) used in this study consisted of low-density polyethylene lay-flat tubing filled with high purity triolein (Huckins et al., 1999). The polyethylene tubing acts as a barrier or membrane between the sampled matrix and the lipid triolein inside, which can be thought to mimic bioavailable tissues in living organisms. Thus, the device simulates the process of bio-concentration (Bonetta et al., 2009). The PSD samplers were prepared as described in Roslund et al. (2019) by cutting polyethylene tubing (25 mm in width and 72 ± 1.7 μm in thickness, Cope Plastics Inc., Alton, Illinois, USA) into 48 cm long pieces which were then sealed with a heat-sealer. Each sealed strip of polyethylene tubing was filled with 500 μL of triolein (99% glycerine trioleate, Acros Organics, Belgium)- performance reference compound fluoranthene-D10 (400 ng/mL, Dr. Ehrenstorfer GmgH, Augsburg, Germany)- mixture. The samplers were stored in air-tight glass containers at −20 °C until the deployment.

A sampling unit consisted of a PSD sampler and a 2.5 L zinc container which served as a shelter from rain, wind, and solar radiation. The sampling units were attached with straps to the downspouts or equivalent of houses located at the study sites. The sampling units were placed approximately 2.5–3 m above the ground to prevent potential vandalism and to ensure sufficient air circulation around the unit. At each study site, the sampling unit was placed nearby a window or an entrance in order to monitor the air immediately around a house or an apartment. This allowed for a comparison between the air qualities of the study sites for the participants. At urban sites, the sampling units were placed in the inner courtyards rather than by the city streets, and at the rural sites the units were placed in the backyard side of the house.

After the 28-day exposure, the samplers were collected and placed individually in glass bottles (volume of 50 mL). The samplers were stored at −20 °C until the PAH analyses.

2.3. PAH analyses

The samples were analyzed for 16 EPA (The United States Environmental Protection Agency) priority PAHs as in Roslund et al. (2019), and the analysis gave reliable results for seven PAH species as only the vapor phase PAHs were sampled with the PSDs. We used a deuterated PAH mixture (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12) (Dr.

Ehrenstorfer Gmbh, Germany) as a standard and Anthracene-d10 (Dr. Ehrenstorfer GmbH, Germany) as a recovery standard. The aerial concentrations (ng/m^3) of the detected PAHs were calculated using the uptake rates reported by Cranor et al. (2009). In order to monitor for possible contamination during the deployment of the samplers, field blanks were included during each day of the deployment. The field blanks were prepared and analyzed analogous and simultaneous to the exposed samplers. Similarly, reagent and laboratory contaminations were monitored with laboratory or reagent blanks. One reagent blank was included with each batch of samplers being prepared for analysis. The limit of quantification (LOQ) of the GC-MS analysis was derived from the signal to noise ratio of 10, which was approximately 1 ng per sample for all analytes. This was converted to an actual analyte air concentration (ng/m^3) in the same manner as with the samples (Table S1). To assess the toxicity of a PAH mixture, we calculated benzo(a)pyrene total potency equivalent (BaPtpe) as described in CCME (2010).

2.4. Microbial analyses

Stool samples were collected from the study participants in August 2015. DNA was extracted from 30 to 60 mg of frozen unprocessed stool sample and bacterial communities were analyzed using Illumina MiSeq 16S rRNA gene metabarcoding as described in Nurminen et al. (2018). Raw sequencing data was processed with Mothur (version 1.39.5) (Schloss et al., 2009) as in Roslund et al. (2019). Less abundant OTUs that were represented by 10 or fewer sequences across all experimental units were removed, and samples were subsampled to 4024 sequences for functional metagenome analysis.

Functional orthologs were generated with PICRUST (Langille et al., 2013) from the 16S rRNA OTU data classified against the Greengenes Database (Desantis et al., 2006) according to 97% similarity. PPAR signaling pathway was predicted with KEGG (Kyoto Encyclopaedia of Genes and Genomes, release 89.0) database.

2.5. Land cover category classification

The land cover category data for the sampled sites was based on CORINE land cover data 2012, and the data was processed using geographic information system (GIS) software MapInfo. CORINE land cover data is available from 39 European countries, and it comprises 44 land cover categories. The land cover raster resolution used in the research was 20 m $\times$ 20 m. The calculations included ten buffer-zones, namely 100, 200, 500, 1000, 1500, 2000, 2500, 3000, 4000, and 5000-m radius around any given study site. Due to the close proximity of some measurement sites and the diffusive nature of PAHs, buffers above 1500 m were rejected from the analyses.

2.6. Statistical analyses

Descriptive analyses were performed to determine the distributions of the data thus allowing the selection of appropriate statistical tests. Since the data was not normally distributed, the associations between PAHs and PPAR pathway, and between PAHs and land cover categories were analyzed using generalized linear models (GLM) with quasipoisson distribution with stats package in R computing environment (v3.5.1) (R development core team, 2018). To conceptualize the false discovery rate (FDR), statistical tests were carried out with Benjamini–Hochberg correction (Benjamini and Hochberg, 1995). Each buffer zone was considered as family of hypotheses, i.e. corrected separately to avoid the problem of multiple nested corrections (Kim and van de Wiel, 2008). When analyzing land cover categories, we first excluded categories that did not occur in the surroundings of most study participants. As we hypothesized to find associations between land cover categories and PAHs, and as green spaces were associated with air purification in previous studies (De Nicola et al., 2017; Vieira et al., 2018), we thereafter selected land cover categories that are either green areas or that act as major sources of PAH pollution. In the CORINE data set, these green categories were coniferous, mixed and broad-leaved forests, and transitional woodland and shrub areas. Air pollution associated categories were road and rail network associated land, industrial sites, and detached and apartment buildings (Blasco et al., 2006; Tan et al., 2014).

All the other statistical analyses were performed using statistics program IBM SPSS 24. Differences between urban and rural sites in PAH concentrations were analyzed using two parallel methods. Nonparametric Mann-Whitney *U* test was utilized to determine the rank-based locational differences in PAH concentrations between the groups (rural versus urban sites). It was also used to determine whether farming affected the PAH concentrations. As peak values of each individual PAH were considerably higher in rural sampling sites, the data was divided into four categories from the order of magnitude (lowest to highest value). Each category had 15 values except for the highest value category which had 16 values. Pearson's χ^2 tests were thereafter conducted to examine how PAH concentrations varied between rural and urban areas, i.e., to determine whether the frequencies (urban vs. rural) of the measured PAH concentrations were similar in site categories. Finally, Moses Extreme Reactions Test was conducted to determine whether the dispersion of conducted measurements in the rural group was larger than the dispersion in the urban group. Maps were done with ArcMap 10.2.1. A significance level of 0.05 was used in all analyses.

3. Results

3.1. Gaseous PAHs had similar means but higher variation in rural than urban areas

Seven individual PAH compounds were reliably identified in the samples: acenaphthylene (ANL, 3-ring), acenaphthene (ACP, 3-ring), fluorene (FL, 3-ring), phenanthrene (PHE, 3-ring), anthracene (ANT, 3-ring), fluoranthene (FLT, 4-ring), and pyrene (PYR, 4-ring). Recoveries of the internal standards ranged on average between 75% and 115% except for perylene-D12 with a recovery rate of 42% (Table S2).

The measured total PAH concentrations, i.e. the sum of seven individual PAHs, sequestered by the samplers ranged from 4.0 to 28 ng/m^3 with the mean of 10 ng/m^3. Both the highest and the lowest total PAH concentrations were measured in rural areas (Fig. 2). The variation within rural site measurements was larger than variation within urban sites (Moses Extreme Reactions Test, $P < 0.001$). The difference between the mean PAH concentrations at rural and urban sites was not significant (Fig. 2A).

The frequencies of categories of the measured total PAHs differed between urban and rural sites (Pearson's χ^2-test $P = 0.004$). While the urban sites had more measurements in the middle range of scores (between 7.5 and 11 ng/m^3), the rural site measurements were more concentrated at both ends of the range (4.0–7.5 ng/m^3or 11–28 ng/m^3) (Fig. 2 B). In fact, the highest number of measurements in rural sites fell into the high PAH concentration category (i.e. class 4 in Fig. 2B) followed by the low concentration measurements (i.e. class 1). There were over four times more high-category concentration measurements in rural than in urban sites. On the other hand, there were also 1.5 times more low-category PAH concentration measurements in rural than urban sites. Regardless of the variations in the range of scores, the

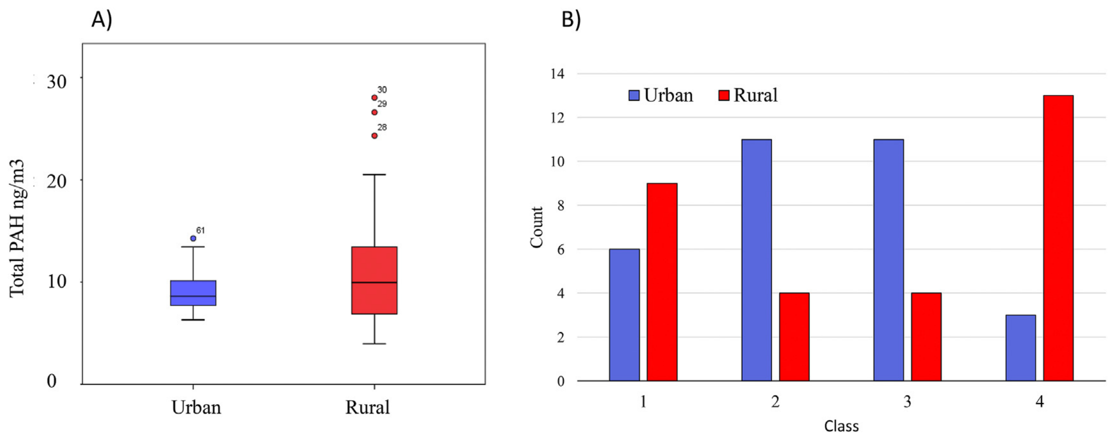

Fig. 2. Total PAH concentrations (ng m^{-3}) were **(A)** differently distributed, but the averages do not differ between the urban (n = 31) and rural (n = 30) sites. **(B)** The frequencies of the PAH measurements in each concentration category showed that both the lowest (Class 1) and highest (Class 4) concentrations were measured in rural sites. Frequencies are categorized from lower PAH concentrations "Class 1" to higher concentrations "Class 4". The boxplots (A) show median, 25 and 75% distributions as the bottom and top of the box, and 5 and 95% distributions as the head of lines, respectively, and outliers as separate dots, while bars (B) show mean ± standard deviation.

rank-based PAH concentrations were independent of the measurement site (i.e. urban vs. rural site, Mann-Whitney *U* test, $P = 0.45$).

The measurements for seven individual PAHs identified in the GC-MS analyses ranged from 0.08 ng/m^3 to 6.47 ng/m^3 at the urban sampling sites and from 0.06 ng/m^3 to 13.48 ng/m^3 at the rural sites (Table 1). The frequencies of the measured PAH concentrations in categories were different for all individual PAHs except for acenaphthene and pyrene (χ^2-test, Table 1). Mean values of the measured PAHs were at the same level but slightly higher in rural than urban sites. However, location of anthracene measurements seemed to affect its concentrations, whereas with the other PAHs the urbanicity or ruralness of the sampling locations did not have an effect in rank-based Mann-Whitney *U*-tests (Table 1).

Among the seven PAH compounds identified phenanthrene and fluorene were the most abundant chemicals in both urban and rural measurements. Phenanthrene accounted for nearly 44% and 45% of the total PAH concentrations in rural and urban sites, respectively. Phenanthrene measurements also had the largest concentration range of all identified PAHs. Fluorene concentrations accounted for nearly 24% in rural and 28% in urban total PAH concentrations. In contrast, anthracene amounts were the lowest with 2.2% and 3.8% at the urban and rural sites, respectively.

Table 1
Comparison of urban and rural PAH concentrations (ng/m^3). Mann-Whitney *U*-tests were used to reveal potential rank-based and χ^2-tests category-based differences between rural and urban sampling sites. Each category contained 15–16 values. The values were divided into categories in order of magnitude.

	Concentration ng m^{-3}			χ^2-test		Mann-Whitney *U* test	
	Min.	Max.	Average	P value	Q value[a]	P value	Q value[a]
Anthracene							
Urban	0.08	0.44	0.20	0.002	0.015	0.041	0.287
Rural	0.06	2.11	0.45				
Pyrene							
Urban	0.14	0.76	0.40	0.633	0.633	0.320	0.642
Rural	0.13	2.22	0.67				
Fluoranthene							
Urban	0.32	1.3	0.73	0.023	0.032	0.367	0.642
Rural	0.26	3.07	1.07				
Acenaphthylene							
Urban	0.26	1.68	0.60	0.009	0.016	0.151	0.528
Rural	0.15	8.12	0.91				
Acenaphthene							
Urban	0.42	1.11	0.66	0.104	0.121	0.686	0.800
Rural	0.35	2.77	0.76				
Fluorene							
Urban	1.89	4.35	2.49	0.007	0.016	0.812	0.812
Rural	1.14	6.71	2.72				
Phenanthrene							
Urban	2.64	6.47	4.06	0.005	0.016	0.564	0.790
Rural	1.8	13.48	5.03				

[a] Benjamini-Hochberg adjusted P value.

3.2. Farming elevated gaseous PAH concentrations

When farming activity on rural measuring sites was considered, a clear connection was found. Out of the 30 rural study sites, 16 had farming activity, and the sites with active day-to-day farming had elevated total PAH-concentrations compared to non-active farming sites or to urban sites (Mann-Whitney *U* test, $P < 0.01$). Interestingly, when farming-activity was ignored from statistical tests, no correlations were found between the PAH concentrations and any available land cover categories in rural sites ($P > 0.10$). This was the case for all individual PAHs as well as for the sum of them.

3.3. Gaseous PAH concentrations correlated with land cover categories in urban area

The PAHs correlated negatively with broad-leaved and mixed forests (Table 2 A; Fig. 3 A and B), and positively with transitional woodland and shrub areas with tree crown cover under 10% in urban areas (Table 2 B). Total PAHs correlated negatively with the areas of broad-leaved forests in buffer zones of 200 and 500 m (Table 2 A), and the results are similar for BaPtpe (Table S3). The areas of broad-leaved and mixed forests correlated negatively particularly with fluorene in buffer zones of 200 and 500 m, and broad-leaved forests correlated negatively with phenanthrene in buffer zones of 500 m (Table 2 A). The transitional areas correlated positively particularly with fluoranthene, acenaphthylene, total PAHs (Table 2 B) and BaPtpe (Table 3S) in buffer zones of 500 m around the sites. Both fluorene and fluoranthene seemed to correlate positively with the road and rail networks and the land associated with them in the city (Table 2C). They seemed to have the only noteworthy correlation with the land use of this type.

There was also a positive correlation between acenaphthylene and detached houses at urban sites (Table 3). The correlation was strong from 500 m up to 1500 m. Interestingly, the opposite was

Table 2
Correlations between the measured PAHs **(A)** broad-leaved and mixed forests, **(B)** transitional woodland and shrub with tree crown cover (cc) < 10% and **(C)** road and rail network associated land coverage around urban sites (n = 31). Generalized linear mixed model statistics are shown as t value, probability P value and Benjamini-Hochberg corrected Q value.

A) meter buffers	Broad-leaved forest					Mixed forest				
	100	200	500	1000	1500	100	200	500	1000	1500
Acenaphthene										
t value	−0.148	−1.282	−1.514	−0.456	−2.009	−1.419	−2.553	−2.850	−0.906	−1.124
P value	0.883	0.210	0.141	0.652	0.054	0.166	**0.016**	**0.008**	0.372	0.270
Q value	0.883	0.420	0.282	0.779	0.216	0.666	0.065	**0.032**	0.744	0.540
Fluorene										
t value	−1.589	−3.128	−2.361	−1.135	−1.749	−2.540	−2.652	−2.777	−2.077	−1.763
P value	0.123	**0.004**	**0.025**	0.266	0.091	**0.017**	**0.013**	**0.010**	**0.047**	0.088
Q value	0.164	**0.016**	**0.050**	0.266	0.121	0.067	**0.026**	**0.038**	0.160	0.121
Fluoranthene										
t value	−0.987	−1.693	−1.674	−0.386	−0.403	−2.268	−2.180	−2.155	−1.600	−1.122
P value	0.332	0.101	0.105	0.702	0.690	**0.031**	**0.038**	**0.040**	0.121	0.271
Q value	0.443	0.135	0.140	0.702	0.690	0.124	0.086	0.079	0.241	0.542
Phenanthrene										
t value	−0.981	−2.287	−2.729	−0.809	−0.623	−1.293	−1.718	−2.083	−0.975	−0.467
P value	0.335	**0.030**	**0.011**	0.425	0.538	0.206	0.096	**0.046**	0.338	0.644
Q value	0.446	0.119	**0.039**	0.567	0.859	0.412	0.149	0.062	0.567	0.859
Total PAHs										
t value	−1.097	−2.461	−2.361	−0.937	−0.778	−1.873	−1.821	−2.093	−1.237	−0.704
P value	0.282	**0.020**	**0.025**	0.357	0.443	0.071	0.079	**0.045**	0.226	0.487
Q value	0.375	0.080	**0.050**	0.476	0.650	0.142	0.158	0.060	0.452	0.650

B)	Transitional woodland and shrub, cc 10%				
meter buffers	100	200	500	1000	1500
Acenaphthylene					
t value	0.496	0.124	3.698	2.291	2.859
P value	0.624	0.902	**<0.001**	**0.029**	**0.008**
Q value	0.624	0.961	**0.001**	**0.039**	**0.012**
Fluoranthene					
t value	1.489	2.113	4.334	2.015	2.367
P value	0.147	**0.043**	**<0.001**	0.053	**0.025**
Q value	0.295	0.087	**0.001**	0.213	0.099
Pyrene					
t value	2.206	2.253	2.422	0.834	1.013
P value	**0.035**	**0.032**	**0.022**	0.411	0.319
Q value	0.142	0.128	0.088	0.877	0.666
Anthracene					
t value	1.690	1.239	2.769	0.779	1.021
P value	0.102	0.225	**0.010**	0.442	0.316
Q value	0.310	0.450	**0.039**	0.627	0.632
PAH total					
t value	1.987	1.560	3.189	1.564	1.933
P value	0.056	0.130	**0.003**	0.129	0.063
Q value	0.142	0.173	**0.014**	0.452	0.252

C)	Road and rail networks associated land				
meter buffers	100	200	500	1000	1500
Fluoranthene					
t value	1.006	1.862	2.173	2.150	2.097
P value	0.323	0.073	**0.038**	**0.040**	**0.045**
Q value	0.431	0.146	0.152	0.132	0.179
Fluorene					
t value	0.365	1.285	2.476	2.312	2.283
P value	0.718	0.209	**0.019**	**0.028**	**0.030**
Q value	0.974	0.418	0.077	0.112	0.120

true for areas of apartment buildings in the city, which had a negative correlation with acenaphthylene (Table 3). In addition, anthracene correlated negatively with apartment buildings, but its correlation was marginally significant for 100 m around the measured sites (Table 3).

3.4. PPAR signaling pathway correlated with land cover categories

High coverage of broad-leaved forests in the vicinity of permanent residences was associated with increased PPAR signaling pathway of the study subjects when all the study subjects (urban and rural subjects) were in the generalized linear model (Table 4; Fig. 3C and D). The strongest associations were observed using 200 m radius and they gradually decreased as the radius increased (Table 4). Similar associations were observed between the PPAR signaling pathway and mixed forests that consist of both broad-leaved and coniferous forests (Table 4), but there was no association between the PPAR signaling pathway and the coverage of pure coniferous forests (Table S4). In addition, we did not find any significant associations between PAH levels in ambient air and PPAR signaling pathway (Table S5).

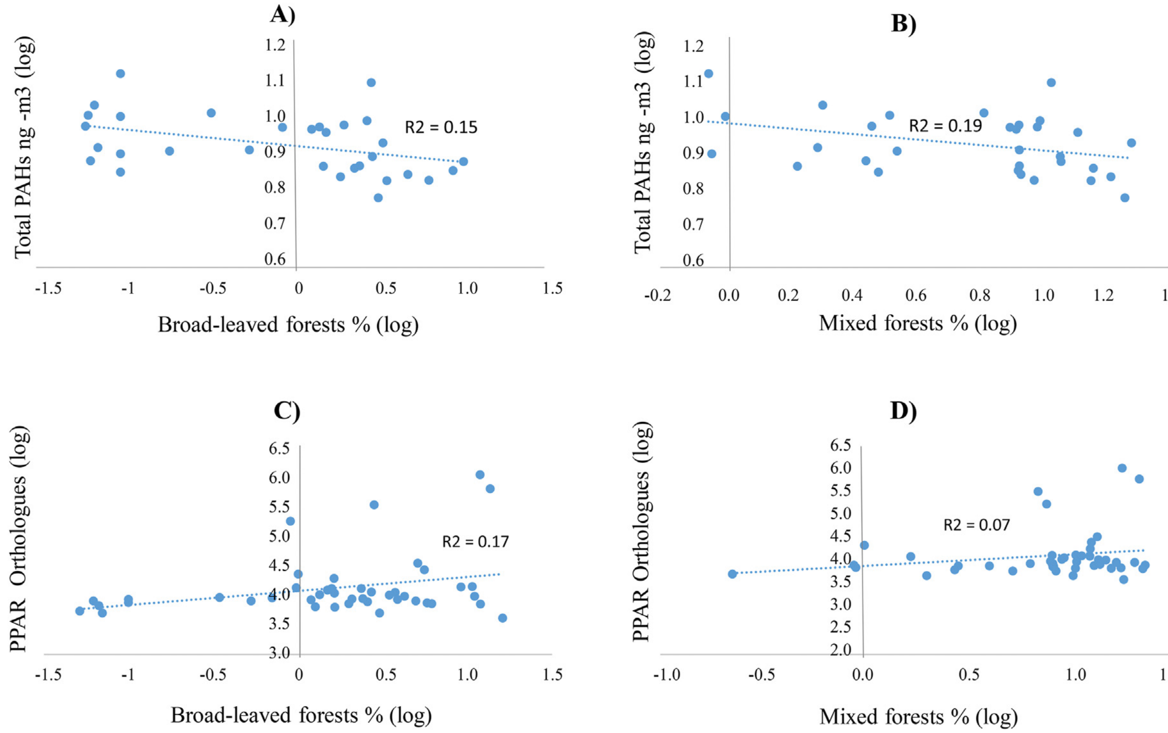

Fig. 3. PAHs correlated negatively with **(A)** broad-leaved and **(B)** mixed forests, and gut bacterial functional orthologs of PPAR signaling pathway correlated positively with the coverage **(C)** Broad-leaved and **(D)** mixed forests within 500 m radiuses around permanent residences. For statistics, see Tables 2 and 4

Table 3
Correlations between PAHs and coverage of the apartment buildings and detached houses at the urban sites (n = 31). Generalized linear mixed model statistics are shown as t value, probability P value and Benjamini-Hochberg corrected Q value.

	Apartment buildings					Detached houses				
meter buffer	100	200	500	1000	1500	100	200	500	1000	1500
Acenaphthylene										
t value	−1.110	−1.879	−3.486	−2.039	−2.305	0.371	2.004	3.487	3.828	3.287
P value	0.276	0.070	**0.002**	0.051	**0.028**	0.713	0.054	**0.002**	**<0.001**	**0.003**
Q value	0.460	0.141	**0.003**	0.068	**0.038**	0.891	0.141	**0.003**	**0.001**	**0.005**
Anthracene										
t value	−2.799	−1.785	−1.779	−1.290	−1.712	−0.395	0.759	1.648	1.650	1.223
P value	**0.009**	0.085	0.086	0.207	0.098	0.696	0.454	0.110	0.110	0.231
Q value	**0.036**	0.339	0.220	0.414	0.390	0.696	0.606	0.220	0.414	0.462

Table 4
Correlations between broad-leaved and mixed forests and PPAR pathway. Generalized linear mixed model statistics are shown as t value, probability P value and Benjamini-Hochberg corrected Q value.

	Broad-leaved forest					Mixed forest				
meter buffers	100	200	500	1000	1500	100	200	500	1000	1500
All the study subjects										
t value	1.953	2.440	2.716	1.687	1.055	2.911	3.474	1.788	1.011	0.266
P value	**0.057**	**0.019**	**0.010**	0.099	0.297	**0.006**	**0.001**	0.081	0.318	0.792
Q value	0.115	**0.038**	**0.038**	0.396	0.594	**0.023**	**0.005**	0.162	0.424	0.792
Urban population										
t value	−0.122	−0.030	−0.398	0.013	0.074	0.568	0.550	0.096	1.124	1.426
P value	0.904	0.976	0.695	0.990	0.941	0.577	0.589	0.925	0.276	0.171
Q value	0.904	0.976	0.925	0.990	0.941	0.846	0.785	0.925	0.368	0.342
Rural population										
t value	1.373	1.767	1.997	0.950	0.338	2.243	2.748	1.198	0.186	−0.636
P value	0.183	0.091	0.058	0.352	0.738	**0.035**	**0.012**	0.244	0.854	0.531
Q value	0.367	0.136	0.117	0.933	0.798	0.141	**0.047**	0.325	0.933	0.798

4. Discussion

4.1. PPAR signaling pathway, forested areas and PAH concentrations

As far as we are aware of, this is the first study finding an association between the coverage of forested areas and PPAR signaling pathway predicted from gut bacterial metagenome. With higher area of broad-leaved and mixed forests in home vicinity, the PPAR signaling pathway increased. Coupled with the simultaneous finding that forested areas had lower PAH concentrations, and our previous finding that with high air PAH concentrations the PPAR signaling pathway may decrease (Roslund et al., 2019), it seems plausible that green areas and air PAH pollution have opposite associations with the health-associated PPAR signaling pathway via gut microbial dynamics. This study thus indicates that the air purification provided by broad-leaved and mixed forests might be important for human health, since disturbances in the PPAR signaling pathway are generally thought to promote inflammation, obesity, diabetes and other hormonally mediated disorders (Braun, 2017; Street et al., 2018; Wang et al., 2011). As several other pollutants are filtered by plants from ambient air and soil (Hansi et al., 2014; Pacwa-Płociniczak et al., 2018; Płociniczak et al., 2013; Terzaghi et al., 2015), and as several pollutants have the capacity to shape commensal bacterial communities and functional metagenome in the gut (Claus et al., 2016; Fouladi et al., 2020), the ecosystem services provided by broad-leaved woody plants in urban areas offer novel possibilities to mitigate pollution-induced adverse health effects.

Since broad-leaved and mixed forests had clear associations and coniferous forests no association with gaseous PAH concentrations and PPAR signaling pathway, our results indicate that all trees are not equal in terms of air purification ability. Since broad-leaved trees have previously been connected to air purification (Terzaghi et al., 2015; Vieira et al., 2018), it is tempting to state that particularly broad-leaved forests can balance health effects caused by anthropogenic pollution. However, previous studies have also observed the opposite: coniferous trees capture more gaseous PAHs while broad-leaved trees capture more particle-bound PAHs (De Nicola et al., 2017) and gaseous PAH concentrations may even be higher in urban forests compared to open areas (Viippola et al., 2016). Due to these contrasting earlier findings, we cannot exclude the possibility that in addition to plausible air purification, broad-leaved forests provide exposure to diverse environmental microbiota that shape gut microbial dynamics. Indeed, recent comparative and intervention studies indicate that when urban dwellers are exposed to diverse environmental microbial communities lurking in soil and organic gardening materials, the exposure modifies commensal microbiota (Grönroos et al., 2019; Hui et al., 2019a), promote immune regulation (Nurminen et al., 2018; Roslund et al., 2020) and affect pollutant degradation in soil (Kauppi et al., 2012; Pacwa-Płociniczak et al., 2018; Parajuli et al., 2017; Roslund et al., 2018). In addition, other chemicals in the atmosphere may disrupt endocrine signaling pathways (Annamalai and Namasivayam, 2015). Since forested areas, several pollutants and environmental microbiota have been observed to contribute to commensal microbiota in separate studies (Parajuli et al., 2020; Roslund et al., 2019, 2020; Ruokolainen et al., 2015), there is an unmet need to estimate associations between several exposomic factors including chemical and biological interference that predict health responses.

The current study serves as a model for future research searching for associations between urban living environment and endocrine signaling regulated partly by gut metagenome. Study participants were elderly people, and it is demonstrated that the gut microbiome of the elderly is distinct from those of other age groups (Greenhalgh et al., 2016). Because interindividual variation in the gut microbial communities and functional genes is greater among younger cohorts (Greenhalgh et al., 2016; Lozupone et al., 2012), our study highlights the need to estimate the associations between forest cover and the functionality of gut microbiota in other studies with broader age range. In this study, we were particularly interested how the land cover categories associated with air pollution and purification are connected to PAH levels. The reason is that land cover categories around households affect primarily outdoor air PAH levels, while indoors gaseous PAH levels are typically affected mostly by heating and cooking sources (Lv et al., 2009; Ohura et al., 2004). Because indoor air quality is associated with health (Lv et al., 2009), future studies should consider the effect of indoor air quality on gut microbiota. According to the questionnaire, study participants were having many outdoor activities, and the most common activity was walking (Table S6). Thus, the study participants were exposed to outdoor air. Indeed, because the measuring campaign was performed during the relatively short boreal summer, time spent outdoors was high among elderly people based on data from previous studies (Pouta et al., 2006; Sievänen, 2011).

The current investigation is the second case study published to date that searched for and found associations between urban pollution and endocrine signaling based on gut metagenome. The difference between the previous study and the current one is in the direct association between air pollution levels and endocrine signaling. The previous study found a direct negative association between air pollution and PPAR signaling pathway predicted from gut metagenome of children (Roslund et al., 2019). In this study we found positive association between land cover categories associated with air purification and PPAR signaling pathway. Based on these studies, it is unfortunate that current estimates of hazardous chronic exposure to PAHs do not consider the possibility of limited endocrine signaling resulting from low-level chronic PAHs exposure.

4.2. PAH concentrations in urban and rural areas

The amounts of ambient gaseous PAHs were found to be very similar in both urban and rural sites on average. The urban PAH levels showed very little variation throughout the city which might be a consequence of the general levels of PAHs emitted from day-to-day activities, e.g. vehicular exhaust or it could just represent the relatively short distances between the sampling sites. Similar finding was reported by Jedynska et al. (2014) who found no difference in PAH concentrations between street and urban background locations in Helsinki, Finland.

The gaseous PAH concentrations measured in this study were comparable with the findings of similar PAH studies (Slezakova et al., 2011; Zhu et al., 2011). The concentrations should not cause a threat in the form of acute toxicity, even though at a local scale farming activity may increase exposure to PAHs in ambient air. However, much higher concentrations have been measured in countries like China and India as these countries are battling to curb and regulate their emissions in industrial as well as domestic sectors (Hong et al., 2016; Li et al., 2014; Salve et al., 2015; Wang et al., 2020). Many earlier studies have assessed that PAH concentrations are higher at urbans sites (Arruti et al., 2012; Hong et al., 2016; Liu et al., 2014a), although some studies have shown that the concentrations are similar between urban and rural areas depending on biofuel combustion and seasonality (Li et al., 2014). Our study adds that the use of heavy agricultural machinery, such as tractors, combine harvesters, and grain dryers, can have negative effects on air quality and contribute to air pollution levels in rural areas. Heavy machinery generally runs on diesel or light fuel oil, and their exhaust products are considered carcinogens and noteworthy polluting agents (Lewtas, 2007).

The timing of this study highlighted the use of grain dryers and their marked PAH emissions; this plausibly increased the ambient PAH levels at sampling sites that were located at farms employing grain dryers. Obviously, this effect is very seasonal and only elevates PAH concentrations during the use of the grain dryer, which generally lasts a few weeks annually. The employment of other agricultural machinery affected the results as well, because elevated PAH levels were also observable at other rural sites with active farming, such as cattle farming, but no grain dryers nearby. The use of heavy machinery affects the emission levels and thus the PAH concentrations throughout the whole year as they generally are used continuously for different purposes during the year. Additionally, other agricultural practices, such as burning of biomass, contribute to the PAH emissions; burning of twigs, tree branches or stumps is a common practice in Finland just like in other countries (Afshar-Mohajer et al., 2016; Noth et al., 2011; Ravindra et al., 2008; Sevimoglu and Rogge, 2016). The use of wood for heating is also common in Finland. The use of wood as a main heating source can result in major emissions, and it can significantly worsen the local air quality. Many studies demonstrate well that atmospheric PAHs are dependent on seasons, precipitation, temperature as well as spatial factors, i.e. geography and topography, of sampling sites (Jedynska et al., 2014; Li et al., 2014b; Liu et al., 2014; Sevimoglu and Rogge, 2016; Viippola et al., 2016). The results of this 28-day study give a reasonable snapshot of amounts of PAHs in measured locations, but they do not allow definite conclusions to be drawn for the annual or average concentrations at any of the sampling sites.

4.3. Land use and air quality in urban areas

Our results indicate that PAH levels are reduced in areas with broad-leaved and mixed type forests at urban areas. This is in accordance with studies indicating that plants capture PAHs from ambient air and improve air quality (De Nicola et al., 2017; Howsam et al., 2000; Nowak et al., 2006). In contrast with this, fluoranthene and pyrene correlated positively with transitional woodland and shrubs with tree crown cover under 10%. This association can be explained by an argument brought forward by Viippola et al. (2016) who found that in summertime gaseous PAH concentrations were higher under park tree canopy near roads than in open-areas. This may happen due to decreased air circulation under tree canopies. In our study, the measuring devices were placed next to houses that had scattered trees nearby. Our results are further put in the context by investigations observing that different types of green spaces have different capacity to improve air quality: highly managed green spaces have low capacity compared to more diverse green spaces with less management (Vieira et al., 2018). This might explain why in our study both broad-leaved and mixed forest-types with more diverse structures correlated negatively with the PAH concentrations but the highly managed transitional woodland and shrubs, such as urban parks, correlated positively.

In our study, fluoranthene and fluorene correlated positively with roads, rail networks and the land associated with them. This is expected as they are both found in exhaust products of vehicles. No correlation was found for phenanthrene and traffic-related land cover category, which is surprising since it is a major component of traffic emissions. The observed correlations and the lack of them might be related to the small variation between PAH levels in different urban sites and the proximity of the different sampling sites. The fact that the correlations are for buffers of 500 m and above is also surprising since traffic emissions are noted to result in very locally elevated PAH readings (Tan et al., 2014). In order to make more meaningful correlations with traffic emissions or volumes, a much larger dataset, i.e. number of sampling sites and the frequency of measurements, would most likely be needed, so that both spatial and temporal variations could be considered. Also, the sampling devices in this study were not positioned to specifically measure pollution from the traffic but to distinguish the PAH concentrations between potentially different exposures by the urban and rural study participants. Finally, acenaphthylene correlated moderately or even strongly with buildings; there was a negative correlation with apartment buildings and a positive one with detached houses. Since acenaphthylene is associated with wood combustion, this could be an indication of higher fireplace usage in detached houses.

5. Conclusions

This study indicates that the presence of broad-leaved and mixed forests in close proximity of households might promote the functional potential of human gut microbiome by increasing orthologues for PPAR signaling pathway. Homes located nearby these forest areas had lower PAH levels, indicating that broad-leaved trees capture gaseous PAHs from the ambient air. These findings together with previous environmental, pollution and health studies, suggest that broad-leaved and mixed forests reduce the negative health risks induced by PAH pollution, and may balance pollution-induced disturbances within commensal microbiota.

The PAH concentrations results tend to highlight the use of farming equipment that operate on fossil fuels and their impact on the local air quality. Significant sources of endocrine disrupting air pollution do exist in urban environment as well as in rural settings.

This study supports initiating further studies to identify exposomic disease determinants and the underlying biological pathways which are driving the epidemic of emerging public health problems, including diabetes, obesity and inflammatory disorders. Eventually this may offer new opportunities for prophylactic treatment practices and urban management in the context of human health.

Credit author statement

Heli K. Vari: designed study (conceptualization), implemented study, generated data, did data curation and formal analysis, wrote the original draft, reviewed and edited the manuscript. Marja I. Roslund: designed study (conceptualization), implemented study, generated data, did data curation and formal analysis, wrote the original draft, reviewed and edited the manuscript. Sami Oikarinen: generated data. Noora Nurminen: implemented study, reviewed and edited the manuscript. Riikka Puhakka: designed study (conceptualization), implemented study, reviewed and edited the manuscript. Anirudra Parajuli: implemented study, generated data, did data curation and formal analysis. Mira Grönroos: designed study (conceptualization), implemented study, reviewed and edited the manuscript. Nathan Siter: generated data. Olli H. Laitinen: designed study (conceptualization), implemented study, reviewed and edited the manuscript. Heikki Hyöty: designed study (conceptualization), took care of funding acquisition and project management. Juho Rajaniemi: generated data, reviewed and edited the manuscript, took care of funding acquisition and project management. Anna-Lea Rantalainen: implemented study, generated data, reviewed and edited the manuscript. Aki Sinkkonen: designed study (conceptualization), implemented study, did data curation and formal analysis, reviewed and edited the manuscript, took care of funding acquisition and project management

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.chemosphere.2020.128965.

7. Data availability

Raw sequence reads for stool metagenome are available in Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) with accession numbers SAMN08991885-SAMN08992045.

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