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Impact of exposure to per- and polyfluoroalkyl substances on fecal microbiota composition in mother-infant dyads

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ABSTRACT

There is growing evidence suggesting that chemical exposure alters gut microbiota composition. However, not much is known about the impact of per- and polyfluoroalkyl substances (PFAS) on the gut microbial community. Here, in a mother-infant study, we set out to identify the gut bacterial species that associate with chemical exposure before (maternal) and after (maternal, infant) birth. Paired serum and stool samples were collected from mother-infant dyads (n = 30) in a longitudinal setting. PFAS were quantified in maternal serum to examine their associations with the microbial compositions (determined by shotgun metagenomic sequencing) in mothers and infants. High maternal exposure to PFAS was consistently associated with increased abundance of *Methanobrevibacter smithii* in maternal stool. Among individual PFAS compounds, PFOS and PFHpS showed the strongest association with *M. smithii*. However, maternal total PFAS exposure associated only weakly with the infant microbiome. Our findings suggest that PFAS exposure affects the composition of the adult gut microbiome.

1. Introduction

The period spanning between conception to 24 months of age represents a critical window for our long term growth and development (Barker, 2007). During these 1000 days, the fetus is exposed to a wide range of molecules that cross the placenta from the mother and exposure to microbes occurs just after birth (Kennedy et al., 2023). Emerging evidence suggests that the succession of gut microbiota during birth and early life are key in influencing and supporting the trajectories of healthy growth and development, including immune system maturation, metabolism, endocrine functions, and neurodevelopment (Gilbert et al., 2018; Robertson et al., 2019).

Host genetic and postnatal environmental factors shape the gut microbial composition in early life (Bäckhed et al., 2015; Rothschild et al., 2018). Intriguingly, the gut microbiome also contributes to the metabolism of ingested xenobiotic compounds and *vice versa* (Koppel et al., 2017; Sunderland et al., 2019). Humans are exposed to tens of thousands of environmental chemicals every day. One specific class of persistent environmental chemicals to which humans are widely exposed to include per- and polyfluoroalkyl substances (PFAS). These have biological half-lives ranging from three to five years (Li et al., 2018; Liew et al., 2018). Human exposure to PFAS occurs predominantly through ingestion of contaminated food and drinking water (Sunderland et al., 2019). Fetuses are already exposed to PFAS *in utero via* placental transfer and in infants the exposure occurs *via* contaminated maternal breast milk (Croes et al., 2012; Lamichhane et al., 2021). Current evidence suggests that high exposure to PFAS during early life can lead to adverse health outcomes in adulthood (Sunderland et al., 2019). For instance, we and others have recently shown that high prenatal PFAS exposure contributes to increased risk and pathogenesis of T1D in offspring (McGlinchey et al., 2020).

PFAS interfere with the synthesis and metabolism of bile acids (BAs),

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which are (Zhao et al., 2015), which are key microbial metabolites that drive early life gut microbial maturation (van Best et al., 2020). In addition, exposure to PFAS alters the concentration of circulatory metabolites, including sphingomyelin, which play a critical role in regulating immune cells (McGlinchey et al., 2020). Thus, exposure to PFAS in early life may affect the maturation of the gut microbiome and immune system *via* bile acid metabolism. Intriguingly, high PFAS exposure has been shown to affect the microbiome diversity in infants (Iszatt et al., 2019; Naspolini et al., 2022). While several studies have demonstrated an association between exposure to a series of various environmental chemicals (bisphenols, phthalates, persistent organic pollutants, heavy metals, and pesticides) and the gut microbiome, our understanding of how PFAS influences the overall viability of gut microbes remains limited (Chiu et al., 2020). Importantly, we do not yet understand the potential of PFAS to alter the human gut microbiome at the individual species level. Recently, in a US pregnancy cohort, impact of milk PFAS concentrations on gut microbiome were accessed at bacterial species level in the infants (Laue et al., 2023). However, not much is known about the impact of PFAS on the paired maternal-child samples. Given the growing global concern about PFAS exposure, our objective here was to identify the gut microbial species that are associated with PFAS exposure during the prenatal and early stages of life in a longitudinal mother-infant cohort. We sought to fill gaps in previous studies by (1) utilizing metagenomics sequencing to enable more precise identification of microbial species, and (2) investigating longitudinal correlations between microbes and PFAS in mother–child pairs.

2. Methods

2.1. Study population

The study cohort of 30 healthy pregnant people were recruited from January 28, 2013, to February 26, 2015 (Yassour et al., 2018). Families were contacted at the fetal ultrasonography visit, which is arranged for all pregnant women in Finland around gestational week 20. Written informed consent was signed by the parents at the beginning of the third trimester. Longitudinal mother and offspring stool samples taken at three sampling points were analyzed including the meconium sample collected in the delivery hospital and another sample collected at the age of 3 months in the offspring, a maternal sample collected at the beginning of the third trimester (gestational sample, G), another maternal sample collected at delivery (D), and a third maternal sample collected 3 months post-delivery (T). Paired serum and stool samples from mothers and offspring child were collected at the age of 3 months postpartum. Selected characteristics of the study subjects are shown in **Table S1**.

2.2. PFAS analyses

Sample preparation and PFAS quantification was carried out as described previously (Salihović et al., 2020). In short, 450 μl acetonitrile with 1% formic acid, and internal standards were added to 150 μl serum and samples subsequently treated with Ostro Protein Precipitation & Phospholipid Removal 96-well plate (Waters Corporation, Milford, USA) Ostro sample preparation in a 96-well plate for protein precipitation and phospholipid removal. The analysis of PFAS was performed using automated column-switching ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Waters, Milford, USA) using an ACQUITY C18 BEH 2.1 × 100 mm × 1.7 μm column and a gradient with 30% methanol in 2 mM NH4Ac water and 2 mM NH4Ac in methanol with a flow rate of 0.3 mL/min. Quantitative analysis of the selected analytes was performed using the isotope dilution method; all standards (i.e., internal standards, recovery standards, and native calibration standards) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). The method's detection limits ranged between 0.02 and 0.19 ng/mL, depending on the analyte (**Table S2**). NIST SRM 1957 reference serum as well as in-house pooled plasma samples were used in quality control, and the results agreed well with the reference values, with average recovery being 97.4% for PFOS, PFOA, PFDA, PFNA, PFHpA, PFuNDA and PFHxS. The sum of total PFAS was calculated in pg/ml.

2.3. Analysis of metabolites

Forty μl of serum sample was mixed with 90 μl of cold MeOH/H2O (1:1, v/v) containing the internal standard mixture (Valine-d8, Glutamic acid-d5, Succinic acid-d4, Heptadecanoic acid, Lactic acid-d3, Citric acid-d4. 3-Hydroxybutyric acid-d4, Arginine-d7, Tryptophan-d5, Glutamine-d5, 1-D4-CA, 1-D4-CDCA, 1-D4-CDCA, 1-D4-GCA, 1-D4-GCDCA, 1-D4-GLCA, 1-D4-GUDCA, 1-D4-LCA,1-D4-TCA, 1-D4-UDCA, PFOA-13C4, PFNA-13C5, PFUdA-13C2, PFHxS-18O2, PFOS-13C4) for protein precipitation. The tube was vortexed and ultra-sonicated for 3 min, followed by centrifugation (10000 rpm, 5 min). After centrifuging, 90 μl of the upper layer of the solution was transferred to the LC vial and evaporated under the nitrogen gas to the dryness. After drying, the sample was reconstituted into 60 μl of MeOH: H2O (70:30).

An Agilent 1290 Infinity LC system coupled with 6545 Q-TOF MS interfaced with a dual jet stream electrospray (dual ESI) ion source (Agilent Technologies, Santa Clara, CA, USA) was used for the analysis. Chromatographic separation was carried out using an Acquity UPLC BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm particle size), fitted with a C18 pre-column (Waters Corporation, Wexford, Ireland). Mobile phase A consisted of H_2O: MeOH (v/v 70:30) and mobile phase B of MeOH with both phases containing 2 mM ammonium acetate as an ionization agent. The flow rate was set at 0.4 mL·min^{-1} with the elution gradient as follows: 0–1.5 min, mobile phase B was increased from 5% to 30%; 1.5–4.5 min, mobile phase B increased to 70%; 4.5–7.5 min, mobile phase B increased to 100% and held for 5.5 min. A post-time of 5 min was used to regain the initial conditions for the next analysis. The total run time per sample was 18 min. The dual ESI ionization source was settings were as follows: capillary voltage was 4.5 kV, nozzle voltage 1500 V, N_2 pressure in the nebulized was 21 psi and the N_2 flow rate and temperature as sheath gas was 11 L·min^{-1} and 379 °C, respectively. In order to obtain accurate mass spectra in MS scan, the *m/z* range was set to 100–1700 in negative ion mode. MassHunter B.06.01 software (Agilent Technologies, Santa Clara, CA, USA) was used for all data acquisition. Data pre-processing was performed using MZmine 2.53 and identification was done using an in-house spectral library, based on *m/z* and retention time.

Quantification of BAs were performed using a 7-point internal calibration curve. The identification process utilized a customized database that follows the Metabolomics Standards Initiative, featuring two identification levels (level 1 and level 2).

2.4. Gut microbiome analysis by shotgun metagenomics

The method is adapted version of descriptions in the related work (Yassour et al., 2018).

2.4.1. Sample collection and DNA extraction

The stool samples were collected at home or in the delivery hospital. The samples collected at home were stored in the households' freezers (−20 °C) until the next visit to the study centre. The samples were then shipped on dry ice to the early dietary intervention and later signs of beta cell autoimmunity (EDIA) Core Laboratory in Helsinki, where the samples were stored at −80 °C until shipping to the University of Tampere for DNA extraction. DNA extractions from stool were carried out using the vacuum protocol of PowerSoil DNA Isolation Kit.

2.4.2. Metagenome library construction

Metagenomic DNA samples were quantified by Quant-iT PicoGreen dsDNA Assay (Life Technologies) and normalized to a concentration of

50 pg μL − 1. Illumina sequencing libraries were prepared from 100 to 250 pg DNA using the Nextera XT DNA Library Preparation kit (Illumina) according to the manufacturer's recommended protocol, with reaction volumes scaled accordingly. Batches of 24, 48, or 96 libraries were pooled by transferring equal volumes of each library using a Labcyte Echo 550 liquid handler. Insert sizes and concentrations for each pooled library were determined using an Agilent Bioanalyzer DNA 1000 kit (Agilent Technologies).

2.5. Metagenomic sequencing

Metagenomic libraries were sequenced on the Illumina HiSeq 2500 platform, targeting ~2.5 Gb of sequence per sample with 101 bp paired-end reads. Reads were quality controlled by trimming low-quality bases, removing reads shorter than 60 nucleotides. Potential human contamination was identified and filtered using the KneadData Tool, v0.5.1 with the hg19 human reference genome. Quality controlled samples were profiled taxonomically using MetaPhlAn 2.0 (Segata et al., 2012; Truong et al., 2015).

2.6. Statistical analysis

The linear discriminant analysis effect size (LEfSe) (Segata et al., 2011) was applied to identify differentially abundant taxa between the groups (high vs. low exposure). In order to minimize the confounding effect of age (mother vs. child), data were analyzed separately in different age cohorts. The high and low exposure groups were defined by the median ΣPFAS value. Wilcoxon rank-sum tests in LEfSe were used to identify significant differences. To account for the co-variates, the difference in the microbiome between the two exposure groups was also compared using linear mixed-effects model with the fixed effect being (~ exposure group + BMI + Age) and the random effect being subject-wise variation using MaAsLin2 package in the statistical programming language R. Spearman correlation coefficients were calculated using the Statistical Toolbox in MATLAB 2017b and p values < 0.05 (two-tailed) were considered significant for the correlations. The individual Spearman correlation coefficients were illustrated as a heat map using the "corrplot" package (version 0.84) for the R (https://www.r-project.org/). The subsequent visualizations were generated using the ggplot2 R package.

3. Results

3.1. Metabolomics analyses of the mother-infant cohort

Thirty mother–child dyads were included in the study (Fig. 1, **Table S1**). Serum concentrations of 20 PFAS compounds were quantified in maternal serum samples drawn during pregnancy (**Table S2**). Serum metabolites were quantified in samples collected at 3 months after delivery from both mothers and their children. We analyzed metabolites from across a wide range of chemical classes, including, bile acids, amino acids, free fatty acids, and other chemical pollutants including benzyl paraben and furoic acid (CMPFPA). Relative abundance of microbial species were characterized by shotgun metagenomics sequencing in longitudinal mother and child stool samples: Infant –birth (meconium) and 3 months of age; Mother – gestational (G, during pregnancy), delivery (D), and three months post-delivery (T).

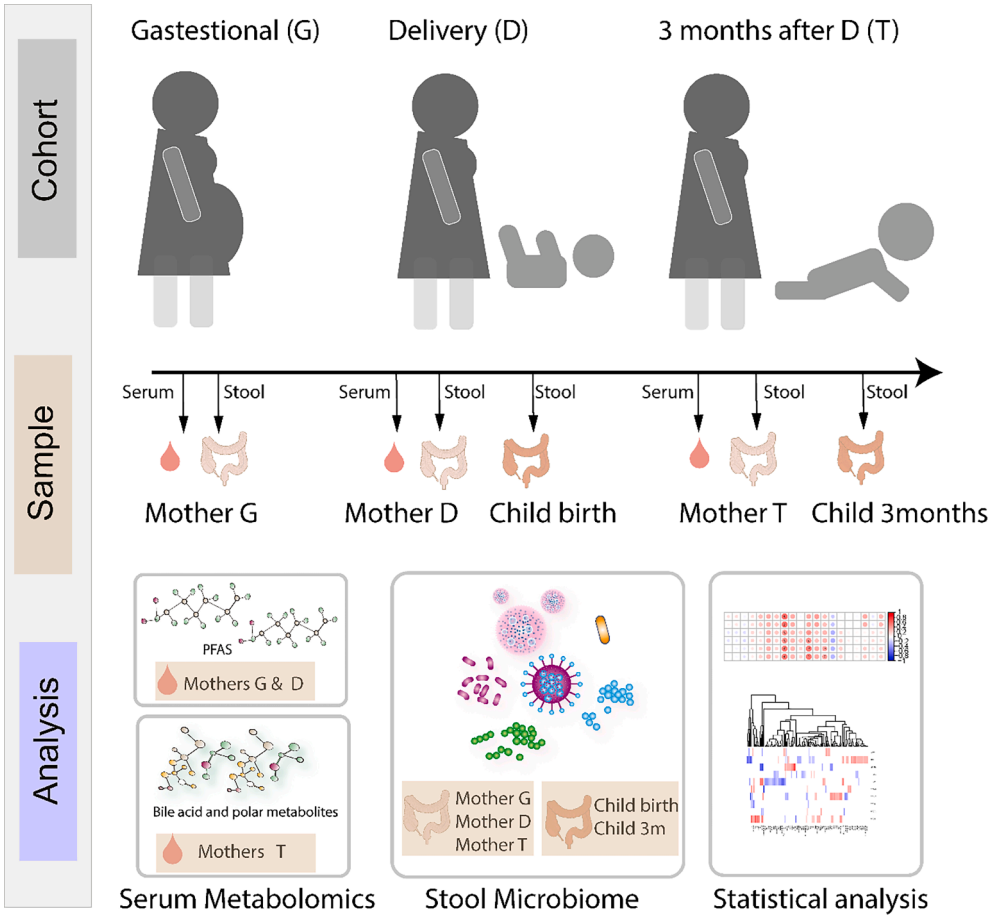

Fig. 1. An overview of the study setting. Mother and child stool sample were obtained from three sampling points: Two offspring samples at birth (meconium), and at 3 months of age, together with three maternal stool samples: gestational (G, during pregnancy at the beginning of the third trimester), delivery (D), and 3 months after the delivery (T). Paired serum and stool samples from mothers and offspring were collected during delivery and at the infant age of 3 month. Selected characteristics of the study subjects are shown in Table S1.

3.2. Impact of PFAS exposure on the gut microbiome

PFAS exposure associated with the longitudinal maternal gut microbiome profile obtained during G, D and T (Fig. 2). To understand the impact of PFAS on the microbial communities in the mothers and in the offspring, we classified the microbiome profiles into two groups (high vs. low exposure) based on total maternal PFAS exposure levels.

We found that during pregnancy, a total of six microbial species differed between higher and lower quartiles of maternal exposure to PFAS (Fig. 2). *Methanobrevibacter smithii*, additional unclassified *Methanobrevibacter* sequences, and *Subdoligranulum variabile* were more abundant in highly exposed group, while *Erysipelotrichaceae bacterium 6145*, *Bacteroides nordii* and *Ruminococcus lactaris* were more abundant in the low exposure group. Comparing high vs. low exposure at the time of delivery revealed elevated levels of 10 microbial species (*Burkholderiales bacterium 1147*, *Veillonella parvula*, *Alistipes senegalensis*, *Coprobacter fastidiosus*, *Parasutterella excrementihominis*, *Eubacterium eligens*, *Peptostreptococcaceae noname unclassified*, *Veillonella dispar*, *Methanobrevibacter smithii*, *Methanobrevibacter unclassified*) in the highly exposed mothers (Fig. 2). Similarly, we compared the maternal gut microbiome difference between the exposures groups in the samples collected 3 months after the delivery. A total of 15 microbial species were different between the high and low exposure groups. There was no persistent trend with respect to microbial differences between the groups (Fig. 2**A**), with the exception for *Methanobrevibacter* (Fig. 2), which was persistently higher in high exposure group as compared to

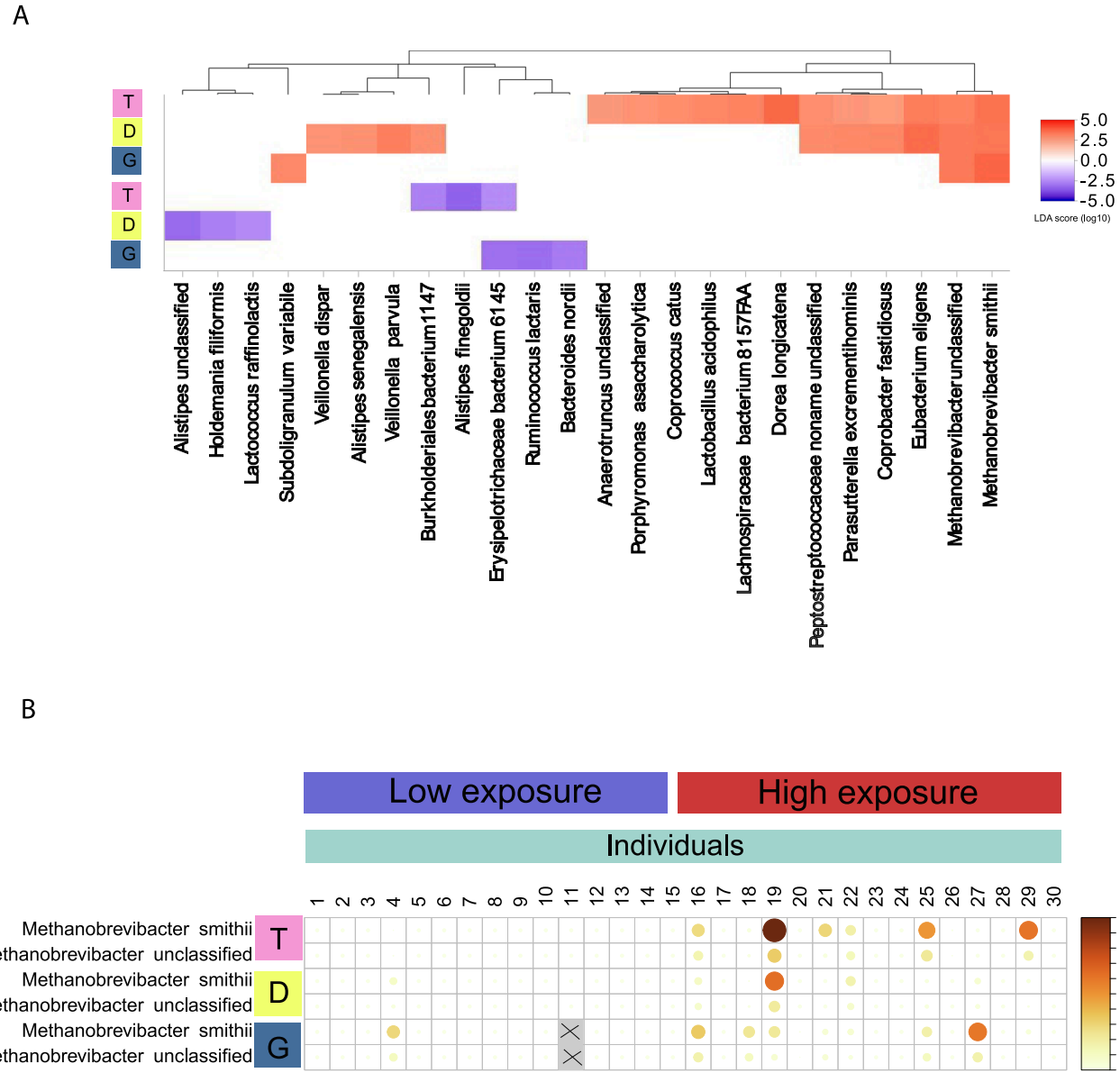

Fig. 2. Comparison of microbiome abundances between the exposure groups in maternal samples. (**A**) Heat map showing the log-transformed linear discriminant analysis (LDA) scores. The heat map highlights the scores for bacterial taxa that were found to be significantly different between high and low exposure groups at different sampling time points.The LDA score indicates the effect size and ranking of each microbial taxon. Statistical significance was evaluated using the Wilcoxon rank-sum tests in LEfSe. The negative values indicate species enriched in the low exposure group and positive values indicate species enriched in the high exposure group. The x-axis clustering was performed using the default settings in the heatmaply R package, which is based on the dissimilarity between rows and columns. (**B**) The relative abundances of microbiome between maternal samples of the exposure groups. The heat map shows how the relative abundances of Methanobrevibacter species in maternal samples change over time. The positive values indicate species enriched in the given exposure group. The values remained persistently higher in the highly exposed stool sample, while these species were missing in most of the low exposure group in the longitudinal settings. Here, X, shaded light grey represents missing sample collected during pregnancy at the beginning of the third trimester. Mother gestational (G), delivery (D), and three months post-delivery (T).

low exposure. In addition, the difference in the *Methanobrevibacter* composition between the exposure groups was compared using a multivariate linear model, co-accounting for age and BMI. We found that *Methanobrevibacter smithii* and *Methanobrevibacter unclassified* differed between the exposures group, however the effect of age and BMI remained insignificant in the maternal longitudinal stool sample (**Table S3**).

Next, we sought to determine associations of individual PFAS on those gut microbes whose abundances differed between the high and low exposure maternal groups (Fig. 2). By using ranked based correlation analysis, we tested for associations between 20 measured PFAS species with those microbial species that were different between the high and low exposure group in two maternal sampling time points (i.e., G, D, or T). PFOS and PFHpS were found to be consistently associated with *Methanobrevibacter* species (Fig. 3). PFECHS showed positive association with *Methanobrevibacter* in the sample collected 3 months post-delivery. In addition, we found that *Erysipelotrichaceae bacterium 6145* was negatively associated with PFOS and PFUnDA during pregnancy, while it remained positively associated with PFPeS at the time of delivery.

Infants are exposed to PFAS due to cumulative exposure *via* breastfeeding during early life. To study the potential impact of PFAS on microbial communities in infants, we compared the maternal PFAS exposure quartiles with the infants' gut microbiome profiles. We found that maternal total PFAS showed only modest association with stool microbiome in the infants (**Supplementary Fig. S1**). The linear discriminant effect size analysis revealed a total of three microbial species (*Subdoligranulum unclassified*, *Bifidobacterium pseudocatenulatum*, and *Propionibacterium avidum*), which were differentially abundant between the high and low maternal exposure groups either at birth or at 3 months of age.

3.3. Metabolomic profiles in mothers were associated with microbiome profile

We also examined whether the abundance of PFAS-associated bacterial species correlated with the levels of circulating metabolites in serum samples collected at 3 months after the delivery from the mothers. We found that *Methanobrevibacter* species (*M smithii* and the *unclassified Methanobrevibacter species*) were negatively associated with glycine-conjugated BAs, particularly with glycodeoxycholic acid (GDCA), glycoursodeoxycholic acid (GUDCA) and glycoursodeoxycholic acid sulfate (GUDCAS) (Fig. 4). We also found a strong correlation between *M. smithii* and the unclassified *Methanobrevibacter species* in the mother's stool samples. The Spearman's rank correlation coefficient between these two species was 0.99 in the stool samples collected three months after delivery. *Alistipes putredinis* was negatively associated with docosahexanoic acid (C22:6) and one BA (iso ursodeoxycholic acid [iso-UDCA] or hyodeoxycholic acid [isoHDCA] or murocholic deoxycholic acid [MCDCA]). In the maternal serum at 3 months post-delivery, we also observed positive associations between *Parasutterella excrementihominis* and microbial-derived metabolite indole-3-acetic acid and two BAs (GUDCA and cholic acid [CA]). In addition, *Erysipelotrichaceae bacterium 6145* showed a significant, positive association with 12-ketolithocholic acid, indicating that *Erysipelotrichaceae* and *Parasutterella* may actively participate in human BA metabolism and homeostasis (Fig. 4).

4. Discussion

Our study revealed that high PFAS exposure in mothers is associated with changes in the composition of their gut microbiome, characterized by consistently higher relative abundances of *Methanobrevibacter* species in highly exposed mothers compared to mothers with lower exposure. We were also able to demonstrate that the microbially-derived metabolite, indole-3-acetic acid and the conjugated BAs (such as GUDCA) are associated with an altered maternal microbiome 3 months after delivery.

Our observations suggest that the abundance of the methanogenic archaea may vary with PFAS exposure, which is in agreement with recent studies reporting that persistent organic pollutant exposure induce changes in human microbiome composition (Iszatt et al., 2019; Naspolini et al., 2022). Maternal exposure to PFAS was previously found associated with a decrease of *Faecalibacterium* and a concomitant increase in *Clostridium, Staphylococcus*, and *Bifidobacterium* bacterial species in infant stool samples (Naspolini et al., 2022). In previous studies PFAS exposure was also associated with changes in stool microbiome diversity in infants, specifically in the bacterial domain (Iszatt et al., 2019; Naspolini et al., 2022).

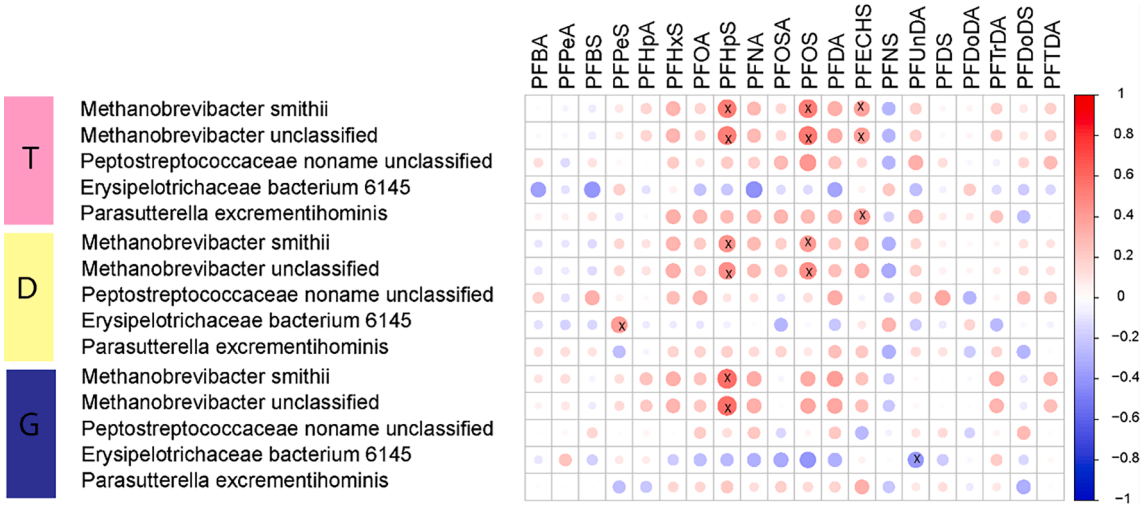

Fig. 3. Associations between the microbiome and individual PFAS in the mothers. Spearman rank correlation between the individual PFAS species (n = 20) with those microbial species that appeared to be affected in a minimum of two longitudinal sampling time points (i.e., G, D, and T) in the mothers. Mother gestational (G), delivery (D), and three months post-delivery (T). Perfluorobutanoic acid (PFBA) Perfluoropentanoic acid (PFPeA) Perfluorobutane sulfonate (PFBS) Perfluoro pentane sulfonate (PFPeS) Perfluoroheptanoic acid (PFHpA) Perfluorohexane sulfonate (PFHxS) Perfluorooctanoic acid (PFOA) Perfluoroheptane sulfonate (PFHpS) Perfluorononanoic acid (PFNA) Perfluorooctane sulfonamide (PFOSA) Linear-Perfluorooctane sulfonate (PFOS) Perfluorodecanoic acid (PFDA) Potassium Perfluoro-4-ethylcyclohexanesulfonate (PFECHS) Perfluorononane sulfonate (PFNS) Perfluoroundecanoic acid (PFUnDA) Perfluorodecane sulfonate (PFDS) Perfluorododecanoic acid (PFDoDA) Perfluorotridecanoic acid (PFTrDA) Perfluorododecane sulfonate (PFDoDS) Perfluorotetradecanoic acid (PFTDA).

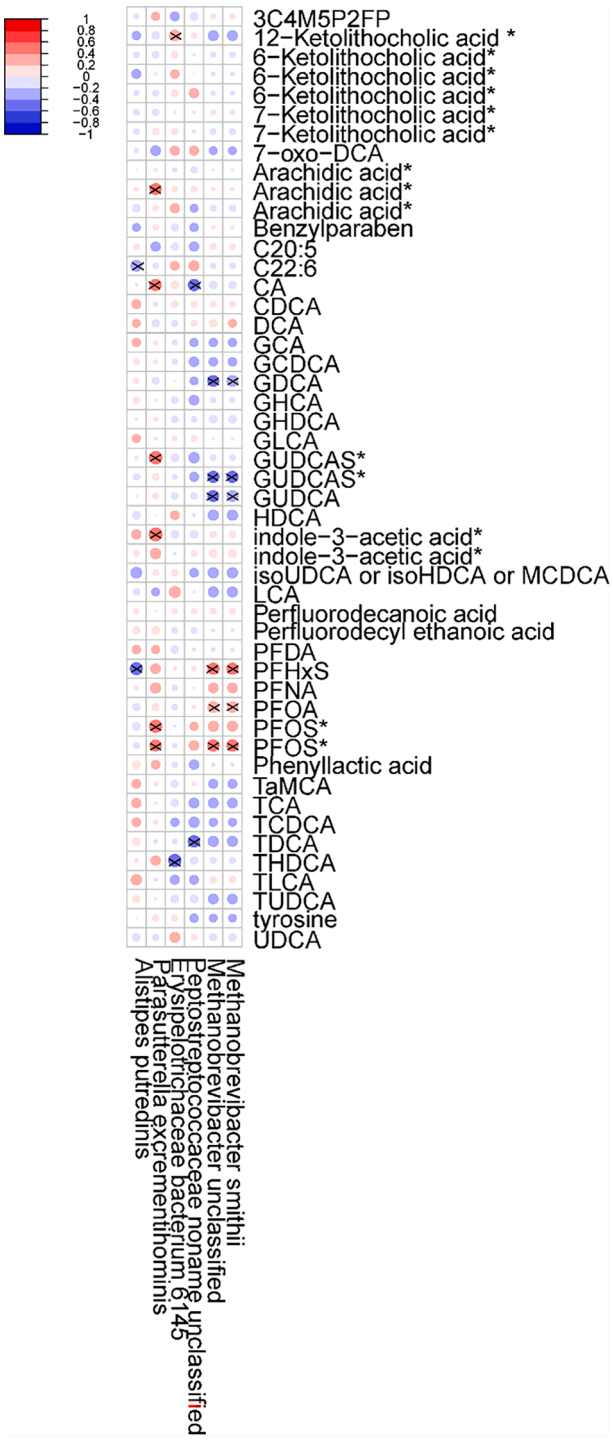

Fig. 4. Associations between microbiome and circulatory metabolite in the mothers 3 months after the delivery. Spearman rank correlation between the polar metabolites with those microbial species that were affected in a minimum of two longitudinal sampling time points (i.e. G, D, and T) in the mothers. Mother gestational (G), delivery (D), and three months post-delivery (T).*indicates the isomers and only metabolites are depicted in the figure. Here, black X, represents significant association (p values < 0.05). 3-Carboxy-4-methyl-5-propyl-2-furanpropanoate (3C4M5P2FP), 7-oxo-deoxycholic acid (7-oxo-DCA), cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (CDCA), glycolithocholic acid (GLCA), glycocholic acid (GCA), glycodeoxycholic acid (GDCA), glycoursodeoxycholic acid (GUDCA) and glycoursodeoxycholic acid sulfate (GUDCAS), docosahexanoic acid (C22:6) and one BA (iso ursodeoxycholic acid (isoUDCA) or hyodeoxycholic acid (isoHDCA) or murocholic deoxycholic acid (MCDCA), lithocholic acid (LCA), Taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), tauroursodeoxycholic acid (TUDCA), taurolithocholic acid (TLCA), ursodeoxycholic acid (UDCA), Perfluorodecanoic acid (PFDA), Perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), Perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS).

Here, we focused on mother-infant dyads, and the metagenomics sequencing approach allowed us to investigate microbiome samples at subspecies resolution (Yassour et al., 2018). We found that PFAS exposure appeared associated particularly with the *Methanobrevibacter* species in maternal sample but not in the infant microbiome, which could be due to the absence of *Methanobrevibacter* colonization/succession in the infant gut. Interestingly, we identified two specific PFAS, being PFOS and PFHpS, which were strongly associated with the

Methanobrevibacter, a dominant methanogenic archaea in the adult human gut. These methanogens are functionally important due to their ability to consume molecular hydrogen and contribute to host energy harvest (Hansen et al., 2011). Importantly, *Methanobrevibacter* strains have been reported to be proinflammatory pathobionts associated with multiple sclerosis (Jangi et al., 2016), childhood obesity (Mbakwa et al., 2015) and urinary tract infection (Grine et al., 2019). Meanwhile, *Methanobrevibacter* were also found to be enriched in Sardinian centenarians (Wu et al., 2019).

The gut microbiome has a profound influence on the human metabolome (Dekkers et al., 2022). We found that microbial species (archaea) which appear associated with PFAS exposure, were negatively associated with glycine-conjugated BAs, in particular with GDCA, GUDCA and GUDCAS. The BA pool in humans is also regulated by the gut microbes, secondary BAs in particular (Ridlon et al., 2014). Functionally, PFAS have been suggested to impact the intestinal reabsorption of BAs (Beggs et al., 2016; Chiang, 2017; Zhao et al., 2015). Previous studies indicated that BA and methanogens in the human colon are interlinked (Florin and Jabbar, 1994). Recently, gut-associated archaea M. *smithii* have have also been found to have bile salt hydrolase (BSH) activities, which are responsible for conjugation of amino acids with BAs (Jones et al., 2008).

We acknowledge some limitations to our study, including the small sample size in our longitudinal setting. However, our study is unique in reporting associations between PFAS exposure levels and the human gut microbiome at the individual species level in paired mother–child dyad. Our study suggests that PFAS exposure may affect human health through the host-microbiome-metabolome co-axis. Specifically, the association between *M. smithii* and conjugated BAs suggests that methanogenic archaea may play a role in mediating the interplay of BA metabolism, reabsorption, and the impact of PFAS exposure. Nevertheless, additional research is required to investigate the underlying mediation and mechanisms to validate this relationship. We also recognize that confounding covariates must be taken into account and systematic integration of numerous cofactors are required to draw strong conclusions regarding cause-and-effect relationships between microbiome constituents and phenotype.

5. Conclusions

Taken together, our results suggest that the PFAS exposure may influence adult gut microbiota composition, while the effect on the infant microbiome appear weak. The changes in microbiome composition indicate that methanogenic archaea were consistently increased in the highly-exposed group. At the individual PFAS level, PFOS and PFHpS showed the strongest connection with the altered methanogenic archaea.

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.

CRediT authorship contribution statement

Santosh Lamichhane: Conceptualization, Formal analysis, Funding acquisition, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing. **Taina Härkönen:** Resources, Writing - review & editing. **Tommi Vatanen:** Resources, Writing - review & editing. **Tuulia Hyötyläinen:** Investigation, Methodology, Resources, Data curation. **Mikael Knip:** Funding acquisition, Investigation, Resources, Supervision. **Matej Orešič:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Metagenomic sequencing data have been deposited to the SRA database under BioProject ID PRJNA475246. The metabolomics datasets were submitted to MetaboLights under accession number MTBLS875.

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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.2023.107965.

References

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M.T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y.S., Kotowska, D., Colding, C., Tremaroli, V., Yin, Y., Bergman, S., Xu, X., Madsen, L., Kristiansen, K., Dahlgren, J., Wang, J., 2015. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell. Host. Microbe 17, 690–703.

Barker, D.J., 2007. The origins of the developmental origins theory. J. Intern. Med 261, 412–417.

Beggs, K.M., McGreal, S.R., McCarthy, A., Gunewardena, S., Lampe, J.N., Lau, C., Apte, U., 2016. The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicol. Appl. Pharmacol 304, 18–29.

Chiang, J.Y. Recent advances in understanding bile acid homeostasis. F1000Res 2017;6: 2029.

Chiu, K., Warner, G., Nowak, R.A., Flaws, J.A., Mei, W., 2020. The impact of environmental chemicals on the gut microbiome. Toxicol. Sci 176, 253–284.

Croes, K., Colles, A., Koppen, G., Govarts, E., Bruckers, L., Van de Mieroop, E., Nelen, V., Covaci, A., Dirtu, A.C., Thomsen, C., Haug, L.S., Becher, G., Mampaey, M., Schoeters, G., Van Larebeke, N., Baeyens, W., 2012. Persistent organic pollutants (POPs) in human milk: a biomonitoring study in rural areas of Flanders (Belgium). Chemosphere 89, 988–994.

Dekkers, K.F., Sayols-Baixeras, S., Baldanzi, G., Nowak, C., Hammar, U., Nguyen, D., Varotsis, G., Brunkwall, L., Nielsen, N., Eklund, A.C., Bak Holm, J., Nielsen, H.B., Ottosson, F., Lin, Y.T., Ahmad, S., Lind, L., Sundström, J., Engström, G., Smith, J.G., Ärnlöv, J., Orho-Melander, M., Fall, T., 2022. An online atlas of human plasma metabolite signatures of gut microbiome composition. Nat. Commun 13, 5370.

Florin, T.H., Jabbar, I.A., 1994. A possible role for bile acid in the control of methanogenesis and the accumulation of hydrogen gas in the human colon. J. Gastroenterol. Hepatol 9, 112–117.

Gilbert, J.A., Blaser, M.J., Caporaso, J.G., Jansson, J.K., Lynch, S.V., Knight, R., 2018. Current understanding of the human microbiome. Nat. Med 24, 392–400.
Grine, G., Lotte, R., Chirio, D., Chevalier, A., Raoult, D., Drancourt, M., Ruimy, R., 2019. Co-culture of Methanobrevibacter smithii with enterobacteria during urinary infection. EBioMedicine 43, 333–337.
Hansen, E.E., Lozupone, C.A., Rey, F.E., Wu, M., Guruge, J.L., Narra, A., Goodfellow, J., Zaneveld, J.R., McDonald, D.T., Goodrich, J.A., Heath, A.C., Knight, R., Gordon, J.I., 2011. Pan-genome of the dominant human gut-associated archaeon, Methanobrevibacter smithii, studied in twins. Proc. Natl. Acad. Sci. U. S. A 108 (Suppl 1), 4599–4606.
Iszatt, N., Janssen, S., Lenters, V., Dahl, C., Stigum, H., Knight, R., Mandal, S., Peddada, S., González, A., Midtvedt, T., Eggesbø, M., 2019. Environmental toxicants in breast milk of Norwegian mothers and gut bacteria composition and metabolites in their infants at 1 month. Microbiome 7, 34.
Jangi, S., Gandhi, R., Cox, L.M., Li, N., von Glehn, F., Yan, R., Patel, B., Mazzola, M.A., Liu, S., Glanz, B.L., Cook, S., Tankou, S., Stuart, F., Melo, K., Nejad, P., Smith, K., Topçuolu, B.D., Holden, J., Kivisäkk, P., Chitnis, T., De Jager, P.L., Quintana, F.J., Gerber, G.K., Bry, L., Weiner, H.L., 2016. Alterations of the human gut microbiome in multiple sclerosis. Nat. Commun 7, 12015.
Jones, B.V., Begley, M., Hill, C., Gahan, C.G., Marchesi, J.R., 2008. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. Proc. Natl. Acad. Sci. U. S. A 105, 13580–13585.
Kennedy, K.M., de Goffau, M.C., Perez-Muñoz, M.E., Arrieta, M.C., Bäckhed, F., Bork, P., Braun, T., Bushman, F.D., Dore, J., de Vos, W.M., Earl, A.M., Eisen, J.A., Elovitz, M. A., Ganal-Vonarburg, S.C., Gänzle, M.G., Garrett, W.S., Hall, L.J., Hornef, M.W., Huttenhower, C., Konnikova, L., Lebeer, S., Macpherson, A.J., Massey, R.C., McHardy, A.C., Koren, O., Lawley, T.D., Ley, R.E., O'Mahony, L., O'Toole, P.W., Pamer, E.G., Parkhill, J., Raes, J., Rattei, T., Salonen, A., Segal, E., Segata, N., Shanahan, F., Sloboda, D.M., Smith, G.C.S., Sokol, H., Spector, T.D., Surette, M.G., Tannock, G.W., Walker, A.W., Yassour, M., Walter, J., 2023. Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. Nature 613, 639–649.
Koppel, N., Maini Rekdal, V., Balskus, E.P., 2017. Chemical transformation of xenobiotics by the human gut microbiota. Science 356.
Lamichhane, S., Siljander, H., Duberg, D., Honkanen, J., Virtanen, S.M., Orešič, M., Knip, M., Hyötyläinen, T., 2021. Exposure to per- and polyfluoroalkyl substances associates with an altered lipid composition of breast milk. Environ. Int 157, 106855.
Laue, H.E., Moroishi, Y., Palys, T.J., Christensen, B.C., Criswell, R.L., Peterson, L.A., Huset, C.A., Baker, E.R., Karagas, M.R., Madan, J.C., Romano, M.E., 2023. Early-life exposure to per- and polyfluoroalkyl substances and infant gut microbial composition. Environ. Epidemiol 7, e238.
Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C.H., Tallving, P., Jakobsson, K., 2018. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup. Environ. Med 75, 46–51.
Liew, Z., Goudarzi, H., Oulhote, Y., 2018. Developmental exposures to perfluoroalkyl substances (PFASs): An update of associated health outcomes. Curr. Environ. Health. Rep 5, 1–19.
Mbakwa, C.A., Penders, J., Savelkoul, P.H., Thijs, C., Dagnelie, P.C., Mommers, M., Arts, I.C., 2015. Gut colonization with methanobrevibacter smithii is associated with childhood weight development. Obesity. (Silver. Spring) 23, 2508–2516.
McGlinchey, A., Sinioja, T., Lamichhane, S., Sen, P., Bodin, J., Siljander, H., Dickens, A. M., Geng, D., Carlsson, C., Duberg, D., Ilonen, J., Virtanen, S.M., Dirven, H., Berntsen, H.F., Zimmer, K., Nygaard, U.C., Orešič, M., Knip, M., Hyötyläinen, T., 2020. Prenatal exposure to perfluoroalkyl substances modulates neonatal serum phospholipids, increasing risk of type 1 diabetes. Environ. Int 143, 105935.
Naspolini, N.F., Meyer, A., Moreira, J.C., Sun, H., Froes-Asmus, C.I.R., Dominguez-Bello, M.G., 2022. Environmental pollutant exposure associated with altered early-life gut microbiome: Results from a birth cohort study. Environ. Res 205, 112545.
Ridlon, J.M., Kang, D.J., Hylemon, P.B., Bajaj, J.S., 2014. Bile acids and the gut microbiome. Curr. Opin. Gastroenterol 30, 332–338.
Robertson, R.C., Manges, A.R., Finlay, B.B., Prendergast, A.J., 2019. The Human Microbiome and Child Growth - First 1000 Days and Beyond. Trends. Microbiol 27, 131–147.
Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P. I., Godneva, A., Kalka, I.N., Bar, N., Shilo, S., Lador, D., Vila, A.V., Zmora, N., Pevsner-Fischer, M., Israeli, D., Kosower, N., Malka, G., Wolf, B.C., Avnit-Sagi, T., Lotan-Pompan, M., Weinberger, A., Halpern, Z., Carmi, S., Fu, J., Wijmenga, C., Zhernakova, A., Elinav, E., Segal, E., 2018. Environment dominates over host genetics in shaping human gut microbiota. Nature 555, 210–215.
Salihović, S., Dickens, A.M., Schoultz, I., Fart, F., Sinisalu, L., Lindeman, T., Halfvarson, J., Orešič, M., Hyötyläinen, T., 2020. Simultaneous determination of perfluoroalkyl substances and bile acids in human serum using ultra-high-performance liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem 412, 2251–2259.
Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome. Biol 12, R60.
Segata, N., Waldron, L., Ballarini, A., Narasimhan, V., Jousson, O., Huttenhower, C., 2012. Metagenomic microbial community profiling using unique clade-specific marker genes. Nat. Methods 9, 811–814.
Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J. Expo. Sci. Environ. Epidemiol 29, 131–147.
Truong, D.T., Franzosa, E.A., Tickle, T.L., Scholz, M., Weingart, G., Pasolli, E., Tett, A., Huttenhower, C., Segata, N., 2015. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat. Methods 12, 902–903.
van Best, N., Rolle-Kampczyk, U., Schaap, F.G., Basic, M., Olde Damink, S.W.M., Bleich, A., Savelkoul, P.H.M., von Bergen, M., Penders, J., Hornef, M.W., 2020. Bile acids drive the newborn's gut microbiota maturation. Nat. Commun 11, 3692.
Wu, L., Zeng, T., Zinellu, A., Rubino, S., Kelvin, D.J., Carru, C., 2019. A cross-sectional study of compositional and functional profiles of gut microbiota in sardinian centenarians. mSystems 4.
Yassour, M., Jason, E., Hogstrom, L.J., Arthur, T.D., Tripathi, S., Siljander, H., Selvenius, J., Oikarinen, S., Hyöty, H., Virtanen, S.M., Ilonen, J., Ferretti, P., Pasolli, E., Tett, A., Asnicar, F., Segata, N., Vlamakis, H., Lander, E.S., Huttenhower, C., Knip, M., Xavier, R.J., 2018. Strain-level analysis of mother-to-child bacterial transmission during the first few months of life. Cell. Host. Microbe 24, 146–154.e144.
Zhao, W., Zitzow, J.D., Ehresman, D.J., Chang, S.C., Butenhoff, J.L., Forster, J., Hagenbuch, B., 2015. Na+/Taurocholate cotransporting polypeptide and apical sodium-dependent bile acid transporter are involved in the disposition of perfluoroalkyl sulfonates in humans and rats. Toxicol. Sci. 146, 363–373.