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Natalie Shen	April 19, 2023
	Date

The Impact of Phthalate Exposure on the Gut Microbiota of Pregnant African American People in Atlanta, Georgia

By

Natalie Shen
Master of Public Health

Gangarosa Department of Environmental Health

Donghai Liang, PhD, MPH
Committee Chair

Anne L. Dunlop, MD, MPH
Committee Member

Yijuan Hu, PhD
Committee Member

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By

Natalie Shen

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Thesis Committee Chair: Donghai Liang, PhD, MPH

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Abstract

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By Natalie Shen

Phthalates, commonly found in household and personal care products, are endocrine disruptors that negatively impact reproductive health and fetal development. They are also associated with gut microbiota modulation, which can increase the risk of pre-eclampsia and preterm birth. Pregnant African Americans have higher concentrations of urinary phthalate metabolites and experience more preterm births compared to Caucasians, yet few studies have investigated the impact of phthalate exposure on the gut microbiota and its implications for pregnancy and birth outcomes in this understudied population. We used data from the Atlanta AA Maternal-Child cohort between 2016-2020 (N = 193). Maternal rectal swabs and urine samples were collected at 8-14 weeks and 24-30 weeks gestation. Gut microbiota profiling was performed via 16S rRNA gene sequencing of rectal swabs while phthalate exposure was measured by urinary metabolite concentration and dichotomized into high and low exposure levels using the median threshold. We compared relative abundance of bacterial genera and alpha-diversity between high and low phthalate exposure groups. We used univariate Spearman correlation and multivariable linear regression to examine the associations between phthalate metabolite concentrations and gut bacterial abundance. We observed a significantly increased alpha-diversity in those with higher MBzP exposures. Additionally, Verrucomicrobiota was negatively associated with MiBP, while Proteobacteria was positively associated with MBP and MEHP. *Peptostreptococcus* was positively associated with MEP, while *Bifidobacterium* was negatively associated with MEHP. Our findings provide evidence that maternal gestational phthalate exposure may be associated with altered gut microbiota alpha-diversity. Moreover, higher phthalate exposure is associated with increased abundance of some pathogenic bacterial taxa and decreased abundance of some beneficial bacterial taxa. These negative associations between beneficial bacteria and phthalate metabolites provide insight for potential clinical applications to prevent or treat phthalate-induced disease during pregnancy. While our study sheds light on the potential impact of phthalate metabolites on human health, future research should incorporate more diverse populations, assess dietary and plastic-product use patterns, and employ more advanced bacterial sequencing methods to confirm and expand upon these findings.

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1. Introduction

1.1 Phthalates

Phthalates, diesters of 1,2-benzendicarboxylic acid (phthalic acid), are non-persistent ubiquitous xenobiotics commonly used in industrial applications[1,2]. As plasticizers, phthalates are widely found in many household and personal-care items, such as vinyl flooring, plastic packaging, soaps, shampoos, cosmetics, and hairsprays[3]. Therefore, exposure to phthalates in humans is widespread, with inhalation, dermal contact, and ingestion being the major routes of exposure in adults[4], and additionally consuming breastmilk in infants[5]. Phthalates are known as endocrine disruptors, which are chemicals that interfere with the hormonal balance in the body[6]. As a result, they often impact reproductive health[7]. In women, phthalates can compromise ovarian function, pubertal development, and pregnancy outcomes. Multiple studies have confirmed that exposure to di-(2-ethylhexyl) phthalate (DEHP), a widely used phthalate ester, leads to disruption of primordial germ cells development and survival, as well as oocyte maturation and activation[8,9]. Another study illustrated the negative association between various phthalate metabolites and breast development and menstrual cycle initiation[10]. Because of its close ties with reproductive health, phthalate exposure also negatively impacts pregnancy and fetal development[4]. Specifically, an *in vivo* study demonstrated that pregnant mice exposed to DEHP experienced repressed placental angiogenesis[11], and an observational study on females exposed to high levels of phthalates recorded decreased rates of pregnancy and increased miscarriage[12]. Similarly, a study by Latini et al. found that increased DEHP exposure was associated with increased rates of preterm birth[13]. In addition to pregnancy outcomes, murine studies have also shown that prenatal DEHP exposure can increase adiposity and impair social

interaction in offspring, indicating potential disruption of fetal metabolism and neuronal development[14,15].

1.2 Phthalates and the gut microbiota

Phthalates are also associated with modulation of the gut microbiota[16], which may elucidate their mechanism of action in maternal and infant health outcomes. Mice exposed to phthalate-containing microplastics were found to have significantly decreased beta-diversity (i.e., inter-group dissimilarity) compared to the control group, suggesting that phthalates changed gut microbial composition[17]. Dysbiosis (i.e., homeostatic disruption of the gut microbiota) is associated with various negative health effects, such as inflammatory bowel diseases, diabetes, and weight gain[18]. Dysbiosis of the gut microbiota has been observed in multiple studies to be associated with adverse pregnancy and birth outcomes. A recent study found that women with pre-eclampsia (PE) experienced decreased alpha-diversity compared to healthy controls in the third trimester of pregnancy[19]. Furthermore, fecal microbiota transplantation into mice using samples from PE patients resulted in a dramatic increase in murine blood pressure, which increased after gestation[20]. Another study demonstrated that the intestinal microbiota, rather than the vaginal microbiota, was the varying factor in preterm birth events[21]. Negative impacts of maternal phthalate exposure on *in utero* penile development has been observed as well[22]. Although there have been studies that investigate the effects of phthalates on the gut microbiota, most existing research involve only animal models and/or exposure to DEHP[23-25]. Therefore, we conducted this study to bridge the research gap by utilizing data from a human cohort study and different phthalate metabolites to gain a broader understanding of phthalate impact on the human gut microbiota.

1.3 Phthalate health disparities

In the United States, there are great racial health disparities, especially among African Americans (AAs) compared to Caucasians. Based on the 2020 National Vital Statistics report, AA individuals are more likely to experience pre-term birth (14.36%) versus white individuals (9.10%)[26]. Additionally, AA and Hispanic people have higher concentrations of certain urinary phthalate metabolites compared to Caucasians across pregnancy[27]. However, there are few studies investigating the impact of phthalate exposure in pregnant AA people on the gut microbiota, as well as its implication for pregnancy and birth outcomes in this context. Thus, the purpose of this analysis is to determine the cross-sectional association between phthalate exposure (assessed using urinary phthalate metabolites) and the maternal gut microbiota among pregnant people in the Atlanta African American Maternal-Child Cohort. The results of this study may elucidate a potential gut microbiota target of phthalate-induced physiological changes. This study may also inform future targeted interventions for prenatal care in preventing adverse health effects and birth outcomes related to phthalate exposure or gut dysbiosis in the AA population.

2. Methods

2.1 Study participants

This study leverages participant data from the prospective Atlanta African American Maternal-Child Cohort consisting of pregnant people recruited from Emory University Hospital Midtown and the Grady Memorial Hospital[28]. Inclusion criteria for enrolled individuals included 1) self-identification as AA, 2) 8-14 weeks gestation with a verified singleton pregnancy, 3) between 18 and 40 years old, 4) able to comprehend written and spoken English, and 5) experiencing no current chronic medical condition. Biological samples (i.e., urine, rectal swabs)

were collected at the first (8-14 weeks) and second (24-30 weeks) clinical visits. Questionnaire data related to socio-demographics (i.e., age, education) was collected at the first clinical visit while health survey (i.e., medication and substance use) and clinical data (i.e., parity, gestational weeks) were collected at both clinical visits. For the current analysis, 193 individuals who had complete data related to sociodemographic and health history, as well as phthalate exposure assessment and gut microbiota, were included in the final analysis. Signed informed consent was obtained from all participants and the study was approved by the Emory University Institutional Review Board (IRB ID 1017).

2.2 Urinary phthalate metabolite identification

To assess maternal exposure to phthalates, urine samples were collected at enrollment clinical Visit 1 (8-14 weeks) and follow-up clinical Visit 2 (24-30 weeks). Eight urinary phthalate metabolites were assessed, namely Monoethyl phthalate (MEP), Mono-n-butyl phthalate (MBP), Monoisobutyl phthalate (MiBP), Monobenzyl phthalate (MBzP), Mono(2-ethlyhexyl) phthalate (MEHP), Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and Mono(2-ethly-5-carboxypentyl) phthalate (MECPP). As outlined previously, a volume of 0.5 ml urine sample was mixed with isotopically-labeled 13C stable analogues of the phthalate metabolites and 2000 units of β-glucuronidase in 1 mM ammonium acetate (pH 5) buffer[29]. The samples were subsequently incubated overnight at 37 °C. Afterwards, sample enzymatic activity was terminated by adding 0.15 M sodium phosphate buffer. Then, Bond Elut solid phase extraction (SPE) cartridge (Agilent Technologies, Inc., Santa Clara, CA) was utilized to extract the phthalate metabolites. The dried extracts were mixed with Milli-Q water for reconstitution and target phthalate metabolites were separated using high performance liquid chromatography on a Betasil Phenyl (3 μ,150 × 2.1 mm) (Thermo

Scientific, San Jose, CA) analytical column. An Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Inc) was used to analyze the resulting metabolites via tandem mass spectrometry. From this, metabolite concentrations were calculated from a regression analysis of the area of the analyte ion divided by the area of the internal standard ion and the calibrant concentrations. The analyses included quality assurance and control procedures (i.e., analyses of NIST reference materials and quality control samples). The limits of detection (LOD) (ng/mL) for the eight target phthalate metabolites were as follows: 1.0 (MEP), 4.0 (MBP), 2.0 (MiBP), 0.2 (MBzP), 0.2 (MEHP), 0.4 (MEOHP), 0.4 (MEHHP), and 5.25 (MECPP). Any values obtained below the LOD were assigned the LOD divided by the square root of two[30]. Urinary creatinine concentration (mg/dL) was also measured to correct urinary phthalate levels for urinary dilution by diving the raw phthalate values by the creatinine level[31]. The concentration values were then log-normalized. The median values for each phthalate metabolite concentration were obtained and used as the threshold for creating categorical high and low exposure variables for use in comparing alpha- and beta-diversity indices between groups. The use of median values was employed to establish categorical levels, allowing better comparability with prior publications that utilized median phthalate concentrations in analyses[32,33].

2.3 Fecal sample collection

Maternal rectal swabs were collected at enrollment clinical Visit 1 (8-14 weeks) and follow-up clinical Visit 2 (24-30 weeks). Following the protocol by Corwin et al., they were self-collected, stored in MoBio bead tubes (MoBio Laboratories, Inc., Carlsbad, CA), and frozen upright on dry ice until transported to the lab for storage until DNA extraction[28].

2.4 Fecal sample processing

Bacterial DNA was extracted from the rectal swab samples using the MoBio isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) in accordance with the HMP Standard Operating Protocol[34]. Hypervariable regions (V3/V4+) of a small subunit of the ribosomal RNA gene (16S rRNA) were then amplified using polymerase chain reaction (PCR) with primers unique to this project [319F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′]. Quantified libraries were pooled and sequenced at 10pM loading density with 20% PhiX spike-in (FC-110-3001) on the Illumina MiSeq platform using v3 600 cycle MiSeq Reagent chemistry (Illumina, catalog # MS-102-3003)[35].

2.5 Bioinformatic processing

The raw fastq sequencing files were imported to Qiime2 (v2017.12) and denoised using dada2[36,37]. Reads were truncated for quality control and amplicon sequence variants (ASVs) were characterized using SILVA 138.1 classifier with 99% identity threshold[36,38]. ASV feature tables were then analyzed using the *phyloseq* R package[39]. 16S rRNA gene approaches are limited in their ability to reliably assign many bacterial genera to the species level; as such, we glommed taxa at the genus level for analyses. For samples run in duplicate (N = 2), the read counts were averaged between the samples. Finally, the samples were rarified at the 750 read count level, resulting in 193 unique participants included in the final analyses.

2.6 Statistical analysis

All statistical analyses were performed using R (v4.2.0) and SAS ® OnDemand (v3.81)[40,41]. A descriptive summary of participant demographic and clinical information was presented in participants across the two sample collection time points (Visit 1 and Visit 2). Wilcoxon test (for continuous variables) and chi-square test (for categorical variables) was used

to test for significant differences in these measurements between Visit 1 and Visit 2 participants. Chi-square tests were also used to evaluate differences in the associations between exposure categories across Visit 1 and Visit 2 participants.

To evaluate the associations between phthalate exposure and alpha-diversity, we calculated Inverse Simpson, Shannon, and Chao1 indices using the *phyloseq* R package[39]. The differences in alpha diversity between high and low phthalate exposure groups were analyzed using the Wilcoxon test. Alpha-diversity is the measure of community richness (number of taxa) and/or evenness (abundance distribution across taxa)[42]. Additionally, a principal coordinate analysis (PCoA) using the Bray-Curtis dissimilarity index for beta-diversity of the high and low phthalate exposure groups was created using the *phyloseq* R package, where points closer together denote bacterial communities that are more similar in taxa presence and abundance[43]. Beta-diversity, or dissimilarity, is the measure of diversity and variability between communities[44].

To obtain a global view of gut bacterial abundance in this population, the relative abundance of phyla (i.e., the highest bacterial taxonomic rank) per visit were calculated using the following equation:

(Taxa read count)/(Total bacterial read count per visit)*100

Phyla at less than 4% relative abundance in each visit were grouped together in the stacked barplot, created using the *ggplot2* R package[45]. The Firmicutes/Bacteroidota (F/B) ratio, as an important marker of gut microbiota homeostasis, was also calculated for each visit using their relative abundance measurements[46,47].

A correlation analysis was performed for each visit to determine the relationship between gut bacteria and phthalate exposure, excluding bacteria with more than a 90% zero-count. Due to

the non-normal distribution of both gut bacterial read counts and phthalate metabolite concentrations, the non-parametric Spearman's correlation (rho, ρ) was utilized, calculated using the *Hmisc* R package, along with the corresponding p-values[48]. These correlations were visualized by heatmaps, created using the *ComplexHeatmap* R package[49].

Finally, we used a multivariate linear regression model to determine the association between the maternal gut microbiota (read counts) and continuous phthalate metabolite levels (urinary concentrations) at each visit, excluding bacteria with more than a 90% zero-count. A directed acyclic graph (DAG) created using Daggity[50] (not shown) was utilized to evaluate confounders, confirming the addition of age at enrollment, insurance type, education, tobacco use, alcohol use, and marijuana use as covariates in the linear regression model. The model also included the following *a priori* covariates determined using literature search and biological relevance: prenatal BMI, prenatal and gestational oral antibiotic use, and parity[51,52]. P-values were adjusted for multiple testing using the Benjamini-Hochberg method[53]. The following equation was the final, fully adjusted model used in the analysis:

$$\begin{aligned} Y = \ & \beta_0 + \beta_1 Phthalate\ metabolites + \beta_2 Age + \beta_3 Prenatal\ BMI + \beta_4 Education \\ & + \beta_5 Insurance\ type + \beta_6 Antibiotic\ use + \beta_7 Parity + \beta_8 Tobacco\ use \\ & + \beta_9 Alcohol\ use + \beta_{10} Marijuana\ use + \varepsilon \end{aligned}$$

3. Results

3.1 Participant characteristics

Demographic, clinical, and behavioral characteristics of the 193 study participants are presented in **Table 1**. 167 participants were included in Visit 1, while 123 were included in Visit 2. There were no statistically significant differences between Visit 1 and Visit 2 groups for demographic, clinical, and behavioral characteristics ($P > 0.05$).

3.2 Phthalate metabolites

In total, eight urinary phthalate metabolites were assessed, as listed in **Table 2a-2b**: Monoethyl phthalate, MEP; Mono-n-butyl phthalate, MBP; Monoisobutyl phthalate, MiBP; Monobenzyl phthalate, MBzP; Mono(2-ethlyhexyl) phthalate, MEHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEOHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MEHHP; and Mono(2-ethly-5-carboxypentyl) phthalate, MECPP. Their histogram distributions are shown in **Figure S1**. Non-parametric Wilcoxon test revealed no significant differences of metabolite concentrations between Visit 1 and Visit 2 ($P > 0.05$). There was a significant difference between high and low MEHP concentration across Visit 1 and Visit 2 ($P < 0.01$). However, there were no significant differences between high and low concentration levels of the other metabolites across visits ($P > 0.05$).

3.3 Gut microbiota community structure

The phyla relative abundance of the gut microbiota across Visit 1 and Visit 2 among the 193 participants are displayed in **Figure 1**. Due to an abundance of low and zero read counts, taxa containing less than 4% relative abundance in each visit group were grouped together. The two most abundant phyla at both visits were Bacteroidota and Firmicutes, with 46.21% and 41.11% in Visit 1 and 35.65% and 42.96% in Visit 2, respectively. The other phyla detected in the gut microbiota samples in Visit 1 and Visit 2 include Actinobacteriota (4.73% and 4.11%), Campylobacter (3.55% and 5.70%), Fusobacteria (4.09% and 3.16%), and Proteobacteria (3.29% and 1.87%). There was a 5.10% decrease in the relative abundance of Bacteroidota from Visit 1 to Visit 2, while Firmicutes increased by 7.31%. The Visit 1 and Visit 2 Firmicutes/Bacteroidota ratios were 0.77 and 1.04, respectively.

3.4 Gut bacterial diversity

Across visits, the alpha-diversity indices were similar (i.e., Chao1, Shannon, and Inverse Simpson) ($P > 0.05$, Visit 1 vs. Visit 2) (**Table 3a**). When examining Shannon diversity indices by exposure status, those with high compared to low MBzP exposure had significantly higher Shannon diversity in Visit 2, while there were no differences in the Shannon diversity indices for all other phthalate exposures at either visit (**Table 3b**). Corresponding box plots of the Shannon alpha-diversity index across high and low exposure categories to each phthalate exposure at Visit 1 and Visit 2 are shown in **Figure 2**. Beta-diversity was calculated using the Bray-Curtis dissimilarity index for high and low phthalate exposure for each visit are displayed in PCoA plots (**Figure 3**). Based on the distribution patterns of the PCoA plots and lack of clustering within high and low exposure strata, there were no differences in the gut microbiota beta-diversity between high and low phthalate exposure groups. However, there were obvious clusters within the PCoA plots, potentially by other means, and thus future studies can investigate other factors that may explain the grouping patterns observed in the plots.

3.5 Correlation analysis

At the phylum level (**Figure 4**), among Visit 1 participants, Verrucomicrobiota was negatively correlated with MiBP ($\rho = -0.19$, $P = 0.02$) while Proteobacteria was positively correlated with MBP ($\rho = 0.18$, $P = 0.02$). Among Visit 2 participants, Firmicutes phylum was negatively correlated with MiBP ($\rho = -0.21$, $P = 0.02$). At the genus level (**Figure 5**), *Peptostreptococcus* was positively correlated with MEP ($\rho = 0.23$, $P < 0.01$), *Fusobacterium* was positively correlated with MEHP ($\rho = 0.23$, $P < 0.01$), and *Bifidobacterium* was negatively correlated with MEHP ($\rho = -0.19$, $P = 0.01$) among Visit 1 participants. On the other hand,

among Visit 2 participants, *Streptococcus* was positively correlated with MEHHP (ρ = 0.21, P = 0.02) and *Fenollaria* was negatively correlated with MEHHP (ρ = -0.18, P = 0.02).

3.6 Regression modeling

Overall, several significant associations between gut bacteria and phthalate metabolites were observed at different taxonomic levels; however, many of these associations did not reach statistical significance after adjustment for multiple testing.

At the phylum level (**Table 4**), there was a significant association between the relative abundance of Verrucomicrobiota and MiBP (β = -0.35, 95% CI: -0.65, -0.06, P = 0.02, P_{BH} = 0.17) at Visit 1, whereas at Visit 2 there was a significant association between the relative abundance of Bacteroidota and MiBP (β = 0.53, 95% CI: 0.14, 0.91, P = 0.01, P_{BH} = 0.05) and MEHP (β = 0.31, 95% CI: 0.02, 0.61, P = 0.04, P_{BH} = 0.12) and between the relative abundance of Proteobacteria and MEHP (β = 0.49, 95% CI: 0.062, 0.93, P = 0.03, P_{BH} = 0.12).

At the class level (not shown), there was a significant association between Bacteroidia and MiBP (β = 0.64, 95% CI: 0.14, 0.91, P = 0.01, P_{BH} = 0.08), Clostridia and MEP (β = 0.55, 95% CI: 0.11, 0. 98, P = 0.01, P_{BH} = 0.14), and Gammaproteobacteria and MEHP (β = 0.31, 95% CI: 0.02, 0.61, P = 0.03, P_{BH} = 0.17) among the Visit 2 participants.

At the order level (not shown), there was a significant association between Peptostreptococcales Tissierellales and MEP (β = 0.51, 95% CI: 0.13, 0.90, P = 0.01, P_{BH} = 0.15), Bifidobacteriales and MEHP (β = -0.36, 95% CI: -0.66, -0.06, P = 0.02, P_{BH} = 0.32), and Lactobacillales and MEHHP (β = 0.51, 95% CI: 0.06, 0.96, P = 0.03, P_{BH} = 0.45) among the Visit 1 participants. Among those in Visit 2, there was a significant association between Bacteroidales and MEHP (β = 0.02, 95% CI: 0.61, 0.04, P = 0.04, P_{BH} = 0.27), and between

Lachnospirales and MEHHP (β = 0.70, 95% CI: 0.11, 1.30, P = 0.02, P_{BH} = 0.35), and MECPP (β = 0.80, 95% CI: 0.11, 1.49, P = 0.03, P_{BH} = 0.39).

At the family level (not shown), there was a significant association between *Peptostreptococcaceae* and MEP (β = 0.61, 95% CI: 0.25, 0.97, P = 0.001, P_{BH} = 0.03), S*treptococcaceae* and MBzP (β = 0.39, 95% CI: 0.07, 0.7, P = 0.02, P_{BH} = 0.40), and *Bifidobacteriaceae* and MEHP (β = -0.36, 95% CI: -0.66, -0.06, P = 0.02, P_{BH} = 0.43) among the Visit 1 participants. Among those in Visit 2, there was a significant association between *Rikenellaceae* and MEOHP (β = -0.49, 95% CI: -0.80, -0.18, P = 0.002, P_{BH} = 0.05) and MEHHP (β = -0.40, 95% CI: -0.71, -0.09, P = 0.01, P_{BH} = 0.26), and between *Coriobacteriaceae* and MECPP (β = 0.67, 95% CI: 0.12, 1.23, P = 0.02, P_{BH} = 0.43).

At the genus level (not shown), there was a significant association between *Bifidobacterium* and MEHP (β = -0.31, 95% CI: -0.53, -0.09, P = 0.006, P_{BH} = 0.17), *Peptoniphilus* and MEP (β = 0.51, 95% CI: 0.15, 0.86, P = 0.005, P_{BH} = 0.08), and *Peptostreptococcus* and MEP (β = 0.52, 95% CI: 0.18, 0.87, P = 0.003, P_{BH} = 0.08) among the Visit 1 participants. Among those in Visit 2, there was a significant association between *Veillonella* and MiBP (β = -0.55, 95% CI: -0.90, -0.19, P = 0.003, P_{BH} = 0.08), *Collinsella* and MBP (β = 0.67, 95% CI: 0.12, 1.23, P = 0.02, P_{BH} = 0.47), and between *Prevotella* and MiBP (β = 0.80, 95% CI: 0.15, 1.45, P = 0.02, P_{BH} = 0.22).

4. Discussion

Using both targeted phthalate exposure assessment and gut microbiome profiling, we detected numerous significant associations between phthalate metabolites and gut bacterial taxa (at the level of phylum, class, order, family, and genus) among participants from the Atlanta

African American Maternal-Child cohort. These findings point to the gut microbiome as a potential biological mechanism by which prenatal exposure to phthalates impact pregnancy outcomes and can also inform future development of targeted interventions to mitigate harmful effects of these plasticizers. Overall, the results from this study highlight that bacteria from taxa commonly associated with pathogenicity were correlated or associated with higher exposure to phthalate metabolites. However, it is worth noting that when looking at the overall abundance of phyla in the participant cohort's gut microbiotas, they were found to be relatively normal and not significantly different from what has been reported in previous literature. For instance, the most abundant phyla in the gut microbiota of AA Atlanta pregnant cohort were Bacteroidota (or Bacteroidetes) and Firmicutes across both Visits, along with Fusobacteriota, Campylobacter, Actinobacteriota in lesser abundance. This is supported by previous research on pregnant populations that also observed that Bacteroidota and Firmicutes, along with Proteobacteria and Actinobacteria, were the most abundant phyla at the first and third trimesters[54]. Similarly, these phyla are also predominant in healthy, non-pregnant people[55]. Bacteroidota and Firmicutes have both been established as beneficial bacteria and balance of the Firmicutes/Bacteroidetes (F/B) ratio has been established as a marker of gut microbiota homeostasis and overall health status[56]. Our study found low F/B ratios of 0.77 and 1.04 across Visit 1 and Visit 2, whereas a previous study reported a F/B ratio of 0.6 in the elderly compared to 10.9 in adults[57]. This contrast highlights that the participants in this study have overall low gut bacterial abundance of Firmicutes in relation to Bacteroidota, which has been associated with inflammatory bowel disease[47]. Many Bacteroidota (or Bacteroides) species are able to provide nutrients and vitamins to the host by metabolizing polysaccharides and oligosaccharides[58] while Firmicutes contains

probiotic genera (i.e., *Lactobacillus*, *Ruminococcus*) that can promote gut mucosal function[59] and can also convert polysaccharides into host nutrient metabolites[60].

Those with greater exposure to MBzP had significantly increased Shannon diversity (1.71 ± 0.48 [high exposure group] vs. 1.54 ± 0.39 [low exposure group], $P < 0.05$), suggesting that higher phthalate exposure, specifically to monobenzyl phthalate, is associated with an increase in gut microbial diversity. Although there are no prior studies on the specific impact of MBzP on the gut microbiota, another phthalate metabolite, DEHP, has been observed to increase alpha diversity of exposed mice as well[61]. Despite their significant difference, both diversity measurements from our study are considered low, since the Shannon index ranges from 1.5-3.5[62]. MBzP is a monoester metabolite of the parent compound, benzylbutyl phthalate (BzBP), which in high doses, can lead to developmental and reproductive health outcomes in rodents[63]. However, due to the paucity of research in this area, there is no corroborating prior evidence to support our findings.

At the phylum level, Verrucomicrobiota was negatively associated with MiBP while Proteobacteria was positively correlated with MBP. Members of the Verrucomicrobiota and Firmicutes phylum have been found to be a biomarker of healthy gut[59], as they have anti-inflammatory properties and are protective for gut barrier functions and insulin sensitivity, among other qualities [64]. Thus, its negative correlation with MiBP, which has been associated with gestational diabetes mellitus in pregnant women[65], offers some insight into potential clinical applications among pregnant people and prevention or treatment of gestational diabetes. On the other hand, members of the Proteobacteria phylum have pro-inflammatory properties[66], such as being predictive of neonatal necrotizing enterocolitis[67] and a signature of several intestinal and extraintestinal human diseases[68]. It is positively correlated with MBP, of which prenatal

exposure has been found to be associated with earlier onset of spermatogenesis in male murine offspring[69] and lower birth weight, birth length, and gestational age[70], revealing that prenatal exposure to MBP might be adjacently-related to an increased abundance of Proteobacteria and these both synergistically result in adverse health outcomes for both mother and offspring. Our study also observed a significant positive association between Bacteroidota and MiBP and MEHP and between phylum Proteobacteria and MEHP. Although Bacteroidota are generally considered probiotic bacteria, some members can have a pathogenic effect and cause infection if the opportunity arises (i.e. compromised gastrointestinal barrier function)[71], which may partially explain its positive association with MEHP, due to its relation with Bis(2-ethylhexyl)phthalate (DEHP) as a metabolite, which has been demonstrated to substantially decrease microbial abundance in wastewater. Translated to human health, a change in the gut microbial abundance and diversity (i.e., gut dysbiosis) can lead to increased disease susceptibility due to compromised immune function[72]. However, a disease-state of the human host can also result in modulation of the gut microbiota[73], suggesting that MEHP, DEHP, and/or MiBP exposure may have contributed to the initial dysbiosis, which then resulted in an increase in potentially pathogenic Bacteroidota and Proteobacteria phyla.

At the class level, Bacteroidia and MiBP, Clostridia and MEP, Gammaproteobacteria and MEHP were all significantly positively associated. Bacteroidia. Clostridia are commonly decreased in those experiencing pregnancy complications[74] and are in high abundance in healthy individuals during early pregnancy[75], which points to MEP as a potential confounder in this study. On the other hand, Gammaproteobacteria and Bacteroidia are increased in disease states of pre-eclampsia[74] and Gammaproteobacteria are also positively associated with intrahepatic cholestasis of pregnancy (ICP)[76], a liver disorder that causes buildup of bile acids in the blood[77].

Furthermore, a previous study found evidence to support that maternal phthalate exposure during early pregnancy can be a risk factor of ICP[78]. Taken together, our results point to a possible therapeutic target of phthalate-associated ICP by modulating the abundance of Gammaproteobacteria in the maternal gut microbiota.

At the order level, there was a significant positive association between Bacteroidales and MEHP. However, a rat study by Yu et al. observed a decrease in Bacteroidales order and *Lactobacillus* genus with exposures to DEHP, which also dysregulated bile acid metabolism[79]. Although our study found conflicting evidence for the association between Bacteroidales and MEHP, a metabolite of DEHP, this gives insight into the potential for DEHP and/or MEHP to be related to the modulation of this bacterial taxa group. Members of Bacteroidales are mainly gut commensals and protect the host from pathogenic infections while providing nutrients for other gut microbiota residents[58].

At the family level, there was a significant positive association *Coriobacteriaceae* and MECPP. Similar to our study, Shum et al.[80] found that DEHP exposure enhanced abundance of *Coriobacteriaceae* members *(i.e., Collinsella),* which are often found in the mucosa of Crohn's disease patients but has also been found associated with good metabolic health in overweight and obese individuals[81]. Thus, these conflicting conclusions about the effects of *Coriobacteriaceae* warrant further studies investigating this specific bacterial family and phthalates to elucidate their temporality and mechanistic properties in relation to each other.

At the genus level, there was a significant positive association between *Peptostreptococcus* and MEP. There have also been multiple members of the *Peptostreptococcus* genus implicated in adenoma and colorectal cancer, namely *Peptostreptococcus anaerobius* and *P. stomatis.* Due to its significant positive association with MEP in our study, there could be a

potential for MEP to mechanistically benefit from Peptostreptococcus, or vice versa, in producing toxic effects in the host. Our study findings also report a negative association between *Veillonella* and MiBP. Similarly, prior research has demonstrated a decrease of *Veillonella* in the intestinal tract of newborns exposed to DEHP[82]. *Fusobacterium* was positively correlated with MEHP in our study. Although part of the normal gut flora, they are considered opportunistic pathogens and can lead to production of cytokines and other pro-inflammatory response molecules[83]. *Bifidobacterium* genus was negatively correlated with MEHP in this study. It is considered non-pathogenic and rarely causes infection in patients[84], which aligns with its inverse relationship with MEHP. *Streptococcus* was positively correlated with MEHHP. Exhibiting a dose-response behavior, prenatal exposure to MEHHP has been observed to be associated with an increased risk of miscarriage[85] and autistic traits in young children[86]. Previous reports have found gut microbiota-derived *Streptococcus* implicated in a variety of adverse health effects, such as join pain and inflammation[87] and gastric cancer[88], and bacterial infection of neonates[89], despite being found in the majority of healthy women. As both *Streptococcus* and MEHHP are negatively related to pregnancy and health-related outcomes, future studies can further investigate their synergistic effects and how novel interventions targeting these factors can mitigate their health impact.

Although this study is the first to analyze multiple urine phthalate biomarkers and gut microbiota within a pregnant AA cohort, there are some important limitations. First, this is a cross-sectional study, so temporality between phthalate exposure and gut microbiota changes cannot be established, and findings cannot reflect the effects of long-term exposure. Furthermore, much of phthalate exposure is via dermal and ingestion pathways; however, we were unable to assess dietary patterns or usage of phthalate-contaminated products or include

related covariates in our regression model, so results and conclusions from this study should be drawn with caution. There is also limited generalizability as this study analyzes a specific pregnant and racial demographic. Further research that incorporates racial diversity is required to understand maternal health effects of phthalates in a more heterogenous population. However, a strength of this study is that it explores the associations between phthalate exposure and the gut microbiota in a traditionally understudied population. Additionally, the rectal swabs were sequenced using 16S rRNA-specific primers, which limits resolution in taxonomic assignment, direct functional determination of the various taxa, and may have resulted in lower read counts compared to using stool samples or shotgun metagenomic sequencing. Lastly, to the best of our knowledge, this is the first study to investigate the impact of phthalate exposure and maternal gut microbiota in pregnant African American individuals, which helps to address current research gaps in the literature, but also is limited in corroborating evidence. Future studies should incorporate racial heterogeneity, or look at other individual racial groups, to determine if the associations and correlations from this study are observed in other subpopulations. Furthermore, in order to infer more accurately the functions of the gut microbiota and their role in phthalate exposure sequelae, the use of shotgun metagenomic sequencing can be employed rather than solely relying on 16S rRNA sequences. Lastly, as dietary patterns and plastic-product usage is another large component of phthalate exposure, future studies can incorporate more data collection coverage in those areas as another way to determine extent of phthalate exposure on a more granular level.

5. Conclusion

This study analyzed the cross-section of phthalate exposure and the gut microbiota in a traditionally understudied population, which bears a disproportionate exposure to phthalates. In

conclusion, there are several opportunistic pathogens whose relative abundance in the gut microbiota was positively associated with urinary phthalate metabolites, as well as multiple probiotic bacteria negatively associated with urinary phthalate metabolites. Although there was still some conflicting evidence with previous literature, this study highlights the potential for certain gut microbiota bacteria to be novel therapeutic targets to prevent or treat the harmful effects of phthalate exposure, specifically in the pregnant population, which would benefit not only maternal health, but also birth and infant health outcomes. Furthermore, the information gained from the study can aid future studies in elucidating a potential gut microbiota mechanism of phthalates in the human host.

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List of Tables

Table 1. Demographic and clinical characteristics table of 193 African American pregnant participants from Atlanta, GA across Visit 1 (N = 167) and Visit 2 (N = 123). Statistics are listed as mean ± SD or frequency (%). Wilcoxon (continuous) or chi-square (categorical) tests were performed to assess differences in distribution of characteristics across visits. $P < 0.05$ indicates significant difference at the 0.05 alpha level.

Characteristics	Visit 1 (N = 167)	Visit 2 (N = 123)	P-value
Prenatal BMI (kg/m^2)	28.87 ± 7.48	28.04 ± 7.17	0.39
Underweight (< 18.5)	3 (1.8%)	4 (3.3%)	
Healthy weight (18.5-24.99)	60 (35.9%)	47 (38.2%)	
Overweight (25.0-29.99)	40 (24.0%)	30 (24.4%)	
Obese (30+)	64 (38.3%)	42 (34.1%)	
Age at enrollment [years]	25.72 ± 4.65	25.73 ± 4.93	0.88
Education level			0.77
Less than high school	23 (13.8%)	16 (13.0%)	
High school	71 (42.5%)	48 (39.0%)	
Some college or more	73 (43.7%)	59 (48.0%)	
Insurance type			0.46
Low-income Medicaid	49 (29.3%)	40 (32.5%)	
Medicaid at start of pregnancy	84 (50.3%)	53 (43.1%)	
Private	34 (20.4%)	30 (24.4%)	
Prenatal or gestational oral antibiotic use			0.32
No	99 (59.3%)	80 (65.0%)	
Yes	68 (40.7%)	43 (35.0%)	
Parity			0.86
Nulliparous	71 (42.5%)	51 (41.5%)	
Multiparous	96 (57.5%)	72 (58.5%)	
Tobacco use in month prior to pregnancy			0.65
No	135 (80.8%)	102 (82.9%)	
Yes	32 (19.2%)	21 (17.1%)	
Alcohol use in month prior to pregnancy			0.71
No	149 (89.2%)	108 (89.1%)	
Yes	18 (10.8%)	15 (12.2%)	
Marijuana use in month prior to pregnancy			0.69
No	98 (58.7%)	75 (61.0%)	
Yes	69 (41.3%)	48 (39.0%)	

Note: BMI, body mass index

Table 2. Descriptive summary of urinary phthalate metabolite levels of 193 African American pregnant participants from Atlanta, GA across Visit 1 (N = 167) and Visit 2 (N = 123). Raw metabolite values were corrected for urinary creatinine and log normalized. P < 0.05 indicates significance at the 0.05 alpha level. A) Continuous phthalate statistics are listed as log(Mean) ± SD. Wilcoxon tests were performed to assess differences between urinary phthalate metabolite levels across visits. B) High and low exposure levels were assigned based on the median threshold of continuous phthalate metabolite data. Statistics are listed as counts (N) and frequency (%). Chi-square tests were performed to assess differences in distribution between high and low exposure groups across visits.

A. **Phthalate Metabolites**	**Visit 1** log(Mean) ± SD	**Visit 2** log(Mean) ± SD	**P-value**
Monoethyl phthalate, MEP	-0.19 ± 0.46	-0.17 ± 0.53	0.82
Mono-n-butyl phthalate, MBP	-1.24 ± 0.43	-1.16 ± 0.44	0.14
Monoisobutyl phthalate, MiBP	-1.28 ± 0.41	-1.24 ± 0.38	0.40
Monobenzyl phthalate, MBzP	-1.45 ± 0.45	1.44 ± 0.50	0.94
Mono(2-ethlyhexyl) phthalate, MEHP	-1.96 ± 0.53	-1.88 ± 0.53	0.13
Mono(2-ethyl-5-oxohexyl) phthalate, MEOHP	-1.67 ± 0.41	-1.64 ± 0.40	0.35
Mono(2-ethyl-5-hydroxyhexyl) phthalate, MEHHP	-1.46 ± 0.42	-1.52 ± 0.40	0.22
Mono(2-ethly-5-carboxypentyl) phthalate, MECPP	-1.28 ± 0.33	-1.26 ± 0.33	0.77
B. Phthalate Metabolite Exposure Levels	**Visit 1** N (%)	**Visit 2** N (%)	**P-value**
Monoethyl phthalate, MEP			0.18
High	79 (47.3%)	68 (55.3%)	
Low	88 (52.7%)	55 (44.7%)	
Mono-n-butyl phthalate, MBP			0.58
High	80 (47.9%)	63 (51.2%)	
Low	87 (52.1%)	60 (48.8%)	
Monoisobutyl phthalate, MiBP			0.35
High	79 (47.3%)	65 (52.8%)	
Low	88 (52.7%)	58 (47.2%)	
Monobenzyl phthalate, MBzP			0.88
High	84 (50.3%)	63 (51.2%)	
Low	83 (49.7%)	60 (48.8%)	
Mono(2-ethlyhexyl) phthalate, MEHP			0.01*
High	71 (42.5%)	72 (58.5%)	
Low	96 (57.5%)	51 (41.5%)	
Mono(2-ethyl-5-oxohexyl) phthalate, MEOHP			0.27
High	77 (46.1%)	65 (52.8%)	
Low	90 (53.9%)	58 (47.2%)	
Mono(2-ethyl-5-hydroxyhexyl) phthalate, MEHHP			0.27
High	87 (52.1%)	56 (45.5%)	
Low	80 (47.9%)	67 (54.5%)	
Mono(2-ethly-5-carboxypentyl) phthalate, MECPP			0.99
High	83 (49.7%)	61 (49.6%)	
Low	84 (50.3%)	62 (50.4%)	

Note: *P < 0.05; SD, standard deviation

Table 3. Alpha diversity across Visit 1 (N = 167) and Visit 2 (N = 123) in 193 African American pregnant participants from Atlanta, GA. No reads were excluded after initial rarefication to obtain the most representative measure of gut microbiota diversity. Wilcoxon test was used for statistical testing between groups. P < 0.05 indicates significant difference at the 0.05 alpha level. A) Descriptive summary of genus-level alpha diversity indices (Chao1, Shannon, and Inverse Simpson) of maternal gut microbiota samples across visits, listed using mean ± SD and median measurements. B) Shannon alpha diversity measurements across high and low phthalate metabolite exposure levels across visits.

A. Alpha Diversity Index	**Visit 1**		**Visit 2**		**P-value**
	Mean ± SD	Median	Mean ± SD	Median	
Chao1	12.1 ± 5.2	12	11.4 ± 4.9	11	0.3
Shannon	1.4 ± 0.5	1.5	1.4 ± 0.5	1.5	0.24
Inverse Simpson	3.5 ± 1.5	3.3	3.3 ± 1.4	3	0.2

B. Phthalate Metabolites	**Shannon Diversity Index**		**P-value**
	High Exposure Mean ± SD	**Low Exposure** Mean ± SD	
Visit 1			
MEP	1.64 ± 0.40	1.72 ± 0.43	0.07
MBP	1.71 ± 0.40	1.65 ± 0.43	0.42
MiBP	1.69 ± 0.40	1.68 ± 0.43	0.96
MBzP	1.69 ± 0.40	1.67 ± 0.43	0.98
MEHP	1.69 ± 0.40	1.67 ± 0.42	0.74
MEOHP	1.72 ± 0.45	1.68 ± 0.39	0.84
MEHHP	1.67 ± 0.45	1.69 ± 0.38	0.9
MECPP	1.69 ± 0.46	1.68 ± 0.37	0.63
Visit 2			
MEP	1.60 ± 0.44	1.66 ± 0.45	0.4
MBP	1.67 ± 0.46	1.58 ± 0.43	0.27
MiBP	1.71 ± 0.40	1.54 ± 0.47	0.06
MBzP	1.71 ± 0.48	1.54 ± 0.39	0.03*
MEHP	1.69 ± 0.45	1.58 ± 0.44	0.17
MEOHP	1.70 ± 0.40	1.55 ± 0.47	0.08
MEHHP	1.66 ± 0.42	1.57 ± 0.47	0.45
MECPP	1.62 ± 0.45	1.63 ± 0.44	0.92

Note: *P < 0.05; MEP, monoethyl phthalate; MBP, mono-n-butyl phthalate; MiBP, monoisobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono(2-ethlyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethly-5-carboxypentyl) phthalate

Table 4. Multivariate linear regression model results of the phylum-level gut microbiota associations with each phthalate metabolite as the main predictor. The model adjusted for prenatal BMI, maternal age, education level, insurance type, prenatal and gestational oral antibiotic use, parity, tobacco use, alcohol use, and marijuana use. Beta coefficients for each phthalate metabolite are reported along with their 95% confidence intervals and P-values. P < 0.05 indicates significance at the 0.05 alpha level.

	MEP		MBP		MiBP		MBzP		MEHP		MEOHP		MEHHP		MECPP	
Phylum	**β (95% CI)**	**P-value**	**β (95% CI)**	**P-value**	**β (95% CI)**	**P-value**	**β (95% CI)**	**P-value**	**β (95% CI)**	**P-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
Visit 1																
Actinobacteria	-0.04 (-0.44, 0.36)	0.85	0.13 (-0.32, 0.57)	0.58	-0.16 (-0.63, 0.30)	0.49	-0.27 (-0.70, 0.16)	0.22	-0.26 (-0.61, 0.09)	0.14	0.001 (-0.44, 0.45)	1	0.03 (-0.40, 0.46)	0.91	0.03 (-0.53, 0.58)	0.93
Bacteroidota	0.23 (-0.02, 0.47)	0.07	0.12 (-0.16, 0.40)	0.40	0.17 (-0.12, 0.45)	0.25	0.13 (-0.14, 0.39)	0.34	0.16 (-0.06, 0.37)	0.16	0.14 (-0.14, 0.41)	0.33	0.14 (-0.12, 0.41)	0.29	0.14 (-0.20, 0.48)	0.42
Campylobacterota	-0.08 (-0.48, 0.33)	0.71	0.22 (-0.24, 0.67)	0.35	0.12 (-0.35, 0.59)	0.61	-0.14 (-0.57, 0.30)	0.53	-0.14 (-0.50, 0.22)	0.44	-0.11 (-0.56, 0.34)	0.62	-0.22 (-0.65, 0.22)	0.32	-0.09 (-0.66, 0.47)	0.74
Firmicutes	0.02 (-0.11, 0.15)	0.74	-0.003 (-0.15, 0.14)	0.96	-0.01 (-0.16, 0.14)	0.88	-0.04 (-0.18, 0.09)	0.54	0.01 (-0.10, 0.12)	0.84	0.02 (-0.12, 0.17)	0.74	0.07 (-0.07, 0.20)	0.35	0.08 (-0.10, 0.26)	0.39
Fusobacteriota	-0.23 (-0.62, 0.17)	0.26	0.24 (-0.21, 0.68)	0.29	0.05 (-0.41, 0.51)	0.84	0.35 (-0.08, 0.77)	0.11	0.13 (-0.22, 0.48)	0.47	0.007 (-0.44, 0.45)	0.98	0.04 (-0.39, 0.46)	0.86	-0.01 (-0.57, 0.54)	0.96
Proteobacteria	0.14 (-0.23, 0.52)	0.45	0.39 (-0.02, 0.80)	0.07	0.37 (-0.06, 0.80)	0.09	0.06 (-0.34, 0.47)	0.75	0.08 (-0.25, 0.41)	0.65	0.09 (-0.33, 0.50)	0.68	0.02 (-0.38, 0.43)	0.91	-0.18 (-0.70, 0.34)	0.49
Verrucomirobiota	-0.26 (-0.53, 0.002)	0.05	-0.17 (-0.46, 0.13)	0.27	-0.35 (-0.65, -0.06)	0.02*	-0.27 (-0.55, 0.01)	0.06	-0.07 (-0.31, 0.16)	0.53	-0.12 (-0.41, 0.17)	0.42	-0.15 (-0.43, 0.13)	0.30	-0.15 (-0.52, 0.20)	0.42
Visit 2																
Actinobacteria	-0.18 (-0.73, 0.38)	0.52	0.25 (-0.45, 0.95)	0.47	-0.46 (-1.12, 0.20)	0.17	0.24 (-0.40, 0.88)	0.46	-0.11 (-0.62, 0.40)	0.68	-0.09 (-0.75, 0.57)	0.79	0.06 (-0.59, 0.71)	0.86	0.12 (-0.63, 0.87)	0.75
Bacteroidota	0.16 (-0.17, 0.49)	0.34	0.07 (-0.35, 0.49)	0.73	0.53 (0.14, 0.91)	0.01*	0.19 (-0.20, 0.57)	0.33	0.31 (0.02, 0.61)	0.04*	0.29 (-0.10, 0.68)	0.14	0.27 (-0.11, 0.65)	0.16	0.3 (-0.15, 0.74)	0.19
Campylobacterota	0.05 (-0.55, 0.65)	0.87	0.38 (-0.37, 1.14)	0.32	0.236 (-0.49, 0.96)	0.52	0.52 (-0.16, 1.21)	0.13	0.54 (-0.002, 1.07)	0.05	0.464 (-0.24, 1.17)	0.19	0.35 (-0.35, 1.05)	0.32	0.06 (-0.76, 0.87)	0.89
Firmicutes	-0.01 (-0.19, 0.16)	0.87	-0.01 (-0.22, 0.21)	0.95	-0.13 (-0.34, 0.07)	0.21	-0.04 (-0.24, 0.16)	0.71	-0.06 (-0.22, 0.10)	0.46	-0.07 (-0.27, 0.13)	0.50	0.01 (-0.19, 0.21)	0.93	-0.01 (-0.24, 0.22)	0.94
Fusobacteriota	-0.06 (-0.58, 0.46)	0.82	-0.1 (-0.76, 0.56)	0.75	-0.1 (-0.73, 0.53)	0.75	-0.09 (-0.69, 0.52)	0.76	-0.14 (-0.62, 0.34)	0.56	0.25 (-0.36, 0.87)	0.41	0.29 (-0.31, 0.90)	0.34	0.37 (-0.34, 1.07)	0.30
Proteobacteria	0.03 (-0.46, 0.51)	0.91	0.12 (-0.50, 0.73)	0.71	-0.32 (-0.90, 0.26)	0.28	-0.14 (-0.70, 0.42)	0.62	0.49 (0.06, 0.93)	0.03*	0.3 (-0.27, 0.873)	0.30	0.45 (-0.11, 1.01)	0.11	0.57 (-0.08, 1.22)	0.08

Note: *P < 0.05; MEP, monoethyl phthalate; MBP, mono-n-butyl phthalate; MiBP, monoisobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono(2-ethlyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethly-5-carboxypentyl) phthalate

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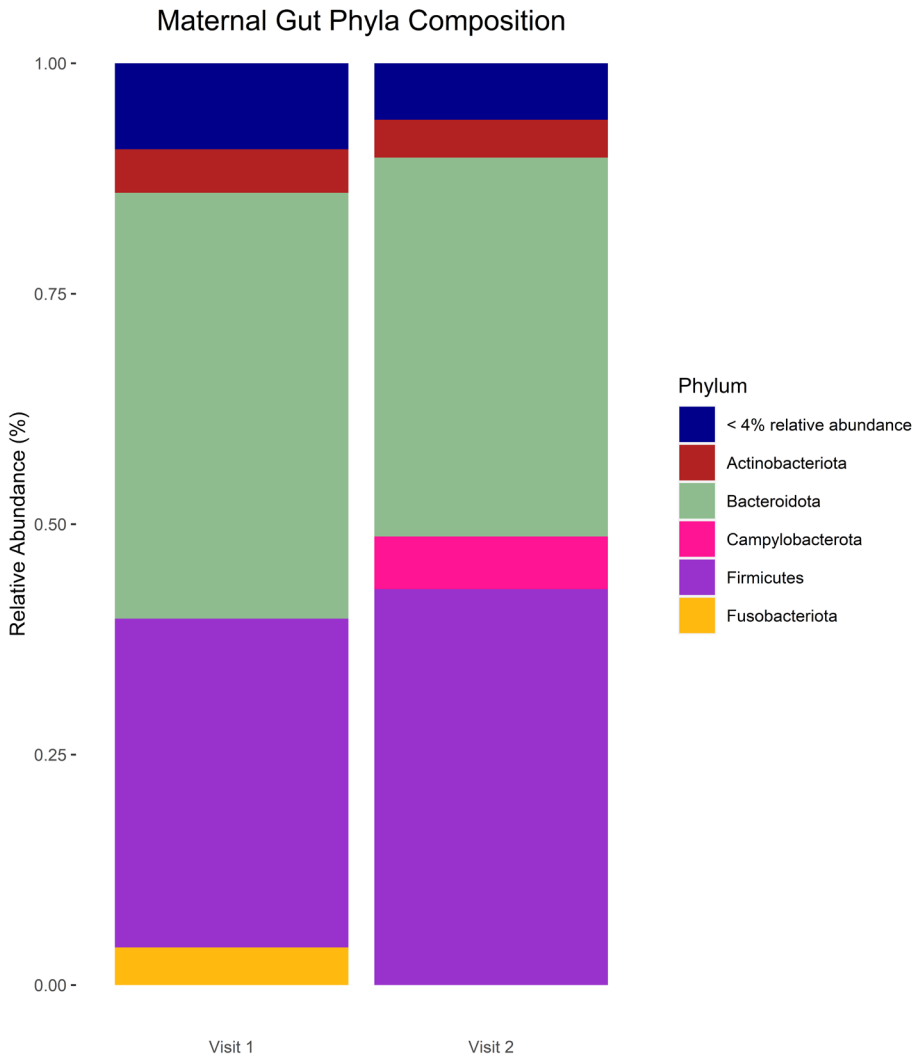

Figure 1. Summarized maternal gut phyla composition of 193 African American pregnant participants from Atlanta, GA across visit 1 (N = 167) and Visit 2 (N = 123). Taxa with less than 4% relative abundance were grouped together at each visit.

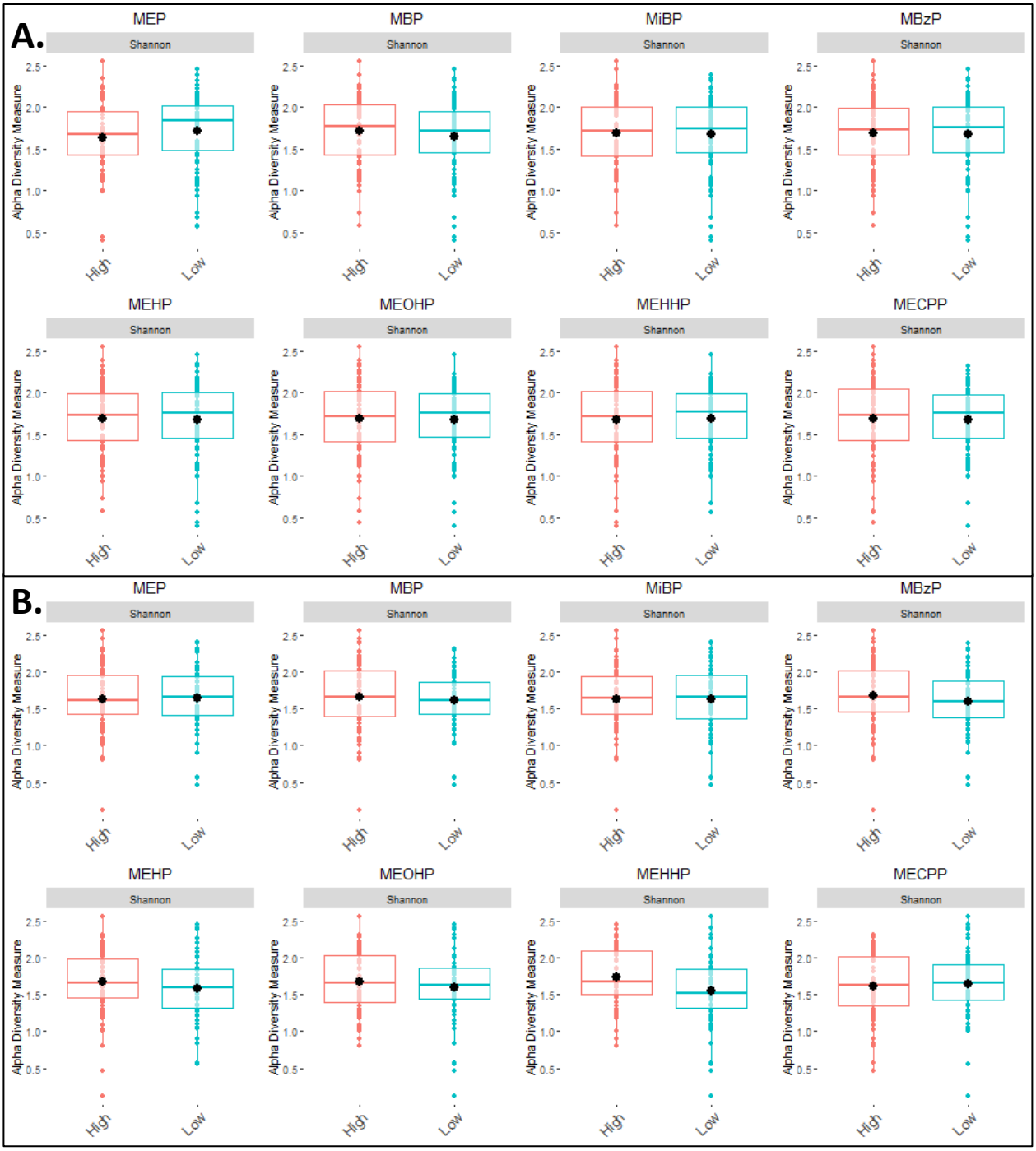

Figure 2. Box plots of genus-level Shannon diversity index measurements across high and low phthalate metabolite exposure among maternal fecal samples collected at A) Visit 1 (N = 167) and B) Visit 2 (N = 123). MEP, monoethyl phthalate; MBP, mono-n-butyl phthalate; MiBP, monoisobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono(2-ethlyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethly-5-carboxypentyl) phthalate.

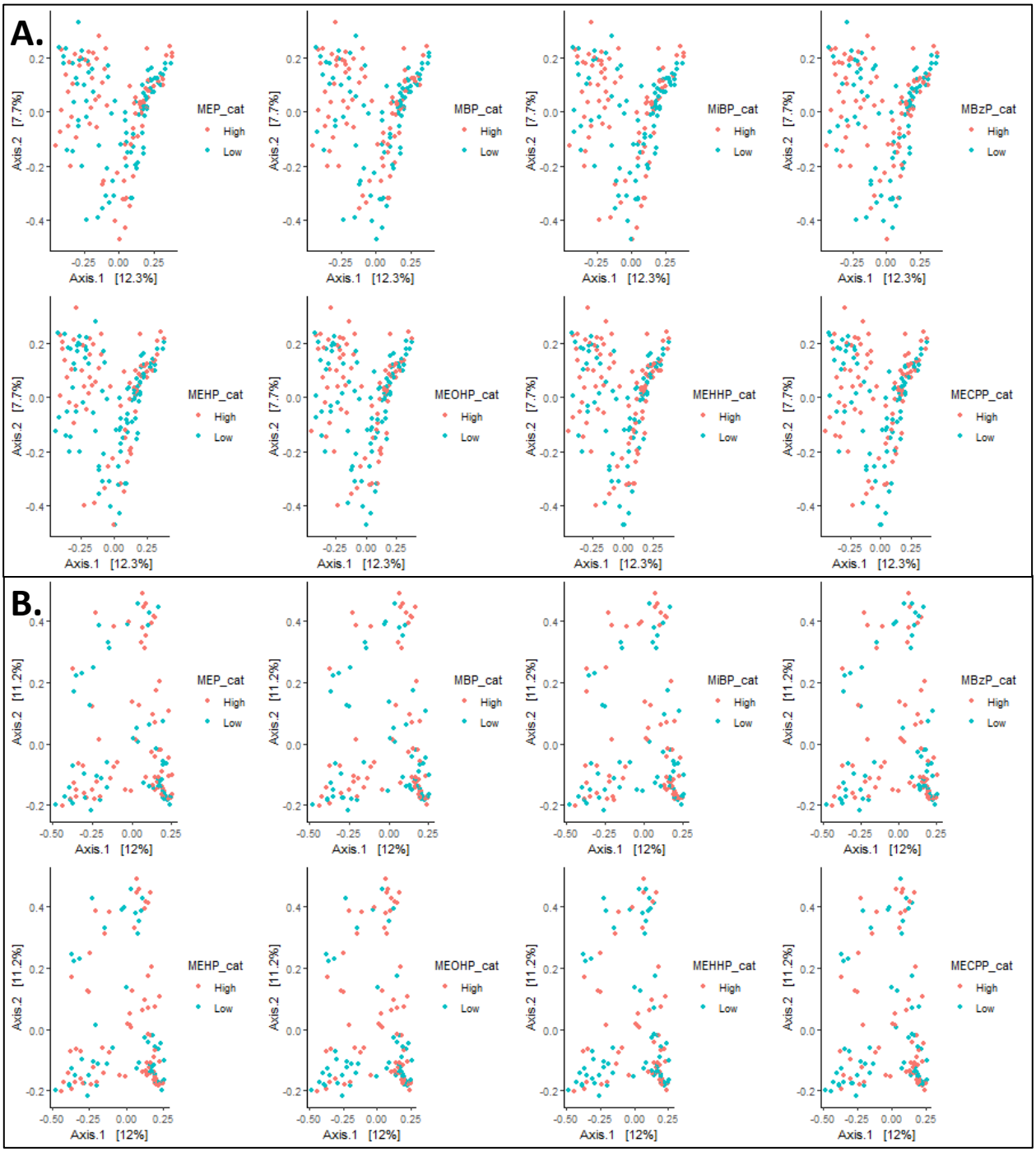

Figure 3. Principal coordinate analysis (PCoA) plot of Bray-Curtis dissimilarity index according to high and low urinary phthalate metabolite exposure among maternal fecal samples collected at A) Visit 1 (N = 167) and B) Visit 2 (N = 123). MEP, monoethyl phthalate; MBP, mono-n-butyl phthalate; MiBP, monoisobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono(2-ethlyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethly-5-carboxypentyl) phthalate.

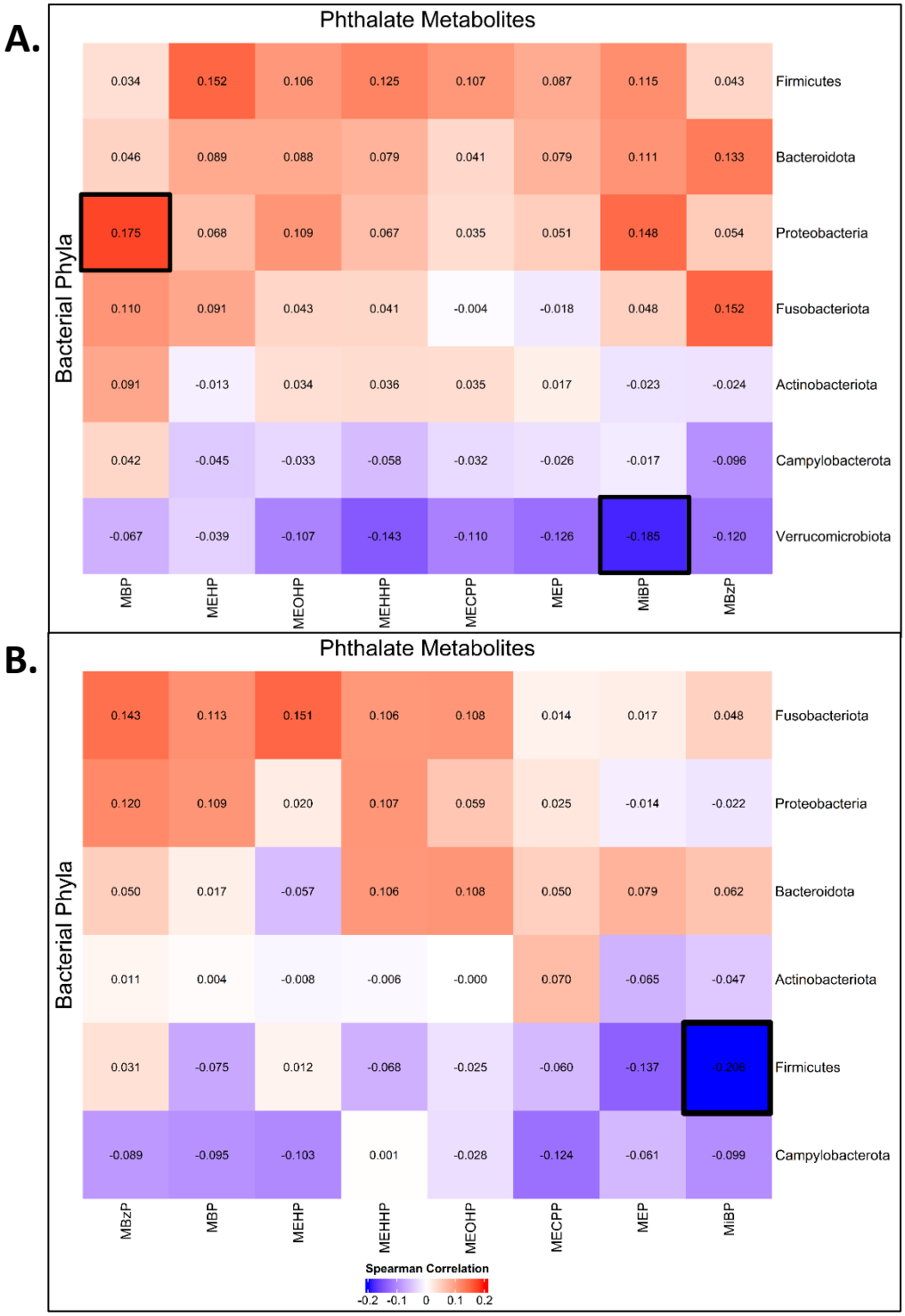

Figure 4. Spearman's rho correlation coefficients between phylum-level gut microbiota and phthalate metabolites at A) Visit 1 (N = 167) and B) Visit 2 (N = 123). Blue indicates negative correlations; red indicates positive correlations. P-values were approximated by using the t or F distributions and correlations are considered significant at $P < 0.05$, indicated by boxed outlines. MEP, monoethyl phthalate; MBP, mono-n-butyl phthalate; MiBP, monoisobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono(2-ethlyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethly-5-carboxypentyl) phthalate.

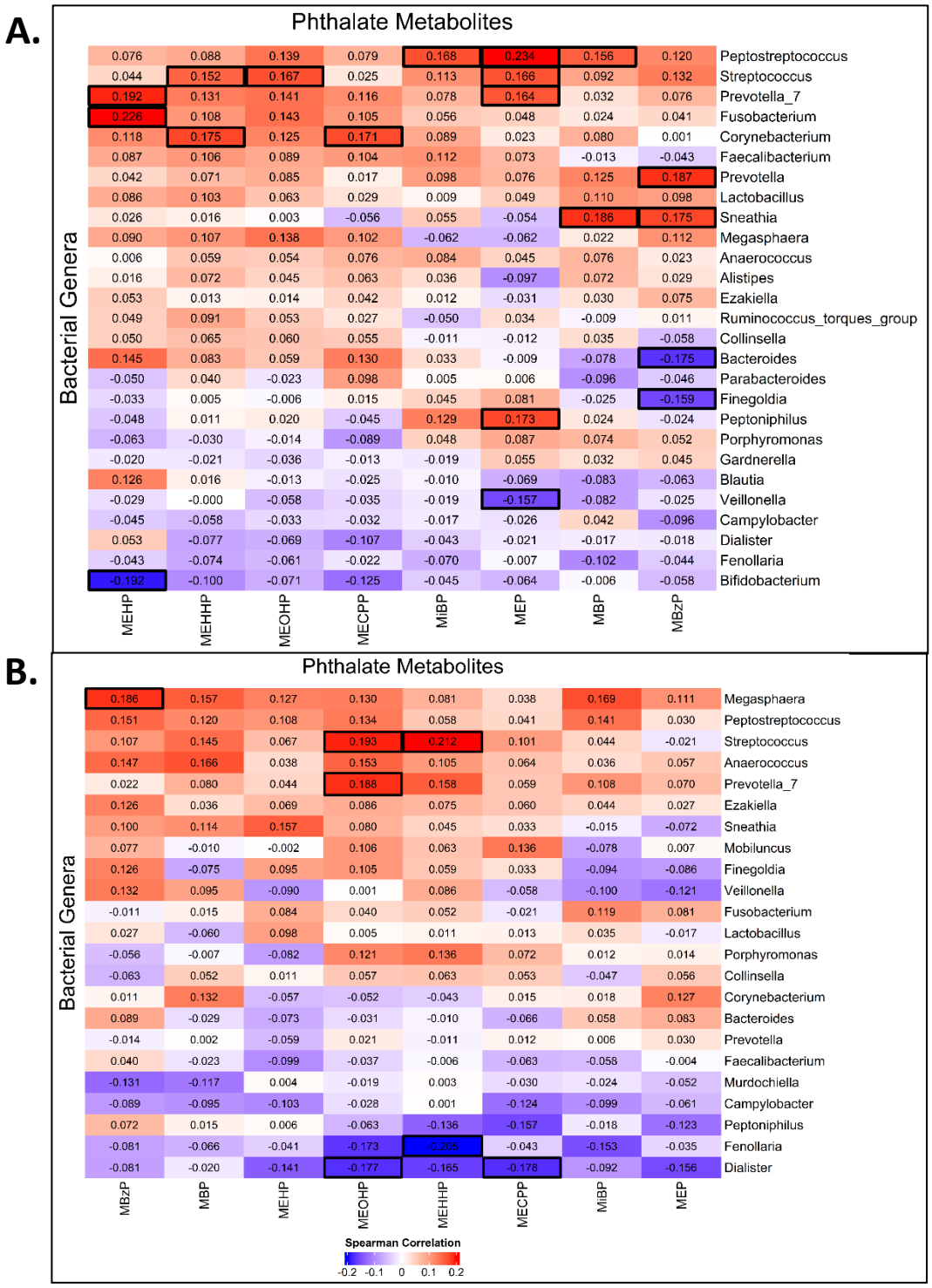

Figure 5. Spearman's rho correlation coefficients between genus-level gut microbiota and phthalate metabolites at A) Visit 1 (N = 167) and B) Visit 2 (N = 123). Blue indicates negative correlations; red indicates positive correlations. P-values were approximated by using the t or F distributions and correlations are considered significant at $P < 0.05$, indicated by boxed outlines. MEP, monoethyl phthalate; MBP, mono-n-butyl phthalate; MiBP, monoisobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono(2-ethlyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethly-5-carboxypentyl) phthalate.

Supplementary Figure

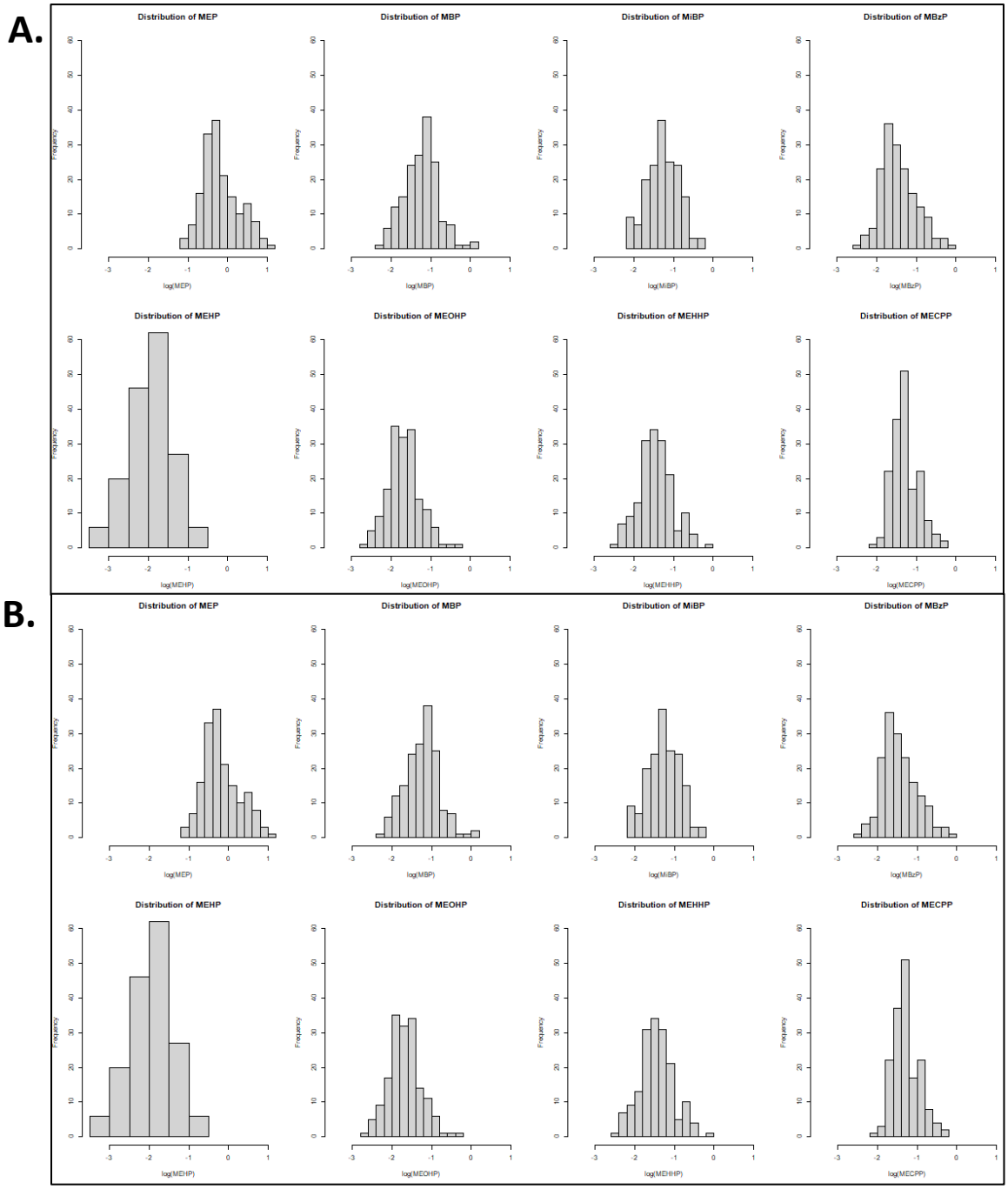

Figure S1. Distribution of urinary phthalate metabolites in African American pregnant participants from Atlanta, GA across A) Visit 1 (N = 167) and B) Visit 2 (N = 123) after creatinine correction and log normalization. MEP, monoethyl phthalate; MBP, mono-n-butyl phthalate; MiBP, monoisobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono(2-ethlyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate; MECPP, mono(2-ethly-5-carboxypentyl) phthalate.