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Influence of Early Life Factors on the Breast Milk and Fecal Microbiota of Mother-Newborn Dyads

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Abstract: Maternal gut and breast milk (BM) are key in vertically transmission bacteria to infants, shaping their gut microbiota in early life. Although the establishment of early gut microbiota is known, the role of the combined influence of maternal factors and newborn characteristics are not explored. In this study we aimed to assess the influence of maternal BMI and total body fat, age, delivery mode, and newborn sex on the diversity and composition of the BM and gut microbiota (GM) in mother- newborn dyads. In this cross-sectional study, of the 986 pregnant women candidates, 53 participated, and finally, 40 mother-newborn dyads exclusively breastfeeding at 20-28 days post-partum were included. Metataxonomic profiling of DNA extracted from BM and fecal samples was conducted using 16S rRNA sequencing. Globally, the findings offer valuable insights that excessive adiposity, age and C-section delivery influence on a lower abundance of specific taxa in the BM, maternal gut, and gut newborns. Also, the simultaneous analysis of maternal factors and newborn characteristics shows that maternal age and newborn sex explain an important variation in the microbiota composition. These results add to understanding of the intricate interplay between maternal factors and the microbial communities that influence early-life gut and BM microbiota.

Keywords: mom-newborn dyad; gut microbiota; breast milk; newborn sex; age; nutrition status; delivery mode; early-life microbiota

1. Introduction

The neonatal period is a crucial stage for the establishment of the gut microbiota (GM), which plays a key role in short- and long-term health outcomes [1]. The first significant exposure to microbiota is during birth when a neonate ingests native microorganisms from the maternal vagina and gut [2,3]. Subsequent exposure to breast milk (BM) provides additional bacteria diversity, possibly from several sources, including the maternal skin, newborn oral cavity, or maternal gut via the entero-mammary pathway [4,5]. While previous studies have investigated the influence of factors like maternal nutritional status and delivery mode on GM and BM microbiota diversity [6], evidence remains limited for others like maternal age and newborn sex.

Obesity has been associated with GM imbalance, potentially affecting BM microbiota [7–9]. Consequently, newborns born to mothers with obesity may inherit an altered microbiota characterized by reduced diversity, decreased *Bifidobacterium* abundance, and increased

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Staphylococcus abundance, predisposing them to childhood obesity [10]. Additionally, emerging evidence suggests that maternal age can shape the composition of pregnant women's GM, with women over 35 years exhibiting an increase in the opportunistic *Prevotella bivia* compared to younger women [11]. Furthermore, BM from women over 30 years tends to display greater microbiota diversity than BM from younger women [12,13]. Delivery mode also impacts maternal gut [14], and BM microbiota [15,16], and it is a well-known effect on newborn GM, infants born via C-section displayed a GM resembling maternal skin microbiota [17].

Moreover, recent evidence suggests that sexual dimorphism plays a role in BM microbiota and neonatal GM. Less diversity has been observed in BM intended for male neonates, with a higher abundance of *Streptococcus* bacteria [17,18]. Similarly, male neonates tend to harbor less diverse GM and display a higher abundance of Streptococcaceae family bacteria [19].

However, existing knowledge on these factors comes primarily stems from individual studies, with limited information on maternal age and newborn sex. Given the multifaceted interplay of these factors, it is necessary to evaluate them collectively. Therefore, the present study aimed to assess how maternal BMI, total body fat, age, delivery mode, and newborn sex collectively influence GM and BM diversity and composition in mother-newborn dyads. By elucidating these relationships, the study seeks to provide valuable insights into the determinants of both maternal and newborn gut microbiota, as well as breast milk microbiota composition.

2. Materials and Methods

2.1. Study Design and Population

This cross-sectional study recruited pregnant women from the *Instituto Mexicano del Seguro Social* (IMSS). Inclusion criteria comprised women aged 18 to 35 years, first-time mothers without chronic diseases, and those experiencing uncomplicated pregnancy. Newborns included were at term delivery (≥ 37 weeks gestation), weighed ≥ 2,500 g at birth, and exclusive breastfeeding. Participants with mastitis symptoms, newborns with clinical conditions, those using mixed feeding, antibiotic treatment within 15 days before sample collection, or probiotic supplementation during the sampled period were excluded. The study was conducted in accordance with the Declaration of Helsinki ant the National Commission for Scientific Research of IMSS (R-2017-785-055) approved this study. Written informed consent was obtained from each participant prior to sample collection. The flowchart detailing participants' eligibility assessment and sample analysis is detailed in Figure S1 described in Supplementary Material.

Forty participants were stratified based on primarily 1) maternal body index (BMI) at 20-28 days post-partum (20-28DPP) categorized as normal weight vs. overweight/obesity per World Health Organization guidelines [20] and total body fat mass grouped as adequate body fat < 30 % vs. excessive body fat ≥ 30 %. Other clinical variables were also used, like 2) maternal age (young ≤ 30 vs. mature > 30 years), 3) delivery mode (vaginal vs. C-section), and 4) newborn sex (female vs. male). The maternal age categories were chosen because currently, there are increasing numbers of women who delay childbearing, and around the World, the mean age of women at the birth of their first child has crossed the 30 years threshold [21,22].

2.2. Procedures

A home visit was scheduled between 20-28DPP, during which participants completed a questionnaire providing demographic data. BM was expressed under aseptic conditions using an electric breast pump (Medela Lactina 0162011, Medela, U.S.A.), with nipple and areola cleaning with a 0.5 % chlorhexidine solution (Famicare, Laboratorio Boniquet de México, México) before expression. The BM samples from both breasts were collected in sterile glass bottles after mixing; a 15 mL aliquot was transferred into a sterile conical tube (Axygen Scientific, U.S.A.) and stored at 4 °C for transportation. Fecal samples were self-collected by participants using an OMNIgene Gut kit (DNAgenotek, Canada) and stored at ambient temperature. At the laboratory, both BM and fecal samples were frozen at -80 °C until microbiota composition analysis.

During the home visit, trained clinical personnel conducted anthropometric measurements of the mother and newborn, as detailed in Supplementary material.

2.3. 16S rRNA Gene Sequencing and Data Processing

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DNA extraction from BM and fecal samples was followed by amplification of the V4 region of the bacterial 16S rRNA gene and sequencing on the Illumina MySeq 2x250-bp platform. Data processing for BM and GM composition and diversity was conducted as outlined in the Supplemental Material.

2.4. Statistical Analysis

Statistical analysis was performed using R software (version 4.1.2; R Core Team 2021) in the RStudio environment (version 1.4.1717, RStudio team 2021). The Gaussian distribution of anthropometric parameters and alpha diversity were computed using the Shapiro-Wilk test. Alpha diversity between groups was compared using parametric T-test or non-parametric U Mann-Whitney test. Significant differences in beta diversity between groups were determined by permutational multivariate analysis of variance (PERMANOVA) with 1,000 permutations. Variation of community structure explained by the maternal and newborn characteristics was depicted using the Vegan package *envfit* function. According to Lefse analysis, genera with an LDA score above a threshold of 2.0 were considered differentially abundant. Statistical significance was set at a $p < 0.05$.

Eleven participants in each group of normal weight or overweight/obesity were estimated to provide 80 % study power to identify a difference of 11.2 % of abundance in Firmicutes with an assumption of a Standard Deviation (SD) of 9.2 % with an α-value = 0.05 according to the paper of Verdam *et al*. 2013 [23]. However, we decided to augment the sample size and included more lactating women to reach almost twice the calculated sample size and exploring the data analysis.

3. Results

3.1. Demographic Characteristics

Table 1 summarizes the demographic characteristics of the participants. The median age at enrollment was 29.6 years, with a mean pregnancy body weight gain of 10.51 kg. According to BMI, over half of the participants were classified as overweight or obese before pregnancy and at 20-28DPP. However, considering total body fat, a larger proportion (72 %) of women exhibited excessive body fat at 20-28DPP. Vaginal delivery occurred in approximately 53 % of the mothers, with female newborns accounting for 45 % of the total sample.

Table 1. Demographic characteristics of the mother-newborn dyad participants.

Characteristics	Mean ± SD or median (minimum – maximum)
Maternal	
Age (y)	29.60 (19 – 35)
Height (m)	1.59 ± 0.06
Body weight (kg)	
Pre-gestational	63.74 ± 10.51
20-28PPD	64.75 ± 10.64
Last gestational	74.25 ± 10.39
Gain during pregnancy	10.51 ± 4.59
BMI (kg/m^2)	
Pre-gestational	25.3 ± 3.99
20-28PPD	25.71 ± 4.07
Total body fat (%)	33.03 ± 6.44
Delivery mode (V/C, %)	53%/47%
Newborn	
Gestational age (weeks)	39.3 ± 1.08
Length (cm)	
At birth	50 (48 – 53)
At 20-28PND	52.32 ± 1.79
Gain at 20 20-28PND	2.27 ± 1.67
Body weight (kg)	

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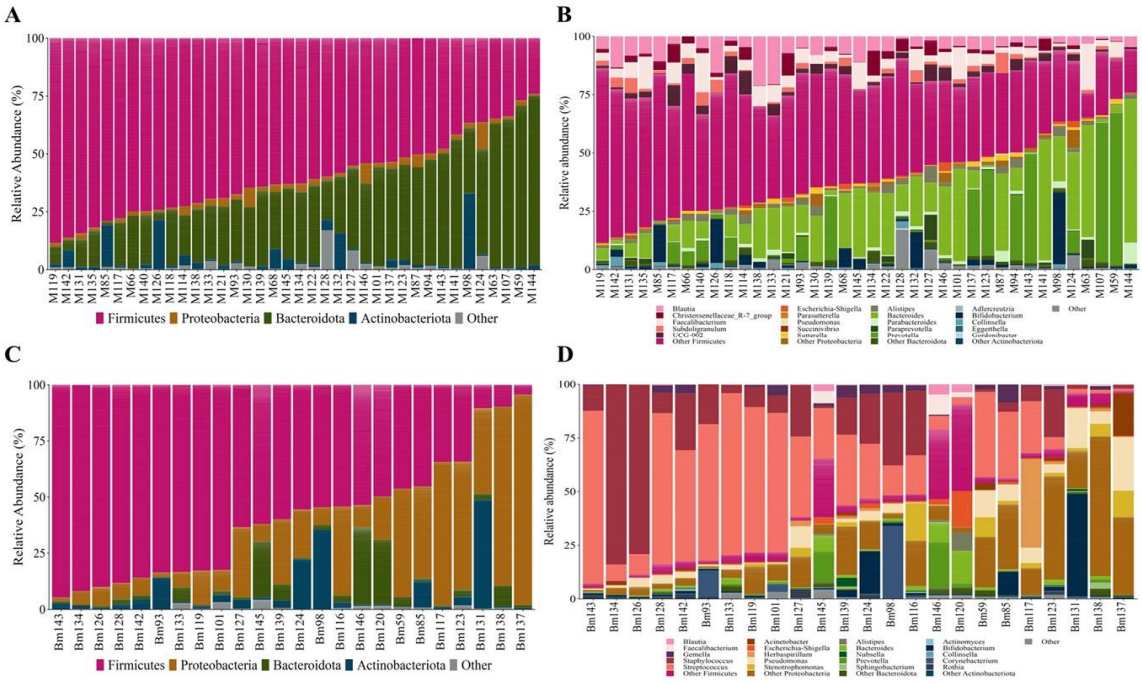
At birth	3.07 ± 0.33
At 20-28PND	3.81 ± 0.46
Gain at 20-28PND [1]	0.74 ± 0.47
Head circumference (cm)	36.55 ± 1.01
Newborn sex F/M (%)	45/55

Data were analyzed and expressed as mean ± standard deviation for normally distributed data or median (minimum–maximum) for non-normal distribution data, as appropriate. BMI: body mass index; C: cesarean; F: female; M: male; PND: Postnatal day; PPD: Post-partum day; SD: Standard Deviation; V: vaginal; N = 40. [1] Gain at 20-28PND = At 20-28PND - at birth.

3.2. Microbial Composition

The maternal gut microbiota comprised 15 distinct phyla, Firmicutes and Bacteroidota accounting for 91.7 % of the reads; minor phyla included Actinomicrobiota, Proteobacteria, and Verrucomicrobiota, among others (Figure 1A & Supplementary Table 1). Out of the 304 genera detected, those belonging to the Firmicutes phylum were most prevalent (Figure 1B & Supplementary Table 2). The maternal GM core included genera such as *Blautia*, *Bacteroides*, *Faecalibacterium*, *Dorea*, *Anaerostipes*, *Fusicatenibacter*, *Bifidobacterium*, *Parabacteroides*, *Coprococcus* and *Escherichia-Shigella*.

The BM microbiota comprised 23 phyla, with Firmicutes dominant, followed by Proteobacteria, Actinomicrobiota, and Bacteroidota (Figure 1C & Supplementary Table 3). Among the 402 genera that comprise the BM microbiota (Figure 1D & Supplementary Table 4), the most abundant were *Streptococcus* and *Staphylococcus*, both belonging to the Firmicutes phylum. The BM core included *Streptococcus*, *Staphylococcus*, *Escherichia-Shigella*, *Bifidobacterium*, and *Gemella*.



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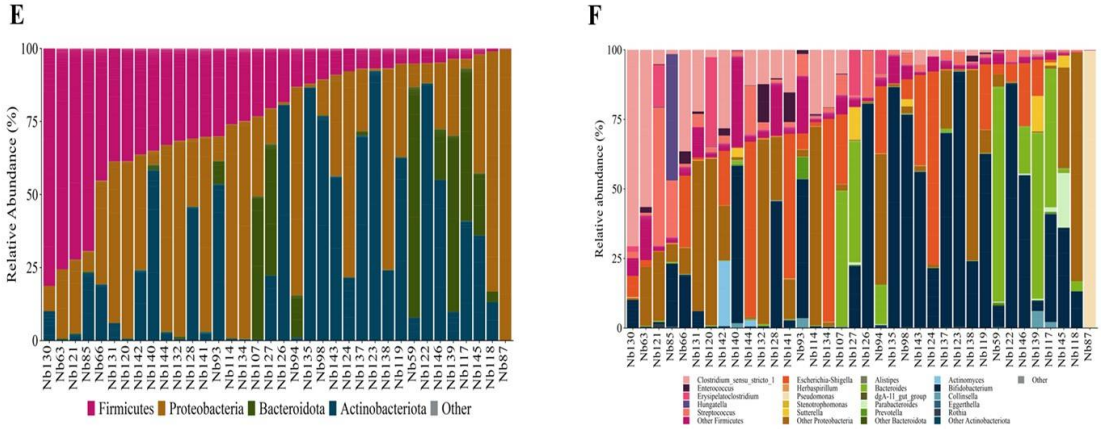

Figure 1. Microbiota composition from the maternal gut at the phylum level (A) and at the genus level (B), from BM at the phylum level (C) and at the genus level (D), and from the newborn gut at the phylum level (E) and genus level (F). Relative abundance bar plot of each sample at the phylum or genus levels. The vertical axis represents the relative abundance, and the horizontal axis is the sample code of the participant. M: Mother; Bm: Breast Milk; Nb: Newborn. Genera belonging to the Firmicutes are shown in pink color, Proteobacteria in orange color, Bacteroidota in green color, Actinobacteria in blue, and other distinct genera color are shown in gray color.The newborn GM was primarily made up of the phyla Proteobacteria, Actinomicrobiota, Firmicutes, and Bacteroidota comprising the 99.95 % of the composition (Figure 1E & Supplementary Table 5). The most abundant genera were *Bifidobacterium*, *Escherichia-Shigella*, *Pseudomonas*, and notably other genera from the Proteobacteria phylum, with abundances under 1 %, but collectively comprising 18.26% of the total composition (Figure 1F & Supplementary Table 6). The neonatal GM core detected consisted of *Bifidobacterium*, *Escherichia-Shigella*, *Streptococcus*, and *Staphylococcus*.

3.3. Factors Influencing Mother-Newborn Gut and BM Microbiota Composition

3.3.1. Maternal BMI and Total Body Fat

Maternal BMI did not significantly affect the richness and diversity of the mothers' and newborns' GM and BM microbiota. Nevertheless, mothers GM with excessive adiposity had a lower abundance of certain genera, such as *Fusobacterium*, along with a higher abundance of bacteria belonging to the Firmicutes phylum (Figure 2A). Moreover, BM microbiota from mothers with excessive adiposity (Figure 2B) displayed a lower abundance of specific genera belonging to the Proteobacterium phylum, accompanied by a diminished abundance of fatty acid-producing bacteria like *Faecalibacterium*, *Anaerostipes*, and *Butyrivibrio*. Furthermore, we notice that newborns born to mothers with excessive adiposity (Figure 2C) showed a lower abundance of core member *Staphylococcus*.

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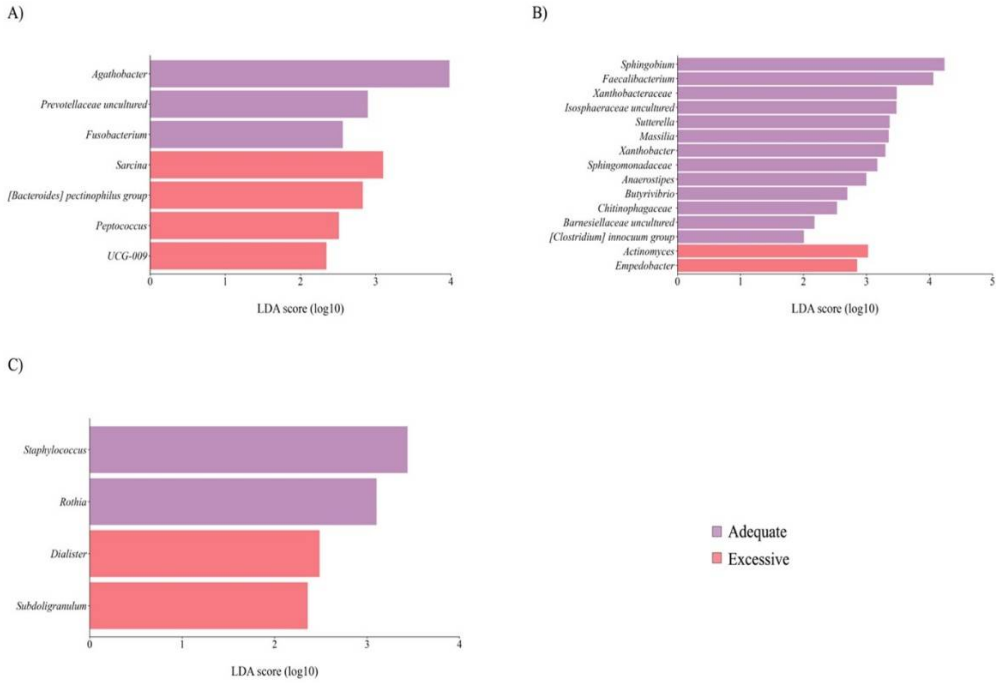

Figure 2. Taxon difference between adequate and excessive maternal total body fat groups. Linear discriminant analysis effect size of maternal fecal (A), breast milk (B), and newborn fecal (C) samples according to maternal total body fat. Pink color bars represent differentially abundant taxa in the adequate adiposity group, while purple color bars represent differentially abundant taxa in the excessive maternal adiposity group. Taxa with significant differences and a minimum linear discriminant analysis (LDA) score of 2.0 are shown.

3.3.2. Maternal Age

No differences in the richness and diversity of maternal GM and BM microbiota were found. However, a significant clustering in maternal GM was noted using the weighted UniFrac distance (R^2 = 0.09, P = 0.01). Mature women exhibited a distinct microbial profile with enrichment of core member *Bacteroides* and other genera belonging to Bacteroidota phylum, while younger women showed enrichment of certain genera from the Firmicutes phylum, including *Clostridium sensu stricto 1* and *Roseburia*, among others (Figure 3A). Subtle changes in BM microbiota were observed, with a higher abundance of minority genera like lactic acid bacteria *Leuconostoc* and the archaeal *Methanobrevibacter* in BM from mature women (Figure 3B). Notably, newborns GM from mature mothers showed lower richness (P = 0.01) and a trend towards lower diversity (P = 0.06) compared to neonates from younger women. Although no distinction between beta diversity was observed, the GM of newborns from mature women displayed a lack of enrichment in any taxa and instead had a decreased abundance of Bacteroidales order, including the prevalent genus *Bacteroides* compared to those from younger mothers (Figure 3C).

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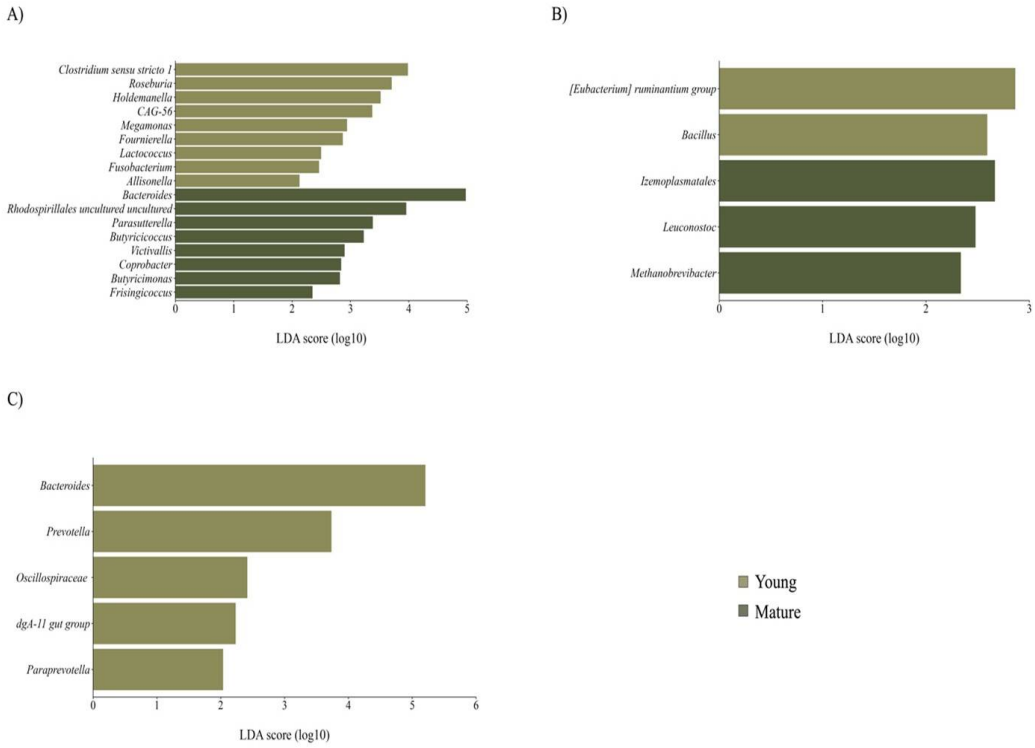

Figure 3. Taxon difference between young and mature age groups. Linear discriminant analysis effect size of maternal fecal (A), BM (B), and newborn fecal (C) samples according to maternal age. Light green bars represent differentially abundant taxa in the young age group, while dark green bars represent differentially abundant taxa in the mature age group. Taxa with significant differences and a minimum linear discriminant analysis (LDA) score of 2.0 are shown.

3.3.3. Delivery Mode

While delivery mode did not affect the richness, diversity, or overall structure of the maternal GM and BM microbiota, women who had undergone cesarean delivery were associated with specific alterations in maternal and BM microbiota composition. Women who underwent C-section exhibited a lower abundance in genera from the *Ruminococcaceae* family, accompanied by an enrichment observed in a minor genus such as *Erysipelatoclostridium* compared to those women with vaginal delivery (Figure 4A). In addition, BM from women with C-section delivery showed a reduced abundance of the minority genera belonging to the Firmicutes (Figure 4B). Regarding newborn's GM, while delivery mode did not affect richness, it influenced diversity ($P < 0.01$) and the community structure (unweighted UniFrac: $R^2 = 0.04$, $P = 0.01$ and weighted UniFrac: $R^2 = 0.08$, $P < 0.01$). While GM of C-section newborns indicated higher abundances of bacteria from order Oscillospirales, those born vaginally exhibited a higher abundance of bacteria from the *Lachnospiraceae* family (Figure 4C).

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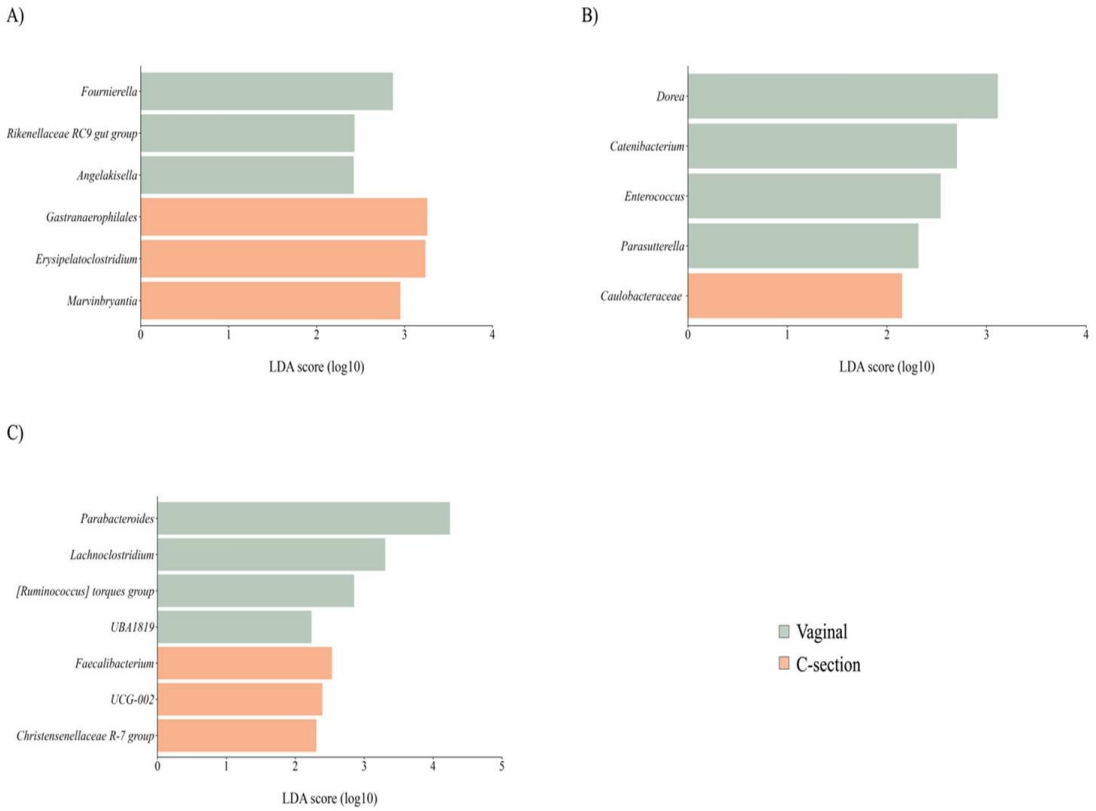

Figure 4. Taxon difference between vaginal and C-section delivery mode groups. Linear discriminant analysis effect size of maternal fecal (A), BM (B), and newborn fecal (C) samples according to delivery mode. Green color bars represent differentially abundant taxa in the vaginal delivery group, while orange bars represent differentially abundant taxa in the C-section delivery group. Taxa with significant differences and a minimum linear discriminant analysis (LDA) score of 2.0 are shown.

3.3.4. Newborn Sex

Newborn sex did no impact alpha diversity of maternal gut, BM, or newborn gut microbiota. However, differential analysis revealed distinct microbial profiles. The maternal GM of female newborns displayed higher abundance in different minority genera, such as *Stenotrophomonas* and *Paludicola* (Figure 5A). In BM samples from mothers with male newborns, there was an enrichment in the core member *Bifidobacterium* (Figure 5B), among others. Finally, we identified differences in composition between females and males in their gut microbiota (Figure 5C).

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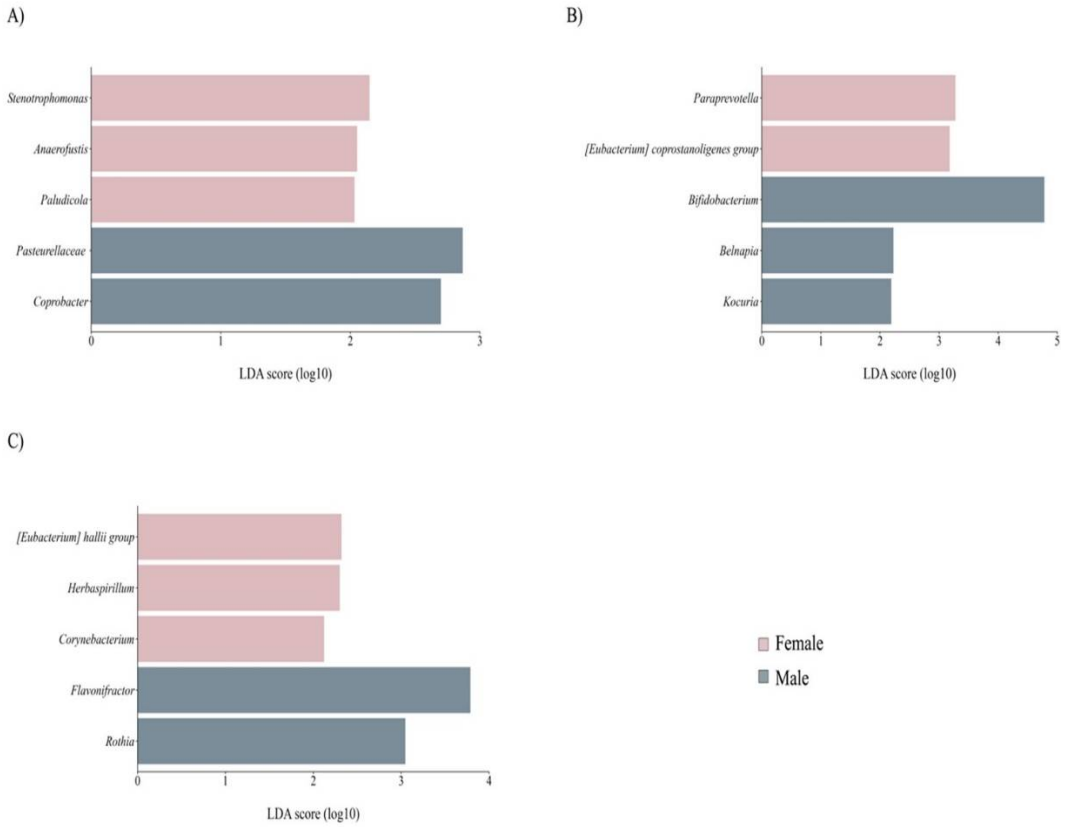

Figure 5. Taxon difference between female and male newborn sex groups. Linear discriminant analysis effect size of maternal fecal (A), BM (B), and newborn fecal (C) samples according to delivery mode. Pink color bars represent differentially abundant taxa in the female newborn sex group, while orange color bars represent differentially abundant taxa in the male newborn sex group. Taxa with significant differences and a minimum linear discriminant analysis (LDA) score of 2.0 are shown.

3.4. Exploring the Impact of Maternal and Neonatal Factors on GM and BM Microbiota

Multivariate analysis revealed significant associations between maternal and neonatal factors and the composition of gut and BM microbiota. Maternal age emerged as a key determinant, contributing to 20.9 % (P = 0.022) and 14.4 % (P = 0.075) of the variation in maternal GM community, as demonstrated by unweighted and weighted UniFrac analyses, respectively (Table 2). Furthermore, as maternal age explained 19.4 % (P = 0.038) of the total variation in neonatal gut microbiota structure as determined by unweighted UniFrac distance; delivery mode also contributed to 16.3 % of the variability but did not reach statistical significance (P = 0.060). Notably, the newborn sex accounted for 29.3 % of the variation in the BM microbiota community (P = 0.028).

Table 2. Envfit analysis on the maternal gut, BM, and newborn GM structure according to UniFrac and weighted UniFrac distances associated with mother-newborn characteristics.

	r^2	*p*-Value
Mother gut		
Unweighted UniFrac		
Age, y	0.209	**0.022**
Total body fat, %	0.004	0.944
Delivery mode	0.010	0.830
Sex	0.021	0.707
Weighted UniFrac		
Age, y	0.144	0.075
Total body fat, %	0.002	0.971

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Delivery mode	0.033	0.593
Sex	0.041	0.499
Breast milk		
Unweighted UniFrac		
Age, y	0.030	0.719
Total body fat, %	0.016	0.850
Delivery mode	0.163	0.173
Sex	0.139	0.230
Weighted UniFrac		
Age, y	0.086	0.378
Total body fat, %	0.049	0.623
Delivery mode	0.059	0.530
Sex	0.293	**0.028**
Newborn gut		
Unweighted UniFrac		
Age, y	0.194	**0.038**
Total body fat, %	0.004	0.945
Delivery mode	0.163	0.060
Sex	0.019	0.743
Weighted UniFrac		
Age, y	0.048	0.465
Total body fat, %	0.018	0.740
Delivery mode	0.084	0.231
Sex	0.030	0.614

The model was constructed based on PCoA ordination using UniFrac distance for community richness and weighted UniFrac for community abundance. The r^2 represents the proportion of variance explained by ordination. *P* values are based on 999 random permutations; significant values are in boldface.

4. Discussion

Our findings align with previous evidence linking the mother's total body adiposity and delivery mode to changes in the GM composition in both mothers and newborns, as well as BM. Furthermore, our study highlights the impact of maternal age and newborn sex on the GM of mother-newborn dyads and BM, respectively. These findings represent a significant advancement in our understanding of how various factors influence microbiota in the maternal-infant context, providing new perspectives for future research.

Our study aligns with previous research, revealing that maternal GM reflects typical adult composition [24], and sheds light on post-partum women's GM, a less explored area [25]. Notably, differences in lactating and non-lactating women's GM imply persistent post-pregnancy effects [26,27]. Our findings suggest a potential GM imbalance persisting between 20-28 days post-partum, possibly aiding microbial translocation to mammary glands [28,29]. The wide variety of genera within Firmicutes phylum, particularly during lactation, may support maternal gut health via immune stimulation and reduced inflammation through short-chain fatty acids (SCFAs) and lactate production [30,31]. While the lactating women's GM core remains unclear, certain genera identified are common in healthy adults [32] and may influence gamma-aminobutyric acid production [33], linked to post-partum depression risk reduction [34].

In our study, despite variations in abundance among BM samples from participants, identified taxa are in line with the findings of most studies [15]. Our findings support the notion that breastfeeding provides more than just nutrition; it serves as a natural reservoir of bacterial signatures that are beneficial for newborns' gastrointestinal and immune system development [35,36].

In neonates, the GM displayed bacterial signatures indicative of early life [37]. Given that Proteobacteria phylum includes a wide variety of Gram (-) potential pathogenic bacteria [38], their presence in the neonatal GM may reflect an evolutionary strategy aimed at stimulating the immune system, increasing its tolerance, and preventing the overgrowth of gut pathogens [39]. In addition,

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we observed that *Bifidobacterium was* notably represented alongside bacteria from the *Enterobacteriaceae* family. This family can create anaerobic conditions [40], allowing the settlement of strictly anaerobic bacteria like *Bifidobacterium*, *Clostridia*, and *Bacteroides* [1]. *Bifidobacterium*, as a core member in newborn GM, plays a vital role in metabolizing milk oligosaccharides and promoting the maturation of the gastrointestinal and immune system [41].

Our findings demonstrated evidence of the interplay between perinatal factors and the microbiota profiles of gut mothers, BM, and gut newborns. For instance, women with excessive body fat showed alterations in the abundance of several bacterial genera, primarily belonging to the Firmicutes phylum. These changes could potentially influence the host's energy balance [42] and contribute to a persistent dysbiotic state, with implications for the intergenerational transmission of obesity [43]. Furthermore, women with obesity exhibited a lower abundance of *Fusobacterium* in the GM, which contradicts its association with obesity and unhealthy metabolism [44,45]. Considering that the GM during pregnancy resembles that of individuals with obesity or diabetes [46], supports our hypothesis that maternal GM changes could persist until 28 days after childbirth. Additionally, in light that the *Fusobacterium* includes Gram-negative opportunistic anaerobic bacteria [47] that are part of the endogenous microbiota of the oral cavity [48], and that maternal oral microbiota undergoes changes during pregnancy, including an increase in the presence of pathogenic bacteria in the oral cavity [49,50]. The fact of the lower abundance of *Fusobacterium* in the GM of women with obesity suggests the possibility of an alteration in the oro-intestinal microbiota axis, as both mutually influence each other through microbial transmission [51]. However, further studies are needed to confirm this hypothesis. It is relevant to mention that although the identified taxa represent a minority, it would be interesting to evaluate whether changes in composition due to nutritional status have the potential to modify the functionality of the maternal GM during lactation. Our findings support the limited impact of BMI and total body fat on BM microbiota, as indicated by a recent review [52]. However, our observation of a reduced abundance of certain bacteria within the Proteobacteria phylum, along with decreased levels of SCFAs-producing bacteria exhibited by the BM from women with excessive body fat, are particularly relevant. This reduction may potentially affect the establishment of the newborn GM, particularly in infants born to mothers with obesity, thereby elevating the risk of inflammatory diseases during childhood, such as atopy or childhood overweight, as suggested in the literature [53–57]. When studying the neonatal microbiota, we consistently observed that maternal BMI did not influence diversity and structure, but total body fat did influence the abundance of different taxa, particularly affecting the core member *Staphylococcus*. The decreased abundance of *Staphylococcus* in newborns born to women with elevated adiposity may appear contradictory to previous studies associating Staphylococcus, specifically *S. aureus*, with obesity [10,58–60]. However, these discrepancies could be attributed to the diverse species within the *Staphylococcus* genus, such as *S. epidermidis*, which is vertically transmitted from mother to newborn through BM [36,61]. Given that *Staphylococcus* is part of both the core microbiota of BM and newborn gut, we suggest further investigation using more specific approaches.

According to maternal age, we observed differences in the gut microbiota between younger and older mothers, which also extended to the gut microbiota of their newborns. Maternal GM exhibited distinct clustering patterns, with an enrichment of genera within the Bacteroidota phylum among mature women. Surprisingly, offspring born to mature women not only displayed a lower richness but also showed a decreased abundance of Bacteroides, contrary to expectations based on maternal profiles. This reduction was evident among other members within the Bacteroidales order, which play crucial roles in immune system development [62,63]. We observe subtle differences in BM, suggesting the possibility that maternal age may influence neonatal gut microbiota independently of BM microbiota. However, we acknowledge studies that have found differences in terms of age [9,12,13]. These distinctions in BM microbiota could be due to several factors, including changes in the mammary gland over a woman's lifespan [9,64], alongside methodological variations. Therefore, it would be interesting to evaluate whether these changes are due to intrinsic aging processes linked with progressive loss of intestinal and immune homeostasis [65] or factors associated with age such as modifications in diet, social environment, medication use, and decreased physical activity [66,67].

In relation to delivery mode, we noted a decrease in the abundance of certain obligate anaerobes, such as members of the Ruminococcaceae family, in women who underwent C-sections, consistent with previous findings [14]. This alteration could be attributed to abdominal trauma resulting from

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the C-section procedure, potentially affecting the maternal gut microenvironment, and leading to a reduction in the abundance of anaerobic bacteria. Moreover, disparities were noted between BM samples from women who had vaginal deliveries and those who underwent cesarean sections, with the latter exhibiting lower levels of anaerobic bacteria. Therefore, alterations in the maternal gut environment may influence the composition of less prevalent microbiota members found in BM. While these changes in the gut and BM microbiota may be imperceptible, they undoubtedly impact the neonatal GM. Cesarean section is recognized as a factor capable of disrupting the establishment and development of the GM [17,68]. Our study identified distinct patterns in the abundance of SCFA-producing bacteria, with a lower abundance of *Lachnospiraceae* bacteria in the gut microbiota of neonates delivered by cesarean section, consistent with existing evidence [68]. The altered profile caused by C-section may disrupt microbial community ecology during establishment [69], along with functional repertoires involved in metabolic and immune responses [68,70]. However, the long-term effects of C-sections in our study population need to be assessed, considering the increasing use of elective C-sections. It's crucial to determine whether observed differences are specifically linked to C-sections or related factors such as antibiotic treatment, medications, or exposure to controlled environments [71]. According to newborn sex, the multivariate analysis allowed us to propose that neonatal sex explains a portion of the variation in the microbiota composition from BM. For instance, BM from women with males showed enrichment in *Bifidobacterium*, a keystone genus involved in GM establishment. This finding supports the hypothesis that BM may be sex-specific and provide additional protection to male newborns in response to "male disadvantage" [72,73], although the underlying mechanism remains unknown. Furthermore, we observed a higher abundance of *Stenotrophomonas*, an efficient estrogen degrader, and *Pladulicolla*, a genus belonging to the *Ruminococcocacea* family, positively associated with systemic non-ovarian estrogen, and related to the ability to metabolize steroids [74,75], in samples from women who had females. Moreover, we identified differential composition between females and males, possibly due to changes related to sex hormones, although these became more evident after puberty [76].

Our study presents the following points to be highlighted and certain limitations. We are among the pioneering studies to conduct a comprehensive assessment of several factors including maternal BMI and total body fat, age, delivery mode, and newborn sex, and their influence on the diversity and composition of both maternal and newborn gut and BM microbiota simultaneously. We meticulously controlled for variables such as newborns' gestational age, parity, lactation stage, and feeding mode during our analysis, albeit resulting in the exclusion of a considerable number of mother-newborn dyads. To enhance accuracy, we incorporated total adiposity measurements alongside BMI, recognizing the limitations of BMI as a sole indicator. We also implemented rigorous procedures to ensure the robustness of our methodology. Thorough cleaning and emptying of both breasts were conducted to minimize potential biases in sampling, and stringent quality control measures were applied, including the use of negative controls throughout the sample extraction, library preparation, and sequencing processes to identify and eliminate contaminant reads.

However, limitations include the use of 16S rRNA sequencing, which may introduce technical biases, and the exclusion of other potential microbial niches. Additionally, the sample size is limited, warranting caution in interpretation, and highlighting the need for larger cohorts to validate and expand upon our findings.

5. Conclusions

Overall, we provided valuable insights into the determinants of both maternal and newborn gut microbiota as well as BM microbiota composition; mainly, we contribute new evidence highlighting the influence of maternal age and newborn sex. Maternal BMI and total body fat, age, delivery mode, and newborn sex were found to have significant associations with microbial profiles. These findings contribute to understanding the complex interplay between maternal factors and the microbial communities that shape early-life gut and BM microbiota. However, further research is essential to fully elucidate the mechanisms underlying these associations and their long-term implications for interventions or therapeutic strategies targeting maternal and neonatal microbiome. Specifically, future studies should incorporate longitudinal designs and employ more comprehensive characterization of microbial species to unravel the related health outcomes.

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Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Supplementary Supplementary Material, Figure S1 and Tables S1, S2, S3, S4, S5 and S6.

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