

Figure S1. Flow diagram of study participants. *Low weight gain (LWG) data were not used in the analysis.

Table S1. Poisson regression model for top 10 genera relative abundance based on pre-gestational BMI and weight gain over pregnancy.

Pre-gestational BMI				
Genus		IRR	95 % CI	<i>p</i>
<i>Streptococcus</i>	NW vs OW	1.21	1.12-1.30	<0.001
<i>Staphylococcus</i>	NW vs OW	0.89	0.83-0.96	0.002
<i>Ralstonia</i>	NW vs OW	1.16	1.03-1.32	0.017
<i>Acinetobacter</i>	NW vs OW	0.94	0.83-1.06	0.326
<i>Gemella</i>	NW vs OW	0.97	0.81-1.16	0.718
<i>Pseudomonas</i>	NW vs OW	0.55	0.44-0.68	<0.001
<i>Lactobacillus</i>	NW vs OW	0.94	0.75-1.19	0.611
<i>Escherichia/Shigella</i>	NW vs OW	1.50	1.02-2.17	0.037
<i>Bifidobacterium</i>	NW vs OW	4.67	2.53-8.64	<0.001
<i>Stenotrophomonas</i>	NW vs OW	12.0	4.90-29.20	<0.001
Weight gain over pregnancy				
<i>Streptococcus</i>	NWG vs EWG	1.38	1.27-1.51	<0.001
<i>Staphylococcus</i>	NWG vs EWG	1.00	0.92-1.09	0.942
<i>Ralstonia</i>	NWG vs EWG	0.53	0.46-0.61	<0.001
<i>Acinetobacter</i>	NWG vs EWG	0.80	0.68-0.93	0.004
<i>Gemella</i>	NWG vs EWG	1.36	1.09-1.70	0.006
<i>Pseudomonas</i>	NWG vs EWG	8.14	5.33-12.42	<0.001
<i>Lactobacillus</i>	NWG vs EWG	0.60	0.46-0.79	<0.001
<i>Escherichia/Shigella</i>	NWG vs EWG	0.19	0.14-0.26	<0.001
<i>Bifidobacterium</i>	NWG vs EWG	3.20	1.71-5.98	<0.001
<i>Stenotrophomonas</i>	NWG vs EWG	0.24	0.11-0.54	<0.001

Poisson regression models were run for top 10 genera based on pre-gestational BMI and weight gain, adjusted for mode of birth and breastfeeding practices at one month. $p < 0.05$ was considered statistically significant. IRR, Incidence rate ratio. CI, Confidence interval. (NW) Normal weight and (OW) Overweight. (EWG) Excessive weight gain and (NWG) Normal weight gain.

Table S2. Characteristics of population participant based pre-gestational BMI (n=135)

	All	NW (18.5 – 24.9 kg/m ²) n=97	OW (> 25 kg/m ²) n=38	<i>p</i>
Maternal characteristics				
Maternal age (years)	34.44 ± 3.80	34.25 ± 3.82	34.95 ± 3.74	0.338
Gestational age (weeks)	40 [39-40]	40 [39-40]	40 [39-40]	0.604
Pre-gestational BMI (kg/m ²)	22.84 [21.01-25.39]	21.63 [20.46-22.96]	27.25 [25.97-29.69]	<0.001
Weight gain (kg) over pregnancy*	12 [9.5-15]	12 [10-15]	10 [7.8-14]	0.023
Low weight gain (LWG)	43 (31.9)	36 (37.1)	7 (18.4)	0.004
Normal weight gain (NWG)	60 (44.4)	45 (46.4)	15 (39.5)	
Excessive weight gain (EWG)	32 (23.7)	16 (16.5)	16 (42.1)	
Intrapartum antibiotic exposure (%)	54 (40)	35 (36.1)	19 (50)	0.138
Antibiotic during pregnancy (%)	42 (31.1)	27 (27.8)	15 (39.5)	0.189
Antibiotic treatment 1 month (%)	11 (8.2)	8 (8.3)	3 (7.9)	0.934
Infant characteristics				
Gender Female (%)	76 (56.3)	55 (56.7)	21 (55.3)	0.965
Birth mode: vaginal birth (%)	84 (62.2)	65 (67)	19 (50)	0.046
Height at birth (cm)	49.86 ± 2.10	49.75 ± 2.16	50.16 ± 1.95	0.309
Weight at birth (g)	3.32 ± 0.44	3.26 ± 0.43	3.48 ± 0.45	0.009
Antibiotic treatment 1 month (%)	8 (5.9)	5 (5.2)	3 (7.9)	0.544
Breastfeeding duration (months)	8.37 ± 3.92	8.43 ± 3.89	8.20 ± 4.04	0.755
Exclusive Breastfeeding (1 month)	110 (81.5)	79 (81.4)	31 (81.6)	0.985

Categorical data results are shown as number of case (percentage %). Normally distributed data are shown as mean ± SD and non-normal data as median [interquartile range= IQR]. (NW) Normal weight, (OW) Overweight. (NWG) Normal weight gain, (EWG) Excessive weight gain.* Low weight gain (LWG) data were not used in the analysis.

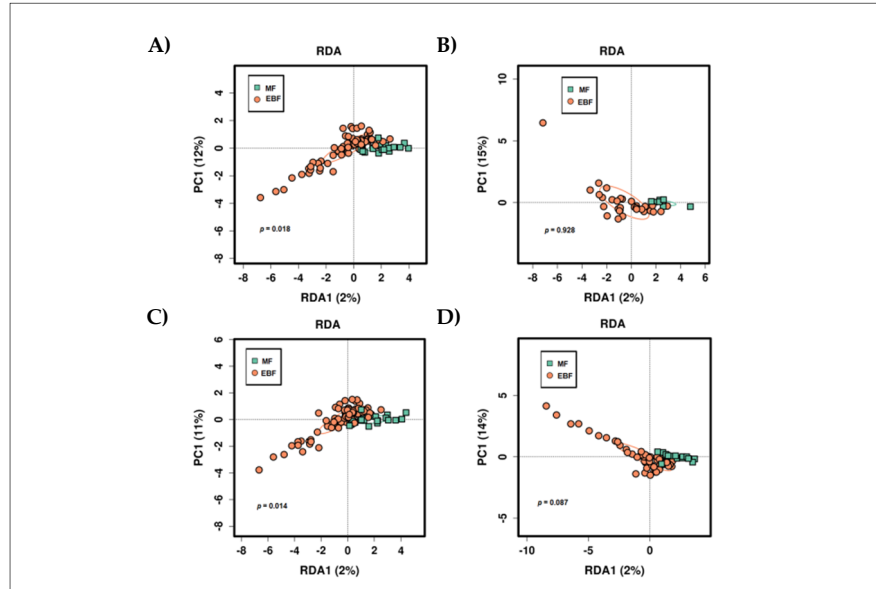


Figure S2. Impact of pre-gestational BMI and weight gain over pregnancy on breast milk microbiota according to breastfeeding practices. Multivariate redundancy discriminant analysis (RDA) showed significant differences in breast milk microbial communities between breastfeeding practices depending on pre-gestational BMI: (A) NW mothers (B) and OW mothers. Weight gain over pregnancy: (C) NWG mothers and (D) EWG mothers. (EBF) Exclusive breastfeeding and (MF) Mixed feeding.

Table S3. Comparison of breast milk microbiota composition at top 10 genera, by breastfeeding practices and pre-gestational BMI and Weight gain.

Genus	EBF_NW	EBF_OW	MF_NW	MF_OW	<i>p</i>
<i>Streptococcus</i>	28.99 [8.49-47.91]	16.69 [6.42-35.92]	27.84 [10.92-48.37]	26.35 [7.02-61.14]	0.363
<i>Staphylococcus</i>	19.69 [5.25-38.86]	23.07 [4.47-40.50]	26.91 [13.12-57.48]	56.36 [8.09-67.54]	0.323
<i>Ralstonia</i>	1.14 [0-21.62]	0.095 [0.01-3.59]	0.075 [0-2.11]	0.01 [0-4.82]	0.216
<i>Acinetobacter</i>	0.39 [0.07-1.54]	0.20 [0.02-26.59]	0.18 [0-6.82]	0.11 [0.02-1.81]	0.502
<i>Gemella</i>	1.39 [0.16-4.42]	1.00 [0.29-6.21]	1.86 [0.46-5.90]	4.58 [0.92-5.17]	0.804
<i>Pseudomonas</i>	0.57 [0.21-1.89]	0.63 [0.11-2.13]	0.12 [0.03-0.48]	0.09 [0.04-0.25]	<0.01
<i>Lactobacillus</i>	0.51 [0.03-4.18]	0.92 [0.07-2.80]	0.48 [0-1.30]	0.2[0-1.11]	0.319
<i>Escherichia/Shigella</i>	0.05 [0-0.17]	0.04 [0-0.25]	0.05 [0-0.18]	0 [0-0.14]	0.738
<i>Bifidobacterium</i>	0.30 [0.05-1.10]	0.075 [0.03-0.58]	0.07 [0.01-0.20]	0.05 [0-0.63]	0.033
<i>Stenotrophomonas</i>	0.08 [0-0.32]	0.045 [0-0.29]	0.04 [0-0.08]	0.01 [0-0.04]	0.186
Genus	EBF_NWG	EBF_EWG	MF_NWG	MF_EWG	<i>p</i>
<i>Streptococcus</i>	31.04 [11.23-51.44]	17.67 [6.81-29.73]	27.83 [12.93-44.33]	26.35 [6.50-54.40]	0.298
<i>Staphylococcus</i>	21.98 [6.55-41.65]	21.44 [4.04-31.49]	44.01 [24.02-67.54]	26.39 [16.05-78.23]	0.049
<i>Ralstonia</i>	0.44 [0-9.05]	0.10 [0.02-29.48]	0.01 [0-0.08]	0.03[0-10.29]	0.225
<i>Acinetobacter</i>	0.13 [0.03-1.16]	0.34 [0.06-7.98]	0.11 [0.01-4.35]	0.02 [0-0.16]	0.076
<i>Gemella</i>	3.08 [0.17-6.78]	1.08 [0.29-4.42]	4.72 [0.20-6.91]	5.35 [2.68-13.29]	0.402
<i>Pseudomonas</i>	0.50 [0.14-1.76]	0.86 [0.15-1.65]	0.09 [0.02-0.37]	0.10 [0.04-0.30]	0.019
<i>Lactobacillus</i>	0.82 [0.03-2.59]	1.09 [0.04-7.88]	0.58 [0.02-1.07]	0.39 [0-1.23]	0.438
<i>Escherichia/Shigella</i>	0.06 [0-0.19]	0.07 [0.01-0.18]	0.08 [0-0.18]	0.02 [0.01-28.78]	0.983
<i>Bifidobacterium</i>	0.15 [0.03-1.10]	0.24 [0.04-1.04]	0.06 [0.02-0.27]	0.08 [0.03-0.19]	0.436
<i>Stenotrophomonas</i>	0.05 [0-0.16]	0.08 [0-0.59]	0.03 [0-0.07]	0 [0-0.03]	0.063

Relative abundances of dominant genus within each breastfeeding practices and pre-gestational BMI and weight gain over pregnancy are in bold. Data is presented as median [interquartile range= IQR]. Relative abundances were compared by Mann-Whitney test.

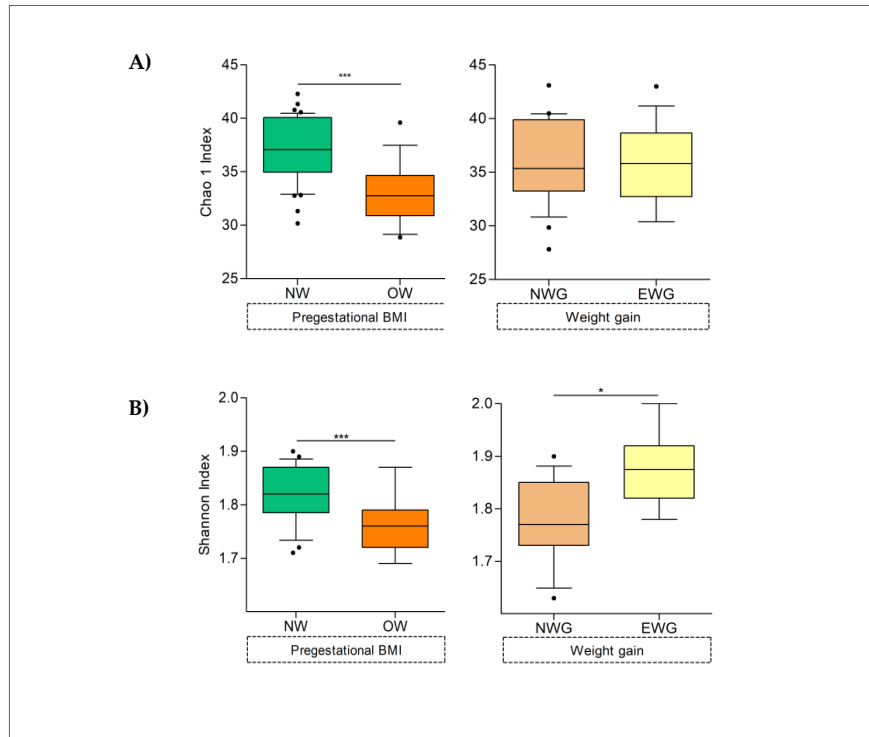


Figure S3. Microbial richness and diversity is associated by pre-gestational BMI and weight gain influence. Diversity (Shannon index) and richness (Chao1 index) of breast milk microbiota according to pre-gestational BMI and weight gain categories. Microbial richness and diversity indexes at ASV. (A) Richness and according to pre-gestational BMI and weight gain. (B) Diversity according pre gestational BMI and weight gain. Richness and diversity values were adjusted for total bacterial load. Whiskers represented 5-95 percentile interval. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (NW) Normal weight, (OW) Overweight. (EWG) Excessive weight gain and (NWG) Normal weight gain

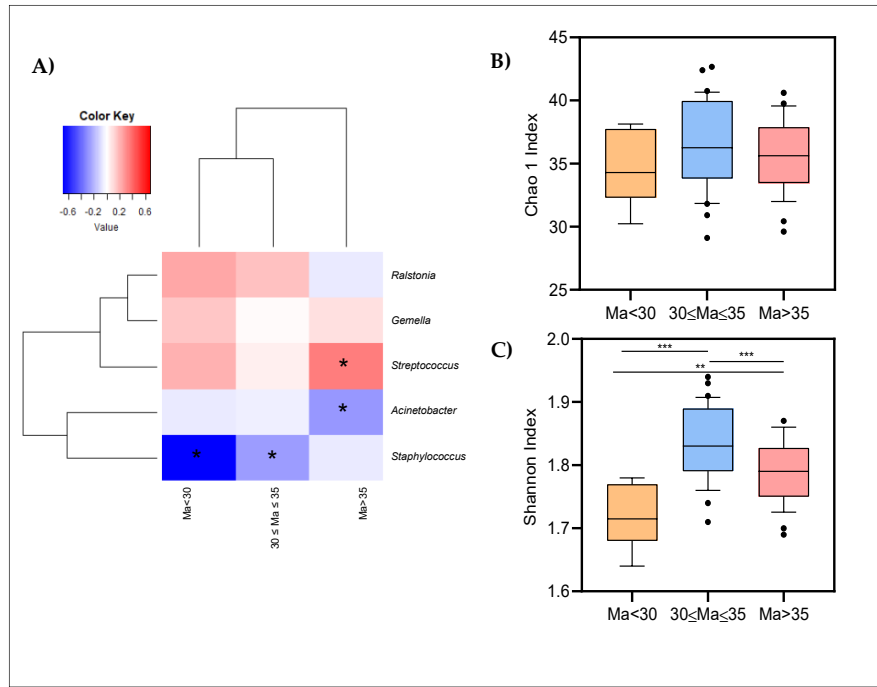


Figure S4. Impact of maternal age on breast milk microbiota. (A) Maternal age was associated with the relative abundances of top 5 genera (Spearman's rank correlation). (B, C) Microbial richness and diversity indexes at ASV. Richness and diversity values were adjusted for total bacterial load. Whiskers represented 5-95 percentile interval. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.