

Online Supplemental Material

Supplement Figure Legends

Supplemental Figure 1. Effect of delivery mode on faecal microbiota in exclusively breast-fed infants. (A) NMDS plots of faecal microbial profiles from infants born via normal delivery (N) or caesarean section (C). A total of 34, 27 and 25 N infants were compared to 25, 20 and 16 C infants at months 1, 3 and 7. Only breast-fed infants were considered for analysis to exclude confounding effects of feeding. (B) No significant difference was observed in faecal metabolite profiles between the delivery modes.

Supplemental Figure 2. Effect of antibiotics administered during the first year. Impact of antibiotic treatment on *beta*-diversity of infant faecal microbiota at month 1 (A), 3 (B), 7 (C) and 12 (D). Grey data sets include infants that had not been treated with antibiotics before time of sampling and red dots indicate treated infants. No significant difference was detected between microbiota of antibiotics-treated and non-treated infants at any measured time point. This hold true when antibiotic-treated infants were compared to non-treated infants of the same feeding group.

Supplemental Figure 3. Mean gain in body weight and size in breast (B), intervention (F+) and placebo (F-) groups. Monthly assessment of body weight (A) and size (B) revealed no difference between the three main study populations, intervention (F+, n=48), placebo (F-, n=49) and breast-fed (B, n=9).

Supplemental Figure 4. Identification of the 10 most abundant OTUs in interventional and placebo formula-fed infants from birth until first year of age. *Bacteroides fragilis* (OTU18) and *Blautia* sp. (OTU8) were significantly higher in the placebo group than in the interventional group. Among the 10 most abundant OTUs, 4 were classified as *Bifidobacterium* spp. in the interventional group (OTU1, 2, 4, 7) and three in the placebo

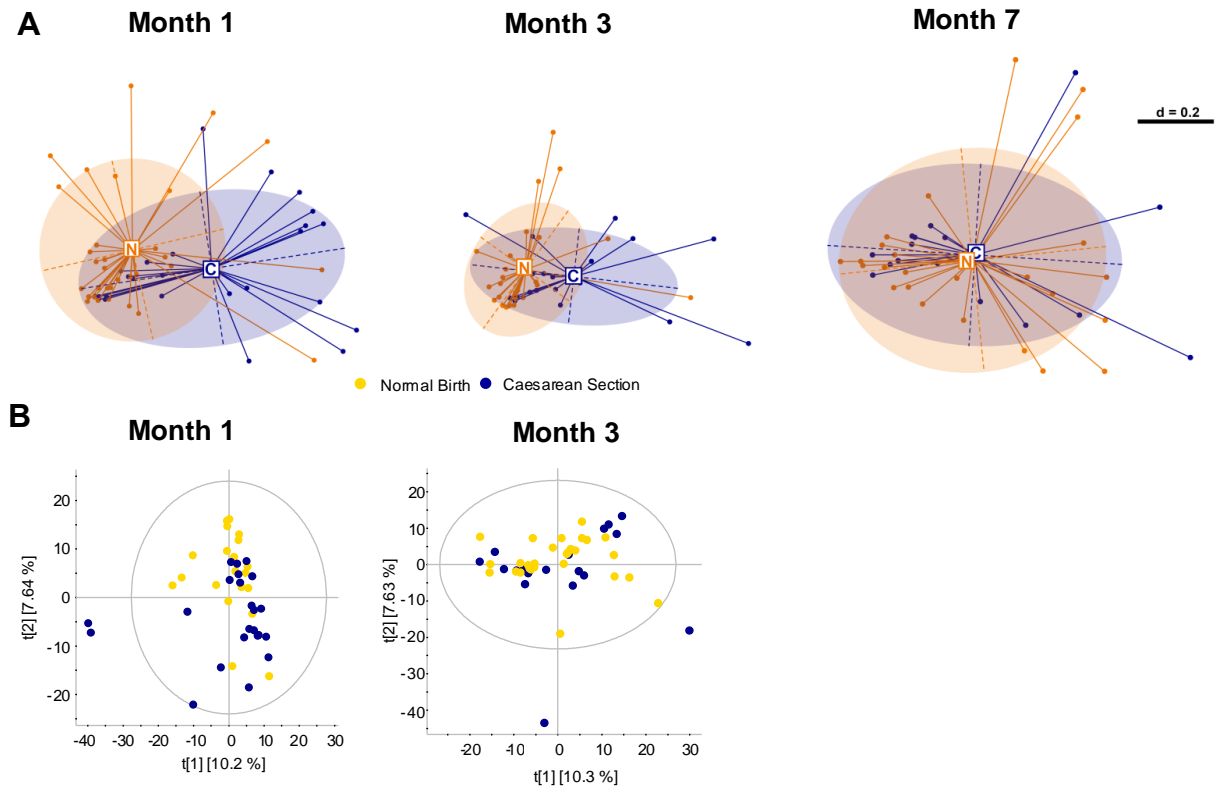
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group (OTU1, 2, 636). Percentages in brackets indicate mean relative abundance within the first year of life. Months 1, 3, 5, 7, 9, and 12 are encoded by colours from light to dark. * <0.05 , ** <0.005 , *** <0.0005

Supplemental Figure 5. *Bifidobacterium bifidum* BF3, *breve* BR3, *longum* BG7 and *longum* subsp. *infantis* BT1 strain-specific analysis. (I) Primer pairs were tested for specificity against available strains (lines: M=marker; 1=*Bifidobacterium bifidum* DSM20082; 2=*Bifidobacterium bifidum* DSM20239; 3=*Bifidobacterium bifidum* DSM20215; 4=*Bifidobacterium breve* DSM20091; 5=*Bifidobacterium longum* DSM20097; 6=*Bifidobacterium longum* subsp. *infantis* DSM20090; 7=*Bifidobacterium longum* subsp. *infantis* DSM20218; T=type strain; F=formula strain). (II) Formula strains were cultured on *Bifidobacterium*-selective medium followed by DNA extraction and strain-specific PCR to determine the presence of viable cells. (III) Strain-specific PCR from milk powder was performed to test the original product used for feeding. The first two lines are isolates from starter formula, the second two lines from follow-up formula.

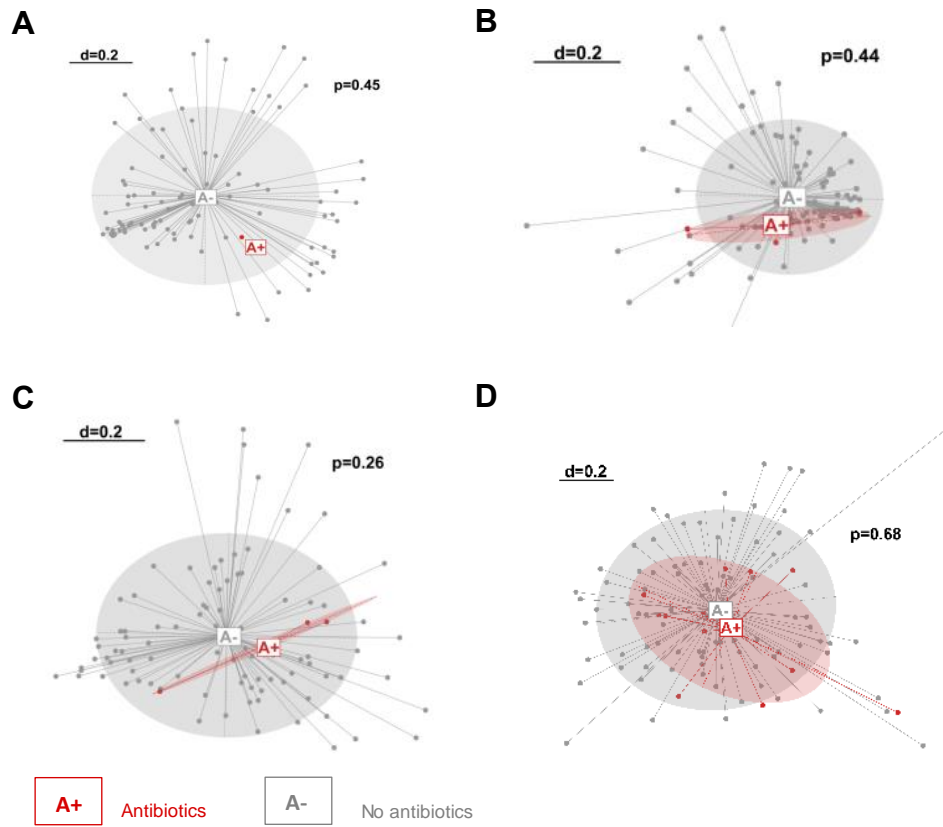
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Supplemental Figure 1



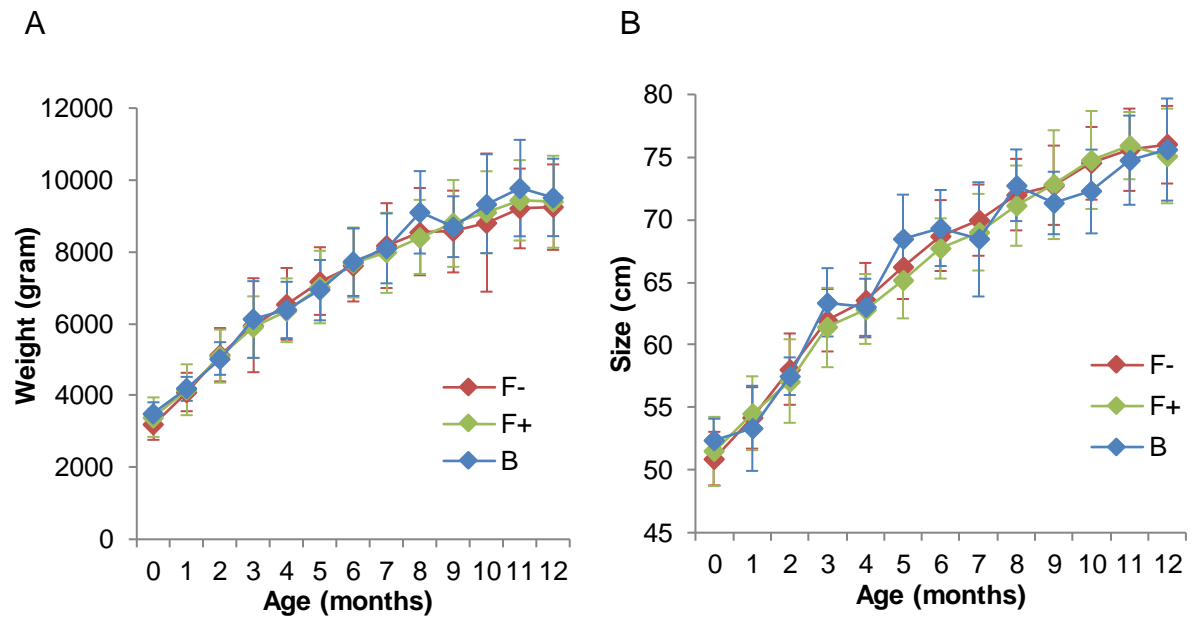
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Supplemental Figure 2



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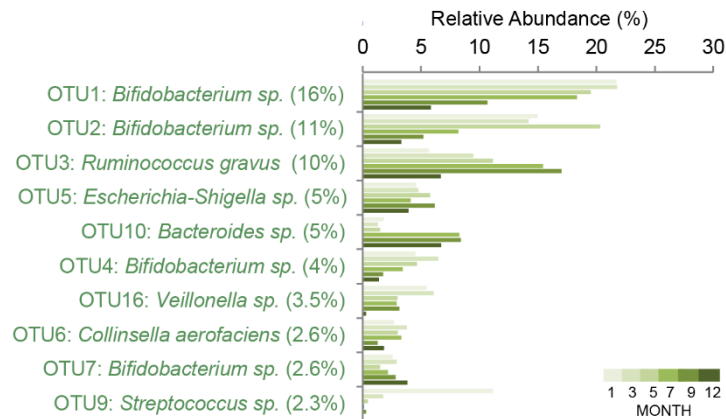
Supplemental Figure 3



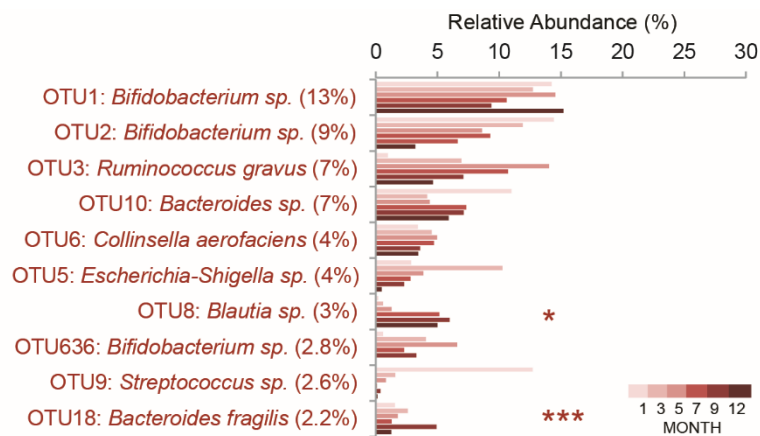
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Supplemental Figure 4

A

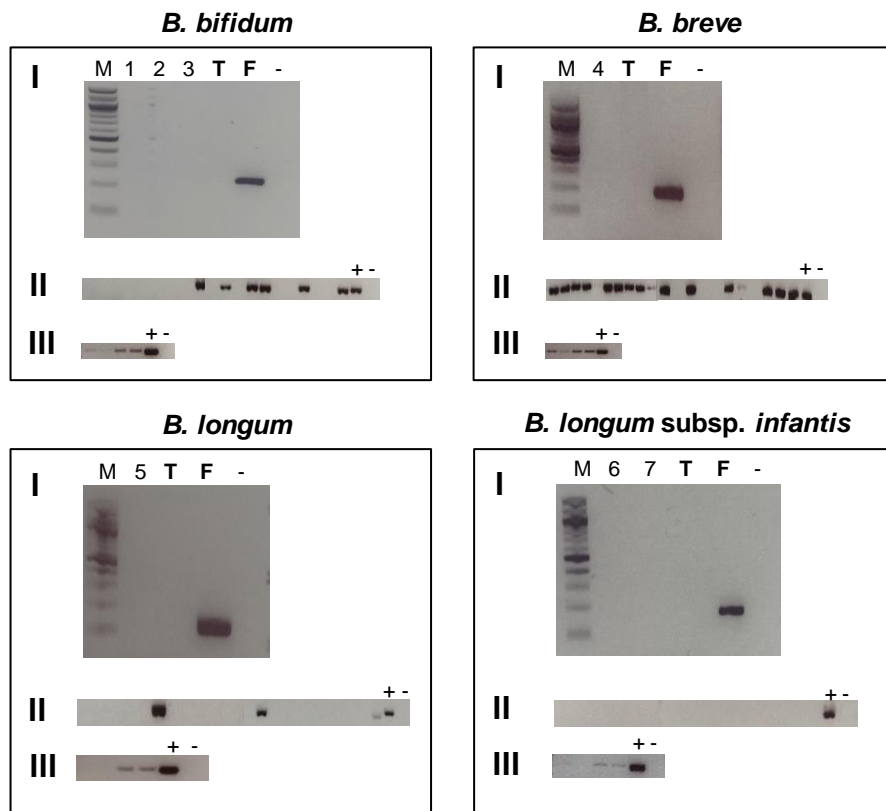


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Supplemental Figure 5



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Supplemental Tables

Supplemental Table 1. Primers used for strain-specific PCR¹

Strain	Forward Sequence	Reverse Sequence	T _m (°C)	Product Size (nt)	Target Gene
<i>B. bifidum</i> CBT BF3	TCATCAGGGGGACACCGTTC	AGCGAGTAGAGTGGAGGGGG	62	187	2629604485 hypothetical protein
<i>B. breve</i> CBT BR3	CGATAACTCACGGCATTGTG	CCATAATTGCTACCGCCAGT	60	225	2629607000 oligosaccharide repeat unit polymerase
<i>B. longum</i> CBT BG7	GATTCGCTTCCCGATTCCCT	GTTCTTCAGCATCTCGCCCT	60	237	2629608866 type III restriction enzyme
<i>B. longum</i> subsp. <i>infantis</i> CBT BT1	GGTCACCACTGTGCCAATCA	CGATGCTCGCCAAAACAC	60	94	2629609788 hypothetical protein

¹ Sequences of primers for strain-specific analyses including melting temperature (T_m) and product size (in nucleotides nt) are shown. Moreover, the genes targeted by the primer pairs are indicated.

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Supplemental Table 2. List of the six most abundant *Bifidobacterium* spp. in exclusively breast- and formula-fed infants ¹

OTU	Rank	Name	Sequence Identity (%)
1	1	<i>Bifidobacterium longum</i> subsp. <i>suis</i>	100.00
	2	<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	100.00
	3	<i>Bifidobacterium longum</i> subsp. <i>longum</i>	99.74
	4	<i>Bifidobacterium saguini</i>	98.19
	5	<i>Bifidobacterium breve</i>	97.42
	6	<i>Bifidobacterium scardovii</i>	97.16
	7	<i>Bifidobacterium merycicum</i>	97.16
2	1	<i>Bifidobacterium breve</i>	100.00
	2	<i>Bifidobacterium saguini</i>	98.19
	3	<i>Bifidobacterium boum</i>	97.44
	4	<i>Bifidobacterium scardovii</i>	97.43
	5	<i>Bifidobacterium longum</i> subsp. <i>suis</i>	97.42
	6	<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	97.42
	7	<i>Bifidobacterium pseudolongum</i> subsp. <i>globosum</i>	97.19
	8	<i>Bifidobacterium pseudolongum</i> subsp. <i>pseudolongum</i>	97.19
4	1	<i>Bifidobacterium bifidum</i>	100.00
	2	<i>Bifidobacterium thermacidophilum</i> subsp. <i>thermacidophilum</i>	97.17
	3	<i>Bifidobacterium thermacidophilum</i> subsp. <i>porcinum</i>	97.17
	4	<i>Bifidobacterium thermophilum</i>	97.17
	5	<i>Bifidobacterium saguini</i>	97.16
7	1	<i>Bifidobacterium adolescentis</i>	100.00
	2	<i>Bifidobacterium stercoris</i>	100.00
	3	<i>Bifidobacterium faecale</i>	100.00
	4	<i>Bifidobacterium ruminantium</i>	99.74
	5	<i>Bifidobacterium catenulatum</i>	98.20
	6	<i>Bifidobacterium kashiwanohense</i>	98.20
	7	<i>Bifidobacterium pseudocatenulatum</i>	98.20
	8	<i>Bifidobacterium boum</i>	97.95
	9	<i>Bifidobacterium dentium</i>	97.70
	10	<i>Bifidobacterium callitrichos</i>	97.67
636	1	<i>Bifidobacterium scardovii</i>	96.40
1670	1	<i>Bifidobacterium kashiwanohense</i>	97.69
	2	<i>Bifidobacterium catenulatum</i>	97.69
	3	<i>Bifidobacterium pseudocatenulatum</i>	97.69

¹ Relative abundances of these OTUs are shown in Figure S5 for the interventional and placebo infants. and in Figure 5 for breast-fed infants. Coloured cells indicate species that may potentially represent the strains added to the formula (i.e. their sequence matched that of the supplemented species. among others). All species with a sequence identity >97% are listed (top 10 hits only are shown if more species fulfilled this criterion).

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Supplemental Table 3. Metabolite- and OTU-based features of feeding groups (B, breast-fed; F, formula; F+, bifidobacteria-supplemented formula, F-, placebo formula) at month 1, 7, and 12 of age ranked from high to low importance by significance on OPLS-DA loadings plot. ¹

	ID	Feed	Order	m/z or taxonomy	Lipid Maps classification	HMDB classification
MONTH 1	Cluster_4611	B	1	552,3366878		
	Cluster_1816	B	2	621,7141547		
	Cluster_5216	B	3	621,2105299		
	Cluster_0644	B	4	256,0821987		
	Cluster_5825	B	5	694,2352887		
	Cluster_3214	B	6	376,2631225		Benzopyrans
	Cluster_5970	B	7	713,2216027		
	Cluster_5136	B	8	613,1922285		
	Cluster_2992	B	9	341,293116		
	Cluster_2002	B	10	721,2122736		
	Cluster_3467	F	1	407,245529		
	Cluster_3230	F	2	377,2296393		Lipids
	Cluster_0971	F	3	386,7162076		
	Cluster_3644	F	4	427,3609756		Lipids
	Cluster_3593	F	5	421,2189653		
	Cluster_3745	F-	1	440,2844395		
	OTU_10	F-	2	Bacteroides sp.		
	Cluster_3789	F-	3	445,3706539	Sterol Lipids [ST]	Prenol Lipids
	Cluster_4023	F-	4	472,4172805		
	Cluster_5412	F-	5	644,4008303		
	OTU_18	F-	6	Bacteroides fragilis		
	OTU_96	F-	7	Odoribacter sp.		
	Cluster_5175	F-	8	616,3489547		
	OTU_68	F-	9	Alistipes sp.		
	Cluster_6345	F-	10	764,4230286		
	Cluster_0385	F+	1	510,3339711		
	Cluster_4506	F+	2	537,386372		
	OTU_4	F+	3	Bifidobacterium sp.		
	Cluster_6749	F+	4	813,5680134	Glycerophospholipids [GP]	
	Cluster_1515	F+	5	520,762328		
	OTU_142	F+	6	Lactococcus sp.		
	Cluster_3067	F+	7	353,2506538		
	Cluster_2260	F+	8	144,0825079		
	Cluster_3573	F+	9	417,3345252		
MONTH 7	Cluster_4141	B	2	491,330764		
	Cluster_4188	B	3	497,1767158		
	Cluster_3864	B	4	453,2158723		
	Cluster_4611	B	5	552,3366878		
	Cluster_3868	B	6	453,2406183		
	Cluster_3207	B	7	375,2921489	Sterol Lipids [ST]	Steroids and Steroid Derivatives
	Cluster_3088	B	8	357,2827519		Fatty Acids and Conjugates
	Cluster_4029	B	9	473,2536532	Sterol Lipids [ST]	Steroids and Steroid Derivatives
	Cluster_3946	B	10	462,3440454	Sphingolipids [SP]	Sphingolipids
	Cluster_3580	F	1	418,3461543		
	Cluster_3425	F	2	401,3093941	Sterol Lipids [ST]	Prenol Lipids
	Cluster_3573	F	3	417,3345252		
	Cluster_0971	F	4	386,7162076		
	Cluster_3493	F	5	410,320276		
	Cluster_3683	F	6	431,3553964	Sterol Lipids [ST]	Prenol Lipids
	Cluster_3644	F	7	427,3609756		Lipids
	Cluster_3230	F	8	377,2296393		Lipids
	Cluster_4577	F	9	547,4244787		
	Cluster_3803	F	10	447,3479744		
MONTH 12	Cluster_2524	B	1	261,1469315	primary amine	Amino Acids and Derivatives
	Cluster_4871	B	2	583,2605075		
	Cluster_4197	B	3	498,0621014		
	Cluster_2250	B	4	118,0879665		
	Cluster_1342	B	5	466,1913538	Polyketides [PK]	
	Cluster_2753	B	6	301,1126443		
	Cluster_2352	B	7	206,0829788		
	Cluster_3088	B	8	357,2827519		Fatty Acids and Conjugates
	Cluster_4611	B	9	552,3366878		
	Cluster_3207	B	11	375,2921489	Sterol Lipids [ST]	Steroids and Steroid Derivatives
	OTU_29	F	1	Flavonifractor sp.		
	Cluster_3642	F	2	427,3608033		Lipids
	Cluster_3613	F	3	423,3295783		Fatty Acid Esters
	Cluster_3666	F	4	430,331642	Fatty Acyls [FA]	
	Cluster_3781	F	5	445,371389	Sterol Lipids [ST]	Prenol Lipids
	Cluster_3783	F	6	445,3707973	Sterol Lipids [ST]	Prenol Lipids
	Cluster_3689	F	7	431,3536514	Sterol Lipids [ST]	Prenol Lipids
	Cluster_3493	F	8	410,320276		
	Cluster_3914	F	9	458,3999067	Sterol Lipids [ST]	
	Cluster_3683	F	10	431,3553964	Sterol Lipids [ST]	Prenol Lipids

¹ With an error of 0.005 Da. the features were assigned using the MassTRIX webserver to classify them to the LipidMaps and HMDB compound classes. m/z = mass-to-charge ratio of positive electrospray ionization. Red color indicates OTUs specific for the non-supplemented formula group. Green color indicates OTUs specific for bifidobacterium supplemented formula group.