

Supplementary information for the manuscript entitled:

The effect of prebiotic fortified infant formulas on microbiota composition and dynamics in early life

Klaudyna Borewicz, Maria Suarez-Diez, Christine Hechler, Roseriet Beijers, Carolina de Weerth, Ilja Arts, John Penders, Carel Thijs, Arjen Nauta, Cordula Lindner, Ellen van Leusen, Elaine E. Vaughan, Hauke Smidt

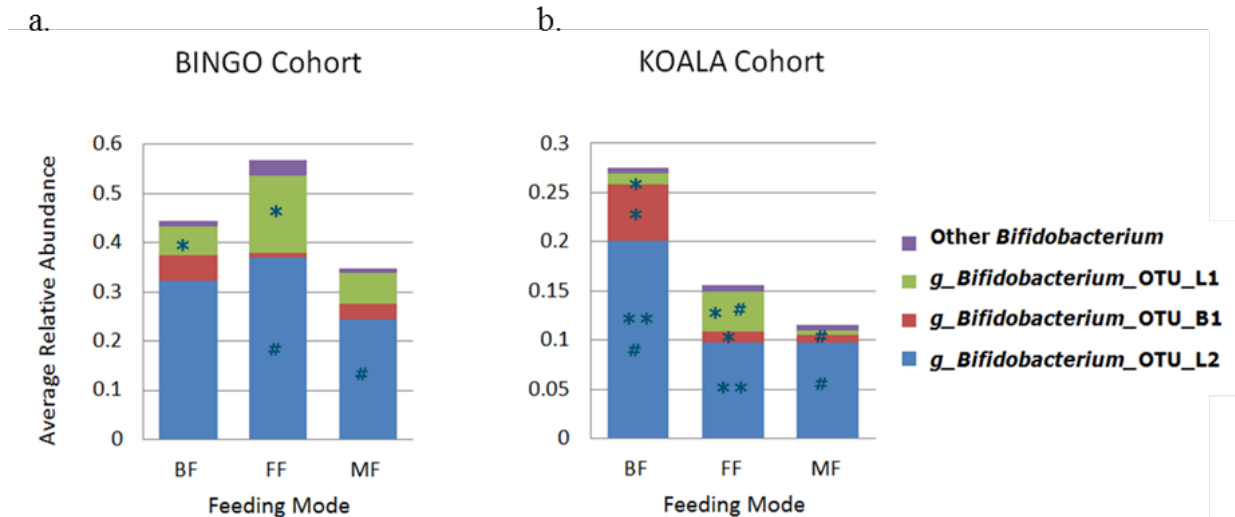


Fig. S1. Relative abundance of bifidobacterial Operational Taxonomic Units (OTUs) in different feeding modes. **a.** Differences of the main *Bifidobacterium* OTUs between formula fed (FF) and breastfed (BF) infants in the BINGO cohort are not significant for OTUs L2 and B1 but significant for OTU L1 ($p < 0.05$); **b.** There are significant differences in the main *Bifidobacterium* OTUs between BF and FF infants in the KOALA cohort (OTU L2 FDR < 0.05 ; OTU L1 and B1 $p < 0.05$). In both cohorts mixed feeding (MF) resulted in decrease in relative abundance of bifidobacteria, and there was a significant decrease for OTU L2 ($p < 0.05$) between FF and MF in the BINGO cohort and between BF and MF in the KOALA cohort, and a significant decrease in OTU L1 in MF compared to FF in the KOALA cohort. Symbols *, #, ** indicate pairs of taxa that were compared and were significantly different in Kruskal - Wallis test ($p < 0.05$)

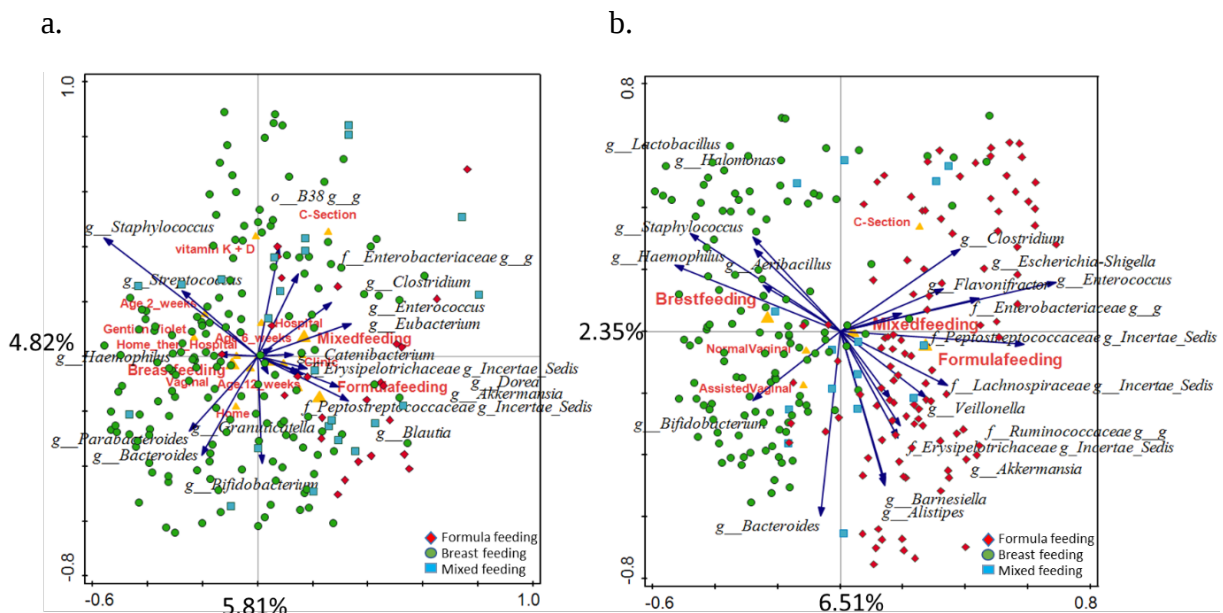


Fig. S2. RDA showing factors with a significant effect on fecal microbiota of infants. Sample are colour coded by feeding mode. Displayed taxa include ten best fitting species, in addition to the microbial groups that differed significantly (Wilcoxon test, FDR < 0.05) between BF and FF groups. **a.** BINGO cohort; **b.** KOALA cohort

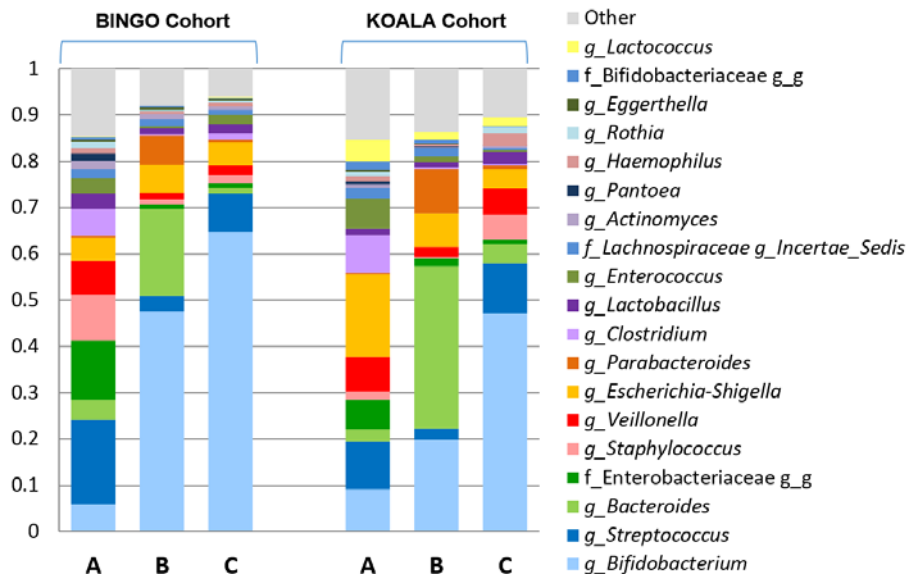


Fig. S3. The average relative contribution of the main bacterial genus-level taxa defining each of three clusters obtained by DMM modelling of fecal microbial composition of infants included in the BINGO and KOALA cohorts. When the taxonomic assignment could not be made at genus level, the lowest classifiable taxonomy assignment is used instead. A - Cluster A characterised by mixed microbial composition; B - Cluster B characterised by high relative abundance of both *Bifidobacterium* and *Bacteroides*; C - Cluster C characterised by high relative abundance of *Bifidobacterium*

Table S1. Average relative abundance of major bacterial taxa detected in BINGO and KOALA cohorts. Microbial groups that significantly differ in relative abundance (Wilcoxon test, FDR<0.05) between BF and FF groups are indicated with * (BINGO) and # (KOALA).

Study	BINGO Cohort									KOALA Cohort		
Feeding Mode	BF			FF			MF			BF	FF	MF
Age (weeks)	2	6	12	2	6	12	2	6	12	4	4	4
<i>g_Bifidobacterium</i> #	0.3467	0.4614	0.5111	0.4519	0.6218	0.5528	0.2806	0.4321	0.3217	0.3192	0.1718	0.1216
<i>g_Bacteroides</i>	0.0831	0.1212	0.1230	0.0652	0.0225	0.0357	0.0759	0.0877	0.1314	0.2134	0.2316	0.3195
<i>g_Streptococcus</i>	0.0844	0.0711	0.0437	0.0238	0.0314	0.0680	0.1788	0.1223	0.0305	0.0375	0.0454	0.0348
<i>g_Escherichia-Shigella</i> #	0.1108	0.0647	0.0769	0.0380	0.1038	0.0790	0.0431	0.0617	0.0912	0.1185	0.1947	0.2095
<i>f_Enterobacteriaceae g_g</i> #	0.1199	0.0524	0.0390	0.0910	0.0061	0.0401	0.0634	0.0919	0.0816	0.0396	0.0555	0.0126
<i>f_Lachnospiraceae Incertae_Sedis</i> #	0.0390	0.0268	0.0360	0.0229	0.0084	0.0191	0.0416	0.0593	0.0856	0.0188	0.0276	0.0146
<i>g_Lactobacillus</i> #	0.0105	0.0383	0.0271	0.0480	0.0210	0.0278	0.0201	0.0337	0.0564	0.0228	0.0050	0.0204
<i>g_Clostridium</i> #	0.0277	0.0162	0.0290	0.0167	0.0275	0.0249	0.0677	0.0422	0.0392	0.0256	0.0318	0.0076
<i>g_Enterococcus</i> #*	0.0223	0.0133	0.0090	0.0042	0.0295	0.0583	0.0507	0.0237	0.0146	0.0020	0.0194	0.0181
<i>g_Blautia</i> *	0.0002	0.0035	0.0032	0.0245	0.0523	0.0316	0.0468	0.0032	0.0486	0.0053	0.0037	0.0232
<i>g_Veillonella</i> #	0.0237	0.0205	0.0321	0.0167	0.0128	0.0149	0.0260	0.0118	0.0408	0.0274	0.0504	0.0508
<i>g_Staphylococcus</i> #*	0.0683	0.0136	0.0028	0.0257	0.0035	0.0019	0.0535	0.0018	0.0003	0.0218	0.0006	0.0011
<i>g_Pantoea</i>	0.0029	0.0128	0.0009	0.1201	0.0000	0.0020	0.0006	0.0012	0.0031	0.0015	0.0040	0.0001
<i>g_Parabacteroides</i> *	0.0131	0.0182	0.0136	0.0006	0.0002	0.0003	0.0269	0.0011	0.0111	0.0668	0.0316	0.0904
<i>f_Erysipelotrichaceae Incertae_Sedis</i> #*	0.0032	0.0036	0.0050	0.0230	0.0027	0.0089	0.0072	0.0000	0.0101	0.0074	0.0109	0.0098
<i>g_Actinomyces</i>	0.0085	0.0225	0.0043	0.0025	0.0009	0.0015	0.0020	0.0070	0.0026	0.0023	0.0005	0.0212
<i>g_Collinsella</i>	0.0014	0.0004	0.0006	0.0000	0.0314	0.0013	0.0000	0.0000	0.0032	0.0011	0.0008	0.0009
<i>f_Peptostreptococcaceae Incertae_Sedis</i> #*	0.0001	0.0003	0.0006	0.0019	0.0057	0.0115	0.0013	0.0000	0.0037	0.0001	0.0040	0.0018
<i>f_Lachnospiraceae g_g</i>	0.0069	0.0052	0.0004	0.0000	0.0006	0.0005	0.0002	0.0008	0.0071	0.0015	0.0010	0.0000
<i>g_Haemophilus</i> #*	0.0041	0.0057	0.0055	0.0000	0.0000	0.0000	0.0008	0.0000	0.0005	0.0098	0.0004	0.0062
<i>g_Catenibacterium</i> *	0.0000	0.0000	0.0000	0.0090	0.0050	0.0001	0.0000	0.0000	0.0000	0.0010	0.0000	0.0000
<i>g_Akkermansia</i> #*	0.0000	0.0000	0.0000	0.0000	0.0011	0.0050	0.0000	0.0008	0.0003	0.0000	0.0225	0.0008
<i>g_Dorea</i> *	0.0000	0.0001	0.0000	0.0016	0.0026	0.0023	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000
<i>g_Flavonifractor</i> #	0.0000	0.0020	0.0020	0.0014	0.0000	0.0002	0.0002	0.0000	0.0000	0.0034	0.0047	0.0000
<i>g_Halomonas</i> #	0.0003	0.0005	0.0002	0.0000	0.0000	0.0011	0.0002	0.0012	0.0002	0.0007	0.0000	0.0001
<i>f_Ruminococcaceae g_g</i> #	0.0000	0.0003	0.0001	0.0017	0.0002	0.0005	0.0000	0.0000	0.0000	0.0033	0.0044	0.0003
<i>g_Eubacterium</i> *	0.0000	0.0000	0.0000	0.0014	0.0004	0.0000	0.0000	0.0000	0.0000	0.0004	0.0002	0.0000
<i>g_Aeribacillus</i> #	0.0001	0.0002	0.0001	0.0000	0.0000	0.0005	0.0000	0.0005	0.0000	0.0004	0.0000	0.0001
<i>g_Alistipes</i> #	0.0002	0.0001	0.0001	0.0000	0.0000	0.0001	0.0000	0.0003	0.0003	0.0013	0.0058	0.0029
<i>g_Barnesiella</i> #	0.0000	0.0000	0.0002	0.0000	0.0003	0.0000	0.0002	0.0000	0.0000	0.0018	0.0145	0.0031
<i>g_Granulicatella</i> *	0.0000	0.0000	0.0001	0.0000	0.0000	0.0004	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
TOTAL	0.9775	0.9750	0.9666	0.9918	0.9918	0.9900	0.9877	0.9840	0.9842	0.9545	0.9432	0.9715
Other taxa	0.0225	0.0250	0.0334	0.0082	0.0082	0.0104	0.0123	0.0160	0.0158	0.0488	0.0615	0.0285

Table S2. Selected V4 16S rRNA gene based OTUs showing a list of matching taxa with the highest total NCBI scores (NCBI Blast on 12/11/2018)

<i>g_Bifidobacterium_B1: Total BLAST Score: 262</i> TACGTAGGGCGCAAGCGTTATCCGGATTATTGGGCGTAAAGGGCTCGTAGGCGGCTCGTCGCGTCCGGT GCCTGTTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGACGGCCCAGAGACCTGCCTTCGCCATCGGTG
NR_044771.1 <i>Bifidobacterium bifidum</i> strain KCTC 3202 16S ribosomal RNA, partial sequence NR_117505.1 <i>Bifidobacterium bifidum</i> strain KCTC 3202 16S ribosomal RNA gene, partial sequence NR_117764.1 <i>Bifidobacterium bifidum</i> strain DSM 20456 16S ribosomal RNA gene, partial sequence NR_113873.1 <i>Bifidobacterium bifidum</i> strain NBRC 100015 16S ribosomal RNA gene, partial sequence
<i>g_Bifidobacterium_L1: Total BLAST Score: 262</i> TACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTCGTAGGCGGTTTCGTCGCGTCCGGT GCCTGTTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTGACGGCCCAGAGACCTGCCTTCGCCATTGGTG
NR_036857.1 <i>Bifidobacterium ruminantium</i> strain Ru 687 16S ribosomal RNA gene, partial sequence NR_041348.1 <i>Bifidobacterium tsurumiense</i> strain OMB115 16S ribosomal RNA gene, partial sequence NR_116746.1 <i>Bifidobacterium stercoris</i> strain Eg1 16S ribosomal RNA gene, partial sequence NR_118589.1 <i>Bifidobacterium stercoris</i> strain Eg1 16S ribosomal RNA gene, partial sequence NR_114397.1 <i>Bifidobacterium moukalabense</i> strain GG01 16S ribosomal RNA gene, partial sequence NR_133982.1 <i>Bifidobacterium faecale</i> strain CU3-7 16S ribosomal RNA, partial sequence NR_037115.2 <i>Bifidobacterium dentium</i> strain B764 16S ribosomal RNA gene, partial sequence NR_074802.2 <i>Bifidobacterium adolescentis</i> strain ATCC 15703 16S ribosomal RNA, complete sequence
<i>g_Bifidobacterium_L2: Total BLAST Score: 262</i> TACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTCGTAGGCGGTTTCGTCGCGTCCGGT GCCTGTTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAACGGCCCAGAGACCTGCCTTCGCCATTGGTG
NR_043437.1 <i>Bifidobacterium longum</i> subsp. <i>infantis</i> strain ATCC 15697 16S ribosomal RNA gene, partial sequence NR_037118.1 <i>Bifidobacterium gallicum</i> strain P6 16S ribosomal RNA gene, partial sequence NR_040783.1 <i>Bifidobacterium breve</i> strain DSM 20213 16S ribosomal RNA gene, partial sequence NR_041875.1 <i>Bifidobacterium catenulatum</i> strain DSM 16992 16S ribosomal RNA gene, partial sequence NR_117506.1 <i>Bifidobacterium longum</i> strain KCTC 3128 16S ribosomal RNA gene, partial sequence NR_112779.1 <i>Bifidobacterium kashiwanohense</i> strain HM2-2 16S ribosomal RNA gene, partial sequence NR_037117.1 <i>Bifidobacterium pseudocatenulatum</i> strain B1279 16S ribosomal RNA gene, partial sequence NR_113174.1 <i>Bifidobacterium stellenboschense</i> strain AFB23-3 16S ribosomal RNA gene, partial sequence NR_145535.1 <i>Bifidobacterium longum</i> subsp. <i>suillum</i> strain Su 851 16S ribosomal RNA, partial sequence NR_159261.1 <i>Bifidobacterium callitrichidarum</i> strain TRI 5 16S ribosomal RNA, partial sequence