Consumption of a Bifidobacterium bifidum Strain for 4 Weeks Modulates Dominant Intestinal Bacterial Taxa and Fecal Butyrate in Healthy Adults

Giorgio Gargari[1], Valentina Taverniti[1], Silvia Balzaretti[1], Chiara Ferrario[1], Claudio Gardana[2], Paolo Simonetti[2], Simone Guglielmetti[1]#

Department of Food Environmental and Nutritional Sciences (DeFENS), Division of Food Microbiology and Bioprocesses[1], and Division of Nutrition[2], Università degli Studi di Milano, Milano, Italy

Running Head: Fecal microbiota modulation by *B. bifidum*

#Address correspondence to Simone Guglielmetti, simone.guglielmetti@unimi.it.

G.G. and V.T. contributed equally to this work.

Figure S1. Analyses of the fecal microbiota diversity in (panels A and C) and among (panel B) the Probiota-Bb fecal samples. **A**, bacterial richness estimated through rarefaction curves using Chao1 coefficient as α-diversity predictors. **B**, biodiversity according to Chao1 coefficient of α-diversity, before and after the treatments. **C**, Principal Coordinate Analysis (PCoA) based on Weighted Unifrac expressing the β-diversity of samples. Axes of the two panels are the two most informative components explaining the differences among samples (percent of explained variation is shown between brackets on each axis). Each sample is represented by the overall microbiota composition of a single fecal specimen. Samples are separated into four categories: before and after probiotic treatment (left panel), and before and after placebo treatment (right panel). $\sum|v|$, the sum of absolute Euclidean distances of paired points calculated as the sum of square variances of the coordinates of each point before and after a treatment ($|v| = \sqrt{[(x_i-x_j)^2+(y_i-y_j)^2]}$, where «i» indicates before treatment and «j» after treatment). Paired points are the sample before and the sample after a treatment for a specific subject. For coordinates PC1 vs PC2, absolute distances were not significantly different between the probiotic and the placebo treatments.

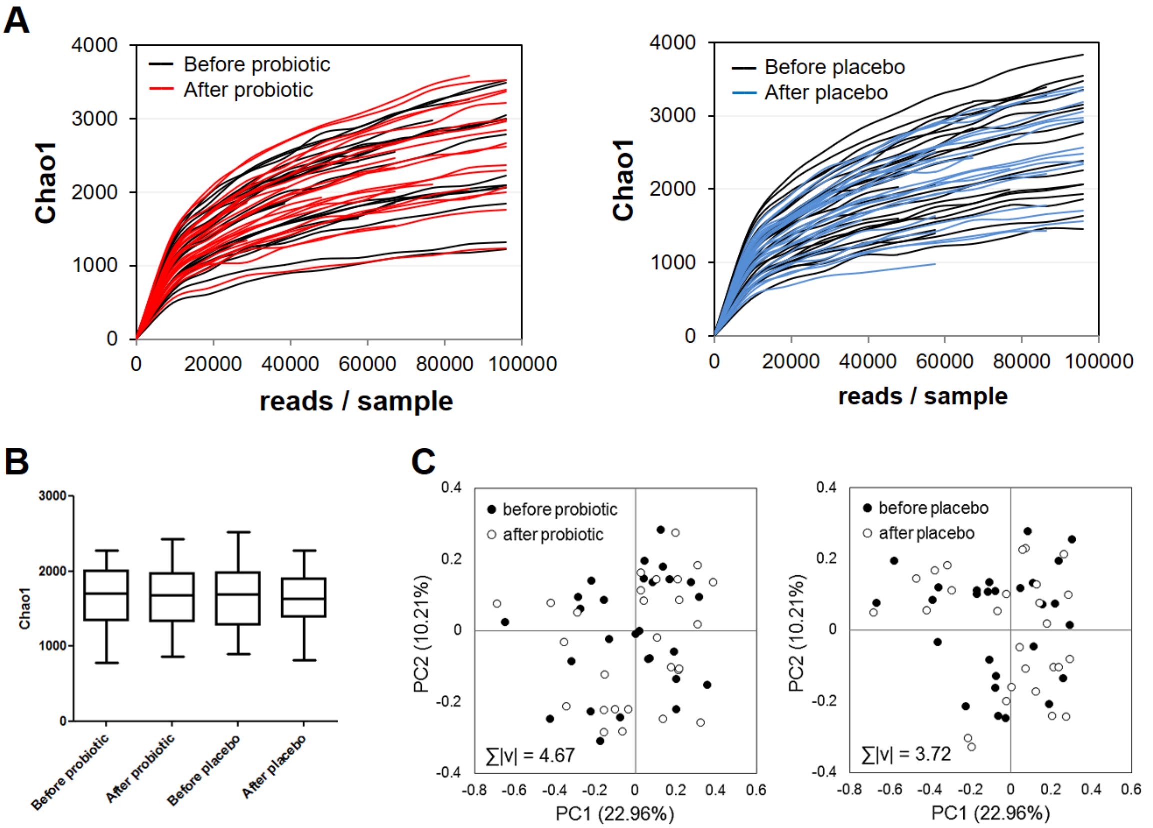

Fig. S2. Analyses of enterotypes in samples of the PROBIOTA-Bb trial. **A**. Principal Coordinated Analysis (PCoA; the first two principal components are shown); clustering was based on genus relative abundance using JSD distance and the Partitioning Around Medoids (PAM) algorithm. The optimal number of clusters was determined through the Calinski-Harabasz (CH) index and the Silhouette coefficient. **B**, intra-subject enterotype shifts observed during the study; red, blue, and dotted-black arrows indicate shifts that occurred across the probiotic, placebo, and wash-out phases respectively. **C**, Tukey boxplots of the dominant bacterial genera distribution in the two identified enterotypes.

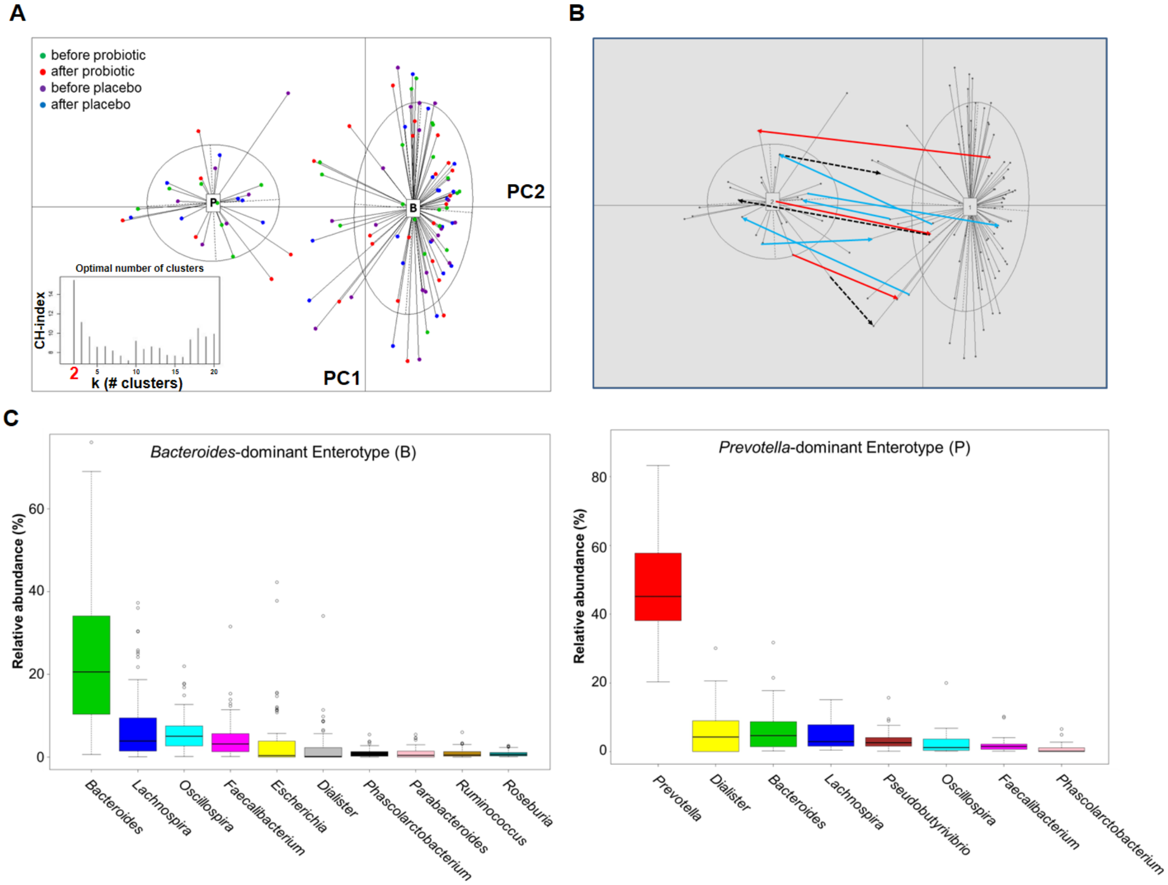

Figure S3. Tukey boxplots representing bacterial taxonomic richness of enterotypes assessed with a Chao1 estimator of α-diversity. *Ba*, *Bacteroides*-dominated enterotypes; *Pr*, *Prevotella*-dominated enterotype. Statistically significant difference is according to Mann Whitney test; ***, $P < 0.001$.

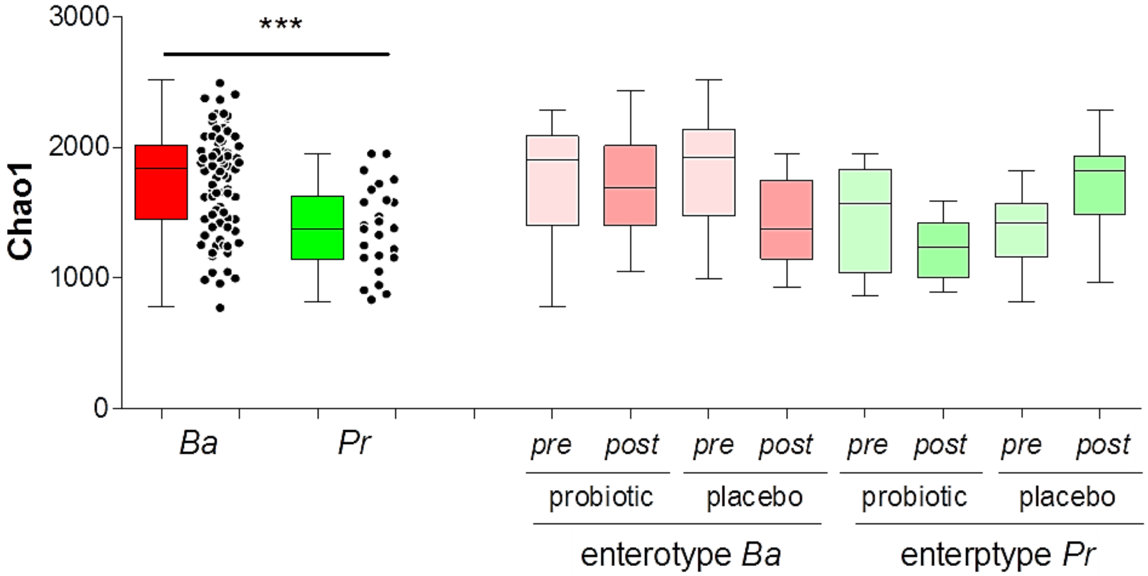

Table S1. Bacterial taxa that were significantly modified by probiotic or placebo treatments. Mean bacterial relative abundances are shown at baseline (before treatment) and post (after treatment), and at visit 1 (V1) and 3 (V3). Visits are according to Fig. 1. p values are according to Wilcoxon-Mann-Whitney test with Benjamini-Hochberg correction to determine statistically significant differences before and after the treatment. Bacterial taxa with a relative abundance higher than 1% are shown in bold. The taxonomic lineage of each taxon is shown; k, kingdom; p, phylum; c, class; o, order; f, family; g, genus.

ALL SUBJECTS	p values	baseline	post
PROBIOTIC			
k_Bacteria;Other	**0.030**	**1.536%**	**2.812%**
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_;g_	0.018	0.162%	0.364%
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae	**0.041**	**14.179%**	**11.973%**
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella	**0.034**	**14.158%**	**11.963%**
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae	**0.010**	**3.987%**	**5.919%**
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_undefined	**0.013**	**3.823%**	**5.640%**
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;Other	0.015	0.120%	0.158%
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_Alistipes	0.013	0.044%	0.122%
p_Firmicutes;c_Bacilli;o_Bacillales;f_Staphylococcaceae;g_Staphylococcus	0.023	0.000%	0.005%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_;g_undefined	0.044	0.000%	0.000%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;Tissierellaceae;g_Peptoniphilus	0.029	0.001%	0.002%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae	0.005	0.405%	0.838%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_undefined	0.006	0.389%	0.810%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;Other	0.006	0.016%	0.027%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_Christensenella	0.042	0.000%	0.001%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Dehalobacteriaceae	0.042	0.002%	0.005%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Dehalobacteriaceae;g_Dehalobacterium	0.046	0.001%	0.004%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae	**0.039**	**12.212%**	**15.271%**
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Anaerotruncus	0.029	0.026%	0.031%
p_Firmicutes;c_Clostridia;o_Clostridiales;Other	**0.008**	**1.289%**	**1.794%**
p_Firmicutes;c_Clostridia;Other	0.030	0.107%	0.215%
p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_;Eubacterium;	0.010	0.023%	0.056%
p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;Other	0.038	0.004%	0.017%
p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae	0.014	0.133%	0.245%
p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae;g_Bilophila	0.010	0.051%	0.110%

PLACEBO			
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;Other	**0.036**	**1.095%**	**0.457%**
p_Firmicutes;c_Bacilli	0.012	0.030%	0.104%
p_Firmicutes;c_Bacilli;o_Gemellales;f_Gemellaceae;g_undefined	0.038	0.000%	0.001%
p_Firmicutes;c_Bacilli;o_Lactobacillales	0.030	0.027%	0.094%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Carnobacteriaceae;g_Granulicatella	0.008	0.000%	0.002%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus	0.019	0.001%	0.002%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Streptococcus	0.019	0.022%	0.087%
p_Firmicutes;c_Bacilli;o_Turicibacterales;f_Turicibacteraceae;g_Turicibacter	0.006	0.001%	0.008%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Peptococcaceae	0.025	0.001%	0.006%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_undefined	0.021	0.024%	0.010%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Veillonella	0.017	0.018%	0.038%
p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Holdemania	0.032	0.003%	0.015%
p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;g_Haemophilus	0.012	0.002%	0.019%
BEFORE TREATMENTS (samples at visit V1 versus V3)	p values	V1	V3
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24;7;g_undefined	0.010	0.025%	0.033%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia	0.004	0.936%	0.413%
p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Bulleidia	0.012	<0.001%	<0.001%

Table S2. Bacterial taxa that were significantly modified by probiotic or placebo treatments in enterotypes. Mean bacterial relative abundances are shown before treatment (baseline) and after treatment (post). p values are according to Wilcoxon test with Benjamini-Hochberg correction to determine statistically significant differences before and after the treatment. Bacterial taxa with a relative abundance higher than 1% are shown in bold. The taxonomic lineage of each taxon is shown; k, kingdom; p, phylum; c, class; o, order; f, family; g, genus.

Bacteroides-dominated enterotype	p values	baseline	post
PROBIOTIC			
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_;g_	0.011	0.026%	0.072%
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_;Paraprevotellaceae;g_;Prevotella;	0.021	0.001%	0.367%
p_Firmicutes;c_Clostridia;o_Clostridiales;Other;Other	**0.039**	**1.169%**	**1.686%**
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;Other	0.005	0.012%	0.032%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_undefined	0.034	0.549%	0.655%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_Christensenella	0.022	0.001%	0.001%
p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae;g_Bilophila	0.044	0.061%	0.146%
PLACEBO			
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;Other;Other	**0.036**	**1.657%**	**0.457%**
p_Firmicutes;c_Bacilli;o_Gemellales;f_Gemellaceae;g_undefined	0.038	0.001%	0.001%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Carnobacteriaceae;g_Granulicatella	0.008	0.001%	0.002%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus	0.019	0.001%	0.002%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Streptococcus	0.019	0.025%	0.087%
p_Firmicutes;c_Bacilli;o_Turicibacterales;f_Turicibacteraceae;g_Turicibacter	0.006	0.001%	0.008%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_undefined	0.021	0.032%	0.010%
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Veillonella	0.017	0.017%	0.038%
p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Holdemania	0.032	0.003%	0.015%
p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;g_Haemophilus	0.012	0.002%	0.019%

Prevotella-dominated enterotype	p values	baseline	post
PROBIOTIC			
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;Other	0.024	0.051%	0.023%
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella	**0.014**	**34.416%**	**23.547%**
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;Other	0.002	0.057%	0.208%
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_undefined	**0.005**	**3.118%**	**6.478%**
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_Alistipes	0.024	0.063%	0.172%
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_;Barnesiellaceae;g_undefined	**0.003**	**0.388%**	**1.092%**
PLACEBO			
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Veillonella	0.031	0.010%	0.084%

Table S3. Levels of short chain fatty acids in the fecal samples. Data are reported as mg per kg of feces. -, not detectable.

Subject	**Phase**	**Pyruvate**	**Lactate**	**Acetate**	**Succinate**	**Propionate**	**Butyrate**	**Isobutyrate**	**Isovalerate**	**Valerate**
S02	Before Probiotic	-	0.77	60.76	6.86	13.97	11.82	6.55	0.46	1.15
S03		0.32	0.55	48.09	1.97	26.18	15.02	-	1.90	5.21
S04		0.38	-	48.16	1.91	6.62	2.43	-	1.11	1.79
S05		0.29	0.41	45.62	1.86	24.70	14.18	-	1.81	4.69
S07		0.26	-	53.96	1.42	17.56	35.63	-	1.44	3.87
S08		0.33	0.93	68.78	1.95	27.35	17.92	-	3.54	4.65
S13		0.19	0.24	50.64	1.35	12.21	21.31	-	0.96	2.26
S14		0.39	0.10	55.40	1.56	23.03	10.99	-	5.62	6.51
S15		0.33	1.81	11.26	1.54	2.32	-	-	1.55	2.00
S16		0.25	0.74	34.82	1.29	13.21	7.53	-	5.21	4.18
S17		-	0.23	23.95	0.85	9.09	3.71	2.60	2.19	2.62
S19		0.27	0.22	19.41	1.62	9.52	5.82	-	1.78	2.64
S20		-	0.23	33.17	0.86	18.85	8.09	4.46	1.06	1.63
S23		-	0.28	103.38	0.72	38.66	18.21	11.16	2.37	6.07
S25		-	0.44	2.77	0.39	1.00	0.67	-	0.43	0.60
S26		-	0.56	53.49	1.25	25.25	22.22	13.35	2.05	2.66
S28		-	0.19	40.23	0.39	16.29	6.60	3.70	0.42	0.97
S32		-	0.95	38.44	0.55	11.18	9.18	4.93	0.96	2.29
S33		-	0.18	17.55	0.45	6.14	1.61	1.86	2.74	1.46
S34		-	0.15	44.40	0.90	9.68	4.51	2.73	1.67	1.56
S35		-	0.24	44.48	0.46	5.07	10.21	5.44	0.94	2.61
S36		-	0.16	37.61	0.59	11.39	6.16	3.28	1.38	2.41
S37		-	0.30	93.50	0.84	37.23	20.43	12.63	3.02	4.40
S38		-	0.14	48.60	0.44	15.82	17.06	11.29	4.34	3.25
S39		-	0.17	37.38	0.70	9.41	2.45	2.31	2.75	2.00
S02	After Probiotic	-	0.16	51.95	0.87	11.69	11.98	7.54	0.82	1.38
S03		0.37	0.46	72.93	1.46	33.96	20.33	-	10.18	6.95
S04		0.38	1.02	22.04	1.61	8.78	2.03	-	2.40	2.10
S05		0.30	0.27	64.70	13.36	29.99	17.95	-	8.98	5.71
S07		0.22	-	45.78	1.83	15.27	27.40	-	1.77	2.70
S08		0.36	1.51	47.32	2.32	6.77	3.99	-	2.48	2.72
S13		0.27	0.25	8.31	1.44	6.37	3.76	-	2.46	2.57
S14		0.35	1.92	76.83	1.70	26.52	9.40	-	5.26	5.71
S15		0.36	1.96	42.40	1.56	7.59	1.32	-	3.91	2.53
S16		0.22	0.36	41.66	1.56	14.03	19.97	-	3.18	3.34
S17		-	0.22	10.39	0.46	2.40	0.65	-	0.65	0.84
S19		0.34	0.29	33.83	1.82	13.34	9.99	-	2.93	3.08
S20		-	0.24	110.13	1.07	49.00	15.48	9.41	2.68	4.23
S23		-	0.74	32.00	2.46	10.27	8.17	4.31	0.43	1.06
S25		-	0.16	40.94	0.52	12.79	11.48	7.77	3.92	3.47
S26		-	0.42	41.81	1.17	17.25	18.09	10.81	3.77	2.73
S28		-	0.28	52.20	0.41	35.10	13.17	7.41	0.49	2.09
S32		-	0.26	12.12	0.94	7.84	4.28	2.76	1.96	1.67
S33		-	0.32	18.60	0.53	3.58	1.66	1.11	1.63	1.06
S34		-	0.23	30.22	0.75	7.91	6.47	4.05	2.14	1.55
S35		-	0.19	63.94	0.42	16.85	21.64	11.98	1.03	4.10
S36		-	0.17	42.63	0.74	20.16	10.47	5.92	1.71	2.15
S37		-	0.09	77.39	0.98	30.90	15.21	9.51	2.59	3.94
S38		-	0.08	43.43	0.49	-	11.00	8.21	4.32	2.54
S39		-	0.09	47.98	0.62	9.89	3.36	2.57	2.53	1.57

Subject	Phase	Pyruvate	Lactate	Acetate	Succinate	Propionate	Butyrate	Isobutyrate	Isovalerate	Valerate
S02	Before placebo	-	0.14	20.48	0.77	5.37	2.19	1.25	1.59	1.51
S03		0.40	0.78	39.45	1.72	9.91	5.16	-	2.77	2.73
S04		0.26	0.47	18.12	1.71	14.82	8.51	-	2.29	2.62
S05		-	0.47	160.15	19.39	39.77	57.22	32.31	0.59	2.49
S07		0.44	0.95	47.04	1.73	14.18	2.41	-	3.19	3.25
S08		0.25	0.55	6.59	1.97	2.72	3.22	-	1.51	1.85
S13		0.25	0.35	21.65	1.47	16.16	-	-	5.11	2.35
S14		0.27	0.28	35.84	3.57	9.57	16.89	-	0.72	2.37
S15		0.29	0.31	34.95	1.71	9.55	0.49	-	4.47	3.89
S16		0.26	0.30	50.38	1.21	17.72	17.77	-	2.06	2.91
S17		-	0.25	64.07	0.42	42.39	6.27	3.25	0.77	0.65
S19		0.26	0.34	19.33	1.75	15.32	8.71	-	2.64	2.53
S20		-	0.22	119.78	0.74	43.94	21.10	11.86	8.34	8.38
S23		-	0.15	4.98	0.59	4.28	0.73	0.33	1.27	1.04
S25		-	0.26	49.38	1.36	17.00	8.11	5.29	1.76	2.43
S26		-	0.08	15.19	0.44	3.07	2.90	1.34	1.06	0.92
S28		-	0.41	51.50	0.78	14.17	7.25	5.28	3.57	2.99
S32		-	0.19	25.33	0.49	10.60	6.17	3.42	0.69	0.98
S33		-	0.25	43.62	0.59	11.55	9.91	5.23	1.35	3.39
S34		-	0.14	18.16	0.63	5.21	0.53	0.95	1.80	1.26
S35		-	0.18	45.81	0.42	19.61	13.07	6.52	0.68	1.46
S36		-	0.06	43.04	0.52	12.40	11.14	7.18	2.26	2.40
S37		-	0.24	13.37	0.65	2.98	3.52	2.29	1.45	1.08
S38		0.34	0.66	26.48	2.03	22.92	4.61	-	1.19	2.30
S39		-	0.19	39.75	9.83	21.80	9.93	5.79	0.59	1.03
S02	After placebo	-	0.14	12.89	0.72	2.37	-	-	0.72	0.80
S03		0.32	1.22	44.58	1.90	7.99	8.59	-	1.84	2.83
S04		0.33	0.45	50.94	1.78	32.35	23.72	-	2.05	7.03
S05		-	0.13	54.62	0.84	10.84	10.32	6.11	0.58	1.22
S07		0.37	0.72	85.83	2.29	53.83	25.93	-	12.23	10.80
S08		0.27	0.44	100.66	1.55	25.59	46.09	-	11.89	3.69
S13		0.39	3.73	39.93	1.63	1.55	-	-	1.00	1.98
S14		0.23	0.25	26.11	2.74	13.60	12.94	-	1.37	3.02
S15		0.26	0.43	13.30	1.34	7.44	3.08	-	2.29	3.13
S16		0.27	0.25	29.15	1.47	6.75	6.30	-	1.18	1.87
S17		-	0.23	40.35	0.44	34.27	9.05	4.77	0.21	1.27
S19		0.28	0.26	48.10	1.66	30.41	22.20	-	3.00	6.60
S20		-	0.22	96.93	0.81	39.93	20.78	13.14	2.40	5.98
S23		-	0.51	10.79	0.64	7.33	1.89	1.47	1.55	1.10
S25		-	0.31	40.49	0.60	11.36	5.75	3.92	2.30	2.64
S26		-	0.84	34.78	0.76	7.99	4.99	2.96	1.95	1.56
S28		-	0.11	34.66	0.40	10.20	7.77	4.13	0.19	1.26
S32		-	0.15	19.44	1.28	6.87	4.49	2.30	0.68	0.83
S33		-	0.16	24.86	0.59	4.17	1.10	0.44	0.93	1.72
S34		-	0.10	10.02	0.57	4.28	1.49	1.92	3.31	2.15
S35		-	0.53	63.90	0.60	33.36	17.38	8.52	1.13	2.50
S36		-	0.12	40.79	0.66	9.57	9.41	6.61	3.30	2.22
S37		-	0.27	15.25	0.48	2.69	2.71	1.39	1.06	0.92
S38		0.32	1.01	66.25	1.81	43.33	10.98	-	2.45	3.83
S39		-	0.14	61.15	1.44	36.84	18.10	10.55	1.93	3.07

Table S4. Effects of the probiotic and placebo treatments on fecal short chain fatty acid (SCFA) concentration. Mean SCFA concentration at baseline (before treatment) and post (after treatment) are reported as mg per kg of feces. p values are according to Wilcoxon-Mann-Whitney test with Benjamini-Hochberg correction to determine statistically significant differences before and after the treatments. The asterisk (*) indicates statistically significant differences ($p < 0.05$).

	All subjects			SCFA group L			SCFA group H		
	p values	baseline	post	p values	baseline	post	p values	baseline	post
PROBIOTIC									
Acetate	0,833	101,44	106,58	0.268	30.97	40.65	0.365	62.02	51.13
Butyrate	0,271	22,78	27,51	**0.025 ***	**4.93**	**8.71**	**0.042 ***	**18.62**	**13.39**
Isovalerate	0,853	5,37	6,15	0.217	1.73	2.23	0.465	2.50	3.92
Lactate	0,653	0,82	1,27	0.808	0.41	0.41	0.919	0.39	0.54
Propionate	0,711	38,70	44,49	0.068	9.27	14.95	0.102	23.81	17.18
Succinate	0,508	5,65	2,90	0.502	0.89	0.93	0.278	1.84	2.55
Valerate	0,426	5,88	7,40	0.502	2.06	2.41	0.365	4.06	3.45
PLACEBO									
Acetate	0,937	111,58	113,16	0.179	29.53	40.23	0.125	98.60	55.27
Butyrate	0,916	27,38	26,92	0.083	5.97	10.89	0.375	25.59	11.61
Isovalerate	0,120	5,1708	7,42	0.393	2.00	2.72	0.125	2.94	1.09
Lactate	0,989	1,00	1,170	0.338	0.33	0.57	0.250	0.31	0.21
Propionate	0,874	39,17	39,82	0.203	11.58	16.82	0.125	35.95	22.95
Succinate	0,164	3,27	4,108	0.473	1.65	1.21	0.875	5.44	0.89
Valerate	0,853	7,35	7,179	0.128	2.11	3.03	0.250	3.61	2.58

Table S5. Bacterial taxa that were significantly modified by probiotic or placebo treatments in SCFA groups. Mean bacterial relative abundances are shown before treatment (baseline) and after treatment (post). p values are according to Wilcoxon test with Benjamini-Hochberg correction to determine statistically significant differences before and after the treatment. All bacterial taxa significantly modified by the two treatments have a relative abundance lower than 1%. The taxonomic lineage of each taxon is shown; k, kingdom; p, phylum; c, class; o, order; f, family; g, genus.

SCFA group L	p values	baseline	post
PROBIOTIC			
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_SMB53	0.048	0.060%	0.147%
PLACEBO			
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_;Odoribacteraceae;g_Odoribacter	0.036	0.102%	0.199%
p_Firmicutes;c_Bacilli;o_Gemellales;f_Gemellaceae;g_	0.045	0.001%	0.001%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Carnobacteriaceae;g_Grnulicatella	0.025	0.001%	0.002%
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Streptococcus	0.006	0.013%	0.103%
p_Firmicutes;c_Bacilli;o_Turicibacterales;f_Turicibacteraceae;g_Turicibacter	0.014	0.002%	0.008%
p_Proteobacteria;c_Bet_prePROroteobacteria;Other	0.037	0.001%	0.002%

SCFA group H	p values	baseline	post
PROBIOTIC			
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Tissierellaceae;g_WAL_1855D	0.035	0.001%	0.005%
PLACEBO			
/			