

SUPPLEMENTARY MATERIAL

Methods

Supplementary material 1: Inclusion and exclusion criteria

Patients with constipation-predominant IBS were not included as restricting substrates that increase small bowel water content might worsen constipation. Participants were naïve to the LFD and were not already restricting lactose and wheat in their diet. Patients with a medical condition that could have impacted gut microbiota (e.g. inflammatory bowel disease, coeliac disease, diabetes) were excluded as were those considered unable to comply with study protocol (e.g. severe psychiatric illness, current eating disorder). Other exclusion criteria were IBS symptoms related only to alcohol or caffeine consumption, procedures or use of medication that could affect colonic luminal environment (e.g. bowel preparation, antibiotics, probiotics, prebiotics, lactulose), or change to IBS medication type or dose, within 4-weeks of starting the trial and pregnancy or lactation. Patients that violated the protocol during the trial were withdrawn and excluded from per protocol analysis.

Supplementary material 2: Dietary interventions

Low FODMAP diet

The low FODMAP diet restricts fermentable carbohydrates including fructo-oligosaccharides (fructans e.g. wheat, onion, garlic), α -galacto-oligosaccharides (beans and pulses containing non-selectively fermented raffinose, stachyose and verbascose), disaccharides (lactose e.g. cow's milk), monosaccharides (excess fructose e.g. honey and mango), and the polyols sorbitol (e.g. avocado and sweetcorn) and mannitol (e.g. cauliflower and sweet potato) [1].

Sham diet

The Sham diet was designed to be a detailed exclusion diet of similar difficulty to the low FODMAP diet restricting a similar number of foods without impacting nutrient intake, fibre content or FODMAP intake. Carbohydrate sources are modified within each food group e.g. rice is disallowed but potato is allowed, citrus fruits with pith are disallowed but apples and bananas are allowed, for the allowed and non-allowed categories there is a mixture of fruit and vegetables ensuring that the diet neither promotes high FODMAP eating nor restricts any class of FODMAPs.

Supplementary material 3: Microbiota analysis

FISH was performed using a previously described method [2]. Prior to storage, 50% w/v glycerol was added to the stool aliquot to preserve cells during freeze/thaw. Bacterial cells were extracted from stool and fixed in paraformaldehyde before hybridization with fluorescent oligonucleotide DNA probes specific for bifidobacteria (Bif164[3]) and total bacteria (EUB338, EUB338II and EUB338III[4]). Cells were counted manually on a Nikon Eclipse E400 microscope at 1000 magnification by an independent researcher blinded to treatment allocation.

Sequencing was performed by amplification of the V4 region of bacterial 16S rRNA using PCR and Illumina adapter sequences using an Illumina MiSeq platform and a custom pipeline based on Quantitative Insights Into Microbial Ecology (QIIME) 1.9.0 [5]. Both α -diversity (Chao-1) and β -diversity (Bray-Curtis) were measured. A 95000 rarefaction threshold and a filter to remove lower abundance OTUs with a minimum fraction count below 0.0005 were applied before taxonomy assignment [6]. To identify key differences in microbial relative abundance, any genus with <1% abundance in all patient samples was removed before statistical analysis.

Supplementary material 4: Urine analysis

The first urine sample of the day (mid-stream) was collected and 1 ml stored at -80°C until analysis. A 2:1 urine:phosphate buffer (0.2 M sodium dibasic, 0.05 M sodium phosphate monobasic dihydrate, 2 mM sodium azide in 50:50 MilliQ H₂O:deuterium oxide containing 1 mM sodium 3-(trimethylsilyl)propionate-d₄) mix was prepared (adapted from[7]). ¹H NMR was performed under automation at 298 Kelvin and 700 MHz. Spectra were acquired into Topspin™ software (Bruker Biospin, Germany), normalized then aligned with MVAPACK [8]. Metabolite profiles were compared using supervised orthogonal partial-least squared discriminant analysis (OPLS-DA) using bespoke software [9]. S-plots were used to identify the spectral co-ordinates of metabolites that most contributed to separation between groups. The co-ordinates were then assigned to known metabolites using Chenomx® software.

Results

Supplementary material 5: Recruitment and adherence

Two patients withdrew prior to the 1-week timepoint and therefore did not provide compliance data. In the 67 patients who provided compliance data, across the 4-week intervention period 21/22 (95%) patients met compliance thresholds for diet and 21/22 (95%) for supplement in the control group, 20/22 (91%) met compliance thresholds for diet and 22/22 (100%) for supplement in LFD, and 20/23 (87%) met compliance thresholds for diet and 23/23 (100%) for supplement in LFD/B-GOS.

Supplementary material 6: IBS Quality of life scores, and perceived stress scores in intention-to-treat population at follow-up in patients receiving sham dietary advice, low FODMAP dietary advice or low FODMAP dietary advice plus B-GOS prebiotic

	Control (n=23)		LFD (n=22)		LFD/B-GOS (n=24)		
IBS-QoL Sub-scale	EMM	SE	EMM	SE	EMM	SE	*p-value
Dysphoria	70.3	(3.5)	75.5	(3.6)	71.3	(3.4)	0.544
Interference with activity	71.8	(3.5)	71.2	(3.5)	76.2	(3.4)	0.554
Body image	69.2	(3.3)	72.0	(3.3)	72.9	(3.3)	0.720
Health worry	68.5	(3.6)	75.2	(3.6)	70.9	(3.5)	0.422
Food avoidance	62.7	(4.2)	56.8	(4.3)	63.2	(4.1)	0.500
Social reaction	73.0	(3.5)	72.6	(3.6)	72.9	(3.4)	0.996
Sexual	86.2	(2.5)	90.2	(2.6)	88.3	(2.4)	0.560
Relationships	79.0	(3.2)	80.7	(3.3)	77.1	(3.1)	0.730
Overall score	71.9	(2.7)	73.8	(2.7)	72.9	(2.6)	0.885
MCID n (%)	7	(30)	8	(36)	5	(21)	0.703**
Perceived Stress Scale	16.1	(1.0)	16.2	(1.1)	16.7	(1.1)	0.905

Values are presented as estimated marginal means (EMM) (std error (SE)) except MCID, which is the number (%) achieving minimal clinically important difference (MCID) in total score from baseline per group. *ANCOVA analysis. ** Pearson Chi² analysis. LFD, low FODMAP diet. B-GOS, B-galactooligosaccharide.

Discussion: IBS-QoL

Whilst we did not show any change in QoL, two studies have reported that the LFD improved IBS-QoL. One was non-blinded ^[10], and the other recruited more participants than the current study (n=103) and therefore had more power to detect a difference compared with a sham diet ^[11].

Supplementary material 7: Adverse events and acceptability

There were no serious adverse events reported. In the control group 13 patients reported a total of 16 adverse events (five GI related, three cold or flu and eight other (one incidence each of itchy rash from gardening, eye infection, sore jaw from grinding teeth, increased anxiety, candidiasis, swelling in legs, sprained ankle, and sweaty after taking sachet)), in the LFD group five patients reported a total of seven adverse events (2 GI related, 3 cold or flu

and 2 other (one incidence each of feeling as though a UTI was starting at final visit and heartburn)). In the LFD/B-GOS group six patients reported a total of six adverse events (4 GI related and 2 other (one incidence each of muscular cramp and arthritic pain)). The number of patients reporting adverse events was significantly different across groups (χ^2 (2, N=64) = 6.769, $p=0.034$), with higher values in the control group. However, the number of patients reporting GI-related adverse events was not different across groups (χ^2 (2, N=64) = 1.392, $p=0.499$).

Acceptability of the intervention diets (sham (n=21), LFD (n=42)) was reported in the *per protocol* population. Compared to habitual diet, time spent shopping for food was perceived to take longer by fewer patients on the sham diet (12/21 (57%)) than the LFD (37/42 (88%)), $p=0.009$). However similar percentages reported that compared to habitual diet meal preparation was more difficult (sham 16/21 (76%), LFD 36/42 (86%), $p=0.483$), dining out was more difficult (sham 18/21 (86%), LFD 40/42 (95%), $p=0.323$), and the diet was more troublesome (sham 16/21 (76%), LFD 37/42 (88%), $p=0.280$) (full data provided in supplementary material 8).

Supplementary material 8: Acceptability of the sham diet and the low FODMAP diet compared to habitual diet in patients with irritable bowel syndrome

Acceptability of intervention diet compared with habitual diet, n (%)	Sham diet (n=21)	Low FODMAP diet (n=42)	p-value*
Meal preparation was more difficult	16 (76.2)	36 (85.7)	0.483
Time spent food shopping was longer	12 (57.1)	37 (88.1)	0.009
Time spent preparing food was longer	8 (38.1)	20 (47.6)	0.593
Dining out was more difficult	18 (85.7)	40 (95.2)	0.323
Flavor of food was less palatable	5 (23.8)	25 (59.5)	0.009
Cost of groceries was higher	5 (23.8)	33 (78.6)	<0.001
Understanding written information was easy	21 (100)	37 (88.1)	0.16
The diet was less convenient than normal diet	0 (0)	5 (11.9)	0.16
The diet was more troublesome than normal diet	16 (76.2)	37 (88.1)	0.28
Benefits of diet outweighed the burden	14 (66.7)	28 (66.7)	1.000

*p Values calculated by χ^2

Supplementary material 9: Absolute abundance (log₁₀ cells/ g dry weight) of Bifidobacteria and Total bacteria in the control, LFD and LFD/B-GOS groups.

	Control (n=20)		LFD (n=21)		LFD/B-GOS (n=21)		p*
Bifidobacteria							
Baseline	9.92	(0.21)	9.85	(0.38)	9.84	(0.43)	0.477
Week 1	9.86	(0.26)	9.69	(0.66)	9.69	(1.17)	0.202
Week 4	9.77	(0.41)	9.62	(0.62)	9.49	(0.73)	0.023^a
Change from baseline							
Week 1	-0.09	(0.20)	-0.14	(0.53)	-0.16	(0.71)	0.374
Week 4	-0.10	(0.35)	-0.13	(0.56)	-0.34	(0.40)	0.008^b
Total bacteria							
Baseline	11.12	(0.25)	11.09	(0.17)	11.04	(0.20)	0.790
Week 1	11.05	(0.16)	11.04	(0.11)	11.01	(0.24)	0.873
Week 4	11.06	(0.25)	10.99	(0.18)	10.98	(0.24)	0.473
Change from baseline							
Week 1	-0.04	(0.12)	-0.05	(0.14)	-0.04	(0.13)	0.861
Week 4	-0.06	(0.14)	-0.03	(0.21)	-0.12	(0.18)	0.451

Data are presented as Median (IQR).

*p calculated using Kruskal-Wallis test.

Bonferroni post-hoc

a: Control vs LFD p=0.559; control vs LFD/B-GOS p=0.018, LFD vs LFD/B-GOS p=0.465

b: Control vs LFD p=0.238; control vs LFD/B-GOS p=0.006; LFD vs LFD/B-GOS p=0.530

Supplementary material 10: Microbiota diversity

At 4-weeks, there was no difference in Chao-1 (α -diversity) between control (267.2 (48.7)) and LFD (280.7 (55.7), p=0.732) or LFD/B-GOS (288.4 (51.7), p=0.696), nor between LFD and LFD/B-GOS groups (p=0.666). There was also no difference in Shannon index (α -diversity) between control (5.5 (0.6)) and LFD (5.6 (0.5), p=1.000) or LFD/B-GOS (5.6 (0.6), p=1.000), nor between LFD and LFD/B-GOS groups (p=0.936). There was no difference in Bray-Curtis dissimilarity index (β -diversity) at 4-weeks between control and LFD (t-test statistic=0.51, p=1.000), control and LFD/B-GOS (t-test statistic=1.06, p=1.000), or LFD and LFD/B-GOS (t-test statistic=-0.67, p=1.000).

Supplementary material 11: 16S RNA sequencing data showing proportion of phyla and genera >1% abundant in all patients receiving sham dietary advice, low FODMAP dietary advice or low FODMAP dietary advice plus B-GOS prebiotic at baseline and follow up.

Mean % (SD)	Control					LFD					LFD/B-GOS					Baseline 4-weeks	
	BL (n=16)		FU (n=16)		p*	BL (n=18)		FU (n=20)		p*	BL (n=19)		FU (n=20)		p*	p**	p***
Phylum																	
Euryarchaeota	0.30	(0.69)	0.20	(0.55)	0.317	0.34	(0.61)	0.07	(0.15)	0.028	0.40	(0.63)	0.31	(0.68)	0.092	0.531	0.409
Bacteria Other	0.03	(0.06)	0.03	(0.07)	0.317	0.08	(0.16)	0.10	(0.13)	0.212	0.04	(0.08)	0.05	(0.10)	1.000	0.672	0.064
Actinobacteria	3.16	(3.74)	4.22	(3.07)	0.198	2.42	(2.00)	1.90	(1.31)	0.098	4.26	(5.58)	1.82	(1.25)	0.058	0.796	p<0.001^a
Bacteroidetes	23.85	(15.04)	20.21	(13.01)	0.331	21.45	(13.14)	22.89	(9.16)	0.384	26.12	(16.27)	25.81	(12.71)	0.811	0.622	0.373
Cyanobacteria	0.03	(0.07)	0.01	(0.03)	0.564	0.06	(0.21)	0.02	(0.07)	0.257	0.04	(0.16)	0.04	(0.11)	0.317	0.481	0.759
Elusimicrobia	0.00	(0.00)	0.00	(0.00)	1.000	0.00	(0.00)	0.00	(0.00)	1.000	0.37	(1.63)	0.03	(0.13)	0.317	0.409	1.000
Firmicutes	70.96	(14.32)	73.90	(12.56)	0.510	73.44	(12.63)	73.30	(8.97)	0.679	66.77	(14.54)	70.27	(12.84)	0.711	0.393	0.683
Fusobacteria	0.00	(0.00)	0.00	(0.00)	1.000	0.00	(0.00)	0.00	(0.00)	1.000	0.01	(0.05)	0.00	(0.00)	0.317	0.409	-
Lentisphaerae	0.00	(0.00)	0.00	(0.00)	1.000	0.02	(0.07)	0.02	(0.07)	0.317	0.00	(0.00)	0.01	(0.02)	1.000	0.138	0.686
Proteobacteria	1.31	(1.02)	1.06	(0.98)	0.220	1.41	(1.36)	1.23	(0.65)	0.810	1.16	(1.45)	1.19	(1.23)	0.924	0.286	0.794
Spirochaetes	0.00	(0.00)	0.00	(0.00)	1.000	0.00	(0.00)	0.00	(0.00)	1.000	0.00	(0.00)	0.00	(0.00)	1.000	1.000	-
Synergistetes	0.00	(0.00)	0.02	(0.08)	1.000	0.02	(0.05)	0.04	(0.14)	0.257	0.00	(0.00)	0.00	(0.00)	1.000	0.138	0.936
TM7	0.01	(0.03)	0.02	(0.04)	0.317	0.01	(0.02)	0.01	(0.02)	1.000	0.01	(0.02)	0.00	(0.00)	0.317	0.992	0.250
Tenericutes	0.03	(0.10)	0.06	(0.18)	0.102	0.08	(0.21)	0.04	(0.07)	0.458	0.05	(0.12)	0.04	(0.08)	0.739	0.358	0.440
Verrucomicrobia	0.24	(0.48)	0.19	(0.47)	0.043	0.61	(0.80)	0.31	(0.60)	0.090	0.65	(1.09)	0.39	(0.79)	0.678	0.524	0.460
Genus																	
Phylum_Archaea																	
Methanobrevibacter	0.30	(0.69)	0.20	(0.55)	0.317	0.27	(0.44)	0.05	(0.08)	0.028	0.38	(0.59)	0.28	(0.64)	0.106	0.526	0.396
Methanosphaera	0.00	(0.00)	0.00	(0.00)	0.317	0.07	(0.25)	0.03	(0.09)	1.000	0.02	(0.10)	0.03	(0.11)	0.317	0.760	0.426
Phylum_Actinobacteria																	
Bifidobacterium	1.97	(2.66)	2.63	(2.61)	0.777	1.31	(1.45)	0.96	(1.03)	0.107	3.02	(4.14)	0.93	(1.26)	0.010	0.704	0.002^b
Other (f__Coriobacteriaceae)	0.17	(0.27)	0.26	(0.38)	0.104	0.23	(0.19)	0.28	(0.27)	0.095	0.32	(0.50)	0.33	(0.35)	0.699	0.321	0.975
Collinsella	0.78	(1.22)	1.13	(1.02)	0.141	0.74	(0.92)	0.50	(0.56)	0.033	0.79	(1.29)	0.44	(0.34)	0.141	0.994	0.004^c
Phylum_Bacteroidetes																	

Mean % (SD)	Control					LFD					LFD/B-GOS					Baseline 4-weeks	
	BL (n=16)		FU (n=16)		p*	BL (n=18)		FU (n=20)		p*	BL (n=19)		FU (n=20)		p*	p**	p***
Other (o__Bacteroidales)	0.08	(0.31)	0.05	(0.19)	0.317	0.00	(0.00)	0.00	(0.00)	0.317	0.00	(0.01)	0.02	(0.08)	0.317	0.986	0.430
Other (f__Barnesiellaceae)	0.65	(0.74)	0.55	(0.61)	0.036	0.56	(0.52)	0.39	(0.33)	0.047	0.52	(0.69)	0.46	(0.60)	0.469	0.747	0.566
Odoribacter	0.21	(0.27)	0.12	(0.11)	0.133	0.22	(0.28)	0.17	(0.16)	0.605	0.17	(0.17)	0.22	(0.19)	0.507	0.923	0.132
[Prevotella][f__Paraprevotellaceae]	0.22	(0.61)	0.08	(0.28)	0.180	0.14	(0.61)	0.09	(0.34)	0.655	0.40	(1.08)	0.70	(1.53)	0.109	0.610	0.225
Paraprevotella	0.13	(0.31)	0.05	(0.10)	0.785	0.12	(0.28)	0.09	(0.17)	0.785	0.12	(0.23)	0.20	(0.46)	0.042	0.867	0.310
Bacteroides	11.31	(11.53)	8.91	(7.62)	0.173	10.41	(7.82)	13.86	(9.50)	0.039	15.15	(10.49)	15.41	(12.85)	0.733	0.374	0.094
Parabacteroides	0.69	(0.62)	0.73	(0.75)	0.823	0.76	(0.65)	1.16	(2.13)	0.586	2.25	(5.89)	0.89	(0.57)	0.909	0.522	0.484
Prevotella	8.91	(16.5)	8.18	(15.46)	1.000	7.09	(13.60)	5.08	(7.25)	0.678	5.95	(14.91)	5.82	(11.25)	0.401	0.883	0.561
Other (f__Rikenellaceae)	0.29	(0.44)	0.38	(0.58)	0.565	0.51	(0.59)	0.50	(0.53)	0.950	0.22	(0.25)	0.30	(0.36)	0.299	0.252	0.745
Human (f__Rikenellaceae)	0.00	(0.00)	0.00	(0.00)	1.000	0.41	(0.99)	0.29	(0.60)	0.715	0.04	(0.14)	0.04	(0.16)	0.655	0.108	0.407
Other (f__Rikenellaceae)	0.98	(1.20)	0.88	(0.86)	0.928	0.91	(0.86)	0.92	(1.12)	0.652	0.68	(0.71)	1.04	(1.07)	0.232	0.673	0.640
S24-7 (o__Bacteroidales)	0.24	(0.69)	0.18	(0.40)	0.180	0.20	(0.50)	0.24	(0.63)	0.336	0.48	(1.16)	0.60	(1.23)	0.916	0.666	0.946
Phylum_Elusimicrobia																	
Other (f__Elusimicrobiaceae)	0.00	(0.00)	0.00	(0.00)	0.317	0.00	(0.00)	0.00	(0.00)	0.317	0.37	(1.63)	0.03	(0.14)	1.000	0.409	0.479
Phylum_Firmicutes_Class_Bacilli																	
Lactobacillus	0.18	(0.65)	0.04	(0.15)	0.414	0.03	(0.08)	0.01	(0.03)	0.276	0.01	(0.03)	0.01	(0.04)	1.000	0.732	0.327
Lactococcus	0.21	(0.85)	0.01	(0.02)	0.074	0.02	(0.04)	0.03	(0.06)	0.084	0.02	(0.05)	0.02	(0.04)	0.953	0.683	0.872
Streptococcus	0.84	(1.44)	0.66	(0.82)	0.759	0.44	(0.70)	0.72	(1.82)	0.465	0.35	(0.43)	0.53	(0.76)	0.200	0.557	0.679
Phylum_Firmicutes_Class_Clostridia																	
Other (o__Clostridiales)	1.49	(2.37)	2.84	(4.39)	0.086	2.31	(2.62)	1.73	(1.70)	0.925	1.39	(2.30)	1.36	(1.99)	0.484	0.123	0.348
Other (f__Mogibacteriaceae)	0.34	(0.78)	0.19	(0.29)	0.301	0.13	(0.09)	0.14	(0.11)	0.265	0.10	(0.09)	0.11	(0.10)	0.813	0.545	0.447
Other (f__Christensenellaceae)	0.28	(0.47)	0.23	(0.33)	0.496	0.29	(0.45)	0.44	(1.22)	0.593	0.19	(0.32)	0.27	(0.50)	0.779	0.413	0.632
Other (f__Clostridiaceae)	0.05	(0.09)	0.09	(0.11)	0.363	0.15	(0.33)	0.06	(0.12)	0.210	0.03	(0.03)	0.04	(0.10)	0.420	0.608	0.319
Clostridium	0.38	(0.41)	0.48	(0.33)	0.717	0.51	(0.42)	1.07	(1.32)	0.078	0.35	(0.42)	0.59	(0.59)	0.082	0.434	0.150
SMB53 (f__Clostridiaceae)	0.03	(0.13)	0.04	(0.07)	1.000	0.14	(0.30)	0.22	(0.31)	0.122	0.04	(0.16)	0.04	(0.10)	0.713	0.044^d	0.052
Other (f__Clostridiaceae)	0.00	(0.00)	0.10	(0.34)	0.068	0.00	(0.00)	0.14	(0.37)	0.009	0.03	(0.13)	0.03	(0.10)	0.916	0.164	0.506
Other (f__Lachnospiraceae)	1.58	(0.76)	1.59	(1.08)	0.660	1.22	(0.66)	1.47	(0.75)	0.024	1.59	(1.51)	1.57	(1.12)	0.816	0.241	0.881
Ruminococcus (f__Lachnospiraceae)	2.41	(2.10)	2.15	(1.34)	0.969	1.42	(0.95)	1.47	(1.08)	0.507	2.19	(1.38)	2.03	(1.83)	0.896	0.167	0.839

Mean % (SD)	Control					LFD					LFD/B-GOS					Baseline 4-weeks	
	BL (n=16)		FU (n=16)		p*	BL (n=18)		FU (n=20)		p*	BL (n=19)		FU (n=20)		p*	p**	p***
Anaerostipes	0.18	(0.21)	0.34	(0.47)	0.116	0.32	(0.53)	0.49	(1.01)	0.594	0.22	(0.27)	0.16	(0.24)	0.445	0.889	0.246
Blautia	5.65	(3.93)	6.47	(4.05)	0.294	5.65	(4.20)	5.22	(3.11)	0.327	5.58	(3.27)	5.42	(4.20)	0.740	0.985	0.359
Butyrivibrio	0.01	(0.03)	0.01	(0.05)	0.317	0.27	(0.85)	0.54	(1.86)	0.109	0.00	(0.00)	0.00	(0.00)	1.000	0.336	0.902
Clostridium	0.19	(0.31)	0.19	(0.26)	0.666	0.19	(0.33)	0.28	(0.51)	0.673	0.55	(1.26)	0.49	(1.28)	0.310	0.906	0.669
Coprococcus	6.26	(4.62)	6.43	(2.91)	0.660	6.29	(2.92)	4.69	(2.79)	0.014	5.74	(2.86)	4.56	(2.87)	0.085	0.732	0.099
Dorea	0.58	(0.46)	0.59	(0.34)	0.505	0.73	(0.53)	0.63	(0.57)	0.094	0.52	(0.27)	0.54	(0.34)	0.392	0.607	0.982
Lachnobacterium	0.61	(0.81)	0.30	(0.63)	0.152	0.50	(0.82)	0.46	(0.83)	0.833	0.05	(0.16)	0.12	(0.26)	0.340	0.007^e	0.334
Lachnospira	2.04	(2.09)	2.09	(2.08)	0.659	2.05	(1.51)	2.30	(1.37)	0.257	1.70	(1.91)	2.64	(1.62)	0.045	0.452	0.345
Phylum_Firmicutes_Class_Clostridia																	
Roseburia	4.92	(3.84)	6.47	(4.78)	0.209	6.03	(4.99)	7.28	(6.08)	0.327	6.22	(6.07)	6.65	(4.92)	0.777	0.869	0.793
Other (f__Lachnospiraceae)	5.11	(2.73)	5.64	(2.94)	0.157	5.13	(1.85)	5.67	(2.49)	0.320	5.12	(1.94)	5.93	(3.55)	0.176	0.953	0.918
Other (f__Peptostreptococcaceae)	0.38	(0.51)	0.48	(0.53)	0.623	0.34	(0.34)	0.31	(0.26)	0.263	0.38	(0.48)	0.44	(1.03)	0.118	0.821	0.729
Other (f__Ruminococcaceae)	1.76	(2.50)	1.52	(2.09)	0.343	2.11	(2.31)	1.88	(2.23)	0.468	1.28	(1.43)	1.33	(1.26)	0.740	0.454	0.972
Faecalibacterium	9.94	(5.74)	12.20	(6.53)	0.209	11.99	(5.74)	12.25	(4.40)	0.896	10.38	(5.45)	10.83	(6.04)	0.663	0.498	0.897
Oscillospira	2.06	(1.45)	2.44	(2.55)	0.929	3.52	(2.92)	2.94	(1.49)	0.653	2.95	(2.49)	3.48	(2.38)	0.332	0.279	0.259
Ruminococcus	3.49	(3.58)	3.48	(3.23)	0.944	4.34	(1.99)	3.98	(4.20)	0.248	3.39	(4.40)	2.94	(2.37)	0.679	0.234	0.762
Subdoligranulum	0.16	(0.24)	0.32	(0.65)	0.308	0.15	(0.25)	0.28	(0.68)	0.196	0.12	(0.22)	0.14	(0.23)	0.209	0.657	0.551
Other (f__Ruminococcaceae)	3.99	(2.76)	4.14	(2.79)	0.752	4.95	(2.85)	4.57	(3.54)	0.395	3.59	(2.16)	4.66	(2.97)	0.059	0.347	0.542
Other (f__Veillonellaceae)	0.00	(0.00)	0.00	(0.00)	1.000	0.02	(0.09)	0.21	(0.92)	0.317	0.00	(0.01)	0.82	(3.64)	0.414	0.219	0.347
Acidaminococcus	0.99	(2.10)	0.24	(0.87)	1.000	0.24	(0.61)	0.36	(0.89)	0.715	0.59	(1.82)	0.22	(0.85)	0.180	0.761	0.799
Anaerovibrio	0.46	(1.83)	0.44	(1.78)	0.317	0.00	(0.00)	0.00	(0.00)	1.000	0.00	(0.00)	0.00	(0.00)	1.000	0.315	1.000
Dialister	1.49	(1.86)	2.69	(3.72)	0.343	1.66	(2.31)	2.02	(3.51)	0.889	1.99	(1.74)	1.71	(1.72)	0.045	0.539	0.050^f
Megamonas	0.00	(0.00)	0.00	(0.00)	1.000	1.40	(5.94)	0.83	(3.40)	0.317	2.13	(7.68)	1.66	(5.87)	0.593	0.208	0.403
Megasphaera	2.24	(7.42)	0.69	(2.57)	0.109	0.85	(3.04)	0.34	(1.13)	0.180	2.37	(5.97)	1.03	(3.01)	0.144	0.514	0.270
Mitsuokella	0.00	(0.00)	0.08	(0.33)	0.317	0.00	(0.00)	0.00	(0.00)	1.000	0.46	(1.34)	0.17	(0.38)	0.465	0.023^g	0.451
Phascolarctobacterium	8.01	(9.29)	5.41	(6.98)	0.202	5.91	(6.22)	6.21	(6.10)	0.875	3.49	(4.68)	5.75	(7.05)	0.101	0.218	0.231
Succiniclasticum	0.00	(0.00)	0.00	(0.00)	1.000	0.00	(0.00)	0.00	(0.00)	1.000	0.07	(0.30)	0.00	(0.01)	0.705	0.161	0.156
Veillonella	1.34	(3.52)	1.07	(3.52)	0.959	0.44	(0.56)	0.46	(0.75)	0.590	0.22	(0.36)	0.34	(0.74)	0.244	0.537	0.539

Mean % (SD)	Control					LFD					LFD/B-GOS					Baseline 4-weeks	
	BL (n=16)		FU (n=16)		p*	BL (n=18)		FU (n=20)		p*	BL (n=19)		FU (n=20)		p*	p**	p***
Other (o__Clostridiales)	0.96	(0.62)	1.19	(0.66)	0.276	1.18	(0.69)	1.17	(0.55)	0.243	0.83	(0.55)	1.01	(0.85)	0.191	0.230	0.953
Catenibacterium	0.02	(0.06)	0.11	(0.28)	0.109	0.02	(0.07)	0.03	(0.09)	0.285	0.04	(0.12)	0.11	(0.32)	0.345	0.784	0.495
Phylum_Proteobacteria																	
Other (c__Alphaproteobacteria)	0.09	(0.16)	0.04	(0.09)	0.572	0.26	(0.99)	0.07	(0.13)	0.435	0.07	(0.16)	0.13	(0.23)	0.258	0.464	0.291
Sutterella	0.71	(0.61)	0.41	(0.38)	0.049	0.63	(0.44)	0.62	(0.53)	0.755	0.24	(0.26)	0.39	(0.32)	0.072	0.003^h	0.307
Bilophila	0.11	(0.11)	0.08	(0.08)	0.480	0.14	(0.17)	0.12	(0.12)	0.403	0.14	(0.25)	0.16	(0.23)	0.319	0.973	0.128
Desulfovibrio	0.11	(0.31)	0.11	(0.37)	0.180	0.06	(0.15)	0.08	(0.17)	0.496	0.08	(0.20)	0.13	(0.24)	0.408	0.995	0.870
Succinivibrio	0.04	(0.18)	0.12	(0.48)	0.317	0.01	(0.02)	0.05	(0.22)	0.317	0.31	(1.24)	0.28	(1.18)	0.180	0.805	0.380
Escherichia	0.15	(0.23)	0.15	(0.25)	0.814	0.13	(0.19)	0.05	(0.04)	0.084	0.18	(0.34)	0.08	(0.10)	0.234	0.831	0.119
Serratia	0.00	(0.00)	0.03	(0.11)	1.000	0.00	(0.00)	0.09	(0.38)	1.000	0.02	(0.09)	0.00	(0.00)	0.180	0.334	0.293
Phylum_Verrucomicrobia																	
Other (f__Cerasicoccaceae)	0.00	(0.00)	0.00	(0.00)	1.000	0.01	(0.04)	0.07	(0.30)	0.715	0.00	(0.01)	0.00	(0.00)	0.317	0.073	0.474
Akkermansia	0.24	(0.48)	0.19	(0.47)	0.043	0.60	(0.81)	0.24	(0.55)	0.023	0.65	(1.09)	0.39	(0.79)	0.678	0.531	0.333

BL: baseline, FU: follow up Data are displayed as the mean (SD) percentage abundance of all samples available at that timepoint

p*Wilcoxon signed rank test within group comparison. P** Kruskal Wallis H-test between group comparison at baseline. P*** ANCOVA between groups comparison at 4-weeks adjusting for baseline. Bonferroni post-hoc: a) Control vs LFD p=0.003; Control vs LFD/B-GOS p<0.001; LFD vs LFD/B-GOS p=1.000; b) Control vs LFD p=0.015, control vs LFD/B-GOS p=0.002; LFD vs LFD/B-GOS p=1.000, c) Control vs LFD p=0.018; control vs LFD/B-GOS p=0.005; LFD vs LFD/B-GOS p=1.000, d) Control vs LFD p=0.114; control vs LFD/B-GOS p=1.000; LFD vs LFD/B-GOS p=0.075, e) Control vs LFD p=1.000; control vs LFD/B-GOS p=0.022; LFD vs LFD/B-GOS p=0.021 f) Control vs LFD p=0.316, control vs LFD/B-GOS p=0.046; LFD vs LFD/B-GOS p=1.000, g) Control vs LFD p=1.000; control vs LFD/B-GOS p=0.060, LFD vs LFD/B-GOS p=0.049, h) Control vs LFD p=1.000; control vs LFD/B-GOS p=0.013, LFD vs LFD/B-GOS p=0.010. LFD, low FODMAP diet. B-GOS, β -galactooligosaccharide.

Supplementary material 12: Fecal short-chain fatty acids mg/100g dry weight of stool at baseline, week-1 and week-4 for IBS patients receiving sham dietary advice, low FODMAP dietary advice or low FODMAP dietary advice plus B-GOS prebiotic

	Baseline						
Mean (SD)	Control (n=18)		LFD (n=20)		LFD-B-GOS (n=21)		P*
Acetate	1310.3	(708.0)	1631.8	(987.2)	1846.5	(1993.3)	0.483
Propionate	464.5	(297.0)	595.7	(620.6)	702.0	(1033.6)	0.605
Butyrate	388.1	(279.9)	552.5	(466.3)	615.0	(741.7)	0.417
Valerate	54.2	(33.8)	54.3	(32.2)	101.2	(197.7)	0.368
Isobutyrate	46.2	(15.7)	42.6	(21.5)	62.3	(50.2)	0.145
Isovalerate	55.7	(17.6)	52.8	(22.5)	88.3	(106.7)	0.170
Total SCFA	2318.9	(1226.1)	2929.8	(1919.7)	3415.2	(4053.7)	0.468
Dry weight (%)	26.1	(6.4)	24.8	(8.4)	25.6	(8.8)	0.885
pH	6.6	(0.4)	6.4	(0.4)	6.6	(0.4)	0.181
	Week-1						
Mean (SD)	Control (n=20)		LFD (n=21)		LFD-BGOS (n=20)		
Acetate	1686.2	(798.1)	1181.1	(622.9)	1243.6	(851.2)	0.08
Propionate	552.4	(312.1)	366.4	(193.7)	412.5	(240.4)	0.058
Butyrate	571.5	(393.3)	343.9	(170.4)	327.6	(249.0)	0.014^a
Valerate	67.1	(33.1)	50.1	(32.4)	52.8	(30.4)	0.203
Isobutyrate	51.1	(21.5)	44.2	(21.4)	45.9	(21.2)	0.564
Isovalerate	63.2	(24.4)	55.2	(26.9)	55.5	(23.9)	0.524
Total SCFA	2991.4	(1399.7)	2040.9	(955.4)	2138.0	(1320.9)	0.033^b
Dry weight (%)	25.4	(8.2)	27.7	(6.8)	28.4	(5.6)	0.363
pH	6.4	(0.5)	6.7	(0.4)	6.6	(0.4)	0.017^c
	Week-4						
Mean (SD)	Control (n=21)		LFD (n=21)		LFD-BGOS (n=20)		
Acetate	1640.3	(785.4)	1354.8	(941.1)	1309.3	(709.2)	0.376
Propionate	584.1	(383.1)	423.1	(305.8)	421.7	(252.5)	0.178
Butyrate	600.3	(434.8)	416.2	(292.6)	374.6	(235.5)	0.076
Valerate	68.3	(37.7)	50.5	(32.4)	57.4	(42.0)	0.308
Isobutyrate	50.1	(22.3)	48.8	(25.9)	53.3	(29.1)	0.85
Isovalerate	58.1	(21.7)	60.2	(28.2)	62.9	(31.9)	0.856
Total SCFA	3001.2	(1452.0)	2353.6	(1540.2)	2279.1	(1210.2)	0.202
Dry weight (%)	25.5	(10.0)	24.8	(7.7)	26.3	(7.2)	0.852
pH	6.4	(0.6)	6.8	(0.5)	6.8	(0.6)	0.030^d

Data are presented as Mean (SD).

*p calculated using ANOVA.

a) Control vs LFD p=0.039; control vs LFD/B-GOS p=0.027, LFD vs LFD/B-GOS p=1.000, b) control vs LFD p=0.051; control vs LFD/B-GOS p=0.099, LFD vs LFD/B-GOS p=1.000, c) Control vs LFD p=0.017; control vs LFD/B-GOS p=0.149, LFD vs LFD/B-GOS p=1.000, d) control vs LFD p=0.047; control vs LFD/B-GOS p=0.097, LFD vs LFD/B-GOS p=1.000

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