

Fecal Clostridiales distribution and short-chain fatty acids reflect bowel habits in irritable bowel syndrome

Giorgio Gargari¹, Valentina Taverniti¹, Claudio Gardana¹, Cesare Cremon², Filippo Canducci³,
Isabella Pagano², Maria Raffaella Barbaro², Lara Bellacosa², Anna Maria Castellazzi⁴, Chiara
Valsecchi⁵, Sara Carlotta Tagliacarne⁴, Massimo Bellini⁶, Lorenzo Bertani⁶, Dario Gambaccini⁶,
Santino Marchi⁶, Michele Cicala⁷, Bastianello Germanà⁸, Elisabetta Dal Pont⁸, Maurizio Vecchi⁹,
Cristina Ogliari⁹, Walter Fiore¹⁰, Vincenzo Stanghellini², Giovanni Barbara², Simone
Guglielmetti^{1*}

¹ Division of Food Microbiology and Bioprocesses, Department of Food, Environmental and
Nutritional Sciences, University of Milan, Milan, Italy.

²Department of Medical and Surgical Sciences, Centre for Applied Biomedical Research,
University of Bologna, Bologna, Italy.

³Dipartimento di biotecnologie e scienze della vita, Università degli Studi dell'Insubria, Varese,
Italy.

⁴Department of Clinical Surgical Diagnostic and Pediatric Sciences, University of Pavia, Pavia,
Italy.

⁵Department of Pediatrics, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

⁶Gastroenterology Unit, Department of Gastroenterology, University of Pisa, Pisa, Italy, Italy.

⁷Gastroenterology Unit, University Campus Bio-Medico of Rome, Rome, Italy.

⁸Gastroenterology Unit, S. Martino Hospital, Belluno, Italy.

⁹Gastroenterology and Digestive Endoscopy Unit, IRCCS Policlinico San Donato, San Donato
Milanese, Italy.

¹⁰Sofar S.p.A., Trezzano Rosa, Italy.

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

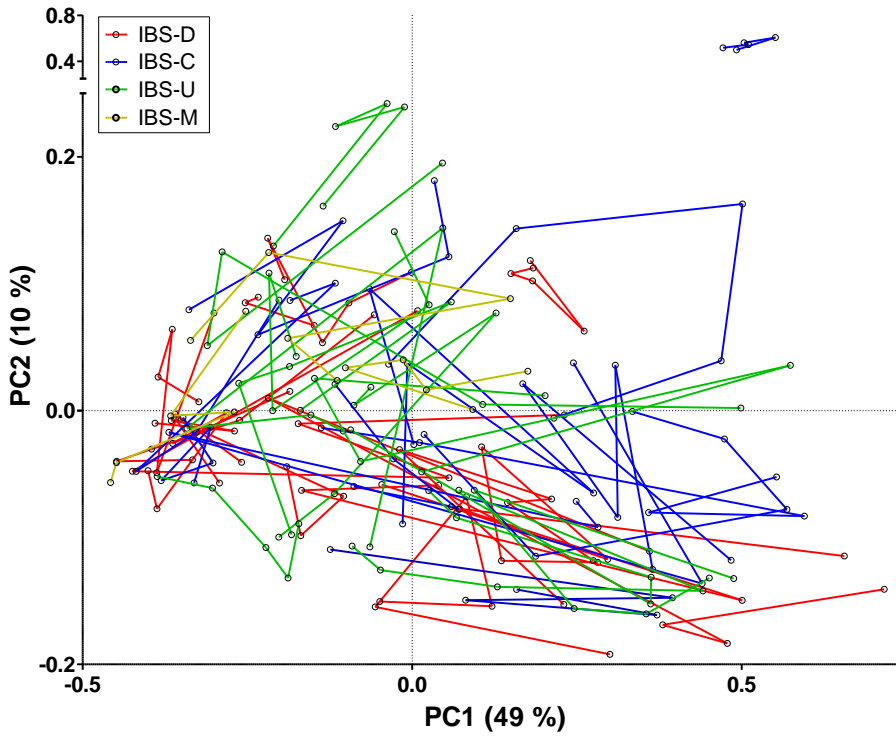
Running Head: Fecal microbial ecosystem of IBS subtypes.

Supplementary Table 1. Demographic data for the IBS (A) and control (B) populations under study. F, female; M, male.

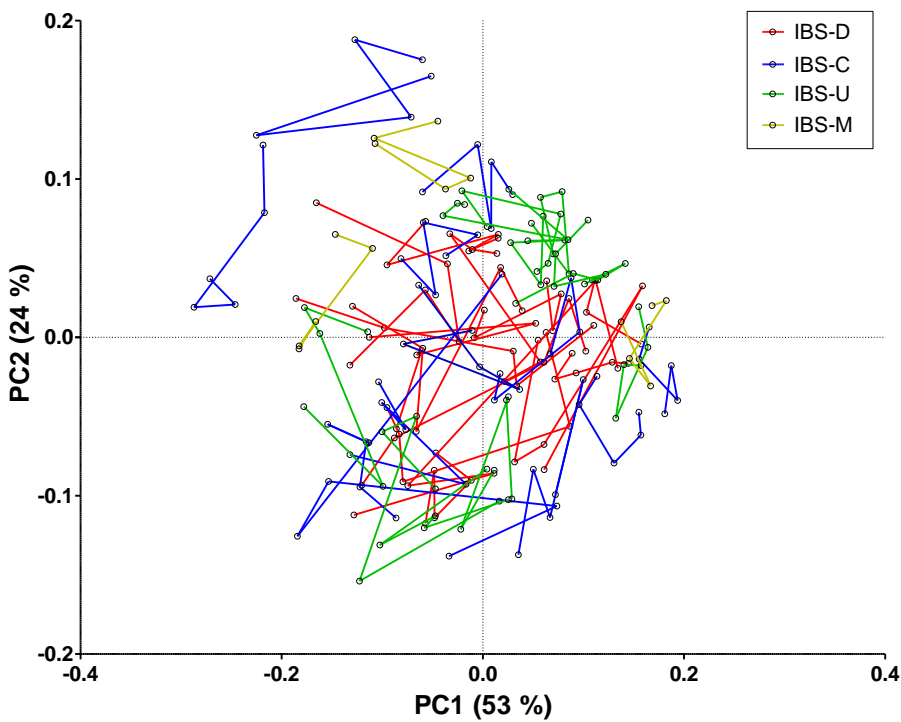
A				B		
Patient	Sex	Age	IBS subtype	Volunteer	Sex	Age
S01	F	26	U	C01	M	49
S02	M	26	D	C02	M	47
S03	F	20	D	C03	F	45
S04	F	64	U	C04	F	22
S05	M	27	U	C05	M	45
S06	F	63	C	C06	M	26
S07	F	35	C	C07	F	40
S08	M	25	C	C08	F	54
S09	M	25	C	C09	F	57
S10	F	45	Mix	C10	F	42
S11	F	24	U	C11	M	28
S12	M	50	C	C12	F	45
S13	F	38	D	C13	F	38
S14	F	47	D	C14	F	73
S15	M	21	Mix	C15	M	42
S16	F	57	C	C16	M	19
S17	F	46	D			
S18	F	41	C			
S19	F	39	U			
S20	M	40	U			
S21	F	43	D			
S22	F	47	D			
S23	M	42	C			
S24	F	24	U			
S25	F	46	U			
S26	M	41	U			
S27	F	51	D			
S28	M	36	D			
S29	M	52	D			
S30	F	34	C			
S31	M	60	C			
S32	F	35	C			
S33	F	41	U			
S34	M	57	D			
S35	F	33	Mix			
S36	F	31	C			
S37	F	66	U			
S38	M	52	D			
S39	F	52	D			
S40	F	36	D			

Supplementary figure 1. Principal coordinates analysis of weighted (A) and unweighted (B) Unifrac distances based on 16S rRNA gene profiling data. Lines connect samples belonging to the same patient. The first two coordinates (PC1 and PC2) are displayed with the percentage of variance explained in brackets.

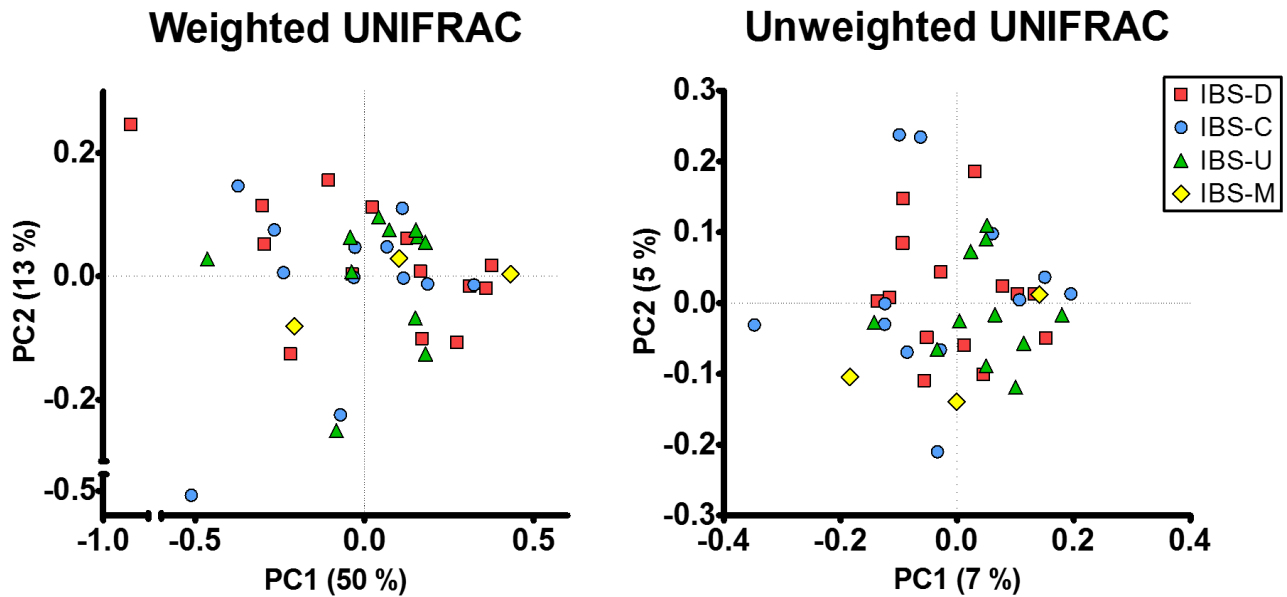
A



B

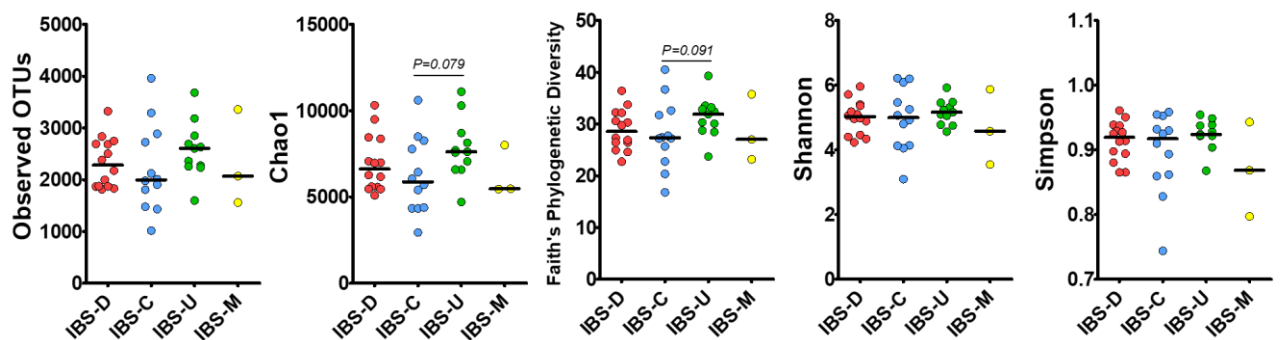


Supplementary figure 2. Principal coordinates analysis of Unifrac distances based on 16S rRNA gene profiling data of a single fecal sample collected from 40 IBS patients. The first two coordinates (PC1 and PC2) are displayed with the percentage of variance explained in brackets.

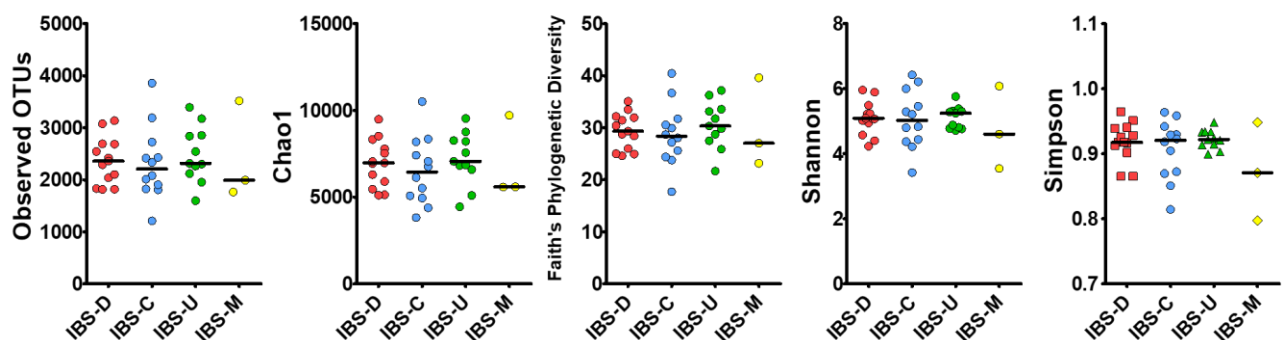


Supplementary figure 3. Within-subject (alpha) diversity of fecal samples from IBS patients determined through four different estimators. Scatter dot plots show data from a single fecal sample per subject (panel A; n = 40) and medians of the data from five fecal samples per subject (panel B, n = 39). Statistical significances are according to Mann-Whitney test.

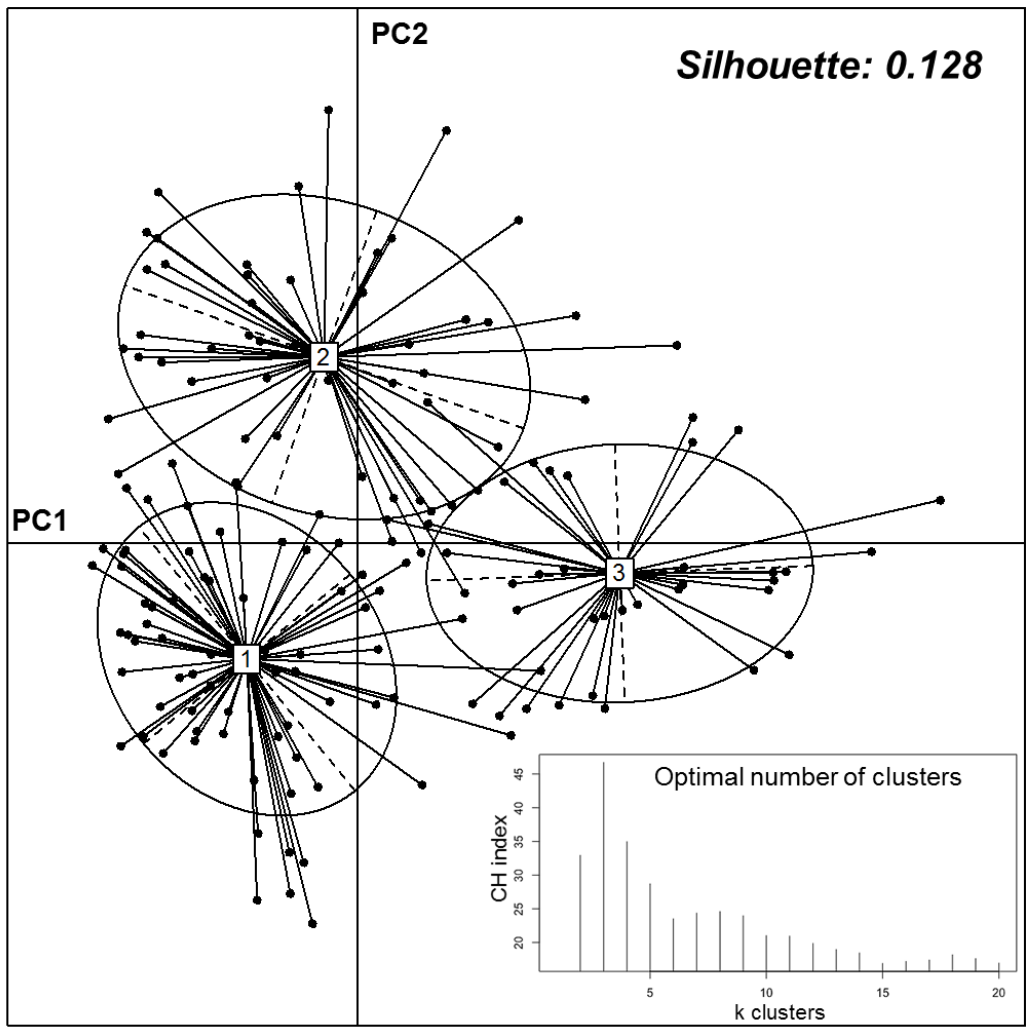
A



B

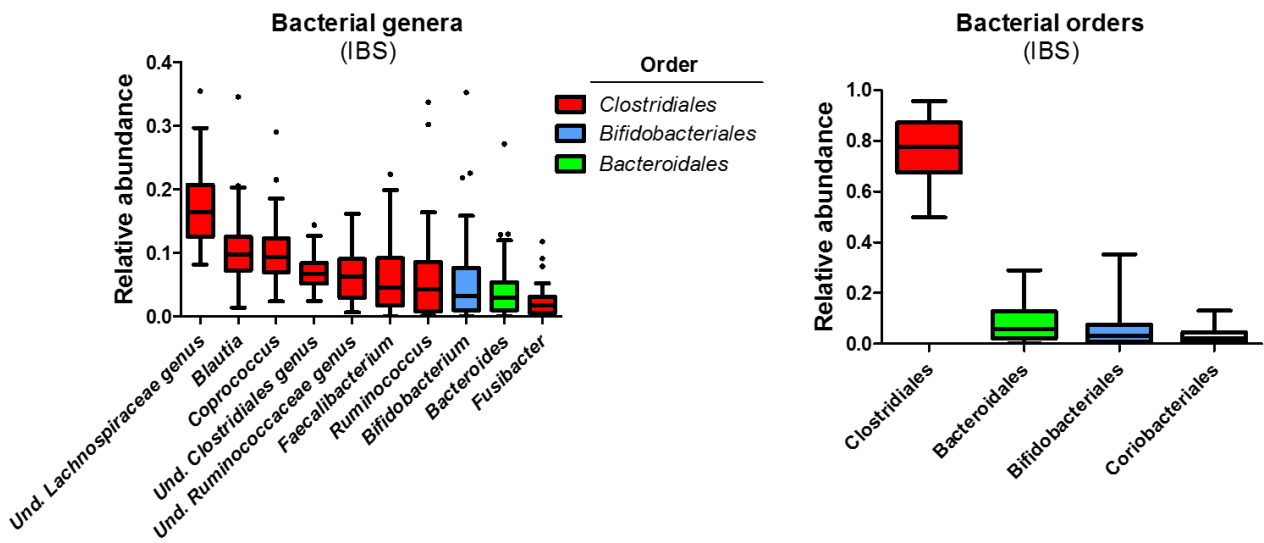


Supplementary figure 4. Analyses of enterotypes in IBS fecal samples. A, Principal Coordinates Analysis (PCoA; the first two principal components are shown). The optimal number of clusters was determined through the Calinski-Harabasz (CH) index and the Silhouette coefficient.

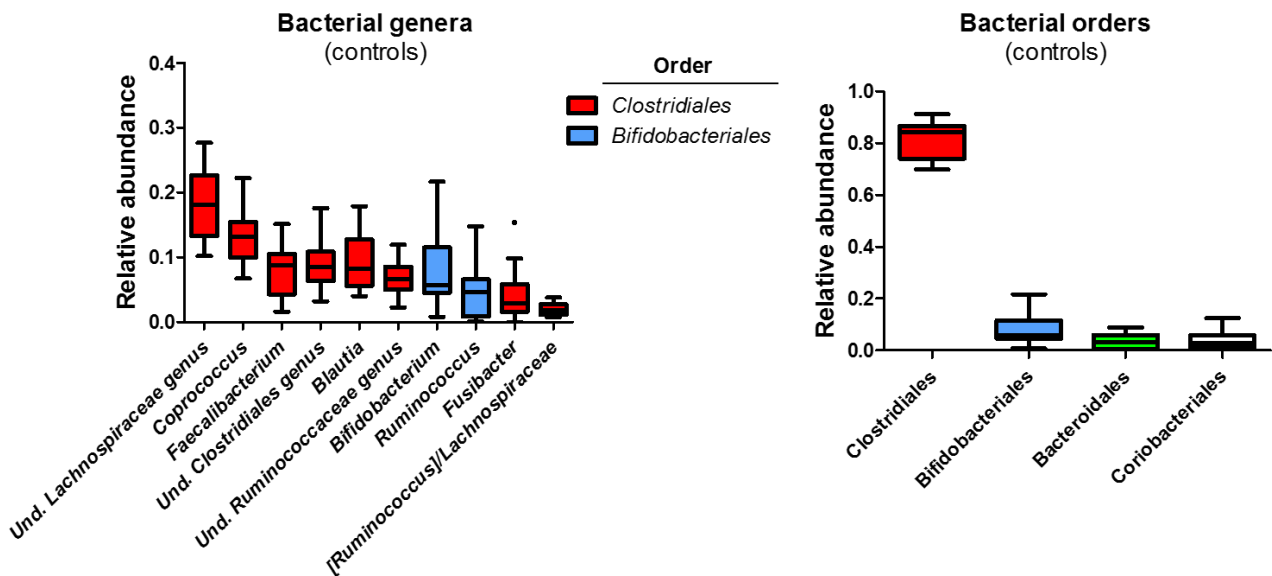


Supplementary figure 5. Tukey boxplots of the dominant bacterial genera and orders in IBS fecal samples (n=40; panel A) and healthy control subjects (n=16; B).

A



B



Supplementary figure 6. OTUs distinguishing IBS subtypes determined by using the DESeq2 negative binomial distribution method on 16S rRNA gene profiling data of a single fecal sample per patient. The colors in the heatmap represents the mean of normalized relative abundances of the reported OTUs. The taxonomic lineage of each taxon is shown; p, phylum; c, class; o, order; f, family; g, genus; s, species. Positive fold changes (shown on a red background) indicate OTU overrepresentation in IBS-U (panels A and B) and IBS-C (panel C); negative fold changes (shown on a blue background) indicate an increase of OTU relative abundance in IBS-C (panel A) and IBS-D (panel B and C).

A

OTU	Normalized Base Mean		log2 Fold Change	padj	Taxonomy
	IBS-U	IBS-C			
OTU841419			-2.40	3.5E-02	p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_;s_
OTU560981			-2.32	7.5E-03	p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_Slackia;s_
ncur_OTU34595			-2.21	1.6E-02	p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium;s_adolescentis
nr_OTU333			1.80	4.6E-02	p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium;s_longum
OTU1646183			2.45	1.9E-02	p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_;s_
OTU355291			-2.70	1.2E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_
OTU197072			2.33	2.6E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_
OTU846127			2.35	1.3E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae;g_Parabacteroides;s_
OTU535549			3.06	7.8E-04	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_fragilis
OTU524318			3.49	1.3E-04	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_
OTU552988			-2.37	1.6E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
OTU1110312			-2.12	3.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
OTU815179			-2.02	2.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
OTU700540			-1.97	4.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
OTU560873			-1.92	3.9E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
OTU310178			-3.80	1.1E-04	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_;s_
OTU410242			-2.40	7.9E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_;s_
OTU192240			-3.49	1.3E-04	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_;s_
OTU181466			-2.05	3.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_;s_
OTU297182			-2.17	4.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU182903			-1.96	3.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU369827			-3.86	1.7E-05	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU342947			-3.05	7.8E-04	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU199374			-2.85	7.9E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU367946			-2.08	1.2E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU147100			-1.97	3.6E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU626544			-1.86	4.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU334215			-2.25	4.7E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_

OTU581933			-1.77	3.3E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
OTU369602			-5.28	4.2E-07	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU192079			-2.34	4.7E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU174516			2.39	2.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_Clostridium;s_
OTU529740			2.35	3.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU197760			2.35	7.2E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU345111			1.95	3.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_;s_
OTU192226			2.93	7.8E-04	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_[Ruminococcus];s_
OTU550013			3.18	1.3E-04	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia;s_
ncur_OTU47801			1.71	3.2E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
ncur_OTU75122			2.53	6.7E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU185659			2.60	4.7E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Acidaminococcus;s_
OTU342427			1.95	1.4E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Veillonella;s_dispar
OTU1820513			-2.40	1.4E-02	p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella;s_
OTU791348			-3.63	1.7E-05	p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae;g_Desulfovibrio;s_D168
OTU359809			3.57	1.3E-04	p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella;s_
OTU825033			2.05	4.7E-02	p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_;s_
OTU1671681			2.15	1.1E-02	p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;g_Haemophilus;s_parainfluenzae
OTU968675			2.82	2.4E-03	p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;g_Haemophilus;s_parainfluenzae
OTU661526			-2.40	1.4E-02	p_Synergistetes;c_Synergistia;o_Synergistales;f_Synergistaceae

B

IBS-U vs IBS-D OTU	Normalized Base Mean		log2 Fold Change	padj	Taxonomy
	IBS-U	IBS-D			
OTU841419			-2.98	3.3E-03	p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_s_
ncur_OTU47315			2.54	3.5E-03	p_Actinobacteria;c_Actinobacteria
OTU568118			-4.07	2.6E-06	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella;s_copri
OTU369449			-2.85	2.5E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_
OTU107044			-2.50	6.4E-03	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae
OTU524318			2.75	5.3E-03	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_
OTU535549			3.16	5.4E-04	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_fragilis
OTU297182			-3.03	1.1E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU199374			-3.36	5.8E-04	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_s_
OTU916143			-3.65	7.4E-05	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Phascolarctobacterium;s_
OTU524884			-5.94	1.2E-10	p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_[Eubacterium];s_biforme
OTU584978			2.68	4.7E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_s_
OTU582089			2.02	2.5E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
ncur_OTU72889			2.15	1.4E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
ncur_OTU36690			2.23	1.4E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU287608			2.77	6.4E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU183532			3.05	5.8E-04	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU1820513			-2.33	2.1E-02	p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella;s_

C

IBS-C vs IBS-D OTU	Normalized Base Mean		log2 Fold Change	padj	Taxonomy
	IBS-C	IBS-D			
ncur_OTU47315			1.83	3.9E-02	p_Actinobacteria;c_Actinobacteria
nr_OTU225			2.01	2.3E-02	p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium;s_adolescentis
ncur_OTU34595			2.06	1.1E-02	p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Bifidobacterium;s_adolescentis
OTU302545			2.44	7.1E-03	p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_;s_
OTU560981			1.88	1.6E-02	p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_Slackia;s_
OTU850905			-1.95	1.7E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Barnesiellaceae];g_;s_
OTU195508			-2.05	1.8E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_caccae
OTU577294			-2.27	1.2E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrimonadaceae;g_Parabacteroides;s_distasonis
OTU349809			-2.18	4.1E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrimonadaceae;g_Parabacteroides;s_distasonis
OTU568118			-3.90	5.9E-06	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella;s_copri
OTU530653			-3.31	4.6E-04	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella;s_copri
OTU355291			2.22	2.6E-02	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_
OTU4336943			2.92	6.6E-04	p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_;s_
OTU766768			-2.34	4.6E-02	p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Enterococcaceae;g_Enterococcus;s_
OTU245625			-1.42	4.5E-02	p_Firmicutes;c_Clostridia;o_Clostridiales
nr_OTU145			-2.02	1.1E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
ncur_OTU34892			-1.64	3.5E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae
OTU174516			-2.55	7.3E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_Clostridium;s_
OTU562038			-2.24	9.2E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU332349			-2.01	1.3E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
nr_OTU48			-1.98	1.6E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU584463			-1.82	4.8E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU197760			-1.82	2.7E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU564400			-1.73	4.1E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU508875			-1.73	3.5E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
ncur_OTU13517			-1.86	1.6E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_;s_
ncur_OTU65094			-1.61	4.1E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_;s_
OTU550013			-1.83	3.9E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia;s_
nr_OTU390			-1.89	2.7E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Peptostreptococcaceae;g_;s_
OTU189899			-2.92	3.6E-03	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU185659			-2.59	2.4E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Acidaminococcus;s_
OTU340113			-1.78	2.7E-02	p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Dialister;s_

OTU916143		-2.55	6.7E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Phascolarctobacterium;s_
OTU524884		-4.47	5.9E-06 p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_[Eubacterium];s_biforme
OTU197105		-2.12	9.4E-03 p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_[Eubacterium];s_biforme
OTU383885		1.79	3.6E-02 p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;s_
ncur_OTU48808		1.74	2.1E-02 p_Firmicutes;c_Clostridia;o_Clostridiales
ncur_OTU1883		1.83	9.4E-03 p_Firmicutes;c_Clostridia;o_Clostridiales
OTU567011		1.37	2.7E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU311693		1.77	1.4E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
nr_OTU150		1.82	2.0E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU178511		1.88	1.4E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU197427		1.93	4.6E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
nr_OTU440		1.94	4.2E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
nr_OTU249		2.01	3.4E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU416341		2.05	2.2E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU368412		2.40	5.8E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU358439		2.61	6.7E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU815179		2.73	3.6E-04 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU191421		3.12	6.6E-04 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU552988		3.33	6.3E-05 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU360890		3.80	6.8E-05 p_Firmicutes;c_Clostridia;o_Clostridiales;f_g_s_
OTU552235		1.59	4.1E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_s_
OTU289454		1.74	1.6E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_s_
OTU410242		2.68	6.6E-04 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_s_
OTU310178		2.93	2.7E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae;g_s_
OTU192240		2.04	3.3E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_s_
OTU181466		2.25	8.2E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_s_
nr_OTU61		1.55	1.1E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
OTU368950		1.69	3.3E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
nr_OTU262		1.30	4.1E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_s_
nr_OTU26		2.12	8.2E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_s_
OTU538947		1.41	4.5E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia;s_
OTU436032		1.51	4.0E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia;s_
OTU594227		1.75	2.7E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia;s_
nr_OTU148		2.03	1.3E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia;s_
OTU187112		2.10	6.1E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia;s_
OTU196791		2.14	1.2E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Dorea;s_
nr_OTU434		2.33	8.0E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae

OTU751584		1.41	4.5E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU688800		1.75	3.1E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU367946		1.90	8.2E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
nr_OTU157		1.95	1.7E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU296941		2.04	3.0E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
ncur_OTU4880		2.23	8.2E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU147100		2.58	1.0E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU342947		3.06	3.6E-04 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
OTU369827		4.34	1.3E-07 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
nr_OTU300		1.47	1.9E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
OTU364341		2.28	6.7E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
OTU547479		1.61	4.1E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU582652		1.88	1.6E-02 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
ncur_OTU32182		1.98	6.9E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
ncur_OTU21274		2.06	7.3E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU183532		2.70	2.3E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU287608		3.09	6.9E-04 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
OTU369602		3.25	4.2E-03 p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
ncur_OTU84083		1.71	2.2E-02 p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_;s_
OTU970951		-2.21	1.0E-02 p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_;s_
OTU825033		-2.16	1.4E-02 p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_;s_
OTU968675		-2.20	1.6E-02 p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;g_Haemophilus;s_parainfluenzae
OTU791348		3.42	1.3E-05 p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae;g_Desulfovibrio;s_D168
OTU661526		2.83	7.5E-04 p_Synergistetes;c_Synergistia;o_Synergistales;f_Synergistaceae



Supplementary figure 7. Levels of short chain fatty acids determined in a single fecal sample per IBS patient (n=40) and healthy control (n=25; data from Gargari et al., 2016). Statistical significances are according to Mann-Whitney test; *, P<0.05; **, P<0.01.

