

Multi-omics gut microbiome signatures in obese women: role of diet and uncontrolled eating behavior

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Supplementary Material

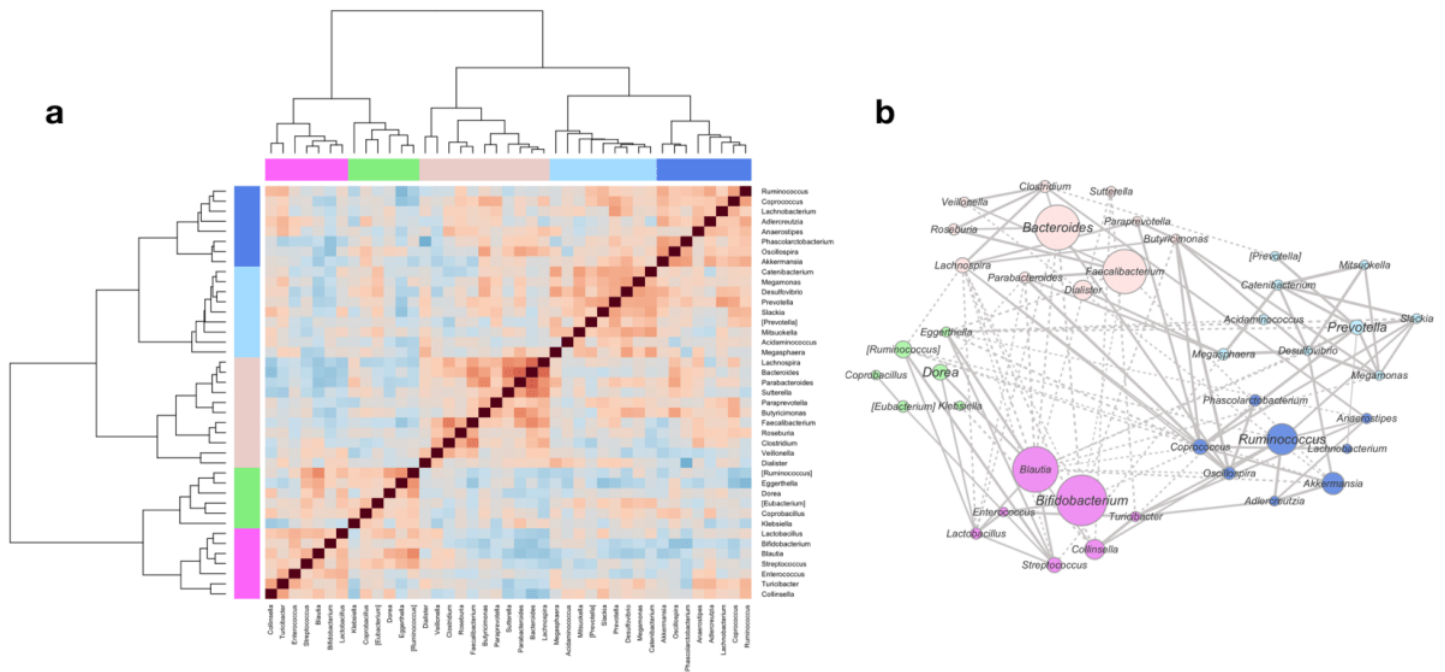


Figure S1. Assignment of bacterial co-abundance groups (CAGs). CAGs were assigned according to the heat plot (a) showing Kendall correlations between genera clustered by the Spearman correlation coefficient and Ward linkage hierarchical clustering. (b) Wiggum plot correlation between the five CAGs identified. Circle size is proportional to the genus relative abundance and the connections between nodes represent significant correlations between genera (Kendall, FDR < 0.05). Positive correlations are represented by continuous lines, while negative correlations are represented by dashed lines. Thickness of the lines is proportional to the significance of the correlation.

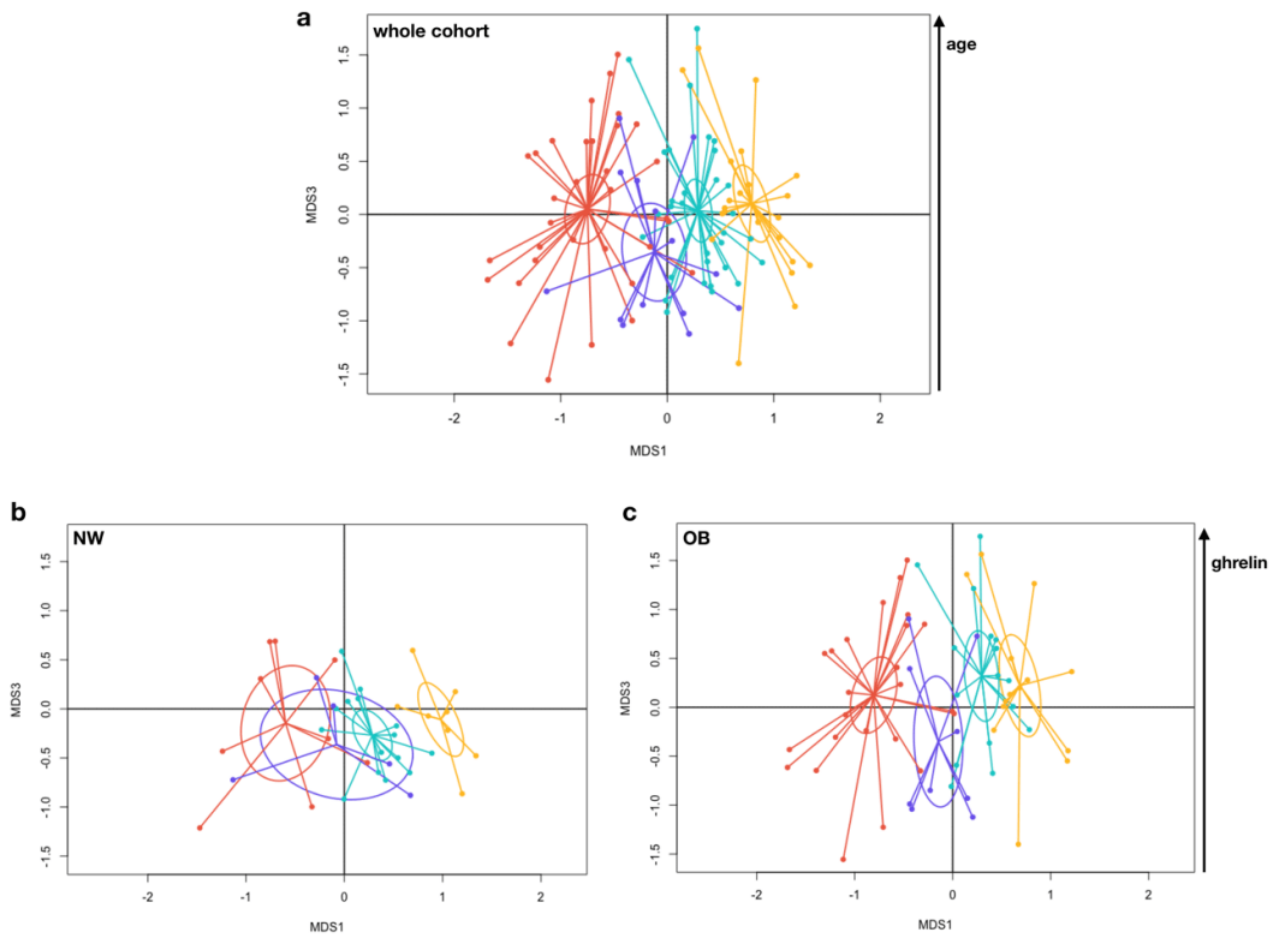


Figure S2. Associations of the microbiota profiles with host metadata. Unweighted UniFrac PCoA of the fecal microbiota in the whole cohort (a), as well as in normal-weight women (NW, b) and overweight/obese women (OB, c), stratified according to the four microbiota clusters (C1-C4, see Fig. 3). The host variables significantly associated with the axes are highlighted. MDS, multidimensional scaling.

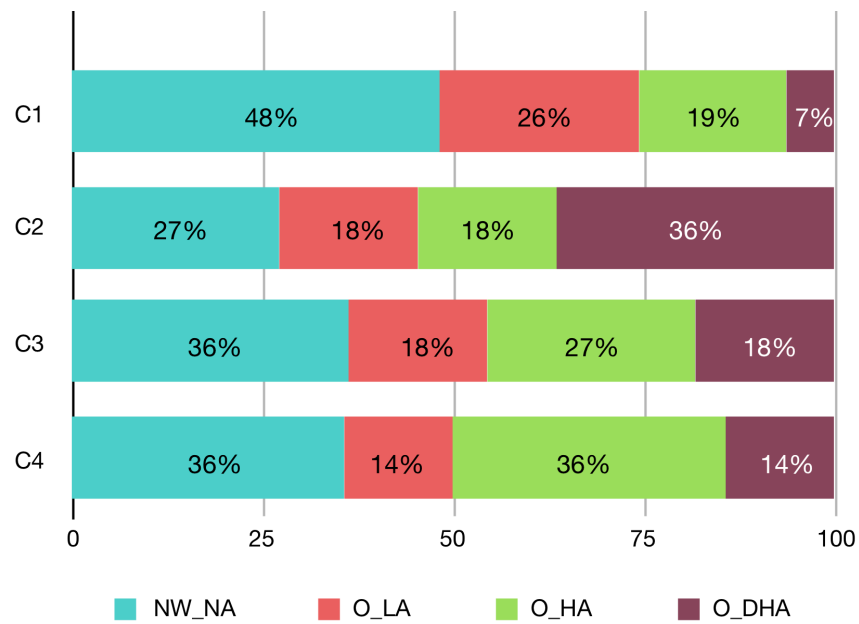


Figure S3. Distribution of eating behavior-based study groups across the four microbiome clusters (C1-C4). Women were stratified based on addictive eating behavior into 4 groups: NW_NA (normal-weight, non-addicted), O_LA (overweight/obese, low-addicted), O_HA (overweight/obese, high-addicted), and O_DHA (overweight/obese, high-addicted with food addiction diagnosis). See Methods for further details on stratification.

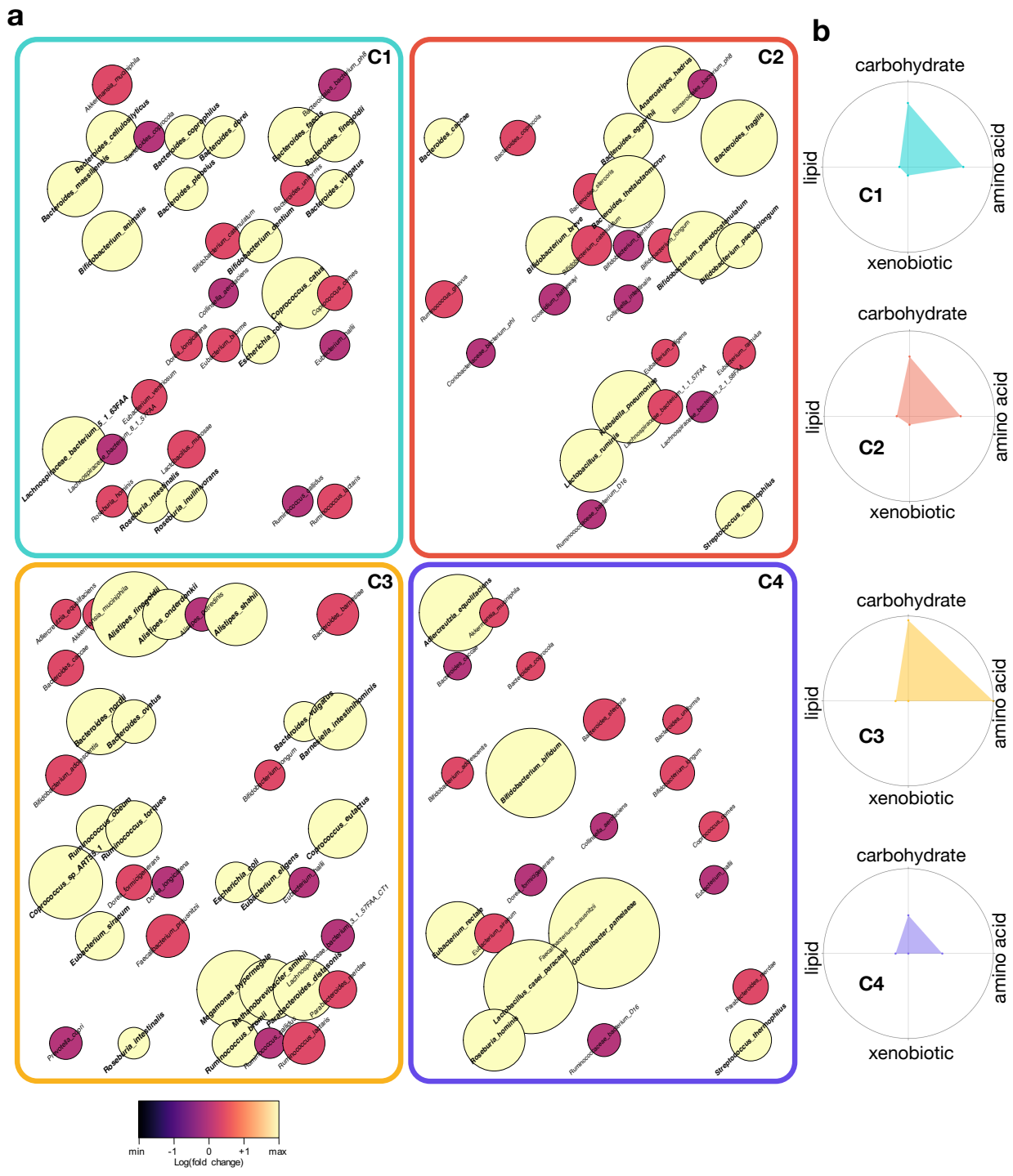


Figure S4. The transcriptionally active fraction of the gut microbiome differs according to obesity and uncontrolled eating behavior. (a) Bubble charts showing the transcriptionally active microbial species within the four microbiome configurations (clusters C1-C4). Circle sizes and colors indicate the transcriptional over-abundance of a given species compared to its average normalized abundance in the whole cohort using log₂ transformation. (b) Spider plots showing the number of active species for each metabolism (i.e., carbohydrate, lipid, amino acid and xenobiotic metabolism). Species were counted as “active” when present with log₂ normalized abundance > 1 in at least 1/3 of the pathways comprised in the specific metabolic category.

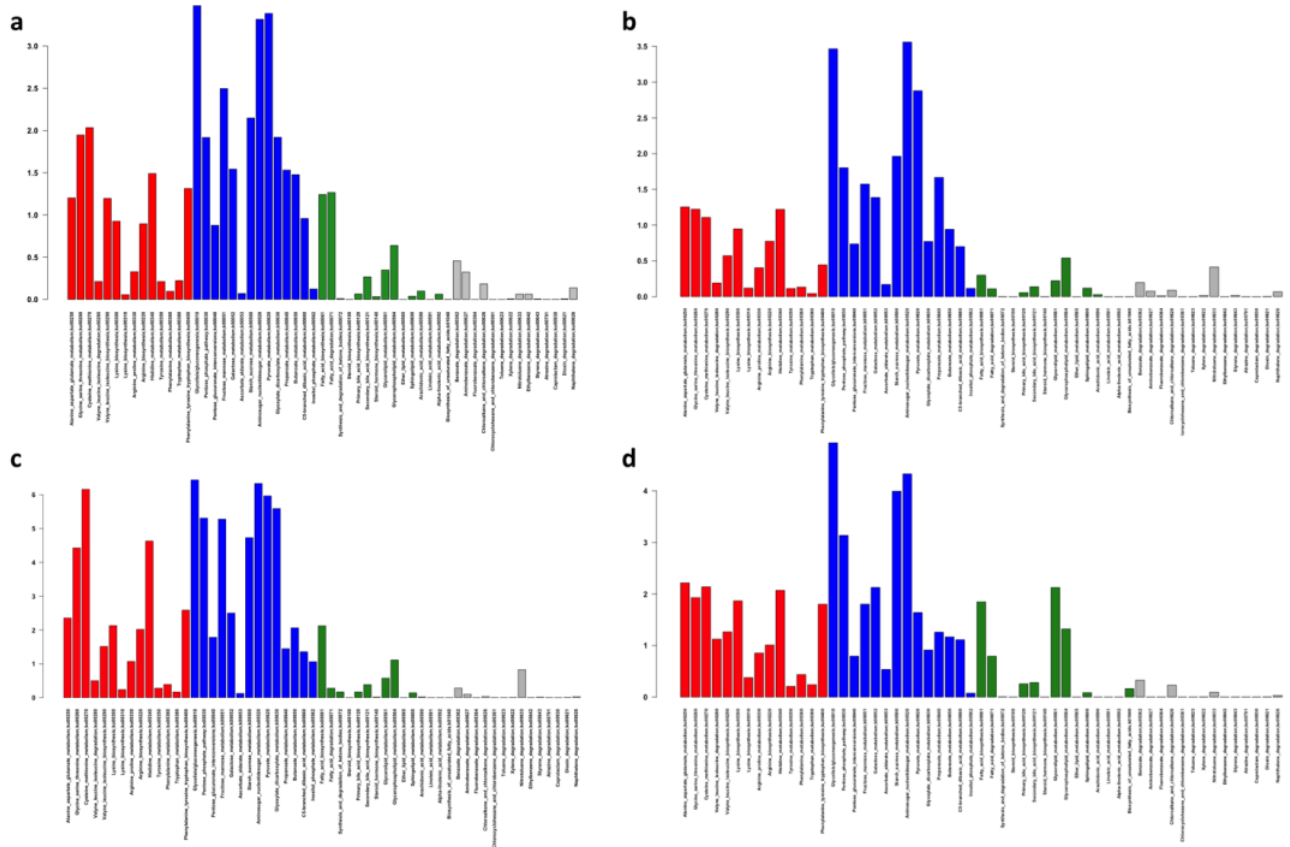


Figure S6. Metabolic activities of the four microbiota clusters according to metatranscriptomic analysis. Expression levels of the 57 identified KEGG pathways related to amino acid (red), carbohydrate (blue), lipid (green), and xenobiotic (grey) metabolisms that were most prevalently transcribed among the species detected by metagenomics, for the microbiome clusters C1 (a), C2 (b), C3 (c) and C4 (d).

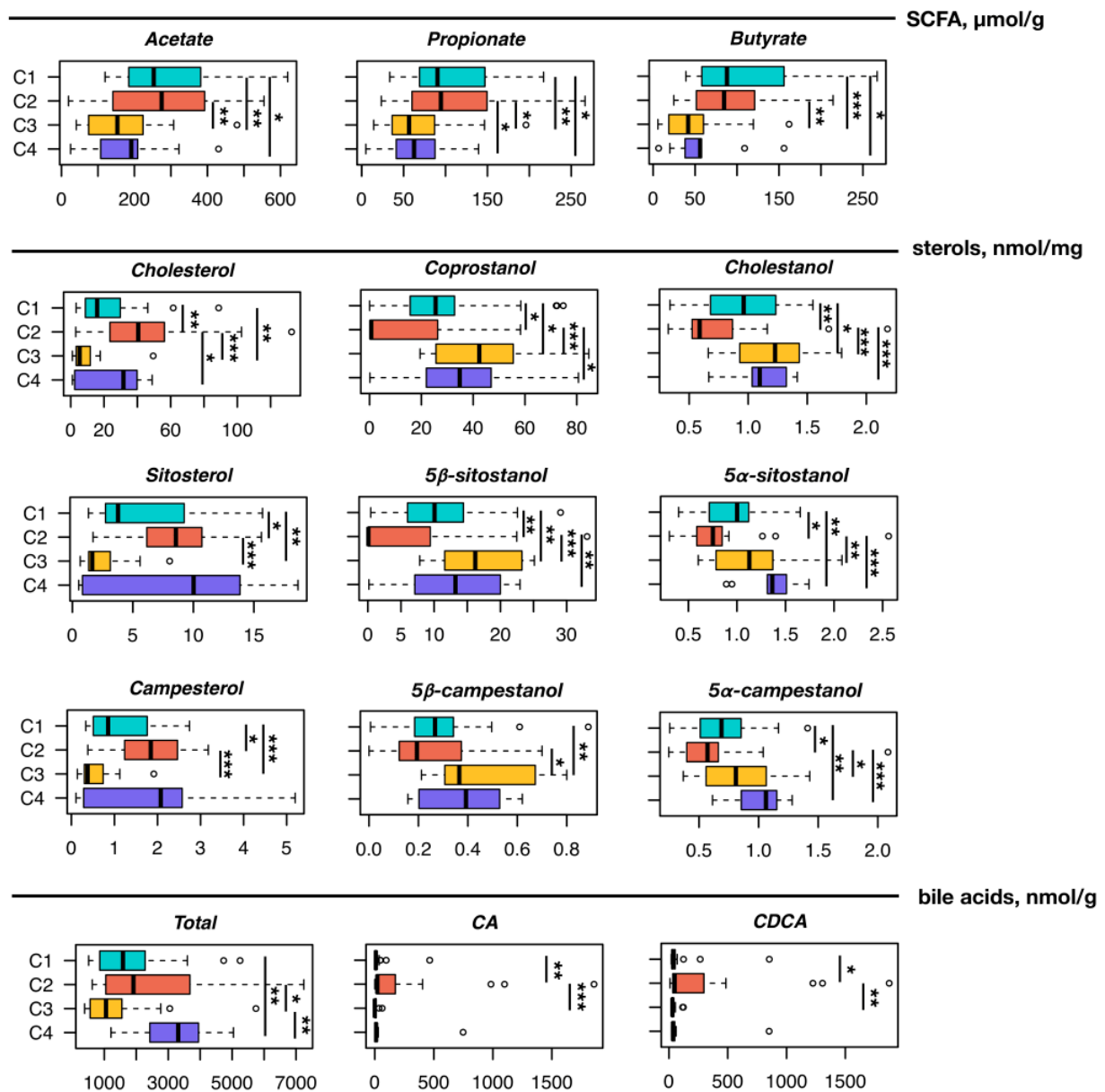


Figure S7. Fecal lipidomic profiles of the four microbiota clusters (C1-C4). Boxplots showing the amount distribution of short-chain fatty acids (SCFAs, upper panel), sterols (middle panel) and bile acids (lower panel) significantly different between the four microbiota clusters. CA, cholic acid; CDCA, chenodeoxycholic acid; GDCA, glycocholic acid; Glyco, glycoconjugates. See [50] for further details on fecal lipidomics analysis. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; Wilcoxon test.

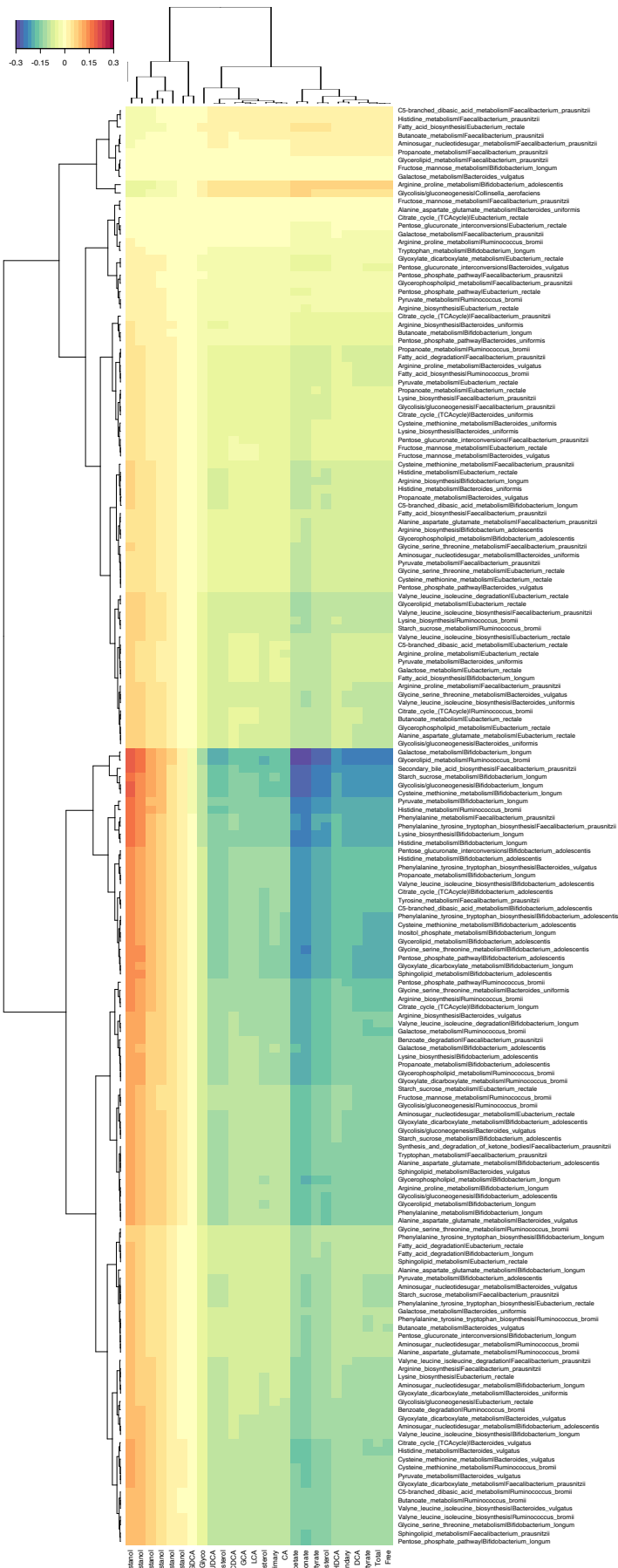


Figure S8. Associations between the transcriptionally active fraction of the gut microbiome and lipidomics measures. Hierarchical clustering obtained with complete linkage method and Pearson correlation as distance, performed on the sparse partial least square (sPLS) regression of transcriptionally active pathways and species of the gut microbiome and host lipids. CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GDCA, glycodeoxycholic acid; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid.

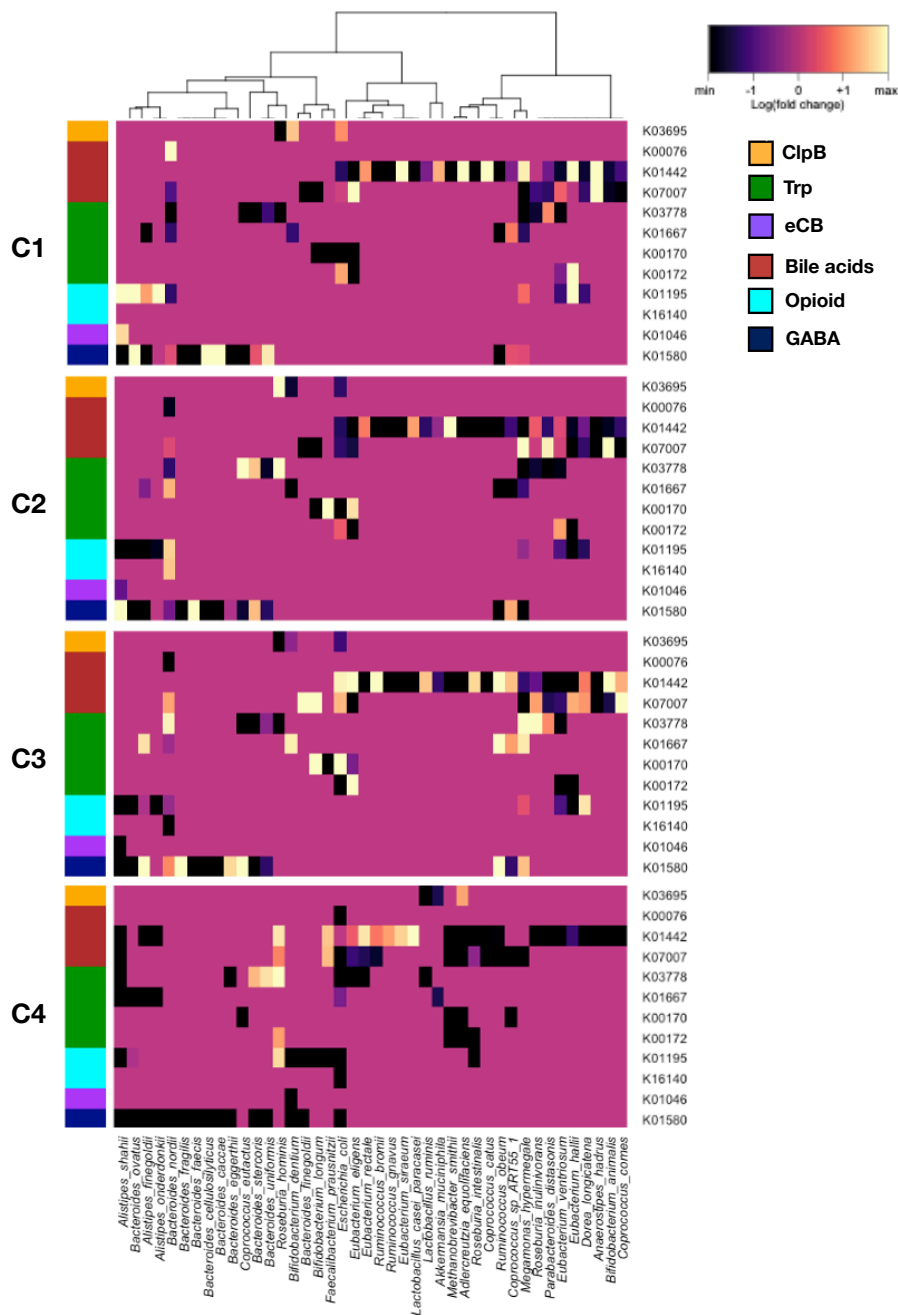


Figure S9. Transcriptional activities of microbiome configurations specifically related to food intake, energy expenditure and neuroendocrine signaling. Average RNA:DNA ratio for each cluster (C1-C4) with respect to the whole cohort, of a selection of key genes involved in ClpB (orange), bile acid (brown), tryptophan (green), opioid (cyan), eCB (violet), and GABA (navy) production, divided by the assigned microbial species. Phylogenetic tree on the top of the heatmap was built using hierarchical Ward linkage clustering based on the Spearman correlation coefficients of the average DNA-normalized transcript abundance profiles for the species with detectable transcriptomes in the whole cohort.

Table S1. Genus-level distribution and differences in the gut microbiota profiles among the four clusters (C1-C4). For each genus, the mean relative abundance and sem (standard error of the mean) are reported, along with Wilcoxon test *p* values (significant values in bold).

Genus	relative abundances								p values					
	mean C1	sem C1	mean C2	sem C2	mean C3	sem C3	mean C4	sem C4	C1 vs C2	C1 vs C3	C1 vs C4	C2 vs C3	C2 vs C4	C3 vs C4
<i>Anaerotruncus</i>	0.0038	0.0013	0.0049	0.0017	0.03	0.0077	0.019	0.0094	0.8	0.00004	0.07	0.0001	0.1	0.1
<i>Coprobacillus</i>	0.023	0.0094	0.032	0.013	0.048	0.021	0.2	0.11	0.7	0.6	0.03	0.4	0.02	0.2
<i>Eggerthella</i>	0.026	0.011	0.32	0.081	0.054	0.034	0.13	0.048	8.00E-06	0.3	0.002	0.0006	0.3	0.03
<i>Butyricimonas</i>	0.034	0.0076	0.062	0.03	0.14	0.056	0.0054	0.0027	0.08	0.03	0.02	0.002	0.5	0.0009
<i>Lachnobacterium</i>	0.035	0.017	0.013	0.0061	0.081	0.058	0.0046	0.0027	0.03	0.8	0.05	0.03	0.7	0.05
[<i>Prevotella</i>]	0.057	0.049	0	0	0.13	0.11	0	0	0.07	0.2	0.2	0.005	NA	0.06
<i>Slackia</i>	0.059	0.019	0.042	0.021	0.18	0.093	0.16	0.11	0.1	0.1	0.4	0.007	0.7	0.09
<i>Desulfovibrio</i>	0.061	0.034	0.022	0.016	0.2	0.081	0.0062	0.0054	0.4	0.1	0.3	0.03	0.7	0.06
<i>Veillonella</i>	0.094	0.026	0.3	0.2	0.01	0.0033	0.024	0.014	0.5	0.0004	0.009	0.004	0.05	0.7
<i>Adlercreutzia</i>	0.19	0.057	0.074	0.028	0.13	0.033	0.29	0.063	0.0002	0.5	0.04	0.005	0.0001	0.02
<i>Turicibacter</i>	0.21	0.1	0.16	0.073	0.091	0.026	0.28	0.095	0.02	0.5	0.3	0.2	0.04	0.08
<i>Catenibacterium</i>	0.26	0.17	0.13	0.13	0.5	0.29	0	0	0.4	0.1	0.1	0.01	0.3	0.01
<i>Anaerostipes</i>	0.3	0.042	0.19	0.043	0.23	0.057	0.24	0.082	0.02	0.1	0.1	0.3	0.3	0.7
<i>Parabacteroides</i>	0.4	0.064	0.6	0.17	0.42	0.066	0.079	0.035	0.2	0.7	0.0001	0.1	0.07	0.00004
<i>Phascolarctobacterium</i>	0.48	0.1	0.25	0.12	1.06	0.27	0.33	0.22	0.01	0.2	0.05	0.002	0.8	0.01
<i>Roseburia</i>	0.62	0.11	0.46	0.099	0.21	0.049	0.24	0.061	0.1	0.001	0.02	0.4	0.8	0.4
<i>Clostridium</i>	0.67	0.077	0.83	0.14	0.37	0.09	0.25	0.074	0.6	0.006	0.002	0.04	0.02	0.4
[<i>Ruminococcus</i>]	0.99	0.14	2.94	0.59	0.84	0.16	1.37	0.25	0.001	0.4	0.1	0.0008	0.1	0.04
<i>Akkermansia</i>	1.5	0.31	0.39	0.21	6.74	1.71	4.9	1.88	0.00009	0.03	0.9	0.00006	0.05	0.3
<i>Lachnospira</i>	1.54	0.36	1.48	0.41	1.13	0.22	0.32	0.14	0.3	0.8	0.0004	0.4	0.04	0.002
<i>Collinsella</i>	1.73	0.21	2.02	0.42	1.95	0.33	4.92	0.91	0.4	0.8	0.001	0.5	0.003	0.005
<i>Coprococcus</i>	1.75	0.3	0.33	0.074	2.38	0.5	0.94	0.31	3.00E-07	0.4	0.03	2.00E-06	0.03	0.02
<i>Prevotella</i>	2.23	0.72	0.18	0.12	1.89	0.7	0.083	0.034	0.002	0.6	0.08	0.0002	0.3	0.02
<i>Bifidobacterium</i>	5.55	0.9	11.14	1.38	4.59	1.05	17.84	3.51	0.001	0.4	0.0002	0.00008	0.05	0.0002
<i>Ruminococcus</i>	5.87	0.46	2.45	0.71	5.36	0.91	6.47	1.11	1.00E-06	0.1	0.7	0.00009	0.001	0.3
<i>Blautia</i>	7.33	0.81	10.68	1.51	3.86	0.61	8.39	1.32	0.1	0.001	0.6	0.00007	0.5	0.003
<i>Bacteroides</i>	8.44	0.98	9.07	1.62	7.68	1.82	2.84	1	0.5	0.2	0.0004	0.9	0.09	0.02
<i>Faecalibacterium</i>	10.43	0.83	7.61	1.03	5.99	0.97	3.05	0.75	0.05	0.0004	6.00E-07	0.5	0.03	0.04

Table S2. Food description and average frequency of daily consumption for each microbiota cluster (C1 to C4).

Food	Description	Food category	Microbiota group			
			C1	C2	C3	C4
bisquits and sweet snacks	Biscuits, packaged cakes or pastries	Snacks	0.385	0.417	0.215	0.567
cheese	Grated, sliced or spreadable cheese	Cheese	0.25	0.312	0.288	0.333
chocolate	Chocolate candy and bars	Snacks	0.244	0.231	0.182	0.183
eggs	Boiled or fried eggs	Eggs	0.11	0.099	0.092	0.088
fried food	Fried food	Other	0.111	0.077	0.082	0.11
fried potatoes	Fried potatoes	Vegetable	0.084	0.099	0.067	0.076
homemade sandwiches	Hamburger, hotdog, kebab, wrap, falafel	Cereal	0.198	0.149	0.206	0.152
ice cream	Ice cream, milk and fruit based bars	Snacks	0.138	0.135	0.123	0.062
meat	Fresh and fried meat	Meat Products	0.088	0.117	0.086	0.138
milk and yoghurt	Milk and plain sweetened yoghurt	Dairy Products	0.481	0.664	0.464	0.683
not homemade sandwiches	Hamburger, hotdog, kebab, wrap, falafel	Cereal	0.076	0.064	0.092	0.112
olive oil	Vegetable dressing	Fat and oils	0.658	0.784	0.561	0.776
pizza	Pizza	Cereal	0.146	0.14	0.185	0.133
sausages	Cold cuts and preserved meat product	Meat Products	0.038	0.072	0.029	0.043
seasonings and condiments	Butter, margarine on bread, pesto, ragù	Condiments	0.214	0.244	0.089	0.217
slide ham	Cold cuts and preserved meat product	Meat Products	0.116	0.139	0.12	0.167
sweet and salty snacks	Packaged cakes or savoury pastries and fritters	Snacks	0.204	0.244	0.155	0.16
sweetened drinks	Sweetened soft beverages	Drinks	0.118	0.195	0.092	0.1
wine	Alcoholic beverages	Drinks	0.189	0.172	0.273	0.16

Table S3. Summary of dietary groups and microbiota clusters for each of the 100 enrolled women. Women were stratified based on addictive eating behavior into 4 groups: NW_NA (normal-weight, non-addicted), O_LA (overweight/obese, low-addicted), O_HA (overweight/obese, high-addicted), and O_DHA (overweight/obese, high-addicted with food addiction diagnosis). For microbiota clusters and dietary groups, see Fig. 3 and Fig. 5, respectively.

SampleID	Group	Microbiota cluster	Diet group
NEU10B	O_LA	C3	D1
NEU116B	O_HA	C2	D3
NEU117B	O_NA	C4	D3
NEU118B	O_NA	C2	D1
NEU119B	O_LA	C2	D1
NEU11B	O_NA	C4	D1
NEU127B	O_LA	C4	D3
NEU129B	O_NA	C2	D2
NEU12B	O_LA	C2	D1
NEU134B	O_HA	C3	D2
NEU139B	O_NA	C1	D3
NEU13B	O_LA	C1	D3
NEU144B	O_NA	C3	D2
NEU14B	O_LA	C4	D1
NEU152B	O_NA	C1	D1
NEU153B	O_LA	C4	D1
NEU155B	O_LA	C3	D3
NEU160B	O_NA	C2	D1
NEU161B	O_LA	C1	D1
NEU163B	O_LA	C4	D1
NEU164B	O_LA	C2	D3
NEU167B	O_NA	C3	D2
NEU16B	O_HA	C2	D1
NEU170B	O_NA	C1	D2
NEU172B	O_NA	C2	D1
NEU174B	O_HA	C2	D2
NEU176B	O_NA	C1	D2
NEU177B	O_LA	C3	D3
NEU178B	O_HA	C3	D1
NEU179B	O_LA	C1	D1
NEU17B	O_HA	C2	D1
NEU180B	O_LA	C1	D2
NEU18B	O_HA	C1	D1
NEU19B	O_HA	C3	D1
NEU1B	O_NA	C3	D3
NEU20B	O_HA	C3	D3
NEU21B	O_HA	C4	D1
NEU22B	O_HA	C2	D1
NEU23B	O_HA	C2	D1
NEU25B	O_NA	C1	D1
NEU26B	O_NA	C1	D1
NEU27B	O_LA	C3	D3
NEU28B	O_NA	C1	D3
NEU29B	O_NA	C2	D1
NEU2B	O_NA	C2	D1
NEU30B	O_NA	C1	D1
NEU31B	O_LA	C1	D3
NEU32B	O_HA	C3	D2
NEU33B	O_HA	C3	D3
NEU34B	O_NA	C2	D3
NEU35B	O_LA	C2	D3
NEU36B	O_NA	C2	D2
NEU37B	O_NA	C2	D1
NEU38B	O_NA	C2	D1
NEU39B	O_LA	C2	D3
NEU3B	O_LA	C1	D3
NEU4B	O_LA	C4	D1
NEU5B	O_NA	C2	D1
NEU6B	O_NA	C2	D1
NEU7B	O_NA	C3	D3
NEU8B	O_HA	C1	D1
NEU9B	O_LA	C2	D1

SampleID	Group	Microbiota cluster	Diet group
NC120B	NW_NA	C1	D1
NC121B	NW_NA	C2	D2
NC122B	NW_NA	C1	D3
NC123B	NW_NA	C1	D3
NC124B	NW_NA	C2	D3
NC125B	NW_NA	C2	D3
NC126B	NW_NA	C1	D1
NC128B	NW_NA	C2	D1
NC130B	NW_NA	C2	D1
NC131B	NW_NA	C1	D1
NC132B	NW_NA	C1	D3
NC133B	NW_NA	C3	D3
NC135B	NW_NA	C2	D1
NC136B	NW_NA	C1	D1
NC137B	NW_NA	C4	D2
NC138B	NW_NA	C4	D1
NC140B	NW_NA	C1	D3
NC141B	NW_NA	C3	D2
NC142B	NW_NA	C1	D2
NC143B	NW_NA	C4	D2
NC145B	NW_NA	C1	D2
NC146B	NW_NA	C3	D3
NC147B	NW_NA	C1	D1
NC148B	NW_NA	C2	D2
NC149B	NW_NA	C4	D1
NC150B	NW_NA	C3	D1
NC151B	NW_NA	C1	D2
NC154B	NW_NA	C4	D1
NC156B	O_HA	C4	D3
NC157B	NW_NA	C3	D1
NC159B	NW_NA	C1	D2
NC162B	NW_NA	C3	D1
NC165B	NW_NA	C2	D2
NC166B	NW_NA	C1	D1
NC168B	NW_NA	C3	D2
NC171B	NW_NA	C1	D1
NC173B	NW_NA	C3	D2
NC175B	NW_NA	C2	D1

Table S5. Summary of the main distinctive features of the four microbiota clusters in terms of clinical, dietary and lipidomic profiles. BITE, Bulimic Investigatory Test Edinburgh; NDT, non-distinctive tracts; SCFAs, short-chain fatty acids.

	microbiota profile			
	C1	C2	C3	C4
clinical characteristics	NDT	↑ uric acid, BITE severity score	NDT	↑ BITE symptoms score ↓ uric acid
diet	↑ fiber intake	↑ energy intake ↓ fiber, fat intake	NDT	↑ energy, carbohydrate intake ↓ fat intake
lipidomics	↑ SCFAs	↑ SCFAs, cholesterol, bile acids	↑ converted sterols ↓ SCFAs	↑ bile acids ↓ SCFAs