Integrating Microbiome Analysis, Metabolomics, Bioinformatics, and Histopathology to Elucidate the Protective Effects of Pomegranate Juice against Benzo-alpha-pyrene–Induced Colon Pathologies

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Supplementary Material

1. **Supplementary Data Files:**

   Data file S1: Microbiome abundance and relative abundance data (as analyzed by Mothur), with raw read counts, normalized read counts, normalized week 4/week 0 ratios (for non-zero values), and taxa that are unique to either week 0 or week 4 samples.

   Data file S2: Metabolomic analysis results, for all samples, normalized to xylitol, and double-normalized week 4/week 0 ratios.

   Data file S3: Microbiome-metabolome correlation matrices

2. **Supplementary Tables:**

   Table S1: Results of GC-MS analysis of primary metabolites from different groups

   Table S2: Updated names of some bacterial phyla according to the International Code of Nomenclature of Prokaryotes (ICNP)_taxonomy 2022

   Table S3: List of used R packages

3. Supplementary Figures:

Table S1: Results of GC-MS analysis of primary metabolites from different treatment groups in week 0 and week 4. The data are expressed as relative percentile (mean ± standard deviation, n = 3).

Class	Metabolite	Wk0				Wk4			
		Wk0_U	Wk0_P	Wk0_B	Wk0_PB	Wk4_U	Wk4_P	Wk4_B	Wk4_PB
Acids	Butyric acid	0.033 ± 0.026	0.017 ± 0.027	0.033 ± 0.025	0.015 ± 0.019	0.039 ± 0.031	0.019 ± 0.029	0.047 ± 0.035	0.016 ± 0.025
	Isovaleric acid	0.017 ± 0.008	0.021 ± 0.007	0.023 ± 0.016	0.015 ± 0.011	0.028 ± 0.009	0.042 ± 0.019	0.033 ± 0.012	0.034 ± 0.012
	Propionic acid	0.001 ± 0.000	0.014 ± 0.037	0.074 ± 0.073	0.041 ± 0.056	0.057 ± 0.066	0.020 ± 0.037	0.010 ± 0.028	0.029 ± 0.043
	Valeric acid	0.037 ± 0.023	0.044 ± 0.015	0.047 ± 0.039	0.020 ± 0.013	0.066 ± 0.020	0.056 ± 0.028	0.070 ± 0.013	0.063 ± 0.038
	Lactic acid	0.374 ± 0.214	0.345 ± 0.356	0.218 ± 0.176	0.140 ± 0.030	0.393 ± 0.213	0.232 ± 0.085	0.270 ± 0.207	0.383 ± 0.332
	Caproic acid	0.028 ± 0.020	0.027 ± 0.013	0.019 ± 0.013	0.008 ± 0.004	0.024 ± 0.008	0.014 ± 0.005	0.019 ± 0.007	0.026 ± 0.015
	Glycolic acid	0.025 ± 0.006	0.030 ± 0.007	0.027 ± 0.004	0.022 ± 0.012	0.027 ± 0.013	0.031 ± 0.016	0.019 ± 0.004	0.026 ± 0.015
	alpha-Hydroxybutyric acid	0.010 ± 0.003	0.011 ± 0.002	0.009 ± 0.002	0.008 ± 0.004	0.009 ± 0.003	0.009 ± 0.005	0.006 ± 0.003	0.012 ± 0.010
	beta-Lactic acid	0.019 ± 0.015	0.013 ± 0.008	0.023 ± 0.009	0.029 ± 0.032	0.006 ± 0.003	0.005 ± 0.001	0.006 ± 0.003	0.004 ± 0.002
	2-Hydroxyvaleric acid	0.025 ± 0.011	0.022 ± 0.004	0.029 ± 0.010	0.039 ± 0.010	0.036 ± 0.036	0.026 ± 0.014	0.036 ± 0.019	0.036 ± 0.028
	2-Aminobutyric acid	0.043 ± 0.014	0.054 ± 0.022	0.047 ± 0.017	0.049 ± 0.012	0.013 ± 0.010	0.040 ± 0.086	0.007 ± 0.007	0.050 ± 0.071
	gamma-Hydroxybutyric acid	0.320 ± 0.197	0.255 ± 0.283	0.394 ± 0.236	0.203 ± 0.134	0.462 ± 0.260	0.335 ± 0.253	0.524 ± 0.268	0.350 ± 0.232
	2-Hydroxyisocaproic acid	0.016 ± 0.006	0.015 ± 0.004	0.015 ± 0.004	0.019 ± 0.005	0.014 ± 0.012	0.015 ± 0.010	0.010 ± 0.005	0.019 ± 0.014
	Succinic acid	0.257 ± 0.061	0.142 ± 0.057	0.196 ± 0.053	0.323 ± 0.199	0.088 ± 0.051	0.076 ± 0.029	0.158 ± 0.062	0.104 ± 0.083
	4-Hydroxybutyric acid	0.004 ± 0.004	0.002 ± 0.000	0.005 ± 0.004	0.006 ± 0.005	0.004 ± 0.003	0.005 ± 0.001	0.007 ± 0.005	0.007 ± 0.005
	Decanoic acid	0.013 ± 0.004	0.010 ± 0.007	0.010 ± 0.005	0.007 ± 0.003	0.015 ± 0.012	0.013 ± 0.010	0.010 ± 0.004	0.021 ± 0.019
	Tartaric acid	0.018 ± 0.008	0.026 ± 0.008	0.020 ± 0.005	0.009 ± 0.008	0.004 ± 0.001	0.004 ± 0.003	0.004 ± 0.002	0.004 ± 0.003
	beta-Phenyllactic acid	0.007 ± 0.004	0.005 ± 0.001	0.007 ± 0.001	0.007 ± 0.002	0.005 ± 0.003	0.005 ± 0.005	0.005 ± 0.002	0.010 ± 0.011

Class	Metabolite	Wk0				Wk4			
		Wk0_U	Wk0_P	Wk0_B	Wk0_PB	Wk4_U	Wk4_P	Wk4_B	Wk4_PB
	3-Hydroxyphenylacetic acid	0.007 ± 0.001	0.008 ± 0.003	0.008 ± 0.003	0.008 ± 0.002	0.021 ± 0.002	0.013 ± 0.004	0.018 ± 0.002	0.012 ± 0.003
	p-Hydroxyphenylacetic acid	0.034 ± 0.004	0.030 ± 0.004	0.034 ± 0.008	0.032 ± 0.006	0.016 ± 0.007	0.010 ± 0.005	0.010 ± 0.004	0.016 ± 0.005
	Ribofuranose (isomer 1)	0.157 ± 0.032	0.125 ± 0.024	0.144 ± 0.049	0.149 ± 0.027	0.039 ± 0.015	0.028 ± 0.009	0.036 ± 0.010	0.047 ± 0.019
	3-Hydroxyphenyl-propionic acid	0.067 ± 0.012	0.053 ± 0.014	0.071 ± 0.020	0.080 ± 0.016	0.143 ± 0.060	0.205 ± 0.053	0.300 ± 0.092	0.111 ± 0.046
	Azelaic acid	0.011 ± 0.004	0.011 ± 0.001	0.011 ± 0.005	0.009 ± 0.002	0.005 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.006 ± 0.001
	5-Hydroxyindoleacetic acid	0.024 ± 0.006	0.012 ± 0.004	0.020 ± 0.006	0.039 ± 0.031	0.040 ± 0.039	0.059 ± 0.020	0.092 ± 0.057	0.046 ± 0.036
	Dehydroabietic acid	0.015 ± 0.008	0.019 ± 0.006	0.026 ± 0.005	0.030 ± 0.018	0.024 ± 0.023	0.010 ± 0.004	0.014 ± 0.005	0.018 ± 0.008
	Total	**1.564**	**1.310**	**1.511**	**1.307**	**1.577**	**1.274**	**1.717**	**1.452**
Alcohols	Ethylene glycol	0.024 ± 0.035	0.013 ± 0.033	0.001 ± 0.001	0.013 ± 0.034	0.002 ± 0.001	0.022 ± 0.040	0.032 ± 0.036	0.035 ± 0.044
	Propylene glycol	0.015 ± 0.004	0.015 ± 0.005	0.011 ± 0.001	0.015 ± 0.003	0.018 ± 0.005	0.019 ± 0.006	0.018 ± 0.005	0.020 ± 0.007
	1,3 Propanediol	0.023 ± 0.012	0.018 ± 0.013	0.026 ± 0.014	0.017 ± 0.009	0.034 ± 0.012	0.023 ± 0.013	0.034 ± 0.014	0.021 ± 0.016
	1,4-Butanediol	0.251 ± 0.153	0.355 ± 0.120	0.314 ± 0.126	0.276 ± 0.114	0.053 ± 0.036	0.071 ± 0.097	0.029 ± 0.018	0.122 ± 0.153
	1-Hexadecanol	0.040 ± 0.057	0.036 ± 0.034	0.024 ± 0.015	0.050 ± 0.049	0.041 ± 0.048	0.079 ± 0.122	0.029 ± 0.037	0.030 ± 0.030
	Octadecan-1-ol	0.026 ± 0.034	0.025 ± 0.021	0.017 ± 0.007	0.031 ± 0.026	0.027 ± 0.026	0.049 ± 0.066	0.026 ± 0.018	0.021 ± 0.016
	Total	**0.379**	**0.461**	**0.393**	**0.401**	**0.175**	**0.263**	**0.169**	**0.248**
Aliphatic hydro-carbons	Nonane	0.079 ± 0.019	0.093 ± 0.030	0.086 ± 0.017	0.068 ± 0.011	0.112 ± 0.042	0.101 ± 0.034	0.122 ± 0.014	0.092 ± 0.041
	Total	**0.079**	**0.093**	**0.086**	**0.068**	**0.112**	**0.101**	**0.122**	**0.092**
Aromatic hydrocarbons	Ethylbenzene	0.009 ± 0.009	0.022 ± 0.011	0.024 ± 0.008	0.018 ± 0.005	0.028 ± 0.016	0.004 ± 0.006	0.026 ± 0.019	0.012 ± 0.015
	p-Xylene	0.730 ± 1.124	1.314 ± 1.696	0.856 ± 1.082	0.978 ± 1.047	0.855 ± 1.307	2.263 ± 2.024	0.868 ± 1.203	1.638 ± 1.594
	Total	**0.738**	**1.336**	**0.880**	**0.995**	**0.884**	**2.267**	**0.894 ±**	**1.650**
Amino acids	l-Alanine	0.060 ± 0.083	0.005 ± 0.002	0.003 ± 0.001	0.005 ± 0.002	0.010 ± 0.012	0.028 ± 0.051	0.011 ± 0.006	0.038 ± 0.069

Class	Metabolite	Wk0				Wk4			
		Wk0_U	Wk0_P	Wk0_B	Wk0_PB	Wk4_U	Wk4_P	Wk4_B	Wk4_PB
	L-Valine	0.011 ± 0.007	0.013 ± 0.004	0.014 ± 0.014	0.012 ± 0.004	0.004 ± 0.003	0.015 ± 0.017	0.002 ± 0.002	0.019 ± 0.020
	Valine	0.274 ± 0.078	0.451 ± 0.105	0.315 ± 0.074	0.349 ± 0.124	0.056 ± 0.039	0.120 ± 0.236	0.033 ± 0.012	0.196 ± 0.209
	l-Leucine	0.125 ± 0.052	0.254 ± 0.116	0.157 ± 0.057	0.148 ± 0.088	0.021 ± 0.015	0.029 ± 0.035	0.016 ± 0.007	0.099 ± 0.112
	Leucine	0.021 ± 0.006	0.015 ± 0.006	0.018 ± 0.008	0.018 ± 0.004	0.011 ± 0.003	0.009 ± 0.003	0.012 ± 0.003	0.014 ± 0.004
	Isoleucine	0.092 ± 0.045	0.165 ± 0.060	0.109 ± 0.042	0.112 ± 0.071	0.010 ± 0.007	0.031 ± 0.071	0.009 ± 0.005	0.038 ± 0.039
	beta-Alanine	0.005 ± 0.002	0.005 ± 0.001	0.005 ± 0.001	0.003 ± 0.001	0.009 ± 0.003	0.006 ± 0.001	0.008 ± 0.001	0.006 ± 0.001
	Serine	0.019 ± 0.013	0.026 ± 0.013	0.016 ± 0.007	0.016 ± 0.010	0.004 ± 0.003	0.0050. ± 007	0.003 ± 0.002	0.005 ± 0.005
	L-Threonine	0.008 ± 0.002	0.008 ± 0.002	0.015 ± 0.007	0.009 ± 0.003	0.011 ± 0.006	0.008 ± 0.007	0.014 ± 0.009	0.010 ± 0.007
	Glycine	0.284 ± 0.081	0.424 ± 0.166	0.322 ± 0.072	0.319 ± 0.102	0.396 ± 0.162	0.462 ± 0.181	0.396 ± 0.118	0.435 ± 0.208
	Homoserine	0.009 ± 0.003	0.014 ± 0.011	0.007 ± 0.002	0.007 ± 0.003	0.001 ± 0.001	0.009 ± 0.017	0.001 ± 0.000	0.008 ± 0.011
	Aspartate	0.014 ± 0.005	0.017 ± 0.006	0.016 ± 0.005	0.018 ± 0.005	0.005 ± 0.002	0.008 ± 0.005	0.005 ± 0.002	0.016 ± 0.011
	DL-Phenylalanine	0.015 ± 0.009	0.014 ± 0.007	0.017 ± 0.010	0.016 ± 0.005	0.002 ± 0.001	0.008 ± 0.015	0.002 ± 0.001	0.006 ± 0.006
	2-Aminoadipic acid	0.042 ± 0.045	0.020 ± 0.021	0.044 ± 0.031	0.020 ± 0.031	0.005 ± 0.004	0.013 ± 0.014	0.003 ± 0.002	0.011 ± 0.014
	Phenylalanine	0.040 ± 0.019	0.063 ± 0.021	0.035 ± 0.013	0.038 ± 0.027	0.006 ± 0.004	0.027 ± 0.066	0.004 ± 0.002	0.015 ± 0.016
	Tyrosine	0.119 ± 0.055	0.154 ± 0.065	0.087 ± 0.037	0.105 ± 0.096	0.011 ± 0.006	0.082 ± 0.185	0.011 ± 0.004	0.058 ± 0.061
	Total	**1.138**	**1.648**	**1.181**	**1.196**	**0.561**	**0.862**	**0.528**	**0.974**
Amino Sugars	N-Acetyl-D-glucosamine (isomer 2)	0.039 ± 0.011	0.045 ± 0.010	0.04 ± 0.007	0.050 ± 0.002	0.010 ± 0.004	0.009 ± 0.003	0.013 ± 0.003	0.012 ± 0.003
	Total	**0.039**	**0.045**	**0.04**	**0.05**	**0.01**	**0.009**	**0.013**	**0.012**
Esters	Tributyl acetylcitrate	0.003 ± 0.065	0.052 ± 0.078	0.003 ± 0.006	0.074 ± 0.108	0.038 ± 0.111	0.140 ± 0.237	0.020 ± 0.045	0.038 ± 0.063
	Total	**0.033**	**0.052**	**0.003**	**0.074**	**0.038**	**0.140**	**0.020**	**0.038**

Class	Metabolite	Wk0				Wk4			
		Wk0_U	Wk0_P	Wk0_B	Wk0_PB	Wk4_U	Wk4_P	Wk4_B	Wk4_PB
Fatty acids/esters	Myristic acid	0.117 ± 0.111	0.310 ± 0.105	0.248 ± 0.033	0.247 ± 0.057	0.369 ± 0.048	0.370 ± 0.072	0.346 ± 0.048	0.393 ± 0.063
	Pentadecanoic acid ester	0.029 ± 0.011	0.030 ± 0.008	0.031 ± 0.003	0.037 ± 0.012	0.026 ± 0.005	0.022 ± 0.007	0.028 ± 0.006	0.023 ± 0.005
	Palmitic acid	0.575 ± 0.069	0.544 ± 0.182	0.483 ± 0.035	0.714 ± 0.392	0.534 ± 0.082	0.474 ± 0.118	0.483 ± 0.065	0.448 ± 0.104
	Linoleic acid	0.394 ± 0.061	0.320 ± 0.067	0.325 ± 0.069	0.495 ± 0.145	0.314 ± 0.086	0.251 ± 0.086	0.318 ± 0.060	0.225 ± 0.080
	9-Octadecenoic acid ester	0.287 ± 0.073	0.230 ± 0.095	0.241 ± 0.064	0.388 ± 0.340	0.250 ± 0.068	0.208 ± 0.071	0.231 ± 0.041	0.167 ± 0.054
	trans-13-Octadecenoic acid	0.073 ± 0.014	0.066 ± 0.022	0.060 ± 0.014	0.088 ± 0.074	0.072 ± 0.018	0.052 ± 0.016	0.064 ± 0.012	0.054 ± 0.017
	Oleic acid	0.008 ± 0.006	0.003 ± 0.001	0.006 ± 0.003	0.016 ± 0.013	0.018 ± 0.021	0.019 ± 0.015	0.030 ± 0.023	0.011 ± 0.009
	Stearic acid	0.175 ± 0.032	0.196 ± 0.115	0.157 ± 0.020	0.269 ± 0.240	0.192 ± 0.039	0.182 ± 0.050	0.176 ± 0.024	0.175 ± 0.043
	Arachidonic acid	0.011 ± 0.002	0.013 ± 0.005	0.010 ± 0.004	0.014 ± 0.005	0.006 ± 0.002	0.008 ± 0.005	0.005 ± 0.001	0.007 ± 0.002
	Eicosanoic acid	0.008 ± 0.002	0.007 ± 0.006	0.007 ± 0.003	0.014 ± 0.023	0.008 ± 0.001	0.006 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
	1-Monopalmitin	0.080 ± 0.028	0.125 ± 0.029	0.099 ± 0.010	0.120 ± 0.027	0.196 ± 0.022	0.206 ± 0.052	0.183 ± 0.013	0.182 ± 0.024
	9-Octadecenoic acid, -2-propyl ester	0.014 ± 0.005	0.008 ± 0.004	0.013 ± 0.005	0.021 ± 0.012	0.079 ± 0.049	0.066 ± 0.023	0.085 ± 0.030	0.052 ± 0.023
	1-Monooleoylglycerol	0.057 ± 0.025	0.039 ± 0.014	0.044 ± 0.014	0.090 ± 0.052	0.427 ± 0.170	0.372 ± 0.165	0.480 ± 0.078	0.298 ± 0.068
	Stearic acid, 2,3-propyl ester	0.054 ± 0.038	0.082 ± 0.038	0.054 ± 0.008	0.087 ± 0.054	0.100 ± 0.032	0.137 ± 0.070	0.086 ± 0.017	0.107 ± 0.037
	Total	**1.883**	**1.974**	**1.779**	**2.600**	**2.590**	**2.372**	**2.521**	**2.147**
Nitrogenous compounds	Uracil	0.116 ± 0.052	0.096 ± 0.035	0.117 ± 0.064	0.119 ± 0.034	0.007 ± 0.008	0.010 ± 0.009	0.013 ± 0.009	0.018 ± 0.005
	Adenosine	0.003 ± 0.001	0.001 ± 0.001	0.003 ± 0.001	0.004 ± 0.001	0.010 ± 0.004	0.014 ± 0.004	0.011 ± 0.005	0.013 ± 0.003
	Total	**0.119**	**0.097**	**0.121**	**0.122**	**0.017**	**0.023**	**0.024**	**0.031**
Phenolic acids	m-Hydroxybenzoic acid	0.080 ± 0.093	0.099 ± 0.182	0.091 ± 0.088	0.017 ± 0.020	0.140 ± 0.139	0.091 ± 0.132	0.124 ± 0.110	0.087 ± 0.165
	protocatechuic acid	0.028 ± 0.056	0.033 ± 0.044	0.004 ± 0.009	0.052 ± 0.076	0.024 ± 0.059	0.071 ± 0.108	0.012 ± 0.027	0.036 ± 0.043

Class	Metabolite	Wk0				Wk4			
		Wk0_U	Wk0_P	Wk0_B	Wk0_PB	Wk4_U	Wk4_P	Wk4_B	Wk4_PB
	4-Hydroxyhydrocinnamic acid	0.007 ± 0.001	0.005 ± 0.002	0.008 ± 0.002	0.008 ± 0.003	0.014 ± 0.005	0.034 ± 0.008	0.038 ± 0.023	0.019 ± 0.014
	Benzenepropanoic acid, 4-hydroxy-3-methoxy	0.010 ± 0.003	0.004 ± 0.003	0.007 ± 0.005	0.008 ± 0.006	0.004 ± 0.002	0.010 ± 0.003	0.003 ± 0.001	0.016 ± 0.018
	Hydrocaffeic acid	0.021 ± 0.008	0.009 ± 0.003	0.014 ± 0.008	0.026 ± 0.018	0.016 ± 0.018	0.029 ± 0.017	0.046 ± 0.019	0.015 ± 0.013
	Total	**0.145**	**0.151**	**0.125**	**0.111**	**0.198 ±**	**0.235**	**0.223**	**0.174**
Sterols	Coprostanol	0.069 ± 0.033	0.053 ± 0.011	0.089 ± 0.064	0.053 ± 0.025	0.109 ± 0.056	0.064 ± 0.026	0.102 ± 0.016	0.060 ± 0.022
	Total	**0.069**	**0.053**	**0.089**	**0.053**	**0.109**	**0.064**	**0.102**	**0.060**
Sugar acids	L-Threonic acid	0.076 ± 0.046	0.083 ± 0.024	0.078 ± 0.028	0.043 ± 0.047	0.008 ± 0.002	0.007 ± 0.007	0.007 ± 0.003	0.012 ± 0.011
	Ribonic acid	0.005 ± 0.002	0.006 ± 0.001	0.005 ± 0.001	0.004 ± 0.002	0.004 ± 0.001	0.004 ± 0.002	0.003 ± 0.001	0.006 ± 0.004
	Total	**0.081**	**0.089**	**0.083**	**0.047**	**0.012**	**0.011**	**0.010**	**0.019**
Sugar alcohols	L-Threitol	0.006 ± 0.003	0.003 ± 0.001	0.005 ± 0.002	0.004 ± 0.001	0.004 ± 0.004	0.004 ± 0.003	0.002 ± 0.000	0.006 ± 0.004
	Pinitol	0.238 ± 0.213	0.082 ± 0.086	0.096 ± 0.135	0.016 ± 0.010	0.154 ± 0.211	0.101 ± 0.121	0.008 ± 0.001	0.465 ± 0.382
	1,5-Anhydro-D-sorbitol	0.007 ± 0.003	0.010 ± 0.004	0.007 ± 0.003	0.006 ± 0.005	0.001 ± 0.000	0.006 ± 0.014	0.001 ± 0.000	0.002 ± 0.002
	Ribitol	0.209 ± 0.037	0.168 ± 0.050	0.169 ± 0.053	0.220 ± 0.100	0.040 ± 0.022	0.022 ± 0.006	0.024 ± 0.007	0.034 ± 0.015
	Myoinositol-1	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.002 ± 0.004	0.002 ± 0.001
	Myoinositol-2	0.034 ± 0.022	0.013 ± 0.012	0.014 ± 0.017	0.003 ± 0.001	0.014 ± 0.011	0.011 ± 0.006	0.015 ± 0.011	0.050 ± 0.039
	Myoinositol-3	0.021 ± 0.041	0.023 ± 0.030	0.004 ± 0.007	0.037 ± 0.051	0.020 ± 0.046	0.054 ± 0.080	0.010 ± 0.022	0.029 ± 0.029
	Maltitol	0.023 ± 0.018	0.012 ± 0.003	0.011 ± 0.004	0.014 ± 0.006	0.108 ± 0.030	0.277 ± 0.079	0.145 ± 0.081	0.316 ± 0.301
	Total	**0.540**	**0.314**	**0.308**	**0.301**	**0.343**	**0.477**	**0.207**	**0.904**
Sugars	Glycerol	0.829 ± 0.106	0.875 ± 0.081	0.899 ± 0.191	1.097 ± 0.187	0.410 ± 0.076	0.378 ± 0.139	0.335 ± 0.074	0.393 ± 0.196
	Xylulose	0.003 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.005 ± 0.003	0.006 ± 0.004	0.008 ± 0.004	0.007 ± 0.004	0.007 ± 0.006
	Arabinose-1	0.183 ± 0.117	0.181 ± 0.120	0.203 ± 0.104	0.141 ± 0.058	0.060 ± 0.027	0.055 ± 0.066	0.055 ± 0.022	0.043 ± 0.027

Class	Metabolite	Wk0				Wk4			
		Wk0_U	Wk0_P	Wk0_B	Wk0_PB	Wk4_U	Wk4_P	Wk4_B	Wk4_PB
	Arabinose-2	0.433 ± 0.050	0.342 ± 0.093	0.378 ± 0.081	0.380 ± 0.104	0.106 ± 0.030	0.061 ± 0.017	0.080 ± 0.026	0.094 ± 0.022
	Ribose	0.259 ± 0.082	0.193 ± 0.027	0.239 ± 0.091	0.254 ± 0.055	0.046 ± 0.014	0.034 ± 0.007	0.040 ± 0.010	0.063 ± 0.029
	Ribofuranose (isomer 2)	0.082 ± 0.020	0.068 ± 0.021	0.070 ± 0.022	0.068 ± 0.027	0.035 ± 0.012	0.022 ± 0.006	0.025 ± 0.013	0.031 ± 0.012
	Arabinose-3	0.681 ± 0.192	0.594 ± 0.179	0.605 ± 0.099	0.643 ± 0.113	0.115 ± 0.018	0.075 ± 0.051	0.150 ± 0.036	0.092 ± 0.016
	Fructofuranose (isomer 1)	0.037 ± 0.010	0.034 ± 0.020	0.030 ± 0.022	0.020 ± 0.004	0.006 ± 0.004	0.006 ± 0.001	0.005 ± 0.002	0.010 ± 0.003
	Glucopyranose-1	0.080 ± 0.012	0.061 ± 0.018	0.054 ± 0.026	0.070 ± 0.009	0.011 ± 0.003	0.011 ± 0.003	0.010 ± 0.002	0.019 ± 0.007
	Glucose	0.004 ± 0.004	0.002 ± 0.001	0.004 ± 0.002	0.003 ± 0.002	0.018 ± 0.011	0.020 ± 0.011	0.012 ± 0.003	0.018 ± 0.012
	Talofuranose (isomer 1)	0.203 ± 0.181	0.211 ± 0.104	0.132 ± 0.070	0.201 ± 0.141	0.036 ± 0.024	0.029 ± 0.011	0.038 ± 0.017	0.093 ± 0.040
	Glucose-1	0.020 ± 0.006	0.012 ± 0.006	0.016 ± 0.004	0.020 ± 0.007	0.025 ± 0.009	0.030 ± 0.012	0.033 ± 0.018	0.062 ± 0.020
	Glucose-2	2.463 ± 0.212	1.893 ± 0.235	2.252 ± 0.771	2.171 ± 0.538	0.303 ± 0.147	0.234 ± 0.058	0.339 ± 0.096	0.675 ± 0.259
	Glucopyranose-2	0.478 ± 0.056	0.411 ± 0.118	0.387 ± 0.103	0.517 ± 0.230	0.093 ± 0.044	0.053 ± 0.014	0.060 ± 0.019	0.080 ± 0.028
	Glucopyranose	3.403 ± 0.182	2.548 ± 0.437	3.181 ± 1.076	2.909 ± 0.608	0.426 ± 0.190	0.337 ± 0.080	0.487 ± 0.122	0.956 ± 0.344
	Trehalose	0.008 ± 0.002	0.006 ± 0.002	0.008 ± 0.002	0.008 ± 0.002	0.005 ± 0.002	0.005 ± 0.002	0.007 ± 0.003	0.006 ± 0.002
	Cellobiose (isomer 1)	0.049 ± 0.015	0.034 ± 0.025	0.068 ± 0.036	0.047 ± 0.013	0.057 ± 0.030	0.053 ± 0.011	0.087 ± 0.028	0.061 ± 0.032
	Cellobiose (isomer 2)	0.066 ± 0.022	0.048 ± 0.039	0.106 ± 0.067	0.058 ± 0.024	0.096 ± 0.048	0.088 ± 0.018	0.144 ± 0.057	0.097 ± 0.049
	Total	**9.282**	**7.517**	**8.636**	**8.612**	**1.856**	**1.499**	**1.915**	**2.803**

Table S2: Updated names of some bacterial phyla according to the International Code of Nomenclature of Prokaryotes (ICNP)_taxonomy 2022*

SILVA v. 123 phylum name	Updated name (ICNP 2022)
Firmicutes	Bacillota
Proteobacteria	Pseudomonadota
Actinobacteria	Actinomycetota
Bacteroidetes	Bacteroidota
Tenericutes	Mycoplasmatota
Verrucomicrobia	Verrucomicrobiota
Fusobacteria	Fusobacteriota

*** Source:** Oren A, Garrity GM. Valid publication of the names of forty-two phyla of prokaryotes. *Int J Syst Evol Microbiol* (2021) 71(10). Epub 2021/10/26. doi: 10.1099/ijsem.0.005056.

Table S3: List of used R packages

R package	Main use
ggplot2 and ggpubr	Used for generating box and beanplots (Figures 2E, 3, S1, S2), with statistical tests included in the plots
dunn.test	Alternative *post hoc* test for multiple comparisons of non-parametric data
pheatmap	Used for generating heatmaps (Figures 11 and S14-S16)
factoextra	Used for calculating principal components and plotting PCA and loading plots
coin	For permutational-based statistical tests (in case of ties)

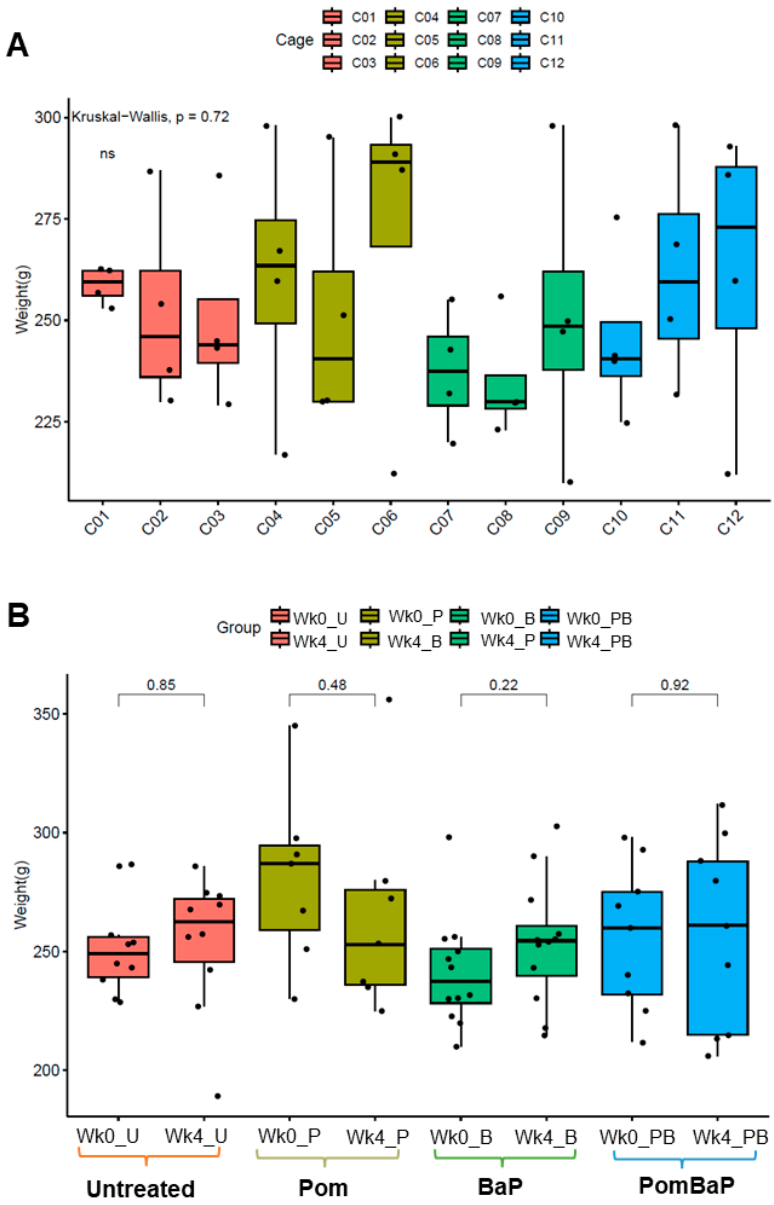

Figure S1. Animal weight and weight variation. A. Boxplots representing the weights of 48 Sprague-Dawley rats, measured at week 0, plotted against the 12 cages (n = 4 rats per cage). Kruskal-Wallis *p* value is indicated: no significant difference is observed between the animal weights per cage. **B.** Change in rat weights with between week 0 and week 4 in different treatment groups (only rats that survived the entire experiment are compared). Data were analyzed for significance by paired t-test, and *p* values are indicated for each pair of groups.

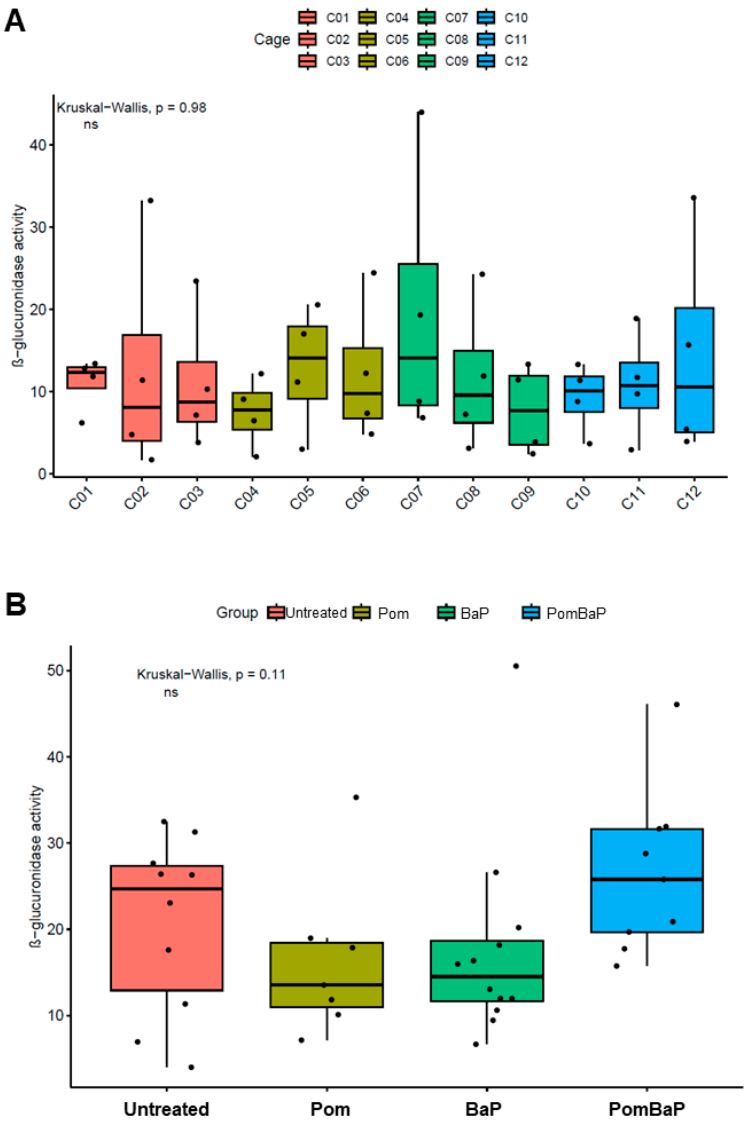

Figure S2. Fecal glucuronidase activity at the start and end of the experiment. A. Boxplots representing the fecal glucuronidase activity (expressed as mg of PNPG/mg feces per minute), determined in week 0, of 48 Sprague-Dawley rats, randomized over 12 cages (n = 4 rats per cage). Statistical significance was assessed by the Kruskal-Wallis test. No *post hoc* comparisons were conducted since the medians were not significantly different between cages. **B.** β-Glucuronidase enzyme activity in different treatment groups (only rats that survived till the end of the experiment), determined on week 4, no significant difference was observed between the groups, and thus no *post hoc* comparisons were conducted.

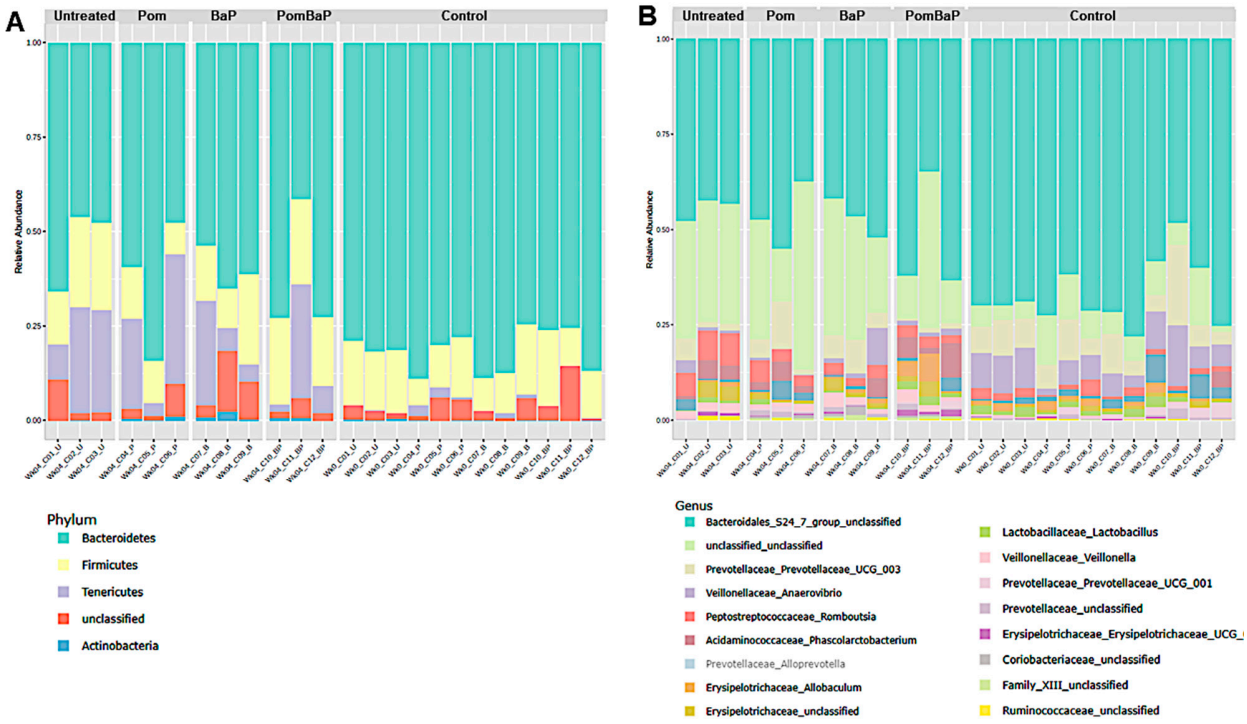

Figure S3. Taxonomic profile of the fecal microbiome of experimental animals. Summary of the relative abundance of different taxonomic units among microbial communities detected in the fecal samples at the phylum **(A)** and genus **(B)** levels.

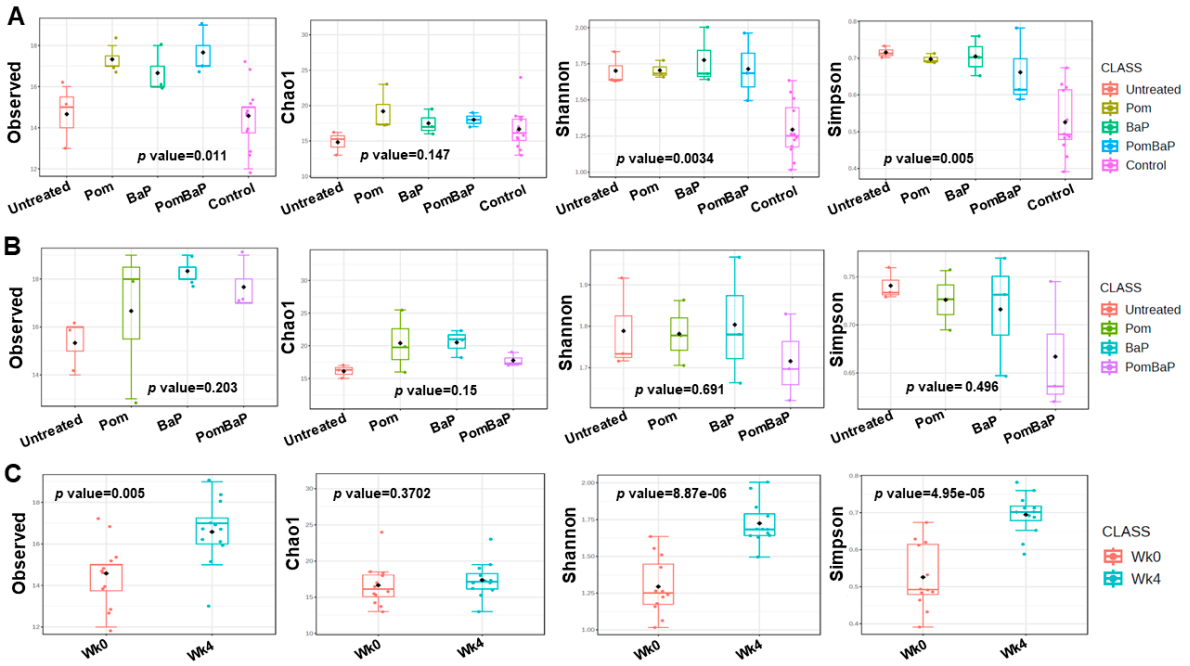

Figure S4. Different alpha diversity metrics (observed OTUs, Chao1, Shannon, and Simpson diversity indices), for microbiome samples at genus level, in different treatment groups, including control samples **(A)**, different treatment groups after excluding the control samples **(B),** and between the two age groups, Wk0 and Wk4 **(C)**.

Differences were analyzed for statistical significance by the Kruskal-Wallis test. *Post hoc* pairwise Wilcoxon tests for Shannon and Simpson indices **(A panels)**, using FDR *p* value adjustment, indicated that pairwise comparisons between the control and each of the following treatment groups: Untreated, Pom, and BaP, were statistically significant (*p* value = 0.029, 0.022, and 0.022, respectively for Shannon index; and 0.022, 0.022, and 0.029, respectively for Simpson index); while the difference between control and PomBaP groups was not statistically significant (Wilcoxon test *p* value = 0.077 for Shannon index and *p* value = 0.341, for Simpson index). Pairwise comparisons between the control and each of the following treatment groups: Pom, BaP, and PomBaP were statistically significant (*p* value = 0.029, 0.048, 0.024), while the difference between the control and untreated groups was not statistically significant (*p* value = 0.926) for observed OTUs.

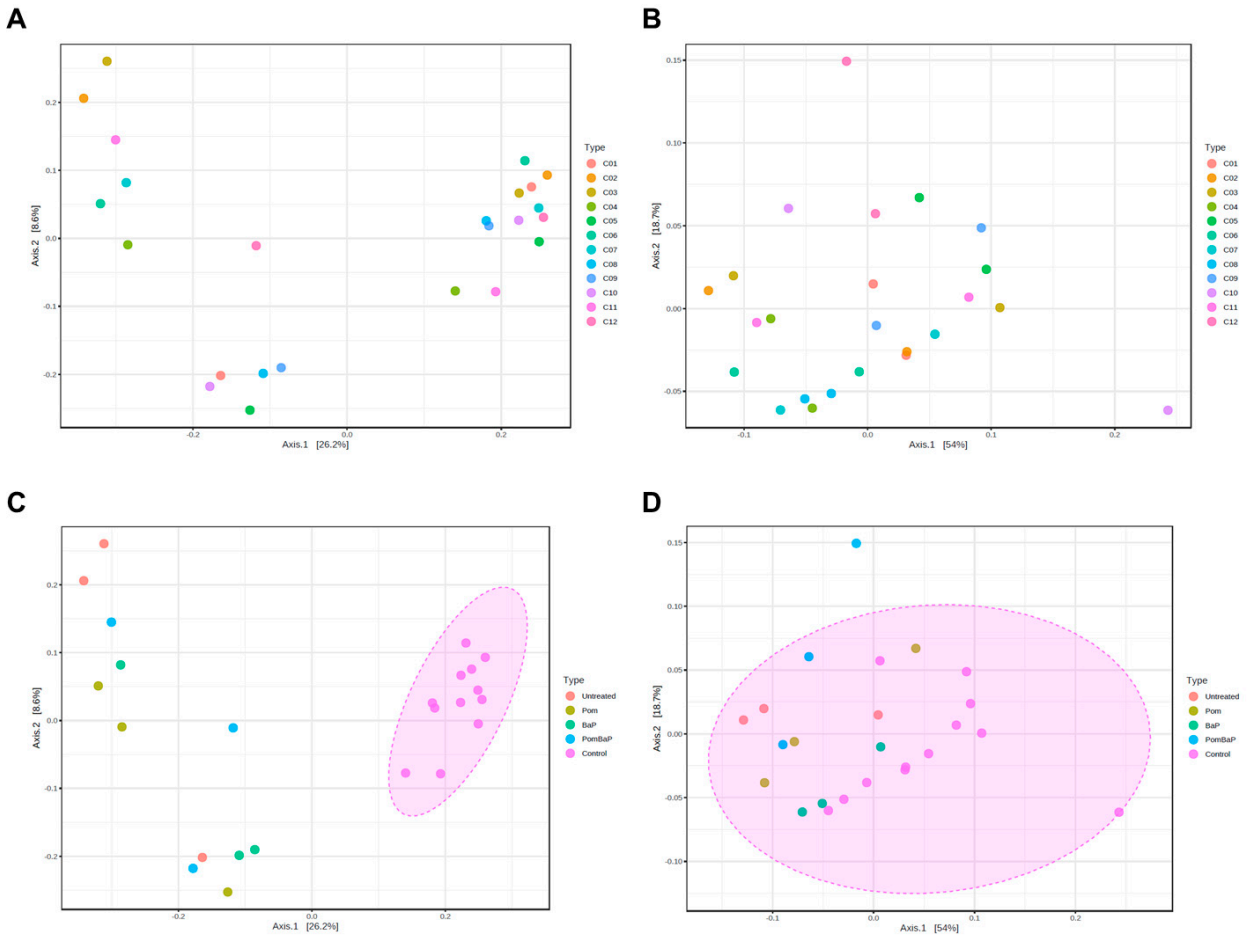

Figure S5. Microbiome beta-diversity analysis, visualized by PCA of Bray-Curtis distances, in which each sample is colored by the cage it was sampled from **(A,B)**. **A.** The distances are calculated at the feature level —PERMANOVA F-value = 0.665, R-squared = 0.379, *p* value: 0.999. **B.** The distances are calculated at the genus level—PERMANOVA F-value = 0.833, R-squared = 0.433, *p* value = 0.718. No significant cage-based clustering was observed in either case. Corresponding group-based clusters (Fig. 4) from feature- and genus-level analysis (**C and D,** respectively) are shown for comparison.

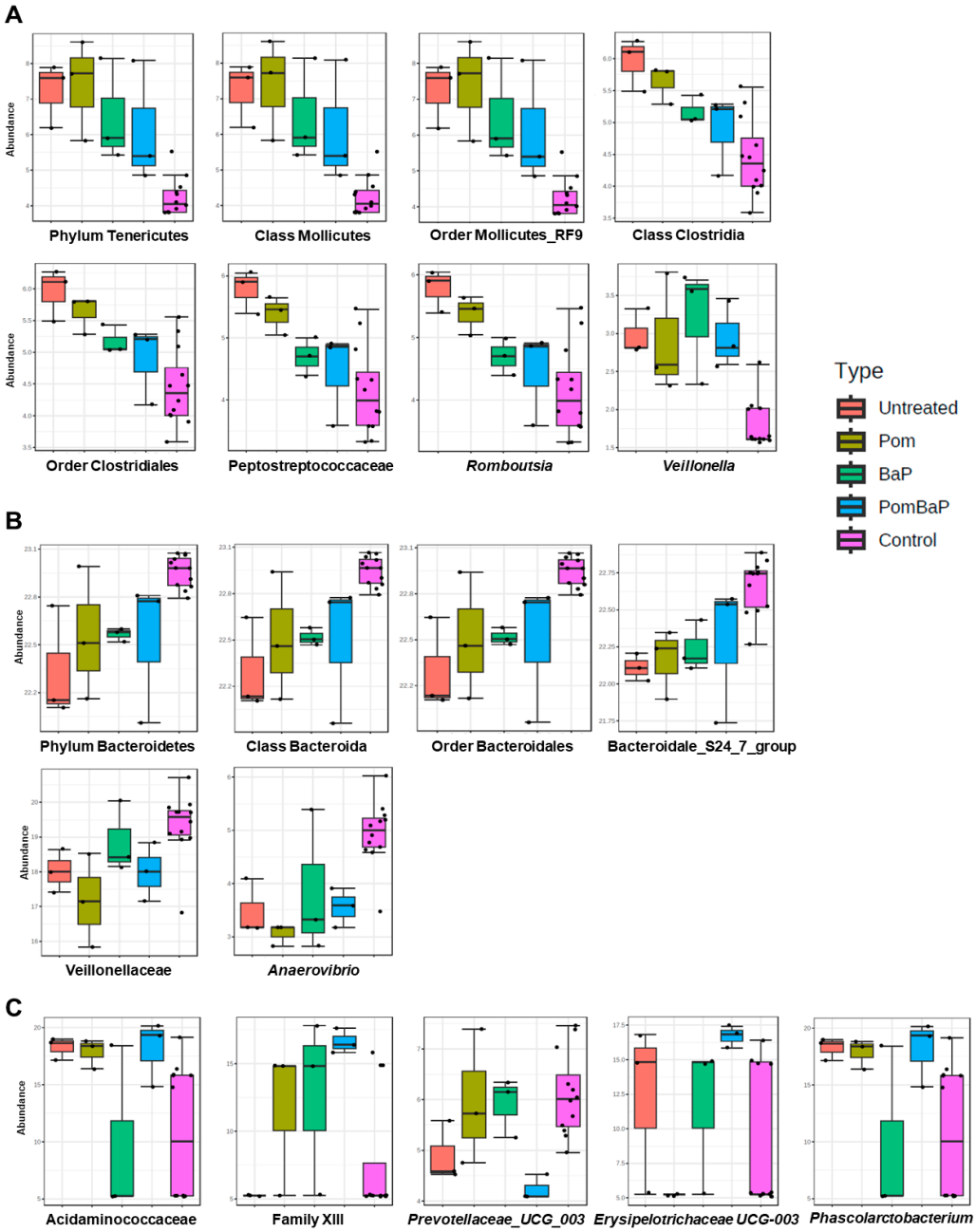

Figure S6. Full list of taxa with significant differences in relative abundance among the five groups. Boxplots describing differential relative abundance of nineteen significant taxa in different samples (influenced by both age and treatment factors). The significance was identified by linear discriminant analysis effect size (LEfSe) analysis, with *p* value cutoff of 0.05, and *p* value adjustment by the FDR method. **Y-axis:** Relative abundance expressed as log-transformed counts.

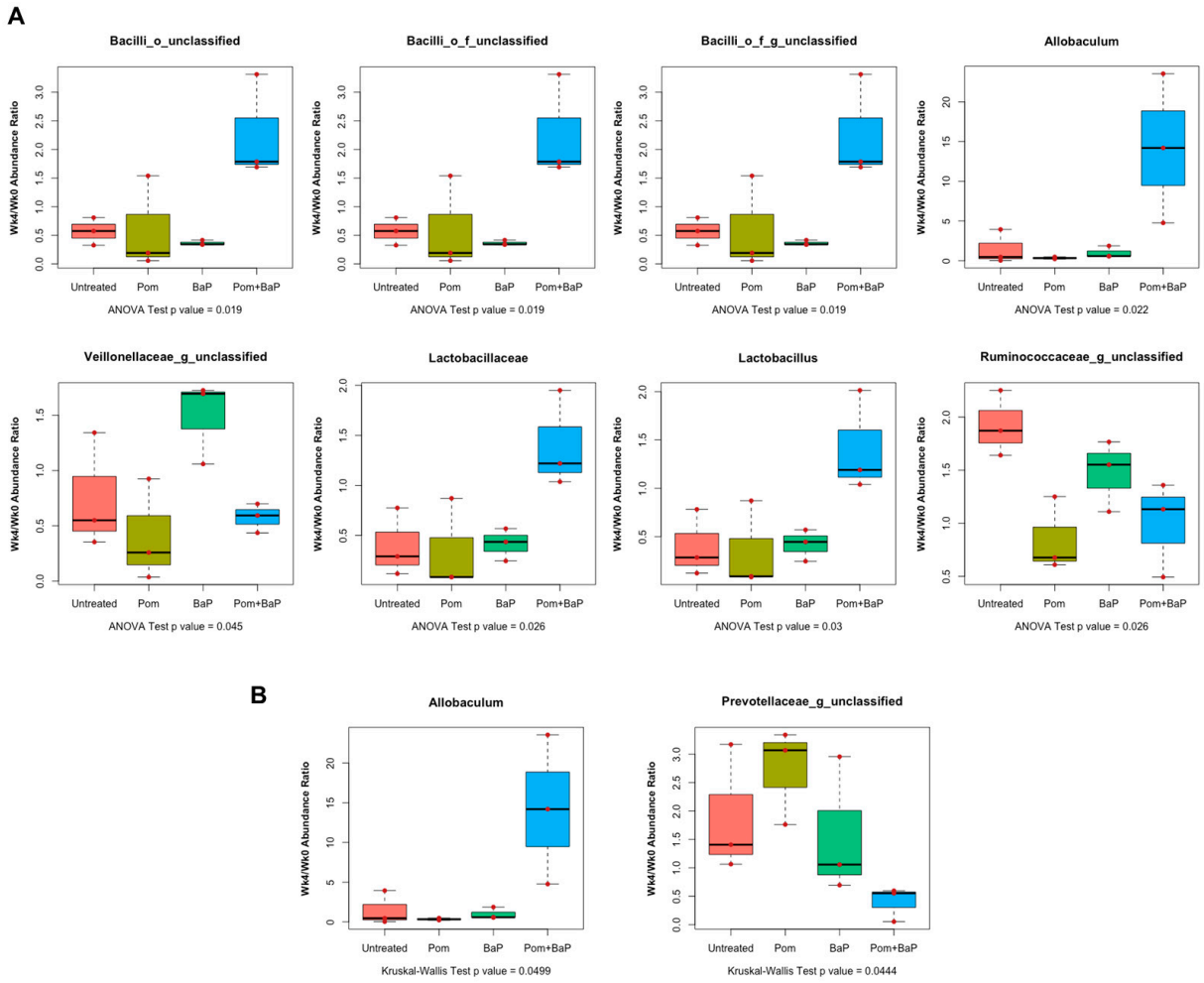

Figure S7. Significantly different taxa between different treatment groups of week 4 (n = 3 cages per group), normalized to their corresponding week 0 samples.

A. Significantly different taxa analyzed by ANOVA (p value < 0.05). **B.** Significantly different taxa analyzed by the Kruskal-Wallis test (p value < 0.05).

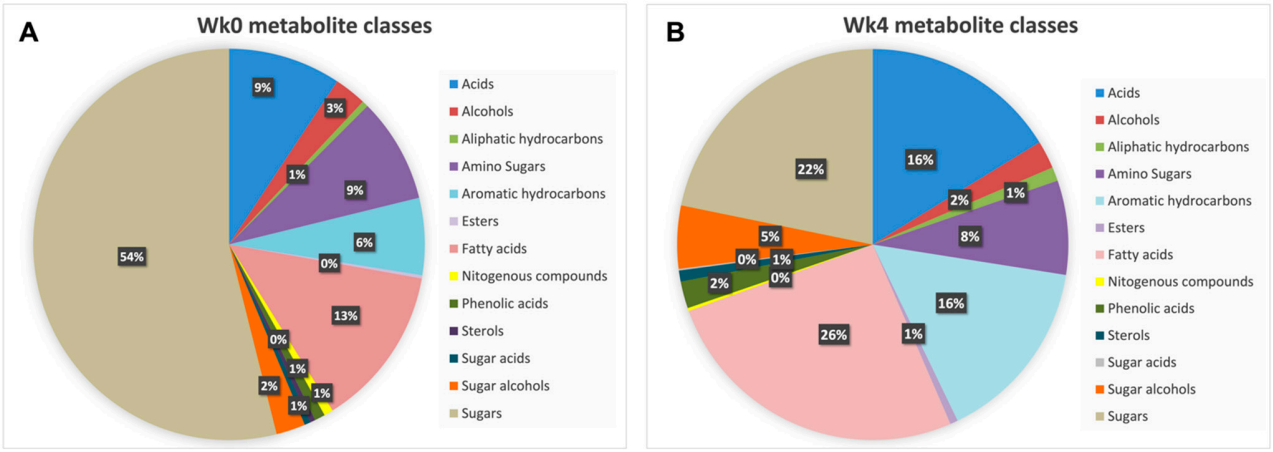

Figure S8. Distribution of the different detected metabolite classes, normalized to xylitol, from rat feces collected on week 0 **(A)** and week 4 **(B)**.

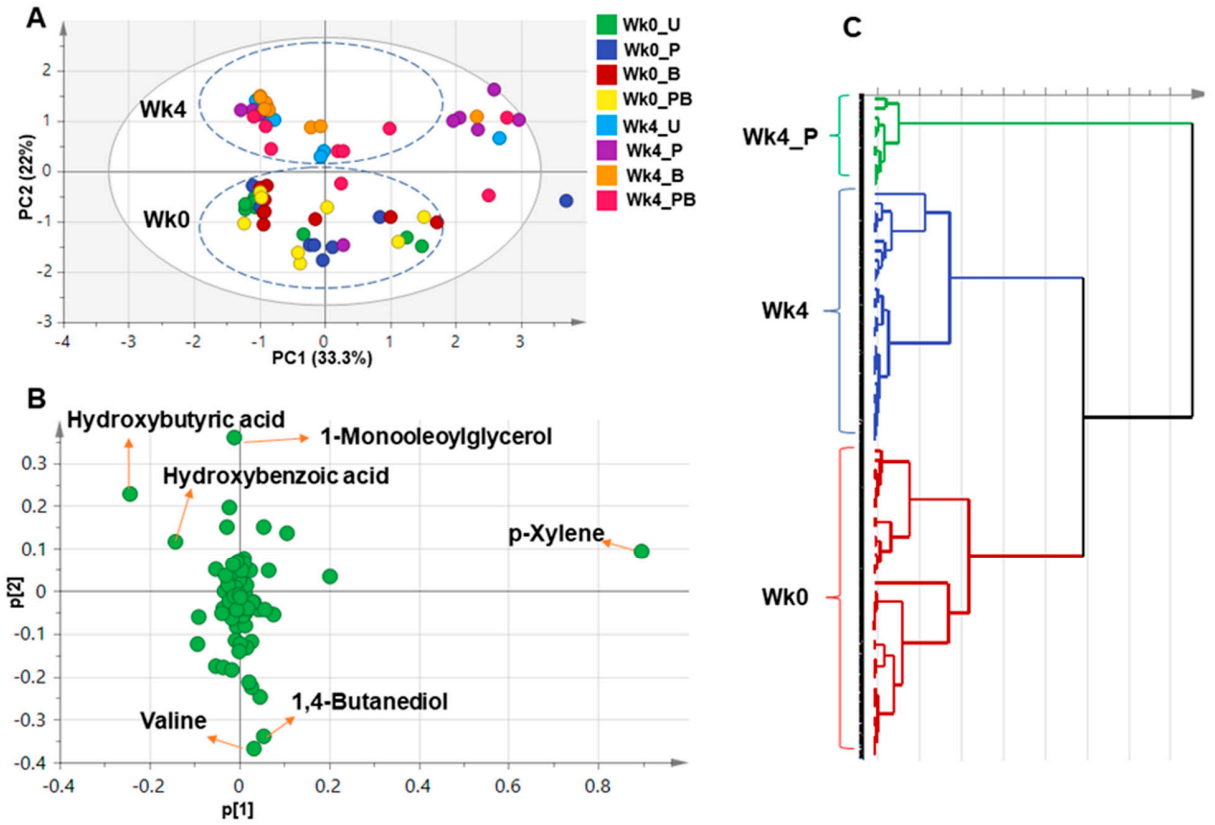

Figure S9. PCA for four sample groups from the start and end of the experiment: four groups representing week 0, and four representing week 4), with sugars removed. This model was explained by 7 components with total variance = 0.855 and prediction power = 0.183). **A.** Score plot of PC1 vs. PC2, which both explain 55% of the variance. A complete separation of week 0 from week 4 samples is observed. **B.** Loading plot for PC1 and PC2 showing the major metabolites (trimethylsilylated) contributing to sample clustering. **C.** Hierarchical clustering analysis (HCA) of GC dataset shows three main clusters. **U** = untreated, **P** = pomegranate, **B** = benzo[a]pyrene, **PB** = pomegranate+benzo[a]pyrene

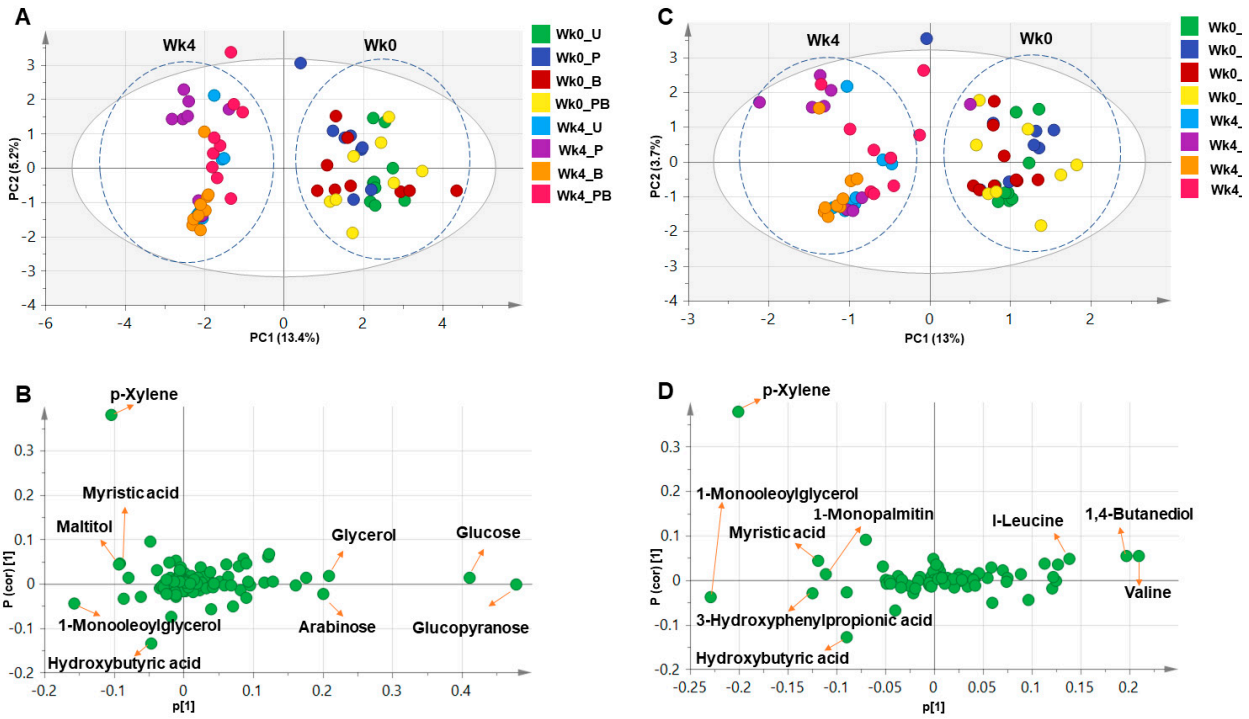

Figure S10. OPLS analyses of the sample groups, including and excluding sugars.

A-B. OPLS for eight sample groups (4 representing Wk0, and 4 representing Wk4).
A. Score plot of PC1 vs. PC2, complete separation of Wk0 from Wk4 samples,this model was explained by 7 components with total variance = 0.479 and prediction power = 0.181). **B.** S-Plot for PC1 and PC2 showing the major metabolites contributing to sample clustering.
C-D: OPLS for eight sample groups, with class 'Sugars' excluded- (4 representing Wk0, and 4 representing Wk4). **C.** Score plot of PC1 vs. PC2, complete separation of Wk0 from Wk4 samples, this model was explained by 7 components with total variance=0.414 and prediction power = 0.104. **D.** S-Plot for PC1 and PC2 showing the major metabolites (trimethylsilylated) contributing to sample clustering. **U** = untreated, **P** = pomegranate, **B** = benzo[a]pyrene, **PB** = pomegranate+benzo[a]pyrene.

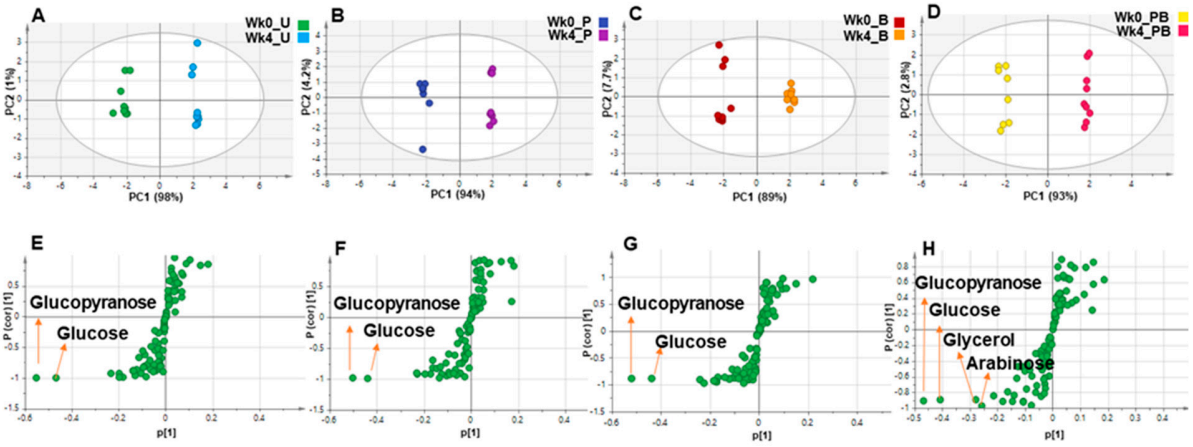

Figure S11. OPLS-DA for normalized groups before (Wk0) and at the end (Wk4) of the experiment: untreated, Pom, BaP, and POM+BaP groups (**A, B, C, and D,** respectively). S-Plots with the major metabolites (trimethylsilylated) contributing for the variability between Wk0 and Wk4 of the untreated, Pom, BaP, and POM+BaP groups (**E, F, G, and H** respectively). **U** = untreated, **P** = pomegranate, **B** = benzo[a]pyrene, **PB** = pomegranate+benzo[a]pyrene

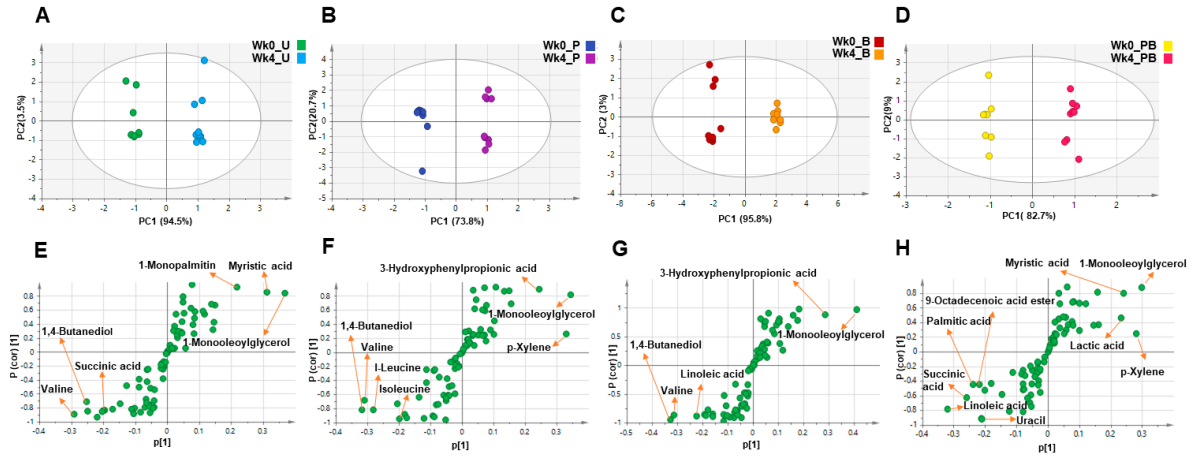

Figure S12. OPLS-DA for normalized groups before (Wk0) and at the end (Wk4) of the experiment—with sugars excluded: untreated, Pom, BaP, and POM+BaP groups (**A, B, C, and D**, respectively). S-Plots with the major metabolites (trimethylsilylated) contributing for the variability between Wk0 and Wk4 of the untreated, Pom, BaP, and POM+BaP groups (**E, F, G, and H** respectively). **U** = untreated, **P** = pomegranate, **B** = benzo[a]pyrene, **PB** = pomegranate+benzo[a]pyrene

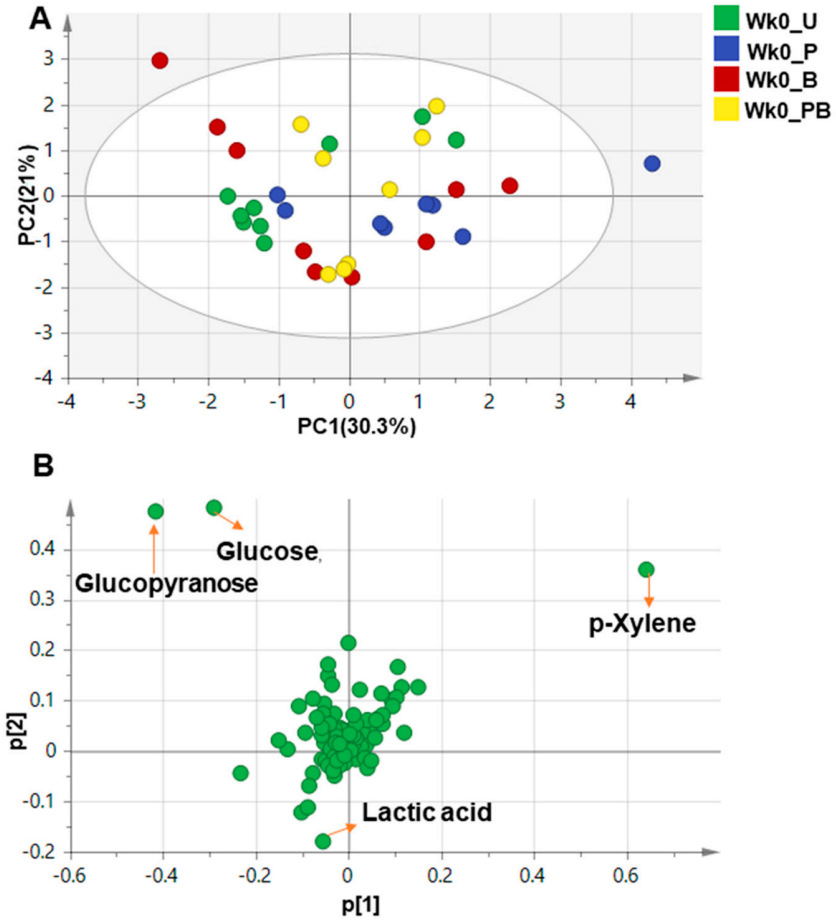

Figure S13. PCA for four sample groups, representing Wk0. This model was explained by seven components with total variance = 0.87 and prediction power = 0.35. **A**: Score plot of PC1 vs. PC2, no clear segregation between the examined samples. **B**: Loading plot for PC1 and PC2 showing the major metabolite (trimethylsilylated) contributing to the divergence of the outlier samples. **U** = untreated, **P** = pomegranate, **B** = benzo[a]pyrene, **PB** = pomegranate+benzo[a]pyrene.

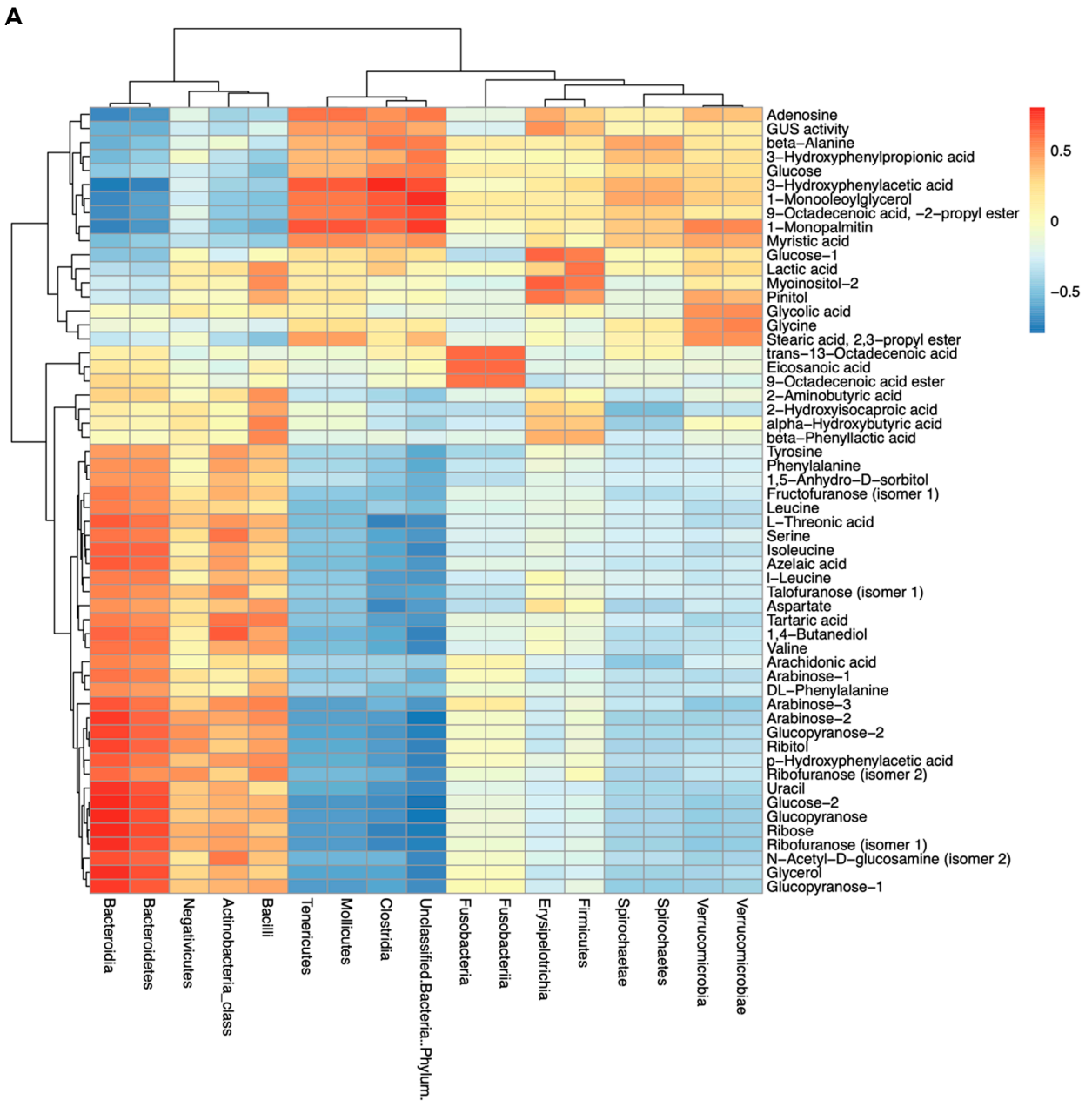

Figure S14. Microbiome-metabolome correlations. Correlation between microbiome profiles (% abundance of different taxa) and metabolome normalized levels was calculated by the Spearman correlation method. **A.** Correlation between microbial phyla/classes and metabolites. Correlation between microbial phyla/classes and metabolite classes is displayed in **Fig. 11B.** The heatmap is colored based on Spearman correlation coefficients r_s.

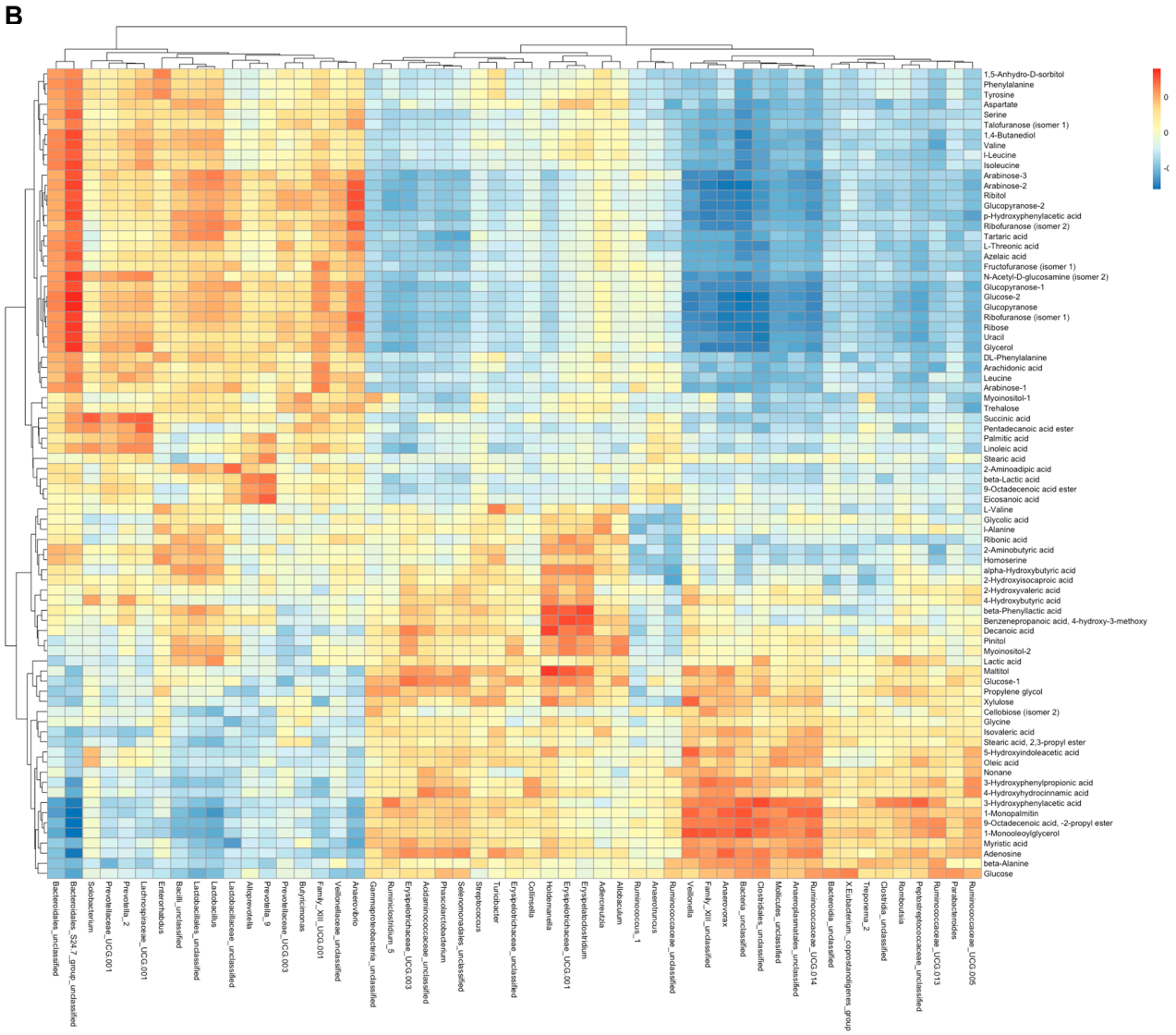

Figure S14. Microbiome-metabolome correlations. Correlation between microbiome profiles (% abundance of different taxa) and metabolome normalized levels was calculated by the Spearman correlation method. **B.** Correlation between microbial genera and metabolites. The heatmap is colored based on Spearman correlation coefficients r_s.

C

Figure S14. Microbiome-metabolome correlations. Correlation between microbiome profiles (% abundance of different taxa) and metabolome normalized levels was calculated by the Spearman correlation method. **C.** Correlations between microbial genera and metabolite classes. Correlation between microbial phyla/classes and metabolite classes is displayed in **Fig. 11B.** The heatmap is colored based on Spearman correlation coefficients r_s.

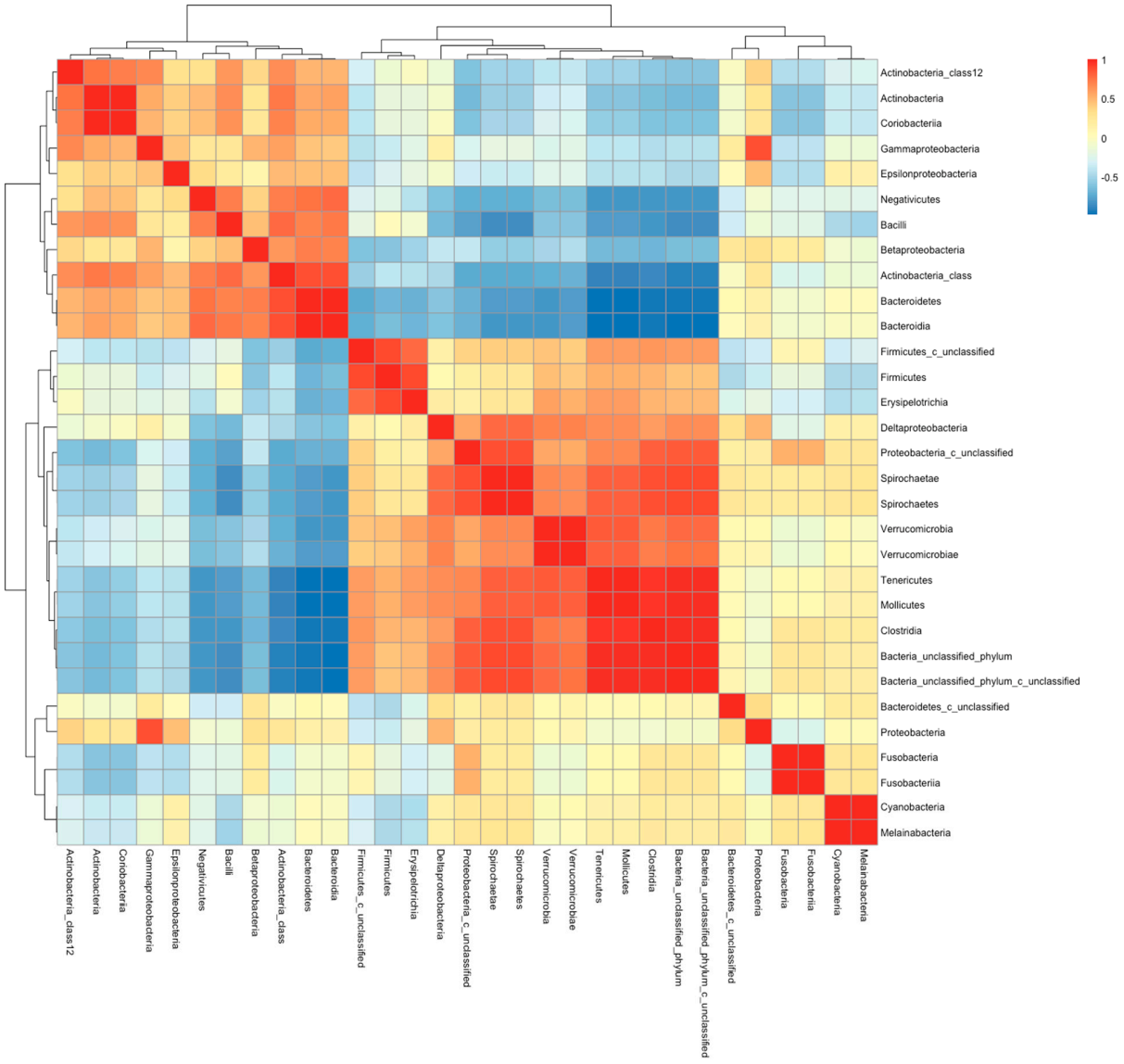

Figure S15. Correlation matrix of microbial phyla and classes based on their correlation with metabolites. The heatmap is colored based on Spearman correlation coefficients r_s.

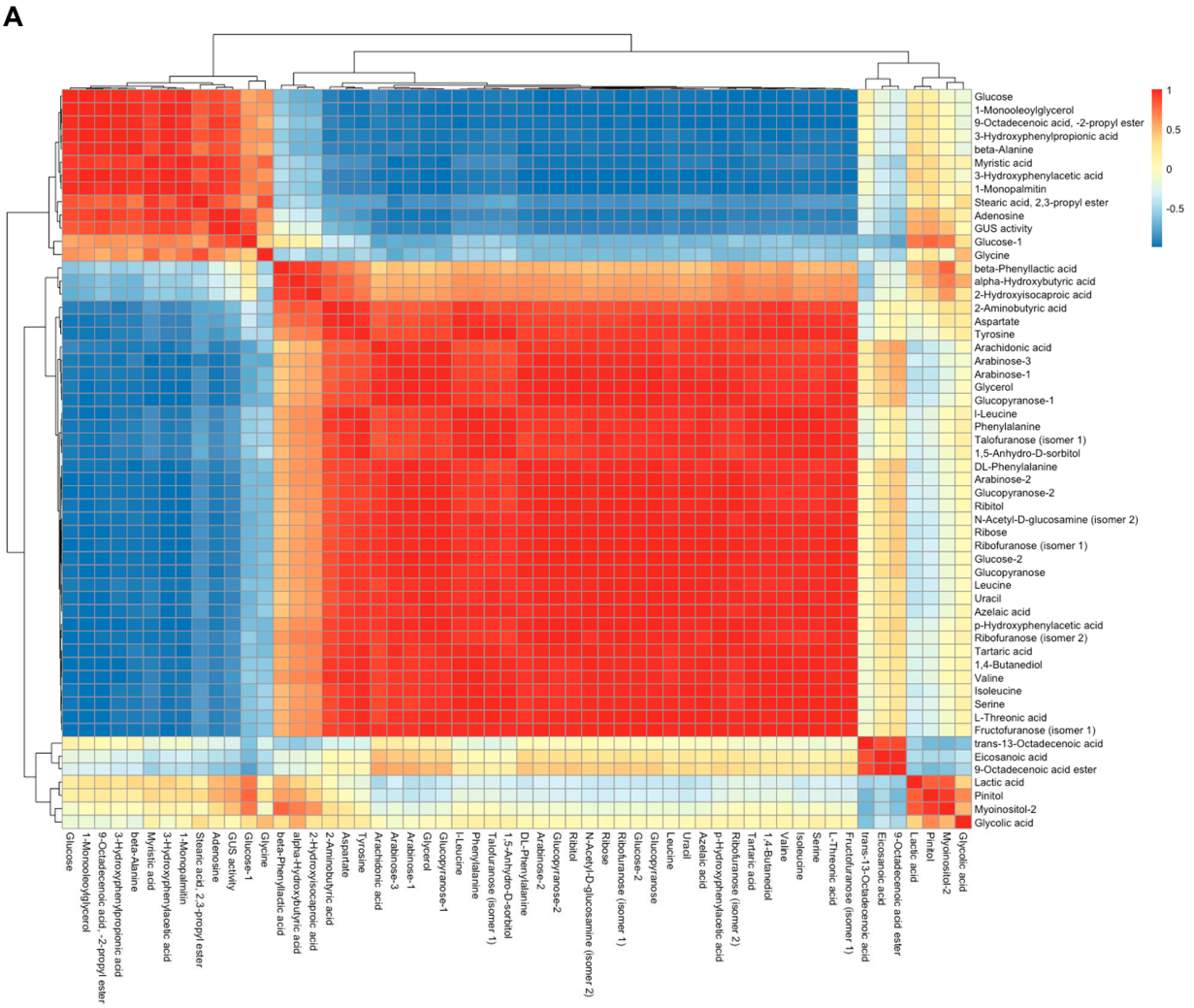

Figure S16. Correlation matrix of metabolites based on their correlation with microbial taxa.
A. Correlation between metabolites based on their correlation with phyla/classes.
The heatmap is colored based on Spearman correlation coefficients r_s.

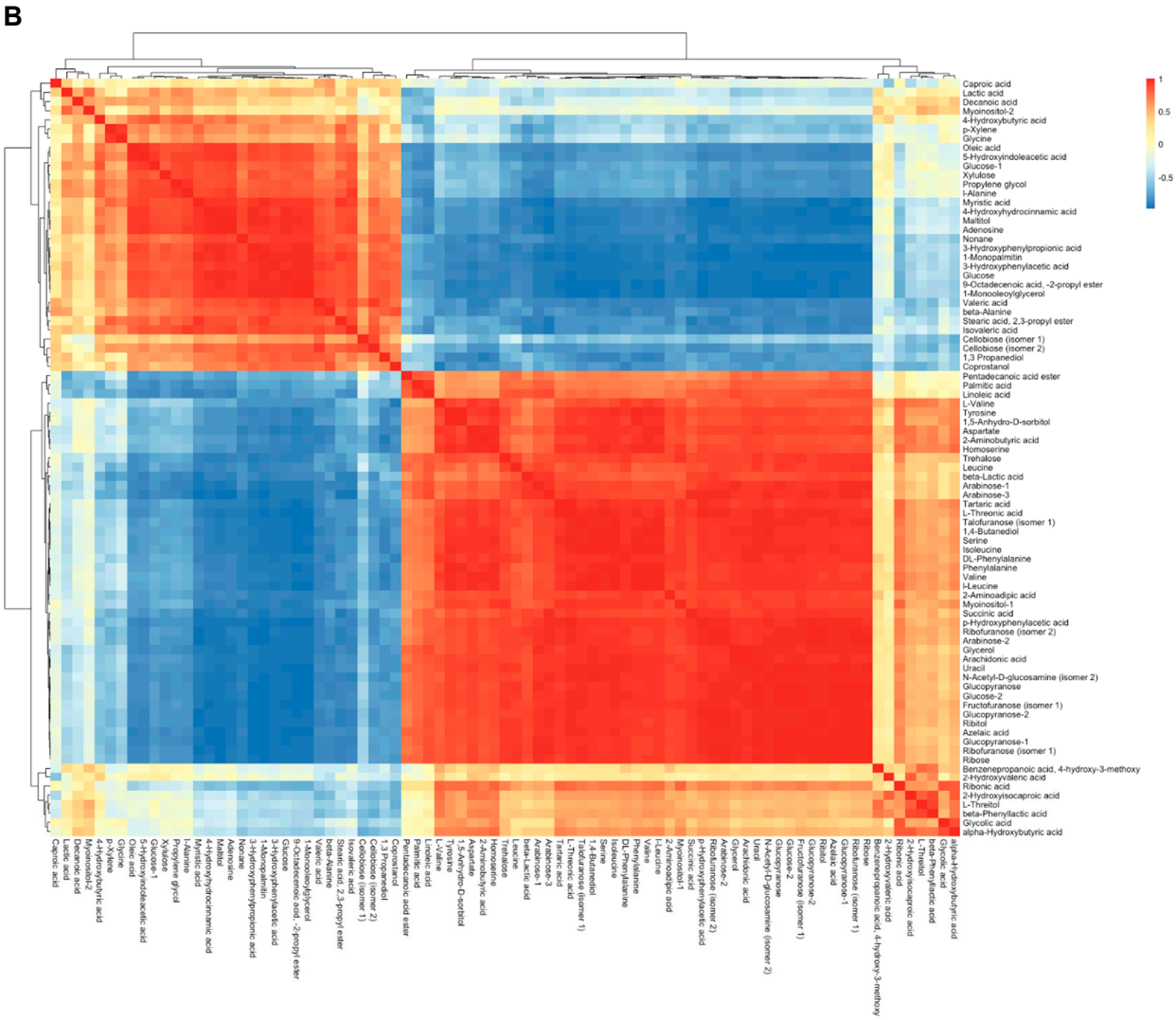

Figure S16. Correlation matrix of metabolites based on their correlation with microbial taxa.
B. Correlation between metabolites based on their correlation with bacterial genera.
The heatmap is colored based on Spearman correlation coefficients r_s.