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3 **Single exposure of food-derived polyethylene and polystyrene microplastics** 4 **profoundly affects gut microbiome in an *in vitro* colon model.**

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6 **Running title: Microplastics exacerbate proteobacteria colonization of the large intestine.**

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36 **Table S1.** Primers pairs employed for PCR and qPCR reactions and quantifications.

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Bacterial taxa	Target	Sequence 3'-5'	Bp*	MT** (°C)	Reference
<i>Eubacteria</i>	V3-V4 16S	Eub518-R: ATTACCGCGGCTGCTGG Eub338-F: ACTCCTACGGGAGGCAG	147	57.6 63.5	Lane et al., 1992
<i>Firmicutes</i>	V3-V4 16S	Firm-F: GGAGYATGTGGTTAATT Firm-R: ACTCCTACGGGAGGCAG	300	60.5 63.5	Guo et al., 2008
<i>Bacteroidetes</i>	V3-V4 16S	Bact-F: GGARCATGTGGTTAATT Bact-R: AGCTGACGACAACCATG	250	58.9 59.4	Guo et al., 2008
<i>Lactobacillales</i>	V3-V4 16S	F-Lac: GCAGCAGTAGGGAATCT R-Lac: GCATTYCACCCTACACA	340	59.8 58.3	Walter et al., 2001
<i>Bifidobacteriaceae</i>	RecA	RecA-F: CGTYTCBCAGCCGGAYA RecA-R: CCAVGVCRCCGGTTCATC	220	60.3 59.2	Masco et al 2004
<i>Enterobacteriaceae</i>	V3-V4 16S	Enterb-F: TGCCGTAACCTCGGGAG Enterb-R: TCAAGACCAGTGTTCAG	450	64.2 60.3	Bartosch et al., 2004
<i>Clostridium</i> group I	V3-V4 16S	CloSI-F: TACHRAGGAGGAAGCCAC CloSI-R: GTTCTTCCTAATCTACGCAT	148	54.6 53.0	Bartosch et al., 2004
<i>Clostridium</i> group IV	V3-V4 16S	CloIV-F: TTAACACAATAAGTWATC CloIV-R: ACCTTCCTCCGTTTTGTC	400	58.1 57.9	Goldberg et al., 2014
ATOP group	V3-V4 16S	ATOP-F: GGGTTGAGAGACCGACC ATOP-R: CGGRGCTTCTTCTGCAG	190	57.6 59.4	Zhou and Helmstetter, 1994
BPP group	V3-V4 16S	BPP-F: GAGAGGAAGGTCCCCA BPP-R: CGCKACTTGGCTGGTTCA	140	60.5 59.9	Guo et al., 2016
<i>Escherichia coli</i>	FtsZ	EcFtsZ-F: GGTATCCTGACCGTTGCT EcFtsZ-R: ATACCTCGGCCAGAACT	250	59.4 57.3	Matsuki et al., 2004
<i>Akkermansia muciniphila</i>	V3-V4 16S	AkM1: CAGCACGTGAAGGTGGG AkM2: CCTTGCGGTTGGCTTCA	327	63.5 59.4	Pachikian et al., 2011
<i>Bifidobacterium longum</i>	V3-V4 16S	Blon-F: GATTCTGGCTCAGGATGA Blon-R: CTGATAGGACGCGACCC	220	62.1 61.4	Chen et al., 1996
<i>Faecalibacterium prausnitzii</i>	V3-V4 16S	Fprau223-F: GATGCGCTCGCTCCGA Fprau420-R: CCGAAGACCTTCTTCCTC	199	63.7 58.8	Wang et al., 1996
<i>Desulfovibrio</i> spp.	V3-V4 16S	Dsv691-f: CCGTAGATATCTGGAGG Dsv681-r: ACATCTAGCATCCATCGT	135	63.0 61.3	Fite et al. 2004

37 *Base pairs, **Melting Temperature.

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39 **Table S2.** ANOVA R Models for multivariate analysis from microbiomics datasets. Test of SS

40 Whole Model versus SS residuals.

	MultipleR	MultipleR ²	Adjusted	SS	df	MS	SS	df	MS	F	p*
1) Eubacteria											
Time Effect											
BL	0.000000	0.000000	-0.133333	0.000000E+00	2	0.000000E+00	7.079592E+19	15	4.719728E+18	0.000000	1.000000
24 h	0.552996	0.305804	0.213245	2.898392E+19	2	1.449196E+19	6.579544E+19	15	4.386363E+18	3.303867	0.064731
48 h	0.576708	0.332592	0.243605	5.892891E+19	2	2.946445E+19	1.182517E+20	15	7.883450E+18	3.737508	0.048188
Matrix Effect											
M0166	0.000000	0.000000	-0.133333	0.000000E-01	2	0.000000E-01	2.267637E+13	15	1.511758E+12	0.000000	1.000000
M0033	0.287775	0.082814	-0.039477	1.056865E+13	2	5.284323E+12	1.170497E+14	15	7.803315E+12	0.677189	0.522912
Water	0.401888	0.161514	0.049716	3.013428E+13	2	1.506714E+13	1.564392E+14	15	1.042928E+13	1.444696	0.266820
2) Firmicutes											
Time Effect											
BL	0.000000	0.000000	-0.133333	6.400000E+01	2	3.200000E+01	1.819020E+17	15	1.212680E+16	0.000000	1.000000
24 h	0.859847	0.739336	0.704581	4.368000E+17	2	2.184000E+17	1.540000E+17	15	1.026667E+16	21.27273	0.091642
48 h	0.954655	0.911366	0.899549	8.614900E+18	2	4.307450E+18	8.378300E+17	15	5.585533E+16	77.11797	0.032507
Matrix Effect											
M0166	0.947900	0.898515	0.884984	5.572357E+18	2	2.786178E+18	6.293840E+17	15	4.195893E+16	66.40251	0.225928
M0033	0.881244	0.776590	0.746802	1.365105E+18	2	6.825523E+17	3.927140E+17	15	2.618093E+16	26.07059	0.399315
Water	0.879533	0.773579	0.743389	5.180646E+17	2	2.590323E+17	1.516340E+17	15	1.010893E+16	25.62410	0.427862
3) Bacteroidetes											
Time Effect											
BL	0.000000	0.000000	-0.133333	5.000000E-01	2	2.500000E-01	1.145625E+15	15	7.637500E+13	0.0000	1.000000
24 h	0.897394	0.805315	0.779357	6.254400E+16	2	3.127200E+16	1.512000E+16	15	1.008000E+15	31.0238	0.088535
48 h	0.985741	0.971686	0.967910	2.071930E+17	2	1.035965E+17	6.037500E+15	15	4.025000E+14	257.3826	0.144383
Matrix Effect											
M0166	0.998893	0.997786	0.997491	2.647668E+17	2	1.323834E+17	5.873750E+14	15	3.915833E+13	3380.720	0.434662
M0033	0.938351	0.880502	0.864569	1.388043E+17	2	6.940213E+16	1.883788E+16	15	1.255858E+15	55.263	0.365202
Water	0.990501	0.981093	0.978572	1.493343E+17	2	7.466713E+16	2.877875E+15	15	1.918583E+14	389.178	0.095589
4) Lactobacillales											
Time Effect											
BL	0.000000	0.000000	-0.133333	0.000000E+00	2	0.000000E+00	2.267637E+13	15	1.511758E+12	0.000000	1.000000
24 h	0.287775	0.082814	-0.039477	1.056865E+13	2	5.284323E+12	1.170497E+14	15	7.803315E+12	0.677189	0.522912
48 h	0.401888	0.161514	0.049716	3.013428E+13	2	1.506714E+13	1.564392E+14	15	1.042928E+13	1.444696	0.266820
Matrix Effect											
M0166	0.318418	0.101390	-0.018425	2.849012E+13	2	1.424506E+13	2.525053E+14	15	1.683368E+13	0.846223	0.448523
M0033	0.189464	0.035897	-0.092650	1.150027E+12	2	5.750137E+11	3.088717E+13	15	2.059144E+12	0.279249	0.760198
Water	0.134699	0.018144	-0.112770	2.360300E+11	2	1.180150E+11	1.277288E+13	15	8.515251E+11	0.138592	0.871685

5) <i>Bifidobacteriaceae</i>											
Time Effect											
BL	0.00000	0.00000	-0.13333	0.00000E+00	2	0.00000E+00	2.230147E+14	15	1.486765E+13	0.00000	1.00000
24 h	0.149190	0.022258	-0.108108	5.537144E+12	2	2.768572E+12	2.432374E+14	15	1.621582E+13	0.170733	0.844662
48 h	0.712777	0.508052	0.442458	1.176112E+14	2	5.880561E+13	1.138834E+14	15	7.592228E+12	7.745501	0.004891
Matrix Effect											
M0166	0.806176	0.649920	0.603242	1.688808E+14	2	8.444039E+13	9.096788E+13	15	6.064525E+12	13.92366	0.00381
M0033	0.375877	0.141284	0.026788	3.090391E+13	2	1.545196E+13	1.878329E+14	15	1.252219E+13	1.23397	0.319061
Water	0.146534	0.021472	-0.108998	6.612293E+12	2	3.306146E+12	3.013347E+14	15	2.008898E+13	0.16458	0.849765
6) <i>Bifidobacterium longum</i>											
Time Effect											
BL	0.225715	0.050947	-0.075593	6.533524E+11	2	3.266762E+11	1.217074E+13	15	8.113830E+11	0.402617	0.675581
24 h	0.042025	0.001766	-0.131332	2.055542E+10	2	1.027771E+10	1.161826E+13	15	7.745504E+11	0.013269	0.986830
48 h	0.691985	0.478843	0.409356	4.896943E+12	2	2.448472E+12	5.329665E+12	15	3.553110E+11	6.891067	0.007538
Matrix Effect											
M0166	0.804316	0.646924	0.599847	1.155764E+13	2	5.778820E+12	6.307894E+12	15	4.205263E+11	13.74187	0.00406
M0033	0.375877	0.141284	0.026788	1.479725E+12	2	7.398626E+11	8.993717E+12	15	5.995812E+11	1.23397	0.319061
Water	0.146534	0.021472	-0.108998	3.031924E+11	2	1.515962E+11	1.381705E+13	15	9.211369E+11	0.16458	0.849765
7) <i>BPP (Bacteroides-Prevotella-Porphyrromonas) group</i>											
Time Effect											
BL	0.00000	0.00000	-0.133333	2.560000E+02	2	1.280000E+02	1.922308E+18	15	1.281539E+17	0.00000	1.00000
24 h	0.187338	0.035096	-0.093558	6.217585E+16	2	3.108793E+16	1.709433E+18	15	1.139622E+17	0.272792	0.764948
48 h	0.408941	0.167232	0.056197	3.034763E+17	2	1.517381E+17	1.511221E+18	15	1.007481E+17	1.506115	0.253471
Matrix Effect											
M0166	0.307944	0.094830	-0.025860	1.062565E+17	2	5.312825E+16	1.014242E+18	15	6.761614E+16	0.785733	0.473672
M0033	0.414107	0.171484	0.061016	2.246907E+17	2	1.123454E+17	1.085579E+18	15	7.237195E+16	1.522333	0.243925
Water	0.114453	0.013099	-0.118487	4.039254E+16	2	2.019627E+16	3.043141E+18	15	2.028760E+17	0.099550	0.905838
8) <i>Clostridium group IV (e.g. Faecalibacterium prausnitzii)</i>											
Time Effect											
BL	0.00000	0.00000	-0.133333	0.000000E+00	2	0.000000E+00	1.378380E+16	15	9.189200E+14	0.00000	1.00000
24 h	0.437832	0.191697	0.083923	1.210744E+15	2	6.057318E+14	5.105190E+15	15	3.403460E+14	1.778695	0.202678
48 h	0.642602	0.412937	0.334662	1.760748E+15	2	8.803739E+14	2.503217E+15	15	1.668812E+14	5.275454	0.018414
Matrix Effect											
M0166	0.731085	0.534485	0.472417	6.196395E+15	2	3.098197E+15	5.396803E+15	15	3.978969E+14	8.611203	0.003232
M0033	0.502948	0.252957	0.153351	2.648680E+15	2	1.324400E+15	7.822207E+15	15	5.214805E+14	2.539727	0.012226
Water	0.358457	0.128491	0.012290	1.205020E+15	2	6.025098E+14	8.173198E+15	15	5.448798E+14	1.105766	0.356482
9) <i>Faecalibacterium prausnitzii</i>											
Time Effect											
BL	0.895409	0.801757	0.727415	1.596821E+08	3	5.322738E+07	3.948321E+07	15	4.935401E+06	10.78481	0.003486
24 h	0.963647	0.928616	0.901848	2.141098E+10	3	7.136692E+09	1.645881E+09	15	2.057325E+08	34.69019	0.000062
48 h	0.952920	0.908056	0.873577	6.192059E+13	3	2.064020E+13	6.269681E+12	15	7.837101E+11	26.33652	0.001169
Matrix Effect											
M0166	0.952016	0.906335	0.875114	5.985196E+13	2	2.992598E+13	6.185374E+12	15	1.030896E+12	29.0291	0.000066
M0033	0.944186	0.891488	0.855317	7.013020E+11	2	3.506510E+11	8.536245E+10	15	1.422707E+10	24.6467	0.044322
Water	0.785621	0.617200	0.489600	9.87865E+07	2	4.793933E+07	5.946598E+07	15	9.910996E+06	4.8370	0.096337
10) <i>Akkermansia muciniphila</i>											
Time Effect											
BL	0.00000	0.00000	-0.133333	0.000000E+00	2	0.000000E+00	4.537891E+12	15	3.025260E+11	0.00000	1.00000
24 h	0.788178	0.621224	0.570721	1.523536E+12	2	7.617681E+11	9.289368E+11	15	6.192912E+10	12.30064	0.000688
48 h	0.912914	0.833412	0.811200	2.452892E+12	2	1.226446E+12	4.903015E+11	15	3.268677E+10	37.52118	0.000001
Matrix Effect											
M0166	0.900561	0.811010	0.785811	8.489157E+12	2	4.244579E+12	1.978234E+12	15	1.318823E+11	32.18460	0.000004
M0033	0.781679	0.611021	0.559158	2.642952E+12	2	1.321476E+12	1.682514E+12	15	1.121676E+11	11.78127	0.079043
Water	0.677546	0.459069	0.386945	1.948856E+12	2	9.744282E+11	2.296381E+12	15	1.530921E+11	6.36498	0.099118
11) <i>Enterobacteriaceae</i>											
Time Effect											
BL	0.00000	0.00000	-0.133333	0.000000E+00	2	0.000000E+00	3.340986E+14	15	2.227324E+13	0.00000	1.00000
24 h	0.256908	0.066002	-0.058531	2.032942E+13	2	1.016471E+13	2.876841E+14	15	1.917894E+13	0.529993	0.599233
48 h	0.684687	0.468796	0.397969	1.670589E+15	2	8.352946E+14	1.892982E+15	15	1.261988E+14	6.618880	0.008699
Matrix Effect											
M0166	0.700560	0.490785	0.422889	1.890712E+15	2	9.453562E+14	1.961715E+15	15	1.307810E+14	7.228543	0.006335
M0033	0.353002	0.124611	0.007892	2.751194E+13	2	1.375597E+13	1.932713E+14	15	1.288476E+13	1.067616	0.368561
Water	0.224185	0.050259	-0.076373	1.903893E+13	2	9.519465E+12	3.597778E+14	15	2.398518E+13	0.396889	0.679265
12) <i>Escherichia coli</i>											
Time Effect											
BL	0.00000	0.00000	-0.133333	0.000000E+00	2	0.000000E+00	1.240026E+10	15	8.266838E+08	0.00000	1.00000
24 h	0.544784	0.296789	0.203028	9.174333E+10	2	4.587168E+10	2.173761E+11	15	1.449174E+10	3.165368	0.001308
48 h	0.548379	0.300719	0.207482	5.890431E+10	2	2.945216E+10	1.369736E+11	15	9.131575E+09	3.225310	0.048373
Matrix Effect											
M0166	0.513174	0.263347	0.165127	9.921201E+10	2	4.960601E+10	2.775227E+11	15	1.850152E+10	2.681186	0.011035
M0033	0.290695	0.084503	-0.037563	7.299893E+09	2	3.694946E+09	7.908586E+10	15	5.272391E+09	0.692275	0.045734
Water	0.435497	0.189658	0.081612	2.373545E+09	2	1.186772E+09	1.014134E+10	15	6.760894E+08	1.755348	0.026545
13) <i>Clostridium group I (C. perfringens) group</i>											
Time Effect											
BL	0.00000	0.00000	-0.133333	3.576279E-07	2	1.788139E-07	4.282469E+08	15	28549794	0.00000	1.00000
24 h	0.491618	0.241688	0.140580	2.183754E+09	2	1.091877E+09	6.851665E+09	15	45677663	2.39039	0.125562
48 h	0.759856	0.577381	0.521032	8.363981E+09	2	4.181990E+09	6.122079E+09	15	408138614	10.24650	0.001565
Matrix Effect											
M0166	0.639613	0.409104	0.330318	7.569758E+09	2	3.784879E+09	1.093349E+10	15	728899031	5.192597	0.019334
M0033	0.269314	0.072530	-0.051133	1.054227E+08	2	5.271135E+07	1.348080E+09	15	89871972	0.586516	0.568524
Water	0.167147	0.027938	-0.101670	3.220211E+07	2	1.610106E+07	1.120426E+09	15	74695067	0.215557	0.808546
14) <i>ATOP group (Atopobium-Collinsella) group</i>											
Time Effect											
BL	0.316089	0.099912	-0.237621	1.120715E+12	3	3.735717E+11	1.009628E+13	15	1.262035E+12	0.2960	0.827430
24 h	0.606082	0.367335	0.130086	2.908888E+13	3	9.696295E+12	5.010006E+13	15	6.262508E+12	1.5483	0.006117
48 h	0.996961	0.993931	0.991655	3.853460E+14	3	1.284487E+14	2.353017E+12	15	2.941271E+11	436.7114	0.000552
Matrix Effect											
M0166	0.886362	0.785637	0.714183	1.345509E+13	2	6.727547E+12	3.671258E+12	15	6.118764E+11	10.9949	0.000676
M0033	0.906753	0.822202	0.762936	7.762247E+12	2	3.881123E+12	1.678560E+12	15	2.797600E+11	13.8730	0.004344
Water	0.996969	0.993947	0.991930	1.080148E+14	2	5.400739E+13	6.577784E+11	15	1.096297E+11	492.6345	0.097541
15) <i>Desulfovibrio spp.</i>											
Time Effect											
BL	0.00000	0.00000	-0.133333	0.000000E+00	2	0.000000E+00	1.040250E+12	15	6.935000E+10	0.00000	1.00000
24 h	0.731678	0.535353	0.473400	2.918601E+13	2	1.459301E+13	2.533128E+13	15	1.688752E+12	8.64129	0.003187
48 h	0.947972	0.898650	0.885137	1.293567E+14	2	6.467836E+13	1.458888E+13	15	9.725923E+11	66.50100	0.000000
Matrix Effect											
M0166	0.940627	0.884780	0.869417	1.793157E+14	2	8.965786E+13	2.335132E+13	15	1.556755E+12	57.59280	0.000000
M0033	0.836884	0.700374	0.660424	2.832568E+13	2	1.416284E+13	1.211797E+13	15	8.078644E+11	17.53121	0.000119
Water	0.950880	0.904173	0.891396	7.938836E+12	2	3.969418E+12	8.413837E+11	15	5.609224E+10	70.76590	0.000000

41 * $p < 0.05$. M0.166 = digested homogeneous mix of polyethylene and polystyrene microplastics in the
42 concentration of 0.166 g; M0.033 = digested homogeneous mix of polyethylene and polystyrene microplastics
43 in the concentration of 0.033 g; water = blank control; T1 = intermediate time point of 24 h of colonic
44 fermentation after the baseline; EP = endpoint of 48 h of colonic fermentation after the baseline.
45

46 <

qPCR Target & Sample	Quantification (cells/mL)	Shift as Log ₂ (F/C)		MANOVA	FDR*
	Baseline ± SD**	T1 (24 h)	EP (48 h)	p “time”	-Log ₁₀ (p)
<i>Eubacteria</i>					
mix 0.166	4.35E+09 ± 6.24E+08	0.57	0.81 ^A	0.225928	0.646030
mix 0.033	4.35E+09 ± 6.24E+08	0.35	0.43 ^{AB}	0.522912	0.281571
water	4.35E+09 ± 6.24E+08	-0.37	-0.45 ^B	0.266820	0.573781
p “matrix”		0.064731	0.048188		
<i>Firmicutes</i>					
mix 0.166	1.20E+09 ± 8.56E+07	0.39	0.92 ^A	0.225928	0.646029
mix 0.033	1.20E+09 ± 8.56E+07	0.18	0.53 ^A	0.399315	0.398648
water	1.20E+09 ± 8.56E+07	-0.16	-0.23 ^B	0.427862	0.368696
p “matrix”		0.091642	0.032507		
<i>Bacteroidetes</i>					
mix 0.166	8.37E+08 ± 1.10E+07	-0.11	-0.62	0.434662	0.361848
mix 0.033	8.37E+08 ± 1.10E+07	-0.17	-0.45	0.365202	0.437466
water	8.37E+08 ± 1.10E+07	-0.40	-0.07	0.095589	1.019592
p “matrix”		0.088535	0.144383		
<i>Lactobacillales</i>					
mix 0.166	6.14E+05 ± 7.30E+04 ^b	1.03 ^a	1.77 ^{aA}	0.044852	1.348218
mix 0.033	6.14E+05 ± 7.30E+04	0.13	0.67 ^{AB}	0.760198	0.119073
water	6.14E+05 ± 7.30E+04	-0.15	0.07 ^B	0.871685	0.059640
p “matrix”		0.522912	0.026682		
<i>Bifidobacteriaceae</i>					
mix 0.166	8.46E+06 ± 3.93E+05 ^a	-0.51 ^a	-3.07 ^{bb}	0.000381	3.419075
mix 0.033	8.46E+06 ± 3.93E+05	-0.43	-0.72 ^A	0.319061	0.496126
water	8.46E+06 ± 3.93E+05	-0.26	-0.31 ^A	0.849765	0.070701
p “matrix”		0.844662	0.004891		
BPP (<i>Bacteroides-Prevotella-Porphyromonas</i>) group					
mix 0.166	3.80E+07 ± 1.29E+06	-0.20	-0.83	0.473672	0.324522
mix 0.033	3.80E+07 ± 1.29E+06	0.05	-0.45	0.243925	0.612743
water	3.80E+07 ± 1.29E+06	0.14	0.30	0.905838	0.042949
p “matrix”		0.764948	0.253471		
<i>Clostridium</i> group IV (e.g. <i>Faecalibacterium prausnitzii</i>)					
mix 0.166	4.84E+07 ± 9.90E+06 ^a	-2.38 ^b	-4.19 ^{cC}	0.003232	2.490528
mix 0.033	4.84E+07 ± 9.90E+06 ^a	-0.79 ^a	-1.28 ^{bb}	0.012226	1.912715
water	4.84E+07 ± 9.90E+06	-0.54	-0.64 ^A	0.356482	0.447962
p “matrix”		0.202678	0.018414		
<i>Bifidobacterium longum</i>					
mix 0.166	2.61E+06 ± 1.74E+05 ^a	-0.86 ^b	-3.31 ^{cB}	0.000406	3.391473
mix 0.033	2.61E+06 ± 1.74E+05	-0.40	-1.31 ^A	0.319061	0.496126
water	2.61E+06 ± 1.74E+05	-0.88	-0.93 ^A	0.849765	0.070701
p “matrix”		0.966830	0.007538		
<i>Akkermansia muciniphila</i>					
mix 0.166	1.70E+06 ± 8.17E+05 ^a	-1.23 ^{bb}	-2.05 ^{cB}	0.000004	5.397949
mix 0.033	1.70E+06 ± 8.17E+05	-0.45 ^{AB}	-0.72 ^A	0.079043	0.245212
water	1.70E+06 ± 8.17E+05	-0.25 ^A	-0.50 ^A	0.099118	0.092295
p “matrix”		0.000688	0.000001		
<i>Faecalibacterium prausnitzii</i>					
mix 0.166	8.26E+06 ± 7.13E+05 ^a	-1.36 ^{bb}	-3.46 ^{cC}	0.000066	4.180456
mix 0.033	8.26E+06 ± 7.13E+05 ^a	-1.08 ^{aAB}	-1.53 ^{bb}	0.044322	1.353380
water	8.26E+06 ± 7.13E+05	-0.95 ^A	-0.83 ^A	0.096337	1.016206
p “matrix”		0.000062	0.000169		
<i>Enterobacteriaceae</i>					

mix 0.166	2.90E+06 ± 4.53E+05 ^c	1.51 ^b	3.17 ^{aA}	0.006335	2.198253
mix 0.033	2.90E+06 ± 4.53E+05	0.27	1.15 ^B	0.368561	0.433490
water	2.90E+06 ± 4.53E+05 ^b	0.43	0.87 ^B	0.679265	0.167960
<i>p</i> “matrix”		0.599233	0.008699		
<i>Escherichia coli</i> (total)					
mix 0.166	1.89E+04 ± 1.39E+03 ^b	3.13 ^{aA}	3.34 ^{aA}	0.011035	1.957227
mix 0.033	1.89E+04 ± 1.39E+03 ^b	0.68 ^{bB}	2.22 ^{aAB}	0.045734	1.339760
water	1.89E+04 ± 1.39E+03 ^b	0.37 ^{bB}	1.53 ^{aB}	0.026545	1.576011
<i>p</i> “matrix”		0.001308	0.048373		
<i>Desulfovibrio</i> spp.					
mix 0.166	1.25E+06 ± 2.82E+05 ^b	1.77 ^{aA}	2.71 ^{aA}	0.000775	3.110698
mix 0.033	1.25E+06 ± 2.82E+05 ^b	1.32 ^{aA}	1.48 ^{aAB}	0.000119	3.924533
water	1.25E+06 ± 2.82E+05	-0.16 ^B	0.88 ^B	0.096212	1.016770
<i>p</i> “matrix”		0.003187	0.000001		
ATOP (<i>Atopobium</i> – <i>Collinsella</i>) group					
mix 0.166	5.81E+05 ± 6.85E+04 ^c	1.43 ^{bA}	4.32 ^{aA}	0.000676	3.170053
mix 0.033	5.81E+05 ± 6.85E+04 ^b	1.99 ^{aA}	2.00 ^{aB}	0.004344	2.362110
water	5.81E+05 ± 6.85E+04	0.41 ^B	0.90 ^B	0.097541	1.010812
<i>p</i> “matrix”		0.006117	0.000552		
<i>Clostridium</i> group I (e.g. <i>Clostridium perfringens</i>)					
mix 0.166	6.33E+03 ± 9.08E+02 ^b	2.14 ^a	3.16 ^{aA}	0.019334	1.713678
mix 0.033	6.33E+03 ± 9.08E+02	0.51	0.81 ^B	0.568524	0.245251
water	6.33E+03 ± 9.08E+02	0.19	0.55 ^B	0.808546	0.092295
<i>p</i> “matrix”		0.125562	0.001565		

47 *FDR = False Discovery Rate as $-\text{Log}_{10}(p)$; **SD = Standard Deviation; mix 0.166 = digested
48 homogeneous mix of polyethylene and polystyrene microplastics in the concentration of 0.166 g; mix 0.033
49 = digested homogeneous mix of polyethylene and polystyrene microplastics in the concentration of 0.033 g;
50 water = blank control; T1 = intermediate time point of 24 h of colonic fermentation after the baseline; EP =
51 endpoint of 48 h of colonic fermentation after the baseline. For each bacterial target, values with different
52 letters are significantly different among a sample and among a time point, with lower- or upper-case letter,
53 respectively, by *post hoc* Duncan multiple range test ($p < 0.05$).
54

55 **Table S4.** *Firmicutes* to *Bacteroidetes* ratio (F/B) of the human colon microbiota after exposure to
56 microplastics mixes.

Sample	Baseline	T1 (24 h)	EP (48 h)	MANOVA
				<i>p</i> “time”
mix 0.166	1.44 ± 0.11 ^b	2.13 ± 0.05 ^b	4.00 ± 0.16 ^{aA}	0.009624
mix 0.033	1.44 ± 0.11 ^b	1.91 ± 0.13 ^{ab}	2.72 ± 0.29 ^{aA}	0.000522
water	1.44 ± 0.11	1.76 ± 0.06	1.23 ± 0.10 ^B	0.072141
<i>p</i> “matrix”		0.175213	0.001543	

57 Values are expressed as means ± Standard Deviation. Mix 0.166 = digested homogeneous mix of polyethylene
58 and polystyrene microplastics in the concentration of 0.166 g; mix 0.033 = digested homogeneous mix of
59 polyethylene and polystyrene microplastics in the concentration of 0.033 g; water = blank control; T1 =
60 intermediate time point of 24 h of colonic fermentation after the baseline; EP = endpoint of 48 h of colonic
61 fermentation after the baseline. For each bacterial target, values with different letters are significantly different

62 among a sample and among a time point, with lower- or upper-case letter, respectively, by *post hoc* Duncan
 63 multiple range test ($p < 0.05$).

64

65 **Table S5.** ANOVA R Models for multivariate analysis from metabolomics datasets. Test of SS

66 Whole Model versus SS residuals.

Compound	MultipleR	MultipleR ²	Adjusted	SS	df	MS	SS	df	MS	F	p
Acetic acid	0,980293	0,960974	0,944713	7,482123	5	1,496425	0,303859	12	0,025322	59,0968	0,000000
Propanoic acid	0,918544	0,843723	0,778607	5,462733	5	1,092547	1,011828	12	0,084319	12,9573	0,000172
Butanoic acid	0,913451	0,834394	0,765391	5,002784	5	1,000557	0,992929	12	0,082744	12,0922	0,000240
Pentanoic acid	0,867342	0,752281	0,649065	3,473243	5	0,694649	1,143703	12	0,095309	7,2884	0,002370
Hexanoic acid	0,988695	0,977518	0,968151	9,764394	5	1,952879	0,224568	12	0,018714	104,3537	0,000000
Octanoic acid	0,982922	0,966136	0,952026	8,169703	5	1,633941	0,286354	12	0,023863	68,4722	0,000000
Nonanoic acid	0,996306	0,992625	0,989552	9,196908	5	1,839382	0,068332	12	0,005694	323,0207	0,000000
Phenol	0,839791	0,705249	0,582436	0,707580	5	0,141516	0,295725	12	0,024644	5,74247	0,006218
p-Cresol	0,834259	0,695989	0,569318	0,787919	5	0,157584	0,344167	12	0,028681	5,49445	0,007367
Phenol, 2,4-*	0,979848	0,960102	0,943478	1,579604	5	0,315921	0,065641	12	0,005470	57,75391	0,000000
Indole	0,925571	0,856682	0,796966	1,323337	5	0,264667	0,221386	12	0,018449	14,34600	0,000104
Phenol, p-tert-**	0,877341	0,769728	0,673781	1,404497	5	0,280899	0,420170	12	0,035014	8,02245	0,001573

67 *Phenol, 2,4-bis(1-methylethyl)-; ** Phenol, p-tert-butyl-

68

69 **Table S6.** Absolute quantification of selected VOCs at the beginning of colonic fermentation.

Compound	mg/kg \pm SD
Acetic acid	1.544 \pm 1.172
Propanoic acid	0.537 \pm 0.387
Butanoic acid	0.346 \pm 0.228
Pentanoic acid	0.237 \pm 0.224
Hexanoic acid	0.153 \pm 0.147
Octanoic acid	0.061 \pm 0.070
Nonanoic acid	0.034 \pm 0.037
Phenol	0.150 \pm 0.033
p-Cresol	1.159 \pm 0.491
Phenol, 2,4-bis(1-methylethyl)-	0.582 \pm 0.148
Indole	2.943 \pm 0.832
Phenol, p-tert-butyl-	0.101 \pm 0.044

70

71 **Table S7.** Significance of Spearman-rank correlations.

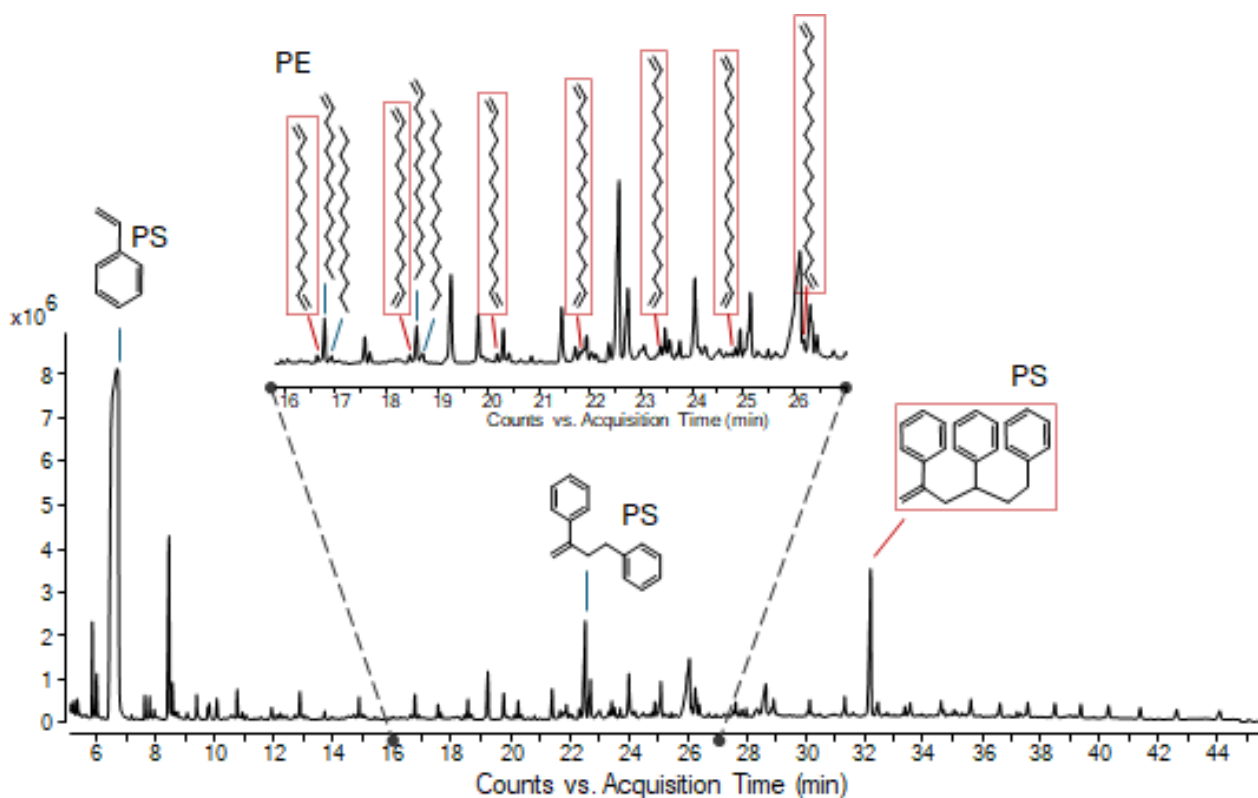
Volatile Organic Compounds	qPCR Bacterial targets								
	Clos IV	Lactob	BPP	B long	Bifido	Enterob	E coli	Clos I	ATOP
Acetic acid	<u>0.833333</u>	-0.216667	0.583333	<u>0.800000</u>	<u>0.800000</u>	-0.450000	-0.183333	-0.233333	<u>-0.850000</u>
Propanoic acid	0.616667	-0.150000	0.266667	0.583333	0.583333	-0.450000	-0.200000	-0.150000	<u>-0.750000</u>
Butanoic acid	-0.333333	0.333333	-0.433333	-0.466667	-0.466667	-0.016667	0.166667	0.033333	0.100000
Pentanoic acid	0.300000	0.033333	0.033333	0.250000	0.250000	-0.416667	-0.133333	-0.100000	-0.450000
Hexanoic acid	0.542857	0.142857	0.142857	0.771429	0.771429	0.600000	0.257143	0.085714	-0.028571
Octanoic acid	-0.485714	0.142857	-0.714286	-0.600000	-0.600000	0.600000	0.428571	0.485714	-0.142857
Nonanoic acid	-0.485714	0.142857	-0.714286	-0.600000	-0.600000	0.600000	0.428571	0.485714	-0.142857
Phenol	<u>-0.800000</u>	0.116667	-0.633333	<u>-0.683333</u>	<u>-0.683333</u>	0.316667	0.133333	0.366667	0.616667
p-Cresol	<u>-0.850000</u>	0.300000	-0.533333	<u>-0.883333</u>	<u>-0.883333</u>	0.516667	0.483333	0.400000	<u>0.716667</u>
Phenol, 2,4-bis(1-methylethyl)-	<u>-0.750000</u>	0.216667	-0.483333	<u>-0.800000</u>	<u>-0.800000</u>	<u>0.800000</u>	<u>0.766667</u>	0.633333	0.483333
Indole	<u>-0.800000</u>	0.300000	-0.650000	<u>-0.716667</u>	<u>-0.716667</u>	0.466667	0.183333	0.233333	<u>0.733333</u>
Phenol, p-tert-butyl-	<u>-0.828571</u>	-0.085714	-0.257143	<u>-0.600000</u>	<u>-0.600000</u>	0.771429	0.085714	0.314286	<u>0.885714</u>

72 Underlined values are significant at $p < 0.05$.

73

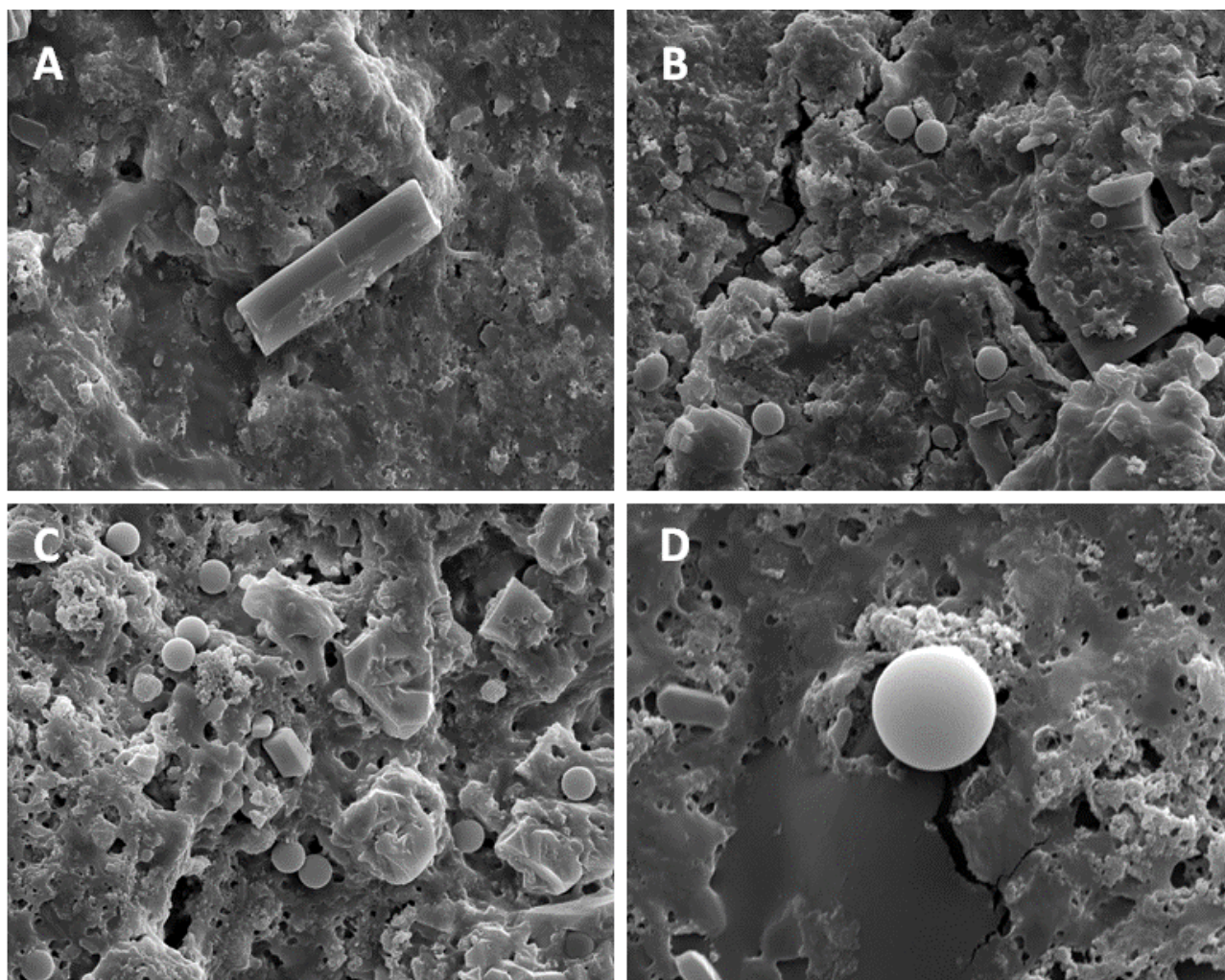
74 **Supplementary Figures**

75



76

77 **Figure S1.** Example of total ion pyrogram from Py-GC-MS of filtered sample after Fenton pre-
78 treatment. The chemical structure of pyrolysis markers of PE and PS are reported and those used for
79 quantitation are framed (styrene trimer for PS and C14-C20 n- α,ω -alkadienes for PE).
80



81
82 **Figure S2.** Scanning electron microscope (SEM) images of fecal slurries into MICODE vessels.
83 Panel A, donor fecal slurry (water = blank control) containing objects referring to plastics, with
84 dimensions on average larger than the added MPs. Panel B, Fecal slurry added with 0,033 g of MPs
85 mix. Panel C, Fecal slurry added with 0,166 g of MPs mix. Panel D, zoom on a single MP particle.
86 Panels A-C are acquired at 6500 X, panel D at 15,000 X.

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