Accumulation of polybrominated diphenyl ethers and microbiome response in the great pond snail *Lymnaea stagnalis* with exposure to nylon (polyamide) microplastics

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SUPPLEMENTARY INFORMATION

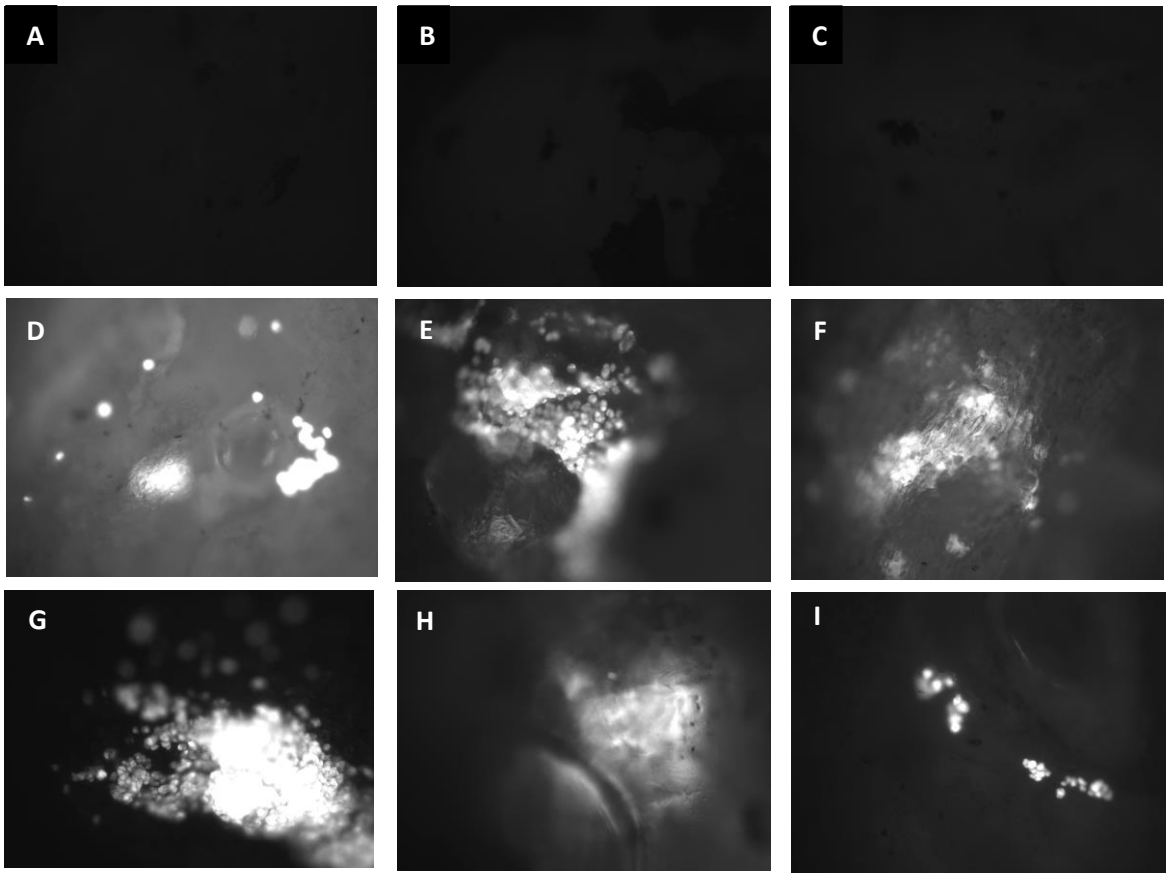

Fig S1. Microscope images showing fluorescently labelled nylon particles within the guts of snails: blank controls (no microplastics) = A-C, microplastic controls = D-F and 3000 ng g^{-1} PBDEs with microplastics = G-I.

DNA extraction procedure

Three snails were analysed per treatment. Frozen snails were defrosted, removed from the shells, then rinsed in phosphate buffered saline prior to DNA extraction. In order to capture the entire snail microbiome, DNA was extracted from a whole snail through the application of both chemical and enzymatic lysis. Per 250 mg of snail tissue 100 µl of lysis buffer (10 ppmL 1 M Tris pH7.5, 1 ppmL 0.5 M EDTA, 2 ppmL 10% SDS and 4 ppmL 5 M NaCl, made up in molecular grade water) and 20 µl proteinase K solution (20 mg ml^{-1}) was added. Tissue was ground and mixed with a disposable polypropylene tissue pestle and hand-held tissue grinder. To ensure complete cell lysis, samples were incubated at 37°C overnight. When samples were fully lysed, proteins were removed through the addition of 600 µl 5M NaCl (per 250 mg of snail weight), mixed well through vortexing and allowed to precipitate for 10 mins at room temperature. 700 µl of lysate was moved to a clean tube and centrifuged at 20000 x g for 10 minutes. Supernatant was transferred to a new tube, mixed by inversion and DNA precipitated through the addition of 650 µl absolute ethanol. DNA was pelleted through centrifugation at 20000 x g for 10 minutes. Ethanol was removed and pelleted DNA was cleaned using 400 µl 70% ethanol. Pellet was centrifuged again at 20000 x g for 2 minutes and ethanol aspirated. Pelleted DNA was air dried to remove residual ethanol and resuspended in 500 µl molecular grade water. Sample DNA required an additional cleaning step performed through the application of Genomic DNA Clean & Concentrator kit (Zymo research) under the manufacturer's recommended protocol. Resultant DNA was quantified using the nanodrop 8000 UV-Vis spectrophotometer (ThermoFisher scientific).

Table S1. The OTUs which show the most change in abundance between the highest nominal PBDE concentration (3000) and control treatment, without microplastics (a) or with microplastics (b). Significance at the P > 0.05 level was determined through the application of a Kruskal Wallis test. Significant OTUs are highlighted with an asterisk. Taxonomic identity is given at the highest resolution, using a 97% threshold. ^abundance based on rarefied values

(a) Without Microplastics

	Average^	Standard deviation	ratio	Average^ in 3000 ng g^{-1}	Average^ in solvent control	Cumulative sum	Kruskal-Wallis chi-squared	df	P value	Taxonomic Identity
OTU5512*	2.22E-02	1.51E-02	1.4768	357.3333	74.6667	0.2779	3.8571	1	0.04953*	Enterobacteriaceae
OTU4432*	2.16E-02	1.46E-02	1.4751	355.6667	81	0.316	3.8571	1	0.04953*	Enterobacteriaceae
OTU32	1.04E-01	5.78E-02	1.7932	2851.333	1621.667	0.1831	2.3333	1	0.1266	Enterobacteriaceae (*Klebsiella*)
OTU2245	6.07E-03	6.32E-03	0.9605	84	35	0.4158	0.047619	1	0.8273	Enterobacteriaceae (*Serratia marcescens*)
OTU8733*	3.15E-02	2.48E-02	1.272	146.6667	547	0.2387	3.8571	1	0.04953*	Flavobacteriaceae
OTU12263	1.12E-02	1.57E-02	0.7155	16.3333	154	0.3568	1.1905	1	0.2752	Leptotrichiaceae (*Streptobacillus moniliformis*)
OTU3412	1.19E-02	7.02E-03	1.6896	170.6667	172.6667	0.337	0.047619	1	0.8273	Pasteurellales
OTU16390	8.45E-03	9.13E-03	0.9255	15	119.3333	0.4051	2.3333	1	0.1266	Rhodospirillaceae
OTU10409	8.48E-03	7.39E-03	1.148	98.3333	172	0.3901	0.42857	1	0.512	Bacteroidales
OTU3010	1.04E-02	7.17E-03	1.4502	144.6667	124.6667	0.3752	0.047619	1	0.8273	Neisseriaceae

(b) With Microplastics

	Average^	Standard deviation	ratio	Average^ in 3000 ng g^{-1}	Average^ in solvent control	Cumulative sum	Kruskal-Wallis chi-squared	df	P value	Taxonomic Identity
OTU5512	1.68E-02	1.43E-02	1.1786	140.5	252.6667	0.2882	0	1	1	Enterobacteriaceae
OTU4432	1.49E-02	1.29E-02	1.155	118	230.6667	0.3094	0.33333	1	0.5637	Enterobacteriaceae
OTU32	1.87E-01	9.03E-02	2.0676	892.5	3265.667	0.2644	3	1	0.08326	Enterobacteriaceae (*Klebsiella*)
OTU2245	1.47E-02	1.43E-02	1.032	218	53.6667	0.3302	0	1	1	Enterobacteriaceae (*Serratia marcescens*)
OTU8733	1.36E-02	4.48E-03	3.0278	172.5	140.3333	0.3494	0	1	1	Flavobacteriaceae
OTU12263	6.09E-03	3.26E-03	1.8703	87.5	10	0.4297	3	1	0.08326	Leptotrichiaceae (*Streptobacillus moniliformis*)
OTU3010	1.28E-02	1.15E-02	1.1192	197.5	69	0.3676	0.33333	1	0.5637	Neisseriaceae
OTU3412	1.26E-02	1.05E-02	1.2007	214.5	120.6667	0.3854	0.33333	1	0.5637	Pasteurellales
OTU16395	7.82E-03	7.29E-03	1.0735	103.5	19	0.4124	0	1	1	Rhizobiaceae
OTU10409	1.12E-02	7.45E-03	1.5026	212	69.6667	0.4013	3	1	0.08326	Bacteroidales
OTU999	6.13E-03	6.72E-03	0.9129	78	0	0.421	1.5	1	0.2207	Desulfobacteraceae

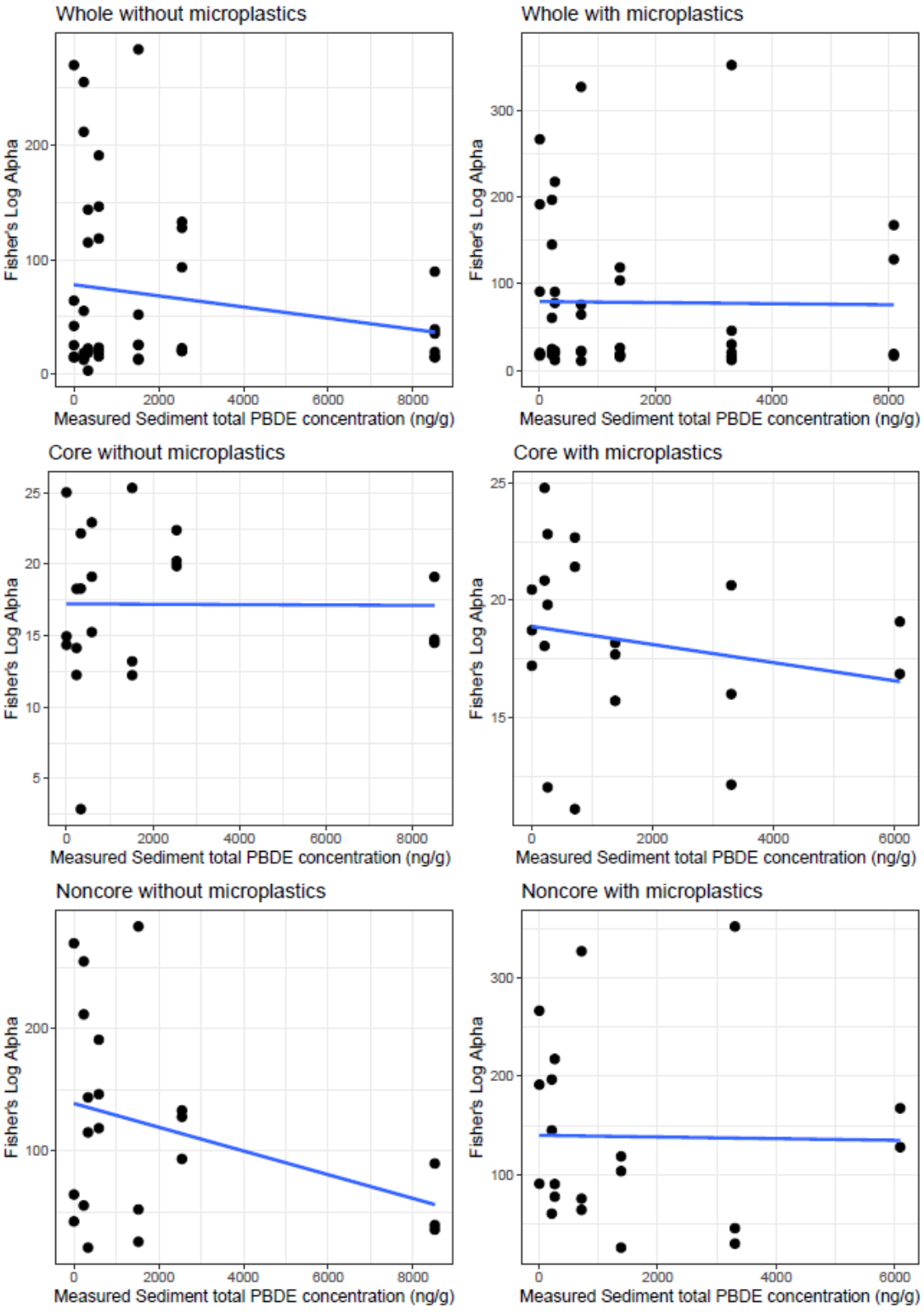

Fig S2. Linear regression of Fisher's log alpha diversity in relation to total sediment PBDE concentration (all congeners combined). Each data point represents an individual snail. Only one sediment concentration value was measured per treatment, therefore these regression lines are to provide a visual representation of the data only.

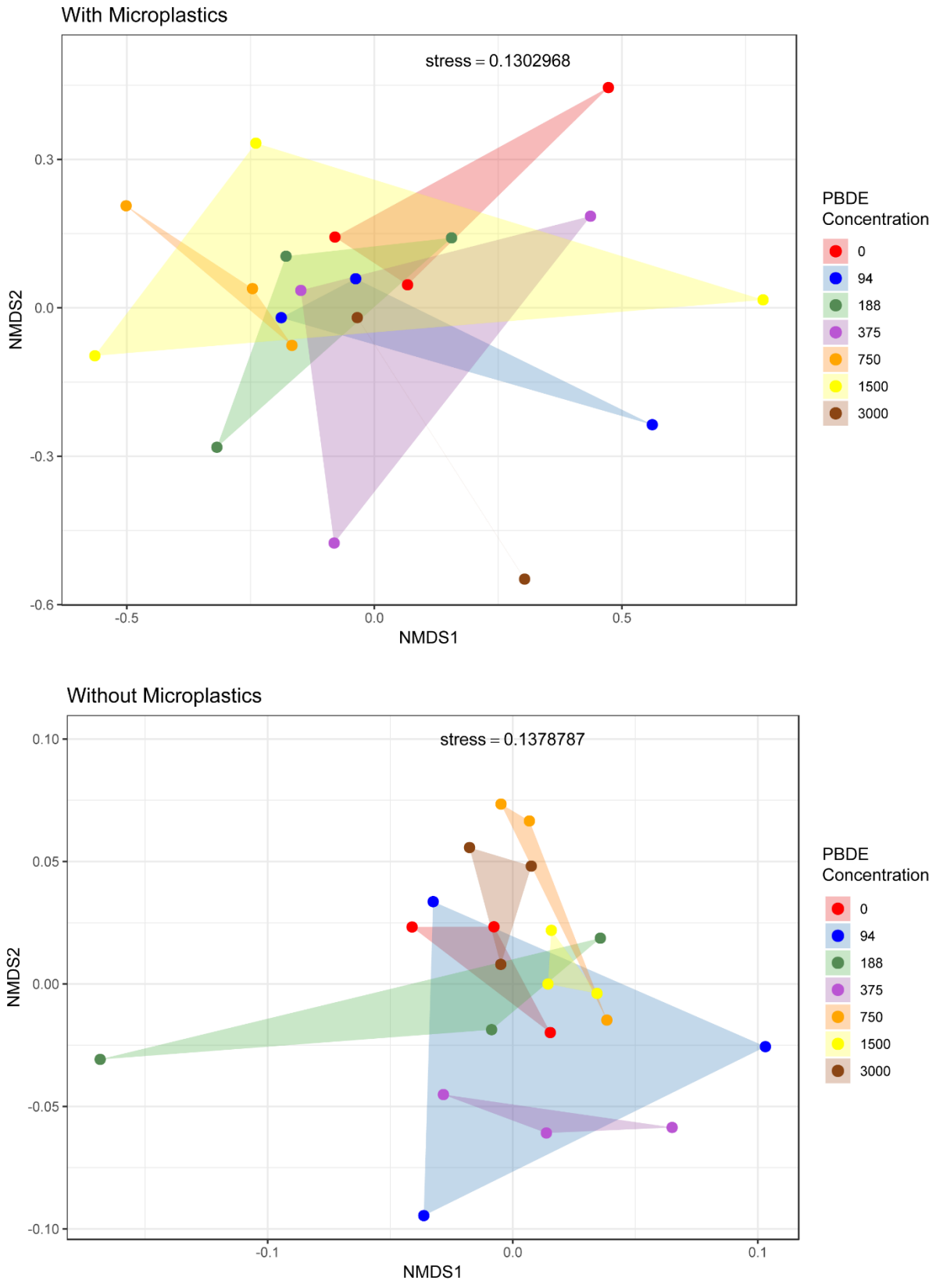

Fig S3. NMDS plots showing community dissimilarity for each PBDE treatment, with or without microplastics.

Fig. S4. Class composition of bacterial communities at each nominal PBDE concentration (ng g^{-1}), with and without microplastics (present/absent). Relative abundance was calculated as rarefied number of sequences in OTU/total sequences in each sample (= 6359), relative abundances per treatment (*N*=3) are plotted on Y axis. For ease of representation classes of an abundance of <0.02 (2%) from an individual sample were excluded. *Note one sample was removed from this treatment due to inefficient sequencing, therefore *N*=2.

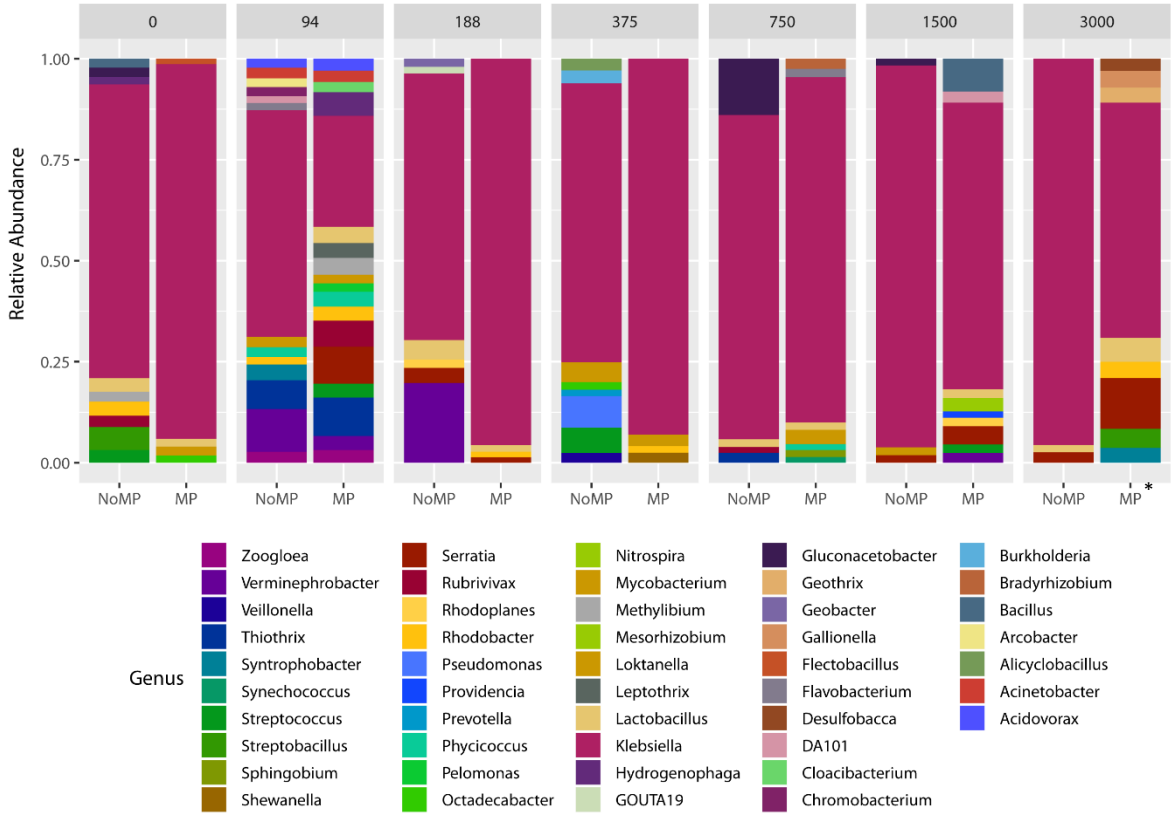

Fig S5. Genus composition of bacterial communities at each nominal PBDE concentration (ng g^{-1}), with and without microplastics (present/absent). Relative abundance was calculated as rarefied number of sequences in OTU/total sequences in each sample (= 6359), relative abundances per treatment (*N*=3) are plotted on Y axis. For ease of representation genera of an abundance of <0.03 (3%) from an individual sample were excluded. *Note one sample was removed from this treatment due to inefficient sequencing, therefore *N*=2.