

**Supplementary information: Influence of Microplastics on Microbial Structure,
Function and Mechanical Properties of Stream Periphyton.**

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Supplementary information (SI)

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Supplementary Tables

Table S1:

Composition of the exposure medium PERIQUIL (Stewart et al 2013).

Component	Concentration (mM)
<i>Salts</i>	
CaCl ₂	0.20
Ca(NO ₃) ₂	0.10
MgSO ₄	0.15
NaHCO ₃	1.20
KNO ₃	0.10
Na ₂ SiO ₃	0.05
<i>Nutrients</i>	
K ₂ HPO ₄	5.00 x 10 ⁻³
NH ₄ NO ₃	0.10
<i>Trace elements</i>	
CoCl ₂	5.00 x 10 ⁻³
H ₃ BO ₃	0.05
Na ₂ MoO ₄	8.00 x 10 ⁻⁵
CuSO ₄	1.63 x 10 ⁻³
MnCl ₂	1.22 x 10 ⁻³
ZnSO ₄	1.58 x 10 ⁻⁴
FeCl ₃	9.00 x 10 ⁻⁴
<i>Metal ligand</i>	
Na ₂ EDTA	0.02
<i>Buffer</i>	
MOPS, pH	7.5
NaOH	10.00

Table S2:
16S and 18S frame shift primers used in the current study

Name	5' - 3'	L	nextera adapter	seq primer site	shift	linker
16S-F_nex0	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGACCTACGGGNNGCWGCAG	52	TCGTCGGCAGCGTC	AGATGTGTATAAGAGACAG		GA
16S-F_nex1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNGACCTACGGGNNGCWGCAG	53	TCGTCGGCAGCGTC	AGATGTGTATAAGAGACAG	N	GA
16S-F_nex2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGACCTACGGGNNGCWGCAG	54	TCGTCGGCAGCGTC	AGATGTGTATAAGAGACAG	NN	GA
16S-F_nex3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGACCTACGGGNNGCWGCAG	55	TCGTCGGCAGCGTC	AGATGTGTATAAGAGACAG	NNN	GA
16S-R_nex0	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGACTACHVGGGTATCTAATCC	57	GTCTCGTGGGCTCGG	AGATGTGTATAAGAGACAG		CA
16S-R_nex1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNCAGACTACHVGGGTATCTAATCC	58	GTCTCGTGGGCTCGG	AGATGTGTATAAGAGACAG	N	CA
16S-R_nex2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNCAGACTACHVGGGTATCTAATCC	59	GTCTCGTGGGCTCGG	AGATGTGTATAAGAGACAG	NN	CA
16S-R_nex3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNCAGACTACHVGGGTATCTAATCC	60	GTCTCGTGGGCTCGG	AGATGTGTATAAGAGACAG	NNN	CA
18S-F_nex0	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGACGGTAATTCCAGCTCYV	52	TCGTCGGCAGCGTC	AGATGTGTATAAGAGACAG		GA
18S-F_nex1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNGACGGTAATTCCAGCTCYV	53	TCGTCGGCAGCGTC	AGATGTGTATAAGAGACAG	N	GA
18S-F_nex2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGACGGTAATTCCAGCTCYV	54	TCGTCGGCAGCGTC	AGATGTGTATAAGAGACAG	NN	GA
18S-F_nex3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGACGGTAATTCCAGCTCYV	55	TCGTCGGCAGCGTC	AGATGTGTATAAGAGACAG	NNN	GA
18S-R_nex0	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACCGTCAATHCTTYAART	54	GTCTCGTGGGCTCGG	AGATGTGTATAAGAGACAG		CA
18S-R_nex1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNCACCGTCAATHCTTYAART	55	GTCTCGTGGGCTCGG	AGATGTGTATAAGAGACAG	N	CA
18S-R_nex2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNCACCGTCAATHCTTYAART	56	GTCTCGTGGGCTCGG	AGATGTGTATAAGAGACAG	NN	CA
18S-R_nex3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNCACCGTCAATHCTTYAART	57	GTCTCGTGGGCTCGG	AGATGTGTATAAGAGACAG	NNN	CA

Table S3

p-values of two-tailed t-test, two-sample unequal variance (heteroscedastic) for

"Experiment 2", bMP								"Experiment 1", sMP							
Eukaryotes				Prokaryotes				Eukaryotes				Prokaryotes			
ASV	Control-MPaged	Control-bMP	ASV	Control-bMPaged	Control-bMP	ASV	Control-sMPaged	Control-sMP	ASV	Control-sMPaged	Control-sMP	ASV	Control-sMPaged	Control-sMP	
ASV1	0.061834	0.001689	ASV16	0.033273	0.206071	ASV1	0.045477	0.108414	ASV9	0.00134	0.010687	ASV47	0.058689	0.040032	
ASV2	0.000378	0.571101	ASV26	0.016295	0.050094	ASV2	0.043689	0.356827	ASV59	0.04311	0.508326	ASV4	5.66E-06	0.026377	
ASV3	0.106473	1.85E-06	ASV32	0.014064	0.361638	ASV7	0.009277	0.26394	ASV78	0.029498	0.511839	ASV11	0.108079	0.012294	
ASV4	0.007782	0.003657	ASV34	0.012932	0.274086	ASV12	0.007821	0.9865	ASV10	0.156565	0.037972	ASV15	0.107864	0.036626	
ASV5	0.05973	0.000537	ASV39	0.001755	0.343264	ASV16	0.041542	0.866676	ASV117	0.024555	0.560398	ASV3	0.05973	0.000537	
ASV9	0.006175	0.526615	ASV47	0.001113	0.654635	ASV18	0.033579	0.539005	ASV132	0.004715	0.571131	ASV17	0.001132	0.042295	
ASV12	0.001132	0.042295	ASV56	0.011748	0.802452	ASV24	0.000318	0.88525	ASV134	0.375918	0.025262	ASV20	0.002302	0.090599	
ASV13	0.002302	0.090599	ASV67	0.702045	0.036863	ASV26	0.01053	0.446031	ASV152	0.014561	0.736717	ASV1	0.061834	0.001689	
ASV15	0.009956	0.033527	ASV71	0.011525	0.457835	ASV27	0.504756	0.0333	ASV184	0.093174	0.000985	ASV22	0.493775	5.93E-09	
ASV16	0.712122	0.006358	ASV99	0.039481	0.011255	ASV28	0.017375	0.026239	ASV213	0.020448	0.093821	ASV27	0.000223	0.325343	
ASV17	0.002583	0.331472	ASV111	0.192523	0.039357	ASV29	0.011196	0.932311	ASV223	0.27219	0.04546	ASV28	0.885204	0.00583	
ASV20	0.94638	0.0044	ASV112	0.000417	0.251537	ASV37	0.004782	0.966843	ASV260	0.138716	0.001577	ASV32	0.086884	0.002252	
ASV22	0.493775	5.93E-09	ASV125	0.022474	0.385636	ASV48	0.370205	0.023807	ASV282	0.632218	0.009474	ASV34	0.527137	0.000895	
ASV27	0.000223	0.325343	ASV126	0.980013	0.034768	ASV44	0.000161	0.001056	ASV284	0.018317	0.265279	ASV44	0.001715	0.992727	
ASV45	0.568771	0.008409	ASV200	0.015229	0.122535	ASV49	0.003691	0.017831	ASV290	0.075445	2.84E-05	ASV45	0.568771	0.008409	
ASV46	0.056678	0.008901	ASV203	0.001397	0.037336	ASV53	0.001234	0.305979	ASV292	0.019983	0.023573	ASV46	0.056678	0.008901	
ASV48	0.060022	0.004483	ASV250	0.216592	0.009599	ASV54	0.001524	0.049612	ASV293	0.277441	0.049405	ASV49	0.06753	0.002885	
ASV49	0.06753	0.002885	ASV258	0.722686	0.027699	ASV55	0.727646	0.022146	ASV355	0.08574	0.039155	ASV56	9.8E-05	0.013881	
ASV56	9.8E-05	0.013881	ASV265	0.006878	0.02565	ASV62	0.001564	0.824572	ASV356	0.005834	0.006287	ASV58	0.041455	0.002188	
ASV58	0.041455	0.002188	ASV267	0.131633	0.02245	ASV65	0.046699	0.402215	ASV388	0.818455	0.010846	ASV80	0.037272	0.007128	
ASV80	0.037272	0.007128	ASV273	0.251564	0.00632	ASV66	0.530321	0.001718	ASV396	0.046067	0.947822	ASV82	0.001598	0.052754	
ASV82	0.001598	0.052754	ASV275	0.048864	0.089973	ASV69	0.003402	0.674293	ASV447	0.011276	0.633403	ASV83	0.006673	0.186211	
ASV83	0.006673	0.186211	ASV279	0.009085	0.151477	ASV71	0.000987	0.239352	ASV463	0.047802	0.800138	ASV84	0.761798	0.001361	
ASV84	0.761798	0.001361	ASV282	0.031407	0.105612	ASV75	0.020107	0.146801	ASV491	0.180562	0.04879	ASV86	0.003059	0.161877	
ASV86	0.003059	0.161877	ASV323	0.011528	0.209782	ASV81	0.443906	0.001627	ASV548	0.043466	0.343436	ASV98	0.006974	0.483745	
ASV98	0.006974	0.483745	ASV331	0.048492	0.234501	ASV83	0.339374	0.028203	ASV579	0.016795	0.167885	ASV119	0.186403	0.006098	
ASV119	0.186403	0.006098	ASV340	0.595306	0.023114	ASV84	0.000546	0.506902	ASV614	0.047619	0.211806	ASV143	0.968676	0.002559	
ASV143	0.968676	0.002559	ASV351	0.014379	0.13164	ASV85	0.197928	0.01517	ASV617	0.171168	0.043294	ASV150	0.00591	0.056548	
ASV150	0.00591	0.056548	ASV354	0.005411	0.483334	ASV86	0.042842	0.244339	ASV183	0.005635	0.003030				
ASV183	0.005635	0.003030	ASV355	0.016526	0.28978	ASV94	0.01892	0.829715	ASV240	0.008076	0.653087				
ASV240	0.008076	0.653087	ASV356	0.008154	0.241993	ASV95	0.04194	0.185855	ASV366	0.032552	0.350617				
			ASV397	0.036205	0.950932	ASV98	0.006257	0.049255	ASV406	0.015434	0.090623				
			ASV423	0.01601	0.026301	ASV103	0.194018	0.041056	ASV428	0.017839	0.008392				
			ASV450	0.153109	0.034034	ASV111	0.018663	0.928144	ASV456	0.123726	0.014321				
			ASV467	0.02426	0.475322	ASV118	0.003921	0.006364	ASV467	0.02426	0.475322				

ASV497	0.043962	0.93721	ASV120	0.001679	0.002341
ASV517	0.010037	0.692498	ASV122	0.029195	0.47127
ASV533	0.314728	0.00186	ASV124	0.020006	0.565881
ASV577	0.488394	0.015717	ASV125	0.002108	0.262878
ASV581	0.045735	0.07632	ASV129	0.476972	0.030656
ASV598	0.256831	0.004748	ASV132	0.288881	0.000984
ASV600	0.736031	0.029814	ASV134	8.31E-05	0.257433
ASV610	0.014301	0.236993	ASV135	0.027172	0.343945
ASV614	0.043865	0.086498	ASV136	0.635128	0.043287
ASV615	0.081761	0.047613	ASV138	0.008242	0.008689
ASV616	0.024804	0.904327	ASV139	0.028319	0.057449
ASV617	0.5821	0.018527	ASV140	0.046124	0.204548
ASV628	0.012979	0.54841	ASV142	0.001151	0.802301
ASV640	0.037767	0.159701	ASV143	0.619387	0.046279
ASV653	0.978577	0.039315	ASV150	0.042458	0.862604
ASV659	0.062935	0.015851	ASV161	0.012635	0.312392
ASV660	0.017187	0.350617	ASV176	0.021888	0.065713
ASV714	0.041915	0.302013	ASV178	0.10925	0.025154
ASV744	0.215083	0.039907	ASV179	0.035705	0.878922
ASV753	0.015997	0.182148	ASV180	0.628991	0.046404
ASV776	0.021991	0.985214	ASV182	0.001982	0.4741
ASV779	0.325309	0.037169	ASV184	0.006076	0.304125
ASV807	0.634387	0.040311	ASV187	0.003137	0.282279
ASV830	0.600874	0.026909	ASV191	0.008326	0.924103
ASV841	0.059226	0.044372	ASV197	0.006641	0.982398
ASV876	0.01799	0.343436	ASV212	0.035226	0.42585
ASV897	0.041246	0.101138	ASV218	0.018706	0.300183
ASV917	0.012157 na		ASV220	0.902104	0.048116
ASV922	0.184608	0.03749	ASV229	0.007246	0.235622
ASV1044	0.043321	0.350617	ASV244	0.041753	0.198831

Table S4

Prokaryotic ASV accounting for > 1 % on average of all counts in at least one exposure in «Experiment 1»/sMP exposure communities. Full data set is available in repository (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA744749>).

OTU	Phylum	Class	Order	Family	Genus	Species	average % of all counts per treatment		
							control	sMP	sMPaged
ASV9	Cyanobacteria	Cyanobacteriia	Leptolyngbyales	Leptolyngbyaceae	Chamaesiphon	NA PCC-7430	3.09	1.90	1.61
ASV16	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	1.90	1.62	2.04
ASV19	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	NA	1.62	2.07	1.68
ASV25	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	NA	1.60	1.27	1.77
ASV26	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	Pirellula	NA	1.73	1.36	1.38
ASV30	Armatimonadota	Armatimonadia	Armatimonadales	Armatimonadaceae	Armatimonas	NA	1.71	1.38	1.37
ASV31	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	Pirellula	NA	1.44	1.08	1.67
ASV32	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	Arcicella	NA	1.36	1.62	1.26
ASV34	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NA	NA	1.33	1.53	1.34
ASV40	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	1.13	1.46	1.44
ASV42	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Luteolibacter	NA	1.07	1.07	1.55
ASV45	Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	1.02	1.27	1.45
ASV47	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	1.01	1.34	1.31
ASV48	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	Methylotenera	NA	1.37	1.16	1.10
ASV52	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	1.20	1.24	1.00
ASV53	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	Runella	NA	1.17	1.28	0.89
ASV54	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NA	NA	0.89	1.11	1.11
ASV55	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	0.84	1.09	0.99

ASV56	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	NA	0.88	1.08	1.06
ASV58	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	Runella	NA	0.88	1.18	0.95
ASV59	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	Phreatobacter	NA	0.75	0.89	1.20
ASV62	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Luteolibacter	NA	0.87	0.80	0.98
ASV63	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	NA	0.71	1.04	0.93
ASV64	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	1.13	0.69	0.93
ASV65	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	0.73	0.92	1.00
ASV74	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	0.75	1.19	0.69
							32.18	32.65	32.73

Table S5

Number of ASV associated to prokaryotic genera in «Experiment 1»/sMP exposure communities. All ASV were assigned on genus level.

Family	Genus	# of ASV
<i>Spirosomaceae</i>	<i>Arcicella</i>	1
<i>Armatimonadaceae</i>	<i>Armatimonas</i>	4
<i>Bdellovibrionaceae</i>	<i>Bdellovibrio</i>	1
<i>Amoebophilaceae</i>	<i>Candidatus Amoebophilus</i>	1
<i>Leptolyngbyaceae</i>	<i>Chamaesiphon PCC-7430</i>	1
<i>Chitinophagaceae</i>	<i>Edaphobaculum</i>	1
<i>Elsteraceae</i>	<i>Elstera</i>	1
<i>Spirosomaceae</i>	<i>Emticicia</i>	45
<i>Flavobacteriaceae</i>	<i>Flavobacterium</i>	186
<i>Saprospiraceae</i>	<i>Haliscomenobacter</i>	88
<i>Hyphomonadaceae</i>	<i>Hyphomonas</i>	4
<i>Spirosomaceae</i>	<i>Lachhabitans</i>	1
<i>Rubritaleaceae</i>	<i>Luteolibacter</i>	2
<i>Xanthomonadaceae</i>	<i>Lysobacter</i>	1
<i>Methylophilaceae</i>	<i>Methylotenera</i>	3
<i>Parachlamydiaceae</i>	<i>Neochlamydia</i>	1
<i>Sphingobacteriaceae</i>	<i>Pedobacter</i>	1
<i>Rhizobiales Incertae</i>		
<i>Sedis</i>	<i>Phreatobacter</i>	1
<i>Pirellulaceae</i>	<i>Pir4 lineage</i>	1
<i>Pirellulaceae</i>	<i>Pirellula</i>	3
<i>Reyranellaceae</i>	<i>Reyranella</i>	1
<i>Rhodobacteraceae</i>	<i>Rhodobacter</i>	1
<i>Spirosomaceae</i>	<i>Runella</i>	3
<i>Sandaracinaceae</i>	<i>Sandaracinus</i>	1
<i>Sphingomonadaceae</i>	<i>Sphingomonas</i>	1
<i>Sphingomonadaceae</i>	<i>Sphingopyxis</i>	4

Table S6:

Eukaryotic ASV accounting for > 1 % on average of all counts in at least one exposure in «Experiment 1»/sMP exposure communities. Full data set is available in repository (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA744749>).

OTU	Phylum	Class	Order	Family	Genus	Species	average % of all counts per treatment		
							control	sMP	sMPaged
ASV1	Diatomea	Bacillariophyceae	NA	NA	Achnanthidium	NA	18.62	17.48	21.68
ASV2	Diatomea	Bacillariophyceae	NA	NA	Achnanthidium	NA	10.02	9.60	11.92
ASV3	Diatomea	Bacillariophyceae	NA	NA	Gomphonema	NA	9.37	9.59	9.94
ASV4	Cryptomycota	NA	NA	NA	NA	NA	4.65	6.37	2.58
ASV5	Diatomea	Bacillariophyceae	NA	NA	Achnanthidium	NA	4.63	4.21	5.36
ASV6	Diatomea	Bacillariophyceae	NA	NA	Gomphonema	NA	2.46	2.50	2.51
ASV7	Diatomea	Bacillariophyceae	NA	NA	Achnanthidium	NA	2.07	2.22	2.59
ASV8	Diatomea	Bacillariophyceae	NA	NA	Achnanthidium	NA	1.99	1.98	2.30
ASV9	Diatomea	Bacillariophyceae	NA	NA	Nitzschia	NA	2.00	2.14	1.75
ASV10	NA	NA	NA	NA	NA	NA	1.35	1.68	1.58
ASV11	Diatomea	Bacillariophyceae	NA	NA	Nitzschia	NA	1.11	1.43	1.37
ASV12	Diatomea	Bacillariophyceae	NA	NA	Gomphonema	NA	1.13	1.13	1.43
ASV13	Diatomea	Bacillariophyceae	NA	NA	Achnanthidium	NA	1.11	1.23	1.06
ASV14	NA	Tubulinea	NA	NA	BOLA868	NA	1.13	1.13	1.12
ASV15	Ciliophora	Intramacronucleata	Conthreep	Oligo-hymenophorea	NA	NA	0.90	1.70	0.51
ASV16	Diatomea	Bacillariophyceae	NA	NA	Nitzschia	NA	1.16	1.19	0.94
ASV17	NA	Trebouxiophyceae	NA	NA	NA	NA	1.08	1.06	0.80
ASV18	NA	NA	NA	NA	NA	NA	1.02	0.91	0.46
ASV22	NA	Chrysophyceae	Chromulinales	NA	Spumella	NA	0.02	0.00	2.23
					Sum		65.81	67.56	72.14

Table S7

Number of ASV assigned to eukaryotic genera in «Experiment 1»/sMP exposure communities. 103 ASV were not assigned on genus level, 19 of these ASV belonged to ciliate family Oligohymenophorea.

Genus	# of ASV
<i>Achnanthidium</i>	31
<i>Amphileptus</i>	1
<i>Amphora</i>	3
<i>Aspidisca</i>	3
<i>BOLA868</i>	3
<i>Chilodonella</i>	4
<i>Chromulina</i>	1
<i>Cocconeis</i>	2
<i>Cryptocaryon</i>	1
<i>Dictyococcus</i>	9
<i>Fistulifera</i>	1
<i>Fragilaria</i>	1
<i>Freshwater Choanoflagellates 1</i>	2
<i>Gomphonema</i>	14
<i>Hemiurosomoida</i>	2
<i>Leptophys</i>	1
<i>Melosira</i>	1
<i>Navicula</i>	1
<i>Nitzschia</i>	15
<i>Ochromonas</i>	3
<i>Paracercomonas</i>	1
<i>Paraphysomonas</i>	4
<i>Phascolodon</i>	2
<i>Planothidium</i>	12
<i>Poteriospumella</i>	1
<i>Pseudochilonopsis</i>	1
<i>Pseudodiffugia</i>	1
<i>Rhizomastix</i>	8
<i>Rhizophydium</i>	2
<i>Rhogostoma</i>	1
<i>Sorodiplophys</i>	1
<i>Spumella</i>	2
<i>Telotrochidium</i>	2
<i>Ulnaria</i>	2
<i>Vorticella</i>	5

Table S8

Prokaryotic ASV accounting for > 1 % on average of all counts in at least one exposure in «Experiment 2»/bMP exposure communities.

OTU	Phylum	Class	Order	Family	Genus	Species	average % of all counts per treatment		
							control	sMP	sMPaged
ASV1	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Inhella	NA	1.48	1.52	1.34
ASV2	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas	NA	0.97	1.28	1.08
ASV3	Bacteroidota	Bacteroidia	Sphingobacteriales	NS11-12 marine group	NA	NA	1.05	1.17	1.08
ASV5	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Luteolibacter	NA	1.05	1.22	0.94
ASV6	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	Hirschia	NA	0.92	0.86	1.19
ASV7	Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobactraceae	NA	NA	1.10	0.87	1.12
ASV8	Verrucomicrobiota	Chlamydiae	Chlamydiales	Parachlamydiaceae	NA	NA	0.98	1.05	0.88
ASV9	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	NA	NA	1.06	1.13	0.89
ASV11	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	Emticicia	NA	1.05	0.74	0.85
ASV13	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	Cellvibrio	NA	0.81	0.98	0.84
ASV16	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	NA	NA	1.04	0.81	0.70
ASV62*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	NA	0.07	0.09	1.03
					sum		11.59	11.72	11.94

*present in 3 control samples, 2 sMP samples, and 5 sMPaged samples.

Table S9

Number of ASV associated to prokaryotic genera in «Experiment 2»/bMP exposure communities. All were ASV assigned on genus level.

Family	Genus	# of ASV
<i>Moraxellaceae</i>	<i>Acinetobacter</i>	1
<i>Spirosomaceae</i>	<i>Arcicella</i>	1
<i>Xanthomonadaceae</i>	<i>Arenimonas</i>	1
<i>Armatimonadaceae</i>	<i>Armatimonas</i>	30
<i>Bdellovibrionaceae</i>	<i>Bdellovibrio</i>	3
<i>Unknown Family</i>	<i>Calothrix KVSF5</i>	2
<i>Cellvibrionaceae</i>	<i>Cellvibrio</i>	1
<i>Crocinitomicaceae</i>	<i>Crocinitomix</i>	1
<i>Cyanobiaceae</i>	<i>Cyanobium PCC-6307</i>	1
<i>Cytophagaceae</i>	<i>Cytophaga</i>	1
<i>Spirosomaceae</i>	<i>Emticicia</i>	2
<i>Flavobacteriaceae</i>	<i>Flavobacterium</i>	363
<i>Saprospiraceae</i>	<i>Haliscomenobacter</i>	46
<i>Hyphomonadaceae</i>	<i>Hirschia</i>	1
<i>Hyphomonadaceae</i>	<i>Hyphomonas</i>	43
<i>Comamonadaceae</i>	<i>Inhella</i>	1
<i>Spirosomaceae</i>	<i>Lacihabitans</i>	94
<i>Rubritaleaceae</i>	<i>Luteolibacter</i>	2
<i>Xanthomonadaceae</i>	<i>Lysobacter</i>	33
<i>Methylophilaceae</i>	<i>Methylophilus</i>	3
<i>Methylophilaceae</i>	<i>Methylotenera</i>	21
<i>Microscillaceae</i>	<i>OLB12</i>	2
<i>Phaselicystidaceae</i>	<i>Phaselicystis</i>	1
<i>Pirellulaceae</i>	<i>Pirellula</i>	14
<i>Sphingomonadaceae</i>	<i>Porphyrobacter</i>	8
<i>Reyranellaceae</i>	<i>Reyranella</i>	3
<i>Alteromonadaceae</i>	<i>Rheinheimera</i>	19
<i>Sphingomonadaceae</i>	<i>Rhizorhapis</i>	6
<i>Rhodobacteraceae</i>	<i>Rhodobacter</i>	8
<i>Rickettsiaceae</i>	<i>Rickettsia</i>	9
<i>Rubritaleaceae</i>	<i>Rubritalea</i>	1
<i>Spirosomaceae</i>	<i>Runella</i>	1
<i>Chitinophagaceae</i>	<i>Rurimicrobium</i>	2
<i>Chitinophagaceae</i>	<i>Sediminibacterium</i>	3
<i>Rubinisphaeraceae</i>	<i>SH-PL14</i>	1
<i>Sphingobacteriaceae</i>	<i>Solitalea</i>	16
<i>Sphingomonadaceae</i>	<i>Sphingopyxis</i>	30
<i>Chitinophagaceae</i>	<i>Terrimonas</i>	3

Table S10

Eukaryotic ASV accounting for > 1 % on average of all counts in at least one exposure in «Experiment 2»/bMP exposure communities.

OTU	Phylum	Class	Order	Family	Genus	Species	average % of all counts per treatment		
							control	bMP	bMPaged
ASV1	Diatomea	Bacillariophyceae	NA	NA	Gomphonema	NA	13.39	10.68	15.20
ASV2	NA	NA	NA	NA	NA	NA	5.55	5.83	7.83
ASV3	Diatomea	Bacillariophyceae	NA	NA	Achnanthidium	NA	6.09	3.79	5.52
ASV4	Diatomea	NA	NA	Melosirids	Melosira	NA	3.75	3.14	3.07
ASV5	NA	NA	NA	NA	NA	NA	2.07	3.28	2.93
ASV6	Ciliophora	Intramacronucleata	Conthreep	Oligo-hymeno-phorea	NA	NA	2.57	3.38	1.71
ASV7	Diatomea	NA	Fragilariales	NA	Ulnaria	NA	2.25	1.72	2.20
ASV8	NA	NA	NA	NA	NA	NA	1.74	1.59	1.74
ASV9	Ciliophora	Intramacronucleata	Conthreep	Oligo-hymeno-phorea	NA	NA	2.02	2.24	1.10
ASV10	NA	Tubulinea	NA	NA	BOLA868	NA	1.52	1.95	1.39
ASV11	NA	NA	NA	NA	NA	NA	1.22	1.83	1.77
ASV12	Cercozoa	Thecofilosea	Incertae Sedis_10	NA	NA	NA	1.09	1.42	1.78
ASV13	Diatomea	NA	NA	NA	NA	NA	1.26	1.03	1.73
ASV14	Ciliophora	Intramacronucleata	Conthreep	Oligo-hymeno-phorea	NA	NA	1.36	2.00	1.01
ASV15	Diatomea	Bacillariophyceae	NA	NA	Nitzschia	NA	1.15	0.86	1.45
ASV16	Diatomea	Bacillariophyceae	NA	NA	Encyonema	NA	1.23	0.93	1.19
ASV17	Ciliophora	Intramacronucleata	Conthreep	Oligo-hymeno-phorea	NA	NA	1.40	1.25	0.75
ASV18	Ciliophora	Intramacronucleata	Conthreep	Oligo-hymeno-phorea	NA	NA	1.07	1.14	0.85
ASV19	Ciliophora	Intramacronucleata	Litostomatea	Haptoria	NA	NA	1.18	1.22	0.84
ASV20	Chytridio-mycota	Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	Rhizophydium	NA	0.82	1.46	0.81
ASV21	NA	Tubulinea	NA	NA	BOLA868	NA	0.97	1.09	0.81
ASV22	Ciliophora	Intramacronucleata	Conthreep	Oligo-hymeno-phorea	Telotrochidium	NA	0.56	1.70	0.67
ASV23	Diatomea	Bacillariophyceae	NA	NA	Nitzschia	NA	0.94	0.69	1.08
ASV26	Apusomo-nadidae	NA	NA	NA	Amastigomonas	NA	0.82	1.07	0.74

ASV27	Ciliophora	Intramacronucleata	Spirotrichea	Euplotia	Aspidisca	NA	0.87	1.05	0.55
						Sum	55.94	55.65	57.65

Table S11

Number of ASV assigned to eukaryotic genera in «Experiment 2»/bMP exposure communities. 137 ASV were not assigned on genus level, 62 of these ASV belonged to ciliate family *Oligohymenophorea*.

Genus	# of ASV
<i>Achnanthidium</i>	3
<i>Amastigomonas</i>	1
<i>Amphora</i>	1
<i>Aspidisca</i>	15
<i>Bicosoeca</i>	1
<i>BOLA868</i>	5
<i>Chrysamoeba</i>	2
<i>Cocconeis</i>	3
<i>Cryptocaryon</i>	1
<i>Cyclidium</i>	1
<i>Dictyococcus</i>	2
<i>Encyonema</i>	2
<i>Fistulifera</i>	1
<i>Fragilaria</i>	1
<i>Gomphonema</i>	13
<i>Hemiurosomoida</i>	1
<i>Leptophys</i>	3
<i>Melosira</i>	1
<i>Navicula</i>	23
<i>Nitzschia</i>	19
<i>Ochromonas</i>	2
<i>Paracercomonas</i>	4
<i>Paraphysomonas</i>	2
<i>Phascolodon</i>	3
<i>Planothidium</i>	22
<i>Pseudodifflugia</i>	2
<i>Pseudovorticella</i>	2
<i>Pterocystis</i>	6
<i>Rhizomastix</i>	2
<i>Rhizophydium</i>	3
<i>Rhogostoma</i>	2
<i>Sellaphora</i>	2
<i>Telotrochidium</i>	3
<i>Tetrahymena</i>	2

<i>Tokophrya</i>	3
<i>Ulnaria</i>	3
<i>Vermamoeba</i>	1
<i>Vorticella</i>	6

Supplementary Figures

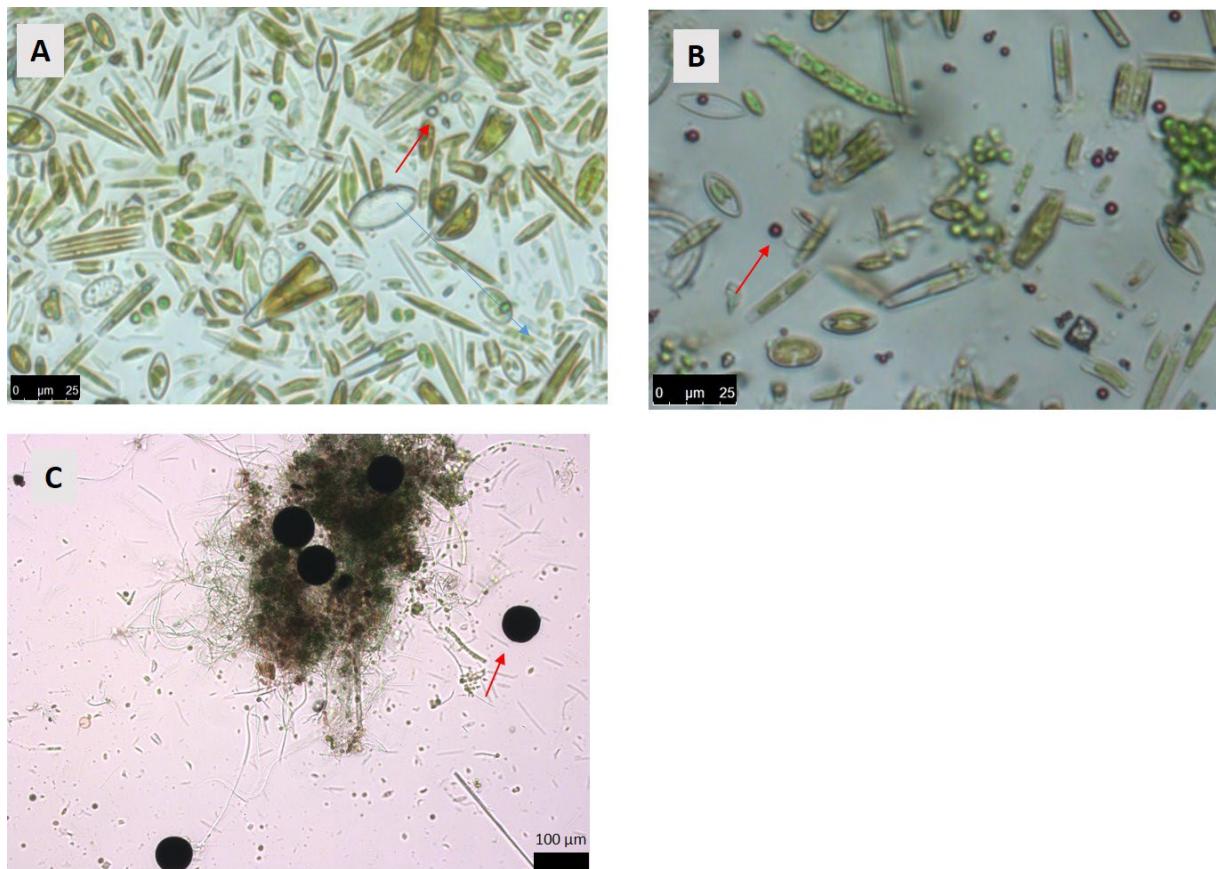


Figure S1:

Images highlighting size differences between periphyton organisms and sMP (A), rMP (B) and bMP (C), respectively. Red arrow indicates the respective MP type. Pictures were taken with a light microscope, (DMI 6000 B, Leica).

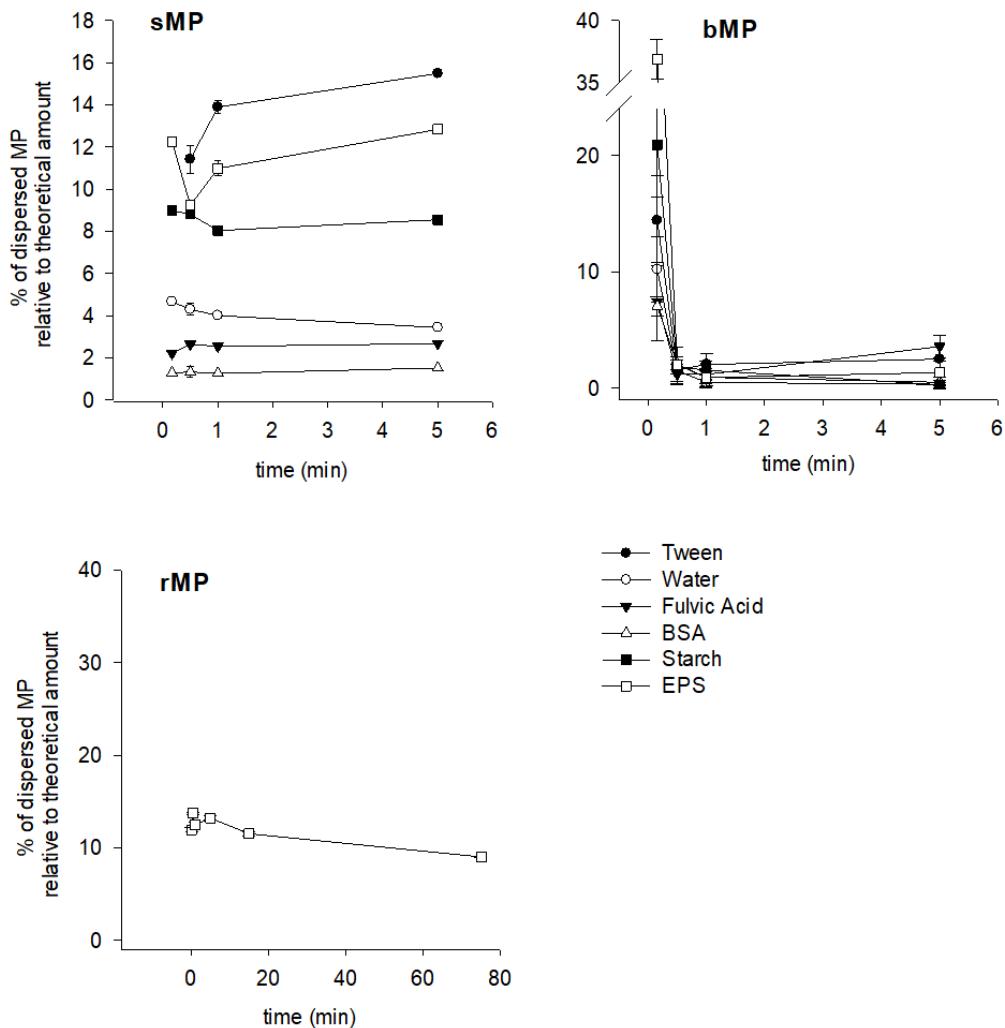


Figure S2:

Stability of MP suspensions. The percentage of dispersed sMP, bMP and rMP relative to the calculated concentration in Tween 80 (0.01% w/w, black circles), water alone (white circles), fulvic acid (40 mg mL⁻¹, black triangle), BSA (5 mg mL⁻¹, white triangle), starch (5 mg mL⁻¹, black square) and EPS (white square) over time. rMP were only particle type tested in EPS. Each dot represents the mean of n=3 replicates, whiskers indicate the standard error of the mean.

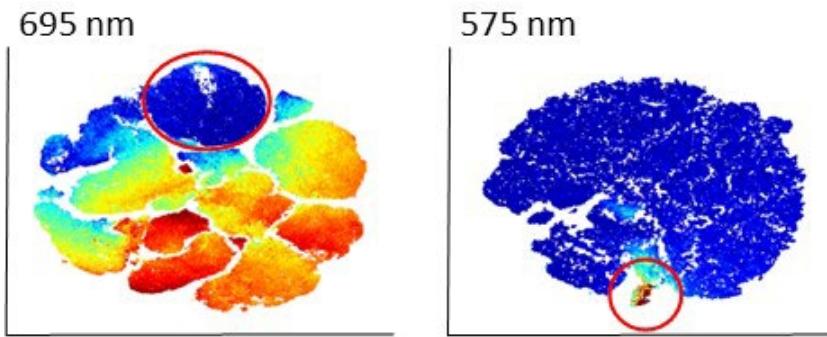


Figure S3:

ViSNE maps of Experiment 1 (left panel) and Experiment 3 (right panel) colored according to fluorescence intensity at 695 and 575 nm measured by flow-cytometer. Blue and red shading indicated low and high intensities. Red circles highlight the sMP/sMPaged and rMP cluster, respectively.

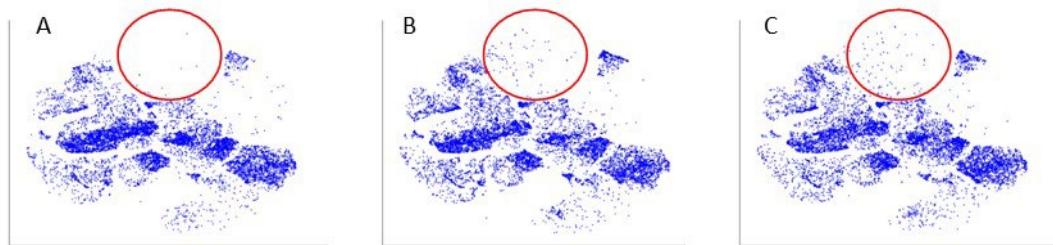


Figure S4:

ViSNE maps of Control (A), sMP (B) and sMPaged (C) treatment of experiment 1 after 28 days of exposure. Red circles highlight the location of sMP/sMPaged cluster in the viSNE map. In control treatment, 0.2 % of all measured cells fall in this cluster (false positive counts).

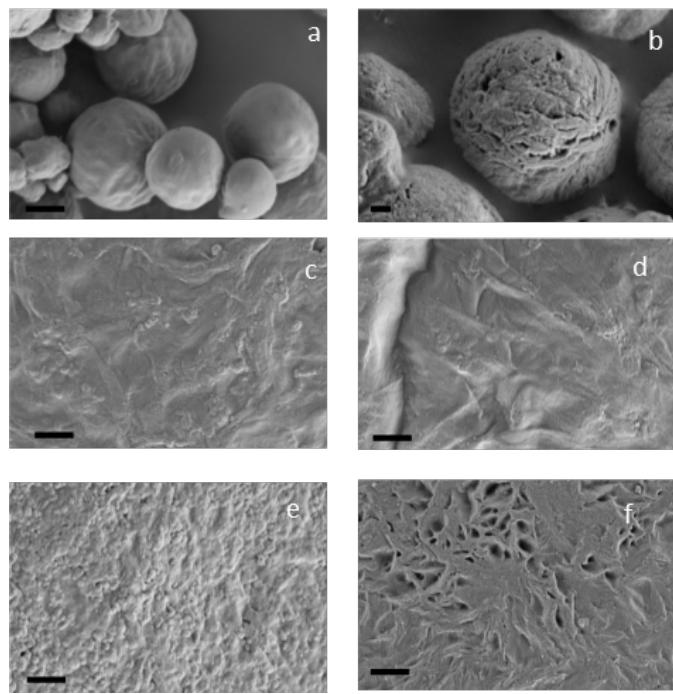


Figure S5:

Electron microscopic pictures of sMP (a) and sMP_{aged} (b) and bMP (c) and bMP_{aged} (d, e, f). Scale bars indicate 300 nm in (b) and 1 μm in all other images.

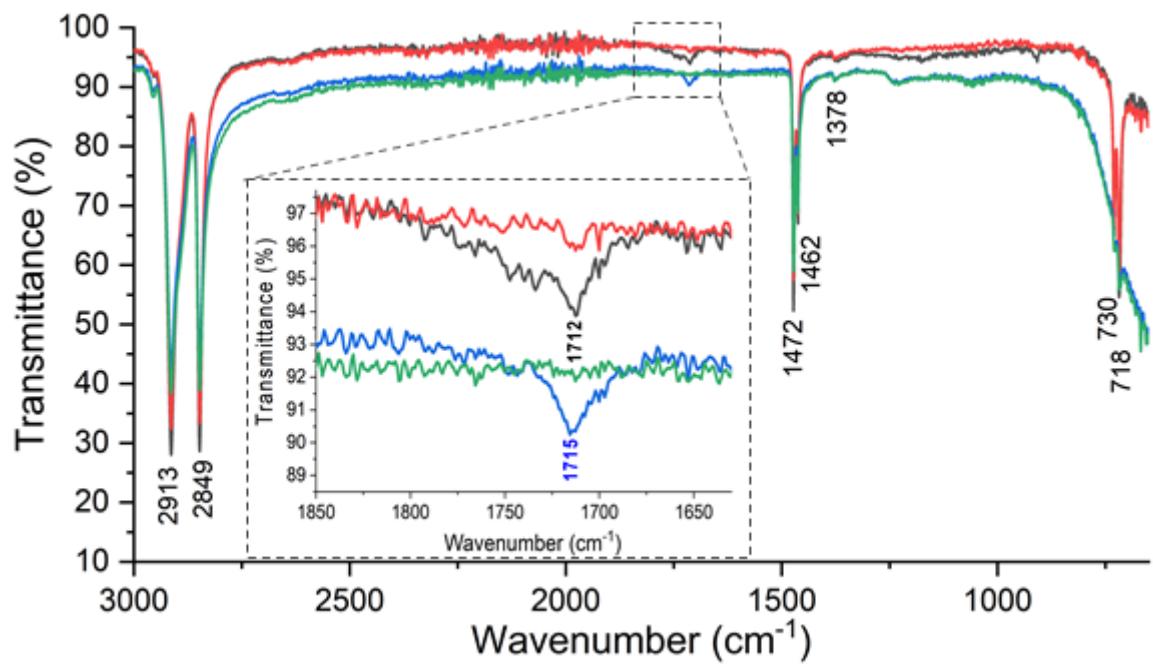


Figure S6:

Attenuated total reflection – Fourier transform infrared measurements of native small (sMP, red), and big (bMP, green) MP resulted in the characteristic spectra of polyethylene. Chemical alteration in the surface structure of aged small (sMP_{aged}, black) and big (bMP_{aged}, blue) MP were evidenced by the appearance of an absorption band at 1712 and 1715 cm⁻¹, respectively. This band is specific for ketone groups.

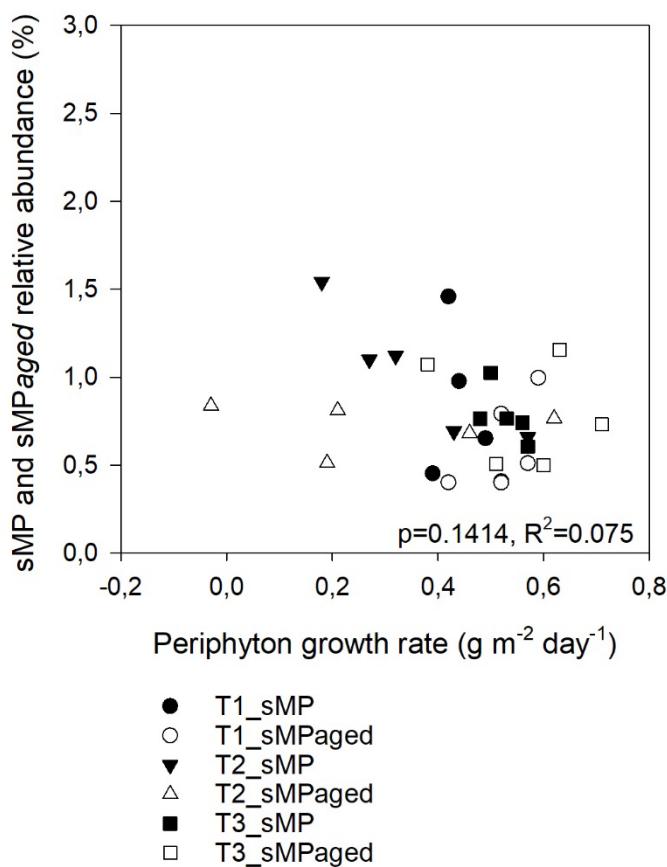


Figure S7:

Relation between sMP and sMPaged relative abundance (%) as measured with FC and viSNE with periphyton growth rate at T1, T2 and T3. Growth rate was calculated by dividing the difference between periphyton biomass of i) T1-T0, ii) T2-T1 and iii) T3-T2 by exposure time. The in the case of i) and ii) 7 days and in the case of iii) by 14 days. Statistical dependence via linear regression was tested using R, showing no significant relationship.

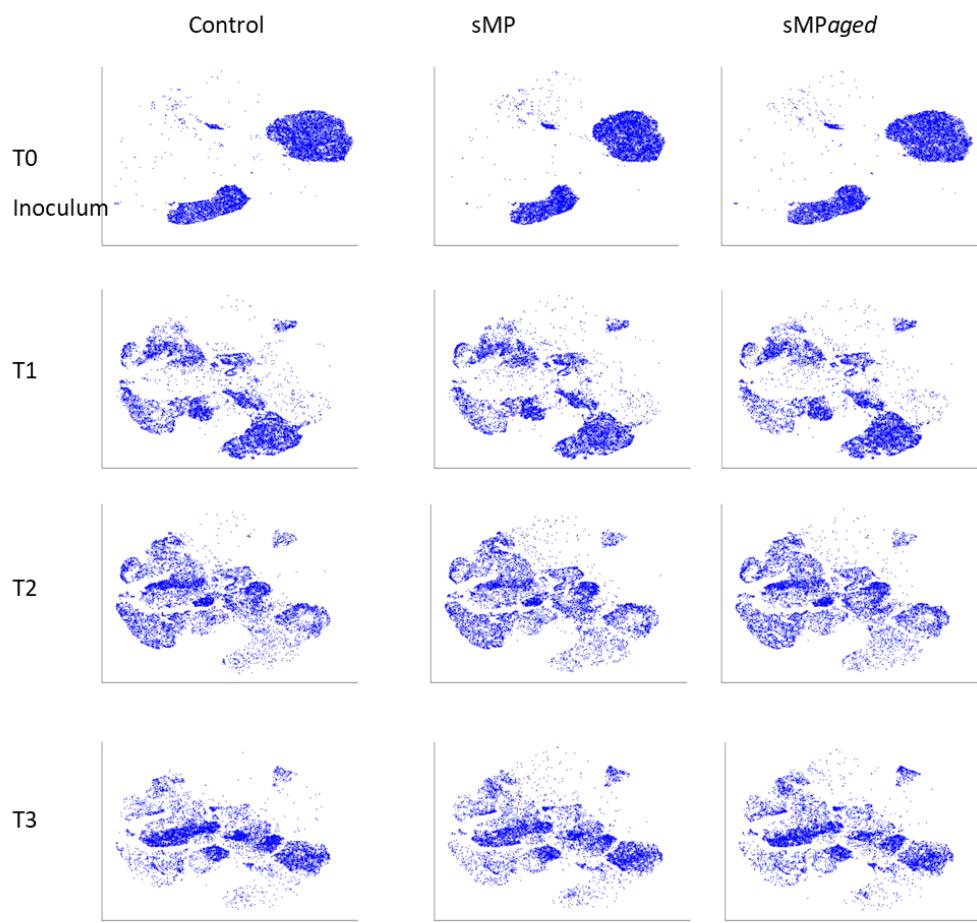


Figure S8:

ViSNE maps of the Experiment 1 control (first column), sMP (second column) and sMPaged (third column) per time point inoculum (first row), T1 (7 days, second row), T2 (21 days, third row) and T3 (28 days, forth row) of exposure.

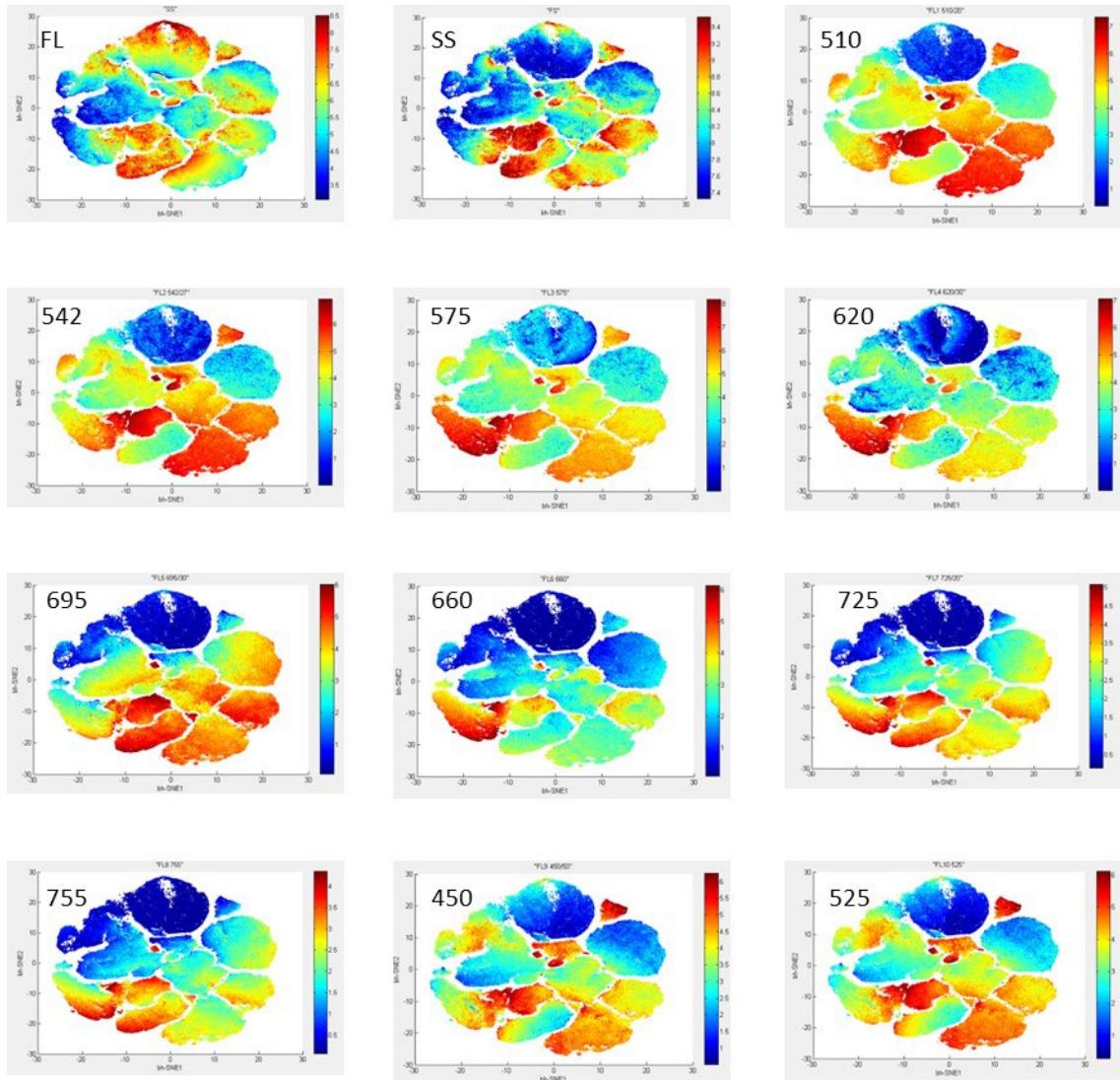


Figure S9:

VisNE maps of Experiment 1 colored according to optical scatter intensity (FS, forward scatter and SS sideward scatter) and fluorescence intensity at specific wavelengths [nm] measured by flow-cytometer. Color code from blue and red indicate low to high intensity.

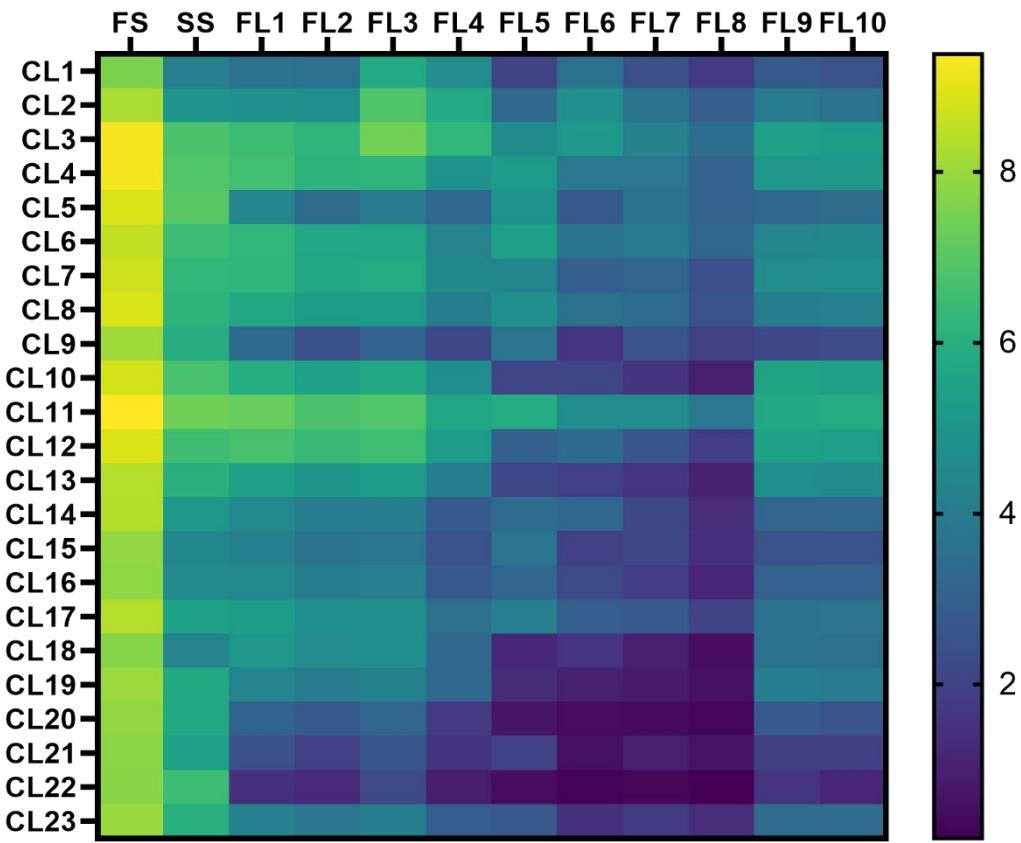


Figure S10.

Heat map of optical scatter and fluorescence intensities of each subpopulation (CL-Cluster 1 – 23) defined in Figure 6. Laser and filter wavelength [nm] are shown on top of the figure. FS – Forward Scatter, SS – Sideward Scatter, FL1- 510 nm, FL2 – 542 nm, FL3-575 nm, FL4 – 620 nm, FL5 – 695 nm, FL7 – 660 nm, FL8 – 725 nm, FL9 – 450 nm and FL10 – 525 nm.

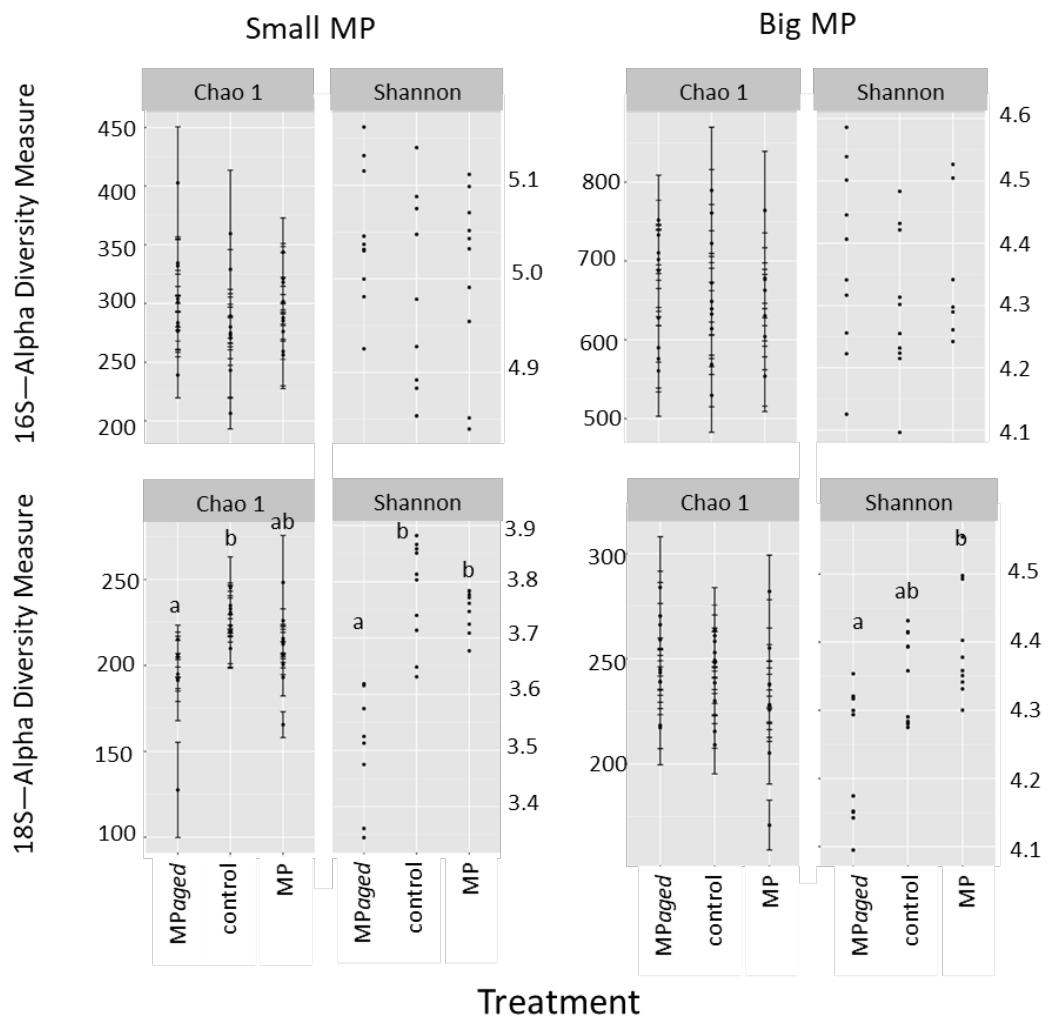


Figure S11:

Alpha diversity indicators Chao1 and Shannon for 16S (upper row) and 18s (lower row) periphyton community of Experiment 1(left column) and Experiment 2 (right column). Statistical differences ($p<0.05$) are indicated by letters within the graph. Different letters on bars indicate statistical significance in pairwise comparisons performed with R. Letters shared in common between experimental conditions indicate no significant difference.

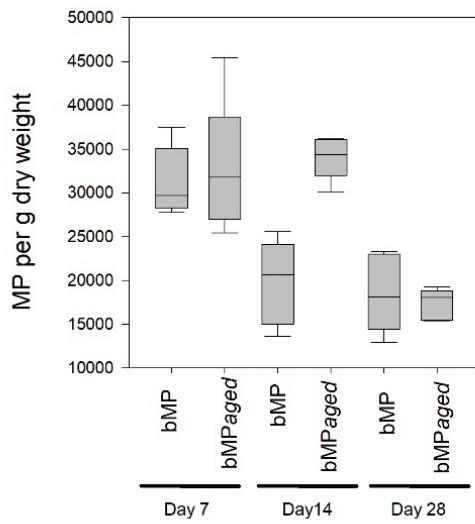


Figure S12:

bMP and bMPaged per biomass at 7, 14 and 28 days of exposure. Statistical differences were tested using mixed with MP per biomass as dependent variable and experimental time and treatment as fixed variables *nlme* package in R version 3.6.1. Results showed significant differences between MP:biomass ratio over time and between treatments ($p<0.05$) indicating a dilution of bMP and bMPaged with increasing biomass.

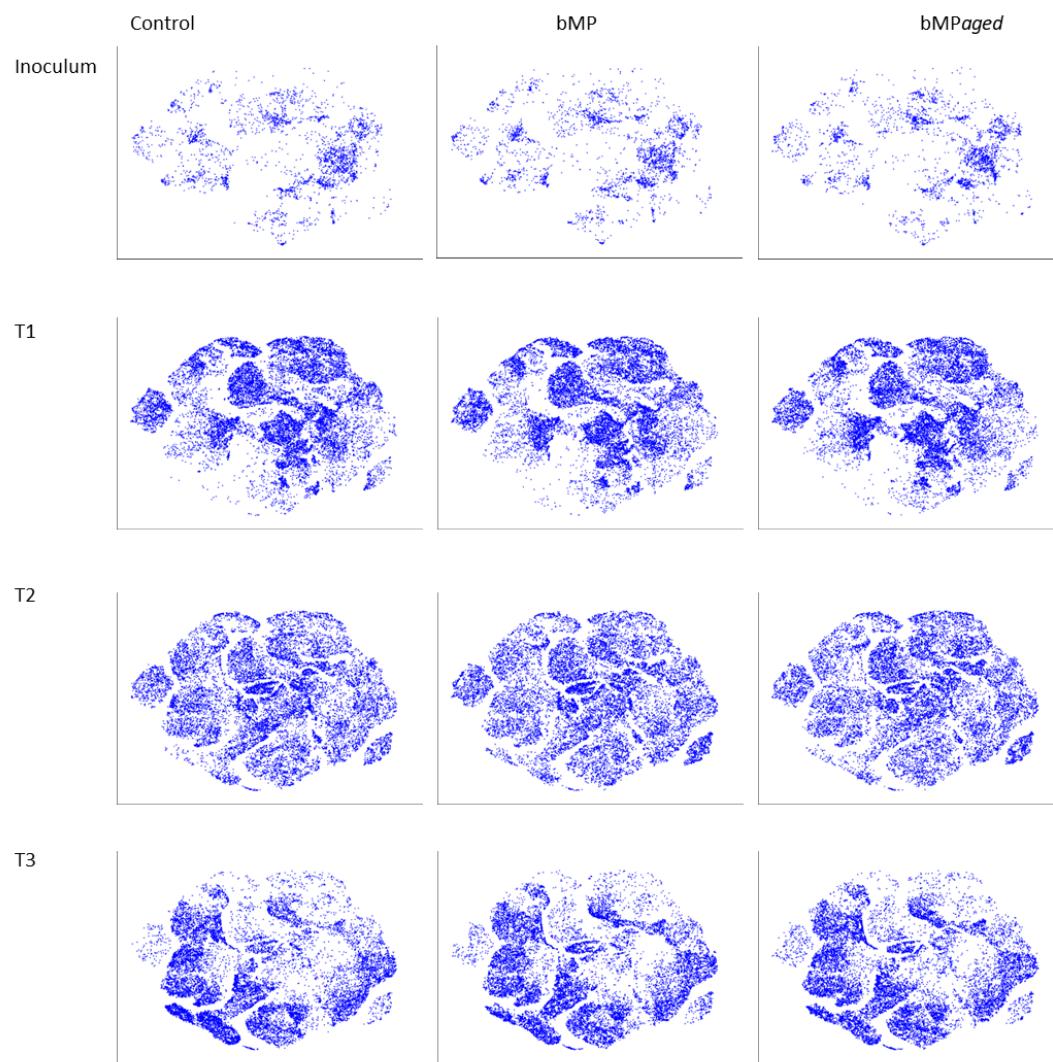


Figure S13:

VisNE maps of the control (first column), bMP (second column) and bMPaged (third column) per time point inoculum (first row), T1 (7 days, second row), T2 (21 days, third row) and T3 (28 days, forth row) of exposure.

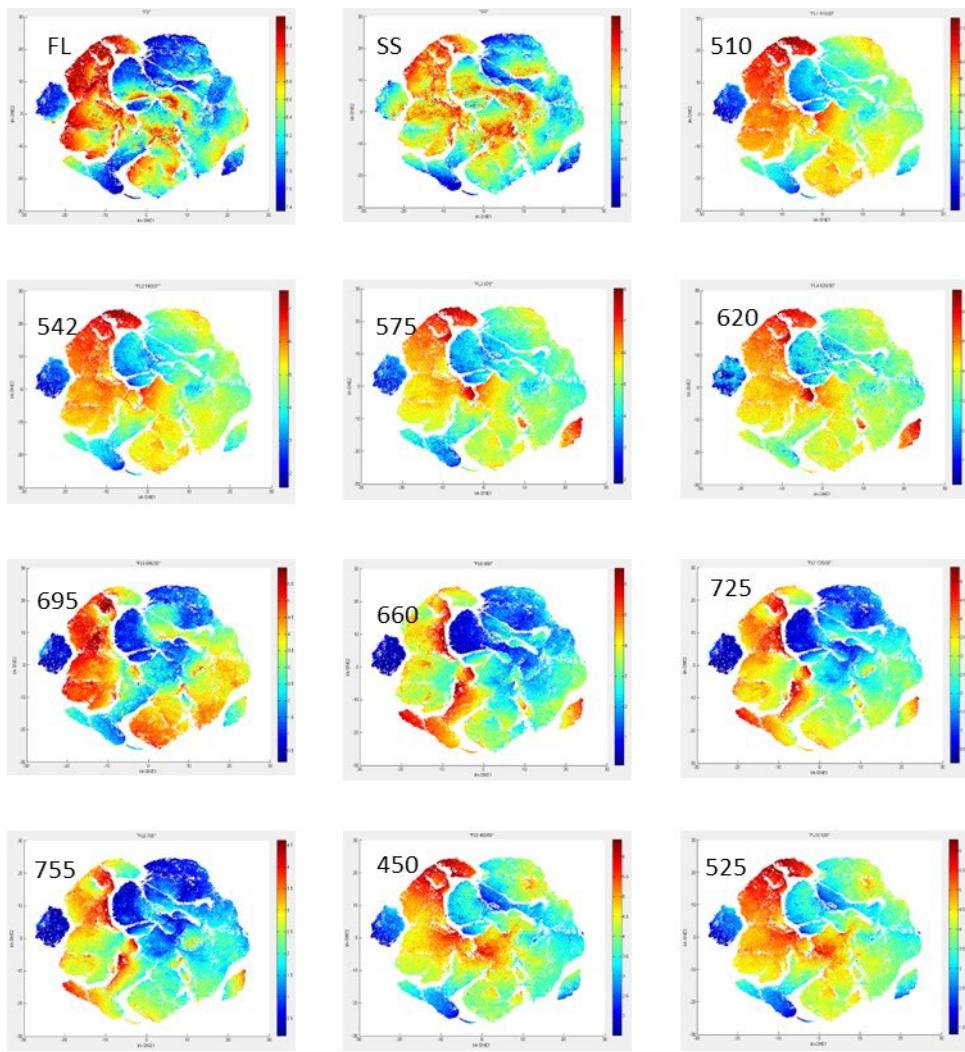


Figure S14:

ViSNE maps of Experiment 2 colored according to optical scatter intensity (FS, forward scatter and SS sideward scatter) and fluorescence intensity at specific wavelengths [nm] measured by flow-cytometer. Color code from blue and red indicate low to high intensity

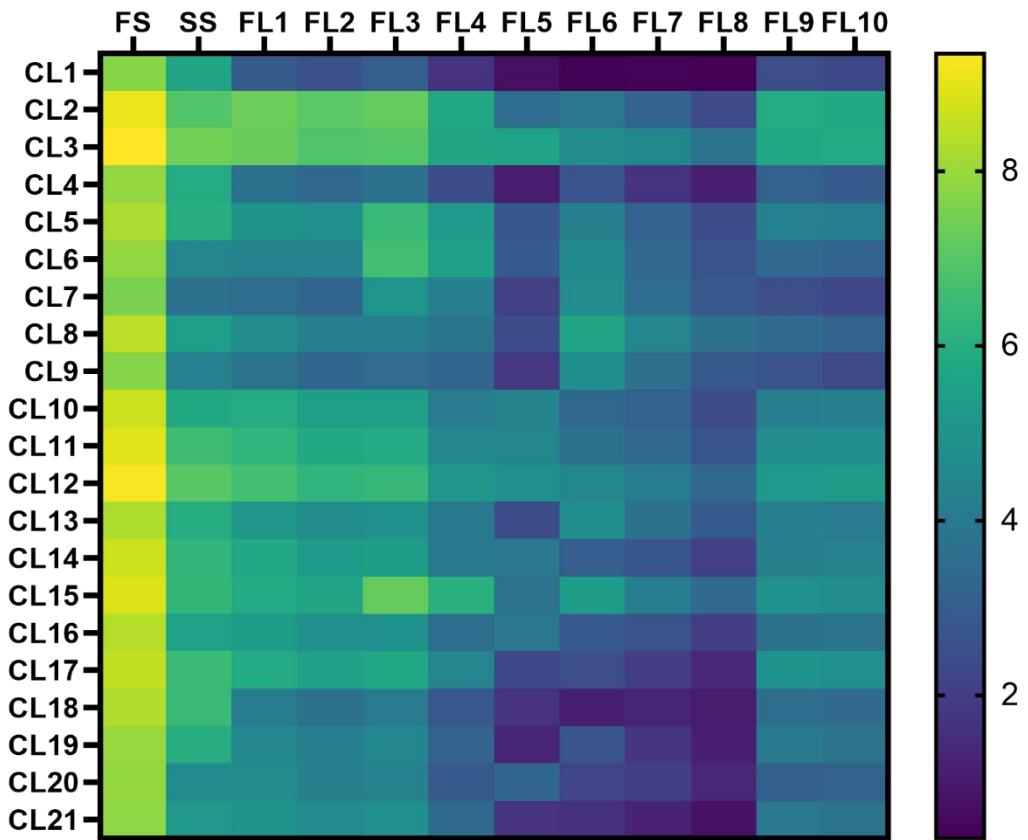


Figure S15.

Heat map of optical scatter and fluorescence intensities of each subpopulation of Experiment 2 (CL-Cluster 1 – 21) defined in Figure 6. Laser and filter wavelength [nm] are shown on top of the figure. FS – Forward Scatter, SS – Sideward Scatter, FL1- 510 nm, FL2 – 542 nm, FL3-575 nm, FL4 – 620 nm, FL5 – 695 nm, FL7 – 660 nm, FL8 – 725 nm, FL9 – 450 nm and FL10 – 525 nm.

Supplementary Text

Text S1:

Calculations for the estimation of the percentage of dispersed MP.

To calculate the number of MP per g MP powder, the following equation was used:

Equation S1:

$$c_{theoretic} = 1/V \times \rho$$

Where $c_{theoretic}$ is the number of MP per g MP powder, ρ the specific density of each MP type (see Table 1) and V is the volume of the MP. V was calculated using the following equation:

Equation S2:

$$V = \frac{4}{3} \times \pi \times r^3$$

With r the specific radius of each MP (2.5 μm and 69 μm was used for sMP and bMP, respectively).

For the estimation of the % of the dispersed MP of the initially weighted number of MP, the following equation was used:

Equation S3:

$$\% \text{ MP} = \frac{c_m}{c_t} \times 100$$

Where c_m is the measured concentration of MP and c_t the theoretical concentration estimated with equation 1.

Text S2:

Estimation of relative volume of MP in periphyton

To estimate the relative volumne of sMMP/sMPaged, bMP/bMPaged and rMP in the mature biofilms we first estimated the volume of the peripyhton by multiplying microcosm size, length and periphyotn hight (as estimated in Experiment 3 as proxi).

Next, we calculated the total volume of MP within the microcosms as follows:

The volumne of on particle was estimated using Eqution S2 and multiplied by particular density of the respective particle type, which results in the weight of one particle (g). The reciprocal of the weight of one partilce results in particles per g MP powder. This value allowed us to calculate the absolut number of particles in each exposure conditions by multiplying with the weighted mass of MP poweder. Finally we estimated the total particle volumne per microcosm by multipling with the volume of the respcitve particle type.

The relation between periphyton and particle volume is expressed in %.

Text S3

Phenotypic characterization of periphyton in experiment 1 by flow cytometry/viSNE

Overall, phenotypes diversified in the process of periphyton formation and growth (Figure 3). The inoculum was dominated by large cells with strong chlorophyll fluorescence (CL5, 33-34 %) and small cells with medium chlorophyll fluorescence (CL9, 61-62 %) (not shown in Figure 3, plots of the inoculum are included in Supplemental Figure 3b). After seven days, large cells with strong overall fluorescence (CL7, 34-38 %) appeared along with an increase in most other groups (Figure 3). CL7 decreased substantially again till day 14. Further changes were observed till day 28 with CL8, CL15, and CL17 now accounting for > 50% of all counts representing small to medium-sized cells with medium to strong chlorophyll fluorescence. CL1, CL2 and CL3 show a signature known for cyanobacterial species (combination of elevated FL3, FL4 and FL6) accounting for ~0.2% (inoculum, d0), ~2% (d7), ~28% (d14) and 7% (d28) successively of all counts. The change in abundance is dominated by CL2.

Text S4

Phenotypic characterization of periphyton in Experiment 2 by flow cytometry/viSNE

In the process of biofilm formation and growth, proportions of the 21 identified groups fluctuated, while a diversification as for sMP was not observed (Figure 3). Further, no particular pattern in changes over time in scattering and fluorescence properties was noted. CL5, CL6 and CL15 show a signature typical for cyanobacterial species (combination of elevated FL3, FL4 and FL6; ref) and account for ~6% (inoculum, d0), ~ 2 % (d7), ~5 % (d14) and 2 % (d28) successively of all counts.

Text S5

Discussion: Comparing NGS and FC&viSNE as methods for the community composition characterization

While in sMP/sMP_{aged} the periphyton was composed of three mayor subpopulations, which diversified over time (e.g. clusters divided over time), the bMP/bMP_{aged} communities were more diverse at the beginning and varied at different time points. When comparing the results from the phylogenetic and phenotypic community analysis, we found similarities in the cyanobacterial fraction. In experiment 1, three phenotypic groups (CL1, CL2 and CL3) showed a signature known for cyanobacterial species accounting for ~2% of the counts on day 7. Changes in abundance were dominated by CL2. At the same time, the highest relative

abundance derived by NGS was detected for ASV9 (cyanobacterium genus *Chamaesiphon*) in control communities and was significantly reduced in sMP and sMPaged communities. While the differences for CL2 were not significant between the treatments, the relative abundance is in the same order of magnitude. In experiment 2, also three phenotypic groups (CL5, CL6 and CL15) showed a signature typical for cyanobacterial species accounting for ~ 2 % of the counts on day 7. Based on NGS analyses, two cyanobacterial ASV (50 and 148) were identified accounted for 0.7 % on average. These are first indications that for certain phenotypes identified by flow cytometry, corresponding genetic sequences may be identified by NGS.