Supplementary Information for

Impact of Upstream Chlorination on Filter Performance and Microbial Community Structure of GAC and Anthracite Biofilters

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This file includes:

1 text, 13 figures, and 7 tables addressing experimental procedure and additional data

Text S1. Sample preparation, DNA extraction, and qPCR analysis of FLA

Water samples were collected in sterile 10-L polypropylene carboys and transported to the laboratory for subsequent sample concentration by ultrafiltration. 10 mL of sterile 10% sodium polyphosphate (Fisher Scientific, USA) was added to the 10-L sample. Before concentration, the ultrafilter was preconditioned with a 5% solution of sterile calf serum (Life Technologies, USA) and rinsed with 2 L of spare sample. Sample concentration was performed using the tangential flow ultrafiltration (TFU) set-up shown in Fig. S3. The TFU set-up used a Rexeed-25S hollow fiber filter (Asahi Kasei Medical Co., Ltd, U.S.) and a peristaltic pump (Masterflex L/S precision pump system, Cole-Palmer, USA) connected by Tygon R-3603 tubing (3/8" ID x ½" OD, Cole-Palmer, USA). The tubing passing through the pump was Masterflex platinum-cured silicone (L/S 36, Cole-Palmer, USA). The 10-L samples were filtered at a rate of 0.6 – 1.2 L/min to a volume of 200 mL. In a separate TFU run, microorganisms attached to the ultrafilter were eluted using a 500-mL buffer solution (Tween 80 (Fisher Scientific) + Antifoam A (Sigma Aldrich) + 10% sodium polyphosphate) until a 150 mL concentrate was collected. The sample retentate and filter eluate were combined (total volume = 350 mL) and centrifuged at 800×g for 15 min at 24°C. The resulting pellet was resuspended in 0.40 mL sterile reagent water and stored at -80°C. Nucleic acid extraction and purification of samples followed using a MagAttract Virus Mini M48 Kit on a BioRobot M48 workstation (Qiagen, Germany) to a final volume of 100 μL. A ten-fold dilution was next applied to this extract and a 5 μL aliquot was used for real time quantitative polymerase chain reaction (qPCR) (Applied Biosystems, CA, U.S.). To amplify and quantify the pathogenic FLA target DNA sequences, the following primers, probes, and standards were used.[1]

Naegleria fowleri

NaeglR344: 5'- CACTAGAAAAAGCAAACCTGAAAGG

NaeglF192: 5'- GTGCTGAAACCTAGCTATTGTAACTCAGT

NfowlP: 5'- 6FAM-ATAGCAATATATTCAGGGGAGCTGGGC—BBQ

N. fowl Standard: Clone Plasmid DNA TIB MOLBIOL Reference # pLCNF 2818

Acanthamoeba spp.

AcantF900: 5'- CCCAGATCGTTTACCGTGAA

AcantR1100: 5'- TAAATATTAATGCCCCCAACTATCC

AcantP1000: 5'- 6FAM-CTGCCACCGAATACATTAGCATGG—BBQ

Acant Standard: Clone Plasmid DNA TIB MOBIOL Reference # pLCACAN 2817

Balamuthia mandrillaris

BalaF1451: 5'- TAACCTGCTAAATAGTCATGCCAAT

BalaR1621: 5'- CAAACTTCCCTCGGCTAATCA

BalaP1582: 5'- 6FAM-AGTACTTCTACCAATCCAACCGCCA—BBQ

Bala Standard: Clone Plasmid DNA TIB MOBIOL Reference # pLCBMAN 2818

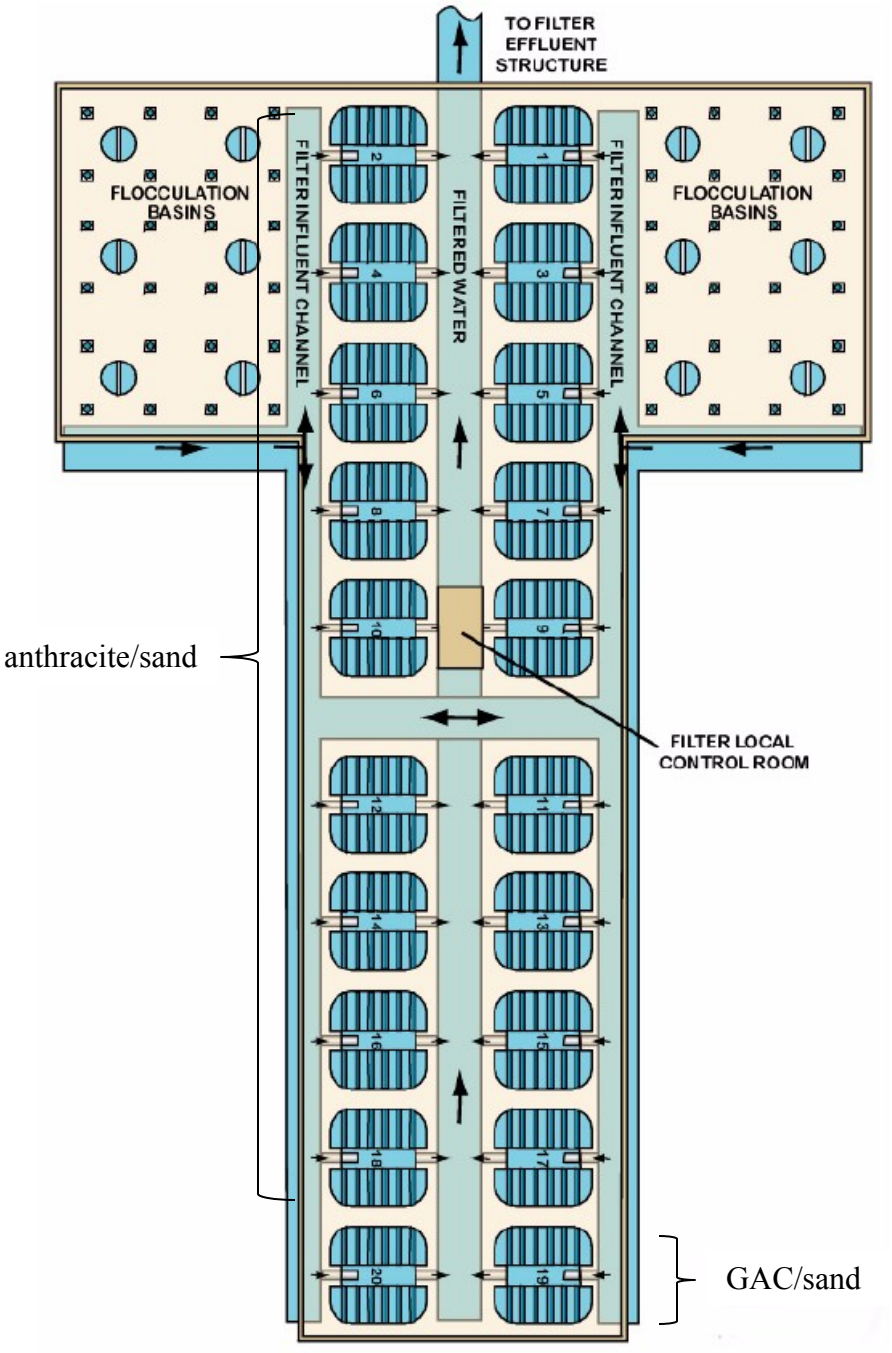

Fig. S1. Scheme for the full-scale filtration system at River Mountains WTF

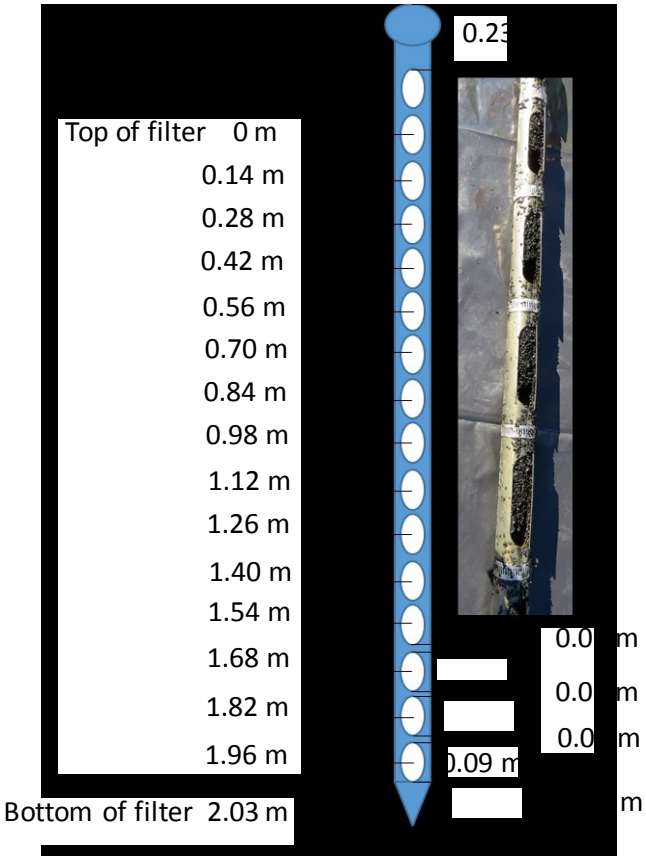

Fig. S2. Wheat thief sampling device

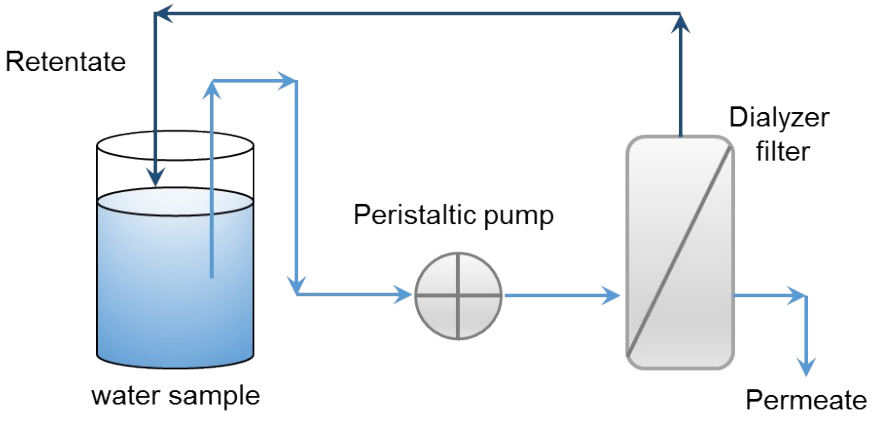

Fig. S3. Tangential flow ultrafiltration set-up

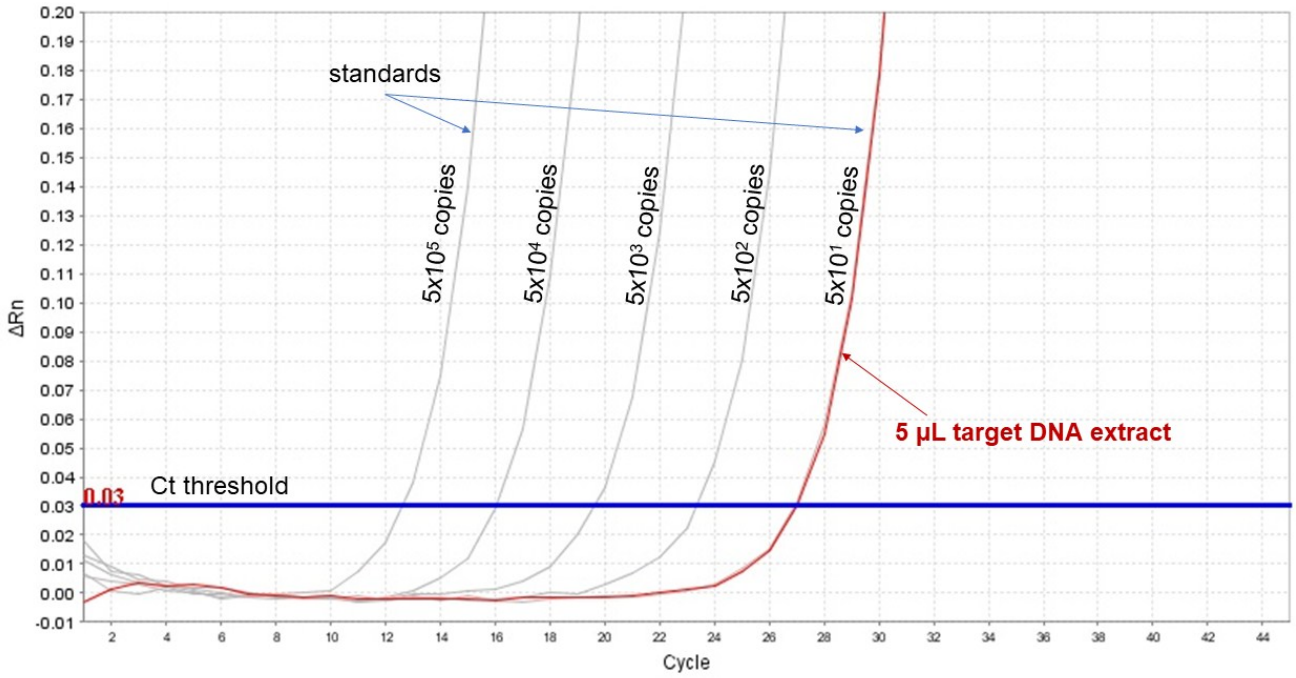

Fig. S4. Amplification plot (qPCR) showing *Acanthamoeba* spp. target DNA detection in the GAC filter effluent (Filter 19).

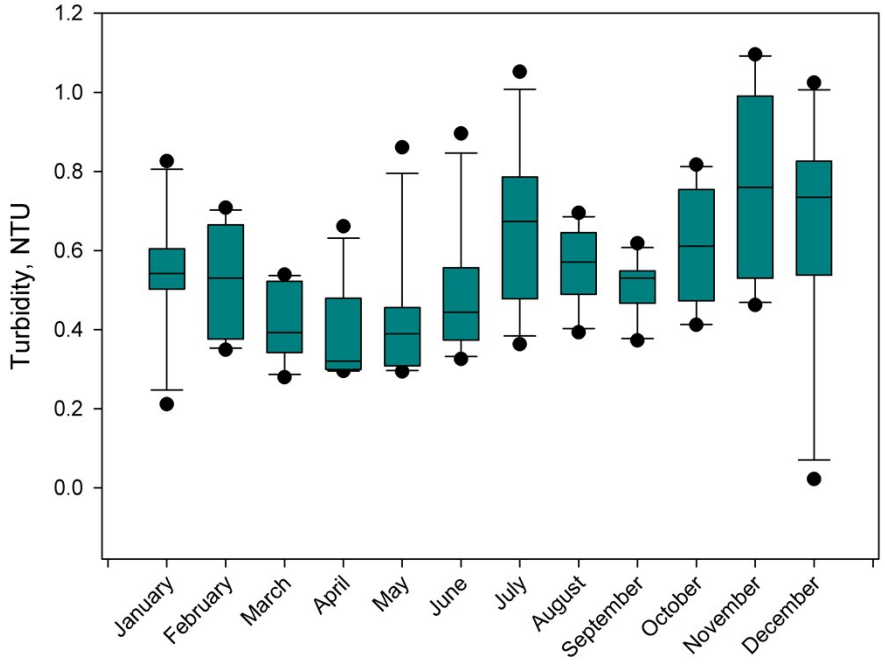

Fig. S5. Raw water turbidity at River Mountains WTF. The plots were generated from monthly average data from 2007 – 2017. The top and bottom of the boxes represent the 75th and 25th percentiles, the top and bottom whiskers represent the 90th and 10th percentiles, the black circles are the 5th and 95th percentiles, and the solid line in the box is the median. Typical lake turnover occurs in November.

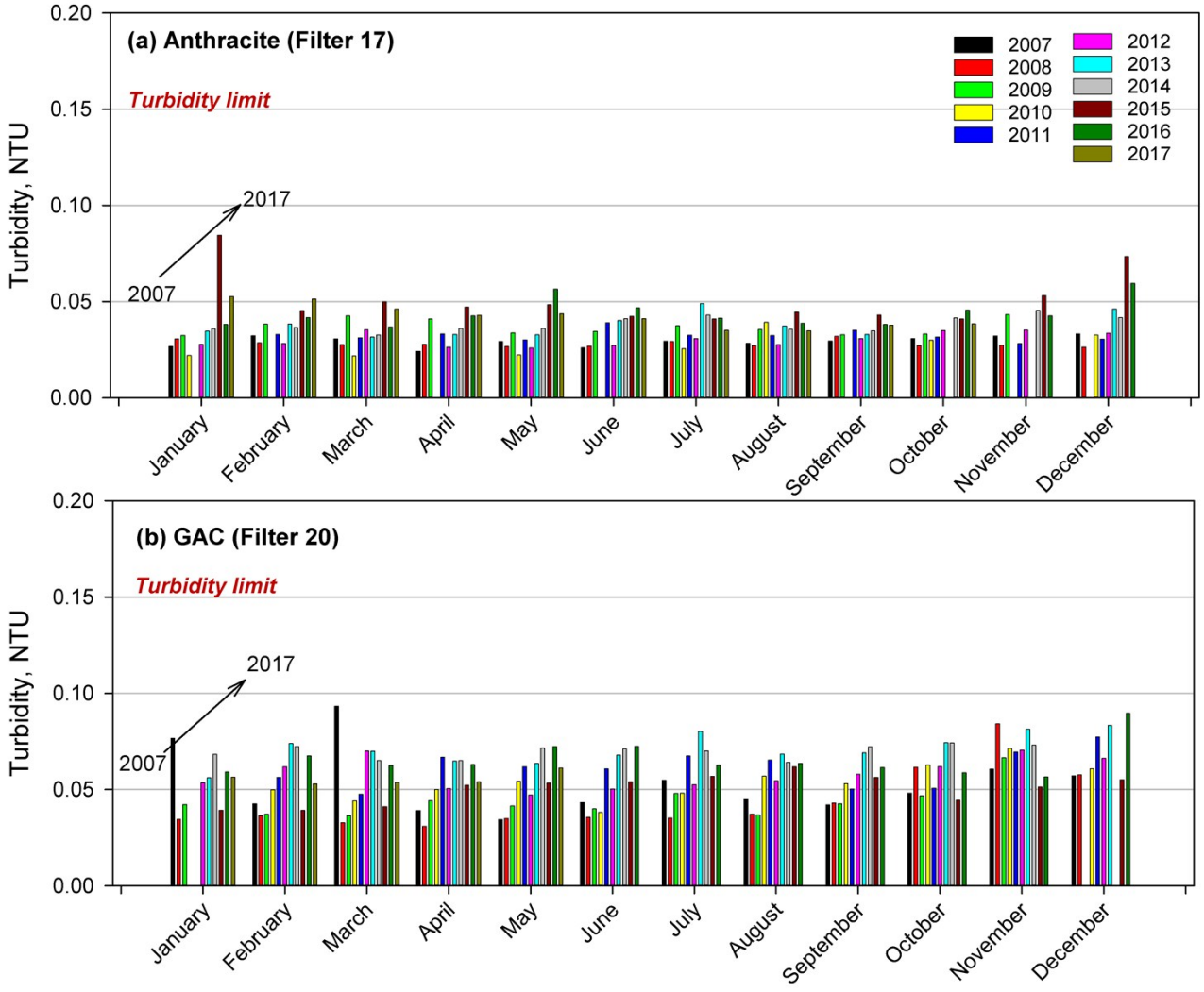

Fig. S6. Comparison of turbidity in effluent of (a) chlorinated anthracite (Filter 17) and (b) GAC biofilter (Filter 20). Data measured from 2007 – 2017. The target turbidity according to the US EPA's LT2 = 0.15 NTU.

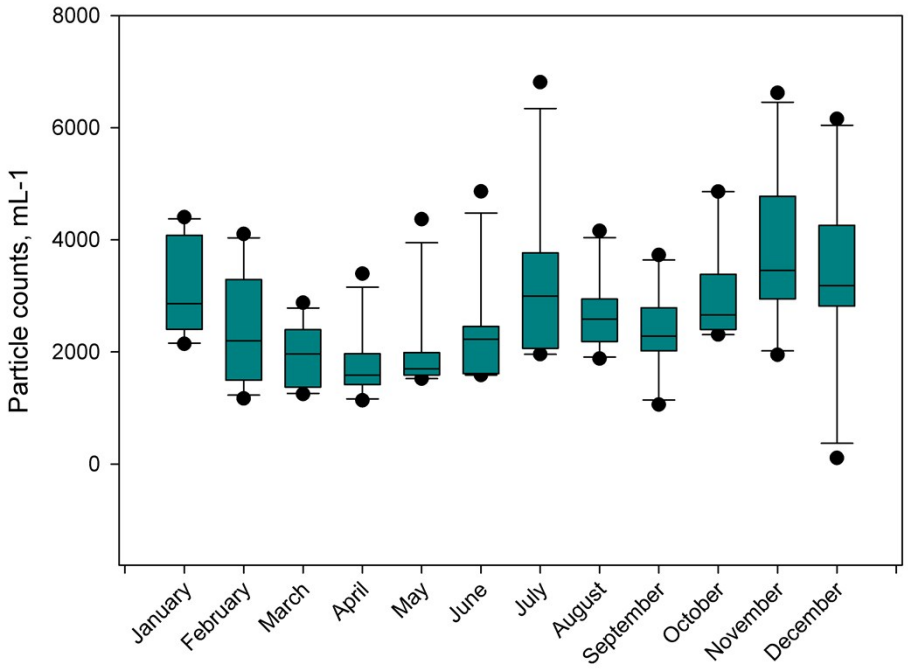

Fig. S7. Raw water particle counts at River Mountains WTF. The plots were generated from monthly average data from 2007 – 2017. The top and bottom of the boxes represent the 75th and 25th percentiles, the top and bottom whiskers represent the 90th and 10th percentiles, the black circles are the 5th and 95th percentiles, and the solid line in the box is the median.

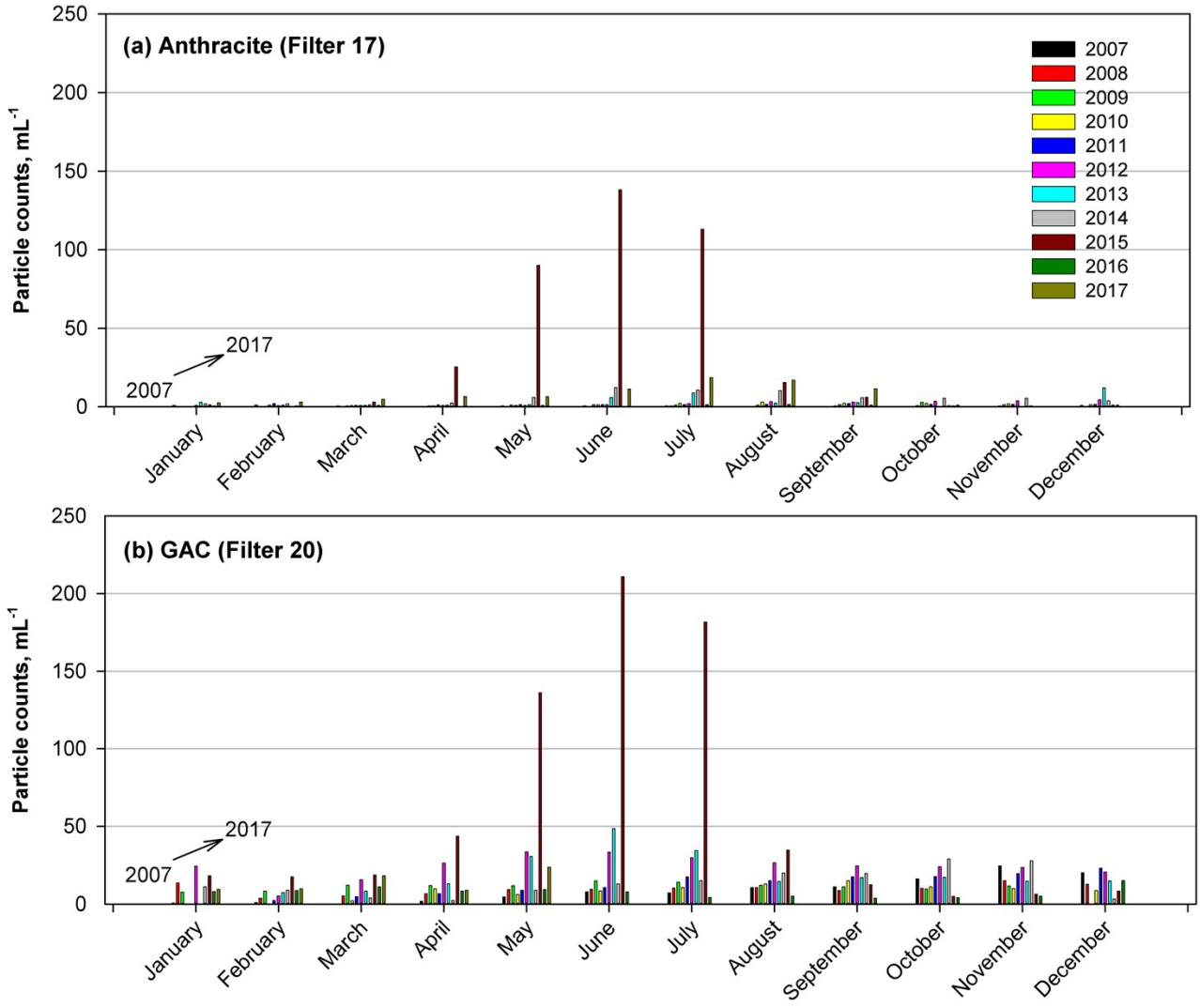

Fig. S8. Comparison of particle counts in effluent of (a) chlorinated anthracite (Filter 17) and (b) GAC biofilter (Filter 20). Data measured from 2007 – 2017.

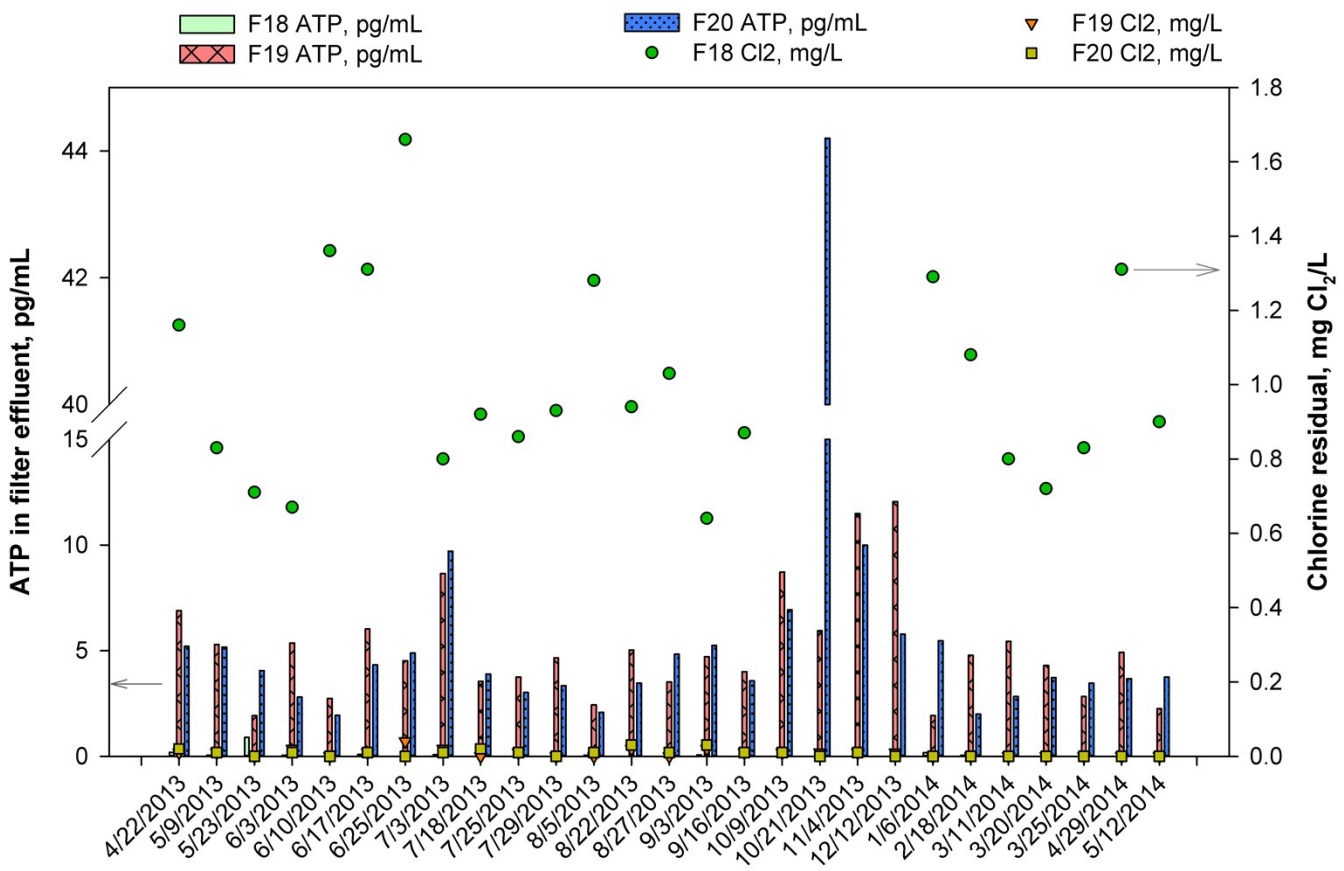

Fig. S9. Cellular ATP and chlorine residual measurements in the effluents from the anthracite (i.e., F18) and GAC filters (F19 and F20). The cellular ATP concentrations for the anthracite filter (i.e., F18) were ~0 pg/mL for all but one sample (5/23/2013).

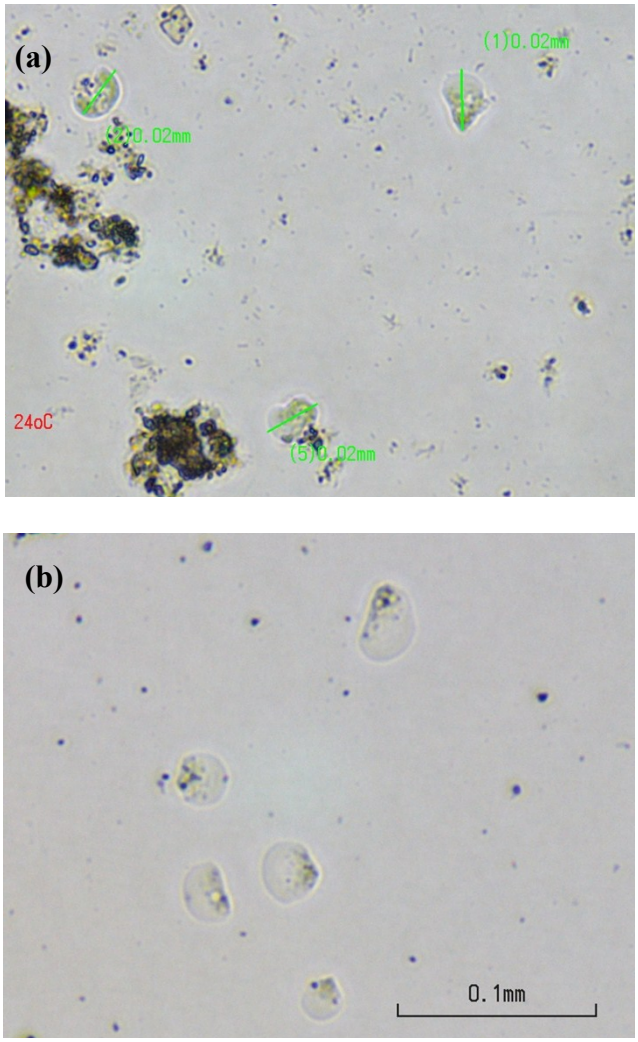

Fig. S10. Microscopic image of the viable free-living amoeba from (a) raw water samples and (b) GAC filter media (Filter 19).

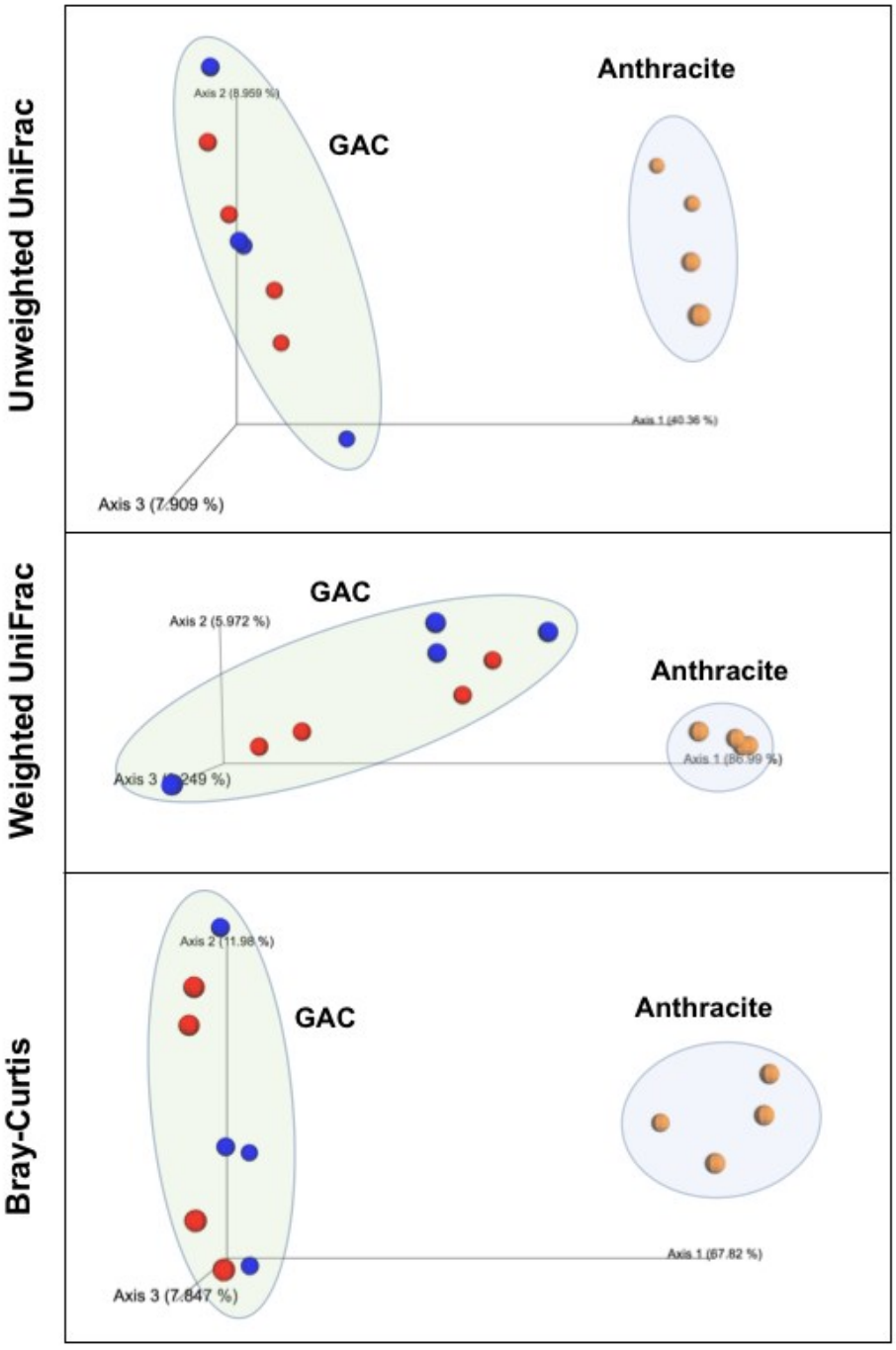

Fig. S11. Principal coordinate analyses (PCoAs) of 16S rRNA gene sequence data from the anthracite filter (Filter 18 - orange) and GAC filter (Filter 19 – red; Filter 20 – blue).

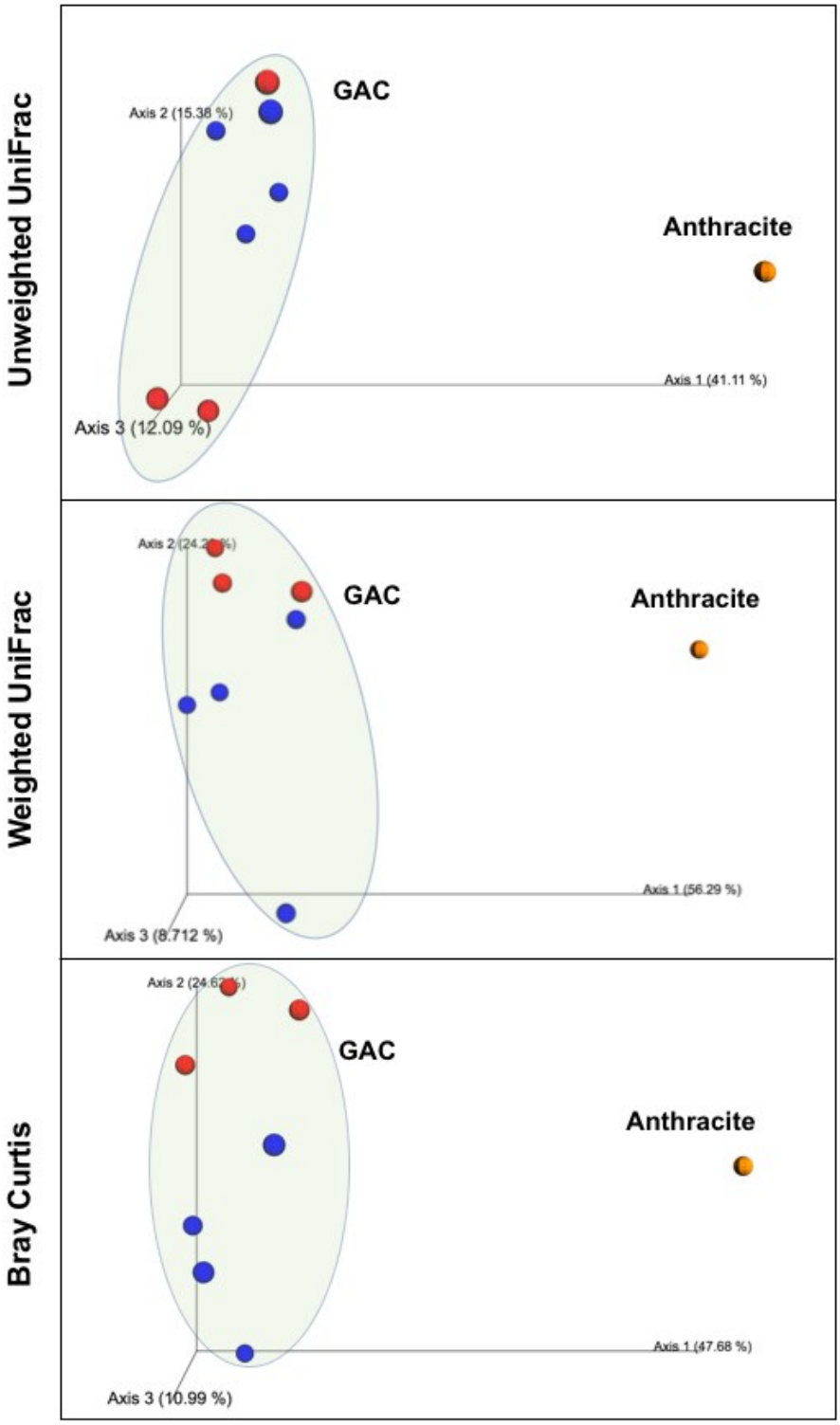

Fig. S12. Principal coordinate analyses (PCoAs) of 18S rRNA gene sequence data from the anthracite filter (Filter 18 - orange) and GAC filter (Filter 19 – red; Filter 20 – blue).

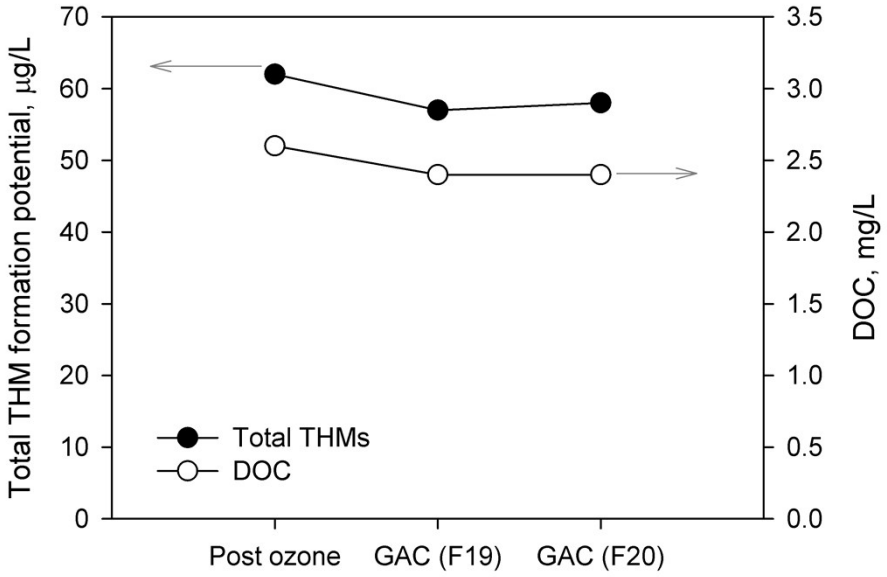

Fig. S13. DOC and total trihalomethane formation potentials of ozonated and GAC filter effluent samples (Filters 19 – 20). Cl_2 residual after 24 h formation potential test of grab samples = 1.1 – 1.4 mg/L as Cl_2.

Table S1. Raw water quality characteristics at River Mountains WTF*

Parameter	Value
DOC, mg/L	2.58 (0.08)
UV_{254} Absorbance, cm^{-1}	0.054 (0.001)
pH	8.1 (0.2)
Temperature, °C	16.1 (1.8)
Turbidity, NTU	0.377 (0.098)
Conductivity, µS/cm	946 (35)
Total dissolved solids, mg/L	587 (10)
Alkalinity, mg HCO_3^-/L	138 (3)
Total hardness, mg $CaCO_3$/L	287 (9)
Bromide, µg/L	68 (6)
Nitrate, µg-N/L	386 (43)
Orthophosphate, µg-P/L	3.5 (1.0)

** taken from January – October 2017. Values in parentheses are standard deviations.*

Table S2. Species alpha-diversity indices from analysis of 16S rRNA gene sequence data

Sample	Simpson	Shannon	Chao1
Anthracite			
F18: 0 m	0.60	2.5	84.1
F18: 0 m + BW	0.64	2.4	76.9
F18: 2 m	0.77	2.9	73.6
F18: 2 m + BW	0.70	2.9	62.1
GAC			
F19: 0.8 m	0.87	4.1	147.2
F19: 0.8m + BW	0.81	3.7	124.8
F19: 1.6 m	0.92	4.6	161.9
F19: 1.6 m + BW	0.94	5.0	144.6
F20: 0.8 m	0.84	3.7	99.8
F20: 0.8 m + BW	0.85	4.1	145.1
F20: 1.6 m	0.89	4.2	128.6
F20: 1.6 m + BW	0.94	5.0	165.8

** Calculated using QIIME2[2] after rarefaction at a sequencing depth of 6816 sequences (maximum sequencing depth for inclusion of all samples); Notes: Simpson (evenness, 0-1) and Shannon (species richness and evenness): greater value, greater sample diversity; Chao1: greater value; greater species richness.*

Table S3. Species alpha-diversity indices from analysis of 18S rRNA gene sequence data

Sample	Simpson	Shannon	Chao1
Anthracite			
F18: 0 m	0.53	1.9	37.0
GAC			
F19: 0.8 m	0.80	2.9	33.0
F19: 0.8 m + BW	0.65	2.0	19.0
F19: 1.6 m + BW	0.69	2.2	21.3
F20: 0.8 m	0.80	3.1	33.0
F20: 0.8 m + BW	0.66	2.6	36.0
F20: 1.6 m	0.76	2.9	33.5
F20: 1.6 m + BW	0.75	2.7	27.0

** Calculated using QIIME2[2] after rarefaction at a sequencing depth of 6358 sequences (maximum sequencing depth for inclusion of all samples); Notes: Simpson (evenness, 0-1) and Shannon (species richness and evenness): greater value, greater sample diversity; Chao1: greater value; greater species richness. No data were reported for other F18 samples (bottom, after BW) because of amplification problems due to insufficient biomass.*

Table S4. Impact of filter media type on bacterial community structure. Significance of results was assessed using permutational analysis of variance (PERMANOVA) of unweighted/weighted UniFrac distances and Bray Curtis dissimilarities.

Group 1	Group 2	p value		
		Unweighted UniFrac	Weighted UniFrac	Bray Curtis
Filter 18 (anthracite)	Filter 19 (GAC)	0.034	0.026	0.030
Filter 18 (anthracite)	Filter 20 (GAC)	0.034	0.028	0.027
Filter 19 (GAC)	Filter 20 (GAC)	0.369	0.429	0.135

Table S5. Impact of filter media type on invertebrate community structure. Significance of results was assessed using permutational analysis of variance (PERMANOVA) of unweighted/weighted UniFrac distances and Bray Curtis dissimilarities.

Group 1	Group 2	p value		
		Unweighted UniFrac	Weighted UniFrac	Bray Curtis
Filter 18 (anthracite)	Filter 19 (GAC)	0.246	0.236	0.271
Filter 18 (anthracite)	Filter 20 (GAC)	0.178	0.211	0.212
Filter 19 (GAC)	Filter 20 (GAC)	0.102	0.058	0.050

Table S6. Most abundant OTUs from 16S rRNA gene sequence data at the genus (family) level in anthracite (F18) and GAC (F19-20) filters. *confidence value of <51%; Unknown = unable to make confident determination; Unclassified = taxonomic information retrieved from NCBI contains missing information; BW = backwash; 0, 0.8, 1.6 and 2.0 m are filter depths. color scheme: green (highest) → red (lowest).

Class	Genus	F18 0 m	F18 0 m BW	F18 2 m	F18 2 m BW	F19 0.8 m	F19 0.8m BW	F19 1.6 m	F19 1.6 m BW	F20 0.8 m	F20 0.8 m BW	F20 1.6 m	F20 1.6 m BW
Betaproteobacteria	Unknown (Undibacterium*)	67.53	58.93	44.48	54.60	0.15	0.04	0.07	0.03	0.06	0.11	0.12	0.03
Unknown	Unknown	5.80	17.71	24.17	3.52	1.50	3.02	1.53	2.06	1.41	2.00	1.61	2.64
Betaproteobacteria	Massilia	5.65	5.65	10.41	15.89	10.12	13.96	8.03	8.30	29.50	35.63	20.30	12.86
Gammaproteobacteria	Pseudomonas	3.98	1.05	0.28	0.00	0.43	0.00	0.10	0.03	1.05	0.04	0.28	0.00
Betaproteobacteria	Hydrogenophaga	2.66	1.04	1.65	0.71	0.00	0.00	0.02	0.08	0.00	0.00	0.00	0.08
Betaproteobacteria	Herbaspirillum	2.46	4.21	7.65	4.92	35.91	43.82	12.85	11.13	23.90	10.43	16.51	1.92
Alphaproteobacteria	Unknown	2.02	1.78	1.27	3.62	5.32	3.21	14.68	13.17	4.80	6.49	6.61	17.01
Betaproteobacteria	Unknown	1.46	1.13	0.66	0.89	1.75	0.98	2.37	1.59	1.08	1.59	2.46	3.37
Unknown	Unknown	1.37	1.12	0.68	2.59	5.93	7.75	8.66	8.90	2.69	4.71	6.64	7.59
Betaproteobacteria	Unknown (Comamonadaceae)	1.22	2.28	1.61	2.87	15.50	5.69	15.58	10.15	17.25	12.27	18.49	9.44
Betaproteobacteria	Methylophilus	0.89	0.28	0.51	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alphaproteobacteria	Sphingomonas	0.70	0.50	0.47	0.50	1.39	1.66	1.51	1.70	2.42	3.68	3.28	1.55
Alphaproteobacteria	Sphingopyxis	0.69	0.54	1.48	0.85	4.65	5.75	3.56	5.11	2.31	2.75	3.28	1.71
Alphaproteobacteria	Brucella	0.52	1.61	1.89	1.85	0.01	0.13	0.02	0.00	0.02	0.00	0.00	0.00
Unclassified	Unclassified	0.37	0.18	0.18	0.83	1.16	2.00	2.40	3.18	1.00	1.95	1.14	1.36
Alphaproteobacteria	Hyphomicrobium	0.32	0.10	0.15	0.55	2.87	1.28	4.32	5.63	1.86	2.35	3.95	5.54
Alphaproteobacteria	Bradyrhizobium	0.23	0.11	0.18	0.55	1.68	1.87	4.38	4.83	1.43	1.55	4.17	6.92
Betaproteobacteria	Oxalicibacterium	0.23	0.73	1.40	0.39	0.04	0.00	0.01	0.02	0.04	0.01	0.04	0.02
Alphaproteobacteria	Unknown	0.08	0.02	0.04	0.23	0.25	0.48	0.75	1.38	0.33	2.45	1.08	3.17
Unknown	Unknown	0.00	0.00	0.00	0.00	4.76	1.82	5.73	5.81	1.84	2.90	1.32	5.49
Gammaproteobacteria	Perlucidibaca	0.00	0.00	0.00	0.00	0.56	0.00	0.14	0.00	1.60	0.06	0.36	0.00
Gammaproteobacteria	Legionella	0.00	0.00	0.00	0.00	0.02	0.09	0.02	0.08	0.00	0.00	0.03	0.05

Table S7. Most abundant OTUs from 18S rRNA gene sequence data at the genus (family) level in anthracite (F18) and GAC (F19-20) filters. Unknown = unable to make confident determination; Unclassified = taxonomic information retrieved from NCBI contains missing information; No Hit = no match when run against RTL Genomics database; BW = backwash; 0.8 and 1.6m are filter depths. No data for other F18 samples (bottom, after BW) because of amplification problems due to insufficient biomass. color scheme: green (highest) → red (lowest).

Class	Genus	F18 0 m	F19 0.8 m	F19 0.8 m + BW	F19 1.6 m	F20 0.8 m	F20 0.8 m + BW	F20 1.6 m	F20 1.6 m + BW
Maxillopoda	Leptodiaptomus	66.51	8.00	0.00	0.00	10.66	0.03	0.02	0.00
Bivalvia	Dreissena	9.98	1.29	0.00	0.00	0.48	0.00	1.10	0.00
Maxillopoda	Unknown (Cyclopidae)	8.59	0.00	0.00	0.00	0.00	0.00	0.02	0.00
No Hit	No Hit	6.71	67.96	88.89	88.16	62.57	72.26	61.22	45.88
Maxillopoda	Mesocyclops	2.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ostracoda	Cypridopsis	1.03	0.87	0.00	0.00	0.00	0.00	0.00	0.00
Dinophyceae	Unknown	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Unclassified	Unclassified	0.47	0.62	0.01	0.16	1.74	0.68	0.03	0.19
Unknown	Unknown	0.47	2.00	1.57	4.79	3.13	1.90	0.83	1.36
Maxillopoda	Unknown	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chromadorea	Eumonhystera	0.31	0.03	0.00	0.00	3.04	2.77	0.00	26.77
Oomycetes	Aphanomyces	0.12	4.13	0.00	0.00	0.06	0.00	0.00	0.00
Unclassified	Unknown	0.11	0.22	0.14	0.35	1.04	0.28	2.85	3.51
Unclassified	Unknown (Heterophryidae)	0.10	0.60	0.30	0.00	1.87	0.24	1.96	4.62
Ostracoda	Unknown	0.06	0.00	0.00	0.00	1.37	0.00	0.00	0.00
Oomycetes	Pythiopsis	0.02	2.53	0.01	0.00	1.04	0.00	0.80	0.00
Unclassified	Enchytraeus	0.00	2.67	0.00	0.00	0.41	0.05	0.00	1.10
Unknown	Unknown	0.00	0.00	0.00	0.00	0.00	2.48	0.02	0.06
Coccidia	Unclassified (Eimeriidae)	0.00	0.05	0.03	0.00	0.66	0.17	0.88	0.14
Heterolobosea	Unclassified (Valkampfiidae)	0.00	0.35	0.44	0.25	0.21	0.22	0.33	1.35
Litostomatea	Loxophyllum	0.00	0.00	6.07	0.49	0.00	6.28	14.54	3.24
Oligohymenophorea	Trichodina	0.00	0.00	0.00	0.00	0.00	1.53	0.00	0.00
Oomycetes	Unknown	0.00	0.72	0.00	0.00	1.58	0.00	0.11	0.00
Phyllopharyngea	Unclassified	0.00	0.00	0.00	0.00	0.00	0.96	0.00	0.04
Unclassified	Bodomorpha	0.00	0.15	0.00	0.86	0.24	4.29	1.56	3.38
Unclassified	Cochliopodium	0.00	0.03	0.02	0.36	0.00	0.62	0.10	0.85
Unclassified	Ancyromonas	0.00	0.21	0.00	0.00	0.00	0.19	1.94	0.70
Unclassified	Unknown	0.00	0.00	0.00	0.00	6.35	1.34	4.05	1.42
Unclassified	Unknown (Vannellidae)	0.00	4.16	0.00	0.00	0.35	0.00	2.37	0.03
Unclassified	Vannella	0.00	1.16	0.00	0.00	0.00	0.00	0.04	0.00
Unknown	Unknown	0.00	0.37	2.53	0.01	1.55	1.33	1.53	0.55
Unclassified	Unclassified	0.00	0.28	0.00	4.57	0.00	0.84	0.33	4.74

References

1. Y. Qvarnstrom, G. S. Visvesvara, R. Sriram and A. J. da Silva, Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*, *J. Clin. Microbiol.*, 2006, **44**, 3589-3595.
2. J. G. Caporaso, Quantitative insights into microbial ecology (QIIME) 2. https://qiime2.org. (Accessed April 2018).