

## **Supplementary material**

### **Kinetics of mercury accumulation by freshwater biofilms**

*Perrine Dranguet*<sup>A,B</sup> *Vera I. Slaveykova*<sup>A</sup> and *Séverine Le Faucheur*<sup>A,C</sup>

<sup>A</sup>University of Geneva, Faculty of Sciences, Earth and Environment Sciences, Department F.-A. Forel for Environmental and Aquatic Sciences, Environmental Biogeochemistry and Ecotoxicology, Uni Carl Vogt, 66 Bvd Carl-Vogt, CH 1211, Geneva, Switzerland.

<sup>B</sup>Present address: Département de sciences biologiques, Université de Montréal, Pavillon Marie-Victorin CP6128, Succ. Centre-Ville, Montréal, Québec H3C 3J7, Canada

<sup>C</sup>Corresponding author. Email: [severine.lefaucheur@unige.ch](mailto:severine.lefaucheur@unige.ch)

**Table S1. Targets and sequences of the primers used to characterise the bacterial communities in biofilms using qPCR and amplicon sequencing**

Molecular tool	Primers	Target	Sequence (5'-3')	References
qPCR	P338f	All bacteria	GCATGGCYGYCGTCAG	[1]
	P518r		CGACGCCATCTTCATTCACAT	
	<i>merAF</i>	Hg resistance	ATTCCAGCTCCAATAGCG	[2]
	<i>merAR</i>		GACTACGATGGTATCTAATC	
	<i>hgcAR</i>	Hg methylation	TCCGTAGGTGAACCTGCGG	[3]
	<i>hgcAR</i>		TCCTCCGCTTATTGATATGC	
Amplicon sequencing	1053F	Universal small subunit ribosomal rRNA gene	GCATGGCYGYCGTCAG	[4]
	1319R		CGACGCCATCTTCATTCACAT	
	D512F	Nuclear small ribosomal subunit 18S	ATTCCAGCTCCAATAGCG	[5]
	D978R		GACTACGATGGTATCTAATC	

**Table S2. Taxonomic ranks of the major microorganisms living in both biofilms (B1 and B2), as well as the number of sequences and their abundance (%) calculated with OTUs assigned to (a) bacteria and (b) microalgae prior (T0) and after 24h (T24) exposure to Hg**

The abundance was calculated by dividing the specific of number of sequences assigned to an OTU to the total number of sequences, multiplied by 100

(a)

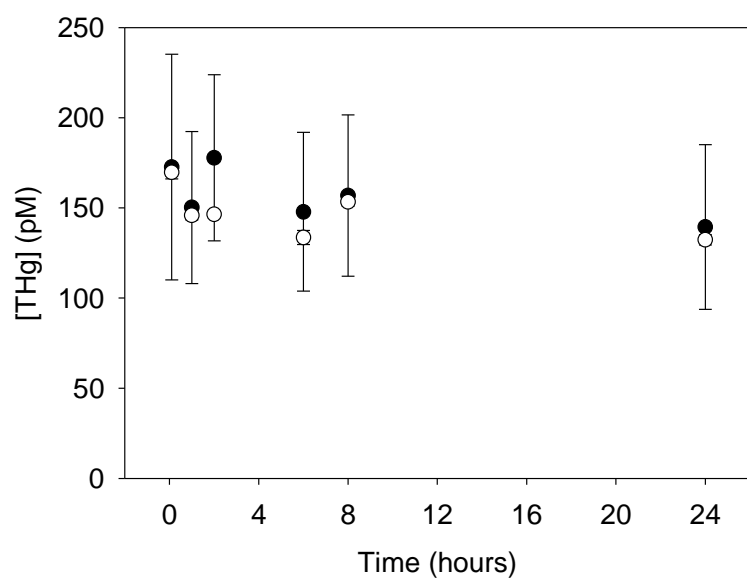
	OTUs						Number of Sequence	Abundance (%)*
	Phylum	Class	Order	Family	Genus	Species		
T0-B1	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Caulobacterales</i>	<i>Caulobacteraceae</i>	<i>Brevundimonas</i>	Unknown	57	1.9
	<i>Cyanobacteria</i>	<i>Cyanophyceae</i>	Unknown	Unknown	Unknown	Unknown	62	2.1
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Erythrobacteraceae</i>	<i>Porphyrobacter</i>	Unknown	67	2.3
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Hyphomonadaceae</i>	<i>Hyphomonas</i>	Unknown	69	2.3
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	Unknown	Unknown	72	2.4
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Sphingomonas</i>	Unknown	86	2.9
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	Unknown	Unknown	Unknown	Unknown	124	4.2
	<i>Proteobacteria</i>	Unknown	Unknown	Unknown	Unknown	Unknown	124	4.2
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	Unknown	Unknown	Unknown	277	9.3
T24-B1	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Hyphomonadaceae</i>	<i>Hyphomonas</i>	Unknown	66	2.3
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Sphingomonas</i>	Unknown	66	2.3
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	Unknown	Unknown	71	2.5
	<i>Cyanobacteria</i>	<i>Cyanophyceae</i>	<i>Chroococcales</i>	<i>Synechococcaceae</i>	<i>Synechococcus</i>	Unknown	83	2.9
	<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	<i>Verrucomicrobiales</i>	<i>Verrucomicrobiaceae</i>	<i>Verrucomicrobium</i>	Unknown	84	2.9
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Rhodobacter</i>	Unknown	86	3.0
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Sphingomonas</i>	Unknown	130	4.5
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	Unknown	Unknown	Unknown	Unknown	135	4.7
	<i>Proteobacteria</i>	Unknown	Unknown	Unknown	Unknown	Unknown	176	6.2
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	Unknown	Unknown	Unknown	260	9.1
T0-B2	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Caulobacterales</i>	<i>Caulobacteraceae</i>	Unknown	Unknown	71	2.3
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	Unknown	Unknown	71	2.3
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Sphingomonas</i>	Unknown	72	2.3
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Caulobacterales</i>	<i>Caulobacteraceae</i>	<i>Brevundimonas</i>	Unknown	97	3.1
	<i>Cyanobacteria</i>	<i>Cyanophyceae</i>	<i>Chroococcales</i>	<i>Synechococcaceae</i>	<i>Synechococcus</i>	Unknown	115	3.7
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Hyphomonadaceae</i>	<i>Hyphomonas</i>	Unknown	134	4.3
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Sphingomonas</i>	Unknown	147	4.7
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	Unknown	Unknown	Unknown	Unknown	176	5.6
	<i>Proteobacteria</i>	Unknown	Unknown	Unknown	Unknown	Unknown	207	6.6
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	Unknown	Unknown	Unknown	284	9.0
T24-B2	<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	<i>Verrucomicrobiales</i>	<i>Verrucomicrobiaceae</i>	<i>Verrucomicrobium</i>	Unknown	63	1.5
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	Unknown	Unknown	Unknown	Unknown	95	2.3
	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Hyphomonadaceae</i>	<i>Hyphomonas</i>	Unknown	115	2.8
	<i>Cyanobacteria</i>	<i>Cyanophyceae</i>	<i>Oscillatoriales</i>	Unknown	Unknown	Unknown	121	2.9
	<i>Proteobacteria</i>	Unknown	Unknown	Unknown	Unknown	Unknown	292	7.1
	<i>Cyanobacteria</i>	<i>Cyanophyceae</i>	Unknown	Unknown	Unknown	Unknown	382	9.3
	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	Unknown	Unknown	Unknown	416	10.1

\* The total number of sequences was 2974 and 2861 for B1, and 3145 and 4116 for B2, at T0 and T24h, respectively.

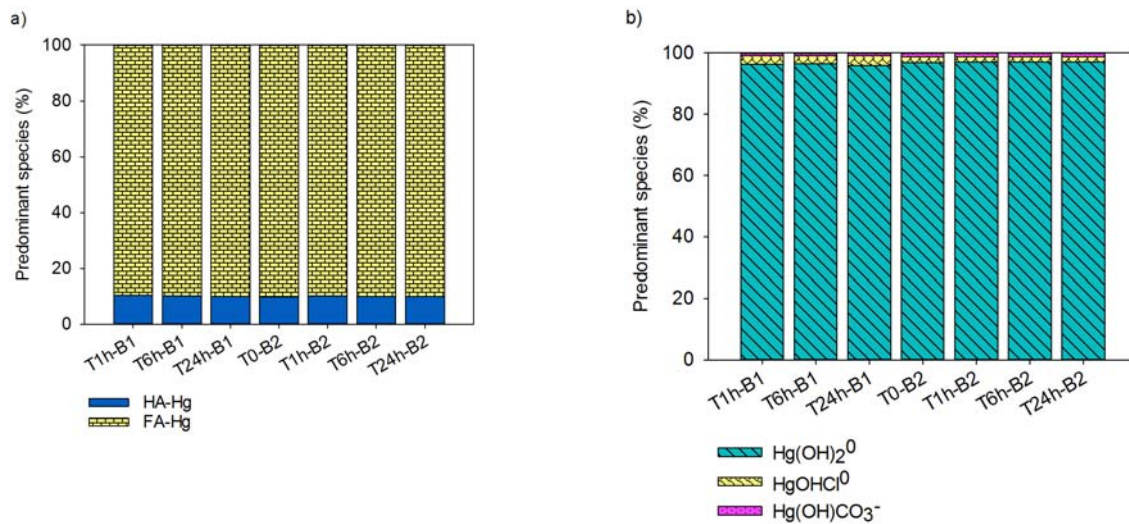
(b)

	OTUs						Number of Sequence	Abundance (%) **
	Phylum	Class	Order	Family	Genus	Species		
T0-B1	Bacillariophyta	Bacillariophyceae	Unknown	Unknown	Unknown	Unknown	76	2.7
	Chlorophyta	Chlorophyceae	Sphaeropleales	Mychonastaceae	Mychonastes	Unknown	84	3.0
	Ochrophyta	Eustigmatophyceae	Eustigmatales	Loboceae	Pseudotetraedriella	Pseudotetraedriellakamillae	95	3.4
	Bacillariophyta	Fragilariophyceae	Fragilariales	Fragilariaceae	Diatoma	Diatomatenuae	100	3.6
	Chlorophyta	Unknown	Unknown	Unknown	Unknown	Unknown	114	4.1
	Bacillariophyta	Bacillariophyceae	Unknown	Unknown	Unknown	Unknown	152	5.5
	Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	Hylodesmus	Hylodesmussingaporensis	187	6.7
	Ochrophyta	Chrysophyceae	Chromulinales	Chromulinaceae	Unknown	Unknown	204	7.3
Chlorophyta	Chlorophyceae	Oedogoniales	Oedogoniaceae	Oedogonium	Unknown	1103	39.7	
T24-B1	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	73	2.8
	Ochrophyta	Eustigmatophyceae	Eustigmatales	Loboceae	Pseudotetraedriella	Pseudotetraedriellakamillae	88	3.3
	Ochrophyta	Chrysophyceae	Chromulinales	Chromulinaceae	Unknown	Unknown	92	3.5
	Chlorophyta	Chlorophyceae	Sphaeropleales	Mychonastaceae	Mychonastes	Unknown	117	4.4
	Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	Hylodesmus	Hylodesmussingaporensis	127	4.8
	Bacillariophyta	Bacillariophyceae	Unknown	Unknown	Unknown	Unknown	148	5.6
	Bacillariophyta	Fragilariophyceae	Fragilariales	Fragilariaceae	Diatoma	Diatomatenuae	232	8.8
	Chlorophyta	Chlorophyceae	Oedogoniales	Oedogoniaceae	Oedogonium	Unknown	290	11.0
Ochrophyta	Chrysophyceae	Chromulinales	Chromulinaceae	Unknown	Unknown	947	35.9	
T0-B2	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	63	1.9
	Bacillariophyta	Bacillariophyceae	Cymbellales	Cymbellaceae	Cymbella	Unknown	92	2.8
	Chlorophyta	Chlorophyceae	Unknown	Unknown	Unknown	Unknown	563	17.4
	Chlorophyta	Chlorophyceae	Oedogoniales	Oedogoniaceae	Oedogonium	Unknown	1141	35.3
Bacillariophyta	Bacillariophyceae	Unknown	Unknown	Unknown	Unknown	1381	42.6	
T24-B2	Bacillariophyta	Coccolithophyceae	Thalassiosirales	Thalassiosiraceae	Stephanodiscus	Unknown	53	2.0
	Ochrophyta	Chrysophyceae	Chromulinales	Chromulinaceae	Unknown	Unknown	53	2.0
	Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	Hylodesmus	Hylodesmussingaporensis	74	2.9
	Ochrophyta	Chrysophyceae	Chromulinales	Chromulinaceae	Unknown	Unknown	195	7.5
	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	280	10.8
	Bacillariophyta	Bacillariophyceae	Cymbellales	Cymbellaceae	Cymbella	Unknown	290	11.2
	Bacillariophyta	Bacillariophyceae	Unknown	Unknown	Unknown	Unknown	417	16.1
	Chlorophyta	Chlorophyceae	Oedogoniales	Oedogoniaceae	Oedogonium	Unknown	756	29.2

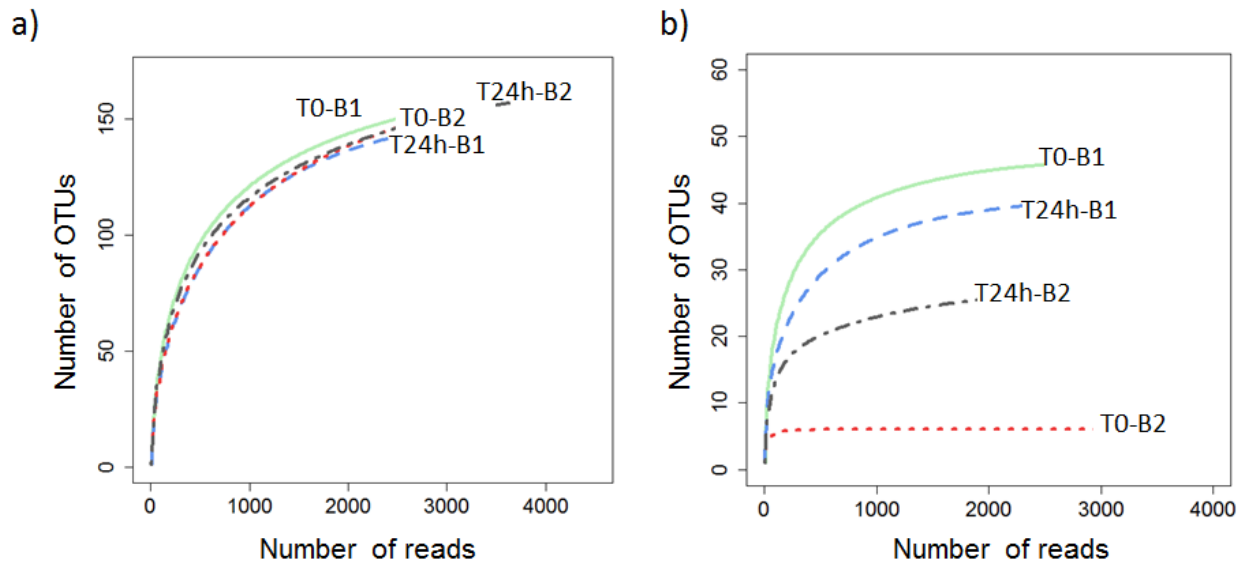
\* The total number of sequences was 2776 and 2639 for B1, and 3240 and 2586 for B2, at T0 and T24h, respectively.



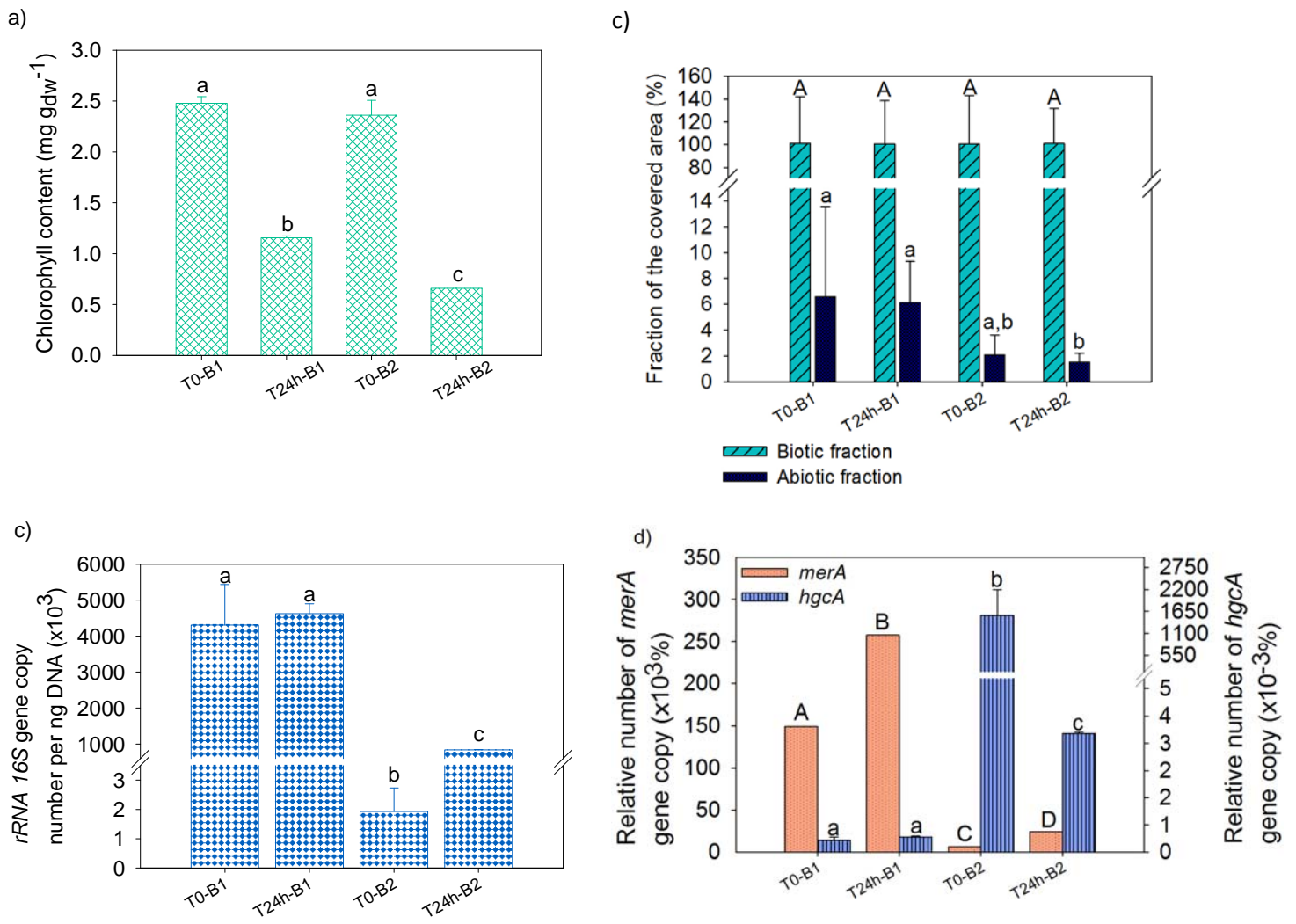
**Fig. S1.** Total Hg concentration [THg] in the media as a function of exposure time for biofilms B1 (filled circle) and B2 (white circle).



**Fig. S2.** Modelled IHg speciation (a) bound to humic (HA) and fulvic (FA) acids, and (b) not bound to DOM.

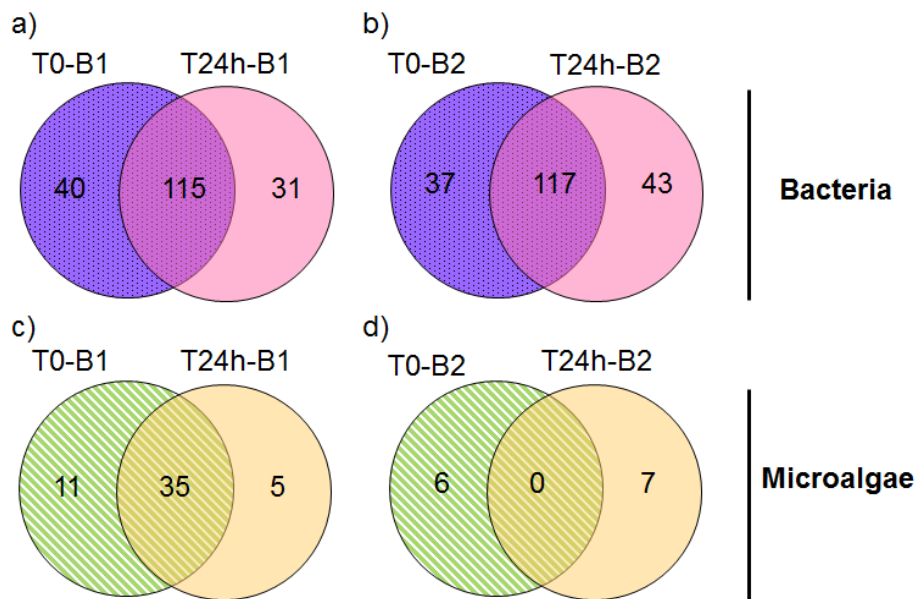


**Fig. S3.** Rarefaction curves from the amplicon sequencing of (a) bacteria and (b) microalgae in the 85-day-old (B1) and 127-day-old (B2) biofilms prior (T0) and after 24 h Hg exposure (T24h).

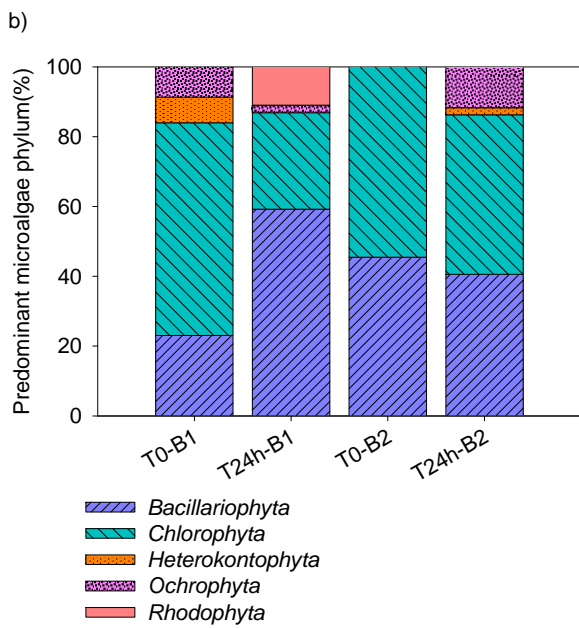
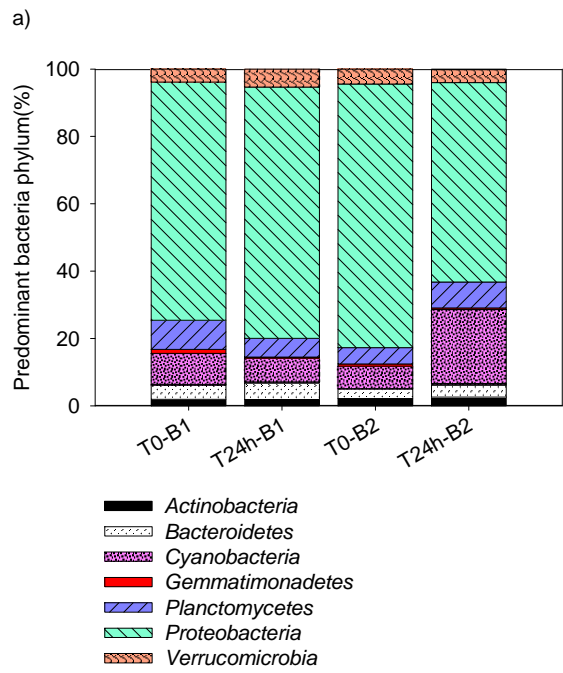


**Fig. S4.** Composition of the 85-day-old (B1) and 127-day-old (B2) biofilms, prior Hg exposure (T0) and after 24 h Hg exposure (T24h) with (a) chlorophyll content ( $n = 3$ ), (b) abiotic and biotic fractions measured with fluorescence microscopy ( $n = 10$ ), (c) *rRNA 16S* gene copy numbers ( $n = 3$ ) and (d) *merA* and *hgcA* gene copy numbers per ng 16S gene copy numbers ( $n = 3$ ). Letters indicate a significant difference in the biofilm characteristics between exposure times obtained with Tukey's HSD test ( $p < 0.05$ ).

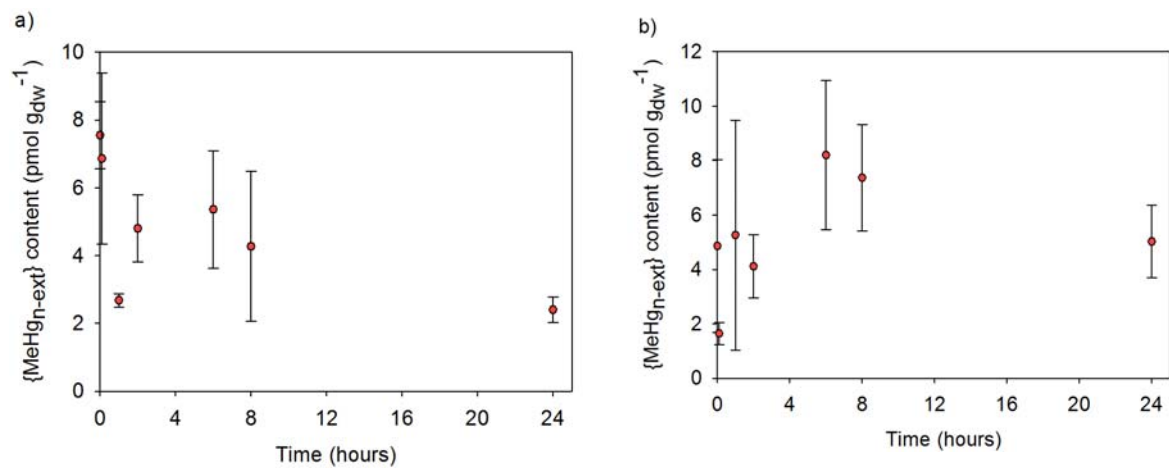




**Fig. S5.** Venn diagram showing the shared and specific OTUs in the 85-day-old (B1) and 127-day-old (B2) biofilms prior (T0) and after 24 h Hg exposure (T24h) for (a) bacteria in B1, (b) bacteria in B2, (c) microalgae in B1 and (d) microalgae in B2.



**Fig. S6.** Composition of (a) the bacterial and (b) the microalgal communities in the 85-day-old (B1) and 127-day-old (B2) biofilms prior (T0) and after 24 h Hg exposure (T24h).



**Fig. S7.** Concentrations of non-extractable methylmercury  $\{MeHg\}_{n-ext}$  as a function of time in (a) 85-day-old (B1) and (b) 127-day-old (B2) biofilms.

## References

- [1] S. El Fantroussi, L. Verschuere, W. Verstraete, E.M. Top, Effect of phenylurea herbicides on soil microbial communities estimated by analysis of *16S rRNA* gene fingerprints and community-level physiological profiles, *Appl. Environ. Microb.* **1999**, *65*, 982.
- [2] J.K. Schaefer, J. Yagi, J.R. Reinfelder, T. Cardona, K.M. Ellickson, S. Tel-Or, T. Barkey, Role of the bacterial organomercury lyase (MerB) in controlling methylmercury accumulation in mercury-contaminated natural waters, *Environ. Sci. Technol.* **2004**, *38*, 4304.
- [3] J.K. Schaefer, R.M. Kronberg, F.M.M. Morel, U. Skyllberg, Detection of a key Hg methylation gene, *hgcA*, in wetland soils, *Env. Microbiol. Rep.* **2014**, *6*, 441.
- [4] G. Bicheux, L. Morin, G. Le Moal, G. Coffe, D. Balestrino, N. Charbonnel, J. Bohatier, C. Forestier, Pyrosequencing assessment of prokaryotic and eukaryotic diversity in biofilm communities from a French river, *Microbiologyopen.* **2013**, *2*, 402.
- [5] J. Zimmermann, R. Jahn, B. Gemeinholzer, Barcoding diatoms: evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols, *Org. Divers. Evol.* **2011**, *11*, 173.