Electronic Supplementary Information

Long-term exposure to silver nanoparticles affects periphyton community structure and function

Carmen Gil-Allué [a,b], Ahmed Tlili [a,*], Kristin Schirmer [a,b,d], Mark O. Gessner [c,e], and Renata Behra[a]

[a] Department of Environmental Toxicology, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

[b] School of Architecture, Civil and Environmental Engineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

[c] Department of Experimental Limnology, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Stechlin, Germany

[d] Department of Environmental Science, ETH Zurich, Zurich, Switzerland

[e] Department of Ecology, Berlin Institute of Technology (TU Berlin), Berlin, Germany

[*] Corresponding author: Ahmed Tlili

Address: Department of Environmental Toxicology, Eawag

Überlandstrasse 133, P.O. Box 611. 8600 Dübendorf, Switzerland

Phone: + 41 58 765 5330

Email: ahmed.tlili@eawag.ch

Content

Table S1. Composition of the exposure medium PERIQUIL [1]

Component	Concentration (mM)
Salts	
$CaCl_2$	0.20
$Ca(NO_3)_2$	0.10
$MgSO_4$	0.15
$NaHCO_3$	1.20
KNO_3	0.10
Na_2SiO_3	0.05
Nutrients	
K_2HPO_4	5.00×10^{-3}
NH_4NO_3	0.10
Trace elements	
$CoCl_2$	5.00×10^{-5}
H_3BO_3	0.05
Na_2MoO_4	8.00×10^{-5}
$CuSO_4$	1.63×10^{-4}
$MnCl_2$	1.22×10^{-3}
$ZnSO_4$	1.58×10^{-4}
$FeCl_3$	9.00×10^{-4}
Metal ligand	
Na_2 EDTA	0.02
Buffer	
MOPS (pH 7.5)	10.00
NaOH	7.00

Table S2. Water chemistry of exposure medium sampled weekly during 21 days of exposures from all microcosms after medium exchange. * Nominal concentrations lower than those measured are likely due to the precipitation of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) and quartz (SiO_4) as predicted by the software Visual MINTEQ v3.0 Beta (http://vminteq.lwr.kth.se/).

Time (days)	Treatment	pH	Ca (mg L^{-1})	Cl (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Na (mg L^{-1})	Total N (mg N L^{-1})	NO_3^- (mg N L^{-1})	PO_4^{-3} (µg L^{-1})	H_4SiO_4 (mg L^{-1})	SO_4^{-2} (mg L^{-1})
	Control (intended)	7.5	12	14.4	4.3	3.6	192	147	5.6	475	11.2	14.4
0	Control	7.7	13.2	15.2	4.7	4.4	195	143	5.8	160*	5.3*	16.9
7	Control	8.0 ± 0.1	29.7 ± 3.4	15.1 ± 0.2	4.8 ± 0.3	4.4 ± 0.1	197 ± 3	140 ± 1	4.7 ± 0.7	3.0 ± 0.7	ND	16.3 ± 0.1
	AgNP	8.0 ± 0.1	33.4 ± 3.4	15.1 ± 0.1	4.7 ± 0.2	4.6 ± 0.1	197 ± 2	140 ± 1	5.1 ± 0.4	3.1 ± 1.4	ND	15.4 ± 0.2
	$AgNO_3$	8.0 ± 0.1	35.0 ± 5.3	15.1 ± 0.2	4.6 ± 0.1	4.6 ± 0.1	196 ± 3	140 ± 1	4.6 ± 0.9	3.3 ± 2.4	ND	15.9 ± 0.5
14	Control	7.8 ± 0.1	13.4 ± 0.3	15.2 ± 0.1	4.3 ± 0.1	4.5 ± 0.1	187 ± 1	157 ± 21	3.8 ± 1.0	3.5 ± 0.7	ND	15.0 ± 0.7
	AgNP	7.8 ± 0.1	14.6 ± 1.4	15.1 ± 0.1	4.2 ± 0.1	4.5 ± 0.1	187 ± 1	141 ± 1	3.8 ± 0.8	3.3 ± 0.8	ND	13.9 ± 0.6
	$AgNO_3$	7.8 ± 0.1	13.7 ± 0.7	15.2 ± 0.1	4.4 ± 0.1	4.6 ± 0.1	188 ± 1	139 ± 4	3.9 ± 1.5	2.3 ± 1.4	ND	15.3 ± 0.9
21	Control	7.7 ± 0.1	12.6 ± 0.2	14.8 ± 0.1	5.0 ± 0.6	4.4 ± 0.1	185 ± 2	140 ± 2	3.5 ± 0.8	2.7 ± 2.1	ND	15.8 ± 1.1
	AgNP	7.8 ± 0.1	12.6 ± 0.2	14.7 ± 0.2	4.6 ± 0.1	4.5 ± 0.1	185 ± 1	141 ± 1	2.9 ± 0.8	2.1 ± 0.2	ND	12.7 ± 0.6
	$AgNO_3$	7.8 ± 0.1	12.5 ± 0.2	14.6 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	185 ± 1	138 ± 1	2.4 ± 1.6	3.9 ± 1.9	ND	14.2 ± 0.7

Table S3. Primer sequences used to amplify conserved 16S and 18S rDNA fragments for DGGE analyses.

Primer		Sequence	Reference
18S	Euk1Af	5'−CTGGTTGATCCTGCCAG−3'	[2]
	Euk516r-GC	5'−GCCCGGGGCGCGCCCCGGGCG GGGCGGGGGCACGGGGGGACCA GACTTGCCCTCC−3'	[3]
16S	341f-GC	5'−CGCCCGCCGCGCGCGGCGGGC GGGGCGGGGGCACGGGGGGCCT ACGGGAGGCAGCAG−3'	[4]
	907rM	5'−CCGTCAATTCMTTTGAGTTT−3'	[5]

Table S4. Speciation of silver in the range of AgNP and $AgNO_3$ concentrations used in the exposure medium, as calculated by Visual MINTEQ v3.0 Beta. Results show constant silver speciation within this range.

	Silver concentrations	
Silver species	**0.1 µM**	**10 µM**
Ag^+	52.35 %	52.96 %
AgCl (aq)	44.55 %	43.99 %
$AgCl_2^-$	1.66 %	1.60 %
$AgNH_3^+$	1.15 %	1.16 %
$Ag(NH_3)_2^+$	0.12 %	0.12 %
$AgNO_3$ (aq)	0.06 %	0.06 %
$AgSO_4^-$	0.10 %	0.10 %

Table S5. Concentration of chlorophyll *a*, chlorophyll *b*, fucoxanthin and lutein in periphyton exposed to silver. Four additional unidentified pigments (UP) were UP 1, which is similar to chlorophyll *c*, and UP 2 to 4, which are xanthophylls. ND = not detected. Data are means ± standard deviations (N = 3 microcosms).

		Proportion (%)							
Treatment	**Sampling day**	**Chlorophyll *a***	**Chlorophyll *b***	**Fucoxanthin**	**Lutein**	**UP 1**	**UP 2**	**UP 3**	**UP 4**
Control	7	37.6 ± 1.2	21.4 ± 2.3	36.1 ± 5.5	4.9 ± 4.3	ND	ND	ND	ND
	21	44.3 ± 8.3	12.3 ± 3.1	17.5 ± 1.6	5.8 ± 3.5	9.9 ± 0.7	4 ± 1.1	4.7 ± 0.7	4.8 ± 0
0.1 µM AgNP	7	42.9 ± 3.4	14.2 ± 12.4	37.8 ± 8.5	5 ± 0.7	ND	ND	ND	ND
	21	38.3 ± 2.1	13.9 ± 1.6	17.7 ± 3.7	6.2 ± 1.6	9.2 ± 1.2	4.1 ± 0.5	5.5 ± 1.3	5.1 ± 0.2
1 µM AgNP	7	38.8 ± 0.9	19.9 ± 1.8	39 ± 0.6	2.3 ± 3.3	ND	ND	ND	ND
	21	35.4 ± 1.6	18.9 ± 4.3	15.2 ± 5.3	8.1 ± 2.5	6.5 ± 2.4	3.6 ± 0.1	7.9 ± 2.1	3.9 ± 1.1
10 µM AgNP	7	41.5 ± 1.2	28.7 ± 2.9	26 ± 4.3	3.9 ± 0.2	ND	ND	ND	ND
	21	41.4 ± 3.3	19.9 ± 4.3	9.6 ± 1	9.1 ± 1.9	3.2 ± 0.6	3.9 ± 0.7	9.9 ± 1	2.9 ± 0.2
0.1 µM $AgNO_3$	7	41.1 ± 1.5	21.1 ± 2.3	32.8 ± 4.2	5 ± 0.6	ND	ND	ND	ND
	21	35.1 ± 4.6	13.6 ± 0.5	16.7 ± 0.7	7.8 ± 2.8	9.2 ± 0.3	6.2 ± 0.5	6.1 ± 0.7	5.2 ± 0.1

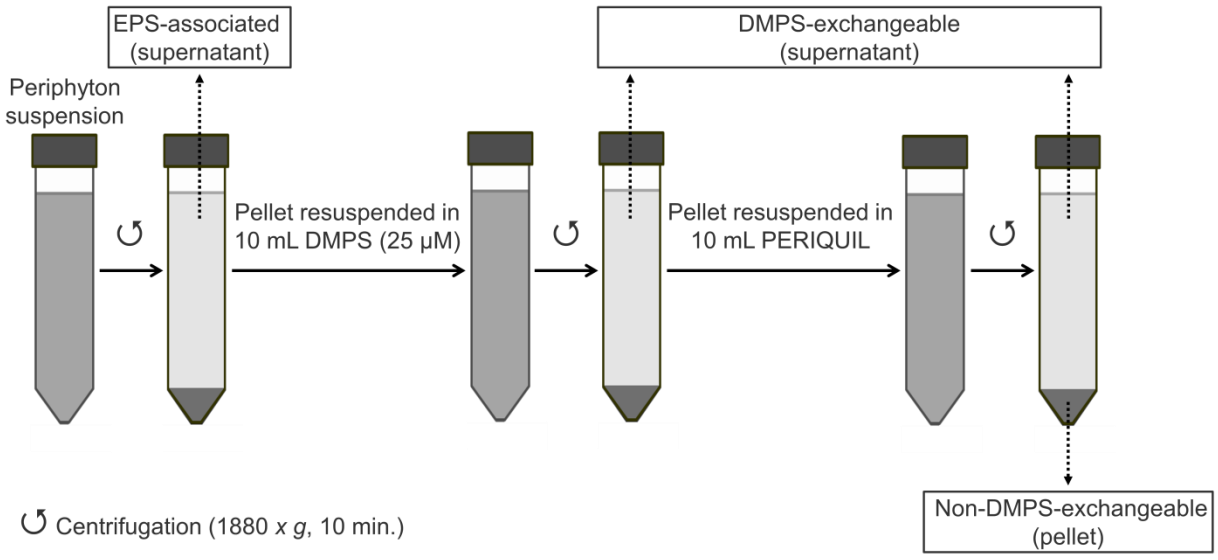

Figure S1. Diagram of the periphyton fractionation procedure to quantify the distribution of silver in EPS-associated (loosely sorbed), DMPS-exchangeable (strongly sorbed to periphyton), and non-DMPS-exchangeable (strongly associated or internalized in periphyton biomass) fractions.

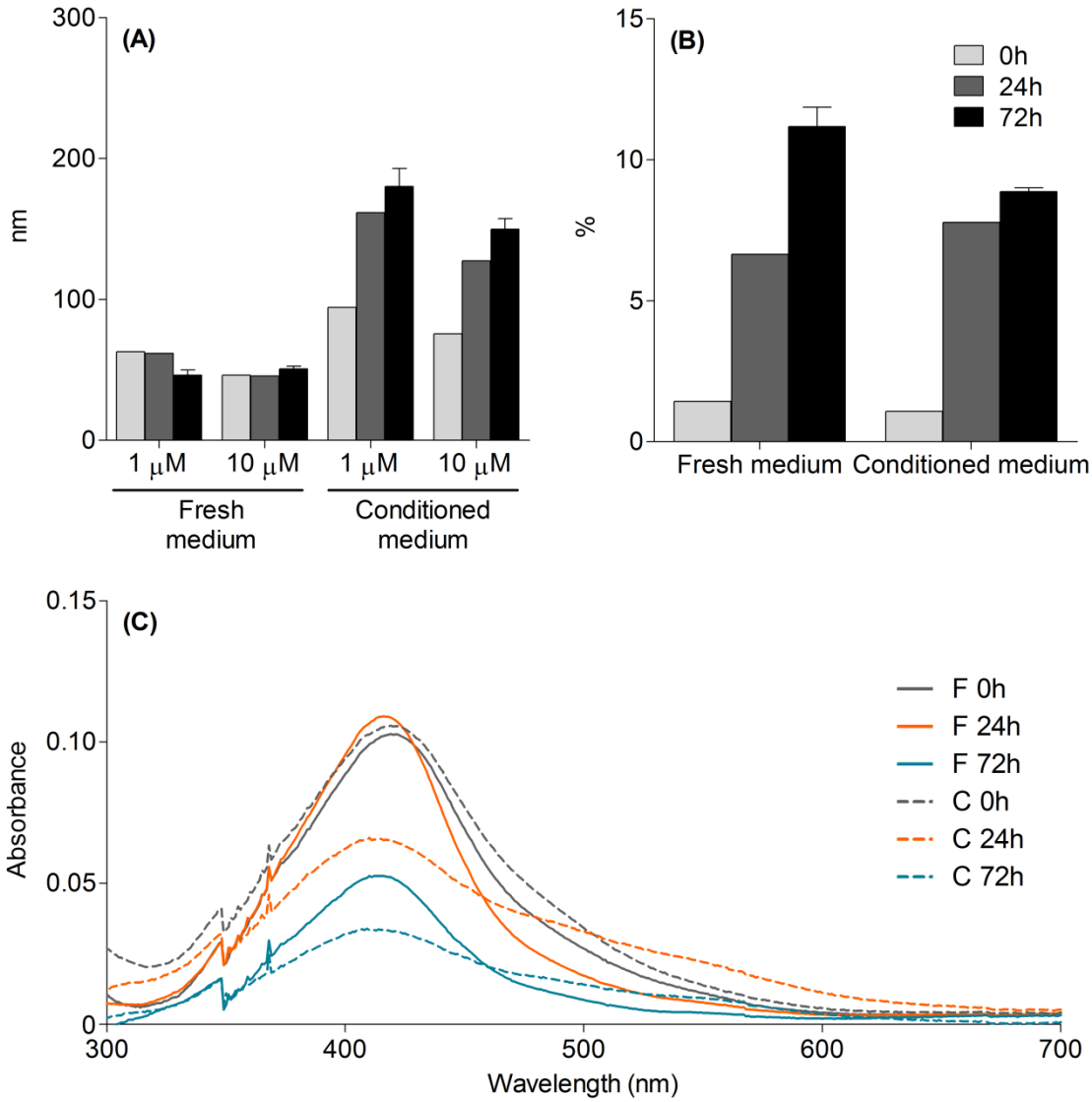

Figure S2. AgNP size distributions (A) measured as the hydrodynamic diameter by nanoparticle tracking analysis (NTA) of 1 and 10 µM AgNP suspensions, and percentage of dissolved Ag(I) (B) measured by ultrafiltration in 10 µM AgNP suspensions in fresh and conditioned medium after 0, 24, and 72 hours of exposure. The optical properties of 10 µM AgNP suspensions (C) were determined from their UV-VIS absorbance spectra in fresh (F) and conditioned (C) medium after 0, 24 and 72 hours of exposure.

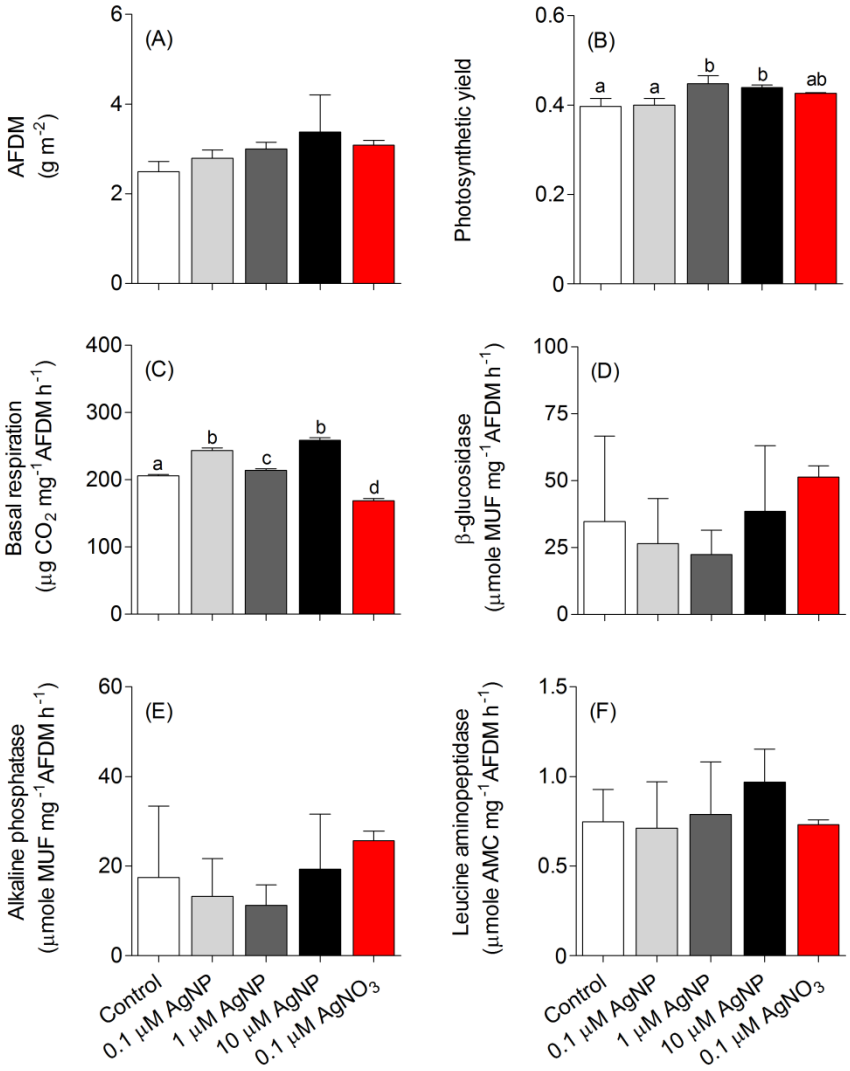

Figure S3. Effects of 0.1, 1 and 10 µM AgNP and 0.1 µM $AgNO_3$ on periphyton ash-free dry mass (AFDM) (A), photosynthetic yield (B), basal respiration (C), and the extracellular enzymes β-glucosidase (D), alkaline phosphatase (E) and leucine aminopeptidase (F), after 7 days of exposure. Different letters above the bars denote significant difference (N = 3; $p < 0.05$, Tukey's test)

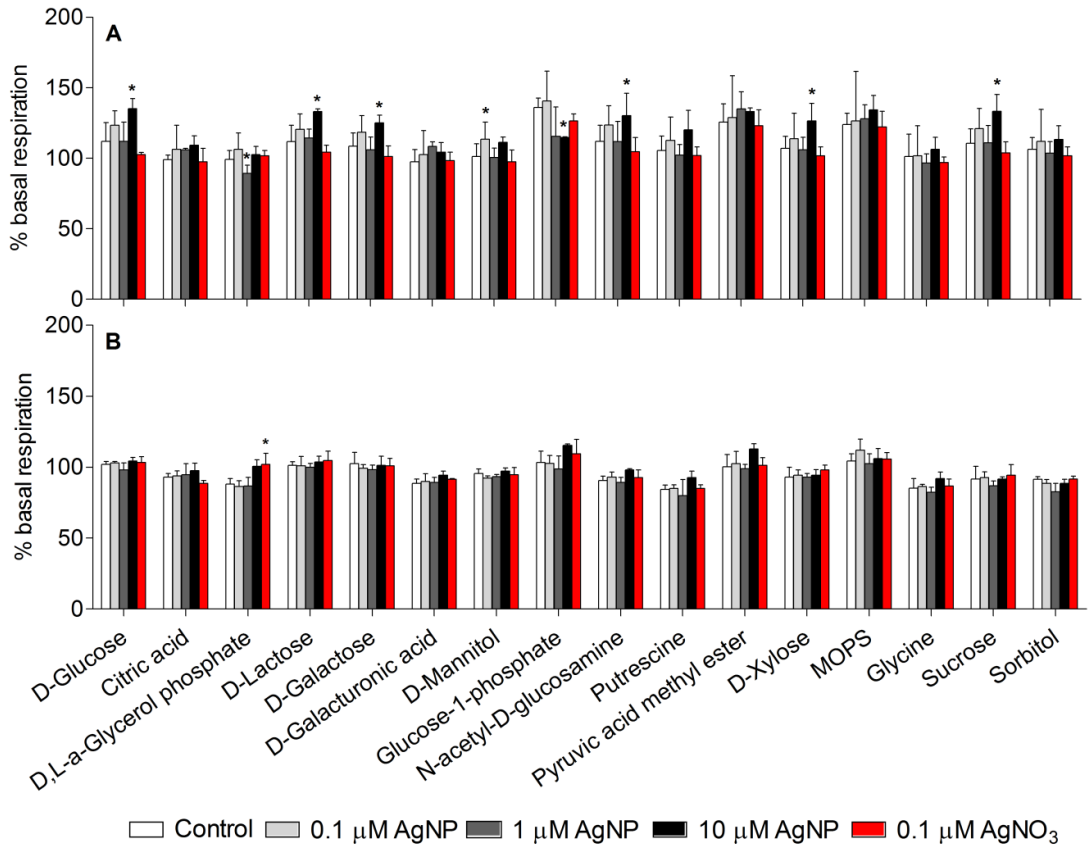

Figure S4. Community-level physiological profiles (CLPP) of periphyton determined by substrate-induced respiration (SIR) after 7 (A) and 21 (B) days of exposure to 0.1, 1 or 10 µM of AgNP, or to 0.1 µM of $AgNO_3$. Data are means ± standard deviations (N= 3 microcosms). Asterisks above the bars indicate significant difference from the control ($p < 0.05$; Tukey's test) for each carbon substrate tested.

References

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