Nanosilver impacts on aquatic microbial decomposers and litter decomposition assessed as pollution-induced community tolerance (PICT)

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This Supplementary Information section is 8 pages long and contains five supplemental tables and one supplemental figure.

SUPPORTING INFORMATION

Table S1 - Physical and chemical characteristics of stream water collected in mid-September at Kleine Lonau, a third-order softwater stream in the Harz Mountains, Germany, where leaf litter was deployed for microbial colonization.

Parameter	Value
pH	7.2
O_2 (mg L^{-1})	8.8
NO_3^- (mg N L^{-1})	0.67
NO_2^- (μg N L^{-1})	2
NH_4^+ (μg N L^{-1})	22
PO_4^{3-} (μg P L^{-1})	5

Table S2 - Physical and chemical characteristics of water collected from microcosms when renewing medium every 5th day for 25 days of exposure to AgNPs (NP) or AgNO₃ (Ag⁺).

Treatment (µg L⁻¹)	Day	N-NO₂⁻ (mg L⁻¹)	N-NO₃⁻ (mg L⁻¹)	N-NH₄⁺ (mg L⁻¹)	TN (mg L⁻¹)	SRP (mg L⁻¹)	TP (mg L⁻¹)	DOC (mg L⁻¹)	Oxygen (mg L⁻¹)	pH
0		0.015	0.020	0.057	1.87	0.022	0.129	54.3	6.5	5.95
50 NP		0.000	0.014	0.052	1.91	0.012	0.105	52.9	6.7	6.14
100 NP	0	0.000	0.014	0.045	1.55	0.013	0.059	55.5	6.4	6.52
200 NP		0.000	0.013	0.041	1.98	0.010	0.060	54.2	6.2	6.55
20 Ag⁺		0.000	0.000	0.045	1.33	0.014	0.050	51.4	5.7	6.65
0		0.005	0.006	0.020	1.16	0.013	0.064	19.2	7.9	6.49
50 NP		0.005	0.006	0.021	1.38	0.015	0.080	19.3	8.1	6.62
100 NP	5	0.005	0.006	0.022	1.29	0.017	0.091	19.8	7.8	6.66
200 NP		0.006	0.009	0.049	1.26	0.018	0.101	20.5	7.7	6.69
20 Ag⁺		0.005	0.006	0.021	1.18	0.016	0.077	17.4	8.0	6.67
0		0.004	0.011	0.044	0.97	0.011	0.063	14.2	7.9	6.49
50 NP		0.003	0.005	0.042	1.07	0.012	0.069	15.6	8.1	6.62
100 NP	10	0.004	0.011	0.052	1.30	0.011	0.068	15.5	7.8	6.66
200 NP		0.004	0.013	0.056	1.11	0.013	0.075	16.3	7.7	6.69
20 Ag⁺		0.005	0.203	0.047	0.96	0.011	0.080	14.2	8.0	6.67
0		0.004	0.007	0.046	1.44	0.012	0.060	13.6	7.4	6.60
50 NP		0.004	0.012	0.042	1.01	0.012	0.061	14.0	7.4	6.56
100 NP	15	0.004	0.004	0.028	0.98	0.011	0.066	13.3	8.4	6.62
200 NP		0.004	0.035	0.068	1.07	0.013	0.065	14.4	7.5	6.67
20 Ag⁺		0.004	0.035	0.022	1.01	0.011	0.071	13.1	8.0	6.79
0	20	0.003	0.016	0.042	0.83	0.010	0.077	13.5	7.5	6.52

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50 NP		0.003	0.024	0.025	0.75	0.008	0.051	14.3	6.8	6.58
100 NP		0.003	0.271	0.076	0.76	0.010	0.079	13.5	7.5	6.64
200 NP		0.004	0.017	0.041	0.76	0.007	0.060	14.0	6.8	6.66
20 Ag$^+$		0.003	0.017	0.033	0.82	0.012	0.084	15.6	7.5	6.73
0		0.006	0.026	0.196	2.47	0.006	0.181	11.3	7.3	7.38
50 NP		0.013	0.208	0.112	2.13	0.006	0.176	14.4	7.0	7.35
100 NP	25	0.010	0.149	0.106	1.80	0.009	0.131	15.2	7.5	7.35
200 NP		0.012	0.305	0.051	1.41	0.005	0.039	32.9	7.4	7.36
20 Ag$^+$		0.009	0.130	0.042	1.44	0.007	0.069	40.8	7.0	7.37

Table S3 - Percent contribution of fungal taxa to total conidial production on leaves in the inoculum and after exposure for 25 days in microcosms to AgNPs (NP) at different concentrations ($\mu g\ L^{-1}$) or to $AgNO_3$ (Ag^+, $\mu g\ L^{-1}$). -, not detected

Fungal taxon	Inoculum	0	50 NP	100 NP	200 NP	20 Ag+
Alatospora acuminata	0.43	-	0.87	0.70	1.03	0.99
Alatospora pulchella	-	0.26	-	-	-	0.25
Anguillospora crassa	0.36	-	-	-	-	-
Anguillospora filiformis	0.21	0.43	-	-	-	-
Articulospora tetracladia	0.78	2.09	2.83	3.95	4.62	9.16
Clavariopsis aquatica	0.92	-	0.89	3.74	5.26	1.30
Cylindrocarpon sp.	0.14	0.28	0.20	-	0.36	0.24
Dimorphospora foliicola	0.85	-	-	-	-	-
Flagellospora curvula	70.2	80.9	86.5	84.3	77.6	77.1
Fusarium sp.	0.71	-	-	-	-	-
Heliscus lugdunensis	1.14	-	0.28	0.19	0.21	0.23
Lemonniera aquatica	0.28	0.53	0.10	0.30	0.10	0.76
Tetrachaetum elegans	23.5	5.52	1.64	4.49	3.17	2.92
Tetracladium marchalianum	0.36	9.98	6.65	2.40	5.67	7.04
Tricladium chaetocladium	0.14	-	-	-	-	-
Total no. of species	14	8	9	8	9	10

Table S4 - ANOVA results from the effects of AgNPs or ionic Ag on leaf mass loss, fungal sporulation rate, ergosterol concentration, bacterial production, bacterial abundance, microbial respiration and LAP activity on decomposing leaves (one-way ANOVA). Effect size was calculated between groups, where η^2 (Eta squared) = Treatment

RESPONSE VARIABLE	SOURCE OF VARIATION	SS	DF	MS	F	P	Effect size (%)
Leaf mass loss	Treatment	244.6	4	61.15	2.85	0.061	43.2
	Residual	322.0	15	21.47			
	Total	566.7	19				
Fungal Sporulation rate	Treatment	5.53×10^{10}	4	1×10^{10}	13.72	<0.0001	78.5
	Residual	1.51×10^{10}	15	1×10^{9}			
	Total	7.04×10^{10}	19				
Ergosterol concentration	Treatment	34242	4	8561.0	10.71	0.0003	74.1
	Residual	11991	15	799.4			
	Total	46233	19				
Bacterial production	Treatment	3.482	4	0.8705	4.22	0.017	52.9
	Residual	3.093	15	0.2062			
	Total	6.575	19				
Bacterial abundance	Treatment	4.77×10^{21}	4	1×10^{21}	8.29	0.001	68.9
	Residual	2.16×10^{21}	15	1×10^{20}			
	Total	6.92×10^{21}	19				
Microbial respiration	Treatment	73988	4	18497	7.05	0.002	65.3
	Residual	39352	15	2623			
	Total	113340	19				
LAP activity	Treatment	22.62	4	5.656	1.67	0.209	30.1
	Residual	50.92	15	3.395			
	Total	73.55	19				

Sum of Squares/Total Sum of Squares.
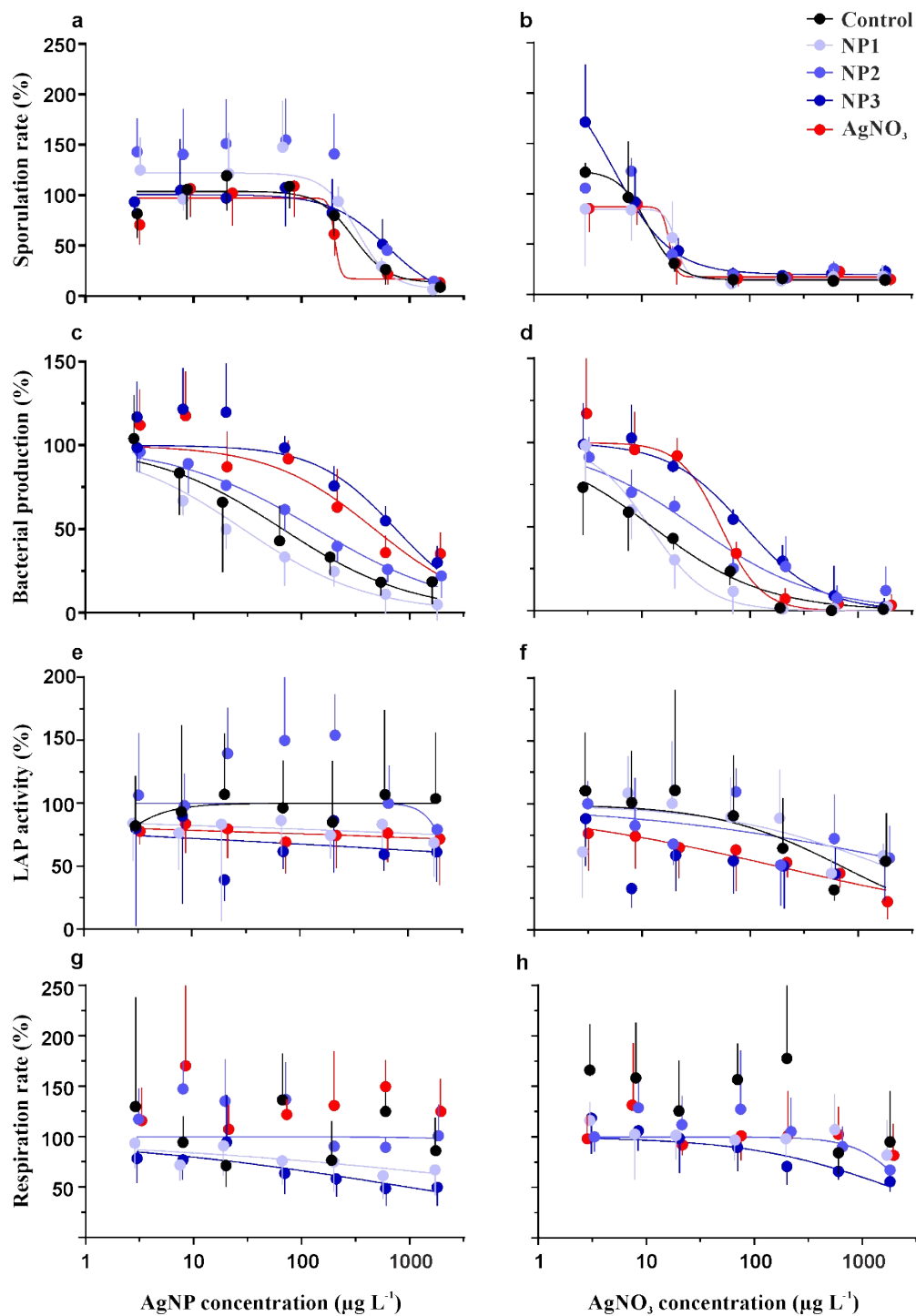
Figure S1 - Dose-response curves for short-term exposure (12 h) to AgNPs (a, c, e, g) or AgNO$_3$ (b, d, f, h), showing responses of fungal sporulation (a, b), bacterial production (c, d), enzyme activity (e, f) and microbial respiration (g, h) associated with decomposing leaf litter in microcosms. Control = 0 µg L^{-1}, NP1 = 50 µg L^{-1}; NP2 = 100 µg L^{-1}; NP3 = 200 µg L^{-1}, and AgNO$_3$ = 20 µg L^{-1}.

Table S5 – R^2 values for the fit of each dose-response curve for bacterial production, fungal sporulation rate and potential LAP activity in control communities and pre-exposed communities to AgNP (NP1 = 50 µg L^{-1}; NP2 = 100 µg L^{-1}; NP3 = 200 µg L^{-1}) or $AgNO_3$ (20 µg L^{-1}), obtained via in short-term bioassays with increasing concentrations of AgNP or $AgNO_3$.

Communities	Bacterial production		Sporulation rate		Potential LAP activity	
	AgNPs	$AgNO_3$	AgNPs	$AgNO_3$	AgNPs	$AgNO_3$
Control	0.66	0.82	0.73	0.83	-	0.30
NP1	0.85	0.88	0.74	0.61	-	0.23
NP2	0.87	0.86	0.75	0.96	-	0.21
NP3	0.68	0.90	0.61	0.85	-	-
AgNO₃	0.68	0.84	0.69	0.85	-	0.44