Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information

Silver nanoparticles alter bacterial assembly and antibiotic resistome in biofilm during colonization

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I. Texts:

Text S1. Determination of the physicochemical properties of culturing water

The concentrations of total Ag and dissolved Ag in the culturing water during the exposure period were analyzed as previously described.[1, 2] Briefly, total Ag concentrations in water samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) following acid digestion in 0.15 M HNO_3. Dissolved Ag in water samples were determined using ICP-MS after the ultrafiltration through a 3-kDa centrifugal filter (Amicon Ultra-15 3 kDa, Millipore). In addition, the water quality physicochemical parameters including dissolved oxygen (DO), conductivity, oxidation-reduction potential (ORP), pH, salinity, and total dissolved solids (TDS) were analyzed with a portable water analyzer (YSI Professional Plus meter, Yellow Springs, Ohio, USA). Total nitrogen (TN), total phosphorous (TP), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), and Chlorophyll a (Chla) in water samples were determined according to the standard methods.[3]

Text S2. The temporal dynamics of physicochemical properties of culturing water

The water physicochemical parameters during the exposure time (day 7 to day 35) in different treatments are summarized in **Table S8**. The measured total Ag concentrations in each microcosm were close to the respective nominal concentration on the initial day (**Table S8**). Not surprisingly, total Ag and dissolved Ag were significantly higher in Ag dosed groups than the control throughout the whole exposure period. Notably, the total Ag concentrations in 0.1 mg/L AgNPs dosed microcosms maintained relatively stable and close to the nominal concentration during 0 to 21 days, suggesting the high stability of AgNPs in exposure water. However, we observed a sharp decrease of Ag concentration in 0.1 mg/L AgNPs dosed water on day 35, as well as decreasing trends of total Ag concentration in other Ag treatments, we suppose the growth of bacteria in microcosms might account for this phenomenon, for bacteria might assimilate or adsorb more AgNPs/dissolved Ag from water with the enrichment of bacteria.

The water parameters including salinity, conductivity, TDS, TN, NO_3^--N, NH_4^+-N, and TP showed clear decreasing trends in all treatments during the exposure period. In contrast, the concentration of Chla in water for each treatment generally increased over time. Notably, the nutrient parameters such as TN, NO_3^--N, NH_4^+-N, and TP were generally lower in control group than in the Ag exposed groups, whereas, Chla concentrations were usually higher in control group than in the Ag exposed groups, especially at the early or middle stages. In addition, water parameters such as DO, pH and ORP were also found to be significantly influenced by Ag exposure in the present study. These results indicated that the presence of AgNPs could significantly affect the water quality during biofilm formation.

II. Tables:

Table S1. Basic physicochemical properties of source water.

Parameter	**Value**
Temperature (℃)	7.6 ± 0.1
Dissolved Oxygen (mg/L)	10.48 ± 1.22
pH	8.25 ± 0.05
Oxidation-Reduction Potential (mV)	311.7 ± 12.4
Salinity (%)	0.016 ± 0.001
Conductivity (μs/cm)	219.4 ± 0.4
Total dissolved solids (mg/L)	215.2 ± 2.9
Total nitrogen (mg/L)	1.67 ± 0.13
Total phosphorous (mg/L)	0.08 ± 0.01
Ammonia nitrogen (mg/L)	0.60 ± 0.09
Nitrate nitrogen (mg/L)	0.48 ± 0.07
Nitrite nitrogen (mg/L)	0.03 ± 0.01
Chla (μg/L)	15.1 ± 0.8

Table S2. Primers for antibiotic resistance genes, mobile genetic elements and 16S rRNA gene sequence used in this study.

Gene Name	**Forward Primer**	**Reverse Primer**	**Classification**	**Mechanism**
16S rRNA	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG		
aadA1	AGCTAAGCGCGAACTGCAAT	TGGCTCGAAGATACCTGCAA	Aminoglycoside	antibiotic deactivate
aadA5-01	ATCACGATCTTGCGATTTTGCT	CTGCGGATGGGCCTAGAAG	Aminoglycoside	antibiotic deactivate
aadD	CCGACAACATTTCTACCATCCTT	ACCGAAGCGCTCGTCGTATA	Aminoglycoside	antibiotic deactivate
strA	CCGGTGGCATTTGAGAAAAA	GTGGCTCAACCTGCGAAAAG	Aminoglycoside	antibiotic deactivate
strB	GCTCGGTCGTGAGAACAATCT	CAATTTCGGTCGCCTGGTAGT	Aminoglycoside	antibiotic deactivate
aph6ia	CCCATCCCATGTGTAAGGAAA	GCCACCGCTTCTGCTGTAC	Aminoglycoside	antibiotic deactivate
ampC/blaDHA	TGGCCGCAGCAGAAAGA	CCGTTTTATGCACCCAGGAA	Beta_Lactamase	antibiotic deactivate
bla1	GCAAGTTGAAGCGAAAGAAAAGA	TACCAGTATCAATCGCATATACACCTAA	Beta_Lactamase	antibiotic deactivate
blaCTX-M-01	GGAGGCGTGACGGCTTTT	TTCAGTGCGATCCAGACGAA	Beta_Lactamase	antibiotic deactivate
blaSHV-01	TCCCATGATGAGCACCTTTAAA	TTCGTCACCGGCATCCA	Beta_Lactamase	antibiotic deactivate
mphA-01	CTGACGCGCTCCGTGTT	GGTGGTGCATGGCGATCT	MLSB	antibiotic deactivate
mphB	CGCAGCGCTTGATCTTGTAG	TTACTGCATCCATACGCTGCTT	MLSB	antibiotic deactivate
vatE-01	GGTGCCATTATCGGAGCAAAT	TTGGATTGCCACCGACAAT	MLSB	antibiotic deactivate
ermB	TAAAGGGCATTTAACGACGAAACT	TTTATACCTCTGTTTGTTAGGGAATTGAA	MLSB	cellular protection
ermC	TTTGAAATCGGCTCAGGAAAA	ATGGTCTATTTCAATGGCAGTTACG	MLSB	cellular protection
ermJ/ermD	GGACTCGGCAATGGTCAGAA	CCCCGAAACGCAATATAATGTT	MLSB	cellular protection
sul1	CAGCGCTATGCGCTCAAG	ATCCCGCTGCGCTGAGT	Sulfonamide	cellular protection
sul2	TCATCTGCCAAACTCGTCGTTA	GTCAAAGAACGCCGCAATGT	Sulfonamide	cellular protection
vanSB	GCGCGGCAAATGACAAC	TTTGCCATTTTATTCGCACTGT	Vancomycin	cellular protection
vanXD	TAAACCGTGTTATGGGAACGAA	GCGATAGCCGTCCCATAAGA	Vancomycin	cellular protection
tetO-01	ATGTGGATACTACAACGCATGAGATT	TGCCTCCACATGATATTTTTCCT	Tetracycline	cellular protection

tetQ	CGCCTCAGAAGTAAGTTCATACACTAAG	TCGTTCATGCGGATATTATCAGAAT	Tetracycline	cellular protection
tetA-01	GCTGTTTGTTCTGCCGGAAA	GGTTAAGTTCCTTGAACGCAAACT	Tetracycline	efflux pump
tetB-01	AGTGCGCTTTGGATGCTGTA	AGCCCCAGTAGCTCCTGTGA	Tetracycline	efflux pump
floR	ATTGTCTTCACGGTGTCCGTTA	CCGCGATGTCGTCGAACT	Multidrug	efflux pump
cmr	CGGCATCGTCAGTGGAATT	CGGTTCCGAAAAAGATGGAA	Multidrug	efflux pump
marR-01	GCGGCGTACTGGTGAAGCTA	TGCCCTGGTCGTTGATGA	Multidrug	efflux pump
mexF	CCGCGAGAAGGCCAAGA	TTGAGTTCGGCGGTGATGA	Multidrug	efflux pump
acrR-01	GCGCTGGAGACACGACAAC	GCCTTGCTGCGAGAACAAA	Multidrug	efflux pump
tolC-01	GGCCGAGAACCTGATGCA	AGACTTACGCAATTCCGGGTTA	Multidrug	efflux pump
cmlA1-01	TAGGAAGCATCGGAACGTTGAT	CAGACCGAGCACGACTGTTG	Chloramphenicol	efflux pump
qnrA	AGGATTTCTCACGCCAGGATT	CCGCTTTCAATGAAACTGCAA	Others	other/unknown
tnpA-01	CATCATCGGACGGACAGAATT	GTCGGAGATGTGGGTGTAGAAAGT	Transposase	transposase
cIntI-1(class1)	GGCATCCAAGCAGCAAG	AAGCAGACTTGACCTGA	Integron	integrase
intI-1(clinic)	CGAACGAGTGGCGGAGGGTG	TACCCGAGAGCTTGGCACCCA	Integron	integrase

Table S3. The numbers of different kinds of bacterial ASVs in different treatments

Group	**Time (d)**	**Number of ASVs in water samples**	**Number of ASVs in biofilm samples**
CK	7	2193±179 ^{a}	940±257 ^{a}
AL	7	1206±210 ^{b}	400±50 ^{b}
AH	7	322±22 ^{c}	309±89 ^{b}
NL	7	669±139 ^{d}	433±31 ^{b}
NH	7	448±52 ^{c}	303±38 ^{b}
CK	14	1902±229 ^{a}	1045±94 ^{a}
AL	14	956±353 ^{b}	679±146 ^{a}
AH	14	310±31 ^{c}	286±38 ^{b}
NL	14	747±66 ^{b}	722±200 ^{a}
NH	14	182±32 ^{d}	174±28 ^{b}
CK	21	1660±48 ^{a}	1694±40 ^{a}
AL	21	1101±188 ^{a}	1356±109 ^{ab}
AH	21	453±153 ^{b}	354±60 ^{c}
NL	21	1240±68 ^{a}	1083±97 ^{bd}
NH	21	196±3 ^{c}	165±27 ^{e}
CK	35	1517±344 ^{a}	1419±206 ^{a}
AL	35	1247±116 ^{a}	1108±221 ^{ab}
AH	35	1081±301 ^{a}	736±82 ^{b}
NL	35	915±201 ^{a}	733±140 ^{b}
NH	35	402±241 ^{b}	317±87 ^{c}

Data are shown as Mean ± SD (n = 3). CK represents control treatment; AL represents 0.1 mg/L Ag^+ treatment; AH represents 1 mg/L Ag^+ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment.

Different letters indicate significant differences among treatments (at the same time) as determined by ANOVA followed by Tukey's test ($P < 0.05$).

Table S4. DeSeq2 analysis ($P_{adj} < 0.05$) showing the significantly changed phyla in Ag exposed surrounding water when compared to the controls.

	AL				AH				NL				NH			
	7 d	14 d	21 d	35 d	7 d	14 d	21 d	35 d	7 d	14 d	21 d	35 d	7 d	14 d	21 d	35 d
Cyanobacteria	-	+		-	-	-	-			-	-	-	-	-	-	
Proteobacteria	+								+							
Planctomycetes	-				-	-	-	-	-	-	-			-	-	
Chlamydiae															-	
unclassified_Bacteria	+							+								
Bacteroidetes	+				+	+			+							
Chloroflexi	-		-		-			-			-				-	
Acidobacteria		-	-			-	-	-		-	-			-	-	
Actinobacteria	-		+		-	-	-		-				+	+		
Hydrogenedentes		-			-	-	-		-	-	-		-	-	-	
Firmicutes										+		-				
Armatimonadetes				-				-			-	-			-	
Verrucomicrobia			+		-	-				-	+		-	-	-	
Gemmatimonadetes			-				-	-			-				-	
Patescibacteria								-							-	
Dependentiae							-									
BRC1				-				-								

AL represents 0.1 mg/L Ag^+ treatment; AH represents 1 mg/L Ag^+ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment.

The '+' symbol indicates that a phylum is significantly increased in Ag treatments compared to control, whereas the '-' symbol indicates that a phylum is significantly decreased in Ag treatments compared to control.

Table S5. DeSeq2 analysis ($P_{adj} < 0.05$) showing the significantly changed phyla in Ag exposed biofilms when compared to the controls.

	AL				AH				NL				NH			
	7 d	14 d	21 d	35 d	7 d	14 d	21 d	35 d	7 d	14 d	21 d	35 d	7 d	14 d	21 d	35 d
Bacteroidetes	+				+	+	+	+	+				+			
Cyanobacteria	-		-		-	-	-		-	-			-	-	-	
Proteobacteria	+		+		+			+	+	+						
Firmicutes									-				-			
Actinobacteria						-	-	-	-	-			+	+		
Fusobacteria					+				+							
unclassified_Bacteria						-				+						
Chlamydiae			+													
Patescibacteria			-				-				-				-	-
Planctomycetes				+		-	-	-	-	-					-	-
Verrucomicrobia			-					-	-						-	
Armatimonadetes			-	-				-				-				-

AL represents 0.1 mg/L Ag^+ treatment; AH represents 1 mg/L Ag^+ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment.

The '+' symbol indicates that a phylum is significantly increased in Ag treatments compared to control, whereas the '-' symbol indicates that a phylum is significantly decreased in Ag treatments compared to control.

Table S6. Analysis of similarities (ANOSIM) evaluating community dissimilarity based on Bray-Curtis distances.

Source	Group	R	P-value	P_{adj_BH}
Surrounding water				
	AL vs. CK	0.3987	0.001	0.00125
	AH vs. CK	0.9164	0.001	0.00125
	NL vs. CK	0.4785	0.001	0.00125
	NH vs. CK	0.9799	0.001	0.00125
Biofilm				
	AL vs. CK	0.3555	0.002	0.00286
	AH vs. CK	0.6459	0.001	0.00167
	NL vs. CK	0.3823	0.003	0.00333
	NH vs. CK	0.8133	0.001	0.00167

CK represents control treatment; AL represents 0.1 mg/L Ag$^+$ treatment; AH represents 1 mg/L Ag$^+$ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment.

Table S7. Analysis of similarities (ANOSIM) evaluating resistome dissimilarity based on Bray-Curtis distances.

Source	Group	R	P-value	P_{adj_BH}
Surrounding water				
	AL vs. CK	0.1205	0.049	0.054
	AH vs. CK	0.3797	0.001	0.004
	NL vs. CK	0.1445	0.022	0.031
	NH vs. CK	0.6045	0.001	0.004
Biofilm				
	AL vs. CK	0.0590	0.125	0.178
	AH vs. CK	0.1155	0.045	0.094
	NL vs. CK	0.084	0.122	0.178
	NH vs. CK	0.2383	0.008	0.08

CK represents control treatment; AL represents 0.1 mg/L Ag$^+$ treatment; AH represents 1 mg/L Ag$^+$ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment.

Table S8. Data of physicochemical properties of surrounding environment during exposure.

Group	**Time (d)**	**DO (mg/L)**	**pH**	**ORP (mV)**	**Salinity (%)**	**CON. (μs/cm)**	**TDS (mg/L)**	**TN (mg/L)**	**NO$_3^-$-N (mg/L)**	**NH$_4^+$-N (mg/L)**	**NO$_2^-$-N (mg/L)**	**TP (mg/L)**	**Chla (μg/L)**	**Total Ag (μg/L)**	**Dis. Ag (μg/L)**
CK	7	8.0±0.7^{a}	8.7±0.1^{a}	235±3^{a}	***0.4±0.1^{ab}***	662±12^{a}	***472±8^{a}***	16.5±1.3^{a}	13.6±0.3^{a}	1.5±0.3^{a}	0.2±0.1^{a}	***1.2±0.1^{ab}***	86.8±5.3^{a}	1.6±0.1^{a}	0.29±0.04^{a}
AL	7	6.1±0.2^{bc}	8.7±0.1^{a}	238±1^{ab}	***0.4±0.1^{a}***	670±6^{a}	***479±4^{a}***	17.2±0.2^{a}	13.5±0.7^{a}	1.8±0.2^{a}	0.3±0.1^{a}	***1.3±0.1^{ab}***	1.0±0.2^{b}	23.2±6.8^{a}	0.34±0.03^{a}
AH	7	6.5±0.1^{bc}	8.7±0.1^{a}	268±4^{c}	***0.4±0.1^{a}***	670±7^{a}	***477±4^{a}***	16.9±0.3^{a}	14.2±0.2^{a}	1.6±0.2^{a}	0.7±0.1^{b}	***1.4±0.1^{a}***	0.5±0.1^{b}	625.6±44.0^{b}	15.0±0.9^{b}
NL	7	6.6±0.3^{b}	8.7±0.1^{a}	258±5^{d}	***0.3±0.1^{b}***	613±14^{b}	***438±10^{a}***	16.7±0.4^{a}	13. 6±0.7^{a}	1.6±0.2^{a}	0.2±0.1^{a}	***1.2±0.2^{ab}***	0.9±0.4^{b}	37.9±8.5^{a}	1.8±0.2^{a}
NH	7	5.7±0.1^{c}	8.7±0.1^{a}	246±4^{be}	***0.3±0.0^{ab}***	618±6^{b}	***440±8^{a}***	16.8±0.4^{a}	13.4±0.4^{a}	1.5±0.1^{a}	0.3±0.1^{a}	***1.3±0.1^{b}***	0.4±0.2^{b}	1036.5±135.6^{c}	118.8±7.0^{c}
CK	14	9.1±0.6^{a}	9.2±0.1^{a}	244±2^{a}	***0.3±0.1^{a}***	611±39^{a}	***408±27^{a}***	14.6±0.6^{a}	12.2±1.5^{ab}	0.9±0.3^{ab}	0.3±0.1^{a}	0.4±0.2^{a}	228.7±23.3^{ab}	1.6±0.5^{a}	0.3±0.1^{a}
AL	14	12.5±1.0^{b}	9.5±0.1^{b}	237±4^{b}	***0.3±0.1^{a}***	638±19^{a}	***427±14^{ab}***	15.9±1.0^{ab}	12.6±0.8^{ab}	0.8±0.5^{a}	0.4±0.1^{a}	0.5±0.1^{a}	270.7±25.7^{c}	33.6±20.4^{a}	1.0±1.4^{a}

AH	14	5.1±0.2^{c}	8.8±0.1^{c}	253±3^{c}	***0.4±0.1***^{a}	725±11^{b}	***483±4***^{b}	17.3±0.2^{b}	11.8±1.1^{a}	1.7±0.3^{b}	0.7±0.1^{b}	1.4±0.1^{b}	0.8±0.3^{c}	539.6±73.9^{b}	21.0±1.2^{b}
NL	14	8.0±0.7^{ad}	9.1±0.1^{ad}	242±3^{ab}	***0.3±0.1***^{a}	647±8^{a}	***433±4***^{ab}	16.0±0.4^{ab}	13.1±1.6^{ab}	1.0±0.4^{ab}	0.3±0.1^{a}	0.6±0.1^{a}	199.6±46.3^{a}	30.1±3.0^{a}	1.8±0.2^{ab}
NH	14	5.0±0.4^{de}	8.8±0.1^{ce}	240±4^{ab}	***0.3±0.1***^{a}	***682±30***^{ab}	***459±19***^{ab}	17.0±0.2^{b}	15.0±0.2^{b}	1.4±0.1^{ab}	0.4±0.1^{a}	1.3±0.2^{bc}	0.7±0.3^{cd}	1011.2±64.0^{c}	118.9±16.6^{c}
CK	21	9.7±1.0^{a}	9.4±0.1^{a}	212±2^{a}	***0.2±0.1***^{a}	***482±26***^{a}	***322±21***^{a}	8.0±1.4^{a}	3.7±0.5^{a}	0.5±0.2^{a}	0.3±0.1^{a}	0.2±0.1^{a}	204.8±29.0^{a}	2.0±0.2^{a}	0.3±0.2^{a}
AL	21	10.5±1.4^{a}	9.4±0.1^{a}	210±3^{a}	***0.2±0.1***^{a}	***500±22***^{a}	***333±15***^{a}	9.2±0.5^{a}	4.8±0.7^{a}	0.6±0.1^{a}	0.2±0.1^{a}	0.2±0.1^{a}	208.9±8.3^{a}	27.3±1.0^{a}	0.9±0.2^{a}
AH	21	5.9±0.3^{b}	8.9±0.1^{b}	219±3^{a}	***0.4±0.1***^{a}	***702±66***^{a}	***466±44***^{a}	18.2±0.8^{bc}	15.0±0.3^{b}	1.4±0.2^{b}	0.6±0.1^{b}	1.1±0.1^{b}	15.8±6.3^{b}	468.1±38.1^{b}	15.7±1.4^{a}
NL	21	11.0±2.2^{a}	9.5±0.1^{a}	200±5^{b}	***0.2±0.1***^{a}	***502±18***^{a}	***332±13***^{a}	7.2±0.2^{a}	4.2±0.7^{ac}	0.7±0.1^{ab}	0.3±0.1^{a}	0.2±0.1^{a}	202.4±41.4^{a}	24.6±10.2^{a}	0.7±0.2^{a}
NH	21	7.2±1.7^{ab}	9.2±0.1^{ab}	200±5^{b}	***0.3±0.1***^{a}	***677±56***^{a}	***451±38***^{a}	16.8±1.0^{c}	14.3±0.1^{c}	0.9±0.7^{ab}	0.4±0.1^{c}	0.9±0.3^{bc}	26.5±17.6^{bc}	921.0±62.4^{c}	60.9±14.7^{b}
CK	35	8.1±1.0^{a}	9.3±0.1^{a}	220±2^{a}	***0.2±0.1***^{ab}	400±16^{a}	***265±11***^{a}	3.1±0.4^{a}	0.0±0.1^{a}	0.3±0.2^{ab}	0.0±0.1^{a}	***0.1±0.1***^{a}	216.0±7.8^{a}	1.4±0.2^{a}	0.23±0.03^{a}
AL	35	8.7±0.6^{a}	9.5±0.1^{a}	218±0.1^{a}	***0.2±0.1***^{a}	384±12^{a}	***254±10***^{a}	3.1±0.2^{a}	0.0±0.1^{a}	0.4±0.2^{ab}	0.0±0.1^{a}	***0.1±0.1***^{a}	204.1±22.1^{a}	14.6±4.4^{a}	0.4±0.1^{a}
AH	35	9.6±0.7^{a}	9.5±0.1^{a}	218±3^{a}	***0.2±0.1***^{ab}	421±12^{a}	***279±9***^{a}	10.2±0.3^{bc}	5.4±0.3^{b}	0.5±0.1^{a}	0.4±0.1^{b}	***0.2±0.1***^{a}	216.1±31.3^{a}	166.2±10.9^{b}	0.4±0.1^{a}
NL	35	8.4±0.7^{a}	9.4±0.1^{a}	215±2^{a}	***0.2±0.1***^{ab}	389±13^{a}	***260±8***^{a}	7.4±1.9^{b}	0.3±0.3^{a}	0.2±0.1^{b}	0.0±0.1^{a}	***0.2±0.1***^{a}	221.2±10.3^{a}	29.9±12.7^{a}	0.24±0.04^{a}
NH	35	7.5±1.5^{a}	9.2±0.1^{a}	220±5^{a}	***0.2±0.1***^{b}	496±53^{b}	***334±37***^{a}	12.4±2.3^{c}	9.3±3.0^{c}	0.4±0.2^{ab}	0.8±0.3^{bc}	***0.2±0.2***^{a}	196.1±28.4^{a}	209.7±33.9^{b}	5.6±1.8^{b}

Data are shown as Mean ± SD (n = 3). CK represents control treatment; AL represents 0.1 mg/L Ag^+ treatment; AH represents 1 mg/L Ag^+ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment. On the initial day, the total Ag concentrations in CK, AL, AH, NL, and NH were 1.2 ± 0.1, 97.4 ± 0.4, 976.3 ± 4.1, 96.6 ± 0.9, 1001.6 ± 6.2 μg/L, respectively.

Abbreviation: DO, dissolved oxygen; ORP, Oxidation-Reduction Potential; CON., conductivity; TDS, total dissolved solids; TN, total nitrogen; NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonia nitrogen; NO_2^--N, nitrite nitrogen; TP, total phosphorous; Chla, Chlorophyll a; Dis. Ag, dissolved Ag.

Different letters indicate significant differences ($P < 0.05$) among treatments (at the same time) as determined with ANOVA followed by Tukey's test or non-parametric Kruskal-Wallis test (highlighted using bold/italic/underlined text).

Table S9. Mantel correlations between biofilm total Ag and bacterial communities, antibiotic resistance genes, and mobile genetic elements in the biofilm.

	Microbial communities		**Antibiotic resistance genes**		**Mobile genetic elements**	
	R	***P*-value**[a]	**R**	***P*-value**[a]	**R**	***P*-value**[a]
Total Ag	0.2311	< 0.001	0.0598	0.1607	0.06388	< 0.1885

[a] *P*-value was derived from 9999 permutations.

III. Figures:

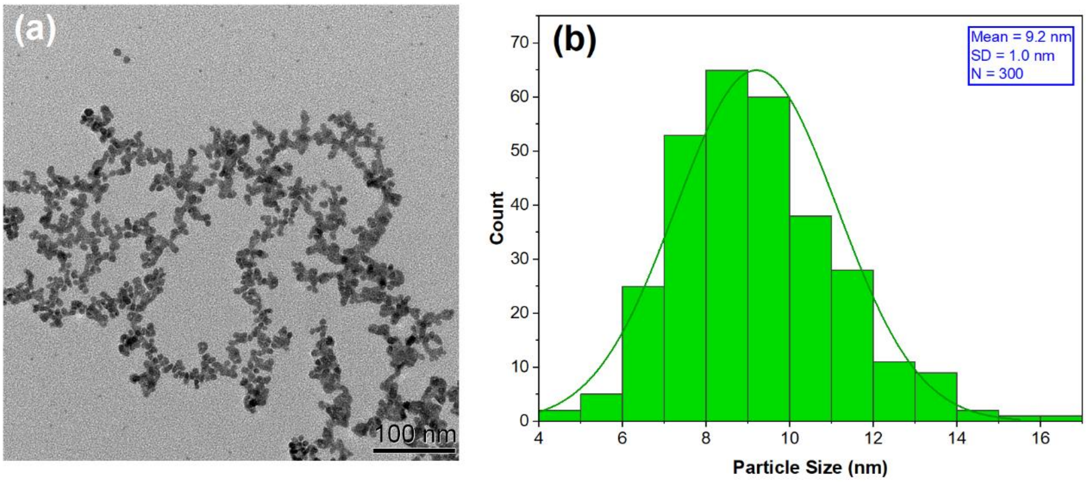


Fig. S1. The TEM morphology and size of silver nanoparticles (**a**), and the particle size distribution of the silver nanoparticles (**b**).

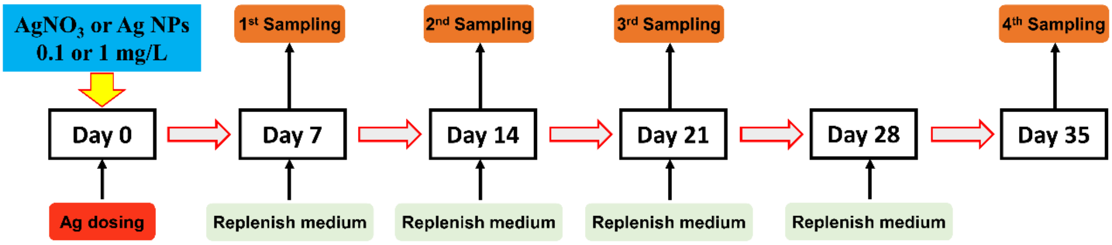
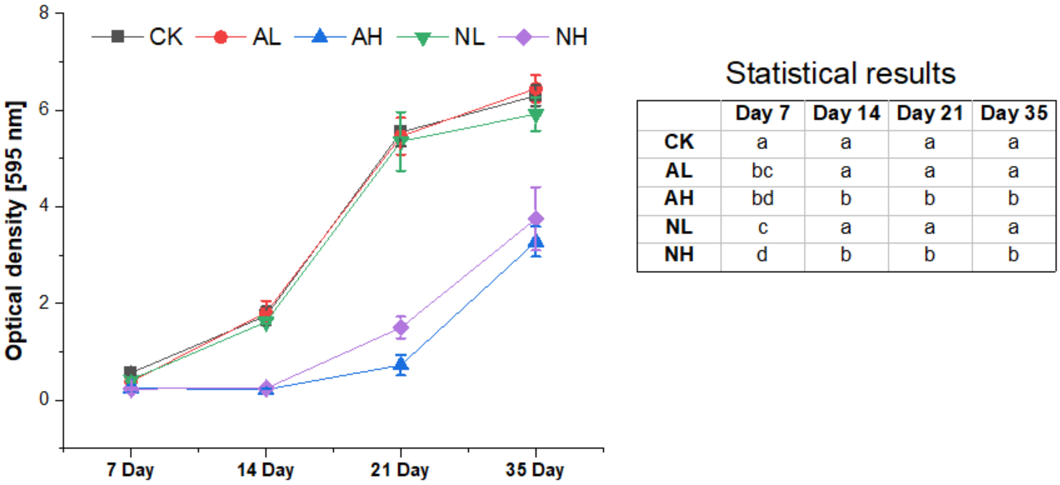


Fig. S2 Schematic diagram showing the experimental design.



Statistical results

	Day 7	Day 14	Day 21	Day 35
CK	a	a	a	a
AL	bc	a	a	a
AH	bd	b	b	b
NL	c	a	a	a
NH	d	b	b	b

Fig. S3. Impacts of Ag exposure on microbial biomass on glass slide measured as optical density at 595 nm. CK represents control; AL represents 0.1 mg/L Ag^+ treatment; AH represents 1 mg/L Ag^+ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment. Different letters indicate significant differences ($P < 0.05$) among treatments (at the same time) as determined with ANOVA followed by Tukey's test.

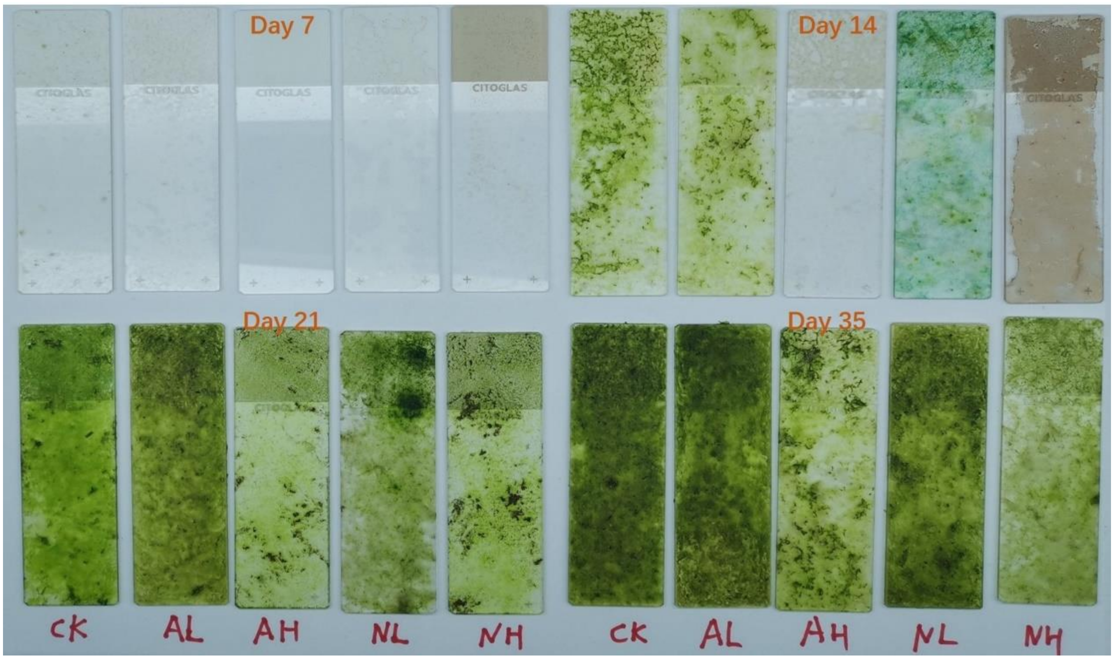


Fig. S4. Graphs showing the biofilm on the glass slides during biofilm formation. CK represents control; AL represents 0.1 mg/L Ag^+ treatment; AH represents 1 mg/L Ag^+ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment.

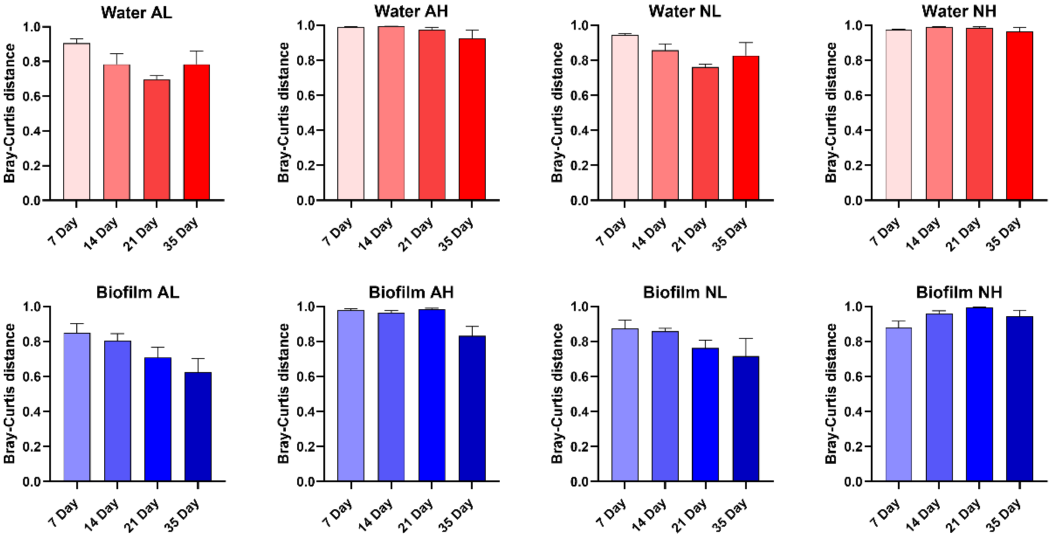


Fig. S5. The Bray-Curtis distance between microbial communities in Ag treatments and control. AL represents 0.1 mg/L Ag^+ treatment; AH represents 1 mg/L Ag^+ treatment; NL represents 0.1 mg/L AgNPs treatment; NH represents 1 mg/L AgNPs treatment.

References:

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3. E.W. Rice, R.B. Baird and A.D. Eaton, Standard Method for Examination of Water and Wastewater, *American Public Health Association*, 2017, 23rd Edition.