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


# Gut-microbial adaptation and transformation of silver nanoparticles mediated the detoxification of *Daphnia magna* and offsprings

Yingdong Li[1,2], Wen-Xiong Wang[3*], Hongbin Liu[1,2,4*]

[1]Department of Ocean Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

[2]Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, China

[3]School of Energy and Environment, State Key Laboratory of Marine Pollution, Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory, City University of Hong Kong, Kowloon, Hong Kong, China

[4]Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory, The Hong Kong University of Science and Technology, Hong Kong, China

**Keywords:** Metatranscriptomic analysis, Zooplankton, *Daphnia magna*, Gut microbe, Ag nanoparticles

***AUTHOR FOR CORRESPONDENCE:** Hongbin Liu, liuhb@ust.hk; Wen-Xiong Wang, wx.wang@cityu.edu.hk

## Materials and Methods

### Synthesis and characterization of AgNPs

Citrate-capped AgNPs (20 nm) were prepared as described previously [1]. $NaBH_4$ (5 mM, 6 mL) was added to a solution containing $AgNO_3$ (100 mM, 250 µL) and citrate (100 mM, 250 µL) to give a total volume of 100 mL, and stirred vigorously for 30 min. The resulting yellow solution was stirred for another 30 min, after which the resulting AgNPs that were synthesized, were purified by ultrafiltration (Amicon Ultra15 3 K, Millipore, MA) to remove the soluble by-products. This stock suspension of AgNPs was stored at 4°C prior to use. These AgNPs were characterized via transmission electron microscopy (TEM) (Fig. S1).

### Validating the toxicity of AgNPs and $Ag^+$

Zooplankton were exposed to AgNPs or $Ag^+$ to verify their toxicity and calculate the lethal concentration for 50% of *D. magna* within 48 h (i.e., 48 h $LC_{50}$). In brief, the guts of the *D. magna* were evacuated for 30 min, after which the zooplankton were transferred into a beaker containing either AgNPs or $Ag^+$ at a density of one individual per 10 mL. Four replicates were used in each treatment. *D. magna* were exposed to ADaM medium only (control), or they were treated with AgNPs at 0.1, 0.5, 0.75, 0.85, 1, 1.5, 2, 3, 4, 5, 9, 10, 15 or 20 µg $L^{-1}$ or $Ag^+$ at 0.1, 0.5, 0.75, 1, 1.25, 1.5, 1.75 or 2 µg $L^{-1}$. The number of surviving *D. magna* was then recorded after exposure to AgNPs or $Ag^+$ for 48 h. The 48 h $LC_{50}$ of *D. magna* for these different treatments was then calculated using the trimmed Spearman-Kärber method, based on the actual concentration of AgNPs or $Ag^+$.

### Preparation of axenic *D. magna* cultures

*D. magna* was made into an axenic culture using antibiotics according to previous reports [2]. In brief, control *D. magna* eggs in their external membrane, were treated with 0.25% ampicillin (Sigma, Germany) for 30 min to remove all associated bacteria. Then, 5% of the eggs were selected at random and crushed into detritus before being filtered through a 0.22 µm membrane for PCR detection of any remaining bacteria. The remaining eggs were rinsed with sterile ADaM to remove any remaining ampicillin and then transferred to a sterile six-well plate for inoculation with the prepared microflora as well as hatching.

## Respiration rate measurement

Individual *D. magna* were weighed and then enclosed in a 200 µL 24-well glass-bottom multiplate. After a 10 min period of acclimation, the concentration of oxygen in the incubation chambers was recorded (mg $L^{-1}$) at 15-sec intervals for 6 h using an SDR SensorDish® Reader. The oxygen consumption was calculated as the linear slope of the change in $O_2$ over time during the incubation period (all linear regressions were significant $P < 0.0001$). These respiration rates were corrected against a control chamber filled with zooplankton-free water and normalized with the weight of each individual.

## WGCNA procedures

First, the hierarchical clustering of samples was analyzed using the flashClust function based on the gene expression profile in WGCNA. The average connectivity degrees of different modules and their independence were then tested using the pickSoftThreshold function with a set of candidate powers (ranging between 1 and 30). A suitable power value was selected if the degree of independence was >0.9. The WGCNA algorithm was then performed to construct the hierarchical clustering tree of genes, and those with high topological overlap similarity (i.e., not less than 10), were clustered into different co-expression modules. Finally, we calculated the eigengene (the average expression level of all genes in each co-expression module), hierarchically clustered the modules, and merged similar modules (abline = 0.25).

## Reference:


1. Yan, N.; Tang, B. Z.; Wang, W.-X., In vivo bioimaging of silver nanoparticle dissolution in the gut environment of zooplankton. *ACS nano* **2018,** *12*, (12), 12212-12223.
2. Li, Y.; Yan, N.; Wong, T. Y.; Wang, W.-X.; Liu, H., Interaction of antibacterial silver nanoparticles and microbiota-dependent holobionts revealed by metatranscriptomic analysis. *Environmental Science: Nano* **2019,** *6*, (11), 3242-3255.

Table S1 Primers for qPCR detection

| Gene | Forward (5'-3') | Reverse (5'-3') | Affiliation |
|---|---|---|---|
| *dsrA* (dissimilatory sulfite reductase alpha subunit) | ACSCACTGGAAGCACG CCGG | GTGGMRCCGTGCAKRTT GG | Gut microbiota |
| butyryl-CoA CoA transferase | GCIGAICATTTCACITGG AAYWSITGGCAYATG | CCTGCCTTTGCAATRTCI ACRAANGC | Gut microbiota |
| *narG* (nitrate reductase / nitrite oxidoreductase, alpha subunit) | TAYGTSGGGCAGGARA AACTG | CGTAGAAGAAGCTGGT GCTGTT | Gut microbiota |
| *nirK* (nitrite reductase) | TCATGGTGCTGCCGCG KGACGG | GAACTTGCCGGTKGCCC AGAC | Gut microbiota |
| GAPDH (glyceraldehyde 3-phosphate dehydrogenase) | CCTGCCAAGTATGATG ACATCAA | AGCCCAGGATGCCCTTT AGT | Gut microbiota |
| 16S rRNA | TCCTACGGGAGGCAGC AGT | GGACTACCAGGGTATCT AATCCTGTT | Gut microbiota |

Table S2 Disentangling the results for the holobiont system

| Samples | Assigned to host library (% reads from holobiont) | Assigned to bacterial library (% reads from holobiont) | Shared (% reads from holobiont) | Unassigned (% reads from holobiont) |
|---|---|---|---|---|
| Normal-1 | 72.22 | 16.37 | 1.87 | 9.54 |
| Normal-2 | 70.92 | 16.74 | 1.25 | 11.09 |
| Normal-3 | 74.28 | 16.93 | 1.45 | 7.34 |
| Low Ag-1 | 77.20 | 14.55 | 1.68 | 6.57 |
| Low Ag-2 | 79.56 | 9.22 | 1.99 | 9.23 |
| Low Ag-3 | 74.25 | 14.90 | 2.11 | 8.74 |
| High Ag-1 | 85.17 | 0.08 | 0 | 14.75 |
| High Ag-2 | 81.49 | 0.10 | 0 | 18.41 |
| High Ag-3 | 80.71 | 0.13 | 0 | 19.16 |
| F1 low Ag-1 | 90.11 | 0.15 | 0 | 9.74 |
| F1 low Ag-2 | 88.27 | 0.06 | 0 | 11.67 |
| F1 low Ag-3 | 85.04 | 0.05 | 0 | 14.91 |
| F3 low Ag-1 | 75.45 | 14.98 | 3.45 | 6.12 |
| F3 low Ag-2 | 75.77 | 13.71 | 4.78 | 5.74 |
| F3 low Ag-3 | 79.85 | 11.87 | 4.11 | 4.17 |
| DOM-1 | 73.39 | 9.78 | 6.51 | 10.32 |
| DOM-2 | 69.18 | 10.14 | 7.21 | 13.47 |
| DOM-3 | 67.27 | 9.62 | 8.22 | 14.89 |
| DOM-Ag-1 | 60.64 | 18.64 | 9.45 | 11.27 |
| DOM-Ag-2 | 58.14 | 19.17 | 8.21 | 14.48 |
| DOM-Ag-3 | 60.24 | 17.92 | 7.69 | 14.15 |
| DOM-AgNPs-1 | 64.68 | 17.64 | 8.81 | 8.87 |
| DOM-AgNPs-2 | 66.73 | 16.48 | 5.14 | 11.65 |
| DOM-AgNPs-3 | 61.34 | 19.15 | 6.54 | 12.97 |

Table S3 Illumina sequencing statistics of mRNA dataset

| Sample | Base number (Gb) | Raw reads | Clear reads |
|---|---|---|---|
| Normal-1 | 37.42 | 113,581,610 | 113,578,438 |
| Normal-2 | 35.66 | 102,373,718 | 102,371,140 |
| Normal-3 | 36.28 | 109,114,324 | 103,112,170 |
| Low Ag-1 | 28.32 | 71,704,2032 | 71,682,274 |
| Low Ag-2 | 34.28 | 97,305,120 | 97,288,722 |
| Low Ag-3 | 36.38 | 10,857,398 | 10,841,168 |
| High Ag-1 | 36.14 | 105,978,704 | 105,765,148 |
| High Ag-2 | 26.42 | 73,809,582 | 73,754,428 |
| High Ag-3 | 36.16 | 106,604,802 | 106,577,722 |
| FLAg-1 | 42.04 | 118,324,904 | 118,285,154 |
| FLAg-2 | 32.22 | 91,997,456 | 91,975,422 |
| FLAg-3 | 50.06 | 140,721,142 | 139,551,374 |
| TLAg-1 | 38.08 | 108,365,374 | 108,132,654 |
| TLAg-2 | 28.24 | 81,319,188 | 81,118,454 |
| TLAg-3 | 44.26 | 127,019,062 | 126,556,878 |
| DOM-1 | 30.04 | 85,389,630 | 85,153,331 |
| DOM-2 | 28.07 | 79,743,122 | 79,354,784 |
| DOM-3 | 42.11 | 119,921,154 | 119,431,312 |
| DOM-Ag-1 | 36.67 | 104,811,922 | 104,633,646 |
| DOM-Ag-2 | 30.15 | 86,112,456 | 85,803,112 |
| DOM-Ag-3 | 36.41 | 104,974,388 | 104,674,214 |
| DOM-AgNPs-1 | 36.17 | 102,793,422 | 102,453,312 |
| DOM-AgNPs-2 | 36.24 | 102,822,314 | 102,514,334 |
| DOM-AgNPs-3 | 42.12 | 119,961,368 | 119,633,112 |

Table S4 Summary of assembly

| Sample | N50 (*Daphnia*) | Coding regions (*Daphnia*) | N50 (Gut bacteria) | Coding regions (Gut bacteria) |
|---|---|---|---|---|
| Normal-1 | 1,842 | 95,897 | 1,174 | 17,712 |
| Normal-2 | 1,733 | 93,987 | 1,038 | 15,573 |
| Normal-3 | 1,697 | 94,113 | 974 | 16,635 |
| TLAg-1 | 1329 | 74,821 | 725 | 12,304 |
| TLAg-2 | 1,398 | 83,521 | 744 | 10,963 |
| TLAg-1 | 1,266 | 72,327 | 511 | 9,347 |
| FLAg-1 | 1,369 | 80,354 | - | 54 |
| FLAg-2 | 1,442 | 84,154 | - | 30 |
| FLAg-3 | 1,310 | 81,327 | - | 11 |
| High Ag-1 | 1,424 | 84,233 | - | 24 |
| High Ag-2 | 1,382 | 81,354 | - | 37 |
| High Ag-3 | 1,354 | 83,665 | - | 19 |
| Low Ag-1 | 1,381 | 80,512 | 625 | 19,091 |
| Low Ag-2 | 1,457 | 82,118 | 611 | 15,211 |
| Low Ag-3 | 1,416 | 85,682 | 574 | 18,719 |
| DOM-1 | 2,353 | 75,913 | 1,324 | 22,993 |
| DOM-2 | 2,113 | 73,441 | 1,027 | 19,347 |
| DOM-3 | 2,621 | 77,335 | 1,434 | 24,334 |
| DOM-Ag-1 | 3,651 | 81,567 | 1,967 | 27,487 |
| DOM-Ag-2 | 3,447 | 79,396 | 2,074 | 26,311 |
| DOM-Ag-3 | 3,761 | 82,334 | 2,133 | 28,673 |
| DOM-AgNPs-1 | 4,512 | 86,754 | 2,164 | 28,679 |
| DOM-AgNPs-2 | 4,932 | 87,245 | 2,278 | 30,348 |
| DOM-AgNPs-3 | 4,785 | 86,819 | 2,184 | 28,688 |

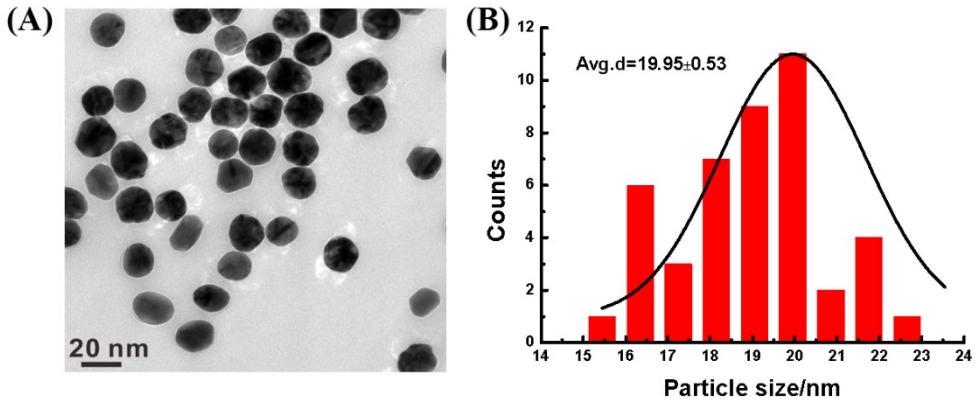


Figure S1. TEM examination of the AgNPs synthesized (A), and graph showing the average particle size of the AgNPs with the line represent a best fit curve (B).

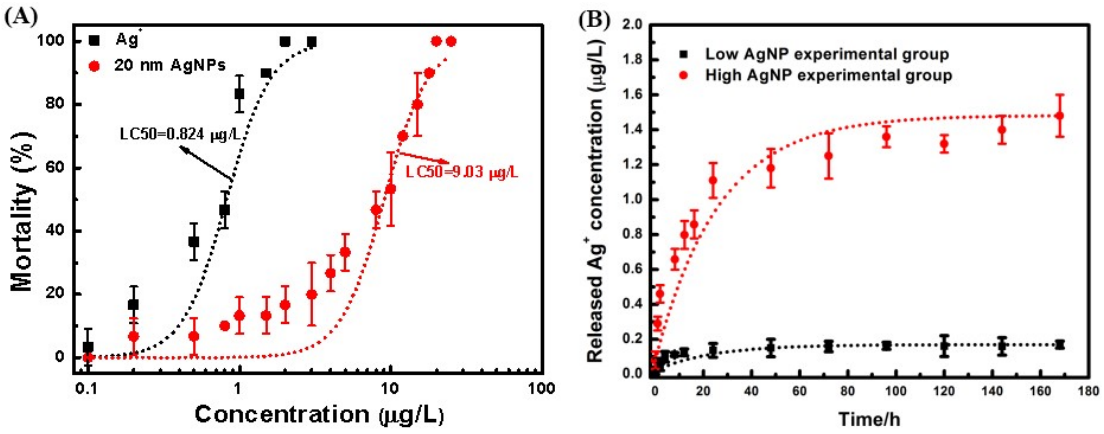


Figure S2. The toxicity of AgNPs and $Ag^{+}$ to *D. magna* (A), and the concentration of $Ag^{+}$ released during the dissolution of AgNPs over a period of 180 h (B).

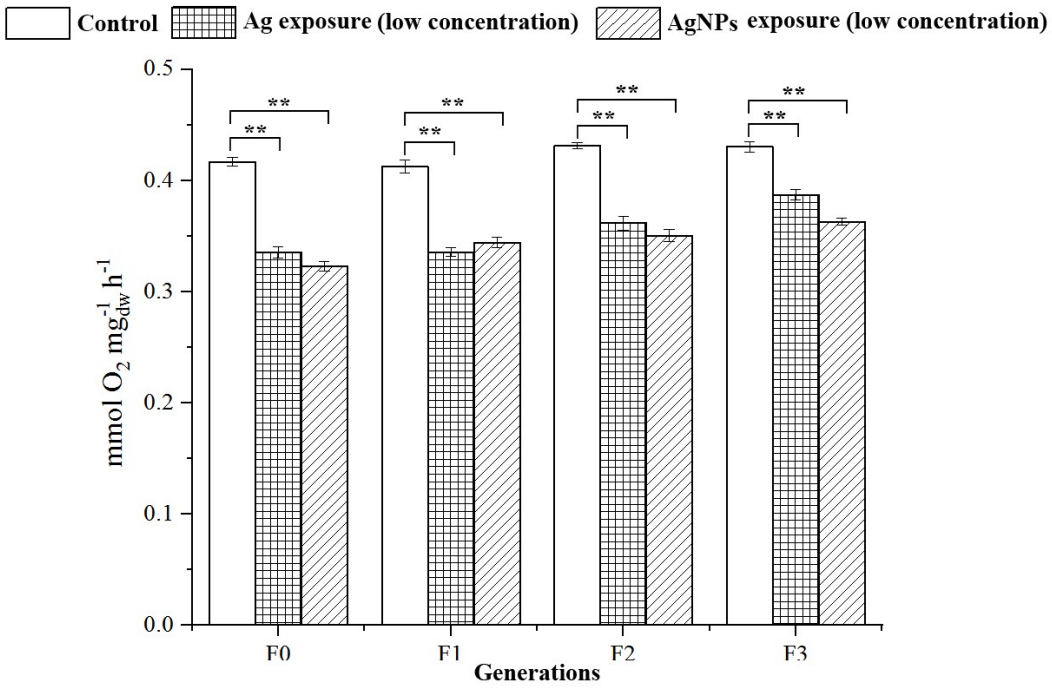


Figure S3. The oxygen consumption rate of zooplankton under multi-generational exposure of silver ion or AgNPs. ** indicates the significant differences ($p$<0.01) in T-test between two comparision groups.

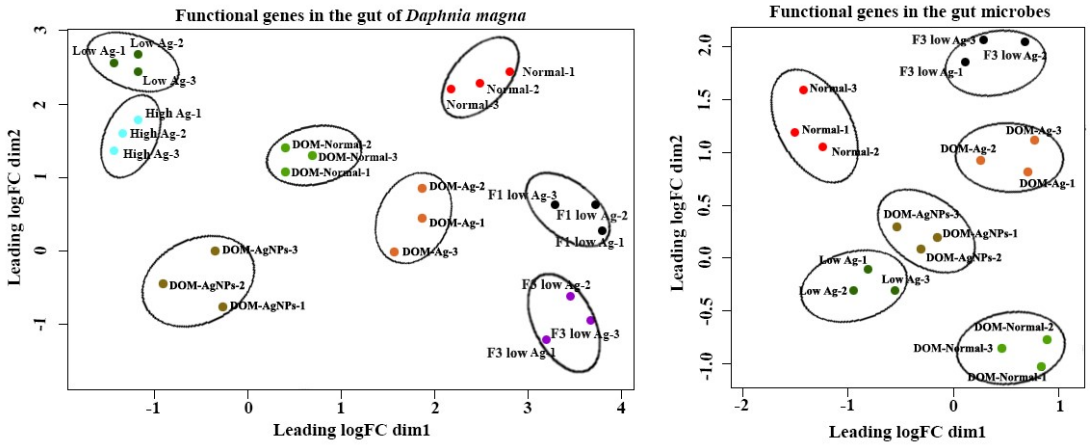


Figure S4. The biological coefficient of variation (BCV) of the transcriptomic data affiliated to *D. magna* (A), and the gut microbiota (B), using normalized gene expression counts for each experimental group.

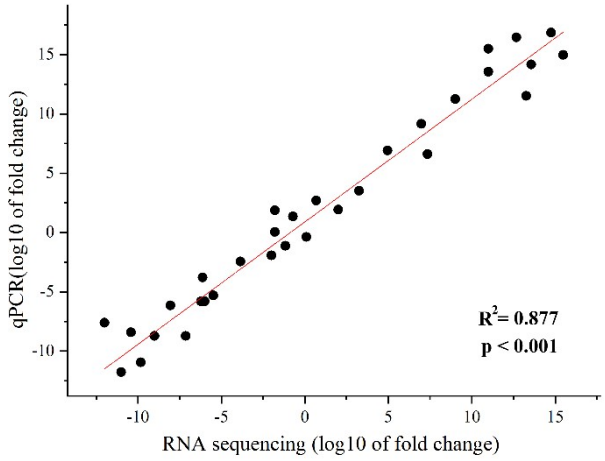


Figure S5. qPCR verification of the expression of selected genes in the gut microbiota.

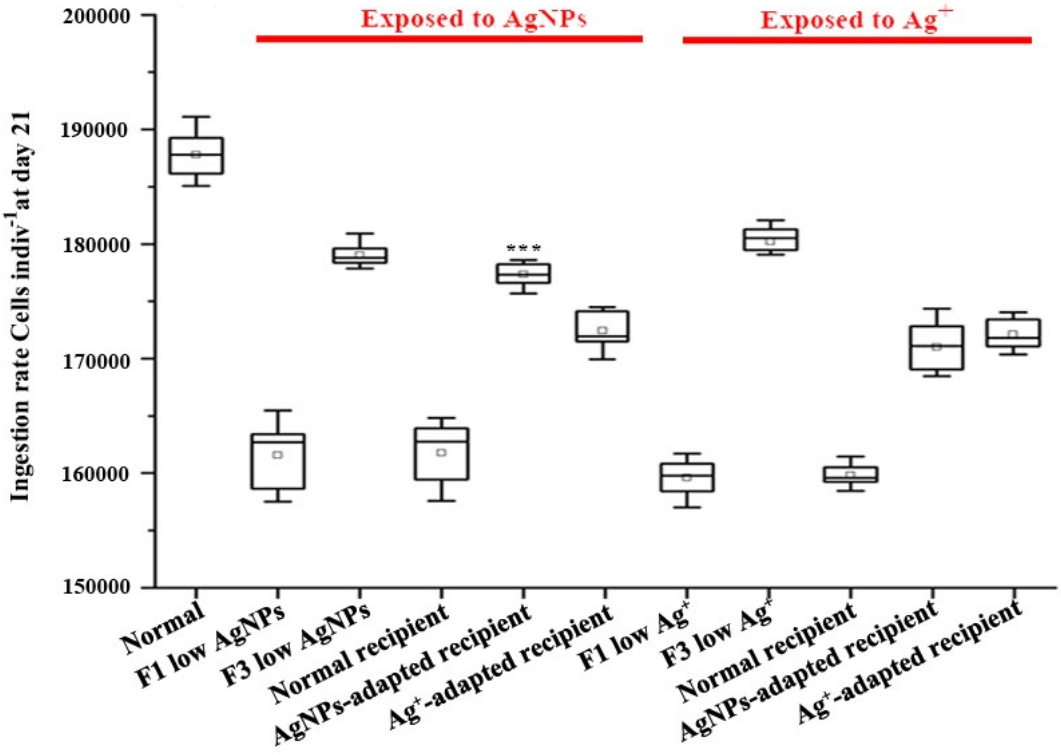


Figure S6. The ingestion rate of *D. magna* exposed to AgNPs and $Ag^+$.

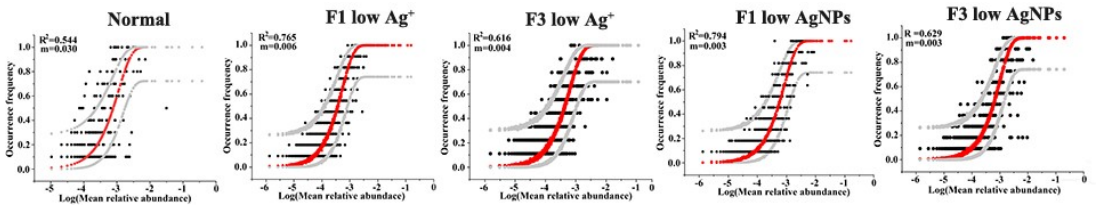


Figure S7. The neutral community model (NCM) based calculation of gut microbial community from different treatments.

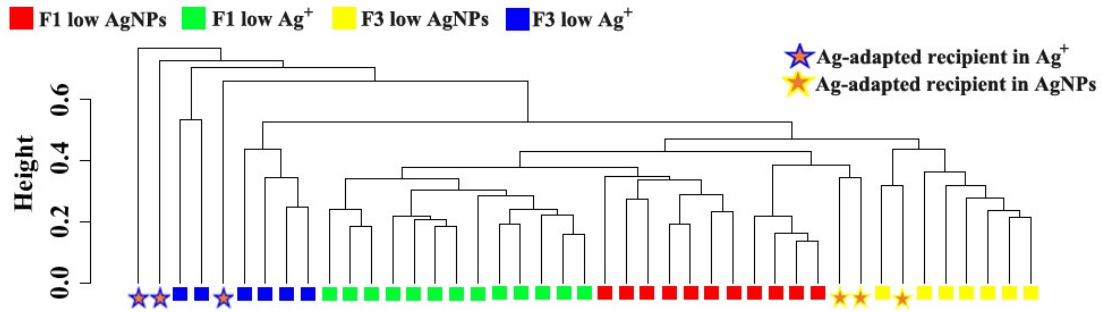


Figure S8. The Bray-Curtis similarity between gut-microbial communities

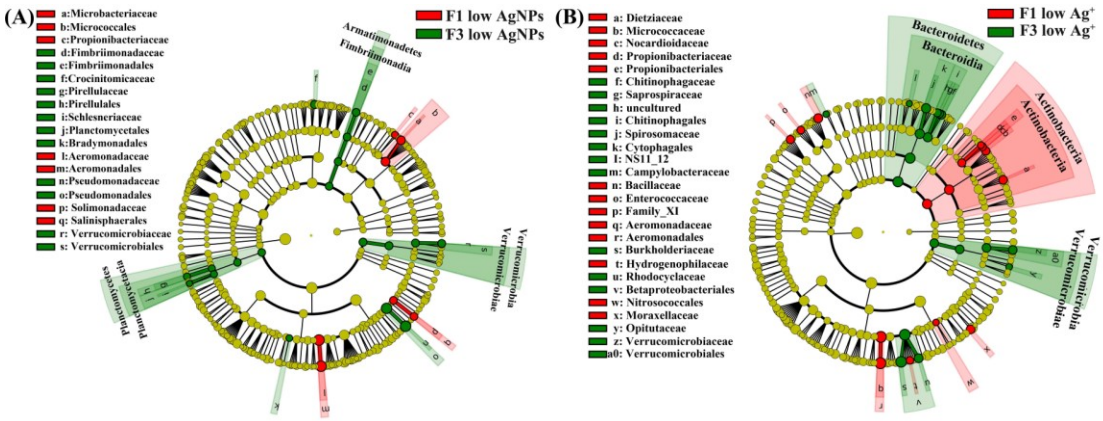


Figure S9. The cladogram indicates the phylogenetic distribution (at family level) of the gut microbial lineages between first generation low AgNPs and third generation low AgNPs (A), and first generation low $Ag^+$ and third generation low $Ag^+$ (B).

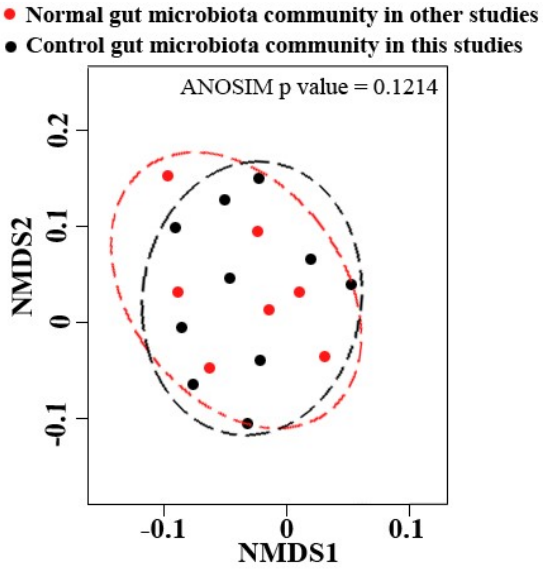


Figure S10. The gut-microbial community similarity between this study and other studies.

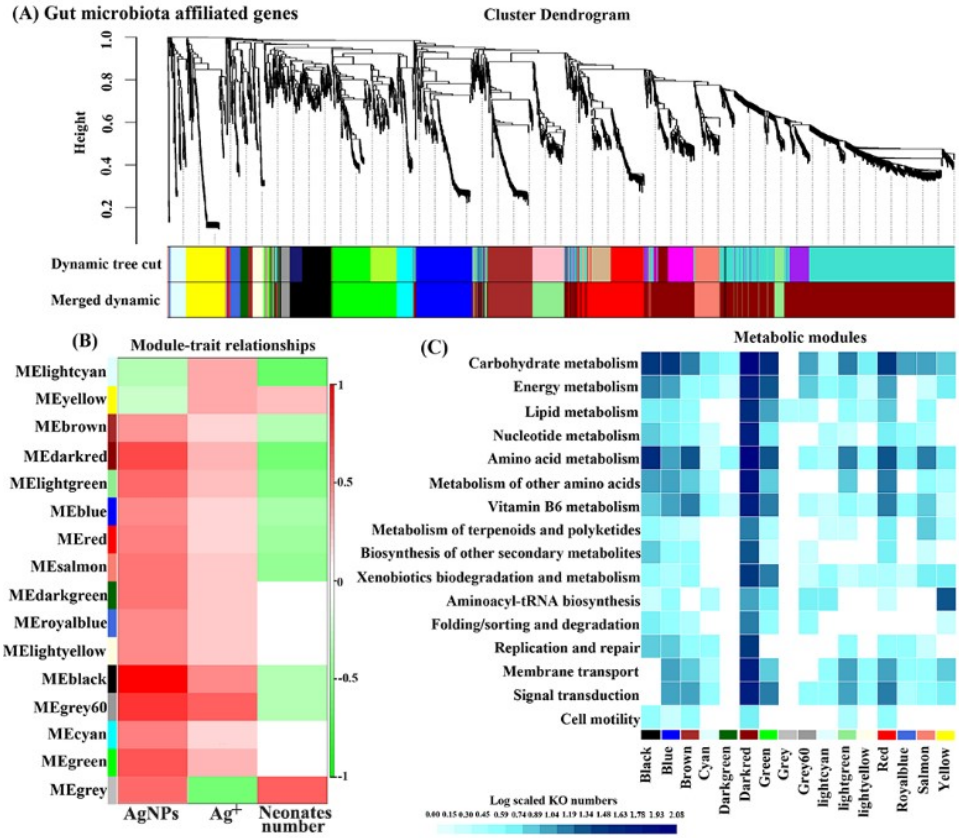


Figure S11. The weighted gene co-expression network analyses of gut microbiota and *D. magna*. Cluster dendrograms showing the expression pattern of gut microbiota affiliated genes in the different metabolic modules (A). WGCNA demonstrating the correlation between calculated metabolic modules and experimental conditions for gut microbiota (B), and the gut microbial metabolic modules that were positively correlated with the reproduction rate of zooplankton were highlighted with red pentagram. Heatmaps for the number of genes clustered into different metabolic modules in the metabolic category level for gut microbiota (C).

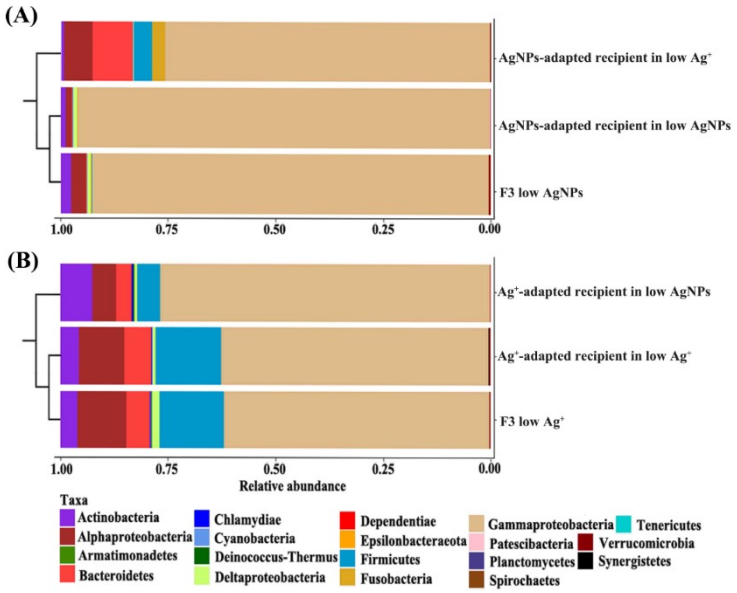


Figure S12. The microbial community composition at phylum and class level for proteobacteria with community similarities among samples for recipient with AgNPs-adapted gut-microbiota (A) and $Ag^{+}$-adapted gut-microbiota (B) based on the UPGMA clustering with the abundance of 16S ASVs. The cluster dendrograms were constructed based on Bray-Curtis similarity using vegan package in R.

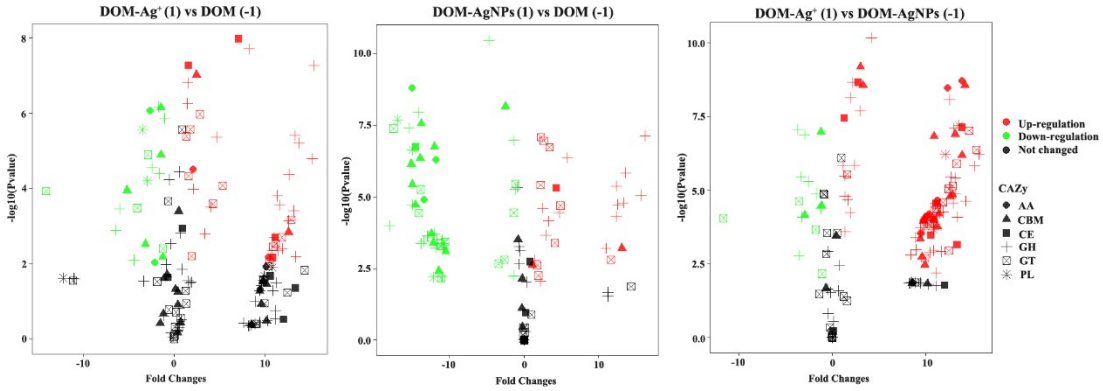


Figure S13. The differentially expressed genes for carbohydrate-active enzymes (CAZymes). Auxiliary Activities (AA): redox enzymes that act in conjunction with CAZymes; Carbohydrate-Binding Modules (CBM): adhesion to carbohydrates; Carbohydrate Esterases (CE) : hydrolysis of carbohydrate esters; Glycoside Hydrolases (GH) : hydrolysis and/or rearrangement of glycosidic bonds; GlycosylTransferases (GT) : formation of glycosidic bonds; Polysaccharide Lyases (PL) : non-hydrolytic cleavage of glycosidic bonds.