

Article

Supplementary Material: Microbial Population Dynamics in Model Sewage Treatment Plants and the Fate and Effect of Gold Nanoparticles

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Table S1. Number of sequences obtained and quality scores of the sequences for the 1st experiment (control/dispersant experiment).

| GENEWIZ NGS DATA REPORT | | | | | | | |
|-------------------------|-------------|---------|------------|---------|---------|--------|---------|
| Sample | Length (bp) | # Reads | Bases (bp) | Q20 (%) | Q30 (%) | GC (%) | N (ppm) |
| 1 | 249.52 | 98,436 | 24,561,461 | 97.28 | 95.89 | 53.28 | 1.42 |
| 2 | 249.57 | 97,808 | 24,410,236 | 97.13 | 95.59 | 54.20 | 2.54 |
| 3 | 249.53 | 108,208 | 27,001,593 | 93.27 | 90.48 | 53.16 | 2.22 |
| 4 | 249.55 | 113,318 | 28,278,197 | 93.34 | 90.56 | 53.17 | 0.00 |
| 5 | 249.53 | 103,112 | 25,729,944 | 93.26 | 90.47 | 53.23 | 0.00 |
| 6 | 249.53 | 104,504 | 26,077,044 | 92.97 | 90.05 | 53.30 | 0.00 |
| 7 | 249.54 | 100,426 | 25,060,715 | 93.36 | 90.61 | 53.10 | 1.60 |
| 8 | 249.53 | 102,456 | 25,565,843 | 92.96 | 90.03 | 53.23 | 2.97 |
| 9 | 249.54 | 100,540 | 25,088,593 | 93.18 | 90.33 | 53.27 | 0.20 |
| 10 | 249.53 | 104,496 | 26,074,971 | 93.19 | 90.33 | 53.52 | 0.69 |
| 11 | 249.55 | 106,570 | 26,594,453 | 96.91 | 95.37 | 53.36 | 0.75 |
| 12 | 249.56 | 109,128 | 27,234,016 | 96.87 | 95.32 | 53.43 | 0.18 |
| 13 | 249.54 | 105,718 | 26,381,097 | 96.95 | 95.43 | 53.26 | 0.00 |
| 14 | 249.56 | 111,004 | 27,701,636 | 96.80 | 95.18 | 53.70 | 0.00 |
| 15 | 249.57 | 106,562 | 26,594,728 | 96.93 | 95.38 | 53.42 | 0.83 |
| 16 | 249.52 | 101,180 | 25,246,477 | 96.76 | 95.13 | 53.52 | 3.56 |
| 17 | 249.57 | 103,002 | 25,706,130 | 96.88 | 95.34 | 53.41 | 0.00 |
| 18 | 249.56 | 109,834 | 27,410,112 | 97.01 | 95.51 | 53.43 | 0.22 |
| 19 | 249.55 | 112,472 | 28,067,249 | 96.63 | 94.96 | 54.20 | 1.50 |
| 20 | 249.61 | 110,260 | 27,521,466 | 96.68 | 95.05 | 53.59 | 0.00 |
| 21 | 249.57 | 110,580 | 27,597,078 | 96.76 | 95.15 | 53.86 | 1.38 |

Table S2. Alpha diversity index of bacterial microbiomes in the 1st experiment (a) and 2nd experiment (b).

| Day | Sample Type | Ace | Chao1 | Shannon | Simpson | Goods_Coverage | |
|------------|-------------|------------|--------|---------|---------|----------------|------|
| a | -8 | Control | 496.15 | 498.50 | 7.51 | 0.99 | 1.00 |
| | -4 | Control | 505.37 | 505.77 | 7.28 | 0.98 | 1.00 |
| | | Control | 507.24 | 505.67 | 7.28 | 0.98 | 1.00 |
| | | Control | 514.84 | 524.25 | 7.33 | 0.99 | 1.00 |
| | | Control | 500.98 | 502.56 | 7.31 | 0.99 | 1.00 |
| | | Control | 507.01 | 510.04 | 7.06 | 0.98 | 1.00 |
| | 0 | Control | 496.80 | 497.96 | 7.09 | 0.98 | 1.00 |
| | | Control | 503.19 | 505.16 | 7.17 | 0.98 | 1.00 |
| | | Control | 500.64 | 503.07 | 7.18 | 0.98 | 1.00 |
| | | Control | 491.18 | 501.07 | 6.88 | 0.98 | 1.00 |
| | 3 | Control | 485.94 | 486.12 | 6.86 | 0.98 | 1.00 |
| | | Dispersant | 497.92 | 498.92 | 6.95 | 0.98 | 1.00 |
| | | Dispersant | 511.50 | 516.44 | 7.00 | 0.98 | 1.00 |
| | | Control | 479.29 | 482.00 | 6.84 | 0.98 | 1.00 |
| | 9 | Control | 485.43 | 487.10 | 6.81 | 0.98 | 1.00 |
| | | Dispersant | 487.70 | 499.46 | 6.93 | 0.98 | 1.00 |
| Dispersant | | 499.36 | 505.77 | 7.01 | 0.98 | 1.00 | |
| Dispersant | | 499.36 | 505.77 | 7.01 | 0.98 | 1.00 | |
| b | -8 | Control | 142.72 | 143.00 | 6.13 | 0.98 | 1.00 |
| | -4 | Control | 147.96 | 147.50 | 6.15 | 0.98 | 1.00 |
| | | Control | 145.50 | 146.00 | 6.08 | 0.98 | 1.00 |
| | | Control | 147.54 | 147.67 | 6.05 | 0.97 | 1.00 |
| | | Control | 145.45 | 144.75 | 6.13 | 0.98 | 1.00 |
| | | Control | 148.99 | 151.00 | 6.08 | 0.98 | 1.00 |
| | 0 | Control | 152.65 | 155.00 | 6.03 | 0.97 | 1.00 |
| | | Control | 151.21 | 151.00 | 6.08 | 0.98 | 1.00 |
| | | Control | 151.42 | 152.50 | 6.03 | 0.97 | 1.00 |
| | | Control | 151.42 | 152.50 | 6.03 | 0.97 | 1.00 |
| | 3 | Dispersant | 153.36 | 153.25 | 5.97 | 0.97 | 1.00 |
| | | Dispersant | 148.79 | 148.62 | 5.88 | 0.97 | 1.00 |
| | | Au-NPs | 154.56 | 154.62 | 5.95 | 0.97 | 1.00 |
| | | Au-NPs | 151.37 | 151.62 | 5.83 | 0.97 | 1.00 |
| | 9 | Dispersant | 145.93 | 147.11 | 5.58 | 0.96 | 1.00 |
| | | Dispersant | 143.94 | 145.67 | 5.61 | 0.95 | 1.00 |
| Au-NPs | | 152.35 | 152.00 | 5.62 | 0.95 | 1.00 | |
| Au-NPs | | 148.82 | 147.50 | 5.75 | 0.96 | 1.00 | |

Table S3. Number of sequences obtained and quality scores of the sequences for the 2nd experiment (dispersant and AuNPs experiment).

| GENEWIZ NGS 16S-EZ ANALYSIS REPORT | | | | | | | |
|------------------------------------|-------------|---------|------------|---------|---------|--------|---------|
| Sample | Length (bp) | # Reads | Bases (bp) | Q20 (%) | Q30 (%) | GC (%) | N (ppm) |
| FW01-1-16S | 249.48 | 70,434 | 17,571,669 | 96.36 | 94.86 | 53.17 | 0.57 |
| FW02-2-16S | 249.43 | 33,228 | 8,288,110 | 94.29 | 92.20 | 53.17 | 0.48 |
| FW03-3-16S | 249.49 | 63,492 | 15,840,926 | 96.27 | 94.72 | 53.13 | 0.57 |
| FW04-4-16S | 249.46 | 49,244 | 12,284,184 | 96.12 | 94.51 | 53.09 | 0.00 |
| FW05-5-16S | 249.48 | 60,292 | 15,041,497 | 96.24 | 94.65 | 53.10 | 6.12 |
| FW06-6-16S | 249.54 | 58,266 | 14,539,872 | 96.49 | 94.98 | 53.45 | 0.14 |
| FW07-7-16S | 249.52 | 33,598 | 8,383,422 | 96.40 | 94.87 | 53.63 | 0.36 |
| FW08-8-16S | 249.56 | 63,900 | 15,946,803 | 95.87 | 94.17 | 53.36 | 1.25 |
| FW09-9-16S | 249.50 | 51,558 | 12,863,469 | 96.92 | 95.58 | 53.37 | 1.32 |
| FW10-10-16S | 249.43 | 28,894 | 7,207,084 | 95.05 | 93.20 | 53.45 | 0.00 |
| FW11-11-16S | 249.49 | 55,262 | 13,787,192 | 96.86 | 95.50 | 53.58 | 0.07 |
| FW12-12-16S | 249.51 | 38,796 | 9,679,822 | 96.94 | 95.60 | 53.44 | 1.65 |
| FW13-13-16S | 249.52 | 52,862 | 13,190,057 | 96.93 | 95.58 | 53.51 | 1.59 |
| FW14-14-16S | 249.45 | 44,378 | 11,070,042 | 97.12 | 95.87 | 53.41 | 1.81 |
| FW15-15-16S | 249.55 | 27,144 | 6,773,743 | 97.01 | 95.70 | 53.46 | 0.30 |
| FW16-16-16S | 249.52 | 49,148 | 12,263,374 | 96.57 | 95.14 | 53.46 | 1.30 |
| FW17-17-16S | 249.47 | 63,582 | 15,861,860 | 96.56 | 95.10 | 53.39 | 2.71 |

Table S4. Measured dissolved organic carbon concentration in influent and effluent of the STPs and the elimination rate throughout the two experiments.

| Experiment 1 (Control vs. Dispersant) | | | | | | |
|---------------------------------------|------------------------|--------------------|------------------------|---------------------------|--------------------|------------------------|
| Day | Control—Replicate 1 | | | Control—Replicate 2 | | |
| | Influent [mg/L] | Effluent [mg/L] | DOC-Elimination [%] | Influent [mg/L] | Effluent [mg/L] | DOC-Elimination [%] |
| -6 | 175 | 6.4 | 96 | 143 | 6.8 | 95 |
| -4 | 148 | 7.9 | 95 | 125 | 5.4 | 96 |
| 0 | 139 | 4.8 | 97 | 122 | 3.7 | 97 |
| 2 | 136 | 4.8 | 96 | 126 | 3.9 | 97 |
| 4 | 132 | 4.7 | 96 | 124 | 3.7 | 97 |
| 6 | 128 | 4.5 | 96 | 121 | 3.4 | 97 |
| 9 | 165 | 4.0 | 98 | 142 | 3.6 | 97 |
| Day | Dispersant—Replicate 1 | | | Dispersant— - Replicate 2 | | |
| | Influent [mg/L] | Effluent [mg/L] | DOC-Elimination [%] | Influent [mg/L] | Effluent [mg/L] | DOC-Elimination [%] |
| -6 | 146 | 6.8 | 95 | 151 | 6.5 | 96 |
| -4 | 117 | 5.4 | 95 | 134 | 6.1 | 95 |
| 0 | 123 | 3.2 | 97 | 161 | 3.7 | 98 |
| 2 | 116 | 3.4 | 97 | 130 | 4.5 | 97 |
| 4 | 118 | 3.9 | 97 | 123 | 4.0 | 97 |
| 6 | 121 | 4.4 | 96 | 116 | 3.5 | 97 |
| 9 | 158 | 3.9 | 98 | 136 | 3.2 | 98 |
| Experiment 2 (Dispersant vs. AuNPs) | | | | | | |
| Day | Dispersant—Replicate 1 | | | Dispersant—Replicate 2 | | |
| | Influent [mg/L] | Effluent [mg/L] | DOC-Elimination [%] | Influent [mg/L] | Effluent [mg/L] | DOC-Elimination [%] |
| -6 | 151 | 5.9 | 96 | 154 | 4.0 | 97 |
| -4 | 135 | 3.8 | 97 | 152 | 3.8 | 97 |
| 0 | 143 | 4.4 | 97 | 153 | 0.3 | 100 |
| 2 | 129 | 3.6 | 97 | 143 | 0.3 | 100 |
| 4 | 126 | 4.0 | 97 | 138 | 3.9 | 97 |
| 6 | 133 | 3.8 | 97 | 132 | 3.8 | 97 |
| 9 | 129 | 3.4 | 97 | 128 | 3.7 | 97 |
| Day | AuNP—Replicate 1 | | | AuNP—Replicate 2 | | |
| | Influent [mg/L] | Effluent [mg/L] | DOC-Elimination [%] | Influent [mg/L] | Effluent [mg/L] | DOC-Elimination [%] |
| -6 | 152 | 3.9 | 97 | 152 | 3.9 | 97 |
| -4 | 147 | 4.4 | 97 | 149 | 3.9 | 97 |
| 0 | 145 | 4.3 | 97 | 148 | 4.1 | 97 |
| 2 | 137 | 4.2 | 97 | 149 | 4.1 | 97 |
| 4 | 160 | 4.1 | 97 | 145 | 3.8 | 97 |
| 6 | 117 | 3.9 | 97 | 135 | 3.8 | 97 |
| 9 | 117 | 3.4 | 97 | 119 | 3.5 | 97 |

Table S5. Measured pH values in the denitrification, nitrification and settling tank of the STPs throughout the two experiments.

| Experiment 1 (Control vs. Dispersant) | | | | | | |
|----------------------------------------------|-------------------------------|----------------------|----------------------|-------------------------------|----------------------|----------------------|
| Day | Control—Replicate 1 | | | Control—Replicate 2 | | |
| | Denitrification | Nitrification | Settling Tank | Denitrification | Nitrification | Settling Tank |
| −6 | 7.5 | 7.5 | 7.3 | 7.5 | 7.5 | 7.4 |
| −4 | 7.4 | 7.3 | 7.3 | 7.3 | 7.4 | 7.3 |
| 0 | 7.3 | 7.4 | 7.3 | 7.4 | 7.3 | 7.3 |
| 2 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 |
| 6 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.2 |
| 9 | 7.3 | 7.3 | 7.2 | 7.3 | 7.3 | 7.2 |
| Experiment 1 (Control vs. Dispersant) | | | | | | |
| Day | Dispersant—Replicate 1 | | | Dispersant—Replicate 2 | | |
| | Denitrification | Nitrification | Settling Tank | Denitrification | Nitrification | Settling Tank |
| −6 | 7.4 | 7.4 | 7.3 | 7.5 | 7.5 | 7.4 |
| −4 | 7.4 | 7.4 | 7.3 | 7.4 | 7.3 | 7.3 |
| 0 | 7.3 | 7.4 | 7.3 | 7.3 | 7.3 | 7.4 |
| 2 | 7.3 | 7.4 | 7.3 | 7.3 | 7.3 | 7.3 |
| 6 | 7.4 | 7.4 | 7.3 | 7.3 | 7.3 | 7.2 |
| 9 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.2 |
| Experiment 2 (Dispersant vs. AuNPs) | | | | | | |
| Day | Dispersant—Replicate 1 | | | Dispersant—Replicate 2 | | |
| | Denitrification | Nitrification | Settling tank | Denitrification | Nitrification | Settling tank |
| −6 | 7.4 | 7.5 | 7.4 | 7.6 | 7.6 | 7.5 |
| −4 | 7.3 | 7.2 | 7.2 | 7.4 | 7.3 | 7.1 |
| 0 | 7.2 | 7.0 | 7.0 | 7.3 | 7.0 | 7.0 |
| 2 | 7.2 | 7.0 | 7.0 | 7.3 | 7.0 | 7.0 |
| 6 | 7.3 | 7.1 | 7.1 | 7.3 | 7.1 | 7.1 |
| 9 | 7.2 | 7.0 | 6.9 | 7.2 | 7.1 | 7.0 |
| Experiment 2 (Dispersant vs. AuNPs) | | | | | | |
| Day | AuNP—Replicate 1 | | | AuNP—Replicate 2 | | |
| | Denitrification | Nitrification | Settling tank | Denitrification | Nitrification | Settling tank |
| −6 | 7.5 | 7.3 | 7.3 | 7.5 | 7.3 | 7.2 |
| −4 | 7.4 | 7.1 | 7.1 | 7.4 | 7.0 | 6.9 |
| 0 | 7.3 | 7.0 | 7.0 | 7.4 | 6.9 | 6.8 |
| 2 | 7.3 | 6.9 | 6.9 | 7.3 | 6.8 | 6.8 |
| 6 | 7.2 | 7.1 | 7.0 | 7.3 | 6.9 | 6.8 |
| 9 | 7.2 | 7.0 | 6.9 | 7.3 | 7.0 | 6.8 |

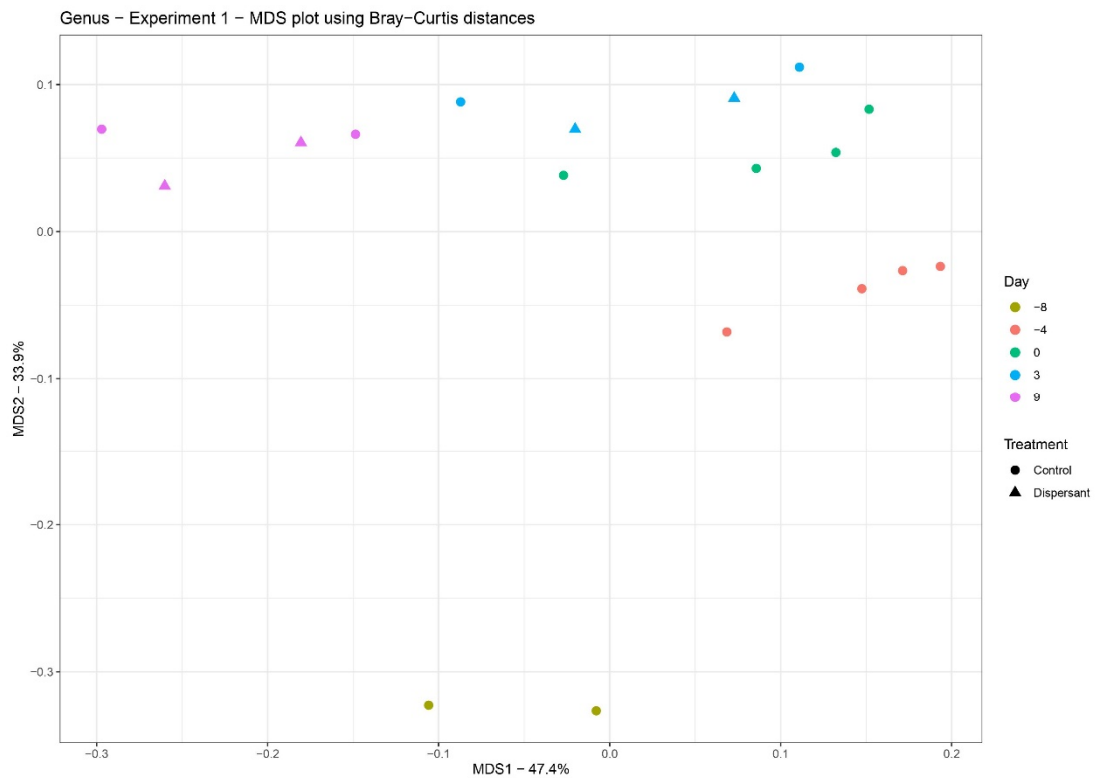


Figure S1. PCoA of bacterial communities, evaluated to genus level, using Bray-Curtis distance. Symbols represent control (●) and dispersant (Δ), and color codes represent the different sampling points: yellow (day -8), red (day -4), green (day 0), blue (day 3) and purple (day 9).

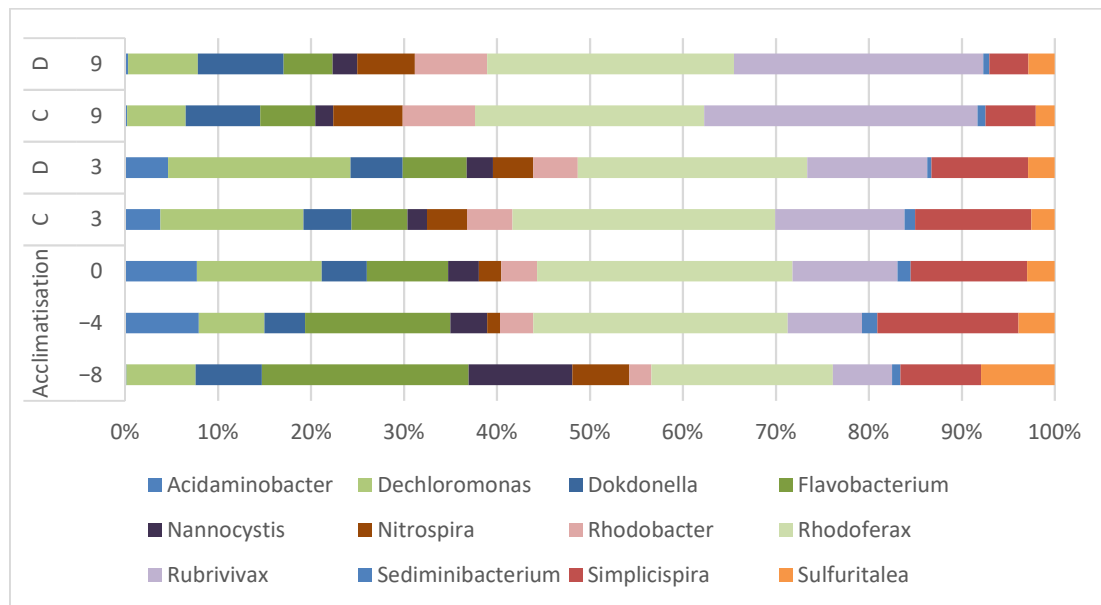


Figure S2. Mean abundances at genus level of the sludge community from collection of fresh sludge until day 9 (C = control treatment and D = dispersant treatment).

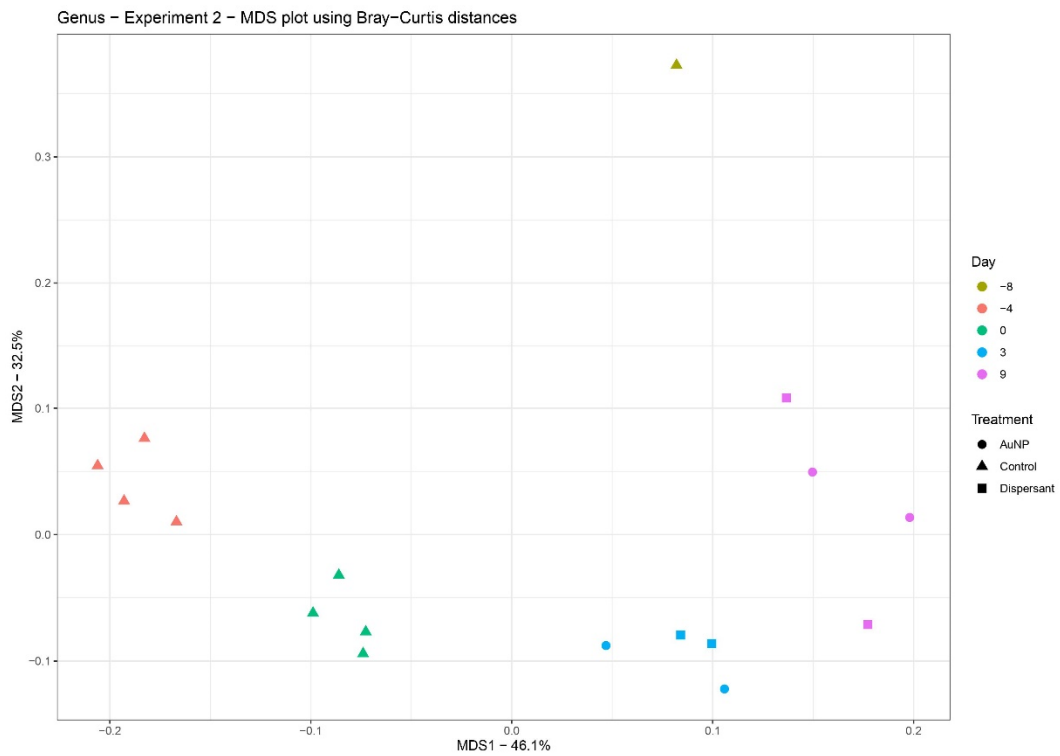


Figure S3. PCoA of bacterial communities, evaluated to genus level, using Bray-Curtis distance. Symbols represent control (Δ), applied with AuNPs (\bullet) and dispersant (\blacktriangleright), and color codes represent the different sampling points: yellow (day -8), red (day -4), green (day 0), blue (day 3) and purple (day 9).

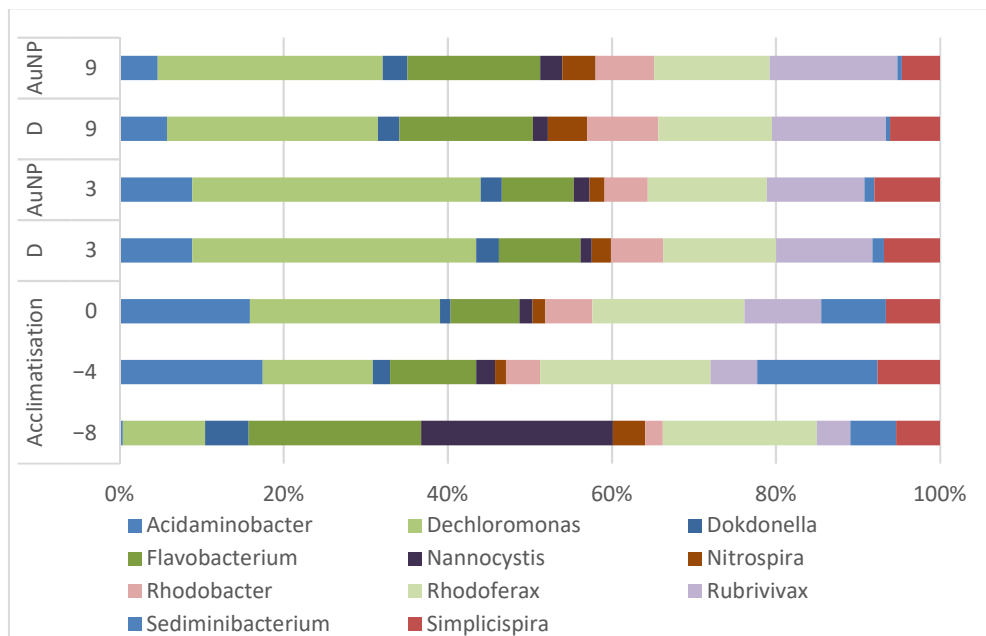


Figure S4. Mean abundance at genus level of the sludge, during the experiment course, before and after the application of dispersant (D) and AuNPs.