Electronic Supplementary Information

Important ecological processes are affected by the accumulation and trophic transfer of nanoplastics in a freshwater periphyton-grazer food chain

Manuel Holzer\textsuperscript{a}, Denise M. Mitrano\textsuperscript{b}, Louis Carles\textsuperscript{a}, Bettina Wagner\textsuperscript{a} and Ahmed Tlili\textsuperscript{a,*}

\textsuperscript{a}Department of Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), 8600 Dübendorf, Switzerland

\textsuperscript{b}Department of Environmental Systems Science, ETH Zurich, Universitatstrasse 16, 8092 Zurich, Switzerland

*Corresponding author: Ahmed Tlili

Address: Eawag, Department of Environmental Toxicology
Überlandstrasse 133. 8600 Dübendorf, Switzerland
Phone: + 41 58 765 5330
Email: ahmed.tlili@eawag.ch
Table S1. Composition of the exposure medium PERIQUIL

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Table S2. Read processing reports for 16S and 18S rRNA genes. Read counts for each sample for each of the processing steps. The mean length of the reads is also indicated in the table. The composition of Mock communities used as positive controls is described in Carles et al. (https://doi.org/10.1016/j.watres.2021.117486).

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<td>21836</td>
<td>20358</td>
<td>19579</td>
<td>19512</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B44-18S</td>
<td>Biofilm</td>
<td>15</td>
<td>R04</td>
<td>10269</td>
<td>9501</td>
<td>8631</td>
<td>8619</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S4
<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Condition</th>
<th>Snail</th>
<th>Day</th>
<th>R</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B45-18S</td>
<td>Biofilm</td>
<td>Snail</td>
<td>15</td>
<td>R05</td>
<td>38270</td>
<td>36072</td>
<td>35635</td>
<td>35563</td>
</tr>
<tr>
<td>B46-18S</td>
<td>Biofilm</td>
<td>nanoPS×Snail</td>
<td>15</td>
<td>R01</td>
<td>17913</td>
<td>16656</td>
<td>15704</td>
<td>15673</td>
</tr>
<tr>
<td>B47-18S</td>
<td>Biofilm</td>
<td>nanoPS×Snail</td>
<td>15</td>
<td>R02</td>
<td>82992</td>
<td>79019</td>
<td>77282</td>
<td>77142</td>
</tr>
<tr>
<td>B48-18S</td>
<td>Biofilm</td>
<td>nanoPS×Snail</td>
<td>15</td>
<td>R03</td>
<td>46135</td>
<td>42008</td>
<td>40386</td>
<td>40313</td>
</tr>
<tr>
<td>B49-18S</td>
<td>Biofilm</td>
<td>nanoPS×Snail</td>
<td>15</td>
<td>R04</td>
<td>39904</td>
<td>37229</td>
<td>35516</td>
<td>35441</td>
</tr>
<tr>
<td>B50-18S</td>
<td>Biofilm</td>
<td>nanoPS×Snail</td>
<td>15</td>
<td>R05</td>
<td>38808</td>
<td>35678</td>
<td>34702</td>
<td>34589</td>
</tr>
<tr>
<td>NegExtr2-18S</td>
<td>Negative control</td>
<td></td>
<td></td>
<td></td>
<td>1687</td>
<td>1628</td>
<td>77</td>
<td>69</td>
</tr>
<tr>
<td>PosPCR-18S</td>
<td>Positive control</td>
<td></td>
<td></td>
<td></td>
<td>94705</td>
<td>92083</td>
<td>90376</td>
<td>90323</td>
</tr>
</tbody>
</table>

**Total = 2527059**
**Table S3.** Two-way ANOVA results of periphyton endpoints at the end of the feeding trial at day 14.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Snail effect</th>
<th>Nanoplastics effect</th>
<th>Nanoplastics × Snail effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFDW</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>$F = 9.5, P &lt; 0.007$</td>
<td>$F = 36.8, P &lt; 0.0001$</td>
<td>ns</td>
</tr>
<tr>
<td>Photosynthetic efficiency</td>
<td>$F = 53.4, P &lt; 0.0001$</td>
<td>$F = 14.1, P &lt; 0.002$</td>
<td>ns</td>
</tr>
<tr>
<td>Lipid content</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Carbon:Nitrogen</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Carbon:Phosphorus</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Nitrogen:Phosphorus</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table S4. Measured endpoints (mean ± SD, n = 5) at day 0 and day 14 (i.e., start and end of the feeding trial) of periphyton (previously exposed for 24 hours to medium without (control) or with the filtrate of 0.5 mg nanoparticles L⁻¹ suspension (f-nanoplastics)) in the presence or absence of the grazers (snail).

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Day 0</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>f-nanoplastics</td>
</tr>
<tr>
<td>AFDW (g m⁻²)</td>
<td>4.0 ± 0.8</td>
<td>5.1 ± 1.0</td>
</tr>
<tr>
<td>chlorophyll-a (mg g⁻¹ AFDW)</td>
<td>17.2 ± 2.4</td>
<td>16.3 ± 2.5</td>
</tr>
<tr>
<td>Physiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>photosynthetic efficiency (quantum yield φ')</td>
<td>0.55 ± 0.06</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>Nutrient value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lipid content (mg glycerol g⁻¹ AFDW)</td>
<td>308 ± 99</td>
<td>195 ± 38</td>
</tr>
</tbody>
</table>
Table S5. Two-way ANOVA results of periphyton endpoints from the control experiment with the filtrate of the 0.5 mg L\(^{-1}\) nanoplastics suspension

<table>
<thead>
<tr>
<th></th>
<th>Snail effect</th>
<th>f-nanoplastics effect</th>
<th>f-nanoplastics × Snail effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFDW</td>
<td>(F = 28.5, P &lt; 0.0001)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>(F = 9.4, P = 0.007)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Photosynthetic efficiency</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Lipid content</td>
<td>(F = 6.4, P = 0.022)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Two-way ANOVA</td>
<td>nanoplastics effect</td>
<td>Snail effect</td>
<td>nanoplastics × Snail effect</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------</td>
<td>--------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><strong>Prokaryotes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chao 1</td>
<td>$F = 11.0, P = 0.0044$</td>
<td>$F = 42.32, P &lt; 0.0001$</td>
<td>$F = 8.244, P = 0.0111$</td>
</tr>
<tr>
<td>Shannon index</td>
<td>$F = 9.4, P = 0.0072$</td>
<td>$F = 49.76, P &lt; 0.0001$</td>
<td>$F = 9.888, P = 0.0063$</td>
</tr>
<tr>
<td><strong>Eukaryotes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chao 1</td>
<td>ns</td>
<td>$F = 40.11, P &lt; 0.0001$</td>
<td>$F = 8.168, P = 0.0114$</td>
</tr>
<tr>
<td>Shannon index</td>
<td>ns</td>
<td>$F = 11.49, P = 0.0037$</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Pairwise comparisons**

<table>
<thead>
<tr>
<th>Result</th>
<th>Chao 1</th>
<th>Shannon index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prokaryotes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. nanoplastics</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Control vs. Snail</td>
<td>$F = 9.3, P &lt; 0.0001$</td>
<td>$F = 10.2, P &lt; 0.0001$</td>
</tr>
<tr>
<td>Control vs. nanoplastics×Snail</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>nanoplastics vs. Snail</td>
<td>$F = 9.8, P &lt; 0.0001$</td>
<td>$F = 10.1, P &lt; 0.0001$</td>
</tr>
<tr>
<td>nanoplastics vs. nanoplastics×Snail</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Snail vs. nanoplastics×Snail</td>
<td>$F = 6.2, P = 0.0024$</td>
<td>$F = 6.2, P = 0.0023$</td>
</tr>
<tr>
<td><strong>Eukaryotes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. nanoplastics</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Control vs. Snail</td>
<td>$F = 9.2, P &lt; 0.0001$</td>
<td>ns</td>
</tr>
<tr>
<td>Control vs. nanoplastics×Snail</td>
<td>$F = 5.8, P = 0.0041$</td>
<td>$F = 5.1, P = 0.012$</td>
</tr>
<tr>
<td>nanoplastics vs. Snail</td>
<td>$F = 6.8, P = 0.0009$</td>
<td>ns</td>
</tr>
<tr>
<td>nanoplastics vs. nanoplastics×Snail</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Snail vs. nanoplastics×Snail</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Table S6. Two-way ANOVA and pairwise comparisons results of periphyton alpha-diversity at day 14, corresponding to the end of the feeding trial.
**Table S7.** Two-way PERMANOVA and pairwise comparisons results of periphyton β-diversity at day 14, corresponding to the end of the feeding trial. Weighted UniFrac distances were used to evaluate β-diversity.

<table>
<thead>
<tr>
<th>Two-way PERMANOVA</th>
<th>nanoplastics effect</th>
<th>Snail effect</th>
<th>nanoplastics × Snail effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prokaryotes</td>
<td>ns</td>
<td>$F = 23.4, P &lt; 0.01$</td>
<td>$F = 4.4, P &lt; 0.05$</td>
</tr>
<tr>
<td>Eukaryotes</td>
<td>ns</td>
<td>$F = 11.3, P &lt; 0.01$</td>
<td>$F = 2.6, P &lt; 0.05$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pairwise comparisons</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prokaryotes</td>
<td>Control vs. nanoplastics</td>
</tr>
<tr>
<td>Control vs. Snail</td>
<td>$F = 14.9, P &lt; 0.05$</td>
</tr>
<tr>
<td>Control vs. nanoplastics×Snail</td>
<td>$F = 6.7, P &lt; 0.05$</td>
</tr>
<tr>
<td>nanoplastics vs. Snail</td>
<td>$F = 21.4, P &lt; 0.05$</td>
</tr>
<tr>
<td>nanoplastics vs. nanoplastics×Snail</td>
<td>$F = 12.2, P &lt; 0.05$</td>
</tr>
<tr>
<td>Snail vs. nanoplastics×Snail</td>
<td>$F = 4.1, P &lt; 0.05$</td>
</tr>
</tbody>
</table>

Eukaryotes  | Control vs. nanoplastics | ns                  |
| Control vs. Snail              | $F = 5.1, P < 0.05$  |
| Control vs. nanoplastics×Snail | $F = 8.6, P < 0.05$  |
| nanoplastics vs. Snail        | $F = 5.4, P < 0.05$  |
| nanoplastics vs. nanoplastics×Snail | $F = 9.8, P < 0.05$  |
| Snail vs. nanoplastics×Snail  | $F = 2.8, P < 0.05$  |
Table S8. Two-way ANOVA results of the abundance of the top-ten prokaryotic and eukaryotic phyla in periphyton at day 14, corresponding to the end of the feeding trial.

<table>
<thead>
<tr>
<th>Prokaryotes</th>
<th>phyla</th>
<th>nanoplastics effect</th>
<th>Snail effect</th>
<th>nanoplastics × Snail effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidobacteria</td>
<td>ns</td>
<td></td>
<td>$F = 16.2, P &lt; 0.001$</td>
<td>$F = 5.2, P &lt; 0.05$</td>
</tr>
<tr>
<td>Armatimonadetes</td>
<td>ns</td>
<td>$F = 25.6, P &lt; 0.001$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Deinococcus-Thermus</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Gemmatimonadetes</td>
<td>ns</td>
<td>$F = 77.4, P &lt; 0.001$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Parcubacteria</td>
<td>$F = 11.8, P &lt; 0.01$</td>
<td>$F = 96.5, P &lt; 0.001$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>ns</td>
<td>$F = 5.2, P &lt; 0.05$</td>
<td>$F = 11.4, P &lt; 0.01$</td>
<td>ns</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>ns</td>
<td>ns</td>
<td>$F = 9.6, P &lt; 0.01$</td>
<td>ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eukaryotes</th>
<th>phyla</th>
<th>nanoplastics effect</th>
<th>Snail effect</th>
<th>nanoplastics × Snail effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphelida</td>
<td>ns</td>
<td>$F = 5.9, P &lt; 0.05$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Bacillariophyta</td>
<td>ns</td>
<td>$F = 19.4, P &lt; 0.001$</td>
<td>$F = 5.2, P &lt; 0.05$</td>
<td>ns</td>
</tr>
<tr>
<td>Cercozoa</td>
<td>ns</td>
<td>$F = 9.9, P &lt; 0.01$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td>ns</td>
<td>ns</td>
<td>$F = 4.8, P &lt; 0.05$</td>
<td>ns</td>
</tr>
<tr>
<td>Chytridiomycota</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Ciliophora</td>
<td>$F = 6.6, P &lt; 0.05$</td>
<td>$F = 30.6, P &lt; 0.001$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Discosea</td>
<td>$F = 7.7, P &lt; 0.05$</td>
<td>$F = 9.1, P &lt; 0.01$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Nematoda</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Rotifera</td>
<td>ns</td>
<td>$F = 8.5, P &lt; 0.05$</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Stramenopiles</td>
<td>ns</td>
<td>$F = 26.4, P &lt; 0.001$</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table S9. Reproductive output (mean ± SD) of snails during 14 days of feeding on periphyton previously exposed for 24 hours to medium without (Control) or with the filtrate (f-nanoplastics) of 5 mg nanoplastics L⁻¹ suspension. Means and SD are calculated from five replicate microcosms per treatment, each containing four snails.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of egg clutches</th>
<th>Number of eggs</th>
<th>Number of eggs per clutch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17 ± 6 (87)</td>
<td>220 ± 74 (1101)</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>f-nanoplastics</td>
<td>11 ± 5 (55)</td>
<td>150 ± 64 (748)</td>
<td>14 ± 4</td>
</tr>
</tbody>
</table>