



HAL
open science

Seasonal Differences and Grazing Pressure Alter the Fate of Gold Nanoparticles in a Microcosm Experiment

Christina Bergemann, Astrid Avellan, Brittany Perrotta, Emily Bernhardt,
Marie Simonin

► **To cite this version:**

Christina Bergemann, Astrid Avellan, Brittany Perrotta, Emily Bernhardt, Marie Simonin. Seasonal Differences and Grazing Pressure Alter the Fate of Gold Nanoparticles in a Microcosm Experiment. *Environmental Science and Technology*, 2023, 57 (37), pp.13970-13979. 10.1021/acs.est.3c01839 . hal-04208811

HAL Id: hal-04208811

<https://hal.science/hal-04208811>

Submitted on 20 Dec 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Seasonal differences and grazing pressure alter the fate of gold**
2 **nanoparticles in a microcosm experiment**

3 Authors: Christina M. Bergemann^{1,2,3}, Astrid Avellan^{1,4,5}, Brittany G. Perrotta^{1,6}, Emily S.
4 Bernhardt^{1,2}, Marie Simonin^{1,2,7*}

5 ¹ Center for the Environmental Implications of Nanotechnology (CEINT), Duke University,
6 Durham, North Carolina 27708, USA

7 ² Biology Department, Duke University, Durham, North Carolina 27708, USA

8 ³ Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

9 ⁴ Department of Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh,
10 Pennsylvania 15289, USA

11 ⁵ Géosciences Environnement Toulouse, CNRS, Toulouse University, CNES, IRD, UPS, 31400
12 Toulouse, France

13 ⁶ Department of Biology, Center for Reservoir and Aquatic Systems Research, Baylor University,
14 Waco, Texas 76706, USA

15 ⁷ Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

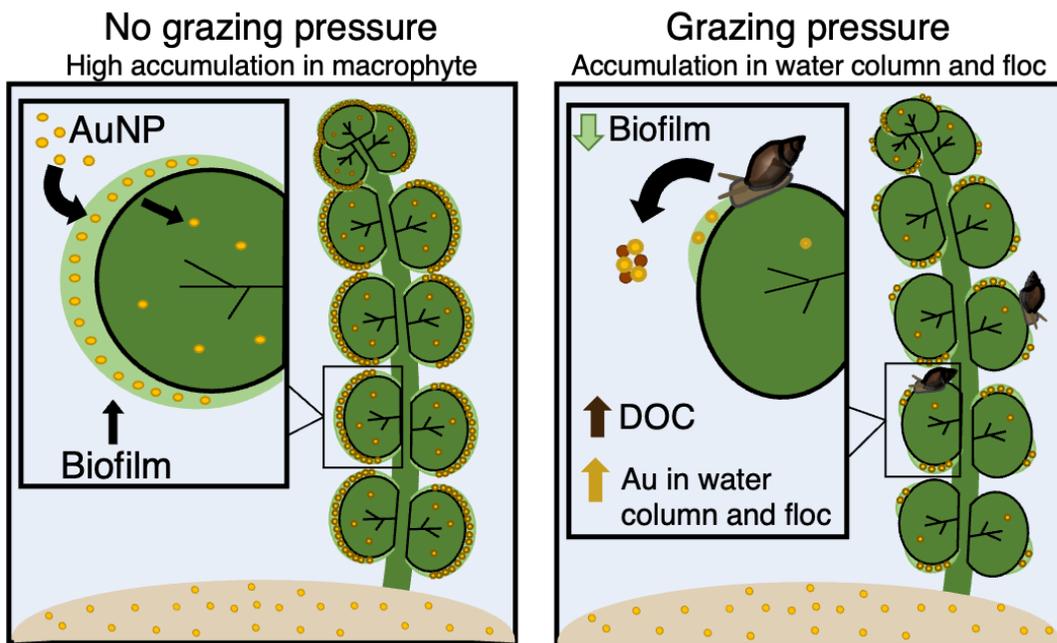
16

17 **Abstract:**

18 Gold nanoparticles (AuNPs) are used as models to track and predict NP fates and effects in
19 ecosystems. Previous work found that aquatic macrophytes and their associated biofilm primarily

20 drove the fate of AuNPs within aquatic ecosystems and that seasonality was an important abiotic
21 factor in the fate of AuNPs. Therefore, the present work aims to study if grazers, by feeding on
22 these interfaces, modify the AuNP fate and if this is altered by seasonal fluctuations. Microcosms
23 were dosed with 44.8 $\mu\text{g/L}$ of AuNP weekly for 4 weeks and maintained in environmental
24 chambers simulating Spring and Fall light and temperature conditions. We discovered that
25 seasonal changes and the presence of grazers significantly altered the fate of Au. Higher
26 temperatures in the warmer season increased dissolved organic carbon (DOC) content in the water
27 column, leading to stabilization of Au in the water column. Additionally, snail grazing on biofilm
28 growing on the *Egeria densa* surface led to a transfer of Au from macrophytes to the organic matter
29 above the sediments. These results demonstrate that climate and grazers significantly impacted the
30 fate of Au from AuNPs, highlighting the role that grazers might have in a large and biologically
31 more complex ecosystem.

32 **Graphical abstract:**



33

34 **Keywords: Snails, nanomaterials, emerging contaminants, macrophytes, microcosms**

35 **Synopsis:**

36 This study highlights how food web complexity and seasonal changes influence nanomaterial fate
37 in aquatic systems, which can impact aquatic plant growth and alter water quality.

38 **Introduction:**

39 Engineered nanomaterial use is increasing in a wide variety of applications ranging from
40 drug delivery to agriculture use¹⁻³. While many studies have shown that nanomaterials can cause
41 toxicity^{4,5}, further research is needed to fully understand how these chemicals interact within the
42 environment and in the presence of other stressors. Gold nanoparticles (AuNPs) are frequently
43 used as a nanomaterial model⁶⁻⁹ but use is also growing in the fields of biomedicine, technology,
44 and pharmaceuticals¹⁰⁻¹². Previous research has shown that citrate capped AuNPs have high
45 chemical stability in abiotic, surface waters and overall low toxicity in aquatic environments,
46 suggesting low environmental consequences if AuNPs enter aquatic ecosystems^{13,14}. Though inert
47 in sterile conditions, AuNPs can undergo oxidation in the presence of biofilms¹³ where they can
48 interact with other biological systems¹⁴⁻¹⁷. Therefore, AuNPs are used as a model for
49 understanding how biological responses can impact the chemical and physical processes and
50 influence the fate and transport of Au within systems of varying complexity.

51 Our previous work demonstrated that low-level, chronic AuNP enrichment led to
52 significant increases in algal blooms and hypoxia¹⁵ in large-scale freshwater mesocosm setups. We
53 hypothesize that this interaction was largely modulated by primary consumers and a macrophyte
54 *Egeria densa*, but the large-scale experimental design limited our ability to test this interaction
55 implicitly. Other work using *E. densa* has shown that AuNPs are not taken up through shoot or

56 root uptake and adsorption of AuNPs on plant surfaces was influenced by dissolved organic carbon
57 present in the water column¹⁸. Yet, we found that the dominant fate of AuNPs was deposition on,
58 and/or absorption into the macrophyte *Egeria densa* resulting in suppression of growth and
59 productivity¹⁵. Due to this suppression, we concluded that algae within the water column were able
60 to outcompete rooted macrophytes for nutrients, shifting the mesocosms from clear water to an
61 algal bloom state¹⁵. It has also been shown that an increase in algae and epiphyton is capable of
62 suppressing macrophyte growth leading to algal blooms¹⁹. Furthermore, it was found that Au from
63 supposedly inert AuNPs present at the macrophyte interface had largely undergone oxidation due
64 to microbial activity within the biofilms attached to macrophyte leaves leading to Au
65 dissolution^{13,15}. The Au fate and transformation at the macrophyte interface seem to be one of the
66 major drivers for the ecosystem responses¹³. To add to this complexity, biofilm grazers are also
67 present at this interface and will feed on the macrophyte leaves and biofilm, accumulating Au
68 within their tissues²⁰. Snail grazing has also been shown to significantly impact the water column
69 quality through nitrogen, phosphorus, and carbon excretions^{20,21}. Grazing on the surface of
70 macrophytes can significantly reduce algal assemblages and improve macrophyte growth by
71 grazing on epiphyton and increasing light attenuation^{22,23}. Therefore, grazing activity at this
72 interface may thus modulate AuNP fate and transformation, and potentially impact their overall
73 effect at the ecosystem level.

74 In this present work, we aimed at understanding the extent to which biofilm grazers (snails)
75 could alter the fate of Au in simplified systems regarding its association with the different
76 compartments of the microcosms (*Egeria densa*, biofilm, sediment floc, or water column). We
77 hypothesized that grazers may reduce the amount of AuNP associated with macrophytes by
78 reducing biofilm growth. If true, grazing may alter AuNPs fate, transport, and form of Au within

79 the microcosm. We used the pulmonate, *Physa gyrina*, which is an air-breathing freshwater snail
80 that primarily consumes filamentous algae, detritus, and senescent plant tissue²⁴. *P. gyrina* was
81 chosen as a grazer for this experiment due to their ability to consume large amounts of filamentous
82 algae while not directly consuming live plant tissue since we wanted to test Au toxicity and
83 concentration in aquatic macrophytes. Furthermore, aquatic snails are readily found in freshwater
84 habitats that are susceptible to runoff and chemical exposures and have shown to accumulate
85 nanoparticles in their tissues^{25,26}.

86 Additionally, we wanted to understand how grazer activity and seasonal differences will
87 influence the impact and fate of AuNP in a simplified aquatic ecosystem. Previous research has
88 shown that gold nanoparticles impacted *E. densa* growth rates and ecosystem metabolism rates
89 more substantially in the spring months than in the fall¹⁵. In order to manipulate both biotic and
90 abiotic conditions while controlling the exposure and growth conditions parameters, we used
91 microcosms to test our ecological questions with a high replication, while simplifying the chemical
92 and biological complexity of the system to isolate the grazers contribution in the observation. Our
93 simplified food web treatments included three biotic factors: phytoplankton, the aquatic
94 macrophyte *Egeria densa*, and the aquatic snail *Physa gyrina*. The microcosms were randomly
95 sorted into two treatment groups: control and AuNP addition. The AuNP treatments were dosed
96 each week for four weeks to investigate the effects of chronic AuNP exposure. Both treatment
97 groups received two seasonal conditions, fall (12.5°C average) and spring (25°C average), and
98 with and without grazing pressure. We hypothesized that spring would have higher biological
99 metabolism causing stress between organisms through competition. Higher competition coupled
100 with AuNP stress could result in less *E. densa* growth and productivity.

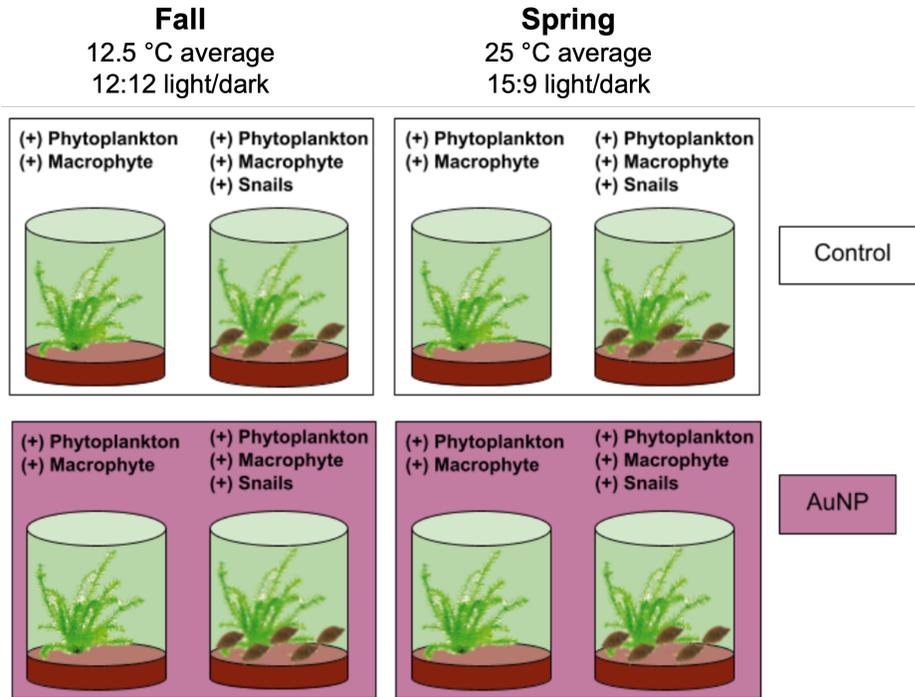
101 **Materials and Methods:**

102 Nanoparticle characterization and concentration

103 Citrate-coated gold nanoparticles were synthesized by the Center for the Environmental
104 Implication of Nanotechnology (CEINT). In filtered (0.45 μ m), control mesocosms water (pH 7.5,
105 conductivity 80 μ S/cm), AuNPs have a hydrodynamic diameter of 10.86 ± 1.44 nm and zeta
106 potential in ultrapure water of -13 ± 6.24 mV. Full characterization of gold nanoparticles used in
107 this experiment has previously been reported^{8,22}. Each week 31.36 μ g of AuNP were added to
108 each microcosm for a total of 125.44 μ g added over the four weeks of the experiment. To dose,
109 each microcosm received 196 μ L of 160 ppm AuNP stock solution pipetted across the water
110 surface for even dispersal of nanoparticles.

111 Experimental Design and microcosm setup

112 Microcosms were built into acid-washed one-quart Ball® glass mason jars (16.36 x 8.59
113 cm). Water and *E. densa* were collected in July 2017 from a control mesocosm setup from the
114 previous experiment in Duke Forest. Each microcosm was inoculated with phytoplankton
115 communities collected from a local wetland and filtered through a 0.25 mm sieve to remove large
116 organisms and debris. *Physa gyrina* were purchased from Carolina Biological Supply. We used
117 two seasonal conditions (spring vs fall), two AuNP conditions (AuNP vs Control), and two food
118 web conditions. The two food web conditions created were Phytoplankton + Macrophyte, and
119 Phytoplankton + Macrophyte + Snails. This full factorial design resulted in 8 treatments with 48
120 microcosms in total (2 food webs x 2 seasons x 2 AuNP x 6 technical replicates). Macrophyte
121 shoots were added to a bin with DI water and loaded onto a shaker for one hour at medium speed
122 to ensure *E. densa* did not contain any phytoplankton or organisms. Shoots were examined again
123 before placing them into the microcosms.



124

125 Figure 1: Experimental design presenting food web, seasons, and AuNP treatments.

126 Each microcosm was assembled using 100 g of Quikrete pool filter sand thoroughly rinsed with
 127 DI water and dried at 100°C, 700 mL of filtered (0.25mm) mesocosm water, 1 mL of
 128 phytoplankton communities, and five shoots of rinsed *E. densa* with a total wet biomass of 6g.
 129 Microcosms were then capped with Parafilm® to allow the exchange of gases as well as light
 130 infiltration and placed in their respective controlled environmental growth chamber. Macrophytes
 131 and phytoplankton were acclimated to the microcosms for one week. During the acclimation
 132 period, microcosms were dosed with 0.1433 mg of Nitrogen (N) as KNO₃ and 0.05697 mg of
 133 phosphorus (P) as KH₂PO₄. Nutrient addition continued weekly for the duration of the experiment
 134 for a total of 0.7165 mg of N and 0.2849 mg of P over five weeks. After the one-week acclimation
 135 period, snails and AuNP treatments were added.

136 For the snail treatments, five *P. gyrina* were added to the microcosms. After receiving snails from
137 Carolina Biological, snails were kept in holding aquariums filled with the same water, sand,
138 phytoplankton, and *E. densa* as the microcosms, allowing them to clear their intestinal tract for 48
139 hours and acclimate to the experimental conditions²⁵. For each microcosm that received snails,
140 five snails were removed from the holding aquariums, lengths and weights were recorded, and
141 unique enamel paint markings were applied to each snail before placing the snails in their
142 respective microcosms. The size variation for individual snails was maintained for each microcosm
143 replicate (Table S1). To control for any effects on the systems from the addition of the enamel
144 paint, glass beads were tagged in the same way as the snails and placed in all microcosms that did
145 not contain snails. During the experiment, we replaced dead snails and removed any snail egg
146 masses to keep the number of snails and snail biomass consistent across all treatments. Microcosms
147 were monitored twice a week for egg clutches and snail death. Egg clutches were removed with a
148 disposable transfer pipette with the tip cut. When snail death occurred, they were removed and
149 replaced with a snail similar in size and weight, and the timing of exposure in the microcosms for
150 each snail was tracked if they were introduced later in the experiment.

151 *Incubation chambers*

152 Our seasonality treatments were maintained by running the experiment in two controlled
153 environmental growth chambers that were set to mimic the average seasonal conditions recorded
154 during our previous field mesocosm experiment. In the spring treatment, the chamber oscillated
155 between a 15-hour day at 30°C and a 9-hour night at 20°C. In the fall treatment, the chamber
156 cycled between a 12-hour day at 15°C and a 12-hour night at 10°C. All work was performed inside
157 of the incubators, and microcosms were maintained on wire shelving units equipped with

158 fluorescent lights. Jars were randomized within these shelving units, with replicates rotated twice
159 a week to eliminate any shading effects.

160 Sample collection and analysis

161 After 28 days of AuNP exposure, microcosms were disassembled. Water samples were
162 collected and dissolved organic matter and total nitrogen (TN) were measured using TOC- VCPH
163 Analyzer with a TNM-1 module (Shimadzu, Kyoto, Japan), and ammonium and phosphate were
164 analyzed using Lachat QuikChem 8500 autoanalyzer. Au concentration was determined using
165 ICP-MS after acidification of the samples with HNO₃.

166 We determined the biomass and tissue Au concentrations of macrophytes, macrophyte
167 associated biofilm, and snails. To remove the biofilm from *E. densa* shoots, shoots from each
168 microcosm were placed into 100 mL of ultrapure water in a 250 mL Nalgene bottle and vigorously
169 shaken by hand for 30 seconds. The cleaned shoots were then placed in aluminum tins and dried
170 at 60°C for 48 hours to determine total dry biomass. The biofilm that was removed from the *E.*
171 *densa* shoots was transferred to a weigh boat and dried at 60°C for 48 hours and weighed. Snails
172 were removed from their microcosms and placed into a 15 mL conical tube and stored in the freezer
173 until mass and Au content were measured. After thawing, snails were removed from their shell
174 and dried at 60°C for 24 hours. After weighing all dried biomass samples were ground to a fine
175 powder and weighed before digestion for metal analysis.

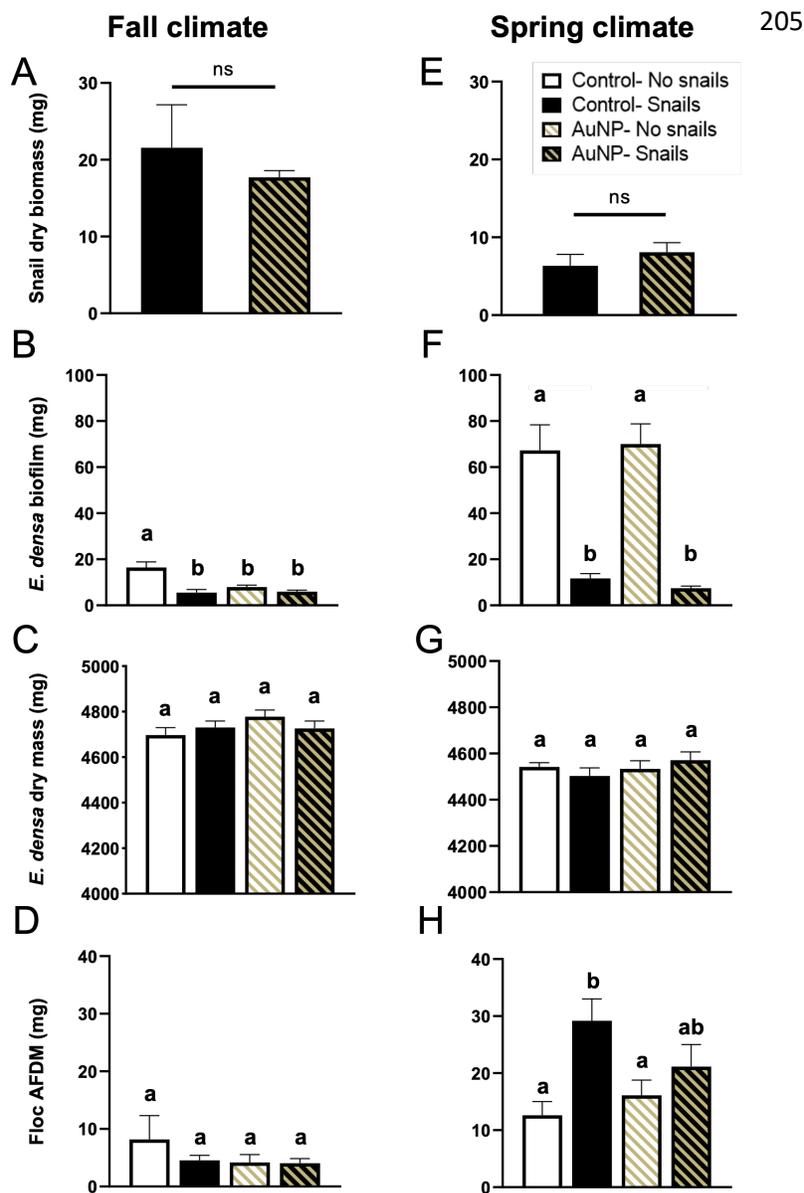
176 We expected unconsolidated organic matter, or floc, which accumulated on top of the sand
177 throughout the experiment to be an important reservoir of Au. The constituents of the floc that
178 accumulated varied by treatment. The Phytoplankton + Macrophyte mainly consisted of decaying
179 *E. densa* debris, and Phytoplankton+Macrophyte+Snails was a combination of *E. densa* debris and

180 snail excrement. At the end of the experiment, transfer pipettes were used to collect all the floc
181 from each replicate. Floc samples were then homogenized, and subsamples were concentrated onto
182 a 0.7 μm pore glass fiber filter attached to a filtering apparatus. All filters were dried at 60°C for
183 48 hours and weighed to determine dry floc mass. After weighing, each filter was cut in half and
184 re-weighed. Half of the filter was subsequently combusted at 500°C for 1 hour in a muffle furnace
185 to determine the ash-free dry mass (AFDM). The second half was then processed to determine
186 AuNP content.

187 All dried solid samples were subsequently digested and analyzed for their Au content. The
188 glass fiber filters used to determine AFDM were cut into small pieces and transferred into Teflon
189 vials with 1 mL of 15 N hydrofluoric acid and 2 mL of 15 N nitric acid. Samples were heated for
190 30 min and allowed to air dry under a fume hood until all acid was removed. Rehydrated snails,
191 biofilm, pre-digested filters, and *E. densa* powders were then digested in 70% HNO₃ overnight
192 and heated for 30 minutes at 95°C. HCl 30% was added to match a 3:1 HNO₃: HCl volume ratio
193 and heated additionally at 95°C for 30 minutes. All digested samples were diluted to 2% HNO₃,
194 filtered at 0.45 μm , and analyzed for Au concentration by ICP-MS (Agilent 7700 for plant and floc
195 samples and Agilent 7900 for snail and biofilm samples). Spike and certified reference material
196 recoveries were 95.6 \pm 4%, and detection limits were determined as (mean of 3 different reagent
197 blanks) + (3 \times SD of 10 replicate analyses of the reagent blank solution).

198 Statistical analyses

199 Endpoints were analyzed using either a one-way ANOVA to test for AuNP and snail
200 treatment effects or a t-test when testing for snail effects or AuNP effects separately. One-way
201 ANOVAs were followed by a Tukey posthoc test. All statistical analyses were performed in
202 GraphPad Prism 8.0.1



206

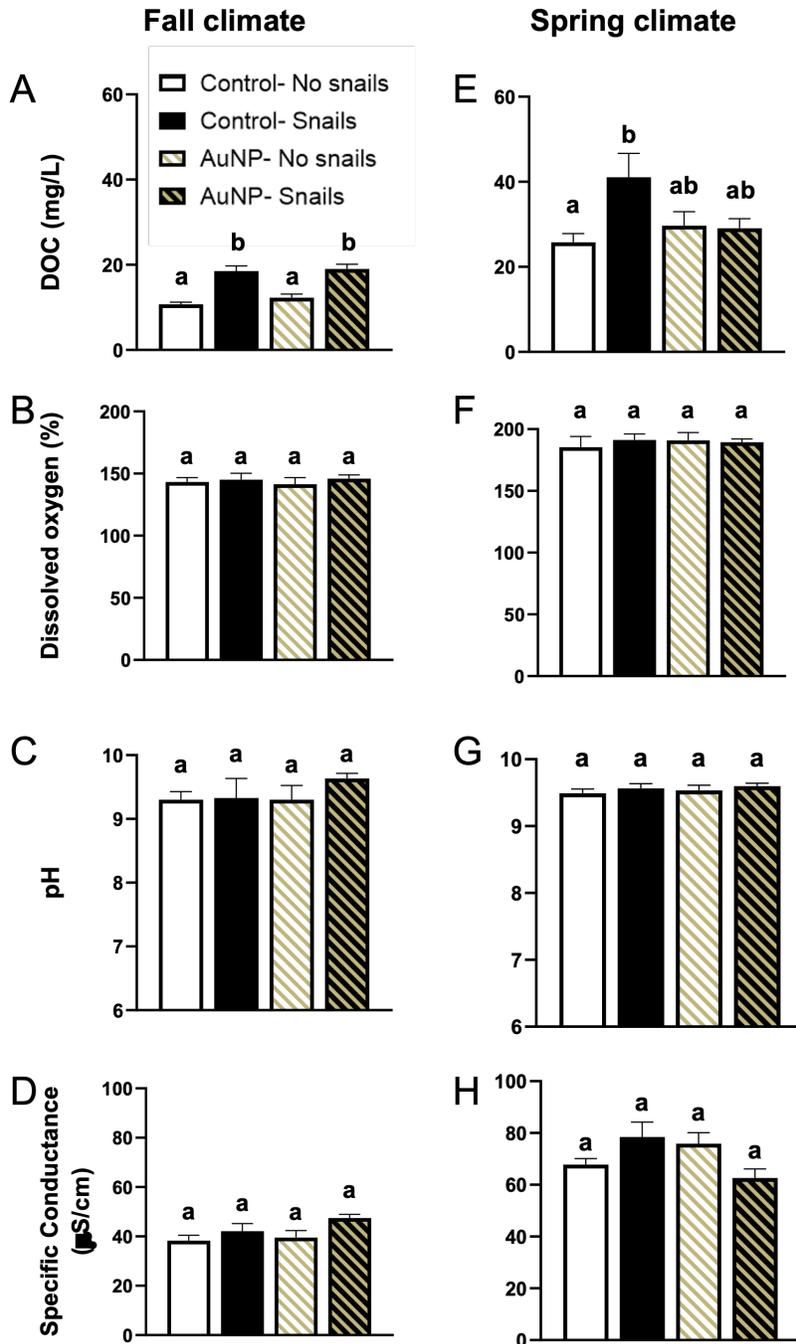
207 Figure 2: Total dry biomass of each compartment in the microcosms at the end of the experiment
 208 (28 days). Panels A-D and E-H represent the biomass in the fall and spring microcosms,
 209 respectively. Data shown are the averages of six technical replicates \pm SEM. Note the y-axis range

210 on panels C and G graphs. Letters represent a significant difference ($P < 0.05$) from Tukey's post
211 hoc test. Asterisks indicate statistical significance from a Student's t-test, * $P < 0.05$, ** $P < 0.001$,
212 *** $P < 0.001$.

213 To assess how seasonal changes, AuNP, and snail presence influenced biotic growth after
214 28 days of AuNP exposure, total dry biomass was measured for each compartment in the
215 microcosms (Fig 2).

216 Limited effects of AuNP on biomass pools or water chemistry

217 Overall, AuNP exposure had limited effects on biomass pools or water chemistry in either
218 the fall or spring microcosms or with snail grazing presence. However, in the fall microcosms, the
219 amount of biofilm significantly decreased with the addition of AuNP compared to control without
220 snails (-52%, $P = 0.003$) (Fig. 2B). While no effects were seen on the snail, *E. densa*, or floc
221 biomass in the fall microcosms (Fig. 2 C-D). Additionally, no AuNP effects were seen in the spring
222 microcosms on biomass in any of the compartments measured. Additionally, AuNP did not alter
223 water chemistry parameters in either season (Fig. 3). Furthermore, we did not observe a difference
224 in *E. densa* photosynthetic and respiration rates with Au present compared to control (Fig. S6).



225

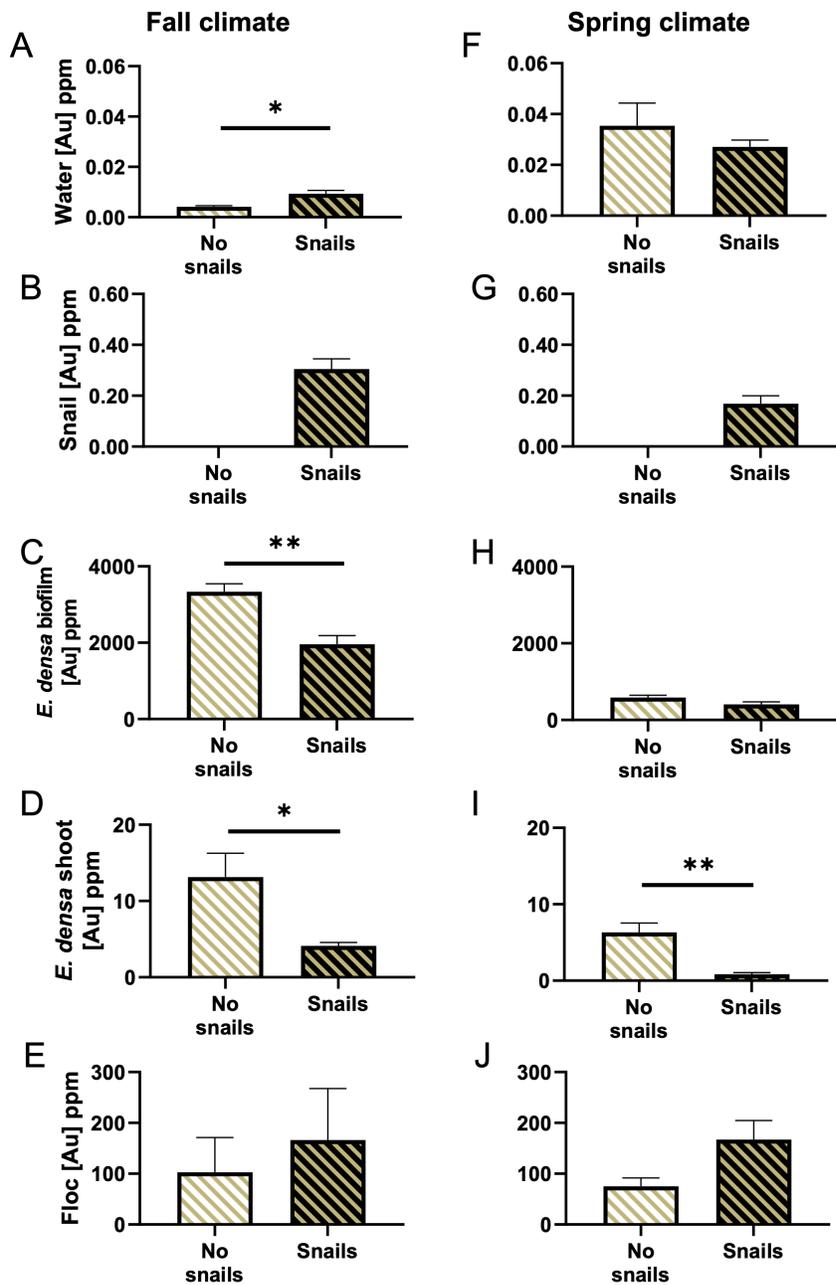
226 Figure 3: Dissolved organic carbon (DOC), Dissolved oxygen (DO), pH, and Specific
 227 Conductance in the water column of the microcosms. Data shown are the averages of six technical
 228 replicates \pm SEM. Letters represent a significant difference ($P < 0.05$) from Tukey's post hoc test.

229 *Strong seasonal and grazer effects on biomass pools and water chemistry*

230 Seasonal differences had a substantial impact on the amount of biomass for all
231 compartments. Snail and *E. densa* biomass decreased in the spring conditions compared to the fall
232 (-63%, $P < 0.001$ and -4.1%, $P = < 0.001$, respectively) (Fig. S1) while biofilm and floc organic
233 matter (OM) increased by 3.7-fold and 4.3-fold in the spring conditions compared to the fall ($P <$
234 0.001 and $P = < 0.001$, respectively) (Fig. S1). Overall, the spring season had an increase in DOC,
235 total nitrogen (TN), ammonium nitrogen ($\text{NH}_4\text{-N}$), and phosphorus ($\text{PO}_4\text{-P}$) in the water column
236 compared to the fall ($P < 0.001$, Fig. S1-S2). Additionally, dissolved oxygen (DO) and specific
237 conductance also increased in the spring compared to the fall by 32% and 70%, respectively
238 ($P < 0.001$, Fig. S3), while pH remained similar between the two seasonal conditions.

239 In the fall conditions, snail grazing reduced the amount of biofilm by 66% in the controls
240 and 25% in the AuNP treatments ($P < 0.001$ and $P = 0.77$, respectively) (Fig. 2B). Snail presence
241 did not influence the biomass of *E. densa* or floc; however, DOC within the water column
242 increased in the control treatments by 73% ($P < 0.001$) and AuNP treatments by 55% ($P < 0.001$)
243 with the addition of snails (Fig. 3A). Further, snail presence increased the amount of total nitrogen
244 and phosphate in both control and AuNP treatments (Fig. S4).

245 In the spring conditions, snail grazing reduced the amount of biofilm by 97% in the control
246 and 89% in the AuNP treatments ($P < 0.001$ and $P < 0.001$, respectively) (Fig. 2F). Snail presence
247 also increased the amount of floc (OM) in the control treatments by 1.3-fold ($P = 0.009$, Fig. 2H).
248 Snail presence also increased the amount of DOC in the control only treatment by 59% ($P = 0.03$,
249 Fig. 3E) and had no significant impact on TN or phosphorus amounts (Fig. S2).



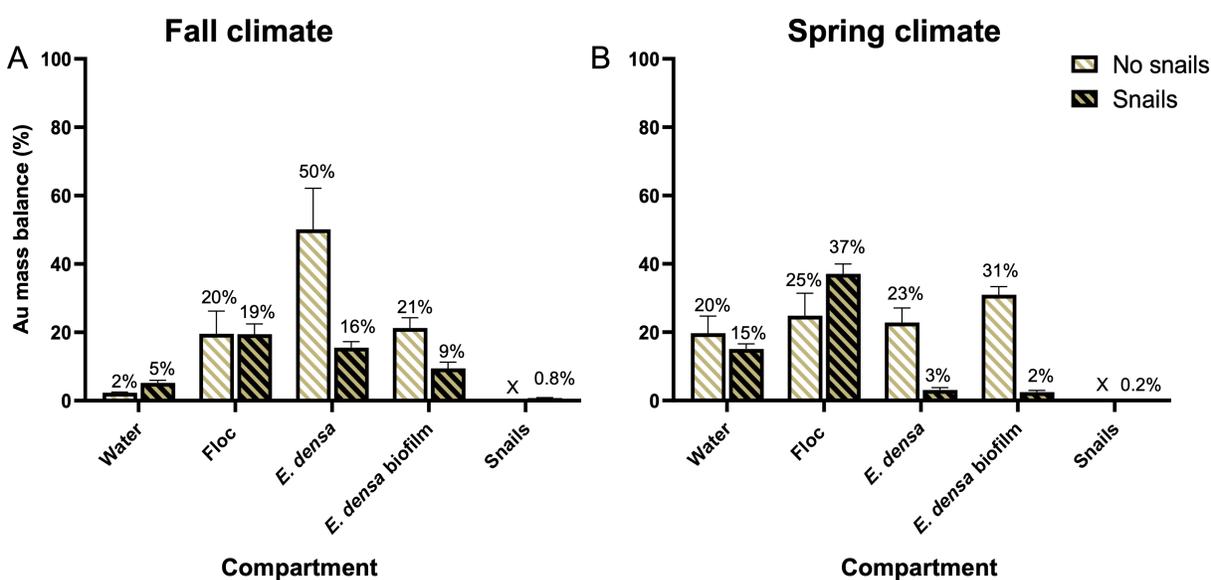
250

251 Figure 4: Au concentration in each compartment in the microcosms. Data shown are the averages
 252 of six technical replicates \pm SEM. Asterisks indicate statistical significance from a Student's t-test,
 253 * $P < 0.05$, ** $P < 0.001$, *** $P < 0.001$.

254 Seasonal differences and grazing altered AuNP fate

255 Seasonal changes and snail grazing also played a significant role in the AuNP concentration
256 found in each compartment in the microcosms. In the fall microcosms, the presence of snails
257 significantly increased the Au concentration in the water column by 1.23 fold (Fig. 4A, $P=0.005$),
258 while decreasing the concentration found in the biofilm by 41% (Fig. 4C, $P=0.001$) and *E. densa*
259 shoots by 69% (Fig. 4D, $P=0.02$).

260 In the spring microcosms, snail presence decreased the Au concentration in the *E. densa*
261 shoots by 86% (Fig. 4I, $P=0.001$). There was also a trend for higher Au concentrations in the floc
262 in the fall and spring conditions with the presence of snails ($P=0.63$ and $P=0.09$).



263
264 Figure 5: Au recovery in microcosm compartments. Microcosms without snails showed a higher
265 recovery of Au than microcosms with snails present. Panel A and B represent the percentage of
266 AuNP recovered in each abiotic or biotic compartment within the microcosms for fall and spring

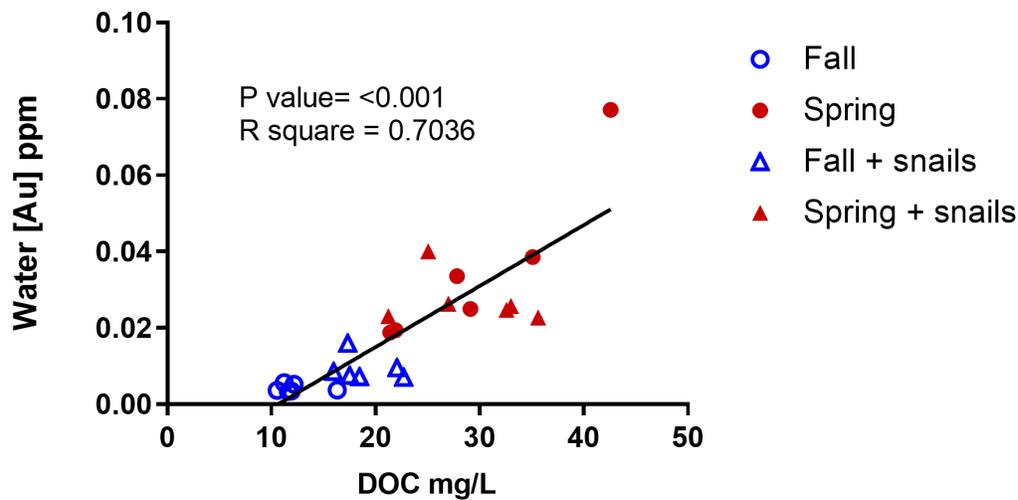
267 seasons, respectively. Data shown are the averages of six technical replicates \pm SEM. Percentages
268 were calculated based on the total amount of Au added to microcosms.

269 *Au mass balance and recovery*

270 Au mass balance was calculated in the microcosms with almost complete recovery in the
271 treatments without snails for both fall and spring conditions. While 96-104% of Au was recovered
272 in the microcosms without snails, 50-60% of Au was recovered in the treatments with snails (SI
273 Fig. 5).

274 In the fall microcosms, snail presence altered the fate of Au compared to the treatments
275 without snails. The majority of Au added to the treatment without snails was found associated with
276 *E. densa* (shoots and roots combined), followed by biofilm and floc. In the treatment with snails,
277 most of the Au recovered was found in the floc and *E. densa* with a substantial portion not being
278 recovered.

279 In the spring microcosms without snails, similar amounts of Au were recovered in the
280 different compartments, with values ranging on average from 20% in the water column to 31% in
281 the biofilm. In the treatments with snails, Au was mainly accumulated in the floc and water, with
282 a large portion not being recovered. In the spring season, the Au concentration in the water column
283 was higher than in the fall.



284

285 Figure 6: Correlation of Au concentration vs. DOC in the water column. P-value based on a
 286 Pearson correlation test.

287 Environmental drivers associated with AuNP fate

288 More Au accumulated in the water column in the spring microcosms with and without
 289 snails compared to the fall microcosms. To better understand what was driving this accumulation,
 290 correlations of different water parameters were investigated in relation to the Au water column
 291 concentration. The strongest correlation to the accumulation of Au in the water column was the
 292 DOC concentration in the water column with an R² value of 0.7036. (P<0.001, Figure 6).

293

294 **Discussion**

295 After 28 days of chronic AuNP exposure, the different seasonal conditions did not alter
 296 AuNP effects but they did have a significant impact on the biomass, nutrients, and competitive
 297 interactions between the *E. densa* and their biofilm. In the spring conditions without snails, biofilm
 298 growth was abundant, which could cause stress to the macrophyte by increasing competition for

299 nutrients and light²³. Higher biofilm growth and macrophyte stress with the warmer season could
300 result in higher DOC concentrations in the water column that was observed^{27,28}. Although the
301 spring temperature range was well within the tolerant range for both the macrophyte *E. densa* and
302 the snail *P. gyrina*, both species showed visual stress from these conditions. The *E. densa* had a
303 slight overall decrease in biomass in the spring conditions compared to the fall, while *P. gyrina*
304 saw a higher death rate and lower biomass.

305 Concerning the impact of the AuNP chronic addition, Simonin et al have shown that an
306 association of AuNP with the macrophyte tissues could reduce the photosynthetic and respiratory
307 rates of *E. densa*¹⁵. In this study, we found that *E. densa* was a large sink of Au, similar to what
308 was described in more complex mesocosm systems¹⁶. However, we did not observe a decrease in
309 photosynthetic or respiratory rates. This could have been due to the total duration of the microcosm
310 experiment, which only lasted five weeks, compared to the mesocosm experiment which lasted
311 nine months. Also, we did not observe a difference in biomass from the *E. densa* stems that were
312 placed in any microcosms meaning they did not undergo substantial growth. These factors could
313 result in similar photosynthetic and respiratory rates despite having the Au addition.

314 Little impact was thus observed on the primary producer's and consumer's biomasses.
315 However, while the *E. densa* biomass and photosynthetic activity were not altered by any of the
316 treatments, we did see a reduction in biofilm growth in the fall microcosms exposed to AuNP
317 without the presence of snails. Since biofilm growth decreased in the colder season and narrower
318 window of light, the addition of AuNP could have posed as an additional stressor, further reducing
319 the amount of biofilm growth. Although a reduction of biofilm was not observed in the spring
320 microcosms, this could have been due to the abundant growth of biofilm in ideal conditions as

321 well as the higher amount of DOC in the water column leading to the stabilization of Au in the
322 water column, thus reducing deposition on the biofilm, as discussed below.

323 Although the impacts of AuNP were minimal on biomass, the fate of AuNP was
324 dramatically altered with the addition of the aquatic snail, *P. gyrina*. The primary mechanisms by
325 which *P. gyrina* altered Au fate in the microcosms were likely by the consumption of biofilm that
326 accumulated on the leaves of the aquatic macrophyte, *E. densa*. In our study, *P. gyrina* reduced
327 the biofilm amount by 89-97% in the spring season and 25-66% in the fall. Macrophyte associated
328 biofilm growth has been shown to transform AuNP into oxidized, dissolved Au¹³, which could be
329 more readily available to other aquatic organisms, but also increase its stability among the water
330 column. With the addition of *P. gyrina*, biofilm growth in the spring conditions resulted on average
331 a 20% reduction of Au associated with the *E. densa* tissue and an accumulation in the floc at the
332 bottom of the microcosms. In the fall conditions, the addition of snails also decreased the amount
333 of biofilm growth and accumulation in *E. densa*; however, the reduction of biofilm was smaller
334 due to less growth overall. Remarkably, the addition of snails had the most dramatic reduction of
335 Au associated with *E. densa* in the fall conditions with an average reduction of 34%.

336 The addition of snails also increased the amount of DOC found in the water column
337 possibly due to snail excretions. This accumulation of DOC in the water column likely contributed
338 to the stabilization of Au seen in the water column, as suggested by the strong correlation observed
339 between these two traits. The presence of DOC in the water column has been shown to stabilize
340 AuNP and reduce adsorption into macrophyte tissues^{18,25}. Similarly, organic matter released by *E.*
341 *densa* in mesocosms has been shown to stabilize AgNP in the water column²⁹.

342 While Au in both seasons without snails had full recovery (96-104%, Fig. S5), microcosms
343 with snails had lower recovery (50-60%, Fig. 5S). There are several possible scenarios why lower

344 Au recovery was seen with snails, such as a result of Au attaching to the shells of the snails, which
345 were not measured for Au concentration. Similar to biofilm growth on aquatic macrophytes,
346 biofilm growth can also occur on hard substrates such as the shell of mollusks³⁰. Since snail death
347 was observed in both seasons, the removal of dead snails could also be a sink for Au both within
348 tissues and shells. Further, aquatic snails can secrete extracellular polysaccharides, which can also
349 be a sink for metals^{31,32}. Alternatively, it could be due to the heterogeneity of Au associated with
350 floc. While the majority of the floc was recovered from the microcosms complete recovery was
351 not possible, which could account for the remaining Au.

352 This study highlights how critical climate and ecosystem complexity can alter the fate and
353 effects of emerging chemicals. Using simplified systems allowed us to highlight the significant
354 role of aquatic grazers in altering the fate of Au in freshwater environments. The addition of only
355 five snails grazing on epiphyton significantly shifted the fate of Au from the macrophyte tissues
356 to other compartments within the microcosms. This shift in fate can have significant impacts on
357 water quality by increasing macrophyte growth, reducing periphyton buildup, and possibly
358 reducing the frequency of algal blooms. Although the use of microcosms allowed for higher
359 replication and ease of testing different climate conditions, this setup is likely far from any natural
360 ecosystem. Therefore, future studies should focus on using large scale mesocosms or field sites.

361 ASSOCIATED CONTENT

362 **Supporting Information**

363 Additional information on overall differences in biomass and water chemistry between the fall and
364 spring microcosms, as well as overall Au recovery and photosynthesis measurements in *E. densa*.
365 (PDF)

366 AUTHOR INFORMATION

367 **Corresponding Author**

368 *Marie Simonin - Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers,
369 France; Email: marie.simonin@inrae.fr

370 **Present Addresses**

371 Christina Bergemann - Nicholas School of the Environment, Duke University, Durham, NC
372 27708, USA

373 Astrid Avellan - Géosciences Environnement Toulouse, CNRS, Toulouse University, CNES, IRD,
374 UPS, 31400 Toulouse, France

375 Marie Simonin - Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers,
376 France

377

378 ACKNOWLEDGEMENTS

379 We would like to thank Dr. Ryan King, Eva May, Nia Bartolucci, Benjamin Wernette, Gary
380 Dwyer, Jeffrey A. Back, and Jenny Rocca for their help in the experimental setup, sample
381 collection, and overall feedback. Funding was supported by the National Science Foundation
382 (NSF) and the Environmental Protection Agency (EPA) under NSF Cooperative Agreement EF-
383 0830093 and DBI-1266252, Center for the Environmental Implications of Nanotechnology
384 (CEINT).

385

386 REFERENCES

- 387 1. Ghosh, P., Han, G., De, M., Kim, C. K. & Rotello, V. M. Gold nanoparticles in delivery
388 applications. *Adv. Drug Deliv. Rev.* **60**, 1307–1315 (2008).
- 389 2. Lowry, G. V., Avellan, A. & Gilbertson, L. M. Opportunities and challenges for
390 nanotechnology in the agri-tech revolution. *Nat. Nanotechnol.* **14**, 517–522 (2019).
- 391 3. Li, L., Xu, Z., Kah, M., Lin, D. & Filser, J. Nanopesticides: A Comprehensive Assessment of
392 Environmental Risk Is Needed before Widespread Agricultural Application. *Env. Sci Technol*
393 **53**, 59 (2019).
- 394 4. Miralles, P., Church, T. L. & Harris, A. T. Toxicity, Uptake, and Translocation of Engineered
395 Nanomaterials in Vascular plants. *Env. Sci Technol* **46**, 19 (2012).
- 396 5. Garner, K. L. & Keller, A. A. Emerging patterns for engineered nanomaterials in the
397 environment: A review of fate and toxicity studies. *J. Nanoparticle Res.* **16**, (2014).
- 398 6. Ferry, J. L. *et al.* Transfer of gold nanoparticles from the water column to the estuarine food
399 web. *Nat. Nanotechnol.* **4**, 441–444 (2009).
- 400 7. Wray, A. T. & Klaine, S. J. Modeling the influence of physicochemical properties on gold
401 nanoparticle uptake and elimination by *Daphnia magna*. *Environ. Toxicol. Chem.* **34**, 860–872
402 (2015).
- 403 8. C. Surette, M. & A. Nason, J. Effects of surface coating character and interactions with natural
404 organic matter on the colloidal stability of gold nanoparticles. *Environ. Sci. Nano* **3**, 1144–
405 1152 (2016).
- 406 9. Smith, B. M., Pike, D. J., Kelly, M. O. & Nason, J. A. Quantification of Heteroaggregation
407 between Citrate-Stabilized Gold Nanoparticles and Hematite Colloids. *Environ. Sci. Technol.*
408 **49**, 12789–12797 (2015).

- 409 10. Santhoshkumar, J., Rajeshkumar, S. & Venkat Kumar, S. Phyto-assisted synthesis,
410 characterization and applications of gold nanoparticles – A review. *Biochem. Biophys. Rep.*
411 **11**, 46–57 (2017).
- 412 11. Elahi, N., Kamali, M. & Baghersad, M. H. Recent biomedical applications of gold
413 nanoparticles: A review. *Talanta* **184**, 537–556 (2018).
- 414 12. Farzin, M. A., Abdoos, H. & Saber, R. AuNP-based biosensors for the diagnosis of pathogenic
415 human coronaviruses: COVID-19 pandemic developments. *Anal. Bioanal. Chem.* **414**, 7069–
416 7084 (2022).
- 417 13. Avellan, A. *et al.* Gold nanoparticle biodissolution by a freshwater macrophyte and its
418 associated microbiome. *Nat. Nanotechnol.* **13**, 1072–1077 (2018).
- 419 14. Alkilany, A. M. & Murphy, C. J. Toxicity and cellular uptake of gold nanoparticles: What we
420 have learned so far? *J. Nanoparticle Res.* **12**, 2313–2333 (2010).
- 421 15. Simonin, M. *et al.* Engineered nanoparticles interact with nutrients to intensify eutrophication
422 in a wetland ecosystem experiment HHS Public Access. *Ecol Appl* **28**, 1435–1449 (2018).
- 423 16. Avellan, A. *et al.* Differential Reactivity of Copper-and Gold-Based Nanomaterials Controls
424 Their Seasonal Biogeochemical Cycling and Fate in a Freshwater Wetland Mesocosm. *Env.*
425 *Sci Technol* **2020**, 30 (2020).
- 426 17. Perrotta, B. G. *et al.* Copper and gold nanoparticles increase nutrient excretion rates of primary
427 consumers. *Environ. Sci. Technol.* **54**, 10170–10180 (2020).
- 428 18. Glenn, J. B. & Klaine, S. J. Abiotic and Biotic Factors That Influence the Bioavailability of
429 Gold Nanoparticles to Aquatic Macrophytes. *Env. Sci Technol* **17**, 17 (2013).
- 430 19. Yang, L., Venneti, S. & Nagrath, D. Glutaminolysis: A Hallmark of Cancer Metabolism. *Annu*
431 *Rev Biomed Eng* **19**, 163–194 (2017).

- 432 20. Perrotta, B. *et al.* Copper and Gold Nanoparticles Increase Nutrient Excretion Rates of Primary
433 Consumers. *Environmental Science and Technology*. **2020**, 10170–10180.
- 434 21. Hall, R. O., Tank, J. L. & Dybdahl, M. F. Exotic snails dominate nitrogen and carbon cycling
435 in a highly productive stream. *Front. Ecol. Environ.* **1**, 407–411 (2003).
- 436 22. Nifong, R. L. Experimental effects of grazers on autotrophic species assemblages across a
437 nitrate gradient in Florida springs. *Aquat. Bot.* **139**, 57–64 (2017).
- 438 23. Yang, L. *et al.* Mesocosm experiment reveals a strong positive effect of snail presence on
439 macrophyte growth, resulting from control of epiphyton and nuisance filamentous algae:
440 Implications for shallow lake management. *Sci. Total Environ.* **705**, 135958 (2020).
- 441 24. Newman, R. M., Kerfoot, W. C. & Hanscom, Z. *Watercress Allelochemical Defends High-*
442 *Nitrogen Foliage Against Consumption: Effects on Freshwater Invertebrate Herbivores. III*
443 *Source: Ecology* vol. 77 2312–2323 (1996).
- 444 25. Oliver, A. L. S. *et al.* Does water chemistry affect the dietary uptake and toxicity of silver
445 nanoparticles by the freshwater snail *Lymnaea stagnalis*? *Environ. Pollut.* **189**, 87–91 (2014).
- 446 26. Kulacki, K. J., Cardinale, B. J., Keller, A. A., Bier, R. & Dickson, H. How do stream organisms
447 respond to, and influence, the concentration of titanium dioxide nanoparticles? A mesocosm
448 study with algae and herbivores. *Environ. Toxicol. Chem.* **31**, 2414–2422 (2012).
- 449 27. Zhang, P. *et al.* Effects of Rising Temperature on the Growth, Stoichiometry, and Palatability
450 of Aquatic Plants. *Front. Plant Sci.* **9**, 1947 (2019).
- 451 28. Reitsema, R. E., Meire, P. & Schoelynck, J. The future of freshwater macrophytes in a
452 changing world: Dissolved organic carbon quantity and quality and its interactions with
453 macrophytes. *Front. Plant Sci.* **9**, 629 (2018).

- 454 29. Unrine, J. M., Colman, B. P., Bone, A. J., Gondikas, A. P. & Matson, C. W. Biotic and Abiotic
455 Interactions in Aquatic Microcosms Determine Fate and Toxicity of Ag Nanoparticles. Part 1.
456 Aggregation and Dissolution. (2012) doi:10.1021/es204682q.
- 457 30. Minton, R. L., Creech, C. C. & Jackson, D. W. Bacterial diversity and abundance in shell
458 biofilms from the freshwater snail *Pleurocera canaliculatum*(cerithioidea: Pleuroceridae). *Pol.*
459 *J. Microbiol.* **64**, 181–184 (2015).
- 460 31. Ballance, S. *et al.* Influence of sediment biofilm on the behaviour of aluminum and its
461 bioavailability to the snail *Lymnaea stagnalis* in neutral freshwater. *Can. J. Fish. Aquat. Sci.*
462 **58**, 1708–1715 (2001).
- 463 32. Jugdaohsingh, R. *et al.* Mucus Secretion by the Freshwater Snail *Lymnaea stagnalis* Limits
464 Aluminum Concentrations of the Aqueous Environment. *Environ. Sci. Technol.* **32**, 2591–
465 2595 (1998).
- 466