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# Dissolved organic matter controls of arsenic bioavailability to bacteria

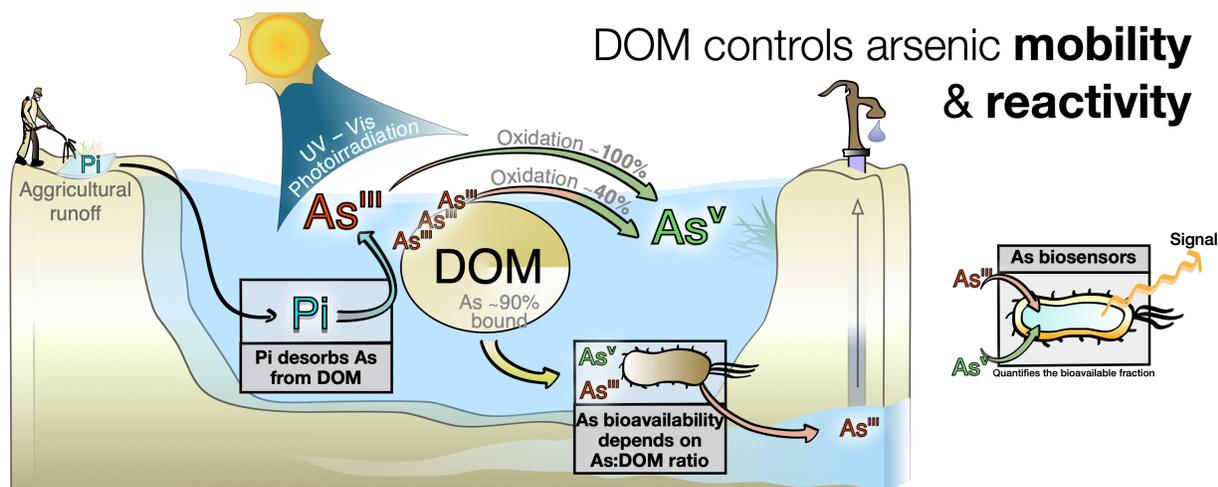
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Graphical abstract

15 **ABSTRACT**

16 **The presence of arsenic in irrigation and drinking waters is a threat to worldwide human**  
17 **health. Dissolved organic matter (DOM) is a ubiquitous and photoreactive sorbent of**  
18 **arsenic, capable of both suppressing and enhancing its mobility. Microbes can control the**  
19 **mobilization of mineral-bound arsenic, through redox processes thought to occur**  
20 **intracellularly. The role that DOM plays on the bioavailability of arsenic to microbes is**  
21 **often invoked but remains untested experimentally. Here, using a whole-cell biosensor, we**  
22 **tested the role of DOM on As (III) and As(V) bioavailability. Using cation amendments, we**  
23 **explored the nature of As-DOM interactions. We found As bioavailability to be dependent**  
24 **on [As]/[DOM] ratio and on the strength of As binding to DOM which varied as a function**  
25 **of time. We further tested the role of DOM on As(III) photooxidation and showed that As**  
26 **(III) photooxidation rate is limited by the strength of its interactions with DOM and**  
27 **sensitive to ionic competitive desorption. Our study demonstrates the dynamic control that**  
28 **photoreactive DOM poses on the bioavailability and reactivity of As in the environment**  
29 **and highlights the kinetic controls that DOM can possibly exert on As toxicity at various**  
30 **levels in foodwebs.**

**Keywords:** biosensor; bioavailability; arsenic; water quality; photooxidation

31 Arsenic (As), classified as a group 1 carcinogen (1), is estimated to affect the food  
32 and drinking waters of over 140 million people worldwide (2). The effects of human-  
33 mediated As release through mining, farming and manufacturing processes (1, 3) are  
34 observed at local, or even regional scales (3). Microbes, ubiquitous organisms  
35 operating at a global scale (4), are capable of affecting As mobility via catabolic (e.g.  
36  *aio, aso, aox, arr...*) or resistance (e.g.  *ars, arsM...*) pathways (5, 6). Therefore,  
37 conditions limiting As availability to microbes will reduce As remobilization.

38 Several environmental drivers of As bioavailability have been proposed (7) but very  
39 few have been directly tested. Such drivers include changes in redox (8), nutrients  
40 (9), cations (10) and solar radiation, known to profoundly affect organic material  
41 degradation, nutrient release and organic matter mineralization (11). Recently,  
42 Langner *et al.* (12) have presented spectroscopic evidence of covalent bonds between  
43 trivalent arsenic (As(III)) and natural organic matter (NOM) suggesting that NOM  
44 strongly governs the bioavailability and thus the mobility of As in anoxic peat.

45 DOM acts as a ubiquitous environmental sorbent of As through the formation of  
46 covalent (12) and ternary complexes with As (13, 14), thereby preventing solid phase  
47 sorption (7) and maintaining high As levels in soils (15) and in water (16-18). With  
48 the rise of terrestrially-derived DOM concentration of surface waters over the past  
49 few decades (19), we can expect that the role that DOM plays on As cycling will be  
50 increasing. The current mechanistic understanding of the interactions between As  
51 species and DOM includes: i) specific binding of As(III) to sulfur (12), and to amino  
52 (20) functional groups within DOM; ii) indirect cationic bridging of As(V) with calcium  
53 (14) and of As(III) with iron (21), and iii) weak electrostatic complex formation of  
54 As(III) and As(V) with phenolate or carboxylic functional groups (13, 21).  
55 Furthermore, previous experiments during which we evaluated As bioavailability in  
56 17 lakes sampled around Giant Mine (YK, Canada) and exhibiting a wide range of As  
57 and DOC concentrations, identified a role for DOC on As bioavailability (22).  
58 Surprisingly, we found that newly added As to lake water samples was less

59 bioavailable than older, “legacy” arsenic, already present in the system. This led us  
60 to hypothesize that As-DOM complex aging is important in controlling its  
61 bioavailability.

62 In this study, we hypothesized that DOM acts as a strong predictor of As  
63 bioavailability to microbes and predicted that strong As binding to DOM would reduce  
64 the pool of As available for microbial uptake. Using an As-specific biosensor (22), we  
65 monitored for changes in As(III) and As(V) bioavailability by altering [As]/[DOM],  
66 equilibration time, DOM origin, and investigated the role of DOM photoirradiation on  
67 As redox state and bioavailability. We found As bioavailability to be dependent on  
68 [As]/[DOM] ratio and on the strength of As binding to DOM which varied as a function  
69 of time.

70

## 71 **1. MATERIALS AND METHODS**

### 72 **1.1. Reagents**

73 All media and reagents were made and kept in pre-sterilized, acid washed containers.  
74 Purification of ultrapure water (Milli-Q), preparation/preservation of the 10 mM As  
75 standards, and constituents of growth (LB & MGP) and exposure media (MGP & MIP)  
76 have been previously described (22). Dissolved organic matter (DOM) standards were  
77 sourced from the International Humic Substance Society (IHSS): i) Suwannee River  
78 Natural Organic Matter 2R101N (NOM), ii) Suwannee River Humic Acid 3S101H  
79 (SRHA), iii) Suwannee River Fulvic Acid 3S101F (SRFA), and iv) Elliott Soil Fulvic Acid  
80 5S102F (ESFA). These represented a wide-range in sulfur (0.41 to 1.78% (w/w)),  
81 carboxyl (9.13 to 13.24 mg•g<sup>-1</sup> C) and phenolic (2.27 to 3.72 mg•g<sup>-1</sup> C) functional  
82 groups (23-25). Properties of the DOM origins used to produce this RDA can be found  
83 in Supplementary Information (Supplementary Table). DOM standards were prepared  
84 in acid washed volumetric flasks at 100 mg•L<sup>-1</sup> by dissolving 0.0050 g in 50 mL of  
85 ultra-pure (Milli-Q) water and incubated at 37 °C and 200 RPM for 24 h. Orbital

86 shaking/incubation ensured complete dissolution of the standards in Milli-Q water  
87 without adjustment of ionic strength or pH. Filtropur 0.2  $\mu\text{m}$  PES membranes filters  
88 (Sarstedt 83.1826.001) were used to ensure sterility of the standards and prevent  
89 microbial degradation during storage. Refraining from using buffering and  
90 preservation agents drove the pH to approximately 4.5. All DOM standards were  
91 prepared following the above noted method, and kept in dark containers at 4  $^{\circ}\text{C}$  for  
92 no longer than 3 weeks.

### 93 **1.2. Containers**

94 Containers used in this study include: i) Simport Scientific polypropylene 3 mL  
95 containers (Fisher Scientific 22-040-408) for all DOM incubation treatments that did  
96 not involve photoirradiation; ii) Globe Scientific Spectrophotometer polymethyl  
97 methacrylate 4.5 mL UV grade (280–800 nm) cuvettes (Fisher Scientific 111157) for  
98 all photoreactor assays; iii) Nalgene® High Density Polyethylene (HDPE) 500 mL  
99 bottles for all dialysis experiments, and Trace Metal Grade Corning® tubes for storage  
100 and analyses; and iv) Fisherbrand glass bottles (FB800100) for all media and reagent  
101 storage.

### 102 **1.3. Biosensor Culture and Exposure Protocol**

103 Biosensor construct, speciation protocol, and instrumentation used to incubate and  
104 quantify fluorescent output and culture yield of the biosensors are fully described  
105 elsewhere (22). Briefly, the biosensing construct was inspired by the design of J.  
106 Stocker (26), for which we used two ArsR binding sites shown to provide optimal  
107 detection while minimizing noise. The sensing-reporting sequence (ArsRBS2-  
108 mCherry) was constructed by custom gene synthesis (Integrated DNA Technologies)  
109 and cloned into the XmaI and XbaI restriction sites of the high copy pUCP19 shuttle  
110 vector upstream of the sequence encoding for mCherry. The reporter plasmid was  
111 transformed into *E. coli* NEB10-beta (New England BioLabs) – a level 1, non-  
112 pathogenic, non-regulated host. The MGP growth and exposure media, comprised of  
113 glycerol and organic phosphate, were used in all biosensor exposure assays. Use of

114 these media was found to allow for the uptake of over 94% of the added As(III) and  
115 As(V) additions (22).

116 For all assays, As concentration was set at 200 nM because this concentration falls  
117 at a mid-point in the linear range of the biosensor's calibration curve and has  
118 previously led to reproducible quantification (22). Furthermore, this concentration is  
119 environmentally relevant as the World Health Organization set a guideline of 133 nM  
120 (10 µg/L) for As in drinking water (27). Biosensor exposure protocol consists of two  
121 steps. First, all working solutions (DOM, ions, and As) were prepared at a final  
122 concentration of 2× and added to the containers in the following order: (i) Milli-Q  
123 water, (ii) DOM, (iii) cations (if required), and (iv) arsenic. Time of interaction prior  
124 to exposing the biosensor cells was assay dependent and conducted in the dark and  
125 at room temperature. Second, pre-incubated working solutions were then  
126 subsampled into three separate wells on the 96 well plate then analyzed by three  
127 individually grown biosensor cultures. Exposure occurred in the following order: (i)  
128 100 µL subsample of the pre-incubated 2× concentrated working solution, (ii) 80 µL  
129 of 2× concentrated exposure medium (MGP or MIP), and (iii) 20 µL of MGP grown  
130 biosensor cells for a final biosensor cell concentration of 10%.

#### 131 **1.4. Data analysis**

132 The biosensor fluorescent output present in all figures has been corrected for  
133 autofluorescence, noise and culture health using R programming language. First,  
134 autofluorescence is a background interference that differs by DOM origins, bacteria  
135 cultures, reagents, and by well in the exposure plate. Standardization consists of  
136 subtracting endpoint output signal (20 h) of each well from the initial output (T<sub>0</sub>) of  
137 that same well (**Fig. S7a**). Second, we refer to background noise as the signal  
138 produced by biosensor cells that are not exposed to As; even in the absence of the  
139 inducer (As) there is a basal expression of fluorescence that must be controlled for.  
140 Removal of background noise consists of the difference between signal output of the  
141 treatment (e.g. As + SRHA) and of the no-As control at the same timepoint (20 h)

142 and of the same biosensor culture (**Fig. S7b**). Third, differences in biosensor fitness  
143 or yield can arise when working with environmental samples (**Fig. S1**). Normalization  
144 consists of dividing the corrected output signal by the culture's optical density  
145 (OD600) at the same time point (**Fig. S7c**). Finally, assuming no-DOM controls  
146 represents 100% bioavailability, we can convert the biosensor's fluorescent output  
147 signal to percent biouptake. This correction involves dividing the normalized  
148 biosensor output of the treatments (e.g. As + SRHA) by output signal of the As-only  
149 calibration point control and multiply by 100 (**Fig. S7d**).

### 150 **1.5. Photoirradiation**

151 A Luzchem Photoreactor was used for all photoirradiation assays. These consisted of  
152 a 22 h pre-incubation assay followed by a 4 h irradiation step subsampled at 0 h (T0),  
153 2 h (T2) and 4 h (T4). Similarly to the exposure protocol previously described,  
154 samples were prepared, incubated, and irradiated at 2× their final concentration (400  
155 nM As(III) & 20 mg/L DOM), then diluted to 1× upon exposure to the biosensors.  
156 The power per unit area (irradiance) for wide-band UV radiation was measured using  
157 GOLDILUX ultraviolet meters, UVA (315–400 nm), and UVB spectrum (280–315 nm).  
158 Irradiance intensity for UVA averaged  $0.037 \text{ J}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and for UVB averaged  $0.129$   
159  $\text{J}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Spectral components of light from 200 nm to 1000 nm were analysed using  
160 a ThorLabs compact CCS200 (**Fig. S5**).

### 161 **1.6. Chemical analysis**

162 Cross-analysis of HPLC-ICP-MS and the biosensor speciation technique was  
163 performed on the same-day and on the same samples. Subsamples for As speciation  
164 analysis were preserved using HNO<sub>3</sub> at 0.3% (2 μL of 70% HNO<sub>3</sub> in 398 μL Milli-Q  
165 plus 100 μL of sample). Details regarding HPLC-ICP-MS speciation analysis are  
166 detailed elsewhere (22).

## 167 **1.7. Dialyses experiments**

168 All dialysis experiments, used to quantify the fraction of As bound to DOM, were  
169 performed in triplicate and consisted of adding DOM ( $10 \text{ mg}\cdot\text{L}^{-1}$ ) and As (200 nM,  
170 either As(III) or As(V)) to the inside of a dialysis bag (pore size 500 Da). Similarly to  
171 the biosensor exposure assays, no buffering agents were used. For experiments  
172 involving major ions,  $10 \text{ mM Na}^+$  or  $\text{Ca}^{2+}$  were added both inside the dialysis bag and  
173 in the external solution, at the same ionic strength to prevent crossing of As induced  
174 by osmotic gradient. Quantification of As species levels (ICP-MS) for the external  
175 solution, was performed at both T0 and T24 h whereas measurement for the internal  
176 solution was only quantified after a 24 h contact time. Here, quantification of [As]  
177 inside the dialysis bag represents both bound and unbound As, whereas quantification  
178 of the outside fraction represents only the “free” or unbound fraction. All  
179 concentrations in  $\mu\text{g}\cdot\text{L}^{-1}$  used to determine the bound fraction were first converted  
180 to pmols to account for differences between internal (3 mL) and external (300 mL)  
181 volumes before percent conversions (**Fig. S3**).

182

## 183 **1.8. FEEM analysis**

184 Measurement of Fluorescence Excitation/Emission Matrices (FEEM), were performed  
185 in triplicate by mixing DOM ( $10 \text{ mg}\cdot\text{L}^{-1}$ ) and As (200 nM, either As(III) or As(V)) to  
186 a 3 mL quartz Suprasil cells, at room temperature, in the dark. No buffering or  
187 acidifying agents were used. Fluorescence Excitation/Emission Matrices were  
188 regularly measured on a HITACHI F4500 spectrofluorometer. The excitation  
189 wavelength ranged from 320 to 460 nm, with 10 nm step and an excitation slit of 1  
190 nm. The corresponding emission spectra were acquired from 350 to 550 nm with a  
191 scan speed of  $2400 \text{ nm}\cdot\text{min}^{-1}$  and a slit of 1 nm. The photomultiplier tension was  
192 fixed at 950 V and the integration time set at 0.1 s. The extraction of the 5 nm

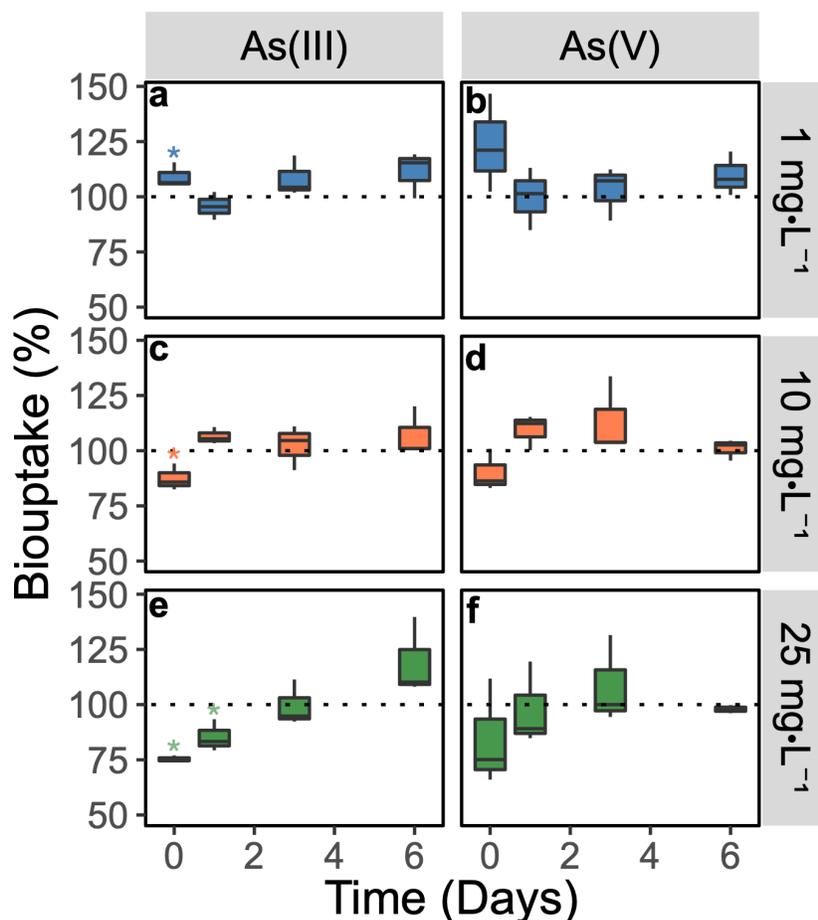
193 stepped emission was obtained by FL-Solution software. Each experiment was  
194 performed in triplicate.

195

## 196 **2. RESULTS AND DISCUSSION**

### 197 **2.1. DOM kinetically controls As bioavailability to bacteria**

198 In a first series of experiments, we tested the role of i) DOM concentrations and ii)  
199 the duration of As-DOM pre-incubation, prior to exposure to the biosensor, on the  
200 bioavailability of As(III) and As(V). Here, DOM was provided as Suwannee River  
201 Humic Acid from IHSS over a range of concentrations representative of what can  
202 typically be found in natural surface waters (streams, lakes, wetlands) and  
203 porewaters (28).



204  
 205 **Fig. 1** | DOM control on As(III) and As(V) bioavailability over time. DOM control on As(III) and As(V)  
 206 bioavailability over time. Boxplots represent biological replicates ( $n = 3$ ) containing either 200 nM of  
 207 As(III) (a, c, e) or 200 nM As (V) (b, d, f) in the presence of a DOM (SRHA) gradient (vertical facets).  
 208 Axes include room-temperature pre-incubation time (X-axis) and output signals of treatments are  
 209 indicated as percent deviation from our no-DOM controls (Y-axis). Dotted line represents biosensor  
 210 output signal of the no-DOM controls. Stars represent a significant decrease from the 200 nM (no-  
 211 DOM) control incubated for the same period of time determined using raw fluorescent signals and  
 212 TukeyHSD post-hoc analysis on a oneway ANOVA.

213 First, we observed that without a pre-incubation step, addition of 25 mg/L SRHA  
 214 decreased As(III) and As(V) bioavailability by 25% and 16%, respectively (significant  
 215 decrease of 25% for As(III),  $p < 0.0001$ ) (**Fig. 1e**), supporting our previous  
 216 observation with natural lake water samples (22). Second, in the presence of [SRHA]  
 217  $\geq 10 \text{ mg}\cdot\text{L}^{-1}$ , As(III) and As(V) bioavailability increased with increasing pre-  
 218 incubation time (**Fig. 1e**), to values comparable to the no-DOM control (**Fig. 1c-f**).  
 219 Here, the pre-incubation time required to reach control values ranged from 1 ([SRHA]  
 220  $= 10 \text{ mg}\cdot\text{L}^{-1}$ ) to 3 days ([SRHA]  $= 25 \text{ mg}\cdot\text{L}^{-1}$ ). After a 6 day pre-incubation time in

221 the presence of [SRHA] = 25 mg•L<sup>-1</sup>, As(III) bioavailability was greater than in the  
222 no-DOM control. This corresponded to a significant increase of 45% of As  
223 bioavailability over a 6-day period (p<0.001). Note that increasing DOM  
224 concentration also stimulated biosensor cells yield (**Fig. S1c-f**), probably due to the  
225 presence of nutrients within the DOM pool but this stimulation was not time  
226 dependent and did not affect accuracy of the As-specific biosensor signal (**Fig. 1c-f**).

227 Our data showed that DOM hampered As bioavailability at low As (III) to DOM ratio  
228 (8 nmol As(III) • mg<sup>-1</sup> DOM) and during a short time period after As and DOM were  
229 placed together (t < 30 min). This observation is in line with what was predicted in  
230 the literature. Indeed, both higher stability constants (29) and stronger binding (21)  
231 have been reported when working at pH and [As]/[DOM] ratios similar to the values  
232 used in this study. Using a two-site ligand binding model, Liu and Cai (2010) have  
233 shown that humic acids have a limited number of strong As binding sites that are  
234 sensitive to increases in [DOM] (29). Furthermore, Bushmann *et al.* (21) proposed  
235 that the limited number of strong As binding sites on DOM is sensitive to competition  
236 and/or conformational changes of humic macromolecules, which may explain the  
237 time-dependent nature of our results.

238 To document a possible change in DOM conformational properties over time and in  
239 the presence of As, we used FEEM (Fluorescence Excitation/Emission Matrices).  
240 Though widely used to quantify DOM, contour maps of the 3D FEEM can also be used  
241 to characterize changes in DOM's fluorescent components (30). Using FEEM, we  
242 predicted that time-dependent changes in fluorescence would correspond to changes  
243 in the nature of the interactions between As and DOM. Using colour distance matrix  
244 and k-mean cluster analyses, we confirmed a change in fluorescence spectrum  
245 intensity and profile over time, both peaking in intensity after a 2-day incubation and  
246 shifting in profile following a 6-day incubation (**Fig. S2**). Changes in the molecular  
247 conformation of DOM over time can result from modifications of the surface charge  
248 (31), and from the intramolecular cationic bridging (32). We recognize that

249 fluorescence data presented here offer little mechanistic insights into the underlying  
250 interactions between As and DOM. That being said, quantification of changes in  
251 fluorophore intensity (or quenching) through time does present evidence of the  
252 dynamic nature of As-DOM interactions (**Fig. S2**), which is also reflected in the  
253 response of the biosensor (**Fig. 1**).

254 It is generally recognized that cationic metal-DOM interactions strengthen over time.  
255 Arsenic, an oxyanion under most environmentally relevant conditions, would  
256 predictably offer different binding dynamics. We propose that increasing As(III)  
257 bioavailability over time resulted from a series of ligand exchange within the DOM  
258 pool, induced by DOM conformational changes; this transfer of As(III) from the few  
259 sites at which it formed strong bonds, towards sites that are more abundant but  
260 exhibiting weaker electrostatic affinity, made it more accessible to the biosensor and  
261 hence bioavailable. Contrary to what appears to be currently accepted in the  
262 literature for bacteria (33) and derived from DGT experiments (34), our data suggest  
263 that inorganic As species do remain bioavailable in the presence of DOM, through  
264 time, even when abiotic conditions favour As complexation.

265 In addition to free As species, As weakly bound to DOM also represents a pool of  
266 labile As that is bioavailable to microbes. Furthermore, microbial cells themselves  
267 may play a role in actively releasing weakly-bound As from DOM. Possible  
268 mechanisms include: i) internalization of co-transported As during microbial  
269 consumption of DOM (35); ii) release of weakly-bound As induced by extracellular  
270 electron transfer (36) and shuttled by quinone moieties within the DOM pool (37,  
271 38); and iii) uptake of As through siderophores channels (e.g. ABC-type exporters  
272 (39)) that are possibly upregulated by phosphate/iron starvation during biosensor  
273 growth. Determining the mechanisms involved will require further work.

274

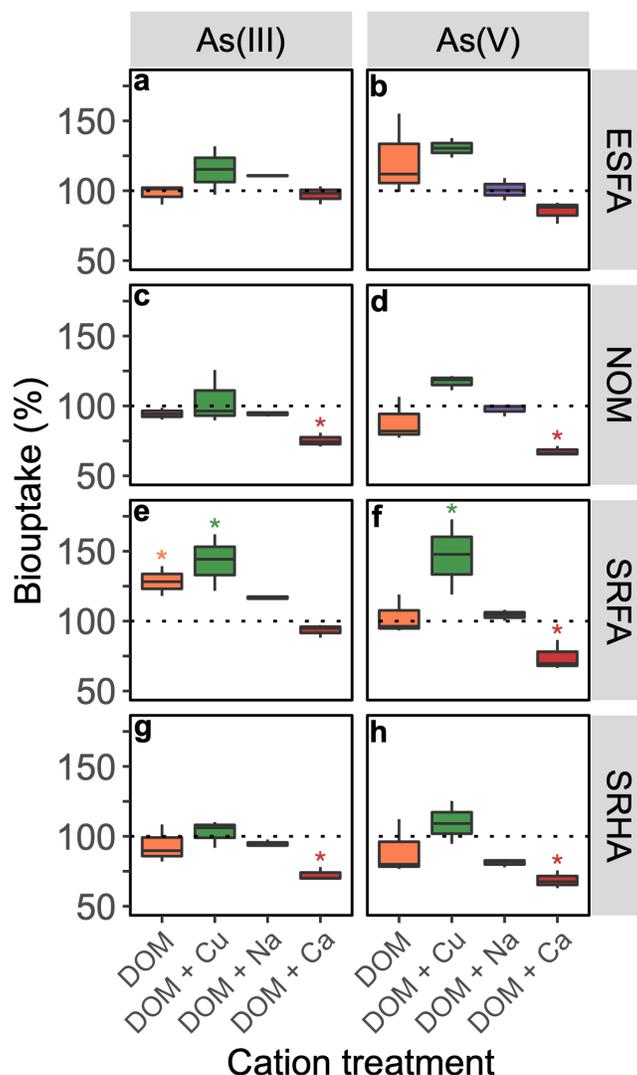
## 275 2.2. The nature of As binding to DOM controls its bioavailability

276 Our interest in identifying the mechanism involved in DOM's control on As  
277 bioavailability required more targeted experiments. Carboxylic groups are one of the  
278 main contributors to DOM's negative charge (40) and thus promote strong surface  
279 and inner-sphere complexes with cationic metals (21, 41, 42). Moreover, cations are  
280 known to affect the sorption of As to DOM by changing its affinity to functional  
281 moieties such as carboxylic, phenolic, amino and sulfhydryl groups (12, 21, 29).  
282 These associations can induce aggregation and structural re-orientation of the  
283 organic matter by increasing the compression and rigidity of the structure while  
284 favoring a hydrophobic core (43).

285 We first tested the effect of cations on As bioavailability in the presence of DOM (20  
286 nmol As•mg<sup>-1</sup> DOM), by adding 10 mM Na<sup>+</sup>, 10 mM Ca<sup>2+</sup>, or 5 μM Cu<sup>2+</sup>. Mono- and  
287 di-valent cations (Na<sup>+</sup> and Ca<sup>2+</sup>) were selected for their ubiquity in the environment  
288 and pronounced effects on DOM charge (44) and structure (45), respectively. Cu<sup>2+</sup> is  
289 a trace metal with high affinity for DOM (46), and is expected to form strong covalent  
290 bonds at complexation sites (47, 48). The concentration of cations was limited by  
291 their toxicities and chosen to maintain relevance to concentrations commonly found  
292 in freshwaters. We found that across all tested commercially available DOM  
293 treatments, As(III) and As(V) bioavailability variably increased in the presence of  
294 Cu<sup>2+</sup>, was unaffected in the presence of Na<sup>+</sup>, and consistently decreased in the  
295 presence of Ca<sup>2+</sup> (**Fig. 2**). These data suggest that the mechanisms involved in  
296 controlling As bioavailability are conserved across DOM samples.

297 Experiments performed in the presence of copper support our finding that strong  
298 binding of As to DOM limits its bioavailability. In this case, we suspect that the  
299 presence of Cu<sup>2+</sup> prevented As binding, maintaining its accessibility and bioavailability  
300 to the biosensor. Although the mechanism remains unclear, we speculate that it  
301 relates to the nature of the DOM conformational changes differentially induced by  
302 calcium and copper. Copper, like calcium, electrostatically interacts with

303 deprotonated carboxylic groups of DOM (46, 49). Copper (49), like calcium,  
304 electrostatically interacts with deprotonated carboxylic groups of DOM (46).  
305 However,  $\text{Ca}^{2+}$  interactions with DOM differ from that of copper in the strength and  
306 specificity of the bonds it forms with DOM (46, 49). Indeed, calcium is thought to  
307 better penetrate DOM structure (50), thereby forming strong inner-sphere ionic  
308 bridges (14, 42, 50), creating more space for water molecules (51), and changes in  
309 DOM conformation (43, 52).



310  
 311 **Fig. 2 |** As bioavailability in the presence of DOM of varying origins. Changes in As bioavailability (Y-  
 312 axis) were plotted over various cation treatments (X-axis). Environmentally relevant concentrations of  
 313 Na<sup>+</sup> (10 mM), Ca<sup>2+</sup> (10 mM), Cu<sup>2+</sup> (5 μM), and DOM (10 mg•L<sup>-1</sup>) were used. The origin of the DOM  
 314 used is identified by the vertical facet labels. Each box represents the mean and standard deviation of  
 315 biological triplicate samples containing 200 nM of As(III) (a, c, e, g) or 200 nM As(V) (b, d, f, h).  
 316 Percent conversions were based on presence vs absence of organic matter. Dotted line represents  
 317 biosensor output signal of the no-DOM controls at the same ionic strength. Significant decrease from  
 318 the no-DOM controls were determined using TukeyHSD post-hoc analysis on a one-way ANOVA.

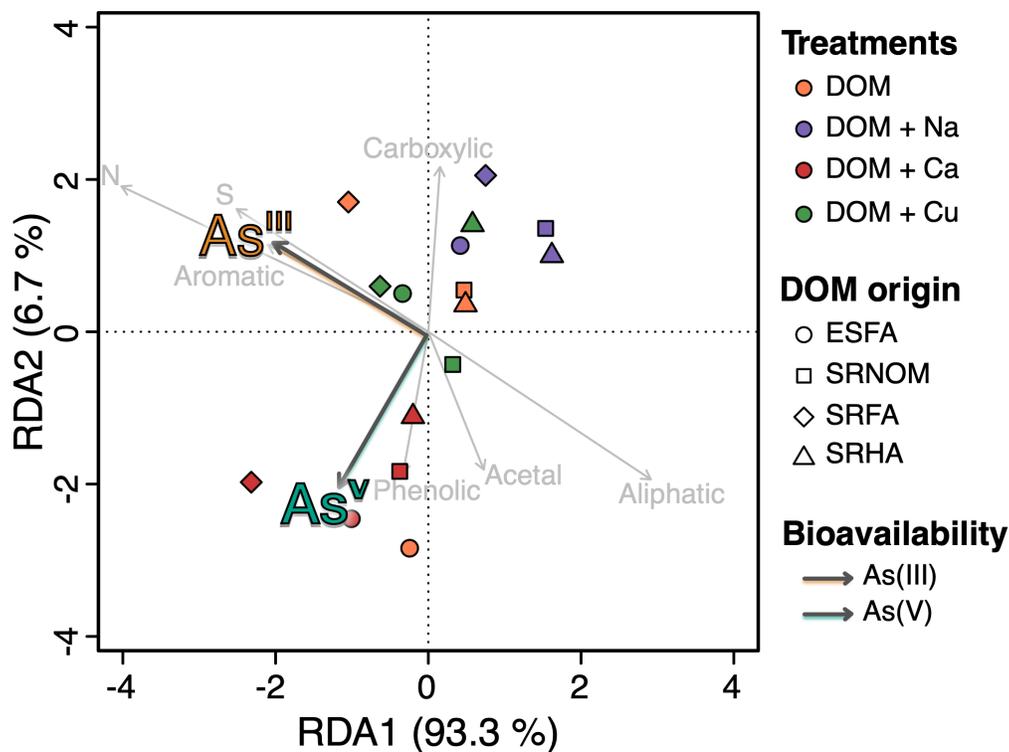
319 Second, to test the extent to which As(V) binding to DOM affected its bioavailability,  
 320 we subjected the exposure solution to a 500 Da dialysis membrane bag in the  
 321 presence and absence of SRHA, Ca<sup>2+</sup> or Na<sup>+</sup>. In the absence of cations, >95% of  
 322 As(V) was bound to SRHA, yet remained bioavailable (**Fig. S3**). Although the addition  
 323 of Na<sup>+</sup> and Ca<sup>2+</sup> decreased the extent to which As(V) was bound to DOM (**Fig. S3**),

324 only in the presence of  $\text{Ca}^{2+}$  did we observe a 25% decrease in the bioavailability of  
325 As bound to DOM (**Fig. 2**). These results confirm that microbes are not limited to the  
326 unbound fraction of As but are also be capable of accessing weak electrostatically  
327 held As.

328 We used FEEM to test the effects of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  amendments on As(V)-SRHA  
329 treatments (**Fig. S4**). Both colour distance matrix and k-mean cluster analysis  
330 confirmed that all three  $\text{Ca}^{2+}$  treatments increased fluorescence intensity beyond that  
331 of the  $\text{Na}^+$  treatments and of the no-cation controls, suggesting an important role of  
332  $\text{Ca}^{2+}$  in changing the structural properties of DOM. These findings are in line with  
333 reports indicating that  $\text{Ca}^{2+}$  affects DOM structure, namely its compression and  
334 rigidity (43, 52). Following the two-site ligand binding model proposed by Buschmann  
335 et al. (2006) and further characterized by Liu and Cai (2010), it is conceivable that  
336 changes to the molecular structure and arrangement of DOM evoked by 10 mM  $\text{Ca}^{2+}$ ,  
337 led to the release of weakly but electrostatically bound As towards newly  
338 available/accessible, high affinity sites. The consistent yet modest (ca. 25%)  
339 decrease in As(III) and As(V) bioavailability in the presence of  $\text{Ca}^{2+}$  (**Fig. 2**), supports  
340 the low number of strong As sites proposed by other studies (21, 29). Unfortunately,  
341 we do not yet have spectroscopic evidence supporting a change in bioavailability  
342 induced by conformational changes of DOM under our experimental conditions.

343 Finally, we performed a redundancy analysis (RDA) that assigns predictor variables  
344 to bioavailability measurements to explore the broad relationships existing between  
345 As species bioavailability and the various experiments performed in this study (**Fig.**  
346 **3**). This analysis highlights that the drivers of As(III) and As(V) bioavailability in the  
347 presence of DOM are likely different, underscoring the relevance of our approach of  
348 performing speciation using a biosensor. Here, most variability was driven by  
349 predictor variables that align with RDA1 axis (N, S and aliphatic content of DOM) and  
350 with As(III) bioavailability vector. The magnitude of As(III) bioavailability in the  
351 presence of DOM appears to be less dependent on the presence of cations than As(V).

352 Indeed, and despite low coverage of the RDA2 axis (6.7%), alignment of As (V)  
 353 bioavailability vectors with cationic treatment groupings and predictor variables  
 354 suggest a stronger cationic interference on As (V) bioavailability, best characterized  
 355 by Q1 (carboxylic) and Q2 (phenolic) pools of binding sites.

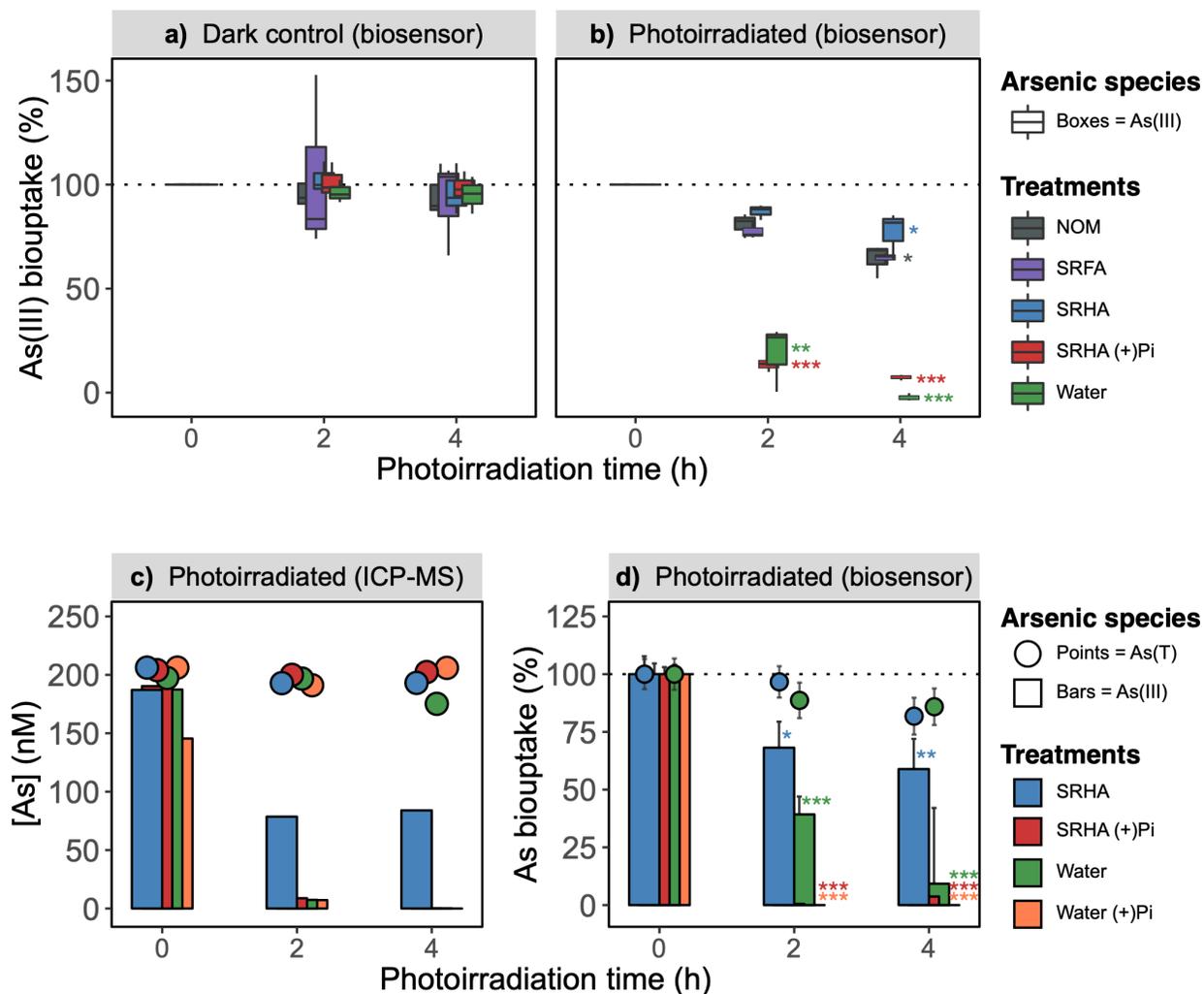


356  
 357 **Fig. 3 |** RDA triplot assigning predictor variables to drivers of bioavailability. Each RDA point  
 358 represents ordination of biosensor outputs (**Fig. 2**) separated by Euclidian distances as a metric of  
 359 variation. Overlaid are both bioavailability drivers (colored vectors) and explanatory variables (grey  
 360 vectors), pointing in the direction of most rapid change. The angle between vectors of explanatory  
 361 variables and bioavailability drivers reflect their linear correlation. Properties of the DOM origins used  
 362 to produce this RDA can be found in Supplementary Information (**Supplementary Table**).

### 363 2.3. Arsenic photoreactivity and bioavailability in the presence of DOM

364 DOM can profoundly affect As(III) behavior in the environment. Here, DOM can  
 365 compete with mineral adsorption sites, and enhance mobility of this contaminant by  
 366 maintaining As in dissolved or colloidal forms (53). One important variable controlling  
 367 the fate of DOM in natural surface waters is its photochemical reactivity which has  
 368 also been involved in As redox transformations (13, 54). Moreover, the effects of As-  
 369 DOM photoirradiation on As bioavailability have yet to be characterized. Such

370 information is relevant for situations when: i) groundwater reaches surface waters,  
 371 ii) ice cover melts, iii) summer anoxia in DOM-rich wetlands/bogs, or iv) episodic  
 372 draining and flooding of rice paddy soils. In this last series of experiments, we tested  
 373 the role of As (III)-DOM photoreactivity on the bioavailability of As species (**Fig. 4**).



374  
 375 **Fig. 4 |** DOM affects the extent of As(III) photooxidation. HPLC-ICP-MS mass balance c) was used for  
 376 validation of biosensor speciation technique a), b) and d) where a decrease in As(III) uptake over time  
 377 suggests As(III) oxidation. Biosensor fluorescence was converted to percent biouptake (Y-axis) by  
 378 comparing output signal of the irradiated treatments to the pre-irradiated (T0) controls. Biosensor  
 379 endpoints present the mean and standard deviation of three independent biological triplicates. Panels  
 380 b) and c) present an independent same-day mass balance using ICP-MS and biosensor outputs  
 381 respectively. Dotted line represents biosensor output signal at T0. A significance code of (\*)  
 382 represents a p-value between 0.01 and 0.05 and of (\*\*\*) for a p-value >0.001. Analysis of  
 383 photoreactor light spectrum (**Fig. S5**) including supplemental controls for this figure, can be found in  
 384 supplementary information (**Fig. S6**).

385 Our 2-step experimental treatment required the pre-incubation of a As(III) solution  
386 under UV-vis radiations (with or without DOM), prior to exposure to the biosensor  
387 assay. Upon exposure, only As(III) can be detected because, as per our protocol, 10  
388 mM  $\text{PO}_3^-$  is added to the bioassay medium to prevent As(V) uptake (22). We observed  
389 that virtually none of the As(III) remained bioavailable after 4 h of irradiation in the  
390 absence of DOM (**Fig. 4bd**). In this case, a decrease of As(III) bioavailability could  
391 be attributed to i) conditions that directly prevents the biouptake of As(III) or ii) its  
392 oxidation to As(V). Our same-day control experiments analyzed using ICP-MS,  
393 confirmed the virtually complete As(III) oxidation to As(V) (**Fig. 4c**).

394 To maintain environmental relevance, no UVC bulbs were installed in our  
395 photoreactor. Yet in the absence of DOM and in the presence of light, oxidation of  
396 As(III) to As(V) in water alone was consistently observed in over two dozen separate  
397 replicated experiments. We performed a series of control experiments, to reasonably  
398 rule out the role of atmospheric gases (e.g.  $\text{N}_2$ ,  $\text{O}_2$ ,  $\text{CO}_2$ ), the acid from the As stock  
399 solution preservative ( $\text{HNO}_3$ ), and the nature of the cuvette container as sources  
400 and/or acceptors of electrons in the As(III) photooxidation process (**Fig. S6**,  
401 **Supplementary Method**). We sought to investigate whether water could be a  
402 source of  $\text{HO}\bullet$  and  $\text{H}\bullet$  radicals likely involved in As(III) photooxidation. Our  
403 spectroradiometer measurements confirmed that mostly visible spectral components  
404 (**Fig. S5a**) were emitted in the reactor with little spectral irradiance ( $0.166 \text{ J}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )  
405 in the UV regions (**Fig. S5b**). The photooxidation of As(III) at environmentally  
406 relevant levels (nM) requires only a relatively small number of photons emitted at  
407 water's ionization energy threshold ( $\sim 6.5 \text{ eV}$ ) (55). In our incubation experiments,  
408 what appears as inconsequential spectral peaks (**Fig. S5e**) in the UV range ( $\sim 6.1$   
409 eV), may provide the energy required to produce hydroxyl radicals needed to catalyze  
410 this reaction at the low As levels used. We unfortunately cannot properly report  
411 quantum efficiency yields considering CCD spectrometers sensors provide inaccurate

412 absolute intensity measurements due to the nature of their integrated analog  
413 (photons) to digital (volts) conversion process.

414 Surprisingly, the presence of DOM, amended as NOM, SRFA or SRHA greatly limited  
415 the extent to which As(III) was photooxidized to As(V) (**Fig. 4**). Hydroxyl radicals,  
416 regardless of their origins (water or DOM), can affect C-H and S-H bonds (56). This  
417 photochemical-induced conformational change of DOM could enhance binding of As-  
418 DOM. In either case, we suspected that the presence of DOM both attenuated photon  
419 flux via shading and limited As(III) photoreactivity via As-DOM binding.

420 Finally, we tested the role of a possible association between As(III) and DOM on  
421 As(III) photooxidation by adding phosphate to an As(III)-DOM solution prior to its  
422 photoirradiation. Our prediction was that addition of  $\text{PO}_4^{3-}$ , which is known to limit  
423 As(III) binding to DOM (57), would favour As(III) photooxidation by increasing the  
424 pool of unbound As(III) in solution. Indeed, in the presence of DOM +  $\text{PO}_4^{3-}$ , ICP-MS  
425 measurements indicated rates of As(III) photooxidation, similar to those observed in  
426 water alone. Our finding only partially supports previous literature claiming that  
427 As(III) oxidation is mediated by the transfer of electron from DOM (13). We  
428 cautiously conclude that differences among published results on the role of DOM on  
429 As photooxidation likely result from variable As-DOM binding conditions due to  
430 variation in Pi content (e.g., buffer), pH, and ionic strength of the exposure solutions.  
431 Overall, further experiments are required to determine the nature of the interaction  
432 between As and DOM in the presence of  $\text{PO}_4^{3-}$ . That being said, our data support our  
433 hypothesis that the nature and strength of As binding to DOM controls As  
434 bioavailability and reactivity.

435

### 436 **3. CONCLUSION**

437 It is generally accepted and often implied that the bioavailable fraction of As is the  
438 “free” or unbound one (58, 59). The work presented here showed that the  
439 bioavailable fraction of As is comprised of both labile and weakly complexed to DOM  
440 fractions. Though we found that 20 nmol As•mg<sup>-1</sup> DOM ratio appears sufficient to  
441 saturate the low number of strong As binding sites in several types of commercially  
442 available DOM, further work is warranted to characterize the nature of weak vs strong  
443 As binding sites. Characterizing the strength and specificity of As-DOM interaction is  
444 fundamental in improving our understanding of microbially driven mobilization of As.  
445 This is an important gap of knowledge, which once addressed, will improve effective  
446 management of As contamination. Indeed, environmental changes brought by  
447 climate change such as variations in temperatures and precipitations already affect  
448 the physicochemical properties of natural waters making large-scale predictions on  
449 the fate of As-DOM complexes difficult (11). In the context of global water quality  
450 assessment, our work emphasizes the importance of considering the effects of  
451 climate change and of agricultural practices as they affect the levels of nutrients and  
452 their remobilization. Changes in DOM concentrations and nutrient levels may affect  
453 the fraction of As bound to DOM, which also control the bioavailability of As to the  
454 microbes responsible for its transformation and mobilization. Most importantly, this  
455 work calls for additional investigations on the kinetic controls that DOM exerts on As  
456 toxicity at various levels in food webs.

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463 **Author contributions**

464 MPP, AJP, VL, BM initiated and designed the experiments; MPP, VL, and CR carried  
465 out the experiments; MPP wrote the R scripts for data analyses; MPP wrote the  
466 manuscript with support from AJP, VL, BM, and CR; AJP, VL, and BM supervised the  
467 project. All authors have given approval to the final version of the manuscript.

468 **Appendix A. Supplementary Information**

469 Biosensor culture yield, fluorescence spectrum/intensity, bound vs bioavailable  
470 arsenic, photoreactor light spectrum, additional control experiments, and chemical  
471 properties of DOM extracts.

472 **Abbreviations**

473 As(III), arsenite; As(V), arsenate; DOM, dissolved organic matter; ESFA, Elliott  
474 Soil Fulvic Acid; HDPE, high density polyethylene; HPLC, high performance liquid  
475 chromatography; ICP-MS, inductively coupled mass spectroscopy; MGP, Mops  
476 Glycerophosphate; MIP, Mops Inorganic Phosphate; NOM, Natural Organic Matter;  
477 NSERC, Natural Sciences and Engineering Research Council of Canada; pMP01,  
478 Arsenic biosensor; RDA, redundancy analysis; SRFA, Suwannee River Fulvic Acid;  
479 SRHA, Suwannee River Humic Acid.

480 **Declaration of interest statement**

481 Authors declare no competing financial interest.

482

483 **4. REFERENCES**

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