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1 Diatom immigration drives biofilm recovery after chronic copper exposure

2

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8

9 Summary

- 10 1. The impact of immigration on the recovery of diatom assemblages after chronic
11 exposure to copper was investigated in laboratory microcosms.
- 12 2. We examined the recovery trajectories of copper-contaminated biofilms after
13 reducing copper stress and with or without the possibility of immigration from
14 unimpaired communities.
- 15 3. The biofilms mixed with unimpaired communities returned to a "control"
16 assemblage structure within 6 weeks, with recovery patterns depending on the
17 endpoint considered (i.e. 2 weeks for relative abundances of diatom species but 6
18 weeks for total diatom biomass). In contrast, no recovery was observed in
19 assemblages placed under control conditions without external immigrants.
- 20 4. Immigration has important effects on the recovery of quantitative and qualitative
21 characteristics of biofilms.

22 Keywords: Diatoms; Biofilm; Recovery; Copper; Immigration; Re-colonization

23

24 Introduction

25 In the early 90s, Stevenson & Peterson (1989, 1991) studied the emigration and

26 immigration of benthic diatoms as determinants of periphytic communities in streams.

27 Since their work, the influence of immigration in freshwater benthic systems has mainly

28 been considered in relation to recovery following sharp changes in flow regime, i.e.

29 spate (Peterson & Stevenson, 1992; Peterson, 1996; Stevenson, 1990) or episodes of

30 drought (Bambakidis, 2009; Benenati *et al.*, 1998).

31 Translocation studies are increasingly used to assess the recovery potential of riverine

32 periphytic communities (biofilms). They are generally performed by transferring

33 biofilms from contaminated sites to uncontaminated sites upstream of the

34 contamination source (Dorigo *et al.*, 2010; Morin *et al.*, 2010a; Tolcach & Gómez, 2002;

35 Rotter *et al.*, 2011), but sometimes by transplanting contaminated communities to a

36 neighbouring river with similar environmental conditions (Ivorra *et al.*, 1999).

37 Depending on the study, the time for full recovery of diatom assemblage structure has

38 been estimated at from as little as two (Tolcach & Gómez, 2002) to over nine weeks

39 (Dorigo *et al.*, 2010). The contribution of immigration to recovery after episodes of

40 contamination is often questioned in translocation experiments, and ecotoxicological

41 studies in general (e.g. Ivorra *et al.*, 1999; Morin *et al.*, 2010a). However, it remains

42 virtually impossible to discriminate between the effects of re-growth from remaining

43 viable cells and colonization by new immigrants.

44 Here we performed an experimental study designed to assess the relative contribution
45 of drift to the recovery of diatom assemblages from copper exposure. A series of
46 laboratory channels were used to grow biofilms under control and Cu-contaminated
47 conditions, prior to simulating the recovery of exposed assemblages with and without
48 connectivity to unimpacted biofilms (i.e. allowing or not species immigration from a
49 pristine community). Total diatom biomass and species relative abundances were
50 monitored. In addition, a range of descriptors for assessing diatom population dynamics
51 (daily rates of increase in diatom density, estimation of population growth) were
52 determined in order to identify the ecological importance to recovery of immigration as
53 distinct from re-growth from remnant cells.

54

55 Methods

56 *Experimental setup*

57 A suspension of natural biofilm from a pristine site located upstream of any pollution
58 inputs in the Morcille river (Beaujolais region, Eastern France) was inoculated into 12
59 laboratory channels ($l \times w \times d = 63 \times 11 \times 4$ cm). These channels were arranged in a
60 blocked design into four sets of triplicate channels, two of which were grown under
61 control conditions (see details in Lambert *et al.*, 2012) while the other two were
62 supplemented with copper at about $20 \mu\text{g L}^{-1}$ for four weeks to obtain mature biofilms
63 before the experiment. The channels were placed in parallel, at a light intensity of
64 approximately 3500 lux (13:11 light:dark regime), under continuous water flow in
65 recirculating mode (centrifugal pumps Rena Flow 600, 1.2L min^{-1}). The copper
66 concentrations used in this experiment were sufficient to produce effects on the

67 assemblages (allowing for the study of recovery), but still environmentally realistic
68 (such concentrations are occasionally measured downstream in the Morcille river: see
69 Montuelle *et al.*, 2010). In each channel, microscope glass slides were immersed to allow
70 biofilm settlement and harmonize sampling conditions (similar substratum
71 characteristics, constant surface area) throughout the experiment. The diatom
72 assemblage inoculum was composed of at least 57 species, dominated by *Nitzschia*
73 *hantzschiana* Rabenhorst (more than 80% relative abundance) followed by
74 *Achnantheidium minutissimum* (Kützing) Czarnecki, *Nitzschia capitellata* Hustedt,
75 *Fragilaria capucina* Desmazières var. *capucina* and *Planothidium lanceolatum*
76 (Brébisson ex Kützing) Lange-Bertalot.

77 During the 6-weeks of recovery, four different treatments (each with three replicates)
78 were tested (Fig. 1): I) Control treatment: a set of n = 3 uncontaminated channels kept
79 under uncontaminated water; II) Cu treatment: a set of n = 3 Cu-contaminated channels
80 kept exposed to about 20 µg Cu L⁻¹; III) ExCu1 treatment: set of channels with biofilms
81 grown under control conditions placed in the upstream half-section of the channels and
82 biofilms grown under Cu-contaminated conditions placed in the downstream section,
83 filled with uncontaminated water; IV) ExCu2 treatment: set of biofilms grown under Cu-
84 contaminated conditions and filled with uncontaminated water. The ExCu1 and ExCu2
85 treatments were used to assess the recovery of diatom assemblages following a
86 decrease in Cu exposure, with (ExCu1) or without (ExCu2) the possibility of diatom
87 immigration from "control" assemblages to the impacted biofilms.

88

89

90 *Water analyses*

91 All physicochemical parameters were monitored every two weeks, before and after the
92 water was changed. For water changes, groundwater containing a very low Cu
93 concentration ($<0.3 \mu\text{g L}^{-1}$) was adjusted to the conductivity (i.e. $600 \mu\text{S cm}^{-1}$) and
94 nutrient concentrations (i.e. about 4 mg Si L^{-1} , $5 \text{ mg NO}_3 \text{ L}^{-1}$, $0.05 \text{ mg PO}_4 \text{ L}^{-1}$) of the
95 sampling site. In the Cu-contaminated channels, the water was supplemented with
96 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to obtain the nominal concentration of $20 \mu\text{g Cu L}^{-1}$.

97 Conductivity, pH, temperature and oxygen concentration were measured *in situ* in each
98 series of channels using suitable probes (WTW Meters, Weilheim, Germany). Water
99 samples were taken to analyse nutrient content (NO_2 , NO_3 , NH_4 , PO_4 , SiO_2) and copper
100 concentration. Nutrients were determined following French standards (AFNOR
101 operating procedures and protocols). Dissolved Cu concentrations were analysed by
102 ICP-MS (X Series II, Thermo Electron, UK) after filtration of a 30-mL sample ($0.45 \mu\text{m}$
103 PVDF, Whatman) and acidification with 0.5% nitric acid (Suprapur, Merck).

104

105 *Algal biomass and diatom analysis*

106 Biofilm samples (five glass slides) were collected in each replicate channel every two
107 weeks from week 0 to week 6). In the ExCu1 treatment, channel samples were always
108 collected in the "downstream" section, corresponding to biofilms previously exposed to
109 copper (Fig. 1). The five slides were carefully scraped using a razor blade and the
110 biofilms pooled and suspended in a standard volume of mineral water (Volvic, France).
111 After homogenization of the resulting suspensions, aliquots were taken for fluorescence
112 measurements (as a proxy of total algal biomass and of diatoms) and diatom taxonomic

113 identification. Chlorophyll-*a* fluorescence (Chl) emitted by diatoms was determined by
114 measurements using a PhytoPAM fluorometer (Walz, Effeltrich, Germany) equipped
115 with the PhytoEDF emitter-detector unit, performed on fresh material (as described in
116 Morin *et al.*, 2010b). Data were then converted into $\mu\text{g Chl cm}^{-2}$. Subsamples for diatom
117 identification were preserved in a formalin solution (4%, v/v) and prepared according
118 to standard NF EN 13946. Briefly, organic matter was removed from the samples by
119 digestion in boiling hydrogen peroxide (30% H₂O₂) followed by rinsing and successive
120 cycles of centrifugation and re-suspension in distilled water to remove any residual
121 peroxide. Clean material was then pipetted onto cover-slips and allowed to dry before
122 being mounted with a high refractive index mountant (Naphrax, Brunel Microscopes
123 Ltd, UK) for microscopic identification (x1000 magnification on a Leica DMRB
124 photomicroscope, Wetzlar, Germany). At least 400 individual diatoms were identified to
125 the lowest taxonomical level possible. The primary references used were Krammer &
126 Lange-Bertalot (1986 - 1991) and the Diatoms of Europe series (A.R.G. Gantner Verlag
127 K.G, Ruggell). Relative abundance of the taxa identified was recorded (RA, in %); when
128 deformed individuals were observed, their percentage was also determined.

129

130 *Data treatment*

131 Between-channel differences in physicochemical variables were tested with one-way
132 ANOVA and Tukey's *post hoc* HSD test using Statistica software (v5.1, StatSoft, France).
133 Linear mixed effects (LME) models were applied on the biological endpoints (total
134 diatom biomass, species richness, proportion of diatom deformities) to differentiate
135 between the effects of sampling date, treatment and their combination. Treatments and
136 dates were set as fixed effects and the samples taken from each artificial channel as
137 random effects. The analyses were performed using the nlme library implemented

138 within the R environment (<http://cran.r-project.org/>).

139 Samples were ordinated by cluster analysis (Bray Curtis similarities) on the basis of

140 diatom species composition (PC-Ord v4.25, MjM Software, USA).

141 Diatom biomass values measured through fluorescence were used to convert taxon

142 counts (RA) into biomass values for the dominant species and abnormal valves

143 (expressed in $\mu\text{g Chl cm}^{-2}$). Based on these data, daily rates of increase (r , in cell

144 doublings/day) during the exponential growth phase were calculated using the slope of

145 the line relating time and natural log biomass according to Wood *et al.* (2005).

146 Probability values of the linear regressions were calculated to determine whether

147 growth was significant, before investigating differences between treatments in the total

148 diatom biomass, dominant species found, and abnormal forms.

149 Two-week population growth (written as Δpop) was determined for each treatment and

150 pair of consecutive sampling dates to estimate the impact of immigration. Δpop was

151 determined by two factors: net fecundity (reproduction - death) and net immigration

152 (immigration - emigration). Net fecundity and immigration may differ according to

153 environmental conditions (Control vs. Cu), thus ANOVA comparisons were only

154 performed for ExCu1 and ExCu2 to assess differences in net immigration. As they shared

155 a comparable contamination history, the differences in growth between ExCu1 and

156 ExCu2 corresponded to the immigration that occurred only in ExCu1 (immigration of

157 species from the "upstream" to the "downstream" slides, only the latter being sampled),

158 and were thus taken as an estimate of the potential influence of immigration.

159

160

161

162 Results

163 *Water*

164 Physicochemical variables varied little among treatments (Table 1), with the exception
165 of SiO₂ and Cu concentrations which differed significantly (ANOVA, df=25; $P=0.003$,
166 $F=6.36$ and $P<0.001$, $F=44.80$, respectively). Silicate concentrations were lower in
167 Control samples due to marked decreases (*ca.* 70%) between water changes, compared
168 to ExCu2 and Cu channels; intermediate values were recorded in ExCu1. Copper
169 concentrations were significantly higher in the Cu-treated channels ($16.6 \pm 1.4 \mu\text{g L}^{-1}$),
170 and no statistically significant difference was found between Control, ExCu1 and ExCu2
171 channels, even though the two latter channels had slightly higher concentrations of Cu
172 ($4.2 \pm 0.9 \mu\text{g L}^{-1}$).

173

174 *Diatom assemblages*

175 Biomass of diatoms (Fig. 2) represented $87.5 \pm 0.5\%$ of that of the overall phototrophic
176 assemblage in the Control channels, against $36.0 \pm 1.4\%$ in Cu-exposed channels.
177 Statistical differences in biomass were observed between treatments ($P<0.001$), and a
178 significant increase with time was noted ($P=0.022$ in week 2, $P<0.001$ in weeks 4 and 6).
179 The proportion of diatoms increased in ExCu1 from $42.1 \pm 0.3\%$ initially to $82.7 \pm 3.7\%$ in
180 week 6, and reached a comparable diatom biomass to Controls ($P=0.058$), whereas in
181 ExCu2 it was stable and significantly lower ($P<0.001$), at $44.1 \pm 2.3\%$ throughout the
182 experiment.

183 Species richness was significantly lower in the Controls than in the other treatments
184 (18 ± 1 vs 21 ± 1 , $P=0.024$), and tended to decrease over time (time effect on week 6, 17 ± 1

185 species, $P < 0.001$). The classification based on diatom species relative abundance (Fig. 3)
186 clearly distinguished two sets of data:

187 1- Control and ExCu1 biofilms had comparable assemblages (upper branch of the cluster
188 in Fig. 3); within this group, the samples were then classified according to sampling date.
189 The assemblage was composed mainly of *Achnantheidium minutissimum* ($41 \pm 2\%$),
190 *Fragilaria capucina* ($29 \pm 1\%$), *Nitzschia hantzschiana* ($19 \pm 2\%$), and a number of
191 accompanying *Achnantheidium* species (*A. affine* [Grunow] Czarnecki, *A. gracillimum*
192 [Meister] Lange-Bertalot and *A. macrocephalum* [Hustedt] Round & Bukhtiyarova)
193 accounting for about 5% of cumulated abundances.

194 2- Cu and ExCu2 communities composed the lower branch of the cluster, in which ExCu2
195 samples were all grouped together, except one (week 2, that was grouped with Cu
196 samples). Relative abundance of species was less even, as *Achnantheidium minutissimum*
197 ($53 \pm 4\%$) and *Nitzschia hantzschiana* ($30 \pm 4\%$) tended to dominate whereas *Fragilaria*
198 *capucina* abundances markedly decreased ($6 \pm 1\%$). In the sub-cluster of ExCu2 samples
199 (all but one, corresponding to week 2), separation was due to a dominance of *A.*
200 *minutissimum* ($67 \pm 4\%$) over *N. hantzschiana* ($14 \pm 1\%$) and *F. capucina* ($8 \pm 3\%$).

201 Initial percentages of diatom deformities were higher in Cu channels than in the
202 Controls ($p < 0.001$, Fig. 2). Linear mixed effects analysis revealed significant
203 treatment*date effects, as well as statistically higher abundances of deformed cells on
204 week 6 ($p = 0.003$).

205

206 *Rates of increase in cell numbers and population dynamics*

207 Daily rates of increase (Fig. 4) in total diatom numbers were positive in all treatments,
208 except for Cu channels where no significant growth was noticeable. Rates of increase (r

209 values) were highest in ExCu1 channels (three times the Control values) and were
210 related mainly to increased growth of *Achnanthydium minutissimum* and *Fragilaria*
211 *capucina* populations. In ExCu2, *Nitzschia hantzschiana* declined (i.e. r values were
212 negative); the highest r values were recorded for *A. minutissimum*. This species grew
213 significantly in all channels, even Cu-contaminated ones. Values of r for abnormal
214 diatom cells were positive in all Control, ExCu1 and ExCu2 channels, whereas their
215 quantity remained stable in Cu channels (no significant growth). This growth of
216 abnormal cells in Control, ExCu1 and ExCu2 channels led all the treatments to reach
217 similar proportions of diatom deformities at the end of the experiment, i.e. $2.6 \pm 0.1\%$
218 (Fig. 2), which is significantly higher than the initial percentages detailed above.

219 Table 2 summarizes mean population growth parameters for all the treatments over the
220 experimental period. Subscripts in equations refer to the water conditions to indicate
221 that net fecundity (nF) of the standing crop, as well as immigration (I) and emigration
222 (E), differed according to environmental factors. No significant self-generated
223 detachment (Boulêtreau *et al.*, 2006) was observed in the biofilms, suggesting that the
224 emigration component was probably comparable between treatments. Two-week Δpop
225 was always lower under Cu conditions, agreeing with the r calculations. Comparisons
226 between ExCu1 and ExCu2 population kinetics indicated statistical differences (linked to
227 significant immigration) for total diatom biomass and *Fragilaria capucina* (respectively
228 $P=0.006$ and $P<0.001$; $df=23$, $F=9.22$).

229

230

231

232 Discussion

233 *Structural recovery after copper pressure ceased*

234 Diatoms dominated in the Control channels but were less well represented in Cu-
235 exposed channels (Fig. 2). Chronic exposure to copper is known to cause a shift towards
236 dominance by green algae (Serra & Guasch, 2009; Tlili *et al.*, 2010): indeed, Cu samples
237 on week 0 were composed of $47 \pm 5\%$ green algae. ExCu1 communities, due to increased
238 diatom cell doublings (Fig. 4), progressively reached the Control algal composition,
239 while ExCu2 communities remained close to Cu-exposed composition.

240 Focussing on assemblage structure of diatoms in more detail, a selection of species less
241 susceptible to copper exposure occurred in the biofilms exposed to Cu, i.e.
242 *Achnantheidium minutissimum* or *Nitzschia capitellata* (Duong *et al.*, 2008; Morin *et al.*,
243 2008b, 2012; Verb & Vis, 2005). Figure 3 indicates that two weeks were more than
244 enough for ExCu1 assemblages to attain structure similar to the Controls, in accordance
245 with the most optimistic *in situ* translocation scenarios (Tolcach & Gómez, 2002).

246 Without any external supply of colonists, ExCu2 assemblage structure tended to diverge
247 from the Cu-stressed state but did not reach the Control composition within the time-
248 course of the experiment. It followed a trajectory that was probably driven by *in situ*
249 differential reproduction of species selected by previous Cu exposure and subsequently
250 favoured by the new (uncontaminated) environmental conditions. The slower increase
251 in periphytic biomass in ExCu2 channels did not lead to such a strong decrease in local
252 contamination (i.e. Cu concentration in the biofilm) as in ExCu1 (Lambert *et al.*, 2012),
253 thus maintaining somewhat greater toxic pressure. This could explain why the
254 succession trajectory of ExCu2 assemblages were mainly driven by a massive growth of
255 *Achnantheidium minutissimum*, a pioneer species (Medley & Clements, 1998, Stevenson,

256 1990) that is known to cope with metal contaminations (Morin *et al.*, 2012), and to the
257 decline of the copper-tolerant *Nitzschia hantzschiana*, which is probably less competitive
258 than other species when water contamination ceases. Although deriving from a natural
259 inoculum, the original assemblage was quite simple from the outset. The selection that
260 occurred, along with the laboratory conditions used, meant that the assemblages studied
261 here were closer to “model assemblages” than to the intricate structures that can be
262 expected in nature. However, the trends observed here can be expected to be similar in
263 more complex assemblages.

264

265 Interestingly, the percentage of diatom abnormalities remained fairly stable in the
266 ExCu2 and Cu channels throughout the experiment. No significant increase in *r* values
267 was observed in Cu treatments (Fig. 4), suggesting that species affected by deformities
268 were unable to reproduce, which is additional evidence for metal-induced genetic
269 impairments (Falasco *et al.*, 2009) which are likely to affect cell division (Schmid, 1984).
270 Accrual of abnormal diatoms in ExCu2 (positive *r* values) apparently contradicts this
271 statement, but in this case can be compared to the clear kinetics of increasing
272 deformities observed in Control and ExCu1 channels. Long-term culture under
273 laboratory conditions has been shown to be responsible for cell deformations (Estes &
274 Dute, 1994; Torgan *et al.*, 2006), which could explain the antagonistic results above.
275 Nevertheless, the relative abundance of deformed diatoms had stabilized at about
276 $2.6 \pm 0.1\%$ by week 6 in all treatments.

277

278 *The role of immigration in diatom assemblage recovery*

279 It is almost impossible to distinguish between biotic and abiotic drivers in *in situ*
280 experiments, but the abiotic drivers are generally considered as the major structuring

281 components for natural communities (Sabater, 2009; Stevenson *et al.*, 1996). In this
282 respect, biofilm recovery after toxicant reduction would result in a rearrangement in the
283 abundances of the remaining viable organisms (differential re-growth), whereas re-
284 colonization by upstream drift would rebuild an assemblage comprising those taxa that
285 coped with the toxic stress plus immigrants. The residual biofilm including the surviving
286 species would probably make the surface rougher and stickier than bare surfaces, and
287 thus produce autogenic habitats favouring quicker re-colonization (Peterson *et al.*,
288 1990). Moreover, the initially dominant species may enhance the ultimate development
289 of late-successional species through facilitation (M. Bottin, pers. comm.).

290 In this study, we differentiated between the effects of a reduction in toxic stress and of
291 inputs of immigrants on the recovery process. Whatever the treatment (ExCu1, ExCu2),
292 the biofilms remaining after one month of Cu exposure contained enough viable
293 organisms to ensure there was no lag time in re-growth (data not shown). We found
294 clear differential trajectories from previously-exposed to unexposed diatom assemblage
295 structure in the ExCu1 and ExCu2 channels. In ExCu1, connection with unstressed
296 assemblages led to extremely rapid recovery. Conversely, recovery was delayed for
297 ExCu2 biofilms, which were still unable to attain a comparable diatom species
298 composition to Control biofilms even after 6 weeks under uncontaminated conditions.
299 Iserentant & Blancke (1986) attributed changes in diatom assemblages after
300 transplantation to differential development/proliferation of the species that had already
301 settled. However, the quick recovery observed here of ExCu1 in terms of species
302 composition (within 2 weeks for diatom species relative abundances and 6 weeks for
303 diatoms chlorophyll *a*) demonstrates that immigration is important in the dynamics of
304 recovery. The role of species drift in recovery thus has to be considered, even if the
305 present very optimistic scenario probably resulted from the experimental design

306 promoting dispersal and high connectivity (the proximity and high density of potential
307 immigrants, very few water renewals, and intermediate water flow) enhancing potential
308 immigration and subsequent algal succession (McCormick & Stevenson, 1991; Rolland *et*
309 *al.*, 1997; Stevenson, 1983).

310 Higher reproduction rates in Control and ExCu1 channels can be inferred from increased
311 silica consumption (Table 1). *In situ* reproduction of immigrants, although not easily
312 measurable, is assumed to have substantially contributed to the above-mentioned
313 recovery process. Indeed, biofilm diatoms show rapid reproduction (Baars, 1983; Morin
314 *et al.*, 2008a), resulting in an increase in the density of the newly-introduced organisms.
315 Studies on interspecific variation in diatom immigration (Stevenson & Peterson, 1991)
316 indicated that *Achnantheidium minutissimum* immigration was double that for *Fragilaria*
317 species; thus the massive growth of *F. capucina* in ExCu1 probably resulted from
318 immigration and subsequent reproduction of highly-productive individuals originating
319 from unimpaired biofilms. Ultimately, immigration and *in situ* reproduction of both
320 persistent and re-colonizing species, although undistinguishable, play a major role in
321 periphytic recovery after exposure to copper pollution.

322

323 *Ecological importance of preserving longitudinal connectivity in streams*

324 Current freshwater management plans (e.g. EU Water Framework Directive -
325 2000/60/EC-, EU River Basin Management Plans, or Wenger, 2002) underline the
326 importance of preserving river corridors as major “monitoring tools” for ecological
327 restoration of freshwater hydroecosystems. Indeed, the proximity of unimpacted areas
328 within catchments is believed to increase effective recovery of connected impaired sites.
329 In this study, we indirectly assessed the gain in remediation efforts by characterizing the

330 differential recovery of diatom assemblages (ExCu1 and ExCu2) after a chronic exposure
331 to copper. Water contamination by micropollutants is recognized as a major issue in the
332 EU, and our results support the qualitative importance of maintaining river corridors.
333 Overall, both immigration and *in situ* reproduction, although undistinguishable,
334 enhanced periphytic recovery after exposure to copper pollution (ExCu1 treatment). In
335 the wider perspective of restoring ecosystem integrity, maintaining intact reaches
336 upstream would maximize the potential for downstream sites to recover from past
337 contamination or after unexpected toxicant releases.

338 The findings reported here also demonstrated that diatom assemblages can recover
339 largely by propagule immigration and *in situ* reproduction of recolonizing species. As
340 might be expected, the immigrants here were species known as pioneers that are
341 resistant to disturbances (e.g. *Achnantheidium minutissimum*; Peterson & Stevenson,
342 1992) and common in the upstream site from which they originated (Morin *et al.*, 2010a,
343 Roubex *et al.*, 2011). These results could also highlight risks of high dispersability and
344 potential to establish of undesirable diatoms, such as invasive species that immigrate
345 readily, grow quickly and are highly competitive (Keller & Lodge, 2009; Friberg *et al.*,
346 2011). In a context of global change, such exotic species could be favoured by multiple
347 stresses reinforced by environmental warming, and consequently outcompete or even
348 replace native species (Coste & Ector, 2000).

349

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358

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494 TABLES

495 **Table 1: Mean values (\pm SE, n = 3) of physicochemical parameters of the water in the four series of**
 496 **channels, determined during the 6-week recovery period. Letters in superscript following SiO₂ and**
 497 **Cu values indicate statistical differences between treatments (Tukey's HSD test; $P < 0.01$).**

	Control	Ex-Cu1	Ex-Cu2	Cu
Conductivity ($\mu\text{S cm}^{-1}$)	106 \pm 12	97 \pm 11	99 \pm 11	106 \pm 12
O ₂ (mg L ⁻¹)	7.9 \pm 0.6	8.0 \pm 0.7	8.4 \pm 0.8	7.9 \pm 0.8
pH	7.9 \pm 0.1	7.9 \pm 0.1	7.8 \pm 0.1	7.8 \pm 0.1
Temperature ($^{\circ}\text{C}$)	20.6 \pm 0.4	21.2 \pm 0.5	20.9 \pm 0.5	20.7 \pm 0.4
NH ₄ ($\mu\text{g L}^{-1}$)	17.4 \pm 2.8	17.0 \pm 3.3	20.3 \pm 5.4	18.7 \pm 4.1
NO ₂ ($\mu\text{g L}^{-1}$)	23.1 \pm 4.9	30.3 \pm 7.2	41.7 \pm 7.2	41.4 \pm 8.0
NO ₃ (mg L ⁻¹)	2.3 \pm 0.7	2.6 \pm 0.8	2.6 \pm 0.8	2.3 \pm 0.7
PO ₄ ($\mu\text{g L}^{-1}$)	52.9 \pm 16.3	78.3 \pm 14.3	63.3 \pm 18.7	60.0 \pm 6.7
SiO ₂ (mg L ⁻¹)	2.1 \pm 0.6 ^a	4.8 \pm 0.5 ^{a,b}	6.9 \pm 1.3 ^b	7.0 \pm 1.3 ^b
Cu ($\mu\text{g L}^{-1}$)	1.4 \pm 0.5 ^a	3.4 \pm 1.2 ^a	4.9 \pm 1.6 ^a	16.6 \pm 1.4 ^b

498

499 **Table 2: Mean (\pm SE) values of 2-week variations in population and the corresponding formula of the Δpop .**
 500 **$\Delta\text{pop} = nF$ (net fecundity: reproduction - death) + I (immigration) - E (emigration). Letters in subscript refer**
 501 **to specific environmental conditions (CC: Control conditions; CU: Copper conditions; EX: ExCu1 and 2**
 502 **conditions). I_{EX} values in italics are non-significant. NHAN: *Nitzschia hantzschiana*, ADMI: *Achnanthydium*
 503 ***minutissimum*, FCAP: *Fragilaria capucina*.****

Two-week Δpop ($\mu\text{gChl cm}^{-2}$)	Control	Cu	ExCu1	ExCu2	Immigration
Equation	$nF_{\text{CC}} - E_{\text{CC}}$	$nF_{\text{CU}} - E_{\text{CU}}$	$nF_{\text{EX}+I_{\text{EX}} - E_{\text{EX}}}$	$nF_{\text{EX}} - E_{\text{EX}}$	I_{EX}
Total diatoms	5.3 \pm 1.9	0.3 \pm 0.4	6.8 \pm 1.4	1.7 \pm 0.7	5.1 \pm 1.6
NHAN	-0.7 \pm 0.7	-0.2 \pm 0.3	0.2 \pm 0.6	-0.3 \pm 0.2	<i>0.5\pm0.7</i>
ADMI	3.9 \pm 0.8	0.6 \pm 0.4	3.5 \pm 0.5	1.6 \pm 0.8	<i>1.8\pm0.9</i>
FCAP	1.6 \pm 0.7	-0.0 \pm 0.1	2.5 \pm 0.4	0.1 \pm 0.1	2.4 \pm 0.4

504

505

506 FIGURES

507 **Figure 1: Experimental set-up during exposure and recovery periods. Biofilms grown under**
508 **control conditions are in white (no Cu added in water) and under contaminated conditions in grey**
509 **(channels supplemented with Cu).**

510

511 **Figure 2: Proportion of diatoms and relative abundance of the dominant diatom species and**
512 **abnormal individuals over the course of the experiment. All data are expressed in % (in the case of**
513 **total diatoms, % of the total algal community).**

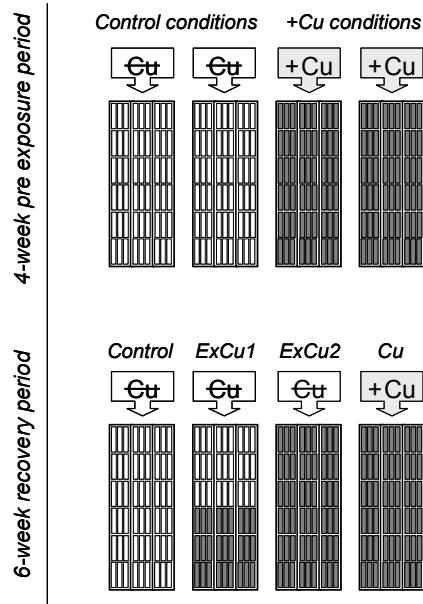
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515 **Figure 3: Cluster analysis of the samples based on diatom species relative abundance. Only the**
516 **taxa representing more than 1% relative abundance in at least one sample are taken into account.**
517 **See Fig. 2 for abundance of the dominant species on each sampling date.**

518

519 **Figure 4: Mean rates of increase (in cell doublings/day) over the 6-week experimental period of**
520 **the global diatom assemblage (Total diatoms), dominant species and abnormal cells. n.s.: non-**
521 **significant ($P>0.05$).**

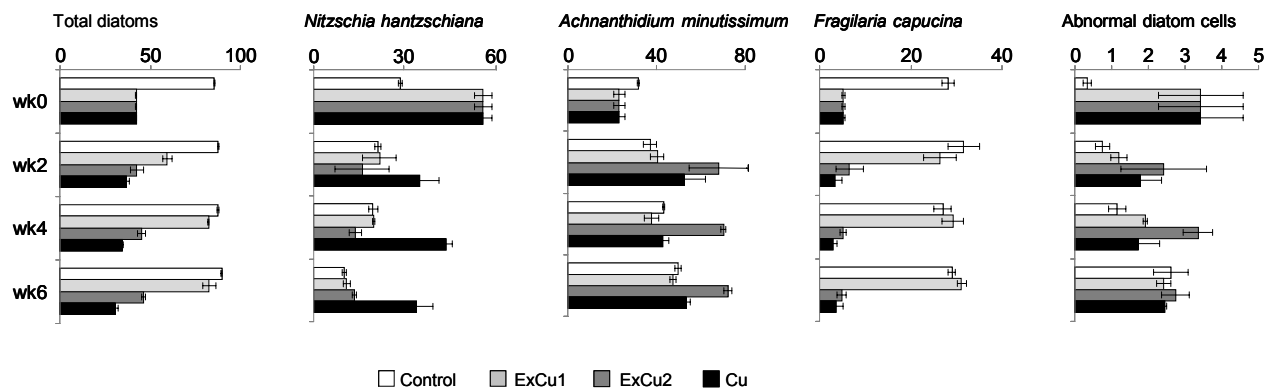
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524 Fig. 1

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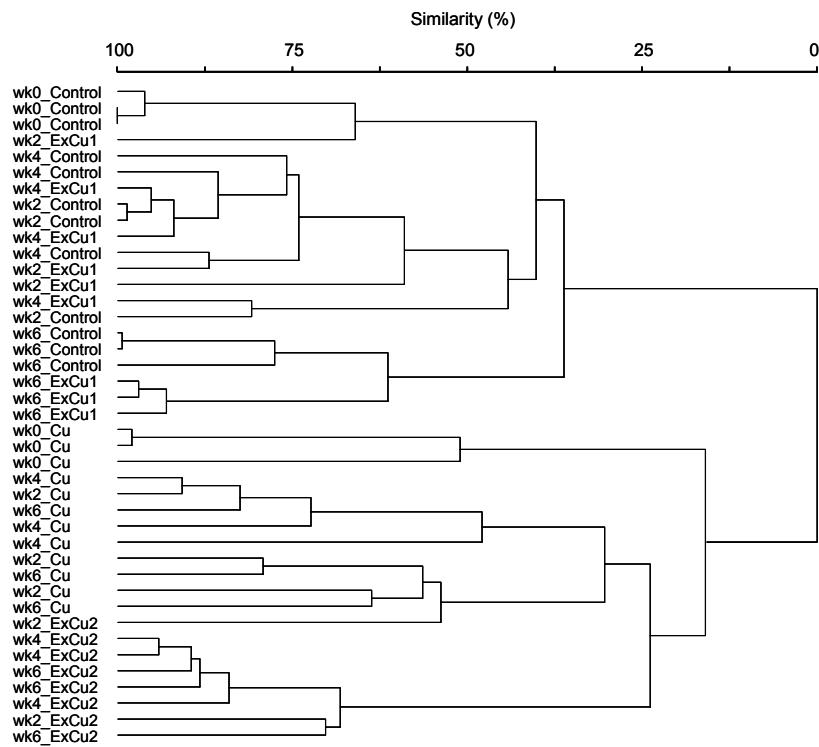


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527 Fig. 2

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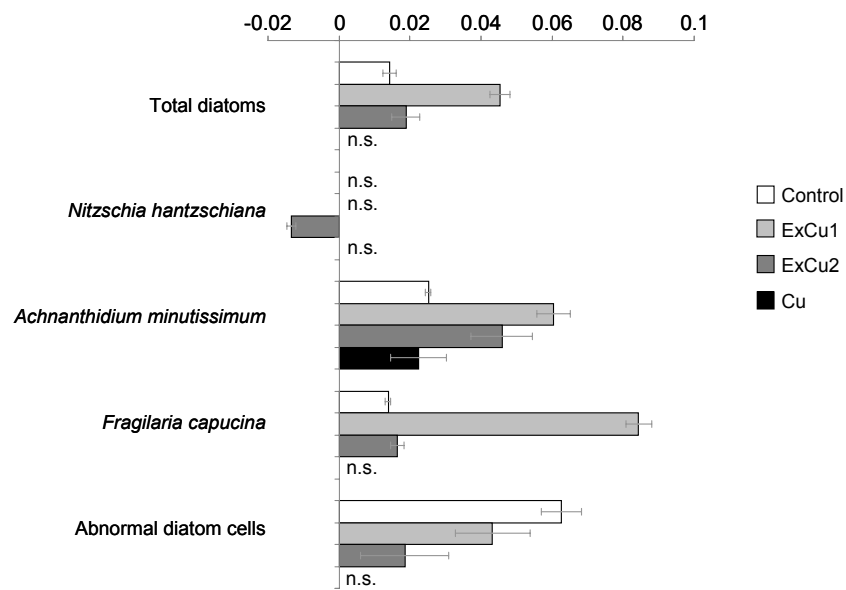
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531 Fig. 3

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534 Fig. 4

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