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Diatom immigration drives biofilm recovery after chronic copper exposure

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Summary

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

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

Introduction

In the early 90s, Stevenson & Peterson (1989, 1991) studied the emigration and immigration of benthic diatoms as determinants of periphytic communities in streams. Since their work, the influence of immigration in freshwater benthic systems has mainly been considered in relation to recovery following sharp changes in flow regime, i.e. spate (Peterson & Stevenson, 1992; Peterson, 1996; Stevenson, 1990) or episodes of drought (Bambakidis, 2009; Benenati *et al.*, 1998).

Translocation studies are increasingly used to assess the recovery potential of riverine periphytic communities (biofilms). They are generally performed by transferring biofilms from contaminated sites to uncontaminated sites upstream of the contamination source (Dorigo *et al.*, 2010; Morin *et al.*, 2010a; Tolcach & Gómez, 2002; Rotter *et al.*, 2011), but sometimes by transplanting contaminated communities to a neighbouring river with similar environmental conditions (Ivorra *et al.*, 1999).

Depending on the study, the time for full recovery of diatom assemblage structure has been estimated at from as little as two (Tolcach & Gómez, 2002) to over nine weeks (Dorigo *et al.*, 2010). The contribution of immigration to recovery after episodes of contamination is often questioned in translocation experiments, and ecotoxicological studies in general (e.g. Ivorra *et al.*, 1999; Morin *et al.*, 2010a). However, it remains virtually impossible to discriminate between the effects of re-growth from remaining viable cells and colonization by new immigrants.

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

Methods

Experimental setup

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

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

During the 6-weeks of recovery, four different treatments (each with three replicates) were tested (Fig. 1): I) Control treatment: a set of $n = 3$ uncontaminated channels kept under uncontaminated water; II) Cu treatment: a set of $n = 3$ Cu-contaminated channels kept exposed to about $20 \mu\text{g Cu L}^{-1}$; III) ExCu1 treatment: set of channels with biofilms grown under control conditions placed in the upstream half-section of the channels and biofilms grown under Cu-contaminated conditions placed in the downstream section, filled with uncontaminated water; IV) ExCu2 treatment: set of biofilms grown under Cu-contaminated conditions and filled with uncontaminated water. The ExCu1 and ExCu2 treatments were used to assess the recovery of diatom assemblages following a decrease in Cu exposure, with (ExCu1) or without (ExCu2) the possibility of diatom immigration from "control" assemblages to the impacted biofilms.

Water analyses

All physicochemical parameters were monitored every two weeks, before and after the water was changed. For water changes, groundwater containing a very low Cu concentration ($<0.3 \mu\text{g L}^{-1}$) was adjusted to the conductivity (i.e. $600 \mu\text{S cm}^{-1}$) and 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 sampling site. In the Cu-contaminated channels, the water was supplemented with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to obtain the nominal concentration of $20 \mu\text{g Cu L}^{-1}$.

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

Algal biomass and diatom analysis

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

identification. Chlorophyll-*a* fluorescence (Chl) emitted by diatoms was determined by measurements using a PhytoPAM fluorometer (Walz, Effeltrich, Germany) equipped with the PhytoEDF emitter-detector unit, performed on fresh material (as described in Morin *et al.*, 2010b). Data were then converted into $\mu\text{g Chl cm}^{-2}$. Subsamples for diatom identification were preserved in a formalin solution (4%, v/v) and prepared according to standard NF EN 13946. Briefly, organic matter was removed from the samples by digestion in boiling hydrogen peroxide (30% H_2O_2) followed by rinsing and successive cycles of centrifugation and re-suspension in distilled water to remove any residual peroxide. Clean material was then pipetted onto cover-slips and allowed to dry before being mounted with a high refractive index mountant (Naphrax, Brunel Microscopes Ltd, UK) for microscopic identification (x1000 magnification on a Leica DMRB photomicroscope, Wetzlar, Germany). At least 400 individual diatoms were identified to the lowest taxonomical level possible. The primary references used were Krammer & Lange-Bertalot (1986 - 1991) and the Diatoms of Europe series (A.R.G. Gantner Verlag K.G, Ruggell). Relative abundance of the taxa identified was recorded (RA, in %); when deformed individuals were observed, their percentage was also determined.

Data treatment

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

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

Samples were ordinated by cluster analysis (Bray Curtis similarities) on the basis of diatom species composition (PC-Ord v4.25, MjM Software, USA).

Diatom biomass values measured through fluorescence were used to convert taxon counts (RA) into biomass values for the dominant species and abnormal valves (expressed in $\mu\text{g Chl cm}^{-2}$). Based on these data, daily rates of increase (r , in cell doublings/day) during the exponential growth phase were calculated using the slope of the line relating time and natural log biomass according to Wood *et al.* (2005).

Probability values of the linear regressions were calculated to determine whether growth was significant, before investigating differences between treatments in the total diatom biomass, dominant species found, and abnormal forms.

Two-week population growth (written as Δpop) was determined for each treatment and pair of consecutive sampling dates to estimate the impact of immigration. Δpop was determined by two factors: net fecundity (reproduction - death) and net immigration (immigration - emigration). Net fecundity and immigration may differ according to environmental conditions (Control vs. Cu), thus ANOVA comparisons were only performed for ExCu1 and ExCu2 to assess differences in net immigration. As they shared a comparable contamination history, the differences in growth between ExCu1 and ExCu2 corresponded to the immigration that occurred only in ExCu1 (immigration of species from the "upstream" to the "downstream" slides, only the latter being sampled), and were thus taken as an estimate of the potential influence of immigration.

Results

Water

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

Diatom assemblages

Biomass of diatoms (Fig. 2) represented $87.5 \pm 0.5\%$ of that of the overall phototrophic assemblage in the Control channels, against $36.0 \pm 1.4\%$ in Cu-exposed channels. Statistical differences in biomass were observed between treatments ($P<0.001$), and a significant increase with time was noted ($P=0.022$ in week 2, $P<0.001$ in weeks 4 and 6). The proportion of diatoms increased in ExCu1 from $42.1 \pm 0.3\%$ initially to $82.7 \pm 3.7\%$ in week 6, and reached a comparable diatom biomass to Controls ($P=0.058$), whereas in ExCu2 it was stable and significantly lower ($P<0.001$), at $44.1 \pm 2.3\%$ throughout the experiment. Species richness was significantly lower in the Controls than in the other treatments (18 ± 1 vs 21 ± 1 , $P=0.024$), and tended to decrease over time (time effect on week 6, 17 ± 1

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

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

2- Cu and ExCu2 communities composed the lower branch of the cluster, in which ExCu2 samples were all grouped together, except one (week 2, that was grouped with Cu samples). Relative abundance of species was less even, as *Achnantheidium minutissimum* ($53 \pm 4\%$) and *Nitzschia hantzschiana* ($30 \pm 4\%$) tended to dominate whereas *Fragilaria capucina* abundances markedly decreased ($6 \pm 1\%$). In the sub-cluster of ExCu2 samples (all but one, corresponding to week2), separation was due to a dominance of *A. minutissimum* ($67 \pm 4\%$) over *N. hantzschiana* ($14 \pm 1\%$) and *F. capucina* ($8 \pm 3\%$).

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

Rates of increase in cell numbers and population dynamics

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

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

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

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,

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

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

The role of immigration in diatom assemblage recovery

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

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

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

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

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

Ecological importance of preserving longitudinal connectivity in streams

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

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

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

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TABLES

Table 1: Mean values ($\pm$ SE, n = 3) of physicochemical parameters of the water in the four series of channels, determined during the 6-week recovery period. Letters in superscript following SiO₂ and 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 (°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

Table 2: Mean ($\pm$ SE) values of 2-week variations in population and the corresponding formula of the Δpop . $\Delta\text{pop} = nF$ (net fecundity: reproduction – death) + I (immigration) – E (emigration). Letters in subscript refer to specific environmental conditions (CC: Control conditions; CU: Copper conditions; EX: ExCu1 and 2 conditions). I_{EX} values in italics are non-significant. NHAN: *Nitzschia hantzschiana*, ADMI: *Achnanthes 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	0.5 $\pm$ 0.7
ADMI	3.9 $\pm$ 0.8	0.6 $\pm$ 0.4	3.5 $\pm$ 0.5	1.6 $\pm$ 0.8	1.8 $\pm$ 0.9
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

FIGURES

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

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

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

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

Fig. 1

Fig. 2

Fig. 3

Fig. 4