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1 Recovery potential of periphytic communities in a river impacted by a vineyard watershed

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8 Abstract

9 Vineyard areas are important causes of water contamination, especially by pesticides  
10 and residues. These compounds can markedly disturb aquatic communities particularly  
11 photosynthetic organisms that are targeted by herbicides. Biofilms and diatoms were used as  
12 bioindicators for quality assessment in the Morcille watershed, an area impacted by  
13 Beaujolais vineyards (SE France), during the pesticide spreading period (April-May 2008).  
14 Biofilms were allowed to settle on glass slides for 4 or 8 weeks at three sites along a 7-km  
15 long gradient of trophic (mainly orthophosphate) and pesticide pollution. After a 4-week  
16 colonisation, samples from the two contaminated downstream sites were transferred upstream  
17 to the clean site for four weeks while others were left in the same place.

18 *In vivo* fluorescence measurements indicated that the periphytic communities were  
19 dominated by diatoms. Going downstream, biofilm biomass and diatom species richness  
20 decreased; normalized diatom indices (including the French standard BDI) expressed the  
21 increase in trophic status quite well. The species composition of the assemblages was used to  
22 discriminate between the effects of nutrients and toxicants, which increased simultaneously as  
23 the river continued downstream.

24 The way in which the biofilm samples transferred upstream recovered was quite  
25 different depending on the location of the original site in the contamination gradient. Most of  
26 the quantitative descriptors reached a level comparable to that of the reference communities,

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27 but the diatom assemblages (cell density per surface unit, taxonomic composition) varied  
28 between dates and along the gradient. These communities did not entirely recover a reference  
29 structure but the increase in diversity, associated with the settlement of sensitive species,  
30 suggested an elevated recovery potential.

31

32 Keywords

33 River biofilms; diatoms; pesticides; Biological Diatom Index; community analysis; diuron;  
34 recovery

35

## 36 Introduction

37 Aquatic life constitutes the ultimate indicator of conditions in aquatic systems, and  
38 many studies undertaken to assess the degradation of habitats and water quality have been  
39 based on key organisms (primary producers, benthic invertebrates and fishes). In particular,  
40 periphytic diatoms are widely used for monitoring purposes because of their value as  
41 indicators of organic pollution, eutrophication and acidification (the major diatom-based  
42 indices are reviewed in Besse-Lototskaya et al. submitted). More recently, *in situ* surveys of  
43 toxic pollutants like heavy metals (Ivorra et al. 2002; Gold et al. 2003; Morin et al. 2008a) or  
44 pesticides (De Jonge et al. 2008; Morin et al. accepted) have provided data in favour of an  
45 extension of the application domain of diatoms indices.

46 Reaching a 'good ecological status' (i.e. close to reference conditions) for most surface waters  
47 by 2015, as imposed by the European Water Framework Directive (2000/60/EC), should lead  
48 to the rehabilitation of many impacted sites. In this context, growing interest is now given to  
49 studying recovery trajectories and community resilience in aquatic environments. Due to their  
50 key ecological role in streams and rivers, studying and understanding biofilm resilience is a  
51 matter of importance. Since polluted sites are difficult to remediate and generally necessitate  
52 long-term surveillance, the assessment of the consequences of site rehabilitation on periphytic  
53 communities could be helped by rapid alternative methods. The use of translocation  
54 approaches (from up- to downstream of a point source of contamination and vice-versa) has  
55 thus been proposed to provide an *in situ* assessment of the impacts on un-exposed  
56 microbenthic communities or of the resilience of chronically exposed communities after  
57 mimicking a reduction of pollution pressure by biofilm translocation (Ivorra et al. 1999;  
58 Tolcach and Gómez 2002; Dorigo et al, submitted).

59 Considering the lack of knowledge about the recovery trajectory of microbenthic  
60 communities moved from contaminated sites, we propose here an *in situ* survey for studying

61 biofilm and diatom recovery potential in the Morcille River (France) which exhibits  
62 increasing pesticide contamination with vineyard pressure (Gouy et al. 1998; Lagacherie et al.  
63 2006; Rabiet et al. 2008), accompanied by an elevation in nutrients (Dorigo et al. 2007) and  
64 metals (Rabiet et al. 2008). Along this gradient, microbial biofilms differ in structure  
65 (Montuelle et al. 2006; Dorigo et al. 2007; Dorigo et al. 2009) as well as in tolerance to  
66 pesticides, notably diuron (Dorigo et al. 2007) and in diuron-mineralization potential (Pesce et  
67 al. submitted; Pesce et al. 2009).

68 We studied the structural changes in assemblages induced by the transfer of periphytic  
69 communities from 2 contaminated sites on the Morcille River, respectively draining areas of  
70 51.6 and 79% of vineyard cover, to a clean site upstream. We expected a recovery of the  
71 translocated communities, either in structure or in diversity. Biofilm biomass, diatom  
72 taxonomic composition, and commonly used indices were used to characterize their response  
73 to the gradient of agricultural contamination and to the simulated improvement of water  
74 quality.

## 76 Materials and methods

### 77 *Location of the study sites*

78 The translocation experiments were conducted in Spring (April and May) 2008 along  
79 the Morcille River, located in the Beaujolais vineyard area, eastern France (46.150°N,  
80 4.600°E, belonging to national river type 3 “Massif Central sud”, Figure 1). The Morcille  
81 River is a small first-order stream (7 km long) subjected to strong agricultural pressure,  
82 essentially exerted by vineyards that occupy almost 80% of the 8.5 km<sup>2</sup> catchment area. Three  
83 sites were selected along the gradient of increasing percentage contribution of vineyards to the  
84 catchment area: Saint-Joseph (vineyard percentage cover: 6.7%), Les Versauds (51.6%) and  
85 Saint-Ennemonde (79%).

86 With pesticide concentrations below the quantification limits in 2007, Saint-Joseph  
87 was considered as a “clean” site, whereas total pesticide concentrations at Les Versauds and  
88 Saint-Ennemond in Spring sometimes exceeded 2 µg/L and 5 µg/L, respectively (Rabiet et al.  
89 2008).

90

91 *Stream water physicochemical characteristics of the sites.*

92 Sampling was performed in Spring 2008, during the intense pesticide application  
93 period. During the experiment, pH, electrical conductivity and dissolved oxygen were  
94 measured *in situ* (WTW, Weilheim, Germany). Water samples were taken, cooled to 4°C and  
95 brought back to the laboratory for nutrient (bimonthly) and pesticide analyses, twice a month  
96 at Saint-Joseph and Les Versauds and mid-survey at Saint-Ennemond. Nitrite, nitrate,  
97 ammonia, orthophosphate and suspended solids concentrations were determined following  
98 French standard operating procedures and protocols (Association Française de  
99 NORmalisation, AFNOR).

100 Using standardized protocols, the 8 most frequently found pesticides and some of their  
101 degradation products were analyzed in the water samples by ESI-LC-MS/MS (API 4000,  
102 Applied Biosystems) at the Water Chemistry Laboratory in Cemagref (Lyon). An exhaustive,  
103 complementary, screening of 379 substances was also performed at Les Versauds on the 30<sup>th</sup>  
104 May by the Laboratoire Départemental d’Analyses de la Drôme (LDA, Valence, France).

105

106 *Collection of periphytic communities*

107 At each site, large glass slides (300 cm<sup>2</sup> area for both slides) fixed in perforated plastic  
108 boxes were used as artificial substrates allowing algal colonisation. After a 4-week immersion  
109 (2 April to 6 May), slides were removed i) for collection to characterize the 4-week-old  
110 communities (3 slides per site, called “1 month April”), ii) for a 4-week translocation to the

111 site Saint-Joseph (2 slides per site, called “translocated slides”), and iii) 3 slides remained for  
112 4 more weeks at their respective sites (2 April to 29 May; called “2 months”).  
113 Simultaneously, new slides were caged to characterize the 4-week-old communities settling  
114 between 6 and 29 May at the three sites (called “1 month May”).

115

#### 116 *Biofilm analyses and diatom species composition*

117 The proportions of the different algal groups (i.e. green algae, diatoms and  
118 cyanobacteria) were estimated by *in vivo* chlorophyll *a* fluorescence measurements  
119 (Leboulanger et al. 2006) using a Phyto-PAM (Phytoplankton analyzer Phyto-PAM, Heinz  
120 Walz GmbH, Effeltrich, Germany) directly on randomly-selected points of the colonized  
121 slides. Then the biofilm was scraped off each replicate slide, suspended in a standard volume  
122 of mineral water and subsampled for further analyses.

123 A 20-ml aliquot was used to determine the dry weight (DW) and ash-free dry mass  
124 (AFDM) of the biofilm, expressed as mg/cm<sup>2</sup>. After filtration of the suspension through  
125 individual, previously dried and weighed, glass fibre filters (pore size: 1.2 µm; Sartorius,  
126 Göttingen, Germany), the samples were dried for 1 hour at 105°C for DW calculations. Then  
127 the filters were ashed at 500°C for 1 hour (Nabertherm P320 furnace) and weighed to  
128 determine the mineral content. AFDM was calculated by subtracting the mineral matter from  
129 the total dry weight.

130 Ten ml of the suspension were filtered through a Whatman GF/C filter, then extracted  
131 with acetone for 24 hours before spectrophotometric analyses. Chlorophyll *a* concentrations  
132 were calculated after Lorenzen (1967).

133 An aliquot of 5 ml was preserved with 1 ml of formalin solution for diatom cell  
134 density enumeration and taxonomic identification. Enumeration was done from 125 µL of  
135 each preserved sample using a Nageotte counting chamber: the total number of cells counted

136 in 10 fields (1.25 ml each, 0.5 mm depth) using light microscopy at 400x magnification  
137 (photomicroscope Leica DMRB, Wetzlar, Germany) was then recorded as cells per unit area  
138 of substrate (number of diatom cells/cm<sup>2</sup>). Subsamples assigned to taxonomic analyses were  
139 prepared according to Charles et al. (2002), i.e. digestion in boiling hydrogen peroxide (30%  
140 H<sub>2</sub>O<sub>2</sub>) and hydrochloric acid (35%) followed by three cycles of centrifugation of the sample  
141 and pellet rinsing with distilled water. After the last treatment, the pellet was once again  
142 resuspended in distilled water, and the suspension deposited onto coverslips then mounted  
143 onto slides after air drying, using the high refractive index (1.74) medium Naphrax (Brunel  
144 Microscopes Ltd, UK). Diatom counts were conducted at a magnification of 1000x;  
145 individual fields were scanned until at least 400 valves had been identified using taxonomic  
146 literature from central Europe (Krammer and Lange-Bertalot 1986 - 1991). From the specific  
147 composition of each sample, the indices BDI v.2006 (Biological Diatom Index, Coste et al.  
148 2009) and SPI (Specific Polluosensitivity Index, Coste in Cemagref 1982) were calculated  
149 using Omnidia software (Lecoite et al. 1993).

150

#### 151 *Data treatment*

152 Biofilm characteristics and diatom index values were checked for normality and  
153 variance equality before analyzing the dataset using one-way ANOVA with STATISTICA  
154 software (v. 5.1, StatSoft, 1998). After having completed ANOVA, Tukey's post hoc tests  
155 were performed in order to determine which groups of data significantly differed from each  
156 other.

157 Non-metric multi-dimensional scaling (NMDS), an indirect ordination method based  
158 on the dissimilarities in species community structure of the samples, was performed using the  
159 labdsv package (<http://ecology.msu.montana.edu/labdsv/R/labdsv>) for the R statistical  
160 environment (Ihaka and Gentleman 1996).

161

## 162 Results

### 163 *Physical and chemical characteristics of the sites (table 1)*

164 During the survey, pH was quite stable over time and between sites. From up- to  
165 downstream, there was a longitudinal increase in conductivity, DOC, nitrite, orthophosphate  
166 and pesticides. Conductivity, DOC, nitrite and orthophosphate indicate the progressive  
167 increase of human inhabitants from up- to downstream in the watershed and pesticides are  
168 connected to the vineyard area.

169 Routine analyses gave pesticide concentrations that were always below the  
170 quantification limits at Saint-Joseph; they increased downstream to reach the value of 8.4  
171 µg/L at Saint-Ennemond. Differences were observed between April and May at Les Versauds  
172 (1.9 and 2.6 µg/L). The contamination of the water was mainly due to herbicides (diuron and  
173 its metabolite DCMU) and, to a lesser extent, by fungicides (azoxystrobin, dimethomorph,  
174 procymidone, tebuconazole). At the half-course of the experiment, the complementary survey  
175 performed by the LDA at Les Versauds recorded 21 substances reaching a total concentration  
176 of 8.2 µg/L, mainly herbicides (90%, data not shown). Among the compounds found were  
177 high concentrations of diuron (4.1 µg/L) as well as the substances routinely analyzed, but also  
178 the pyridazinone herbicide norflurazon (0.3 µg/L) together with its breakdown product  
179 norflurazon desmethyl (2.6 µg/L), terbumeton desethyl (0.4 µg/L) and dichlorobenzamide  
180 (0.2 µg/L).

181

### 182 *Global descriptors of the biofilm (table 2)*

183 Significant differences were observed between DW ( $p<0.01$ ) and AFDM ( $p<0.05$ )  
184 along the gradient and between April and May. Both indicators of biomass established in a  
185 one-month period showed a significant decrease along the gradient in April. In May DW and

186 AFDM were lower at Saint-Joseph, and no statistical difference was observed between sites.

187 The translocated samples presented intermediate values of biomass between Saint-Joseph and  
188 the site from which they were transferred.

189 The PhytoPAM measurements indicated that the biofilms were almost exclusively  
190 made up of the pigment class corresponding to diatoms (average 90% of the photosynthetic  
191 activity). Chlorophyll *a* concentrations and diatom cell densities were strongly correlated  
192 ( $R^2=0.934$ ,  $p<0.01$ ) and their variations closely paralleled those of DW and AFDM. The cell  
193 densities after a 1-month colonization, and 2 months at the same site or after translocation, are  
194 given in Figure 2.

195

#### 196 *Diatom communities*

197 A total number of 120 species representing 40 genera were identified, but the  
198 assemblages were always dominated by *Planothidium lanceolatum* (Brébisson ex Kützing)  
199 Lange-Bertalot, this species representing about 60% of the relative abundances in all samples.  
200 From the NMDS performed using the 40 species occurring at more than 1% relative  
201 abundance in at least one sample (Figure 3), samples presenting strong similarities in  
202 community structure were grouped.

203 Clear differences were observed between in the assemblages collected in both April  
204 and May (noted 1m/April and 1m/May in Figure 3). April samples from Les Versauds and  
205 Saint-Ennemond were characterized by increasing abundances of *Planothidium lanceolatum*,  
206 *P. frequentissimum* (Lange-Bertalot) Lange-Bertalot, *Cocconeis placentula* Ehrenberg var.  
207 *placentula* and by decreasing proportions of *Achnantheidium minutissimum* (Kützing)  
208 Czarnecki and *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot, in comparison with the  
209 communities grown at Saint-Joseph (see Table 3). In May, higher relative abundances (in  
210 decreasing order of abundance) of *Nitzschia linearis* (Agardh) W. M. Smith var. *linearis*,

211 *Hantzschia amphioxys* (Ehrenberg) Grunow, *Luticola cohnii* (Hilse) D.G. Mann, *Surirella*  
212 *angusta* Kützing and *Nitzschia palea* (Kützing) W. Smith were found downstream. The  
213 communities translocated from Saint-Ennemond to the upstream site tended to shift towards  
214 typical Saint-Joseph community structure (with higher proportions of *A. minutissimum* and *R.*  
215 *abbreviata*), whereas the composition of the biofilms translocated from Les Versauds did not  
216 diverge as much from the assemblages that settled in April or during the 2-month  
217 colonization.

218         Whatever the sampling date and the duration of colonization, BDI and SPI values  
219 decreased significantly from up- to downstream ( $p < 0.01$ , see Table 2). In cases of  
220 translocation, the values returned to levels comparable to those of Saint-Joseph.

221

## 222 Discussion

### 223 1) *Potential of diatoms in the assessment of multi-contamination.*

224 The BDI (Lenoir and Coste 1996; Coste et al. 2009) and SPI (Coste in Cemagref 1982)  
225 indices were originally designed to assess alterations in trophic status. The values of diatom  
226 indices along the gradient for 1-month-old communities in April and May indicate the  
227 efficiency of the index for the diagnosis of trophic pollution, with significant differences  
228 between the index values along the stream (Table 2) and changes in water quality class (as  
229 defined by the WFD; good/moderate boundary: BDI=14 for this national type and stream  
230 order) between Saint Joseph (good status), Les Versauds (good to moderate) and Saint  
231 Ennemond (moderate). This was confirmed by the organic pollution tolerance scales of the  
232 species in the assemblages. According to Lange-Bertalot (1979), larger proportions of  
233 “pollution-sensitive” species were found at Saint-Joseph (25.4%), as compared to Les  
234 Versauds (18.2%) or Saint-Ennemond (15.9%). Comparably, the trophic conditions as  
235 assessed by Steinberg and Schiefele’s method (1988) or the saprobity state (van Dam et al.

236 1994), confirmed the gradient of both organic and inorganic pollution along the Morcille  
237 River with 14.1 down to 6.2% of species sensitive to trophic conditions and 8.9 to 16.4% of  
238  $\alpha$ meso- to polysaprobous species between up- and downstream. In the present context of  
239 multi-contamination (nutrients and chemicals increasing simultaneously along the gradient) of  
240 the Morcille River, it is difficult to determine whether the changes in periphytic community  
241 structure are due to effects of pesticides or to other factors like organic or inorganic nutrients.  
242 Indeed, Dorigo et al. (2007) were unable to unambiguously distinguish between the effects of  
243 nutrients and xenobiotics on the community structure of both prokaryotic and eukaryotic  
244 microorganisms in biofilms. The shift in community structure they observed was concomitant  
245 with an increased diuron-induced tolerance, revealing that pesticide contamination was  
246 probably a major driving factor. In the present study, we prove that the BDI was able to  
247 demonstrate the specific effects of increasing trophic status along the gradient, whereas it was  
248 almost impossible for Dorigo to unequivocally explain the changes in microbial assemblages  
249 using non-taxonomic methods.

250 Complementary ecological information was obtained by a precise study of diatom species.  
251 Indeed, most of the taxa present in the Morcille River have already been described as metal-  
252 tolerant (Gold et al. 2002; Ivorra et al. 2002; Szabó et al. 2005; Morin et al. 2008a; Morin et  
253 al. 2008b). This may result from the chronic contamination of the stream by As and Cu,  
254 already found at Saint-Joseph in significant concentrations (e.g. in Spring 2006, total As was  
255 around 4 $\mu$ g/L and total Cu around 2 $\mu$ g/L, see Rabiet et al. 2008). Among the diatoms  
256 recorded, several subdominant species (e.g. *A. minutissimum*, *Eolimna minima* (Grunow)  
257 Lange-Bertalot, *Navicula lanceolata* (Agardh) Ehrenberg) found at Saint-Joseph but not  
258 downstream have already been described as pesticide-sensitive (Hamilton et al. 1987; Pérès et  
259 al. 1996; Morin et al. accepted), as well as less abundant ones like *Eunotia minor* (Kützing)  
260 Grunow in Van Heurck or *Encyonema minutum* (Hilse in Rabenhorst) D.G. Mann (Morin et

261 al. accepted). Their extinction could not have resulted from the trophic gradient, as they are  
262 known to tolerate quite elevated organic and inorganic nutrient concentrations (van Dam et al.  
263 1994). In contrast, some of the species that preferentially developed at Les Versauds  
264 (especially in May) and Saint-Ennemond (*P. lanceolatum*, *P. frequentissimum*, *C. placentula*)  
265 have already been recorded under herbicide concentrations > 5 µg/L (Kosinski 1984; Pérès et  
266 al. 1996; Morin et al. accepted). To date, only few studies have provided data about diatom  
267 sensitivity or tolerance to pesticides, but substantial evidence has been given by these works  
268 in favour of diatom use for the assessment of pesticide contamination. Hamilton et al. (1988),  
269 Pérès et al. (1996) and Schmitt-Jansen and Altenburger (2005) found a remarkable decrease in  
270 diatom numbers under atrazine and isoproturon contamination. Growth inhibition of the  
271 periphytic communities exposed to pesticides has also been observed by Kasai and Hanazato  
272 (1995), Tang et al. (1997) and Leboulanger et al. (2001). However, the nature and the  
273 intensity of interactions between the various xenobiotics in this environment are quite difficult  
274 to determine precisely: antagonistic effects were observed between nutrients (favouring the  
275 development of the community) and toxicants (drastically reducing diatom biomass)(Lozano  
276 and Pratt 1994). An improved identification of pesticide specific effects, based on more  
277 precise indicators is needed, such as PICT approaches (Blanck et al. 1988; Dorigo et al,  
278 2007). Globally, further studies in environments with various xenobiotic loads, in different  
279 hydro-ecoregions (*sensu* Tison et al. 2005), would be necessary to implement diatom-based  
280 indices for such pollution.

281 Other characteristics of diatom communities like quantitative estimates (e.g. diatom cell  
282 densities, AFDM or chlorophyll *a* as global indicators of periphytic biomass), the impacts of  
283 the toxicants were underlined (see Figure 2 and Table 2). Finally, not only the thickness of  
284 biofilm, but also its adhesion to the substrate, differed between the sites. When scraping the  
285 glass slides, the biofilms from Les Versauds and Saint-Ennemond appeared to be much more

286 tightly attached to the substrates than those from Saint-Joseph. This could also be considered  
287 as a response of the biofilm to pesticide exposure. Indeed, Guasch et al. (2003) demonstrated  
288 the influence of the physiognomy of the communities on their tolerance to atrazine pollution,  
289 the loosely attached algae being more sensitive than compact periphyton from the same reach.

290

## 291 2) *Temporal variability of water contamination and periphytic community responses.*

292 In this study the example of the site Les Versauds underlined the limits of spot  
293 measurements of pesticides to analyse dose / response effects. Here the bimonthly frequency  
294 of sampling is maybe not sufficient and we might have missed high contamination events,  
295 especially during floods, as described by Rabiet et al. (2008). The exhaustive pesticide  
296 analysis on this particular site by the LDA also proved that a wider range of chemicals should  
297 be tested to assess the diatoms exposure to the toxics. In particular, the high concentrations of  
298 norflurazon desmethyl that were measured should be considered: no data are available in the  
299 literature concerning its effect on biofilms, but its parent compound norflurazon has been  
300 previously shown to affect the growth of freshwater benthic algal species (Blanck et al. 1984).  
301 For these reasons, we were not able to characterise precisely the real exposure of benthic  
302 microorganisms.

303 However, the assemblages developed at Les Versauds in April and May were quite  
304 different in terms of community structure and SPI values, diatom cell densities and even  
305 chlorophyll *a* concentrations. The decrease of these last estimates in May suggests that  
306 pesticide contamination, which only increased slightly from April (average total pesticides:  
307 5.7 µg/L) to May (7.5 µg/L), reached a threshold above which the effect became noticeable.  
308 As nutrient availability has been shown to mitigate the effects of toxicants (Lozano and Pratt  
309 1994), the trophic level is likely to reduce the sensitivity of the biofilms towards xenobiotics,  
310 until contamination reaches a critical level. It is difficult to establish whether the increase in

311 trophic level affects sensitivity by interfering with the bioavailability of the toxicant, or by  
312 protecting the algae in some way from herbicide exposure. The shift in diatom community  
313 structure from up- to downstream may partly contribute to a protective effect towards  
314 contamination. Guasch et al. (1998) underlined the fact that the diatom taxa occurring under  
315 different levels of eutrophication have different sensitivities to pesticides, which can be linked  
316 to species' tolerance to high trophic levels, but also to physiological and structural parameters  
317 of the biofilms. Inverse correlations also occur between the sensitivity to toxicants and  
318 biomass accrual (age, succession stage as well as thickness)(e.g. Sabater et al. 2007), but  
319 environmental factors like irradiance are important as well (Guasch et al. 1997).

320         Assuming that pesticide contamination was too low in April would also mean that the  
321 translocation experiment of the communities was probably done too prematurely in the  
322 pesticide treatment season, so the periphytic communities of Les Versauds and Saint-  
323 Ennemonde had not had the time to adapt to the prevailing levels of pesticides. The dramatic  
324 cell loss between 1-month-old (April) samples and 2-month-old biofilms could be the result  
325 of this increase in pesticide contamination, but other processes could have taken place  
326 simultaneously, such as suspended sediment scour (Francoeur and Biggs 2006) or self-  
327 generated detachment (Boulêtreau et al. 2006) due to senescence, especially in the context of  
328 the Morcille River where discharge can undergo great variations. However, the use of  
329 artificial, caged samplers that decreased local flow velocity (divided 10 times inside the  
330 plastic racks) may have severely limited the abrasive effects of the current.

331

### 332 3) *Recovery potential of diatom assemblages.*

333         The samples transferred from Les Versauds and Saint-Ennemonde to the upstream site  
334 recovered at different rates, particularly depending on the descriptors taken into consideration.  
335 Most of the quantitative parameters (DW, AFDM, chlorophyll *a*) of the translocated biofilms

336 showed rapid recovery, with values close to those of Saint-Joseph after a 2-month  
337 colonization. Using the cell density data, the samples originating from the less contaminated  
338 Les Versauds site proved to have quicker recovery trajectories than those transferred from  
339 Saint-Ennemond.

340         The intermediate values of BDI for the translocated biofilms between Saint-Joseph  
341 and the downstream sites, as well as the shift in diatom community structure towards the  
342 “reference” assemblages, indicate the occurrence of a recovery process. However, recovery  
343 was not attained within 1 month and a longer translocation time would be necessary. Dorigo  
344 et al. (submitted) observed that translocation over comparable periods of time was too short to  
345 recover a reference community structure analysed by molecular finger print (PCR-DGGE),  
346 whether for eukaryote or for prokaryote assemblages. They also found with PICT assays, in  
347 the transferred biofilms, EC50 values intermediate between those obtained with up- and  
348 downstream biofilms. This seemed to indicate the persistence of a toxic pressure. It was  
349 shown that detectable concentrations of xenobiotics were still adsorbed in the biofilms even  
350 after several weeks of translocation, which would have affected the cells exposed to this  
351 residual internal contamination. Ivorra et al. (1999) and Dorigo et al. (submitted) have in fact  
352 demonstrated that transferred biofilms did not completely release metals accumulated after 2-  
353 to 9-weeks of translocation to a new environment. We cannot exclude that such  
354 accumulations are possible with pesticides, but in their attempt to measure diuron  
355 accumulation in biofilms from the Morcille River, Tlili et al. (2008) did not observe any  
356 accumulation of pesticide within the matrix. They hypothesized that the sorption depended on  
357 the physicochemical properties of the chemical (log K<sub>ow</sub>) or that accumulation was followed  
358 by rapid release from the biofilm to the water phase when replaced in uncontaminated water.

359         Whatever the age of the biofilms, immigration and emigration of diatoms play an  
360 important role in diatom accumulation (Stevenson and Peterson 1989; Stevenson and Peterson

361 1991), and certainly took place in our translocation experiment. Therefore, the trajectories of  
362 structure assemblage recovery observed in the present study are partly due to immigration and  
363 emigration of species and not only to the new water conditions. At Saint-Ennemond in  
364 particular, diatom cell densities in April were outstandingly low and 3-fold higher after  
365 translocation with higher proportions of the rapid colonizer *Achnanthydium minutissimum*  
366 (that was rather found in Saint-Joseph). In this case, immigration may have prevailed over  
367 multiplication rates of pre-established species in the process of translocated community  
368 development. Complementary experiments are needed to assess the real importance of cell  
369 import and export in the evolution of diatom community structure. However, especially in the  
370 context of small rivers like the Morcille River the effects of diatom immigration and  
371 emigration cannot be dissociated from the recovery potential of the ecosystem. Indeed, as the  
372 distance between the up- and downstream sites is no longer than 7 km, an improvement of the  
373 water physicochemical quality following stream restoration associated to the drift of species  
374 usually found upstream, would probably result in comparable shifts in community structure as  
375 observed by our translocation experiment.

376

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383

### 384 References

- 385 Alvarez, D. A., Petty, J. D., Huckins, J. N., Jones-Lepp, T. L., Getting, D. T., Goddard, J. P.  
386 and Manahan, S. E. 2004. Development of a passive, *in situ*, integrative sampler for  
387 hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem. 23,  
388 1640-8.
- 389 Besse-Lototskaya, A., Verdonschot, P. F. M., Coste, M. and Van de Vijver, B. submitted.  
390 Evaluation of European diatom trophic indices. Ecol. Indicators.
- 391 Biggs, B. J. F., Stevenson, R. J. and Lowe, R. L. 1998. A habitat matrix conceptual model for  
392 stream periphyton. Arch. Hydrobiol. 143, 21-56.
- 393 Blanck, H., Wallin, G. and Wängberg, S. A. 1984. Species-dependent variation in algal  
394 sensitivity to chemical compounds. Ecotoxicol. Environ. Saf. 8, 339-51.
- 395 Blanck, H., Wängberg, S. A. and Molander, S. 1988. Pollution-Induced Community  
396 Tolerance - A new ecotoxicological tool. In: Functional Testing of Aquatic Biota for  
397 estimating Hazards of Chemicals. Cairns, J. and Pratt, J.R. (Editors), Philadelphia, pp. 219-30.
- 398 Bony, S., Gillet, C., Bouchez, A., Margoum, C. and Devaux, A. 2008. Genotoxic pressure of  
399 vineyard pesticides in fish: Field and mesocosm surveys. Aquat. Toxicol. 89, 197-203.
- 400 Boulêtreau, S., Garabétian, F., Sauvage, S. and Sánchez-Pérez, J.-M. 2006. Assessing the  
401 importance of a self-generated detachment process in river biofilm models. Freshwat. Biol.  
402 51, 901-12.
- 403 Cemagref. 1982. Etude des méthodes biologiques d'appréciation quantitative de la qualité des  
404 eaux. In. Rapport Q.E. Lyon A.F. - Bassin Rhône-Méditerranée-Corse, p. 218.
- 405 Coste, M., Boutry, S., Tison-Rosebery, J. and Delmas, F. 2009. Improvements of the  
406 Biological Diatom Index (BDI): Description and efficiency of the new version (BDI-2006).  
407 Ecol. Indicators. 9, 621-50.

- 408 De Jonge, M., Van de Vijver, B., Blust, R. and Bervoets, L. 2008. Responses of aquatic  
409 organisms to metal pollution in a lowland river in Flanders: A comparison of diatoms and  
410 macroinvertebrates. *Sci. Total Environ.* 407, 615-29.
- 411 Dorigo, U., Bérard, A., Rimet, F., Bouchez, A. and Montuelle, B. submitted. Resilience of a  
412 river periphyton contaminated by pesticides: an *in situ* assessment.
- 413 Dorigo, U., Leboulanger, C., Bérard, A., Bouchez, A., Humbert, J. F. and Montuelle, B. 2007.  
414 Lotic biofilm community structure and pesticide tolerance along a contamination gradient in a  
415 vineyard area. *Aquat Microb Ecol.* 50, 91-102.
- 416 Dorigo, U., Lefranc, M., Leboulanger, C., Montuelle, B. and Humbert, J. F. 2009. Spatial  
417 heterogeneity of periphytic microbial communities in a small pesticide-polluted river. *FEMS*  
418 *Microbiol. Ecol.* 67, 491-501.
- 419 Faessel, B., Roger, M. C. and Cazin, B. 1993. Incidence de rejets ponctuels et diffus sur les  
420 communautés d'invertébrés benthiques d'un cours d'eau du Beaujolais : l'Ardières. *Annls*  
421 *Limnol. (Int. J. Limnol.)* 29, 307-23.
- 422 Francoeur, S. N. and Biggs, B. J. F. 2006. Short-term effects of elevated velocity and  
423 sediment abrasion on benthic algal communities. *Hydrobiologia.* 561, 59-69.
- 424 Gold, C., Feurtet-Mazel, A., Coste, M. and Boudou, A. 2002. Field transfer of periphytic  
425 diatom communities to assess short-term structural effects of metals (Cd, Zn) in rivers. *Water*  
426 *Res.* 36, 3654-64.
- 427 Gold, C., Feurtet-Mazel, A., Coste, M. and Boudou, A. 2003. Impacts of Cd and Zn on the  
428 development of periphytic diatom communities in artificial streams located along a river  
429 pollution gradient. *Arch. Environ. Contam. Toxicol.* 44, 189-97.
- 430 Gouy, V., Gril, J. J., Laillet, B., Garon-Boucher, C., Dubernet, F. and Cann, C. 1998. Suivi du  
431 transfert des produits phytosanitaires sur les bassins versants et exemple de modélisation  
432 globale. *Ingénieries* 13, 3-14.

- 433 Guasch, H., Muñoz, I., Rosés, N. and Sabater, S. 1997. Changes in atrazine toxicity  
434 throughout succession of stream periphyton communities. J. Appl. Phycol. 9, 132-46.
- 435 Guasch, H., Ivorra, N., Lehmann, V., Paulsson, M., Real, M. and Sabater, S. 1998.  
436 Community composition and sensitivity of periphyton to atrazine in flowing waters: the role  
437 of environmental factors. J. Appl. Phycol. 10, 203-13.
- 438 Guasch, H., Admiraal, W. and Sabater, S. 2003. Contrasting effects of organic and inorganic  
439 toxicants on freshwater periphyton. Aquat. Toxicol. 64, 165-75.
- 440 Hamilton, P. B., Jackson, G. S., Kaushik, N. K. and Solomon, K. R. 1987. The impact of  
441 atrazine on lake periphyton communities, including carbon uptake dynamics using track  
442 autoradiography. Environ. Pollut. 46, 83-103.
- 443 Hamilton, P. B., Jackson, G. S., Kaushik, N. K., Solomon, K. R. and Stephenson, G. L. 1988.  
444 The impact of two applications of atrazine on the plankton communities of *in situ* enclosures.  
445 Aquat. Toxicol. 13, 123-40.
- 446 Hatakeyama, S., Fukushima, S., Kasai, F. and Shiraishi, H. 1994. Assessment of herbicide  
447 effects on algal production in the Kokai River (Japan) using a model stream and *Selenastrum*  
448 bioassay. Ecotoxicology 3, 143-56.
- 449 Huckins, J. N., Manuweera, G. K., Petty, J. D., Mackay, D. and Lebo, J. A. 1993. Lipid-  
450 containing semipermeable membrane devices for monitoring organic contaminants in water.  
451 Environ. Sci. Technol. 27, 2489-96.
- 452 Ihaka, R. and Gentleman, R. 1996. R: A language for data analysis and graphics. J. Comput.  
453 Graph. Statist. 5, 299-314.
- 454 Ivorra, N., Hettelaar, J., Kraak, M. H. S., Sabater, S. and Admiraal, W. 2002. Responses of  
455 biofilms to combined nutrient and metal exposure. Environ. Toxicol. Chem. 21, 626-32.

- 456 Ivorra, N., Hettelaar, J., Tubbing, G. M. J., Kraak, M. H. S., Sabater, S. and Admiraal, W.  
457 1999. Translocation of microbenthic algal assemblages used for *in situ* analysis of metal  
458 pollution in rivers. Arch. Environ. Contam. Toxicol. 37, 19-28.
- 459 Kasai, F. 1999. Shifts in herbicide tolerance in paddy field periphyton following herbicide  
460 application. Chemosphere 38, 919-31.
- 461 Kasai, F. and Hanazato, T. 1995. Effects of the triazine herbicide, simetryn, on freshwater  
462 plankton communities in experimental ponds. Environ. Pollut. 89, 197-202.
- 463 Kosinski, R. J. 1984. The effect of terrestrial herbicides on the community structure of stream  
464 periphyton. Environmental Pollution Series A, Ecological and Biological 36, 165-89.
- 465 Krammer, K. and Lange-Bertalot, H. 1986 - 1991. Bacillariophyceae 1. Teil: Naviculaceae.  
466 876 p.; 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae, 596 p.; 3. Teil: Centrales,  
467 Fragilariaceae, Eunotiaceae, 576 p.; 4. Teil: Achnanthaceae. Kritische Ergänzungen zu  
468 *Navicula* (Lineolatae) und *Gomphonema*. 437 p. G. Fischer Verlag., Stuttgart.
- 469 Lagacherie, P., Diot, O., Domange, N., Gouy, V., Floure, C., Kao, C., Moussa, R., Robbez-  
470 Masson, J. M. and Szleper, V. 2006. An indicator approach for describing the spatial  
471 variability of artificial stream networks with regard to herbicide pollution in cultivated  
472 watersheds. Ecol. Indicators 6, 265-79.
- 473 Lange-Bertalot, H. 1979. Pollution tolerance of diatoms as a criterion for water quality  
474 estimation. Nova Hedw. 64, 285-304.
- 475 Leboulanger, C., Quiblier, C. and Dufour, P. 2006. Rapid assessment of multiple-limiting  
476 factors of phytoplankton biomass: bioassays, *in vivo* chlorophyll-a fluorescence, and factorial  
477 design. Arch. Hydrobiol. 166, 433-51.
- 478 Leboulanger, C., Rimet, F., Heme de Lacotte, M. and Bérard, A. 2001. Effects of atrazine and  
479 nicosulfuron on freshwater microalgae. Environ. Int. 26, 131-5.

- 480 Lecointe, C., Coste, M. and Prygiel, J. 1993. Omnidia - Software for taxonomy, calculation of  
481 diatom indices and inventories management. *Hydrobiologia* 269/270, 509-13.
- 482 Lenoir, A. and Coste, M. 1996. Development of a practical diatom index of overall water  
483 quality applicable to the French national water Board network. In: Whitton, B. A. and Rott, E.  
484 (Editors) Use of algae for monitoring rivers II. Studia Student. G.m.b.H., Innsbruck Austria,  
485 pp. 29-43.
- 486 Lorenzen, C. J. 1967. Determination of chlorophyll and pheopigments: spectrophotometric  
487 equations. *Limnol. Oceanogr.* 12, 343-6.
- 488 Lozano, R. B. and Pratt, J. R. 1994. Interaction of toxicants and communities - the role of  
489 nutrients. *Environ. Toxicol. Chem.* 13, 361-8.
- 490 Mazzella, N., Dubernet, J.-F. and Delmas, F. 2007. Determination of kinetic and equilibrium  
491 regimes in the operation of polar organic chemical integrative samplers: Application to the  
492 passive sampling of the polar herbicides in aquatic environments. *J. Chromatogr. A* 1154, 42-  
493 51.
- 494 Montuelle, B., Gouy, V., Roger, M. C., Margoum, C., Besson, M., Guillard, C., Chovelon, J.  
495 M., Devaux, A., Durrieux, C., Tran Minh, C., Gillet, C., Leboulanger, C., Faure, R.,  
496 Herbreteau, B., Marote, P. and Clemens, A. 2006. Evaluation de gains biologique et  
497 écologique associés à une réduction d'intrants polluants en milieu aquatique. In: Rapport final  
498 Cemagref - CPER 2003-2006, Région Rhône Alpes. Cemagref; Fresnes.
- 499 Morin, S., Bottin, M., Mazzella, N., Macary, F., Delmas, F., Winterton, P. and Coste, M.  
500 accepted. Linking diatom community structure to pesticide input as evaluated through a  
501 spatial contamination potential (Phytopixal): a case study in the Neste system (South-West  
502 France). *Aquat. Tox.*
- 503 Morin, S., Duong, T. T., Dabrin, A., Coynel, A., Herlory, O., Baudrimont, M., Delmas, F.,  
504 Durrieu, G., Schäfer, J., Winterton, P., Blanc, G. and Coste, M. 2008a. Long term survey of

- 505 heavy metal pollution, biofilm contamination and diatom community structure in the Riou-  
506 Mort watershed, South West France. Environ. Pollut. 151, 532-42.
- 507 Morin, S., Duong, T. T., Herlory, O., Feurtet-Mazel, A. and Coste, M. 2008b. Cadmium  
508 toxicity and bioaccumulation in freshwater biofilms. Arch. Environ. Contam. Toxicol. 54,  
509 173-86.
- 510 Pérès, F., Florin, D., Grollier, T., Feurtet-Mazel, A., Coste, M., Ribeyre, F., Ricard, M. and  
511 Boudou, A. 1996. Effects of the phenylurea herbicide isoproturon on periphytic diatom  
512 communities in freshwater indoor microcosm. Environ. Pollut. 94, 141-52.
- 513 Péry, A. R. R., Béthune, A., Gahou, J., Mons, R. and Garric, J. 2005. Body residues: a key  
514 variable to analyze toxicity tests with *Chironomus riparius* exposed to copper-spiked  
515 sediments. Ecotoxicol. Environ. Saf. 61, 160-7.
- 516 Pesce, S., Martin-Laurent, F., Rouard, N. and Montuelle, B. 2009. Potential for microbial  
517 diuron mineralisation in a small wine-growing watershed: from treated plots to lotic receiver  
518 hydrosystem. Pest. Manag. Sci. 65, 651-7.
- 519 Pesce, S., Martin-Laurent, F., Rouard, N., Robin, A. and Montuelle, B. submitted. Evidence  
520 for adaptation of riverine sediment microbial communities to diuron mineralization: incidence  
521 of run-off and soil erosion. J. Soil Sediment.
- 522 Rabiet, M., Margoum, C., Gouy, V., Carluer, N. and Coquery, M. 2008. Transfert des  
523 pesticides et métaux dans un petit bassin versant viticole. Étude préliminaire de l'influence des  
524 conditions hydrologiques sur le transport de ces contaminants. Ingénieries Special issue  
525 "Azote, phosphore et pesticides. Stratégies et perspectives de réduction des flux", 65-75.
- 526 Sabater, S., Guasch, H., Ricart, M., Romaní, A., Vidal, G., Klünder, C. and Schmitt-Jansen,  
527 M. 2007. Monitoring the effect of chemicals on biological communities. The biofilm as an  
528 interface. Anal. Bioanal. Chem. 387, 1425-34.

- 529 Schmitt-Jansen, M. and Altenburger, R. 2005. Toxic effects of isoproturon on periphyton  
530 communities - a microcosm study. *Estuar. Coast. Shelf Sci.* 62, 539-45.
- 531 Steinberg, C. and Schiefele, S. 1988. Biological indication of trophy and pollution of running  
532 waters. *Z. Wasser Abwasser Forsch.* 21, 227-34.
- 533 Stevenson, R. J. and Peterson, C. G. 1989. Variation in benthic diatom (Bacillariophyceae)  
534 immigration with habitat characteristics and cell morphology. *J. Phycol.* 25, 120-9.
- 535 Stevenson, R. J. and Peterson, C. G. 1991. Emigration and immigration can be important  
536 determinants of benthic diatom assemblages in streams. *Freshwat. Biol.* 26, 279-94.
- 537 Szabó, K., Kiss, K. T., Taba, G. and Ács, É. 2005. Epiphytic diatoms of the Tisza River,  
538 Kisköre Reservoir and some oxbows of the Tisza River after the cyanide and heavy metal  
539 pollution in 2000. *Acta Bot. Croat.* 64, 1-46.
- 540 Tang, J. X., Hoagland, K. D. and Siegfried, B. D. 1997. Differential toxicity of atrazine to  
541 selected freshwater algae. *Bull. Environ. Contam. Toxicol.* 59, 631-7.
- 542 Tison, J., Park, Y. S., Coste, M., Wasson, J. G., Ector, L., Rimet, F. and Delmas, F. 2005.  
543 Typology of diatom communities and the influence of hydro-ecoregions: A study on the  
544 French hydrosystem scale. *Water Res.* 39, 3177-88.
- 545 Tlili, A., Dorigo, U., Montuelle, B., Margoum, C., Carluer, N., Gouy, V., Bouchez, A. and  
546 Bérard, A. 2008. Responses of chronically contaminated biofilms to short pulses of diuron.  
547 An experimental study simulating flooding events in a small river. *Aquat. Toxicol.* 87, 252-  
548 63.
- 549 Tolcach, E. R. and Gómez, N. 2002. The effect of translocation of microbenthic communities  
550 in a polluted lowland stream. *Verh. Internat. Verein. Limnol.* 28, 254-8.
- 551 van Dam, H., Mertens, A. and Sinkeldam, J. 1994. A coded checklist and ecological indicator  
552 values of freshwater diatoms from the Netherlands. *Neth. J. Aquat. Ecol.* 28, 117-33.
- 553

554 Table 1: Mean values and standard errors (SE) corresponding to physical and chemical  
 555 parameters of the 3 sites of the Morcille River during the experimental period (5 samplings  
 556 per site, except from Saint-Ennemond where pesticides were sampled once).

557

Parameter	Saint-Joseph		Les Versauds		Saint-Ennemond	
	mean	SE	mean	SE	mean	SE
pH	7.1	0.3	7.3	0.1	7.3	0.2
Conductivity ( $\mu\text{S}/\text{cm}$ )	128.8	3.6	190.9	15.5	210.5	10.9
Temperature ( $^{\circ}\text{C}$ )	n.m.		12.0	0.7	12.6	0.8
DOC (mg/L)	2.3	0.6	2.9	0.1	3.8	0.2
Nitrite (mg/L)	<0.02		0.030	0.005	0.059	0.011
Nitrate (mg/)	5.9	0.2	7.5	0.3	6.5	0.4
Ammonium (mg/L)	0.07	0.05	0.05	0.01	0.07	0.01
Orthophosphate (mg/L)	0.12	0.02	0.23	0.02	0.29	0.02
Suspended solids (mg/L)	16.0	2.0	10.0	3.0	8.7	3.7
Diuron (DIU) ( $\mu\text{g}/\text{L}$ )	<q.l.		1.00	0.52	6.65	-
3-(3,'-dichlorophenyl)-1 methylurea (DCMU) ( $\mu\text{g}/\text{L}$ )	<q.l.		0.22	0.07	1.22	-
Azoxystrobine (AZS) ( $\mu\text{g}/\text{L}$ )	<q.l.		0.04	0.02	<q.l.	-
Tebuconazole (TBZ) ( $\mu\text{g}/\text{L}$ )	<q.l.		0.18	0.05	0.05	-
Dimethomorphe (DMM) ( $\mu\text{g}/\text{L}$ )	<q.l.		1.31	0.80	0.32	-
Procymidone (PCM) ( $\mu\text{g}/\text{L}$ )	<q.l.		0.09	0.01	0.18	-

558

559 n.m. not measured ; q.l. quantification limit =  $0.02\mu\text{g}/\text{L}$  for DIU and DCMU,  $0.025\mu\text{g}/\text{L}$  for

560 AZS,  $0.04\mu\text{g}/\text{L}$  for DMM and TBZ, 0.08 for PCM.

Table 2: Mean ( $\pm$  SE) values of biofilm descriptors and diatom indices. \*  $p < 0.05$ , \*\*  $p < 0.01$ , the superscript letters indicate statistically different groups according to the HSD Tukey test.

	Dry weight <sup>**</sup> (mg/cm <sup>2</sup> )	Ash-free dry matter <sup>*</sup> (mg/cm <sup>2</sup> )	Chlorophyll <i>a</i> <sup>**</sup> ( $\mu$ g/cm <sup>2</sup> )	Cell density <sup>**</sup> (cell/cm <sup>2</sup> )	BDI <sup>**</sup>	SPI <sup>**</sup>
<i>Colonisation: 1 month (April)</i>						
Saint-Joseph	0.78 $\pm$ 0.12 <sup>c</sup>	0.15 $\pm$ 0.03 <sup>c</sup>	0.76 $\pm$ 0.10 <sup>a,b</sup>	46 600 $\pm$ 2500 <sup>a</sup>	15.1 $\pm$ 0.3 <sup>c</sup>	16.4 $\pm$ 0.1 <sup>c</sup>
Les Versauds	0.27 $\pm$ 0.02 <sup>a,b</sup>	0.06 $\pm$ 0.00 <sup>a,b</sup>	0.98 $\pm$ 0.15 <sup>b</sup>	172 700 $\pm$ 12 400 <sup>c</sup>	14.1 $\pm$ 0.2 <sup>a,b</sup>	16.0 $\pm$ 0.5 <sup>b,c</sup>
Saint-Ennemond	0.15 $\pm$ 0.00 <sup>a</sup>	0.03 $\pm$ 0.00 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	10 800 $\pm$ 1200 <sup>a</sup>	13.4 $\pm$ 0.1 <sup>a</sup>	14.9 $\pm$ 0.3 <sup>a</sup>
<i>Colonisation: 1 month (May)</i>						
Saint-Joseph	0.34 $\pm$ 0.14 <sup>a</sup>	0.07 $\pm$ 0.03 <sup>a,b</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	7100 $\pm$ 1500 <sup>a</sup>	14.7 $\pm$ 0.1 <sup>b,c</sup>	17.1 $\pm$ 0.2 <sup>c</sup>
Les Versauds	0.26 $\pm$ 0.040 <sup>a,b</sup>	0.06 $\pm$ 0.01 <sup>a,b</sup>	0.04 $\pm$ 0.00 <sup>a</sup>	4300 $\pm$ 200 <sup>a</sup>	13.5 $\pm$ 0.4 <sup>a</sup>	13.5 $\pm$ 0.4 <sup>a</sup>
Saint-Ennemond	0.38 $\pm$ 0.03 <sup>a,b,c</sup>	0.06 $\pm$ 0.00 <sup>a,b</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	4200 $\pm$ 300 <sup>a</sup>	11.2 $\pm$ 0.4 <sup>a</sup>	9.1 $\pm$ 0.5 <sup>a</sup>
<i>Colonisation: 2 months</i>						
Saint-Joseph	0.57 $\pm$ 0.03 <sup>a,b,c</sup>	0.16 $\pm$ 0.01 <sup>c</sup>	0.83 $\pm$ 0.46 <sup>a,b</sup>	122 000 $\pm$ 20 700 <sup>b</sup>	14.7 $\pm$ 0.1 <sup>b,c</sup>	17.1 $\pm$ 0.3 <sup>c</sup>
Les Versauds	0.21 $\pm$ 0.01 <sup>a,b</sup>	0.07 $\pm$ 0.00 <sup>a,b</sup>	0.21 $\pm$ 0.06 <sup>a,b</sup>	30 000 $\pm$ 900 <sup>a</sup>	13.8 $\pm$ 0.2 <sup>a,b</sup>	16.4 $\pm$ 0.2 <sup>b,c</sup>
Saint-Ennemond	0.62 $\pm$ 0.17 <sup>b,c</sup>	0.10 $\pm$ 0.03 <sup>a,b,c</sup>	0.06 $\pm$ 0.01 <sup>a</sup>	24 000 $\pm$ 4700 <sup>a</sup>	13.1 $\pm$ 0.1 <sup>a</sup>	13.7 $\pm$ 0.0 <sup>a</sup>
<i>Translocation</i>						
Les Versauds $\rightarrow$ Saint-Joseph	0.36 $\pm$ 0.08 <sup>a,b,c</sup>	0.11 $\pm$ 0.02 <sup>b,c</sup>	0.80 $\pm$ 0.16 <sup>a,b</sup>	153 700 $\pm$ 12 700 <sup>b,c</sup>	14.3 $\pm$ 0.1 <sup>a,b,c</sup>	17.0 $\pm$ 0.2 <sup>c</sup>
Saint-Ennemond $\rightarrow$ Saint-Joseph	0.41 $\pm$ 0.00 <sup>a,b,c</sup>	0.12 $\pm$ 0.01 <sup>b,c</sup>	0.14 $\pm$ 0.05 <sup>a,b</sup>	37 000 $\pm$ 500 <sup>a</sup>	14.2 $\pm$ 0.3 <sup>a,b,c</sup>	16.2 $\pm$ 0.3 <sup>b,c</sup>

Table 3: Mean relative abundances of the 15 dominant species (i.e. representing more than 3% relative abundances in at least one sample).

Species abbreviations: ADMI: *Achnanthydium minutissimum*, APED: *Amphora pediculus*, CPLA: *Cocconeis placentula*, EOMI: *Eolima minima*, HAMP: *Hantzschia amphyoaxis*, LCOH: *Luticola cohnii*, NGRE: *Navicula gregaria*, NLAN: *N. lanceolata*, NLIN: *Nitzschia linearis*, NPAL: *N. palea*, PLFR: *Planothidium frequentissimum*, PTLA: *Planothidium lanceolatum*, RABB: *Rhoicosphenia abbreviata*, SANG: *Surirella angusta*, SBRE: *S. brebissonii*

	PTLA	RABB	ADMI	NLIN	PLFR	HAMP	SBRE	NLAN	NGRE	SANG	APED	LCOH	CPLA	NPAL	EOMI
<i>Colonisation: 1 month (April)</i>															
Saint-Joseph	42.8	4.9	14.3	1.1	5.5	-	0.1	6.6	1.8	0.1	0.6	-	0.1	0.1	1.5
Les Versauds	51.4	0.3	13.8	0.2	11.3	-	-	1.1	1.9	0.1	1.7	-	1.4	0.1	3.6
Saint-Ennemond	60.7	0.3	0.6	0.3	13.5	0.1	0.1	1.0	1.8	1.2	5.7	0.2	1.1	1.0	1.6
<i>Colonisation: 1 month (May)</i>															
Saint-Joseph	22.3	14.7	8.0	3.0	2.9	0.1	0.1	7.3	3.6	0.1	0.8	0.1	3.7	0.6	3.1
Les Versauds	26.9	3.4	3.1	7.7	6.7	0.4	0.1	6.2	5.9	0.7	2.8	0.1	2.5	2.3	2.0
Saint-Ennemond	11.0	0.7	0.4	13.9	2.0	11.2	0.6	3.5	2.9	5.8	2.4	5.6	3.6	4.5	0.8
<i>Colonisation: 2 months</i>															
Saint-Joseph	60.3	3.8	7.7	0.6	5.7	-	-	1.7	1.2	0.1	2.1	-	0.4	-	1.4
Les Versauds	68.0	0.1	1.4	0.7	12.9	-	0.1	0.5	0.7	0.5	1.4	-	5.5	0.3	1.2
Saint-Ennemond	57.6	0.1	0.9	2.6	9.1	2.0	7.7	0.2	1.2	0.7	1.5	2.0	5.3	0.5	1.1
<i>Translocation</i>															
Les Versauds → Saint-Joseph	67.2	4.0	5.3	0.6	9.5	-	-	0.7	0.4	-	1.4	-	2.4	0.1	1.4
Saint-Ennemond → Saint-Joseph	50.2	10.1	5.5	1.2	8.3	-	0.1	3.3	2.1	0.1	2.1	-	1.2	0.2	1.0

Figure 1: Location of the study sites along the Morcille River.

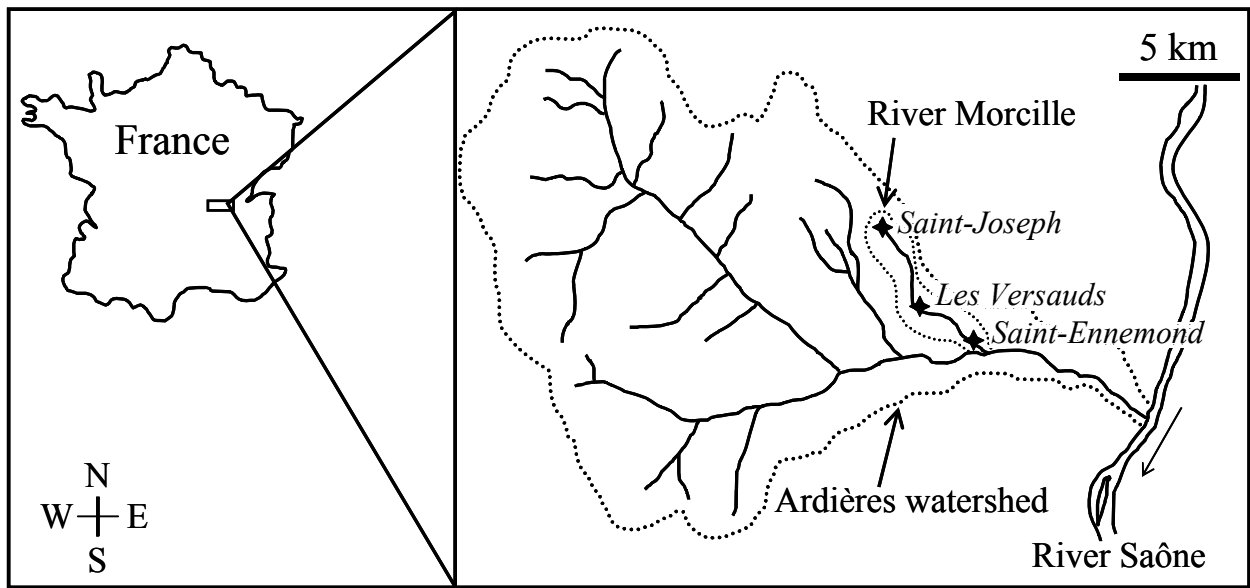


Figure 2: Diatom cell densities corresponding to 1-month-old biofilms grown in April (1), in May (2), 2-month-old biofilms (3) and translocated biofilms (4). The letters refer to the different groups defined in Table 2.

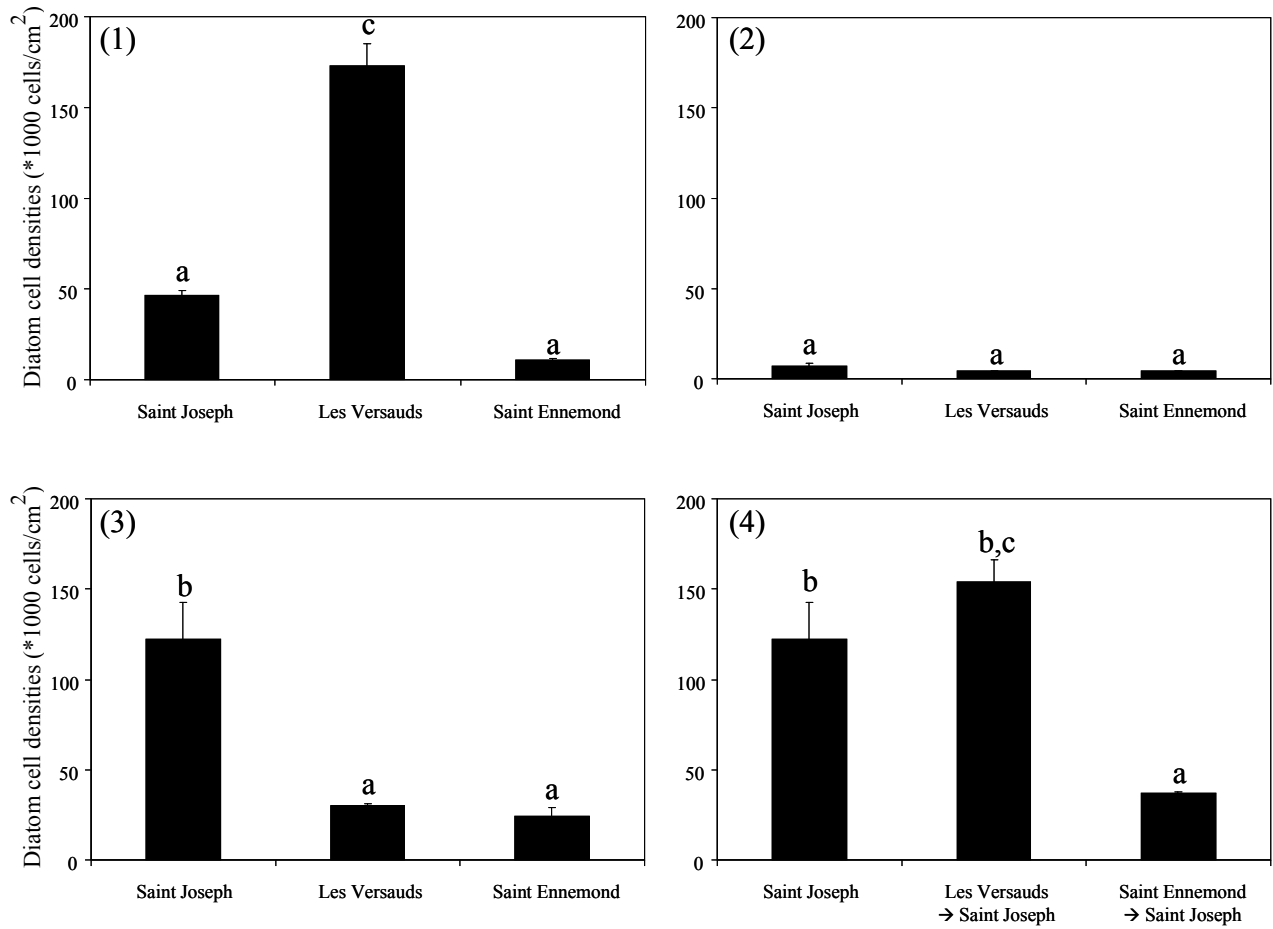


Figure 3: Plot of the non-metric multi-dimensional scaling. ●: Saint-Joseph, ▲: Versauds, ■: Saint-Ennemond, △: Versauds → Saint-Joseph, □: Saint-Ennemond → Saint-Joseph. The labels refer to the colonization period (1m: 1 month, 2m: 2 months).

