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

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7

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,

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.

75

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 km² 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-Ennemond (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 30th
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 cm² 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/cm². 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/cm²). Subsamples assigned to taxonomic analyses were
139 prepared according to Charles et al. (2002), i.e. digestion in boiling hydrogen peroxide (30%
140 H₂O₂) 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 (Lecointe 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 *Achnanthydium 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 α 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 μ g/L and total Cu around 2 μ 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_{ow}) 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

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

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.

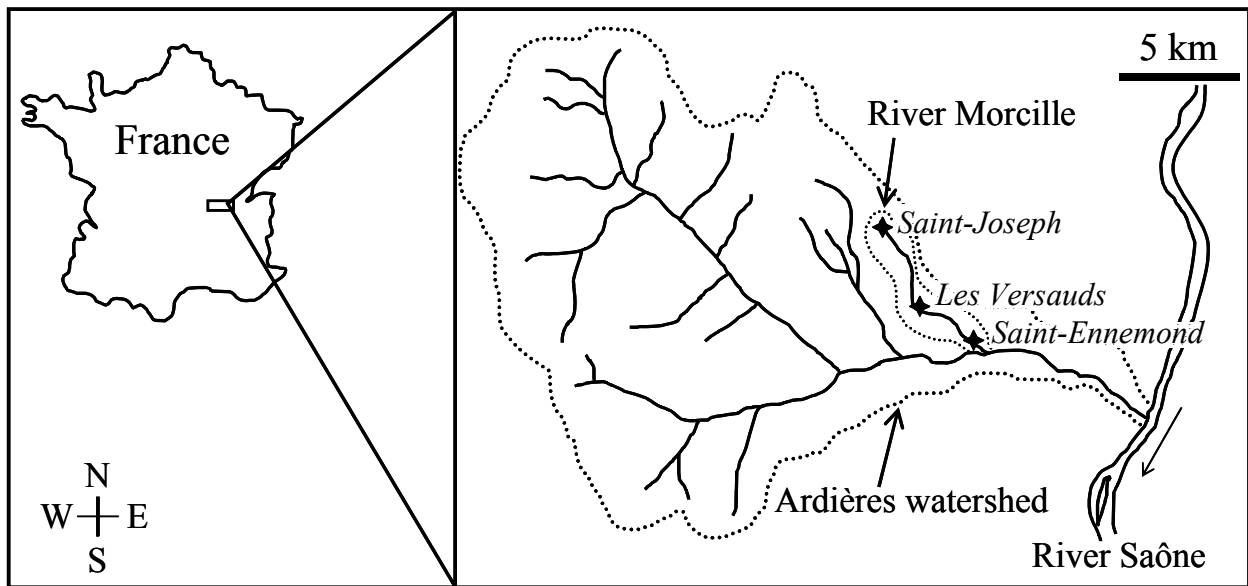


Figure 2: Diatom cell densities corresponding to 1-month-old biofilms grown in April (1), in May (2), 2-month-old biofilms (3) and translocated biofilms (4). The letters refer to the different groups defined in Table 2.

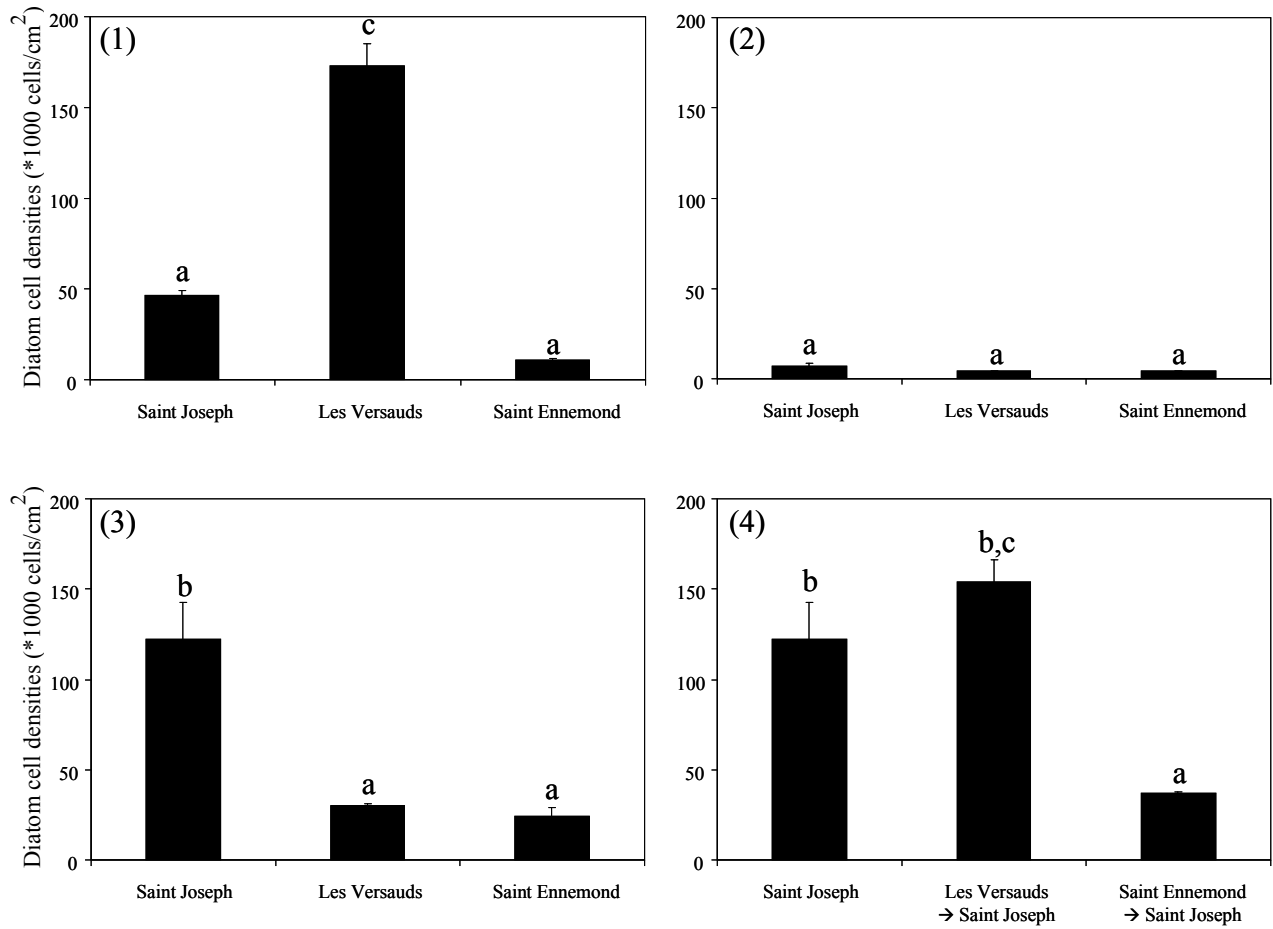


Figure 3: Plot of the non-metric multi-dimensional scaling. ●: Saint-Joseph, ▲: Versauds, ■: Saint-Ennemond, △: Versauds → Saint-Joseph,

□: Saint-Ennemond → Saint-Joseph. The labels refer to the colonization period (1m: 1 month, 2m: 2 months).

