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

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Abstract

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

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

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

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

Keywords

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

Introduction

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

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

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

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

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

Materials and methods

Location of the study sites

The translocation experiments were conducted in Spring (April and May) 2008 along the Morcille River, located in the Beaujolais vineyard area, eastern France (46.150°N, 4.600°E, belonging to national river type 3 “Massif Central sud”, Figure 1). The Morcille River is a small first-order stream (7 km long) subjected to strong agricultural pressure, essentially exerted by vineyards that occupy almost 80% of the 8.5 km² catchment area. Three sites were selected along the gradient of increasing percentage contribution of vineyards to the catchment area: Saint-Joseph (vineyard percentage cover: 6.7%), Les Versauds (51.6%) and Saint-Ennemond (79%).

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

Stream water physicochemical characteristics of the sites.

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

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

Collection of periphytic communities

At each site, large glass slides (300 cm² area for both slides) fixed in perforated plastic boxes were used as artificial substrates allowing algal colonisation. After a 4-week immersion (2 April to 6 May), slides were removed i) for collection to characterize the 4-week-old communities (3 slides per site, called “1 month April”), ii) for a 4-week translocation to the

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

Biofilm analyses and diatom species composition

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

A 20-ml aliquot was used to determine the dry weight (DW) and ash-free dry mass (AFDM) of the biofilm, expressed as mg/cm². After filtration of the suspension through individual, previously dried and weighed, glass fibre filters (pore size: 1.2 µm; Sartorius, Göttingen, Germany), the samples were dried for 1 hour at 105°C for DW calculations. Then the filters were ashed at 500°C for 1 hour (Nabertherm P320 furnace) and weighed to determine the mineral content. AFDM was calculated by subtracting the mineral matter from the total dry weight.

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

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

in 10 fields (1.25 ml each, 0.5 mm depth) using light microscopy at 400x magnification (photomicroscope Leica DMRB, Wetzlar, Germany) was then recorded as cells per unit area of substrate (number of diatom cells/cm²). Subsamples assigned to taxonomic analyses were prepared according to Charles et al. (2002), i.e. digestion in boiling hydrogen peroxide (30% H₂O₂) and hydrochloric acid (35%) followed by three cycles of centrifugation of the sample and pellet rinsing with distilled water. After the last treatment, the pellet was once again resuspended in distilled water, and the suspension deposited onto coverslips then mounted onto slides after air drying, using the high refractive index (1.74) medium Naphrax (Brunel Microscopes Ltd, UK). Diatom counts were conducted at a magnification of 1000x; individual fields were scanned until at least 400 valves had been identified using taxonomic literature from central Europe (Krammer and Lange-Bertalot 1986 - 1991). From the specific composition of each sample, the indices BDI v.2006 (Biological Diatom Index, Coste et al. 2009) and SPI (Specific Polluosensitivity Index, Coste in Cemagref 1982) were calculated using Omnidia software (Lecointe et al. 1993).

Data treatment

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

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

Results

Physical and chemical characteristics of the sites (table 1)

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

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

Global descriptors of the biofilm (table 2)

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

AFDM were lower at Saint-Joseph, and no statistical difference was observed between sites. The translocated samples presented intermediate values of biomass between Saint-Joseph and the site from which they were transferred.

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

Diatom communities

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

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

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

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

Discussion

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

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

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

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

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

Other characteristics of diatom communities like quantitative estimates (e.g. diatom cell densities, AFDM or chlorophyll *a* as global indicators of periphytic biomass), the impacts of the toxicants were underlined (see Figure 2 and Table 2). Finally, not only the thickness of biofilm, but also its adhesion to the substrate, differed between the sites. When scraping the 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.

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 Ennemond 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.

332 3) *Recovery potential of diatom assemblages.*

333 The samples transferred from Les Versauds and Saint-Ennemond 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

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

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

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

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

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

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/L)	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-(3,4-dichlorophenyl)-1-methylurea (DCMU) ($\mu\text{g}/\text{L}$)	<q.l.		0.22	0.07	1.22	-
Azoxystrobin (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	-
Dimethomorph (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	-

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 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: *Achnanthes minutissimum*, APED: *Amphora pediculus*, CPLA: *Cocconeis placentula*, EOMI: *Eolima minima*, HAMP: *Hantzschia amphioxys*, LCOH: *Luticola cohnii*, NGRE: *Navicula gregaria*, NLAN: *N. lanceolata*, NLIN: *Nitzschia linearis*, NPAL: *N. palea*, PLFR: *Planorbulina frequentissima*, PTLA: *Planorbulina lanceolata*, 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).