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Assessing the disturbance caused by an industrial discharge using field transfer of epipelic biofilm

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ABSTRACT

A translocation experiment of epipelic biofilm was performed in order to explore the effects on this biological complex when exposed to different water qualities. To carry out such an experiment we employed artificial substrata placed at two sites within a stream that receives a textile effluent: at site 1, located upstream from this influx, and at site 2, downstream. After a 4-week colonization, the substrata at each site were switched in location between sites 1 and 2.The analysis of the epipelon was performed once a week between April and July 2008. In order to evaluate the disturbance on the biofilms we assessed structural (biofilm composition, chlorophyll "a" and ash-free dry weight) and metabolic (net and gross primary production, respiration, and assimilation rate) features. With the metabolic variables that showed significant differences, resistance was calculated. The taxonomic and metabolic variables analyzed responded differently in accordance with the type of environmental challenge presented. In this regard, the biofilm developing at the site upstream from the textile effluent that was later transferred to the downstream site proved to be more resistant to the environmental perturbations with respect to its composition, but not at the level of its metabolic descriptors. Indeed, in the translocated and nontranslocated biofilms growing downstream we observed diatom species with morphological deformations in their frustules, fact that clearly reflects the environmental stress at this site. On the other hand, the biofilm transferred in the opposite direction, in turn, rapidly exhibited tendencies to compensate for its lower biological integrity, but responded more slowly at the metabolic level. Finally, the observation of the changes occurred in the biofilms as a consequence of the worsening and improvement of the water quality could be efficiently evaluated through this experiment.

1. Introduction

Considering that many global and regional environmental changes (loss of biodiversity, resource depletion, and land degradation) are largely caused by human activities, there is a consensus among scientists that the global environment is already incapable of absorbing "natural" and anthropogenic stresses from our growing human population at current levels of consumption (Tong and Soskolne, 2007). The bounds over which the system changes from its normal or expected condition as a result of most natural events are narrow in comparison with the changes that result from human actions such as row crop agriculture, timber harvest, or urbanization (Karr, 1999). In this context it is essential to understand the way in which communities respond to disturbances (e.g. the speed employed to return to their previous conditions after a disturbance) as well as to know their resistance capacity (Begon et al., 2006). In addition to classical analyses of pollution, effort is being made to identify and design new approaches and tools for diagnostic use (de la Torre et al., 2000).

The biofilms are key elements in the self-depuration processes which occur in rivers (Sabater et al., 2002) and they have properties that make them particularly useful in evaluating the rates at which recovery occurs in lotic ecosystems following disturbances (Burns and Ryder, 2001). In this way the biofilms have been widely used for routine monitoring being very useful as "early warning systems" after disturbances (Sabater et al., 2007).

According to Steinman and McIntire (1990) disturbance is an event that changes the local environmental conditions and the biological properties of the natural systems. In our study we analyzed how metabolic and structural parameters of the epipelic biofilm respond to the disturbance generated by a complex mixture of pollutants coming from a textile industry. The measurement of functional biofilm descriptors provides a fundamental insight about the river health that is not available through structural attributes and characteristically they provide an integrated response to a broad range of disturbances (Bunn et al., 1999; Burns and Ryder, 2001).

In order to accomplish the epipelic biofilm assessment we carried out a translocation experiment in a lowland stream located in the

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Pampean plain, Don Carlos stream, which receives the textile industry effluent. Tolcach and Gómez (2002) employed this methodology in Don Carlos stream to analyze the changes in some structural variables, such as species composition, diversity and biomass of the epipelic biofilm. In the present study we analyzed structural descriptors in conjunction with functional descriptors of the epipelic biofilms in order to assess the biotic integrity of this biologic complex. Furthermore we estimated the resistance of the epipelic biofilm in relation with the disturbance generated by the textile effluent. We hypothesized that the taxonomic and nontaxonomic descriptors respond differently in accordance with the type of environmental challenge presented, and that the nontaxonomic descriptors are more sensitive than taxonomic in reflecting alterations in the water quality upon deterioration of the environmental conditions at hand.

The proper knowledge to these topics will provide the necessary tools for an improvement of the management of the aquatic resources, particularly focused to the restoration measures or rehabilitation of the aquatic systems.

2. Materials and methods

2.1. Study area

The translocation experiment was conducted in the Don Carlos stream, a lowland stream located in the Argentine Pampean plain, which flows into the Río de la Plata estuary. For this study two sampling sites were selected: site 1 located 100 m upstream from a textile plant and site 2 located 30 m downstream from that factory's effluent (Fig. 1). Site 1 is located in an area where horticulture is carried out, so that the water at this site has high amounts of nutrients, mostly phosphorus, and at site 2 receives an intermittent discharge from the textile plant. Previous studies carried out in this stream have provided useful information on the quality of the habitat and the spatial and seasonal variability of physical, chemical and biological parameters (Tolcach and Gómez, 2002; Gómez and Licursi, 2003; Sierra and Gómez, 2007; Gómez et al., 2008).

2.2. Experimental design

Plastic trays (135 cm^2 surface) were used as an artificial substratum for biofilm attachment. Each sampling tray was perforated over its entire perimeter (with holes 0.2–0.3 mm diameter) at about 1 cm from the upper edge in order to allow the free circulation of water over the epipelon. Likewise, a central hole was made in each tray that permitted it to be anchored to the river bottom with stakes. For these trays to remain submerged, cement was added to the lower half of their volume. Twenty-four artificial substrata were placed at each of the two sampling sites and remained at those locations for the 4-week period recommended in the literature to insure an optimal colonization by the microbenthic organisms (Descy and Coste, 1990; Stevenson and Bahls, 1999). After this colonization period the translocation was carried out: 12 of the substrata at each site were switched in location between sites 1 and 2.

The twelve substrata that remained in their original sites 1 and 2 throughout the experiment were designated A and B, respectively (i.e., the reference or resident biofilms); while the twelve moved from site 1 to site 2 and from site 2 to site 1, A–B and B–A, respectively (Fig. 2).

The experiment was conducted from April to July 2008 and involved a total of 6 sampling times. Two days after the translocation (called week 1), we began the measurements of the physical, chemical, and biological parameters at the two sites. The following measurements were carried out weekly (week 2, week 3, week 4 and week 5), the last one taking place nine weeks after the beginning of the experiment (week 9). During the course of the experiment scarce precipitations were recorded and the main flow differences at site 2 were due to the discharge of the industrial effluent.

The biofilm developed in the sampling units reached a width of 1–1.5 cm (Fig. 3).

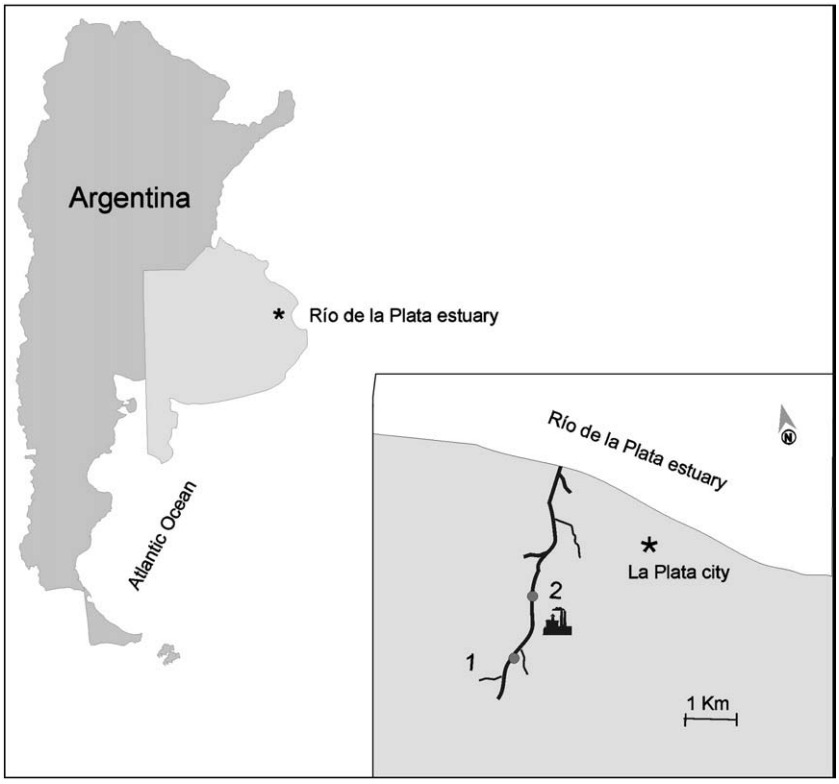

Fig. 1. Location of the Don Carlos stream and sampling sites.

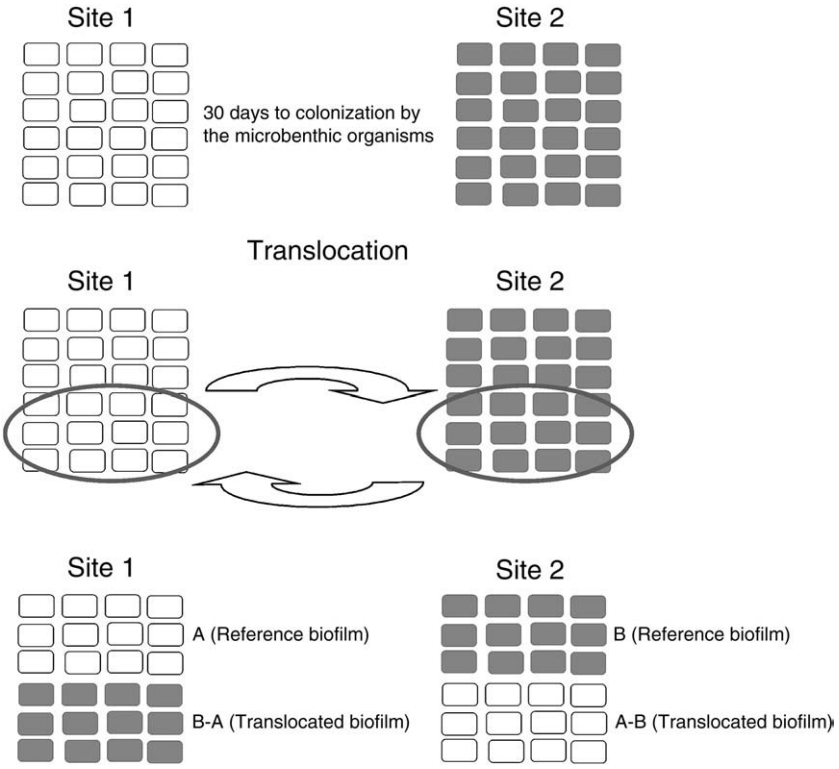

Fig. 2. Scheme that shows the design of the experience. A: control substrates colonized at site 1. B: control substrates colonized at site 2. B–A: substrates colonized at site 2 and that were translocated to site 1. A–B: substrates colonized at site 1 and that were translocated to site 2.

2.3. Water and sediment quality

On each sampling date the following physical and chemical variables were measured: conductivity (Lutron CD-4303), photosynthetic active radiation–PAR–(Li-Cor 250), temperature, and pH (Hanna HI 8633). Water samples were also collected for the analysis of nutrients, BOD_5, and chemical oxygen demand (Tabatabai, 1974; Mackereth et al., 1978; American Public Health Association, APHA, 1998). On the first sampling date, samples of sediment were collected at both sites for analysis of heavy metals (Pb, Cu, Cr, Cd, Ni) and phthalates, which measurements were performed by atomic-absorption spectrophotometry (American Public Health Association, APHA, 1998; EPA SW 846, 1986).

2.4. Biological descriptors

Net primary production (NPP) and respiration (R) were measured in situ. Five mm in thickness acrylic chambers (length, 35 cm; width, 28.5 cm; height, 10 cm; volume (about 6 l)) were used in triplicate. All chambers were filled with stream water, and in three of them were set the reference sediment trays (two per chamber), while in the other three chambers the translocated substrates were placed. A final chamber containing stream water and two uncolonized sampling trays was used as a control. Here the trays were present in order to keep water volume the same as in the other chambers. The purpose of this control was to correct the changes in oxygen concentration as a result of the metabolic activity of the plankton. Dissolved-oxygen levels were measured (ISY 52 oxymeter) at the beginning and end of the incubation period. To determine R, the chambers were covered with a black plastic sheet and incubated for 60 min. To determine the NPP the chambers were incubated for 30 min in sunlight. The water inside the chambers was not recirculated during the incubations because the current velocity in the stream was low. To test whether or not the concentration of nutrients decreased during the incubation period, water samples were taken from inside the chambers at the beginning and at the end of the incubation in first metabolism experiment. Since we found no significant alterations in the nutrient levels after the incubation on that occasion, we were reassured that incubation times were sufficiently short to avoid that problem.

The values of NPP and R of the epipelic biofilms were calculated according to Fellows et al. (2006). To facilitate comparison with other studies, these NPP and R estimates were converted from $mg\,O_2\,cm^{-2}\,h^{-1}$ to $mg\,O_2\,cm^{-2}\,d^{-1}$ (Hill et al., 1997; Fellows et al., 2006). The gross primary production (GPP) was calculated as the sum of the NPP and the R values, and the assimilation rate (AR), which is a measure of the fraction of the biofilm biomass that is photosynthetically active, was calculated by dividing the NPP-per hour values by the amount of chlorophyll "a" (Crossey and La Point, 1988).

On weeks 1 and 9 samples were collected to determine chlorophyll "a" (Ch "a"), ash-free dry weight (AFDW), and specific composition. After measuring the metabolic variables 1 cm^2 of epipelic biofilm was collected by pipetting 5 ml of the superficial layer (5–10 mm) of the sediment that accumulated in the sampling units (Sierra and Gómez, 2007). The samples obtained were kept in the cold and dark during their transportation to the laboratory.

For chlorophyll analysis two aliquots 5 ml were filtered using Whatman GF/C glass fiber filters and immersed in 90% acetone for 24 h in the dark at 4 °C. The extract was read with a spectrophotometer and the Ch "a" concentration was obtained according to Steinman and Lamberti (1996). Two 5-ml aliquots were taken for estimating ash-free dry weight (AFDW) which was measured as the difference in weight between the dried mass at 60 °C for 24 h and combusted at 550 °C for 4 h (Bourassa and Cattaneo, 1998).

Samples dedicated to the study of the community composition of the biofilms (three 5-ml aliquots) were fixed with formaldehyde (4% [v/v]), and quantified in a 5-ml sedimentation chamber under an inverted microscope (Olympus CK2). The following keys were used for species identification: Hustedt (1930), Desikachary (1950), Frenguelli (1941), Bourrely (1966, 1968, 1970), Krammer and Lange-Bertalot (1986, 1988, 1991a,b), Tell and Conforti (1986),

Fig. 3. Artificial substrates after the period of colonization a) biofilm A. b) biofilm B.

Streble and Krauter (1987), Lopreto and Tell (1995), and Komarek and Anagnostidis (1999, 2005). For the identification of diatoms, samples were cleaned with hydrogen peroxide and then mounted on microscope slides with Naphrax®. To estimate the bacterial density in the epipelic biofilm, one 4-ml aliquot was fixed with formaldehyde (2% [v/v]) and sonicated for 90 s. After appropriate dilution (dilution factor of 400) fixed samples were stained for 5 m with DAPI (4,6-diamidino-2 phenylindole; 2 µg ml-1), filtered through 0.2-µm irgalan black stained polycarbonate filters (Nucleopore, Newton, Massachusetts) and bacteria were counted under a fluorescence microscope (Olympus BX 50) at 1000× magnification (Romaní, 2001). Twenty fields were counted per filter for a total of 400–1500 organisms. The bacterial density was expressed per cm^2 of sediment-grain surface area. This method of estimation was performed as described by Marxsen and Witzel (1991) and Romaní and Sabater (2001).

Table 1
Physicochemical characteristics of the sampling sites. Data are expressed as means ± standard deviations.

Parameters	Site 1	Site 2
T (°C)	15.7 ± 3.3	23.6 ± 0.9
Conductivity (µS cm^{-1})	859.9 ± 55.6	964.7 ± 101.5
pH	8.3 ± 0.3	7.9 ± 0.3
PAR water (µmol s^{-1} m^{-2})	481.5 ± 256.1	859.3 ± 453.0
PAR air (µmol s^{-1} m^{-2})	1068.9 ± 625.7	1362.1 ± 614.5
Nitrate (mg l^{-1})	1.2 ± 0.8	1.5 ± 1.9
Nitrite (mg l^{-1})	0.1 ± 0.03	0.1 ± 0.3
Ammonium (mg l^{-1})[a]	0.05 ± 0.03	0.3 ± 0.1
Phosphate (mg l^{-1})[a]	0.9 ± 0.1	0.1 ± 0.1
BOD_5 (mg l^{-1})	3.8 ± 4.5	61[b]
COD (mg l^{-1})	7.6 ± 5.2	7 ± 1.5

[a] Significant differences at $p<0.05$.
[b] At site 2 BOD_5 is reported only on the last measurement day because in the previous measurements there had been substances in the water that had interfered with the quantification.

Table 2
Morphometric and hydraulic characteristics, cover of aquatic plants, and the granulometric composition at the indicated sampling sites.

	Site 1	Site 2
Depth (m)	0.5	0.35
Width (m)	1.5	1
Flow (m s^{-1})	0.11	1.8
Discharge (m^3 s^{-1})	0.15	0.1
Granulometric composition		
Silt (%)	45	25
Clay (%)	23	11
Sand (%)	29	37
Gravel (%)	3	27
Cover of aquatic plants (%)	56	44

2.5. Data analysis

The Kruskal–Wallis test was performed to detect significant differences in the physicochemical variables at both sites of the Don Carlos stream and between the biological variables of the reference and translocated biofilms. With the parameters that showed significant differences a Tukey-multiple-comparison test was used to identify which samples were statistically different.

With the metabolic variables that showed significant differences, resistance was calculated at each sampling date, employing the modified Uehlinger (2000) equation:

$$R_{x=}1-[(X_r-X_t)/X_r] \quad (1)$$

where X_r is the value of the metabolic variable corresponding to the reference biofilm (A and B) and X_t is the value of the metabolic variable corresponding to the translocated biofilm (A–B and B–A).

The percent community similarity (PS_c) was determined by means of the formula proposed by Whittaker (Stevenson and Bahls, 1999):

$$PS_c = 100-0.5\sum_{i=1}^{s}|a_i-b_i| \quad (2)$$

where:

a_i percentage of specie *i* in sample A
b_i percentage of specie *i* in sample B.

Table 3
Concentrations of heavy metals and phthalic esters in streambed sediments at sites 1 and 2 of the Don Carlos stream.

Heavy metals	Site 1	Site 2
Pb (µg g^{-1})	10.30	13.30
Ni (µg g^{-1})	11.25	10.00
Cr (µg g^{-1})	9.00	9.30
Cd (µg g^{-1})	<0.125	<0.125
Cu (µg g^{-1})	19.0	23.0
Phthalic-acid esters (phthalates)		
Benzyl butyl phthalate (ng g^{-1})	<1.0	<1.0
Diethyl phthalate (ng g^{-1})	<1.0	<1.0
Dimethyl phthalate (ng g^{-1})	<1.0	<1.0
Di-n-butyl phthalate (ng g^{-1})	<1.0	20.0
Water content (%)	29.93	43.03

This index varies between 0% (no similarity) and 100% (maximum similarity) and was applied to determine the degree of similarity between the biofilm which remained always in its place (control) and the translocated biofilm. Thus the index value gives a measure of the degree to which the composition of the translocated biofilm remained the characteristics developed at the site of colonization, i.e. resistance to change in response to changes in the water quality.

3. Results

3.1. Water and sediment quality

Information about physical and chemical variables of the sampling sites, and their significant differences, are shown in Table 1. With the exception of phosphate and ammonium, the water chemistry and

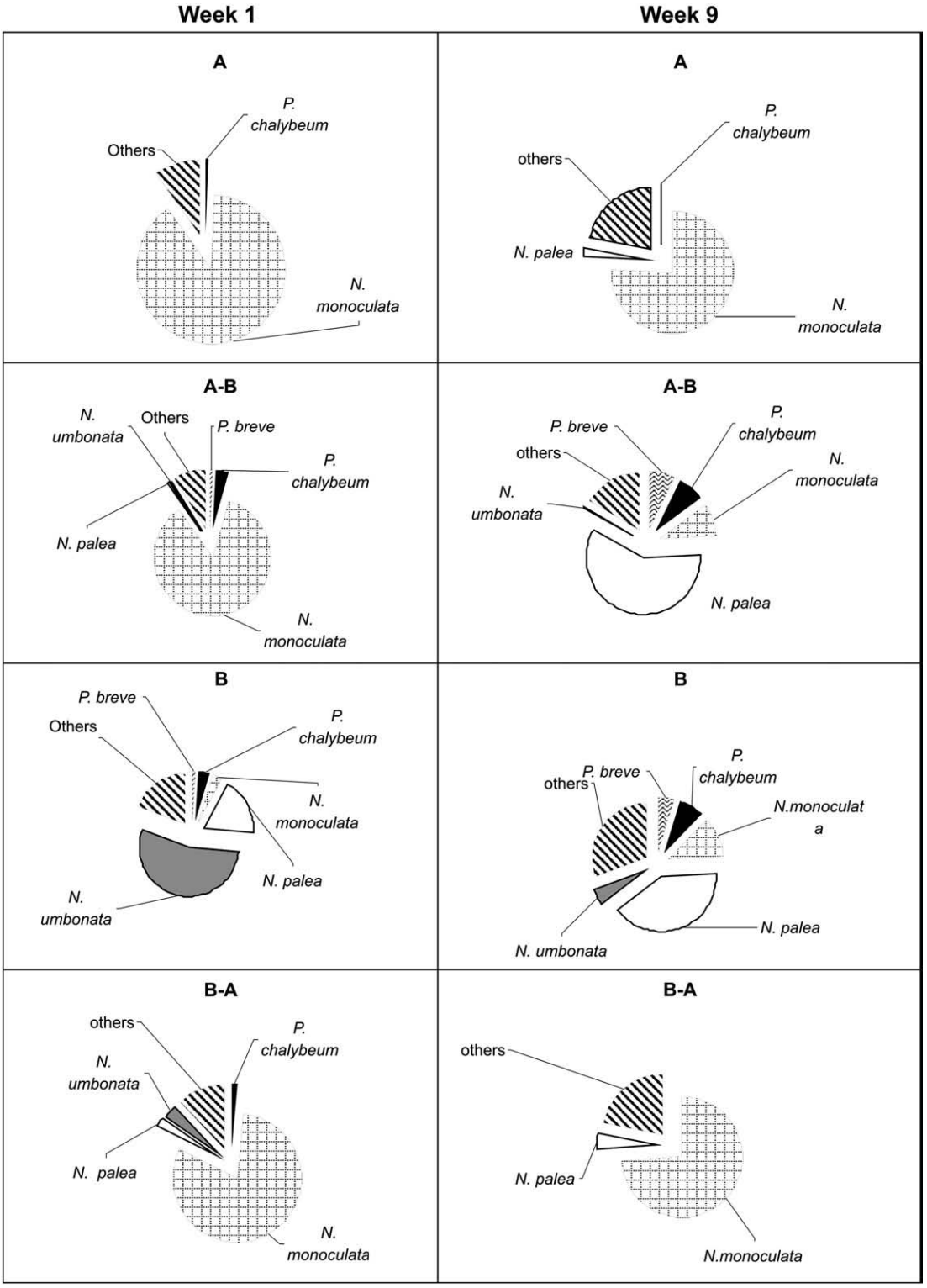

Fig. 4. Algae species with a relative abundance above 5% in the local and translocated biofilms.

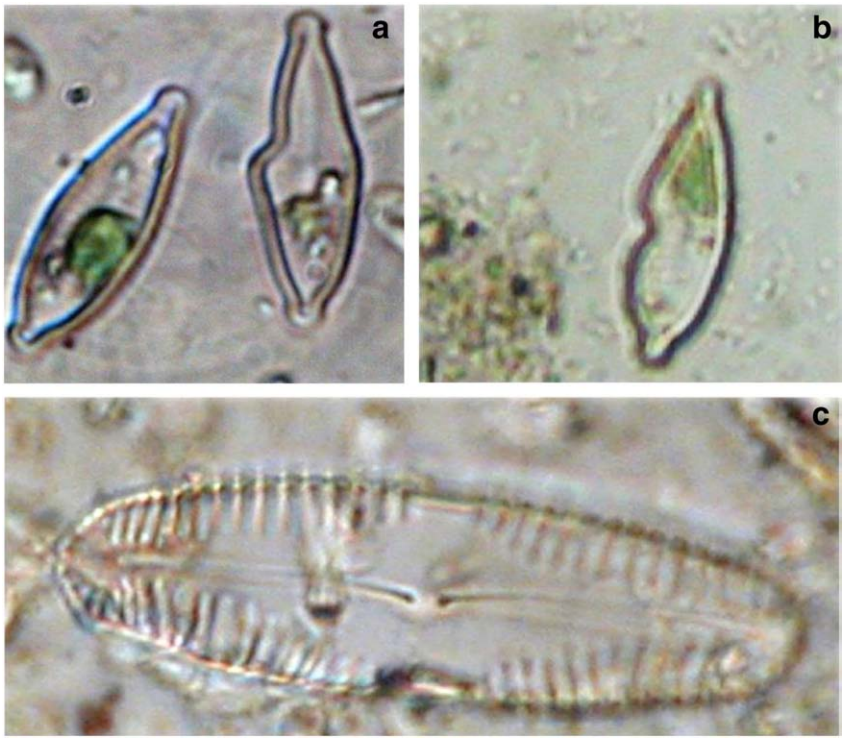

Fig. 5. Abnormal valves of *Gomphonema parvulum* (a and b) and *Pinnularia gibba* (c) in the biofilms development at site 2.

physical variables were not significantly different between the sampling sites. The temperature which was 1.5 times higher at site 2 is a direct consequence of the effluent coming from textile industry. The high values of this variable could have contributed to increase of DBO_5 at site 2. Despite only one value of DBO_5 was obtained downstream from the textile plant is clear that the effluent had a high proportion of organic substances. Moreover the streambed and banks at site 2 were modified by canalization and cleaning activities. These changes and the intermittent discharge from the textile industry have modified some morphometric and hydraulic characteristics such as the transparency, flow and granulometric composition (Table 2). For example at site 1 the proportion of fine sediments, silt and clay, was higher than the proportion of coarse sediments, while at site 2 the streambed consisted of a consolidated bottom ($CaCO_3$ concretions) with a major proportion of sand and gravel. This granulometric composition favoured the transparency in the water column and therefore the higher PAR values at site 2.

Although the levels of heavy metals measured in the sediment at both sites were all within the average natural range for sedimentary rocks and soils (Frink, 1996), the levels of Pb and Cu were higher at site 2. As concerns the phthalate, the levels of di-n-butyl phthalate at site 2 somewhat exceeded the range expected for sediments and was more than 20-fold higher than at site 1 (Table 3).

3.2. Epipelic biofilm

A total number of 65 taxa were identified during the study. Biofilm A was dominated by *Navicula monoculata*, while biofilm B by *Nitzschia umbonata*, *Nitzschia palea*, *Phormidium chalybeum* and *Phormidium breve*. The best represented consumers in this biofilm were the nematodes in week 9. Biofilm A–B (substrata translocated from site 1 to site 2) maintained a high proportion of *N. monoculata* in week 1 but in week 9 showed a major representation of *N. palea*, with the development of cyanophytes, mainly *P. chalybeum* and *P. breve*. Biofilm B–A (substrata translocated from site 2 to site 1) was dominated by *N. monoculata* in week 1, keeping its high proportion towards week 9. On the other hand, a marked decrease of the cyanophytes was noticed in relation to biofilm B (Fig. 4).

The microinvertebrate fraction in the control and translocated biofilms was mainly represented by nematodes and ciliates. In biofilm A the relative abundance of nematodes and ciliates remained below 20% in both weeks, 1 and 9. On the other hand in biofilm B the proportion of nematodes varied between 63% and 76% and the proportion of ciliates ranged from 31% to 22%. While the proportion of nematodes in biofilm A–B was 49% in week 1 and 71% in week 9, the proportion of ciliates decreased, ranging between 49% and 24%. Finally in biofilm B–A the proportion of nematodes ranged from 69% to 38% and the relative abundance of ciliates varied from 28% to 34%.

In week 9 we observed in biofilms B and A–B such diatom species as *Gomphonema parvulum*, *Craticula ambigua*, *Pinnularia gibba*, and *Nitzschia umbonata* with morphological deformations in their frustules (abnormal patterns of striations and/or deformities in the outline of the valves) (Fig. 5).

The percent community similarity (PS_c) indicated that the similarity between the local biofilms (A *vs.* B) and between biofilm B and B–A was less than 20% (Table 4), i.e. that the biofilm transferred toward the site less polluted more rapidly acquired the characteristic of the latter. On the other hand this index showed a high similarity between biofilm A and A–B in week 1 and low similarity in week 9. This fact reflects that the biofilm transferred from less polluted site to more polluted site kept the characteristics developed at the site of colonization for more time.

Table 4
Percent community similarity (PS_c) between the reference biofilms (A and B) and the translocated biofilms (A–B and B–A). W1: week 1; W9: week 9.

PS_c (%)	W1	W9
A *vs.* B	6.1	10
A *vs.* A–B	90	14
B *vs.* A–B	11	75
B *vs.* B–A	15	19
A *vs.* B–A	86	90

Table 5
Mean values of chlorophyll "a" (Ch "a") and ash-free dry weight (AFDW) and bacterial density of the local and translocated biofilm.

	A		A–B		B		B–A	
	W1	W9	W1	W9	W1	W9	W1	W9
Ch "a" (mg m^{-2})	300.7 ± 270.7	257.1 ± 349.7	211.4 ± 406.3	124.8 ± 106.6	57.57 ± 84.6	45.7 ± 6.3	53.5 ± 69.1	101.4 ± 40.6
AFDW (g m^{-2})	447 ± 402.3	553 ± 652	356.2 ± 404.6	370 ± 310.4	378 ± 550.5	351.6 ± 490.6	260 ± 335.4	458 ± 183
Bacteria (cell $10^6 cm^{-2}$)	0.85	0.81	1.62	2.29	2.14	2.88	1.38	1.41

The bacterial fraction had the highest density in biofilms B and A–B (Table 5) even the presence of mats of *Beggiatoa* spp. at site 2 contributed to increase the proportion of bacteria and the thickness of these biofilms.

A comparison between the reference substrata and those translocated (A *vs.* B–A and B *vs.* A–B) revealed that the Ch "a" content of the biofilm transferred to site 1 (B–A) trended to increase toward the last week 9, duplicating its concentration. The Ch "a" content of the biofilm transferred to site 2 (A–B) was higher than that of biofilm B in both weeks, 1 and 9. The AFDW varied to a lesser degree than did the Ch "a" content in both weeks (Table 5).

The net primary production (NPP) of biofilm A varied between 0.20 and 2.45 $g O_2 m^{-2} d^{-1}$ (Fig. 6), while that of the biofilm transferred to the site with the worse water quality (A–B) was promptly adversely affected, dropping to values below 0 in week 1. From this week on, that biofilm followed a pattern comparable to the local one (B). The NPP of the transferred biofilm B–A, however, though being initially negative, after reciprocal transfer began to increase and became positive towards the fourth week. By the end of the experiment this translocated biofilm reached a value of 1.45 $g O_2 m^{-2} d^{-1}$, similar to that of the local one (A). The ANOVA revealed significant differences ($p<0.05$) between biofilms A and A–B; whereas with biofilm B–A, that parameter underwent increases that were also statistically significant ($p<0.05$) to B. The negative values of NPP at site 2 are a consequence of the high O_2 consumption; during the sunlight incubations the O_2 produced by algal fraction did not exceed the O_2 consumed therefore when the data are included in the NPP equation negative values are obtained.

As to the respiration (R), the ANOVA showed no significant differences between the biofilms. In the first and last weeks, the R of

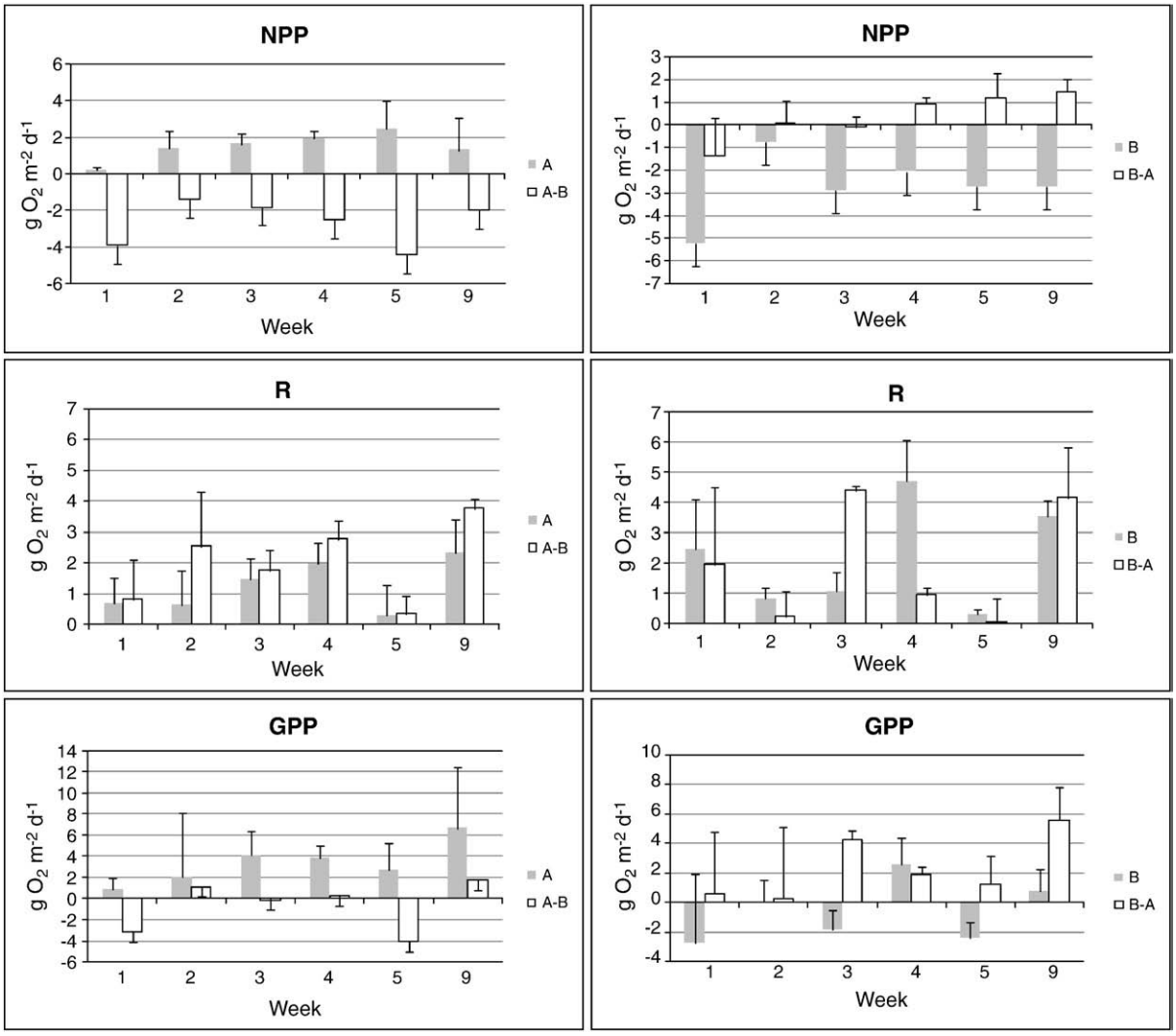

Fig. 6. Variation of the metabolic variables along the transplant experience.

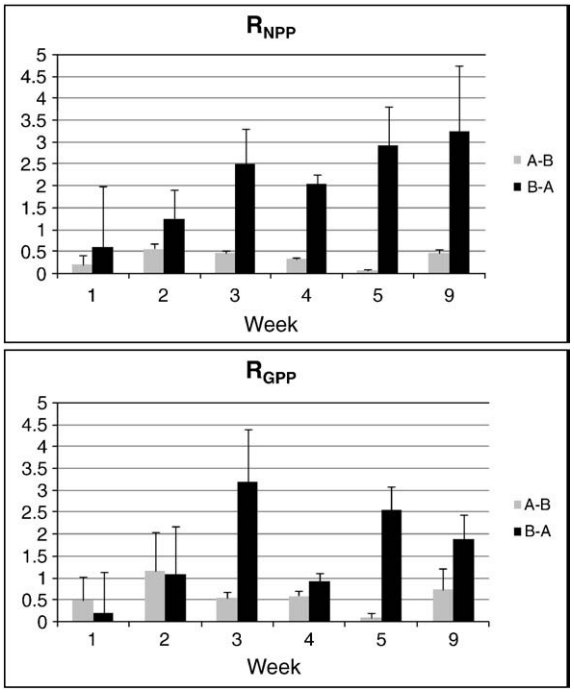

Fig. 7. Resistance of net primary production (R_{NPP}) and gross primary production (R_{GPP}) in the translocated biofilms.

biofilm B–A was 3-fold higher than that of the local biofilm (A) (Fig. 6); while the *R* of the biofilm transferred to site 2 (A–B) by the first week was 3-fold lower than that of the resident biofilm (B).

The gross primary production (GPP) was significantly different between biofilms A and A–B and between those same A and B themselves. Biofilms A and B–A had a similar range of variation during the study period. For the biofilms that remained at site 2, the GPP showed negative values because the NPP was lower than the *R* or else reached zero (Fig. 6).

The analysis of the resistance of the NPP and the GPP showed that the biofilms translocated from site 1 to site 2 exhibited less resistance to the change in water quality (Fig. 7). Both variables R_{NPP} and R_{GPP} of biofilm A–B were significantly lower than the resistance of biofilm B–A ($p<0.05$).

The biofilms placed at site 2 had negative values of AR, while the biofilm transferred to site 1 (B–A), reached a similar value of AR in relation to biofilm A on week 9 (Fig. 8).

4. Discussion

Biofilms in lotic ecosystems are subjected to many different kinds of disturbances, including floods, desiccation, organic nutrient enrichment, and exposure to toxic compound (Steinman and McIntire, 1990). In our research the event that changed the physical, chemical and biological conditions of the section analyzed was an industrial effluent, characterized by chronic disturbance (Singh, 1998). Variables such as temperature, PAR, conductivity, ammonium concentration, BOD_5, some heavy metals and phthalate increased downstream from the industrial effluent. Phthalates are mainly used as plasticizers in the manufacture of plastics, and especially of poly vinyl chloride but are also present in a wide range of industrial and domestic products. In our study di-n-butyl phthalate (DBP) at site 2 was more than 20-fold higher than at site 1. In studies on aquatic biota, mean concentrations of DBP tend to be less than 0.2 mg kg^{-1} wet weight; however, in polluted areas, concentrations of up to 35 mg kg^{-1} have been measured (Acey et al., 1987; Al-Omran and Preston, 1987). More studies are needed to analyze the effects of those elements on the biofilms because according to Bruns and Krauss (1999) new stressors usually have a multiplying effect, i.e. they are added to the effects of natural systems, or they themselves act in combination, with the result that the "tolerance level" of the organisms' ability to cope or to adjust to them is exceeded.

Also Gómez et al. (2008) observed differences in some related variables with the quality of the habitat at site 2 of Don Carlos stream, for example the current velocity which was significantly different between the two sites as a consequence of the volume of the water discharged by the industrial plant into the stream. It is widely recognized that this variable is one of the most important factors that determines the structure and function of stream biofilms (Ghosh and Gaur, 1998; Sabater et al., 2006; Tien et al., 2009). This variable has been found to be positively related to periphyton colonization (Reisen and Spencer, 1970) but a negative relationship has been also observed (Antoine and Benson-Evans 1982) even an inverse relationship between current velocity *vs.* biomass and density has been found (Ghosh and Gaur, 1998; Sabater et al., 2006). It is possible that the increase in flow velocity at site 2 determines a decrease in both biomass and density, but given the complex mixture of chemical compounds present in the water and the hydraulic alterations of the streambed and banks, a more specific study to establish the effect that this variable has on the structural and functional characteristics of the biofilm is necessary.

The different biologic descriptors of the biofilm analyzed in our study showed different changes to the anthropic stress varying in the intensity and the time required to reveal it. The communities react to the enrichment with organic matter or nutrients or an exposure to toxic substances by changing their community composition, usually favouring the most tolerant taxa (Sabater et al., 2007; Hill et al., 2000). In the Don Carlos stream the biofilms growing at site 2, increased the proportion of species tolerant to pollution, such as *Nitzschia palea*, *N. umbonata Phormidium breve*, *P. chalybeum* (Sládecek, 1973; Gómez, 1998; Gómez and Licursi, 2003). Other species appeared that thrive in environments rich in organic matter, such as the filamentous bacterium of *Beggiatoa* spp. (Streble and Krauter, 1987). These sulfur-reducing filamentous, that cover the bed of the Don Carlos stream downstream from the industrial effluent, would imply the occurrence of changes in the aquatic food webs (Williams and Unz, 1989). Indeed these bacteria contributed to increase the thickness of the biofilm, fact that could be linked with the observations of Sabater et al. (2002) who appointed that biomass thickness is protective against the toxicity of different compounds.

The shift in the composition of the translocated biofilms (A–B and B–A) agrees with the observations of Morin et al. (2010) who noticed that the communities translocated from lower to better water quality tended to shift towards typical community structure developing at site with the better water quality. We noted that biofilm B–A was quickly invaded by *N. monoculata* (one of the species that dominated biofilm A) which represented over 70% of the autotrophic component in week 1. In the biofilm transferred in the reverse direction (A–B) *N. palea* (one of the species that characterized biofilm B) required 9 weeks to represent 59% of the algal component in the biofilm. Apart from the time required by the different species to increase or decrease

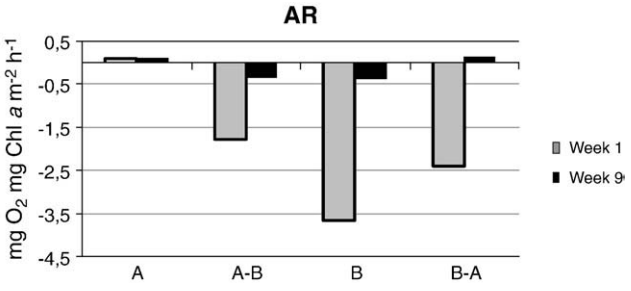

Fig. 8. Assimilation rate (AR) of the local and translocated biofilm.

their proportion in the different biofilms, it is important to consider the factors that could influence on the trajectories of structure assemblage recovery. In this way Morin et al. (2010), pointed out that not only the new water conditions had an effect on such a trajectory but also the immigration and emigration of different species. Even immigration may have prevailed over multiplication rates of pre-established species in the process of translocated community development.

After translocation we observed in biofilm A–B an increase in the microinvertebrate proportion, mainly in the relative abundance of nematodes. Their main food is bacteria, detritus, and diatom cells (Traunspurger, 2000). Although the information about the diet of this group is scarce, one of the few studies available has shown that the activity of bacteria was influenced by the presence of nematodes; feeding of bacteria by nematodes increased bacterial activity and burrowing by nematodes may also result in new spaces for the bacteria, and nematode excreta could be used as substratum by bacteria (Traunspurger, 2000). Also a greater proportion of nematodes feeding of bacteria was found in polluted sections of a Italian river, than in unpolluted (Zullini, 1976). Considering this information is possible to think that low water quality at site 2 contributed to increase of the nematode and bacteria proportion or vice versa.

Another characteristic reflected by the biofilm as a consequence of the environmental stress that the aquatic communities were suffering at site 2, was the presence of abnormal frustules on diatoms. Such teratological changes have been related with effects of environmental stress, which can be both, chemical and physical (Adshead-Simonsen et al., 1981; Gómez and Licursi, 2003; Falasco et al., 2009). Our results are coincident with Gómez and Licursi (2003) who pointed out that the increased pollution and toxic waste, particularly heavy metals, in the Don Carlos stream stimulated the manifestation of diatom anomalies.

Biomass has been widely used to evaluate the enrichment with nutrients in aquatic systems (Hill et al., 2000). In this regard, some authors have reported an increase in the periphytic biomass in response to an increase in nutrients (Leland, 1995; Tate, 1990), while others have observed a decrease in that biomass as a consequence of pollutants such as metals and herbicides (Sigmon et al., 1977; Clark et al., 1979). In our study, the Chl "a" at site 2, with low water quality, followed a similar pattern to that cited by the last authors: the amount of Chl "a" in biofilm B was lower than was registered in biofilm A. Although the algal fraction was represented in biofilm B and A–B by different taxonomic groups, the low level of the assimilation rate at site 2 with its poor water quality would indicate that the Ch "a" of these biofilms has ceased to be functional as a photoreceptor pigment in transforming solar energy into chemical energy under those conditions. On the other hand, site 1 of the Don Carlos stream had a high concentration of phosphorus, which condition favoured an increase in the Chl "a" of biofilm A.

In spite of the PS_c indicated that the taxonomic composition of the biofilm transferred from lower to better water quality (B–A) exhibited a lower resistance to change than did the biofilm translocated in the opposite direction, both metabolic parameters, NPP and GPP, reflected an opposite pattern. In this way R_{NPP} and R_{GPP} were highest when the microcommunity was transferred to the site with the better water quality. This observation is consistent with those of Steinman and McIntire (1990), who pointed out that communities developing in a high-stress environment appear to be more resistant to disturbance than those growing in a low-stress environment.

Although some authors have suggested that the respiration as a functional indicator could be sensitive enough to constitute a test for detecting contaminations with toxic compounds (Hill et al., 2000), in our study the metabolic parameter most sensitive to alterations in water quality was the NPP. This parameter clearly reflected not only the direction of biofilm translocation – a qualitative sensitivity – but also the time intervals required for the transplanted biofilms to acquire the characteristics of the resident biofilms in the new locations — a semiquantitative one.

Finally, we can summarize the main changes detected in the translocated biofilms pointing out that the metabolic responses were more drastically evidenced in the sense of the worsening of the water quality in spite of the fact that the specific composition was more resistant to the change. In the inverse sense of the transference an opposite pattern occurred as the resistance to the change of metabolic variables was high, while at the level of specific composition the change was less resistant, with clear differences in week 1. The accumulation of toxic compounds discharged by the effluent could probably be retained in the biofilm during the colonization period causing the inability to recognize and manifest at a metabolic level the improvement in the water condition in the same time interval of biofilm A–B.

5. Conclusions

The biofilm developing at the site upstream from the textile effluent and that was later transferred to the downstream site proved to be more resistant to the environmental perturbations with respect to its composition, but not at the level of its metabolic descriptors. The biofilm transferred in the opposite direction, in turn, rapidly exhibited tendencies to compensate for its lower biological integrity, but responded more slowly at the metabolic level. From these results we conclude that the taxonomic and metabolic variables respond differently in accordance with the type of environmental challenge presented. With respect to the biological composition, the community residing in the site of higher environmental quality would be more resistant to the degradation of the milieu in which it lives and would thus withstand the insult longer, but the vigor of the organisms comprising that community is more sensitive, perceiving and rapidly manifesting at a metabolic level the perturbation encountered. By contrast, a community that resides in surroundings where the perturbations persist over time and then undergoes a betterment in its milieu – which modifications accordingly improve that community's condition – expresses those changes rapidly at the compositional level, but this new taxonomic structure takes more time to recognize and manifest metabolically the change at hand because the organisms have to recover their physiologic vigor.

Although the combination of structural and functional information, at population, community and ecosystem levels can provide realistic results of the effects of different disturbances on the fluvial ecosystems, more detailed analyses are necessary on the intrinsic mechanisms operating in the biofilm for a better interpretation of its responses in relation to this type of disturbance.

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