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Research Paper

Temporal Dynamics of River Biofilm in Constant Flows: A Case Study in a Riverside Laboratory Flume

key words: epilithon, periphyton, bacterial community fingerprinting, community metabolism, experimental flume

Abstract

A 15-week experiment was performed in a riverside laboratory flume (with diverted river water) to check variations of river biofilm structure (biomass, algal and bacterial compositions) and function (community gross primary production GPP and respiration) under constant flow while water quality went through natural temporal variations. One major suspended matter pulse coinciding with one river flood was recorded after 10 weeks of experiment. Epilithic biofilm first exhibited a 10-week typical pattern of biomass accrual reaching 33 g ash-free dry matter (AFDM) m⁻² and 487 mg chlorophyll-*a* m⁻² and then, experienced a shift to dominance of loss processes (loss of 60% AFDM and 80% chlorophyll-*a*) coinciding with the main suspended matter pulse. Algal diversity remained low and constant during the experiment: *Fragilaria capucina* and *Encyonema minutum* always contribute over 80% of cell counts. DGGE banding patterns discriminated between two groups that corresponded to samples before and after biomass loss, indicating major changes in the bacterial community composition. GPP/R remained high during the experiment, suggesting that photoautotrophic metabolism prevailed and detachment was not autogenic (*i.e.*, due to algal senescence or driven by heterotrophic processes within the biofilm). Observational results suggested that silt deposition into the biofilm matrix could have triggered biomass loss.

1. Introduction

Disturbance is a major source of spatial and temporal heterogeneity in the structure and dynamics of natural communities. Among physical processes of disturbance, flow is the main agent of natural disturbance in lotic ecosystems (POWER and STEWART, 1987; BIGGS *et al.*, 2005). The discharge of a river or stream is made up of both the water that exerts

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forces compared as wind does in terrestrial environments and, any solid particles contained within it. The redistribution of solid particles such as suspended particulate matter (*e.g.*, silt) or larger objects transported by water (*e.g.*, cobbles) can strike and abrade the substratum over which the water flows. As sessile assemblage colonizing pebble surfaces, epilithic biofilms are directly exposed to both increased in flow velocity and material movement.

Many studies confirm that flow is the main factor controlling spatial patterns of epilithic biomass among streams (BIGGS, 1996), temporal patterns in epilithic biomass (BIGGS and THOMSEN, 1995), and heterogeneity in biofilm community composition and biodiversity in streams (BESEMER *et al.*, 2009). Models applied to long time-series suggested that, in natural conditions, epilithic biomass dynamics can roughly be considered as the result of equilibrium between phototrophic growth and discharge-dependant loss (UEHLINGER *et al.*, 1996; BOULÉTREAU *et al.*, 2006; BOULÉTREAU *et al.*, 2008). Models that account for epilithic detachment only considered the effect of fluid forces, using current velocity or discharge as variables (HORNER *et al.*, 1983; MOMO, 1995; UEHLINGER *et al.*, 1996; SARAVIA *et al.*, 1998). However, several studies have suggested that epilithon removal during floods is not likely to be explained by water velocity alone (HORNER *et al.*, 1990; UEHLINGER, 1991; BIGGS, 1996; FRANCOEUR and BIGGS, 2006).

Models show that during long and stable inter-flood periods, other factors can interact and produce complex patterns of epilithic biomass. Among processes known to become significant at promoting biomass loss (BIGGS, 1996) without significant flow events is autogenic sloughing which has rarely been examined in the literature. Autogenic sloughing occurs when parts or clusters of biofilm become thicker, less cohesive and therefore less resistant to floating and drifting, resulting in sloughing of a mature biofilm. Loss of cohesiveness can be consecutive to gas bubble production associated to metabolism activity (photosynthesis or respiration).

We aim to study the evolution of biofilm biomass, community composition and metabolism in a river discriminating the potential effect of changing water flow from other physical or chemical changes due to water flow variations (such as suspended materials, water temperature or nutrient content). To maintain constant flow conditions during a sufficiently long-time period, we studied river biofilm dynamics in an artificial indoor channel.

2. Methods

2.1. Experimental Design

The experiment was conducted during 4 months in an indoor channel (11 m long, 50 cm wide, 20 cm deep, slope: 1/1000, Fig. 1) located on the right bank of the River Garonne at Toulouse, France (43°35'15"N–1°26'17"E). The flume bed and walls are respectively made of opaque PVC 20 mm thick and Plexiglas® 10 mm thick. The experimental flume from GODILLOT *et al.* (2001) and LABIOD *et al.* (2007) was adapted for the present study to be run using a partially re-circulating system open to the River Garonne, so that water followed natural physico-chemical changes, whereas hydraulics were kept constant.

2.1.1. Hydraulic Characteristics

One pump (Selfinox 200/80T, ITT Flygt, France) continuously supplied water from the river to the downstream tank (3300 L) (Fig. 1). One second submerged pump (Omega 10-160-4, Smedegard, Denmark) ran water to the upstream tank (1500 L) through a 100 mm main. The upstream tank fed the downstream tank by gravity. Pumps were robust enough to work equally well with both clear and muddy water. Apparatus to guide (grid) and stabilize (convergent) the flow of water were placed in the inlet reservoir to ensure a quasi uniform entrance flow. Water discharge was measured by an electromagnetic flow meter

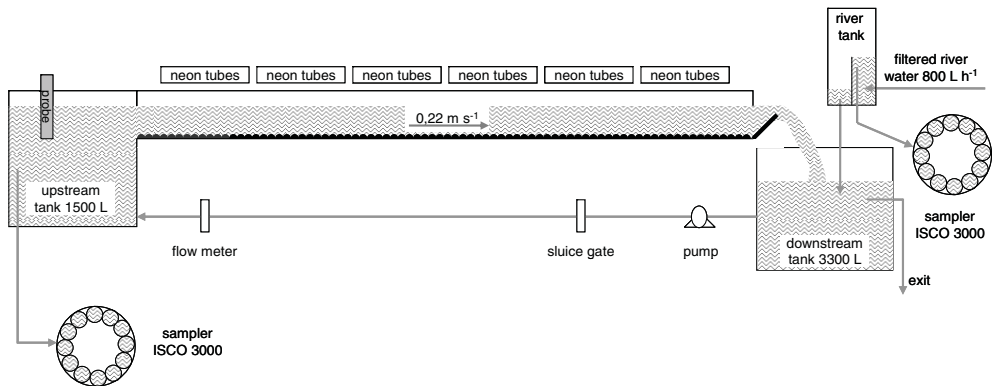


Figure 1. Schematic of the experimental design.

and water discharge and depth regulated with a sluice gate and a by-pass. A moderate current velocity $\pm SD$ ($0.225 \pm 0.002 \text{ m s}^{-1}$) was ensured to limit external shear stress on biofilm and to enhance colonisation (STEVENSON and PETERSON, 1991; GODILLOT *et al.*, 2001). A water depth $h = 10 \text{ cm}$ above substratum was maintained to reduce edge effects associated with side boundary layers. The downstream tank was fed by a water discharge of 800 L h^{-1} thus allowing for a complete turnover of water in the system every 4 h.

2.1.2. Environmental Conditions

River water was treated to exclude macroinvertebrate grazers and fish and to reduce the supply of suspended matter: large particles were eliminated by two centrifugal separators, and the water was then filtered 3 times through 90, 20 and $10 \mu\text{m}$ pore-sized filters. Using $10 \mu\text{m}$ filtration prevented the presence of macroorganisms, although seeding by isolated algal cells from the river drift was enabled.

Light was supplied by six 1.5 m long racks of 5 neon tubes, with “daylight” (Philips TLD 58 W) and fluorescent tubes (Sylvania Gro-Lux 58 W, especially designed for enhancing photosynthesis as they emit in the visible red) evenly disposed. Photoperiod was set at 16 hours of day and 8 hours of night. The incident light, measured with a LI-190SA quantum sensor and a LI-1000 data logger (LI-COR Inc. Lincoln, Nebraska) varied between 140 and $180 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR on the channel bottom ensuring photosynthetic activity saturation (BOTHWELL *et al.*, 1993). Water temperature, conductivity, pH, dissolved oxygen and turbidity in the upstream tank were measured hourly during the whole experiment (YSI 6600 probe, YSI Incorporated, USA). Furthermore, water was sampled weekly (automatic samplers ISCO 3000) in the upstream tank (flume water) and in the river tank (river water) to assess water composition and compare flume water to river water. Samplers were programmed to collect 500 mL hourly from 7:00 to 6:00, and the 3 samples mixed to give 3 composite samples as chosen to convey daily changes of the water chemistry: the first 8 hours of the day (7:00–14:00), the second 8 hours of the day (15:00–22:00) and the third 8 hours of the night (23:00–6:00).

2.1.3. Substrate Characteristics and Layout

The flume bed was completely covered by 3750 artificial substrata, which mimic natural cobbles. Each substrate consisted of a chemically inert sand-ballasted polyurethane resin hemisphere (37 mm diameter, 20 mm high), a shape and a texture shown to provide good conditions for biofilm adhesion and growth (NIELSEN *et al.*, 1984). In order to eliminate any possible toxicity (*e.g.*, solvents), substrates were immersed in river water for 3 weeks, then washed with tap water and sterilized by autoclave ($120 \text{ }^\circ\text{C}$, 20 min) before being positioned side by side in the flume, without any fixation, except at both ends and at the middle of the flume.

2.2. Experimental Procedure and Monitoring

2.2.1. Inoculum and Start-up

To collect diverse biofilm communities, pebbles (average size 10 cm²) with biofilm were collected in SW France streams (Orige, Gave de Pau) and rivers (Garonne, Tarn) displaying a wide range of hydroecological conditions. These pebbles were incubated for a maximum of 3 weeks in a second flume that ran with conditions comparable to those of the experiment. A biofilm suspension was produced by scraping the upper surface of 15 pebbles taken at random with a toothbrush, and adding 1 L of filtered (0.22 µm) water. The biofilm suspension was homogenized (tissue homogenizer) to remove macrofauna and to approach grazer-free conditions. During the 3 first weeks, the flume was run using the closed recirculation: water was renewed weekly, and just after renewal, it was seeded with the prepared biofilm suspension. The first date of seeding phase was defined as the start of the experiment (day 0 of the incubation period).

2.2.2. Water Analyses

Concentrations of ammonium, nitrite, nitrate, phosphate, total phosphorus, silicate and suspended chlorophyll-*a* were quantified by either manual or continuous-flow colorimetric methods (APHA, 1992). Dissolved organic carbon was measured using a Shimadzu TOC5000 carbon analyzer (Kyoto, Japan). Suspended matter (SM) was measured by weighing the dried matter (105 °C, 4 h) collected by filtration onto a 0.45 µm pore-size membrane filter (Whatman nitrate cellulose membrane). Suspended matter size in the river water was measured once by laser diffraction at the end of the experiment using a laser Mastersizer Micro (Malvern Instruments Ltd, Malvern, UK) without disaggregation pre-treatment.

2.2.3. Biofilm and Drift Sampling

Biofilm was sampled in a 5 m long area of the experimental flume, every week after the seeding phase (13 sampling dates). The 3 rows of substrates closer to the walls were not sampled to avoid edge effect. For each sampling, 11 substrates were randomly sampled and kept in sterile vials at 4 °C: six substrates were randomly used for metabolism, and then, for ash-free dry mass (AFDM), 4 substrates for chlorophyll-*a* and bacterial composition (randomly selected) and 1 substrate for algal composition. Every substrate sampled was replaced with a new one, pink-coloured to avoid sampling in further occasions.

Drifting material was sampled weekly with a 50 µm nylon mesh placed weekly at the end of the flume for 10 minutes. Six samples were taken consecutively during one hour. Material from the 2 first meshes (*i.e.*, 20 minutes, 4 times water renewal time in the flume) was excluded from the analyses, and only the other 4 were processed, to measure the quantity of newly dislodged drifting material (DM and AFDM), after suspending in 0.5 L water.

2.2.4. Metabolism

Biofilm metabolism (primary production and respiration) was measured weekly within 4 hours of sampling, using a rapid respirometer method based on changes in oxygen over time (McIntire *et al.*, 1965). Measurements were conducted in 2 transparent Altuglass chambers (18 cm long, 18 cm wide, 5 cm deep, 1.3 L-total volume with pipes, measurement cell and substrates) closed with a 2 cm thick transparent Altuglass lid, filled with filtered water (0.2 µm, Whatman nitrate cellulose membrane), which recirculated at 600 mL min⁻¹ thanks to an aquarium pump. Note that pumping is not intended to recreate the water velocity that prevails in the flume. Three substrates (0.0065 m²) collected in the flume were placed in each chamber, and incubated at the same temperature measured in the flume one hour before. Light was kept constant at 150 µmol m⁻² s⁻¹ by means of a Phyto Claude 400 W lamp. Dissolved oxygen within each chamber was monitored using oxygen probes (WTW Oxi340i) over 2 hours every minute. Net community metabolism (in g O₂ m⁻² d⁻¹) was determined by measuring dissolved oxygen changes

over a 30-min period of daylight (maximal slope). Following the incubation, chambers were exposed to the dark and the rate of community respiration (R in $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) (autotrophic and heterotrophic respirations) was determined as the change in oxygen concentration over a 90-min second incubation period (maximal slope). A dark box was used to prevent light penetration into the chambers. This technique allowed incubations to be relatively short in order to reduce containment effects such as nutrient depletion and supersaturation of oxygen that can be problematic when using enclosed chambers to measure metabolism (BOTT *et al.*, 1997). The gross primary production (GPP in $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) was calculated by adding net community metabolism to community respiration. The net productivity (in $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) was calculated by adding GPP multiplied by the ratio of day hours (16/24) to R . Biomass-specific GPP ($\text{mg O}_2 (\text{mg chlorophyll-}a)^{-1} \text{ d}^{-1}$), biomass-specific R ($\text{mg O}_2 (\text{mg chlorophyll-}a)^{-1} \text{ d}^{-1}$), and biomass-specific net productivity ($\text{mg O}_2 (\text{mg chlorophyll-}a)^{-1} \text{ d}^{-1}$) were calculated by dividing respectively GPP, R and the net productivity by the chlorophyll- a biomass. The ratio of GPP to R (GPP/ R) was calculated and used as an integrative parameter of the physiological state of the community (FISHER and LIKENS, 1973).

2.2.5. Epilithic and Drift Biomass

Substrates used for metabolism measurement were removed from the chambers and used to determine DM and AFDM biomass. They were dried (80°C , overnight), weighed (W_1) and scraped. One fraction (around 25 mg) of scraped dry matter was weighed before and after combustion (500°C , overnight) to determine AFDM. Substrates were finally cleaned, dried and weighed (W_2) to obtain DM by difference between W_1 and W_2 .

The production of drift biomass was expressed as a percentage of biomass that detached per day, by multiplying drift concentration (g AFDM L^{-1}) by the flow (L d^{-1}) divided by the total amount of biomass in the flume (g AFDM). The biofilm suspension (collected with meshes) was homogenised (tissue homogenizer) and a 50 mL aliquot of the homogenised biofilm suspension was centrifuged ($2300 \times g$, 20 min). The pellet was dried (80°C , overnight) and weighed to determine DM, and weighed before and after combustion (500°C , overnight) to determine AFDM of the drift material.

For chlorophyll- a determination, biofilm was scraped from the upper surface of 4 substrates with a sterile toothbrush, and suspended in filtered ($0.2 \mu\text{m}$, Whatman cellulose nitrate membrane) water (50 or 100 mL according to biomass). Suspensions were homogenised (tissue homogenizer) and 10 mL aliquots were centrifuged ($12000 \times g$, 20 min, 4°C). After removing the supernatant, the 4 pellets were stored at -80°C , and chlorophyll a measured spectrophotometrically using trichromatic equations (JEFFREY *et al.*, 1997) after extraction of the ground pellet (following ultrasonic digestion) with acetone 90% (4 h darkness, room temperature). Four other 10 mL aliquots of biofilm suspensions were centrifuged ($12000 \times g$, 20 min, 4°C) and, after removing the supernatant, the pellet was kept at -80°C for the determination of bacterial community composition.

2.2.6. Bacterial Composition

On each occasion, we selected at random three out of the four pellets obtained by centrifugation. DNA was extracted using a MO BIO (USA) Kit on 10–50 mg of biofilm pellets, and quantified by fluorimetry (Fluoroskan Ascent II; Labsystems, Helsinki, Finland) (excitation 485 nm, emission 538 nm) using SYBR Green (Sigma-Aldrich, St. Louis, MO, USA; dilution 1:10000). Extracted DNA was amplified by PCR using the universal *Bacteria* primers 341F-GC and 907R (MUYZER *et al.*, 1997) and 20 ng of template DNA. The PCR products from 3 distinct reactions were pooled and loaded in an acrylamide gel containing a 35 to 70% urea and formamide gradient (100% corresponding to 7 M of urea and 40% of deionised formamide).

DGGE (Denaturing Gradient Gel Electrophoresis) was carried out using D-Code Universal Mutation Detection System (BioRad). The 45 samples (3 replicates \times 15 dates) were dispatched on 3 gels putting one replicate per gel. The electrophoresis was run for 18 h at 100 V at 60°C . The gel was stained for 30 min with SYBR Green (Sigma) and gel image captured using a CCD camera and Biocapt software (Vilber Lourmat) and analyzed using Bio 1D software (Vilber Lourmat). DGGE profiles were compared with regard to the presence or absence of bands using the Jaccard similarity index (JACCARD, 1912) and dendrogram construction according to the unweighed pair group method with average means (UPGMA) (MICHENER and SOKAL, 1957). Statistical differences between groups determined from the dendrograms were tested using random permutation (Monte Carlo test, 10000 permutations) (KROPF *et al.*, 2004).

2.2.7. Algal Composition

Biofilm was removed from the upper surface of 1 substrate as for chlorophyll-*a* determinations. The biofilm suspension was preserved with neutralized glutaraldehyde (1% final concentration) and stored at 4 °C in darkness until examination at 600 to 1000 ×. Taxa were identified to the lowest practical taxonomic level, usually to species but often to genus. For practical reason, nine of 13 samples were selected at random for analysis to observe changes in taxonomic composition.

3. Results

3.1. Water Characteristics

Conductivity, dissolved oxygen and pH (mean ± *SD*) remained fairly stable within the flume during the experiment ($197 \pm 21 \mu\text{S cm}^{-1}$, $10.4 \pm 1.4 \text{ mg L}^{-1}$ and 8.6 ± 0.2 respectively) (Table 1A). Temperature in the flume varied widely during the study, from 8.1 to 20.3 °C. Turbidity averaged 6.5 NTU and exhibited two 3 and 2 day peaks reaching 16 and 19 NTU after 53 and 57 incubation days respectively, followed by one clear 3 day increase on the 66th incubation day reaching 175 NTU (Table 1A and Fig. 2). At the end of the experiment, most of the suspended particles were fine silt-sized particles (>2 μm and <20 μm) corresponding to 33% of the total SM, coarse silt-sized particles (>20 μm and <50 μm) corresponding to 45% and very fine sand (>50 μm and <100 μm) corresponding to 14%. Clay-sized particles (<2 μm) and fine and medium sand (>100 μm and <500 μm) represented less than 3% and around 5% respectively. As no major failure of the centrifugation/filtration device occurred, particle aggregation, floc formation and drifting of biofilm pieces is likely to explain the unexpected proportion of particles larger than 10 μm.

Concentrations in nitrate, ammonia, nitrite and DOC exhibited similar variations in both river and flume waters (Table 1B). Silicate, phosphate and total phosphorus showed higher

Table 1. (A) Recorded mean and standard deviations (*SD*) of temperature (TEMP), conductivity (COND), dissolved oxygen (DO), pH and turbidity (TURB) in the flume and (B) measured values of suspended chlorophyll *a* (chl *a*), nitrate (NO₃), nitrite (NO₂), ammonium (NH₄), phosphate (PO₄), total phosphorus (total P) and silicate (Si) in the flume and the river.

(A)

variable unit	TEMP °C	COND mS cm ⁻¹	DO mg L ⁻¹	DO %	pH –	TURB NTU*
mean	15.2	0.196	10.6	106	8.6	6.7
<i>SD</i>	3.6	0.023	1.3	10	0.2	13.1

* NTU: Nephelometric Turbidity Units

(B)

variable unit	chl <i>a</i> μg L ⁻¹	NO ₃ mg N L ⁻¹	NO ₂ mg N L ⁻¹	NH ₄ mg N L ⁻¹	PO ₄ mg P L ⁻¹	Si mg L ⁻¹	total P mg P L ⁻¹	DOC mg L ⁻¹
condition	flume river	flume river	flume river	flume river	flume river	flume river	flume river	flume river
mean	3.9 2.5	1.135 1.200	0.007 0.011	0.017 0.029	0.003 0.007	1.434 3.564	0.012 0.022	2.052 2.181
<i>SD</i>	2.4 2.3	0.357 0.400	0.002 0.003	0.009 0.015	0.002 0.003	0.973 1.560	0.008 0.011	0.306 0.456

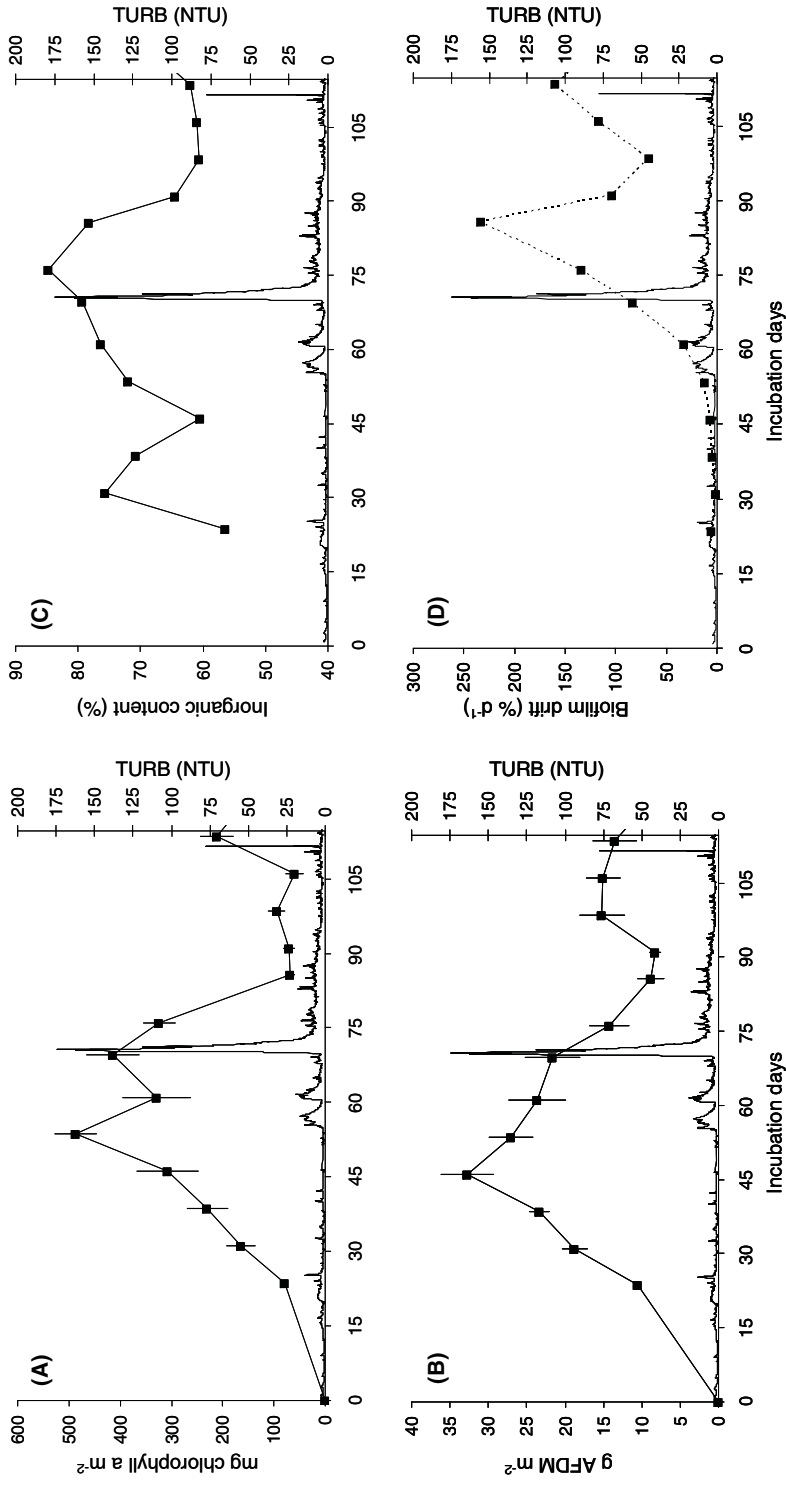


Figure 2. Temporal changes of epilithic biomass (mean \pm standard error) in (A) mg of chlorophyll-*a* per square meter ($n = 4$), (B) g of ash-free dry matter (AFDM) per square meter ($n = 6$), (C) inorganic content (%) and (D) of drift AFDM biomass in % of epilithic AFDM. Temporal changes in turbidity (NTU) are reported on each graph (solid line).

variations in the flume than in the river, and chlorophyll-*a* the reverse pattern. Mean chlorophyll-*a* was around 1.5 times higher in the experimental flume than in the river suggesting production by the epilithic biofilm. Nevertheless mean ammonia, phosphorus, silicate and total phosphorus were 1.7, 2.1, 2.5 and 1.8 times lesser in average in the experimental flume than in the river, suggesting consumption and adsorption in the flume by the epilithic biofilm.

3.2. Biomass of Epilithic and Drifting Biofilm

Epilithic biomass showed a colonisation phase followed by exponential growth to 487 mg chlorophyll-*a*, and 33 g AFDM m⁻² after 65 and 71 incubation days respectively (Fig. 2A and 2B). Both variables were significantly correlated ($r^2 = 0.64$; $n = 12$; $P = 0.0018$). The peak of biomass arrived at 9 weeks for AFDM and at 10 weeks for chlorophyll-*a*. In the 3 following weeks, biomass decreased to 356 mg chlorophyll *a* and 24 g AFDM m⁻². After 10 weeks, biomass followed an abrupt and statistically significant decline, with losses of 60% of AFDM and 80% of chlorophyll-*a* (Mann-Whitney $P < 0.05$). The inorganic content increased to 85% during the first 10 weeks and progressively decreased to 61% during the following weeks (Fig. 2C). Drifting particulate organic matter was first low, averaging 5% of biomass per day after 6 incubation weeks (Fig. 2D). Then, it exponentially increased to 233% during the 5 successive weeks, before decreasing to 67% in two weeks later.

3.3. Metabolism

GPP and R varied respectively between 3.8 and 9.6 and between 0.6 and 2.2 g O₂ m⁻² d⁻¹. Biomass-specific GPP slightly decreased from 57 to 13 mg O₂ (mg chlorophyll-*a*)⁻¹ d⁻¹ until the day 71 coinciding with the first biomass increase and suddenly increased to 122 mg O₂ (mg chlorophyll-*a*)⁻¹ d⁻¹ coinciding with the biomass loss (Fig. 3A). Biomass-specific R exhibited the same pattern as biomass-specific GPP decreasing from 15.9 to 1.8 mg O₂ (mg chlorophyll-*a*)⁻¹ d⁻¹ and increasing to 28.2 mg O₂ (mg chlorophyll-*a*)⁻¹ d⁻¹ concomitantly with the biomass loss (Fig. 3B). Biomass accruals coincided with general decreases in biomass-specific net productivity, from 22.1 to 3.2 mg O₂ (mg chlorophyll-*a*)⁻¹ d⁻¹ between the days 22 and 71, and from 53.0 to 13.9 mg O₂ (mg chlorophyll-*a*)⁻¹ d⁻¹ between the days 92 and 106 (Fig. 3C). Between these drops, biomass-specific net productivity increased rapidly. Biofilm communities were dominated by autotrophic processes, with a mean GPP/R ratio of 5 (Fig. 3D). The highest ratios (between 6.7 and 8.1) coincided with periods of maximal biomass whereas the lowest ratio (2.5) occurred just before the biomass loss on 71st day.

3.4. Bacterial Composition

Bacterial community composition (BCC) was studied using DGGE on three gels. Irrespective of the gel, dendrograms based on Jaccard similarity index of DGGE banding patterns discriminated between two groups of samples that shared respectively 35, 32 and 40% of the detected bands (Fig. 4). These differences were statistically significant (Monte Carlo test, 10000 permutations, $P < 0.01$). Nevertheless groups varied slightly between gels. In every gel, the first group included the 6 samples collected during the first 65 days (first biomass accrual phase), the second group samples collected from the 85th incubation day (samples after biomass loss). Dates between days 43 and 80 were either located in the first group (gel 3), either in the second group (gel 1) or in both groups (gel 2). Number of bands was similar for each cluster in all gels, with 27–40, 26–33 and 29–36 OTUs (Operational

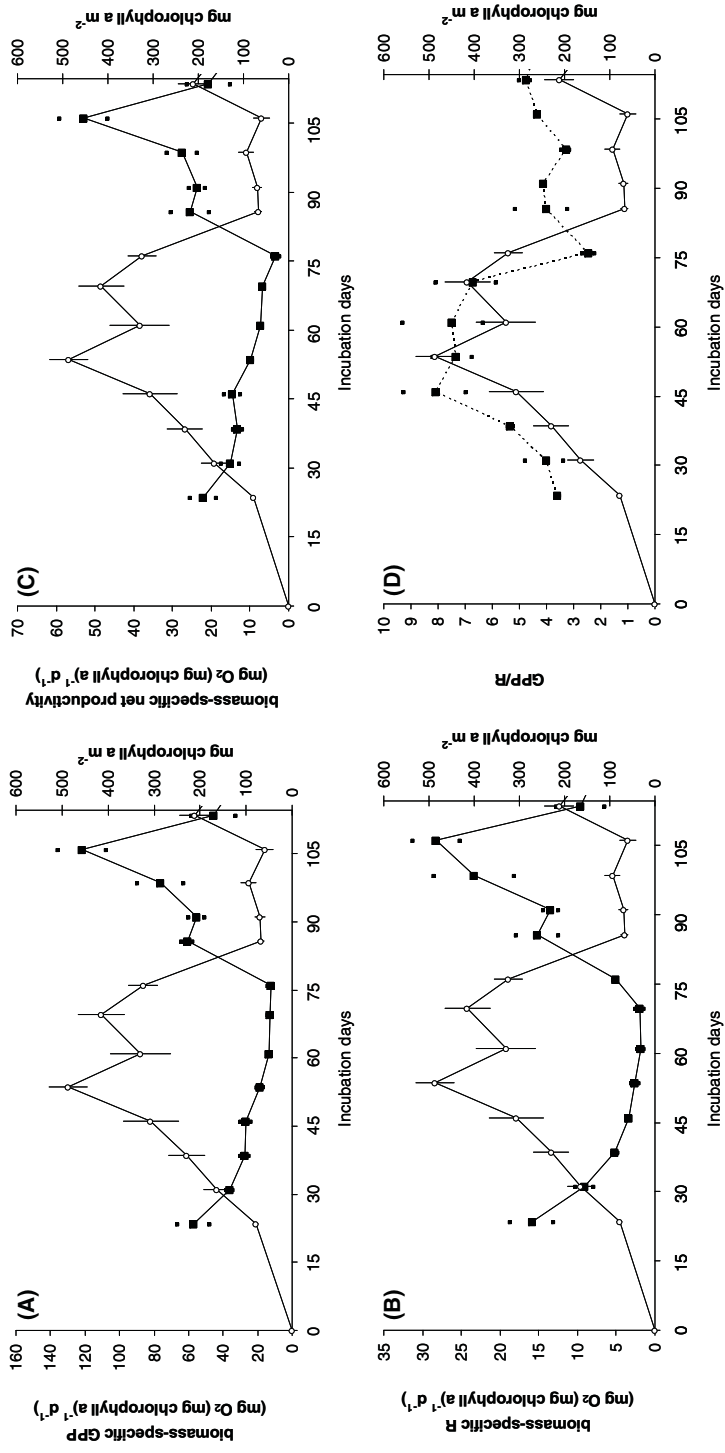


Figure 3. Temporal metabolism changes in (A) biomass-specific GPP ($\text{mg O}_2 (\text{mg chlorophyll-}a)^{-1} \text{d}^{-1}$), (B) biomass-specific R ($\text{mg O}_2 (\text{mg chlorophyll-}a)^{-1} \text{d}^{-1}$), (C) biomass-specific net productivity ($\text{mg O}_2 (\text{mg chlorophyll-}a)^{-1} \text{d}^{-1}$) and (D) GPP/R. For A, B and C, $n = 2$. Chlorophyll- a biomass (mean \pm standard error in $\text{mg of chlorophyll-}a \text{ m}^{-2}$) is reported on each graph (circle).

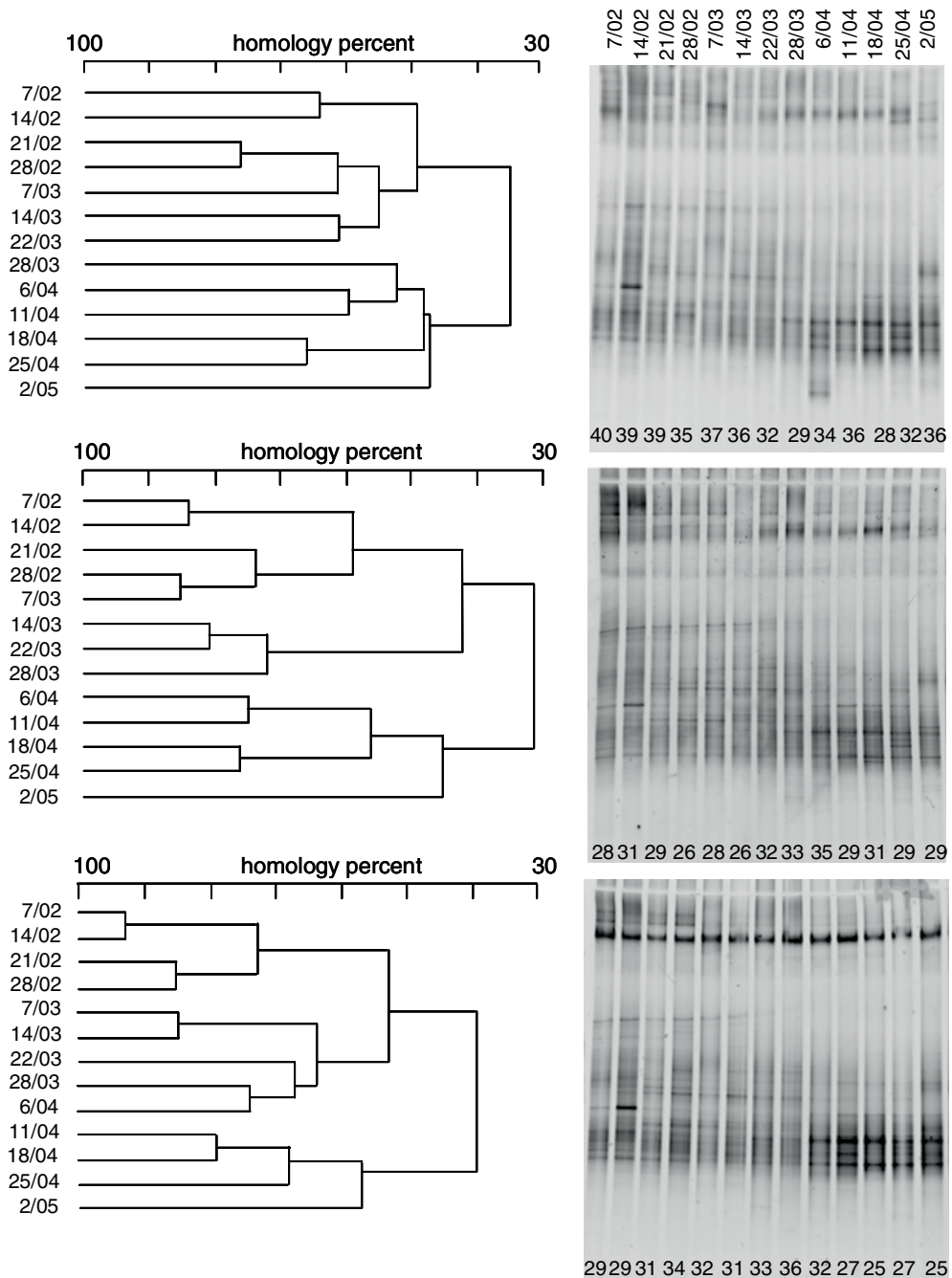


Figure 4. UPGMA Jaccard distance dendrograms (left column) generated from the DGGE profiles of the bacterial community (right column) present in epilithic biofilm samples collected between days 22 and 120. Richness is given for each sample at the bottom of each band.

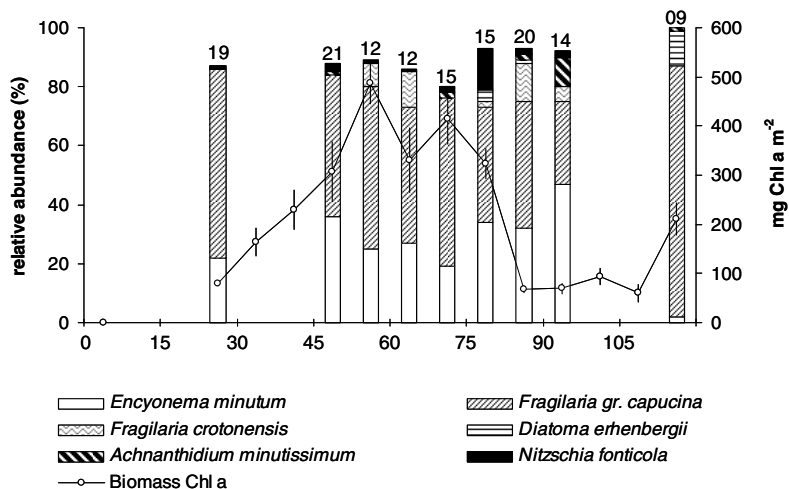


Figure 5. Temporal changes of the cumulative relative abundance of main algal taxa (>5%). Richness is given for each sample at the bottom of each bare. Associated mean (\pm standard error) biomass in mg of chlorophyll-*a* per square meter is reported on each graph (circle).

Taxonomic Units) being present per sample in the first group and 28–36, 24–35 and 22–30 OTUs per sample in the second group. The mean band richness was lower in gels 2 ($S = 29$) and 3 ($S = 30$) than in the gel 1 ($S = 35$).

3.5. Algal Composition

Species richness was relatively low, varying from 9 to 21 taxa (Fig. 5). The algal community was dominated by diatoms, which represented 97 to 100% of the total abundance. Two taxa strongly dominated the algal community: *Fragilaria capucina* DESMAZIERE var. *vaucheriae* (KÜTZ) LANGE-BERTALOT represented 28 to 85% and *Encyonema minutum* HILSE ex. RABENHORST represented 2 to 47% of the total community. Apart from these two species, only 4 species reached abundances higher than 5% at least once: *Achnanthidium minutissimum* KÜTZ., *Fragilaria crotonensis* KITTON and *Nitzschia fonticola* GRUN., and *Diatoma ehrenbergii* KÜTZ.. The last sample was quite different from others: with the lowest diversity, it was largely dominated by *Fragilaria capucina* (85%) and by *Diatoma ehrenbergii*, which did not exceed 5% in other samples. No substantial changes in algal composition and species richness were observed during the main biomass loss

4. Discussion

A 15 week experiment using a laboratory flume was designed to check variations of epilithic biofilm structure and function under constant flow (mean current velocity (\pm SD) of $0.225 (\pm 0.002) \text{ m s}^{-1}$). Flow can be considered constant at the studied scale even if biofilm development could alter the local physical environment including flow conditions (BATTIN *et al.*, 2003). For that matter, another part of the present study dealing with such biofilm-

flow interactions showed that friction velocity (u^*) and equivalent roughness length (k_s), two parameters describing the local flow near the bed, evolved with biomass accretion (GRABA *et al.* in press). Driven by the pluvial-nival flow regime (hydrologic regime characterised by rain supply year-around in addition to spring snowmelt) of the River Garonne, a nutrient-enriched river according to DODDS *et al.* (1998), water quality experienced natural changes. The main limitation of the experimental device was observed analysing algal and bacterial diversity. Diatoms dominated the assemblage consistently with the typical benthic algal composition of the River Garonne (EULIN and LE COHU, 1998). Algal species richness however was 5 times lower than natural community richness at the same period in the River Garonne. Despite cautions taken in the present experiment by (i) the introduction of a large pool of biofilm origins during the seeding phase and (ii) the 10 μm pore-size filtering designed to ensure seeding by algal propagules, poor algal diversity was observed. It could be inherent to partially re-circulating and filtering system that did not ensure continuous enrichment but is more likely to be due to flume characteristics (*e.g.*, low substratum heterogeneity, constant light intensity and flow regime) that reduced environmental variability. Consistently, band richness of DGGE profiles was slightly lower than those previously obtained for natural assemblages in the same river (LYAUTEY *et al.*, 2003; LYAUTEY *et al.*, 2005). The studied biofilm should be considered as a simplified assemblage selected by the selected experimental settings.

Irrespective of a limited pool of algal species (dominated by diatoms), autotrophic energy fixation prevailed in the community metabolism during the whole experiment as proved by the GPP/R values always held higher than 1. The reduction or the increase in light penetration as the mat develops or recedes explained that biomass-specific GPP decreased when biomass increased and clearly increased during the recovery phase (RIBER and WETZEL, 1987; STEVENSON and GLOVER, 1993). The general pattern of biomass observed in the flume was similar to patterns observed *in situ* and in accordance with the benthic theoretical curve described in BIGGS (1996). One 10-week accrual phase and one consecutive 2 week loss phase were observed before to recovery at an intermediate level during 2 weeks. The AFDM peak and time taken to reach this peak were comparable to values measured in the River Garonne (BOULÉTREAU *et al.*, 2006). In the course of the loss phase, a drop of 80% was observed in epilithic chlorophyll-*a*. The concomitant increase in drifting material proved that this drop was partly due to biofilm removal.

Whether the algal pool could maintain the production constant asks the question of the role of the reduced algal diversity on the primary production of the assemblage. This question fits in with the new challenge in functional ecology to describe how biodiversity dynamics, ecosystem processes and abiotic factors interact (LOREAU *et al.*, 2001). The competitive exclusion principle applied to ecosystem function implies that resources would be used more efficiently when more species coexist (HARDIN, 1960). In our experiment, a poorly diversified assemblage proved however to support a phototrophic biofilm development comparable to those of natural assemblages in the river. It can be hypothesized that stable conditions of light and flow could have favoured adapted taxa that supported biofilm growth. Conversely, exhibiting low resistance, the selected assemblage was not fitted when the disturbance occurred.

No evidence of macrofauna (specimens in samples, bioturbation prints or adult emergence in the flume) was observed. If grazing cannot be completely excluded by microfauna such as protozoans it does not totally explain the observed disturbance. Note that reduced grazing pressure that resulted from scarce macrograzer evenness could also have contributed to poor algal and bacterial diversity that is discussed above. Nevertheless, two parameters, temperature and suspended matter, exhibited relevant variations: temperature underwent a seasonal trend to warming and punctual suspended matter pulses coincided with flood events in the River Garonne. By considering BOULÉTREAU *et al.* (2006), the concomitance of the biomass peak and high temperature conditions could be favourable to the

assumption of autogenic sloughing. But GPP/R was higher than 2 during the whole experiment and biomass-specific GPP was particularly low just before biomass loss, limiting intensive oxygen bubble production and discrediting a photosynthetic origin of autogenic sloughing. As for heterotrophic degradation, LYAUTEY *et al.* (2005) have shown that architectural changes were accompanied by changes in the BCC, fitting with a conceptual model proposing a shift from N-autotrophy to N-heterotrophy when epilithic biomass reaches a threshold (TEISSIER *et al.*, 2007). In the present study, BCC clearly changed between before and after biomass loss but biomass-specific R exhibited the weakest values at this time. No marked metabolism shift to from phototrophic to heterotrophic activity could support any putative autogenic sloughing.

Abrasion by suspended material can result in epilithon scouring (HORNER *et al.*, 1990; UEHLINGER, 1991; BIGGS, 1996). Abrasion impact is expected to be still more effective with sand and coarse silt-sized particles than with clay and fine silt-sized particles. Abrasion impact is most effective at the top of substrates where mean flow structures were numerically demonstrated to be more energetic (*e.g.*, COCEAL *et al.*, 2006). If sand and coarse silt-sized particles represented 64% of the SM composition, biomass removal was gradual and mainly impacted the base of substrates. The gradual increase of the biofilm inorganic content (coinciding with the turbidity peak) suggested silt deposition to be responsible for biomass loss too. Silt particles are demonstrated to be transported thanks to mechanisms such as siltation, rolling, sliding and subsequently be trapped into biofilm matrix and on mucilaginous surfaces of diatoms stalks (HOAGLAND *et al.*, 1982; GRAHAM, 1990). The mucilage produced by organisms offers many potential binding sites for a variety of colloidal, organic and inorganic elements (FLEMMING, 1995). The biofilm response to the major turbidity pulse was delayed as would be expected if small particles were adsorbed and incorporated into the biofilm matrix, thus making sloughing easier, especially if oxygen bubbles are incorporated. Flocs and aggregates are demonstrated to form on the surfaces of bubbles that subsequently collapse (WOTTON, 2007). Fine sediment suspension and deposition were demonstrated to prevent attachment of algal cells to the substrate and to affect production by reducing light penetration (*e.g.*, VAN NIEUWENHUYSE and LA PIERRE, 1986; DAVIES-COLLEY *et al.*, 1992; WOOD and ARMITAGE, 1997) and by reducing nutrient availability due to adsorption/desorption on inorganic particles and weakest diffusion.

The impacts of fluid and solid forces on epilithic communities during flood disturbances are always mixed in natural conditions. The present experimental design that keeps current velocity constant with natural changes in suspended matter concentration allows to quantify and understand the isolated natural impact of suspended material on changes in epilithic community structure and function. For lack of control in the present study, suspended matter can only be suspected to have affected the biofilm pattern because a turbidity event occurred concomitantly with biomass loss. Validating the hypothesis of siltation impact would require experimenting on the micrometer scale by using tools such as confocal microscopes and micro sensors to consider biofilm local shading of primary producers and/or local chemical limitations. This original data set provides a weekly characterization of structural and functional changes in epilithon biomass. It is a good candidate to improve modelling by implementing new processes (*e.g.*, solid impact on detachment).

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6. References

- A. P. H. A. 1992: Standard Methods for the Examination of Water and Wastewater, 20th edition. – American Public Health Association, Washington, DC, 1220 pp.
- BATTIN, T. J., L. A. KAPLAN, J. D. NEWBOLD and C. M. E. HANSEN, 2003: Contributions of microbial biofilms to ecosystem processes in stream mesocosms. – *Nature* **426**: 439–442.
- BESEMER, K., G. SINGER, I. HÖDL and T. J. BATTIN, 2009: Bacterial community composition of stream biofilms in spatially variable-flow environments. – *Appl. Environ. Microbiol.* **75**: 7189–7195.
- BIGGS, B. J. F. and H. A. THOMSEN, 1995: Disturbance in stream periphyton by perturbations in shear stress: time to structural failure and difference in community resistance. – *J. Phycol.* **31**: 233–241.
- BIGGS, B. J. F., 1996: Patterns in benthic algae of streams. – *In*: STEVENSON, R. J., M. L. BOTHWELL and R. L. LOWE (eds.), *Algal ecology: freshwater benthic ecosystems*. Academic Press, San Diego, 31–56.
- BIGGS, B. J. F., V. I. NIKORA and T. H. SNELDER, 2005: Linking scales of flow variability to lotic ecosystem structure and function. – *Riv. Res. Appl.* **21**: 283–298.
- BOTHWELL, M. L., D. SHERBOT, A. C. ROBERGE and R. J. DALEY, 1993: Influence of natural ultraviolet radiation on lotic periphytic diatom community growth, biomass accrual, and species composition: short-term versus long-term effects. – *J. Phycol.* **29**: 24–35.
- BOTT, T. L., J. T. BROCK, A. BAATTRUP-PEDERSEN, P. A. CHAMBERS, W. K. DODDS, K. T. HIMBEAULT, J. R. LAWRENCE, D. PLANAS, E. SNYDER and G. M. WOLFAARDT, 1997: An evaluation of techniques for measuring periphyton metabolism in chambers. – *Can. J. Fish. Aquat. Sci.* **54**: 715–725.
- BOULÉTREAU, S., F. GARABÉTIAN, S. SAUVAGE and J.-M. SANCHEZ-PEREZ, 2006: Assessing the importance of a self-generated detachment process in river biofilm models. – *Freshw. Biol.* **51**: 901–912.
- BOULÉTREAU, S., O. IZAGIRRE, F. GARABÉTIAN, S. SAUVAGE, A. ELOSEGI and J.-M. SANCHEZ-PEREZ, 2008: Identification of a minimal adequate model to describe the biomass dynamics of river epilithon. – *Riv. Res. Appl.* **24**: 36–53.
- COCEAL, O., T. G. THOMAS, I. P. CASTRO and S. E. BELCHER, 2006: Mean flow and turbulence statistics over groups of urban-like cubical obstacles. – *Boundary Layer Meteorol.* **121**: 491–519.
- DAVIES-COLLEY, R. J., C. W. HICKEY, J. M. QUINN and P. A. RYAN, 1992: Effects of clay discharge on streams: 1. optical properties and epilithon. – *Hydrobiologia* **248**: 215–234.
- DODDS, W. K., J. R. JONES and E. B. WELCH, 1998: Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen. – *Water Res.* **32**: 1455–1462.
- EULIN, A. and R. LE COHU, 1998: Epilithic diatom communities during the colonization of artificial substrates in the River Garonne (France). Comparison with the natural communities. – *Fundam. Appl. Limnol. (Arch. Hydrobiol.)* **143**: 79–106.
- FISHER, S. G. and G. E. LIKENS, 1973: Energy flow in Bear brook, New Hampshire: an integrative approach to stream ecosystem metabolism. – *Ecol. Monograph* **43**: 431–439.
- FLEMMING, H. C., 1995: Sorption sites in biofilms. – *Water Sci. Technol.* **32**: 27–33.
- FRANCOEUR, S. N. and B. J. F. BIGGS, 2006: Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. – *Hydrobiologia* **561**: 59–69.
- GODILLOT, R., B. CAUSSADE, T. AMÉZIANE and J. CAPBLANCO, 2001: Interplay between turbulence and periphyton in rough open-channel flow. – *J. Hydraul. Res.* **39**: 227–239.
- GRABA, M., F. Y. MOULIN, S. BOULÉTREAU, F. GARABÉTIAN, A. KETTAB, O. EIFF, J.-M. SANCHEZ-PEREZ and S. SAUVAGE, 2010: Effect of near-bed turbulence on chronic detachment in artificial rough, open-channel flow: experimental and modeling approaches. – *Water Resour. Res.* in press.
- GRAHAM, A. A., 1990: Siltation of stone-surface periphyton in rivers by clay-sized particles from low concentrations in suspension. – *Hydrobiologia* **199**: 107–115.
- HARDIN, G., 1960: The competitive exclusion principle. – *Science* **131**: 1292–1297.
- HOAGLAND, K. D., S. C. ROEMER and J. R. ROSOWSKI, 1982 – Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). – *Am. J. Bot.* **69**: 188–213.
- HORNER, R. R., E. B. WELCH and R. B. VEENSTRA, 1983: Development of nuisance periphytic algae in laboratory streams in relation to enrichment and velocity. – *In*: WETZEL, R. G. (ed.), *Periphyton of freshwater ecosystems*. Dr. W. JUNK publishers, The Hague, 121–134.
- HORNER, R. R., E. B. WELCH, M. R. SEELEY and J. M. JACOBY, 1990: Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. – *Freshw. Biol.* **24**: 215–232.
- JACCARD, P., 1912: The distribution of flora in the alpine zone. – *New Phytol.* **11**: 37–50.

- JEFFREY, S. W., R. F. C. MANTOURA and S. W. WRIGHT, 1997: Phytoplankton pigments in oceanography. Unesco, Paris.
- KROPF, S., H. HEUER, M. GRÜNING and K. SMALLA, 2004: Significance test for comparing complex microbial community fingerprints using pairwise similarity measures. – *J. Microb. Methods* **57**: 187–195.
- LABIOD, C., R. GODILLOT and B. CAUSSADE, 2007: The relationship between stream periphyton and near-bed turbulence in rough open-channel flow. – *Ecol. Modell.* **209**: 78–96.
- LOREAU, M., S. NAEEM, P. INCHAUSTI, J. BENGTSOON, J. P. GRIME, D. HECTOOR, D. U. HOOPER, M. A. HUSTON, D. RAFFAELLI, B. SCHMID, D. TILMAN and D. A. WARDLE, 2001: Biodiversity and ecosystem functioning: current knowledge and future challenges. – *Science* **294**: 804–808.
- LYAUTEY, E., S. TEISSIER, J. Y. CHARCOSSET, J. L. ROLS and F. GARABÉTIAN, 2003: Bacterial diversity of epilithic biofilm assemblages of an anthropized river section, assessed by DGGE analysis of a 16S rDNA fragment. – *Aquat. Microb. Ecol.* **33**: 217–224.
- LYAUTEY, E., C. R. JACKSON, J. CAYROU, J. L. ROLS and F. GARABÉTIAN, 2005: Bacterial community succession in natural river biofilm assemblages. – *Microb. Ecol.* **50**: 589–601.
- MCINTIRE, C. D. and H. K. PHINNEY, 1965: Laboratory studies of periphyton production and community metabolism in lotic environments. – *Ecol. Monograph* **35**: 237–258.
- MICHENER, C. D. and R. R. SOKAL, 1957: A quantitative approach to a problem in classification. – *Evolution* **11**: 130–162.
- MOMO, F. R., 1995: A new model for periphyton growth in running waters. – *Hydrobiologia* **299**: 215–218.
- MUYZER, G. and K. SMALLA, 1998: Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. – *Antonie Van Leeuwenhoek* **73**: 127–141.
- NIELSEN, T. S., W. H. FUNK, H. L. GIBBONS and R. M. DUFFNER, 1984: A comparison of periphyton growth on artificial and natural substrates in the upper Spokane River. – *Northwest Sci.* **58**: 243–248.
- POWER, M. E. and A. J. STEWART, 1987: Disturbance and recovery of an algal assemblage following flooding in a Oklahoma stream. – *Am. Nat.* **117**: 333–345.
- RIBER, H. H. and R. G. WETZEL, 1987: Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton. – *Limnol. Oceanogr.* **32**: 1181–1194.
- SARAVIA, L. A., F. MOMO and L. D. BOFFI LISSIN, 1998: Modelling periphyton dynamics in running water. – *Ecol. Modell.* **114**: 35–47.
- STEVENSON, R. J. and R. GLOVER, 1993: Effects of algal density and current on ion transport through periphyton communities. – *Limnol. Oceanogr.* **38**: 1276–1281.
- STEVENSON, R. J. and C. G. PETERSON, 1991: Emigration and immigration can be important determinants of benthic diatom assemblages in streams. – *Freshw. Biol.* **26**: 279–294.
- TEISSIER, S., M. TORRE, F. DELMAS and F. GARABÉTIAN, 2007: Detailing biogeochemical N budgets in riverine epilithic biofilms. – *J. N. Am. Benthol. Soc.* **26**: 178–190.
- UEHLINGER, U., 1991: Spatial and temporal variability of the periphyton biomass in a prealpine river (Necker, Switzerland). – *Fundam. Appl. Limnol. (Arch. Hydrobiol.)* **123**: 219–237.
- UEHLINGER, U., H. BÜRHER and P. REICHERT, 1996: Periphyton dynamics in a floodprone prealpine river: evaluation of significant processes by modelling. – *Freshw. Biol.* **36**: 249–263.
- VAN NIEUWENHUYSE, E. E. and J. D. LA PERRIERE, 1986: Effects of placer gold mining on the primary productivity in subarctic streams of Alaska. – *Water Resour. Bull.* **22**: 91–99.
- WOOD, P. J. and P. D. ARMITAGE, 1997: Biological effects of fine sediments in the lotic environment. – *Environ. Manage.* **21**: 203–217.
- WOTTON, R. S., 2007: Do benthic biologists pay enough attention to aggregates formed in the water column of streams and rivers. – *J. N. Am. Benthol. Soc.* **26**: 1–11.

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