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Biofilm early stage development in two nutrient-rich streams with different urban impacts

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Abstract

The aim of this study was to describe the colonization of the biofilm during its early stages under different concentrations of nutrients and organic matter, specifically in urban streams influenced by agriculture and urbanization. We hypothesized that in a stream with higher concentrations of nutrients and organic matter, the initial biomass growth would be faster, and the changes in the structure of the community would be greater. Sterile glass substrates were placed in 2 urban streams that differed in nutrient and organic matter concentrations; samples were collected during their first week of colonization to measure total biomass, bacterial biomass, chlorophyll *a*, activity of the electron transfer system, and the community composition. Results show that biofilm development in both streams began within a few hours and differed under different conditions of nutrients; in the stream with a better water quality, the colonization dynamics consisted of 2 increments of bacterial biomass linked with an increase of algal biomass. In the urban stream with higher nutrient and organic matter concentrations, biofilm development was slower and consisted of a simultaneous increase of bacteria and algae, consistent with a lower electron transfer system activity. Therefore, the dynamics of the colonization process in addition to those characteristics of the fully developed biofilm could have potential applications in water monitoring of urban streams.

KEYWORDS

attached algae, bacteria, electron transfer activity, succession, urban streams

1 | INTRODUCTION

The colonization of streambed substrata by biofilms (i.e., assemblages of bacteria, archaea, fungi, algae, and protozoans) has been described as a successional pattern resulting from the progressive response of the organisms to physical and chemical factors (Stevenson, Bothwell, Max, & Lowe, 1996). In streams, initial biofilm colonizers are usually bacteria, followed by algae (frequently pennate diatoms) and in later stages are followed by other, sometimes planktonic species (Stock & Ward, 1989). The biomass development of these assemblages is exponential during the accrual phase and fits a logistic model (Díaz Villanueva, Font, Schwartz, & Romani, 2011; Liu, Zhang, & Xu, 2014; Stevenson et al., 1996; Stock & Ward, 1989).

Biofilms integrate the effects of environmental conditions over extended periods of time, mainly because of their small size and rapid growth, species richness, and the physiological variety of the organisms of which they are formed. Their role as an interface between the overlying water and the sediments in the river ecosystem makes them a suitable compartment for evaluating the effect of various chemicals on river ecosystems (Sabater et al., 2007). The colonization process is dependent on multiple factors, such as hydrological variability (Battin, Kaplan, Newbold, Cheng, & Hansen, 2003; Besemer, Singer, Hödl, & Battin, 2009; Coundoul et al., 2015), nutrient availability (Horner & Welch, 1981; Romaní, Giorgi, Acu a, & Sabater, 2004), temperature (Díaz Villanueva et al., 2011), substrata type (e.g., Lee, Nam, Kim, Lee, & Lee, 2008; Romaní et al., 2004), and the internal dynamics associated with the heterogeneity of the microbial composition of the biofilm (Leff, Van Gray, Martí, Merbt, & Romaní, 2016). Particularly, nutrient enrichment and organic matter of anthropogenic origin are major determinants of the structure of biofilms in rivers,

generally favouring biofilm growth (Chen, 2015; Cochero, Romaní, & Gómez, 2013; Díaz Villanueva et al., 2011; Ferragut and Bicudo, 2012; Kobayashi et al., 2009), although excess nutrients can affect the interaction between algal and bacterial production (Scott, Back, Taylor, & King, 2008).

The diffusion and the bioavailability of metals, nutrients, and other ions from the water column into the biofilm, and vice versa, are influenced by an increase in the density and complexity of the biofilms with ageing (Jan Stevenson & Glover, 1993; Johnson, Tuchman, & Peterson, 1997; Rose & Cushing, 1970). Although most current biomonitoring protocols suggest the use of a mature biofilm (i.e., at least 4 weeks old) before sampling (Biggs, 2000; Kelly, 2001), younger biofilms are quickly influenced by variations of water quality (Ivorra, Bremer, Guasch, Kraak, & Admiraal, 2000) and could provide with a sensitive indicator of water quality pollution in a short period of time.

There has been extensive work conducted employing fully developed stream biofilms to measure variations in water quality (some examples include Ancion, Lear, Dopheide, & Lewis, 2013; Duong et al., 2008; Romaní, Guasch, & Balaguer, 2016; Sabater et al., 2007; Tien, Chuang, & Chen, 2009) and some particularly investigating the combined succession of bacteria and algae during the initial colonization phase in streams (Battin et al., 2003; Besemer et al., 2009; Sobczak & Burton, 1996; Szabó et al., 2008). But only a few field studies have focused on biofilm development during its early stages under different water qualities in streams exposed to urban pollution (Merbt, Auguet, & Casamayor, 2011). The aim of this study was to characterize the colonization of the biofilm during its early stages (hours-days) in nutrient-rich streams in urban environments influenced by agriculture and urbanization. We hypothesized that in a stream with higher concentrations of nutrients and organic matter, the initial biomass growth would be faster, and the changes in the structure of the community would be greater. We also examined the relationship between the biological variables and the physical and chemical characteristics of the water during biofilm development, to assess their capacity as early change indicators of water quality.

2 | MATERIALS AND METHODS

2.1 | Experimental design and sampling sites

Frosted glass substrates (5 cm^2) were sterilized in a Ficoinox SL-9000 automatic autoclave and glued to acrylic trays attached to bricks; three bricks per site were prepared, with 120 glass substrates each.

Two reaches in first-order streams were exposed to different water qualities as a consequence of their land use. The first reach, with a lower urban impact (hereafter "LI," 34°58′26.58″S/58°3′13.13″W), runs through a peri-urban area with a moderate agricultural impact, mainly run-off from greenhouses; the second stream reach with a higher urban impact (hereafter "HI," 34°53′24.85″S/58°2′56.07″W) runs through a dense urban area, located downstream from a gated community and the discharges of a meat packing industry. Both reaches have similar geomorphology and hydrological characteristics, low velocity streams (2–3 cm/s), similar turbidity, do not have riparian vegetation, and are rich in nutrients, particularly phosphorous and nitrogen (Cochero, Licursi, & Gómez, 2017, 2015; Feijoó & Lombardo, 2007; Giorgi, Feijoó, & Tell, 2005). A significantly lower water quality, due to higher concentrations of nutrients, organic matter, and heavy metals (such as Zn, Pb, Cu, and Cd), have been reported at the HI when compared with the LI site in previous studies (Armendáriz, Cortese, Rodriguez, & Rodrigues Capítulo, 2017; Cochero et al., 2017; López van Oosterom, Ocon, Armendariz, & Rodrigues Capitulo, 2015; Ronco, Camilión, & Manassero, 2001; Sierra, Gómez, Marano, & Siervi, 2013).

Bricks were submerged at 9:00 a.m. and fixed to the streambed at each site (three replicates per site), making sure they were submerged under at least 10 cm of water at all times. Samples were collected at times 0.5 hr (T0), 3 hr (T1), 6 hr (T2), 24 hr (T3), 48 hr (T4), and 168 hr (1 week, T5). Glass substrates were separated from the bricks using pliers. From each brick, three subsamples were collected to measure the biological variables. Statistical replicates then consisted of the mean values obtained from each of the three subsamples.

2.2 | Variables measured

2.2.1 | Physical and chemical parameters

A Horiba U50 multiparametric sensor was used to measure dissolved oxygen (mg/L and %), temperature (°C), conductivity (μS/cm), pH, and turbidity (nephelometric turbidity units) at each site. Triplicate nutrient samples, biochemical oxygen demand, and chemical oxygen demand were collected in both sites at times T0 and T5. Ammonium, nitrites, nitrates soluble reactive phosphorous (mg/L), and total phosphorous (mg/L) were analysed according to standard methods (American Public Health Association, 1999). Dissolved inorganic nitrogen (mg/L) was calculated as the sum of the concentrations of the three nitrogen forms.

2.2.2 | Bacterial density

Glass substrates were collected in sterile glass vials with 3 ml of distilled autoclaved water; 3 ml of stream water samples was also collected, and both were fixed with a paraformaldehyde/glutaraldehyde 4% solution. Bacterial density from the glass substrates was estimated after sonication (three 2-min cycles) and appropriate dilution (1:100 to 1:400) of the samples. Raw water samples were diluted accordingly as well, and all diluted samples were stained for 10 min with DAPI (4′,6-diamidino-2-phenylindole) to a final concentration of 1 μg/ml (Porter & Feig, 1980) and filtered through a 0.2-μm black polycarbonate filter (GE Osmonics). Bacteria in 20 fields per replicate were counted using an epifluorescence microscope (Olympus BX-50; U-MWU2 filter, with an Olympus Q-Color5 imaging system) under 1,000× magnification.

2.2.3 | Chlorophyll *a*

Glass substrates (or 3 ml of stream water for the water samples) were collected in sterile vials with 3 ml acetone 90% and transported in coolers to the laboratory. Glass substrates were sonicated for three 2-min cycles to detach the biofilm. The suspension from the sonication and the raw water samples were kept in the dark at 4 °C for 24 hr to secure pigment extraction by the acetone. The extract was measured through high-performance liquid chromatography in a

Shimadzu CRB-6A. Separation was carried out with a reverse-phase column (VP-ODS/C8, 250 × 4.6 mm) with a gradient elution (A: 80/20, methanol–ammonium acetate 0.5 M, pH 7.2; B: 90/10, acetonitrile–water; and C: ethyl acetate), a 1 ml/min flow, and a fluorescence detector (Shimadzu RF-10AXL, 430-nm excitation/600-nm emission).

2.2.4 | Electron transfer system activity

The electron transfer system (ETS) activity represents a measure of the overall respiration of the biofilm and was assayed by measuring the reduction of the electron transport acceptor INT (2–3 tetrazolium chloride) into INT-formazan (iodonitrotetrazolium formazan; Blenkinsopp & Lock, 1990). Glass substrates collected from the field were immediately placed in vials with a 3 ml 0.02% INT solution (Sigma-Aldrich). Samples were incubated in the dark for 24 hr, and INT-formazan was extracted with cold methanol for a minimum of 1 hr at 4 °C in the dark. Absorbance at 480 nm was measured spectrophotometrically (Labomed UV-VIS Auto 2602), using a stock solution of 30 mg/ml INT-formazan in methanol to obtain the ETS activity values (μg INT-formazan cm^{-2} hr^{-1}).

2.2.5 | Community composition

Glass substrates were placed in sterile vials with 3 ml distilled autoclaved water and formalin 2% vol/vol. Density of consumers and producers of the microbenthic community (size <1 mm) was estimated using a Sedgwick–Rafter chamber (American Public Health Association, 1999) in an optical microscope (Olympus BX 50) at 200×. Identified taxa were classified in diatoms, chlorophytes, cyanobacteria, euglenophytes, and ciliates. The percentage of empty diatom frustules was also quantified at T0 and T5.

2.2.6 | Ash-free dry mass

Glass substrates were placed in sterile vials with 3 ml distilled autoclaved water and formalin 2% vol/vol. Samples were dried for 48 hr at 60 °C, cooled at room temperature, and weighed to determine the dry mass. Samples were then ashed in a muffle furnace for 4 hr at 550 °C and reweighed. Ash-free dry mass (AFDM) was estimated as the difference between the weight of the dry mass and the weight of the mass after being muffled.

2.2.7 | Statistical analyses

Differences in water quality among the two sites were analysed through a multivariate analysis of variance. Variations in the physical, chemical, and biological variables throughout the experiment and between sites were examined by a repeated-measures analysis of variance (RM-ANOVA, site and time as within-subject factors). Values were first transformed to log (x + 1) to ensure normality, which was previously assessed by the Shapiro–Wilk test (Shapiro & Wilk, 1965); homogeneity of variance was tested by using Cochran's C test (Cochran, 1951). The Student–Newman–Keuls post hoc test was used to test for multiple comparisons, and p values were corrected for false positives by the Benjamini–Hochberg correction (Benjamini & Yekutieli, 2001). Partial eta^2 (η^2) was computed as a measure of the effect size, and pairwise comparisons were conducted to establish if any significant effects of the treatments were present per date.

Growth curves for each biological variable were also fitted by non-linear regression to a logistic (sigmoid) model with three parameters:

$$Y = K/\left(1 + e^{-(X-X50/b)}\right),$$

where K is the Ln of the carrying capacity, b the growth rate, y the dependent variable, X the independent variable (time), and X_{50} the time to reach 50% of the carrying capacity. Non-parametric correlation analyses (Spearman's ρ coefficient) were also performed between the biological variables to establish possible relationships between them and between biological and physical and chemical variables.

3 | RESULTS

3.1 | Site comparison

The physical and chemical parameters measured at both sites during the experiment are summarized in Table 1. The HI site had higher concentrations of soluble reactive phosphorous (3:1), total phosphorous (1.4:1), dissolved inorganic nitrogen (1.6:1), total suspended solids (TSS; 1.5:1), biochemical oxygen demand (9.5:1), and chemical oxygen demand (5:1) than the LI site (one-way ANOVA, $p < .05$). The HI site also had lower pH and dissolved oxygen concentrations, with higher temperature and conductivity ($p < .05$).

As for the biological variables, the water at the HI site had a higher bacterial density, whereas chlorophyll *a* concentration was similar in both sites.

3.2 | Substratum colonization

The total biomass, measured as AFDM (mg/cm^2), was similar in both sites (Figure 1), ranging from 0.14 to 1.37 mg/cm^2 throughout the experiment. Only at T2 significant differences were found between both sites, being higher at the HI site than the LI site (Table 2). The AFDM fits significantly to a logistic function at the HI site (Table 3; $p < .05$).

Mean bacterial density ranged from 8.5×10^8 to 7.7×10^9 cells/mg AFDM at the LI site, and from 3.8×10^8 to 5.6×10^{10} cells/mg AFDM at the HI site (Figure 1). After 30 min of exposure (T0), bacterial colonization in the substrate from both sites had already started, although there were no significant differences between sites. Bacterial density at the LI site peaked twice, once at T1 and again at T3, whereas in the HI site (Figure 1), it remained low till T3, it increased at T4, and it peaked to a maximum at T5. There were no significant differences in bacterial density between sites at T0 and T3, but at T1 and T2, it was higher at the LI site (RM-ANOVA $p < .05$; Table 2). On the other hand, at T4 and T5, the bacterial density at the HI site surpassed the values at the LI site (RM-ANOVA $p < .05$; Table 2).

Algal colonization was measured in the LI site as soon as T0, with mean chlorophyll *a* values of 7.7 μg/mg AFDM, whereas at the HI site, chlorophyll *a* values at T0 were almost zero (Figure 1). Chlorophyll *a* values in the biofilm were higher at the LI site throughout sampling times T0 to T4 when comparing them with the HI site (RM-ANOVA $p < .05$; Table 2). Mean chlorophyll *a* values in the LI site increased

TABLE 1 Mean (±*SD*) physical and chemical parameters and biological variables (bacterial density and chlorophyll *a*) measured in the water of the LI site and the HI site during the experiment and the significance (*p* values) obtained for the ANOVA

	LI site	HI site	
	Coordinates		
	34°55′21.93″S/58° ′58.89″W	34°57′52.48″S/58° ′17.29″W	
	Main land use		
Variable	Low urban impact	High urban impact	Significance (*p* value)
Temperature (°C)	12.2 (±0.5)	13.3 (±0.2)	.10
pH	8.4 (±0.1)	7.9 (±0.1)	**<.01**
Conductivity (μS/cm)	872.5 (±9)	1265 (±10.3)	**<.01**
DO (mg/L)	8.2 (±0.4)	5.2 (±0.2)	**<.01**
DO (%)	79.7 (±4.5)	51.4 (±2.5)	**<.01**
Turbidity (NTU)	77 (±6.8)	88.3 (±12)	.52
TSS (mg/L)	0.4 (±0)	0.6 (±0)	**.02**
Velocity (cm/s)	2.7 (±1.1)	2.4 (±0.9)	.66
SRP (mg/L)	0.51 (±0.21)	1.51 (±0.71)	**.02**
TP (mg/L)	0.83 (±0.42)	1.15 (±0.05)	**.04**
$N\text{-}NO_3^-$ (mg/L)	0.55 (±0.2)	0.47 (±0.04)	.06
$N\text{-}NO_2^-$ (mg/L)	0.12 (±0.12)	0.12 (±0.07)	.99
$N\text{-}NH_4^+$ (mg/L)	0.17 (±0.24)	0.79 (±0.77)	.39
DIN (mg/L)	0.84 (±0.17)	1.38 (±0.73)	**<.01**
BOD_5 (mg/L)	8.0 (±1.0)	78.0 (±2.5)	**.04**
COD (mg/L)	17 (±0)	84.5 (±6.36)	**.04**
Bacterial density (cells/ml)	2.9×10^9 ($\pm 2.7E \times 10^8$)	6.2×10^9 ($\pm 6.1 \times 10^8$)	**<.01**
Chlorophyll *a* (μg/L)	4.43 (±4.28)	4.12 (±1.89)	.89

Note. Significant differences are highlighted in bold. ANOVA = analysis of variance; BOD_5 = biochemical oxygen demand; COD = chemical oxygen demand; DIN = dissolved inorganic nitrogen; DO = dissolved oxygen; HI = higher urban impact; LI = lower urban impact; NTU = nephelometric turbidity units; SRP = soluble reactive phosphorous; TP = total phosphorous; TSS = total suspended solids.

from T0 to T2, remained similar through T3 and T4 and decreased at T5. In the HI site, chlorophyll *a* remained low until T3 to later increase at T4 and T5.

Both bacterial growth rate and chlorophyll *a* concentration at both sites fitted significantly to logistic curve ($p < .01$). X_{50} for bacterial growth and chlorophyll *a* were lower at the LI site (0.6 and 1.4 hr, respectively) when compared with the HI site (19.3 and 18.6 hr).

3.2.1 | ETS activity

At both sites, the ETS activity measured in the biofilm increased from T0 to T4 and decreased at T5 (Figure 1). Except for T0, ETS activity was significantly higher at the biofilm from the LI site.

Concordantly with the bacterial and algal increments, ETS activity at both sites fitted to a logistic function ($p < .01$), where X_{50} was 1.12 hr at the LI site and 17.28 hr at the HI site (Table 3).

3.2.2 | Algal composition

Algal density was higher at the LI site than the HI site (Table 2); the maximum algal density at the LI site was at T2, whereas at the HI site, the maximum density was measured at T3 (Figure 2).

The dominant taxonomic group at both sites was the diatoms (Figure 2), representing from 36% to 89% of the overall composition at the LI site and from 56% to 84% at the HI site. It is noticeable that there was a large percentage of empty diatom frustules at both sites at T0 (>68%), but at T5, the proportion of empty frustules was not significant at either site. The increase in diatom density during the experiment at both sites fits a logistic function with similar rates and X_{50} values (Tables 3, $p < .05$).

At the LI site, euglenophyte abundance reached up to a maximum of 61% (at T1), whereas cyanobacteria gradually increased their abundance from T1 to T5 up to a 22% maximum relative abundance (Figure 2). At the HI site, euglenophytes reached their highest relative abundance value at T2 (25%), whereas cyanobacteria were present throughout the experiment with their highest value of 22% at T5 (Figure 2).

3.2.3 | Relationships between biological and physical and chemical variables

ETS activity was significantly related to both algal and bacterial biomass at both sites, although only at the HI site there was a correlation between bacterial and algal biomasses. Chlorophyll *a* was positively correlated to the total algal density only at the LI site ($p < .05$; Table S1).

The total biomass of the biofilm, the ETS activity, the concentration of chlorophyll *a*, and the proportion of cyanobacteria were positively correlated to water velocity. These biological variables also correlated negatively with the amount of TSS in the water ($p < .05$; Table S2).

The development of both diatoms and euglenophytes was positively correlated to temperature and dissolved oxygen, while being negatively affected by conductivity; TSS also correlated negatively

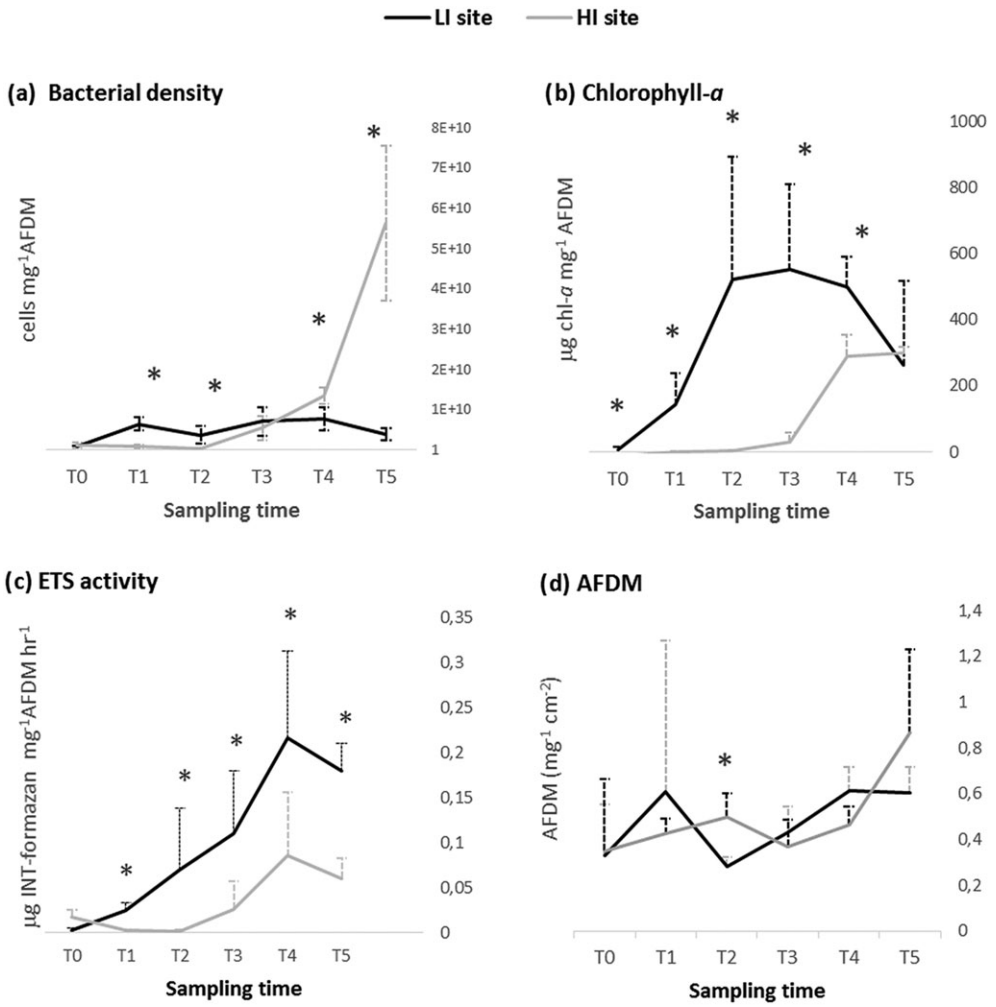

FIGURE 1 (a) Bacterial density, (b) chlorophyll *a*, (c) electron transfer system (ETS) activity, and (d) ash-free dry mass (AFDM) variations in the biofilm colonizing the glass tiles at the lower urban impact (LI) site (black line) and upper urban impact (HI) site (grey line). Whiskers represent *SD* values, and asterisks (*) mark significant differences between sites at a specific date (two-way repeated-measures analysis of variance, $p < .05$)

the diatom density. The density of chlorophytes was only negatively correlated to conductivity ($p < .05$; Table S2).

4 | DISCUSSION

Biofilm development in both streams was measurable within a few hours; in the stream with a higher water quality (lower nutrients and organic matter concentration and higher dissolved oxygen), the colonization dynamics showed two increments of bacterial biomass linked with an increase of algal biomass. In the highly impacted urban stream, with a lower water quality, biofilm development was slower and with a simultaneous increase of bacterial and algal biomasses, related with a lower ETS activity.

4.1 | Colonization patterns

The high bacterial densities in both sites are within the reported values for the area, both in the biofilm and in the stream water (Cochero et al., 2017, 2013). After only 30 min, bacteria had already settled on the glass substrates at both streams, and the chlorophyll *a* concentration was already measurable (7.7 µg/mg AFDM) at the less polluted site, while being almost zero at the highly polluted site. This fast initial colonization at the stream with a higher water quality is in agreement with the results found for previous research conducted in an oligotrophic stream (Pohlon, Marxsen, & Küsel, 2010), where a bacterial density of 4×10^4 DAPI-stained cells/cm^2 and an initial algal density of 10 live cells/cm^2 were measured within the first hour of the colonization process.

As reported in previous studies, the initial algal colonization was characterized by a dominance of diatoms following a logistic increase in density and with a large proportion of empty diatom frustules in the first 24 hr, which diminished by the end of the colonization period (Stevenson, 1984, 1983).

Although there are studies that have investigated the combined succession of bacteria and algae during the initial colonization phase in streams (Battin et al., 2003; Besemer et al., 2009; Sobczak & Burton, 1996; Szabó et al., 2008), only a few have been conducted in streams exposed to urban pollution (Duong et al., 2008; Merbt et al., 2011). Our results show that the dynamics of the biofilm colonization in the less polluted urban stream was similar to that reported for biofilms exposed to water from unpolluted streams (Battin et al., 2003; Stock & Ward, 1989), where two stages of growth in the bacterial assemblage are linked to a period of algal development. After the first algae

TABLE 2 Two-way RM-ANOVA results (factors: time, site, and Time * Site) for the bacterial density, chlorophyll *a*, ETS activity, and the density of algal groups identified

Biological variable		Factor			Post hoc (SNK test)	
		Time	Site	Time * Site	Site	Time
Bacterial density (cells/mg AFDM)	*F*	16.49	12.55	26.11		
	p value	**<.001**	**<.001**	**<.001**	LI	T0 < T1 = T3 = T4 > T2 = T5
	η^2	.81	.41	.81	HI	T0 = T1 = T2 = T3 = T4 < T5
Chlorophyll *a* (mg chlorophyll *a* mg^{-1}AFDM)	*F*	7.52	21.32	4.44		
	p value	**<.001**	**<.001**	**.012**	LI	T0 < T2 = T5 < T1 = T3 = T4
	η^2	.59	.44	.42	HI	T0 = T1 = T2 = T3 < T4 = T5
AFDM (mg/cm^2)	*F*	1.89	0.04	0.78		
	p value	.13	.84	**.04**	LI	T0 = T1 = T2 = T3 = T4 = T5
	η^2	.28	.02	.14	HI	T0 = T1 = T2 = T3 = T4 < T5
ETS activity (μg INT mg^{-1} AFDM hr^{-1})	*F*	13.15	19.30	1.88		
	p value	**<.001**	**<.001**	**.132**	LI	T0 < T1 < T2 = T3 < T4 = T5
	η^2	.76	.43	.27	HI	T0 = T1 = T2 = T3 = T4 = T5
Chlorophytes (cells/mg AFDM)	*F*	9.422	.436	3.134		
	p value	**<.001**	.515	**.026**	LI	T0 = T1 = T2 = T3 = T4 = T5
	η^2	.663	.018	.395	HI	T0 = T1 = T2 = T5 < T3 = T4
Diatoms (cells/mg AFDM)	*F*	3.119	13.156	2.479		
	p value	**.026**	**<.001**	**.060**	LI	T2 > T0 = T1 = T3 = T4 = T5
	η^2	.394	.354	.341	HI	T3 = T4 > T0 = T1 = T2 = T5
Euglenophytes (cells/mg AFDM)	*F*	2.594	13.025	2.371		
	p value	.052	**<.001**	**.040**	LI	T2 > T0 = T1 = T3 = T4 = T5
	η^2	.351	.352	.331	HI	T0 = T1 = T2 = T3 = T4 = T5
Cyanophytes (cells/mg AFDM)	*F*	10.490	12.719	7.424		
	p value	**<.001**	**.002**	**<.001**	LI	T5 > T0 = T1 = T2 = T3 = T4
	η^2	.686	.346	.607	HI	T0 = T1 = T2 = T3 = T4 = T5
Total algal density (cells/mg AFDM)	*F*	3.117	19.272	3.207		
	p value	**.026**	**<.001**	**.023**	LI	T2 > T0 = T1 = T3 = T4 = T5
	η^2	.394	.445	.401	HI	T0 = T1 = T2 = T3 = T4 = T5

Note. Significant differences are highlighted in bold; post hoc test results are also shown for the time factor by site (SNK test) and partial η^2 as a measure of the effect size. AFDM = ash-free dry mass; ETS = electron transfer system; INT = 2–3 tetrazolium chloride; RM-ANOVA = repeated-measures analysis of variance; SNK = Student–Newman–Keuls.

TABLE 3 Parameters for the logistic model of each variable of the biofilm at each site

Site	Variable	Parameters (logistic model)			
		K	*r*	X_{50}	*p*
LI	Bacterial density (cells/mg AFDM)	22.50	−0.88	0.61	.01*
	ETS activity (μg INT mg^{-1} AFDM hr^{-1})	−2.39	−1.63	1.13	.01*
	Chlorophyll *a* (mg^{-1} AFDM)	6.10	−2.05	1.42	.00*
	AFDM (mg/cm^2)				.33
	Chlorophytes (cells/mg AFDM)				.80
	Diatoms (cells/mg AFDM)	12.24	−1.38	0.96	.02*
	Euglenophytes (cells/mg AFDM)				.09
	Cyanophytes (cells/mg AFDM)				.62
	Total algal density (cells/mg AFDM)	12.97	−0.95	0.66	.05*
HI	Bacterial density (cells/mg AFDM)	24.16	−27.87	19.32	.00*
	ETS activity (μg INT mg^{-1} AFDM hr^{-1})	−2.78	−24.93	17.28	.00*
	Chlorophyll a (mg^{-1} AFDM)	5.79	−26.95	18.68	.00*
	AFDM (mg/cm^2)	−0.67	−0.33	0.22	.05*
	Chlorophytes (cells/mg AFDM)				.36
	Diatoms (cells/mg AFDM)	10.76	−1.32	0.92	.06*
	Euglenophytes (cells/mg AFDM)	9.51	−15.91	11.03	.00*
	Cyanophytes (cells/mg AFDM)				.33
	Total algal density (cells/mg AFDM)				.13

Note. Asterisks mark significant values. AFDM = ash-free dry mass; ETS = electron transfer system; *K* = Ln of the carrying capacity; *r* = growth rate; X_{50} = time (h) at which the variable reaches 50% of the carrying capacity.

settle, they create favourable conditions for bacteria to colonize further in between algal cells by their excretion of photosynthetically derived nutrients, mainly dissolved organic carbon (Pohlon et al., 2010; Sobczak, 1996). The initial bacterial colonization started within the first 3 hr, with an increment in algal biomass between 3 and 6 hr and a second bacterial development 24 hr after the initial colonization.

Contrary to our expectations, however, this process was slower in the biofilm from the heavily polluted site, with higher concentrations of phosphorous, nitrogen, and organic matter. The colonization process had a lower metabolic activity per cell, and the bacterial and algal accruals were simultaneous. This synchronous development of the bacterial and algal assemblages suggests that it develops relying on

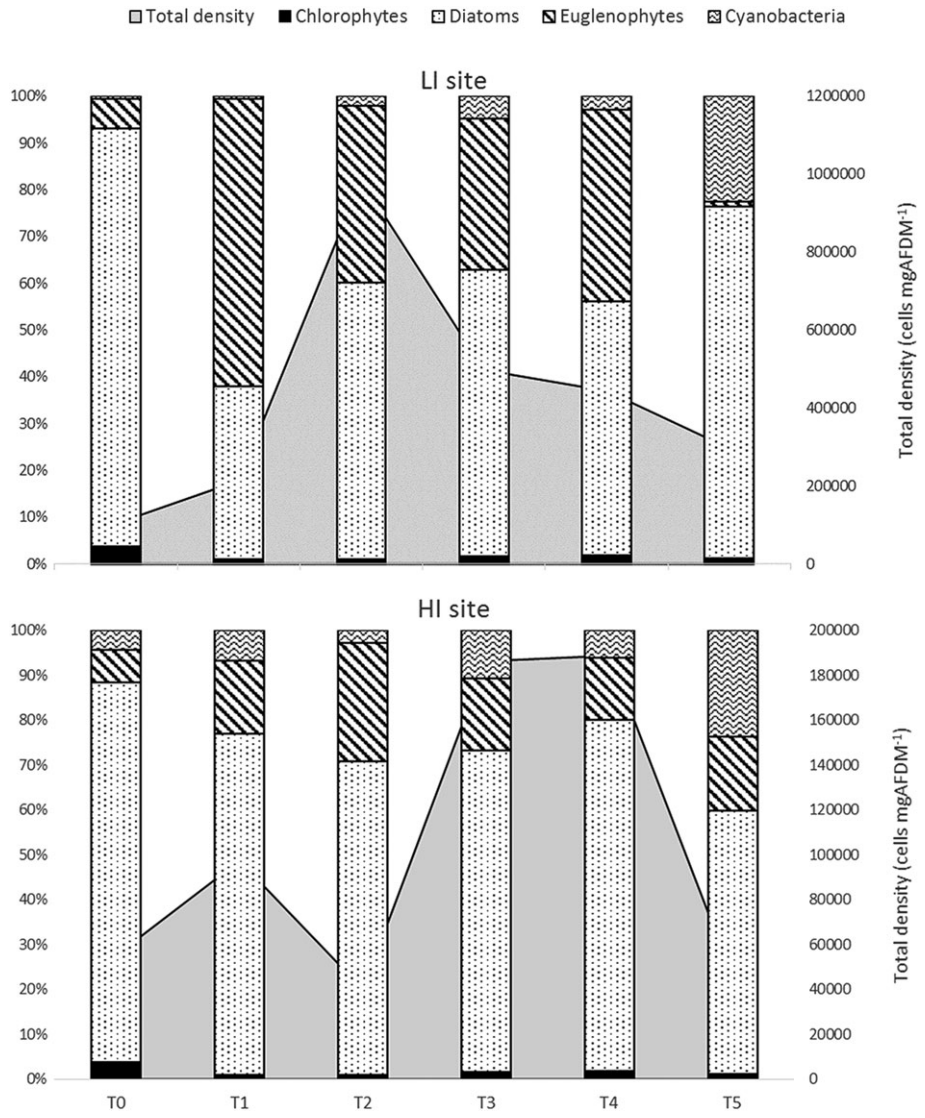

FIGURE 2 Relative abundance of the higher taxonomic algal groups in the biofilm colonizing the glass tiles at the lower urban impact (LI) site and upper urban impact (HI) site, during all sampling dates (left axis, bars) and the total density (right axis, shaded area). AFDM = ash-free dry mass

allochthonous nutrients rather than algal-derived sources (Findlay & Howe, 2012).

4.2 | Water quality and biofilm development

Although the total biomass of the biofilm (measured as AFDM) was not a significant indicator of anthropogenic impact, chlorophyll *a* and ETS activity were lower at the highly polluted site. Even though the bacterial density at this latter site was higher at the end of the experiment, its metabolic activity remained lower than at the less polluted site. Because the metabolic activity of stream biofilms is tightly linked to the characteristics of the stream water (Sabater et al., 2007), this low metabolic activity in the heavily polluted stream could be the consequence of the presence of toxicants resulting from the urban activities (i.e., heavy metals and pesticides; Bernot et al., 2010; Paul & Meyer, 2001; Walsh et al., 2005). Urban stream pollution is usually associated not only with nutrient and organic matter increments, which could enhance biofilm development significantly, but also with an increasing amount of toxicants (i.e., heavy metals and pesticides) as part of the "urban stream syndrome" (Walsh et al., 2005); results from previous studies for the study sites have reported the presence of where heavy metals in the stream with the lower water quality (Armendáriz et al., 2017; Cochero et al., 2017; López van Oosterom et al., 2015; Ronco et al., 2001; Sierra et al., 2013). Contrary to our initial expectation, our results show that biofilm development was actually delayed in the heavily polluted site; this supports the idea that the biofilm rapidly reflects the overall water quality (Navarro, Guasch, & Sabater, 2001; Sabater et al., 2007), rather than just the nutrient concentrations of the water.

Chlorophyll *a* values at the end of the experiment were higher than those reported in fully grown biofilms in similar nutrient-rich streams with a lesser urban impact (Cochero et al., 2017; Sierra et al., 2013), although chlorophyll is expected to diminish in mature biofilms during the loss phase (Stevenson et al., 1996). Nutrient concentrations and organic matter did not correlate significantly with any of the biological variables measured, likely because these physical and chemical parameters were relatively constant throughout the experiment. On the other hand, water velocity fluctuated along with

multiple variables measured in the biofilm, such as the total biomass and the metabolic (ETS) activity. The role of current velocity as a determinant factor in stream biofilm development and structure is well known (e.g., Battin et al., 2003; Besemer et al., 2007; Horner & Welch, 1981). However, at low-to-intermediate flow velocities (e.g., less than 30 cm/s; Passy & Larson, 2011), it can be secondary to nutrient availability when determining biofilm structure, due to the strong nutrient dependence of sensitive forms.

Even though the effect of herbivores was not analysed in this experiment, their effect on biofilm development is largely recognized (e.g., Haglund & Hillebrand, 2005; Mulholland & Elwood, 2011; Rosemond, 1993); in nutrient-richer streams, there is usually a larger proportion of tolerant invertebrates (Ocon, Oosterom, Mu oz, & Rodrigues-Capítulo, 2013; Roberts, Sabater, & Beardall, 2004), which can consume the settling algae and bacteria very rapidly. If a larger abundance of tolerant taxa was present in the highly polluted site during the experiment, their consumption could also contribute to a slower development of the biofilm in that site.

Bacterial and algal colonization rates vary depending on water chemistry and can be measured in such a short period of time as the one used in this experiment. Therefore, we believe that biofilm early development should be further explored as a possible tool for water quality management.

5 | CONCLUSIONS

The early colonization dynamics in the stream with a higher water quality consisted of two increments of bacterial biomass linked with an increase of algal biomass, whereas in the urban stream with a lower water quality, biofilm development was slower and consisted of a simultaneous increase of bacteria and algae.

The study focused only on the early colonization pattern, rather than on the dynamics of the fully developed biofilm. Our results show that this initial development reflects the overall conditions of the stream water, by integrating the effects of multiple stressors present in the environment. The colonization process, considering not only the bacterial and algal biomasses when fully mature but also their growth rates at early stages, should be explored further to examine their possible applications in water monitoring of urban streams.

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REFERENCES

Ancion, P.-Y., Lear, G., Dopheide, A., & Lewis, G. D. (2013). Metal concentrations in stream biofilm and sediments and their potential to explain biofilm microbial community structure. *Environmental Pollution*, *173*, 117–124. https://doi.org/10.1016/J.ENVPOL.2012.10.012

American Public Health Association, 1999. Standard methods for the examination of water and wastewater. Stand. Methods Exam. Water Wastewater 1496. doi:ISBN 9780875532356

Armendáriz, L. C., Cortese, B., Rodriguez, M., & Rodrigues Capítulo, A. (2017). Ecosystem services of runoff marshes in urban lowland basins: Proposals for their management and conservation. *Knowledge and Management of Aquatic Ecosystems*, *32*. https://doi.org/10.1051/kmae/2017022

Battin, T. J., Kaplan, L. A., Newbold, J. D., Cheng, X., & Hansen, C. (2003). Effects of current velocity on the nascent architecture of stream microbial biofilms effects of current velocity on the nascent architecture of stream microbial biofilms. *Applied and Environmental Microbiology*, *69*, 5443–5452. https://doi.org/10.1128/AEM.69.9.5443

Benjamini, Y., & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics*. https://doi.org/10.2307/2674075

Bernot, M. J., Sobota, D. J., Hall, R. O., Mulholland, P. J., Dodds, W. K., Webster, J. R., … Wilson, K. (2010). Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology*, *55*, 1874–1890. https://doi.org/10.1111/j.1365-2427.2010.02422.x

Besemer, K., Singer, G., Hödl, I., & Battin, T. J. (2009). Bacterial community composition of stream biofilms in spatially variable-flow environments. *Applied and Environmental Microbiology*, *75*, 7189–7195. https://doi.org/10.1128/AEM.01284-09

Besemer, K., Singer, G., Limberger, R., Chlup, A.-K., Hochedlinger, G., Hodl, I., … Battin, T. J. (2007). Biophysical controls on community succession in stream biofilms. *Applied and Environmental Microbiology*, *73*, 4966–4974. https://doi.org/10.1128/AEM.00588-07

Biggs, B.J., 2000. New Zealand periphyton guideline: Detecting, monitoring and managing enrichment of streams. Detect. Monit. Manag. Enrich. Streams 1–124.

Blenkinsopp, S. A., & Lock, M. A. (1990). The measurement of electron transport system activity in river biofilms. *Water Research*, *24*, 441–445. https://doi.org/10.1016/0043-1354(90)90226-V

Chen, J. (2015). *Effects on biofilm growth due to the interactions between different levels of dissolved organic carbon and nutrients*. University of British Columbia.

Cochero, J., Licursi, M., & Gómez, N. (2015). Changes in the epipelic diatom assemblage in nutrient rich streams due to the variations of simultaneous stressors. *Limnologica*, *51*, 15–23. https://doi.org/10.1016/j.limno.2014.10.004

Cochero, J., Licursi, M., & Gómez, N. (2017). Effects of pulse and press additions of salt on biofilms of nutrient-rich streams. *Science Total Environment*, *579*, 1496–1503. https://doi.org/10.1016/j.scitotenv.2016.11.152

Cochero, J., Romaní, A., & Gómez, N. (2013). Delayed response of microbial epipelic biofilm to nutrient addition in a Pampean stream. *Aquatic Microbial Ecology*, *69*, 145–155. https://doi.org/10.3354/ame01630

Cochran, W. G. (1951). Testing a linear relation among variances. *Biometrics*, *7*, 17–32. https://doi.org/10.2307/3001601

Coundoul, F., Bonometti, T., Graba, M., Sauvage, S., Sanchez Pérez, J. M., & Moulin, F. Y. (2015). Role of local flow conditions in river biofilm colonization and early growth. *River Research and Applications*, *31*, 350–367. https://doi.org/10.1002/rra.2746

Díaz Villanueva, V., Font, J., Schwartz, T., & Romani, A. M. (2011). Biofilm formation at warming temperature: Acceleration of microbial colonization and microbial interactive effects. *Biofouling*, *27*, 59–71. https://doi.org/10.1080/08927014.2010.538841

Duong, T. T., Morin, S., Herlory, O., Feurtet-Mazel, A., Coste, M., & Boudou, A. (2008). Seasonal effects of cadmium accumulation in periphytic diatom communities of freshwater biofilms. *Aquatic Toxicology*, *90*, 19–28. https://doi.org/10.1016/j.aquatox.2008.07.012

Feijoó, C. S., & Lombardo, R. J. (2007). Baseline water quality and macrophyte assemblages in Pampean streams: A regional approach. *Water Research*, *41*, 1399–1410. https://doi.org/10.1016/j.watres.2006.08.026

Ferragut, C., & de Campos Bicudo, D. (2012). Effect of N and P enrichment on periphytic algal community succession in a tropical oligotrophic reservoir. *Limnology*, *13*, 131–141. https://doi.org/10.1007/s10201-011-0367-z

Findlay, S., Howe, K., 2012. Bacterial–algal relationships in streams of the Hubbard Brook Experimental Forest Author(s): Stuart Findlay, Karin Howe and Dominique Fontvielle Reviewed work(s): Published by: Ecological Society of America Stable URL: http://www.jstor.org/stable 74, 2326–2336.

Giorgi, A., Feijoó, C., & Tell, G. (2005). Primary producers in a Pampean stream: Temporal variation and structuring role. *Biodiversity and Conservation*, *14*, 1699–1718. https://doi.org/10.1007/s10531-004-0694-z

Haglund, A. L., & Hillebrand, H. (2005). The effect of grazing and nutrient supply on periphyton associated bacteria. *FEMS Microbiology Ecology*, *52*, 31–41. https://doi.org/10.1016/j.femsec.2004.10.003

Horner, R. R., & Welch, E. B. (1981). Stream periphyton development in relation to current velocity and nutrients. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*, 449–457. https://doi.org/10.1139/f81-062

Ivorra, N., Bremer, S., Guasch, H., Kraak, M. H. S., & Admiraal, W. (2000). Differences in the sensitivity of benthic microalgae to Zn and Cd regarding biofilm development and exposure history. *Environmental Toxicology and Chemistry*, *19*, 1332. https://doi.org/10.1897/1551-5028(2000)019%3C1332:DITSOB%3E2.3.CO;2

Jan Stevenson, R., & Glover, R. (1993). Effects of algal density and current on ion transport through periphyton communities. *Limnology and Oceanography*, *38*, 1276–1281. https://doi.org/10.4319/lo.1993.38.6.1276

Johnson, R. E., Tuchman, N. C., & Peterson, C. G. (1997). Changes in the vertical microdistribution of diatoms within a developing periphyton mat. *J. North Am. Benthol. Soc.*, *16*, 503–519. https://doi.org/10.2307/1468140

Kelly, M. G. (2001). Use of similarity measures for quality control of benthic diatom samples. *Water Research*, *35*, 2784–2788. https://doi.org/10.1016/S0043-1354(00)00554-6

Kobayashi, Y., Kim, C., Yoshimizu, C., Kohzu, A., Tayasu, I., & Nagata, T. (2009). Longitudinal changes in bacterial community composition in river epilithic biofilms: Influence of nutrients and organic matter. *Aquatic Microbial Ecology*, *54*, 135–152. https://doi.org/10.3354/ame01258

Lee, J. W., Nam, J. H., Kim, Y. H., Lee, K. H., & Lee, D. H. (2008). Bacterial communities in the initial stage of marine biofilm formation on artificial surfaces. *Journal of Microbiology*, *46*, 174–182. https://doi.org/10.1007/s12275-008-0032-3

Leff, L., Van Gray, J.B., Martí, E., Merbt, S.N., Romaní, A.M., 2016. Aquatic biofilms and biogeochemical processes. in: *Aquatic biofilms: Ecology, water quality and wastewater treatment*. Caister Academic Press, pp. 89–108. doi:10.21775/9781910190173.05

Liu, Y., Zhang, W., & Xu, H. (2014). Colonization dynamics of periphytic diatoms in coastal waters of the Yellow Sea, northern China. *Acta Oceanologica Sinica*, *33*, 160–165. https://doi.org/10.1007/s13131-014-0466-x

López van Oosterom, M. V., Ocon, C., Armendariz, L. C., & Rodrigues Capitulo, A. (2015). Structural and functional responses of the oligochaete and aeolosomatid assemblage in lowland streams: A one-way-pollution-modelled ecosystem. *Journal of Limnology*, *74*. https://doi.org/10.4081/jlimnol.2015.1142

Merbt, S.N., Auguet, J., Casamayor, E.O., 2011. *Biofilm recovery in a wastewater treatment plant-influenced stream and spatial segregation of ammonia-oxidizing microbial populations*. 56, 1054–1064. doi:https://doi.org/10.4319/lo.2011.56.3.1054, 56, 1054–1064

Mulholland, P.J., Elwood, J.W., 2011. Top-down and bottom-up control of stream periphyton: Effects of nutrients and herbivores Author(s): Amy D. Rosemond, Patrick J. Mulholland, Jerry W. Elwood Published by: Ecological Society of America Stable URL: http://www.jstor.org/stable/1940. *America (NY). 74*, 1264–1280.

Navarro, E., Guasch, H., & Sabater, S. (2001). Use of microbentbic algal communities in ecotoxicological tests for the assessment of water quality: The Ter river case study. 1, 41–48.

Ocon, C., Oosterom, M. V. L., Mu oz, M. I., & Rodrigues-Capítulo, A. (2013). Macroinvertebrate trophic responses to nutrient addition in a temperate stream in South America. *Fundamental and Applied Limnology/Archiv. für Hydrobiologie*, *182*, 17–30. https://doi.org/10.1127/1863-9135/2013/0382

Passy, S.I., Larson, C.A., 2011. Succession in stream biofilms is an environmentally driven gradient of stress tolerance 414–424. doi:https://doi.org/10.1007/s00248-011-9879-7

Paul, M. J., & Meyer, J. L. (2001). Streams in the urban landscape. *Annual Review of Ecology and Systematics*, *32*, 333–365. https://doi.org/10.1146/annurev.ecolsys.32.081501.114040

Pohlon, E., Marxsen, J., & Küsel, K. (2010). Pioneering bacterial and algal communities and potential extracellular enzyme activities of stream biofilms. *FEMS Microbiology Ecology*, *71*, 364–373. https://doi.org/10.1111/j.1574-6941.2009.00817.x

Porter, K. G., & Feig, Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora1. *Limnology and Oceanography*, *25*, 943–948. https://doi.org/10.4319/lo.1980.25.5.0943

Roberts, S., Sabater, S., & Beardall, J. (2004). Benthic microalgal colonization in streams of differing riparian cover and light availability. *Journal of Phycology*, *40*, 1004–1012. https://doi.org/10.1111/j.1529-8817.2004.03-333.x

Romaní, A. M., Giorgi, A., Acu a, V., & Sabater, S. (2004). The influence of substratum type and nutrient supply on biofilm organic matter utilization in streams. *Limnology and Oceanography*, *49*, 1713–1721. https://doi.org/10.4319/lo.2004.49.5.1713

Romaní, A. M., Guasch, H., & Balaguer, M. D. (2016). *Aquatic biofilms: Ecology, water quality and wastewater treatment*. Girona: Caister Academic Press. https://doi.org/10.21775/9781910190173

Ronco, A., Camilión, C., & Manassero, M. (2001). Geochemistry of heavy metals in bottom sediments from streams of the western coast of the Rio de la Plata estuary, Argentina. *Environmental Geochemistry and Health*, *23*, 89–103. https://doi.org/10.1023/A:1010956531415

Rose, F. L., & Cushing, C. E. (1970). Periphyton: Autoradiography of Zinc-65 adsorption. *Science*, *168*(80), 576–577. https://doi.org/10.1126/science.168.3931.576

Rosemond, A. D. (1993). Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. *Oecologia*, *94*, 585–594. https://doi.org/10.1007/BF00566976

Sabater, S., Guasch, H., Ricart, M., Romaní, A., Vidal, G., Klünder, C., & Schmitt-Jansen, M. (2007). Monitoring the effect of chemicals on biological communities. The biofilm as an interface. *Analytical and Bioanalytical Chemistry*, *387*, 1425–1434. https://doi.org/10.1007/s00216-006-1051-8

Scott, J. T., Back, J., Taylor, J. M., & King, R. S. (2008). Does nutrient enrichment decouple algal–bacterial production in periphyton? *J. North Am. Benthol. Soc.*, *27*, 332–344. https://doi.org/10.1899/07-108.1

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*, 591–611. https://doi.org/10.1093/biomet/52.3-4.591

Sierra, M.V., Gómez, N., Marano, A., Di Siervi, M., 2013. Caracterización funcional y estructural del biofilm epipélico en relación al aumento de la urbanización en un arroyo de la Llanura Pampeana (Argentina). *Ecología Austral 23*, 108–118.

Sobczak, W. V. (1996). Epilithic bacterial responses to variations in algal biomass and labile dissolved organic carbon during biofilm colonization. *J. North Am. Benthol. Soc.*, *15*, 143–154. https://doi.org/10.2307/1467944

Sobczak, W. V., & Burton, T. M. (1996). Epilithic bacterial and algal colonization in a stream run, riffle, and pool: A test of biomass covariation. *Hydrobiologia*, *332*, 159–166. https://doi.org/10.1007/BF00031921

Stevenson, R. J. (1983). Effects of current and conditions simulating autogenically changing microhabitats on benthic diatom immigration. *Ecology*, *64*, 1514–1524. https://doi.org/10.2307/1937506

Stevenson, R. J. (1984). How currents on different sides of substrates in streams affect mechanisms of benthic algal accumulation. *International Review der Gesamten Hydrobiology Und Hydrogr.*, *69*, 241–262. https://doi.org/10.1002/iroh.19840690214

Stevenson, R.J., Bothwell, M.L. (Max L.., Lowe, R.L., 1996. *Algal ecology: Freshwater benthic ecosystems*. Academic Press.

Stock, M. S., & Ward, A. K. (1989). Establishment of a bedrock epilithic community in a small stream: Microbial (algal and bacterial) metabolism and physical structure. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*, 1874–1883. https://doi.org/10.1139/f89-236

Szabó, K. É., Makk, J., Kiss, K. T., Eiler, A., Ács, É., Tóth, B., … Bertilsson, S. (2008). Sequential colonization by river periphyton analysed by microscopy and molecular fingerprinting. *Freshwater Biology*, *53*, 1359–1371. https://doi.org/10.1111/j.1365-2427.2008.01967.x

Tien, C. J., Wu, W. H., Chuang, T. L., & Chen, C. S. (2009). Development of river biofilms on artificial substrates and their potential for biomonitoring water quality. *Chemosphere*, *76*, 1288–1295. https://doi.org/10.1016/j.chemosphere.2009.06.013

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., & Morgan, R. P. (2005). The urban stream syndrome: Current knowledge and the search for a cure. *J. North Am. Benthol. Soc.*, *24*, 706–723. https://doi.org/10.1899/04-028.1

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