RESEARCH ARTICLE

Interactive effects of discharge reduction and fine sediments on stream biofilm metabolism

Ana Victoria Pérez-Calpe [ID] [1]*, **Aitor Larrañaga** [1], **Daniel von Schiller** [ID] [2], **Arturo Elosegi** [ID] [1]

1 Department of Plant Biology and Ecology, University of the Basque Country (UPV/EHU), Bilbao, Spain,
2 Department of Evolutionary Biology, Ecology and Environmental Science, University of Barcelona, Barcelona, Spain

* anavictoria.perez@ehu.eus

Abstract

Discharge reduction, as caused by water diversion for hydropower, and fine sediments deposition, are prevalent stressors that may affect multiple ecosystem functions in streams. Periphytic biofilms play a key role in stream ecosystem functioning and are potentially affected by these stressors and their interaction. We experimentally assessed the interactive effects of discharge and fine sediments on biofilm metabolism in artificial indoor channels using a factorial split-plot design with two explanatory variables: water discharge (20, 39, 62, 141 and 174 $cm^3 s^{-1}$) and fine sediments (no sediment or 1100 $mg L^{-1}$ of sediments). We incubated artificial tiles for 25 days in an unpolluted stream to allow biofilm colonization, and then placed them into the indoor channels for acclimation for 18 days. Subsequently, we manipulated water discharge and fine sediments and, after 17 days, we measured biofilm chlorophyll-a concentration and metabolism. Water velocity (range, 0.5 to 3.0 $cm s^{-1}$) and sediment deposition (range, 6.1 to 16.6 $mg cm^{-2}$) increased with discharge, the latter showing that the effect of increased inputs prevailed over sloughing. In the no-sediment treatments, discharge did not affect biofilm metabolism, but reduced chlorophyll-a. Sediments, probably as a consequence of nutrients released, promoted metabolism of biofilm and chlorophyll-a, which became independent of water discharge. Our results indicate that pulses of fine sediments can promote biofilm algal biomass and metabolism, but show interactive effects with discharge. Although discharge reduction can affect the abundance of basal resources for food webs, its complex interactions with fine sediments make it difficult to forecast the extent and direction of the changes.

Introduction

Stream ecosystems are affected by multiple anthropogenic stressors [1]. Among these, damming and water diversion stand out as detrimental activities for stream biological communities [2–4] and ecosystem functioning [5]. The number of water diversion schemes is rising in response to escalating water demands [6,7]. Stream discharge reduction caused by water diversion reduces the width of the wet channel [8], affects water chemistry [9], alters transport and deposition of sediments [10], and impacts multiple ecosystem functions such as leaf litter breakdown [11–13], nutrient retention [14] and stream metabolism [15].

Fine sediments are also considered an important stressor and often included among the most prevalent pollutants in streams [16]. High inputs of fine sediments can occur as a consequence of natural processes [17,18], but are often exacerbated by human activities such as forestry or agriculture [19]. Suspended fine sediments reduce the light that reaches the stream bottom [20], can abrade biofilms [21], damage organisms gills [22,23] and interact with dissolved nutrients and other pollutants [24–26]. Additionally, fine sediments tend to settle on stream beds, where they cause siltation [27], reduce the supply of oxygen and light to the bottom and damage primary producers [28], macroinvertebrates [29] and fish [30].

Periphytic biofilms (hereafter biofilms) consist of complex communities of microorganisms that include bacteria, algae, fungi and protozoa, and live attached to rocks or other surfaces [31]. They play a key role in stream ecosystem functioning [32] and are an important food resource for invertebrates and fish [33]. The abundance, composition and activity of biofilms is regulated by factors such as light, current, nutrients and grazing [34]. Therefore, biofilms are highly sensitive to environmental changes and can be potentially affected by multiple anthropogenic stressors [35,36].

The response of biofilm to discharge reduction and fine sediments deposition is complex. In fast-flowing streams, water diversion reduces water velocity and shear stress, thus promoting biofilm growth and activity [37–39]. When natural discharge is low, further reductions can detrimentally affect biofilm by reducing nutrient exchange [34,40]. Besides, water diversion reduces the amount of fine sediments entering at reach, as most sediments are diverted with the water. At the same time, however, discharge reduction promotes the deposition rate of those sediments in the reach as a consequence of reduced water velocity, thus impacting benthic biota [41]. The final outcome will depend on factors such as water velocity, the characteristics of fine sediments or the type of organisms. Biofilms can be damaged by sediments via abrasion or burial [21], but can also benefit from fine sediments as a source of nutrients, especially phosphorus [42]. These complex interactions call for controlled experiments to examine how discharge and fine sediments affect biofilm structure and functioning.Here, we experimentally assessed the interactive effects of discharge and fine sediments on biofilm algal biomass and metabolism. The experiment was carried out in artificial stream channels, which were subject to a gradient of water discharge in presence or absence of fine sediments. We tested the following three hypotheses:

i. Algal biomass and metabolism will be lower at low discharge because of limited nutrient exchange.

ii. Addition of fine sediments will reduce algal biomass and metabolism because it hinders algal attachment and limits light availability.

iii. Water discharge and fine sediments will interact, algal biomass and metabolism being lowest in the channels with sediments and lowest water discharge.

Materials and methods

Experimental design

The indoor artificial stream facility of the University of the Basque Country (Leioa, Spain) consists of 30 indoor methacrylate channels (length-width-depth: 200-15-20 cm) grouped in six blocks of five channels. From a primary tank, filtered (1 mm mesh) rainwater is fed to six 200-L tanks (hereafter 'block tanks') that supply water to each block of five channels. In each block, water was recirculated by a pump and run as a closed system (Fig 1). Discharge can be adjusted for each channel individually. In each channel, water depth was set at 3.4 ± 0.1 cm
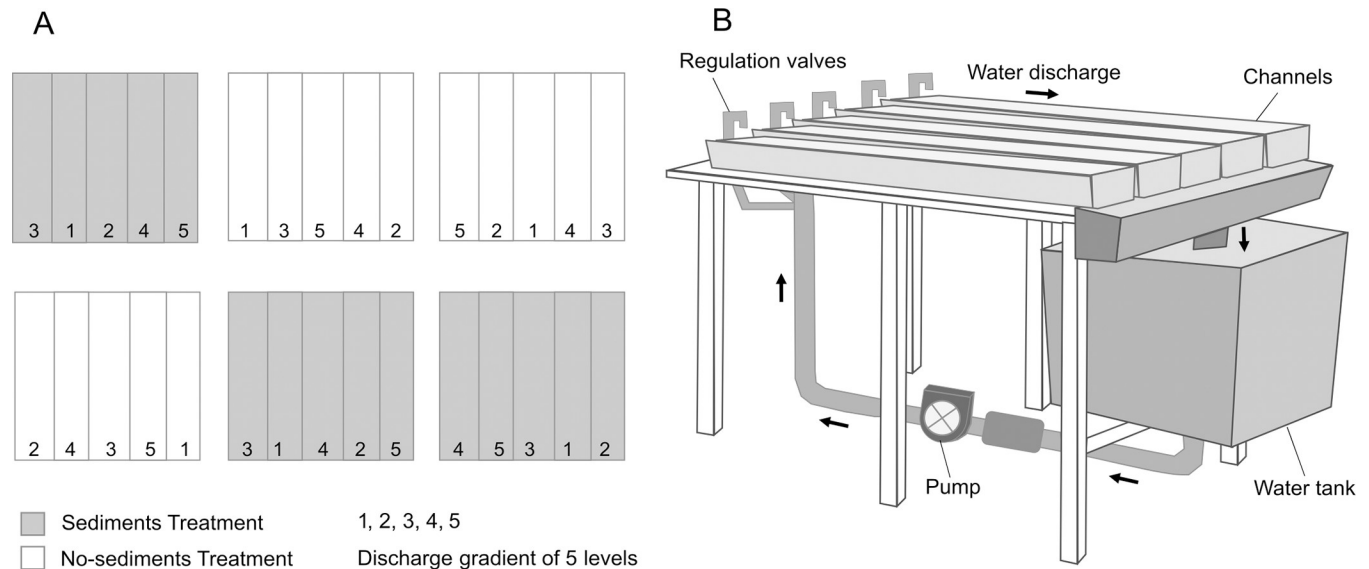
Fig 1. Experimental setup. (A) Schematic drawing of channel blocks setup and experimental levels of the factors discharge and fine sediments. (B) Detail of a channel block.

(mean ± SE) by means of a small dam at the lowermost end. LED lights (36 W 65000k, Aquael, Poland) with a 12/12 light/dark cycle and an intensity of 27.1 ± 1.0 µmol m^{-2} s^{-1} provided lighting. The bottom of the channels was covered by a 2-cm layer of commercial aquarium gravel of 8–16 mm average size (Karlie Flamingo, Germany). We used marble tiles (33.6 cm^2 of surface area) as standard biofilm substrata. To allow biofilm colonization, these tiles were incubated in an unpolluted and oligotrophic reach of the Urumea River (N Iberian Peninsula; 43˚ 12'40.6" N, 1˚54'06.2" W), attached on plastic trays with no protection from grazers and tied to the river bottom for 25 days.

After the incubation period, tiles were collected, transported to the artificial stream facility, and randomly distributed across the channels (12 tiles per channel). Additionally, to ensure biofilm development, we scraped several cobbles at the Urumea River, and the slush produced was split and uniformly distributed among the artificial channels. To allow biofilm acclimation, discharge was kept constant (discharge = 85.2 ± 2.48 cm^3 s^{-1}; water velocity = 1.7 ± 0.06 cm s^{-1}) in all the channels for 18 days. To avoid nutrient depletion, the water in each block tank was renewed every week during the acclimation and the experimental period.

After the acclimation period, we started a factorial experiment with a split-plot design, which lasted for 17 days. Water discharge was set in five levels (19.8 ± 1.3, 39.0 ± 3.8, 62.4 ± 5.1, 141.3 ± 8.6 and 173.7 ± 7.1 cm^3 s^{-1}) and fine sediments in two levels: no fine sediments and 1100 mg L^{-1} of fine sediments, a concentration that is commonly found in the Urumea River during floods [43], as well as during forestry operations. Water discharge levels were randomly assigned to each channel within each block. Water discharge was measured on days 1, 10 and 14, from the time to fill a container at the lower end of each channel; water velocity was estimated from the ratio between water discharge and average channel width and depth, measured with a ruler along each channel. Water discharge and velocity remained constant during the experiment. Sediments were added to three randomly selected block tanks in 2 pulses (days 1 and 10). Sediments were distributed through the water pump and circulated through the treatment channels, where they settled rapidly, turbidity returning to background values a few hours after the addition.

The fine sediments used in this experiment were obtained from the recently emptied Eno-bieta Reservoir (43°12'50.5" N 1°47'31.0" W), located in the Urumea basin upstream from the biofilm collection point. These sediments were dried, ground and sieved through 200 μm. Their organic matter content was $21.0 \pm 0.11\%$ and their C:N molar ratio 49:1. Sediment leach-ate was characterized in the laboratory by mixing 2.0 ± 0.01 gr (n = 5) of dried sediments with 0.2 L of deionized water and kept at 20°C with light (120 μmol cm^{-2}) and with a constant movement (70 rpm in an orbital shaker Multitron II, INFORS HT, Bottmingen, Switzerland) for 24 h to mimic channel conditions. This leachate had a content of 0.093 ± 0.003 mg g^{-1} of ammonium (N-NH$_4^+$), 0.012 ± 0.0002 mg g^{-1} of nitrate (N-NO$_3^-$), 0.36 ± 0.003 mg g^{-1} of total dissolved nitrogen (TDN), 3.96 ± 0.001 mg g^{-1} of dissolved organic carbon (DOC) and 0.008 ± 0.001 mg g^{-1} of soluble reactive phosphorus (SRP). Thus, sediment leachate contrib-uted 20.9 ± 0.2 mg of N (sum of NH$_4^+$ and NO$_3^-$) and 1.7 ± 0.2 mg of P (from SRP) to each 5-channel block per sediment pulse. These quantities correspond to concentrations of 1.06 ± 0.03 mg L^{-1} of N and 0.08 ± 0.01 mg L^{-1} of P. See next section for analytical methods.

Water quality

Water quality was analysed six times: on the first day of the experimental period, before and after renewing water (days 7 and 14) and on the last day of the experiment. We measured tem-perature, pH, electrical conductivity and dissolved oxygen concentration and saturation in the block tanks with a hand-held probe (Multi 3630 IDS, WTW, Germany). Water samples were collected from the block tanks, filtered through 0.7-μm pore size glass fibre filter (Millipore GF/F, Ireland) and stored at -20°C until analysis. The concentration of nitrate (N-NO$_3^-$), sul-phate (SO$_4^{2-}$) and chloride (Cl$^-$), was measured by capillary electrophoresis (Agilent CE, Agi-lent Technologies, USA) [44]. The concentration of soluble reactive phosphorus (SRP) (molybdate method [45]) and ammonium (N-NH$_4^+$) (salicylate method [46]) were determined colorimetrically on a UV-1800 UV–vis Spectrophotometer (Shimadzu, Shimadzu Corpora-tion, Kyoto, Japan). Total dissolved nitrogen (TDN) and total dissolved organic carbon (DOC) were determined by catalytic oxidation on a Shimadzu TOC-L$_{CSH}$ analyser coupled to a TNM-L unit (Shimadzu, Shimadzu Corporation, Kyoto, Japan).

Response variables

At the end of the experiment biofilm variables were measured on the tiles. We measured chlo-rophyll-a (chl-a) concentration as a proxy of algal biomass by in vivo fluorimetry (Bentho-Torch, bbe Moldaenke Gmbh, Germany) in six randomly selected tiles in each channel. BenthoTorch is a non-intrusive tool that quantifies the total algal biomass through the stimula-tion of cell pigments and the reading of red fluorescent light emitted [47]. We summed the val-ues of chlorophyll for green algae, cyanobacteria and diatoms, thus calculating total chl-a concentration [48].

Biofilm metabolism was estimated in 0.21-L glass chambers hermetically closed without recirculation. We enclosed one tile per chamber (6 random replicates per channel, 3 incubated in light conditions, 3 in dark conditions), filled them with water from the corresponding tank and incubated them for 1 h submersed in the channel. After incubation, we measured dis-solved oxygen using a portable fibre optic oxygen meter with a syringe-like probe (Microsen-sor NTH-PSt7 on Microx 4, PreSens, Germany) by inserting its needle through the hermetic membrane. Metabolism metrics (i.e., gross primary production GPP, community respiration CR and net community metabolism NCM) were calculated following Acuña et al. [49]. We also calculated gross primary production per unit of algal biomass (i.e., GPP/Chl-a) as a proxy of metabolic efficiency [50,51].

Finally, we quantified the total amount of sediments deposited in the channels throughout the experiment by washing all the substrate within a container and measuring the turbidity (NTU) of the homogenised solution with a hand-held turbidimeter (AQ4500 Aquafast IV, Thermo Scientific Orion, USA). Turbidity (NTU) was converted to sediment concentration (g L^{-1}) using an empirical equation (*sediment concentration* = 0.0036 * *turbidity* + 0.0971, r^2 = 0.99, p < 0.001) established in the laboratory by measuring of turbidity of several solutions with a known concentration of the fine sediments (0, 0.1, 0.2, 0.5, 1, 2 and 4 gr L^{-1}).

Data analysis

We analysed the differences among treatments in chlorophyll-a concentration (μg cm^{-2}) and biofilm metabolism metrics (GPP, CR, NCM; mg O_2 h^{-1} and GPP/Chl-a; mg O_2 mg chl-a^{-1} h^{-1}) using Linear Mixed-Effects Models (LMEM) with REML (function lme, in R package nlme [52]). Sediments (Yes vs. No) was set as fixed factor, Water discharge as a continuous explanatory variable, and blocks and channels nested within blocks, as random factors. We included a variance structure (varIdent in the nlme function) in the models to account for the variance heterogeneity between levels of the factor Sediments. The significance of each source of variation was tested by means of ANOVA. Chlorophyll-a concentration and GPP/Chl-a were log-transformed to meet homoscedasticity. All analyses were performed using R software, v. 3.4.0 [53].

Results

Water quality

The values of water temperature (22.4 ± 0.2˚C) and pH (7.5 ± 0.1) were stable during the experiment, with no differences among levels of the treatments (Table 1). A small, but significant, increase in dissolved oxygen concentration and saturation was observed in the sediment treatment (8.7 ± 0.1 mg O_2 L^{-1} and 100.3 ± 0.8%) with respect to the no-sediment treatment (8.5 ± 0.1 mg O_2 L^{-1} and 98.4 ± 1.3%) (Table 1). Electrical conductivity was lower in the sediment treatment (73.9 ± 2.8 μS cm^{-2}) than in the no-sediment treatment (104.7 ± 14.3 μS cm^{-2})

Table 1. Water quality values for each sediment treatment during the experiment.

Variable	(unit)	No-sediment	Sediment	F-value	P-value
T	(˚C)	22.3 ± 0.2	22.3 ± 0.2	0.22	0.644
pH	-	7.5 ± 0.1	7.5 ± 0.1	0.70	0.410
DO	(%)	99.2 ± 1.3	100.9 ± 0.7	7.02	**0.013**
DO	(mg L^{-1})	8.6 ± 0.1	8.7 ± 0.1	8.60	**0.007**
EC	(μS cm^{-1})	105.8 ± 13.0	75.2 ± 2.8	20.30	**<0.001**
SRP	(μg P L^{-1})	18.1 ± 2.9	18.4 ± 2.9	0.08	0.783
NO_3^-	(mg N L^{-1})	0.6 ± 0.06	0.4 ± 0.08	8.61	**0.007**
NH_4^+	(μg N L^{-1})	22.2 ± 0.7	13.2 ± 0.2	2.82	0.104
TDN	(mg N L^{-1})	1.8 ± 0.1	1.6 ± 0.1	3.90	0.058
DOC	(mg C L^{-1})	3.2 ± 0.2	3.6 ± 0.3	3.38	0.076
Cl^-	(mg L^{-1})	5.7 ± 0.1	5.7 ± 0.1	0.07	0.796
SO_4^{2-}	(mg L^{-1})	5.6 ± 0.4	3.9 ± 0.1	17.30	**<0.001**

T, temperature; DO, dissolved oxygen concentration and saturation; EC, electrical conductivity; SRP, soluble reactive phosphorus; NH_4^+, ammonium; NO_3^-, nitrate; TDN, total dissolved nitrogen; DOC, dissolved organic carbon; Cl^-, chloride and SO_4^{2-}, sulphate. Values shown are mean ± SE. P-values and F-values were obtained by ANOVA. Degrees of freedom are 1, 29 for all variables. Significant P-values are shown in bold.

([Table 1](#)), suggesting potential sorption of dissolved ions by added sediments. Most measured solutes did not differ between sediment and no-sediment treatments ([Table 1](#)). However, NO_3^- and SO_4^{-2} were significantly lower in the sediment treatment than in the no-sediment treatment (1.6 ± 0.3 and 2.7 ± 0.3 mg L^{-1} vs. 4.0 ± 0.1 and 5.3 ± 0.4 mg L^{-1}, respectively), whereas DOC concentration was significantly higher in the sediment treatment (3.9 ± 0.2 vs. 3.2 ± 0.2 mg L^{-1}). Water renewal affected water quality: temperature, pH, dissolved oxygen, SO_4^{2-} and DOC decreased (an average of 0.5˚C, 0.5, 7.7% and 0.5 mg O$_2$ L^{-1}, 1.1 mg L^{-1}, 1.5 mg C L^{-1}, respectively) and, SRP and NO_3^- increased (an average of 17.5 µg P L^{-1} and 0.2 mg N L^{-1}, respectively) The rest of parameters showed no changes with water ([S1 Dataset](#)). Note that these changes were caused by water renewal, not by sediments, which were added to the corresponding treatments in days 1 and 10.

Hydraulics and sediments

Water discharge correlated with water velocity ([Table 2](#)), which ranged from 0.5 to 3.0 cm s^{-1} ([Fig 2A](#)). Sediments and discharge had a significant effect on deposited sediments ([Table 2](#), [Fig 2B](#)). The amount of sediments deposited in the sediment treatment (6.1 to 16.6 mg cm^{-2}) was higher than in the no-sediment treatment (1.7 to 2.3 mg cm^{-2}). Discharge affected sediment deposition only in the sediment treatment. Sediments deposited increased with discharge, as a consequence of higher mass of sediments entering the channels, since all channels in a block received the same concentration but different mass of sediments.

Table 2. Results of the Linear Mixed-Effects Models (LMEM) with water discharge as continuous explanatory variable, sediments as fixed factor and chlorophyll-a (Chl-a), gross primary production (GPP), community respiration (CR), net community metabolism (NCM), gross primary production per unit of chlorophyll-a (GPP/Chl-a), water velocity and deposited sediments as response variables.

Variable		d.f.	F-value	P-value	Sign of coef.
Water velocity	Discharge	1, 22	254.22	<**0.001**	+
	Sediments	1, 4	4.08	0.113	
	Discharge × Sediments	1, 22	0.24	0.629	
Deposited sediments	Discharge	1, 4	401.11	<**0.001**	+
	Sediments	1, 22	8.15	**0.009**	+
	Discharge × Sediments	1, 22	60.39	<**0.001**	+
Chl-a	Discharge	1, 22	3.79	0.064	
	Sediments	1, 4	11.97	**0.026**	+
	Discharge × Sediments	1, 22	6.67	**0.017**	+
GPP	Discharge	1, 22	0.21	0.647	
	Sediments	1, 4	43.40	**0.003**	+
	Discharge × Sediments	1, 22	0.85	0.366	
CR	Discharge	1, 22	2.31	0.143	
	Sediments	1, 4	20.26	**0.011**	+
	Discharge × Sediments	1, 22	0.21	0.647	
NCM	Discharge	1, 22	1.09	0.306	
	Sediments	1, 4	40.93	**0.003**	+
	Discharge × Sediments	1, 22	0.47	0.499	
GPP/Chl-a	Discharge	1, 22	1.65	0.211	
	Sediments	1, 4	0.85	0.408	
	Discharge × Sediments	1, 22	5.90	**0.023**	-

P-values, F-values and degrees of freedom (d.f.) were obtained by ANOVA. Significant P-values of main and interaction effects are shown in bold. The sign of the coefficient is indicated when the source of the variation is significant.
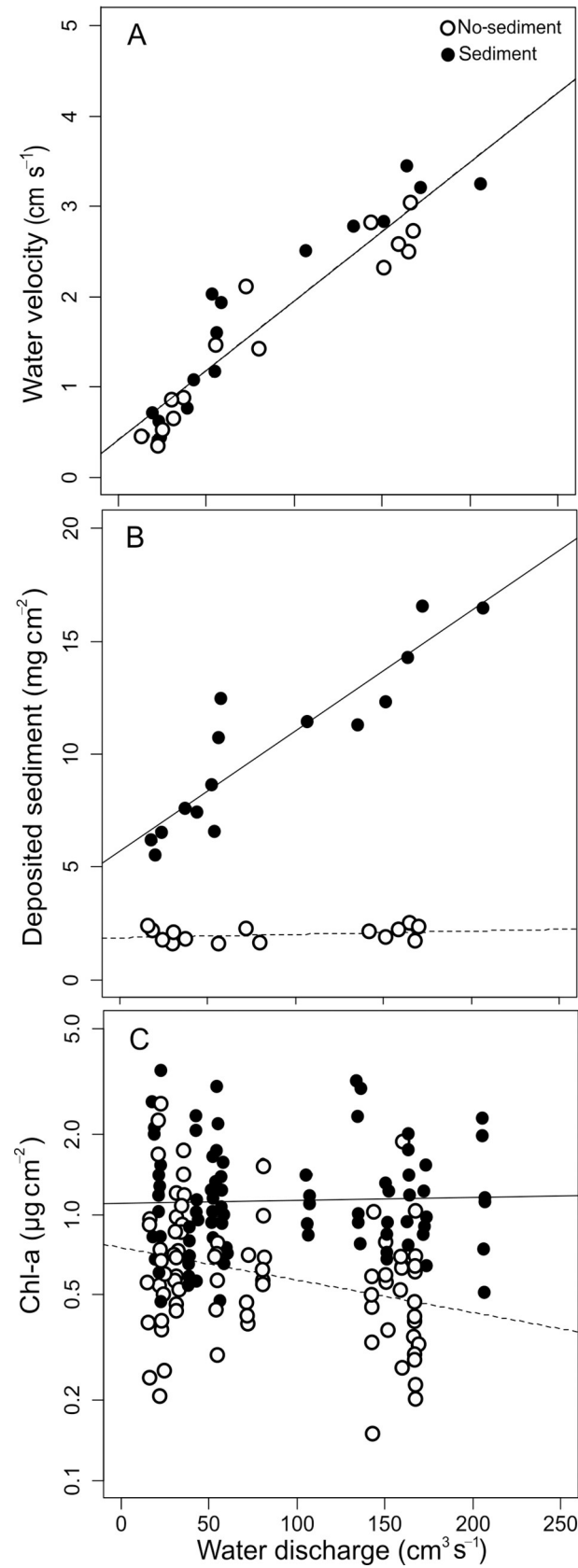
https://doi.org/10.1371/journal.pone.0246719.t002

Fig 2. (A) Relationship between water discharge and velocity, (B) amount of deposited sediments in the channels and (C) chlorophyll-a concentration on tiles. Filled and empty dots correspond to channels with and without added sediments, respectively. Continuous and broken trend lines are built with the LMEM coefficients for channels with and without added sediments, respectively. When the interaction term is not significant a single line is shown. Note that in panels A and B each dot corresponds to a channel, and in panel C to a single tile.

Biofilm

For the relationship between chl-a and discharge the LMEM showed a significant change of slope from the no-sediment to the sediment treatment (Table 2, p = 0.017). In the no-sediment treatment, chl-a concentration decreased significantly when water discharge increased, from 0.8 ± 0.3 μg cm^{-2} in the channels with lowest discharge to 0.5 ± 0.1 μg cm^{-2} in the channels with the highest discharge (Fig 2C). In the sediment treatment, on the other hand, chl-a concentration was higher (1.3 ± 0.1 μg cm^{-2}) and constant along the discharge range. These results indicate that the sediments promoted biofilm chl-a and counteracted the negative effects of high discharge (Fig 2, Table 2).

The biofilm metabolism metrics did not change with discharge but increased significantly with the addition of fine sediments (Table 2, Fig 3). GPP rose from 41.6 ± 6.3 mg O$_2$ h^{-1}m^{-2} in the no-sediment treatments to 92.1 ± 11.9 mg O$_2$ h^{-1} m^{-2} in the sediment treatments, CR from 13.2 ± 2.0 to 19.2 ± 4.5 mg O$_2$ h^{-1} m^{-2}, and NCM from 28.4 ± 5.6 to 72.9 ± 12.2 mg O$_2$ h^{-1} m^{-2}. The interaction between water discharge and the addition of fine sediments was not statistically significant for any metabolism metric. The GPP/Chl-a ratio showed no significant main effects of discharge or sediments; however, the significant interaction between both factors indicated that in the absence of sediments, increasing discharge resulted in a higher metabolic efficiency (p = 0.023, Table 2, Fig 4).

Discussion

Our experiment assessed the interactive effects of water discharge and fine sediments on biofilm metabolism. We expected both, discharge reduction and sediments, to exert negative individual effects, as well as an interaction effect of both stressors, but these predictions were not supported by our results. Contrary to our expectations, both discharge reduction and fine sediments promoted biofilm biomass, their interaction resulting in unchanged biomass across all discharge levels in the sediment treatments. On the other hand, metabolism was positively affected by fine sediments, but unaffected by discharge.

According to our first hypothesis, we expected discharge reduction to negatively affect biofilm biomass and metabolism because of limited nutrient exchange. On the contrary, we observed a weak increase in chl-a and no changes in metabolism metrics with varying water discharge. The literature shows contrasting effects of water discharge on biofilms. Some studies reported no response for algal biomass [54–56] as well as for metabolism [14,54], whereas others showed that algal biomass decreased both above and below optimum velocities, a fact that would be explained by shear stress at high velocities, by nutrient limitation at low ones. This is the type of response reported by Biggs and Stokseth [57] where the algal biomass peaked at a velocity of 30 cm s^{-1}. Similarly, in a flume experiment, Hondzo and Wang [58] reported that shear stress reduced biomass and photosynthetic activity above 15 cm s^{-1}, whereas Liu and Lau [38] reported optimum biofilm growth at 1.5 cm s^{-1}. The discrepancies among studies are large and probably caused by differences in experimental conditions. Our water velocities were in the lower range of those so far mentioned, with a range between 0.5 and 3 cm s^{-1}, but even so, we found an inverse relationship between chlorophyll and velocity. This effect could be explained by the fact that our biofilm was dominated by loose algal filaments, which are the
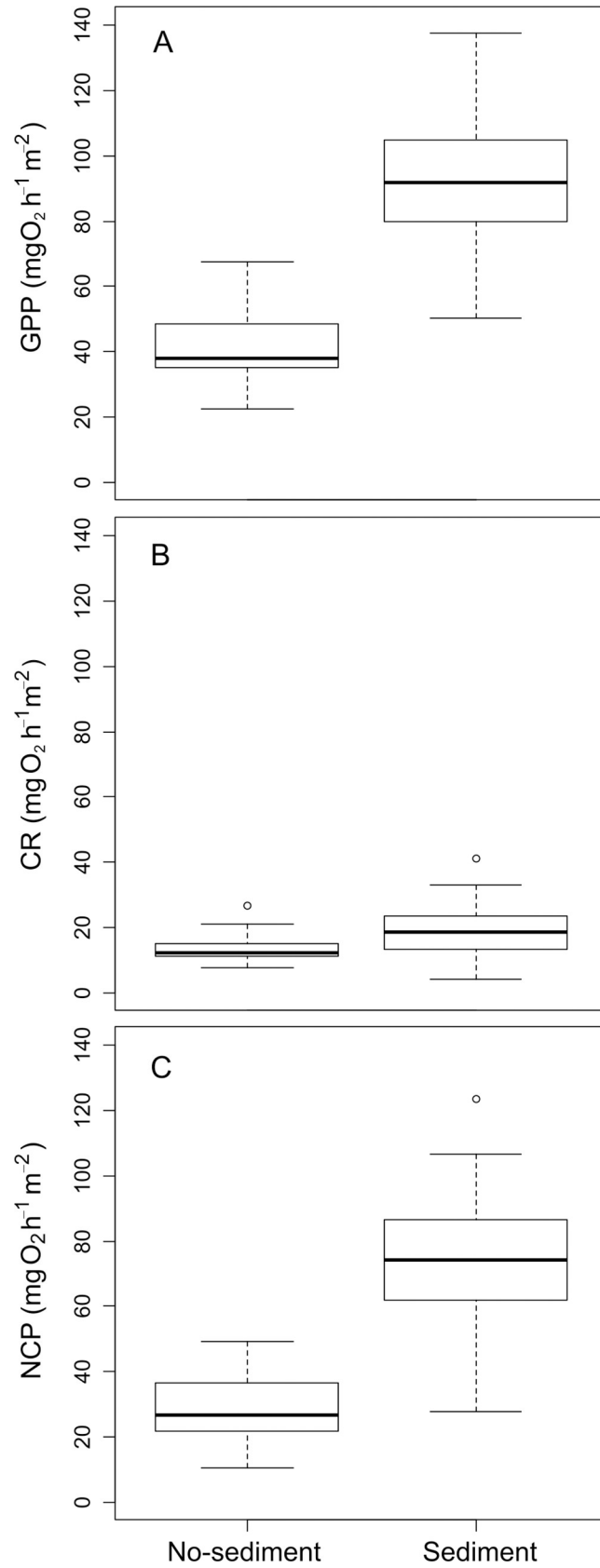
Fig 3. Differences in metabolism (A: Gross Primary Production, B: Community respiration and C: Net Community Metabolism) between channels with and without added sediments. The boxes display first and third quartiles, thick lines are medians, whiskers are range, and open circles are outliers.

https://doi.org/10.1371/journal.pone.0246719.g003

growth forms dominant at low flow velocities [59]. The long and loose filaments in our experiment seemed especially prone to sloughing.

Our second hypothesis predicted that fine sediments would reduce biofilm biomass and metabolic activity, but we observed the opposite effect. The literature shows contrasting effects of fine sediments on biofilm. Several studies showed [41,60–62] fine sediments to reduce biofilm biomass and metabolism, but some [65,66] reported increased biofilm, which was explained as a consequence of shifts in the dominant growth forms towards those (e.g., motile algae) more resistant to physical disturbance. We did not study algal composition of biofilms in our experimental channels, but unlike biomass, which showed clear differences between sediment and no-sediment treatments, by the end of the experiment we did not see any visual difference in the appearance of biofilm. Alternatively, the effects of sediments on biofilm could be caused by nutrients, as their leachates had high concentrations of N and P, important nutrients for algae [42,63,64]. This fertilisation effect would, nevertheless, not depend strictly on the amount of sediments deposited in each channel, since the sediments were added into the block tank and dissolved nutrients from leachates would be distributed across all the channels with
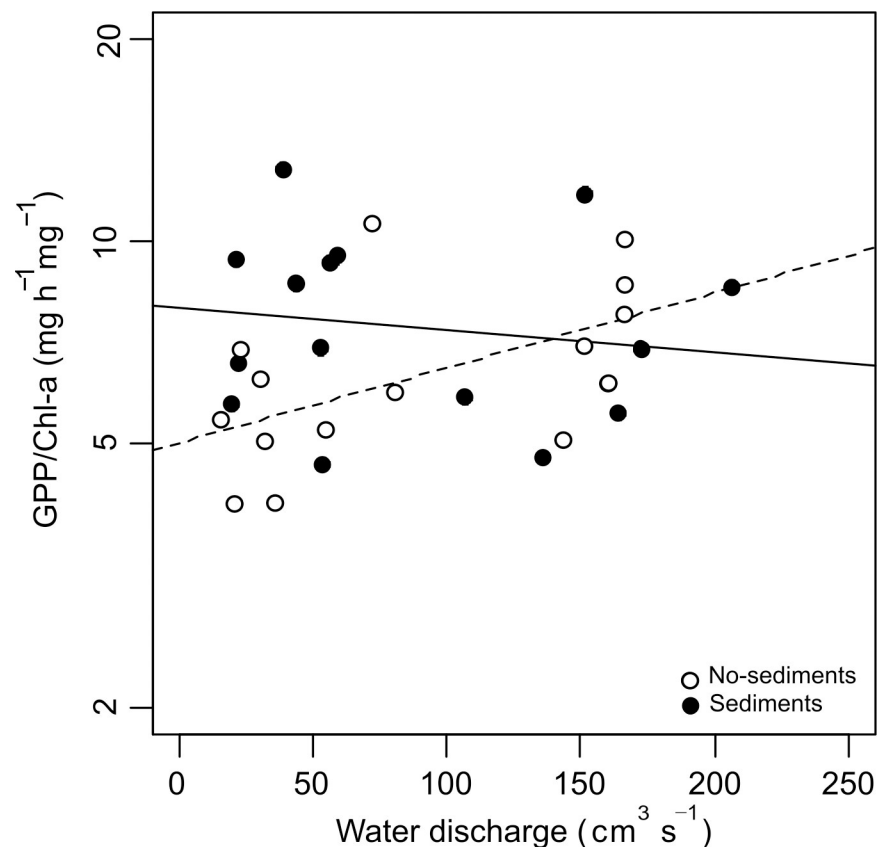

Fig 4. Gross primary production per unit biofilm biomass (GPP/Chl-a). Filled and empty dots correspond to channels with and without added sediments, respectively. The interaction between discharge and sediments is significant. Continuous and broken trend lines made with the LMEM coefficients for channels with and without added sediments, respectively.

https://doi.org/10.1371/journal.pone.0246719.g004

the same concentration. The leaching of nutrients will of course depend on the type of sediments. The one used in our experiment, coming from a reservoir, could be unusually high in nutrients, but many other sediments also will act as fertilisers, as they are often linked to agricultural practices [17,19].

Our third hypothesis predicted algal biomass and metabolism to be lowest in the sediment treatments with lowest discharge. However, although our results showed a significant interaction, it consisted of sediments eliminating the effect of discharge on biomass. This response is consistent with a fertilizing effect that is stronger than the sloughing effect, at least under the experimental conditions. The effects of nutrients and flow velocity on algal biomass tend to interact in complex ways, flow velocity promoting turbulence and the diffusion of nutrients into biofilms [40], until shear stress increases so as to produce algal sloughing. In a recent study, Baattrup-Pedersen et al. [65] measured the metabolic and biomass response of periphytic biofilm to fine sediments, nutrients and discharge reduction and, although they found negative effects of sediments on chl-a concentration and GPP, they concluded that after 1 week, nutrient enrichment to some extent mitigated these negative effects. The metabolic efficiency for GPP showed a significant response to the interaction of discharge and sediments. At high discharge, it was similar in sediment and no-sediment treatments, whereas at low discharge it was higher in the sediment treatment. This difference could be probably explained by a greater stimulatory effect of sediment on GPP than on chl-a. At low discharges and sediment treatment, the positive effect of sediment would compensated any sediment-shading effect, whereas, with no-sediment treatment, the low efficiency with a high chl-a would result from self-shading of algal biomass [63,66]. Then, higher discharges would reduce biomass, but the remaining algae would be more efficient in absence of sediments. However, with higher sediment deposition the stimulatory effect would be overtaken by the sediment-shading effect that would reduce the metabolic efficiency [66] matching to the no-sediment metabolic efficiency.

In conclusion, discharge reduction and sediment inputs can have interactive effects on stream biofilm biomass and metabolism. Nonetheless, the direction and magnitude of the responses may be strongly site-specific and difficult to forecast, as they likely depend on the range of water velocities, on the composition of the fine sediments, as well as on the composition and biomass of benthic biofilms.

Supporting information

S1 Dataset. Excel spreadsheet containing the underlying numerical data for all figures and tables.
(XLSX)

Acknowledgments

Author Contributions

Conceptualization: Aitor Larrañaga, Daniel von Schiller, Arturo Elosegi.

Formal analysis: Ana Victoria Pérez-Calpe, Aitor Larrañaga.

Funding acquisition: Aitor Larrañaga, Daniel von Schiller, Arturo Elosegi.

Investigation: Ana Victoria Pérez-Calpe, Aitor Larrañaga, Daniel von Schiller, Arturo Elosegi.

Methodology: Aitor Larrañaga, Daniel von Schiller, Arturo Elosegi.

Resources: Arturo Elosegi.

Supervision: Aitor Larrañaga, Daniel von Schiller, Arturo Elosegi.

Visualization: Ana Victoria Pérez-Calpe, Aitor Larrañaga.

Writing – original draft: Ana Victoria Pérez-Calpe, Aitor Larrañaga, Daniel von Schiller, Arturo Elosegi.

Writing – review & editing: Ana Victoria Pérez-Calpe, Aitor Larrañaga, Daniel von Schiller, Arturo Elosegi.

References

1. Sabater S, Elosegi A, Ludwig R. Multiple stressors in river ecosystems: Status, Impacts ans Prospects for the Future. Sabater S, Elosegi A, Ludwig R, editors. Elsevier; 2018. 406 p.

2. Bunn SE, Arthington AH. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environ Manage. 2002; 30(4):492–507. https://doi.org/10.1007/s00267-002-2737-0 PMID: 12481916

3. Martínez A, Larrañaga A, Basaguren A, Pérez J, Mendoza-Lera C, Pozo J. Stream regulation by small dams affects benthic macroinvertebrate communities: from structural changes to functional implications. Hydrobiologia [Internet]. 2013; 711(1):31–42. Available from: https://link.springer.com/article/10.1007/s10750-013-1459-z.

4. Benejam L, Saura-Mas S, Bardina M, Solà C, Munné A, García-Berthou E. Ecological impacts of small hydropower plants on headwater stream fish: from individual to community effects. Ecol Freshw Fish. 2016; 25:295–306.

5. Elosegi A, Sabater S. Effects of hydromorphological impacts on river ecosystem functioning: a review and suggestions for assessing ecological impacts. Hydrobiologia. 2013; 712(1):129–43.

6. Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, et al. Global threats to human water security and river biodiversity. Nature. 2010; 467(7315):555–61. https://doi.org/10.1038/nature09440 PMID: 20882010

7. Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PTJ, et al. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol Rev. 2019; 94(3):849–73. https://doi.org/10.1111/brv.12480 PMID: 30467930

8. McKay SF, King AJ. Potential ecological effects of water extraction in small, unregulated streams. River Res Appl. 2006;1032–7.

9. Dewson ZS, James ABW, Death RG. A review of the consequences of decreased flow for instream habitat and macroinvertebrates. J North Am Benthol Soc. 2007; 26(3):401–15.

10. Poff NL, Allan JD, Bain MB, Karr JR, Prestegaard KL, Richter BD, et al. The Natural Flow Regime. Bioscience [Internet]. 1997; 47(11):769–84. Available from: http://www.jstor.org/stable/1313099.

11. Mendoza–Lera C, Larrañaga A, Pérez J, Descals E, Martínez A, Moya O, et al. Headwater reservoirs weaken terrestrial-aquatic linkage by slowing leaf-litter processing in downstream regulated reaches. River Res Appl [Internet]. 2012 Jan 1; 28(1):13–22. Available from: https://doi.org/10.1002/rra.1434.

12. González JM, Mollá S, Roblas N, Descals E, Moya Ó, Casado C. Small dams decrease leaf litter breakdown rates in Mediterranean mountain streams. Hydrobiologia [Internet]. 2013; 712(1):117–28. Available from: https://link.springer.com/article/10.1007%2Fs10750-012-1144-7.

13. Arroita M, Aristi I, Díez J, Martinez M, Oyarzun G, Elosegi A. Impact of water abstraction on storage and breakdown of coarse organic matter in mountain streams. Sci Total Environ. 2015; 503–504:233–40. https://doi.org/10.1016/j.scitotenv.2014.06.124 PMID: 25039020

14. Arroita M, Flores L, Larrañaga A, Martínez A, Martínez-Santos M, Pereda O, et al. Water abstraction impacts stream ecosystem functioning via wetted-channel contraction. Freshw Biol. 2017; 62(2):243–57.

15. Aristi I, Arroita M, Larrañaga A, Ponsatí L, Sabater S, von Schiller D, et al. Flow regulation by dams affects ecosystem metabolism in Mediterranean rivers. Freshw Biol. 2014; 59(9):1816–29.

16. USEPA. Nutrient criteria technical guidance manual: lakes and reservoirs. Washington DC; 2000.

17. Wood PJ, Armitage PD. Biological effects of fine sediment in the lotic environment. Environ Manage. 1997; 21(2):203–17. https://doi.org/10.1007/s002679900019 PMID: 9008071

18. Buendia C, Vericat D, Batalla RJ, Gibbins CN. Temporal Dynamics of Sediment Transport and Transient In-channel Storage in a Highly Erodible Catchment. L Degrad Dev [Internet]. 2016 May 1; 27 (4):1045–63. Available from: https://doi.org/10.1002/ldr.2348.

19. Syvitski JPM, Vörösmarty CJ, Kettner AJ, Green P. Impact of Humans on the Flux of Terrestrial Sediment to the Global Coastal Ocean. Science (80-). 2005 Apr 15; 308(5720):376–80. https://doi.org/10.1126/science.1109454 PMID: 15831750

20. Davies-Colley RJ, Hickey CW, Quinn JM, Ryan PA. Effects of clay discharges in streams. Optical propierties and epilithon. Hydrobiologia. 1992; 248:215–34.

21. Francoeur SN, Biggs BJF. Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. Hydrobiologia. 2006; 561:59–69.

22. Kemp P, Sear D, Collins A, Naden P, Jones I. The impacts of fine sediment on riverine fish. Hydrol Process. 2011; 25(11):1800–21.

23. McKenzie M, Mathers KL, Wood PJ, England J, Foster I, Lawler D, et al. Potential physical effects of suspended fine sediment on lotic macroinvertebrates. Hydrobiologia [Internet]. 2019;0123456789. Available from: https://doi.org/10.1007/s10750-019-04131-x.

24. Chase JW, Benoy GA, Culp JM. Combined effects of nutrient enrichment and inorganic sedimentation on benthic biota in an experimental stream system. Water Qual Res J Canada. 2017; 52(3):151–65.

25. Magbanua FS, Townsend CR, Hageman KJ, Piggott JJ, Matthaei CD. Individual and combined effects of fine sediment and glyphosate herbicide on invertebrate drift and insect emergence: a stream mesocosm experiment. Freshw Sci [Internet]. 2016 Mar 1; 35(1):139–51. Available from: https://doi.org/10.1086/684363.

26. Wagenhoff A, Lange K, Townsend CR, Matthaei CD. Patterns of benthic algae and cyanobacteria along twin-stressor gradients of nutrients and fine sediment: A stream mesocosm experiment. Freshw Biol. 2013; 58(9):1849–63.

27. Rehg KJ, Packman AI, Ren J. Effects of suspended sediment characteristics and bed sediment transport on streambed clogging. Hydrol Process. 2005; 19(2):413–27.

28. Izagirre O, Serra A, Guasch H, Elosegi A. Effects of sediment deposition on periphytic biomass, photosynthetic activity and algal community structure. Sci Total Environ. 2009; 407(21):5694–700. https://doi.org/10.1016/j.scitotenv.2009.06.049 PMID: 19666189

29. Kaller MD, Hartman KJ. Evidence of a threshold level of fine sediment accumulation for altering benthic macroinvertebrate communities. Hydrobiologia [Internet]. 2004; 518(1):95–104. Available from: https://doi.org/10.1023/B:HYDR.0000025059.82197.35.

30. Bilotta GS, Brazier RE. Understanding the influence of suspended solids on water quality and aquatic biota. Water Res [Internet]. 2008; 42(12):2849–61. Available from: https://doi.org/10.1016/j.watres.2008.03.018 PMID: 18462772

31. Romaní AM, Guasch H, Balaguer MD. Aquatic Biofilms. Ecology, water quality and wastewater treatment. Norfolk, UK: Caister Academic Press; 2016. 244 p.

32. Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packmann AI. The ecology and biogeochemistry of stream biofilms. Nat Rev Microbiol. 2016; 14(4):251–63. https://doi.org/10.1038/nrmicro.2016.15 PMID: 26972916

33. Sabater S, Armengol J, Comas E, Sabater F, Urrizalqui I, Urrutia I. Algal biomass in a disturbed Atlantic river: water quality relationships and environmental implications. Sci Total Environ [Internet]. 2000; 263 (1):185–95. Available from: http://www.sciencedirect.com/science/article/pii/S0048969700007026.

34. Allan JD, Castillo MM. Stream Ecology. Structure and functioning of running waters. 2nd editio. Springer; 2007.

35. Sandin L, Solimini AG. Freshwater ecosystem structure–function relationships: from theory to application. Freshw Biol [Internet]. 2009 Oct 1; 54(10):2017–24. Available from: https://doi.org/10.1111/j.1365-2427.2009.02313.x.

36. Sabater S, Timoner X, Borrego C, Acuña V. Stream biofilm responses to flow intermittency: from cells to ecosystems. Front Environ Sci. 2016; 4:14.

37. Chester H, Norris R. Dams and flow in the Cotter River, Australia: Effects on instream trophic structure and benthic metabolism. Hydrobiologia. 2006; 572(1):275–86.

38. Lau YL, Liu D. Effect of flow rate on biofilm accumulation in open channels. Water Res [Internet]. 1993; 27(3):355–60. Available from: http://www.sciencedirect.com/science/article/pii/004313549390034F.

39. Ponsatí L, Acuña V, Aristi I, Arroita M, García-Berthou E, Von Schiller D, et al. Biofilm responses to flow regulation by dams in mediterranean rivers. River Res Appl. 2015; 22(August 2014):1085–95.

40. Horner RR, Welch EB. Stream Periphyton Development in Relation to Current Velocity and Nutrients. Can J Fish Aquat Sci. 1981; 38(4):449–57.

41. Matthaei CD, Piggott JJ, Townsend CR. Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. J Appl Ecol [Internet]. 2010 Apr 28 [cited 2011 Jul 30]; 47(3):639–49. Available from: http://doi.wiley.com/10.1111/j.1365-2664.2010.01809.x.

42. Perry WB, Stanford JA. Algal growth stimulus by phosphorus in Flathead Lake, Montana, sediments (Selenastrum capricornutum). Northwest Sci. 1982; 56(1):48–52.

43. Zabaleta A, Martínez M, Uriarte JA, Antigüedad I. Factors controlling suspended sediment yield during runoff events in small headwater catchments of the Basque Country. Catena. 2007; 71(1):179–90.

44. Environmental Protection Agency. Method 6500. Dissolved Inorganic Anions in Aqueous Matrices by Capillary Electrophoresis. 2007.

45. Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 1962; 27:31–6.

46. Reardon J, Foreman JA, Searcy RL. New reactants for the colorimetric determination of ammonia. Clin Chim Acta. 1966; 14:403–5. https://doi.org/10.1016/0009-8981(66)90120-3 PMID: 5970959

47. Harris TD, Graham JL. Preliminary evaluation of an in vivo fluorometer to quantify algal periphyton biomass and community composition. Lake Reserv Manag. 2015; 31(2):127–33.

48. Echenique-Subiabre I, Dalle C, Duval C, Heath MW, Couté A, Wood SA, et al. Application of a spectro-fluorimetric tool (bbe BenthoTorch) for monitoring potentially toxic benthic cyanobacteria in rivers. Water Res. 2016; 101:341–50. https://doi.org/10.1016/j.watres.2016.05.081 PMID: 27286469

49. Acuña V, Guasch H, Giorgi A, Izagirre O. Flujo de energía en el ecosistema. Metabolismo fluvial. Fundación. Elosegi A, Sabater S, editors. Conceptos y técnicas en ecología fluvial. Bilbao; 2009.

50. Lamberti GA, Resh VH. Stream Periphyton and Insect Herbivores: An Experimental Study of Grazing by a Caddisfly Population. Ecology [Internet]. 1983 Oct 1; 64(5):1124–35. Available from: https://doi.org/10.2307/1937823.

51. Kendrick MR, Huryn AD. Discharge, legacy effects and nutrient availability as determinants of temporal patterns in biofilm metabolism and accrual in an arctic river. Freshw Biol [Internet]. 2015 Nov 1; 60 (11):2323–36. Available from: https://doi.org/10.1111/fwb.12659.

52. Pinheiro J, Bates DM, DebRoy S, Sarkar D, R Core Team. Linear and Nonlinear Mixed Effects Models [Internet]. 2019. Available from: https://cran.r-project.org/package=nlme.

53. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2017. Available from: https://www.r-project.org.

54. Biggs BJF, Hickey CW. Periphyton responses to a hydraulic gradient in a regulated river in New Zealand. Freshw Biol. 1994; 32(1):49–59.

55. Death RG, Dewson ZS, James ABW. Is structure or function a better measure of the effects of water abstraction on ecosystem integrity? Freshw Biol. 2009; 54(10):2037–50.

56. Baekkelie KAE, Schneider SC, Hagman CHC, Petrin Z. Effects of flow events and nutrient addition on stream periphyton and macroinvertebrates: An experimental study using flumes. Knowl Manag Aquat Ecosyst. 2017;2017-Janua(418).

57. Biggs BJF, Stokseth S. Hydraulic habitat suitability for periphyton in rivers. Regul Rivers Res Manag. 1996; 12(2–3):251–61.

58. Hondzo M, Wang H. Effects of turbulence on growth and metabolism of periphyton in a laboratory flume. Water Resour Res. 2002; 38(12):13-1–13–9.

59. Biggs BJF, Goring DG, Nikora VI. Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. J Phycol. 1998; 34(4):598–607.

60. Yamada H, Nakamura F. Effect of fine sediment deposition and channel works on periphyton biomass in the Makomanai River, northern Japan. River Res Appl. 2002; 18(5):481–93.

61. Aspray KL, Holden J, Ledger ME, Mainstone CP, Brown LE. Organic sediment pulses impact rivers across multiple levels of ecological organization. Ecohydrology. 2017; 10(6):1–15.

62. Louhi P, Richardson JS, Muotka T. Sediment addition reduces the importance of predation on ecosystem functions in experimental stream channels. Can J Fish Aquat Sci. 2017; 74(1):32–40.

63. Guasch H, Martí E, Sabater S. Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. Freshw Biol. 1995; 33(3):373–83.

64. Aristi I, Casellas M, Elosegi A, Insa S, Petrovic M, Sabater S, et al. Nutrients versus emerging contaminants—Or a dynamic match between subsidy and stress effects on stream biofilms. Environ Pollut. 2016; 212:208–15. https://doi.org/10.1016/j.envpol.2016.01.067 PMID: 26845368

65. Baattrup-Pedersen A, Graeber D, Kallestrup H, Guo K, Rasmussen JJ, Riis T. Effects of low flow and co-occurring stressors on structural and functional characteristics of the benthic biofilm in small streams. Sci Total Environ [Internet]. 2020; 733:139331. Available from: https://doi.org/10.1016/j.scitotenv.2020.139331 PMID: 32454294

66. Mori T, Miyagawa Y, Onoda Y, Kayaba Y. Flow-velocity-dependent effects of turbid water on periphyton structure and function in flowing water. Aquat Sci [Internet]. 2017 Nov; 80(1):6. Available from: https://doi.org/10.1007/s00027-017-0552-1.