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# Nutrients versus emerging contaminants—Or a dynamic match between subsidy and stress effects on stream biofilms☆

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ABSTRACT

Freshwater ecosystems are threatened by multiple anthropogenic stressors, which might be differentiated into two types: those that reduce biological activity at all concentrations (toxic contaminants), and those that subsidize biological activity at low concentrations and reduce it at high concentrations (assimilable contaminants). When occurring in mixtures, these contaminants can have either antagonistic, neutral or synergistic effects; but little is known on their joint effects. We assessed the interaction effects of a mixture of assimilable and toxic contaminants on stream biofilms in a manipulative experiment using artificial streams, and following a factorial design with three nutrient levels (low, medium or high) and either presence or absence of a mixture of emerging contaminants (ciprofloxacin, erythromycin, diclofenac, methylparaben, and sulfamethoxazole). We measured biofilm biomass, basal fluorescence, gross primary production and community respiration. Our initial hypotheses were that biofilm biomass and activity would: increase with medium nutrient concentrations (subsidy effect), but decrease with high nutrient concentrations (stress effect) (i); decrease with emerging contaminants, with the minimum decrease at medium nutrient concentrations (antagonistic interaction between nutrients subsidy and stress by emerging contaminants) and the maximum decrease at high nutrient concentrations (synergistic interaction between nutrients and emerging contaminants stress) (ii). All the measured variables responded linearly to the available nutrients, with no toxic effect at high nutrient concentrations. Emerging contaminants only caused weak toxic effects in some of the measured variables, and only after 3–4 weeks of exposure. Therefore, only antagonistic interactions were observed between nutrients and emerging contaminants, as medium and high nutrient concentrations partly compensated the harmful effects of emerging contaminants during the first weeks of the experiment. Our results show that contaminants with a subsidy effect can alleviate the effects of toxic contaminants, and that long-term experiments are required to detect stress effects of emerging contaminants at environmentally relevant concentrations.



## 1. Introduction

River ecosystems are commonly threatened by multiple stressors, such as chemical pollution, flow regulation, geomorphological alterations, climate change and invasive species (Jackson et al., 2016; Vörösmarty et al., 2010). Chemical pollution is on its own a complex stressor, as many contaminants can reach rivers from both point and diffuse sources, and often appear in complex mixtures whose joint effects can be contrasting and convey ecological surprises (Culp et al., 2000; Dehedin et al., 2013; Jackson et al., 2016; Paine et al., 1998; Roessink et al., 2008). Depending on their effects on biota, contaminants can be grouped in two main types: those that reduce biological activity (toxic contaminants, such as pesticides), and those that subsidize biological activity at low concentrations but reduce it at high concentrations (assimilable contaminants, such as nutrients) (Odum et al., 1979). In addition, when occurring in mixtures contaminants can have either additive (i.e. response to multiple contaminants is equal to the sum

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of their individual effects), or multiplicative effects (i.e. the response exceeds the sum of their individual effects). Multiplicative interactions can be further synergistic (having a positive feedback) or antagonistic (having a negative feedback) (Brennan and Collins, 2015).

Nutrients are considered as assimilable chemical contaminants, as they cause a typical hump-shape response on biological activity (Bernot et al., 2010; Dunck et al., 2015; Niyogi et al., 2007; Wagenhoff et al., 2011). Most studies on the subsidy-stress effects of nutrients are experimental (Bernot et al., 2010; Cabrini et al., 2013; Stelzer et al., 2003), but there are also correlational field studies (Dunck et al., 2015; Izagirre et al., 2008; Wagenhoff et al., 2011; Woodward et al., 2012). Defining the causes behind the harmful effect of nutrients is not simple, and the prevalence of either the subsidy or the stress effect does not only depend on the concentration, but also on the exposure time. For example, in a review on the effects of nitrogen pollution in aquatic ecosystems, the threshold between subsidy and toxic effects for nitrogen compounds were 0.1 mg $NH_4^+$ $L^{-1}$ and 17 mg $NO_3^-$ $L^{-1}$ in acute toxicity tests (96 h), but of 0.05 and 1.1 mg $L^{-1}$ in chronic toxicity tests (>30 d) (Camargo and Alonso, 2006). Overall, medium concentrations of nutrients subsidize primary production (Biggs, 2000) and may change stoichiometry (Liess and Hillebrand, 2006) of stream biofilm; whereas at high concentrations of nutrients primary production becomes nutrient saturated, communities dominated by eutrophic (nutrient-tolerant) species, and anoxic conditions tend to occur at night (Wagenhoff et al., 2013), thus impacting biota.

Emerging contaminants are substances that have been detected in the environment, but which are currently not included in routine monitoring programmes and whose environmental fate and ecotoxicological effects are not well understood (Pal et al., 2010). Because of that, research on their toxic effects on biological activity has been blooming over the past decade (González et al., 2012; Navarro-Ortega et al., 2015). However, most studies are based on short-term (24–96 h) acute toxicity tests studying the survival of algae, invertebrate or fish at very high concentrations (Brausch and Rand, 2011; Cleuvers, 2004; Franz et al., 2008; Grung et al., 2008). There are fewer long-term studies on the effects of emerging contaminants at environmentally relevant concentrations, or on the effects of mixtures of emerging contaminants and other stressors such as nutrients or flow intermittency (Brennan and Collins, 2015; Corcoll et al., 2015). In the study by Corcoll and others, deleterious effects on biological activity of stream biofilms were reported after 10 days of exposure to a mixture of 5 different pharmaceuticals at environmentally relevant concentrations, and the resulting interaction between flow intermittency and emerging contaminants was process-specific, as an antagonistic effect was observed for bacteria but a synergistic effect was observed for algae. In regards to mixtures of contaminants, some studies reported stress effects to overwhelm the subsidy effects caused by assimilable contaminants (Wagenhoff et al., 2012, 2011); whereas others reported the opposite (Koelmans et al., 2001; Morin et al., 2010; Traas et al., 2004). Furthermore, the resulting effects have been also reported to be process-specific (Aristi et al., 2015).

Given this background, our goal was to assess the interaction effects of a mixture of assimilable and toxic contaminants on biofilms in a manipulative experiment using artificial streams. The experiment followed a factorial design with 3 levels of nutrients (low, medium and high, expected to cause respectively no effects, subsidy, and stress) and 2 levels of a mixture of emerging contaminants (absence/presence), which included three antibiotics (ciprofloxacin, erythromycin, sulfamethoxazole), one anti-inflammatory (diclofenac), and one preservative (methylparaben) with bactericidal and fungicidal properties. The compounds included in the mixture were selected because of their widespread occurrence in polluted urban rivers in the Mediterranean region and high ecotoxicological relevance (Kuzmanović et al., 2015). Specifically, the concentrations of each compound in the mixture mimicked the worst case scenario (the highest concentrations observed during low flow situation in the lower Llobregat river) (González et al., 2012; Gorga et al., 2015), and were expected to have low to no effects on the aquatic biota based on reported ecotoxicological studies for these chemicals (Brausch and Rand, 2011; Grung et al., 2008). These studies are however based on single-compound assays on target organisms, therefore neglecting possible synergistic effects in contaminant mixtures. Our hypotheses were that stream biofilm biomass and activity will: increase with medium nutrient concentrations (subsidy effect), but decrease with high nutrient concentrations (stress effect) (i); decrease with emerging contaminants at all nutrient concentrations, with the minimum decrease at moderate nutrient concentrations (antagonistic interaction between nutrients and emerging contaminants) and the maximum decrease at high nutrient concentrations (synergistic interaction between nutrients and emerging contaminants) (ii). The rationale behind this second hypothesis is the backbone of this manuscript, as we believe that the minor stress effects expected from emerging contaminants at our environmentally relevant concentrations would be detectable at low nutrient concentrations, while they would be partly compensated by the subsidy effect at medium nutrient concentrations, and exacerbated by the stress effect exerted by high nutrient concentrations.

## 2. Methods

### 2.1. Experimental design

The experiment was performed in the indoor Experimental Streams Facility of the Catalan Institute for Water Research (Girona, EU) between June 11th and July 18th, 2014. Each of the 18 artificial streams was assigned to one of six treatments following a randomized complete block design (with 3 replicates per treatment; and one replicate per block of 6 artificial streams). A factorial design was followed, with three nutrient levels [low (L), medium (M) or high (H)] and two levels for the mixture of emerging contaminants [no emerging (NE) or emerging (E)]. Nutrient treatments consisted of a mixture of phosphate, nitrate and ammonium at different concentrations, whereas the treatment with emerging contaminants consisted in a mixture of the 5 previously described contaminants (ciprofloxacin, erythromycin, sulfamethoxazole, diclofenac, and methylparaben). The exposure to treatments lasted for 28 days, therefore allowing the assessment of short-to long-term effects of both the separate effects of nutrients and emerging contaminants and their interaction. For each type of contaminant (emerging and nutrients), the experimental design only allowed the assessment of their cumulative effects, not their individual effects.

### 2.2. Experimental conditions

Each stream consisted of an independent methacrylate channel (l–w – d = 200 cm–10 cm–10 cm), and a 70 L water tank from which water can be recirculated. Each stream received a constant flow of 50 mL $s^{-1}$, and operated under a scheme of combined flow-recirculation (118 min) and flow-open (2 min) every 2 h. The water exchange rate was 4.28% per hour, so water of each artificial stream was completely renewed once a day. Mean water velocity was 0.88 ± 0.03 cm $s^{-1}$, and water depth over the plane bed ranged between 2.2 and 2.5 cm. Each artificial stream was filled with 5 L of sand extracted from an unpolluted segment of the Llémena River

(Sant Esteve de Llémena, Girona, EU) ($d_{50} = 0.74$ mm). The sand was sterilized with a Presoclave-II 30L autoclave (120 °C for 2 h) (JP Selecta S.A., Barcelona, Spain), and evenly distributed in the artificial streams to create a plane bed that facilitated the growth of biofilm. At complete water saturation, the porosity of the sand yielded a water content of 25% of the wet weight. Source water for the artificial streams was rainwater, filtered through activated carbon filters. Daily cycles of photosynthetic active radiation (PAR) were defined as 12 h daylight + 12 h darkness, and were simulated by LED lights (Lightech, Girona, Spain). PAR was held constant at 173.99 ± 33 μE $m^{-2}$ $s^{-1}$ during the daytime, and was recorded every 10 min using 4 quantum sensors located across the whole array of streams (sensor LI-192SA, LiCOR Inc, Lincoln, USA). Water temperature was held constant at 20 °C by means of a cryo-compact circulator (Julabo CF-31, Seelbach, Germany), and recorded every 10 min using VEMCO Minilog (TR model, AMIRIX Systems Inc, Halifax, NS, Canada) temperature data loggers (−5 to 35 °C, ±0.2 °C). Overall, physico-chemical conditions in the artificial streams (water velocity, temperature, and light cycles) emulated those of the Llémena River during late spring and under low flow conditions.

Biofilm was inoculated twice per week during the colonization period using combined inocula from epilithic (growing on surface of rocks) and epipsammic (growing on the surface of sand) biofilms from the same unpolluted segment of the Llémena River. Specifically, biofilm inocula was obtained after scraping 10–12 cobbles and washing around 10 L of fine sediments. Biofilm colonization was allowed in the artificial streams for 20 days before the exposure to treatments. During the colonization period, nutrients were held constant at 0.040, 1.7 and 0.040 mg $L^{-1}$ of phosphate ($P{-}PO_4^{3-}$), nitrate ($N{-}NO_3^-$) and ammonium ($N{-}NH_4^+$) by means of injection of concentrated solutions ($KH_2PO_4$, $NaNO_3$, and $NH_4Cl$, respectively) and using a peristaltic pump (IPC pump, Ismatec, Switzerland). During the experimental period, the artificial streams under the L nutrients treatment remained even, but the M and H nutrient treatments received higher rates of injection of the same concentrated solutions. Target nutrient concentrations for the M nutrients treatment were 0.2 mg $L^{-1}$ of phosphate ($P{-}PO_4^{3-}$), 5 mg $L^{-1}$ of nitrate ($N{-}NO_3^-$) and 0.2 mg $L^{-1}$ of ammonium ($N{-}NH_4^+$), whereas for the H nutrient treatment were 1, 25 and 1 mg $L^{-1}$ of phosphate ($P{-}PO_4^{3-}$), nitrate ($N{-}NO_3^-$) and ammonium ($N{-}NH_4^+$). As previously stated for emerging contaminants, the concentrations of the H treatment mimicked the worst-case scenario, i.e. the highest concentrations observed during low flow situation in the lower Llobregat river (Aguilera et al., 2012).

In the treatments with emerging contaminants, a mixture of 5 compounds was continuously added to the artificial streams using a peristaltic pump (IPC pump, Ismatec, Switzerland) to achieve approximate constant concentrations: 0.1 μg $L^{-1}$ of methylparaben, and 1 μg $L^{-1}$ of ciprofloxacin, diclofenac, erythromycin and sulfamethoxazole. The working standard mixture of emerging contaminants was prepared every 2–3 days at a concentration of 100 mg $L^{-1}$ in 10% methanol: water (v: v). The total concentration of methanol reaching the artificial streams was 400 ng $L^{-1}$. The same concentration of methanol was added in treatments without emerging contaminants. High purity (>97%) standard solutions of the target compounds and their deuterated counterparts (used as surrogate standards) were obtained from Sigma–Aldrich (St Louis, U.S.A.), Aldrich (Milwaukee, U.S.A.), Dr. Ehrenstorfer (Wesel, Germany) Fluka (Buchs, Switzerland) and CDN Isotopes (Pointe-Claire, Quebec, Canada).

### 2.3. Water chemistry

Dissolved oxygen, pH and specific conductivity were measured weekly by noon in each artificial stream using WTW (Weilheim, Germany) hand-held probes. Background concentrations of nutrient and emerging contaminants were measured weekly from water collected from the channel outlet to assess the possible differences between nominal and observed concentrations. Water was filtered immediately through 0.7 μm glass fiber filters (Whatman GF/F, Kent, UK) into pre-washed polyethylene containers for nutrients; and through 0.45 μm (Whatman GD/X) into amber glass bottles for emerging contaminants. The concentration of $P{-}PO_4^{3-}$ was determined colorimetrically using a fully automated discrete analyzer Alliance Instruments Smartchem 140 (AMS, Frépillon, France). The concentrations of $N{-}NO_3^-$ and $N{-}NH_4^+$ were determined on a Dionex ICS-5000 ion chromatograph (Dionex Corporation, Sunnyvale, U.S.A.). The concentration of dissolved organic carbon (DOC) was measured on a Shimadzu TOC-V CSH coupled to a TNM module (Shimadzu Corporation, Kyoto, Japan).

Emerging contaminants were analyzed using a method based on an online pre-concentration with EQuan MAXTM technology coupled to a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific, San José, U.S.A.) (on-line SPE-LC/MS/MS) equipped with an electrospray ionization source (ESI). The system consists of two quaternary pumps: a loading pump (Accela™ 600 pump) and an elution pump (Accela 1250 pump) both of Thermo Fisher Scientific. A divert valve was programmed to control loading and elution of the two LC columns. The first column, also called EQuan, was used for sample pre-concentration and the second for chromatographic separation. Briefly, 2 mL of water sample, containing a mixture of internal standards, were pre-concentrated during the loading stage and the target compounds were retained. Subsequently, the analytes were eluted from the EQquan column (Hypersil GOLD aQ 20 mm × 2.1 mm i.d., 12 μm particle size) and separated on an analytical column (KINETEX C18 50 mm × 2.1 mm i.d., 1.7 μm particle size). The masses were monitored at two different transitions for each compound by SRM (Selected Reaction Monitoring) (SRM1: quantifier transition and SRM2: qualifier transition). Quantification was performed using the internal standard method based on the peak areas obtained for each analyte and its corresponding internal standard analog. The list of SRM monitored, as well as the quality parameters such as calibration range, correlation coefficients, recoveries and precision data at two different levels are shown in Table 1. Note that the limits of detection (LODs) and quantification (LOQs) were estimated experimentally from real samples as the concentration of analyte that provides a signal-to-noise ratio of 3 and 10, respectively.

### 2.4. Biofilm biomass and activity

The response of biofilm to different treatments was assessed weekly in terms of biomass [ash-free dry mass (AFDM)], basal fluorescence ($F_0$), and metabolism [gross primary production (GPP), and community respiration (CR)]. $F_0$, GPP and CR were measured in situ, one measurement per artificial stream at each time, whereas AFDM measurements were carried out at the laboratory immediately after sample collection. The community composition of the algal component of the biofilm was estimated microscopically by means of a Nikon light microscope, at 400x. The algae of all different groups present in each channel were identified at the genus or species level, and qualitatively ranged from 1 to 5 according to their relative abundance in the sample.

AFDM was used as an estimate of total biomass in the biofilm. For its determination, one biofilm sample was taken per artificial stream at each time with a sample corer of 1.2 cm diameter, from which only the uppermost 1 cm was considered for analysis. Samples were dried at 60 °C to constant weight, combusted at 450 °C for 4 h, and reweighted. $F_0$ was measured with a portable

**Table 1**
Quality parameters of SPE-LC/MS/MS analysis for the target emerging contaminants.

| Compound | Internal standard | Linear range ng $L^{-1}$ | $R^2$ | LOD ng $L^{-1}$ | LOQ ng $L^{-1}$ | Recovery (%) | | Intra-day | | Inter-day | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 1 μg $L^{-1}$ | 0.1 μg $L^{-1}$ | 1 μg $L^{-1}$ | 0.1 μg $L^{-1}$ | 1 μg $L^{-1}$ | 0.1 μg $L^{-1}$ |
| Erythromycin | Erythromycin-N,$N_{13}C_2$ | 1–908 | 0.9988 | 0.01 | 0.91 | 99.5 | 91.6 | 4.0 | 6.4 | 3.2 | 6.1 |
| Sulfamethoxazole | Sulfamethoxazole-$d_3$ | 4–2012 | 0.9984 | 0.50 | 4.02 | 100.2 | 104.0 | 3.5 | 4.0 | 3.9 | 4.6 |
| Diclofenac | Diclofenac-$d_4$ | 1–375 | 0.9989 | 0.06 | 0.94 | 88.7 | 106.4 | 1.4 | 5.3 | 3.0 | 5.2 |
| Ciprofloxacin | Ciprofloxacin-$d_8$ | 4–1999 | 0.9998 | 0.35 | 4.00 | 98.6 | 100.0 | 1.1 | 4.9 | 5.7 | 11.7 |
| Methylparaben | Methylparaben-$d_4$ | 1–375 | 0.9989 | 0.06 | 0.94 | 88.7 | 106.4 | 1.4 | 5.3 | 3.0 | 5.2 |

pulse amplitude modulate fluorometer (Diving-PAM; WALZ, Effeltrich, Germany) to evaluate the structural and functional changes in the algal component of the biofilm. $F_0$, used as a surrogate of algal biomass (Schmitt-Jansen et al., 2008), is a variable commonly used to evaluate the algal response to environmental stressors such as toxicants, light stress or desiccation (Corcoll et al., 2012; Sabater et al., 2007; Timoner et al., 2012). Nevertheless, $F_0$ levels must be interpreted with caution because a fluorescence increase is not always linked to a biomass increase, particularly when dealing with PSII inhibitors (Corcoll et al., 2012).

GPP and CR were measured to evaluate biofilm activity. These metabolic rates are key processes for turnover of organic matter, inorganic materials, and energy in a river. Thus, net ecosystem metabolism (NEM) and CR were measured by means of oxygen variations in cylindrical recirculating chambers (Acuña et al., 2015, 2008), which enclosed trays containing 160 $cm^3$ of streambed sediments, one per artificial stream. These trays were located in the artificial streams during the entire experiment and the conditions within were equivalent to those experienced outside because of the used mesh size (diameter of 1 mm), so that epipsammic biofilms developed within the trays under the same conditions. These trays were moved into the chambers only during metabolism measurements, and later returned to the corresponding artificial stream. The chambers were made of acrylic glass (volume 0.96 L), and provided with a submersible water circulation pump to avoid the existence of zones of low diffusion within the chamber. The incubations for each metabolism rate lasted for 60 min, and were carried out inside an incubator chamber (Radiber AGP-700-ESP, Barcelona, Spain) at the same temperature as in the artificial streams. NEM was measured under a PAR of 168 ± 2 μE $m^{-2}$ $s^{-1}$, similar to the irradiance received by artificial streams, and CR was measured in darkness. DO concentration inside the chambers was measured continuously and logged at 15 s intervals with oxygen sensors (PreSens OXY-10mini, Regensburg, Germany). Metabolism rates were calculated, with GPP estimated as the sum of NEM and CR (Acuña et al., 2008).

### 2.5. Data analysis

First of all, we used one-way analysis of variance (ANOVA) with blocks (see section 2.1) as fixed factor to test for differences among experimental arrays for all variables before treatment onset. After that, differences between time and treatments were tested with 3-way repeated measurements ANOVA with time, nutrient treatment and emerging pollutant treatment as fixed factors, and arrays as random factor (n = 72). Interactions between the considered fixed factors were also tested, and Post hoc Tukey tests were done for each sampling day to see whether general trends changed or not. Results for the interaction of nutrients and emergent contaminants allowed the identification of those interactions that were additive (i.e. non-significant interaction) and those that deviate from additive (i.e. significant interaction) (Piggott et al., 2015). Pearson moment correlation analysis was used with the averaged values of each treatment to identify the direction and strength of the relationships between variables. The similarity between the algal communities in the different treatments was estimated by means of a Jaccard index, and used to describe the potential effects of the treatment on the community composition. Normality of all variables was checked with the Kolmogrov–Smirnov test, and variables were log-transformed when necessary. All analyses were considered significant at $P < 0.05$, and were performed with the R software (version 3.1.1; R Development Core Team, Vienna, Austria).

## 3. Results

### 3.1. Experimental conditions

Temperature was held constant during the entire experiment and between the different treatments. Thus, air temperature in the Experimental Streams Facility room averaged 19.61 ± 0.73 °C, whereas water temperature in the artificial streams averaged 19.80 ± 0.42 °C in all treatments. PAR cycles were also steady throughout the experiment, as well as the hydraulics. Dissolved oxygen was steady throughout the entire experiment and among treatments, with values between 9.99 and 10.41 mg $L^{-1}$. Environmental conditions (dissolved oxygen, pH and specific conductivity) showed no statistically significant differences between arrays before the onset of the treatments. The experimental phase involved an increase in conductivity, as it increased in those treatments with nutrients from 206 ± 19 μS $cm^{-1}$ (treatment L) to 233 ± 29 in treatment M and 350 ± 64 μS $cm^{-1}$ in treatment H.

Water chemistry was steady throughout the entire experiment. The achieved concentrations of ammonium and phosphate were lower (80–90% and 25–80% less, respectively) than the nominal concentrations in all studied levels, whereas the achieved concentration of nitrate in L and M was similar to the nominal one, but not in H, which was 10% lower (Table 2). On the other hand, the achieved concentrations of emerging contaminants were in most cases lower than the nominal concentrations (15–40%), but higher in the case of ciprofloxacin (Table 2). These differences between the nominal and achieved emerging contaminants concentrations were most likely caused by natural attenuation within the artificial streams, that is, a combined effect of sorption to sediments and to

**Table 2**
Average (±SD) of nutrient and emerging contaminant concentrations (if any) in each treatment (n = 18, resulting from 3 replicates per treatment and 6 surveys over the experiment).

| | Low | Medium | High |
|---|---|---|---|
| Ammonium (mg N–$NH_4^+$ $L^{-1}$) | 0.008 ± 0.003 | 0.050 ± 0.018 | 0.194 ± 0.015 |
| Nitrate (mg N–$NO_3^-$ $L^{-1}$) | 1.720 ± 0.230 | 5.099 ± 1.143 | 22.89 ± 0.608 |
| Phosphate (mg P–$PO_4^{3+}$ $L^{-1}$) | 0.014 ± 0.006 | 0.113 ± 0.046 | 0.779 ± 0.133 |
| Erythromycin (μg $L^{-1}$) | 0.625 ± 0.104 | 0.635 ± 0.087 | 0.636 ± 0.131 |
| Sulfamethoxazole (μg $L^{-1}$) | 0.805 ± 0.141 | 0.849 ± 0.125 | 0.756 ± 0.193 |
| Diclofenac (μg $L^{-1}$) | 0.812 ± 0.115 | 0.834 ± 0.111 | 0.754 ± 0.166 |
| Ciprofloxacin (μg $L^{-1}$) | 1.234 ± 0.133 | 1.336 ± 0.144 | 1.171 ± 0.216 |
| Methylparaben (μg $L^{-1}$) | 0.037 ± 0.032 | 0.039 ± 0.034 | 0.032 ± 0.034 |

**Table 3**
Results for type III tests of fixed-effect in ash free dry mass (AFDM), basal fluorescence ($F_0$), gross primary production (GPP) and community respiration (CR), indicating the F value and its significance (in parenthesis). Note that N stands for nutrients, E for emerging contaminants, D for day of experiment; and N*E, N*D, E*D and N*E*D for their interactions.

| | AFDM | $F_0$ | GPP | CR |
|---|---|---|---|---|
| Intercept | 9.176 (0.000) | 7.05 (0.000) | 8.91 (0.000) | 8.09 (0.000) |
| N | 5.66 (0.001) | 35.51 (0.000) | 5.469 (0.000) | 10.84 (0.000) |
| E | 3.18 (0.049) | 2.87 (0.066) | 1.98 (0.149) | 7.75 (0.001) |
| D | 2.04 (0.159) | 31.65 (0.000) | 0.03 (0.868) | 1.62 (0.209) |
| N*E | 0.57 (0.685) | 0.58 (0.676) | 0.49 (0.741) | 1.44 (0.233) |
| N*D | 1.29 (0.285) | 2.27 (0.113) | 0.44 (0.649) | 0.14 (0.873) |
| E*D | 1.15 (0.287) | 4.45 (0.039) | 2.72 (0.105) | 1.30 (0.260) |
| N*E*D | 0.05 (0.952) | 0.46 (0.636) | 0.78 (0.465) | 1.26 (0.292) |

the methacrylate, as well as to photo-transformation and biotransformation.

### 3.2. Biofilm structure and function

Before treatment implementation, biofilm biomass averaged 47 ± 10 g AFDM $m^{-2}$ and $F_0$ averaged 499 ± 57. After treatment implementation, AFDM was influenced by nutrients and emerging contaminants (Table 3) (Fig. 1a). Similarly, $F_0$ increased over the experiment, and was influenced by nutrients (Table 3) (Fig. 1b). The analysis of interaction between treatments indicated that the interaction did not deviate significantly from an additive for AFDM, although the interaction component of the linear mixed model was significant for $F_0$ between time and emerging contaminants (Table 3). However, the interaction of the treatments nutrients and emerging contaminants yielded different results over time for AFDM and $F_0$. For example, the values of $F_0$ did not differ between the treatment nutrients M and the interaction between nutrients and emerging contaminants (E_M) at day 14, but this was not the case for day 28 (Fig. 2). Specifically, at day 14 the interaction was indicative of negative antagonistic (less negative than predicted additively), whereas at day 28 was positive antagonistic (less positive than predicted additively). Post-hoc analysis between treatments for specific dates also revealed that $F_0$ had lower values at treatments receiving emerging contaminants than those that did not at the end of the experiment (days 21 and 28) (Fig. 1). All of the treatments had a number of 3–12 algal taxa. The differences between the algal communities in the different treatments, estimated by means of the Jaccard index of similarity, were minor. There were not differences in community composition related to the different treatments.

Before treatment implementation, GPP averaged 16.0 ± 2.3 g $O_2$ $m^{-2}$ $d^{-1}$, CR −3.4 ± 0.3 g $O_2$ $m^{-2}$ $d^{-1}$, and NEM 12.6 ± 1.9 g $O_2$ $m^{-2}$ $d^{-1}$ (Fig. 1c and d). After treatment implementation, GPP was influenced by nutrients but neither by emerging contaminants nor by the interaction between nutrients, emerging contaminants and time (Table 3) (Fig. 1c). CR was influenced by both nutrients and emerging contaminants, but not by any interaction (Table 3) (Fig. 1d). The assessed metabolic rates (NEM, GPP, and CR) were significantly correlated among them ($R^2 > 0.54$; $P < 0.0001$). Similarly to what described for AFDM and $F_0$, the interaction of the treatments nutrients and emerging contaminants yielded different results over time for GPP and CR. Post-hoc analysis between treatments for specific dates also revealed that there were differences in the response to emerging contaminants at different levels of nutrients by day 28, as emerging contaminants affected negatively GPP and CR in the L and M nutrient treatments, but not in the H nutrient treatment. However, this was not the case in previous sampling dates (7, 14, and 21).

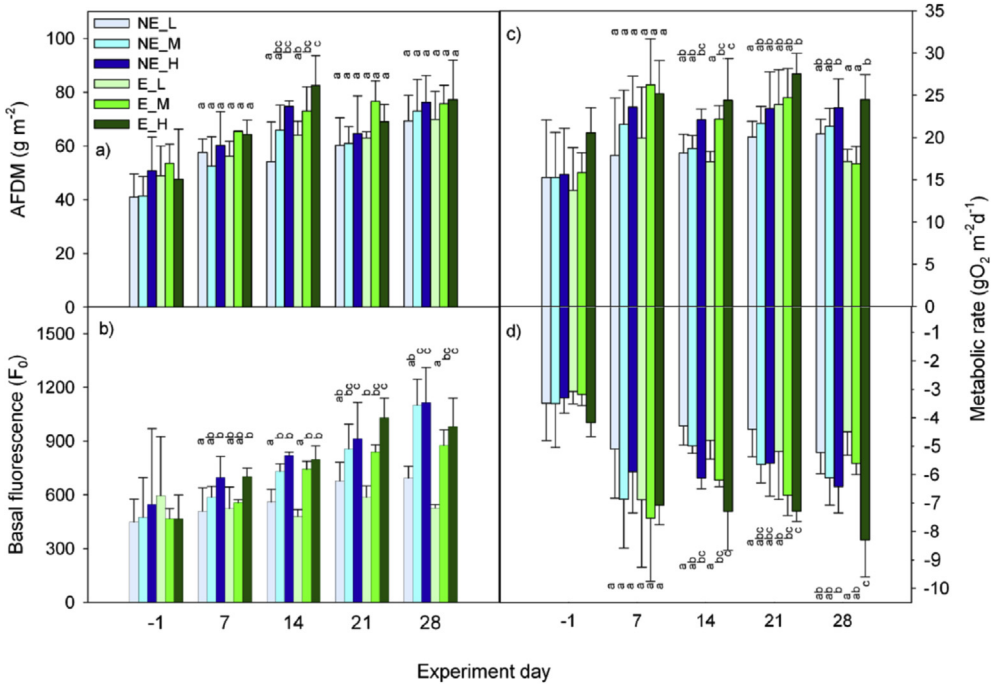


**Fig. 1.** Changes in biofilm variables, expressed as the means (±SD) of the values per treatment and per time of a) ash free dry mass (AFDM), b) basal fluorescence ($F_0$), c) gross primary production (GPP), and d) community respiration (CR). Note that NE_L stands for treatment with no emerging contaminants and low nutrient concentrations, NE_M for treatment with no emerging contaminants and medium nutrient concentrations, NE_H for treatment with no emerging contaminants and high nutrient concentrations, E_L for treatment with emerging contaminants and low nutrient concentrations, E_M for treatment with emerging contaminants and medium nutrient concentrations, and E_H for treatment with emerging contaminants and high nutrient concentrations. Post-hoc Tukey test results for each day of the experiment after treatment implantation are shown in letters.

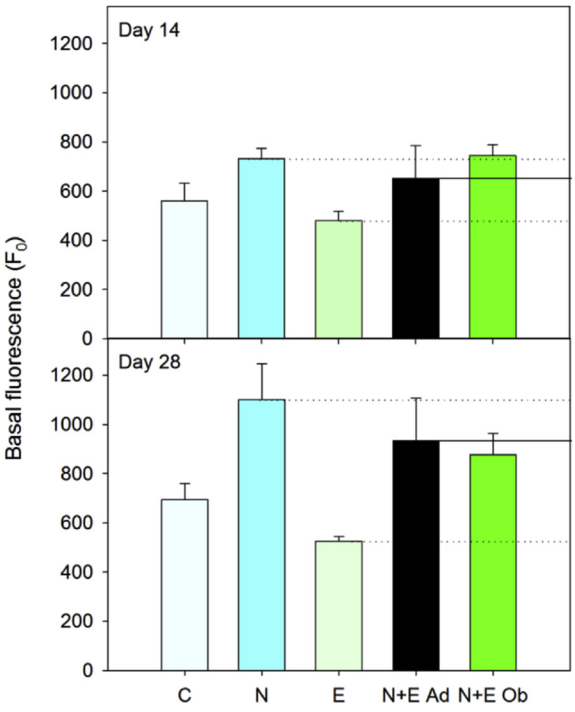


**Fig. 2.** Basal fluorescence (mean ± SD) of treatments NE_L (as control–C), NE_M (as nutrient treatment–N), E_L (as emerging contaminants treatment–E), and E_M (as interaction between nutrients and emerging contaminants treatment–N + E Obs) at days 14 and 28. Note that N + E Ad stands for the estimated response to the nutrients and emerging contaminants treatment if the interaction was additive, which was estimated as the additive sum of individual effects for treatments N and E relative to C (Piggott et al., 2015). Given that we had 3 replicates per treatment, there were 9 estimates of the individual effects of treatment nutrients (N–C), and 9 estimates of the individual effects of treatment emerging contaminants (E–C), and this resulted in 243 estimates of the additive response, which were used to calculate the mean ± SD.

## 4. Discussion

### 4.1. Subsidy-stress effects by nutrients

Because of the concentrations used, we expected the measured stream biofilm variables to increase with medium nutrient concentrations (subsidy effect), but decrease with high nutrient concentrations (stress effect), resulting in a hump-shape response similar to that reported from field and laboratory experiments at similar concentration ranges (Camargo and Alonso, 2006; Wagenhoff et al., 2013, 2012). However, all the measured variables responded linearly to nutrient concentrations, thus showing no evidence of stress effect at high nutrient concentrations. The subsidy effect resulted in similar increases in all variables, which were significantly inter-correlated (AFDM with CR, and $F_0$ with GPP). This coupling between variables indicative of biomass and variables indicative of function has been also observed at the ecosystem scale for both GPP (Hill et al., 2001) and CR (Acuña et al., 2004). The unexpected subsidy effect at high nutrient concentrations might be most likely related with the duration of the experiment, as harmful effects by nutrients are not direct effects but indirect effects through the eutrophication of the ecosystem (Dodds and Oakes, 2004). Even in conditions of high nutrient concentration, eutrophication is a process that requires time to allow for biofilm accrual (Boulêtreau et al., 2006), and only then do night-time anoxia, and biofilm senescence (Izagirre et al., 2008) become a problem. In fact, the attained biofilm biomass in our experimental streams seven weeks after the inoculation was still lower than those commonly found in eutrophic watercourses (150–200 g AFDM $m^{-2}$; (Izagirre et al., 2008)), suggesting that the duration of the experiment fell short to exert an stress effect on the biofilms. An alternative explanation for the subsidy effects observed at high nutrient concentrations is that primary producer's sloughing did not occur under the low flow velocities in our experimental setting.

### 4.2. Stress effects by emerging contaminants

As expected from the low but environmentally realistic concentrations of emerging contaminants used in our experiment, stress effects were weak, detected only in some of the measured variables (AFDM, $F_0$ and CR), and interestingly only after 3–4 weeks of exposure. This means that used concentrations were not high enough to cause short-term stress effects on the stream biofilms, but only long-term effects. In this direction, other studies also reported stress effects after some weeks of exposure to low concentrations of toxic contaminants such as pharmaceuticals, beta-blockers or pesticides (Muñoz et al., 2009; Ricart et al., 2010; Rosi-Marshall et al., 2013). Regardless of the timing of the response, it is important to note the different response of GPP and CR to the presence of emerging contaminants at the end of the experiment (day 28), as the reduction observed in GPP surpassed that of CR (16 and 10%, respectively). Although this is a minor difference between autotrophic and heterotrophic processes, similar results have been reported at both the mesocosms (Corcoll et al., 2015) and the ecosystem scale (Aristi et al., 2015). Due to the relatively short experimental time, we ignore if these differences between autotrophic and heterotrophic processes would increase over time, but if that was the case, this would imply that stream biofilms under stress by emerging contaminants might drift to the heterotrophy with unbalanced metabolic rates (i.e. high respiration rates).

### 4.3. Interaction between nutrients and emerging contaminants

The stress effects caused by emerging contaminants were not even for the different assessed nutrient concentrations, as expected negative effects were never observed when biofilms received the highest nutrient concentrations. This result did not confirm our initial hypothesis of decreases with emerging contaminants at all nutrient concentrations, with the minimum decrease at moderate nutrient concentrations (antagonistic interaction between nutrients and emerging contaminants), and the maximum decrease at high nutrient concentrations (synergistic interaction between nutrients and emerging contaminants). Thus, only an antagonistic interaction was observed, as medium and high nutrient concentrations compensated the stress effects of emerging contaminants. Interestingly, this compensatory effect was not analogous for $F_0$ and GPP, and decreased over the course of the experiment. In the case of $F_0$, compensation occurred at all nutrient levels at day 14, at medium and high nutrient levels at day 21, and at none nutrient level at day 28. In contrast, the effects of emerging contaminants on GPP were compensated at all nutrient levels at days 14 and 21, but only at high nutrient level at day 28. Our results therefore indicate that used concentrations of emerging contaminants only had an effect on the long-term, and that the time of appearance of these effects was delayed by the compensatory effect of nutrients. Similarly, other manipulative experiments have reported compensatory effects of nutrients on the toxic effects of pesticides (Barreiro Lozano and Pratt, 1994; Guasch et al., 2004; Roessink et al., 2008; Traas et al., 2004). In the same direction, a correlative field study also reported similar compensations in biofilms affected by pesticides (Morin et al., 2010). Interestingly, a recent review on multiple

stressors effects on freshwater ecosystems stressed that most studies reported antagonistic interactions, and suggested that a possible explanation for more antagonistic responses by freshwater biota to stressors is that the inherent greater environmental variability of smaller aquatic ecosystems fosters greater potential for acclimation and co-adaptation to multiple stressors (Jackson et al., 2016). Overall, several studies have reported antagonistic effects between assimilable and toxic contaminants, but to our knowledge, no one studied the temporal dynamics of this interaction, which might be relevant to consider when predicting the long-term effects of multiple stressors.

## 5. Conclusions

Our results indicate that emerging contaminants can pose a threat to non-target aquatic organisms at concentrations observed in polluted rivers, that assimilable contaminants such as nutrients can alleviate the stress effect of emerging contaminants, and that long-term experiments are required to detect stress effects of emerging contaminants at environmentally relevant concentrations.

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## References

Acuña, V., Casellas, M., Corcoll, N., Timoner, X., Sabater, S., 2015. Increasing extent of periods of no flow in intermittent waterways promotes heterotrophy. Freshw. Biol. 60, 1810–1823.
Acuña, V., Giorgi, A., Muñoz, I., Uehlinger, U., Sabater, S., 2004. Flow extremes and benthic organic matter shape the metabolism of a headwater mediterranean stream. Freshw. Biol. 49, 960–971.
Acuña, V., Wolf, A., Uehlinger, U., Tockner, K., 2008. Temperature dependence of stream benthic respiration in an Alpine river network under global warming. Freshw. Biol. 53, 2076–2088.
Aguilera, R., Marcé, R., Sabater, S., 2012. Linking in-stream nutrient flux to land use and inter-annual hydrological variability at the watershed scale. Sci. Total Environ. 440, 72–81.
Aristi, I., vonSchiller, D., Arroita, M., Barceló, D., Ponsatí, L., García-Galán, M.J., Sabater, S., Elosegi, A., Acuña, V., 2015. Mixed effects of effluents from a wastewater treatment plant on river ecosystem metabolism: subsidy or stress? Freshw. Biol. 60 (7), 1398–1410.
Barreiro Lozano, R., Pratt, J.R., 1994. Interaction of toxicants and communities: the role of nutrients. Environ. Toxicol. Chem. 13, 361–368.
Bernot, M.J., Sobota, D.J., Hall, R.O., Mulholland, P.J., Dodds, W.K., Webster, J.R., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Gregory, S.V., Grimm, N.B., Hamilton, S.K., Johnson, S.L., Mcdowell, W.H., Meyer, J.L., Peterson, B., Poole, G.C., Valett, H.M., Arango, C., Beaulieu, J.J., Burgin, A.J., Crenshaw, C., Helton, A.M., Johnson, L., Merriam, J., Niederlehner, B.R., O'Brien, J.M., Potter, J.D., Sheibley, R.W., Thomas, S.M., Wilson, K., 2010. Inter-regional comparison of land-use effects on stream metabolism. Freshw. Biol. 55, 1874–1890.
Biggs, B.J.F., 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. J. North Am. Benthol. Soc. 19, 17–31.
Boulêtreau, S., Garabetian, F., Sauvage, S., Sanchez-Perez, J.M., 2006. Assessing the importance of a self-generated detachment process in river biofilm models. Freshw. Biol. 51, 901–912.
Brausch, J.M., Rand, G.M., 2011. A review of personal care products in the aquatic environment: environmental concentrations and toxicity. Chemosphere 85, 1518–1532.
Brennan, G., Collins, S., 2015. Growth responses of a green alga to multiple environmental drivers. Nat. Clim. Chang. 5.
Cabrini, R., Canobbio, S., Sartori, L., Fornaroli, R., Mezzanotte, V., 2013. Leaf packs in impaired streams: the influence of leaf type and environmental gradients on breakdown rate and invertebrate assemblage composition. Water, Air, Soil Pollut. 224, 1967–1979.
Camargo, J.A., Alonso, A., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ. Int. 32, 831–849.
Cleuvers, M., 2004. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicol. Environ. Saf. 59, 309–315.
Corcoll, N., Bonet, B., Leira, M., Montuelle, B., Tlili, A., Guasch, H., 2012. Light history influences the response of fluvial biofilms to Zn exposure. J. Phycol. 48, 1411–1423.
Corcoll, N., Casellas, M., Huerta, B., Guasch, H., Acuña, V., Rodríguez-Mozaz, S., Serra-Compte, A., Barceló, D., Sabater, S., 2015. Effects of flow intermittency and pharmaceutical exposure on the structure and metabolism of stream biofilms. Sci. Total Environ. 503–504, 159–170.
Culp, J.M., Podemski, C.L., Cash, K.J., 2000. Interactive effects of nutrients and pollutants from pulp mill effluents on riverine benthos. J. Aquat. Ecosyst. Stress Recover 8, 67–75.
Dehedin, A., Maazouzi, C., Puijalon, S., Marmonier, P., Piscart, C., 2013. The combined effects of water level reduction and an increase in ammonia concentration on organic matter processing by key freshwater shredders in alluvial wetlands. Glob. Chang. Biol. 19, 763–774.
Dodds, W.K., Oakes, R.M., 2004. A technique for establishing reference nutrient concentrations across watersheds affected by humans. Limnol. Oceanogr. Methods 2, 333–341.
Dunck, B., Lima-Fernandes, E., Cássio, F., Cunha, A., Rodrigues, L., Pascoal, C., 2015. Responses of primary production, leaf litter decomposition and associated communities to stream eutrophication. Environ. Pollut. 202, 32–40.
Franz, S., Altenburger, R., Heilmeier, H., Schmittjansen, M., 2008. What contributes to the sensitivity of microalgae to triclosan? Aquat. Toxicol. 90, 102–108.
González, S., López-Roldán, R., Cortina, J.-L., 2012. Presence and biological effects of emerging contaminants in Llobregat river basin: a review. Environ. Pollut. 161, 83–92.
Gorga, M., Insa, S., Petrovic, M., Barceló, D., 2015. Occurrence and spatial distribution of EDCs and related compounds in waters and sediments of Iberian rivers. Sci. Total Environ. 503–504, 69–86.
Grung, M., Källqvist, T., Sakshaug, S., Skurtveit, S., Thomas, K.V., 2008. Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. Ecotoxicol. Environ. Saf. 71, 328–340.
Guasch, H., Navarro, E., Serra, A., Sabater, S., 2004. Phosphate limitation influences the sensitivity to copper in periphytic algae. Freshw. Biol. 49, 463–473.
Hill, W.R., Mulholland, P.J., Marzolf, E.R., 2001. Stream ecosystem responses to Forest leaf emergence in Spring. Ecology 82, 2306–2319.
Izagirre, O., Agirre, U., Bermejo, M., Pozo, J., Elosegi, A., 2008. Environmental controls of whole-stream metabolism identified from continuous monitoring of Basque streams. J. North Am. Benthol. Soc. 27, 252–268.
Jackson, M.C., Loewen, C.J.G., Vinebrooke, R.D., Chimimba, C.T., 2016. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob. Chang. Biol. 22 (11), 180–189.
Koelmans, A.A., Van der Heijde, A., Knijff, L.M., Aalderink, R.H., 2001. Integrated modelling of eutrophication and organic contaminant fate and effects in aquatic ecosystems. A Rev. Water Res. 35, 3517–3536.
Kuzmanović, M., Ginebreda, A., Petrović, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. Sci. Total Environ. 503, 289–299.
Liess, A., Hillebrand, H., 2006. Role of nutrient supply in grazer-periphyton interactions: reciprocal influences of periphyton and grazer nutrient stoichiometry. J. North Am. Benthol. Soc. 25, 632–642.
Morin, S., Pesce, S., Tlili, A., Coste, M., Montuelle, B., 2010. Recovery potential of periphytic communities in a river impacted by vineyard watershed. Ecol. Indic. 10, 419–426.
Muñoz, I., López-Doval, J., Ricart, M., Villagrassa, M., Brix, R., Geiszinger, A., Ginebreda, A., Guasch, H., de Alda, M.L., Romaní, A.M., Sabater, S., Barceló, D., 2009. Bridging levels of pharmaceuticals in river water with biological community structure in the Llobregat river basin (northeast Spain). Environ. Toxicol. Chem. 28, 2706–2714.
Navarro-Ortega, A., Acuña, V., Bellin, A., Burek, P., Cassiani, G., Choukr-Allah, R., Dolédec, S., Elosegi, A., Ferrari, F., Ginebreda, A., Grathwohl, P., Jones, C., Rault, P.K., Kok, K., Koundouri, P., Ludwig, R.P., Merz, R., Milacic, R., Muñoz, I., Nikulin, G., Paniconi, C., Paunović, M., Petrovic, M., Sabater, L., Sabaterb, S., Skoulikidis, N.T., Slob, A., Teutsch, G., Voulvoulis, N., Barceló, D., 2015. Managing the effects of multiple stressors on aquatic ecosystems under water scarcity. The GLOBAQUA project. Sci. Total Environ. 503–504, 3–9.
Niyogi, D.K., Koren, M., Arbuckle, C.J., Townsend, C.R., 2007. Stream communities along a catchment land-use gradient: subsidy-stress responses to partoral development. Environ. Manage 39, 213–225.
Odum, E.P., Finn, J.T., Franz, E.H., 1979. Perturbation theory and the subsidy-stress gradient. Bioscience 29, 349–352.
Paine, R.R.T., Tegner, M.M.J., Johnson, E.A., 1998. Compounded perturbations yield ecological surprises. Ecosystems 1, 535–545.
Pal, A., Gin, K.Y.-H., Lin, A.Y.-C., Reinhard, M., 2010. Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. Sci. Total Environ. 408, 6062–6069.
Piggott, J.J., Townsend, C.R., Matthaei, C.D., 2015. Reconceptualizing synergism and antagonism among multiple stressors. Ecol. Evol. 5, 1538–1547.
Ricart, M., Guasch, H., Alberch, M., Barceló, D., Bonnineau, C., Geiszinger, A., Farré, M., Ferrer, J., Ricciardi, F., Romaní, A.M., Morin, S., Proia, L., Sala, L.,

Sureda, D., Sabater, S., 2010. Triclosan persistence through wastewater treatment plants and its potential toxic effects on river biofilms. Aquat. Toxicol. 100, 346–353.
Roessink, I., Koelmans, A.A., Brock, T.C.M., 2008. Interactions between nutrients and organic micro-pollutants in shallow freshwater model ecosystems. Sci. Total Environ. 406, 436–442.
Rosi-Marshall, E.J., Kincaid, D.W., Bechtold, H.A., Royer, T.V., Rojas, M., Kelly, J.J., 2013. Pharmaceuticals suppress algal growth and microbial respiration and alter bacterial communities in stream biofilms. Ecol. Appl. 23, 583–593.
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. Anal. Bioanal. Chem. 387, 1425–1434.
Schmitt-Jansen, M., Veit, U., Dudel, G., Altenburger, R., 2008. An ecological perspective in aquatic ecotoxicology: approaches and challenges. Basic Appl. Ecol. 9, 337–345.
Stelzer, R.S., Heffernan, J., Likens, G.E., 2003. The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. Freshw. Biol. 48, 1925–1937.
Timoner, X., Acuña, V., von Schiller, D., Sabater, S., 2012. Functional responses of stream biofilms to flow cessation, desiccation and rewetting. Freshw. Biol. 57, 1565–1578.
Traas, T.P., Janse, J.H., Van den Brink, P.J., Brock, T.C.M., Aldenberg, T., 2004. A freshwater food web model for the combined effects of nutrients and insecticide stress and subsequent recovery. Environ. Toxicol. Chem. 23, 521–529.
Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, a, Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. Nature 467, 555–561.
Wagenhoff, A., Lange, K., Townsend, C.R., Matthaei, C.D., 2013. Patterns of benthic algae and cyanobacteria along twin-stressors gradients of nutrients and fine sediments: a stream mesocosm experiment. Freshw. Biol. 58, 1849–1863.
Wagenhoff, A., Townsend, C.R., Matthaei, C., 2012. Macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients: a stream mesocosm experiment. J. Appl. Ecol. 49, 892–902.
Wagenhoff, A., Townsend, C.R., Phillips, N., Matthaei, C.D., 2011. Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. Freshw. Biol. 56, 1916–1936.
Woodward, G., Gessner, M.O., Giller, P.S.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B.G.G., Tiegs, S.D.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, V., Graca, M.A.S., Fleituch, T., Lacoursiere, J.O.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.-M., Chauvet, E., Graça, M., Fleituch, T., Lacoursiere, J.O.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.-M., Chauvet, E., 2012. Continental-Scale effects of nutrient pollution on stream ecosystem functioning. Science 336, 1438–1440 (80-. ).