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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