Does Grazing Pressure Modify Diuron Toxicity in a Biofilm Community?

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Abstract Herbicides affect the structure and functional parameters of fluvial biofilm. Diuron is toxic to primary producers and disrupts endocrine activity. Here, we studied the interaction between this toxicant and several biological compartments in a simple food chain composed of herbivores (the snail *Physella [Costatella] acuta*) and biofilm. We used indoor experimental channels to which Diuron was added at a realistic concentration (2 μg/L). Bacterial survival and chlorophyll-*a* and photosynthetic activity were analyzed in the biofilm. We monitored biomass, mortality, reproduction, and motility as end points in the freshwater snail *P. acuta*. Our results showed that bacterial survival and photosynthetic activity were sensitive to Diuron. Snails were not affected by the herbicide at the concentration tested. No significant interactions between the toxicant and grazers were observed on the biofilm. Reproductive traits, however, were slightly affected, indicating a possible endocrine disruption.

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Herbicides are among the most common pollutants in rivers. They reach these water courses via runoff from crop areas, spray drift, leaching, or accidental spills (Thurman et al. 1991). Diuron concentrations in European rivers range between 0.01 and 36 μg/L (Azevedo et al. 2001; Dorigo et al. 2007; Pesce et al. 2006; Tlili et al. 2008). Rodríguez-Mozaz et al. (2004) reported a maximum 0.239 μg/L concentration of Diuron in the Llobregat River, although its presence and concentration vary with the season and rain intensity and frequency. Diuron (*N*-[3,4-dichlorophenyl]-*N*,*N*-dimethylurea; CAS No. 330-54-1) is a biologically active pollutant that belongs to the phenylamide family of herbicides. It is a nonionic compound with moderate water solubility (42 mg/L at 20°C) and a moderate octanol–water partition coefficient (log $K_{ow} = 2.6$), as well as a negligible rate of hydrolysis at neutral pH. These chemical features enhance its toxicity to primary producers. Diuron inhibits photosynthesis by blocking electron transport in photosystem II (PSII) and the formation of ATP and NADPH (Corbett 1974; Hayes 1975).This mode of action affects nontarget organisms like river algae and, by extension, river biofilms. Biofilms can be defined as a biological community of microorganisms and associated microfauna that are attached to a surface and embedded in a mucopolysaccharide matrix (Lock 1993). According to various studies, Diuron inhibits photosynthesis in biofilms at a concentration of 4.7 μg/L in marine periphyton (Molander and Blanck 1992), at 7 μg/L in the green alga *Raphidocelis subcapitata* (Ma et al. 2006), and at 10 μg/L in freshwater biofilms (Arrhenius et al. 2004). Recent studies have also shown that nontarget organisms inhabiting biofilm communities, such as bacteria, might be affected by this toxicant. Pesce et al. (2006) demonstrated that 10 μg/L affected the diversity of a natural riverine bacterial community. A reduction of bacterial abundance and an increase in

leucine–aminopeptidase activity was described by Ricart et al. (2009) in biofilms exposed to exponentially increasing concentrations of Diuron (from 0.07 to 710 μg/L).

In running water, biofilms uptake dissolved and particulate matter and, therefore, take part in the self-purification capacity of rivers. Biofilms also have an important role as primary producers and as a food resource for freshwater grazers, so toxicant effects could be perceived in higher levels of the river ecosystem.

The toxic effects of Diuron have also been reported on freshwater invertebrates, although at higher concentrations. The growth and survival of worms are affected by a Diuron concentration of 3.5 mg/L, whereas midge growth decreases at 7.1 mg/L and amphipod mortality occurs at 28.5 mg/L (Nebeker and Schuytema 1998). The LC_{50} for Diuron was found to be 15.3 mg/L in *Lymnaea* spp. after a 96-h exposure (Christian and Tate 1983). At a concentration of 3.6 mg/L in a 24-h experiment, Diuron caused mortality in a stonefly population (Sanders and Cope 1968). This herbicide has also been reported to disrupt endocrine activity in recombinant yeast assays at much lower concentrations (0.26 mg/L) (Noguerol et al. 2006), which indicates that Diuron might have sublethal effects on several animals.

We assessed the effects of an environmental concentration of Diuron on biofilm structure and function in the presence of a grazer, the freshwater snail *Physella (Costatella) acuta* (Draparnaud, 1805), which is common in the rivers of Catalonia and in other European rivers. Mollusks are well-known biofilm consumers (Hunter 1980); they are also sensitive to endocrine disruptors (Matthiessen and Gibbs 1998).

Our working hypothesis was that grazing produces synergistic effects on the toxicant in biofilms that enhance its action. Previous experiences support this hypothesis: Muñoz et al. (2001) described the metabolic and structural changes of algae in biofilms due to the interaction of the herbicide atrazine at 14 μg/L and grazing in an 18-day experiment. Biofilm biomass accumulation protects against toxicants due to layers of polysaccharide-rich materials (Sabater et al. 2007). Grazing might regulate this accumulation by eliminating overlaying and senescent cells and exposing younger biofilm layers to the toxicant, as well as inhibiting any adaptation of the organisms to the toxicant. We monitored the dynamics of algae and bacteria in experimental artificial channels, as well as the growth, behavior, and reproductive ability of the grazer. The experimental design aimed to clarify how fluvial biofilms subjected to grazing are affected by realistic concentrations of Diuron and thereby help to provide reliable predictions of the impact of herbicides on natural river ecosystems.

Material and Methods

The experiment was conducted in a system of 12 indoor artificial Perspex channels (100 cm long, 10 cm wide, and 10 cm high) over 2 months. Each channel received 8 L of dechlorinated tap water. This water was circulated from glass tanks at a flux of 0.024 ± 0.004 L/s and the velocity in the channels was 1.2 ± 0.21 cm/s. The water was propelled from the tanks to the channels by means of submersible water pumps (EHEIM compact 1000) and returned to the tank by gravity. Tank water was replaced twice a week and kept refrigerated at 18°C. Photosynthetically available radiation was provided by fluorescent lamps (PHILIPS TLD 36 W/540). The lamps provided 120 $\mu E/m^2/s$ in a 12-h light/12-h dark regime.

Experimental Design

The bottom of each channel was covered with sandblasted glass substrata, which were used for algal colonization. Large glass plates (12 × 9 cm) were interspersed between several rows of small glass tiles (1.2 × 1.2 cm). The latter were used as sampling units for biofilm parameters, whereas the larger plates were used to ensure enough biofilm material for snail nutrition. The glass tiles used for the analyses were replaced with analogous but noncolonized glass tiles in order to prevent physical stress for the snails. Before the application of treatment conditions in the channels, biofilm inoculum was added to all channels to initiate biofilm colonization. The biofilm inoculum was collected from stones from an unpolluted stream and added to the aquaria twice a week during the colonization phase (4 weeks). Stones were sonicated and scraped; then detached biofilm was collected in 250 mL of water and a fraction of this volume was inoculated in each aquarium.

Four experimental conditions, with three replicates, were distributed at random among the 12 channels: biofilm (channels C, control condition), biofilm exposed to Diuron (channels D, to assess the effect of toxicant on biofilm), biofilm with snails (channels S, to assess the effect of herbivores), and, finally, biofilm with both snails and Diuron (channels SD, to test the interaction of grazing and toxicant).

Individual *P. acuta* freshwater snails were collected from an unpolluted stream and acclimated in a glass aquarium with dechlorinated tap water prior to the experiment. During this acclimation period, snails were fed with TetraMin fish food (Iannacone et al. 2002). After 4 weeks of biofilm colonization in the channels, 15 snails were added to the corresponding treatments (S and SD). This was considered day 0. The initial density of snails per channel was as described in Muñoz et al. (2000) in order to prevent nutritional deficiencies. The initial snail biomass

was 2.69 ± 1.11 mg dry weight, indicating that the grazers were large enough to ensure an initial population of homogeneous sexual maturity.

Diuron (Riedel-de-Haën Seelze, Germany) was added to the channels receiving toxicants (D and SD) on day 0 of the experiment at a nominal concentration of 2 μg/L. The toxicant was replaced with each water change. Diuron concentrations were analyzed twice a week before and after the water change by means of high-performance liquid chromatography–mass spectrometry (HPLC-MS) (Rodríguez-Mozaz et al. 2004). Temperature, conductivity, oxygen concentration, pH, and cation and anion concentrations were measured in the channels on an analogous regular basis. Twice a week, coinciding with each water change, and during the overall experimental period, phosphate was added in reactive soluble form to ensure availability. Ammonia levels were measured once a week in order to detect harmful concentrations.

Water samples for the HPLC-MS analysis of Diuron were preconcentrated by solid-phase extraction. Prefiltered water samples (500 mL) were preconcentrated on Li-Chrolut RP-18 cartridges (500 mg, 3 mL) from Merck. The cartridges were first conditioned with 5 mL of methanol and 5 mL of water, at a flow rate of 4 mL/min. After loading the sample at a rate of 5 mL/min, the cartridges were dried in a Baker LSE 12G apparatus (J.T. Baker, Deventer, The Netherlands) connected to a vacuum system set at –15 psi. Elution was performed using a total volume of 8 mL of methanol. The extracts were then evaporated to dryness under a stream of N_2 and reconstituted to a final volume of 300 μL with 80% acetonitrile.

Biofilm Parameters

The potential toxicity of Diuron on biofilms was assessed by determining bacterial survival, photosynthetic efficiency [(PSII) Fluorescence Yield], and chlorophyll-*a* concentrations.

The abundance and biomass of live and dead bacteria were analyzed using three randomly selected colonized glass tiles per channel. The analysis was performed at the beginning (day 1) and end (day 29) of toxicant exposure. A double staining technique (Live/Dead BacLight Bacterial Viability Kit; Molecular Probes) was used to distinguish live from dead bacteria (Boulos et al. 1999). Each tile was transferred to a previously sterilized vial filled with 10 mL of autoclaved deionized water and then sonicated for 1 min. Afterward, the samples were diluted 10 times in sterile Milli-Q water and the double stain was added. After 15 min, samples were filtered onto a black polycarbonate filter (Nuclepore Track-Etched Membrane; Whatman, UK) and frozen until observation. Counting was done with an epifluorescence microscope (Nikon Eclipse, 600 W) using required filters (450–490-nm and 510–560-nm excitation wavelength for counting live and dead bacteria, respectively) at 1000× magnification. At least 20 randomly chosen microscopy fields were counted for each glass tile. The percentage of live bacteria was calculated as the abundance of live bacteria with respect to the total (live and dead) bacterial count.

The effective quantum yield (efficiency at PSII) was measured with a MiniPAM Photosynthesis Yield Analyzer (Heinz Walz, Germany) at the end of the experiment (29 days after toxicant exposure). PSII quantum yield is defined as a measure of the photosynthetic efficiency of the community (Schreiber 2004). Five randomly collected colonized glass tiles from each channel were placed in a Petri dish filled with 10 mL of channel water. The measurements were done by the emitter–detector unit placed at the sample surface. The distance between the sample surface and the fiber-optic sensor was 2 mm. The efficiency at PSII was automatically calculated by the instrument following the procedure described by Genty et al. (1989).

The chlorophyll-*a* concentrations of the biofilm were analyzed in five colonized glass tiles, which were randomly taken from each channel on days 0, 7, 21, and 29. Pigment was extracted in 90% acetone overnight and subsequently subjected to short sonication (2 min). Concentrations were estimated from spectrophotometric measurements following the procedure of Jeffrey and Humphrey (1975).

Grazer Parameters

Snail mortality was monitored daily. The number of dead individuals was recorded and, to ensure analogous grazing pressure, these were replaced by new individuals with the same length of shell. According to regression analysis (explained below), length of shell and biomass are correlated, so all individuals added to the channels had similar biomass. New individuals were marked in order to differentiate them from those originally placed in the channels. This procedure was not carried out during the last 2 weeks of the experiment to ensure sufficient exposure to the toxicant.

Biomass was measured, in initial and final organisms, after the removal of the body from the shell and subsequent drying to constant weight at 70°C. The initial biomass was calculated from a group of eight individuals randomly selected from the initial pool. In addition, a correlation between shell length and body biomass was calculated thanks to a regression analysis that allowed biomass to be estimated without damaging the animal (data not shown). Only those snails present in the channels from the beginning of the experiment were used to estimate the final population biomass.

Snail motility was selected as a behavioral parameter to indicate the possible sublethal effects of Diuron. Motility

was estimated as the distance covered by a snail in a fixed time and is expressed as speed (cm/s). For this purpose, a reticulated template with squares of 5 × 5 mm was placed on the bottom of each channel, and the speed of four randomly chosen snails per channel was calculated. Motility was analyzed on days 18 and 29.

The total number of clutches and the number of eggs per clutch were analyzed to assess the potentially disruptive effects of Diuron on endocrine activity. The channels were observed daily in order to detect and note new clutches. At the end of the experiment, all clutches were carefully removed from each channel in order to count the number of eggs with a stereomicroscope. To study embryological development, the clutches were monitored for 2 more weeks under the same treatment conditions; clutches were observed twice a week.

Statistical Analysis

The effects of Diuron on chlorophyll-*a* and the percentage of live bacteria were analyzed by means of analysis of variance (three-way ANOVA) with time, toxicant, and the presence of grazers as factors. Differences in photosynthetic efficiency were analyzed using two-way ANOVA, the factors being toxicant and grazing. Differences in mortality, number of clutches, number of eggs per clutch, and biomass were determined by one-way ANOVA, with toxicant as the factor, and by two-way ANOVA for motility, with time and toxicant as factors. Statistical significance was set at $p < 0.05$ and the analyses were performed using the SPSS program.

Results

Physicochemical Parameters

Oxygen, pH, temperature, and conductivity of the water remained constant throughout the experiment (Table 1). Nutrient concentrations decreased 3 days after replacing the water in the channels. Initial concentrations were 0.012 ± 0.005 mg/L for P-PO_4 and 1.944 ± 0.183 mg/L for N-NO_3. After 3 days, nutrient consumption was evident, with concentrations of 0 mg/L for P-PO_4 and 0.087 ± 0.118 mg/L for N-NO_3. Concentrations of ammonia in the channels were between 0.025 and 0.05 mg/L.

Diuron concentrations ranged between 2 ± 0.76 µg/L a few hours after adding the herbicide and 0.6 ± 0.15 µg/L before the water change 3 days later.

Biofilm Parameters

The herbicide significantly affected photosynthetic efficiency (Fig. 1). The algal PSII fluorescence yield decreased after 29 days ($F = 204.089$, $p < 0.0001$, df = 1) in the channels receiving Diuron. However, grazing caused a significant increase in the PSII fluorescence yield ($F = 10.361$, $p = 0.002$ df = 1) at the end of the exposure period. Grazers did not enhance the negative effect of Diuron on photosynthetic efficiency.

Table 1 Water characteristics during the experiment; mean value and standard deviation (shown in parentheses)

	O_2 (mg/L)	Cond (µS/cm)	T (°C)	pH
Control	9.37 (0.55)	490.53 (81.48)	18.61 (1.32)	8.47 (0.27)
Diuron	9.50 (0.50)	490.18 (81.17)	18.52 (1.39)	8.45 (0.16)
Snails	9.48 (0.49)	485.18 (76.84)	18.49 (1.30)	8.47 (0.21)
Diuron + snails	9.53 (0.48)	492.02 (82.07)	18.47 (1.37)	8.44 (0.16)

Chlorophyll-*a* concentrations increased over time. These increases were significant in all treatments from day 21 ($F = 13.98$, $p < 0.0001$, df = 3). Treatments with grazers showed significantly lower chlorophyll concentrations ($F = 4.385$, $p = 0.04$, df = 1) at the end of the experiment (Fig. 2). However, neither the presence of the toxicant ($F = 0.07$, $p > 0.05$, df = 1) nor the combination of grazing and toxicant ($F = 0.03$, $p > 0.05$, df = 3) significantly affected chlorophyll-*a* concentrations.

After 1 day of exposure (Fig. 3a), total bacteria ranged from 163 × 10^6 ± 49 × 10^6 (S channels) to 278 × 10^6 ± 29 × 10^6 (D channels) bacteria/cm^2. The percentage of dead bacteria was higher in treatments with toxicant (D and SD) than without ($F = 14.612$, $p = 0.022$, df = 1). On day 28 (Fig. 3b), total bacteria ranged from 170 × 10^6 ± 1 × 10^6 (SD channels) to 205 × 10^6 ± 70 × 10^6 (C channels)

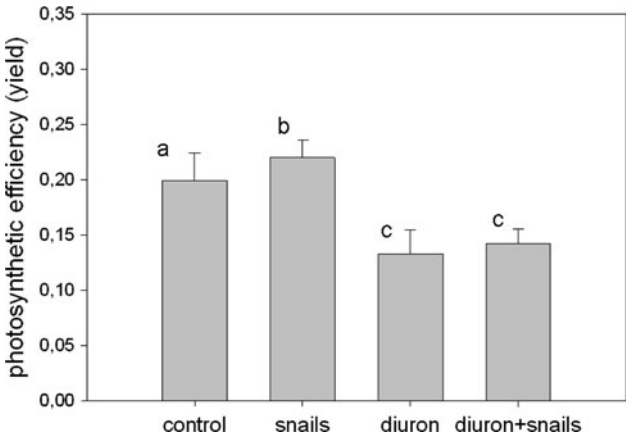

Fig. 1 Photosynthetic efficiency measured as yield in PhytoPAM. On day 29, snails enhanced photosynthetic efficiency, whereas Diuron showed negative effects. *Letters* indicates significance; *bars* with *different letter* indicate significant differences

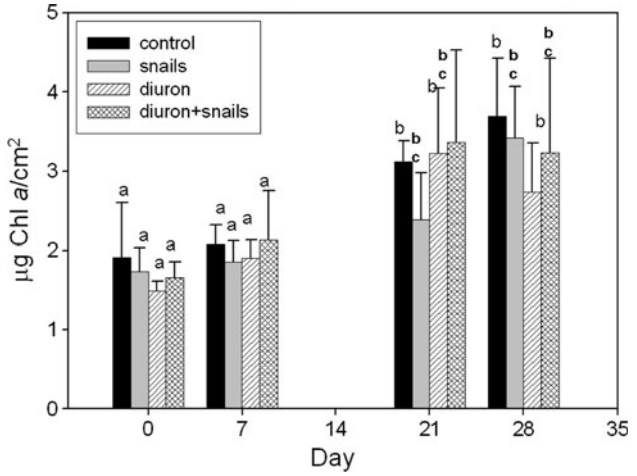

Fig. 2 Mean chlorophyll-*a* concentrations and standard deviations during the experiment. Despite the general increase in chlorophyll-*a* concentrations, in the last 2 weeks lower concentrations were observed in channels with snails (SD and S). Bars with *different letter* indicate significant differences

bacteria/cm^2 and a recovery in the bacterial communities of exposed channels was observed, so the percentage of dead bacteria was well above 50% and similar in all treatments ($F = 0.753$, $p = 0.002$, df = 1). Grazing activity did not produce significant effects on bacteria, neither at the beginning nor at the end of the experiment.

Grazer Parameters

Physella acuta did not show higher mortality in response to the addition of Diuron ($F = 3.769$, $p > 0.05$, df = 1). Snail biomass was not significantly different between treatments ($F = 0.085$, $p > 0.05$, df = 1). The initial biomass was 2.69 ± 1.11 mg and reached 2.73 ± 0.30 mg and 2.66 ± 0.33 mg in S and SD channels, respectively, by the end of the experiment; no significant differences between initial and final biomass were found ($F = 0.003$, $p > 0.05$, df = 1). In Table 2, we show the mean velocity of snails for each treatment, measured on two different days; this parameter did not show significant differences between treatments ($F = 1.282$, $p > 0.05$, df = 1) and days ($F = 3.157$, $p > 0.05$, df = 1).

The mean number of clutches in S and SD channels was 5.33 ± 1.53 and 2.67 ± 2.08, respectively. The mean number of eggs per clutch at the end of the experiment was 17.12 ± 6.35 and 22.20 ± 1.71 in S and SD channels, respectively (Table 3). The differences between treatments were not significant for either parameter ($F = 3.2$, $p > 0.05$, df = 1and $F = 1.79$, $p > 0.05$, df = 1, respectively). In spite of this lack of statistical significance, the number of clutches was lower but the egg *per* clutch ratio was higher in the channels receiving the toxicant.

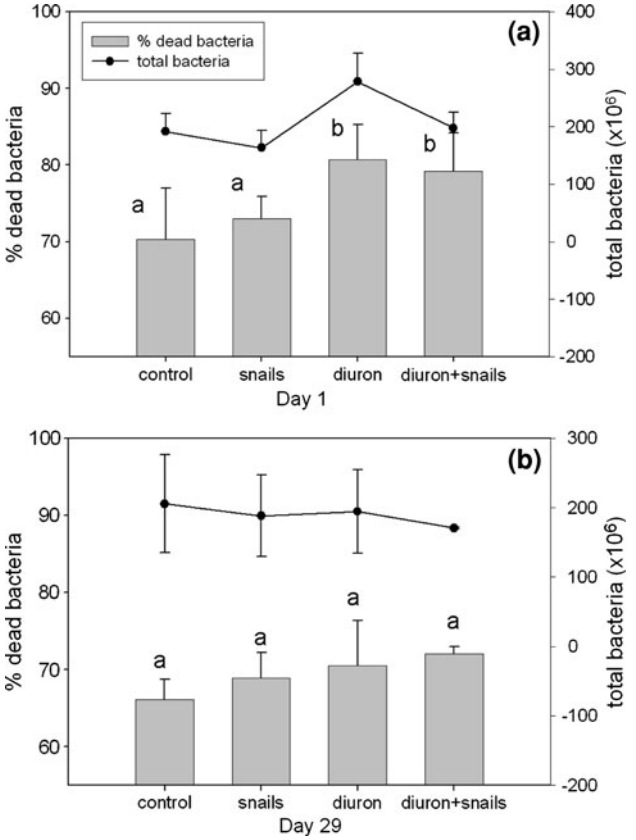

Fig. 3 Total bacteria (*line*) and percentage of dead bacteria (in *bars* showing mean and standard deviation). **a** On day 1, channels with Diuron showed a significant increase in the percentage of dead bacteria. **b** On day 29, the percentage of dead bacteria is similar in all treatments and lower in treatments with Diuron compared to day 1. Bars with the same letter indicate significant similarity

Table 2 Snail motility (measured as velocity cm/s) on days 18 and 29

	Condition	Mean velocity (cm/s) (SD)
Day 18	Snails	5.35 (3.25)
	Diuron + snails	5.72 (2.78)
Day 29	Snails	4.40 (2.89)
	Diuron + snails	4.65 (1.59)

Table 3 Mean number of egg clutches and number of eggs/clutch in channels with snails but without toxicant and with snails and toxicant; standard deviation in parentheses

Condition	No. of clutches	No. of eggs/clutch
Snails	5.33 (1.53)	17.12 (6.35)
Diuron + snails	2.67 (2.12)	22.20 (1.71)

During clutch monitoring, 19% and 25% of clutches in the S and SD channels, respectively, showed eggs with well-formed snails. The rest of the clutches had eggs

without evidence of segmentation or the blastulas had few cells.

Discussion

The concentration of Diuron chosen in our experiment (2 ± 0.76 µg/L) reproduces a near-natural scenario, similar to that found in rivers and to its potential effects on biofilm. Although the pesticide was continuously added to our experimental channels, exposure could be considered discontinuous because the community was subjected to spikes of maximum concentration every 2–3 days. A decrease in Diuron concentrations in experimental systems has been described in several studies (Hayes 1975; Sumpono et al. 2003). Pesce et al. (2006) hypothesized that the decrease in herbicide concentrations is caused by molecular adsorption or absorption to surfaces. Following this line of thought, Diuron loss in our experiments would be related to adsorption in the channels or tube surfaces and to biological activity. These observations highlight the relevance of continuous toxicant addition and monitoring to ensure exposure.

Diuron affected photosynthetic efficiency (effect visible after 29 days). This effect has previously been reported only in experiments of similar duration but with higher concentrations (Arrhenius et al. 2004; Molander and Blanck 1992). In our experimental design, it is possible that prolonged exposure contributed to the damage and dysfunction of the photosynthetic system despite the low toxicant concentration. Grazing contributed to enhanced biofilm photosynthetic activity as previously described in several studies (Kaehler and Froneman 2002; Steinman 1996). Grazing induced change in species composition, resetting the community to the earliest successional states and enhancing the fast-growing opportunistic taxa.

Our results indicate that at the tested concentration, this toxicant did not have significant effects on biofilm chlorophyll-*a* concentrations; on the contrary, these concentrations increased throughout the experiment. Hartgers et al. (1998), in an experiment with three herbicides, including Diuron, found a significant increase in chlorophyll-*a* concentrations at day 14. These authors related this finding to an increase in the abundance of several tolerant species. Ricart et al. (2009) also described an increase in chlorophyll concentrations in biofilms after exposure to Diuron. Molander and Blanck (1992) and Tlili et al. (2008) found similar results in marine and freshwater biofilms, respectively, exposed for 3–4 weeks to low concentrations of Diuron. In our study, a taxonomic determination was not done, but the selection of resistant taxa, in sufficient numbers to make the chlorophyll concentration equivalent in exposed and unexposed channels, would also be possible. Another hypothesis, in addition to a change in community, is a physiological change of exposed algae. Teisseire et al. (1999) found, in the aquatic plant *Lemna minor*, an increment of chlorophyll-*a* induced by Diuron related to the formation of shade-type chloroplasts; a response that was also observed in biofilms (Bérard and Pelte 1999). These chloroplasts are characterized by ultrastructural modifications such as broader grana and a higher stacking degree of thylakoids and were less efficient in photosynthetic quantum conversion (Liechtenthaler et al. 1980). Only the snails' grazing activity produced a decrease in chlorophyll concentrations at the end of our experiment.

The percentage of bacterial survival was also affected by Diuron but not by grazing activity. Channels with this herbicide immediately recorded higher bacterial mortality, although densities recovered by the end of the experiment. The recovery of bacterial density could be due to the selection of resistant strains. Dorigo et al. (2007) described how biofilm bacterial diversity shifted in response to pesticide contamination in rivers. The two samplings programmed in the present work were probably insufficient to detect the real toxicant effects on this compartment. With this information alone, it was not possible to know if the abundance decrease observed at the beginning of the experiment was punctual or became permanent over a period of time.

Diuron did not directly affect the snail *P. acuta*. Mortality, biomass, and motility did not significantly change with respect to the control treatment; neither did the herbicide significantly affect the number of clutches and number of eggs per clutch, which, however, showed distinct averages. Snails exposed to Diuron laid fewer clutches but with more eggs than nonexposed snails. Previous Diuron toxicity experiments on pulmonate snails determined that this herbicide exerts a toxic effect at concentrations higher than that tested in our experiment (Christian and Tate 1983; Nebeker and Schuytema 1998). Therefore, although the concentration in our study did not have lethal effects, it could have sublethal effects on reproductive traits, perhaps by disrupting endocrine activity. Noguerol et al. (2006) reported endocrine-disrupting effects from 0.26 mg/L of Diuron in *in vitro* experiments. Other substances at much higher toxicant concentrations have reportedly had sublethal effects on snails and their reproductive parameters. Oehlmann et al. (2000) observed a greater number of clutches and eggs in individuals exposed to 25 µg/L of bisphenol A and octylphenol compared to controls. Czech et al. (2001) observed a significant decrease in the reproductive parameters of *Lymnaea stagnalis* exposed to 100 µg/L of nonylphenol. In both experiments, the exposure period was longer than 1 month.

Finally, in contrast to our initial hypothesis, no significant interactions were detected between toxicant and

grazers affecting chlorophyll-*a* concentrations, photosynthetic efficiency, or bacterial survival; only the reduction of photosynthetic efficiency can be attributed to the presence of Diuron. Therefore, no additive or synergic effects between the two factors occurred. Grazing activity regulates biofilm biomass and actively removes overlaying and senescent cells from the biofilm, thereby facilitating toxicant exposure (Steinman 1996). In this regard, Muñoz et al. (2001) found that grazing and the herbicide atrazine (14 μg/L) had additive effects on photosynthetic activity, measured as ^{14}C incorporation. Nonetheless, the cited study was different respect to pesticide type and concentration. Perhaps the lower concentration tested in the present work was not enough to enhance synergistic effects of grazing and toxicant on biofilm photosynthetic efficiency.

This study contributes to our knowledge of the effects of Diuron, at realistic concentrations, on related organisms in a food chain: biofilm components and herbivores. In natural systems, organisms are generally exposed to a mixture of pollutants; transposing such conditions is difficult but experimental studies like the present open the way for a better interpretation of the effects of toxicants on the biological community.

Conclusion

To date, the concentration of Diuron tested in this experiment is the lowest reported to affect biofilms; it is a realistic concentration that can be found in freshwater ecosystems. Our results indicate that at a concentration of 2 μg/L, Diuron is harmful to freshwater biofilms; however, its effect on invertebrates is unclear. No clear interaction between toxicant and grazer on the biofilm was found. Nevertheless, Diuron might alter the quality and abundance of biofilm and reduce its bioavailability for subsequent trophic levels.

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