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Impacts of agricultural irrigation on nearby freshwater ecosystems: The seasonal influence of triazine herbicides in benthic algal communities

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HIGHLIGHTS

- Agricultural impacts via run off impacted on nearby freshwater algal communities.
- Triazine herbicides impacts changed through the agricultural year.
- Traditional methods do not capture the seasonality of triazine concentrations.
- Algal tolerance informed about the source of herbicides: application vs. background.
- Assessment of herbicide tolerance is a promising monitoring tool.

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ABSTRACT

A small hydrological basin (Lerma, NE Spain), transformed from its natural state (steppe) to rain-fed agriculture and recently to irrigation agriculture, has been monitored across four seasons of an agricultural year. The goal of this study was to assess how and whether agricultural activities impacted the nearby freshwater ecosystems via runoff. Specifically, we assessed the toxicity of three triazine herbicides, terbuthylazine, atrazine and simazine on the photosynthetic efficiency and structure of algal benthic biofilms (i.e., phototropic periphyton) in the small creek draining the basin. It was expected that the seasonal runoff of the herbicides in the creek affected the sensitivity of the periphyton in accord with the rationale of the Pollution Induced Community Tolerance (PICT): the exposure of the community to pollutants result in the replacement of sensitive species by more tolerant ones. In this way, PICT can serve to establish causal linkages between pollutants and the observed biological impacts.
The periphyton presented significantly different sensitivities against terbuthylazine through the year in accord with the seasonal application of this herbicide in the crops nowadays. The sensitivity of already banned herbicides, atrazine and simazine does not display a clear seasonality. The different sensitivities to herbicides were in agreement with the expected exposures scenarios, according to the agricultural calendar, but not with the concentrations measured in water, which altogether indicates that the use of PICT approach may serve for long-term monitoring purposes. That will provide not only causal links between the occurrence of chemicals and their impacts on natural communities, but also information about the occurrence of chemicals that may escape from traditional sampling methods (water analysis). In addition, the EC50 and EC10 of periphyton for terbuthylazine or simazine are the first to be published and can be used for impact assessments.

1. Introduction

Agriculture uses half of the total land in Europe (Stoate et al., 2009). Traditional agriculture has been replaced by intensive works which maximize the impact on the soil and nearby freshwater ecosystems (De Almeida Azevedo et al., 2000; Loos et al., 2009; Arroita et al., 2013). One way to increase crop production is by implementing irrigation, which affects both physical (altering water flow) and chemical (altering nutrient and pollutant concentrations) conditions in rivers by the runoff of excess waters (Abrahao et al., 2011a, 2011b; Merchán et al., 2013). In the Mediterranean climate irrigation is more intense during spring and summer, in those seasons natural rivers have lower flows so the impacts of the runoff waters from irrigation may be maximized.

This study is focused on the impacts of the triazine family of herbicides which is widely used in Europe. Due to environmental concerns, some triazines have been banned (such as atrazine, simazine and propazine European Commission (SANCO/10496/2003-final), 2003;

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European Commission (SANCO/10495/2003-final), 2003). However, these herbicides are still detected in the environment together with the triazines in use nowadays such as terbuthylazine. This is due to their long retention time in the soil and aquifers, which leads to perdurable leaching and long-lasting levels in different ecosystems even years after their prohibition.

The Lerma basin (within Ebro depression in Spain), recently transformed from its natural state (gypsum soils covered by scrubland and steppe-like vegetation) to rain-fed agriculture, has been monitored during a whole agricultural season (one year). The goal of this study was to assess the impacts of triazines from agricultural runoff on the function and structure of algal benthic communities present in the small creek draining the basin (periphyton). In previous years, the creek contained noticeable concentrations of both atrazine and simazine (banned) and terbuthylazine (still in use). These data are available at the website of the Ebro Hydrological Confederation (www.chebro.es).

Among the various methods and tools available to assess the impact of pollutants, the Pollution Induced Community Tolerance (PICT) approach offers the means to partially isolate and identify the effects of individual toxicants within an ecosystem subjected to multiple stressors. The rationale behind the PICT is that the exposure to a toxicant will eliminate or hinder the success of sensitive species and favor the development of the more tolerant ones (Blanck et al., 1988), and this will be measured as an increase of the community tolerance against such toxicant.

The hypothesis of this work was that the different exposures to the herbicides through the year due to the seasonal agriculture practices would result in changes in the algal community. The sensitivity of the algal communities to the same herbicide in different seasons would depend on the exposure during the growing period. A previous similar seasonal study showed that the structural and functional responses of algal communities to pesticides are likely to reflect past selection pressures (Dorigo et al., 2004).

The sources of herbicides were expected to be: a) the background released from soils (for the two banned herbicides, atrazine and simazine), or b) the direct application on irrigated crops (for terbuthylazine). Triazine herbicide main application takes place at the beginning of spring just after the seeding of the summer cereals. The applications can be extended until the plants reach a certain growth stage, about 30 cm tall in the case of corn, which is the main crop of the region. As a result, a peak of herbicide discharge is usually registered in spring and summer in the Ebro basin yearly (www.chebro.es). In the period of study these applications were between the end of April to mid May (information provided by local farmers).

2. Materials and methods

2.1. Study site

The study was carried out in a small agricultural basin, Lerma (7.3 km^2) located in the Ebro hydrological basin (north-east of Spain). Here 49% of the area has been transformed from natural steppe to crops dominated by corn (40%) and winter cereals (18%). Agriculture works are the only anthropogenic activity in the watershed, which offers an exceptional opportunity to study the impact of agricultural use of soil in the water quality (Pesce et al., 2008).

2.2. Physicochemical water analysis

Water flow (L/s), temperature (°C) and nitrate concentration (NO_3^-, mg/L) of the creek were measured in-situ by a water quality station (from Geological Survey of Spain, IGME). Water pH was measured in situ periodically with a multi-parameter probe (model Pro-Plus from YSI, USA). The concentration of the six main macro nutrients applied in soil fertilization were analyzed from periodical water samplings (4–6 per season), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S).

2.3. Algal biofim sampling and analysis

Benthic algal communities grew on artificial substrates placed on a creek downstream of the basin at different agricultural time periods: pre-herbicide application (autumn and winter), mid-herbicide application (spring) and post application (summer). Communities grew on artificial substrata (small pieces of methacrylate 7.55 × 2 × 0.15 cm, similar to microscopy glass slides) that were fixed in plastic holders. These were anchored to rocks on the middle of the creek bed at ca. 15 cm depth. The substrates were placed at the same point every season. They were removed when the algal biofilm reached an average thickness of around 0.75 mm and a steady-state fluorescence of photosystem II of 1000 (Fs PAM quantification at constant light, measured by a Mini-PAM of Walz©). This ensured that the toxicity tests were performed on algal biofilm communities of similar biomass and physical dimensions.

Chlorophyll *a* content was analyzed with Jeffrey and Humphrey's (1975) method and calculated with the adjusted formula of Ritchie (2006). Taxonomic identification was done in three replicate samples using a light microscope. Cells were counted according to Utermöhl (1958) technique. Diversity indices, Shannon and Weaver (1963) and the inverse of Simpson index, D or $1 - \lambda$ (Lande, 1996) were calculated. The abundance of species in each community was calculated based on the number of individuals of each species and the total number of individuals in the community. Species representing less than 1% of total abundance were not included in the analyses.

2.4. Herbicide analysis

Two different methods were used to assess the herbicide levels in the water: passive sampling with Chemcatcher® devices and discrete water samples. 1 L of water was collected at the end of each sampling period to analyze the concentration of triazines and 10 other pesticides by chromatography (SBSE/GC/MS/HPLC). Moreover, three passive samplers of Chemcatcher (Kingston et al., 2000) were placed in the creek close to the algal biofilms on the creek (Fig. 1). Each Chemcatcher device consists of a sampler disk (Empore® 3M, SDB-RPD, stryrene-divinylbenzene-reverse phase sulfonated; 47 mm Ø, 145 µm thick, 8 nm pore size) covered by a protective membrane (Supor® 200, PALL, 47 mm Ø, 145 µm thick, 0,2 µm pore size) fitted into a methacrylate holder by grabbing its edges. The disks integrated the pesticides present in the water into its matrix over a certain exposure time, whereas the protective membrane acted as a diffusion-limiting media and minimized bio-fouling due to its low-protein binding properties (Schäfer et al., 2008; Vrana and Ian, 2009). The disks and protective membranes were conditioned following the procedure of Vermeirssen et al. (2009). The sampling window during winter, spring and summer period was the last 18 days of algal biofilm incubation in the creek (Fig. 1), while the sampling window during autumn was 60 days (due to problems of access to the sampling site). At the end of the sampling period, the disks were removed from the holder with forceps and submerged into 7 mL of acetone. The presence of triazines and 10 other pesticides was analyzed following Vermeirssen et al. (2009) at Labaqua Laboratories (Alicante-Spain).

2.5. Dose–response test in flow-through artificial channels

The tolerance of periphyton (measured as the effect of triazines on the photosynthetic efficiency) against each herbicide was measured in mesocosm (i.e., flow-through artificial channels) by dose–response test. The concentration of herbicide required to reduce 50% and 10% of the photosynthetic performance of the benthic algal community (EC_{50}, EC_{10}) was used to compare the community tolerance between the different seasons. The three herbicides were provided by Sigma-Aldrich in powder form. The stocks were freshly prepared two days before experimentation and stored at −20 °C. Terbuthylazine and atrazine stock solutions were prepared with acetone (32 mM) whereas simazine was dissolved in methanol (1 mM) due to its poor solubility in acetone.

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