Agriculture, Ecosystems and Environment 221 (2016) 79–86

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

# Pollution-induced community tolerance for *in situ* assessment of recovery in river microbial communities following the ban of the herbicide diuron

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ARTICLE INFO

*Article history:*
Received 12 August 2015
Received in revised form 6 January 2016
Accepted 7 January 2016
Available online 5 February 2016

*Keywords:*
Agricultural pesticide use
Biofilms
Ecological recovery
Microbial ecotoxicology
Periphyton
PICT

ABSTRACT

Changes in agricultural practices related to environmental policies such as the European Water Framework Directive should lead to a decrease in pesticide contamination levels in rivers draining agricultural areas. However, there is still a need for biological tools to evaluate resulting ecological changes, namely to evaluate ecological recovery following the improvement of surface water chemical quality. Accordingly, the main purpose of this study was to assess the relevance of a pollution-induced community tolerance (PICT) approach in natural phototrophic biofilms to estimate microbial community recovery following a decrease in diuron contamination due to the ban of the use of this herbicide on vineyards. To this end, we performed a 3-year field study (2009–2011) in the Morcille River (France), located in the French Beaujolais wine region. This river was frequently contaminated by diuron (among other organic and inorganic pesticides), with increasing concentrations downstream. Following the ban on diuron, imposed in December 2008, a progressive decrease in diuron concentrations was observed in the Morcille River. While the mean annual tolerance levels measured at the low contaminated station remained relatively constant over years, a clear and significant decreasing trend was observed at the medium and high contaminated stations during the survey. This temporal evolution revealed a direct link between a change in agricultural practice due to the ban of diuron and an increase in the sensitivity of phototrophic biofilms to this herbicide. This finding brings further new evidence that PICT has great potential to detect microbial community recovery following chemical restoration due to changes in the agricultural use of pesticides.



## 1. Introduction

In a context of increasing pressure on global water resources, the restoration of impacted aquatic ecosystems has become a major concern, prompting the EU to propose the European Water Framework Directive (WFD, 2000) that initially aimed to achieve good chemical and ecological status of water bodies by 2015. A key aspect of the WFD is to go beyond the estimation of pollutant concentration to take into account the ecological effects of pollution on ecosystem structure and functions. Even though WFD monitoring for the first river basin management plan was focussed on characterizing the present status of a water body, the ultimate aim is to assess the improvement of ecological status following restoration measures (Hering et al., 2010), which poses the scientific challenge of translating data on biotic communities into information for assessing ecological recovery.

Among other measures, specific measures were adopted to regulate chemical pollutants. A first list of 33 priority substances was thus established and many of them have since been banned in several EU states. This is the case of the photosystem II (PSII) herbicide diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), which was banned in France in December 2008 (Official Journal of the French Republic, #204; 04.09.2007) in application of EC directive 2007/417/EC. Before its ban, this herbicide was frequently used in agricultural and urban environments leading to severe contamination of surface waters. This was notably the case in small streams draining wine-growing areas (*e.g.* Louchart et al., 2001; Rabiet et al., 2010). A recent study, performed in the French Beaujolais wine region demonstrated that banning diuron, which was frequently applied to vineyard soils, resulted in a progressive decrease in diuron concentrations in a small adjacent stream (Morcille River) over a four-year survey period (Pesce et al., 2013).

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http://dx.doi.org/10.1016/j.agee.2016.01.009

The authors showed that chemical restoration following chronic diuron exposure ran paralleled with a progressive fall in sediment diuron-mineralizing capacities, revealing a corresponding biological recovery of microbial communities. This finding points to benthic microbial assemblages as useful potential indicators of ecological status as previously stated (Burns and Ryder, 2001; Sabater et al., 2007).

Several field studies have shown that *in situ* diuron exposure can enhance the tolerance of phototrophic biofilms towards diuron (Dorigo et al., 2007; Pesce et al., 2010b). This is in line with the concept of pollution-induced community tolerance (PICT), first introduced by Blanck et al. (1988), which postulates that chronic exposure to pollutants would exert a selection pressure on natural communities, thus eliminating sensitive species and thereby increasing tolerance at the community level. When studying the effects of phenylureas or other PSII-inhibitor herbicides, the PICT method is generally applied using photosynthetic activity as endpoint (Pesce et al., 2011) and, increasingly, using multiwavelength-excitation PAM fluorometry for the toxicity assessment (Schmitt-Jansen and Altenburger, 2008). PICT approaches based on short-term photosynthetic bioassays were successfully applied to assess the potential of natural phototrophic biofilms to recover after a decrease in herbicide exposure, using *in situ* translocation from polluted sites to reference sites (Dorigo et al., 2010a,b; Rotter et al., 2011). These studies showed a decrease in tolerance capacities towards the most predominant herbicide in the rivers studied (diuron and prometryn, respectively) after just a few weeks at the reference sites. Accordingly, it was concluded that biofilm recovery capacity could offer a suitable management tool for evaluating recovery processes in freshwater ecosystems, especially in PICT-based approaches (Dorigo et al., 2010a,b; Rotter et al., 2011). This is in line with the conclusion from a group of European researchers dealing with aquatic microbial ecotoxicology, who recently underlined the relevance of PICT for ecological risk assessment of chemicals in aquatic systems (Tlili et al., 2016).

However, biofilm recovery is an ecological process that is at least partially driven by immigration of non-exposed organisms (Morin et al., 2012). One limit of translocation procedures for microbial community recovery studies consists in the fact that immigration processes, and consequently recovery trajectories, are probably facilitated since the exposed biofilms are transplanted into river sections colonized by indigenous communities. This enhanced recovery in presence of non-previously-exposed (*i.e.* "pristine") communities was also clearly revealed in a microcosm study showing with a PICT approach that recovery processes (*i.e.* a decrease in level of tolerance to copper) were only observed in phototrophic biofilms in contact with pristine biofilms (Lambert et al., 2012). Moreover, recovery studies based on translocation manipulations are generally relatively short (*i.e.* several weeks) and performed in sites exhibiting marked pollution gradients. Consequently, the use of the PICT approach needs to be validated in a context of long-term and progressive change in chemical river quality, as previously shown in a marine environment (Blanck and Dahl, 1998).

Considering this background, the present study aimed to assess the relevance of PICT approaches in natural phototrophic biofilms to estimate microbial community recovery following a decrease in herbicide exposure. Accordingly, we performed a 3-year field study (2009–2011) in the Morcille River (France), which was frequently contaminated by diuron (among other organic and inorganic pesticides), with increasing concentrations along an upstream to downstream gradient (*e.g.* Montuelle et al., 2010; Rabiet et al., 2010). During the 3-year survey period, biofilm samples were collected monthly at three sampling stations distributed along the Morcille River and which are respectively located in the upstream (*i.e.* "low contaminated"), intermediate (*i. e.* "medium contaminated") and downstream (*i. e.* "high contaminated") sections. Temporal and spatial analysis of the capacity of phototrophic communities to tolerate diuron was performed using short-term photosynthetic bioassays. An extensive chemical survey was performed concomitantly to determine whether the evolution in tolerance levels reflected the post-ban decrease in diuron exposure levels in this stream.

Firstly, and according to the steady low level of diuron exposure recorded in the upstream river section, it was expected that the tolerance levels measured at this station would have been low and relatively stable over the survey period. Confirmation of this hypothesis would support the choice of considering biofilms from the upstream low contaminated station as reference communities. Secondly, we hypothesized that the tolerance of phototrophic biofilms towards diuron, which was previously shown as significantly higher in the high contaminated station when diuron was still used (Pesce et al., 2010b), would have progressively decreased in the medium and high contaminated sections in response to the decrease in diuron exposure level following the ban of its use in vineyard treatments. Confirmation of this hypothesis would support the relevance of the PICT approach in our study case. Finally, we discussed possible improvements and perspectives for the development of such approaches in water ecological quality assessment.

## 2. Materials and methods

### 2.1. Study site

The Morcille River (southeastern France, longitude 4°60′E, latitude 46°15′N) is located within the Beaujolais area, which is a large wine-producing region in France. It is a small first-order stream (7 km long), and the surface of its watershed is about 8.8 $km^2$. The head of the watershed basin is essentially covered by forest, whereas vineyard is predominant in the downstream section of the watershed, covering about 85% of the catchment's total area (Rabiet et al., 2015). The Morcille banks are well-vegetated with trees from upstream to downstream and no industrial activities are present on the site. The Morcille River flows continuously and varies widely over the year, with instantaneous water flow ranging from 0.005 $m^3/s$ (low-water period) to more than 0.5 $m^3/s$ (during storms) (Rabiet et al., 2015).

The Morcille watershed, which is part of the Long-Term Ecological Research Rhône Basin (LTER ZABR), is dedicated to the long-term study of the environmental impact of diffuse pollution by metals and pesticides (*e.g.* Dorigo et al., 2007; Montuelle et al., 2010; Rabiet et al., 2015). Even if most of chemicals enter the Morcille River *via* diffuse pollution from adjacent fields (which are separated from the river by grass buffer strips), several point sources exist, such as small agricultural ditches collecting concentrated subsurface lateral flow and drain discharges.

Heavy metal contamination in the Morcille is mainly due to copper and arsenic (Montuelle et al., 2010; Rabiet et al., 2015) with recorded concentrations remaining relatively constant over the last 10 years. Before its ban, the herbicide diuron was the most commonly used herbicide on the Beaujolais vineyards, the main application period being April–September, according to climatic conditions. Accordingly, diuron was the most predominant pesticide in the Morcille River (Rabiet et al., 2010) with dissolved concentrations being sometimes higher than 10 μg/L (Pesce et al., 2009). The Morcille River has been subjected for many years to a chronic contamination by the herbicide norflurazon, a carotenoid-biosynthesis inhibitor banned in France since 2003, and its desmethyl metabolite, revealing its persistence in the environment. In the most contaminated section of the Morcille River (*i.e.* downstream section), mean annual concentrations of norflurazon