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Transient effects following peak exposures towards pesticides – An explanation for the unresponsiveness of *in situ* measured functional variables☆

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ABSTRACT

Invertebrate-mediated leaf litter decomposition is frequently used to assess stress-related implications in stream ecosystem integrity. *In situ* measures such as the mass loss from leaf bags or the feeding of caged invertebrates deployed for days or weeks may, however, fail to detect transient effects due to recovery or compensatory mechanisms. We assessed the relevance of transient effects using the peak exposure towards an insecticide (i.e., etofenprox) as a model scenario at three levels of complexity. These were 1) the assessment of the decomposition realised by invertebrate communities in stream mesocosms over 21 days via leaf bags, 2) 7-days lasting *in situ* bioassays quantifying the leaf consumption of *Gammarus fossarum*, and 3) a laboratory experiment determining the daily feeding rate of the same species over 7 days. Etofenprox did not trigger a significantly altered decomposition by invertebrate communities during the leaf bag assay, while *in situ* bioassays detected a significant reduction in gammarids' feeding rate at the highest tested concentration. The laboratory bioassay suggests that observed mismatches might be explained by recovery and post-exposure compensation. As leaf-shredding invertebrates are likely in a vulnerable state following transient effects, biomonitoring for implications of peak exposures and other pulsed stress events must happen at an adequate temporal resolution.

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1. Introduction

The use of functional variables as a measure for the impact of stressors in the integrity of ecosystems is becoming increasingly popular. In this context, the decomposition of allochthonous organic material (i.e., leaf litter in leaf bags) is frequently used to determine effects of point and non-point sources of chemical stressors including wastewater treatment plant effluents and agricultural edge of field spray drift or run off, respectively (Englert et al., 2013; Schäfer et al., 2007). However, as multiple species and trophic levels interactively perform this ecosystem process, differing sensitivities in combination with functional redundancy within local communities might buffer the translation of behavioural or structural into functional responses (Pascoal et al., 2005; Rasmussen et al., 2012). A potential methodological solution to this is the use of *in situ* bioassays, which involve locally relevant leaf-shredding organisms caged individually or in small groups together with pre-weighed portions of leaves and thus allow only for limited interactions with the stream's community (Matthiessen et al., 1995; Schulz and Liess, 1999). These assays may be of limited ecological relevance if used as exclusive measure for impacts at the functional level. However, they might help to explain effect patterns observed during studies involving leaf bags or even to uncover incidences of stress affecting the functional performance of involved organisms that are not detectable using the leaf bag method (Englert et al., 2013).

For experimental ease and to integrate functional responses over a period of time considered relevant by the experimenter, *in situ* bioassays and leaf bags are usually deployed in the field for

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several days and weeks, respectively (Englert et al., 2013). Such durations may, however, allow for recovery or even post-exposure compensation (e.g., by increased leaf consumption) of shredders if detrimental effects are only transient (Agatz et al., 2014). Such phenomena may be particularly relevant for peak exposures towards chemical stressors, which are often observed in agricultural stream ecosystems and might explain the lack of effects in studies assessing these exposure scenarios (Rasmussen et al., 2012; Schulz, 2004).

The present study assessed the relevance of such transient effects for the quantification of functional responses as a consequence of a 6-h pyrethroid insecticide (i.e., etofenprox) peak exposure – in both mesocosm and laboratory experiments. A tiered testing approach was followed, which included in a first step the assessment of potential effects of this exposure scenario on the leaf litter decomposition realised by a complex community of invertebrates in stream mesocosms. This leaf bag study was supplemented by *in situ* bioassays (i.e., caged organisms within the mesocosms; cf. Hopkin, 1993) quantifying the mortality and feeding rate of a key species in the leaf litter decomposition in central European streams (i.e., *Gammarus fossarum*; Dangles et al., 2004). Selection of this particular species was further prompted by the reported correlation between the *in situ* measured feeding rates of gammarids and local leaf litter decomposition (Maltby et al., 2002). In a third step, the etofenprox peak exposures were simulated under laboratory conditions, while the feeding rate of *G. fossarum* was determined on a daily basis, allowing for the identification of potential post-exposure recovery and compensation (Agatz et al., 2014).

2. Materials and methods

2.1. Stream mesocosm experiment

The present investigation was part of a larger study, which was conducted at the Landau Stream Mesocosm Facility (LSMF; http://uni-ko-ld.de/hy) at the University of Koblenz-Landau, Campus Landau (Germany). The LSMF consists of 16 independent channels (length = 45 m, width = 0.4 m and average water depth = 0.26 m). The channels were equipped with artificial sediment (medium to coarse sand – 50% with a grain size of 0–0.5 mm and 50% 0.2–1.0 mm – and in total 5% vol. white peat) and aquatic macrophytes (*Elodea nuttallii* and *Myriophyllum spicatum*) from pristine streams during October 2013. Established invertebrate communities were introduced passively with leaf material and aquatic macrophytes or actively from pristine streams and covered essential functional feeding groups including shredders that are fundamental for the decomposition of leaf litter (Cummins et al., 1973). Each channel received dried leaves as food for shredders, while the amount of leaves was held constant during the entire experiment (105 ± 22 leaves/m^2).

Etofenprox is a pyrethroid insecticide, which acts as sodium channel modulator (Stenersen, 2004) and is used in cultures such as rape (BVL, 2017) and rice (Tanabe and Kawata, 2009). The substance dissipates from soils and water bodies moderately fast to fast, while it features high toxicity to aquatic animals such as *Daphnia magna* (48-h EC_{50} of 1.2 μg/L; FOOTPRINT, 2017). In the present study, etofenprox was applied as commercial formulation (Trebon 30 EC, BASF SE) using peristaltic pumps in June 2014 at nominal concentrations of 0, 0.05, 0.50 and 5.00 μg/L ($n = 4$) for 6 h. As etofenprox has already been detected in surface waters at concentrations of up to 0.2 μg/L and for up to 7 h (Tanabe and Kawata, 2009; Tanabe et al., 2001), the exposure duration and all but the highest concentration applied during the present study can be considered field-relevant. Concentrations were verified using an ultra-high performance liquid chromatography system with mass spectrometry (HPLC-MS with Thermo Orbitrap Exactive; Thermo Fisher Scientific; Wieczorek et al., 2018; Table 1). The stream channels were fed with chlorine-free municipal tap water and were run in recirculation conditions except for the application phase (i.e., 3 h prior to and 48 h following etofenprox application), during which flow-through conditions were used. During flow-through conditions water from an adjacent storage pond was used. A more detailed description of the experimental procedure and results (besides those data addressed in the present work) are provided elsewhere (Wieczorek et al., 2018).

2.2. Leaf bag study

To quantify the invertebrate-mediated leaf litter decomposition within the mesocosms, *Alnus glutinosa* (black alder) leaves were collected during the time of abscission (October 2013) from a group of trees (49°12′N, 8°8′E). These leaves were stored frozen until further processing and were also used during the additional experiments performed here (see below). Before their deployment in the mesocosms, leaves were dried at 60 °C for 48 h. Subsequently, 4.0 and 2.0 (±0.1) g of the dried leaf material were placed in coarse (mesh size of 10 mm) and fine (mesh size of 0.5 mm) leaf bags. As coarse leaf bags only allow for the combined assessment of invertebrate- and microorganism-mediated leaf litter decomposition, fine leaf bags were used to correct the loss in coarse bags for the latter. Three sets of each leaf bag type were randomly deployed in each of the 16 channels shortly before the peak exposure was initiated. After 21 days, leaf bags were retrieved, cleaned from sediment particles as well as invertebrates, dried and weighted as described above.

2.3. In situ bioassays

Effects of the etofenprox peak exposure scenario on the *in situ* measured survival and feeding rate of *G. fossarum* within the mesocosms were assessed following Bundschuh et al. (2011). Briefly, leaf discs were cut from frozen black alder leaves and microbially colonized following a method known to establish near-natural microbial communities on the leaf material (Zubrod et al., 2015). Subsequently, leaf discs were dried, weighed to the nearest 0.01 mg and re-soaked in tap water before *in situ* bioassays were deployed in the mesocosms. One week before the etofenprox exposure, individuals of *G. fossarum* were kick-sampled from a pristine stream. In the laboratory, gammarids were adapted stepwise to the experimental conditions by increasing the share of water from the mesocosm facility. The *in situ* bioassays consisted of ten cages, each containing one size-standardized individual of

Table 1
Nominal and measured etofenprox concentrations (in μg/L; $n = 4/3$) in the mesocosm (comprising leaf bag and *in situ*; Wieczorek et al., 2018) and laboratory assays. The limit of detection (LOD) for the mesocosm experiment was 0.006 μg/L. LOD was not determined for the laboratory assay, thus non-detects are reported as concentrations below the lowest calibration level (LCL), which was 0.01 μg/L. n.d. not determined.

Assay	Nominal concentration	Mean (±standard deviation) measured concentration
Mesocosm	0	<LOD
	0.05	0.04 (±0.02)
	0.5	0.32 (±0.06)
	5	6.50 (±1.34)
Laboratory	0	<LCL
	0.1	n.d.
	0.5	0.26 (±0.02)

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