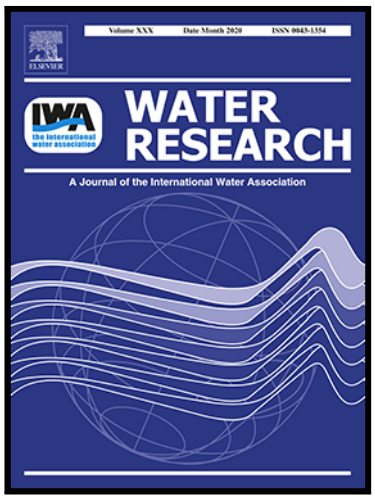
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Paradise lost? Pesticide pollution in a European region with considerable amount of traditional agriculture

Verena C. Schreiner , Moritz Link , Stefan Kunz , Eduard Szöcs , Andreas Scharmüller , Bernadette Vogler , Birgit Beck , Karina P. Battes , Mirela Cimpean , Heinz P. Singer , Juliane Hollender , Ralf B. Schäfer

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Highlights:

- Pesticide concentrations and toxicity similar along agricultural gradient.
- Sum concentrations in Romanian streams ranged from 0.02 to 37 µg L^{-1}.
- Agricultural intensity not related to pesticide concentration and toxicity.
- Toxicity towards invertebrates and algae driven by few pesticides.
- Catchment size and ratio agricultural land use were related to invertebrate toxicity.

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Paradise lost? Pesticide pollution in a European region with considerable amount of traditional agriculture

Verena C. Schreiner[a,*], Moritz Link[a], Stefan Kunz[a], Eduard Szöcs[a,b], Andreas Scharmüller[a], Bernadette Vogler[c], Birgit Beck[c], Karina P. Battes[d], Mirela Cimpean[d], Heinz P. Singer[c], Juliane Hollender[c] and Ralf B. Schäfer[a]

[a]iES Landau, Institute for Environmental Sciences, University Koblenz-Landau, Fortstraße 7, 76829 Landau in der Pfalz, Germany

[b]Present address: BASF SE, 67056 Ludwigshafen am Rhein, Germany

[c]Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

[d]Department of Taxonomy and Ecology, Faculty of Biology and Geology, Babeș-Bolyai University, 5-7 Clinicilor Str, 400006 Cluj-Napoca, Romania

* corresponding author, e-mail: schreiner-verena@uni-landau.de

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Abstract

Pesticide contamination of agricultural streams has widely been analysed in regions of high intensity agriculture such as in Western Europe or North America. The situation of streams subject to low intensity agriculture relying on human and animal labour, as in parts of Romania, remains unknown. To close this gap, we determined concentrations of 244 pesticides and metabolites at 19 low-order streams, covering sites from low to high intensity agriculture in a region of Romania. Pesticides were sampled with two passive sampling methods (styrene-divinylbenzene (SDB) disks and polydimethylsiloxane (PDMS) sheets) during three rainfall events and at base flow. Using the toxic unit approach, we assessed the toxicity towards algae and invertebrates. Up to 50 pesticides were detected simultaneously, resulting in sum concentrations between 0.02 and 37 µg L^{-1}. Both, the sum concentration as well as the toxicities were in a similar range as in high intensity agricultural streams of Western Europe. Different proxies of agricultural intensity did not relate to in-stream pesticide toxicity, contradicting the assumption of previous studies. The toxicity towards invertebrates was positively related to large scale variables such as the catchment size and the agricultural land use in the upstream catchment and small scale variables including riparian plant height, whereas the toxicity to algae showed no relationship to any of the variables. Our results suggest that streams in low intensity agriculture, despite a minor reported use of agrochemicals, exhibit similar levels of pesticide pollution as in regions of high intensity agriculture.

Keywords: monitoring, pesticide toxicity, anthropogenic stressors, streams, drivers, Romania

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1. Introduction

Agricultural pesticides enter streams through pathways such as deposition from spray drift (Schulz et al., 2001), run-off caused by heavy rainfall events (Weibel et al., 1964) and drainage from crop-land (Bennett et al., 2005). In stream ecosystems, pesticides can be a major cause of biodiversity loss (Beketov et al., 2013). Most studies on pesticide pollution in streams have been conducted in areas characterised by high intensity agriculture in Western Europe, North America and Australia (Rasmussen et al., 2012; Schäfer et al., 2012; Stone et al., 2014; Szöcs et al., 2017; Waite and Van Metre, 2017).

High intensity agriculture is not only characterised by high pesticide pollution in streams, but also associated with habitat loss due to channelisation and substrate homogenisation, and the extensive use of fertilisers (MEA, 2005; Vörösmarty et al., 2010). In addition, human labour has largely been replaced by heavy machinery in high intensity agriculture. By contrast, low intensity agriculture, here defined based on remnants of traditional non-mechanised agriculture, is largely relying on human or animal labour (e.g. horse ploughs) (Kovács-Hostyánszki et al., 2016; Lovász and Gurzău, 2013), and a presumed low usage of agrochemicals (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016). Regions with low intensity agriculture are rare in Europe, but can still be found in Eastern Europe, for example in parts of Romania. One of these regions, Transylvania, has been described as hosting some of the last relatively pristine farmlands in Europe but also inhabits areas of high intensity agriculture (Fischer et al., 2012). The importance of human labour in some parts of the agricultural sector of Romania is reflected in the highest proportion of agricultural workforce (over 25 % of the population) in the EU (EU average: 4 %) (Eurostat, 2017). Romania is among the EU countries with the lowest pesticide sales (30 % of the EU average, corresponding to 1.3 g of active ingredient per hectare of arable land in 2016) (Eurostat, 2018a, 2018b), suggesting that agrochemical use is particularly scarce in low intensive agriculture (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016). However, to which extent these lower sales and the remnants of low intensity agriculture are reflected in lower pesticide pollution of selected agricultural streams in Romania has not been studied.

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Previous studies mainly focused on the pesticide pollution of large rivers, where concentrations of single pesticides of up to 240 ng L^{-1} were detected (Ferencz and Balog, 2010; Moldovan et al., 2018). Studies covering pesticide pollution in streams across a range of low to high intensity agriculture could allow us to disentangle the effects of pesticide pollution on ecosystems from other agricultural stressors because across a wide gradient of agricultural intensity, pesticides and other agricultural stressors should be non-correlated.

The identification of variables directly or indirectly influencing pesticide pollution (hereafter: drivers) improves our capacity to predict the pollution of streams by pesticides and the potential risk to organisms. Several studies, mainly in high intensity agricultural areas have examined the drivers of pesticide toxicity in streams (Rasmussen et al., 2011; Szöcs et al., 2017). These studies identified the ratio of agricultural land use within the catchment as an important driver of pesticide toxicity at catchment scale (Rasmussen et al., 2011; Szöcs et al., 2017). Furthermore, the presence of intact buffer strips can reduce pesticide contamination at the local scale (Rasmussen et al., 2011; Stehle et al., 2016).

In this study, we determined pesticide concentrations in streams adjacent to a gradient of low to high intensity agriculture in Transylvania, Romania. This study presents first data on pesticide contamination in streams adjacent to low intensity, traditional agriculture. Additionally, we assessed the pesticide toxicity to freshwater invertebrates and algae using the sum toxic unit approach. Thereby, we aimed to determine if the low agricultural intensity of selected sites results in lower stream pesticide contamination, as claimed in previous studies (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016). To capture pesticide peaks related to heavy rainfall events, we used short-term passive sampling complemented by long-term passive sampling for compounds usually bound or absorbed to sediment particles, because active sampling was unfeasible due to the number and distribution of sampling sites. Moreover, we examined the drivers of pesticide toxicity to invertebrates and algae such as local and catchment-scale explanatory variables including the ratio of agricultural land use in the catchment, riparian buffer width and different proxies for agricultural intensity. We hypothesised that local variables are the most important drivers for both toxicity indices

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because the entry of pesticides into the stream can be mitigated or amplified by local parameters, e.g. vegetation is reducing spray drift, riparian buffer strips are reducing surface runoff, and agricultural intensity is related to the use of pesticides. Companion studies in a subset of sites provide information on the response of aquatic-terrestrial food webs to the pesticide exposure and other environmental gradients (Graf et al., 2020, 2019).

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2. Materials and Methods

2.1. Sampling sites

The study was conducted from April to June 2016 in Romania around the city of Cluj-Napoca, Transylvania (Fig. 1, Table S1). We selected 100 m stream sections with adjacent agricultural land use continuously ranging from high to low intensity. High intensity agriculture was characterised by the use of heavy machinery and fields larger than 3000 m² (median field size 8600 m²). Low intensity agriculture was characterised by relying on human or animal labour (e.g. horse ploughs) and fields being mostly smaller than 1500 m², though occasionally (< 20 %) fields larger than 3000 m² occurred (median field size 1360 m²). Medium intensity agriculture was defined by the use of both machinery as well as occasional human or animal labour and most fields larger than 1500 m² (median field size 2950 m²). We assumed that the gradient of agricultural intensity across the sampling sites reflected a gradient of pesticide use. All 19 streams had agricultural land use on at least one side of the stream, were located upstream of urban land use to minimize the influence from wastewater and non-agricultural pesticide uses and were characterised by similar stream sizes (3rd to 4th Strahler order).

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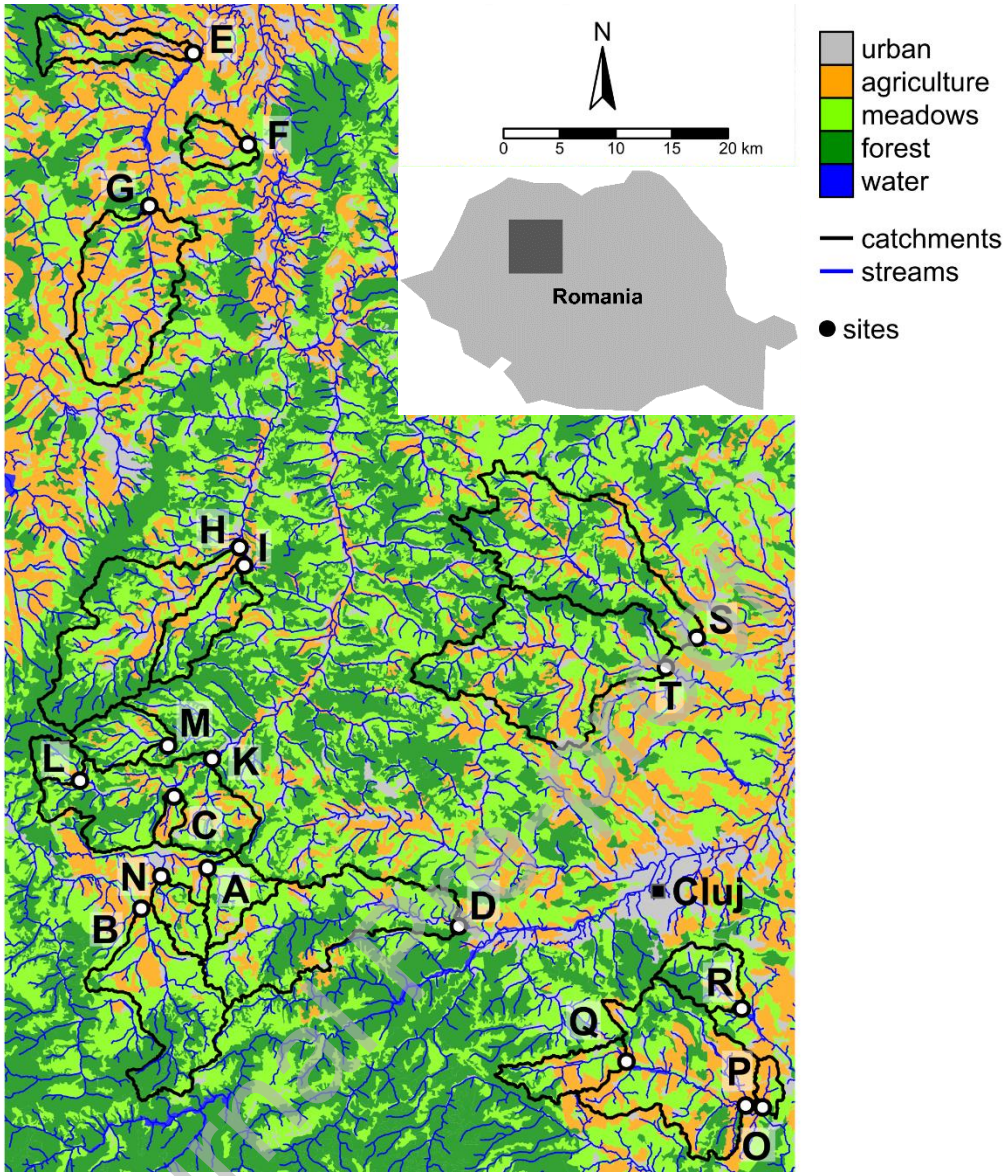


Fig. 1: Stream sampling sites A to T and their catchments in Transylvania, Romania, with different land use categories. The map was created using the R package tmaps (Tennekes et al., 2019).

2.2. Short term passive sampling followed by analysis using LC-HRMS(/MS)

To capture a high number of pesticides, we used two complementing passive sampling methods, with different limits of quantifications (LOQ). Most (91 %) of the pesticides were sampled with short term passive sampling achieving relatively high LOQs (analytical LOQs: 0.02 – 500 µg L^{-1}) using Empore styrene-divinylbenzene (SDB; type reverse-phase sulfonate) disks (3M Company) (Table S2). The disks were deployed three times, one to two

days before a forecasted heavy rainfall event (min. 10 mm day^{-1}; forecast by Norwegian Meteorological Institute and Norwegian Broadcasting Corporation, 2016) and retrieved two days after the rainfall event, resulting in a total exposure time of five to six days (Table S3). One additional set was deployed over six days in the middle of the monitoring campaign in the absence of rainfall to collect the base flow pesticide exposure.

We conditioned the disks by shaking in methanol (LC grade) and ultrapure water (30 min each) and subsequently stored them in ultrapure water. One day before deployment they were fixed to stainless steel holders (Fernández et al., 2014) (Fig. S1A) and transported submerged in ultrapure water to the sites. To check for possible transport contamination, two additional disks per sampling event were treated accordingly but not deployed. To represent whole stream sections and to reduce the probability of total loss (i.e. both replicates), two replicates were deployed at each site with a minimum distance of 20 m. After retrieval, the disks were stored at -20 °C in 6 mL of acetone (LC grade) until further processing. We used the following protocol to extract the analytes: (i) disks submerged in acetone were shaken for 30 min, (ii) the separated liquid phase was evaporated to 1 mL under a gentle nitrogen flow, (iii) 6 mL of methanol (LC grade) was added to the disks, which were again shaken for 30 min, (iv) the methanol extract was added to the acetone. If both replicates at one site could be retrieved intact, they were pooled. One half of the extract was used further, by adding 54 isotope-labelled standards (Table S4) and filtering using PTFE syringe filters (pores 0.45 µm, BGB Analytik), whereas the other half was kept as a reference sample for possible re-analysis (split by solvent weight). The samples were evaporated to 50 or 100 µL depending on the included number of disks and reconstituted with 450 or 900 µL of ultrapure water, respectively, to reach one disk equivalent per mL. Finally, the extract was centrifuged (30 min, 4000 rpm), and the supernatant was used for chemical analysis. All standards of the calibration row and spiked samples (matrix-matched calibration) were treated identically to correct for potential evaporation and filtration losses.

The extracts of the event samplings were analysed with an injection volume of 100 µL using a QExactive Plus Orbitrap system (Thermo Fisher Scientific Corporation) producing high-

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resolution mass spectrometry (HRMS) and MS/MS data. The chromatographic separation was achieved with an Atlantis T3 5 µm 3.0x150 mm column (Waters) using methanol and ultrapure water acidified with 0.1 % of formic acid as mobile phases (gradient see Table S5). A full scan with a resolution of 140,000 (at m z^{-1} = 200) in the range of 100 to 1000 m z^{-1} followed by five data-dependent MS/MS scans with a resolution of 17,500 was acquired separately for positive and negative ionisation (settings see Table S6). Mass accuracy was determined below 5 ppm throughout all measurements. The safe identification of the single compounds was achieved by matching the MS/MS fragments to those of the reference standards. The extracts of the base flow samples were analysed using the same methods as the event samples, with the exception that an Exactive Orbitrap system (Thermo Fisher Scientific Corporation) was used and fewer pesticides having higher LOQs were quantified (Table S2). With the Exactive Orbitrap system, the identification of the compounds in base flow samples was achieved by matching MS data (m z^{-1}; isotope pattern) and retention times to those of the reference standards.

For quantification of the pesticides and their metabolites in the event samples 228 reference standards and 54 internal isotope-labelled standards were used. In base flow samples quantification was limited to 51 pesticides, which were detected in event samples using their reference standards and 54 internal isotope-labelled standards (Tables S2, S4). We selected pesticides authorised in the last ten years in Romania and added some already established in the analytical method (Moschet et al., 2015). Quantification was conducted using extracted ion chromatograms (5 ppm) of the target compounds and the corresponding internal isotope-labelled standards in TraceFinder 3.3 (Thermo Fisher Scientific Corporation). Quality assurance as well as quality control and performance during the TraceFinder quantification as well as the subsequent calculation of the mass of each pesticide accumulated on the respective disk (m_{sorb}) were done according to Moschet et al. (2015).

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2.3. Long term passive sampling followed by analysis using GC-MS/MS

The passive sampling with SDB disks was complimented by passive sampling using polydimethylsiloxane sheets (PDMS, AlteSil 0.5 mm thickness, Altec). This was done because we aimed to sample pesticides with known high toxicity including pyrethroid and organophosphate insecticides (Table S2), whose detection require particularly low LOQs (Moschet et al., 2014a). Indeed, chlorpyrifos and chlorpyrifos-methyl were measured using both passive sampling methods but were only detected in the PDMS sheets, due to a 5,000-fold lower LOQ (Table S2). Handling and processing of the PDMS sheets were done according to Moschet et al. (2014a). Shortly, the PDMS sheets were prepared by pre-extracting them with ethyl acetate (HP grade) in a Soxhlet system and stored dry. The PDMS sheets (12.5x10 cm) were deployed in the streams twice, each for four weeks, fixed to aluminium stakes (Fig. S1B). When retrieving the PDMS sheets, they were gently cleaned and stored rolled in glass vials at -20 °C until further handling. The area where the sheet was fixed to the aluminium stakes in the stream was removed and the remaining sheet was cut in half (5x10 cm). One half was used for extraction, while the other half was kept as a reference for potential re-analysis. The sheets were extracted using pressurized liquid extract (PLE) (Dionex Accelerated Solvent Extraction 350, Thermo Fisher Scientific Corporation) using methanol (LC grade) at 120 °C with a static time of 10 min at a pressure of 105 bar. Afterwards, seven isotope labelled standards were added (Table S7) and the eluates were evaporated to dryness at 50 °C using rotation at 150 mbar. The extract was reconstituted in 500 µL hexane (LC grade) and filtered through silica gel (top) and C18 Isolute (bottom) in a glass pipette and washed with 2 mL hexane (LC grade) to reduce the matrix. The elution was conducted with 10 mL of acetonitrile (LC grade) and the eluate evaporated to dryness at 50 °C and a pressure of 117 mbar. The filtered extract was reconstituted in 1 mL of hexane, centrifuged (30 min, 4000 rpm), and the supernatant was used for further analysis. All standards of the calibration row and spiked samples (matrix-matched calibration) were treated identically to correct for potential evaporation and filtration losses.

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A total of 17 pesticides and one metabolite (Table S2) were quantified by a GC-APCI-MS/MS instrument (gas chromatograph 7890B coupled to a triple quadrupole mass spectrometer 6495 using atmospheric pressure chemical ionisation, both Agilent). Analytical details can be found in Rösch et al. (2019). The mass of each pesticide accumulated on the respective sheet (m_{sorb}) was calculated using MassHunter (version B.07.00; Agilent Technologies).

2.4. Calculating pesticide concentrations

The water concentrations (c_{calc}) of the pesticides were calculated as:

$$c_{calc_i} = \frac{m_{sorb_i}}{R_i\, t} \tag{1}$$

where m_{sorb} is the mass of the pesticide *i* accumulated on the disk or the sheet, R_i is the respective sampling rate (L day^{-1}; Table S2) and *t* is the assumed uptake time in days into the receiving phase. Given that most of the pesticide mass sorbed to a disk during peak exposure samples originates from the peak, we set the uptake time to the estimated peak duration of two days to calculate the peak concentration for rainfall event samples (Schreiner et al., 2020). For base flow samples, we used the whole deployment period (here t = 6), assuming relatively constant exposure and consequently sorption (for discussion see below). The sampling rates R_i for the compounds sampled via SDB-disks were experimentally determined (Table S2). Since no experimental sampling rates were available for compounds sampled via PDMS sheets, we used one average, experimentally-determined sampling rate from compounds with hydrophobicities similar to the sampled pyrethroid and organophosphate insecticides [details see Moschet et al. (2014a)]. Due to missing sampling rates for the other compounds, 55 pesticides (Table S2) were considered for further analysis, all of them quantified in base as well as peak exposure samples. Most of the omitted pesticides (except for all metabolites and a few herbicides) occurred in less than 5 % of the samples. Including omitted pesticides using a fixed sampling rate as a proxy for all, resulted in an only minor change in the sum concentrations and the resulting toxicity indices (see

related R-script). For example, a fixed sampling rate of 0.2 L day^{-1}, which is in the lower range of sampling rates, had a minor impact, though for a given concentration lower sampling rates result in a higher calculated concentration (for a detailed discussion, see below).

2.5. Calculating potential pesticide toxicity

To assess the potential, cumulative toxicity of the detected pesticides within one sample, we used the logarithmic sum of toxic units (sumTU):

$$sumTU = log\left(\sum \frac{c_{calc_i}}{EC_{50_i}}\right) \quad (2)$$

where c_{calci} is the estimated concentration of the pesticide *i* and EC_{50i} is the concentration of pesticide *i* at which 50 % of the test organisms (see below) were affected. To account for the risk for different trophic levels in freshwater ecosystems, we calculated sumTUs for (1) the most sensitive freshwater invertebrate (hereafter: invertebrates) and (2) the most sensitive freshwater algae (hereafter: algae; selected species see Table S2) (Fließgewässerbewertung, 2018; Horton et al., 2018; Schmidt-Kloiber and Hering, 2015). The EC_{50} values were compiled from several databases. Primarily, we used the data from Malaj et al. (2014), which was complemented by data from Lewis et al. (2016) and, if data was missing, from the United States Environmental Protection Agency (EPA) ECOTOX Knowledgebase (EPA, 2018). This sequence of data use was based on different levels of quality control of the databases. Furthermore, all toxicity data were checked for plausibility (e.g. removing outliers, checking for water solubility). For each species where several EC_{50} values were available in the EPA Ecotox database, we used the lowest value ($n < 3$) or the median ($n \geq 3$). Selected test durations were 48 to 96 hours. Due to different numbers of available EC_{50} values, the sumTU analysis was based on 53 and 47 pesticides for $sumTU_{invertebrates}$ and $sumTU_{algae}$, respectively (Table S2).

Site-specific sumTUs were computed for each of the different samplings combining the compounds from the PDMS and SDB passive samplers that were deployed simultaneously (Table S3).

2.6. Characterisation of sampling sites

We measured several physicochemical and habitat-specific variables during the period where the rainfall events occurred (June 2016). These variables included the buffer width and the ratio of stream substrate smaller than 2 mm which can be used as a proxy for sediment input caused by erosion (Lemm and Feld, 2017). Additionally, we measured the average field size in a 200 m long section lateral to the sampling site, which was used as a proxy for the intensity of agricultural land use (Pe'er et al., 2014), based on Google Earth images (Google Earth, 2019) that were temporally closest to the field study. The agricultural intensity was also estimated by determining the ratio of large fields (> 3000 m²) in this 200 m section. Given that the ratio exhibited a high correlation to the average field size ($r = 0.89$, $p < 0.001$), we only included the average field size in the analysis. Moreover, the agricultural intensity of a site was categorised (three levels: low, medium, high agricultural intensity, hereafter called factorial agricultural intensity) based on field size and personal on-site observations of the used agricultural methods. Finally, by using the geospatial algorithm ATRIC (Bhowmik et al., 2015) and CORINE land cover data (European environmental agency, 2019), we derived variables such as catchment size and the ratio of different land use types in the whole catchment (Table S1). In addition, the ratio of agricultural land use in a 200 m wide buffer was calculated as described in Waite and Van Metre (2017).

2.7. Data analysis

For further analysis, we used maximum sum concentrations as well as toxicities at each site over the four sampling events, because we assumed that organisms are subject to environmental selection by the strongest stress event (Fernández et al., 2015; Schäfer et al., 2011). The maximum $sumTU_{algae}$ and $sumTU_{invertebrates}$ correlated highly and moderately with

the maximum sum concentration ($r = 0.96$, $p < 0.001$ and $r = 0.48$, $p = 0.039$), respectively. Hence, we restricted the analysis to the toxicity indices.

To investigate the effects of flow velocity and water temperature on the calculated concentrations of six pesticides (details below), we used single Pearson's correlations.

We selected and combined several monitored variables (Table S8) resulting in eight explanatory variables (Table 1) considered as potentially important drivers of in-stream pesticide toxicity (Rasmussen et al., 2011; Stehle et al., 2016; Szöcs et al., 2017), which exhibited no relevant inter-correlation (all pairwise $r < 0.62$). The most important drivers of the $sumTU_{invertebrates}$ and $sumTU_{algae}$ were identified using regression analysis with elastic net regularisation (Zou and Hastie, 2005) because this technique allows for a low sample size to explanatory variables ratio. The optimal regularisation (i.e. parameters α and λ of the elastic net) was determined as the model with the least variables within one standard error from the model with minimum cross-validation error (Bruce and Bruce, 2017). Given that the elastic net approach prohibits the inclusion of categorical variables, we analysed if pesticide pollution in terms of concentration, toxicity and detected pesticides differed between levels of agricultural intensity separately using a type II ANOVA with F-tests or Chi-square-tests.

All statistical analyses and visualisations were conducted in R (version 3.3.3, (R Core Team, 2017)) using the additional packages vegan, plotmo and glmnet (Friedman et al., 2018; Milborrow, 2019; Oksanen et al., 2018). We provide the complete computer code and all raw data on a Github repository (https://github.com/rbslandau/schreinerromania).

Table 1: Explanatory variables selected as potential drivers of toxicity to invertebrates and algae with range, median and standard deviation (SD). See methods for details on variables.

Explanatory variable [unit]	**range**	**median**	**SD**
Ratio of agricultural land use in upstream catchment [%] [a]	7 - 61	17.7	17.9
Catchment size [km²]	8 - 177	35.0	60.8

Ratio of agricultural land use within a 200 m buffer [%] [a]	0 - 100	51.2	33.2
Average (geometric mean) field size of all fields that fully or partially extended into a 50 m wide and 200 m long stream buffer. Considered as proxy for agricultural intensity (Pe'er et al., 2014) [m²] [b]	497 – 46,029	3,159	10,726
Minimum riparian buffer width of both stream banks [m] [b]	1 – 50	10	14.8
Direct distance between stream and landscape-level (Calculated based on bank height as well as horizontal distance from stream to landscape level), shortest distance of potential runoff [m]	2.1 – 9.1	4.24	2.11
Average riparian plant height in an approx. 5 m buffer [m]	0.9 – 7.5	2.9	2.09
Fine sediment (< 2 mm), proxy for sediment input (Lemm and Feld, 2017) [%]	0 - 90	25	25.6

[a] based on CORINE Land Cover (European environmental agency, 2019) types 211, non-irrigated arable land; 221, vineyards; and 222, fruit trees, and berry plantations.

[b] was log-transformed for elastic net analysis.

Single values used to get displayed combined variables in Table S8.

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3. Results and Discussion

3.1. Pesticide concentrations in streams

We analysed 195 pesticides in a region assumed to have areas of low pesticide exposure (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016). The sampled streams covered a gradient of low to high intensity agriculture, with agricultural practices ranging from human and animal labour to heavy machinery agriculture (Table S1). We found 47 % of the analysed pesticides (91 pesticides) and 23 metabolites (Table S2, all data at https://github.com/rbslandau/schreinerromania). During rainfall events, on average 31.3 (± 7.6) pesticides were detected, with a maximum of 66 different compounds (50 pesticides, 16 metabolites) in a single sample (Fig. 2, Tables S9, S10). The number of detected pesticides was independent of the factorial agricultural intensity (ANOVA; LRT = 0.40, p-value = 0.82). Still, the number of detected pesticides across the whole gradient of agricultural intensity matched those from a study in Switzerland conducted in catchments with high intensity agriculture (Moschet et al., 2014b). This study used composite water samples and incorporated a comparable number of pesticides as in our study. In contrast, routine monitoring in several European countries where high intensity agriculture dominates, only detected between 5 and 15 pesticides (Moschet et al., 2014b; Schreiner et al., 2016). The lower number of detected pesticides in routine monitoring can be explained by a reduced set of analysed pesticides and the lack of event sampling, known to increase the number of detected pesticides (Leu et al., 2004; Weibel et al., 1964). This is also reflected in this study, where during base flow only between 30 to 50 % of the number of pesticides during rainfall event samples were detected (Table S9).

The sum concentrations in rainfall event samples ranged from 0.02 to 37 µg L^{-1} (Tables S11, S12, Fig. 2), with the herbicide 2,4-D detected in calculated concentrations of up to 36.2 µg L^{-1} (Table S2). A previous study identified misuse during application as the driver of such high concentrations as in the case of 2,4-D (Wittmer et al., 2010). We observed the washing of agricultural equipment at the site with the highest 2,4-D calculated concentrations and surveys in the study area reported insufficient education of most farmers regarding

pesticide use (Gurzău et al., 2008; Lovász and Gurzău, 2013). Despite covering a gradient of agricultural intensity, the maximum sum concentration was not significantly influenced by the factorial agricultural intensity (ANOVA; F = 0.59, p-value = 0.57). The detected range of sum concentrations was, however, similar to streams in intensive agriculture in Western Europe and the US (Fernández et al., 2014; Guibal et al., 2018; Gustavsson et al., 2017; Moschet et al., 2014b; Nowell et al., 2018; Ulrich, 2015) and exceed previous results for large rivers in Transylvania by orders of magnitude (Ferencz and Balog, 2010; Moldovan et al., 2018). The sum concentration was on average 7 times higher for peak than for base flow samples, again indicating the relevance of sampling rainfall events. The sum concentration of single peak flow samples (7 % of the peak flow samples, Table S12) was slightly below those of base flow samples at the same site. This was only the case where two rainfall events were only one week apart with no or little new pesticide use. We assumed that the compound uptake from peak exposure samples was driven by the peak duration, whereas from base flow samples it was rather constant during the whole deployment time and considered this in the calculation of concentrations. Assuming that both types of samples were related to the estimated peak duration of two days (equation 1, $t = 2$), the peak exposure sum concentration would still be 2.3 times higher on average (details see below) than those of the base flow samples. However, using this shorter period for samples without a clear indication of rainfall events leads to an overestimation of the calculated concentrations. The differences between peak and base sum concentrations are lower than reported in previous studies, where differences up to a factor of 100 were detected for several single pesticides (e.g. Leu et al., 2004). These previous studies were conducted exclusively in high intensity agriculture and included a lower spectrum of analysed compounds in smaller streams. Additionally, these studies used temporally resolved active sampling, which is more suitable than passive sampling to detect peak concentrations, but was not possible in this study with various catchments and monitoring over a long time period.

As reported by previous studies (Gustavsson et al., 2017; Moschet et al., 2014b; Schreiner et al., 2016), the mixtures of simultaneously detected compounds were dominated by

herbicides both in terms of concentration and number of compounds, which can be attributed to herbicides dominating pesticide sales in Romania (> 40 %) (Eurostat, 2018a). Additionally, herbicides are typically highly water-soluble (Lewis et al., 2016) and consequently more easily transported into streams.

The high detected sum concentrations, as well as a large number of pesticides, with no relationship to the factorial agricultural intensity, provide strong evidence against the claimed negligible pesticide use in remnants of pristine farmlands in Transylvania (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016). Even at the site with the lowest ratio of agriculture within the catchment (7 %) and the lowest agricultural intensity (based on smallest fields and observed agricultural practices), 17 different pesticides were detected with a maximum sum concentration of 0.09 µg L^{-1}, which is in the lower range of concentrations detected in high intensity agriculture in Western Europe (Fernández et al., 2014; Moschet et al., 2014b). Our findings suggest that the presumed lower pesticide use in low intensity agriculture is not reflected in a lower pesticide pollution in adjacent streams. This may be explained, besides potential incorrect disposal of pesticide, by insufficient education of farmers resulting in the disregard of application recommendations and the imprecise application due to the use of self-maintained backpack sprayers, overall resulting in higher pesticide pollution despite lower overall use (Gurzău et al., 2008; Lovász and Gurzău, 2013).

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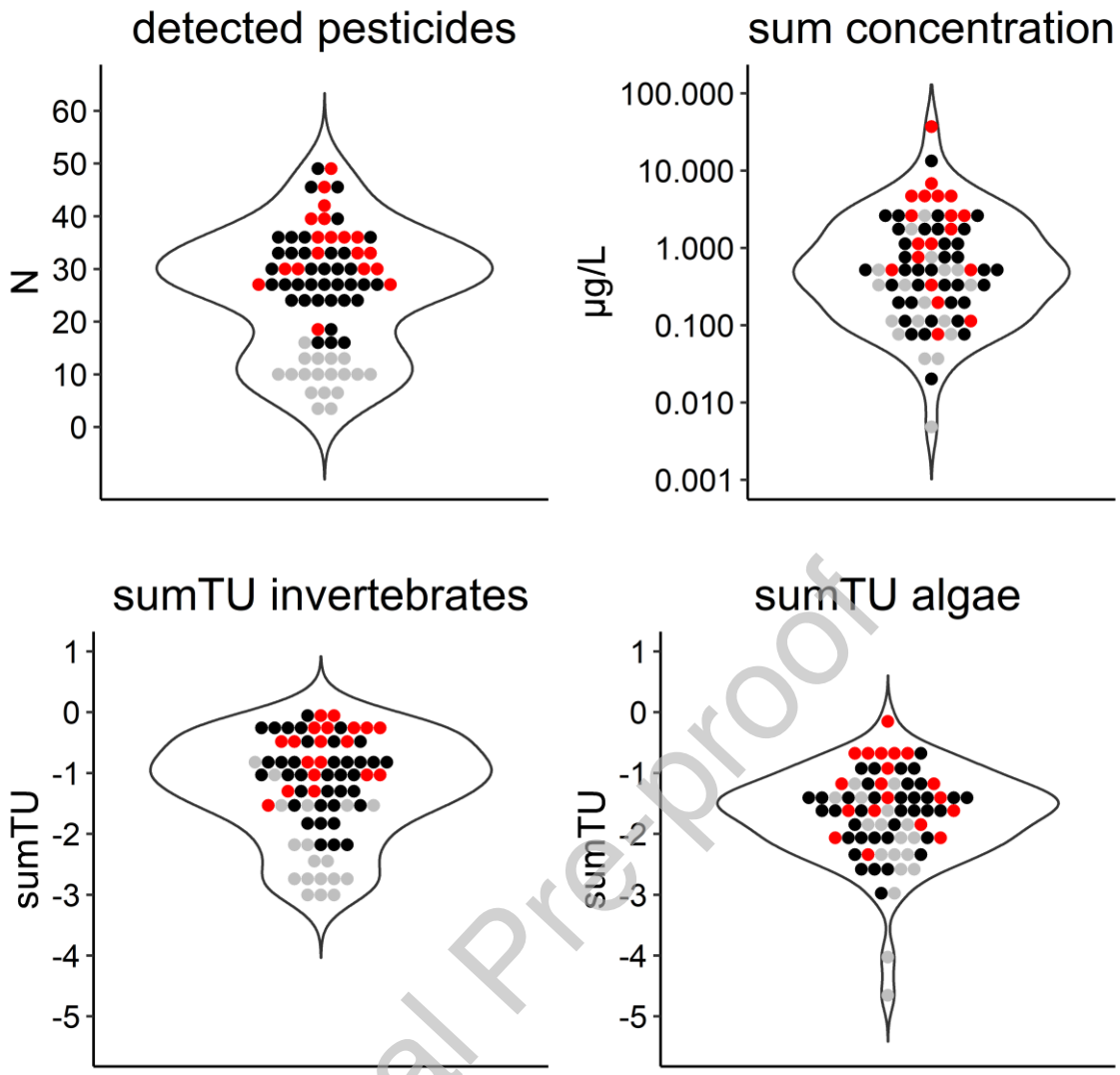


Fig. 2: Violin plots (Wickham, 2016) illustrating the occurrence frequency of detected pesticides, sum concentrations and the toxicity indices $sumTU_{invertebrates}$ and $sumTU_{algae}$ (both on a logarithmic scale). Each dot represents a single sample (four samples per site): grey = base flow (without rainfall event), black = rainfall event, red = maximum per site (only during rainfall events).

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3.2. Assessment of toxicity towards invertebrates and algae

Similar to the sum concentration, the toxicity indices were in the same range as previously reported for high intensity agriculture (Fernández et al., 2015; Gustavsson et al., 2017; Rasmussen et al., 2012) and were not significantly influenced by the factorial agricultural intensity (ANOVA; $sumTU_{invertebrates}$: F = 0.23, p-value = 0.79; $sumTU_{algae}$: F = 1.17, p-value = 0.34).

The maximum $sumTU_{invertebrates}$ per site spanned from -1.62 to -0.01 (on a logarithmic scale, Fig. 2, Table S12), a range that is known to alter macroinvertebrate communities and consequently to affect whole stream ecosystems e.g. Schäfer (2019). Moreover, complementary studies in our region using the maximum $sumTU_{invertebrates}$ per site found that the pesticide toxicity was associated with changes in spider communities and their diet in adjacent riparian ecosystems, most likely a consequence of alterations in freshwater insect emergence which constitutes an important prey for riparian spiders (Graf et al., 2020, 2019).

The maximum $sumTU_{algae}$ per site ranged from -2.36 to -0.15 (Fig. 2, Table S12), a level implying risks for algae by reducing growth and photosynthetic activity, potentially affecting an important base of the stream food web, the primary production (Gustavsson et al., 2017; Rasmussen et al., 2015). The range of $sumTU_{algae}$ is higher than those of $sumTU_{invertebrates}$, which suggests higher variability in herbicide than insecticide pollution, the dominating pesticide groups that determine the corresponding toxicity. This may be because substitution of pesticides through mechanical labour including manual removal, horse ploughs or heavy agricultural machines is more feasible for herbicides than insecticides (Eurostat, 2017; Kovács-Hostyánszki et al., 2016; Lovász and Gurzău, 2013). However, in contrast with this explanation, we did not find a relationship of this gradient with agricultural intensity, i.e. average field size.

The $sumTU_{invertebrates}$ and $sumTU_{algae}$ were mainly driven by few pesticides. On average two to three (2.3 ± 0.8) pesticides contributed to 75 % of the $sumTU_{algae}$ (Table S13), with terbuthylazine, metribuzin and 2,4-D dominating (Table S14). Fewer compounds (1.5 ± 0.7) accounted for 75 % of the $sumTU_{invertebrates}$ (Table S13), where the insecticides diazinon and

imidacloprid dominated (Table S14). Some years before our study, these compounds were among those used by most farmers in Transylvania (Gurzău et al., 2008; Lovász and Gurzău, 2013), and in 2017 2,4-D and imidacloprid were among the pesticides with highest sale amounts regarding their pesticide types (herbicides and insecticides; among herbicides only glyphosate was sold in higher amounts, but was not detectable using the presented methods) (Institutul Național de Statistică, 2018). During the field campaign in 2016 diazinon was no longer approved for field applications in the EU and one year later not listed among the pesticides sold in Romania (Institutul Național de Statistică, 2018).

The few relevant pesticides per mixture support the approach of most routine monitoring programs to concentrate on pre-selected compounds (Moschet et al., 2014b; Schreiner et al., 2016), which enables cost and labour efficient analysis, but may also strongly underestimate risks if relevant compounds are missing (Malaj et al., 2014).

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3.3. Passive sampling of pesticides

Calculated concentrations derived from passive sampling are associated with uncertainties. First, flow velocity, below 0.2 m s^{-1}, is known to have a strong effect on sampling rates (Booij et al., 2016, Moschet et al., 2015; Vermeirssen et al., 2008), even though this effect can be masked by other factors like water matrix or different analytical methods (Schreiner et al., 2020). Since sampling rates across studies seem to be independent of the flow velocity over a range of 0.1 and 0.9 m s^{-1} (Schreiner et al., 2020), we consider flow velocity differences between the determination of sampling rates and the deployment of passive samplings as minor. Nonetheless, in four sites the flow velocities were below 0.2 m s^{-1} during either deployment or retrieval (min. 0.03 m s^{-1}). During rainfall events, however, the flow velocity was presumably always higher than 0.2 m s^{-1}, because rainfall events typically lead to a strong increase in flow velocity. This assumption is supported by an observed minimum increase in the water level of 50 cm at the respective sites. To however, increase the quality of the results, we discarded single samplers, where flow velocities were during deployment as well as during retrieval below 0.1 m s^{-1}.

Therefore, we assumed that flow velocity differences at the single sites only had a minor influence on the resulting calculated concentrations. Indeed, the calculated concentrations of the six most relevant pesticides (2,4-D, terbuthylazine, metribuzin, diazinon, imidacloprid and thiacloprid; details above) exhibited only a weak relationship to the measured flow velocity during deployment and retrieval of the passive samplers (all $|r| < 0.22$, $p > 0.05$). Since only one sampling rate for all compounds sampled via PDMS sheets was available (see above), effects of flow velocity may overlap with inaccuracies related to sampling rates. The two sampling periods of PDMS sheets differed approximately 7-fold in precipitation (Table S3), most likely leading to different average flow velocities. However, these differences did not translate to a clear trend of calculated concentration differences between the periods, suggesting a continuous exposure with very low concentrations of the insecticides sampled via PDMS sheets (pyrethroid and organophosphate) (Liu et al., 2004). Finally, PDMS compounds only accounted for a maximum of 7 % of the $sumTU_{invertebrate}$ and were irrelevant

for $sumTU_{algae}$ (max. 0.6 %, Table S15) when assessed together with the related peak exposure samples of the SDB disks. Hence, we suggest that potential concentration inaccuracies due to flow are largely irrelevant for the maximum toxicity assessment. When regarding all sampling events individually, however, the PDMS compounds were clearly relevant for $sumTU_{invertebrate}$ during base flow (Table S15).

Moreover, under laboratory conditions, higher water temperature increases sampling rates (Vrana et al., 2006). Although this may have influenced the uptake, we suggest that this influence was minor because the six most relevant pesticides (2,4-D, terbuthylazine, metribuzin, diazinon, imidacloprid and thiacloprid; details above) correlated weakly to the average temperature during deployment and retrieval of the passive samplers (all $|r| < 0.33$, $p > 0.01$). In addition, the effect of stream turbidity or the general load of suspended matter on the uptake of compounds is unclear. These factors are usually associated with rainfall events and thus the flow velocity. Since the flow velocity was largely irrelevant when estimating maximum toxicities (see above same section), we suggest a similar minor relevance for turbidity and load of suspended matter. In general, the pesticide concentrations occurring during the passive sampler deployment are likely to be more relevant for the estimation of peak concentrations and the related toxicities than the above-discussed variables influencing sampling rates.

Another uncertainty when using passive sampling to assess toxicity risks is that the pesticide peak duration can vary. Usually, depending on several parameters including stream size, topography and soil structure, peak durations range between one and three days (e.g. Wittmer et al., 2010). Based on this we calculated peak concentrations for the SDB disks from peak exposure samples (Table S3) using an estimated peak duration of two days ($t = 2$, equation 1). A previous study showed that the calculated peak concentrations using this approach may approximate 50 % of the maximum concentration (Schreiner et al., 2020). For the PDMS sheets, we used a time span corresponding to their exposure time, because they were independent of pulses associated with rainfall events. Based on this, our approach is

biased towards acute toxic effects. Lipophilic compounds may be more relevant when using chronic toxicity data to evaluate data on chronic exposure.

Overall, we assume that the calculated concentrations estimated from passive samplers were reliable, especially when using the estimated peak duration. A previous study showed that using this approach calculated concentrations from passive samplers matched well with concentrations based on event-driven samplers (Fernández et al., 2014). When using the whole exposure duration, of passive samplers, however, calculated concentrations from passive sampling were lower in contrast to event-driven samples or the maximum concentration (Bundschuh et al., 2014; Schreiner et al., 2020).

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3.4. Drivers of pesticide toxicity

A wide gradient of pesticide toxicity, for both the $sumTU_{invertebrates}$ as well as the $sumTU_{algae,}$ allows examining variables directly or indirectly influencing pesticide toxicity, referred to as drivers. The relationship of the drivers selected by the elastic net approach contrasted in several cases those of previous studies. This might be due to our relatively small sample size of 19 sites, which contrasts other studies with several hundred observations (Schulz, 2004; Szöcs et al., 2017). Additionally, previous studies were conducted in other regions, potentially characterised by different soil structures.

In contrast to our hypothesis that local scale variables are most important, the catchment scale variables catchment size, as well as the fraction of agricultural land use within the catchment, were the most relevant drivers of $sumTU_{invertebrates}$ (Table S16). Both variables were positively related to the $sumTU_{invertebrates}$ (Fig. S2). The positive relationship between $sumTU_{invertebrates}$ and agricultural land use within the catchment matches earlier studies (Rasmussen et al., 2011; Szöcs et al., 2017). Catchment size and either pesticide toxicity or exceedance of regulatory threshold concentrations were not (Szöcs et al., 2017) or negatively (Schulz, 2004; Stehle and Schulz, 2015) related in previous studies. The contrasting results of our study compared to those with negative relationships might be due to our study having a much narrower range of catchment areas.

In contrast to previous studies (Bunzel et al., 2014) as well as our hypothesis, the only local scale variable driving the $sumTU_{invertebrates}$ was the riparian plant height. An increasing $sumTU_{invertebrates}$ with increasing riparian plant height (from 0.9 – 7.5 m) also contrasts previous findings, where a decrease in pesticide pollution with increasing plant height was attributed to higher organic carbon contents in soils of riparian areas (Aguiar et al., 2015). Additionally, higher plants usually block spray drift (Schulz et al., 2001), an entryway of pesticides of lower relevance in this study due to sampling during runoff caused by heavy rainfall. The pattern in our study may be explained by farmers spraying vegetated riparian areas with the aim to remove potential insect pests, given that a deviation from application recommendations have been observed in our study region.

Two other local scale variables, riparian buffer width and agricultural land use within this buffer were selected as drivers for $sumTU_{invertebrates}$ though the relationships were weak (Fig. S2). This weak relationship of buffer width and the $sumTU_{invertebrates}$ contrasts previous studies and our hypothesis of local scale variables being most important. The relevance of buffers may be low if their effects are neutralised by erosion rills (Bunzel et al., 2014; Stehle et al., 2016), which were not detected across the 100 m section of the sampling sites. Our results suggest that the accumulation of pesticides of a multiplicity of fields in the whole catchment is more relevant than local riparian buffers. Notwithstanding, the agricultural intensity, in terms of average field size (Pe'er et al., 2014) was not selected as a driver for the $sumTU_{invertebrates}$, which can be explained by the fact that the number of pesticides as well as sum concentrations were in similar ranges as previously detected in streams of high intensity agriculture (see above). Also, the factorial agricultural intensity based on the observed agricultural practices and to the average field size was not related to the $sumTU_{invertebrates}$ (ANOVA; $F = 0.23$, p-value = 0.79). This suggests that pesticide pollution, at least in our study region is independent of agricultural intensity. Even sites with low agricultural intensity in terms of small field size and lacking access for heavy machines had $sumTU_{invertebrates}$ of up to -0.17. Overall, the ratio of agricultural land use was more important for invertebrate toxicity than agricultural intensity.

The $sumTU_{algae}$ is, according to the elastic net approach, not driven by any of the analysed variables (Table S16). The lack of relevant drivers on catchment as well as local scale may be explained by the physico-chemical properties such as higher water solubility and lower lipophilicity of herbicides, which dominated the $sumTU_{algae}$. These physico-chemical properties may be associated with a stronger independence of pesticide input from rainfall events.

Additionally, the lack of identifiable relationships between $sumTU_{algae}$ and the analysed drivers might be due to a possible substitution of herbicide applications through mechanical labour (see above), indicating another not identified or measured variable driving the $sumTU_{algae}$ or an insufficient number of observations.

4. Conclusions

- Agricultural intensity evaluated based on field size and use of machines, in an Eastern European region is not associated with lower stream pesticide pollution in terms of concentration and toxicity.
- The levels of pesticide pollution are similar to levels found in high intensity agriculture in Western Europe and North America, despite a reported lower use.
- The absence of agricultural sites without pesticide pollution hampers the discrimination of the effects of pesticide pollution from those of other agricultural stressors. Given our results, it seems unlikely that such sites can be found in Central or Eastern Europe.

Author contributions

VCS and RBS designed the study and discussed the results; RBS, KPS and MC selected sampling sites; KPB and MC helped with local logistics; VCS, SK and ES conducted fieldwork; ML provided geospatial information; VCS and AS provided the toxicity data; VCS, BV and HPS conducted the chemical analysis of the SDB disks, VCS, BB and JH conducted the chemical analysis of the PDMS sheets; VCS calculated sum concentrations as well as toxicities and conducted statistical analysis; HPS and JH gave recommendations for sampling and chemical analysis; all authors revised the manuscript.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

References

Aguiar, T.R., Bortolozo, F.R., Hansel, F.A., Rasera, K., Ferreira, M.T., 2015. Riparian buffer zones as pesticide filters of no-till crops. Environmental Science and Pollution Research 22, 10618–10626. https://doi.org/10.1007/s11356-015-4281-5

Beketov, M.A., Kefford, B.J., Schäfer, R.B., Liess, M., 2013. Pesticides reduce regional biodiversity of stream invertebrates. PNAS 110, 11039–11043. https://doi.org/10.1073/pnas.1305618110

Bennett, E.R., Moore, M.T., Cooper, C.M., Smith, S., Shields, F.D., Drouillard, K.G., Schulz, R., 2005. Vegetated agricultural drainage ditches for the mitigation of pyrethroid-associated runoff. Environmental Toxicology and Chemistry 24, 2121–2127. https://doi.org/10.1897/04-357R.1

Bhowmik, A.K., Metz, M., Schäfer, R.B., 2015. An automated, objective and open source tool for stream threshold selection and upstream riparian corridor delineation. Environmental Modelling & Software 63, 240–250. https://doi.org/10.1016/j.envsoft.2014.10.017

Bruce, P., Bruce, A., 2017. Practical Statistics for Data Scientists: 50 Essential Concepts, 1st ed. O'Reilly Media.

Bundschuh, M., Goedkoop, W., Kreuger, J., 2014. Evaluation of pesticide monitoring strategies in agricultural streams based on the toxic-unit concept — Experiences from long-term measurements. Science of The Total Environment 484, 84–91. https://doi.org/10.1016/j.scitotenv.2014.03.015

Journal Pre-proof

Bunzel, K., Liess, M., Kattwinkel, M., 2014. Landscape parameters driving aquatic pesticide exposure and effects. Environmental Pollution 186, 90–97. https://doi.org/10.1016/j.envpol.2013.11.021

EPA, 2018. The ECOTOXicology knowledgebase (ECOTOX) [WWW Document]. URL https://cfpub.epa.gov/ecotox/index.cfm (accessed 11.8.18).

EU, 2020. EU Pesticides database - European Commission [WWW Document]. URL https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN (accessed 5.5.20).

European environmental agency, 2019. Corine Land Cover (CLC) 2018, Version 20 [WWW Document]. URL https://land.copernicus.eu/pan-european/corine-land-cover/clc2018 (accessed 10.15.19).

Eurostat, 2018a. Pesticide sales Last update: 22-11-2018 Eurostat - Data Explorer [WWW Document]. URL http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=aei_fm_salpest09&lang=en (accessed 12.11.18).

Eurostat, 2018b. Utilised agricultural area by categories Thousand ha [WWW Document]. URL http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=tag00025&lang=en (accessed 12.11.18).

Eurostat, 2017. Farmers in the EU - statistics - Statistics Explained [WWW Document]. URL https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Farmers_in_the_EU_-_statistics (accessed 1.15.19).

Ferencz, L., Balog, A., 2010. A PESTICIDE SURVEY IN SOIL, WATER AND FOODSTUFFS FROM CENTRAL ROMANIA. Carpathian Journal of Earth and Environmental Sciences 5, 111–118.

Fernández, D., Vermeirssen, E.L.M., Bandow, N., Muñoz, K., Schäfer, R.B., 2014. Calibration and field application of passive sampling for episodic exposure to polar organic pesticides in streams. Environmental Pollution 194, 196–202. https://doi.org/10.1016/j.envpol.2014.08.001

Fernández, D., Voss, K., Bundschuh, M., Zubrod, J.P., Schäfer, R.B., 2015. Effects of fungicides on decomposer communities and litter decomposition in vineyard streams. Science of The Total Environment 533, 40–48. https://doi.org/10.1016/j.scitotenv.2015.06.090

Fischer, J., Hartel, T., Kuemmerle, T., 2012. Conservation policy in traditional farming landscapes: Conserving traditional farming landscapes. Conservation Letters 5, 167–175. https://doi.org/10.1111/j.1755-263X.2012.00227.x

Fließgewässerbewertung, 2018. PERLODES [WWW Document]. URL http://www.fliessgewaesserbewertung.de/download/berechnung/ (accessed 11.8.18).

Friedman, J., Hastie, T., Tibshirani, R., Simon, N., Narasimhan, B., Qian, J., 2018. glmnet: Lasso and Elastic-Net Regularized Generalized Linear Models.

Google Earth, 2019.

Graf, N., Battes, K.P., Cimpean, M., Dittrich, P., Entling, M.H., Link, M., Scharmüller, A., Schreiner, V.C., Szöcs, E., Schäfer, R.B., 2019. Do agricultural pesticides in streams influence riparian spiders? Science of The Total Environment. https://doi.org/10.1016/j.scitotenv.2018.12.370

Graf, N., Battes, K.P., Cimpean, M., Entling, M.H., Frisch, K., Link, M., Scharmüller, A., Schreiner, V.C., Szöcs, E., Zubrod, J.P., Schäfer, R.B., 2020. Relationship between agricultural pesticides and the diet of riparian spiders in the field. Environmental Sciences Europe 32. https://doi.org/10.1186/s12302-019-0282-1

Guibal, R., Lissalde, S., Leblanc, J., Cleries, K., Charriau, A., Poulier, G., Mazzella, N., Rebillard, J.-P., Brizard, Y., Guibaud, G., 2018. Two sampling strategies for an overview of pesticide contamination in an agriculture-extensive headwater stream. Environmental Science and Pollution Research 25, 14280–14293. https://doi.org/10.1007/s11356-017-9883-7

Gurzău, A.E., Coman, A., Gurzău, E.S., Penes, M., Dumitrescu, D., Marchean, D., Chera, I., 2008. pesticides use in Rural Settings in Romania. World Academy of Science, Engineering and Technology 44, 2008.

Journal Pre-proof

Gustavsson, M., Kreuger, J., Bundschuh, M., Backhaus, T., 2017. Pesticide mixtures in the Swedish streams: Environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation. Science of The Total Environment 598, 973–983. https://doi.org/10.1016/j.scitotenv.2017.04.122

Horton, T., Kroh, A., Ahyong, S., et al., 2018. World Register of Marine Species (WoRMS). WoRMS Editorial Board.

Institutul Național de Statistică, 2018. Pesticide plasate pe piaţă, în anul 2017. ISSN 2285-9594, https://insse.ro/cms/ro/content/pesticide-plasate-pe-pia%C5%A3%C4%83-%C3%AEn-anul-2017.

Kovács-Hostyánszki, A., Földesi, R., Mózes, E., Szirák, Á., Fischer, J., Hanspach, J., Báldi, A., 2016. Conservation of Pollinators in Traditional Agricultural Landscapes – New Challenges in Transylvania (Romania) Posed by EU Accession and Recommendations for Future Research. PLOS ONE 11, e0151650. https://doi.org/10.1371/journal.pone.0151650

Lemm, J.U., Feld, C.K., 2017. Identification and interaction of multiple stressors in central European lowland rivers. Science of The Total Environment 603–604, 148–154. https://doi.org/10.1016/j.scitotenv.2017.06.092

Leu, C., Singer, H., Stamm, C., Müller, S.R., Schwarzenbach, R.P., 2004. Simultaneous Assessment of Sources, Processes, and Factors Influencing Herbicide Losses to Surface Waters in a Small Agricultural Catchment. Environ. Sci. Technol. 38, 3827–3834. https://doi.org/10.1021/es0499602

Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Human and Ecological Risk Assessment: An International Journal 22, 1050–1064. https://doi.org/10.1080/10807039.2015.1133242

Liu, W., Gan, J.J., Lee, S., Kabashima, J.N., 2004. Phase distribution of synthetic pyrethroids in runoff and stream water. Environmental Toxicology and Chemistry 23, 7–11. https://doi.org/10.1897/03-183

Journal Pre-proof

Lovász, M.-E., Gurzău, E.S., 2013. Case Study on Habits of Pesticides Use on Small Farms 4.

Malaj, E., Ohe, P.C. von der, Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. PNAS 111, 9549–9554. https://doi.org/10.1073/pnas.1321082111

MEA, M.E.A., 2005. Ecosystems and human well-being: wetlands and water. World resources institute, Washington, DC 5.

Milborrow, S., 2019. plotmo: Plot a Model's Residuals, Response, and Partial Dependence Plots.

Moldovan, Z., Marincas, O., Povar, I., Lupascu, T., Longree, P., Rota, J.S., Singer, H., Alder, A.C., 2018. Environmental exposure of anthropogenic micropollutants in the Prut River at the Romanian-Moldavian border: a snapshot in the lower Danube river basin. Environmental Science and Pollution Research 25, 31040–31050. https://doi.org/10.1007/s11356-018-3025-8

Moschet, C., Vermeirssen, E.L.M., Seiz, R., Pfefferli, H., Hollender, J., 2014a. Picogram per liter detections of pyrethroids and organophosphates in surface waters using passive sampling. Water Research 66, 411–422. https://doi.org/10.1016/j.watres.2014.08.032

Moschet, C., Vermeirssen, E.L.M., Singer, H., Stamm, C., Hollender, J., 2015. Evaluation of in-situ calibration of Chemcatcher passive samplers for 322 micropollutants in agricultural and urban affected rivers. Water Research 71, 306–317. https://doi.org/10.1016/j.watres.2014.12.043

Moschet, C., Wittmer, I., Simovic, J., Junghans, M., Piazzoli, A., Singer, H., Stamm, C., Leu, C., Hollender, J., 2014b. How a Complete Pesticide Screening Changes the Assessment of Surface Water Quality. Environ. Sci. Technol. 48, 5423–5432. https://doi.org/10.1021/es500371t

Norwegian Meteorological Institute, Norwegian Broadcasting Corporation, 2016. Yr – Weather forecasts for Norway and the world from the Norwegian Meteorological

Institute and the Norwegian Broadcasting Corporation [WWW Document]. yr.no. URL https://www.yr.no/ (accessed 11.9.18).

Nowell, L.H., Moran, P.W., Schmidt, T.S., Norman, J.E., Nakagaki, N., Shoda, M.E., Mahler, B.J., Van Metre, P.C., Stone, W.W., Sandstrom, M.W., Hladik, M.L., 2018. Complex mixtures of dissolved pesticides show potential aquatic toxicity in a synoptic study of Midwestern U.S. streams. Science of The Total Environment 613–614, 1469–1488. https://doi.org/10.1016/j.scitotenv.2017.06.156

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2018. vegan: Community Ecology Package.

Pe'er, G., Dicks, L.V., Visconti, P., Arlettaz, R., Báldi, A., Benton, T.G., Collins, S., Dieterich, M., Gregory, R.D., Hartig, F., Henle, K., Hobson, P.R., Kleijn, D., Neumann, R.K., Robijns, T., Schmidt, J., Shwartz, A., Sutherland, W.J., Turbé, A., Wulf, F., Scott, A.V., 2014. EU agricultural reform fails on biodiversity. Science 344, 1090–1092. https://doi.org/10.1126/science.1253425

R Core Team, 2017. R: A language and environment for statistical computing.

Rasmussen, J.J., Baattrup-Pedersen, A., Wiberg-Larsen, P., McKnight, U.S., Kronvang, B., 2011. Buffer strip width and agricultural pesticide contamination in Danish lowland streams: Implications for stream and riparian management. Ecological Engineering 37, 1990–1997. https://doi.org/10.1016/j.ecoleng.2011.08.016

Rasmussen, J.J., Wiberg-Larsen, P., Baattrup-Pedersen, A., Cedergreen, N., McKnight, U.S., Kreuger, J., Jacobsen, D., Kristensen, E.A., Friberg, N., 2015. The legacy of pesticide pollution: An overlooked factor in current risk assessments of freshwater systems. Water Research 84, 25–32. https://doi.org/10.1016/j.watres.2015.07.021

Rasmussen, J.J., Wiberg-Larsen, P., Baattrup-Pedersen, A., Friberg, N., Kronvang, B., 2012. Stream habitat structure influences macroinvertebrate response to pesticides. Environmental Pollution 164, 142–149. https://doi.org/10.1016/j.envpol.2012.01.007

Journal Pre-proof

Rösch, A., Beck, B., Hollender, J., Singer, H., 2019. Picogram per liter quantification of pyrethroid and organophosphate insecticides in surface waters: a result of large enrichment with liquid–liquid extraction and gas chromatography coupled to mass spectrometry using atmospheric pressure chemical ionization. Anal Bioanal Chem 411, 3151–3164. https://doi.org/10.1007/s00216-019-01787-1

Schäfer, R.B., 2019. Responses of freshwater macroinvertebrates to pesticides: insights from field studies. Current Opinion in Environmental Science & Health 11, 1–7. https://doi.org/10.1016/j.coesh.2019.06.001

Schäfer, R.B., Bundschuh, M., Rouch, D.A., Szöcs, E., von der Ohe, P.C., Pettigrove, V., Schulz, R., Nugegoda, D., Kefford, B.J., 2012. Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services. Science of The Total Environment 415, 69–78. https://doi.org/10.1016/j.scitotenv.2011.05.063

Schäfer, R.B., von der Ohe, P.C., Kühne, R., Schüürmann, G., Liess, M., 2011. Occurrence and Toxicity of 331 Organic Pollutants in Large Rivers of North Germany over a Decade (1994 to 2004). Environ. Sci. Technol. 45, 6167–6174. https://doi.org/10.1021/es2013006

Schmidt-Kloiber, A., Hering, D., 2015. www.freshwaterecology.info – An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecological Indicators 53, 271–282. https://doi.org/10.1016/j.ecolind.2015.02.007

Schreiner, V.C., Bakanov, N., Kattwinkel, M., Könemann, S., Kunz, S., Vermeirssen, E.L.M., Schäfer, R.B., 2020. Sampling rates for passive samplers exposed to a field-relevant peak of 42 organic pesticides. Science of The Total Environment 740, 140376. https://doi.org/10.1016/j.scitotenv.2020.140376

Schreiner, V.C., Szöcs, E., Bhowmik, A.K., Vijver, M.G., Schäfer, R.B., 2016. Pesticide mixtures in streams of several European countries and the USA. Science of The Total Environment 573, 680–689. https://doi.org/10.1016/j.scitotenv.2016.08.163

Journal Pre-proof

Schulz, R., 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution. Journal of Environmental Quality 33, 419–448.

Schulz, R., Peall, S.K.C., Hugo, C., Krause, V., 2001. Concentration, load and toxicity of spraydrift-borne azinphos-methyl at the inlet and outlet of a constructed wetland. Ecological Engineering 18, 239–245. https://doi.org/10.1016/S0925-8574(01)00073-8

Stehle, S., Dabrowski, J.M., Bangert, U., Schulz, R., 2016. Erosion rills offset the efficacy of vegetated buffer strips to mitigate pesticide exposure in surface waters. Science of The Total Environment 545–546, 171–183. https://doi.org/10.1016/j.scitotenv.2015.12.077

Stehle, S., Schulz, R., 2015. Agricultural insecticides threaten surface waters at the global scale. PNAS 112, 5750–5755. https://doi.org/10.1073/pnas.1500232112

Stone, W.W., Gilliom, R.J., Ryberg, K.R., 2014. Pesticides in U.S. Streams and Rivers: Occurrence and Trends during 1992–2011. Environmental Science & Technology 48, 11025–11030. https://doi.org/10.1021/es5025367

Szöcs, E., Brinke, M., Karaoglan, B., Schäfer, R.B., 2017. Large scale risks from agricultural pesticides in small streams. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.7b00933

Tennekes, M., Gombin, J., Jeworutzki, S., Russell, K., Zijdeman, R., Clouse, J., Lovelace, R., Nowosad, J., Muenchow, J., 2019. tmap: Thematic Maps.

Ulrich, 2015. Datenlage zur Belastung der Kleingewässer durch Pestizide in Deutschland: ein Statusbericht | HyWa.

Vermeirssen, E.L.M., Asmin, J., I. Escher, B., Kwon, J.-H., Steimen, I., Hollender, J., 2008. The role of hydrodynamics, matrix and sampling duration in passive sampling of polar compounds with EmporeTM SDB-RPS disks. Journal of Environmental Monitoring 10, 119–128. https://doi.org/10.1039/B710790K

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global

Journal Pre-proof

threats to human water security and river biodiversity. Nature 467, 555–561. https://doi.org/10.1038/nature09440

Vrana, B., Mills, G.A., Dominiak, E., Greenwood, R., 2006. Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water. Environmental Pollution 142, 333–343. https://doi.org/10.1016/j.envpol.2005.10.033

Waite, I.R., Van Metre, P.C., 2017. Multistressor predictive models of invertebrate condition in the Corn Belt, USA. Freshwater Science 36, 901–914. https://doi.org/10.1086/694894

Weibel, S.R., Anderson, R.J., Woodward, R.L., 1964. Urban land runoff as a factor in stream pollution. Journal (Water Pollution Control Federation) 914–924.

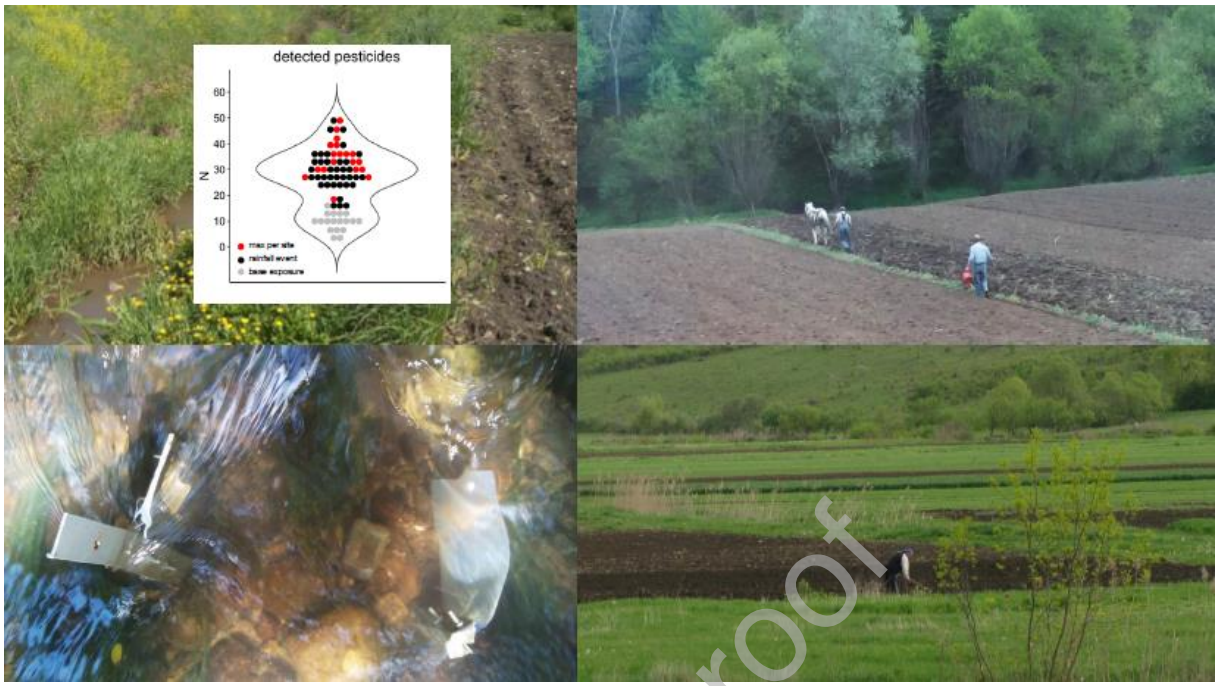
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis.

Wittmer, I.K., Bader, H.-P., Scheidegger, R., Singer, H., Lück, A., Hanke, I., Carlsson, C., Stamm, C., 2010. Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. Water Research 44, 2850–2862. https://doi.org/10.1016/j.watres.2010.01.030

Zou, H., Hastie, T., 2005. Regularization and variable selection via the elastic net. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 67, 301–320. https://doi.org/10.1111/j.1467-9868.2005.00503.x

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GRAPHICAL ABSTRACT



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