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Pesticides are the dominant stressors for vulnerable insects in lowland streams

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ABSTRACT

Despite elaborate regulation of agricultural pesticides, their occurrence in non-target areas has been linked to adverse ecological effects on insects in several field investigations. Their quantitative role in contributing to the biodiversity crisis is, however, still not known. In a large-scale study across 101 sites of small lowland streams in Central Europe, Germany we revealed that 83% of agricultural streams did not meet the pesticide-related ecological targets. For the first time we identified that agricultural nonpoint-source pesticide pollution was the major driver in reducing vulnerable insect populations in aquatic invertebrate communities, exceeding the relevance of other anthropogenic stressors such as poor hydro-morphological structure and nutrients. We identified that the current authorisation of pesticides, which aims to prevent unacceptable adverse effects, underestimates the actual ecological risk as (i) measured pesticide concentrations exceeded current regulatory acceptable concentrations accaptable concen in 81% of the agricultural streams investigated, (ii) for several pesticides the inertia of the authorisation process impedes the incorporation of new scientific knowledge and (iii) existing thresholds of invertebrate toxicity drivers are not protective by a factor of 5.3 to 40. To provide adequate environmental quality objectives , the authorization process needs to include monitoring-derived information on pesticide effects at the ecosystem level. Here, we derive such thresholds that ensure a protection of the invertebrate stream community.

1. Introduction

The ongoing biodiversity crisis is caused by a variety of anthropogenic stressors including pesticides (European Environmental Agency, 2015). However, great uncertainty remains about the respective contribution of various stressors to ecosystem degradation. This debate also relates to agricultural pesticides as some investigations have identified strong impacts of nonpoint-source pesticide pollution on streams in Australia (Beketov et al., 2013), Europe (Beketov et al., 2013; Liess and Von Der Ohe, 2005), North America (Chiu et al., 2016) and South America (Hunt et al., 2017) while others only identified comparatively low impacts of pesticides (Noges et al., 2016). Accordingly, the question remains how severe the effects of pesticides are compared to other stressors

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and, more specifically, at which concentrations ecosystem effects occur and which species and functional parameters are affected. Only with this knowledge it is possible to prioritize and manage environmental stressors effectively.

The regulatory authorisation of agricultural pesticides is supposed to prevent unacceptable effects in the environment. For example in Australia, the EU and the US, an extensive test-system based assessment scheme to protect communities in non-target aquatic ecosystems has been established (Australian Environment Agency, 2009; EFSA, 2013; US Goverment, 2004). This regulatory framework is based on the concept of scaling the effects of individual pesticides in single-species test systems or model ecosystems to the effect in the ecosystem. On this basis, pesticide concentrations are determined at which damage to aquatic communities can be excluded. However, the combined effects of natural and anthropogenic stressors present in the ecosystem are not systematically included. Nor has there been any validation of the prediction of ecosystem effects to date.

In this investigation we therefore performed a monitoring in a large geographical area that allows us to quantify all relevant anthropogenic stressors with high temporal resolution. Additionally, we identified the stream invertebrate community as a measure of ecological quality. On this basis, we aimed (i) to model the relative contribution of environmental variables determining the occurrence of aquatic invertebrates and to attribute measured pesticide pressure to ecological status, (ii) to evaluate the protectivity of the aquatic pesticide risk assessment and (iii) to derive evidence-based thresholds for the effects of pesticides considering the presence of additional stressors relevant to the ecosystem.

2. Materials and methods

2.1. Site selection

A total of 101 stream sections distributed over Germany were sampled in April and July for 2018 and 2019 (see map in SI Fig. 1), 11 sites were monitored both years. The initial selection comprised 124 stream sections, however, we omitted those stream sections that were affected by drought (lack of flow, drying out) or where the automatic rain Event-Driven Samplers (EDS) did not function (EDS: SI chapter 3). The catchment areas of the monitoring sites were characterized by a gradient of agricultural land use (agricultural land cover in hydrological catchment 0 - 100%) and less than 5% of urban areas to focus on agricultural diffuse source pollution. 86 streams were located in agricultural environments (agricultural land cover in hydrological catchment > 20%, referred to as “agricultural” streams) whereas 15 streams were located in areas with less agricultural influence (agricultural land cover in catchment < 20%, see SI chapter 1 for land use analyses). Catchment sizes were generally below 30 km^2 to represent small lowland streams where those with a catchment greater than 10 km^2 (n = 60) correspond to the reporting requirements of the WFD (Commision, 2000); stream sections with a catchment size of less than 10 km^2 (n = 41) corresponding to the "edge-of-field" surface waters of the EU-EFSA risk assessment of plant protection products for aquatic organisms (EFSA, 2013). Detailed site characteristics are listed in Tab. SI 1.

2.2. Water sampling and chemical analyses

Streams were sampled from April to July in 2018 and 2019 during the main application period of pesticides in spring and early summer for most crops (Szöcs et al., 2017). During this time period grab samples (n = 520) were taken regularly in a three-week cycle. This sampling method followed the monthly sampling in governmental monitoring practices under the WFD regardless of weather conditions. EDS samples (n = 320) were taken with automated (MAXX TP5, Rangendingen, Germany) and bottle samplers (Liess and Von Der Ohe, 2005) in order to capture runoff-induced exposure peaks associated with heavy rainfall (Liess et al., 1999), (see Fig. SI 5). Small streams with agricultural catchment area are subject to short-term water level rise (Liess and Von Der Ohe, 2005) with the occurrence of storm events exceeding approximately 10 mm/d (Schulz et al., 1998). EDS sampling for a duration of 3 hours and 20 minutes was triggered by a rise of water level of more than 5 cm so that waves did not trigger the sampling and every runoff event could be captured (further details see SI chapter 3). The samples were cooled to 4°C in the sampler until they were transported to the laboratory after a maximum of 48 h. The total of 840 samples of both field campaigns 2018 (n = 411) and 2019

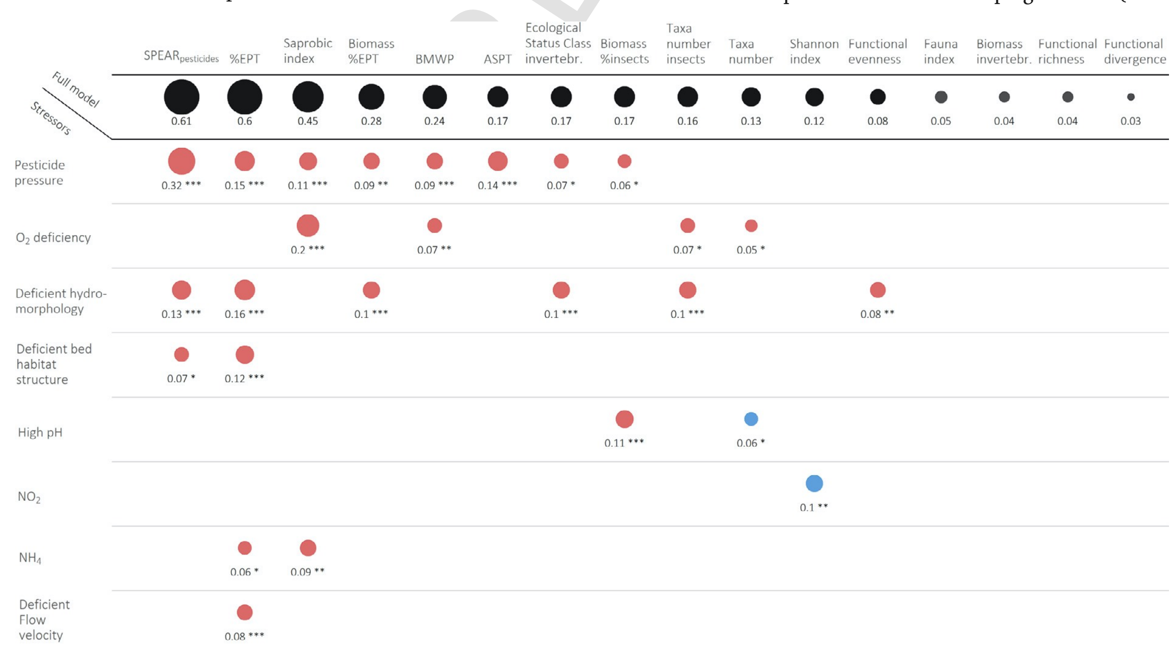

Fig. 1. Relative importance of stressors for biological endpoints - multiple linear regression to determine the explained variance, R^2 (numbers below dots). Significance levels p < 0.05*; < 0.01**; < 0.001***. Red dots indicate a deterioration of the biological endpoints with increasing stress, blue dots an improvement.

(n = 429) were analysed for pesticides, trace elements and nutrients.

For pesticide analysis, water samples were filtered and analysed via direct injection into LC-MS/MS without enrichment by multiple-reaction-monitoring (Reemtsma et al., 2013) (details see SI chapter 4). The target analysis tested for 75 pesticides (active substances) and 33 pesticide metabolites. Pyrethroid insecticides and the herbicide Glyphosate were not included due to analytical limitations. The compound selection was established by prioritization according to active substance-related sale quantities, the consideration of current environmental quality standards (EQS) and the regulatory acceptable concentrations (RAC) (Brinke et al., 2017), (see Tab. SI 2).

To test for further urban toxicants, the samples of 2018 were additionally subjected to LC—HRMS/MS screening analytics (details see SI chapter 6). This screening analyses tested for 257 substances, which were grouped into 16 compound classes including pharmaceuticals, industrial chemicals, rubber additives, stimulants, corrosion inhibitors, plastic additives, sweeteners, biocides, UV filters, bitterns, repellents, per- and polyfluorinated compounds, food ingredients, surfactants, dyes and flame retardants (see Tab. SI 4).

The concentrations of trace elements (arsenic, cadmium, copper, zinc, lead, mercury) were analysed in water samples using Agilent's ICP-MS 8000 Triple Quad. At the site the samples were pre-filtered (20 ml, 0.45 µm) for arsenic, cadmium, copper, zinc, lead, while mercury samples were bottled unfiltered in a stabilizing solution of nitric acid and potassium dichromate.

2.3. Scaling concentrations for toxicity

Concentrations of pesticides and trace elements were converted to invertebrate toxicity by calculating Toxic Units (TUs), where measured substance concentrations are normalized to their respective LC_{50} in acute standard laboratory test systems (Sprague, 1969). These LC_{50} values were derived from *Daphnia magna* or *Chironomus* sp. whose acute sensitivity, when considering a wide range of organic toxicants, is approximately equal or slightly less than the acute sensitivity of many insects (Morrissey et al., 2015; von der Ohe and Liess, 2004). For the TU calculation, the LC_{50} of the most sensitive species was considered and retrieved from the Pesticide Property Data Base (PPDB) and in few cases the US EPA ECOTOXicology knowledgebase, if the PPDB lacked respective data (see Tab. SI 3), (Lewis et al., 2016). In case no experimental data was available (0% of target analytes, 57% of non-target analyte LC_{50} values, mostly urban contaminants also including rubber additives as street-runoff indicators), Quantitative Structure Activity Relationship (QSAR)-derived effect concentrations were used to estimate TUs (Busch et al., 2016).

Pesticide peak exposure (TU_{max}) in streams toxic to invertebrates was determined by the maximum single substance insecticidal toxicity measured (Liess and Von Der Ohe, 2005) (TU_{max}, see Tab. SI 1). Extending this calculation method, we identified that exceptionally toxic samples, that are highly unusual in the exposure profile of the respective stream, did not reflect the ecological situation ($SPEAR_{pesticides}$) and were therefore not considered in the TU_{max} calculation. These exceptional exposure peaks, encountered in 20% of streams (n = 20), were defined by a TU_{max} exceeding the mean TU_{max} of the five subsequent samples (ranked by TU_{max}) by a factor of more than 100. An inclusion of exceptionally high single pulses led to a weaker correlation between the toxic pressure and the ecological effect on vulnerable species ($SPEAR_{pesticides}$) (R^2 = 0.34 versus R^2 = 0.43 with and without high pulses considered). The authors are not aware of studies that have identified the reduced significance of an exceptionally high toxicant pulse compared to many, significantly lower pulses. In contrast, the great ecotoxicological significance of several successive toxicity pulses was recognized; the "culmination" of low-dose pesticide effects (Liess et al., 2013). Analogously, the typical peak pesticide mixture toxicity (TU_{sum}) was determined by summing all individual substance TUs detected in a sample. To assess regulatory thresholds, pesticide concentrations were also scaled by the RACs instead of the LC_{50} values (see SI chapter 11). The toxicity of urban toxicants was determined in the same way as for pesticides (see Tab. SI 4). The toxicity of trace elements was calculated using literature LC_{50} values (Liess et al., 2017; Tsui and Wang, 2005), see Tab. SI 3). Here, the local maximum of summed TUs (TU_{sum}) including all trace elements per sample is considered in the multiple linear regression.

2.4. Further abiotic parameters

Ortho-phosphate, nitrate, nitrite and ammonium concentrations were determined in all grab and EDS samples using either colorimetric tests by "Visicolor" (MColortest, Merck KGaA; Darmstadt, Germany) or a UV spectrophotometer (PF-12 and visocolor ECO tests, Machery-Nagel, Düren, Germany) in 2018 and a UV spectrophotometer (DR 1900, Hach Lange GmbH; Düsseldorf, Germany) in 2019. Furthermore, total phosphorus (TP) and total nitrogen (TN) contents of all water samples were analysed (ICP-MS 8800 Triple Quad from Agilent). Oxygen, temperature, water level was continuously measured throughout the sampling period from April to June in a 3-minute interval using multi-parameter probes (LogTrans7-compact measuring system SENSOdive CTDO2, UIT; Dresden, Germany and O2-Log3055-INT and CTD3100–10 Logger, Driesen + Kern, Bad Bramstedt, Germany). PH was measured with every grab samples using pH-metre (Greisinger G 1500, Regenstauf, Germany and Xylem Analytics WTW Multi 3620 IDS Set G, Weilheim, Germany). The continuous discharge was derived from a stage-discharge relation calculated based on manually measured reference values for flow velocities and water depth for a subset of 31 streams. Hydromorphology was recorded in-situ according to the official procedure by the German Länderarbeitsgemeinschaft Wasser (LAWA) quantifying all hydromorphological criterions required under the WFD. These include amongst others meandering of the watercourse, variation in stream depth and width as well as riparian conditions (Commision, 2000). Additionally, bed habitat structure described the presence of potential holding substrate for invertebrates (Gieswein et al., 2017). This parameter represents the combined fraction of coarse particulate organic matter, plants, debris and stones > 2 mm in the stream bed. See SI chapter 2 for site-specific data and variable aggregation.

2.5. Invertebrate sampling

Benthic macroinvertebrates were sampled at the beginning of June towards the end of the main pesticide application period for most crops and therefore suitable for ecological effect identification (Liess and Von Der Ohe, 2005) (SI Invertebrate list). Standardized multi-habitat sampling(Meier et al., 2006) as prescribed under the WFD ensured comparable observations. A 50 m long section of each stream was divided into its substrate types on a percentage basis. A total of 20 subsamples (100%) were subdivided into frequencies of the occurring substrate types (smallest unit 5%). Each unit (5%) was sampled by kick sampling ten times using a net with a surface of 0.0625 m^2 and a mesh size 0.5 mm. Sampled organisms were separated the from coarse organic debris using a column sieve set, preserved in 90% ethanol, and later determined in the laboratory generally down to the lowest taxonomic level possible under the binocular. The invertebrate determination level, abundance and occurrence at sampling sites is provided in the SI chapter 8.

2.6. Biological metrics of invertebrates

We applied a wide range of biological indicator systems to assess the ecological effects of the stressors measured. Some of the invertebrate based indicators selected were developed to unspecifically respond to stressors. These are taxa number, number of insect taxa, insect and EPT% biomass - estimated using average taxa body volumes approximated by simple geometries (cylinder, ellipsoid, rotational ellipsoid or cone depending on taxon body shape) and a density of 1.06 g/mL (SMIT et al., 1993), Shannon taxa diversity (Shannon and Weaver, 1949), proportion of ephemeroptera, plecoptera and trichopteran (Lenat, 1988), Ecological Status Class (ESC) as multimetric index applied under the WFD considering individual indicators for morphological structure, organic pollution and acidification (Commision, 2000), the biological monitoring working party (BMWP) index and the Average Score Per Taxon (ASPT) indicating general water quality(Armitage et al., 1983), the Fauna Index (Lorenz et al., 2004) and the 3 functional diversity components richness, divergence and evenness (Mason et al., 2005) considering the traits body size, feeding type, locomotion and aquatic stages (Schmidt-Kloiber and Hering, 2015; Usseglio-Polatera et al., 2000). As indicators responding to specific stressors we included the $SPEAR_{pesticides}$ (Liess and Von Der Ohe, 2005) index that relates to the toxic pressure of pesticides on invertebrates and can be calculated with an online tool (https://systemecology.de/indicate/) and the Saprobic index related to the organic pollution that is linked to oxygen deficiency (Kolkwitz and Marsson, 1909; Rolauffs et al., 2013).

We defined the desired ecological status related to pesticides as for other invertebrate metrics under the WFD; with 4 boundaries separating the 5 even quality classes equal EQR (Ecological Quality Ratio) values of 0.8, 0.6, 0.4, and 0.2 (EU Commission, 2008) and classified the resulting ecological status into the usual 5 quality classes ranging from "high" to "bad" related to $SPEAR_{pesticides}$ (for details of approach and classes see SI chapter 9).

2.7. Statistical analyses

All statistical analyses were performed with the statistical software R (version 3.6.1, (R-Core Team, 2019)). Multiple linear regression was performed with all predictors for each of the above listed biological metrics of invertebrates. These include: pesticide pressure, dissolved oxygen, hydromorphology, bed habitat structure, pH, ortho-phosphate, nitrate, nitrite, ammonium, total phosphorus, total nitrogen, flow velocity, temperature, rubber additive concentration, discharge, urban toxicity, metal toxicity, stream width and stream depth (see Tab. SI 1). All predictors were checked for homoscedasticity and normality, some of which were log-transformed if necessary. Different aggregations for individual predictors were investigated to explain all biological indicators by single linear regressions. Those yielding highest coefficients of determinations compared to other aggregations were chosen (details see SI chapter 2). If parameters were only available for a subset of streams (rubber additive concentration, discharge and urban contaminants toxicity) regression analyses was reduced to the respective stream section subset.

Intercorrelation of environmental parameters was tested using the variance inflation factor (VIF). Parameters with VIF-scores greater than two were omitted. The selection of the total model was carried out by an automated forward model selection analysis and the Akaike Information Criterion (stepAIC, R-package "MASS")(Venables and Ripley, 2002). The total model is composed of significant parameters only and the explained variance is given by the adjusted R^2. The contribution of each significant parameter to the total explained variance was evaluated with the metric approach "lmg", which uses R^2 for the evaluation (Hierarchical Partitioning (Chevan and Sutherland, 1991), R-package "relaimpo" (Grömping, 2006)).The visualisation of the data and linear regression models were performed in R using ggplot2 (Wickham, 2016).

3. Results and discussion

3.1. Assessment of anthropogenic stressors

3.1.1. Determining relevant anthropogenic stressors

The 101 streams selected are a representative cross-section of small lowland streams in Central Europe (see SI chapter 1). They cover a wide gradient of agricultural pollution, include 11 small wastewater treatment plants (WWTPs) with less than 3000 population equivalents and a number of diffuse domestic discharges identified by wastewater markers. We used multiple linear regression to identify those anthropogenic stressors that determine invertebrate community composition (see SI chapter 3 for stressor distribution, chapter 8 for invertebrates sampled). Stressors with the highest explanatory power were (i) pesticide toxic pressure during exposure peaks, (ii) oxygen deficiency and (iii) poor hydromorphology (Fig. 1). Stressors showing no or only minor associations with invertebrate-related endpoints include urban toxicants such as pharmaceuticals, heavy metals, and street run-off. Agricultural pesticides, related to the substance of the peak exposure events with the highest exposure to effect concentration ratio, the TU_{max} (maximum TU), were on average 91 times more toxic than urban contaminants (related to the sum of all toxicants (TU_{sum}) 76 times more toxic). We also found that TUs measured at 11 stream sections with WWTPs were similar to those without WWTPs (SI chapter 7) comparable to a study related to WWTP in Switzerland (Munz et al., 2017). Agricultural non-point-source pesticide pollution was thus identified as a major driver of invertebrate community composition in the ecosystems under investigation (see chapter 3.3.2. on the ecological processes of the low-concentration effects of pesticides).

Non-additive interactions between stressors were investigated limited to relevant stressor combinations so as not to reduce statistical power. These were interactions between those stressors already known to act synergistically: toxicants and water temperature (Arambourou and Stoks, 2015; Verheyen and Stoks, 2020) and oxygen deficiency (Ferreira et al., 2008; Gupta et al., 1983; Van der Geest et al., 2002). We also added the remaining stressor that proved to be relevant for many of the ecological endpoints; the deficiency of morphological structure. Interactions between these three stressor combinations were all additive; none resulted in measurable antagonistic and synergistic ecological effects. Other investigations yielded comparable results for the minor relevance of interactions (Birk et al., 2020; Gieswein et al., 2017) explaining them with community adaptation processes which reduce non-additive stressor interactions (Romero et al., 2019).

3.1.2. Assessment of ecological endpoints

Ecological endpoints best responding to the measured anthropogenic stressors were: (i) the $SPEAR_{pesticides}$ index, identifying the degradation of invertebrate communities by pesticide toxicity (Liess and Von Der Ohe, 2005), (ii) the proportion of vulnerable insects%EPT (Ephemeroptera, Plecoptera, Trichoptera), identifying the general degradation of the community (Lenat, 1988) and (iii) the saprobic index, identifying the oxygen deficiency (Kolkwitz and Marsson, 1909) (Fig. 1). Other common indicators of community disturbance were only marginally associated with any of the anthropogenic stressors quantified, namely the BMWP and ASPT (Armitage et al., 1983). Also the Ecological Status Class (ESC) for the biological quality element invertebrates under the EU water framework directive (WFD) (Völker et al., 2016) seems unable to reflect anthropogenic stressor effects in small lowland streams. An extended list of endpoints and their association to stressors is displayed in Fig. 1.

Our results show that indicators of function were only marginally associated with any of the anthropogenic stressors quantified. These include invertebrate biomass, taxa number and also diversity indices as functional richness, evenness and divergence (Mason et al., 2005). Similar results were revealed for other small lowland streams (Voß and Schäfer, 2017). The weak association of anthropogenic stressors and several indicators of function is likely due to compensatory processes (Frost et al., 1995). Obviously such "integrating endpoints" that describe a system in its entirety (i.e. total abundance or biomass) are subject to compensatory processes and therefore respond less to stressors compared to "differentiating endpoints" (Liess and Foit, 2010). The loss of sensitive species may be compensated through tolerant species (Dornelas et al., 2019). Accordingly, "differentiating endpoints" that include structural community measures and can reflect declines of the fraction of vulnerable taxa – increased by competitive processes between taxa (Liess et al., 2013) – , show strong associations with stressors. These measures describe biological systems by grouping its elements (individuals and populations) according to contrasting traits (Liess and Foit, 2010). Examples are the endpoints $SPEAR_{pesticides}$,%EPT, and the Saprobic index that differentiate community composition according to the vulnerability of taxa towards pesticides, general stressors or oxygen depletion. It follows that measures describing the community without reference to competitive processes, the "integrating endpoints" such as total invertebrate biomass, taxa number and the Shannon index are not capable of indicating anthropogenic stress. It is precisely the exclusive use of integrating endpoints that carries the risk of overlooking actual stressor effects and signs of ecological degradation. One example is a recent comprehensive meta-study that reported an increase in freshwater insect abundances over the last decades, based only on integrating endpoints (Klink et al., 2020). Accordingly, total biodiversity without reference to contrasting traits such as size, longevity or sensitivity may not be a sensitive indicator of global change.

3.1.3. Characterization of the agricultural pesticide pollution

In terms of pesticide toxic pressure, regular grab samples, mainly taken during base-flow conditions, revealed a background contamination with an average of 17 detected pesticides and 10 pesticide metabolites per sample, whereas event-driven sampling (EDS) revealed an increased average of 31 pesticides and 11 metabolites per sample. Pesticide concentrations (95% percentiles) sampled by EDS events exceeded grab sample derived background concentrations by a factor of 54 on averaging, with a median of 6.3. A detailed overview of the detected pesticides and their concentrations is reported in the SI chapter 4.

Pesticides contributing dominantly to the toxic pressure of peak events on invertebrates included the neonicotinoids thiacloprid (mean share of TU_{sum} = 46.6%), imidacloprid (9.5%) and clothianidin (3.6%) as well as the biocide fipronil (9.9%) and the carbamates methiocarb (5.1%) and pirimicarb (4.8%). These 6 pesticides drove the invertebrate toxicity in 91.3% of the peak exposure events when considering the pesticide with the highest exposure to effect concentration ratio, the TU_{max}. On average, TU_{max} accounted for 69% of the invertebrate mixture toxicity assuming concentration addition (TU_{sum}). Accordingly, we show that the pesticide causing the highest toxic pressure out of the complex mixture of numerous pesticides is a good proxy of the total toxic pressure from a peak event. This was also confirmed by the linear regression depicted in Fig. 3A which showed no improved association between the toxic pressure and $SPEAR_{pesticides}$ when using TU_{sum} instead of TU_{max} (both $R^2 = 0.43$). This finding matches previous studies, which compared the relevance of the dominant compound to the mixture for the environmental impact of pesticides in agricultural streams (Knillmann et al., 2018; Liess and Von Der Ohe, 2005; Schäfer et al., 2007). Here it is necessary to recognize that the dominant compound in each event can be a different one. Several such pesticide peak exposure pulses with at least a tenth of the TU_{max} occurred on average 3.7 times per site and sampling period.

3.2. Current risk assessment underestimates exposure and effects of pesticides

3.2.1. Exceedances of regulatory acceptable concentrations (RACs)

The authorisation of a pesticide requires that its application results in an environmental exposure below the safe level for non-target organisms within the ecosystem (EFSA, 2013). Exposure models are applied to derive predicted environmental concentrations (PEC). The level of exposure considered to be safe is determined in a tiered approach identifying regulatory acceptable concentrations (RAC) for each pesticide. Our monitoring-based findings show that these regulatory requirements (PEC < RAC) are often not met in reality:

The measured environmental concentration (MEC) was higher than the predicted environmental concentrations (MEC > PEC, Fig. 2B). For 11 out of 16 pesticides that frequently exceeded RACs (selection see Tab. SI 2) we observed PECs being exceeded in more than 1% of EDS samples (Fig. 2B).

The RACs in place during the monitoring were exceeded in the majority of streams (Fig. 2A). Even pesticides no longer approved at the time of the investigation (2018, 2019) were present in concentrations above their RAC (SI Tab. 2). At least one exceedance of a RAC was detected in the 81% of sites in catchments with agricultural land use exceeding 20% (Fig. 2A). More than 5 RAC exceedances within one sampling period were identified in 41% of agricultural streams. EDS with a total $n = 296$ from agricultural streams revealed RAC exceedances in 59%, grab samples with a total $n = 440$ in 26% of samples. This is similar to the results obtained by the most comprehensive meta-study to date, which found that 45% of the 1566 cases of measured insecticide concentrations in EU surface waters exceeded their respective RACs (Stehle and Schulz, 2015). On the substance level, 37 pesticides and 2 metabolites exceeded their RAC (Fig. 2B, for the 20 pesticides with most exceedances, Tab. SI 2 for all substances). Moreover, in this current investigation we identified 41% of the 17 streams with less than 20% of agricultural land use where RACs were still exceeded. 4 out of 7 streams without any agriculture or known point sources within their catchment showed RAC exceedances of 3 pesticides (Imidacloprid, Clothianidin, Fipronil; see Fig. SI 4A). From the 7 sites within nature conservation areas 4 sites show RAC exceedances, 5 sites show AC_{field} exceedances (explanation see 3.3.1, data: Table SI 1, Fig. SI 4). Although the authorisation of spray applications for 3 neonicotinoids had already expired in 2019, similar high exceedances as in 2018 were measured (Clothianidin, Imidacloprid, Thiamethoxam).

3.2.2. Reasons for non-compliance with regulatory thresholds

For the 20 pesticides that most frequently exceeded the RACs, the following potential reasons for non-compliance with the regulatory thresholds were identified (Fig. 2B and SI Chapter 4).

(i) For 11 of these pesticides PECs were exceeded, possibly either due to unauthorised application rates, faulty exposure modelling, failure to consider multiple applications in the river basin, or overestimation of the predicted effectiveness of risk reduction measures (Thiacloprid, Terbuthylazin, Nicosulfuron,

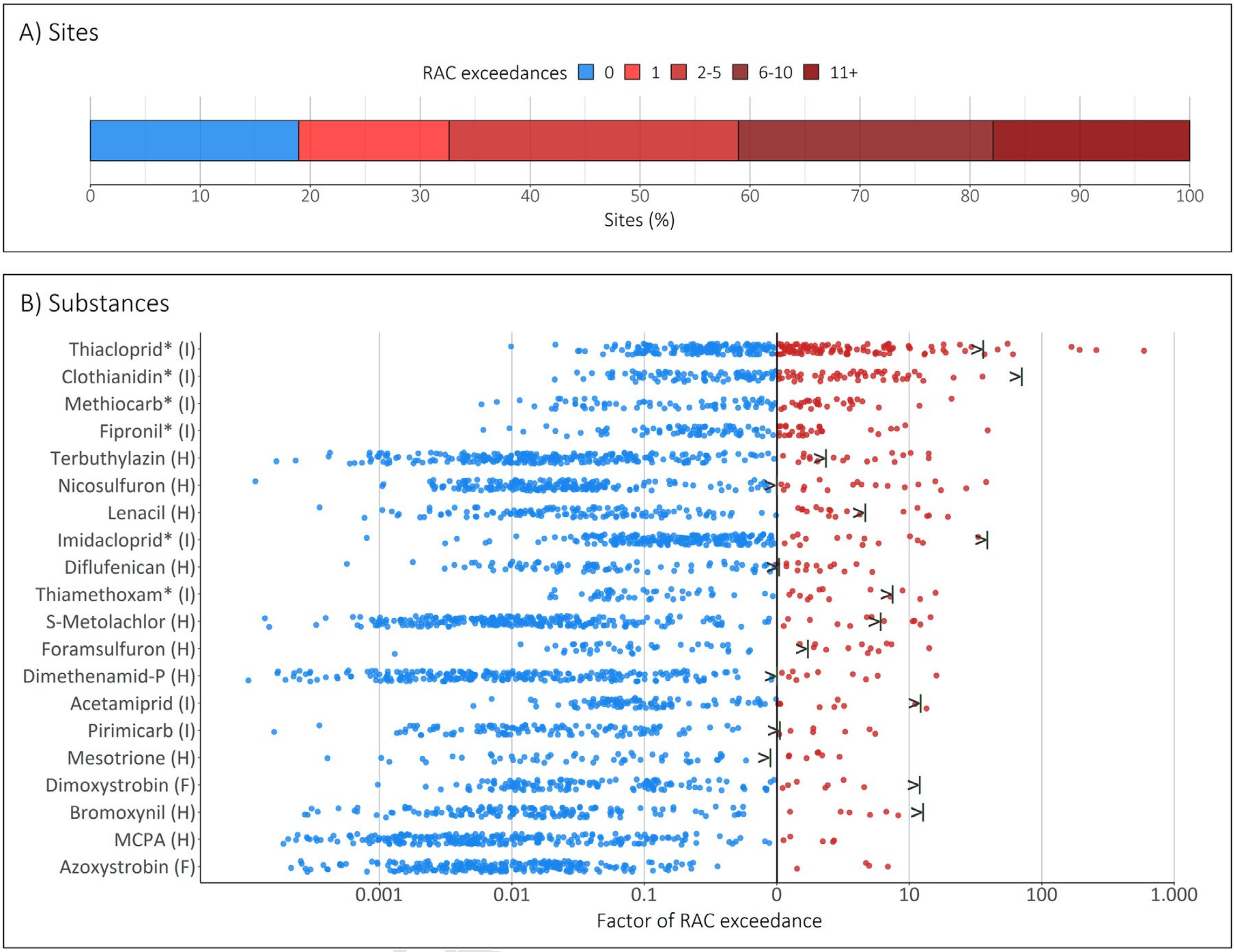

Fig. 2. Measured exceedances of regulatory acceptable concentrations (RAC), Event-driven samples (EDS) from streams with >20% agricultural land use within the catchment. A) RAC exceedances per site and year (n = 95). No exceedances in 19% of sites, 1 in 14%, 2–5 in 23%, and more than 11 in 18%. B) Substance-related RAC exceedances in EDS samples (n = 296) of RACs for those 20 pesticides with most exceedances. Regulatory approval of marked (*) substances expired by December 2020. The ratio of predicted environmental concentrations (PEC) to the respective RAC including risk mitigation measures is shown by black “>|” symbols. For MCPA and Azoxystrobin no single PEC value could be identified.

Lenacil, Diflufenican, Thiamethoxam, S-Metolachlor, Foramsulfuron, Dimethenamid-P, Pirimicarb, Mesotrione).

(ii) Due to regulatory updated effect information, the RAC has been lowered for 8 pesticides after approval of available products. However, this updated information did not have an impact on the authorised products already on the market. This leads to the situation, that products are available for use even if the expected PEC is above the updated RAC and an authorisation would not have been granted (EU Commission, 2011). However, due to the inertia of the risk assessment practice where re-evaluation is generally intended only every 10 to 15 years, this incorporation of new knowledge had not been performed for several products containing the pesticides Thiacloprid, Clothianidin, Methiocarb, Imidacloprid, Thiamethoxam, Acetamiprid, Dimoxystrobin and Bromoxynil.

(iii) The measured environmental concentrations of 2 pesticides exceed their RAC without having a PEC assigned as authorisation assumed that there is no discharge into streams. For Methiocarb, no PEC run-off was modelled due to the exclusive use as seed treatment. Although this assumption has proven wrong years ago, the new assessment practice in place did not have an impact on authorized products already on the market. Fipronil on the other hand is only approved for biocidal and veterinary use and therefore has no PEC for agricultural use assigned.

3.2.3. Contradiction to the pesticide regulation and the water framework directive (WFD)

The environmental situation as revealed in the current investigation related to agricultural streams shows an impairment of vulnerable populations, represented by a reduction of the $SPEAR_{pesticides}$ index. This situation does not comply with the Regulation (EU) 546/2011 that states “Member States shall ensure that use of plant protection products does not have any long-term repercussions for the abundance and diversity of non-target species.” (EU Commission, 2011). This also contradicts the requirements of the EU regulation 1107/2009 that pesticides must not exert “unacceptable effects on the environment” considering “particularly contamination of surface waters,“ with regards to “non-target species” and “impact on biodiversity and the ecosystem“ (EU Parliament, 2009). As required by the European parliament, no authorization to pesticides shall be granted “unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on the viability of exposed species … occurs” (EU Commission, 2011). Whereas unacceptability is defined within the specific protection goal for the “ecological threshold option” as “negligible population-level effects” on the “most sensitive populations”. “The term negligible is used since it is difficult to demonstrate that no effect is occurring” (EFSA, 2013). Furthermore, the responsible authorities themselves are questioning the extent to which these environmental protection requirements are being implemented in practice. For example, the European Court of Auditors noted "limited progress in measuring and reducing risks” of plant protection products (European Court of Auditors, 2020). Furthermore, the German Federal Environment Agency (UBA) criticizes “the current intensity of chemical plant protection in Germany as ecologically unsustainable and thus threatening the achievement of key targets of environmental protection and nature conservation policies” (Frische et al., 2018).

The Water Framework Directive (WFD) also requires a good chemical status of water bodies by not exceeding Environmental Quality Standards (EQS). The respective exceedances of these thresholds point a similar picture, see SI chapter 10 and SI Table 2.

3.3. Deriving protective thresholds for pesticides

3.3.1. Deriving the acceptable concentration (AC_{field})

The extensive dataset generated here allows to identify field-based safe concentrations at which no unacceptable adverse ecological effects on invertebrate communities are expected, the field validated Acceptable Concentration (AC_{field}). For the first time, this enables a validation of regulatory effect thresholds. The AC_{field} is based on 3 components: (i) the indicator system $SPEAR_{pesticides}$, (ii) an identification of the desired ecological status related to pesticides, (iii) the quantification of the uncertainty of the exposure-effect relationship.

(i) As a specific biological indicator, we applied the $SPEAR_{pesticides}$ index that uses pesticide-specific traits (pesticide sensitivity, generation time, migration ability, presence during the time of contamination) characterising the aquatic invertebrate community to establish a link between test-system based toxicity (LC_{50}; *D. magna, C. riparius*) and ecological impact (Liess and Von Der Ohe, 2005). The index responds primarily to toxic pressure and is largely independent of other environmental factors as shown earlier (Knillmann et al., 2018; Liess et al., 2008) and also here (Fig. 1). The approach has been successfully applied in various geographical regions including Europe (Knillmann et al., 2018; Schäfer et al., 2012), Australia (Burgert et al., 2011) and South America (Hunt et al., 2017) enabling a widespread adoption of the presented approach.

(ii) To define the ecological status related to pesticides we derived an EQR (Ecological Quality Ratio) following the respective EU-WFD procedure (EU Commission, 2008) and as detailed within the methods section and the SI chapter 9. The respective quality classes are indicated in Fig. 3A, where the boundary between a “good” and “moderate” status was set to a $SPEAR_{pesticides}$ value of 0.6 resulting in 83% of agricultural streams that did not reach the pesticide related ecological targets.

(iii) The uncertainty of the exposure-effect relationship is quantified by the variance of the relationship (Fig. 3A). Causes for this variance are likely to include site-specific environmental factors and their interaction with pesticides as well as inaccurate exposure and effect assessment. The linear regression between toxic pressure (TU_{max}) and community response ($SPEAR_{pesticides}$) intersects the transition between the “good” and “moderate” quality class at a log TU_{max} of −3.27, identifying the threshold where 50% of sites below the regression line fail to meet a “good” ecological quality for invertebrates (Fig. 3A). To establish a reliable ecosystem-based exposure-effect relationship we assume that all the variance observed is not related to the effects of pesticides but to other factors. This approach will considerably underestimate the true impact of pesticides. Accordingly, the $SPEAR_{pesticides}$ benchmark for an acceptable ecological status is reduced by the variance observed and should therefore be considered a conservative indicator of pesticide exposure (1.645σ corresponding to a one-sided confidence level of 95%, see Fig. 3A, line *a*). Thus, a log TU_{max} of −3.27 marks the toxic pressure at which only 5% of sites will show an unacceptable $SPEAR_{pesticides}$ with a 95% confidence level (Fig. 3A & B, line $b_{5\%}$). With this framework we consider the pesticide effects and as well as the related variability existing in the field and transform an adaptive cause-effect relationship of toxic pressure

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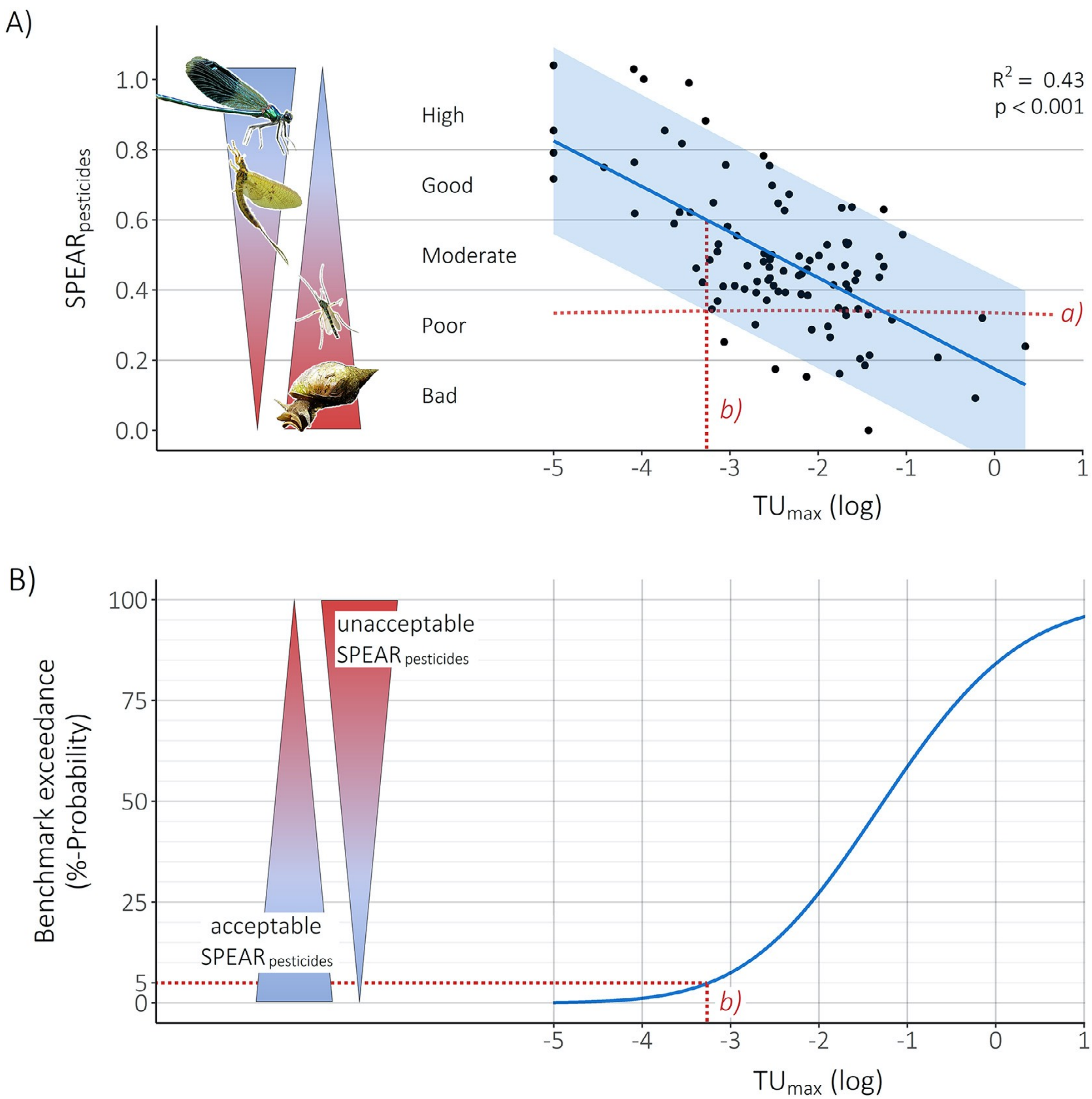

Fig. 3. Field-based adaptive (A) and benchmark-related (B) cause-effect relationship for pesticides. A) Adaptive cause-effect relationship of toxic pressure (TU_{max}) and ecological effect ($SPEAR_{pesticides}$) observed in the 101 streams. The blue band corresponds to the 90% prediction interval. Line $a_{95\%}$ depicts the $SPEAR_{pesticides}$ benchmark to identify unacceptable pesticide effects with a confidence of 95% ("good"-"moderate" benchmark reduced by 1.645σ of the linear regression). Line $b_{5\%}$ represents the log TU_{max} threshold of -3.27, where 5% of streams show an unacceptable ecological status according to $SPEAR_{pesticides}$ with a confidence of 95%. B) Benchmark-related ecological cause-effect relationship: Resulting probability of exceeding the $SPEAR_{pesticides}$ benchmark as a function of TU_{max}.

($SPEAR_{pesticides}$) into a benchmark-related ecological cause-effect relationship (95% of streams protected), termed the AC_{field}. Accordingly, the threshold value for a pesticide that adversely affects invertebrates equals the substance-specific acute LC_{50} divided by an extrapolation factor of about 2000 (AC_{field} see Tab. SI 2). This measure describes the typical short-term exposure of primarily invertebrate-toxic pesticides at which no adverse effect on the invertebrate community is expected in 95% of the streams. The relationship displayed in Figure 3B additionally allows to identify the toxic pressure of a pesticide that relates to any percentage of streams affected.

The approach presented here is based on the assumption that the extrapolation factor from the laboratory-based LC_{50} to the field-effect is similar for all pesticides. Only then is it possible to include all peak loads to derive a common extrapolation factor, regardless of the dominant pesticide in a given mixture. The exceptionally good association between toxic pressure (TU) and invertebrate response ($SPEAR_{pesticides}$) in an ecological context shows that this assumption can obviously be made. Furthermore, pesticides that do not cause the highest toxicity are also contributing to the overall ecological impact. As for other environmental factors, for the ecological assessment they are considered as a constant effect-determining factor that is included in the extrapolation factor. The good correlation identified in Fig. 3A indicates that these assumptions are valid for the majority of the pesticides investigated. Nevertheless, significant deviations from this rule may occur in individual cases, so that the AC_{field} values are merely an indication of the ecological potency of a toxicant. With this restriction in mind a prospective assessment of the ecosystem impact of new pesticides is possible. Accordingly, this approach integrates prior knowledge into the derivation of ecologically effective concentrations in a similar way as other studies have based the probability of occurrence of taxa on habitat suitability (Vermeiren et al., 2020) and toxicant concentration (Liess and Von Der Ohe, 2005). The AC_{field} allows an effect assessment for a pesticide on the basis of the other pesticides typically present in agricultural streams. Therefore, the AC_{field} can only be compared with the RAC when considering that RAC values were derived without taking into account the presence of other pesticides.

The AC_{field} that is available for 22 primarily invertebrate-toxic pesticides identifies an extrapolation factor related to acute LC_{50} values of about 2000 protecting 95% of streams; a factor exceeding the acute regulatory Tier 1 "assessment factor" (100) by 20. To protect 99% of streams the respective extrapolation factor would amount to 18,000, a log TU_{max} of −4.25 (Fig. 3B). However, the exposure to RAC ratio was found to explain $SPEAR_{pesticides}$ equally well as the exposure to LC_{50} ratio ($R^2 = 0.44$ versus $R^2 = 0.43$, see Fig. SI 8A). This shows that the RAC values are related to the ecological effect as shown in the cause-effect relationship in Fig. SI 8A. Nonetheless, their compliance would cause unacceptable effects in 14% of agricultural stream sections; 86% would be protected (Fig. SI 8B). To protect 95% or 99% of streams, respectively, the RAC for invertebrate-toxicity driving pesticides (SI chapter 11) required an additional assessment factor of 5.3 or 40.2. It must to be taken into account that these results refer primarily to the pesticides with the greatest RAC exceedances. These include particularly 4 different neonicotinoids as well as fipronil, methiocarb and terbuthylazine (Fig. Si 4).

3.3.2. Mechanisms for the observed low-concentration effects of pesticides

We hypothesize the following ecological processes as the reason for the high field sensitivity of vulnerable species and the associated increased extrapolation factor identified here:

(i) The multitude of pesticides present in the streams may not only result in additive effects (Loewe and Muischnek, 1926) but also in a synergistic increase of pesticide toxicity due to the presence of additional toxicants that may exceed the additive effects by a factor of up to 660 as identified in laboratory investigations (Liess et al., 2020) or by an increase of single-substance toxicity by more than one order of magnitude as identified in field investigations (Rydh Stenström et al., 2021),

(ii) Environmental stressors may act synergistically when they occur together. Examples include the combined effects of nutrients, suspensions and temperature frequently producing synergistic effects on abundance at the population level of periphyton communities (Piggott et al., 2015) and the combined effects of nutrients, suspensions and chloride inducing invertebrate drift in streamside mesocosms (Beermann et al., 2018). Additionally, stressors such as predator pressure, competition and suboptimal environmental conditions may increase the sensitivity of populations to pesticides by a factor of up to 100 as revealed in microcosm (Liess et al., 2016) and mesocosm studies (Liess and Beketov, 2011).

(iii) Repeated insecticide pulses leading to multiple exposure of individuals within a generation (within a spray season for annual species), increases the impact compared to a single insecticide pulse (Wiberg-Larsen et al., 2021). Also repeated pesticide pulses leading to multiple exposure of populations between generations (between spray seasons for annual species), increases the impact compared to a single insecticide pulse and may result in a multigenerational culmination of low-concentration effects (Liess et al., 2013).

The effect-determining factors and their related processes described here are generally not considered in the aquatic risk assessment. Thus, neither for individual-based lower-tier studies nor for mesocosm-based higher-tier studies effect-determining factors are taken into account that are comparable in their expression with the field. Calibration of existing assessment factors by means of traditional higher-tier studies has been successfully carried out (Brock et al., 2016; Rico et al., 2019), but does not allow prediction of pesticide effects in the field. We therefore suggest to calibrate the assessment factors applied in pesticide regulation integrating field-based findings. For example, a relevant candidate for such an exercise is the insecticide chlorantraniliprole, a pesticide that may replace the widely used neonicotinoids and could therefore gain high relevance in the near future (Schmidt-Jeffris and Nault, 2016). For chlorantraniliprole the RAC is a factor of 50 higher than the respective AC_{field}. Accordingly, regular authorities could review the derivation of the current RAC in order to avoid future environmental problems with this pesticide.

Conclusions

• In this study of 101 small lowland stream sections, we revealed for the first time the prime relevance of agricultural pesticide pressure for the composition of invertebrate communities.
• The diversity and number of vulnerable species was already reduced at very low pesticide concentrations, so that most of agricultural streams and even sites in nature conservation areas did not meet the pesticide-related ecological targets.
• We revealed that the current authorisation of pesticides underestimates the actual ecological risk, as measured pesticide concentrations exceeded current regulatory threshold levels in most of the agricultural streams. In addition existing thresholds were not protective for invertebrates.

• By including monitoring-derived information on pesticide effects within the ecosystem we identified pesticide threshold concentrations that will ensure an appropate protection of the invertebrate stream community.
• Future research should extend this concept developed in this study to other groups of aquatic organisms such as amphibians, fish, plant and fungi communities, and also to terrestrial ecosystems. This identification of field-validated **A**cceptable **C**oncentrations for the ecosystem (**AC**$_{\text{field}}$) can then be used to review the existing thresholds of the Pesticide Risk Assessment (R**AC**) and the Water Framework Directive (M**AC**-EQS).

Additional resources

Additional information on methods and supplementary results are available in the document SI 1. A comprehensive overview of the site parameter (SI 2), pesticide measurements and characteristics (SI 3), urban contaminants measurements and characteristics (SI 4) and invertebrate occurrence and characteristics (SI 5) is presented in the Supporting Information (SI). A visualisation of the distribution of measured pesticide concentrations is available in the exposure classifier (https://www.ufz.de/kgm/index.php?en=48130). All raw data is publicly available under Liess et al. 2021 (Data publication simultaneous to this paper via the data publisher PANGAEA, under embargo and will be publicly available on the 30.09.2022 at https://doi.org/10.1594/PANGAEA.931673. Title: The lowland stream monitoring dataset (KgM, Kleingewässer-Monitoring) 2018, 2019.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117262.

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