Potential propagation of agricultural pesticide exposure and effects to upstream sections in a biosphere reserve

Science of the total environment

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Abstract

In the last decades, several studies have shown that pesticides frequently occur above water quality thresholds in small streams draining arable land and are associated with changes in invertebrate communities. However, we know little about the potential propagation of pesticide effects from agricultural stream sections to least impacted stream sections that can serve as refuge areas. We sampled invertebrates and pesticides along six small streams in south-west Germany. In each stream, the sampling was conducted at an agricultural site, at an upstream forest site (later considered as "refuge"), and at a transition zone between forest and agriculture (later considered as "edge"). Pesticide exposure was higher and the proportion of pesticide-sensitive species ($SPEAR_{pesticides}$) was lower in agricultural sites compared to edge and refuge sites. Notwithstanding, at some edge and refuge sites, which were considered as being least impacted, we estimated unexpected pesticide toxicity (sum toxic units) exceeding thresholds where field studies suggested adverse effects on freshwater invertebrates. We conclude that organisms in forest sections within a few kilometres upstream of agricultural areas can be exposed to ecologically relevant pesticide levels. In addition, although not statistically significant, the abundance of pesticide-sensitive taxa was slightly lower in edge compared to refuge sites, indicating a potential influence of adjacent agriculture. Future studies should further investigate the influence of

spatial relationships, such as the distance between refuge and agriculture, for the propagation of pesticide effects and focus on the underlying mechanisms.

1. Introduction

Several studies over the past two decades have assessed pesticide pollution of freshwater habitats (Liess et al., 2021; Schäfer, 2019; Schulz, 2004). They found that pesticides regularly exceed ecological quality thresholds (Szöcs et al., 2017) and are a major factor shaping macroinvertebrate communities in stream sections draining arable land (Beketov et al., 2013; Chiu et al., 2016; Hunt et al., 2017; Liess et al., 2021; Miller et al., 2020; Schäfer et al., 2012). Through flows of matter, energy and organisms, agricultural stream sections can be connected to least impacted stream sections (Loreau et al., 2003), which, in the face of almost ubiquitous human influence, are defined as sites that are relatively free from human influence, for example without agricultural land use. Via these flows, either pesticides or their effects might propagate to the adjacent ecosystems (Schiesari et al., 2018). For example, pesticides can be transported to adjacent ecosystems, via flows of air, water or organisms (Hageman et al., 2006; Harding et al., 2006; Richmond et al., 2018). Among these flows, organisms occupy a special position, as they can move against the flow direction of water and air. The flow of organisms between patches of different states of pollution can moderate pesticide concentrations of the patches (e.g. biovector-transport; Richmond et al., 2018; Schiesari et al., 2018). In addition, dispersing organisms can alleviate or exacerbate the effects of pesticides. For instance, organisms from less or non-polluted patches can recolonise polluted patches, thereby fostering recovery of vulnerable species and alleviating the pollutants effects on communities (Orlinskiy et al., 2015). A field study attributed the recovery of eight out of eleven invertebrate populations from an insecticide pulse to immigration from less or non-affected connected patches (Liess and Schulz, 1999). We refer to these patches as “refuges”. An analysis of multiple field studies suggested that certain presumed pesticide-vulnerable taxa can occur even in highly polluted stream sections if upstream refuges are present (Knillmann et al., 2018). The authors attributed their occurrence to dispersal and stress-resistant traits, such as asynchronous life cycles and resistant aquatic or terrestrial life stages. Similarly, a meta-analysis of field studies found that the presence of non-polluted upstream refuges supports the persistence of pesticide-sensitive species in polluted downstream sections (Schäfer et al., 2012). Together, these studies suggest that through dispersal from refuge stream sections the effects in polluted stream sections can be alleviated.

However, the dispersal processes that alleviate pesticide effects in polluted downstream sections may incur costs for the refuge populations. For example, the propagation of effects from polluted to non-polluted systems was predicted in several studies with metapopulation models (Chaumot et al., 2003; Spromberg et al., 1998; Willson and Hopkins, 2013). A metapopulation model focusing specifically on pesticide effects in streams estimated a reduction of up to 25 % population size of a freshwater insect in the non-polluted patch (Schäfer et al., 2017). This reduction occurred as a result of density-dependent depletion of source organisms via dispersal and associated mortality. Besides, pesticide effects may propagate to least impacted habitats, when organisms disperse from polluted patches to non-polluted stream sections and genetically exchange with refuge organisms, but related studies are scarce. Empirical studies quantifying to which extent the effects of pollutants such as pesticides propagate to least impacted upstream sections are lacking. Therefore, our understanding of the spatial dynamics of pesticide effects is low, compromising our ability to predict or explain community dynamics in least impacted habitat patches. Assuming that effect propagation is relevant, such knowledge would inform pesticide management and might contribute to the conservation and protection of biodiversity.

Following the concept of edge effects in landscape ecology (Fischer and Lindenmayer, 2007), the transition zone, termed edge, between ecosystems is characterised by the bi-directional extension of flows of organisms, matter and energy into the adjacent system. Thereby, the edge of one ecosystem is most influenced by the adjacent ecosystem. Hence, edge habitats are frequently characterised by the biotic and abiotic conditions, including pesticide pollution, of the adjacent ecosystems. For example, changes in resource availability, quality and/or structure at edges can drive community responses at edges of terrestrial habitats (Ries et al., 2004; Wimp and Murphy, 2021). But also the repeated disturbance by anthropogenic activities, such as pesticide drift from agriculture, has been found to lower the diversity-enhancing properties of edges in comparison with edges maintained by natural processes in terrestrial landscapes (meta-analysis, ground beetles in forested edges; Magura et al., 2017). To our knowledge, studies on potential edge effects in freshwaters are lacking.

We examined six small streams for pesticide exposure and pesticide effects in agricultural sites and related upstream edges and refuges. To detect potential exposure and effects, we compared edges of refuges (hereafter “edge”) to agricultural downstream areas (hereafter “agriculture”) and the core zones of refuges (hereafter “refuge”). The study was conducted in south-west Germany in a region where streams originate in forested areas of a biosphere reserve and subsequently run through an agricultural landscape with presumed high pesticide inputs and effects on invertebrates. Given that refuges mainly drain forested areas without pesticide use, we hypothesised the absence of pesticide exposure at edges and refuges, whereas, we hypothesised high pesticide exposure in the downstream agricultural sites (hypothesis 1). In addition, we hypothesised that dispersal processes result in the propagation of pesticide effects to the edge sites, measurable as a reduction in pesticide-sensitive invertebrate species at edges compared to refuges further upstream (hypothesis 2).

2. Material and Methods

2.1. Study area

We conducted the study in the summer of 2019 in Rhineland-Palatinate, south-west Germany. The study region is located in the transition area between low mountain ranges and lowlands. We selected six streams (Fig. 1; from south to north: Russbach, Otterbach, Hainbach, Modenbach, Triefenbach, Kropsbach). Within each stream, we took samples at three different sampling sites, i.e. “refuge”, “edge” and “agricultural” sites. The agricultural sites were predominantly characterised by vineyards. Refuge sites were defined as sites without known pesticide use in the upstream catchment and were located in the upstream forest section of each stream. The edge sites were located at the edge of the forest in the transition zone to agriculture (Fig. 1). The site types were characterised by different elevations, with the forested refuge sites being about 100 – 200 m higher than the agricultural sites (Table SI 1). The terrain around the agricultural sites is slightly flatter than the terrain of the forested refuge and edge sites but still characterised by low hills. The distances between the agricultural sites and the edge sites ranged from 3.2 to 4.8 km, except for two streams with 1.4 and 1.6 km (Table SI 1). The distances between the edge and the refuge sites were 1.1 to 1.5 km, except for one stream (0.6 km; Table SI 1). The variability in distances owes to differences in stream courses, lengths and accessibilities that influenced site selection. The distance between refuge and edge was selected to exceed the maximum gammarid dispersal, a dominant organism group in this region and assuming that pesticide effects propagate via dispersal. *Gammarus sp.* can reportedly disperse up to 6 m per day and have an average life expectancy of six months (Elliott, 2003), resulting in an upper dispersal limit of approximately 1 km under normal conditions (e.g. no catastrophic drift). However, depending on the network structure, genetic exchange in the stream catchment can also take place over greater distances (Alp et al., 2012).

The present study supplemented the national pesticide monitoring of Germany ("Kleingewässer-Monitoring" or "KgM") in 2019 (Liess et al., 2021). A subset of our sites (six agricultural sites and the Modenbach refuge site) was part of the KgM monitoring and hereafter referred to as "subset of KgM sites".

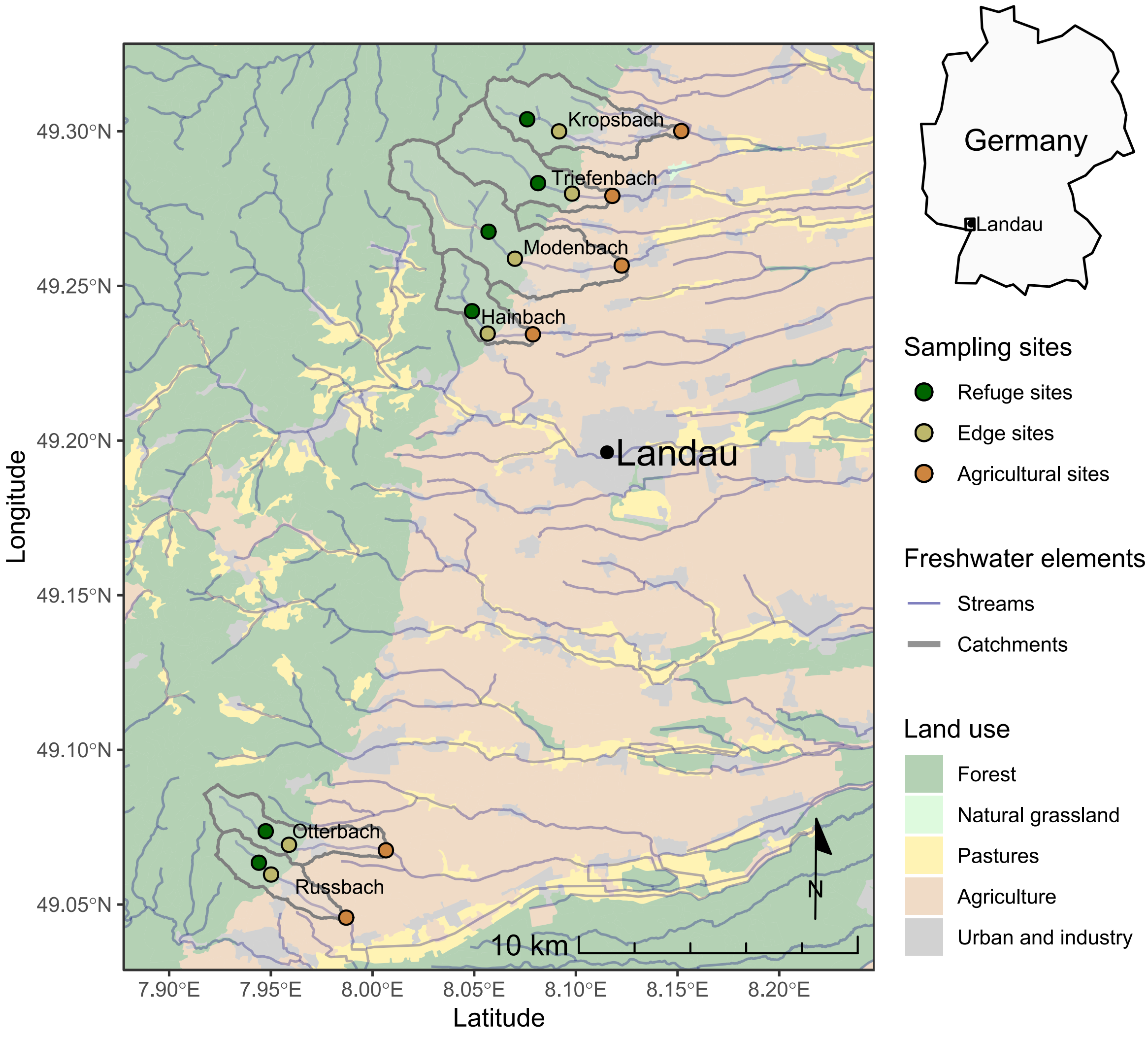

Figure 1: Overview of the sampling sites, i.e. refuge, edge and agricultural sites at the six streams and their catchments in Rhineland-Palatinate, Germany, with different land use categories based on the CORINE land cover 2018 (Copernicus Land Monitoring Service, 2019).

2.2. Habitat characterisation

We recorded physicochemical habitat properties to control for factors other than pesticide exposure that may shape invertebrate assemblages. We measured water temperature, electrical conductivity, dissolved oxygen and pH using a multi-parameter portable meter (WTW® Multi 3630 IDS Set G; Xylem Analytics, Rye Brook, US) and flow using a flow meter (Höntzsch, Waiblingen, Germany) at all sampling sites directly after pesticide field-sampling in June 2019 (11./12). In addition, we recorded stream depth and width and measured concentrations of ammonium-nitrogen (NH4-N), nitrate-nitrogen (NO3-N), nitrite-nitrogen (NO2-N) and phosphate-phosphor (PO4-P) using a portable spectrophotometer (DR1900, Hach Lange, Düsseldorf, Germany) and the corresponding cuvette tests (Hach Lange, Düsseldorf, Germany). If nutrient measurements were below the limit of quantification (LOQ), we set values to 0.5 times the LOQ.

2.3. Sampling and chemical analysis of pesticides

To characterise pesticide exposure and test hypothesis 1, we took grab samples of surface water at all sampling sites (n = 18) in early (11./12.) June 2019 and analysed their pesticide concentrations. In addition, grab samples were taken every three weeks from the beginning of April to the end of July (n = 28) at the subset of KgM sites (Halbach et al., 2021; Liess et al., 2021). Using these KgM data allowed us to assess the representativeness of the June sample for the baseline toxicity during the main pesticide application season (Szöcs et al., 2017). Details of chemical analyses are described in Halbach et al. (2021). Briefly, after filtering with a syringe filter (glass fibre filter with 0.45 µm regenerated cellulose acetate (Altmann Analytik, Munich, Germany)), the samples were analysed for 74 pesticides (in Rhineland-Palatinate, 2019) via direct injection of the aqueous samples into LC-MS/MS (Agilent 1290 infinity liquid chromatography system; Agilent Technologies, Santa Clara, USA; coupled to a QTrap6500+tandem mass spectrometer equipped with an electrospray ionization interface; Sciex, Framingham, USA) and multiple-reaction-monitoring (Halbach et al., 2021). To control instrumental performance, we spiked 1 mL of the filtered samples with internal standards. For further details such as on quantification and qualification procedures see Halbach et al. (2021).

2.4. Determining macroinvertebrate community composition

To characterise community composition and test hypothesis 2, macroinvertebrate communities were sampled quantitively in early (03.-06.) June 2019, the period when pesticide effects on the macroinvertebrate community are most likely (Liess et al., 2021; Liess and Ohe, 2005; Szöcs et al., 2017). Sampling was done following the standardised multi-habitat sampling method (Meier et al., 2006) with minor modifications. Briefly, we collected five Surber kick-samples (0.124 m² area, 0.5 mm mesh; HYDRO-BIOS Apparatebau, Altenholz, Germany) within a stream section of approximately 25 m, sampling each substrate relative to its abundance. The sampled organisms were preserved in ethanol until identification in the laboratory. We identified all macroinvertebrates with a stereomicroscope (4.7x-

42.8x SZX9; Olympus, Tokyo, Japan) to the lowest taxonomic level attainable (see Table SI 2 for identification literature). Species-level was achieved for most taxa of the orders Ephemeroptera, Trichoptera, Amphipoda, for some Diptera and for some taxa of the phylum Mollusca.

For the subset of KgM sites, community data originated from the KgM where the same multi-habitat method was employed (Liess et al., 2021). We accounted for differences in the number of habitat samples per site (KgM; 10 samples, non-KgM: 5 samples) by standardising the abundance to the same area sampled (i.e. 1 m²). Note that the current study was conducted in sandstone streams characterised by relatively homogeneous stream beds with fine substrate (Table SI 3) and consequently a low habitat diversity (Fernández et al., 2015; Voß and Schäfer, 2017). Hence, we suggest that both samplings captured the main habitats and communities. However, to exclude potential bias, we restrict comparisons of the taxonomic richness to refuge and edge sites (same type of habitat sampling) and focus on a metric ($SPEAR_{pesticides}$) that is based on relative community composition, which should be relatively unbiased to sampling intensity.

2.5. Data processing and analysis

2.5.1. Estimating pesticide toxicity

To estimate the toxicity of pesticide exposure for invertebrates, we calculated the logarithmic sum of toxic units (sumTU), which corresponds to the potential mixture toxicity of all detected pesticides within one sample (Sprague, 1969). They were calculated as:

$$\mathrm{sumTU} = log10\left(\sum_{i=1}^{n} \frac{C_i}{EC_{50_i}}\right)$$

where n is the total number of pesticides targeted by analytical measurements, C_i is the measured environmental concentration of pesticide i and EC_{50_i} is the effect concentration of pesticide i that affected 50% of test organisms in acute standard laboratory tests for the most sensitive freshwater invertebrate. We obtained effect concentrations (EC_{50i}) from Standardtox (Version 0.0.1; Scharmüller, 2021; Scharmüller et al., 2020), which constitutes a collection of quality-checked and aggregated ecotoxicological test results from the ECOTOX Knowledgebase (US EPA, 2021). We selected effect concentrations corresponding to active ingredients and 24 to 96 h test duration and used the geometric mean if multiple toxicity data were available for the most sensitive species. Data gaps were filled from the Pesticide Property DataBase (PPDB) (Lewis et al., 2016) (see Table SI 4 for EC_{50i} data and corresponding references). Concentrations of spinosad a and d were summed up and considered as spinosad during TU calculation because of an absence of individual EC_{50} data. We consider sumTU's above -3 to be associated with ecological effects on freshwater invertebrates as suggested by Schäfer et al. (2012), whereas we consider sumTU's below -4 as indicative for reference sites free from pesticide effects as suggested by Becker et al. (2020).

2.5.2. Comparing invertebrate communities between site types

To compare the invertebrate communities between refuge, edge and agricultural sites, we calculated the taxonomic richness (i.e. number of different taxa per site) and total abundance (i.e. number of macroinvertebrate individuals per m²). Moreover, we assessed the compositional similarity of the macroinvertebrate communities using the Jaccard index, a commonly used similarity index (Chao et al., 2004; Le et al., 2021). The Jaccard index was calculated as:

$$\text{Jaccard index} = \frac{n_{ab}}{(n_a + n_b - n_{ab})}$$

where n_{ab} is the number of taxa that both sites a and b have in common, whereas n_a and n_b are the number of taxa present in sites a and b, respectively. The Jaccard index ranges from zero (no shared taxon) to one (identical composition).

Moreover, we particularly focused our analysis on a relative measure of community composition, the $\text{SPEAR}_{\text{pesticides}}$, which has been developed to identify pesticide effects (Knillmann et al., 2018; Liess et al., 2021; Liess and Ohe, 2005). This metric has been successfully applied in multiple studies to link estimated pesticide toxicity to the loss of pesticide-sensitive species in communities (Chiu et al., 2016; Hunt et al., 2017; Liess et al., 2008; Schäfer et al., 2007). We calculated the $\text{SPEAR}_{\text{pesticides}}$ according to Liess and Ohe (2005) and with the updates of Knillmann et al. (2018) and Liess et al. (2021) as:

$$\text{SPEAR}_{\text{pesticides}} = \frac{\frac{\sum_{i=1}^{n} log\,(4x_i + 1) * y_i}{\sum_{i=1}^{n} log\,(4x_i + 1)} * 100}{SPEAR_{reference}}$$

where n is the total number of taxa in a sample, x_i is the abundance of taxon i given as individuals per m² and y_i is the risk classification parameter for taxon i (1 – at risk, 0 – not at risk). The risk classification was retrieved from the online $\text{SPEAR}_{\text{pesticides}}$ calculation tool "indicate" (Version 2.2.1, Indicate, 2021) and is based on species traits. We excluded the trait "dependence on the presence of refuges" from the risk classification given that this trait has been implemented to remove the effects of refuges (Knillmann et al., 2018). As suggested by Liess et al. (2021), we standardised $SPEAR_{pesticides}$ values to a $SPEAR_{reference}$ value (44) determined for reference sites in the framework of the KgM (for details see Liess et al., 2021). The standardised $SPEAR_{pesticides}$ ranges from zero to one and represents the proportion of pesticide-sensitive species present in a site relative to reference sites.

2.5.3. Statistical analysis

To compare estimated pesticide toxicity and community composition across the three site types (refuge, edge, agriculture), we modelled sumTU, taxonomic richness, total abundance, Jaccard index and $SPEAR_{pesticides}$ separately as response variable explained by site type. Given that sites within a stream are likely more similar than between streams, we accounted for the nested structure of the data using linear mixed models (LMM) with stream as random factor (Zuur et al., 2009). Similarly, we modelled $SPEAR_{pesticides}$ as response variable explained by sumTU and site type using a LMM with stream as random factor to evaluate the relationship between the proportion of pesticide-sensitive species and estimated pesticide toxicity. All statistical analyses and figures were produced in R version 4.1.2. (R Core Team, 2021). For LMM, we used the lme4 package 1.1-27.1 (Bates et al., 2015). LMM were fitted using restricted maximum likelihood (REML). To test for significance of single effects in LMM, we applied a type III analysis of variance with Kenward-Roger's method available in the lmerTest package 3.1-3 (Kuznetsova et al., 2017). This method has been shown to perform well for small sample sizes (Luke, 2017). We tested for pairwise differences between sites using the Kenward-Roger estimation of degrees of freedom and adjustment by the Tukey method available in the emmeans package 1.7.2 (Lenth, 2022). For visualisation, we used the ggplot2 package 3.3.5 (Wickham, 2016) and the effects package 4.2-1 (Fox and Weisberg, 2019, 2018). We provide all raw data and the R script on GitHub at https://github.com/rbslandau/schneeweiss_refuge_1. Pesticide- and site-specific results are provided in tables SI 4 to 7.

3. Results

3.1. Habitat characteristics of refuge, edge and agricultural sites

The environmental conditions were similar at edge and refuge sites. The agricultural sites were on average slightly deeper and approximately 2 °C warmer. Similarly, the nutrient concentrations and conductivity (1.6-1.8 fold) were higher, whereas the dissolved oxygen was lower (4-5 %) in agricultural compared to edge and refuge sites (Table 1).

Table 1: Environmental variables characterising refuge, edge and agricultural sites (measured for six streams). Nutrient concentrations indicate the amount of nitrogen or phosphor in the respective compound (i.e. NH4-N, NO3-N, NO2-N, PO4-P). If nutrient measurements were below the limit of quantification (LOQ), we set values to 0.5 times the LOQ (LOQ of NH4-N: 0.015-2 mg/L; NO3-N: 0.23-13.5 mg/L; NO2-N: 0.015-0.6 mg/L; PO4-P: 0.05-1.5 mg/L).

Variable [unit]	Site type	Minimum	Maximum	Median	Mean	SD
Stream width [m]	Refuge	0.80	2.70	1.17	1.48	0.76
	Edge	1.18	2.90	1.83	1.92	0.62
	Agriculture	0.85	2.40	1.66	1.69	0.54
Stream depth [cm]	Refuge	4.50	25.00	13.50	13.25	7.87
	Edge	6.00	17.00	10.00	10.67	3.88
	Agriculture	11.00	28.00	12.50	15.33	6.44
Flow velocity [m/s]	Refuge	0.07	0.40	0.15	0.19	0.13
	Edge	0.09	0.39	0.21	0.22	0.12
	Agriculture	0.10	0.26	0.19	0.19	0.06
Water temperature [°C]	Refuge	11.10	15.60	13.65	13.32	1.53
	Edge	12.90	14.50	13.70	13.75	0.60
	Agriculture	14.50	16.30	15.70	15.60	0.64
Dissolved oxygen [%]	Refuge	89.10	95.00	91.75	92.02	2.59
	Edge	91.40	95.00	93.45	93.43	1.35
	Agriculture	81.10	91.90	89.10	87.92	4.22
Conductivity [µS/cm]	Refuge	124.00	207.00	145.00	157.67	36.41
	Edge	117.00	218.00	186.00	172.00	38.66
	Agriculture	188.00	393.00	243.00	274.17	93.48
pH	Refuge	6.49	7.84	7.38	7.26	0.51
	Edge	5.75	7.93	7.51	7.20	0.81
	Agriculture	6.73	8.22	7.66	7.61	0.54
Ammonium [mg/L]	Refuge	0.01	0.05	0.03	0.03	0.02
	Edge	0.01	0.30	0.04	0.08	0.11
	Agriculture	0.05	0.97	0.07	0.22	0.37
Nitrate [mg/L]	Refuge	0.38	2.53	0.93	1.11	0.73
	Edge	0.50	1.57	0.95	0.98	0.34
	Agriculture	0.34	2.98	1.49	1.50	0.88
Nitrite [mg/L]	Refuge	0.01	0.04	0.03	0.02	0.01
	Edge	0.01	0.05	0.02	0.02	0.02
	Agriculture	0.02	0.08	0.04	0.04	0.02
Phosphate [mg/L]	Refuge	0.03	0.07	0.03	0.03	0.02
	Edge	0.03	0.30	0.04	0.09	0.11
	Agriculture	0.03	0.19	0.06	0.07	0.06

3.2. Estimated pesticide toxicity in refuge, edge and agricultural sites

Agricultural sites were characterised by a higher level of pesticide exposure compared to edge and refuge sites both in terms of number of detected pesticides and total concentrations (Fig. 2 A,B). The average number of detected pesticides was approximately 3.5-fold higher in agricultural sites than in edge and refuge sites (6, 6 and 22 pesticides in refuge, edge and agricultural sites; Table SI 8). Similarly, the total pesticide concentration was approximately 100-fold higher (0.002, 0.003 and 0.391 µg/L in refuge, edge and agricultural sites; Table SI 8). The estimated toxicity in terms of sumTU ranged from -3.7 to -0.9 at agricultural sites (Fig. 2 C; Table SI 8) with five sites exceeding sumTU of -3, suggesting ecological effects on invertebrates (threshold definition section 2.5.1.). This level of toxicity was significantly higher compared to edge and refuge sites (LMM, p=0.015, Table SI 9,10; pairwise differences of site type: agriculture – refuge: p = 0.03, agriculture – edge: p = 0.02, edge – refuge: p = 0.95). The estimated pesticide toxicity at edge and refuge sites only occasionally exceeded the sumTU of -3 (Fig. 2 C), but, when considering additional samples covering April to July (at KgM sites), the quality criterion for reference sites (sumTU below -4; section 2.5.1.) was violated in 7 of the 16 grab samples (Fig. SI 11). The estimated pesticide toxicity for June (sampling temporally closest to invertebrate sampling) was similar to other samplings throughout the season of pesticide application from April to July (One-way ANOVA with sumTU as response and sampling date as predictor, p=0.97; Table SI 10, Fig. SI 11). Although the number of detected compounds and the total concentrations of herbicides and fungicides were generally higher than those of insecticides, the estimated toxicity to invertebrates was driven by insecticides in all site types (Fig. SI 12; Table SI 13). Except for two herbicides (chloridazon, prosulfuron), all pesticides showed common occurrence at the three site types or showed a gradient in occurrence from agricultural to edge to refuge sites (Table SI 14). The estimated toxicity at agricultural sites of this study (median sumTU = -1.93 of 2019 June samples) was slightly higher than in other regions of Germany monitored in the KgM, comprising catchments with a wide variety of crop types (median sumTU = -2.65 of 2019 June samples, calculated from the KgM raw data following the toxicity estimation detailed in the methods).

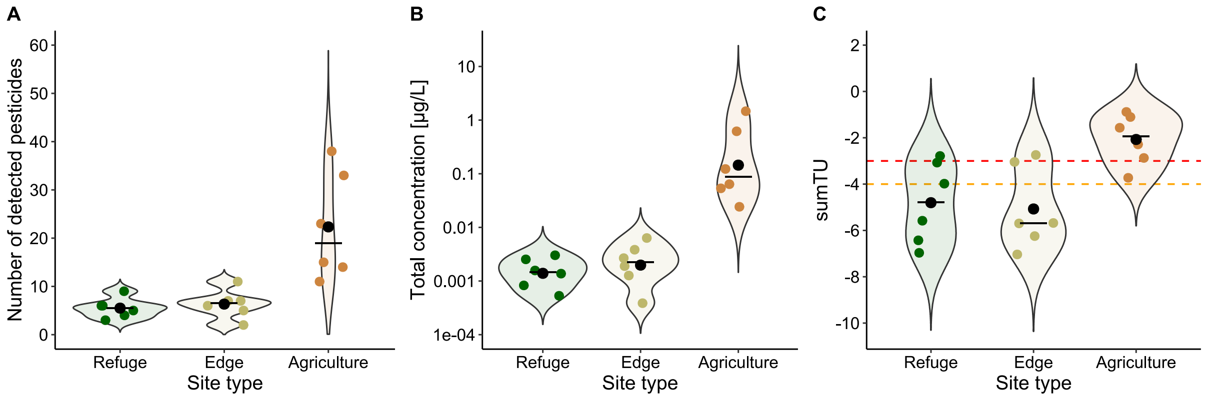

Figure 2: Violin plots (Wickham, 2016) visualising the number of detected pesticides (A), the total pesticide concentration in µg/L (B, on a logarithmic scale) and the estimated invertebrate toxicity (logarithmic sum toxic unit, sumTU) (C) analysed in surface water grab samples from refuge, edge and agricultural sites. Each coloured dot represents a single sample taken in each of the six streams, with the colours representing the site types. The black dot and line represent the mean and the median, respectively, for the six streams per site type. The red and orange lines represent the thresholds for potential effects on invertebrates (-3) and reference sites (-4), respectively (for details see section 2.5.1.).

3.3. Community composition in refuge, edge and agricultural sites

Taxonomic richness and total abundance were similar across site types (LMM, factor site type not significant at p = 0.35 and p = 0.66 for richness and abundance, respectively; Table SI 9,10). Notwithstanding, a slight trend towards an increase in average taxonomic richness from refuge over edge to agricultural sites was observed (35, 39 and 41; Fig. 3 A; Table SI 8). Similarly, average total abundance increased from refuge over edge to agricultural sites (approximately 5800, 7600 and 12300 individuals/m^2; Fig. 3 B; Table SI 8). The higher values in agricultural and edge sites were driven by few individual dipteran species (e.g. from *Tanytarsini Gen. sp.*) and gammarids (Fig. SI 15). Communities of refuge and edge sites were more similar, in terms of the Jaccard index, to each other than to agricultural sites (Fig. 3 C; Table SI 8; LMM, factor pairwise site type comparisons significant at p = 0.03), but edge and agricultural communities were on average slightly more similar than agricultural and refuge communities (0.4 vs 0.3; Table SI 8), though not significant (pairwise differences of pairwise site type comparisons: agriculture – refuge vs edge – refuge: p = 0.02, agriculture – edge vs edge – refuge: p = 0.20, agriculture – edge vs agriculture – refuge: p = 0.36). The abundance of pesticide-sensitive taxa in terms of $SPEAR_{pesticides}$ values differed across site types (Fig. 3 D, LMM, factor site type significant at p = 0.005; Table SI 9,10) with significantly lower values in agricultural sites compared to edge and refuge sites (approximately 50 % lower, pairwise differences of site type: agriculture – refuge: p = 0.006, agriculture – edge: p = 0.016, edge – refuge: p = 0.838).

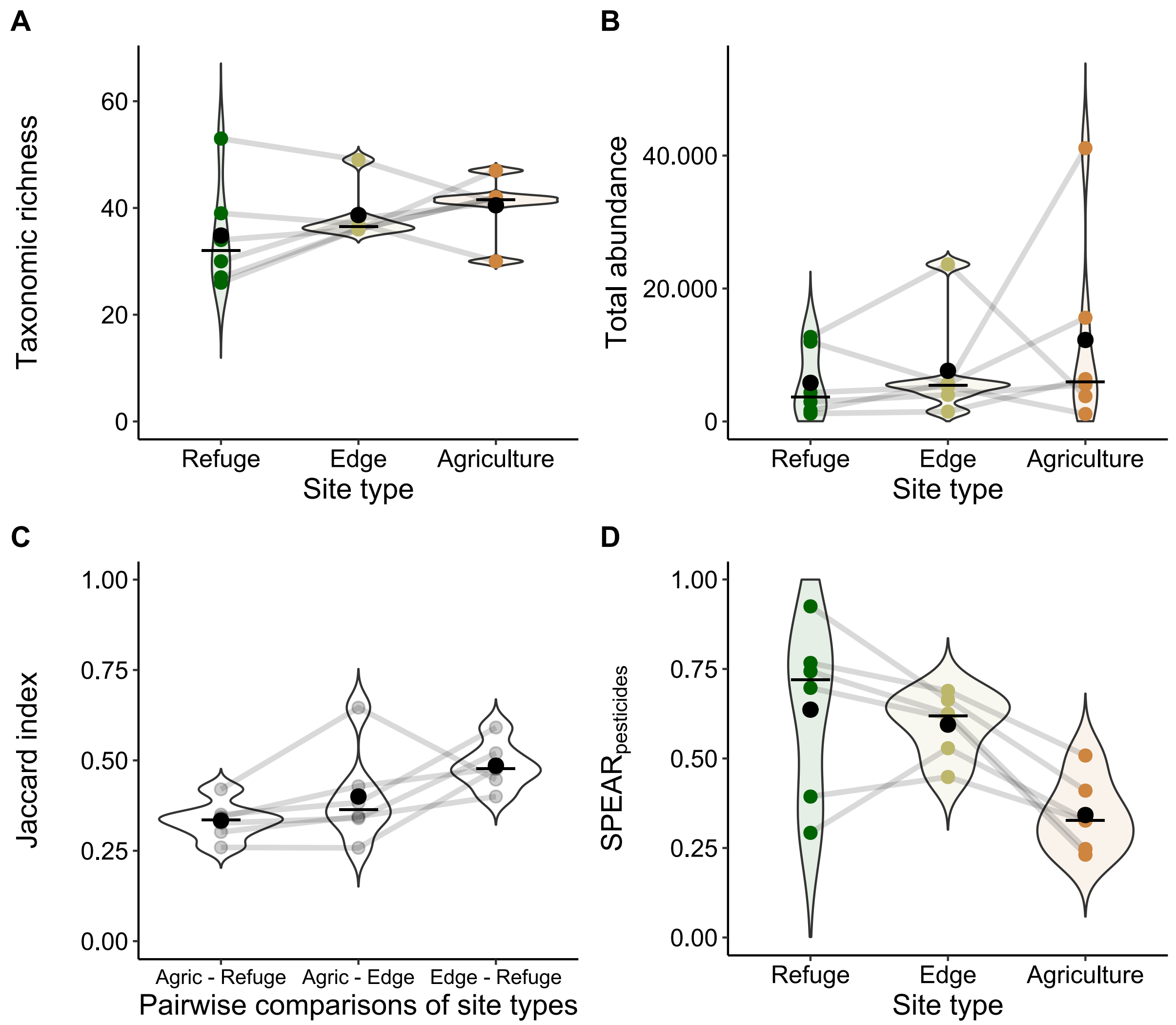

Figure 3: Violin plots (Wickham, 2016) visualising taxonomic richness (A), total abundance of individuals per m² (B), Jaccard index (C), and $SPEAR_{pesticides}$ (D) calculated for quantitative macroinvertebrate community samples at refuge, edge and agricultural sites. Each coloured dot represents a single sample taken in each of the six streams, with the colours representing the site types and grey lines connecting site types in the same stream. The grey dots represent pairwise comparisons of site types. The black dot and line represent the mean and the median, respectively, for the six streams per site type.

3.4. $SPEAR_{pesticides}$ - sumTU relationship

Site type described a significant amount of variation in the $SPEAR_{pesticides}$ values (LMM, factor site type significant at $p = 0.04$; Table SI 9,10), whereas sumTU did not (LMM, factor sumTU not significant at $p = 0.86$). Note that the factor site type captured different segments of the toxicity gradient (see 3.2.). When including sumTU and the interaction term (both not significant) in the model, agricultural sites showed a decrease of $SPEAR_{pesticides}$ with increasing sumTU, whereas edge and refuge sites showed a mild increase, with edge sites exhibiting slightly lower values of sensitive species (Fig. 4).

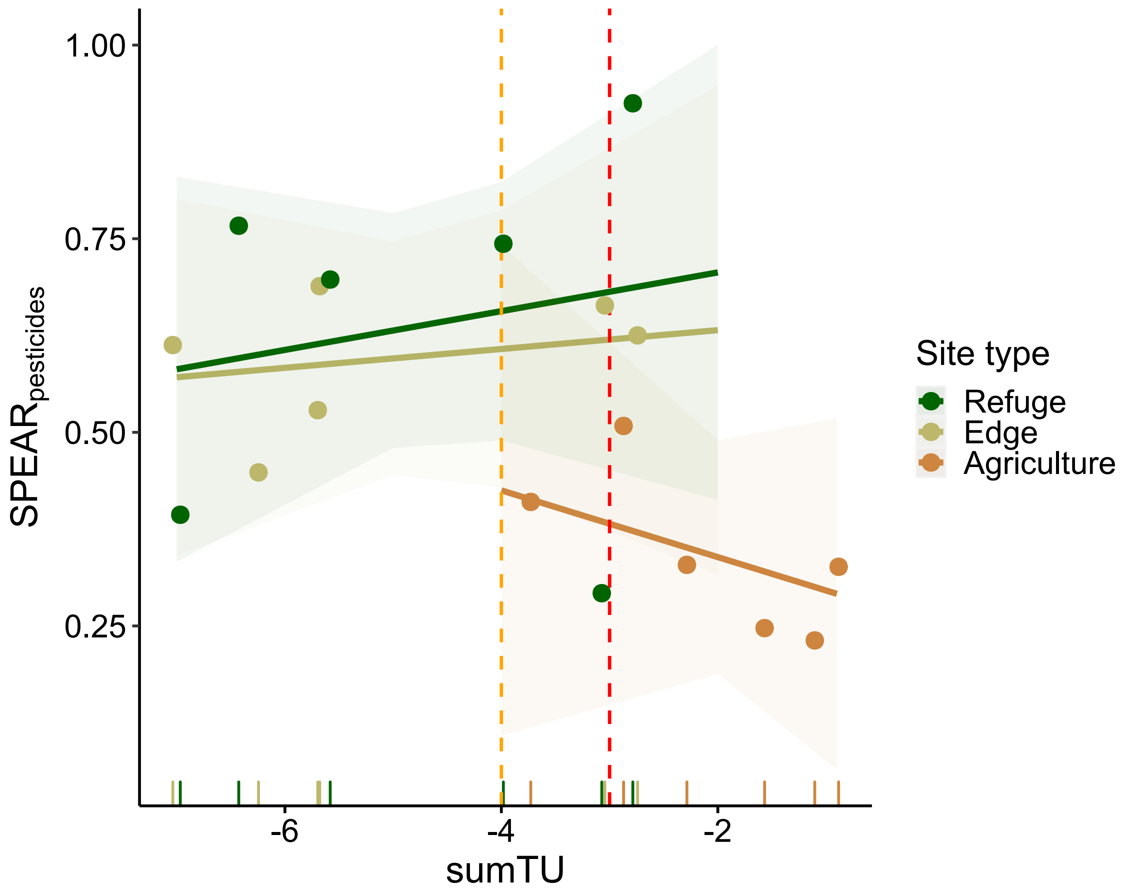

Figure 4: Linear mixed model regression lines (Fox and Weisberg, 2018; Wickham, 2016) visualising SPEAR$_{pesticides}$ in response to the estimated invertebrate toxicity (logarithmic sum toxic units, sumTU), site type and the interaction between them. The model includes stream as random factor. Each coloured dot represents a single sample taken in each of the six streams, with the colours representing the site types. The red and orange lines represent the thresholds for potential effects on invertebrates (-3) and reference sites (-4), respectively (for details see section 2.5.1.).

4. Discussion

4.1. Pesticides occur in forested sections

We hypothesised the absence of pesticide exposure at edge and refuge sites and a high pesticide exposure at downstream agricultural sites (hypothesis 1). We found, in line with this hypothesis, differences in estimated pesticide toxicity between site types, with agricultural sites displaying significantly higher values than edge and refuge sites. This can be explained by the proportion of agricultural land use in the catchment being a dominant driver of pesticide exposure in small streams (Rasmussen et al., 2011; Schreiner et al., 2021; Szöcs et al., 2017). Nevertheless, contrary to our hypothesis, at refuge and edge sites we estimated occasionally relevant pesticide toxicity, defined as a sumTU above -3 (definition section 2.5.1.). Throughout the sampling period from April to July, only 56%

of samples taken at refuge and edge sites met the quality criterion for reference sites (sumTU below -4; Fig. SI 11). In general, the estimated pesticide toxicity levels were similar across the whole sampling period (based on KgM sites), though they varied strongly between single samplings in individual sites which is presumably driven by single substances (Fig. SI 11). Hence, we consider the June sampling as largely representative of the general baseline exposure. The baseline exposure was determined independently of weather conditions using regular three-weekly grab sampling. This sampling method is likely to miss rain-driven pesticide pulses, thereby underestimating pesticide toxicity (Rabiet et al., 2010; Spycher et al., 2018; Xing et al., 2013). However, given that runoff from surfaces treated with pesticides is a major route of precipitation-driven pesticide input (Leu et al., 2004; Liess et al., 1999; Szöcs et al., 2017), in particular in our region characterised by terrain with steep slopes, we suggest that concentrations and toxicity were in particular underestimated in agricultural sites. Overall, we conclude that agricultural sites exhibited significantly higher pesticide toxicity than edge and refuge sites.

Interestingly, most pesticides were detected more frequently in the agricultural sites than in refuge and edge sites in June (Table SI 14). Therefore, we suggest that agricultural land use was also the main source of contamination for refuge and edge sites. We consider it unlikely that the pesticide concentrations are a legacy of past use because exposure should then be more stable and independent from current use patterns. However, companion studies displayed clear temporal trends linked to current use (Halbach et al., 2021; Weisner et al., 2022) and a recent large-scale study for Germany also demonstrated clear seasonal trends, very likely linked to pesticide use (Szöcs et al., 2017). We exclude forest-related pesticide use as a main source of contamination for refuge and edge sites, given that, to our knowledge, the use of pesticides in forests in this region has been limited to rare cases of local application and, in the case of insecticides, to other (i.e. pyrethroids) than the detected compounds over the last decade (Landtag RLP, 2019). We expect that non-agricultural pesticide use (e.g. in urban areas such as roads or residential areas) contributes negligibly to the pesticide residues in the refuges because urban areas were absent or covered only a minor proportion of area in the upstream catchments of edge and refuge sites. Notwithstanding, a recent study suggests that already very low proportions of urban land (< 5 %) in the catchment can result in pesticide residues in streams of undeveloped areas (Sandstrom et al., 2022). Hence, urban use may have contributed to the exposure. Due to the topography of the study region (forested refuge sites are located at approximately 100 – 200 m higher elevation than agricultural sites, Table SI 1), surface water runoff, erosion, drain flow, or leaching can be excluded as transport pathways for pesticide residues originating from downstream agricultural pesticide use. We suggest that compounds were most likely transported in the air to the forested sites, which were located in the proximity of only 0.6 to 1.5 km to the forest-agricultural edges. In the air, pesticides can generally be transported as vapor, droplets or associated with particles or dust and field measurements can provide information on the relative importance of these processes (Plimmer, 1990). Transport as vapor was generally most relevant for persistent and relatively volatile compounds (Daly and Wania, 2005; Hageman et al., 2006; Plimmer, 1990). Most of the compounds driving estimated toxicity in refuge and edge sites (Table SI 13) were persistent in soils but non-volatile (dimension of henrys law constants: 10^{-4} to 10^{-11} Pa m^3/mol, Lewis et al., 2016). They may have been transported as droplets or associated with particles or dust. Several previous studies point to the importance of the transport of pesticides via air and rain into untreated areas (Décuq et al., 2022; Kreuger et al., 2006) and also into protected (forested) areas in Germany (Kruse-Plaß et al., 2020). However, within our study the transport paths remain subject to speculation and a study measuring different transport paths would be required for clarification.

Overall, we suggest that regional transport of pesticides can lead to pesticide exposure in forest sections within a few kilometres upstream of agricultural areas. Several earlier studies also reported pesticide exposure in sites with low or negligible agriculture in upstream catchments. For instance, a large-scale study in Germany reported water quality threshold exceedances, i.e. considerable pesticide exposure, even for sites without agriculture in the catchment (Szöcs et al., 2017). Moreover, several studies reported pesticide exposure in seemingly pristine regions throughout the world (Daly and Wania, 2005; Guida et al., 2018; Hageman et al., 2006; Le Noir et al., 1999; Usenko et al., 2005; Wang et al., 2019; Zhan et al., 2021). Future studies, ideally in catchments with known agricultural and non-agricultural pesticide use, should further scrutinise the influence of pesticides transported to least impacted ecosystems.

4.2. Adjacent agricultural habitats influence edge communities

We hypothesised that dispersal processes result in the propagation of pesticide effects to the edge area (hypothesis 2). We found a significantly lower proportion of pesticide-sensitive species at agricultural sites compared to edge and refuge sites. However, the proportion of pesticide-sensitive species in edges was only slightly, and statistically non-significantly, lower compared to refuges further upstream. An analysis of over 100 sites of which our sites represent a subset (Liess et al., 2021) found that pesticides are the main driver of the community composition depicted by $SPEAR_{pesticides}$. Hence, we suggest that pesticides are also an important driver in the agricultural sites in our study, given that they displayed high estimated pesticide toxicity. Nevertheless, in contrast to previous studies (Schäfer et al., 2012), $SPEAR_{pesticides}$ was not associated with sumTU in our study. The sample size was relatively small (18 sites, but from only 6 streams) and may have prohibited to detect an association between $SPEAR_{pesticides}$ and estimated pesticide toxicity. More importantly, our study was primarily designed to capture differences between site types rather than a pesticide toxicity gradient across streams. Indeed, the factor site type explained a considerable amount of variation in $SPEAR_{pesticides}$ and was closely associated with the pesticide gradient. In refuge and edge sites, the estimated toxicity barely crossed the threshold where effects are likely (-3), whereas in agricultural sites most values were above this threshold. Indeed, in the agricultural sites, the $SPEAR_{pesticides}$ and sumTU were clearly negatively related (Fig. 4). In edge and refuge sites, where the sumTU gradient ranged from approximately -7 to -3, the association between $SPEAR_{pesticides}$ and sumTU was very weak and rather positive as identified previously (Liess et al., 2021). This suggests that the effect threshold applied, i.e. sumTU of -3, is largely protective, which matches the findings of previous studies (Orlinskiy et al., 2015; Schäfer et al., 2012). The $SPEAR_{pesticides}$ values varied to a similar extent for edge and refuge sites and pesticide levels were comparable.

Despite the difference of pesticide-sensitive species in edges compared to refuge was only minor and non-significant, the other metrics (Jaccard Index, taxonomic richness, total abundance) also indicated an influence of the agricultural sites on the edges. For instance, the results for the Jaccard index show that edge communities share on average slightly more taxa with agricultural communities than refuge communities with agricultural communities, though not significant. Similarly, there was no statistical evidence that taxonomic richness and total abundance differed across sites, but they showed a rather increasing trend from refuge over edge to agriculture. This contrasts with the decreasing trend of pesticide-sensitive species in terms of $SPEAR_{pesticides}$. The loss of species with pesticide-vulnerability traits (“losers”), such as long generation time and high physiological sensitivity (Liess and Beketov, 2011) seems to be balanced by an increase in species with tolerance traits (“winners”), resulting in a species turnover rather than a loss (Dornelas et al., 2019). We conclude that the loss of pesticide-sensitive species may not always translate to a loss in species diversity. However, the loss of pesticide-sensitive species can be associated with the loss of functional and genetic diversity, and may affect ecosystem functioning and stability (Cadotte et al., 2011). In line with our findings, a loss of functional diversity with a simultaneous increase in taxonomic diversity was found for fish communities in anthropogenically disturbed habitats (Villéger et al., 2010). In contrast, another field study in our region found a decline in the taxonomic diversity of macroinvertebrate communities with environmental stress, whereas functional diversity remained stable (Voß and Schäfer, 2017). However, this study was conducted in autumn and hence associated with a relatively small taxon pool (Voß and Schäfer, 2017). Generally, the relationship between functional diversity and taxonomic richness is complex and context-dependent (Cadotte et al., 2011). If colonisation matches species loss, functional diversity can change whereas species diversity remains stable (Cadotte et al., 2011). In our study, agricultural sites are bidirectionally connected (with stream sections up- and downstream), while upstream refuges near the stream source are only unidirectionally connected with downstream sections. This particular spatial context may allow for higher net immigration based on mass effects in terms of density-dependent organism dispersal from adjacent habitats with high reproductive success (Shmida and Wilson, 1985), as well as higher gains from drift into agricultural sites compared to refuge sites. In addition, specific habitat characteristics at the agricultural sites such as higher nutrient input as well as higher water temperature (Table 1) may support a higher diversity and abundance (van Klink et al., 2020). Furthermore, the dense shading of the upstream forested sites could hinder higher diversity and abundance while favouring the presence of specialists. However, the agricultural sites also exhibited diverse riparian vegetation, including trees, and we did not monitor shading of sites.

To sum up, although indicators for taxonomic and functional diversity established different relationships with site type, they both suggested an influence of agricultural stream sections on the edges. Given a relatively low sample size and related uncertainty, further research is needed to quantify the extent and unravel the mechanisms of how pesticide effects propagate to refuges. For example, it remains open, whether pesticide effects in edge and refuge sites may be detected at lower levels of biological organisation, such as the organism and sub-organism level. Furthermore, effect propagation is likely to depend on spatial characteristics, such as the distance between agriculture and refuge patches as well as the size of the refuge, as found in previous studies (Orlinskiy et al., 2015; Trekels et al., 2011; Willson and Hopkins, 2013). In our field survey, the distances between refuge, edge and agricultural sites varied slightly for the six streams and refuges were large in proportion to the agricultural land involved within the catchments (Fig.1; Table SI 1). Future studies are required, to understand the influence of spatial patterns and relationships for the propagation of pesticide effects. Finally, given that the information provided by taxonomic richness and the functional metric ($\mathrm{SPEAR}_{\mathrm{pesticides}}$) differed, we suggest that both, taxonomic and functional metrics, should be considered when studying pesticide effects in the context of biodiversity change (Cadotte et al., 2011; Dornelas et al., 2019).

5. Conclusion

We found significantly higher potential pesticide toxicity (sumTU) and altered functional community composition ($\mathrm{SPEAR}_{\mathrm{pesticides}}$) associated with pesticide exposure at agricultural compared to edge and refuge sites. Notwithstanding, at some edge and refuge sites, which were considered as being least impacted, we estimated unexpected pesticide toxicity exceeding thresholds where field studies on the association of estimated pesticide toxicity and invertebrate community composition suggested adverse effects on freshwater invertebrates. We conclude that the regional transport of pesticide residues can result in ecologically relevant pesticide exposure in forest sections within a few kilometres upstream of agricultural areas. In addition, we found that the majority of edge sites were characterised by a slightly lower abundance of pesticide-sensitive species (lower $\mathrm{SPEAR}_{\mathrm{pesticides}}$) compared to refuge sites, indicating a potential influence of adjacent agriculture. However, future studies are required to unravel the extent to which pesticide effects propagate to refuges and to scrutinise underlying mechanisms, and particularly to understand the spatial patterns and relationships for the propagation of pesticide effects. Furthermore, pesticide effects in edge and refuge communities need to be studied in the future at lower levels of biological organisation, such as the organism and sub-organism level.

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Appendices: Supporting Information

The supporting information for this article is available in the online version at: DOI We provide all raw data and the R script on GitHub at https://github.com/rbslandau/schneeweiss_refuge_1. The complete KgM raw data from Liess et al. (2021) and Halbach et al. (2021) are currently under embargo and publicly available on the 30.09.2022 at https://doi.org/10.1594/PANGAEA.931673.

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