Agricultural pesticides pose a continuous ecotoxicological risk to aquatic organisms in a tropical horticulture catchment

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ABSTRACT

Pesticides are used throughout the tropics in large amounts to protect crops against pests and weeds. These chemicals may be lost to the aquatic environment and impair its ecological status and the water quality for human consumption. Despite intensive use of pesticides in many developing countries, the knowledge of pesticide levels in aquatic ecosystems is often very limited. In this study, we try to fill this gap for an horticultural catchment of 35.9 km^2 in Costa Rica, which is intensively used by small-holder farming for potatoes and vegetable production. We continuously monitored pesticides in the Tapezco river during two consecutive rainy seasons with passive sampling systems and screened for a broad set of polar and non-polar pesticides. Spatially distributed measurements revealed high pesticide concentrations of many fungicides, herbicides and insecticides throughout the watershed. Concentration ranges revealed little spatio-temporal variation. From an ecotoxicological point of view, the insecticide levels – notably chlorpyrifos and cypermethrin - were most critical. The observed concentration levels exceeded chronic environmental quality standards more than 100-fold at all sites. These high insecticide levels were partially reflected in the community composition of the macroinvertebrates. Available data revealed a poor status at two upstream locations according to the Costa Rican Biological Monitoring Working Party (BMWP-CR) Index and the SPEAR$_{pesticides}$ index. However, the indices indicated a good quality at most downstream sites despite their high pesticide levels. The wide-spread occurrence of high pesticide levels demonstrated that the relevant sources and pathways existed throughout the catchment. Field observations and survey data showed the relevance of point sources due to poor pesticide handling as well as diffuse losses from fields, which are strongly enhanced by the steep terrain and linear structures like gullies connecting fields with the stream. Mitigation measures to reduce pesticide losses have to account for these different source-flowpath combinations.

1. Introduction

Pesticides are used globally to protect agricultural crops against damage by insects, diseases or weeds. However, these chemicals have also negative environmental effects such as impairing aquatic ecosystems. There is an imbalance though at the global scale, regarding the use of these highly potent chemicals and the research in investigating the environmental consequences: much research is carried out in North America and Europe and established monitoring programs assess the actual status of the respective water bodies (Ryberg and Gilliom 2015,

Chow et al., 2020, Halbach et al., 2021). The use of these chemicals is considerably more intensive in some tropical low- and middle-income countries (LMIC), alone because of the year-round application, but knowledge about pesticide occurrence and its environmental impact is much more limited (Echeverria-Saenz et al., 2021). This fact is all the more worrisome as tropical regions harbor a substantial part of global biodiversity.

Costa Rica is one of these LMICs that applies a lot of plant protection products, and the country's pesticide use is above average even for tropical regions. At the same time, the natural conditions enhance the

risk for pesticide transfer to water bodies (Vryzas et al., 2020, Echeverria-Saenz et al., 2021). This risk has been clearly demonstrated since more than 20 years (Castillo et al., 1997, Castillo et al., 2000, Wesseling et al., 2001). In recent years, several studies have documented the pesticide pollution more broadly across a range of different crops and regions (Echeverria-Saenz et al., 2021) such as fruit monocultures (Arias-Andres et al., 2018), rice and sugar plantations (Carazo-Rojas et al., 2018) or other specific crops (e.g. melons (Perez-Villanueva et al., 2021, Ramírez-Morales et al., 2021, Rodriguez-Rodriguez et al., 2021)). All studies reveal widespread occurrence of pesticides with crop-specific differences between the substances detected.

This well-documented presence of pesticides in streams across different environments and agricultural systems poses ecotoxicological risks to the aquatic ecosystem. Several of the studies mentioned above have evaluated such risks by comparing the measured environmental concentrations (MECs) to ecotoxicological effect concentrations or environmental quality standards (EQS) and partially revealed very high risks.

An important question is to which degree this ecotoxicological risk translates in actual ecological effects. In temperate streams, the ecotoxicological risk has been linked to the loss of sensitive species from the macroinvertebrate community (Beketov et al., 2013) based on the SPEAR$_{pesticides}$ index. Recent ecological studies in Panama also found relationships between pesticide levels and the composition of the macroinvertebrate community. However, the SPEAR$_{pesticides}$ index in its original form seemed not to be adequate to the tropical streams while a local macroinvertebrate index (Biological Monitoring Working Party, BMWP-PAN) yielded promising results (Cornejo et al., 2019). Experiments also suggest that leaf litter decomposition may be compromised in tropical streams due to pesticide pollution (Cornejo et al., 2021) possibly due to negative impacts on detritivores (Boyero et al., 2021, Cornejo et al., 2021).

Despite these recent advances quantifying pesticide pollution in tropical streams, there remain important scientific gaps. The existing studies for the region are all based on grab sampling, for example. However, it is well documented that pesticide concentration can fluctuate strongly in time (Thurman et al., 1991, Leu et al., 2005, La Cecilia et al., 2021) such that grab samples provide only a very coarse representation of the actual chemical status.

To better represent the pesticide levels in time, composite samples can be taken, for example over pre-defined periods of time (Spycher et al., 2018). Such an approach requires active sampling devices, which are costly and may be difficult to be deployed because of terrain conditions, for security reasons or because access is difficult. Passive sampling devices are useful alternatives under such conditions. They can consist of bottles, which get passively filled (Jonsson et al., 2019, Schönenberger et al., 2020) or by membrane-based systems that adsorb the chemicals of interest to a solid phase (Moschet et al., 2015, Ahrens et al., 2018). Combined with broad chemical analytical methods, it is possible to obtain a comprehensive view of the pesticide pollution in streams. Passive samplers can also be easily deployed at several locations providing spatial resolution, which is often absent. Here we collected continuous samples in time with different passive devices, throughout an agricultural catchment and during two rainy seasons. We aimed at tackling some of the sampling related deficits mentioned above by conducting an intensive field study in Costa Rica with the following specific objectives:

1. Providing a broad and comprehensive pesticide exposure assessment throughout the river Tapezco catchment during two consecutive rainy seasons of different meteorological characteristics using passive sampling.
2. Evaluate the pesticide levels in the streams with regard to their ecotoxicological risk.
3. Compare the ecotoxicological risk assessment to existing biological data in the stream network.

Additionally, by combining the spatio-temporal patterns of pesticide occurrence in the streams with the field observations during weekly visits we aim at gaining insight about the relevance of pesticide input pathways. This shall provide guidance for proposing adequate mitigation measures to reduce the pesticide inputs into the streams.

2. Material and methods

2.1. Study area

The Tapezco catchment in the highland plateau in the province of Alajuela in the Zarcero canton covers an area of 35.9 km^2 and ranges from 1209 to 2243 meters above sea level. About 20% of the catchment area are intensively used for small-scale horticultural farming to mainly grow potatoes and vegetables (Ramírez et al., 2016). Forests and pastures for dairy farming cover the majority of the remaining areas (Fig. 1).

The crops are often cultivated on steep fields (average slopes = 10.6%, maximum slope > 60%, see Tab. S1). Contour farming dominates, however, to avoid stagnant water during heavy precipitation also tramlines are installed along the flow lines (Ramírez et al., 2016) (see Fig. S1).

Pesticides are intensively used with average application rates about 22 kg a.i./ha/crop cycle with a maximum of 58 kg a.i./ha/crop cycle (Ramírez et al., 2016). This intensity is similar to rates elsewhere in the country (Polidoro et al., 2008, Echeverria-Saenz et al., 2012, Carazo-Rojas et al., 2018, Rämö et al., 2018).

The catchment has a tropical climate with an average annual rainfall of 1925 mm/yr, (1950 to 2016; National Meteorological Institute from Costa Rica) and a decreasing precipitation trend with increasing altitude (Schaub 2016). The average temperature is 17°C. Generally, there is a pronounced rainy season from May to October and a dryer period from November to April.

2.2. Water level and rainfall

Rainfall data was obtained from the national measuring network (Zarcero-Palmira (10°11′31′′ N, 84°23′35′′W) at an altitude of 1736 meters above sea level, National Meteorological Institute). Water levels were measured during the sampling period at all sampling sites in 2015 and 2016 continuously with HOBO® U20L water level loggers (Onset, Switzerland) in 5 min and 15 min intervals, respectively.

2.3. Sampling

Pesticide sampling was conducted in two successive years (2015/2016) during three sampling periods. Five sites (Fig. 1) were sampled in 2015 during two and a half months (30-Jul and 07-Oct, period ΔT1). In 2016, the same sites plus three additional ones were sampled during four and a half months (25-May to 11-Oct). To compare directly with the previous year, we distinguish two periods (ΔT2a: 25. May – 2. Aug., ΔT2b: 2. Aug. to 11 Oct). The sub-catchments corresponding to the eight sampling sites are characterized in Tab. S1, Figs. S2 and S3. Study sites were selected to include upper (SC1-SC3), middle (SC4-SC6) and lower (sub-)catchments (SC7-SC8) of the Tapezco river basin. These sub-catchments represented areas with contrasting fractions of horticulture (9 to 56%, see Tab. S1). However, it was not possible to instrument a reference site without horticultural land use.

Three different passive sampling devices types were deployed simultaneously next to each other at all sampling sites. They included two membrane-based passive samplers (SDB, PDMS) and a passive sampling bottle. This latter device is a submersed, air-tight bottle that gets filled with water at a rate proportional to the water level above the bottle inlet (Water-level proportional sampler WLP, see Schönenberger et al., (2020)). The sampling rate can be controlled by a valve or other flow resistance for the outflowing air that is pushed out by the
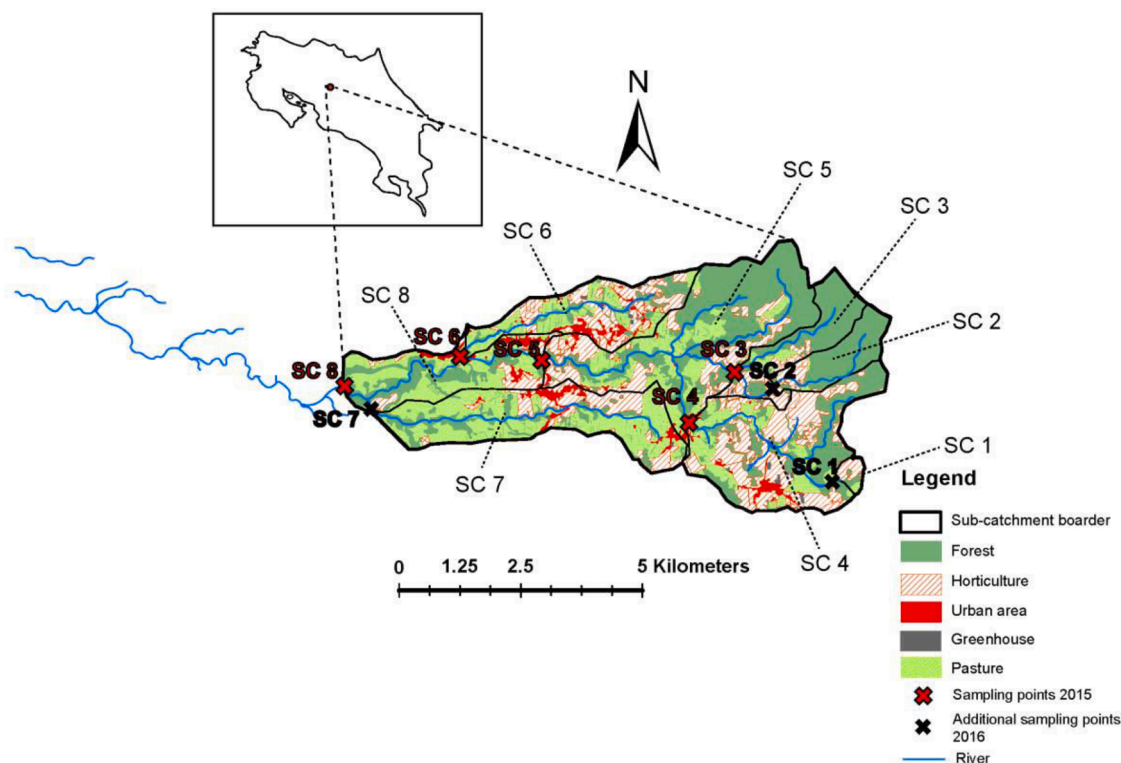
Fig. 1. Map of the Tapezco river catchment with the eight sampling sites (red crosses: sampled 2015 and 2016, black crosses: sampled in 2016 only), the respective sub-catchment (SC1 – SC8), and land use distribution.

hydrostatic pressure. The two membrane-based samplers were exchanged every two weeks, while the WLP was collected weekly.

The SDB disks were applied in polar configuration as described in Moschet et al., (2015) and Vermeirssen et al., (2009). The SDB disks were overlaid by polyethersulfone (PES) filter membranes (Ø 47 mm, pore size: 0.45 µm, Supor, PALL, Switzerland). Before assembly, the SDB disks and the PES filter membranes were conditioned as described in detail in Weiss (2021). The PES filter was discarded and the SDB disks were transported on ice to the lab and stored at -20°C. The SDB disks were further processed as previously described (Vermeirssen et al., 2009).

PDMS sheets from Altecweb (AlteSil™, translucent and talc free, 0.5 ± 0.05 mm thick, 60 × 60 cm^2 area, United Kingdom) with a size of 5 × 10 cm^2 were used as described in Moschet et al., (2014a) following the guideline for non-polar chemicals (Smedes and Booij 2012). The sheets were conditioned via soxhlet extraction in ethyl acetate (purity > 99.7%, Honeywell, Switzerland) for 100 h, dried and stored in methanol. To transport the PDMS sheets to the field, they were wrapped in aluminum foil. After exposure, the PDMS sheets were collected, transported on ice and stored at -20°C.

The WLP (for a schematic drawing see Fig. S4) was used in two configurations. In 2015, it was equipped with an HPLC capillary as flow resistance (HPLC capillary: Ø 0.13 mm, length: 1.3 m). In 2016, a precision valve was used for that purpose (Göldi Präzisionsmechanik AG, Schlieren, Switzerland, https://goeldi-mechanik.ch/kontakt/). Both the HPLC capillary and the precision valve were placed outside the water and were connected to the air-outlet of the sampling flask via a vinyl tube. An amber, wide-neck screw thread glass bottle (1 L, Roth AG, Switzerland, order nr. HT12.1) was used to collect the sample.

Samples were processed as described in Kern et al. (2009) and Ruff et al. (2015). Briefly, the samples were pressure filtered (Grade GF/D, Ø 47 mm, pore size 2.7 µm, Whatman, Huberlab, Switzerland) and stored in the dark at 4°C within 48 h after sample collection. After blending two weekly samples for the biweekly probe, 1 L of sample was buffered to a neutral pH by adding ammonia or formic acid. To each sample, 100 ng of 59 semi-polar and polar isotopically labeled internal standards (ILIS) were added (SI Tab. S3) and all the samples were concentrated via SPE with a multilayer cartridge as described in Kern et al. (2009) and Vogler (2013).

For the WLP samples the SPE was conducted at IRET-UNA, Costa Rica. The SPE multilayer cartridges and the SDB and PDMS samples were stored at -20°C until shipment on ice to Eawag in Switzerland. At Eawag, samples were placed again at -20°C until work-up for chemical analysis.

Further details on the sampling procedures are provided in Weiss (2021).

2.3.1. Benthic macroinvertebrates

The macroinvertebrates data stem from a parallel study that lasted from August 2013 to February 2016. Samples were collected every six months resulting in six sampling campaigns. The first three campaigns included the sites SC1, SC4 and SC5. From August 2014 on, SC8 was additionally included. Physico-chemical properties of the sampling sites are provided in Tab. S2.

Macroinvertebrates were collected according to the Costa Rican National Regulation for the Classification of Freshwaters (La Gaceta Official Newspaper, 2007) by sampling all available habitats within a 50 m stretch of each stream using a D net (250 µm) for a period of five minutes. This semi-quantitative method is based on the RIVPACS methodology (Wright, Sutcliffe & Furse, 2000). To ensure uniformity and decrease bias related to the user of the sampling device, only one person took all the macroinvertebrate samples. Organisms were preserved in ethanol (75%), sorted under a dissecting microscope and identified to the family and/or genus level using regional taxonomical keys (Pennak 1989, Merritt et al., 2008, Springer et al., 2010). The macroinvertebrate raw data are presented in Echeverría-Sáenz and Weiss (2021).

2.4. Chemical analyses

A set of 258 polar and semi-polar pesticides and transformation products (PPTP) in the SDB and WLP samples was analyzed with high-resolution liquid chromatography tandem mass spectrometry (LC-HR MS/MS) (Moschet et al., 2013). These PPTP included 247 compounds from previous studies (Moschet et al., 2015, Ruff et al., 2015) plus 11 compounds, which were added because of reported application in the study area (Ramírez et al., 2016). The monitored PPTP spectrum consisted of 95 herbicides, 55 fungicides, and 40 insecticides. Additionally 58 transformation products (TP) and 10 substances from other substance classes were included (Tab. S5). Further 18 non-polar insecticides (Tab. S6) were measured via atmospheric pressure chemical ionization gas chromatography tandem mass spectrometry (GC-APCI-MS/MS) according to Rösch et al. (2019) in PDMS sheet extracts.

Analysis of polar and semi-polar compounds: For chromatographic separation, a reversed phase C18 column (XBridge, 3.5 μm, 2.1 × 50 mm, Waters, Ireland) was used, and a mass spectrometer (QExactive, Thermo Fisher Scientific Corporation, U.S.) was applied for electrospray ionization (ESI) detection. Full scans were acquired with a resolution of 140,000 (at m/z = 200) in the range of 100 to 1000 m/z followed by top five data-dependent MS/MS (resolution 17,500) in positive and negative ionization mode separately. For measuring, 10 μL of every sample was injected. The chromatographic gradient was set equivalent to Moschet et al. (2013).

Data analysis was realized with TraceFinder (version 3.3, Thermo Fisher Scientific Corperation, U.S.). Chromatographic peaks of target analytes were automatically detected (mass accuracy < 5 ppm, peaks with a minimum of 5 data points) by using the retention times (RT) of the target analytes, confirmed with MS/MS fragments, and comparing them with RT and fragments of analyte identical reference standards (STD). Additionally, each peak was reviewed manually in all samples for further quality control.

Quantification was performed with a twelve-point calibration curve using STD together with ILIS as internal standards. For 44 compounds, structurally identical ILIS were available (SI-Tab. S3/4). The remaining analytes were quantified using structurally non-identical ILIS with similar RT.

For the quantification of the masses in the SDB extracts, an external twelve-point serial calibration was prepared in 1 mL (90:10, methanol:nanopure water (NPW)) by using STD mixes with all target PPTP, to which 100 ng (per sample) of the ILIS were added. For the quantification of WLP samples, another twelve-point internal serial calibration was prepared in 1 L NPW. The WLP calibration samples were then enriched via SPE (enrichment factor: 1000) and extracted in the same way as the environmental samples. Before SPE, 100 ng (per sample) of the ILIS were added to these calibration samples. For the compounds without structurally identical ILIS, the concentrations were corrected by relative recovery.

Atmospheric pressure chemical ionization (APCI) gas chromatography tandem mass spectrometry:

For chromatographic separation, a fused silica column (Rtx-5MS, 30 m, 0.25 μm film thickness, 0.25 mm i.d., Restek, BGB, Switzerland) and a mass spectrometer (triple quadrupole, MS/MS, Agilent 6495, Switzerland) were used as described by Rösch et al. (2019). For measuring, 3 μL of each sample were injected using a deactivated liner (borosilicate glass, 4 mm i.d., Restek) at 250°C. As carrier gas, helium (99.999%, Carbagas, Switzerland) was used with a flow of 3 mL/min.

The substances were measured in positive ionization mode with NPW as a modifier. The NPW was pumped constantly (50 μL/min) into a small open vial, placed in the ionization source. The mass spectrometer was used in dynamic multiple reaction monitoring mode. For each target substance and ILIS, a minimum of two transitions were measured, from which the most sensitive transition was used as quantifier and the remaining transition as qualifier in agreement with Rösch et al (2019).

Peaks were integrated with the Masshunter Qualitative and Quantitative analysis software (version: B.07.00, Agilent, Switzerland) (Rösch et al., 2019). For quantification of the environmental samples, a matrix-matched ten-point calibration series was prepared using STD. Ten conditioned PDMS sheets were spiked with the individual amounts of STD. Afterwards, the spiked sheets were extracted via ASE, ILIS were added, and the calibration samples were processed equivalently to the environmental samples (Tab. S4). The quantification was based as well on internal standard calibration as described previously (Rösch et al., 2019).

The limits of quantification (LOQ, lowest quantifiable mass in ng per L water sample) are presented in Tab. S5 for WLP samplers and the SDB disks and Tab. S6 for PDMS sheets. Further details can be found in (Weiss 2021).

Calculating time-averaged concentration for SDB disks and PDMS sheets

The masses absorbed to the sorbent-based samplers, $m_{sorbent}$, were used to calculate time-averaged concentrations (C_{TIA}) in water as described in Vrana et al. (2005):

$$C_{TIA} = \frac{m_{sorbent}}{R_s \ \Delta t} \tag{1}$$

where R_s is the sampling rate [L/d] obtained from the literature, and Δt is the sampling duration. For 68 polar and semi-polar PPTP, experimentally determined R_s values were available (Tab. S7). If more than one R_S value per target compound was found, the average was used as previously suggested (Curchod et al., 2020). For all PPTP without available R_s values, the average R_s, 0.094 L/d, for all 68 available PPTP was used as an approximation. An uncertainty factor of one-order of magnitude (i.e., R_s multiplied and divided by a factor of three) was applied to all R_s values as previously described (Curchod et al., 2020).

For the target analytes monitored with the PDMS sheets, no compound-specific R_s values were available from the literature. Therefore, the average R_s value of PCBs and PAHs of 5.83 L/d for PDMS sheets of 50 cm² size was used (Rusina et al., 2010, Smedes and Booij 2012) as described in Moschet et al. (2014a).

2.4.1. Risk quotients (RQs)

For a single compound, RQs were calculated for each sample by dividing the measured concentration (MEC) by the respective water quality standard (EQS, see below) (EFSA Panel on Plant Protection Products and their Residues 2013):

$$RQ = \frac{MEC}{EQS} \tag{2}$$

To have a consistent set of EQS values across all compounds (Tab. S9), we relied on EQS values derived according to the Technical Guidance for deriving Environmental Quality Standards, No. 27 (European Commission 2018) provided by the Swiss Ecotox Center (https://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards/). For 18 compounds, no EQS values were available and were excluded from the risk assessment.

2.4.2. Mixture effects

The risk quotients for mixtures (RQ_{mix}) were determined as well for each sample for organism groups of different trophic levels as the sum of risk quotients of all compounds relevant for the respective group of organisms (Junghans et al., 2013):

$$RQ_{mix} = \sum_i \frac{MEC_i}{EQS_i} \tag{3}$$

where, MEC_i is the MEC for each detected PPTP, i, and EQS_i the corresponding EQS for each detected PPTP.

2.4.3. Biological indices

The macroinvertebrate communities were sampled at four sampling

sites (SC1, SC4, SC5, SC8) as described in Echeverría-Sáenz and Weiss (2021). The Costa Rican Biological Monitoring Working Party (BMWP-CR) index (La Gaceta Official Newspaper 2007) was derived as shown in Echeverría-Sáenz and Weiss (2021). This is a macro-invertebrate based biotic index adapted and validated for use in Costa Rica. The index results in a water quality classification which can reflect the effects of different types of stressors or pollutants.

Additionally, the SPEAR_pesticides was calculated to have a pesticide-specific macroinvertebrate index. It was determined based on Knillmann et al. (2018) and Liess and Ohe (2005) as

$$SPEAR_{pesticide} = \frac{\sum_{i=1}^{n} \log(x_i + 1) \times y}{\sum_{i=1}^{n} \log(x_i + 1)} \qquad (4)$$

Here, n is the total number of taxa in each sample, x_i the abundance of taxon i (Echeverría-Sáenz and Weiss 2021) and y is an indicator set to 1 if taxon i is classified as "at risk" dependent on its biological trait information (Liess and von der Ohe 2005, Knillmann et al., 2018). The biological trait information used for the calculation of the SPEAR_pesticides, are presented in Echeverría-Sáenz and Weiss (2021). Results derived from this index must be interpreted cautiously, because it has not yet been validated for the Neotropical region.

3. Results and discussion

3.1. Pesticide exposure

A large number of polar and semi-polar pesticides and transformation products were detected across the eight monitoring sites. From the 275 analytes, 99 and 87 polar to semi-polar PPTP were detected with the WLP and the SDB disks, respectively. The majority (N = 77) was detected by both methods, for 59 compounds quantitative comparions were possible. However, the WLP sampling provided a less complete set of samples because of technical difficulties. Therefore, we base the results and analyses on the two membrane-based methods where not mentioned otherwise.

Insecticides were the most numerous group of pesticides found in both sampling years and during the three study periods (Fig. 2) followed by fungicides and herbicides. This distribution among pesticide groups was very stable in time and varied little across sites. Only two sub-catchments (SC 2, 3) resulted in consistently lower herbicide and TP numbers. In these areas, more than 20 pesticides were detected per sample. At the other sites, this number generally exceeded 40. These results suggest a rather homogeneous pesticides use within the entire catchment. This is plausible given that no obvious spatial crop patterns were observed in the field or during farmer interviews in a related study (Staudacher et al., 2020).

The sum concentrations in the single samples varied widely between 170 and 3100 ng/L. No significant spatial differences between the sites were observed. In contrast, the values varied significantly in time (2-way ANOVA, difference between periods, p = 0.003; see Fig. S7). On average, the values were lower in 2015, which was a pronounced El Niño year (see precipitation data, Fig. S5). The median and average concentrations in 2015 amounted to 750 ng/L, and 825 ng/L, respectively across five sites for ΔT1. In 2016, the values were 1010 ng/L and 1400 ng/L for the same sites and same period (ΔT2b). Similar values were measured when considering all eight sites monitored in 2016 (median = 1240 ng/L, average = 1380 ng/L). These differences between years indicate that the drier weather conditions caused by El Niño reduced either pest pressure and therefore the applied amounts or the pesticide transfer to the stream network. The available application data do not indicate a reduced use under El Niño conditions (Tab. S10). Comparing the sum concentrations of 14-d samples with rainfall data show for most of the cases a trend of increasing sum concentrations with increasing rainfall (Tab. S11). Despite the fact that few of these regressions are significant individually, the overall pattern suggest a rainfall impact (11 positive slopes out of 13, binomial test, p = 0.02).
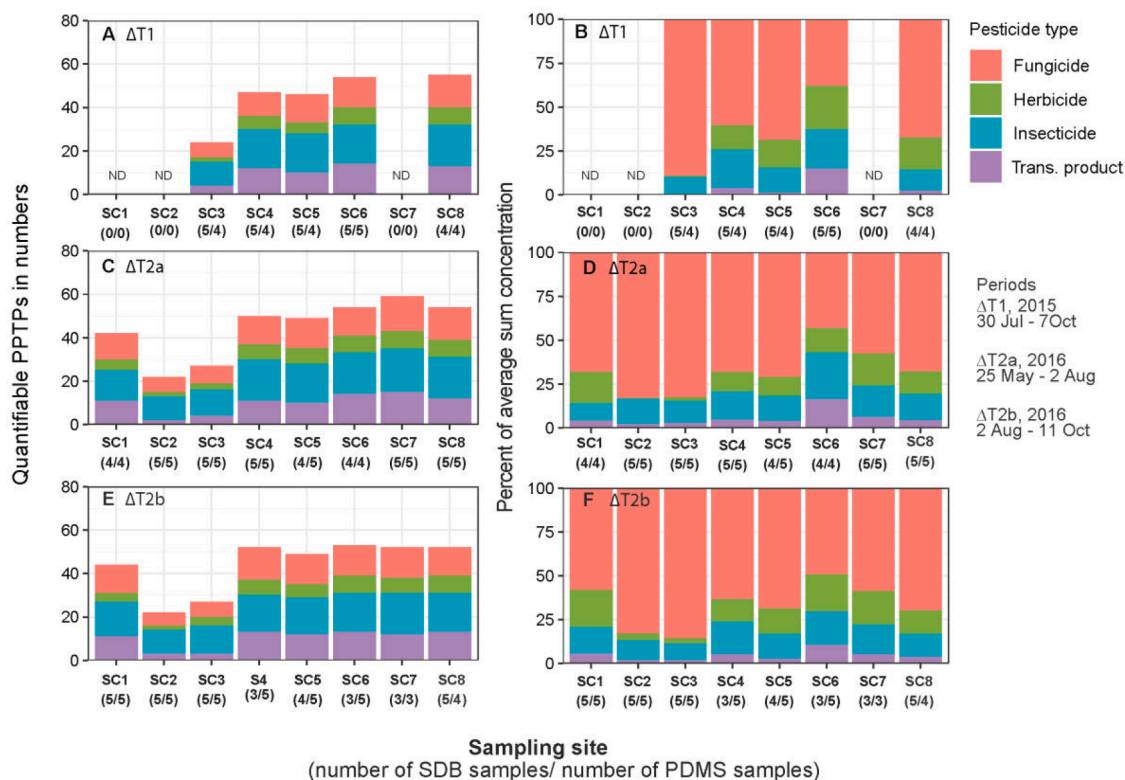
Fig. 2. Spatial (SC1-C8) and temporal (ΔT1, ΔT2a and ΔT2b) occurrence of PPTP types. Left panels: average number of PPTP per sample during the different study periods. Right panels: relative contribution to the total sum concentrations during the different study periods. The numbers in brackets show the number of samples used for evaluation from the SDB and the PDMS, respectively. ND = no samples collected.

Irrespective of the year and sites, the concentrations sums were strongly dominated by fungicides instead of insecticides as found for the number of compounds. Fungicides contributed between 50 and 75% of the total concentration sums (Fig. 2). Of course, single pesticides contributed very differently to the total concentration sums. The pesticide measured in the highest concentrations was the fungicide carbendazim with a 14-d maximum concentration of about 1500 ng/L. Three compounds had a median 14-d average concentration exceeding 100 ng/L (carbendazim, dimethomorph, flutolanil), for 11 compounds this median ranged between 10 and 100 ng/L (Fig. 3, Tab. S12). Among the nonpolar insecticides, chlorpyrifos and cypermethrin were detected at the highest concentrations, exceeding 1 ng/L in most cases with maximums of 34 and 87 ng/L, respectively (Tab. S12).

Despite some temporal variation of the concentration sums as mentioned above, the concentration levels of individual pesticides remained fairly stable across time (Fig. 3). Also the spatial differences were small: an analysis of variance across all concentration data revealed that the compounds explain about 50% of the observed concentration variability. The sites and the periods (ΔT1, ΔT2a and ΔT2b) explain each less than 1%.

The concentrations reported here correspond to the values derived from the SDB disks. Relying on literature-based sampling rates R_s for calculating these concentrations (see Eq. 1), adds uncertainty to the results. Given that we have collected samples with different devices, we used the available WLP data for checking the plausibility and robustness of the SDB-derived concentrations. Up to 16 samples could be used for this comparison depending on the compound (see Tab. S13).

For most compounds, the concentration levels obtained by membrane-based SDBs and the values measured directly in the water samples of the WLPs agreed fairly well. For 45 out of 58 compounds (Fig. S9), the median concentrations between the two approaches did not deviate more than a factor of three. For almost 95% of the compounds, the values were within one order of magnitude. Only for six pesticides, the WLP values exceeded the membrane-based concentrations to a larger extent. For more than 50% of the pesticides with sufficient data points (N ≥ 5), there was a significant correlation between the two sampling methods (p < 0.05, Tab. S13) explaining on average 40% of the variance. For the insecticide chlorpyrifos, it was possible to compare across all three methods. They were all well correlated (WLP-PDMS: p = 0.034, WLP-SDB: p = 0.024; SDB – PDMS: p = 4×10^{-6}; Tab. S13, Fig. S10), but the concentration levels estimated with the SDB disks were lower than those obtained with the other methods (Fig. S11).

Overall, these comparisons suggest that the SDB concentration levels reflect the actual concentration levels mostly within a factor of three.

This level of uncertainty needs to be considered when comparing our findings to other studies and to ecotoxicological quality standards as presented in the next section. Additionally, one has to be aware that these concentrations reflect 14-d averages. They don't reflect peak concentrations, which may be considerably higher (Spycher et al., 2018, La Cecilia et al., 2021).

The compound spectrum found in the Tapezco catchment compares well to recent studies on vegetable and potato production in other parts of Costa Rica (Perez-Villanueva et al., 2021, Rodriguez-Rodriguez et al., 2021). Compounds like the fungicide carbendazim, the insecticides carbofuran, chlorpyrifos and cypermethrin and the herbicides diuron and linuron were detected across the different study sites indicating similar use patterns on these crops. Hence, our results support these previous findings but add substantially in demonstrating the occurrence of many more pesticides that could not be measured in other studies because of a restricted list of analytes.

The absolute concentration levels were generally lower in our study compared to other results. This holds especially true for the maximum concentrations. This can be at least partially explained by the different sampling scheme (grab sampling versus 14-d time-averaged concentrations). Actually, several studies from Costa Rica demonstrate that maximum concentrations may exceed average or median values by a factor of 5 to 20 (Perez-Villanueva et al., 2021, Ramírez-Morales et al., 2021), or even 85 in extreme cases (Carazo-Rojas et al., 2018). This suggests that also in the Tapezco river acute concentrations may have been substantially higher than what we report here as time-averaged values.

A recent review on pesticide measurements in Costa Rica (Echeverria-Saenz et al., 2021) lists about 80 active ingredients that were reported from different climatic regions reflecting crops from small-holder vegetable production to large-scale fruit plantations. This list agrees well with other reports from Central America (e.g., Cornejo et al., 2019). We found a similar number of compounds in one single catchment. About half of the compounds reported in this study seems to have not been reported for Costa Rica before (Echeverria-Saenz et al., 2021). Out of the 44 common active ingredients, the detection frequency is much higher in our study for 37 compounds. This was probably caused by two main factors: first, due to the integrative sampling procedure and second due to possibly lower LOQ in this study. This last aspect is especially relevant for the toxic pyrethroid insecticides (see below): while the pesticides of this class have only occasionally be reported previously, we detected several of them in many samples (e.g., cypermethrin, Tab. S6, Tab. S12, Tab. S14) in the sub-ng/L range. While concentrations were low in absolute terms, due to the toxicity of these
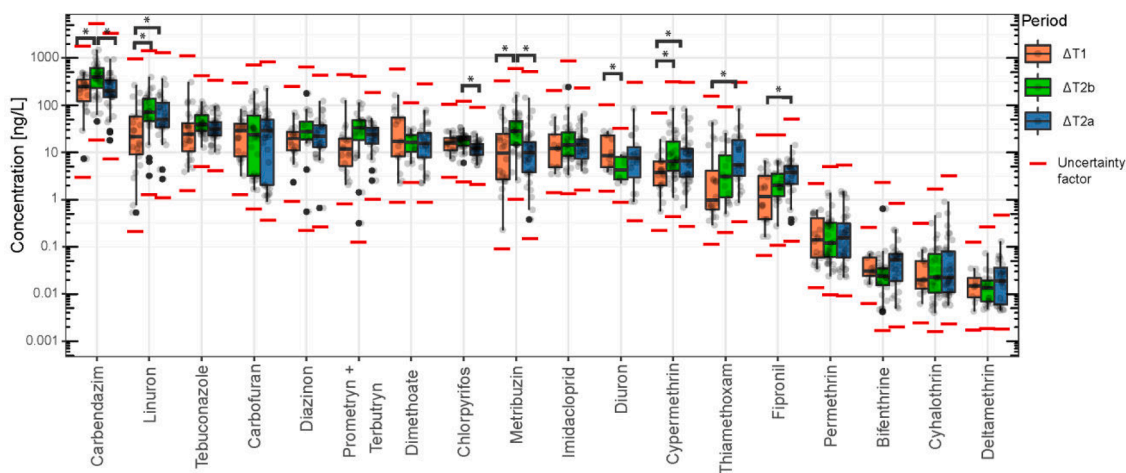
Fig. 3. Boxplot of the concentrations of the 18 pesticides that exceeded at least once the chronic EQS. The data are split according to the sampling periods ΔT1, ΔT2a and ΔT2b. * significant differences between time periods. Red lines represent uncertainties of minima and maxima values based on a factor of three for the sampling rate (RS) values in both directions according to Curchod et al. (2020) and Moschet et al. (2014b).

compounds this has consequences for the ecotoxicological evaluation (see below).

3.2. Ecotoxicological risk assessment

To evaluate the ecotoxicological risk, we compare the pesticide concentrations to a consistent set of environmental quality standards (EQSs, see Tab. S9). In the context of this work, these values do not have a legal meaning but allow for a consistent comparison to identify compounds and use classes that dominate the ecotoxicological impact on the stream organisms.

Little spatial differences were observed across the entire Tapezco catchment (Fig. S12). The number of chronic EQS exceedances of single compounds mostly varied between 20 and 40 for the three 2.5 months observations periods that we distinguished. Only the two sub-catchments SC2 (which is nested into and therefore influencing SC 3) and SC 3 showed slightly lower numbers. This agrees with the lower number of pesticides measured at these sites. Even acute quality standards were exceeded around 5 to 10 times during these periods despite comparing against time-averaged concentrations for 14 d periods (Fig. S13). Insecticides contributed by far the largest share (Fig. S14). Chlorpyrifos and cypermethrin were the most critical compounds across the entire catchment and during both years. Herbicides and fungicides only occasionally exceeded the chronic EQS values and never did so for the acute values.

These results are directly reflected in the findings on mixture toxicity demonstrating the largest ecotoxicological risk occurring for invertebrates (Fig. 4). This held true for both years and across all monitoring sites. The risk existed irrespective of the uncertainty of the observed concentrations (see above). For the other two groups of organisms (vertebrates, primary producers), the ecotoxicological risk was substantially lower with average risk quotients around one.

For all three groups of organisms the level of ecotoxicological risk did not vary strongly over time or between the two years. Despite a tendency for lower risks for invertebrates in the drier El Niño year 2015, the average chronic risk ratio exceeded a value of 10 for most of the time. These findings clearly demonstrate a long-lasting pesticide pollution continuing for months. The data furthermore show little spatial variance across sub-catchments implying that a large fraction of the invertebrates in the stream network are exposed to a high ecotoxicological risk.

These findings support results from other studies across different regions in Costa Rica demonstrating the ecotoxicological risk to several groups of organisms due to agricultural pesticides. Such risks were found for plants through herbicides and for invertebrates and fish through insecticides (Arias-Andres et al., 2018, Echeverria-Saenz et al., 2021), strong risks for fish (Carazo-Rojas et al., 2018) or fish and invertebrates

(Rodriguez-Rodriguez et al., 2021). The observed risk quotients reported in different studies however, are difficult to be compared directly. First, the risk is often dominant by few compounds per study. Accordingly, the reported risk may strongly depend on the list of analytes included in the analytical procedure. Additionally, different studies often use different ecotoxicological endpoints to evaluate measured concentrations.

3.3. Biological status

Given the pronounced ecotoxicological risk for the invertebrates, one can expect to observe also impacted invertebrate communities in the Tapezco catchment. At four of the monitoring sites (SC 1, 4, 5, 8), biological data is available that provides respective insights (Tab. S2, Tab. S15). In agreement with the chemical data and the ecotoxicological risk assessment, the BMWP-CR and the SPEAR$_{pesticides}$ index indicate impaired conditions at three out of the four sites. However, at the most downstream site (SC8), both indices show a good biological status while the risk quotients of most samples revealed a large ecotoxicological risk (Tab. S16). This mismatch is similar to findings of previous work where no relationships between pesticide exposure and the two indices could be found (Rasmussen et al., 2016). This observation raises questions how pesticide impact is captured by the two biological indices and how other factors such as stream morphology or urban areas influence them. For example, both indices were correlated with altitude (see Figs. S17, S18) revealing an increasing status at the downstream locations. Given the prevailing pesticide exposure at the outlet of the catchment, the good biological status at that site might also suggests that local factors such as riparian areas (see Fig. S16) can compensate for the chemical pollution and render the pesticide effect less critical (Hunt et al., 2017). However, the data available in this study does not allow for a causal analysis and we need to leave this important question about the reasons behind the mismatch between pesticide exposure levels and the biological status open.

3.4. Lessons for mitigation

Despite the more than 100-fold differences in size (between 30 and 3560 ha) and some differing landscape features such as slopes (Fig. S3), there was a pronounced similarity between the pesticide exposure levels and the respective ecotoxicological assessment across the eight sub-catchments (Fig. S12). The sources and pathways causing pesticide pollution were obviously distributed evenly across the entire area of the catchment, indicating that the generally prevailing horticultural practice caused relevant pesticide transfer.

Relevant commonalities across the areas included the same set of key
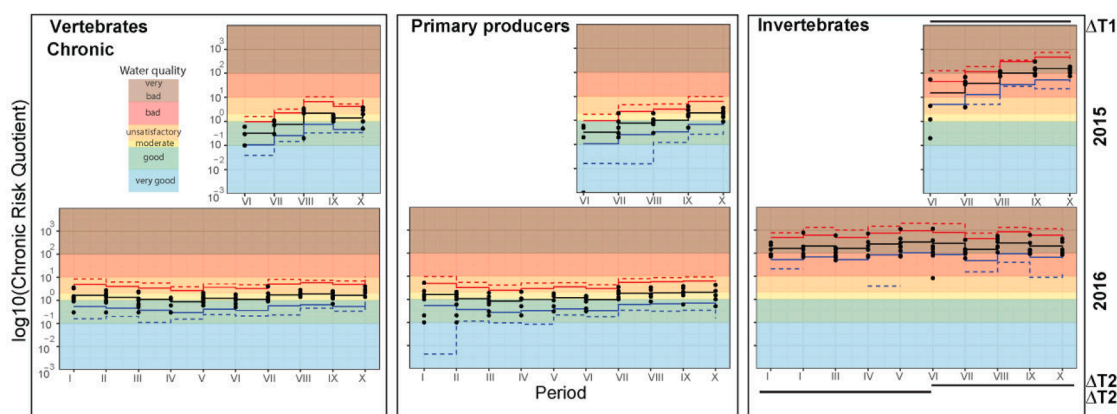
Fig. 4. Temporal development of chronic risk quotients from mixtures of pesticides relevant for vertebrates, primary producers, and invertebrate, respectively. The solid black lines represent the average RQmix values obtained across the five sites in 2015 and the eight sites in 2016, during the biweekly sampling interval (roman numerals). The upper solid red lines represent the RQmix according to upper uncertainty limit and lower solid blue lines according to the lower uncertainty limit.

pesticides that dominated the ecotoxicological risk (chlorpyrifos, cypermethrin). Therefore, their substitution could be one mitigation option. Without these most critical substances, the insecticide levels were generally still 10-fold and more too high (see Fig. S13). Hence, substitution by itself may only contribute partially to a real improvement.

Another aspect that deserves attention based on visual field observations is the connectivity of the field with the stream network. Despite the presence of riparian forests along substantial fractions of the stream network (Tab. S1), linear structures such as roads and culverts establish the connectivity between field and the river (Fig. S20). In addition, the field characteristics and their management also enhance the link between fields and water bodies. Many fields contain artificial structures to drain the surface runoff (Fig. S1),to get rid of excess rainfall that cannot infiltrate during the intensive rainfall bursts. This concentrated flow contributes to the gully formation at the edge of fields establishing connectivity with roads and the stream network, which has been proven to diminish the efficiency of riparian buffer strips (Bereswill et al., 2012). Additionally, the manual spraying of the fields causes a high density of compacted tramlines that generate surface runoff and are pesticide sources at the same time (Fig. S1 right). The sequence of linear structure elements on the fields and outside may strongly enhance the transport of pesticides as it has been demonstrated in other regions (Remund et al., 2021, Schönenberger et al., 2021, Schönenberger and Stamm 2021).

Interviews with farmers on their pesticide use practices (Staudacher et al., 2020) supported by anecdotal evidence and field observations during this study clearly demonstrate poor handling of spray equipment and spray solution as another important pesticide source. This fits together with our measurements demonstrating that rainfall could not have been the only driver for pesticide transport.

The apparent discrepancy between the ecotoxicological risk derived from the pesticide concentrations and the biological indices raised questions about their interpretation for management purposes. If it were true that the ecological status of the macroinvertebrate community was indeed good at the most downstream location, it would be a key question to understand which ecological factors might compensate for impairment through pesticides. Such knowledge could help to take measures to improve the ecological status also elsewhere.

5. Conclusions

Due to the time-integrated and spatially distributed sampling in the Tapezco catchment in Costa Rica, we demonstrate that pesticide pollution of streams in agricultural areas is not only occurring during a few critical periods and critical locations in the landscape. On the contrary, our results clearly demonstrate that pesticide occurrence in catchments with intensive horticultural production is a problem that prevails in space and time implying that it is caused by the normal practice of the production system. Accordingly, aquatic organisms throughout the stream network are exposed to a high ecotoxicological risk. Regarding mitigation, this implies that measures have to target the common practices by farmers and farm workers. There is a need to reach out to this broad group of people.

The quantitative results and visual observations indicate that rain-driven diffuse losses from fields and poor handling of pesticides including their residues pose problems that require improvements. Apart from fostering good practices on individual farms and their fields, the connectivity network consisting of in-field structures, gullies outside fields and the road infrastructure may also require integrated measures at the landscape level.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Author contributions

Frederik T. Weiss, Clemens Ruepert, Rik I.L. Eggen and Christian Stamm contributed to the study conception and design. Material preparation, data collection and analysis were performed by Frederik T. Weiss, Silvia Echeverría-Sáenz and Christian Stamm. The first draft of the manuscript was written by Frederik T. Weiss, all authors commented on previous versions of the manuscript and Christian Stamm wrote the final version. All authors read and approved the final manuscript.

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Availability of data and materials

The data are publicly available on the Eawag Research Data Institutional Collection (https://doi.org/10.25678/0004R4; https://doi.org/10.25678/0006DM; https://doi.org/10.25678/0006CK) and at https://doi.org/10.5281/zenodo.4818488 (macroinvertebrate data).

Declaration of Competing Interests

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

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Supplementary materials

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