OCCURRENCE, MONITORING AND EFFECTS OF PESTICIDES AND THEIR MIXTURES IN AGRICULTURAL STREAMS

by

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LIST OF PUBLICATIONS

The present thesis is a cumulative dissertation based on the following peer-reviewed publications:

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ANNOTATION

This cumulative dissertation is based on four scientific publications written by multiple authors. For this reason, the first-person plural is used throughout this thesis.

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SUMMARY

Stream ecosystems are one of the most threatened ecosystems worldwide due to their exposure to diverse anthropogenic stressors. Pesticides appear to be the most relevant stressor for agricultural streams. Due to the current mismatch of modelled and measured pesticide concentrations, monitoring is necessary to inform risk assessment or improve future pesticide approvals. Knowing if biotic stress responses are similar across large scales and long time frames could ultimately help in estimating protective stressor thresholds.

This thesis starts with an overview of entry pathways of pesticides to streams as well as the framework of current pesticide monitoring and gives an outline of the objectives of the thesis. In chapter **2**, routine monitoring data based on grab sampling from several countries is analysed to identify the most frequently occurring pesticide mixtures. These mixtures are comprised of relatively low numbers of pesticides, of which herbicides are dominating. The detected pesticide mixtures differ between regions and countries, due to differences in the spectrum of analysed compounds and limits of quantification. Current routine monitoring does not include sampling during pesticide peaks associated with heavy rainfall events which likely influences the detected pesticide mixtures. In chapter **3**, sampling rates of 42 organic pesticides for passive sampling are provided together with recommendations for the monitoring of field-relevant peaks. Using this information, in chapter **4** a pesticide gradient is established in an Eastern European region where agricultural intensity adjacent to sampled streams ranges from low to high. In contrast to current routine monitoring, rainfall events were sampled and a magnitude of pesticides were analysed. This led to the simultaneous detection of numerous pesticides of which one to three drive the pesticide toxicity. The toxicity, however, showed no relationship to the agricultural intensity. Using microcosms, the stress responses of fungal communities, the hyphomycetes, and the related ecosystem function of leaf decomposition, is investigated in chapter **5**. Effects of a field-relevant fungicide mixture are examined across three biogeographical regions for three consecutive cycles of microbial leaf colonisation and decomposition. Despite different initial communities, stress responses as well as recoveries were similar across biogeographical regions, indicating a general pattern.

Overall, this thesis contributes to an improved understanding of occurrence and concentrations of pesticides mixtures in streams, their monitoring and impact on an ecosystem function. We showed that estimated pesticide toxicities reach levels that affect non-target organisms and thereby potentially whole ecosystems. Routine monitoring, however, likely underestimates the threat by pesticides. Effects leading to a loss in biodiversity or functions in streams ecosystems can be reduced by reassessing approved pesticides with ongoing targeted monitoring and increased knowledge of effects caused by these pesticides.

ZUSAMMENFASSUNG

Bäche gehören zu den gefährdetsten Ökosystemen, da sie diversen anthropogenen Stressoren ausgesetzt sind, wobei Pestizide für landwirtschaftliche Bäche am relevantesten erscheinen. Aufgrund der Diskrepanz zwischen modellierten und gemessenen Pestizidkonzentrationen ist Monitoring nötig um zukünftige Risikobewertungen und Zulassungen zu verbessern. Festzustellen ob biotische Stressreaktionen über große räumliche und zeitliche Skalen ähnlich sind, ist nötig um Schwellenwerte zum Schutz vor Stressoren abzuschätzen.

Diese Doktorarbeit beginnt mit einem Überblick über Pestizideintrittspfade in Bäche, sowie dem momentanen Stand des Pestizidmonitorings gefolgt von der Zielsetzung der Doktorarbeit. In Kapitel **2** werden Ergebnisse aus Schöpfproben von Routinemonitoring mehrerer Länder analysiert um die häufigsten Pestizidmischungen zu identifizieren. Diese Mischungen werden von wenigen Pestiziden gebildet, wobei Herbizide dominieren. Die nachgewiesenen Mischungen unterscheiden sich regional, da Nachweisgrenzen und Stoffumfang variieren. Aktuelles Routinemonitoring umfasst bisher keine Probenahmen während durch Starkregenereignisse hervorgerufene Pestizidspitzen, die wahrscheinlich Pestizidmischungen beeinflussen. In Kapitel **3** werden Sammelraten für 42 Pestizide bei der Benutzung von Passivsammlern vorgestellt und Empfehlungen zum Monitoring von feldrelevanten Pestizidspitzen gegeben. Damit konnte in Kapitel **4** ein Pestizidgradient in einer osteuropäischen Region aufgestellt werden in der die Landwirtschaftsintensität von niedrig bis hoch reicht. Dabei wurden Regenereignisse beprobt und eine Vielzahl von Pestiziden analysiert. Dies führte zu vielen gleichzeitig nachgewiesenen Pestiziden, von denen ein bis drei die Pestizidtoxizität bestimmten. Diese zeigte jedoch keinen Zusammenhang zur landwirtschaftlichen Intensität. Durch Mikrokosmenexperimente wurde in Kapitel **5** die Stressantwort von Pilzgemeinschaften, den Hyphomyceten, und deren assoziierter Ökosystemfunktion des Laubabbaus untersucht. Effekte einer feldrelevanten Fungizidmischung wurde über drei biogeographische Regionen sowie drei aufeinanderfolgende Zyklen von mikrobieller Laubkolonisation und -abbau untersucht. Trotz anfänglich unterschiedlichen Gemeinschaften waren Stressantworten sowie Erholungen in den untersuchten Regionen ähnlich, was auf ein generelles Muster hindeutet.

Insgesamt trägt diese Doktorarbeit zum verbesserten Verständnis von Vorkommen und Konzentrationen von Pestizidmischungen, deren Monitoring sowie ihren Auswirkungen auf eine Ökosystemfunktion bei. Wir konnten zeigen, dass die abgeschätzten Pestizidtoxizitäten potentiell Nichtzielorganismen und somit ganze Ökosystem beeinflussen. Routinemonitoring unterschätzt diese Gefahr bisher jedoch wahrscheinlich. Effekte, welche Verluste in Biodiversität sowie Funktionen hervorrufen, können verringert werden indem zugelassene Pestizide mit anhaltendem Monitoring neu bewertet werden und die Datenlage zu Pestizidwirkungen verbessert wird.

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1 INTRODUCTION AND OBJECTIVES

1.1 ANTHROPOGENIC STRESSORS IMPACTING STREAM ECOSYSTEMS

Streams and rivers are the lotic part of the surface freshwater and represent less than 0.0002 % of the total world water resources (Shiklomanov, 1993). Despite representing a minor share of inhabitable areas, streams and rivers are important ecosystems with a high biodiversity (Allan and Flecker, 1993; Strayer and Dudgeon, 2010). Some factors causing this high biodiversity are the complexity of the available habitats (Allan and Flecker, 1993; Thienemann, 1954) as well as the range of expression of variables such as temperature, conductivity or flow velocity (Vannote et al., 1980). Depending on the position within the stream network and the manifestation of different variables, diverse nutrition sources and related feeding groups are occurring (Vannote et al., 1980; Webster, 2007). The stream's integrity is crucial not only for maintaining its biodiversity but also for several ecosystems services from which human societies are profiting such as water purification (Harrison et al., 2014). At the same time, streams as the smallest lotic systems are among the most threatened ecosystems worldwide. This is mainly due to their close link to the surrounding landscape, connections within the stream network and their low water volume limiting dilution (Gomi et al., 2002; MEA, 2005; Vaughn, 2010; Vörösmarty et al., 2010).

Stream ecosystems have been threatened since the existence of the earliest human societies. Optimization of utilizable areas, flood control, power water mills, and the creation of dams to ensure water supplies are some of the main alterations made to stream habitats over the millennia (Allan and Flecker, 1993; Strayer, 2006; Strayer and Dudgeon, 2010). This in turn has led to the degradation of more than one third of sampled European streams (Degerman et al., 2007; Schäfer et al., 2016; Schinegger et al., 2012). Moreover, streams are simultaneously endangered by many other stressors (Ormerod et al., 2010; Schäfer et al., 2016; Schinegger et al., 2012; Terrado et al., 2016; Vörösmarty et al., 2010). Among these are abiotic stressors like increasing temperature and altered precipitation regimes due to climate change (Knouft and Ficklin, 2017; Woodward et al., 2010), increasing salinity levels due to mining as well as discharge of wastewater including de-icing salts (Cañedo-Argüelles et al., 2013; Kaushal et al., 2018), and biotic stressors like invasive species (Allan and Flecker, 1993; Lodge et al., 1998). Within the era of the Anthropocene, a newly postulated geological epoch dominated by human impacts (Crutzen, 2002; Monastersky, 2015), more and more xenobiotics are released into the environment (Bernhardt et al., 2017; Schwarzenbach et al., 2006). This leads to a nearly ubiquitous occurrence of xenobiotics in streams.

Since these diverse stressors are usually co-occurring (Ormerod et al., 2010; Schäfer et al., 2016; Schinegger et al., 2012; Vörösmarty et al., 2010), discriminating effects on stream ecosystems caused by single stressors is hardly possible. To enable the estimation of effects

by a single stressor, a wide gradient of this stressor would be necessary, ideally ranging from low to very high stressor intensity, with other stressors occurring across the whole gradient with more or less constant stress intensity. This thesis aimed to establish a wide pesticide gradient in a region with a broad range of agricultural intensity to discriminate pesticide effects from those of other agricultural stressors.

1.2 AGRICULTURAL LAND USE AS A SOURCE OF STRESSORS TO STREAMS

Agriculture is the origin of several stressors affecting stream ecosystems, like the above-mentioned habitat degradation or pesticides, a group of xenobiotics. With a constantly increasing human population (Census, 2020), global crop yields are expected to increase simultaneously to meet the rising global food demand, specifically of animal proteins (Tilman et al., 2011). In order to achieve this, agricultural land use is likely to expand to yet least-impacted areas. The expansion of arable land further strains local conditions and ecosystems, which is aggravated by climate change (Angelsen, 1999; Laurance et al., 2014; Tilman et al., 2001).

In addition, the intensification of existing agricultural areas has been proven to increase crop yield (Godfray et al., 2010; Matson et al., 1997; Struik and Kuyper, 2017). In the course of agricultural intensification, human and animal labour were commonly replaced by heavy machinery in Western Europe and the USA in the first half of the 20th century (Schmitz and Moss, 2015). In other regions of the world, however, low intensity, non-mechanical agriculture still prevails locally. With the mechanisation of agricultural processes, field sizes as well as the productivity of farmers could be increased, leading to a decreased proportion of the population working in agriculture (Hazell and Wood, 2008; Pe'er et al., 2014; Schmitz and Moss, 2015). Also, the use of fertilisers as well as irrigation of agricultural areas have shown to clearly increase the yield of crops (Matson et al., 1997; Stewart et al., 2005).

Besides these measures, the reduction of competition by weeds or crop loss through the management of insect or fungal pests is a successful method to increase crop yield. These methods can be labour- and cost-efficiently accomplished by using pesticides (Matthews, 2008; Oerke, 2006; Schreinemachers and Tipraqsa, 2012; Seufert et al., 2012). The continuous increase in crop yields, combined with the impacts of climate change on the dispersion of crop pests (Kattwinkel et al., 2011; Tilman et al., 2001), is likely to lead to an increase in the use of pesticides by a factor of 2.7 from 2000 to 2050 (Tilman et al., 2001).

Since most pesticides are applied in open field agricultural areas, they often unintentionally enter non-target ecosystems via different pathways (Carter, 2000). Freshwater ecosystems like streams are especially endangered non-target ecosystems since they collect water, which is the vector of several distribution pathways of pesticides. The dominating pathway of

pesticides to streams are runoff events associated with heavy rainfall (Bereswill et al., 2012; Weibel et al., 1964). During runoff, pesticides are transported on the surface up to two weeks after their application if precipitation of more than 8 - 10 mm per day occurs, depending on topography and wetting of the soil (Carter, 2000). The related pesticide pulses in streams can reach concentrations up to a factor 100 higher than those at base flow conditions (Leu et al., 2004; Rasmussen et al., 2015; Reilly et al., 2012). Additionally, pesticides can enter streams via drainage water (Bennett et al., 2005) or through leaching (Kellogg et al., 2002) from adjacent fields. Besides these diffuse entryways, point sources like wastewater treatment plants (WWTP) (Köck-Schulmeyer et al., 2013; Münze et al., 2017) as well as the deposal of tank fillings or misuse during application (Wittmer et al., 2010) can heavily increase in-stream pesticide concentrations. Beyond that, streams but also all other non-target ecosystems can potentially be exposed diffusely by spray drift (Schulz et al., 2001) but also volatilisation followed by precipitation (Houbraken et al., 2016). In addition, pesticides on top or within plant material like leaves can enter various ecosystems, especially when the plant material itself is transported during leaf fall in autumn or extreme storms (Kreutzweiser et al., 2007).

During the approval process of pesticides, their predicted concentrations in streams are modelled and potential environmental risks are estimated using ecotoxicological tests. Pesticides are approved if predicted environmental concentrations are below regulatory acceptable concentrations since this should avoid unwanted side effects (Boivin and Poulsen, 2017). Risk estimates, however, are often inaccurate (Schäfer et al., 2019), which can be seen in the fact that up to two-thirds of samppling sites facing exceedances of regulatory thresholds (Stehle and Schulz, 2015; Szöcs et al., 2017). These exceedances of pesticides threaten most of the freshwater systems on a continental-to-global scale (Ippolito et al., 2015; Malaj et al., 2014; Stone et al., 2014). The mismatch between modelled and measured pesticide concentrations (Knäbel et al., 2014, 2012) could be caused by inaccurate predictions of pesticide exposure in the context of various possible entry pathways, but also on inaccuracies when estimating pesticide applications. For example, one crop is usually treated with several pesticides of different groups before and during its growing period to protect against different pests and to avoid the development of resistances (Matthews, 2008; Whalon et al., 2008). During these applications, multiple pesticides can not only be applied after each other but also simultaneously if they are already mixed in the application device (Luiz et al., 2013). Additionally, agricultural land use consists of a multiplicity of crops with various approved and used pesticides (Oerke, 2006; Roßberg, 2013). Based on these factors and since streams drain whole catchments the occurring pesticide concentration regimes as well as mixtures can be complex (Altenburger et al., 2015; Moschet et al., 2014; Schäfer et al., 2013).

1.3 PESTICIDE MONITORING IN STREAMS

Based on the complexity of pesticide occurrence in number and concentration as well as the mismatches to concentration predictions, pesticide monitoring in streams is pivotal. Monitoring represents an important source to estimate in-stream pesticide exposure, which is necessary to evaluate potential risks to freshwater ecosystems beyond risk assessment during pesticide approval and to inform environmental management. To this end, this thesis presents the most frequently occurring pesticide mixtures in several countries. Various sampling methods can be used to conduct pesticide monitoring. Routine governmental monitoring, as required by the European Water Framework Directive (WFD) (European Union, 2013), usually relies on grab sampling of water. The sampling follows a time schedule, with minimal requirements of one sample per month, analysing a very limited number of compounds (priority compounds), adapted in terms of time schedule as well as analysed compounds for single river basins (Whalley et al., 2018). Using this method, the long-term situation of pesticide pollution in streams can be estimated with limited labour and budget. However, when relying on grab sampling, peaks in concentrations as well as numbers of pesticides are usually missed (Bundschuh et al., 2014; Stehle et al., 2013), since peaks are typically associated with heavy rainfall events (see chapter **1.2**).

Besides the disadvantage of missing peak exposure conditions, routine monitoring usually incorporates a limited number of compounds during analysis, which increases the risk of missing compounds potentially affecting stream ecosystems (Malaj et al., 2014; Moschet et al., 2014). Limiting the number of analysed compounds is usually done to keep labour and finances in check. Minimum requirements of the spectrum of analysed compounds are defined by the WFD complemented by the European Watch List, which comprise in total 60 compounds of which 26 are pesticides (European Union, 2018, 2013). The approval of some of these pesticides has, however, expired and monitoring therefore includes legacy pesticides. Besides these general regulatory requirements which are equal all over the European Union, the list of analysed compounds is required to be expanded for each river basin resulting in rather different compound spectra over time and states/regions (Whalley et al., 2018).

In addition to the mainly conducted grab sampling, methods have been developed to collect event-driven water samples during peak exposure conditions associated with heavy rainfall events. These methods were in Germany until today mainly incorporated in scientific studies and are within the framework of the National Action Plan for plant protection currently tested to be included in pesticide monitoring of German streams (Wick et al., 2019). One simplified method is based on bottles passively filled by elevated water levels (Fernández et al., 2014; Liess et al., 1996). This enables a cost-efficient and robust monitoring of peak concentrations

but requires high flexibility in terms of immediate sample retrieval to limit degradation of compounds. Automatic event-driven samplers can, depending on their configuration, filter, concentrate and cool collected water at the stream site, which provides temporal flexibility regarding sample retrieval (Wick et al., 2019). These are, however, costly both in procurement and maintenance and can be, due to their complexity, susceptible to malfunctions. Both types of event-driven samplers collect suspended particulate matter (SPM) and microorganisms like bacteria to different degrees. This could cause alterations of the actual concentrations since the compounds might adsorb and desorb from SPM and microorganisms might actively degrade compounds (Liess et al., 1996; Singh et al., 1999).

All methods collecting water samples, grab and event-driven sampling, have in common that the water sample can be analysed afterwards using different analytical methods. The most cost- and labour-efficient method is a direct measurement of the compounds. In the process, the resulting limits of quantification (LOQ) are device- as well as matrix dependent and might not be sufficiently low to detect exceedances of concentrations defined in regulation for each compound. Compounds from water samples can be concentrated using liquid-liquid or solid-phase extraction before chemical analysis, increasing the related labour but decreasing the LOQ (European Union, 2013; Wick et al., 2019). The selected extraction method is, however, strongly based on which compounds or chemical groups should be included in chemical analysis. A wide spectrum of compounds can, due to their variance in compound properties, strongly increase the required complexity of extraction methods to achieve appropriate LOQs (e.g. Moschet et al., 2013).

Alternatively, pesticide monitoring can be conducted using passive sampling (Moschet et al., 2015; Vrana et al., 2005). Pesticides are concentrated during the deployment of the passive sampler in its sorbent, which corresponds to an on-site solid-phase extraction. Passive samplers, like Chemcatchers using styrene-divinylbenzene (SDB) disks, can be used to calculate time-weighted average (TWA) concentrations of diverse compounds over the deployment time (Booij et al., 2007). Depending on the desired deployment time, different configurations can be used. For short deployment times, which are usually limited by the biofouling of the disks or when single compounds reach the equilibrium of uptake and release, only the sorption phase of a passive sampler is used. This leads to relatively high sampling rates (i.e. the rate of a compound accumulated in the sorbent) resulting in low LOQs. Therefore, the passive sampler can collect compound masses in highly fluctuating concentration regimes like e.g. during peaks associated with heavy rainfall (Fernández et al., 2014; Mutzner et al., 2019). Since we assume that organisms are subject to environmental selection by the strongest stress event (Fernández et al., 2015; Schäfer et al., 2011), details on how the masses sorbed to the passive sampler can be used to estimate peak concentrations are presented in this thesis. Longer deployment times are achieved by

shielding the sorption phase of the passive sampler with a diffusion-limiting membrane. This membrane is, besides protecting the sorption phase from biofouling and mechanical disturbances, leading to lower sampling rates (Moschet et al., 2015; Vermeirssen et al., 2009). With this configuration, TWA concentrations over longer time frames, like four weeks, can be estimated. In concert with lower sampling rates, the time integrative windows are longer, i.e. masses of past compound occurrences are stored longer in the passive sampler. The lower sampling rates, however, come with the cost that short concentration peaks of compounds are usually missed. Both configurations have in common that each compound requires individual sampling rates to precisely calculate TWA concentrations. Sampling rates are usually estimated with calibration experiments, which are labour-intensive but enable exact results (e.g. Mutzner et al., 2019; Vermeirssen et al., 2009). The estimation of sampling rates using *in-situ* calibrations on the other hand, where time-integrative water samples are collected simultaneously, can be unreliable since high fluctuating compound concentrations are not similarly sampled using the passive and the active sampler (Moschet et al., 2015).

1.4 BIOTIC RESPONSES TO ANTHROPOGENIC STRESSORS IN STREAMS

The multiplicity of anthropogenic stressors is known to lead to individual and combined biotic responses in streams. Among these stressors, pesticides are one of the major risks towards freshwater ecosystems (MEA, 2005). Diverse taxonomic groups in stream ecosystems are affected, including fish, macroinvertebrates, fungi and diatoms (Beketov et al., 2013; Fernández et al., 2015; Hering et al., 2006; Mancini et al., 2019; Schäfer, 2019; Stendera et al., 2012). These effects on one single species or an entire organism group can cascade through the whole food web, causing substantial changes in the whole stream ecosystem. Top-down effects can occur when higher trophic levels like predators or grazers are affected and their control of abundance as well as biomass of respective lower trophic levels is altered (Huryn, 1998). When low trophic levels are altered or inhibited, bottom-up effects can occur (Rosemond et al., 1993; Wallace et al., 1997). Ecosystem functions like primary production or leaf litter decomposition are the basic food sources of the green and brown food web, respectively (Vannote et al., 1980; Webster, 2007). Since they are known to be affected by anthropogenic stressors (López-Rojo et al., 2019), they are likely to cause bottom-up effects if altered or inhibited.

Studies have shown that communities exposed to stressors lose sensitive species. Despite this biodiversity loss, communities can increase their tolerance towards the shaping stressor and maintain their function up to a certain point, following the concept of pollution-induced community tolerance (PICT) (Blanck et al., 1988; Clements, 1999; Tlili et al., 2015). PICT can be a result of genetic, epigenetic or physiological adaptations, usually in combination with a species turnover within the respective community. The related developed tolerance can even

lead to increased performance of adapted communities exposed to their shaping stressor (Feckler et al., 2018; Gardeström et al., 2016) and is likely to be passed, potentially increased, to following generations. If, however, the biodiversity loss reaches a critical point, where a loss of crucial species occurs, communities collapse and the maintenance of ecosystem functions might fail (Cao et al., 2018; Gardeström et al., 2016; Tlili et al., 2015).

To avoid the collapse of communities, failure of ecosystem functions and, in the long term, irreversible effects in stream ecosystems which might cascade to adjacent areas, effect thresholds of stressors such as pesticides need to be derived. This is attempted during pesticide approval through regulatory acceptable concentrations, which are currently however often exceeded (see chapter **1.2**), or environmental quality standards (European Union, 2013). Until today most of these thresholds are based on single species laboratory experiments using organisms from laboratory cultures or few more complex studies with simplified communities. These, however, do not necessarily reflect field-realistic situations, where species and communities are exposed to a wide range of shaping conditions, even without considering the occurrence of multiple stressors (chapter **1.1**). Based on this, the general transferability of thresholds derived using the above-mentioned approaches remains open to speculation. A reliable estimation of stressor thresholds requires similar stress responses detected over larger spatial scales or time frames, estimating universal responses. These universal responses of single species or whole communities can help in training models as well as extrapolating and forecasting responses for further communities or to other stressors including additional pesticide compounds. This can improve the management of ecosystems to reduce anthropogenic effects (Brudvig, 2017; Clements and Rohr, 2009) and reduce the number of tests during risk assessment, which in turn can reduce the necessary test organisms (Scholz et al., 2013). To estimate universal responses of aquatic fungal communities, this thesis presents their stress responses across three biogeographical regions exposed to a field-realistic fungicide mixture.

1.5 OBJECTIVES AND OUTLINE OF THIS THESIS

This thesis aims to contribute to an improved understanding of occurrence and concentrations of pesticides mixtures in streams and their impact on an ecosystem function. The main objectives are to (i) identify the most common pesticide mixtures, (ii) improve the suitability of passive sampling to monitor field-relevant pesticide peaks, (iii) investigate pesticide toxicity during peak exposure scenarios across a gradient of low to high intensity agricultural streams and (iv) study community responses towards ongoing pesticide stress across several biogeographical regions. The results of this thesis can ultimately aid the scientific community in informing future ecological risk assessment, improving pesticide monitoring during peak exposures, establishing a wide pesticide gradient to identify drivers and estimating first universal responses to pesticide stress. Fig. 1.1 provides an overview of the research objectives addressed in this thesis.

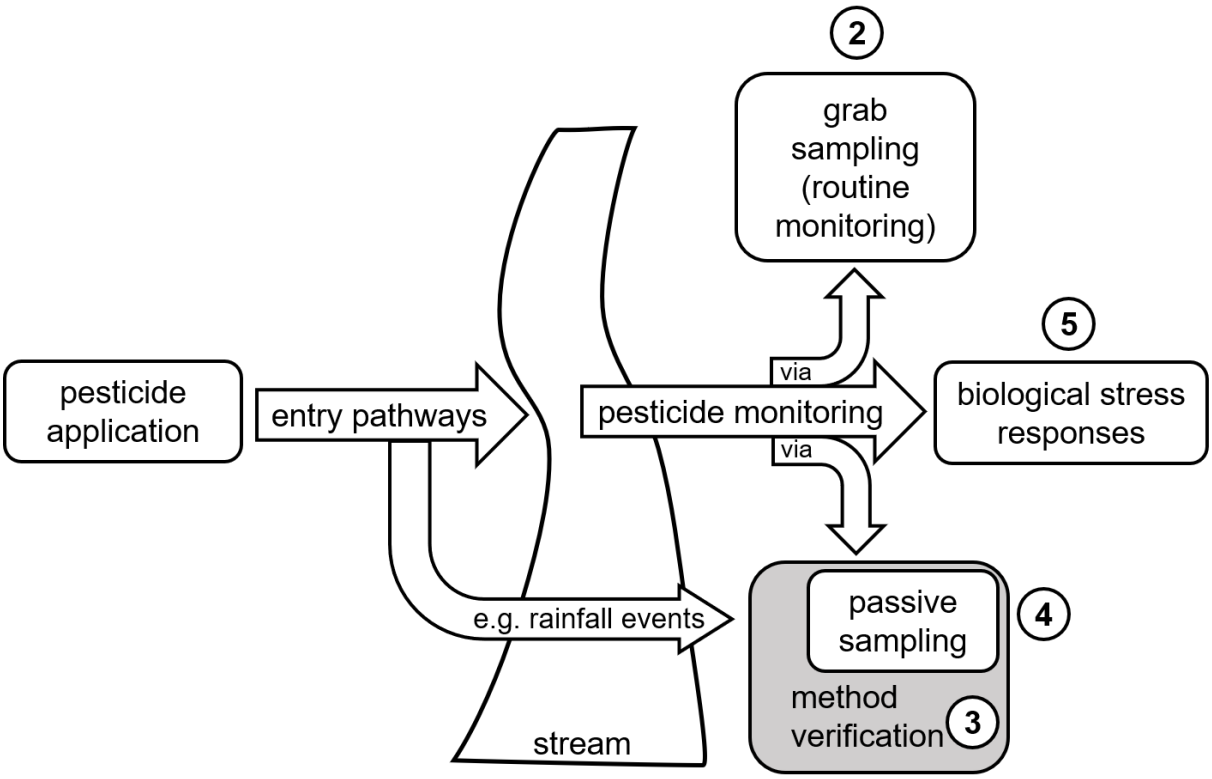

Fig. 1.1: Simplified overview of topics included in this thesis including application, entry pathways to streams and monitoring of pesticides as well as biological stress responses based on monitoring results. Numbers in circles refer to the chapters presented in this thesis.

Routine monitoring uses grab sampling to assess the exposure of streams to pesticides, which usually occur as mixtures. Chapter **2** presents a large scale analysis of pesticide monitoring data and addresses the following aims:

- Identify the most common pesticide compounds as well as mixtures detected in stream routine monitoring programs of the USA, Germany, France and the Netherlands.
- Determine the relationship of stream characteristic and analytical differences to the number of detected pesticides and size of resulting mixtures.
- Compare the composition and size of pesticide mixtures across the analysed countries.

→ This study can inform future ecological risk assessment aiming to estimate the effects of the most common pesticide mixtures. Additionally, the study identifies gaps in current risk assessment based on grab sampling.

Rainfall events have been demonstrated to cause peaks in concentrations as well as the number of pesticides in streams. To use passive sampling under field conditions, a calibration experiment to determine sampling rates was necessary (chapter **3**). Based on this, passive samplers could be used to monitor pesticides during peak conditions associated with heavy rainfall events (chapter **4**). Specific aims of these studies were:

- Estimate sampling rates for styrene-divinylbenzene (SDB) disk passive samplers of 42 organic pesticides under a field-realistic peak exposure.
- Investigate relationships of sampling rates from different calibration studies and with compound properties to possibly predict sampling rates.
- Determine pesticide concentrations and potential toxicities towards invertebrates and algae using passive sampling in streams of a region with a gradient of low to high intensity agriculture.
- Identify relevant drivers on local and catchment-scale for pesticide toxicity.

→ The first study improves pesticide monitoring using passive sampling of field-relevant pesticide peaks, which represent the strongest pesticide stress event capable of shaping stream communities. The second study aims to establish a wide pesticide gradient in agricultural streams to identify drivers of pesticide exposure and ultimately intends to discriminate effects on stream ecosystems from pesticide and other agricultural stressors.

Microbial communities can be used as easy to handle surrogate organisms for complex studies due to their relatively short generation times. Based on this, they can be used to estimate long term effects of stressors such as pesticide exposure, with the possibility to compare several biogeographical regions (chapter **5**). Specific goals of this study were:

- Examine stress responses of aquatic fungal communities, the hyphomycetes, and the related microbial leaf decomposition across three consecutive leaf colonisation cycles to a fungicide exposure.
- Determine if stress responses show similarities over the biogeographical regions Central Plains (Denmark), Western Highlands (Germany) and Fenno-Scandian Shield (Sweden).

→ Similar stress responses identified in this study can help in estimating universal stress responses for the stressor pesticide toxicity and improve modelling and future effect predictions.

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2 PESTICIDE MIXTURES IN STREAMS OF SEVERAL EUROPEAN COUNTRIES AND THE USA

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2.1 ABSTRACT

Given the multitude of pesticides used in agriculture, adjacent streams are typically exposed to pesticide mixtures. Previous studies analysed the ecological risks of a few pesticide mixtures or were limited to an individual region or crop, whereas a large scale analysis of pesticide mixtures is missing. We analysed routine monitoring data from Germany, France, the Netherlands and the USA comprising a total of 4,532 sites and 56,084 sampling occasions with the aim to identify the most frequently detected pesticides, their metabolites and mixtures. The most frequently detected compounds were dominated by herbicides and their metabolites. Mixtures mostly comprised of two up to five compounds, whereas mixtures in the USA and France had clearly less compounds than those of Germany and the Netherlands. The number of detected pesticides and thereby the size of mixtures is positively correlated to the number of measured pesticides ($r = 0.57$). In contrast, a low relationship was found to the ratio of agricultural areas within the catchment ($r = 0.17$), and no relationship was found to the size of the catchment ($r = 0.06$). Overall, our study provides priority mixtures for different countries that may be used for future ecotoxicological studies to improve risk assessment for stream ecosystems.

2.2 INTRODUCTION

Pesticide use in agricultural areas is associated with their unintended release into adjacent non-target ecosystems such as rivers and streams (Schulz, 2004). Given the high number of authorised organic, active ingredients (approved in 2016: EU: 460 (EC, 2015); USA: 473 (US EPA, 2016) pesticides typically occur as mixtures in streams (Altenburger et al., 2015; Malaj et al., 2014; Moschet et al., 2014b; Schäfer et al., 2013; Stone et al., 2014). This is the result of (i) the use of different pesticides on different crops within one catchment area (Oerke, 2006; Roßberg, 2013), (ii) the use of different pesticides on one crop to avoid the development of resistances in pest species (Whalon et al., 2008) and (iii) the use of pesticide products that feature mixtures of several active ingredients (Altenburger et al., 2013; Relyea, 2008; Roßberg, 2013).

Several studies analysed the effects of pesticide mixtures on aquatic ecosystems. One set of studies followed general design principles and selected, for example, (i) one herbicide, one insecticide and one fungicide (Halstead et al., 2014), (ii) several compounds known to be toxic individually (Bundschuh et al., 2013; Grimme et al., 1996), and (iii) several pesticides with the same mode of action (Knauert et al., 2008). Another set of studies aimed to simulate real-world conditions by using (iv) one realistic mixture with 25 compounds that were assumed to occur in a catchment area where cereals, maize and sugar beet are grown (Backhaus et al., 2003; Junghans et al., 2006) or (v) a pesticide mixture that was detected in a stream (Tiam et al., 2014). These different studies assessed effects of pesticide mixtures for a few (model)

mixtures. Empirically testing the effects of all possibly occurring mixtures is rendered impossible by the huge number of possible combinations. Nevertheless, accounting of mixture effects is considered important because they can strongly exceed the effects of single compounds (Brack et al., 2015), consequently resulting in an underestimation of mixtures effects in real-world streams.

In this context, the concentration addition model is typically used to estimate effects from chemical concentration data of compounds with the same mode of action or affecting the same target in organisms. Studies showed that the concentration addition model estimated effects of pesticide mixtures with ±10 % deviation on algae reproduction (e.g. Faust et al., 2001; Silva et al., 2002), suggesting that the underlying premise of additivity holds. By contrast, other studies stressed the limitations of this model especially when accounting for the effects of mixtures with two to ten compounds on single species. Here, the effects of 70 % of the analysed mixtures showed a deviation of up to 300 %, with up to a 12-fold exceedance of the predicted effect (Cedergreen et al., 2008; Nørgaard and Cedergreen, 2010). Consequently, disparities remain regarding the predictive performance of current mixture models and model evaluation would benefit from identifying most frequently occurring realistic mixtures against which mixture models could be validated.

The identification of priority mixtures has been suggested to be a central task for future risk assessment (European Commission, 2011; Lydy et al., 2004). Previous studies identifying environmental mixtures were mostly limited to (i) small spatial scales such as a few sites in a region, (ii) specific crops (Belden et al., 2007) or (iii) or a few pesticides and individual pesticide groups, respectively (Petersen et al., 2012; Reilly et al., 2012; Stehle et al., 2012).

In this study, we analysed large-scale pesticide data to identify the most frequently found (i) individual pesticide compounds and (ii) mixtures of pesticides in rivers and streams. Data on pesticide occurrence originated from routine monitoring programs based on grab sampling of France, Germany, the Netherlands and the USA. We scrutinised whether the number of detected pesticides and compounds in mixtures depended on (iiia) characteristics of the sampling sites or sampling methods such as the size of the upstream catchment area from a sampling site or the number of analysed compounds. To allow for a comparison between countries (iiib) we repeated parts of the analysis for a restricted dataset of pesticides analysed in all countries. Overall, our study provides priority mixtures for different countries that may inform risk assessment.

2.3 MATERIALS AND METHODS

2.3.1 OVERVIEW ON DATA SETS AND PRE-PROCESSING

We compiled pesticide monitoring data of lotic surface waters from databases from Germany, France, the Netherlands, and the USA (Table 2.1, Fig. A.1; details on differences in harvested crops given in Table A.1). We retrieved the data from France from EIONET (Reporting Obligations Database (ROD); River quality (EWN-1) -Eionet, 2014), the data from the Netherlands from www.bestrijdingsmiddelenatlas.nl and the data from Germany were provided by the regional water quality authorities. The US dataset was generated by harmonizing and combining datasets from the National Water-Quality Assessment Program (NAWQA Data Export, 2014) and the Water Quality Data Portal (WQP, 2014). Sites within a 10 m distance from both datasets were considered as identical and entries from them were merged. The data from France, the Netherlands and the USA covered the country-level, whereas the German data were restricted to four German states (Rhineland-Palatinate, North Rhine-Westphalia, Saxony and Baden-Württemberg). Nevertheless, we refer to this data as Germany to enhance readability. The used chemical concentrations originated exclusively from grab water samples.

Data pre-processing consisted of the following steps (see Fig. A2 for a graphical overview): (I) To obtain a spatially-balanced monitoring data set for each region and country, and thus to enhance comparability, we used the Generalized Random Tessellation Stratified method (GRTS; Stevens and Olsen, 2004); R package: spsurvey (Kincaid et al., 2015)) and randomly sampled subsets with maximised spatial balance (Olsen et al., 2012). The subset size was chosen as the maximum number of sites that showed no spatial clustering (as measured by the $\chi 2$ statistic). This method reduced the used number of sites per country (Table 2.1). (II) Non-detects and duplicate entries were removed after assigning a Chemical Abstract Service (CAS) registry number to each chemical. (III) We limited the data to the years of 2008-2012 (only for the German states of Baden-Württemberg and Rhineland-Palatinate the years of 2006-2010 and for North Rhine-Westphalia the years of 2005-2009 were used), because these data had an increased number of sampling occasions compared to preceding years. These steps resulted in a total of 4,532 sites with 56,084 sampling occasions. On average, 12 sampling occasions were performed per site, ranging from 6 in the USA to 27 in France. Up to 779 different pesticides and their metabolites were included in the analysis, with the data set from Netherlands contributing most with 637 different pesticides and their metabolites (Table 2.1, Fig. 2.1). Differences in the analysed pesticides and their metabolites between the different countries were illustrated using multidimensional scaling based on the binary Jaccard distance.

Table 2.1: Overview of data sets analysed with information of detection rates and numbers of compounds and mixtures within the different countries.

	DE	FR	NL	US
Sites remaining after GRTS [%]	72	63	70	62
Sites after GRTS	1037	950	320	2225
Sampling occasions after GRTS	12,177	25,586	5,112	13,209
Median sampling occasions per site	8	26	8.5	3
Analysed compounds	297	292	637	324
Mean No. compounds analysed per sampling occasion	85.1	27.6	83.4	36.2
Detected compounds	205	115	267	127
No. most frequent compounds	132	25	69	14
mean size mixtures all compounds, ± SD	7.0 ± 4.8	3.0 ± 1.6	4.8 ± 3.0	3.2 ± 1.2
sites with compounds, all compounds [%]	85.1	78.1	90.3	23.5
sites with mixtures, all compounds [%]	49.4	12.7	65.3	16.2
sampling occasion with pesticide exposure, all compounds [%]	69.3	32.7	82.1	26.1
Detected core compounds	40	40	38	29
No. most frequent core compounds	33	14	19	9
mean size mixtures core compounds, ± SD	4.7 ± 2.5	2.5 ± 1.0	3.6 ± 1.8	2.5 ± 0 7
Max size mixture, core compounds	20	9	14	6
sites with compounds, core compounds [%]	80.3	73.1	85.3	21.4
sites with mixtures, core compounds [%]	36.2	7.6	36.1	15.0
sampling occasion with pesticide exposure, core compounds [%]	59.9	24.8	60.2	24.6

DE: Germany; FR: France, NL: Netherlands, US: United States of America. “No. most frequent (core) compounds”: number of compounds after establishing level of most frequent compounds (c.f. Fig. A.2). Compounds = pesticides + metabolites, GRTS = Generalized

Random Tessellation Stratified, SD = standard deviation. For the same table with differentiation between the German states see Table A.2.

2.3.2 IDENTIFYING MOST FREQUENTLY DETECTED PESTICIDES AND MIXTURES

We calculated the relative occurrence (*p*) of each pesticide and metabolite (compound) (*i*) for sampling occasions as well as at sites as:

$$p_i = \frac{\sum y_i}{n} \tag{2.1}$$

where *n* is the number of sampling occasions or sites and *y* is 1 if compound was found in a site or on a sampling occasion, otherwise 0. Additionally, we calculated the percentage of sites and sampling occasions were at least one compound was detected (percentage of sites and sampling occasions where $\Sigma p_i > 0$). We identified most frequent mixtures composed of different types of pesticides (herbicides, insecticides and fungicides).

Compounds that occurred at less than 5 % of sites were omitted from further analysis as they lead to an inflation of the number and occurrence frequency of mixtures. For example, consider the case of two compounds A and B occurring on 100 sampling occasions and the compounds X, Y, and Z each occurring on 4 sampling occasions. This could result in multiple ternary (ABX, ABY, ABZ) or quaternary (ABXY, ABXZ, ABYZ) mixtures with low relative occurrence frequency. Subsequently, for each mixture the absolute number of compounds (size), the number of the different pesticide types and the occurrence frequency at sites as well as sampling occasions was calculated.

For the German data set, the analysis was firstly conducted separately for the four German states and subsequently the results were aggregated weighted by the number of analysed sites or sampling occasions.

2.3.3 CALCULATION OF SIZE AND RELATIVE LAND COVER OF CATCHMENT AREAS IN GERMANY

For each site analysed in Germany, we quantified land cover types in its catchment by following a four step procedure: (i) Extraction of the stream network from a digital elevation model (DEM) (ASTER GDEM, NASA, and METI, 2009) that shows the highest concordance with a mapped stream network of the German state, using the open-source software algorithm ATRIC (Bhowmik et al., 2015), (ii) Snapping the sites to the nearest segment of the extracted stream network (see Bhowmik et al., 2015 for details), (iii) Automatically delineating the upstream catchment polygon for each fitted site from the DEM using ATRIC and (iv) overlaying the catchment polygons with the CORINE land cover datasets (EEA, 2007) and subsequently

calculating the percentage of six land cover types (arable land, permanent crop, forest, meadows, water bodies and other). The analysis was limited to Germany because only for Germany mapped stream networks were readily available. Besides, in the case of the Netherlands, geomorphology does not allow for derivation of stream networks from a DEM.

2.3.4 ASSOCIATIONS WITH MONITORING CHARACTERISTICS

We scrutinised whether characteristics of the monitoring programmes influence the detection of pesticides and its mixtures using the following response variables: size of mixtures and number of detected compounds. We correlated (Pearson's correlation) these response variables with the number of analysed pesticides and metabolites per sampling occasion and the size of catchment areas of sampling sites. For Germany, we also correlated the response variables with the areal proportion of agriculture, of arable land and of permanent crop land within the upstream catchment. This was done using a cubic regression spline with a Poisson distribution.

2.3.5 DIRECT COMPARISON OF MIXTURES FROM DIFFERENT COUNTRIES – CORE COMPOUNDS

Given that the compound spectrum varied between countries (Fig. 2.1), we analysed the data for 44 core compounds that were measured in all countries and German states. Most of these (29) were herbicides and metabolites with a herbicide as parent compound. Additionally, eleven insecticides and four fungicides were part of the core compounds (Table A.3). These core compounds enabled a direct comparison of mixtures from different countries.

We tested for differences in the size of mixtures between the countries as well as for differences in mixtures composition using analysis of variance (ANOVA) followed by a Tukey-HSD (Honestly Significant Difference) test for pairwise comparison.

Pre-processing of data, statistical analysis and visualisations were performed using R, version 3.1.1 (R Core Team, 2014).

2.4 RESULTS

2.4.1 (I) MOST FREQUENTLY DETECTED PESTICIDES AND METABOLITES

The spectrum of analysed pesticides and metabolites varied strongly between countries (Fig. 2.1 a, b). The monitoring data of France and Germany showed a high concordance in the total number of analysed compounds (Germany: 297, France: 292, Table 2.1) and identity of analysed compounds in comparison to the Netherlands and the USA (shown with different colours in Fig. 2.1). The different spectrum of analysed pesticides and metabolites resulted, in several compounds among the most frequent pesticides and metabolites that were country-specific, particularly for the Netherlands, such as Bitertanol, Flonicamid and Flutolanil

(Table 2.2). In addition, pesticide detections varied strongly between the countries across sampling occasions (26 % for USA to 82 % Netherlands) and sites (24 % for USA to 90 % for the Netherlands (Table 2.1).

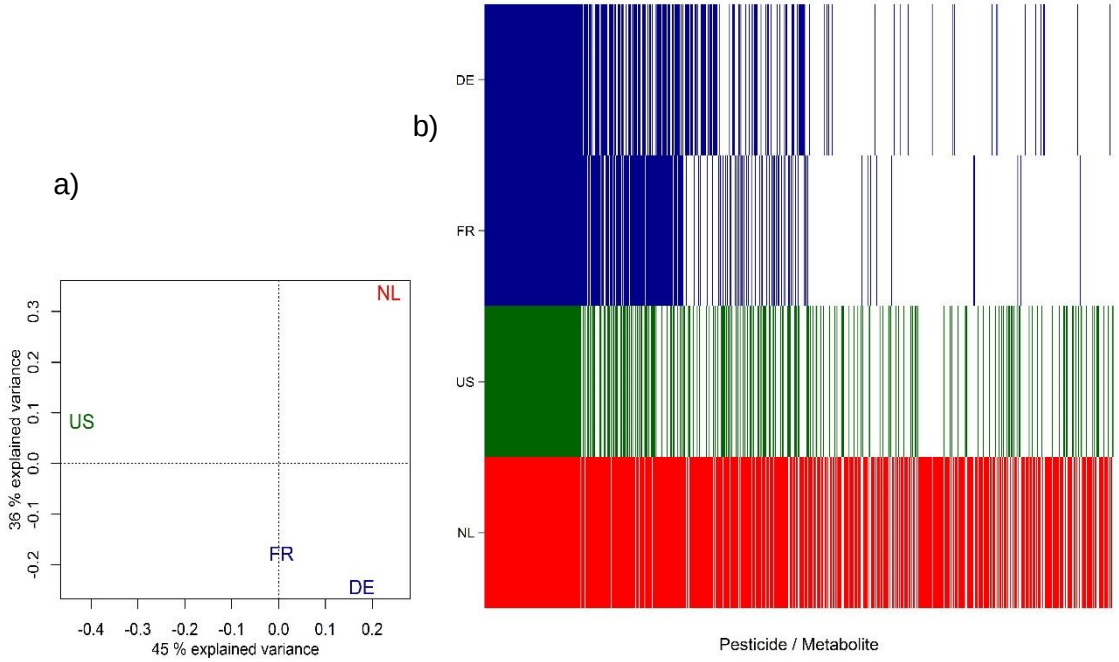

Fig. 2.1: Compound spectra of the different countries a) Multidimensional scaling of the analysed pesticides and their metabolites in the different countries. b) Comparison of the analysed pesticides and metabolites from the different countries. Each line represents one compound. France and Germany were coded with the same colours in both graphs to highlight concordance of the analysed compounds (see a). For number of analysed pesticides and metabolites in each country, see Table 2.1. DE: Germany; FR: France, NL: Netherlands, US: United States of America. See Fig. A.3 for differentiation between German states.

The most frequently detected compounds, occurring at least at 10 % of sites, were mainly herbicides and their metabolites belonging to the chemical classes of phenylurea (Diuron (DCMU), Isoproturon), chlorotriazine (Terbuthylazine, Atrazine) and organophosphorus herbicides (Glyphosate) (Table 2.2). In some countries, fungicides (Propiconazole, Germany; Boscalid, Germany; Carbendazim, the Netherlands) and insecticides (Lindane (γ-HCH), France; Fipronil, USA; Imidacloprid, the Netherlands) were among the most frequently detected pesticides. Although 34 % and 19 % of the analysed compounds were insecticides and fungicides, both pesticide types were less frequently detected in comparison to herbicides.

Table 2.2: List of the most frequently detected pesticides and metabolites with their relative occurrence at sites of the different countries. The compounds are ordered alphabetically. Each listed compound occurred in at least one country at a minimum of 10 % of the sites.

compound	CAS	pesticide type	DE	FR	US	NL
1,2,3,4,5,6-Hexachlorocyclohexane	58899	IN	8.7	11.2	0.7*	10
2,4-D	94757	HB	9.1*	4.9*	3.0*	18.4*
2,6-Dichlorobenzamide	2008584	HB	0.2	0.1	0	28.4
AMPA	1066519	M	12.2	13.2	2.3	37.8
Atrazine	1912249	HB	24.3	42.0	19.2*	11.3
Azoxystrobin	131860338	FU	18.4*	0.7*	0*	27.8*
Bentazon	25057890	HB	23.2*	7.7*	0.1*	37.5*
Bitertanol	55179312	FU	0	0	0	15.9
Boscalid	188425856	FU	38.6*	0*	0*	12.8*
Carbendazim	10605217	FU	16.3*	1.5	0*	55.3
Chloridazon	1698608	HB	13.7*	0.8*	0*	29.4*
Chlorpropham	101213	HB	0*	0.2*	0*	31.9*
Chlorpyrifos	2921882	IN	19.2	6.6*	1.7*	3.8*
Chlortoluron	15545489	HB	13.0*	4.0*	0	8.4
Clomazone	81777891	HB	19.8*	0.1*	0*	2.8*
Desethylatrazine	6190654	M	12.2	5.5	3.0	2.2
Desethylterbuthylazine	30125634	M	34.3	0.5	0	4.4
Dichlobenil	1194656	HB	0	0.4	0*	11.9
Diflufenican	83164334	HB	33.4*	0.3*	0	0.9*
Dimethachlor	50563365	HB	19.8*	0.1*	0	0
Dimethenamid	87674688	HB	20.9	7.5	1.2*	15.3
Dimethoate	60515	IN	7.6*	0.2*	0.6*	20*
Dimethomorph	110488705	FU	1.4*	1.1*	0*	18.4*
Diuron	330541	HB	45.1*	55.3	1.3*	38.1
Epoxiconazole	133855988	FU	10.2*	1.5*	0	6.9*
Ethofumesate	26225796	HB	23.6*	1.3*	0*	32.5*
Flonicamid	158062670	IN	0	0*	0*	11.9*
Flufenacet	142459583	HB	23.7*	0*	0*	1.3*
Fluroxypyr	69377817	HB	0*	0.1*	0*	14.7*
Flurtamone	96525234	HB	23.1*	0.1*	0	0.3
Flutolanil	66332965	FU	0*	0*	0*	19.1*
Glyphosate	1071836	HB	9.7*	12.1*	0*	30*

compound	CAS	pesticide type	DE	FR	US	NL
Hexachlorobenzene	118741	FU	15.5	4.0	0.4	2.5
Irgarol 1051	28159980	FU	26.4	0	0*	0
Isoproturon	34123596	HB	62.0*	53.6*	0	37.5*
Linuron	330552	HB	2.0	10*	0*	28.4*
MCPA	94746	HB	22.5*	43.2*	0.4*	44.4*
Mecoprop	93652	HB	24.9*	5.4*	0	38.4
Metalaxyl	57837191	FU	7.9	0.5	0.1*	21.3
Metamitron	41394052	HB	10.5*	0.7*	0	12.8*
Metazachlor	67129082	HB	45.5*	2.3*	0	19.1*
Metolachlor	51218452	HB	31.1*	7.9	11.6*	36.6
Metribuzin	21087649	HB	3.2*	0.1*	3.6*	11.6*
Napropamide	15299997	HB	18.3*	0.4*	0*	0*
p,p'-DDD	72548	IN	10.5	5.9	0.5	0.9
p,p'-DDT	50293	IN	20.9	7.5	0.9*	3.1
Pencycuron	66063056	FU	0.8*	0*	0*	14.4*
Pendimethalin	40487421	HB	12.7*	0.6*	2.6*	5.0*
Pirimicarb	23103982	IN	7.9*	0.1*	0*	24.1*
Pronamide	23950585	HB	14.3*	3.1*	0.5*	13.4*
Propamocarb	24579735	FU	0.6*	0*	0*	11.6*
Propiconazole	60207901	FU	17.4*	0.1*	0.2*	3.8*
Prosulfocarb	52888809	HB	2.2*	0.4*	0	15.9*
Quinmerac	90717036	HB	15.6*	0*	0	0.3*
Simazine	122349	HB	29.0	19.9	1.6*	15.3
Tebuconazole	107534963	FU	15.6	2.6*	0*	17.5*
Terbuthylazine	5915413	HB	55.1*	0.1	0	33.1*
Terbutryn	886500	HB	37.4	2.9	0	5.6
Terbuthylazine, 2-Hydroxy	66753079	M	10.9	0	0	0

DE: Germany; FR: France, NL: Netherlands, US: United States of America. IN: insecticide, HB: herbicide, FU: fungicide, M: metabolite. * indicates that the respective pesticide was approved during the time frame of the data used for this study (EC, 2015). See Table A.4 for differentiation between German states.

2.4.2 (II) MOST FREQUENTLY DETECTED MIXTURES

The 10 most frequently detected mixtures were mostly binary or ternary and composed of herbicides and consisted of compounds that represented the most frequent individual compounds in the countries. The number of compounds constituting the 10 most frequent mixtures ranged from 5 in France to 12 in Germany (Table 2.3).

Table 2.3: List of the most frequent mixtures from the different countries with the ratio of occurrence at sites and sampling occasions as well as the number of compounds (size). Order of compounds based on CAS numbers.

[%] occurrence site	[%] occurrence sampling occasions	No. compounds	Compounds
			GERMANY
7.6	0.1	2	Diuron (HB), Isoproturon (HB)
3.0	0.1	2	Atrazine (HB), Desethylatrazine (M)
2.4	0.2	2	Boscalid (FU), Isoproturon (HB)
2.0	0.2	2	Isoproturon (HB), Metazachlor (HB)
1.9	0.2	2	Boscalid (FU), Terbuthylazine (HB)
1.9	0.1	2	Isoproturon (HB), Terbuthylazine (HB)
1.5	0.1	2	Isoproturon (HB), Terbutryn (HB)
1.5	0.1	2	Irgarol 1051 (FU), Isoproturon (HB)
1.5	0.1	2	Simazine (HB), Terbuthylazine (HB)
1.4	0.1	2	Isoproturon (HB), Diflufenican (HB)
			FRANCE
18.0	1.5	2	Diuron (HB), Isoproturon (HB)
13.6	1.1	2	Diuron (HB), MCPA (HB)
10.1	0.6	2	Atrazine (HB), Diuron (HB)
9.2	0.7	2	Atrazine (HB), Isoproturon (HB)
8.5	0.5	2	Isoproturon (HB), MCPA (HB)
7.2	0.4	2	Atrazine (HB), MCPA (HB)
6.4	0.4	3	Diuron (HB), Isoproturon (HB), MCPA (HB)
6.0	0.3	3	Atrazine (HB), Diuron (HB), Isoproturon (HB)
5.2	0.3	3	Atrazine (HB), Diuron (HB), MCPA (HB)
4.1	0.2	2	Simazine (HB), Diuron (HB)

[%] occurrence site	[%] occurrence sampling occasions	No. compounds	Compounds
			NETHERLANDS
7.2	1.5	2	AMPA (M), Glyphosate (HB)
4.7	0.3	2	Carbendazim (FU), Imidacloprid (IN)
3.8	0.6	2	Diuron (HB), Isoproturon (HB)
3.4	0.4	2	Bentazon (HB), Isoproturon (HB)
3.4	0.2	2	Carbendazim (FU), Isoproturon (HB)
3.1	0.2	2	Carbendazim (FU), Diuron (HB)
3.1	0.3	2	Bentazon (HB), Mecoprop (HB)
3.1	0.3	2	Mecoprop (HB), MCPA (HB)
2.8	0.2	3	Bentazon (HB), Mecoprop (HB), MCPA (HB)
2.5	0.3	3	Carbendazim (FU), Imidacloprid (IN), Flonicamid (IN)
			USA
5.3	2.5	2	Atrazine (HB), Metolachlor (HB)
3.5	3.2	3	Atrazine (HB), Acetochlor (HB), Metolachlor (HB)
1.9	0.7	2	Atrazine (HB), Desethylatrazine (M)
1.3	0.3	4	Atrazine (HB), Acetochlor (HB), Metolachlor (HB), Desethylatrazine (M)
1.2	0.8	4	Alachlor (HB), Atrazine (HB), Acetochlor (HB), Metolachlor (HB)
1.0	0.3	2	Atrazine (HB), Acetochlor (HB)
1.0	0.3	4	Atrazine (HB), Metribuzin (HB), Acetochlor (HB), Metolachlor (HB)
0.9	0.5	5	Alachlor (HB), Atrazine (HB), Metribuzin (HB), Acetochlor (HB), Metolachlor (HB)
0.8	0.2	3	Atrazine (HB), Metolachlor (HB), Desethylatrazin (M)
0.7	0.4	4	AMPA (M), Atrazine (HB), Acetochlor (HB), Metolachlor (HB)

HB: herbicide, IN: insecticide, FU: fungicide, M: metabolite, No.: number. See Table A.5 for differentiation between German states.

2.4.3 (IIIA) ASSOCIATIONS WITH MONITORING CHARACTERISTICS

The number of detected compounds as well as mixture size (Table 2.1) correlated moderately positive with the total number of analysed compounds per sampling occasion (Fig. 2.2). Both correlated negligibly with catchment size for all countries, and only weakly with the fraction of arable land or of total agricultural area within the catchment areas of Germany (Table 2.4). However, the mean number of detected pesticides increased from 3 to 7 compounds when the fraction of total agricultural area within the catchment area increased from 20 % to 40 % (Fig. A.4).

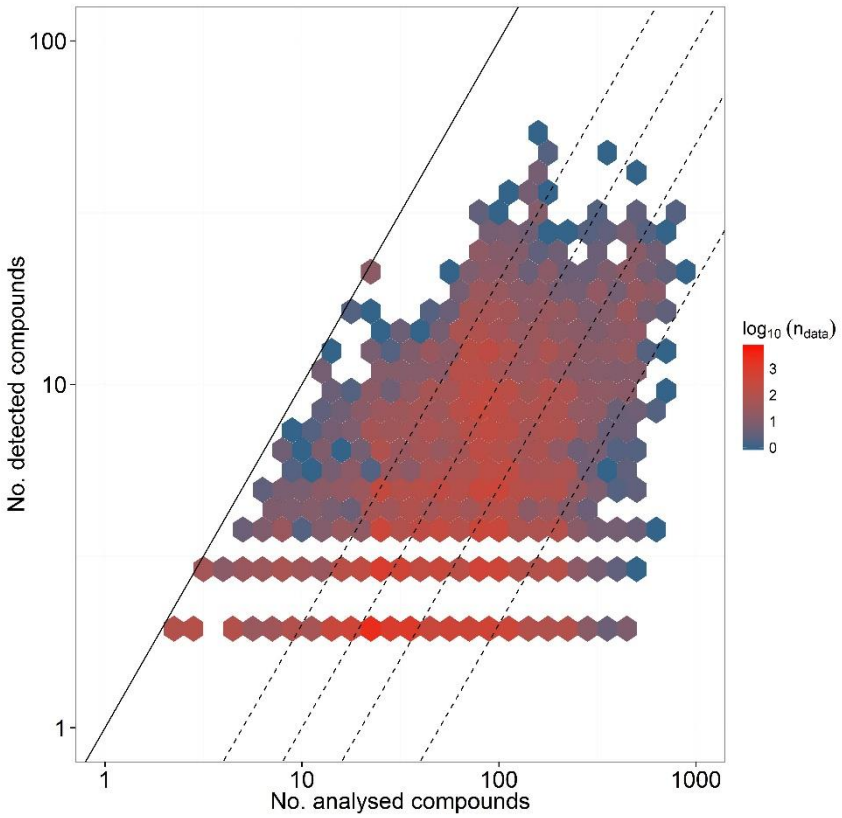

Fig. 2.2: Relationship between number of detected and of analysed compounds (on a log-scale). Solid line indicates a 1:1 ratio of detected: analysed compounds, dashed lines indicate 1:5, 1:10, 1:20 and 1:50 ratios. Colours indicate the number of individual sampling occasions with this respective relationship.

Table 2.4: Correlation coefficients and corresponding confidence intervals (CI) concerning associations with monitoring characteristics.

	r	95 % CI	n
no. analysed compounds ~ n detected compounds	0.57	0.56 - 0.57	56084
no. analysed compounds ~ size mixture	0.54	0.54 - 0.55	56084
catchment size ~ n detected compounds	0.06	0.05 – 0.07	56084
catchment size ~ size mixture	0.06	0.05 – 0.07	56084
% arable land in catchment area ~ n detected compounds	0.17	0.15 - 0.19	12177
% arable land in catchment area ~ size mixture	0.18	0.16 - 0.20	12177
% agricultural area in catchment area ~ n detected compounds	0.19	0.17 - 0.21	12177
% agricultural area in catchment area ~ size mixture	0.20	0.18 - 0.22	12177

no.: number, all correlations with arable land and agricultural area in catchment area only refer to data from Germany.

2.4.4 (IIIB) CORE COMPOUNDS – COMPOSITION AND SIZE OF DETECTED MIXTURES

The pesticide mixtures for the core compounds that were analysed in all countries consisted mainly of herbicides (Fig. 2.3), where Atrazine, Simazine and the metabolite AMPA with a herbicide as parent compound were dominating (see Table A.6 for occurrence of core compounds). For France, herbicide mixtures accounted for 94 % of mixtures, whereas for Germany, only 48 % of mixtures were solely comprised of herbicides, due to frequent mixtures with fungicides (e.g. Metalaxyl, Propiconazole) and insecticides (Chlorpyrifos).

For all countries, insecticides contributed negligibly to mixtures, although one quarter of the analysed core compounds were insecticides (Table A.3). Considering that only four of the 44 analysed core compounds were fungicides, they were comparatively overrepresented in the mixtures of Germany and Netherlands with 41 % and 18 % of all mixtures containing fungicides (Fig. 2.3).

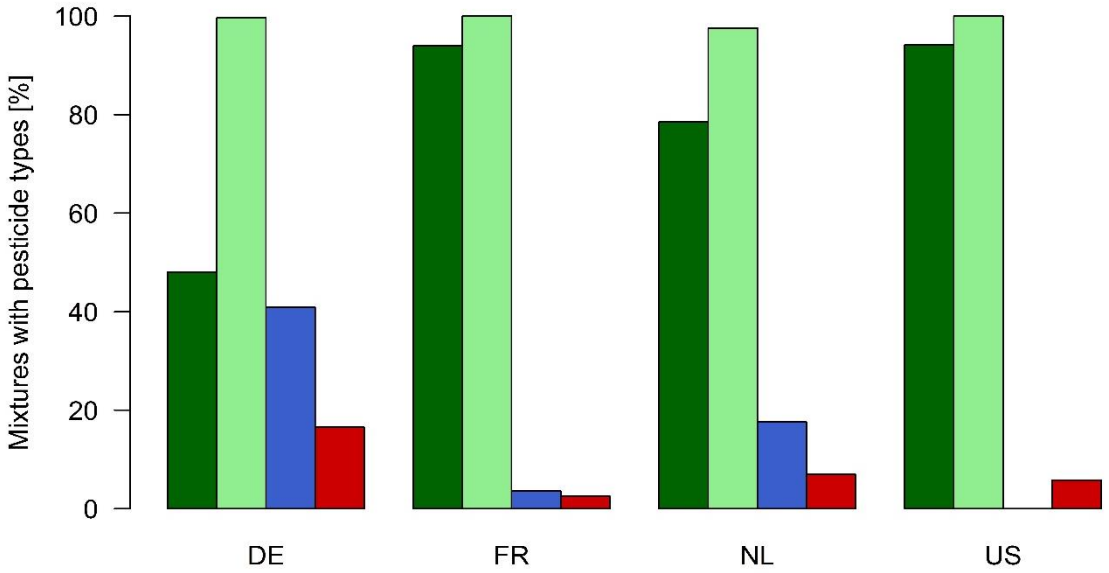

Fig. 2.3: Relative amount of mixtures from core compounds for the main pesticide types. DE: Germany; FR: France, NL: Netherlands, US: Unites States of America. Dark green: mixtures of only herbicides, light green: herbicides in mixture, blue: fungicide in mixtures, red: insecticides in the mixtures. Metabolites were assigned the pesticide type of their parent compound. See Fig. A.5 for differentiation between German states.

Generally, the relative occurrence of mixtures decreased with an increase of mixture size (Fig. 2.4). Binary and tertiary mixtures dominated in surface waters as detected in all countries. Only for the German data, larger mixtures occurred also frequently, which was mainly based on mixtures from the German state Baden-Württemberg (Fig. A.6). Baden-Württemberg also had significantly larger mixture sizes compared to the other countries and German states (all $p < 0.001$, all 95 % confidence intervals exclude 0).

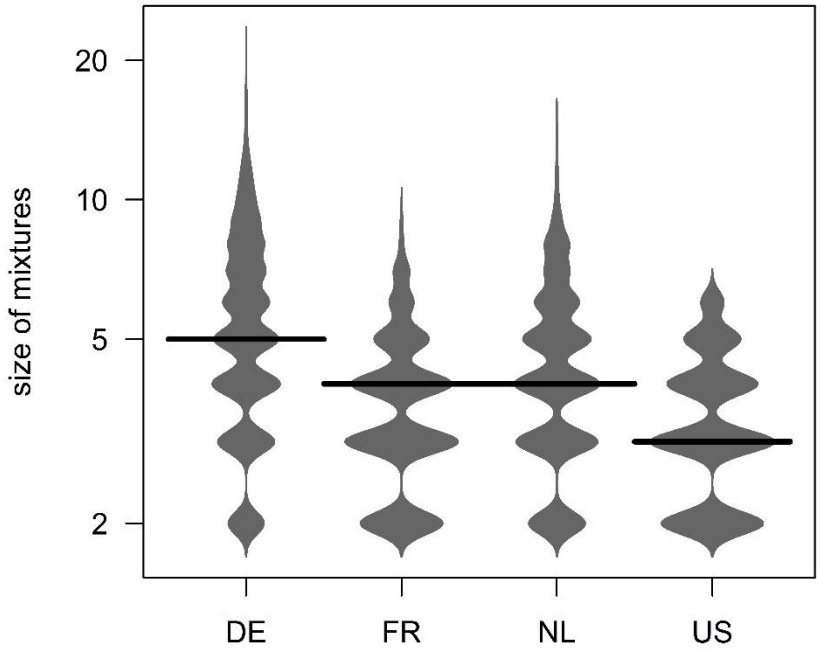

Fig. 2.4: Distribution of mixture size for the different countries for the core compounds. The black solid line gives the median. Y-axis on logarithmic scale. DE: Germany, FR: France, NL: Netherlands, US: United States of America. See Fig. A.6 for differentiation between German states.

2.5 DISCUSSION

2.5.1 (I) AND (II) MOST FREQUENTLY DETECTED PESTICIDES AND MIXTURES

Herbicides and metabolites with herbicides as parent compounds were the most frequently detected pesticide group in our study, of which Isoproturon, MCPA and Atrazine were the most frequent herbicides. This result is in accordance with several other studies that identified herbicides as the most frequently detected compound group (e.g. Belden et al., 2007; Gilliom et al., 2006; Moschet et al., 2014b; Stone et al., 2014). With approximately 83,000 tonnes, the combined herbicide use in France, Germany and the Netherlands was a factor of 12 higher than insecticide and 50 % higher than fungicide use (BVL, 2013; CBS, 2015; EC, 2015; UIPP, 2014). Based on these application quantities, herbicides enter streams usually in relatively high concentrations (e.g. Moschet et al., 2014a; Neumann et al., 2002), which together with their typical high water solubility and persistence simplifies detection in chemical analysis, especially in comparison to insecticides. Despite herbicides in the USA being applied 2.5 times more frequently than insecticides (FAOSTAT, 2015), presumably due to different climate conditions than in Europe, the ratio of herbicide to insecticide detections was similarly low as for the European countries. The low detection rate of insecticide is discussed below (same section). In our study, Glyphosate was not considered in the analysis for the USA, although it is frequently applied, due to a lack of data from the regular monitoring. Other monitoring

programs included Glyphosate and detected it frequently (Battaglin et al., 2014). The exclusion of the Glyphosate and its metabolites in the regular monitoring can be attributed to its difficult analysis, where the high polarity complicates detection using liquid chromatography, and high costs using alternative methods (Stone et al., 2014).

Fungicides were in our study detected in all countries except for the USA, in contrast to other studies which detected fungicides in the USA (e.g. Stone et al., 2014). This lack of detection in the USA may be explained by the fact that fungicides were rarely part of large scale monitoring programs used in our analysis (Reilly et al., 2012). Additionally, the usual application pattern of fungicides leads to relatively low but continuous concentrations of these compounds in streams (Reilly et al., 2012). The limits of quantification (LOQ) for the USA for fungicides in our study were in average 12-fold higher as those of other countries, which might contribute to the low detection frequency. The streams in the German state Baden-Württemberg showed a high percentage of mixtures with fungicides (93 %) in comparison to other countries and German regions (0 – 24 %) (Fig. A.5). This is mainly due to the most frequently detected fungicides Metalaxyl and Propiconazole, which occurred at 58 % and 90 % of the sites respectively (Table A.5). In Baden-Württemberg, the compounds were analysed in almost all sites (98 % for both) and all sampling occasions (94 % and 92 % for Metalaxyl and Propiconazole). In the other regions and countries, except for the German state Saxony where the monitoring was similar to that of Baden-Württemberg, they were analysed in less than 66 % and 36 % of sites and sampling occasions. In the other countries the rather high detection rate of Metalaxyl and Propiconazole can also be attributed to the comparatively low LOQ of 1 ng L^{-1} for both compounds that was only reached for Baden-Württemberg and was for example 15-fold higher in Saxony. The LOQ from these compounds in the other German states and countries ranges from 5-fold higher in Rhineland-Palatinate up to 80-folds higher in France (detailed information on the LOQs see Table A.7). Finally, differences in agricultural land use and consequently in pesticide use may partially explain differences in detection patterns. A study in Switzerland showed that by decreasing the LOQ in pesticide analysis, the number of detected compounds could be increased up to 67 % corresponding to 30 to 50 individual compounds in this study (Moschet et al., 2014b). This decrease of LOQs can be necessary to appropriately evaluate potential ecological risks from pesticides. For our dataset, the ratio of LOQ and LC_{50} of the most sensitive taxa differed strongly from 0.0003 (10^{th} percentile) to 4.1 (90th percentile) (Table A.8). Given that ecological thresholds for toxicants have been found as low as 0.001 for the ratio between measured concentration to LC_{50} of the most sensitive taxa, decreasing the LOQs is still required for many compounds for a comprehensive ecological risk assessment (Schäfer et al., 2012). Additionally, concentrations that may not pose an individual risk can contribute to ecological risks from mixtures, strengthening the conclusion that relatively low LOQs are required for a comprehensive assessment.

Insecticides were the least frequently detected compound group. The most frequently detected insecticides were DDT, Pirimicarb and Chlorpyrifos. The low detection frequencies of insecticides may be due to their general relatively low concentrations (Moschet et al., 2014b; Stehle et al., 2012), resulting, even for similar LOQs as herbicides, in lower detection frequencies. Moreover, insecticides often occur as episodic short time exposure, and can rarely be detected via grab sampling, which is the dominant sampling in routine monitoring (Stehle et al., 2012). Besides, most insecticides, especially pyrethroids and organophosphates, are lipophilic (Casida and Quistad, 2004) and bind to sediments and may not be detected in grab water samples that are aimed to sample compounds existing in the water column (Domagalski et al., 2010; Hill, 1989).

The most frequently detected mixtures from the different countries consisted of two or three compounds with mainly herbicides and metabolites with a herbicide as parent compound. This small size of frequently detected mixtures is partly also due to the limitation to compounds detected at more than 5 % of sites. Without this limitation the average size of the mixtures would be higher. The single compounds of the most frequent mixtures reflect the most frequent single compounds from all analysed surface waters. One study in Swiss streams detected mixtures of four pesticides when relying on routine monitoring methods, whereas approximately 40 compounds, dominated by herbicides, were detected using a broader pesticide spectrum at analysis (Moschet et al., 2014b). Frequently detected mixtures in corn and soybean growing areas showed comparable number of compounds to our study (two to four compounds and were exclusively composed of herbicides (Belden et al., 2007). Mixtures with Acetochlor, Metolachlor and Atrazine dominated the most frequently detected mixtures in this study from the USA as well as in our results from the US monitoring data. Mixtures with these compounds were absent in other countries, which can be explained by to the fact that the herbicide Acetochlor is not authorised in the EU. Compounds such as Diuron, Atrazine, Simazine and Isoproturon that were often contained in frequently detected mixtures were also detected in a different climate zone, for example Mediterranean streams (Proia et al., 2013; Ricart et al., 2010).

2.5.2 (IIIA) ASSOCIATIONS OF DETECTED COMPOUNDS AND MIXTURES WITH MONITORING CHARACTERISTICS

Our results show that the number of detected pesticides and size of mixtures were correlated to the number of analysed compounds. On average, to detect one pesticide, between 5 and 20 pesticides had to be analysed (Fig. 2.2). Other studies also confirmed that the number of analysed compounds, among other factors, influences the number of detections (Malaj et al., 2014; Moschet et al., 2014b). Due to analysis of a high number of randomly detected compounds might not be feasible during routine monitoring, a selection of compounds motivated by current use of pesticides, sales or crop-related use recommendations should be

included to analysis (Vijver et al., 2008). If the routine monitoring had followed this approach, the number of detected compounds would most likely be higher (Moschet et al., 2014b). In one study, for example, all of the analysed pesticides were detected, which indicates that an increase of analysed pesticides could have increased the number of detected pesticides (Proia et al., 2013).

The number of detected compounds and size of mixtures were not associated with the size of the upstream catchment (r = 0.06). We expected that a larger catchment size would result in a higher number of detected pesticides due to (i) higher amount of pesticide use in a larger catchment (Blanchoud et al., 2004), and (ii) a typically larger variety of crops in larger catchments, associated with a higher diversity of applied pesticides. The lack of such a relationship with catchment size may be a result of dilution, i.e. that water body size also increases with catchment size and dilutes pesticide concentrations. Additionally, increasing catchment size is related to longer stream distances and consequently transport times of compounds, and increasing transport time may lead to different degradation and transformation processes as well as partitioning into the sediment phase (Guo et al., 2000), which in turn decreases concentrations, and consequently detection frequencies. On the other hand, catchment size may be less relevant than the distance of the water body to the field margin, which may be unrelated to catchment size. For example, one of the main determinants of pesticide input into streams is the distance of the crop from the water, e.g. width of buffer strips (Dabrowski et al., 2002; de Snoo and de Wit, 1998). However, this reduction of pesticide input in streams of buffer strips is reduced by erosion rills (Bereswill et al., 2012). Another possible factor that was omitted from analysis due to a lack of data is the flow velocity regime in the different streams and stream systems. This might be a factor determining, in addition to the duration a compound occurs in a stream and the related dilution factor and degradation, the amount and grain size of sediments, which might influence adsorption from compounds and subsequently the detection rate of pesticides in grab samples.

In contrast to the size of the catchment upstream of the sampling site, the fraction of agricultural area was weakly correlated with the number of detected pesticides and size of mixtures in Germany (r = 0.17). Nevertheless, the number of detected pesticides increased from 3 to 7 when the agricultural area in the catchment area exceeded 20 % based on the larger area with pesticide use. Other studies in different countries found a clear footprint of agriculture in terms of effects in stream ecosystems for a higher ratio of agriculture within the catchment of 40 % in Germany and France (Feld, 2012) and the USA (Waite, 2014).

Besides, several other monitoring characteristics may influence the detection rates of routine monitoring relying on grab sampling. In general, pesticides with a high octanol-water partition coefficient (log K_{ow}) (such as the insecticides Chlorpyrifos or Cyhalothrin) are detected less

frequently in grab sampling due to their low mobility in the water phase and a high adsorption rate to sediments (Domagalski et al., 2010; Hill, 1989). Also compounds with a low water solubility such as Atrazine and DDT, whose authorisation has expired more than a decade before the earliest sampling data from Europe included in the analysis, were still frequently detected, which can be explained by their persistence and remobilisation during floods (Altenburger et al., 2015; Gilliom et al., 2006). To reliably detect pesticides with a high log K_{ow}, other sampling methods such as sediment or passive sampling, with a receiving phase tailored to lipophilic compounds (Moschet et al., 2014a), can be more suitable (Moschet et al., 2014a, 2014b; Schäfer et al., 2011). But also automated time-weighted or flow-proportional sampling could improve detection of pesticides, due to its sampling also during peak exposures during or after rainfall events (e.g. Bundschuh et al., 2014; Müller et al., 2003; Petersen et al., 2012; Rabiet et al., 2010).

Overall, we suggest that grab sampling largely underestimates the number and concentrations of pesticides occurring in streams (Moschet et al., 2014b; Petersen et al., 2012), potentially also leading to a smaller size of mixtures, particularly for insecticides.

2.5.3 (IIIB) DIFFERENCES IN PESTICIDE DETECTIONS BETWEEN COUNTRIES

The size of mixtures in countries differed between Germany and the Netherlands on the one hand (mean size of mixtures of 7.0 and 4.8, respectively) and USA and France on the other hand (mean mixture size of 3.2 and 3.0, respectively). These groups also differed in the number of analysed compounds per sampling occasion. Whereas in Germany and the Netherlands over 80 compounds were analysed, in the USA and France only 30 compounds were analysed (Table 2.1). This stresses again, as already shown in (iiia) and other studies (Malaj et al., 2014; Moschet et al., 2014b), that a high number of analysed compounds is crucial for a representative picture of the pesticide load of streams. Even when restricting the analysis to the core group of pesticides measured in all countries, these differences prevailed, though to a lower degree. France and the USA had a mean size of mixtures of 2.5 core compounds, whereas average mixtures in Germany and the Netherlands contained 4.7 and 3.6 compounds. These differences in the size of mixtures of core compounds may be caused by differences in the LOQ between the different countries. For 52 % of all compounds, the LOQs were lowest in Germany, potentially increasing the detection frequency. The USA had the lowest LOQ for only 5 % of compounds and, presumably partly related to this, the lowest detection frequencies.

The low number of core compounds detected in the USA and France compared to Germany and the Netherlands could be caused by: (i) soil properties, (ii) the slope and (iii) the distance of agricultural areas, but also by (iv) crop type. For instance, in the USA and France legumes are grown on relatively large area (36 % and 12 %; FAOSTAT, 2014; Table A.1) in comparison

to Germany and the Netherlands (0.5 % and 6 %; FAOSTAT, 2014) and legumes were shown to reduce runoff during rainfall events and the related pesticide input in streams by up to 95 % for full gown plants (Garcia-Estringana et al., 2013). Finally, agricultural areas in the USA are often dominated by large fields and crop monocultures (average farms of 95 ha) and compared to the other countries (average farms: France 54 ha, Germany 56 ha, the Netherlands 26 ha) a lower farm density (Eurostat, 2015; MacDonald, 2013). Based on the assumption of a lower farm density and of a homogeneous selection of pesticides within a farm, the number of different pesticides in streams could be lower due to the lower number of pesticides applied.

This study provides priority pesticides and pesticide mixtures from streams of Germany, France, the Netherlands and the USA. Using these priority mixtures in ecotoxicological risk assessment could help to improve the estimation of mixture effects in aquatic ecosystems. Additionally, this study suggests that through improved routine pesticide monitoring, by increasing the number of analysed pesticides, improving analytical performance in terms of lowering LOQs and the use of alternative sampling methods to grab sampling, monitoring would provide a more realistic picture of the exposure situation and the number of detected pesticides would likely increase.

2.6 CONCLUSIONS

Pesticides in streams typically occur in mixtures of two to five compounds, in which herbicides are clearly dominating. The size of detected mixtures is influenced by the number of analysed compounds, the LOQs, but also the proportion of agriculture in the upstream catchment and the sampling method. We identify frequently detected pesticides which may inform the ecological risk assessment for stream ecosystems. Nevertheless, a comprehensive assessment of exposure to pesticide mixtures, would require a decrease of the LOQ for many compounds and widening the spectrum of compounds considered in monitoring programs.

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3 SAMPLING RATES FOR PASSIVE SAMPLERS EXPOSED TO A FIELD-RELEVANT PEAK OF 42 ORGANIC PESTICIDES

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3.1 ABSTRACT

Pesticide concentrations in agricultural streams are often characterised by a low level of baseline exposure and episodic peak concentrations associated with heavy rainfall events. Traditional sampling methods such as grab sampling, which are still largely used in governmental monitoring, typically miss peak concentrations. Passive sampling represents a cost-efficient alternative but requires the additional determination of sampling rates to calculate time-weighted average (TWA) water concentrations from the accumulated pesticide mass in the sampler. To date, sampling rates have largely been determined in experiments with constant exposure, which does not necessarily reflect field situations. Using Empore styrene-divinylbenzene (SDB) passive sampler disks mounted in metal holders, we determined sampling rates for 42 organic pesticides, of which 27 sampling rates were lacking before. The SDB disks were in an artificial channel system exposed to a field-relevant pesticide peak. We used an open-source algorithm to estimate coefficients of equations for the accumulated pesticide mass in disks and to determine exposure time-dependent sampling rates. These sampling rates ranged from 0.02 to 0.98 L d 1 and corresponded to those from previous studies determined with constant exposure. The prediction of sampling rates using compound properties was unreliable. Hence, experiments are required to determine reliable sampling rates. We discuss the use of passive sampling to estimate peak concentrations. Overall, our study provides sampling rates and computer code to determine these under peak exposure designs and suggests that passive sampling is suitable to estimate peak pesticide concentrations in field studies.

3.2 INTRODUCTION

Pesticides enter streams via various pathways. In agricultural areas, surface run-off associated with heavy rainfall events (> 8 mm precipitation per day) is the dominant entry path in terms of concentrations (Bereswill et al., 2012; Weibel et al., 1964). Runoff leads to in-stream pulse pesticide concentrations that can exceed those during base flow conditions by a factor of up to 100 (Leu et al., 2004; Rasmussen et al., 2015; Reilly et al., 2012). Such concentration pulses can adversely affect stream organisms, thereby contributing to the loss of freshwater biodiversity (Beketov et al., 2013) and ecosystem functions (Schäfer et al., 2012), and may propagate to adjacent ecosystems (Schulz et al., 2015).

Collecting information on pulse pesticide concentrations associated with heavy rainfall events requires targeted sampling methods (Szöcs et al., 2017). Grab water sampling at fixed dates, as conducted in governmental monitoring programs, typically misses pulse concentrations and is mainly suitable to determine baseline concentrations of pesticides. A grab sampling program that aims at capturing pulse concentrations associated with heavy rainfall events would be subject to high uncertainty regarding the timing of rainfall events and the timing of pulse

concentrations. Consequently, a related sampling scheme would require high flexibility regarding working hours. Event-driven samplers represent an alternative. However, automatic event-driven samplers imply high procurement and maintenance costs. Simplified event-driven samplers that rely on bottles, which are passively filled during rainfall events (Liess et al., 1996), imply lower procurement and maintenance costs. However, these samplers demand higher flexibility, given that they require immediate sample retrieval after an event to prevent degradation of the sampled compounds. In addition, both of these systems sample, though to different degrees, suspended particulate matter (SPM) (Liess et al., 1996). Compounds can adsorb or desorb from SPM thereby modifying the concentration of analytes in the sample, which incurs some imprecision concerning the determination of the dissolved water concentration.

Passive sampling represents a cost-efficient technique, which can achieve low limits of quantification (LOQ) for some compounds with less labour and time compared to the methods discussed above that rely on the post-sampling extraction of compounds from water samples (Bundschuh et al., 2014; Taylor et al., 2020). Moreover, passive samplers only sample the compounds in the dissolved phase and degradation of compounds in the sampler has not been reported, though fouling of the sorbent phase might have an effect (Allan and Jenssen, 2019; Schäfer et al., 2008). Therefore, passive sampling methods have gained popularity for sampling toxicants such as pesticides in streams and have been recommended for use in governmental routine monitoring (Brack et al., 2017; Moschet et al., 2014; Szöcs et al., 2017).

To estimate water concentrations from the mass of a chemical in the receiving phase of a passive sampler requires knowledge of compound-dependent so-called sampling rates, which provide data on the water volume sampled per day. To date, sampling rates of passive samplers have usually been determined in experiments using fairly constant water concentrations (e.g. Charlestra et al., 2012; Fernández et al., 2014; Morin et al., 2013, Townsend et al., 2018; Vermeirssen et al., 2013). Studies estimating sampling rates (hereafter: calibration studies) based on concentration pulses in laboratory experiments are lacking and require different approaches to modelling of the data compared to classical calibration studies with constant exposure. Notwithstanding, sampling rates have been determined in-situ under field conditions (e.g. Komarova et al., 2009; Moschet et al., 2015; Stephens et al., 2009), though deployment times were typically longer and the exact exposure profile that was sampled integratively by the sampler remained uncertain.

We conducted a laboratory study with a field-relevant pulse concentration design and established a computer code for data modelling to determine sampling rates of 42 organic pesticides on Empore styrene-divinylbenzene (SDB) passive sampler disks, of which 27 sampling rates were lacking previously. To mimic field conditions, the exposure scenario

during this calibration study consisted of a baseline exposure and a pulse with a 10 fold higher peak (maximum) concentration over two days following the exposure patterns found in previous studies (Leu et al., 2004; Rasmussen et al., 2015; Reilly et al., 2012). The SDB disks mounted in metal holders were used without a diffusion-limiting protective membrane to achieve higher sampling rates, which are more appropriate to capture short term peak concentrations (Vrana et al., 2010). We estimated sampling rates of two combined sets of parallel deployed SDB disks using an algorithm optimised for dynamic fitting. One set of SDB disks was deployed for the whole experimental duration (deployment time of 7 d) and the second set two days into the 7 d period which equals one day before the start of the pulse (deployment time of 5 d). These deployment times correspond to field-realistic deployment times as conducted in previous studies (e.g. Fernández et al., 2014; Novic et al., 2017; Stephens et al., 2009). We examined the relationships between exposure time-dependent sampling rates and different chemical properties of the investigated pesticides such as the water solubility, octanol-water partition coefficient as well as organic carbon soil absorption coefficient. Finally, we compared our time-dependent sampling rates to those from previous studies and give recommendations for field application of passive samplers when the aim is to assess the risk to stream ecosystems.

3.3 THEORY OF MODELLING OF THE SAMPLING PROCESS

We used a first-order differential equation to describe instantaneous uptake of compound mass (m_{sorb}) to the SDB disk sorbent [ng]. In doing so, we estimate the time-independent instantaneous sampling rate (R_{s_t0}), that has classically been used in several publications (equation 3.1, Table B.1) (Booij et al., 2007; Mutzner et al., 2019):

$$\frac{\mathrm{d}m_{sorb}}{\mathrm{d}t} = R_{S\_t0} \cdot \left(c_w(t) - \frac{m_{sorb}}{m_{samp} \cdot K} \right) \tag{3.1}$$

where m_{samp} refers to the mass of the passive sampler sorbent (332 x 10^{-6} kg), K the sorbent-water distribution coefficient [L kg^{-1}] and $c_w(t)$ the water concentration of the respective compounds [ng L^{-1}] at time point t [d]. A related wide spread approach to determine sampling rates is based on an assumed linear relationship between m_{sorb} and the time-weighted average (TWA) water concentration c_{TWA}, where the sampling rate represents the slope in a regression equation (e.g. Gunold et al., 2008). These approaches only (R_{s_t0}) or mainly (R_s linear relationship) consider the mass sorbed to the sorbent during the deployment period of the passive sampler, and therefore neglect or underestimate release kinetics. During the field application of passive samplers over several days, however, the uptake of single compounds may approach or reach the equilibrium of uptake and release and hence depart from the linear uptake regime.

Based on this, we additionally used a first-order kinetic model to calculate the mass transfer processes of uptake and release of compounds to and from the sorbent of the SDB disks (equation 3.2). We fitted differential equation 3.2 to estimate the uptake (k_{ws}; L d^{-1}) and release rate constants (k_{sw}; d^{-1}):

$$\frac{\mathrm{d}m_{sorb}}{\mathrm{d}t} = k_{ws} \cdot c_w(t) - k_{sw} \cdot m_{sorb}(t) \tag{3.2}$$

Using the fitted rate constants k_{ws} and k_{sw} of equation 3.2, we calculated an exposure time-dependent sampling rate (R_{s_5d}) for a field-relevant exposure period of 5 d (t_{exp}):

$$R_{s\_5d} = \frac{\left(1 - exp^{-k_{sw} \cdot t_{exp}}\right) \cdot k_{ws}}{k_{sw} \cdot t_{exp}} \tag{3.3}$$

Equation 3.3 together with the fitted rate constants of equation 3.2 can also be used to calculate sampling rates for very short exposure periods, approximating the instantaneous sampling rate R_{s_t0} when setting t_{exp} to e.g. 0.1 d, where data quality will determine the precision of the approximation. If study designs and exposure periods of the passive samplers differ, other time-dependent sampling rates can be calculated using the rate constants estimated in this study (Table 3.1).

We used a duration of 5 d to calculate the time-dependent sampling rate R_{s_5d} as this approximates the duration of a field exposure of passive samplers deployed in streams aiming to sample peak concentrations induced by heavy rainfall events (Fernández et al., 2014; Novic et al., 2017; Stephens et al., 2009). During passive sampler deployment of several days, the net uptake may decrease over time, since the compound masses that have been sorbed in the sampler have the potential to desorb. This especially applies to compounds approaching or reaching the equilibrium with the water concentration during the deployment time. Some compounds can approach or reach equilibrium already after deployment times of 24 h (Mutzner et al., 2019). In the setting of our study (deployment periods of 5 or 7 d), this was the case for 17 compounds such as 2,4-D and Nicosulfuron (Tables B.1, B.2, Fig. B.1). In such cases, if c_{TWA} is estimated from passive samplers deployed in field studies for several days using R_{s_t0} or the sampling rates based on a linear relationship, the c_{TWA} would be underestimated. Therefore, all further analysis is based on the field-relevant time-dependent sampling rates R_{s_5d}, which were calculated in the present study.

3.4 MATERIAL & METHODS

3.4.1 EXPERIMENTAL DESIGN

The calibration study was conducted in an artificial channel system with a total volume of 700 L (Fig. B.2) that was run with water of an adjacent stream (Chriesbach, Switzerland; Latitude 47.41 N, Longitude 8.61 E). To reduce water hardness and avoid calcareous scaling of the passive samplers, a cation exchanger (minionic Aquarienfilterbau) was used, reducing the hardness by about 40 %. The mean flow velocity during the experiment was 0.14 ± 0.06 m s^{-1} with a mean temperature of 14.9 ± 0.2 °C and a pH of 7.99 ± 0.02.

The experiment was conducted over 7 d as a field-relevant peak exposure design. At the start of the experiment (7 d data) and after two days (5 d data) 47 mm SDB disks (type: reverse-phase sulfonate; 3M Company, USA) with an exposed sorbent area of 12.57 cm^2 were placed in the channels at two time points (Table B.3, details see Supplementary data). A solution containing all analysed compounds (see Table 3.1; chemical solution prepared with PESTANAL analytical standards, Merck, Germany) was spiked continuously to the channels using an HPLC-pump, aiming to reach a nominal baseline concentration of 50 ng L^{-1}. After 3 d the field-relevant 10-fold concentration (i.e. 500 ng L^{-1}; e.g. Nowell et al., 2018; Petersen et al., 2012; Zubrod et al., 2019) was spiked by adding 10-fold the daily dose as a single dose to the channel system. This dosing served to mimic a run-off scenario associated with a heavy rainfall event. A 10-fold maximum, i.e. peak concentration was selected because previous studies detected a 10- to 100-fold increase of pesticide concentrations during pesticide peak concentration events, typically initiated by runoff following heavy rainfall, compared to baseline scenarios (Leu et al., 2004; Rasmussen et al., 2015; Reilly et al., 2012). As water was continuously added to the channel system, the compound pulse was washed out over approximately the next three days and concentrations returned to baseline conditions (Fig. 3.1). Solvent content was kept below 0.015 % during the pulse concentration (factor 10 lower during baseline exposure) to minimise biofouling of passive samplers, which was observed in previous experiments (personal observations) and its potential influence on sampling rates. Duplicate SDB disks were retrieved every one or two days (Table B.3) and processed as described below. Water samples (volume 0.6 L) were syphoned out of the channel system into aluminium bottles more frequently, especially close to the spiked pulse (Fig. 3.1, details Table B.4), and stored at -20 °C until further processing.

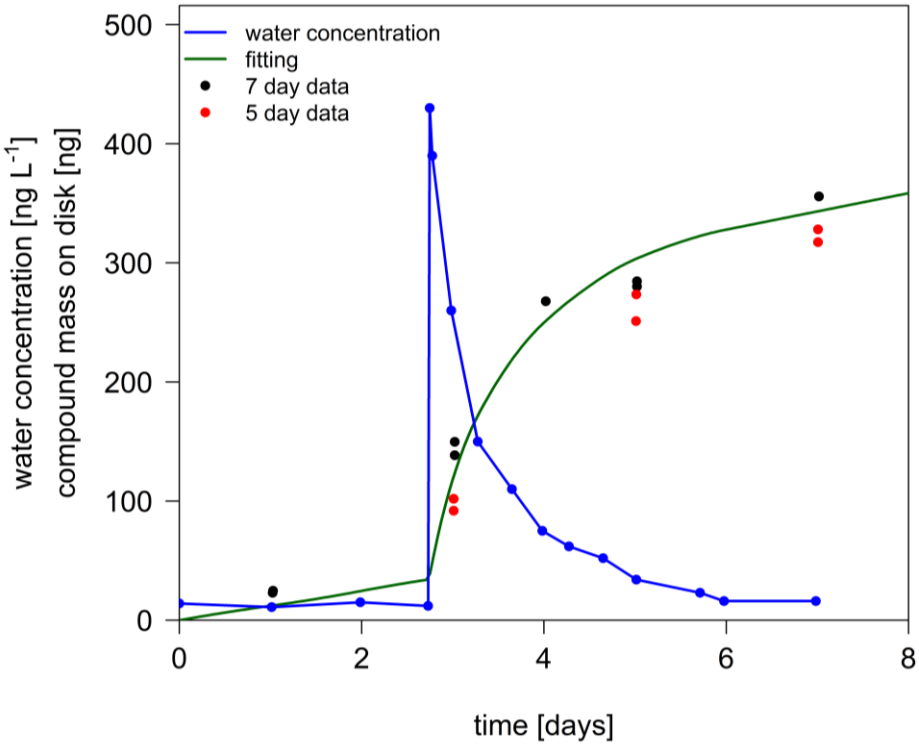

Fig. 3.1: Diazinon concentration in the channel and mass in the passive sampler disks. Figures of fitted models of all analysed compounds are in Fig. B.3.

3.4.2 PROCESSING OF SDB DISKS

SDB disks were conditioned by shaking them in methanol (LC grade) and ultrapure water (30 min each) in an overhead shaker and subsequently stored in ultrapure water at 4 °C. On the day of deployment in the channels, they were fitted into stainless steel holders with a single 40 mm opening (Vermeirssen et al., 2012). To check for possible handling contamination, one additional disk was prepared per day of deployment and handled accordingly. Chemical analysis showed these blanks were free from compound residuals.

After retrieving the disks from the channels, they were put in 6 mL of acetone (LC grade) and stored at -20 °C until further handling. The disks were extracted as follows: (i) disks submerged in acetone were shaken for 30 min, (ii) the liquid phase was transferred and evaporated to 1 mL under a gentle nitrogen flow, (iii) 6 mL of methanol (LC grade) was added to the disks and they were shaken for 30 min, (iv) the methanol extract was added to the acetone fraction, (v) half of the extract (split by solvent weight) was further used by adding isotope labelled standards (Table B.5) and filtered using 13 mm 0.45 µm polytetrafluoroethylene (PTFE) syringe filters (BGB Analytik), whereas the other half was stored as a back-up sample, (vi) the samples were evaporated to 50 µL and 450 µL ultrapure water was added, (vii) the extract was centrifuged at 4000 rpm for 30 min and the supernatant was used for chemical analysis. Selected standards of the calibration row were treated identically and used to correct for potential evaporation and filtration losses.

3.4.3 PROCESSING OF WATER SAMPLES FOR LARGE-VOLUME INJECTION

Water concentrations of a subset of the compounds were analysed using large-volume injection. Frozen water samples were defrosted and subsequently sonicated for 10 min. We added methanol (LC grade) to the aqueous sample resulting in a solvent content of 10 %. Isotope labelled standards (Table B.5) were added to the samples. Subsequently, the samples were filtered using 13 mm 0.45 µm PTFE syringe filters. Selected standards of the calibration row were treated identically and used to correct for potential evaporation and filtration losses. Five mL of each sample were enriched online using a Hypersil Gold aQ column 12 µm 20 x 2.1 mm (Thermo Fisher Scientific Corporation; gradient see Table B.6).

3.4.4 PROCESSING OF WATER SAMPLES USING SOLID PHASE EXTRACTION

To quantify compounds that did not achieve sufficient LOQs using the large-volume injection system (Table B.7), water samples were additionally pre-concentrated via solid phase extraction (SPE) with Oasis HLB 6 cc 500 mg extraction cartridges (Waters). Conditioning of SPE cartridges was conducted using 5 mL of methanol (HP grade) and equilibration with 10 mL ultrapure water. An aliquot of 0.5 L water spiked with isotope labelled standards (Table B.5) was loaded into an SPE cartridge at a flow rate of approx. 7 mL min^{-1}. Subsequently, cartridges were dried under nitrogen flow for 2 h. Elution was conducted with 6 mL methanol:ethyl acetate (1:1, v/v, methanol LC grade, ethyl acetate HP grade) followed by 2 mL methanol (LC grade). Eluates were evaporated to 50 µL under a gentle nitrogen flow at room temperature and 450 µL ultrapure water was added, to reach a solvent content of 10 %. Extracts were centrifuged at 4000 rpm for 30 min and the supernatants were used for chemical analysis. Selected standards of the calibration row were treated identically and used to correct for potential evaporation and filtration losses

3.4.5 CHEMICAL ANALYSIS

Compounds extracted from the SDB disks as well as the differently processed water samples were quantified using an Exactive (LC-HRMS) Orbitrap system (Thermo Fisher Scientific Corporation, gradient see Table B.8, settings see Table B.9) producing high-resolution mass spectrometry (HRMS) data. A full scan with a resolution of 140,000 in the range of 100 to 1000 m z^{-1} was conducted simultaneously for positive and negative ionisation. Chromatographic separation was achieved with an Atlantis T3 5 µm 3.0x150 mm column (Waters) using methanol and ultrapure water acidified with 0.1 % formic acid as mobile phases. The calibration row of the analysed compounds (PESTANAL analytical standards, Merck, Germany) was linear from the respective LOQ (Table S7) to 2000 µg L-1 for the analysis of SDB disks and 750 ng L-1 for water samples (single higher values were extrapolated). Data evaluation using isotope labelled standards was done with TraceFinder 3.3 (Thermo Fisher Scientific Corporation). Quality assurance as well as quality control and performance during

the TraceFinder quantification and the subsequent calculation of the mass of each compound absorbed to the SDB disks (m_{sorb}, ng) and water concentrations (c_w, ng L^{-1}) were done according to Moschet et al. (2015).

3.4.6 DATA ANALYSIS

Fitting of equations 3.1 and 3.2 was done using an algorithm of the R package FME (Soetaert and Petzoldt, 2016) that was suitable for the simultaneous fitting of all single data points of the 5 d and 7 d dataset (in total nine observations, duplicated, excluding deployment controls). Information on the separate fittings of the 5 d (four observations, duplicated, excluding deployment controls) and 7 d dataset (five observations, duplicated, excluding deployment controls). Information on the separate fittings of the 5 d and 7 d dataset see Text B.1.

To identify compound properties suitable to predict sampling rates of not yet calibrated compounds, we conducted regression analysis using the Least Absolute Shrinkage and Selection Operator (LASSO) with the R package glmnet (Friedman et al., 2010). The optimal model was selected based on a fixed alpha (α = 1) and a best-fit lambda value obtained by cross-validation. We used two different models to investigate relationships of the time-dependent sampling rates (R_{s_5d}) to the compound properties. First, we selected compound properties as predictors, which were available for all 42 analysed pesticides: logarithmic octanol-water partitioning coefficient (log K_{ow}), molecular mass, water solubility at 20 °C, and the octanol-water distribution coefficient at pH = 8 reflecting the partitioning of neutral species (log D_{ow}) under our experimental conditions. We calculated the log D_{ow} according to Carmichael (2014), using the dissociation constant (pK_a) and log K_{ow} values (see Text B.2), whereas for non-charged compounds, we used the log K_{ow}. The second model included the non-linear organic carbon soil adsorption coefficient log K_{foc} as well as the dissociation constant *pKa* as additional predictors for 16 pesticides (Table 3.1, compound properties retrieved from Lewis et al. (2016). We ran two related analyses to ensure that the results were not biased by the type of sampling rate and by compounds departing from the linear uptake regime. First, we repeated the analysis and replaces R_{s_5d} by the instantaneous sampling rate R_{s_t0}. Second, we repeated the analysis exclusively with the 25 compounds clearly remained in the linear uptake regime (i.e. k_{ws} / k_{sw} > 15; see Table B.2).

Furthermore, we investigated the match of the time-dependent sampling rates from this study to those of several previous studies, mostly determined under different calibration conditions (e.g. constant water concentrations) or using other modelling approaches. The match over available sampling rates was evaluated based on the difference of the regression from the identity line (1:1-line) using the root mean-square error from the R package cvq2 (Thalheim, 2013).

Calculating water concentrations c_{calc} from the mass of the respective compounds sorbed to the passive sampler m_{sorb} was done using equation 3.4.

$$c_{calc} = \frac{m_{sorb}}{R_S \cdot t_{acc}} \tag{3.4}$$

where t_{acc} is the time frame of accumulation of the compound in the receiving phase and R_S is the sampling rates (here time the time-dependent sampling rate R_{s_5d}). Equation 3.4 is a simplification derived from equations 3.1 and 3.2 (see Text B.3).

Depending on which time frame t_{acc} is set to, different water concentrations can be calculated. If t_{acc} is set to the whole deployment period of the passive sampler (in this study 5 or 7 d), a c_{TWA} is calculated. By setting t_{acc} to the estimated peak duration of 2 d where the majority of compound mass is sorbed, following the approach of Fernández et al. (2014), the pulse concentration can be estimated. We compared the resulting water concentrations to give recommendations for field application of passive samplers. Statistical analyses and visualisations were conducted in R (version 3.5.2) (R Core Team, 2018) with the additional packages data.table and lubridate (Dowle et al., 2019; Spinu et al., 2018). We provide the computer code to determine sampling rates including both algorithms and all raw data on Github (https://github.com/rbslandau/schreiner_calib).

Table 3.1: Compounds analysed in this study, their chemical properties (retrieved from Lewis et al. (2016)) and the modelled time-dependent sampling rates (R_{s_5d}) in L d^{-1} as well as the related rate constants k_{ws} (uptake rate) and k_{sw} (release rate). NA: no value available.

Compound	CAS[a] number	type[b]	K_{foc}[c,d] [mL g^{-1}]	K_{oc}[e] [mL g^{-1}]	log K_{ow}[f,g]	MW[h,g] [g mol^{-1}]	Sw[i,g] [mg L^{-1}]	pK_a[k,d]	class	log D_{ow}[l,g]	R_{s_5d}[m] [L d^{-1}]	k_{ws}[n] [L d^{-1}]	k_{sw}[n] [d^{-1}]	RSE[o] [ng]
2-4-D[p]	94757	H	24	39	-0.8	220	24000	3.4	acid	-5.4	0.05	0.11	0.38	5
2-n-Octyl-4-isothiazolin-3-on-(OIT)	26530201	F	NA	140	2.5	210	500	NA	NA	2.5	0.42	0.50	0.07	6
Acetamiprid[p]	135410207	I	110	200	0.8	220	3000	0.7	acid	-6.5	0.39	0.51	0.11	22
Alachlor[p]	15972608	H	2000	340	3.1	270	240	0.6	acid	-4.3	0.61	0.66	0.03	20
Azoxystrobin	131860338	F	420	590	2.5	400	6.7	NA	NA	2.5	0.77	0.78	<0.01	22
Benthiavalicarb-isopropyl	177406687	F	180	NA	2.6	380	13	NA	NA	2.6	0.86	0.86	<0.01	51
Boscalid	188425856	F	770	NA	3.0	340	4.6	NA	NA	3.0	0.45	0.55	0.09	67
Carbendazim[p]	10605217	F	230	NA	1.5	190	8	4.2	base	1.5	0.35	0.47	0.13	23
Clothianidin[p]	210880925	I	160	120	0.9	250	340	11	base	-2.2	0.46	0.46	<0.01	32
Cyproconazol	94361065	F	36	NA	3.1	300	93	NA	NA	3.1	0.63	0.73	0.06	17
Cyprodinil[p]	121552612	F	2300	NA	4	230	13	4.4	base	4.0	0.74	0.74	<0.01	23
Diazinon[p]	333415	I	640	610	3.7	300	60	2.6	acid	-1.7	0.96	0.97	<0.01	23
Dichlorvos	62737	I	NA	50	1.9	220	18000	NA	NA	1.9	0.98	0.98	<0.01	18
Difenoconazol[p]	119446683	F	3760	NA	4.4	410	15	1.1	acid	-2.6	0.44	0.44	<0.01	50

Compound	CAS[a] number	type[b]	K_{foc}[c,d] [mL g^{-1}]	K_{oc}[e] [mL g^{-1}]	log K_{ow}[f,g]	MW[h,g] [g mol^{-1}]	Sw[i,g] [mg L^{-1}]	pK_a[k,d]	class	log D_{ow}[l,g]	R_{s_5d}[m] [L d^{-1}]	k_{ws} [L d^{-1}]	k_{sw} [d^{-1}]	RSE[n] [ng]
Dimethenamid	87674688	H	69	NA	2.2	280	1200	NA	NA	2.2	0.65	0.65	<0.01	32
Dimethoat	60515	I	28	NA	0.8	230	23000	NA	NA	0.8	0.38	0.56	0.16	16
Dimethomorph[p]	110488705	F	350	NA	2.7	390	29	-1.3	acid	-6.6	0.75	1.09	0.16	51
Epoxiconazol	135319732	F	1100	NA	3.3	330	7.1	NA	NA	3.3	0.80	0.86	0.03	15
Fenamidone	161326347	F	390	NA	2.8	310	7.8	NA	NA	2.8	0.83	0.83	<0.01	80
Fluopicolide	239110157	F	320	NA	2.9	380	2.8	NA	NA	2.9	0.86	0.86	<0.01	83
Fluopyram	658066354	F	280	NA	3.3	400	16	NA	NA	3.3	0.91	0.91	<0.01	49
Imidacloprid	138261413	I	230	NA	0.6	260	610	NA	NA	0.6	0.47	0.65	0.14	22
Iprovalicarb	140923177	F	NA	110	3.2	320	18	NA	NA	3.2	0.52	0.71	0.13	32
Mandipropamid	374726622	F	850	NA	3.2	410	4.2	NA	NA	3.2	0.64	0.64	<0.01	95
MCPA[p]	94746	H	74	NA	-0.8	200	29000	3.7	acid	-5.1	0.06	0.12	0.33	6
Metalaxyl	57837191	F	160	160	1.8	280	8400	NA	NA	1.8	0.60	0.71	0.07	22
Methidathion	950378	I	NA	400	2.6	300	240	NA	NA	2.8	0.48	0.48	<0.01	70
Myclobutanil[p]	88671890	F	520	NA	2.9	290	130	2.3	acid	-2.8	0.75	0.81	0.03	36
Nicosulfuron[p]	111991094	H	21	30	0.6	410	7500	4.8	acid	-2.6	0.04	0.05	0.10	1
Pencycuron	66063056	F	4900	NA	4.7	330	0.3	NA	NA	4.7	0.31	0.33	0.02	18
Picoxystrobin	117428225	F	900	1000	3.6	370	3.1	NA	NA	3.6	0.38	0.38	<0.01	53

Compound	CAS[a] number	type[b]	K_{foc}[c,d] [mL g^{-1}]	K_{oc}[e] [mL g^{-1}]	log K_{ow}[f,g]	MW[h,g] [g mol^{-1}]	Sw[i,g] [mg L^{-1}]	pK_a[k,d]	class	log D_{ow}[l,g]	R_{s_5d}[m] [L d^{-1}]	k_{ws} [L d^{-1}]	k_{sw} [d^{-1}]	RSE[n] [ng]
Piperonyl-butoxide	51036	S	NA	9000	4.8	340	14	NA	NA	4.8	0.63	0.63	<0.01	24
Prochloraz[p]	67747095	F	1400	500	3.5	380	27	3.8	base	3.5	0.69	0.69	<0.01	22
Propamocarb	24579735	F	NA	NA	0.8	190	900000	9.5	base	-0.7	0.02	0.02	0.07	2
Propiconazol	60207901	F	NA	NA	3.6	340	84	1.1	base	3.6	0.74	0.74	<0.01	21
Prosulfocarb	52888809	H	1700	NA	4.5	250	13	NA	NA	4.5	0.46	0.56	0.07	21
Pyrimethanil[p]	53112280	F	300	NA	2.8	200	120	3.5	base	2.8	0.69	0.71	0.01	18
Tebuconazol[p]	107534963	F	770	NA	3.7	310	36	5	base	3.7	0.67	0.73	0.03	27
Thiabendazol[p]	148798	F	2100	4000	2.4	200	30	4.7	base	2.4	0.12	0.21	0.25	11
Thiacloprid	111988499	I	620	NA	1.3	250	180	NA	NA	1.3	0.58	0.87	0.18	23
Thiamethoxam	153719234	I	NA	56	-0.1	290	4100	NA	NA	-0.1	0.31	0.49	0.20	16
Trifloxystrobin	141517217	F	2300	NA	4.5	410	0.6	NA	NA	4.5	0.37	0.37	<0.01	9

[a] CAS: Chemical Abstracts Service;

[b] compound type: F: fungicide, H: herbicide, I: insecticide, S: synergist used with insecticides;

[c] soil adsorption coefficient corrected for soil organic carbon content, Freundlich absorption coefficient (non-linear relationship);

[d] compound properties included in the regression analysis using the Least Absolute Shrinkage and Selection Operator (LASSO) for a subset of 16 of the 42 compounds;

[e] soil absorption coefficient corrected for soil organic carbon content (linear relationship);

[f] logarithmic octanol-water partitioning coefficient;

[g] compound properties included in the regression analysis using the LASSO for all 42 compounds;

[h] molecular weight;

[i] water solubility at 20 °C;

[k] negative 10th logarithmic of the dissociation constant at 25 °C;

[l] distribution coefficient at 25 °C and pH = 8 reflecting the partitioning of neutral species; calculated from pKa and log Kow according to Carmichael (2014). For non-charged compounds, the log Kow was used;

[m] sampling rate, referring to an exposed sorbent area of 12.57 cm² and a disk exposure period of 5 d, instantaneous sampling rates (Rs_t0) see Table B.1.

[n] rate constants related to the time-dependent sampling rate R_{S_5d};

[o] Residual Standard Error of the best fit model that predicted the rates constants;

[p] compounds included in the selection using LASSO using the properties marked with the symbol d.

3.5 RESULTS AND DISCUSSION

3.5.1 USING PASSIVE SAMPLING TO ESTIMATE PESTICIDES RISKS IN AGRICULTURAL STREAMS

Maximum pulse, i.e. peak concentrations of pesticides typically associated with heavy rainfall events, can be biologically more relevant to organisms than baseline concentrations (Ashauer et al., 2016; Stehle et al., 2013). In our study, the duration of the pulse was in the range of two to three days until the concentrations returned to the level of baseline concentration (Fig. 3.2). In field studies, similar pulse durations of one to three days were found depending on the geogenic background, topography, stream size and soil structure (Blume et al., 2007; Haga et al., 2005; Leu et al., 2004; Wittmer et al., 2010). Calculating pulse concentrations based on the approximate pulse duration, here two days, yielded to concentrations of approximately 250 ng L^{-1} corresponding to 50 % of the maximum, i.e. peak concentration (disks exposed for 7 d: 51 % ± 14; disks exposed for 5 d: 54 % ± 13). By contrast, using the actual exposure time of the passive sampler disks, we calculated c_{TWA} concentrations of approximately 85 ng L^{-1}, corresponding to 20 % of the peak concentration (disks exposed for 7 d: 15 % ± 4; disks exposed for 5 d: 21 % ± 5). Given the known duration of the pesticide pulse in our study, the pulse concentration related to its duration of two days more appropriately reflects the pesticide exposure. Setting the peak duration to two days is further supported by Fernández et al. (2014), who found a reasonable match between peak-associated concentrations from passive samplers and a simplified event-driven sampler that is designed to take a flow-proportional sample over several hours after a rainfall event (Liess et al., 1996). If applying a similar approach to peaks of a given height with different pulse durations, the calculated concentrations determined using passive sampling would represent a higher (shorter peak) or lower (longer peak) fraction of the occurring maximum concentration. For typical pulse durations of more than one day (Leu et al., 2004; Wittmer et al., 2010), however, the maximum concentration would be underestimated. For a given peak duration, the calculated concentrations of higher and lower peaks with respect to the baseline exposure would represent a lower and higher fraction of the actual maximum concentration, respectively.

Using the estimated peak duration of here two days in studies with a similar setting as presented here, allows to link the estimated pulse concentrations to pesticide risks during pulses. The risk of pesticides towards different organism groups in freshwater ecosystems is often assessed by comparing exposure concentrations to effect concentrations (EC) determined in laboratory toxicity studies. Those studies are typically conducted with constant exposures of durations between 24 and 96 hours, frequently 48 hours for invertebrates (e.g. Lewis et al., 2016; Nowell et al., 2018; Rasmussen et al., 2015). Hence, these EC concentrations relate to similar time frames as the pulse durations and the related calculated pulse concentrations c_{calc}. Notwithstanding, the effects on organisms are influenced by

toxicodynamic and toxicokinetic processes (Galic et al., 2014; Rubach et al., 2010) and to which extent the average or maximum concentration allows to predict an effect depends on related parameters. In other words, pulses with the same peak or average concentration but different exposure patterns (e.g. duration, shape) can imply different effects in organisms (Ashauer et al., 2013). Hence, this needs to be taken into account when using passive sampling results for risk assessment. Of course, this also applies to other sampling methods such as grab sampling or automated event-driven sampling, though, of these methods, grab sampling is less likely to capture pesticide pulses (Bundschuh et al., 2014; Stehle et al., 2013).

Regarding the potential interference of peak and baseline, if the accumulated pesticide mass is falsely attributed to a pulse, this leads to an overestimation of the pesticide concentration. Hence, if pesticide pulses are incorporated in the calculations of water concentrations by using the estimated pulse duration, the occurrence of a pulse event requires validation. Given that in agricultural areas pesticide pulses are often associated with heavy rainfall, validation can be achieved through constant monitoring of flow, conductivity or water level. In addition, visual inspection of the site can provide evidence of a rainfall event (e.g. deposed material, condition of the riparian area).

Besides adapting water concentration calculations by using estimated pulse durations, passive sampling of short-term exposure also requires adjustments for passive sampler configuration. Passive samplers have typically been designed to determine average concentrations over several weeks to months. For example, the receiving phase of the device used in our study, which is similar to the Chemcatchers', was in other studies often shielded with a diffusion-limiting membrane. This membrane protects the receiving phase, i.e. the SDB disk for example from biofouling but also mechanical disturbances and allows for a longer deployment under field conditions. The associated lower sampling rates (e.g. Moschet et al., 2015; Shaw et al., 2009; Stephens et al., 2009; Vermeirssen et al., 2009) thereby enable sampling over a longer time period without exhausting the sorbent phase. These longer deployment times can, depending on the research question, accurately display TWA concentrations over e.g. a specific part of the vegetation period. Previous studies used this configuration also to sample pulses associated with heavy rainfall events of herbicides occurring in high concentrations (Novic et al., 2017; Stephens et al., 2009). Pulses of short durations (two days) or lower concentration might be missed due to lag phases, as reported for SDB disks covered with a diffusion-limiting membrane (e.g. Shaw et al., 2009; Vermeirssen et al., 2012). When studies like ours, however, aim to sample field-relevant pulses (e.g. a few hours to days), disks without a shielding membrane are commonly used, also called "naked" SDB disks (Fernández et al., 2014; Mutzner et al., 2019). This especially applies when a wide ranges of pesticides, including insecticides are targeted because these usually occur in low to medium concentrations (e.g. Moschet et al., 2014). Naked SDB disks achieve higher sampling rates are achieved, which

comes at the advantages of lower LOQs and a faster response towards repeated concentration changes, but at the risk of saturation of the sorbent if deployed for longer time frames.

In general, the use of passive sampling under field conditions is subject to several uncertainties, independently of the sampler's configuration and the approach to calculate water concentrations. Variables that can be controlled and are traceable under laboratory conditions such as pH, turbidity or the load of organic material can be highly variable under field conditions, especially when sampling pulse events. These can in turn have effects on uptake of pesticides to the sorbent phase (e.g. Novic et al., 2017; Charlestra et al., 2012; Vrana et al., 2006). During field application of passive sampling, especially when comparing a range of sampling sites, information on a variety of physicochemical variables should therefore be collected because these can help to interpret the results and to reduce uncertainties.

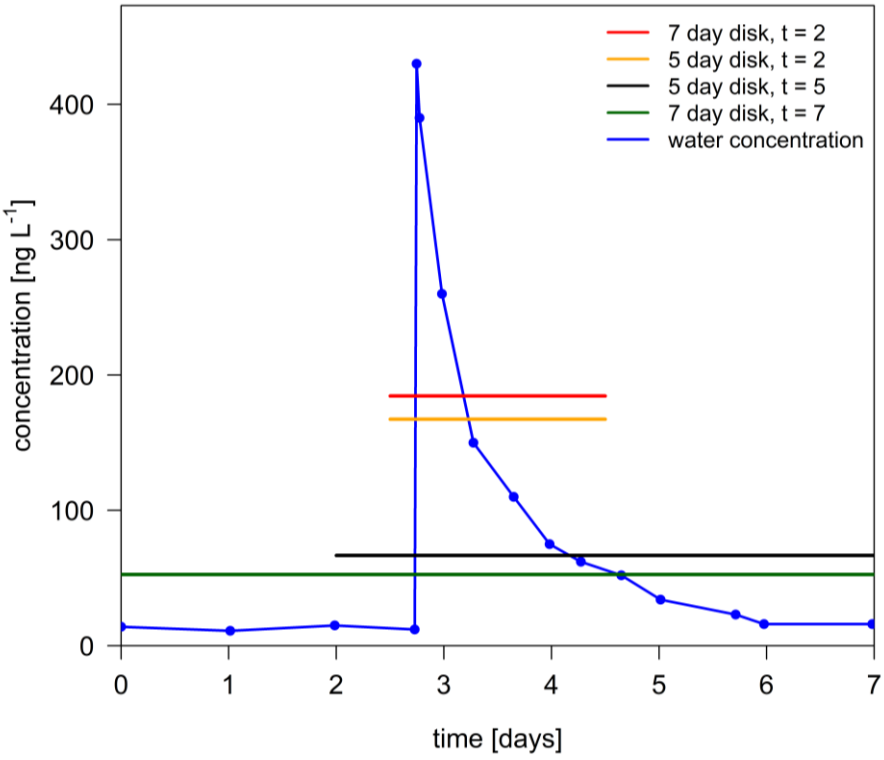

Fig. 3.2: Water concentration gradient in the channel and the calculated time-weighted average (TWA) as well as pulse concentrations (calculation according to equation 4) based on the passive sampler with the longest exposure time for the example of diazinon. Graphical presentation for all compounds in Fig. B.6.

3.5.2 POOR RELATIONSHIPS BETWEEN SAMPLING RATES AND COMPOUND PROPERTIES

We compared relationships between the time-dependent sampling rate (R_{s_5d}) and compound properties, because such relationships may help predict sampling rates for additional compounds (with more or less accuracy) that have not been determined experimentally yet, as done in Fernández et al. (2014). In our study, the compound properties were a poor predictor of sampling rates. Using the LASSO approach for model selection, neither a compound property of those available for all compounds (log K_{ow}, log D_{ow}, molecular mass, and water solubility) nor of those only available for a subset of compounds (log K_{foc}, and *pKa*, Table 3.1), was selected in the best-fit model to predict time-dependent sampling rates. The same result was obtained when we used the time-independent instantaneous sampling rate or limited the analysis to compounds that remained in the linear uptake regime (Table B.2). These results partially contradict those of previous studies with compounds of similar physico-chemical properties (e.g. Mutzner et al., 2019; Shaw et al., 2009; Vermeirssen et al., 2013). Although log K_{ow} was according to the LASSO approach no reliable predictor for sampling rates, it exhibited a moderate correlation to the sampling rate (Fig. B.5 a; $n = 42$, $r = 0.5$, corresponding $R^2 = 0.25$, RSE = 0.25 L d^{-1}, $p < 0.001$). This is in accordance with previous studies ($R^2 = 0.26$ in Fernández et al. (2014)), where the log K_{ow} was used to predict unknown sampling rates.

Compared to the log K_{ow}, previous studies detected stronger relationships of the sampling rate with the log D_{ow} for a pH similar to that in the respective experiment than to log K_{ow} ($R^2 > 0.5$; e.g. Mutzner et al., 2019; Vermeirssen et al., 2013). This is presumably because the log D_{ow} more accurately reflects the uptake into the SDB disks when considering partitioning of neutral species (Bäuerlein et al., 2012; Harman et al., 2012). In our study, however, the correlation between the sampling rate and log D_{ow} was weak (Fig. B.5 b; $n = 42$; $r = 0.36$, corresponding $R^2 = 0.13$, RSE = 0.24 L d^{-1}, $p = 0.019$). The weak relationship could be caused by the incorporation of a broad spectrum of pesticides from various chemical classes, resulting in a log D_{ow} range of -6.6 to 4.8, whereas other studies were restricted to only approximately half of that range (e.g. Mutzner et al., 2019; Shaw et al., 2009). However, the relationship can also be influenced by retrieving chemical properties from different sources. We retrieved the values for the chemical properties from the Pesticide Property DataBase (Lewis et al., 2016) that is based on experimental data and data verification. By contrast, several previous studies (e.g. Mechelke et al., 2019; Mutzner et al., 2019) retrieved the data on chemical properties from models that estimate the value of a chemical property using the chemical structure of the respective compound (ChemAxon, 2019). When re-analysing the relationship using such data, our sampling rates showed a stronger relationship to log D_{ow} (Fig. B.5 c; $n = 42$; $R^2 = 0.35$, RSE = 0.21 L d^{-1}, $p < 0.001$). Hence, even relatively basic chemical variables can vary strongly with the data source and influence the relationship with sampling rates.

Overall, our results call for caution in using compound properties to predict sampling rates with reasonable accuracy, which is in line with previous publications (Moschet et al., 2015; Vermeirssen et al., 2012). Whenever reliable sampling rates are needed, they should be determined in calibration studies.

3.5.3 COMPARISON TO SAMPLING RATES OF PREVIOUS STUDIES

Time-dependent sampling rates (R_{s_5d}) estimated in this study ranged from 0.02 L d^{-1} (propamocarb) to 0.98 L d^{-1} (dichlorvos; Table 3.1). For 15 of the 42 compounds of this study sampling rates were available from previous studies (Table B.2). All of these studies used constant exposure designs, i.e. pure baseline concentrations, and we did not find complementary studies where pulse concentrations were used to determine sampling rates in laboratory studies. In terms of the root mean-square error (RMSE = 0.14 L d^{-1}, n = 22, Fig. 3.3), where we measured the correspondence from the relationship to the identity line (1:1-line), the sampling rates of this and previous studies were in good agreement (factor < 2), except for single outliers, which are discussed below. This suggests that sampling rates from different exposure designs and using various approaches to estimate sampling rates could be comparable, possibly due to consistent interactions between the compound and the sampling phase. This seems to apply (1) across the whole range of sampling rates, because pesticides such as 2,4-D, MCPA and Diazinon were similar and (2) independently from the uptake regime of the compound (8 of the 15 compounds approached equilibrium and had similar matches, Table B.2). Based on these observations, previously reported sampling rates that are similar can be used with some confidence to calculate water concentrations. However, if sampling rates differ between studies or only individual study sampling rates are available, the calculated water concentrations should be treated with caution.

Given that the calibration experiments differed in many conditions in addition to the exposure design (e.g. different flow velocities, design of sampler holders and estimating sampling rates, see below) including several not reported uncertainties (e.g. pH, salinity, dissolved organic carbon), the match between the sampling rates can be considered good. We suggest that this good match with previously reported sampling rates confirms that the SDB disks are reliable under different exposure patterns and can be applied during peak concentrations. However, studies designed to compare different exposure designs, including constant and pulse exposures, are required to provide empirical support for our suggestion. This is because our results are based on observations from only 15 compounds, for which sampling rates of previous studies were available. Given the relatively large effort required to conduct calibration experiments, calibration of passive samplers could be conducted in combination with ecotoxicological mesocosm studies, which often imply pesticide pulse concentration scenarios (Beketov and Liess, 2008; Wieczorek et al., 2018). This would provide sampling rates under

field-relevant conditions at a moderate additional effort. Another alternative would be the in-situ calibration of passive samplers, i.e. deployment under field conditions in concert with frequent water sampling (Harman et al., 2012; Novic et al., 2017; Stephens et al., 2009).

The sampling rates from different studies matched relatively well despite different flow velocities. Previous studies showed that flow velocity in the range between 0 and 0.2 m s^{-1} and sampling rates was positively correlated (Booij and Chen, 2018; Kaserzon et al., 2013; Vermeirssen et al., 2008). When, however, comparing sampling rates across different studies (see Fig. 3.3), the effect of flow velocity seems to have only a minor and non-consistent influence on the sampling rates. Even though flow velocity differed up to 0.7 m s^{-1} (ranging from 0.13 m s^{-1} to 0.85 m s^{-1}), sampling rates for compounds like diazinon or 2,4-D were relatively similar across different calibration experiments, including ours (Fig. 3.3). The effects of flow velocity, however, might be masked by differences in experimental setups (i.e. experimental system and duration, used medium), but also how and if the water matrix is incorporated and the quality of the chemical analysis (Vermeirssen et al., 2013).

Notwithstanding, the sampling rates between some studies and for some compounds differed strongly. The sampling rates of two compounds, alachlor and tebuconazole, from a study using a circular tank were with 0.32 L d^{-1} (alachlor) and 0.19 L d^{-1} (tebuconazole) (Gunold et al., 2008), approximately 50 % and 70 % lower than those from our study. The sampling rate of tebuconazole of our study matched that of a previous study with a similar setup except for the exposure design (Schreiner et al., accepted). The difference to the study of Gunold et al. (2008) for alachlor and tebuconazole may be explained by differences in flow velocity as well as direction. Although Gunold et al. (2008) reported flow velocities of 0.135 and 0.4 m s^{-1} the actual flow velocities were likely much lower due to their experimental system, where the passive samplers were moved by a carousel and the water phase was hence moved as well (R. Gunold, personal communication). Besides probably irrelevant differences in flow velocity from this study to Gunold et al. (2008), different holder designs are likely to result in different sampling rates. Previous studies reported a 50 % decline in sampling rates for the thick sampler holder used by Gunold et al. (2008) compared to a thinner sampler holder because they are speculatively responsible to create a thicker water boundary layer (Lobpreis et al., 2008). This difference matches well with the difference that we observed when considering the same flow velocities. Additionally, Gunold et al. (2008) used SDB-XC disks in contrast to the other studies, including ours, that used SDB-RPS disks. Previous studies found a higher uptake of polar compounds into SDB-RPS disks than into SDB-XC disks, resulting in higher sampling rates (Sánchez-Bayo et al., 2013; Vermeirssen et al., 2009). Moreover, Gunold et al. (2008) estimated the sampling rates using a linear relationship which additionally may lead to an underestimation, though this effect should be minor because in our study both compounds remained in the linear uptake regime (Table B.1).

The identification of the drivers of differences is hampered by the fact that sampling rate differences between studies are inconsistent. The sampling rate of carbendazim was similar in our study, Vermeirssen et al. (2009) and other previous studies (Mutzner et al., 2019; Vermeirssen et al., 2013). In contrast, the sampling rate of diazinon of our study as well as previous studies (Mutzner et al., 2019; Vermeirssen et al., 2013) was about double that reported in Vermeirssen et al. (2009; 0.42 L d^{-1}). The differences of sampling rates of diazinon could be explained by differences in study design like the usage of different passive sampler holders (see above) or the duration of the experiment. Carbendazim, however, had despite these differences and despite approaching equilibrium a similar sampling rate across studies, which might be caused by its compound properties (carbendazim is a base, whereas diazinon is an acid).

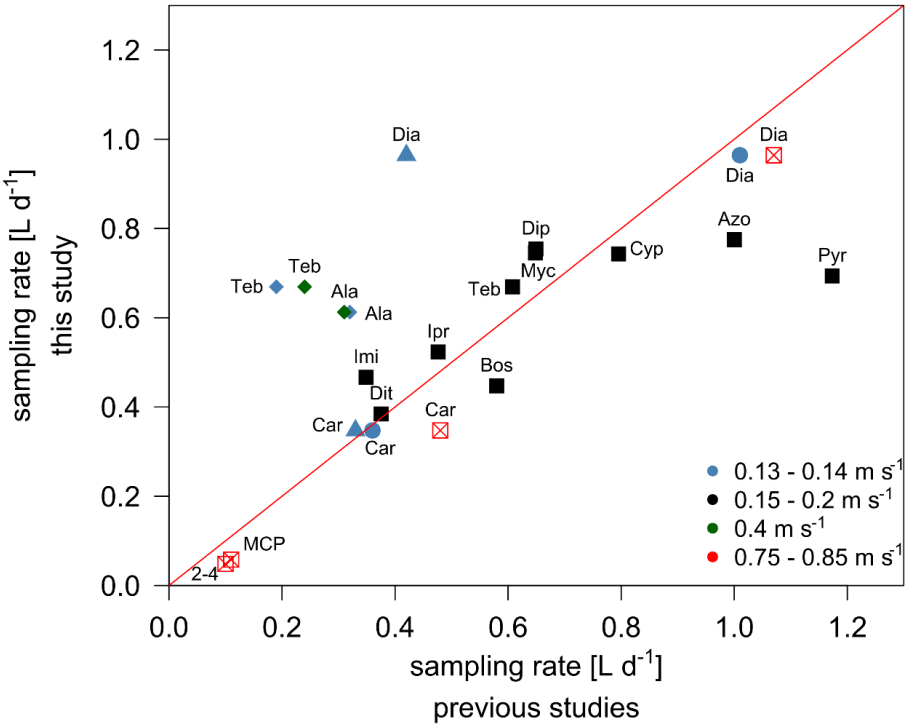

Fig. 3.3: Comparison of sampling rates [L d^{-1}] calculated in this (time-dependent sampling rate over 5 d) and in previous studies. Colour of the points refers to flow velocity of the previous studies, see in Figure legend. Shape of the points refers to the respective study: square: Schreiner et al., accepted, SDB-RPS disks, thin metal holder, time-dependent sampling rate over 5 d; dots: Vermeirssen et al., 2009, SDB-RPS disks, deep Teflon holder, linear relationship used to estimate sampling rates; triangle: Vermeirssen et al., 2013, SDB-RPS disks, thick metal holder, time-dependent sampling rate over 24 h; diamond: Gunold et al., 2008, SDB-XC disks, deep Teflon holder, linear relationship used to estimate sampling rates; crossed box: Mutzner et al., 2019, SDB-RPS disks, thin metal holder, time-dependent sampling rate over 24 h. Compound

abbreviations: 2-4: 2,4-D, Ala: Alachlor, Azo: Azoxystrobin, Bos: Boscalid, Car: Carbendazim, Cyp: Cyprodinil, Dia: Diazinon, Dip: Dimethomorph, Dit: Dimethoat, Imi: Imidacloprid, Ipr: Iprovalicarb, MCP: MCPA, Myc: Myclobutanil, Pyr: Pyrimethanil, Teb: Tebuconazol. Red line: identity line (1:1 line).

3.6 CONCLUSIONS

We determined SDB disk sampling rates for 42 organic pesticides under a baseline exposure with a field-relevant pulse in terms of concentration and duration. We provide two open-source algorithms (https://github.com/rbslandau/schreiner_calib) in the programming language R that allow for estimating sampling rates from pulsed exposures under various data conditions. The relatively good match of sampling rates determined under different exposure designs suggests that sampling rates from different calibration designs including pulse and constant exposures can be used complementarily. Passive sampling based on SDB disks can be suitable for collecting information on pesticide pulses associated with heavy rainfall events. In this context, the estimated pulse duration in the field is the variable that has a strong influence on the estimated water concentrations. Overall, passive sampling with SDB disks can be used, subject to plausible information on the pulse duration, to estimate risks to freshwater communities and ecosystems, which are globally threatened.

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4 PARADISE LOST? PESTICIDE POLLUTION IN A EUROPEAN REGION WITH CONSIDERABLE AMOUNT OF TRADITIONAL AGRICULTURE

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4.1 ABSTRACT

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

4.2 INTRODUCTION

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

High intensity agriculture is not only characterised by high pesticide pollution in streams, but also associated with habitat loss due to channelisation and substrate homogenisation, and the extensive use of fertilisers (MEA, 2005; Vörösmarty et al., 2010). In addition, human labour has largely been replaced by heavy machinery in high intensity agriculture. By contrast, low intensity agriculture, here defined based on remnants of traditional non-mechanised agriculture, is largely relying on human or animal labour (e.g. horse ploughs) (Kovács-Hostyánszki et al., 2016; Lovász and Gurzău, 2013), and a presumed low usage of agrochemicals (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016). Regions with low intensity agriculture are rare in Europe, but can still be found in Eastern Europe, for example

in parts of Romania. One of these regions, Transylvania, has been described as hosting the last relatively pristine farmlands in Europe which also inhabits areas of high intensity agriculture (Fischer et al., 2012). The importance of human labour in some parts of the agricultural sector of Romania is reflected in the highest proportion of agricultural workforce (over 25 % of the population) in the EU (EU average: 4 %) (Eurostat, 2017). Romania is among the EU countries with the lowest pesticide sales (30 % of the EU average, corresponding to 1.3 g of active ingredient per hectare of arable land in 2016) (Eurostat, 2018a, 2018b), suggesting that agrochemical use is particularly scarce in low intensive agriculture (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016). However, to which extent these lower sales and the remnants of low intensity agriculture are reflected in lower pesticide pollution of selected agricultural streams in Romania has not been studied. Previous studies mainly focused on the pesticide pollution of large rivers, where concentrations of single pesticides of up to 240 ng L^{-1} were detected (Ferencz and Balog, 2010; Moldovan et al., 2018). Studies covering pesticide pollution in streams across a range of low to high intensity agriculture could allow us to disentangle the effects of pesticide pollution on ecosystems from other agricultural stressors, because of the availability of agricultural landscapes with no or low pesticide use but the presence of other agricultural stressors.

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

In this study, we determined pesticide concentrations in streams adjacent to a gradient of low to high intensity agriculture in Transylvania, Romania, and assessed the pesticide toxicity to freshwater invertebrates and algae using the sum toxic unit approach. Thereby, we examined if the low agricultural intensity of selected sites results in lower stream pesticide contamination. To capture pesticide peaks related to heavy rainfall events, we used short-term passive sampling complemented by long-term passive sampling for compounds usually bound or absorbed to sediment particles, because active sampling was unfeasible due to the number and distribution of sampling sites. Moreover, we examined the drivers of pesticide toxicity to invertebrates and algae such as local and catchment-scale explanatory variables including the ratio of agricultural land use in the catchment, riparian buffer width and different proxies for agricultural intensity. We hypothesised local variables as the most important drivers for both toxicity indices because the entry of pesticides into the stream can be mitigated or amplified

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

4.3 MATERIALS AND METHODS

4.3.1 SAMPLING SITES

The study was conducted from April to June 2016 in Romania around the city of Cluj-Napoca, Transylvania (Fig. 4.1, Table C.1). We selected 100 m stream sections with adjacent agricultural land use continuously ranging from high to low intensity. High intensity agriculture was characterised by fields larger than 3000 m² and use of heavy machinery, whereas low intensity agriculture was characterised by small, garden-like fields relying on human or animal labour (e.g. horse ploughs). We assumed that the gradient of agricultural intensity across the sampling sites reflected a gradient of pesticide use. All 19 streams had agricultural land use on at least one side of the stream, were located upstream of urban land use to minimize the influence from wastewater and non-agricultural pesticide uses and were characterised by similar stream sizes (3rd to 4th Strahler order).

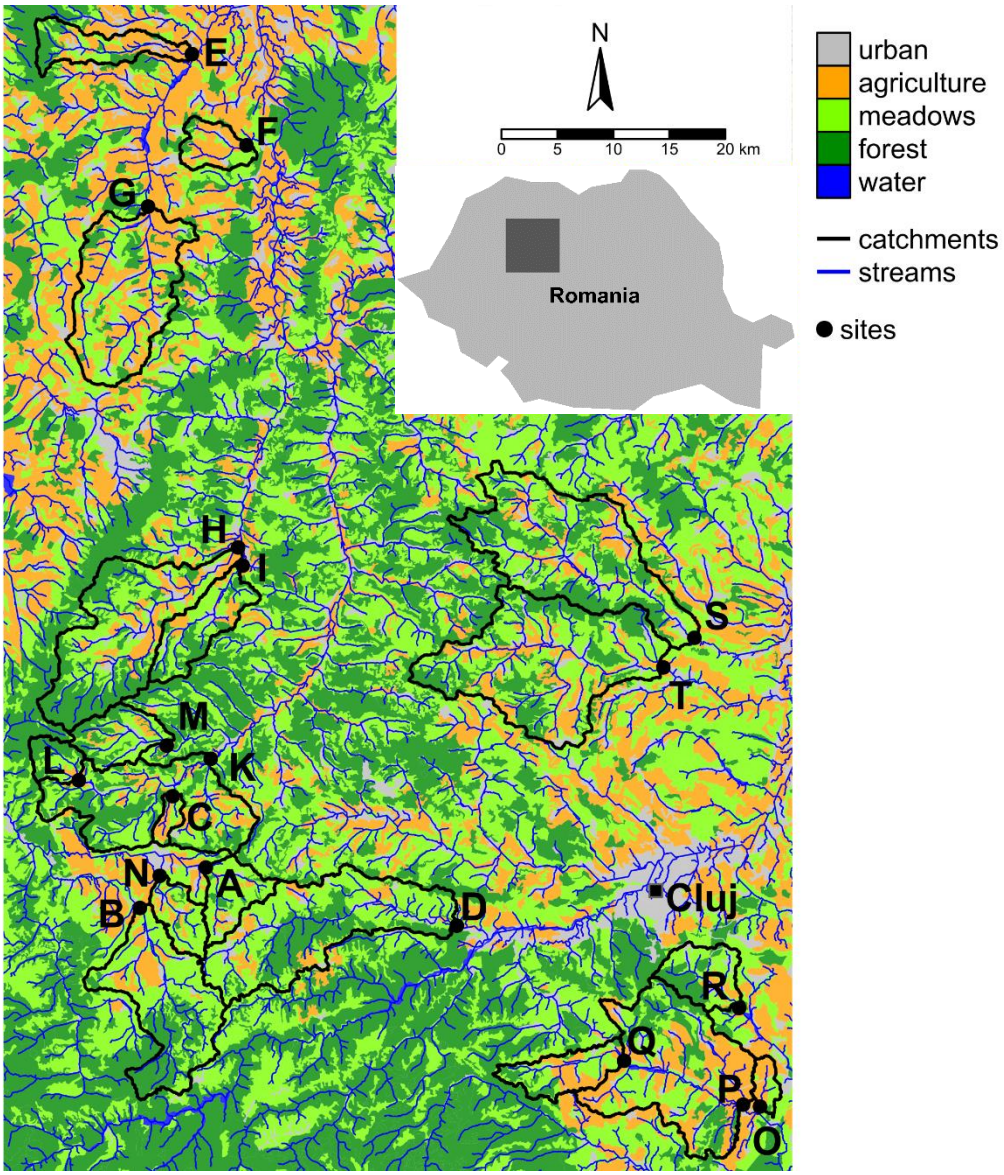

Fig. 4.1: Stream sampling sites A to T and their catchments in Transylvania, Romania, with different land use categories. The map was created using the R package tmaps (Tennekes et al., 2019).

4.3.2 SHORT TERM PASSIVE SAMPLING FOLLOWED BY ANALYSIS USING LC-HRMS(/MS)

To capture a high number of pesticides, we used two complementing passive sampling methods, with different limits of quantifications (LOQ). Most (91 %) of the pesticides were sampled with short term passive sampling achieving relatively high LOQs (analytical LOQs: 0.02 – 500 µg L^{-1}) using Empore styrene-divinylbenzene (SDB)-RPS disks (3M Company) (Table C.2). The disks were deployed three times, one to two days before a forecasted heavy rainfall event (min. 10 mm day^{-1}; forecast by Norwegian Meteorological Institute and NRK,

2016) and retrieved two days after the rainfall event, resulting in a total exposure time of five to six days (Table C.3). One additional set was deployed over six days in the middle of the monitoring campaign in the absence of rainfall to collect the base flow pesticide exposure.

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

The extracts of the event samplings were analysed with an injection volume of 100 µL using a QExactive Plus Orbitrap system (Thermo Fisher Scientific Corporation) producing high-resolution mass spectrometry (HRMS) and MS/MS data. The chromatographic separation was achieved with an Atlantis T3 5 µm 3.0x150 mm column (Waters) using methanol and ultrapure water acidified with 0.1 % of formic acid as mobile phases (gradient see Table C.4). A full scan with a resolution of 140,000 (at m z^{-1} = 200) in the range of 100 to 1000 m z^{-1} followed by five data-dependent MS/MS scans with a resolution of 17,500 was acquired separately for positive and negative ionisation (settings see Table C.5). Mass accuracy was determined below 5 ppm throughout all measurements. The safe identification of the single compounds was achieved by matching the MS/MS fragments to those of the reference standards. The base-flow samples were measured using an Exactive Orbitrap system (Thermo Fisher Scientific Corporation) producing HRMS data using the above-mentioned settings, but incorporating fewer pesticides and having higher LOQs (Table C.2). A total of 228 pesticides and their metabolites were quantified (Table C.2). We selected pesticides authorised in the last ten years in Romania and added some already established in the analytical method (Moschet et al., 2015). Quantification was conducted using extracted ion chromatograms (5 ppm) with internal standards in

TraceFinder 3.3 (Thermo Fisher Scientific Corporation). Quality assurance as well as quality control and performance during the TraceFinder quantification as well as the subsequent calculation of the mass of each pesticide accumulated on the respective disk (m_{sorb}) were done according to Moschet et al. (2015).

4.3.3 LONG TERM PASSIVE SAMPLING FOLLOWED BY ANALYSIS USING GC-MS/MS

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

A total of 17 pesticides and one metabolite (Table C.2) were quantified by a GC-APCI-MS/MS instrument (gas chromatograph 7890B coupled to a triple quadrupole mass spectrometer 6495 using atmospheric pressure chemical ionisation, both Agilent). Analytical details can be found in Rösch et al. (2019). The mass of each pesticide accumulated on the respective sheet (m_{sorb}) was calculated using MassHunter (version B.07.00; Agilent Technologies).

4.3.4 CALCULATING PESTICIDE CONCENTRATIONS

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

$$c_{calc_i} = \frac{m_{sorb_i}}{R_i \, t} \tag{4.1}$$

where m_{sorb} is the mass of the pesticide *i* accumulated on the disk or the sheet, R_i is the respective sampling rate (L day^{-1}; Table C.2) and *t* is the assumed uptake time in days into the receiving phase. Given that most of the pesticide mass sorbed to a disk during peak exposure samples originates from the peak, we set the uptake time to the estimated peak duration of two days to calculate the peak concentration for rainfall event samples (Schreiner et al., 2020). For base exposure samples, we used the whole deployment period (here $t = 6$), assuming relatively constant exposure and consequently sorption (for discussion see below).

The sampling rates R_i for the compounds sampled via SDB-disks were experimentally determined (Table C.2). Since no experimental sampling rates were available for compounds sampled via PDMS sheets, we used one average, experimentally-determined sampling rate from compounds with hydrophobicities similar to the sampled pyrethroid and organophosphate insecticides (details see Moschet et al., 2014a). Due to missing sampling rates for the other compounds, 55 pesticides (Table C.2) were further considered, all of them quantified in base as well as peak exposure samples. Most of the omitted pesticides (except for all metabolites and a few herbicides) occurred in less than 5 % of the samples. Including omitted pesticides using a fixed sampling rate as a proxy for all, resulted in an only minor change in the sum concentrations and the resulting toxicity indices (see related R-script). For example, a fixed sampling rate of 0.2 L day^{-1}, which is in the lower range of sampling rates, had a minor impact, though for a given concentration lower sampling rates result in a higher calculated concentration (for a detailed discussion, see below).

4.3.5 CALCULATING POTENTIAL PESTICIDE TOXICITY

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

$$sumTU = log\left(\sum \frac{c_{calc_i}}{EC_{50_i}}\right) \tag{4.2}$$

where c_{calci} is the estimated concentration of the pesticide *i* and EC_{50i} is the concentration of pesticide *i* at which 50 % of the test organisms (see below) were affected. To account for the risk for different trophic levels in freshwater ecosystems, we calculated sumTUs for (1) the

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

Site-specific sumTUs were computed for each of the different samplings combining the compounds from the PDMS and SDB passive samplers that were deployed simultaneously (Table C.3).

4.3.6 CHARACTERISATION OF SAMPLING SITES

We measured several physicochemical and habitat-specific variables during the period where the rainfall events occurred (June 2016). These variables included the buffer width and the ratio of stream substrate smaller than 2 mm which can be used as a proxy for sediment input caused by erosion (Lemm and Feld, 2017). Additionally, we measured the average field size in a 200 m long section lateral to the sampling site, which was used as a proxy for the intensity of agricultural land use (Pe'er et al., 2014), based on Google Earth images (Google Earth, 2019) that were temporally closest to the field study. The agricultural intensity was also estimated by determining the ratio of large fields (> 3000 m^2) in this 200 m section. Given that the ratio exhibited a high correlation to the average field size ($r = 0.89$, $p < 0.001$), we only included the average field size in the analysis. Moreover, the agricultural intensity of a site was categorised (three levels: low, medium, high agricultural intensity, hereafter called factorial agricultural intensity) based on field size and personal on-site observations of the used agricultural methods. Finally, by using the geospatial algorithm ATRIC (Bhowmik et al., 2015) and CORINE land cover data (European environmental agency, 2019), we derived variables such as catchment size and the ratio of different land use types in the whole catchment (Table C.1). In addition, the ratio of agricultural land use in a 200 m wide buffer was calculated as described in Waite and Van Metre (2017).

4.3.7 DATA ANALYSIS

For further analysis, we used maximum sum concentrations as well as toxicities at each site over the four sampling events, because we assumed that organisms are subject to environmental selection by the strongest stress event (Fernández et al., 2015; Schäfer et al., 2011). The maximum $sumTU_{algae}$ and $sumTU_{invertebrates}$ correlated highly and moderately with the maximum sum concentration ($r = 0.96$, $p < 0.001$ and $r = 0.48$, $p = 0.039$), respectively. Hence, we restricted the analysis to the toxicity indices.

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

We selected and combined several monitored variables (Table C.6) resulting in eight explanatory variables (Table 4.1) considered as potentially important drivers of in-stream pesticide toxicity (Rasmussen et al., 2011; Stehle et al., 2016; Szöcs et al., 2017), which exhibited no relevant inter-correlation (all pairwise $r < 0.62$). The most important drivers of the $sumTU_{invertebrates}$ and $sumTU_{algae}$ were identified using regression analysis with elastic net regularisation (Zou and Hastie, 2005) because this technique allows for a low sample size to explanatory variables ratio. The optimal regularisation (i.e. parameters α and λ of the elastic net) was determined as the model with the least variables within one standard error from the model with minimum cross-validation error (Bruce and Bruce, 2017). Given that the elastic net approach prohibits the inclusion of categorical variables, we analysed if pesticide pollution in terms of concentration, toxicity and detected pesticides differed between levels of agricultural intensity separately using a type II ANOVA with F-tests or Chi-square-tests.

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

Table 4.1: Explanatory variables selected as potential drivers of toxicity to invertebrates and algae with range, median and standard deviation (SD). See methods for details on variables.

Explanatory variable [unit]	range	median	SD
Ratio of agricultural land use in upstream catchment [%] [a]	7 - 61	17.7	17.9
Catchment size [km²]	8 - 177	35.0	60.8
Ratio of agricultural land use within a 200 m buffer [%] [a]	0 - 100	51.2	33.2
Average (geometric mean) field size of all fields that fully or partially extended into a 50 m wide and 200 m long stream buffer. Considered as proxy for agricultural intensity (Pe'er et al., 2014) [m²] [b]	497 – 46,029	3,159	10,726
Minimum riparian buffer width of both stream banks [m] [b]	1 – 50	10	14.8
Direct distance between stream and landscape-level (Calculated based on bank height as well as horizontal distance from stream to landscape level), shortest distance of potential runoff [m]	2.1 – 9.1	4.24	2.11
Average riparian plant height in an approx. 5 m buffer [m]	0.9 – 7.5	2.9	2.09
Fine sediment (< 2 mm), proxy for sediment input (Lemm and Feld, 2017) [%]	0 - 90	25	25.6

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

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

Single values used to get displayed combined variables in Table C.6.

4.4 RESULTS AND DISCUSSION

4.4.1 PESTICIDE CONCENTRATIONS IN STREAMS

We analysed 195 pesticides in a region assumed to have areas of low pesticide exposure (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016). The sampled streams covered a gradient of low to high intensity agriculture, with agricultural practices ranging from human and animal labour to heavy machinery agriculture (Table C.1). We found 47 % of the analysed pesticides (91 pesticides) and 23 metabolites (Table C.2, all data at https://github.com/rbslandau/schreinerromania). During rainfall events, on average 31.3 (± 7.6) pesticides were detected, with a maximum of 66 different compounds (50 pesticides, 16 metabolites) in a single sample (Fig. 4.2, Table C.7). The number of detected pesticides was independent of the factorial agricultural intensity (ANOVA; LRT = 0.40, p-value = 0.82). Still, the number of detected pesticides across the whole gradient of agricultural intensity matched those from a study in Switzerland conducted in catchments with high intensity agriculture (Moschet et al., 2014b). This study used composite water samples and incorporated a comparable number of pesticides as in our study. In contrast, routine monitoring

in several European countries where high intensity agriculture dominates, only detected between 5 and 15 pesticides (Moschet et al., 2014b; Schreiner et al., 2016). The lower number of detected pesticides in routine monitoring can be explained by a reduced set of analysed pesticides and the lack of event sampling, known to increase the number of detected pesticides (Leu et al., 2004; Weibel et al., 1964). This is also reflected in this study, where during base flow only between 30 to 50 % of the number of pesticides during rainfall event samples were detected (Table C.7).

The sum concentrations in rainfall event samples ranged from 0.02 to 37 µg L^{-1} (Table C.8, Fig. 4.2), with the herbicide 2,4-D detected in calculated concentrations of up to 36.2 µg L^{-1} (Table C.2). A previous study identified misuse during application as the driver of such high concentrations as in the case of 2,4-D (Wittmer et al., 2010). We observed the washing of agricultural equipment at the site with the highest 2,4-D calculated concentrations and surveys in the study area reported insufficient education of most farmers regarding pesticide use (Gurzău et al., 2008; Lovász and Gurzău, 2013). Despite covering a gradient of agricultural intensity, the maximum sum concentration was not significantly influenced by the factorial agricultural intensity (ANOVA; F = 0.59, p-value = 0.57). The detected range of sum concentrations was, however, similar to streams in intensive agriculture in Western Europe and the US (Fernández et al., 2014; Guibal et al., 2018; Gustavsson et al., 2017; Moschet et al., 2014b; Nowell et al., 2018; Ulrich, 2015) and exceed previous results for large rivers in Transylvania by orders of magnitude (Ferencz and Balog, 2010; Moldovan et al., 2018). The sum concentration was on average 7 times higher for peak than for base exposure samples, again indicating the relevance of sampling rainfall events. The sum concentration of single peak flow samples (7 % of the peak flow samples, Table C.8) was slightly below those of base flow samples at the same site. This was only the case where two rainfall events were only one week apart with no or little new pesticide use. We assumed that the compound uptake from peak exposure samples was driven by the peak duration, whereas from base exposure samples it was rather constant during the whole deployment time and considered this in the calculation of concentrations. Assuming that both types of samples were related to the estimated peak duration of two days (equation 4.1, $t = 2$), the peak exposure sum concentration would still be 2.3 times higher on average (details see below) than those of the base flow samples. However, using this shorter period for samples without a clear indication of rainfall events leads to an overestimation of the calculated concentrations. The differences between peak and base sum concentrations are lower than reported in previous studies, where differences up to a factor of 100 were detected for several single pesticides (e.g. Leu et al., 2004). These previous studies were conducted exclusively in high intensity agriculture and included a lower spectrum of analysed compounds in smaller streams. Additionally, these studies used temporally resolved active sampling, which is more suitable than passive

sampling to detect peak concentrations, but was not possible in this study with various catchments and monitoring over a long time period.

As reported by previous studies (Gustavsson et al., 2017; Moschet et al., 2014b; Schreiner et al., 2016), the mixtures of simultaneously detected compounds were dominated by herbicides both in terms of concentration and number of compounds, which can be attributed to herbicides dominating pesticide sales in Romania (> 40 %) (Eurostat, 2018a). Additionally, herbicides are typically highly water-soluble (Lewis et al., 2016) and consequently more easily transported into streams.

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

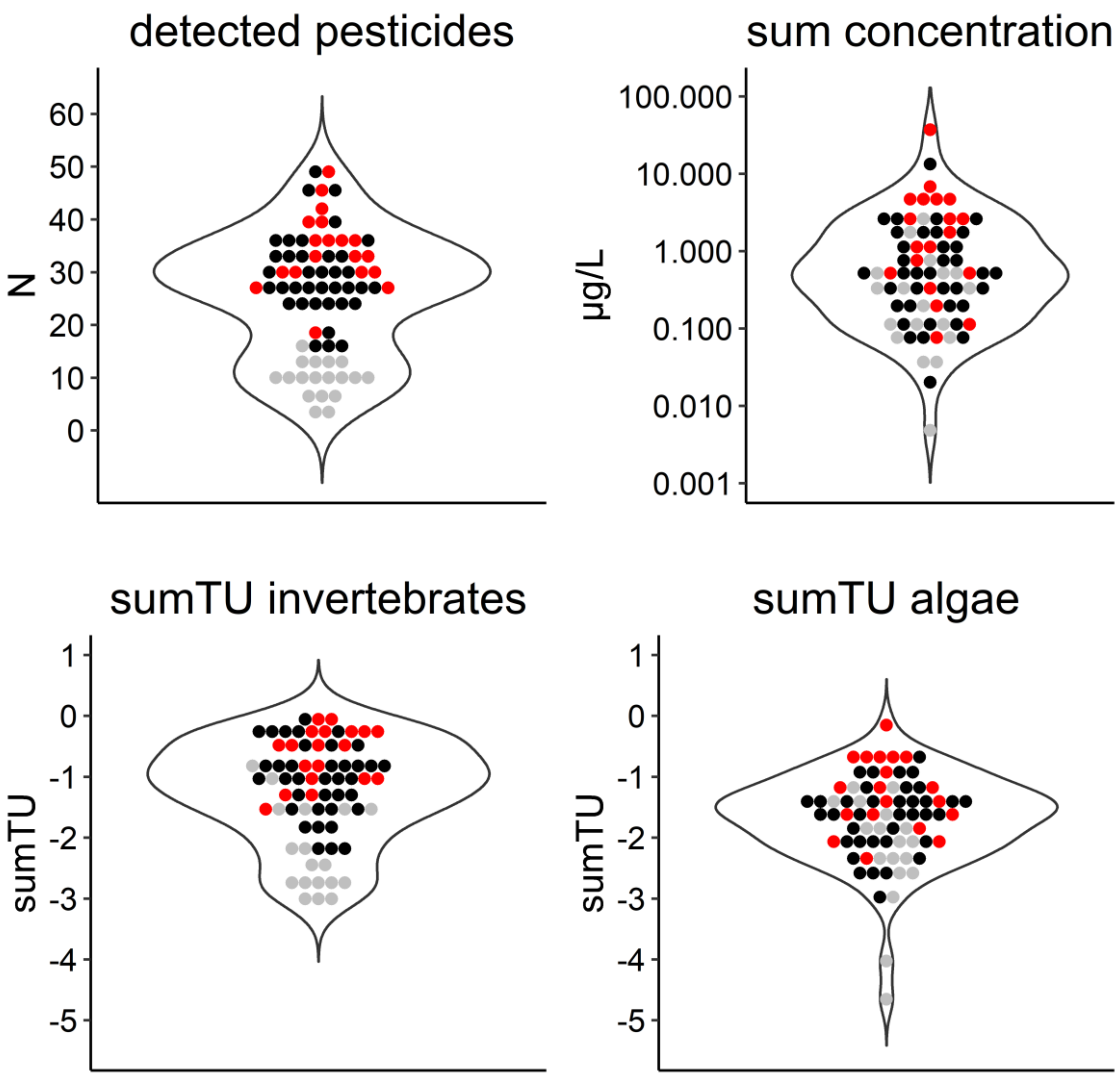

Fig. 4.2: Violin plots (Wickham, 2016) illustrating the occurrence frequency of detected pesticides, sum concentrations and the toxicity indices $sumTU_{invertebrates}$ and $sumTU_{algae}$ (both on a logarithmic scale). Each dot represents a single sample (four samples per site): grey = base exposure (without rainfall event), black = rainfall event, red = maximum per site (only during rainfall events).

4.4.2 ASSESSMENT OF TOXICITY TOWARDS INVERTEBRATES AND ALGAE

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

The maximum $sumTU_{invertebrates}$ per site spanned from -1.62 to -0.01 (on a logarithmic scale, Fig. 4.2, Table C.8), a range that is known to alter macroinvertebrate communities and consequently to affect whole stream ecosystems e.g. Schäfer (2019). Moreover,

complementary studies in our region using the maximum $sumTU_{invertebrates}$ per site found that the pesticide toxicity was associated with changes in spider communities and their diet in adjacent riparian ecosystems, most likely a consequence of alterations in freshwater insect emergence which constitutes an important prey for riparian spiders (Graf et al., 2020, 2019).

The maximum $sumTU_{algae}$ per site ranged from -2.36 to -0.15 (Fig. 4.2, Table C.8), a level implying risks for algae by reducing growth and photosynthetic activity, potentially affecting an important base of the stream food web, the primary production (Gustavsson et al., 2017; Rasmussen et al., 2015). The range of $sumTU_{algae}$ is higher than those of $sumTU_{invertebrates}$, which suggests higher variability in herbicide than insecticide pollution, the dominating pesticide groups that determine the corresponding toxicity. This may be because substitution of pesticides through mechanical labour including manual removal, horse ploughs or heavy agricultural machines is more feasible for herbicides than insecticides (Eurostat, 2017; Kovács-Hostyánszki et al., 2016; Lovász and Gurzău, 2013). However, in contrast with this explanation, we did not find a relationship of this gradient with agricultural intensity, i.e. average field size.

The $sumTU_{invertebrates}$ and $sumTU_{algae}$ were mainly driven by few pesticides. On average two to three (2.3 ± 0.8) pesticides contributed to 75 % of the $sumTU_{algae}$ (Table C.9), with terbuthylazine, metribuzin and 2,4-D dominating (Table C.10). Fewer compounds (1.5 ± 0.7) accounted for 75 % of the $sumTU_{invertebrates}$ (Table C.9), where the insecticides diazinon and imidacloprid dominated (Table C.10). These compounds were among those used by most farmers in Transylvania (Gurzău et al., 2008; Lovász and Gurzău, 2013), even though diazinon was in the EU during the field campaign no longer approved for field applications (EU, 2020). The few relevant pesticides per mixture support the approach of most routine monitoring programs to concentrate on pre-selected compounds (Moschet et al., 2014b; Schreiner et al., 2016), which enables cost and labour efficient analysis, but may also strongly underestimate risks if relevant compounds are missing (Malaj et al., 2014).

4.4.3 PASSIVE SAMPLING OF PESTICIDES

Calculated concentrations derived from passive sampling are associated with uncertainties. First, flow velocity, below 0.2 m s^{-1}, is known to have a strong effect on sampling rates (Moschet et al., 2015; Vermeirssen et al., 2008), even though this effect can be masked by other factors like water matrix or different analytical methods (Schreiner et al., 2020). Since sampling rates across studies seem to be independent of the flow velocity over a range of 0.1 and 0.9 m s^{-1} (Schreiner et al., 2020), we consider flow velocity differences between the determination of sampling rates and the deployment of passive samplings as minor. Nonetheless, in four sites the flow velocities were below 0.2 m s^{-1} during either deployment or retrieval (min. 0.03 m s^{-1}). During rainfall events, however, the flow velocity was presumably

always higher than 0.2 m s^{-1}, because rainfall events typically lead to a strong increase in flow velocity. This assumption is supported by an observed minimum increase in the water level of 50 cm at the respective sites. To however, increase the quality of the results, we discarded single samplers, where flow velocities were during deployment as well as during retrieval below 0.1 m s^{-1}.

Therefore, we assumed that flow velocity differences at the single sites only had a minor influence on the resulting calculated concentrations. Indeed, the calculated concentrations of the six most relevant pesticides (2,4-D, terbuthylazine, metribuzin, diazinon, imidacloprid and thiacloprid; details above) exhibited only a weak relationship to the measured flow velocity during deployment and retrieval of the passive samplers (all $|r| < 0.22$, $p > 0.05$). The effect of flow velocity on the PDMS sheets, however, has not yet been investigated (Moschet et al., 2014a). Since only one sampling rate for all compounds was available (see above), effects of flow velocity may overlap with inaccuracies related to sampling rates. The two sampling periods of PDMS sheets differed approximately 7-fold in precipitation (Table C.3), most likely leading to different average flow velocities. However, these differences did not translate to a clear trend of calculated concentration differences between the periods, suggesting a continuous exposure with very low concentrations of the insecticides sampled via PDMS sheets (pyrethroid and organophosphate) (Liu et al., 2004). Finally, PDMS compounds only accounted for a maximum of 7 % of the $sumTU_{invertebrate}$ and were irrelevant for $sumTU_{algae}$ (max. 0.6 %, Table C.11) when assessed together with the related peak exposure samples of the SDB disks. Hence, we suggest that potential concentration inaccuracies due to flow are largely irrelevant for the maximum toxicity assessment. When regarding all sampling events individually, however, the PDMS compounds were clearly relevant during base exposure (Table C.11).

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

Another uncertainty when using passive sampling to assess toxicity risks is that the pesticide peak duration can vary. Usually, depending on several parameters including stream size, topography and soil structure, peak durations range between one and three days (e.g. Wittmer et al., 2010). Based on this we calculated peak concentrations for the SDB disks from peak exposure samples (Table C.3) using an estimated peak duration of two days (t = 2, equation 4.1). A previous study showed that the calculated peak concentrations using this approach may approximate 50 % of the maximum concentration (Schreiner et al., 2020). For the PDMS sheets, we used a time span corresponding to their exposure time, because they were independent of pulses associated with rainfall events. Based on this, our approach is biased towards acute toxic effects. Lipophilic compounds may be more relevant when using chronic toxicity data to evaluate data on chronic exposure.

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

4.4.4 DRIVERS OF PESTICIDE TOXICITY

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

In contrast to our hypothesis that local scale variables are most important, the catchment scale variables catchment size, as well as the fraction of agricultural land use within the catchment, were the most relevant drivers of $sumTU_{invertebrates}$ (Table C.12). Both variables were positively related to the $sumTU_{invertebrates}$ (Fig. C.2). The positive relationship between $sumTU_{invertebrates}$ and agricultural land use within the catchment matches earlier studies (Rasmussen et al., 2011; Szöcs et al., 2017). Catchment size and either pesticide toxicity or exceedance of regulatory threshold concentrations were not (Szöcs et al., 2017) or negatively (Schulz, 2004; Stehle and Schulz, 2015) related in previous studies. The contrasting results of our study compared to those with negative relationships might be due to our study having a much narrower range of catchment areas.

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

Two other local scale variables, riparian buffer width and agricultural land use within this buffer were selected as drivers for $sumTU_{invertebrates}$ though the relationships were weak (Fig. C.2). This weak relationship of buffer width and the $sumTU_{invertebrates}$ contrasts previous studies and our hypothesis of local scale variables being most important. The relevance of buffers may be low if their effects are neutralised by erosion rills (Bunzel et al., 2014; Stehle et al., 2016), which were not detected across the 100 m section of the sampling sites. Our results suggest that the accumulation of pesticides of a multiplicity of fields in the whole catchment is more relevant than local riparian buffers. Notwithstanding, the agricultural intensity, in terms of average field size (Pe'er et al., 2014) was not selected as a driver for the $sumTU_{invertebrates}$, which can be explained by the fact that the number of pesticides as well as sum concentrations were in similar ranges as previously detected in streams of high intensity agriculture (see above). Also, the factorial agricultural intensity based on the observed agricultural practices and to the average field size was not related to the $sumTU_{invertebrates}$ (ANOVA; $F = 0.23$, p-value = 0.79). This suggests that pesticide pollution, at least in our study region is independent of agricultural intensity. Even sites with low agricultural intensity in terms of small field size and lacking access for heavy machines, had $sumTU_{invertebrates}$ of up to -0.17. Overall, the ratio of agricultural land use was more important for invertebrate toxicity than agricultural intensity.

The $sumTU_{algae}$ is, according to the elastic net approach, not driven by any of the analysed variables (Table C.12). The lack of relevant drivers on catchment as well as local scale may be explained by the physico-chemical properties such as higher water solubility and lower lipophilicity of herbicides, which dominated the $sumTU_{algae}$. These physico-chemical properties may be associated with a stronger independence of pesticide input from rainfall events.

Additionally, the lack of identifiable relationships between $sumTU_{algae}$ and the analysed drivers might be due to a possible substitution of herbicide applications through mechanical labour

(see above), indicating another not identified or measured variable driving the sumTU_{algae} or an insufficient number of observations.

4.5 CONCLUSION

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

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5 SIMILAR RECOVERY TIME OF MICROBIAL FUNCTIONS FROM FUNGICIDE STRESS ACROSS BIOGEOGRAPHICAL REGIONS

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5.1 ABSTRACT

Determining whether the structural and functional stress responses of communities are similar across space and time is paramount for forecasting and extrapolating the consequences of anthropogenic pressures on ecosystems and their services. Stream ecosystems are under high anthropogenic pressure; however, studies have only examined the response of stream communities across large scales over multiple generations. We studied the responses of leaf-associated microbial communities in streams within three European biogeographical regions to chemical stress in a microcosm experiment with multiple cycles of fungicide pollution and resource colonisation. Fungal community composition and the ecosystem function leaf decomposition were measured as response variables. Microbial leaf decomposition showed similar recovery times under environmental levels of fungicide exposure across regions. Initially, the decomposition declined (between 19 to 53 %) under fungicide stress and recovered to control levels during the third cycle of pollution and colonisation. Although community composition and its stress response varied between regions, this suggests similar functional community adaptation towards fungicide stress over time. Genetic, epigenetic and physiological adaptations, as well as species turnover, may have contributed to community adaptation but further studies are required to determine if and to which extent these mechanisms are operating. Overall, our findings provide the first evidence of a similar functional response of microbial leaf decomposition to chemical stress across space and time.

5.2 INTRODUCTION

Human activities are altering ecosystems globally at an unprecedented magnitude. In this context, streams are among the ecosystems with the highest risk of biodiversity loss, and such losses may hamper the perpetuation of crucial ecosystem services. Major anthropogenic stressors for stream ecosystems include habitat degradation, climate change, nutrient enrichment, and chemical pollution (e.g., pesticides) (Vörösmarty et al., 2010). Several of these stressors, such as climate change and nutrient enrichment, are close to or are already exceeding their planetary boundaries (i.e. the stressor-related global biophysical thresholds) (Steffen et al., 2015). Crossing these boundaries could result in irreversible systemic state shifts with adverse consequences for human societies (Barnosky et al., 2012). However, a planetary boundary for man-made chemical pollution has not yet been quantified. Although the physico-chemical properties of certain pollutants allow for their long-range transport and occurrence on a global scale (MacLeod et al., 2014), chemical pollution predominantly affects local processes (Rockström et al., 2009). Setting thresholds, such as planetary boundaries, as well as to reliably extrapolate results across regions would require a similar local stress response (Brudvig, 2017; Clements and Rohr, 2009).

Few studies have examined ecological stress responses in stream ecosystems across large biogeographical scales (Boyero et al., 2016; Chauvet et al., 2016; Pont et al., 2006; Schäfer et al., 2012; Utz et al., 2009; Woodward et al., 2012). Similar responses from regional to global scales have been observed in assemblages of macroinvertebrate and fish communities to urbanisation (Utz et al., 2009), pesticides (Schäfer et al., 2012), and hydromorphological disturbances (Pont et al., 2006). For macroinvertebrate-mediated leaf decomposition, which is an important energy-providing function in stream ecosystems(Webster, 2007), similar responses towards nutrient enrichment were found on a European scale(Woodward et al., 2012), whereas the impacts of temperature changes at European(Chauvet et al., 2016) and worldwide scales (Boyero et al., 2016) were partially contradictory. Moreover, it remains largely unexplored if stress responses across regions show a similar temporal pattern. This limits our ability to identify general mechanisms underlying community responses such as evolutionary (e.g. genetic adaptation) and ecological processes (e.g. competition) as well as eco-evolutionary feedbacks (Cadotte and Tucker, 2017). Microbial communities represent an ideal organism group to study temporal and spatial stress response patterns because of their high reproduction rates and rapid adaptation to stress (Graham et al., 2016).

We examined the functional (i.e., leaf decomposition) and structural (i.e., community dynamics) stress responses of microbial communities in different European biogeographical regions over multiple cycles of pollution and resource colonisation. We used a model system consisting of leaf-associated microbial decomposer communities and a fungicide mixture as a stressor. One part of these microbial communities is the polyphyletic fungal group of aquatic hyphomycetes that are crucial for leaf decomposition in streams (Pascoal and Cássio, 2004). Several representatives of this group are cosmopolitan (Duarte et al., 2016), thereby allowing for comparisons of taxa-specific spatial responses. Fungicides were chosen as the stressor because they are designed to suppress fungal pathogens and are known to affect non-target fungi, such as aquatic hyphomycetes (Fernández et al., 2016). In addition, fungicides are transported into surface waters during or after their application in catchments (Bereswill et al., 2012). The involvement of multiple hyphomycete colonisation and decomposition cycles enabled studying potential microbial community acclimatisation and adaptation to fungicide stress. The experiment was performed with communities from three biogeographical regions (the Central Plains, Denmark; the Western Highlands, Germany; and the Fenno-Scandian Shield, Sweden) to detect if functional as well as structural stress responses caused by fungicides are similar across space.

Using this experimental design, we found an initial reduction in leaf decomposition by fungicide exposure, followed by recovery within a similar time period. This similar recovery period suggests functional adaptation towards fungicide pollution over the course of the experiment. The structural responses in terms of shifts in the aquatic hyphomycete community composition,

however, varied across the biogeographical regions, with the number of colonisation and decomposition cycles, fungicide exposure or the interaction of these factors acting as the main drivers of the effects.

5.3 RESULTS

5.3.1 GENERAL EXPERIMENTAL DESIGN

The experiments were conducted in three biogeographical regions (Central Plains, Silkeborg, Denmark; Western Highlands, Landau/Pfalz, Germany; and Fenno-Scandian Shield, Uppsala, Sweden; European Environment Agency, 2016) over seven weeks following the same protocol (for details, see the Methods section). Briefly, three consecutive sets of leaf material were used that corresponded to three microbial colonisation and decomposition cycles (Fig. 5.1 a, b). While the first leaf set was colonised by microbial communities in unpolluted streams, the other two leaf sets were colonised in microcosm systems by the leaf-associated microbial community present on the leaves of the previous cycle. With this setup, potential adaptations of the whole aquatic hyphomycete communities under fungicide stress were captured over the three consecutive cycles. However, adaptation on the community level integrates various factors including: genetic, epigenetic and physiological (e.g. phenotypic plasticity) adaptations as well as species turnover, of which we only measured the latter indirectly through sporulation.

Vegetative growth and reproduction both contribute to the community dynamics in aquatic hyphomycetes, where reproduction is typically triggered through resource (leaf) availability, which can be periodical (Bärlocher, 1992). Our setup allowed us to track the implications on reproduction because the colonisation of new resources through sporulation is usually more sensitive towards stress than vegetative growth (Lecerf and Chauvet, 2008). Several studies using both sporulation and molecular methods found that the strongest dynamic in community composition and sporulation of aquatic hyphomycetes typically occurs within the first two to three weeks after the initial colonisation (Bärlocher, 1992; Pascoal and Cássio, 2004). Therefore, we set the duration of each cycle to three weeks.

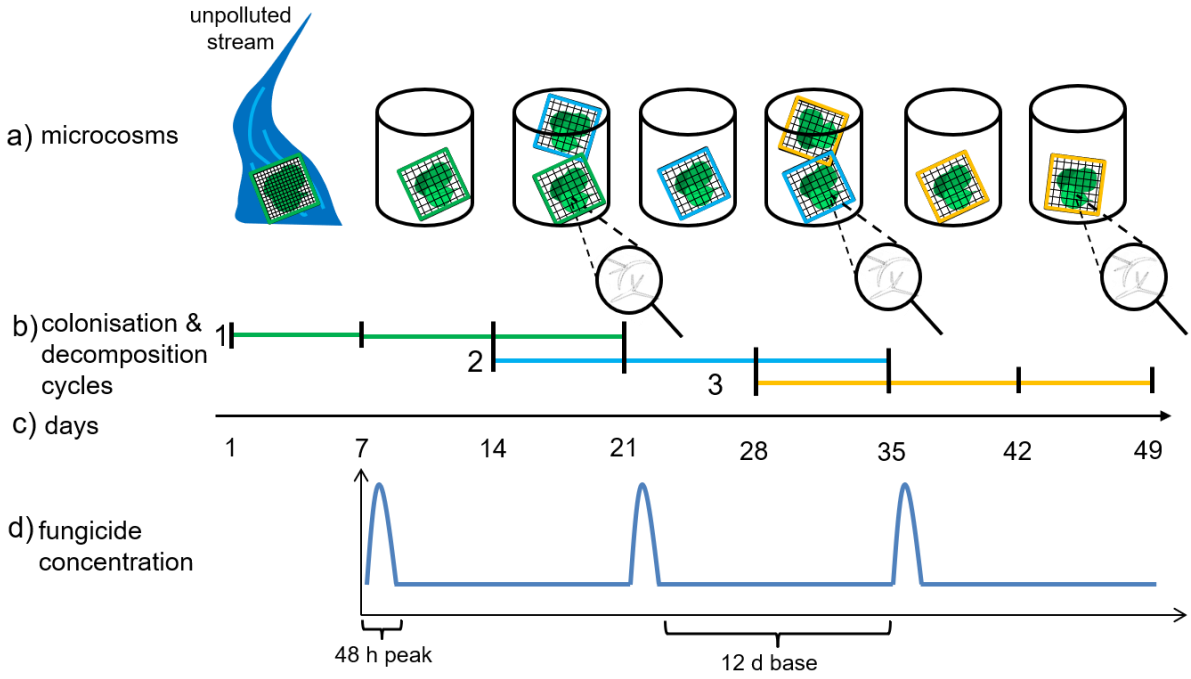

Fig. 5.1: Schematic illustration of the experimental design. Coloured lines in the middle of the figure indicate leaf sets, which correspond to the three colonisation and decomposition cycles (b). The first leaf set (green) was colonised in unpolluted streams for 1 week and subsequently used in the experiment. Leaf sets 2 and 3 (blue and orange, respectively) were colonised in the microcosms by the microbial community from previous leaf sets for 1 week (overlapping bars) (a, c). At the end of each cycle, the decomposed leaf mass was quantified, and the aquatic hyphomycete community composition was determined based on fungal spore production. The fungicide exposure pattern (three 48-hour peaks, each followed by 12-day baseline exposures) is depicted in the lower panel (d).

The microcosms were subjected to two treatments: a control treatment and a fungicide treatment. The latter consisted of 48-hour peak exposures followed by 12-day base exposures with fungicide concentrations of 10 % of the peak (Fig. 5.1 d). This exposure pattern mimicked the higher levels of pesticide toxicity identified in a meta-analysis of global pesticide levels (Schäfer et al., 2012) and the subsequent baseline exposure resulting from drainage within catchments (Bundschuh et al., 2014) (information on the fungicide mixture is presented in Table D.1).

5.3.2 FUNCTIONAL RESPONSES

Leaf decomposition showed a different temporal stress response across biogeographical regions (interaction of factors “cycle” and “region”: likelihood ratio = 19.2, $p < 0.001$). This was

mainly due to different effect sizes related to the initial reduction in leaf decomposition by fungicide exposure. The effect size for Germany in the first cycle was twofold higher (approximately 55 %) than for Denmark and Sweden (approximately 25 and 20 %, respectively), and the reduction was significant in all regions (Fig. 5.2; Table D.2). This effect attenuated during the subsequent cycles and leaf decomposition approached control levels during the third cycle in all regions (Fig. 5.2; Table D.2). Thus, despite different effect sizes, the overall effect pattern and recovery time were similar across regions. Indeed, the functional effect pattern and recovery time over the cycles matched with additional data from the South Eastern Highlands (Australia), which highlights the potential transferability of the functional responses to other regions (Table D.4 and Fig. D.1).

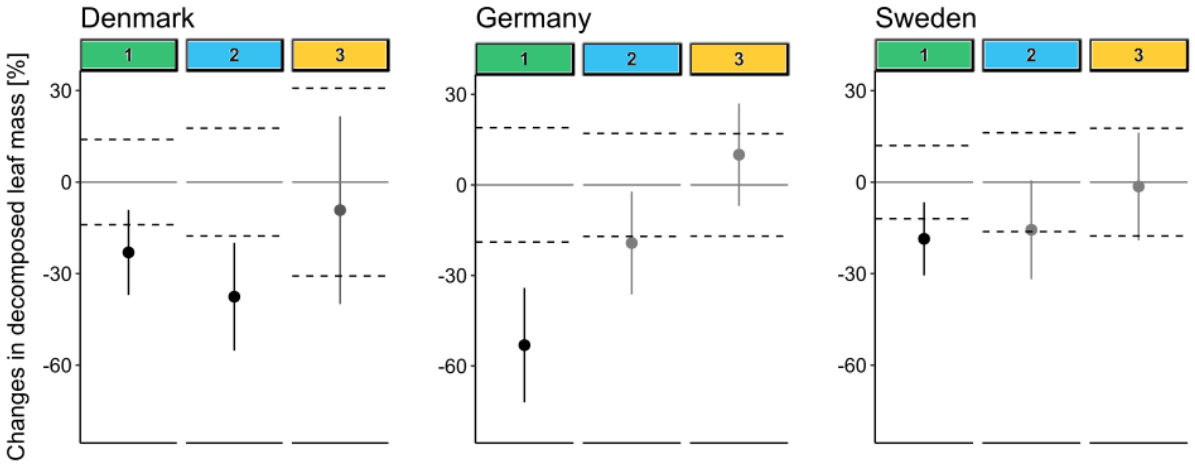

Fig. 5.2: Mean percentage change in decomposed leaf mass. Changes in the decomposed leaf mass under fungicide stress compared with the respective controls (in %, with 95 % confidence intervals; solid horizontal lines represent the controls, and dashed lines indicate the corresponding 95 % confidence intervals) for the different cycles (numbers on top; colour code refers to Fig. 5.1; Denmark and Germany n = 7 and Sweden n = 6). Significant differences at α = 0.05 between the controls and the fungicide treatments (t-test; Table D.2) are depicted with asterisks.

5.3.3 STRUCTURAL RESPONSES

Cosmopolitan hyphomycete taxa occurring in all three experiments showed contrasting responses to fungicide stress and the cycles in terms of spore production (type II analysis of variance, ANOVA). For example, the spore production of *Articulospora tetracladia* (Ingold) decreased with fungicide exposure over the cycles in Denmark but not in Germany and Sweden. The same taxon also responded to cycles in the absence of fungicides in Sweden but not in Denmark and Germany. The sporulation of *Tetrachaetum elegans* (Ingold)

decreased over the cycles in the absence and presence of fungicides in Germany but not in Denmark and generally sporulated at very low densities in Sweden (Table D.3).

Redundancy analyses (RDAs) revealed different drivers of structural responses among the biogeographical regions (Fig. 5.3). In Denmark, the interaction between cycles with fungicide exposure was significant and explained approximately 9 % of the variation. Although care should be taken when interpreting the main effects in the presence of interactions, the cycle seemed to have the highest individual influence on the community composition (explained variance: 41 %; Table 5.1), which was also the only significant variable for the community composition in Germany that explained 17 % of the variance. In Sweden, however, fungicide exposure was the main driver of the community changes (explained variance: 22 %), and only a minor impact of the cycle (8 %) and a non-significant interaction between the cycle and fungicide exposure were observed (Table 5.1).

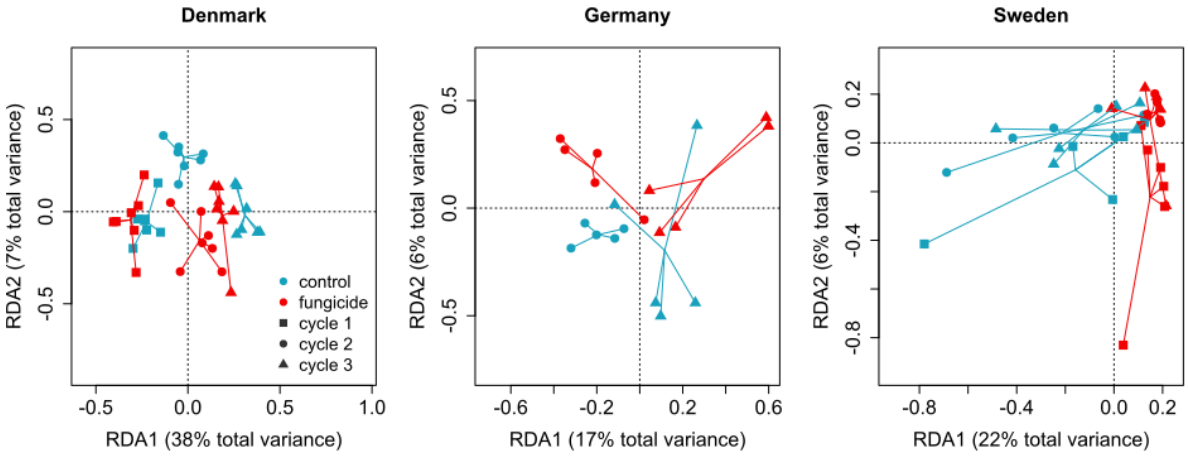

Fig. 5.3: Hyphomycete community composition across cycles and treatments. Each point within the RDAs represents the community of one replicate. Replicates of one treatment are connected through lines (Denmark n = 7, Germany n = 5 and Sweden n = 6). Because of technical difficulties, data are not available for the hyphomycete community from the first cycle in Germany.

Table 5.1: Explained variance and p-values of the RDA variables tested by type III ANOVAs, separated by biogeographical region and explanatory variables. Bold p-values indicate statistical significance.

Region	Explanatory variable	df	t	p-value	Explained variance (%)
Denmark	Fungicide	1	1.2	0.263	2.9
	Cycle	2	13.7	**0.001**	41.3
	Fungicide × Cycle	2	3.6	**0.002**	9.3
Germany	Fungicide	1	1.2	0.299	6.3
	Cycle	1	3.6	**0.001**	16.5
	Fungicide × Cycle	1	1.1	0.365	5.1
Sweden	Fungicide	1	9.1	**0.001**	21.6
	Cycle	2	1.4	0.224	8.0
	Fungicide × Cycle	2	0.4	0.917	1.5

5.4 DISCUSSION

We found similar effect patterns and recovery time periods of leaf decomposition from fungicide exposure across biogeographical regions. More specifically, leaf decomposition declined during the first cycle, but this effect was attenuated during the following two cycles (Fig. 5.2). The initial response is typical for leaf-associated microbes inhabiting unpolluted systems when exposed towards organic fungicides (Rasmussen et al., 2012) that predominantly inhibit the biosynthesis of ergosterol, RNA and proteins (Fungicide Resistance Action Committee, 2017) and consequently affect the growth or activity of aquatic hyphomycetes by compromising their leaf decomposition ability. The similar time spans of recovering leaf decomposition during the following cycles in all regions (Fig. 5.2) suggest that the microbial communities, which originated from unpolluted streams, adapted to fungicide stress and ultimately recovered their functional performance irrespective of slight differences in the applied fungicide mixture (Table D.1). This can be explained by the concept of pollution-induced community tolerance (PICT), which assumes that communities increase their overall tolerance as a result of genetic, epigenetic or physiological (e.g. phenotypic plasticity) adaptations and/or species turnover and thus functional responses are not, or minimally, affected under stress (Blanck et al., 1988). Although our data suggest that a PICT was developed during the present study, identification of the dominant underlying mechanisms (e.g. genetic and species turnover) resulting in community adaptation requires future studies (Feckler et al., 2018; Gardeström et al., 2016). The functional pattern was similar despite different fungal communities in the test systems, and relatively stable decomposition patterns without fungicides across the cycles in Germany,

whereas slight and stronger declines in decomposition over time were observed in Sweden and Denmark, respectively (Fig. D.2).

The similarity in the pattern of the functional response and its recovery time span was not reflected in the structural responses, neither by means of the cosmopolitan occurring fungal species nor by taxa richness of the aquatic hyphomycete communities across biogeographical regions. Chemical stress can act as a strong selection pressure (Lopes et al., 2009) and an environmental filter, through which sensitive taxa are replaced by tolerant competitors. If environmental filtering (here fungicide stress) is the only driver for the community structure, then cosmopolitan aquatic hyphomycete taxa should show a similar response across spatial scales (Kraft et al., 2015). However, the cosmopolitan hyphomycete taxa occurring in communities from all biogeographical regions (Table D.3) responded differently to fungicide exposure and the cycles. This observation supports Cadotte and Tucker (Cadotte and Tucker, 2017), who stressed the importance of other mechanisms, such as ecological processes (e.g. competition and predation) and the interaction of biotic processes with environmental filtering, which may be the main drivers of responses at the community level.

The taxa richness of the hyphomycete communities declined in all biogeographical regions. This, as well as shifts in the composition of the hyphomycete communities, exhibited partially different drivers among the regions (Fig. 5.3; Table 5.1; Table D.5). Sporulation within all three biogeographical regions was mostly driven by one or two hyphomycete taxa per replicate, which contributed between 50 and 100 % of the total sporulation. When assessing the taxon richness based on sporulation, Denmark and Sweden showed comparable tendencies. The sporulating aquatic hyphomycete taxa decreased by up to 50 % over the cycles in both the fungicide treatment and the control (Table D.5, Fig. D.3). This decrease was driven by the elimination of hyphomycete taxa with low sporulation rates, which seems common in microcosms (Drake, 1991). Taxa with lower relative sporulation rates were potentially outcompeted by those with higher sporulation rates when colonising the leaves of the next cycle (Treton et al., 2004). However, the sporulation peaks vary based on the taxon and the environment (Gessner et al., 2007; Pascoal and Cássio, 2004), and under field conditions, low sporulating taxa from the first cycle might sporulate more strongly subsequently. Notwithstanding, non-sporulation does not translate to absence, and the taxa may still have been present and contributed to decomposition during all cycles (Duarte et al., 2017). The pattern of decreases in sporulating taxa may also apply to the communities from Germany, although the lack of data from the first colonisation cycle prohibits a definite interpretation (see the Methods section). Nevertheless, the minor importance of fungicide exposure for community dynamics in Denmark and Germany was reflected in a relatively similar taxa richness and composition across the treatments and cycles (Fig. 5.3; Tables D.3, D.5, Fig. D.2).

This similarity suggests that the taxa thriving under experimental conditions may also exhibited a higher tolerance towards fungicides and may have driven decomposition. Similarly, the Swedish communities consisted of the same taxa irrespective of the fungicide treatment (Table D.3). Nevertheless, the sporulation of fungal taxa was reduced by fungicides, suggesting fungicide stress was the main driver for this structural response (Fig. 5.3, Table 5.1).

The difference in the effect sizes of leaf decomposition between Sweden and Germany during the first cycle and its faster attenuation in Sweden compared to Denmark (Fig. 5.2) might be explained by the higher dissolved organic carbon content of the stream water used as a test medium in Sweden (48 mg L^{-1}) compared with Germany (< 1 mg L^{-1}) and Denmark (1.3 mg L^{-1}) (Pradhan et al., 2016). The associated enzymatic inventory of certain aquatic hyphomycetes allows them to degrade and utilise organic compounds (including xenobiotics) as an alternative source of nutrients (Krauss et al., 2011). Energetic investments in detoxification processes and the supply of additional nutrients could, to some extent, have reduced the fungicide stress experienced by the whole community and facilitated higher leaf decomposition.

Although general conclusions derived from communities of only one single stream per biogeographical region and stressor concentration are considered speculative, our findings represent the first evidence of a similar functional response of microbial leaf decomposition to chemical stress across space and time. Such knowledge on similar stressor responses could help to forecast future stress responses, which is important to inform managers of ecosystems aiming to reduce human-induced effects through mitigation measures (Brudvig, 2017; Clements and Rohr, 2009), but also to model ecosystem response towards future stress. Additionally, comparing stress responses across large biogeographical scales should inform efforts to establish planetary boundaries for chemical pollution. Finally, our study implies that the future risk assessment of chemicals needs to focus on both, ecosystem function and community composition, as their response patterns can differ.

Future studies should scrutinise the response to multiple concentrations levels and mechanisms of community adaptation. While microbial communities have been used as model systems for eco-evolutionary processes and stress adaptations, the translation of the results to higher trophic levels that drive important stream ecosystem functions (e.g., insects or vertebrates) remains open. Although community adaptations of higher trophic levels to stress are likely to occur over longer time periods, because of longer generation times, the steadily increasing stress load under climate change and increasing human populations may exceed the time frame required for adaption, exacerbating functional losses (Delcour et al., 2015). Moreover, although microbial systems are informative regarding the potential occurrence of similar responses, higher-level studies linking short-term, eco-genomic approaches with

ecological models are required. Such studies may ultimately help to determine the effect thresholds for ecosystem management and the planetary boundaries for chemical stressors.

5.5 METHODS

5.5.1 FUNGICIDE SELECTION AND APPLICATION SCENARIOS

A mixture of three to four fungicides with distinct modes of toxic action was used during the experiment (inhibitors of ergosterol, RNA and protein synthesis (Fungicide Resistance Action Committee, 2017); see Table D.1). Microcosms were spiked individually using fungicide mixture solutions shortly before test initiation and after media renewal between the peak and baseline exposure scenario (see below) to achieve the nominal concentrations. Each of the fungicides contributed equally to the toxic potential of the mixtures based on the effective concentration that reduces the growth of test organisms (hyphomycetes when data were available; otherwise, *Pseudokirchneriella subcapitata*; Table D.1). The fungicides were applied as short-term episodic peaks (48 h) at levels representing the upper end of pesticide toxicity in the field identified in a meta-analysis (Malaj et al., 2014). These peaks were interspersed by base exposures at 10-fold lower concentrations (12 d; see Fig. 5.1; for more details see Supplementary Information) (Reilly et al., 2012). A fungicide-free control treatment was run in parallel to this fungicide treatment. The test medium (pre-filtered stream water) in the microcosms together with the respective fungicide concentrations was renewed after each peak exposure until 7 d after base exposure to ensure constant water quality and exposure to the fungicides. Liquid chromatography high-resolution mass spectrometry was used to verify the fungicide concentrations in the respective microcosm experiments (see Tables D.6, D.7).

5.5.2 LEAF DECOMPOSITION

Senescent but undecomposed leaves (*Alnus glutinosa* (L.) Gaertn.) were collected shortly before leaf fall and stored at -20°C (for coordinates, see Table D.9). The soluble leaf components were leached from the leaves in aerated ultrapure water for 48 h before the experiment. This leaching can result in a mass loss of up to 30 % (Petersen and Cummins, 1974). Next, the leaves were dried to constant weight at 60°C, and sets of leaf material were prepared (per replicate: 4 g (Germany; microcosm volume: 4 L) or 2 g (Denmark and Sweden; microcosm volume: 1 L), all ± 0.01 g) and packed into nylon fine-mesh leaf bags (mesh size: 2 mm). For the first colonisation and decomposition cycle, leaf bags (Denmark and Germany: n = 14, Sweden: n = 12) were submerged for 7 days in unpolluted streams for microbial colonisation upstream of any urban or agricultural influence (Fig. 5.1; for the coordinates, see Table D.10). Afterwards, the leaf bags were retrieved, and the leaf material was introduced into the microcosms after the removal of invertebrates and sediment under running tap water. The microcosms contained pre-filtered water from the respective stream sites (to reduce confounding effects on aquatic hyphomycetes caused by changes in water quality; the abiotic

water parameters are shown in Table D.8) and were spiked with the fungicide mixtures of the respective concentrations. For cycles 2 and 3, the leaf bags were directly inserted into the microcosms (after re-soaking in ultrapure water to avoid floating) to allow for colonisation by the microbial assemblages from the previous cycle (Fig. 5.1 c, d). The experiment was conducted under aeration in darkness and at a temperature as close as technically possible to the mean temperature of the stream where the leaves were colonised (Table D.8). At the end of each cycle (i.e., at days 21, 35, and 49; Fig. 5.1), the remaining leaf material was retrieved from the microcosms, leaf discs were cut for the aquatic hyphomycete analyses (see below), and the remaining leaf material was dried to constant weight at 60°C and weighed to the nearest 0.01 g. The decomposed leaf mass (DLM) per degree day was calculated for each replicate as follows:

$$DLM = \frac{\left(\frac{S_i(0) - S_i(t)}{S_i(0)}\right) * 100}{\frac{\left(\sum_j^t \bar{T}_i(j)\right)}{n_j}} \quad (5.1)$$

where S is the leaf mass as a function of deployment time t; T is the mean temperature for a day j of each replicate i; $S_i(0)$ is the leaf mass at the start of each cycle, and n_j refers to the number of experiment days.

We conducted an additional experiment with the same setup in the South Eastern Highlands (Canberra, Australia) (Mackay, 2016) to assess the transferability of the data on the functional response obtained in Europe. In this additional experiment, only the decomposed mass of *Eucalyptus camaldulensis* (Dehnh.) leaves was quantified following the same test protocol (Fig. 5.1). Because of the absence of information on the hyphomycete community composition, we present these data solely in the Supplementary Information. Due to technical difficulties, data on the decomposed leaf mass of cycle 1 are missing.

5.5.3 AQUATIC HYPHOMYCETE COMMUNITY DYNAMICS

We evaluated the aquatic hyphomycete community dynamics based on the number of produced fungal spores because sporulation should have a higher sensitivity to stress than vegetative growth (Lecerf and Chauvet, 2008). To this end, five leaf discs (⌀ 1 cm) were cut from randomly chosen leaves of each replicate and pooled as one sample (Denmark: n = 7, Germany: n = 5, Sweden: n = 6) at the end of each cycle (i.e., at days 21, 35, and 49; Fig. 5.1). To induce the sporulation of aquatic hyphomycetes, the leaf discs were submerged in water of the respective replicate and orbitally shaken at 120 rpm in darkness at the respective experimental temperature (Table D.8). After 72 h, conidia were prevented from agglomerating

using 0.5 % Tween80 and fixed in 2 % formalin, and the samples were stored at 4°C. Directly before identification, an aliquot of the conidia suspension was homogeneously filtered over a gridded membrane filter (0.45 µm), and retained conidia were stained with lactophenol cotton blue. At least 300 conidia were identified for each replicate (100-400x magnification) primarily using the key provided by Gulis et al. (2005). The number of counted conidia was normalised to the total filter surface, sample volume and dry weight of the respective set of leaf discs. Because of a malfunction of the orbital shaker, conidial identification was not possible for the first cycle in Germany.

5.5.4 DATA ANALYSIS

For the statistical analyses of the decomposed leaf mass, a linear mixed-effect model was fitted, where the response was the percentage difference of the fungicide treatment to the fungicide-free control across all biogeographical regions. This model included the factors “region” (categorical; Denmark, Germany, and Sweden) and “cycle” (categorical; 1, 2, and 3) and their interaction, with the replicates nested within the regions. Subsequently, we fitted separate models for the different biogeographical regions using the absolute decomposition values, to identify potentially contrasting drivers of changes in decomposition and simplify interpretation. The factors “cycle” and “fungicide exposure” (categorical; control and treated) and their interaction were included as explanatory variables in all these models. The factor cycle was used as a fixed factor, since the trajectory was of interest, and not used as random factor for temporally dependent data. The effects of these explanatory variables as well as their interactions on the decomposed leaf material were analysed by type II ANOVAs using F-tests. To distinguish significant differences between the fungicide treatment and the respective control, t-tests of each cycle and biogeographical region were run separately to focus on the differences within and not between the cycles. The p-values were adjusted for multiple testing using the multivariate t distribution according to Hothorn et al. (2001). Additionally, the percent change compared with the respective control treatment and the 95 % confidence intervals are provided.

Differences in the aquatic hyphomycete community composition were analysed - separately for the different regions - using RDAs with Hellinger transformation to achieve standardisation and circumvent problems associated with the Euclidean distance for ecological data (Legendre and Gallagher, 2001). We run separate models because the communities differed vastly between the regions, which on the one hand reduces the power to detect responses of taxa occurring in single regions and on the other hand may mask potentially contrasting responses of the same taxa in different regions. The proportion of variance explained by the explanatory variables was first calculated by individual models for either the cycle or the fungicide treatment and then calculated using both variables together, including their interaction. The significance

of the RDAs was checked using permutational type III ANOVAs (Legendre et al., 2011). Analyses of the changes in the occurrence of single hyphomycete taxa were analogous with the analyses of the remaining leaf mass. All statistical analyses and graphs were conducted in R (version 3.3.3; R Core Team, 2017) and supplemented by the required add-on packages. The term “significant(ly)” is exclusively used in the sense of “statistical significance” at a level of 0.05.

5.5.5 DATA AVAILABILITY

The datasets analysed during the current study are available in the GitHub repository https://github.com/rbslandau/schreiner_simstress.

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6 GENERAL DISCUSSION AND OUTLOOK

6.1 PATTERNS OF DETECTED PESTICIDES IN STREAMS

Herbicides dominate pesticide mixtures in streams in terms of detected compounds as well as concentrations (Fig. 2.3, Table D.2 (only available on attached CD)). We were able to detect similar patterns in two studies, where we (i) compiled routine pesticide monitoring data based on grab sampling (chapter **2**) and (ii) conducted monitoring using passive sampling during base flow and rainfall events (chapter **4**). Our results were in line with findings of several other studies, suggesting a widespread pattern (e.g. Gilliom et al., 2006; Moschet et al., 2014b; Stone et al., 2014). The dominance of herbicides is not unexpected since they overall are the most frequently used and sold pesticide group in terms of amounts (Atwood and Paisley-Jones, 2017; Eurostat, 2018; Köhler and Triebskorn, 2013). Due to their high application amounts, persistence and water solubility, which facilitates transport processes, herbicides enter streams in relatively high concentrations. This enables detection with a wide range of methods despite relatively high LOQs or during base flow conditions like sampled in routine monitoring.

In general, pesticides detected commonly or in considerable concentrations were sold in high quantities (Moschet et al., 2014b) or were among those used regularly (chapter **4**). For example, neonicotinoid insecticides such as imidacloprid and thiacloprid were detected widely spread in single studies at sampling times while they were still approved for field application (Hladik et al., 2014; Moschet et al., 2014b, chapter **4**). However, when analysing routine monitoring data insecticides such as neonicotinoids as well as fungicides were only detected scarcely or in specific countries (chapter **2**). This can be attributed to several reasons like the lack of sampling during rainfall events, limitations of the analysed compound spectrum and insufficiently low LOQs (these factors are discussed below). Additionally, several insecticides like pyrethroids and organophosphates are lipophilic (Casida and Quistad, 2004) and thus more likely to adsorb to particles, which hampers detection in the water phase (Domagalski et al., 2010; Moschet et al., 2014a). Based on this and their high potential toxicity, targeted sampling of these compounds is required (Knäbel et al., 2012; Moschet et al., 2014a, chapter **4**).

6.2 DRIVERS OF PESTICIDE EXPOSURE IN STREAMS

The occurrence and concentrations of pesticides in streams is related to their entry pathways. Variables directly or indirectly influencing the entry of pesticides and therefore the pesticide exposure in streams can be summed up as drivers. Since the majority of pesticides are applied in agriculture, agricultural land use in the upstream catchment of a sampling site influences the pesticide exposure in related streams. The agricultural land use can affect the number of detected pesticides (chapter **2**, Fig. A.4) and their toxicity towards invertebrates (chapter **4**,

Fig. C.2). These results are in line with other studies (e.g. Rasmussen et al., 2011; Szöcs et al., 2017). Even a relatively small ratio of agriculture (such as 7 %) can result in rather large pesticide mixtures (17 compounds) with a considerable sum concentration and estimated toxicity (chapter **4**).

Even though pesticide exposure responded consistently to the ratio of agricultural land use in upstream catchments over different studies, various studies detected heterogeneous relationships to the catchment size. In chapter **4**, we detected a positive relationship between catchment size and pesticide toxicity towards invertebrates (Fig. C.2). This could be explained by an accumulation of pesticides from diverse agricultural areas and thus larger absolute amounts of pesticides in streams (Blanchoud et al., 2004). In chapter **4**, we incorporated a relatively small sampling size with a rather narrow range of catchment areas. However, when incorporating data from larger monitoring campaigns, we as well as other studies found no (chapter **2**, Szöcs et al., 2017) or a negative relationship between catchment size and pesticide exposure in streams (Schulz, 2004; Stehle and Schulz, 2015). No or negative relationships might be caused by a dilution of pesticides since the size of the water body and its catchment are directly related. Specifically, during routine monitoring with varying LOQs and sampling of base exposures (discussed in chapter **6.3**), dilution might be a crucial point in missing occurring pesticides. Additionally, with increasing catchment size, pesticides potentially exhibit a longer travel time in the stream, leading to degradation and sorption to sediment or SPM. Identifying these mechanisms and clarifying the relationship between pesticide exposure and catchment size would be subject for future research.

The agricultural field size can influence the number of pesticides simultaneously detected in the stream. Since each field is usually cultivated with one crop, catchments with larger fields have a lower number of different crops. In regions with more homogenous crop cultivation, as e.g. in the USA (Eurostat, 2015), a lower number of pesticides are applied simultaneously, resulting in smaller pesticide mixtures (chapter **2**, Fig. 2.4). While field size can be a proxy for agricultural intensity (Pe'er et al., 2014), a gradient of low to high intensity agriculture showed no relationship to the pesticide exposure in streams (chapter **4**). The detected numbers, concentrations as well as toxicities of pesticides (Fig. 4.2) correspond to those detected in high intensity agriculture of Western Europe and the USA (Fernández et al., 2014; Guibal et al., 2018; Gustavsson et al., 2017; Moschet et al., 2014b; Nowell et al., 2018; Ulrich, 2015). Since we only analysed a limited number of sampling sites on a small spatial scale, no final conclusions of the relationship between agricultural intensity and pesticide exposure in streams can be drawn. Additional studies covering a larger spatial scale covering a similar range of agricultural intensity would be necessary to obtain a better picture of this relationship.

The major entry pathway of pesticides to streams is surface runoff caused by heavy rainfall. One driver which can influence this entry pathway is the buffer width, since runoff from fields has to pass buffers to enter streams. According to previous studies, increasing buffer width is negatively related to the pesticide exposure in streams (Dabrowski et al., 2002; de Snoo and de Wit, 1998). This effect can, however, be neutralised by the occurrence of erosion rills, since these facilitate the direct input of the runoff (Bereswill et al., 2012). Even though no erosion rills were detected across a 100 m section of the sampled stream sites, we only detected a weak relationship of the buffer width to the toxicity towards invertebrates (chapter **4**). The compound properties can have an additional effect on the retention ability of buffers. While pesticides with a low log K_{ow} are transported to streams unrelated to the buffer width, pesticide loads of compounds with higher log K_{ow} values in runoff are reduced with increasing buffer width (Reichenberger et al., 2007).

Besides the width of riparian buffers, their vegetation or the vegetation of areas where surface runoff is flowing can be relevant for pesticide exposure in streams (Hawes and Smith, 2005). Firstly, in fully vegetated buffers the development of erosion rills is reduced since plants and their roots stabilise the soil and thereby reduce erosion (Gyssels et al., 2005). Secondly, trees in combination with smaller plants like grass can reduce the pesticide concentrations of surface runoff (Hawes and Smith, 2005). Since pesticides in surface runoff stem from agricultural fields, they can be altered depending on the cultivated crop. Some crops, such as legumes, are known to reduce the volume of surface runoff during heavy rainfall events and fully grown legumes can reduce the related pesticide input up to 95 % (Garcia-Estringana et al., 2013). Based on the widespread cultivation of legumes in the USA, this might be another explanation for their relatively small pesticide mixtures in comparison to other analysed countries (chapter **2**). Not only runoff can be altered by vegetation, but also the entry pathway of spray drift can be reduced by taller plants between application and non-target areas (Schulz et al., 2001).

Based on the above observations, a wide riparian buffer with pronounced vegetation is a known mitigation measure to reduce pesticide exposure in streams (e.g. Lerch et al., 2017; Popov et al., 2006). Vegetated riparian buffers, however, are no general guarantee for reduced pesticide toxicity in streams. In chapter **4**, we detected contrasting tendencies to previous observations: increased pesticide toxicity with increasing average plant height (Fig. C.2). This detected relationship is likely due to misapplication and insufficient education of farmers regarding pesticide use, which was reported for the study region (Gurzău et al., 2008; Lovász and Gurzău, 2013).

6.3 LIMITATIONS OF CURRENT ROUTINE MONITORING

While routine monitoring based on grab samples (chapter **2**) shows that pesticide mixtures are rather small, i.e. comprised of few compounds (Belden et al., 2007, Fig. 2.4), pesticide monitoring incorporating a multiplicity of pesticides using active (Moschet et al., 2014b) or passive sampling (chapter **4**, Fig. 4.2) shows different results. Since routine monitoring is limiting its analysis to pre-defined compounds, it is likely that the actual pesticide exposure and its related potential risks on stream ecosystems are underestimated (Malaj et al., 2014; Moschet et al., 2014b). Varying monitoring programs between (Malaj et al., 2014, Fig. 2.1) but even within countries (Szöcs et al., 2017, Fig. A.3) hamper the comparability and interpretation of routine monitoring data. We demonstrate in chapter **2** that the number of detected compounds and therefore the size of pesticide mixtures is related to the number of analysed compounds (Fig. 2.2, Table 2.4). Since also non-detected compounds may pose threats to stream ecosystems, the reliable identification of possible threats by pesticides calls for an extension of the list of analysed compounds. An improved compound selection could be based on variables like sales numbers or application recommendations of respective pesticides, crop cultivation in the sampled area as well as, may be most importantly, exposure probability of non-target areas and toxicity to non-target organisms (Vijver et al., 2008; Wick et al., 2019).

Generally, limiting the number of analysed compounds in monitoring to increase cost and labour efficiency is reasonable. Several previous studies detected a high correlation between the most toxic compound and the toxicity of all compounds following the concept of concentration addition (Junghans et al., 2006; Schäfer et al., 2013). Additionally, studies were able to find clear effects of the most toxic compound on macroinvertebrate communities and the ecosystem function of leaf litter decomposition (Liess and Ohe, 2005; Münze et al., 2015; Rasmussen et al., 2012; Schäfer et al., 2007). This is in line with the findings of chapter **4**, where we showed that only one to three pesticides drive the pesticide toxicity to invertebrates and algae (Table C.9). A similar number of pesticides has been identified as relevant in previous studies (Gustavsson et al., 2017; Vallotton and Price, 2016).

Besides differences in number and selection of analysed compounds, analytical methods during monitoring programs of different regions or countries cause additional uncertainties. The analytical results might vary between laboratories of different regions and states, which can lead to a difference of the LOQs by nearly two orders of magnitude for one analysed compound (Table A.7). This can cause different detection frequencies of single compounds, drawing an incomplete picture of the actual pesticide exposure and could lead to compound compositions and concentrations, which are difficult to compare (Senseman et al., 2003). Effects on stream ecosystems, especially caused by insecticides, can be detected up to a factor 0.001 lower than the LC_{50} concentration of the most sensitive taxon within this pesticide

group (Schäfer et al., 2012). Based on this, analytical LOQs should be at least as low as the concentrations where effects can be detected to achieve a reliable characterisation of risk from pesticides (Lepom et al., 2009; Moschet et al., 2014b).

Besides uncertainties of routine monitoring such as the selection and analytics of pesticides, current routine sampling is conducted unrelated to rainfall events. Despite the fact that surface runoff induced by rainfall events is the dominating pathway of pesticide to streams causing pesticide peaks, targeted sampling of rainfall events is currently lacking (Bereswill et al., 2012; Weibel et al., 1964). Regardless of not considering rainfall events in their monitoring schedule, routine monitoring samples taken shortly after rainfall events show a slight increase of pesticide exposure (Szöcs et al., 2017). However, when directly comparing base to peak flow conditions using passive sampling, we were able to detect a 7-fold increase in sum concentration and two to three times more pesticide compounds (chapter **4**, Fig. 4.2). Previous studies comparing base and peak flow conditions using temporally resolved or event-driven active sampling even detected concentration differences from several single compounds of up to a factor of 100 (Leu et al., 2004; Rasmussen et al., 2015; Wittmer et al., 2010). This increase in pesticide occurrence and concentration during peak exposure events results in a clear increase in pesticide toxicity. The higher toxicity levels during peaks presumably affect stream ecosystems, since organisms are most likely affected by the strongest stress event which usually are peak events (Ashauer et al., 2016; Schäfer et al., 2011; Stehle et al., 2013). Therefore, the sampling of rainfall events is pivotal, as already suggested by several previous publications (Bundschuh et al., 2014; Petersen et al., 2012; Stehle and Schulz, 2015; Szöcs et al., 2017). The feasibility of sampling rainfall events using automatic event-driven samplers on a Germany wide scale is currently analysed based on the data of the National Action Plan for plant protection (Wick et al., 2019; https://www.ufz.de/kgm/) and can inform future monitoring programs.

In chapter **3**, we provide measures to successfully apply passive sampling with Empore styrene-divinylbenzene (SDB) disks to sample pesticides during rainfall events and estimated related peak concentrations. We used a configuration of SDB disks in which the sorbent phase is directly exposed to the stream water, leading to high sampling rates, which enables a faster response towards concentration changes. With our experimental setup, we were able to show the suitability of passive sampling to capture rainfall events, which until today was solely tested in single field studies (Fernández et al., 2014). When following the standard procedure of passive sampling, the accumulated mass in the sorbent is converted to concentrations for the whole deployment time of the passive sampler, which equals a time-weighted average concentration (Booij et al., 2007). This however, can lead to an underestimation of the ecologically relevant peak concentrations (Fig. 3.3). In order to calculate the peak concentration, we based the calculation on the approximate peak duration, instead of

incorporating the whole deployment time. Using this approach, knowing if a peak actually occurred is crucial to avoid misinterpretation of the pesticide amount accumulated in the passive sampler. Basing the occurrence of peaks solely on forecasts of heavy rainfall events might lead to false assumptions since rainfall can occur relatively scattered. Therefore, the peak exposure events associated with rainfall events should be validated at the respective sampling site. This can be realised through monitoring flow or water level, which directly respond to increasing water quantity from rainfall, but also by monitoring conductivity which is altered through inorganic materials transported in surface runoff (Robson et al., 1993). If direct monitoring is not feasible, a visual inspection of the condition of the riparian area at the sampling site can provide evidence of a rainfall event. Among other factors, plants in the riparian area might be bent, material will be deposited or the stream substrate will be turned over.

If a peak occurs, its duration usually ranges between one and three days, depending on several parameters including stream size, geogenic background, topography and soil structure (Blume et al., 2007; Haga et al., 2005; Leu et al., 2004; Wittmer et al., 2010). Therefore, local measurements as well as experiences of the approximate peak durations in the study area are necessary to more accurately estimate the peak concentrations. Similar to the approach in our studies (chapter **3**, **4**), Fernández et al. (2014) found a good match between peak concentrations collected with an event-driven sampler (analogous to Liess et al., 1996) and calculated from passive samplers for an estimated peak duration of two days. This good match may be due to the majority of compound masses being transported and therefore sorbed during the peak.

A timeframe of two days corresponds to the estimated peak duration in multiple field settings (chapter **4**, Fernández et al., 2014) and equals the duration of several standard laboratory tests, which assess the acute pesticide risk for different groups of freshwater organisms (EPA, 2018; Lewis et al., 2016). Using effect concentrations (EC) calculated from these tests and the concentrations occurring during peaks the potential risk towards the freshwater system can be estimated with the toxic unit approach, as done in chapter **4** (see below) and several other studies previously (e.g. Panizzi et al., 2016; Schäfer, 2019).

6.4 BIOTIC RESPONSES TO PESTICIDES IN STREAMS

Independent of the method used to determine pesticide concentrations and to estimate the associated toxicities, the effects of pesticides on stream organisms are influenced by different toxicodynamic and toxicokinetic processes (Galic et al., 2014; Rubach et al., 2010). Despite similar absolute or average concentrations, different exposure scenarios can result in different biotic responses (Ashauer et al., 2013), since the concentration of the respective compound

at the site of action is relevant. This concentration can, in turn, be affected by uptake into, distribution and metabolisation in, as well as removal out of the respective organism.

The pesticide concentrations monitored in chapter **4** correspond to estimated toxicity levels which, according to previous studies, may cause alterations of invertebrate communities (Beketov et al., 2013; Schäfer, 2019) or a reduction of the primary production related to algae (Gustavsson et al., 2017; Rasmussen et al., 2015). Despite the complexity of toxicodynamic and toxicokinetic processes, two complementary studies were able to detect effects on organisms at the sampling sites monitored in chapter **4**. The studies showed that the community composition of riparian spiders, as well as their species richness and abundance, was influenced by the in-stream pesticide toxicity, possibly related to the availability of insect emergence as an important part of their diet (Graf et al., 2020, 2019).

These studies show that spiders respond towards pesticides in streams, but to derive or estimate universal stress responses of single spider species or whole communities additional studies across a large spatial scale would be necessary. To estimate universal stress responses, diverse studies analysing responses of one community type such as macroinvertebrates towards a single stressor have to be reviewed. Studies following this approach (e.g. Schäfer, 2019) can detect similar pesticide thresholds across geographical regions which could imply first evidence of a universal response. Causalities or evidence that responses are based on one single stressor, however, cannot be established reviewing field studies (Schäfer, 2019).

To determine universal responses of a community type to a stressor without additional pressure by multiple stressors, laboratory studies have to be conducted. The universal stress responses can either be derived by comparing several laboratory studies (e.g. Feckler and Bundschuh, in press) or conducting studies in laboratories covering a large spatial scale. In chapter **5**, we analysed the effects of a field-relevant fungicide mixture on a model community of aquatic hyphomycetes and its function, the leaf decomposition, across several biogeographic regions. Besides conducting this study in laboratories covering a large spatial scale, we investigated stress responses over three consecutive cycles of resource colonisation, to estimate long term effects mimicking field conditions with ongoing exposure to the analysed stressor. Despite varying initial communities, the effect patterns of the leaf decomposition towards a fungicide exposure were similar in all tested biogeographical regions (Fig. 5.2). This shows, together with results from other studies (Fernández et al., 2015; Rasmussen et al., 2012; Zubrod et al., 2015), that stressors like fungicides can equally impact different initial communities across large spatial scale, with aquatic hyphomycetes dominating in our study. Despite covering large spatial scales these comparable results only cover the European continent. Future studies investigating initial stress responses of communities

associated with leaf decomposition should expand to other regions, to also cover additional climate zones. In addition to similar initial stress responses, the recovery times until the ecosystem function of leaf decomposition again reached control levels were also similar across the investigated biogeographical regions (Fig. 5.2). The maintenance of leaf decomposition by hyphomycete communities in presence of a stressor like fungicides has been previously detected and indicates an adaptation process of the related communities (Feckler et al., 2018; Gardeström et al., 2016; Rossi et al., 2019).

Our results could contribute to a first step in estimating the universal stress responses for hyphomycete communities to a chemical stressor such as the investigated fungicide mixture. To however derive local, continental or global scale thresholds based on universal responses and towards a broad range of pesticides, information on different trophic levels and organism sizes including vertebrates, insects and higher plants would be necessary, which are currently lacking. Only covering a wide range of organisms would enable the derivation of a range of universal thresholds, which aim to protect whole stream ecosystems since these are inhabited by a multiplicity of organisms. Higher trophic level or larger organisms, however, show slower stress responses, partially based on their longer generation times, which hampers the realisation of experiments as presented in chapter **5**. Until today, related experiments using other organisms are lacking, but are important future steps to enable the estimation of universal stress responses for a wider range of pesticides. Since current thresholds such as regulatory acceptable concentrations are currently often exceeded (chapter **1.2**), irreversible effects on the biodiversity of stream ecosystems are likely. Our results (chapter **5**) together with those of other studies (Feckler et al., 2018; Gardeström et al., 2016), demonstrate the occurrence of PICT, where even damaged communities can maintain relevant ecosystem functions. Additional stress caused by higher pesticide toxicity levels or further stressors, however, can lead to a collapse of the communities ultimately leading to loss of ecosystem functions (Cao et al., 2018; Gardeström et al., 2016; Tlili et al., 2015).

Investigating the health of stream ecosystems is done using biological monitoring of diverse communities including several taxonomic groups such as fish or macroinvertebrates (e.g. European Commission, 2003). A sole focus on the biodiversity (e.g. Budnick et al., 2019) can detect small differences between single streams caused by various variables shaping communities, such as habitat structure or altitude. Alterations in functions as well as traits (Lamouroux et al., 2002; Liess and Ohe, 2005; Voß and Schäfer, 2017) can reveal substantial changes in stream ecosystems caused by anthropogenic stressors. Especially the monitoring of functions such as leaf decomposition can be conducted cost-efficiently and allows the use of less highly trained personnel (von Schiller et al., 2017). As demonstrated in diverse studies and described by the concept of PICT (chapter **5**, Blanck et al., 1988; Feckler et al., 2018; Tlili et al., 2015), functional as well as structural responses can be decoupled when regarding

microbial communities (Feckler and Bundschuh, in press). Based on this, only combined monitoring of functions as well as biodiversity can estimate the whole status of stream ecosystems and detect alterations caused by stressors such as pesticides.

6.5 CONCLUSIONS

Our results support previous studies that highlighted the fact that pesticides in streams occur in concentrations that affect non-target organisms and thereby potentially the whole stream ecosystem. The combination of these results suggest that the current risk assessment is underestimating the pesticide concentrations occurring in streams (Knäbel et al., 2014, 2012), possibly since complex pesticide applications within catchments are modelled imprecisely and the potential occurrence of misapplications. Consequently, current risk assessment offers insufficient protection causing unwanted effects on non-target organisms (e.g. Schäfer, 2019). Based on this, an improvement in the current risk assessment would be crucial to eliminate future effects on non-target areas and organisms. This could include controlled application in selected catchments to estimate all effects caused by pesticides before final approval (Schäfer et al., 2019).

Additionally, the risk posed by agricultural pesticides could be reduced by combining several risk mitigation strategies. These include reducing the quantity of applied pesticides (Frische et al., 2016) which, according to some studies, can be realised without a major decrease in yields (Lechenet et al., 2017) when e.g. using biological plant protection measures (Pedneault and Provost, 2016; Riddick, 2017). Furthermore, improved technical measures during pesticide application (Berger and Laurent, 2019; Cengiz et al., 2018; Palardy and Centner, 2017) or improved education of farmers can reduce pesticide amounts in non-target areas.

The success of improved risk assessment and risk mitigation strategies could be evaluated through targeted monitoring of pesticides in streams. This would allow on-going control of pesticides, including identification of exceedances that would finally lead to a review of currently approved pesticides. Our results suggest using a homogenised and standardised list of core compounds with comparable LOQs in pesticide monitoring when aiming to compare pesticide exposures over a larger scale. The list of core compounds should be improved in the future in comparison to the one presented in this thesis, by overcoming most limitations of the current routine monitoring, which includes consideration of currently underestimated pesticide groups such as fungicides and insecticides. When, however, aiming to estimate local threats by pesticides, the list of analysed compounds can be adjusted, based on their potential local occurrences as well as threats considering applications which are carried out in the target catchments. Additionally, compounds should be analysed using methods achieving LOQs sufficiently low to detect exceedances of thresholds, along with the incorporation of sampling during rainfall events, to sample stress events which shape stream ecosystems. To achieve this, methods like passive sampling could be used instead of taking water samples, as already suggested earlier. The results of the monitoring need to be incorporated in decisions of the risk assessment of pesticides (Schäfer et al., 2019).

Stream ecosystems exposed to anthropogenic stress are likely to recover when threats from pesticides are reduced and other stressors such as habitat degradation are eliminated through restoration measures (Gore, 1985). This is possible due to strong recovery capacities of stream ecosystems, which partly rely on refuge stream sections and dispersal abilities of several stream organisms (Knillmann et al., 2018). The resilience of single streams is, however, only assured as long as only reversible effects occur in their whole networks and re-colonisation from refuge communities is possible. Globally however, single streams might be already impacted irreversibly since meeting large scale thresholds can be achieved despite local exceedances.

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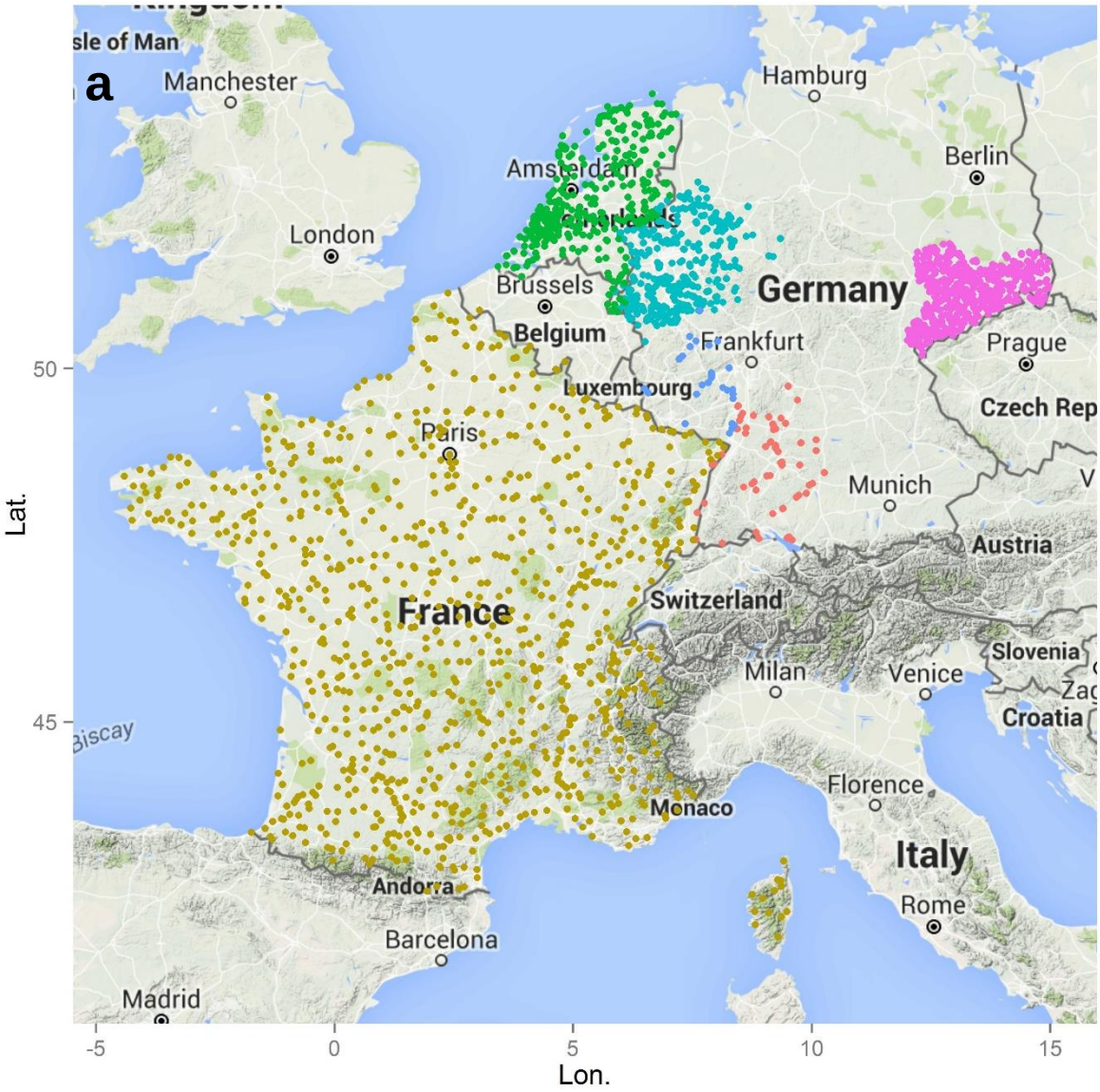
7 APPENDIX

7.1 SUPPLEMENTARY MATERIAL

A SUPPLEMENTARY MATERIAL: PESTICIDE MIXTURES IN STREAMS OF SEVERAL EUROPEAN COUNTRIES AND THE USA



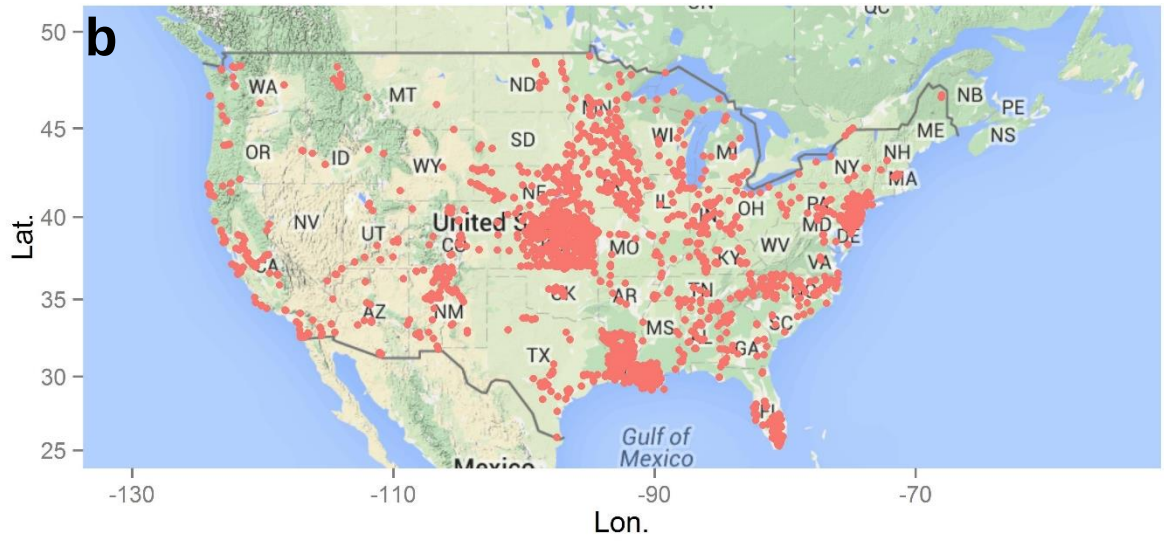


Fig. A.1: Maps showing the sites of the different countries after applying GRTS. a) European sampling sites. Green: the Netherlands; olive: France; aqua: North Rhine-Westphalia, Germany; red: Baden-Württemberg, Germany; blue: Rhineland-Palatinate, Germany; pink: Saxony, Germany. Source of Basemap: google (2015) GeoBasis-DE/BKG (2009), b) Sampling sites from the USA. Sites from Hawaii and Alaska not shown. Source: google (2015) INEGI

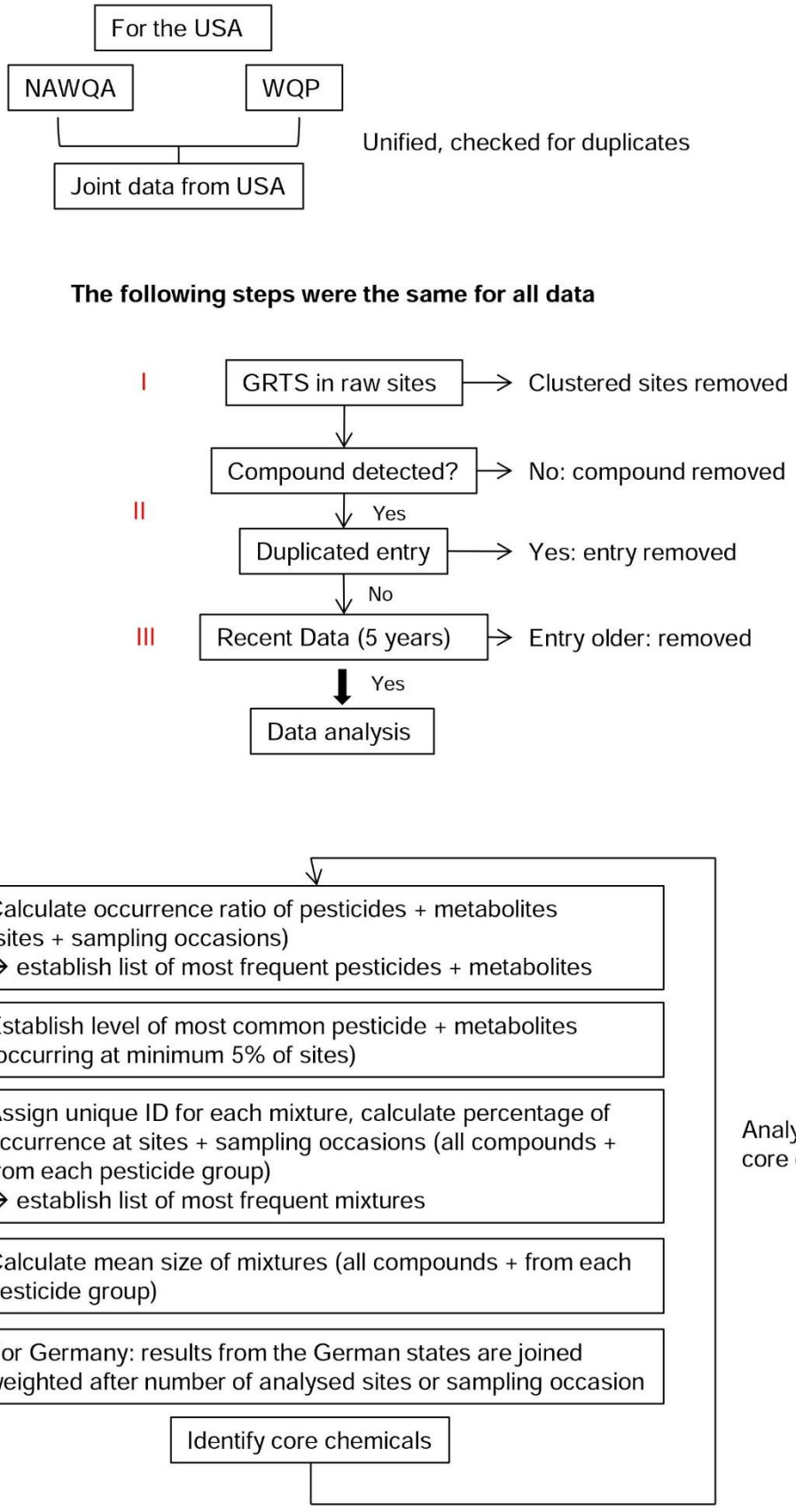


Fig. A.2: Flow chart visualizing quality control and data analysis steps for the datasets of the different countries and German states. Roman numerals refer to section "**2.3.1** Overview on data sets and pre-processing".

AGRICULTURAL LAND USE IN DIFFERENT COUNTRIES

Agricultural land use varied within the different countries with France dominated by wheat and legumes, Netherlands by forage and silage from grasses and maize, the USA by maize and soybeans and Germany by forage and silage from rye grass and wheat (“FAOSTAT,” 2014; more detailed information Table A.1). Additionally, to these dominating crops, a large area in the Netherlands is used to grow bulbs which are treated heavily with pesticides (van Wijngaarden et al., 2004). The German states display state-specific differences in crops. The state of Rhineland-Palatinate has the highest ratio of vineyards in Germany and a high portion of vegetables. Baden-Württemberg has several areas with orchards and corn. Sachsen has a relatively high ratio of cultivating rapeseed. In North Rhine-Westphalia a high ratio of sugar beet and rapeseed is cultivated (Statistische Ämter des Bundes und der Länder, 2015).

Table A.1: Crop production in the different countries, all information in % of the whole country area (“FAOSTAT,” 2014). Crops with more than 9 % upwards are shown.

crop	DE	FR	NL	US
wheat	16	28	10	15
legumes	0.5	12	6	36
barley		10		
maize		9	17	14
soybeans				23
forage and silage from grasses	10		39	
potatoes			11	
forage and silage from rye grass	21			
forage and silage from oilseeds	13			

DE: Germany; FR: France, NL: Netherlands, US: Unites States of America.

Table A.2: Overview of data sets analysed with information of detection rates and numbers of compounds and mixtures within the different countries and German states.

	BW	NRW	SN	RLP	FR	NL	US
Sites remaining using GRTS [%]	72	71	73	61	63	70	62
No. sites after GRTS	60	350	600	27	950	320	2225
Sampling occasions	1,560	3,016	6,949	652	25,586	5,112	13,209
Median sampling occasions per site	21.5	3	13	9	26	8.5	3
Analysed compounds	97	169	169	200	292	637	324
Mean No. compounds analysed per sampling occasion	66.2	52.5	105.6	62.2	27.6	83.4	36.2
Detected compounds	64	85	144	110	115	267	127
No. most frequent compounds	52	18	62	88	25	69	14
mean size mixtures all compounds, ± SD	9.4 ± 3.3	3.7 ± 2.3	6.6 ± 4.6	7.8 ± 8.3	3.0 ± 1.6	4.8 ± 3.0	3.2 ± 1.2
sites with compounds, all compounds [%]	95.0	69.4	92.8	96.3	78.1	90.3	23.5
sites with mixtures, all compounds [%]	89.7	23.8	59.4	69.5	12.7	65.3	16.2
sampling occasion with exposure, all compounds [%]	91.4	44.9	73.2	88.0	32.7	82.1	26.1
Detected core compounds	38	35	40	29	40	38	29
No. most frequent core compounds	33	15	23	25	14	19	9
mean size mixtures core compounds, ± SD	6.6 ± 1.9	3.2 ± 1.6	3.9 ± 2.2	4.7 ± 3.2	2.5 ± 1.0	3.6 ± 1.8	2.5 ± 0 7
Max size mixture, core compounds	14	10	16	20	9	14	6
sites with compounds, core compounds [%]	91.7	64.1	88.0	96.3	73.1	85.3	21.4
sites with mixtures, core compounds [%]	89.4	16.0	41.8	56.6	7.6	36.1	15.0
sampling occasion with exposure, core compounds [%]	90.6	36.3	60.9	82.5	24.8	60.2	24.6

RLP: Rhineland-Palatinate, Germany; NRW: North Rhine-Westphalia, Germany; SN: Saxony, Germany; BW: Baden-Württemberg, Germany; FR: France, NL: Netherlands, US: Unites States of America. “No. most frequent (core) compounds”: number of compounds after establishing level of most frequent compounds (c.f. Fig. A.3). Compounds = pesticides + metabolites.

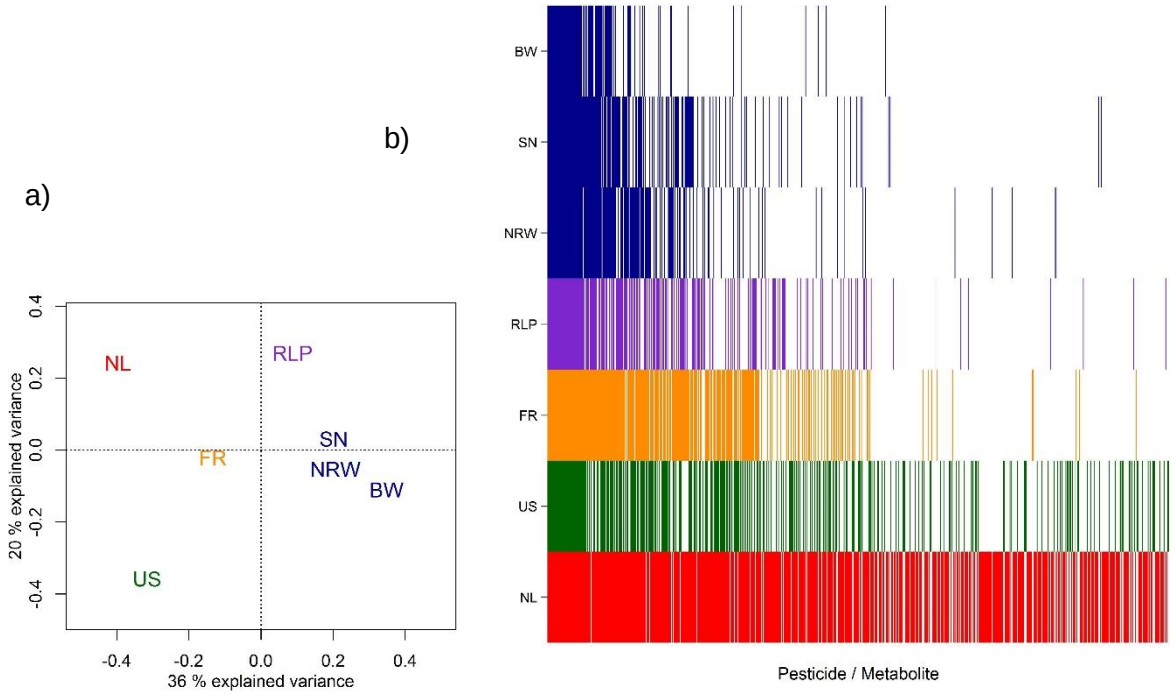


Fig. A.3: a) Multidimensional scaling of the analysed pesticides and their metabolites in the different countries and German states. b) Comparison of the analysed pesticides and metabolites from the different countries. Each line represents one compound. Same colours indicate the relative concordance of the compound spectrum, according to a). For number of analysed pesticides and metabolites in each country, see Table A.1. RLP: Rhineland-Palatinate, Germany; NRW: North Rhine-Westphalia, Germany; SN: Saxony, Germany; BW: Baden-Württemberg, Germany; FR: France, NL: Netherlands, US: United States of America.

Table A.3: List of core compounds used for mixture analysis.

compound	CAS	pesticide type
AMPA	1066519	M
Glyphosate	1071836	HB
Malathion	121755	IN
Simazine	122349	HB
Azoxystrobin	131860338	FU
Propazin	139402	HB
Flufenacet	142459583	HB
Trifluralin	1582098	HB
Alachlor	15972608	HB
Chloridazon	1698608	HB
Atrazine	1912249	HB
Cyanazin	21725462	HB
Bentazon	25057890	HB
Ethofumesate	26225796	HB
Chlorpyrifos	2921882	IN
Parathion-methyl	298000	IN
Disulfoton	298044	IN
Bromacil	314409	HB
Diuron	330541	HB
Linuron	330552	HB
Pendimethalin	40487421	HB
Chlorfenvinphos	470906	IN
trans-Chlordan	5103742	IN
Metolachlor	51218452	HB
Hexazinon	51235042	HB
Parathion-ethyl	56382	IN
Metalaxyl	57837191	FU
Terbutylazin	5915413	HB
Propiconazol	60207901	FU
Dimethoate	60515	IN
Desethylatrazine	6190654	M

compound	CAS	pesticide type
Dichlorvos	62737	IN
Prometryn	7287196	HB
Heptachlor	76448	IN
Mevinphos	7786347	IN
Hexachlorbutadien	87683	HB
Pentachlorphenol	87865	FU
Terbutryn	886500	HB
Mecoprop	93652	HB
2,4,5-T	93765	HB
MCPA	94746	HB
2,4-D	94757	HB
MCPB	94815	HB
2,4-DB	94826	HB

HB: herbicide, IN: insecticide, FU: fungicide, M: metabolite.

Table A.4: List of the most frequent pesticides and metabolites with their relative occurrence at sites of the different countries and German states. The compounds are ordered alphabetically. Each listed compound occurred in at least one country at a minimum of 10 % of the sites and German states.

compound	CAS	pesticide type	BW	NRW	SN	RLP	FR	NL	US
1-Chlornaphthalin	90131	IN	0	0	15.2	0	0	0	0
1,2,3,4,5,6-Hexachlorocyclohexane	58899	IN	0	2.9	13.3	0	11.2	10	0.7
2-Methyl-4,6-dinitrophenol	534521	IN	0	15.4	0	0	0.1	7.2	0
2,4-D	94757	HB	8.3	9.7	6.7	55.6	4.9	18.4	3
2,6-Dichlorobenzamide	2008584	HB	0	0	0.3	0	0.1	28.4	0
4-Para-nonylphenol	1044050	FU	10	0	0	0	0	0	0
Alachlor	15972608	HB	15	0.6	1.2	0	9.5	0.6	3
Ametryn	834128	HB	16.7	0	0.3	0	0.1	0	1.1
AMPA	1066519	M	8.3	9.4	10.8	85.2	13.2	37.8	2.3
Atrazine	1912249	HB	90	6.6	26.2	66.7	42	11.3	19.2
Azoxystrobin	131860338	FU	5	1.1	28.5	48.1	0.7	27.8	0
Bentazon	25057890	HB	20	10.9	28.2	81.5	7.7	37.5	0.1
Bitertanol	55179312	FU	0	0	0	0	0	15.9	0
Boscalid	188425856	FU	0	4.3	62.2	44.4	0	12.8	0
Bromacil	314409	HB	5	0.3	1.2	11.1	0.1	0.9	0.7
Bromoxynil	1689845	HB	0	1.7	2.5	25.9	0.1	0	0
Carbendazim	10605217	FU	0	0	26.2	44.4	1.5	55.3	0
Chlorfenvinphos	470906	IN	18.3	0	0.5	0	2.9	2.8	0
Chloridazon	1698608	HB	15	14	14	0	0.8	29.4	0
Chlorpropham	101213	HB	0	0	0	0	0.2	31.9	0
Chlorpyrifos	2921882	IN	6.7	0.3	32.2	3.7	6.6	3.8	1.7
Chlortoluron	15545489	HB	3.3	4.3	18.5	25.9	4	8.4	0
Clomazone	81777891	HB	0	0	34.2	0	0.1	2.8	0
Clothianidin	210880925	IN	0	0	7.8	18.5	0	0.3	0

compound	CAS	pesticide type	BW	NRW	SN	RLP	FR	NL	US
Cyprodinil	121552612	FU	0	0	4.5	29.6	0.3	7.5	0
Desethylatrazine	6190654	M	88.3	4.6	7.5	44.4	5.5	2.2	3
Desethylterbutylazin	30125634	M	83.3	15.7	41.2	14.8	0.5	4.4	0
Diazinon	333415	IN	53.3	0	1.7	0	0.3	5.6	1.2
Dichlobenil	1194656	HB	0	0	0	0	0.4	11.9	0
Dichlorprop	120365	HB	0	7.7	7	88.9	3.3	0.9	0
Diflufenican	83164334	HB	46.7	1.1	52.2	3.7	0.3	0.9	0
Dimethachlor	50563365	HB	0	0	34	3.7	0.1	0	0
Dimethenamid	87674688	HB	0	2.6	34.7	0	7.5	15.3	1.2
Dimethoate	60515	IN	10	0.3	10.3	37	0.2	20	0.6
Dimethomorph	110488705	FU	0	0	0.3	44.4	1.1	18.4	0
Dinoseb	88857	HB	0	0	0	11.1	0.1	0.3	0
Disulfoton	298044	IN	16.7	0.3	0.2	0	0.1	0.3	0
Diuron	330541	HB	26.7	47.1	44	85.2	55.3	38.1	1.3
Epoxiconazole	133855988	FU	1.7	2.9	14.7	25.9	1.5	6.9	0
Ethofumesate	26225796	HB	6.7	14	30.3	37	1.3	32.5	0
Fenarimol	60168889	FU	0	0	0	18.5	0	0.3	0
Fenhexamid	126833178	FU	0	0	0.3	40.7	0	2.5	0
Fenpropimorph	67564914	FU	0	0	11.5	7.4	0.1	5	0
Flazasulfuron	104040780	HB	0	0	0	14.8	0	0	0
Flonicamid	158062670	IN	0	0	0	0	0	11.9	0
Fluazifop	69335917	HB	0	0	0	25.9	0	0.3	0
Fludioxonil	131341861	FU	0	0.3	0	33.3	0	0.3	0
Flufenacet	142459583	HB	8.3	5.4	36.5	11.1	0	1.3	0
Fluquinconazol	136426545	FU	0	0	5.2	29.6	0.1	0	0
Fluroxypyr	69377817	HB	0	11.1	0	18.5	0.1	14.7	0
Flurtamone	96525234	HB	0	1.4	39	3.7	0.1	0.3	0
Flutolanil	66332965	FU	0	0	0	0	0	19.1	0

compound	CAS	pesticide type	BW	NRW	SN	RLP	FR	NL	US
Glyphosate	1071836	HB	8.3	8	7.5	85.2	12.1	30	0
Haloxyfop	69806344	HB	0	2.3	0	22.2	0	0.9	0
Hexachlorobenzene	118741	FU	0	4.3	24.3	0	4	2.5	0.4
Hexazinon	51235042	HB	38.3	3.1	1	3.7	0.1	0	0.9
Hydroxyatrazine	2163680	M	0	0	13	0	3.6	1.3	1.8
Imidacloprid	105827789	IN	0	0.9	0.8	22.2	0	0	0.4
Ioxynil	1689834	HB	0	0.6	0.8	18.5	0	0.3	0
Iprodion	36734197	FU	0	0	0	22.2	0	9.1	0.7
Iprovalicarb	140923177	FU	0	0	0	40.7	0	0	0
Irgarol 1051	28159980	FU	68.3	0	38.5	7.4	0	0	0
Isoproturon	34123596	HB	65	42	73	70.4	53.6	37.5	0
Kresoxim	143390890	FU	0	0	1	29.6	0	4.4	0
Linuron	330552	HB	0	2.6	1.3	14.8	10	28.4	0
Malathion	121755	IN	21.7	0	0.3	0	0.2	3.1	0.1
MCPA	94746	HB	36.7	25.1	16.8	81.5	43.2	44.4	0.4
Mecoprop	93652	HB	55	26.9	17.8	88.9	5.4	38.4	0
Metalaxyl	57837191	FU	58.3	1.4	5.3	37	0.5	21.3	0.1
Metamitron	41394052	HB	15	12.6	7.8	33.3	0.7	12.8	0
Metazachlor	67129082	HB	80	11.4	61	66.7	2.3	19.1	0
Methabenzthiazuron	18691979	HB	0	1.4	0.5	22.2	0.9	3.1	0
Methoxyfenozid	161050584	IN	0	0	0	22.2	0	9.1	0
Metobromuron	3060897	HB	1.7	0.6	0.3	14.8	0.1	0.9	0
Metolachlor	51218452	HB	81.7	16.3	35.2	22.2	7.9	36.6	11.6
Metribuzin	21087649	HB	0	3.4	2.3	25.9	0.1	11.6	3.6
Myclobutanil	88671890	FU	0	0	0	44.4	0	0.3	1.4
Napropamide	15299997	HB	65	0	25.2	0	0.4	0	0
o,p'-DDT	789026	IN	0	0	10.5	0	6.7	0.6	0
p,p'-DDD	72548	IN	0	0	18.2	0	5.9	0.9	0.5

compound	CAS	pesticide type	BW	NRW	SN	RLP	FR	NL	US
p,p'-DDT	50293	IN	0	0	36.2	0	7.5	3.1	0.9
Parathion	56382	IN	10	0	1.8	7.4	0.2	0.3	0
Penconazol	66246886	FU	70	0	0.2	37	0	2.2	0
Pencycuron	66063056	FU	0	0.9	0.5	7.4	0	14.4	0
Pendimethalin	40487421	HB	81.7	0	12.5	29.6	0.6	5	2.6
Pirimicarb	23103982	IN	70	0	6	14.8	0.1	24.1	0
Prometryn	7287196	HB	28.3	0.6	5.7	3.7	0	0	0
Pronamide	23950585	HB	0	1.4	22.7	25.9	3.1	13.4	0.5
Propamocarb	24579735	FU	0	0	1	0	0	11.6	0
Propazin	139402	HB	0	0.6	3.7	11.1	0.2	0.3	0.4
Propiconazol	60207901	FU	90	0	19.5	33.3	0.1	3.8	0.2
Propoxur	114261	IN	0	0	5	25.9	0.4	6.9	0
Prosulfocarb	52888809	HB	0	1.7	0.8	0	0.4	15.9	0
Pyraclostrobin	175013180	FU	0	0.3	0.3	25.9	0	4.1	0
Pyrimethanil	53112280	FU	0	0	9.5	40.7	0.3	9.7	0
Quinmerac	90717036	HB	0	14.3	17	37	0	0.3	0
Quizalofop	76578126	HB	0	0	0	22.2	0	0	0
Simazine	122349	HB	83.3	7.7	50.2	66.7	19.9	15.3	1.6
Spirodiclofen	148477718	IN	0	0	0	14.8	0	0	0
Spiroxamin	118134308	FU	0	0	10.8	37	0	0	0
Tebuconazole	107534963	FU	0	4	21.8	63	2.6	17.5	0
Tebufenozid	112410238	IN	0	0	0	18.5	0	0.3	0
Tebutam	35256850	HB	23.3	0	0	0	0.6	0	0
Terbuthylazine	5915413	HB	83.3	22.3	70.8	66.7	0.1	33.1	0
Terbutryn	886500	HB	85	10.9	48.2	37	2.9	5.6	0
Terbutylazin, 2-Hydroxy	66753079	M	0	0	18.8	0	0	0	0
Thiacloprid	111988499	IN	0	0	0.7	18.5	0	6.9	0
Triadimenol	55219653	FU	0	0	1.3	33.3	0	5	0

compound	CAS	pesticide type	BW	NRW	SN	RLP	FR	NL	US
Trifloxystrobin	141517217	FU	0	0	0.5	22.2	0	1.6	0
Trifluralin	1582098	HB	13.3	0.3	3.3	0	3.5	0	1

RLP: Rhineland-Palatinate, Germany; NRW: North Rhine-Westphalia, Germany; SN: Saxony, Germany; BW: Baden-Württemberg, Germany; FR: France; NL: Netherlands; US: Unites States of America. IN: insecticide, HB: herbicide, FU: fungicide, M: metabolite.

Table A.5: List of the most frequent mixtures from the different countries and German states with the ratio of occurrence at sites and sampling occasions as well as the number of compounds (size). Order of compounds based on CAS numbers.

[%] occurrence site	[%] occurrence sampling occasions	No. compounds	compounds
			BADEN-WÜRTTEMBERG, GERMANY
20.0	1.7	5	Simazine (HB), Atrazine (HB), Propiconazol (FU), Desethylatrazine (M), Terbutryn (HB)
20.0	1.0	6	Simazine (HB), Atrazine (HB), Desethylterbutylazin (M), Propiconazol (FU), Desethylatrazin (M), Terbutryn (HB)
16.7	1.5	7	Simazine (HB), Atrazine (HB), Desethylterbutylazine (M), Terbutylazin (HB), Propiconazol (FU), Desethylatrazine (M), Terbutryn (HB)
13.3	0.6	9	Simazine (HB), Atrazine (HB), Irgarol 1051 (FU), Desethylterbutylazin (M), Terbutylazin (HB), Propiconazol (HB), Desethylatrazin (M), Metazachlor (HB)
11.7	0.4	6	Simazine (HB), Atrazine (HB), Pendimethalin (HB), Propiconazol (HB), Desethylatrazin (M), Terbutryn (HB)
11.7	0.7	7	Simazine (HB), Atrazine (HB), Irgarol 1051 (FU), Desethylterbutylazine (M), Propiconazole (HB), Desethylatrazine (M), Terbutryn (HB)
10.0	0.8	2	Atrazine (HB), Desethylatrazine (M)
10.0	0.4	5	Atrazine (HB), Desethylterbutylazine (M), Propiconazole (HB), Desethylatrazine (M), Terbutryn (HB)
10.0	0.4	8	Simazine (HB), Atrazine (HB), Desethylterbutylazine (M), Terbutylazin (HB), Propiconazol (HB), Desethylatrazine (M), Metazachlor (HB), Terbutryn (HB)
10.0	1.1	8	Simazine (HB), Atrazine (HB), Irgarol 1051 (FU), Desethylterbutylazine (M), Terbutylazin (HB), Propiconazol (HB), Desethylatrazine (M), Terbutryn (HB), Terbutryn (HB)

[%] occurrence site	[%] occurrence sampling occasions	No. compounds	compounds
			NORTH RHINE-WESTPHALIA, GERMANY
12.0	2.1	2	Diuron (HB), Isoproturon (HB)
4.3	0.6	4	Desethylterbutylazine (M), Diuron (HB), Metolachlor (HB), Terbutylazin (HB)
3.7	0.5	2	Diuron (HB), Terbutryn (HB)
3.4	0.6	2	AMPA (M), Glyphosate (HB)
2.9	0.3	2	Mecoprop (HB), MCPA (HB)
2.9	0.5	2	Desethylterbutylazin (M), Diuron (HB)
2.3	0.3	2	Flufenacet (HB), Isoproturon (HB)
2.0	0.3	2	Diuron (HB), Mecoprop (HB)
2.0	0.2	2	Diuron (HB), Terbutylazin (HB)
2.0	0.3	2	AMPA (M), Isoproturon (HB)
			SAXONY, GERMANY
4.0	0.4	2	Boscalid (FU), Isoproturon (HB)
3.2	0.3	2	Boscalid (FU), Terbutylazin (HB)
3.0	0.3	2	Isoproturon (HB), Metazachlor (HB)
2.5	0.2	2	Isoproturon (HB), Diflufenican (HB)
2.5	0.2	2	Isoproturon (HB), Terbutylazin (HB)
2.3	0.2	2	Isoproturon (HB), Terbutryn (HB)
2.3	0.2	2	Irgarol 1051 (FU), Isoproturon (HB)
2.2	0.2	2	Simazine (HB), Terbutylazin (HB)
2.0	0.2	2	Diflufenican (HB), Flurtamone (HB)
1.8	0.2	2	Diuron (HB), Isoproturon (HB)

[%] occurrence site	[%] occurrence sampling occasions	No. compounds	compounds
			RHINELAND-PALATINATE, GERMANY
40.7	3.8	2	AMPA (M), Glyphosate (HB)
18.5	1.1	2	AMPA (M), Isoproturon (HB)
18.5	1.4	2	AMPA (M), Mecoprop (HB)
11.1	0.8	3	AMPA (M), Diuron (HB), Isoproturon (HB)
11.1	0.8	2	AMPA (M), Bentazon (HB)
11.1	0.8	2	AMPA (M), Chlortoluron (HB)
11.1	0.8	3	AMPA (M), Chlortoluron (HB), Isoproturon (HB)
11.1	0.5	4	AMPA (M), Glyphosate (HB), Mecoprop (HB), MCPA (HB)
11.1	3.2	3	Chlortoluron (HB), Diuron (HB), Isoproturon (HB)
7.4	0.5	2	Isoproturon (HB), Mecoprop (HB)
			FRANCE
18.0	1.5	2	Diuron (HB), Isoproturon (HB)
13.6	1.1	2	Diuron (HB), MCPA (HB)
10.1	0.6	2	Atrazine (HB), Diuron (HB)
9.2	0.7	2	Atrazine (HB), Isoproturon (HB)
8.5	0.5	2	Isoproturon (HB), MCPA (HB)
7.2	0.4	2	Atrazine (HB), MCPA (HB)
6.4	0.4	3	Diuron (HB), Isoproturon (HB), MCPA (HB)
6.0	0.3	3	Atrazine (HB), Diuron (HB), Isoproturon (HB)
5.2	0.3	3	Atrazine (HB), Diuron (HB), MCPA (HB)
4.1	0.2	2	Simazine (HB), Diuron (HB)

[%] occurrence site	[%] occurrence sampling occasions	No. compounds	compounds
			NETHERLANDS
7.2	1.5	2	AMPA (M), Glyphosate (HB)
4.7	0.3	2	Carbendazim (FU), Imidacloprid (IN)
3.8	0.6	2	Diuron (HB), Isoproturon (HB)
3.4	0.4	2	Bentazon (HB), Isoproturon (HB)
3.4	0.2	2	Carbendazim (FU), Isoproturon (HB)
3.1	0.2	2	Carbendazim (FU), Diuron (HB)
3.1	0.3	2	Bentazon (HB), Mecoprop (HB)
3.1	0.3	2	Mecoprop (HB), MCPA (HB)
2.8	0.2	3	Bentazon (HB), Mecoprop (HB), MCPA (HB)
2.5	0.3	3	Carbendazim (FU), Imidacloprid (IN), Flonicamid (IN)
			USA
5.3	2.5	2	Atrazine (HB), Metolachlor (HB)
3.5	3.2	3	Atrazine (HB), Acetochlor (HB), Metolachlor (HB)
1.9	0.7	2	Atrazine (HB), Desethylatrazine (M)
1.3	0.3	4	Atrazine (HB), Acetochlor (HB), Metolachlor (HB), Desethylatrazine (M)
1.2	0.8	4	Alachlor (HB), Atrazine (HB), Acetochlor (HB), Metolachlor (HB)
1.0	0.3	2	Atrazine (HB), Acetochlor (HB)
1.0	0.3	4	Atrazine (HB), Metribuzin (HB), Acetochlor (HB), Metolachlor (HB)
0.9	0.5	5	Alachlor (HB), Atrazine (HB), Metribuzin (HB), Acetochlor (HB), Metolachlor (HB)
0.8	0.2	3	Atrazine (HB), Metolachlor (HB), Desethylatrazine (M)
0.7	0.4	4	AMPA (M), Atrazine (HB), Acetochlor (HB), Metolachlor (HB)

HB: herbicide, IN: insecticide, FU: fungicide, M: metabolite.

Table A.6: List of the most frequently occurring core pesticides and metabolites with their relative occurrence at sites of the different countries and German states. The compounds are ordered alphabetically. Each listed compound occurred in at least one country or German state at a minimum of 10 % of the sites.

compound	CAS	pesticide type	BW	NRW	SN	RLP	FR	NL	USA
2,4-D	94757	HB	8.3	9.8	6.8	55.6	4.9	18.4	3.0
Alachlor	15972608	HB	15.0	0.6	1.2	0	9.5	0.6	3.0
Aminomethylphosphonic acid	1066519	metabolite	8.3	9.5	11.1	85.2	13.2	37.8	2.3
Atrazine	1912249	HB	90.0	6.6	26.8	66.7	42.0	11.3	19.2
Azoxystrobin	131860338	FU	5.0	1.1	29.2	48.1	0.7	27.8	0
Bentazon	25057890	HB	20.0	10.9	28.9	81.5	7.7	37.5	0.1
Bromacil	314409	HB	5.0	0.3	1.2	11.1	0.1	0.9	0.7
Chlorfenvinphos	470906	IN	18.3	0	0.5	0	2.9	2.8	0
Chloridazon	1698608	HB	15.0	14.1	14.4	0	0.8	29.4	0
Chlorpyrifos	2921882	IN	6.7	0.3	33.0	3.7	6.6	3.8	1.7
Desethylatrazine	6190654	metabolite	88.3	4.6	7.7	44.4	5.5	2.2	3.0
Dimethoate	60515	IN	10.0	0.3	10.6	37.0	0.2	20.0	0.6
Disulfoton	298044	IN	16.7	0.3	0.2	0	0.1	0.3	0
Diuron	330541	HB	26.7	47.4	45.1	85.2	55.3	38.1	1.3
Ethofumesate	26225796	HB	6.7	14.1	31.1	37.0	1.3	32.5	0
Flufenacet	142459583	HB	8.3	5.5	37.4	11.1	0	1.3	0
Glyphosate	1071836	HB	8.3	8.0	7.7	85.2	12.1	30.0	0
Hexazinon	51235042	HB	38.3	3.2	1.0	3.7	0.1	0	0.9
Linuron	330552	HB	0	2.6	1.4	14.8	10.0	28.4	0
Malathion	121755	IN	21.7	0	0.3	0	0.2	3.1	0.1
MCPA	94746	HB	36.7	25.3	17.3	81.5	43.2	44.4	0.4
Mecoprop	93652	HB	55.0	27.0	18.3	88.9	5.4	38.4	0
Metalaxyl	57837191	FU	58.3	1.4	5.5	37.0	0.5	21.3	0.1
Metolachlor	51218452	HB	81.7	16.4	36.1	22.2	7.9	36.6	11.6

compound	CAS	pesticide type	BW	NRW	SN	RLP	FR	NL	USA
Parathion	56382	AK	10.0	0	1.9	7.4	0.2	0.3	0
Pendimethalin	40487421	HB	81.7	0	12.8	29.6	0.6	5.0	2.6
Prometryn	7287196	HB	28.3	0.6	5.8	3.7	0	0	0
Propazin	139402	HB	0	0.6	0	11.1	0.2	0.3	0.4
Propiconazol	60207901	FU	90.0	0	20.0	33.3	0.1	3.8	0.2
Simazine	122349	HB	83.3	7.8	51.5	66.7	19.9	15.3	1.6
Terbuthylazine	5915413	HB	83.3	22.4	72.6	66.7	0.1	33.1	0
Terbutryn	886500	HB	85.0	10.9	49.4	37.0	2.9	5.6	0
Trifluralin	1582098	HB	13.3	0.3	3.4	0	3.5	0	1.0

RLP: Rhineland-Palatinate, Germany; NRW: North Rhine-Westphalia, Germany; SN: Saxony, Germany; BW: Baden-Württemberg, Germany; FR: France; NL: Netherlands; US. United States of America. IN: insecticide, HB: herbicide, FU: fungicide.

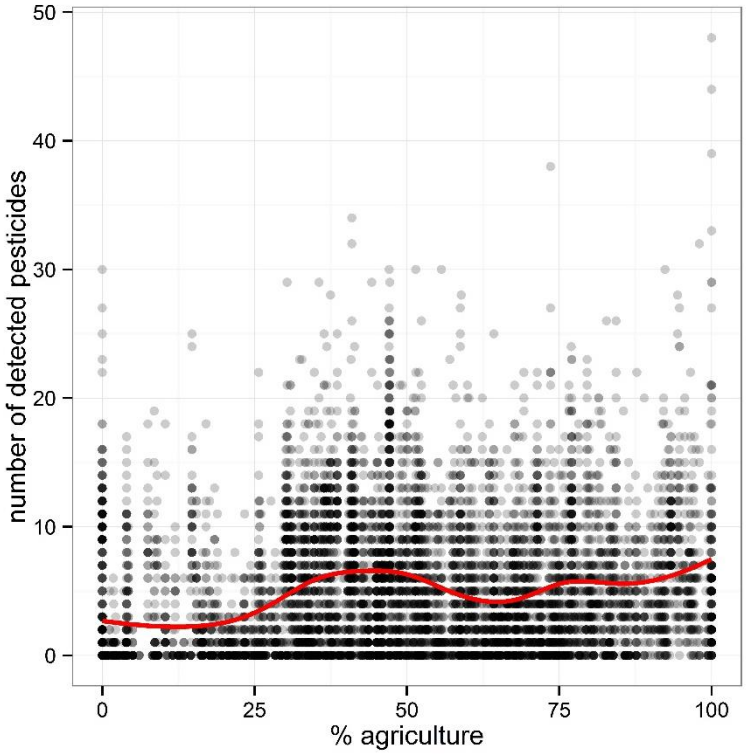


Fig. A.4: Relationship between the number of detected pesticides and the ratio of agriculture within the upstream catchment area in Germany. Smoothed regression spline in red. Colour intensity of the dots reflects number of sampling occasions.

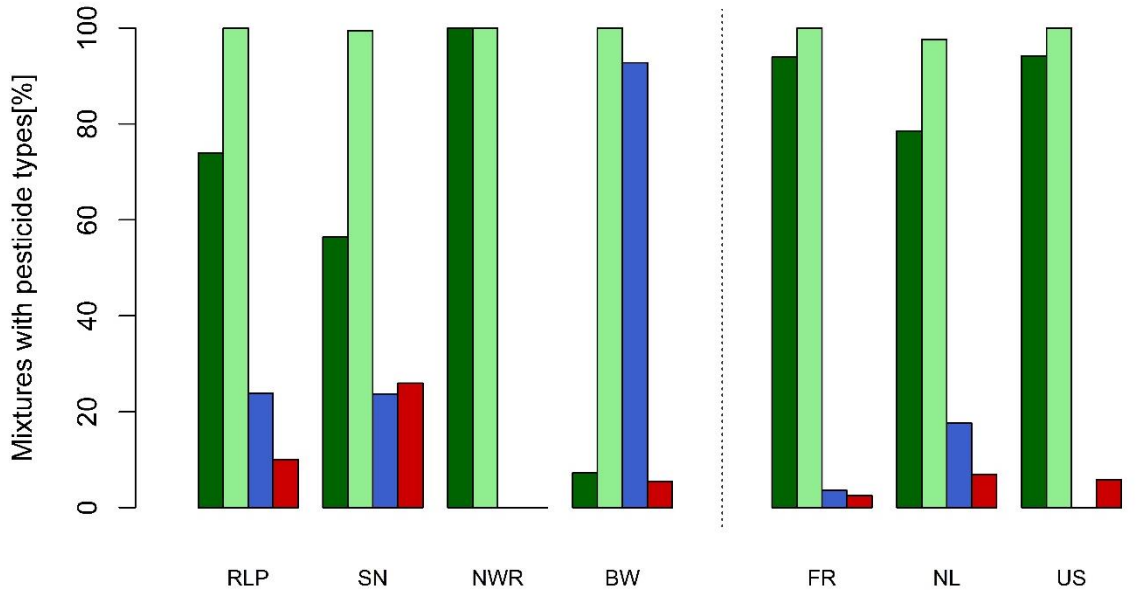


Fig. A.5: Relative amount of mixtures with occurrence of the main pesticide groups. Detected mixtures based on core compounds. RLP: Rhineland-Palatinate, Germany; NRW: North Rhine-Westphalia, Germany; SN: Saxony, Germany; BW: Baden-Württemberg, Germany; FR: France, NL: Netherlands, US: Unites States of America. Green: mixtures of only herbicides, light green: herbicides in the mixtures, blue: fungicides in the mixtures, red: insecticides in the mixtures. Metabolites were added to the respective pesticide group of the parent compound.

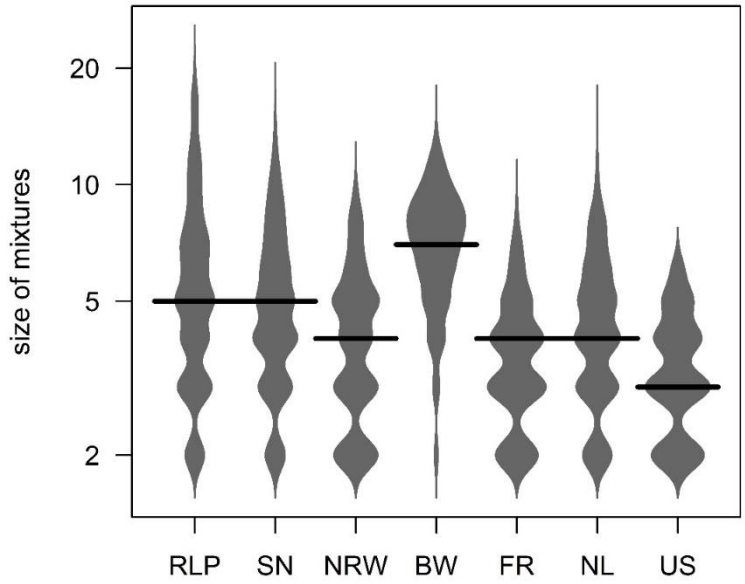


Fig. A.6: Distribution of mixture size for the different countries. The black solid line gives the median. Y-axis on logarithmic scale. RLP: Rhineland-Palatinate, Germany; NRW: North Rhine-Westphalia, Germany; SN: Saxony, Germany; BW: Baden-Württemberg, Germany; FR: France, NL: Netherlands; US: United States of America.

Table A.7: Limits of Quantification (LOQs) in mg L^{-1} of the fungicides Metalaxyl and Protioconazole for data of our study.

	Metalaxyl	Propiconazole
BW	0.001	0.001
NRW	0.03	NA
RLP	0.006	0.005
SN	0.015	0.015
FR	0.05	0.08
NL	0.005	0.005
USA	0.05	0.03

RLP: Rhineland-Palatinate, Germany; NRW: North Rhine-Westphalia, Germany; SN: Saxony, Germany; BW: Baden-Württemberg, Germany; FR: France, NL: Netherlands; US: United States of America.

Table A.8: Limits of Quantification (LOQs) for the frequently detected compounds (see Table 2.2) and the related LC_{50} of the most sensitive species. As LOQ the respective highest of the countries was used.

compound	pesticide type	LOQ [mg L^{-1}]	LC_{50} [mg L^{-1}]	LOQ/ LC_{50}	Species	Source
1,2,3,4,5,6-Hexachlorocyclohexane	IN	0.003	0.002	1.5	*Pteronarcys californica*	1
2,4-D	HB	0.08	0.6	0.133	*Daphnia magna*	1
2,6-Dichlorobenzamide	HB	0.3	856	0.0004	*Daphnia magna*	1
AMPA	M	0.2	NA	NA	NA	
Atrazine	HB	0.02	0.08	0.250	*Daphnia magna*	1
Azoxystrobin	FU	0.03	0.3	0.100	*Daphnia magna*	1
Bentazon	HB	0.05	62	0.001	*Chironomus riparius*	1
Bitertanol	FU	0.01	13	0.001	*Daphnia magna*	1
Boscalid	FU	0.03	5	0.006	*Daphnia magna*	1
Carbendazim	FU	0.006	0.02	0.3	*Daphnia magna*	1
Chloridazon	HB	0.03	0.2	0.2	*Pseudokirchneriella subcapitata*	1
Chlorpropham	HB	0.09	3.7	0.02	*Daphnia magna*	1
Chlorpyrifos	IN	0.01	0.0000003	33333	*Chironomus riparius*	1
Chlortoluron	HB	0.04	0.009	4.4	*Pseudokirchneriella subcapitata*	1
Clomazone	HB	0.1	5.2	0.02	*Daphnia magna*	1
Desethylatrazine	M	0.05	2	0.03	*Pseudokirchneriella subcapitata*	5
Desethylterbuthylazine	M	0.04	0.01	4.0	*Pseudokirchneriella subcapitata*	5
Dichlobenil	HB	0.01	2.8	0.004	*Hyalella azteca*	1
Diflufenican	HB	0.06	0.3	0.2	*Pseudokirchneriella subcapitata*	3
Dimethachlor	HB	0.2	24	0.008	*Daphnia magna*	2
Dimethenamid	HB	0.03	16	0.002	*Daphnia magna*	1
Dimethoate	IN	0.2	0.005	40	*Daphnia magna*	1
Dimethomorph	FU	0.01	10.6	0.001	*Daphnia magna*	1
Diuron	HB	0.03	0.09	0.3	*Acropora tumida*	1

compound	pesticide type	LOQ [mg L^{-1}]	LC_{50} [mg L^{-1}]	LOQ/LC_{50}	Species	Source
Epoxiconazole	FU	0.2	NA	NA	NA	
Ethofumesate	HB	0.02	64	0.0003	*Daphnia magna*	1
Flonicamid	IN	0.02	98.6	0.0002	*Daphnia magna*	1
Flufenacet	HB	0.04	30.9	0.001	*Daphnia magna*	2
Fluroxypyr	HB	0.03	100	0.0003	*Daphnia magna*	1
Flurtamone	HB	2	13	0.2	*Daphnia magna*	2
Flutolanil	FU	0.005	6.8	0.001	*Daphnia magna*	1
Glyphosate	HB	0.2	3	0.07	*Daphnia magna*	1
Hexachlorobenzen	FU	0.005	0.00001	500	*Artemia salina*	1
Irgarol 1051	FU	0.005	1.6	0.003	*Artemia salina*	1
Isoproturon	HB	0.03	0.04	0.8	*Pseudokirchneriella subcapitata*	1
Linuron	HB	0.05	0.1	0.5	*Daphnia magna*	1
MCPA	HB	0.05	180	0.0003	*Daphnia magna*	1
Mecoprop	HB	0.05	10	0.005	*Daphnia magna*	1
Metalaxyl	FU	0.05	28	0.002	*Daphnia magna*	1
Metamitron	HB	0.03	34.9	0.001	*Pseudokirchneriella subcapitata*	5
Metazachlor	HB	0.03	0.02	1.5	*Pseudokirchneriella subcapitata*	5
Metolachlor	HB	0.03	0.7	0.04	*Chironomus plumosus*	1
Metribuzin	HB	0.04	4.2	0.01	*Daphnia magna*	1
Napropamide	HB	0.01	14.3	0.001	*Daphnia magna*	1
p,p'-DDD	IN	0.003	0.003	1.00	*Daphnia pulex*	1
p,p'-DDT	IN	0.003	0.0004	7.5	*Daphnia pulex*	1
Pencycuron	FU	0.09	0.7	0.1	*Daphnia magna*	3
Pendimethalin	HB	0.01	0.3	0.03	*Daphnia magna*	1
Pirimicarb	IN	0.005	0.007	0.7	*Daphnia magna*	1
Pronamide	HB	0.03	5.6	0.005	*Daphnia magna*	1

compound	pesticide type	LOQ [mg L^{-1}]	LC_{50} [mg L^{-1}]	LOQ/LC_{50}	Species	Source
Propamocarb	FU	0.01	2.6	0.004	*Pseudokirchneriella subcapitata*	3
Propiconazole	FU	0.08	1.2	0.07	*Litopenaeus vannamei*	1
Prosulfocarb	HB	0.04	25	0.002	*Pseudokirchneriella subcapitata*	3
Quinmerac	HB	0.03	9.1	0.003	*Pseudokirchneriella subcapitata*	3
Simazine	HB	0.03	1.1	0.03	*Daphnia magna*	1
Tebuconazole	FU	0.03	2.9	0.01	*Daphnia magna*	1
Terbuthylazine	HB	0.08	5	0.02	*Daphnia magna*	1
Terbutryn	HB	0.03	2.7	0.01	*Daphnia magna*	1
Terbuthylazine, 2-Hydroxy	M	0.005	225	0.00002	*Daphnia magna*	4
Propamocarb	FU	0.01	2.6	0.004	*Pseudokirchneriella subcapitata*	3

Group of the respective species: *Acropora tumida*: coral; *Artemia salina*: crustacea; *Daphnia spp.*: crustacea; *Hyalella azteca*: crustacea; *Pseudokirchneriella subcapitata*: algae; *Pteronarcys californica*: insect; *Chironomus spp.*: insect; *Litopenaeus vannamei*: crustacea. Sources of LC_{50} values: 1: EPA, 2015; 2: Lewis et al., 2016; 3: Schüürmann et al., 2011; 4: Malaj et al., 2014. Sources other than EPA (2015) were used, when no LC_{50} was available.

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B SUPPLEMENTARY MATERIAL: SAMPLING RATES FOR PASSIVE SAMPLERS EXPOSED TO A FIELD-RELEVANT PEAK OF 42 ORGANIC PESTICIDES

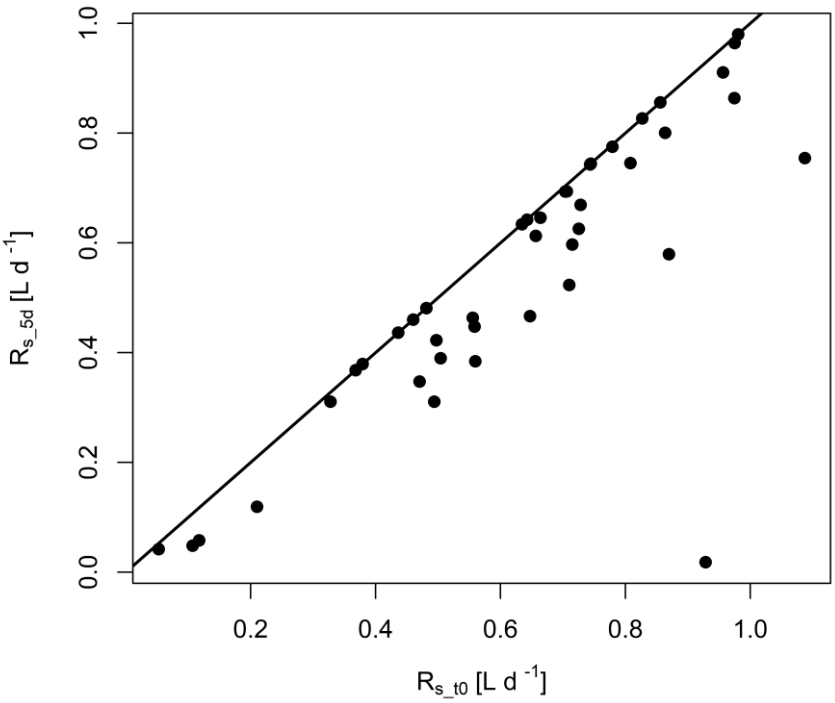


Fig. B.1: Relationship of the instantaneous R_{s_t0} [L d^{-1}] to the time-dependent R_{s_5days} [L d^{-1}] sampling rates calculated in this study. Compounds clearly diverging from the 1:1 line (black line) are approaching or reaching equilibrium of ab- and desorbance from and to the sorbent phase.

Table B.1: The instantaneous sampling rates R_{s_t0} [L d^{-1}], the sorbent-water distribution coefficients K [L kg^{-1}] and the Residual Standard Error (RSE) [ng] of the best fit model.

Compound	R_{s_t0}[a] [L d^{-1}]	K [L kg^{-1}]	RSE[b] [ng]
2-4-D	0.11	1	5
2-n-Octyl-4-isothiazolin-3-one-(OIT)	0.50	22	6
Acetamiprid	0.50	14	22
Alachlor	0.66	70	20
Azoxystrobin	0.78	973	22
Benthiavalicarb-isopropyl	0.86	912183	51
Boscalid	0.56	18	67
Carbendazim	0.47	11	23
Clothianidin	0.46	126589	32
Cyproconazol	0.73	36	17
Cyprodinil	0.74	640239	23
Diazinon	0.98	594	23
Dichlorvos	0.98	12290312	18
Difenoconazol	0.44	841813	50
Dimethenamid	0.66	159	31
Dimethoat	0.56	10	16
Dimethomorph	1.09	21	51
Epoxiconazol	0.86	85	15
Fenamidone	0.83	19174	80
Fluopicolide	0.97	53	81
Fluopyram	0.96	131	49
Imidacloprid	0.65	14	22
Iprovalicarb	0.71	17	32
Mandipropamid	0.64	1410924	95
MCPA	0.12	1	6
Metalaxyl	0.71	29	22
Methidathion	0.48	24123	70
Myclobutanil	0.81	74	36
Nicosulfuron	0.05	2	1
Pencycuron	0.33	46	18

Compound	R_{s_t0}[a] [L d^{-1}]	K [L kg^{-1}]	RSE[b] [ng]
Picoxystrobin	0.38	212743	53
Piperonyl-butoxide	0.63	10366	24
Prochloraz	0.70	326	22
Propamocarb	0.93	0	3
Propiconazol	0.74	1645656	21
Prosulfocarb	0.56	22	21
Pyrimethanil	0.71	295	18
Tebuconazol	0.73	64	27
Thiabendazol	0.21	2	11
Thiacloprid	0.87	15	23
Thiamethoxam	0.49	7	16
Trifloxystrobin	0.37	60676	9

[a] instantaneous sampling rate, referring to an exposed sorbent area of 12.57 cm^2 and a sorbent mass of 332 x 10^{-6} kg;

[b] Residual Standard Error of the best fit model which predicted the instantaneous sampling rate and the sorbent-water distribution coefficient.

Table B.2: List of compounds, which reached equilibrium during the deployment time of passive samplers within this study as well as for which sampling rates from previous studies were available.

Compound	Reached equilibrium[a]	Sampling rate available
2-4-D	X	X
2-n-Octyl-4-isothiazolin-3-one-(OIT)	X	
Acetamiprid	X	
Alachlor		X
Azoxystrobin		X
Benthiavalicarb-isopropyl		
Boscalid	X	X
Carbendazim	X	X
Clothianidin		
Cyproconazol	X	
Cyprodinil		X
Diazinon		X
Dichlorvos		
Difenoconazol		
Dimethenamid		
Dimethoat	X	X
Dimethomorph	X	X
Epoxiconazol		
Fenamidone		
Fluopicolide		
Fluopyram		
Imidacloprid	X	X
Iprovalicarb	X	X
Mandipropamid		
MCPA	X	X
Metalaxyl		
Methidathion		
Myclobutanil		X
Nicosulfuron	X	

Compound	Reached equilibrium[a]	Sampling rate available
Pencycuron		
Picoxystrobin		
Piperonyl-butoxide		
Prochloraz		
Propamocarb	X	
Propiconazol		
Prosulfocarb	X	
Pyrimethanil		X
Tebuconazol		X
Thiabendazol	X	
Thiacloprid	X	
Thiamethoxam		
Trifloxystrobin	X	

[a] Compounds reaching equilibrium had rations between uptake rate k_{ws} and release rate constants k_{sw} smaller than 15.

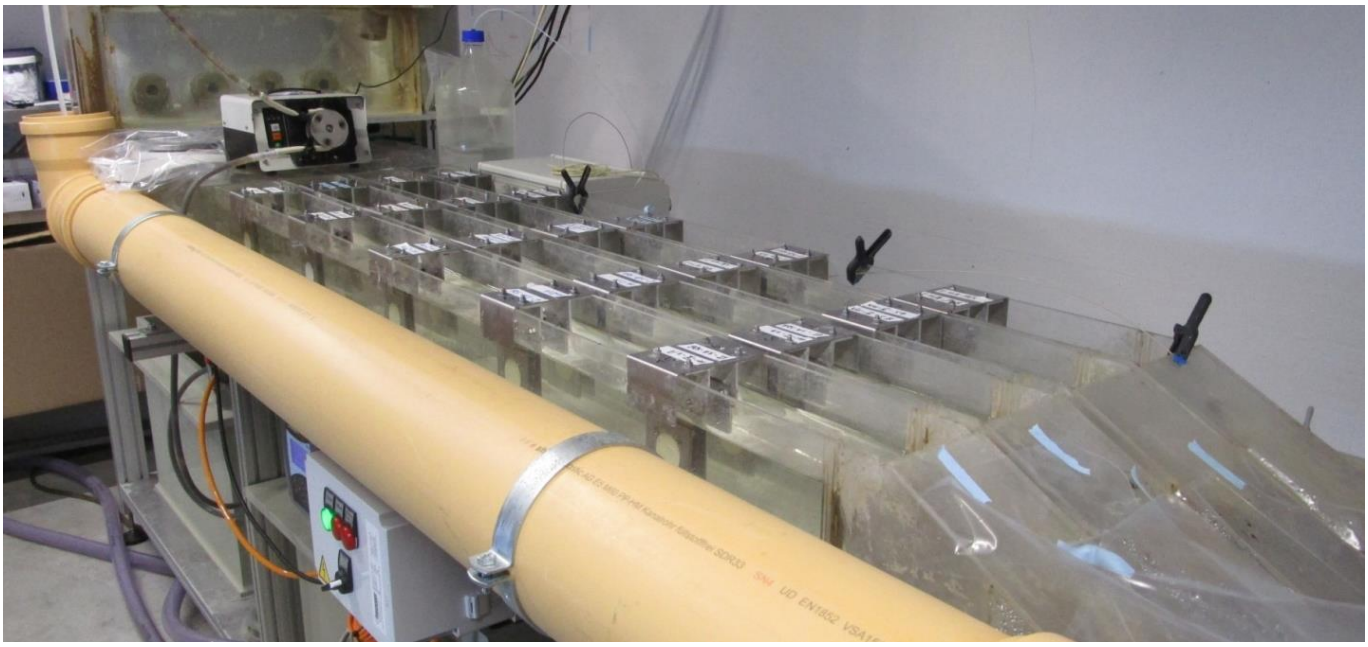


Fig. B.2: Channel system, where the experiment was conducted. Passive samplers were deployed in metal holders. Photo by Etiënne L. M. Vermeirssen.

Table B.3: Times of deployment and retrieval of Empore styrene-divinylbenzene (SDB) disks.

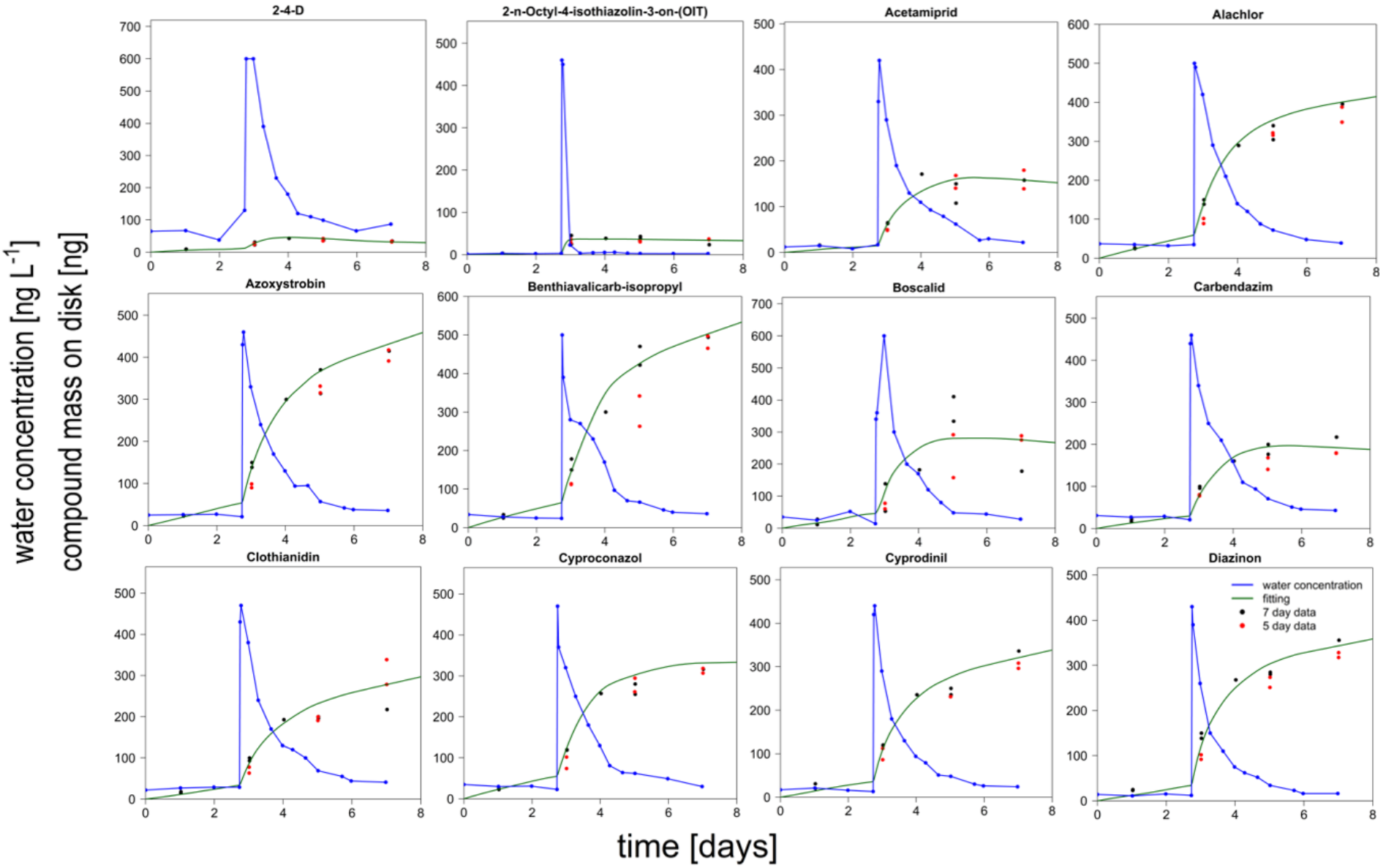
Exact time of water sample taken	Days after deployment 7-day data	Days after deployment 5-day data
15/12/2016 15:25	0.00	NA
16/12/2016 15:20	1.02	NA
17/12/2016 15:10	NA	0.00
18/12/2016 15:15	3.03	1.02
19/12/2016 15:15	4.02	1.29
20/12/2016 15:15	5.02	3.02
22/12/2016 15:15	7.02	5.02

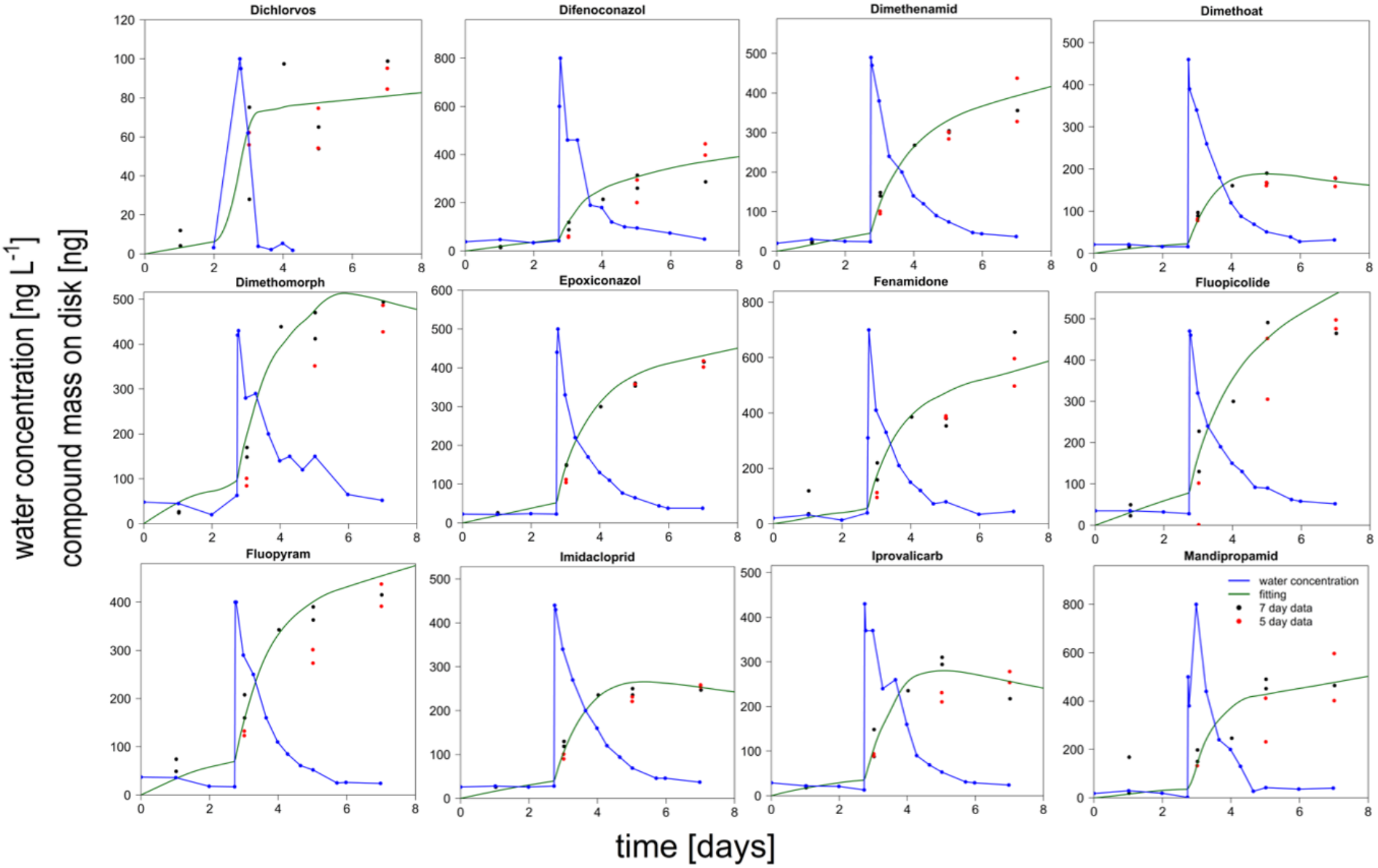
The number of days “0” refers to the time of deployment of the respective set of passive samplers. At each time point duplicated SDB disks were removed from the artificial channel system.

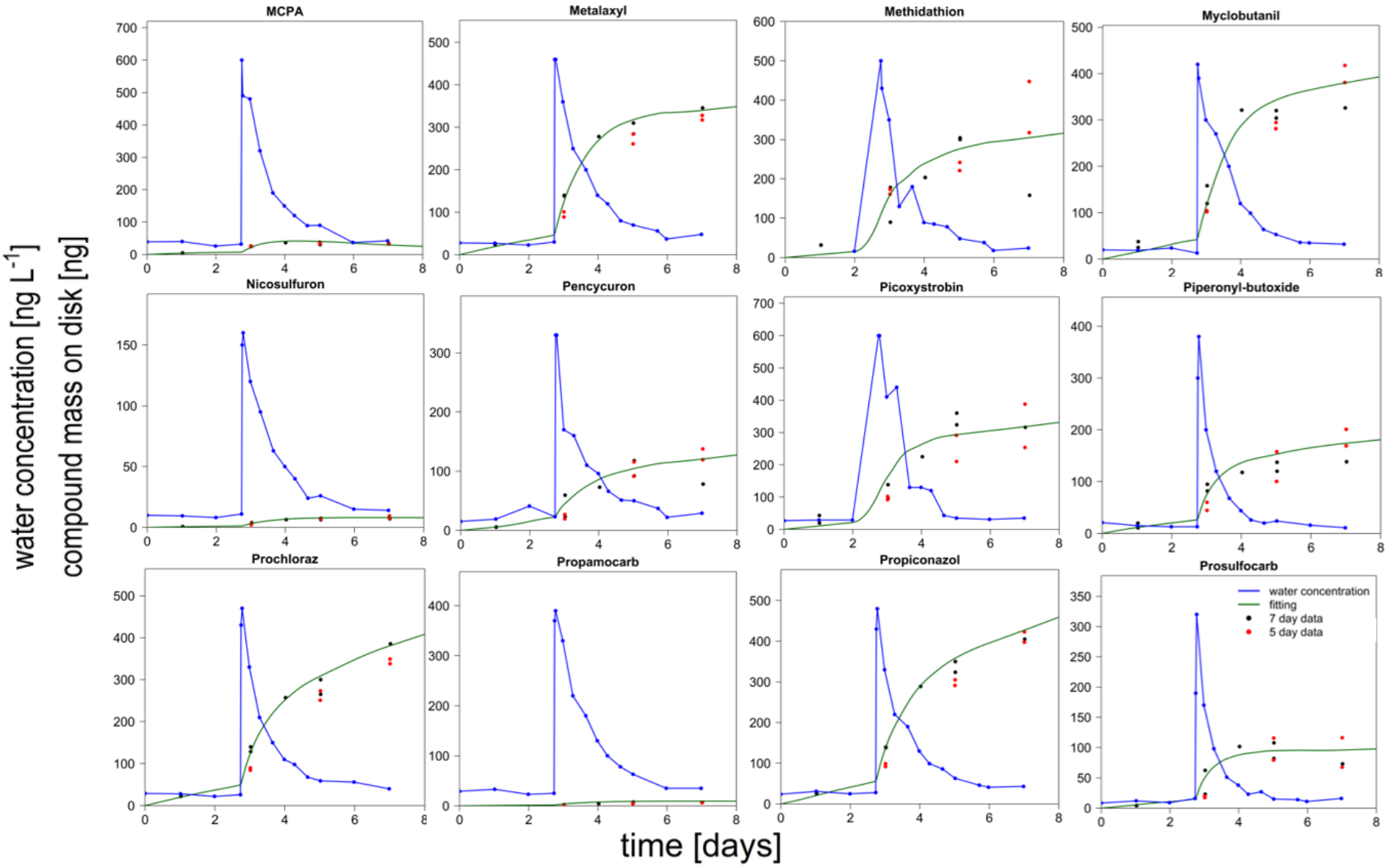
Table B.4: Time points where water samples were taken.

Exact time of water sample taken	Days after deployment 7-day data	Days after deployment 5-day data
15/12/2016 15:25	0.00	NA
16/12/2016 15:45	1.01	NA
17/12/2016 15:05	1.99	0.00
18/12/2016 08:55	2.73	0.74
18/12/2016 09:20	2.75	0.76
18/12/2016 10:00	2.77	0.79
18/12/2016 15:00	2.98	1.00
18/12/2016 22:00	3.27	1.29
19/12/2016 07:00	3.65	1.66
19/12/2016 15:00	3.98	2.00
19/12/2016 22:00	4.27	2.29
20/12/2016 07:00	4.65	2.66
20/12/2016 15:45	5.01	3.03
21/12/2016 08:30	5.71	3.73
21/12/2016 14:50	5.98	3.99
22/12/2016 15:00	6.98	5.00

The peak concentration was added on the 18.12.2016 at 9 am. The number of days “0” refers to the time of deployment of the respective set of passive samplers.


2-4-D
2-n-Octyl-4-isothiazolin-3-on-(OIT)
Acetamiprid
Alachlor
Azoxystrobin
Benthiavalicarb-isopropyl
Boscalid
Carbendazim
Clothianidin
Cyproconazol
Cyprodinil
Diazinon
water concentration
fitting
7 day data
5 day data
water concentration [ng L-1]
compound mass on disk [ng]
time [days]


Dichlorvos
Difenoconazol
Dimethenamid
Dimethoat
Dimethomorph
Epoxiconazol
Fenamidone
Fluopicolide
Fluopyram
Imidacloprid
Iprovalicarb
Mandipropamid
water concentration
fitting
7 day data
5 day data
water concentration [ng L-1]
compound mass on disk [ng]
time [days]


MCPA
Metalaxyl
Methidathion
Myclobutanil
Nicosulfuron
Pencycuron
Picoxystrobin
Piperonyl-butoxide
Prochloraz
Propamocarb
Propiconazol
Prosulfocarb
water concentration
fitting
7 day data
5 day data
water concentration [ng L-1]
compound mass on disk [ng]
time [days]

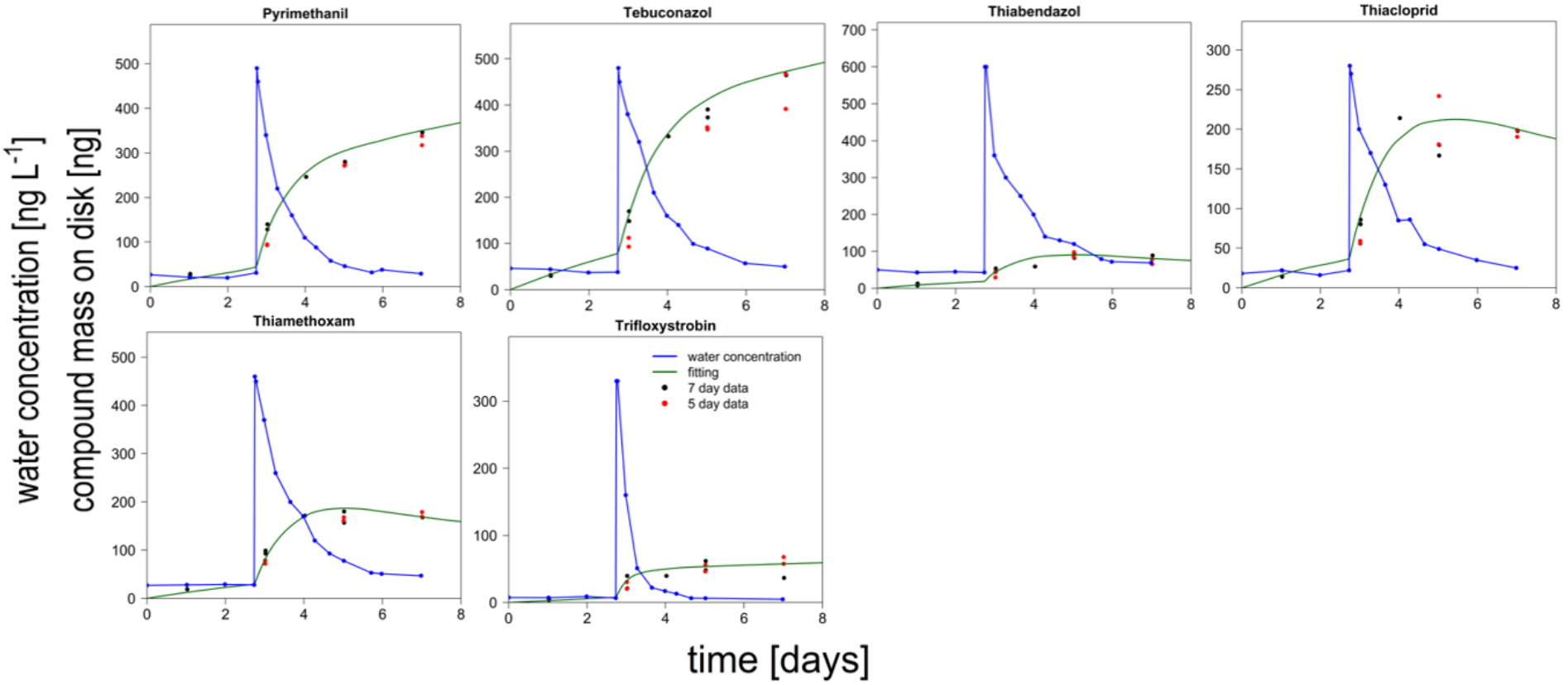


Fig. B.3: Compound concentrations in the channel and masses in the passive sampler disks.

Table B.5: List of isotope labelled standards that were spiked to Empore styrene-divinylbenzene (SDB) disk (75 µg L^{-1}), large volume injection and SPE samples (both 100 ng L^{-1}).

isotope labelled standard
2-4-D-D3
Alachlor-D13
Atrazin-2-Hydroxy-D5
Atrazin-Desethyl-15N3
Azoxystrobin-d4
Carbendazim-D4
Clothianidin-D3
Cyprodinil-D5
Diazinon-D10
Diflufenican-D3
Dimethenamid-D3
Dimethoat-D6
Diuron-D6
Epoxiconazole-D4
Imidacloprid-D4
Isoproturon-D6
MCPA-D3
Metalaxyl-D6
Nicosulfuron-D6
Prochloraz-D7
Propamocarb-D7
Propiconazol-D5
Pyrimethanil-D5
Simazin-D5
Tebuconazole-D6
Terbutylazin-D5
Thiamethoxame-D3

Table B.6: Gradient used for the inline enrichment using a Hypersil Gold aQ column 12 µm 20 x 2.1 mm.

time [min]	flow rate [mL min^{-1}]	mobile phases
0	1	98 % ultrapure water with 0.1 % of formic acid 2 % methanol with 0.1 % of formic acid
1.5	1	98 % ultrapure water with 0.1 % of formic acid 2 % methanol with 0.1 % of formic acid
1.51	0.1	98 % ultrapure water with 0.1 % of formic acid 2 % methanol with 0.1 % of formic acid
17	0.1	98 % ultrapure water with 0.1 % of formic acid 2 % methanol with 0.1 % of formic acid
17.1	1	2 % ultrapure water with 0.1 % of formic acid 98 % methanol with 0.1 % of formic acid
20	1	2 % ultrapure water with 0.1 % of formic acid 98 % methanol with 0.1 % of formic acid
20.01	1	98 % ultrapure water with 0.1 % of formic acid 2 % methanol with 0.1 % of formic acid
25	1	98 % ultrapure water with 0.1 % of formic acid 2 % methanol with 0.1 % of formic acid

Table B.6: Limits of quantification (LOQ) of different methods used to determine water concentrations.

Compound	LOQ [ng L^{-1}] Large volume injection	LOQ [ng L^{-1}] Solid phase extraction	LOQ [µg L^{-1}] SDB disks
2-4-D	>100	35	8
2-n-Octyl-4-isothiazolin-3-one-(OIT)	0.5	NA	1
Acetamiprid	8	NA	4
Alachlor	>100	25	3
Azoxystrobin	20	NA	0.5
Benthiavalicarb-isopropyl	10	NA	3
Boscalid	>100	10	10
Carbendazim	20	NA	0.2
Clothianidin	20	NA	15
Cyproconazol	>100	20	15
Cyprodinil	10	NA	0.5
Diazinon	10	NA	20

Compound	LOQ [ng L^{-1}] Large volume injection	LOQ [ng L^{-1}] Solid phase extraction	LOQ [µg L^{-1}] SDB disks
Dichlorvos	>100	1	3
Difenoconazol	>100	30	2
Dimethenamid	20	NA	2
Dimethoat	15	NA	0.3
Dimethomorph	>100	20	20
Epoxiconazol	20	NA	3
Fenamidone	30	10	4
Fluopicolide	25	NA	1
Fluopyram	15	NA	7
Imidacloprid	7	NA	0.1
Iprovalicarb	10	NA	3
Mandipropamid	15	1	15
MCPA	>100	25	3
Metalaxyl	20	NA	0.3
Methidathion	15	NA	1
Myclobutanil	10	NA	1
Nicosulfuron	>100	8	0.5
Pencycuron	15	NA	5
Picoxystrobin	>100	20	20
Piperonyl-butoxide	>100	10	5
Prochloraz	8	NA	2
Propamocarb	>100	15	1
Propiconazol	20	NA	5
Prosulfocarb	8	NA	1
Pyrimethanil	20	NA	10
Tebuconazol	>100	25	15
Thiabendazol	35	NA	5
Thiacloprid	>100	15	0.8
Thiamethoxam	25	NA	0.5
Trifloxystrobin	>100	4	2

Table B.8: Gradient used for the chromatographic separation on the analytical column (Atlantis T3 5 µm 3.0x150 mm column).

time [min]	flow rate [mL min^{-1}]	mobile phases
0	0.3	95 % ultrapure water with 0.1 % of formic acid 5 % methanol with 0.1 % of formic acid
1.5	0.3	95 % ultrapure water with 0.1 % of formic acid 5 % methanol with 0.1 % of formic acid
12.5	0.3	0 % ultrapure water with 0.1 % of formic acid 100 % methanol with 0.1 % of formic acid
20	0.3	0 % ultrapure water with 0.1 % of formic acid 100 % methanol with 0.1 % of formic acid
20.5	0.3	95 % ultrapure water with 0.1 % of formic acid 5 % methanol with 0.1 % of formic acid
25	0.3	95 % ultrapure water with 0.1 % of formic acid 5 % methanol with 0.1 % of formic acid

Table B.9: Settings for the Exactive (LC-HRMS) Orbitrap system.

Parameter	Description
Mode	Orbitrap MS
Ionization	ESI, positive and negative
Spray voltage	4,000 V
Capillary temperature	280 °C
Scan range	100 – 1,000 m z^{-1}

TEXT B.1: ADDITIONAL INFORMATION ON TWO EXPOSURE DURATIONS

The calibration experiment included two deployment scenarios of Empore styrene-divinylbenzene (SDB) disks, in which the disks were deployed three (7 day data) and one day (5 day data) before the pulse started (Table B.3). In addition to, estimating the sampling rates using both datasets combined, we also estimated them separately. We compared results from both deployment scenarios to examine how different exposure periods involving different durations of baseline exposure influence the determination of sampling rates and the estimation of (peak) pesticide water concentration.

Fitting of equation 3.2 in the main text was done using two different algorithms. One algorithm (hereafter: algorithm 1) of the R package FME (Soetaert and Petzoldt, 2016) was suitable for the simultaneous fitting of the single data points of the 5 d and 7 d datasets (hereafter: combined dataset), as well as for fitting of the 7 d data alone, for which a sufficient amount of

observations were available. To fit the 5 d data with fewer observations, an algorithm (hereafter: algorithm 2) based on the R package simecol (Petzoldt, 2018) was used, which, however, was unsuitable for fitting the combined datasets. We compared both algorithms for the 7 day data and they provided almost identical estimates of time-dependent sampling rates (Pearsons correlation coefficient $r = 0.99$, $p < 0.001$, $n = 42$, Fig. B.4). Moreover, the results of these algorithms were compared with results obtained in a different software environment, namely ModelMaker (version 4.0; Cherwell Scientific Ltd.) to confirm the reliability of sampling rate estimates.

The time-dependent sampling rates modelled for the three different datasets (i.e. combined dataset with algorithm 1, 7-day data with algorithms 1 and 2, 5-day data with algorithm 2) showed high congruence (Fig. B.4, Table B.10), hence we only considered sampling rates of the combined dataset in the manuscript.

COMPARISON OF DIFFERENT DATASETS

The time-dependent sampling rates derived for the 5 d, 7 d, as well as the combined datasets, were very congruent with a maximum difference of 10 % (Table B.10). This high congruence of sampling rates with only marginal, non-systematic (i.e. lacking specific trends) differences between the different datasets was reflected in the uptake rate k_{ws} (Table B.10). The release rate k_{sw}, however, showed higher differences (Table B.10). The congruence of the release constants and the fitting would very likely be improved with a higher number of observations (Vermeirssen et al., 2013).

Given that both rate constants are fitted in a dependent manner (the increase of one rate constant leading to an increase of the other) and the release rate being at minimum one order of magnitude lower than the uptake rate, different pairs of rate constants can result in similar sampling rates. This may especially be the case in our study with a limited number of observations available for modelling uptake and release. The time-dependent sampling rates for thiabendazole, for example, were 0.12 L day^{-1} for all three datasets, although the uptake and release rate constants differed by 65 % and 41 % (percentage difference of minimum and maximum value), respectively. This implies that sampling rates (R_s) are robust and can be used to compare different studies and rate constants are associated with uncertainty.

The fact that different datasets lead to comparable sampling rates indicates that passive sampling can be reliably calibrated with short-time peak concentration scenarios similar to the one presented in this study. The experiment further shows that the influence of a previous one- or three-day baseline exposure on the sampling rates is insignificant since the majority of the pesticide mass was carried in the pesticide pulse.

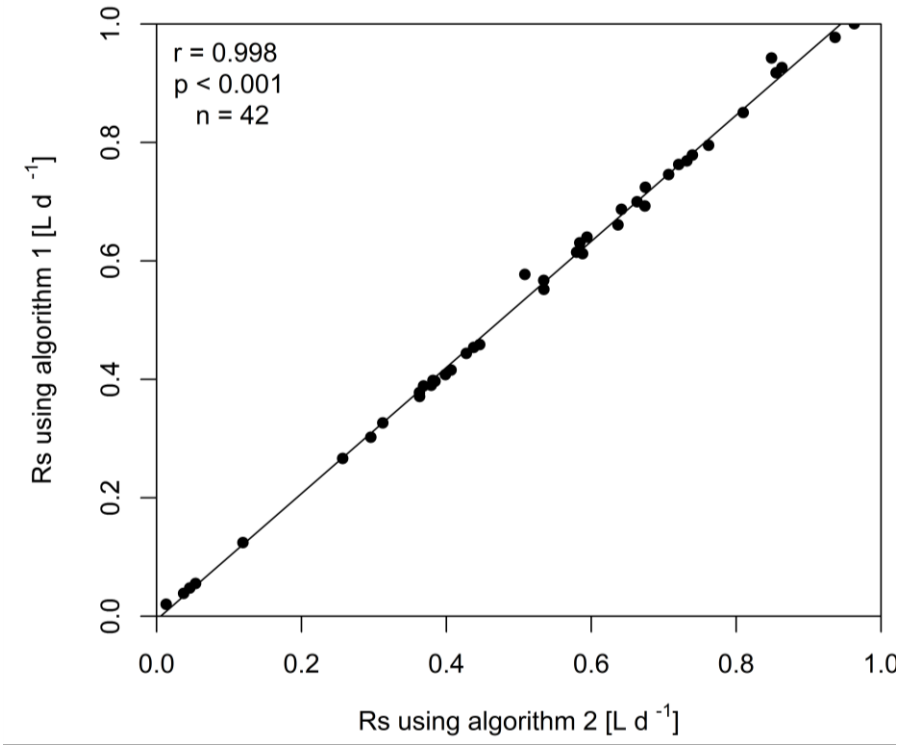


Fig. B.4: Relationship of modelled time-dependent sampling rates of the 7 day dataset using algorithms 1 and 2. Pearsons correlation coefficient r = 0.999, p < 0.001, n = 42. Black line: correlation.

Table B.10: Modelled time-dependent sampling rates (R_{S_5d}) in L day^{-1} as well as rates constants k_{ws} and k_{sw} from the different datasets and used algorithms.

	combined dataset algorithm 1			5-day dataset algorithm 2			7-day dataset algorithm 1			7-day dataset algorithm 2		
Compound	R_{S_d5}	k_{ws}	k_{sw}	R_{S_5d}	k_{ws}	k_{sw}	R_{S_5d}	k_{ws}	k_{sw}	R_{S_5d}	k_{ws}	k_{sw}
2-4-D	0.05	0.11	0.38	0.05	0.12	0.41	0.05	0.10	0.37	0.05	0.10	0.35
2-n-Octyl-4-isothiazolin-3-one-(OIT)	0.42	0.50	0.07	0.43	0.45	0.02	0.42	0.55	0.12	0.41	0.54	0.12
Acetamiprid	0.39	0.51	0.11	0.40	0.49	0.08	0.37	0.54	0.16	0.36	0.52	0.15
Alachlor	0.61	0.66	0.03	0.62	0.66	0.02	0.63	0.68	0.04	0.58	0.61	0.02
Azoxystrobin	0.77	0.78	0.00	0.79	0.79	0.00	0.78	0.84	0.03	0.74	0.77	0.01
Benthiavalicarb-isopropyl	0.86	0.86	0.00	0.82	0.83	0.00	0.92	0.95	0.02	0.86	0.86	0.00
Boscalid	0.45	0.55	0.09	0.45	0.45	0.00	0.45	0.68	0.18	0.44	0.64	0.16
Carbendazim	0.35	0.47	0.13	0.33	0.44	0.12	0.38	0.48	0.10	0.36	0.44	0.08
Clothianidin	0.46	0.46	0.00	0.51	0.51	0.00	0.40	0.55	0.14	0.38	0.51	0.12
Cyproconazol	0.63	0.73	0.06	0.65	0.76	0.06	0.61	0.74	0.08	0.58	0.66	0.06
Cyprodinil	0.74	0.74	0.00	0.74	0.74	0.00	0.76	0.75	-0.01	0.72	0.72	0.00
Diazinon	0.96	0.97	0.00	0.95	0.95	0.00	1.00	1.05	0.02	0.96	0.98	0.01
Dichlorvos	0.98	0.98	0.00	1.03	1.03	0.00	0.98	0.83	-0.08	0.94	0.94	0.00
Difenoconazol	0.44	0.44	0.00	0.48	0.48	0.00	0.39	0.46	0.07	0.37	0.41	0.05
Dimethenamid	0.65	0.65	0.00	0.67	0.67	0.00	0.64	0.74	0.07	0.59	0.68	0.06

Compound	combined dataset algorithm 1			5-day dataset algorithm 2			7-day dataset algorithm 1			7-day dataset algorithm 2		
	R_{S_d5}	k_{ws}	k_{sw}	R_{S_5d}	k_{ws}	k_{sw}	R_{S_5d}	k_{ws}	k_{sw}	R_{S_5d}	k_{ws}	k_{sw}
Dimethoat	0.38	0.56	0.16	0.38	0.55	0.16	0.39	0.57	0.16	0.38	0.53	0.15
Dimethomorph	0.75	1.09	0.16	0.75	1.02	0.13	0.77	1.16	0.18	0.73	1.00	0.13
Epoxiconazol	0.80	0.86	0.03	0.82	0.87	0.02	0.80	0.89	0.05	0.76	0.82	0.03
Fenamidone	0.83	0.83	0.00	0.81	0.81	0.00	0.85	0.62	-0.15	0.81	0.81	0.00
Fluopicolide	0.86	0.86	0.00	0.82	0.82	0.00	0.94	1.19	0.12	0.85	1.07	0.10
Fluopyram	0.91	0.91	0.00	0.88	0.88	0.00	0.93	1.14	0.08	0.86	1.01	0.06
Imidacloprid	0.47	0.65	0.14	0.47	0.59	0.09	0.46	0.72	0.20	0.45	0.67	0.18
Iprovalicarb	0.52	0.71	0.13	0.52	0.54	0.02	0.58	0.84	0.21	0.51	0.79	0.19
Mandipropamid	0.64	0.64	0.00	0.64	0.64	0.00	0.66	0.64	-0.02	0.64	0.64	0.00
MCPA	0.06	0.12	0.33	0.06	0.13	0.36	0.06	0.11	0.33	0.05	0.10	0.30
Metalaxyl	0.60	0.71	0.07	0.59	0.65	0.04	0.61	0.77	0.10	0.59	0.71	0.08
Methidathion	0.48	0.48	0.00	0.54	0.54	0.00	0.41	0.55	0.13	0.40	0.54	0.12
Myclobutanil	0.75	0.81	0.03	0.78	0.78	0.00	0.69	0.99	0.15	0.67	0.94	0.14
Nicosulfuron	0.04	0.05	0.10	0.04	0.05	0.04	0.04	0.06	0.20	0.04	0.06	0.18
Pencycuron	0.31	0.33	0.02	0.36	0.36	0.00	0.27	0.40	0.18	0.26	0.37	0.16
Picoxystrobin	0.38	0.38	0.00	0.37	0.37	0.00	0.40	0.36	-0.05	0.38	0.38	0.00
Piperonyl-butoxide	0.63	0.63	0.00	0.69	0.69	0.00	0.57	0.74	0.11	0.53	0.67	0.09

Compound	combined dataset algorithm 1			5-day dataset algorithm 2			7-day dataset algorithm 1			7-day dataset algorithm 2		
	$RS_{\_d5}$	k_{ws}	k_{sw}	$RS_{\_5d}$	k_{ws}	k_{sw}	$RS_{\_5d}$	k_{ws}	k_{sw}	$RS_{\_5d}$	k_{ws}	k_{sw}
Prochloraz	0.69	0.69	0.00	0.68	0.68	0.00	0.72	0.72	0.00	0.67	0.67	0.00
Propamocarb	0.02	0.02	0.07	0.01	0.01	0.01	0.02	0.03	0.11	0.01	0.01	0.03
Propiconazol	0.74	0.74	0.00	0.75	0.75	0.00	0.75	0.81	0.03	0.71	0.73	0.01
Prosulfocarb	0.46	0.56	0.07	0.48	0.48	0.00	0.44	0.66	0.17	0.43	0.62	0.16
Pyrimethanil	0.69	0.71	0.01	0.70	0.70	0.00	0.70	0.73	0.02	0.66	0.67	0.00
Tebuconazole	0.67	0.73	0.03	0.67	0.69	0.01	0.69	0.77	0.05	0.64	0.68	0.02
Thiabendazole	0.12	0.21	0.25	0.12	0.26	0.35	0.12	0.18	0.17	0.12	0.17	0.14
Thiacloprid	0.58	0.87	0.18	0.61	0.91	0.17	0.55	0.91	0.22	0.53	0.83	0.19
Thiamethoxam	0.31	0.49	0.20	0.32	0.47	0.16	0.30	0.54	0.26	0.30	0.51	0.24
Trifloxystrobin	0.37	0.37	0.00	0.41	0.41	0.00	0.33	0.40	0.09	0.31	0.38	0.08

Algorithm 1 is based on the FME package (Soetaert and Petzoldt, 2016). Algorithm 2 is based on the simecol R package (Petzoldt, 2018).

TEXT B.2: CALCULATION OF LOG D_{ow} ACCORDING TO CARMICHAEL (2014)

To calculate the *log* D_{ow} according to Carmichael (2014), we used experimental log K_{ow} and pK_a values retrieved from ppdb (Lewis et al., 2016). Calculation of the Log D_{ow} was done using equation B.1 for acids and B.2 for bases:

$$log\ D_{ow} = log\ K_{ow} - log\left[1 + \ 10^{(pH - pK_a)}\right] \quad \text{(B.1)}$$

$$log\ D_{ow} = log\ K_{ow} + log\left[1 + \ 10^{(pK_a - pH)}\right] \quad \text{(B.2)}$$

TEXT B.3: TRANSFORMATION OF EQUATIONS

Water concentrations (c_{calc}) from the compound mass sorbed to the sorbent (m_{sorb}) during the exposure time (t_{exp}) can be calculated, by transforming equations 3.1 (for instantaneous sampling rates) and 2 (for time-dependent sampling rates).

Calculation of water concentration based on instantaneous sampling rate (R_{s_t0}) based on equation 3.1:

$$c_{calc} = \frac{m_{sorb}}{m_{samp} \cdot k \cdot \left(1 - exp^{\left(-\frac{R_{s\_t0} \cdot t_{exp}}{m_{samp} \cdot k}\right)}\right)} \quad \text{(B.3)}$$

where k is the sorbent-water distribution coefficient and m_{samp} refers to the mass of the passive sampler sorbent (332 x 10^{-6} kg).

Calculation of water concentration for time-dependent sampling based on equation 3.2:

$$c_{calc} = \frac{m_{sorb} \cdot k_{sw}}{1 - exp^{(-k_{sw} \cdot t_{exp})} \cdot k_{ws}} \quad \text{(B.4)}$$

where k_{ws} is the uptake and k_{sw} the release rate constant.

Both these equations can be transformed to the simplified equation 3.4 given in the main text. For time frames relevant to this study (2 – 7 d), the different equations result in a similar c_{calc}, therefore we only report the results for equation 3.4.

a)

b)

c)

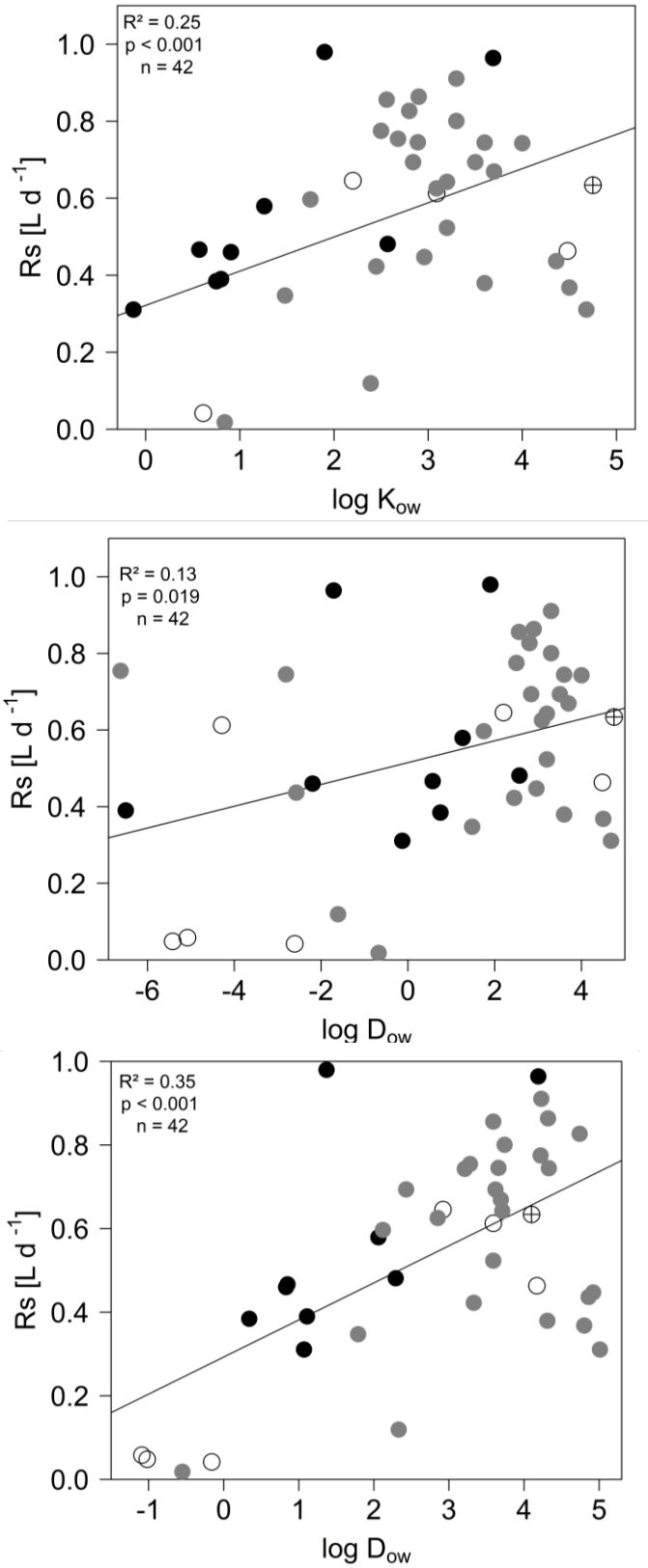
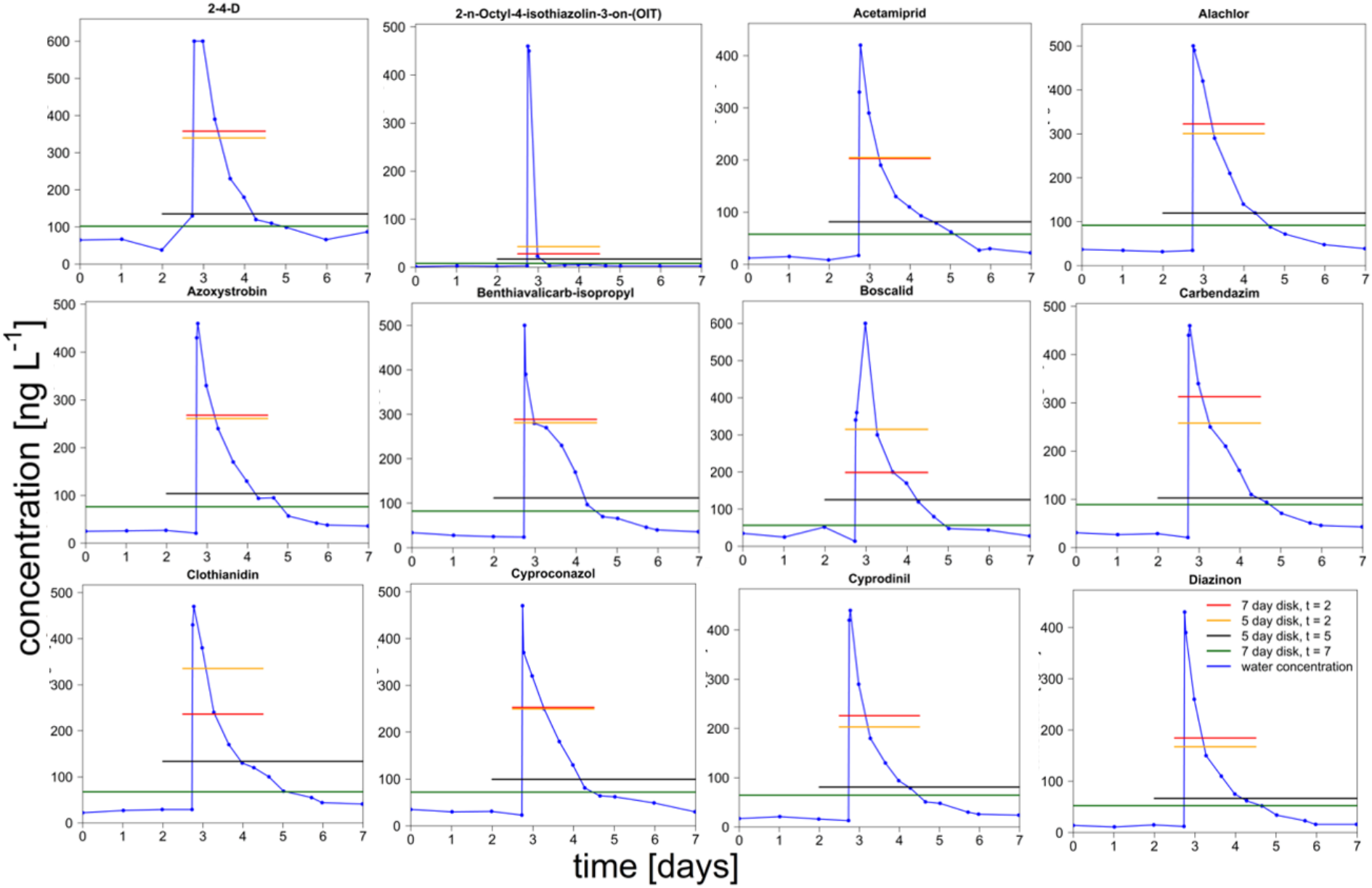


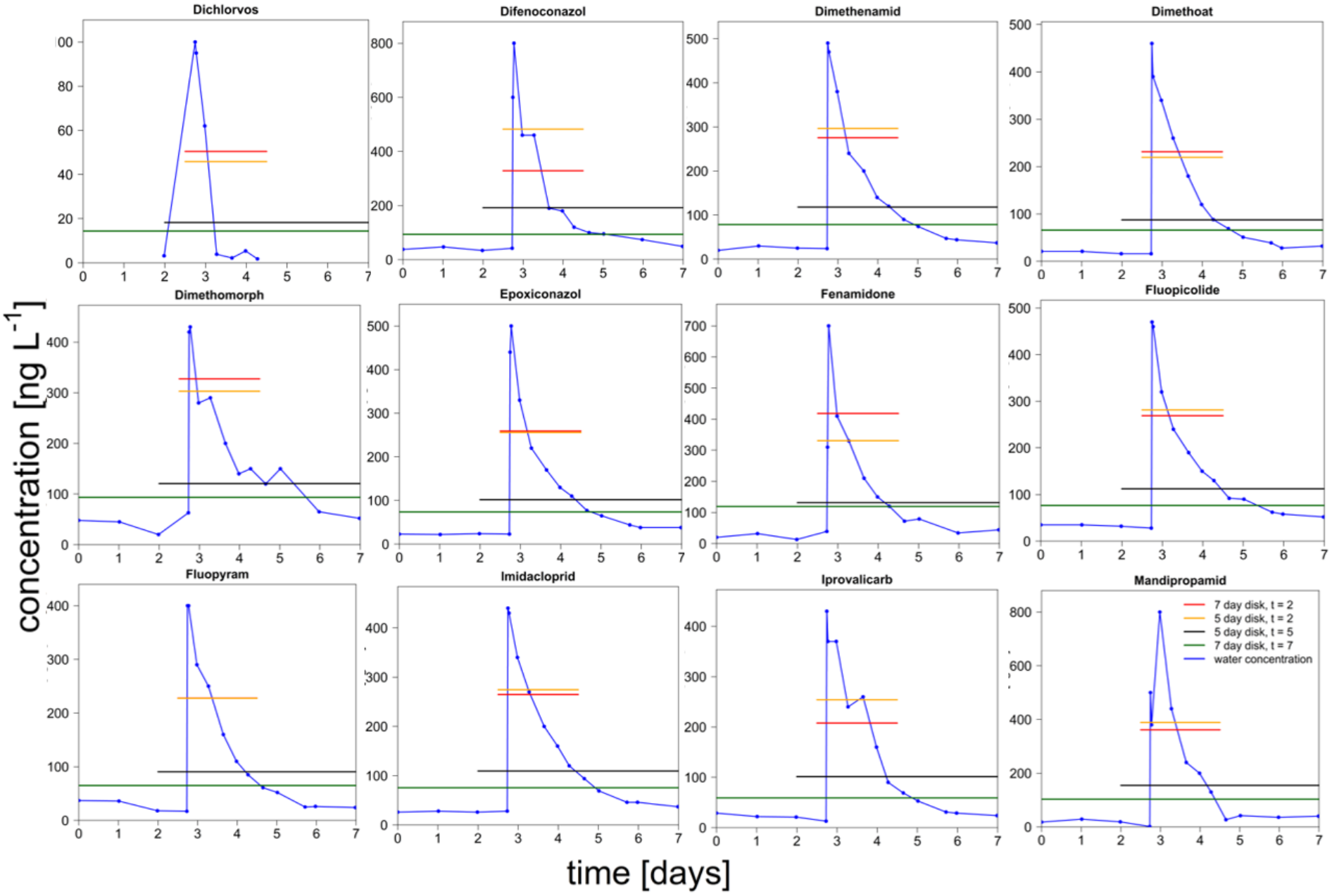
Fig. B.5: Relationship of sampling rates R_S [L day^{-1}] with a) log K_{ow}, b) log D_{ow} from ppdb (Lewis et al., 2016) based on experimental values (at pH = 8) and c) log D_{ow} from chemicalize.com (ChemAxon, 2019) based on estimations from chemical structure (at pH = 8). Direct comparison of log K_{ow} and log D_{ow} from ppdb and chemicalize.com see Tabel B.6. White: herbicides, grey: fungicides, black: insecticides, crossed: synergist.

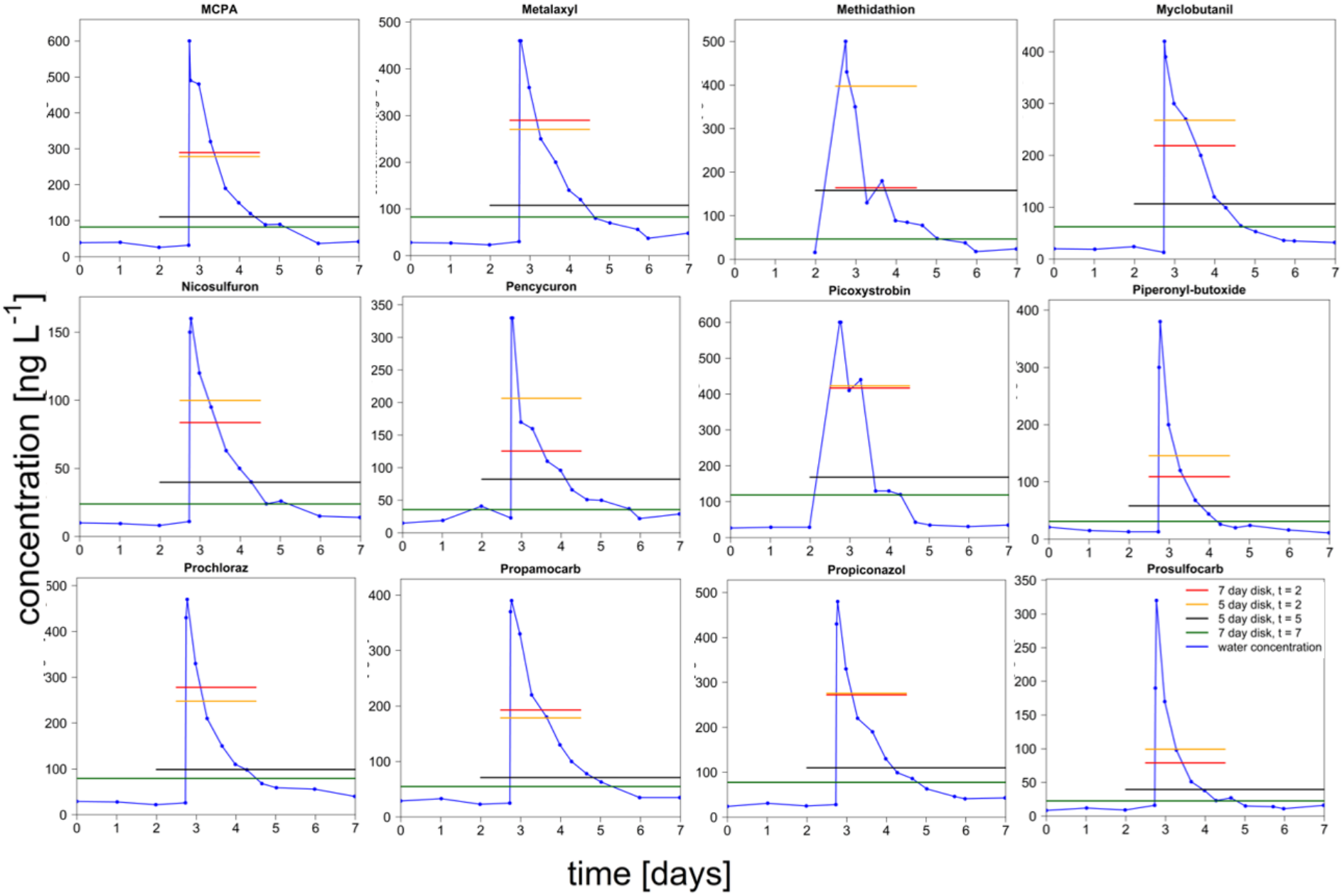
Table B.11: Direct comparison of log K_{ow} and log D_{ow} at pH = 8 values retrieved from ppdb (Lewis et al., 2016) and chemicalize.com (ChemAxon, 2019). Log D_{ow} indicated as retieved from ppdb was calculated according to Carmichael (2014) based on log K_{ow} and pK_a retrieved from ppdb. For non-charged compounds, the log K_{ow} was used.

	log K_{ow}		log D_{ow}	
Compound	ppdb	chemicalize.com	ppdb	chemicalize.com
2-4-D	2.81	2.50	-1.79	-1.02
2-n-Octyl-4-isothiazolin-3-one-(OIT)	2.45	3.33	2.45	3.33
Acetamiprid	0.80	1.11	-6.50	1.11
Alachlor	3.09	3.59	-4.29	3.59
Azoxystrobin	2.50	4.22	2.50	4.22
Benthiavalicarb-isopropyl	2.56	3.59	2.56	3.59
Boscalid	2.96	4.92	2.96	4.92
Carbendazim	1.48	1.80	1.48	1.79
Clothianidin	0.91	0.88	-2.20	0.83
Cyproconazol	3.09	2.85	3.09	2.85
Cyprodinil	4.00	3.21	4.00	3.21
Diazinon	3.69	4.19	-1.71	4.19
Dichlorvos	1.90	1.37	1.90	1.37
Difenoconazol	4.36	4.86	-2.57	4.86
Dimethenamid	2.20	2.92	2.20	2.92
Dimethoat	0.75	0.34	0.75	0.34
Dimethomorph	2.68	3.28	-6.62	3.28
Epoxiconazol	3.30	3.74	3.30	3.74
Fenamidone	2.80	4.74	2.80	4.74
Fluopicolide	2.90	4.33	2.90	4.32
Fluopyram	3.30	4.23	3.30	4.23
Imidacloprid	0.57	0.87	0.57	0.85
Iprovalicarb	3.20	3.59	3.20	3.59
Mandipropamid	3.20	3.71	3.20	3.71
MCPA	3.25	2.41	-1.02	-1.09
Metalaxyl	1.75	2.12	1.75	2.12
Methidathion	2.57	2.29	2.57	2.29
Myclobutanil	2.89	3.66	-2.81	3.66

	log K_{ow}		log D_{ow}	
Compound	ppdb	chemicalize.com	ppdb	chemicalize.com
Nicosulfuron	0.61	0.78	-2.61	-0.16
Pencycuron	4.68	5.01	4.68	5.01
Picoxystrobin	3.60	4.31	3.60	4.31
Piperonyl-butoxide	4.75	4.10	4.75	4.10
Prochloraz	3.50	3.62	3.50	3.62
Propamocarb	0.84	0.77	-0.67	-0.55
Propiconazol	3.60	4.33	3.60	4.33
Prosulfocarb	4.48	4.17	4.48	4.17
Pyrimethanil	2.84	2.43	2.84	2.43
Tebuconazol	3.70	3.69	3.70	3.69
Thiabendazol	2.39	2.33	-1.61	2.33
Thiacloprid	1.26	2.06	1.26	2.06
Thiamethoxam	-0.13	1.07	-0.13	1.07
Trifloxystrobin	4.50	4.80	4.50	4.80


2-4-D
2-n-Octyl-4-isothiazolin-3-on-(OIT)
Acetamiprid
Alachlor
Azoxystrobin
Benthiavalicarb-isopropyl
Boscalid
Carbendazim
Clothianidin
Cyproconazol
Cyprodinil
Diazinon
concentration [ng L-1]
time [days]
7 day disk, t = 2
5 day disk, t = 2
5 day disk, t = 5
7 day disk, t = 7
water concentration


Dichlorvos
Difenoconazol
Dimethenamid
Dimethoat
Dimethomorph
Epoxiconazol
Fenamidone
Fluopicolide
Fluopyram
Imidacloprid
Iprovalicarb
Mandipropamid
7 day disk, t = 2
5 day disk, t = 2
5 day disk, t = 5
7 day disk, t = 7
water concentration
concentration [ng L-1]
time [days]


MCPA
Metalaxyl
Methidathion
Myclobutanil
Nicosulfuron
Pencycuron
Picoxystrobin
Piperonyl-butoxide
Prochloraz
Propamocarb
Propiconazol
Prosulfocarb
concentration [ng L-1]
time [days]
7 day disk, t = 2
5 day disk, t = 2
5 day disk, t = 5
7 day disk, t = 7
water concentration

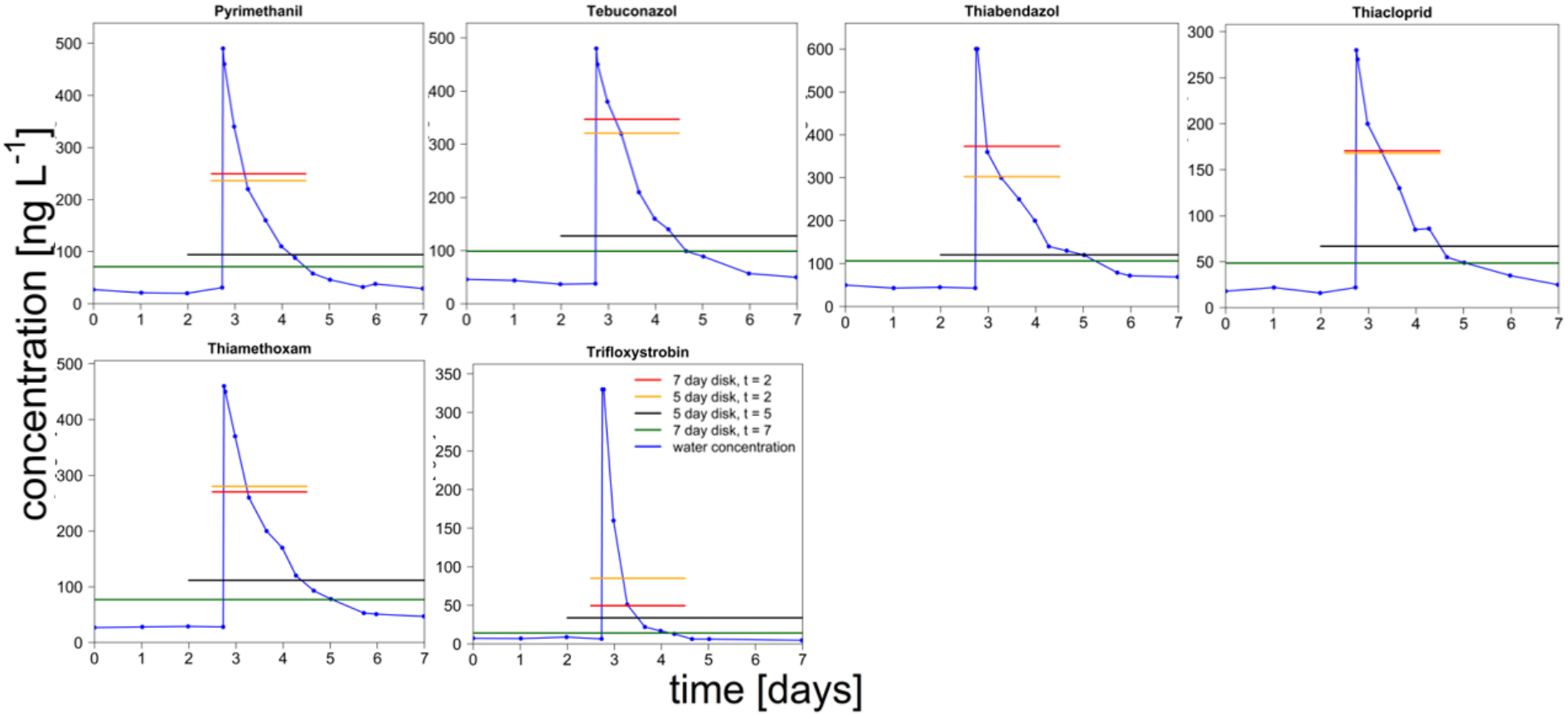


Fig. B.6: Water concentration gradient in the channel and the calculated time-weighted average (TWA) as well as pulse concentrations (calculation according to equation 3.4) based on the passive sampler with the longest exposure time for all analysed compounds

SUPPLEMENTARY REFERENCES

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C SUPPLEMENTARY MATERIAL: PARADISE LOST? PESTICIDE POLLUTION IN A EUROPEAN REGION WITH CONSIDERABLE AMOUNT OF TRADITIONAL AGRICULTURE

Table C.1: Coordinates, elevation, catchment size and agricultural land use in the catchment of sampling sites.

site	latitude[a]	longitude[a]	elevation [m]	catchment size [km²]	agricultural land use [%][b]	agricultural intensity[c]
A	46.84921	23.07857	565	29.3	12.0	low
B	46.82658	22.99440	576	102.3	12.1	medium
C	46.91014	23.0544	361	7.6	58.7	high
D	46.76527	23.36033	415	134.7	9.7	medium
E	47.49242	23.22602	168	35.0	39.6	high
F	47.41272	23.27111	183	21.8	53.0	low
G	47.37907	23.14218	178	117.8	45.6	low
H	47.0970	23.17982	253	126.2	17.7	medium
I	47.08473	23.18643	259	41.9	16.0	medium
K	46.93456	23.11216	317	133.8	16.9	high
L	46.93583	22.94882	448	15.9	6.9	low
M	46.95094	23.05741	369	33.0	9.6	high
N	46.84914	23.02365	553	23.2	21.4	medium
O	46.57814	23.6671	465	9.3	61.3	medium
P	46.58483	23.64969	472	171.7	38.1	high
Q	46.63419	23.52278	527	33.2	32.8	high
R	46.66156	23.66503	527	32.7	10.1	high
S	46.95959	23.69000	304	157.1	16.7	low
T	46.94083	23.64793	316	176.8	20.3	medium

[a] coordinate reference system WGS84.

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

[c] based on average field sizes and observations of agricultural practice during field work.

Table C.2: Information on measured compounds including detection status, limits of quantification, sampling rates and EC_{50} values. only available on attached CD.

Table C.3: Sampling times of Empore styrene-divinylbenzene (SDB) disks and polydimethylsiloxane sheets (PDMS) sheets.

Passive sampler type	Sampling event	Description	Deployment time frame	Duration [d]	Event type	Total amount of rain over whole exposure period [mm] [a, b]
SDB disk	1	Base exposure 1	11. - 18.05.2016	6	Base flow	2
SDB disk	2	Peak exposure 1	22. - 28.05.2016	5	Rainfall event	51
SDB disk	3	Peak exposure 2	02. - 09.06.2016	6	Rainfall event	29
SDB disk	4	Peak exposure 3	15. - 22.06.2016	6	Rainfall event	13
PDMS sheet	1	[c]	28.04. - 22.05.2016	23	Base flow	17
PDMS sheet	2	[c]	22.05. - 21.06.2016	29	Base flow with periodic rainfall events	125

[a] Rainfall amount from weather station in Cluj-Napoca retrieved from (Weather Underground, 2017).

[b] Additionally the stream banks were visually inspected for signs of floods.

[c] combined with respective sampling event of SDB disk.

A

B

Fig. C.1: Images of passive samplers deployed in the stream. A: One replicate of Empore styrene-divinylbenzene (SDB) disk in metal holder. B: polydimethylsiloxane sheets (PDMS) sheet. Photos by Verena C. Schreiner.

Table C.4: Gradient of mobile phases during LC-HRMS(/MS) analysis of Empore styrene-divinylbenzene (SDB) disks.

time [min]	flow velocity [mL min^{-1}]	mobile phases
0	0.3	95 % ultrapure water with 0.1 % of formic acid 5 % methanol with 0.1 % of formic acid
1.5	0.3	95 % ultrapure water with 0.1 % of formic acid 5 % methanol with 0.1 % of formic acid
17.5	0.3	5 % ultrapure water with 0.1 % of formic acid 95 % methanol with 0.1 % of formic acid
25	0.3	5 % ultrapure water with 0.1 % of formic acid 95 % methanol with 0.1 % of formic acid
25.5	0.3	95 % ultrapure water with 0.1 % of formic acid 5 % methanol with 0.1 % of formic acid
29.5	0.3	95 % ultrapure water with 0.1 % of formic acid 5 % methanol with 0.1 % of formic acid

Table C.5: Settings for the QExactive Plus (LC-HRMS/MS) and the Exactive (LC-HRMS) Orbitrap system.

Parameter	Description
Ionization	ESI, positive and negative
Spray voltage	4,000 V (positive) 3,000 V (negative)
Capillary temperature	320°C
Scan range	100 – 1,000 m z^{-1}
sheath and aux gas (nitrogen):	40 and 10 (arbitrary units)

Table C.6: Single variables which were combined to gain variables used in Table 4.1 in the main text.

original variable	Combined variable	unit	min.	max.	median	mean	SD
bank height - vertical distance to landscape level	direct distance between stream and landscape level	m	1	6	3	3.00	1.49
horizontal distance to landscape level	direct distance between stream and landscape level	m	1	8.5	2	3.16	2.01
riparian cover forest	average riparian plant height[a]	%	0	40	10	13.03	13.01
riparian cover reed	average riparian plant height	%	0	92.5	0	9.61	24.57
riparian cover shrubs	average riparian plant height	%	0	47.5	12.5	17.11	14.98
riparian cover forbs	average riparian plant height	%	0	75	30	33.55	19.73
riparian cover meadow	average riparian plant height	%	0	77.5	20	24.21	19.86
riparian without vegetation	average riparian plant height	%	0	12.5	0	0.93	3.03
riparian cover agriculture	average riparian plant height	%	0	10	0	1.05	2.68
riparian height forest	average riparian plant height	m	0	17	6.5	7.55	5.79
riparian height reed	average riparian plant height	m	0	2	0	0.35	0.68
riparian height shrubs	average riparian plant height	m	0	6	3.5	3.11	1.80
riparian height forbs	average riparian plant height	m	0	8.25	1	1.25	1.78
riparian height meadow	average riparian plant height	m	0	1.25	0.4	0.46	0.28
riparian height agriculture	average riparian plant height	m	0	0.15	0	0.01	0.04

[a] all values refer to an approx. 5 m riparian buffer

SD: standard deviation, min: minimum, max: maximum.

Table C.7: Number of detected compounds (pesticides considered for calculation of sum concentration/ metabolites) in each sample as well as across all samples based on the complete compound screening.

site	Base exposure 1	Peak exposure 1	Peak exposure 2	Peak exposure 3	Across all samplings
A	10 (9/1)	31 (17/4)	32 (17/7)	33 (18/5)	43 (24/8)
B	12 (11/1)	22 (13/6)	25 (15/6)	33 (19/7)	42 (26/8)
C	15 (14/1)	39 (20/10)	40 (21/10)	41 (24/8)	51 (27/11)
D	11 (9/1)	33 (18/8)	38 (22/11)	45 (24/9)	56 (31/11)
E	14 (13/1)	38 (19/13)	52 (28/12)	54 (28/14)	65 (32/15)
F	13 (11/1)	36 (20/9)	47 (24/12)	54 (27/12)	58 (28/13)
G	10 (9/1)	61 (30/15)	59 (29/14)	63 (31/17)	72 (34/18)
H	10 (9/1)	40 (22/9)	44 (25/9)	48 (25/11)	58 (30/12)
I	8 (7/1)	44 (22/10)	41 (23/9)	46 (23/9)	55 (26/11)
K	12 (11/1)	40 (21/10)	34 (19/10)	36 (18/9)	47 (25/12)
L	4 (4/NA)	23 (12/5)	23 (10/6)	24 (11/7)	34 (17/9)
M	7 (6/1)	32 (19/9)	39 (20/9)	40 (19/9)	50 (26/11)
N	11 (10/1)	44 (23/10)	39 (20/11)	37 (20/10)	48 (26/11)
O	7 (6/1)	39 (20/12)	NA	44 (22/13)	46 (23/14)
P	16 (15/1)	47 (25/12)	62 (32/14)	66 (33/16)	71 (37/16)
Q	13 (12/1)	43 (23/13)	37 (21/10)	40 (21/10)	49 (27/13)
R	3 (3/NA)	36 (18/8)	34 (17/9)	46 (25/10)	54 (28/12)
S	10 (8/1)	43 (22/9)	42 (20/10)	44 (18/11)	57 (24/14)
T	NA	55 (23/16)	42 (21/9)	48 (24/11)	63 (27/17)

NA: not available.

Table C.8: Sum concentration [µg L^{-1}] (conc) as well as $sumTU_{invertebrate}$ ($sumTU_{iv}$) and $sumTU_{algae}$ ($sumTU_{al}$) for each sample event as well as maximum across all sampling events. Respectively based on 55, 53 and 47 pesticides from both passive sampling methods (different number of missing EC_{50} values).

	Base exposure 1			Peak exposure 1			Peak exposure 2			Peak exposure 3			Across all samplings		
site	conc [µg L^{-1}]	$sumTU_{iv}$	$sumTU_{al}$	conc [µg L^{-1}]	$sumTU_{iv}$	$sumTU_{al}$	conc [µg L^{-1}]	$sumTU_{iv}$	$sumTU_{al}$	conc [µg L^{-1}]	$sumTU_{iv}$	$sumTU_{al}$	conc [µg L^{-1}]	$sumTU_{iv}$	$sumTU_{al}$
A	0.04	-2.81	-2.95	0.75	-1.45	-1.68	0.17	-1.06	-2.17	0.11	-1.48	-2.52	0.75	-1.06	-1.68
B	0.10	-3.11	-2.26	0.13	-1.93	-2.43	0.12	-1.35	-2.18	0.08	-1.36	-2.68	0.13	-1.35	-2.18
C	0.44	-2.28	-1.57	2.90	-0.86	-0.74	1.29	-1.52	-1.10	0.50	-0.81	-1.72	2.90	-0.81	-0.74
D	0.11	-1.61	-2.27	0.57	-0.80	-1.52	0.30	-0.75	-1.55	0.43	-0.18	-1.56	0.57	-0.18	-1.52
E	0.17	-2.17	-2.03	0.50	-0.42	-1.68	1.09	-0.41	-1.34	0.38	-0.79	-1.34	1.09	-0.41	-1.34
F	1.56	-1.57	-1.12	2.93	-1.11	-1.13	5.20	-0.51	-0.70	1.86	-0.81	-1.12	5.20	-0.51	-0.70
G	0.39	-2.90	-1.90	5.30	-0.35	-0.64	2.75	-0.17	-0.83	2.31	-0.21	-1.00	5.30	-0.17	-0.64
H	2.69	-1.48	-1.15	6.79	-1.07	-0.78	1.12	-1.14	-1.47	0.60	-0.47	-1.55	6.79	-0.47	-0.78
I	0.51	-2.81	-1.46	13.30	-1.35	-0.57	36.80	-1.03	-0.15	0.86	-0.85	-1.31	36.80	-0.85	-0.15
K	0.11	-1.44	-2.27	0.48	-1.03	-1.70	0.37	-0.58	-1.71	0.09	-1.60	-2.25	0.48	-0.58	-1.70
L	0.005	-2.55	-4.03	0.09	-1.40	-2.57	0.08	-2.11	-2.36	0.02	-2.08	-3.00	0.09	-1.40	-2.36
M	0.81	-2.35	-1.47	0.98	-2.09	-1.39	0.99	-0.26	-1.26	0.39	-0.88	-1.50	0.99	-0.26	-1.26
N	0.06	-2.67	-2.51	2.00	-1.20	-1.13	0.62	-0.95	-1.53	0.29	-1.27	-1.88	2.00	-0.95	-1.13
O	0.29	-2.96	-2.00	2.65	-1.02	-1.44	NA	NA	NA	0.49	-0.93	-2.05	2.65	-0.93	-1.44
P	0.28	-0.90	-1.77	1.74	-0.47	-1.03	4.34	-0.04	-0.69	2.03	-0.34	-0.85	4.34	-0.04	-0.69
Q	0.09	-1.13	-2.48	0.21	-0.33	-2.07	0.23	-0.89	-1.96	0.34	-0.31	-2.00	0.34	-0.31	-1.96
R	0.04	-2.77	-4.66	0.20	-1.88	-2.10	0.11	-1.73	-1.94	0.17	-1.63	-1.75	0.20	-1.63	-1.75
S	0.54	-2.75	-1.92	1.50	-0.39	-1.36	4.11	-0.20	-1.10	2.35	-0.34	-1.34	4.11	-0.20	-1.10
T	NA	NA	NA	0.84	-0.10	-1.48	2.75	-0.01	-0.92	0.66	-0.75	-1.46	2.75	-0.01	-0.92

NA: not available.

Table C.9: Number of pesticides contributing to 75 % of $sumTU_{invertebrate}$ ($sumTU_{iv}$) and $sumTU_{algae}$ ($sumTU_{al}$).

site	Base exposure 1		Peak exposure 1		Peak exposure 2		Peak exposure 3	
	$sumTU_{iv}$	$sumTU_{al}$	$sumTU_{iv}$	$sumTU_{al}$	$sumTU_{iv}$	$sumTU_{al}$	$sumTU_{iv}$	$sumTU_{al}$
A	2	2	2	3	1	3	1	2
B	1	2	4	3	1	2	1	4
C	1	3	2	1	2	1	2	2
D	2	2	3	1	1	4	1	2
E	1	4	1	2	2	1	2	3
F	1	2	1	1	2	2	2	3
G	1	2	1	3	3	3	1	2
H	1	2	2	4	3	3	1	1
I	1	3	1	1	2	2	2	2
K	1	2	1	2	1	3	1	2
L	4	2	2	2	2	3	1	3
M	1	2	2	2	2	3	2	2
N	1	2	2	2	1	2	1	2
O	1	2	3	2	NA	NA	2	3
P	1	4	1	2	1	2	1	1
Q	1	2	1	1	1	2	1	3
R	2	2	2	1	1	3	1	3
S	1	1	2	3	1	3	1	3
T	NA	NA	2	2	1	4	2	2

NA: not available

Table C.10: The ten strongest contributors to $sumTU_{invertebrate}$ and $sumTU_{algae}$.

	$sumTU_{invertebrate}$		$sumTU_{algae}$	
	compound	% samples where pesticide has highest TU	compound	% samples where pesticide has highest TU
1	Diazinon	67.6	Terbuthylazin	47.3
2	Imidacloprid	17.6	Metribuzin	18.9
3	alpha-Cypermethrin[a]	4.1	2,4-D	17.6
4	Terbuthylazin	4.1	Atrazin	4.1
5	Acetamiprid	2.7	Diuron	2.7
6	Chlorpyrifos	1.4	Chlortoluron	1.4
7	Dichlorvos	1.4	Irgarol	1.4
8	Dimethoat	1.4	Isoproturon	1.4
9	Thiacloprid	1.4	MCPA	1.4
10	NA		Propiconazol	1.4

[a] Only alpha-Cypermethrin was detected via polydimethylsiloxane sheets (PDMS) sheets, on contrast to all other compounds detected using Empore styrene-divinylbenzene (SDB) disks. Alpha-Cypermethrin was only in base exposure samples the most important pesticides.

Table C.11: Contribution (in %) of pesticides samples via polydimethylsiloxane sheets (PDMS) to sum concentration, $sumTU_{invertebrate}$ and $sumTU_{algae}$.

	sum concentration				$sumTU_{invertebrate}$				$sumTU_{algae}$			
site	Base 1	Peak 1	Peak 2	Peak 3	Base 1	Peak 1	Peak 2	Peak 3	Base 1	Peak 1	Peak 2	Peak 3
A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B	0.01	NA	NA	NA	21.9	NA	NA	NA	< 0.01	NA	NA	NA
C	< 0.01	< 0.01	< 0.01	0.01	6.5	1.5	6.9	1.4	< 0.01	< 0.01	< 0.01	< 0.01
D	< 0.01	< 0.01	< 0.01	< 0.01	NA	0.1	0.1	0.03	NA	< 0.01	< 0.01	< 0.01
E	0.02	0.04	0.02	0.05	15.6	1.4	1.3	3.3	< 0.01	0.01	< 0.01	< 0.01
F	< 0.01	< 0.01	< 0.01	< 0.01	2.6	0.2	0.06	0.1	< 0.01	< 0.01	< 0.01	< 0.01
G	< 0.01	< 0.01	< 0.01	< 0.01	13.7	0.04	0.03	0.03	< 0.01	< 0.01	< 0.01	< 0.01
H	< 0.01	< 0.01	0.01	0.01	6.6	5.9	6.8	1.5	< 0.01	< 0.01	< 0.01	< 0.01
I	< 0.01	< 0.01	< 0.01	< 0.01	33.4	0.8	0.4	0.2	< 0.01	< 0.01	< 0.01	< 0.01
K	0.01	< 0.01	< 0.01	0.01	1.0	0.4	0.1	1.4	< 0.01	< 0.01	< 0.01	< 0.01
L	0.6	NA	NA	NA	83.9	NA	NA	NA	0.4	NA	NA	NA
M	< 0.01	NA	NA	NA	7.7	NA	NA	NA	< 0.01	NA	NA	NA
N	0.05	< 0.01	0.01	0.01	67.4	3.0	1.7	3.5	0.01	< 0.01	< 0.01	< 0.01
O	NA	< 0.01	NA	< 0.01	NA	1.0	NA	0.8	NA	< 0.01	NA	< 0.01
P	0.02	< 0.01	< 0.01	< 0.01	2.2	0.6	0.2	0.4	< 0.01	< 0.01	< 0.01	< 0.01
Q	0.02	0.01	0.01	0.01	0.7	0.1	0.4	0.1	< 0.01	< 0.01	< 0.01	< 0.01
R	< 0.01	0.01	0.01	0.01	NA	2.61	1.8	1.5	NA	< 0.01	< 0.01	< 0.01
S	< 0.01	NA	NA	NA	9.7	NA	NA	NA	< 0.01	NA	NA	NA
T	NA	< 0.01	< 0.01	< 0.01	NA	0.06	0.05	0.3	NA	< 0.01	< 0.01	< 0.01

NA: sample lost or no compound was detected via PDMS.

Table C.12: Standardised regression coefficients and parameters of the best fit model of the elastic net. The size of the regression coefficient may be interpreted as variable importance regarding maximum toxicity. Variables without a value were not selected for the best fit model.

Variable	$sumTU_{invertebrates}$	$sumTU_{algae}$[a]
Alpha + lambda of best fit model	α = 0.2; λ = 0.29	α = 0.25; λ = 0.91
agricultural land use within catchment	0.080	Not included
catchment size	0.118	Not included
agricultural land use within buffer	0.039	Not included
field size	Not included	Not included
buffer width	0.101	Not included
slope distance	Not included	Not included
riparian plant height	0.059	Not included
fine substrate	Not included	Not included

[a]all variables in this model were shrunk to zero.

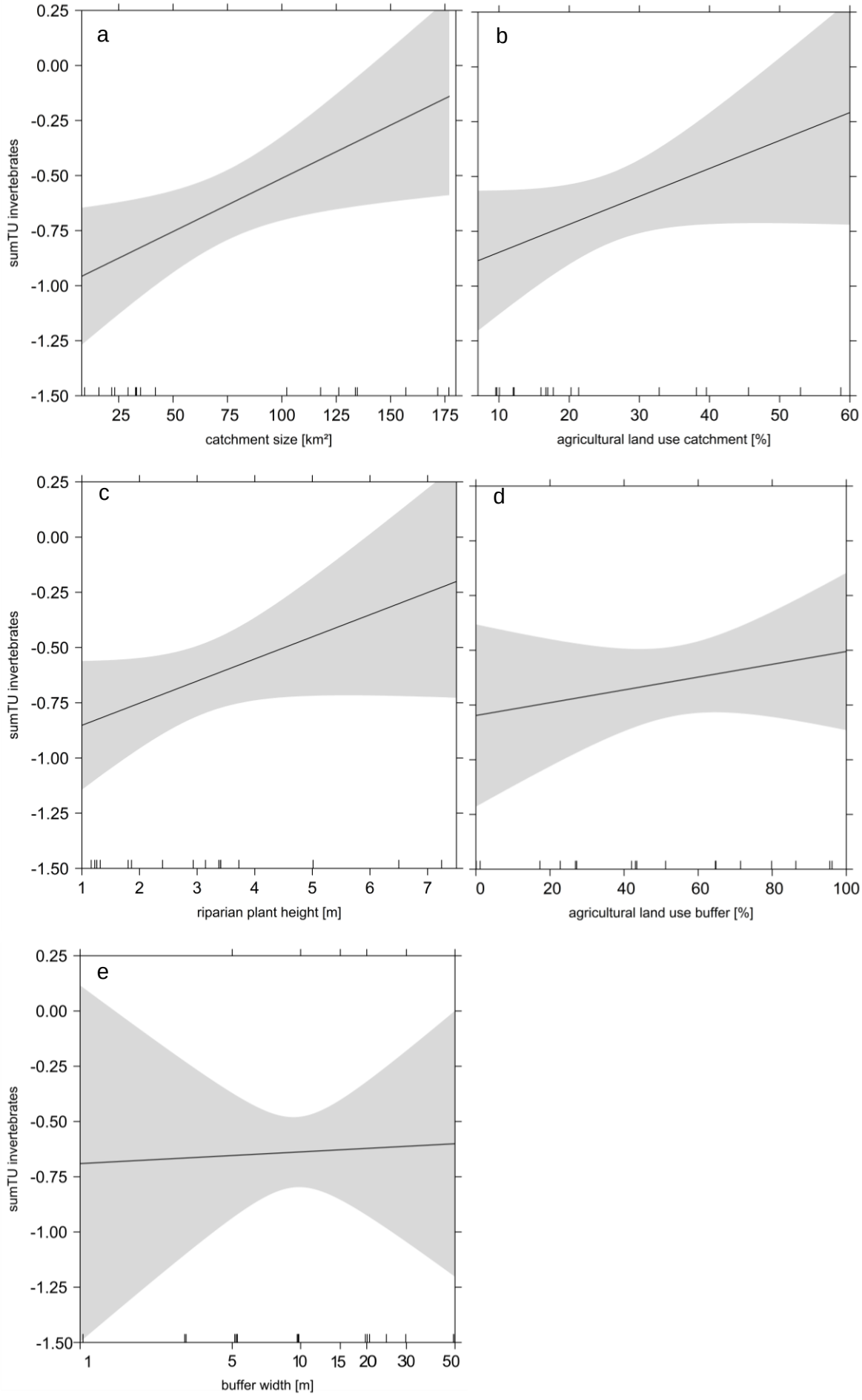
a
b
c
d
e
sumTU invertebrates
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
25 50 75 100 125 150 175
catchment size [km²]
10 20 30 40 50 60
agricultural land use catchment [%]
1 2 3 4 5 6 7
riparian plant height [m]
0 20 40 60 80 100
agricultural land use buffer [%]
1 5 10 15 20 30 50
buffer width [m]

Fig. C.2: Predictor effect plots (Fox and Weisberg, 2018) of the relationship of $sumTU_{invertebrates}$ to a) catchment size, b) agricultural land use within the catchment, b) riparian plant height, d) agricultural land use within a buffer and e) buffer width. Only variables which were selected by the elastic net approach (Table C.12) are presented. Grey areas around the black correlation indicate 95 % confidence bands for the explanatory variable (i.e. the predictor). Dashes at the y-axis show the marginal distribution of the explanatory variable. The plot displays fitted values of the response based on partial effects of the explanatory variable with the other variables held at a typical (i.e. average) value. Since the original value of the response depends on multiple explanatory variables, plotting these would mislead.

SUPPLEMENTARY REFERENCES

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D SUPPLEMENTARY MATERIAL: SIMILAR RECOVERY TIME OF MICROBIAL FUNCTIONS FROM FUNGICIDE STRESS ACROSS BIOGEOGRAPHICAL REGIONS

FUNGICIDE CONCENTRATIONS

A mixture of the fungicides metalaxyl, prothioconazole, pyrimethanil, and prochloraz was used as a model stressor during the experiments. These four fungicides have partially dissimilar modes of action and inhibit RNA synthesis in ribosomes (metalaxyl), methionine synthesis (pyrimethanil), or ergosterol synthesis (prothioconazole and prochloraz) (Fungicide Resistance Action Committee, 2017). The concentrations of the single compounds in the mixture were chosen to reach equal toxicity equivalents, which were calculated using the logarithmic sum of toxic units (sumTU):

$$sumTU = log\left(\sum_{i=1}^{n} \frac{c_i}{EC_{50_i}}\right) \quad \text{(D.1)}$$

where c_i is the concentration of the fungicide *i* and EC_{50i} is the concentration at which 50 % of the test organisms of the reference species (Table D.1) were affected by exposure to fungicide *i*.

The concentrations displayed in Table D.1 are nominal peak concentrations and equal a sumTU of -1, whereas the base concentration was 10 % of this value, i.e., sumTU of -2. Because of the technical difficulties regarding solubility, prochloraz was only applied during the first colonisation and decomposition cycle in Germany and was not applied in the Swedish experiment. Despite differences in the compounds used in the mixture, which consisted of three and four fungicides, the same modes of toxic action were implicated in all exposures because prothioconazole and prochloraz both inhibit ergosterol synthesis (Fungicide Resistance Action Committee, 2017). When discarding prochloraz from the mixture, the concentrations of the other pesticides were adjusted to sustain the chosen stressor intensity (see Table D.1). We suggest that this difference was irrelevant for the observed patterns, which is supported by the fact that i) functional effects were equal or less pronounced in Denmark and ii) structural responses were similar to those in Germany. If the inclusion of prochloraz had a major influence, Denmark should have displayed a deviating pattern from Germany and Sweden, which was not the case.

Table D.1: EC_{50} values and nominal peak concentrations of the fungicides used in the mixtures comprising three and four different compounds. Base concentrations were at 10 % of the peak concentrations.

Fungicide	EC_{50} [µg L^{-1}]	Reference species[a]	Source	Concentration [µg L^{-1}]; 4 pesticides[d]	Concentration [µg L^{-1}]; 3 pesticides
Metalaxyl	743	*Pseudokirchneriella subcapitata*[b]	EPA, 2014	18.6	24.8
Prothioconazole	126	Several hyphomycete species[c]	Dijksterhuis et al., 2011	3.2	4.2
Prochloraz	8	Several hyphomycete species[c]	Dijksterhuis et al., 2011	0.2	-
Pyrimethanil	1200	*Pseudokirchneriella subcapitata*[b]	Lewis et al., 2016	30.0	40.0

[a]If available, hyphomycete species were chosen as reference organisms, if not available we used *Pseudokirchneriella subcapitata*.

[b]EC_{50} values for *Pseudokirchneriella subcapitata* are based on 72-h acute toxicity tests after OECD 201 (OECD, 2011).

[c]Data calculated from mean EC_{50} values of several hyphomycete species of pesticides from same substance group as the used ones.

[d]The pesticide mixture with four pesticides consisted of metalaxyl, prothioconazole, pyrimethanil, and prochloraz, while in the mixture with three pesticides prochloraz was discarded due to technical difficulties in some regions and during some cycles.

Table D.2: Percentage change in the decomposed leaf mass between the fungicide treatment and respective controls (%; with 95 % confidence intervals), sample sizes, as well as the t-test results separated by biogeographical regions and cycles. Bold p-values indicate statistical significance.

Region	Cycle	Percentage change	95 % confidence interval	n	df	t-ratio	p-value
Denmark	1	-23.1	-37.0 to -9.1	7	36	-2.4	**0.023**
	2	-37.6	-55.3 to -19.9	7	36	-3.1	**0.004**
	3	0.0	-40.0 to 21.6	7	36	-0.4	0.671
Germany	1	-53.1	-72.1 to -34.1	7	36	-4.0	**0.003**
	2	-19.3	-36.3 to -2.2	7	36	-1.6	0.114
	3	10.0	-7.0 to 26.9	7	36	0.8	0.404
Sweden	1	-18.6	-30.6 to -6.6	6	30	-2.2	**0.033**
	2	-15.6	-31.8 to 0.6	6	30	-1.4	0.174
	3	-1.4	-19.1 to 16.3	6	30	-0.1	0.908

Table D.3: Influence of explanatory variables on the sporulation of cosmopolitan (occurring in all analysed biogeographical regions) and non-cosmopolitan (occurring in two biogeographical regions) aquatic hyphomycete taxa tested by type II ANOVAs, separated by biogeographical region and explanatory variables. Only statistical significant explanatory variables are shown.

	Hyphomycete	Region	Explanatory variable	df	F	p-value
Cosmopolitan taxa	*Articulospora tetracladia*	Denmark	Fungicide × Cycle	2	15.2	< 0.001
		Germany			NS[a]	
		Sweden	Cycle	2	8.2	0.002
	Flagellospora curvula	Denmark			NS	
		Germany			NS	
		Sweden			NS	
	Tetrachaetum elegans	Denmark			NS	
		Germany	Cycle	1	11.2	0.004
		Sweden			LS[b]	
Non- cosmopolitan taxa	*Alatospora sp.*	Denmark			NF[c]	
		Germany			LS	
		Sweden	Fungicide	1	14.0	< 0.001
	Anguillospora sp	Denmark			LS	
		Germany			NF	
		Sweden	Cycle	2	10.8	< 0.001
	Mycocentrospora clavata	Denmark			NS	
		Germany			NF	
		Sweden			LS	
	Tetracladium sp.	Denmark	Cycle	2	13.8	< 0.001
		Germany			NS	
		Sweden			NF	
	Tetracladium marchalianum	Denmark	Cycle	2	6.5	0.004
		Germany			NS	
		Sweden			NF	

[a]NS: no significant explanatory variables
[b]LS: sporulation too low to build a stable model
[c]NF: not found

ADDITIONAL EXPERIMENT IN THE SOUTH EASTERN HIGHLANDS (AUSTRALIA)

The colonisation and decomposition cycles 2 and 3 showed a non-significant decrease in leaf decomposition in the fungicide treatment compared to the respective controls ($p \geq 0.081$; Fig. D.1). However, and in accordance with the experiments in the other regions, the differences between control and fungicide treatment decreases from cycle 2 to 3 (Table D.4).

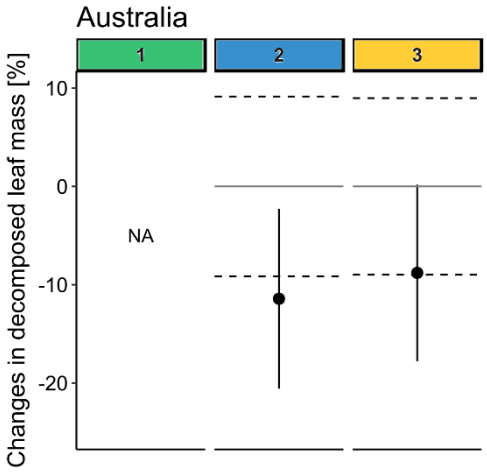

Fig. D.1: Percentage change in the decomposed leaf mass between the fungicide treatment and the respective controls (%; with 95 % confidence intervals, solid horizontal lines represent controls, dashed lines indicate corresponding 95 % confidence intervals) for the different cycles (numbers on top; n = 7) from the Australian experiment.

Table D.4: Percentage change in the decomposed leaf mass between the fungicide treatment and respective controls (%; with 95 % confidence intervals), sample sizes, as well as the type II ANOVA results from the Australian experiment.

Region	Cycle	Percentage change	95 % confidence interval	n	df	t-ratio	p-value
Australia	2	-11.4	-20.6 to -2.2	7	24	-1.8	0.081
	3	-8.8	-17.8 to -0.2	7	24	-1.4	0.166

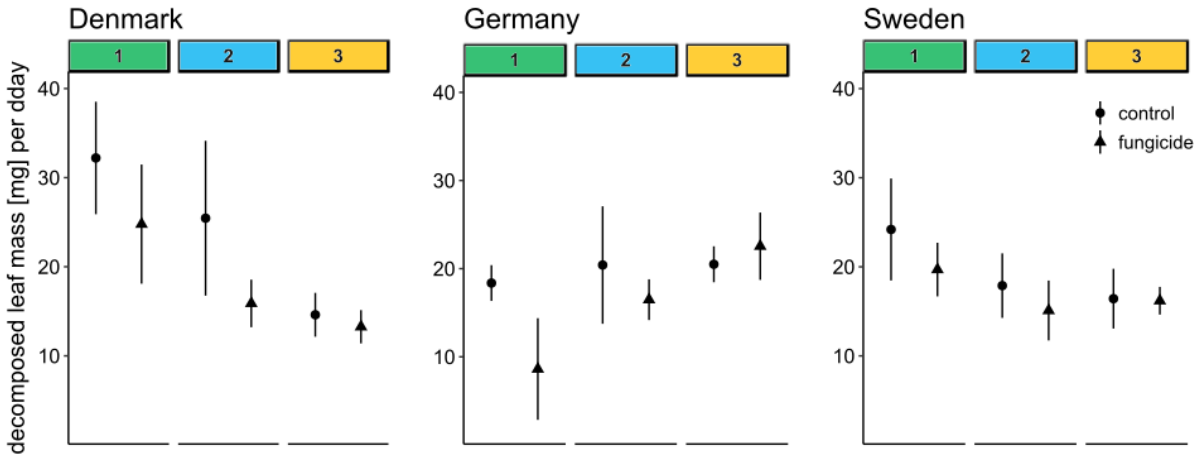

Fig. D.2: Mean decomposed leaf mass [mg] (with 95 % confidence intervals) per degree day (dday) for the different cycles (numbers on top) and treatments (circle: control; triangle: fungicide treatment).

Table D.5: Influence of explanatory variables on the aquatic hyphomycete taxa richness tested by type III ANOVAs, separated by biogeographical regions and explanatory variables. Bold p-values indicate statistical significance.

Region	Explanatory variable	df	LRT	p-value
Denmark	Fungicide	1	0.0	1.00
	Cycle	2	34.7	**< 0.001**
	Fungicide × Cycle	2	4.1	0.126
Germany	Fungicide	1	1.8	0.184
	Cycle	1	0.5	0.481
	Fungicide × Cycle	1	0.0	0.879
Sweden	Fungicide	1	2.8	0.093
	Cycle	2	13.2	**0.001**
	Fungicide × Cycle	2	1.9	0.387

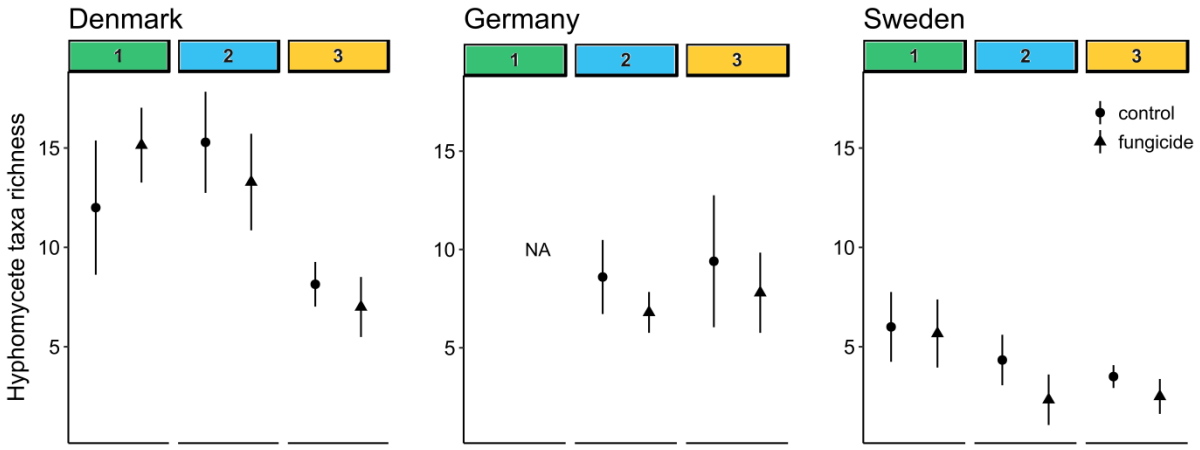

Fig. D.3: Mean hyphomycete taxa richness (with 95 % confidence intervals) for the different cycles (numbers on top) and treatments (circle: control; triangle: fungicide treatment)

FUNGICIDE ANALYSIS

Water samples were collected from four randomly chosen replicates per treatment at different time points during peak and base exposures (for time points, see Table D.7) and concentrated using solid phase extraction (SPE; HLB 3 cc 60 mg extraction cartridges). SPE columns were conditioned with 5 mL acetone and 5 mL acetonitrile (both HPLC grade) and equilibrated with 5 mL ultrapure water. An aliquot of 0.1 L water was loaded into the SPE column at a velocity of 3 - 4 mL min^{-1}. The columns were dried under a nitrogen flow for 30 min and stored at -20°C until analysis. Before elution, the columns were dried accordingly and eluted with 4 mL acetonitrile followed by 4 mL acetone (both liquid chromatography grade). The eluate was evaporated to dryness under a gentle nitrogen flow at room temperature and reconstituted in 0.5 mL methanol:water (1:1, v/v, methanol liquid chromatography grade, ultrapure water). Finally, the extract was centrifuged (3000 rpm, 7 minutes), and the supernatant was used for further analysis. Depending on the nominal concentrations, samples were 5- or 50-fold diluted with methanol:water (1:1, v/v) to fit within the linear range of the calibration curve.

The samples and standards were analysed using a liquid chromatography high-resolution mass-spectrometry (LC-HRMS) Orbitrap system according to Fernández et al. (2016). Quantitative analyses were performed by interpolating the data according to the respective matrix-matched calibration curve. All studied fungicides showed a linear range from 0.5 to 200 µg L^{-1} in pure solvents (methanol:water) as well as in the different matrices studied. Additionally, the matrix effect was calculated via post-extraction spike using pre-filtered stream water from each biogeographical region. The effect of the matrix on the performance of the method differed between the stream water samples from each biogeographical region, and it was affected also by the sample dilution factor (5- and 50-fold dilution). Consequently, different

limits of quantification were obtained (LOQs; Table D.6). Because prothioconazole has a high degradation rate (Lewis et al., 2016) and was applied at relatively low nominal concentrations during this study, the pesticide was not detectable in the samples based on the applied methods.

Table D.6: Limits of quantifications (LOQs) of the individual fungicides (Chemical Abstract Service, CAS, given in brackets) in ultrapure water and stream water that was used as test medium in the individual experiments from the different geographical regions (Denmark, Germany, and Sweden). The LOQ values represent the lowest calibration limit in each matrix, adjusted to the extraction method considering a sample volume of 0.1 L.

		Metalaxyl (57837-19-1)	Prochloraz (67747-09-5)	Pyrimethanil (53112-28-0)
Region	Dilution step	LOQ [µg L^{-1}]	LOQ [µg L^{-1}]	LOQ [µg L^{-1}]
-	Ultrapure water	0.005	0.0025	0.0025
Denmark & Germany	Undiluted	0.025	0.050	0.025
	1:5 dilution	0.005	0.050	0.025
	1:50 dilution	0.005	0.0025	0.005
Sweden	Undiluted	0.050	0.025	0.025
	1:5 dilution	0.025	0.005	0.025
	1:50 dilution	0.005	0.005	0.025

Table D.7: Mean measured pesticide concentrations (with standard deviations, from four randomly chosen replicates), separated by biogeographical regions and sampled time points during the peak and the base exposure. Nominal initial peak concentrations in the mixtures containing three or four fungicides were: metalaxyl 24.8 and 18.6 µg L^{-1}, prothioconazole 4.2 and 3.2 µg L^{-1}, prochloraz 0.2 µg L^{-1} (only in the mixture containing four fungicides), and pyrimethanil 40.0 and 30.0 µg L^{-1}. Note that prothioconazole was not detectable due to its high degradation rate and therefore is not reported.

Region	Exposure	Hours after peak application	Metalaxyl [µg L^{-1}]	Prochloraz [µg L^{-1}]	Pyrimethanil [µg L^{-1}]
Denmark	peak	0	22.1 ± 2.0	0.10 ± 0.02	30.5 ± 2.6
	base	50	2.3 ± 0.4	0.008 ± 0.005	3.9 ± 0.6
Germany	peak[a]	0	19.5 ± 8.1	0.08 ± 0.01	34.1 ± 6.0
	peak[b]	0	29.0 ± 7.0	NA	49.9 ± 10.3
	peak	24	32.3 ± 7.3	NA	41.9 ± 7.9
	peak	48	27.3 ± 3.3	NA	31.5 ± 3.9
	base	50	1.7 ± 0.3	NA	2.7 ± 0.6
	base	86	1.6 ± 0.2	NA	2.4 ± 0.5
	base	122	2.8 ± 0.4	NA	2.2 ± 0.2
	base	242	0.09 ± 0.01	NA	1.2 ± 0.5
Sweden	peak	0	28.0 ± 10.4	NA	35.7 ± 10.4
	peak	24	24.9 ± 10.5	NA	20.1 ± 5.7
	peak	48	21.9 ± 6.2	NA	21.4 ± 4.5
	base	50	1.2 ± 0.3	NA	3.3 ± 0.6
	base	86	0.9 ± 0.2	NA	2.1 ± 0.4
	base	122	0.8 ± 0.1	NA	1.9 ± 0.3

[a]The fungicide mixtures of the peak exposure of cycle 1 in Germany included prochloraz, which was excluded in later cycles due to technical difficulties.

[b]The fungicide mixtures of the peak exposures of cycles 2 and 3 in Germany consisted only of the fungicides prothioconazole, pyrimethanil and metalaxyl.

NA = not applied.

All fungicides were below the level of detection in all control samples and thus are not listed in Table D.7.

WATER QUALITY PARAMETERS

The average water temperature was recorded every 30 min during microbial colonisation of the leaf material in streams and in each control of the randomly distributed microcosms during the experiment to calculate the decomposed leaf mass per degree day (Table D.8).

The ion contents in Germany were measured using a field photometer PF-12 as well as related VISOCOLOR ECO kits, and they were measured in Sweden using an automated photometric analyser. In Denmark, the biological oxygen demand (BOD5) and concentrations of ammonia-N and ortho-phosphate were measured according to their European Standards (DS/EN 1899 1999, DS 11732 2005, and DS/EN 1189 1999, respectively). Nitrate-N was analysed using the Lachat-method (Lachat Instruments, USA, QuickChem. No. 10-107-06-33-A, salicylate method).

Table D.8: Abiotic water parameters. Temperature and pH values are given during colonisation of the leaf material in the field and the experimental part of the experiment, while water quality parameters were measured directly after water exchanges.

		Australia	Denmark	Germany	Sweden
During colonisation	Temperature [°C]	18	9	8	9
	pH	7.2	7.4	7.4	6.6
During experiment	Temperature [°C]	18	15	18	11
	pH	NM	7.1	7.1	7.1
	Conductivity [µS/cm]	55	NM	84	455
	DOC [mg L^{-1}]	NM	1.3	< 1	48
	NH4-N [mg L^{-1}]	NM	0.026	< 0.1	0.023
	NO2-N [mg L^{-1}]	NM	NM	< 0.01	
	PO4-P [mg L^{-1}]	NM	0.01	< 0.2	0.013
	NO3-N [mg L^{-1}]	NM	0.54	< 0.1	
	Sum NO2+NO3 [µg L^{-1}]	NM	NM	NM	11
	BOD5 [mg L^{-1}]	NM	0.84	NM	NM

NM: Not measured.

Table D.9: Coordinates and times for leaf collection in the different biogeographical regions.

Region	Time	Latitude	Longitude
Australia	February 2015	34°55'20"S	149°10'49"E
Denmark	Autumn 2014	56°00'16"N	10°07'26"E
Germany	October 2013	49°12'07"N	8°08'37"E
Sweden[a]	Autumn 2014	49°12'39"N	8°13'15"E

[a]Leaves were imported from Landau, Germany.

Table D.10: Coordinates of colonisation of leaves from cycle 1.

Region	Name of stream	Latitude	Longitude
Australia	Cotter River	35°24'14"S	148°51'23"E
Denmark	Hulbaek	56°00'16"N	10°07'26"E
Germany	Sulzbach	49°15'43"N	7°57'36"E
Sweden	Pinglaström	59°46'19"N	17°45'19"E

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7.2 AUTHOR CONTRIBUTIONS

ARTICLE 1

TITLE: Pesticide mixtures in streams of several European countries and the USA

AUTHORS: Verena C. Schreiner, Eduard Szöcs, Avit Kumar Bhowmik, Martina G Vijver & Ralf B. Schäfer

STATUS: Published in 2016 in SCIENCE OF THE TOTAL ENVIRONMENT, Volume 573, pp. 680–689.

CONTRIBUTIONS: SCHREINER designed study, conducted calculations and data analyses, discussed results, drafted the manuscript.
SZÖCS conducted calculations and data analyses, edited manuscript.
BHOMWIK conducted catchment and land use extraction, edited manuscript.
VIJVER provided data, edited manuscript.
SCHÄFER designed study, discussed results, edited manuscript.

ARTICLE 2

TITLE: Sampling rates for passive samplers exposed to a short pulse exposure of 42 organic pesticides

AUTHORS: Verena C. Schreiner, Nikita Bakanov, Mira Kattwinkel, Sarah Könemann, Stefan Kunz, Etiënne L.M. Vermeirssen & Ralf B. Schäfer

STATUS: Published in 2029 in SCIENCE OF THE TOTAL ENVIRONMENT, Volume 740, 140376

CONTRIBUTIONS: SCHREINER designed study, conducted chemical analyses, wrote R-script, discussed results, drafted the manuscript.
BAKANOV assisted with the analytical devices, edited manuscript.
KATTWINKEL wrote R-script, edited manuscript.
KÖNEMANN conducted the calibration experiment, edited manuscript.
KUNZ conducted chemical analyses, edited manuscript.
VERMEIRSSEN designed study, conducted the calibration experiment, discussed results, edited manuscript.
SCHÄFER designed study, discussed results, edited manuscript.

ARTICLE 3

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

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

STATUS: Submitted in 2020 to WATER RESEARCH.

CONTRIBUTIONS: SCHREINER designed study, conducted field work, provided the toxicity data, conducted the chemical analysis of the SDB disks and the PDMS sheets, conducted statistical analysis, discussed results, drafted the manuscript.
LINK provided geospatial information, edited manuscript.
KUNZ and SZÖCS conducted field work, edited manuscript.
SCHARMÜLLER provided the toxicity data, edited manuscript.
VOGLER conducted the chemical analysis of the SDB disks, edited manuscript.
BECK conducted the chemical analysis of the PDMS sheets, edited manuscript.
BATTES and CAMPEAN selected sampling sites, helped with local logistics, edited manuscript.
SINGER and HOLLENDER gave recommendations for sampling and chemical analysis, edited manuscript.
SCHÄFER designed study, selected sampling sites, discussed results, edited manuscript.

CHAPTER 7

ARTICLE 4

TITLE: Similar recovery time of microbial functions from fungicide stress across biogeographical regions

AUTHORS: Verena C. Schreiner, Alexander Feckler, Diego Fernández, Katharina Frisch, Katherine Muñoz, Eduard Szöcs, Jochen P. Zubrod, Mirco Bundschuh, Jes J. Rasmussen, Ben J. Kefford, Josepha Axelsen, Nina Cedergreen & Ralf B. Schäfer

STATUS: Published in 2018 in SCIENTIFIC REPORTS, Volume 8, Article number 17021.

CONTRIBUTIONS: SCHREINER designed study, performed experiment, conducted statistical analyses, performed fungicide analyses, discussed results, drafted the manuscript.
FECKLER performed experiment, discussed results, contributed in drafting the manuscript.
FERNÁNDEZ designed study, performed experiment, edited manuscript.
FRISCH performed experiment, performed fungicide analyses, edited manuscript.
MUÑOZ performed fungicide analyses, edited manuscript.
SZÖCS conducted statistical analyses, edited manuscript.
ZUBROD designed study, performed experiment, discussed results, edited manuscript.
BUNDSCHUH designed study, performed experiment, discussed results, edited manuscript.
RASMUSSEN designed study, performed experiment, discussed results, edited manuscript.
KEFFORD performed experiment, discussed results, edited manuscript.
AXELSEN performed experiment, edited manuscript.
CEDERGREEN, edited manuscript.
SCHÄFER designed study, discussed results, contributed in drafting the manuscript.

7.3 DECLARATION

I, the author of this work, hereby declare that this PhD thesis entitled "OCCURRENCE, MONITORING AND EFFECTS OF PESTICIDES AND THEIR MIXTURES IN AGRICULTURAL STREAMS" contains no material which has been submitted at any university or other tertiary institution for scientific examination.

The work has been prepared independently. All used aids and references as well as involved contributors are clearly declared. I did not use the assistance of a doctoral consultant or similar persons in return for payment.

I am aware that a violation of the above-mentioned points can have legal consequences including the withdrawal of the doctoral degree.

Neustadt / Weinstraße, _____

30th June 2020 Verena C. Schreiner

7.4 CURRICULUM VITAE

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EDUCATION

07/2014 – present **Ph. D. Environmental Sciences**, University of Koblenz-Landau, Landau
Working Group Quantitative Landscape Ecology
Occurrence, monitoring and effects of pesticide mixtures in agricultural streams.

10/2010 – 12/2012 **M. Sc. Organismic Biology**, Philipps University of Marburg
CORE SUBJECTS: conservation biology, ecology, zoology
THESIS: Variation in diet of *Isoperla sp*. (Plecoptera) and *Rhyacophila sp*. (Trichoptera) larvae across an elevational gradient.

10/2007 – 09/2010 **B. Sc. Biologie**, Ruprecht-Karls University of Heidelberg
CORE SUBJECTS: zoology, aquatic ecology, ecotoxicology
THESIS: Der Heidelberger Mühlbach – ein Biotop in Gefahr: Ökologie und Ökotoxikologie eines vom Menschen stark beeinflussten Baches.

WORK EXPERIENCE AND TEACHING

07/2014 – present **Research Assistant**, University of Koblenz-Landau, Landau
Field studies in Romania and Germany (Palatinate Forest), micro- and mesocosm experiments, pesticide analysis, project “Kleingewässer-monitoring” in cooperation with UFZ, data analysis, Ph.D. research.

04/2019 – present **Teaching Assistant**, University of Koblenz-Landau, Landau
Course: Measurement of environmental parameters – limnology (B. Sc. level, German)

08/2017 - present **Teaching Assistant**, University of Koblenz-Landau, Landau
Course: Indicator organisms - macroinvertebrates (M. Sc. level, English)

10/2016 – 02/2018 **Teaching Assistant**, University of Koblenz-Landau, Landau
Single lectures: Aquatic Ecotoxicology (M. Sc. level)

08/2016 – 09/2016 **Research Stay**, Eawag, Dübendorf, Switzerland
Preparing and measuring passive sampler (SDB and PDSM) samples

WORK EXPERIENCE AND TEACHING (CONTINUED)

03/2014 – 06/2014 **Research Assistant**, University of Koblenz-Landau, Landau
Mesocosm experiment in artificial streams

01/2013 – 10/2013 **Research Assistant**, Senckenberg, Gelnhausen, Germany
Analysing temperature and chloride thresholds of benthic invertebrates, assessment of the biodiversity of benthic invertebrates and ground beetles, handling chironomid cultures

06/2011 – 07/2012 **Teaching Assistant**, Philipps University of Marburg
Courses: Systematics of animals, animal adaption, zoological knowledge (M. Sc. level; in German)

04/2010 – 07/2010 **Teaching Assistant**, Ruprecht-Karls University of Heidelberg
Course: Biodiversity of animals – zoological identification course (B. Sc. level; in German)

PUBLICATIONS AND CONFERENCE CONTRIBUTIONS

PEER-REVIEWED PUBLICATIONS

[1] **Schreiner, V.C.**, Link, M., Kunz, S., Szöcs, E., Scharmüller, A., Vogler, B., Beck, B., Battes, K.P., Cimpean, M., Singer, H.P., Hollender, J., Schäfer, R.B., submitted. Paradise lost? Pesticide pollution in a European region with relatively traditional agriculture. Water Research.

[2] Le, T.D.H., **Schreiner, V.C.**, Kattwinkel, M., Schäfer, R.B., submitted. Invertebrate turnover along gradients of anthropogenic salinisation in rivers of two German regions. Science of the Total Environment.

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PUBLICATIONS AND CONFERENCE CONTRIBUTIONS (CONTINUED)

PEER-REVIEWED PUBLICATIONS (CONTINUED)

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In addition, I conducted reviews for the journals *Environmental Pollution, Environmental Toxicology and Chemistry*, *SN Applied Sciences* and *Water Research*.

ORAL PRESENTATIONS (ONLY AS PRESENTING AUTHOR)

[1] **Schreiner, V.C.**, Bundschuh, M., Feckler, A., Foit, K., Gunold, R., Knillmann, S., Leese, F., Liebmann, L., Liess, M., Link, M., Reemtsma, T., Salis, R., von Tümpling, W., Vormeier, P., Weisner, O., Schäfer, R.B., 2020. Ökosystemfunktion Blattabbau. Abschlussworkshop Kleingewässermonitoring, Leipzig, Germany.

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[3] **Schreiner, V.C**., Link, M., Amelung, G., Schäfer, R.B., 2019. A field based experiment on the responses of different microbial communities and related ecosystem functions to fungicides. SFS Annual Meeting, Salt Lake City, USA.

[4] **Schreiner, V.C**., Link, M., Amelung, G., Schäfer, R.B., 2019. Responses of fungal communities and their associated leaf decomposition in viticultural streams. 6th Fresh Blood for FreshWater Conference, Tihany, Hungary.

[5] **Schreiner, V.C**., Link, M., Kunz, S., Szöcs, E., Vogler, B., Beck, B., Battes, K.P., Cimpean, M., Vermeissen, E.L. M., Singer, H., Hollender, J., Schäfer, R.B., 2018. Macroinvertebrate communities across a gradient of multiple stressors from agricultural land use in Romanian streams. 28th Annual Meeting SETAC Europe, Rome, Italy.

PUBLICATIONS AND CONFERENCE CONTRIBUTIONS (CONTINUED)

ORAL PRESENTATIONS (ONLY AS PRESENTING AUTHOR) (CONTINUED)

[6] **Schreiner, V.C**., Kunz, S., Szöcs, E., Vogler, B., Beck, B., Battes, K.P., Cimpean, M., Vermeissen, E.L. M., Singer, H., Hollender, J., Schäfer, R.B., 2017. Pesticide loads across an agricultural gradient in Romanian streams. 22th Annual Meeting SETAC GLB, Neustadt an der Weinstraße, Germany.

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POSTER

[1] **Schreiner, V.C.**, Kunz, S., Szöcs, E., Vogler, B., Beck, B., Vermeissen, E.L.M., Singer, H., Hollender, J., Schäfer, R.B., 2018. Pesticides across an agricultural gradient in Romanian streams. Training Course on Passive Sampling, Lausanne, Switzerland.

[2] **Schreiner, V.C.**, Szöcs, E., Bundschuh, M, Schäfer, R.B., 2015. Most common plant protection product mixtures in freshwater systems of the USA, France and Germany [poster spotlight] 25th Annual Meeting SETAC Europe, Barcelona, Spain.