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Exposure and effect assessment of pesticides in running waters

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Prof. Dr. Matthias Liess, UFZ Leipzig (Betreuer der Doktorarbeit)

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Freiberg, den 28. Oktober 2017

Ronald Münze

This doctoral thesis is based on the following peer-reviewed research articles:

1 **Münze, R.**, Orlinskiy, P., Gunold, R., Paschke, A., Kaske, O., Beketov, M.A., Hundt, M., Bauer, C., Schüürmann, G., Möder, M., Liess, M., 2015. Pesticide impact on aquatic invertebrates identified with Chemcatcher® passive samplers and the SPEAR$_{\text{pesticides}}$ index. *Science of the Total Environment* 537, 69–80.

2 **Münze, R.**, Hannemann, C., Orlinskiy, P., Gunold, R., Paschke, A., Foit, K., Becker, J., Kaske, O., Paulsson, E., Peterson, M., Jernstedt, H., Kreuger, J., Schüürmann, G., Liess, M., 2017. Pesticides from wastewater treatment plant effluents affect invertebrate communities. *Science of the Total Environment* 599-600, 387–399.

3 Orlinskiy, P., **Münze, R.**, Beketov, M., Gunold, R., Paschke, A., Knillmann, S., Liess, M., 2015. Forested headwaters mitigate pesticide effects on macroinvertebrate communities in streams: Mechanisms and quantification. *Science of the Total Environment* 524-525, 115–123.

Contents

Abstract

Pesticides negatively affect biodiversity and ecosystem functions of surface waters. The current pesticide risk assessment is afflicted with uncertainties. This PhD thesis (i) introduces an enhanced version of the Chemcatcher® passive sampler for the quantification of pesticides in running waters, (ii) reveals the contribution of wastewater treatment plants (WWTP) to insecticide loads in running waters and the associated consequences for stream macroinvertebrates, and (iii) confirms the recolonisation process of in-stream drift hypothesised for downstream community recovery from pulsed pesticide contamination. After an introduction into the fields of pesticides, running water ecology, and pesticide monitoring in running waters, the thesis continues with three studies on pesticide exposure and effects in lotic ecosystems.

In the first study, the pesticide stress of stream macroinvertebrates was determined using the bioindicator $\mathrm{SPEAR}_{\mathrm{pesticides}}$ and modified Chemcatcher® passive samplers. To inhibit the manifestation of a biofilm on the sorbent medium of the sampling device, a diffusion-limiting membrane was installed atop the receiving phase. The results show that Chemcatchers with a shielding membrane reliably quantify pesticide pollution. It is concluded that monitoring programmes using this passive sampler version can benefit from the properties of the membrane.

In the second study, the ecological impact of WWTP-induced pesticide pollution was assessed. WWTPs discharge pesticides from agricultural and urban uses into receiving waters. The results show that both macroinvertebrate community structure and organic matter breakdown are adversely affected by pesticides from WWTP effluents. It is concluded that further wastewater treatment steps are needed to eliminate pesticides from treated wastewater prior to its release into aquatic ecosystems.

In the third study, the impact mitigation potential of stream reaches not contaminated with pesticides was examined. In-stream drift is the main source of recolonising taxa and has been hypothesised to be the main driving force behind downstream community recovery from short-term pesticide stress. The results confirm that forested stretches accelerate the recovery of downstream macroinvertebrate communities by replenishing taxa vulnerable to pesticides. It is concluded that riparian forest needs to be protected (and newly grown where applicable), accounting for its ecological importance for lotic biodiversity in agricultural landscapes. Overall, the combined use of Chemcatcher® passive samplers and $\mathrm{SPEAR}_{\mathrm{pesticides}}$ is recommended for the assessment of pesticide exposure and effects in running waters.

Zusammenfassung

Pestizide wirken sich negativ auf die Artenvielfalt und Ökosystemfunktionen von Oberflächengewässern aus. Die derzeitige Pestizid-Risikobewertung ist mit Unsicherheiten behaftet. Diese Dissertation (i) stellt eine erweiterte Version des Chemcatcher®- Passivsammlers für die Quantifizierung von Pestiziden in Fließgewässern vor, (ii) zeigt den Beitrag von Kläranlagen (KA) zu Pestizidfrachten in Fließgewässern und die sich daraus ergebenden Konsequenzen für Makroinvertebraten auf und (iii) bestätigt die Trift als Hauptprozess bei der Wiederbesiedelung flussabwärts gelegener Gewässerabschnitte nach kurzzeitiger Pestizidbelastung. Im Anschluss an eine Einführung in die Bereiche Pestizide, Fließgewässerökologie und Pestizid-Monitoring in Fließgewässern setzt die Dissertation mit drei Studien zur Exposition und Auswirkung von Pestiziden in Fließgewässer-Ökosystemen fort.

In der ersten Studie wurde der Pestizidstress für Makroinvertebraten mit Hilfe des Bioindikators $\text{SPEAR}_{\text{pesticides}}$ und modifizierten Chemcatcher®-Passivsammlern festgestellt. Um die Bildung eines Biofilms auf dem Sorptionsmedium des Sammlers zu unterbinden, wurde eine die Diffusion einschränkende Membran über der Sammelphase installiert. Die Ergebnisse zeigen, dass Chemcatchers mit einer schützenden Membran zuverlässig Pestizidbelastung quantifizieren. Es wird geschlussfolgert, dass Monitoring-Programme bei Verwendung dieser Passivsammler-Version von den Membraneigenschaften profitieren können.

In der zweiten Studie wurden die ökologischen Auswirkungen von KA-induzierter Pestizidbelastung bewertet. Kläranlagen stoßen Pestizide aus landwirtschaftlichem und urbanem Einsatz in ihre Vorfluter aus. Die Ergebnisse zeigen, dass sowohl die Struktur der Makroinvertebraten-Gemeinschaft als auch der Abbau organischen Materials durch Pestizide aus KA-Einleitungen nachteilig beeinflusst werden. Es wird geschlussfolgert, dass weitere Reinigungsstufen benötigt werden, um Pestizide aus behandeltem Abwasser vor dessen Einleitung in aquatische Ökosysteme zu entfernen.

In der dritten Studie wurde das Potenzial von unbelasteten Fließgewässerabschnitten bewertet, die Umweltauswirkungen von Pestiziden zu reduzieren. Trift ist die Hauptquelle für rekolonisierende Taxa und ist als die treibende Kraft hinter der Erholung flussabwärts lebender Gemeinschaften von kurzzeitigem Pestizidstress vermutet worden. Die Ergebnisse bestätigen, dass bewaldete Gewässerabschnitte die Erholung flussabwärts lebender Gemeinschaften durch die Bereitstellung pestizidsensitiver Taxa beschleunigen. Es wird geschlussfolgert, dass bewaldete Ufer geschützt (und nach Möglichkeit neu angelegt) werden müssen, um ihrer ökologischen Bedeutung für die Artenvielfalt in Fließgewässern landwirtschaftlicher Gebiete Rechnung zu tragen. Die kombinierte Verwendung von Chemcatcher®-Passivsammlern und $\text{SPEAR}_{\text{pesticides}}$ wird für die Bewertung von Pestizidexposition und -auswirkungen empfohlen.

Abbreviations/Glossary

Acaricide	-	Pesticide designed to harm ticks and mites
Algicide	-	Pesticide designed to hinder the growth of algae
Allochthonous	-	Originating in a place other than where it is found
AUSRIVAS	-	**AUS**tralian **RIV**er **A**ssessment **S**cheme
Autochthonous	-	Originating in the place where it is found
Biocide	-	Pesticide used for non-agricultural purposes
BMWP	-	Biological Monitoring Working Party
CPOM	-	Coarse particulate organic matter
Ecotone	-	Transition zone between ecological systems
EPT (index)	-	Ephemeroptera, Plecoptera, and Trichoptera
FPOM	-	Fine particulate organic matter
Fungicide	-	Pesticide designed to prevent fungal infections
Herbicide	-	Pesticide designed to harm unwanted plants
Hyporheic zone	-	Region beneath and alongside the stream bed
IBI	-	Index of Biological Integrity
Insecticide	-	Pesticide designed to harm pest insects
K_{OC}	-	Soil organic carbon-water partition coefficient
K_{OW}	-	Octanol-water partition coefficient
LC_{50}	-	Median lethal concentration
Molluscicide	-	Pesticide designed to harm molluscs
msPAF	-	**m**ulti-**s**ubstance **P**otentially **A**ffected **F**raction of species
Nematicide	-	Pesticide designed to harm plant-parasitic nematodes
Priority substance	-	Chemical presenting a significant risk to the aquatic environment
RIVPACS	-	**R**iver **I**n**V**ertebrate **P**rediction **A**nd **C**lassification **S**ystem
SPEAR	-	**SPE**cies **A**t **R**isk
Taxon/Taxa	-	Taxonomic unit/units (e.g., species, family, etc.)
TU_{max}	-	*Toxic Unit* value representing maximum toxicity, exerted by one individual compound within a mixture of substances
TU_{sum}	-	*Toxic Unit* value representing the sum toxicity of chemicals
WFD	-	Water Framework Directive
WWTP	-	Wastewater treatment plant

CHAPTER 1

Introduction, Methods, and Objectives

"In every respect, the valley rules the stream."

Hugh Bernard Noel Hynes

Pesticide use and fate

Major groups and configurations

Pesticides are biologically highly effective substances, intentionally released into the environment in order to protect human goods and objects from detrimental effects of ‘pests’. In agricultural production, plant protection products (sensu EC, 2009a) help maximise yields by preventing cultivated plants from, e.g., weed competition (*herbicides*), plant diseases (*fungicides*), and pest infestation (*acaricides*, *insecticides*, *molluscicides*, and *nematicides*). In settlement, industrial, and recreational areas, ‘biocides’ (sensu EC, 2012) assist in vegetation management programmes and inhibit plant root penetration of roof sealings (*herbicides*; Bucheli et al., 1998; NAS, 1968); they prevent biofouling and biodegradation of building materials and underwater structures (*algicides*, *fungicides*, *herbicides*, *insecticides*, and *molluscicides*; Schultz et al., 2007; Thomas et al., 2002); and they fight commensal rodents such as mice and rats (*rodenticides*; Larsen, 2003), among many other things.

The history of pest control began some 4500 years ago, when the Sumerians started to kill pest insects using sulphur (McKinney et al., 2007). In modern agriculture, a wide range of active substances from several chemical classes are deployed. With respect to (i) the physicochemical properties of the active substance, (ii) the particular mode of action, (iii) the intended application method, and (iv) some production and marketing criteria (Webb, 2002), pesticides are manufactured in various types of formulation, such as concentrate, granule, wettable powder, liquid, emulsion, and suspension (BVL, 2017). Furthermore, to enhance shelf life, ease of use, application, and biological performance of the active ingredients, pesticidal products usually contain various inert additives that act as, e.g., preservatives, stabilisers, solvents, emulsifiers, diluents, defoamers, and surfactants (USEPA, 2015; Webb, 2002).

Pesticidal products contain one or several active ingredients; in turn, many active substances are incorporated in more than one product (BVL, 2017). In Germany, the number of commercial products (in 2015: 766) and registered trade names (in 2015: 1490) usually by far exceed the number of active substances approved (in 2015: 277; BVL, 2016). At present, 272 active substances are available on the German domestic market. The majority of current-use herbicides, fungicides, and insecticides (the most relevant product types applied to agricultural fields) are formulated as concentrates and granules to be emulsified/suspended before application, followed by ready-to-use emulsions and liquids (BVL, 2017).

In Germany, 52% of land is used for agriculture (Destatis, 2016). With organic farming accounting for only 6.5% of total agricultural land, conventional farming methods are the predominant form of agrarian production (BMEL, 2017). For the past 20 years, the overall demand for pesticides has been rising; however, not all product types were equally concerned (UBA, 2016a; Fig. A). In 2014, pesticide sales in Germany accounted for approximately 12% of all sales in the European Union (EC, 2016).

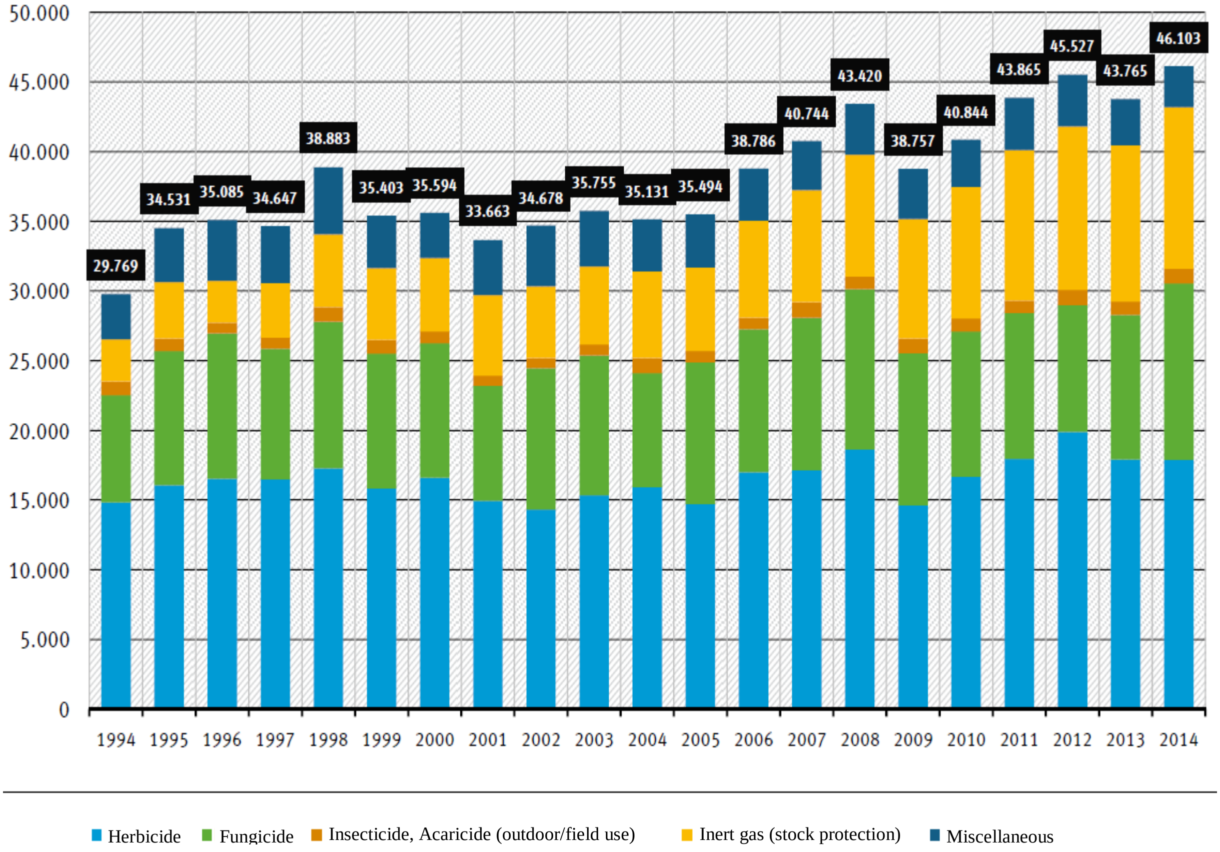

Fig. A. Development of domestic sales of plant protection products in Germany. Modified from UBA, 2016a.

Regulatory background

In the European Union, the regulation of pesticides, biocides, and their active substances is mandatory due the environmental risks associated with their high biological effectiveness (EC, 2012, 2009a). Prior to their release into the environment, all active substances and final products need to obtain the EU-wide authorisation for use from the European Commission and its member states. First, the manufacturer of a given active ingredient submits an application to a chosen EU member state. This member state performs an initial risk assessment that is later reviewed by the European Food Safety Authority (EFSA); on the basis of their conclusion, the European Commission and its member states decide whether this active substance is to be approved. Then, the manufacturer or importer of a given pesticidal product containing an approved active substance submits an application to a chosen EU member state. This time, the EFSA and the European Commission decide on the compound's maximum residue level in food and feed as proposed by the applicant; in the case of a positive conclusion, the respective EU member state can authorise the product to be placed on the market (EFSA, 2017).

In Germany, four federal authorities share the responsibilities during the pesticide authorisation procedure. Following positive conclusions regarding the potential risks for operators and consumers (Federal Institute for Risk Assessment – BfR), for crops and beneficial organisms (Federal Research Centre for Cultivated Plants – JKI), and for natural ecosystems (Federal Environment Agency – UBA), the Federal Office of Consumer Protection and Food Safety (BVL) issues a nationwide licence to the applicant (UBA, 2013). Numerous aspects concerning the use of pesticides and biocides in Europe are covered by the respective pieces of EU legislation, such as the list of approved active substances (EC, 2011; updated in 2014), data requirements for the authorisation of active substances and final plant protection products (EC, 2013a, 2013b), the regulations for the classification, labelling, and packaging of chemicals (EC, 2008), the demand for a sustainable use of pesticides (EC, 2009b), and the maximum acceptable residue levels in food and feed (EC, 2005).

Pesticidal modes of action

Herbicides fight plants that compete with crops for light, space, nutrients, and further resources. Many herbicides disrupt the process of photosynthesis, such as *benzothiadiazole* (e.g., bentazon), *phenylarbamate* (e.g., phenmedipham), *phenylurea* (e.g., diuron and isoproturon), *triazine* (e.g., atrazine and simazine) and *triazinone* (e.g., metribuzin) *herbicides*. Others impair the biosynthesis of plant lipids, proteins, and pigments: *Benzofuran* (e.g., ethofumesate), *cyclohexanedione* (e.g., alloxydim), and *thiocarbamate* (e.g., butylate) *herbicides* inhibit fatty acid biosynthesis; *sulphonylurea* (e.g., amidosulfuron and chlorsulfuron) *herbicides* block amino acid biosynthesis; and *pyrazole* (e.g., pyrazoxyfen) and *triazole* (e.g., amitrole) *herbicides* hinder the biosynthesis of carotenoids. A third herbicidal mode of action is the regulation of plant growth: *phenoxy* (e.g., dichlorprop) and *pyridine* (e.g., triclopyr) *herbicides* mimic natural phytohormones, while *carbamate* (e.g., chlorpropham), *chloroacetamide* (e.g., alachlor and metolachlor), and *benzamide* (e.g., propyzamide) *herbicides* interfere with normal cell division and development. These and additional modes of action are operated by further classes of herbicides (Copping, 1998; Faggiano et al., 2010).

Fungicides protect crops from pathogens that cause plant diseases. ‘Protectant’ fungicides, such as *dithiocarbamate fungicides* (e.g, mancozeb) and inorganic compounds (e.g., copper and sulphur), act on crop surfaces as a barrier to fungal invasion, while ‘eradicant’ fungicides fight fungal diseases that are already established: *Benzimidazole* (e.g., carbendazim) and *phenylurea* (e.g., pencycuron) *fungicides* interfere with normal cell division; *imidazole* (e.g., prochloraz), *morpholine* (e.g., fenpropimorph), *pyridine* (e.g., pyrifenox), and *triazole* (e.g., epoxiconazole and propiconazole) *fungicides* inhibit sterol biosynthesis in fungal cell membranes; *phenylamide fungicides* (e.g., metalaxyl) affect protein biosynthesis; *dicarboxamide fungicides* (e.g., iprodione) block fungal osmoregulation; *carbamate fungicides* (e.g., propamocarb) interfere with cell membrane permeability; and *strobilurin* (e.g., azoxystrobin and picoxystrobin) and *carboxamide* (e.g., boscalid) *fungicides* hinder fungal respiration. These and additional modes of action are operated by further classes of fungicides (Copping, 1998; FRAC, 2017).

The majority of **insecticides** are nerve poisons that act upon various signal transmission processes in the nervous system of insects. *Organochlorine* (e.g., endosulfan and lindane) and *pyrethroid* (e.g., esfenvalerate) *insecticides* interfere with the shutdown and recovery processes of the sodium channels after excitation. *Carbamate* (e.g., carbofuran and pirimicarb) and *organophosphate* (e.g., chlorpyrifos and dimethoate) *insecticides* inhibit the enzyme acetylcholinesterase (AChE) that normally inactivates the signal transmitter acetylcholine after excitation; this effect is reversible with *carbamates* and irreversible with *organophosphates*. *Neonicotinoid insecticides* (e.g., imidacloprid and thiacloprid) activate the acetylcholine receptors like the original excitatory signal transmitter, acetylcholine. In contrast, *organochlorines* and fipronil affect the receptors of the inhibitory signal transmitter *gamma*-Aminobutyric acid (GABA), preventing the chloride channels from ending excitation stimuli. All these insecticidal effects cause hyper-excitation that subsequently leads to exhaustion and death of the insects concerned (Copping, 1998; Matsumura, F., 2009). Additional modes of action, such as respiration impairment, growth regulation, and membrane disruption, are operated by further classes of insecticides including bacteria (IRAC, 2017).

Environmental fate of pesticides

How and when a pesticidal product is applied to the field depends on chemical formulation, application method, crop stage, intended target, and weather conditions (Leonard, 1988). Likewise, its environmental fate is governed by a similar set of factors: (i) the pesticide's physicochemical properties that determine persistence and mobility within and across environmental compartments (air, soil, water, and biota), (ii) application method, (iii) soil conditions, and (iv) regional climate (reviewed by Alletto et al., 2010). Generally, three main fate processes can be distinguished: **adsorption/desorption** (to and from crop foliage, plant residues, and soil), **distribution** (absorption into crops and pests, volatilisation into air, washoff from foliage, leaching into the soil, and runoff from fields), and **degradation/transformation** (photolysis, hydrolysis, and microbiological activity). Wind drift during the spraying process represents an additional means of distribution for pesticides (Nowell et al., 2000; Reichenberger et al., 2007; Fig. B).

Variations in formulation and application method not only have implications for the activity and efficiency of pesticides (Bonham et al., 2009; Van Timmeren et al., 2011) but they also affect the fate and water pollution potential of these chemicals (Davis et al., 1996). The likelihood of a certain fate pathway to occur is influenced by the specific area of pesticide placement on the field. For example, for pesticides incorporated into the soil (*in-furrow application*), transformation is a major loss process whereas volatilisation and surface runoff are negligible. Highly volatile compounds are usually soil-incorporated to avoid their loss to the atmosphere (Capel et al., 2001; Caro et al., 1973). In contrast, for pesticides deposited on crop surfaces and bare soil, important loss processes include volatilisation, plant surface wash-off, and field runoff (Davis et al., 1996; Reddy et al., 1994; Wauchope et al., 2004). In the case of the latter two, the extent of the loss is determined by the chronological sequence of wash-off and runoff (Burgoa and Wauchope, 1995).

Plant protection products can enter surface waters via non-point (diffuse) and point sources. Diffuse pesticide input comes from air-borne (i.e., spray drift, volatilisation, and atmospheric deposition) and water-driven transport processes (i.e., surface runoff, drain flow, leaching through the soil, and groundwater discharge); point source pathways include farmyard runoff, wastewater treatment plant effluents, and spillages (Reichenberger et al., 2007). Upon entry into a water course, behaviour and fate of a pesticide are controlled by the characteristics of the chemical (e.g., water solubility and persistence) and the characteristics of the stream (e.g., current velocity, concentration of suspended particles, and constituents causing transformation; Capel et al., 2001). Potential in-stream processes include sorption and desorption, bioaccumulation, degradation, and transformation of pesticides (Fig. B, inset).

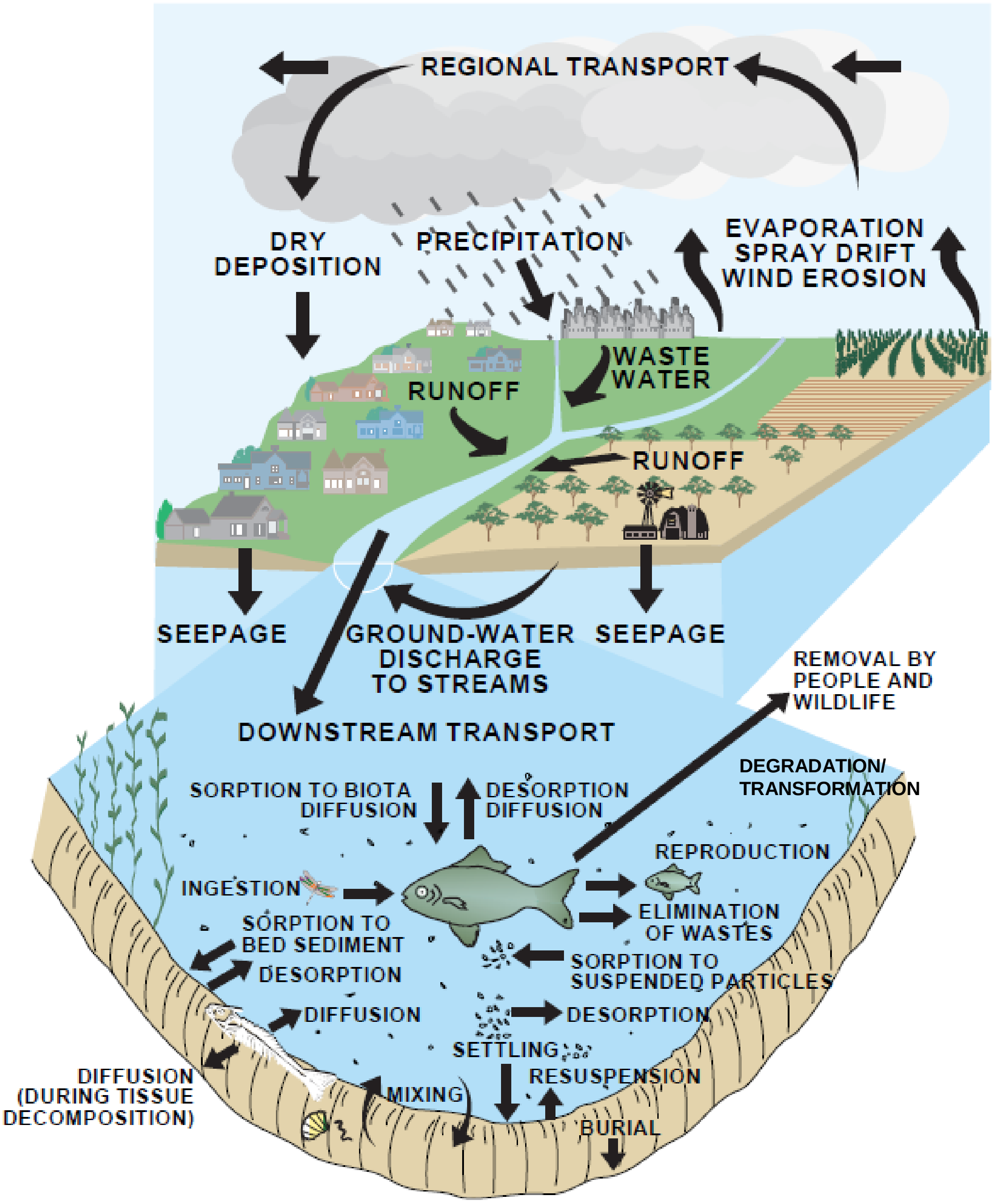

Fig. B. Pesticide movement in the hydrologic cycle. *Inset*: Pesticide movement to and from sediment and aquatic biota in rivers and streams. Modified from Nowell et al., 2000, with kind permission of the authors.

Running water ecosystems

Running waters in the landscape context

Riverine ecosystems are open ecosystems that are tightly interconnected with the landscape areas they run through. Geological features, land cover, and human land-use activities strongly affect the properties of flowing waters, such as stream morphology (i.e., channel shape, size, and slope), hydrology (e.g., water level, current velocity, and solute transport), water chemistry (e.g., contents of oxygen, nutrients, and pollutants), riparian vegetation (e.g., magnitude of stream exposure to sunlight and organic matter input), and in-stream structures and surfaces (e.g., composition of the channel bottom substrate; presence of logs and boulders). Regional proximity effects influence the occurrence and variety of the biota in rivers and streams (Allan, 2004; Armitage and Blackburn, 2010; Hynes, 1975; Roth et al., 1996; Vannote et al., 1980; Ward et al., 2002).

Stream habitat conditions and aquatic communities are also determined by longitudinal variations in channel morphology and riparian land. From the headwaters to the mouths of river networks, i.e., with increasing stream order (see Strahler, 1954), several ecosystem parameters gradually change, such as channel size, current velocity, water temperature, riparian vegetation, and canopy cover (Vannote et al., 1980; Warren et al., 2013). In small headwaters, autochthonous primary production is limited by the shading of riparian vegetation, and organic carbon is mostly provided in the form of leaf litter and woody debris from the outside (coarse particular organic matter; CPOM). In midsized streams, allochthonous energy input is less important, and in-stream primary production by algae and aquatic vascular plants is high. Finally, in large rivers, the terrestrial carbon input is rather insignificant, and primary production is reduced by channel depth and water turbidity; thus, energy is largely available in the form of fine particular organic matter (FPOM). In accordance with the longitudinal and unidirectional in-stream transport of particulate organic carbon, the faunal community composition differs along the *river continuum* (Newbold et al., 1982; Vannote et al., 1980; Fig. C), with the headwaters constituting the basis for biodiversity in the whole riverine network (Finn et al., 2011).

The interactions of water courses with the groundwater constitute the vertical dimension of lotic ecosystems (Ward, 1989). In the hyporheic zone, water, nutrients, organic matter, and fauna are being exchanged across the *ecotone* (‘stress line’; Clements, 1905) between streams and groundwater (Brunke and Gonser, 1997; Hynes, 1983; Stanford and Ward, 1993).

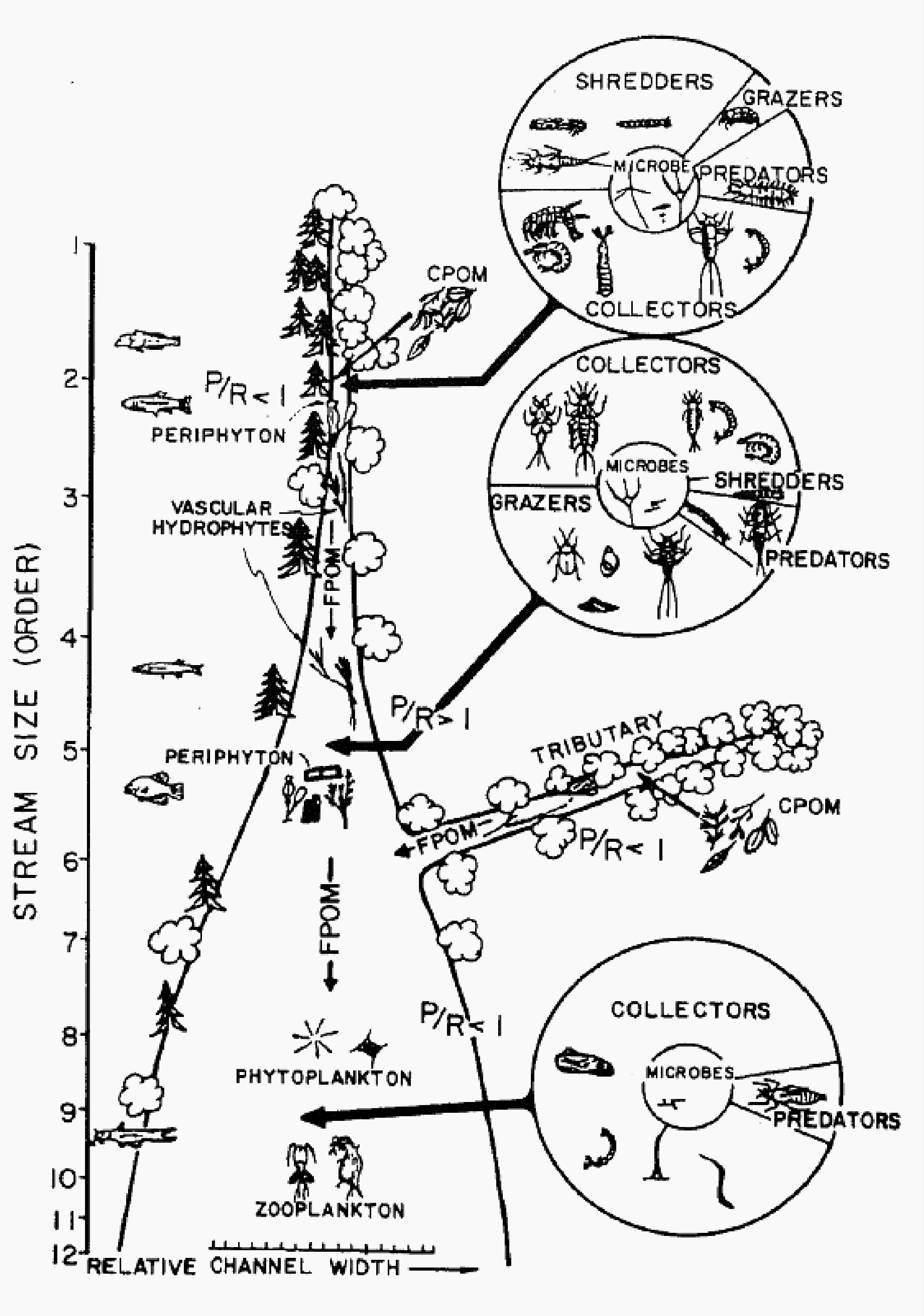

Fig. C. Relationship between stream size and the progressive shift in structural and functional attributes of lotic communities. Reproduced from Vannote et al., 1980.

In addition to the three landscape dimensions outlined above, the temporal dimension describes seasonal and between-years changes in the physicochemical parameters and the biota of rivers and streams (Ward, 1989; Fig. D). For example, annual fluctuations in precipitation patterns can lead to variations in current velocity and stream depth, with considerable implications for water temperature, oxygen content, pH, and the lotic fauna (Bunn et al., 1986).

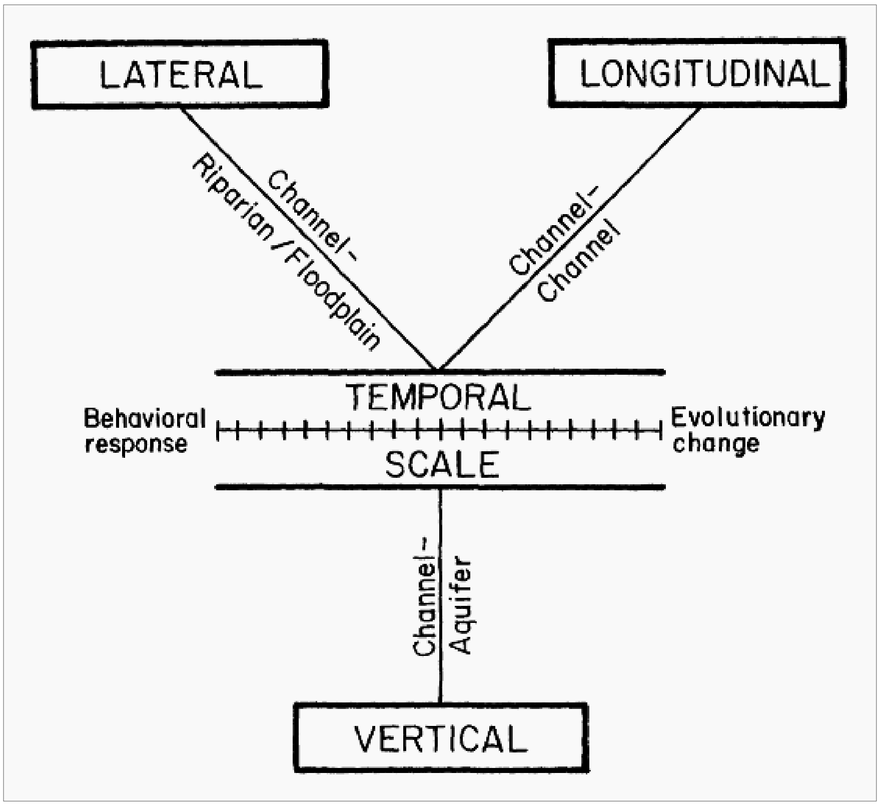

Fig. D. A conceptualisation of the four-dimensional nature of lotic ecosystems. Reproduced from Ward, 1989.

The biota of running waters

Rivers and streams account for less than 0.01% of all global freshwater reserves (Shiklomanov, 1993). Yet, due to their high spatial and temporal heterogeneity, fluvial ecosystems sustain a very diverse array of aquatic life (Giller and Malmqvist, 1998). Important stream inhabitants include bacteria, fungi, algae, bryophytes (e.g., liverworts and mosses), macrophytes, microinvertebrates (e.g., protozoa and rotifers), macroinvertebrates, and vertebrates, such as fish (Downes et al., 2008).

Lotic macroinvertebrate communities comprise platyhelminthes (flatworms), nematodes (roundworms), annelids (segmented worms), molluscs (e.g., snails, clams, and mussels), and arthropods (e.g., insects, water mites, and Crustacea). Because the majority of them are benthic, i.e., dwelling on and in the stream bed, they are commonly referred to as ‘macrozoobenthos’. The stream macrozoobenthos is dominated by the class of insects; most of them enter a terrestrial adult stage following their aquatic larval development (Downes et al., 2008), which can take up to five years to completion (some Odonata; Silsby, 2001).

Benthic macroinvertebrates play vital functional roles within aquatic food webs and nutrient cycles (Wallace and Webster, 1996). Pursuing different foraging strategies, they have been classified into four functional feeding groups: **Shredders** feed on CPOM and the adhering biofilm, breaking the CPOM down into FPOM and thereby promoting downstream energy flow; **grazers** (or scrapers) detach periphyton and biofilm layers from pebbles, rocks, woody debris, and submerged vegetation; **collectors** take advantage of the FPOM and biofilm pieces dislodged by shredders and grazers in upper reaches, and they also salvage dead organisms and other organic detritus; finally, **predators** prey on live organisms, such as other insect larvae (Cummins 1973). According to the *River Continuum concept* (Vannote et al., 1980; Fig C), benthic macroinvertebrate communities respond structurally and functionally to longitudinal resource gradients, approaching equilibrium with the dynamic physical conditions of riverine systems. By maximising the respiratory carbon utilisation, the organic carbon turnover length (i.e., the downstream travel distance of particulate organic carbon) is being kept to a minimum – the shorter the longitudinal distances required for equilibrium, the smaller the downstream energy losses (Newbold et al., 1982; Vannote et al., 1980).

Ecosystem services and functions of running waters

Freshwater streams provide a variety of ecosystem services to humankind and the environment, including (i) *provisioning services* (material and energy outputs, e.g., water source for agricultural, industrial, and domestic purposes; food source; and transportation route), (ii) *maintenance services* (biota mediating/moderating the ambient environment, e.g., regulation of the climate and floods; nutrient cycling; and waste degradation), (iii) *cultural services* (non-material benefits, e.g., recreation, aesthetics, and spirituality), and (iv) *supporting services* (habitats for the freshwater flora and fauna; ecosystem functions, e.g., soil formation, primary production, and organic matter breakdown; Biggs et al., 2017; MEA, 2005).

For example, a major ecosystem function of running waters is the decomposition of leaf litter. This process ensures the continuous flow of carbon and energy along the *river continuum* (Newbold et al., 1982; Vannote et al., 1980; Webster, 2007). The amount of allochthonous CPOM from riparian vegetation that will be available for utilisation by stream biota is determined by the retentive properties of the watercourse (e.g., size, discharge, riffle-pool sequences, and substrate features) and the structure and composition of the riparian zone (e.g., leaf litter quantity and woody debris input; Speaker et al., 1984; Webster et al., 1999). Once in the water, CPOM quickly leaches soluble components; the remaining CPOM surfaces are being colonised and metabolised by microorganisms in transport (Cummins, 1974), which leads to increased nitrogen and protein contents of stream leaf litter (Kaushik and Hynes, 1971). This *leaf conditioning* is vital for the process of matter breakdown, making the plant material palatable for coarse particle feeders or *shredders* (e.g., Bärlocher and Kendrick, 1975; Cummins et al., 1973). The high significance of shredding macroinvertebrates for this key ecosystem-level process is well-known (e.g., Cuffney et al., 1990, 1984; Hieber and Gessner, 2002).

Monitoring of pesticide exposure and effects in running waters

Chemical monitoring (Exposure monitoring)

Precipitation-induced pesticide input into surface waters happens episodically, and the in-stream presence of pesticides is only brief; hence, linking any observed changes in lotic communities to pesticide exposure is not possible unless pesticide peak concentrations are adequately incorporated into the investigation (Friberg et al., 2003). However, various spatial and temporal factors affect pesticide peak concentrations in small running water bodies, making the monitoring of pesticide contamination a great challenge (Lorenz et al., 2017).

During runoff events, the pesticide loss rate from agricultural fields depends on the extent of surface runoff and pesticide availability. The former is determined by the duration and intensity of precipitation, the properties of the soil (e.g., particle size and organic-matter content), the slope gradient of the field, and vegetation cover. The latter is determined by the timing and intensity of the first rainfall after pesticide application, the choice of crop, application rate and method, formulation type, resistance or susceptibility of the chemical to degradation and volatilisation, and the kinetics of the transformation reactions in the field (Capel et al., 2001; Leonard, 1988). For example, when deposited on crop foliage, half-lives of currently licensed pesticides (see BVL, 2017) vary immensely, ranging from 0.2 days (pyrethrins) to 20 days (metolachlor; Fantke et al., 2014). Single precipitation events commonly cause runoff losses of only 1-2% of the pesticide amount applied; however, very heavy rainfall can lead to much higher loss rates (Wauchope, 1978).

Chemical surface water quality is determined in different ways. Grab sampling, (automatic) composite sampling (USEPA, 2014), and event-driven sampling (Liess and von der Ohe, 2005) are used to obtain stream water samples (*active sampling*). In contrast, in-situ sampling devices are used to quantify selected target compounds according to the design of the monitoring programme (*passive sampling*; Greenwood et al., 2007a). Passive sampling devices, such as the Chemcatcher® passive sampler, enable an integrative sampling of pesticides in the water phase over longer periods of time (Allan et al., 2009; Gunold et al., 2008; Schäfer et al., 2008; Shaw and Mueller, 2009), whilst also providing estimates of the bioavailable fractions of the pesticides concerned (Holvoet et al., 2007). The Chemcatcher® passive sampler, hereafter referred to as Chemcatcher, is designed to monitor polar and semi-polar organic chemicals in surface waters (Greenwood et al., 2007b; Stephens et al., 2005). Furthermore, Chemcatchers are able to preconcentrate the target substances in-situ, leading to lower limits of detection/quantification for various chemical compounds in comparison to conventional grab sampling.

Biological monitoring (Effect monitoring)

Biological monitoring, or biomonitoring, can be defined as “the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program” (Matthews et al., 1982). Biomonitoring is considered the most appropriate means of assessing stream health (Karr, 1999) and can be performed at several levels of biological organisation (Allan et al., 2006). On the community level, benthic macroinvertebrates have become the favoured group of organisms to be used in this regard because they (i) reflect local stream conditions due to their relatively sessile mode of life, (ii) have aquatic life-cycle stages long enough to integrate short-term environmental deviations from local standard stream conditions over extended periods of time, (iii) sensitively and quickly respond to a wide range of pollutants, and (iv) are pervasive, abundant, and relatively easily sampled and identified (Barbour et al., 1999). Consequentially, benthic macroinvertebrates can be used for the biomonitoring of various types of environmental stress, such as pesticides (e.g., Schulz and Liess, 1999), eutrophication (e.g., Hering et al., 2006), acidification (e.g., Dangles and Guérold, 2009), and hydromorphological degradation (e.g., Lorenz et al., 2004).

More than a century ago, Kolkwitz and Marsson (1909) proposed a first list of aquatic insects that would serve as bioindicators for organic pollution; since then, a wide range of different biomonitoring concepts and methods involving benthic macroinvertebrates has been developed (Bonada et al., 2006; Metcalfe, 1989): **Diversity indices** (e.g., EPT index and Shannon’s index) evaluate community structure using species richness, evenness, and abundance; **biotic indices** (e.g., BMWP score and Trent index) assess stream water quality using certain indicator organisms and their physiological responses to particular types of water pollution, and **similarity indices** (e.g., Bray-Curtis dissimilarity and Jaccard's index) compare the structure of up- and downstream communities in connection with suspected point source water pollution (reviewed by Metcalfe, 1989, and Washington, 1984). **Multimetric indices** (e.g., IBI) assess biological integrity combining several different metrics, thereby incorporating information from ecosystem, community, population, and individual levels (Karr and Chu, 1997; Ofenböck et al., 2004). Multivariate **predictive models** (e.g., RIVPACS and AUSRIVAS) compare expected and observed stream fauna values (Smith et al., 1999; Wright, 1993). **Trait-based approaches** (e.g., SPEAR) use selected life history traits of the macrozoobenthos to link community patterns, anthropogenic stressors, and ecosystem processes in running waters (Baird et al., 2008; Dolédec et al., 1999; Liess and von der Ohe, 2005; Schäfer et al., 2011; Verberk et al., 2008).

The community level has been found to be appropriate for the investigation into pollution effects (Cairns 1974; Hawkes, 1957). Community endpoints, such as species richness and diversity, are closely interlinked with ecosystem function and services (Clements and Rohr, 2009). However, attributing a given ecological effect to a specific stressor among a multitude of environmental variables is challenging. The co-occurrence of various natural and anthropogenic *confounding factors*, such as habitat characteristics, biotic (e.g., species interactions) and abiotic (e.g., hydrodynamics) implications, and contaminant composition, can both mask and increase the effects of pesticide pollution on benthic macroinvertebrate communities (Alexander et al., 2013; Becker and Liess, 2015; Friberg et al., 2003; Johnson et al., 2006; Kattwinkel and Liess, 2014; Knillmann et al. 2012a, 2012b).

Trait-based biomonitoring approaches, such as the pesticide-specific SPEAR index, are able to circumvent these difficulties (Liess and von der Ohe, 2005; Liess et al., 2008), and they can be applied across large spatial scales (Schäfer et al., 2007; Schletterer et al., 2010;

Statzner et al., 2001). Baird et al. (2008) define *traits* as “the physiological, morphological, and ecological attributes of species or other taxonomic entities, which describe their physical characteristics, ecological niche, and functional role within an ecosystem”. Suboptimal living conditions (e.g., frequent disturbances) encourage trait similarity among benthic invertebrates (Thienemann, 1918); an organism’s trait profile is largely determined by the biotic and abiotic characteristics of that organism’s habitat (*Habitat Templet concept*; Southwood, 1977; adapted to running waters by Townsend & Hildrew, 1994; Fig. E). Freshwater lotic insects often occur with the same families and genera in geographically separated areas (Giller and Malmqvist, 1998).

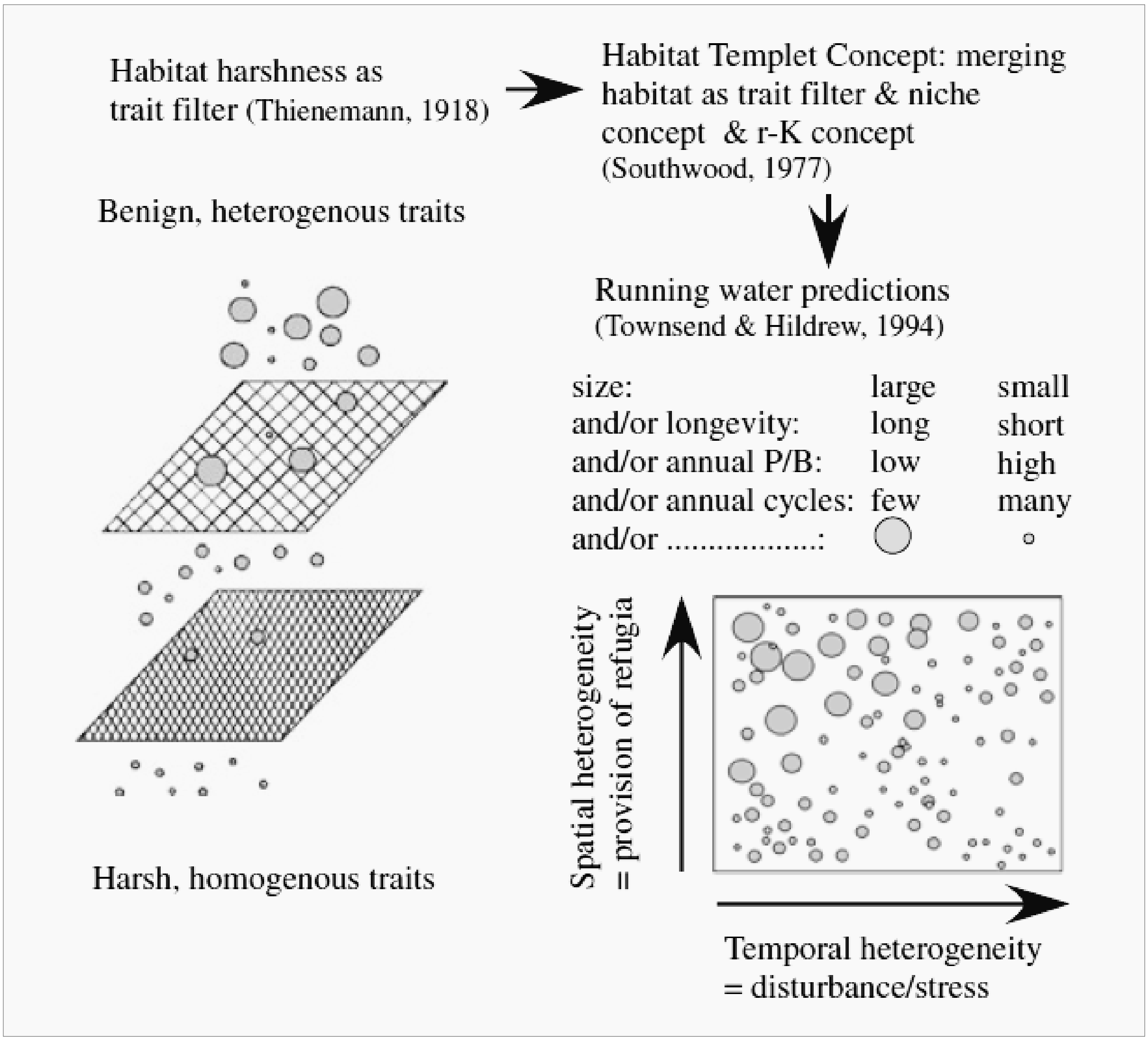

Fig. E. Three pivotal ideas from theoretical ecology that initiated the use of biological traits in assessments of the effects of human-caused stressors on running water ecosystems. Reproduced from Statzner and Bêche, 2010.

The bioindicator system SPEAR$_{pesticides}$

For the biomonitoring of pesticides in running waters, Liess and von der Ohe (2005) developed the trait-based SPEAR$_{\text{pesticides}}$ bioindicator (**SPE**cies **A**t **R**isk). Following the observation of pesticide-related effects at the ecosystem level, in a *top-down approach*, relevant traits and processes were identified that can be used for the biomonitoring of pesticide pollution; using these traits in a *bottom-up approach*, ecosystem effects of pesticides can be predicted and verified using SPEAR$_{\text{pesticides}}$ in small-scale experiments (Liess and Beketov, 2011) and large-scale ecosystem studies (e.g., Liess et al., 2008; Rasmussen et al., 2013; Schäfer et al., 2007; Fig. F). The index is based on four selected life-history traits of aquatic macroinvertebrates: the physiological sensitivity to insecticides and other pesticides with insecticidal mode of action (see Wogram and Liess, 2001), generation time, migration ability (both representing the recovery potential), and the presence of aquatic life cycle stages during the periods of maximum pesticide application, i.e., a high probability of exposure to pesticide pollution (Liess and von der Ohe, 2005).

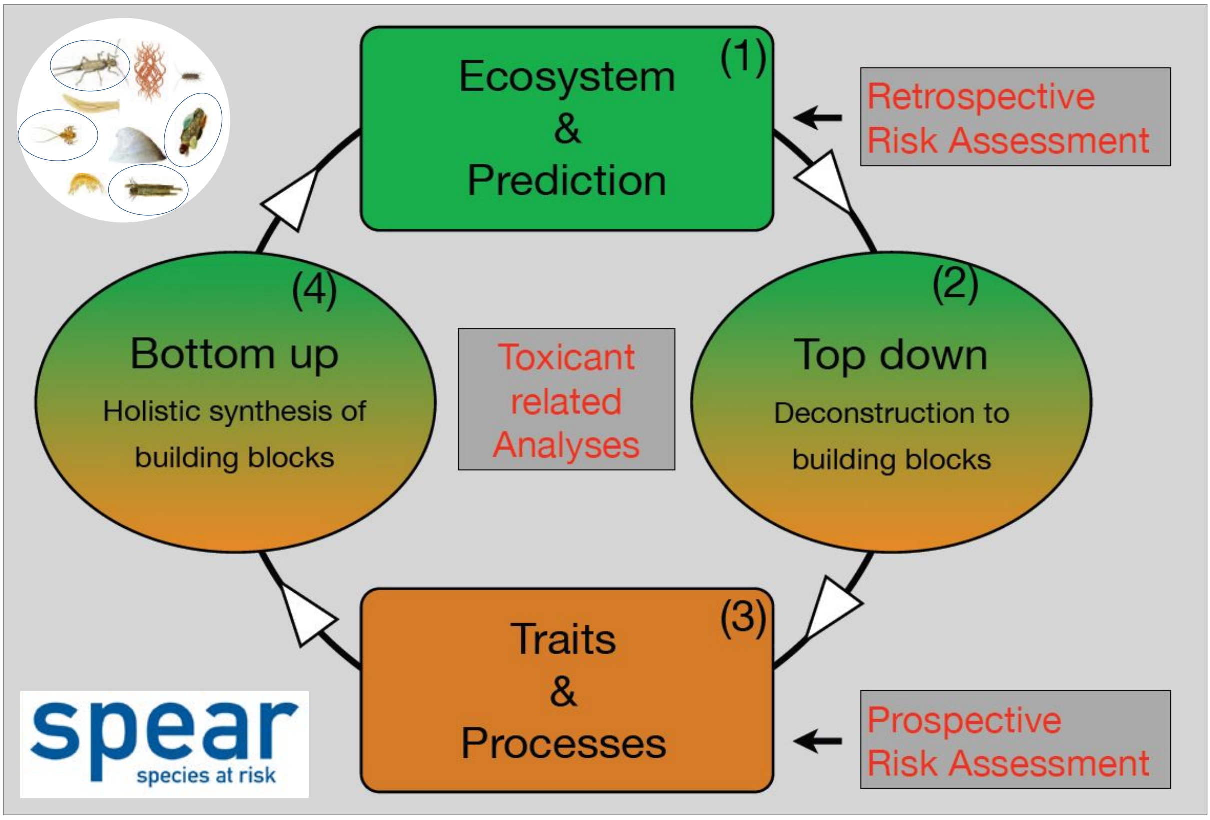

Fig. F. Top-down and bottom-up approach for risk assessment activities using SPEAR. Modified from Liess, 2013.

To classify a given aquatic macroinvertebrate taxon as vulnerable to pesticides (i.e., a *species at risk* = *SPEAR*), the respective decision criteria have been specified as follows: physiological sensitivity > -0.36; generation time (in years) ≥ 0.5; migration ability = *no*; and exposed aquatic life cycle stages = *yes*. SPEAR indicator values give the relative abundance of taxa vulnerable to pesticide pollution, and they are computed according to the following equation:

$$\mathrm{SPEAR}_{\mathrm{pesticides}} = \frac{\sum_{i=1}^{n} \log(x_i + 1)\, y}{\sum_{i=1}^{n} \log(x_i + 1)} \cdot 100$$

where n is the total number of taxa in a sample, x_i is the abundance of taxon i (given as individuals m^{-2}), and y is set to 1 if taxon i is classified as ‘at risk’, i.e., vulnerable to pesticides, and set to 0 otherwise.

Methods and Objectives of this thesis

Methods

The field investigations took place from 2010 to 2013; they comprised the quantification of pesticide pollution, the sampling of macrozoobenthos, and the deployment of leaf litter bags at selected sampling sites. Physicochemical habitat characteristics were also determined at the sampling sites. For the third study (see CHAPTER 4), additional data from a previous investigation by Liess and von der Ohe (2005) were also used.

To assess pesticide exposure, Chemcatchers were deployed at the sampling sites to obtain time-weighted average concentrations of pesticides over the length of the respective study periods. In two studies (see CHAPTERS 3 and 4), event-driven samplers (see Liess and von der Ohe, 2005) were additionally deployed to capture peak pesticide concentrations from precipitation-induced runoff events.

To determine the toxicity of the pesticides detected, the LC_{50} values of the most sensitive test organisms according to the literature, *Daphnia* ssp. and *Chironomus* ssp., were used. The *Toxic Unit (TU) concept* (Sprague, 1970) was applied to link pesticide toxicity to the site-specific macroinvertebrate composition, expressed by the respective SPEAR indicator value calculated.

To assess the ecological effects of pesticides on faunal assemblages, the macroinvertebrate community structure at the sampling sites was determined using the SPEAR$_{\text{pesticides}}$ index (see Liess and von der Ohe, 2005). Considering the potential effects of the sampling effort on the study results (Bady et al., 2005), adequate amounts of time were devoted to the collection and identification of the benthos. Collected macroinvertebrates were identified to family level, which is sufficient for freshwater biomonitoring in general (Marshall et al., 2006; Waite et al., 2004) and for the application of SPEAR$_{\text{pesticides}}$ in particular (Beketov et al., 2009). Furthermore, leaf litter breakdown was analysed to assess the ecological effects of pesticides on community functioning. For this, leaf litter bags were deployed in the streams under investigation. Detailed information on the sampling procedures for chemicals and stream benthos, the derivation of LC_{50} and TU values, and the processing of benthos samples, chemical samples, and leaf litter bags can be found in the respective Materials and Methods sections of CHAPTERS 2 to 4 and in the associated Supplementary Materials (see Appendices A to C).

Study sites

The studies have been primarily conducted at rather small to medium headwater streams and ditches in agricultural landscapes in central Germany, selected according to the research question of the respective project (see CHAPTERS 2 to 4). The study area is part of the TERENO Harz/Central German Lowland Observatory, the main region for the hydrological research conducted at the Helmholtz Centre for Environmental Research (UFZ; Wollschläger et al., 2017). In the first study (see CHAPTER 2), chemical and biological samples were obtained from 19 independent locations that were unaffected by chemical input other than pesticide runoff from adjacent agricultural fields (Fig. G). In the second study (see CHAPTER 3), 14 sampling locations were pairwise situated up- and downstream of seven wastewater treatment plant effluent discharge points. Finally, in the third study (see CHAPTER 4), 37 sampling locations were chosen in a way that they were not influenced by chemical input other than pesticide runoff from adjacent agricultural fields. Here, 23 of those sites had a forested upstream reach within a distance of up to11 km with no agricultural land further upstream. Detailed information on the characteristics of all study sites can be found in the respective Materials and Methods sections of CHAPTERS 2 to 4.

Fig. G. Typical small running water body in agricultural surroundings. Photo: R. Münze

Objectives

$\text{SPEAR}_{\text{pesticides}}$ has been successfully deployed numerous times to indicate pesticide pollution in aquatic environments, both in agricultural landscapes across biogeographical regions (Hunt et al., 2017; Liess et al., 2008; Rasmussen et al., 2013; Schäfer et al., 2007) as well as in mesocosm studies (Liess and Beketov, 2011). However, the following three aspects of the deployment of $\text{SPEAR}_{\text{pesticides}}$ had not yet been explored before:

(i) The application of the index in connection with a modified version of the established Chemcatcher® passive sampler;

(ii) The application of the index around WWTP outlets to evaluate the ecological impact of effluent-borne pesticides on whole macroinvertebrate communities; and

(iii) The application of the index downstream of forested stream reaches to assess the potential of riparian forest to mitigate pesticide impact.

In the first study (see CHAPTER 2), the effects of pesticide pollution on lotic macroinvertebrates were examined using $\text{SPEAR}_{\text{pesticides}}$ and Chemcatchers whose sampling phases were covered with a diffusion-limiting membrane. Previously, Chemcatchers had been successfully used without a membrane (Schäfer et al., 2008). However, the absence of such a membrane impairs the uptake characteristics of and the hydrodynamic conditions on the receiving phase of the sampler; hence, ideally, Chemcatchers equipped with a diffusion-limiting membrane are used (Shaw and Mueller, 2009). The research question was whether, in combination with the $\text{SPEAR}_{\text{pesticides}}$ index, membrane-covered Chemcatchers would perform just as well as their uncovered counterparts (see Schäfer et al., 2008). The ecosystem process of leaf litter decomposition, which is associated with benthic community structure, was also assessed (see Gessner and Chauvet, 2002; Young et al., 2008).

In the second study (see CHAPTER 3), pesticides from WWTP effluents were quantified and related to macroinvertebrate community structure in receiving waters, using membrane-covered Chemcatchers and $\text{SPEAR}_{\text{pesticides}}$. Treated wastewater transport a wide range of anthropogenic chemicals into receiving waters, among them pesticides (Le et al., 2017; Neale et al, 2017). Previously, ecological effects on stream macroinvertebrates caused by pesticides from WWTP effluents were derived using the modelled pesticide runoff potential (Bunzel et al., 2013). The research question was whether the combination of membrane-covered Chemcatchers and the $\text{SPEAR}_{\text{pesticides}}$ index would detect in-stream exposure and effects of effluent-borne pesticides. Event-driven samplers were used complementarily for the quantification of peak pesticide concentrations. Furthermore, the ecosystem process of leaf litter decomposition, which is associated with benthic community structure, was also assessed (see Gessner and Chauvet, 2002; Young et al., 2008).

Finally, in the third study (see CHAPTER 4), the influence of forested headwaters on pesticide-impacted macroinvertebrate communities was assessed using membrane-covered Chemcatchers and $\text{SPEAR}_{\text{pesticides}}$. Previously, several relevant studies indeed showed higher proportions of taxa vulnerable to pesticides in the presence of forested upstream reaches (e.g., Bunzel et al., 2014; von der Ohe and Goedkoop, 2013); however, the underlying mechanisms had remained unclear. The research question was whether the combination of membrane-covered Chemcatchers and the $\text{SPEAR}_{\text{pesticides}}$ index would identify the specific forest characteristics responsible for the recovery of downstream macroinvertebrate communities from pesticide impact. Event-driven samplers were used complementarily for the quantification of peak pesticide concentrations.

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CHAPTER 2

Pesticide impact on aquatic invertebrates identified with Chemcatcher® passive samplers and the SPEAR$_{pesticides}$ index

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Abstract

Pesticides negatively affect biodiversity and ecosystem function in aquatic environments. In the present study, we investigated the effects of pesticides on stream macroinvertebrates at 19 sites in a rural area dominated by forest cover and arable land in Central Germany. Pesticide exposure was quantified with Chemcatcher® passive samplers equipped with a diffusion-limiting membrane. Ecological effects on macroinvertebrate communities and on the ecosystem function detritus breakdown were identified using the indicator system $\text{SPEAR}_{\text{pesticides}}$ and the leaf litter degradation rates, respectively. A decrease in the abundance of pesticide-vulnerable taxa and a reduction in leaf litter decomposition rates were observed at sites contaminated with the banned insecticide Carbofuran (*Toxic Units* ≥ -2.8), confirming the effect thresholds from previous studies. The results show that Chemcatcher® passive samplers with a diffusion-limiting membrane reliably detect ecologically relevant pesticide pollution, and we suggest Chemcatcher® passive samplers and $\text{SPEAR}_{\text{pesticides}}$ as a promising combination to assess pesticide exposure and effects in rivers and streams.

Keywords: Pesticides; Carbofuran; Passive sampling; Chemcatcher®; Diffusion-limiting membrane; Macroinvertebrates; SPEAR; Leaf litter degradation

1 Introduction

Freshwater ecosystems are affected by a multitude of human activities leading to catchment disturbance and water pollution, with pesticides, nutrients, polycyclic aromatic hydrocarbons (PAH), and brominated flame retardants being the most important contaminants (Malaj et al., 2014; Sarriquet et al., 2006; UNEP, 2010; Vörösmarty et al., 2010). Surface water pollution leads to adverse effects on the benthic fauna (Liess and von der Ohe, 2005; Ippolito et al., 2015) and on ecosystem functions (Bowmer, 2013; Peters et al., 2013). Field studies on pesticide exposure and community-level effects that include more than one water body have been performed by Liess and von der Ohe (2005) and by Schäfer et al. (2012), but such studies are generally rare (Beketov and Liess, 2012). This scarcity of investigations stems from the challenging nature of pesticide exposure assessment at the ecosystem level. Edge-of-field runoff, the dominant non-point source entry route for pesticides into surface waters, occurs in pulses and leads to short-term contamination (Liess and Schulz, 1999; Wauchope, 1978). Furthermore, pesticide loads in running waters are subject to (i) the pesticide amounts applied to fields, (ii) the timing and intensity of rain, (iii) the pesticide-specific octanol-water partition coefficient K_{OW} (Bach et al., 2000; Burgoa and Wauchope, 1995; Kreuger and Törnqvist, 1998; Neumann et al., 2002; Schulz, 2004), and (iv) the heterogeneous soil hydrology at the catchment level (Doppler et al., 2012; Freitas et al., 2008; Leu et al., 2004).

In an attempt to reduce the cost and complexity of surface water monitoring, a range of passive samplers has been introduced recently (Greenwood et al., 2007a). These samplers can be deployed for an integrative sampling of pesticides in the water phase, providing less variable data in much shorter times and at much lower monetary expenses (Allan et al., 2009; Gunold et al., 2008; Kot et al., 2000; Kreuger, 1998; Schäfer et al., 2011, 2008b; Shaw and Mueller, 2009; Stephens et al., 2005; Vrana et al., 2005). However, passive samplers have rarely been used to capture short-term pollution events, such as pesticide input via edge-of-field runoff (Fernandez et al., 2014; Greenwood et al., 2007a).

In addition to metal species, most passive samplers are designed to monitor non-polar organic compounds (Lohmann et al., 2012; Schulze et al., 2011; Vrana et al., 2005). However, many currently used pesticides are polar and semi-polar compounds (Jansson and Kreuger, 2010). The Chemcatcher® passive sampler, hereafter referred to as Chemcatcher, is one of two passive samplers designed to monitor polar organic compounds in surface waters and can be configured with or without a diffusion-limiting membrane overlaying the receiving sorbent phase (Greenwood et al., 2007b; Schäfer et al., 2008a; Stephens et al., 2005). Comparing these two Chemcatcher configurations in a mesocosm experiment, Schäfer et al. (2008a) proposed the use of ‘naked’ receiving sorbent phases, i.e., Chemcatchers without a diffusion-limiting membrane, when monitoring short-term contaminations. Moreover, Schäfer et al. (2008b) reported community-level responses using ‘naked’ receiving sorbent phases in the only field study with Chemcatchers using an ecological endpoint (macroinvertebrate community structure; $\mathrm{SPEAR}_{\mathrm{pesticides}}$).

Chemcatchers without protecting membranes have a shorter response time, i.e., the analyte uptake commences instantly after exposure. However, for exposure periods longer than a week at ambient temperatures above approx. 10 °C, considerable biofouling occurs directly on the receiving phase, which can alter the uptake characteristics of the sampler. Another problem can arise through a larger influence of (changing) hydrodynamic conditions on the exchange surface of the sampler. From their uptake simulation study, Shaw and Mueller (2009) concluded that Chemcatchers should be exposed with a membrane, as they predict time-weighted average concentrations closely when deployed beyond the lag period of several hours.

At the beginning of our study, a newly designed Chemcatcher body became available (Greenwood et al., 2007b; Lobpreis et al., 2008) in which the depth of the cavity at the 'sampler face' is reduced from 20 to 5 mm to increase sampling rates (decrease the sampler response time). With this new sampler design, the use of a diffusion-limiting membrane appeared advisable (i) to balance the samplers' sensitivity (short response time) with a reduction in the impact of hydrodynamics on the sampling rates (comparable results) and (ii) to extend the time of exposure until pesticides reach the distribution equilibrium between the water and the receiving phase. This last reason is evident based on the evaluation of time-weighted average concentrations (c_{TWA}) using sampling rates because this approach requires linear uptake kinetics throughout exposure. On the basis of the performance of Chemcatchers without a diffusion-limiting membrane (Gunold et al., 2008; Stephens et al., 2005), the advanced properties of the latest Chemcatcher version, and the suggestion by Shaw and Mueller (2009) to use a diffusion-limiting membrane, we hypothesised that Chemcatchers equipped with a diffusion-limiting membrane are also capable of detecting ecologically relevant pesticide concentrations.

The stressor specific $\mathrm{SPEAR}_{\mathrm{pesticides}}$ index reliably uncovers community-level responses to pesticide stress (Liess et al., 2008; Liess and von der Ohe, 2005; Schäfer et al., 2012, 2008b, 2007; Schletterer et al., 2010; von der Ohe and Goedkoop, 2013). Complementary to structural integrity (e.g., macroinvertebrate community composition), the inclusion of functional integrity (e.g., ecosystem processes) into the assessment of stream ecosystem health has been suggested repeatedly (Bunn and Davies, 2000; Gessner and Chauvet, 2002; Rasmussen et al., 2012a; Woodward et al., 2012). We chose the $\mathrm{SPEAR}_{\mathrm{pesticides}}$ index and the shredder feeding guild (Cummins, 1973) for the evaluation of the pesticide impact and the ecosystem function 'leaf litter degradation', respectively. Using ecosystem-level endpoints (USEPA, 2003), the present study sought to establish the 'shielded' Chemcatcher, i.e., the membrane-equipped version, as a reliable alternative pesticide monitoring tool for future investigations into the effects of pesticides on aquatic biota.

2 Materials and Methods

2.1. Study area

The present study was conducted in the Bode river catchment in Central Germany (SI Fig. S1). The area is part of the TERENO Harz/Central German Lowland Observatory (TERENO, 2011). The sampling sites were located in rural areas of the Harz and Börde regions dominated by forest and arable land. The potential for pesticide contamination of the investigated rivers was classified as low to medium in a previous study (Kattwinkel et al., 2011). The most important crops are cereal (wheat, barley, rye; approx. 60%) and rapeseed (approx. 20%; STALA, 2011).

In total, the samples were taken from 19 sites in 6 perennial rivers (Bode, 6 sites; Eine, 2; Mulde, 1; Selke, 5; Wipper, 3; Ziethe, 2) exposed to diffuse pesticide input from adjacent agricultural fields. The streams were of Strahler stream orders 1 to 5 and were between 5 and 10 m wide, except for the Mulde, with a width of approx. 40 m. Any neighbouring sampling sites along the same water body were at least 3 km apart, with the exception of the two Ziethe sites. Here, the downstream sampling site was affected by a wastewater effluent discharge and was therefore distinctly different from the upstream site despite the close proximity. Sampling was conducted 1 to 6 m off the river bank, in terms of the stream width, at a depth of 0.3 to 0.6 m. The prevailing current velocities ranged from 0.05 to 0.59 m s^{-1}. All sampling sites featured soft- and hard-bottom substrates in varying proportions, and hard substrate patches (i.e., gravel) were chosen for the macroinvertebrate sampling to ensure comparability. Coarse particulate organic matter (CPOM) and algae were found frequently (SI Table S1). Additional abiotic characteristics considered in the present study, such as pH, oxygen content, and total organic carbon (TOC), were recorded by the governmental agency for flood protection and water management in Saxony-Anhalt (LHW) and are shown in SI Table S2.

2.2. Pesticide measurements

Pesticide measurements in the streams were performed using Chemcatchers (Kingston et al., 2000) in the polar configuration (Stephens et al., 2005), but with the disposable sampler housing made from polycarbonate (2nd generation; Greenwood et al., 2007b). Duplicate samplers were deployed in June 2010 for 2 weeks during the peak pesticide application period when there was a high chance of a heavy rainfall event according to the weather forecast. Trip blanks were used to detect possible background contamination of the passive samplers originating from the preparation process or from field sources other than the respective surface waters.

Time-weighted average concentrations (c_{TWA}) of pesticides in the water phase were calculated from a generic sampling rate for all target compounds (Escher et al., 2011) and were corrected for different hydrodynamic conditions using the average flow velocity at each site. The average flow velocities of the investigated streams were estimated from the gypsum depletion in passive flow monitors (PFM; O'Brien et al., 2009) using the calibration function from the work of O'Brien et al. (2011), which was verified in our laboratory. For details on the sampling rate evaluation of the different target compounds, see the supplementary information (SI Text S1).

Chemcatchers were the sampling devices of choice for this study because they reliably detect polar and semi-polar pesticides in surface waters (Schäfer et al., 2008a, 2008b; Shaw et al., 2010; Stephens et al., 2005). An SDB-RPS Empore disk modified with sulfonic acid groups was applied as receiving phase to detect hydrophilic and semi-hydrophilic chemicals with log $K_{\mathrm{OW}} < 4$ (Gunold et al., 2008; Moschet et al., 2015; Sánchez-Bayo & Hyne, 2014; Stephens et al., 2005; Vermeirssen et al., 2008). An overlaying diffusion-limiting membrane made from hydrophilic polyethersulfone (PES) with a pore size of 0.2 µm was used.

2.3. Chemicals and reagents

The methanol and acetone used for the solvent extraction of SDB-RPS Empore disks were GC grade solvents from Merck (Darmstadt, Germany). For cleaning laboratory instruments and passive sampler housings, analytical grade methanol and acetone were used as was sodium sulphate ($NaSO_4$) for sample preparation (Merck, Darmstadt, Germany). The standard chemicals and deuterated standards (Pirimicarb-d_6 and Alachlor-d_{13}) added to the samplers as performance reference compounds are listed in SI Table S3. Ammonium formate (Fluka, Sigma-Aldrich, Seelze, Germany), water (J.T. Baker, Griesheim, Germany), and methanol (Merck, Darmstadt, Germany), all of LC-MS grade quality, were used for LC-MS measurements.

2.4. Preparation, clean-up and extraction of Chemcatcher passive samplers

Chemcatchers with polycarbonate (2nd generation) housing provided by the University of Portsmouth (Portsmouth, UK) were equipped with three machine screws each to ensure watertight fastening of the upper and lower parts (SI Fig. S2). This minor technical adjustment proved necessary to prevent water from circumventing the PES membrane during exposure. Prior to preparation, the Chemcatcher bodies were soaked in Decon 90 overnight and washed in a laboratory dishwasher, followed by the removal of detergent residues with methanol. An Empore SDB-RPS (styrene-divinylbenzene reversed-phase sulfonate) extraction disk from Varian (Walton-on-Thames, UK) was soaked in methanol overnight, kept in distilled water afterwards to prevent it from drying, and then placed on the lower part of the Chemcatcher housing as the receiving phase. A Supor® 200 PES diffusion-limiting membrane from Pall Corporation (Port Washington, NY, USA) was soaked in methanol overnight and rinsed with distilled water. Then, the PES membrane was placed over the pre-soaked SDB-RPS disk and fixed on the upper part of the Chemcatcher housing. After fastening the receiving phase and membrane between the housing parts using three machine screws, 1 mL of distilled water was poured on top of the membrane to prevent drying of the conditioned passive sampling devices during storage and transport to the sites. The housings were closed with the cap and wrapped in aluminium foil prior to exposure in the field. At each site, the Chemcatchers were fixed with cable ties to a metal lattice (face downwards) approx. 0.1 - 0.2 m above the stream bed to ensure that the samplers did not fall dry during the period of deployment.

After 14 days of exposure, the Chemcatcher bodies were retrieved from the streams, capped and wrapped in aluminium foil. During transport and until processing in the laboratory, the samplers were refrigerated at 4 °C. For extraction of the receiving phase, the Chemcatcher body was dismantled, and the PES membrane was discarded. Then, the SDB-RPS disk was extracted with 5 mL methanol for 15 min in an ultrasonic bath, followed by a 15 min extraction with 5 mL acetone and again with 5 mL of a methanol:acetone mixture (1:1). The extracts were combined, and 50 µL of the internal standard hexachlorobenzene-$^{13}C_6$ (10 ng µL^{-1} in methanol) was added to correct for analyte loss during evaporation. The extract was passed through a glass solid phase extraction cartridge filled halfway with sodium sulphate ($NaSO_4$) to remove any traces of water. At the outlet of the cartridge, the extract was filtered through a Minisart® SRP4 syringe filter from Sartorius (Göttingen, Germany), employing a polypropylene-reinforced poly

(1,1,2,2-tetrafluoroethylene) (PTFE) membrane with a pore size of 0.45 µm. All sampler extracts were reduced to approximately 400 µL using a Turbovap 2 evaporation system from Biotage (Uppsala, Sweden). After the addition of 50 µL of the internal standard Biphenyl-D10 (10 ng µL^{-1} in methanol), the extract was adjusted to 500 µL and split into two 2 mL crimp-top vials with 250 µL glass inserts from Agilent (Waldbronn, Germany).

2.5. Instrumental analysis setup

All pesticides were quantified on an Agilent 1100 liquid chromatograph (Waldbronn, Germany) coupled with an API 2000 tandem mass spectrometer from AB Sciex (Darmstadt, Germany). Chromatographic separation was conducted with an HPLC column ‘Aqua’ (C_{18}, 50 mm x 2mm i. d., 5 µm particle size, 12.5 nm pores; Phenomenex, Aschaffenburg, Germany). The injection volume was 5 µL; prior to injection, the samples and standards in the methanol were mixed with HPLC water in equal parts. Methanol and water containing 2 mmol ammonium formate were used as solvents for gradient elution (SI Table S4). Mass spectral analysis was performed with positive electrospray ionisation and using multiple reaction monitoring mode. The respective ion transitions for each compound and the instrumental conditions used are shown in SI Table S5.

2.6. Exposure quantification – calculation of Toxic Units (TU)

The use of *TU* allows for a comparison of toxicity levels among different sampling sites (see EC, 2012). First, c_{TWA} were calculated from the pesticide amounts found in the passive samplers based on the flow velocity monitored with PFM, as described in detail in the supplementary information (SI Text S1). Then, individual *TU* values were calculated for all compounds according to the acute (48 h) LC_{50} for the most sensitive standard test organisms ($TU_{\mathrm{sensitive}}$), *Daphnia* spec. and *Chironomus* spec. (EFSA, 2013). We compared the LC_{50} values from three data sources: the TU Calculator (part of the SPEAR Calculator desktop application; UFZ, 2014a), the Pesticide Properties Database (PPDB; University of Hertfordshire, 2014), and the ECOTOX Database (USEPA, 2014). The TU Calculator offers median LC_{50} values for *Daphnia magna* on the basis of ECOTOX data (USEPA, 2014). In case the LC_{50} values for *D. magna* in the PPDB (University of Hertfordshire, 2014) were lower than the median values in the ECOTOX database, they were preferred only if classified as ‘EU Regulatory & Evaluation Data (A)’ or if originating from ‘UK CRD and ACP

Evaluation Documents (B)'; additionally, only the highest data confidence levels 5 ('Verified data used for regulatory purposes') and 4 ('Verified data') were considered. In the case of Isoproturon, the lowest acute (48 h) LC_{50} for *D. magna* was given by LANUV (2014). Acute toxicity data on *Chironomus* spec. were obtained from ECOTOX (USEPA, 2014). In the case of neonicotinoid insecticides, a reference organism other than *Daphnia* spec. is strongly suggested (Beketov and Liess, 2008; Brock and Van Wijngaarden, 2012; Schäfer et al., 2013). As toxicity data from standard tests appear to be rare for this class of insecticides, computing the median acute (48 h) LC_{50} from five tested neonicotinoids (Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, and Thiamethoxam) was considered appropriate. Here, the standard test organism was *Chironomus* spec. (SI Table S6).

The highest TU value from each sampling site ($TU_{\max}$) was used to quantify pesticide-induced water toxicity at the respective sites (Liess and von der Ohe, 2005). A log-transformation was chosen to provide a suitable resolution for illustration purposes (Eq. 1):

$$TU = \max_{i=1}^{n}\left[\log\left(\frac{c_i}{LC_{50_i}}\right)\right] \tag{1}$$

where TU is the highest value of n pesticides detected at each sampling site ($TU_{\max}$), c_i is the concentration (µg L^{-1}) of the respective pesticide i, and LC_{50i} is the pesticide's corresponding median lethal concentration (48 h, µg L^{-1}) for the chosen reference organism.

Pesticide input from agricultural field runoff occurs in pulses rather than continuously (Liess et al., 1999). We estimated peak exposure concentrations with regards to the results of Schäfer et al. (2008b), who reported a Chemcatcher performance comparable to that of event-driven water samplers (EDS) with respect to the exposure and effect assessment. In that study, c_{TWA} were approximately one-fifth of EDS concentrations. Applying the factor of 5 to all TU values, we derived estimated pulse water concentrations from the passive sampler data, making our effect assessment comparable to the results of studies using EDS (e.g., Bereswill et al., 2013; Liess and von der Ohe, 2005; Schäfer et al., 2007). Equation 1 was modified appropriately (Eq. 2):

$$TU = \max_{i=1}^{n}\left[\log\left(\frac{c_i * 5}{LC_{50_i}}\right)\right] \tag{2}$$

Comparing the results of studies using different sampling techniques, we were able to evaluate the performance of the Chemcatchers in the present study. Moreover, we linked the ecological relevant concentrations to the ecological effects observed.

2.7. Invertebrate sampling, leaf litter breakdown, and environmental parameters

Stream macroinvertebrates were sampled in June 2010 immediately after the deployment period of the passive samplers. The benthos was collected with a Surber sampler (32 x 32 cm; mesh size: 500 µm). Before sampling, the types of stream substrates were assessed following a field protocol described in the river assessment manual (Meier et al., 2006). Three replicate samples were taken from each site.

The sampled benthos contents were immediately washed through a test sieve system (mesh sizes: 8 mm, 4 mm, 2 mm, and 500 µm; Retsch GmbH, Haan, Germany) and collected in white deep draw trays. All visible specimens from the larger sieve fractions (8 mm and 4 mm) were individually transferred into plastic containers and preserved with 70% ethanol. The smaller sieve fractions (2 mm and 500 µm) were completely transferred into plastic containers and preserved in 90% ethanol because of their higher relative water content. Identification of the macroinvertebrates at the family level (see Beketov et al., 2009) was carried out using a stereo-microscope (Carl Zeiss, Germany, Stemi 2000; magnification: 50 x) and identification keys for *Amphipoda*, *Bivalvia*, *Gastropoda*, *Hirudinea*, *Isopoda*, *Oligochaeta*, *Phyllopoda* (Stresemann et al., 1992), *Coleoptera* (Bouchard, 2004), *Diptera* (Sundermann and Lohse, 2006), *Ephemeroptera* (Elliott and Humpesch, 2010), *Heteroptera* (Savage, 1989), *Odonata* (Theischinger and Endersby, 2009), *Plecoptera* (Brittain and Saltveit, 1996), and *Trichoptera* (Edington and Hildrew, 1995; Wallace et al., 2003).

To measure leaf litter breakdown, 3 g of loose dried birch leaf mass were placed in fine (nylon) and coarse (polyethylene) mesh cases. Each fine mesh case, serving as control for microbial induced degradation, was then put into a coarse mesh case whose enclosed leaf mass was accessible to shredder invertebrate taxa. Triplicate coarse mesh cases were deployed for approx. 21 days alongside the passive samplers. The correction for handling losses was done using additionally prepared mesh cases that were only very briefly deployed in the streams (see also Benfield, 1996). After retrieval, the leaves were carefully extracted from all mesh cases, gently cleaned with tap water, and dried for 4 days at 60 °C to a constant weight, using a drying oven (Type UT 6060, Heraeus Instruments GmbH, Hanau, Germany). At 6 sites (rivers Bode, Eine, Selke, and Ziethe), the mesh cases were either washed away or damaged during the time of deployment. At another 3 sites (Wipper), satisfactorily cleaning the leaf mass from infiltrated soft sediment after retrieval was impossible, and the loss of leaf mass could not be determined. Using data from the 10 remaining sampling sites (rivers Bode, Mulde, Selke, and Ziethe), the leaf litter breakdown rate coefficient k was calculated for each site (Eq. 3):

$$k = \frac{-\ln\left(\frac{W_t}{W_0}\right)}{t} \tag{3}$$

where W_t is the remaining leaf mass (sum of the weight remainder from the coarse mesh case and the weight loss from the respective fine mesh case), W_0 is the initial leaf mass, and t is the time of deployment.

Additionally, we used physicochemical parameters from environmental monitoring data provided by the government agency for flood protection and water management in Saxony-Anhalt (LHW), whose monitoring sites are located in close proximity to our sampling sites. The following parameters were selected: temperature, pH, electrical conductivity (EC), total dissolved oxygen (TDO), total organic carbon (TOC), NH_4, NO_2, NO_3, PO_4, and water hardness (SI Table S2).

2.8. Assessment of ecological effects and data analyses

At any sampling site, the natural diversity of the respective biological community is reflected by a specific combination of environmental parameters and inhabiting taxa that can obscure the effects of pesticides (Liess et al., 1999). Disentanglement of the effects of pesticides from those of other stressors can be achieved with the bio-indicator $\text{SPEAR}_{\text{pesticides}}$ (Species At Risk), which was developed to differentiate between the effects of pesticides and those of other environmental factors (Liess and von der Ohe, 2005) and has repeatedly linked pesticide contamination to changes in aquatic communities because of its independence from other stressors (Liess et al., 2008; Liess and von der Ohe, 2005; Schäfer et al., 2012, 2007; Schletterer et al., 2010).

The $\text{SPEAR}_{\text{pesticides}}$ index is based on a dichotomic classification of aquatic taxa into ‘species at risk’ and ‘species not at risk’ on the grounds of specific characteristics (for details, see Liess and von der Ohe, 2005). The $\text{SPEAR}_{\text{pesticides}}$ values in the present study were calculated using the publicly available SPEAR Calculator desktop application (UFZ, 2014a; Eq. 4).

$$\text{SPEAR}_{\text{pesticides}} = \frac{\sum_{i=1}^{n} \log(x_i + 1)\, y}{\sum_{i=1}^{n} \log(x_i + 1)} 100 \tag{4}$$

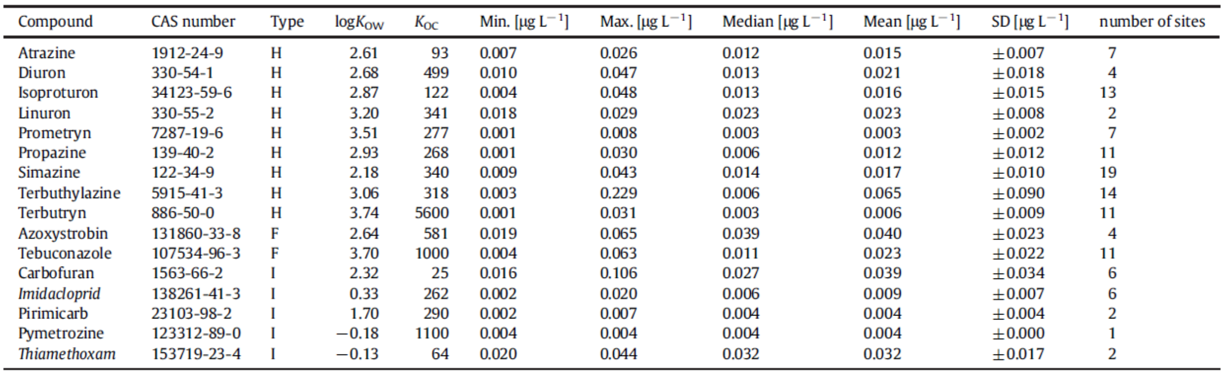
where *n* is the number of taxa, x_i is the abundance of taxon *i* (given as individuals m^{-2}) and y is 1 if taxon *i* is classified as ‘at risk’ and 0 otherwise. The SPEAR values obtained were then plotted against the respective *TU* values.

Correlation models were established for the relationships between $\text{SPEAR}_{\text{pesticides}}$ and *TU* and between *k* and $\text{SPEAR}_{\text{pesticides}}$, respectively. To further assess the effects of pesticides on *k*, we assigned a functional feeding group (scrapers, collectors, and shredders), according to Cummins et al. (2005), Cummins (1973) and USEPA (2012), to each macroinvertebrate family and analysed the relationship between *k* and the site-specific proportion of shredder taxa. Multiple linear regression analyses (stepwise backward elimination and stepwise forward selection) were used to identify the predictive variables among all environmental factors. For the identification of the pesticide effect threshold and the leaf litter decomposition in respect thereof (see 3.2., Effect assessment), additional statistical significance analyses were carried out. Initially, we checked for normal distribution using Shapiro-Wilk’s normality test and for homoscedasticity of residuals using Levene’s homogeneity-of-variance test. If data were normally distributed, Welch’s t-test (corrected for non-homogeneity of variance) was performed; otherwise, Wilcoxon’s rank sum test was used. The open source statistics software applications RStudio for Windows (version 0.98.1028; RStudio, 2014) and R for Windows (version 3.0.3; R Core Team, 2014) were used for the computations and graphics.

3 Results

3.1. Exposure assessment

The sampler concentrations from the 19 sites in central Germany were converted into water toxicity levels and successfully linked to macroinvertebrate compositions. We detected 16 of the 25 measured compounds, with 9 herbicides, 2 fungicides and 5 insecticides present in various mixtures of up to 15 compounds per sample (Tables 1 and 2). The most toxic compounds per sample were the following: Carbofuran (6 sampling sites; mean *TU* = -2.5), Imidacloprid (2; -3.7), Pirimicarb (1; -4.0), Atrazine (2; -4.7), Isoproturon (1; -4.7), and Simazine (7; -5.0). Herbicide-dominated pesticide mixtures led to lower site-specific TU_{max} values (-5.1 to -4.7), whereas the presence of insecticides caused higher TU_{max} values (-4.0 to -1.9; Table 3 and Fig. 1).



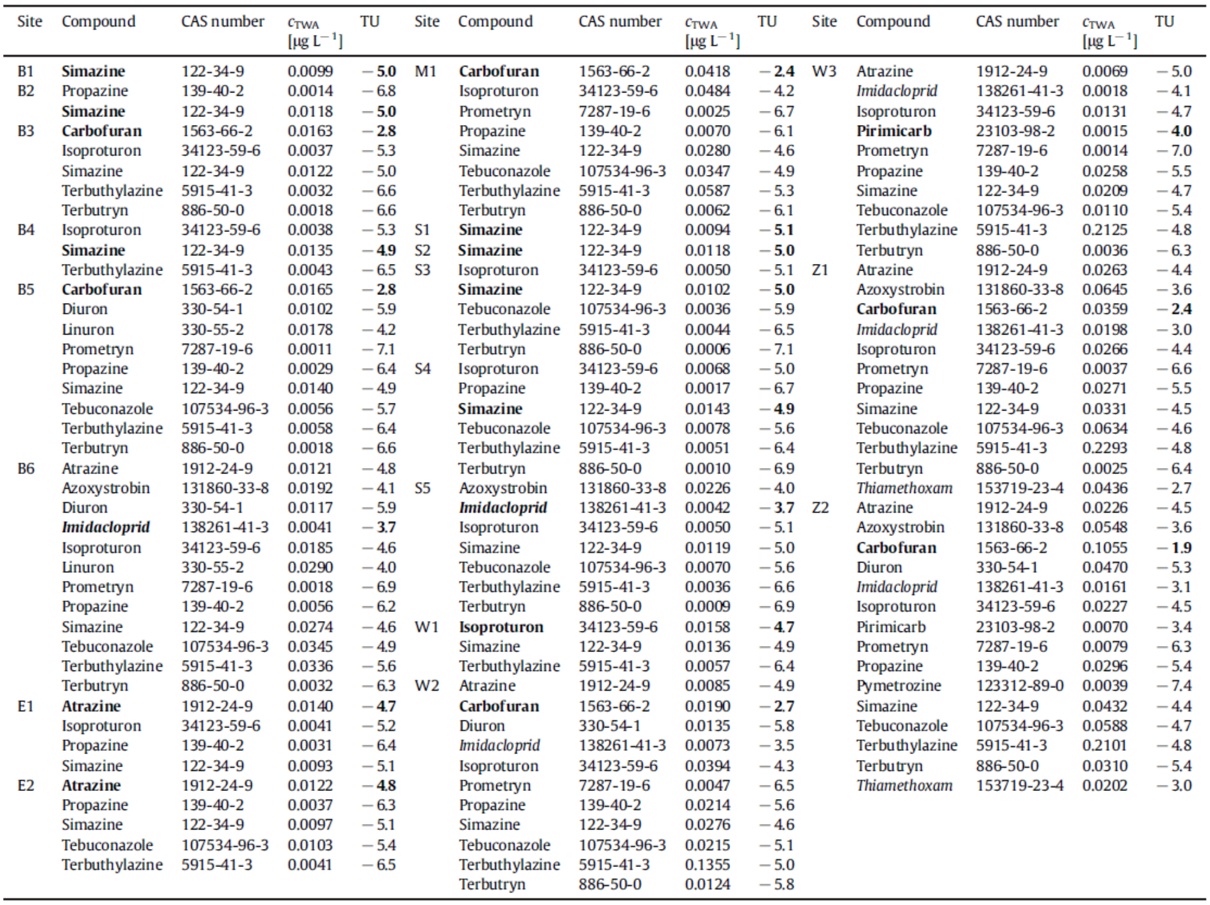
Compound	CAS number	Type	$\log K_{OW}$	K_{OC}	Min. [μg L^{-1}]	Max. [μg L^{-1}]	Median [μg L^{-1}]	Mean [μg L^{-1}]	SD [μg L^{-1}]	number of sites
Atrazine	1912-24-9	H	2.61	93	0.007	0.026	0.012	0.015	±0.007	7
Diuron	330-54-1	H	2.68	499	0.010	0.047	0.013	0.021	±0.018	4
Isoproturon	34123-59-6	H	2.87	122	0.004	0.048	0.013	0.016	±0.015	13
Linuron	330-55-2	H	3.20	341	0.018	0.029	0.023	0.023	±0.008	2
Prometryn	7287-19-6	H	3.51	277	0.001	0.008	0.003	0.003	±0.002	7
Propazine	139-40-2	H	2.93	268	0.001	0.030	0.006	0.012	±0.012	11
Simazine	122-34-9	H	2.18	340	0.009	0.043	0.014	0.017	±0.010	19
Terbuthylazine	5915-41-3	H	3.06	318	0.003	0.229	0.006	0.065	±0.090	14
Terbutryn	886-50-0	H	3.74	5600	0.001	0.031	0.003	0.006	±0.009	11
Azoxystrobin	131860-33-8	F	2.64	581	0.019	0.065	0.039	0.040	±0.023	4
Tebuconazole	107534-96-3	F	3.70	1000	0.004	0.063	0.011	0.023	±0.022	11
Carbofuran	1563-66-2	I	2.32	25	0.016	0.106	0.027	0.039	±0.034	6
Imidacloprid	138261-41-3	I	0.33	262	0.002	0.020	0.006	0.009	±0.007	6
Pirimicarb	23103-98-2	I	1.70	290	0.002	0.007	0.004	0.004	±0.004	2
Pymetrozine	123312-89-0	I	−0.18	1100	0.004	0.004	0.004	0.004	±0.000	1
Thiamethoxam	153719-23-4	I	−0.13	64	0.020	0.044	0.032	0.032	±0.017	2

Table 1 Pesticides detected in the present study. CAS: Chemical Abstracts Service; KOW: octanol–water partitioning coefficient; KOC: soil organic carbon–water partitioning coefficient; SD: standard deviation; H: herbicide; F: fungicide; I: insecticide; italics: neonicotinoid.
Chemical properties according to UFZ (2014b), Sangster (2013) and University of Hertfordshire (2014).

3.2. Effect assessment

At the investigated sites, 65 families of aquatic macroinvertebrates were identified, with a mean of 23 families per site (Table 4). The $\text{SPEAR}_{\text{pesticides}}$ values ranged from 4.22 to 62.33 and corresponded to the range of water quality classes from bad (< 11) to high (≥ 44) according to the EU Water Framework Directive (WFD; Beketov et al., 2009). $\text{SPEAR}_{\text{pesticides}}$ was significantly negatively correlated with $TU_{\text{sensitive}}$ (linear regression, $p < 0.001$, adjusted $R^2 = 0.59$; Fig. 1). The ecological effects on the community composition expressed by $\text{SPEAR}_{\text{pesticides}}$ were significant at $TU \geq -2.8$ (Wilcoxon's rank sum test, $p < 0.01$). The leaf litter decomposition rate *k* ranged from 0.004 to 0.044 d^{-1} and correlated significantly with $TU_{\text{sensitive}}$ (linear regression, $p < 0.05$, adjusted $R^2 = 0.32$; Fig. 2) and even more significantly with $\text{SPEAR}_{\text{pesticides}}$ (linear regression, $p < 0.01$, adjusted $R^2 = 0.53$; Fig. 3). However, the *k* values from sites with $TU \leq -2.8$ were not significantly different from the values from sites with $TU > -2.8$ (Wilcoxon's rank sum test, $p > 0.05$).

The stepwise backward elimination and the stepwise forward selection each produced 4 environmental variables deemed predictive for SPEAR$_{pesticides}$. While backward elimination determined $TU_{sensitive}$ (p = 0.003), oxygen content (p = 0.003), temperature (p = 0.02), and electrical conductivity (p = 0.03) to be predictive, forward selection identified $TU_{sensitive}$ (p < 0.001), NO_3 (p < 0.01), pH (p = 0.036), and stream depth (p = 0.043).



Site	Compound	CAS number	c_{TWA} [µg L^{-1}]	TU	Site	Compound	CAS number	c_{TWA} [µg L^{-1}]	TU	Site	Compound	CAS number	c_{TWA} [µg L^{-1}]	TU
B1	**Simazine**	122-34-9	0.0099	**−5.0**	M1	**Carbofuran**	1563-66-2	0.0418	**−2.4**	W3	Atrazine	1912-24-9	0.0069	−5.0
B2	Propazine	139-40-2	0.0014	−6.8		Isoproturon	34123-59-6	0.0484	−4.2		*Imidacloprid*	138261-41-3	0.0018	−4.1
	Simazine	122-34-9	0.0118	**−5.0**		Prometryn	7287-19-6	0.0025	−6.7		Isoproturon	34123-59-6	0.0131	−4.7
B3	**Carbofuran**	1563-66-2	0.0163	**−2.8**		Propazine	139-40-2	0.0070	−6.1		**Pirimicarb**	23103-98-2	0.0015	**−4.0**
	Isoproturon	34123-59-6	0.0037	−5.3		Simazine	122-34-9	0.0280	−4.6		Prometryn	7287-19-6	0.0014	−7.0
	Simazine	122-34-9	0.0122	−5.0		Tebuconazole	107534-96-3	0.0347	−4.9		Propazine	139-40-2	0.0258	−5.5
	Terbuthylazine	5915-41-3	0.0032	−6.6		Terbuthylazine	5915-41-3	0.0587	−5.3		Simazine	122-34-9	0.0209	−4.7
	Terbutryn	886-50-0	0.0018	−6.6		Terbutryn	886-50-0	0.0062	−6.1		Tebuconazole	107534-96-3	0.0110	−5.4
B4	Isoproturon	34123-59-6	0.0038	−5.3	S1	**Simazine**	122-34-9	0.0094	**−5.1**		Terbuthylazine	5915-41-3	0.2125	−4.8
	Simazine	122-34-9	0.0135	**−4.9**	S2	**Simazine**	122-34-9	0.0118	**−5.0**		Terbutryn	886-50-0	0.0036	−6.3
	Terbuthylazine	5915-41-3	0.0043	−6.5	S3	Isoproturon	34123-59-6	0.0050	−5.1	Z1	Atrazine	1912-24-9	0.0263	−4.4
B5	**Carbofuran**	1563-66-2	0.0165	**−2.8**		**Simazine**	122-34-9	0.0102	**−5.0**		Azoxystrobin	131860-33-8	0.0645	−3.6
	Diuron	330-54-1	0.0102	−5.9		Tebuconazole	107534-96-3	0.0036	−5.9		**Carbofuran**	1563-66-2	0.0359	**−2.4**
	Linuron	330-55-2	0.0178	−4.2		Terbuthylazine	5915-41-3	0.0044	−6.5		*Imidacloprid*	138261-41-3	0.0198	−3.0
	Prometryn	7287-19-6	0.0011	−7.1		Terbutryn	886-50-0	0.0006	−7.1		Isoproturon	34123-59-6	0.0266	−4.4
	Propazine	139-40-2	0.0029	−6.4	S4	Isoproturon	34123-59-6	0.0068	−5.0		Prometryn	7287-19-6	0.0037	−6.6
	Simazine	122-34-9	0.0140	−4.9		Propazine	139-40-2	0.0017	−6.7		Propazine	139-40-2	0.0271	−5.5
	Tebuconazole	107534-96-3	0.0056	−5.7		**Simazine**	122-34-9	0.0143	**−4.9**		Simazine	122-34-9	0.0331	−4.5
	Terbuthylazine	5915-41-3	0.0058	−6.4		Tebuconazole	107534-96-3	0.0078	−5.6		Tebuconazole	107534-96-3	0.0634	−4.6
	Terbutryn	886-50-0	0.0018	−6.6		Terbuthylazine	5915-41-3	0.0051	−6.4		Terbuthylazine	5915-41-3	0.2293	−4.8
B6	Atrazine	1912-24-9	0.0121	−4.8		Terbutryn	886-50-0	0.0010	−6.9		Terbutryn	886-50-0	0.0025	−6.4
	Azoxystrobin	131860-33-8	0.0192	−4.1	S5	Azoxystrobin	131860-33-8	0.0226	−4.0		*Thiamethoxam*	153719-23-4	0.0436	−2.7
	Diuron	330-54-1	0.0117	−5.9		***Imidacloprid***	138261-41-3	0.0042	**−3.7**	Z2	Atrazine	1912-24-9	0.0226	−4.5
	Imidacloprid	138261-41-3	0.0041	**−3.7**		Isoproturon	34123-59-6	0.0050	−5.1		Azoxystrobin	131860-33-8	0.0548	−3.6
	Isoproturon	34123-59-6	0.0185	−4.6		Simazine	122-34-9	0.0119	−5.0		**Carbofuran**	1563-66-2	0.1055	**−1.9**
	Linuron	330-55-2	0.0290	−4.0		Tebuconazole	107534-96-3	0.0070	−5.6		Diuron	330-54-1	0.0470	−5.3
	Prometryn	7287-19-6	0.0018	−6.9		Terbuthylazine	5915-41-3	0.0036	−6.6		*Imidacloprid*	138261-41-3	0.0161	−3.1
	Propazine	139-40-2	0.0056	−6.2		Terbutryn	886-50-0	0.0009	−6.9		Isoproturon	34123-59-6	0.0227	−4.5
	Simazine	122-34-9	0.0274	−4.6	W1	**Isoproturon**	34123-59-6	0.0158	**−4.7**		Pirimicarb	23103-98-2	0.0070	−3.4
	Tebuconazole	107534-96-3	0.0345	−4.9		Simazine	122-34-9	0.0136	−4.9		Prometryn	7287-19-6	0.0079	−6.3
	Terbuthylazine	5915-41-3	0.0336	−5.6		Terbuthylazine	5915-41-3	0.0057	−6.4		Propazine	139-40-2	0.0296	−5.4
	Terbutryn	886-50-0	0.0032	−6.3	W2	Atrazine	1912-24-9	0.0085	−4.9		Pymetrozine	123312-89-0	0.0039	−7.4
E1	**Atrazine**	1912-24-9	0.0140	**−4.7**		**Carbofuran**	1563-66-2	0.0190	**−2.7**		Simazine	122-34-9	0.0432	−4.4
	Isoproturon	34123-59-6	0.0041	−5.2		Diuron	330-54-1	0.0135	−5.8		Tebuconazole	107534-96-3	0.0588	−4.7
	Propazine	139-40-2	0.0031	−6.4		*Imidacloprid*	138261-41-3	0.0073	−3.5		Terbuthylazine	5915-41-3	0.2101	−4.8
	Simazine	122-34-9	0.0093	−5.1		Isoproturon	34123-59-6	0.0394	−4.3		Terbutryn	886-50-0	0.0310	−5.4
E2	**Atrazine**	1912-24-9	0.0122	**−4.8**		Prometryn	7287-19-6	0.0047	−6.5		*Thiamethoxam*	153719-23-4	0.0202	−3.0
	Propazine	139-40-2	0.0037	−6.3		Propazine	139-40-2	0.0214	−5.6					
	Simazine	122-34-9	0.0097	−5.1		Simazine	122-34-9	0.0276	−4.6					
	Tebuconazole	107534-96-3	0.0103	−5.4		Tebuconazole	107534-96-3	0.0215	−5.1					
	Terbuthylazine	5915-41-3	0.0041	−6.5		Terbuthylazine	5915-41-3	0.1355	−5.0					
						Terbutryn	886-50-0	0.0124	−5.8					

Table 2 Pesticide concentrations detected in the present study. CAS: Chemical Abstracts Service; c_{TWA}: time-weighted average concentrations; bold: compounds providing TU_{max}; italics: neonicotinoid.

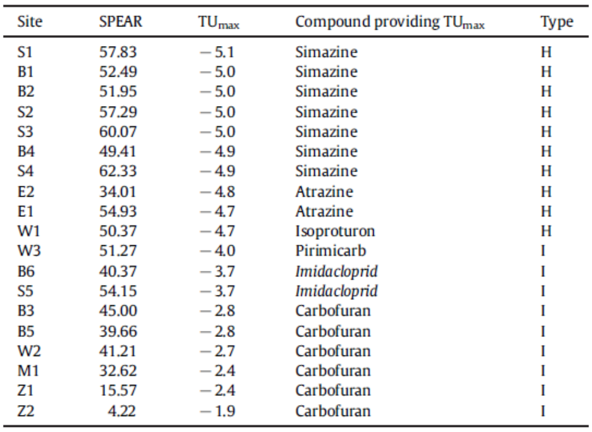
4 Discussion

Peak water phase concentrations are highly suitable for linking pesticide concentrations to ecological effects on aquatic communities (Bundschuh et al., 2014; Liess and von der Ohe, 2005; Schäfer et al., 2008b). However, the use of passive samplers and sediment samples has also been reported to be appropriate for this purpose (Schäfer et al., 2011, 2008b). Accumulating only the bioavailable pesticide fractions in the water phase, passive samplers provide a more realistic basis for the biological monitoring of surface waters, contrary to EDS that collect whole water samples. Moreover, passive samplers can detect runoff events in which water levels do not rise sufficiently high to trigger EDS (≥ 5 cm; Liess and von der Ohe, 2005). An additional benefit of using passive samplers (such as the Chemcatcher with the appropriate collecting phase) is their ability to preconcentrate target substances in-situ and, if a diffusion-limiting membrane is employed, over longer time periods. This feature enables the achievement of much lower limits of detection/quantification for many target analytes compared to conventional grab samples.

4.1. Exposure quantification validation

The concentrations obtained from passive samplers in our study were much lower than those obtained by EDS techniques in other studies (SI Tables S7, S8a - S8d). This finding is in accordance with previous investigations that showed the need for correction factors to enable a comparison of concentrations from passive samplers with those from EDS (Schäfer et al., 2008b, 2007). Our *TU* values calculated from Chemcatcher-based c_{TWA}, *multiplied by the factor of 5* derived by Schäfer et al. (2008b) with a similar deployment period (10 to 13 days), yielded a dose-response relationship similar to those reported in previous studies using EDS techniques to measure pesticide exposure (e.g., Bereswill et al., 2013; Liess and von der Ohe, 2005; Schäfer et al., 2007). In a study with a much longer deployment period (approx. 6 months), Bundschuh et al. (2014) reported a factor of 6 for the derivation of peak pesticide concentrations from time-proportional automated water samplers.

Remarkably, half of all detected pesticides had been banned in the EU by the year of the study (2010): Atrazine (authorisation, including the grace period, expired in 2007; mean $TU_{\text{max}} = -4.7$), Carbofuran (2008; -2.5), Diuron (2008; -5.7), Linuron (2004; -4.1), Prometryn (2007; -6.7), Propazine (2007; -6.1), Simazine (2007; -4.9), and Terbutryn (2007; -6.4; BVL, 2014; EC, 2007, 2004a, 2004b, 2002; PAN, 2013). In the case of the WFD priority substances Atrazine, Diuron, Simazine, and Terbutryn (EC, 2013, 2000), residues can still be found in the environment because of the nature of the historical treatment procedures as well as the chemical properties of the compounds (Hillenbrand et al., 2007). Moreover, these residues have been detected recently (e.g., HLUG, 2013). Another possible explanation for the presence of forbidden pesticides in agricultural surface waters is the use of unauthorised chemicals on fields (BMU, 2005). However, we did not investigate the origin of the compounds detected in the present study.



Site	SPEAR	TU_{max}	Compound providing TU_{max}	Type
S1	57.83	− 5.1	Simazine	H
B1	52.49	− 5.0	Simazine	H
B2	51.95	− 5.0	Simazine	H
S2	57.29	− 5.0	Simazine	H
S3	60.07	− 5.0	Simazine	H
B4	49.41	− 4.9	Simazine	H
S4	62.33	− 4.9	Simazine	H
E2	34.01	− 4.8	Atrazine	H
E1	54.93	− 4.7	Atrazine	H
W1	50.37	− 4.7	Isoproturon	H
W3	51.27	− 4.0	Pirimicarb	I
B6	40.37	− 3.7	*Imidacloprid*	I
S5	54.15	− 3.7	*Imidacloprid*	I
B3	45.00	− 2.8	Carbofuran	I
B5	39.66	− 2.8	Carbofuran	I
W2	41.21	− 2.7	Carbofuran	I
M1	32.62	− 2.4	Carbofuran	I
Z1	15.57	− 2.4	Carbofuran	I
Z2	4.22	− 1.9	Carbofuran	I

Table 3 TU values of the most toxic compounds and the respective SPEAR values, calculated from the field data collected.

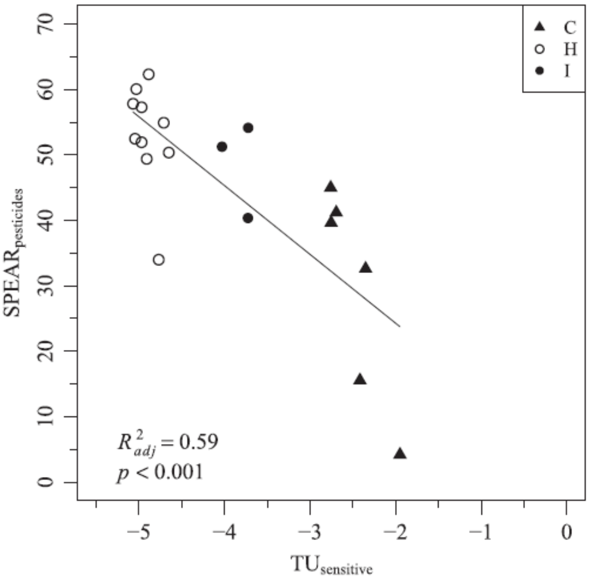
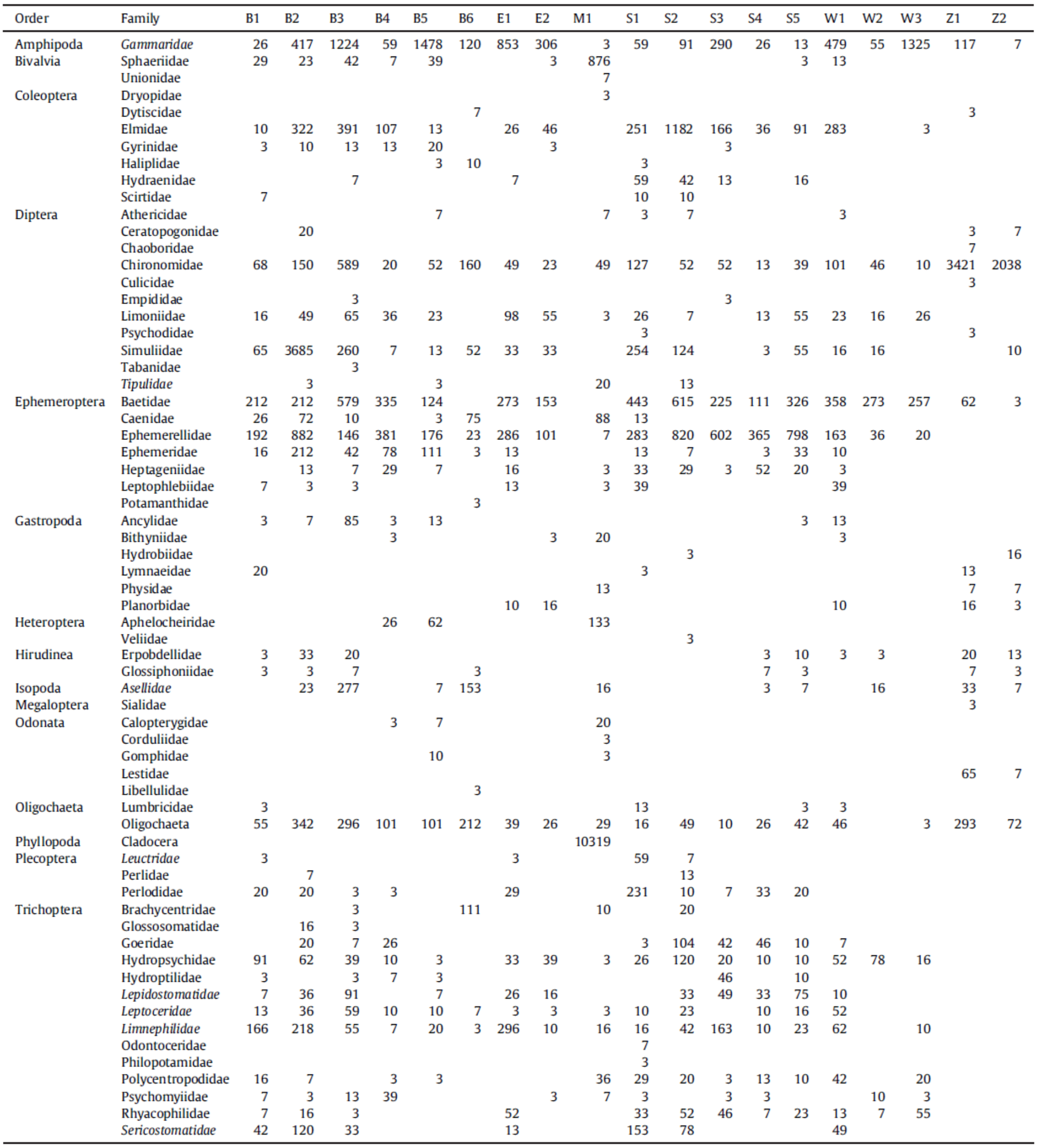


Fig. 1 Relationship between the structures of the stream macroinvertebrate assemblage expressed as SPEAR$_{pesticides}$ and the water toxicity at the sampling sites expressed as TU$_{sensitive}$. The linear regression is significant with $p < 0.001$ and adjusted $R^2 = 0.59$. C: Carbofuran; H: herbicide; I: insecticide other than Carbofuran (2× Imidacloprid and 1× Pirimicarb).

4.2. Concentration-response dependence and compounds responsible for the effects

The relationship between SPEAR and $TU_{sensitive}$ identified in the present study was similar to the dependencies reported in previous field studies with EDS applying SPEAR$_{pesticides}$ (Bereswill et al., 2013; Liess and von der Ohe, 2005; Rasmussen et al., 2011; Schäfer et al., 2007). Because of the lack of observations between $TU \leq -3.7$ (no ecological effects) and $TU \geq -2.8$ (impact of Carbofuran), the threshold for effective concentrations can be set to the mean of both values. This value, $TU = -3.3$, is between the previously established effect thresholds of $TU = -3.5$ (Beketov et al., 2009; Bereswill et al., 2013) and $TU = -3.0$ (Liess and von der Ohe, 2005; Schäfer et al., 2012, 2007). From the variables deemed predictive for SPEAR$_{pesticides}$ by stepwise backward elimination and stepwise forward selection, respectively, only $TU_{sensitive}$ was present in both sets of variables, even yielding the highest p value in each group. We concluded that $TU_{sensitive}$ was the driving force behind the change in community composition, i.e., decreased abundances of taxa vulnerable to pesticides (SPEAR). Carbofuran (6 sampling sites; mean $TU = -2.5$), Imidacloprid (2; -3.7), and Pirimicarb (1; -4.0) determined the water toxicity at sites with insecticides present ($TU \geq -4.0$), but only Carbofuran caused TU values above the aforementioned thresholds. Neonicotinoids are frequently found in surface waters (Kreuger et al., 2010; Main et al., 2014; Morrissey et al., 2015, Starner and Goh, 2012; Van Dijk et al., 2013), and Imidacloprid and Thiamethoxam were detected in the present study. While the Chemcatcher was not originally designed to collect compounds with log $K_{OW} < 2$ (Kingston et al., 2000), successful sampling of more polar compounds with an SDB-RPS Empore disk as the receiving phase was reported previously (Stephens et al., 2005; Tran et al., 2007; Vermeirssen et al., 2008), and compounds with log $K_{OW} < 2$ were also accumulated in quantifiable amounts by the Chemcatchers in the present study (Tables 1 and 2).

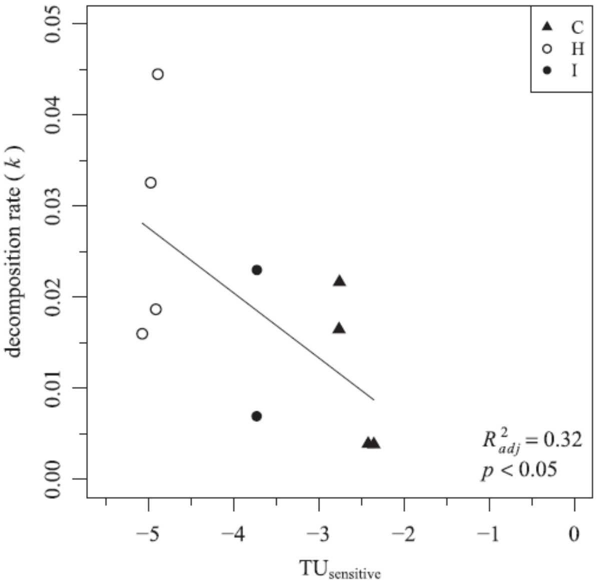


Order	Family	B1	B2	B3	B4	B5	B6	E1	E2	M1	S1	S2	S3	S4	S5	W1	W2	W3	Z1	Z2
Amphipoda	*Gammaridae*	26	417	1224	59	1478	120	853	306	3	59	91	290	26	13	479	55	1325	117	7
Bivalvia	Sphaeriidae	29	23	42	7	39			3	876					3	13				
	Unionidae									7										
Coleoptera	Dryopidae									3										
	Dytiscidae						7												3	
	Elmidae	10	322	391	107	13		26	46		251	1182	166	36	91	283		3		
	Gyrinidae	3	10	13	13	20			3				3							
	Haliplidae					3	10				3									
	Hydraenidae			7				7			59	42	13		16					
	Scirtidae	7									10	10								
Diptera	Athericidae					7				7	3	7				3				
	Ceratopogonidae		20																3	7
	Chaoboridae																		7	
	Chironomidae	68	150	589	20	52	160	49	23	49	127	52	52	13	39	101	46	10	3421	2038
	Culicidae																		3	
	Empididae			3									3							
	Limoniidae	16	49	65	36	23		98	55	3	26	7		13	55	23	16	26		
	Psychodidae										3								3	
	Simuliidae	65	3685	260	7	13	52	33	33		254	124		3	55	16	16			10
	Tabanidae			3																
	Tipulidae		3			3				20		13								
Ephemeroptera	Baetidae	212	212	579	335	124		273	153		443	615	225	111	326	358	273	257	62	3
	Caenidae	26	72	10		3	75			88	13									
	Ephemerellidae	192	882	146	381	176	23	286	101	7	283	820	602	365	798	163	36	20		
	Ephemeridae	16	212	42	78	111	3	13			13	7		3	33	10				
	Heptageniidae		13	7	29	7		16		3	33	29	3	52	20	3				
	Leptophlebiidae	7	3	3				13		3	39					39				
	Potamanthidae						3													
Gastropoda	Ancylidae	3	7	85	3	13									3	13				
	Bithyniidae				3				3	20						3				
	Hydrobiidae											3								16
	Lymnaeidae	20									3								13	
	Physidae									13									7	7
	Planorbidae							10	16							10			16	3
Heteroptera	Aphelocheiridae				26	62				133										
	Veliidae											3								
Hirudinea	Erpobdellidae	3	33	20										3	10	3	3		20	13
	Glossiphoniidae	3	3	7			3							7	3				7	3
Isopoda	*Asellidae*		23	277		7	153			16				3	7		16		33	7
Megaloptera	Sialidae																		3	
Odonata	Calopterygidae				3	7				20										
	Corduliidae									3										
	Gomphidae					10				3										
	Lestidae																		65	7
	Libellulidae						3													
Oligochaeta	Lumbricidae	3									13				3	3				
	Oligochaeta	55	342	296	101	101	212	39	26	29	16	49	10	26	42	46		3	293	72
Phyllopoda	Cladocera									10319										
Plecoptera	*Leuctridae*	3						3			59	7								
	Perlidae		7									13								
	Perlodidae	20	20	3	3			29			231	10	7	33	20					
Trichoptera	Brachycentridae			3			111			10		20								
	Glossosomatidae		16	3																
	Goeridae		20	7	26						3	104	42	46	10	7				
	Hydropsychidae	91	62	39	10	3		33	39	3	26	120	20	10	10	52	78	16		
	Hydroptilidae	3		3	7	3							46		10					
	Lepidostomatidae	7	36	91		7		26	16			33	49	33	75	10				
	Leptoceridae	13	36	59	10	10	7	3	3	3	10	23		10	16	52				
	Limnephilidae	166	218	55	7	20	3	296	10	16	16	42	163	10	23	62		10		
	Odontoceridae										7									
	Philopotamidae										3									
	Polycentropodidae	16	7		3	3				36	29	20	3	13	10	42		20		
	Psychomyiidae	7	3	13	39				3	7	3		3	3			10	3		
	Rhyacophilidae	7	16	3				52			33	52	46	7	23	13	7	55		
	Sericostomatidae	42	120	33				13			153	78				49				

Table 4 Abundances of stream macroinvertebrate families sampled in the present study, given as individuals m^{-2} per sampling site (B1-Z2). B1–B6: Bode river; E1–E2: Eine river; M1: Mulde river; S1–S5: Selke river; W1–W3:Wipper river; Z1–Z2: Ziethe river; italics: shredder taxon.

In the absence of insecticides, the herbicides Atrazine (2 sampling sites, mean $TU_{\max}$ = -4.7), Isoproturon (1; -4.7), and Simazine (7; -5.0) caused only low water toxicity levels ($TU < -3.3$; Table 3 and Fig. 1). Except for the insecticide Carbofuran, all banned substances were classified as herbicides and did not show any detectable effect on the invertebrate community composition (mean *TU* ranging from -6.7 to -4.1).

The leaf litter decomposition in rivers and streams is governed by a wide range of factors (Webster and Benfield, 1986), and shredding invertebrates are hereby of high importance (e.g., Benfield and Webster, 1985; Martínez et al., 2013; Mbaka and Schäfer, 2015; Rasmussen et al., 2012a, 2012b). Shredders feeding on CPOM account for approx. 20-25% of the leaf litter degradation (Anderson and Sedell, 1979; Iversen et al., 1982; Petersen and Cummins, 1974; Taylor and Chauvet, 2014). Microbial decomposers contribute to a similar (20%; Anderson and Sedell, 1979; Iversen et al., 1982; Petersen and Cummins, 1974) or higher extent (60%; Taylor and Chauvet, 2014). In fact, the ‘leaf conditioning’ by bacteria and fungi is an indispensable prerequisite for the feeding activity of shredding invertebrates (Bärlocher and Kendrick, 1975; Cummins et al, 1973), with the fungal community composition determining the leaf palatability (Arsuffi and Suberkropp, 1989, 1984; Suberkropp et al., 1983; Gulis and Suberkropp, 2003). Hence, fungicides acting on the microbial decomposers indirectly affect the habitat or food choices of shredding invertebrates (Bundschuh et al., 2011; Flores et al., 2014; Rasmussen et al., 2012a, 2012b; Zubrod et al., 2015, 2011), and the reduction of shredders has been linked to a slow-down of leaf decomposition processes (e.g., Flores et al., 2014; Rasmussen et al., 2008; Wallace et al., 1982). In the present study, shredders were found at sites with and without Carbofuran impact, at mean proportions of 19% (total of taxa) and 21% (total of SPEAR). On average, 53% of the shredder taxa were classified as SPEAR. These proportions were similar to the whole set of sites investigated (20%, 23%, and 55%, respectively). Direct and indirect effects of pesticides on the leaf litter breakdown are hard to distinguish (Flores et al., 2014; Zubrod et al., 2011). Moreover, low food quality, as induced by indirect effects of pesticides, can trigger higher food intake in shredder taxa seeking to compensate for the decreased nutritional benefit (Cummins and Klug, 1979; Rasmussen et al., 2012a; Simpson and Abisgold, 1985). Hence, the leaf litter degradation detected in the present study could not fully reflect the presence or absence of shredding invertebrates.



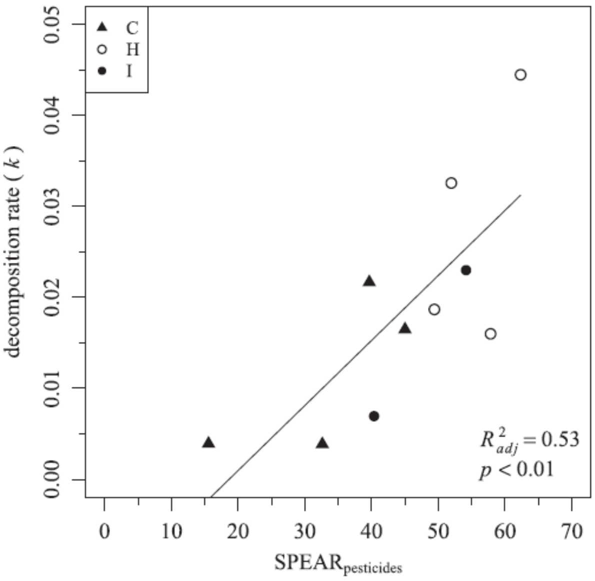


Fig. 2 Relationship between the leaf litter breakdown rate k [d^{-1}] and the water toxicity at the sampling sites expressed as $TU_{\text{sensitive}}$ for 10 sampling sites. The linear regression is significant with $p < 0.05$ and adjusted $R^2 = 0.32$.

Fig. 3 Relationship between the leaf litter breakdown rate k [d^{-1}] and the stream macroinvertebrate assemblage expressed as $\text{SPEAR}_{\text{pesticides}}$ for 10 sampling sites. The linear regression is significant with $p < 0.01$ and adjusted $R^2 = 0.53$.

C: Carbofuran; H: herbicide; I: insecticide other than Carbofuran (Imidacloprid).

Nevertheless, the leaf litter decomposition rate k was correlated to pesticide toxicity ($p < 0.05$, adjusted $R^2 = 0.32$; Fig. 2) and even more strongly correlated to decreased abundances of taxa vulnerable to pesticides (SPEAR; $p < 0.01$, adjusted $R^2 = 0.53$; Fig. 3). The latter is in line with the studies of Schäfer et al. (2012, 2007) who also showed leaf breakdown rates to be correlated to the abundances of macroinvertebrates classified as SPEAR. Even though the k values did not significantly differ in the presence ($TU \geq -2.8$) and absence ($TU < -2.8$; Wilcoxon's rank sum test, $p > 0.05$) of Carbofuran, according to Gessner and Chauvet (2002), the breakdown rates established at sites where Carbofuran was present can be considered 'indicative of severely compromised stream functioning' (mean k_{impacted} : mean $k_{\text{not impacted}} = 0.486$).

The assessment of stream community responses to pesticides is complicated because of manifold interactions between pesticides and nutrients (Alexander et al., 2013). Moderate eutrophication, stimulating microbial activity, can lead to higher macroinvertebrate abundances and elevated organic matter breakdown rates (Greenwood et al., 2007; Gulis et al., 2006), and more severe organic pollution, inducing hypoxia and acidification, can reach levels detrimental to aquatic organisms (Camargo and Alonso, 2006). However, we could not observe an effect of nutrients on invertebrate abundances in the present study. This is in line with the study of Bunzel et al. (2013) who particularly investigated the inter-correlation between the Saprobic Index and SPEAR, reporting only a slight overlapping between the two indices.

In the present study, we used Chemcatchers equipped with a diffusion-limiting membrane and successfully linked pesticide pollution to effects on aquatic macroinvertebrates using the pesticide quantities obtained. We demonstrated that despite the prolonged response time or lag phase, Chemcatchers with a diffusion-limiting membrane deliver sufficient information on short-term pesticide pollution and are suitable for relating benthic community structures to pesticide contamination. Very recently, in a large field study in Switzerland, Moschet et al. (2015) showed the feasibility of the in-situ calibration of Chemcatchers. However, they also stated that their resultant field sampling rates for compounds that originate from sources with a changing release pattern (e.g., pesticides from fields) need to be used with much more caution than those for substances with only moderately fluctuating concentrations (e.g., pharmaceuticals originating from wastewater effluents). In-situ R_S values were reported for a flow velocity range of 0.05 - 0.8 m s^{-1} and could hence be applied to all sampling sites within our study. For substances not investigated by Moschet et al. (2015), the median R_S value of the study (0.07 L d^{-1}) was chosen. Application to our field data yielded no superior results compared to our approach of using a flow-adjusted generic R_S value.

For the calculation of c_{TWA}, we propose the use of a generic sampling rate (as suggested by Escher et al. (2011) in another context), additionally corrected for specific hydrodynamic conditions at each sampling site, which would make the use of Chemcatchers even easier.

5 Conclusions

Assessment of pesticide exposure in the water phase by applying Chemcatchers in a polar configuration with a diffusion-limiting membrane was proven feasible. Pesticide effect assessment was performed with the $SPEAR_{pesticides}$ index, and ecological effects of pesticides on stream macroinvertebrates could be shown. We suggest that future field studies and monitoring programmes, focusing on identifying exposure and ecological effects of pesticides in the field and the effectiveness of mitigation strategies, would benefit from the ease of use of Chemcatcher® passive samplers and the robustness of the $SPEAR_{pesticides}$ index.

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[*] project title: Prüfung und Validierung der Einsatzmöglichkeiten neuartiger Passivsammler für die Überwachung prioritärer Schadstoffe unter der WRRL, der MSRL und im Rahmen von HELCOM und OSPAR

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CHAPTER 3

Pesticides from wastewater treatment plant effluents affect invertebrate communities

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Abstract

We quantified pesticide contamination and its ecological impact up- and downstream of seven wastewater treatment plants (WWTPs) in rural and suburban areas of central Germany. During two sampling campaigns, time-weighted average pesticide concentrations (c_{TWA}) were obtained using Chemcatcher® passive samplers; pesticide peak concentrations were quantified with event-driven samplers. At downstream sites, receiving waters were additionally grab sampled for five selected pharmaceuticals. Ecological effects on macroinvertebrate structure and ecosystem function were assessed using the biological indicator system $\mathrm{SPEAR}_{\mathrm{pesticides}}$ (SPEcies At Risk) and leaf litter breakdown rates, respectively. WWTP effluents substantially increased insecticide and fungicide concentrations in receiving waters; in many cases, treated wastewater was the exclusive source for the neonicotinoid insecticides acetamiprid and imidacloprid in the investigated streams. During the ten weeks of the investigation, five out of the seven WWTPs increased in-stream pesticide toxicity by a factor of three. As a consequence, at downstream sites, SPEAR values and leaf litter degradation rates were reduced by 40% and 53%, respectively. The reduced leaf litter breakdown was related to changes in the macroinvertebrate communities described by $\mathrm{SPEAR}_{\mathrm{pesticides}}$ and not to altered microbial activity. Neonicotinoids showed the highest ecological relevance for the composition of invertebrate communities, occasionally exceeding the Regulatory Acceptable Concentrations (RACs). In general, considerable ecological effects of insecticides were observed above and below regulatory thresholds. Fungicides, herbicides and pharmaceuticals contributed only marginally to acute toxicity. We conclude that pesticide retention of WWTPs needs to be improved.

Keywords: Wastewater treatment plants; Pesticides; Chemcatcher® passive samplers; Macroinvertebrates; $\mathrm{SPEAR}_{\mathrm{pesticides}}$; Leaf litter degradation

1 Introduction

Pesticide contamination of surface waters is known to affect the structure (Liess and von der Ohe, 2005) and biodiversity of invertebrate communities (Beketov et al., 2013). Pesticides are intended to protect agricultural production from pest organisms; however, their residues reach far beyond their target areas via atmospheric, overland, subsurface, and groundwater routes

(Groenendijk et al., 1994). Surface runoff and wastewater effluents are amongst the most important entry pathways for pesticides into aquatic environments (Holvoet et al., 2007; Köck-Schulmeyer et al., 2013; Liess et al., 1999). Pesticide input from farmyards into sewage systems mainly originates from field sprayer filling and cleaning activities on paved surfaces, the direct disposal of unused product residues, accidental spillages, and non-agricultural uses (Bach et al., 2000; Kreuger, 1998). Pesticide residues from non-agricultural uses found in sewage systems have origins in, for example, grass management activities (e.g., golf courses, parks), industrial vegetation control (e.g., highways, railroads) and pest control in private homes and gardens (Barceló and Hennion, 2003).

The impact of diffuse (non-point) pesticide pollution on aquatic macroinvertebrates has been studied frequently (e.g., Kuzmanović et al., 2016; Liess et al., 2008; Münze et al., 2015; Orlinskiy et al., 2015). In contrast, the majority of studies investigating the environmental impact of wastewater treatment plants (WWTPs) have focused on the quantification of pesticides (Barco-Bonilla et al., 2010; Peschka et al., 2006), the effects of nutrients (Grantham et al., 2012; Gücker et al., 2006; Spänhoff et al., 2007), emerging water contaminants (De Castro-Català et al., 2015; Muñoz et al., 2009; Neale et al., 2017), and differing flow conditions, i.e., the dilution potential of receiving waters (Burdon et al., 2016; Englert et al., 2013; Kolpin et al., 2004). Most investigations that have examined ecological effects on aquatic macroinvertebrates have focused on a single taxon (e.g., Bundschuh et al., 2011; Köck-Schulmeyer et al., 2013).

To our knowledge, only two studies have linked pesticides in WWTP effluents to ecological effects on whole stream macroinvertebrate assemblages. Bunzel et al. (2013) employed the modelled pesticide runoff potential, and Ashauer (2016) used measured micropollutant mixtures from a single WWTP to explain changes in community composition. Expanding on those investigations, we measured the in-stream concentrations of pesticides up- and downstream of seven WWTPs. This allowed us to pinpoint the contribution of wastewater-borne pesticides to alterations in macroinvertebrate community structure.

The aim of our investigation was (1) to assess WWTP-related pesticide pollution in receiving waters with a focus on insecticides, and (2) to link contamination levels to effects on macroinvertebrate community structure and function. Pesticides were quantified using both passive and event-driven samplers. Effects on the composition of the macroinvertebrate community (structural endpoint) were assessed with the biological indicator $\mathrm{SPEAR}_{\mathrm{pesticides}}$ (Liess and von der Ohe, 2005). In addition, leaf litter degradation (functional endpoint) was included in our study because structural approaches in the assessment of stream health are ideally complemented by functional measures (Woodward et al., 2012). For this, we calculated the breakdown rate, *k*, and analysed the shredder-feeding guild (Cummins, 1973). We hypothesised that pesticides discharged with WWTP effluents affect the structure of the macroinvertebrate community and leaf litter degradation in receiving waters.

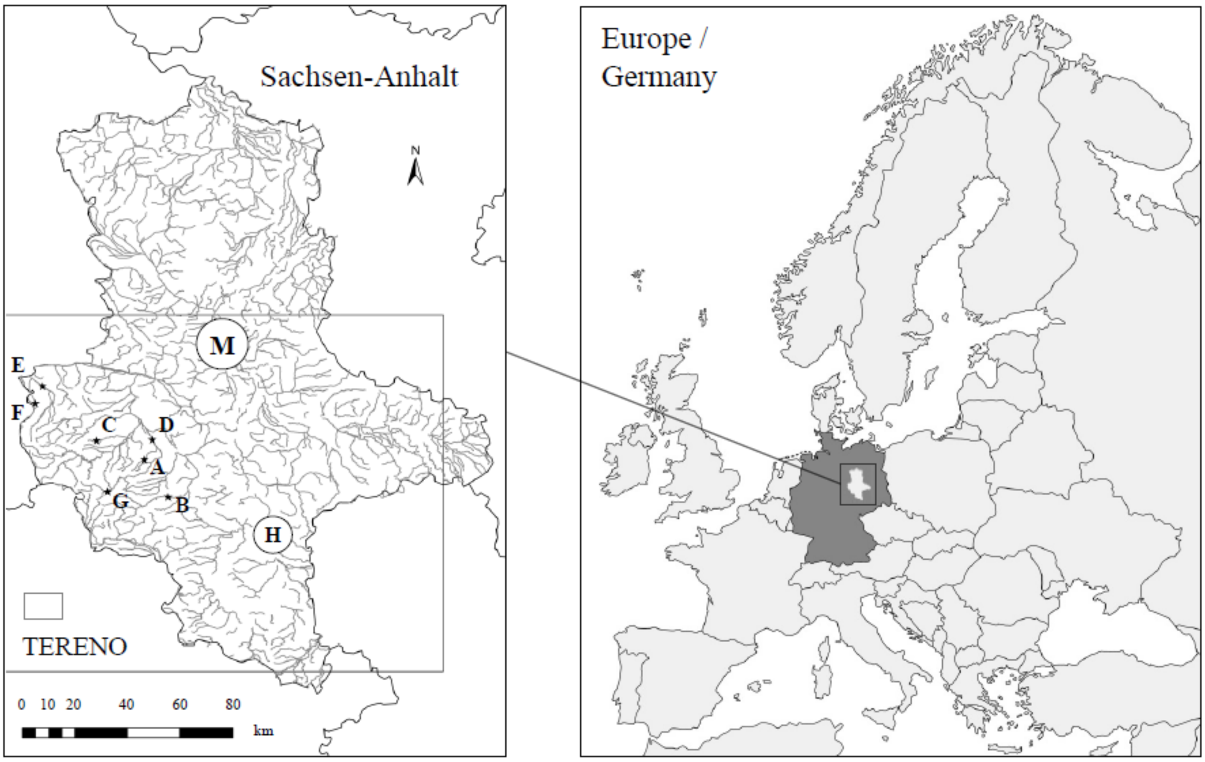


Fig. 1 Map of the study area in Sachsen-Anhalt, Germany. M: Magdeburg; H: Halle/Saale.
A: Ballenstedt WWTP; B: Biesenrode WWTP; C: Blankenburg/Harz WWTP; D: Hoym WWTP;
E: Osterwieck WWTP; F: Stapelburg WWTP; G: Straßberg WWTP.

2 Materials and Methods

2.1 Study area

The present study was conducted in the Bode River catchment in Sachsen-Anhalt, Germany (Fig. 1). This region is part of the TERENO Harz/Central German Lowland Observatory (Wollschläger et al., 2017). The most important crops in the area are cereals (wheat, barley, rye) and rapeseed (STALA, 2014), and the potential for pesticide contamination via agricultural field runoff is low to medium (Kattwinkel et al., 2011). We selected seven rural/suburban WWTPs in agricultural catchment areas (Ballenstedt, Biesenrode, Blankenburg/Harz, Hoym, Osterwieck, Stapelburg, and Straßberg; Fig. 1). They were characterised by a tertiary treatment level (including nitrification, denitrification, and phosphorous removal; LAU, 2012), a zero probability of stormwater overflow into receiving waters (i.e., the absence of a combined sewer overflow; Tibbetts, 2005), and receiving waters with a structural quality class that is typical for streams within agriculturally dominated landscapes in Germany (3 = 'moderately altered' and 4 = 'considerably altered'; classification according to LAWA, 2000). The Hoym and Straßberg WWTPs shared the same receiving water (Selke River); however, these sites were approximately 50 km apart and were therefore treated as independent sampling sites. The GPS coordinates of the effluent discharge points were obtained from the Sachsen-Anhalt State Office of Environmental Protection (LAU, 2012). The streams' structural quality classes were identified using a GIS data shapefile provided by the Sachsen-Anhalt State Agency for Flood Protection and Water Management (LHW). Information on the presence of combined sewer overflows was obtained from the individual WWTPs. Data on the number of farmyards connected to the sewage systems were not available. All receiving waters were perennial rivers and streams of the orders 1 and 3 according to Strahler (1954), with widths of 1.1 m to 6.0 m and depths of 0.1 m to 0.25 m at the sampling sites. The mean stream flow velocity during the investigation period ranged from 0.04 m s^{-1} to 0.19 m s^{-1}. Information on the WWTPs and receiving waters is summarised in SI Table 1.

At each WWTP, samples were taken 50 m upstream (serving as control sites) and 50 m downstream of the effluent discharge point in order to enable the mixing of effluent discharge and stream water and to enable ecological effects. At each sampling site, organic pollution by ammonium (NH_4), nitrite (NO_2), nitrate (NO_3), and phosphate (PO_4) was identified using a Spectroquant® Multy colorimeter (Merck KGaA, Darmstadt, Germany). Electrical conductivity (EC) along with pH and total dissolved oxygen (TDO) were recorded using an ExStik® II pH/Conductivity Meter (EC500) and an ExStik® II Dissolved Oxygen Meter (DO600), respectively (Extech Instruments Corp., Nashua, NH, USA). For the biochemical oxygen demand (BOD) and the total organic carbon (TOC), grab samples were analysed by IFB Halle GmbH (Halle Lettin, Germany) and at the UFZ, respectively (SI Tables 2a, 2b).

2.2 Monitoring of chemical exposure

In total, 88 pesticides frequently found in surface waters (McKnight et al., 2015; Moschet et al., 2014) were included in the chemical analyses: 32 herbicides, four herbicide metabolites, 30 fungicides, one fungicide metabolite, 18 insecticides, two plant growth regulators, and one acaricide (SI Table 3). In addition, we measured five pharmaceuticals that are ubiquitous in WWTP effluents and surface waters (Ginebreda et al., 2010; Pérez and Barceló, 2007): the anti-epileptic drug carbamazepine, the anti-inflammatory drugs diclofenac and ibuprofen, and the iodinated X-ray contrast media iopamidol and iopromide.

We simultaneously deployed passive samplers (Kingston et al., 2000) and event-driven samplers (EDSs; Liess and von der Ohe, 2005) to capture both time-weighted average concentrations (c_{TWA}) and peak concentrations of pesticides. The passive samplers were processed after a two-week deployment period in April/May and a three-week deployment period in June/July 2013 (hereafter referred to as May and July, respectively). Each sampling campaign covered a heavy rainfall event, and the EDS samples were collected and processed directly after each event. Using two differing sampling techniques, we were able to expand the spectrum of analytes in order to adjust the time-integrated concentrations to the peak concentrations and to utilise different analytical methods with respect to the polarity of the compounds detected. Hydrophilic and semi-hydrophilic analytes were identified with both sampling techniques. The hydrophobic analytes (pyrethroids) were measured in the EDS water samples using solid-phase extraction (SPE; see Barco-Bonilla et al., 2010).

2.2.1 Passive sampling for pesticides

Chemcatcher® passive samplers (Kingston et al., 2000) in the polar configuration (Stephens et al., 2005), hereafter referred to as Chemcatchers, were chosen for the time-integrative sampling of hydrophilic and semi-hydrophilic compounds. Chemcatchers provide the c_{TWA} of chemicals obtained from variable water phase concentrations during the time of deployment (Hawker, 2010). The 3rd-generation housings made from polytetrafluoroethylene (PTFE; 'Teflon') were obtained from the University of Portsmouth, UK (SI Figs. 1a, 1b). An Empore SDB-RPS (styrene-divinylbenzene reversed-phase sulfonate) extraction disk from Varian (Walton-on-Thames, UK), modified with sulfonic acid groups, was applied as the receiving sorbent phase. The utilisation of an overlying diffusion-limiting membrane was recommended by Shaw and Mueller (2009) and has been successfully put into practice (Münze et al., 2015). Hence, in the present study, we used a membrane made from hydrophilic polyethersulfone (PES) with a pore size of 0.2 µm. Passive flow monitors (PFM; O'Brien et al., 2009) were used to monitor the average stream flow velocities, which are required for the calculation of the c_{TWA} of pesticides in the water phase. For a detailed description of Chemcatcher sampling procedures, see Münze et al. (2015).

2.2.2 Event-driven sampling for pesticides

Event-driven samplers (EDSs; Liess and von der Ohe, 2005) were deployed alongside the Chemcatchers to capture peak pesticide concentrations in the water phase induced by a heavy rainfall event. At each sampling site, we attached a pair of brown glass bottles, each with a capacity of 1 L, to a stainless steel pole, one bottle on top of the other. The sampling device was positioned in the stream such that the opening of the water inlet of the lower bottle was approximately 5 cm above the stream water level. We used stainless steel tubes (4 mm diameter) for the water inlet and the air outlet. The water levels of the investigated streams were checked regularly, and the position of the EDSs in relation to the stream water level was adjusted when necessary. The EDS design and the sampling principle followed those described by Liess and von der Ohe (2005). However, because of the shallow water at our sampling sites, we changed the orientation of the bottles from vertical to horizontal with some necessary adjustments. To allow maximum filling, the air outlet was extended deeply into the bottle, pointing close to the upper wall. Outside the bottle, a silicon tube with a length of 15 cm on top of the air outlet provided sufficient distance between the openings of the water inlet and the air outlet. In addition, we inserted a 100-µL pipette tip (Eppendorf AG, Hamburg, Germany) into the top end of the silicon tube, thus reducing the diameter of the air outlet and expanding the bottle’s filling time from 1 min to 4 min (SI Fig. 2). We ensured that the new horizontal sampling design did not cause any unwanted water exchange with the surroundings when completely underwater. For this, we submersed an EDS filled with a defined NaCl solution in a 10-L bucket containing tap water. A water current in the bucket was simulated using a Rena Air 50 pump (MARS Fishcare Europe, Annecy Cedex, France). After 48 h, the EDS still contained more than 99% of the original NaCl solution.

2.2.3 Grab sampling for pharmaceuticals

The stream sites investigated in the present study were selected so that we could exclude any input of pharmaceuticals upstream of the WWTP effluent discharge points (i.e., no application of liquid manure and no additional WWTPs upstream). Grab samples were taken 50 m downstream of the effluent discharge points using brown glass bottles with a capacity of 1 L.

Water samples were kept refrigerated at 4 °C during transport and until they were processed in the laboratory.

2.3 Preparation and analysis of chemical samples

Upon Chemcatcher retrieval, the Empore SDB-RPS disks were removed and extracted according to Münze et al. (2015). The methanolic extracts were transferred into 1-mL crimp-top vials (Agilent Technologies, Waldbronn, Germany). The Chemcatcher extracts were frozen and shipped to Uppsala, Sweden, for analysis.

Upon EDS retrieval, all collected water samples were filtered using Whatman grade 5 qualitative filter papers (GE Healthcare UK Ltd., Little Chalfont, UK). Subject to availability, a maximum of 250 mL of filtrate from each site was frozen and shipped to Uppsala for direct water analysis. Upon receipt of the shipment, the water samples were adjusted to a pH of 4.9 to 5.1 using acetic acid and ammonium acetate, and the Chemcatcher extracts were diluted by a factor of 200 with Milli-Q purified water (pH 5). An internal standard mixture consisting of atrazine-d_5, ethion, isoproturon-d_6, propamocarb-d_7, quinmerac-d_4, and terbuthylazine-d_5 was added to each sample at a concentration of 100 ng L^{-1}, and the water samples were filtered using RC filters before analysis.

The remaining EDS contents were passed slowly through Chromabond® C18ec SPE cartridges, pre-conditioned according to the instructions of the manufacturer (Macherey-Nagel, Düren, Germany). After adding the sample and drying the SPE packing for 10 min using a gentle air stream, the cartridges were frozen and shipped to Uppsala for analysis. Upon receipt of the shipment, the SPE cartridges were eluted using 3 mL acetone, 3 mL acetone:hexane (1:1, v/v) and 3 mL hexane. The eluent was dried with Na_2SO_4 and evaporated under a gentle stream of nitrogen; it was then adjusted to a final volume of 1 mL using cyclohexane:acetone (9:1, v/v).

The pesticide analyses were carried out at the Swedish University of Agricultural Sciences, Uppsala, Sweden. Chemcatcher extracts and water samples were analysed using LC-MS/MS, and the SPE extracts were analysed by GC-MS. The pharmaceutical analyses were performed using LC-MS/MS according to the German (draft) standard DIN 38407-47 by the accredited commercial laboratory SGS Institute FRESENIUS GmbH, Taunusstein, Germany. The analytical procedures are described in SI Text 1.

2.4 Quantification of exposure

Chemcatcher data were available from all sampling sites. The EDS data were not available in an equal quantity because some field samples delivered an insufficient water volume for chemical analysis. Low water sample volumes can be explained by locally differing precipitation patterns or occasional EDS operating failures triggered by floating materials that might have temporarily blocked the water inlet or the air outlet of the bottles.

To analyse the pesticide quantities obtained with different sampling methods, some harmonisation steps were required. In the first step, the accumulated total concentrations from the Chemcatchers were transformed into c_{TWA} using Eq. 1 with flow-dependent sampling rates:

$$c_{TWA} = \frac{m_{\mathrm{S}}}{R_{\mathrm{S}} \cdot t} \qquad (1)$$

where m_{S} is the mass of the pesticide accumulated by the SDB-RPS Empore disk, R_{S} is the sampling rate [L d^{-1}] and t is the exposure time [d] of the Chemcatcher at the sampling site. The compound-specific sampling rates usually did not significantly vary among the investigated analytes; hence, overall sampling rates may be applied, as suggested by Escher et al. (2011). The evaluation of experimental sampling found in the literature shows that the sampling rates can be high or low. Low sampling rates are expected for compounds with $\log K_{\mathrm{OW}} < 1$ and high water solubility because the difference in their chemical potential between the water and the receiving phase of the Chemcatcher is rather low, thus showing little tendency for phase transfer. In addition, compounds that are prone to microbiological degradation in the receiving phase during exposure were assigned to the group with low sampling rates. Hence, different regression equations were applied for the estimation of R_{S} (Eqs. 2 and 3):

$$R_S\ (\mathrm{high})\ = 0.85 \cdot v + 0.014 \qquad (2)$$

$$R_S\ (\mathrm{low})\ = 0.47 \cdot v + 0.0004 \qquad (3)$$

where v is the stream flow velocity in m s^{-1}. Average flow velocities were obtained using passive flow monitors (O'Brien et al., 2009) and calculated according to Eq. 4 (O'Brien et al., 2011):

$$v = \frac{r_{\mathrm{PFM}} - 0.065}{16.4} \tag{4}$$

where r_{PFM} is the daily loss of plaster from the passive flow monitor [g d^{-1}]. For further details, see Münze et al. (2015; particularly SI Text S1).

In each sampling campaign, the EDS peak pesticide concentrations in stream water exceeded the c_{TWA} from the Chemcatcher measurements by a median factor of two. Therefore, in the second step, we converted the Chemcatcher c_{TWA} into peak concentrations by multiplying the c_{TWA} by two. In the literature, such 'correction factors' have been reported (factor of three, Fernández et al., 2014; factor of five, Schäfer et al., 2008; factor of six, Bundschuh et al., 2014).

To enable the comparison of the toxicity levels of different compounds among the sampling sites, in the last step, all established peak concentrations were converted into Toxic Units (*TU*; see EC, 2012). The pesticide toxicity of a sample was determined as the highest *TU* value of all detected compounds ($TU_{\max}$; Liess and von der Ohe, 2005). A log-transformation was conducted to provide a suitable resolution for illustration purposes (Eq. 5):

$$TU = \max_{i=1}^{n}\left[\log\left(\frac{c_i}{LC_{50_i}}\right)\right] \tag{5}$$

where *TU* is the highest value of *n* pesticides detected at each sampling site ($TU_{\max}$), c_i is the peak concentration of pesticide *i*, and LC_{50i} is the pesticide's corresponding median lethal concentration (48 h) for the chosen reference organism.

In general, the *TU* values were calculated using the most sensitive standard test organism, *Daphnia* ssp. or *Chironomus* ssp. This criterion meant that in most cases, *TU* calculations were based on the acute (48 h) LC_{50} values of *Daphnia* spp. In contrast, for neonicotinoids, *Chironomus* spp. was the more sensitive standard test organism. However, toxicity data were available for only five neonicotinoids, with median acute (48 h) LC_{50} values ranging from 19.9 µg L^{-1} to 72.1 µg L^{-1}. Because of this scarcity of data and to achieve stable results for the *TU* calculation, we applied the median (48 h) LC_{50} value of 22 µg L^{-1} in order to calculate *TU* values for the neonicotinoids detected in the present study (acetamiprid, clothianidin, imidacloprid, and thiacloprid). LC_{50} values were determined by referencing several databases (SI Table 4). A detailed description of the selection criteria for the acute (48 h) LC_{50} values is given by Münze et al. (2015).

2.5 Assessment of ecological effects

2.5.1 Macroinvertebrate community composition

Directly after the Chemcatcher deployment periods in May and July 2013, the stream benthos was sampled up- and downstream of the effluent discharge points. All specimens were identified to the family level as described in Münze et al. (2015). To disentangle effects of pesticides from those of other environmental factors, we applied the stressor-specific bioindicator system $\text{SPEAR}_{\text{pesticides}}$ (SPEcies At Risk), which quantitatively links pesticide contamination to the composition of the macroinvertebrate community in streams (Liess and von der Ohe, 2005). The $\text{SPEAR}_{\text{pesticides}}$ values were calculated using the publicly available SPEAR Calculator desktop application (version 0.8.0; UFZ, 2014; http://www.systemecology.eu/spear/). $\text{SPEAR}_{\text{pesticides}}$ gives the relative abundance of vulnerable species within a community according to Eq. 6:

$$\text{SPEAR}_{\text{pesticides}} = \frac{\sum_{i=1}^{n} \log(x_i + 1)\, y}{\sum_{i=1}^{n} \log(x_i + 1)} \cdot 100 \qquad (6)$$

where n is the total number of taxa in a sample, x_i is the abundance of taxon i (given as individuals m^{-2}), and y is set to 1 if taxon i is classified as 'at risk', i.e., vulnerable to pesticides, and set to 0 otherwise.

2.5.2 Leaf litter breakdown

We estimated this relevant ecosystem function at all sampling sites with triplicate leaf litter bags containing loose dried birch leaves. Each of the bags (coarse mesh cases made from polyethylene; mesh size: 8 mm) contained 3 g of dried leaf mass and a smaller fine mesh case (made from nylon; mesh size: 75 µm) that also contained 3 g of dried leaf mass, accounting for invertebrate- and microorganism-induced leaf litter breakdown, respectively. Handling losses were corrected using additional leaf litter bags that were only briefly deployed in the streams but equally processed otherwise. The water temperature was recorded throughout the three-week deployment periods using DK310 TempLog 'rugged' temperature data loggers (Driesen & Kern, Bad Bramstedt, Germany). Upon litter bag retrieval, all remaining contents were carefully extracted, cleaned and dried at 60 °C in a drying oven (Type UT 6060, Heraeus Instruments GmbH, Hanau, Germany) for four days to a constant weight.

At six sampling sites in May (Biesenrode, Hoym, and Straßberg WWTPs) and at one site in July (downstream of the Hoym WWTP), mesh cases were either washed away or damaged whilst deployed in the streams. Using data from eight sampling sites in May (Ballenstedt, Blankenburg, Osterwieck, and Stapelburg WWTPs) and 13 sampling sites in July (all but downstream of the Hoym WWTP), the leaf litter breakdown rate, k, was calculated for each site according to Eq. 7:

$$k = \frac{-\ln\left(\frac{W_t}{W_0}\right)}{t} \qquad (7)$$

where W_t is the remaining dried leaf mass after the end of the deployment period (sum of the remaining weight from the coarse mesh case and the weight lost from the respective fine mesh case), W_0 is the initial dried leaf mass, and t is the time of deployment [d].

The effects of pesticides on k were further assessed using the functional feeding group of shredders according to Cummins (1973), Cummins et al. (2005), and USEPA (1999). No distinction was made between obligate and facultative shredders (Cummins and Klug, 1979). Finally, the relationship between TU_{max} and the site-specific proportion of shredder taxa classified as SPEAR was analysed.

2.6 Statistical analysis

To quantify the pesticide input induced by the WWTPs, we analysed the differences in the down- and upstream peak concentrations individually for several groups of pesticides: (i) fungicides; (ii) herbicides in two subgroups, i.e., priority substances under the European

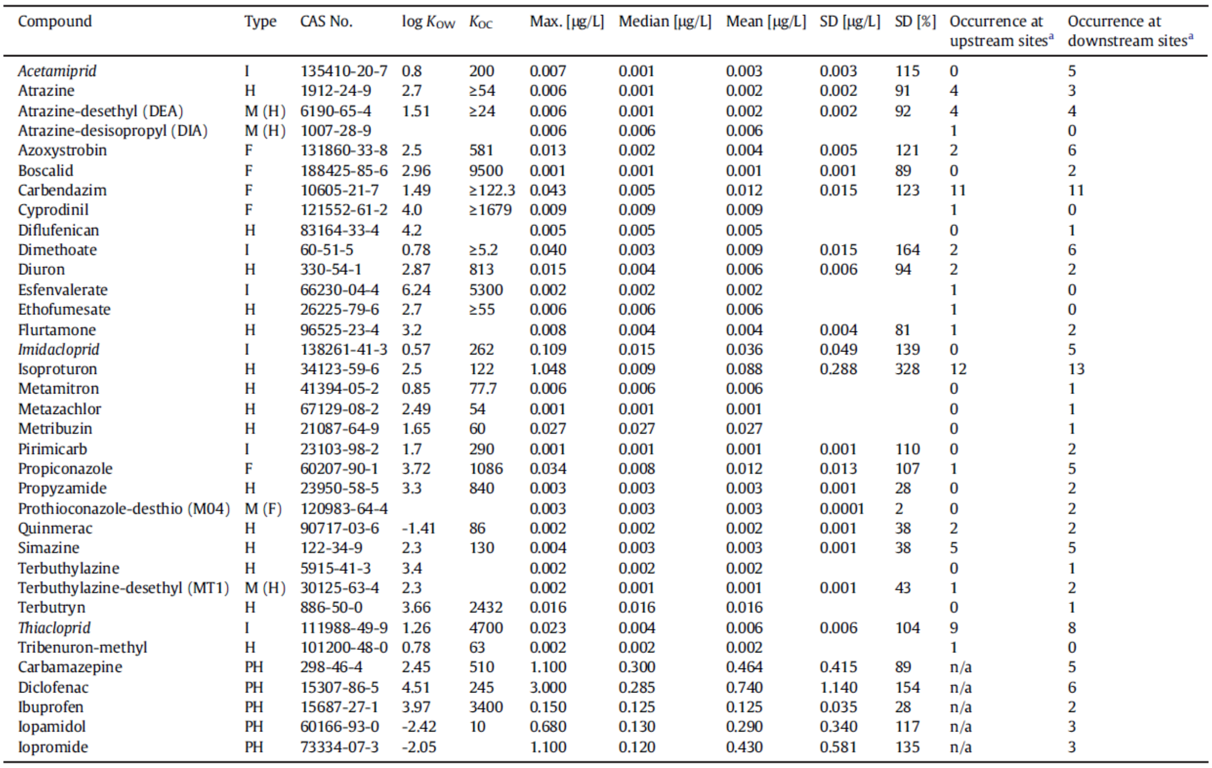
Water Framework Directive (WFD; EC, 2013, 2000) and common herbicides; (iii) insecticides in two subgroups, i.e., neonicotinoids due to their ubiquity and high risk potential (Roessink et al., 2013; Van Dijk et al., 2013) and non-neonicotinoid insecticides; and (iv) pesticide metabolites. The normal distribution of residuals and homoscedasticity in the data were checked using the Shapiro-Wilk test and Levene's test, respectively. Pesticide toxicity related to WWTP effluents was quantified as the ratio of downstream to upstream TU_{max} values, and the pesticide-driven changes in ecological endpoints (i.e., the community composition, $\text{SPEAR}_{\text{pesticides}}$, and the leaf decomposition rate, k) were quantified as percentages. For the impact assessment, we considered only those downstream samples in which the increase in TU_{max} was at least twofold in comparison to the respective upstream samples (50% of events) in order to focus on the effects of WWTPs. The statistical significance of concentration differences, of the deviation in TU_{max} and of the changes in ecological endpoints was determined using a one-sided Mann-Whitney *U*-test and a one-sided t-test, subject to the normality and homoscedasticity of the data.

To identify which of the measured environmental parameters had a relevant effect on $\text{SPEAR}_{\text{pesticides}}$ and k, we applied linear mixed-effect models (LMMs) using stepwise forward selection. The variables 'stream' and 'sampling campaign' were considered random effects in a crossed design in order to avoid pseudoreplication due to the nature of our study design, i.e., repeated measurements within the same stream (up- and downstream of WWTPs) and over time (in May and in July). The variables NH_4, NO_2, NO_3, PO_4, TOC, and stream flow velocity were log-transformed to meet the requirements of the test (normal residual distribution and homogeneity of variance). Testing the main effects first, we compared the null model to all possible one-way models that comprised a single main effect using maximum likelihood ratio tests. The main effects were then ranked according to their significance. Effects were sequentially added to the null model in this order if their addition significantly improved the model according to a likelihood ratio test. Subsequently, the same process was repeated with two-way and three-way interactions. For an interpretation of results, we used hierarchical partitioning (Chevan and Sutherland, 1991), which showed the individual weights of the variables concerned. The open-source statistics software applications RStudio for Windows (version 0.98.1028; RStudio, 2014) and R for Windows (version 3.2.3; R Core Team, 2015) were employed for all computations and graphics. The LMMs were fitted using the lme4 package 1.1-10 (Bates et al., 2015).

3 Results and Discussion

3.1 Pesticides in streams

In total, 45 pesticides (19 herbicides, 14 fungicides, seven insecticides, and five metabolites) were quantified in various mixtures of up to 26 compounds per sample. In July, the number of pesticides detected was 33% higher than in May (Tables 1a, 1b; SI Fig. 3). Generally, the investigated WWTPs increased the number and concentrations of pesticides in the receiving waters. In May, WWTP effluents added 6 ± 2 (mean and standard error) compounds to the in-stream pesticide pollution from upstream reaches; in July, they contributed 2 ± 2 substances.



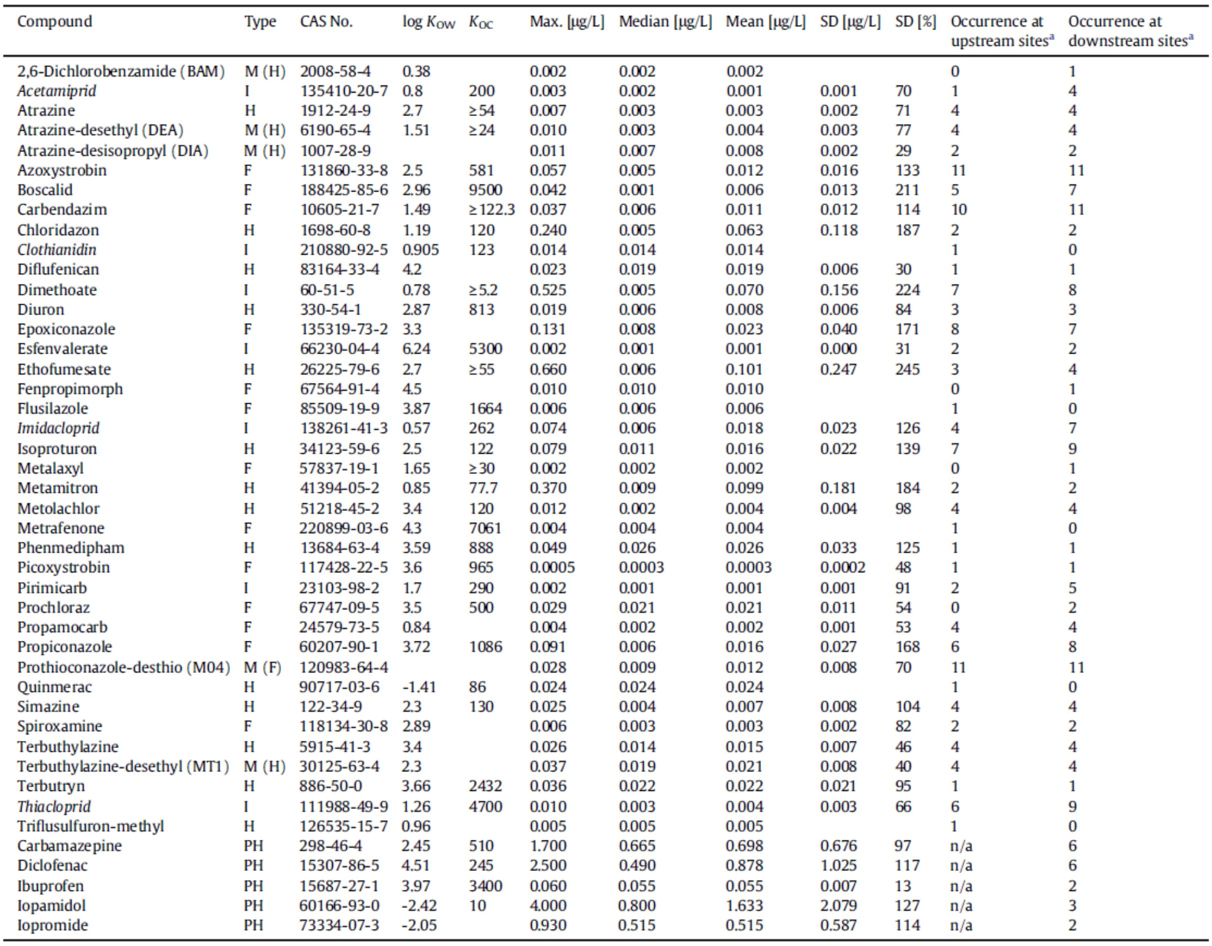
Compound	Type	CAS No.	log K_{OW}	K_{OC}	Max. [µg/L]	Median [µg/L]	Mean [µg/L]	SD [µg/L]	SD [%]	Occurrence at upstream sites[a]	Occurrence at downstream sites[a]
Acetamiprid	I	135410-20-7	0.8	200	0.007	0.001	0.003	0.003	115	0	5
Atrazine	H	1912-24-9	2.7	≥54	0.006	0.001	0.002	0.002	91	4	3
Atrazine-desethyl (DEA)	M (H)	6190-65-4	1.51	≥24	0.006	0.001	0.002	0.002	92	4	4
Atrazine-desisopropyl (DIA)	M (H)	1007-28-9			0.006	0.006	0.006			1	0
Azoxystrobin	F	131860-33-8	2.5	581	0.013	0.002	0.004	0.005	121	2	6
Boscalid	F	188425-85-6	2.96	9500	0.001	0.001	0.001	0.001	89	0	2
Carbendazim	F	10605-21-7	1.49	≥122.3	0.043	0.005	0.012	0.015	123	11	11
Cyprodinil	F	121552-61-2	4.0	≥1679	0.009	0.009	0.009			1	0
Diflufenican	H	83164-33-4	4.2		0.005	0.005	0.005			0	1
Dimethoate	I	60-51-5	0.78	≥5.2	0.040	0.003	0.009	0.015	164	2	6
Diuron	H	330-54-1	2.87	813	0.015	0.004	0.006	0.006	94	2	2
Esfenvalerate	I	66230-04-4	6.24	5300	0.002	0.002	0.002			1	0
Ethofumesate	H	26225-79-6	2.7	≥55	0.006	0.006	0.006			1	0
Flurtamone	H	96525-23-4	3.2		0.008	0.004	0.004	0.004	81	1	2
Imidacloprid	I	138261-41-3	0.57	262	0.109	0.015	0.036	0.049	139	0	5
Isoproturon	H	34123-59-6	2.5	122	1.048	0.009	0.088	0.288	328	12	13
Metamitron	H	41394-05-2	0.85	77.7	0.006	0.006	0.006			0	1
Metazachlor	H	67129-08-2	2.49	54	0.001	0.001	0.001			0	1
Metribuzin	H	21087-64-9	1.65	60	0.027	0.027	0.027			0	1
Pirimicarb	I	23103-98-2	1.7	290	0.001	0.001	0.001	0.001	110	0	2
Propiconazole	F	60207-90-1	3.72	1086	0.034	0.008	0.012	0.013	107	1	5
Propyzamide	H	23950-58-5	3.3	840	0.003	0.003	0.003	0.001	28	0	2
Prothioconazole-desthio (M04)	M (F)	120983-64-4			0.003	0.003	0.003	0.0001	2	0	2
Quinmerac	H	90717-03-6	-1.41	86	0.002	0.002	0.002	0.001	38	2	2
Simazine	H	122-34-9	2.3	130	0.004	0.003	0.003	0.001	38	5	5
Terbuthylazine	H	5915-41-3	3.4		0.002	0.002	0.002			0	1
Terbuthylazine-desethyl (MT1)	M (H)	30125-63-4	2.3		0.002	0.001	0.001	0.001	43	1	2
Terbutryn	H	886-50-0	3.66	2432	0.016	0.016	0.016			0	1
Thiacloprid	I	111988-49-9	1.26	4700	0.023	0.004	0.006	0.006	104	9	8
Tribenuron-methyl	H	101200-48-0	0.78	63	0.002	0.002	0.002			1	0
Carbamazepine	PH	298-46-4	2.45	510	1.100	0.300	0.464	0.415	89	n/a	5
Diclofenac	PH	15307-86-5	4.51	245	3.000	0.285	0.740	1.140	154	n/a	6
Ibuprofen	PH	15687-27-1	3.97	3400	0.150	0.125	0.125	0.035	28	n/a	2
Iopamidol	PH	60166-93-0	-2.42	10	0.680	0.130	0.290	0.340	117	n/a	3
Iopromide	PH	73334-07-3	-2.05		1.100	0.120	0.430	0.581	135	n/a	3

Table 1a Compounds detected in May (30 pesticides and five pharmaceuticals).

CAS: Chemical Abstracts Service; SD: standard deviation; K_{OW}: octanol-water partitioning coefficient; K_{OC}: soil organic carbon-water partitioning coefficient; F: fungicide; H: herbicide; I: insecticide; M: metabolite; PH: pharmaceutical; Italics: neonicotinoid.

[a] For pesticides, both Chemcatcher and EDS results are considered.

The resulting increase in the downstream pesticide concentrations (overall: 0.021 ± 0.101 µg L^{-1}) was significant for insecticides ($p < 0.01$), particularly neonicotinoids ($p < 0.001$), and for fungicides ($p < 0.001$; one-sided Mann-Whitney *U*-test; Fig. 2). At the downstream sampling sites, the highest toxicity values, TU_{max}, were provided by acetamiprid, imidacloprid, and thiacloprid (neonicotinoid insecticides), esfenvalerate (pyrethroid insecticide), azoxystrobin and carbendazim (fungicides), and isoproturon (herbicide; Tables 2a, 2b; SI Figs. 4, 5).



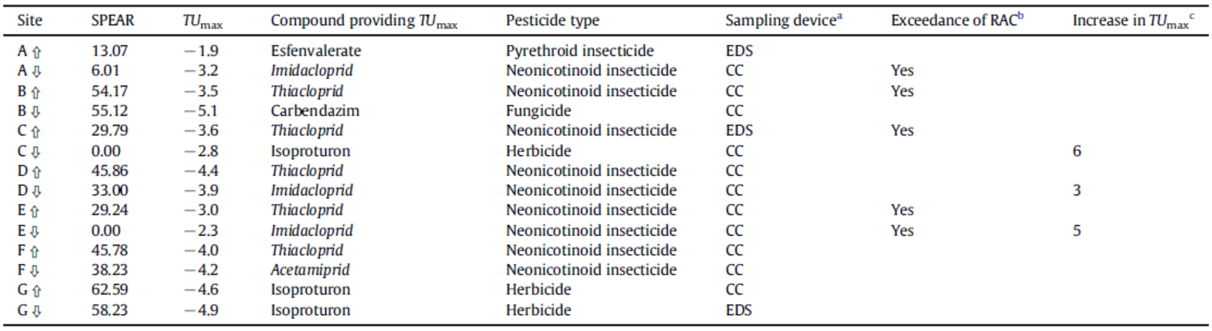
Compound	Type	CAS No.	log K_{OW}	K_{OC}	Max. [µg/L]	Median [µg/L]	Mean [µg/L]	SD [µg/L]	SD [%]	Occurrence at upstream sites[a]	Occurrence at downstream sites[a]
2,6-Dichlorobenzamide (BAM)	M (H)	2008-58-4	0.38		0.002	0.002	0.002			0	1
Acetamiprid	I	135410-20-7	0.8	200	0.003	0.002	0.001	0.001	70	1	4
Atrazine	H	1912-24-9	2.7	≥54	0.007	0.003	0.003	0.002	71	4	4
Atrazine-desethyl (DEA)	M (H)	6190-65-4	1.51	≥24	0.010	0.003	0.004	0.003	77	4	4
Atrazine-desisopropyl (DIA)	M (H)	1007-28-9			0.011	0.007	0.008	0.002	29	2	2
Azoxystrobin	F	131860-33-8	2.5	581	0.057	0.005	0.012	0.016	133	11	11
Boscalid	F	188425-85-6	2.96	9500	0.042	0.001	0.006	0.013	211	5	7
Carbendazim	F	10605-21-7	1.49	≥122.3	0.037	0.006	0.011	0.012	114	10	11
Chloridazon	H	1698-60-8	1.19	120	0.240	0.005	0.063	0.118	187	2	2
Clothianidin	I	210880-92-5	0.905	123	0.014	0.014	0.014			1	0
Diflufenican	H	83164-33-4	4.2		0.023	0.019	0.019	0.006	30	1	1
Dimethoate	I	60-51-5	0.78	≥5.2	0.525	0.005	0.070	0.156	224	7	8
Diuron	H	330-54-1	2.87	813	0.019	0.006	0.008	0.006	84	3	3
Epoxiconazole	F	135319-73-2	3.3		0.131	0.008	0.023	0.040	171	8	7
Esfenvalerate	I	66230-04-4	6.24	5300	0.002	0.001	0.001	0.000	31	2	2
Ethofumesate	H	26225-79-6	2.7	≥55	0.660	0.006	0.101	0.247	245	3	4
Fenpropimorph	F	67564-91-4	4.5		0.010	0.010	0.010			0	1
Flusilazole	F	85509-19-9	3.87	1664	0.006	0.006	0.006			1	0
Imidacloprid	I	138261-41-3	0.57	262	0.074	0.006	0.018	0.023	126	4	7
Isoproturon	H	34123-59-6	2.5	122	0.079	0.011	0.016	0.022	139	7	9
Metalaxyl	F	57837-19-1	1.65	≥30	0.002	0.002	0.002			0	1
Metamitron	H	41394-05-2	0.85	77.7	0.370	0.009	0.099	0.181	184	2	2
Metolachlor	H	51218-45-2	3.4	120	0.012	0.002	0.004	0.004	98	4	4
Metrafenone	F	220899-03-6	4.3	7061	0.004	0.004	0.004			1	0
Phenmedipham	H	13684-63-4	3.59	888	0.049	0.026	0.026	0.033	125	1	1
Picoxystrobin	F	117428-22-5	3.6	965	0.0005	0.0003	0.0003	0.0002	48	1	1
Pirimicarb	I	23103-98-2	1.7	290	0.002	0.001	0.001	0.001	91	2	5
Prochloraz	F	67747-09-5	3.5	500	0.029	0.021	0.021	0.011	54	0	2
Propamocarb	F	24579-73-5	0.84		0.004	0.002	0.002	0.001	53	4	4
Propiconazole	F	60207-90-1	3.72	1086	0.091	0.006	0.016	0.027	168	6	8
Prothioconazole-desthio (M04)	M (F)	120983-64-4			0.028	0.009	0.012	0.008	70	11	11
Quinmerac	H	90717-03-6	-1.41	86	0.024	0.024	0.024			1	0
Simazine	H	122-34-9	2.3	130	0.025	0.004	0.007	0.008	104	4	4
Spiroxamine	F	118134-30-8	2.89		0.006	0.003	0.003	0.002	82	2	2
Terbuthylazine	H	5915-41-3	3.4		0.026	0.014	0.015	0.007	46	4	4
Terbuthylazine-desethyl (MT1)	M (H)	30125-63-4	2.3		0.037	0.019	0.021	0.008	40	4	4
Terbutryn	H	886-50-0	3.66	2432	0.036	0.022	0.022	0.021	95	1	1
Thiacloprid	I	111988-49-9	1.26	4700	0.010	0.003	0.004	0.003	66	6	9
Triflusulfuron-methyl	H	126535-15-7	0.96		0.005	0.005	0.005			1	0
Carbamazepine	PH	298-46-4	2.45	510	1.700	0.665	0.698	0.676	97	n/a	6
Diclofenac	PH	15307-86-5	4.51	245	2.500	0.490	0.878	1.025	117	n/a	6
Ibuprofen	PH	15687-27-1	3.97	3400	0.060	0.055	0.055	0.007	13	n/a	2
Iopamidol	PH	60166-93-0	-2.42	10	4.000	0.800	1.633	2.079	127	n/a	3
Iopromide	PH	73334-07-3	-2.05		0.930	0.515	0.515	0.587	114	n/a	2

Table 1b Compounds detected in July (39 pesticides and five pharmaceuticals).

CAS: Chemical Abstracts Service; SD: standard deviation; K_{OW}: octanol-water partitioning coefficient; K_{OC}: soil organic carbon-water partitioning coefficient; F: fungicide; H: herbicide; I: insecticide; M: metabolite; PH: pharmaceutical; Italics: neonicotinoid.

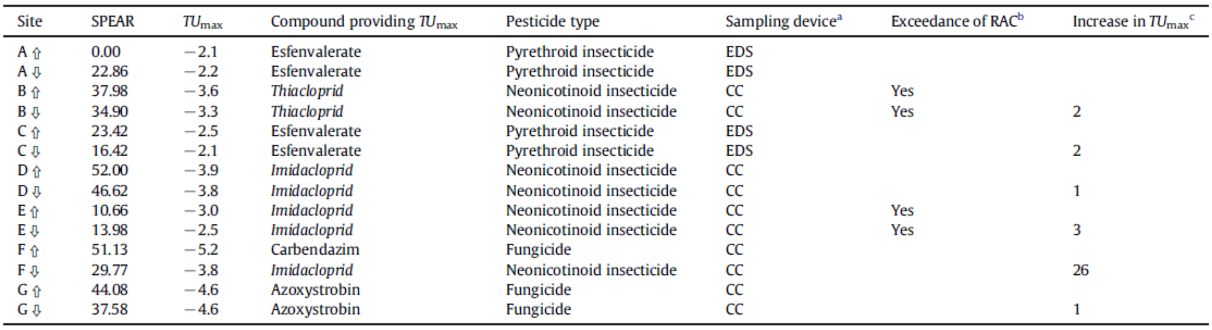
[a] For pesticides, both Chemcatcher and EDS results are considered.

In 50% of events, WWTP effluents increased the in-stream pesticide toxicity by a median factor of three (one-sided Mann-Whitney U-test, $p < 0.01$; Fig. 3; Tables 2a, 2b). This was caused by (i) higher concentrations of a specific compound (esfenvalerate, imidacloprid, isoproturon, and thiacloprid; 57% of samples) or (ii) the occurrence of a substance exclusively introduced by the WWTP effluents (imidacloprid; 43% of samples). Treated wastewater was frequently found to be the exclusive source for acetamiprid and imidacloprid in the investigated streams. Neonicotinoids and pyrethroids seemed to have comparable ecological effects at a similar toxicity level. However, in contrast to neonicotinoids, esfenvalerate was detected less frequently. This might be due to the fact that the pyrethroids' limit of detection is close to those concentrations that are ecologically relevant. Future studies into this aspect are needed.



Site	SPEAR	TU_{max}	Compound providing TU_{max}	Pesticide type	Sampling device^{a}	Exceedance of RAC^{b}	Increase in TU_{max}^{c}
A ⇧	13.07	−1.9	Esfenvalerate	Pyrethroid insecticide	EDS		
A ⇩	6.01	−3.2	*Imidacloprid*	Neonicotinoid insecticide	CC	Yes	
B ⇧	54.17	−3.5	*Thiacloprid*	Neonicotinoid insecticide	CC	Yes	
B ⇩	55.12	−5.1	Carbendazim	Fungicide	CC		
C ⇧	29.79	−3.6	*Thiacloprid*	Neonicotinoid insecticide	EDS	Yes	
C ⇩	0.00	−2.8	Isoproturon	Herbicide	CC		6
D ⇧	45.86	−4.4	*Thiacloprid*	Neonicotinoid insecticide	CC		
D ⇩	33.00	−3.9	*Imidacloprid*	Neonicotinoid insecticide	CC		3
E ⇧	29.24	−3.0	*Thiacloprid*	Neonicotinoid insecticide	CC	Yes	
E ⇩	0.00	−2.3	*Imidacloprid*	Neonicotinoid insecticide	CC	Yes	5
F ⇧	45.78	−4.0	*Thiacloprid*	Neonicotinoid insecticide	CC		
F ⇩	38.23	−4.2	*Acetamiprid*	Neonicotinoid insecticide	CC		
G ⇧	62.59	−4.6	Isoproturon	Herbicide	CC		
G ⇩	58.23	−4.9	Isoproturon	Herbicide	EDS		

Table 2a TU_{max} and SPEAR values at the up- and downstream sampling sites in May.



Site	SPEAR	TU_{max}	Compound providing TU_{max}	Pesticide type	Sampling device^{a}	Exceedance of RAC^{b}	Increase in TU_{max}^{c}
A ⇧	0.00	−2.1	Esfenvalerate	Pyrethroid insecticide	EDS		
A ⇩	22.86	−2.2	Esfenvalerate	Pyrethroid insecticide	EDS		
B ⇧	37.98	−3.6	*Thiacloprid*	Neonicotinoid insecticide	CC	Yes	
B ⇩	34.90	−3.3	*Thiacloprid*	Neonicotinoid insecticide	CC	Yes	2
C ⇧	23.42	−2.5	Esfenvalerate	Pyrethroid insecticide	EDS		
C ⇩	16.42	−2.1	Esfenvalerate	Pyrethroid insecticide	EDS		2
D ⇧	52.00	−3.9	*Imidacloprid*	Neonicotinoid insecticide	CC		
D ⇩	46.62	−3.8	*Imidacloprid*	Neonicotinoid insecticide	CC		1
E ⇧	10.66	−3.0	*Imidacloprid*	Neonicotinoid insecticide	CC	Yes	
E ⇩	13.98	−2.5	*Imidacloprid*	Neonicotinoid insecticide	CC	Yes	3
F ⇧	51.13	−5.2	Carbendazim	Fungicide	CC		
F ⇩	29.77	−3.8	*Imidacloprid*	Neonicotinoid insecticide	CC		26
G ⇧	44.08	−4.6	Azoxystrobin	Fungicide	CC		
G ⇩	37.58	−4.6	Azoxystrobin	Fungicide	CC		1

Table 2b TU_{max} and SPEAR values at the up- and downstream sampling sites in July.

Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites. WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg. TU_{max}: highest Toxic Unit value of all pesticides detected at each sampling site, log-transformed; RAC: Regulatory Acceptable Concentration (UBA, 2016).

[a] Sampling technique that obtained the compound concentration leading to TUmax (CC: Chemcatcher, EDS: event-driven sampler).

[b] Details are found in SI Table 5.

[c] Increase in downstream TUmax compared to the respective upstream TUmax, based on the values before log-transformation.

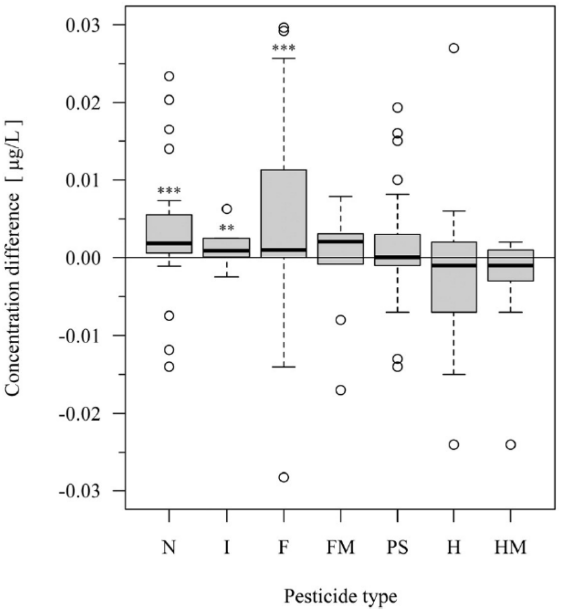
Both up- and downstream of the effluent discharge points, imidacloprid and thiacloprid occasionally exceeded the legally permissible levels of pesticides in water (Regulatory Acceptable Concentrations; RACs) derived by UBA (2016) according to the guidelines developed by EFSA (2013). In May, at the Blankenburg/Harz WWTP, a downstream isoproturon concentration above the maximum allowable surface water concentration of 1.0 µg L^{-1} (EC, 2013) outweighed the toxic pressure of imidacloprid (SI Fig. 4; SI Table 5). Isoproturon has been one of the most extensively used herbicides for weed control in cereals in Europe (Bach et al., 2000), but its approval was withdrawn in 2016 (EC, 2016).

A dilution effect of WWTP effluents was observed where downstream pesticide concentrations fell below the upstream pollution level (33% of all pairwise downstream/upstream comparisons), leading to lower *TU* values of the respective compounds. As a result, in some cases, the downstream pesticide toxicity, $TU_{\max}$, actually originated from agricultural field runoff in upstream reaches (SI Fig. 4 [b], Straßberg WWTP; SI Fig. 5 [f], Ballenstedt WWTP).

Five of the compounds detected are classified as priority substances under the WFD (EC, 2013, 2000): atrazine, diuron, isoproturon, simazine, and terbutryn. Apart from isoproturon, these substances were not authorised for use in Europe during the time of the present study (EC, 2007, 2004a, 2004b, 2002). They occurred irregularly at up- and downstream sampling sites and showed little ecotoxicological relevance (see also Fig. 2). Residues of ‘legacy pesticides’ are commonly found in aquatic environments (McKnight et al., 2015; Münze et al., 2015; Rasmussen et al., 2015).

3.2 Pharmaceuticals in streams

Pharmaceuticals and personal care products (PPCPs) are known to occur in WWTP effluents and surface waters (e.g., Kümmerer, 2009), where they may affect the same target pathways or biological compartments in lower vertebrates, invertebrates, and plants (Fent et al., 2006). In contrast to pesticides, with their pulsed short-term presence in surface waters (Liess et al., 1999), PPCPs are more continuously emitted into aquatic environments (Ternes, 1998), making them ‘pseudo-persistent’ contaminants (Petrovic et al., 2009). Some pharmaceuticals such as ibuprofen are known to be effectively removed from wastewater; however, other substances such as carbamazepine, diclofenac, iopamidol, and iopromide have low removal rates and are therefore often detected in WWTP effluents (Clara et al., 2005; Joss et al., 2006; Strenn et al., 2004).



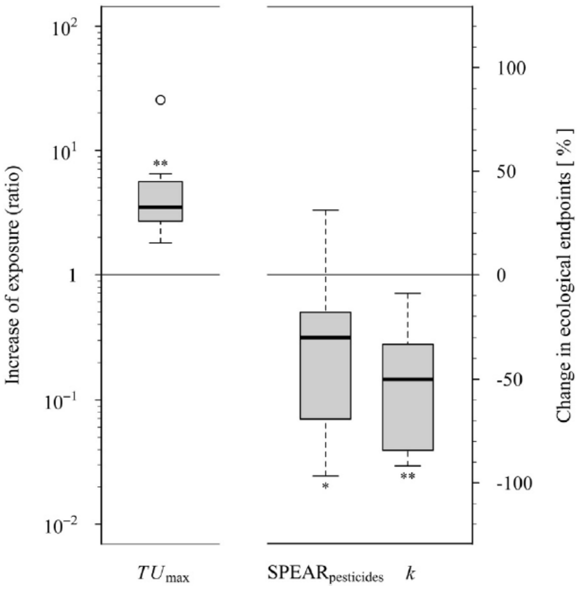


Fig. 2 WWTP-induced input of pesticides quantified as the difference between the down- and upstream concentrations. Different types of pesticides and metabolites were considered separately. N: neonicotinoid insecticide (acetamiprid, imidacloprid, and thiacloprid); F: fungicide; I: non-neonicotinoid insecticide; PS: priority substance under the WFD (the herbicides atrazine, diuron, isoproturon, simazine, and terbutryn); FM: fungicide metabolite; H: herbicide; HM: herbicide metabolite. The WWTP-induced input of insecticides and fungicides into receiving waters was significant (one-sided Mann-Whitney U test; neonicotinoids and fungicides: $p < 0.001$ [***], non-neonicotinoid insecticides: $p < 0.01$ [**]).

Fig. 3 WWTP-induced changes in TUmax, SPEARpesticides, and k. Only downstream samples with a significant increase in pesticide toxicity were considered (50% of events). Median TU_{max} increased by a factor of three (shown on a logarithmic scale; one-sided Mann-Whitney U test, p b 0.01 [**]). Species vulnerable to pesticides, expressed as SPEARpesticides, significantly declined (by 40%; one-sided t-test, $p < 0.05$ [*]). Leaf litter degradation, expressed as the daily breakdown rate, k, significantly decreased (by 53%; one-sided t-test, $p < 0.01$ [**]).

In the present study, the five measured pharmaceuticals were detected at concentrations of up to 4.0 µg L^{-}1 (iopamidol; Tables 1a, 1b; SI Figs. 4, 5). With mean *TU* values between -7.5 (X-ray contrast media) and -4.9 (diclofenac), they imposed only a very low acute toxic pressure upon macroinvertebrates. Such *TU* levels are not expected to alter the invertebrate community structure over the long term (Liess and von der Ohe, 2005). This is in accordance with many studies reporting that PPCPs are unlikely to pose a risk for acute toxic effects in surface waters (Carlsson et al., 2006; Cleuvers, 2004, 2003; Fent et al., 2006; Gros et al., 2010; Ternes and Hirsch, 2000); only a few investigations have concluded otherwise (De Castro-Català et al., 2015; Hernando et al., 2006). Nevertheless, there is still a possibility that PPCPs may exert chronic effects in surface waters (Fent et al., 2006; Ferrari et al., 2003;

Ginebreda et al., 2010; Gros et al., 2010; Huerta et al., 2015; Muñoz et al., 2009). However, for the WWTPs investigated here, we expect that all observed effects were caused by pesticides and not by pharmaceuticals. We explain the high relevance of pesticides in the present study by the fact that the selected WWTPs were located in an agricultural catchment.

3.3 Macroinvertebrate community composition

In total, 51 families from 14 orders of aquatic macroinvertebrates were identified, with 43 families in May and 39 families in July (families per site: 15 ± 0; SI Tables 6a, 6b). There was a congruence of 61% in the aquatic fauna during the two sampling campaigns. The percentage of taxa vulnerable to pesticides ($\text{SPEAR}_{\text{pesticides}}$) ranged from 0 to 62.59 in May and from 0 to 52.0 in July. In 50% of events, WWTP effluents increased the in-stream pesticide toxicity by a median factor of three. Here, the $\text{SPEAR}_{\text{pesticides}}$ values significantly declined (by 40%) compared to those in the upstream reaches (one-sided t-test, $p < 0.05$; Fig. 3).

The stepwise forward selection identified TU_{max} ($p < 0.0001$, df = 1, $Chi^2 = 16.9$), NH_4 ($p = 0.0004$, df = 1, $Chi^2 = 12.4$), TDO ($p = 0.03$, df = 1, $Chi^2 = 4.8$) and NO_2 ($p = 0.04$, df = 1, $Chi^2 = 4.0$) as predictor variables for $\text{SPEAR}_{\text{pesticides}}$. No interactions between TU_{max} and the other parameters were found by the LMM. The hierarchical partitioning showed that TU_{max} explained 46.9% of the variance, followed by NO_2 (23.7%), NH_4 (23.1%), and TDO (6.3%; SI Fig. 6). In contrast, the macroinvertebrate community composition was not influenced by the following parameters: NO_3, PO_4, TOC, BOD, pH, water temperature, and stream flow velocity. Pesticides and nutrients concurrently enter surface waters; distinguishing these groups of chemicals with regard to their effects on stream macroinvertebrates is challenging (Alexander et al., 2013). However, with both the explanatory weight and the significance level being the highest among the variables suggested, we considered TU_{max} the driving force behind the observed changes in the community structure (according to $\text{SPEAR}_{\text{pesticides}}$). Furthermore, in the aforementioned 50% of events, the downstream concentrations of NO_2, NH_4, and TDO were not significantly higher than their upstream counterparts ($p > 0.05$), in contrast to the significant increase in TU_{max} ($p < 0.001$). In both sampling campaigns, $\text{SPEAR}_{\text{pesticides}}$ was significantly negatively correlated with TU_{max} (LMM; May: $p < 0.001$, $R^2 = 0.69$; July: $p < 0.001$, $R^2 = 0.66$; Fig. 4). Ecological impact was not restricted to pesticide concentrations above the RACs proposed by UBA (2016; SI Table 5).

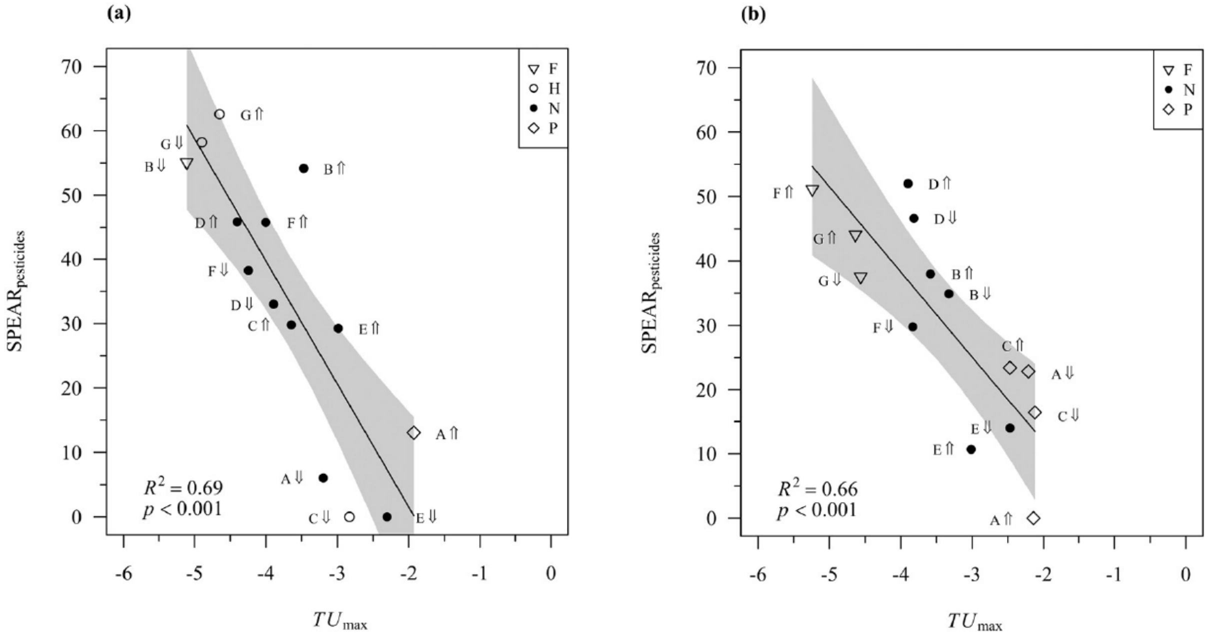


Fig. 4 Relationship between the macroinvertebrate community composition expressed as SPEAR$_{pesticides}$ and pesticide-driven water toxicity expressed as TU$_{max}$ in May (a) and in July (b).

Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; Arrows pointing downwards indicate downstream sampling sites. Pesticide symbols indicate the pesticide class providing TU$_{max}$ (F: fungicide; H: herbicide; N: neonicotinoid (acetamiprid, imidacloprid, or thiacloprid); P: pyrethroid (esfenvalerate)). Shaded areas show 95% confidence intervals. The linear regressions (LMM) are significant (May: $p < 0.001$, $R^2 = 0.69$; July: $p < 0.001$, $R^2 = 0.66$). WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.

Contrary to Ashauer (2016), who concluded “that those macroinvertebrates that are vulnerable to pesticide pollution are also vulnerable to pollution by micropollutants from WWTPs”, we identified pesticide toxicity as the cause of the observed ecological effects on stream macroinvertebrates. Our study of seven WWTPs enabled us to compare multiple up- and downstream sampling sites in this regard. We observed that the highest toxicity was always exerted by pesticides, and the respective TU_{max} values correlated well with the SPEAR$_{pesticides}$ index, the integrative biological measure of pesticide effects. At up- and downstream sampling sites, TU_{max} was equally related to SPEAR$_{pesticides}$; the residuals were distributed around the regression line without any indication that hidden factors or substances released from WWTPs systematically increased biological effects downstream of the effluent discharge points (Fig. 4). The same held true for the relationship between the leaf litter degradation expressed as the daily breakdown rate, k, and SPEAR$_{pesticides}$ and also for the relationship between k and TU_{max} (Figs. 5, 6; see Section 3.4, Decomposition of leaf litter).

The occurrence of community alterations at TU_{max} values between -4 and -3 corresponds to the ecologically relevant pesticide toxicity identified in previous studies at the ecosystem level (Beketov et al., 2009; Bereswill et al., 2013; Liess and von der Ohe, 2005; Münze et al., 2015; Schäfer et al., 2012). Numerous environmental stressors act upon the fauna in surface waters; hence, the individual sensitivity to toxicants is fundamentally higher at the ecosystem level than under laboratory conditions (Liess et al., 2016).

3.4 Decomposition of leaf litter

3.4.1 Macroinvertebrates (coarse mesh cases)

For both sampling campaigns, the average leaf litter breakdown rate, *k*, was 0.023 ± 0.003 d^{-1}, with a mean total leaf mass loss of 44 ± 4% at upstream sites and 33 ± 4% at downstream sites. The leaf litter breakdown rates observed in the present study were consistent with values reported for birch leaves (Collen et al., 2004; Petersen and Cummins, 1974). In 50% of events, WWTP effluents increased the in-stream pesticide toxicity by a median factor of three. Here, the *k* values significantly decreased (by 53%) compared to those in the upstream reaches (one-sided t-test, $p < 0.01$; Fig. 3).

The stepwise forward selection identified $\mathrm{SPEAR}_{\mathrm{pesticides}}$ ($p < 0.0001$, df = 1, $Chi^2 = 23.5$), NO_2 ($p = 0.0007$, df = 1, $Chi^2 = 11.5$), EC ($p = 0.003$, df = 1, $Chi^2 = 9.0$), and TDO ($p = 0.02$, df = 1, $Chi^2 = 5.4$) as predictor variables for *k*. The hierarchical partitioning showed that $\mathrm{SPEAR}_{\mathrm{pesticides}}$ explained 44.3% of the variance, followed by NO_2 (32.5%), EC (18.3%), and TDO (4.9%; SI Fig. 7). In both sampling campaigns, *k* was significantly positively correlated with $\mathrm{SPEAR}_{\mathrm{pesticides}}$ (LMM; May: $p < 0.001$, $R^2 = 0.82$; July: $p < 0.001$, $R^2 = 0.77$; Fig. 5) and significantly negatively correlated with TU_{max} (LMM; May: $p < 0.01$, $R^2 = 0.58$; July: $p < 0.001$, $R^2 = 0.70$; Fig. 6). The influence of water temperature on *k* was negligible, presumably due to the relatively homogeneous temperature distributions at the selected sites (see SI Tables 2a, 2b). This might explain the absence of the relationship between leaf litter decomposition and water temperature that has been reported elsewhere (Friberg et al., 2009; Martinez et al., 2014).

Decomposition of organic matter in aquatic environments is not stressor-specific and responds to a wide variety of habitat conditions (Gessner and Chauvet, 2002; Webster and Benfield, 1986). The presence of shredding invertebrates and microbial decomposers is of vital importance to the process (Bärlocher and Kendrick, 1975; Cummins et al., 1973).

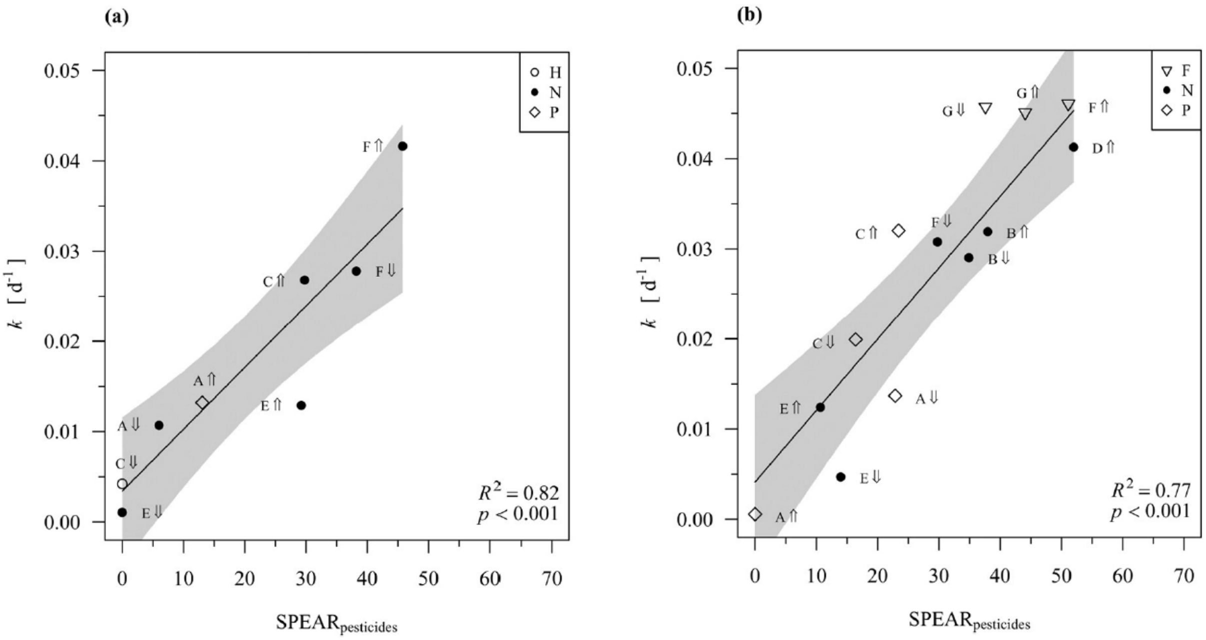


Fig. 5 Relationship between the leaf litter breakdown rate, *k*, and macroinvertebrate community composition, expressed as SPEAR$_{pesticides}$, in May (a) and in July (b).

Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites. Because of the loss or damage of leaf litter bags at six sites in May and at one site in July, data for eight and 13 sites were available for analysis, respectively.

Pesticide symbols indicate the pesticide class providing TU$_{max}$ (F: fungicide; H: herbicide; N: neonicotinoid (acetamiprid, imidacloprid, or thiacloprid; P: pyrethroid (esfenvalerate)).

Shaded areas show 95% confidence intervals.

The linear regressions (LMM) are significant (May: $p < 0.001$, $R^2 = 0.82$; July: $p < 0.001$, $R^2 = 0.77$).

WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.

Of all macroinvertebrate families collected in the present study, approximately 25% belonged to the shredder feeding guild, and approximately half of these shredder families are classified as SPEAR taxa (May: 50%; July: 54%). ‘SPEAR shredders’ comprised the families *Leuctridae*, *Nemouridae*, and *Taeniopterygidae* from the order *Plecoptera*, as well as the families *Lepidostomatidae*, *Leptoceridae*, *Limnephilidae*, and *Sericostomatidae* from the order *Trichoptera*. We observed a significant negative correlation between the proportion of pesticide-sensitive ‘SPEAR shredder’ families and TU_{max} (LMM; $p < 0.001$, $R^2 = 0.38$; not shown). This reduction in shredders may be part of the cause for the relationships between *k* and SPEAR$_{pesticides}$ and between *k* and TU_{max}. A reduction in shredders is known to slow leaf decomposition processes (Flores et al., 2014; Rasmussen et al., 2008; Wallace et al., 1982).

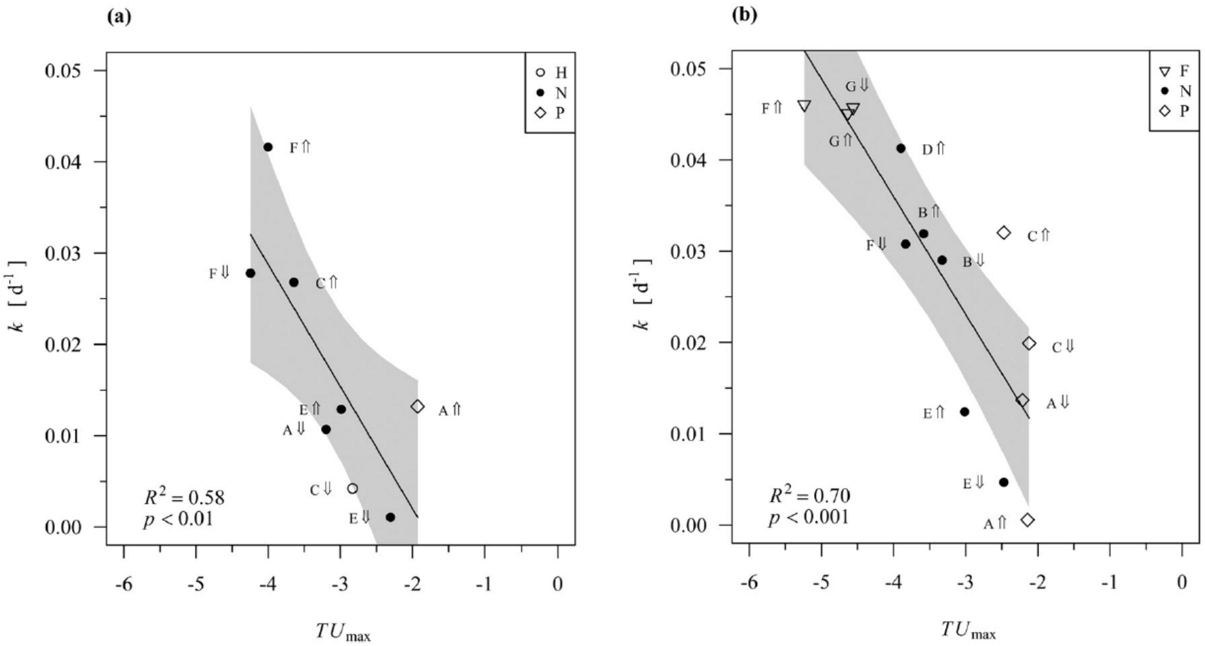


Fig. 6 Relationship between the leaf litter breakdown rate, *k*, and pesticide toxicity, expressed as TU_{max}, in May (a) and in July (b).

Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites. Because of the loss or damage of leaf litter bags at six sites in May and at one site in July, data for eight and 13 sites were available for analysis, respectively.
Pesticide symbols indicate the pesticide class providing TU_{max} (F: fungicide; H: herbicide; N: neonicotinoid (acetamiprid, imidacloprid, or thiacloprid; P: pyrethroid (esfenvalerate)).
Shaded areas show 95% confidence intervals.
The linear regressions (LMM) are significant (May: $p < 0.01$, $R^2 = 0.58$; July: $p < 0.001$, $R^2 = 0.70$).
WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.

3.4.2 Microorganisms (fine mesh cases)

Fungicides acting on microbial decomposers have also been shown to affect the breakdown of leaf litter in streams (Fernández et al., 2015; Rasmussen et al., 2012; Zubrod et al., 2011). However, despite the observed significant effluent-based input of fungicides (Fig. 2), our results do not support a considerable influence of a fungicide-induced decrease in leaf litter degradation; the mean leaf mass loss from fine mesh cases was very similar at up- and downstream sampling sites, resulting in almost identical mean *k* values within each sampling campaign (May: $k = 0.017 \pm 0.003$ d^{-1} and 0.018 ± 0.006 d^{-1}; July: $k = 0.023 \pm 0.002$ d^{-1} and 0.022 ± 0.001 d^{-1}). Furthermore, *k* derived from fine mesh cases did not correlate with TU_{max} (LMM; $p = 0.51$, $R^2 = 0.02$; not shown).

This is in accordance with the findings of Schäfer et al. (2012), who reported a similarly low R^2 value for the relationship between microbial leaf breakdown and TU_{max} ($R^2 = 0.04$; Schäfer et al., 2012; Appendix A). Hence, the toxicant-related reduction in leaf litter degradation was related to the proportion of 'SPEAR shredder' families rather than microbial activity. Accordingly, leaf degradation was mostly governed by the community composition of macroinvertebrates based on ecological traits that make species sensitive to pesticides (Liess and von der Ohe, 2005).

4 Conclusion

WWTP effluents substantially increased insecticide and fungicide concentrations in receiving waters. Neonicotinoids showed the highest ecological relevance for the composition and the ecological function of invertebrate communities. Therefore, pesticide emissions from WWTPs need to be reduced so that the resulting in-stream toxicity in receiving waters does not exceed the ecotoxicologically relevant *TU* range between -4 and -3. This can be achieved by applying recently developed strategies such as ozonation and activated carbon treatment as shown by Eggen et al. (2014).

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CHAPTER 4

Forested headwaters mitigate pesticide effects on macroinvertebrate communities in streams: Mechanisms and quantification

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Abstract

Pesticides impact invertebrate communities in freshwater ecosystems, leading to the loss of biodiversity and ecosystem functions. One approach to reduce such effects is to maintain uncontaminated stream reaches that can foster recovery of the impacted populations. We assessed the potential of uncontaminated forested headwaters to mitigate pesticide impact on the downstream macroinvertebrate communities in 37 streams, using SPEAR$_{\text{pesticides}}$ index. Pesticide contamination was measured with runoff-triggered techniques and Chemcatcher$^{®}$ passive samplers. The data originated from 3 field studies conducted between 1998 and 2011. The proportion of vulnerable species decreased after pesticide exposure even at low toxicity levels ($-4 < TU_{max} \leq -3$). This corresponds to pesticide concentrations down to 3-4 orders of magnitude below the LC_{50} value for standard test organisms. The toxicity of pesticides and the length of the forested reaches together explained 78% of variation in the community composition (SPEAR$_{\text{pesticides}}$). The proportion of vulnerable species doubled within the measured length of the forested stream section (0.2-18 km), whereas other characteristics of the forest or abiotic water parameters did not have an effect within the measured gradients. The presence of forested headwaters was not associated with reduced pesticide exposure 3 km downstream and did not reduce the loss of vulnerable taxa after exposure. Nevertheless, forested headwaters were associated with the absence of long-term pesticide effects on the macroinvertebrate community composition. We conclude that although pesticides cause the loss of vulnerable aquatic invertebrates even at low toxicity levels, forested headwaters can enhance the recovery of their populations in agricultural landscapes.

Keywords: Recolonisation; Riparian forest; Recovery; Agriculture; Pesticides; Invertebrates; SPEAR

1 Introduction

Pesticides are a major stressor for freshwater communities on a continental scale (Malaj et al., 2014). Despite continued efforts to avoid long-term effects of pesticides in the environment through an improved pesticide registration process in Europe (EC, 2009; EC, 2013), field studies conducted within the last decade have consistently shown the effects of pesticides on stream invertebrates (Bereswill et al., 2013; Liess and von der Ohe, 2005; Rasmussen et al., 2013; Schäfer et al., 2012). Pesticide contamination of streams and rivers that exceeds toxic thresholds for invertebrates was also reported in studies from Australia (Sánchez-Bayo and Hyne, 2014; Schäfer et al., 2011) and North America (Kuo et al., 2012; Stone et al., 2014). The effects of such contamination were associated with reduced regional biodiversity (Beketov et al., 2013) and impaired ecosystem functions (Rasmussen et al., 2012b; Schäfer et al., 2012, 2007).

The main pesticide application period in Central and Northern Europe occurs between spring and midsummer (Bundschuh et al., 2014; Liess et al., 1999). After strong rainfall events pesticides enter lotic environments through field runoff and drainage systems, resulting in pulses of exposure for aquatic organisms (Liess and Schulz, 1999; Reichenberger et al., 2007). The SPEAR index (Species At Risk) for pesticides ($\text{SPEAR}_{\text{pesticides}}$) was specifically developed (Liess and von der Ohe, 2005) and validated (Beketov et al., 2009; Schäfer et al., 2007) to detect the effects of such contamination pulses on macroinvertebrate community composition. Using this index, it was shown that the effect of pesticides occurs below the thresholds predicted even by the most conservative first tier risk assessment in the EU (Liess and von der Ohe, 2005; Rasmussen et al., 2012a; Schäfer et al., 2012). Furthermore, pesticide application is predicted to increase in temperate regions due to climate change, potentially exacerbating their impact on non-target organisms (Kattwinkel et al., 2011; Noyes et al., 2009). Thus, developing mitigation measures in parallel to improving the pesticide regulation is crucial to prevent damage to the environment.

$\text{SPEAR}_{\text{pesticides}}$ has been applied to identify impact-reducing factors on a landscape level. Several previous studies have shown that upstream forested reaches (UFR) alleviate the impact of pesticides on the macroinvertebrate community composition at downstream sites (Bunzel et al., 2014; Liess and von der Ohe, 2005; Schäfer et al., 2007; von der Ohe and Goedkoop, 2013). There was a higher proportion of pesticide-vulnerable taxa at the sites with UFR compared to the sites without UFR. However, to apply such knowledge to the development of mitigation strategies, a better understanding of the underlying mechanisms is required.

The increased proportion of pesticide-vulnerable taxa downstream of the forested reaches was mainly observed several months after contamination and was explained by enhanced recolonisation processes (Liess and von der Ohe, 2005). Most previous studies did not specifically consider the influence of the UFR during the main period of pesticide application, except for Schäfer et al. (2007), who observed the influence of forested stream reaches less than 1 month after pesticide exposure. In a different study, Schäfer et al. (2012) showed that the thresholds for community-level pesticide effects were higher in streams with UFR. However, it remained unknown whether the higher threshold is related to reduced mortality of vulnerable invertebrates after pesticide exposure or enhanced recolonisation. Furthermore, Harding et al. (2006) showed that forested headwaters can change physicochemical habitat characteristics downstream. However, none of the previous studies using the SPEAR approach investigated exactly which characteristics of the forest influence the vulnerable taxa downstream of the forested reach.

Hence, the aim of our study was to investigate the influence of the UFR and associated parameters on pesticide-impacted communities. We examined the $\text{SPEAR}_{\text{pesticides}}$ values before and shortly after (< 1.5 months) the measured contamination in 37 streams during the main period of pesticide application. Additionally, to identify the most important parameters associated with the UFR, we analyzed the influence of selected characteristics of the forest (length of forested reach, surface area, direct and downstream distance to sampling site) and abiotic conditions at the sampling sites on macroinvertebrate community composition, using $\text{SPEAR}_{\text{pesticides}}$.

2 Materials and Methods

2.1 Study area and sampling schedule

We compiled data on pesticide contamination, physicochemical habitat characteristics and macroinvertebrate community composition collected in 37 streams, with one sampling site per stream. The data were collected in three field studies conducted between April and June in 1998-2000 (Liess and von der Ohe, 2005), 2010 (Münze et al., 2015) and 2011 (unpublished data). From the study of Liess and von der Ohe (2005), we selected the sampling year with the highest contamination for repeatedly sampled sites. The streams were located in a crop-growing area between the cities of Braunschweig and Halle in Central Germany (Fig. 1). The dominant crops in the area were winter wheat, winter barley, sugar beets, and rape seed. Sites were selected so that no dredging occurred at least one year before or during the sampling and there were no sources of industrial or municipal effluents in the entire watercourse upstream of the investigated site. Arable land was present in the catchment upstream of all sampling sites. Twenty-three sampling sites additionally had upstream forested reaches (UFR), whereas 17 did not. UFR were defined as forested riparian corridors at least 100 m wide and 200 m long. They were located on average 3.3 km and a maximum of 11 km upstream of the sampling site. To ensure that UFRs were not contaminated with agricultural pesticides, they were chosen so that no arable land was present upstream of these reaches. We assessed the macroinvertebrate community composition once before the peak measured pesticide contamination and once after. That corresponded to invertebrate sampling in April/May or in May/June, respectively. However, the 5 streams from the field study in 2010 were sampled only once, after the pesticide input was measured, in June. Thus, these data were not included in the analysis involving $\text{SPEAR}_{\text{pesticides}}$ values before contamination, but were included in all other analyses.

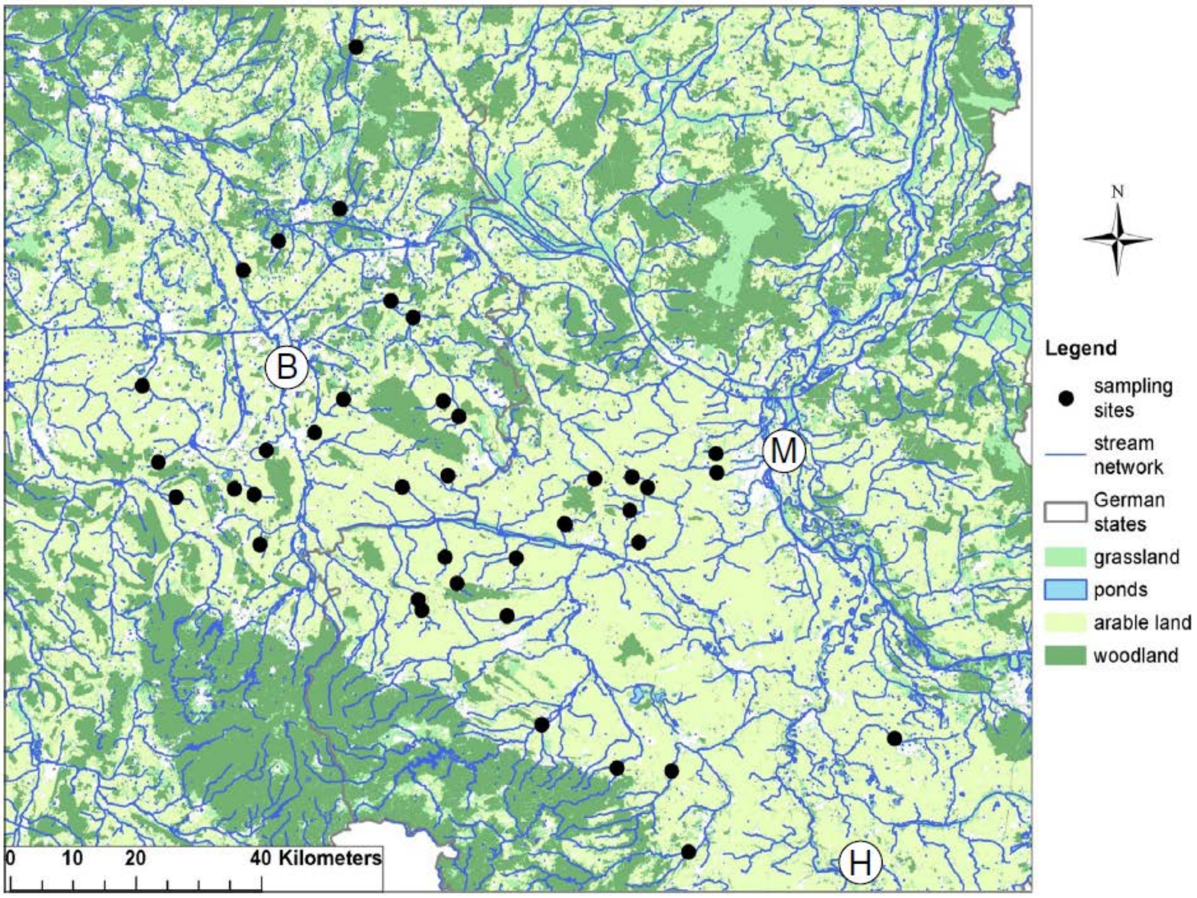


Fig. 1 Location of the sampling sites: cities with over 200,000 inhabitants are marked with letters: H: Halle; M: Magdeburg; B: Braunschweig.

2.2 Environmental variables

Physicochemical parameters were measured in the field during the invertebrate sampling following the pesticide contamination. In the field investigations in 1998-2000 and in 2011, dissolved oxygen concentrations, pH and conductivity were measured using Extech dissolved oxygen and conductivity meters (DO600 and EC500 ExStik® II, Extech Instruments Corp., Waltham, USA). The phosphate and nitrate concentrations were measured using colorimetric tests by Visicolor® (Macherey&Nagel, Düren, Germany). For the study in 2010, the nutrient concentrations and conductivity measurements were obtained from the monitoring stations of the municipal water management agency (LHW Saxony-Anhalt) located near the sampling sites. In the study conducted in 2011, the nutrient concentrations were not measured (11 out of 37 sites).

2.3 Pesticide concentrations

The runoff containing the highest pesticide concentrations occurred between April and mid-June, following rainfall events of ≥ 10 mm/day. Pesticide contamination was quantified using runoff-triggered sampling techniques (Liess and von der Ohe, 2005) and duplicate Chemcatcher® passive samplers in the polar configuration (Stephens et al., 2005). In 1998-2000, automated runoff samplers and runoff-triggered samplers were used, in 2011, runoff-triggered and Chemcatcher® and in 2010, only Chemcatcher®. For an overview of sampling methods applied at each site, please refer to the supplementary information (Table S1).

The runoff-triggered and automated samplers are described in detail by Liess and von der Ohe (2005). The automated sampler collected 500 mL samples every 8 minutes during 1 hour as soon as runoff was indicated by conductivity and water level sensors (a decline in conductivity by more than 10% in 10 minutes and water level rise by at least 5 cm). The runoff-triggered samplers, fixed bottles, were filled with water within 1 hour through a thin glass or silicone tube, as soon as the water level rose by at least 5 cm. The obtained water samples during the sampling campaign 1998-2000 were pre-filtered on site, cooled to 4°C and analyzed with a gas chromatograph (GC)/electron capture detector (GC NP 5990, Series II; Hewlett-Packard, Avondale, PA,USA). The water samples collected in 2011 were stored at 4°C and delivered to an analytical laboratory within 24 hours for further analysis on GC/coupled to mass spectrometer (MS) and a liquid chromatograph (LC)/MS (Eurofins Umwelt Ost GmbH, Germany).

A detailed description of the Chemcatcher® preparation and analysis is provided in the handling protocol from the patent holder (University of Portsmouth, 2012). Briefly, the conditioning of the samplers before deployment was performed according to the simplified procedure (Vermeirssen et al., 2009). The SDB-RPS receiving phase (styrene-divinylbenzene - reversed phase sulfonate) and the Supor® 200 PES (polyethersulfone) diffusion limiting membranes from Pall Corporation (Port Washington, NY, USA) were soaked overnight in GC grade methanol and for 2 hours in HPLC water. The PTFE sampler housings were cleaned and soaked in methanol overnight.

The passive samplers were deployed in duplicate for 3 weeks when heavy rainfalls were expected according to the weather forecast. Gypsum passive flow monitors (PFM; O'Brien et al., 2009) were deployed together with passive samplers to obtain average flow velocities (O'Brien et al., 2011). Upon retrieval, the samplers were transported and stored at 4°C until extraction. The receiving phase was extracted by a succession of GC-grade methanol, acetone and a methanol-acetone 1:1 mixture in an ultrasonic bath for a total of 45 minutes. This procedure gave one extract per sample, which was evaporated until 0.4mL on Turbovap 2 evaporation system from Biotage (Uppsala, Sweden). The extract was transferred to the sample vial and adjusted to 1 mL. Solutions of 10 ng/µL alachlor D-13 and pirimicarb D-6 in methanol were used as standards for extraction control and volume control, respectively. The extracts were analyzed using LC/MS (Agilent 1100 liquid chromatograph from Agilent Technologies, Waldbronn, Germany coupled with an API 2000 Mass Spectrograph from AB Sciex Darmstadt, Germany).

The time-weighted average concentrations of pesticides from passive samplers (c_{TWA}) were determined according to the following equation (Schäfer et al., 2008):

$$CTWA = \frac{\mathrm{m}}{Rs \times T} \tag{1}$$

where Rs is the compound sampling rate (L/day); T is the time of Chemcatcher exposure (days); and m is the accumulated mass of the compound (µg). Due to the lack of compound-specific sampling rates in the literature for the majority of the sampled compounds and flow velocities, we estimated generic Rs values, as recommended by Escher et al. (2011).

To estimate Rs, we used literature Rs values for hydrophilic pesticides and polar Chemcatcher configuration (SDB-RPS, PES) at different flow velocities (Table S2). The compound-specific sampling rates found in literature could be sub-divided into high and low values which were used to derive two regression equations (Eq. 2 and 3 below). All measured compounds were assumed to have a high sampling rate, unless (i) they are known to have a low sampling rate from the literature (Table S2), (ii) have log $K_{\mathrm{OW}} < 1$, or (iii) are unstable in water within the time of the Chemcatcher® exposure (Table S3).

$$Rs\ (high) = 0.85 \times \mathrm{V} + 0.014 \tag{2}$$

$$Rs(low) = 0.47 \times \mathrm{V} + 0.0004 \tag{3}$$

where V is the flow velocity in cm/s.

For each sampling site, the generic *Rs* values were calculated, using the measured flow velocity and the empirical equations above. Considering pulsed contamination and the semi-polar nature of pesticides detected with the passive samplers (log $K_{\mathrm{OW}} < 4$), the pesticide exposure was estimated to last 24 hours, as indicated by the work of Gallé (2013). Accordingly, we assumed the time of exposure of 1 day for the calculation of c_{TWA}.

2.4 Quantification of pesticide toxicity

To estimate the toxicity of the detected pesticides to invertebrate communities, we computed the toxic units (TU) for each sampling event, according to Liess and von der Ohe (2005) (Eq. 4):

$$TU = max_{i=1}^{n}\ log\left(\frac{Ci}{LC50i}\right) \tag{4}$$

where C_i is the concentration of compound *i* and LC_{50i} is the 48 hour lethal concentration for 50% of the population of the reference organism.

Regarding the reference organism, we applied the LC_{50} values based on the most sensitive of the standard test species *Daphnia sp.* or insect larvae of *Chironomus sp.* as defined in the current EU regulation on plant protection products (EC, 2013). Using only *Daphnia sp.*, as in some of the previous studies (Liess and von der Ohe, 2005; Schäfer et al., 2007), was not appropriate due to the presence of neonicotinoids in our samples. Neonicotinoids are much less toxic to *Daphnia sp.* than to insects. Therefore, insect larvae should be used to estimate the toxicity of these compounds to aquatic invertebrate communities (Schäfer et al., 2013). There was no data on the standard insect species *Chironomus sp.* for the insecticides Indoxacarb and Thiacloprid. Thus, the respective LC_{50} values for the TU calculation were based on other insect larvae for these compounds, as recommended in the European Commission Guidance Document for Aquatic Toxicology (EC, 2002). The list of LC_{50} values used for each substance is shown in the supplementary material (Table S4). The median LC_{50} for each compound and species was calculated from the values taken from the ecotoxicology database of the US Environmental Protection Agency (USEPA, 2014). If the data was not available in this database, the Pesticide Properties Database of the University of Hertfordshire was used instead (University of Hertfordshire, 2013).

For interpretation of the TU, we assumed that the sites with a TU $\leq$ -4.5 represented control sites, where no pesticide effects are expected. Thus, all the TU values below -4.5 were set to -4.5. We based this assumption on thresholds for pesticide effects derived by Schäfer et al. (2012) from 8 field studies in Europe and Australia. Other TU calculation methods, such as: (i) using the LC_{50} of the most sensitive invertebrate for each compound (Schäfer et al., 2013) and (ii) estimating additive effects of the pesticide mixture (TU_{sum}, SI text) lead to the same results (see examples in Table S6).

2.5 Determination of pesticide effects

Macroinvertebrate communities were sampled once per month in April, May and June in 1998-2000, in May and June in 2011 and only in June in 2010 with a Surber sampler (area 0.062 m^2; 1-mm mesh). Four samples were collected from different habitat types at each site and pooled, so that there was only one representative sample per site. The invertebrates were stored in plastic tubes with 70% ethanol. Then the taxa were identified to the family level for the samples collected in 2010 and 2011. The data on macroinvertebrates from the study in 1998-2000 were converted to the family level to make it comparable with the other two studies. The effects of pesticides on macroinvertebrates were quantified with the pesticide-specific SPEAR bioindicator concept developed by Liess and von der Ohe (2005). The index $SPEAR_{pesticides}$ was calculated using the online software “SPEAR calculator” (http://www.systemecology.eu/SPEAR/index.php, version 10.10.2012).

2.6 Spatial analysis

Spatial analysis was performed in ArcGIS 10.1 using the shape files from the ATKIS database (scale 1:25,000), which were provided by the German Federal Agency for Cartography and Geodesy (Bundesamt für Kartographie und Geodäsie, Leipzig, Germany). The parameters of the forested upstream reaches, such as the surface area of the forest patch (A), the length of the UFR (L), the distance (D) between the sampling site (S) and the forest patch, were measured for each site (Fig. 2). Additionally, to analyze the availability of undisturbed habitat (in terms of pesticide exposure) in the vicinity of the sampling sites, we measured the shortest direct distance to undisturbed habitat, which was defined as a UFR on the same or on a different stream.

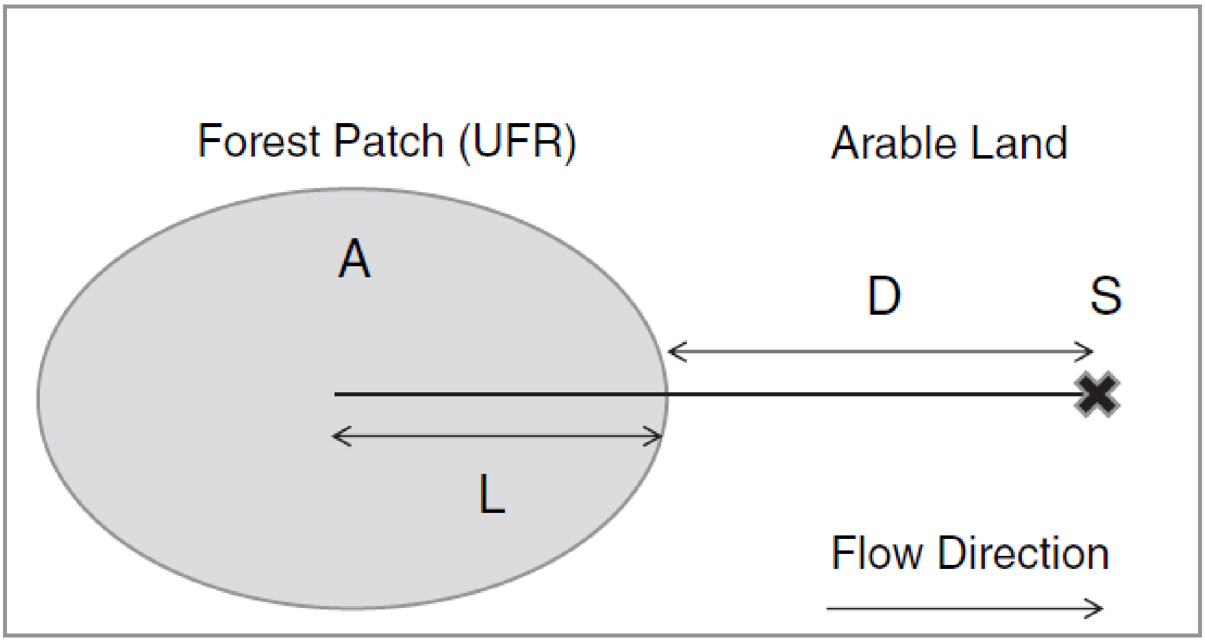


Fig. 2 A schematic representation of selected measured parameters: the surface area of the forest patch (A), the length of the stream inside the forest patch (L), the distance between the sampling site and the forest patch (D). A stream flowing from the forest patch into arable land is shown with a thick black line, where S is the sampling site.

2.7 Hydromorphology

The GIS shape files on hydromorphological characteristics of each sampling site were obtained from the municipal water management authorities (LHW, 2009; NLWKN, 2008). Structural habitat parameters were estimated for 100 m sections of streams as described in LAWA (2000). We selected the stream bed structure as a representative parameter for stream hydromorphology. This parameter was chosen instead of the combined parameter total hydromorphological quality because of the lack of data on the combined parameter for the state of Lower Saxony. In addition, characteristics of the stream bed structure were the most important structural parameters influencing macroinvertebrate communities in a previous study (von der Ohe and Goedkoop, 2013). The stream bed quality is described in terms of its departure from the potential natural state using a seven-point scale from 1: unchanged, to 7: completely changed (LAWA, 2000). The structural stream bed quality class was treated as a numeric variable for simplicity, as it follows a logical order of decreasing habitat quality from 1 to 7.

2.8 Data analysis

To determine which factors affected the invertebrate community, we performed simple regression analysis with $SPEAR_{pesticides}$ as a response variable (see results in Table S7). The relationship between $SPEAR_{pesticides}$ and the UFR length (and UFR area) was non-linear. For the presentation of the non-linear relationship (Fig. 4) we selected the best fit model using the Bayesian information criterion in the mselect function (R package drc) (Ritz and Streibig, 2005).

To identify the most important parameters for the macroinvertebrate community, we additionally performed multiple linear regression analysis with selected environmental parameters as independent variables. The following parameters were selected: UFR length, distance from the forest patch to the site, the direct distance to undisturbed habitat, stream bed structure and the toxic unit (TU). The selection was made as follows: (i) we included all measured forest parameters to identify their influence on $SPEAR_{pesticides}$, and (ii) we included additional environmental parameters that were significantly associated with $SPEAR_{pesticides}$ in single regression analysis (TU and hydromorphology). We simplified the multiple linear regression model using stepwise removal of insignificant terms, as outlined by Crawley (2012). All models were checked for normality of residuals, homogeneity of variances and influential data points. The percent explained variance was calculated for significant explanatory variables using the R package “relaimpo” (Grömping, 2006), and the “LMG” method (Lindeman et al., 1980). For the multiple linear regression analysis we assumed a linear relationship between SPEAR and UFR length, because including quadratic or cubic terms to account for curvature did not significantly change the outcome of the model (Crawley, 2012; Dormann and Kühn, 2004).

To determine the short-term effect of pesticide exposure on the macroinvertebrate community the decrease in SPEAR$_{pesticides}$ value from before to after measured contamination was compared between the sites with different levels of contamination: reference sites (TU > -4.5), sites with low ($-4 \leq TU \leq -3$) and high ($TU > -3$) contamination levels. Any significant differences from the reference sites were identified by a one-tailed Kruskal-Wallis multiple comparison test with a Bonferroni correction (to control for type I error), using the function “kruskalmc” in the “pgirmess” R package (Giraudoux and Giraudoux, 2013). For comparisons between the two groups of sites with and without UFR in terms of measured parameters a t-test with a Holm correction for multiple comparisons was used. Welch’s t-test was applied for unequal sample sizes. Holm correction was preferred to Bonferroni in this case, to avoid Type II error (Quinn and Keough, 2002). All calculations were performed in R, version 2.13.0 (R Development Core Team, 2011).

3 Results

3.1 Description of the streams and macroinvertebrate communities

The investigated streams were similar to each other in terms of their pH, dissolved oxygen content and temperature (Table 1; standard deviation < 20%). The differences in other environmental parameters, such as conductivity and the concentrations of nitrate and phosphate, were more pronounced (Table 1; standard deviations 38-67%) but did not show any significant relationships with community structure as measured by SPEAR$_{pesticides}$ (see section 3.3).

Parameter	Unit	Min	Max	Mean	SD	% SD	Comparison between sites with and without UFR[a]
Conductivity	µS	130	1866	1007	378.7	38	>0.05
Nitrate	mg/L	3.5	36.3	13.24	7.14	54	>0.05
Orthophosphate	mg/L	0.0	0.60	0.18	0.12	67	>0.05
D.O.	%	74	120	91.6	11.0	12	>0.05
pH	–	7.2	8.6	8.1	0.37	4.6	>0.05
Water temperature	°C	8.0	19.4	15.3	2.9	19	>0.05
Forest length	km	0.2	18.3	1.5	6.8	–	–
Forest area	Log(km^2)	−1.2	3.4	1.4	1.22	–	–
Distance from the forest	km	0.1	11	3.3	2.5	–	–
Direct distance to undisturbed stream sections	km	0.1	12	4.4	3	67	3×10^{-3}
Depth	cm	4	65	19.1	11.8	61	>0.05
Width	cm	60	180	114.6	36.6	31	>0.05
Stream bed structure	Class	7	2	5.6	–	–	>0.05

Table 1 Physicochemical characteristics of the sampling sites.
SD: Standard deviation.
a Comparisons between two groups of sites with and without the upstream forested reaches (UFRs) using Welch's t-test and Holm correction for multiple comparisons.

In addition to comparing individual streams, we compared two groups of sites: with UFR and without UFR to identify potential dissimilarities in environmental parameters. There were no significant differences in physicochemical parameters between the sites with and without the UFR (Table 1; Welch's t-test, $p > 0.05$), including the pesticide toxicity (section 3.2). The two groups of sites differed only in terms of the direct distance to the nearest undisturbed aquatic habitat (defined in section 2.6). The mean direct distance to undisturbed habitat was 6.6 km for the sites without UFR and 2.7 km for the sites with UFR. However, there was no relationship between the distance to undisturbed habitat and $\text{SPEAR}_{\text{pesticides}}$ indicator in simple or in multiple regression analysis (section 3.4).

A total of 68 families of benthic invertebrates were identified at the 37 sampling sites. The mean number of families per site was 15. The invertebrate communities were similar in terms of the most abundant taxa. At all sites, the most abundant taxa included *Gammaridae*, *Chironomidae* and *Baetidae*. Most of the taxa (78%) were found at both categories of sites, with and without the UFR. The other 22% of the taxa that differed in their occurrence between the two categories of sites were present in very low abundances only, at most 3% of the total abundance of the invertebrates at the respective sites.

3.2 Pesticide exposure

Pesticide contamination was detected at all sampling sites following rainfall events of more than 10 mm per day. The detected compounds included fungicides, herbicides, insecticides and one molluscicide. The toxicity of the pesticides in the water samples ranged from the TU of -0.4 to below -4.5. The highest TU values were associated with the insecticide Parathion and the fungicide Azoxystrobin, whereas reference sites (TU ≤ -4.5) were contaminated with compounds of low toxicity such as the herbicides Simazine and Terbuthylazine (Table 2). There was no significant difference in TU between the sites with and without UFR (Welch's t-test, $p > 0.05$).

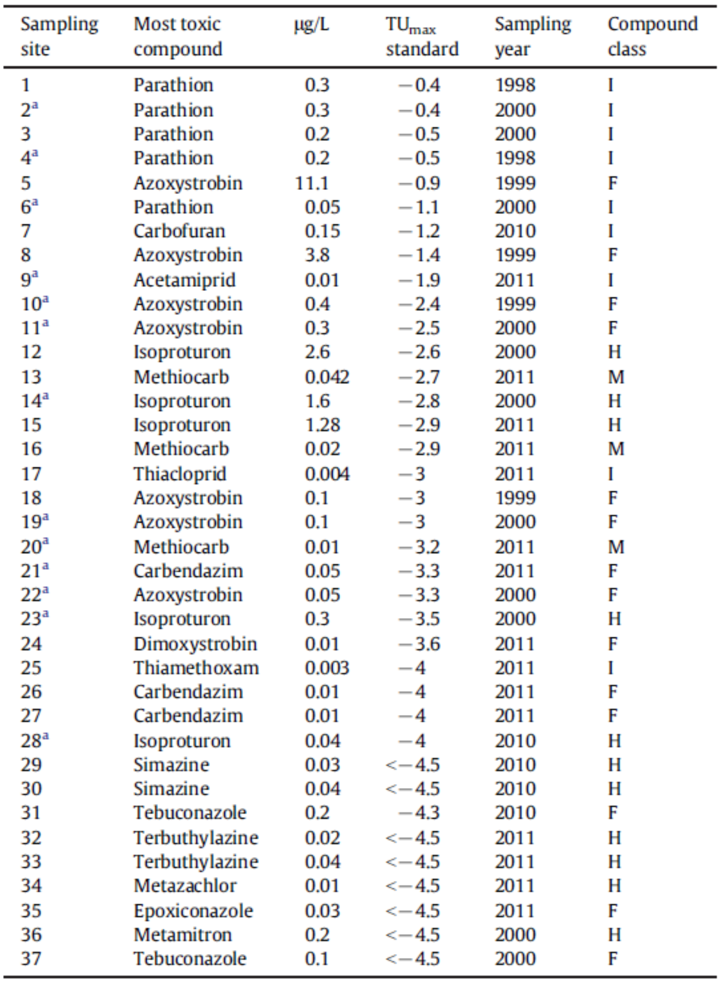
3.3 The reduction in $\text{SPEAR}_{\text{pesticides}}$ in response to contamination

The community composition of benthic invertebrates changed in response to the measured pesticide exposure. The proportion of vulnerable species decreased at the majority of the sampling sites shortly (< 1.5 months) after contamination. This was reflected in the decrease of $\text{SPEAR}_{\text{pesticides}}$, compared to the pre-contamination values. Out of all environmental parameters, the change in $\mathrm{SPEAR}_{\mathrm{pesticides}}$ depended only on TU (linear regression, $p = 0.0003$, $R^2 = 0.34$). Also the presence of the UFR did not affect the change in the SPEAR value (Welch's t-test, $p = 0.82$). $\mathrm{SPEAR}_{\mathrm{pesticides}}$ decreased by 38% on average if pesticide contamination was high (TU > -3), by 11% at the sites with a low pesticide contamination ($-4 \leq \mathrm{TU} \leq -3$) and by 4% at the control sites ($\mathrm{TU} \leq -4.5$), compared to the pre-contamination period. The means of both the low and the high contamination groups were significantly different from the control (Kruskal-Wallis multiple comparison test, $p < 0.05$).

3.4 TU and the length of upstream forested reaches determine $\mathrm{SPEAR}_{\mathrm{pesticides}}$

We tested the influence of selected environmental variables on $\mathrm{SPEAR}_{\mathrm{pesticides}}$ after pesticide exposure using a multiple linear regression analysis. These variables were: TU, the length of the upstream forested reach, structural stream bed quality, downstream distance from the riparian forest to the sampling site and the direct distance to undisturbed habitat (for the selection procedure see section 2.8). The analysis showed that $\mathrm{SPEAR}_{\mathrm{pesticides}}$ was significantly influenced only by the TU of the detected pesticides and the length of the forest patch upstream of the investigated sites (0.2-18 km). These two parameters explained 78% of the variance in $\mathrm{SPEAR}_{\mathrm{pesticides}}$ (Table 3). Most of this variance (44%) was explained by the TU, whereas the length of the UFR explained 34%. There was no correlation between the UFR length and TU, indicating that riparian forest patches did not reduce pesticide exposure downstream. The surface area of the UFR had a similar effect on $\mathrm{SPEAR}_{\mathrm{pesticides}}$ as the length of the UFR. However, the two parameters were highly inter-correlated ($r = 0.88$). As the length correlated with $\mathrm{SPEAR}_{\mathrm{pesticides}}$ better than the surface area (Table S7), we used only this parameter in all subsequent analyses.

$\mathrm{SPEAR}_{\mathrm{pesticides}}$ was not strongly influenced by other environmental parameters within the measured ranges. The distance of the sampling site to the UFR on the same stream (0.1-10 km, mean 3.3 km) and the direct distance to the potential undisturbed habitat (0.1-12 km, mean 4.4 km) did not have an effect. There was a significant relationship between hydromorphology and $\mathrm{SPEAR}_{\mathrm{pesticides}}$ in simple regression analysis ($R^2 = 0.16$, $p = 0.02$, section 2.8, Fig. S3). However, the influence of hydromorphology on $\mathrm{SPEAR}_{\mathrm{pesticides}}$ was weak in comparison to TU and UFR length, as can be seen from the R^2 and p values of the single regressions (Table S7). In the multiple linear regression analysis (described above), hydromorphology was not included in the best fit regression model, because it did not cause a significant increase in total explained variance. Regarding the relationships between the measured environmental parameters, there was an inter-correlation between hydromorphology and the UFR length ($R^2 = 0.30$, $p = 0.01$).

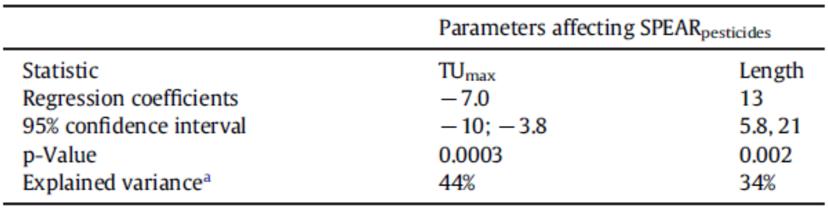


Sampling site	Most toxic compound	µg/L	TU_{max} standard	Sampling year	Compound class
1	Parathion	0.3	−0.4	1998	I
2[a]	Parathion	0.3	−0.4	2000	I
3	Parathion	0.2	−0.5	2000	I
4[a]	Parathion	0.2	−0.5	1998	I
5	Azoxystrobin	11.1	−0.9	1999	F
6[a]	Parathion	0.05	−1.1	2000	I
7	Carbofuran	0.15	−1.2	2010	I
8	Azoxystrobin	3.8	−1.4	1999	F
9[a]	Acetamiprid	0.01	−1.9	2011	I
10[a]	Azoxystrobin	0.4	−2.4	1999	F
11[a]	Azoxystrobin	0.3	−2.5	2000	F
12	Isoproturon	2.6	−2.6	2000	H
13	Methiocarb	0.042	−2.7	2011	M
14[a]	Isoproturon	1.6	−2.8	2000	H
15	Isoproturon	1.28	−2.9	2011	H
16	Methiocarb	0.02	−2.9	2011	M
17	Thiacloprid	0.004	−3	2011	I
18	Azoxystrobin	0.1	−3	1999	F
19[a]	Azoxystrobin	0.1	−3	2000	F
20[a]	Methiocarb	0.01	−3.2	2011	M
21[a]	Carbendazim	0.05	−3.3	2011	F
22[a]	Azoxystrobin	0.05	−3.3	2000	F
23[a]	Isoproturon	0.3	−3.5	2000	H
24	Dimoxystrobin	0.01	−3.6	2011	F
25	Thiamethoxam	0.003	−4	2011	I
26	Carbendazim	0.01	−4	2011	F
27	Carbendazim	0.01	−4	2011	F
28[a]	Isoproturon	0.04	−4	2010	H
29	Simazine	0.03	<−4.5	2010	H
30	Simazine	0.04	<−4.5	2010	H
31	Tebuconazole	0.2	−4.3	2010	F
32	Terbuthylazine	0.02	<−4.5	2011	H
33	Terbuthylazine	0.04	<−4.5	2011	H
34	Metazachlor	0.01	<−4.5	2011	H
35	Epoxiconazole	0.03	<−4.5	2011	F
36	Metamitron	0.2	<−4.5	2000	H
37	Tebuconazole	0.1	<−4.5	2000	F

Table 2 Characterisation of the pesticide exposure at investigated sites: most important pesticide per sample in order of decreasing toxicity.

F: fungicide; H: herbicide; I: insecticide, M: molluscicide.

[a] Sites with an upstream forested reach.

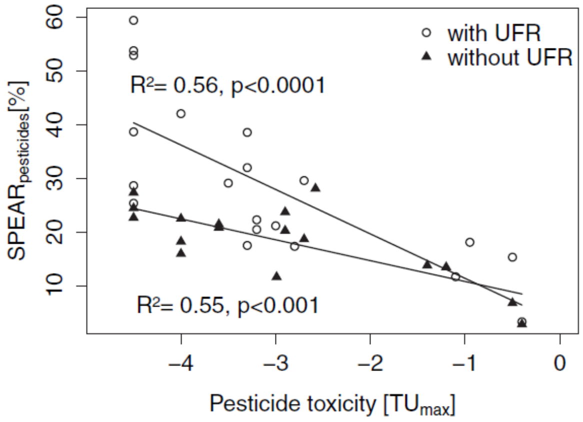


	Parameters affecting $SPEAR_{pesticides}$	
Statistic	TU_{max}	Length
Regression coefficients	−7.0	13
95% confidence interval	−10; −3.8	5.8, 21
p-Value	0.0003	0.002
Explained variance[a]	44%	34%

Table 3 The environmental variables with a significant influence on the $SPEAR_{pesticides}$ after contamination, as determined by the multiple linear regression analysis including the toxic units (TU_{max}), riparian forest length (Length), distance between the forest and the sampling site, stream bed structure and the direct distance to undisturbed habitat.

The simplified regression model was: $SPEAR_{pesticides} = -36 + 13\text{Length} - 7\text{TUmax}$ ($R^2 = 0.75$).

Additionally, we examined whether SPEAR$_{\text{pesticides}}$ based on macroinvertebrate data collected before the contamination was affected by environmental parameters and the presence of the UFR. From the environmental parameters, only the UFR length/surface area and the TU had an effect on SPEAR$_{\text{pesticides}}$ (linear regressions, $p < 0.05$). The presence of UFR was also associated with higher SPEAR$_{\text{pesticides}}$ values before the contamination (Welch's t-test, $p < 0.05$). Furthermore, for the sites without UFR, there was a significant correlation between SPEAR$_{\text{pesticides}}$ before contamination and TU measured during the contamination period (linear regression, $R^2 = 0.36$, $p = 0.009$). No such relationship was found for the sites with UFR (Figs. S2 and S3). Assuming that pesticide contamination in terms of TU was similar in the previous years (Bundschuh et al., 2014; Liess and von der Ohe, 2005), this result indicates that there are long-lasting effects of pesticides at the sites without UFR.



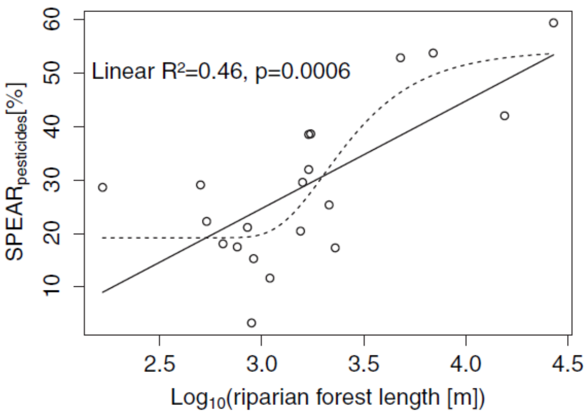


Fig. 3 Linear regressions for the proportion of vulnerable taxa (SPEAR$_{\text{pesticides}}$) and the TU for the sites with upstream forested reaches (UFR, $R^2 = 0.56$, $p < 0.0001$) and without UFR ($R^2 = 0.55$, $p < 0.001$) shortly after pesticide exposure (< 1.5 months).

Fig. 4 The relationship between the SPEARpesticides and the length of the upstream forested reach shortly (< 1.5 months) after pesticide exposure. The best fit model, four-parameter Weibull type I ($f(x) = 19 + 34 \exp(-\exp(-13(\log(x)-0.52)))$) and the linear function ($f(x) = 21.5x - 38.9$) are shown with a dashed and a solid line, respectively.

3.5 The influence of UFR on pesticide-vulnerable taxa

SPEAR$_{\text{pesticides}}$ values tended to be higher at all contamination levels (TU) if UFR were present (Fig. 3). The positive correlation between UFR length and SPEAR$_{\text{pesticides}}$ after contamination is shown in Fig. 4. The relationship between SPEAR and the UFR length was best described by a 4 parameter type 1 Weibull model. This model indicates that there is a minimum and a maximum length beyond which the UFR length has no effect on $\text{SPEAR}_{\text{pesticides}}$.

The observed positive effect of the UFR on $\text{SPEAR}_{\text{pesticides}}$ was related to its influence only on pesticide-vulnerable taxa. The presence of the UFR did not affect the overall number of taxa (Welch's t-test, $p = 0.80$) or the total abundance of vulnerable and invulnerable taxa (Welch's t-test, $p > 0.05$). However, the forest presence was associated with a higher number of pesticide-vulnerable taxa at downstream sites both before and after contamination (Welch's t-test, $p < 0.05$). Furthermore, the abundance of Trichoptera and Ephemeroptera that are classified as SPEAR increased significantly with forest length ($R^2 = 0.23$, $p = 0.02$ and $R^2 = 0.45$, $p < 0.001$, respectively).

In contrast, the species that are not vulnerable to pesticides (SPEnotAR) were not supported by the UFR. The number of invulnerable species was lower at the sites with UFR compared to the sites without UFR (Welch's t-test, $p < 0.01$), whereas their abundances were unaffected. Furthermore, the length of the UFR also did not affect either the invulnerable species numbers or abundance. Therefore, pesticide-vulnerable taxa were the main reason for the effect of the UFR on the $\text{SPEAR}_{\text{pesticides}}$ indicator.

4 Discussion

4.1 The influence of forested reaches on pesticide effects

By applying the $\text{SPEAR}_{\text{pesticides}}$ index, we showed that the presence of riparian forest upstream of contaminated sites mitigates pesticide effects on lotic invertebrate communities. $\text{SPEAR}_{\text{pesticides}}$ values were higher at the sites with UFR than at the sites without UFR at comparable toxicity levels shortly after the measured contamination (< 1.5 months). In addition, the index values were higher at the UFR sites also before contamination. This observation indicates that the influence of UFR on macroinvertebrate community composition is a result of long-term processes, as we discuss below (see section 4.2). The analysis of the influence of UFR characteristics combined with further abiotic parameters on $\text{SPEAR}_{\text{pesticides}}$ revealed that TU and the length of UFR explained most of the variation in $\text{SPEAR}_{\text{pesticides}}$ (78%) after pesticide exposure.

The positive effect of the length of UFR on $\text{SPEAR}_{\text{pesticides}}$ was previously shown in several studies (Bunzel et al., 2014; Liess and von der Ohe, 2005; von der Ohe and Goedkoop, 2013). In addition to the previous investigations, we found that: 1) the distance from the riparian forest (measured along the stream) does not affect $\text{SPEAR}_{\text{pesticides}}$ within the tested range (0.2-11 km) and 2) the length is likely the most important parameter of the UFR for pesticide-vulnerable taxa. Additionally, the total surface area of the UFR had a similar effect, but our results indicate that it is probably less important than the length. Further physicochemical parameters of the sampling sites had no significant effect on $\text{SPEAR}_{\text{pesticides}}$ in multiple linear regression analysis. Conversely, in simple linear regression analysis, we found a weak significant relationship between hydromorphology and $\text{SPEAR}_{\text{pesticides}}$. This result can have two explanations: 1) an observed inter-correlation of UFR length and hydromorphology; or 2) a restrictive effect of the heavily degraded habitat on some of the pesticide-vulnerable species (Bunzel et al., 2014; Rasmussen et al., 2011).

In addition to the influence of the forest, we analyzed the change in the macroinvertebrate community from before to after the contamination period. The proportion of vulnerable species decreased significantly in response to contamination starting at a TU between -4 and -3 in both categories of sites. Similar thresholds for pesticide effects were shown by Schäfer et al. (2012) when comparing the stream community composition under pesticide exposure to reference sites. Interestingly, the loss in vulnerable taxa was determined only by the TU and was independent of the UFR or other environmental parameters in our study. Therefore, the presence of UFR did not reduce the loss of vulnerable SPEAR taxa after pesticide exposure.

4.2 Enhanced recolonisation at the sites with UFR

$\text{SPEAR}_{\text{pesticide}}$ values were significantly higher at the sites with UFR than at the sites without UFR before pesticide exposure. The $\text{SPEAR}_{\text{pesticides}}$ also remained higher at the sites with UFR after contamination at similar TU. Because the relative loss of taxa due to contamination did not differ between the two categories of sites, the reason for higher $\text{SPEAR}_{\text{pesticides}}$ is the higher pre-contamination value, rather than a reduced initial effect of pesticides. Riparian forest fragments embedded in agricultural landscapes are known to serve as a source of stream invertebrates for the recolonisation of disturbed sites (Harding et al., 2006; Liess and von der Ohe, 2005; Sedell et al., 1990), which takes at least several months to several years for single taxa (Wallace, 1990). Our study also suggests that recolonisation is the most likely explanation for the higher proportion of SPEAR taxa downstream of the forest, due to (i) the long-term effects of pesticides shown for the sites without UFR, but not with UFR, and (ii) the positive effect of the UFR on vulnerable taxa.

We detected a significant correlation between $\text{SPEAR}_{\text{pesticides}}$ before contamination and the TU measured during the contamination period for the sites without UFR. In contrast, there was no such correlation for the sites with UFR. Assuming similar pesticide contamination (TU) in previous years, as shown by Liess and von der Ohe (2005) and Bundschuh et al. (2014), the observed correlation indicates long-term pesticide effects at the sites without UFR. Several studies have reported pesticide impacts on vulnerable macroinvertebrates up to one year after contamination (Caquet et al., 2007; Liess and Beketov, 2011). In the absence of external recolonisation sources, univoltine species, such as most of the SPEAR taxa, can take several years to recover from pesticide application (Wallace, 1990). Hence, the undisturbed populations in the UFR most likely served as a source for recolonisation in our study, whereas the sites without UFR were prone to long-term effects.

Liess and von der Ohe (2005) observed a correlation between the TU and $\text{SPEAR}_{\text{pesticides}}$ before contamination also for the sites with UFR, indicating long-term effects at these sites as well. The likely explanation is that the forested reaches in that study had on average only half the length of the UFR considered in our study, highlighting the importance of the length of the riparian forest for recolonisation. Another difference is in the definition of the UFR. Unlike Liess and von der Ohe (2005), we considered only the forested reaches without agriculture upstream to exclude the possibility of pesticide contamination within the forested reach. Thus, it is possible that forested reaches which may receive pesticide inputs do not have the same effect as uncontaminated forested reaches considered in our study.

Regarding the relation between UFR and the invertebrate community composition, we observed that the abundances and numbers of SPEAR taxa belonging to Ephemeroptera and Trichoptera were positively affected by the forest. These larvae are most commonly found in drift samples (Waters, 1972) and are able to drift several km downstream during the summer months (Hemsworth and Brooker, 1981; Neves, 1979). The dispersal of flying adults from forested refugia may also play a role in the recolonisation process (Wallace, 1990). However, uncontaminated forested reaches in neighboring streams did not have an effect on $\text{SPEAR}_{\text{pesticides}}$ in our study. Also Liess and von der Ohe (2005) suggested drift as the main mechanism of recolonisation, as the forested reaches located downstream of the sampling sites had no effect on the macroinvertebrate community.

4.3 Potential influence of stream parameters, associated with the UFR

Alternative explanations for the higher abundances of vulnerable Ephemeroptera and Trichoptera downstream of the forest can be: 1) the improvement of the physicochemical conditions and 2) an increased detritus input due to the forest cover in the upper catchment (Harding et al., 2006; Niyogi et al., 2007). However, the physicochemical water parameters were not affected by the forest due to the large distances between our sampling sites and the UFR (on average 3.3 km). Liess and von der Ohe (2005) also found no influence of the forest on water parameters at an average of 2.5 km downstream from the forest. Only improved stream bed hydromorphology was associated with UFR length in our study. The positive effect of forest cover on stream hydromorphology is well known (Kail et al., 2009). However, the influence of UFR on pesticide-vulnerable taxa cannot be explained by improved hydromorphology. This is because the influence of the hydromorphology on $\text{SPEAR}_{\text{pesticides}}$ was much weaker relative to the influence of the UFR length on $\text{SPEAR}_{\text{pesticides}}$ ($R^2 = 0.16$, $p = 0.02$ and $R^2 = 0.46$, $p = 0.0006$, respectively).

Additionally, streams with UFR may be associated with greater inputs of allochthonous materials (Webster, 2007), that can promote recovery (Bond et al., 2006). We did not measure the amount of detritus in the streams. Thus, we cannot exclude the possibility that in addition to serving as a source of organisms, UFR enhance recovery by increasing detritus availability at downstream sites (Wipfli and Gregovich, 2002). But, in this case, non-SPEAR taxa, such as *Tipulidae* would also profit from UFR, which is not the case in our study. Nevertheless, this aspect demands a more detailed investigation in the future.

5 Conclusions and recommendations

Forested headwaters mitigate the effects of pesticides on vulnerable invertebrates in streams. In contrast, uniform agricultural landscapes devoid of refuge habitats are a pre-condition for the local extinction of such vulnerable species. Our study indicates that a strategic placement of uncontaminated forest patches in the headwaters of streams may serve as a species-protection measure. We suggest that UFR serve as a source of organisms for the recolonisation of pesticide-impacted sites downstream. Sampling macroinvertebrates throughout the year and drift sampling would further specify the dynamics of recovery from pesticide impacts.

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CHAPTER 5

Discussion and Conclusions

"No man ever steps in the same river twice,
for it's not the same river and he's not the same man."

Heraclitus of Ephesus

Pesticide stress alters lotic macroinvertebrate communities

Running waters – Ecosystems under pressure

More than one-third of all renewable freshwater on Earth is used for agricultural, industrial, and domestic purposes (Schwarzenbach et al., 2006), and freshwater biodiversity is facing major threats from combined and interacting influences of overexploitation, water pollution, flow modification, habitat degradation, and species invasion (reviewed by Dudgeon et al., 2006). Exploitation and land-use alterations force profound changes upon riverine systems (Malmqvist and Rundle, 2002; MEA, 2005); hence, lotic macroinvertebrate communities not only have to cope with chemical pollution (von der Ohe et al., 2011) but also with, e.g., hydromorphological degradation (Feld, 2004), substrate instability (Cobb et al., 1992), eutrophication (Smith et al., 1999), and salinisation (Kefford, 2000; Fig. H). For example, many German rivers and streams suffer from artificial channel morphology and eutrophication (UBA, 2015a). Changes in channel bank/bed structure and increased nutrient contents each have their own impact on stream macroinvertebrates (Rasmussen et al., 2011a, 2012; von der Ohe and Goedkoop, 2013).

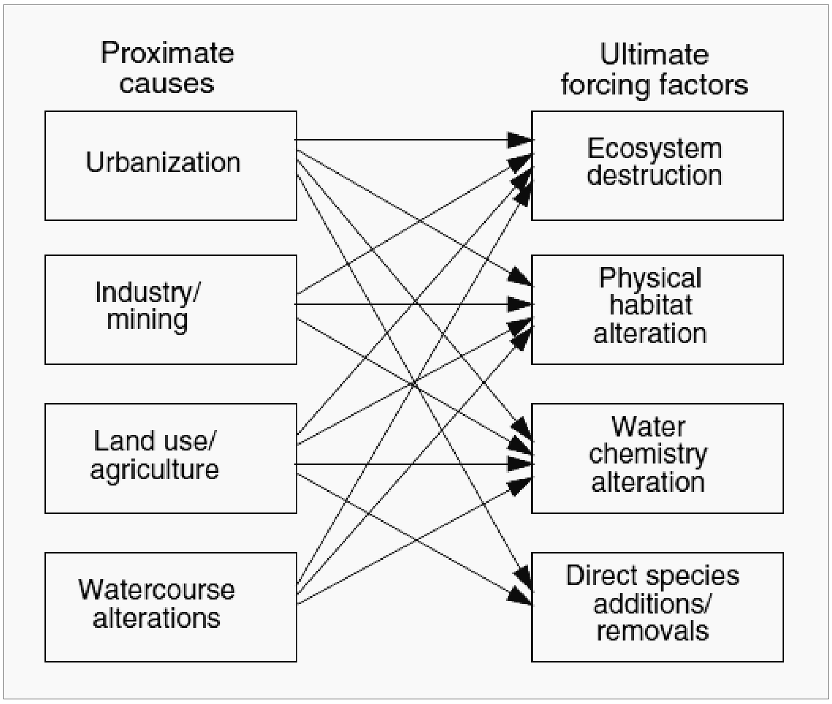

Fig. H. The four main proximate causes of ecosystem change in streams and rivers and their link with factors that ultimately lead to change.
Reproduced from Malmqvist and Rundle, 2002.

Small running water bodies are of high ecological importance (EEA, 2012). They support comparatively more aquatic life than do large rivers (Biggs et al., 2014) although differences among small water courses are also known (Probst et al., 2005). Unfortunately, all over the world, the aquatic biota in agricultural landscapes is exposed to and endangered by pesticide pollution (Beketov et al., 2013; Ippolito et al., 2015; Szöcs et al., 2017). Insecticides are of the utmost significance for aquatic macroinvertebrates (Schulz and Liess, 1999; Purcell and Giberson, 2007; Wallace et al., 1989), particularly in small streams with little dilution (Burgoa and Wauchope, 1995): Sharing the same neurological and respiratory mechanisms as the terrestrial target insects, aquatic insects lack adequate detoxification systems (Sánchez-Bayo, 2011). Insecticides also indirectly affect macroinvertebrates by interfering with aquatic food webs (Relyea and Hoverman, 2008), and so do fungicides and herbicides (Bundschuh et al., 2011; Geiszinger et al., 2009; Zubrod et al., 2015).

Detection of pesticide pollution in running waters

In the studies presented in CHAPTERS 2 to 4, the Chemcatchers and event-driven samplers performed well. The first study (see CHAPTER 2) was designed to test membrane-equipped Chemcatchers regarding the quantification of water-borne pesticides. Because of the good results, this Chemcatcher configuration was also used in the other two investigations (see CHAPTERS 3 and 4). In the second study, the change in the orientation of the event-driven samplers from vertical (see Liess and von der Ohe, 2005) to horizontal (see CHAPTER 3) was crucial due to the relatively shallow depths of the streams investigated. This approach had not been tested before in the field; however, the technical modifications associated with the change in orientation proved to be effective.

During rainfall events, not only hydrophilic but also hydrophobic pesticides ($\log K_{\mathrm{OW}} > 4$) are being mobilised (Kronvang et al., 2004). Some hydrophobic compounds (e.g., esfenvalerate) were detected in agricultural edge-of-field runoff and wastewater treatment plant effluents (see CHAPTERS 2 and 3). Generally, a wide spectrum of active substances was found in running waters during the study periods. Ecotoxicologically relevant $\mathrm{TU}_{\mathrm{max}}$ values were provided by all major product types, i.e., insecticides, fungicides, and herbicides. However, as one would expect, this group of most important compounds was dominated by insecticides: three *neonicotinoids* (acetamiprid, imidacloprid, and thiacloprid), one *pyrethroid* (esfenvalerate), two *carbamates* (carbofuran and methiocarb), and one *organophosphate* (parathion). This is in accordance with previous studies that have shown the adverse effects on aquatic macroinvertebrates caused by neonicotinoids (Beketov et al., 2008; Van Dijk et al., 2013), pyrethroids (Lauridsen and Friberg, 2005; Palmquist et al., 2008), and insecticides not licenced for use (*legacy pesticides*; McKnight et al., 2015; Rasmussen et al., 2015). Additionally, lotic communities were also affected by one *benzimidazole* (carbendazim) and two *strobilurin* (azoxystrobin and dimoxystrobin) *fungicides* as well as one *urea herbicide* (isoproturon). This is in accordance with the literature that shows that fungicides and herbicides, although less toxic to aquatic macroinvertebrates than insecticides, can affect the macrozoobenthos (e.g., Dewey, 1986; Zubrod et al., 2014). Meanwhile, the high risk for aquatic ecosystems posed by the herbicide isoproturon (a priority substance under the WFD; EC, 2013a, 2000) has resulted in the withdrawal of its approval (EC, 2016).

Effects of pesticides on lotic macroinvertebrate composition

In the studies presented in CHAPTERS 2 to 4, the highest site-specific TU value (= TU_{max}) was used to assess the ecological impact of water-borne pesticides. Macroinvertebrate community alterations, expressed as SPEAR values, were clearly linked to pesticide-driven water toxicity, expressed as TU. This is in accordance with previous studies conducted under field (e.g., Liess and von der Ohe, 2005; Schäfer et al., 2012, 2008, 2007) and artificial conditions (e.g., Hose et al., 2003; Liess and Beketov, 2011). The reliable distinction of pesticide pollution effects from other stresses or natural variability is the main difficulty in assessing the dose-response relationships in large-scale studies (Liess et al. 2008). In this regard, $SPEAR_{pesticides}$ outperforms other monitoring concepts (McKnight et al., 2012; Smetanová et al., 2014; Schletterer et al., 2010). Because of its high specificity with regard to pesticides, $SPEAR_{pesticides}$ is one of the 28 indicators used to assess the progress of the German National Action Plan for the sustainable use of pesticides (NAP; BMEL, 2013).

Pesticides in surface waters usually occur in mixtures (Vijver and van den Brink, 2014). For mixtures of chemicals, the *concentration addition* model (= TU_{sum}) can be used to quite adequately predict effect concentrations, particularly for mixtures of compounds with the same or similar modes of action (Deneer, 2000; Deneer et al., 1988b). This concept can also work quite accurately if the chemicals concerned have different mechanisms of action (Altenburger et al., 1996; Hermens et al., 1984); however, the concept’s principle (i.e., a joint action of the respective substances by acting of dilutions of each other) is not generally applicable for substances featuring dissimilar modes of action (Altenburger et al., 2004;

Deneer, 2000; Deneer et al., 1988a). Furthermore, for biotic endpoints, the explanatory power of TU_{sum} is similar to that of TU_{max} (Schäfer et al., 2012, 2011; von der Ohe et al., 2009).

The acute toxic pressure of chemical mixtures on macroinvertebrate communities can also be estimated by calculating the **m**ulti-**s**ubstance **p**otentially **a**ffected **f**raction of species (msPAF; de Zwart and Posthuma, 2005). Incorporating the *Species-Sensitivity Distribution concept* (Posthuma et al., 2002), this approach combines the alternative models of *concentration addition* (same/similar modes of action) and *response addition* (dissimilar modes of action). A few field studies on the effects of pesticides on stream macroinvertebrates have used the *msPAF concept* (Schäfer et al., 2013; Smetanová et al., 2014). However, several difficulties and uncertainties are associated with this method, such as the scarcity of toxicity data and the fact that important ecological parameters are not taken into account (Smetanová et al., 2014). Furthermore, in agricultural areas, ecotoxicological effects are mainly driven by one single compound (or very few) out of the whole set of contaminants (Belden et al., 2007; Schäfer et al, 2007).

Temporal variations in macroinvertebrate life cycles, food availability, and climate conditions naturally lead to fluctuations in community structure (Resh and Rosenberg, 1989). Nevertheless, macroinvertebrate communities remain quite stable over the years in streams not impacted by anthropogenic stressors (Robinson et al., 2000) and streams recovering from pesticide disturbance (Hutchens et al., 1998). However, running waters in agricultural landscapes usually receive repetitive pesticide inputs year after year. Hence, at the study sites referred to in CHAPTERS 2 to 4, one can assume that the pesticide-driven water toxicity during the study periods resembled the situation in previous growing seasons (see Bundschuh et al., 2014; Liess and von der Ohe, 2005), and chronic effects resulting from earlier pesticide contaminations cannot be excluded (see CHAPTER 4). The selection pressure of baseline pesticide toxicity not only leads to reduced abundances of pesticide-sensitive taxa (McKnight et al, 2012; von der Ohe et al., 2009; Wallace et al., 1989) but also to modifications at the genetic level (*microevolution*) promoted by intraspecific competition (Becker and Liess, 2015). However, adaptation comes at the expense of fitness costs, which affects the interspecific interactions of taxa (Jansen et al., 2011). In turn, a high level of interspecific competition restrains a community's adaptation potential, i.e., the more diverse the community, the smaller the chances of developing toxicant resistance (Becker and Liess, 2017, 2015).

Effects of pesticides on leaf litter decomposition

The degradation of fallen leaves is a vital component of terrestrial and aquatic ecosystem functioning (Gessner et al., 2010). In the studies presented in CHAPTERS 2 and 3, decreasing breakdown rates were clearly linked to macroinvertebrate community alterations induced by pesticide-driven water toxicity. This relationship can be explained by the decrease of shredder abundances within the community. This is in accordance with previous studies conducted under field (Schäfer et al., 2007) and artificial conditions (e.g., Cuffney et al., 1990; Zubrod et al., 2014). Further to the direct effects of pesticides diminishing shredder abundances, indirect effects of fungicides hinder or reduce fungal leaf colonisation, thereby affecting the leaf palatability (Bundschuh et al., 2011; Gulis and Suberkropp, 2003). Moreover, pesticide mixtures might produce interactive effects, and distinguishing between pesticidal effects on fungi performance and those on shredder performance is complicated (Flores et al., 2014).

Future challenges

Environmental risk assessment (ERA)

Following the first successes of synthetic organic insecticides, the consequences of their widespread application for human and environmental health soon became apparent; particularly Rachel Carson’s book *Silent Spring* (Carson, 1962) influenced public attitudes and led to the creation of regulating authorities and procedures (Casida and Quistad, 1998). However, although based on internationally agreed testing methods (OECD, 2017), current ERA practices fail to sufficiently protect the aquatic non-target biota from pesticide harm (e.g., Becker and Liess, 2017; Beketov et al., 2013; Bundschuh et al., 2014; Schäfer et al., 2012). The reasons for this are manifold:

Standard laboratory single species tests are quick, inexpensive, and comparable; yet, they lack environmental relevance, and the actual consequences of chemical exposure for ecosystem structure and function remain uncertain (Lagadic et al., 1994; Tlili et al., 2015). At the ecosystem level, whole aquatic communities are exposed to a whole cocktail of pesticides; in pesticide mixtures, a given compound’s toxicity might be enhanced by co-occurring substances (Norgaard & Cedergreen 2010; Vijver and van den Brink, 2014). Furthermore, the sensitivity of organisms towards pesticide stress is increased by intraspecific (Foit et al., 2012; Knillmann et al 2012a) and interspecific competition (Kattwinkel and Liess, 2014;

Knillmann et al 2012b) as well as abiotic factors, such as climate and acidification (Christensen et al., 2006). In fact, the occurrence of synergistic effects of anthropogenic and natural stressors is considered the standard field scenario rather than an exception (reviewed by Holmstrup et al., 2010). This means that effective pesticide concentrations in the field are much lower than those derived using laboratory tests. As a consequence, extrapolations from single-species-tests to community-level effects are laden with uncertainties (Calow and Forbes, 2003). The same holds true for extrapolations from the individual to the population level (Liess, 2002) and for extrapolations from one taxon to another; even closely related freshwater species respond differently to pesticide stress (Schulz and Liess, 1995). Nevertheless, extrapolation is seen as a practical necessity in ERA (Forbes and Calow, 2002).

Sub-lethal effects of pesticide pollution (reviewed by Desneux et al., 2007) are not included in ERA (Beketov and Liess, 2008; Lauridsen and Friberg, 2005); neither are indirect effects of pesticides (Preston, 2002) nor the effects of pesticides not currently licenced for use (*legacy pesticides*; Rasmussen et al., 2015). In contrast to direct effects on, e.g., abundance and metabolism of sensitive taxa (reviewed by Schäfer et al., 2011), indirect effects become noticeable at lower and higher trophic levels (Geiszinger et al., 2009; Relyea and Hoverman, 2008), and they might also be more common than direct effects (Rohr et al., 2006). Furthermore, legacy pesticides still affect surface waters, e.g., by moving from groundwater to adjoining rivers and streams (McKnight et al., 2015).

In addition to these practical aspects of ERA, many of the assumptions used in pesticide exposure estimation models, such as standard water body dimensions, often do not match the actual landscape characteristics (Ohliger and Schulz, 2010). For example, the width:depth ratio of 3.33 supposed for small water bodies in agricultural areas (EC, 2007) is not consistent with the ratios of 10 (Wogram, 2010) and 15 (Ohliger and Schulz, 2010) established in the field, potentially resulting in a profound underestimation of the actual extent of exposure (Ohliger and Schulz, 2010).

Wastewater treatment plants

In modern wastewater treatment plants (WWTPs), raw wastewater is processed using a minimum of three treatment stages (*tertiary treatment*). In the first (mechanical) step, grit, coarse solids, and large floating objects are removed to prevent damage and enhance operation. Furthermore, settleable organic and inorganic solids are eliminated by sedimentation. The objective of the second (biological) step is the removal of biodegradable organics and suspended solids from the primary effluent, using aerobic biological processes. In the third step, the secondary effluent is treated to remove further wastewater constituents such as nitrogen, phosphorus, and heavy metals (Pescod, 1992).

However, hundreds of anthropogenic organic micropollutants (*emerging contaminants*), such as pesticides, biocides, pharmaceuticals, personal care products, medical diagnostic media, illicit drugs, and household cleaning agents, pass the WWTP treatment process nearly unaffected. Their omnipresence in surface waters is of great environmental concern and urgently calls for an advanced wastewater treatment (e.g., Geissen et al., 2015; Kümmerer, 2011; Petrie et al., 2015; Verlicchi et al., 2010).

Pesticides and biocides enter sewage systems on numerous pathways (Gerecke et al., 2002; Fig. J), and losses from agricultural and nonagricultural uses are of similar importance (Wittmer et al., 2011, 2010). During rainfall events, the WWTP discharge of pesticides and biocides is intensified (Singer et al., 2010). In the second study (see CHAPTER 3), pesticides released with WWTP effluents were shown to alter macroinvertebrate community composition and to impair ecosystem function downstream of the effluent discharge points. Overall, neonicotinoid insecticides were the ecologically most relevant compounds; in most cases, they had entered the investigated water bodies with treated wastewaters, either exclusively or at concentrations exceeding the agricultural runoff from upstream reaches. WWTP-induced eutrophication was not observed to influence the macrozoobenthos. Such a relationship has been reported in studies that had focused on WWTP-induced nutrient influxes (Englert et al., 2013; Grantham et al., 2012; Gücker et al., 2006).

The presence of pesticides in WWTP effluents can be tackled from several sides. From a farmer’s point of view, adhering to the ‘Code of Good Agricultural Practice’ (BMEL, 2010) is the most obvious way. For example, the cleaning of field sprayers on paved surfaces is one of the main causes for pesticides in raw wastewater (Bach et al., 2000) notwithstanding that in-field sprayer cleaning is required under the German Plant Protection Act (BMJV, 2012; updated in 2016). In fact, cleaning the spraying equipment on paved surfaces is a violation of the application instruction NW468 that demands pesticides not to enter sewage systems (BVL, 2017).

From a technical perspective, WWTPs need to be equipped with additional treatment processes. It is widely recognised that today’s WWTPs urgently need to meet the challenge of eliminating pesticides and other organic contaminants from liquid wastes (EC, 2013a; Lofrano and Brown, 2010; UBA, 2015b; Stalter et al., 2013). Recently developed strategies, such as ozonation, powder activated carbon treatment, advanced oxidation processes, nanofiltration, reverse osmosis, and membrane bioreactors, have been shown to effectively reduce micropollutants in aquatic environments (Eggen et al., 2014; Luo et al., 2014). Another mitigation measure can be the abandonment of combined sewage systems and combined sewer overflows when building new municipal WWTPs (Tibbetts, 2005).

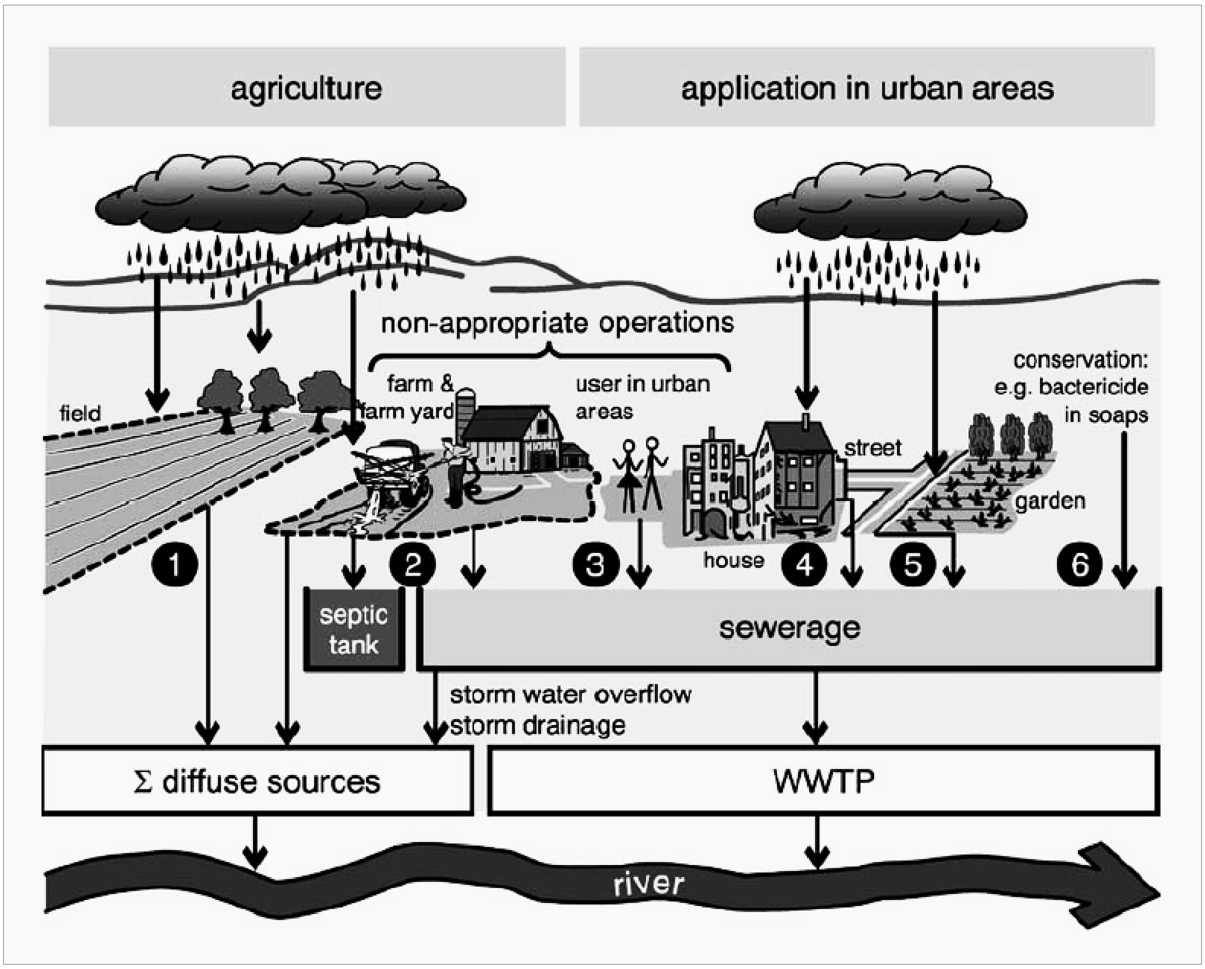

Fig. J. Major pathways for pesticide transport into surface waters involving WWTPs [(2) – (6)]:
(1) Field: spray drift, surface runoff, leaching, and drainage.
(2) Farm and Farmyard: improper operations (e.g., cleaning and filling of sprayers, driving with seeping sprayers): drainage to the sewerage, to the septic tank or into surface waters.
(3) Like (2) for pesticide users in urban areas.
(4) Pesticides in building material: leaching.
(5) Applications on lawns, streets, road embankments: runoff.
(6) Protection of materials: e.g., product ingredients that get into the sewerage.
Modified from Gerecke et al., 2002.

Mitigation of pesticide impact

According to the EU regulation 1107/2009 (EC, 2009), any unacceptable pesticide effects on the environment is to be avoided or at least minimised. Nevertheless, pesticides severely jeopardise terrestrial and aquatic biodiversity in agrarian landscapes (Geiger et al., 2010), impacting soil and freshwater quality as well as ecosystem functioning and services, such as, community diversity, nutrient cycling, pest control, pollination, and ecosystem resilience (reviewed by Chagnon et al., 2015). Further to the impact on aquatic macroinvertebrates described above, disastrous effects have also been observed in, e.g., amphibians (Brühl et al., 2013; Relyea, 2003), birds (Goulson, 2014; Hart et al., 2006), butterflies (Forister et al., 2016; Longley and Sotherton, 1997), earthworms (Dittbrenner et al., 2010; Pelosi et al., 2014), honey bees (Goulson, 2013; Woodcock et al., 2017), and wild bees (Whitehorn et al., 2012; Woodcock et al., 2017). Hence, the implementation of mitigation measures is indispensable.

Forested patches within agricultural landscapes can deliver various ecosystem services to humans and also mitigate the drawbacks of agricultural practices (Decocq et al., 2016), such as agrochemical contamination and biodiversity loss (Dale and Polasky, 2007). With drift being the most important source of recolonising stream fauna (Williams and Hynes, 1976), forested upstream reaches have been hypothesised to considerably contribute to pesticide mitigation at impacted downstream reaches (Liess and von der Ohe, 2005; Schäfer et al., 2007, Schriever et al., 2007). In the third study (see CHAPTER 4), it was shown that riparian forest in upstream stretches indeed accelerates the recovery of downstream communities by providing source populations of sensitive taxa for recolonisation. While lotic communities' capacity to withstand natural and anthropogenic disturbances (*resistance*) is only moderate to low (Downes et al., 2008), their capacity to recover from disturbances (*resilience*) is rather high (Yount and Niemi, 1990). Biomonitoring is an important tool for the documentation of 'environmental recovery' and also for the evaluation of the effectiveness of any mitigation measures implemented (Barbour et al., 1999).

Various pesticide mitigation strategies are being implemented today, such as vegetated buffer strips, wind breaking structures, conservation tillage, ecotoxicologically more favourable products, and awareness campaigns for farmers and pesticide operators (reviewed by Reichenberger et al., 2007). However, the realisation of some of these measures can be quite challenging: Although riparian buffer strips effectively mitigate pesticide input into running waters (Rasmussen et al 2011b), their efficiency can be hampered by erosion rills and reduced vegetation cover (Bereswill et al., 2012; Ohliger and Schulz 2010). While spray drift during pesticide application can be reduced using coarse sprays, product retention on plant surfaces is better with smaller droplets. Furthermore, an increased droplet size requires a larger spray volume to produce the same number of droplets per unit area; e.g., to double the droplet diameter, an eight-fold increase in spray volume is required (Matthews, 2002). *Conservation tillage*, i.e., not removing crop residues before planting, can reduce pesticide runoff and erosion losses. However, its efficiency is determined by soil conditions, and *conventional tillage*, i.e., turning the soil before planting, can reduce pesticide leaching to groundwater (Reichenberger et al., 2007). Raising awareness among farmers and pesticide operators can efficiently reduce pesticide losses to surface waters, but initial big responses might be short-lived (Kreuger and Nilsson, 2001; Peschka et al., 2006).

In 2013, the European Commission’s reform of its common agricultural policy (CAP) introduced a set of direct payments to farmers in order to promote the implementation of measures designed to help meet environmental requirements (*greening*; EC, 2013b). With respect to the protection of running waters from pesticide input, an important aspect of the new CAP is the creation of ecological focus areas, which includes, e.g., the conversion of arable land into permanent pasture, the creation of buffer zones along water courses, and the management of uncultivated buffer strips and field margins (EC, 2013c).

Climate change

While the current policy- and market-driven agricultural changes in Europe may benefit terrestrial and aquatic biodiversity (reviewed by Stoate et al., 2009), global warming is going to have predominantly adverse effects (Bellard et al 2012; Geyer et al., 2011) even though they might be less severe in northern temperate ecosystems (Sala et al., 2000). In particular lotic macroinvertebrates are under threat by climate change (Woodward et al., 2016, 2010). Rising temperatures will (i) cause shifts in crop location, crop type, and crop pest distribution; (ii) increase rainfall frequency and intensity as well as pesticide volatilisation, degradation, and runoff, and (iii) alter key physiological mechanisms and toxicant kinetics in exposed biota, i.e., enhance the bioavailability and toxicity of water-borne pesticides and other contaminants (reviewed by Noyes et al., 2009). The subsequent increase in pesticide applications and water pollution is widely accepted (Kattwinkel et al., 2011; Noyes et al., 2009). Taxa already living at the edge of their tolerance ranges could be especially struggling to acclimatise to a warming world (Noyes et al., 2009).

Conclusions

The diffusion-limiting membrane atop the receiving phase of the Chemcatcher has proven to be a valuable amendment of this sampling device. The shielding cover inhibits the establishment of a biofilm on the sorbent medium while still allowing the time-integrative sampling of waterborne pesticides and relating them to benthic community structure. The membrane-equipped Chemcatcher is recommended for future investigations into pesticide effects on lotic ecosystems.

SPEAR$_{\text{pesticides}}$ is known to reliably indicate pesticide pollution in running waters. In the studies presented in CHAPTERS 2 to 4, the index (i) complemented chemical measurements using Chemcatchers with a diffusion-limiting membrane, enabling the assessment of pesticide effects on benthic macroinvertebrate communities, (ii) responded to pesticide emissions from WWTPs, enabling the determination of the ecological impact of WWTP-related pesticide inputs, and (iii) showed community recovery from pesticide stress by in-stream drift of sensitive taxa from uncontaminated areas, highlighting the ecological importance of riparian forest along water courses in agrarian areas.

The reduction of pesticide input into running waters from diffuse and point sources is urgently required. All parties involved are encouraged to collaborate in order to ensure food security without sacrificing floral and faunal biodiversity in and around cultivated fields: Farmers are to strictly comply with best management practices. Regulating authorities are to improve ERA procedures, aligning them with ‘real life’ scenarios rather than bioassay results. WWTPs need to be comprehensively equipped with further adequate treatment steps so that pesticides can be efficiently eliminated from treated wastewater. The pesticide industry is to make their products safer for the environment. Farmers and authorities are required to implement mitigation measures efficiently, especially in the face of climate change. Adaptation of farming practices to altering environmental conditions is vital for the conservation of intact freshwater ecosystems in agricultural landscapes.

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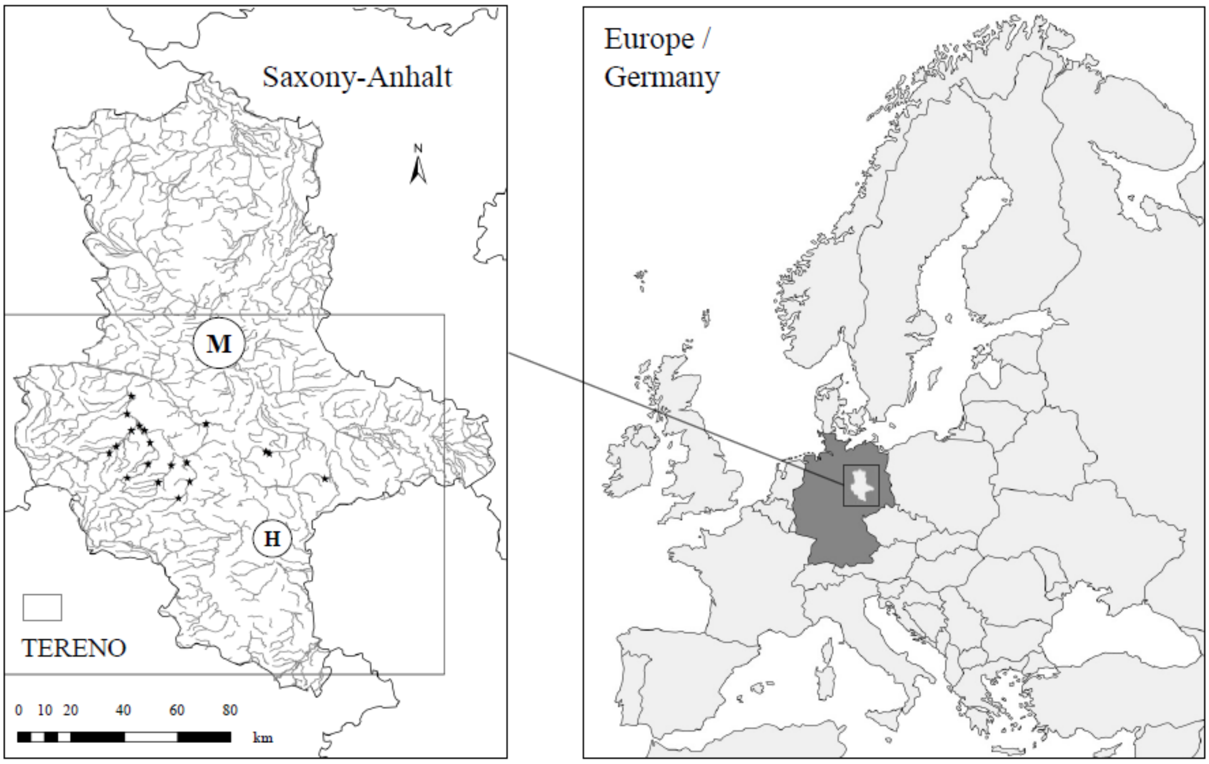
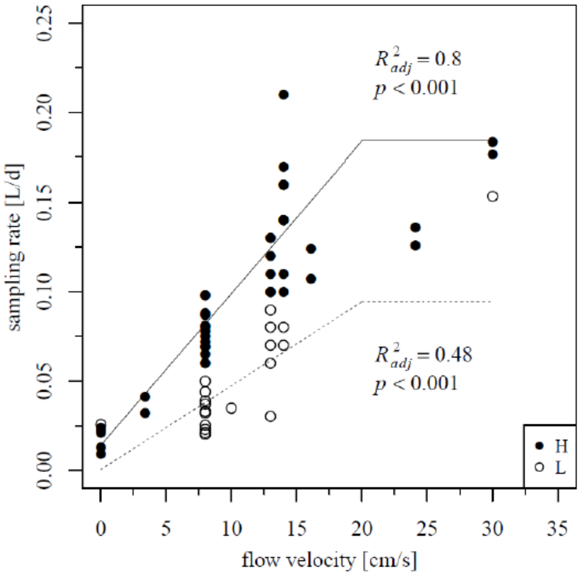
APPENDIX A

Supplementary Material to CHAPTER 2



SI Fig. S1: Map of the study area. M - Magdeburg; H - Halle/Saale

SI Fig. S2: Chemcatcher with the disposable sampler housing made from polycarbonate (2nd generation; J.L. Greenwood et al., 2007). Additional machine screws were used to hold the housing parts more closely together (Photo: R. Gunold).



SI Fig. S3: Experimentally obtained sampling rates and regression fits.
H: compound with a high sampling rate; L: compound with a low sampling rate.

Stream	Strahler stream order	Width [m]	Sampling depth [m]	Current velocity [m s^{-1}]	Gravel [%]	Algae [%]	CPOM [%]	number of sites
Bode	4	10.0	0.4	0.21	30	10	30	6
Eine	2	5.0	0.4	0.27	25	5	20	2
Mulde	5	40.0	0.5	0.05	10	10	30	1
Selke	3	5.0	0.3	0.40	30	5	20	5
Wipper	3	5.0	0.4	0.18	20	0	30	3
Ziethe	1	5.0	0.6	0.06	10	10	20	2

SI Table S1: Physical stream characteristics at the sampling sites; mean values.
CPOM: coarse particulate organic matter.

Stream	temp [°C]	pH	EC [µS cm^{-1}]	O_2 [mg L^{-1}]	TOC [mg L^{-1}]	NH_4 [mg L^{-1}]	NO_2 [mg L^{-1}]	NO_3 [mg L^{-1}]	PO_4 [mg L^{-1}]	hardness [°dH]	number of sites
Bode	10,7	7,8	1397	11,1	4,4	0,24	0,12	11,6	0,08	25,3	6
Eine	14,8	8,1	1157	9,6	4,0	0,16	0,07	32,4	0,28	30,1	2
Mulde	17,3	7,4	362	9,6	5,8	0,12	0,30	16,6	0,20	7,2	1
Selke	14,8	8,0	692	10,0	3,0	0,04	0,06	14,2	0,21	15,3	5
Wipper	16,7	7,8	764	8,5	7,8	0,36	0,25	22,0	0,50	20,8	3
Ziethe	14,6	7,7	1670	7,4	6,4	0,28	0,60	39,6	0,26	48,8	2

SI Table S2: Chemical stream parameters as recorded by the governmental agency for flood protection and water management in Saxony-Anhalt (LHW); mean values. temp: temperature; EC: electrical conductivity; O_2: total dissolved oxygen (TDO); TOC: total organic compounds; NH_4: ammonium; NO_2: nitrite; NO_3: nitrate; PO_4: phosphate; hardness: water hardness.

Compound	CAS number	MW [Da]	Purity [%]	Supplier	Use	log K_{OW}	log S_W [mol L^{-1}]	R_S model [low/high]	Remark	Reference
Alachlor	15972-60-8	269,77	99.4	E	H	3,52	-3,05	high		
Ametryn	834-12-8	227,34	98.2	E	H	2,98	-3,09	high		
Atrazine	1912-24-9	215,69	98.4	E	H	2,61	-3,89	high		
Atrazin-desethyl	6190-65-4	187,64	96.0	E	M	1,51	-1,76	high		
Atrazine-desisopropyl	1007-28-9	173,61	98.7	E	M	1,15	-2,41	high		
Azoxystrobin	131860-33-8	403,40	99.6	S	F	2,64	-4,78	low	not stable in water	MacBean, 2012
Carbofuran	1563-66-2	221,26	99.5	E	I	2,32	-2,84	low	degradation / hydrolysis	Gunold et al., 2008
Cyprodinil	121552-61-2	225,30	99.9	S	F	3,90	-4,24	high		
Dimethoate	60-51-5	229,26	98.0	E	I	0,78	-0,98	low	log $K_{OW} < 1$	
Diuron	330-54-1	233,10	98.0	S	H	2,68	-3,29	low	retained by PES membrane	Vermeirssen et al., 2012
Fenpropidin	67306-00-7	273,46	94.0	E	F	2,59	-4,64	high		
Imidacloprid	138261-41-3	255,67	99.9	S	I	0,33	-2,70	low	log $K_{OW} < 1$	
Isoproturon	34123-59-6	206,29	99.5	E	H	2,87	-3,54	high		
Linuron	330-55-2	249,10	99.5	E	H	3,20	-3,52	low	degradation / hydrolysis	Gunold et al., 2008
Pirimicarb	23103-98-2	238,29	99.6	S	I	1,70	-1,90	high		
Promethryn	7287-19-6	241,37	98.0	E	H	3,51	-3,86	high		
Propazine	139-40-2	229,72	98.0	E	H	2,93	-4,43	high		
Pymetrozine	123312-89-0	217,24	99.0	E	I	-0,18	-2,91	low	log $K_{OW} < 1$	
Simazine	122-34-9	201,67	99.6	E	H	2,18	-4,12	high		
Sulfotep	3689-24-5	322,32	94.0	E	I	3,99	-4,51	high		
Tebuconazole	107534-96-3	307,83	99.0	E	F	3,70	-3,98	high		
Terbuthylazine	5915-41-3	241,37	99.0	E	H	3,06	-4,43	high		
Terbutryn	886-50-0	229,72	98.7	S	H	3,74	-4,04	high		
Thiacloprid	111988-49-9	252,73	99.9	S	I	1,26	-3,14	low	low values in the literature	Schäfer et al., 2008
Thiamethoxam	153719-23-4	291,72	98.5	E	I	-0,13	-1,85	low	log $K_{OW} < 1$	
Alachlor D13	1015856-63-9	282,85	99,0	E	PRC					
Pirimicarb D6	1015854-66-6	244,32	98.5	E	PRC					

SI Table S3: Standard chemicals and deuterated standards (Pirimicarb-d6 and Alachlor-d13) used as performance reference compounds in the present study.
CAS: Chemical Abstracts Service; MW: molecular weight; K_{OW}: octanol-water partition coefficient; S_W: solubility in water at 20-25 °C; R_S model: equations for the calculation of overall sampling rates for compounds with *high* and *low* affinity to the Chemcatcher, respectively, based on the average flow velocity of the sampled stream during exposure;
E: Dr. Ehrenstorfer, Augsburg, Germany; S: Sigma-Aldrich, Seelze, Germany; H: herbicide; F: fungicide; I: insecticide; M: metabolite; PRC: performance reference compound.

Time [min]	Flow rate [µL min^{-1}]	30 % methanol + 70 % $H_2$0 2 mM ammonium formate [%]	90 % methanol + 10 % $H_2$0 2 mM ammonium formate [%]
0.2	200	85	15
0.5	200	85	15
6.0	200	0	100
19.0	200	0	100
20.0	200	85	15
30.2	200	85	15

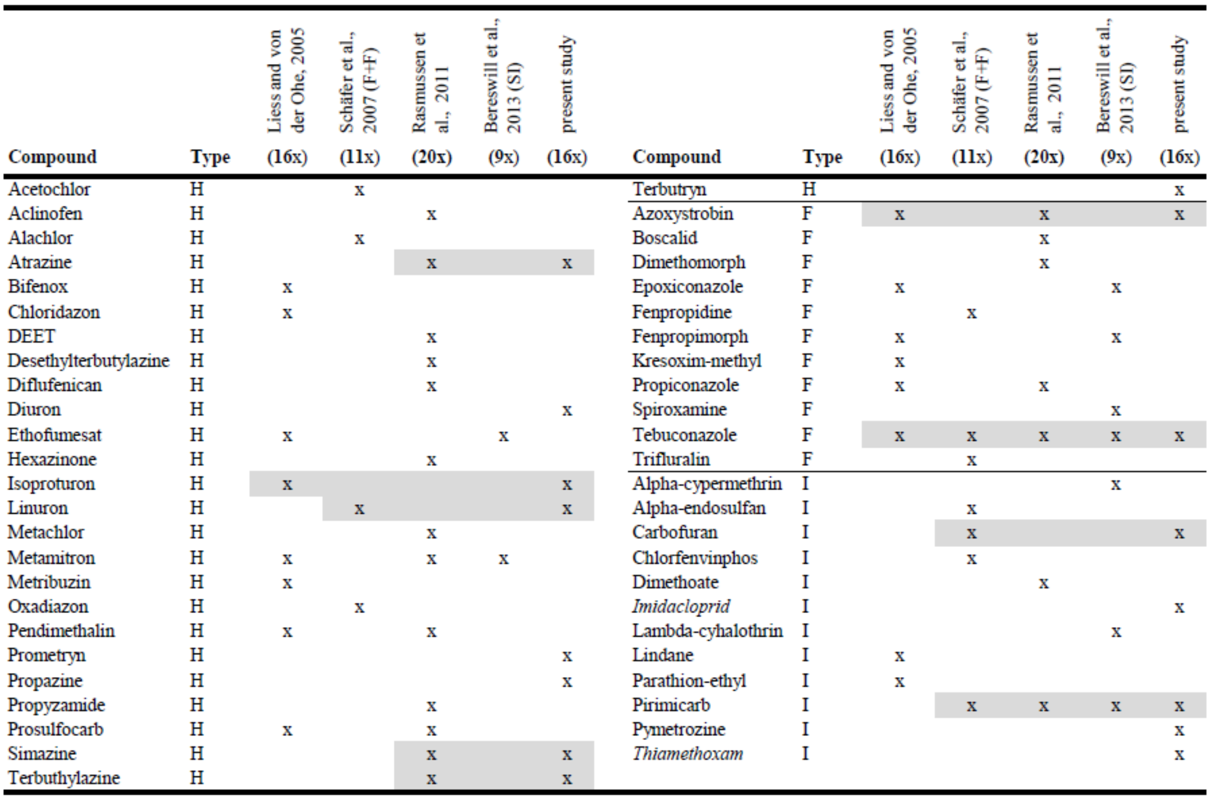
SI Table S4: Composition of the mobile phase used for the LC measurements.

Compound	1^{st} mass transition		DT	DP	EP	CE	CXP	2^{nd} mass transition		DT	DP	EP	CE	CXP
	[m z^{-1}]	[m z^{-1}]	[ms]	[V]	[V]	[V]	[V]	[m z^{-1}]	[m z^{-1}]	[ms]	[V]	[V]	[V]	[V]
Alachlor	270.1	238.1	50	15	11	15	12	270.1	162.2	25	15	11	25	8
Ametryn	228.1	186.2	25	36	11	25	10	228.1	96.1	25	36	10.5	35	4
Atrazine	215.9	174.0	25	25	10	28	2	215.9	104.0	25	25	10	40	2
Atrazine-desethyl	188.1	146.1	50	24	12	26	6	188.1	104.1	25	24	12	36	6
Atrazine-desisopropyl	174.1	104.2	50	61	10.5	31	4	174.1	96.0	25	61	11	27	6
Azoxystrobin	404.0	371.9	25	36	9.5	19	20	404.0	343.9	25	31	10	29	18
Carbofuran	222.1	123.0	25	16	10	29	6	222.1	165.1	25	16	9	17	8
Cyprodinil	226.1	93.0	25	30	12	50	2	226.1	77.1	25	30	12	67	7
Dimethoate	229.8	199.0	50	11	6	16	4	229.8	125.1	50	11	6	30	4
Diuron	233.0	72.0	25	20	8	40	2	233.0	160.0	25	20	10	35	2
Fenpropidin	274.2	147.1	25	51	10	37	8	274.2	117.1	25	51	9.5	65	6
Imidacloprid	255.9	209.0	50	22	4	32	3.5	255.9	175.0	50	22	4	32	3.5
Isoproturon	207.0	72.0	25	22	12	40	10	207.0	165.0	25	22	12	22	10
Linuron	249.0	160.0	25	20	10	28	7	249.0	182.0	25	20	11	26	8
Pirimicarb	239.1	72.0	25	16	7	31	10	239.1	182.0	25	16	6.5	21	10
Prometryn	242.0	158.0	25	25	10	35	6	242.0	200.0	25	25	10	28	6
Propazine	229.9	146.0	25	25	10	35	7	800.0	500.0	5	25	7	31	10
Pymetrozine	217.9	105.0	25	56	9.5	27	6	218.0	79.0	25	51	10	47	7
Simazine	202.0	132.0	50	20	8	29	2	202.0	124.0	25	20	8	25	2
Sulfotep	323.0	115.0	25	46	12	39	6	323.0	97.1	25	46	12	45	4
Tebuconazole	308.0	70.0	25	21	12	39	10	308.0	125.0	25	21	10	47	6
Terbuthylazine	230.0	174.1	25	41	11	23	8	230.0	104.2	25	41	9.5	43	4
Terbutryn	242.3	186.1	25	21	11.5	25	10	242.3	68.1	25	26	12	57	4
Thiacloprid	252.9	126.0	50	22	10	30	4	252.9	73.0	50	22	10	94	4
Thiamethoxam	292.0	211.0	25	51	8.5	17	10	292.0	181.0	25	56	8.5	31	10
Alachlor D13	283.1	175.0	25	5	6	30	4	283.1	251.0	25	5	6	18	4
Pirimicarb D6	245.1	78.0	25	16	10	40	7	245.1	185.0	25	16	10	25	7

SI Table S5: Analytes investigated in the present study and parameters used for mass spectrometry. DT: dwell time; DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: cell exit potential.

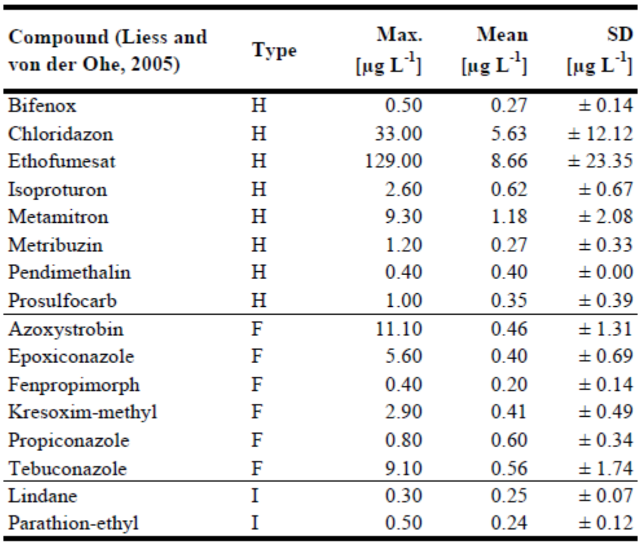
Compound	Type	Class	Acute (48 h) LC_{50} [µg L^{-1}]	Most sensitive standard test organism	Reference
Atrazine	H	triazine	720	Chironomus tentans	USEPA, 2014
Azoxystrobin	F	strobilurin	230	Daphnia magna	University of Hertfordshire, 2014
Carbofuran	I	carbamate	9.4	Daphnia magna	University of Hertfordshire, 2014
Diuron	H	phenylurea	8400	Daphnia magna	UFZ, 2014a
Imidacloprid	I	neonicotinoid	22	Chironomus spec.	USEPA, 2014
Isoproturon	H	urea	710	Daphnia magna	LANUV, 2014
Linuron	H	urea	310	Daphnia magna	University of Hertfordshire, 2014
Pirimicarb	I	carbamate	16.25	Daphnia magna	UFZ, 2014a
Prometryn	H	triazine	14145	Daphnia magna	UFZ, 2014a
Propazine	H	triazine	8160	Daphnia magna	UFZ, 2014a
Pymetrozine	I	pyridine	87000	Daphnia magna	University of Hertfordshire, 2014
Simazine	H	triazine	1100	Daphnia magna	University of Hertfordshire, 2014
Tebuconazole	F	triazole	2790	Daphnia magna	University of Hertfordshire, 2014
Terbuthylazine	H	triazine	13100	Daphnia magna	UFZ, 2014a
Terbutryn	H	triazine	7100	Daphnia magna	UFZ, 2014a
Thiamethoxam	I	neonicotinoid	22	Chironomus spec.	USEPA, 2014

SI Table S6: Acute (48 h) LC50 values for the most sensitive standard test organisms (Daphnia spec. and Chironomus spec.) for the compounds detected in the present study. For Imidacloprid and Thiamethoxam, the median acute (48 h) LC_{50} from five tested neonicotinoids (Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, and Thiamethoxam) was computed.
H: herbicide; F: fungicide; I: insecticide; Italics: neonicotinoid.



Compound	Type	Liess and von der Ohe, 2005 (16x)	Schäfer et al., 2007 (F+F) (11x)	Rasmussen et al., 2011 (20x)	Bereswill et al., 2013 (SI) (9x)	present study (16x)	Compound	Type	Liess and von der Ohe, 2005 (16x)	Schäfer et al., 2007 (F+F) (11x)	Rasmussen et al., 2011 (20x)	Bereswill et al., 2013 (SI) (9x)	present study (16x)
Acetochlor	H		x				Terbutryn	H					x
Aclinofen	H			x			Azoxystrobin	F	x		x		x
Alachlor	H		x				Boscalid	F			x		
Atrazine	H			x		x	Dimethomorph	F			x		
Bifenox	H	x					Epoxiconazole	F	x			x	
Chloridazon	H	x					Fenpropidine	F		x			
DEET	H			x			Fenpropimorph	F	x			x	
Desethylterbutylazine	H			x			Kresoxim-methyl	F	x				
Diflufenican	H			x			Propiconazole	F	x		x		
Diuron	H					x	Spiroxamine	F				x	
Ethofumesat	H	x			x		Tebuconazole	F	x	x	x	x	x
Hexazinone	H			x			Trifluralin	F		x			
Isoproturon	H	x				x	Alpha-cypermethrin	I				x	
Linuron	H		x			x	Alpha-endosulfan	I		x			
Metachlor	H			x			Carbofuran	I		x			x
Metamitron	H	x		x	x		Chlorfenvinphos	I		x			
Metribuzin	H	x					Dimethoate	I			x		
Oxadiazon	H		x				*Imidacloprid*	I					x
Pendimethalin	H	x		x			Lambda-cyhalothrin	I				x	
Prometryn	H					x	Lindane	I	x				
Propazine	H					x	Parathion-ethyl	I	x				
Propyzamide	H			x			Pirimicarb	I		x	x	x	x
Prosulfocarb	H	x		x			Pymetrozine	I					x
Simazine	H			x		x	*Thiamethoxam*	I					x
Terbuthylazine	H			x		x							

SI Table S7: Pesticides detected during five recent field investigations on the impact of pesticides on the non-target biota. Compounds highlighted have been detected in more than one study.
F + F: France and Finland; SI: Supplementary Information; H: herbicide; F: fungicide;
I: insecticide; Italics: neonicotinoid.



Compound (Liess and von der Ohe, 2005)	Type	Max. [µg L^{-1}]	Mean [µg L^{-1}]	SD [µg L^{-1}]
Bifenox	H	0.50	0.27	± 0.14
Chloridazon	H	33.00	5.63	± 12.12
Ethofumesat	H	129.00	8.66	± 23.35
Isoproturon	H	2.60	0.62	± 0.67
Metamitron	H	9.30	1.18	± 2.08
Metribuzin	H	1.20	0.27	± 0.33
Pendimethalin	H	0.40	0.40	± 0.00
Prosulfocarb	H	1.00	0.35	± 0.39
Azoxystrobin	F	11.10	0.46	± 1.31
Epoxiconazole	F	5.60	0.40	± 0.69
Fenpropimorph	F	0.40	0.20	± 0.14
Kresoxim-methyl	F	2.90	0.41	± 0.49
Propiconazole	F	0.80	0.60	± 0.34
Tebuconazole	F	9.10	0.56	± 1.74
Lindane	I	0.30	0.25	± 0.07
Parathion-ethyl	I	0.50	0.24	± 0.12

SI Table S8a: Pesticide concentrations from the study of Liess and von der Ohe (2005).
SD: standard deviation; H: herbicide; F: fungicide; I: insecticide.

Compound (Schäfer et al., 2007; France data)	Type	Max. [µg L^{-1}]	Compound (Schäfer et al., 2007; Finland data)	Type	Max. [µg L^{-1}]
Acetochlor	H	1.92	Azoxystrobin	F	not detected
Alachlor	H	0.81	Cyprodinil	F	not detected
Linuron	H	0.10	Trifluralin	F	0.001
Oxadiazon	H	0.07	Alpha-cypermethrin	I	not detected
Fenpropidine	F	0.06	Alpha-endosulfan	I	not detected
Tebuconazole	F	0.07	Deltamethrin	I	not detected
Alpha-endosulfan	I	0.08	Lambda-cyhalothrin	I	not detected
Carbofuran	I	0.72	Malathion	I	not detected
Chlorfenvinphos	I	0.12	Sulfotep	I	not detected
Pirimicarb	I	0.07	Tau-fluvalinate	I	not detected

SI Table S8b: Pesticide concentrations from the study of Schäfer et al. (2007).
H: herbicide; F: fungicide; I: insecticide.

Compound (Rasmussen et al., 2011)	Type	Min. [µg L^{-1}]	Max. [µg L^{-1}]
Aclinofen	H	0.14	0.14
Atrazine	H	0.01	0.02
DEET	H	0.05	0.05
Desethylterbutylazine	H	0.01	0.11
Diflufenican	H	0.02	0.15
Hexazinone	H	0.06	0.06
Metachlor	H	0.01	0.05
Metamitron	H	0.12	0.12
Pendimethaline	H	0.02	0.97
Propyzamide	H	0.01	0.43
Prosulfocarb	H	0.01	0.07
Simazine	H	0.03	0.03
Terbutylazine	H	0.01	0.60
Azoxystrobin	F	0.05	0.51
Boscalid	F	0.07	0.72
Dimethomorf	F	0.01	0.08
Propiconazole	F	0.04	0.27
Tebuconazole	F	0.02	0.24
Dimethoate	I	0.01	0.18
Pirimicarb	I	0.01	0.32

SI Table S8c: Pesticide concentrations from the study of Rasmussen et al. (2011).
H: herbicide; F: fungicide; I: insecticide.

Compound (Bereswill et al., 2013; SI, water phase data)	Type	Max. [µg/L]	Mean [µg/L]	SD [µg/L]
Ethofumesate	H	21.00	3.60	± 6.00
Metamitron	H	0.19	0.19	± 0.00
Epoxiconazole	F	0.25	0.17	± 0.06
Fenpropimorph	F	0.10	0.08	± 0.02
Spiroxamine	F	0.24	0.15	± 0.08
Tebuconazole	F	0.60	0.31	± 0.19
Alpha-cypermethrin	I	0.09	0.09	± 0.00
Lambda-cyhalothrin	I	0.55	0.26	± 0.21
Pirimicarb	I	0.18	0.18	± 0.00

SI Table S8d: Pesticide concentrations from the study of Bereswill et al. (2013).
SD: standard deviation; H: herbicide; F: fungicide; I: insecticide.

Compound	CAS number	MW	log K_{OW}	log S_W	R_S-model	$R_S = mv + n$		Flow velocity	Sampling rate R_S	Reference (sampling rates)
		[Da]		[mol L^{-1}]	[low/high]	m	n	v [m s^{-1}]	[L day^{-1}]	
Ametryn	834-12-8	227.34	2.98	-3.09	high	0.0085	0.014	14.0	0.140	Shaw et al., 2009
								14.0	0.140	Shaw and Mueller, 2009
Atrazine	1912-24-9	215.69	2.61	-3.89	high	0.0085	0.014	0.0	0.013	O'Brien et al., 2011
								0.4	0.023	Tran et al., 2007
								3.4	0.041	O'Brien et al., 2011
								8.0	0.072	O'Brien et al., 2011
								8.0	0.088	Vermeirssen et al., 2012
								13.0	0.120	Vermeirssen et al., 2009
								14.0	0.140	Shaw et al., 2009
								14.0	0.170	Shaw and Mueller, 2009
								16.1	0.124	O'Brien et al., 2011
								24.1	0.136	O'Brien et al., 2011
								30.0	0.177	Stephens et al., 2009
Atrazine, hydroxy-	2163-68-0	197.24	1.75	-4.52	low	0.0047	0.0004	8.0	0.037	Vermeirssen et al., 2012
Atrazine, desethyl-	6190-65-4	187.64	1.51	-1.76	high	0.0085	0.014	8.0	0.065	Vermeirssen et al., 2012
								13.0	0.100	Vermeirssen et al., 2009
Atrazine, -deisopropyl	1007-28-9	173.61	1.15	-2.41	low	0.0047	0.0004	8.0	0.044	Vermeirssen et al., 2012
Benzotriazole	95-14-7	119.13	1.44	-1.06	low	0.0047	0.0004	8.0	0.025	Vermeirssen et al., 2012
Benzotriazole, 5-methyl-	136-85-6	133.15	1.61	-1.64	high	0.0085	0.014	8.0	0.060	Vermeirssen et al., 2012
Caffeine	58-08-2	194.19	-0.07	-0.95	low	0.0047	0.0004	8.0	0.039	Vermeirssen et al., 2012
Carbamazepine	298-46-4	236.28	2.45	-3.76	high	0.0085	0.014	8.0	0.098	Vermeirssen et al., 2012
Carbendazim	10605-21-7	191.19	1.52	-3.83	high	0.0085	0.014	8.0	0.078	Vermeirssen et al., 2012
								13.0	0.100	Vermeirssen et al., 2009
Chloridazon	1698-60-8	221.65	1.14	-2.74	high	0.0085	0.014	8.0	0.075	Vermeirssen et al., 2012
Clomazone	81777-89-1	239.70	2.50	-2.34	high	0.0085	0.014	0.4	0.022	Tran et al., 2007
Diazinon	333-41-5	304.35	3.81	-3.88	low	0.0047	0.0004	8.0	0.033	Vermeirssen et al., 2012
								13.0	0.060	Vermeirssen et al., 2009
Diclofenac	15307-79-6	296.15	1.17	-6.29	low	0.0047	0.0004	8.0	0.050	Vermeirssen et al., 2012
								13.0	0.080	Vermeirssen et al., 2009
Diuron	330-54-1	233.10	2.68	-3.29	low	0.0047	0.0004	0.4	0.026	Tran et al., 2007
								8.0	0.032	Vermeirssen et al., 2012
								13.0	0.030	Vermeirssen et al., 2009
								14.0	0.080	Shaw et al., 2009
								14.0	0.070	Shaw and Mueller, 2009
								30.0	0.153	Stephens et al., 2009
Fipronil	120068-37-3	410.40	4.00	-5.33	high	0.0085	0.014	14.0	0.160	Shaw et al., 2009
Hexazinone	51235-04-2	252.32	1.36	-0.88	high	0.0085	0.014	14.0	0.100	Shaw et al., 2009
								14.0	0.140	Shaw and Mueller, 2009
Irgarol	28159-98-0	253.38	3.38	-3.67	high	0.0085	0.014	8.0	0.080	Vermeirssen et al., 2012
								13.0	0.130	Vermeirssen et al., 2009
Isoproturon	34123-59-6	206.29	2.87	-3.54	high	0.0085	0.014	8.0	0.069	Vermeirssen et al., 2012
								13.0	0.100	Vermeirssen et al., 2009
Mecoprop	93-65-2	214.65	3.13	-2.38	low	0.0047	0.0004	8.0	0.021	Vermeirssen et al., 2012
								13.0	0.070	Vermeirssen et al., 2009
Metolachlor	51218-45-2	283.80	3.13	-4.13	high	0.0085	0.014	0.4	0.021	Tran et al., 2007
								8.0	0.098	Vermeirssen et al., 2012
								14.0	0.210	Shaw et al., 2009
Phenazone	60-80-0	188.23	0.38	-0.56	high	0.0085	0.014	8.0	0.069	Vermeirssen et al., 2012
Promethryn	7287-19-6	241.37	3.51	-3.86	high	0.0085	0.014	0.0	0.009	O'Brien et al., 2011
								3.4	0.032	O'Brien et al., 2011
								8.0	0.060	O'Brien et al., 2011
								16.1	0.107	O'Brien et al., 2011
								24.1	0.126	O'Brien et al., 2011
Simazine	122-34-9	201.67	2.18	-4.12	high	0.0085	0.014	0.4	0.024	Tran et al., 2007
								14.0	0.140	Shaw et al., 2009
								14.0	0.160	Shaw and Mueller, 2009
								30.0	0.183	Stephens et al., 2009
Sulcotrione	99105-77-8	328.77	2.31	-3.30	low	0.0047	0.0004	8.0	0.023	Vermeirssen et al., 2012
Sulfamethoxazole	723-46-6	253.28	0.89	-2.62	low	0.0047	0.0004	8.0	0.020	Vermeirssen et al., 2012
								13.0	0.090	Vermeirssen et al., 2009
Tebuthiuron	34014-18-1	228.30	1.79	-1.96	high	0.0085	0.014	14.0	0.110	Shaw et al., 2009
								14.0	0.160	Shaw and Mueller, 2009
Terbuthylazine	5915-41-3	229.72	3.06	-4.43	high	0.0085	0.014	8.0	0.087	Vermeirssen et al., 2012
								13.0	0.130	Vermeirssen et al., 2009
Terbutryn	886-50-0	241.37	3.74	-4.04	high	0.0085	0.014	8.0	0.081	Vermeirssen et al., 2012
								13.0	0.110	Vermeirssen et al., 2009
Thiacloprid	111988-49-9	252.73	1.26	-3.14	low	0.0047	0.0004	10.0	0.035	Schäfer et al., 2008

SI Table S9: Experimental sampling rates from calibration studies used for RS modelling. CAS: Chemical Abstracts Service; MW: molecular weight; K_{OW}: octanol-water partition coefficient; S_W: solubility in water at 20-25 °C; R_S model: experimental data contributed to the evaluation of the low and high sampling rate model, respectively.

SI Text S1

Using overall sampling rates for the calculation of time-weighted average concentrations

In the present study, Chemcatcher passive samplers were deployed for the detection of the time-weighted average concentration (c_{TWA}) of hydrophilic pesticides (mainly hydrophilic pesticides) in streams located in an agricultural area (see SI Table S5). Because the sampling sites were allocated to various river zones based on the topography of these zones, the passive samplers were exposed to a wide range of hydrodynamic conditions. The velocity of the water that passes by a passive sampler strongly affects the extent of the aqueous boundary layer above the sampler’s surface. In this layer, the water is regarded as quiescent, and for dissolved compounds, the only transport process therein is diffusion.

Various studies on the uptake kinetics of hydrophobic compounds into passive samplers concluded that the diffusion through this aqueous boundary can be seen as the limiting factor during the transport process from the water phase into the receiving phase of the sampler (Bayen et al., 2009; Gobas and MacKay, 1987; Vaes et al., 1996). In contrast, the uptake kinetics of hydrophilic compounds into passive samplers is primarily controlled by the resistance of the sampler. Hydrodynamic conditions at the sampling site and, hence, the extent of the aqueous boundary layer should therefore have a minor influence on the uptake characteristics.

However, studies on passive sampling of hydrophilic compounds observed deviant uptake rates when the flow regime was altered. This effect was reported by Green and Abraham (2000) for the passive sampling of Diuron with a C_{18} Empore disk, a common receiving phase for a wide range of Chemcatcher applications. This result at least indicates a significant impact of the hydrodynamic conditions on the uptake kinetics even for hydrophilic compounds.

In general, the uptake behaviour at any specific sampling site is monitored with the elimination of performance reference compounds (PRC; Huckins et al., 1993). Suitable analytes for this approach have properties similar to the target compounds; therefore, their stable labelled isotopes are in common use (not present in the investigated stream). Typically, the hydrogen or carbon atoms of selected target compounds are replaced by deuterium or carbon-13, respectively.

Within the scientific community, the PRC approach is widely regarded as appropriate for the in-situ correction of the c_{TWA} of hydrophobic compounds, while for hydrophilic compounds, the PRC concept apparently has to be verified for every new target compound and its deuterated surrogate. Hence, this study used passive flow monitors (PFM) to monitor the flow velocity in the vicinity of the Chemcatchers at each sampling site, as suggested by O’Brien and co-workers (O’Brien et al., 2009). Using this approach, the c_{TWA} of detected pesticides were calculated as a function of the average flow velocity measured with the PFM and the amount of pesticides found in the passive sampler. For flow velocities greater than 0.034 m s^{-1}, the amount of plaster released from the PFMs is a linear function of the current. Hence, the average flow velocity v (m s^{-1}) was calculated from the daily loss of plaster r_{PFM} (g d^{-1}) using the following equation (O'Brien et al., 2011):

$$v = \frac{r_{\mathrm{PFM}} - 0.065}{16.4}$$

The PFM used in this study were prepared with dental plaster from another supplier than that suggested by the authors of this method (O’Brien et al., 2009). To correct for possible deviation of the linear relationship between plaster mass loss and flow velocity, we conducted our own calibration experiment in the range of 0 - 0.6 m s^{-1}. The results proved the applicability of the method with a random error of less than 10 %.

Without the implementation of the PRC approach, the calculation of the c_{TWA} of a certain compound requires an experimentally derived sampling rate (R_{S}), which is valid for the hydrodynamic condition prevalent during calibration in the laboratory. These calibration experiments are time and work intensive; therefore, especially for novel pesticides, relevant data in the literature are scarce.

For the range of pesticides investigated in the present study, suitable R_{S} values could not be obtained from the literature for most of the compounds and flow velocities (see SI Table S9). This lack of calibration data was overcome by using an overall sampling rate, as promoted by Escher et al. (2011). This alternative approach is capable of calculating c_{TWA} for the majority of hydrophilic compounds without the need for extensive calibration experiments, yielding reasonable accuracy. The method was adapted to the present study by compiling all R_{S} values available for this Chemcatcher configuration from the literature, with the flow velocities prevalent during the calibration experiments being used as correction factors. Other possible influences, such as temperature (Greenwood et al., 2007) or housing geometry (Lobpreis et al., 2008), are regarded as negligible and were therefore not used for the correction of sampling rates. As observed in previous studies (Vermeirssen et al., 2012, 2009), sampling rates increase linearly with flow velocity but show constant values when water currents exceed more than approx. 0.2 m s^{-1}. This finding was challenged by a recent field study that denied any significant influence on sampling rates for flow velocities in the range of 0.05 - 0.8 m s^{-1} (Moschet et al., 2015). However, in that investigation, the average flow velocities were ≥ 0.2 m s^{-1}, except for 7 of 44 sampling sites, with a minimum average flow velocity of 0.125 L d^{-1}.

Furthermore, other factors impair the apparent sampling rates. Compounds with high affinity for the overlaying PES membrane, as well as those with a low tendency of accumulation in the SDB-RPS sorbent, show comparatively reduced sampling rates (Shaw et al., 2009; Shaw and Mueller, 2009; Vermeirssen et al., 2012, 2009). Compounds with high water solubility and log $K_{\mathrm{OW}} < 1$ show a similar effect, causing a negligible tendency of diffusion from the water to the receiving phase. Another reason for apparently lower sampling rates is microbiological degradation or hydrolysis that reduces the compound's concentration in the receiving phase until the passive sampler is retrieved from the sampling site (Gunold et al., 2008; Vermeirssen et al., 2009). The sampling rates of compounds with accordant properties were estimated with another regression equation (see SI Tables S3 and S9). This second equation showed a reduced slope but likewise maximum R_{S} values at a flow velocity of approx. 0.2 m s^{-1} (Stephens et al., 2009; Vermeirssen et al., 2012, 2009). The experimental sampling rate values, the regression fits, and the applied regression equations are given in SI Fig. S3.

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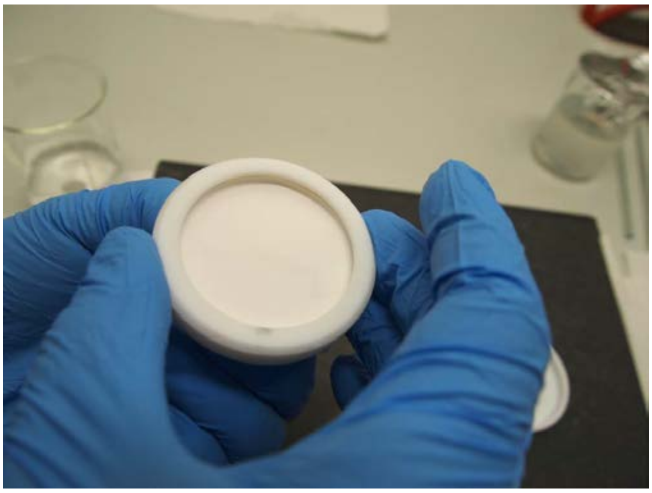
APPENDIX B

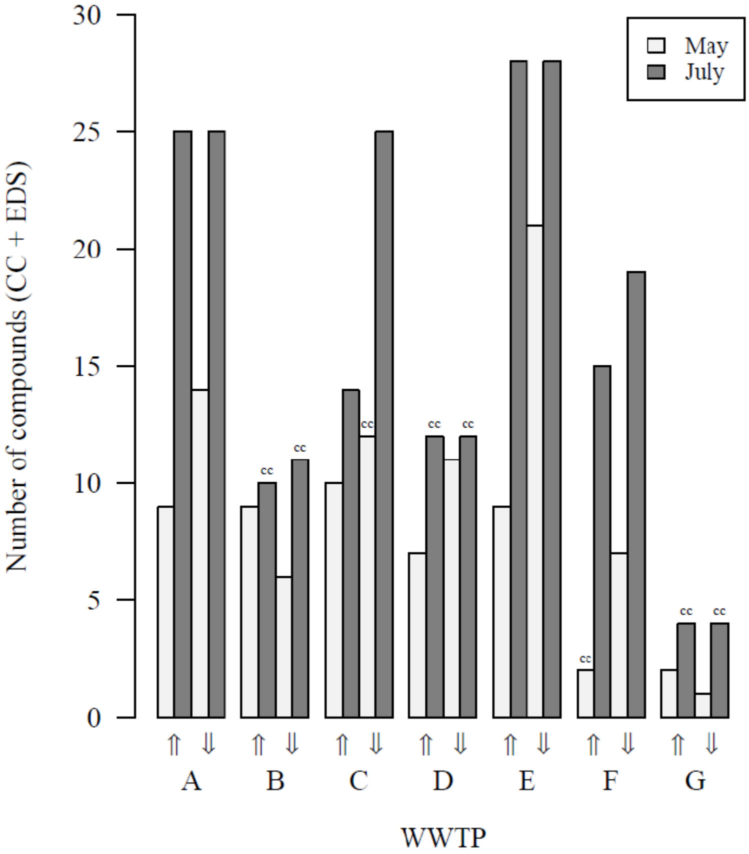
Supplementary Material to CHAPTER 3



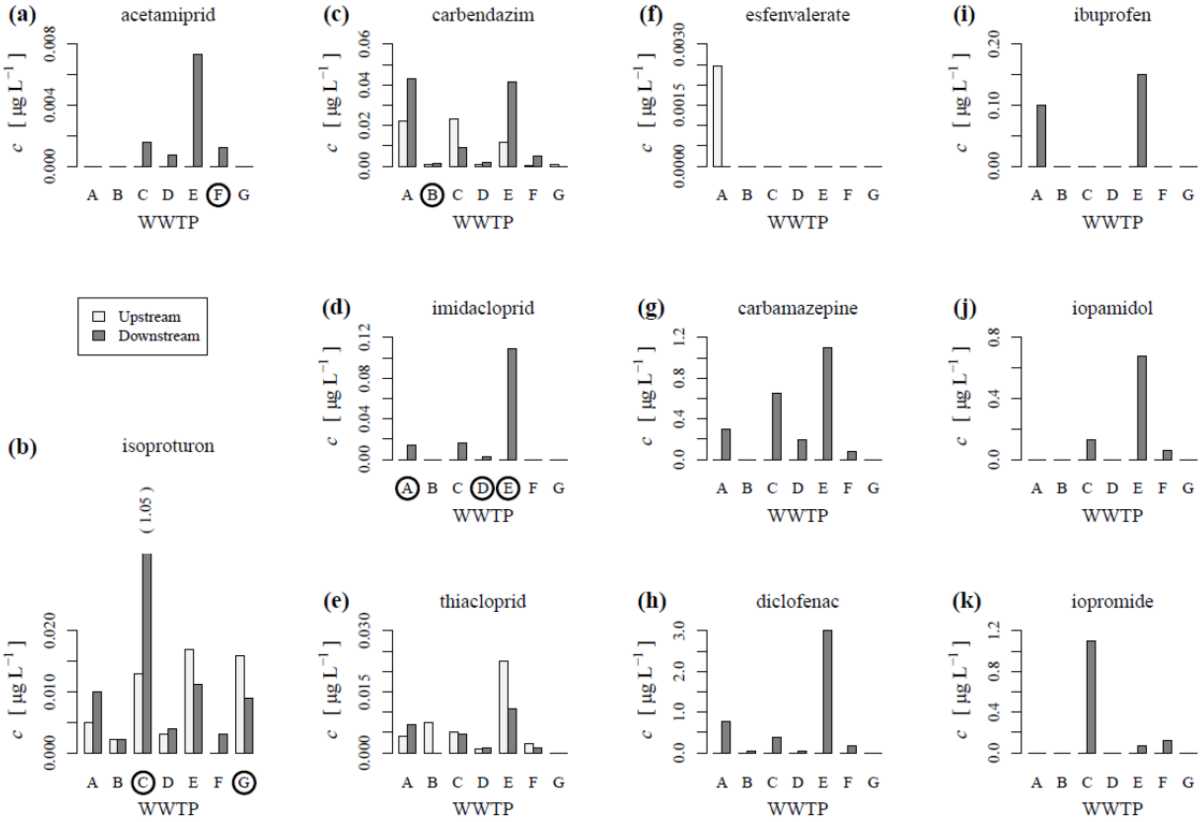
SI Fig. 1a Chemcatcher passive sampler with 3rd-generation housing made from polytetrafluoroethylene (PTFE; 'Teflon'). Photo: O. Kaske

SI Fig. 1b Field sampling with Chemcatcher passive samplers. Photo: R. Münze

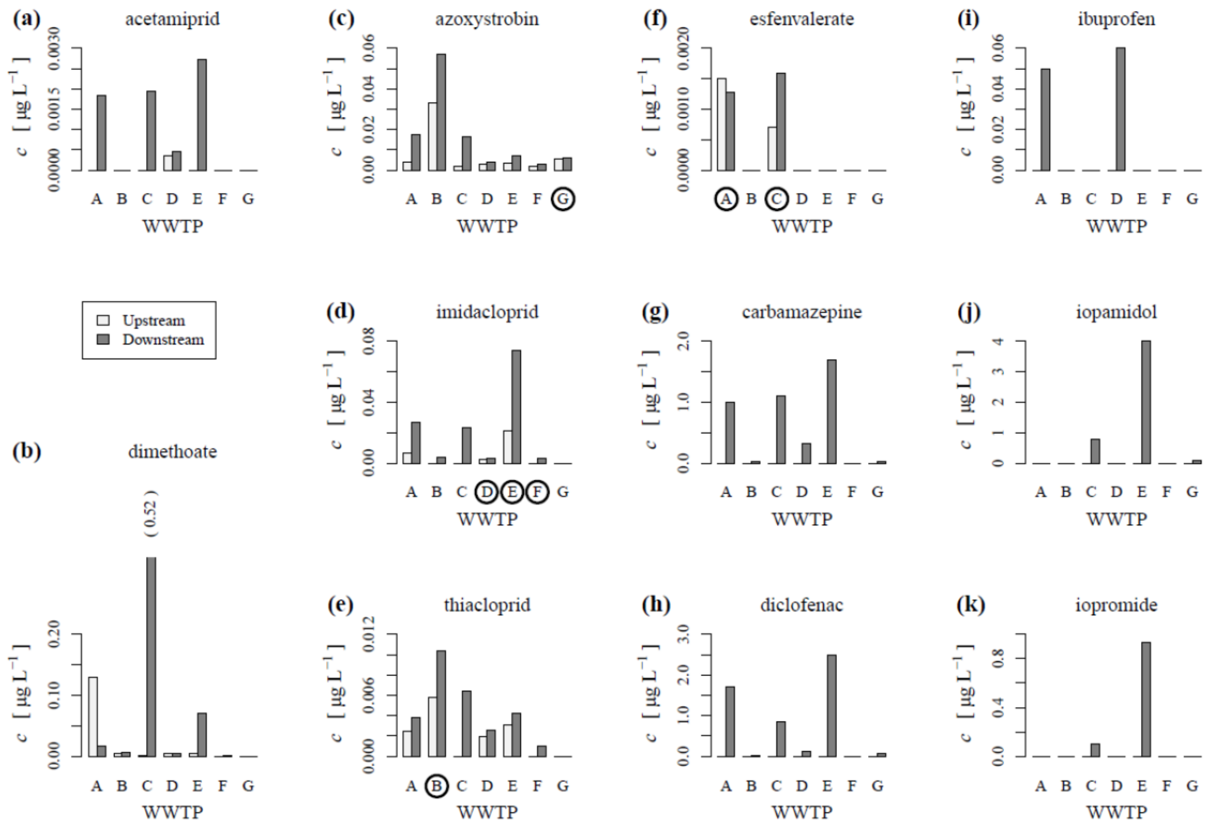
SI Fig. 2 Event-driven sampler as used in the present study. Photo: R. Münze



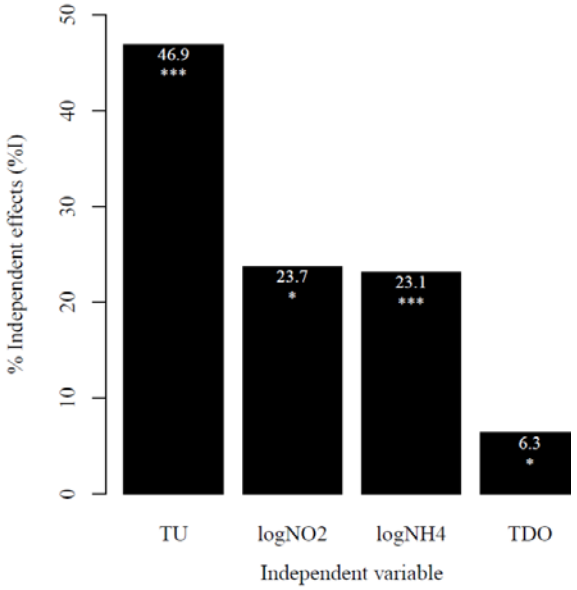
SI Fig. 3 Numbers of pesticides detected in both sampling campaigns. Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites. Where EDS data were not available, only Chemcatcher data could be considered (indicated by ‘CC’). WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.

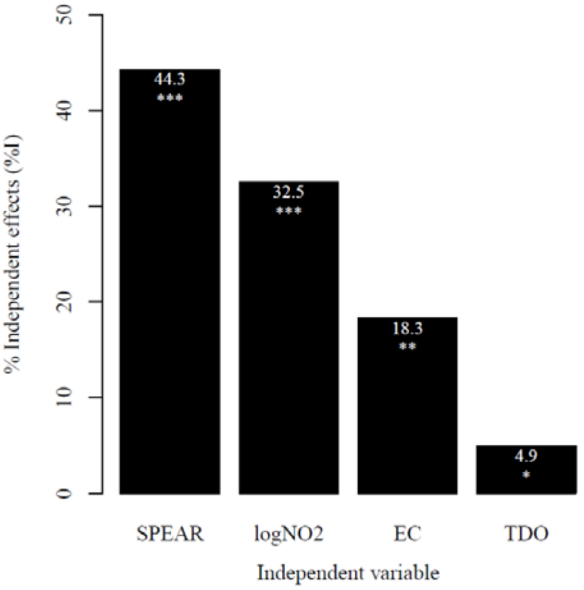


SI Fig. 4 Important pesticides at downstream sampling sites in May. Both upstream and downstream peak concentrations are shown (a – f).
Circles indicate downstream sites where the respective compounds provided TU_{max}.
Pesticide input from the WWTPs C, D, and E was ecologically relevant (significant changes in downstream TU_{max}, SPEAR$_{\text{pesticides}}$, and k). Pharmaceutical input (only measured downstream) is shown for illustration purposes (g – k). WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.



SI Fig. 5 Important pesticides at downstream sampling sites in July. Both upstream and downstream peak concentrations are shown (a – f).
Circles indicate downstream sites where the respective compounds provided TU_{max}.
Pesticide input from the WWTPs B, C, E, and F was ecologically relevant (significant changes in downstream TU_{max}, $SPEAR_{pesticides}$, and k). Pharmaceutical input (only measured downstream) is shown for illustration purposes (g – k). WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.





SI Fig. 6 Hierarchical partitioning: weights of the explanatory variables for $SPEAR_{pesticides}$ (left side).
TU: Toxic Unit ($p < 0.0001$, df = 1, *Chi2* = 16.9);
NO_2 ($p = 0.04$, df = 1, *Chi2* = 4.0);
NH_4 ($p = 0.0004$, df = 1, *Chi2* = 12.4);
TDO: total dissolved oxygen ($p = 0.03$, df = 1, *Chi2* = 4.8).

SI Fig. 7 Hierarchical partitioning: weights of the explanatory variables for the leaf litter breakdown rate, *k*.
SPEAR: $SPEAR_{pesticides}$ index ($p < 0.0001$, df = 1, *Chi2* = 23.5);
NO_2 ($p = 0.0007$, df = 1, *Chi2* = 11.5);
EC: electrical conductivity ($p = 0.003$, df = 1, *Chi2* = 9.0);
TDO: total dissolved oxygen ($p = 0.02$, df = 1, *Chi2* = 5.4).

WWTP					Receiving water							
WWTP	**PT [L]**	**Treatment levels**	**Sewage system**	**Combined sewage overflow (CSO)**	**Strahler stream order**	**Structural quality class**	**Stream width [m]**		**Stream depth [m]**		**Velocity [m s^{-1}]** (mean of May and July)	
							upstream	downstream	upstream	downstream	upstream	downstream
A	10000	3	combined	no (retention basin)	1	3	2.2	1.6	0.1	0.15	0.09	0.1
B	1435	3	separate	n/a	3	3	6.0	6.0	0.2	0.1	0.15	0.12
C	30000	3	combined	no (dilution in the maturation pond)	1	4	1.1	1.1	0.1	0.15	0.13	0.14
D	10000	3	separate	n/a	3	4	6.0	6.0	0.2	0.2	0.19	0.17
E	12800	3	combined	no (dilution in the maturation pond)	1	4	2.6	2.0	0.12	0.2	0.04	0.09
F	2800	3	separate	n/a	1	4	1.8	1.2	0.15	0.15	0.11	0.15
G	2700	3	combined	no (discharge to a percolation area)	3	4	3.0	2.0	0.25	0.25	0.08	0.17

SI Table 1 Characteristics of the WWTPs, with physical properties of receiving waters at the sampling sites. WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg. PT: total number of inhabitants and population equivalents

Site	NH_4 [µg L^{-1}]	NO_2 [µg L^{-1}]	NO_3 [mg L^{-1}]	PO_4 [mg L^{-1}]	TOC [mg L^{-1}]	BOD [mg L^{-1}]	EC [µS]	pH	TDO [mg L^{-1}]	Temp [°C]	Width [m]	Depth [m]	Velocity [cm s^{-1}]
A ⇧	981.00	289.00	4.60	0.34	7.96	9.00	428.00	7.80	5.90	10.67	2.20	0.10	7.74
A ⇩	1163.00	358.00	4.60	0.28	9.29	8.50	588.00	7.40	5.60	10.93	1.60	0.15	8.32
B ⇧	129.00	145.00	14.60	0.08	4.58	9.30	363.00	7.90	8.80	8.60	6.00	0.20	16.69
B ⇩	381.00	140.00	15.90	0.00	6.55	14.80	364.00	7.90	8.60	8.68	6.00	0.10	13.09
C ⇧	529.00	136.00	2.80	0.00	2.10	0.00	497.00	8.20	5.80	12.20	1.10	0.10	14.01
C ⇩	1355.00	275.00	0.00	0.96	4.05	10.80	708.00	7.40	5.40	12.72	1.10	0.15	15.63
D ⇧	1002.00	84.00	9.30	0.12	3.88	0.10	503.00	8.10	6.40	10.00	6.00	0.20	21.03
D ⇩	688.00	85.00	9.10	0.16	4.22	0.20	524.00	7.90	6.60	10.13	6.00	0.20	17.14
E ⇧	193.00	164.00	19.00	0.08	4.95	0.40	676.00	8.30	5.90	10.90	2.60	0.12	3.57
E ⇩	29313.00	156.00	5.50	3.68	18.97	0.10	1157.00	7.50	6.30	11.05	2.00	0.20	7.38
F ⇧	158.00	64.00	8.80	0.00	2.20	0.00	530.00	8.10	5.80	9.93	1.80	0.15	13.12
F ⇩	101.00	62.00	9.10	0.08	3.63	2.20	536.00	8.20	5.20	9.56	1.20	0.15	14.67
G ⇧	94.00	54.00	4.10	0.00	4.20	0.20	207.00	8.00	9.00	9.10	3.00	0.25	9.18
G ⇩	173.00	54.00	4.80	0.00	3.69	3.10	229.00	7.90	6.10	9.38	2.00	0.25	27.10

SI Table 2a Physicochemical parameters of receiving waters in May.
WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg. Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites. TOC: total organic carbon; BOD: biochemical oxygen demand; EC: electrical conductivity; TDO: total dissolved oxygen; Temp: water temperature; Width: stream width; Depth: stream depth.

Site	NH_4 [µg L^{-1}]	NO_2 [µg L^{-1}]	NO_3 [mg L^{-1}]	PO_4 [mg L^{-1}]	TOC [mg L^{-1}]	BOD [mg L^{-1}]	EC [µS]	pH	TDO [mg L^{-1}]	Temp [°C]	Width [m]	Depth [m]	Velocity [cm s^{-1}]
A ⇧	1778.00	367.00	28.20	0.66	4.52	7.80	1171.00	8.30	10.13	14.60	2.20	0.10	10.79
A ⇩	1973.00	314.00	20.20	0.59	4.88	13.50	1116.00	7.90	9.12	15.40	1.60	0.15	11.52
B ⇧	880.00	96.00	12.00	0.36	4.88	0.30	437.00	8.10	10.20	13.80	6.00	0.30	13.64
B ⇩	661.00	100.00	12.20	0.33	5.15	6.00	440.00	8.20	9.86	14.15	6.00	0.30	10.70
C ⇧	736.00	42.00	4.10	0.24	2.71	0.10	825.00	8.50	9.16	14.01	1.10	0.10	11.28
C ⇩	2546.00	92.00	3.40	1.76	5.51	2.30	878.00	7.90	7.53	16.32	1.10	0.15	12.15
D ⇧	1657.00	87.00	10.60	0.28	5.58	0.20	650.00	8.20	9.00	14.27	6.00	0.20	17.67
D ⇩	1896.00	101.00	10.30	0.39	4.16	0.10	651.00	8.20	9.33	15.38	6.00	0.20	17.48
E ⇧	2009.00	290.00	25.70	0.11	4.28	4.70	710.00	8.20	9.22	14.69	2.60	0.12	4.55
E ⇩	6945.00	241.00	8.80	0.89	6.14	6.00	921.00	7.60	8.58	15.25	2.00	0.20	9.65
F ⇧	2128.00	64.00	10.90	0.26	5.99	8.50	621.00	7.90	9.29	11.38	1.80	0.15	9.76
F ⇩	1650.00	61.00	10.50	0.42	5.30	11.70	627.00	8.10	8.71	11.84	1.20	0.15	15.42
G ⇧	984.00	56.00	2.20	0.23	4.42	8.50	200.00	7.90	9.89	13.68	3.00	0.25	7.10
G ⇩	2616.00	61.00	2.30	0.19	4.16	6.90	245.00	7.90	9.69	13.72	2.00	0.25	7.34

SI Table 2b Physicochemical parameters of receiving waters in July.
WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg. Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites. TOC: total organic carbon; BOD: biochemical oxygen demand; EC: electrical conductivity; TDO: total dissolved oxygen; Temp: water temperature; Width: stream width; Depth: stream depth.

				May			July		
Compound	**Type**	**CAS No.**	**PS**	CC	Water	SPE	CC	Water	SPE
2,6-dichlorobenzamide (BAM)	M (H)	2008-58-4			-			+	
acetamiprid	I	135410-20-7		+	+		+	-	
alachlor	H	15972-60-8	PS		-			-	
alpha-cypermethrin	I	67375-30-8	PS			-			-
amidosulfuron	H	120923-37-7			-			-	
atrazine	H	1912-24-9	PS		+			+	
atrazine-desethyl (DEA)	M (H)	6190-65-4			+			+	
atrazine-desisopropyl (DIA)	M (H)	1007-28-9			+			+	
azoxystrobin	F	131860-33-8		+	+		+	+	
beta-cyfluthrin	I	68359-37-5				-			-
boscalid	F	188425-85-6		+	-		+	+	
carbendazim	F	10605-21-7		+	+		+	+	
carbofuran	I	1563-66-2			-			-	
carfentrazone-ethyl	H	128639-02-1			-			-	
chlorfenvinphos	I	470-90-6	PS		-			-	
chloridazon	H	1698-60-8			-			+	
clomazone	H	81777-89-1			-			-	
clothianidin	I	210880-92-5			-			+	
cyazofamid	F	120116-88-3			-			-	
cybutryne	H	28159-98-0	PS		-			-	
cyflufenamid	F	180409-60-3			-			-	
cyfluthrin	I	68359-37-5				-			-
cycloxydim	H	101205-02-1			-			-	
cyprodinil	F	121552-61-2		-	+		-	-	
deltamethrin	I					-			-
dichlorvos	I		PS		-			-	
difenoconazole	F			-	-		-	-	
diflufenican	H	83164-33-4		+	-		-	+	
dimethoate	I	60-51-5		+	+		+	+	
diuron	H	330-54-1	PS		+			+	
epoxiconazole	F	135319-73-2		-	-		+	+	
esfenvalerate	I	66230-04-4				+			+
ethofumesate	H	26225-79-6			+			+	
fenpropidin	F			-	-		-	-	
fenpropimorph	F	67564-91-4		-	-		+	-	
fludioxonil	F				-			-	
flupyrsulfuron-methyl	H				-			-	
flurprimidol	P				-			-	
flurtamone	H	96525-23-4			+			-	
flusilazole	F	85509-19-9			-			+	
flutriafol	F				-			-	
foramsulfuron	H				-			-	
fuberidazole	F				-			-	
hexazinone	H				-			-	
hexythiazox	A				-			-	
imazalil	F				-			-	

SI Table 3 Target compounds in the present study.
(Continued overleaf.)

imidacloprid	I	138261-41-3		+	+		+	+	
isoproturon	H	34123-59-6	PS	+	+		+	+	
lambda-cyhalothrin	I					-			-
mandipropamid	F				-			-	
methabenzthiazuron	H				-			-	
metalaxyl	F	57837-19-1			-			+	
metamitron	H	41394-05-2			+			+	
metazachlor	H	67129-08-2			+			-	
methiocarb	I			-	-		-	-	
metolachlor	H	51218-45-2			-			+	
metrafenone	F	220899-03-6			-			+	
metribuzin	H	21087-64-9			+			-	
pendimethalin	H				-			-	
penconazole	F				-			-	
phenmedipham	H	13684-63-4			-			+	
picoxystrobin	F	117428-22-5		-	-		+	-	
pirimicarb	I	23103-98-2		+	-		+	+	
prochloraz	F	67747-09-5		-	-		+	-	
propamocarb	F	24579-73-5			-			+	
propiconazole	F	60207-90-1		+	+		+	+	
propyzamide	H	23950-58-5			+			-	
prothioconazole-desthio (M04)	M (F)	120983-64-4		+	+		+	+	
pyraclostrobin	F			-	-		-	-	
quinmerac	H	90717-03-6			+			+	
rimsulfuron	H				-			-	
silthiofam	F				-			-	
simazine	H	122-34-9	PS		+			+	
spiroxamine	F	118134-30-8			-			+	
sulfosulfuron	H				-			-	
tau-fluvalinate	I					-			-
terbuthylazine	H	5915-41-3			+			+	
terbuthylazine-desethyl (MT1)	M (H)	30125-63-4			+			+	
terbutryn	H	886-50-0	PS		+			+	
thiacloprid	I	111988-49-9		+	+		+	+	
thiamethoxam	I				-			-	
thiophanate-methyl	F				-			-	
tolylfluanid	F				-			-	
tribenuron-methyl	H	101200-48-0			+			-	
trifloxystrobin	F			-	-		-	-	
triflusulfuron-methyl	H	126535-15-7			-			+	
trinexapac-ethyl	P				-			-	
triticonazole	F				-			-	

SI Table 3 Target compounds in the present study (continued).
Successful quantifications are indicated by a positive sign (“+”) for each sampling campaign and each sampling technique; a negative sign (“-“) indicates substances that were measured but not detected.
CAS: Chemical Abstracts Service; PS: priority substance; CC: Chemcatcher;
SPE: solid phase extraction; A: acaricide; F: fungicide; H: herbicide; I: insecticide;
M: metabolite; P: plant growth regulator; Italics: neonicotinoid.

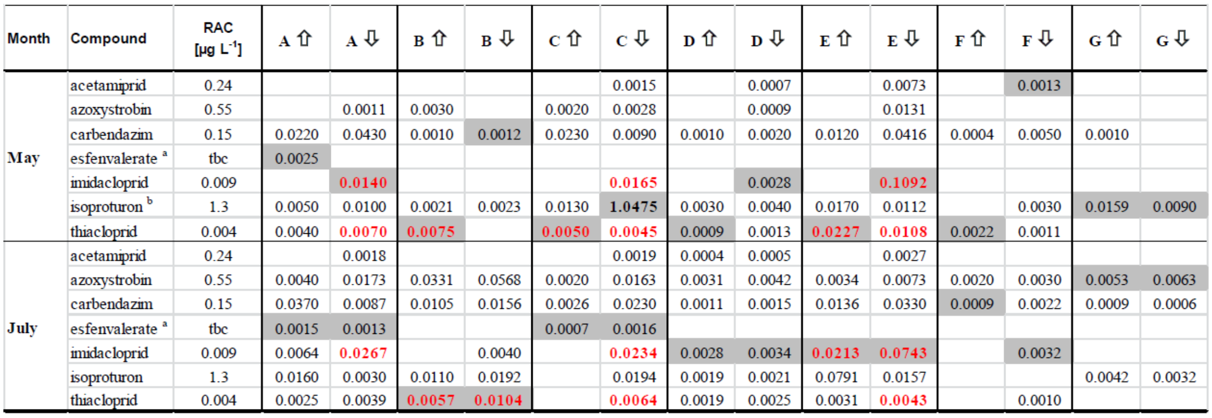
Compound	**Type**	**Acute (48 h) LC_{50} [µg L^{-1}]**	**Standard organism**	**Source**
2,6-dichlorobenzamide (BAM)	M	180000	Daphnia magna	University of Hertfordshire, 2015
acetamiprid	I	22	Chironomus spp.	USEPA, 2015
atrazine	H	720	Chironomus tentans	USEPA, 2015
atrazine-desethyl (DEA)	M	720	Chironomus tentans	Shipitalo & Owens, 2003
atrazine-desisopropyl (DIA)	M	720	Chironomus tentans	Shipitalo & Owens, 2003
azoxystrobin	F	230	Daphnia magna	University of Hertfordshire, 2015
boscalid	F	5330	Daphnia magna	University of Hertfordshire, 2015
carbamazepine	PH	111000	Daphnia spp.	Han et al., 2006
carbendazim	F	150	Daphnia magna	University of Hertfordshire, 2015
chloridazon	H	132000	Daphnia magna	University of Hertfordshire, 2015
clothianidin	I	22	Chironomus spp.	USEPA, 2015
cyprodinil	F	32	Daphnia magna	UFZ, 2014
diclofenac	PH	22430	Daphnia spp.	Ferrari et al., 2004
diflufenican	H	240	Daphnia magna	University of Hertfordshire, 2015
dimethoate	I	248.9	Chironomus tentans	USEPA, 2015
diuron	H	8400	Daphnia magna	UFZ, 2014
epoxiconazole	F	8690	Daphnia magna	University of Hertfordshire, 2015
esfenvalerate	I	0.21	Daphnia magna	UFZ, 2014
ethofumesate	H	14000	Daphnia magna	University of Hertfordshire, 2015
fenpropimorph	F	2240	Daphnia magna	University of Hertfordshire, 2015
flurtamone	H	13000	Daphnia magna	University of Hertfordshire, 2015
flusilazole	F	3400	Daphnia magna	University of Hertfordshire, 2015
ibuprofen	PH	101200	Daphnia spp.	Cleuvers, 2004
imidacloprid	I	22	Chironomus spp.	USEPA, 2015
iopamidol	PH	10000000	Daphnia spp.	Steger-Hartmann et al., 1998
iopromide	PH	10000000	Daphnia spp.	Steger-Hartmann et al., 1998
isoproturon	H	710	Daphnia magna	LANUV, 2014
metalaxyl	F	40400	Daphnia magna	UFZ, 2014
metamitron	H	5700	Daphnia magna	University of Hertfordshire, 2015
metazachlor	H	33000	Daphnia magna	University of Hertfordshire, 2015
metolachlor	H	16625	Daphnia magna	UFZ, 2014
metrafenone	F	92000	Daphnia magna	USEPA, 2015
metribuzin	H	49000	Daphnia magna	University of Hertfordshire, 2015
phenmedipham	H	410	Daphnia magna	University of Hertfordshire, 2015
picoxystrobin	F	24	Daphnia magna	University of Hertfordshire, 2015
pirimicarb	I	16.25	Daphnia magna	UFZ, 2014
prochloraz	F	4300	Daphnia magna	University of Hertfordshire, 2015
propamocarb	F	106000	Daphnia magna	USEPA, 2015
propiconazole	F	4900	Daphnia magna	UFZ, 2014
propyzamide	H	5600	Daphnia magna	UFZ, 2014
prothioconazole-desthio (M04)	M	10000	Daphnia magna	University of Hertfordshire, 2015
quinmerac	H	100000	Daphnia magna	University of Hertfordshire, 2015
simazine	H	1100	Daphnia magna	University of Hertfordshire, 2015
spiroxamine	F	6100	Daphnia magna	University of Hertfordshire, 2015
terbuthylazine	H	13100	Daphnia magna	UFZ, 2014
terbuthylazine-desethyl (MT1)	M	42000	Daphnia magna	University of Hertfordshire, 2015
terbutryn	H	7100	Daphnia magna	UFZ, 2014
thiacloprid	I	22	Chironomus spp.	USEPA, 2015
tribenuron-methyl	H	894000	Daphnia magna	University of Hertfordshire, 2015
triflusulfuron-methyl	H	960000	Daphnia magna	University of Hertfordshire, 2015

SI Table 4 Acute (48h) LC_{50} values for the most sensitive standard test organisms. (Details overleaf.)

SI Table 4 Acute (48h) LC_{50} values for the most sensitive standard test organisms, given for the 45 pesticides and five pharmaceutical substances (bold) detected.

F: fungicide; H: herbicide; I: insecticide; M: metabolite; PH: pharmaceutical;

Italics: neonicotinoid.



Month	Compound	RAC [µg L^{-1}]	A ⇧	A ⇩	B ⇧	B ⇩	C ⇧	C ⇩	D ⇧	D ⇩	E ⇧	E ⇩	F ⇧	F ⇩	G ⇧	G ⇩
May	acetamiprid	0.24						0.0015		0.0007		0.0073		0.0013		
	azoxystrobin	0.55		0.0011	0.0030		0.0020	0.0028		0.0009		0.0131				
	carbendazim	0.15	0.0220	0.0430	0.0010	0.0012	0.0230	0.0090	0.0010	0.0020	0.0120	0.0416	0.0004	0.0050	0.0010	
	esfenvalerate [a]	tbc	0.0025													
	imidacloprid	0.009		**0.0140**				**0.0165**		0.0028		**0.1092**				
	isoproturon [b]	1.3	0.0050	0.0100	0.0021	0.0023	0.0130	**1.0475**	0.0030	0.0040	0.0170	0.0112		0.0030	0.0159	0.0090
	thiacloprid	0.004	0.0040	**0.0070**	**0.0075**		**0.0050**	**0.0045**	0.0009	0.0013	**0.0227**	**0.0108**	0.0022	0.0011		
July	acetamiprid	0.24		0.0018				0.0019	0.0004	0.0005		0.0027				
	azoxystrobin	0.55	0.0040	0.0173	0.0331	0.0568	0.0020	0.0163	0.0031	0.0042	0.0034	0.0073	0.0020	0.0030	0.0053	0.0063
	carbendazim	0.15	0.0370	0.0087	0.0105	0.0156	0.0026	0.0230	0.0011	0.0015	0.0136	0.0330	0.0009	0.0022	0.0009	0.0006
	esfenvalerate [a]	tbc	0.0015	0.0013			0.0007	0.0016								
	imidacloprid	0.009	0.0064	**0.0267**		0.0040		**0.0234**	0.0028	0.0034	**0.0213**	**0.0743**		0.0032		
	isoproturon	1.3	0.0160	0.0030	0.0110	0.0192		0.0194	0.0019	0.0021	0.0791	0.0157			0.0042	0.0032
	thiacloprid	0.004	0.0025	0.0039	**0.0057**	**0.0104**		**0.0064**	0.0019	0.0025	0.0031	**0.0043**		0.0010		

SI Table 5 Regulatory Acceptable Concentrations (RACs) according to UBA (2016), with exceedances observed.

WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.

Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites.

Bold and red: RACs exceeded;

Highlighted in grey: Concentration of the compound providing TU_{max};

[a] RAC currently under re-evaluation (UBA, 2016);

[b] The maximum allowable concentration or short-term Environmental Quality Standard (MAC-EQS) according to EC (2013) was exceeded at the Blankenburg/Harz WWTP.

Order	**Family**	**A ⇧**	**A ⇩**	**B ⇧**	**B ⇩**	**C ⇧**	**C ⇩**	**D ⇧**	**D ⇩**	**E ⇧**	**E ⇩**	**F ⇧**	**F ⇩**	**G ⇧**	**G ⇩**
Amphipoda	Gammaridae		3		7	1149	94	146	140	169	169	661	986	36	104
Bivalvia	Sphaeriidae									52	42				
Coleoptera	Dytiscidae									3					3
	Elmidae					514	173	88	26	3	16	46		42	3
	Gyrinidae				3			13	3						
Diptera	*Athericidae*				7									13	3
	Chironomidae	153	531	62	81	10	36	68	42	166	169	49	1022	26	65
	Empididae											3			
	Limoniidae				7	10		3				10	3	13	3
	Muscidae								3						
	Ptychopteridae											3			
	Simuliidae								3				3		7
	Stratiomyidae												3		
	Tipulidae	3	10					3	3						
Ephemeroptera	*Baetidae*									3					
	Baetis rhodani			202	205	2015	3	3	10			169	179	78	52
	Ephemerellidae			23	55							283	254	16	20
	Ephemeridae			16	65					16				7	13
	Heptageniidae			52	33			26	3			10	3	75	49
	Leptophlebiidae			46	10					13				20	16
	Siphlonuridae									3					
Gastropoda	Ancylidae						3	3							
	Lymnaeidae						3						7		
Heteroptera	Corixidae		3				124								
Hirudinea	Erpobdellidae	36	59	13				3	36	501	264		36		
	Glossiphoniidae							7			3				
Isopoda	Asellidae	36	7						3	1654	339				
Oligochaeta	Oligochaeta	10	7	20	3	7	3		29	251	1729	3	7	3	
Plecoptera	*Chloroperlidae*													16	26
	Nemouridae	3						3							
	Perlodidae			23	13			3						7	3
	Taeniopterygidae							7						3	
Trichoptera	*Glossosomatidae*											439	163		
	Goeridae				3	153		91	7				13	81	
	Hydropsychidae	10	20	3	13		1650	173	16	10	23	98	23	23	
	Lepidostomatidae								3	7		133		3	
	Limnephilidae			3	7	23		114	46	33		94	49	117	49
	Odontoceridae													23	16
	Polycentropodidae			3				3							3
	Psychomyiidae	3				3									
	Rhyacophilidae		3	3	3	16		7	3				3	3	
	Sericostomatidae					3		143	98			68	36	23	16
Turbellaria	Planaria			3		120	264				3	10	52	3	7

SI Table 6a Abundances of stream macroinvertebrate families sampled in May, given as individuals m^{-2} per sampling site (A-G).

WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.

Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites.

Italics: taxon regarded as vulnerable to pesticides (SPEAR).

Order	**Family**	**A ⇧**	**A ⇩**	**B ⇧**	**B ⇩**	**C ⇧**	**C ⇩**	**D ⇧**	**D ⇩**	**E ⇧**	**E ⇩**	**F ⇧**	**F ⇩**	**G ⇧**	**G ⇩**
Acari	Hydrachnidia			10	88						3			3	
Amphipoda	Gammaridae	163	72	488	98	977	1309	661	166	228	719	827	817	983	488
Coleoptera	Dytiscidae	3													3
	Elmidae			52	140	290	133	94	81				3	75	72
	Haliplidae			3											
	Hydrophilidae									3					
	Scirtidae					3									
Diptera	Chironomidae	140	52	173	169	10	3	68	329	335	32731	7	16	146	36
	Limoniidae		7	16	3	3	3	3	3		7	3	46	10	7
	Simuliidae			3	7		3					7		42	36
Ephemeroptera	*Baetidae*						3								
	Baetis rhodani	1689	911	303	52	228	576	36			182	345	570	306	150
	Ephemerellidae		3	208	169	1813	1152	511	85	10	186	518	283	781	898
	Ephemeridae			7	29			3						3	3
	Heptageniidae			13		3		10	7					3	
	Leptophlebiidae			55	3									3	
Gastropoda	Ancylidae												3		
	Hydrobiidae					143									
	Lymnaeidae					72	212						7		
Heteroptera	Corixidae	10	3				20								
Hirudinea	Erpobdellidae	29	75				7			91	72		7		
	Glossiphoniidae					3				3	3				
Isopoda	Asellidae	20	49					13		42	114				
Oligochaeta	Oligochaeta	3		208	329	39	326	3			654	3			3
Plecoptera	*Leuctridae*			13	7			91	46					36	23
	Nemouridae							3	3						
Trichoptera	*Glossosomatidae*											202			
	Goeridae			3	7				3				3	10	7
	Hydropsychidae			3	3	3	413	36	49		3				7
	Hydroptilidae		7												
	Lepidostomatidae							3				3			
	Leptoceridae			23	36		3	3	3						
	Limnephilidae		7	3		3		46	7		3	42	7	36	13
	Odontoceridae											10			
	Polycentropodidae							3						10	3
	Psychomyiidae		3												
	Rhyacophilidae			3		23		7				3	3		3
	Sericostomatidae				10			3	3			7	10	13	3
Turbellaria	Planaria	7		33	39	163	166			3	3	7	42	16	52

SI Table 6b Abundances of stream macroinvertebrate families sampled in July, given as individuals m^{-2} per sampling site (A-G).

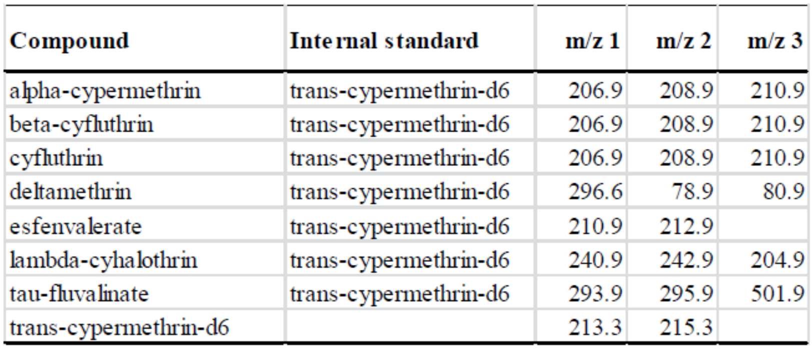
WWTPs A: Ballenstedt; B: Biesenrode; C: Blankenburg/Harz; D: Hoym; E: Osterwieck; F: Stapelburg; G: Straßberg.

Arrows pointing upwards indicate sampling sites upstream of WWTP effluent discharge points; arrows pointing downwards indicate downstream sampling sites.

Italics: taxon regarded as vulnerable to pesticides (SPEAR).

	Compound	Internal standard	Precursor ion	Product ion	Fragmentor [V]	Collision energy [V]	Cell acceleration [V]	Precursor ion	Product ion	Fragmentation [V]	Collision energy [V]	Cell acceleration [V]
*	2,6-dichlorobenzamide	isoproturon-d6	190.0	173.0	120	19	3	190.0	145.0	120	32	3
	acetamiprid	isoproturon-d6	223.1	126.1	100	20	3	223.1	73.1	100	60	3
*	alachlor	isoproturon-d6	270.1	238.1	60	4	3	270.1	162.1	60	17	3
*	amidosulfuron	isoproturon-d6	370.0	261.1	100	8	3	370.0	217.9	100	25	2
*	atrazine-desetyl	isoproturon-d6	188.1	146.1	100	17	2	188.1	104.1	100	27	2
*	atrazine-desisopropyl	isoproturon-d6	174.0	104.0	100	22	5	174.0	132.0	100	14	5
*	atrazine	atrazine-d5	216.1	174.1	10	16	2	216.1	104.0	100	32	2
	atrazine-d5		221.1	179.1	100	16	3					
	azoxystrobin	isoproturon-d6	404.1	372.1	80	7	3	404.1	344.1	80	22	3
	boscalid	terbutylazin-d5	343.0	307.0	140	20	3	343.0	140.0	140	10	3
	carbendazim	isoproturon-d6	192.1	160.1	80	17	5	192.1	132.1	80	35	5
*	carbofuran	isoproturon-d6	222.1	165.1	60	4	4	222.1	123.1	60	22	5
*	carfentrazone-ethyl	isoproturon-d6	429.0	412.1	80	4	4	429.0	345.9	80	27	5
*	chlorfenvinphos	isoproturon-d6	359.0	170.0	100	48	5	359.0	155.0	100	7	3
*	chloridazon	isoproturon-d6	222.0	104.1	120	22	3	222.0	92.1	120	27	3
*	clomazone	isoproturon-d6	240.1	125.1	80	28	2	240.1	89.1	80	55	3
*	*clothianidin*	isoproturon-d6	250.0	169.1	60	8	3	250.0	132.0	60	10	3
*	cyazofamid	isoproturon-d6	325.1	108.0	60	8	3	325.1	44.1	60	33	3
*	cybutryne	isoproturon-d6	254.1	198.1	100	20	3	254.1	156.1	100	30	3
*	cycloxidim	ethion	326.2	280.1	100	6	4	326.2	180.1	100	17	4
*	cyflufenamid	terbutylazine-d5	413.1	241.1	100	18	5	413.1	359.1	100	5	5
	cyprodinil	terbutylazin-d5	226.1	108.1	140	26	2	226.1	108.1	140	40	3
*	dichlorvos	isoproturon-d6	221.0	109.1	110	15	3	221.0	95.0	110	45	3
	difenoconazol	terbutylazin-d5	408.1	253.1	120	25	2	406.1	251.0	120	25	2
	diflufenican	terbutylazin-d5	395.1	266.1	140	22	5	395.1	218.0	140	55	4
	dimetoat	isoproturon-d6	230.0	171.0	60	10	3	230.0	125.0	60	22	2
*	diuron	isoproturon-d6	233.0	160.0	100	30	3	233.0	72.1	100	20	3
	epoxiconazol	isoproturon-d6	330.1	121.0	100	17	5	330.1	101.0	100	50	5
	ethion		385.0	143.0	70	25	4					
*	ethofumesate	isoproturon-d6	287.1	259.0	140	3	2	287.1	121.1	140	12	5
	fenpropidin	isoproturon-d6	274.2	147.1	160	35	4	274.2	119.1	160	38	4
	fenpropimorph	isoproturon-d6	304.3	147.1	140	28	3	304.3	117.1	140	60	3
*	fludioxonil	isoproturon-d6	266.0	229.1	60	6	5	266.0	158.1	60	31	5
*	flupyrsulfuron-methyl	isoproturon-d6	466.1	182.1	100	22	5	466.1	139.1	100	50	5
*	flurprimidol	isoproturon-d6	313.1	270.1	100	24	2	313.1	269.1	100	33	2
*	flurtamone	isoproturon-d6	334.1	247.1	120	27	5	334.1	178.1	120	50	5
*	flusilazole	isoproturon-d6	316.1	247.1	140	14	3	316.1	165.1	140	28	5
*	flutriafol	isoproturon-d6	302.1	123.1	100	32	2	302.1	70.1	100	17	2
*	foramsulfuron	isoproturon-d6	453.1	272.1	100	4	5	453.1	182.1	100	17	5
*	fuberidazole	isoproturon-d6	185.1	157.1	140	22	4	185.1	156.1	140	32	5
*	hexazinone	isoproturon-d6	253.2	171.1	80	12	5	253.2	71.1	80	37	5
*	hexythiazox	ethion	353.1	228.1	100	6	5	353.1	168.1	100	22	4
*	imazalil	isoproturon-d6	297.1	255.0	110	15	5	297.1	159.0	110	22	5
	imidacloprid	isoproturon-d6	256.1	209.1	90	22	5	256.1	175.1	90	22	2
	isoproturon	isoproturon-d6	207.1	72.1	100	26	3	207.1	46.1	100	18	3
	isoproturon-d6		213.1	78.1	100	26	3					
*	mandipropamid	isoproturon-d6	412.1	356.1	100	4	3	412.1	328.1	100	6	3
*	metalaxyl	isoproturon-d6	280.2	220.1	80	7	3	280.2	192.2	80	13	3
*	metamitron	isoproturon-d6	203.0	175.1	100	14	2	203.0	104.1	100	23	3
*	metazachlor	isoproturon-d6	278.0	210.1	70	3	4	278.0	134.1	70	12	7
*	methabenzthiazuron	isoproturon-d6	222.1	165.1	80	16	2	222.1	150.1	80	42	3
	methiocarb	isoproturon-d6	226.1	169.1	60	7	3	226.1	121.1	60	15	3
*	metrafenone	terbutylazine-d5	411.1	209.1	90	8	5	409.1	227.0	90	13	5
*	metribuzin	isoproturon-d6	215.0	187.1	100	16	3	215.0	84.1	100	20	3
*	penconazole	isoproturon-d6	284.1	159.0	90	32	3	284.1	70.1	90	22	2
*	pendimethalin	Ethion	282.1	212.1	70	6	2	282.1	194.0	70	16	3
*	phenmedipham	isoproturon-d6	318.1	168.1	70	7	4	318.1	136.0	70	27	4
	picoxystrobin	isoproturon-d6	368.1	205.1	60	3	2	368.1	145.1	60	25	3
	pirimicarb	isoproturon-d6	239.1	182.1	90	12	3	239.1	72.1	90	27	3
	pirimicarb-d6	isoproturon-d6	245.1	185.2	100	10	3	245.2	78.2	100	20	3
	prochloraz	terbutylazin-d5	378.0	310.0	60	5	3	376.0	308.0	60	5	2
*	propamocarb	propamocarb-d7	198.1	102.1	100	17	2	198.1	74.0	100	27	3
	propamocarb-d7		196.1	103.1	100	17	3					
	propiconazole	isoproturon-d6	344.1	161.0	100	32	5	342.1	159.0	100	32	5
*	propyzamide	isoproturon-d6	256.0	189.9	60	8	5	256.0	173.0	60	18	5
	prothioconazole-destio	isoproturon-d6	312.1	125.1	80	38	3	312.1	70.1	80	28	2
	pyraclostrobin	terbutylazin-d5	388.1	194.1	70	6	3	388.1	163.1	70	25	3
*	quinmerac	quinmerac-d4	222.0	204.0	70	12	5	222.0	141.1	70	37	4
	quinmerac-d4		226.0	208.0	70	10	5					
*	rimsulfuron	isoproturon-d6	432.1	325.1	100	11	5	432.1	182.1	100	30	5
*	silthiofam	isoproturon-d6	268.1	139.0	120	17	3	268.1	73.1	120	32	3
*	simazine	isoproturon-d6	202.1	132.1	100	16	2	202.1	124.1	100	17	2
*	S-metolachlor	isoproturon-d6	284.0	252.1	100	12	2	284.0	176.1	100	25	2
*	spiroxamine	isoproturon-d6	298.3	144.2	110	21	3	298.3	100.2	110	37	3
*	sulfosulfuron	isoproturon-d6	471.1	261.1	100	14	3	471.1	211.1	100	13	3
*	terbutryn	isoproturon-d6	242.1	116.1	100	30	3	242.1	91.1	100	27	2
	terbuthylazine-d5		253.1	179.1	100	24	3					
*	terbuthylazine	terbutylazine-d5	230.0	174.1	100	14	2	230.0	146.1	100	22	3
*	terbuthylazine-desethyl	isoproturon-d6	202.1	146.1	80	15	3	202.1	104.0	80	33	3
	thiacloprid	isoproturon-d6	253.0	126.1	100	18	5	253.0	73.0	100	60	5
*	*thiamethoxam*	isoproturon-d6	292.0	211.1	60	7	3	292.0	181.0	60	22	3
*	thiophanate-methyl	isoproturon-d6	343.1	311.0	80	4	4	343.1	151.1	80	15	5
*	tolylfluanid	isoproturon-d6	347.0	238.0	60	5	5	347.0	137.0	60	25	6
*	tribenuron-methyl	isoproturon-d6	396.1	181.1	90	16	5	396.1	155.1	90	12	4
	trifloxystrobin	isoproturon-d6	409.1	186.0	100	10	4	409.1	145.1	100	50	5
*	triflusulfuron-methyl	isoproturon-d6	493.1	264.1	100	27	3	493.1	96.1	100	57	3
*	trinexapac-ethyl	isoproturon-d6	253.1	207.1	80	6	3	253.1	185.1	80	5	4
*	triticonazole	isoproturon-d6	318.1	125.1	120	40	3	318.1	70.1	120	27	3

SI Table 7 Instrumental conditions for LC-MS/MS analysis of pesticides in Chemcatcher extracts and water samples. Compounds with an asterisk were included in the direct water analysis only. Italics: neonicotinoid.



Compound	Internal standard	m/z 1	m/z 2	m/z 3
alpha-cypermethrin	trans-cypermethrin-d6	206.9	208.9	210.9
beta-cyfluthrin	trans-cypermethrin-d6	206.9	208.9	210.9
cyfluthrin	trans-cypermethrin-d6	206.9	208.9	210.9
deltamethrin	trans-cypermethrin-d6	296.6	78.9	80.9
esfenvalerate	trans-cypermethrin-d6	210.9	212.9	
lambda-cyhalothrin	trans-cypermethrin-d6	240.9	242.9	204.9
tau-fluvalinate	trans-cypermethrin-d6	293.9	295.9	501.9
trans-cypermethrin-d6		213.3	215.3	

SI Table 8 Fragments used in GC-MS analysis of pesticides in SPE extracts.

SI Text 1

1 Pesticide analyses

1.1 Chemicals and reagents

During the preparation and solvent extraction of the SDB-RPS Empore disks, GC-grade methanol and acetone from Merck (Darmstadt, Germany) were used. The passive sampler housings were cleaned using analytical grade methanol and acetone, and sodium sulphate, $NaSO_4$, was used for sample preparation (Merck, Darmstadt, Germany). The final extracts were transferred into 1 mL crimp-top vials from Agilent (Waldbronn, Germany). Ammonium formate (Fluka, Seelze, Germany), water (J.T. Baker, Griesheim, Germany), and methanol (Merck, Darmstadt, Germany), all of LC-MS-grade quality, were used for the LC-MS measurements. Solid-phase extraction (SPE) was conducted with endcapped octadecyl-modified silica in the form of 6 ml/500 mg Chromabond® C18ec SPE cartridges from Macherey-Nagel (Düren, Germany).

For the sample analyses in Uppsala, Sweden, gradient-grade methanol, 2-propanol for HPLC, acetone for pesticide residue analysis and p.a.-grade hexane were all obtained from Sigma-Aldrich (Steinheim, Germany). Pesticide-grade cyclohexane was purchased from Fisher Scientific (Loughborough, UK). Formic acid and ammonium acetate ≥ 98% of p.a. quality were also purchased from Sigma-Aldrich (Steinheim, Germany). Sodium sulphate, Na_2SO_4, and anhydrous and 25% ammonium solutions were obtained from Merck (Darmstadt, Germany) and > 90% HPLC-grade acetic acid from Scharlau (Barcelona, Spain). Deionised water was produced by a Milli-Q Advantage A10 Ultrapure Water Purification

System (Millipore, Billerica, MA, USA). The HPLC syringe filter 17 mm with regenerated cellulose (RC) and a pore size of 0.2 µm was purchased from Scantec Nordic (Gothenburg, Sweden).

The standards for the LC analysis were obtained either as certified standard mixes (purity > 90%) in acetonitrile from Restek (Bellefonte, PA, USA) or as individual compounds (purity > 95%) from Dr. Ehrenstorfer (Augsburg, Germany) and Neochema (Bodenheim, Germany). Stock solutions were dissolved in acetonitrile and further diluted in Milli-Q water (pH 5) for the calibration curve. Atrazine-d_5, ethion, isoproturon-d_6, pirimicarb-d_6, propamocarb-d_7, quinmerac-d_4 and terbuthylazine-d_5 were used as internal standards and were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The working solution of internal standards was prepared in methanol. The concentration ranges of the calibration curves were 1 to 256 ng L^{-1}, 5 to 1280 ng L^{-1}, or 25 to 6400 ng L^{-1}, depending on the substance. For the pesticides analysed using GC, the certified standards (purity > 90%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions were prepared in acetone, and working solutions for the calibration curve were prepared in a mixture of cyclohexane:acetone (9:1, v/v). The concentration ranges of the calibration curves varied with each substance but were all within 0.1 to 140 ng L^{-1}.

1.2 Instrumental analysis setup (Uppsala, Sweden)

The analysis of the diluted Chemcatcher extracts and the water samples was performed using online solid-phase extraction coupled with HPLC-MS/MS according to the method described by Jansson and Kreuger (2010), with slight modifications. An Agilent 1260 HPLC system with a quaternary pump, a binary pump, a micro degasser, a thermostatted wellplate sampler and a thermostatted column compartment coupled with a Jetstream interface to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used. The injection volume was 500 µL. The preconcentration of the samples was performed with two SPE columns in succession, Strata C_{18}-E and Strata X, both 20 × 2 mm and 20-25 µm (Phenomenex, Torrance, CA, USA). Milli-Q water with 5% methanol was used for loading the SPE apparatus. Chromatographic separation was achieved on a Zorbax Eclipse Plus C_{18}, 100 × 3 mm and 3.5 µm (Agilent Technologies, Santa Clara, CA, USA), with mobile phase A (2/6/92 methanol/2-propanol/10 mM ammonium formate pH 4) and mobile phase B (100% methanol) starting with 0% B from 0 to 3.1 min followed by a linear gradient to 100% B at 19 min and 100% B for 19 to 27 min. The analysis was performed with positive electrospray ionisation (ESI+) and operated in multiple reaction monitoring (MRM) mode with at least two transitions for each analyte (SI Table 7). The ion source parameters were as follows: nebulizer gas 45 psi, capillary voltage 3.5 kV, gas temperature 300 °C, drying gas flow 5 L min^{-1}, and sheath gas flow 11 L min^{-1}. The system was controlled by MassHunter B.04.01.

For the analysis of the SPE extracts, a 7890A GC equipped with a multimode inlet operated in solvent vent mode coupled to a 5975C mass detector (Agilent Technologies, Santa Clara, CA, USA) was used. A DB-5MS UI capillary column, 30 m × 0.25 mm and 0.25 µm film thickness (Agilent Technologies, Santa Clara, CA, USA) was connected, and the temperature programme was 70 °C for 1 min and then 11.5 °C per min to 280 °C, which was held for 8 min. Helium was used as carrier gas at a flow rate of 1.2 mL min^{-1}, and the transfer line was set to 280 °C. Electron capture negative ionisation (ECNI) using methane as buffer gas was used for ionisation. The analysis was run in selected ion monitoring (SIM) mode, scanning two or three fragments for each analyte (SI Table 8). The system was controlled by Chemstation software (version E.02.01). Analyses of method blanks and spiked matrix samples were included in each series of samples for quality control.

2 Pharmaceutical analyses

The analyses of pharmaceuticals were performed by the accredited commercial laboratory SGS Institute FRESENIUS GmbH, Taunusstein, Germany. The water samples were analysed using LC-MS/MS according to the German (draft) standard DIN 38407-47. This standard has been developed in the course of the project "German standard methods for the examination of water, waste water and sludge – Jointly determinable substances (group F) – Part 47: Determination of selected active pharmaceutical ingredients and other organic substances in water and waste water – Method using high performance liquid chromatography and mass spectrometric detection (HPLC-MS/MS or -HRMS) after direct injection (F 47)".

2.1 Instrumental analysis setup (Taunusstein, Germany)

Aliquots of 10 µL to 100 µL of the water samples were injected directly onto the LC column (Waters ACQUITY UPLC BEH Phenyl 1.7 µm 2.1 × 100mm) by means of an autosampler CTC Pal-DLW combined with an Agilent 1290 infinity LC pump and thermostat. For all target pharmaceuticals except for ibuprofen, the mobile phase was formed using a mixture of

5 mM ammonium formiate aqueous solution and 5 mM ammonium formiate methanolic solution. In the case of ibuprofen, 2 mM ammonium acetate aqueous solution (with 0.1% acetic acid) and acetonitrile were used to form the eluent. All considered compounds with the exception of ibuprofen were introduced into the mass spectrometer after positive electrospray ionisation (ESI+). For ibuprofen, the negative ESI-mode was applied. An AB Sciex 5500 QTrap mass spectrometer was employed for the detection/quantification, using the molecule ions and fragment ions according to DIN 38407-47.

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APPENDIX C

Supplementary Material to CHAPTER 4

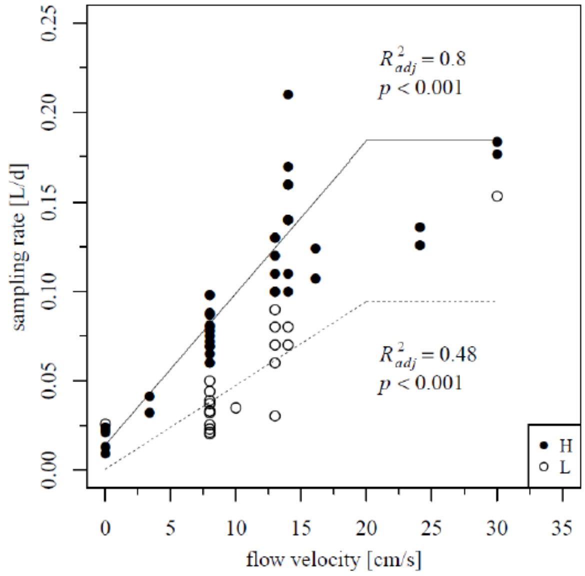


Fig. S1: Linear dependence of the sampling rate (R_S) on the average flow velocity until 20cm/s (Vermeirssen et al., 2012, 2009) for substances with known high (H) and a low (L) sampling rates taken from the literature (for exact values see Table S3). Taken from Münze et al., 2015.

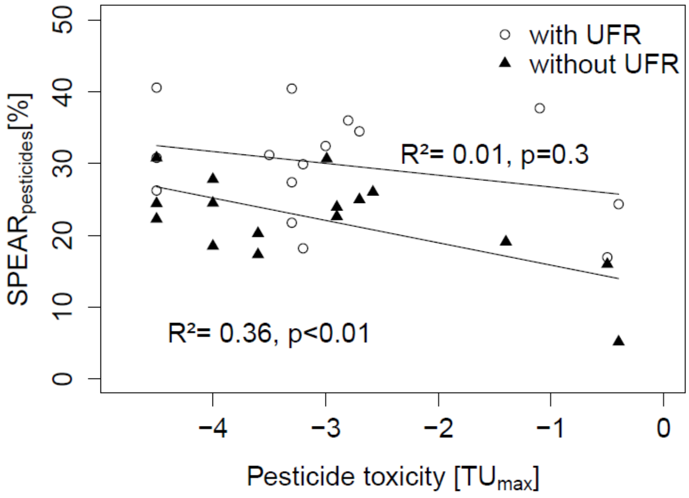


Fig. S2: Relationship between SPEAR$_{pesticides}$ before contamination and TU$_{max}$, measured during the pesticide contamination period for the sampling sites with and without upstream forested reaches (UFR). Sites from 2010 are missing, as macroinvertebrates were not sampled before contamination during that year (see section 2.5)

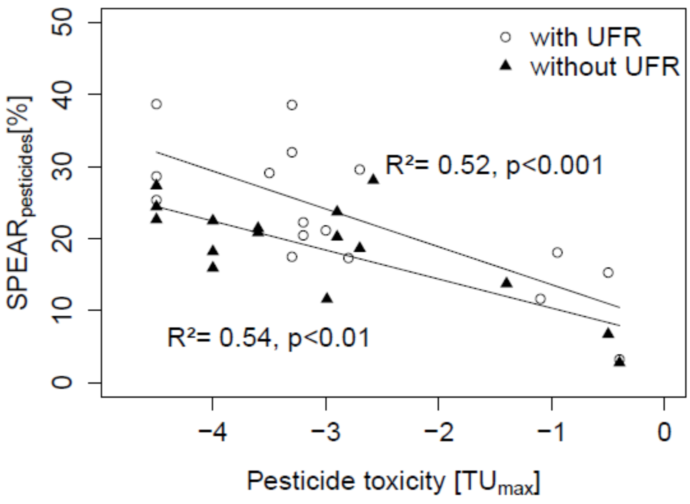
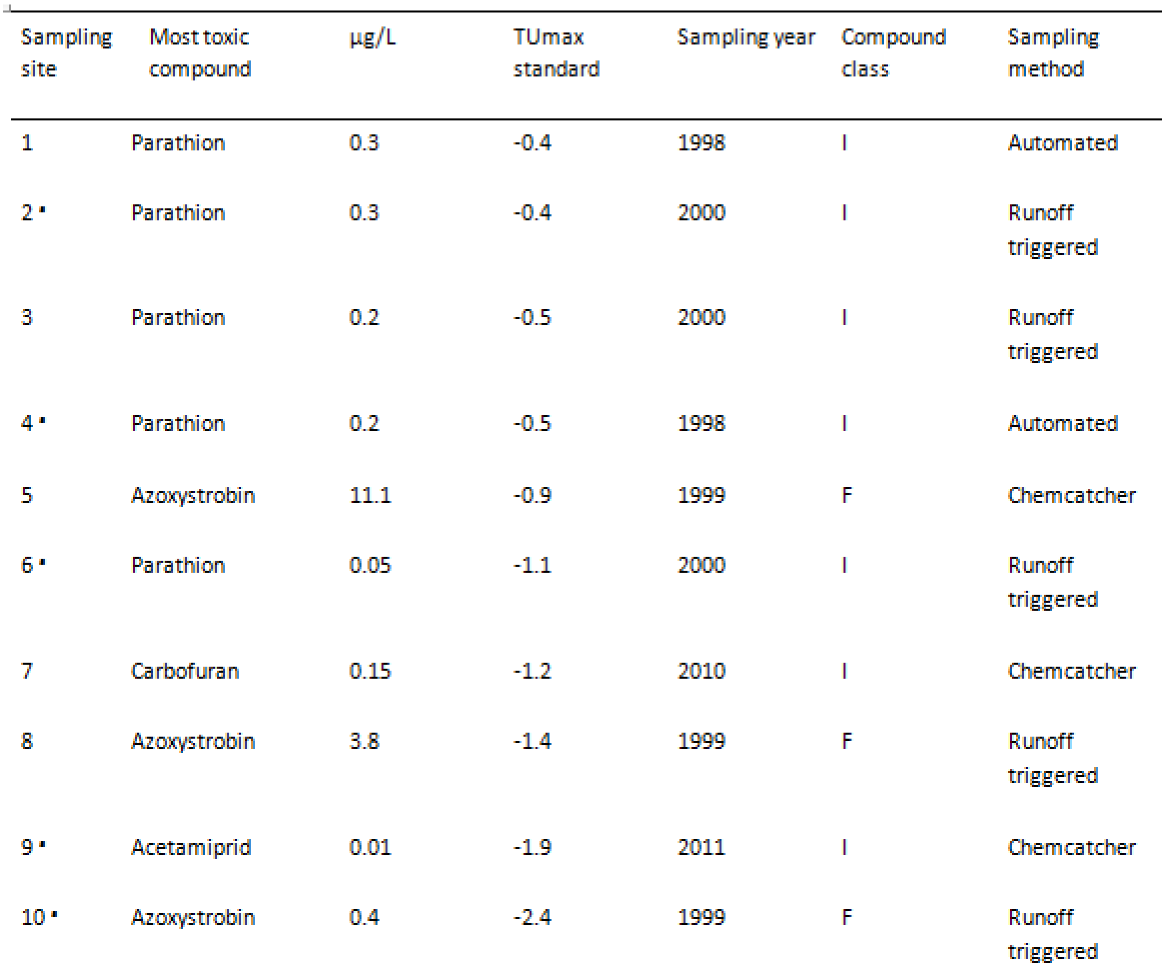


Fig. S3: Relationship between $\mathrm{SPEAR}_{\mathrm{pesticides}}$ during contamination and $\mathrm{TU}_{\mathrm{max}}$ for the sampling sites with and without upstream forested reaches (UFR) without the sites in 2010.

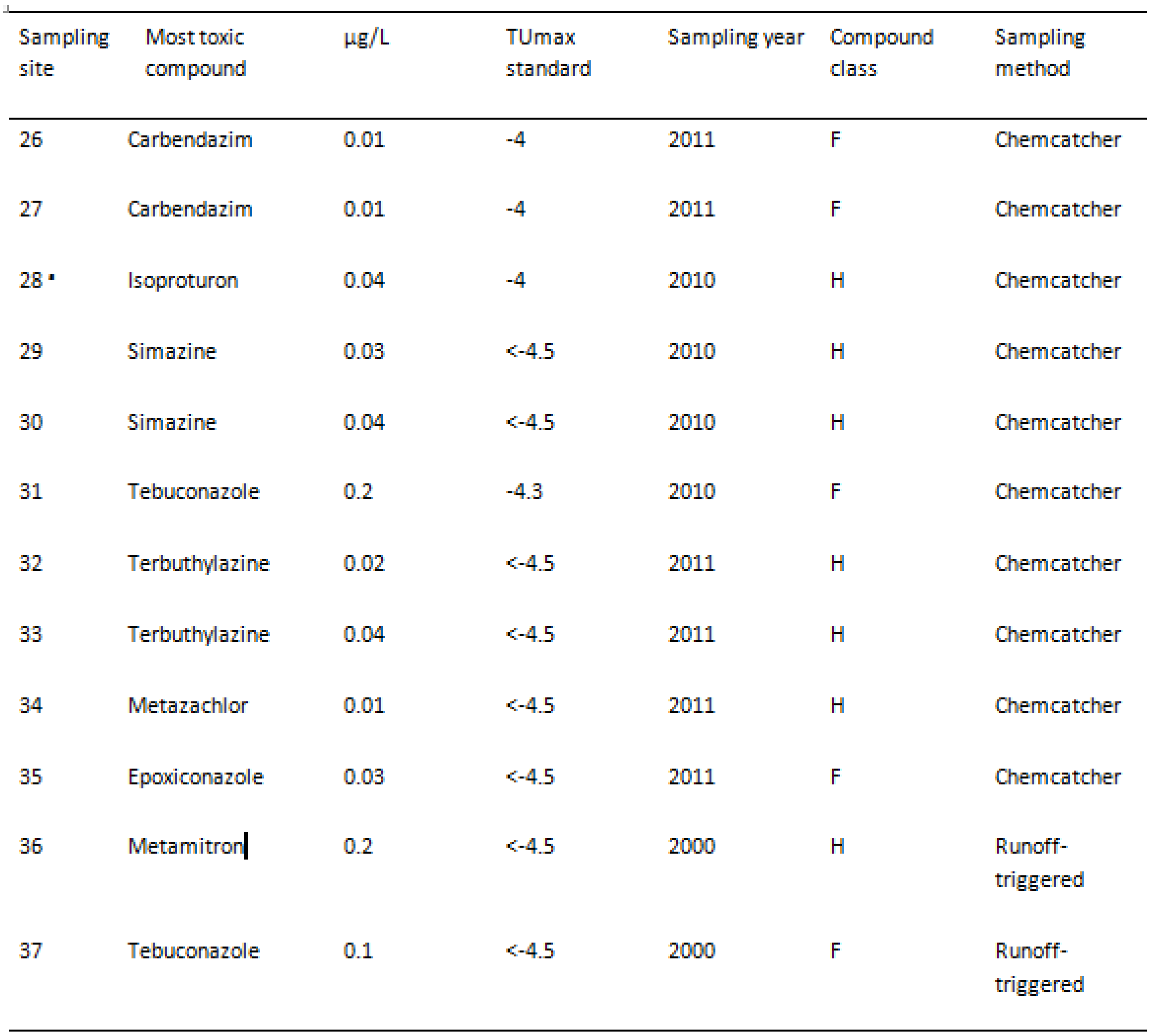


Sampling site	Most toxic compound	µg/L	TUmax standard	Sampling year	Compound class	Sampling method
1	Parathion	0.3	-0.4	1998	I	Automated
2 ▪	Parathion	0.3	-0.4	2000	I	Runoff triggered
3	Parathion	0.2	-0.5	2000	I	Runoff triggered
4 ▪	Parathion	0.2	-0.5	1998	I	Automated
5	Azoxystrobin	11.1	-0.9	1999	F	Chemcatcher
6 ▪	Parathion	0.05	-1.1	2000	I	Runoff triggered
7	Carbofuran	0.15	-1.2	2010	I	Chemcatcher
8	Azoxystrobin	3.8	-1.4	1999	F	Runoff triggered
9 ▪	Acetamiprid	0.01	-1.9	2011	I	Chemcatcher
10 ▪	Azoxystrobin	0.4	-2.4	1999	F	Runoff triggered

Table S1 Pesticide sampling methods applied at each sampling site. (Continued overleaf.)

Sampling site	Most toxic compound	µg/L	TUmax standard	Sampling year	Compound class	Sampling method
11 ▪	Azoxystrobin	0.3	-2.5	2000	F	Runoff triggered
12	Isoproturon	2.6	-2.6	2000	H	Runoff triggered
13	Methiocarb	0.042	-2.7	2011	M	Runoff triggered
14 ▪	Isoproturon	1.6	-2.8	2000	H	Runoff triggered
15	Isoproturon	1.28	-2.9	2011	H	Chemcatcher
16	Methiocarb	0.02	-2.9	2011	M	Runoff-triggered
17	Thiacloprid	0.004	-3	2011	I	Chemcatcher
18	Azoxystrobin	0.1	-3	1999	F	Runoff-triggered
19 ▪	Azoxystrobin	0.1	-3	2000	F	Runoff-triggered
20 ▪	Methiocarb	0.01	-3.2	2011	M	Chemcatcher
21 ▪	Carbendazim	0.05	-3.3	2011	F	Chemcatcher
22 ▪	Azoxystrobin	0.05	-3.3	2000	F	Runoff-triggered
23 ▪	Isoproturon	0.3	-3.5	2000	H	Runoff-triggered
24	Dimoxystrobin	0.01	-3.6	2011	F	Chemcatcher
25	Thiamethoxam	0.003	-4	2011	I	Runoff-triggered

Table S1 Pesticide sampling methods applied at each sampling site. (Continued overleaf.)

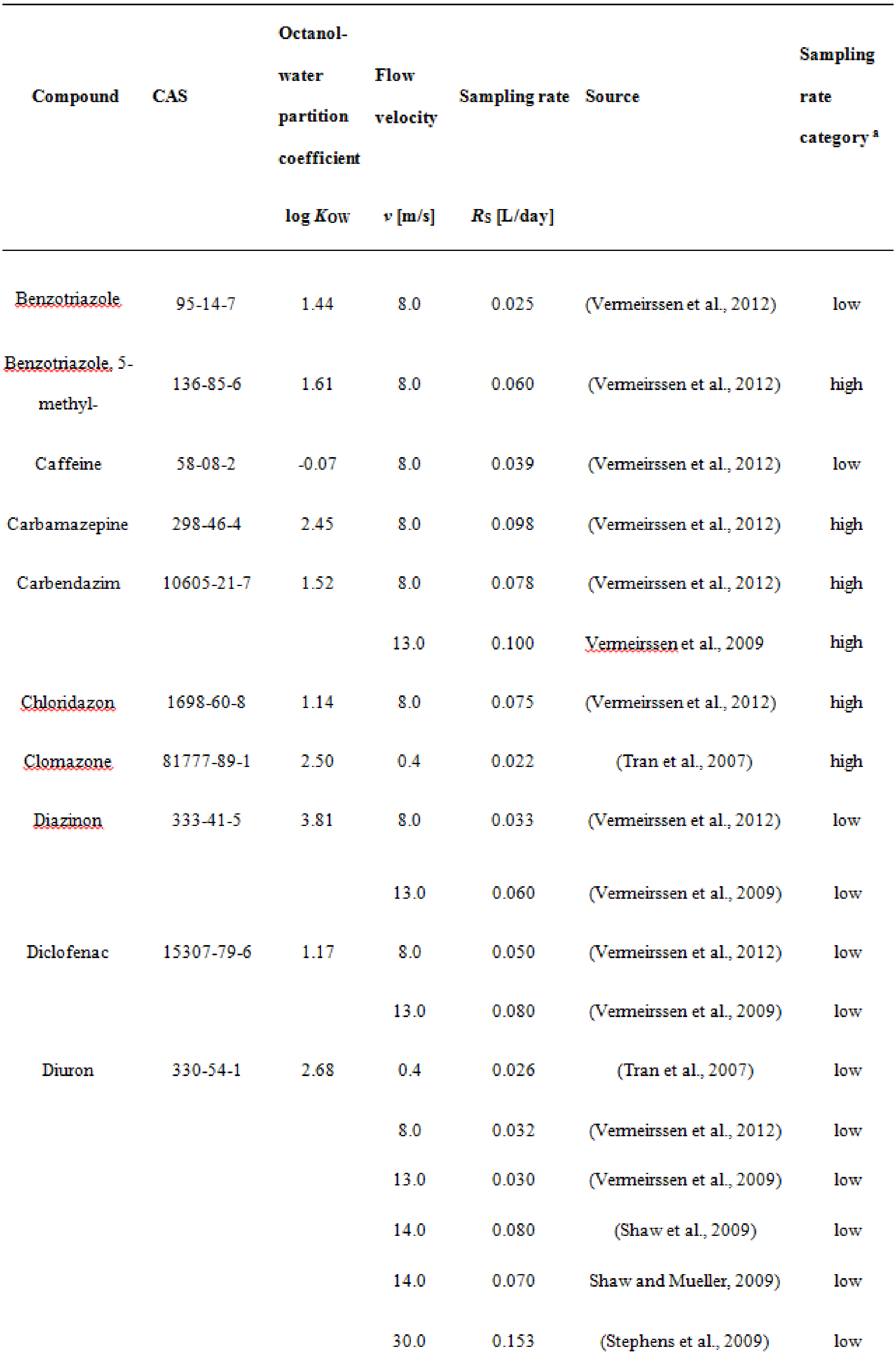


Sampling site	Most toxic compound	µg/L	TUmax standard	Sampling year	Compound class	Sampling method
26	Carbendazim	0.01	-4	2011	F	Chemcatcher
27	Carbendazim	0.01	-4	2011	F	Chemcatcher
28	Isoproturon	0.04	-4	2010	H	Chemcatcher
29	Simazine	0.03	<-4.5	2010	H	Chemcatcher
30	Simazine	0.04	<-4.5	2010	H	Chemcatcher
31	Tebuconazole	0.2	-4.3	2010	F	Chemcatcher
32	Terbuthylazine	0.02	<-4.5	2011	H	Chemcatcher
33	Terbuthylazine	0.04	<-4.5	2011	H	Chemcatcher
34	Metazachlor	0.01	<-4.5	2011	H	Chemcatcher
35	Epoxiconazole	0.03	<-4.5	2011	F	Chemcatcher
36	Metamitron	0.2	<-4.5	2000	H	Runoff-triggered
37	Tebuconazole	0.1	<-4.5	2000	F	Runoff-triggered

Table S1 Pesticide sampling methods applied at each sampling site (continued). In 1998-2000, runoff-triggered samplers and automated runoff samplers were used. In 2011, both techniques were applied, Chemcatcher® were only applied where water level did not rise by at least 5 cm during the sampling campaign. In 2010, only Chemcatcher® passive samplers were used. Runoff-triggered and automated samplers were shown to be comparable by Liess and von der Ohe (2005), whereas the concentrations obtained by Chemcatcher were adjusted to represent peak concentrations during runoff events, as described in section 2.3 of the present article.

Compound	**CAS**	**Octanol-water partition coefficient**	**Flow velocity**	**Sampling rate**	**Source**	**Sampling rate category**[a]
		log *K*OW	***v* [m/s]**	***R*S [L/day]**		
Ametryn	834-12-8	2.98	14.0	0.140	(Shaw et al., 2009)	high
			14.0	0.140	Shaw and Mueller, 2009)	high
Atrazine	1912-24-9	2.61	0.0	0.013	(O'Brien et al., 2011)	high
			0.4	0.023	(Tran et al., 2007)	high
			3.4	0.041	(O'Brien et al., 2011)	high
			8.0	0.072	(O'Brien et al., 2011)	high
			8.0	0.088	(Vermeirssen et al., 2012)	high
			13.0	0.120	(Vermeirssen et al., 2009)	high
			14.0	0.140	(Shaw et al., 2009)	high
			14.0	0.170	Shaw and Mueller, 2009)	high
			16.1	0.124	(O'Brien et al., 2011)	high
			24.1	0.136	(O'Brien et al., 2011)	high
			30.0	0.177	(Stephens et al., 2009)	high
Atrazine, hydroxy-	2163-68-0	1.75	8.0	0.037	(Vermeirssen et al., 2012)	low
Atrazine, desethyl-	6190-65-4	1.51	8.0	0.065	(Vermeirssen et al., 2012)	high
			13.0	0.100	Vermeirssen et al., 2009	high
Atrazine, -deisopropyl	1007-28-9	1.15	8.0	0.044	(Vermeirssen et al., 2012)	low

Table S2 Literature values for compound properties and sampling rates R_S. (Continued overleaf.)

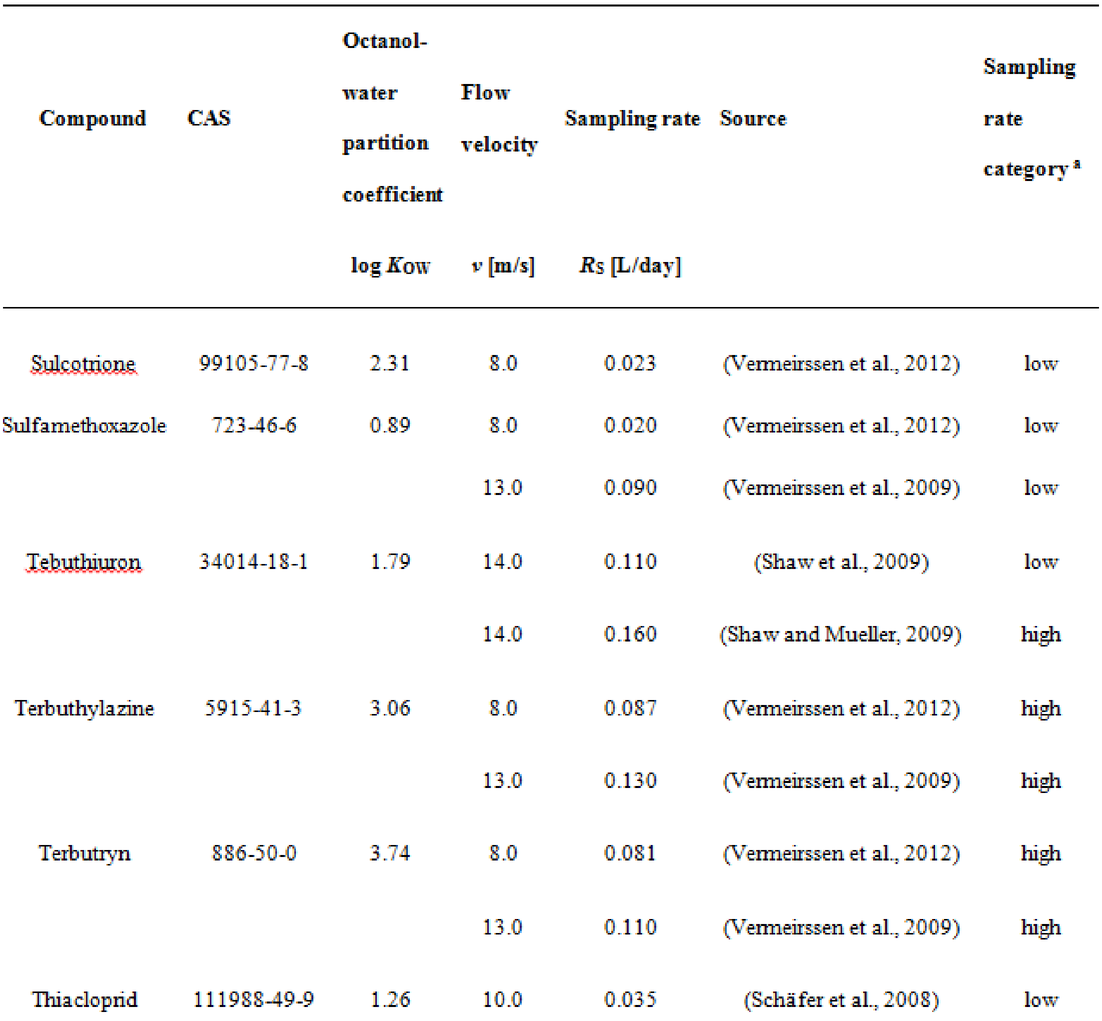


Compound	CAS	Octanol-water partition coefficient log K_{OW}	Flow velocity v [m/s]	Sampling rate R_S [L/day]	Source	Sampling rate category ^{a}
Benzotriazole	95-14-7	1.44	8.0	0.025	(Vermeirssen et al., 2012)	low
Benzotriazole, 5-methyl-	136-85-6	1.61	8.0	0.060	(Vermeirssen et al., 2012)	high
Caffeine	58-08-2	-0.07	8.0	0.039	(Vermeirssen et al., 2012)	low
Carbamazepine	298-46-4	2.45	8.0	0.098	(Vermeirssen et al., 2012)	high
Carbendazim	10605-21-7	1.52	8.0	0.078	(Vermeirssen et al., 2012)	high
			13.0	0.100	Vermeirssen et al., 2009	high
Chloridazon	1698-60-8	1.14	8.0	0.075	(Vermeirssen et al., 2012)	high
Clomazone	81777-89-1	2.50	0.4	0.022	(Tran et al., 2007)	high
Diazinon	333-41-5	3.81	8.0	0.033	(Vermeirssen et al., 2012)	low
			13.0	0.060	(Vermeirssen et al., 2009)	low
Diclofenac	15307-79-6	1.17	8.0	0.050	(Vermeirssen et al., 2012)	low
			13.0	0.080	(Vermeirssen et al., 2009)	low
Diuron	330-54-1	2.68	0.4	0.026	(Tran et al., 2007)	low
			8.0	0.032	(Vermeirssen et al., 2012)	low
			13.0	0.030	(Vermeirssen et al., 2009)	low
			14.0	0.080	(Shaw et al., 2009)	low
			14.0	0.070	Shaw and Mueller, 2009)	low
			30.0	0.153	(Stephens et al., 2009)	low

Table S2 Literature values for compound properties and sampling rates R_S. (Continued overleaf.)

Compound	**CAS**	**Octanol-water partition coefficient**	**Flow velocity**	**Sampling rate**	**Source**	**Sampling rate category** [a]
		log K_{OW}	**v [m/s]**	**R_S [L/day]**		
Fipronil	120068-37-3	4.00	14.0	0.160	(Shaw et al., 2009)	low
Hexazinone	51235-04-2	1.36	14.0	0.100	(Shaw et al., 2009)	high
			14.0	0.140	(Shaw and Mueller, 2009)	high
Irgarol	28159-98-0	3.38	8.0	0.080	(Vermeirssen et al., 2012)	high
			13.0	0.130	(Vermeirssen et al., 2009)	high
Isoproturon	34123-59-6	2.87	8.0	0.069	(Vermeirssen et al., 2012)	high
			13.0	0.100	(Vermeirssen et al., 2009)	high
Mecoprop	93-65-2	3.13	8.0	0.021	(Vermeirssen et al., 2012)	low
			13.0	0.070	(Vermeirssen et al., 2009)	high
Metolachlor	51218-45-2	3.13	0.4	0.021	(Tran et al., 2007)	high
			8.0	0.098	(Vermeirssen et al., 2012)	high
			14.0	0.210	(Shaw et al., 2009)	high
Phenazone	60-80-0	0.38	8.0	0.069	(Vermeirssen et al., 2012)	high
Promethryn	7287-19-6	3.51	0.0	0.009	(O'Brien et al., 2011)	high
			3.4	0.032	(O'Brien et al., 2011)	high
			8.0	0.060	(O'Brien et al., 2011)	high
			16.1	0.107	(O'Brien et al., 2011)	high
			24.1	0.126	(O'Brien et al., 2011)	high
Simazine	122-34-9	2.18	0.4	0.024	(Tran et al., 2007)	high
			14.0	0.140	(Shaw et al., 2009)	high
			14.0	0.160	(Shaw and Mueller, 2009)	high
			30.0	0.183	(Stephens et al., 2009)	high

Table S2 Literature values for compound properties and sampling rates R_S. (Continued overleaf.)

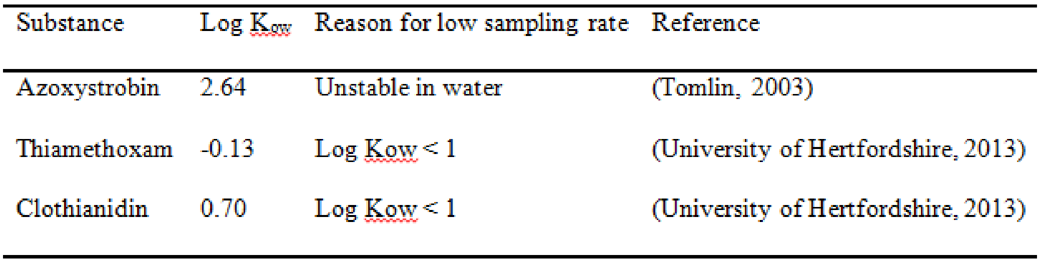


Compound	CAS	Octanol-water partition coefficient	Flow velocity	Sampling rate	Source	Sampling rate category [a]
		log *Kow*	*v* [m/s]	R_S [L/day]		
Sulcotrione	99105-77-8	2.31	8.0	0.023	(Vermeirssen et al., 2012)	low
Sulfamethoxazole	723-46-6	0.89	8.0	0.020	(Vermeirssen et al., 2012)	low
			13.0	0.090	(Vermeirssen et al., 2009)	low
Tebuthiuron	34014-18-1	1.79	14.0	0.110	(Shaw et al., 2009)	low
			14.0	0.160	(Shaw and Mueller, 2009)	high
Terbuthylazine	5915-41-3	3.06	8.0	0.087	(Vermeirssen et al., 2012)	high
			13.0	0.130	(Vermeirssen et al., 2009)	high
Terbutryn	886-50-0	3.74	8.0	0.081	(Vermeirssen et al., 2012)	high
			13.0	0.110	(Vermeirssen et al., 2009)	high
Thiacloprid	111988-49-9	1.26	10.0	0.035	(Schäfer et al., 2008)	low

Table S2 Literature values for compound properties and sampling rates R_S (continued) at different average flow velocities (v), which were used to derive the regression equations for the low and high sampling rates (see methods section 2.3).

[a] Sampling rate categories (Figure S1) with following regression equations:

R_S (high) = 0.85 × v + 0.014 and R_S (low) = 0.47 × v + 0.0004



Substance	Log K_{ow}	Reason for low sampling rate	Reference
Azoxystrobin	2.64	Unstable in water	(Tomlin, 2003)
Thiamethoxam	-0.13	Log Kow < 1	(University of Hertfordshire, 2013)
Clothianidin	0.70	Log Kow < 1	(University of Hertfordshire, 2013)

Table S3 Additional pesticides sampled with Chemcatcher (SDB-RPS) with missing literature data on R_S and assumed low sampling rates (R_S).

Most sensitive species				Standard species		
Substance	LC_{50} µg/L	Species		LC_{50} µg/L	Species	
Acetamiprid	3.35	*Cheumatopsyche brevilineata*	[b]	20	*Chironomus riparus*	[b]
Azoxystrobin	98	*D.magna*	[b]	98	*D.magna*	[b]
Bentazone	1000	*D.magna*	[b]	1000	*D.magna*	[b]
Bifenox	350	*D.magna*	[b]	350	*D.magna*	[b]
Carbendazim	103	*D.magna*	[b]	103	*D.magna*	[b]
Chloridazon	94000	*D.magna*	[b]	94000	*D.magna*	[b]
Clothianidin	4.4	*Cheumatopsyche brevilineata*	[b]	22	*Chironomus riparus*	[b]
Diflufenican	240	D.magna	[c]	240	*D.magna*	[c]
Dimoxystrobin	39.4	*D.magna*	[c]	39.4	*D.magna*	[c]
Epoxiconazol	8690	*D.magna*	[c]	8690	*D.magna*	[c]
Ethofumesate	179000	*D.magna*	[b]	179000	*D.magna*	[b]
Fluoxastrobin	480	*D.magna*	[c]	480	*D.magna*	[c]
Indoxacarb	48.5	*Baetis rhodani*	[b]	48.5	*Baetis rhodania*	[a,b]
Isoproturon	1000	*D. magna*	[b]	1000	*D. magna*	[b]
Kresoxim-methyl	186	*D. magna*	[b]	186	*D. magna*	[b]
Metamitron	103775	*D. magna*	[b]	103775	*D. magna*	[b]
Metazachlor	33000	*D. magna*	[b]	33000	*D. magna*	[b]
Metholachlor	4100	*Chironomus plumosus*	[b]	4100	*Chironomus plumosus*	[b]
Methiocarb	16	*Pteronarcys californica*	[b]	19	*D.magna*	[b]
Metribuzin	4200	*D.magna*	[b]	4200	*D.magna*	[b]
Parathion	0.68	*D. pulex*	[b]	0.68	*D. pulex*	[b]

Table S4 The 48 hour LC_{50} values used for the calculation of TU. (Continued overleaf.)

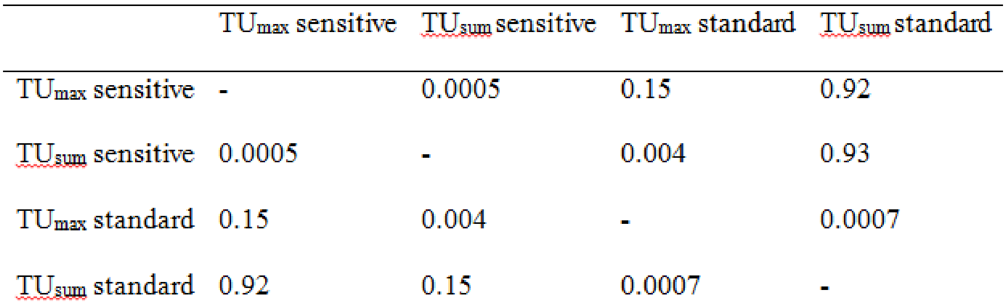
Most sensitive species			Standard species	
Substance	LC_{50} µg/L	Species	LC_{50} µg/L	Species
Picoxystrobin	24	*D.magna* [c]	24	*D.magna* [c]
Pirimicarb	17	*D.magna* [b]	17	*D.magna* [b]
Propiconazole	10000	*D.magna* [c]	10000	*D.magna* [c]
Pyraclostrobin	16	*D.magna* [b]	16	*D.magna* [b]
Tebuconazole	4000	*D.magna* [b]	4000	*D.magna* [b]
Terbutylazine	21200	*D.magna* [b]	21200	*D.magna* [b]
Thiacloprid	5.27	*Cheumatopsyche brevilineata* [b]	5.27	*Cheumatopsyche brevilineata*[a] [a,b]
Thiamethoxam	35	*Chironomus sp.* [b]	35	*Chironomus sp.* [b]

Table S4 The 48 hour LC_{50} values used for the calculation of TU most sensitive and TU standard for the detected pesticides (continued).

[a] non-standard insect species was taken, when no acute test for standard test species was available (EC, 2002) in the databases of USEPA (2014) and University of Hertfordshire (2013);

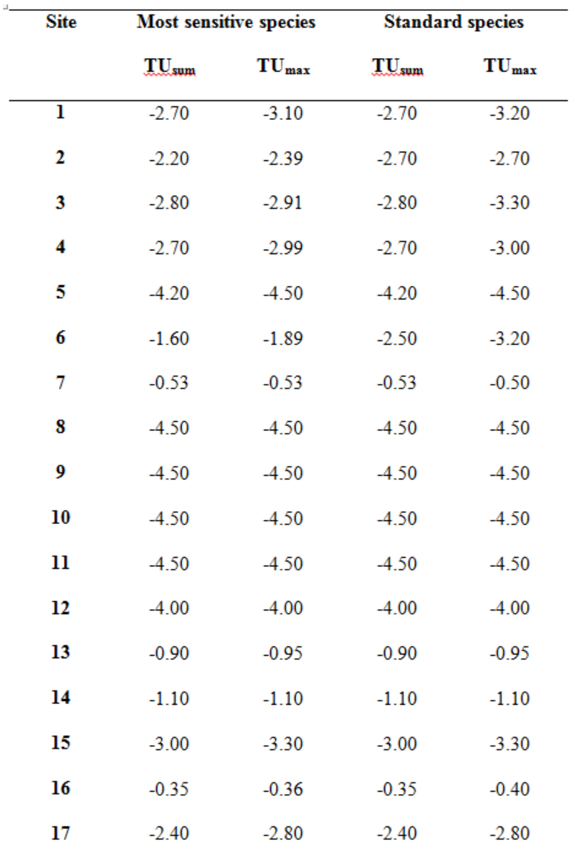
[b] Source: ECOTOX Database (USEPA, 2014);

[c] Source: Pesticide Properties Database (University of Hertfordshire, 2013)

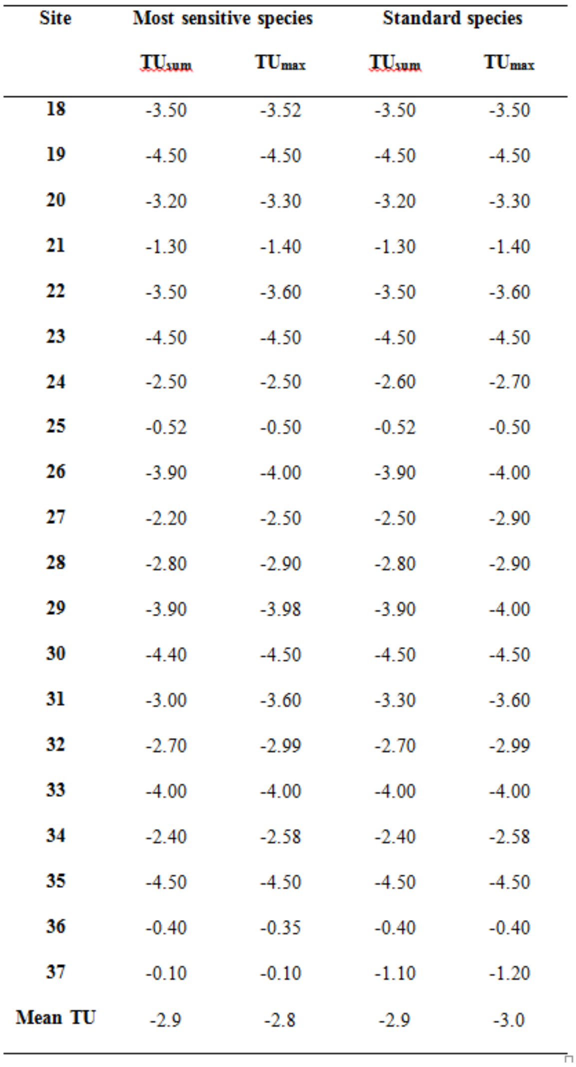


	TU_{max} sensitive	TU_{sum} sensitive	TU_{max} standard	TU_{sum} standard
TU_{max} sensitive	-	0.0005	0.15	0.92
TU_{sum} sensitive	0.0005	-	0.004	0.93
TU_{max} standard	0.15	0.004	-	0.0007
TU_{sum} standard	0.92	0.15	0.0007	-

Table S5a P-values calculated using pairwise t test comparisons and Holm adjustment for multiple comparisons for the comparison of different TU calculation methods.

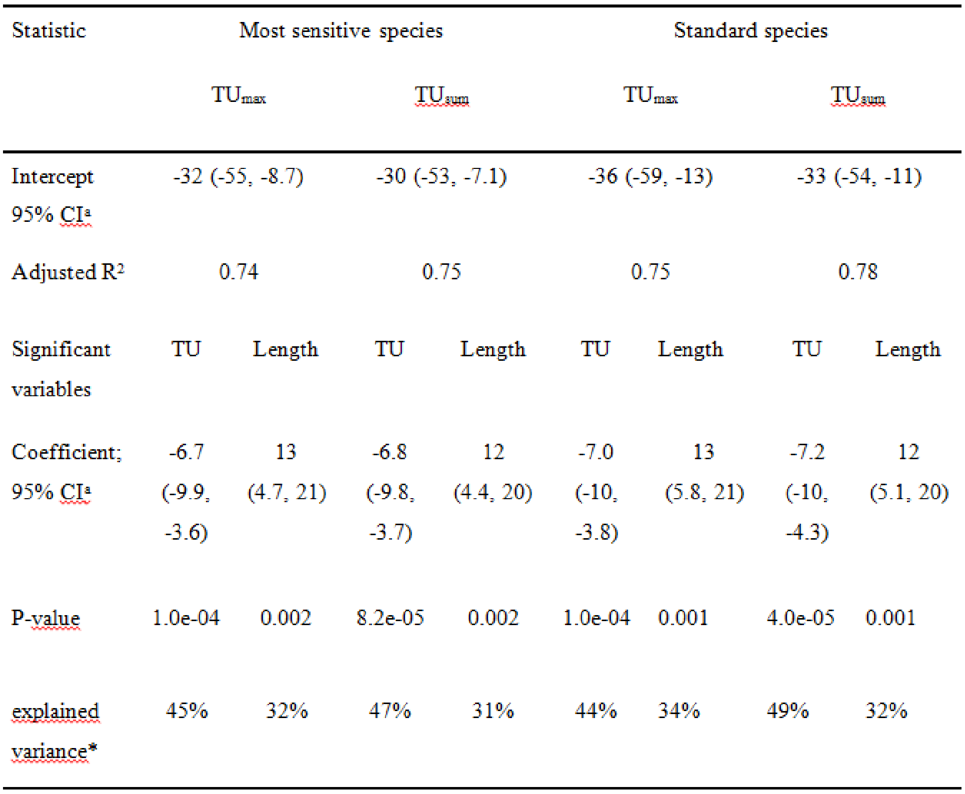


Site	Most sensitive species		Standard species	
	TU_{sum}	TU_{max}	TU_{sum}	TU_{max}
1	-2.70	-3.10	-2.70	-3.20
2	-2.20	-2.39	-2.70	-2.70
3	-2.80	-2.91	-2.80	-3.30
4	-2.70	-2.99	-2.70	-3.00
5	-4.20	-4.50	-4.20	-4.50
6	-1.60	-1.89	-2.50	-3.20
7	-0.53	-0.53	-0.53	-0.50
8	-4.50	-4.50	-4.50	-4.50
9	-4.50	-4.50	-4.50	-4.50
10	-4.50	-4.50	-4.50	-4.50
11	-4.50	-4.50	-4.50	-4.50
12	-4.00	-4.00	-4.00	-4.00
13	-0.90	-0.95	-0.90	-0.95
14	-1.10	-1.10	-1.10	-1.10
15	-3.00	-3.30	-3.00	-3.30
16	-0.35	-0.36	-0.35	-0.40
17	-2.40	-2.80	-2.40	-2.80



Site	Most sensitive species		Standard species	
	TU_{sum}	TU_{max}	TU_{sum}	TU_{max}
18	-3.50	-3.52	-3.50	-3.50
19	-4.50	-4.50	-4.50	-4.50
20	-3.20	-3.30	-3.20	-3.30
21	-1.30	-1.40	-1.30	-1.40
22	-3.50	-3.60	-3.50	-3.60
23	-4.50	-4.50	-4.50	-4.50
24	-2.50	-2.50	-2.60	-2.70
25	-0.52	-0.50	-0.52	-0.50
26	-3.90	-4.00	-3.90	-4.00
27	-2.20	-2.50	-2.50	-2.90
28	-2.80	-2.90	-2.80	-2.90
29	-3.90	-3.98	-3.90	-4.00
30	-4.40	-4.50	-4.50	-4.50
31	-3.00	-3.60	-3.30	-3.60
32	-2.70	-2.99	-2.70	-2.99
33	-4.00	-4.00	-4.00	-4.00
34	-2.40	-2.58	-2.40	-2.58
35	-4.50	-4.50	-4.50	-4.50
36	-0.40	-0.35	-0.40	-0.40
37	-0.10	-0.10	-1.10	-1.20
Mean TU	-2.9	-2.8	-2.9	-3.0

Table S5b Toxic unit per sampling site, calculated using the TU_{sum} and TU_{max} approaches and different reference species: (i) *D. magna*, (ii) the most sensitive species per compound (Schäfer et al., 2013), and (iii) the most sensitive out of the standard test species *Daphnia* sp. and *Chironomus* sp. (EC, 2013).



Statistic	Most sensitive species				Standard species			
	TU_{max}		TU_{sum}		TU_{max}		TU_{sum}	
Intercept 95% CI[a]	-32 (-55, -8.7)		-30 (-53, -7.1)		-36 (-59, -13)		-33 (-54, -11)	
Adjusted R^2	0.74		0.75		0.75		0.78	
Significant variables	TU	Length	TU	Length	TU	Length	TU	Length
Coefficient; 95% CI[a]	-6.7 (-9.9, -3.6)	13 (4.7, 21)	-6.8 (-9.8, -3.7)	12 (4.4, 20)	-7.0 (-10, -3.8)	13 (5.8, 21)	-7.2 (-10, -4.3)	12 (5.1, 20)
P-value	1.0e-04	0.002	8.2e-05	0.002	1.0e-04	0.001	4.0e-05	0.001
explained variance*	45%	32%	47%	31%	44%	34%	49%	32%

Table S6 Results of the multiple linear regression analysis with $SPEAR_{pesticides}$ and selected independent variables, with TU calculated using 6 different methods. The TU_{max} and TU_{sum} were calculated on the basis of most sensitive species, most sensitive standard species, and *D. magna*. Only significant terms and the percent variance explained (*method by Lindemann et al., 1980) by these terms are shown.

[a] CI: Confidence interval

Parameter	Adjusted R^2	*p* value
Nitrate	-	> 0.05
Orthophosphate	-	> 0.05
Depth	-	> 0.05
Width	-	> 0.05
Distance (downstream) from forest	-	> 0.05
Dissolved oxygen	-	> 0.05
pH	-	> 0.05
Conductivity	-	> 0.05

Table S7 Linear regression analysis of potential relationships. (Continued overleaf.)

Stream bed structure	0.16	0.02
Aerial distance to the nearest potential undisturbed reach	-	> 0.05
Forest area	0.39	0.002
Forest length	0.46	0.0006
Toxic unit (TU standard)	0.43	7x10^-6

Table S7 Linear regression analysis of potential relationships (continued) between SPEAR$_{\text{pesticides}}$ and water quality, habitat and forest characteristics for all sites (with and without upstream forested reaches combined).

SI Text

Estimation of pesticide toxicity to the macroinvertebrate communities

To estimate the toxicity of the detected pesticides to invertebrate communities, we computed the toxic units (TU) for each sampling event, according to equation 1 below. However, to make sure that our results are not changed by different TU calculation methods, we also computed TU using other commonly applied methods.

First, we compared two approaches: assuming that the most toxic compound determines the effects of the mixture (TU$_{\text{max}}$) (as in the main text of the article) and estimating the additive effects of the mixture (TU$_{\text{sum}}$). For the first approach, TU$_{\text{max}}$ is determined as the highest TU per sample (Eq. 1).

$$TU_{max} = max_{i=1}^{n}\, log\left(\frac{C_i}{LC_{50i}}\right) \tag{1}$$

where C_i is the concentration of compound i and LC_{50i} is the 48 hour lethal concentration for 50% of the population of the reference organism.

In the second approach, TU$_{\text{sum}}$ is calculated as the sum of the ratio of the concentration to the LC_{50} values over all the compounds in the sample (Eq. 2).

$$TU_{sum} = log\sum_{i=1}^{n}\left(\frac{C_i}{LC_{50i}}\right) \tag{2}$$

where the C_i is the concentration of each pesticide and the LC_{50i} is the 48 hour lethal concentration for 50% of the population of the reference organism.

Regarding the choice of the standard test species, we used LC_{50} values of standard test species *Daphnia sp.* or *Chironomus sp.* (EC, 2013). We did not select *D.magna* alone because neonicotinoids were present in our samples, and they are considerably more toxic to insects than to *Daphnids* (Beketov and Liess, 2008; Schäfer et al., 2013). Schäfer et al. (2013) used the LC_{50} of the most sensitive invertebrate species available for each compound in the mixture to estimate TU. This method was suggested as an improvement to basing the TU calculation only on LC_{50} of *D.magna*, to account for the specific mechanism of action of some modern pesticides, like the neonicotinoids. However, basing the TU calculation on different species for every compound may lead to discrepancies in toxicity estimates for similar compounds that are evaluated with data from assays of different species. Therefore, we suggested a different method, which on the one hand reduces this possible bias, and on the other hand accounts for the specific toxicity of neonicotinoids to insects. We suggested to use LC_{50} based on the most sensitive of the standard test species *Daphnia sp.* or insect larvae *Chironomus sp.* as defined in the current EU regulation on plant protection products (EC, 2013).

We compared our approach to calculating TU_{sum} and to the most sensitive species approach (see Schäfer et al., 2013). Thus, we calculated the TU with 4 methods: (i) using the most sensitive species per compound (Schäfer et al., 2013) – **TU_{max} and TU_{sum} sensitive** –, and (ii) using the standard test species *Daphnia* sp. and *Chironomus* sp. (EC, 2013) – **TU_{max} and TU_{sum} standard** –. The insecticides indoxacarb and thiacloprid were exceptions, because no data on the standard insect species *Chironomus* sp. were available. Thus, the respective LC_{50} values for the TU_{max}/TU_{sum} standard calculation were based on other insect larvae for these compounds, as recommended in the European Commission Guidance Document for Aquatic Toxicology (EC, 2002). The median LC_{50} for each compound and species was calculated from the values taken from the ecotoxicology database of the US Environmental Protection Agency (USEPA, 2014). If the data was not available in this database, the Pesticide Properties Database of the University of Hertfordshire was used instead (University of Hertfordshire, 2013). The list of LC_{50} values for *D. magna*, the standard species, and most sensitive species used for each substance is shown in Table S4.

Estimating additive toxicity (Eq. 1 above) gave slightly higher TU (±0.2 TU) than using the highest TU per sample (Eq. 1) (Table S5a and Table S5b). Using the most sensitive species approach yielded very similar TU values to the standard test species approach we suggested (Table S5b). The results of other analyses conducted within the study were the same regardless of the TU calculation method (for an example, see the results in Table S6). The similarity in TU values regardless of the reference organism was due to low concentrations of neonicotinoids in our study. Only at 3 sampling sites, all sampled in 2011, neonicotinoids caused the highest TU per sample (Table 2 in the main text). Thus, to compare the methods, they should be tested further on datasets with a greater share of neonicotinoids.

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