Pesticide sources and landscape elements driving aquatic pesticide exposure and effects on macroinvertebrate communities

by

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from Meissen

Accepted Dissertation thesis for the partial fulfilment of the requirements for a
Doctor of Natural Sciences
Fachbereich 7: Natur- und Umweltwissenschaften
Universität Koblenz-Landau

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Date of the oral examination: 25th July 2014

This doctoral thesis is based on the following scientific publications:

1. Bunzel, K.; Kattwinkel, M.; Liess, M. (2013): Effects of organic pollutants from wastewater treatment plants on aquatic invertebrate communities. Water Research 47 (2), Pages 597-606, doi: 10.1016/j.watres.2012.10.031

2. Bunzel, K.; Liess, M.; Kattwinkel, M. (2014): Landscape parameters driving aquatic pesticide exposure and effects. Environmental Pollution 186, Pages 90-97, doi: 10.1016/j.envpol.2013.11.021

3. Bunzel, K.; Kattwinkel, M.; Schauf, M.; Thrän, D. (2014): Energy crops and pesticide contamination: Lessons learned from the development of energy crop cultivation in Germany. Biomass & Bioenergy, doi: 10.1016/j.biombioe.2014.08.016

Summary

The adoption of the EU Water Framework Directive (WFD) in 2000 marked the beginning of a new era of European water policy. However, more than a decade later, the majority of European rivers are still failing to meet one of the main objectives of the WFD: the good ecological status. Pesticides are a major stressor for stream ecosystems. This PhD thesis emphasises the need for WFD managers to consider all main agricultural pesticide sources and influencing landscape parameters when setting up River Basin Management Plans and Programmes of Measures. The findings and recommendations of this thesis can help to successfully tackle the risk of pesticide contamination to achieve the WFD objectives.

A total of 663 sites that were situated in the German Federal States of Saxony, Saxony-Anhalt, Thuringia and Hesse were studied (Chapter 3 and 4). In addition to an analysis of the macroinvertebrate data of the governmental WFD monitoring network, a detailed GIS analysis of the main agricultural pesticide sources (arable land and garden allotments as well as wastewater treatment plants (WWTPs)) and landscape elements (riparian buffer strips and forested upstream reaches) was conducted. Based on the results, a screening approach was developed that allows an initial rapid and cost-effective identification of those sites that are potentially affected by pesticide contamination. By using the trait-based bioindicator $SPEAR_{pesticides}$, the insecticidal long-term effects of the WWTP effluents on the structure of the macroinvertebrate community were identified up to at least 1.5 km downstream (in some cases even 3 km) of the WWTPs. The results of the German Saprobic Index revealed that the WWTPs can still be important sources of oxygen-depleting substances. Furthermore, the results indicate that forested upstream reaches and riparian buffer strips at least 5 m in width can be appropriate measures in mitigating the effects and exposure of pesticides.

There are concerns that the future expansion of energy crop cultivation will lead to an increased pesticide contamination of ecosystems in agricultural landscapes. Therefore, the potential of energy crops for pesticide contamination was examined based on an analysis of the development of energy crop cultivation in Germany and a literature search on perennial energy crops (Chapter 5). The results indicate that the future large-scale expansion of energy crop cultivation will not necessarily cause an increase or decrease in the amounts of pesticides that are released into the environment. The potential effects will depend on the future design of the agricultural systems. Instead of creating energy monocultures, annual energy crops should be integrated into the existing food production systems. Financial incentives and further education are needed to encourage the use of sustainable crop rotations, innovative cropping systems and perennial energy crops, which may contribute to crop diversity and generate lower pesticide demands than do intensive farming systems.

Zusammenfassung

Die Verabschiedung der Europäischen Wasserrahmenrichtlinie (WRRL) in 2000 markierte den Beginn einer neuen Ära in der europäischen Wasserpolitik. Mehr als ein Jahrzehnt später, verfehlt jedoch weiterhin die Mehrheit der europäischen Flüsse den guten ökologischen Zustand, eines der wichtigsten WRRL-Ziele. Ein bedeutender Belastungsfaktor für Fließgewässerökosysteme sind Pflanzenschutzmittel (PSM). Die vorliegende Doktorarbeit unterstreicht die Notwendigkeit, alle wichtigen landwirtschaftlichen PSM-Quellen und beeinflussenden Landschaftsfaktoren bei der Erstellung von WRRL-Bewirtschaftungsplänen und Maßnahmenprogrammen zu berücksichtigen. Die Ergebnisse und Empfehlungen dieser Doktorarbeit verbessern das Verständnis für eine zielgerichtete Bekämpfung von PSM-Belastungen zur Erreichung der WRRL-Ziele.

Insgesamt wurden 663 Messstellen in den Bundesländern Sachsen, Sachsen-Anhalt, Thüringen und Hessen untersucht (Kapitel 3 und 4). Neben einer Analyse der Makrozoobenthos-Daten aus dem WRRL-Monitoringnetz, erfolgte eine detaillierte GIS-Analyse der wichtigsten landwirtschaftlichen PSM-Quellen (Ackerland, Kleingärten sowie kommunale Abwasserreinigungsanlagen) sowie Landschaftsfaktoren (Gewässerrandstreifen und bewaldete Abschnitte im Oberlauf). Basierend auf den Ergebnissen wurde eine Screening-Methode zur schnellen und kostengünstigen Identifizierung von potenziell mit PSM belasteten Stellen entwickelt. Mit Hilfe des Bioindikators $\text{SPEAR}_{\text{pesticides}}$ konnten insektizide Langzeitwirkungen der Abwässer von Abwasserreinigungsanlagen auf die Struktur der Makrozoobenthos-Gemeinschaft bis in 1,5 km Entfernung flussabwärts (in einigen Fällen sogar 3 km) aufgezeigt werden. Die Ergebnisse für den Deutschen Saprobienindex zeigen zudem, dass Abwasserreinigungsanlagen weiterhin eine bedeutende Quelle für sauerstoffzehrende Substanzen sind. Als geeignete Maßnahmen zur Verminderung der Belastung und der Auswirkungen von PSM wurden Gewässerrandstreifen (mindestens 5 m breit) und bewaldete Oberläufe identifiziert.

Es wird befürchtet, dass die zukünftige Ausdehnung des Energiepflanzenanbaus zu einem Anstieg der diffusen PSM-Belastung von Ökosystemen in Agrarlandschaften führen könnte. Diese Fragestellung wurde im Rahmen der vorliegenden Doktorarbeit basierend auf einer Analyse der Entwicklung des Energiepflanzenanbaus in Deutschland und anhand einer Literaturrecherche zu mehrjährigen Energiepflanzen untersucht (Kapitel 5). Die Ergebnisse zeigen, dass eine großflächige Ausdehnung des Energiepflanzenanbaus nicht unbedingt zu einer Erhöhung oder Verringerung der Menge an PSM, die in die Umwelt gelangen, führen muss. Die potenziellen Auswirkungen hängen vielmehr von der zukünftigen Ausgestaltung der Agrarsysteme ab. Anstelle des Anbaus von einjährigen Energiepflanzen in Monokulturen, sollten diese in die bereits vorhandenen Nahrungsmittelanbausysteme integriert werden. Zudem könnten finanzielle Anreize sowie eine verstärkte Aus- und Fortbildung der Bauern dazu beitragen, die Nutzung von nachhaltigen Fruchtfolgen, innovativen Anbausystemen und mehrjährigen Energiepflanzen zu erhöhen. Dies würde die Vielfalt der Feldfrüchte erhöhen und könnte helfen, den PSM-Bedarf der bisherigen intensiven Nahrungsmittelanbausysteme zu verringern.

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List of Abbreviations

EPT	Ephemeroptera, Plecoptera, and Trichoptera
EU	European Union
GIS	Geographic information systems
PE	Population Equivalent
PoM	Programme of Measure
PSM	Pflanzenschutzmittel
RBMP	River Basin Management Plan
RP	Runoff Potential
SPEAR	SPEcies At Risk
SRC	Short-Rotation Coppice
TU	Toxic Units
WFD	Water Framework Directive
WRRL	Wasserrahmenrichtlinie
WWTP	Wastewater Treatment Plant

1. Introduction

1.1. State of Europe's streams

In 2000, the adoption of the European Water Framework Directive (WFD) marked the beginning of a new era of European water policy [1]. In the past, the quality of a water body was traditionally assessed in terms of chemical parameters such as pH, dissolved oxygen, nutrients or concentrations of frequent pollutants. The WFD shifted the focus towards a more holistic understanding of aquatic ecology and the interaction between abiotic stressors and the responses of biological indicators. Accordingly, the aim of the WFD is to achieve not only a good chemical status but also a good ecological status of all of the European water bodies by 2015.

River basin management is a continuous and iterative process. For this reason, the WFD requires EU Member States to develop River Basin Management Plans (RBMPs), which must be revised and reported every six years. Each RBMP contains information about the current status of the water bodies and their WFD objectives and defines a “Programme of Measures” (PoM). The PoM lists an integrated set of measures that are necessary to reach the WFD objectives. The first reporting of the RBMPs by the EU Member States was due at the end of 2009. While nearly half of the European rivers (by total river length) reached a good chemical status, only a minority achieved a good ecological status [2] (Fig. 1.1). For example, only approximately 14% of the total length of German rivers and streams are currently in a good ecological status [3]. Extensive changes in the hydromorphology and excessive nutrient loads are considered the dominant causes for the inferior ecological status class of European rivers [2].

The chemical status is measured exclusively in terms of compliance with environmental quality standards for a limited set of 33 European-wide priority substances. However, Schäfer et al. [4] questioned this approach by suggesting that most of the compounds that are responsible for the potential acute effects on aquatic organisms are not considered in the current set of priority substances. Schäfer et al. [4] analysed the detection frequencies and concentrations of 331 organic compounds in four large German rivers and found that only two of the substances that were most relevant for the acute toxic effect to the standard test organisms (*Pimephales promelas, Daphnia magna, and Pseudokirchneriella subcapitata*) were priority substances (alachlor and diuron). Therefore, Schäfer et al. [4] suggested that organic toxicants, especially pesticides, may be underestimated in their importance for the ecological conditions in river systems.

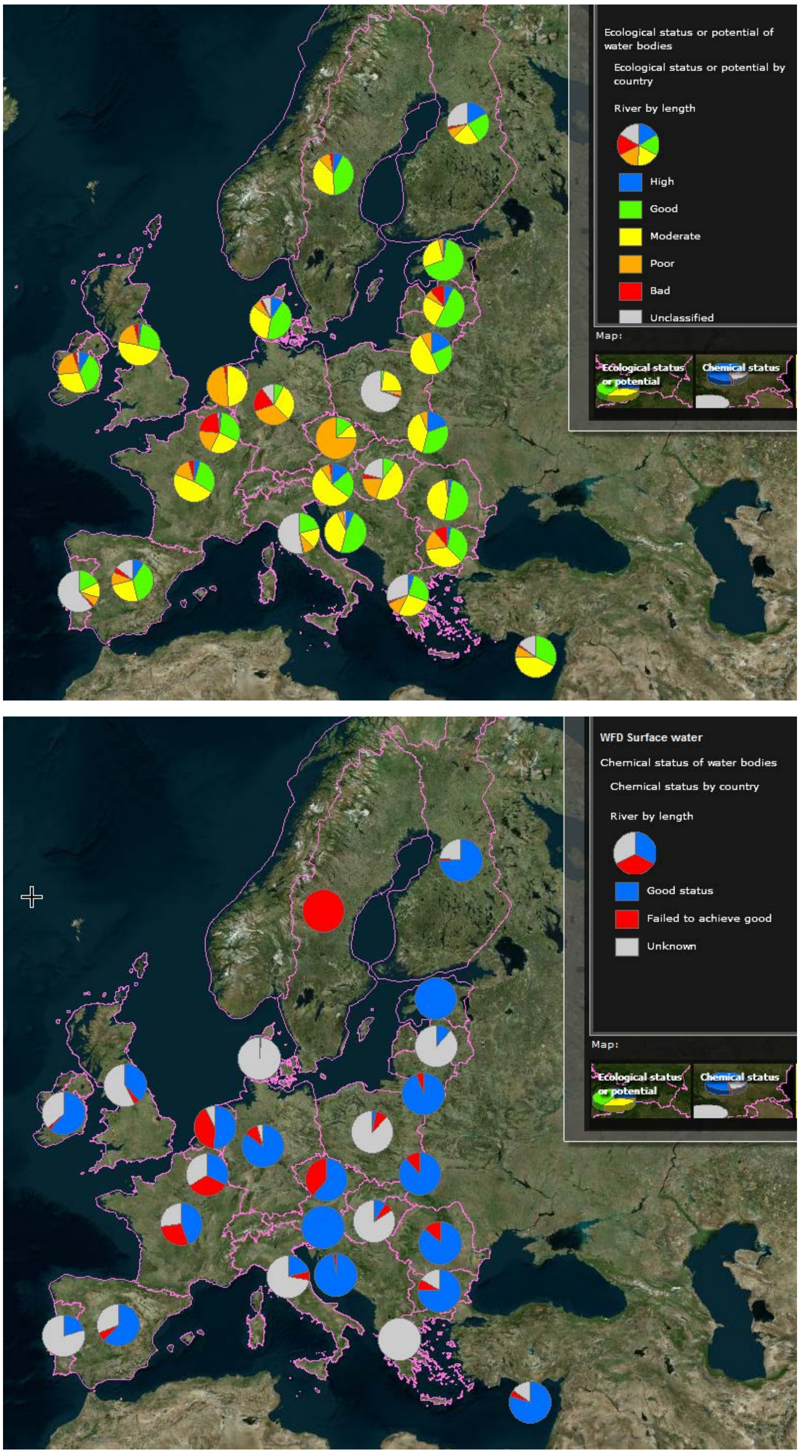

Fig. 1.1: Ecological and chemical status or potential of the European rivers as reported in the RBMPs of 2009. Each segment of pie charts is proportional to the percentage of total river length assigned to the respective class and country (based on EEA [5]).

Additionally, von der Ohe et al. [6] demonstrated that organic substances pollute European rivers more than previously thought. From 2000 to 2008, von der Ohe et al. [6] analysed a dataset of more than 750,000 analytical measurements of 500 organic compounds. For the catchments of four major European rivers, a total of 73 compounds were classified as risks to the environment with two-thirds being pesticides. Likewise, for small European streams (<0.25 m^3/s), Kattwinkel et al. [7] estimated in a modelling study that the macroinvertebrate community in one-third of these streams fails to meet the requirements for good ecological status due to pollution with insecticides.

In summary, pesticide contamination could be an important stressor for aquatic ecosystems and an obstacle for the achievement of a good ecological status of European rivers. To successfully tackle the problem of pesticide contamination, water authorities require a sound knowledge and understanding of the various pesticide sources and suitable mitigation measures. This knowledge would enable these authorities to efficiently target the restricted monitoring capacities and to develop appropriate PoMs.

1.2. Pesticide use and energy crop cultivation in Europe

The use of synthetic pesticides in agricultural crops is the most widespread method to control pests such as weeds, insects and fungal diseases. However, due to their intrinsic properties, pesticides can be harmful to non-target organisms and can be found as contaminants in agricultural soils, ground and surface waters [8-10]. In the EU-27, approximately 270,000 tons of active ingredients were used in or sold to the agricultural sector for crops and seeds in 2010 [11]. The use of pesticides in agriculture varies considerably from year to year, depending on the development of weeds, plant diseases and insect populations, which, in turn, depend on the weather conditions (Fig. 1.2).

The EU sets rules for the sustainable use of pesticides to reduce the risks and impacts of pesticides on people’s health and the environment (Directive 2009/128/EC) [12]. Against this background, there are concerns that the current expansion of energy crop cultivation may counteract the efforts to reduce the dependency on pesticide use [13, 14].

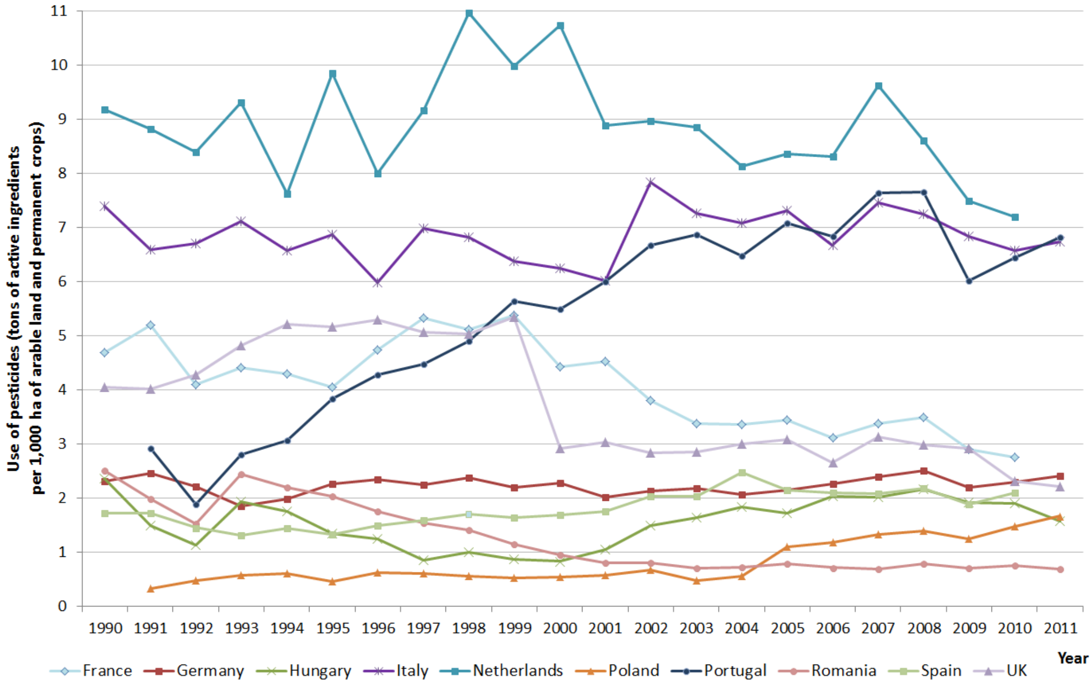

Fig. 1.2: Quantity of pesticides that were used in or sold to the agricultural sector for crops and seeds in selected European countries (selection criteria for countries: total amount of pesticides >5,000 tons in 2010; pesticides: herbicides, insecticides, fungicides, and bactericides; expressed in metric tons of active ingredients; based on FAO [11]).

In the last ten years, the production of biomass on farmland for energy purposes started to become a significant contributor to renewable energy production in Europe. From 2005 to 2008, the European area under cultivation of energy crops increased from 3.5 to 5.5 million ha. This area corresponded to approximately 5% of the arable land and consisted mainly of rapeseed for the production of biodiesel [13, 15]. The sharpest increase of energy crop cultivation was recorded in Germany, from 0.9 million ha in 2000 to 2.1 million ha in 2012 (mainly rapeseed for biodiesel and maize for biogas) [16]. The cultivation of energy crops usually takes place in intensive agricultural production systems, which require, among other things, the application of pesticides. In this respect, main concerns are associated with the increased cultivation of pesticide-intensive energy crops, such as rapeseed and sugar beet, especially when grown in monoculture or on formerly set-aside land or converted grassland.

1.3. Main sources of agricultural pesticide contamination of streams

Pesticide contamination of streams can result from a variety of sources, ranging from diffuse, landscape-level causes such as agricultural runoff to discrete point sources such as wastewater treatment plants (WWTPs) [17-19]. There are a considerable number of studies describing the role of pesticide input from diffuse agricultural sources and the subsequent negative effects on aquatic communities (e.g., decrease in leaf-litter decomposition rates and in the relative abundance and number of sensitive species in the community) [20-22].

In addition, in the last decade, an increasing number of studies detected agricultural pesticides in the effluents of municipal WWTPs [18, 23-26]. Agricultural pesticides that enter WWTPs originate mainly from the filling or cleaning of spraying equipment on farmyards, from which the pesticides are washed off by rain or wash water and then enter the sewage system [18, 24]. However, most of these studies investigated only a small number of WWTPs (one to five), and all of these studies focused exclusively on the detection and quantitative evaluation of these pesticides. What is missing so far is the link between the pesticide input and the potential effects on the aquatic community in the receiving streams.

1.4. Landscape elements as mitigation measures for pesticide contamination

In addition to mitigation options with respect to pesticide handling and application, there are two landscape elements that have been suggested to reduce pesticide exposure and the effects on the aquatic community: riparian buffer strips and forested upstream reaches.

Several studies have demonstrated the effectiveness of riparian buffer strips as suitable measures for reducing diffuse pesticide input from adjacent fields [17, 27-30]. Vegetated buffer strips are designed to remove pesticides, sediment, and nutrients from the surface runoff through processes such as filtration, adsorption, and deposition. However, their effectiveness depends on many factors (e.g., buffer width and slope, soil and vegetation type) and is highly variable [17, 30, 31]. For example, in a vineyard region in southwest Germany, Bereswill et al. [32] identified edge-of-field runoff via the concrete path network and erosion rills as the main pathway for pesticide input into streams. Via this pathway, the runoff was transported rapidly into the stream without significant pesticide reduction. According to Bereswill et al. [32], broad vegetated buffer strips hardly influenced the pesticide in-stream concentrations under these local circumstances.

Furthermore, a considerable amount of literature has been published on the role of macroinvertebrate drift and aerial recolonisation as main processes for the recolonisation of downstream macroinvertebrate communities after pulsed disturbances such as floods or chemical spills [33-37]. For example, several studies have suggested that forested upstream reaches can be potential sources for the recolonisation of macroinvertebrate communities after pesticide contamination [20-22].

1.5. Monitoring of pesticide contamination in streams

The detection of pesticides in streams is expensive, time-consuming and requires a very complex sampling design, making this detection hardly practical especially for large numbers of sampling sites. Discrete samples that were collected for laboratory tests only characterise the condition at the time of sampling; the probability of missing the short pulses of pesticides is high [38]. Furthermore, the wide variety of pesticides and their exceedingly low concentrations make it difficult to cover the entire range in chemical analyses. A promising approach to assess pesticide contamination in streams is the use of bioindicators. Bioindicators allow for the integration of different time periods, can indicate indirect biotic effects of pollutants and are mostly easy and inexpensive to survey [39].

Benthic macroinvertebrates are a widely acknowledged indicator for the health of stream ecosystems and one of the biological quality elements that are used to assess the ecological status according to the WFD [1]. At the beginning of the twentieth century, Kolkwitz and Marsson [40] presented a first practical system for water quality assessment using biological indicators (Saprobic system). Kolkwitz and Marsson [40] observed that the structure of the biological community changes downstream of a major source of organic pollution.

Therefore, they related the occurrence of certain taxa to the degree of organic pollution.

Today, the German Saprobic Index reflects the saprobic condition of a water body and is the core metric of organic pollution within the official German WFD assessment system for macroinvertebrates. The metric value increases with increasing intensity of decomposition of organic material, which is coupled to decreasing oxygen concentrations and thus to a change in the macro-invertebrate community towards species that are more tolerant of low-oxygen conditions [41].

In general, macroinvertebrate species are often stressed by a multitude of natural and anthropogenic factors, which makes it difficult to assess the effects of one specific stressor. A promising approach to solve this problem is the use of species traits (ecological, physiological, and behavioural traits) [42].

For pesticides, Liess and von der Ohe [20] developed the trait-based bioindicator $SPEAR_{pesticides}$ to quantify the effects of insecticidal toxicity of pesticides on macroinvertebrate communities in streams. The index $SPEAR_{pesticides}$ incorporates the physiological sensitivity of macroinvertebrate species to organic toxicants, post-contamination recovery, and the presence of sensitive stages during the main application time of pesticides. Based on these biological traits, the taxa are classified as pesticide-sensitive species (SPEcies At Risk - SPEAR) or pesticide-tolerant species (SPEnotAR). Subsequently, the relative abundance of sensitive taxa ($SPEAR_{pesticides)}$ in the community is calculated as follows [21]:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n} \log(x_i+1) \cdot y}{\sum_{i=1}^{n} \log(x_i+1)} \times 100 \qquad (1.1)$$

where n is the number of taxa; x_i is the abundance of taxon i, and y is: 1 if taxon is classified as SPEAR, otherwise 0.

$SPEAR_{pesticides}$ has been applied successfully in different types of streams and geographical regions [21, 43-45]. Several studies claim that the index is independent of other environmental factors [20, 44, 46].

The second SPEAR index, $SPEAR_{organic}$, was developed to indicate continuous exposure to organic toxicants (e.g., petrochemicals and synthetic surfactants) and is based exclusively on the trait of taxon-specific sensitivity to organic toxicants [47]. $SPEAR_{organic}$ is calculated as the arithmetic mean of species' sensitivities weighted by the log(x+1) transformed abundance of the respective species [47]. The values of species' sensitivities (taxon-specific $S_{organic}$) reflect the taxon-specific sensitivity to organic toxicants in general (for details see [48]). So far, $SPEAR_{organic}$ has been only successfully applied along one large-river continuum in southwestern Siberia.

In the last years, some studies raised points of criticism on the current SPEAR approach [49]. For example, Rubach et al. [50] criticised that the species' sensitivity $S_{organic}$ relies on Daphnia margna as benchmark organism and does not consider the mode of action of the different pesticides. Rasmussen et al. [51] found that $SPEAR_{pesticides}$ responds to characteristics of the physical habitat.

2. Research questions and aim of the thesis

Against the described background, this thesis investigates the effect of pesticides on macroinvertebrate communities by including the main agricultural pesticide sources and landscape elements. The following research questions have been addressed (Fig. 2.1):

Pesticide sources:

(1) Are there significant effects of pesticides from municipal WWTP effluents on the downstream macroinvertebrate community? (Chapter 3)

(2) Will the future expansion of energy crop cultivation cause an increase or decrease in the amount of pesticides that are released into the environment? (Chapter 5)

Landscape elements as mitigation measures:

(3) What is the minimum width of riparian buffer strips that is necessary to significantly reduce the effects of diffuse pesticide input from adjacent fields on the structure of the macroinvertebrate community? (Chapter 4)

(4) How does the number of forested upstream reaches enhance the recovery potential of macroinvertebrate communities at downstream sites that are disturbed by pulsed pesticide contamination? (Chapter 4)

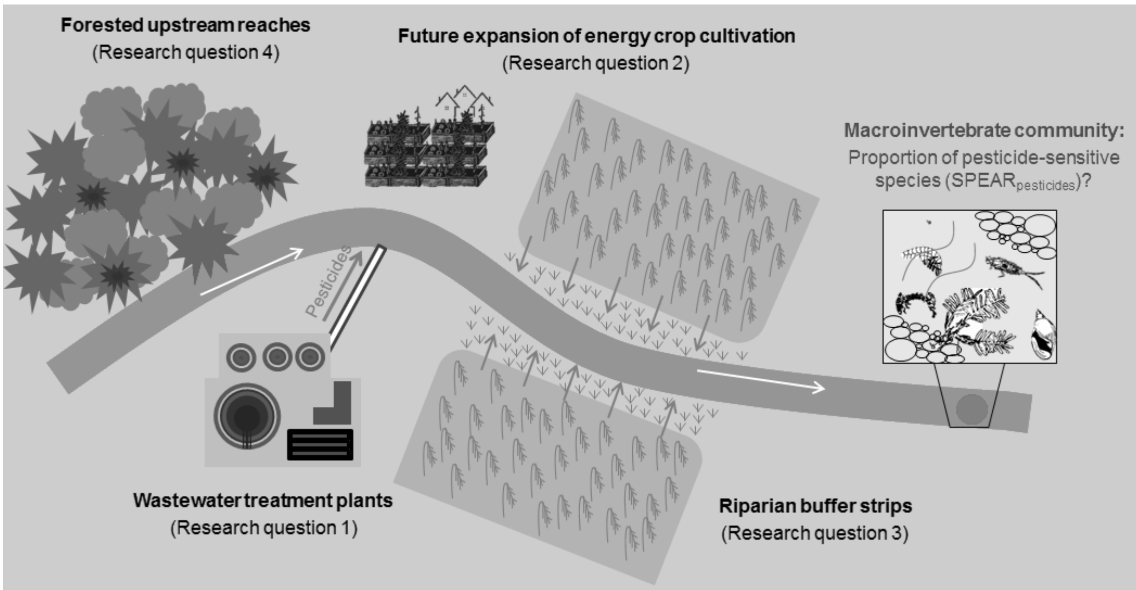

Fig. 2.1: Scheme of the research questions of the thesis.

In the first study, governmental monitoring data from 328 sites in the German Federal State of Hesse were evaluated (Chapter 3). The sites were sampled by independent consultants from March to April 2005 or 2006. The bioindicator $SPEAR_{pesticides}$ was used to detect and quantify the effects of insecticide toxicity of pesticides from municipal WWTP effluents on the downstream macroinvertebrate community. The effluent of WWTPs is often a complex mixture of different oxygen-consuming and/or hazardous organic substances. To enable a better differentiation between the stressors, two additional indices were calculated: $SPEAR_{organic}$ (indicating continuous exposure to organic toxicants) and German Saprobic Index (reflecting oxygen deficiency).

The second study assessed the effects of pesticides on the macroinvertebrate community by including the main agricultural pesticide sources (arable land and municipal WWTPs) and landscape elements (riparian buffer strips, forested upstream reaches) (Chapter 4). Furthermore, a screening approach was developed that allows an initial fast and cost-effective identification of sites potentially affected by pesticide contamination. A total of 663 sampling sites of the governmental WFD monitoring network of the German Federal States of Saxony, Saxony-Anhalt, Thuringia and Hesse were analysed. The sites were sampled by independent consultants from March to June 2005 or 2006. Similar to the first investigation, the bioindicator $SPEAR_{pesticides}$ was used to quantify the effects of insecticidal toxicity of pesticides on the macroinvertebrate community. In addition, a detailed GIS analysis of the different pesticide sources (arable land and garden allotments as well as WWTPs) and landscape elements (riparian buffer strips and forested upstream reaches) was conducted. The GIS-based runoff potential (RP) model was used to assess the potential exposure of sites to runoff-induced pesticide input.

In the third study, the potential of energy crops for pesticide contamination was examined. Based on an analysis of the development of energy crop cultivation in Germany, Europe's leading country in agricultural bioenergy production, and a literature research on perennial energy crops, general conclusions and recommendations were developed for the future large-scale expansion of energy crop cultivation from a phytosanitary point of view (Chapter 5).

3. Effects of organic pollutants from wastewater treatment plants on aquatic invertebrate communities

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Water Research (2013), 47 (2), Pages 597-606

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3.1. Abstract

Pesticides are a major stressor for stream ecosystem health. They enter surface waters from diffuse agricultural sources but also from point sources such as municipal wastewater treatment plants (WWTPs). However, to date, no studies have focused on the ecological effects of pesticide-contaminated WWTP effluent on macroinvertebrate communities. On the basis of governmental monitoring data of 328 sites in Hesse, Germany, we identified insecticidal long-term effects on the structure of the macroinvertebrate community up to 3 km downstream of WWTPs. The effects were quantified using the trait-based $SPEAR_{pesticides}$ index, which has been shown to be an effective tool for identifying community effects of pesticide contamination. In addition, based on the German Saprobic Index, we revealed that WWTPs are still an important source of oxygen-depleting organic pollution, despite the extensive technological improvements in wastewater management over several centuries. In general, our findings emphasize the need to take municipal WWTPs into consideration in the management of river basins under the EU Water Framework Directive to achieve good ecological and chemical status for European streams and rivers.

3.2. Introduction

The adoption of the EU Water Framework Directive (WFD; Directive 2000/60/EC) in 2000 marked the beginning of a new era of European water policy, with aquatic ecosystems becoming the central focus for water management [1]. The aim of the WFD is not only to achieve good chemical status but also good ecological status for all water bodies by 2015.

However, only few years before this deadline, in Germany, for example, about 90% of rivers and streams are still at risk of failing to meet this objective [52]. To date, hydromorphological degradation and lack of river continuity have been considered as the most common features that result in classification into an inferior status class [53]. However, Kattwinkel et al. [7] estimated recently that pollution with insecticides probably prevents invertebrate communities in one-third of small European streams (<0.25 m^3/s) from meeting the requirements for a good ecological status.

To develop an effective strategy to reduce pesticide contamination, it is important to evaluate the various sources of pesticide input into surface waters. A considerable number of investigations have been published on pesticide input from diffuse agricultural sources and the subsequent negative effects on aquatic communities [20-22].

In addition to this, over the last decade, an increasing number of studies have emphasized the importance of WWTPs as point sources of pesticides by their detection of not only significant amounts of herbicides but also insecticides and fungicides [18, 23-26]. However, most of these previous studies investigated only a small number of WWTPs (one to five) and all of them focused exclusively on the detection and quantitative evaluation of pesticides. To date, no study has linked the pesticide input from WWTP effluent to benthic macroinvertebrate communities in the receiving streams.

According to the WFD, benthic macroinvertebrates are one of the elements that reflect biological quality and should be used to assess ecological status; they are also a widely acknowledged indicator of the health of stream ecosystems. Macroinvertebrate species are often stressed by a multitude of natural and anthropogenic factors, which makes it difficult to assess the effects of one specific stressor. A promising approach to solve this problem is the use of species traits (ecological, physiological and behavioural traits) [42]. Recently, the trait-based bioindicator system SPEAR (SPEcies At Risk) was developed which calculates the fraction of species sensitive to a specific type of contaminant (e.g., pesticides and general organic toxicants) in the macroinvertebrate community [20]. In the case of pesticides, the $SPEAR_{pesticides}$ index has been shown to be an effective tool for the evaluation of effects of pesticides (insecticidal toxicity) on macroinvertebrates in streams [46].

Against this background, the aim of the study reported herein was to evaluate the long-term insecticidal effects of WWTP effluent on the downstream macroinvertebrate community using governmental monitoring data from 328 sites in the Federal State of Hesse, Germany. In addition, we investigated the on-site quality of the physical habitat as a confounding factor that potentially alters the effects of pesticides on the macroinvertebrate community. Furthermore, we investigated the effect of organic pollution to verify the statement of German water authorities that nowadays organic pollution from WWTPs only rarely poses a problem due to the extensive technical improvements in wastewater management over several centuries [52].

3.3. Material and Methods

3.3.1. Study area

The sampling sites are situated in the German Federal State of Hesse, which is located in the centre of Germany (Fig. 3.1). Hesse is characterized by low mountain ranges with an elevation up to 950 m and consists mainly of richly wooded uplands. Besides forest (approximately 40% of the total area), agriculture is the most common land use (approximately 43% of the total area, consisting of 27% arable land and 16% permanent grassland).

The Hessian River Basin Analysis 2004 identified hydromorphological degradation, high nutrients level, and pesticide contamination as the main stressors in Hessian streams [54]. In Hesse, approximately 90% of the farms are connected to the municipal wastewater treatment system, which includes approximately 740 WWTPs. The majority of the sewer network consists of a combined sewer system that collects stormwater and domestic wastewater in a single pipe system and then directs it to the next WWTP. During intensive rainfall, these combined sewer systems discharge excess untreated water, via overflows, directly into streams [55].

3.3.2. Macroinvertebrate data and biological indices

In 2005 and 2006, the Hessian State Office for Environment and Geology (HLUG) commissioned independent consultants to undertake an extensive operational WFD monitoring to verify the results of the Hessian River Basin Analysis and to support the development of the river basin management plan by 2009.

For the present study, we selected a total of 328 sampling sites from this dataset on macroinvertebrates provided by HLUG. We applied the following criteria to obtain a relatively harmonized dataset. First, the sites were situated at siliceous streams in the lower mountain range, by far the most common stream type in Hesse (two-third of the WFD-relevant watercourse length of Hesse). Second, the stream sites were characterized by stream width smaller than 10 m (1-5 m: 88% of sites; 5-10 m: 12% of sites). Third, the sampling of the community took place before the main period of application of insecticides and fungicides in Germany (which occurs from May to July) to reflect possible long-term effects from the previous year. The majority of the sites were sampled in March or April 2005, and were supplemented by 24 sites sampled in March or April 2006. Finally, the sites were at a minimum of 1,500 m from the stream source.

Some of the sampling sites were located at 100-500 m intervals from each other at the same stream which could potentially produce a pseudoreplication effect, due to the spatial correlation in structural and biological conditions. To minimise the potential for pseudoreplication, while also retaining as much information as possible, we decided on a compromise. We chose one of the sites at random if the following criteria were met: structural quality class differs to a maximum of one class, similar land uses in the vicinity of the sampling site, and no tributary joins the stream between these sites. Otherwise, we kept both sides in the dataset.

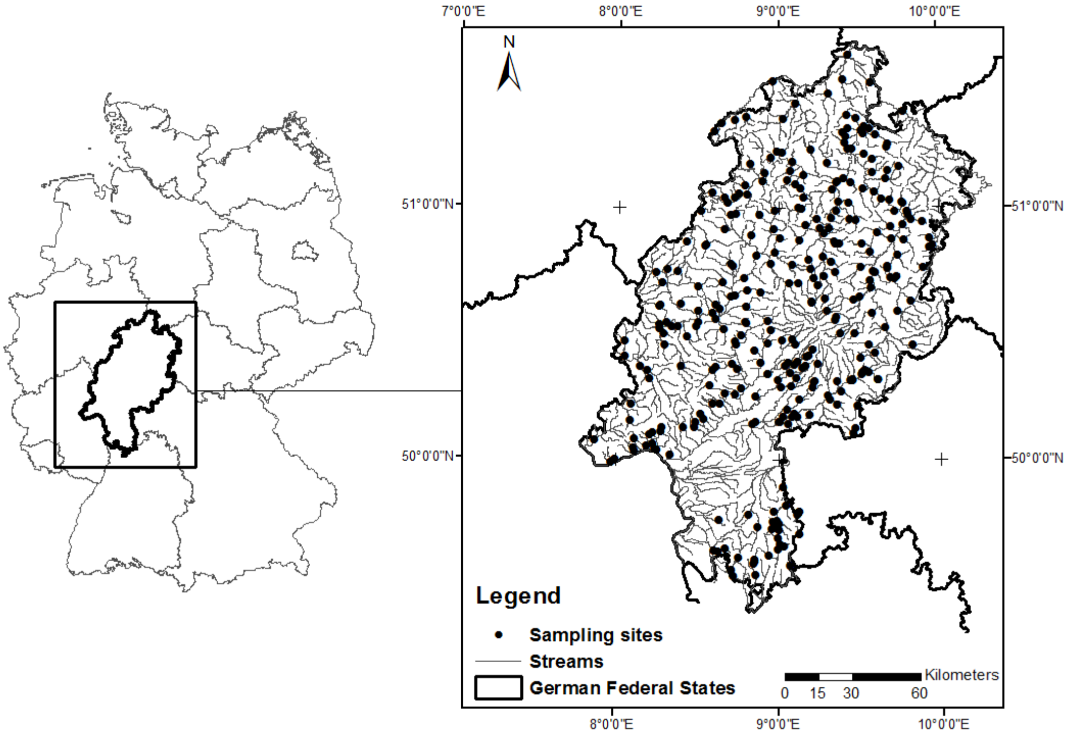

Fig. 3.1: Location of the study area (Federal State of Hesse) in Germany and distribution of the 328 investigated sampling sites.

We used the index $SPEAR_{pesticides}$ to detect and quantify the effects of agricultural pesticides (insecticide toxicity) on the macroinvertebrate community. In this study, we considered the insecticidal effects of insecticides and fungicides. Herbicides were not taken into account due to their generally low toxicity on macroinvertebrates. Insecticides and fungicides are in general applied on a seasonal basis. Thus, $SPEAR_{pesticides}$ not only considers the physiological sensitivity of the species in relation to organic toxicants but also includes biological traits that are known to influence post-contamination recovery (generation time and migration ability). Furthermore, it takes into account whether the species is present in the water during the main application period to determine likelihood of exposure.

Based on the ecological and physiological traits, the identified taxa were classified according to their vulnerability towards pesticides into sensitive species (SPEAR) and tolerant species (SPEnotAR). Afterwards, the relative abundance of sensitive taxa in the community ($SPEAR_{pesticides}$) was calculated for each site:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n} \log(x_i+1) \cdot y}{\sum_{i=1}^{n} \log(x_i+1)} \cdot 100 \quad (3.1)$$

where *n* is the number of taxa; x_i is the abundance of taxon *i*, and *y* is: 1 if taxon is classified as SPEAR, otherwise 0.

Increasing values of the index $SPEAR_{pesticides}$ indicate increasing proportion of pesticide-sensitive species; showing decreasing insecticidal effects of pesticides at a given site. To facilitate the inclusion of the $SPEAR_{pesticides}$ index into the monitoring programmes of the WFD, Beketov et al. [56] proposed boundaries of ecological status classes for small European streams which were also found to be valid for medium-size and large rivers (at least between High ($SPEAR_{pesticides}$=44%), Good ($SPEAR_{pesticides}$=33%), and Moderate ($SPEAR_{pesticides}$=22%)) [44].

$SPEAR_{pesticides}$ has been applied successfully in different geographical regions to specifically indicate the insecticidal effects of pesticides on macroinvertebrate communities while being non-responsive to other environmental factors [20-22]. Furthermore, Schletterer et al. [44] showed that $SPEAR_{pesticides}$ is independent from channel width and related river longitudinal factors and is potentially applicable across different types of watercourses.

As a second SPEAR index, we calculated $SPEAR_{organic}$, which indicates continuous exposure to organic toxicants (e.g., petrochemicals and synthetic surfactants) and is based exclusively on the trait of taxon-specific sensitivity to organic toxicants [47]. The higher the value of $SPEAR_{organic}$, the higher the proportion of species that are sensitive to continuously present organic toxicants.

Both SPEAR indices were calculated using the SPEAR Calculator (available online at http://www.systemecology.eu/SPEAR/index.php).

In recent decades, considerable effort has been expended on the technical improvement of German WWTPs to reduce the deterioration of many streams due to organic pollution. Recently, the German water authorities have stated that degradable substances from WWTP effluent only rarely pose a problem for the health of stream ecosystems [52]. To assess this statement, we calculated the German Saprobic Index by using the software Asterics 3.3.1 (available for download at http://www.fliessgewaesserbewertung.de). The German Saprobic

Index reflects the saprobic condition of a water body and is the core metric of organic pollution within the official German WFD assessment system for macroinvertebrates. The metric value increases with increasing intensity of decomposition of organic material, which is coupled to decreasing oxygen concentrations and thus to a change in the macroinvertebrate community towards species that are more tolerant of low-oxygen conditions [41].

3.3.3. Diffuse agricultural pollution

To elucidate the relative contributions of WWTPs as point sources and of diffuse agricultural sources to the overall level of pesticide contamination, we used a GIS-based runoff model. The model enables sampling sites to be ranked on the basis of their potential exposure to runoff-induced pesticide input from adjacent arable land. This runoff potential (RP) model [22] represents a simplified version of a model by the Organisation for Economic Co-operation and Development [57].

As a first step, the dissolved amount of a generic substance that potentially reaches a stream site (gLOAD) was calculated for each rainfall event during the main period of insecticide and fungicide application (from May to July) as follows:

$$gLOAD = \sum_{i=1}^{n} A_i \cdot D_{generic} \cdot \left(1 - \frac{I_i}{100}\right) \cdot \frac{1}{1+\frac{K_{OC,generic} \cdot OC_i}{100}} \cdot f(s_i) \cdot \frac{f(P_i,T_i)}{P_i} \qquad (3.2)$$

It is assumed that the loss of the generic substance results from a single application in the vicinity of the stream, which is defined as a two-sided 100-m stream corridor that extends for 1,500 m upstream of the site (including all branches and tributaries) [22]. A short description of the factors and their parameterization can be found in Table 3.1.

Table 3.1: Parameters for the calculation of gLOAD (Eq. 3.2) (index *i* refers to different polygons of arable land within one stream corridor).

Factor	Description	Parameterization and data source
A_i	Size of arable polygon within stream corridor (in ha)	Shapefile from ATKIS database (scale 1:25,000), provided by the German Federal Agency for Cartography and Geodesy
$D_{generic}$	Rate of application of generic substance	No data, therefore set to a constant value of 1 g/ha for all crops
$K_{OC,generic}$	Soil organic carbon sorption coefficient of the generic compound	No data, therefore set to a constant value of 100, which represents a highly mobile compound
OC_i	Organic soil carbon content of polygon (in %)	Shapefile of organic matter in topsoil in Germany (scale 1:1,000,000), calculated as 58% of humus content, provided by the German Federal Institute for Geosciences and Natural Resources
I_i	Crop- and growth phase-specific plant interception at the time of the rainfall event (in %)	Average from crop statistics per administrative district, assumed to be distributed uniformly in the district, provided by the Statistical Office of Hesse; interception values were assigned to all present crop types modified after Linders et al. [58]
s_i	Mean slope of polygon (in %)	Shapefile created by using Slope function in ArcGIS 10, based on Digitalized Elevation Map (DEM), provided by the German Federal Institute for Geosciences and Natural Resources
$f(s_i)$	Influence of slope	according to OECD [57] if $s_i \leq 20\% = 0.00143 * s_i^2 + 0.02153 * s_i$ if $s_i > 20\% = 1$
P_i	Precipitation level (in mm)	Daily recorded precipitation from May to July 2004 or 2005, assumed to result from one rainfall event, interpolated from relevant weather stations of Germany's National Meteorological Service (DWD)
T_i	Soil texture of polygon (sandy or loamy)	Shapefile of soil map of Germany (BUEK 1000, scale 1,000,000), provided by the German Federal Institute for Geosciences and Natural Resources; decision on sandy or loamy based on sand and clay content of the soil type
$f(P_i,T_i)$	Volume of surface	specified according to OECD [57]

runoff (in mm)	if T_i=sandy:
	 $-5.86*10^{-6}*P_i^3+2.63*10^{-3}*P_i^2-1.14*10^{-2}*P_i-1.164*10^{-2}$
	 if T_i=loamy:
	 $-9.04*10^{-6}*P_i^3+4.04*10^{-3}*P_i^2+4.16*10^{-3}*P_i-6.11*10^{-2}$

The RP of a stream site is then calculated as:

$$RP = \log_{10}(max_{i=1}^{n}(gLOAD_i)) \qquad (3.3)$$

where *n* is the number of rainfall events that occur during the main period of insecticide and fungicide application, $gLOAD_i$ is the amount of a generic substance that potentially reaches a stream site during rainfall event *i* as given in Eq. (3.2).

According to Kattwinkel et al. [7], the generic indicator RP was classified into five classes (from RP≤-3: very low, to RP>0: very high).

The RP model was developed to reflect the vulnerability of sites to potential runoff exposure to a variety of substances. Therefore, a set of generalized compound characteristics was used instead of specific compound properties or use patterns (e.g., constant application rate of 1 g/ha, constant soil organic carbon sorption coefficient of 100, and no degradation of the substance) (Tab. 3.1). Owing to these simplifications, values of runoff losses represent relative predictions of potential runoff inputs.

Schriever et al. [45] showed a significant positive correlation between predicted RP and measured pesticide runoff losses for small streams (width <5 m) draining arable land in the region of Braunschweig, Germany (1998: R^2=0.6, p<0.01, n=10; 1999: R^2=0.72, p<0.001, n=12; 2000: R^2=0.77, p<0.001, n=13). In addition, Schriever et al. [22] established a spatial link between RP and long-term shifts in the composition of macroinvertebrate communities ($SPEAR_{pesticides}$). The RP model has furthermore been supported by studies comparing RP estimations with $SPEAR_{pesticides}$ values calculated from field data in France, Finland, and Australia [46, 59].

3.3.5. Wastewater treatment plants

A shapefile of the Hessian WWTPs was provided by the Hessian State Office for Environment and Geology (status as of 2007). In addition, the data were cross-checked with the ATKIS shapefile for the category of “WWTPs” and aerial pictures provided by the Federal Agency for Cartography and Geodesy (www.geodatenzentrum.de). The distance between each sampling site and the closest WWTP situated upstream was determined using ArcGIS 10 on the basis of the HLUG shapefile. The variable “upstream distance to next WWTP” (distance between the sampling site and the closest WWTP situated upstream of the respective sampling site) represented a categorical variable (five groups: “<1.5 km”, “1.5–3 km”, “3–5 km”, “5–10 km”, and “>10 km”).

Furthermore, we included information regarding the sizes of the WWTPs (Population Equivalent, PE) and treatment steps of the WWTPs in the analysis. The data was derived from the regular report on “Removal of municipal wastewater in Hesse” published by the Hessian Ministry for Environment, Energy, Agriculture and Consumer Protection [55]. The capacities of the 59 investigated WWTPs (situated <3 km upstream of site) were as follows: 12 WWTPs <1,000 PE, 27 WWTPs ≥1,000 to 5,000 PE, 6 WWTPs ≥5,000 to 10,000 PE, and 14 WWTPs ≥10,000 to 50,000 PE. Of the 59 WWTPs, 32% used only a biological treatment, 19% used both biological treatment combined with nitrogen removal (nitrification) and a further 22% of the WWTPs used nitrification/denitrification methods for nitrogen removal in conjunction with biological treatments. Another 27% of WWTPs had biological treatment as well as nitrogen (nitrification/denitrification) and phosphorus removal. Due to missing data, it was not possible to investigate other relevant parameters like number of farms connected to the WWTP, proportion of the WWTP effluent compared to the water load of the receiving stream or proportion of the combined sewage system.

3.3.6. Hydromorphological degradation

Hydromorphological degradation in the streams was quantified using a shapefile of the structural quality classes of Hessian streams provided by the Hessian State Office for Environment and Geology. The assessment of the structural quality classes took place in the years 1998 and 1999 and was carried out in 100-m stream sections in accordance with the on-site method as described elsewhere [60]. More recent data was not available at the time of this study. It can be assumed that the structural quality of Hessian streams remained relatively constant until 2006 (Asmis [61], personal communication).

The quality of the water body structure is given as its deviation from the potential natural state using a seven-point scale (from 1: unchanged, to 7: completely changed). The overall structural quality class is the average of the six main hydromorphological parameters (course development, longitudinal profile, cross profile, bed structure, bank structure, and area surrounding the water body).

3.3.7. Differentiation between various types of stressors

The effluent of WWTPs is often a complex mixture of different types of oxygen-consuming and/or hazardous organic substances. Consequently, downstream of a WWTP, macroinvertebrate species may be influenced by more than one stressor. For example, one species may be sensitive to pesticides and low-oxygen conditions (e.g., *Drusus annulatus*) or tolerant to both (e.g., *Erpobdella octoculata*). Therefore, a clear differentiation between the two stressors oxygen deficiency and toxicants (pesticides and general organic toxicants) can only be done based on taxa defined as being sensitive exclusively to one of the two stressors.

We addressed this problem by conducting a subsequent analysis with two adapted taxon lists. First, we elaborated the effects of toxicants (pesticides and general organic toxicants) and therefore considered only taxa not sensitive to oxygen deficiency. Approximately 80% of the sites investigated had a German Saprobic Index of less than 2, that is, they have good saprobic water quality according to the German WFD classification system (Fig. 3.2). Therefore, all 157 taxa with a German Saprobic Value of less than 2 were defined as taxa sensitive to low-oxygen conditions and excluded from the original sampling taxon list (total of 442 taxa). In addition, 167 taxa with an unknown German Saprobic Value were excluded. The shortened taxon list (total of 118 taxa) was used to recalculate the indices $SPEAR_{pesticides}$ and $SPEAR_{organic}$. In a second analysis, all 189 taxa sensitive to organic toxicants [according to the SPEAR approach [20], median of sensitivity to organic toxicants >-0.36] were excluded from the original taxon list to emphasize the effects of oxygen deficiency. This second shortened taxon list (total of 253 taxa) was used to recalculate the German Saprobic Index.

3.3.8. Statistics

For the comparison of two groups of sites, we used Student's t-test, Welch's t-test or Wilcoxon rank-sum test, depending on the normality or homogeneity of variances of the data.

For the comparison of several groups of sites, we conducted Kruskal–Wallis one-way analysis of variance, analysis of covariance (ANCOVA) or pairwise t-tests, depending on the normality or homogeneity of variances of the data. For non-normally distributed data, Kruskal–Wallis one-way analysis of variance was used to test whether one of the observed groups was different from at least one of the others. When the obtained value of a Kruskal–Wallis test was significant at the level of 0.05, a non-parametric multiple-comparison test was used to determine which groups were different by pairwise comparisons that were adjusted appropriately (R-package pgirmess, function kruskalmc, http://giraudoux.pagesperso-orange.fr/#pgirmess). In case of normally distributed data with homogeneous variances, analysis of covariance (ANCOVA) was performed. Pairwise t-tests were applied for data with a normal distribution but unequal variance (adjustment method for multiple testing: Holm's sequential Bonferroni). All tests were conducted using R version 2.14.1.

We assessed the correlation between $SPEAR_{pesticides}$ and RP by calculating the Pearson product-moment correlation coefficient r. The influences of the categorical variables "upstream distance to next WWTP" and "structural quality class" and the continuous variable "RP" on $SPEAR_{pesticides}$ were investigated using ANCOVA. We simplified the models by stepwise removal of non-significant terms until the minimal adequate model was reached.

3.4. Results and discussion

3.4.1. No significant insecticidal effects of diffuse agricultural sources of pesticides detected

We assessed the relevance of pesticide contamination from diffuse agricultural sources by calculating the RP at all 328 sampling sites. The analysis of the ATKIS land use data revealed that meadow or pasture dominated the 1.5-km corridors upstream of the sites (on average, approximately 40% grassland compared with approximately 16% arable land). Only 27 of the 328 investigated sites were characterized by more than 50% arable land in the respective upstream corridors.

The predicted runoff exposure of the 328 sites ranged from no RP (20% of sites) to a maximum RP of -0.5 (Fig. 3.2). The majority of the sites (72%) were characterized by a very low to medium RP (≤-1), whereas high RP values (>-1) were assigned to only 8% of the sites (Fig 3.2). According to ecological status classes of Beketov et al. (2009), most of the investigated sites had high (>44%), good (33-44%) or moderate values of $SPEAR_{pesticides}$ (22–33%), and only 26% were classified as poor or bad ($SPEAR_{pesticides}$ <22%) (Fig. 3.2). The spatial distribution of the results is presented in Appendix A1.

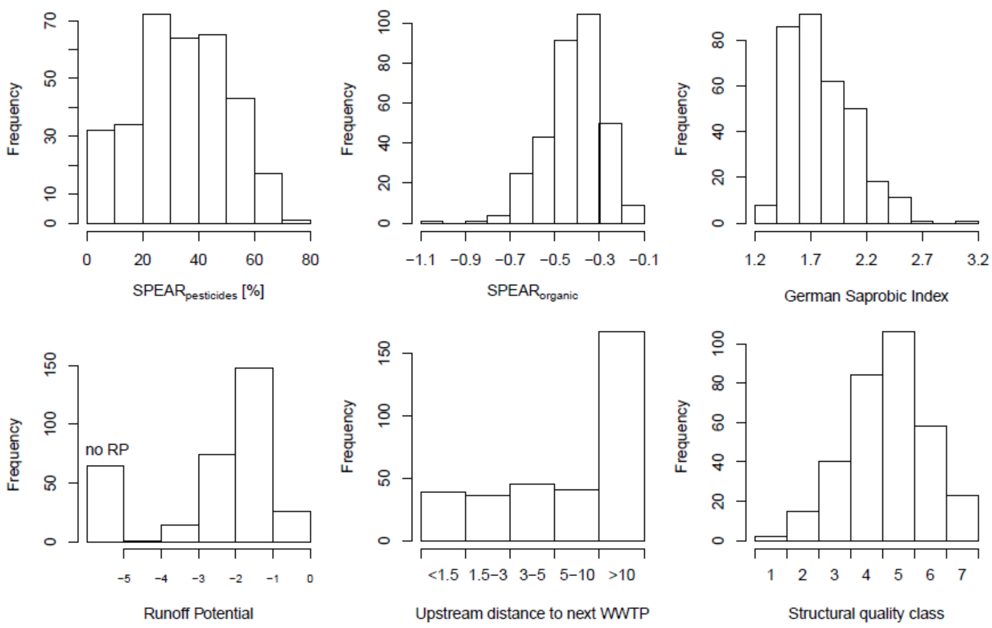

Fig. 3.2: Histograms of the three macroinvertebrate indices ($SPEAR_{pesticides}$, $SPEAR_{organic}$ and German Saprobic Index) and the investigated variables (“Runoff Potential“, “upstream distance to next WWTP”, and “structural quality class”) for all 328 sampling sites.

Comparison of the values of $SPEAR_{pesticides}$ with the corresponding RP values showed no significant negative correlation between them ($r=-0.11$, $p>0.05$). For comparison, Schriever et al. [22] found a significant decrease in median values of $SPEAR_{pesticides}$ for sites with RP>-3 as compared with sites with RP≤-3.

Also the ANCOVA showed that in contrast to the variables “upstream distance to next WWTP” and the “structural quality class” ($p<0.001$), “RP” had only a marginal effect on the index $SPEAR_{pesticides}$ ($p=0.09$). Furthermore, there was no significant interaction between the three variables, which indicated that these variables showed only additive effects.

These findings suggested that surface runoff from adjacent arable land is no major source of pesticide at the investigated sites. A likely explanation for this is the scarcity of adjacent arable land and the frequent occurrence of riparian buffer strips. By including a minimum width for buffer strips in the RP model, Rasmussen et al. [28] were able to increase the explanatory power of the model from 46% to 64%. However, the efficiency of buffer strips is still under discussion. While Rasmussen et al. [28] pointed out the importance of riparian buffer strips for reducing surface runoff from adjacent arable land, Bereswill et al. [32] identified field paths and erosion rills as main pathways for pesticide loss. However, owing to the relatively low proportion of arable land in the upstream corridors in the present study, we decided not to undertake a detailed investigation of the buffer strips.

Another possible explanation for the lack of pesticide effects originating from diffuse agricultural sources is that the macroinvertebrate data were sampled before the main period of application of insecticide and fungicide. Thus, the species had several months to recover from possible effects from the previous year. In line with this hypothesis, Liess and von der Ohe [20] found a lower level of dependence of $SPEAR_{pesticides}$ on measured pesticide contamination [in toxic units (TU), based on acute (48-h) LC50 of *D. magna*] for the data for April, before contamination, compared with that for the data from May and June, directly after contamination with insecticides and fungicides.

3.4.2. Hydromorphological degradation needs to be considered

We investigated the interacting effects of hydromorphological degradation and pesticide contamination on the macroinvertebrate community by analysing the relationship between the overall structural quality class of a given site and its potential exposure to pesticide. Given that the RP results revealed no major influence of pesticide contamination from diffuse sources on $SPEAR_{pesticides}$, we regarded WWTPs as the dominant source of pesticide in our dataset. A preliminary analysis of the 328 sites revealed significantly lower values of $SPEAR_{pesticides}$ for sites with a WWTP within a distance of 3 km upstream than at sites with no WWTP within 3 km upstream (median values of $SPEAR_{pesticides}$: WWTP<3 km: 22%; WWTP≥3 km: 39%, Wilcoxon rank-sum test: $p<0.001$). Consequently, we grouped the 328 sites into sites with or without potential pesticide contamination on the basis of the presence of a WWTP within a distance of 3 km upstream.

The analysis of the relationship between overall structural quality class and $SPEAR_{pesticides}$ showed that, for both groups, the median of $SPEAR_{pesticides}$ decreased with increasing hydromorphological degradation of the stream site (Fig. 3.3a). Furthermore, sites with a structural quality class of 3, 4, 5 or 6 had a significantly lower value for $SPEAR_{pesticides}$ when there was a WWTP within 3 km upstream (grey boxes). Subdivision of the sites into those that were unchanged to clearly changed (1–5) and those that were strongly to completely changed (6–7) revealed significantly lower values of $SPEAR_{pesticides}$ for sites with the higher levels of degradation (Wilcoxon rank-sum test: $p<0.001$, Fig. 3.3b), regardless of whether or not there was a potential source of pesticide contamination present. Within these two groups, the value of $SPEAR_{pesticides}$ was higher at sites without a WWTP than at sites with a WWTP within 3 km upstream.

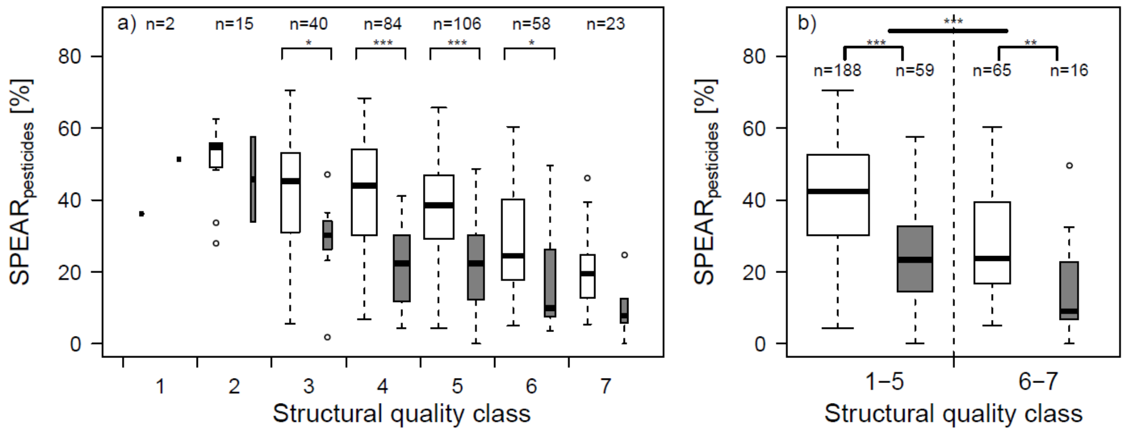

Fig. 3.3: a) Relationship between overall structural quality class and SPEAR$_{pesticides}$ for all 328 sampling sites, which were grouped into 253 sites without (white) and 75 sites with a WWTP within 3 km upstream (grey). Asterisks indicate significant differences between sites with or without WWTP per overall structural quality class (Student's t-tests: *p<0.05, ***p<0.001).

b) Boxplot of overall structural quality class and SPEAR$_{pesticides}$ for all 328 sampling sites, which were subdivided as follows: sites with overall structural quality class from 1 to 5, and from 6 to 7. Asterisks indicate significant differences between sites with or without WWTP per overall structural quality class group of 1 to 5, and 6 to 7 (Student's t-tests or Wilcoxon rank-sum test: *p<0.05, **p<0.01, ***p<0.001).

A possible explanation for the significantly lower values of SPEAR$_{pesticides}$ for sites with a strongly or completely altered structural quality class might be that many Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa are considered sensitive to insecticides and fungicides according to the SPEAR approach. Many EPT taxa have relatively strict habitat requirements and thus are vulnerable to the loss of hydromorphological and habitat heterogeneity [62]. Also Rasmussen et al. [63] found that SPEAR$_{pesticides}$ responds to characteristics of the physical habitat, mainly due to the decrease in abundance and species richness of EPT taxa. In general, other macroinvertebrate indices, such as EPT index, Average Score per Taxon, and German Saprobic Index, are also correlated to some extent with hydromorphological degradation [64].

Taken together, our findings and those of previous studies demonstrate the need to obtain a better understanding of the interaction of the quality of the physical habitat with pesticide contamination, and the influence of this interaction on the indicator $SPEAR_{pesticides}$. For the time being, we recommend that sites with extremely degraded hydromorphological status (e.g., straight artificial stream beds, pipe culverts or concrete channels with extensive embankment consolidation) should not be considered in calculations of $SPEAR_{pesticides}$ to avoid overestimation of the toxic impact of pesticides.

3.4.3. Insecticidal effects of WWTP effluent cause long-term change in macroinvertebrate community structure

To avoid overestimation of the pesticide contamination due to significant effects of hydromorphological degradation, we excluded all sites with a structural quality class of 6 or 7 from the subsequent analysis. We evaluated the dataset of the remaining 247 sampling sites to investigate how $SPEAR_{pesticides}$ was related to the upstream distance to the next WWTP (Fig. 3.4a, left).

As indicated already by the preliminary analysis of all 328 sites, the values for $SPEAR_{pesticides}$ increase with increasing upstream distance to the next WWTP (Fig. 3.4a, left). There was a significant difference between sites with a WWTP within 1.5 km upstream and sites with a WWTP within 1.5-3 km upstream (pairwise t-tests: $p<0.01$). Furthermore, both, sites with a WWTP within 1.5 km upstream and sites with a WWTP within 1.5-3 km upstream, were significant different from sites with a WWTP in 3-5, 5-10, or >10 km (pairwise t-tests: $p<0.01$). The grouping of sites with and without a WWTP in 3 km upstream revealed a significant reduction of species classified as SPEAR at sites with a WWTP within 3 km upstream as compared with sites without a WWTP (Fig. 3.4a, middle, Student's t-test: $p<0.001$).

A total of 75% of the sites with a WWTP within 3 km upstream had $SPEAR_{pesticides}$ values lower than 33%, which indicated a moderate to bad ecological quality class according to the ecological status class boundaries proposed by Beketov et al. [56]. In contrast, only 31% of the sites with no WWTP within 3 km upstream had a $SPEAR_{pesticides}$ value lower than 33%.

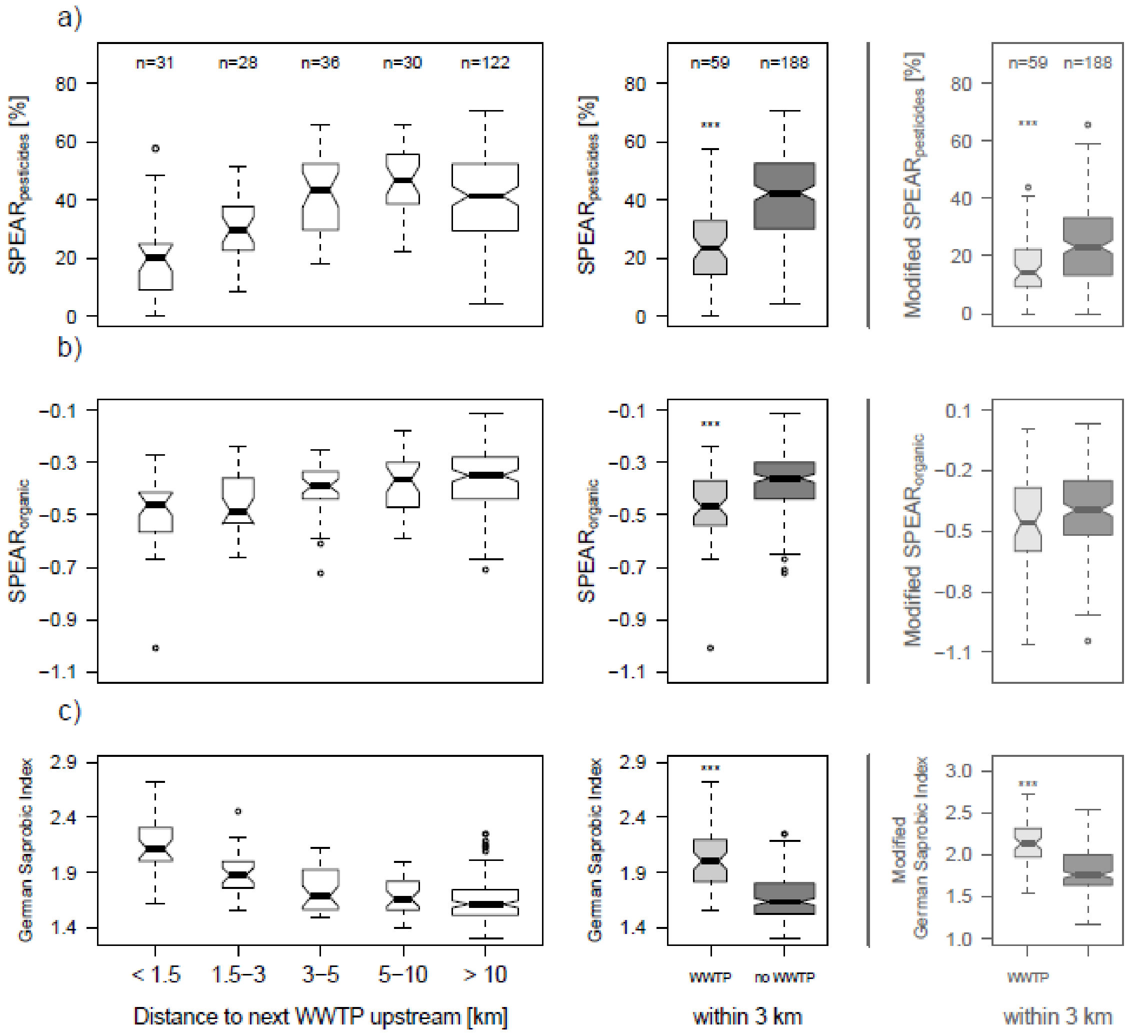

Fig. 3.4: Relationship between a) SPEAR$_{pesticides}$, b) SPEAR$_{organic}$, and c) the German Saprobic Index and the upstream distance to the next WWTP for the 247 sampling sites (overall structural quality class 1–5). The grey-shaded figures (right) show the values of the three modified indices. For the recalculation of SPEAR$_{pesticides}$ and SPEAR$_{organic}$, all taxa with a German Saprobic Value of less than 2 or an unknown German Saprobic Value were excluded from the original taxon list. For the recalculation of the German Saprobic Index, all taxa that were sensitive to organic toxicants (according to the SPEAR approach) were excluded from the underlying taxon list. Asterisks indicate significantly different groups (Student's t-test or Wilcoxon rank-sum test: ***p<0.001).

After the exclusion of the taxa that were potentially sensitive to oxygen deficiency from the original taxon list, values of SPEAR$_{pesticides}$ were still significantly lower for sites with a WWTP within 3 km upstream (Fig. 3.4a right, Wilcoxon rank-sum test: p<0.001).

These findings indicated the long-term change in structure of the macroinvertebrate communities due to insecticidal effects of the WWTP effluent. It is therefore likely that insecticides and fungicides either are not degraded completely in WWTPs or are discharged from the combined sewer system as part of the untreated wastewater that is released during periods of heavy rainfall. Several studies have revealed that combined sewer overflows can be a significant source of organic pollutants for the water systems, especially for substances that are normally removed at high rates by WWTPs [65, 66].

Based on the original taxon list, $SPEAR_{organic}$ was significantly lower at sites with a WWTP within 3 km upstream (Fig. 3.4b, left and middle, Wilcoxon rank-sum test: $p<0.001$). However, when all species that were sensitive to oxygen deficiency were removed, there was no longer a significant difference in $SPEAR_{organic}$ between sites with or without a WWTP (Fig. 3.4b, right). Therefore, in contrast to the seasonal occurrence of agricultural insecticides and fungicides, the continuous exposure to organic toxicants seems to play no important role at the investigated sites. However, this result needs to be interpreted with caution. So far, $SPEAR_{organic}$ has been successfully applied along one large-scale river continuum in southwestern Siberia [47]. Further studies may be necessary to prove its specificity to general organic toxicants.

3.4.4. WWTP effluents still source of organic pollution

The results for the German Saprobic Index showed a clear trend of decreasing German Saprobic Index with increasing upstream distance to the next WWTP (Fig. 3.4c, left). A significant difference could be found between the sites with and without a WWTP within 3 km upstream (Fig. 3.4c, middle, Wilcoxon rank-sum test: $p<0.001$), which indicated the presence of oxygen-consuming substances downstream of WWTPs. A total of 51% of the sites with a WWTP within 3 km upstream had a German Saprobic Index value higher than 2, which indicated a medium ecological quality class according to the German WFD classification system. In contrast, 91% of the sites with no WWTP within 3 km upstream had a good or even high ecological status (Fig. 3.4c, middle).

After the exclusion of all taxa that were potentially sensitive to organic toxicants (according to the SPEAR approach) from the original taxon list, the modified German Saprobic Index was still higher for sites with a WWTP within 3 km upstream (Fig. 3.4c, right, Wilcoxon rank-sum test: $p<0.001$). Thus, WWTPs discharge not only pesticides that have insecticidal effects on the structure of the macroinvertebrate community, but also oxygen-depleting substances. This finding contradicts the view of the German water authorities that oxygen-depleting substances in WWTP effluent only rarely pose a problem for the health of stream ecosystems nowadays [52].

3.4.5. More detailed data on WWTPs needed

The three investigated biological indices $SPEAR_{pesticides}$, $SPEAR_{organic}$ and German Saprobic Index varied considerably for the 59 sampling sites with a WWTP within 3 km upstream. However, no significant part of the variation could be explained by the available data (capacity and applied treatment steps) of the upstream WWTPs. In future small-scale studies on this topic it is therefore recommended to collect data on other relevant parameters like number of farms connected to the WWTP or proportion of the WWTP effluent compared to the water load of the receiving stream. This information can only be gained by conducting a survey of the local WWTP operators.

3.5. Conclusions

We showed that in many streams both pesticides (insecticidal toxicity) and oxygen-depleting substances affect the macroinvertebrate community significantly and cause a change towards species that can tolerate pesticides and/or oxygen deficiency.

For pesticide monitoring, we identified that the bioindicator system $SPEAR_{pesticides}$ is an effective tool for evaluating the insecticidal effects of WWTP effluent on macroinvertebrates in streams.

For pesticide management, our findings emphasize the need to take municipal WWTPs into consideration within the EU WFD. Measures as the implementation of additional treatment steps, e.g, ozonation or adsorption to activated carbon, need to be considered. Furthermore, campaigns and training courses could help to increase the awareness of farmers to avoid improper handling of pesticides, e.g. cleaning of sprayer equipment directly on the treated field instead of cleaning on the farmyards.

3.6. Acknowledgements

We thank the Hessian State Office for Environment and Geology (HLUG) for providing the macroinvertebrate and structural quality class data. In addition, we thank Jeanette Völker and Dietrich Borchardt, Department of Aquatic Ecosystem Analysis, Helmholtz Centre for Environmental Research, for fruitful discussions. Furthermore, we are grateful to Sinje Burgert for providing us with her “Extract upstream sections” ArcGIS tool. We also thank the anonymous reviewers for their helpful comments on a previous version of the manuscript.

This work was made possible by funding from the Helmholtz Association of German Research Centres within the project funding “Biomass and Bioenergy Systems” and supported by Helmholtz Impulse and Networking Fund through Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE).

4. Landscape parameters driving aquatic pesticide exposure and effects

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Environmental Pollution (2014), 186, Pages 90-97

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4.1. Abstract

Pesticide contamination is considered one of the reasons streams fail to achieve good ecological and chemical status, the main objectives of the Water Framework Directive. However, little is known on the interaction of different pesticide sources and landscape parameters and the resulting impairment of macroinvertebrate communities. We evaluated the potential effects of diffuse and point sources of pesticides using macroinvertebrate monitoring data from 663 sites in central Germany. Additionally, we investigated forested upstream reaches and structural quality as landscape parameters potentially mitigating or amplifying the effects of pesticides. Diffuse pesticide pollution and forested upstream reaches were the most important parameters affecting macroinvertebrate communities (pesticide-specific indicator $SPEAR_{pesticides}$). Our results indicate that forested upstream reaches and riparian buffer strips at least 5 m in width can mitigate the effects and exposure of pesticides. In addition, we developed a screening approach that allows an initial, cost-effective identification of sites of concern.

4.2. Introduction

In agricultural areas, pesticides are major stressors in freshwater ecosystems and potentially have adverse effects on aquatic communities. A considerable number of investigations have shown the negative effects of pesticide contamination caused by diffuse agricultural sources on aquatic macroinvertebrate communities [20, 43, 67]. In addition, several studies have detected significant amounts of pesticides in the effluent of wastewater treatment plants (WWTPs) [23, 24, 26]. In a previous study, we showed that pesticides from these point sources can significantly affect the macroinvertebrate community within 3 km downstream of WWTPs [68]. However, we could not detect significant insecticidal effects of diffuse agricultural sources of pesticides, because of the scarcity of adjacent arable land and the frequent occurrence of riparian buffer strips in our data set.

In addition to the various pesticide sources, landscape parameters potentially mitigate or amplify the effects of pesticides on macroinvertebrate communities. For example, previous studies have shown that forested stream sections can partially mitigate the effects of pesticides and considered them as potential sources for recolonisation of downstream sites [20-22]. Furthermore, Rasmussen et al. [51] suggested that hydromorphological degradation often interacts with pesticide pollution in agricultural areas which, in turn, can cause altered effects of these stressors.

In general, there is a lack of studies that investigate the effects of diffuse or point sources of pesticides and provide an overview of the different sources and landscape parameters that influence the effects of pesticides on the macroinvertebrate community. This knowledge would be valuable to water authorities with respect to the Water Framework Directive (WFD) objective of achieving good ecological and chemical status for all water bodies. Information from these studies would allow authorities to target limited monitoring capacities and to develop appropriate mitigation measures for pesticide contamination.

Against this background, the overall aim of the present study was to conduct a comprehensive assessment of the effects of pesticides on macroinvertebrate communities by including all relevant pesticide sources and landscape parameters. We first evaluated the insecticidal effects of diffuse (arable land

and garden allotments, taking into account riparian buffer strips) and point sources (WWTPs) on the macroinvertebrate community using governmental monitoring data from 663 sampling sites. In addition to the 327 Hessian sites used in Bunzel et al. [68], we analysed 336 sites in the German federal states of Saxony, Saxony-Anhalt and Thuringia. We investigated the different pesticide sources, as well as forested upstream reaches and structural quality as landscape parameters that influence the effects of pesticides. In a last step, we set up a screening approach reflecting the risk to macroinvertebrate communities as a result of pesticide contamination.

4.3. Material and methods

4.3.1. Study area

We analysed 663 sampling sites in four central German federal states: Hesse – 327, Saxony – 160, Saxony-Anhalt – 127 and Thuringia – 49 (Fig. 4.1).

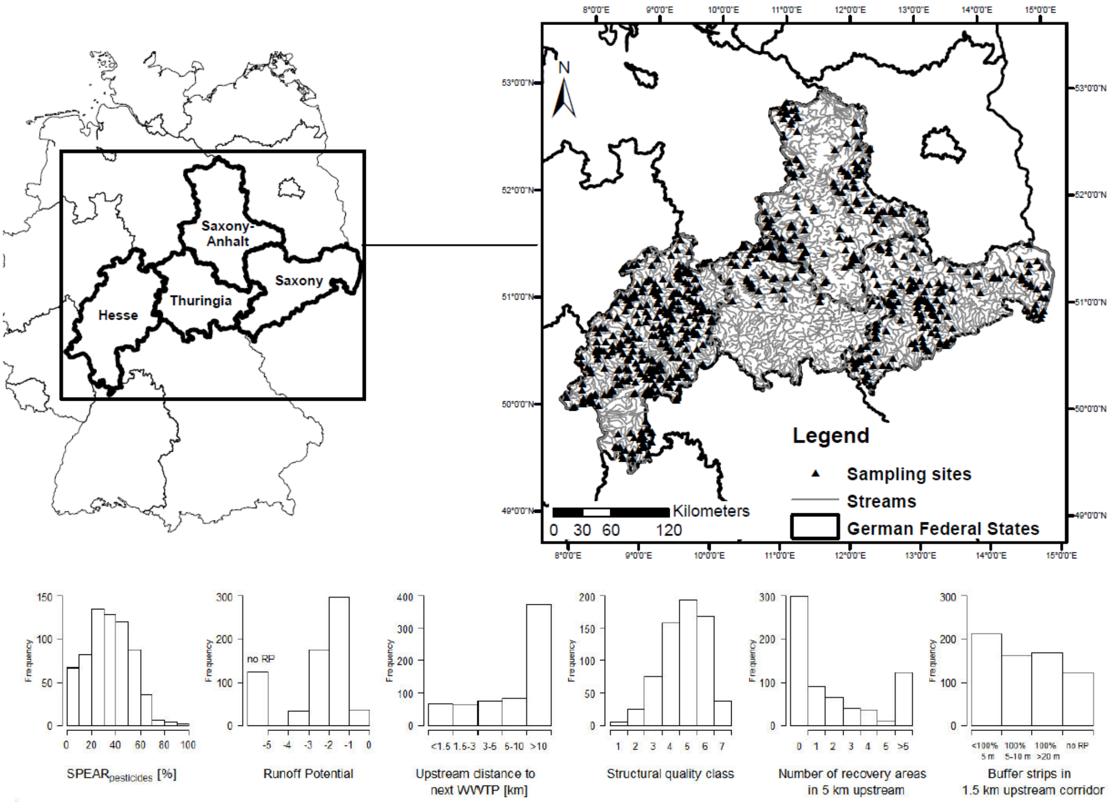

Fig. 4.1: Distribution of sampling sites and histograms of the main variables investigated.

Agriculture is the main land use in all four states; agriculture covers 42% of the total area in Hesse, 55% in Saxony, 62% in Saxony-Anhalt, and 54% in

Thuringia. The next important land use is forest, which covers 40% of the total area in Hesse, 27% in Saxony, 24% in Saxony-Anhalt, and 32% in Thuringia.

4.3.2. Macroinvertebrate data

We used WFD monitoring macroinvertebrate data provided by the respective official authority: Hesse – Hessian State Office for Environment and Geology (HLUG); Saxony – Saxon State Office for the Environment, Agriculture and Geology (LfLUG); Saxony-Anhalt – Saxony-Anhalt State Agency for Flood Protection and Water Management (LHW); and Thuringia – Thuringian Authority of Environment and Geology (TLUG). We applied the following criteria to obtain a relatively harmonised dataset: a) sampling from March to June 2005 or 2006, b) a stream width smaller than 10 m, c) a minimum of 1 500 m from the stream source, d) no lake or reservoir within 1 500 m upstream, e) at least 10 species identified, and f) existing data on structural quality (see Section 4.3.5.). We excluded five sites in Saxony and six sites in Saxony-Anhalt because they were either in a former open-cast mining area or close to recently flooded open-cast mines.

Three-fourth of the sites were sampled in March or April and, therefore, before the main application time for pesticides in Germany. At these sites, the species had several months to recover from possible effects from the previous year. Therefore, the potential alterations in community composition represent rather long-term than acute effects.

We used the macroinvertebrate index $SPEAR_{pesticides}$ to quantify the effects of insecticide toxicity of pesticides on the macroinvertebrate community [20]. Pesticides are generally applied on a seasonal basis. Therefore, $SPEAR_{pesticides}$ incorporates the physiological sensitivity of the species to organic toxicants, post-contamination recovery (generation time and migration ability), and the presence of sensitive aquatic stages during the main application time for pesticides.

Based on these biological traits, the identified taxa were classified as sensitive species (SPecies At Risk - SPEAR) or tolerant species (SPEnotAR) based on their vulnerability to pesticides. Subsequently, the relative abundance of sensitive taxa in the community ($SPEAR_{pesticides}$) was calculated for each site:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n} \log(x_i+1) \cdot y}{\sum_{i=1}^{n} \log(x_i+1)} \times 100 \quad (4.1)$$

where n is the number of taxa; x_i is the abundance of taxon i, and y is: 1 if taxon is classified as SPEAR, otherwise 0.

$SPEAR_{pesticides}$ has been applied successfully to indicate insecticidal effects of pesticides on macroinvertebrate communities in different geographical regions [69]. This index is also potentially applicable across different types of water courses and has been shown to be independent of other environmental factors [20, 44, 46]. The index $SPEAR_{pesticides}$ was calculated using the SPEAR Calculator (available online at www.systemecology.eu/SPEAR/index.php).

4.3.3. Diffuse pesticide contamination from arable land and garden allotments

The detection of pesticides in the field is expensive, time-consuming and needs a very complex sampling design; especially for large numbers of sampling sites, it is hardly practicable. The used WFD monitoring data included no data on pesticides. Therefore, we used the GIS-based runoff potential (RP) model to assess the potential exposure of sites to runoff-induced pesticide input. The RP model is a simplified version of a model by the Organization of Economic Co-operation and Development [57]. It is assumed that the loss of a generic substance results from a single application in a two-sided 100 m stream corridor extending 1 500 m upstream of the site [22]. The dissolved amount of the generic substance that potentially reaches the stream site (gLOAD) was calculated for each rainfall event from May to July (the main period of pesticide application). A detailed description of the underlying formula for the calculation of gLOAD and a short description of the parameterisation can be found in Appendix A2. The final RP was calculated by log-transforming the overall maximum gLOAD per site.

The RP model uses a set of generalised compound characteristics (e.g., a constant application rate of 1 g/ha and a constant soil organic carbon sorption coefficient of 100) instead of specific compound properties. Therefore, RP values represent relative predictions of potential runoff inputs and reflect the vulnerability of sites exposed to a variety of substances to potential runoff. The

RP model has been supported by studies comparing RP values with $SPEAR_{pesticides}$ values calculated from field data from Germany, France, Finland, and Australia [45, 46, 59].

Pesticides are not only used on arable land but also in garden allotments [70]. Therefore, we considered both arable land and garden allotments as potential sources of diffuse pesticide contamination. There are approximately 1.24 million allotments covering 50,000 ha in Germany [71]. In Germany, only pesticides that are labelled “for application in garden allotments” are allowed. However, no training for their proper handling and use is provided. Shapefiles from the ATKIS database (“arable land”, “special crops”, and “garden lands” provided by the German Federal Agency for Cartography and Geodesy), as well as aerial pictures (BING Maps, Google Maps), were used to determine the respective areas.

In addition to RP values, we calculated the percentage of land in the 1.5-km upstream corridor that could cause diffuse pesticide contamination (arable land and garden allotments, continuous variable “percentage area of diffuse pesticide sources”.

As a third variable for diffuse pesticide contamination, we assessed the occurrence of double-sided riparian buffer strips in the 1.5-km upstream corridor based on ATKIS land use data and aerial pictures from BING Maps. The categorical variable “buffer strips in 1.5-km upstream corridor” distinguished four levels: “<100% 5 m” (the 1.5-km upstream corridor has partially less than 5-m wide or no buffer strips), “100% 5-10 m” (the whole 1.5-km upstream corridor has at least 5-m wide buffer strips, partially up to 10 m), “100% >20 m” (broad strip of non-arable land use beside the stream) and “no RP” (no diffuse pesticide sources in the 1.5-km upstream corridor and, therefore, no buffer strips necessary).

4.3.4. Wastewater treatment plants as point sources

The shapefiles of the WWTP locations and the data of the features of the WWTPs were provided by the respective authority (Hesse – HLUG, Saxony – LfULG, Saxony-Anhalt – State Agency for Environmental Protection Saxony-Anhalt (LAU), and Thuringia – TLUG). The data were cross checked with aerial pictures (BING Maps, Google Maps) and with the ATKIS shapefile for "WWTPs". The distance between a sampling site and the next upstream WWTP was determined using ArcGIS 10 (categorical variable "upstream distance to next WWTP": "<1.5 km", "1.5-3 km", "3-5 km", "5-10 km", and ">10 km".

4.3.5. Relevant landscape parameters

We included an analysis of upstream riparian habitat quality in our investigation to study the potential influence of recovery areas on the downstream macroinvertebrate community. In previous studies, the minimum size of these recovery areas varied (e.g., forested stream sections >200 m in length and up to 4 km upstream of the site [20], more than 20% of the 1.5-km upstream stream corridor covered by forest [22] and double-sided riparian forest >100 m in length in the 3 km reach upstream of site [21]). For the present investigation, we considered double-sided riparian forest stretches with a length of 200 m and a width of 50 m on each side of the stream. Identification was based on the ATKIS land use data and BING Maps aerial pictures. The number of forested stretches was counted within 5 km upstream of the sampling sites using ArcGIS 10 (variable "number of recovery areas").

We used the shapefiles of the structural quality classes of the streams to estimate the hydromorphological quality of the sampling sites. The overall structural quality is the average of six main hydromorphological parameters and is given as its deviation from the potential natural state based on a seven-point scale ranging from 1 (unchanged) to 7 (completely changed). The main hydromorphological parameters are course development, longitudinal profile, cross profile, bed structure, bank structure, and area surrounding the water body. The shapefiles were provided by the respective state authority (Hesse – HLUG, Saxony – LfULG, Saxony-Anhalt – LHW, and Thuringia – TLUG). The assessment of the structural quality classes took place in the years 1998 and 1999 (Hesse), 2001 (Saxony), 2007 to 2009 (Saxony-Anhalt) and 1999 to 2007 (Thuringia). Recent data were not available at the time of this study.

4.3.6. Data analysis

The statistical analysis was conducted using R version 2.14.1. The Spearman rank correlation coefficient ρ was applied to test the correlation between different variables. The influences of the continuous variable “Runoff Potential” and the categorical variables “upstream distance to next WWTP“, “structural quality class”, “number of recovery areas”, and “buffer strips in 1.5-km corridor” on $SPEAR_{pesticides}$ were investigated using an analysis of covariance (ANCOVA). We simplified the linear models by stepwise removal of non-significant terms and by grouping the factor levels that were not significantly different from each other.

The RP model calculates no RP values for sites without diffuse sources (no arable land or garden allotments in 1.5-km stream corridor). Therefore, we performed the ANCOVA in two separate groups: 539 sampling sites with diffuse pesticide contamination and 124 sampling sites without diffuse pesticide contamination. Due to non-significant factor levels, we grouped the variable “number of recovery areas” into the following four levels: 1 (more than three recovery areas), 2 (two to three recovery areas), 3 (one recovery area), and 4 (no recovery area). Furthermore, we grouped the variable “structural quality classes”: 1 (structural quality class 1 to 3), 2 (structural quality class 4 to 5) and 3 (structural quality class 6 to 7). There was only a significant difference between sites with and without a WWTP in the 1.5-km upstream distance. Therefore, we distinguished the variable “upstream distance to next WWTP” only between these two levels.

To set up the screening model, we ran another ANCOVA with the same variables but replaced the variable “Runoff Potential” with the variable “percentage area of diffuse pesticide sources”. We found a high positive correlation between both variables ($\rho=0.78$, $p<0.001$). Moreover, for the 539 sites with diffuse pesticide contamination, both variables correlated with $SPEAR_{pesticides}$ to the same extent ($SPEAR_{pesticides}$ and RP: $\rho=-0.35$, $p<0.001$; $SPEAR_{pesticides}$ and “percentage area of diffuse pesticide sources”: $\rho=-0.4$, $p<0.001$).

The determination of the variable “percentage area of diffuse pesticide sources” requires far less effort than the calculation of RP. Therefore, we used the variable “percentage area of diffuse pesticide sources” instead of RP to develop the screening model. This variable also had an assigned value for all sites (0 for sites without diffuse pesticide sources), whereas RP was not defined for sites without diffuse contamination. Therefore, it was possible to do the ANCOVA for all 663 sites together.

Based on the respective landscape parameters, we used the screening model to predict $SPEAR_{pesticides}$ for the 663 sampling sites. Subsequently, we compared the results with the $SPEAR_{pesticides}$ values calculated from the original macroinvertebrate data (Fig. 4.5). We then checked whether the sites were classified above or below the class boundary for good and moderate ecological status ($SPEAR_{pesticides}$=33%) proposed by Beketov et al. [56].

We used the R-packages “hier.part” to quantify the individual importance of an explanatory variable to the multiple regression models (for details see http://cran.r-project.org/web/packages/hier.part/hier.part.pdf). For illustrative purposes, we used the function “ctree” from the R-package “party“ to create a conditional inference tree and, therefore, to group the sites based on their attributes. A conditional inference tree is a tree-based method that estimates a regression relationship by binary recursive partitioning in a conditional inference framework (http://cran.r-project.org/web/packages/party/party.pdf). In the first step, the function tests whether any of the explanatory variables and the response are independent from each other. If independence is detected, the explanatory variable with the strongest association to the response is selected. This association is measured by a p-value corresponding to a test for the partial null hypothesis of a single explanatory variable and the response. In the next step, a binary split in the selected input variable occurs: in one, the value of the selected explanatory variable is over the selected threshold and, in the other, it is below the threshold. A split is implemented when the p-value is less than 0.05. This procedure is recursively repeated. The process results in a tree-like structure of groups (Fig. 4.3).

4.4. Results and discussion

4.4.1. Analysis of sites with diffuse pesticide contamination

We assessed the influence of the different variables on $SPEAR_{pesticides}$ using an ANCOVA. The minimum adequate model for the 539 sites with diffuse pesticide contamination explained approximately 33% of the variation of $SPEAR_{pesticides}$. The following variables related to pesticide sources were found to have a significant influence on $SPEAR_{pesticides}$: “Runoff Potential” ($p<0.001$), “buffer strips in 1.5-km corridor” ($p<0.001$), and “upstream distance to next WWTP” (WWTP or no WWTP in 1.5 km, $p<0.002$). The influencing landscape parameters “number of recovery areas” and “structural quality class” also had a significant effect on $SPEAR_{pesticides}$ ($p<0.001$). We found no significant interaction between the variables, indicating only additive effects of the variables.

Most of the variance of $SPEAR_{pesticides}$ was explained by the variables “number of recovery areas“, “buffer strips in 1.5-km corridor“, and “Runoff Potential“ (Fig. 4.2a and Fig. 4.3).

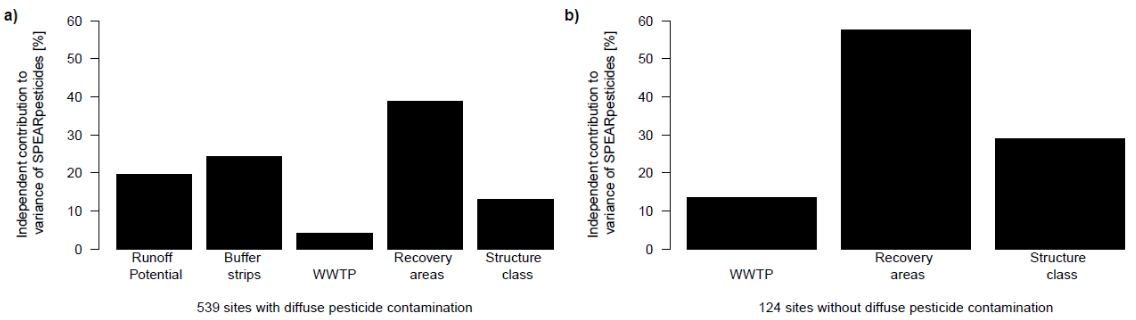

Fig. 4.2: Visualisation of the independent contributions of the different variables to the variance of $SPEAR_{pesticides}$ (result of R-function “hier.part”; a) 539 sampling sites with diffuse pesticide sources and b) 124 sampling sites without diffuse pesticide sources).

The probability of a high $SPEAR_{pesticides}$ value was greatest for sites with more than four recovery areas and with buffer strips of at least 5 m in the whole 1.5-km corridor (Fig. 4.3). On the other hand, comparably low $SPEAR_{pesticides}$ values were observed at sites with RP>-1.5, as well as at sites with no or only one recovery area, continuous or discontinuous buffer strips of less than 10 m width, a structural quality of 4 to 7, and a WWTP in 1.5 km. Structural quality was especially relevant to SPEAR$_{pesticides}$ at sites with low RP values and, therefore, low diffuse pesticide input. The border for significant diffuse pesticide contamination was at a RP value of approximately -1.4 to -1.5 (Fig. 4.3). WWTPs also had an important influence on SPEAR$_{pesticides}$ but only in cases of low diffuse pesticide contamination and medium to bad structural quality.

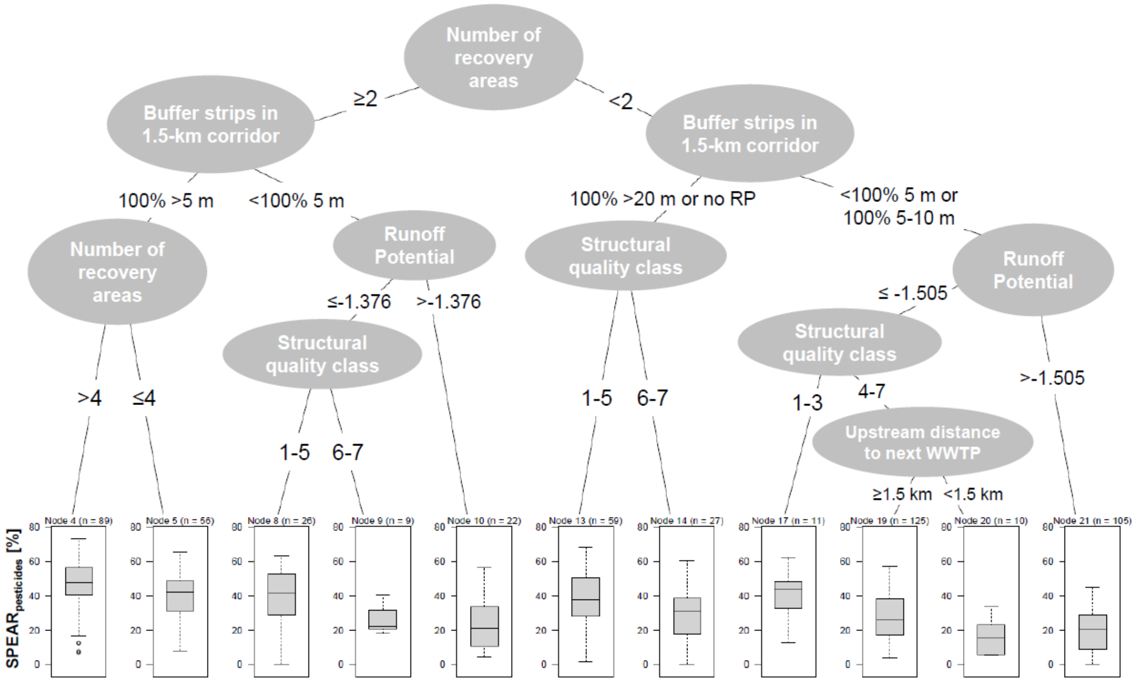

Fig. 4.3: Conditional interference tree for predicting SPEAR$_{pesticides}$ as a function of the explanatory variables “Runoff Potential”, “upstream distance to next WWTP“, “structural quality class”, “number of recovery areas”, and “buffer strips in 1.5-km corridor” (based on the 539 sites with diffuse pesticide sources).

4.4.1.1. Riparian buffer strips can reduce diffuse pesticide input

We found that riparian buffer strips at least 5 m in width had a positive effect on SPEAR$_{pesticides}$. Therefore, we assume that the buffer strips reduce the pesticide input from adjacent arable land or garden allotments. To illustrate this point, we compared the SPEAR$_{pesticides}$ values of sites with comparable RP values but with different buffer strip widths (Fig. 4.4a). We only considered sites with a low to medium hydromorphological degradation and no WWTP in the 1.5-km upstream distance to minimise the effects of these variables.

For each RP class, there is a clear trend of increasing $SPEAR_{pesticides}$ values with increasing buffer strip width (Fig. 4.4a). For RP values between -1 and -2, there was a significant difference between sites where the 1.5-km upstream corridor was not completely accompanied by 5-m wide buffer strips and sites that had 100% of at least 5-m wide buffer strips ($p<0.05$). The highest $SPEAR_{pesticides}$ values had sites with broad buffer strips of more than 20 m. For sites with a low diffuse pesticide contamination (RP<-2), there was no significant effects of buffer strips (Fig. 4.4a). Our results support the findings of other studies that emphasise the use of buffer strips as an effective mitigation measure to reduce pesticide input from adjacent fields [17, 27, 28].

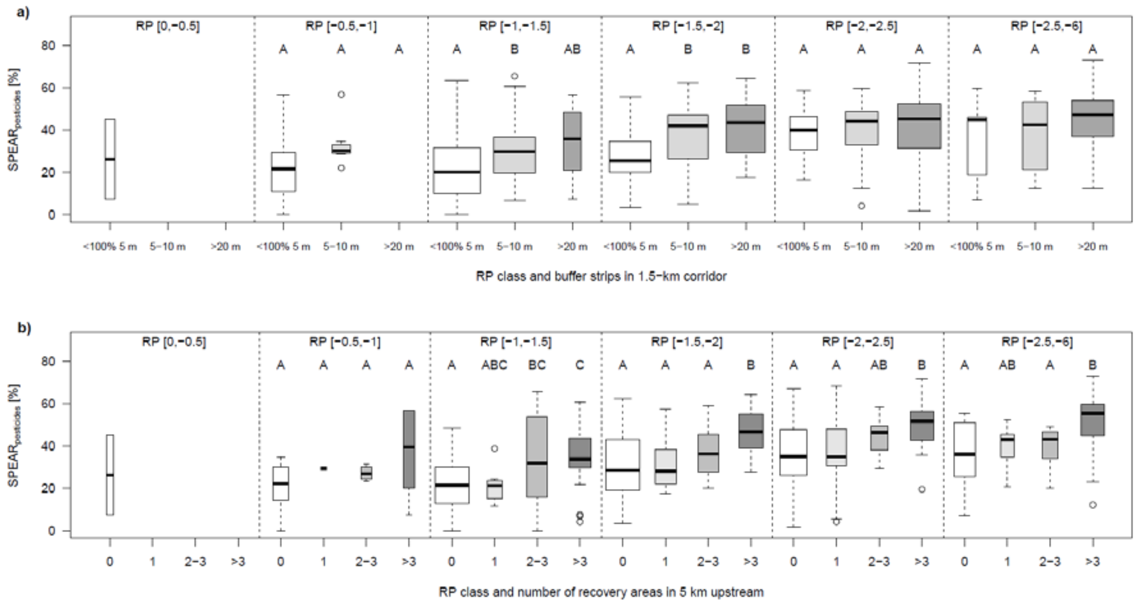

Fig. 4.4: Relationship between $SPEAR_{pesticides}$ and buffer strips in 1.5-km upstream corridor (a) and number of recovery areas in 5-km upstream distance (b) for 330 sampling sites (overall structural quality class 1-5 and no WWTP in the 1.5-km upstream distance). The sites are grouped according to different RP classes. Capital letters indicate significantly different groups per RP class ($p<0.05$).

In Germany, the federal states can pass their own ordinances concerning restrictions regarding buffer widths and the use of pesticides, as well as exceptional rules for the implementation of riparian buffer strips. Whereas Thuringia mandates the buffer strip requirements of the German Federal Water Act [72] which requires 5-m buffer strips in the outer zone, Hesse requires 10-m wide buffer strips. Saxony-Anhalt demands 10-m strips for 1st order streams and

5-m strips for 2^{nd} order streams. Saxony has the strictest requirements: no application of pesticides is allowed on 10-m wide buffer strips for the outer zone and on 5-m strips for streams in built-up land. However, all federal state ordinances allow the local water authorities to deviate from these requirements in cases of unreasonable hardship and overriding reasons of general interest. Therefore, the study area was characterised by a great variety of buffer strips. In general, the results of our study indicate the need for at least 5-m wide buffer strips in not only the outer zone but also in the whole stream system.

4.4.1.2. WWTPs can be important point sources of pesticides

We found a significant difference between sites with and without a WWTP within a 1.5-km upstream distance, especially for sites with low diffuse pesticide contamination (RP≤-1.5) and medium to bad structural quality (Fig. 4.3). A possible explanation for the role of structural quality might be that most of the sites with a WWTP within a 1.5-km upstream distance had a medium (class 4 to 5, 59% of sites) or bad (class 6 to 7, 30%) structural quality. Only 11% of the sites had a structural quality class of 3. Therefore, this dataset could not be used to draw conclusions for sites with a good structural quality of 1 to 3.

The detection limit for significant insecticidal effects of WWTP effluents was at a 1.5-km upstream distance. This result was different to our previous study of the 327 Hessian sites [68], where we showed significant insecticidal effects of WWTPs up to a distance of 3 km upstream. The difference between the results may be explained by the fact that more Hessian sites had large WWTPs (more than 10 000 Population Equivalent (PE)) within a 3-km upstream distance (< 1.5 km: 14 sites, 1.5-3 km: 8 sites) and, therefore, high effluent volumes entering the receiving streams. Saxony, Saxony-Anhalt and Thuringia had rather small or medium WWTPs and only some sites with WWTPs >10 000 PE (<1.5 km: 6 sites, 1.5-3 km: 4 sites). All investigated streams had approximately the same dilution potential (stream width <10 m).

4.4.1.4. Landscape parameters can mitigate or amplify effects of pesticides

There was a clear trend of increasing $SPEAR_{pesticides}$ values for each RP class with increasing number of upstream recovery areas (Fig. 4.4b). Even for sites with low diffuse pesticides contamination (RP<-2.5), the $SPEAR_{pesticides}$ values were higher for sites with one to three recovery areas within a 5-km upstream distance and even higher for sites with more than three recovery areas (Fig. 4.4b). Our findings are consistent with those of previous studies that reported a significant positive effect of forested upstream stretches on $SPEAR_{pesticides}$ [20-22, 73], although significant positive effects were not always found [69]. In regard to pesticides contamination, forested upstream reaches seem to represent potential sources of after-contamination recolonisation of downstream sites.

The $SPEAR_{pesticides}$ values decreased with increasing hydromorphological degradation. In particular, sites with completely changed hydromorphology (structural quality class 6 to 7) (e.g., concrete channels with extensive embankment consolidation or straight artificial stream beds) showed significantly lower $SPEAR_{pesticides}$ values even if there was no obvious upstream source of pesticides (Fig. 4.3). These results are consistent with Rasmussen et al. [51] and Bunzel et al. [68], who found that $SPEAR_{pesticides}$ responds to characteristics of the physical habitat, mainly due to the decrease in species richness and abundance of EPT taxa. Mažeika et al. [74] and Dunbar et al. [62] found that many EPT taxa have relatively strict habitat requirements and are vulnerable to the loss of habitat heterogeneity. Therefore, Rasmussen et al. [51] argued that EPT taxa that are to a great extend pesticide-sensitive according to SPEAR may be partially restricted from colonising agricultural streams with uniform and degraded physical conditions. In contrast, von der Ohe and Goedkoop [73] studied 100 streams in Sweden and found that $SPEAR_{pesticides}$ was not affected by habitat degradation. However, most of the sites were only characterised by a low to medium hydromorphological degradation.

Furthermore, our results indicate that structural quality seemed to play an important role, especially for sites with low RP values and, therefore, low diffuse pesticide input (Fig. 4.3). This finding supports Rasmussen et al. [51], who suggested that potential positive effects of heterogeneous habitat conditions could become less important for sites with high pesticide contamination. In summary, our findings demonstrate the need to take into account the influence of hydromorphological degradation when interpreting $SPEAR_{pesticides}$ to avoid overestimation of the toxic impact of pesticides at highly degraded streams.

4.4.2. Analysis of sites without diffuse pesticide contamination

The minimum adequate model for the 124 sampling sites without diffuse pesticide contamination explained approximately 25% of the variation of $SPEAR_{pesticides}$. The following variables had a significant influence on $SPEAR_{pesticides}$: "upstream distance to next WWTP" (WWTP or no WWTP in 1.5 km, p<0.05), "number of recovery areas", and "structural quality class" (p<0.001). We found no significant interactions between the variables. Most of the variance of $SPEAR_{pesticides}$ was explained by the variable "number of recovery areas", followed by the variable "structural quality class" (Fig. 4.2b).

4.4.3. Screening approach based on landscape parameters

We conducted an ANCOVA for all 663 sites to set up a simple screening approach to estimate the effects of pesticide contamination on macroinvertebrate communities (see Section 4.2.6). The results for the detection limit of significant insecticidal effects of WWTP effluents were not conclusive. Therefore, we opted for a conservative approach using 3 km as the distance to group the variable "upstream distance to next WWTP" ("WWTP in 3 km" and "no WWTP in 3 km"). The minimum adequate model explained approximately 33% of the variation of $SPEAR_{pesticides}$. $SPEAR_{pesticides}$ values can be predicted by the following equation:

$$predicted\ SPEARpesticides\ [\%] = 55.174 + Perc_{diff} + SQC + \mathrm{Rec} + \mathrm{Buff} + \mathrm{WWTP} \quad (4.2)$$

A short description and the coefficients of the different variables can be found in Table 4.1.

Table 4.1: Variables for the prediction of $\mathrm{SPEAR}_{\mathrm{pesticides}}$ (Eq. (4.2)). For categorical variables, the appropriate coefficient for the variable must be chosen depending on the level. Asterisks indicate significantly different groups (ANCOVA: $^{**}p<0.01$, $^{***}p<0.001$).

Variable	Description	Levels	Coefficient
$\mathrm{Perc}_{\mathrm{diff}}$	Percentage area of diffuse pesticides sources (arable land and garden allotments) in 1.5-km upstream corridor	-0.091 x Percentage area**	
SQC	Structural quality class at sampling site	1-3 4-5 6-7	0 -5.33** -8.88***
Rec	Number of forested reaches (double-sided, 200-m long and 50-m wide) in up to 5-km upstream distance	>3 2-3 1 0	0 -6.68*** -9.54*** -13.50***
Buff	Buffer strips in 1.5-km upstream corridor	No buffer needed 100% >20 m 100% 5-10 m <100% 5 m	0 -0.61 -5.29** -9.12***
WWTP	WWTP in 3-km upstream distance	No Yes	0 -4.91***

We compared the predicted $\mathrm{SPEAR}_{\mathrm{pesticides}}$ values with the $\mathrm{SPEAR}_{\mathrm{pesticides}}$ values calculated from the original macroinvertebrate data for the 663 sites (Fig. 4.5). The majority of the sites (73.6%) were properly classified (observed and predicted $\mathrm{SPEAR}_{\mathrm{pesticides}}$ either >33% or <33%). Approximately 12.7% of the sites were underestimated by the model (observed $\mathrm{SPEAR}_{\mathrm{pesticides}}$>33% and predicted $\mathrm{SPEAR}_{\mathrm{pesticides}}$<33%), and 13.7% were overestimated (observed $\mathrm{SPEAR}_{\mathrm{pesticides}}$>33% and predicted $\mathrm{SPEAR}_{\mathrm{pesticides}}$<33%) (Fig. 4.5). Therefore, from a conservative point of view, only the 13.7% of sites where the screening model overestimates the $\mathrm{SPEAR}_{\mathrm{pesticides}}$ values are critical. In these cases, there is a risk of overlooking sites that fail to achieve good ecological status according to $\mathrm{SPEAR}_{\mathrm{pesticides}}$.

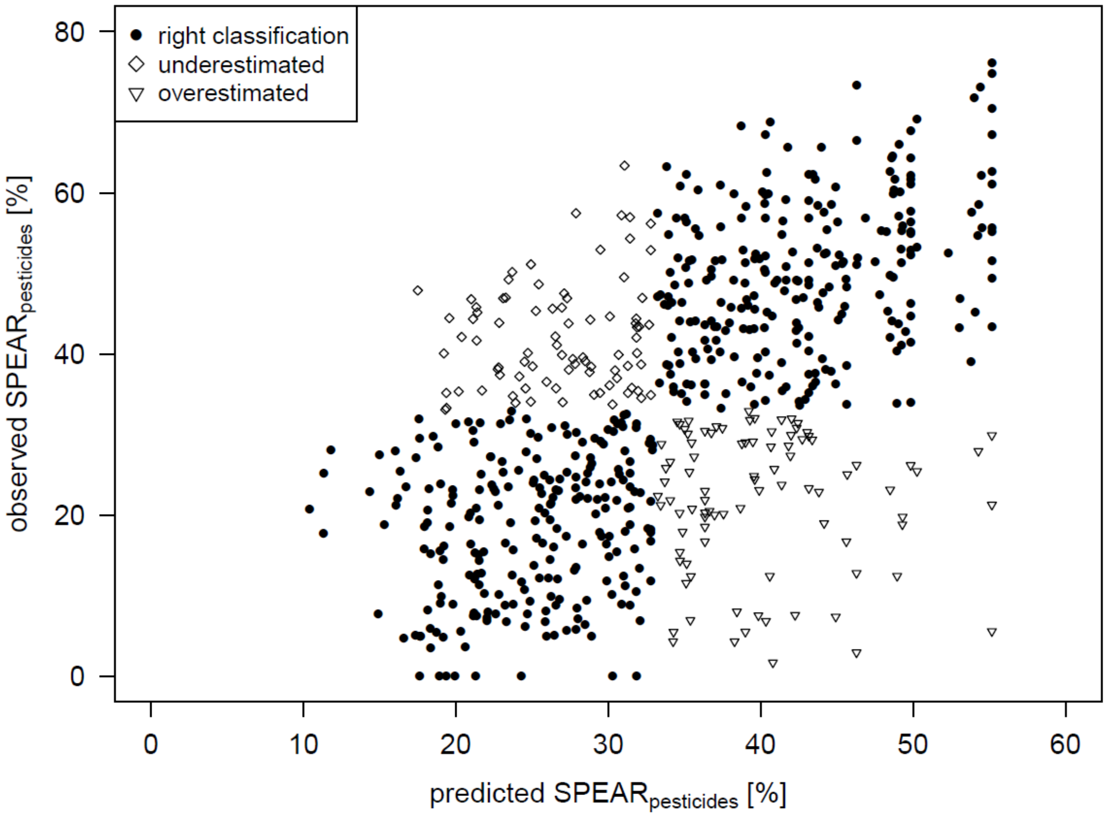

Fig. 4.5: Comparison of the SPEAR$_{pesticides}$ values calculated based on sampling data and predicted SPEAR$_{pesticides}$ values derived by Eq. 4.2) for the 663 sites (right classification: observed and predicted SPEAR$_{pesticides}$ either >33% or <33%; underestimated: observed SPEAR$_{pesticides}$>33% and predicted SPEAR$_{pesticides}$<33%; overestimated: observed SPEAR$_{pesticides}$>33% and predicted SPEAR$_{pesticides}$ <33%).

The misclassification of the screening approach has several possible explanations. First, the pesticide contamination caused by diffuse agricultural sources can vary between sites even if the size of the arable area and its key environmental characteristics in the 1.5-km corridor are the same. For example, agricultural practice can vary between different farms, and not every farmer complies with good agricultural practice (e.g., by taking care of the distance requirements of the respective pesticide). Furthermore, not every WWTP is a significant source of pesticides. The amount of pesticide leaving a WWTP is dependent on several factors, such as the number of farms connected to the WWTP or the retention time of the wastewater in the WWTP. Regarding the hydromorphological degradation of streams, the overall structural quality class represents the average of six hydromorphological parameters. Rasmussen et al. [51] argued that bed structure may play an important role. Therefore, averaging the different hydromorphological parameters may also cause certain inaccuracies. Pesticides may have other possible point sources, such as urban areas [26, 75] or golf courses [76, 77].

4.5. Conclusions

Our study emphasises the need to consider all important pesticide sources and landscape parameters when assessing the effects of pesticide contamination on macroinvertebrate communities. We developed a screening approach that allows an initial fast and cost-effective identification of sites of concern. The approach requires readily available GIS data. Our screening approach can help water authorities to efficiently target the restricted monitoring capacities and to identify sites where a site-specific assessment is necessary. This knowledge is essential for developing effective river basin management plans and for designing appropriate mitigation measures. Our results indicated that riparian buffer strips at least 5 m in width and forested upstream reaches can mitigate the pesticides effects by reducing pesticide input or enhancing the recovery of affected stream regions. Therefore, buffer strips and forested upstream reaches can help to improve the health of aquatic ecosystems, even in landscapes with relatively intensive agriculture.

4.6. Acknowledgments

We thank the following authorities for providing data: Hessian State Office for Environment and Geology; Saxon *S*tate Office for the Environment, Agriculture and Geology; Saxony-Anhalt State Agency for Flood Protection and Water Management; State Agency for Environmental Protection Saxony-Anhalt; Thuringian Authority of Environment and Geology. Furthermore, we are grateful to Eyske Siemens for her preliminary work on the RP calculation.

The work was made possible by funding from the Helmholtz Association of German Research Centres within the project funding “Biomass and Bioenergy Systems” and supported by Helmholtz Impulse and Networking Fund through Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE).

5. Energy crops and pesticide contamination: Lessons learned from the development of energy crop cultivation in Germany

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Biomass & Bioenergy (2014)

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5.1. Abstract

Biomass provides two thirds of the total energy produced from renewables in Europe. The share of bioenergy from energy crops is growing rapidly. Given the environmental pressures arising from pesticide pollution from current agricultural food production, a substantial increase in energy crop cultivation might put additional pressure on biodiversity and soil and water resources. In the present study, we examine the potential of energy crops for pesticide contamination and develop general conclusions and recommendations for the future large-scale expansion of agricultural bioenergy. We base our analysis on the development of energy crop cultivation in Germany, the European country with the largest share of energy crops. Our findings reveal that there will not necessarily be an increase or decrease in the amounts of pesticides released into the environment. Due to the great variety of energy crops, the potential effects will depend rather on the future design of the agricultural systems.

Possible risks are associated with the increased cultivation of pesticide-intensive energy crops, such as rapeseed, especially when grown in monocultures or on formerly set-aside land or converted grassland. Instead, energy crops should be integrated into the existing food production systems. Financial incentives and further education are needed to encourage the use of sustainable crop rotations, innovative cropping systems and perennial energy crops, which may add to crop diversity and generate lower pesticide demands than intensive food farming systems. Optimised cultivation systems with diverse crop rotations could help to improve monotonous agricultural landscapes, increase biodiversity and minimise pesticide exposure.

5.2. Introduction

The European Union (EU) is committed to combatting climate change and increasing the security of its energy supply [78]. Therefore, increasing the share of renewable energy in its total final energy consumption is a key EU policy objective (from 10% and 12.5% in 2007 and 2010, respectively, up to 20% in 2020). Bioenergy has been the backbone of renewable energies in Europe to date (accounting for approximately 60-70% in the last decade) [79]. In addition to the use of woody biomass and residues for energy production, the cultivation of energy crops is of increasing importance. However, aside from the food vs. fuel debate [80, 81], the on-going expansion of energy crop production raises concerns regarding potential environmental impacts on, for example, water resources, soil quality and biodiversity [13-15]. One of the potential impacts that needs further consideration is the possible deterioration of ecosystems in agricultural landscapes caused by pesticide pollution deriving from large-scale energy crop expansion.

In 2008, energy crops were grown on more than 5.5 million ha (approximately 5% of the arable land) in the EU-27, compared to 3.5 million ha in 2005 [13, 15]. On most of this land (82%), rapeseed was grown for the production of biodiesel. The remainder was used for the cultivation of annual crops for ethanol (11%) and biogas production (4%), as well as for plantations of perennial crops (2%). The European Academies Science Advisory Council calculated that approximately 21 million ha of arable land would be needed to achieve the EU 10% target for biofuel use in the transport sector [82].

The cultivation of annual energy crops usually takes place in intensive agricultural production systems, which require, among other things, the application of pesticides. Several studies have reported the negative effects of pesticide contamination by agricultural sources on the structure and biodiversity of terrestrial and aquatic communities [9, 83]. Increased agricultural biomass production for energy purposes could, therefore, put additional pressure on biodiversity in agricultural areas, which would run contrary to EU environmental objectives, such as the management of the Natura 2000 network of protected sites for biodiversity or the ecological quality targets of the Water Framework Directive. On the other hand, innovative energy crops (such as perennials) and cropping systems (such as mixed cultivation) may add to crop diversity and have lower pesticide demands than intensive food farming systems.

Based on the case study of Germany, Europe's leading country in the cultivation of energy crops, we examine the potential of energy crops for pesticide contamination under the following objectives: (1) to describe and analyse the development of energy crop cultivation in Germany; (2) to compile the latest knowledge regarding the pesticide demands of annual and perennial energy crops; and (3) to develop general conclusions and recommendations for policy-makers in the European Union and Member States for future large-scale energy crop development from a phytosanitary point of view.

5.3. Material and methods

5.3.1. Germany as case study

Germany is the European country with the largest share of energy crop areas [15]. Agricultural bioenergy production is well developed and took place on 2.1 million ha (approximately 17.8%) of Germany's arable land in 2012. In the last decade, the German government promoted the cultivation of energy crops by the adoption of specific legislation, i.e., the Renewable Energy Sources Act (EEG), the Market Incentive Programme for Renewable Energies, the Renewable Energy Heat Act, and Biofuel Quota Act, and the establishment of political subsidy tools [84]. The German National Biomass Action Plan sets ambitious targets for the further development of bioenergy by 2020 (share of bioenergy: 8% of total power consumption and 9.7% of total heat usage, 12% of the energy used in transport from biofuels) [85]. In addition, the German government made large amounts of funding available to promote research, development and market introduction programs, e.g., to develop sustainable energy crop cultivation systems and to identify new promising energy crops. Against this background, we chose Germany as case study that exemplifies the risks but also opportunities of the large-scale expansion of energy crop cultivation.

5.3.2. Agricultural data

For both Germany as a whole and its individual 16 Federal States, we analysed statistical data on the development of the different agricultural land use categories (e.g., arable land, permanent grassland, set-aside land) and of the cultivation areas of the main food and energy crops. In this study, we consider silage maize separately from other cereals. Maize is harvested as either grains or silage for food, feed, and energy purposes. Silage maize for animal feed or biogas production is harvested some weeks earlier than grain maize and as whole plant silage. In this study, the term “cereals” refers to the main cereal crops in Germany: winter wheat, winter rye, winter triticale, winter and summer barley, and grain maize.

In Germany, the main development of energy crop cultivation started around 2004 (Fig. 5.1). Therefore, we focused our analysis on the time period 2003 to 2012, the latest year for which data were available for all German regions. The data were provided by the German Federal Statistical Office, the statistical offices of the 16 Federal States, the Federal Office for Agriculture and Food, and the FNR (Fachagentur Nachwachsende Rohstoffe e.V).

In addition, we analysed statistical data of the 46 districts of Lower Saxony and the 12 districts of Mecklenburg-West Pomerania, the German Federal States with the largest current area of silage maize and rapeseed fields, respectively. The cultivation data of Mecklenburg West-Pomerania was available for the years 1999, 2003, 2007, and 2010 and provided by the statistical office of Mecklenburg West-Pomerania. For Lower Saxony, we used data reported annually within the framework of the Common Agricultural Policy (CAP) direct payments (2007 to 2013, provided by the Lower Saxony Chamber of

Agriculture). In order to assess the demand of silage maize as biogas substrate and cattle feed, we examined data on biogas plants (2005 to 2011, Lower Saxony Network for Renewable Resources) and cattle farming (2003, 2007 and 2010, statistical office of Lower Saxony).

5.3.3. Pesticide data for annual energy crops

No nationwide statistics are available on the actual use of pesticides in Germany. Therefore, we examined, in a first step, the statistics of the German Federal Office of Consumer Protection and Food Safety (BVL) regarding the annual sales of plant protection products in Germany (2000 to 2012) [86].

In the next step, we analysed the treatment index for the main annual energy crops in Germany. Since 2003, the treatment index (“Behandlungsindex”, BI) has been the generally agreed-upon indicator of the crop-specific intensity of the use of chemical plant protection products in Germany [87]. The treatment index serves as a benchmark for the evaluation of farmers’ crop-specific chemical pest management within a region with similar conditions and as monitoring tool for the reduction of pesticide use.

The BI is defined as the sum of the applications of all applied pesticides for a specific crop and takes into account the proportion of treated area compared to the total cultivation area of the specific crop (Eq. 5.1). The calculation also accounts for the actual application rate of the specific pesticide in relation to the maximum allowed application rate. The treatment index is calculated for every application of a specific pesticide, irrespective of whether the pesticide is applied as a single application or as part of a tank mix. It is calculated for each crop as follows:

$$BI = \frac{1}{n_{fields}} \sum_{i=1}^{n_{fields}} \sum_{j=1}^{n_{pesticides}} \sum_{k=1}^{n_{applications\ j}} \frac{treated\ area_{i,j,k}}{total\ area_i} \cdot \frac{actual\ application\ rate_{i,j,k}}{maximum\ allowed\ application\ rate_j} \quad (5.1)$$

where n_{fields} is the number of fields where the specific crop is grown, $n_{pesticides}$ is the number of pesticides used for a specific crop, $n_{applications\ j}$ is the number of applications of a specific pesticide *j*.

Since 2007, the calculation of the treatment index is based on a network of reference farms surveyed by the plant protection services of the German Federal States in cooperation with the Julius-Kühn-Institut (JKI). Since 2011, these reference farms have been part of the PAPA (panel pesticide applications) network that was established to comply with the reporting obligations of various national and European legislative frameworks [88]. We analysed the treatment index data of the reference network from 2007 to 2011 ([87]) and of the PAPA network for 2011 and 2012 [89]).

5.3.4. Literature research on perennial energy crops

In Germany, there has been only limited experience with the pesticide requirements of perennial crops because they are grown predominately in small-scale projects rather than on a commercial scale. Therefore, we conducted a literature review to summarise recent knowledge regarding the pesticide demand of the most promising perennial energy crops for Germany: willow and poplar grown in short-rotation coppice and miscanthus. We reviewed English and German peer-reviewed journals and publications by using the databases and library services of Sciencedirect (http://www.sciencedirect.com), Web of Science (www.webofknowledge.com) and Google Scholar (www.scholar.google.de) (time period: 1970 to December 2013). Subsequently, we searched the reference lists of the found publications for relevant papers. In a second step, we used the search engines Google, Yahoo, and Scirus to find relevant reports, books, and conference proceedings.

5.4. Results and discussion

5.4.1. Annual energy crops

5.4.1.1. Strong increase of annual energy crop cultivation

In Germany, the area under cultivation of energy crops has increased considerably in the last ten years. In 2012, energy crops were grown on approximately 2.1 million ha, whereas in 2000, only 0.9 million ha were used to produce biomass for energy production (Fig. 5.1a). The total arable land area of Germany is approximately 11.8 million ha and remained relatively constant in the last 10 years. Although there are a great many potential energy crops, there is a limited number of crops with high production potentials. Therefore, the majority of the agricultural bioenergy production consists of established conventional food crops, with winter rapeseed for biodiesel (*Brassica napus L*., 0.9 million ha in 2012) and silage maize for biogas (*Zea mays L*., 0.8 million ha in 2012) being by far the most important (Fig. 5.1a) [16].

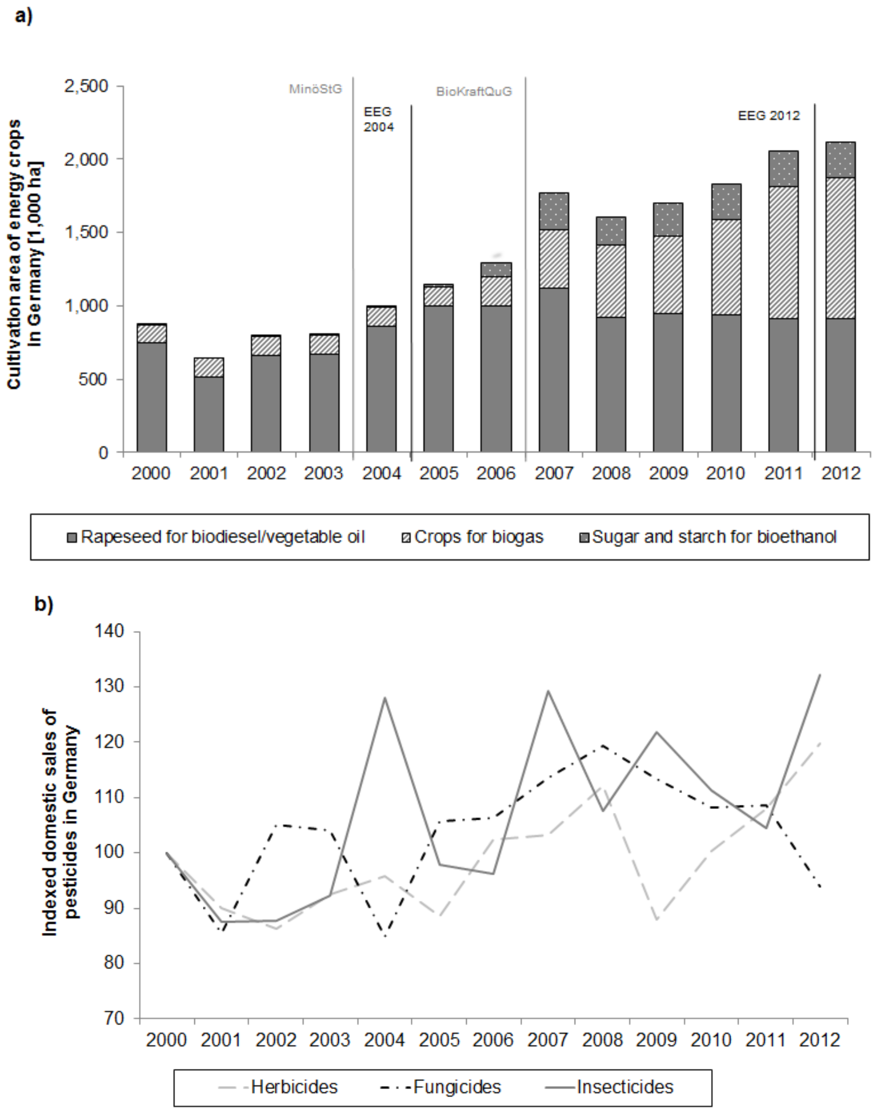

Fig. 5.1: a) Development of the cultivation area of energy crops and b) the domestic sales of pesticides in Germany from 2000 to 2012 (based on Langert [90]; FNR [16]; BVL [86]). EEG 2004 and 2012: Amendments of the German Renewable Energy Sources Act (e.g., 2004: introduction of bonus payments for energy crops as biogas substrate; 2012: maximum combined annual feedstock share of maize silage and cereal grains of 60% of the mass content); MinöStG: amendment of the Mineral Oil Tax Law (e.g., tax exemptions for biofuels); BioKraftQuG: adoption of the Biofuel Quota Act (e.g., minimum quota for biofuels). The y-values in Fig. 5.1b are standardised to the domestic sales of pesticides in the year 2000 (=100).

In Germany, the development of the production of biofuels was mainly encouraged by the amendment of the Mineral Oil Tax Law (MinöStG) at the beginning of 2004 and the adoption of the Biofuel Quota Act (BioKraftQuG) at the beginning of 2007 (Fig. 5.1a). While the first one secured tax exemptions for biofuels (even when mixed with fossil fuels), the second one obliges fuel distributors to sell a minimum quota in the form of biofuels.

The domestic cultivation of crops for biodiesel production in Germany consists almost exclusively of winter rapeseed (87% of the raw materials used in 2011) [91]. Winter rapeseed is the oil plant with the highest yields under German climatic conditions. Other oil plants like sunflower and flax play only a marginal role due to lower yields and therefore lower economic viability [92, 93]. While there was a brief decrease in the area under cultivation of rapeseed for biodiesel from 2000 to 2001 (according to Langert [90] most probably caused by increasing food prices), the area under biodiesel rapeseed increased steadily in the following years (from approximately 0.5 million ha in 2001 to a maximum of 1.1 million ha in 2007) (Fig. 5.1a). At the same time, the German production capacity for biodiesel (domestic and imported feedstock) increased from 0.5 to 4.4 million tons per year [94]. Since 2008, the area under cultivation of rapeseed for biodiesel has remained relatively stable at approximately 0.9 million ha (approximately 70% of the total rapeseed area) (Fig. 5.1a).

For bioethanol production, the domestic cultivation of energy crops increased strongly between 2005 and 2007 (Fig. 5.1a). In recent years, the area remained relatively constant at between 200,000 and 250,000 ha, of which approximately 80% are cultivated with cereals (mainly winter wheat and winter rye) and 20% with sugar beets [95].

The cultivation of crops for biogas production has increased dramatically since the 2004 revision of the EEG’s tariff scheme which introduced bonus payments for the use of energy crops in biogas plants. Between 2003 and 2012, the number of biogas plants increased from 1,750 (0.2 GW) to approximately 7,800 (3.5 GW) and accordingly the cultivation area for biogas substrates from 125,000 to nearly 1 million ha (Fig. 5.1a) [91].

The majority of the crop area for biogas production is used to grow silage maize. A survey of biogas plant operators showed that approximately 73% of the substrate input of renewable resources consists of maize silage, followed by 11% grass silage, 7% whole crop silage from cereals, 3% sugar beets and 1% intercrops (e.g., legumes, forage rye, rye grass and mustard) [96]. Prospective energy crops for biogas production are sorghum and Sudan grass. However, their cultivation is limited to field trails [91]. The dominance of silage maize as substrate for biogas plants is due to the well-established cultivation methods and the high standing crop yields and biogas yields of maize. In 2006, approximately 7% of the silage maize grown in Germany was cultivated for the production of biogas (200,000 ha). The percentage increased to approximately 37% in 2012 (800,000 ha). Nevertheless, the majority of silage maize is still used for the feeding of livestock (on an area that has remained relatively constant at between 1.2 to 1.4 million ha) [16].

5.4.1.2. Slight upward trend in domestic sales of pesticides

The total amount of domestic sales of pesticides (herbicides, fungicides, insecticides and acaricides) varied from approximately 24,000 to 31,000 t between 2000 and 2012 in Germany (Fig. 5.1b). Of the three types, herbicides are the most commonly sold (approximately 14,300 to 19,900 t), followed by fungicides (8,200 to 11,500 t). Insecticides and acaricides (without inert gases) had the lowest share (740 to 1,100 t) (Fig. 5.1b).

Between 2000 and 2012, there was a slight upward trend in the domestic sales of all three groups of pesticides (Fig. 5.1b). Agricultural pesticide use can vary considerably from year to year, depending on the development of weeds, plant diseases and insect populations which, in turn, depend on the weather conditions. While fungal diseases appear mostly in cold and wet conditions, pest insects increase in prevalence in warm and dry periods. For example, the drop in fungicide sales in 2004 could be explained by the extremely hot and dry year of 2003, which caused farmers to build up high stock levels of fungicides that were used up in 2004 [97].

As possible reasons for the increased pesticide sales since the year 2000, Gutsche [97] mentions the increase in arable land due to the re-use of brownfields or set-aside land, the conversion of permanent grassland into arable land and the increased cultivation of rapeseed and maize for energy production. Likewise, the German Federal Environmental Agency considers the increased cultivation of the energy crops maize and rapeseed as one of the possible reasons for the increased domestic sales of pesticides [98].

5.4.1.3. Pesticide demand of annual energy crops

The analysis of the annual treatment index values for Germany shows that the treatment index varies from year to year, depending on the occurrence of diseases and pests (Tab. 5.1).

Table 5.1: Average treatment index values for Germany from 2007 to 2011 (reference farm network, [87]) and for 2011 and 2012 (PAPA network, [89]), for the main energy crops and the main groups of pesticides. According to Roßberg [88], the treatment index values for winter barley are representative for winter rye and triticale. The total treatment index is calculated independently of the pesticide group and does not represent the sum of the three pesticide groups.

		Freier					PAPA	
Crop	Pesticide group	2007	2008	2009	2010	2011	2011	2012
Silage maize	Herbicide	1.8	2.5	1.9	2.0	2.2	1.85	1.92
	Fungicide	0	0	0	0	0	0	0
	Insecticide	0	0	0	0	0	0.04	0.01
	Total	1.8	2.5	1.9	2.0	2.2	1.89	1.93
Sugar beet	Herbicide	3.5	2.7	2.8	2.6	5.8	2.61	2.75
	Fungicide	1.4	1.2	1.2	1.1	1.2	0.93	1.10
	Insecticide	0.1	0.2	0.2	0.4	0.3	0.17	0.31
	Total	5.0	4.1	4.2	4.2	7.3	3.72	4.17
Winter barley	Herbicide	1.5	1.7	1.6	1.7	1.7	1.54	1.63
	Fungicide	1.1	1.3	1.3	1.3	1.4	1.32	1.43
	Insecticide	0.9	0.7	0.3	0.3	0.4	0.35	0.51
	Total	4.1	4.6	4.0	4.0	4.1	3.78	4.13
Winter rapeseed	Herbicide	1.6	1.8	1.7	1.6	1.8	1.68	1.72
	Fungicide	1.5	1.9	2.0	1.9	1.9	1.57	2.03
	Insecticide	2.3	2.3	2.8	2.8	3.1	2.93	2.72
	Total	5.4	5.9	6.4	6.4	6.7	6.18	6.47
Winter wheat	Herbicide	1.9	2.0	1.8	1.8	2.0	1.63	1.73
	Fungicide	1.9	2.2	2.0	1.9	1.8	1.71	1.86
	Insecticide	1.2	1.0	1.0	0.8	1.1	0.81	0.75
	Total	5.7	6.2	5.8	5.4	5.6	4.86	5.16

A major limitation of the treatment index is that it allows no direct comparison of the different crops or conclusions regarding the environmental effects caused by the crop-specific pesticide applications. For example, it sets the applied amount of pesticides in relation to the maximum allowed amount, which differs among crops and pesticides. In addition, it includes no information on the chemical and physical properties influencing pesticide effects on the environment or the toxicity to different species. Nonetheless, some general conclusions on the treatment intensity of different crops can be drawn.

Winter rapeseed has total treatment index values of 5.4 to 6.7 and needs the application of herbicides, fungicides and insecticides (Tab. 5.1). There are frequent occurrences of special rapeseed pests such as the blossom beetle and the rape stem weevil [99]. Therefore, cultivation breaks of three to four years are recommended to prevent diseases and pests from occurring in increasing frequency [100-102].

Sialge maize has total treatment index values of 1.8 to 2.5, with herbicides being the primary group of pesticides applied (Tab. 5.1). No fungicides and very rarely insecticides were applied at the investigated farms. These results are consistent with the findings of Karpenstein-Machan and Weber [102], who interviewed 76 farmers growing energy crops in Lower Saxony. In their survey, only one agricultural company was found to have applied fungicides for the cultivation of maize, and no insecticides were reported to have been used. In recent years, however, there have been an increasing number of reports from German authorities of the local occurrence of the Western corn rootworm and the European corn borer [103]. In case of pest infestation, the affected areas need to be sprayed with insecticides. In addition to the application of insecticides and mechanical methods of treatment, Mielke and Schöber-Butin [104] suggest avoiding long-lasting monocultures of maize. To minimize the risks of increased disease and pest pressure and to prevent soil degradation, maximum shares of maize in the crop rotation between 25 and 66% (depending on the soil type) are recommended [100, 105].

The cereal crops winter wheat and winter barley have total treatment index values of 3.8 to 6.2 (Tab. 5.1). Winter wheat is the most widely grown crop in Germany, accounting for approximately 25% of the arable land in 2011. The continuous growing of winter wheat is problematic due the existence of soil-borne pathogens that lead to reduced yields and increased production costs. Therefore, it is recommended that the maximum share of winter wheat be 33% of the crop rotation and that the maximum share of all cereals be 75 % of the crop rotation [100]. For sugar beets, primarily herbicides are used for plant protection. In the juvenile stage, sugar beets have a low competitiveness against weeds, which can hamper their growth and reduce the yield significantly. The recommendations for the maximum share of sugar beets in the crop rotation vary between 25 and 33% (with regular intercropping) [100].

There have only been a few quantitative studies to date on the differences in plant protection measures for crops that can be used for either food or bioenergy production. Rippel et al. [101] assume that the same types of pesticides will be applied for a specific crop, regardless of its subsequent use. Therefore, Rippel et al. [101] expect only slight changes in the treatment index values of crops that are used for energy instead of food production (Tab. 5.2). For example, the cultivation of cereals as whole crop silage for use in biogas plants and the associated early harvest offers the potential for reduced pesticide use (especially late fungicide treatments). The hypothesis that only minor differences are to be expected is supported by the fact that most farmers decide after the harvest where they will sell their products.

Table 5.2: Possible changes in the treatment indexes for the main energy crops in comparison to the use of the same crop for food production (based on Rippel et al. [101]).

Crop and bioenergy pathway	Change of treatment index
Rapeseed for biodiesel	+/-0
Rapeseed as whole crop silage for biogas	-0.5 to -0.25
Sugar beet for bioethanol	+/-0
Cereals for bioethanol	-0.25 to +0.25
Cereals for combustion	-0.5 to 0
Cereals as whole crop silage (biogas)	-1 to -0.5
Silage maize (main crop) for biogas	-0.25 to +0.25
Silage maize (secondary crop) for biogas	-1 to -0.25

5.4.1.4. Regional expansion of rapeseed and silage maize up to the recommended maximum share

The increase in the cultivation of winter rapeseed and silage maize was not evenly distributed across Germany. Between 2003 and 2007, the main extension of the rapeseed cultivation took place in the Federal States Lower Saxony (+65,000 ha), Saxony Anhalt (+62,000 ha), Mecklenburg-West Pomerania (+40,000 ha) and Brandenburg (+30,000 ha) [106]. As a result, for example, in Mecklenburg-West Pomerania, the share of winter rapeseed increased in some districts up to 28% of the arable land in 2010 and, therefore, reached already the recommended maximum share of rapeseed in the crop rotation of 25-33% (Fig. 5.2).

The same development can be observed for silage maize. For example, in Lower Saxony, the Federal State with the largest area of maize fields, there is a clear trend of increasing shares of silage maize on arable land, especially in the Northern part (Fig. 5.2c). One district even reached a maximum of 71% in 2012 (for comparison: German recommendations vary between 25 and 66% [105]). The continuous cultivation of maize involves risks of increased disease and pest pressure [100] (see Sec. 5.3.1.3). Likewise, many studies have shown that continuous monoculture systems not only increase soil degradation, nutrient leaching and loss of biodiversity but also support the build-up of pathogens and pests and therefore increase the need for disease, pest and weed control [107-109]. As Tilman et al. [83] argue, the number of diseases and the disease incidence increases in proportion to the host abundance. For maize, Deuker et al. [103] notes that the infestation risk for the Western corn rootworm and consequent damage are closely linked to the percentage of maize within the regional crop rotation scheme.

In the 2012 amendment of the EEG, the German government responded to the concerns regarding maize monocultures and introduced a new prerequisite for the payments for electricity from biogas plants: a maximum combined annual feedstock share of maize silage and cereal grains of 60% of the mass content [110].

However, this decision does not take into account the strong regional variation of the maize share in the crop rotation or the fact that maize could diversify crop rotations in areas with, for example, high shares of cereals. Cereals are the dominant crops in Germany (approximately 55% of the total arable land) and reach shares of more than 60% in some districts. For example, in some Southern districts of Lower Saxony, the share of cereals decreased from 60-70% in 2003 to 50-60% in 2012, while the share of silage maize increased about 10% (Fig. 5.2b and c). In accordance with that, a survey by Karpenstein-Machan and Weber [102] of Lower Saxonian farms revealed that energy crops have mainly replaced winter wheat. Therefore, in cereal dominated areas, the integration of maize could help to break up monocultures. Another advantage is that maize does not transfer cereal diseases and therefore can reduce the phytopathogenic potential of soils of winter-cereal-rich crop rotations [102]. In general, crop rotation has been long recognised as a system for controlling pests and diseases that can become established in the soil over time [111-113]. Furthermore, as usually no fungicides and rarely insecticides are used in maize cultivation compared to winter wheat (Tab. 5.1), a reduction in pesticide pollution (especially insecticides and fungicides) can be expected.

An extensive agricultural system that promises not only greater biodiversity in agro-ecosystems but also a reduction in pesticide use is the mixed cropping system. In this system, different annual energy crops are cultivated simultaneously in the same field, e.g., maize and sunflowers or cereals and false flax. For biogas production in particular, mixed crops can be used as whole crop silage without any difficulties. Mixed stands are assumed to have lower disease and pest infection rates and to show a better weed suppression, leading to reduced demand for herbicides [13, 92, 114-117]. However, it is more difficult to mechanise crop mixtures and to optimise their management due to the different demands of the crops for with nutrients, water, and light. Therefore, crop mixtures typically have lower biomass yields than single stands under optimised crop management [114, 115]. Nevertheless, more research is needed for a conclusive assessment of mixed cropping systems.

In general, innovative crop rotations and cropping systems increase the needs for training, diversified farm activities, agricultural equipment, and storage facilities. As Zegada-Lizarazu and Monti [118] note, farmers may regard them riskier and prefer to maintain with their established, often continuous monoculture systems.

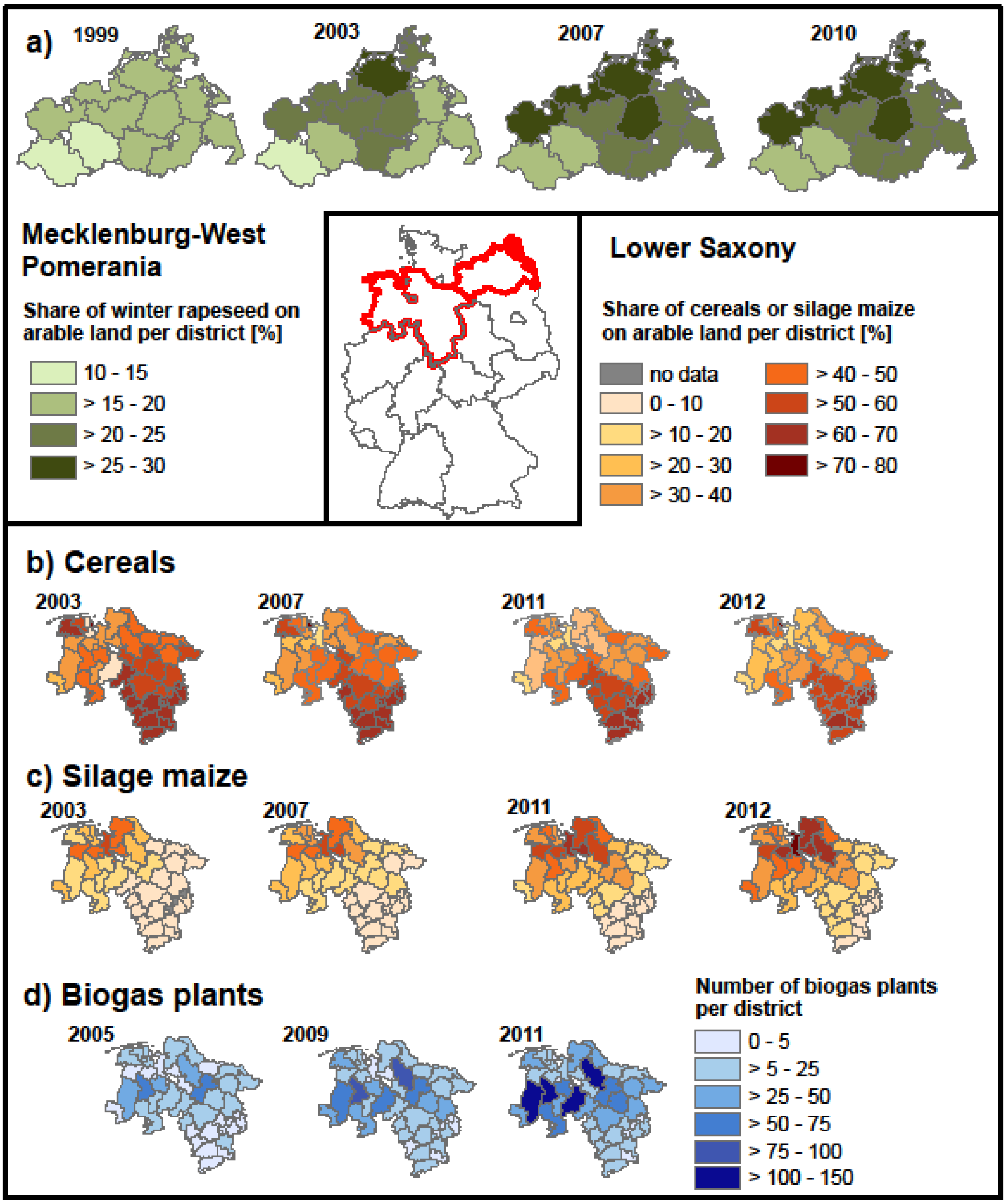

Fig. 5.2: Development of a) the share of winter rapeseed on arable land per district of Mecklenburg-West Pomerania (1999 to 2010, based on Statistical office of Mecklenburg-West Pomerania [119]), b) the share of cereals and c) of silage maize on arable land per district of Lower Saxony (2003 to 2012, reported within framework of CAP direct payments, based on Lower Saxony Chamber of Agriculture [120]), and d) the number of biogas plants (2005 to 2011, based on Lower Saxony Network for Renewable Resources [121]).

5.4.1.5. Re-use of set-aside land and increased conversion of grassland

After the EU set-aside scheme became compulsory in 1992, the amount of set-aside land increased up to 1.36 million ha in Germany (Fig. 5.3) [122]. While farmers were prohibited from growing food or feed crops on these areas, the cultivation of energy crops was permitted. This resulted in a rapid increase of rapeseed cultivation on German set-aside fields (Fig. 5.3). In 2007, due to the recent boom in biofuels and increasing demand for cereal crops, the European Commission suspended set-aside for the 2008 harvest year and abolished it completely in the following year [123, 124]. As a result, the amount of German set-aside fields decreased to approximately 200,000 ha (Fig. 5.3).

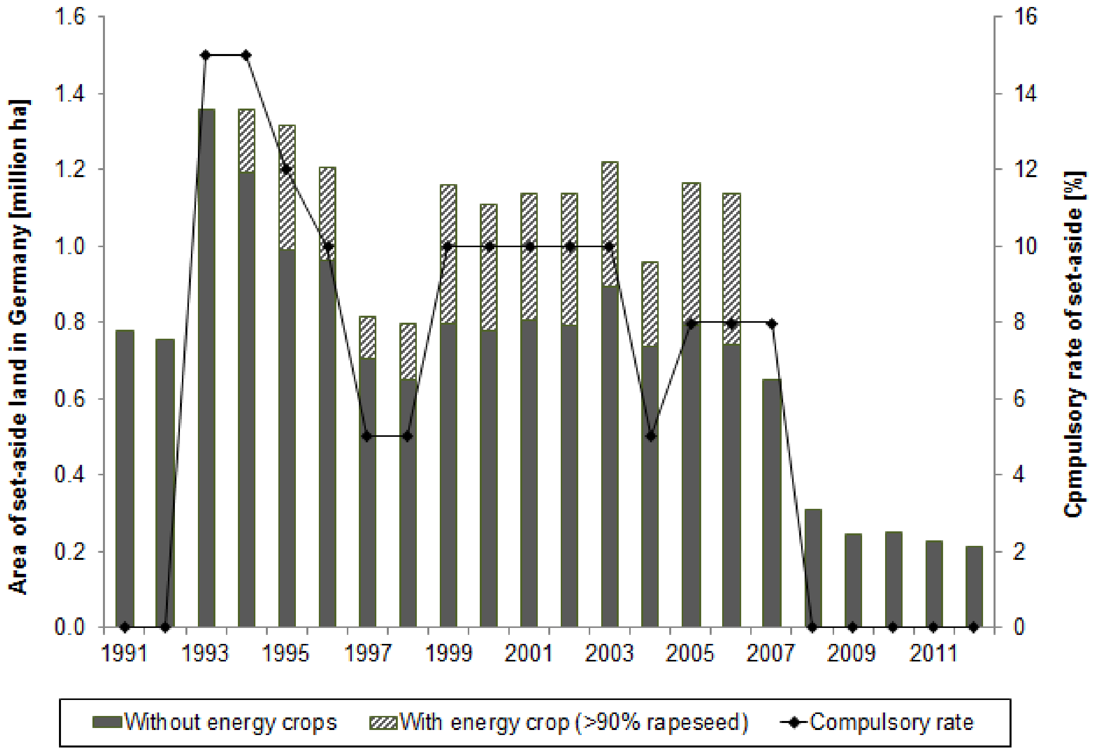

Fig. 5.3: Development of set-aside land with and without energy crops in Germany and compulsory rate of set-aside from 1991 to 2012 (based on Defra [125], FNR [16], and BVL [126]).

Beside set-aside land, also permanent grassland has been put under increasing pressure in the last years in Germany [106]. Beside its function for agricultural production, permanent grassland provides several ecological functions (e.g., carbon storage, soil protection, provision of habitat) and represents an essential part of diverse agricultural landscapes. The conversion of arable land is accompanied by the loss of these functions [127-130].

Furthermore, the conversion of permanent grassland or set-aside land to arable land results in an increase in pesticide pollution. For example, in the first years, new fields have an above-average demand for herbicides [97]. The extent of the total increase in pesticide pollution will depend on whether the new arable land is used to grow energy crops with a comprehensive demand for crop protection such as rapeseed or energy crops with lower pesticide demand such as perennial crops.

In Germany, the area of permanent grassland decreased from about 5 million ha in 2003 to 4.6 million in 2012 [106]. However, there are significant regional differences in the amount of grassland being converted to arable land. High losses of permanent grassland occurred especially in the Federal States Bavaria, Lower Saxony, North Rhine-Westphalia, and Schleswig-Holstein. These four states represent about two-third of Germany's production of cattle and silage maize as well as of Germany's biogas plants in 2012 [96, 131].

Nitsch et al. [132] analysed land use data from Lower Saxony, Mecklenburg-Western Pomerania, North-Rhine Westphalia, and Rhineland-Palatinate. They found that about 53% of the permanent grassland converted to arable land between 2005 and 2007 was occupied by silage maize in 2007. Nitsch et al. [132] suggest that the increased demand for silage maize as biogas substrate, together with the already existing demand as cattle feed, is increasing the pressure to convert grassland to arable land in these areas.

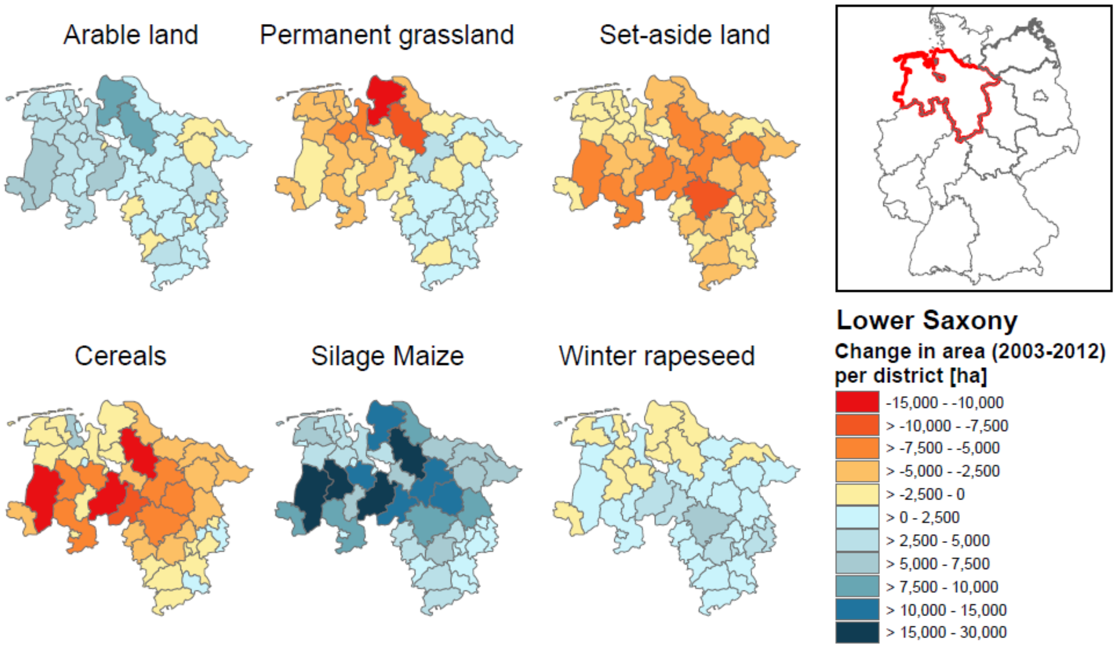

Fig 5.4: Change of the area of arable land, permanent grassland, set-aside land, and the cultivation area for cereals, silage maize, and winter rapeseed for the German Federal State Lower Saxony from 2003 to 2012 (exclusively areas reported within framework of CAP direct payments, based on Lower Saxony Chamber of Agriculture [120]).

The example of Lower Saxony illustrates clearly this relationship. Lower Saxony is the German Federal State with the highest increase in cultivation area for silage maize (from approximately 230,000 ha in 2003 to approximately 515,000 ha in 2012), especially in the North of Lower Saxony where cattle farming is traditionally of great importance (Fig. 5.2c). Biogas plants normally obtain their input materials from nearby fields and animal farms [133]. Therefore, the building of new biogas plants concentrated in the Northern part of Lower Saxony in the last years (Fig. 5.2d). In the same districts, huge areas of permanent grassland were converted and set-aside land put back into production between 2003 and 2012 (Fig. 5.4). While the cultivation area for cereals decreased, the amount of silage maize fields increased strongly. The number of cattle and, accordingly the demand for silage maize as cattle feed, remained relatively stable in the same time period. Therefore, the converted grassland and the re-used set-aside land were most probably used to cultivate silage maize as substrate for the newly built biogas plants.

The drastic decrease of permanent grassland in some regions of Germany suggests that the current instruments (e.g., CAP's cross compliance, national and regional environmental legislation) are not sufficient to protect grassland [132]. Some German Federal States (e.g., Lower Saxony) have already reacted on the strong decline of permanent grassland by enacting supplementary legislation that requires prior authorisation for ploughing of grassland.

However, if set-aside land and permanent grassland is to be preserved, the increased cultivation of energy crops, whilst ensuring the food production is not compromised, may increase the pressure on agricultural productivity and, therefore, lead to a further intensification of agricultural management systems. This intensification could be associated with increased use of external inputs such as pesticides.

5.4.2. Perennial energy crops

5.4.2.1. Perennial energy crops (so far) of minor importance

Perennial energy crops offer several environmental advantages such as reduced soil erosion, improved wildlife cover and lower needs for fertilisers [134, 135]. In addition, their yields are comparable or even higher than annual energy crops (e.g., heating oil equivalent in l/ha*year: short-rotation coppice: 5,120; miscanthus: 6,081; silage maize: 5,280; whole crop cereal silage: 4,013; rapeseed oil: 1,528) [136].

Nevertheless, the cultivation of perennial energy crops on arable land is still in its infancy in Germany. In 2012, short-rotation coppice (willow or poplar) took place on 4,500 ha, compared to 1,000 ha in 2007 [91]. Most of the cultivation is operated by energy companies that burn the wood in their decentralised biomass heating and power stations. A promising perennial grass is miscanthus, which was grown on 2,000 ha in Germany in 2012, mainly for thermal use [91].

There are several reasons for the marginal importance of perennial energy crops in Germany. In contrast to annual crops that fit well into conventional crop rotations, the conversion to perennial crops involves many challenges. Most of the farmers have no practical knowledge of how to establish, maintain and harvest short-rotation coppice and miscanthus plantations. In addition, there are many uncertainties about potential purchasers and markets, and the price and required quality of biomass [137]. Furthermore, the breeding of appropriate planting material is still in progress [100].

Beside plantations of perennial energy crops, the concept of agroforestry, i.e., the cultivation of trees in association with annual crops, is coming back into focus as a promising approach to combine food and energy production [138, 139]. Another currently discussed option is the use of short-rotation coppice as riparian buffer strips to reduce pesticide and nutrient input from adjacent fields and to enhance the landscape diversity and wildlife habitat [140-142].

5.4.2.2. Pesticide demand of perennial energy crops

Several studies emphasise the advantages of perennial crops compared to annual crops in terms of their lower demand for pesticides [13, 14, 143]. For example, the European Environmental Agency classifies perennial bioenergy crops as posing a low environmental risk with respect to the indicator “pesticide pollution of soils and water” (proxy indicator based on qualitative description of crop-specific pest sensitivity in the literature) [13]. In contrast, cereal crops and maize are estimated to pose a moderate level of environmental risk, and rapeseed, sugar beets and potatoes are estimated to pose a high environmental risk [13].

For short-rotation coppice, it is important to keep the plantation weed free until the canopy is closed, usually in summer of the second year. Therefore, mechanical or chemical weed control in the pre-ploughing and post-planting phases is recommended to guarantee that the trees become well established. For sustainability reasons, increased mechanical weed control is preferred over increased use of herbicides [100, 144].

In addition to weed problems, several studies have reported fungal infestations by *poplar or willow* leaf rust (*Melampsora* spp.) that have led to serious yield losses on short-rotation coppice plantations [144-147]. In addition, the German Association for Technology and Structures in Agriculture mentions poplar leaf and shoot blight as an important leaf disease caused by *Venturia populina* [100]. However, adequate control of these leaf diseases through fungicides is not feasible from economic and ecological points of view [100, 144, 148]. Instead, it is recommended that the breeding *of varieties resistant to rust and*

Venturia populina be given priority [144, 149]. Furthermore, there is the possibility of significant yield loss due to insects, such as chrysomelid or longhorn beetles, the goat moth or the willow gall weevil [144, 150]. For Ireland, Styles et al. [151] and Caslin et al. [144] recommend the application of an insecticide in the post-planting phase to control crane flies. However, for Germany, it is assumed that the control of insects is normally not necessary [100]. Likewise, Dimitriou et al. [152] report little or no fungicide and insecticide use on the vast majority of Swedish and UK short-rotation coppice plantations. Zalesny et al. [153] found that pests and insects have not yet had any impact on yields of willow biomass crops in North America.

Like short-rotation coppice, the perennial grass miscanthus needs herbicides for weed control during the establishing phase (the first two years of growth) [151, 154, 155]. So far, no reported plant diseases or insect pests have significantly affected the production of miscanthus in Europe [155, 156]. However, the UK Department for Environment, Food and Rural Affairs (Defra) notes that the common rustic moth and ghost moth larvae feed on miscanthus and may cause problems in the future [155]. Bradshaw et al. [157] suspect that the yellow sugarcane and corn leaf aphids have the potential to damage young miscanthus.

A controversial issue associated with miscanthus is its potential to serve as a refuge or host for the Western corn rootworm, an important maize pest [158]. The larvae can survive to adulthood on *miscanthus* rhizomes, and adult beetles may lay their eggs at the base of *miscanthus* plants grown near maize fields. However, there are other crops, such as sorghum, soybean and cereals, that could also be potential hosts for the Western corn rootworm [103, 159]. In contrast to the concern that perennial crops may enhance pest numbers in existing food crops, Meehan et al. [160] suggests that strategically positioned perennial bioenergy crops could reduce insect damage and insecticide use on neighbouring food and forage crops by providing predatory arthropods (biocontrol services).

5.5. Conclusions

Increasing the usage of renewable energies, including agricultural bioenergy, is an important policy objective of the EU. Given the environmental pressures arising from current agricultural food production, the large-scale expansion of energy crop cultivation needs to be conducted in a sustainable way. Our findings reveal that the growth of energy crops will not necessarily cause an increase or decrease in the amounts of pesticides released into the environment. Due to the great variety of energy crops, the potential effects will depend rather on the future design of the agricultural systems.

Instead of creating energy monocultures, annual energy crops should be integrated into the existing food production systems. Financial incentives and further education are required to encourage the usage of sustainable crop rotations, innovative cropping systems and the cultivation of perennial energy crops, which may add to crop diversity and generate lower pesticide demands than do intensive food farming systems. In addition, a further extension of the cultivation of energy crops should be accompanied by mandatory restrictions to protect the remaining permanent grassland.

5.6. Acknowledgments

We would like to thank the statistical offices of the German Federal States for providing the statistical data on agriculture. Furthermore, we are grateful to Kirsten Murek, Lower Saxony Chamber of Agriculture, for the quick and competent assistance with the Lower Saxonian GAP data. We also thank Dietmar Roßberg, Julius-Kühn-Institut, for his help with the treatment index. We are grateful to the anonymous reviewers for their helpful comments on a previous version of the manuscript. This work was made possible by funding from the Helmholtz Association of German Research Centres within the project funding “Biomass and Bioenergy Systems” and supported by Helmholtz Impulse and Networking Fund through Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE).

6. Discussion of the main results

6.1. Insecticidal effects of WWTP effluents cause long-term changes in the downstream macroinvertebrate community structure

A clear trend of decreasing $SPEAR_{pesticides}$ values with decreasing upstream distance to the next municipal WWTP was found for the investigated 663 sites in the German Federal States of Saxony, Saxony-Anhalt, Thuringia, and Hesse.

For the Hessian sites, there was a significant reduction of species that were classified as pesticide-sensitive according to the SPEAR concept at sites with a WWTP within 3 km upstream compared with sites without a WWTP within 3 km upstream. The results for the Runoff Potential indicate that the surface runoff from adjacent arable land was not a major source of pesticides at any of the investigated sites. A total of 75% of the sites with a WWTP but only 31% of the sites without a WWTP within 3 km upstream had $SPEAR_{pesticides}$ values that were lower than 33%. According to Beketov et al. [56], the $SPEAR_{pesticides}$ values that were lower than 33% relate to a moderate to bad ecological quality. For the sites in Saxony, Saxony-Anhalt and Thuringia, significant insecticidal effects of WWTP effluents were only detected up to a maximum of 1.5 km downstream. A total of 77% of the sites with a WWTP and 47% of the sites without a WWTP within 1.5 km upstream had $SPEAR_{pesticides}$ values that were lower than 33%.

The different distances of influence (1.5 or 3 km) could not be explained by the available technical characteristics (capacity and applied treatment steps) of the WWTPs. However, it seems possible that there are more relevant factors such as the number of farms that were connected to the WWTP or the proportion of the WWTP effluent compared to the water load of the receiving stream. For example, more Hessian sites had large WWTPs (>10,000 Population Equivalent) and, therefore, potentially higher effluent volumes entering the receiving streams.

The acquisition of these additional data would require a survey of the local WWTP operators and was not possible within the frame of this large-scale study. For future small-scale studies, it is recommended to collect these data and to evaluate their influence on the spatial dimension of the effects of the WWTP effluents.

In general, the $SPEAR_{pesticides}$ results show that the insecticidal effects of WWTP effluents can cause long-term changes in the structure of the downstream macroinvertebrate community. The pesticides are either not degraded completely in the WWTPs or are discharged to streams through combined sewer overflows. The majority of the Hessian sewer network consists of a combined sewer system that collects stormwater and domestic wastewater in a single pipe system and then directs the water to the next WWTP [55]. During intensive rainfalls, these combined sewer systems discharge excess untreated water via overflows directly into the streams. Several studies have already identified combined sewer overflows as significant sources of organic pollutants, especially for substances that are normally removed at high rates by the WWTPs [65, 66, 161].

Taken together, the findings of this thesis support previous studies that emphasised the importance of municipal WWTPs as point sources of pesticides [18, 23-26]. In addition, this thesis demonstrates that the amount of pesticides in the WWTP effluents seems to reach sufficiently high levels to significantly affect the downstream macroinvertebrate community up to at least a 1.5 km distance (in some cases even 3 km).

6.2. WWTP effluents are still important sources of organic pollution

For the Hessian sites, a significant difference in the German Saprobic Index values was found for sites with and without a WWTP within 3 km upstream. A total of 51% of the sites with a WWTP within 3 km upstream had a German Saprobic Index value that was higher than 2, indicating a medium ecological quality class according to the German WFD classification system. In contrast, 91% of the sites with no WWTP within 3 km upstream had a good or even high ecological status. This finding contradicts the current view of the German water authorities that oxygen-depleting substances in the WWTP effluents only rarely pose a problem for the health of stream ecosystems [52].

As for the insecticidal effects of the WWTP effluents, a possible reason for the detected organic pollution could be the combined sewer systems that account for the majority of the Hessian sewer network [55]. Several studies have shown that combined sewer systems tend to release higher loads of oxygen-demanding substances, particularly of nutrients, into the receiving stream [66, 162-165]. However, a general recommendation to use separate sewer systems cannot be given. Both of the sewer systems have specific advantages and disadvantages [165-167]. For example, while separate sewer systems have only limited or no risk of sewage overflows, they have much higher construction and management costs than do combined sewer systems. Furthermore, separate sewer systems can be problematic in urban areas where the polluted runoff from the streets containing, e.g., different heavy metals, is discharged directly into the stream. Therefore, a general answer to the question as to which system is better - combined or separate - cannot be given. The answer is case-specific and depends on the characteristics of the region, e.g., the pollutants, the pollution of the stormwater and the temporal and spatial rain variability [167].

In addition to the issue of pesticides and oxygen-depleting substances, recent studies have emphasised the importance of the WWTP effluents as sources for micropollutants such as pharmaceuticals or endocrine disruptors [168-170]. However, the results for the index $SPEAR_{organic}$ indicated that the continuous exposure to organic toxicants seems to play no important role at the investigated sites in Hesse. There were no significantly different $SPEAR_{organic}$ values between the sites with or without a WWTP upstream. However, this result needs to be interpreted with caution. So far, $SPEAR_{organic}$ has been only successfully applied along one large-scale river continuum in southwestern Siberia. Further studies may be necessary to prove the specificity of the index $SPEAR_{organic}$ to general organic toxicants.

6.3. Riparian buffer strips and forested upstream reaches can be efficient mitigation measures for pesticide contamination

The analysis of the 663 sites in central Germany showed that riparian buffer strips that were at least 5 m in width positively affected the index $SPEAR_{pesticides}$.

Therefore, it can be assumed that buffer strips can reduce the pesticide input from adjacent arable land or garden allotments. The highest $SPEAR_{pesticides}$ values were at sites with broad buffer strips that were greater than 20 m. Therefore, the results of this thesis support the findings of other studies that have suggested using riparian buffer strips to reduce the pesticide input from adjacent fields [17, 27, 28]. However, the $SPEAR_{pesticides}$ values varied considerably for sites with approximately the same Runoff Potential, and therefore, the same diffuse pesticide contamination, and buffer strip width (Fig. 4.4). This result is in agreement with the findings of other studies that determined that strip width is not the only important factor for the efficiency of riparian buffer strips [17, 30]. Other important factors include the buffer slope, soil type, and vegetation cover [171, 172].

Furthermore, there was a clear trend of increasing $SPEAR_{pesticides}$ values with the increasing number of forested upstream reaches within 5 km upstream. These findings are consistent with those of previous studies that reported a significant positive effect of forested upstream reaches on the $SPEAR_{pesticides}$ values [20-22, 73]. Therefore, forested upstream reaches could be suitable for enhancing the recovery potential of affected stream sections in landscapes with relatively intensive agriculture.

6.4. Independence of the bioindicator $SPEAR_{pesticides}$ from other environmental stressors requires further evaluation

The studies of Liess and von der Ohe [20], Schäfer et al. [21] and Schletterer et al. [44] found no significant responses of $SPEAR_{pesticides}$ to environmental factors or stressors other than pesticide contamination.

In contrast, the analysis of the Hessian sites showed a strong correlation of $SPEAR_{pesticides}$ with the German Saprobic Index (Spearman rank correlation coefficient $\rho=-0.8$, $p<0.001$). This result may be explained by the fact that a part of the investigated species is sensitive to pesticides and low-oxygen conditions (e.g., *Drusus annulatus*) or tolerant to both of the conditions (e.g., *Erpobdella octoculata*). However, a clear differentiation between the two stressors pesticide contamination and oxygen deficiency can only be based on taxa that respond differently to both of the stressors. Therefore, a subsequent analysis of two adapted taxon lists was conducted. The effects of toxicants (pesticides and general organic toxicants) were elaborated by considering only the taxa that were tolerant to oxygen deficiency (taxa with a German Saprobic Value≥2). The shortened taxon list was used to recalculate the indices $SPEAR_{pesticides}$ and $SPEAR_{organic}$. A second adapted taxa list including only the taxa that were tolerant to organic toxicants (according to the SPEAR approach, with median of sensitivity to organic toxicants s>-0.36, [20]) was used to evaluate the effects of oxygen deficiency (recalculation of the German Saprobic Index). The recalculated $SPEAR_{pesticides}$ and German Saprobic Index values were still significantly lower and higher, respectively, at sites with a WWTP within 3 km upstream. In contrast, the $SPEAR_{organic}$ values were not significantly different between the sites with or without a WWTP after the removal of the taxa that were sensitive to oxygen deficiency (Sec. 6.2).

In addition to the stressor organic pollution, the results of this thesis indicate that the structural stream quality of the sampling site significantly affected the bioindicator $SPEAR_{pesticides}$. The analysis of the 663 sites in Central Germany revealed a clear trend of decreasing $SPEAR_{pesticides}$ values with increasing hydromorphological degradation. In particular, sites with completely changed hydromorphology (structural quality class 6 to 7) (e.g., concrete channels with extensive embankment consolidation or straight artificial stream beds) had significantly lower $SPEAR_{pesticides}$ values even when there was no obvious upstream pesticide source.

These results are consistent with those of Rasmussen et al. [51], who found that $SPEAR_{pesticides}$ responds to characteristics of the physical habitat mainly due to a decrease in the species richness and abundance of EPT taxa. Many EPT taxa have relatively strict habitat requirements and are vulnerable to the loss of habitat heterogeneity [62, 74]. Therefore, Rasmussen et al. [51] argued that EPT taxa that are to a great extent pesticide-sensitive according to SPEAR may be partially restricted from colonising agricultural streams with uniform and degraded physical conditions.

A first attempt to distinguish between the effects of habitat degradation and pesticide stress has been made by von der Ohe and Goedkoop [73] for 100 streams in Sweden. However, instead of using species-specific data on the habitat requirements, von der Ohe and Goedkoop [73] derived their $SPEAR_{habitat}$

index based on the occurrence frequency of the observed species. Starting with the taxon with the highest occurrence, the occurrences of each subsequent taxon were summed, until the cut-off value of 50% was reached. Taxa with high occurrence frequencies were classified as “not at risk”. The findings of von der Ohe and Goedkoop [73] suggest that $SPEAR_{habitat}$ has a high degree of specificity for the effects of habitat degradation. In contrast to the results of this thesis, von der Ohe and Goedkoop [73] found that $SPEAR_{pesticides}$ was not affected by habitat degradation. However, an important limitation of von der Ohe and Goedkoop [73] is that most of the investigated Swedish sites were only characterised by low to medium hydromorphological degradation.

Taken together, the findings of this thesis suggest that further studies needs to be conducted to determine the effect of hydromorphological degradation on the index $SPEAR_{pesticides}$. Similar to the approach that was used in this thesis for organic pollution, a future study could investigate the relationship between the classification of a certain taxa according to the SPEAR concept and according to the German Fauna Index. The German Fauna Index indicates the impact of hydromorphological degradation on stream macroinvertebrates and is based on a stream type-specific list of indicator taxa (species-specific score from -2 (taxon shows a preference for hydromorphological degraded streams) to +2 (taxon shows a preference for streams with near-natural conditions)) [173]. Until final clarification, the influence of hydromorphological degradation needs to be considered when interpreting $SPEAR_{pesticides}$ to avoid overestimating the toxic impact of pesticides. Especially in highly degraded streams, it is currently not possible to distinguish between the stressors pesticide contamination and hydromorphological degradation based on $SPEAR_{pesticides}$.

In summary, the results of this thesis demonstrate the need for additional studies to evaluate the independence of $SPEAR_{pesticides}$ from other environmental stressors (e.g., hydromorphological degradation, organic pollution, and acidification).

6.5. Effects of energy crop expansion will depend on the design of future agricultural systems

The analysis of the development of agricultural bioenergy in Germany showed that the area under the cultivation of energy crops more than doubled in the last ten years (from 0.9 million ha in 2000 to 2.1 million ha in 2012) [16]. Although there exists a wide variety of potential energy crops, the development focused on two previously established conventional crops with high production potentials: winter rapeseed for biodiesel (0.9 million ha in 2012) and silage maize for biogas production (0.8 million ha in 2012) [16]. The increase in the cultivation of these two crops was not evenly distributed across Germany. In regions with a strong increase, the share of these crops reached or even exceeded the recommended maximum share in the crop rotation (rapeseed: 25 to 33%, silage maize: 25 to 66%) [119, 120]. Limits on the share of a specific crop in a crop rotation are recommended to prevent diseases and pests from occurring in increasing frequencies [100-102, 105]. Several studies have demonstrated that continuous monoculture systems not only increase the soil degradation, nutrient leaching and loss of biodiversity but also involve risks of increased disease and pest pressure [83, 107, 108]. However, in cereal dominated areas the cultivation of energy crops such as silage maize could help to break up cereal monocultures.

In addition, the analysis of the statistical land use data showed an increased amount of permanent grassland being converted to arable land in German regions with strongly increased energy crop cultivation, especially of silage maize. The conversion of permanent grassland to arable land is accompanied by the loss of ecological functions such as carbon storage, soil protection and provision of habitat [127-130]. Therefore, a further extension of the cultivation of energy crops should be accompanied by mandatory restrictions to protect the remaining grassland.

The analysis of the treatment index (“Behandlungsindex”, [89]), the generally agreed-upon indicator of the crop-specific pesticide intensity in Germany, showed that winter rapeseed is the energy crop with the highest total treatment index and by far the highest treatment index of insecticides. Maize has the lowest treatment index, with herbicides being the primary group of applied pesticides. Cereal crops and sugar beets have moderate treatment index levels. So far, it is assumed that the same types of pesticides will be applied for a specific annual crop regardless of its subsequent use (food or energy production) [101]. Therefore, Rippel et al. [101] expect only slight changes in the intensity of pesticide use on crops that are used for energy instead of food production.

Compared with the development of annual energy crops, the cultivation of perennial energy crops on arable land is still in its infancy in Germany (4,500 ha of short-rotation coppice and 2,000 ha of Miscanthus in 2012). The results of the literature research on the pesticide demand of these perennial energy crops support the findings of previous studies that emphasise the advantage of perennial energy crops compared with annual crops in terms of a lower pesticide demand [13, 14, 143]. To date, short-rotation coppice and miscanthus only require herbicides during their establishment phase [100, 135, 144, 151, 155]. Diseases and pests have usually no significant impact on the yields of these crops [100, 152, 153, 155, 156]. Furthermore, previous studies have recommended the breeding of resistant varieties rather than the application of insecticides [100, 144, 147].

The results of this study reveal that the future large-scale expansion of energy crop cultivation will not necessary cause an increase or decrease in the amounts of pesticides that are released into the environment. Due to the wide variety of energy crops, the potential effects will depend instead on the future design of the agricultural systems.

7. Conclusions and implications for water managers

The findings of this thesis emphasise the need for WFD managers to consider all main agricultural pesticide sources and influencing landscape parameters when setting up RBMPs and defining PoMs. In addition, the following recommendations can help the WFD manager to successfully tackle the risk of pesticide contamination to achieve the WFD-targets.

7.1. Identification and stressor-specific assessment of the sites of concern

As a first step, the developed screening approach allows an initial quick and cost-effective identification of sites where macroinvertebrate communities may be jeopardised by pesticide contamination. This screening approach is exclusively based on readily available GIS data. Such a screening could be beneficial for water authorities when targeting more efficiently restricted monitoring capacities and identifying sites where site-specific assessment is necessary.

The site-specific assessment needs to be adapted to the stressor pesticide contamination. As several studies have shown, the practice of collecting monthly or quarterly water samples may not be sufficient for detecting pesticides that show high fluctuations in their concentration over time [23, 46, 174]. Therefore, the frequency and the timing of the sampling must be adapted to this time-varying stressor.

In addition to the chemical monitoring, the use of the bioindicator $SPEAR_{pesticides}$ could help to detect macroinvertebrate communities that are impaired by pesticide contamination [20, 21, 46]. While bioindicators have certain advantages, such as the possibility of integrating different time periods and identifying indirect effects, they need to be interpreted with caution. Ideally, stressor-specific bioindicators are clearly linked to a single stressor and are independent of other stressors. However, when applied to field data, there are often correlations between the indicators for different stressors. As the results of this thesis demonstrate, when interpreting the $SPEAR_{pesticides}$ values, at least the effect of other stressors such as oxygen-deficiency and hydromorphological degradation needs to be considered (Sec. 6.4).

Neglecting possible correlations may lead to false conclusions regarding the stressor that impairs the stream. A clear link to a single stressor would only be possible when the different traits or trait combinations are independently distributed across taxa. Otherwise, the selection of traits that are related to one stressor will cause a correlated trait-response for another stressor without any environmental influence. This problem will be particularly severe in regions with small taxon pools where it will be less likely to find taxa that can help in differentiating between stressors. As Verberk et al. [175] pointed out, selection pressures do not act independently on single traits, but rather, on species whose success in a particular environment is controlled by many interacting traits. Therefore, trait-based indicators need to consider the way combinations of traits interact.

After having identified and monitored significant pesticide sources, WFD managers need to plan and design appropriate mitigation measures to either reduce the input of pesticides or to increase the resilience of the aquatic community to periodic pesticide contamination.

7.2. Mitigation measures for the WWTPs as point sources of pesticides

There is an ongoing debate about the need of additional treatment steps, such as ozonation or adsorption to activated carbon, to remove not only pesticides but also other organic micropollutants (e.g., pharmaceutical residues, and cosmetics ingredients or detergents) from the municipal WWTP effluents [176-179]. However, these optional treatment processes are connected to a high level of investment and increased maintenance costs. Therefore, the costs of these treatment steps need to be weighed against the benefits. Especially in agricultural areas with low population densities, it may be a more favourable measure to increase the awareness of farmers with regard to the WWTPs as important sources of pesticide contamination. For example, training courses and campaigns could help to improve the handling of pesticides, e.g., cleaning the sprayer equipment directly on the treated field instead of cleaning in the farmyards.

Furthermore, in the case of combined sewer systems, water managers need to evaluate mitigation measures to reduce the combined sewer overflows into the receiving streams. For example, retention facilities could store the stormwater until a WWTP has the capacity to treat the stored stormwater. Another possibility is the systematic use of green infrastructure to reduce the quantity of stormwater flows into the combined sewer system (e.g., green roofs, porous pavements, and vegetated treatment systems).

7.3. Mitigation measures for diffuse agricultural sources of pesticides

The results of this thesis support the findings of other studies that riparian buffer strips could be used to reduce pesticide input from adjacent fields [17, 27, 28]. However, the effectiveness of buffer strips is very variable. Although the strip width is a significant factor for the buffer mitigation efficacy, factors such as the buffer slope, soil, and vegetation type also play an important role [30, 171, 172]. In addition, the effectiveness of these factors can be strongly reduced through hydraulic by-passes (e.g., rills, gullies, and tile drains) through the buffer zone [17, 30]. Therefore, it is not only important to thoroughly design buffer strips but also to constantly maintain them (e.g., removing sediments and mowing).

The creation of forested upstream reaches as mitigation measures is rather lengthy, costly and complex to put into practice. In addition, studies are lacking that scrutinise the necessary size and design of the forested upstream reaches to enable an efficient recovery of the disturbed downstream stretches.

Another currently discussed option is the use of plantations of fast-growing trees (short-rotation coppice, SRC) as riparian buffer strips [140-142, 180, 181]. During a growth period of approximately 20 years, SRC plantations require no fertilisers, pesticide application or soil cultivation. The aboveground woody biomass can be harvested every 3 to 5 years and used, for example, in the pulp and paper industry or to produce energy. Buffer strips of fast-growing trees could not only be an effective barrier to erosion, nutrient and pesticide input from agricultural fields into streams but could also enhance the landscape diversity and wildlife habitat [140, 141, 181]. Therefore, SRC plantations along water courses could be a sustainable method of combining water protection and energy production targets while improving the landscape structure and biodiversity. However, e.g., in Germany, the German Water Act currently hinders the planting of fast-growing trees in riparian buffer strips (Article 38 prohibits the removal of trees and shrubs, the conversion of grassland and the new planting of trees that are not suitable for the site). Furthermore, there are concerns that SRC plantations close to the stream bank could act as barrier for runoff during flood events. Therefore, in case of an amendment of the German Water Act, clear rules need to be established to ensure legal certainty of SRC buffer strips. Research and development projects on the feasibility of SRC plantations as riparian buffer strips could help to answer the open questions and to provide an incentive to overcome the legal obstacles.

In addition to the already existing agricultural food production systems, there are concerns that the future expansion of energy crop cultivation will lead to an increased pesticide contamination of ecosystems in agricultural landscapes. However, as the results of this thesis suggest, optimised cultivation systems with diverse crop rotations that integrate food and energy crops could not only help to minimise the environmental effects of pesticide exposure but also to improve monotonous agricultural landscapes and increase agricultural biodiversity. Monocultures of pesticide-intensive cultures, such as rapeseed and sugar beet, should be avoided. The recommended limits of the share of certain annual energy crops on crop rotation should not be exceeded. Financial incentives and further education are required to encourage the use of sustainable crop rotations, innovative cropping systems and perennial energy crops, which may add to crop diversity and generate lower pesticide demands than do intensive food farming systems.

The diversity of scenarios for the future agricultural food and energy production makes it impossible to provide a simple answer to the question as to whether the future energy crop expansion will lead to an increase or decrease in the amount of pesticide contamination of agricultural ecosystems. Further research is required to analyse different cultivation scenarios and their effects on the pesticide exposure of the neighbouring agricultural ecosystems. For example, as a follow-up of this thesis, an analyse different cultivation scenarios and their pesticide exposure to aquatic ecosystems using the Runoff Potential model is planned.

8. References

[1] European Parliament. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. In: Official Journal of the European Communities. 2000

[2] European Environment Agency. European waters - assessment of status and pressures. Copenhagen. EEA Report. 2012

[3] German Federal Environmental Agency (UBA). Water Resource Management in Germany - Part 2 Water quality. Dessau-Roßlau. UBA; 2010

[4] Schäfer RB, von der Ohe PC, Kuhne R, Schuurmann G, Liess M. Occurrence and toxicity of 331 organic pollutants in large rivers of north Germany over a decade (1994 to 2004). Environmental Science & Technology 2011;45:6167.

[5] European Environment Agency. Ecological or chemical status or potential of water bodies. [Last Update: 19.02.2012]. 2013 [cited 19.12.2013]. Available from http://discomap.eea.europa.eu/map/WISE/?configfile=http://discomap.eea.europa.eu/map/WISE/config_wfdsurfacewater.xml.

[6] von der Ohe PC, Dulio V, Slobodnik J, De Deckere E, Kühne R, Ebert R-U, et al. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. Science of The Total Environment 2011;409:2064.

[7] Kattwinkel M, Kuhne JV, Foit K, Liess M. Climate change, agricultural insecticide exposure, and risk for freshwater communities. Ecological Applications 2011;21:2068.

[8] Fenner K, Canonica S, Wackett LP, Elsner M. Evaluating Pesticide Degradation in the Environment: Blind Spots and Emerging Opportunities. Science 2013;341:752.

[9] Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmerson M, Morales MB, et al. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic and Applied Ecology 2010;11:97.

[10] Ware GW. Effects of pesticides on nontarget organisms. Residue Reviews 1980;76:173.

[11] Food and Agriculture Organization of the United Nations (FAO). FAO Domain - Ressources. FAO. 2013 [cited 19.12.2013]. Available from http://faostat3.fao.org/faostat-gateway/go/to/download/R/*/E.

[12] European Parliament. Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. In: Official Journal of the European Communities. 2009

[13] European Environment Agency. Estimating the environmentally compatible bioenergy potential from agriculture. Copenhagen. EEA; EEA Technical report 12/2007. 2007

[14] Fernando AL, Duarte MP, Almeida J, Boléo S, Mendes B. Final Report on Task 4.1 Environmental impact assessment of energy crops production in Europe. 2010

[15] Elbersen B, Startisky I, Hengeveld G, Schelhaas M-J, Naeff H, Böttcher H. Atlas of EU biomass potentials - Deliverable 3.3 Spatially detailed and quantified overview of EU biomass potential taking into account the main criteria determining biomass availability from different sources. Biomass Futures Project. 2012

[16] Fachagentur Nachwachsende Rohstoffe e.V. (FNR). Anbau Energiepflanzen (Energy crop cultivation - data and facts). FNR. 2013 [cited 09.10.2013]. Available from http://mediathek.fnr.de/grafiken/daten-und-fakten/anbau.html.

[17] Reichenberger S, Bach M, Skitschak A, Frede HG. Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review. Science of The Total Environment 2007;384:1.

[18] Neumann M, Schulz R, Schäfer K, Müller W, Mannheller W, Liess M. The significance of entry routes as point and non-point sources of pesticides in small streams. Water Research 2002;36:835.

[19] Bach M, Röpke B, Frede H-G. Pesticides in rivers - Assessment of source apportionment in the context of WFD. European Water Management Online. 2005

[20] Liess M, von der Ohe PC. Analyzing effects of pesticides on invertebrate communities in streams. Environmental Toxicology and Chemistry 2005;24:954.

[21] Schäfer RB, Caquet T, Siimes K, Mueller R, Lagadic L, Liess M. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Science of The Total Environment 2007;382:272.

[22] Schriever CA, Hansler-Ball M, Holmes C, Maund S, Liess M. Agricultural intensity and landscape structure: influences on the macroinvertebrate assemblages of small streams in northern Germany. Environmental Toxicology and Chemistry 2007;26:346.

[23] Berenzen N, Hummer S, Liess M, Schulz R. Pesticide peak discharge from wastewater treatment plants into streams during the main period of insecticide application: Ecotoxicological evaluation in comparison to runoff. Bulletin of Environmental Contamination and Toxicology 2003;70:891.

[24] Gerecke AC, Schärer M, Singer HP, Müller SR, Schwarzenbach RP, Sägesser M, et al. Sources of pesticides in surface waters in Switzerland: pesticide load through waste water treatment plants - current situation and reduction potential. Chemosphere 2002;48:307.

[25] Müller K, Bach M, Hartmann H, Spiteller M, Frede H-G. Point- and nonpoint-source pesticide contamination in the Zwester Ohm catchment, Germany. Journal of Environmental Quality 2002;31:309.

[26] Wittmer IK, Bader HP, Scheidegger R, Singer H, Luck A, Hanke I, et al. Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. Water Research 2010;44:2850.

[27] Borin M, Bigon E, Zanin G, Fava L. Performance of a narrow buffer strip in abating agricultural pollutants in the shallow subsurface water flux. Environmental Pollution 2004;131:313.

[28] Rasmussen JJ, Baattrup-Pedersen A, Wiberg-Larsen P, McKnight US, Kronvang B. Buffer strip width and agricultural pesticide contamination in Danish lowland streams: Implications for stream and riparian management. Ecological Engineering 2011;37:1990.

[29] Schulz R. Field Studies on Exposure, Effects, and Risk Mitigation of Aquatic Nonpoint-Source Insecticide Pollution: A Review. Journal of Environmental Quality 2004;33:419.

[30] Zhang X, Liu X, Zhang M, Dahlgren RA, Eitzel M. A review of vegetated buffers and a meta-analysis of their mitigation efficacy in reducing nonpoint source pollution. Journal of Environmental Quality 2010;39:76.

[31] Hickey MBC, Doran B. A review of the efficiency of buffer strips for the maintenance and enhancement of riparian ecosystems. Water Quality Research Journal of Canada 2004;39:311.

[32] Bereswill R, Golla B, Streloke M, Schulz R. Entry and toxicity of organic pesticides and copper in vineyard streams: Erosion rills jeopardise the efficiency of riparian buffer strips. Agriculture, Ecosystems & Environment 2012;146:81.

[33] Svendsen CR, Quinn T, Kolbe D. Review of macroinvertebrate drift in lotic ecosystems. Settle. 2004

[34] Niemi GJ, Devore P, Detenbeck N, Taylor D, Lima A, Pastor J, et al. Overview of case-studies on recovery of aquatic systems from disturbance. Environmental Management 1990;14:571.

[35] Palmer MA, Rensburger PA, Botts PS, Hakenkamp CC, Reid JW. Disturbance and the community structure of stream invertebrates: patch-specific effects and the role of refugia. Freshwater Biology 1995;34:343.

[36] Langford TEL, Shaw PJ, Ferguson AJD, Howard SR. Long-term recovery of macroinvertebrate biota in grossly polluted streams: Re-colonisation as a constraint to ecological quality. Ecological Indicators 2009;9:1064.

[37] Parkyn SM, Smith BJ. Dispersal constraints for stream invertebrates: setting realistic timescales for biodiversity restoration. Environmental Management 2011;48:602.

[38] Stehle S, Knäbel A, Schulz R. Probabilistic risk assessment of insecticide concentrations in agricultural surface waters: a critical appraisal. Environmental monitoring and assessment 2013;185:6295.

[39] Holt EA, Miller SW. Bioindicators: Using organisms to measure environmental impacts. Nature Education Knowledge 2011;2.

[40] Kolkwitz R, Marsson M. Grundsätze für die biologische Beurteilung des Wassers nach seiner Flora und Fauna. Mitteilungen der königlichen Prüfanstalt Wasserversorgung Abwasserbeseitigung Berlin-Dahlem 1902;1:33.

[41] Rolauffs P, Hering D, Sommerhaeuser M, Roediger S, Jaehnig S. Entwicklung eines leitbildorientierten Saprobienindexes für die biologische Fließgewässerbewertung (Biological stream assessment for characterising the

oxygen content based in stream type-specific reference conditions). Umweltbundesamt; UBA-Texte 2003

[42] Statzner B, Beche LA. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biology 2010;55:80.

[43] Schäfer RB, von der Ohe PC, Rasmussen J, Kefford BJ, Beketov MA, Schulz R, et al. Thresholds for the Effects of Pesticides on Invertebrate Communities and Leaf Breakdown in Stream Ecosystems. Environmental Science & Technology 2012;46:5134.

[44] Schletterer M, Fureder L, Kuzovlev VV, Beketov MA. Testing the coherence of several macroinvertebrate indices and environmental factors in a large lowland river system (Volga River, Russia). Ecological Indicators 2010;10:1083.

[45] Schriever CA, von der Ohe PC, Liess M. Estimating pesticide runoff in small streams. Chemosphere 2007;68:2161.

[46] Liess M, Schäfer RB, Schriever CA. The footprint of pesticide stress in communities - species traits reveal community effects of toxicants. Science of The Total Environment 2008;406:484.

[47] Beketov MA, Liess M. An indicator for effects of organic toxicants on lotic invertebrate communities: Independence of confounding environmental factors over an extensive river continuum. Environmental Pollution 2008;156:980.

[48] von der Ohe P, Liess M. Relative Sensitivity Distribution (RSD) of Aquatic Invertebrates to Organic and Metal Compounds. Environmental Toxicology and Chemistry 2004;23:150.

[49] Schaefer RB, Liess M. Species at Risk (SPEAR) Biomonitoring Indicators. In: Férard J-F, Blaise C, editors. Encyclopedia of Aquatic Ecotoxicology: Springer; 2014.

[50] Rubach MN, Baird DJ, Van den Brink PJ. A new method for ranking mode-specific sensitivity of freshwater arthropods to insecticides and its relationship to biological traits. Environmental Toxicology and Chemistry 2010;29:476.

[51] Rasmussen JJ, Wiberg-Larsen P, Baattrup-Pedersen A, Friberg N, Kronvang B. Stream habitat structure influences macroinvertebrate response to pesticides. Environmental Pollution 2012;164:142.

[52] German Federal Environmental Agency (UBA). Water Resource Management in Germany - Part 1 Fundamentals. Dessau-Roßlau. 2010

[53] German Federal Ministry for Environment and Nature Conservation and Nuclear Safety (BMU). Water Framework Directive - Summary of River Basin District Analysis 2004 in Germany. BMU; Environmental Policy. 2005

[54] Hessian Agency for the Environment and Geology (HLUG). EG-WRRL - Bestandsaufnahme oberirdischer Gewässer (EU WFD Basin Analysis of surface waters). HLUG; Wasser in Europa - Wasser in Hessen. 2004

[55] Hessian Ministry for Environment and Energy and Agriculture and Consumer Protection (HMUELV). Beseitigung von kommunalen Abwässern in

Hessen - Lagebericht 2006 (Removal of municipal wastewater in Hesse, Germany - report 2006). Wiesbaden. HMUELV; 2007

[56] Beketov MA, Foit K, Schäfer RB, Schriever CA, Sacchi A, Capri E, et al. SPEAR indicates pesticide effects in streams - Comparative use of species- and family-level biomonitoring data. Environmental Pollution 2009;157:1841.

[57] Organisation for Economic Cooperation and Development (OECD). Report of Phase 1 of the Aquatic Risk Indicators Project. Paris, France. 1998

[58] Linders J, Mensink H, Stephenson G, Wauchope D, Racke K. Foliar interception and retention values after pesticide application. A proposal for standardized values for environmental risk assessment. Pure and Applied Chemistry 2000;72:2199.

[59] Burgert S, Schafer RB, Foit K, Kattwinkel M, Metzeling L, MacEwan R, et al. Modelling aquatic exposure and effects of insecticides - Application to south-eastern Australia. Science of The Total Environment 2011;409:2807.

[60] Working Group on water issues (LAWA). Gewässerstrukturgüte-kartierung in der Bundesrepublik Deutschland: Verfahren für kleine und mittelgroße Fließgewässer (Mapping of the structural quality of water bodies: method for small and medium streams in Germany): Kulturbuch; 2000.

[61] Asmis M. Personal communication. 2012. [Telephone interview Bunzel - Asmis]. Date: 31.08.2012.

[62] Dunbar MJ, Pedersen ML, Cadman DAN, Extence C, Waddingham J, Chadd R, et al. River discharge and local-scale physical habitat influence macroinvertebrate LIFE scores. Freshwater Biology 2010;55:226.

[63] Rasmussen JJ, Baattrup-Pedersen A, Larsen SE, Kronvang B. Local physical habitat quality cloud the effect of predicted pesticide runoff from agricultural land in Danish streams. Journal Environmental Monitoring 2011;13:943.

[64] Davy-Bowker J, Furse M. Hydromorphology – major results and conclusions from the STAR project. Hydrobiologia 2006;566:263.

[65] Welker A. Occurrence and fate of organic pollutants in combined sewer systems and possible impacts on receiving waters. Water Science and Technology 2007;56:141.

[66] Weyrauch P, Matzinger A, Pawlowsky-Reusing E, Plume S, von Seggern D, Heinzmann B, et al. Contribution of combined sewer overflows to trace contaminant loads in urban streams. Water Research 2010;44:4451.

[67] Thiere G, Schulz R. Runoff-related agricultural impact in relation to macroinvertebrate communities of the Lourens River, South Africa. Water Research 2004;38:3092.

[68] Bunzel K, Kattwinkel M, Liess M. Effects of organic pollutants from wastewater treatment plants on aquatic invertebrate communities. Water Research 2013;47:597.

[69] Schäfer RB, Gerner N, Kefford BJ, Rasmussen JJ, Beketov MA, de Zwart D, et al. How to characterize chemical exposure to predict ecologic effects on aquatic communities? Environmental Science & Technology 2013;47:7996.

[70] German Federal Ministry for Food and Agricultural and Consumer Protection (BMELV). Nationaler Aktionsplan zur nachhaltigen Anwendung von Pflanzenschutzmitteln (National action plan for the sustainable usage of pesticides). BMELV; 2013

[71] German Federal Ministry for Transport and Building and Urban Development (BMVBS). Städtebauliche, ökologische und soziale Bedeutung des Kleingartenwesens (Importance of the garden allotments movement regarding ecological and social factors and urban development). Forschungen. BMVBS; Forschungen 2008

[72] German Bundestag. Gesetz zur Ordnung des Wasserhaushalts (German Federal Water Act). In: BGBl. I. 2009

[73] von der Ohe PC, Goedkoop W. Distinguishing the effects of habitat degradation and pesticide stress on benthic invertebrates using stressor-specific metrics. Science of The Total Environment 2013;444:480.

[74] Mažeika S, Sullivan P, Watzin M, Hession WC. Understanding Stream Geomorphic State in Relation to Ecological Integrity: Evidence Using Habitat Assessments and Macroinvertebrates. Environmental Management 2004;34:669.

[75] Overmyer JP, Noblet R, Armbrust KL. Impacts of lawn-care pesticides on aquatic ecosystems in relation to property value. Environmental Pollution 2005;137:263.

[76] Baris RD, Cohen SZ, Barnes NL, Lam J, Ma Q. Quantitative analysis of over 20 years of golf course monitoring studies. Environmental Toxicology and Chemistry 2010;29:1224.

[77] Winter JG, Somers KM, Dillon PJ, Paterson C, Reid RA. Impacts of golf courses on macroinvertebrate community structure in precambrian shield streams. Journal of Environmental Quality 2002;31:2015.

[78] Youngs R. The EU's global climate and energy policies: gathering or losing momentum? In: Goldthau A, editor. The Handbook of Global Energy Policy. West Sussex, UK: John Wiley & Sons Ltd; 2013, p. 421

[79] European Commission. Main tables for energy statistics. European Commission, [Last Update: 09.10.2013]. 2013 [cited 09.10.2013]. Available from http://epp.eurostat.ec.europa.eu/portal/page/portal/energy/data/main_tables.

[80] Srinivasan S. The food v. fuel debate: A nuanced view of incentive structures. Renewable Energy 2009;34:950.

[81] Elbehri A, Segerstedt A, Liu P. Biofuels and the sustainability challenge: A global assessment of sustainability issues, trends and policies for biofuels and related feedstocks. Rome. FAO; 2013

[82] European Academies Science Advisory Council. The current status of biofuels in the European Union, their environmental impacts and future prospects: German National Academy of Science Leopoldina 2012; 2012.

[83] Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. Nature 2002;418:671.

[84] Thraen D, Fritsche UR, Hennig C, Rensberg N, Krautz A. IEA Bioenergy Task 40: Country Report Germany 2011. Leipzig/Darmstadt. 2012

[85] German Federal Ministry for the Environment and Nature Conservation and Nuclear Safety (BMU). National Biomass Action Plan for Germany - Biomass and sustainable energy supply. Berlin. BMU; 2009

[86] German Federal Office of Consumer Protection and Food Safety (BVL). Absatz an Pflanzenschutzmitteln in der Bundesrepublik Deutschland - Ergebnisse der Meldungen gemäß § 64 Pflanzenschutzgesetz für das Jahr 2012 (Domestic sales of pesticides in Germany in 2012). Braunschweig. BVL; Absatz an Pflanzenschutzmitteln in der Bundesrepublik Deutschland. 2013

[87] Freier B, Sellmann J, Strassemeyer J, Schwarz J, Klocke B, Moll E, et al. Network of reference farms for plant protection - Annual Report 2011 and Analysis of Results of 2007 to 2011. Braunschweig. Julius Kühn-Institut FRCfCP; Berichte aus dem Julius Kühn-Institut. 2012

[88] Roßberg D. Erhebungen zur Anwendung von Pflanzenschutzmitteln in der Praxis im Jahr 2011 (Survey on application of chemical pesticides in Germany). Journal für Kulturpflanzen 2013;65:141

[89] Roßberg D. Statistische Erhebungen zur Anwendung von Pflanzenschutzmitteln in der Praxis (PAPA - statistical surveys on the application of pesticides). Julius Kühn-Institut (JKI). 2014 [cited 10.03.2014]. Available from http://papa.jki.bund.de.

[90] Langert M. Der Anbau nachwachsender Rohstoffe in der Landwirtschaft Sachsen-Anhalts und Thüringens - eine innovations- und diffusionstheoretische Untersuchung (The cultivation of renewable resources in the agriculture of Saxony-Anhalt and Thuringia). Sozialwissenschaften und historische Kulturwissenschaften. Halle: Martin-Luther-Universität Halle-Wittenberg; 2007.

[91] Fachagentur Nachwachsende Rohstoffe e.V. (FNR). Daten und Fakten - Bioenergie (Data and facts - Bioenergy). FNR. 2013 [cited 29.08.2013]. Available from http://mediathek.fnr.de/grafiken/daten-und-fakten.html.

[92] Paulsen M, Schochow M. Anbau von Mischkulturen mit Ölpflanzen zur Verbesserung der Flächenproduktivität im ökologische Landbau - Nährstoffaufnahme, Unkrautunterdrückung, Schaderregerbefall und Produktqualitäten (Cultivation of mixed cultures of oil plants for the improvement of the productivity of land in the frame of the organic farming). Trenthorst. Sonderheft. 2007

[93] Fachagentur Nachwachsende Rohstoffe e.V. (FNR). Biokraftstoffe (Pflanzen, Rohstoffe, Produkte) (Biofuels - crops, raw materials, products). Guelzow. FNR; 2007

[94] Fachagentur Nachwachsende Rohstoffe e.V. (FNR). Biokraftstoffe Basisdaten Deutschland (Basic data on biofuels Germany). Guelzow. FNR; 2009

[95] German Federal Ministry for the Environment and Nature Conservation and Nuclear Safety (BMU). Renewable Energy Sources in Figures - National and International Development. Berlin. BMU; 2012

[96] Scheftelowitz M, Daniel-Gromke J, Denysenko V, Sauter P, Naumann K, Krautz A, et al. Stromerzeugung aus Biomasse - Zwischenbericht 03MAP250 (Electricity generation from biomass - interim report). Leipzig. 2013

[97] Gutsche V. Managementstrategien des Pflanzenschutzes der Zukunft im Focus von Umweltverträglichkeit und Effizienz (Pest management strategies on the future with a focus on environmental compatibility and efficiency). Journal für Kulturpflanzen 2012;64:325.

[98] German Federal Environmental Agency (UBA). Data on the environment 2011 edition - Environment and agriculture. Dessau. UBA; Data on the environment 2012

[99] Williams I. The Major Insect Pests of Oilseed Rape in Europe and Their Management: An Overview. In: Williams IH, editor. Biocontrol-Based Integrated Management of Oilseed Rape Pests: Springer Netherlands; 2010, p. 1.

[100] German Association for Technology and Structures in Agriculture (KTBL). Energiepflanzen - Daten für die Planung des Energiepflanzenanbaus (Energy crops - Data for the management of energy crops). Darmstadt: KTBL; 2012.

[101] Rippel R, Brandhuber R, Burger F, Capriel P, Kreuter T, Müller C, et al. Einfluss des Biomasseanbaus für Energiebereitstellung auf den Bodenschutz (Impact of the biomass cultivation for energy production on the soil protection). Landeskultur in Europa - Lernen von den Nachbarn und Bioenergie - eine Sackgasse für die Landeskultur?: Deutsche Landeskulturgesellschaft; 2008, p. 131.

[102] Karpenstein-Machan M, Weber C. Energiepflanzenanbau für Biogasanlagen (Cultivation of energy crops for biogas plants). Naturschutz und Landschaftsplanung 2010;42:312.

[103] Deuker A, Stinner W, Rensberg N, Wagner L, Hummel HE. Regional risks for biogas production in Germany by the maize pest Diabrotica v. virgifera? Journal of Agricultural Science and Technology 2012;A 2:749.

[104] Mielke H, Schöber-Butin B. Pflanzenschutz bei Nachwachsenden Rohstoffen: Kartoffel, Getreide und Mais (Plant protection for re-growing industrial plants: potato, cereals and maize). Berlin and Braunschweig. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. 2001

[105] Pickert J. Produktionstechnik zur Integration in das Ökosystem des Standortes und betriebliche Aspekte (Production technology for the integration in the site-specific ecosystem and operational aspects). In: Hanus H, Heyland K-U, Keller ER, editors. Handbuch des Pflanzenbaus 2. Stuttgart: Hanus, H; 2008, p. 496.

[106] German Federal Statistical Office. Feldfrüchte und Grünland (Arable crops and permanent grassland). 2013 [cited 10.03.2014]. Available from https://www.destatis.de/DE/ZahlenFakten/Wirtschaftsbereiche/LandForstwirtschaftFischerei/FeldfruechteGruenland/FeldfruechteGruenland.html.

[107] Bianchi FJ, Booij CJ, Tscharntke T. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and

natural pest control. Proceedings of the Royal Society B: Biological Sciences 2006;273:1715.

[108] Landis DA, Gardiner MM, van der Werf W, Swinton SM. Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. Proceedings of the National Academy of Sciences 2008;105:20552.

[109] Stewart A, Cromey M. Identifying disease threats and management practices for bio-energy crops. Current Opinion in Environmental Sustainability 2011;3:75.

[110] German Government. Gesetz für den Vorrang Erneuerbare Energien - EEG (Renewable Energy Sources Act). In: Bundesgesetzblatt. 2012

[111] Krupinsky JM, Bailey KL, McMullen MP, Gossen BD, Turkington TK. Managing plant disease risk in diversified cropping systems. Agronomy Journal 2002;94.

[112] Ratnadass A, Fernandes P, Avelino J, Habib R. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agronomy for Sustainable Development 2012;32:273.

[113] Rusch A, Bommarco R, Jonsson M, Smith HG, Ekbom B. Flow and stability of natural pest control services depend on complexity and crop rotation at the landscape scale. Journal of Applied Ecology 2013;50:345.

[114] Fritz M, Heimler F. Towards risk limitation and higher biomass quality: Is mixed cropping an advisable strategy in energy crop production? 15th European biomass conference and exhibition. Berlin: ETA Renewable Energies; 2007.

[115] Dietze M, Fritz M, Deiglmayr K. Abschlussbericht 2008: Entwicklung und Optimierung von standortangepassten Anbausystemen für Energiepflanzen im Fruchtfolgeregime - Teilprojekt Mischfruchtanbau (Final report 2008: Development and optimisation of site-specific cultivation systems of energy crop in crop rotation - Subproject mixed cropping systems). 2008

[116] Paulsen MH, Schochow M, Ulber B, Kuehne S, Rahmann G. Mixed cropping systems for biological control of weeds and pests in organic oilseed crops. In: COR, editor. Aspects of Applied Biology 79: COR; 2006, p. 215.

[117] Ulber B, Kühne S. Schädlingsbefall an Raps in Rein- und Mischfruchtanbau im ökologischen Landbau (Insect pest infestation of oilseed rape in organic sole and mixed cropping systems). In: Paulsen M, Schochow M, editors. Anbau von Mischkulturen mit Ölpflanzen zur Verbesserung der Flächenproduktivität im ökologischen Landbau Braunschweig: Landbauforschung Völkenrode; 2007, p. 96.

[118] Zegada-Lizarazu W, Monti A. Energy crops in rotation. A review. Biomass and Bioenergy 2011;35:12.

[119] Statistical office of Mecklenburg-West Pomerania. Statistical data on arable crops per district of Mecklenburg-West Pomerania. 2014. [Dataset]. [provided by Dr. Dieter Gabka, statistical office of Mecklenburg-West Pomerania].

[120] Lower Saxony Chamber of Agriculture. Statistics on CAP direct payments of Lower Saxony. 2013. [Dataset]. [provided by Kirsten Murek, Lower Saxony Chamber of Agriculture].

[121] Lower Saxony Network for Renewable Resources. Statistical data on biogas plants in Lower Saxony. 2014. [Dataset]. [provided by Kirsten Murek, Lower Saxony Chamber of Agriculture].

[122] European Economic Community. Commission Regulation (EEC) No 1272/88 of 29 April 1988 laying down detailed rules for applying the set-aside incentive scheme for arable land. In: Official Journal of the European Union. 1988

[123] Jack B. Agriculture and EU environmental law. Surrey: Ashgate Publishing Company; 2009.

[124] European Commission. Council Regulation (EC) No 73/2009 of 19 January 2009 establishing common rules for direct support schemes for farmers under the common agricultural policy and establishing certain support schemes for farmers, amending Regulation (EC) No 1290/2005, (EC) No 247/2006, (EC) No 378/2007 and repealing Regulation (EC) No 1782/2003. In: Official Journal of the European Union. 2009

[125] UK Department for Environment and Food and Rural Affairs (Defra). Change in the area and distribution of set-aside in England and its environmental impacts. London. Observatory Research Report. 2007

[126] German Federal Office for Agriculture and Food. Statistical data on set-aside land and cultivation of renewable resources in Germany. 2014. [Dataset]. [provided by Detlev Pfeiffer, German Federal Office for Agriculture and Food].

[127] Guo LB, Gifford RM. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 2002;8:345.

[128] Soussana JF, Loiseau P, Vuichard N, Ceschia E, Balesdent J, Chevallier T, et al. Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use and Management 2004;20:219.

[129] Sala OE, Vivanco L, Flombaum P. Grassland Ecosystems. In: Levin SA, editor. Encyclopedia of Biodiversity (Second Edition). Waltham: Academic Press; 2013, p. 1.

[130] DuPont ST, Culman SW, Ferris H, Buckley DH, Glover JD. No-tillage conversion of harvested perennial grassland to annual cropland reduces root biomass, decreases active carbon stocks, and impacts soil biota. Agriculture, Ecosystems & Environment 2010;137:25.

[131] German Federal Statistical Office. Viehbestand und tierische Erzeugung (Livestock and animal production). Wiesbaden. Fachserie 3 Reihe 4. 2013

[132] Nitsch H, Osterburg B, Roggendorf W, Laggner B. Cross compliance and the protection of grassland – Illustrative analyses of land use transitions between permanent grassland and arable land in German regions. Land Use Policy 2012;29:440.

[133] Epp C, Rutz D, Koettner M, Finsterwalder T. Project Biogas for Eastern Europe: Guidelines for selecting suitable sites for biogas plants (Deliverable D 6.1). Munich. Energies WR; 2008

[134] Börjesson P. Environmental effects of energy crop cultivation in Sweden—I: Identification and quantification. Biomass and Bioenergy 1999;16:137.

[135] Caslin B, Finnan J, Easson L. Miscanthus best practice guidelines. A guide prepared by Teagasc and the Agri-food and Bioscience Institute. Oak Park, Carlow. 2010

[136] Fachagentur Nachwachsende Rohstoffe e.V. (FNR). Bioenergie - die vielfältige erneuerbare Energie (Bioenergy - the manifold reneable energy). Gülzow-Prüzen. (FNR) FNReV; 2013

[137] Paine LK, Peterson TL, Undersander DJ, Rineer KC, Bartelt GA, Temple SA, et al. Some ecological and socio-economic considerations for biomass energy crop production. Biomass and Bioenergy 1996;10:231.

[138] Gruenewald H, Brandt BKV, Schneider BU, Bens O, Kendzia G, Hüttl RF. Agroforestry systems for the production of woody biomass for energy transformation purposes. Ecological Engineering 2007;29:319.

[139] Kuemmel B, Langer V, Magid J, De Neergaard A, Porter JR. Energetic, economic and ecological balances of a combined food and energy system. Biomass and Bioenergy 1998;15:407.

[140] Volk TA, Abrahamson LP, Nowak CA, Smart LB, Tharakan PJ, White EH. The development of short-rotation willow in the northeastern United States for bioenergy and bioproducts, agroforestry and phytoremediation. Biomass and Bioenergy 2006;30:715.

[141] Schultz RC, Collettil JP, Isenhart TM, Simpkins WW, Mize CW, Thompson ML. Design and placement of a multi-species riparian buffer strip system. Agroforestry Systems 1995;29:201.

[142] Christen B, Dalgaard T. Buffers for biomass production in temperate European agriculture: A review and synthesis on function, ecosystem services and implementation. Biomass and Bioenergy 2013;55:53.

[143] Haughton AJ, Bond AJ, Lovett AA, Dockerty T, Sünnenberg G, Clark SJ, et al. A novel, integrated approach to assessing social, economic and environmental implications of changing rural land-use: a case study of perennial biomass crops. Journal of Applied Ecology 2009;46:315.

[144] Caslin B, Finnan J, McCracken A. Short Rotation Coppice Willow - best practice guidelines. 2010

[145] Dillen SY, Djomo SN, Al Afas N, Vanbeveren S, Ceulemans R. Biomass yield and energy balance of a short-rotation poplar coppice with multiple clones on degraded land during 16 years. Biomass and Bioenergy 2013;56:157.

[146] Pei MH, Hunter T, Ruiz C. Occurrence of Melampsora rusts in biomass willow plantations for renewable energy in the United Kingdom. Biomass and Bioenergy 1999;17:153.

[147] Royle DJ, Ostry ME. Disease and pest control in the bioenergy crops poplar and willow. Biomass and Bioenergy 1995;9:69.

[148] Mitchell CP, Stevens EA, Watters MP. Short-rotation forestry – operations, productivity and costs based on experience gained in the UK. Forest Ecology and Management 1999;121:123.

[149] Larsson S. Genetic improvement of willow for short-rotation coppice. Biomass and Bioenergy 1998;15:23.

[150] Gruppe A, Fußeder M, Schopf R. Short rotation plantations of aspen and balsam poplar on former arable land in Germany: defoliating insects and leaf constituents. Forest Ecology and Management 1999;121:113.

[151] Styles D, Thorne F, Jones MB. Energy crops in Ireland: An economic comparison of willow and Miscanthus production with conventional farming systems. Biomass and Bioenergy 2008;32:407.

[152] Dimitriou I, Baum C, Baum S, Busch G, Schulz U, Köhn J, et al. Quantifying environmental effects of Short Rotation Coppice (SRC) on biodiversity, soil and water. 2011

[153] Zalesny RS, Cunningham MW, Hall RB, etc. Woody biomass from short rotation energy crops. Sustainable production of fuels, chemicals, and fibers from forest biomass. Washington, DC, USA: American Chemical Society; 2011, p. 27.

[154] Anderson E, Arundale R, Maughan M, Oladeinde A, Wycislo A, Voigt T. Growth and agronomy of Miscanthus × giganteus for biomass production. Biofuels 2011;2:167.

[155] UK Department for Environment and Food and Rural Affairs (Defra). Planting and growing Miscanthus - Best practice guidelines. 2007

[156] Lewandowski I, Scurlock JMO, Lindvall E, Christou M. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass and Bioenergy 2003;25:335.

[157] Bradshaw JD, Prasifka JR, Steffey KL, Gray ME. First report of field populations of two potential aphid pests of the bioenergy crop Miscanthus x Giganteus. Florida Entomologist 2010;93:135.

[158] Spencer JL, Raghu S. Refuge or Reservoir? The potential impacts of the biofuel crop Miscanthus x giganteus on a major pest of maize. PLoS One 2009;4:e8336.

[159] Wilson TA, Hibbard BE. Host suitability of nonmaize agroecosystem grasses for the western corn rootworm (Coleoptera: Chrysomelidae). Environmental Entomology 2004;33:1102.

[160] Meehan TD, Werling BP, Landis DA, Gratton C. Pest-suppression potential of midwestern landscapes under contrasting bioenergy scenarios. PLoS One 2012;8:e41728.

[161] Gasperi J, Garnaud S, Rocher V, Moilleron R. Priority pollutants in wastewater and combined sewer overflow. Science of The Total Environment 2008;407:263.

[162] Chebbo G, Gromaire MC, Ahyerre M, Garnaud S. Production and transport of urban wet weather pollution in combined sewer systems: the “Marais” experimental urban catchment in Paris. Urban Water 2001;3:3.

[163] Soonthornnonda P, Christensen ER. Source apportionment of pollutants and flows of combined sewer wastewater. Water Research 2008;42:1989.

[164] Burm RJ, Krawczyk DF, Harlow GL. Chemical and Physical Comparison of Combined and Separate Sewer Discharges. Journal (Water Pollution Control Federation) 1968;40:112.

[165] Brombach H, Weiss G, Fuchs S. A new database on urban runoff pollution: comparison of separate and combined sewer systems. Water Science & Technology 2005;51:119.

[166] Welker A. Emissions of pollutant loads from combined sewer systems and separate sewer systems - Which sewer system is better? 11th International Conference on Urban Drainage. Edinburgh, UK; 2008.

[167] De Toffol S, Engelhard C, Rauch W. Combined sewer system versus separate system--a comparison of ecological and economical performance indicators. Water Science and Technology 2007;55:255.

[168] Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U, et al. The challenge of micropollutants in aquatic systems. Science 2006;313:1072.

[169] Englert D, Zubrod JP, Schulz R, Bundschuh M. Effects of municipal wastewater on aquatic ecosystem structure and function in the receiving stream. Science of The Total Environment 2013;454–455:401.

[170] Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, et al. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of The Total Environment 2014;473–474:619.

[171] Norris V. The use of buffer zones to protect water quality: a review. Water Resources Management 1993;7:257.

[172] Correll D. Buffer zones and water quality protection: general principles. In: Haycock N, Burt T, Goulding K, Pinay G, editors. Buffer zones: their processes and potential in water protection (The proceedings of the international conference on buffer zones); 1996, p. 7.

[173] Lorenz A, Hering D, Feld C, Rolauffs P. A new method for assessing the impact of hydromorphological degradation on the macroinvertebrate fauna of five German stream types. Hydrobiologia 2004;516:107.

[174] Liess M, Schulz R, Liess MH-D, Rother B, Kreuzig R. Determination of insecticide contamination in agricultural headwater streams. Water Research 1999;33:239.

[175] Verberk WCEP, van Noordwijk CGE, Hildrew AG. Delivering on a promise: integrating species traits to transform descriptive community ecology into a predictive science. Freshwater Science 2013;32:531.

[176] Reungoat J, Macova M, Escher BI, Carswell S, Mueller JF, Keller J. Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration. Water Research 2010;44:625.

[177] Hernández-Leal L, Temmink H, Zeeman G, Buisman CJN. Removal of micropollutants from aerobically treated grey water via ozone and activated carbon. Water Research 2011;45:2887.

[178] Bundschuh M, Zubrod JP, Seitz F, Stang C, Schulz R. Ecotoxicological evaluation of three tertiary wastewater treatment techniques via meta-analysis and feeding bioassays using Gammarus fossarum. Journal of Hazardous Materials 2011;192:772.

[179] Margot J, Kienle C, Magnet A, Weil M, Rossi L, de Alencastro LF, et al. Treatment of micropollutants in municipal wastewater: Ozone or powdered activated carbon? Science of The Total Environment 2013;461–462:480.

[180] Fortier J, Gagnon D, Truax B, Lambert F. Biomass and volume yield after 6 years in multiclonal hybrid poplar riparian buffer strips. Biomass and Bioenergy 2010;34:1028.

[181] Bärwolff M, Reinhold G, Fürstenau C, Graf T, Jung L, Vetter A. Gewässerrandstreifen als Kurzumtriebsplantagen oder Agroforstsysteme (Short-rotation coppice as riparian buffer strips or agroforestry systems). Dessau-Roßlau. UBA-Texte. 2013

Appendix A1:

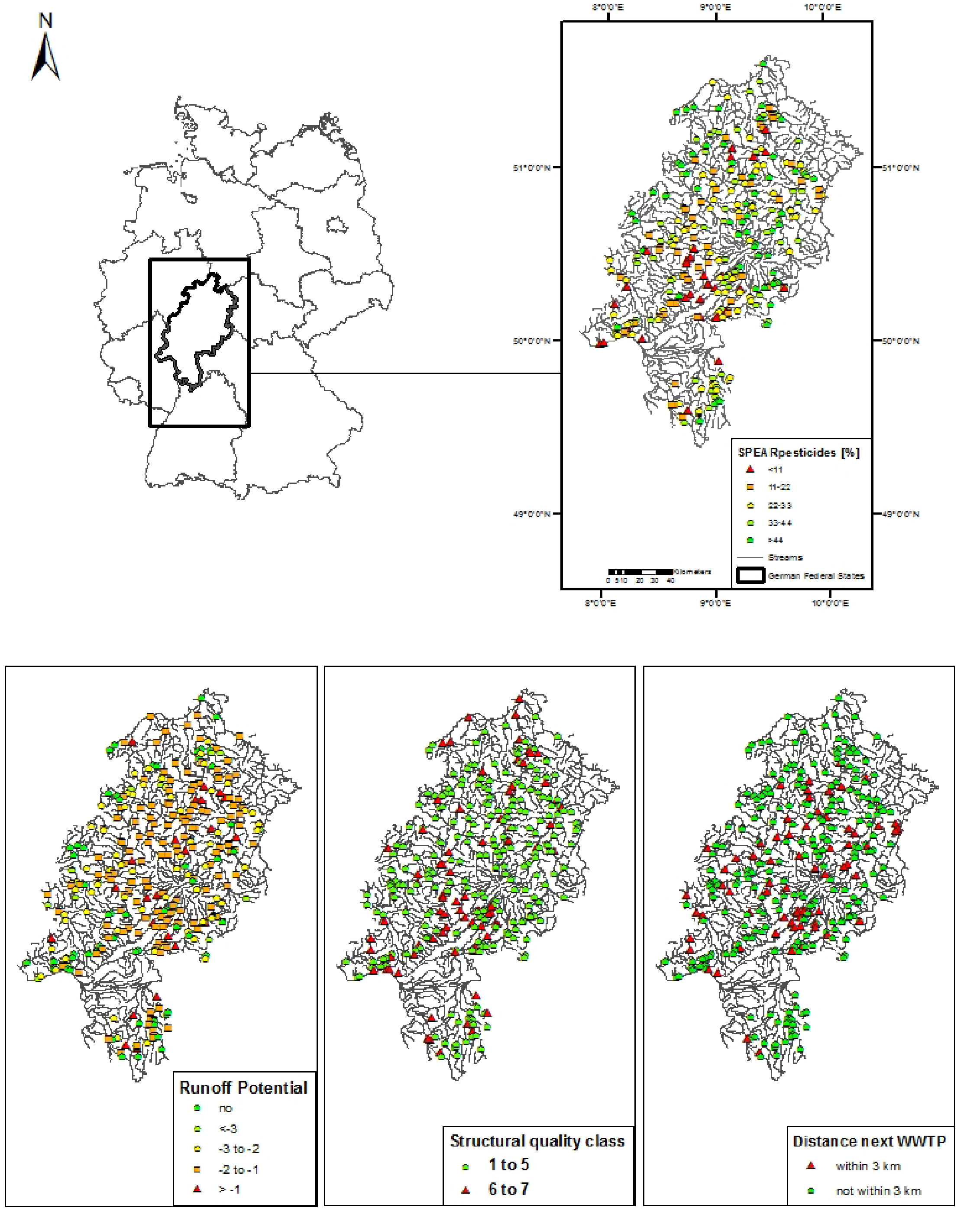

Fig. A1: Spatial distribution of the results for SPEAR$_{pesticides}$, Runoff Potential, structural quality class and distance to next upstream WWTP for the investigated 328 sampling sites in Hesse.

Appendix A2. Runoff Potential model formula and parameterisation

The potential insecticide runoff input into a stream site during a rainfall event (gLOAD [g]) was calculated according to:

$$gLOAD = \sum_{i=1}^{n} A_i \times D_{generic} \times \left(1 - \frac{I_i}{100}\right) \times \frac{1}{1+\frac{K_{OC,generic} \times OC_i}{100}} \times f(s_i) \times \frac{f(P_i,T_i)}{P_i} \quad \text{(A2.1)}$$

The Runoff Potential (RP) of a stream site is then calculated as:

$$RP = \log_{10}(max_{i=1}^{n}(gLOAD_i)) \quad \text{(A2.2)}$$

where *n* is the number of rainfall events that occur during the main application time for pesticides (from May to July), $gLOAD_i$ is the amount of a generic substance that potentially reaches a stream site during rainfall event *i* as given in Eq. (A2.1).

A short description of the factors and their parameterization can be found in Table A2.1.

Table A2.1: Parameters for the calculation of gLOAD (Eq. A2.1) (index i refers to different polygons of arable land within one stream corridor)

Factor	Description	Parameterization and data source
A_i	Size of arable polygon within stream corridor [ha]	Shapefile from ATKIS database (scale 1:25,000), provided by the German Federal Agency for Cartography and Geodesy
$D_{generic}$	Rate of application of generic substance	No data, therefore set to a constant value of 1 g/ha for all crops
$K_{OC,generic}$	Soil organic carbon sorption coefficient of the generic compound	No data, therefore set to a constant value of 100, which represents a highly mobile compound
OC_i	Organic soil carbon content of polygon [%]	Shapefile of organic matter in topsoil in Germany (scale 1:1,000,000), calculated as 58% of humus content, provided by the German Federal Institute for Geosciences and Natural Resources
I_i	Crop- and growth phase-specific plant interception at the time of the rainfall event [%]	Average from crop statistics per administrative district, assumed to be distributed uniformly in the district, provided by the Statistical Offices of Hesse, Saxony, Saxony-Anhalt, Thuringia;

		interception values were assigned to all present crop types modified after Linders et al. [58]
s_i	Mean slope of polygon [%]	Shapefile created by using Slope function in ArcGIS 10, based on Digitalized Elevation Map (DEM), provided by the German Federal Institute for Geosciences and Natural Resources
$f(s_i)$	Influence of slope	according to OECD [57] if $s_i \leq 20\% = 0.00143 * s_i^2 + 0.02153 * s_i$ if $s_i > 20\% = 1$
P_i	Precipitation level [mm]	Daily recorded precipitation from May to July 2004 and May to July 2005, assumed to result from one rainfall event, interpolated from relevant weather stations of Germany's National Meteorological Service (DWD)
T_i	Soil texture of polygon (sandy/loamy)	Shapefile of soil map of Germany (BUEK 1000, scale 1,000,000), provided by the German Federal Institute for Geosciences and Natural Resources; decision on sandy or loamy based on sand and clay content of the soil type

$f(P_i,T_i)$	Volume of surface runoff [mm]	specified according to OECD [57]
		if T_i=sandy:
		$-5.86*10^{-6}*P_i^3+2.63*10^{-3}*P_i^2-1.14*10^{-2}*P_i-1.164*10^{-2}$
		if T_i=loamy:
		$-9.04*10^{-6}*P_i^3+4.04*10^{-3}*P_i^2+4.16*10^{-3}*P_i-6.11*10^{-2}$

Acknowledgments

The work presented here was conducted at the Departments of Bioenergy and System Ecotoxicology at the Helmholtz Centre for Environmental Research (UFZ) in Leipzig, in collaboration with the Institute for Environmental Sciences at the University of Koblenz-Landau. The work was made possible by the funding from the Helmholtz Association of German Research Centres within the project funding “Biomass and Bioenergy Systems” and was supported by the Helmholtz Impulse and Networking Fund through the Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE).

First and foremost, I am greatly obliged to Prof. Dr.-Ing. Daniela Thrän, head of the Department of Bioenergy, for the continuous support and for giving me the opportunity to carry out my PhD research in the very interesting and interdisciplinary field of bioenergy and water protection. I truly appreciate her trust and confidence in me and I am very grateful for the large degree of freedom in the selection of my research questions and approaches.

Furthermore, I would like to thank PD Dr. Matthias Liess for giving me the opportunity to cooperate with the Department of System Ecotoxicology, for his scientific advice and for many stimulating discussions.

I am grateful to Prof. Dr. Ralf Schäfer for supporting my thesis at the University of Koblenz-Landau and for his valuable feedback on several scientific questions and the manuscript of this thesis.

I owe my sincere gratitude to Dr. Mira Kattwinkel for her excellent supervision, the many fruitful discussions and the guidance throughout this thesis, even after she moved about 700 km to Zürich. I have been extremely lucky to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly. I’ve learned a lot from her and without her help I could not have finished my dissertation in three years.

Thanks also to my colleagues of the Departments System Ecotoxicology and Bioenergy for the warm and inspiring atmosphere. I especially thank my office mates Ida Dolciotti, Nadine Gerner, Polina Orlinskiy, and Lena Reiber for lightened up my time in the office, for supporting me through the ups and downs of the day and for spending time with me in the great city of Leipzig. Furthermore, I thank my colleague Saskia Knillmann for her feedback on the draft of this thesis and the nice walks in the Clara-Zetkin park.

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Research experiences

Since Jan. 2011 **Helmholtz-Centre for Environmental Research (UFZ), Leipzig, Germany**

Research assistant in the Departments “Bioenergy“ and “System Ecotoxicology”

- GIS-based modelling of pesticide exposure and ecological risk of aquatic ecosystems in agricultural landscapes
- Systematic assessment of the risks and opportunities associated with the increased cultivation of energy crops (focus: pesticides)

Jan. 2009 – Jan. 2011 **German Biomass Research Centre (DBFZ), Leipzig, Germany**

Research assistant in the Department “Bioenergy Systems“

- Determination of regional and global biomass potentials and their utilisation (material flow analysis)
- Sustainability aspects and system integration of biomass and bioenergy
- Project manager for several joint research projects (e.g.,“Global and regional spatial distribution of biomass potentials“ and “Biomass potentials for a sustainable production of biomethane“)

June 2005 – Jan. 2009 **German Federal Environment Agency (UBA), Dessau, Germany**

Research assistant in the Section “Protection of the Marine Environment”

- Work within the Common Implementation Strategy of the EU Water Framework Directive (WFD)
- German representative in the EU working groups on “Ecological Status“ and “Water Framework Directive and hydromorphological pressures“
- Coordination of the German activities regarding the harmonisation of biological assessment systems throughout the EU (Intercalibration)
- Organisation of an international workshop on “Water Framework Directive and hydropower“

- Assessment of environmental impacts and risks associated with offshore wind farms, consideration of applications for the construction and operation of offshore wind farms in the German exclusive economic zone of the North Sea and the Baltic Sea
- Writing and editorial work for several research reports and papers of the German Federal Environment Agency (e. g., the brochure "Climate change and marine ecosystems")

Education and training

Since Jan. 2011 **University of Koblenz-Landau, Institute for Environmental Sciences, Quantitative Landscape Ecology, Landau, Germany**

Doctoral candidate

Oct. 1998 – Jan. 2005 **TU Bergakademie Freiberg (TU BAF), Freiberg, Germany**

Graduation as Diploma-Geoecologist (1.2 - very good)

Main subjects: Hydrology, Environmental Microbiology/Biotechnology, Environment/Business/Law

Apr. 2004 – Oct. 2004 **"Center of Energy and Environmental Studies" (IVEM) of the Rijksuniversiteit Groningen (RUG), Groningen, the Netherlands**

Master's thesis: "Hydrological consequences of changing from food crops to energy crops – a modelling study for the Netherlands" (Winner of the "Hans-Carl-von-Carlowitz" Award in 2005)

Jan. 2003 – June 2003 **Norwegian University of Science and Technology (NTNU), Trondheim, Norway**

Courses: Design of Marine Production Plants, Atmospheric physics, Biogeography

Aug. 1992 – July 1998 **Gymnasium, Coswig, Germany**

Education: German A Level (1.4 - very good)

Beiträge an den Publikationen

(1) Bunzel, K. (KB); Kattwinkel, M. (MK); Liess, M. (ML) (2013): Effects of organic pollutants from wastewater treatment plants on aquatic invertebrate communities. Water Research 47 (2), Pages 597-606, doi: 10.1016/j.watres.2012.10.031

Konzept	KB 40%, MK 30%, ML 30%
Datenaufnahme und -aufbereitung	KB 100%
Statistische Analyse	KB 80%, MK 20%
Interpretation der Daten	KB 50%, MK 30%, ML 20%
Schreiben des Manuskripts	KB 100%
Korrektur und Kommentierung des Manuskripts	KB 50%, MK 30%, ML 20%

(2) Bunzel, K. (KB); Liess, M. (ML); Kattwinkel, M. (MK) (2014): Landscape parameters driving aquatic pesticide exposure and effects. Environmental Pollution 186, Pages 90-97, doi: 10.1016/j.envpol.2013.11.021

Konzept	KB 60%, MK 30%, ML 10%
Datenaufnahme und -aufbereitung	KB 100%
Statistische Analyse	KB 90%, MK 10%
Interpretation der Daten	KB 80%, MK 20%
Schreiben des Manuskripts	KB 100%
Korrektur und Kommentierung des Manuskripts	KB 60%, MK 30%, ML 10%

(3) Bunzel, K. (KB); Kattwinkel, M. (MK); Schauf, M. (MS); Thrän, D. (DT) (2014): Energy crops and pesticide contamination: Lessons learned from the development of energy crop cultivation in Germany. Biomass & Bioenergy, doi: 10.1016/j.biombioe.2014.08.016

Konzept	KB 70%, MK 30%
Literaturrecherche	KB 80%, MS 20%
Datenaufnahme und statistische Datenanalyse	KB 100%
Interpretation der Daten	KB 70%, MK 30%
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