Sources and effects of multiple stressors including chemical pollution on the ecological quality of river ecosystems

Quellen und Effekte multipler Stressoren einschließlich stofflicher Belastungen auf die ökologische Qualität von Fließgewässerökosystemen

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Nele Markert
born in Osnabrück

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1. Examiner: PD Dr. Christian K. Feld, University of Duisburg-Essen

2. Examiner: Prof. Dr. Henner Hollert, Goethe University Frankfurt

Chair of the Board of Examiners: Prof. Dr. Jens Boenigk, University of Duisburg-Essen

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Summary

Freshwater ecosystems are strongly affected by anthropogenic activities, which can significantly alter environmental conditions with adverse effects on riverine biota. The status of both the freshwater communities and the environmental stressors are subject to frequent monitoring programs. However, despite considerable efforts in monitoring and restoration improving both water quality and habitat conditions, less than 10 % of German rivers have so far achieved a good ecological status as defined by the European Water Framework Directive (WFD). Thus, further reduction of adverse anthropogenic impacts and improvement of the ecological status of river ecosystems are necessary, which require the identification of predominant stressors and the selection of effective management measures. The aim of this thesis was to analyze the sources and effects of multiple stressors on riverine biota using comprehensive datasets of WFD-related monitoring programs in Germany. Firstly, the relative importance of each stressor group for the biological quality elements macroinvertebrates, benthic diatoms and fishes was assessed to provide additional evidence on relevant stressor groups for river basin management. Secondly, the influence of anthropogenic land uses, including urban areas, wastewater treatment plant (WWTP) effluents and intensive agriculture in the catchments, on stressor levels was analyzed to inform the identification of targeted management measures. A particular focus of these analyses has been on micropollutants, such as pesticides, pharmaceuticals and industrial chemicals, and their mixtures, as knowledge gaps remain for the sources and ecological effects of this stressor group in a multi-stressor context. Additionally, the multi-stressor datasets included physico-chemical, hydrological and morphological stressors.

Water quality showed dominating effects on the status of the three organism groups in each multi-stressor analysis. Especially, physico-chemical variables, including concentrations of nutrients, salt ions and oxygen, were often associated with the strongest biological responses. Furthermore, the analyses showed a high relevance of hydrological alterations, particularly of changes in the flow variability as well as the frequency and duration of high and low flow events. Differences were observed for the stressor responses of the three biological quality elements. This included stronger responses of macroinvertebrate and fish communities to the physical water quality (oxygen concentration), whereas benthic diatoms particularly responded to nutrient concentrations. Relative effects of the stressor groups also distinctly differed between the ecological metrics used to describe community characteristics, such as sensitivity, functional traits, community composition and biodiversity, and between individual fish species. Especially, sensitivity metrics (e.g. the Pollution Sensitivity Index on the basis of diatoms or the $SPEAR_{pest}$ Index on the basis of macroinvertebrates, both responding to micropollutants)

showed strong responses, which are well suited for stressor-specific diagnoses of biological deterioration. The percentage of urban areas and WWTP effluents were strongly associated with micropollutant concentrations and calculated ecotoxicological risks, especially for pharmaceuticals. Additionally, further water quality-related variables (oxygen and nutrient concentrations) and hydrological variables (flow variability and high flow frequency) were linked to WWTP effluents. In contrast, the influence of urban areas and WWTP effluents on pesticide levels were less pronounced but still evident for individual substances, for example biocides with applications in urban areas (e.g. facade paints). Stronger associations were found between pesticide concentrations and the percentage of agricultural land, especially when differentiating between individual crop types. Effects of the percentage of forests and grasslands in the catchments were negligible.

The multi-stressor analyses compiled additional evidence of the adverse effects of anthropogenic stressors, water quality deterioration and hydrological alteration in particular, on riverine ecosystems. The results highlight the need for additional management measures addressing both stressor groups to achieve a good ecological status. Furthermore, differences in stressor responses between organism groups and individual fish species indicate the influence of the choice of biological quality elements and metrics on the identification of relevant stressors. Ideally, diagnoses of biological deterioration and selection of targeted management measures should consider all three organism groups and different ecological metrics. For selected stressors (e.g., micropollutants) and organism groups (e.g., fishes), the development of additional stressor-specific metrics is recommended. To reduce individual stressor levels different sources need to be addressed in management plans, with advanced wastewater treatments potentially mitigating water quality deterioration associated with WWTP effluents, whereas additional measures, such as restoring riparian vegetation, are required to reduce diffuse pollution in agricultural areas. Moreover, the analyses revealed uncertainties in the assessment of micropollutants, such as limitations in the number and selection of substances and the frequency of grab samples used for the chemical monitoring, which may lead to an underestimation of ecological effects of this stressor group. Enhanced monitoring programs, particularly considering micropollutants and hydrological variables, may be implemented as part of the investigative monitoring to specifically analyze the stressor's effects at selected sites to facilitate targeted diagnoses of the cause of biological deterioration and evidence-based developments of management measures to achieve and maintain a good ecological status by 2027 and beyond.

Zusammenfassung

Fließgewässerökosysteme werden stark durch anthropogene Aktivitäten beeinflusst, die die Umweltbedingungen erheblich verändern und dadurch die Lebensgemeinschaften in Flüssen beeinträchtigen können. Sowohl der Zustand dieser Lebensgemeinschaften als auch der Zustand der Stressoren in der Umwelt werden durch regelmäßige Monitoringprogramme überwacht. Trotz aufwändiger Monitoring- und Restaurierungsprojekte zur Verbesserung der Wasserqualität und der Habitatbedingungen, erreichten bisher weniger als 10 % der deutschen Flüsse einen guten ökologischen Zustand gemäß der Europäischen Wasserrahmenrichtlinie (WRRL). Daher müssen negative Einflüsse anthropogener Aktivitäten reduziert und der ökologische Zustand weiter verbessert werden. Dies erfordert die Identifikation relevanter Stressoren und die Auswahl effektiver Bewirtschaftungsmaßnahmen. Ziel dieser Arbeit war es daher, die Ursachen und die Effekte verschiedener Stressoren auf die Lebensgemeinschaften anhand der umfassenden Datensätzen der WRRL-bezogenen Monitoringprogramme zu analysieren. Zuerst wurden die relativen Effektanteile der Stressoren auf die biologischen Qualitätskomponenten Makroinvertebraten, benthische Diatomeen und Fische bewertet, um Erkenntnisse über wichtige Stressoren für die Maßnahmenplanung abzuleiten. Weiterhin wurde der Einfluss der Landnutzung in den Einzugsgebieten (u.a. urbane Gebiete, Kläranlagenabwässer und intensive Landwirtschaft) auf die Intensität der Stressoren betrachtet. Der Fokus dieser Analysen lag auf Spurenstoffen, wie Pestiziden, Arzneimitteln und Industriechemikalien, und deren Mischungen, da zu den Quellen und den ökologischen Effekten dieser Stressorgruppe im Multi-Stressor-Kontext noch immer Wissenslücken bestehen. Daneben wurden in den Multi-Stressor-Datensätzen chemisch-physikalische, hydrologische und morphologische Stressoren berücksichtigt.

Die Wasserqualität zeigte dominierende Effekte auf den Zustand der drei betrachteten Organismengruppen. Insbesondere chemisch-physikalische Parameter, einschließlich der Nährstoff-, Salz- und Sauerstoffkonzentrationen, standen dabei stark mit den biologischen Veränderungen im Zusammenhang. Daneben wurde eine hohe Relevanz hydrologischer Veränderungen, insbesondere Veränderungen der Abflussvariabilität sowie der Häufigkeit und Dauer von Hoch- und Niedrigwasserereignissen, beobachtet. Es zeigten sich jedoch deutliche Unterschiede zwischen den drei biologischen Qualitätskomponenten. Die Makroinvertebraten und Fische reagierten stärker auf die physikalische Wasserqualität (Sauerstoff), wohingegen die Diatomeen sich als besonders sensitiv gegenüber Nährstoffen erwiesen. Zusätzlich unterschieden sich die relativen Effekte der Stressoren zum einen zwischen den ökologischen Metrics, die zur Beschreibung der Merkmale der Lebensgemeinschaften (z.B. Sensitivität, funktionale Eigenschaften, Biodiversität) verwendet

wurden, und zum anderen zwischen den einzelnen betrachteten Fischarten. Insbesondere die Sensitivitätsmetrics (z.B. „Pollution Sensitivity Index“ auf Basis der Diatomeen und „$SPEAR_{pest}$ Index“ auf Basis der Makroinvertebraten, beide zur Indikation von Spurenstoffen verwendet) zeigten deutliche und für eine stressorspezifische Indikation geeignete Antworten. Die Anteile urbaner Flächen und Kläranlagenabwässer standen in deutlichem Zusammenhang mit Spurenstoffkonzentrationen und den berechneten ökotoxikologischen Risiken, insbesondere hinsichtlich der Arzneimittel. Daneben wurden weitere Wasserqualitätsparameter (Konzentrationen von Sauerstoff und Nährstoffen) und hydrologische Variablen (Abflussvariabilität und Häufigkeit von Hochwasserereignissen) mit dem Anteil der Kläranlagenabwässer in Verbindung gesetzt. Im Gegensatz dazu war der Einfluss auf Pestizid-Konzentrationen geringer, jedoch für einzelne Substanzen, z.B. Biozide mit Anwendung im urbanen Raum, deutlich erkennbar. Stärkere Zusammenhänge wurden zwischen Pestiziden und dem Anteil landwirtschaftlicher Flächen festgestellt, insbesondere wenn zwischen unterschiedlichen Kulturpflanzen unterschieden wurde. Der Einfluss des Anteils von Waldflächen oder Grünland war vernachlässigbar.

Die Multi-Stressor-Analysen bestätigen negative Auswirkungen anthropogener Stressoren, insbesondere einer verschlechterten Wasserqualität und hydrologischer Veränderungen, auf Fließgewässerökosysteme. Die Ergebnisse verdeutlichen die Notwendigkeit zusätzlicher Maßnahmen zu beiden Stressorgruppen, um in Zukunft einen guten ökologischen Zustand zu erreichen. Darüber hinaus zeigen die Unterschiede zwischen den Organismengruppen, dass die Auswahl der Organismengruppen und der Metrics die Identifikation relevanter Stressoren beeinflussen kann. Idealerweise sollten daher bei der Analyse biologischer Veränderungen und der Identifikation geeigneter Maßnahmen alle drei Organismengruppen und verschiedene Metrics berücksichtigt werden. Für ausgewählte Stressoren (z.B. Spurenstoffe) und Organismengruppen (z.B. Fische) ist die Entwicklung zusätzlicher stressorspezifischer Metrics zu empfehlen. Zur Verringerung negativer Effekte müssen Bewirtschaftungspläne verschiedene Ursachen berücksichtigen. Dabei können eine erweiterte Abwasserbehandlung punktuelle Einträge und weitergehende Maßnahmen wie die Wiederherstellung der Ufervegetation diffuse Einträge aus landwirtschaftlichen Flächen verringern. Die Analysen verdeutlichen Unsicherheiten in der Spurenstoffbewertung, durch z.B. eine limitierte Stoffanzahl und Stichproben-Frequenz. Dies kann zu einer Unterschätzung der ökologischen Effekte dieser Stressorgruppe führen. Ein erweitertes Monitoring, v.a. der Spurenstoffe und der Hydrologie, kann insbesondere das investigative Monitoring ergänzen, um Stressoreffekte an ausgewählten Stellen zu analysieren, Ursachen biologischer Veränderungen zu identifizieren und eine evidenzbasierte Entwicklung von Maßnahmen zur Erreichung und Erhaltung des guten ökologischen Zustands bis 2027 und darüber hinaus zu ermöglichen.

1 Introduction

Freshwater ecosystems encompass a wide range of natural environments – from rivers and lakes to wetlands – and although they cover only a small proportion of the Earth's surface, freshwater ecosystems are exceptional hotspots of biodiversity (Balian et al., 2008; Dudgeon, 2019; Strayer and Dudgeon, 2010). The significance of these ecosystems extends beyond their intrinsic value, as functioning freshwater ecosystems are essential for human well-being (Carpenter et al., 2011; IPBES, 2019; Naiman and Dudgeon, 2011). Nature’s contributions to people include important benefits such as water purification, food supply, energy production, flood protection, climate regulation as well as recreation and cultural values (Díaz et al., 2018; IPBES, 2019; Petsch et al., 2023).

Freshwater ecosystems and the associated biodiversity are increasingly threatened by water quality deterioration, habitat degradation and flow modification with climate change further amplifying these and further environmental challenges (Dudgeon et al., 2006; Palmer et al., 2008; Reid et al., 2019; Strayer and Dudgeon, 2010). Anthropogenic activities and land use changes including urban development, industrial use and intensive agriculture are important drivers of environmental degradation with significant impacts on freshwater communities (Chen et al., 2023; Haase et al., 2023; Roy et al., 2003; Rumschlag et al., 2023; Schürings et al., 2024b). Urban and industrial areas are related to water quality deterioration including point source discharges of nutrients, salt ions and micropollutants such as pharmaceuticals, industrial chemicals and biocides (Kaushal et al., 2021; Royano et al., 2023; Waiser et al., 2011b; Wicke et al., 2021). In contrast, agricultural areas are often associated with diffuse pollution of nutrients and pesticides (Beusen et al., 2016; Halbach et al., 2021; Wolfram et al., 2021) and contribute to hydro-morphological alterations, both changes in riverine habitat characteristics and flow regimes. These can result from, for example, river straightening, bed fixation, water abstraction, increases in impervious surfaces or loss of riparian vegetation (Booth et al., 2016; Feld and Hering, 2007; Sabater et al., 2018).

Over the past decades, habitat restoration and improved wastewater treatment have made considerable progress in combating environmental degradation and biodiversity loss in freshwater ecosystems (Haase et al., 2023; Pharaoh et al., 2023; van Klink et al., 2020). Yet, studies observed that the recovery process of biodiversity has decelerated (Haase et al., 2023; Sinclair et al., 2024; Vaughan, 2023), while others report population declines for many freshwater species (Finn et al., 2023; WWF, 2022). Therefore, environmental challenges remain and necessitate additional measures to retrieve and sustain the resilience and functionality of freshwater ecosystems (Haase et al., 2023; Langhans et al., 2019).

1.1 Multiple Stressors

A significant challenge to the sustainable management of freshwater ecosystems, for example rivers, is the simultaneous presence and interaction of multiple stressors (Birk et al., 2020; Ormerod et al., 2010; Sabater et al., 2021). The term “stressor” here refers to an environmental variable whose range exceeds the natural variation as a result of anthropogenic activities, affecting individual species, communities, or ecosystems (Matthaei et al., 2010; Piggott et al., 2015; Townsend et al., 2008). Common riverine stressors can be categorized into four stressor groups (Perujo et al., 2021; Richter et al., 1996; Rico et al., 2016; Sabater et al., 2019): i) physical (e.g., oxygen depletion and temperature increase), ii) chemical (e.g., increased concentrations of nutrients, salt ions and micropollutants), iii) hydrological (e.g., increased flow variability, high flow frequency or low flow duration) and iv) morphological (e.g., physical alterations of the river bed, banks and the riparian zone).

The effects of multiple stressors on riverine biota have been analyzed in many scientific studies (e.g., Dolédec et al., 2021; Lemm et al., 2021; Liess et al., 2021; Nõges et al., 2016; Sabater et al., 2018; Sarkis et al., 2023; Schinegger et al., 2016). Yet, not all stressors have been considered equally often in previous multi-stressor studies, with some stressors remaining unaddressed or at least underrepresented, for example micropollutants (Nõges et al., 2016; Posthuma et al., 2020) and hydrological alterations of the flow regime (Kakouei et al., 2017; Meißner et al., 2019; Monk et al., 2006). The effects of micropollutants have regularly been analyzed separately from other stressors, whereas multi-stressor studies have often focused on non-chemical stressors (Johnson et al., 2020; Posthuma et al., 2020; Schäfer et al., 2023). Hydrological alteration in this study refers to the complex dynamics of the flow regime of a river, including the frequency, duration and seasonality of high and low flow events as well as the flow variability (Bunn and Arthington, 2002; Meißner et al., 2019; Richter et al., 1996). Despite the significant influence of hydrological alterations on the habitat structure and the riverine communities (Belmar et al., 2019; Dolédec et al., 2021; Waite et al., 2021), hydrology has often been assessed indirectly as a part of hydro-morphological alterations, for example through records of impoundments from hydro-morphological surveys (Meißner et al., 2019)

Biological responses in these studies can significantly vary between individual species and between different biological assemblages, such as macroinvertebrates, phytobenthos or fish (Fierro et al., 2019; Herlihy et al., 2020; Marzin et al., 2012; Villeneuve et al., 2015). Eutrophication, for example, affects the taxonomic composition of different riverine biota, with primary producers and macroinvertebrates often showing particularly strong responses (Dahm et al., 2013; Marzin et al., 2012; Poikane et al., 2020), while oxygen depletion affects the riverine fauna in particular but the flora to a lesser extent (Birk et al., 2020). Hydro-morphological alterations again disrupt the flow regime and essential habitat characteristics influencing all riverine biota, but especially disturb feeding and reproduction of fishes (Gieswein et al., 2017; Schmutz et al., 2015; Waite et al., 2021). Observed differences in the biological responses may depend on specific characteristics of the organisms such as the physiology, mobility, sensitivity to contaminants, reproduction or life cycle (Alric et al., 2021; Marzin et al., 2012; Rico and van den Brink, 2015; Urban et al., 2016). In the environment, differences in species sensitivity can lead to shifts in the community structure, with sensitive species declining while more tolerant species benefit from the influence of certain stressors (Enns et al., 2023; Mondy et al., 2016; Schürings et al., 2022).

Disentangling the effects of multiple stressors in the environment is difficult as many different combination of stressors can co-occur (Lemm et al., 2021; Sabater et al., 2021; Schäfer et al., 2015). For example, in agricultural areas communities are simultaneously influenced by increased concentrations of nutrients and pesticides as well as habitat degradation, with stressor variables often showing strong correlations or even multi-collinearity (Berger et al., 2017; Feld et al., 2016). Furthermore, co-occurring stressors can interact and thus can lead to higher (synergistic) or lower (antagonistic) effects (Folt et al., 1999). For river basin management this is particularly relevant as interactions between stressors can significantly influence the success of management measures (Spears et al., 2021). However, predicting the effects of a large number of stressors or stressor interactions is highly dependent on the study design and remains difficult (Birk et al., 2020; Mack et al., 2022; Schäfer et al., 2023).

1.2 Micropollutants and Chemical Mixtures

One important stressor group in aquatic environments is chemical pollution. This includes micropollutants, i.e. chemical substances that usually are available in low concentrations, for example in the range of nanograms to milligrams per liter (Loos et al., 2013). The term micropollutant covers a wide range of substances including pharmaceuticals, pesticides, personal care products and industrial chemicals (Schwarzenbach et al., 2006), which originate from various point and diffuse sources of pollution, such as municipal and industrial wastewater treatment plants (WWTPs; Finckh et al., 2022; Lainé et al., 2014; Petrie et al., 2015) or surface and groundwater runoff in agricultural areas (Bundschuh et al., 2014; Liess et al., 2008; Wiering et al., 2020). Pesticide applications in agricultural areas can significantly vary between different agricultural practices and cultivated crop types (Andert et al., 2015; Dachbrodt-Saaydeh et al., 2021).

Despite their low concentrations, micropollutants can have significant adverse effects on aquatic organisms (Bradley et al., 2021; Burdon et al., 2019; Schreiner et al., 2021). Similar to effects of other stressors, effects of micropollutants vary between different organism groups depending on their toxic mode of action, for example inhibition of specific receptors, enzymes or biosynthesis processes (Busch et al., 2016; Rico and van den Brink, 2015). Herbicides, for example, particularly affect riverine flora, whereas insecticides often show stronger effects on aquatic invertebrates (Morrissey et al., 2015; Schäfer et al., 2011; Wood et al., 2019). For fishes, effects of pesticides are usually lower compared to the other organism groups, but still evident at sub-lethal level (Nowell et al., 2018; Schäfer et al., 2011; Werner et al., 2021). In contrast, fish communities are often particularly sensitive to pharmaceuticals (Galus et al., 2013; Royano et al., 2023; Schwarz et al., 2017). Acute effects and chronic effects of micropollutants on survival, growth, development, reproduction and behavior have been demonstrated for all organisms groups (Kidd et al., 2007; Melvin and Wilson, 2013; Schwarz et al., 2017). Additionally, indirect effects, such as bottom-up or top-down effects in the food web due to altered community compositions at a specific trophic level, affect aquatic communities (Clance et al., 2023; Fleeger et al., 2003; Kidd et al., 2014; Prosser et al., 2016).

Currently, thousands of chemical substances have been registered globally with numbers further increasing (Wang et al., 2020). Many of these substances are transported into the environment (Schwarzenbach et al., 2006), where they often occur in complex mixtures of spatially and temporally ever-changing compositions (Kortenkamp and Faust, 2018; Scholz et al., 2022). The effects of chemical mixtures may exceed those of the individual substances (Thrupp et al., 2018) or may lead to combined mixture effects, even if each individual component would not cause adverse effects on its own (Silva et al., 2002). Several field studies revealed adverse effects of micropollutant mixtures on individual organisms, community composition as well as structural and functional biodiversity (Heß et al., 2023; Junghans et al., 2019; Liess et al., 2021). The environmental risk assessment in the regulation of chemicals, however, mainly addresses the risks of single substances, but so far does not fully integrate the risks of unintentional mixtures in the environment (Hassold et al., 2021; Kienzler et al., 2016; Kortenkamp and Faust, 2018). In the European Union, the Chemicals Strategy for Sustainability as part of the European Green Deal (European Commission, 2019, 2020a) demands the consideration of mixture effects in the chemical risk assessments. This is already integrated in the Biocidal Product Regulation (528/2012/EC; EU, 2012) but remains missing for effects of unintentional mixtures in the aquatic environment in other legislations such as the Plant Protection Product Regulation (1107/2009/EC; EU, 2009) or the REACH Regulation (1907/2006/EC; EU, 2006) on the registration, evaluation, authorization and restriction of chemicals (European Commission, 2020b).

Predictions of environmental risks of chemical mixtures have commonly been based on two mathematical concepts (e.g., Faust et al., 2001; Gustavsson et al., 2017; Spilsbury et al., 2020): Concentration Addition (CA; Loewe and Muischnek, 1926) and Independent Action (IA; Bliss, 1939). Both concepts require information on the composition of the mixture as well as data on the environmental concentrations and the ecotoxicological effects of the individual substances in the mixture (Backhaus and Faust, 2012; Hassold et al., 2021). The main difference is the assumption of a similar (i.e. CA) as opposed to a different (i.e. IA) mode of action of all individual mixture components, although the predictions of both models were often of a similar order of magnitude (Altenburger et al., 1996; Faust et al., 2001; Spilsbury et al., 2020). Significant deviations from the additivity assumption of both CA and IA were rare and mainly observed for specific groups of chemicals, such as mixtures of heavy metals, or for substances in particularly high concentrations (Cedergreen, 2014; Martin et al., 2021). Since predictions of CA were usually more conservative and robust even for mixtures of substances with unknown or dissimilar mode of action (Altenburger et al., 1996; Faust et al., 2001; Jakobs et al., 2020), CA has been recommended as a pragmatic and precautionary default approach for assessing mixture risks in the environment (Backhaus and Faust, 2012; Faust et al., 2003; Junghans et al., 2006). Combined mixture risks can simply be calculated from the sum of the toxic units (TUs), i.e. the quotient of the environmental concentration and the ecotoxicological effect concentration (Sprague, 1970), of the individual substances in a mixture (Backhaus and Faust, 2012; Spilsbury et al., 2024). Additional assessment factors are applied to account for uncertainties arising from extrapolation between different species, from short-term to long-term effects or from laboratory to field assessments (Backhaus and Faust, 2012; Spilsbury et al., 2024). Although strictly CA should be applied to the same ecotoxicological endpoint, depending on the data availability and the research question, TUs and combined mixture risks may be calculated on the basis of effect concentrations for specific species and organism groups or for the most sensitive species, respectively (Backhaus and Faust, 2012). Risk quotients (RQs), i.e. the quotient of environmental concentrations and official environmental assessment values (e.g., quality standards or predicted no effect concentrations, PNEC), and sums of RQs can also be calculated for a simple and conservative assessment of mixture risks (Backhaus and Faust, 2012; Gustavsson et al., 2017; Langer et al., 2017; Munz et al., 2017). In addition to predicting mixture risks, TUs and RQs can be used to identify substances with a high relative contribution to the combined mixture risks or to identify sites with a particularly high risk of adverse effects of chemical mixtures (Altenburger et al., 2019; Finckh et al., 2022; Ginebreda et al., 2014). This is particularly relevant for the development and prioritization of management measures to reduce ecotoxicological effects of micropollutants and micropollutant mixtures in the environment (Ohe et al., 2011).

1.3 European Water Framework Directive

The European Union has established comprehensive policies addressing the protection and sustainable management of water resources and aquatic ecosystems (Giakoumis and Voulvoulis, 2018). The cornerstone of water policy is the European Water Framework Directive (WFD, 2000/60/EC; EU, 2000). The key objective of the WFD is reaching a good ecological status as well as a good chemical status of all European water bodies in three management cycles ultimately by 2027 (Arle et al., 2016; Carvalho et al., 2019). For heavily modified or artificial water bodies, which non-substitutable use for, for example, water supply, flood protection or hydropower would be significantly affected by management measures, a good ecological potential may be defined instead (Arle et al., 2016; Hering et al., 2010). For simplification, only the term “status” will be used in this thesis.

The assessment and management of water bodies follows a holistic and integrated approach and is grounded on comprehensive monitoring programs (Arle et al., 2016). In Germany, these monitoring programs are defined in the German surface water directive (OGewV, 2016) and additional monitoring guidelines for the federal states (e.g., LAWA, 2021). The monitoring comprises different biological quality elements (BQEs), including fishes, macroinvertebrates, macrophytes, phytobenthos and phytoplankton (Arle et al., 2016). The status of each BQE can be described using ecological metrics of the community composition, abundance and presence/absence of species, taking into account, for example, species that are particularly sensitive or tolerant to certain stressors, or functional traits such as feeding types or habitat preferences (Hering et al., 2006a; Poikane et al., 2020). These ecological metrics are compared to target values of defined reference conditions and are used to assess the ecological status but may also serve as indicators of specific stressors affecting the biological assemblages (Arle et al., 2016; Hering et al., 2006a). However, as the WFD does not specify the assessment methods, a wide range of approaches have been developed for the individual BQEs by different countries (Birk et al., 2012; Hering et al., 2010; Poikane et al., 2020). Besides the BQEs, the ecological status is determined by monitoring supporting quality elements, including physico-chemical quality elements (e.g., water temperature, oxygen and nutrient concentrations), hydro-morphological quality elements (e.g., longitudinal continuity, river bed and bank structure), as well as river basin-specific pollutants (Arle et al., 2016). The actual chemical status is based on the monitoring of selected priority substances for which official Environmental Quality Standards (EQSs) have been derived (Arle et al., 2016).

The development and implementation of these broad scale, comprehensive monitoring programs requires considerable effort, but it is also a major achievement as it provides extensive monitoring datasets covering different BQEs and a wide range of environmental stressors (Carvalho et al., 2019; Hering et al., 2010). These datasets constitute a valuable basis for assessing the state of the BQEs, identifying relevant stressors and selecting appropriate management measures to improve the ecological status (Baattrup-Pedersen et al., 2019; Borja et al., 2006; Carvalho et al., 2019). However, as the objective of reaching a good ecological status has only been achieved in 9 % of rivers in Germany (BMUV/UBA, 2022), additional efforts are needed to understand the causes for not meeting the targets as well as to derive further effective management measures (Baattrup-Pedersen et al., 2019; Poikane et al., 2020).

1.4 Research Motivation

A holistic understanding of and linkage between the assessment of biological assemblages and environmental stressors on the one hand and between the assessment and its implications for river basin management on the other hand is essential to progress towards reaching a good ecological status in surface waters (Carvalho et al., 2019; Poikane et al., 2020). Effective river basin management necessitates the monitoring, analysis, identification and ranking of relevant stressors, their effects on the biological assemblages and their potential sources (Arle et al., 2016; Baattrup-Pedersen et al., 2019).

The aim of this thesis was to analyze the effects of multiple stressors on riverine assemblages using the comprehensive datasets of WFD-related monitoring programs in Germany. The relative importance of each stressor was examined in order to provide information for identifying and prioritizing future management measures. The stressor groups were selected based on evidence of relevance for riverine biota in previous studies (e.g., Lemm et al., 2021; Meißner et al., 2019; Waite et al., 2021) and included water quality, both physico-chemical variables and micropollutants, and hydro-morphological quality, combining hydrological and morphological parameters. A particular emphasis was put on micropollutants and their mixtures as knowledge gaps still remain for the ecological effects of this group, especially in a multi-stressor context (Nõges et al., 2016; Posthuma et al., 2020; Schäfer et al., 2015). In Chapters 2.1 and 2.2, comprehensive multi-stressor datasets were compiled for i) a case study of macroinvertebrates in the Erft and Niers catchments, Germany using detailed micropollutant data from a special monitoring program including a wide range of substances, and for ii) an extended dataset of the BQEs macroinvertebrates, benthic diatoms and fishes across four federal states of Germany. Stressor effects were compared between both the taxa lists of the BQEs and different ecological community metrics to assess their respective stressor responses and their applicability as diagnostic indicators for the impact of specific stressors. In Chapter 2.3, responses of fish communities and species-specific differences were further examined at the species-level, as multi-stressor analyses on the basis of available ecological metrics for this organism group proved to be difficult in the previous chapter.

After analyzing the effects of the selected stressors on the biological assemblages and ranking them according to their relative importance, potential sources of these stressors were examined. This information can help target future management measures aimed at reducing the stressors' impacts on the ecological status. In Chapter 2.3, the stressor groups, including individual micropollutant TUs as well as physico-chemical, morphological and hydrological variables, were set in relation to municipal WWTP effluents using modelled data of the cumulative percentage of wastewater in stream. In Chapter 2.4, the influence of different anthropogenic land uses on micropollutant levels was specifically addressed, namely the impact of the percentage of urban area, forest, grassland and cropland in the catchment, while differentiating between effects of individual crop types, for which variations in agricultural practices and pesticide applications have been reported (Andert et al., 2015; Dachbrodt-Saaydeh et al., 2021).

The central research questions of this thesis were:

- Which stressor groups are of particular relevance for macroinvertebrates, benthic diatoms and fishes? Do the stressor effects differ between biological assemblages or between individual species? What is the relative importance of micropollutants for the ecological status in a multi-stressor context?
- How do wastewater treatment plant effluents contribute to the water quality and hydro-morphological degradation in the receiving surface waters? What is the impact of anthropogenic land use on micropollutant levels, when considering urban areas, forests and agriculture?
- What information can be gained to support the development of tailored management measures addressing both water quality-related stressors and hydro-morphological stressors?

2 Scientific Articles

In the context of this thesis, the following scientific articles have been published in peer-reviewed journals. The inclusion of the following publications in the dissertation does not infringe any copyright.

1. Markert, N., Guhl, B. & Feld, C.K., 2022
   The hierarchy of multiple stressors' effects on benthic invertebrates: a case study from the rivers Erft and Niers, Germany
   Environmental Sciences Europe 34, 100, DOI: 10.1186/s12302-022-00679-z

2. Markert, N., Guhl, B. & Feld, C.K., 2024
   Water quality deterioration remains a major stressor for macroinvertebrate, diatom and fish communities in German rivers
   Science of the Total Environment 907, 167994, DOI: 10.1016/j.scitotenv.2023.167994

3. Markert, N., Guhl, B. & Feld, C.K., 2024
   Linking wastewater treatment plant effluents to water quality and hydrology: effects of multiple stressors on fish communities
   Water Research 260, 121914, DOI: 10.1016/j.watres.2024

4. Markert, N., Schürings, C. & Feld, C.K., 2024
   Water Framework Directive micropollutant monitoring mirrors catchment land use: importance of agricultural and urban sources revealed
   Science of the Total Environment 917, 170583, DOI: 10.1016/j.scitotenv.2024.170583

2.1 The hierarchy of multiple stressors' effects on benthic invertebrates: a case study from the rivers Erft and Niers, Germany

Nele Markert[1,2], Barbara Guhl[1], Christian K. Feld[2]

[1] North Rhine-Westphalian Office of Nature, Environment and Consumer Protection (LANUV NRW), 40208 Düsseldorf, Germany
[2] University of Duisburg-Essen, Faculty of Biology, Aquatic Ecology and Centre for Water and Environmental Research (ZWU), Universitätsstr. 5, 45141 Essen, Germany

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Abstract

A variety of anthropogenic stressors influences the ecological status of rivers worldwide. Important stressors include elevated concentrations of nutrients, salt ions, heavy metals and other pollutants, habitat degradation and flow alteration. Some stressors tend to remain underrepresented in multiple-stressor studies, which in particular is apparent for micropollutants (e.g. pesticides, pharmaceuticals) and alterations of the flow regime. This case study analyzed and compared the effects of 19 different stressor variables on benthic macroinvertebrates in the two German rivers Erft and Niers (Federal State of North Rhine-Westphalia, Germany). The stressors variables were assigned to four stressor groups (physico-chemical stress, mixture toxicity of 42 micropollutants, hydrological alteration and morphological degradation) and were put into a hierarchical context according to their relative impact on the macroinvertebrate community using redundancy analysis and subsequent variance partitioning.

The results suggest a strong and unique effect of physico-chemical stress, yet at the same time reveal also a strong joint effect of physico-chemical and hydrological stressor variables. Morphological degradation showed subordinate effects. Notably, only a minor share of the explained variance was attributed to the mixture toxicity of micropollutants in these specific catchments.

The stressor hierarchy indicates that management measures for improving the ecological status still need to address water quality issues in both rivers. The strong joint effect of physico-chemical stress and hydrological alteration might imply a common source of both stressor groups in these two catchment areas: lignite mining drainage, urban area and effluents of wastewater treatment plants. The findings point at the important role of alterations in the flow regime, which often remain unconsidered in hydro-morphological surveys.

Introduction

Rivers in Europe and worldwide are impacted by multiple stressors, which can adversely affect riverine biota and ecological integrity (Birk et al., 2020; Lemm et al., 2021; Liess et al., 2021; Ormerod et al., 2010). Multiple stressors include eutrophication, salinization, heavy metals and physical habitat degradation and are subject to frequent river monitoring and assessment programs. Yet, some stressors are less frequently monitored and often remain unaddressed, such as micropollutants and hydrological alterations (Kakouei et al., 2017; Meißner et al., 2019; Monk et al., 2006; Poikane et al., 2020).

Micropollutants comprise numerous chemical compounds, for example, pesticides, industrial chemicals, pharmaceuticals, and personal care and household products. Some micropollutants belong to the group of so-called priority substances (e.g., the pesticides Diuron and Lindane), which are mandatorily monitored under the EU Water Framework Directive (EU WFD, 2000/60/EC and 2013/39/EU) in Europe. In the environment micropollutants often occur in complex mixtures of numerous individual substances, which might result in a biologically relevant joint mixture toxicity, even if each individual substance occurs at low (non-toxic) concentrations (Silva et al., 2002; Thrupp et al., 2018). Because of the very high number of micropollutants, however, a comprehensive monitoring of these substances and their complex mixtures remains laborious and very resource-intensive, which may explain, why this stressor group remained under-addressed – or even unaddressed – in previous multiple-stressor studies (e.g., Lemm and Feld, 2017, Villeneuve et al., 2018 and Segurado et al., 2018, but see Lemm et al., 2021, Liess et al., 2021 and Nowell et al., 2018 for multiple-stressor studies including micropollutants). So far, there is still little knowledge about effects of micropollutants in a multiple-stressor context, but evidence from previous studies suggests that ecotoxicological effects of these substances pose a significant risk to riverine biota (Kuzmanović et al., 2016; Langer et al., 2017; Malaj et al., 2014; Posthuma et al., 2020; Waite et al., 2019).

In contrast to micropollutants, there is a huge body of literature on the effects of hydrological and morphological stressors on riverine biota (Elosegi and Sabater, 2013; Gieswein et al., 2017; Lemm et al., 2021; Nõges et al., 2016; Poff and Zimmermann, 2010; Villeneuve et al., 2018). The European Environment Agency recently listed hydro-morphological impacts, such as channelization, disconnection of floodplains or flow regulation, among the top stressor groups affecting Europe’s rivers (EEA, 2018). Hydrological alteration in particular refers to the deviation of river flow and discharge regimes from natural conditions. It covers the extent, timing and frequency of high and low flow conditions as well as its seasonal and annual dynamics (Bunn and Arthington, 2002b; Poff et al., 1997; Richter et al., 1996b). Poff et al. (1997) suggested numerous Indicators of Hydrological Alteration (IHA) that are calculable from time-series data and that have been shown to relate to riverine biological conditions. The degree of hydrological alteration within a river reach might be derived from records of gauging stations provided that a gauging station has been present for several years in – or close to – a river reach of interest. Hydrological alteration is known to severely and adversely impact riverine biota (Bunn and Arthington, 2002a; Kakouei et al., 2017; Meißner et al., 2019). However, the degree of hydrological alteration continues to remain largely unaddressed by hydro-morphological surveys in Europe (Gellert et al., 2014; Raven et al., 1997), which tend to address hydrological stress by mere spot-measures of flow conditions through estimates of flow velocities and its diversity within a river reach. Besides, the degree of hydrological alteration is indirectly derived from its interlinkage with morphological degradation in such surveys. For example, stagnant flow conditions are assigned to reaches directly upstream of weirs or dams.

The ongoing disparity in the coverage of different stressor groups by contemporary standard monitoring schemes render a comparative analysis of the relevance of these stressors difficult. Here, we present an attempt to compare and hierarchically order the impact of multiple-stressor groups (physico-chemical variables, micropollutants, hydrological alteration and morphological degradation) on riverine biota. Effects of micropollutants were included using approaches for assessing risks of mixture toxicity calculated for a comprehensive monitoring dataset of 42 selected substances based on previous findings on important drivers of mixture toxicity (Markert et al., 2020). The aim of this study was to identify a stressor hierarchy, i.e., a hierarchical order of stressors according to their effects on riverine benthic macroinvertebrates. We hypothesized i) that micropollutants would occupy a high rank order because of their potential ecotoxicological effects reported in previous studies; ii) that the ranks of hydrological alteration and morphological degradation would be similar, due to the interlinkage of hydrological and morphological conditions; iii) that the rank of physico-chemical variables would be subordinate due to improved wastewater treatment in Germany.

Methods

Study area

In total, 49 sampling sites of benthic invertebrates are included in this study (Figure 1). The study sites are located in the catchments of the rivers Erft and Niers in the West of North Rhine-Westphalia (NRW), Germany. Both catchments are characterized by urban areas including effluents from wastewater treatment plants (WWTPs) and combined sewage and rainwater discharges as well as lignite mining. Percent urban area is associated with a high proportion of impervious surfaces, which strongly influences hydrological patterns of rivers including the flow variation or the frequency and magnitude of high flow events (Booth et al., 2016; Coleman et al., 2011; Zhou et al., 2014). Urban surface run-off and WWTP effluents are sources of both chemical pollution and thermal load (Booth et al., 2016; Kay et al., 2017), whereas mining and the discharge of mining drainage are associated with increased concentrations of chloride, sulphate and iron as well as with disturbances of the river hydrology and thermal regime (Braukmann and Böhme, 2011; Cadmus et al., 2018; García-Criado et al., 1999; Petruck and Stöffler, 2011; Pusch and Hoffmann, 2000). Therefore, the study area is particularly suited to address the impact of hydrological alteration and chemical pollution. Statistical key parameters of the land use characteristics as well as additional maps including the land use in both catchments are shown in Supplement S1.

The upper part of the Erft catchment (total catchment size: 1,918 km^2) is located in the low mountain range “Eifel” at altitudes around 550 m above sea level. The region’s land cover is characterized by forest and grassland, yet with increasing shares of intensive agriculture and urban areas (including a high number of wastewater treatment plants) along the middle and lower section of the river. Both sections are influenced by lignite mining and associated discharges of drainage water, too (Erftverband, 2018a; MULNV, 2015b). The catchment of river Niers (total catchment size: 1,380 km^2) is entirely located in the lowland; its source close to the city of Mönchengladbach is at 80 m above sea level. The Niers region is also strongly affected by lignite mining, namely by drainage and the related drop of groundwater levels. All of the natural sources in the upper catchment have dried up and the river is artificially fed by discharges of deep groundwater. The upper reach of the Niers is influenced by a high percentage of urban area and agriculture. In this region, the Niers receives rainwater as well as combined sewage discharges. From the WWTP Mönchengladbach onward, the Niers is strongly influenced by WWTP effluents, which contributes a high proportion to the discharge downstream. In the middle and lower regions, the catchment of the Niers consists mainly of agricultural area (MULNV, 2015a).

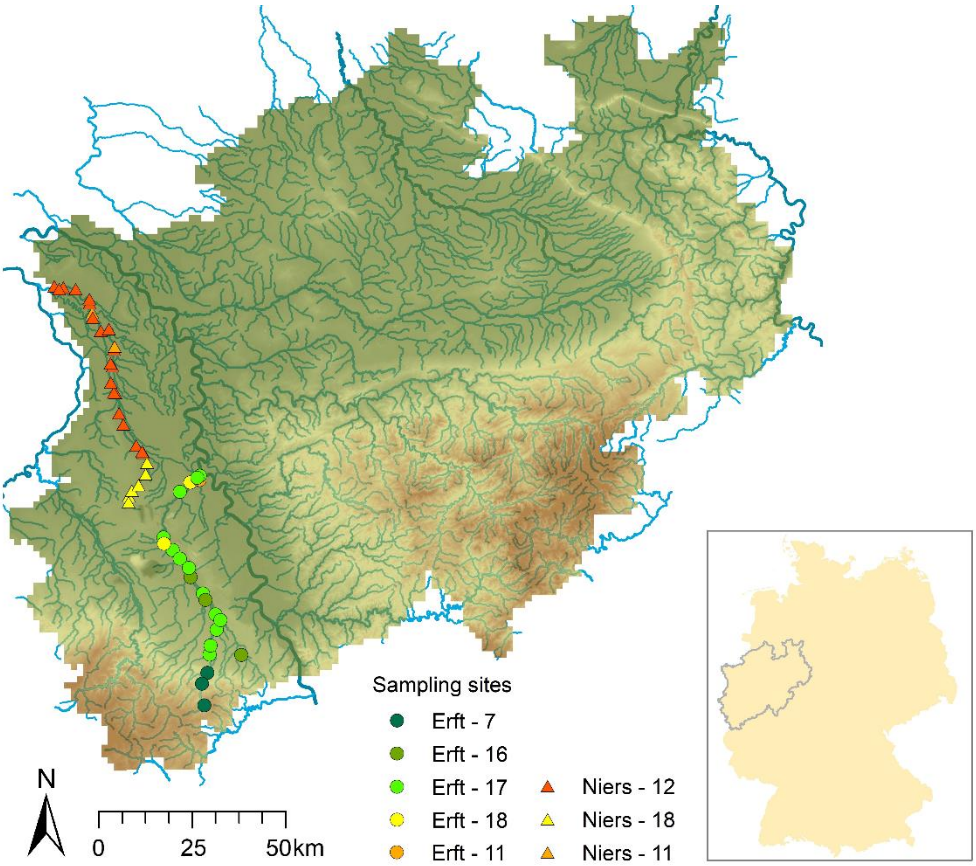

Figure 1: Sampling sites in the catchments of the river Erft and Niers. Sampling sites are located in the river Erft (triangles) and the river Niers (dots) in North Rhine-Westphalia (NRW), western Germany. Map color corresponds to the elevation profile with green colors indicating lowlands and brown colors indicating low mountain ranges (artificial minimum of -299 m in mining area, natural range 9 m to 843 m above sea level). Color of sampling sites show the stream types according to the German river typology for the EU WFD (Pottgiesser and Sommerhäuser, 2004, 2014) (©dl-zero-de/2.0).

Stressor variables

Altogether, 19 stressor variables belonging to four stressor groups were analyzed in this study (Table 1). We focused on environmental variables that constitute directly measurable stressors (e.g., nutrients, salt ions, habitat structure) and were identified as important stressor groups in previous studies (Birk et al., 2020; EEA, 2018). Land use thus was excluded because of its collinearity with several other environmental variables (Supplements S2 for spearman correlations; see also Bradley et al. 2020, Munz et al. 2017 and Kail et al. 2009). Sampling sites for water chemistry (physico-chemical variables and micropollutants) were spatially matched to macroinvertebrate sampling sites using a maximum distance of approx. 5 km up-/ downstream. Gauging stations were spatially matched using a maximum distance of approx. 7.5 km up-/downstream as well as a maximum deviation of catchment sizes of approx. 15 %. Potential confounding factors, such as WWTP effluents or confluences with larger tributaries between the macroinvertebrate sampling sites, the chemical sampling sites and the gauging stations these sampling sites, were checked using ArcGIS. Only samples without signs of confounding factors were included in the dataset. Macroinvertebrate samples of 2017 were matched to chemical and hydrological data from the period of 2016 to 2017 as well as to the most recently available hydro-morphological surveys, which date from 2011 to 2013. In some cases, additional data from 2015 or 2018 were included to reduced data gaps (see descriptions of methods physico-chemical variables and methods macroinvertebrate data below).

Table 1: Statistical key parameters of mean annual values of all stressor variables. Stressor's relevance is expressed as percent samples above the risk threshold (RT). RT of physico-chemical variables were chosen in accordance with the German surface water directive (Oberflächengewässerverordnung, 2016). If different stream type-specific thresholds were available, the strictest value was used. RTs were set to class 3 for morphological quality (1 = natural, 7 = fully degraded). For mixture toxicity of micropollutants a quotient of 1 indicated measured concentrations exceeding the respective ecotoxicological effect concentration. Statistical parameters of each catchment are shown in Supplements S3.

Stressor	Stressor code	Description	Mean	Median	Min.	Max.	RT	%Samples at risk
Physico-chemistry	T	Maximum water temperature [°C]	20.48	20.70	12.50	24.90	-	-
	O_2	Minimum oxygen concentration [mg/L]	8.03	8.10	5.70	10.20	7	22 %[1]
	TP	Mean total phosphate concentration [mg/L]	0.13	0.14	0.02	0.24	0,1	67 %
	TN	Mean total nitrogen concentration [mg/L]	4.38	4.65	0.50	7.91	-	-
	Cl	Mean chloride concentration [mg/L]	72.67	76.60	17.38	237.63	200	2 %
	SO_4	Mean sulphate concentration [mg/L]	83.83	91.39	24.39	252	200	2 %
	Fe	Mean total iron concentration [mg/L]	0.91	0.92	0.15	2.53	0,7	63 %
	NO_2-N	Mean total nitrite nitrogen concentration [mg/L]	0.04	0.04	0.01	0.13	0,05	43 %
	NH_4-N	Mean total ammonium nitrogen concentration [mg/L]	0.12	0.11	0.03	0.37	0,1	55 %

Table 1 (continued)

Stressor	Stressor code	Description	Mean	Median	Min.	Max.	RT	%Samples at risk
Mixture toxicity	$RQ_{mix,acute}$	Risk quotient of acute mixture toxicity	0.23	0.18	0.06	1.29	1	2 %
	$RQ_{mix,chr}$	Risk quotient of chronic mixture toxicity	0.77	0.73	0.20	1.98	1	18 %
Hydrological alteration	fh5	High flow frequency[2]	19.05	20.50	4.00	41.00	-	-
	dl16	Low flow pulse duration[3]	8.89	5.18	3.15	36.72	-	-
	ra5	Number of day rises[4]	0.38	0.38	0.27	0.49	-	-
	MQMNQ	Quotient of the long-term mean and mean low-flow discharge	3.20	2.47	1.54	13.67	-	-
Morphological degradation	HP1	Channel development	6.24	6.00	4.00	7.00	3	100 %
	HP2	Longitudinal profile	5.38	5.00	3.00	6.50	3	96 %
	HP4	Cross profile	5.32	5.00	3.00	7.00	3	94 %
	HP5	Bank structure	5.68	6.00	3.00	7.00	3	96 %

[1] Values are strongly influenced by timing of sampling during the day and only represent an estimation.
[2] Average number of events above the median flow of the flow record
[3] Median of the yearly average durations of flow events below the 25th percentile
[4] Percentage of days in the flow record in which the flow is greater than at the previous day

Physico-chemical variables

Mean annual statistics of physico-chemical variables were calculated using the arithmetic mean of mean concentrations for all nutrients, salt ions and iron as well as the mean of the minimum for the oxygen concentration and mean of the maximum for the water temperature in accordance with the German surface waters directive transposing the WFD into national law (OGewV 2016; Table 1). To avoid data gaps data for the selected period of 2016 and 2017 were supplemented by mean concentrations measured in the four-years-period of 2015 to 2018 for the majority of sampling sites. To exclude a temporal trend of concentrations between 2015 and 2018 the long-term variation of concentrations from 2009 to 2019 was examined for all selected sampling sites prior to the analyses and only sampling sites without visible temporal trends were used for further analyses. For each site a minimum of seven and a maximum of 35 measured values were available for each physico-chemical variable. Concentrations of total nitrogen were imputated using Multivariate Imputation by Chained Equations (default method of predicted mean matching; van Buuren and Groothuis-Oudshoorn, 2011) for two sampling sites.

Micropollutants

A selection of 42 micropollutants of the substance classes pesticides (21 herbicides, two insecticides and two fungicides), pharmaceuticals (13 substances) as well as industrial and household chemicals (four substances) were included in this study. The selection was based on previous analyses of key drivers of mixture toxicity in the Erft catchment (Markert et al., 2020) as well as further studies. A full list of all selected substances as well as number of detections per substance is shown in Supplement S4. Data originated from routine monitoring schemes in accordance with the WFD, where between four and twelve grab samples were taken at each sampling site. For 22 sampling sites in the Erft catchment data from a special monitoring program of the Erftverband (Erftverband, 2018b; Markert et al., 2020) were included. The program covered 13 grab samples taken between March 2016 and March 2017 and included five rain event samples. Additionally, at one sampling site seven grab samples and seven composite samples were taken. Between 25 and 41 substances were measured at each site of the routine monitoring scheme, whereas all 42 selected substances were measured within the special monitoring program over the entire period. On average, each substance was measured at 38 sampling sites (min: 23 sites, max: 49 sites). Concentrations below the limit of quantification (LOQ) were substituted by half of the value of the LOQ (HLOQ) of the respective substance. Effects of micropollutant mixtures were described by the proxy variable RQ_{mix} which is based on toxic units using the concept of concentration addition (Backhaus and Faust, 2012; Kortenkamp et al., 2009; Table 1). An RQ_{mix} above one indicates potential mixture risks for the aquatic communities. Further details on the calculation of the RQ_{mix} can be found in Markert et al. (2020). Acute mixture risks were assessed using yearly maximum concentrations and acute ecotoxicological effect concentration (EC_{50}), chronic mixture risks using measured yearly mean concentrations and chronic effect concentrations (EC_{10} or No Observed Effect Concentration, NOEC), respectively (Supplement S4). For comparisons of the mixture risks, the RQ_{mix} was additionally calculated for the organism groups algae and fish (Supplement S3).

Hydrological alteration

Indicators of Hydrological Alteration were calculated using data of the daily mean discharge from gauging stations (Olden and Poff, 2003; Richter et al., 1996a). To avoid data gaps, data for two sites, which are positioned in-between stations, were supplemented by the median of the discharge of the two gauging stations above and below the sites. The data for two sites close to the Erft estuary below the lower-most gauging station were supplemented by the sum of discharges of that station plus a station in the larger tributary Gillbach entering the Erft upstream of the two sites. Based on previous studies on ecologically relevant IHA (Archfield et al., 2014; Meißner et al., 2019; Olden and Poff, 2003), a selection of 39 IHA was subjected to a principal component analysis (PCA) to identify suitable IHAs for multiple-stressor analyses (Supplement S5). Indicators were selected based on three criteria: high loadings in the PCA, low correlation with other indicators and coverage of the main IHA groups magnitude of flow events, rate of change and the frequency and duration of high-flow and low-flow events. Due to collinearity, only three parameters were finally included in subsequent analyses (Table 1): High flow frequency (fh5, number of events above median flow), the low flow pulse duration (dl16, average duration of events below the 25th percentile of flow in the flow record) describing high and low flow conditions and the number of day rises (ra5, percentage of days with a flow greater than the previous day) describing the flow variation. Full descriptions of the IHA are shown in Supplement S5. In addition to the IHA, the quotient of the long-term mean discharge and mean low-flow discharge (MQ/MNQ) based on regionalized data at the sampling sites were included indicating the flow variation of low flow compared to mean flow conditions. Both the MQ and the MNQ are commonly used for hydrological analyses and were therefore included as additional stressor variable (Kempe and Krahe, 2005).

Morphological degradation

Morphological degradation was assessed using data from the German standard river habitat survey of North Rhine-Westphalia (Gellert et al., 2014). For each sampling site, the quality classes of different main parameters (channel development, longitudinal profile, bed structure, cross profile and bank structure) were recorded at 100 m increments and the median was calculated over different stream course lengths (0.5 km, 1 km, 2 km and 5 km upstream of the biological sampling site). Because correlations between the ecological status using the Ecological Quality Class (EQC) according to the WFD (see description of macroinvertebrate metrics below) and the morphological quality at the different stream course lengths were particularly high for the 1 km medians, these were chosen for further analyses (Spearman correlation plots are included in Supplement S6). The main parameter bed structure (HP3) was excluded due to data gaps (Table 1). Morphological quality was graded from 1 (unaltered, natural reference condition) to 7 (unnatural, completely modified) (Gellert et al., 2014).

Stressor relevance

For each site, stressor values were compared to German environmental quality targets (OGewV 2016), if available, and expressed as percentage of sites at risk, i.e., the share of sites exceeding the target values (Table 1). Percentage sites at risk was particularly high for morphological (95–100 %) and physico-chemical stressors (22–65 %), while it was notably low for sulphate and chloride (2 % each) as well as for acute and chronic invertebrate mixture toxicity (2 and 18 %, respectively). In contrast, the calculated acute and chronic mixture toxicity were distinctly higher for algae (100 % both) and fish (0 and 98 %), respectively

(Supplement S3). Environmental quality targets were unavailable for hydrological stressors.

Macroinvertebrate metrics

Benthic macroinvertebrates were collected during spring and early summer in 2017, except for seven sites at tributaries to the Erft river, which were sampled in spring 2018. To ensure comparability, macroinvertebrate metrics of the latter sites were compared for samples taken in 2015 and 2018, but did not reveal temporal patterns (results not shown here). Macroinvertebrate sampling followed a multi-habitat sampling protocol (Meier et al., 2006), which allows of a standardized sampling of 20 microhabitats according to its coverage on the river bottom. Determination aimed for species level except for oligochaetes and dipterans (for details see the German operational taxa list (Haase et al., 2006). In addition, taxa lists were manually harmonized to eliminate remaining determination bias and subjected to the German assessment software Perlodes Online (Version 5.0.8, gewaesser-bewertung-berechnung.de, 2021) to calculate macroinvertebrate community metrics. Five different metrics types were included: abundance, diversity, sensitivity and function as well as the Ecological Quality Class (EQC) of the EU WFD integrating different river-type specific metrics into one quality score. A predecessor software tool (Asterics v.4.0.4; UBA, 2014) was used to calculate the Index of Biocoenotic Region (IBR) and the Average Score per Taxon (ASPT). Altogether, the responses of 21 metrics were analyzed for multiple stressors' effects (Table 2). Metric selection was based on its ecological meaningfulness as reported by previous studies (Berger et al., 2017; Feld et al., 2020; Lemm et al., 2019; Meißner et al., 2019; Sundermann et al., 2013), and checked for pairwise correlations to reduce redundant information per metric group.

Table 2: Selection of 21 benthic macroinvertebrate metrics included in the redundancy analysis and subsequent variance partitioning.

Group	Metric name	Metric code	Metric description	Reference
Abundance	Total Abundance	Abund	Sum of the abundance of all taxa	Perlodes Online, 2021
Diversity	Number of Taxa	#Taxa	Number of reported taxa	Perlodes Online, 2021
	Evenness	Even	Diversity-Index	
Sensitivity	German Fauna Index	FI	General and morphological degradation	Lorenz et al., 2004
	Number of taxa of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata	#EPTCBO	Number of taxa belonging to sensitive taxonomical groups	Perlodes Online, 2021

Table 2 (continued)

Group	Metric name	Metric code	Metric description	Reference
	Average Score per Taxon	ASPT	Multiple degradation types	Armitage et al., 1983
	Multimetric Index	MMI	River-type specific general degradation	Böhmer et al., 2004
	KLIWA-Index	KLIWA	Temperature tolerance as temperature equivalent [°C]	Halle et al., 2016; Sundermann et al., 2022
	Species at Risk Index	$SPEAR_{pest}$	Sensitivity towards pesticide pollution	Liess and von der Ohe, 2005
Function	Percentage of specific habitat preferences	Pel%, Psa%, Phy%, POM%	Habitat preference (pelal, psammal, phytal, particulate organic matter)	Moog, 1995
	Percentage of specific feeding type preferences	Shr%, Gath%, Graz%, Fil%	Feeding preference (shredder, gatherer, grazer and filterer)	Schweder, 1992
	Rheoindex	RI	Stream flow preference	Banning, 1998
	Index of biocoenotic regions	IBR	Preference for regions of the longitudinal river zonation	UBA, 2014
	Percentage of alien species	Alien%	Alien species	Perlodes Online, 2021
Integrated	Ecological Quality Class	EQC	Ecological Quality Class	Hering et al., 2006a; Perlodes Online, 2021

Statistical analysis

Data processing and analyses were conducted using the open-source software R (Version 4.0.3; R. Core Team, 2013) with R Studio (Version 1.4.1103). IHA were calculated using the package EflowStats (calc_allHIT; Mills and Blodgett, 2017). Stressor gradients and correlations were graphically analyzed with a PCA using the core package stats (prcomp) and the package factoextra (fviz_pca_biplot; Kassambara and Mundt, 2020). This step aimed at identifying the main stressor gradients in the dataset. Collinear stressors were then identified based upon variance inflation factors (vifstep, package usdm; Naimi, 2017) and excluded from subsequent analyses. To identify a stressor hierarchy, the remaining stressor variables were z-transformed and analyzed by a redundancy analysis (RDA) and a subsequent variance partitioning using the package vegan (rda, varpart; Oksanen et al., 2020). Thereby, biological variance was partitioned to the four stressor groups as outlined before. The stream type according to the German river typology for the EU WFD (Pottgiesser and Sommerhäuser, 2004, 2014) was included as a co-variable in all RDAs, to partial out the influence of natural stream type-specific characteristics (e.g., size, geology, altitude, ecoregion). The dataset comprises six different stream types in total ranging from small coarse substrate dominated calcareous highland rivers (Type 7), small and mid-sized gravel-dominated or loess and loam-dominated lowland river (Type 16, 17 and 19) to organic substrate-dominated rivers (Type 11 and 12; Figure 1). RDA models and marginal effects of explanatory variables (stressors, stream types) were tested for significance with an ANOVA permutation test (anova, package vegan; Oksanen et al., 2020). Pairwise-correlations between macroinvertebrate metrics and between metrics and stressor variables were calculated using Spearman Rank correlation (rcorr, package Hmisc; Harrell Jr, 2021).

Results

Stressor gradients and relationships

The PCA of 19 stressor variables revealed a separation of two main gradients of stressor variables along the first two principal components (PC1 and PC2, Figure 2). PC1 is characterized by water quality stressors, with all physico-chemical and mixture toxicity variables showing a high to moderate correlation among each other (correlation strengths not shown). Notably, a high degree of physico-chemical pollution is related to low oxygen contents in the dataset, which is shown by the relevant vectors pointing at opposite directions in the plot (Figure 2). PC2, in contrast, marks a clear hydrological-morphological stressor gradient, with all but two variables (dl16 and MQMNQ) pointing to the bottom of the PCA plot, thus indicating hydrological-morphological stress in terms of a higher frequency of high flow and higher flow variation. The average duration of low flow conditions (dl16) and the relation of long-term mean discharge to mean low-flow discharge indicating the variation of low flow in relation to mean flow, however, appear to be negatively correlated with hydrological alteration, and indicate favorable hydrological conditions (Figure 2). Because of the nearly perpendicular orientation of both stressor gradients in the plot, water quality-related and hydrological-morphological stressor variables were largely independent from each other in both case study catchments.

Several correlations between stressor variables and land use characteristics were observed underpinning the proxy character of land use as a stressor: In both catchments the percentage of urban area and WWTP discharges were positively correlated with different stressor variables, e.g., nutrients, chloride, sulphate, temperature, the RQ_{mix}, fh5 and ra5 (spearman rho = 0.5 - 0.9, Supplements S2). In the Erft catchment iron was also positively correlated to urban area and WWTP effluents (rho = 0.8 and 0.5, respectively). Negative correlations were observed for dl16 and MQ/MNQ (rho = -0.5 - -0.91). Intensive agriculture was positively correlated with HP1, HP2 and ra5 indicating hydrological-morphological stress in the Erft catchment (rho = 0.51 - 0.81), whereas no positive correlation was observed in the Niers catchment.

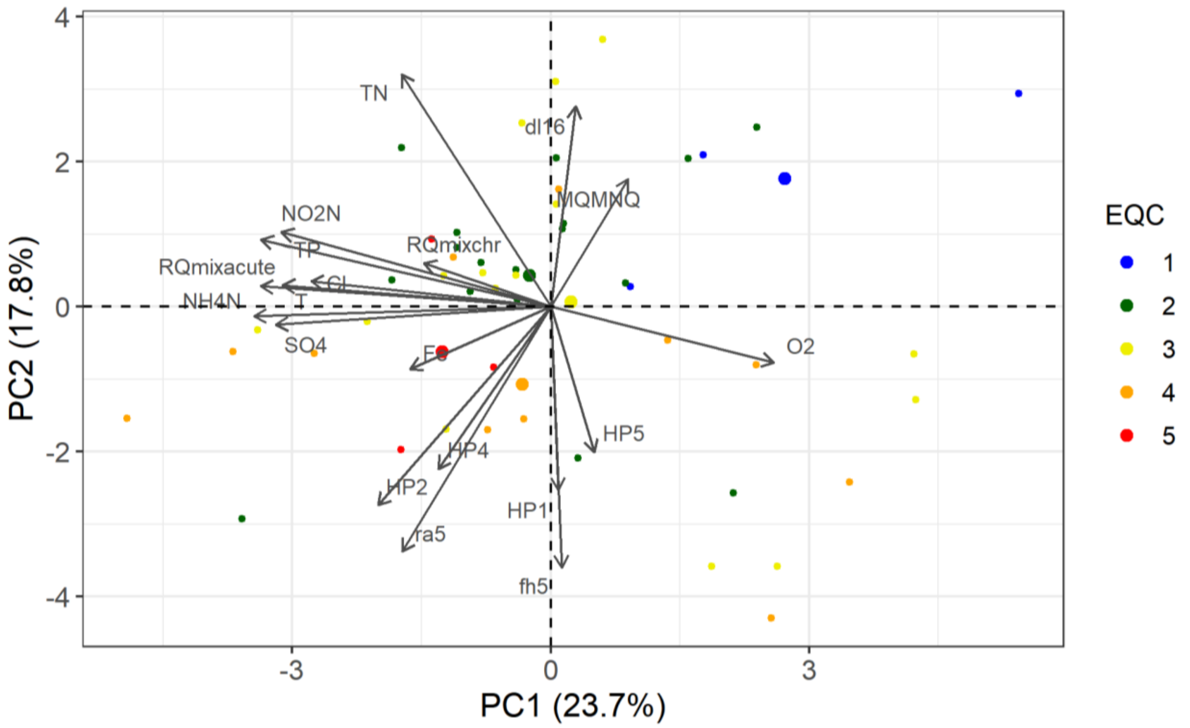

Figure 2: Principal component analyses biplot of all stressor variables. Sampling sites (dots) are color-coded according to their ecological status class (ESC), with enlarged dots indicating the centroid for each ESC. For abbreviations of stressor variables, see Table 1.

Stressor hierarchy

Altogether, the 19 stressor variables explained 51 % (R^2) and 38 % (adjusted R^2), respectively, of the variation in 21 macroinvertebrate metrics (Table 3). Notably, the conditional variance introduced by the co-variable ‘stream type’ accounted for another 28 % of the model’s variance, thus underpinning the important role of natural stream type-specific characteristics such as, for example, stream size, geology or dominant substrate type in both case study catchments. The (individual) marginal effects of the RDA model reveal five stressors and three stressor groups, respectively, having a significant influence at $p < 0.05$ (Table 3). In particular, physico-chemical variables (iron, chloride, sulphate) show a strong influence on the benthic macroinvertebrate community.

The strong influence of physico-chemical variables was confirmed by the partial RDAs (pRDAs) and subsequent variance partitioning (Figure 3). These variables alone accounted for 18 % of the variance (unique effect) and, together with hydrological alteration, contributed another 12 % to the explained variance (joint effect). This strong joint effect of both stressor groups suggests a co-occurrence of physico-chemical and hydrological stress in both case study catchments. In contrast, the effect of morphological degradation was subordinate, while micropollutants appeared to have only minor effects on the macroinvertebrate community in our dataset. In concert, our findings suggest the following ranking of stressor groups: physico-chemistry > hydrological alteration > morphological degradation > micropollutants. This ranking is supported by the constrained variance of the four pRDAs that were run exclusively with the variables of the four stressor groups (Table 3).

Table 3: Statistical key parameters of the Redundancy Analysis. A selection of 21 benthic macroinvertebrate metrics and 19 stressor variables of four stressor groups (physico-chemistry, micropollutants, morphological degradation, hydrological alteration) were analyzed. Significance of the RDA model and of marginal effects of stressor variables were tested using an ANOVA on the results of 999 permutations. Only stressors with significant marginal effects ($p < 0.1$) are shown. Total explained variance of each stressor group was calculated using variance partitioning (pRDA). Individual fractions are shown in Figure 3.

	Inertia	Proportion	p-value	Adjusted R^2
Total model	21	1	0.001	0.38
Conditional (i.e. explained by the co-variable stream type)	5.92	0.28		
Constrained (i.e. explained by stressors)	10.7	0.51		
Unconstrained (i.e. unexplained)	4.38	0.21		
Marginal effects (Permutation)				
Longitudinal profile (HP2)			0.003	
Sulphate (SO_4)			0.009	
Iron (Fe)			0.023	
Chloride (Cl)			0.025	
High flow frequency (fh5)			0.037	
Number of day rises (ra5)			0.071	
Cross profile (HP4)			0.074	
Total constrained variation of stressor groups				
Physico-chemistry				0.33
Mixture Toxicity				0.01
Hydrology				0.21
Morphology				0.17

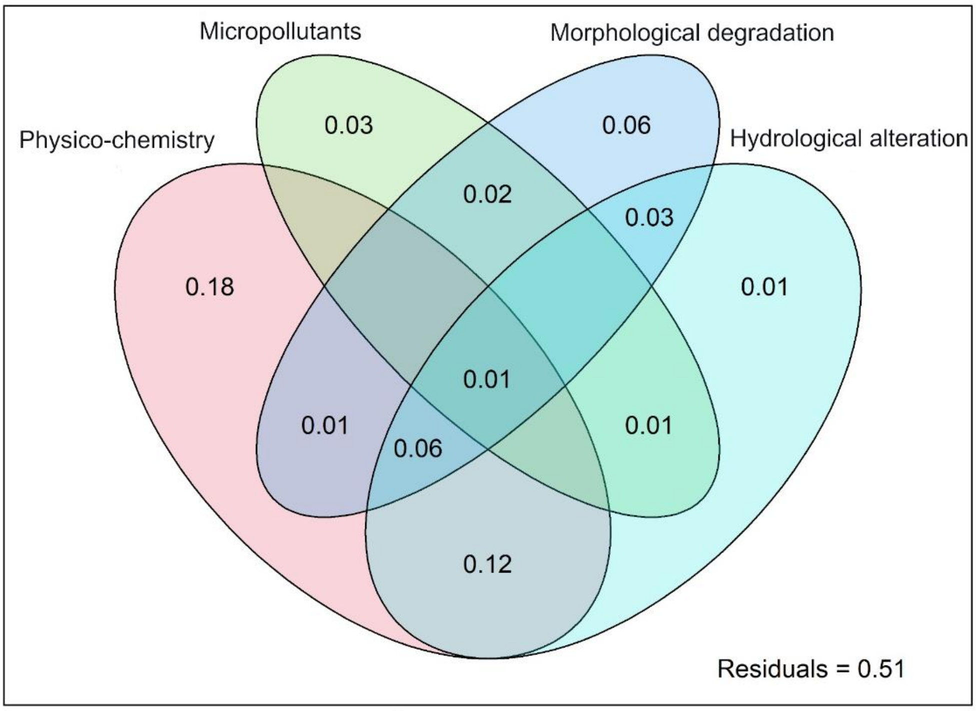

Figure 3: Venn diagram of the variance in macroinvertebrate metrics. Numbers indicate the unique and shared portions (adjusted R^2) of the variance in 21 macroinvertebrate metrics (Table 2) that are explained by physico-chemistry, micropollutants, morphological degradation and hydrological alteration. R^2 values below zero are not shown.

Relationship between stressor variables and macroinvertebrate metrics

The pairwise correlations of stressor variables with macroinvertebrate metrics revealed only modest relationships with a maximum of rho = 0.66, and only for ten out of the total of 21 metrics considered (Table 4). Nevertheless, if correlations below rho = |0.5| are neglected, three metrics (Ecological Quality Class, Index of Biocoenotic Region, Rheoindex) appeared to be particularly related to hydrological alteration, and two more metrics (Nb. of EPTCBO taxa and KLIWA Index) responded non-exclusively to this stressor group. Five metrics were particularly responsive to physicochemical stressor variables, four of which showed a comparatively strong relationship to oxygen. In concert, the number of significantly correlated metrics per stressor group well reflects the stressor group ranking that resulted from the pRDAs and subsequent variance partitioning.

Table 4: Spearman rank correlations between selected macroinvertebrate metrics and stressor variables of the four stressor groups. Only results with Spearman's rho >|0.5| are shown. All correlations are significant at $p < 0.001$. Ecological Quality Class indicates better ecological condition at lower classes, Fauna Index indicates reference conditions at higher values. Index of Biocoenotic Region represents river zonation from crenal to hypopotamal and Rheoindex the proportion of still water and ubiquists to rheobiontic species. KLIWA Index is scaled as a temperature-equivalent [°C].

Metric code	**Physico-chemical stress**				**Hydrological alteration**	**Morphological degradation**
	O_2	**Fe**	**TP**	**NO2-N**	**ra5**	**HP2**
Ecological Quality Class					0.50	
Index of Biocoenotic Region					0.56	
Rheoindex					-0.53	
Nb. of EPTCBO		-0.64			-0.54	
KLIWA Index		0.59	0.59		0.57	0.66
%Alien species	-0.53		0.52			0.53
German Fauna Index	0.63					
%Psa				-0.54		
%POM	-0.54					
%Gath	-0.57					

Discussion

In this study, the stressor groups analyzed showed distinct differences regarding their effect on the macroinvertebrate community. However, our hypotheses of a strong ecological relevance of micropollutants as well as subordinate effects of physico-chemical variables were not confirmed. In contrast, physico-chemical variables were the dominant stressor group with highest unique and joint effects on the macroinvertebrate community, which is in line with some previous multiple-stressor studies, though (e.g. Lemm et al., 2021; Sabater et al., 2016). The physico-chemical variables showing the highest marginal effects are sulphate, chloride and iron. In the Erft catchment, elevated concentrations of sulphate, chloride and iron were observed for sampling sites in the middle and lower reaches of the Erft as well as in tributaries in this region. These sampling sites are influenced by the discharges of drained groundwater in connection with lignite mining activities as well as by a higher percentage of urban area including WWTP discharges. Urban area and WWTP discharges were positively correlated with the concentration of iron and sulphate in the Erft as well as with chloride in the Niers. Thus, the effects of physico-chemical variables might indicate an influence of the lignite mining activities and high percentage of urban area including WWTP discharges in both catchments. Negative effects of salinization caused by mining as well as diffuse pollution from urban area on benthic invertebrates were described in previous studies (Braukmann and Böhme, 2011; Cadmus et al., 2018; Dyer and Wang, 2002; García-Criado et al., 1999; Uieda et al., 2017).

Interestingly, however, neither sulphate nor chloride notably exceeded the environmental quality targets of the German surface waters directive (OGewV, 2016). This finding points at a potential mismatch of environmental quality targets for salinization and the actual biological response to salinization (Halle et al., 2017; Sundermann, 2017). Notably, salinization can have different sources, such as drainage from lignite mining and WWTP discharges, but may also result from the application of fertilizers (e.g., potassium chloride) or road salt used for de-icing. Thus, salinization may be relevant for a large number of surface waters (Cañedo-Argüelles et al., 2013; Halle and Müller, 2015; Schulz and Cañedo-Argüelles, 2019; Thunqvist, 2004). The minimum oxygen concentrations are not fully captured in the routine monitoring (Rajwa-Kuligiewicz et al., 2015) but are strongly influenced by e.g. effluents of WWTPs, drainage from lignite mining and heavy rain events and thus might further point at the relevance of physico-chemical variables in the Erft and Niers catchments.

Micropollutants only explained a minor share of the variance in the invertebrate community in the selected catchments. This result may reflect the small number of sampling sites at risk of acute and chronic invertebrate mixture toxicity. In contrast, distinctly higher mixture risk quotients (RQ_{mix}) were calculated for both algae and fish (Supplement S4), which suggests notably higher ecotoxicological risks for these organism groups. Unfortunately, algal and fish data were not available for our sampling sites and sampling years and thus we were not able to confirm the potentially stronger effect of the selected substances on these organism groups. However, we cannot conclude that micropollutants in general had negligible effects on macroinvertebrates, because mixture toxicity risks for invertebrates might have been underestimated by our dataset for three reasons. First, micropollutant sampling rarely included event-driven or composite samples and hence might largely exclude peak discharge events with peak concentrations of pesticides, insecticides in particular. Indeed, in multiple stressor studies higher effects of pesticides and other chemicals were observed when the analyses were based on data from event-driven monitoring, high-frequent grab or composite sampling (Bradley et al., 2016; Castro-Català et al., 2020; Liess et al., 2021). Measured concentrations and corresponding risk quotients are difficult to compare due to the different sampling campaigns sometimes resulting in different number of detected substances. Second, the selection of 42 micropollutants in this study was based on substances identified as drivers of mixture toxicity in previous studies but reflects only a fraction of available substances (Bundschuh et al., 2014; Malaj et al., 2014; Rabiet et al., 2010; Spycher et al., 2018). Third, mixture risks were mainly calculated using effect concentrations of Daphnia magna, which in case of interspecies differences does not always reflect the highest sensitivity of benthic invertebrates towards the specific substances (Ashauer et al., 2011; Rico and van den Brink, 2015; Von der Ohe and Liess, 2004).

The strong joint effect of physico-chemical and hydrological variables underpins the potential impact of the lignite mining and urban area on macroinvertebrate communities in these catchments. Similar to the physico-chemical variables increases in flow variability (ra5) and the frequency of high flow events (fh5) were related to sampling sites influenced by the lignite mining activities in the middle and lower reaches of the Erft catchment as well as the headwater region of the Niers. Both parameters were positively correlated to the percentage of urban area and WWTP discharges, as well. Urban areas influence the flow regime due to WWTP, combined sewage and rainwater discharges as well as increased surface runoff of sealed surface area leading to increased flow variabilities and higher frequency of high flow events. These effects as well as effects of lignite mining on the hydrological regime were described in previous studies (Booth et al., 2016; Coleman et al., 2011; Meißner et al., 2019; Pusch and Hoffmann, 2000; Zhou et al., 2014). Furthermore, correlation between macroinvertebrate metrics and stressor variables indicated strong responses to the flow variability, i.e., to the Rheoindex and the Index of Biocoenotic Region both indicators of macroinvertebrate preferences for the flow condition and the river zonation which is linked to the hydrological conditions, but also metrics generally reflecting different stressors such as the Ecological Quality Class and the number of EPTCBO taxa. In both cases, an increased flow variability was associated with a poorer classification of the EQC and a reduction of the number of sensitive species belonging the group of EPTCBO taxa. For the interpretation of the correlations, however, it needs to be considered that only pairwise correlations of metrics and stressors were calculated and thus, interactions or co-variance of stressors with other variables not considered in this study were disregarded.

Hydro-morphological degradation is listed among the top stressor groups compromising the ecological status of Europe’s rivers (EEA, 2018). In this study, the morphological degradation might have even been underestimated as the bed structure could not be included in the analyses. Strong adverse effects of hydrological alterations and, in particular, of changes in high flow conditions and/or flow variability parameters, have been previously reported by Meißner et al. 2019, Kakouei et al. 2017, Suren and Jowett 2006, Laini et al. 2018, Konrad et al. 2008 and Clausen and Biggs 1997. In light of this evidence for strong biological effects of hydrological alterations, it is important to note that IHA are not frequently considered in multiple-stressor studies. Instead, hydro-morphological surveys tend to focus on morphological (physical habitat) conditions of the bed, banks and riparian area of rivers (e.g. Gellert et al., 2014). Hydrological alteration then is merely addressed by records of dams or weirs, as a cause of stagnant flow conditions (e.g. Dahm et al., 2013; Hering et al., 2006b; Villeneuve et al., 2018). This study shows that river hydrological alterations constitute an important stressor group that incorporates changes in the magnitude, timing and frequency of both high and low flow conditions. Based upon time series data from gauging stations (Gibbins et al., 2001), Indicators of Hydrological Alteration can be derived to express the changes in the temporal dynamics of the flow regime. These dynamics cannot be derived from mere spot measures and flow estimates during field surveys. Therefore, it is important to incorporate IHA in multiple-stressor studies especially for studies intended as decision-making support for water management. IHA from unimpacted reference sites may help to identify environmental target values, which can be used to guide improvement measures.

Conclusion

Physico-chemical stress and hydrological alteration were the dominant stressor groups for the macroinvertebrate communities in the rivers Erft and Niers. Thus, management measures to improve the ecological quality in both catchments would need to address them jointly. However, multiple-stressor analysis of river data is context-specific and strongly dependent on the selection of catchments and sampling sites, respectively. In order to capture the effects of different stressor groups and to put them into a hierarchical context, it is important to generate appropriate data. With regard to common physico-chemical monitoring schemes, data generation and methodologies seem appropriate. It is important to acknowledge, however, that physico-chemical stress may still be an issue, even in catchments with a high quality of wastewater treatment. Furthermore, event-driven monitoring and high-frequent grab or composite sampling might help to capture pollution events, in particular those involving pesticides. Due to the limitations of the micropollutant monitoring and the varying sensitivities between organism groups and species, it cannot be concluded that micropollutants generally have negligible effects on aquatic communities. To describe hydrological alterations, it is inevitable to compile and analyze time-series data. If available data from the existing gauging stations can be used for this purpose and might be supplemented by additional modelled data. Hydro-morphological surveys alone cannot fill this gap, but can complement data on riverbed, riverbank and riparian habitat conditions.

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2.2 Water quality deterioration remains a major stressor for macroinvertebrate, diatom and fish communities in German rivers

Nele Markert[1,2], Barbara Guhl[1], Christian K. Feld[2,3]

[1] North Rhine-Westphalian Office of Nature, Environment and Consumer Protection (LANUV NRW), 40208 Düsseldorf, Germany

[2] University of Duisburg-Essen, Faculty of Biology, Aquatic Ecology, Universitätsstr. 5, 45141 Essen, Germany

[3] University of Duisburg-Essen, Centre for Water and Environmental Research (ZWU), Universitätsstr. 5, 45141 Essen, Germany

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Abstract

About 60 % of Europe's rivers fail to meet ecological quality standards derived from biological criteria. The causes are manifold, but recent reports suggest a dominant role of hydro-morphological and water quality-related stressors. Yet, in particular micropollutants and hydrological stressors often tend to be underrepresented in multiple-stressor studies. Using monitoring data from four Federal States in Germany, this study investigated the effects of 19 stressor variables from six stressor groups (nutrients, salt ions, dissolved oxygen/water temperature, mixture toxicity of 51 micropollutants, hydrological alteration and morphological habitat quality) on three biological assemblages (fishes, macroinvertebrates, benthic diatoms). Biological effects were analyzed for 35 community metrics and quantified using Random Forest (RF) analyses to put the stressor groups into a hierarchical context. To compare metric responses, metrics were grouped into categories reflecting important characteristics of biological communities, such as sensitivity, functional traits, diversity and community composition as well as composite indices that integrate several metrics into one single index (e.g., ecological quality class).

Water quality-related stressors – but not micropollutants – turned out to dominate the responses of all assemblages. In contrast, the effects of hydro-morphological stressors were less pronounced and stronger for hydrological stressors than for morphological stressors. Explained variances of RF models ranged 23–64 % for macroinvertebrates, 16–40 % for benthic diatoms and 18–48 % for fishes. Despite a high variability of responses across assemblages and stressor groups, sensitivity metrics tended to reveal stronger responses to individual stressors and a higher explained variance in RF models than composite indices. The results of this study suggest that (physico-chemical) water quality deterioration continues to impact biological assemblages in many German rivers, despite the extensive progress in wastewater treatment during the past decades. To detect water quality deterioration, monitoring schemes need to target relevant physico-chemical stressors and micropollutants. Furthermore, monitoring needs to integrate measures of hydrological alteration (e.g., flow magnitude and dynamics). At present, hydro-morphological surveys rarely address the degree of hydrological alteration. In order to achieve a good ecological status, river restoration and management needs to address both water quality-related and hydro-morphological stressors. Restricting analyses to just one single organism group (e.g., macroinvertebrates) or only selected metrics (e.g., ecological quality class) may hamper stressor identification and its hierarchical classification and, thus may mislead river management.

Introduction

Freshwater ecosystems suffer from the impact of multiple stressors, which pose serious threats to riverine biota and constitute major challenges for ecosystem management (Nõges et al., 2016; Reid et al., 2019; Birk et al., 2020). Biological responses to (multiple) stressors can vary between biological assemblages such as macroinvertebrates, benthic diatoms and fishes due to, for example, differences in their life cycles, physiological characteristics, mobility and individual sensitivity to pollution (Marzin et al., 2012; Alric et al., 2021). While often primary producers and macroinvertebrates are commonly reported to respond to eutrophication and general degradation, fishes appear to be particularly sensitive towards hydro-morphological degradation (e.g., Marzin et al., 2012; Dahm et al., 2013; Poikane et al., 2020).

In Europe, the status of riverine biological assemblages and the level of different environmental stressors are subject to frequent surface water monitoring schemes in accordance with the European Water Framework Directive (WFD; EU, 2000). Key environmental stressors of biological responses in European rivers include water quality deterioration (point and diffuse sources of pollution), hydro-morphological pressures (e.g., physical alteration of river channels and riparian zones) and hydrological alteration (e.g., damming and water abstraction; EEA, 2018;2019). There is broad scientific evidence that, for example, excess nutrients and salt ions cause changes in taxonomic composition of several riverine assemblages and reduce their biodiversity (Villeneuve et al., 2015; Reid et al., 2019). Thus, organic pollution, e.g., eutrophication and subsequently oxygen depletion still remain important and often dominating stressors for riverine biota (Carvalho et al., 2019; EEA, 2019; Birk et al., 2020; Valerio et al., 2021), despite notable advances in wastewater treatment over the past decades (Haase et al., 2023). Additionally, effects of hydro-morphological degradation on riverine assemblages have been reported in numerous studies. Anthropogenic alteration of riverine bed and bank structure and of riparian conditions, for example, affect temperature regimes, substrate compositions and flow dynamics and reduce the habitat diversity (Gieswein et al., 2017; Waite et al., 2021; Haase et al., 2023). Despite this large body of evidence on the adverse effects of water quality deterioration and hydro-morphological degradation, knowledge gaps remain for certain stressors within both groups, including micropollutants and hydrological alterations (EEA, 2019; Meißner et al., 2019; Reid et al., 2019; Heß et al., 2023).

The term micropollutants refers to a variety of chemical compounds such as pesticides, pharmaceuticals, personal care products and industrial chemicals, which are widely distributed as a result of diffuse pollution or insufficient wastewater treatment and can have direct toxic effects on various aquatic organisms (Malaj et al., 2014; Posthuma et al., 2020; Bradley et al., 2021; Halbach et al., 2021). In the environment, micropollutants are present in complex mixtures, i.e. spatially and temporally variable combinations of multiple compounds that may contribute to a joint mixture toxicity even if each individual compound occurs at non-toxic concentration (Silva et al., 2002; Escher et al., 2020). Micropollutants and their mixtures negatively affect aquatic ecosystems at different biological endpoints, e.g. survival and reproduction of sensitive species, taxonomic composition and biodiversity (Malaj et al., 2014; Mor et al., 2019; Posthuma et al., 2020; Liess et al., 2021). There is only little knowledge, however, of the response of riverine assemblages to micropollutants in the presence of multiple other stressors (EEA, 2019; Reid et al., 2019; Heß et al., 2023). Hydrological alterations address the timing and dynamics of river flow, for example the degree of changes in magnitude, frequency and duration of flow events. Alterations of the flow variability or the frequency of high and low flow events can significantly influence the structural and functional composition of riverine assemblages and their biodiversity (Meißner et al., 2019; Waite et al., 2021). However, hydrological alterations are often only indirectly derived from hydro-morphological surveys, which is considered inadequate for measuring and quantifying the timing and dynamics of flows (Richter et al., 1996; Meißner et al., 2019).

Considering the remaining knowledge gaps on adverse effects of micropollutants and hydrological alterations on riverine assemblages in combination with other stressors, the aim of this study was to comprehensively analyze the response of riverine assemblages to water quality deterioration and hydro-morphological degradation based on WFD monitoring data across Germany. This study simultaneously addressed the response of three riverine assemblages (macroinvertebrates, diatoms, fishes) to multiple stressors, including mixture effects of ecotoxicologically relevant micropollutants and detailed effects of hydrological alterations. In total, six stressor groups including water quality (nutrients, salts ions, physical parameters, micropollutants) and hydro-morphology (morphological and hydrological alterations) were analyzed. Biological responses to these stressors were assessed separately for sensitivity and functional trait-based metrics, community composition and diversity metrics as well as composite indices integrating several metrics into one single index (e.g. ecological quality class).

We hypothesized that the ranking of stressor groups differed among biological assemblages due to their specific stressor sensitivities (Marzin et al., 2012; Waite et al., 2019; Poikane et al., 2020). More specifically, we expected high effects of water quality deterioration on all assemblages, with nutrient enrichment particularly driving macroinvertebrate and diatom responses, and oxygen depletion and water temperature driving macroinvertebrate and fish responses (Marzin et al., 2012; Dahm et al., 2013; Poikane et al., 2020). Effects of micropollutants were expected to differ between substances for macroinvertebrates (insecticides), diatoms (herbicides) and fishes (pharmaceuticals) due to the toxic mode of actions and species sensitivity (Beckers et al., 2018; Finckh et al., 2022). Morphological and hydrological stressors were expected to show subordinate, but evident effects, that were particularly pronounced for macroinvertebrates and fishes (Marzin et al., 2012; Waite et al., 2019; Poikane et al., 2020; Valerio et al., 2021).

Methods

Study area and data origin

We used a dataset of 249 sampling sites for macroinvertebrates, 195 sampling sites for diatoms and 103 sampling sites for fishes located in mountainous (altitude 200 – 800 m a.s.l) and lowland regions (altitude below 200 m a.s.l) of the Federal States of North Rhine-Westphalia, Bavaria, Saxony and Schleswig-Holstein, Germany (Fig. 1). Due to limited availability and quality of micropollutants data, sites in other Federal States were not included. The drainage area of sampling sites ranged between 12 and 2,261 km² in lowland regions and between 10 and 4,480 km² in mountainous regions.

Environmental stressors

A selection of 19 stressor variables representing six stressor groups were selected for this study (Table 1). The stressor groups (physical water quality parameters, nutrients, salt ions, micropollutants, morphological and hydrological parameters) reflect the key environmental drivers of biological deterioration in European rivers (EEA, 2018; 2019). The biological relevance of these stressor groups has been shown in many recent studies (e.g., Meißner et al., 2019; Birk et al., 2020; Castro-Català et al., 2020; Lemm et al., 2021), although none of these studies included all stressor groups in parallel to compare and hierarchically order multiple-stressor effects on the riverine fauna and flora.

All environmental stressor data originate from WFD-related monitoring schemes between 2014 and 2020 (LAWA, 2021). Sampling of physico-chemical variables, micropollutants and hydrological parameters (derived from gauging stations) were spatially and temporally matched to the respective biological sampling sites, the latter of which served as reference in terms of sampling location and timing (see section 2.3). Water quality stressors were derived and averaged for both the year of the biological sampling and the year before. Each water quality parameter was measured at least once at each sampling site in the period of interest (range between one and 17 measurements). Data were averaged to obtain mean parameter values for further analyses, except for oxygen and temperature, where the minimum and maximum values were used, respectively (Table 1; acc. to the German surface water directive, OGewV, 2016). For an a priori characterization of individual stressor variables and stress levels, the data were compared to available quality targets (QT) for physico-chemical parameters (OGewV, 2016), micropollutants (see section 2.2.1 for details) and morphological quality (see section 2.2.2 for details).

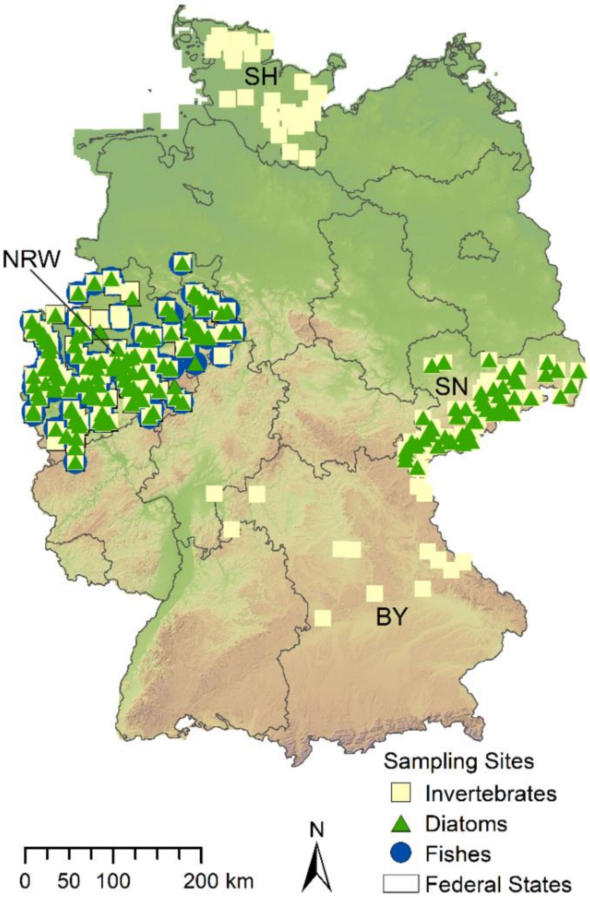

Figure 1: Location of sampling sites in the Federal States of Schleswig-Holstein (SH), North Rhine-Westphalia (NRW), Saxony (SN) and Bavaria (BY), Germany. Background colors indicate lowland regions (in green, altitude < 200 m a.s.l) and mountainous regions (in brown, altitude 200 – 800 m a.s.l). No alpine sites at altitudes > 800 m a.s.l. were sampled. ©GeoBasis-DE/BKG 2023.

Water quality stressors

Water quality-related stressors included the four stressor groups physical water quality parameters (i.e., oxygen and water temperature), nutrients (nitrogen and phosphor compounds), salt ions (sulphate and chloride) and micropollutants (Table 1). Physical water quality parameters were obtained from field measurements, chemical data of nutrients, salt ions and micropollutants were obtained from grab samples of surface water (see OGewV (2016) and LAWA (2019) for details on sampling and analysis).

Altogether, 51 micropollutants were selected from three types of micropollutants: pesticides (N = 30), pharmaceuticals (N = 14) and industrial-, household- and other chemicals (N = 7, Supplementary Material Table A1). Substances were selected based on their ecotoxicological relevance identified in previous studies (see Markert et al. (2020) for details). To address ecotoxicological effects on the biological assemblages, toxic units (TUs) were calculated for each individual substance. TUs express the quotient of mean concentrations and ecotoxicological effect concentrations (NOEC/EC_{10}, Supplementary Material Table A1; European Commission, 2017). To obtain risk quotients (RQ_{mix}) for effects of mixtures of different substances, TUs of each type of micropollutant (pesticides, pharmaceuticals and industrial chemicals) were summed and a safety factor of 10 for chronic effects was applied (see Backhaus and Faust (2012) for methodological details). An RQ_{mix} value above one points at potential mixture toxicity risks, i.e. either one individual or a mixture of several micropollutants exceeding the ecotoxicological effect concentrations (Backhaus & Faust, 2012). Concentrations below the (technical) limit of quantification (LOQ) were substituted by half of the value of LOQ for pharmaceuticals and industrial chemicals and by zero for pesticides. The replacement by zero for pesticides seemed more appropriate as pesticides often show stronger seasonal patterns with peak concentrations during rain events and no/low exposure during dry periods (Vormeier et al., 2023). Between eight and 47 micropollutants were detected at each sampling site (on average between 25 and 30 substances for macroinvertebrate, diatom and fish sites, respectively; Table A1). This inconsistency in the individual coverage of micropollutants at each sampling site resulted from differences in the number and selection of substances in the underlying sampling campaigns. However, because we aimed to capture the joint mixture toxicity of ecotoxicologically relevant micropollutants across different regions in Germany instead of strictly confining to ubiquitous substances, we believe that the data basis was suitable for the purpose of this study.

Morphological and hydrological stressors

The level of morphological degradation at each sampling site was derived from hydro-morphological surveys in accordance with the WFD at a resolution of 100 m river sections (Gellert et al., 2014). At each section physical habitat parameters were recorded and aggregated into five main habitat quality parameters representing the conditions of the stream course (HP1), the longitudinal profile (HP2), the bed structure (HP3), the cross profile (HP4) and the bank structure (HP5, Table 1; Gellert et al., 2014). The habitat quality classification followed a five-class system in line with the WFD: 1 = unchanged, 2 = slightly changed, 3 = moderately changed, 4 = distinctly changed, 5 = completely changed). To account for assemblage-specific aggregated effects of morphological conditions upstream of a biological sampling site (Lorenz & Feld, 2013), we used median morphological quality within a stream length of 1 km upstream of the biological sampling site for the analyses of diatoms, 2 km upstream for macroinvertebrates and 5 km upstream for fishes.

Hydrological stressors were quantified using mean discharge measurements from gauging stations located close to the biological sampling site. Five indicators of hydrological alteration (IHA, Table 1) were calculated representing the five main IHA groups magnitude (mh20), frequency (fh5), duration (dl16), timing (tl1) and rate of change (ra5; Richter et al., 1996; Olden & Poff, 2003; Mills & Blodgett, 2017). The selection of particular (meaningful) indices for each IHA group was informed by previous studies (Olden & Poff, 2003; Archfield et al., 2014; Meißner et al., 2019). Details on the final selection of non-redundant IHA indices are given in Markert et al. (2022).

Table 1: Statistical parameters of stressor variables used for multi-stressor analyses. Quality targets (QT) indicate the threshold between a good and moderate ecological status, i.e. an exceedance of a QT towards the moderate direction suggests a low probability to achieve a good ecological status (OGewV, 2016). QTs are stream type-specific for physico-chemical variables, but applicable across stream types for the other stressor variables. QT was set to 2 (slightly changed) for habitat quality and to 1 for the mixture risk quotient of micropollutants (RQ_{mix}).

	Stressor code	Stressor description	Mean (±SD)	Min	Max	QT	%Sites with QT violation
Physical	T[1]	Maximum Water temperature [°C]	18.3 (±3)	8.6	25.7	-	-
	O_2[1]	Minimum Oxygen conc. [mg/L]	8.4 (±1.3)	2.9	11.5	> 6–8[3]	13 %
Salts	SO_4[1]	Sulphate conc. [mg/L]	60.2 (±41)	10.3	277.8	≤ 75–220[3]	5 %
	Cl[1]	Chloride conc. [mg/L]	59.7 (±81.6)	0.0	902.9	≤ 200[3]	3 %
Nutrients	TP[1]	Total phosphate conc. [mg/L]	0.1 (±0.1)	0.0	0.6	≤ 0.1–0.15[3]	51 %
	NH_4-N[1]	Total ammonium nitrogen conc. [mg/L]	0.1 (±0.2)	0.0	2.6	≤ 0.1–0.2[3]	22 %
Micropollutants	$RQ_{mix,Pest}$	RQ of mixture toxicity of pesticides [-]	I: 0.2 (±1)[2] D: 0.4 (±0.8)[2] F: 0.0 (±0)[2]	0.0 0.0 0.0	10.0 7.6 0.2	≤ 1	2 % 10 % 0 %
	$RQ_{mix,Pharm}$	RQ of mixture toxicity of pharmaceuticals [-]	I: 0.2 (±0.3)[2] D: 0.1 (±0.2)[2] F: 5.8 (±7.4)[2]	0.0 0.0 0.0	2.1 1.9 33.9	≤ 1	2 % 1 % 73 %
	$RQ_{mix,Ind}$	RQ of mixture toxicity of industrial chemicals [-]	I: 0.1 (±0.2)[2] D: 0.1 (±0.1)[2] F: 0.6 (±1.8)[2]	0.0 0.0 0.0	1.1 0.4 9.6	≤ 1	1 % 0 % 12 %

Table 1 (continued)

	Stressor code	Stressor description	Mean (±SD)	Min	Max	QT	%Sites with QT violation
Hydrology	fh5	High flow frequency (flow above median) [events/year]	15.4 (±7.9)	3.5	51.0	-	-
	dl16	Low flow pulse duration (events below 25th percentile) [days]	10.0 (±9.5)	0.8	72.1	-	-
	ra5	Number of day rises (number of positive gain days divided by total number of days [-]	0.3 (±0.1)	0.1	0.5	-	-
	mh20	Specific mean annual maximum flow (divided by catchment area) [m^3/s per km^2]	0.1 (±0.2)	0.0	2.6	-	-
	tl1	Date of annual minimum (Julian calendar) [-]	221.6 (±50.8)	12.3	349.0	-	-
Habitat Quality	HP1	Channel development [-]	4.2 (±1)	1.0	5.0	≤ 2	94 %
	HP2	Longitudinal profile [-]	3.7 (±1)	1.3	5.0	≤ 2	88 %
	HP3	Bed structure [-]	3.2 (±1.2)	1.0	5.0	≤ 2	73 %
	HP4	Cross profile [-]	3.7 (±1.1)	1.0	5.0	≤ 2	85 %
	HP5	Bank structure [-]	3.9 (±1.1)	1.0	5.0	≤ 2	88 %

[1] Arithmetic mean of annual mean concentration (minimum concentration for oxygen, maximum for water temperature) derived from a two-year period (acc. to OGewV, 2016).

[2] RQ_{mix} are separately shown for macroinvertebrates (I), diatoms (D) and fishes (F) due to assemblage-specific differences in ecotoxicological effect concentrations.

[3] QTs vary between stream types (min–max indicated); stream-type-specific percentage of violation of QTs were calculated.

Biotic data

Biological data originated from WFD monitoring schemes of state agencies and regional water boards between 2014 and 2019. If assemblage data of one site were available for multiple years, we chose data from the most recent year that also matched the timing of the other assemblages best. Macroinvertebrates were sampled from March to August according to the German national standard methodology Perlodes (Meier et al., 2006). Field sampling followed a multi-habitat approach targeting representative microhabitats on the river bottom. Within a 20 – 50 m section, 20 sample units from representative mineral and organic substrates were taken using a hand net and kick sampling (see Hering et al. (2004) for methodological details). Random sub-samples were sorted and determined to the lowest taxonomic level possible (Haase et al., 2006). Benthic diatoms were sampled from June to September according to the German national standard methodology Phylib (Schaumburg et al., 2012) from natural or artificial mineral and organic substrates that were representative for the river bottom within a 20 m long sampling section. Fishes were caught from June to October in lowland regions and in spring in mountainous regions using electrofishing along river stretches of a minimum of

200 – 500 m length following standard protocols (CEN, 2003; Dußling, 2009). Fish abundance was calculated as catch per unit of effort (CPUE, i.e. the number of individuals within a 100 m section).

Taxalists were processed and community-based biological metrics were calculated using Perlodes Online (V. 5.0.9, Perlodes Online, 2021) and Asterics (V. 4.0.4, UBA, 2014) for macroinvertebrates, Phylib (V. 6.2.2, Müller, 2022) and Omnidia (V. 6.1.4, Lecointe et al., 1993) for diatoms and fiBS (V. 1.0, Dußling, 2009) and EFI+ (EFI+ Consortium, 2009) for fishes. In total, 56 candidate metrics representing different metric categories were selected for multiple-stressor analyses (see Supplementary Material Table A2 for a full list of metrics; Table 2 for final selection of metrics). The metric selection was informed by guidance documents for official WFD-related status assessments as well as by selections of similar previous multiple-stressor studies (Hering et al., 2006; Dahm et al., 2013; Lemm et al., 2019; Markert et al., 2022). We distinguished four different metric categories to investigate differences in stressor responses across metrics from these categories: i) composite metrics integrating several metrics (e.g., ecological status class); ii) community compositional metrics of biodiversity (e.g., Shannon Wiener Index) and taxonomic composition (e.g., taxon richness); iii) sensitivity metrics that express a community's sensitivity to a particular stressor (e.g., $SPEAR_{pest}$ index for micropollutants. trophic index for nutrient pollution); iv) community functional trait composition (e.g., feeding and habitat preferences).

Data preparation and analysis

Biological and water quality monitoring sites were spatially matched using a maximum allowable distance of 5 km upstream and downstream. Gauging stations were spatially matched using a maximum allowable distance of 7.5 km upstream and downstream as well as a maximum allowable deviation of drainage areas of the biological sampling site and the gauging station of 15 %. Sites were excluded from further analyses if potentially confounding factors (e.g., confluences with tributaries, effluents from wastewater treatment plants) between biological and abiotic sampling sites were evident from manual checks in ArcGIS. Some stressor variables included missing values (i.e., sulphate, total phosphorus, bed habitat structure, mean daily discharge values). We used an imputation algorithm to calculate approximate values for missing ones if the percentage of missing values in the dataset did not exceed 15 % of sampling sites. The imputation was run using the missForest package (Stekhoven, 2022) in R. All statistical data analyses were run in R V.4.0.3 (R Core Team, 2020) within the GUI RStudio V.1.4.1103 (RStudio Team, 2020).

Multiple-stressor analyses

To quantify and hierarchically order the impact of multiple stressors on community metrics of riverine assemblages we calculated Random Forests (RF) regression models (RandomForestSRC V.3.1.1, Ishwaran et al., 2022) for each response variable (metric) according to the cookbook provided by Feld et al. (2016b). RF are a flexible, non-parametric Machine Learning tool and are able to handle large numbers of explanatory variables even in small datasets and have shown high performances in ecological models (Knudby et al., 2010; Visser et al., 2022; see Breiman (2001) for methodological details). Since parameter tuning did not distinctly improve model performances, default parameters were consistently used for all models. The number of trees was set to 2000 resulting in lowest model error. A random variable (between 0 and 1) and a constant variable (integer 1) were added to check for random effects in variable importance (Kaijser et al., 2022). Permutation variable importance of single stressors and joint variable importance of stressor groups were extracted and used to hierarchically order the influence of stressor variables. In a first analysis, RF models were run for each of the 56 candidate metrics (Supplementary Material Table A2). For the final comparison of stressor effects, however, we chose to use metrics with an explained variance equal to or larger than 15 % in the RF models to exclude models with a poor explanatory power (Table 2). The relative influence of the stressor variables were aggregated across the final selection of metrics to the mean relative influence (MRI) for comparisons of stressor responses between metric categories and biological assemblages.

Although RF can handle collinear descriptors, we checked for highly collinear stressors prior analyses using Spearman rank correlation (threshold 0.75; package Hmisc, Harrell Jr, 2019) and variance inflation factors (threshold 4; package usdm, Naimi, 2019). Only nitrite had to be removed due to collinearity with other stressor variables (full correlation matrix in Supplementary Material Table A3). In order to account for potential confounding effects of ecoregional and river size-related covariates on the stressor's importance in RF models, we initially included ecoregion (lowlands, low mountains), altitude (m a.s.l), distance to the river source (km) and catchment size (km^2) during model development. We also checked the model's residuals for patterns between ecoregions and along gradients of the covariates. Neither the effects of covariates on the stressor hierarchy nor the analyses of residual patterns, however, supported the existence of confounding co-variate effects on the outcome of our analyses (see Supplementary Material Table A4 for details). Therefore, no covariates were included in the final RF models.

Table 2: Final selection of community metrics of the three biological assemblages with an explained variance ≥15 % in RF models. Metric categories denote community characteristics that were introduced to investigate patterns in the biological response among categories (see section Biotic data for details). Metric categories: integrating = composite index, sensitivity = sensitivity metric to water quality (WQ) or morphological habitat quality (HQ), functional = metric of functional trait composition, richness/diversity = metric of community taxonomic composition.

Biological assemblage	Code	Metric description	Metric category	Reference
Macro-invertebrate	EQC	Ecological Quality Class	Integrating	Perlodes Online, 2021
	MMI	Multimetric Index	Integrating	Perlodes Online, 2021
	FI	German Fauna Index	Sensitivity (HQ)	Lorenz et al., 2004
	GSI	German Saprobic Index	Sensitivity (WQ)	Rolauffs et al., 2004
	%EPT	Percentage of Ephemeroptera, Plecoptera and Trichoptera	Sensitivity (WQ)	Perlodes Online, 2021
	$SPEAR_{pest}$	Species at Risk for pesticides	Sensitivity (WQ)	Liess & von der Ohe, 2005
	KLIWA	KLIWA Index (temperature preference)	Sensitivity (WQ)	Halle et al., 2016; Sundermann et al., 2022
	RI	Rheoindex	Sensitivity (HQ)	Banning, 1998
	IBR	Index of biocoenotic region	Functional	UBA, 2014
	%Phy	Habitat preferences for phytal (plants)	Functional	Perlodes Online, 2021
	%Lith	Habitat preferences for lithal (gravel/stones)	Functional	Perlodes Online, 2021
	%Graz	Feeding type preference grazer	Functional	Perlodes Online, 2021
	Richness	Richness	Richness/Diversity	Perlodes Online, 2021
	Shannon	Shannon Wiener Index	Richness/Diversity	Shannon, 1948
Diatom	DI	Diatom Index	Integrating	Müller, 2022
	TI	Trophic Index	Sensitivity (WQ)	Rott, 1999
	SI	Saprobic Index	Sensitivity (WQ)	Rott, 1997
	HI	Halobian Index	Sensitivity (WQ)	Ziemann et al., 1999
	IBD	Biological Diatom Index	Sensitivity (WQ)	Coste et al., 2009
	IDG	Generic Diatom Index	Sensitivity (WQ)	Rumeau & Coste, 1988
	IPS	Pollution Sensitivity Index	Sensitivity (WQ)	Cemagref, 1982
	EPID	Eutrophication-Pollution Index	Sensitivity (WQ)	Dell'Uomo, 1996
	DI CH	Swiss Diatom Index	Sensitivity (WQ)	Hürlimann & Niederhauser, 2002
	RI	Rheophilous taxa	Sensitivity (HQ)	Denys, 1991
	Richness	Richness	Richness/Diversity	Müller, 2022
	Shannon	Shannon Wiener Index	Richness/Diversity	Shannon, 1948
	Even	Evenness	Richness/Diversity	Magurran & McGill, 2011

Table 2 (continued)

Biological assemblage	Code	Metric description	Metric category	Reference
Fish	WQINTOL	Water quality intolerance	Sensitivity (WQ)	Solana-Gutierrez et al., 2009
	HINTOL	Habitat quality intolerance	Sensitivity (HQ)	Solana-Gutierrez et al., 2009
	HTOL	Habitat quality tolerance	Sensitivity (HQ)	Solana-Gutierrez et al., 2009
	Rheopar	Rheoparous species	Sensitivity (HQ)	Solana-Gutierrez et al., 2009
	Limnopar	Limnoparous species	Sensitivity (HQ)	Solana-Gutierrez et al., 2009
	%Phy	Habitat preferences for phytal	Functional	Solana-Gutierrez et al., 2009
	%Lith	Habitat preferences for lithal	Functional	Solana-Gutierrez et al., 2009
	Richness	Richness	Richness/Diversity	Dußling, 2009

Results

Ecological relevance of stressors

The comparison of stressor values with available environmental quality targets (QT) showed a high relevance of total phosphorus and ammonium-nitrogen with 51 % and 22 % of the sites exceeding QT, respectively (Table 1). Minimum oxygen concentrations fell below quality targets at 13 % of the sites. For micropollutants, mixture risks varied between the biological assemblages: risks of pesticides were evident for diatoms at 10 % of the sites, whereas for fish risks were calculated for pharmaceuticals (73 % of the sites) and industrial chemicals (12 % of the sites). The calculated mixture risks for invertebrates were neglectable. In contrast, hydro-morphological conditions exceed the QT at between 73 % (HP3: bed structure) and 94 % (HP1: channel development) of sites.

Explained variance of Random Forest models

The explained variance of the 35 final metrics (Table 2) varied between 16 % and 64 %, with a mean value (± SD) of 37 % ± 13 % for macroinvertebrates, 29 % ± 7 % for diatoms and 34 % ± 12 % for fishes (Fig. 2)

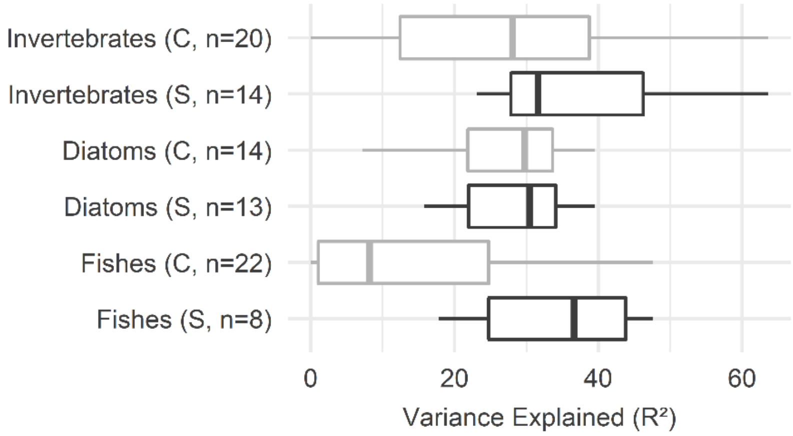

Figure 2: Distribution of explained variances of biological metrics in Random Forests. Results for the initial selection of 56 candidate metrics (C) are shown in grey and for the final selection of 35 metrics (S) in black.

Stressor ranking

Water quality variables showed dominating effects on all biological assemblages, but effects differed for nutrients, salts and physical water quality variables (Fig. 3, Table 3). Effects on macroinvertebrates were dominated by physical water quality (MRI of 26 %) or a combination of physical water quality and salt ions. Contrastingly, diatoms showed a stronger relative influence of nutrients (MRI of 25 %), except for the Halobian Index (HI), which was primarily influenced by salt ions (MRI of 61 %). Fish metrics responded strongly to salt ions (MRI of 26 %) or a combination of physical water quality and nutrients. The relative influence of micropollutants on diatoms (MRI of 20 %) and both faunal assemblages (MRI of 10 %) was lower than the influence of the other physico-chemical water quality variables (combined MRI of 59 % for macroinvertebrates, 51 % for diatoms and 52 % for fishes). Overall, micropollutants exceeded a relative influence of 15 % in 69 % of diatom, 25 % of fish and 21 % of macroinvertebrate RF models. The mean relative influence of hydrological variables was 27 % for diatoms, 27 % for fishes and 18 % for macroinvertebrates. Relative effects of morphological habitat quality were mostly observed for macroinvertebrates (MRI of 14 %) and fishes (MRI of 12 %), but neglectable for diatoms (MRI of 2 %).

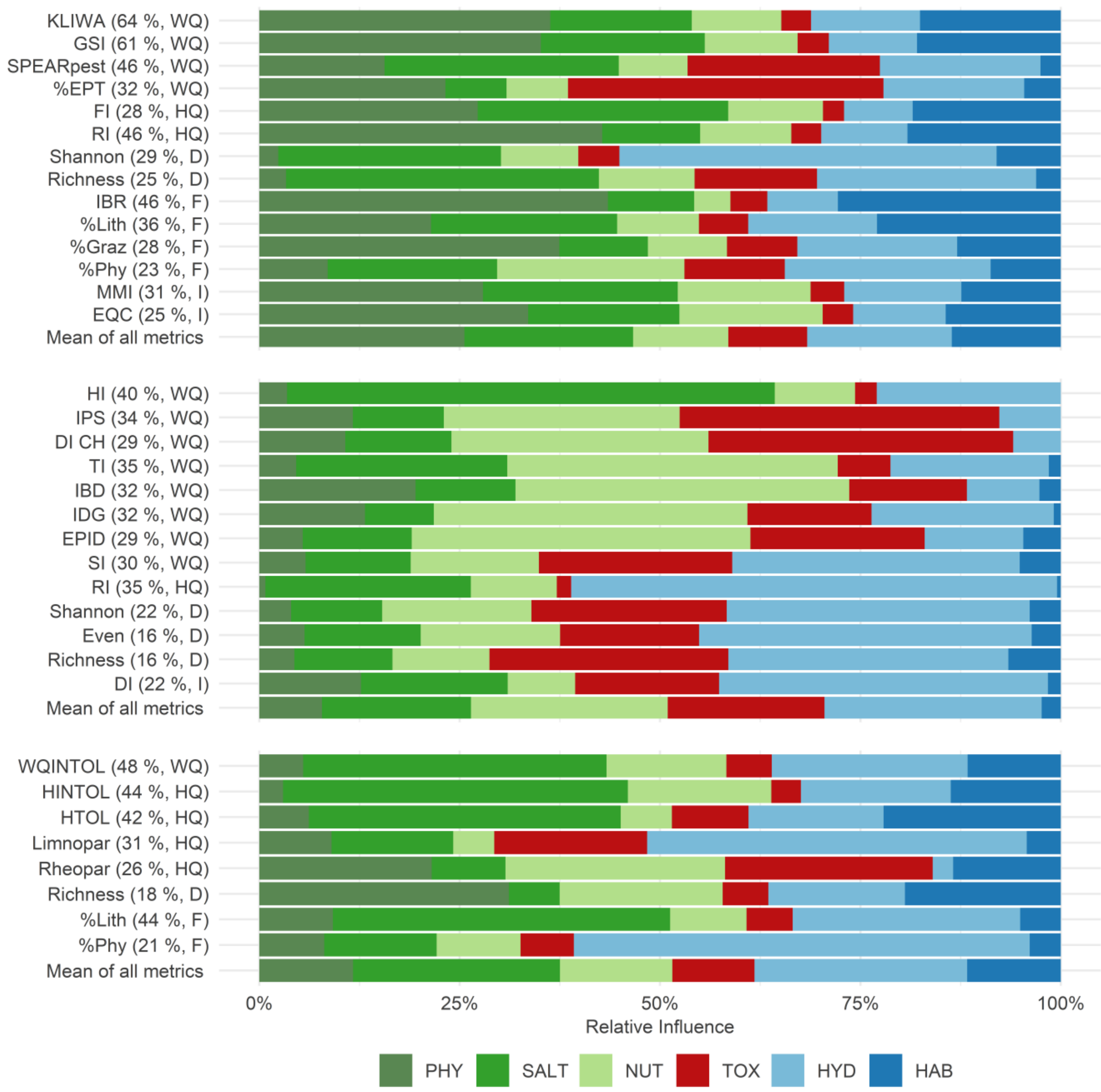

Figure 3: Relative influence (%) of physical water quality stressors (PHY), salt ions (SALT), nutrients (NUT), micropollutant mixture toxicity (TOX), hydrological alteration (HYD) and morphological habitat degradation (HAB) in Random Forest (RF) models on macroinvertebrate (top), diatom (middle) and fish (bottom) community metrics. Explained variance (%) of RF models and metric classification (WQ = Water Quality, HQ = Habitat Quality, D = Richness/Diversity, F = Function, I = Integrating) are provided in parentheses behind metric codes. For full metric names, see Table 2.

Table 3: Mean relative influence (MRI) of stressor groups for biological assemblages and metric categories. Influence was derived from the joint variable importance of stressor groups in Random Forests models (PHY = physical water quality stressors, SALT = salt ions, NUT = nutrients, TOX = mixture toxicity, HYD = hydrological alteration, HAB = morphological habitat modification). For details on metric categories, see section Biotic data.

	Metric category	Number of metrics	Mean Relative Influence MRI (±SD)					
			PHY	SALT	NUT	TOX	HYD	HAB
Macroinvertebrates	All	14	25.6 (±13.8)	21 (±8.9)	11.9 (±4.7)	9.8 (±10.4)	18 (±10.2)	13.6 (±7.6)
	Integrating (composite indices)	2	30.7 (±4)	21.6 (±3.8)	17.2 (±0.9)	4 (±0.3)	13.1 (±2.2)	13.4 (±1.4)
	Sensitivity metrics (Habitat Quality)	2	35 (±11)	21.7 (±13.5)	11.6 (±0.3)	3.2 (±0.8)	9.7 (±1.6)	18.8 (±0.5)
	Sensitivity metrics (Water Quality)	4	27.6 (±9.9)	18.7 (±8.9)	9.8 (±1.9)	17.7 (±17.3)	15.6 (±4)	10.6 (±8.2)
	Functional trait composition	4	27.7 (±15.8)	16.6 (±6.6)	12 (±8)	8 (±3.5)	17.6 (±7.1)	18.1 (±8.8)
	Richness/diversity metrics	2	2.9 (±0.7)	33.4 (±8)	10.8 (±1.6)	10.2 (±7.2)	37.2 (±13.9)	5.5 (±3.5)
Diatoms	All	13	7.8 (±5.3)	18.6 (±13.8)	24.5 (±13.4)	19.6 (±12)	27.1 (±16.4)	2.4 (±2.2)
	Integrating (composite indices)	1	12.7	18.3	8.4	18	41	1.6
	Sensitivity metrics (Habitat Quality)	1	0.8	25.6	10.7	1.8	60.6	0.5
	Sensitivity metrics (Water Quality)	8	9.3 (±5.5)	19.9 (±17.3)	31.5 (±12.4)	20.4 (±13.5)	17 (±10.2)	1.8 (±2.1)
	Richness/diversity metrics	3	4.7 (±0.9)	12.7 (±1.6)	16 (±3.5)	23.8 (±6.2)	38.1 (±3.3)	4.7 (±1.6)
Fishes	All	8	11.7 (±9.6)	25.8 (±16)	14 (±7.6)	10.3 (±7.9)	26.5 (±17.7)	11.7 (±6.9)
	Sensitivity metrics (Habitat Quality)	4	9.9 (±8.1)	26.6 (±16.9)	14.2 (±10.5)	14.6 (±9.9)	21.4 (±18.8)	13.4 (±7.3)
	Sensitivity metrics (Water Quality)	1	5.5	37.9	15	5.6	24.4	11.6
	Functional trait composition	2	8.7 (±0.8)	28 (±19.8)	10 (±0.7)	6.2 (±0.6)	42.6 (±20.2)	4.5 (±0.8)
	Richness/diversity metrics	1	31.1	6.3	20.3	5.7	17	19.4

Differences between metric categories

Across the three biological assemblages, integrating composite indices, e.g., the ecological status class, and functional metrics were mainly influenced by physical water quality (MRI of 25 % and 21 %, respectively), salt ions (MRI of 20 % for both categories) and hydrological alteration (MRI of 22 % and 26 %, respectively). In contrast, richness and diversity metrics responded more strongly to hydrological alteration (MRI of 34 %). Water quality-related sensitivity metrics were dominated by nutrients and salt ions (MRI of 24 % and 21 %), but also responded to mixture toxicity (MRI of 18 %), hydrological alteration (MRI of 17 %) and physical water quality (MRI of 15 %). Habitat quality-related sensitivity metrics strongly responded to hydrological alteration and salt ions (MRI of 25 % and 24 %, respectively). In summary, all metric groups responded comparatively well to water quality stressors (except micropollutants) and hydrological alterations. The influence of micropollutants mixture toxicity was captured best by diversity and water quality-related sensitivity metrics, while morphological habitat degradation was captured best by habitat quality sensitivity and functional metrics.

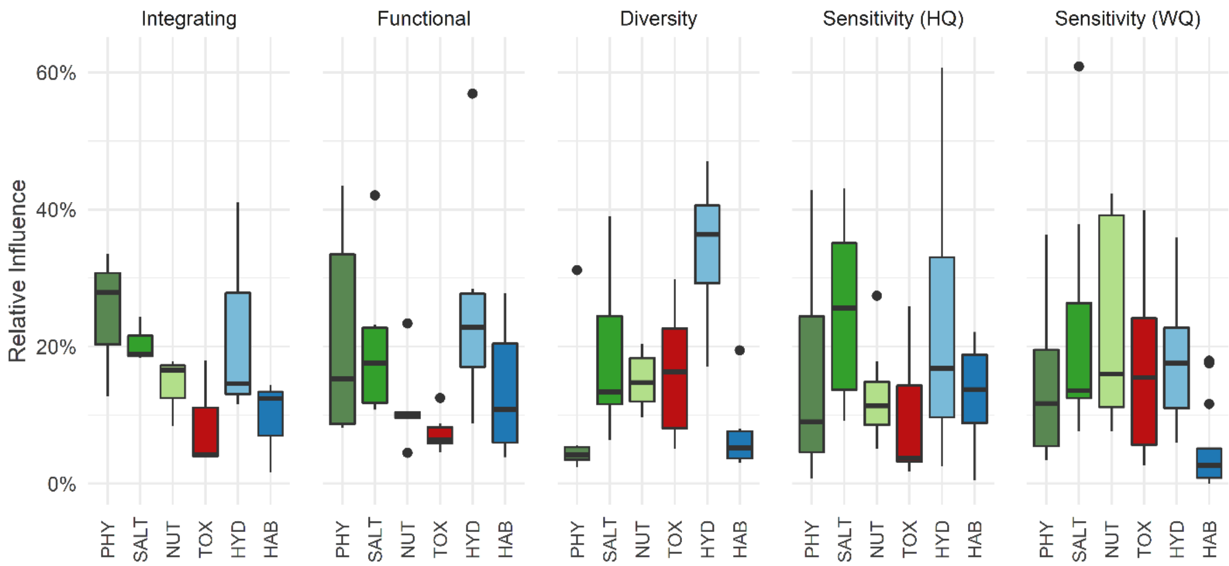

Figure 4: Stressor hierarchy in Random Forest models for metric categories. See section 2.3 for details on metric categories. Sensitivity metrics were divided into water quality (WQ) and habitat quality (HQ). Stressor groups: physical water quality stressors (PHY), salt ions (SALT), nutrients (NUT), mixture toxicity (TOX), hydrological alteration (HYD) and morphological habitat modification (HAB).

Discussion

Dominant stressors across biological assemblages

Among the six stressors groups that were addressed in this study, water quality-related stressors showed a stronger relative influence on biological community metrics than morphological habitat and hydrological stressors. The importance of water quality, physico-chemical stressors in particular, for the ecological status of rivers has already been shown in previous multiple-stressor studies from other European countries (e.g. Marzin et al., 2012; Villeneuve et al., 2015; Herrero et al., 2018; Valerio et al., 2021). Thus, despite the improvements of wastewater treatment in the past decades, water quality deterioration is still a dominant stressor of rivers (Haase et al., 2023).

The relevance of physico-chemical stressors in this study corresponds to the frequent exceedances of environmental quality targets that were observed for nutrients. Interestingly, chloride and sulphate also showed a high relative influence on many biological metrics, although both ions less often exceeded the quality targets. This finding suggests that stricter quality targets might be necessary for reaching a good ecological status in light of salinity effects (Sundermann et al., 2015; Halle et al., 2017; Feld et al., 2023). The quality target for oxygen was rarely undercut in our data, yet it is likely that the data do not reflect real minima, as these usually occur during night time (Feld et al., 2023). Hence, the strong diurnal variability in the minimum of oxygen concentrations is unlikely to be fully captured with discrete field measurements during daytime, as they are usually subjected in routine monitoring schemes (Halliday et al., 2015). Thus, ecological effects of oxygen depletion might have been underestimated in this study.

Differences among biological assemblages

Differences in the response of biological assemblages to individual stressor groups were observable and meaningful. With regard to water quality-related stressors, the flora (diatoms) responded in particular to nutrients, which points at the direct link between nutrient enrichment and primary production. In contrast, the fauna (fishes and macroinvertebrates) was more strongly influenced by physical water quality stressors, oxygen in particular, and salt ions. Oxygen depletion is particularly relevant for breathing animals, while an increased salinity has direct physiological effects on all assemblages, often resulting in the loss of salinity-sensitive species (Schröder et al., 2015). The assemblage-specific differences in the response to certain water quality-related stressor variables confirm the importance of considering both faunal and floral assemblages in multiple-stressor studies (Marzin et al., 2012). Biological responses to micropollutants varied across assemblages and were lower than the effects of physico-chemical and hydro-morphological stressors for the majority of tested metrics, which supports similar findings of previous studies (Kapo et al., 2014; Rico et al., 2016; Sabater et al., 2016). However, although micropollutants were not identified as key stressors of the three biological assemblages in this study, some metrics indicated a strong influence of micropollutants, namely water quality-related sensitivity metrics for diatoms (e.g., Pollution Sensitivity Index IPS, Swiss Diatom Index DI CH) and macroinvertebrates (e.g., $SPEAR_{pest}$). For $SPEAR_{pest}$, the sensitivity of the metric to pesticide exposure was recently re-confirmed again by Liess et al. (2021). In light of the subordinate effects of the mixture toxicity of micropollutant in this study, it should be noted that the assessment of the effects of micropollutants is limited due to the design of the chemical monitoring schemes (e.g., use of grab samples, limitation to selected substances, incomplete and censored data, low sampling frequencies). This may lead to an underestimation of micropollutant concentrations (Spycher et al., 2018; Babitsch et al., 2021; Weisner et al., 2022), which might also apply to this study. In fact, recent multiple stressor studies found stronger biological response to micropollutants (e.g., Waite et al., 2019; Lemm et al., 2021; Waite et al., 2021; Heß et al., 2023).

Among the hydro-morphological stressors, our results suggest that hydrological alteration and morphological degradation address different aspects of physical habitat degradation and thus, require separate consideration in multiple stressor studies. Notable effects of morphological degradation were only observed for macroinvertebrate and fish metrics, but not for diatom metrics (Hering et al., 2006; Marzin et al., 2012). This may point at spatial scaling-related aggregation effects of physical habitat surveys, which often aggregate local and reach-scale habitat conditions into composite indices (Gellert et al., 2014). Such indices may appear to be stronger related to macroorganisms (fish, macroinvertebrates) than to microorganisms (diatoms). Interestingly, hydrological alterations, which also aggregate measures of flow conditions at the gauging stations, showed a comparatively high influence on all biological assemblages in this study. This again points at the need to better incorporate hydrological alterations in multiple stressor studies (Meißner et al., 2019; Castro-Català et al., 2020) and to distinguish between the hydrological and the morphological component within hydro-morphological surveys.

Implications for river basin management

Macroinvertebrates, diatoms and fishes showed notable – and partly expectable – differences in their responses to multiple stressors as described in previous studies (Marzin et al., 2012; Dahm et al., 2013; Villeneuve et al., 2015). However, several patterns were observed for metric categories across the three biological assemblages. Sensitivity metrics responded strongly to water quality-related stressors, physico-chemical variables in particular, and hydrological alteration. In fact, sensitivity metrics are often designed to express the sensitivity (or tolerance) of a community to a particular stressor (e.g., diatom trophic index to indicate nutrient enrichment, diatom halobian index to indicate salinization or macroinvertebrate saprobic index to indicate organic pollution/oxygen depletion). This stressor-specific metric design may explain its high utility for water quality assessment. Functional trait-based metrics (e.g., macroinvertebrate feeding types, fish spawning habitat preferences) were mainly influenced by hydro-morphological degradation and physical water quality (Vitecek et al., 2021). The strong relationship with hydro-morphological degradation may be due to the species traits underlying most of the functional metrics in this study, namely habitat preferences. Metrics of habitat preference describe, for example, preferences of macroinvertebrate communities for specific bottom substrates (e.g., sand, gravel, particulate organic matter) or preferences of fish communities for spawning habitats (Schmidt-Kloiber & Hering, 2015). Generally, metrics of community sensitivity and functional composition are considered more applicable than abundance and diversity metrics as the latter do not reflect changes in sensitive vs. tolerant taxa or alterations of the functional diversity (Feld et al., 2016a; Enns et al., 2023; Worischka et al., 2023). Thus, the detection of multiple-stressors effects should be based upon responses of different biological assemblages and – in addition – upon different metric categories within each assemblage (Marzin et al., 2012; Larras et al., 2017; Meador & Frey, 2018; Dézerald et al., 2020; Herlihy et al., 2020). Our final metric selection included many metrics that target particular stressors such as eutrophication (e.g. diatom trophic indices), salinization (e.g. diatom halobian index) and physical habitat degradation (e.g., macroinvertebrate habitat preferences). These metrics were specifically designed and developed to indicate the effects of the targeted stressor groups. Metrics targeting toxic pollution or hydrological alterations, however, were rare. This misbalance of targeted biological response metrics available for individual stressor groups may bias the identification of key stressors and subsequently the identification of appropriate management and restoration measures (Hering et al., 2010; Lemm et al., 2019). For the future development of metrics, we recommend the development and testing of novel metrics to better address emerging stressors such as micropollutants (Birk et al., 2012; Gieswein et al., 2017). Sensitivity-related metrics seem appropriate, but should include both faunal and floral assemblages due to the different modes of action of micropollutants (Busch et al., 2016). Additionally, future development of metrics should target fish community composition and function as many metrics available for fishes only showed weak stressor responses (Birk et al., 2012; Dahm et al., 2013; Gieswein et al., 2017).

The results of this study suggest that water quality deterioration is still a key factor affecting the ecological status of macroinvertebrates, diatoms and fishes (EEA, 2019). For river basin management, this implies that restoration measures focusing on hydro-morphological condition might not improve ecological conditions as expected, unless additional measures improving water quality are implemented (Palmer et al., 2010; Brettschneider et al., 2023). Furthermore, multiple-stressor analyses require high-quality datasets. To facilitate the use of available WFD monitoring data in multiple-stressor studies, further improvements of the monitoring system are necessary. Especially, the temporal and spatial resolution and the consistency of data across monitoring programs of different stressor groups need to be enhanced (see Carvalho et al. (2019) for a detailed discussion of WFD monitoring). Event-driven and/or high-frequent monitoring of a consistent set and number of ecotoxicological relevant substances might improve the assessment of mixture risks of micropollutants (Castro-Català et al., 2020; Liess et al., 2021). The assessment of hydrological alterations requires time-series data from gauging stations and/or hydrological models both of which should be made readily available.

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2.3 Linking wastewater treatment plant effluents to water quality and hydrology: effects of multiple stressors on fish communities

Nele Markert[1,2], Barbara Guhl[2], Christian K. Feld[1,3]

[1] University of Duisburg-Essen, Faculty of Biology, Aquatic Ecology, Universitätsstr. 5, 45141 Essen, Germany
[2] North Rhine-Westphalian Office of Nature, Environment and Consumer Protection (LANUV NRW), 40208 Düsseldorf, Germany
[3] University of Duisburg-Essen, Centre for Water and Environmental Research (ZWU), Universitätsstr. 5, 45141 Essen, Germany

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Abstract

Wastewater treatment plants (WWTPs) are essential for maintaining a good water quality of surface waters. However, WWTPs are also associated with water quality deterioration and hydro-morphological alteration. Riverine communities respond to these stressors with changes in their community structure, abundance and diversity.

In this study, we used a dataset of 94 monitoring sites across North Rhine-Westphalia, Germany to investigate the influence of WWTPs on the water quality and hydro-morphological quality in river sections downstream of WWTP effluents. More specifically, we analyzed the effects of the percentage of WWTP effluents (in relation to median base flow) on four stressor groups (physico-chemistry, micropollutants, hydrological and morphological alteration) using Linear Mixed Models (LMM). Furthermore, we assessed the impact of a selection of twelve ecologically relevant stressor variables reflecting water quality deterioration and hydro-morphological alteration on reference fish communities using Canonical Correspondence Analysis (CCA).

The percentage of WWTP effluents was correlated with water quality, especially with toxic units of a wide range of pharmaceuticals including diclofenac, venlafaxine and sulfamethoxazole (R^2 up to 0.54) as well as specific pesticides (e.g., terbutryn: $R^2 = 0.33$). The correlation of percent WWTP effluents with hydro-morphological alteration was weaker and most pronounced for the frequency of high flow ($R^2 = 0.24$) and flow variability ($R^2 = 0.19$). About 40 % of the variance in the fish community structure were explained by 12 stressor variables in the CCA models. Water quality and hydrological, but not morphological stressors showed strong albeit highly variable effects on individual fish species. The results indicate that water quality degradation and hydrological alteration are important factors determining the ecological status of fish communities. In this context, WWTP effluents can impose relevant point sources of pollution that affect water quality but also cause alterations of the hydrological regime. Further management measures addressing both stressor groups are needed to improve the ecological status.

Introduction

Wastewater treatment plants (WWTPs) play an important role in limiting the anthropogenic impact to the water quality of surface waters. Over the past decades wastewater treatment has continuously improved; yet WWTPs are still associated with a number of different environmental stressors (Canobbio et al., 2009; Haase et al., 2023). Especially in densely populated areas, they may constitute important point sources of nutrients, salt ions and micropollutants, particularly pharmaceuticals as well as biocides and pesticides applied in public or private spaces (Castelar et al., 2022; McEneff et al., 2014; Müller and Gächter, 2012; Münze et al., 2017). More than 9 billion cubic meters of treated wastewater were released in Germany in 2019 (Destatis, 2023a), which underlines the widespread potential impacts of WWTPs on riverine water and habitat quality, and ultimately on biodiversity.

Besides water quality alterations, WWTPs may impose riverine hydro-morphological degradation, such as hydraulic stress caused by peak flows especially during intensive rainfall events resulting in stormwater overflow and the associated alteration of morphological (habitat) conditions (Canobbio et al., 2009; Uhl and Dittmer, 2005). In the context of climate change, these effects may even be exacerbated by prolonged periods of low flow resulting in a lower dilution and a higher percentage of wastewater contributing to the discharge of rivers, which is associated with increased concentrations of micropollutants and thus an increased likelihood of adverse effects on aquatic organisms (Abily et al., 2021; Kinouchi et al., 2007; Link et al., 2017). However, in periods of low flow WWTPs may also contribute a substantial share to the base flow, thus improving hydrological conditions for riverine biota (Canobbio et al., 2009).

Both water and hydro-morphological conditions are important environmental factors for riverine biota (e.g., Castro-Català et al., 2020; Lemm et al., 2021; Waite et al., 2021). River fish and macroinvertebrates are known to negatively respond to temperature stress, oxygen depletion and salinization (Holguin-Gonzalez et al., 2014; Kinouchi et al., 2007; Schröder et al., 2015). Ecotoxicological risks of micropollutants and mixtures thereof in aquatic environments were frequently classified as high, especially for fish communities (Markert et al., 2020; Royano et al., 2023; Spilsbury et al., 2024). Pharmaceuticals, such as the painkillers diclofenac and ibuprofen in particular have been shown to impose adverse ecotoxicological effects on fishes. Freshwater organisms also strongly respond to hydro-morphological degradation, such as the alteration of river bed and bank structure, or changes in magnitude, timing, duration, frequency and seasonality of flow conditions (Lemm et al., 2021; Marzin et al., 2012; Meißner et al., 2019).

In Europe, the ecological status of freshwater communities as well as the status of the water and hydro-morphological quality of surface waters are assessed through extensive monitoring programs associated with the European Water Framework Directive (WFD; 2000/60/EC). The WFD has set ambitious goals to protect and restore the ecological integrity of freshwater ecosystems. However, the latest status report by the European Environment Agency (EEA) stated that only 38 % and 40 % of the rivers achieved a good chemical and a good ecological status, respectively (EEA, 2018, 2019). Therefore, to improve the chemical and ecological status of rivers, further management actions, for example, advanced wastewater treatment, and restoration projects are required. To derive effective management measures for reducing stressor levels and mitigating their adverse impacts, potential causes of water and hydro-morphological quality degradation need to be identified, though. Here, a comprehensive assessment of biological responses to water quality and hydro-morphological degradation can assist the derivation of appropriate management measures (Hering et al., 2010).

The aim of this study was to assess the influence of WWTP effluents on water and hydro-morphological quality of rivers. More specifically, we quantified the effects of WWTP effluents on riverine environmental variables of four stressor groups: physico-chemical parameters (oxygen, temperature, salt ions and nutrients), micropollutants (pharmaceuticals and pesticides), hydrological (variability, magnitude, frequency, duration and timing of flow events) and morphological parameters (channel development, cross and longitudinal profile, bed and bank structure). Physico-chemical parameters and micropollutants represent important water quality conditions, while the other two stressor groups represent hydro-morphological (habitat) conditions. We expected a strong influence of WWTP effluents on all stressor groups, in particular in rivers with a high contribution of WWTP effluents to the river base flow. To investigate the ecological relevance of the stressors, we analyzed the response of riverine fish communities to the four stressor groups. Fish community structure was expected to notably respond to all stressor groups and hence to indicate the impact that WWTP effluents may impose on riverine environmental and ecological quality.

Methods

Study area

The dataset comprises 94 monitoring sites of fish communities in mountainous (altitude 200–358 m a.s.l) and lowland regions (altitude below 200 m a.s.l) of the Federal State of North Rhine-Westphalia, Germany (Figure 1). The study area covers small, mid-sized and large rivers with a drainage area between 12 and 4,480 km² (for details on catchment size and altitude see Table S1, Supplementary Material). North Rhine-Westphalia is characterized by a high population density (525 inhabitants per km²) and a high proportion of urban and industrial areas, but also includes (intensive) agricultural area (Destatis, 2023b, 2023c). In this dataset, the percentage of urban area in the catchment ranged from 3 % to 55 % (mean of 19 %), the percentage of intensive agriculture from 0.3 % to 72 % (mean of 31 %; Table S1).

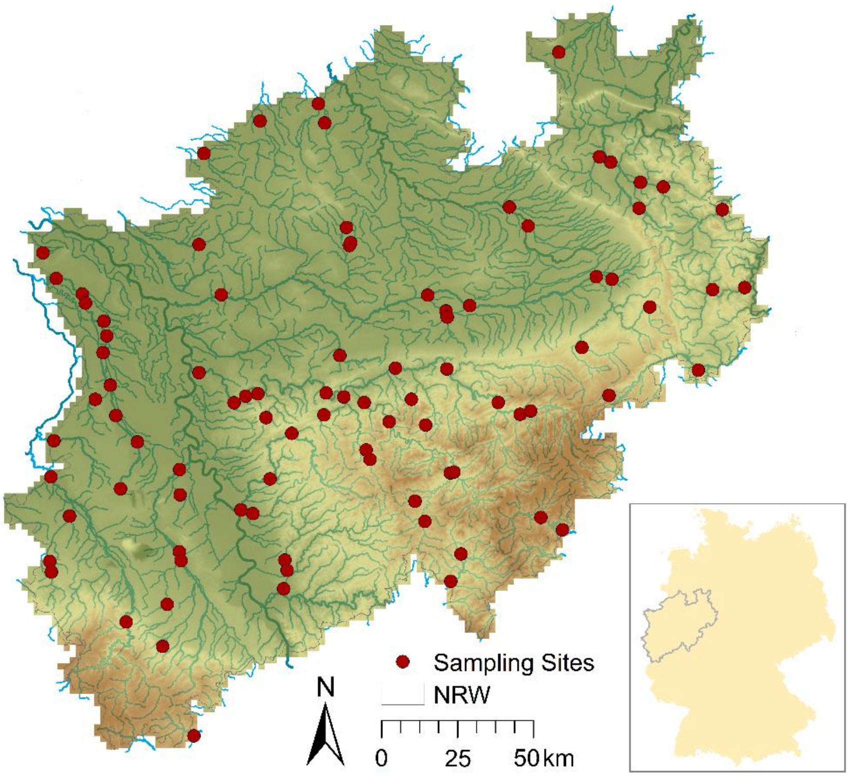

Figure 1: Monitoring sites of fish communities in the Federal State of North Rhine-Westphalia (NRW), Germany. Background colors indicate lowland regions (altitude < 200 m a.s.l., green) and mountainous regions (altitude 200-800 m a.s.l., brown). (©Data licence Germany-Zero-Version 2.0).

Cumulative percentage of wastewater

In order to assess the impact of WWTP effluents, the cumulative percentage of municipal wastewater (CumWW) was included using modelled data from the North Rhine-Westphalian Office of Nature, Environment and Consumer Protection (MUNV, 2020). The CumWW describes the percentage of wastewater, i.e. the mean annual wastewater inflow [m³/s] in relation to the median annual flow (Q_{183}) at the point of effluent discharge including wastewater inflow of all upstream WWTPs (MUNV, 2020). Previous analyses have shown that the median flow (Q_{183}; in the absence of representative data for Q_{183} also 0.5 mean flow, MQ) is a good proxy for the annual flow when assessing the effects of WWTP effluents (MUNV, 2020). Median flow at the point of wastewater discharge was derived from gauging stations using regionalization approaches (MUNV, 2020). Available data on the CumWW were allocated to the fish monitoring sites using Esri ArcGIS 10.6.1.

Environmental stressors

Both water and hydro-morphological quality were analyzed using data on four stressor groups: physico-chemical parameters (n = 6), micropollutants (n = 29), hydrological (n = 5) and morphological parameters (n = 5; Table 1). The stressor groups were selected and parameterized based upon their ecological relevance as identified in previous studies (e.g., Birk et al., 2020; Castro-Català et al., 2020; Lemm et al., 2021; Meißner et al., 2019). Stressor data were derived from WFD-related monitoring programs (LAWA, 2021) between 2014 and 2020. The monitoring sites of physico-chemical parameters, micropollutants and hydrological parameters (using data from gauging stations) were spatially and temporally matched to biological (fish) monitoring sites (details described in the following sections).

Water quality

Data on water quality parameters (Table 1) were obtained from grab samples of surface water according to the Surface Water Ordinance (OGewV, 2016) and the monitoring guideline (LAWA, 2019) in Germany and were derived for both the year of the biological sampling and the year before. An average of 4.5 grab samples were taken per site and year. For each water quality parameter, the average concentration was calculated based on these grab samples, except for oxygen (minimum concentration) and water temperature (maximum temperature; OGewV, 2016). It should be noted that peak concentrations of micropollutants are unlikely to be reliably captured with monitoring schemes using grab samples. The selection of micropollutants was based on their occurrence frequency and ecotoxicological relevance for freshwater communities identified in previous studies (e.g., Ginebreda et al., 2014; Gustavsson et al., 2017; Markert et al., 2020; Munz et al., 2017). Substances with a proportion of data gaps above 50 % of the monitoring sites or a proportion of left-censored data (i.e., values below the limit of quantification, LOQ) above 70 % were removed from the analyses. The final dataset comprised 29 micropollutants (20 pesticides and 9 pharmaceuticals). The number of detected micropollutants at each monitoring site ranged between 9 and 29 (mean of 21). Concentrations below the LOQ were substituted by half of the value of LOQ for pharmaceuticals and by zero for pesticides since pharmaceuticals are commonly ubiquitously and continuously available in regions impacted by wastewater (Loos et al., 2013; McEneff et al., 2014). The replacement by zero for pesticides seemed more appropriate as pesticides often show stronger seasonal patterns with peak concentrations during rain events and no or low exposure during dry periods (Vormeier et al., 2023). To consider the ecotoxicological risks imposed by the micropollutants, Toxic Units (TU) were calculated, which represent the ratio of the mean concentration and the ecotoxicological effect concentrations (NOEC/EC10) of a substance for fishes (see Table S2 for effect concentrations and Markert et al., 2020 for methodological details of TU).

Hydro-morphology

Hydrological stressors were calculated using data on the daily mean discharge at gauging stations close to the biological monitoring site for the year of the biological sampling and the year before. Five indicators of hydrological alteration (IHA, Table 1) were used to describe the characteristics of the hydrological regime in the rivers (Olden and Poff, 2003; Richter et al., 1996; see Mills and Blodgett (2017) for calculation of IHA). The indicators represent the main IHA groups magnitude (mh20), frequency (fh5), duration (dl16), timing (tl1) and rate of change (ra5) and were selected based on previous studies (Archfield et al., 2014; Meißner et al., 2019; Olden and Poff, 2003).

Morphological degradation was assessed on the basis of the German standard river habitat survey of North Rhine-Westphalia with a resolution of 100 m river sections (Gellert et al., 2014). For each site, morphological parameters were aggregated into five main parameters (HP1-HP5, Table 1) describing the stream course, the longitudinal and cross profile as well as the bed and bank structure (Gellert et al., 2014). The main parameters are classified in a five-class system (1 = unchanged, 2 = slightly changed, 3 = moderately changed, 4 = distinctly changed, 5 = completely changed). To account for aggregated effects of morphological conditions, the median of the morphological parameters of the 5 km upstream of the biological monitoring site were used for the analyses (Lorenz and Feld, 2013).

Table 1: Statistical parameters of stressor variables. Quality targets (QT) represent thresholds between a good and a moderate ecological status in accordance with the German Surface Water Ordinance (OGewV, 2016). QTs are stream type-specific for physico-chemical variables. For morphological parameters (HP1-HP5) QT were set to class 2 (slightly changed), for Toxic Units of micropollutants to 0.1. All variables were included in Linear Mixed Models assessing WWTP effects, but only selected variables (bold) were included in multivariate analyses of fish communities due to collinearity. Statistical parameters of stressor variables for each ecoregion are shown in Table S7 (Supplementary Material).

	Stressor variable	Mean	SD	Min	Max	QT	%Sites with QT violation
	Cumulative percentage of wastewater [%]	0.3	0.3	0	1	-	-
Physico-chemistry	Maximum water temperature [°C][a]	18.8	3.3	7.1	26.2	≤ 20–28[a]	5 %
	Minimum oxygen (MinO2) [mg/L][a]	8.3	1.4	4.4	11.6	> 6–8 [a]	15 %
	Ammonium nitrogen (NH4N) [mg/L] [a]	0.1	0.1	0	0.5	≤ 0.1-0.2 [a]	18 %
	Total phosphorus (TP) [mg/L][a]	0.1	0.1	0.0	0.5	≤ 0.1-0.15 [a]	51 %
	Sulphate (SO4) [mg/L][a]	62.9	40.3	10.4	298.3	≤ 75–220 [a]	4 %
	Chloride (Cl) [mg/L][a]	60.7	51	12.5	320.3	≤ 200 [a]	3 %

Table 1 (continued)

Micropollutant Toxic Units	**2,4-D [-]**	**1.50E-06**	**5.20E-06**	**0.00E+00**	**4.70E-05**	**≤ 0.1**	**0 %**
	Azoxystrobin [-]	2.50E-05	5.10E-05	0.00E+00	3.60E-04	≤ 0.1	0 %
	Chlortoluron [-]	6.40E-06	1.30E-05	0.00E+00	5.40E-05	≤ 0.1	0 %
	Clothianidin [-]	1.40E-08	3.70E-08	0.00E+00	2.30E-07	≤ 0.1	0 %
	Dimethenamid [-]	6.50E-05	1.30E-04	0.00E+00	8.60E-04	≤ 0.1	0 %
	Diuron [-]	1.40E-05	5.00E-05	0.00E+00	4.60E-04	≤ 0.1	0 %
	Epoxiconazole [-]	5.70E-04	1.40E-03	0.00E+00	1.10E-02	≤ 0.1	0 %
	Ethofumesate [-]	3.70E-05	1.00E-04	0.00E+00	8.20E-04	≤ 0.1	0 %
	Flufenacet [-]	4.10E-05	6.70E-05	0.00E+00	4.20E-04	≤ 0.1	0 %
	Imidacloprid [-]	8.10E-06	3.70E-05	0.00E+00	3.60E-04	≤ 0.1	0 %
	Isoproturon [-]	2.90E-05	1.70E-04	0.00E+00	1.60E-03	≤ 0.1	0 %
	MCPA [-]	9.40E-06	2.30E-05	0.00E+00	1.80E-04	≤ 0.1	0 %
	Metazachlor [-]	1.30E-06	5.20E-06	0.00E+00	4.80E-05	≤ 0.1	0 %
	Metolachlor [-]	3.10E-06	1.30E-05	0.00E+00	1.20E-04	≤ 0.1	0 %
	Metribuzin [-]	5.00E-07	1.50E-06	0.00E+00	1.20E-05	≤ 0.1	0 %
	Prosulfocarb [-]	8.50E-06	2.00E-05	0.00E+00	1.40E-04	≤ 0.1	0 %
	Tebuconazole [-]	3.80E-04	6.30E-04	0.00E+00	4.70E-03	≤ 0.1	0 %
	Terbutryn [-]	4.10E-05	5.40E-05	0.00E+00	3.10E-04	≤ 0.1	0 %
	Terbuthylazine [-]	4.90E-05	8.50E-05	0.00E+00	5.40E-04	≤ 0.1	0 %
	Thiacloprid [-]	3.50E-06	7.00E-06	0.00E+00	5.10E-05	≤ 0.1	0 %
	Bezafibrate [-]	2.70E-07	2.00E-07	6.40E-08	1.10E-06	≤ 0.1	0 %
	Carbamazepine [-]	1.60E-04	1.70E-04	1.20E-05	6.50E-04	≤ 0.1	0 %
	Clofibric acid [-]	1.10E-03	4.00E-04	4.90E-05	2.20E-03	≤ 0.1	0 %
	Diclofenac [-]	4.90E-01	5.60E-01	2.00E-02	2.90E+00	≤ 0.1	90 %
	Erythromycin [-]	2.00E-06	1.70E-06	5.00E-07	1.10E-05	≤ 0.1	0 %
	Ibuprofen [-]	2.50E-01	1.90E-01	5.90E-02	1.10E+00	≤ 0.1	94 %
	Naproxen [-]	2.90E-05	2.10E-05	9.80E-06	1.00E-04	≤ 0.1	0 %
	Sulfamethoxazole [-]	7.30E-03	6.80E-03	1.00E-03	3.20E-02	≤ 0.1	0 %
	Venlafaxine [-]	6.80E-03	4.60E-03	1.40E-03	2.30E-02	≤ 0.1	0 %
Hydrology	**High flow frequency (fh5) [events/a]**	16.5	8	4.5	46	-	-
	Low flow duration (dl16) [days]	9.6	9.7	2.5	83.2	-	-
	Flow variability (ra5) [-]	0.4	0	0.2	0.5	-	-
	Magnitude of high flow (mh20) [m³/s per km²]	0.1	0.3	0	2.6	-	-
	Timing of low flow (tl1) [-]	229	48	37	360	-	-
Morphology	**Channel development (HP1) [-]**	4.4	0.9	1	5	≤ 2	98 %
	Longitudinal profile (HP2) [-]	3.9	0.9	2	5	≤ 2	95 %
	Bed structure (HP3) [-]	3.5	1	1	5	≤ 2	82 %
	Cross profile (HP4) [-]	3.9	1	1	5	≤ 2	90 %
	Bank structure (HP5) [-]	4	1	2	5	≤ 2	94 %

[a] Quality targets vary between stream types (minimum/maximum QT indicated); stream-type-specific percentage of violation of QTs were calculated for each variable.

Characterization of stressor levels

Quality targets (QT) indicating the threshold between a good and a moderate ecological status were used to characterize the stressor levels (Table 1). For physico-chemical variables these QTs are stream type-specific following the Surface Water Ordinance (OGewV, 2016). For micropollutants QT was set to Toxic Units of 0.1 indicating environmental concentrations exceeding ecotoxicological effect concentrations for fishes while applying a safety factor of 10 for assessing chronic risks (European Commission, 2017). For morphological parameters, the QT was set to 2 indicating an unchanged to slightly changed morphological condition. For hydrological variables no quality targets are available, yet.

Fish communities

Fish communities were sampled in WFD-related monitoring programs by the local state agency and responsible water boards between 2014 and 2020. If multiple samples were available for a site, only the most recent sample was considered for further analyses. Fish were caught using electrofishing in river stretches of a minimum of 200 m length following standard protocols (CEN, 2003; Dußling, 2009). Depending on the ecoregion, sampling occurred in spring (mountainous regions) or between June and October (lowland regions) in accordance with the monitoring guidelines. Fish abundance was expressed as catch per unit of effort (CPUE), which describes the adjusted number of individuals within a 100 m section. The abundance of nine reference species was assessed in the following multi-stressor analyses to include species naturally occurring in the rivers, based on information on reference biocoenoses of the respective stream types present in the study area: river trout (*Salmo trutta fario*), brook lamprey (*Lampetra planeri*), stone loach (*Barbatula barbatula*), common dace (*Leuciscus leuciscus*), common chub (*Squalius cephalus*), common barbel (*Barbus barbus*), European bullhead (*Cottus gobio*), gudgeon (*Gobio gobio*) and roach (*Rutilus rutilus*).

Data preparation

Chemical monitoring sites were spatially matched to monitoring sites of fishes using a maximum distance of 5 km up-/downstream. Gauging stations were spatially matched using a maximum distance of 7.5 km up-/downstream as well as a maximum deviation of catchment sizes of approx. 15 %. Distances were chosen to reduce confounding effects of, for example, adjacent land uses and tributary discharges, based on expert judgement and previous analyses (Markert et al., 2022). Sites where the influence of potential confounding factors (e.g., WWTP effluents or confluences of major tributaries between matched monitoring sites) could not be excluded were removed from the dataset. Data gaps of water quality variables and missing data in the daily mean discharge were imputed using a Random Forest algorithm (missForest, Stekhoven, 2022). Data preparation and analyses were performed using the open-source software R (R V. 4.0.3 in GUI RStudio V. 1.4.1103, R Core Team, 2020).

Statistical analyses

Pairwise-correlations and collinearity between stressor variables and CumWW were analyzed using Spearman rank correlation (Table S3 and S4, R package Hmisc, Harrell Jr, 2019) and variance inflation factors (R package usdm, Naimi, 2019). To identify the main stressor gradients, stressor variables were subjected to a principal components analysis (PCA; Figure S5, R package factoextra, Kassambara and Mundt, 2020). Non-metric multidimensional scaling (NMDS; Figure S6, R package vegan, Oksanen et al., 2022) was used to examine ecoregional patterns in species composition.

Due to expected direct effects of CumWW on the water and hydro-morphological quality, confirmed by high correlation between CumWW and stressor variables in the dataset (Spearman ρ up to 0.78, Table S3), CumWW was not included in analyses of multi-stressor effects on the fish communities. Instead, the statistical relationship between CumWW and stressor variables was assessed using univariate linear mixed models (LMM; R package gamlss, Rigby and Stasinopoulos, 2005) with a Gaussian distribution. Normality of residuals was checked with residual and QQ-plots (plot function in gamlss package). Separate models were calculated for each stressor variable (response) fitted against the CumWW (predictor). Ecoregion and catchment size of the monitoring site were included as random effects to account for the influence of natural stream type-specific characteristics (e.g. river size, altitude). For each model the pseudo-R^2 and confidence intervals of fixed effects were calculated using bootstrapping with 70 % of the data (N = 1000). The pseudo-R^2 was estimated as squared correlation between the fitted and the predicted response based on the fixed effect. Additionally, the pseudo-R^2 was calculated for each stressor group (R package metafor, Viechtbauer, 2010). The effect size of CumWW on individual stressor variables and stressor groups was derived from the individual and the grouped pseudo-R^2, with a positive or negative sign added indicating the direction of the effect based on the regression coefficients.

To quantify the effects of stressor variables on the nine reference fish species, canonical correspondence analysis (CCA; R package vegan, Oksanen et al., 2022) was applied. Since the NDMS on fish communities showed ecoregional differences in fish community composition (Figure S6) CCA models were run separately for mountainous and lowland sites. Catchment size and river type (based on Pottgiesser and Sommerhäuser (2008) with information on the longitudinal fish zonation) were included as conditional variables in CCA models to account for and partial out effects of the natural effects of river size and type (conditional effect). The effects of the four stressor groups (physico-chemistry, micropollutants, hydrology and morphology) were quantified using variance partitioning (R package vegan, Oksanen et al., 2022). Both marginal effects (i.e. explained variance if only one variable was included in the model) and unique effects (i.e. explained variance of one variable if the other variables were included as co-variables) were calculated for each stressor group using the partial models. The significance of CCA models and of individual stressor variables was tested using ANOVA (R package vegan, Oksanen et al., 2022).

Due to high correlations between individual stressor variables (Spearman ρ up to 0.86; Table S3 and S4) only a selection of non-redundant stressor variables was included in the analyses. For physico-chemical, hydrological and morphological variables the selection was based on the percentage of QT exceedances and the main stressor gradients identified in the PCA

(Figure S5). For micropollutants, substances with the highest calculated ecotoxicological risk were included. The final selection (n = 12; Table 1, variables in bold) included concentrations of oxygen, ammonium-N and chloride (physico-chemistry), diclofenac, ibuprofen and venlafaxine (micropollutants), high flow frequency (fh5), flow variability (ra5), magnitude of high flow (mh20; hydrology) as well as channel development (HP1), bed structure (HP3) and cross profile (HP4; morphology).

Results

Effects of wastewater treatment plant effluents on stressor levels

The relationship between CumWW and the stressor variables substantially differed between the groups of stressors (Figure 2). In general, water quality showed a stronger relationship to CumWW than hydro-morphological conditions, with pooled effect sizes of 0.18, 0.34 and 0.17 for physico-chemical variables, pharmaceuticals and pesticides, respectively.

The highest effect sizes were observed for pharmaceuticals, especially sulfamethoxazole ($R^2 = 0.54$), venlafaxine ($R^2 = 0.51$) and diclofenac ($R^2 = 0.50$). For pesticides, effect sizes were smaller but the relationship to CumWW was evident for individual pesticides with strongest effects for terbutryn ($R^2 = 0.33$), ethofumesate ($R^2 = 0.30$) and azoxystrobin ($R^2 = 0.32$). Ammonium-N ($R^2 = 0.34$) and chloride ($R^2 = 0.26$) exhibited the strongest relationship with CumWW among the physico-chemical variables; notably, the oxygen concentration was the only water quality variable with a notable negative relationship with CumWW ($R^2 = -0.12$). Apart from oxygen, negative effects were observed only for clofibric acid and metolachlor, but these showed very weak relationships with CumWW ($R^2 = -0.01$ and -0.02).

The relationship between CumWW and hydro-morphological condition was distinctly lower (hydrology: $R^2 = 0.06$, morphology: $R^2 = 0.02$), but noticeable for certain hydrological variables, i.e. frequency of high flow (fh5, $R^2 = 0.24$) and flow variability (ra5, $R^2 = 0.19$).

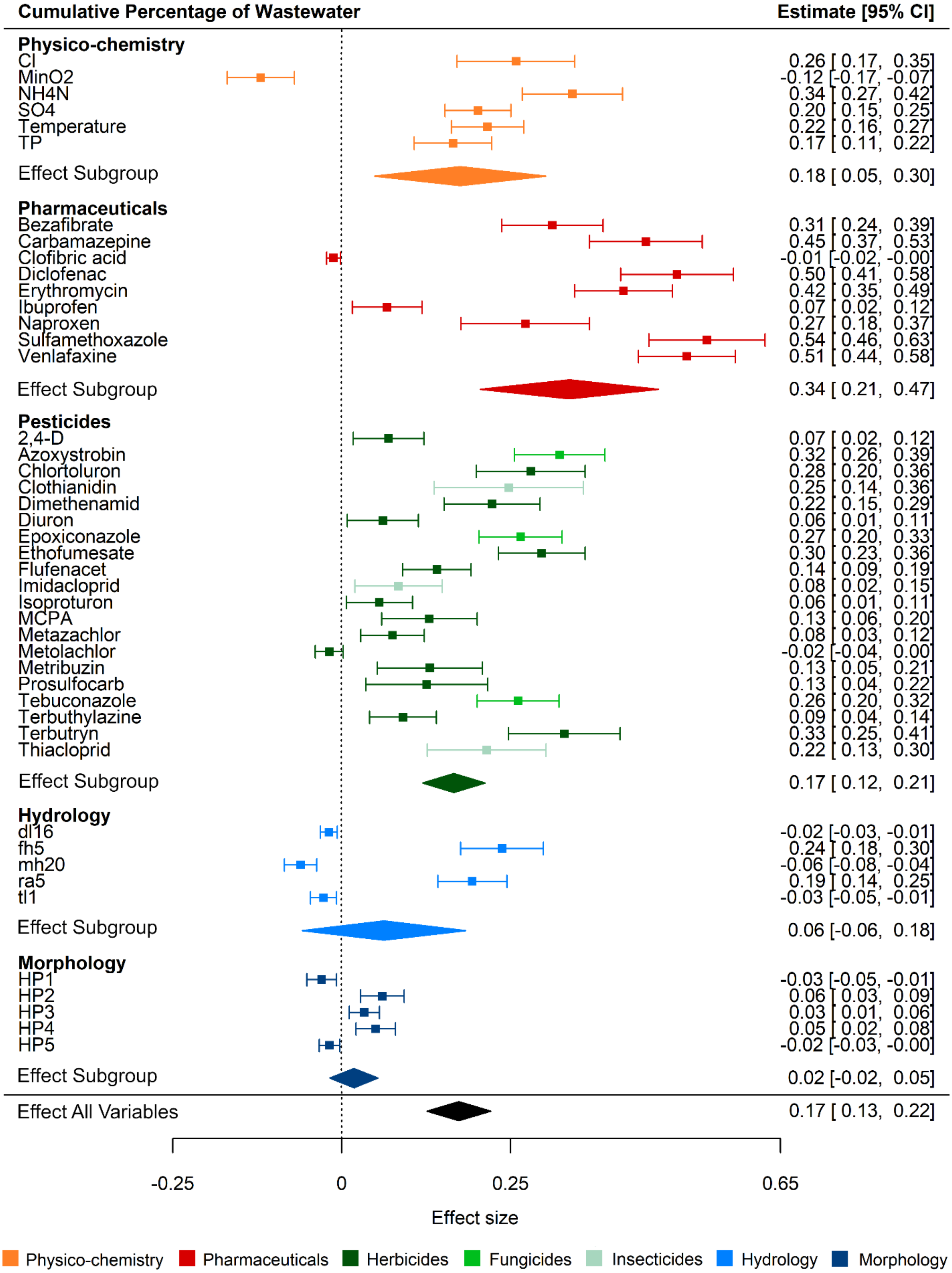

Figure 2: Relationship (effects size) of stressor variables with cumulative percentage of wastewater. Effect sizes (pseudo R²) were derived from univariate linear mixed models (LMM) with bootstrapped samples (n = 1000, 95 % confidence interval in brackets). Positive and negative signs of the effect sizes were included to account for the effect direction (based on regression coefficients).

Ecological relevance of stressor variables

Among the physico-chemical variables, total phosphorus (51 % of sites showing QT violations, Table 1), ammonium-N (18 % of sites) and oxygen (15 % of sites) regularly exceeded QTs, whereas the concentrations of sulphate and chloride mainly stayed within QTs (QTs violated at only 4 % and 3 % of the sites, respectively).

For micropollutants, only the pharmaceuticals diclofenac and ibuprofen (both painkillers) showed a TU above 0.1 set as QT. Ecotoxicological risks of both diclofenac and ibuprofen were calculated for 90 % and 94 % of sites, respectively. Additionally, TU of the pharmaceuticals sulfamethoxazole (antibiotic) and venlafaxine (antidepressant) were just below the risk threshold (TU > 0.01) at 22 % and 30 % of sites, respectively. Figure 3 shows the relationship between the CumWW and the micropollutant TU (effect size derived from LMM) as a function of the micropollutant TU. Micropollutants with a high ecotoxicological relevance (e.g., diclofenac, sulfamethoxazole, venlafaxine) also had the strongest relationship to the CumWW.

Morphological parameters (HP1-HP5) all frequently exceeded the QT, with exceedances ranging from 82 % (bed structure, HP3) to 98 % (channel development, HP1) of sites.

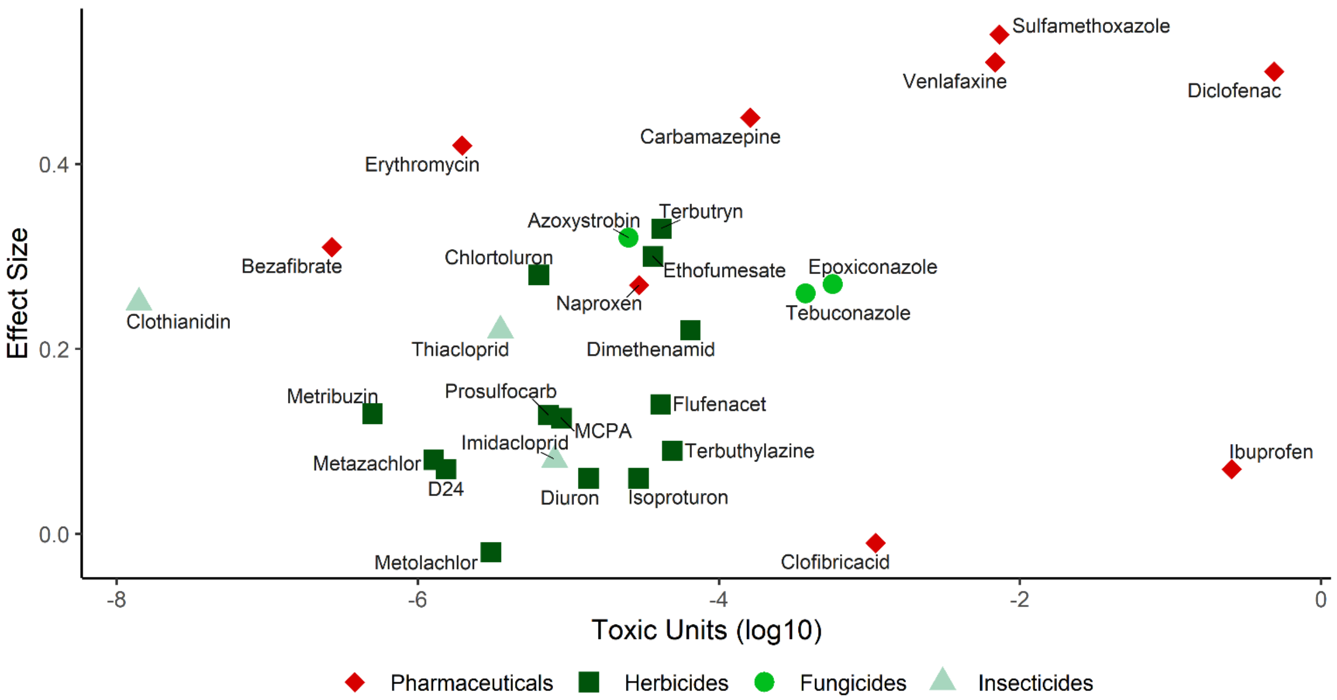

Figure 3: Scatterplot of the average Toxic Unit (TU) of each micropollutant and the effect size of the relationship between the cumulative percentage of wastewater and micropollutant TU. Effect sizes (pseudo R^2) were derived from univariate linear mixed models (Figure 2).

Response of fish communities to environmental stressors

The twelve selected stressor variables together explained 43 % of the variance in the nine reference fish species for both the lowland and the mountainous regions (adjusted R^2 of CCA models of 0.40 and 0.35, respectively; Table 2). Conditional effects of the river size and type amounted to 9 % in both CCA models (adjusted R^2).

For lowland regions, the stressor group's effects as identified by the variance partitioning were similar for physico-chemistry, micropollutants and hydrology (adjusted R^2 of 0.08, 0.09 and 0.07, respectively). In contrast, micropollutants and hydrology showed the highest effects (adjusted R^2 of 0.07 and 0.08) in the mountainous regions. Notably, unique effects of individual stressor groups were lower than their marginal effects, thus indicating a high proportion of shared explained variance among stressor groups (Table 2). The high shared explained variance points at notable correlations between stressor groups and stressor variables therein. The variables diclofenac, ibuprofen, venlafaxine, ammonium-N, magnitude of high flow (mh20) and bed structure (HP3) showed significant effects in the CCA models (ANOVA, $p < 0.05$).

The CCA plots (Figure 4) indicate differences between the reference species, with river trout (*Salmo trutta fario*), European bullhead (*Cottus gobio*) and brook lamprey (*Lampetra planeri*) located opposite to the stressor gradient of water quality (diclofenac, venlafaxine, ammonium-N and (conversely) oxygen; CCA2 in lowlands, CCA1 in mountainous regions). In contrast, roach (*Rutilus rutilus*) and barbel (*Barbus barbus*) were located at the other end of this gradient in both ecoregions. The second gradient showed a stronger dispersion of the variables but was more influenced by hydrological variables (ra5, fh5, mh20). Species with the strongest association to this gradient were mainly stone loach (*Barbatula barbatula*) and roach (*Rutilus rutilus*). However, in comparison to the water quality gradient, the associations were less pronounced.

Table 2: Statistical parameters of Canonical Correspondence Analysis (CCA). CCA analyses were performed with nine indicator fish species and stressor variables each. Due to differences in fish community composition analyses were carried out for the ecoregions lowland and mountainous regions, separately. Catchment size and river type were included as conditional variables. Significance of the CCA models and stressor variables were tested using ANOVA (999 permutations). Only p-values for significant variables are shown ($p < 0.05$). Marginal effects and unique effect (in parentheses) of stressor groups were calculated using variance partitioning.

	Lowland			**Low Mountain Range**		
	Proportion	***P***	**Adjusted R^2**	**Proportion**	***P***	**Adjusted R^2**
Total model	1	0.001 (***)		1	0.001 (***)	
Conditional effects (Catchment size, river type)	0.38		0.09	0.22		0.09
Constrained effects	0.43		0.40	0.43		0.35
Unconstrained effects	0.19			0.35		
Marginal effects (unique effects) of stressor groups						
Physico-chemistry			0.08 (0.01)			0.03 (0.00)
Micropollutants			0.09 (0.03)			0.07 (0.02)
Hydrology			0.07 (0.08)			0.08 (0.03)
Morphology			0.01 (0.01)			0.03 (0.01)
Significant descriptor variables						
NH4N		0.008 (**)			-	
Diclofenac		0.015 (*)			-	
Venlafaxine		0.005 (**)			0.004 (**)	
Ibuprofen		-			0.001 (***)	
mh20		0.01 (**)			0.006 (**)	
HP3		0.002 (**)			0.031 (*)	

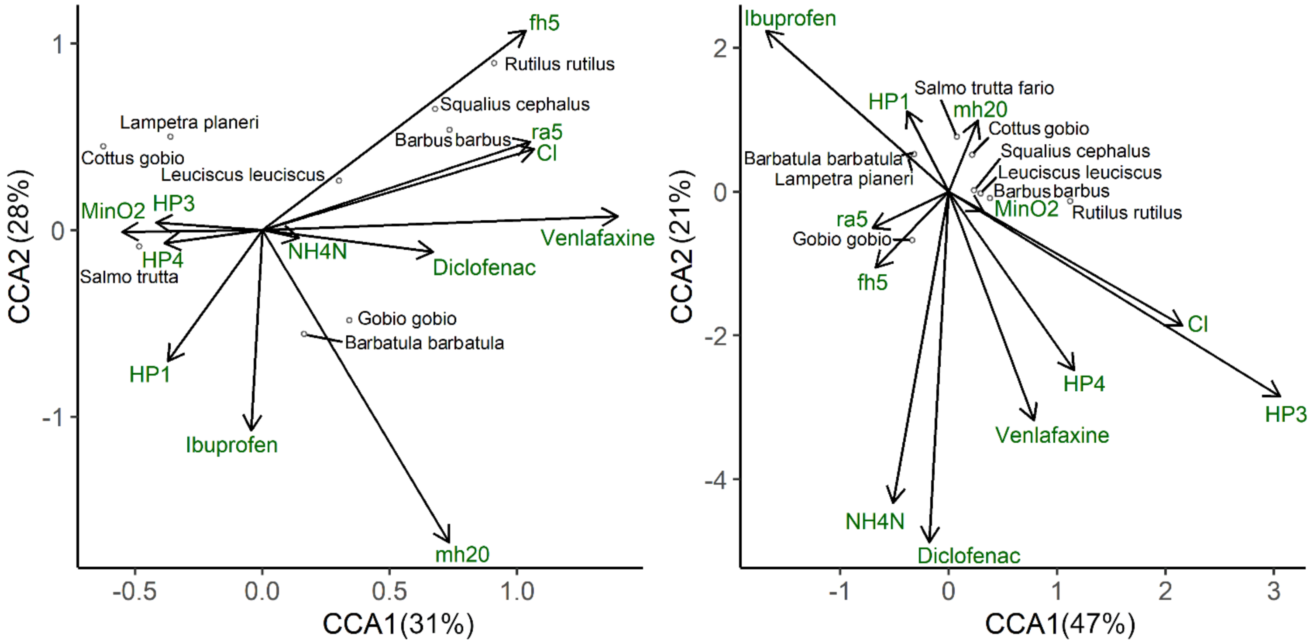

Figure 4: Canonical Correspondence Analysis (CCA) plot of fish species in lowlands (left) and mountainous regions (right) and selected stressor variables. For abbreviation of stressor variables, see Table 1.

Discussion

Wastewater treatment plants as a stressor point source

The percentage of WWTP effluents to the median flow was clearly associated with water quality deterioration in our data. Especially, the TUs of pharmaceuticals (sulfamethoxazole, venlafaxine, carbamazepine and diclofenac) were positively related to CumWW pointing to WWTPs as important point sources of ecotoxicologically relevant pharmaceutical pollution. Diclofenac, sulfamethoxazole, venlafaxine and ibuprofen also showed the highest ecotoxicological risks for fish, with diclofenac and ibuprofen frequently exceeding the QTs. These substances were regularly found in high concentrations in surface waters and were identified as main risk drivers in wastewater-impacted streams in previous studies (Finckh et al., 2022; Royano et al., 2023; Spilsbury et al., 2024). Notably, ibuprofen (and clofibric acid) was not related to CumWW, which may point at the degradability of the substance. In contrast to sulfamethoxazole, carbamazepine and diclofenac, both ibuprofen and clofibric acid have shown to be readily degradable in WWTPs and in the environment (Clara et al., 2005; Joss et al., 2005; Patrolecco et al., 2015; Vieno and Sillanpää, 2014). Still, for ibuprofen widespread and continuous WWTP emissions and high environmental concentrations leading to pseudo-persistence have been reported (Link et al., 2017; Wu et al., 2023). Pharmaceuticals, therefore, may constitute important and ubiquitous stressors in wastewater-impacted rivers (Kay et al., 2017).

The relationship between CumWW and pesticides was less strong when compared to pharmaceuticals but was evident for certain pesticides, for example, terbutryn which is commonly applied as a biocide in facade paint as well as for herbicides (ethofumesate and chlortoluron) and fungicides (azoxystrobin and tebuconazole). The relationship between CumWW and insecticides (e.g., imidacloprid) was slightly lower compared to other pesticide groups. This corresponds to findings of high pesticide concentrations and ecotoxicological risks, mainly of herbicides, in urban and wastewater-impacted areas (Le et al., 2017; Tauchnitz et al., 2020). The results might depend on the season, region and monitoring methods used, though. Previous studies in fact reported high concentrations and ecotoxicological risks of insecticides in wastewater-impacted streams (Finckh et al., 2022; Munz et al., 2017; Münze et al., 2017). In the present study, calculated ecotoxicological risks of pesticides for fish were low, which might indicate a lower relevance of pesticides compared to pharmaceuticals for this organism group but might also be associated with restrictions of the monitoring method: the design of the micropollutant monitoring, for example, the use of single grab samples, limited sampling frequency and restricted number of selected substances, may lead to an underestimation of micropollutant concentrations and associated ecotoxicological risks in this study (Moschet et al., 2014; Weisner et al., 2022). Furthermore, pesticides with particularly high ecotoxicological relevance to fish, such as pyrethroid insecticides (Werner and Young, 2018), were not included in the assessment. Nevertheless, the results confirm that WWTP effluents may contribute to pesticide pollution in surface waters (Finckh et al., 2022; Le et al., 2017; Tauchnitz et al., 2020). In addition to WWTP effluents, diffuse pollution from agricultural areas is a significant source of pesticide pollution (Halbach et al., 2021; Whelan et al., 2022).

Similarly, the physico-chemical water quality including concentrations of nutrients and salt ions is influenced by WWTP effluents as indicated by the relationship with CumWW in this study (Castelar et al., 2022; Kinouchi et al., 2007; Müller and Gächter, 2012) but also other sources including agricultural or industrial (e.g., mining) areas (Müller and Gächter, 2012; Whelan et al., 2022). Both nutrients and oxygen frequently exceeded QTs; ecological risks of salt ions, however, might be underestimated on the basis of the current QT. Salt ions can impose direct and physiological stress to freshwater organisms and therefore distinctly lower QT are currently proposed for the assessment compared to QT set in the Surface Water Ordinance (OGewV, 2016) in Germany (Feld et al., 2023; Sundermann et al., 2015).

The relationship between CumWW and hydro-morphological condition was less pronounced compared to the water quality, yet the effects were stronger for hydrological variables, namely flow variability and frequency of high flow. Although hydrological stressors tended to be underrepresented in previous multi-stressor studies, WWTPs have previously been associated with alterations of the hydrological regimes: especially discharges during intensive rainfall events, including combined sewer overflows, strongly influenced the hydrological regime (Canobbio et al., 2009; Uhl and Dittmer, 2005).

Ecological effects of environmental stressors

Water quality-related stressors, micropollutants and physico-chemical variables (the latter only in lowlands), and the hydrological condition showed the highest effect on the community of reference fish species. These results confirm the importance of both water quality deterioration and hydrological alteration for fish reported in previous studies (Marzin et al., 2012; Meador, 2020; Schinegger et al., 2016). Especially nitrogen compounds as well as pharmaceuticals but also pesticides were commonly associated with changes in fish communities (Meador, 2020; Waite et al., 2021; Weitere et al., 2021). For hydrological variables, changes in flow variability as well as magnitude and frequency of high flows, but also low flows, negatively affected fish abundance, diversity and reproduction (Bower et al., 2022; Poff and Zimmermann, 2010; Stewart-Koster et al., 2011). Effects of WWTP effluents on both the water quality and the hydrological regime (as observed in this study) can, therefore, significantly influence the ecological status of fish communities. Wastewater-impacted surface waters have been previously associated with a decline in the ecological status of fish communities including a reduction in reproductive success and a community shift towards stress-tolerant species downstream of WWTPs (McCallum et al., 2019; Teichert et al., 2016; Weitere et al., 2021). In fact, ecological responses to alterations of water quality and hydrological condition differed between fish species: while abundance of river trout and European bullhead was negatively related to the gradient of water quality degradation, roach was more robust. A high sensitivity of both Salmonidae and Cottidae to different pollutants compared to other fishes including Cyprinids was reported previously (Besser et al., 2020). Additionally, species response to hydrological alterations differs depending on species traits, for example, feeding and substrate preferences and reproductive strategies (Mignien and Stoll, 2023). Therefore, multi-stressor analyses at species level should consider a range of species that show different ecological preferences and life strategies, and thus different sensitivities or tolerances to specific stressors.

It should be noted, however, that the selected stressor variables only explained about 40 % of the biological variance in the CCA models and unique effects of individual stressor groups were only modest. This may be partly explained by the fact that some environmental variables of high relevance to the status of fish communities were not included in this study due to a limited availability of data, for example, the contamination of stream sediments, migration barriers as well as food availability (Hayes et al., 2022; Mueller et al., 2020; Müller et al., 2021).

Additionally, river size and type showed a notable conditional effect (adjusted R^2 of 0.09), thus confirming a river size and type-dependent natural variation, which requires consideration in multi-stressor analyses. Despite these restrictions, the results provide additional evidence that both water quality and hydrological conditions are important environmental determinants of riverine fish community (Marzin et al., 2012; Meador, 2020; Schinegger et al., 2016).

Implications for river basin management

WWTP effluents are important point sources of pollution and distinctly affect both the water quality and the hydrological regime, which may lead to significant changes in fish community structure. For river basin management this implies that additional measures addressing both water quality and hydrological condition are needed to improve the ecological status of surface waters. This is especially important as additional pressures from climate change potentially cause reduced base flows and increase percentages of wastewater (Brettschneider et al., 2023). Advanced wastewater treatment, for example, ozonation, activated carbon treatment and membrane filtration, has been shown to considerably reduce pollution levels and associated ecotoxicological risks (Finckh et al., 2022; Spilsbury et al., 2024; Völker et al., 2019; Yang et al., 2017). However, additional management measures will most likely be required to improve the ecological status as further sources such as diffuse pollution of pesticides from agricultural areas may remain (Neale et al., 2017). Furthermore, combined sewer overflows can contribute significantly to both water quality deterioration and hydrological alteration, which might be reduced, for example, by implementing constructed wetlands, adopting flow control systems or separating less or unpolluted surfaces from combined sewer systems (Holguin-Gonzalez et al., 2014; Köster et al., 2023; Uhl and Dittmer, 2005; Wang et al., 2021).

Generally, knowledge gaps remain for effects of hydrological alterations on river communities. Intensified monitoring of the hydrological condition including all aspects of the hydrological regime is therefore required as fish are sensitive to changes in both high and low flow conditions and flow variability (Bower et al., 2022). Furthermore, additional information on the effects of WWTP effluents and combined sewer overflows on water quality and hydrology are needed (Uhl and Dittmer, 2005). Risk assessment of micropollutants could be improved, albeit at additional cost and effort, by using additional event-based and high-frequent sampling to capture maximum concentrations or automated composite sampling to measure average concentrations, especially for pesticides (Castro-Català et al., 2020; Halbach et al., 2021; Tauchnitz et al., 2020). In Switzerland, for example, composite sampling is routinely performed at selected monitoring sites (Doppler et al., 2020; Spycher et al., 2018).

Conclusion

Using comprehensive monitoring datasets of four stressor groups and nine reference fish species as well as modelled data of the percentage of municipal WWTP effluents this study highlights the impact of WWTP on riverine environmental conditions and ecological status. Strong effects of the percentage of WWTP effluents were identified for water quality parameters, in particular pharmaceuticals, and hydrological condition, in particular high flow frequency and flow variability. Responses of fish communities on the selected stressors were less pronounced but showed stronger effects of water quality degradation and hydrological alteration, as well. For river basin management the results provide additional evidence that:

i) Management measures to improve the ecological status of fish communities need to address both water quality and hydrological alteration.

ii) Advanced wastewater treatment might reduce pollution levels from WWTPs. Additional measures, however, might be required to reduce input from other sources, such as diffuse pollution.

iii) WWTP effluents can significantly influence the hydrological regime in rivers. Further studies and monitoring of the effects of WWTP effluents as well as effects of combined sewer overflows and rainwater discharges on the hydrological condition and the ecological status of aquatic communities are necessary.

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2.4 Water Framework Directive micropollutant monitoring mirrors catchment land use: importance of agricultural and urban sources revealed

Nele Markert[1,2]*, Christian Schürings[1]*, Christian K. Feld[1,3]

[1] University of Duisburg-Essen, Faculty of Biology, Aquatic Ecology, Universitätsstr. 5, 45141 Essen, Germany
[2] North Rhine-Westphalian Office of Nature, Environment and Consumer Protection (LANUV NRW), 40208 Düsseldorf, Germany
[3] University of Duisburg-Essen, Centre for Water and Environmental Research (ZWU), Universitätsstr. 5, 45141 Essen, Germany

*Authors Nele Markert and Christian Schürings contributed equally to the work.

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Abstract

River monitoring programs worldwide consistently unveil micropollutant concentrations (pesticide, pharmaceuticals, and industrial chemicals) exceeding regulatory quality targets with deteriorating effects on aquatic communities. However, both the composition and individual concentrations of micropollutants are likely to vary with the catchment land use, in particular regarding urban and agricultural area as the primary sources of micropollutants.

In this study, we used a dataset of 109 governmental monitoring sites with micropollutants monitored across the Federal State of North Rhine-Westphalia, Germany, to investigate the relationship between high-resolution catchment land use (distinguishing urban, forested and grassland area as well as 22 different agricultural crop types) and 39 micropollutants using Linear Mixed Models (LMMs). Ecotoxicological risks were indicated for mixtures of pharmaceutical and industrial chemicals for 100 % and for pesticides for 55 % of the sites. The proportion of urban area in the catchment was positively related with concentrations of most pharmaceuticals and industrial chemicals (R^2 up to 0.54), whereas the proportions of grassland and forested areas generally showed negative relations. Cropland overall showed weak positive relationships with micropollutant concentrations (R^2 up to 0.29). Individual crop types, particularly vegetables and permanent crops, showed higher relations (R^2 up to 0.46).

The findings suggest that crop type-specific pesticide applications are mirrored in the detected micropollutant concentrations. This highlights the need for high-resolution spatial land use to investigate the magnitude and dynamics of micropollutant exposure and relevant pollution sources, which would remain undetected with highly aggregated land use classifications. Moreover, the findings imply the need for tailored management measures to reduce micropollutant concentrations from different sources and their related ecological effects. Urban point sources, could be managed by advanced wastewater treatment. The reduction of diffuse pollution from agricultural land uses requires additional measures, to prevent pesticides from entering the environment and exceeding regulatory quality targets.

Introduction

Globally, societies face three major planetary crises: biodiversity loss, climate change and chemical pollution (UNEP, 2021). The latter is associated with negative effects on biodiversity, ecosystem functioning (Groh et al., 2022; Sigmund et al., 2022) as well as human health (Fuller et al., 2022) and may impose long-term economic effects for societies (Grandjean and Bellanger, 2017). More than 350,000 chemicals have so far been registered for production and use worldwide (Wang et al., 2020) and many compounds can be found in the environment at environmentally relevant concentrations (Schwarzenbach et al., 2006). Hence, a reduction of chemical pollution is essential to remain within the planetary boundaries, which describe the natural limits for human impact to prevent unacceptable environmental change (Diamond et al., 2015; Persson et al., 2022; Rockström et al., 2009). Aquatic ecosystems in particular are strongly impaired by a multitude of micropollutants including pharmaceuticals (Fekadu et al., 2019), pesticides (Liess et al., 2021; Schäfer et al., 2011) and industrial chemicals (Koumaki et al., 2018), which have previously been associated with ecological degradation (Lemm et al., 2021; Posthuma et al., 2020; Schürings et al., 2024a).

International policies (e.g., European Green Deal) and environmental legislation (European Commission, 2019, 2020; UNEP, 2017) have been developed to promote the sustainable use of chemical substances and achieve a toxic-free environment. Comprehensive programs to monitor chemical pollution already exist (e.g., EU Water Framework Directive (WFD), Directive 2000/60/EC), which, however, cannot adequately address the numerous substances that are applied (Malaj et al., 2014; Moschet et al., 2014; Weisner et al., 2022). The risk assessment of micropollutants is typically based on the comparison of its environmental concentrations with substance-specific ecotoxicological assessment values. For several micropollutants (i.e., priority substances and river basin-specific pollutants) environmental quality standards (EQSs) and further ecotoxicologically derived assessment values are set by the WFD and related national legislations (e.g., the German surface waters directive, OGewV, 2016).

The sources of micropollutants and the pathway of pollution vary between substances, while two major pathways of pollution can be distinguished. Point sources constitute spatially explicit points of pollution, for example, effluents of industrial or municipal wastewater treatment plants (WWTPs) in urban areas (Finckh et al., 2022; Loos et al., 2013). Contrastingly, diffuse sources of pollution cannot be attributed to explicit effluents, but comprise rather broad-scale pathways such as surface and groundwater run-off from agricultural areas into the aquatic environment (Harrison et al., 2019; Wiering et al., 2020). Agricultural practices and pesticide applications vary between crop types (Andert et al., 2015). In particular, the high pesticide application rates for permanent crops and vegetables (Dachbrodt-Saaydeh et al., 2021) result in enhanced and ecotoxicologically relevant concentrations for riverine biota (Bereswill et al., 2012; Schulz, 2001; Xing et al., 2012). In contrast, forage maize cultivations are often highly fertilized (Britz and Witzke, 2014), but associated with rather small amounts of pesticides, nearly exclusively herbicides, whereas the use of pesticides on grassland is very limited (Dachbrodt-Saaydeh et al., 2021; Riedo et al., 2022). Forested areas in general show low relationships to micropollutant concentrations and often relate positively to river health (Goss et al., 2020).

In this study, we investigated the relationships between catchment land use and individual micropollutant concentrations in German rivers. More specifically, we aimed to test whether differences in concentration patterns are observed for specific crop types, revealing crop type-specific pesticide applications that are reported by Andert et al. (2015) and Dachbrodt-Saaydeh et al. (2021). This differentiation between the sources of pollution as well as the source-specific pollutants is deemed of primary importance for water management, because the management of diffuse and point sources would require different management strategies. The following research questions were formulated:

i) Which micropollutants do exceed the environmental quality targets that are set by available environmental regulations and ecotoxicological risks assessments?

ii) Do the monitored micropollutant concentrations reflect the proportions of urban, forested and agricultural areas in the catchment of monitoring sites?

iii) Do agricultural pesticide concentrations relate to specific crop types, thus reflecting crop-specific pesticide application rates?

Materials and methods

Study area

In total, 109 micropollutant monitoring sites were included in this study (Figure 1). The sites are located in the Federal State of North Rhine-Westphalia (NRW), Germany and cover lowland (altitude below 200 m a.s.l) and mountainous regions (altitude 200–800 m a.s.l) as well as small (catchment area 5–100 km^2), mid-sized (catchment area 100–1000 km^2) and large rivers. Catchment area ranged 5–2834 km^2 (median: 326 km^2; see Supplementary Material Table A1 for detailed site characterization).

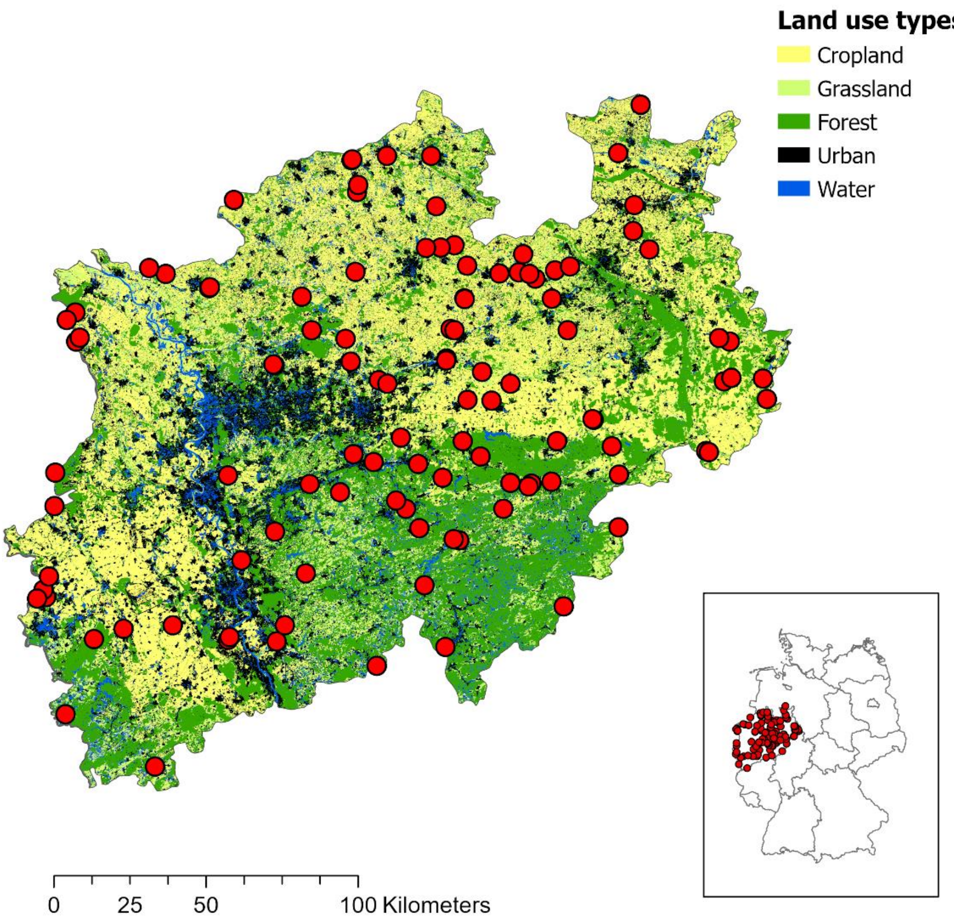

Figure 1: Location of micropollutant monitoring sites in the Federal State of North Rhine-Westphalia (NRW), Germany with adjacent land use derived from Blickensdörfer et al. (2022) and Griffiths et al. (2019) using ESRI ArcGIS Pro.

Micropollutant monitoring and ecotoxicological risk assessment

Data on micropollutant concentrations originate from WFD-related chemical monitoring programs of the North Rhine-Westphalian Office of Nature, Environment and Consumer Protection and regional water boards. Sampling was based upon grab samples of surface water (see OGewV (2016) and LAWA (2019) for details on sampling and analysis) and occurred between 2016 and 2019. For each site one sampling year was selected that temporally matched the reference timing of land use data (2016/2017) best (section 2.3). In total, 39 micropollutants (19 pesticides, 14 pharmaceuticals and six industrial chemicals including personal care products and household chemicals; Table 1) were selected for this study because of their ecotoxicological relevance, i.e. they constitute priority substances, river basin-specific pollutants or candidate substances on the watch list listed by the WFD and were identified as ecotoxicologically relevant by previous studies (e.g., Ginebreda et al., 2014; Gustavsson et al., 2017; Markert et al., 2020).

To quantify the ecotoxicological risk of micropollutants (research question 1), we calculated individual risk quotients (RQs) for each substance, i.e., the quotient of the measured concentration divided by the substance-specific assessment value (Backhaus and Faust, 2012). The estimation of chronic risks during longer exposure periods was based on annual mean concentrations of individual micropollutants (OGewV, 2016). For all substances, the number of measured concentrations ranged between one and 24 values with a mean of four measured values per substance, site and year (Table A2 Supplementary Material). Assessment values were derived from environmental quality standards (EQSs) from the WFD, national legislation (OGewV, 2016) and validated ecotoxicological data (e.g., EQS proposals and predicted no effect concentrations) in accordance with the technical guidance for deriving environmental quality standards (European Commission, 2017; Markert et al., 2020). To account for combined risks of micropollutant mixtures, the sum of individual RQs (SUM RQ) was calculated for each site (Backhaus and Faust, 2012; Markert et al., 2020). RQ and SUM RQ values above one indicate that individual and combined micropollutant concentrations exceed the ecotoxicological effect levels and thus constitute a potential (mixture) risk.

Since both the number of micropollutants and the composition of substances measured at each site varied among the sites, data gaps occurred for individual micropollutants that ranged between 1 and 32 % of the sites (mean across all substances: 12 %). Missing values were imputed using an iterative imputation algorithm based on random forests (missForest), which has previously been shown to perform well for data gaps extending up to 30 % (or even 50 %) of the values (Stekhoven and Bühlmann, 2012; Tang and Ishwaran, 2017). Left-censored data, i.e. concentrations below the limit of quantification (LOQ) were replaced by half of the LOQ value for pharmaceuticals and industrial chemicals and by zero for pesticides. This approach was chosen since pharmaceuticals and industrial chemicals are ubiquitously and continuously released into the aquatic environment (Hernando et al., 2006; McEneff et al., 2014), where substitution with zero might lead to a critical underestimation of concentrations. In contrast, pesticides tend to show seasonal concentrations patterns (Vormeier et al., 2023b), where substitution with half the LOQ might result in arbitrarily high concentration ranges.

Table 1: Statistical parameters and calculated risk quotients of micropollutants

Application of pesticides (‘plant protection products’) refers to substance-related approvals in Germany (BVL, 2023a). Three pesticide subgroups were distinguished: herbicides (H), fungicides (F) and insecticides (I). Risk quotients (RQs) were calculated as quotients of measured concentrations and assessment values in accordance with the technical guidance for deriving environmental quality standards (European Commission, 2017). Individual CAS numbers and assessment values used for ecotoxicological risk assessment of micropollutants are listed in Table A2, Supplementary Material. Concentrations below the limit of quantification (LOQ) were replaced by half of the LOQ value for pharmaceuticals and industrial chemicals and by zero for pesticides.

			Concentration (µg/L)				Risk quotient (RQ)		
	Substance	**Application**	**Min.**	**Max.**	**Mean**	**SD**	**Min.**	**Max.**	**%Sites with RQ > 1**
Industrial chemicals	Benzo(a)pyrene	Polycyclic aromatic hydrocarbon-	0.0003	0.010	0.002	0.001	1.47	56.44	100 %
	Benzotriazole	Diverse, i.a. corrosion inhibitor	0.025	5.267	1.156	0.991	0.001	0.28	0 %
	Bisphenol A	Diverse, mainly plastic production	0.005	0.135	0.040	0.029	0.01	0.40	0 %
	Fluoranthene	Polycyclic aromatic hydrocarbon	0.001	0.028	0.007	0.004	0.11	4.48	44 %
	Galaxolide (HHCB)	Fragrance	0.032	0.243	0.104	0.051	0.007	0.06	0 %
	Triclosan[1]	Disinfecting and preserving agent	0.001	0.014	0.006	0.002	0.06	0.70	0 %
Pharmaceuticals	Azithromycin	Antibiotic agent	0.005	0.079	0.027	0.015	0.28	4.15	60 %
	Bezafibrate	Antiepileptic agent	0.005	0.610	0.040	0.091	0.002	0.27	0 %
	Carbamazepine	Antiepileptic agent	0.005	0.710	0.119	0.124	0.01	1.42	2 %
	Ciprofloxacin	Antibiotic agent	0.005	0.018	0.013	0.002	0.06	0.20	0 %
	Clarithromycin	Antibiotic agent	0.006	0.310	0.039	0.052	0.06	3.10	7 %
	Clindamycin	Antibiotic agent	0.006	0.054	0.021	0.010	0.14	1.23	3 %
	Clofibric acid	Antibiotic agent	0.001	0.062	0.012	0.006	0.0001	0.01	0 %
	Diclofenac	Pain medication	0.005	3.900	0.352	0.586	0.1	78.00	74 %
	Erythromycin	Antibiotic agent	0.003	0.200	0.020	0.023	0.02	1.00	0 %
	Ibuprofen	Pain medication	0.004	0.250	0.028	0.039	0.40	25.00	98 %
	Naproxen	Pain medication	0.002	0.840	0.050	0.108	0.001	0.49	0 %
	Paracetamol	Pain medication	0.005	0.107	0.025	0.021	0.0001	0.002	0 %
	Sulfamethoxazole	Antibiotic agent	0.005	0.600	0.071	0.086	0.008	1.00	0 %
	Venlafaxine	Antidepressant agent	0.005	1.000	0.085	0.126	0.006	1.14	1 %
Pesticides	Aclonifen (H)	Field crop, Vegetable	0.000	0.006	0.001	0.001	0.00	0.05	0 %
	Azoxystrobin (F)	Field crop, Vegetable, Fruit, Wine, Biocide, Hop, Ornamental	0.000	0.781	0.016	0.094	0.00	3.91	3 %
	Chlortoluron (H)	Field crop	0.000	0.042	0.003	0.007	0.00	0.11	0 %
	Clothianidin (I)	Field crop, Vegetable, Ornamental, Biocide	0.000	0.016	0.000	0.002	0.00	0.20	0 %
	2,4-D (H)	Field crop, Fruit, Ornamental	0.000	0.031	0.001	0.003	0.00	0.15	0 %
	Dimethenamid (H)	Field crop, Vegetable, Fruit, Ornamental	0.000	0.617	0.010	0.063	0.00	2.37	1 %
	Diuron[2] (H)	Field crop, Fruit, Wine, Biocide	0.000	0.173	0.006	0.023	0.00	0.87	0 %
	Ethofumesate (H)	Field crop, Vegetable	0.000	0.035	0.001	0.004	0.00	0.01	0 %
	Flufenacet (H)	Crop, Vegetable, Fruit, Ornamental	0.000	0.097	0.008	0.014	0.00	2.42	3 %
	Imidacloprid[2] (I)	Field crop, Vegetable, Fruit, Wine, Biocide, Ornamental, Hop	0.000	0.159	0.006	0.017	0.00	79.50	43 %

Table 1 (continued)

	Substance	Application	Concentration (µg/L) Min.	Max.	Mean	SD	Risk quotient (RQ) Min.	Max.	%Sites with RQ > 1
Pesticides	Isoproturon[2] (H)	Field crop, Ornamental, Biocide	0.000	0.053	0.003	0.009	0.00	0.18	0 %
	MCPA (H)	Field crop, Hop, Fruit, Ornamental	0.000	0.163	0.010	0.022	0.00	0.25	0 %
	Metazachlor (H)	Field crop, Vegetable, Ornamental	0.000	0.195	0.004	0.024	0.00	0.49	0 %
	Metolachlor (H)	Field crop, Vegetable	0.000	0.117	0.004	0.015	0.00	0.58	0 %
	Nicosulfuron (H)	Field crop	0.000	0.020	0.002	0.003	0.00	2.22	4 %
	Tebuconazole (F)	Field crop, Biocide	0.000	0.161	0.004	0.017	0.00	0.28	0 %
	Terbuthylazine (H)	Field crop, Vegetable	0.000	0.243	0.012	0.034	0.00	0.49	0 %
	Terbutryn[2] (H)	Biocide	0.000	0.103	0.008	0.016	0.00	1.59	3 %
	Thiacloprid[2] (I)	Field crop, Vegetable, Fruit, Ornamental	0.000	0.014	0.001	0.002	0.00	1.43	2 %

[1] Triclosan was used as a biocidal active substance for human hygiene, disinfection and preservation but the approval was withdrawn in the EU in 2016 (ECHA, 2023); yet it is used in cosmetic and personal care products (European Commission, 2014).

[2] The substances, diuron, imidacloprid, isoproturon, thiacloprid and terbutryn have been banned for (outdoor) use as 'plant protection product' in the EU since 2002 (terbutryn), 2007 (diuron), 2016 (isoproturon), 2018 (imidacloprid) and 2020 (thiacloprid) (BVL, 2023b), but are still approved as biocidal active substances for preservatives, for example, in facade paint or construction material (diuron, terbutryn, isoproturon; ECHA, 2023), for insecticide (imidacloprid, ECHA, 2023) or as veterinary medicinal products (imidacloprid, EMA, 2021).

Catchment land use

For each sampling site, we quantified the proportions of forested, urban and agricultural terrestrial land use in the catchment area upstream of the site. Catchment delineation was based on a digital elevation model (©dl-zero-de/2.0, Geobasis NRW, 10 m resolution) in ESRI ArcView 3.3 subsequently checked visually for correctness and clipped with altogether 23 different crop types (including grassland) using ESRI ArcGIS Pro 2.9.0 and Spyder (Phyton 3.7.0). Crop type-specific land uses for 2017 were derived from satellite images (Sentinel-2, Landsat 8 and Sentinel-1, 10 m resolution; Blickensdörfer et al., (2022). The proportion of urban and forested areas in the catchment for 2016 were derived from Griffiths et al.(2019) and quantified alike crop type-specific land use. To statistically account for the temporal variation of micropollutant data (2016 – 2019) and land use/cover data (2016 – 2017), the year of micropollutant sampling was included as a random factor in the models (see below). However, the influence is likely minor, as Schürings et al. (2024b) found no major differences between year when comparing the effect of land use on river biota using the land use data of Blickensdörfer et al. (2022) of the years 2017 and 2018.

To quantify and compare catchment land uses, the 23 different crop types (including grassland) and urban and forested area were assigned several categories (Table 2). Except for grassland, all crop types were merged into a category 'cropland' to account for general effects of intensive agricultural land use. In the category, maize and cereals were dominant. Grassland was kept separate because it constitutes a rather extensive form of agricultural land use. To analyze crop type-related effects, the 22 individual crop types were categorized into maize, cereals, oilseeds, permanent crops and vegetables. To further differentiate between different vegetables that are known to be associated with high pesticide application rates (Dachbrodt-Saaydeh et al., 2021) asparagus, strawberries and onions were additionally kept as individual categories (Table 2).

Table 2: Statistical parameters of the proportion of land uses in the catchments upstream of the sampling sites and categorization of crop types into sub-groups.

Land use	**Min. %**	**Max. %**	**Mean %**	**SD %**
Forest	0.04	74.94	31.78	20.07
Urban area	4.05	59.09	20.04	11.51
Grassland	2.89	34.86	12.57	6.54
Cropland	0.11	76.77	29.87	21.66
Individual crop type				
Maize (silage maize, grain maize)	0.00	76.91	29.13	17.61
Cereals (wheat, rye, barley, oat, other cereals)	5.93	87.39	46.75	15.80
Oilseeds (rapeseed, sunflowers)	0.00	25.38	5.56	5.68
Permanent crops (vineyards, hops, orchards)	0.00	27.52	2.66	4.83
Vegetables (potatoes, sugar beets, legumes, strawberries, asparagus, onions, carrots, other vegetables)	1.51	48.96	13.72	10.46
Asparagus	0.00	7.20	1.72	1.65
Strawberries	0.00	24.06	2.72	4.23
Onions	0.00	4.53	0.52	0.95

Statistical analyses

To investigate, whether micropollutant mixture risks are reflected by catchment land uses (research question 2), individual linear regression models of the SUM RQ of industrial chemicals, pharmaceuticals and pesticides (incl. the sub-groups herbicides, fungicides and insecticides) with catchment land uses (cropland, urban area, forest) as predictors were visualized (R package ggplot2 (Wickham, 2016) with lm smooth function; R Core Team, 2020).

For micropollutant-specific analyses, separate linear mixed models (LMMs) were fitted for each possible combination of four land use categories (urban, forest, grassland, cropland as well as individual crop types) and 39 micropollutants, with the micropollutant concentration as response and the proportion of one land use type as the predictor (i.e. the fixed effect in the model). Ecoregion (lowlands, low mountains) and the year of micropollutant sampling were included in each LMM as random effects. No spatial autocorrelation was assumed, as all sites have distinct sub-catchments and additionally, prior studies using the same data basis did not find strong autocorrelation (e.g. Schürings et al., 2024b). A gaussian distribution was selected for LMMs, as preliminary analyses of the data using Generalized Additive Models (GAMs) suggested that a linear relationship of fixed effects can be assumed. LMMs were run in R with the 'gamlss' package (v5.2-0, Rigby and Stasinopoulos, 2005).

In each of the models, 70 % of the data were bootstrapped 1,000 times to calculate a mean-peudo-R^2 (from here on referred to as R^2) for the fixed effect including confidence intervals. The R^2 of the fixed effect was calculated as the squared correlation between the fitted response and the predicted response, solely based on the fixed effect. Alongside the individual R^2 (and confidence intervals) for individual micropollutant concentrations, an overall R^2 was calculated for each group of micropollutants (concentrations of pesticides, pharmaceuticals, and industrial chemicals), using a random effect model with the metafor package (Viechtbauer, 2010). Effect size (Figure 2) was based on the individual and grouped R^2, however, a sign was added to the plot axis to distinguish positive and negative regression coefficients, i.e. positive or negative effects of land uses on micropollutant concentrations. To analyze whether pesticide concentrations reflect crop-specific pesticide application rates (research question 3), additional models were calculated for the individual crop types, i.e., maize, cereals, oilseeds, permanent crops, vegetables as well as asparagus, strawberries and onions, following the same procedure.

Results

Ecotoxicological risk assessment

Six out of the 39 micropollutants frequently (i.e. at more than 10 % of the sampling sites) exceeded regulatory assessment values (RQ > 1) and hence imposed individual ecotoxicological risks: ibuprofen, diclofenac, azithromycin (pharmaceuticals), benzo(a)pyrene, fluoranthene (industrial chemicals), and imidacloprid (pesticide; Table 1). Furthermore, the pharmaceuticals clarithromycin, clindamycin, carbamazepine and venlafaxine as well as the pesticides thiacloprid, azoxystrobin, nicosulfuron, flufenacet, dimethenamid and terbutryn were found in concentrations exceeding the assessment values, although at less than 10 % of the sites. In contrast to individual RQs, when considering chemical mixtures, ecotoxicological risks of pharmaceuticals and industrial chemicals were indicated at 100 % of the sites (SUM RQ > 1, Table A3 Supplementary Material). Mixture Risks of pesticide mixtures were evident at 55 %

of the sites, with insecticides (44 % of the sites) dominating the risk assessment over herbicides (27 %) and fungicides (3 %).

The relationship between risks of chemical mixtures and catchment land use varied among micropollutant groups (Figure 2) but showed strong positive relationships of cropland with herbicides (R^2 = 0.31) and fungicides (R^2 = 0.30) and of urban area with pharmaceuticals (R^2 = 0.38). The proportion of forested area was negatively related with risks of chemical mixtures of all micropollutant groups, which was most pronounced for herbicides (R^2 = 0.45) and fungicides (R^2 = 0.33).

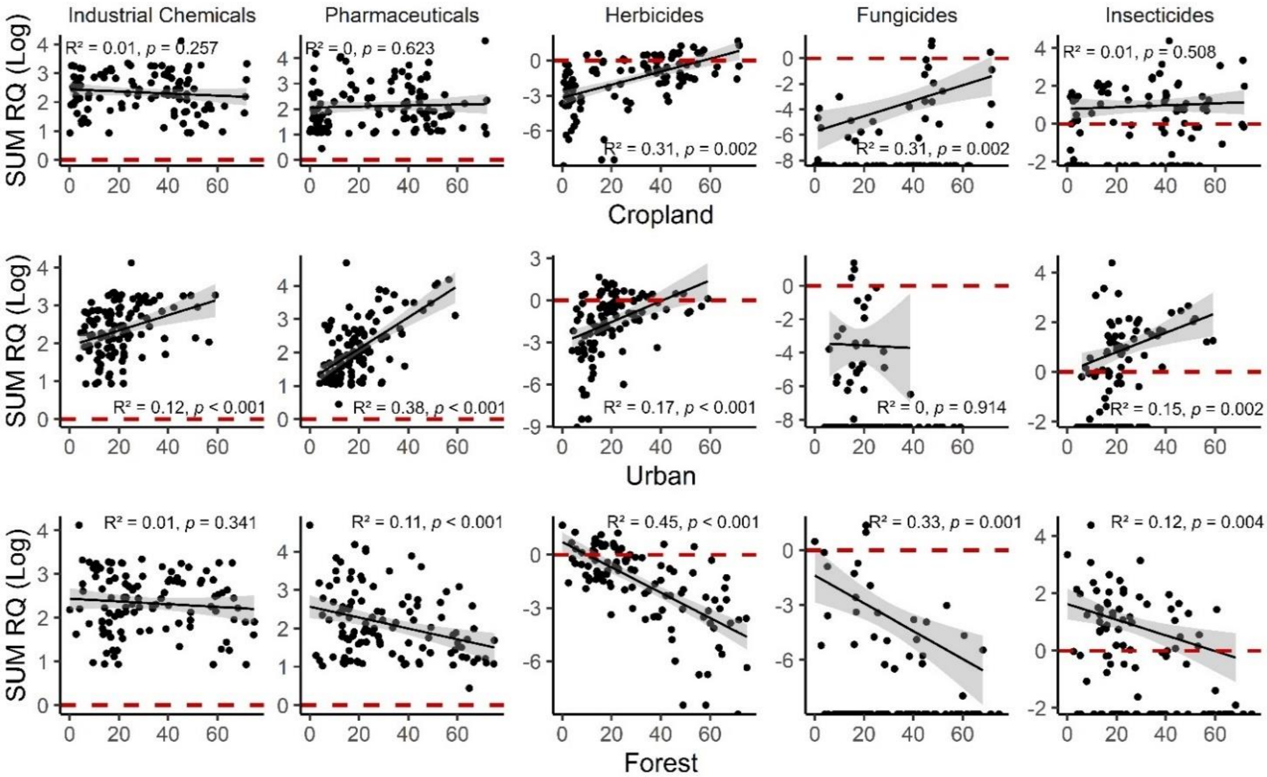

Figure 2: Relationship between the proportion of cropland, urban and forested areas in the catchment and mixture risk quotients (log SUM RQ) of industrial chemicals, pharmaceuticals, herbicides, fungicides and insecticides. The solid line marks the fit of a linear regression model with 95 % confidence interval indicated in gray; dashed red lines mark the threshold of SUM RQ = 1, which translates to 0 along the log-transformed y-axis.

Link between micropollutant concentrations and land use

The proportion of urban and forested areas, cropland and grassland revealed clear differences in their relationship to individual micropollutants and micropollutant groups (Figures 3 and 4). Urban land use (Figure 3a) was positively related to numerous pollutants, particularly to pharmaceuticals (R^2 = 0.31) and industrial chemicals (R^2 = 0.39), while its relationship with pesticides (R^2 = 0.02) was almost negligible. Among the pharmaceuticals, antibiotics (azithromycin: R^2 = 0.54, clindamycin. R^2 = 0.45 and clarithromycin: R^2 = 0.44) revealed the strongest relationship to proportion of urban areas. The effect sizes for industrial chemicals were in a similar range and showed particular strong relationships to galaxolide (R^2 = 0.51) and triclosan (R^2 = 0.48). The strongest individual relationship of a pesticide to urban area was found for terbutryn (R^2 = 0.27).

Cropland showed a weak, but positive relationship to pesticides (pooled R^2 = 0.08), while its relationship to pharmaceuticals and industrial chemicals was negligible (both R^2 = 0.02). The strongest individual relationship between proportion of cropland and pesticides were found for flufenacet (R^2 = 0.29) and nicosulfuron (R^2 = 0.21), individual relationships to pharmaceuticals and industrial chemicals were negligible (R^2 up to 0.04), except for a weak negative relation with ciprofloxacin (R = 0.13).

Grassland (Figure 4a) showed weak and negative relationships to all micropollutant groups, with pooled effect sizes of R^2 = 0.04, R^2 = 0.07 and R^2 = 0.06 for pesticides, pharmaceuticals and industrial chemicals, respectively. Individual effects of the proportion of grassland were most pronounced and negative for the pharmaceutical ciprofloxacin (antibiotic, R^2 = 0.17) and for the pesticide flufenacet (herbicide, R^2 = 0.13). Eventually, forest (Figure 4b) showed weak and negative relationships to all micropollutant groups with pooled effect sizes of R^2 = 0.07, R^2 = 0.08 and R^2 = 0.08 for pesticides, pharmaceuticals, and industrial chemicals, respectively. Again, the strongest individual relationships to the proportion of forested areas were found for ciprofloxacin (R^2 = 0.33) and flufenacet (R^2 = 0.22).

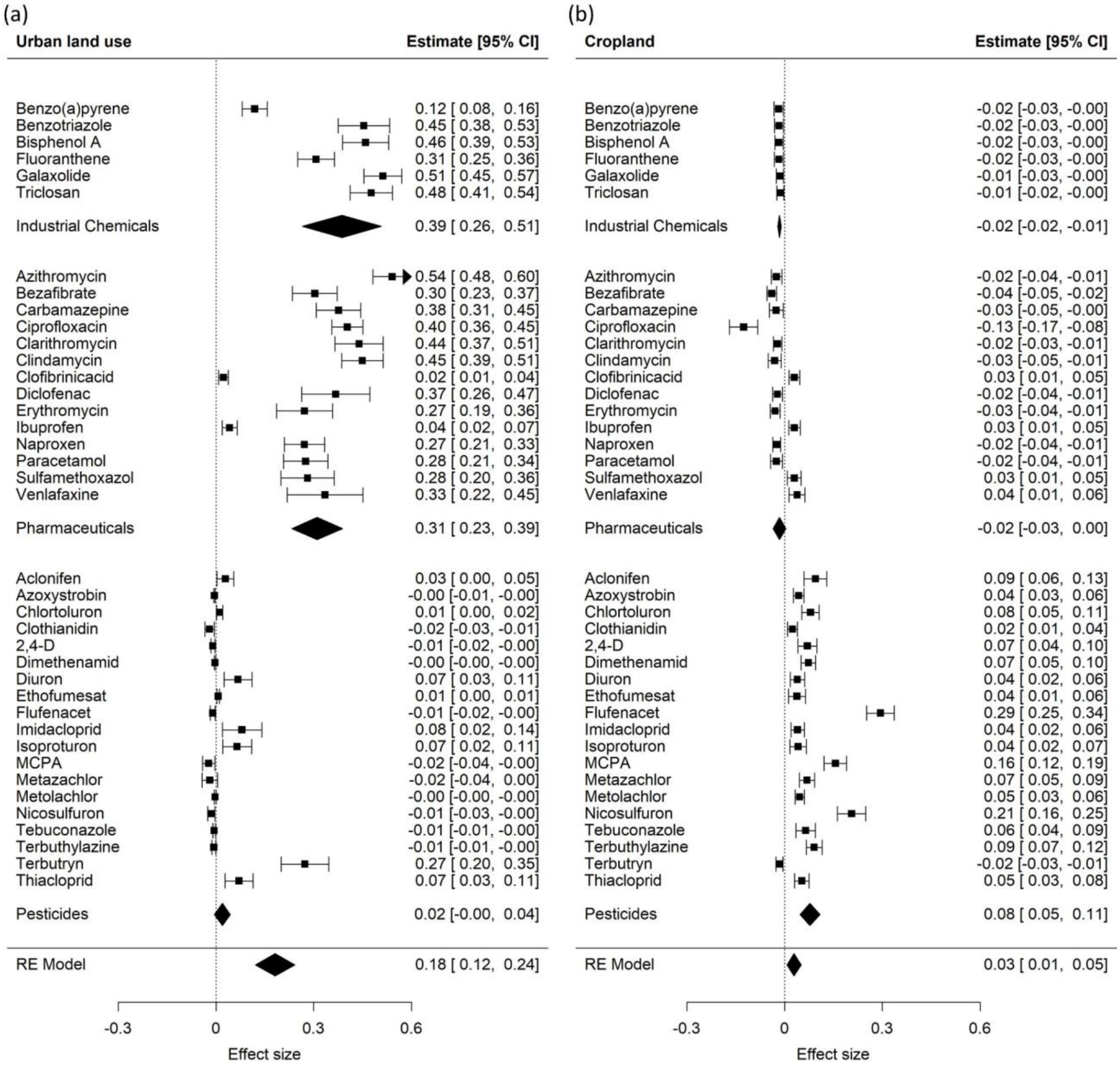

Figure 3: Relationship (effect size) of the proportion of urban areas (a) and cropland (b) in the catchment with micropollutant concentrations. Effect sizes represent model fits (pseudo-R^2) derived from bootstrapped (n = 1,000) univariate linear mixed models (LMM) with 95 % confidence intervals indicated in brackets. Negative signs were added to account for negative relationships (i.e., negative regression coefficients) – although R^2 values are positive by definition.

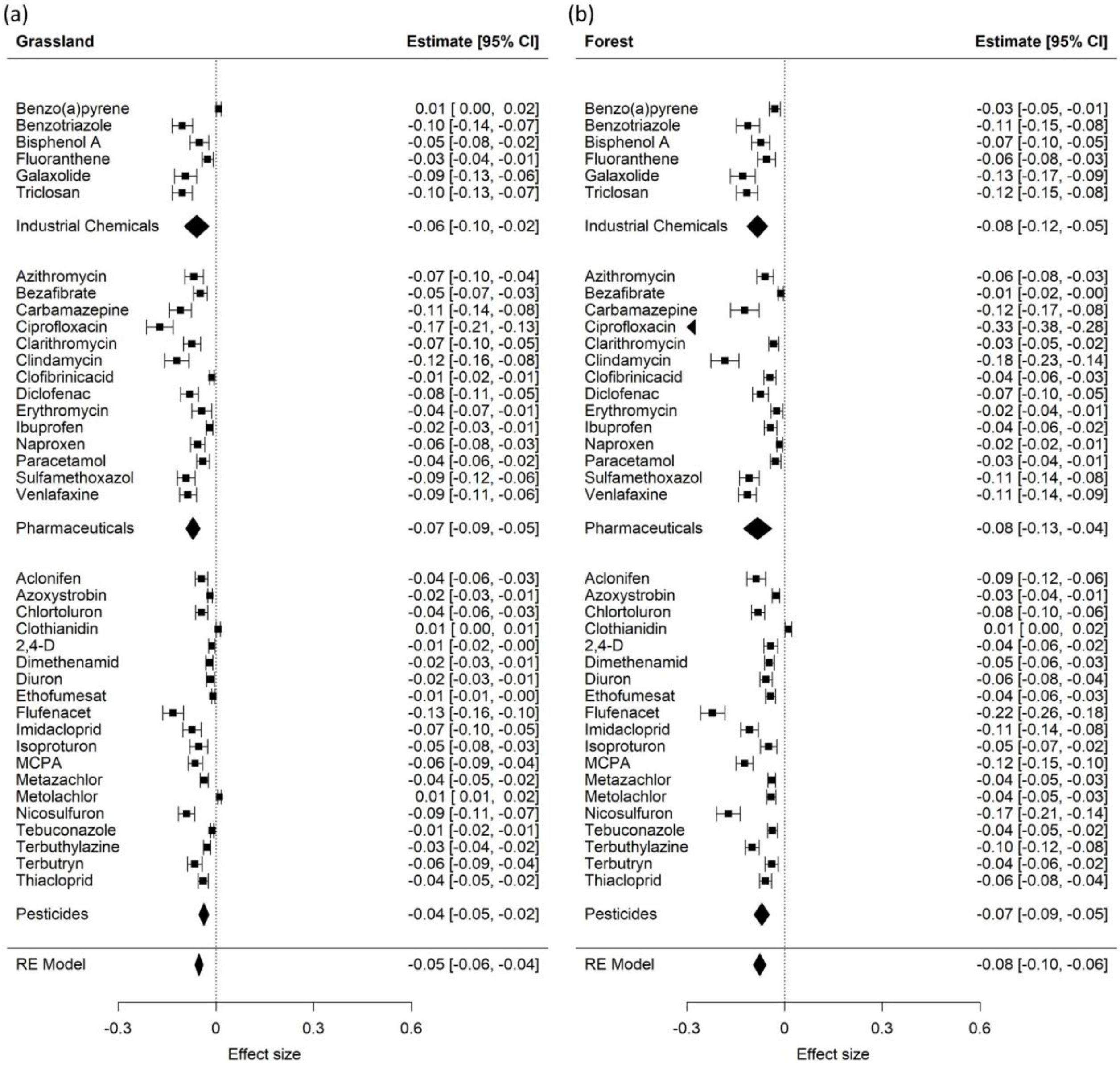

Figure 4: Relationship (effect size) of the proportions of grassland (a) and forested areas (b) in the catchment with micropollutant concentrations. Effect sizes represent model fits (pseudo-R^2) derived from bootstrapped (n = 1,000) univariate linear mixed models (LMM) with 95 % confidence intervals indicated in brackets. Negative signs were added to account for negative relationships (i.e., negative regression coefficients) – although R^2 values are positive by definition.

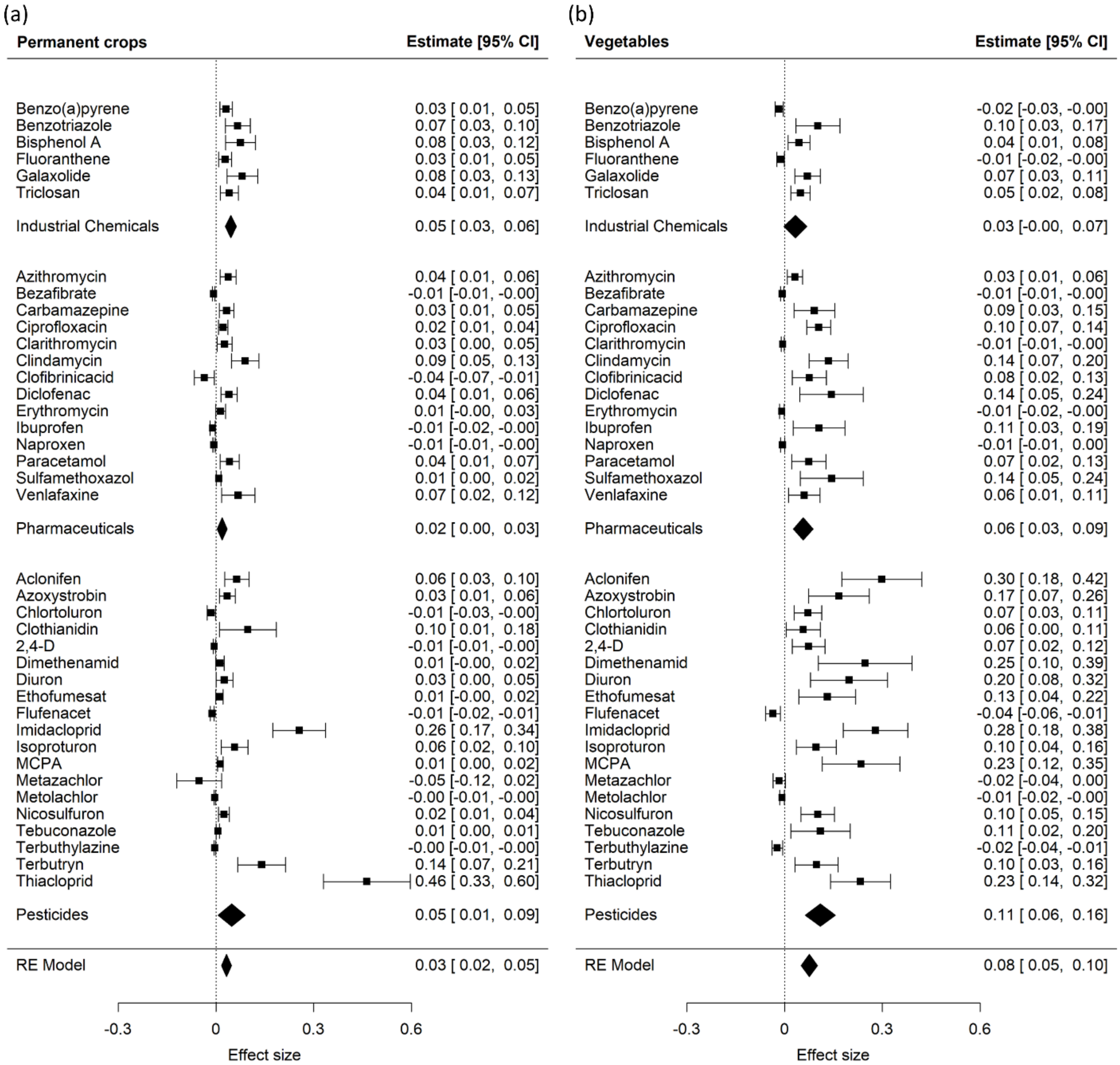

Figure 5: Relationship (effect size) of the proportion of permanent crops (a) and vegetables (b) in the catchment with micropollutant concentrations. Effect sizes represent model fits (pseudo-R^2) derived from bootstrapped (n = 1,000) univariate linear mixed models (LMM) with 95 % confidence intervals indicated in brackets. Negative signs were added to account for negative relationships (i.e., negative regression coefficients) – although R^2 values are positive by definition.

Link between micropollutant concentrations and individual crop types

In contrast to the overall weak effects of the proportion of cropland in the catchment on the majority of micropollutants as described in the previous section, much more pronounced relationships on pesticides were evident for individual crop types. Permanent crops (vineyards, hops and orchards) were strongly related to two insecticides: thiacloprid (R^2 = 0.46) and imidacloprid (R^2 = 0.26.; Figure 5a). Vegetables also showed strong relationships to both insecticides (imidacloprid: R^2 = 0.28, thiacloprid: R^2 = 0.23) and in addition to the herbicides aclonifen ($R^2 = 0.30$) and dimethenamid ($R^2 = 0.25$; Figure 5b). Imidacloprid and thiacloprid were (at the time of the data) approved for, amongst others, applications to fruits and hops (both) and viticulture (imidacloprid), while aclonifen and dimethenamid were approved for various field crops and vegetables (Table 1; BVL, 2023a). Notably, both insecticides imidacloprid and thiacloprid have been banned for (outdoor) use as plant protection product in the EU in 2018 and 2020. Cereals and maize constitute the dominating crop types in this dataset of the Federal State of North Rhine-Westphalia and showed the strongest relationships to flufenacet ($R^2 = 0.29$ and $R^2 = 0.27$, respectively) and nicosulfuron ($R^2 = 0.16$ for both crop types; Figure A4.1, Supplementary Material). These herbicides are approved for field crops including maize (both) and cereals such as winter barley, winter rye, winter soft wheat (only flufenacet; Table 1, BVL, 2023a). Strong relationships were also found between the proportion of strawberry fields and the herbicides dimethenamid ($R^2 = 0.40$) and diuron ($R^2 = 0.33$), and between the proportion of asparagus fields, and the herbicides dimethenamid and MCPA (both $R^2 = 0.33$; Figure A4.2, Supplementary Material). Interestingly, MCPA has been approved only for pome and stone fruits (e.g., apple or peach), but not for other fruits or vegetables (BVL, 2023a). Furthermore, the proportion of onion fields was related to dimethenamid (herbicide, $R^2 = 0.38$), imidacloprid (insecticide, $R^2 = 0.36$) and aclonifen (herbicide, $R^2 = 0.33$; Figure A4.3, Supplementary Material), all of which are approved for – and applied to cultivations of onions (BVL, 2023a). The proportion of oilseeds (e.g., rapeseed, sunflowers) showed comparatively weak relationships with pesticides (max. $R^2 = 0.11$ for 2,4-D; Figure A4.3, Supplementary Material).

Discussion

Micropollutant concentrations exceed regulatory assessment values

Several micropollutants were found to exceed existing regulatory assessment values at multiple sites. Especially, concentrations of pharmaceuticals, the non-steroidal anti-inflammatory drugs diclofenac and ibuprofen, the antibiotic azithromycin as well as concentrations of polycyclic aromatic hydrocarbons, benzo(a)pyrene and fluoranthene, exceeded assessment values, thus indicating a widespread and enhanced ecotoxicological risk for riverine biota (e.g, Beckers et al., 2018; Beek et al., 2016; Markert et al., 2020). For pesticides, ecotoxicological risks were evident for less than 10 % of sites and found only for the insecticide imidacloprid, while other pesticides (thiacloprid, azoxystrobin, nicosulfuron, flufenacet, dimethenamid) exceeded assessment values at less than 5 % of sites. Pesticide risk assessment, however, substantially changed, when risks of chemical mixtures were evaluated, which exceeded the threshold of one (SUM RQ > 1) at 55 % of the sites. Thus, while for pharmaceuticals and industrial chemicals ecotoxicological risk were already driven by single substances, pesticide risks originate primarily from joint mixture risks.

Based on the (mixture) toxicity risk quotients calculated in this study, adverse effects of micropollutant exposure on river biota are very likely. The calculated risks, however, might underestimate actual toxicity risks, because micropollutant monitoring was based on grab sampling. In contrast to high-frequent and event-based monitoring, grab sampling is likely to miss the peak concentrations of micropollutants, pesticides in particular, as they often occur directly after stormwater rain events and with strong seasonal differences (Halbach et al., 2021; Munz et al., 2017; Rumschlag et al., 2019; Weisner et al., 2021; Weisner et al., 2022). When using event-based sampling and increasing sampling efforts (Liess et al., 2021; Rumschlag et al., 2019; Weisner et al., 2022), measured concentrations can exceed concentrations found by grab samples by more than an order of magnitude. Moreover, the detection of (mixture) toxicity risks may also be limited by the selection and number of regularly measured micropollutants and their individual detection limits (Malaj et al., 2014; Moschet et al., 2014; Weisner et al., 2022). Toxicity risk assessment is often biased by missing or left-censored data (i.e. unknown concentrations between zero and the technical limit of quantification; Ohe et al., 2011), which constitutes a main obstacle for multivariate comparisons of effects among sites and in relation to potential sources and biological responses. Despite these sources of uncertainty in the chemical risk assessment, however, our findings confirm those of previous studies (e.g., Finckh et al., 2022; Halbach et al., 2021; Markert et al., 2020): regulatory assessment values for micropollutants are frequently exceeded in the aquatic environment so that freshwater biota are exposed to critical levels of both individual micropollutants and mixtures thereof.

Micropollutant concentrations relate to catchment land uses

Our results point at clear relationships between particular land use types and individual micropollutants as well as micropollutants groups. Cropland was related to pesticide concentrations while relations to pharmaceuticals and industrial chemicals were negligible. This is partly in line with recent studies describing agriculture as a main determinant for pesticide exposure (Szöcs et al., 2017). Previous studies also suggested urban point sources to substantially contribute to pesticide pollution due to the use of pesticides in urban gardens or as biocidal products, for example in façade paints (Münze et al., 2017; Tauchnitz et al., 2020). We, however, found the major part of the monitored pesticides to relate to the proportion of agricultural areas in the catchment, except for terbutryn, which in fact is no longer approved for agricultural use but for biocidal facade paint; thus, this herbicide showed a stronger relationship to proportion of urban areas. Urban areas were found to be strongly associated with individual and mixture risks of pharmaceuticals and industrial chemicals (Bradley et al., 2020; Ebele et al., 2017). Notably, detailed characteristics of urban areas, such as the population density or the proportion of industrial areas, were not specified in this study but may influence the association with micropollutant concentrations (Mandaric et al., 2018). Nonetheless, the proportion of cropland and urban areas in the catchment can apparently explain – and differentiate between – distinct patterns of micropollutant exposure. In contrast, the proportion of forested and grassland areas primarily showed a negative relationship to micropollutants. Despite strong negative correlation between the proportion of forests and cropland (Pearson r = -0.82), this indicates that both forms of extensive land use relate to lower pollution (Dachbrodt-Saaydeh et al., 2021; Goss et al., 2020; Riedo et al., 2022).

Individual pesticide concentrations relate to crop-specific pesticide application

Our findings confirm that individual pesticide concentrations can be linked to individual crop types in the catchment of rivers (Andert et al., 2015; Dachbrodt-Saaydeh et al., 2021; Schürings et al., 2024b). Pesticide concentrations, particularly of insecticides, were strongly related to permanent crops and vegetables, in particular to onion fields. These crop types are associated with intensive pesticide application, in particular with insecticides (Dachbrodt-Saaydeh et al., 2021). Further studies reported a deterioration of riverine biota in agricultural catchments with a high areal coverage of permanent crops, vegetables, vineyards or orchards (Bereswill et al., 2012; Schulz, 2001; Schürings et al., 2024b; Xing et al., 2012). Cereals and maize showed weaker relationships to pesticide concentrations (except for the herbicides flufenacet and nicosulfuron), which suggests a less intensive pesticide application connected to these crop types, except for herbicides (Andert et al., 2015; Roßberg, 2016). Although the uncertainties in the detection of pesticides in our data (see above) prevent us from drawing final conclusions as to the relationship between pesticides, insecticides in particular, and agricultural land uses (Weisner et al., 2022), our findings support the clear demand to distinguish between crop types. The use of rather general categories like 'cropland' in our study showed that relationships between individual herbicides and insecticides, and individual crop types would have been largely overlooked.

Implications for micropollutant risk assessment and management

This study shows that both the proportion of urban and agricultural areas in the catchment of rivers are notably related to the micropollutant exposure in the rivers. Agricultural effects on micropollutant concentrations and joint mixture risks are not uniform and strongly vary between individual crop types. The mere differentiation between cropland and grassland does not adequately represent agricultural stress. Notably, the individual pesticides that were found to be strongly associated with individual crop types largely reflected their approved area of application in Germany (BVL, 2023a). Thus, in the absence of site-specific data on pesticide concentrations, proportion of individual crop types cultivated in the catchment (or at finer scales) may provide a good proxy to inform the assessment of potential toxicity risks (Schürings et al., 2024a). The same areal data could also support the identification of specific pollution sources and the assessment of (mixture) risks of micropollutants in the environment. In order to improve the assessment of (mixture) risks of micropollutants, chemical monitoring programs need to further implement high frequent and event-based monitoring or composite sampling (Bundschuh et al., 2014; Carvalho et al., 2019).

Industrial chemicals and pharmaceuticals were mainly related to proportion of urban areas in the catchment, thus indicating a high relevance of urban point sources, especially wastewater treatment plants (Beek et al., 2016). Therefore, advanced wastewater treatment using ozonation or activated carbon (or a mixture of both) require implementation to reduce the concentrations of micropollutants and hence the ecotoxicological risks originating from them (Bundschuh et al., 2011; Finckh et al., 2022; Kienle et al., 2022; Spilsbury et al., 2024; Triebskorn et al., 2019). However, advanced wastewater treatment cannot remove all micropollutants and neither can it remove the secondary (transformation) products that result, for example, from the ozonation of primary pollutants (Bundschuh et al., 2011).

Intensive agriculture constitutes another major source of micropollutants that imposes strong negative effects on riverine biota (Hughes and Vadas Jr, 2021; Schürings et al., 2022). In contrast to waste water treatment plants, the diffuse pollution (and related ecological risks) from agricultural areas cannot be reduced by selective local measures (Rothe et al., 2021). Instead, agricultural approaches minimizing or eliminating pesticide application, such as integrated pest management, organic farming, agroecology or precision agriculture (Barzman et al., 2015; Gebbers and Adamchuk, 2010; González-Chang et al., 2020; Reganold and Wachter, 2016) are required. Additionally, constructed wetlands, vegetated buffer strips and riparian vegetation have been shown to reduce pesticide exposure in surface waters (Lerch et al., 2017; Stehle et al., 2011; Turunen et al., 2019; Vormeier et al., 2023a). However, these approaches rely on substantial changes in agricultural management and successful implementation of ambitious regulations (Pe'er et al., 2022).

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3 General Discussion

The multi-stressor analyses in this thesis compiled additional evidence of the adverse effects of anthropogenic stressors on riverine ecosystems. The individual studies derived a stressor hierarchy for each of the three organism groups macroinvertebrates, benthic diatoms and fishes and provided detailed discussions on the relative importance of micropollutants in a multi-stressor context (Chapters 2.1, 2.2. and 2.3). Furthermore, anthropogenic sources of the stressors, including WWTP effluents and agricultural activities were analyzed (Chapters 2.3 and 2.4). In the following, the results of these studies and their implications for the development of appropriate management measures are discussed in detail.

3.1 Stressor Hierarchy

Water quality-related stressors showed dominating effects on riverine biota in both multi-stressor datasets compiled for the analyses (Chapter 2.1 and 2.2). Here, physico-chemical variables, including physical water-quality (oxygen and temperature), salt ions and nutrients, caused stronger biological responses than the micropollutant mixtures. In accordance with existing knowledge of the stressor sensitivity and the diagnostic features of the three organism groups (Hering et al., 2006b), differences were observed for the specific stressor responses: diatoms (riverine flora) particularly responded to nutrients, whereas macroinvertebrates and fishes (riverine fauna) showed stronger relative effects of the physical water quality. Stronger effects of physico-chemical variables as compared to the mixture toxicity of micropollutants, morphology and hydrology have also been reported in multi-stressor studies before (Herrero et al., 2018; Lemm et al., 2021; Marzin et al., 2012; Sabater et al., 2016; Valerio et al., 2021). Thus, water quality remains an important stressor for riverine biota, despite improvements in the water quality in recent decades, for example due to increased wastewater treatment (Haase et al., 2023; Pharaoh et al., 2023; Sinclair et al., 2024).

However, the relative effects of the micropollutant mixtures in the multi-stressor context did not reflect the distinct differences in predicted ecotoxicological risks of micropollutants for the three organism groups: Predicted ecotoxicological risks were highest for fishes, mainly caused by widespread exposures to pharmaceuticals for which high environmental concentrations and ecotoxicological risks have often been reported (Castaño-Trias et al., 2023; Finckh et al., 2022; Hernando et al., 2006; Royano et al., 2023; Spilsbury et al., 2024; Waiser et al., 2011a). Risks were also calculated for effects of pesticides, herbicides specifically, on benthic diatoms, whereas the predicted risks for invertebrates were negligible – based on available measured concentrations from WFD-related monitoring programs. Notably, some metrics indicated strong effect of micropollutants, including the Pollution Sensitivity Index and the Swiss Diatom Index for benthic diatoms, partly reflecting the high predicted ecotoxicological risks. Higher effects were also observed for the macroinvertebrate metrics $SPEAR_{pest}$ and %EPT, which are not predicted in the mixture risk assessments, whereas dominant risks for fishes were not reflected in the respective stressor hierarchies. On the one hand, this may indicate that in a multi-stressor context, other variables also influence biological assemblages, potentially superimposing the effects of micropollutant mixtures. Micropollutants may still act as limiting factors for the ecological status in rivers, though (Posthuma et al., 2020). On the other hand, this points at uncertainties in the assessment of environmental concentrations and ecotoxicological risks. For example, monitoring programs using grab samples with a limited temporal sampling frequency have difficulties in capturing short-term exposure and peak concentrations of micropollutants, especially for seasonal and periodic substances such as pesticides (Babitsch et al., 2021; Weisner et al., 2022). The measured concentrations and resulting ecological effects in multi-stressor studies might be higher when considering event-driven monitoring, high-frequent grab or composite sampling as observed in previous studies (Castro-Català et al., 2020; Halbach et al., 2021; Liess et al., 2021; Waite et al., 2021). Furthermore, effects might be underestimated because of the limited number of substances monitored at each sampling site, the lack of sufficient ecotoxicological data at all trophic levels for some substances and species-specific differences in the pollution sensitivity, that may not be reflected in available effect concentrations (Bundschuh et al., 2014; Rico et al., 2016; Spycher et al., 2018; Weisner et al., 2022).

Additionally, ecological metrics may differ in their diagnostic feature for specific stressors as metrics designed to address micropollutants (i.e., Pollution Sensitivity Index and $SPEAR_{pest}$) captured ecological responses to this stressor group. Comparable ecological metrics targeting micropollutants are still rare, especially for fishes (Birk et al., 2012; Heß et al., 2023). Moreover, multi-stressor analyses using individual fish species showed higher relative effects of individual micropollutants, which were at a similar level compared to physico-chemical variables and hydrological condition (Chapter 2.3). In line with the high calculated ecotoxicological risks of pharmaceuticals for this organism group, the result underlines the relevance of micropollutants for the status of fish communities (Meador, 2020; Waite et al., 2021). The stronger responses to micropollutants at species level combined with the overall lower explained variance in the models of fish metrics further suggest that the fish metrics used in Chapter 2.2 may be less suitable for multi-stressor assessments, and point to the need to develop metrics targeting stressor-specific effects on fishes (Birk et al., 2012; Dahm et al., 2013; Gieswein et al., 2017). The misbalance of ecological metrics targeting each individual stressor group may bias the stressor hierarchy and thus the prioritization of relevant stressors for selecting appropriate management measures (Hering et al., 2010; Lemm et al., 2019).

Among the hydro-morphological stressors, hydrological variables showed stronger effects on the three organism groups than the morphological parameters. Especially, alterations of the flow variability and the magnitude, frequency and duration of high and low flow events negatively affect macroinvertebrate, benthic diatom and fish communities (Bower et al., 2022; Kakouei et al., 2017; Laini et al., 2018; Meißner et al., 2019; Stewart-Koster et al., 2011). Thus, alterations of the hydrological regime are important stressors for biological assemblages, which are not adequately addressed by only considering morphological parameters on physical habitat degradation. Hydrological variables describing the river flow regime need to be included in multi-stressor studies to distinguish between effects of hydrological and morphological degradation (Castro-Català et al., 2020; Meißner et al., 2019). Consequently, river basin management also needs to consider alterations of the flow regime since these are closely linked to morphological alterations but might require additional measures (White et al., 2019). Although, morphological alterations, for example river straightening and disconnection of flood plains, were listed among the top stressor groups influencing the ecological status of rivers in Europe (EEA, 2018), effects of morphological parameters were low compared to the other stressor groups for all three organism groups. The gradient of these parameters was small as the morphological condition of most of the sites in the datasets were strongly altered, which could influence the results of the multi-stressor assessments, though (Mack et al., 2022).

3.2 Anthropogenic Sources of Multiple Stressors

The impact of anthropogenic land use, specifically of urban areas including WWTP effluents, agricultural areas including different crop types as well as grasslands and forests, on stressor levels was analyzed in Chapters 2.3 and 2.4. The percentage of municipal WWTP effluents (modelled in relation to the median flow at the point of discharge in the river) showed strong associations with micropollutant concentrations and calculated toxic units. Especially, pharmaceuticals such as diclofenac (nonsteroidal anti-inflammatory drug), sulfamethoxazole (antibiotic), venlafaxine (antidepressant) and carbamazepine (antiepileptic) were positively related to WWTP effluents. These regularly used pharmaceuticals were frequently detected in wastewater-receiving rivers (Fonseca et al., 2020; Ohe et al., 2011; Wolfram et al., 2021) and were associated with high ecotoxicological risks for aquatic organisms, fishes in particular (Bradley et al., 2021; Fonseca et al., 2020; Royano et al., 2023; Spilsbury et al., 2024). Thus, pharmaceuticals were considered to be important and ubiquitous stressors in wastewater impacted rivers (Kay et al., 2017). Similar relationships were observed for the influence of the percentage of urban area on the concentrations of both pharmaceuticals and industrial chemicals, such as bisphenol A (plastics manufacturing) or galaxolide (synthetic fragrance).

The influence of the percentage of urban areas and WWTP effluents on pesticide levels were less pronounced than for other groups of micropollutants but still evident for specific pesticides. Especially, the biocide terbutryn, which is applied as an algicide in facade paint and reaches surface waters via urban stormwater runoff (Burkhardt et al., 2011), showed clear associations with urban areas and WWTP effluents. Nevertheless, different plant protection products, including herbicides (e.g., ethofumesate and chlortoluron), fungicides (e.g., azoxystrobin and tebuconazole) and insecticides (e.g., clothianidin), were also related to the percentage of WWTP effluents and were found in urban and wastewater-impacted areas before (Finckh et al., 2022; Le et al., 2017; Spilsbury et al., 2020; Tauchnitz et al., 2020). Therefore, urban areas and WWTP effluents may constitute important sources of pesticide pollution in surface waters (Finckh et al., 2022; Le et al., 2017; Tauchnitz et al., 2020; Wittmer et al., 2010).

Another significant source of pesticides in the aquatic environment, however, is diffuse pollution from agricultural fields (Halbach et al., 2021; Szöcs et al., 2017; Whelan et al., 2022). The proportion of cropland in the river catchment showed strong individual relationships with herbicides in particular (i.e., flufenacet and nicosulfuron). Further differentiation between individual crop types, such as vegetables or permanent crops (vineyards, hops and orchards), led to stronger associations with pesticide concentrations. Especially insecticides (e.g., thiacloprid and imidacloprid) were strongly related to these crop types, which have been associated with intensive pesticide application (Dachbrodt-Saaydeh et al., 2021). Therefore, distinguishing between cultivation intensities, such as individual crop types, may improve the assessment of agricultural contributions to pesticide pollution and their effects on riverine biota (Schürings et al., 2024a). Notably, a closer assessment of the influence of urban areas on pharmaceutical concentrations by specifically considering the percentage of WWTP effluents led to stronger associations, as well. The relationship with WWTP effluents were distinctly higher than for urban areas, for example for sulfamethoxazole (R^2 of 0.54 vs 0.28), venlafaxine (R^2 of 0.51 vs 0.33) and diclofenac (R^2 of 0.50 vs 0.37). However, these results were derived from linear mixed model analyses of different datasets and therefore, although both included data for the federal state of North Rhine-Westphalia, are only partially suitable for direct comparisons of these associations. Nevertheless, the results show that considering detailed information on stressors and anthropogenic land uses could further improve the assessment of relevant stressor sources than just using data on the percentage of urban or agricultural areas in the river catchments.

In addition to micropollutant concentrations and mixture risks, also other water quality-related variables were influenced by WWTP effluents. Concentrations of oxygen, nutrients and salt ions showed clear relationships with the percentage of WWTP effluents (Castelar et al., 2022; Kinouchi et al., 2007; Müller and Gächter, 2012; Waiser et al., 2011b). For the hydro-morphological condition, the influence of WWTP effluents was particularly pronounced for hydrological variables, especially flow variability and high flow frequency. Hydro-morphological alterations caused by WWTPs have been previously reported, particularly for intensive rain events leading to flash floods and combined sewer overflows, i.e. discharge of untreated rain- and wastewater into the rivers (Canobbio et al., 2009; Uhl and Dittmer, 2005).

Besides agriculture and urban areas there is a large number of further anthropogenic impacts influencing stressor levels and effects in rivers. Both forests and grasslands showed negligible effects on micropollutant concentrations in this study (Chapter 2.4). Other sources, such as industrial areas, have not been specifically addressed here, but may be important influencing factors, for example lignite mining activities in the Erft and Niers catchments (Chapter 2.1).

3.3 Implications for Environmental Monitoring and Management

Water quality deterioration is still a dominant stressor for the ecological status of macroinvertebrates, benthic diatoms and fishes. Additionally, the results revealed high ecological effects of hydrological alterations. For river basin management this emphasizes that despite considerable WWTP improvements and restoration efforts addressing water quality and hydro-morphological stressors in the past decades, both the water quality and the hydrological conditions still need to be addressed in future management measures. Although the habitat quality is certainly not irrelevant for the ecological integrity, further measures focusing on improving the morphological condition alone will most likely not suffice for reaching a good ecological status as long as water quality and hydrological conditions remain deficient (Brettschneider et al., 2019; Brettschneider et al., 2023; Palmer et al., 2010; Sundermann et al., 2013). This is particularly important in the face of additional emerging environmental pressures including climate change, which is predicted to further reduce average annual flows while increasing seasonal extreme events such as droughts and floods (Guerreiro et al., 2018; O'Briain, 2019). Thus, river basin management needs to adapt an evidence-based development of management measures using available information on important stressors and needs to address both already known stressors such as nutrients and emerging stressors including hydrological alterations and micropollutants (Dudgeon et al., 2006; Haase et al., 2023; Reid et al., 2019).

Urban areas including WWTP effluents negatively affected the water quality, both micropollutants and physico-chemical variables, and the hydrological condition in surface waters. Management measures addressing these point sources are therefore needed, such as advanced wastewater treatment. Different processes, for example ozonation, active carbon treatment and membrane filtration, have been shown to reduce the concentrations and consequently the ecotoxicological risks of micropollutants (Finckh et al., 2022; Kienle et al., 2022; Spilsbury et al., 2024; Triebskorn et al., 2019; Wolf et al., 2022; Yang et al., 2017). Reducing the negative impact of combined sewer overflows on water quality and hydrological condition necessitates additional measures, which could include, for example, constructed wetlands to treat and control waste- and stormwater or flow control systems and separation of unpolluted surfaces from combined sewer systems to reduce the amount of water (Köster et al., 2023; Rizzo et al., 2018; Uhl and Dittmer, 2005; Wang et al., 2021; Wolf et al., 2022). Furthermore, agricultural activities distinctly contribute to water quality deterioration and negatively affect riverine biota (Haase et al., 2023; Hughes and Vadas Jr, 2021; Schürings et al., 2022). Reducing diffuse pollution from agricultural fields requires additional management measures (Neale et al., 2017; Rothe et al., 2021). For example, vegetated buffer strips, constructed wetlands and riparian vegetation can reduce pesticide and nutrient runoff into surface waters (Lerch et al., 2017; Palt et al., 2023; Stehle et al., 2011; Turunen et al., 2019; Vormeier et al., 2023). However, reducing the adverse effects of pesticides in aquatic environments will further require a substantial reduction in pesticide applications, replacement of hazardous substances and refinement of agricultural practices, such as the implementation of precision agriculture, integrated pest management or agro-ecology (Barzman et al., 2015; Gebbers and Adamchuk, 2010; González-Chang et al., 2020; Silva et al., 2022).

Anthropogenic sources and their impacts on both stressor levels and ecological effects are generally highly context-dependent since both the biotic and abiotic conditions can distinctly vary in different catchments, though (Burdon et al., 2016; Clements et al., 2012). A multitude of different environmental factors can have detrimental effects on aquatic ecosystems, not all of which have been considered in detail here. For example, river sediments can act as a sink or a source for micropollutants and thus affect the water quality in addition to pollution of the water phase included in the analyses (Baat et al., 2019b; Moran et al., 2017; Müller et al., 2021). Additionally, sediment characteristics and the associated condition of the interstitial space are important factors for the habitat quality (Blöcher et al., 2020; Lemm and Feld, 2017; Villeneuve et al., 2015). Therefore, large-scale analyses of the sources and effects of multiple stressors can help to derive general information on relevant environmental factors and suitable management measures, but both need to be considered in integrated assessments of specific catchments in order to derive targeted management measures (Schuwirth et al., 2018).

Data-driven decisions on management measures require sufficient information on the ecological condition and environmental stressors und thus, necessitate extensive monitoring (Carvalho et al., 2019). As stressor responses varied between different organism groups and between individual (fish) species (Chapters 2.2 and 2.3), all three BQEs and different metrics need to be considered in the identification of relevant stressors and effective management measures (Lemm et al., 2019; Marzin et al., 2012; Sinclair et al., 2024). Especially, metrics reflecting the sensitivity to a particular stressor responded strongly to that variable, consistent with the stressor-specific design of the metric. This was for example the case for a variety of metrics for benthic diatoms, including the Trophic Index for nutrients, the Halobian Index for salinization, the Rheoindex for hydrological alteration or the Pollution Sensitivity Index for micropollutants. Thus, sensitivity metrics as well as functional trait-based metrics (e.g., macroinvertebrates feeding types or fish spawning habitat preferences), which responded particularly to hydro-morphological degradation and physical water quality (Vitecek et al., 2021), are well suited for stressor-specific diagnosis of ecological impairment (Enns et al., 2023; Worischka et al., 2023). In contrast, abundance and diversity metrics often fail to reflect community shifts from sensitive to tolerant taxa as well as alterations of the functional community composition (Enns et al., 2023; Schürings et al., 2022; Sinclair et al., 2024; Worischka et al., 2023). However, targeted metrics are not equally available for all stressors and organism groups (cf. Chapter 3.1; Birk et al., 2012; Carvalho et al., 2019; Poikane et al., 2020). The identification of relevant stressors and the selection of management measures requires a considered choice of metrics for each BQE and the development of additional stressor-specific metrics for future assessments (Birk et al., 2012; Lemm et al., 2019; Sinclair et al., 2024; von der Ohe et al., 2009).

The compilation and analyses of the multi-stressor datasets in this thesis revealed both the advantages and the difficulties of using available environmental monitoring data for multi-stressor analyses. Since the implementation of the WFD, extensive datasets on different BQEs and a wide range of environmental stressors have been compiled and are readily available for analyses. However, the spatial and temporal aggregation of datasets, especially for chemical and biological data, poses a critical challenge for the compilation of multi-stressor datasets and often resulted in large data gaps (Arenas-Sánchez et al., 2019; De Zwart et al., 2009). Thus, there is a need to enhance the spatial and temporal consistency and resolution of data for monitoring programs of different stressors (Carvalho et al., 2019). For micropollutants, due to the myriad of different substances occurring in the aquatic environment, the number and the selection of substances measured varied distinctly between the sampling sites (De Zwart et al., 2009; Weisner et al., 2022). This limitation in the number of micropollutants monitored as well as limitations in the monitoring design, such as the frequency of grab samples or the analytical capacities, may lead to an underestimation of micropollutant concentrations and risks (cf. Chapter 3.1; Babitsch et al., 2021; Spycher et al., 2018; Weisner et al., 2022). Consequently, high-frequent and event-based sampling as well as additional composite samples may improve the assessment of micropollutant concentrations and ecotoxicological risks (Castro-Català et al., 2020; Halbach et al., 2021; Szöcs et al., 2017). Furthermore, advanced monitoring methods, such as effect-based methods (i.e., bioassays), could be implemented to capture ecotoxicological effects of micropollutants independently of the described systematic or analytical limitations of the chemical monitoring of micropollutants (Baat et al., 2019a; Könemann et al., 2018; Neale et al., 2020). Since an expanded monitoring would require extra effort and resources, these approaches may be implemented as part of the investigative monitoring in WFD monitoring programs to specifically analyze micropollutants at selected sites, for example to further diagnose the cause of biological deterioration or before and after the implementation of management measures to assess their (expectable) effectiveness (Carvalho et al., 2019). Furthermore, the high relevance of hydrological alterations for riverine biota in this thesis highlights the importance of considering hydrological conditions (i.e., the magnitude, timing, duration and frequency of flow events as well as the flow variability) alongside the assessment of habitat conditions in hydro-morphological surveys, for example using the Indicators of Hydrological Alteration derived from time-series data from available gauging stations or hydrological models (Kakouei et al., 2017; Meißner et al., 2019; Olden and Poff, 2003).

All in all, much has already been accomplished since adopting the WFD and implementing the first monitoring programs and river basin management plans. Many aspects, including the chemical pollution, have been considerably improved (Haase et al., 2023; Pharaoh et al., 2023; Whelan et al., 2022). However, given the continuing relevance of water quality deterioration for riverine biota in this study, the influence of emerging pressures including climate change and micropollutants, the stagnating recovery of freshwater biodiversity and the slow improvement of the ecological status (Carvalho et al., 2019; Haase et al., 2023; Reid et al., 2019; Vaughan, 2023), challenges remain for environmental monitoring and management. Therefore, targeted assessments of multiple stressors, especially considering water quality deterioration and hydrological alterations, and evidence-based developments of management measures are required to ensure an integrated and sustainable water resource management as well as to achieve and maintain a good ecological status by 2027 and beyond.

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5 Supplementary Information

The supplementary information of the scientific articles has been slightly modified in order to ensure a clear and consistent presentation.

1. The hierarchy of multiple stressors' effects on benthic invertebrates: a case study from the rivers Erft and Niers, Germany

Table S1: Land use characteristics in the Erft and Niers catchment.

	Agriculture Intensive%	Urban% [1]	Cumulative Percentage of Wastewater [2]
Mean	0.47	0.22	0.47
Median	0.50	0.20	0.60
Min	0.10	0.08	0.00
Max	0.73	0.45	1.00
Mean Erft	0.43	0.14	0.50
Median Erft	0.50	0.14	0.61
Min Erft	0.10	0.08	0.00
Max Erft	0.73	0.24	0.87
Mean Niers	0.50	0.29	0.44
Median Niers	0.50	0.28	0.60
Min Niers	0.40	0.11	0.00
Max Niers	0.64	0.45	1.00

[1] The percentage of urban area includes both urban and industrial area as well as lignite mining.

[2] The cumulative percentage of wastewater was calculated on the basis of the yearly mean inflow of wastewater from municipal wastewater treatment plants at the point of discharge including upstream points of discharges, related to 0.5 MNQ.

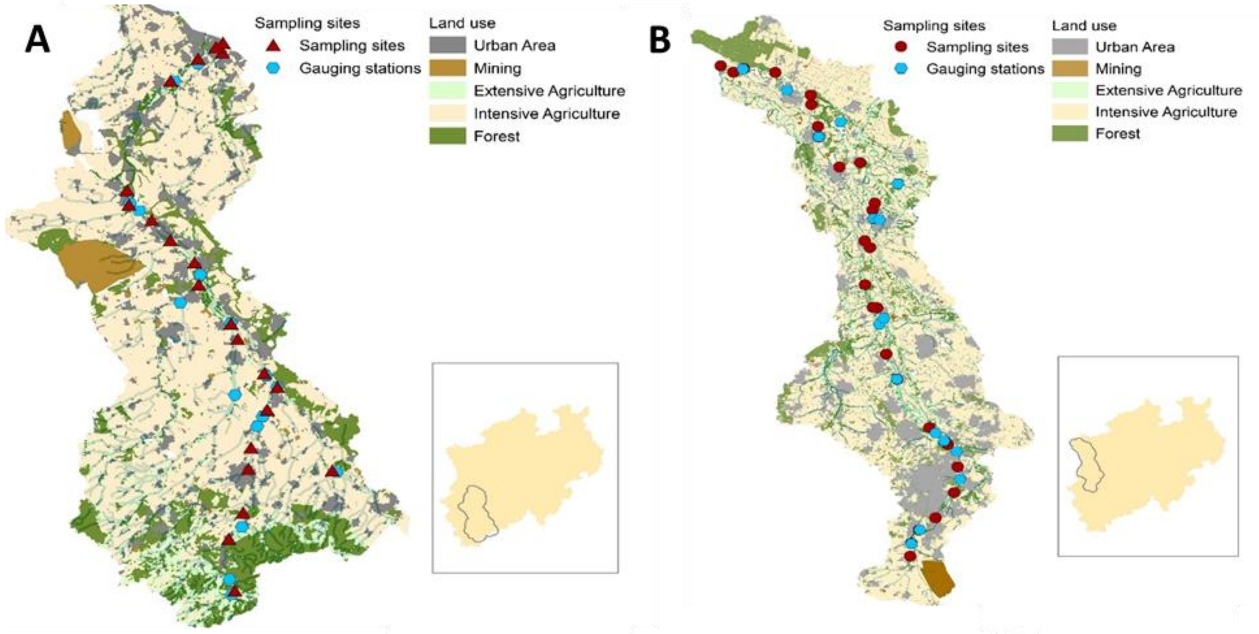

Figure S1.1: Map of the sampling sites of benthic invertebrates, gauging stations as well as land use characteristics in the Erft catchment (A) and the Niers catchment (B) (©Data licence Germany-Zero-Version 2.0).

Table S2: Spearman Correlation rho (> |0.5|) between land use variables (percentages of intensive agriculture and urban area in the catchment, percentage of WWTP discharges) and stressor variables as well as macroinvertebrate metrics.

Stressors														
Erft and Niers	TN	NH4-N	NO2-N	TP	Fe	Cl	T	O2	SO4	RQ $_{mix,acute}$	RQ $_{mix,chr}$	HP1	HP2	fh5
Intensive Agriculture														
Urban Area														
WWTP Discharges		0.51	0.58	0.59		0.60	0.56			0.64	0.75			
Erft														
Intensive Agriculture												0.59	0.52	
Urban Area	-0.61			0.59	0.80				0.51				0.72	
WWTP Discharges				0.64	0.50		0.63							0.63
Niers														
Intensive Agriculture		-0.61	-0.60			-0.56				-0.57	-0.60			
Urban Area	-0.63				-0.56									0.85
WWTP Discharges		0.80	0.81	0.60		0.90	0.55	-0.51		0.87	0.84			

Metrics														
Erft and Niers	Abundance	Nb Taxa	Evenness	EQC	MMI	GSI	IBR	ASPT	Nb EPTCBO	Graz%	SPEAR $_{pest}$	Rheo	Alien%	KLIWA
Intensive Agriculture									-0.51					
Urban Area										-0.77				
WWTP Discharges														
Erft														
Intensive Agriculture		-0.60		0.64	-0.53	0.77	0.73	-0.62	-0.76		-0.54	-0.75	0.82	0.70
Urban Area		-0.81	-0.50			0.63	0.72	-0.52	-0.77	-0.68		-0.48	0.64	0.68
WWTP Discharges														
Niers														
Intensive Agriculture														
Urban Area	0.52													
WWTP Discharges													0.60	0.53

Table S3: Statistical key parameters of all stressor variables for the complete dataset as well as for each catchment.

	TN	NH4-N	NO2-N	TP	Cl	Fe	T	O2	SO4	$RQ_{mix, acute}$	$RQ_{mix, chr}$	HP1	HP2	HP4	HP5	fh5	dl16	ra5	MQ MNQ
Mean	4.38	0.12	0.04	0.13	72.67	0.91	20.48	8.03	83.83	0.23	0.77	6.24	5.38	5.32	5.68	19.05	8.89	0.38	3.20
Median	4.65	0.11	0.04	0.14	76.60	0.92	20.70	8.10	91.39	0.18	0.73	6.00	5.00	5.00	6.00	20.50	5.18	0.38	2.47
Min	0.50	0.03	0.01	0.02	17.38	0.15	12.50	5.70	24.39	0.06	0.20	4.00	3.00	3.00	3.00	4.00	3.15	0.27	1.54
Max	7.91	0.37	0.13	0.24	237.63	2.53	24.90	10.20	252.00	1.29	1.98	7.00	6.50	7.00	7.00	41.00	36.72	0.49	13.67
Mean Erft	4.66	0.10	0.04	0.12	88.07	0.79	20.33	8.50	83.29	0.35	0.76	6.16	5.25	5.23	5.68	20.61	6.49	0.38	4.06
Median Erft	4.90	0.07	0.05	0.13	85.36	0.37	20.65	8.40	83.34	0.27	0.71	6.00	5.00	5.00	6.00	22.00	4.69	0.37	3.04
Min Erft	0.50	0.03	0.01	0.02	17.38	0.15	12.50	6.80	24.39	0.07	0.63	4.50	3.00	4.00	3.00	7.50	3.15	0.27	2.08
Max Erft	7.91	0.25	0.09	0.20	237.63	2.53	24.60	10.20	252.00	1.29	1.17	7.00	6.50	7.00	6.50	31.50	15.45	0.49	13.67
Mean Niers	4.09	0.14	0.05	0.14	60.11	1.00	20.74	7.47	84.26	0.14	0.77	6.31	5.48	5.39	5.69	17.78	10.84	0.39	2.50
Median Niers	4.35	0.12	0.04	0.13	66.08	1.00	21.20	7.74	94.14	0.14	0.76	6.00	6.00	5.50	6.00	20.00	6.85	0.38	1.87
Min Niers	0.06	0.05	0.01	0.04	21.25	0.43	13.60	5.70	41.29	0.06	0.20	4.00	3.00	3.00	3.00	4.00	3.54	0.35	1.54
Max Niers	7.78	0.37	0.13	0.24	91.11	2.03	24.90	9.70	106.32	0.31	1.98	7.00	6.00	7.00	7.00	41.00	36.72	0.44	8.29

Table S3.1: Statistical key parameters of the RQ_{mix} calculated for the organism groups algae and fishes. In addition to the mixture risks for benthic invertebrates, risks for algae and fishes were calculated using the same procedure but ecotoxicological effect concentrations for the respective organism groups.

	$RQ_{mix, acute}$ (Algae)	$RQ_{mix, chr}$ (Algae)	$RQ_{mix, acute}$ (Fish)	$RQ_{mix, chr}$ (Fish)
Mean	20.65	2.53	0.07	10.09
Median	14.38	2.71	0.06	8.61
Min	4.34	1.26	0.01	0.01
Max	58.17	4.81	0.30	31.80

Table S4: Overview of all 42 micropollutants analyzed. Summary statistics and number of detections of micropollutants analyzed in the Erft and Niers catchment in 2016/2017, as well as ecotoxicological effect concentrations used for calculation of toxic units and RQ_{mix}. Ecotoxicological data were derived from online databases (i.e., UBA ETOX, USUS EPA ECOTOX, ECHA information on chemicals or Pesticides Properties DataBase). Data were last updated in 01/2021.

Summary Statistics						
	CAS	**Median [µg/L]**	**Mean [µg/L]**	**Max [µg/L]**	**Min [µg/L]**	**%Detects**
2,4-D	94-75-7	0.03	0.03	0.13	0.01	47 %
Atenolol	29122-68-7	0.03	0.04	0.28	0.01	97 %
Atrazine	1912-24-9	0.03	0.03	0.07	0.01	57 %
Bentazon	25057-89-0	0.01	0.02	0.57	0.01	47 %
Benzotriazole	95-14-7	1.00	1.94	16.00	0.01	96 %
Bezafibrate	41859-67-0	0.03	0.04	0.30	0.01	97 %
Bisphenol A	80-05-7	0.01	0.03	1.40	0.01	47 %
Carbamazepine	298-46-4	0.12	0.19	1.40	0.01	98 %
Chloridazon	1698-60-8	0.03	0.03	0.54	0.01	100 %
Chlortoluron	17254-80-7	0.03	0.04	1.90	0.01	100 %
Clarithromycin	81103-11-9	0.10	0.10	1.80	0.01	89 %
Clofibric acid	882-09-7	0.01	0.02	0.05	0.01	97 %
DEET	134-62-3	0.02	0.03	0.33	0.01	48 %
Diclofenac	15307-86-5	0.26	0.40	3.60	0.01	97 %
Dimethenamid	87674-68-8	0.03	0.04	0.73	0.01	48 %
Diuron	330-54-1	0.03	0.03	0.28	0.01	100 %
Erythromycin	114-07-8	0.10	0.08	0.47	0.01	85 %
Ethofumesate	26225-79-6	0.03	0.03	0.75	0.01	100 %
Flufenacet	142459-58-3	0.03	0.04	0.70	0.00	48 %
Ibuprofen	15687-27-1	0.01	0.02	0.43	0.01	97 %
Imidacloprid	138261-41-3	0.03	0.03	0.10	0.01	74 %
Isoproturon	34123-59-6	0.03	0.06	4.40	0.01	100 %
Linuron	330-55-2	0.03	0.03	0.24	0.01	100 %
MCPA	94-74-6	0.01	0.03	0.50	0.01	47 %
Metoprolol	37350-58-6	0.23	0.30	2.40	0.01	97 %
Metazachlor	67129-08-2	0.03	0.03	0.53	0.01	100 %
Mecoprop	7085-19-0	0.01	0.02	0.10	0.01	47 %
Metamitron	41394-05-2	0.03	0.05	1.50	0.01	100 %
Metolachlor	51218-45-2	0.03	0.03	0.15	0.01	100 %
Metribuzin	21087-64-9	0.03	0.04	3.70	0.01	100 %
Naproxen	22204-53-1	0.02	0.04	0.28	0.01	97 %
Metformin	657-24-9	0.47	0.62	3.30	0.03	44 %
Propiconazol	139-40-2	0.03	0.03	0.10	0.01	48 %

Table S4 (continued)

	CAS	Median [µg/L]	Mean [µg/L]	Max [µg/L]	Min [µg/L]	%Detects
Prosulfocarb	52888-80-9	0.03	0.03	0.52	0.01	48 %
Quinmerac	90717-03-6	0.03	0.05	1.10	0.01	47 %
Sulfamethoxazole	723-46-6	0.03	0.09	0.60	0.01	97 %
TCPP	13674-84-5	0.24	0.27	1.80	0.01	47 %
Tebuconazole	107534-96-3	0.03	0.04	0.28	0.01	48 %
Terbutryn	5915-41-3	0.03	0.04	0.40	0.01	48 %
Terbuthylazin	886-50-0	0.03	0.03	0.25	0.01	100 %
Triclosan	3380-34-5	0.01	0.01	0.04	0.01	47 %
Trimethoprim	738-70-5	0.03	0.03	0.27	0.01	97 %

Effect concentrations													
	Acute Toxicity						**Chronic Toxicity**						
	Algae		**Benthic Invertebrates**		**Fishes**		**Algae**		**Benthic Invertebrates**		**Fishes**		
	EC_{50} [mg/L]	**Species**	**EC_{50} [mg/L]**	**Species**	**EC_{50} [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**Reference**
2,4-D	24.200	*Raphidocelis subcapitata*	134.200	*Daphnia magna*	100.000	*Pimephales promelas*	39.000	*Raphidocelis subcapitata*	46.200	*Daphnia magna*	63.400	*Pimephales promelas*	EFSA 2014, PPDB
Atenolol	110.000	*Raphidocelis subcapitata*	33.400	*Ceriodaphnia dubia*	100.000	*Oncorhynchus mykiss*	10.000	*Raphidocelis subcapitata*	1.480	*Daphnia magna*	3.200	*Pimephales promelas*	AstraZeneka Risk Assessment 2016, VSDB, EQS Dossier Oekotoxzentrum CH 2015
Atrazine	0.059	*Raphidocelis subcapitata*	85.000	*Daphnia magna*	4.500	*Oncorhynchus mykiss*	0.084	*Raphidocelis subcapitata*	0.250	*Daphnia magna*	2.000	*Oncorhynchus mykiss*	PPDB, EQS Draft 2002
Bentazon	21.300	*Raphidocelis subcapitata*	58.000	*Daphnia magna*	127.000	*Oncorhynchus mykiss*	9.890	*Raphidocelis subcapitata*	32.000	*Daphnia magna*	9.000	*Pimephales promelas*	EFSA 2015 peer review Document, ECHA CLH 2019, PPDB, KEMI Report 2008, EQS Oekotoxzentrum CH 2016
Benzo-triazole	189.000	*Desmodesmus subspicatus*	15.800	*Daphnia galeata*	38.000	*Danio rerio*	1.180	*Desmodesmus subspicatus*	0.970	*Daphnia magna*			Ökotoxzentrum EQS Dossier 2016
Beza-fibrate	222.600	*Desmodesmus subspicatus*	0.130	*Cereodaphnia dubia*	171.500	*Danio rerio*	100.000	*Desmodesmus subspicatus*	0.023	*Cereodaphnia dubia*	112.000	*Oncorhynchus mykiss*	EQS Draft 2015 UBA
Bisphe-nol A	2.800	*Raphidocelis subcapitata*	3.900	*Daphnia galeata*	4.600	*Pimephales promelas*	1.360	*Raphidocelis subcapitata*	3.150	*Daphnia magna*	0.016	*Pimephales promelas*	EU EQS Draft 2015
Carba-mazepine	74.000	*Desmodesmus subspicatus*	67.500	*Daphnia magna*	19.900	*Oncorhynchus mykiss*	0.520	*Raphidocelis subcapitata*	0.025	*Ceriodaphnia dubia*	0.862	*Pimephales promelas*	Oekotoxzentrum EQS 2016, GDCh 4/2016, EQS Sheet Oekotoxzentrum CH 2016
Chlorida-zon	0.600	*Raphidocelis subcapitata*	132.000	*Daphnia magna*	34.000	*Oncorhynchus mykiss*	0.100	*Selenastrum bibraianum*	6.230	*Daphnia magna*	3.160	*Oncorhynchus mykiss*	EFSA 2007 peer review Dokument, PPDB, KEMI Report 2008, EQS Dossier Oekotoxzentrum CH 2016
Chlortolu-ron	0.009	*Raphidocelis subcapitata*	67.000	*Daphnia magna*	20.000	*Oncorhynchus mykiss*	0.001	*Scenedesmus quadricauda*	16.700	*Daphnia magna*	0.400	*Oncorhynchus mykiss*	PPDB, INERIS, EQS Dossier Oekotoxzentrum CH 2016

Table S4 (continued)

	Acute Toxicity						Chronic Toxicity						
	Algae		Benthic Invertebrates		Fishes		Algae		Benthic Invertebrates		Fishes		
	EC_{50} [mg/L]	**Species**	**EC_{50} [mg/L]**	**Species**	**EC_{50} [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**Reference**
Clari-thromycin	0.002	*Raphidocelis subcapitata*	18.660	*Ceriodaphnia dubia*	2.000	*Oncorhynchus mykiss*	0.002	*Raphidocelis subcapitata*	0.003	*Daphnia magna*			EQS proposal Oekotoxzentrum 2016, GDCH 4/16, UBA report 61/2017
Clofibric acid	145.000	*Desmodesmus subspicatus*	75.000	*Daphnia magna*			75.000	*Desmodesmus subspicatus*	0.640	*Ceriodaphnia dubia*	0.010	*Oncorhynchus mykiss*	UBA Risk Assessment 2004, LAWA 2004, QSAR Toolbox
DEET	41.000	*Raphidocelis subcapitata*	75.000	*Daphnia magna*	97.000	*Oncorhynchus mykiss*	3.800	*Raphidocelis subcapitata*	7.200	*Daphnia magna*			EU Biocide risk assessment 2010, EQS Dossier Oekotoxzentrum CH 2016
Diclo-fenac	135.400	*Raphidocelis subcapitata*	22.430	*Daphnia magna*	71.000	*Cyprinus carpio*	25.000	*Raphidocelis subcapitata*	8.300	*Daphnia magna*	0.001	*Oncorhynchus mykiss*	UBA EQS Proposal 2011/2018, UBA Text 44/2017, Islas-Flores et al., 2013, Lee et al. 2011, Saucedo-Vence et al. 2014
Dime-thenamid	0.025	*Monoraphidium griffithii*	3.200	*Daphnia magna*	2.600	*Oncorhynchus mykiss*	0.003	*Monoraphidium griffithii*	0.680	*Daphnia magna*	0.120	*Oncorhynchus mykiss*	UBA UQN 2017, EQS Dossier Oekotoxzentrum CH 2019, PPDB
Diuron	0.003	*Synechococcus sp.*	1.400	*Daphnia magna*	6.700	*Cyprinodon variegatus*	0.001	*Synechococcus sp.*	0.096	*Daphnia magna*	0.410	*Oncorhynchus mykiss*	PPDB, EQS Datasheet EU 2005, Oekotoxzentum CH 2016, CLH report Diuron
Erythro-mycin	0.020	*Raphidocelis subcapitata*	10.230	*Ceriodaphnia dubia*	61.000	*Pimephales promelas*	0.010	*Raphidocelis subcapitata*	0.248	*Daphnia magna*	10.000	*Oryzias latipes*	UBA EQS Draft 2015, EQS Dossier Oekotoxzentrum CH 2011
Etho-fumesate	3.900	*Raphidocelis subcapitata*	14.000	*Daphnia magna*	3.623	*Oncorhynchus mykiss*	0.031	*Raphidocelis subcapitata*	0.320	*Daphnia magna*	0.156	*Danio rerio*	EFSA 2016 peer review Dokument, PPDB, EQS Dossier Oekotoxzentrum CH 2016, Kemi Report 2008
Flufe-nacet	0.002	*Raphidocelis subcapitata*	30.900	*Daphnia magna*	2.130	*Lepomis macrochirus*	0.000	*Raphidocelis subcapitata*	3.260	*Daphnia magna*	0.179	*Oncorhynchus mykiss*	PPDB, EQS Dossier UBA 2015, EQS Dossier Oekotoxzentrum CH 2017
Ibuprofen	315.000	*Raphidocelis subcapitata*	34.100	*Daphnia magna*	5.000	*Lepomis macrochirus*	35.000	*Chlorella vulgaris*	0.615	*Daphnia magna*	0.000	*Danio rerio*	EQS Draft 2016
Imida-cloprid	389.000	*Desmodesmus subspicatus*	0.119	*Asellus aquaticus*	211.000	*Oncorhynchus mykiss*	10.000	*Desmodesmus subspicatus*	0.001	*Asellus aquaticus*	1.200	*Oncorhynchus mykiss*	EFSA 2014, RIVM Report 2014, UBA EQS Datasheet 2014
Isopro-turon	0.098	*Raphidocelis subcapitata*	0.580	*Daphnia magna*	37.220	*Oncorhynchus mykiss*	0.018	*Raphidocelis subcapitata*	0.064	*Daphnia magna*	1.000	*Oncorhynchus mykiss*	PPDB, EFSA Conclusion Document 2015, KEMI Report 2008
Linuron	0.002	*Scenedemus subspicatus*	0.120	*Daphnia magna*	3.150	*Oncorhynchus mykiss*	0.006	*Scenedemus subspicatus*	0.180	*Daphnia magna*	0.100	*Oncorhynchus mykiss*	PPDB, EQS Draft UK 2007, EFSA Peer review 2016
MCPA	18.400	*Raphidocelis subcapitata*	155.000	*Daphnia magna*	41.000	*Oncorhynchus mykiss*	8.900	*Pseudokirchneriella subcapitata*	13.000	*Daphnia magna*	15.000	*Pimephales promelas*	EQS Proposal Oekotoxzentrum CH 2016, KEMI Report 2008
Meto-prolol	1.800	*Scenedesmus subspicatus*	8.800	*Ceriodaphnia dubia*	130.000	*Oncorhynchus mykiss*	0.430	*Scenedesmus subspicatus*	3.200	*Daphnia magna*	18.700	*Oncorhynchus mykiss*	EQS proposal Oekotoxzentrum 2016, UBA EQS Draft 2015 UBA, UBA 61/2017

Table S4 (continued)

	Acute Toxicity						Chronic Toxicity						
	Algae		Benthic Invertebrates		Fishes		Algae		Benthic Invertebrates		Fishes		
	EC_{50} [mg/L]	**Species**	**EC_{50} [mg/L]**	**Species**	**EC_{50} [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**EC_{10}/ NOEC [mg/L]**	**Species**	**Reference**
Metazachlor	0.031	*Desmodesmus subspicatus*	33.348	*Daphnia magna*	4.400	*Oncorhynchus mykiss*	0.002	*Desmodesmus subspicatus*	0.100	*Daphnia magna*	2.318	*Oncorhynchus mykiss*	RIVM EQS proposal 2013, EQS Ökotoxzentrum CH 2015, EFSA 2007 peer review 2008, EFSA DAR 2005, KEMI Report 2008
Mecoprop	122.000	*Scenedesmus subspicatus*	91.000	*Daphnia magna*	69.000	*Oncorhynchus mykiss*	27.000	*Pseudokirchneriella subcapitata*	22.200	*Daphnia magna*	11.100	*Oncorhynchus mykiss*	PPDB, EQS Proposal 2010 UK, KEMI Report 2008
Metamitron	0.140	*Selenastrum capricornutum*	25.373	*Daphnia magna*	194.000	*Cyprinus carpio*	0.103	*Selenastrum capricornutum*	17.889	*Daphnia magna*	3.200	*Oncorhynchus mykiss*	EFSA peer review 2008, EFSA DAR 2007, EQS Dossier Metamitron 2016
Metolachlor	0.050	*Raphidocelis subcapitata*	13.000	*Daphnia magna*	3.900	*Oncorhynchus mykiss*	0.025	*Raphidocelis subcapitata*	0.354	*Daphnia magna*	1.000	*Cyprinodon variegatus*	PPDB, QSAR Toolbox, ECOSAR
Metribuzin	0.021	*Raphidocelis subcapitata*	49.300	*Daphnia magna*	77.400	*Oncorhynchus mykiss*	0.003	*Raphidocelis subcapitata*	0.640	*Daphnia magna*	4.430	*Oncorhynchus mykiss*	EQS Draft Oekotoxzentrum CH 2016, EFSA 2005/2006, KEMI Report 2008, CLP 2020
Naproxen	21.000	*Desmodesmus subcapitata*	37.000	*Daphnia magna*	57.000	*Oncorhynchus mykiss*	7.100	*Raphidocelis subcapitata*	0.085	*Ceriodaphnia dubia*	1.000	*Pimephales promelas*	EQS Dossier Oekotoxzentrum CH 2015, AstraZeneka Risk Assessment 2016
Metformin	99.000	*Raphidocelis subcapitata*	64.000	*Daphnia magna*	110.000	*Lepomis macrochirus*	78.000	*Raphidocelis subcapitata*	10.000	*Daphnia magna*	10.000	*Pimephales promelas*	EQS proposal Oekotoxzentrum CH 2016, QSAR Toolbox
Propiconazol	0.390	*Raphidocelis subcapitata*	1.150	*Daphnia magna*	0.830	*Oncorhynchus mykiss*	0.007	*Raphidocelis subcapitata*	0.310	*Daphnia magna*	0.430	*Pimephales promelas*	JRC EQS Draft 2015, KEMI 2008
Prosulfocarb	0.038	*Desmodesmus subspicatus*	0.510	*Daphnia magna*	0.840	*Oncorhynchus mykiss*	0.048	*Selenastrum capricornutum*	0.045	*Daphnia magna*	0.310	*Oncorhynchus mykiss*	EFSA Peer Review 2007, PPDB, KEMI 2008
Quinmerac	244.000	*Raphidocelis subcapitata*	148.700	*Daphnia magna*	86.800	*Oncorhynchus mykiss*	82.000	*Raphidocelis subcapitata*	1.000	*Daphnia magna*	3.160	*Oncorhynchus mykiss*	EFSA peer review 2010, EFSA DAR 2007, KEMI Report 2008
Sulfamethoxazole	0.520	*Raphidocelis subcapitata*	123.100	*Daphnia magna*	890.000	*Pimephales promelas*	0.090	*Raphidocelis subcapitata*	0.006	*"Crustaceans"*	0.010	*"Fish"*	UBA EQS Datasheet 2015, GDCh 04/2016
TCPP	4.500	*Raphidocelis subcapitata*	1.600	*Daphnia magna*	1.100	*Oncorhynchus mykiss*	2.300	*Raphidocelis subcapitata*	0.500	*Daphnia magna*	0.002		UBA Texte 61/2017, EU RA 2008, Danish RA 2014
Tebuconazole	3.200	*Raphidocelis subcapitata*	2.025	*Daphnia magna*	4.400	*Oncorhynchus mykiss*	0.560	*Desmodesmus subspicatus*	0.035	*Daphnia magna*	0.012	*Oncorhynchus mykiss*	EQS Dossier UBA 2017, EFSA EFSA 2007/2014, EQS Dossier Oekotoxzentrum CH 2016
Terbutryn	0.003	*Raphidocelis subcapitata*	5.259	*Daphnia magna*	0.950	*Oncorhynchus mykiss*	0.001	*Raphidocelis subcapitata*	1.300	*Daphnia magna*	0.150	*Oncorhynchus mykiss*	EQS Dossier 2011
Terbuthylazin	0.029	*Raphidocelis subcapitata*	50.900	*Daphnia magna*	0.800	*Pimephales promelas*	0.002	*Raphidocelis subcapitata*	0.019	*Daphnia magna*	0.222	*Oncorhynchus mykiss*	EQS Draft Oekotoxzentrum CH 2016, EFSA 2007/2011, KEMI Report 2008
Triclosan	0.002	*Desmodesmus subspicatus*	0.258	*Daphnia magna*	0.289	*Oryzias latipes*	0.001	*Desmodesmus subspicatus*	0.016	*Daphnia magna*	0.034	*Oncorhynchus mykiss*	UBA Datenblatt 2015, EQS Oekotoxzentrum CH 2017
Trimethoprim	107.200	*Raphidocelis subcapitata*	123.000	*Daphnia magna*	75.000	*Oncorhynchus mykiss*	25.500	*Raphidocelis subcapitata*	6.150	*Daphnia magna*	100.000	*Danio rerio*	EQS Dossier Oekotoxzentrum CH 2015, JRC Third Watch List Report 2020

Table S5: Description of Indicator of Hydrological Alteration and PCA loadings used as a basis for a refined selection (description taken from Olden et Poff, 2003).

	IHA	Description	Calculation	PC1	PC2
Magnitude of flow events					
Average flow conditions	MA3	Variability in daily flows	Coefficient of variation in daily flows	0.96	0.20
	MA5	Skewness in daily flows	Mean daily flows divided by median daily flows	0.94	0.22
	MA11	Spread in daily flow	Range in daily flows (25th and 75th percentiles) divided by median daily flow	0.08	0.18
	MA37	Variability across monthly flows	MA37 is the third quartile minus the first quartile divided by the median of the monthly means	0.95	-0.21
	MA39	Variability across monthly flows	MA39 is the standard deviation times 100 divided by mean of monthly means.	0.97	-0.07
	MA41	Annual runoff	MA41 is the mean of the annual means divided by the drainage area	-0.03	-0.53
High flow conditions	MH14	Median of annual maximum flows	Compute the ratio of annual maximum flow to median annual flow for each year. MH14 is the median of these ratios	0.89	0.33
	MH16	High flow discharge index	Compute the 10 percent exceedance value. MH16 is the 10 percent exceedance value divided by median flow for the entire record	0.96	0.05
	MH20	Specific mean annual maximum flow	MH20 is the mean of the annual maximum flows divided by the drainage area	0.84	0.07
	ML13	Variability across min. monthly flow values	ML13 is the standard deviation times 100 divided by the mean minimum monthly flow for all years	0.95	-0.24
	ML14	Compute the minimum annual flow for each year	ML14 is the mean of ratios of minimum annual flows to median for each year	-0.87	0.15
Low flow condition	ML17	Base flow	Compute the minimum of a 7-day moving average flow for each year and divide them by the mean annual flow for that year. ML17 is the mean of those ratios	-0.94	0.09
	ML18	Variability in base flow	Compute the standard deviation for the ratios of 7-day moving average flows to mean annual flows for each year. ML18 is the standard deviation times 100 divided by the mean of the ratios	0.52	0.08
	ML21	Variability across annual minimum flows	ML21 is the standard deviation times 100 divided by the mean	0.60	-0.25
Frequency of flow events					
Low flow conditions	FL1	Low flood pulse count.	Compute the average number of flow events with flows below a threshold equal to the 25th percentile value for the entire flow record. FL1 is the average number of events	-0.41	0.87
	FL2	Variability in low pulse count	Compute the standard deviation in the annual pulse counts for FL1. FL2 is 100 times the standard deviation divided by the mean pulse count	0.47	-0.33
High flow conditions	FH2	Variability in high pulse count	Compute the standard deviation in the annual pulse counts for FH1. FH2 is 100 times the standard deviation divided by the mean pulse count	0.00	-0.30
	FH3	High flood pulse count	Compute the average number of days per year that the flow is above a threshold equal to three times the median flow for the entire record. FH3 is the mean of the annual number of days for all years	0.94	0.17
	FH4	High flood pulse count	Compute the average number of days per year that the flow is above a threshold equal to seven times the median flow for the entire record. FH4 is the mean of the annual number of days for all years	0.89	0.29
	FH5	Flood frequency	Compute the average number of flow events with flows above a threshold equal to the median flow value for the entire flow record. FH5 is the average number of events	-0.52	0.80
	FH6	Flood frequency	Compute the average number of flow events with flows above a threshold equal to three times the median flow value for the entire flow record. FH6 is the average number of events	0.63	0.56
	FH7	Flood frequency	Compute the average number of flow events with flows above a threshold equal to seven times the median flow value for the entire flow record. FH6 is the average number of events	0.85	0.42

Table S5 (continued)

	IHA	Description	Calculation	PC1	PC2
Duration of flow events					
Low flow conditions	DL1	Annual minimum daily flow	Compute the minimum 1-day average flow for each year. DL1 is the mean of these values	-0.45	-0.43
	DL6	Variability of annual min. daily average flow	Compute the standard deviation for the minimum daily average flow. DL6 is 100 times the standard deviation divided by the mean	0.60	-0.25
	DL11	Annual minimum daily flow divided by the median for the entire record	Compute the minimum daily flow for each year. DL11 is the mean of these values divided by the median for the entire record	-0.88	0.17
	DL16	Low flow pulse duration	Compute the average pulse duration for each year for flow events below a threshold equal to the 25th percentile value for the entire flow record. DL16 is the median of the yearly average durations	0.37	-0.75
	DL17	Variability in low pulse duration	Compute the standard deviation for the yearly average low pulse durations. DL17 is 100 times the standard deviation divided by the mean of the yearly average low pulse durations	0.23	-0.20
High flow conditions	DH5	Annual max. of 90-day moving average flows	Compute the maximum of a 90day moving average flow for each year. DH5 is the mean of these values	-0.33	-0.56
	DH10	Variability of annual maximum of 90-day moving average flows	Compute the standard deviation for the maximum 90-day moving averages. DH10 is 100 times the standard deviation divided by the mean (percent)	0.84	0.28
	DH13	Annual max. of 30-day moving average flows divided by the median	Compute the maximum of a 30-day moving average flow for each year. DL13 is the mean of values divided by median for the entire record	0.96	0.12
	DH15	High flow pulse duration	Compute the average duration for flow events with flows above a threshold equal to the 75th percentile value for each year in the flow record. DH15 is the median of the yearly average durations	0.29	-0.89
	DH16	Variability in high flow pulse duration	Compute the standard deviation for the yearly average high pulse durations. DH16 is 100 times the standard deviation divided by the mean of the yearly average high pulse durations (percent)	0.10	0.37
	DH20	High flow duration	Compute the 75th percentile for the entire flow record. Compute the average duration of events with flows above a threshold equal to the 75th percentile for median annual flows. DH20 is the average duration of events	0.29	-0.89
Rate of change					
	RA1	Rise rate	Compute the change in flow for days in which the change is positive for the entire flow record. RA1 is the mean of these values	-0.20	-0.12
	RA4	Variability in fall rate	Compute the standard deviation for the negative flow changes. RA4 is 100 times the standard deviation divided by the mean	0.83	0.36
	RA5	Number of day rises	Compute the number of days in which the flow is greater than the previous day. RA5 is the number of positive gain days divided by the total number of days in the flow record	-0.77	0.07
	RA6	Change of flow	Compute the log10 of the flows for the entire flow record. Compute the change in log of flow for days in which the change is positive for the entire flow record. RA6 is the median of these values	0.46	0.72
	RA8	Number of reversals	Compute the number of days in each year when the change in flow from one day to the next changes direction. RA8 is the average of the yearly values	-0.40	0.82
	RA9	Variability in reversals	Compute the standard deviation for the yearly reversal values. RA9 is 100 times the standard deviation divided by the mean	0.34	-0.33

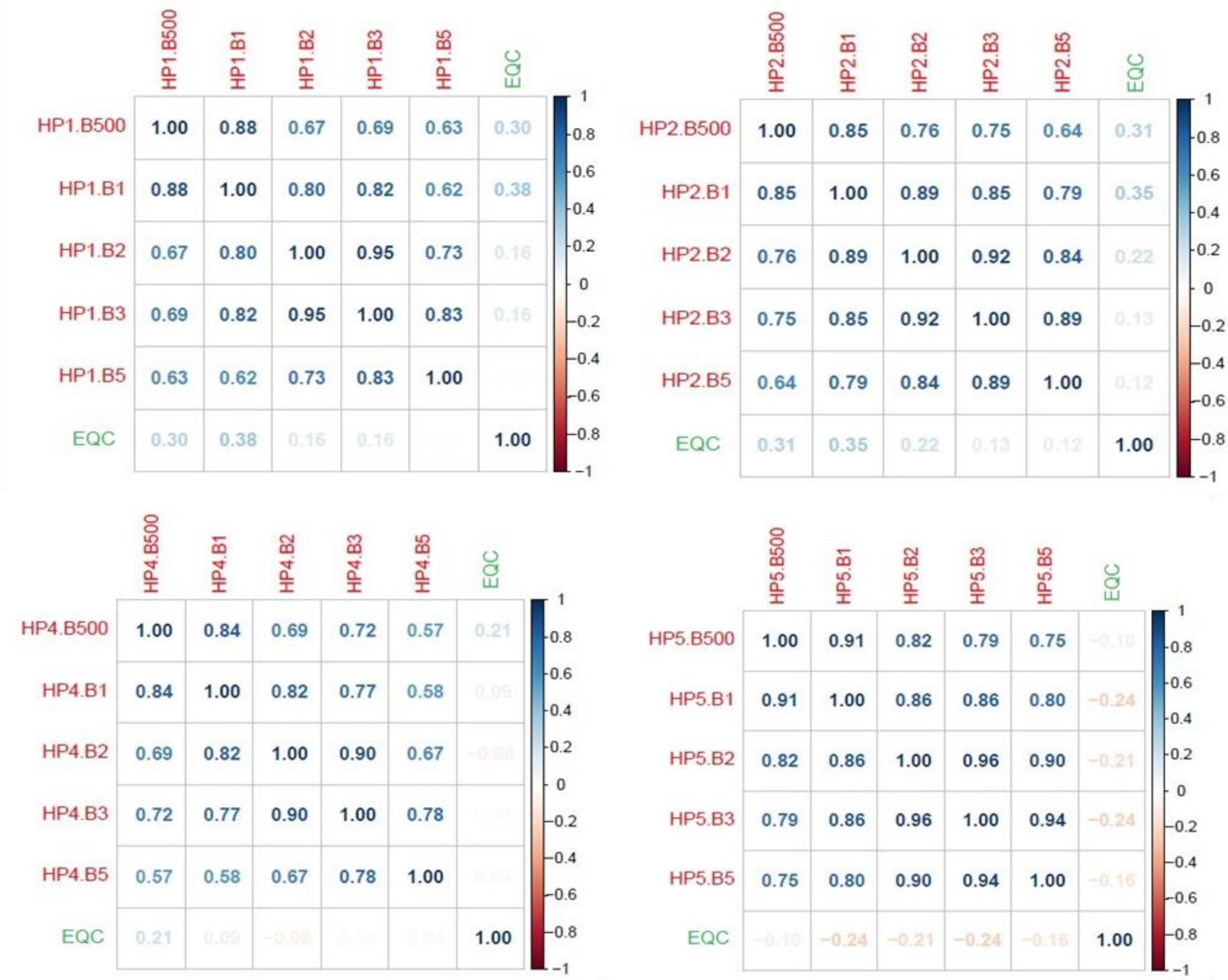

Figure S6: Spearman correlation between the Ecological Quality Class (EQC) and the main parameters of the German standard river habitat survey of North Rhine-Westphalia. Due to data gaps the main parameter HP3 (Bed structure) had to be removed from the dataset. The EQC was calculated on the basis of benthic invertebrate data using the online tool PERLODES Online. Abbreviation: HP1: Channel development, HP2: Longitudinal profile, HP4: Cross profile, HP5: Bank structure; Buffer Length: B500: 500 m, B1: 1 km, B2: 2 km, B3: 3 km, B5: 5 km.

2. Water quality deterioration remains a major stressor for macroinvertebrate, diatom and fish communities in German rivers

Table A1: Summary statistics [µg/l] and ecotoxicological effect concentrations ([mg/l] incl. test species) of micropollutants used for calculating chronic mixture risks. EC_{10} and NOEC values were last updated in 01/2021. Empty values indicate missing values. Abbreviations: substance groups (SG): pesticide (P), pharmaceuticals (A), industrial, household and other chemicals (I).

CAS	Substance	SG	Mean Conc.	Min. Conc.	Max. Conc.	Mean Nb. of Detections	Min. Nb. of Detections	Max. Nb. of Detections
103-90-2	Acetaminophen	A	0.010	0.003	0.230	2.1	0.0	38.0
83905-01-5	Azithromycin	A	0.015	0.005	0.170	1.5	0.0	14.0
41859-67-0	Bezafibrat	A	0.034	0.003	0.820	4.7	0.0	38.0
298-46-4	Carbamazepine	A	0.116	0.003	1.200	6.6	0.0	49.0
85721-33-1	Ciprofloxacin	A	0.010	0.005	0.360	1.7	0.0	22.0
81103-11-9	Clarithromycin	A	0.033	0.002	0.870	3.9	0.0	31.0
18323-44-9	Clindamycin	A	0.009	0.002	0.100	1.5	0.0	20.0
15307-86-5	Diclofenac	A	0.247	0.003	2.700	4.5	0.0	38.0
114-07-8	Erythromycin	A	0.016	0.002	0.320	3.8	0.0	31.0
15687-27-1	Ibuprofen	A	0.028	0.001	1.300	4.5	0.0	38.0
22204-53-1	Naproxen	A	0.029	0.001	0.460	4.8	0.0	38.0
723-46-6	Sulfamethoxazole	A	0.065	0.003	0.620	4.7	0.0	38.0
93413-69-5	Venlafaxin	A	0.046	0.001	0.430	3.1	0.0	24.0
882-09-7	Clofibric acid	A	0.008	0.001	0.050	4.4	0.0	38.0
50-28-2	17β-Estradiol	I	0.000	0.000	0.008	0.8	0.0	35.0
95-14-7	1H-Benzotriazol	I	1.279	0.003	16.000	3.9	0.0	38.0
50-32-8	Benzo(a)pyren	I	0.004	0.000	0.290	4.4	0.0	44.0
80-05-7	Bisphenol A	I	0.029	0.002	4.200	2.7	0.0	36.0
206-44-0	Fluoranthen	I	0.009	0.000	0.410	4.3	0.0	44.0
1222-05-5	HHCB	I	0.040	0.003	0.740	1.7	0.0	26.0
3380-34-5	Triclosan	I	0.004	0.000	0.079	2.9	0.0	38.0
1820573-27-0	Beta-Cyfluthrin	P	0.000	0.000	0.000	0.2	0.0	13.0
28159-98-0	Cybutryne	P	0.000	0.000	0.054	6.0	0.0	44.0
52315-07-8	Cypermethrin	P	0.000	0.000	0.005	1.0	0.0	17.0
52918-63-5	Deltamethrin	P	0.000	0.000	0.000	0.3	0.0	13.0
80844-07-1	Etofenprox	P	0.000	0.000	0.000	0.1	0.0	10.0
138261-41-3	Imidacloprid	P	0.003	0.000	1.700	5.7	0.0	44.0
91465-08-6	lambda-Cyhalothrin	P	0.000	0.000	0.002	0.3	0.0	13.0
52645-53-1	Permethrin	P	0.000	0.000	0.000	0.2	0.0	13.0
94-75-7	2,4-D	P	0.011	0.000	12.000	5.0	0.0	44.0
74070-46-5	Aclonifen	P	0.003	0.000	0.025	3.6	0.0	44.0
131860-33-8	Azoxystrobin	P	0.002	0.000	0.097	5.4	0.0	44.0
15545-48-9	Chlortoluron	P	0.004	0.000	2.100	7.0	0.0	44.0
210880-92-5	Clothianidin	P	0.002	0.000	0.056	5.5	0.0	44.0
333-41-5	Diazinon	P	0.001	0.000	0.015	3.6	0.0	44.0
87674-68-8	Dimethenamid	P	0.005	0.000	0.730	5.1	0.0	49.0
330-54-1	Diuron	P	0.006	0.000	0.500	6.9	0.0	44.0
133855-98-8	Epoxiconazol	P	0.002	0.000	0.092	5.8	0.0	44.0
26225-79-6	Ethofumesate	P	0.004	0.000	0.910	6.3	0.0	40.0
142459-58-3	Flufenacet	P	0.005	0.000	0.380	5.6	0.0	44.0
34123-59-6	Isoproturon	P	0.010	0.000	4.300	6.9	0.0	44.0
94-74-6	MCPA	P	0.026	0.000	11.000	5.5	0.0	44.0
67129-08-2	Metazachlor	P	0.004	0.000	1.000	6.9	0.0	44.0
21087-64-9	Metribuzin	P	0.004	0.000	2.300	6.7	0.0	44.0
111991-09-4	Nicosulfuron	P	0.003	0.000	0.270	4.0	0.0	44.0
52888-80-9	Prosulfocarb	P	0.002	0.000	0.350	5.5	0.0	44.0
51218-45-2	S-Metolachlor	P	0.004	0.000	1.200	6.9	0.0	44.0
107534-96-3	Tebuconazole	P	0.007	0.000	1.300	5.8	0.0	44.0
5915-41-3	Terbuthylazin	P	0.013	0.000	1.000	6.9	0.0	44.0
886-50-0	Terbutryn	P	0.006	0.000	0.600	6.0	0.0	44.0
111988-49-9	Thiacloprid	P	0.001	0.000	0.270	5.1	0.0	44.0

CAS	Subst.	SG	Algae		Benthic invertebrates		Fishes		Reference Note
103-90-2	Acetaminophen	A	22.000	*(Not reported, green algae)*	1.000	*Daphnia magna*	0.460	*Pimephales promelas*	ECHA Registration Dossier, FASS.se Dokument (15.10.2020); Gómez-Oliván et al (2020)
83905-01-5	Azithromycin	A	0.002	*Raphidocelis subcapitata*	0.004	*Ceriodaphnia dubia*	4.600	*Pimephales promelas*	Oekotoxzentrum CH EQS Dossier 2016
41859-67-0	Bezafibrate	A	100.000	*Desmodesmus subspicatus*	0.023	*Ceriodaphnia dubia*	112.000	*Oncorhynchus mykiss*	UBA EQS Draft 2015
298-46-4	Carbamazepin	A	0.520	*Raphidocelis subcapitata*	0.025	*Ceriodaphnia dubia*	0.862	*Pimephales promelas*	Oekotoxzentrum CH EQS 2016, GDCh Paper 4/2016
85721-33-1	Ciprofloxacin	A	1.800	*Chlorella vulgaris*	1.600	*Daphnia magna*	1.000	*Cyprinus carpio*	ACES report 15 , 2018, Oekotoxzentrum CH EQS Dossier 2013, GDCh Paper 4/16
81103-11-9	Clarithromycin	A	0.002	*Raphidocelis subcapitata*	0.003	*Daphnia magna*			Oekotoxzentrum CH Dossier 2016, GDCh Paper 4/16, UBA Report 61/2017
18323-44-9	Clindamycin	A	0.002	*Desmodesmus subspicatus*	0.200	*Daphnia magna*			GDCH 04/16 (LFU), UBA Texte 233/2020
15307-86-5	Diclofenac	A	25.000	*Raphidocelis subcapitata*	8.300	*Daphnia magna*	0.001	*Oncorhynchus mykiss*	UBA EQS Proposal 2011 and 2018, UBA Text 44/2017, Islas-Flores et al. 2013, Lee et al. 2011, Saucedo-Vence et al. 2014
114-07-8	Erythromycin	A	0.010	*Raphidocelis subcapitata*	0.248	*Daphnia magna*	10.000	*Oryzias latipes*	UBA EQS Draft 2015, Oekotoxzentrum CH EQS Dossier 2011
15687-27-1	Ibuprofen	A	35.000	*Chlorella vulgaris*	0.615	*Daphnia magna*	0.000	*Danio rerio*	EQS Draft 2016
22204-53-1	Naproxen	A	7.100	*Raphidocelis subcapitata*	0.085	*Ceriodaphnia dubia*	1.000	*Pimephales promelas*	Oekotoxzentrum CH EQS Dossier 2015, AstraZeneka Risk Assessment 2016
723-46-6	Sulfamethoxazole	A	0.090	*Raphidocelis subcapitata*	0.006	*"Crustaceans"*	0.010	*"Fish" (JRC, RIVM)*	UBA EQS Datasheet 2015, GDCh Paper 04/2016
93413-69-5	Venlafaxin	A	9.800	*Desmodesmus subspicatus*	0.500	*Daphnia magna*	0.0088	*Pimephales promelas*	UBA Texte 233/2020
882-09-7	Clofibric acid	A	75.000	*Desmodesmus subspicatus*	0.640	*Ceriodaphnia dubia*	0.010	*Oncorhynchus mykiss*	UBA Risk Assessment 2004, LAWA 2004
50-28-2	17β-Estradiol	I	0.523	*Desmodesmus subspicatus*	10.000	*Ceriodaphnia dubia*	0.000	*Oncorhynchus mykiss*	UBA EQS Dossier 2011
95-14-7	1H-Benzotriazole	I	1.180	*Desmodesmus subspicatus*	0.970	*Daphnia magna*			Oekotoxzentrum CH EQS Dossier 2016
50-32-8	Benzo(a)pyren	I	0.001	*Raphidocelis subcapitata*	0.001	*Ceriodaphnia dubia*	0.001	*Oncorhynchus mykiss*	EU RAR COAL-TAR PITCH 2008, Annex XV report 2006, RIVM Report 2011
80-05-7	Bisphenol A	I	1.360	*Raphidocelis subcapitata*	3.150	*Daphnia magna*	0.016	*Pimephales promelas*	EU EQS Draft 2015
206-44-0	Fluoranthen	I	0.009	*Raphidocelis subcapitata*	0.001	*Daphnia magna*	0.001	*Pimephales promelas*	RIVM Report 607711007, ECHA 2018: SVHC Proposal, EQS Dossier 2011, EU RAR COAL-TAR PITCH 2008
1222-05-5	HHCB	I	0.201	*Raphidocelis subcapitata*	0.111	*Daphnia magna*	0.068	*Pimephales promelas*	ECHA 2008, EPA 2014, LANUV ECHO Bericht Duftstoffe 2020

Table A1 (continued)

CAS	Subst.	SG	Algae		Benthic invertebrates		Fishes		Reference Note
3380-34-5	Triclosan	I	0.001	*Desmodesmus subspicatus*	0.016	*Daphnia magna*	0.034	*Oncorhynchus mykiss*	UBA Datenblatt 2015, Oekotoxzentrum CH EQS Dossier 2017
1820573-27-0	Beta-Cyfluthrin	P	0.010	*Desmodesmus subspicatus*	0.000023	*Daphnia magna*	0.00001	*Oncorhynchus mykiss*	CLH Report 2018, Biocidal products assessment report 2018
28159-98-0	Cybutryne	P	0.0001	*Nitszchia sp.*			0.004	*Oncorhynchus mykiss*	UBA EQS Dossier 2011
52315-07-8	Cypermethrin	P	0.033	*Raphidocelis subcapitata*	0.00001	*Daphnia magna*	0.00003	*Pimephales promelas*	UBA EQS Dossier 2011, CLH Report 2018, Oekotoxzentrum CH EQS Dossier 2017
52918-63-5	Deltamethrin	P	0.470	*Chlorella vulgaris*	0.0000041	*Daphnia magna*	0.000017	*Pimephales promelas*	JRC Technical Report 2018
80844-07-1	Etofenprox	P	0.056	*Raphidocelis subcapitata*	0.0001	*Daphnia magna*	0.0001	*Danio rerio*	JRC Technical Report 2018, Biocidal Products Assessment Report Austria 2013, EFSA Scientific Report 2008
138261-41-3	Imidacloprid	P	10.000	*Desmodesmus subspicatus*	0.001	*Asellus aquaticus*	1.200	*Oncorhynchus mykiss*	EFSA 2014, RIVM Report 2014, UBA EQS Datasheet 2014
91465-08-6	lambda-Cyhalothrin	P	0.130	*Raphidocelis subcapitata*	0.000002	*Daphnia magna*	0.00003	*Pimephales promelas*	Biocidal products Assessment Report Sweden 2011
52645-53-1	Permethrin	P	0.002	*Raphidocelis subcapitata*	0.000005	*Daphnia magna*	0.00041	*Danio rerio*	JRC Technical Report 2020, Biocidal products Assessment Report Ireland 2014
94-75-7	2,4-D	P	39.000	*Raphidocelis subcapitata*	46.200	*Daphnia magna*	63.400	*Pimephales promelas*	EFSA Dossier 2014, PPDB,
74070-46-5	Aclonifen	P	0.003	*Desmodesmus subspicatus*	0.016	*Daphnia magna*	0.005	*Pimephales promelas*	EQS Dossier 2011, EFSA Dossier 2008, KEMI Report 2008
131860-33-8	Azoxystrobin	P	0.038	*Raphidocelis subcapitata*	0.044	*Daphnia magna*	0.160	*Oncorhynchus mykiss*	KEMI Report 2008
15545-48-9	Chlortoluron	P	0.001	*Scenedesmus quadricauda*	16.700	*Daphnia magna*	0.400	*Oncorhynchus mykiss*	PPDB, INERIS Datasheet, Oekotoxzentrum CH EQS 2016
210880-92-5	Clothianidin	P	15.000	*Raphidocelis subcapitata*	0.120	*Daphnia magna*	20.000	*Pimephales promelas*	JRC Technical Report 2015, CLP Report 2020
333-41-5	Diazinon	P	1.000	*Raphidocelis subcapitata*	0.000	*Daphnia magna*	0.002	*Salvelinus fontinalis*	EFSA 2006, Oekotoxzentum CH EQS Dossier 2016
87674-68-8	Dimethenamid	P	0.003	*Monoraphidium griffithii*	0.680	*Daphnia magna*	0.120	*Oncorhynchus mykiss*	UBA UQN 2017, Oekotoxzentrum CH EQS Dossier 2019, PPDB
330-54-1	Diuron	P	0.001	*Synechococcus sp.*	0.096	*Daphnia magna*	0.410	*Oncorhynchus mykiss*	PPDB, EU EQS Datasheet 2005, Oekotoxzentum CH Dossier 2016, CLH Report
133855-98-8	Epoxiconazole	P	0.008	*Raphidocelis subcapitata*	0.630	*Daphnia magna*	0.003	*Pimephales promelas*	PPDB, EFSA Dossier 2008, ECHA Registrierungsdossier
26225-79-6	Ethofumesat	P	0.031	*Raphidocelis subcapitata*	0.320	*Daphnia magna*	0.156	*Danio rerio*	EFSA 2016, PPDB, Oekotoxzentrum CH EQS Dossier 2016, Kemi Report 2008

Table A1 (continued)

CAS	Subst.	SG	Algae		Benthic invertebrates		Fishes		Reference Note
142459-58-3	Flufenacet	P	0.000	*Raphidocelis subcapitata*	3.260	*Daphnia magna*	0.179	*Oncorhynchus mykiss*	PPDB, UBA EQS Dossier 2015, Oekotoxzentrum CH EQS 2017
34123-59-6	Isoproturon	P	0.018	*Raphidocelis subcapitata*	0.064	*Daphnia magna*	1.000	*Oncorhynchus mykiss*	PPDB, EFSA Conclusion Document 2015 , KEMI Report 2008
94-74-6	MCPA	P	8.900	*Raphidocelis subcapitata*	13.000	*Daphnia magna*	15.000	*Pimephales promelas*	Oekotoxzentrum CH EQS Dossier 2016, KEMI Report 2008
67129-08-2	Metazachlor	P	0.002	*Desmodesmus subspicatus*	0.100	*Daphnia magna*	2.318	*Oncorhynchus mykiss*	RIVM EQS Proposal 2013, Oekotoxzentrum CH EQS Dossier 2015, EFSA 2007 & 2008, EFSA DAR 2005, KEMI Report 2008
21087-64-9	Metribuzin	P	0.003	*Raphidocelis subcapitata*	0.640	*Daphnia magna*	4.430	*Oncorhynchus mykiss*	Oekotoxzentrum CH EQS Dossier 2016, EFSA 2006, EFSA DAR 2005, KEMI Report 2008, CLH Report 2020
111991-09-4	Nicosulfuron	P	100.000	*Desmodesmus subspicatus*	5.200	*Daphnia magna*	10.000	*Oncorhynchus mykiss*	UBA EQS Datasheet 2014, JRC Draft 2016, EFSA 2008, EFSA DAR 2007
52888-80-9	Prosulfocarb	P	0.048	*Raphidocelis subcapitata*	0.045	*Daphnia magna*	0.310	*Oncorhynchus mykiss*	EFSA 2007, PPDB, KEMI Report 2008
51218-45-2	S-Metolachlor	P	0.025	*Raphidocelis subcapitata*	0.354	*Daphnia magna*	1.000	*Cyprinodon variegatus*	PPDB, ECOSAR
107534-96-3	Tebuconazole	P	0.560	*Desmodesmus subspicatus*	0.035	*Daphnia magna*	0.012	*Oncorhynchus mykiss*	EQS Dossier UBA 2017, EFSA 2014, EFSA DAR 2007, Oekotoxzentrum CH EQS Dossier 2016
5915-41-3	Terbuthylazin	P	0.002	*Raphidocelis subcapitata*	0.019	*Daphnia magna*	0.222	*Oncorhynchus mykiss*	Oekotoxzentrum CH EQS Dossier 2016, EFSA 2011, EFSA DAR 2007, KEMI Report 2008
886-50-0	Terbutryn	P	0.001	*Raphidocelis subcapitata*	1.300	*Daphnia magna*	0.150	*Oncorhynchus mykiss*	UBA EQS Dossier 2011
111988-49-9	Thiacloprid	P	32	*Desmodesmus subcapitata*	0.00022	*Chironomus riparius*	0.240	*Oncorhynchus mykiss*	JRC Technical Report 2015, Oekotoxzentrum CH EQS Dossier 2016

Table A2: Overview of biological candidate metrics used for Random Forests.

Metric Group	Code	Organism group	Metric name	Metric description	Random Forest R^2	**Reference**
Integrating	EQC	Macroinvertebrates	Ecological Quality Class	Ecological Quality Class	25.2 %	PERLODES Online Metric (German only): https://gewaesser-bewertung-berechnung.de/files/downloads/perlodes/PerlodesOnline_Dokumentation_Teil_III_Beschreibung_Indizes.pdf
Integrating	MMI	Macroinvertebrates	Multimetric Index	River-type specific general degradation	30.9 %	“
Sensitivity	FI	Macroinvertebrates	German Fauna Index	General and morphological degradation	28.4 %	Lorenz, A., Hering, D., Feld, C. K. & Rolauffs, P. (2004). Hydrobiologia 516: 107-127.
Sensitivity	GSI	Macroinvertebrates	German Saprobic Index	Organic pollution	61.4 %	Rolauffs, P., Hering, D., Sommerhäuser, M., Jähnig, S. & Rödiger, S. (2003). Umweltbundesamt Texte 11/03: 137 S
Sensitivity	%EPT	Macroinvertebrates	Percentage of taxa of Ephemeroptera, Plecoptera and Trichoptera	Number of taxa belonging to sensitive taxonomical groups	32.3 %	PERLODES Online Metric (German only): https://gewaesser-bewertung-berechnung.de/files/downloads/perlodes/PerlodesOnline_Dokumentation_Teil_III_Beschreibung_Indizes.pdf
Sensitivity	$SPEAR_{pest}$	Macroinvertebrates	Species at Risk pesticides	Sensitivity towards pesticide pollution	45.6 %	Liess M, v.d. Ohe P.C. (2005). Environmental Toxicology and Chemistry. 24, (4): 954-965
Sensitivity	KLIWA	Macroinvertebrates	KLIWA Index	Temperature tolerance as temperature equivalent [°C]	63.6 %	Sundermann A, Müller A, Halle M (2022). Limnologica 95:125980, Halle M, Müller A, Sundermann A (2016). KLIWA-Berichte, Heft 20
Sensitivity	RI	Macroinvertebrates	Rheoindex	Stream flow preference	46.4 %	Banning M (1998). Essener ökologische Schriften, 9, Westarp-Wiss, Hohenwarsleben
Functional	IBR	Macroinvertebrates	Index of biocoenotic regions	Preference for regions of the longitudinal river zonation	46.4 %	UBA (2014): ASTERICS - AQEM/STAR Ecological River Classification (Version 4.0.4)
Functional	%Pel	Macroinvertebrates	Percentage of habitat preferences for pelal	Habitat preference for pelal (fine sediment)	6.0 %	PERLODES Online Metric (German only): https://gewaesser-bewertung-berechnung.de/files/downloads/perlodes/PerlodesOnline_Dokumentation_Teil_III_Beschreibung_Indizes.pdf
Functional	%Phy	Macroinvertebrates	Percentage of habitat preferences for phytal	Habitat preference for phytal (plants)	23.1 %	“
Functional	%Lith	Macroinvertebrates	Percentage of habitat preferences for lithal	Habitat preference for lithal (gravel/stones)	36.5 %	“
Functional	%Shr	Macroinvertebrates	Percentage of feeding type preference shredder	Feeding preference for shredder	9.7 %	“
Functional	%Gath	Macroinvertebrates	Percentage of feeding type preference gatherer	Feeding preference for gatherer	0.0 %	“
Functional	%Graz	Macroinvertebrates	Percentage of feeding type preference grazer	Feeding preference for grazer	27.7 %	“
Functional	%Fil	Macroinvertebrates	Percentage of feeding type preference filterer	Feeding preference for filterer	12.5 %	“

Table A2 (continued)

Metric Group	Code	Organism group	Metric name	Metric description	Random Forest R^2	Reference
Richness/ Diversity	Abun-dance	Macroinvertebrates	Abundance	Sum of the abundance of all species/taxa	0.0 %	“
Richness/ Diversity	Rich-ness	Macroinvertebrates	Richness	Number of reported species/taxa	25.5 %	“
Richness/ Diversity	Shan-non	Macroinvertebrates	Shannon Wiener Index	Diversity Index	28.6 %	Shannon, C.E. (1948). Bell Syst. Tech. J., 27, 379–423.
Richness/ Diversity	Even	Macroinvertebrates	Evenness	Diversity Index	12.3 %	PERLODES Online Metric (German only): https://gewaesser-bewertung-berechnung.de/files/downloads/perlodes/PerlodesOnline_Dokumentation_Teil_III_Beschreibung_Indizes.pdf
Integrating	EQC	Diatoms	Ecological Quality Class	Ecological Quality Class	7.2 %	Phylib 6 Technische Dokumentation (2022): Version 6.2.2, https://gewaesser-bewertung- berechnung.de/files/downloads/phylib/ PhylibOnlineTechnischeDokumentation.pdf
Integrating	DI	Diatoms	Diatom Index	River-type specific index of composition, abundance, trophic/ saprobic situation	22.0 %	“
Sensitivity	TI	Diatoms	Trophic Index	Trophic status	35.1 %	Rott, E., Pfister, P., Van Dam H., Pipp, E., Pall, K., Binder, N., Ortler, K. (1999). Bundesministerium für Land- und Forstwirtschaft, Wien, 248 S.
Sensitivity	SI	Diatoms	Saprobic Index	Organic pollution	30.4 %	Rott, E., Hofmann, G., Pall, K., Pfister, P., Pipp, E. (1997). Bundesministerium für Land- und Forstwirtschaft, Wien, 73 S.
Sensitivity	HI	Diatoms	Halobian Index	Salinization	39.5 %	Ziemann (1999). In: Tümpling, W. von, Friedrich, G. (Hrsg.) Methoden der Biologischen Gewässeruntersuchung 2: 310–313
Sensitivity	IBD	Diatoms	Biological Diatom Index	General water quality deterioration	32.4 %	Cemagref, 1995; Coste, M.; Boutry, S.; Tison-Rosebery, J.; Delmas, F. (2009). Ecol. Indic. 2009, 9, 621–650; Omnidia Version 6.1.4; Omnidia Version 6.1.4
Sensitivity	IDG	Diatoms	Generic Diatom Index	Organic pollution, trophic status	32.3 %	Rumeau, A.; Coste, M. (1988). Bulletin Français de la Peche et de la Pisciculture 309 (1988): 1-69; Omnidia Version 6.1.4
Sensitivity	IPS	Diatoms	Pollution Sensitivity Index	Organic pollution	34.1 %	Cemagre (1982): Etude des Methodes Biologiques Quantitatives d'Appreciation de la Qualite des Eaux; Agence de l'eau Rhône Méditerranée Corse: Lyon, France; Omnidia Version 6.1.4
Sensitivity	EPID	Diatoms	Eutrophication/ Pollution Index	Euthrophication and organic pollution	28.6 %	Dell'uomo, A (1996). In: Use of Algae for Monitoring Rivers, pp. 65–72.; Omnidia Version 6.1.4
Sensitivity	DI CH	Diatoms	Swiss Diatom Index	Trophic status	29.1 %	Hürlimann, J. & Niederhauser, P., 2007, Omnidia Version 6.1.4
Sensitivity	ACID	Diatoms	Acidification Index	Acidification	10.0 %	Andrén, C. & Jarlman, A. (2008). Fundamental and Applied Limnology 173 (3):237-253; Omnidia Version 6.1.4
Sensitivity	RI	Diatoms	Rheophilous	Percentage with preference for running water	34.9 %	Denys L. (1991). Geological Survey of Belgium. 1991/02-246.; Omnidia Version 6.1.4

Table A2 (continued)

Metric Group	Code	Organism group	Metric name	Metric description	Random Forest R^2	Reference
Richness/ Diversity	Rich-ness	Diatoms	Richness	Number of reported species/taxa	15.7 %	Phylib 6 Technische Dokumentation (2022): **Version 6.2.2, https://gewaesser-bewertung-berechnung.de/files/downloads/phylib/PhylibOnlineTechnischeDokumentation.pdf**
Richness/ Diversity	Shannon	Diatoms	Shannon Wiener Index	Diversity Index	21.8 %	Shannon, C.E. (1948). Bell Syst. Tech. J., 27, 379–423.
Richness/ Diversity	Even	Diatoms	Evenness	Diversity Index	16.0 %	Magurran, A.E.; McGill, B.J. (2011). Oxford University Press: New York, NY, USA; pp. 1–359.
Integrating	EQC	Fishes	Ecological Quality Class	Ecological Quality Class	4.2 %	FIBS Handbuch: https://gewaesser-bewertung-berechnung.de/files/downloads/fibs/Handbuch_fiBS.pdf
Integrating	EQR	Fishes	Ecological Quality Ratio	River-type specific index of composition, abundance, migration, indicator species and zonation	10.0 %	“
Integrating	QM1	Fishes	Species inventory	Species composition (FIBS QM1)	2.8 %	“
Integrating	QM2	Fishes	Species abundance	Abundance of species and guilds (FIBS QM2)	0.0 %	“
Integrating	QM3	Fishes	Age structure	Percentage of species of age stage 0+ (FIBS QM3)	7.2 %	“
Integrating	QM4	Fishes	Migration Index	Fish migration (FIBS QM4)	7.5 %	“
Integrating	QM5	Fishes	Fish Region Index	Longitudinal river zonation of fish regions (FIBS QM5)	8.9 %	“
Integrating	QM6	Fishes	Dominant Species	Occurrence of indicator species (FIBS QM6)	1.6 %	“
Sensitivity	WQ-INTOL	Fishes	Water quality intolerance	Percentage of species intolerant to oxygen depletion	47.6 %	Solana-Gutierrez J, Garcia de jalon D, Pont D, Bady P, Logez M, Noble R, Schinegger R, Haidvogl G, Melcher A, Schmutz S (2009): Manual for the application of the new European Fish Index - EFI+. EFI+ consortium
Sensitivity	WQ-TOL	Fishes	Water quality tolerance	Percentage of species tolerant to oxygen depletion	0.0 %	“
Sensitivity	H-INTOL	Fishes	Habitat quality intolerance	Percentage of species intolerant to habitat degradation	44.2 %	“
Sensitivity	HTOL	Fishes	Habitat quality tolerance	Percentage of species tolerant to habitat degradation	42.3 %	“
Sensitivity	Rheo-par	Fishes	Rheoparous species	Percentage of species with preference for running water for reproduction	26.1 %	“

Table A2 (continued)

Metric Group	Code	Organism group	Metric name	Metric description	Random Forest R²	Reference
Sensitivity	Limno-par	Fishes	Limnoparous species	Percentage of species with preference for stagnant water for reproduction	30.9 %	"
Functional	%Phy	Fishes	Percentage of habitat preferences for phytal	Preference for phytophilic habitat conditions	20.8 %	"
Functional	%Psa	Fishes	Percentage of habitat preferences for psammal	Preference for psamnophilic habitat conditions	0.8 %	"
Functional	%Lith	Fishes	Percentage of habitat preferences for lithal	Preference for lithophilic habitat conditions	43.7 %	"
Richness/ Diversity	Abunda nce	Fishes	Abundance	Sum of the abundance of all species/taxa	0.0 %	FIBS Handbuch: https://gewaesser-bewertung-berechnung.de/files/downloads/fibs/Handbuch_fiBS.pdf
Richness/ Diversity	Rich-ness	Fishes	Richness	Number of reported species/taxa	17.8 %	"
Richness/ Diversity	Shan-non	Fishes	Shannon Wiener Index	Diversity Index	12.9 %	Shannon, C.E. (1948). Bell Syst. Tech. J., 27, 379–423.
Richness/ Diversity	Even	Fishes	Evenness	Diversity Index	0.0 %	Magurran, A.E.; McGill, B.J. (2011); Oxford University Press: New York, NY, USA; pp. 1–359.

Table A3: Spearman correlation matrix of all stressor variables for macroinvertebrate (A), benthic diatom (B) and fish (C) datasets. Nitrite (NO2-N) was removed due to high correlation (threshold > 0.75).

A: Dataset macroinvertebrates

	O_2	**Temp**	**SO4**	**Cl**	**NH_4-N**	**NO_2-N**	**TP**	**RQ_{mix} Pest**	**RQ_{mix} Pharm**	**RQ_{mix} Ind**	**dl16**	**fh5**	**mh20**	**ra5**	**tl1**	**HP1**	**HP2**	**HP3**	**HP4**	**HP5**
O_2		-0.56	-0.32	-0.25	-0.52	-0.60	-0.45	-0.15	0.01	-0.08	-0.21	0.08	0.23	-0.29	0.18	0.07	-0.32	-0.17	-0.13	-0.04
Temp	-0.56		0.33	0.38	0.33	0.40	0.40	0.21	0.31	0.25	0.04	0.03	-0.17	0.42	-0.05	0.16	0.34	0.28	0.26	0.24
SO4	-0.32	0.33		0.66	0.40	0.49	0.47	0.15	0.36	0.20	-0.04	0.18	-0.43	0.26	-0.10	0.35	0.36	0.38	0.32	0.35
Cl	-0.25	0.38	0.66		0.49	0.49	0.49	0.13	0.47	0.11	-0.15	0.24	-0.27	0.36	-0.14	0.32	0.32	0.35	0.30	0.34
NH_4-N	-0.52	0.33	0.40	0.49		0.81	0.66	0.21	0.25	0.16	0.03	0.20	-0.18	0.24	-0.27	0.15	0.29	0.25	0.24	0.21
NO_2-N	-0.60	0.40	0.49	0.49	0.81		0.64	0.25	0.18	0.15	0.10	0.13	-0.28	0.25	-0.24	0.16	0.33	0.27	0.24	0.17
TP	-0.45	0.40	0.47	0.49	0.66	0.64		0.26	0.26	0.11	-0.01	0.18	-0.18	0.28	-0.24	0.08	0.33	0.29	0.18	0.14
RQ_{mix} Pest	-0.15	0.21	0.15	0.13	0.21	0.25	0.26		-0.09	0.25	-0.01	0.08	-0.35	0.00	-0.01	-0.05	0.05	-0.06	-0.03	-0.09
RQ_{mix} Pharm	0.01	0.31	0.36	0.47	0.25	0.18	0.26	-0.09		0.38	-0.19	0.25	0.04	0.23	-0.04	0.45	0.15	0.28	0.30	0.43
RQ_{mix} Ind	-0.08	0.25	0.20	0.11	0.16	0.15	0.11	0.25	0.38		0.00	0.09	-0.15	0.08	0.10	0.25	0.15	0.09	0.19	0.25
dl16	-0.21	0.04	-0.04	-0.15	0.03	0.10	-0.01	-0.01	-0.19	0.00		-0.71	-0.05	-0.29	0.01	-0.11	0.00	-0.03	-0.02	-0.08
fh5	0.08	0.03	0.18	0.24	0.20	0.13	0.18	0.08	0.25	0.09	-0.71		-0.08	0.31	-0.17	0.19	0.12	0.14	0.11	0.13
mh20	0.23	-0.17	-0.43	-0.27	-0.18	-0.28	-0.18	-0.35	0.04	-0.15	-0.05	-0.08		-0.15	0.16	-0.13	-0.21	-0.11	-0.08	-0.11
ra5	-0.29	0.42	0.26	0.36	0.24	0.25	0.28	0.00	0.23	0.08	-0.29	0.31	-0.15		-0.02	0.09	0.27	0.14	0.16	0.10
tl1	0.18	-0.05	-0.10	-0.14	-0.27	-0.24	-0.24	-0.01	-0.04	0.10	0.01	-0.17	0.16	-0.02		-0.10	-0.18	-0.17	-0.13	-0.13
HP1	0.07	0.16	0.35	0.32	0.15	0.16	0.08	-0.05	0.45	0.25	-0.11	0.19	-0.13	0.09	-0.10		0.50	0.55	0.73	0.75
HP2	-0.32	0.34	0.36	0.32	0.29	0.33	0.33	0.05	0.15	0.15	0.00	0.12	-0.21	0.27	-0.18	0.50		0.65	0.64	0.56
HP3	-0.17	0.28	0.38	0.35	0.25	0.27	0.29	-0.06	0.28	0.09	-0.03	0.14	-0.11	0.14	-0.17	0.55	0.65		0.63	0.63
HP4	-0.13	0.26	0.32	0.30	0.24	0.24	0.18	-0.03	0.30	0.19	-0.02	0.11	-0.08	0.16	-0.13	0.73	0.64	0.63		0.71
HP5	-0.04	0.24	0.35	0.34	0.21	0.17	0.14	-0.09	0.43	0.25	-0.08	0.13	-0.11	0.10	-0.13	0.75	0.56	0.63	0.71	

B: Dataset benthic diatoms

	O_2	**Temp**	**SO4**	**Cl**	**NH_4-N**	**NO_2-N**	**TP**	**RQ_{mix} Pest**	**RQ_{mix} Pharm**	**RQ_{mix} Ind**	**dl16**	**fh5**	**mh20**	**ra5**	**tl1**	**HP1**	**HP2**	**HP3**	**HP4**	**HP5**
O_2		-0.62	-0.52	-0.34	-0.43	-0.56	-0.38	-0.36	-0.25	-0.23	-0.05	-0.08	0.34	-0.30	0.12	-0.12	-0.33	-0.26	-0.21	-0.18
Temp	-0.62		0.42	0.41	0.40	0.47	0.46	0.30	0.42	0.33	0.02	0.14	-0.21	0.44	-0.04	0.27	0.32	0.26	0.34	0.25
SO4	-0.52	0.42		0.68	0.54	0.61	0.56	0.48	0.23	0.20	-0.02	0.28	-0.46	0.36	-0.10	0.31	0.38	0.32	0.31	0.26
Cl	-0.34	0.41	0.68		0.63	0.59	0.59	0.38	0.38	0.11	-0.14	0.35	-0.26	0.44	-0.11	0.31	0.35	0.30	0.34	0.29
NH_4-N	-0.43	0.40	0.54	0.63		0.81	0.68	0.42	0.53	0.31	-0.13	0.40	-0.28	0.32	-0.22	0.33	0.31	0.28	0.31	0.35
NO_2-N	-0.56	0.47	0.61	0.59	0.81		0.63	0.53	0.39	0.27	-0.02	0.32	-0.39	0.32	-0.16	0.35	0.36	0.31	0.34	0.29
TP	-0.38	0.46	0.56	0.59	0.68	0.63		0.48	0.39	0.21	-0.10	0.34	-0.19	0.31	-0.17	0.23	0.30	0.31	0.25	0.22
RQ_{mix} Pest	-0.36	0.30	0.48	0.38	0.42	0.53	0.48		0.19	0.25	-0.03	0.21	-0.41	0.06	-0.17	0.19	0.19	0.16	0.22	0.13
RQ_{mix} Pharm	-0.25	0.42	0.23	0.38	0.53	0.39	0.39	0.19		0.52	-0.01	0.12	0.09	0.31	0.06	0.24	0.15	0.11	0.25	0.19
RQ_{mix} Ind	-0.23	0.33	0.20	0.11	0.31	0.27	0.21	0.25	0.52		0.06	0.09	-0.08	0.13	0.07	0.23	0.24	0.07	0.28	0.21
dl16	-0.05	0.02	-0.02	-0.14	-0.13	-0.02	-0.10	-0.03	-0.01	0.06		-0.67	0.00	-0.28	0.10	0.06	0.00	-0.03	0.00	-0.05
fh5	-0.08	0.14	0.28	0.35	0.40	0.32	0.34	0.21	0.12	0.09	-0.67		-0.23	0.39	-0.19	0.10	0.16	0.18	0.12	0.14
mh20	0.34	-0.21	-0.46	-0.26	-0.28	-0.39	-0.19	-0.41	0.09	-0.08	0.00	-0.23		-0.12	0.25	-0.28	-0.26	-0.16	-0.17	-0.19
ra5	-0.30	0.44	0.36	0.44	0.32	0.32	0.31	0.06	0.31	0.13	-0.28	0.39	-0.12		0.04	0.13	0.25	0.13	0.18	0.12
tl1	0.12	-0.04	-0.10	-0.11	-0.22	-0.16	-0.17	-0.17	0.06	0.07	0.10	-0.19	0.25	0.04		-0.11	-0.07	-0.07	-0.04	-0.11
HP1	-0.12	0.27	0.31	0.31	0.33	0.35	0.23	0.19	0.24	0.23	0.06	0.10	-0.28	0.13	-0.11		0.63	0.61	0.73	0.67
HP2	-0.33	0.32	0.38	0.35	0.31	0.36	0.30	0.19	0.15	0.24	0.00	0.16	-0.26	0.25	-0.07	0.63		0.72	0.60	0.60
HP3	-0.26	0.26	0.32	0.30	0.28	0.31	0.31	0.16	0.11	0.07	-0.03	0.18	-0.16	0.13	-0.07	0.61	0.72		0.59	0.59
HP4	-0.21	0.34	0.31	0.34	0.31	0.34	0.25	0.22	0.25	0.28	0.00	0.12	-0.17	0.18	-0.04	0.73	0.60	0.59		0.67
HP5	-0.18	0.25	0.26	0.29	0.35	0.29	0.22	0.13	0.19	0.21	-0.05	0.14	-0.19	0.12	-0.11	0.67	0.60	0.59	0.67	

C: Dataset Fishes

	O_2	Temp	SO4	Cl	NH_4-N	NO_2-N	TP	RQ_{mix} Pest	RQ_{mix} Pharm	RQ_{mix} Ind	dl16	fh5	mh20	ra5	tl1	HP1	HP2	HP3	HP4	HP5
O_2		-0.64	-0.46	-0.44	-0.51	-0.61	-0.44	-0.38	-0.50	-0.25	-0.15	0.01	0.32	-0.20	0.07	-0.24	-0.35	-0.35	-0.34	-0.19
Temp	-0.64		0.37	0.42	0.47	0.49	0.33	0.20	0.54	0.32	0.00	0.16	-0.19	0.43	-0.09	0.33	0.31	0.30	0.41	0.28
SO4	-0.46	0.37		0.65	0.46	0.57	0.59	0.51	0.24	0.08	0.08	0.11	-0.55	0.21	-0.10	0.22	0.23	0.31	0.32	0.18
Cl	-0.44	0.42	0.65		0.65	0.62	0.60	0.47	0.35	0.09	-0.08	0.27	-0.33	0.21	-0.18	0.29	0.33	0.35	0.33	0.24
NH_4-N	-0.51	0.47	0.46	0.65		0.83	0.68	0.41	0.60	0.08	0.01	0.23	-0.23	0.16	-0.22	0.33	0.32	0.35	0.33	0.29
NO_2-N	-0.61	0.49	0.57	0.62	0.83		0.67	0.55	0.50	0.05	0.10	0.15	-0.29	0.17	-0.29	0.34	0.36	0.39	0.40	0.31
TP	-0.44	0.33	0.59	0.60	0.68	0.67		0.47	0.45	0.10	0.06	0.13	-0.29	0.07	-0.09	0.24	0.21	0.32	0.26	0.18
RQ_{mix} Pest	-0.38	0.20	0.51	0.47	0.41	0.55	0.47		0.12	0.16	0.13	0.08	-0.35	0.10	-0.08	0.10	0.31	0.35	0.23	0.07
RQ_{mix} Pharm	-0.50	0.54	0.24	0.35	0.60	0.50	0.45	0.12		0.47	0.08	0.10	-0.04	0.20	-0.04	0.38	0.20	0.16	0.26	0.27
RQ_{mix} Ind	-0.25	0.32	0.08	0.09	0.08	0.05	0.10	0.16	0.47		0.00	0.13	-0.19	0.19	0.21	0.15	0.27	0.29	0.24	0.11
dl16	-0.15	0.00	0.08	-0.08	0.01	0.10	0.06	0.13	0.08	0.00		-0.67	-0.05	-0.43	0.03	0.12	0.04	0.04	0.06	0.15
fh5	0.01	0.16	0.11	0.27	0.23	0.15	0.13	0.08	0.10	0.13	-0.67		-0.18	0.50	-0.10	0.00	0.10	0.06	0.08	-0.10
mh20	0.32	-0.19	-0.55	-0.33	-0.23	-0.29	-0.29	-0.35	-0.04	-0.19	-0.05	-0.18		-0.26	0.21	-0.19	-0.34	-0.37	-0.21	-0.17
ra5	-0.20	0.43	0.21	0.21	0.16	0.17	0.07	0.10	0.20	0.19	-0.43	0.50	-0.26		0.09	0.14	0.24	0.18	0.16	0.11
tl1	0.07	-0.09	-0.10	-0.18	-0.22	-0.29	-0.09	-0.08	-0.04	0.21	0.03	-0.10	0.21	0.09		-0.13	-0.03	0.01	-0.03	-0.04
HP1	-0.24	0.33	0.22	0.29	0.33	0.34	0.24	0.10	0.38	0.15	0.12	0.00	-0.19	0.14	-0.13		0.60	0.48	0.65	0.67
HP2	-0.35	0.31	0.23	0.33	0.32	0.36	0.21	0.31	0.20	0.27	0.04	0.10	-0.34	0.24	-0.03	0.60		0.72	0.64	0.62
HP3	-0.35	0.30	0.31	0.35	0.35	0.39	0.32	0.35	0.16	0.29	0.04	0.06	-0.37	0.18	0.01	0.48	0.72		0.59	0.55
HP4	-0.34	0.41	0.32	0.33	0.33	0.40	0.26	0.23	0.26	0.24	0.06	0.08	-0.21	0.16	-0.03	0.65	0.64	0.59		0.68
HP5	-0.19	0.28	0.18	0.24	0.29	0.31	0.18	0.07	0.27	0.11	0.15	-0.10	-0.17	0.11	-0.04	0.67	0.62	0.55	0.68	

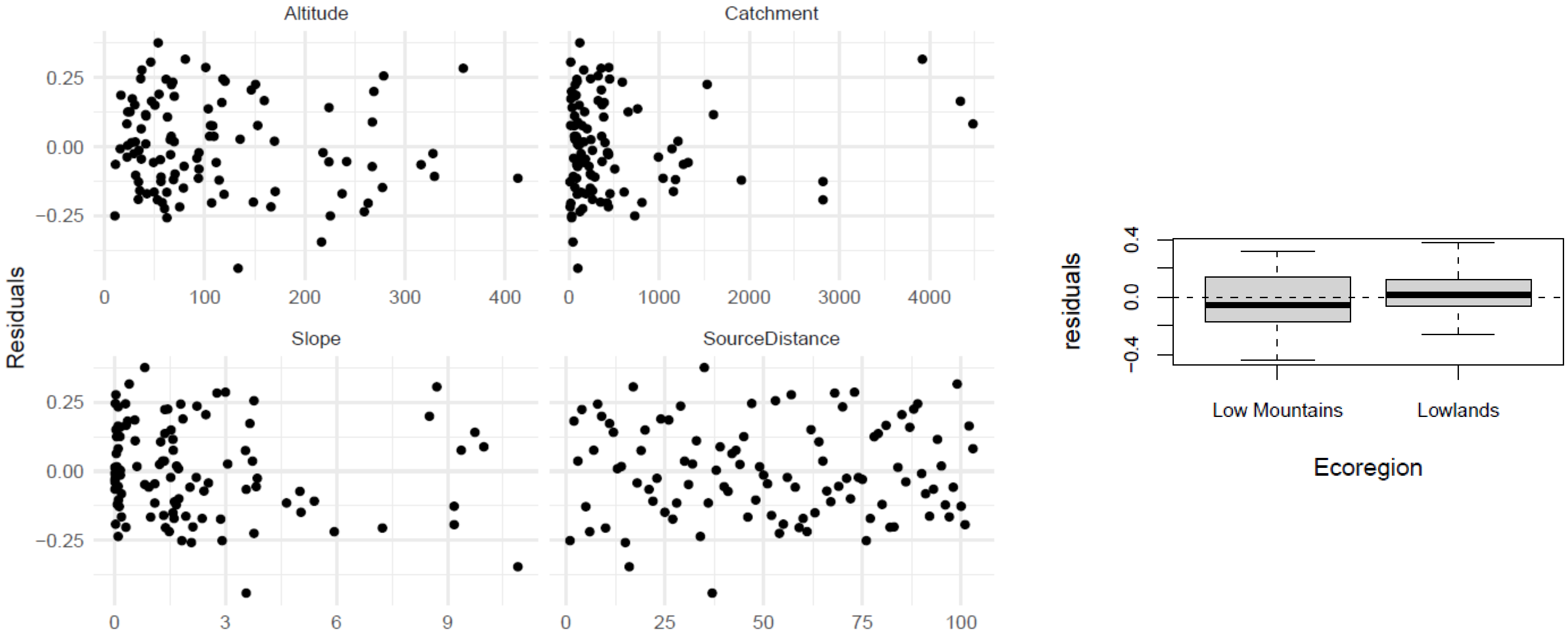

Figure A4: Analyses of Random Forest residuals for effects of co-variables ecoregion, altitude, slope, catchment size and site distance to river source. Example of residual plots of random forest models (Ecological Quality Class).

3. Linking wastewater treatment plant effluents to water quality and hydrology: effects of multiple stressors on fish communities

Table S1: Characteristics of study area. Statistical parameters of catchment size, altitude as well as percentage of intensive agriculture and percentage of urban area in the catchment of the 94 sampling sites of fish communities in the dataset.

	All (n = 94)			**Lowland regions (n = 42)**			**Mountainous regions (n = 52)**		
	Catch-ment [km²]	**Intensive agricul-ture [%]**	**Urban area [%]**	**Catch-ment [km²]**	**Intensive agricul-ture [%]**	**Urban area [%]**	**Catch-ment [km²]**	**Intensive agricul-ture [%]**	**Urban area [%]**
Minimum	12.3	0	3	12.3	5	3	14.7	1	3
Maximum	4480.7	72	55	1603.4	72	43	4480.7	51	55
Median	242.4	36	16	192.7	51	21	337.5	6	16
Mean	531.4	31	19	341.3	52	22	684.9	14	18
Standard Deviation (SD)	859.6	24	12	389.4	14	11	1082.5	14	12

Table S2: Ecotoxicological effect concentrations and number of detections of micropollutants. Ecotoxicological effect concentrations (EC) for chronic toxicity (EC_{10} or NOEC), summary statistics of number of detections (#Det) per micropollutant per site in the sampling period and frequency of detection per micropollutant across all sites. In total 29 micropollutants of the substance groups (SG) pesticides (Pest) and pharmaceuticals (Ph) were included.

Substance	**SG**	**EC [mg/L]**	**Test Species**	**Reference Note**	**Mean #Det**	**Median #Det**	**Min. #Det**	**Max. #Det**
Bezafibrate	Ph	112.00	*Oncorhynchus mykiss*	UBA EQS Draft 2015	4.1	4	0	14
Carbama-zepine	Ph	0.8620	*Pimephales promelas*	Oekotoxzentrum CH EQS 2016, GDCh Paper 4/2016	5.2	4	0	23
Diclofenac	Ph	0.0005	*Oncorhynchus mykiss*	UBA EQS Proposal 2011/2018, Islas-Flores et al. 2013, Lee et al. 2011, Saucedo-Vence et al. 2014	4.1	4	0	14
Erythromycin	Ph	10.000	*Oryzias latipes*	UBA EQS 2015, Oeko-toxzentrum CH EQS 2011	2.8	4	0	14
Ibuprofen	Ph	0.0001	*Danio rerio*	EQS Draft 2016	4.1	4	0	14
Naproxen	Ph	1.0000	*Pimephales promelas*	Oekotoxzentrum CH EQS 2015, AstraZeneka RA 2016	3.6	4	0	13
Sulfame-thoxazole	Ph	0.0100	Not defined	UBA EQS 2015, GDCh Paper 04/2016	4.1	4	0	14
Venlafaxine	Ph	0.0088	*Pimephales promelas*	UBA Texte 233/2020	2.0	1	0	13
Clofibric acid	Ph	0.0103	*Oncorhynchus mykiss*	UBA RA 2004	4.0	4	0	13
Azoxystrobin	Pest	0.1600	*Oncorhynchus mykiss*	KEMI Report 2008	3.3	3.5	0	15
Chlortoluron	Pest	0.4000	*Oncorhynchus mykiss*	PPDB, INERIS Datasheet, Oekotoxzentrum CH EQS 2016	5.0	4	0	15
Clothianidin	Pest	20.0000	*Pimephales promelas*	JRC Technical Report 2015, CLP Report 2020	3.4	4	0	15
2,4-D	Pest	63.4000	*Pimephales promelas*	EFSA Dossier 2014, PPDB	2.8	2	0	15
Dimethen-amid	Pest	0.1200	*Oncorhynchus mykiss*	UBA UQN 2017, Oeko-toxzentrum CH EQS 2019, PPDB	3.0	3	0	23
Diuron	Pest	0.4100	*Oncorhynchus mykiss*	PPDB, EU EQS 2005, Oekotoxzentum CH 2016	5.0	4	0	15
Epoxicon-azole	Pest	0.0030	*Pimephales promelas*	PPDB, EFSA Dossier 2008, ECHA Dossier	3.2	3	0	15
Ethofu-mesate	Pest	0.1560	*Danio rerio*	EFSA 2016, PPDB, Oeko-toxzentrum CH EQS 2016, KEMI Report 2008	4.2	4	0	15

Table S2 (continued)

Substance	SG	EC [mg/L]	Test Species	Reference Note	Mean #Det	Median #Det	Min. #Det	Max. #Det
Flufenacet	Pest	0.1790	*Oncorhynchus mykiss*	PPDB/, UBA EQS 2015, Oeko-toxzentrum CH EQS 2017	3.3	4	0	15
Imidacloprid	Pest	1.2000	*Oncorhynchus mykiss*	EFSA 2014, RIVM Report 2014, UBA EQS Datasheet 2014	4.3	4	0	15
Isoproturon	Pest	1.0000	*Oncorhynchus mykiss*	PPDB, EFSA Document 2015 , KEMI Report 2008	5.0	4	0	15
MCPA	Pest	15.0000	*Pimephales promelas*	Oekotoxzentrum CH EQS 2016, KEMI Report 2008	2.8	2	0	15
Metazachlor	Pest	2.3180	*Oncorhynchus mykiss*	RIVM EQS 2013, Oekotox-zentrum CH EQS 2015, EFSA 2005/2007/2008, KEMI 2008	5.0	4	0	15
Metolachlor	Pest	1.0000	*Cyprinodon variegatus*	PPDB, ECOSAR	5.0	4	0	15
Metribuzin	Pest	4.4300	*Oncorhynchus mykiss*	Oekotoxzentrum CH EQS 2016, EFSA 2005/2006, KEMI Report 2008, CLH Report 2020	5.0	4	0	15
Prosulfocarb	Pest	0.3100	*Oncorhynchus mykiss*	EFSA 2007, PPDB, KEMI Report 2008	3.1	3	0	15
Tebucona-zole	Pest	0.0120	*Oncorhynchus mykiss*	UBA EQS 2017, EFSA 2007/2014, Oekotoxzentrum CH EQS 2016	3.2	3	0	15
Terbuthyl-azine	Pest	0.2220	*Oncorhynchus mykiss*	Oekotoxzentrum CH EQS 2016, EFSA 2007/2011, KEMI 2008	5.0	4	0	15
Terbutryn	Pest	0.1500	*Oncorhynchus mykiss*	UBA EQS 2011	3.5	4	0	15
Thiacloprid	Pest	0.2400	*Oncorhynchus mykiss*	JRC Technical Report 2015, Oekotoxzentrum CH EQS 2016	3.4	4	0	15

Table S3 - 1: Spearman ρ of water quality variables (physico-chemistry) and cumulative percentage of wastewater (CumWW).

	CumWW	Min. O2	Max. T	SO4	NH4N	Cl	Total phosphorus
CumWW	1.00	-0.35	0.45	0.42	0.67	0.67	0.51
Min. O2	-0.35	1.00	-0.65	-0.44	-0.47	-0.36	-0.40
Max. T	0.45	-0.65	1.00	0.36	0.42	0.40	0.26
SO4	0.42	-0.44	0.36	1.00	0.47	0.66	0.58
NH4N	0.67	-0.47	0.42	0.47	1.00	0.65	0.76
Cl	0.67	-0.36	0.40	0.66	0.65	1.00	0.59
Total phosphorus	0.51	-0.40	0.26	0.58	0.76	0.59	1.00

Abbreviations: Cumulative percentage of wastewater (CumWW)
Water quality variables: minimum oxygen concentration, maximum water temperature (T), concentrations of ammonium (NH4), sulphate (SO4), chloride (Cl), total phosphorus.

Table S3 - 2: Spearman ρ of water quality variables (micropollutants toxic units) and cumulative percentage of wastewater (CumWW).

	WW	2,4-D	Azo	Chlo	Clot	Dim.	Diu.	Epo	Etho	Flu	Imi	Iso	MCPA	Meta	Meto	Met	Pro	Teb	Terb	T.az	Thia	Bez	Car	CA	Dicl.	Eryt	Ibu	Nap	Sulf	Venl
WW		0.26	0.44	0.48	0.33	0.42	0.41	0.36	0.50	0.34	0.39	0.40	0.42	0.44	0.28	0.52	0.31	0.39	0.53	0.45	0.37	0.58	0.78	0.00	0.72	0.61	0.26	0.52	0.77	0.69
2,4-D	0.26		0.65	0.34	0.54	0.50	0.11	0.55	0.23	0.52	0.22	0.13	0.76	0.07	0.24	0.38	0.51	0.50	0.27	0.32	0.50	0.23	0.29	0.22	0.19	0.23	0.30	-0.02	0.22	0.46
Azo	0.44	0.65		0.49	0.69	0.62	0.34	0.72	0.54	0.56	0.36	0.33	0.73	0.33	0.29	0.68	0.66	0.63	0.52	0.36	0.59	0.36	0.45	0.04	0.37	0.34	0.32	0.16	0.37	0.52
Chlo	0.48	0.34	0.49		0.59	0.66	0.36	0.58	0.64	0.69	0.25	0.34	0.46	0.48	0.45	0.73	0.42	0.48	0.46	0.58	0.48	0.20	0.45	-0.06	0.45	0.28	0.06	0.23	0.38	0.39
Clot	0.33	0.54	0.69	0.59		0.64	0.40	0.73	0.64	0.64	0.36	0.33	0.56	0.32	0.32	0.69	0.64	0.66	0.60	0.31	0.73	0.18	0.35	0.05	0.28	0.20	0.15	0.04	0.28	0.53
Dim.	0.42	0.50	0.62	0.66	0.64		0.42	0.77	0.58	0.68	0.25	0.46	0.63	0.50	0.39	0.70	0.65	0.73	0.50	0.67	0.71	0.19	0.39	0.01	0.27	0.29	0.22	0.04	0.30	0.45
Diu.	0.41	0.11	0.34	0.36	0.40	0.42		0.39	0.39	0.30	0.30	0.46	0.29	0.64	0.45	0.40	0.30	0.41	0.55	0.39	0.43	0.32	0.41	0.12	0.37	0.29	0.33	0.26	0.38	0.45
Epo	0.36	0.55	0.72	0.58	0.73	0.77	0.39		0.52	0.57	0.21	0.45	0.67	0.49	0.24	0.61	0.68	0.81	0.51	0.38	0.66	0.25	0.36	-0.04	0.29	0.27	0.19	0.13	0.28	0.47
Etho	0.50	0.23	0.54	0.64	0.64	0.58	0.39	0.52		0.49	0.28	0.38	0.41	0.42	0.53	0.77	0.55	0.54	0.47	0.52	0.52	0.18	0.44	-0.13	0.43	0.29	0.07	0.16	0.43	0.39
Flu	0.34	0.52	0.56	0.69	0.64	0.68	0.30	0.57	0.49		0.34	0.35	0.60	0.31	0.31	0.53	0.56	0.72	0.48	0.45	0.58	0.15	0.37	0.14	0.34	0.27	0.18	0.01	0.31	0.47
Imi	0.39	0.22	0.36	0.25	0.36	0.25	0.30	0.21	0.28	0.34		0.57	0.30	0.46	0.15	0.31	0.11	0.28	0.37	0.14	0.27	0.18	0.36	0.50	0.28	0.20	0.28	0.04	0.31	0.43
Iso	0.40	0.13	0.33	0.34	0.33	0.46	0.46	0.45	0.38	0.35	0.57		0.30	0.71	0.39	0.43	0.26	0.57	0.30	0.42	0.40	0.14	0.33	0.29	0.26	0.20	0.26	0.03	0.25	0.25
MCPA	0.42	0.76	0.73	0.46	0.56	0.63	0.29	0.67	0.41	0.60	0.30	0.30		0.29	0.29	0.48	0.65	0.61	0.51	0.46	0.53	0.40	0.38	0.21	0.38	0.44	0.41	0.18	0.37	0.59
Meta	0.44	0.07	0.33	0.48	0.32	0.50	0.64	0.49	0.42	0.31	0.46	0.71	0.29		0.39	0.46	0.29	0.49	0.41	0.48	0.42	0.24	0.36	0.18	0.34	0.20	0.23	0.18	0.33	0.35
Meto	0.28	0.24	0.29	0.45	0.32	0.39	0.45	0.24	0.53	0.31	0.15	0.39	0.29	0.39		0.47	0.33	0.38	0.19	0.54	0.31	0.06	0.16	-0.03	0.15	0.13	0.21	0.09	0.15	0.11
Met	0.52	0.38	0.68	0.73	0.69	0.70	0.40	0.61	0.77	0.53	0.31	0.43	0.48	0.46	0.47		0.61	0.56	0.50	0.58	0.61	0.22	0.45	-0.06	0.40	0.26	0.09	0.12	0.36	0.40
Pro	0.31	0.51	0.66	0.42	0.64	0.65	0.30	0.68	0.55	0.56	0.11	0.26	0.65	0.29	0.33	0.61		0.68	0.35	0.44	0.61	0.15	0.24	-0.03	0.22	0.29	0.18	0.04	0.18	0.41
Teb	0.39	0.50	0.63	0.48	0.66	0.73	0.41	0.81	0.54	0.72	0.28	0.57	0.61	0.49	0.38	0.56	0.68		0.46	0.43	0.63	0.22	0.39	0.07	0.34	0.32	0.23	0.09	0.33	0.48
Terb	0.53	0.27	0.52	0.46	0.60	0.50	0.55	0.51	0.47	0.48	0.37	0.30	0.51	0.41	0.19	0.50	0.35	0.46		0.30	0.49	0.61	0.65	0.09	0.64	0.52	0.38	0.42	0.65	0.72
T.az.	0.45	0.32	0.36	0.58	0.31	0.67	0.39	0.38	0.52	0.45	0.14	0.42	0.46	0.48	0.54	0.58	0.44	0.43	0.30		0.44	0.21	0.42	0.00	0.39	0.36	0.24	0.11	0.37	0.34
Thia.	0.37	0.50	0.59	0.48	0.73	0.71	0.43	0.66	0.52	0.58	0.27	0.40	0.53	0.42	0.31	0.61	0.61	0.63	0.49	0.44		0.23	0.36	0.11	0.24	0.27	0.26	0.03	0.32	0.49
Bez	0.58	0.23	0.36	0.20	0.18	0.19	0.32	0.25	0.18	0.15	0.18	0.14	0.40	0.24	0.06	0.22	0.15	0.22	0.61	0.21	0.23		0.70	0.05	0.74	0.78	0.67	0.76	0.79	0.71
Car	0.78	0.29	0.45	0.45	0.35	0.39	0.41	0.36	0.44	0.37	0.36	0.33	0.38	0.36	0.16	0.45	0.24	0.39	0.65	0.42	0.36	0.70		0.07	0.87	0.69	0.37	0.55	0.91	0.76
CA	0.00	0.22	0.04	-0.06	0.05	0.01	0.12	-0.04	-0.13	0.14	0.50	0.29	0.21	0.18	-0.03	-0.06	-0.03	0.07	0.09	0.00	0.11	0.05	0.07		0.00	0.10	0.26	-0.26	0.00	0.10
Dicl	0.72	0.19	0.37	0.45	0.28	0.27	0.37	0.29	0.43	0.34	0.28	0.26	0.38	0.34	0.15	0.40	0.22	0.34	0.64	0.39	0.24	0.74	0.87	0.00		0.74	0.42	0.70	0.86	0.73
Eryt	0.61	0.23	0.34	0.28	0.20	0.29	0.29	0.27	0.29	0.27	0.20	0.20	0.44	0.20	0.13	0.26	0.29	0.32	0.52	0.36	0.27	0.78	0.69	0.10	0.74		0.55	0.68	0.76	0.69
Ibu	0.26	0.30	0.32	0.06	0.15	0.22	0.33	0.19	0.07	0.18	0.28	0.26	0.41	0.23	0.21	0.09	0.18	0.23	0.38	0.24	0.26	0.67	0.37	0.26	0.42	0.55		0.44	0.41	0.46
Nap	0.52	-0.02	0.16	0.23	0.04	0.04	0.26	0.13	0.16	0.01	0.04	0.03	0.18	0.18	0.09	0.12	0.04	0.09	0.42	0.11	0.03	0.76	0.55	-0.26	0.70	0.68	0.44		0.68	0.54
Sulf	0.77	0.22	0.37	0.38	0.28	0.30	0.38	0.28	0.43	0.31	0.31	0.25	0.37	0.33	0.15	0.36	0.18	0.33	0.65	0.37	0.32	0.79	0.91	0.00	0.86	0.76	0.41	0.68		0.80
Venl	0.69	0.46	0.52	0.39	0.53	0.45	0.45	0.47	0.39	0.47	0.43	0.25	0.59	0.35	0.11	0.40	0.41	0.48	0.72	0.34	0.49	0.71	0.76	0.10	0.73	0.69	0.46	0.54	0.80	

Abbreviations: Cumulative percentage of wastewater (CumWW)

Water quality variables: 2,4-D (24D), Azoxystrobin (Azo.), Chlortoluron (Chlo), Clothianidin (Clot), Dimethenamid (Dim.), Diuron (Diu.), Epoxiconazole (Epo.), Ethofumesate (Etho.), Flufenacet (Flu.), Imidacloprid (Imi.), Isoproturon (Iso.), MCPA, Metazachlor (Meta.), Metolachlor (Meto.), Metribuzin (Met.), Prosulfocarb (Pro.), Tebuconazole (Teb.), Terbutryn (Terb.), Terbuthylazine (T.az.), Thiacloprid (Thia.), Bezafibrate (Bez.), Carbamazepine (Car.), Clofibric acid (CA), Diclofenac (Dicl.), Erythromycin (Eryt.), Ibuprofen (Ibu.), Naproxen (Nap.), Sulfamethoxazole (Sulf.), Venlafaxine (Venl.)

Table S4: Spearman ρ of hydro-morphological variables and cumulative percentage of wastewater.

	CumWW	dl16	fh5	mh20	ra5	tl1	HP1	HP2	HP3	HP4	HP5
CumWW		-0.22	0.47	-0.18	0.37	-0.18	0.15	0.23	0.17	0.22	0.03
dl16	-0.22		-0.76	-0.08	-0.40	-0.02	0.15	0.03	0.08	0.06	0.19
fh5	0.47	-0.76		-0.17	0.48	-0.08	-0.01	0.12	0.05	0.12	-0.10
mh20	-0.18	-0.08	-0.17		-0.26	0.24	-0.22	-0.38	-0.39	-0.23	-0.18
ra5	0.37	-0.40	0.48	-0.26		0.12	0.15	0.27	0.18	0.18	0.12
tl1	-0.18	-0.02	-0.08	0.24	0.12		-0.11	-0.03	0.00	-0.04	-0.02
HP1	0.15	0.15	-0.01	-0.22	0.15	-0.11		0.58	0.46	0.65	0.74
HP2	0.23	0.03	0.12	-0.38	0.27	-0.03	0.58		0.78	0.62	0.61
HP3	0.17	0.08	0.05	-0.39	0.18	0.00	0.46	0.78		0.56	0.52
HP4	0.22	0.06	0.12	-0.23	0.18	-0.04	0.65	0.62	0.56		0.66
HP5	0.03	0.19	-0.10	-0.18	0.12	-0.02	0.74	0.61	0.52	0.66	

Abbreviations: Cumulative percentage of wastewater (CumWW)

Hydro-morphological variables: Low flow duration (flow events below 25th percentile, dl16), High flow frequency (flow events above median flow, fh5), Flow variability (Number of day rises, i.e., number of positive gain days divided by total number of days, ra5), Magnitude of high flow (Mean annual maximum flow divided by catchment area, mh20), Timing of low flow (Date of annual minimum in Julian calendar, tl1), Channel development (HP1), Longitudinal profile (HP2), Bed structure (HP3), Cross profile (HP4), Bank structure (HP5)

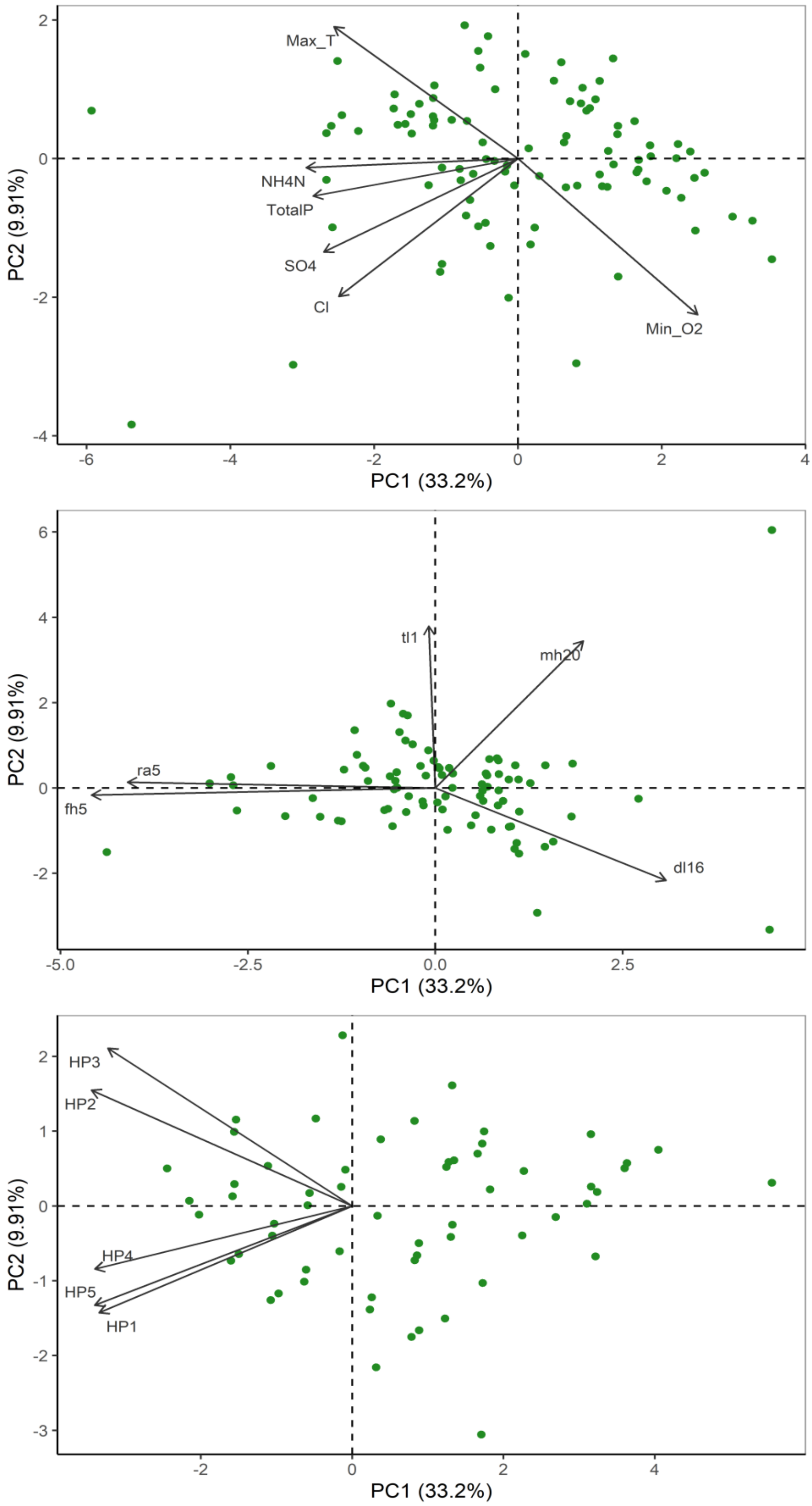

Figure S5: Principal component analysis (PCA) of stressor variables. PCA were plotted for variables of physico-chemistry (top), hydrology (middle) and morphology (bottom), separately. For abbreviations of stressor variables, see Table 1.

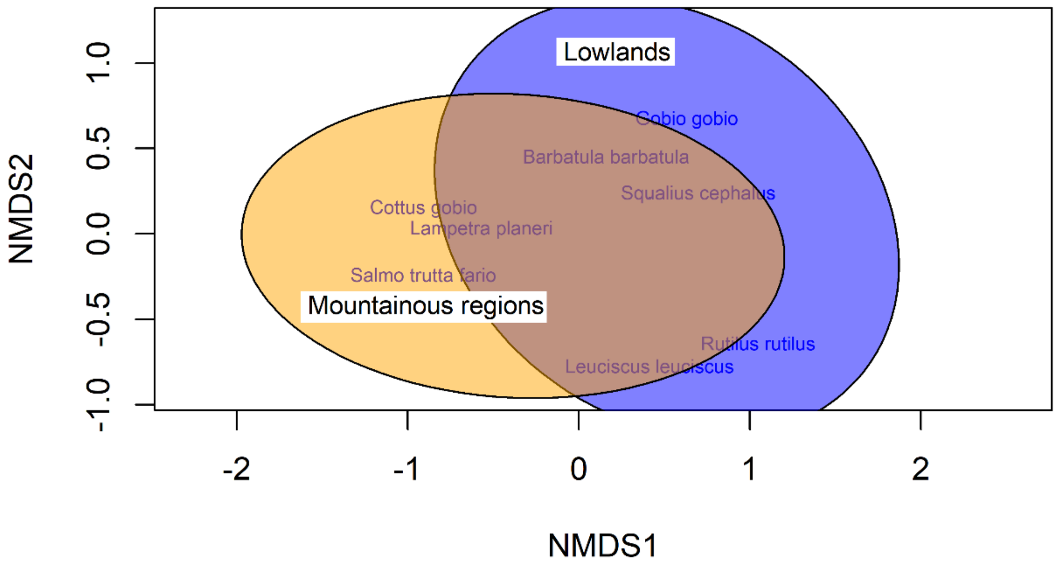

Figure S6: Non-metric multidimensional scaling (NMDS) of nine fish reference species.

NMDS ellipses indicate location of sampling sites in lowlands and mountainous regions. Due to differences in species composition between both ecoregions, subsequent statistical analyses were performed for each ecoregion separately.

4. Water Framework Directive micropollutant monitoring mirrors catchment land use: importance of agricultural and urban sources revealed

Table A1: Statistical parameters of sampling site characteristics

	Minimum	Maximum	Mean	SD
Catchment area [km²]	5	2834	316	503
Altitude [m a.s.l]	12	465	123	101

Table A2: CAS number, ecotoxicological assessment values and number of measured concentrations of micropollutants. Assessment values were derived from environmental quality standards (EQSs) from the national legislation (OGewV, 2016, Monitoring guideline NRW Annex D4) and validated ecotoxicological data (e.g., EQS proposals and predicted no effect concentrations, PNECs). Official assessment values for WFD-related monitoring in NRW, Germany are continuously updated and publicly available (Monitoring guideline NRW Annex D4: https://flussgebiete.nrw.de/monitoring-leitfaden-oberflaechengewaesser). Data from the fourth cycle were used in this study. EQS proposals from the Swiss Ecotox Centre are published online (https://www.oekotoxzentrum.ch/expertenservice/qualitaetskriterien/qualitaetskriterienvorschlaege-oekotoxzentrum). Number of measured values refers to the number (minimum-maximum, mean in parentheses) of measured concentrations per substance, site and sampling year used for calculating annual mean concentrations.

Group	CAS nb.	Substance	Type of value	Assess ment value [µg/L]	Nb. of measured values (Min-Max, Mean)	Reference
Industrial chemicals	50-32-8	Benzo(a) pyrene	EQS	0.00017	1-24 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 8
Industrial chemicals	95-14-7	Benzotriazole	EQS Proposal	19	1-24 (6)	EQS Dossier Oekotoxzentrum Schweiz 2015, deviating from Monitoring guideline NRW Annex D4 (preventive value)
Industrial chemicals	80-05-7	Bisphenol A	EQS Proposal	0.34	1-12 (3)	EQS Dossier SCHEER 2022 (probabilistic method), deviating from Monitoring guideline NRW Annex D4 (preventive value)
Industrial chemicals	206-44-0	Fluoranthene	EQS	0.0063	1-24 (5)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 8
Industrial chemicals	1222-05-5	Galaxolide (HHCB)	Orientation value	4.4	1-6 (3)	Monitoring guideline NRW Annex D4: Orientation value
Industrial chemicals	3380-34-5	Triclosan	EQS	0.02	1-24 (5)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 6
Pharma-ceuticals	83905-01-5	Azithromycin	Orientation value	0.019	1-11 (5)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	41859-67-0	Bezafibrate	Orientation value	2.3	1-24 (3)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	298-46-4	Carba-mazepine	Orientation value	0.5	1-24 (4)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	85721-33-1	Ciprofloxacin	Orientation value	0.089	1-6 (4)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	81103-11-9	Clarithro-mycin	Orientation value	0.1	1-13 (3)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	18323-44-9	Clindamycin	EQS Proposal	0.044	4-12 (6)	EQS Proposal UBA Texte 233/2020, deviating from Monitoring guideline NRW Annex D4 (preventive value)

Table A2 (continued)

Group	CAS nb.	Substance	Type of value	Assessment value [µg/L]	Nb. of measured values (Min-Max, Mean)	Reference
Pharma-ceuticals	882-09-7	Clofibric acid	Orientation value	5	1-24 (3)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	15307-86-5	Diclofenac	Orientation value	0.05	1-24 (3)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	114-07-8	Erythromycin	Orientation value	0.2	1-13 (3)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	15687-27-1	Ibuprofen	Orientation value	0.01	1-14 (4)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	22204-53-1	Naproxen	EQS Proposal	1.7	1-19 (3)	EQS Dossier Oekotoxzentrum Schweiz 2015, deviating from Monitoring guideline NRW Annex D4 (preventive value)
Pharma-ceuticals	103-90-2	Paracetamol	PNEC	46	1-15 (9)	FASS (https://www.fass.se, assessed 01.09.2023), deviating from Annex D4 (preventive value)
Pharma-ceuticals	723-46-6	Sulfa-methoxazol	Orientation value	0.6	1-24 (3)	Monitoring guideline NRW Annex D4: Orientation value
Pharma-ceuticals	93413-69-5	Venlafaxine	EQS Proposal	0.88	1-19 (3)	EQS Proposal UBA Texte 233/2020, deviating from Monitoring guideline NRW Annex D4 (preventive value)
Pesticides	74070-46-5	Aclonifen	EQS	0.12	1-14 (8)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 8
Pesticides	131860-33-8	Azoxystrobin	EQS Proposal	0.2	1-15 (4)	EQS Dossier Oekotoxzentrum Schweiz 2016, deviating from Monitoring guideline NRW Annex D4 (preventive value)
Pesticides	15545-48-9	Chlortoluron	EQS	0.4	1-19 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 6
Pesticides	210880-92-5	Clothianidin	Orientation value	0.08	1-19 (4)	Monitoring guideline NRW Annex D4: Orientation value
Pesticides	94-75-7	2,4-D	EQS	0.2	1-19 (4)	Monitoring guideline Annex D4: EQS, OGewV 2016 Annex 6
Pesticides	87674-68-8	Dimethenamid	EQS Proposal	0.26	1-18 (4)	EQS Dossier Oekotoxzentrum Schweiz 2019, deviating from NRW Annex D4 (preventive value)
Pesticides	330-54-1	Diuron	EQS	0.2	1-19 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 8
Pesticides	26225-79-6	Ethofumesat	Orientation value	3.1	1-16 (4)	Monitoring guideline NRW Annex D4: Orientation value
Pesticides	142459-58-3	Flufenacet	EQS	0.04	1-16 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 6
Pesticides	138261-41-3	Imidacloprid	EQS	0.002	1-19 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 6
Pesticides	34123-59-6	Isoproturon	EQS	0.3	1-19 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 8
Pesticides	94-74-6	MCPA	EQS Proposal	0.66	1-19 (4)	EQS Dossier Oekotoxzentrum Schweiz 2016, deviating from Monitoring guideline NRW Annex D4
Pesticides	67129-08-2	Metazachlor	EQS	0.4	1-19 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 6
Pesticides	51218-45-2	Metolachlor	EQS	0.2	1-19 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 6
Pesticides	111991-09-4	Nicosulfuron	EQS	0.009	1-15 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 6
Pesticides	107534-96-3	Tebuconazole	Orientation value	0.578	1-19 (4)	Monitoring guideline NRW Annex D4: Orientation value
Pesticides	5915-41-3	Terbuthyl-azine	EQS	0.5	1-19 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 6
Pesticides	886-50-0	Terbutryn	EQS	0.065	1-19 (4)	Monitoring guideline NRW Annex D4: EQS, OGewV 2016 Annex 8
Pesticides	111988-49-9	Thiacloprid	Orientation value	0.01	1-19 (4)	Monitoring guideline NRW Annex D4: Orientation value

Table A3: Statistical parameters of SUM RQ values of micropollutant groups

	Min. SUM RQ	**Max. SUM RQ**	**Mean SUM RQ**	**SD SUM RQ**	**% Sites with SUM RQ > 1**
Industrial Chemicals	0.0	169.5	12.9	18.3	100
Pharmaceuticals	0.0	108.5	12.9	11.6	100
Pesticides	0.0	80.1	4.1	5.6	55
Herbicides	0.0	26.7	0.7	1.7	27
Insecticides	0.0	80.0	3.3	5.2	44
Fungicides	0.0	3.9	0.1	0.2	3

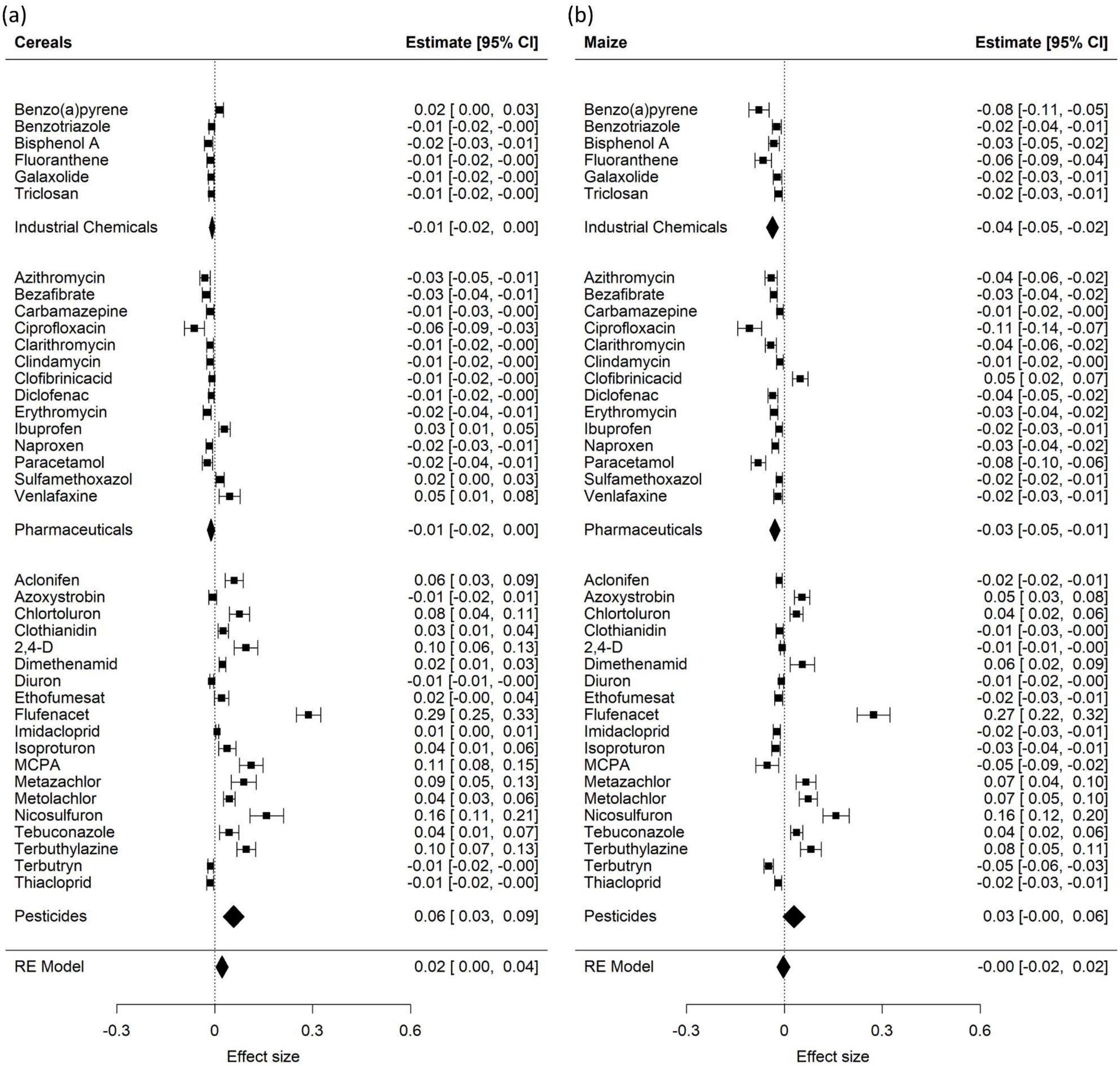

Figure A4.1: Relationship (effect size) of the proportion of cereals (a) and maize (b) in the catchment with micropollutant concentrations. Effect sizes represent model fits (pseudo-R^2) derived from bootstrapped (n = 1,000) univariate linear mixed models (LMM) with 95 % confidence intervals indicated in brackets. Negative signs were added to account for negative relationships (i.e., negative regression coefficients) – although R^2 values are positive by definition.

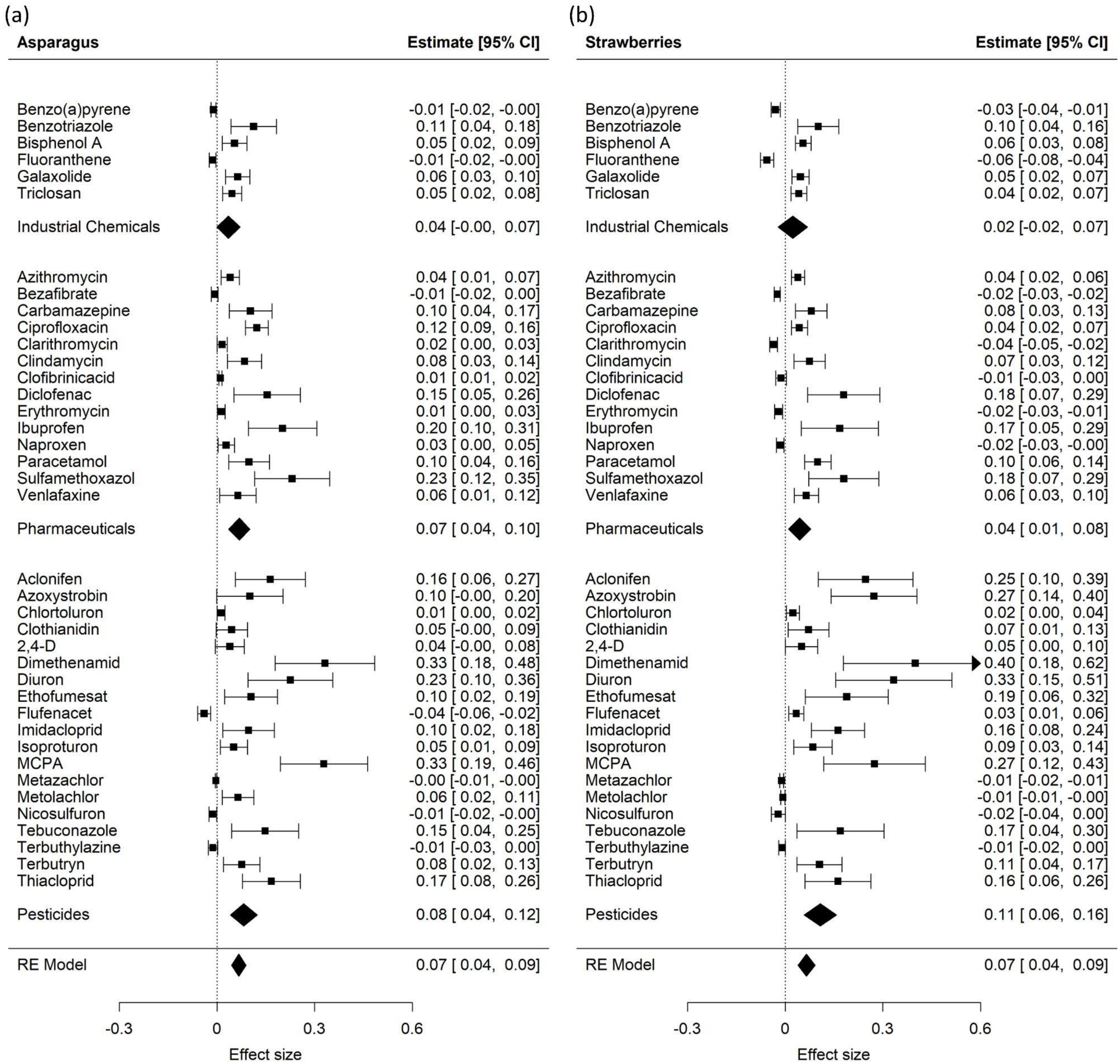

Figure A4.2: Relationship (effect size) of the proportion of asparagus (a) and strawberries (b) in the catchment with micropollutant concentrations. Effect sizes represent model fits (pseudo-R^2) derived from bootstrapped (n = 1,000) univariate linear mixed models (LMM) with 95 % confidence intervals indicated in brackets. Negative signs were added to account for negative relationships (i.e., negative regression coefficients) – although R^2 values are positive by definition.

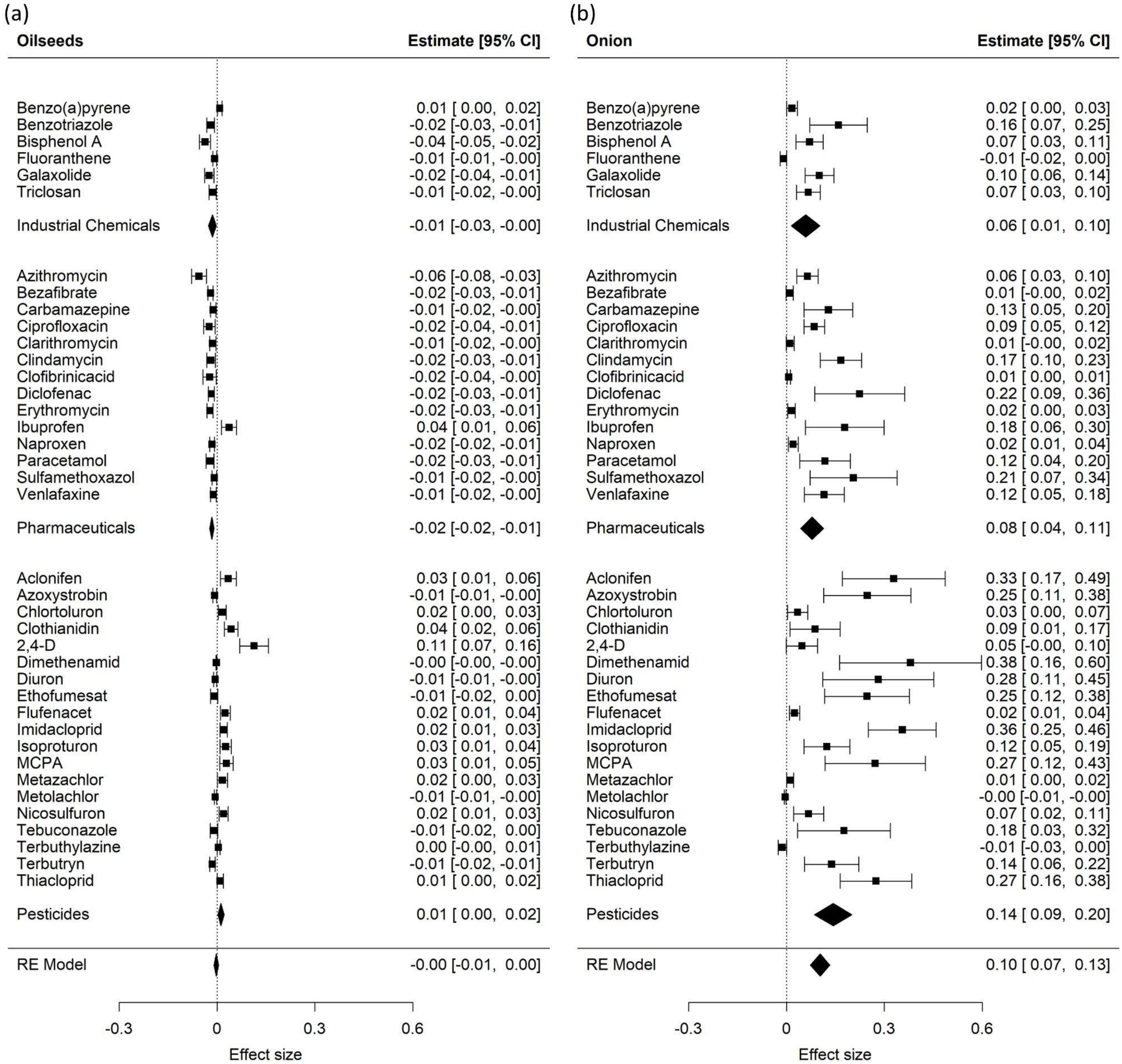

Figure A4.3: Relationship (effect size) of the proportion of oilseeds (a) and onion (b) in the catchment with micropollutant concentrations. Effect sizes represent model fits (pseudo-R^2) derived from bootstrapped (n = 1,000) univariate linear mixed models (LMM) with 95 % confidence intervals indicated in brackets. Negative signs were added to account for negative relationships (i.e., negative regression coefficients) – although R^2 values are positive by definition.

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7 Contributions to Publications

Cumulative Dissertation of Nele Markert

Author contributions

1) Markert, N., Guhl, B. & Feld, C.K., 2022
The hierarchy of multiple stressors' effects on benthic invertebrates: a case study from the rivers Erft and Niers, Germany
Environmental Sciences Europe 34, 100, DOI: 10.1186/s12302-022-00679-z

Contributions:

- Conception: 60 %
- Experimental work: not applicable
- Data preparation and analysis: 100 %
- Species identification: not applicable
- Statistical analysis: 100 %
- Writing the manuscript: 80 %
- Revision of the manuscript: 60 %

2) Markert, N., Guhl, B. & Feld, C.K., 2024
Water quality deterioration remains a major stressor for macroinvertebrate, diatom and fish communities in German rivers
Science of the Total Environment 907, 167994, DOI: 10.1016/j.scitotenv.2023.167994

Contributions:

- Conception: 60 %
- Experimental work: not applicable
- Data preparation and analysis: 100 %
- Species identification: not applicable
- Statistical analysis: 100 %
- Writing the manuscript: 80 %
- Revision of the manuscript: 60 %

3) Markert, N., Guhl, B. & Feld, C.K., 2024
Linking wastewater treatment plant effluents to water quality and hydrology: effects of multiple stressors on fish communities
Water Research 260, 121914, DOI: 10.1016/j.watres.2024

Contributions:

- Conception: 60 %
- Experimental work: not applicable
- Data preparation and analysis: 100 %
- Species identification: not applicable
- Statistical analysis: 100 %
- Writing the manuscript: 80 %
- Revision of the manuscript: 60 %

4) Markert, N., Schürings, C. & Feld, C.K., 2024
Water Framework Directive micropollutant monitoring mirrors catchment land use: Importance of agricultural and urban sources revealed
Science of the Total Environment 917, 170583, DOI: 10.1016/j.scitotenv.2024.170583

Contributions:

- Conception: 40 %
- Experimental work: not applicable
- Data preparation and analysis: 50 %
- Species identification: not applicable
- Statistical analysis: 50 %
- Writing the manuscript: 50 %
- Revision of the manuscript: 40 %

Essen, ____________

Signature of the doctoral candidate | Signature of the doctoral supervisor

8 Declarations

In accordance with § 6 (para. 2, clause g) of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I represent the field to which the topic "Sources and effects of multiple stressors including chemical pollution on the ecological quality of river ecosystems" is assigned in research and teaching and that I support the application of Nele Markert.

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Name and signature of the supervisor, member of the University of Duisburg-Essen

In accordance with § 7 (para. 2, clause d and f) of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I have written the herewith submitted dissertation independently using only the materials listed and have cited all sources taken over verbatim or in content as such.

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In accordance with § 7 (para. 2, clause e and g) of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I have undertaken no previous attempts to attain a doctoral degree, that the current work has not been rejected by any other faculty, and that I am submitting the dissertation only in this procedure.

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