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

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Abstract

Background: A variety of anthropogenic stressors influences the ecological status of rivers wordwide. 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 analysed 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.

Results: 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.

Conclusions: 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.

Keywords: Multiple stressors, Mixture toxicity, Hydrological alteration, Ecological quality, Macroinvertebrates

Background

Rivers in Europe and worldwide are impacted by multiple stressors, which can adversely affect riverine biota and ecological integrity [1–4]. Multiple stressors include eutrophication, salinisation, 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 [5–8].

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

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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 [9, 10]. 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 [11–13], but see Lemm et al. 2021, Liess et al. 2021 and Nowell et al. 2018 [1, 2, 14] 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 [15–19].

In contrast to micropollutants, there is a huge body of literature on the effects of hydrological and morphological stressors on riverine biota [1, 12, 20–23]. The European Environment Agency recently listed hydromorphological impacts, such as channelisation, disconnection of floodplains or flow regulation, among the top stressor groups affecting Europe's rivers [24]. 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 [25–27]. Poff et al. 1997 [26] 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 [6, 7, 27]. However, the degree of hydrological alteration continues to remain largely unaddressed by hydromorphological surveys in Europe [28, 29], 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 [30]. 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 hypothesised (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 (Fig. 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 characterised by urban areas including effluents from wastewater treatment plants (WWTP) 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 [31–33]. Urban surface run-off and WWTP effluents are sources of both chemical pollution and thermal load [32, 34], 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 [35–39]. 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 Additional file 1: Supplement S1.

The upper part of the Erft catchment (total catchment size: 1918 km^2) is located in the low mountain range "Eifel" at altitudes around 550 m above sea level. The region's land cover is characterised 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

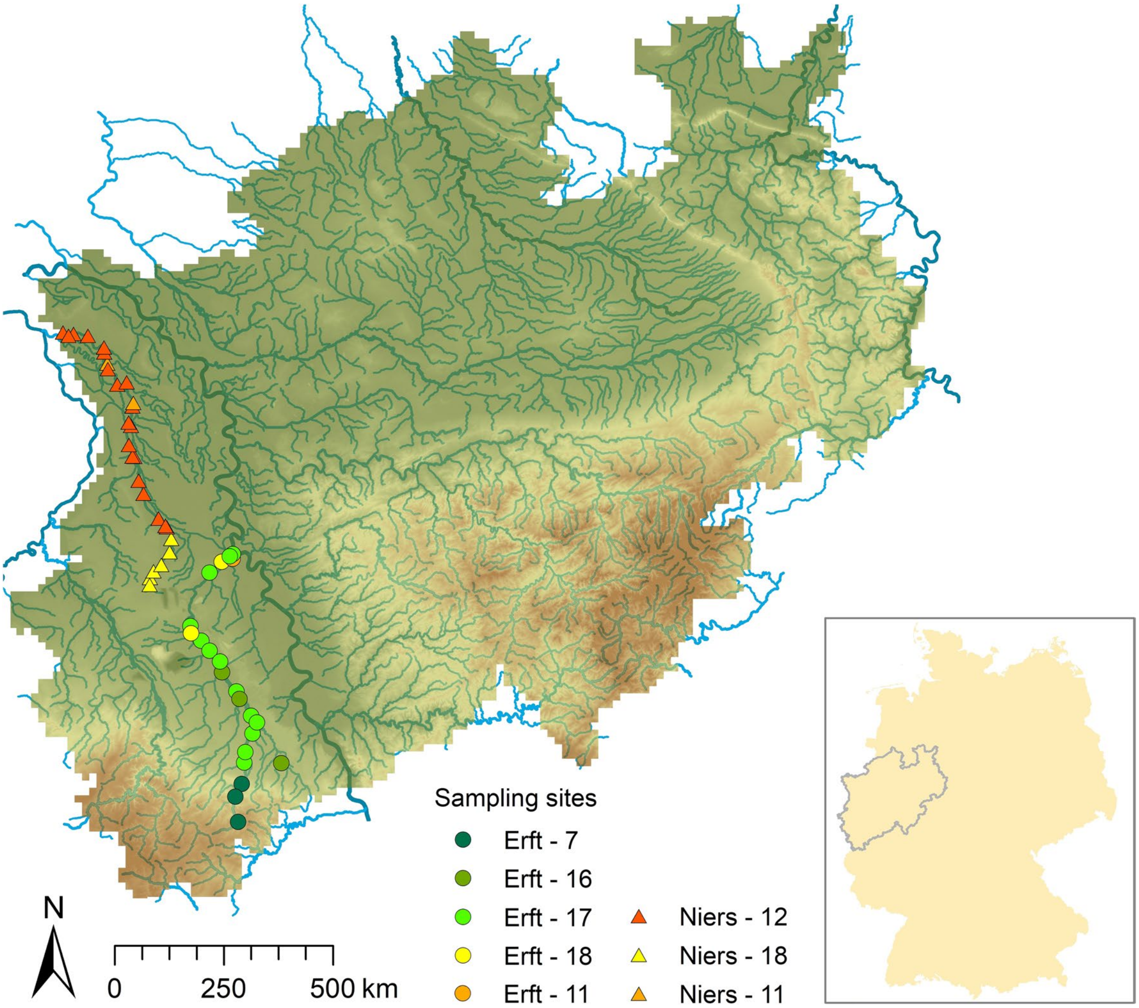

Fig. 1 Sampling sites in the catchments of the river Erft and Niers. Sampling sites are located in the river Erft (dots) and the river Niers (triangles) in North Rhine-Westphalia (NRW), western Germany. Map colour corresponds to the elevation profile with green colours indicating lowlands and brown colours indicating low mountain ranges (artificial minimum of -299 m in mining area, natural range 9 m to 843 m above sea level). Colour of sampling sites show the stream types according to the German river typology for the EU WFD [43, 44] (©Data licence Germany-Zero-Version 2.0)

and associated discharges of drainage water, too [40, 41]. The catchment of river Niers (total catchment size: 1380 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 [42].

Stressor variables

Altogether, 19 stressor variables belonging to four stressor groups were analysed in this study (Table 1). We focussed 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 [3, 24]. Land use thus was excluded because of its collinearity with several other environmental variables (Additional file 1: Supplements S2 for spearman correlations; see also Bradley et al. 2020, Munz et al. 2017 and Kail et al. 2009 [45–47]). Sampling sites for water chemistry (physico-chemical variables

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)

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	–	–
	O2	Minimum oxygen concentration [mg/L]	8.03	8.10	5.70	10.20	7	22%[a]
	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%
	SO4	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%
	NO2-N	Mean total nitrite nitrogen concentration [mg/L]	0.04	0.04	0.01	0.13	0.05	43%
	NH4-N	Mean total ammonium nitrogen concentration [mg/L]	0.12	0.11	0.03	0.37	0.1	55%
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[b]	19.05	20.50	4.00	41.00	–	–
	dl16	Low flow pulse duration[c]	8.89	5.18	3.15	36.72	–	–
	ra5	Number of day rises[d]	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%

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 Additional file 1: Supplements S3

[a] Values are strongly influenced by the timing of sampling during the day and only represent a rough estimation

[b] Average number of events above the median flow of the flow record

[c] Median of the yearly average durations of flow events below the 25th percentile

[d] Percentage of days in the flow record in which the flow is greater than at the previous day

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).

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 4-year 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 imputed using Multivariate Imputation by Chained Equations (default method of predicted mean matching [48]) 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 [30] as well as further studies. A full list of all selected substances as well as number of detections per substance is shown in Additional file 1: Supplement S4. Data originated from routine monitoring schemes in accordance with the WFD, where between four and 12 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 [30, 40] 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 [49, 50] (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 [30]. 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), respectively (Additional file 1: Supplement S4). For comparisons of the mixture risks, the $\mathrm{RQ}_{\mathrm{mix}}$ was additionally calculated for the organism groups algae and fish (Additional file 1: Supplement S3).

Hydrological alteration

Indicators of Hydrological Alteration were calculated using data of the daily mean discharge from gauging stations [25, 51]. 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 [6, 51, 52], a selection of 39 IHA was subjected to a principal component analysis (PCA) to identify suitable IHAs for multiple-stressor analyses (Additional file 1: 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 Additional file 1: Supplement S5. In addition to the IHA, the quotient of the long-term mean discharge and mean low-flow discharge (MQMNQ) based on regionalised 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 [53].

Morphological degradation

Morphological degradation was assessed using data from the German standard river habitat survey of North Rhine-Westphalia [28]. 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 Additional file 1: 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) [28].

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 (Additional file 1: 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 [54], which allows of a standardised 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 [55]. In addition, taxa lists were manually harmonised to eliminate remaining determination bias and subjected to the German assessment software Perlodes Online (Version 5.0.8, [56]) 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 [57]) was used to calculate the Index of Biocoenotic Region (IBR) and the Average Score per Taxon (ASPT). Altogether, the responses of 21 metrics were analysed for multiple stressors' effects (Table 2). Metric selection was based on its ecological meaningfulness as reported by previous studies [6, 58–61], and checked for pairwise correlations to reduce redundant information per metric group.

Statistical analysis

Data processing and analyses were conducted using the open-source software R (Version 4.0.3 [73]) with R Studio (Version 1.4.1103). IHA were calculated using the package EflowStats (*calc_allHIT* [74]). Stressor gradients and correlations were graphically analysed with a PCA using the core package stats (*prcomp*) and the package factoextra (*fviz_pca_biplot* [75]). This step aimed at identifying the main stressor gradients in the dataset. Collinear stressors were then identified based

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

Metric group	Metric name	Metric code	Metric description	Refs.
Abundance	Total Abundance	Abund	Sum of the abundance of all taxa	[62]
Diversity	Number of Taxa	NbTaxa	Number of reported taxa	[62]
	Evenness	Even	Diversity index	
Sensitivity	German Fauna Index	FI	General and morphological degradation	[63]
	Number of taxa of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata	NbEPTCBO	Number of taxa belonging to sensitive taxonomical groups	[62]
	Average Score per Taxon	ASPT	Multiple degradation types	[64]
	Multimetric Index	MMI	River-type specific general degradation	[65]
	KLIWA Index	KLIWA	Temperature tolerance as temperature equivalent [°C]	[66, 67]
	Species at Risk Index	SPEARpest	Sensitivity towards pesticide pollution	[68]
Function	Percentage of specific habitat preferences	Pel%, Psa%, Phy%, POM%	Habitat preference (pelal, psammal, phytal, particulate organic matter)	[69]
	Percentage of specific feeding type preferences	Shr%, Gath%, Graz%, Fil%	Feeding preference (shredder, gatherer, grazer and filterer)	[70]
	Rheoindex	RI	Stream flow preference	[71]
	Index of biocoenotic regions	IBR	Preference for regions of the longitudinal river zonation	[57]
	Percentage of alien species	Alien%	Alien species	[62]
Integrated	Ecological Quality Class	EQC	Ecological Quality Class	[62, 72]

upon variance inflation factors (*vifstep*, package usdm [76]) and excluded from subsequent analyses. To identify a stressor hierarchy, the remaining stressor variables were z-transformed and analysed by a redundancy analysis (RDA) and a subsequent variance partitioning using the package vegan (*rda, varpart* [77]). 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 [43, 44] 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; Fig. 1). RDA models and marginal effects of explanatory variables (stressors, stream types) were tested for significance with an ANOVA permutation test (*anova*, package vegan [77]). Pairwise-correlations between macroinvertebrate metrics and between metrics and stressor variables were calculated using Spearman Rank correlation (*rcorr*, package Hmisc [78]).

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, Fig. 2). PC1 is characterised 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 (Fig. 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 favourable hydrological conditions (Fig. 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.

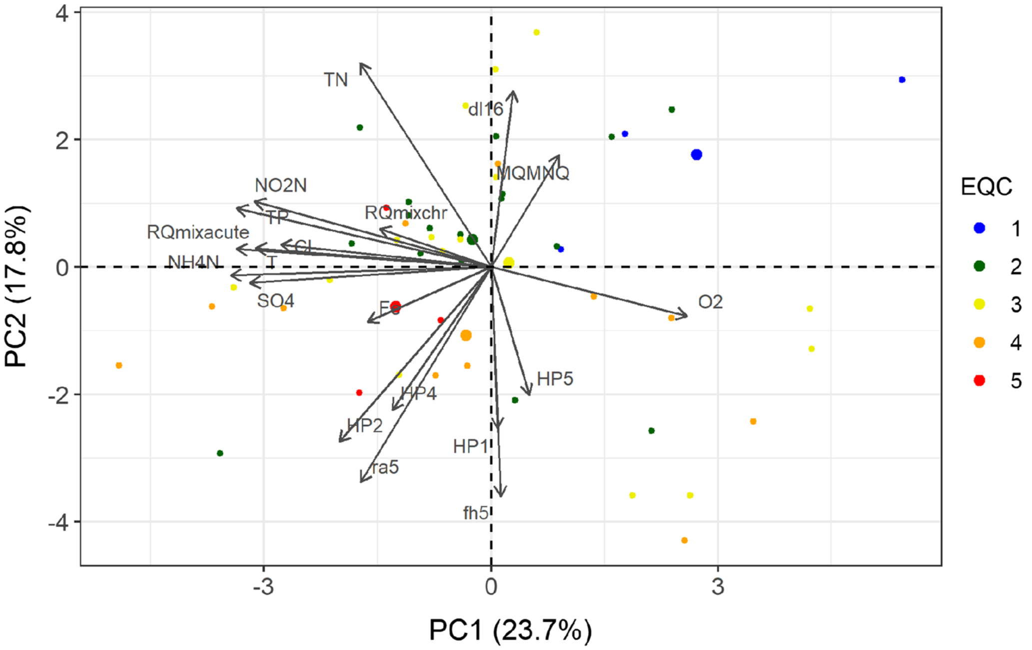

Fig. 2 Principal component analyses biplot of all stressor variables. Sampling sites (dots) are colour-coded according to their Ecological Quality Class (EQC), with enlarged dots indicating the centroid for each EQC. For abbreviations of stressor variables, see Table 1

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 to 0.9, Additional file 1: 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 MQMNQ (rho = − 0.5 to − 0.91). Intensive agriculture was positively correlated with HP1, HP2 and ra5 indicating hydrological–morphological stress in the Erft catchment (rho = 0.51 to 0.81), whereas no positive correlation were observed in the Niers catchment.

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 (Fig. 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

	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 (SO4)			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 (pRDA)				
Physico-chemistry				0.33
Mixture toxicity				0.01
Hydrology				0.21
Morphology				0.17

A selection of 21 benthic macroinvertebrate metrics and 19 stressor variables of four stressor groups (physico-chemistry, micropollutants, morphological degradation, hydrological alteration) were analysed. 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. Individual fractions are shown in Fig. 3

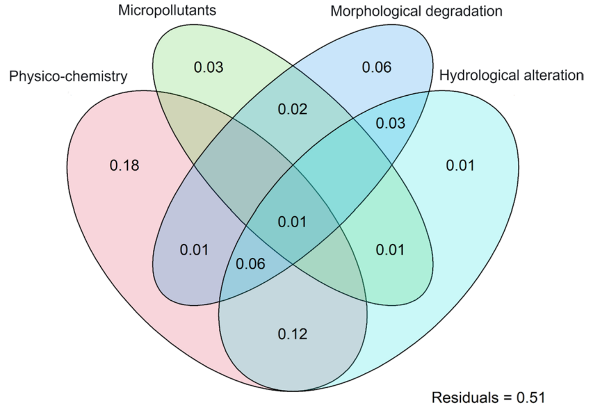

Fig. 3 Venn diagram of the variance in macroinvertebrate metrics. Numbers indicate the unique and shared portions (adjusted R^2) of the variance in 21 macroinvertebrate metrics (Table 2) that are explained by physico-chemistry, micropollutants, morphological degradation and hydrological alteration. R^2 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 physico-chemical 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.

Discussion

In this study, the stressor groups analysed 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. [1, 79]). 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 salinisation caused by mining as well as diffuse pollution from urban area on benthic

Table 4 Spearman rank correlations between selected macroinvertebrate metrics and stressor variables of the four stressor groups

Metric name (metric code)	Physico-chemical stress				Hydrological alteration	Morphological degradation
	O2	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					

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] invertebrates were described in previous studies [36, 37, 39, 80, 81]. 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 salinisation and the actual biological response to salinisation [82, 83]. Notably, salinisation can have different sources, such as drainage from lignite mining and WWTP discharges, but may also result from the application of fertilisers (e.g. potassium chloride) or road salt used for de-icing. Thus, salinisation may be relevant for a large number of surface waters [84–87]. The minimum oxygen concentrations are not fully captured in the routine monitoring [88] but are strongly influenced by, e.g. effluents of WWTP, 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 (Additional file 1: Supplement S3), 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 [2, 89, 90]. 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 [17, 91–93]. 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 [94–96].

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 run-off 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 [6, 31–33, 35]. 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 [24]. 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 [6, 7, 97–100]. 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. [28]). Hydrological alteration then is merely addressed by records of dams or weirs, as a cause of stagnant flow conditions (e.g. [12, 101, 102]). 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 [103], 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 analyse 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.

Abbreviations

EU WFD: EU Water Framework Directive; EC_x: Ecotoxicological effect concentration; IHA: Indicators of Hydrological Alteration; LOQ: Limit of quantification; MNQ: Long-term mean low-flow discharge; MQ: Long-term mean discharge; NRW: North Rhine-Westphalia; PCA: Principal component analysis; RDA: Redundancy analysis; RQ: Risk quotient; RT: Risk threshold; WWTP: Wastewater treatment plant.

Supplementary Information

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Additional file 1: Supplement S1. Land use characteristics in the catchments. **Supplement S2.** Correlation with land use variables. **Supplement S3.** Statistical key parameters of all stressor variables. **Supplement S4.** Overview of micropollutants. **Supplement S5.** Description of Indicators of Hydrological Alteration. **Supplement S6**. Selection of the stream course length for assessing morphological degradation.

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Author contributions

NM compiled and analysed the data and drafted the manuscript. BG coordinated the study. CKF supervised the analyses and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Ecotoxicological data used for calculation of the mixture toxicity are included in the additional files. Complete raw data of stressors and macroinvertebrates are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The manuscript does not report on or involve the use of any animal or human data or tissue.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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