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Contribution of sediment contamination to multi-stress in lowland waters

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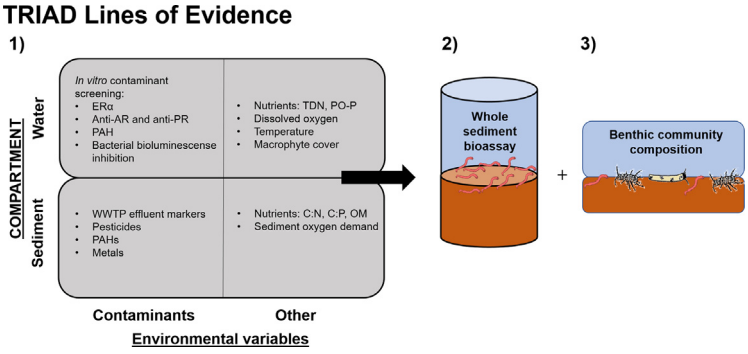
HIGHLIGHTS

- Lowland water bodies are impacted by multiple stressors in water and sediment.
- Nutrients mask the impacts of sediment contaminants in *C. riparius* bioassays.
- All present stressors jointly drove the impoverished benthic invertebrate community.

GRAPHICAL ABSTRACT



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ABSTRACT

Water bodies in densely populated lowland areas are often impacted by multiple stressors. At these multi-stressed sites, it remains challenging to quantify the contribution of contaminated sediments. This study, therefore, aimed to elucidate the contribution of sediment contamination in 16 multi-stressed drainage ditches throughout the Netherlands. To this end an adjusted TRIAD framework was applied, where 1) contaminants and other variables in the sediment and the overlying water were measured, 2) whole-sediment laboratory bioassays were performed using larvae of the non-biting midge *Chironomus riparius*, and 3) the *in situ* benthic macroinvertebrate community composition was determined. It was hypothesized that the benthic macroinvertebrate community composition would respond to all jointly present stressors in both water and sediment, whereas the whole-sediment bioassays would only respond to the stressors present in the sediment. The benthic macroinvertebrate community composition was indeed related to multiple stressors in both water and sediment. Taxa richness was positively correlated with the presence of PO_4-P in the water, macrophyte cover and some pesticides. Evenness, the number of Trichoptera families and the $SPEAR_{pesticides}$ were positively correlated to the C:P ratios in the sediment, whilst negative correlations were observed with various contaminants in both the water and sediment. The whole-sediment bioassays with *C. riparius* positively related to the nutrient content of the sediment, whereas no negative relations to the sediment-associated contaminants were observed, even though the lowered $SPEAR_{pesticides}$ index indicated contaminant effects in the field. Therefore, it was concluded that sediment contamination was identified as one of the various stressors that potentially drove the benthic macroinvertebrate community composition in the multi-stressed drainage ditches, but that nutrients may have masked the adverse effects caused by low and diverse sediment contaminants on *C. riparius* in the bioassays.

1. Introduction

Water bodies in densely populated lowland areas are often impacted by multiple stressors (Allan et al., 1997; Paul and Meyer, 2001; Rico et al.,

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2016). Hydromorphological alterations cause flow changes and habitat degradation, while excess nutrients, and contaminants like pharmaceuticals, metals, polycyclic aromatic hydrocarbons (PAHs), pesticides, and mixtures of other (un)known compounds originating from human activities, pose chemical stress on these lowland water bodies (Bernhardt et al., 2017; Brown et al., 2009; Paul and Meyer, 2001; Waite et al., 2019). Multiple stressors can negatively affect both structure and functioning of aquatic ecosystems (de Vries et al., 2019; van der Lee et al., 2020), but understanding which (combinations of) stressors actually cause the observed deterioration of ecological integrity remains challenging (Clements, 2000; dos Reis Oliveira et al., 2020; Martinez-Haro et al., 2015).

Recently, Lemm et al. (2021) estimated that aqueous contaminants contribute 26 % to aquatic ecosystem deterioration. However, many compounds that are released into the water column eventually end up in sediments (Burton, 2013), making these major repositories of contaminants (Beutel et al., 2008), which can persist for decades (Lorgeoux et al., 2016; Marasinghe Wadige et al., 2017). Consequently, sediment-associated contaminants may reach concentrations that cause adverse effects on the benthic macroinvertebrate communities that live in and on top of the sediment (Reynoldson, 1987; de Castro-Català et al., 2016; Beasley and Kneale, 2002; de Lange et al., 2005), where they play an important role in the functioning of aquatic ecosystems by driving energy flows and nutrient cycling through freshwater food webs (Covich et al., 1999; Diepens et al., 2016). Hence, effects of sediment-associated contaminants on benthic macroinvertebrates may have far-reaching consequences for the structure and functioning of aquatic ecosystems (Brock et al., 2020; de Castro-Català et al., 2016; Peeters et al., 2001). It is thus important to incorporate sediment into the quality assessment of aquatic ecosystems.

The TRIAD approach is the traditional tool for site-specific quality assessment of contaminated sediments (Chapman, 1990). This 'weight of evidence' framework combines three lines of evidence (LoE) including 1) chemical analysis of the sediment, 2) whole sediment bioassays with benthic organisms, and 3) benthic community composition in the field (Chapman et al., 1997). While these fundamental pillars of the TRIAD are nowadays still valid, several amendments, improvements and upgrades have been proposed (Chapman, 2007; Chapman and Hollert, 2006; Höss et al., 2011; Wolfram et al., 2012). Chemical analysis may be improved by considering the bioavailable fraction of the sediment-associated contaminants and the accelerated developments in the field of passive sampling open a wealth of possibilities to do so (de Baat et al., 2020; Escher et al., 2020). In addition, whole-sediment exposure in bioassays have been suggested to most closely represent *in situ* exposure conditions in the laboratory (Feiler et al., 2005; Simpson and Batley, 2003). While the TRIAD has explicitly been designed for sediment quality assessment, it has been argued that due to the strong mutual relationship between water and sediment with many key processes taking place at the sediment-water interface, water and sediment quality should be assessed in tandem to quantify aquatic ecosystem integrity (Brack et al., 2019; Hollert et al., 2002a, 2002b). Likewise, toxic pressure should not be studied in isolation, as it forms an integrated part of the of multi-stress that lowland water bodies and their inhabitants are faced with (de Vries et al., 2019; dos Reis Oliveira et al., 2018), including other stressors like increased nutrient levels (Smith et al., 1999), lowered dissolved oxygen concentrations (Connolly et al., 2004), high sediment oxygen demand (Lee et al., 2018), and habitat degradation (Heino et al., 2009).

This study aimed to elucidate the contribution of sediment contamination to multi-stress in lowland water bodies by applying an adjusted TRIAD framework. To this end 1) bioavailable contaminants and other environmental variables were measured in the sediment and the overlying water, 2) whole sediment laboratory bioassays were performed using larvae of the non-biting midge *Chironomus riparius*, and 3) the *in situ* benthic macroinvertebrate community composition was determined. It was hypothesized that the benthic macroinvertebrate community composition would respond to all jointly present stressors in both water and sediment (Matthaei et al., 2010), whereas the whole-sediment bioassays would only respond to the stressors present in the sediment (de Baat et al., 2019).

2. Materials and methods

2.1. Outline of the study

Sixteen drainage ditches were selected that were impacted to varying degrees by multi-stress, based on land-use information of the surroundings and information provided by the local water authorities (Table S1). The selected water bodies were stagnant, narrow (1.5–8 m), and shallow (0.4–1.2 m). Sampling was conducted from August to October 2018. At each site, various contaminants and other environmental variables were measured in the sediment and the overlying water (Fig. 1: LoE 1 contaminants). To investigate which (groups of) contaminants were present in the water and the sediment, bioavailability-based chemical profiling of groups of legacy and emerging contaminants was conducted using combinations of passive sampling techniques, *in vitro* bioassays, and chemical analyses. In the water, freely-dissolved organic compounds and metals were captured using passive sampling. The organic passive sampler extracts were subjected to bioassays that are indicative of the presence of common contaminants, such as pharmaceuticals and personal care products (PPCPs), pesticides and industrial chemical stress such as PAHs. In the sediment, porewater-equilibrated concentrations of selected wastewater treatment plant (WWTP) effluent markers, commonly used pesticides, PAHs, and freely-dissolved concentrations of metals were determined using passive sampling and chemical analyses. In addition to the contaminant measurements, nutrients, organic matter, dissolved oxygen, sediment oxygen demand (SOD) were measured, and submerged macrophyte cover was estimated as a proxy for habitat structure (Fig. 1: LoE 1 other environmental variables). To isolate the effects of the stressors of the sediment from those in the water column, intact sediment cores were subjected to laboratory whole sediment bioassays with larvae of the non-biting midge *C. riparius* (Fig. 1: LoE 2). Lastly, to measure the biological responses to the stressors present in water and sediment, the *in situ* benthic macroinvertebrate community composition was determined (Fig. 1: LoE 3).

2.2. Methods

2.2.1. Sediment sampling

Ten undisturbed whole sediment cores per site were transported to the laboratory using an acrylic tube (l: 60 cm, d: 6 cm), where they were transferred into short acrylic tubes (l. 15 cm, d: 6 cm) using a sediment core cutter (UWITEC, Mondsee, Austria). Three cores were used on the day of sampling for SOD measurements. The other seven cores were frozen at −20 °C for at least 48 h and subsequently used for contaminant profiling ($n = 2$) and the whole sediment bioassays ($n = 5$). Freezing ensured the elimination of indigenous fauna and is considered the standard for preserving organic characteristics of the sediment, although it cannot be excluded that structural internal modifications may occur when sediments are frozen (Otim, 2019; U.S. EPA, 2001).

2.2.2. Contaminants

2.2.2.1. Water. The presently applied passive sampling procedures, including chemical analysis, were described in detail, by de Baat et al. (2020) as summarized below. Silicone rubber (SR) sheets were obtained from Deltares (Utrecht, The Netherlands) and applied for the sampling of non-polar compounds ($n = 6$). Polar organic chemical integrative samplers (POCIS) containing 0.2 g of Oasis hydrophilic-lipophilic balance sorbent (HLB; Waters, Etten-Leur, The Netherlands) were applied for the sampling of compounds in the more polar range ($n = 4$). POCIS and SR were exposed in stainless steel cages in the middle of the water column for six weeks. Diffusive gradients in thin films (DGT; $n = 3$) containing a 0.15 mL mixed chelex and TiO2 (Metsorb) binding layer were obtained from DGT Research (Lancaster, UK) and were exposed in the field in polyacrylate retainers for 14 days for the passive sampling of metals. After exposure, the passive samplers were rinsed, transported to the laboratory in airtight containers, and stored at −20 °C (POCIS and SR) or 4 °C (DGT) until extraction. POCIS and SR were extracted using organic solvents, and DGT was extracted

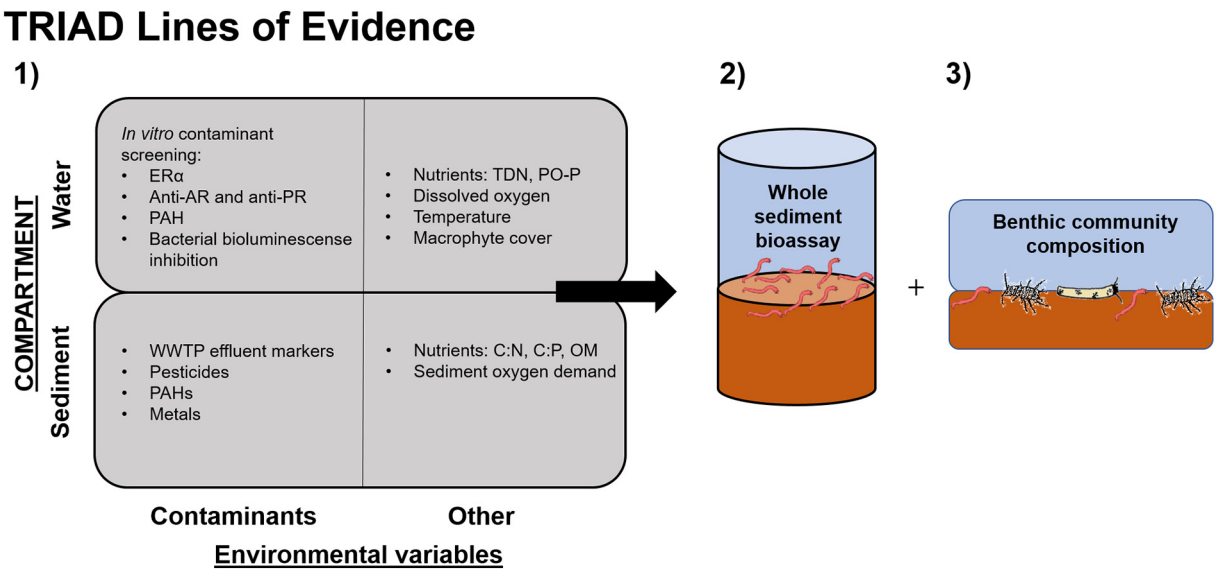

Fig. 1. Graphical outline of the study with three different lines of evidence from the TRIAD framework, including 1) the measurement of contaminants and other environmental variables in the water and sediment), 2) whole sediment laboratory bioassays conducted on intact sediment cores using larvae of *C. riparius*, 3) the *in situ* benthic macroinvertebrate community composition.

using acid. All replicate samplers per site were pooled to obtain one extract per sampler type per site, and extracts were stored at −20 °C until analysis. POCIS and SR extracts were converted into dimethylsulphoxide (DMSO) and subjected to a suite of chemical activated luciferase gene expression (CALUX®) bioassays at the Bio-Detection Systems laboratories (Amsterdam, The Netherlands) to indicate the presence of i) PPCPs (estrogen receptor: ERα CALUX) (Välitalo et al., 2016), ii) pesticides (androgen and progesterone receptor antagonism assays: *anti*-AR and *anti*-PR CALUX) (Pieterse et al., 2015), and iii) the total aryl hydrocarbon receptor (AhR) mediated toxicity from dioxins, furans, PAHs, polychlorinated biphenyls, among others (AhR: PAH CALUX) (Pieterse et al., 2013). The inorganic DGT extracts were also converted into DMSO and used to quantify metal toxicity in the bacterial bioluminescence inhibition assay with *Aliivibro fischeri* (Hamers et al., 2001). The *in vitro* bioassay responses were divided by their respective effect-based trigger values (EBTs) to obtain an effect-based risk quotient, where a quotient ≥1 represents a potential ecotoxicological risk indicated by that particular bioassay (de Baat et al., 2020; Neale et al., 2021). As such, these quotients provided an indication of the toxic pressure exerted by the freely dissolved contaminant concentrations in the water. This, in turn, allowed the calculation of the cumulative effect-based risk quotients as a measure of the cumulative toxic pressure at each site. For further details on the *in vitro* contaminant screening procedures and the effect-based risk quotient calculations see de Baat et al. (2020).

2.2.2.2. Sediment. Pore water-equilibrated concentrations of the selected organic compounds were sampled using solid-phase microextraction (SPME) fibres. The WWTP effluent markers (galaxolide [HHCB], nonylphenol [mixture of isomers], triclosan, and versalide) were selected based on their common occurrence in WWTP effluents (de Baat et al., 2019). Furthermore, 91 pesticides were selected for their common use in the investigated area (Table 1 and Table S2), and phenanthrene and pyrene were selected as model PAHs since they are representative of the presence of complex PAH mixtures (Arp et al., 2011). Two of the collected sediment cores were used for chemical analyses. After thawing, the top 2 cm of one core was collected, homogenized and divided into three subsamples. The methods, including chemical analysis, and calculations of porewater-equilibrated concentrations of the selected compounds were according to de Baat et al. (2019), except for a modification to the analysed pesticides (see S3). To measure the freely-dissolved concentrations of the metals in the sediment that were considered to be relevant for the effects on the macroinvertebrate community (Al, Ba, Cd, Cr, Ni, Pb and Zn), per site, approximately 50 g of sediment from the top 2 cm homogenized sediment layer of the second core was added to each of three 250 mL containers with a DGT (same type as used in the water column) placed on top of the sediment. After 24 h deployment at 20 °C, the DGTs were removed from the sediment, extracted and analysed as described by de Baat et al. (2020).

The toxic stress per contaminant group (WWTP effluent markers, PAH, metals and pesticides) present in the sediment was evaluated using the Toxic Unit (TU) concept which assumes that toxic effects are additive (Sprague, 1971). Freely-dissolved contaminant concentrations (Ci) were converted into TU using the acute (48 h) LC50 of *Daphnia magna* supplemented with EC50 values if no LC50 values were available (Table S4), resulting in the following formula TU contaminant group $_{D.\ magna} = \Sigma\ (Ci/LC50_i)$. A TU of 1 represents a concentration equal to that of the acute LC50 for *D. magna*. Complementary to the TU approach, multi substance potentially affected fraction of species (msPAF) for the sediment was calculated based on the detected contaminant concentrations per site to estimate the joint toxic pressure exerted by all present sediment-associated contaminants (de Zwart and Posthuma, 2005; Wolfram et al., 2012).

2.2.3. Other environmental variables

2.2.3.1. Water. To measure the nutrient concentrations in the water, one surface water sample was collected weekly at each site for six weeks and analysed for total dissolved nitrogen (TDN) and orthophosphate (PO_4-P) on a continuous flow analyzer (SAN + + system, Skalar Analytical B.V., Breda, The Netherlands). The mean nutrient concentration over the six weeks was used for further calculations ($n = 6$). Dissolved oxygen (DO) concentrations were measured with optical HOBO® Dissolved Oxygen loggers U26–001 protected by the antifouling protective guard U26-GUARD-2 (Onset Computer Corporation, Bourne, Massachusetts). Measurements were taken every 10 min, rotating bi-weekly between the sites. DO was expressed as the percentage of time that DO levels were below 10 % saturation, as many macroinvertebrate taxa do not tolerate these low oxygen levels (Connolly et al., 2004). For details on the methods see van der Lee et al. (2020). Water temperature was measured every 10 min for six weeks using HOBO Temperature/Light loggers (model UA-002-64; Onset Computer Corporation, Bourne, Massachusetts). Loggers were placed in the middle of the waterbody 15 cm below the water surface, and mean temperatures were calculated. As a proxy for habitat structure, the percentage

Table 1
Overview of the variables (minimum, maximum) in water and sediment. Bioavailability-based chemical profiling of groups of legacy and emerging contaminants was conducted using combinations of passive sampling techniques, *in vitro* bioassays, and chemical analyses. For the list of contaminants that were measured in the sediment but not detected, see Table S2, for an overview of all variables per site, see Table S5.

Compartment	Stressor	Stressor group	Variable	Minimum	Maximum	Unit
Water	Contaminant	Toxicity index	Σ effect-based risk quotients	1.5	22.8	n/a
		PPCPs	ERα *in vitro* assay	0.1	3.8	ng EEQ/L
		Pesticides	anti-AR *in vitro* assay	2.5	252.4	ug FEQ/L
			anti-PR *in vitro* assay	1.6	49.1	ng REQ/L
		PAHs a.o.	PAH *in vitro* assay	8.4	302.2	ng BEQ/L
		Metals	Bact. biolum. assay	0.0	0.2	TU
		Nutrients	TDN	0.3	5.1	mg/L
			PO-P	0.01	2.38	mg/L
		Dissolved oxygen	Time DO <10 %	0	67	% time
	Other	Macrophyte cover	Macrophyte cover	0	70	%
		Temperature	Temp	14.6	19.4	°C
		Toxicity index	msPAF	23	82	%
			TU	0.000	0.074	n/a
		WWTP effluent	HHCB	0.0	0.14	ug/L
			nonylphenol	0.0	0.58	ug/L
			versalide	0.0	29.79	ug/L
			triclosan	0.0	5.86	ug/L
		Pesticides	ametryn	0.0000	0.0097	ug/L
			chlorpropham	0.0000	0.0355	ug/L
			clomazone	0.0000	0.0922	ug/L
			cycloxydim	0.0000	0.0269	ug/L
			dimethametryn	0.0000	0.0005	ug/L
			fluoxastrobin	0.0000	0.0410	ug/L
			irgarol	0.0000	0.0016	ug/L
			methabenzthiazuron	0.0000	0.0065	ug/L
			metolachlor.s	0.0000	14.403	ug/L
			pentachlorophenol 2	0.0000	0.0004	ug/L
			prosulfocarb	0.0000	0.0352	ug/L
	Contaminant	PAH	phenanthrene	0.0000	0.04	ug/L
			pyrene	0.0000	0.36	ug/L
		Metals	Al	0.00	14.73	ug/L
			Ba	0.00	34.12	ug/L
			Cr	0.00	2.04	ug/L
Sediment			Pb	0.00	75.01	ug/L
			Zn	0.00	53.65	ug/L
			Ni	0.00	3.83	ug/L
	Other	Nutrients	CN	11	25	Molar ratio
			CP	33	4221	Molar ratio
			OM	2	66	%
		Sediment oxygen demand	SOD	0.2	2.0	$g/m^2/day$

submersed macrophyte cover was visually estimated at each site along a trajectory of 50 m (Heino et al., 2009; Thomaz and da Cunha, 2010).

2.2.3.2. Sediment. For the analysis of nutrients in the sediment, the top 2 cm of the sediment in the field was sampled in the first, third, and fifth week of the sampling campaign, and stored at −20 °C. The sediment samples were freeze-dried, dry sieved (2 mm mesh) to remove mollusc shell fragments and dead plant remains, and ground to a fine powder using a ball mill for 5 min at 400 rpm. The organic matter content was determined after heating the dry sediment samples at 550 °C for 4 h ($n = 3$). For total-P, homogenized portions of 200 mg dry sediment were digested with 4 mL HNO_3 (65 %) and 1 mL H_2O_2 (30 %), using a microwave (n = 3). Digestates were diluted and phosphorus concentrations were determined by ICP (Perkin Elmer ICP-OES 8000). For total-C, total-N5–20 mg freeze-dried material was analysed using a Bio Vision isotope ratio mass spectrometer (Elementar UK, Manchester, UK) (n = 3). The C:N and C:P ratios were calculated in molar units. Sediment oxygen demand, including both the respiration rate of benthic communities as well as the chemical oxidation of reduced substances in the sediment, was determined in three replicate sediment cores per site on the day of sampling as described by Rong et al. (2016) and dos Reis Oliveira et al. (2018) with slight modifications. Cores were topped off with tap water, measured for sediment and water height, closed with an airtight cap and wrapped in aluminium foil to ensure darkness, and aerated overnight in a dark climate room at 20 °C. The next day (>12 h), the air supply was stopped and thereafter the oxygen concentration was measured in the overlaying water every 10 min for >12 h using an Oxy-4 MINI (PreSens, Regensburg, Germany). Calculations of SOD were performed according to Rong et al. (2016). The mean SOD over the replicate cores was used for further analysis ($n = 3$).

2.2.4. Whole sediment bioassays

Five of the collected sediment cores per site were used for the whole sediment bioassays. The 28 d whole sediment bioassays were performed with first instar larvae (<24 h) of the non-biting midge *Chironomus riparius*, originating from the University of Amsterdam in-house laboratory culture, based on OECD guideline 218 (OECD, 2004) with slight modifications (de Baat et al., 2019; Marinković et al., 2011). The bioassays were constantly aerated. Five replicates per treatment and ten replicates of a negative laboratory control with artificial sediment were included in the experiment. After 14 d, the sediment cores were covered with fine mesh gauze and checked daily for emerging midges, which were removed after their sex was identified. At the end of the 28 d experiment, the sediments were sieved and the surviving larvae were counted. Survival (the number of emerged adults and surviving larvae), the number of emerged adults, and the emergence time of the adults ($EmT_{50\ males}$ and $EmT_{50\ females}$: the day at which 50 % males and females emerged, respectively) were calculated. Since the emergence time of males and females differs (León Paumen et al., 2008; Vogt et al., 2007), the EmT_{50} was calculated as the mean of the $EmT_{50\ males}$ and $EmT_{50\ females}$. Next, the EmT_{50} was inversed as $1/EmT_{50}$ to represent faster emergence of the larvae.

2.2.5. Benthic community composition

The benthic macroinvertebrate community was sampled on a single occasion. Three subsamples were taken with a pond net (1 mm mesh size, 25 cm width) that was swept over a length of 0.5 m through the top layer of the sediment. The samples were stored overnight at 4 °C with oxygen supply, washed over 1 mm and 250 μm sieves, sorted alive and preserved in 70 % ethanol until identification. Macroinvertebrates were identified to the genus level, except for Oligochaeta (order), Hydracarina (order) and Diptera (family). Mean abundances were used for further analysis ($n = 3$).

To assess the relation between the macroinvertebrate community and the other LoE, various indices were calculated. Species richness was the total number of taxa in a sample (Ludwig and Reynolds, 1988). Evenness was estimated by calculating the Smith and Wilson evenness (E_{var}) index, which describes the species abundance distributions using statistics to avoid dependence on species richness (Heip et al., 1998; Smith and Wilson, 1996). The number of trichopteran families was used as an indication of the ecological quality of the ditches, as recommended by Verdonschot et al. (2012). Lastly, the $SPEAR_{pesticides}$ index was calculated as a trait-based approach to assess the overall sensitivity of a community to pesticide exposure, using the SPEcies At-Risk calculator 2021.02 (Version 2.2.1) as implemented in http://www.systemecology.eu/indicate/ (Knillmann et al., 2018; Liess and von der Ohe, 2005).

2.3. Statistical analysis

The patterns in the benthic macroinvertebrate community composition at the different sites were evaluated using classification and ordination (Chapman et al., 1997). The mean macroinvertebrate taxon abundance was log10 (x + 1) transformed to minimize the effect of high abundances in the multivariate analyses. The sites were clustered, using an initial, non-hierarchical clustering, following the algorithm of Sorensen (1948), for a site-by-site matrix based on the similarity ratio. The initial clustering was optimized by relocative centroid sorting (van Tongeren, 1986). The homogeneity of the resulting clusters was examined by comparing the taxon composition within the respective cluster. The clustering was done with the program FLEXCLUS (van Tongeren, 1986). Complementary, ordination was used to arrange the community composition along gradients. To assess if linear or unimodal techniques should be used for the ordination, a Detrended Correspondence Analysis (DCA) was run. The DCA indicated a short gradient length (< 3) and therefore indirect Principal Component Analysis (PCA) was used (Jongman et al., 1995). Thereafter, the bioassay results and the environmental variables were fitted onto the ordination space of the macroinvertebrate community using indirect gradient analysis. Rather than with direct gradient analysis, in indirect gradient analysis the ordination itself is not influenced by the input of the bioassays and environmental variables, which has the advantage that no prior decision is needed about which variables are relevant (Ter Braak and Prentice, 1988). Prior to the indirect gradient analysis, the bioassay results and the environmental variables were standardized to the mean and divided by variance (z-score) to rule out the effects of differences in unit and concentrations between variables and to give equal weight to all variables. The strength of the relationship (R^2) between community composition and the bioassay results and the environmental variables was derived from multiple regressions. To this end, the bioassay results and the environmental variables of all sites were regressed against the respective scores of the first two ordination axes. The statistical significance of the relationships was assessed using a permutation test with 999 random permutations (Oksanen et al., 2013). Additionally, a Spearman Correlation Analysis was used to test relationships between the different macroinvertebrate based indices, the bioassay results and the environmental variables, all standardized to the z-score. As the data were used twice, Bonferroni correction was applied to correct for multiple hypothesis testing (significance level of 0.05/2 = 0.025). The ordination and correlation were performed in R (R Core Team 2021; v. 3.6.3) using the packages *vegan* (Oksanen et al., 2013) and *Hmisc* (Harrell, 2022) and *ggplot2* (Wickham, 2016) to produce figures, while evenness was calculated using *codyn* (Hallett et al., 2020).

3. Results

3.1. Multi-stress

The selected sampling sites were moderately-to-severely impacted by multi-stress (Table 1; see Table S5 for an entire overview). Specifically, the toxicity indices indicated moderate to severe toxic pressure, with the Σ effect-based risk quotients in the water ranging between 1.5 and 22.8 (where values >1 suggest a potential risk; Neale et al., 2021), the msPAF in the sediment ranging between 23 and 82 % (where the % indicates the amount of species that is affected; de Zwart and Posthuma, 2005) and the TU_{tot} in the sediment ranging between 0 and 0.074 (where values above 0.001 of the acute LC50 were found to affect the share of sensitive taxa in macroinvertebrate communities; von der Ohe and de Zwart, 2013). The SOD range (0.2–2.0 g/m^2/day) and nutrient concentrations (range C:N ratios 11–25 and C:P ratios 33–4221) implied meso- to hypertrophic conditions, while the varying degree of macrophyte cover (0–70 %) reflected a wide range of habitat structure.

3.2. Whole sediment bioassays

The pH, temperature, ammonium concentration, and the performance of the control larvae in the *C. riparius* whole sediment bioassays met the validity criteria of OECD guideline 218 (OECD, 2004) (Table S7). Only site 16 showed lower pH values than the guideline value (pH 5.1 ± 0.6 instead of pH 6–9), but no effects on the outcome of the bioassay were expected (Khosrovyan et al., 2014). Survival of *C. riparius* on the field sediments was generally high (mean ± sd = 85 ± 13 %), with survival below 70 % at only 2 of the 16 sites, indicating that this endpoint was hardly affected by the sediments (Table 2). Emergence was more variable (mean ± sd = 74 ± 20 %) with emergence below 70 % at 5 of the 16 sites. Emergence time (EmT_{50}) was slightly shorter for males (15–23 days) than for females (17–25 days), resulting in an emergence time for males and females combined between 16 and 24 days (Table 2). A higher number of emerged adults was strongly positively correlated with faster emergence (Spearman $r_s = 0.87$, $p < 0.0001$), therefore, the most sensitive endpoint ($1/EmT_{50}$) was included in further analysis (Marinković et al., 2011; León Paumen et al., 2008).

3.3. Benthic macroinvertebrate community composition

A total of 12,044 individuals belonging to 64 macroinvertebrate taxa were collected, while 8 to 26 different taxa were collected per site. Evenness ranged between 0.10 and 0.35 (Table 2) while the number of Trichoptera families ranged between 0 and 4 per site (where >5 families indicates the highest quality; Verdonschot et al., 2012). A wide range of exposure to pesticide pressure was indicated by the $SPEAR_{pesticides}$ index ranging from 0.0 to 0.9 (where <0.2 indicates bad quality and > 0.8 indicates high quality; Liess et al., 2021).

The benthic macroinvertebrate community was most optimally classified in four different clusters, of which two clusters were larger (A2 and A4) and two were smaller (A1 and A3) (Table 2). The clusters are shown in the ordination of the benthic macroinvertebrate community composition (Fig. 2A). Cluster A2 was a species poor cluster (mean ± sd = 13 ± 4, $n = 6$), where mainly Chironomidae were dominant ($p = 0.001$) (Fig. 2A, Table S6). Cluster A4 was composed of multiple taxa (22 ± 4, $n = 7$), including various genera of snails, like *Bithynia* ($p = 0.004$), *Hippeutis* ($p = 0.009$) and *Valvata* ($p = 0.001$). Oligochaeta were abundant in the bottom half of this cluster ($p = 0.005$). Cluster A1 and A3 took an intermediate position regarding the number of different taxa (A1: 24 and 14, A3: 17 for the respective sites in the clusters) (Table 2). Notably, the $SPEAR_{pesticides}$ index was higher in these two clusters (A1: 0.92 and 0.70 $n = 2$: A3: 0.64, $n = 1$) compared to clusters A2

Table 2
Indices based on the benthic macroinvertebrate community composition (*i.e.* number of taxa, Smith and Wilson's evenness index, number of Trichoptera families and Spear index) and bioassay endpoints (*i.e.* survival, emergence and mean emergence time of the males and females) following the clusters of the macroinvertebrates.

		Sites															
		cluster A1		cluster A2						cluster A3	cluster A4						
Group	Variable	1	9	10	11	12	13	15	16	2	3	4	5	6	7	8	14
Benthic community	# Taxa	24	14	9	13	13	8	20	13	17	19	23	25	24	26	25	15
	Evenness	0.30	0.25	0.10	0.16	0.17	0.16	0.35	0.21	0.35	0.13	0.27	0.21	0.25	0.25	0.18	0.13
	# Trichoptera families	2	4	0	1	1	0	1	0	4	0	2	2	1	1	0	1
	Spear index	0.70	0.92	0.08	0.18	0.38	0.50	0.55	0.80	0.64	0.04	0.49	0.42	0.44	0.52	0.47	0.00
Whole sediment bioassay	Survival (%)	92	48	90	82	64	90	86	80	82	84	98	98	96	88	84	90
	Emergence (%)	72	34	90	36	62	80	64	73	50	84	98	98	96	82	76	90
	Emergence time (d)	24	24	19	24	21	21	23	22	24	18	18	19	16	22	20	20

(0.42 ± 0.26, n = 6) and A4 (0.34 ± 0.22, n = 7), indicating that more taxa at these sites were sensitive to toxic contaminants, like pesticides.

3.4. Relation between the different lines of evidence of the TRIAD

The measured variables in sediment and water were related to the ordination of the macroinvertebrate community composition (Fig. 2B, Table 3). Sites in cluster A2, taxa-poor and dominated by Chironomidae, correlated with stressors from WWTP effluent discharges, as indicated by *e.g.* the arrows for PPCPs in the water and higher TU for WWTP effluent markers in the sediment, although none of the correlations were significant (Fig. 2B, Table 3). Sites in cluster A4, inhabited by a relatively high number of taxa including various snails, correlated most strongly with the presence of PO_4-P ($R^2 = 0.56$, $p = 0.011$) and pesticides (anti AR & PR assay: $R^2 = 0.60$, $p = 0.002$) in the water, as well as a higher effect-based risk quotient ($R^2 = 0.54$, $p = 0.012$). Only the arrows for nutrient ratios and organic matter content point in the direction of clusters A1 and A3, indicating lower toxicity and eutrophication levels at these sites than at the other clusters.

Complementary to the ordination, the macroinvertebrate-derived indices were related to the variables measured in sediment and water (Fig. 3, Table S8). The number of taxa at the sites was primarily positively correlated with presence of pesticides (anti-AR & -PR assays: $r_s = 0.58$, $p = 0.017$) and PO_4-P ($r_s = 0.53$, $p = 0.034$) in the water, as well as the macrophyte cover ($r_s = 0.51$, $p = 0.042$). The three other indices revealed a different pattern in relation to the measured variables. Specifically, evenness, the number of Trichoptera families and the $SPEAR_{pesticides}$ positively correlated to the C:P ratios in the sediment ($r_s = 0.68$, $p = 0.003$; $r_s = 0.57$, $p = 0.020$ and $r_s = 0.67$, $p = 0.004$ respectively), and to a lesser extent to the C:N ratios. The number of Trichopteran families was negatively correlated to the SOD ($r_s = -0.55$, $p = 0.027$). Moreover, evenness, the number of Trichoptera families and the $SPEAR_{pesticides}$ showed a negative correlation with various contaminants, including the presence of metals (bact. Biolum. assay: $r_s = -0.71$, $p = 0.002$; rs $= -0.66$, $p = 0.006$ and $r_s = 0.48$, $p = 0.060$) and PPCP (ERα assay: $r_s = -0.60$, $p = 0.014$; $r_s = -0.48$, $p = 0.062$ and $r_s = -0.25$, $p = 0.35$), and to a lesser extent with the effect-based risk quotient in the water and the TU_{PAH} in the sediment.

Lastly, the most sensitive bioassay results were related to the benthic macroinvertebrate community and the variables measured in the sediment. The inversed emergence time ($1/EmT_{50}$) pointed in opposite direction of clusters A1 and A3 (Fig. 2B; $R^2 = 0.40$, $p = 0.047$). The larvae performed best on the sediment from sites with a lower $SPEAR_{pesticides}$ score, harboring a community that was less sensitive to toxic contaminants in the field (Fig. 3, Table S8; $r_s = -0.61$, $p = 0.013$). Moreover, the $1/EmT_{50}$ showed a positive correlation to the TU_{PAH} ($r_s = 0.74$, $p = 0.001$) in the sediment, and a negative correlation to the C:N ratios in the sediment ($r_s = -0.56$, $p = 0.024$). This suggests that faster emergence of *C. riparius* in the bioassay was related higher nutrient content (*i.e.* a lower C:N ratio) in the sediment, as positive effects from toxicants on emergence are not likely and a negative effect of toxicants was observed on the macroinvertebrate community in the field (*i.e.* lower $SPEAR_{pesticides}$).

4. Discussion

The present study aimed to elucidate the contribution of sediment contamination to multi-stress by applying an adjusted TRIAD framework. The studied drainage ditches were impacted by different combinations of multiple stressors. The benthic macroinvertebrate community composition related to these multiple stressors in both water and sediment. The number of taxa at the sites was primarily positively correlated with the PO_4-P concentration in the water, the macrophyte cover, and the presence of some pesticides. Evenness, the number of Trichoptera families and the $SPEAR_{pesticides}$ positively correlated to the C:P ratios in the sediment, whilst negative correlations were observed with various contaminants in both water and sediment. The whole-sediment bioassay with *C. riparius* positively related to the nutrient content of the sediment, whereas no negative relations to the sediment-associated contaminants were observed, even though the low $SPEAR_{pesticides}$ values indicated contaminant effects in the field. The correlation and co-presence of stressors made it difficult to prove causal relationships between individual stressors, bioassay results and macroinvertebrate community composition. Nonetheless, the adjusted TRIAD framework applied in the present study allowed to disentangle, to some extent, the contribution of sediment contamination to multi-stress in these water bodies.

4.1. Potential drivers of benthic macroinvertebrate community composition

Drainage ditches are an important habitat for aquatic macroinvertebrates, supporting a high biodiversity, including rare and threatened species (Bracewell et al., 2019; Verdonschot et al., 2011). However, most drainage ditches waters do not reach this high biodiversity potential, as multiple stressors frequently impact the benthic community composition (Maloney, 2019; Verdonschot et al., 2012). The present sampling sites also showed an impoverished macroinvertebrate community, as indicated by the low number of caddisfly families, a taxonomic metric used as an ecological quality indicator of drainage ditches (Verdonschot et al., 2012). Nonetheless, variation in the benthic macroinvertebrate community in relation to the presence and intensity of the multiple stressors was still observed.

Specifically, sites with the lowest number of taxa, where mostly chironomids were present, and a low $SPEAR_{pesticides}$ index, contained higher concentrations of WWTP effluent markers in the sediment, and higher concentrations of PPCPs in the water. This corresponds to other studies where macroinvertebrate species diversity decreased and chironomids became dominant with increasing organic pollution from WWTPs (Hynes, 1960; Wright et al., 1995). This is supported by the observation that some chironomid species can persist in contaminated sediments where they can thrive due to their rapid development and high tolerance to contamination and low oxygen levels (de Haas et al., 2005). Contrastingly, sites with a comparatively higher number of taxa, but still a low $SPEAR_{pesticides}$ index, characterized by a high abundance of various genera of snails, were likely influenced by stressors originating from agricultural activities, such as high nutrient and pesticide concentrations. At sites with higher taxa richness, the macrophyte cover was also higher, likely attributable to eutrophication, which can result in excessive

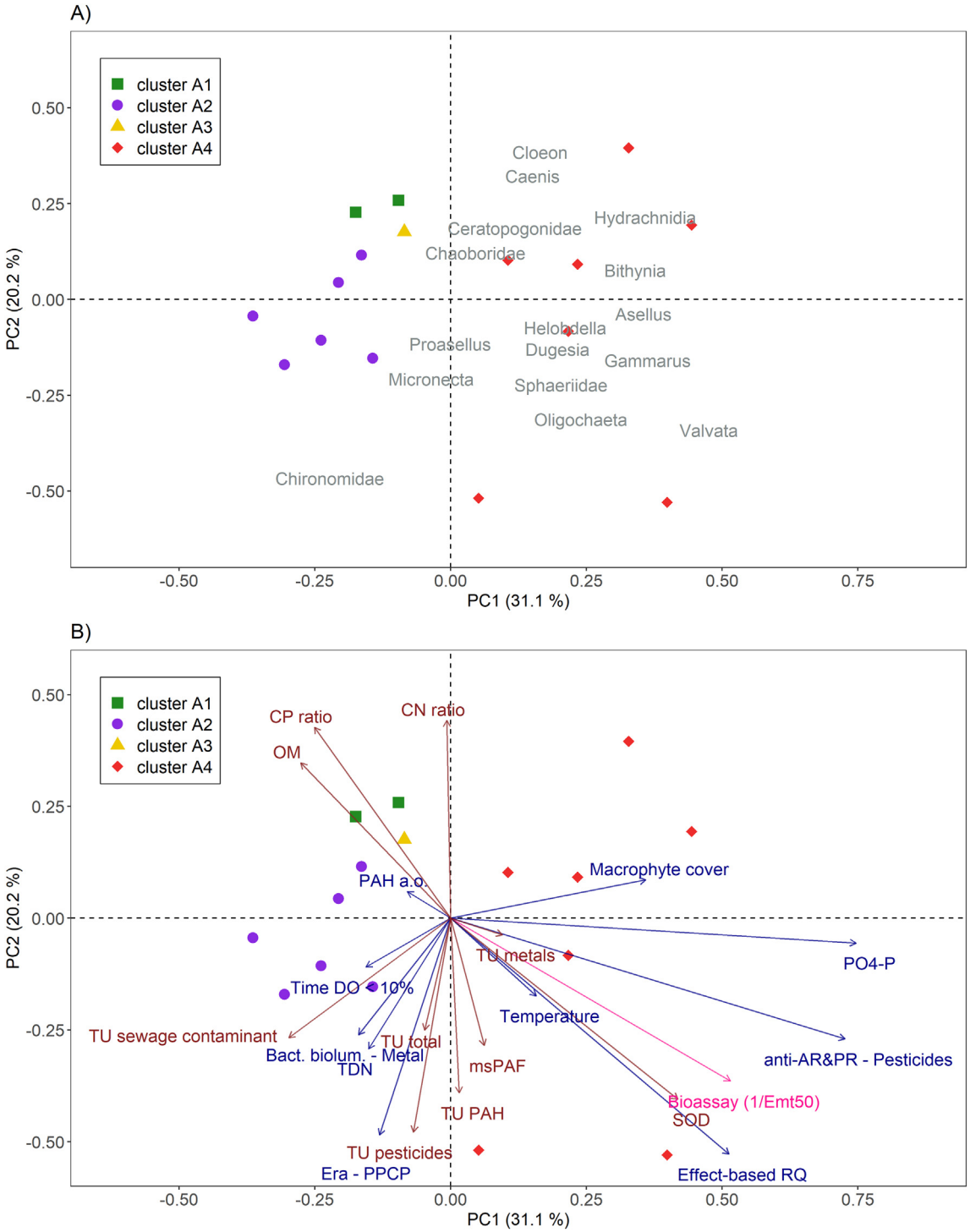

Fig. 2. PCA ordination of sites based on benthic macroinvertebrate community composition with A) Species that have the highest correlation with the ordination, and B) Bioassay (pink) and environmental variables measured in the sediment (brown) and the water column (blue) fitted onto the ordination space of the macroinvertebrate community using indirect gradient analysis. The vector (arrow in the plot) points to the direction where the variables most strongly correlates with the scores of the first two ordination axes of the PCA of the community composition. Different colors of the sites indicate different clusters.

macrophyte growth in drainage ditches (Janse and Van Puijenbroek, 1998). Snails can thrive in such ditches (Guan and Wu, 2021; Qazar, 2016). High food and habitat availability (Verdonschot et al., 2012), coinciding with the relatively low sensitivity of snails to pesticides (Reiber et al., 2020; von der Ohe and Liess, 2004), likely resulted in their higher abundance and richness. At the other sites, the $SPEAR_{pesticides}$ index was higher, which coincided with a lower toxic pressure from contaminants in both the water and sediment. Yet, also these sites were inhabited by only a moderate number of macroinvertebrate taxa. The presence of these few and common taxa still indicated the pressure of one or more stressors (Verdonschot et al., 2012).

4.2. Potential drivers of the whole sediment bioassay responses

To isolate the stressors present in the sediment from those in the water column, whole sediment bioassays were applied. The most sensitive

Table 3
Correlation of the most sensitive bioassay result and the environmental variables with PCA ordination of the macroinvertebrate community composition. The goodness-of-fit statistic is the squared correlation coefficient (R^2) and the *p*-value of the correlation envfit function (999 permutations, $N = 16$).

TRIAD	Compartment	Variable	R^2	p-value
Bioassay	Sediment	1/ EmT_{50}	0.40	0.047
Contaminants and other environmental variables	Water	Effect-based RQ	0.54	0.012
		Era - PPCP	0.25	0.17
		anti-AR&PR - Pesticides	0.60	0.002
		PAH	0.01	0.94
		Bact. biolum. - Metal	0.10	0.45
		TDN	0.11	0.47
		PO4-P	0.56	0.011
		Time DO <10 %	0.04	0.80
		Macrophyte cover	0.14	0.37
		Temperature	0.05	0.69
	Sediment	msPAF	0.08	0.55
		TU total	0.10	0.50
		TU sewage contaminant	0.16	0.29
		TU pesticides	0.23	0.17
		TU PAH	0.15	0.34
		TU metals	0.01	0.93
		CN ratio	0.20	0.22
		CP ratio	0.25	0.14
		OM	0.20	0.22
		SOD	0.34	0.056

endpoint, faster emergence was primarily related to nutrient ratios in the sediment, with a limited and in some cases even positive relation to the various sediment-associated contaminants. This is in contrast with previous work (de Baat et al., 2019), where a clear negative relationship between toxic pressure and the emergence and survival of *C. riparius* was observed using the same bioassay, whilst no relation was apparent with the C:N ratio, even though the measured C:N range was similar to that in the present study. The difference in toxic pressure in the two studies may, however, potentially explain the contrasting results. In the sediments studied by de Baat et al. (2019) the concentrations of compounds, such as PAHs, nonylphenol, and specific pesticides were even higher than in the present study (extremely severe), which likely overruled the positive effects of sediment nutritional value on the measured endpoints of *C. riparius*, as also observed by Heye et al. (2019) and Arambourou et al. (2019). In contrast, at the presently studied sites, the toxic signal was less dominant, allowing the manifestation of positive responses of the chironomid larvae to the nutritional value of the sediments. Likewise, de Haas et al. (2005) showed that a higher food quality, which provides better growth conditions, can overrule the avoidance of *C. riparius* of sediments with higher toxicant concentrations. Moreover, the higher organic matter content (coinciding with the higher C:N ratios) may have decreased the bioavailability of contaminants (Goedkoop et al., 2010), although the relation between OM and the bioassay results were not as strong. Nutrients may thus have masked the adverse effects of sediment contamination in the presently performed *C. riparius* bioassays, which was also observed in studies on various other aquatic organisms (Barmentlo et al., 2018; Ieromina et al., 2014).

4.3. Are C. riparius bioassays effective in isolating the effect of sediment contamination in multi-stressed water bodies?

The positive response of the *C. riparius* larvae to nutrient enrichment at moderately to severely contaminated sites is in line with their characterization as opportunistic tube-dwelling deposit feeders (Armitage et al., 1995) that are fairly tolerant to chemical contamination (van den Berg et al., 2019). The present findings thus question the use of chironomids to isolate the effects of sediment contamination from the other stressors in moderately to severely polluted sediments, as for instance the pesticide toxicity in the sediment was not picked up. OECD guidelines are available for only for a few sediment test species, mainly oligochaetes and chironomids (OECD, 2004; OECD, 2008), and the choice for chironomids is well motivated. Larvae of *C. riparius* collect particles at the sediment surface for building their tube and for feeding (Brennan and McLachlan, 1979). This way they are exposed to sediment-associated contaminants (Leppanen and Kukkonen, 1998), in addition to partitioning based contaminant exposure, which is considered to be the most relevant exposure route (Leslie et al., 2002). Yet, the results of the present study plead for other, preferably more sensitive sediment test organisms. Employing multiple test species in whole sediment bioassays, such as *Hyalella azteca* (ISO 16303, 2013) or *Caenorhabditis elegans* (ISO 10872, 2020), could increase the chance to demonstrate toxic effects of contaminated sediments. However, a systematic approach to improve employing alternative test species and developing a wider choice of test protocols is still lacking. A battery of bioassays that are sensitive to sediment-associated contaminants could, therefore, represent a necessary innovation in the field of sediment quality assessment.

4.4. Perspectives

Contaminated sediments can represent serious ecotoxicological and ecological risks to benthic communities (Bian et al., 2016; Brack et al.,

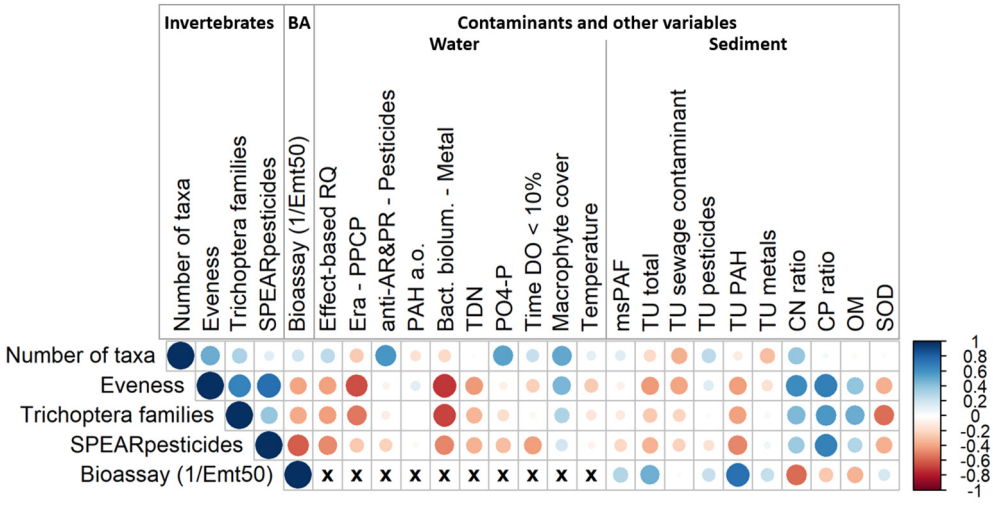

Fig. 3. Spearman Correlation matrix between the different macroinvertebrate based indices, the bioassay (BA) and the contaminants and other variables measured in the water and sediment. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients. In the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors. The bioassay result was not correlated to the variables measured in the water as the test only includes sediment from the site.

2017). In the present study sediment contamination was also identified as one of the multiple stressors that jointly impacts the benthic macroinvertebrate community composition in multi-stressed waters. Due to their response to the sediment nutritional value, it is debatable, however, whether chironomids are the most appropriate test species to isolate the effects of contaminated sediments in multi-stressed sites. Besides the quest for more sensitive test species, a promising avenue to isolate the effects of sediment-associated contaminants would be the application of passive equilibrium sampling in combination with *in vitro* and *in vivo* bioassays (Muz et al., 2020; Niu et al., 2020). Passive equilibrium sampling is performed by bringing a polymer into contact with the sediment to achieve equilibrium with the target contaminants (Mayer et al., 2003). Extracts can then be used for chemical analysis and/or in *in vitro* and *in vivo* bioassays. The loaded polymer can also be used for passive dosing in acute toxicity tests (Kwon et al., 2020). Alternatively, the diagnostic value of individual macroinvertebrate species of the *in situ* community to different stressors could be further explored (Verdonschot and van der Lee, 2020). In conclusion, restoring, exploring, and protecting the ecological potential of the 300,000 km multi-stressed Dutch drainage ditches requires a management approach that simultaneously tackles all jointly present stressors to facilitate a thriving benthic macroinvertebrate community.

CRediT authorship contribution statement

All authors conceptualised the study. NW, GHvdL and MLdB conducted the field work. NW performed chemical and non-chemical analysis of the sediment and conducted the bioassay, GHvdL performed non-chemical analysis of the water and sediment and identified the invertebrates, MLdB performed chemical analysis of the water. GHvdL analysed the data. NW and GHvdL wrote the first draft of the manuscript. All authors critically reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157045.

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