CHAPTER 3

THE CONTRIBUTION OF PESTICIDES TO THE VARIANCE IN COMMUNITY COMPOSITION OF AQUATIC MACROFAUNA IN THE FIELD

O. Ieromina[†], W.J.G.M. Peijnenburg[†,‡], C.J.M. Musters[†], M.G. Vijver[†]
[†]Institute of Environmental Sciences, Leiden University, P.O.Box 9518, 2300 RA, Leiden, The Netherlands; [‡]National Institute for Public Health and Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

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Abstract

Ditches surrounding agricultural fields in the Netherlands serve predominantly the function of flood control, and in addition accommodate aquatic plant and animal species. The studies addressing the effects of pesticides on aquatic biota in the field are scarce. The current study aimed to assess the contribution of pesticides along with other factors to the total variance in community composition of aquatic macrofauna in ditches next to flower bulb fields. Macrofauna samples and environmental data were collected during two consecutive years (2011 - 2012) in ditches next to flower bulb fields and pastures. Watersheds in the nature reserve area next to the polders were sampled as control sites. Data was analyzed with the variance partitioning procedure based on the redundancy analysis (RDA). The total variance in macrofauna community composition was divided into the variance explained by pesticides, environmental factors, the presence of other biota, time, shared variance, and unexplained variance. The total explained variance reached 22.6%. The largest proportion of explained variance (10.1%) was attributed to environmental factors, followed by pesticides (5.4%) and time (4.8%). When each macrofauna group was analyzed separately, the presence of other biota and environmental factors explained the largest proportion of variance in most of the macrofauna groups. Results of the study indicate that environmental factors, biotic interactions and temporal variation influence freshwater macrofauna considerably along with pesticides. We suggest that environmental managers should consider the multiple stressor context of aquatic ecosystems.

Keywords: abiotic factors, biotic factors, freshwater macrofauna, pesticides, RDA

Introduction

Ditches are the representative aquatic ecosystems in the Netherlands and next to their direct function of water level control also have high aquatic biodiversity (Verdonschot et al., 2012). Drainage ditches contain high numbers of aquatic plant and animal species, as well as semi-terrestrial species. Macrofauna in turn plays an important role in the food chain and biochemical cycles in the aquatic ecosystem. Thus, the presence of macrofauna in the sediment enhances the microbial nitrogen cycle by bioturbation. Bioturbation facilitates the transport of inorganic and organic nitrogen between sediment and water (Laverock et al., 2011; Kristensen & Kostka, 2005). This way macrofauna takes part in the processes of nitrification and denitrification, which in turn link nutrients in water to microbial communities in sediments (Stief, 2013). Macrofauna living in the water column feed on unicellular algae and bacteria, consuming fixed nitrogen and controlling the nitrogen pool in the ecosystem (Stief, 2013).

To protect aquatic biodiversity and the ecosystem functions it performs, it is important to understand the effects of chemicals on aquatic biota in the field. The most important reason is that in the realistic environment various abiotic and biotic factors influence the fate of pesticides and the performance of aquatic organisms. Several studies underlined that it is important to consider ecological parameters in ecotoxicological studies (Liess et al., 2003; Clements et al., 2012; Maund et al., 1997). Policy guidelines also emphasized that complexity within the biological communities, and the presence of multiple stress factors in natural ecosystems represents one of the challenges in ecological risk assessment (SCENIHR, SCHER, SCCS, 2012). A number of studies did include ecological factors in the assessment of pesticide effects on aquatic biota in the field. For instance, in the study of Berenzen et al. (2005) the effects of pesticides on aquatic invertebrates in freshwater streams were analysed in combination with environmental factors. Martin et al. (2012) studied the responses of aquatic invertebrates to pesticide runoff accounting for physico-chemical and hydrological parameters, and the vegetation coverage. Bollmohr et al. (2011) studied the effects of pesticides along with environmental factors on benthic communities in estuary. However, to our knowledge, the effects of pesticides on aquatic biota in the field, in combination with abiotic, biotic factors and time, have not been studied before.

In the present study we aimed to quantify what proportion of the total variance in aquatic macrofauna community composition (including crustaceans, annelids, molluscs, fish and insects) can be explained by pesticides, environmental factors (water chemistry parameters and macrophyte coverage), presence of other biota and time. To answer these questions, macrofauna sampling, measurements of water chemistry parameters and pesticide concentrations in ditches of the Dutch polders with intensive flower bulb crops were done during two consecutive years (2011 - 2012). Variance partitioning based on the redundancy analysis (RDA) was used to rank the explanatory factors (pesticides, environmental factors, biota, time and shared variance) with respect to the amount of explained variance.

Materials and methods

Research area

The research area is located in the flower bulb growing area of The Netherlands. There is an elevation gradient in the area: the height above sea level decreases gradually from the nature reserve (the highest site is located 4.26 - 4.5 m above the sea level) towards the polders (the lowest site is located -0.49 m to -0.25 m below the sea level). The nature reserve area is located on the northern part of the polder, so that no contamination comes from the north and north-west side. The water flows mainly in the South-West direction partly due to the pumping of the water coordinated by the water management board "Rijnland" and partly by the natural elevation gradient. A detailed description of the research area is given in Ieromina et al. (2014).

Sampling sites

During the year 2011, macrofauna and water chemistry samples were collected at 14 sites within the area: two sites in watersheds of the nature reserve, two sites in ditches alongside pastures and ten sites in ditches alongside flower bulb fields. Sampling was performed during the period April – November 2011 six times in order to account for seasonal fluctuation in water chemistry parameters and macrofauna life cycles. This period corresponds to the main phase of agricultural activities in the area. The same sites were sampled again in 2012, with four additional sites (two watersheds of nature reserve and two ditches next to pastures), located next to the polder area, sampled 4 times a year. In total 148 macrofauna and water chemistry samples were collected. Coordinates of the sampling sites are given in the Supplemental Data (Table S1).

Pesticide and nutrient measurements

Concentrations of pesticides were measured by Omegam Laboratoria BV. Pesticides were measured according to the standard guidelines (GC-MS and LC-MS/MS analysis). Nutrients were measured according to the following guidelines: NEN 6663 for phosphate and NEN-EN-ISO 13395 for nitrate and nitrite. Water samples for pesticide measurements were collected in watersheds of nature reserve (site D1) and in ditches of the flower bulb area (P1, P2, P3, F1, F2, F3, F5, F6, F8, and F10). Pesticide and nutrient concentrations were found to be below detection limits in samples collected in the nature reserve site D1. Therefore, pesticide and nutrient concentrations were assumed to be below detection limits in other nature reserve sites D2, D3 and D4. Sampling for pesticide and water chemistry measurements and macrofauna sampling were done during the same day. If the concentration of a pesticide was below its limit of detection, half of the detection limit was used in the data analysis (Clarke, 1998). The overview of environmental parameters and pesticide concentrations at the sampling sites is given in the Supplemental Data, (Tables S2 and S3).

Measuring water chemistry parameters

The following water chemistry parameters were measured: temperature (°C), dissolved oxygen (DO, mg/L), pH and conductivity (mS). Temperature and Oxygen were measured with a Z521 Consort Oxygen meter. pH was measured with a Greisinger electronic pH-meter. Conductivity was measured with a Eijkelkamp Agriresearch Equipment conductivity-meter. DOC measurements were done with a non-dispersive infrared detector (NDIR). In addition, the percentage of water surface covered with floating macrophytes was estimated.

Macrofauna sampling and determination

Macrofauna samples were collected with a dipping net (mesh size of 500 µm). The dipping net with a 0.25 m opening was dragged over a total length of 5 m of the upper part of the sediment layer (depth 3-5 cm within the sediment layer). Therefore, 20 sampling units (each sampling unit was 0.25 m×0.25 m) in total were collected from dominating habitats according to the method described in Keizer-Vlek et al. (2011) and Vlek et al. (2006), hence resulting in a multi-habitat sampling strategy.

Larger organisms (for instance: Gastropoda, Coleoptera) were identified in the field or photographed for further identification. After all sampling units were collected, macrofauna samples were rinsed and transferred to plastic sample jars. Samples were preserved with 70% ethanol directly after sampling. Samples were washed in the laboratory, sorted and identified to the lowest taxonomic level feasible. Latin names for species, genus, family, order and class were verified in ITIS (the Integrated Taxonomic Information System, http://www.itis.gov/). The level of identification for each taxonomic group is given in the Supplemental Data (Table S4).

Statistical analysis

Principal component analysis (PCA) of macrofauna community composition

Principal component analysis (PCA) was performed on macrofauna abundance data on the level of order to identify variation patterns in the macrofauna community composition and visualize groups of sampling sites containing similar macrofauna taxa. This analysis was done for macrofauna collected from all sampling locations (N = 145) and separately for macrofauna collected from sampling sites where pesticide concentrations were measured (N = 79). Prior to analysis, all biological data were transformed using the Hellinger transformation (Legendre & Gallagher, 2001). In all multivariate analysis, data were centered by species and not centered by sample (Leps & Smilauer, 2003).

Selecting explanatory variables for Redundancy Analysis (RDA)

Variance partitioning based on RDA was applied to divide the total variance in macrofauna community composition into different components (Borcard et al., 1992; Leps & Smilauer, 2003). Four groups of explanatory variables were defined: pesticides, environmental factors, time, and the presence of other biota. A list of explanatory variables included in each

component of variance is given in Table 1. Variance partitioning analysis was based on the data from sampling sites at which pesticide concentrations were measured (N = 79). In previous studies, the percentage of explained variance obtained in canonical analysis is denoted as R^2 (Peres-Neto et al., 2006). Similarly, in the current manuscript we imply canonical R^2 when referring to the percentages of explained variance.

Table 1. List of response variables and explanatory variables included in four variance components

Response variables	Components of variance	Explanatory variables included in variance components
Total species composition	Pesticides (P)	Chlorprofam, pirimiphos-methyl, tolclophos-methyl, carbendazim, imidacloprid, isoproturon, imazalil, methiocarb, ethiofencarb
	Environmental factors (E)	Temperature, dissolved oxygen, dissolved organic carbon, phosphate, nitrite, nitrate, macrophyte coverage
	Time (T)	number of the year, number of the month
Species composition of different macrofauna groups (orders Hemiptera, Diptera, Ephemeroptera, Trichoptera, Odonata, Coleoptera, Gasterosteiformes, Haplotaxida, Diplostraca, Basommatophora, Heterostropha, Veneroida, Neotaenioglossa, Rhynchobdellida)	Biota (B)*	Hemiptera, Diptera, Ephemeroptera, Trichoptera, Plecoptera, Odonata, Coleoptera, Lepidoptera, Collembola, Megaloptera, Gasterosteiformes, Cypriniformes, Rhynchobdellida, Haplotaxida, Tricladida, Isopoda, Cyclopoida, Diplostraca, Amphipoda, Arguloida, Anostraca, Mysida, Basommatophora, Heterostropha, Architaenioglossa, Neotaenioglossa, Veneroida, Ostracoda, Acari

*When each macrofauna group was analysed separately, additional biota component of variance was included in the analysis

Table 2. Summary of variance partitioning analysis

Response variable	Estimated component of variance	Explanation	Calculation procedure
Total macrofauna	Total Variance	all variance	assumed to be 100%
community composition	P∪E∪T	total explained variance	all groups of variables (P, E, T) included as explanatory variables
	residual variance	unexplained variance	100% - P∪E∪B
	P|E∪T	variance explained by pesticides only	pesticides included as explanatory variables, environmental factors and time-covariables
	E|P∪T	variance explained by environmental factors only	environmental factors included as explanatory variables, pesticides and time-covariables

Table 2. Summary of variance partitioning analysis (*Continued*)

Response variable	Estimated component of variance	Explanation	Calculation procedure
	T|E∪P	variance explained by time only	time included as explanatory variable, environmental factors and pesticides-covariables
	P∩E∩T	shared variance by P, E and T	P∪E∪T -P|E∪T - E|P∪T- T|E∪P (equation 1)
	P*	all variance explained by pesticides	pesticides included as explanatory variables, no covariables
	E*	all variance explained by environmental factors	environmental factors included as explanatory variables, no covariables
	T*	all variance explained by time	time included as explanatory variable, no covariables
	T∩E	shared variance between time and environmental factors	P∩E∩T - (P - P|E∪T)
	T∩P	shared variance between time and pesticides	P∩E∩T - (E - E|P∪T)
	P∩E	shared variance between pesticides and environmental factors	P∩E∩T - (T - T|E∪P)
	PTE	joined variance between P, T and E	P∩E∩T- T∩E - T∩P - P∩E
Composition of different macrofauna groups	P∪E∪T∪B	total explained variance	all groups of variables (P, E, T, B) included as explanatory variables
	P|E∪T∪B	variance explained by pesticides only	pesticides included as explanatory variables, environmental factors, biota and time-covariables
	E|P∪T∪B	variance explained by environmental factors only	environmental factors included as explanatory variables, pesticides, biota and time-covariables
	T|E∪P∪B	variance explained by time only	time included as explanatory variables, environmental factors, biota and pesticides-covariables
	B|E∪P∪T	variance explained by biota only	biota included as explanatory variables, environmental factors, time and pesticides-covariables
	P∩E∩T∩B	shared variance by P, E, T and B	P∪E∪T∪B - P|E∪T∪B - E|P∪T∪B - T|E∪P∪B - B|E∪P∪T (equation 2)

*including variance shared with other factors

Response variable datasets consisted of the total macrofauna community composition and of the composition of different macrofauna groups on the level of order (Table 2).

The variance in total macrofauna community composition was divided into five components: variance explained by pesticides (P|E∪T), environmental factors (E|P∪T), time (T|P∪E), shared variance between pesticides, environmental factors and time (P∩E∩T), and residual (unexplained) variance (Table 2, Supplemental Data, Figure S1).

The variance in each macrofauna group (Hemiptera, Diptera, Ephemeroptera, Trichoptera, Odonata, Coleoptera, Gasterosteiformes, Haplotaxida, Diplostraca, Basommatophora, Heterostropha, Veneroida, Neotaenioglossa, and Rhynchobdellida) was divided into six components: variance explained by pesticides (P|E∪T∪B), environmental factors (E|P∪T∪B), the presence of other biota (abundance of other macrofauna orders except the order being analysed) (B|E∪P∪T), time (T|E∪P∪B), shared variance between pesticides, environmental factors, biota and time (P∩E∩T∩B), and unexplained variance.

Data transformation prior RDA

The number of explanatory variables in each component of variance was different (Table 1). Generally, the number of explanatory variables included in RDA model affects the model outcome: the explained variance increases even when additional variables that only contain noise (i.e. are not related to the response variable) are included (Freedman, 1983, Peres-Neto et al., 2006). To account for different number of explanatory variables in variance components, a PCA was performed on each set of explanatory variables, and sample scores of the first four Principal Components (PC) axes were included in RDA as explanatory variables. Results of PCA on pesticide, environmental factors, biota, and time data datasets can be found in Supplemental Data, Tables S5 and S6. In addition, the correlation between PC sample scores was checked prior the RDA. The correlation coefficient between PC scores was below 0.5.

Missing values in the water chemistry dataset were estimated based on the average values of the variable calculated from the samples collected at the same date/sampling site (Leps & Smilauer, 2003). In addition, we performed PCA on datasets with and without missing values to test if PCA results differed between the two datasets. The results of PCA on datasets with and without estimated missing values were similar (Supplemental Data, Tables S5 and S6). Therefore, further analysis (RDA on PC sample scores) was based on the dataset with estimated missing values, because otherwise the software would replace the missing values with zeros.

Variance partitioning procedure

The Total Variance (TV) in macrofauna community composition represented the sum of unconstrained and constrained eigenvalues. The total explained variance (P∪E∪T for total macrofauna community and P∪E∪T∪B for different macrofauna groups) represented the amount of variance explained by all variance components, or the sum of the constrained eigenvalues. The total explained variance was obtained by constructing a RDA in which all groups of explanatory variables (PC sample scores obtained from PCA

on pesticide, environmental factor, time, and biota datasets) were included in the analysis as explanatory variables.

The percentage of variance explained by pesticides was estimated by constructing a partial RDA in which pesticide data (PC sample scores obtained from PCA on pesticide dataset) were included in the analysis as explanatory variables, and PC sample scores obtained from PCA on environmental, time and biotic datasets were included in the analysis as covariables (Table 2). Similar procedure was repeated to quantify the percentages of variance explained by environmental factors, time and biota. Proportion of variance in macrofauna community composition shared by different factors was estimated based on equations 1 and 2 presented in Table 2. In addition, Ezekiel's R^2 adjustment was applied to the estimated explained variance according to the formula:

$$R2adjusted = 1 - \frac{n - 1}{n - p - 1}(1 - R2)$$ **(Peres-Neto** et al., 2006).

where n = number of samples, p = number of explanatory variables, R^2 = percentage of explained variance. In the manuscript we refer to R^2 adjusted. Multivariate analysis was performed in Canoco software version 4.5 (Lepš & Šmilauer, 2003).

Results

Macrofauna community composition

The order Diptera contained the highest number of species followed by the order Coleoptera (Supplemental Data, Table S4). As a result of Principal Component Analysis (PCA), first four PC explained 61.6% of variance in macrofauna abundance on the level of order (based on the macrofauna data from sampling sites where pesticide concentrations were measured, N = 79) (Supplemental Data, Table S7). Diplostraca contributed mostly to the PC1, Ephemeroptera – to PC2 and Diptera – to PC3. High abundances of crustaceans Diplostraca, annelid Haplotaxida, gastropods Basommatophora and Heterostropha were associated with ditches adjacent to flower bulb fields (Figure 1). On the other hand, watersheds of nature reserve contained high numbers of insects Ephemeroptera, Odonata, Diptera, and Trichoptera. A similar result was found when PCA was performed on macrofauna data collected from all sampling sites (N = 145) (Supplemental Data, Figure S2, Table S8). Average abundances of macrofauna on the level of order and standard deviations are given in Supplemental Data, Table S9.

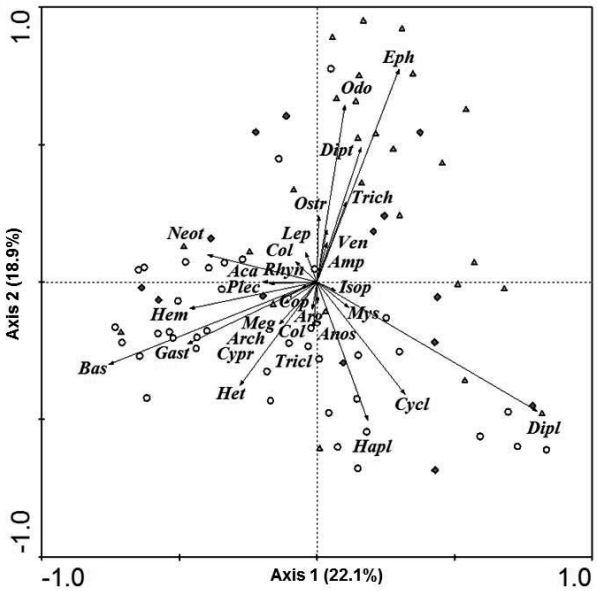

Figure 1. Principal component analysis of Hellinger-transformed macrofauna abundance on the level of Order (N=79). Hem = Hemiptera, Dipt = Diptera, Eph = Ephemeroptera, Trich = Trichoptera, Plec = Plecoptera, Odo = Odonata, Col = Coleoptera, Lep = Lepidoptera, Meg = Megaloptera, Colm = Collembola, Gast = Gasterosteiformes, Cypr = Cypriniformes, Rhyn = Rhynchobdellida, Hapl = Haplotaxida, Tricl = Tricladida, Isop = Isopoda, Ostr = Ostracoda, Cycl = Cyclopoida, Dipl = Diplostraca, Cop = Copepoda, Amp = Amphipoda, Arg = Arguloida, Anos = Anostraca, Mys = Mysida, Bas = Basommatophora, Het = Heterostropha, Ven = Veneroida, Neot = Neotaenioglossa, Arch = Architaenioglossa, Aca = Acari, Tricl = Tricladida. Triangular = sites in watersheds of nature reserve, Circle = sites in ditches next to flower fields, Diamond = sites in ditches next to pastures

Table 3. Components of variance estimated for total macorfauna community composition: total explained variance (P∪E∪T), residual variance, variance explained by pesticides (P|E∪T), environmental factors (E|P∪T), time (T|E∪P), shared variance between pesticides, environmental factors and time (P∩E∩T), shared variance between pesticides and environmental factors (P∩E), pesticides and time (P∩T), time and environmental factors (T∩E), joined variance between three components (TPE). Presented are the percentages of explained variance (R^2) and R^2 adjusted by Ezekiel's transformation (in italic)

Response group	P∪E∪T	Residual variance	P|E∪T	E|P∪T	T|E∪P	P∩E∩T	P∩E	P∩T	T∩E	TPE
Macrofauna community composition	19	81	4.7	8.6	4.2	1.5	1.1	0.3	0.2	-0.1
	22.6	*77.4*	*5.4*	*10.1*	*4.8*	*1.6*	*1.1*	*0.2*	*0.04*	*-0.3*

Variance partitioning of macrofauna community composition

When the total community composition was analyzed, the explained variance reached 22.6% (Table 3). Environmental factors contributed mostly to the explained variance (10.1%), followed by pesticides (5.4%) and time (4.8%). Shared variance between all factors was 1.6% (Table 3).

From all macrofauna groups analyzed, the percentage of explained variance was the highest for Rhynchobdellida and Ephemeroptera (59.2% and 51.8%, respectively). The variance in Diplostraca was least explained least by RDA model (total explained variance was 12.5%) (Table 4). All components explained between 1% and 17% of the total variance in macrofauna, except for the time (the percentage of variance in Ephemeroptera explained by time reached 21%) (Table 4).

Discussion

Macrofauna community composition in the research area

For most of the macrofauna taxonomic groups, the number of species found in the current study was in line with previous field studies performed in the Netherlands (Keizer-Vlek et al., 2011). The following orders showed the highest similarity with the study of Keizer-Vlek et al. (2011) in terms of the number of taxa: Bivalvia, Trichoptera and Diptera (Supplemental Data, Table S4).

As seen in PCA plots (Figure 1, Supplemental Data, Figure S2), higher densities of insect larvae Trichoptera, Odonata, Ephemeroptera and Diptera were associated with nature reserve. Based on the analysis of the water chemistry dataset, concentrations of dissolved oxygen were generally higher in watersheds of nature reserve than in agricultural ditches. While the levels of nutrients and pesticides in water were considerably lower in the nature reserve (Supplemental Data, Tables S2 and S3). Therefore, high water quality of the nature reserve favored sensitive insect species. The high sensitivity of insect larvae to pesticides observed in our study complies with previous findings (Berenzen et al., 2005; Liess & Von der Ohe, 2005).

On the other hand, abundances of insects Hemiptera, gastropods Bassomatophora and Heterostropha, crustacean Diplostraca, annelids Rhynchobdellida and Haplotaxida were larger in ditches of agricultural area. Similarly, large abundances of Gastropoda, Hirudinea and Oligochaeta in contaminated waters were found in the study of Berenzen et al. (2005). Species from these taxonomic groups are described as generally tolerant to organic pollution (Hilsenhoff 1987, 1988; Murdoch et al., 1996). Previous study of Armendáriza et al. (2012) suggested that nutrients increase the biomass of bacteria and algae used by Oligochaeta

Table 4. Components of variance estimated for macrofauna groups: total explained variance (P∪E∪T∪B), residual variance, variance explained by pesticides (P|E∪T∪B), environmental factors (E|P∪T∪B), biota (B|E∪P∪T), time (T|E∪P∪B) and shared variance (P∩E∩T∩B). Presented are the percentages of explained variance (R^2) and R^2 adjusted by Ezekiel's transformation (in italic)

Response group	P∪E∪T∪B	Residual variance	P|E∪T∪B	E|P∪T∪B	B|E∪P∪T	T|E∪P∪B	P∩E∩T∩B
Rhynchobdellida	49.5	50.5	14	9.1	4.8	0.7	20.9
	59.2	*40.8*	*16.6*	*10.7*	*5.6*	*0.6*	*24.9*
Ephemeroptera	43.3	56.7	1.5	9.3	6.4	18.2	7.9
	51.8	*48.2*	*1.6*	*11.0*	*7.5*	*21.6*	*9.3*
Odonata	35.3	64.7	1.4	13.4	14.2	2.3	4
	42.2	*57.8*	*1.5*	*15.9*	*16.8*	*2.6*	*4.6*
Neotaenioglossa	34.1	65.9	4.9	12.8	4.4	3.5	8.5
	40.7	*59.3*	*5.7*	*15.2*	*5.1*	*4.0*	*10.0*
Heterostropha	30.6	69.4	8.1	6.7	7.9	4.6	3.3
	36.5	*63.5*	*9.5*	*7.8*	*9.3*	*5.3*	*3.8*
Gasterosteiformes	29.7	70.3	10.2	5	4.3	2.3	7.9
	35.4	*64.6*	*12.0*	*5.8*	*5.0*	*2.6*	*9.3*
Basommatophora	25.3	74.7	5.9	6.2	8	1.5	3.7
	30.2	*69.8*	*6.9*	*7.2*	*9.4*	*1.6*	*4.2*
Haplotaxida	18.5	81.5	6	7.9	3	0.1	1.5
	22.0	*78.0*	*7.0*	*9.3*	*3.4*	*-0.1*	*1.6*
Hemiptera	17.6	82.4	2.9	6.9	3.5	2.3	2
	20.9	*79.1*	*3.3*	*8.1*	*4.0*	*2.6*	*2.2*
Veneroida	15.8	84.2	1.1	6.1	8.7	5.7	-5.8
	18.8	*81.2*	*1.1*	*7.1*	*10.2*	*6.6*	*-7.2*
Trichoptera	14.6	85.4	1.9	4	8.2	0.8	-0.3
	17.3	*82.7*	*2.1*	*4.6*	*9.6*	*0.8*	*-0.6*
Diptera	13.7	86.3	1.9	6.2	3.9	3.7	-2
	16.2	*83.8*	*2.1*	*7.2*	*4.5*	*4.2*	*-2.6*
Coleoptera	13.3	86.7	1.5	2.5	5	2	2.3
	15.8	*84.2*	*1.6*	*2.8*	*5.8*	*2.2*	*2.6*
Diplostraca	10.6	89.4	1.5	3.7	3.9	0.5	1
	12.5	*87.5*	*1.6*	*4.2*	*4.5*	*0.4*	*1.0*
Average R^2 adjusted	30.0	70.0	5.2	8.4	7.2	3.9	4.5

as a food source. This way, nutrients induce positive effect on Oligochaeta abundance (Armendáriza et al., 2012).

Variance partitioning of macrofauna community composition

When variance partitioning was applied to the total macrofauna community composition, the overall explained variance reached 22.6%. Other field studies reported similar percentages of variance in biological communities explained by different factors. For instance, in Zuellig et al. (2012), the total variance in freshwater algae, fish, and invertebrate communities explained by between-site variance and time was ~30%. The variance in macroinvertebrate community explained by environmental and spatial factors reached ~ 25% in the study of Heino et al. (2012). Out of 22.6% of total explained variance found in our study, the largest proportion of variance (10.1%) was attributed to environmental factors, followed by pesticides (5.4%), and time (4.8%).

First, our results suggest that environmental factors induce the largest effect on macrofauna community composition. Previous studies emphasized the importance of environmental factors in shaping community composition of aquatic biota. For instance, in the study of Larsen et al. (2012), environmental factors were more important than species interactions in structuring fish and invertebrate communities. In the study of Zuellig et al. (2012), environmental factors dominated the inter-annual variance in shaping invertebrate community. Water chemistry parameters vary significantly in ditches next to flower bulb fields. For instance, the average phosphate and nitrate concentration in ditches varied from 0.03 mg/L to 4.10 mg/L and from 0.05 to 0.6 mg/L, respectively. Average DOC levels varied between 48 mg/L and 290 mg/L (Supplemental Data, Table S2). DOC and nutrients relate to food availability for aquatic macrofauna and limit the performance of many aquatic species. Large variation in these important parameters can possibly explain the high contribution of environmental factors to the total variance in macrofauna community composition. As a second conclusion, the contribution of pesticides to the total variance in macrofauna community composition was two times lower then the contribution of environmental factors (5.4%). There are not many studies quantifying the contribution of toxicants to the variance in community composition of aquatic biota. In the study of De Zwart (2006), the toxicants explained 3% of the total variance in fish communities inhabiting rivers, relative to 28% of variance explained by water chemistry parameters and 16% of variance explained by habitat characteristics. Similarly to our study, environmental factors dominated toxicants in structuring community composition of aquatic biota. Third, we observed a relatively high contribution of the time to the total variance (4.8%) that can be explained by seasonal variation in macrofauna community composition. Shared variance between different components of variance explained up to 1.6% of the total variance in macrofauna community composition. Shared variance can be possibly explained by correlation between different factors. For instance, it is documented that the fate of pesticides in the aquatic environment is largely dependent on environmental conditions (Maund et al.,

1997). In addition, nutrients co-occur with pesticides due to their similar origin: pesticides and fertilizers are applied together at the bulb fields.

The average percentage of total explained variance for all macrofauna groups was 30.0%. On average, biota and environmental factors components explained the largest percentage of variance in different macrofauna groups, followed by pesticides, shared variance between all four components and time. This result can possibly be explained by the importance of biotic interactions and site-specific environmental conditions in structuring macrofauna community composition. RDA procedure yielded a negative value for shared variance for Diptera, Trichoptera and Veneroida (Table 4). Such a result for shared variance means that the groups of variables separately explain the variance in the response variable better than when combined together (Legendre & Legendre, 2012).

Variance partitioning based on the redundancy analysis allowed us to quantify the contribution of different field-relevant factors to the total variance in macrofauna community composition. Results of the study indicated that in most of the macrofauna groups, the contribution of environmental factors and presence of other biota to the total variance exceeded the contribution of pesticides, or was equally important.

Conclusions

The entire aquatic macrofauna community composition was highly dependent on environmental factors that made a twofold higher contribution to the total explained variance than pesticides. Based on our results we can conclude that the responses of macrofauna community to pesticides in the field are largely dependent on environmental factors. Policy guidelines developed to protect surface water and preserve aquatic biodiversity should include multi-stressor assessments at tiered levels, taking into account abiotic factors, habitat features, biotic interactions, as well as the differences in responses of distinct macrofauna groups due to their varying ecological preferences.

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References cited

1. Armendáriza L, Ocóna C & Rodrigues AC. 2012. Potential responses of oligochaetes (Annelida, Clitellata) to global changes: Experimental fertilization in a lowland stream of Argentina (South America). *Limnologica - Ecology and Management of Inland Waters*, *42(2):* 118–126.
2. Berenzen N, Kumke T, Schulz HK & Schulz R. 2005. Macroinvertebrate community structure in agricultural streams: impact of runoff-related pesticide contamination *Ecotoxicology and Environmental Safety 60:* 37-46.
3. Borcard D, Legendre P & Drapeau P. 1992. Partialling out the Spatial Component of Ecological Variation. *Ecology, 73:* 1045–1055.
4. Bollmohr S, Brink PJ van den, Wade PW, Day JA & Schulz R. 2011. Environmental variables, pesticide pollution and meiofaunal community structure in two contrasting temporarily open/closed false bay estuaries. SourceWater SA 37. ISSN 0378-4738.
5. Clarke JU. 1998. Evaluation of censored data methods to allow statistical comparisons among very small samples with below detection limit observations. *Environmental Science & Technology 32:* 177-183.
6. De Zwart D, Dyer SD, Posthuma L & Hawkins CP. 2006. Predictive models attribute effects on fish assemblages to toxicity and habitat alteration. *Ecological Applications* 16(4): 1295-310.
7. SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), SCHER (Scientific Committee on Health and Environmental Risks), SCCS (Scientific Committee on Consumer Safety). 2012. Preliminary report on Addressing the New Challenges for Risk Assessment. European Commission.
8. Freedman DA. 1983. A Note on Screening Regression Equations. *American Statistician 37*: 152-155.
9. Heino J, Grönroos M, Soininen J, Virtanen R & Muotka T. 2012. Context dependency and metacommunity structuring in boreal headwater streams. *Oikos 121*: 537–544.
10. Hilsenhoff WL. 1987. An improved biotic index of organic stream pollution. *Great Lakes Entomologist, 20:* 31-39.
11. Hilsenhoff WL. 1988. Rapid Field Assessment of Organic Pollution with a Family-Level Biotic Index. *Journal of the North American Benthological Society, 7(1)*: 65-68.
12. Ieromina O, Peijnenburg WJGM, De Snoo GR & Vijver MG. 2014. Population responses of Daphnia magna, Chydorus sphaericus and Asellus aquaticus in pesticide contaminated ditches around bulb fields. *Environmental Pollution, 192*: 196-203.
13. Keizer-Vlek HE, Goedhart PW & Verdonschot PFM. 2011. Comparison of bioassessment results and costs between preserved and unpreserved macroinvertebrate samples from streams. *Environmental Monitoring and Assessment, 175*: 613–621.
14. Kristensen E & Kostka JE. 2005. Macrofaunal Burrows and Irrigation in Marine Sediment: Microbiological and Biogeochemical Interactions. In Interactions Between Macro- and Microorganisms in Marine Sediments (eds E. Kristensen, R. R. Haese and J. E. Kostka), American Geophysical Union, Washington, D. C. ISBN 0733-9569.
15. Larsen S, Mancini L, Pace G, Scalici M & Tancioni L. 2012. Weak Concordance between Fish and Macroinvertebrates in Mediterranean Streams. *PLOS ONE, 7(12):* e51115.
16. Laverock B, Gilbert JA, Tait K, Osborn AM & Widdicombe S. 2011. Bioturbation: impact on the marine nitrogen cycle. *Biochemical Society Transactions, 39(1):* 315-20.
17. Legendre P & Legendre L. 2012. Numerical ecology. Imprint: Elsevier. ISBN: 978-0-444- 53868-0.
18. Legendre P & Gallagher ED. 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia*, *129*: 271–280.
19. Lepš J & Šmilauer P. 2003. *Multivariate analysis of ecological data using CANOCO*. Cambridge University Press. ISBN: 9780521891080.
20. Liess M, Brown C, Dohmen P, Duquesne S, Hart A, Heimbach F, Jenny Kreuger J, Lagadic L, Maund S, Reinert W, Streloke M, Tarazona JV. 2003. Effects of Pesticides in the Field. EU & SETAC Europe Workshop (2003 : Le Croisic, France). ISBN 1-880611- 81-3.
21. Liess M & Von der Ohe PC. 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry 24(4):* 954–965.

22. Martin S, Bertaux A, Le Ber F, Maillard E & Imfeld G. 2011. Seasonal Changes of Macroinvertebrate Communities in a Stormwater Wetland Collecting Pesticide Runoff From a Vineyard Catchment (Alsace, France). *Archives of Environmental Contamination and Toxicology 62(1):* 29-41.
23. Maund SJ, Sherratt TN, Stickland T, Biggs J, Williams P, Shillabeer N & Jepson PC. 1997. Ecological Considerations in Pesticide Risk Assessment for Aquatic Ecosystems. *Pesticide Science*, *49:* 185–190.
24. Murdoch T, Cheo M & O'K. 1996. The streamkeeper's field guide: watershed inventory and stream monitoring methods. Chapter 6. Pollution tolerance values for families of stream macroinvertebrates. Adopt-A-Stream Foundation. ISBN: 9780965210904.
25. Peres-Neto PR, Legendre P, Dray S & Borcard D. 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology 87(10):* 2614-25.
26. Stief P. 2013. Stimulation of microbial nitrogen cycling in aquatic ecosystems by benthic macrofauna: mechanisms and environmental implications. *Biogeosciences, 10*: 7829- 7846.
27. Verdonschot RCM, Keizer-Vlek HE & Verdonschot PFM. 2012. Biodiversity value of agricultural drainage ditches; a comparative analysis of the aquatic invertebrate fauna of ditches and small lakes. *Aquatic Conservation: Marine and Freshwater Ecosystems 21:* 715-727.
28. Vlek HE, Sporka F & Krno J. 2006. Influence of macroinvertebrate sample size on bioassessment of streams. *Hydrobiologia, 556:* 523-542.
29. Zuellig RE, Carlisle DM, Meador MR & Potapova M. 2012. Variance partitioning of stream diatom, fish, and invertebrate indicators of biological condition. *Journal of Freshwater Science, 31(1):* 182-190.

Supplemental information

Table S1. Coordinates of the sampling sites and the number of samples collected at each site

Site Code	Land use area	Coordinates	Year sampled	N samples
MF1	Flower field	52°17'26.94"N, 4°30'51.04"O	2011-2012	10
MF2	Flower field	52°17'35.36"N, 4°29'54.25"O	2011-2012	10
MF3	Flower field	52°16'28.54"N, 4°29'36.05"O	2011	6
MF4	Flower field	52°16'46.93"N, 4°29'44.32"O	2011-2012	10
MF5	Flower field	52°15'55.66"N, 4°28'27.94"O	2011-2012	10
MF6	Flower field	52°15'13.06"N, 4°28'40.95"O	2011-2012	10
MF7	Flower field	52°15'10.38"N, 4°28'16.64"O	2011-2012	10
MF8	Flower field	52°15'6.26"N, 4°27'53.95"O	2011-2012	10
MF9	Flower field	52°14'44.08"N, 4°27'12.15"O	2011	6
MF10	Flower field	52°15'39.93"N, 4°27'49.28"O	2011-2012	10
MP1	Grassland	52°17'14.80"N, 4°29'32.03"O	2011-2012	10
MP2	Grassland	52°16'38.09"N, 4°29'2.23"O	2011-2012	10
MP3	Grassland	52°21'15.76"N, 4°35'56.81"O	2012	4
MP4	Grassland	52°19'35.52"N, 4°34'28.78"O	2012	4
MD1	Dunes	52°17'36.31"N, 4°29'43.92"O	2011-2012	10
MD2	Dunes	52°17'32.42"N, 4°29'39.89"O	2011-2012	10
MD3	Dunes	52°21'45.23"N, 4°33'21.05"O	2012	4
MD4	Dunes	52°20'45.65"N, 4°34'42.81"O	2012	4

Table S2. Mean and standard deviation (in italic) of water chemistry parameters measured at the sampling sites

Site	T, °C	pH	Conductivity, mS	DO, mg/L	DOC, mg/L	Phosphate, mg/L	Nitrite, mg/L	Nitrate, mg/L
D1	15.25	8.07	249.92	6.17	141.23	0.03	0.01	0.05
	4.71	*1.13*	*220.21*	*2.32*	*132.99*	*0.01*	*0.00*	*0.07*
D2	15.10	7.55	401.12	5.24	187.43	0.03	0.01	0.05
	5.50	*0.47*	*297.15*	*2.40*	*204.33*	*0.01*	*0.00*	*0.07*
D3	15.58	10.54	622.25	6.26	63.33	0.03	0.01	0.05
	3.38	*5.34*	*50.49*	*2.50*	*32.32*	*0.01*	*0.00*	*0.07*
D4	15.05	10.05	617.50	6.40	48.27	0.03	0.01	0.05
	3.27	*4.70*	*51.80*	*2.00*	*5.57*	*0.01*	*0.00*	*0.07*
P1	15.85	7.75	612.59	4.83	163.70	2.46	0.03	0.13
	5.08	*0.40*	*306.96*	*1.64*	*128.36*	*2.48*	*0.03*	*0.19*
P2	14.73	7.69	726.77	5.01	181.97	0.91	0.02	0.19
	4.35	*0.48*	*376.44*	*2.21*	*119.18*	*0.44*	*0.01*	*0.24*
P3	15.78	7.56	760.75	6.37	87.27	*N*	*N*	*N*
	5.87	*0.55*	*66.09*	*5.82*	*68.13*			
P4	14.73	7.46	729.50	5.45	86.88	*N*	*N*	*N*
	5.25	*0.31*	*92.42*	*2.36*	*60.85*			
F1	14.48	7.66	547.54	4.37	215.42	1.83	0.04	0.15
	3.57	*0.31*	*418.78*	*1.67*	*144.03*	*1.38*	*0.01*	*0.08*
F2	14.77	7.68	467.32	3.71	193.96	0.78	0.02	0.06
	3.83	*0.42*	*351.88*	*1.43*	*217.22*	*0.99*	*0.01*	*0.07*
F3	14.74	7.90	450.25	4.67	286.40	2.01	0.05	0.23
	5.86	*0.74*	*634.63*	*2.14*	*112.96*	*1.58*	*0.04*	*0.19*
F4	15.23	8.09	607.27	4.45	290.97	*N*	*N*	*N*
	4.73	*0.62*	*359.19*	*1.91*	*360.86*			
F5	15.67	7.98	712.42	4.76	152.58	1.12	0.04	0.18
	4.46	*0.65*	*358.91*	*2.10*	*124.94*	*0.44*	*0.02*	*0.33*
F6	16.02	8.20	752.94	5.34	233.32	4.10	0.03	0.33
	4.50	*0.99*	*403.71*	*1.61*	*144.41*	*3.24*	*0.03*	*0.49*
F7	15.02	7.99	816.43	5.20	173.37	*N*	*N*	*N*
	4.29	*0.67*	*408.01*	*1.92*	*137.78*			
F8	15.35	8.11	851.24	6.10	179.27	1.96	0.07	0.60
	4.43	*0.75*	*425.48*	*1.47*	*164.93*	*0.79*	*0.07*	*0.66*
F9	14.52	8.13	617.86	4.60	247.11	*N*	*N*	*N*
	5.11	*0.99*	*536.43*	*2.70*	*126.32*			
F10	15.28	7.98	743.59	5.58	153.43	1.48	0.01	0.05
	5.09	*0.69*	*370.61*	*1.72*	*103.93*	*1.32*	*0.01*	*0.04*

N = the parameter was not measured at this sampling site

Table S3. Mean and standard deviation (in italic) of pesticide concentrations (µg/L) measured at the sampling sites

	Chloor	PirM	TolM	Prochloraz	Carb	Ethfc	Imzl	Imdc	Ispr	Meth
D1	0.010	0.005	0.005	0.100	0.010	0.025	0.005	0.024	0.005	0.010
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
D2	0.010	0.005	0.005	0.100	0.010	0.025	0.005	0.024	0.005	0.010
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
D3	0.010	0.005	0.005	0.100	0.010	0.025	0.005	0.024	0.005	0.010
	0	*0*	*0*	*0.045*	*0*	*0*	*0*	*0*	*0*	*0*
D4	0.010	0.005	0.005	0.100	0.010	0.025	0.005	0.024	0.005	0.010
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
P1	0.030	0.035	0.005	0.100	0.650	0.025	0.005	0.046	0.005	0.010
	0.045	*0.064*	*0*	*0*	*1.259*	*0*	*0*	*0.047*	*0*	*0*
P2	0.019	0.030	0.007	0.100	0.124	0.025	0.005	0.026	0.005	0.010
	0.018	*0.066*	*0.005*	*0*	*0.173*	*0*	*0*	*0.002*	*0*	*0*
P3	0.010	0	0.005	0.100	0.460	0.025	0.005	0.030	0.005	0.010
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
F1	0.025	0.005	0.005	0.100	0.110	0.025	0.005	0.025	0.005	0.010
	0.021	*0*	*0*	*0*	*0.042*	*0*	*0*	*0*	*0*	*0*
F2	0.010	0.005	0.006	0.100	0.168	0.025	0.005	0.029	0.005	0.010
	0	*0*	*0.003*	*0*	*0.259*	*0*	*0*	*0.008*	*0*	*0*
F3	0.040	0.007	0.005	0.100	0.186	0.025	0.005	0.025	0.005	0.010
	0.037	*0.003*	*0*	*0*	*0.097*	*0*	*0*	*0*	*0*	*0*
F5	0.016	0.005	0.012	0.100	0.069	0.025	0.014	0.065	0.005	0.010
	0.018	*0*	*0.014*	*0.000*	*0.063*	*0*	*0.016*	*0.118*	*0*	*0*
F6	0.017	0.183	0.007	0.100	0.287	0.025	0.005	0.053	0.006	0.010
	0.021	*0.241*	*0.005*	*0*	*0.263*	*0*	*0*	*0.075*	*0.002*	*0*
F8	0.017	0.005	0.006	0.162	0.277	0.025	0.005	0.138	0.005	0.010
	0.021	*0*	*0.002*	*0.198*	*0.687*	*0*	*0*	*0.359*	*0*	*0*
F10	0.018	0.005	0.005	0.100	0.373	0.025	0.005	0.029	0.005	0.010
	0.020	*0*	*0*	*0*	*0.798*	*0*	*0*	*0.010*	*0*	*0*
LOD (µg/L)	0.02	0.01	0.01	0.2	0.02*	0.05	0.01	0.05*	0.01	0.02

*LOD of carbendazim and imidacloprid for samples collected in autumn 2012 was 0.01 µg/L
Chlor = chlorprofam, Pir-meth = pirimiphos-methyl, Tolc-meth = tolclophos-methyl, Carb = carbendazim, Ethiofen = ethiofencarb, Imidacl = imidacloprid, Ispr = isoproturon, Proch = prochloraz, Imaz = imazalil Meth = methiocarb, LOD = limit of detection

Table S4. The level of identification for each macrofauna Class, the total number of individuals counted in each Order and the number of taxa identified in each Order

Class	Taxonomic identification level	Order	Total N individuals	Total N taxa
Insecta	Species (73.6%), genus	Hemiptera	4217	18
	(22.7%), family (0.4%), order	Diptera	4308	43
	(1.0%)	Ephemeroptera	9078	6
		Trichoptera	220	12
		Plecoptera	6	1
		Odonata	336	13
		Coleoptera	982	25
		Lepidoptera	10	2
		Collembola	308	3
		Megaloptera	1	1
Actinopterygii	Species (100%)	Gasterosteiformes	883	2
		Cypriniformes	32	5
Clitellata	Species (98.7%), genus	Rhynchobdellida	148	4
	(0.6%)	Haplotaxida	4736	3
		Tricladida	31	1
Malacostraca	Species (99.0%), genus	Isopoda	656	1
	(0.9%)	Mysida	22	1
		Amphipoda	240	3
Ostracoda	Order (100%)	Class Ostracoda	202	1
Maxillopoda	Genus (0.45%), order	Cyclopoida	2323	1
	(67.9%), subclass (31.6%)	Subclass Copepoda	1081	1
		Arguloida	17	1
Branchiopoda	Species (8.2%), genus	Diplostraca	72523	4
	(91.8%)	Anostraca	4	1
Mollusca	Species 81.43%), genus	Basommatophora	6785	10
	(18.56%)	Architaenioglossa	13	1
		Veneroida	1485	3
		Heterostropha	4293	3
		Neotaenioglossa	1281	3
Arachnida	Suborder (100%)	Subclass Acari	212	1
Total	Species (32.8%), genus (63.2%), order (2.3%)		116433	174

Table S5. Summary of PCA on pesticide, environmental and macrofauna data. Presented are cumulative percentages of variance explained by the first four Principal Components (PC)

PC axes	1	2	3	4
Pesticides	81.7	91.9	96.5	99.4
Env Estimated*	56	78.2	88.2	94.3
Env Not estimated*	56.8	79.5	90	94.7
Time	100	0	0	0
Hemiptera	22.8	43.1	55	65.5
Diptera	23.9	43.3	54.1	64.6
Ephemeroptera	24.9	41.5	53.1	63.8
Trichoptera	22.5	41.6	52.4	62.4
Odonata	22.3	40.9	52	61.8
Coleoptera	22.4	41.6	52.7	62.6
Gasterosteiformes	22.1	41.3	52.6	62.2
Haplotaxida	24.2	43.5	55.3	65.7
Diplostraca	23.7	39.3	51.5	62.8
Basommatophora	22	40.3	52.2	63.1
Heterostropha	23.2	42.6	54.3	64.3
Veneroida	23	42.4	53.8	63.9
Neotaenioglossa	22.5	42.2	53.6	63.6
Rhynchobdellida	22.1	41	52.1	61.9

*PCA on environmental dataset was performed including (Env Estimated) and excluding (Env Not estimated) the estimated values of water chemistry parameters

Table S6. Summary of PCA on pesticide and environmental data. Presented are cumulative percentages of variance explained by the first four Principal Components (PC), percentages of variance explained by each PC (Individual %) and component loadings.

Dataset	PC axis	1	2	3	4
Pesticide data	Cumulative % Pesticides	81.7	91.9	96.5	99.4
	Individual %	81.7	10.2	4.6	2.9
	Ethiofencarb	0	0	0	0
	Chlorprofam	-0.007	-0.074	-0.037	-0.053
	Pirimiphos-methyl	0.369	-0.055	0.928	0.013
	Tolclophos-methyl	0.042	-0.029	0.118	-0.025
	Prochloraz	-0.040	-0.029	-0.022	0.999
	Carbendazim	1.000	-0.003	-0.022	0.001
	Imazalil	-0.058	-0.025	-0.008	-0.021
	Imidacloprid	0.035	0.999	0.025	0.009
	Isoproturon	0.088	-0.054	0.015	-0.037
	Methiocarb	0	0	0	0
Environmental data*	Cumulative % Estimated	56	78.2	88.2	94.3
	Individual %	56	22.2	10	6.1
	Temperature	0.090	-0.324	0.006	-0.340
	Dissolved oxygen	0.111	-0.349	0.041	-0.877
	DOC	-0.421	0.879	-0.184	-0.121
	Phosphate	0.054	0.409	0.908	-0.044
	Macrophyte coverage	0.986	0.163	-0.044	-0.006
	Nitrite	0.070	0.226	0.458	0.250
	Nitrate	-0.025	0.212	0.381	0.277
Environmental data	Cumulative % Not estimated	56.8	79.5	90	94.7
	Individual %	56.8	22.7	10.5	4.7
	Temperature	0.167	-0.302	0.131	0.827
	Dissolved oxygen	0.131	-0.172	-0.241	-0.460
	DOC	-0.416	0.883	-0.206	0.059
	Phosphate	-0.041	0.440	0.889	-0.054
	Macrophyte coverage	0.985	0.172	-0.028	0.002
	Nitrite	-0.008	0.219	0.454	-0.070
	Nitrate	-0.078	0.210	0.343	-0.427

*PCA on environmental dataset was performed including (Estimated) and excluding (Not estimated) the estimated values of water chemistry parameters

Table S7. Summary of PCA on the Hellinger-transformed macrofauna abundance on the level of Order (N=79): cumulative percentage of variance explained by the first four Principal Components (PC) and component loadings

PC Axis	1	2	3	4
Cumulative %	22.1	40.9	51.9	61.6
Hemiptera	-0.463	-0.097	-0.160	-0.438
Diptera	0.161	0.490	0.701	-0.210
Ephemeroptera	0.299	0.774	-0.427	0.256
Trichoptera	0.107	0.292	0.438	0.018
Plecoptera	-0.173	-0.006	-0.036	-0.039
Odonata	0.101	0.644	0.017	-0.198
Coleoptera	-0.017	-0.100	0.242	-0.235
Lepidoptera	-0.041	0.107	-0.119	0.211
Megaloptera	-0.133	-0.059	-0.024	0.032
Collembola	-0.079	0.074	-0.031	-0.092
Gasterosteiformes	-0.471	-0.226	-0.040	-0.496
Cypriniformes	-0.119	-0.078	0.158	-0.178
Rhynchobdellida	-0.017	0.000	-0.187	0.233
Haplotaxida	0.184	-0.503	0.392	0.536
Tricladida	-0.013	-0.164	0.164	0.053
Isopoda	0.069	-0.032	-0.222	0.338
Ostracoda (Class)	0.007	0.242	0.190	-0.228
Cyclopoida	0.320	-0.408	0.027	0.014
Diplostraca	0.802	-0.471	-0.254	-0.189
Copepoda (Subclass)	-0.058	-0.022	-0.123	0.006
Amphipoda	0.036	0.143	0.380	0.033
Arguloida	-0.060	-0.089	0.016	0.064
Anostraca	0.002	-0.076	0.065	0.013
Mysida	0.116	-0.094	0.123	0.270
Basommatophora	-0.757	-0.300	-0.250	-0.025
Heterostropha	-0.280	-0.377	-0.027	0.556
Veneroida	0.036	0.191	-0.125	0.172
Neotaenioglossa	-0.398	0.098	-0.229	0.375
Architaenioglossa	-0.137	-0.154	0.024	0.029
Acari (Subclass)	-0.198	0.000	0.078	-0.185

Table S8. Summary of PCA on the Hellinger-transformed macrofauna abundance on the level of order (N=145): cumulative percentage of variance explained by the first four Principal Components (PC) and component loadings

PC Axis	1	2	3	4
Cumulative %	9.8	17.7	24.6	31.2
Hemiptera	9.4	17.3	24.8	31.2
Diptera	-0.056	0.030	0.248	0.255
Ephemeroptera	0.741	0.145	0.055	-0.063
Trichoptera	0.419	0.224	-0.173	-0.569
Plecoptera	0.684	0.205	0.017	0.248
Odonata	-0.118	0.404	0.676	-0.106
Coleoptera	0.557	-0.111	0.059	-0.537
Lepidoptera	0.346	0.182	0.163	0.200
Megaloptera	0.201	-0.012	-0.160	-0.267
Collembola	-0.193	0.546	0.555	-0.175
Gasterosteiformes	0.030	0.021	0.541	-0.175
Cypriniformes	-0.209	-0.104	0.380	0.303
Rhynchobdellida	-0.012	-0.039	0.042	0.000
Haplotaxida	-0.413	0.389	-0.207	-0.076
Tricladida	-0.022	0.278	-0.029	0.149
Isopoda	-0.272	-0.004	-0.202	0.145
Ostracoda(Class)	-0.120	0.506	-0.389	-0.203
Cyclopoida*	0.221	-0.218	0.312	-0.114
Diplostraca	-0.287	0.462	-0.328	-0.043
Copepoda(Subclass)	-0.041	0.676	-0.232	-0.005
Amphipoda	0.157	0.224	-0.089	0.254
Arguloida	0.516	0.369	-0.235	0.282
Anostraca	-0.056	-0.223	0.190	0.042
Mysida	0.070	-0.038	-0.001	0.352
Basommatophora	-0.226	0.128	0.133	-0.149
Heterostropha	-0.305	0.136	0.123	-0.024
Veneroida	-0.149	0.359	-0.051	-0.045
Neotaenioglossa	0.211	-0.023	-0.135	-0.593
Architaenioglossa	-0.072	0.301	0.073	-0.150
Acari(Subclass)	-0.062	-0.186	-0.011	0.088

Table S9. Average values and standard deviations (in italic) of macrofauna abundances on the level of order at the sampling sites. Abbreviations can be found in Figure 1.

	Hem	Dipt	Eph	Trich	Plec	Odo	Col	Lep	Meg	Col	Gast	Cypr	Rhyn	Hapl
D1	18.8	31.9	119.1	0.3	0.0	5.4	0.8	0.1	0.0	15.8	2.2	0.1	0.4	7.4
	12.6	*71.4*	*106.6*	*0.9*	*0.0*	*5.6*	*1.0*	*0.3*	*0.0*	*49.3*	*3.5*	*0.3*	*1.0*	*23.1*
D2	21.2	106.5	286.6	2.7	0.0	23.1	5.7	0.0	0.0	3.5	0.1	0.0	0.0	7.4
	29.6	*104.5*	*487.7*	*3.5*	*0.0*	*32.3*	*9.7*	*0.0*	*0.0*	*9.4*	*0.3*	*0.0*	*0.0*	*23.4*
D3	5.8	435.3	5.8	135.3	0.0	0.5	18.3	0.0	0.0	0.0	0.5	0.0	0.0	44.8
	5.7	*757.8*	*3.6*	*181.3*	*0.0*	*1.0*	*25.4*	*0.0*	*0.0*	*0.0*	*0.6*	*0.0*	*0.0*	*64.2*
D4	9.8	8.3	52.0	0.5	0.0	0.0	167.5	0.0	0.0	0.0	4.0	0.0	0.0	38.5
	10.7	*6.3*	*31.1*	*0.6*	*0.0*	*0.0*	*333.7*	*0.0*	*0.0*	*0.0*	*8.0*	*0.0*	*0.0*	*51.9*
P1	44.1	7.5	39.1	0.6	0.2	0.8	2.2	0.0	0.3	0.9	6.3	0.1	0.9	45.8
	56.8	*8.4*	*34.4*	*1.1*	*0.6*	*1.3*	*3.8*	*0.0*	*0.9*	*1.5*	*9.3*	*0.3*	*1.1*	*98.6*
P2	37.8	4.5	45.1	0.4	0.4	0.5	1.0	0.0	1.7	1.9	1.2	0.0	1.0	13.4
	41.3	*9.2*	*69.5*	*0.7*	*1.3*	*0.8*	*1.5*	*0.0*	*5.0*	*5.0*	*2.8*	*0.0*	*1.6*	*15.8*
P3	20.5	3.5	132.5	1.3	0.0	5.0	2.5	1.0	0.3	0.3	1.0	1.0	0.3	17.5
	19.1	*2.6*	*80.8*	*1.9*	*0.0*	*5.0*	*3.3*	*2.0*	*0.5*	*0.5*	*1.4*	*1.4*	*0.5*	*12.6*
P4	11.5	1.5	34.0	0.0	0.0	0.5	3.0	0.8	0.0	0.8	0.5	0.0	2.0	50.0
	11.1	*0.6*	*44.0*	*0.0*	*0.0*	*1.0*	*4.8*	*1.5*	*0.0*	*1.0*	*1.0*	*0.0*	*2.3*	*57.7*
F1	49.0	6.2	12.8	0.1	0.1	0.3	1.2	0.0	0.0	0.1	2.1	0.7	0.0	24.2
	48.2	*12.3*	*12.3*	*0.3*	*0.3*	*0.7*	*1.4*	*0.0*	*0.0*	*0.3*	*6.0*	*1.3*	*0.0*	*26.7*
F2	27.5	3.3	18.6	1.2	0.0	0.0	1.3	0.1	0.2	0.1	6.3	0.0	0.9	13.1
	63.0	*8.1*	*32.0*	*3.5*	*0.0*	*0.0*	*3.8*	*0.3*	*0.4*	*0.3*	*13.0*	*0.0*	*1.4*	*20.2*
F3	1.7	39.5	7.5	0.3	0.0	0.0	2.2	0.0	0.0	0.0	0.7	0.0	0.7	30.7
	2.3	*59.3*	*8.8*	*0.8*	*0.0*	*0.0*	*3.3*	*0.0*	*0.0*	*0.0*	*1.2*	*0.0*	*1.0*	*32.0*
F4	19.5	23.1	21.7	0.0	0.0	0.1	0.9	0.0	1.6	1.7	3.8	0.1	4.4	10.4
	27.4	*65.1*	*28.3*	*0.0*	*0.0*	*0.3*	*1.1*	*0.0*	*4.7*	*4.7*	*4.0*	*0.3*	*6.0*	*15.5*
F5	16.0	1.4	26.8	0.0	0.0	0.0	0.8	0.0	0.0	0.0	9.4	0.0	0.7	69.5
	17.5	*2.0*	*33.1*	*0.0*	*0.0*	*0.0*	*1.1*	*0.0*	*0.0*	*0.0*	*27.6*	*0.0*	*1.1*	*83.1*
F6	47.8	6.9	7.5	1.7	0.0	0.0	2.4	0.0	0.1	3.6	2.6	0.1	1.2	38.6
	63.3	*12.9*	*8.5*	*3.9*	*0.0*	*0.0*	*3.4*	*0.0*	*0.3*	*11.4*	*3.7*	*0.3*	*2.1*	*55.4*
F7	33.2	26.7	58.8	0.3	0.0	0.3	0.7	0.0	0.0	1.7	33.3	0.0	0.3	128.6
	48.8	*62.3*	*53.6*	*0.9*	*0.0*	*0.9*	*0.9*	*0.0*	*0.0*	*5.4*	*70.1*	*0.0*	*0.5*	*240.2*
F8	32.2	4.5	31.0	0.1	0.0	0.1	1.6	0.0	0.2	1.0	12.3	1.3	0.7	11.0
	28.5	*5.8*	*28.9*	*0.3*	*0.0*	*0.3*	*2.9*	*0.0*	*0.6*	*3.2*	*22.2*	*4.1*	*0.7*	*11.9*
F9	51.2	5.3	194.3	0.5	0.0	1.0	0.0	0.0	0.5	0.0	2.8	0.5	3.3	11.8
	36.4	*5.1*	*250.8*	*0.8*	*0.0*	*2.4*	*0.0*	*0.0*	*0.8*	*0.0*	*3.7*	*1.2*	*5.4*	*21.8*
F10	19.1	2.4	30.2	0.1	0.0	0.1	1.9	0.0	0.1	0.0	4.2	0.1	1.2	6.8
	12.4	*3.9*	*38.2*	*0.3*	*0.0*	*0.3*	*3.2*	*0.0*	*0.3*	*0.0*	*8.8*	*0.3*	*2.6*	*13.2*

Tricl	Isop	Ostr	Cycl	Dipl	Cop	Amp	Arg	Anos	Mys	Bas	Het	Ven	Neot	Arch	Aca
0.0	1.0	0.2	0.3	69.7	0.0	0.1	0.0	0.0	0.0	24.7	0.3	61.1	1.1	0.0	1.1
0.0	*3.2*	*0.6*	*0.7*	*131.0*	*0.0*	*0.3*	*0.0*	*0.0*	*0.0*	*28.2*	*0.7*	*127.2*	*3.1*	*0.0*	*1.9*
0.0	0.0	18.0	0.5	22.0	0.0	0.0	0.0	0.0	0.0	18.0	21.4	32.9	2.6	0.0	0.8
0.0	*0.0*	*40.5*	*1.6*	*44.1*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*38.0*	*51.7*	*66.8*	*6.9*	*0.0*	*1.1*
0.0	0.0	0.0	0.5	28.0	0.0	31.0	0.0	0.0	0.0	7.0	18.3	0.8	9.3	0.0	6.5
0.0	*0.0*	*0.0*	*1.0*	*56.0*	*0.0*	*20.7*	*0.0*	*0.0*	*0.0*	*10.9*	*20.8*	*1.5*	*15.3*	*0.0*	*9.3*
0.0	12.5	0.0	0.5	324.3	0.0	4.5	0.0	0.0	0.0	7.0	8.3	0.0	0.3	0.0	0.0
0.0	*13.0*	*0.0*	*1.0*	*273.5*	*0.0*	*4.2*	*0.0*	*0.0*	*0.0*	*2.9*	*15.8*	*0.0*	*0.5*	*0.0*	*0.0*
0.0	2.1	2.0	3.2	223.7	7.8	0.1	0.6	0.0	0.9	27.9	24.7	9.9	6.3	0.1	1.0
0.0	*3.1*	*6.3*	*5.5*	*455.1*	*16.8*	*0.3*	*1.1*	*0.0*	*1.4*	*22.4*	*28.8*	*24.4*	*10.9*	*0.3*	*1.6*
0.2	2.9	0.0	2.1	1032.7	86.5	0.6	0.1	0.0	1.0	35.7	17.5	5.1	4.6	0.0	3.4
0.6	*4.6*	*0.0*	*2.8*	*3241.5*	*271.8*	*1.9*	*0.3*	*0.0*	*1.8*	*52.6*	*16.9*	*5.2*	*4.8*	*0.0*	*8.0*
0.0	5.8	0.0	5.0	290.3	0.0	5.5	0.0	0.0	0.0	10.5	7.8	1.0	8.3	0.0	2.8
0.0	*5.1*	*0.0*	*7.1*	*274.8*	*0.0*	*6.4*	*0.0*	*0.0*	*0.0*	*6.6*	*12.3*	*1.4*	*7.4*	*0.0*	*5.5*
0.0	12.8	0.0	0.3	104.8	0.0	2.0	0.0	0.0	0.0	47.3	9.3	3.8	8.5	0.0	0.5
0.0	*5.4*	*0.0*	*0.5*	*196.9*	*0.0*	*2.2*	*0.0*	*0.0*	*0.0*	*41.7*	*15.2*	*4.1*	*7.7*	*0.0*	*0.6*
0.4	2.0	0.0	6.5	155.1	1.0	1.8	0.1	0.3	5.5	12.5	24.5	5.3	2.4	0.0	4.3
1.3	*2.3*	*0.0*	*9.3*	*311.6*	*3.2*	*2.9*	*0.3*	*0.9*	*10.9*	*18.6*	*23.4*	*9.7*	*3.0*	*0.0*	*6.0*
0.0	1.7	0.0	0.6	25.3	1.2	0.3	0.0	0.0	2.4	63.1	47.8	10.1	31.3	0.0	0.2
0.0	*1.7*	*0.0*	*1.3*	*47.7*	*3.8*	*0.5*	*0.0*	*0.0*	*4.1*	*49.3*	*57.4*	*11.1*	*27.9*	*0.0*	*0.4*
0.3	2.0	0.0	10.0	6.5	0.0	0.2	0.0	0.0	0.8	19.2	17.0	1.5	2.2	0.0	0.8
0.8	*2.1*	*0.0*	*15.8*	*9.5*	*0.0*	*0.4*	*0.0*	*0.0*	*1.3*	*23.0*	*19.9*	*2.8*	*2.7*	*0.0*	*2.0*
1.3	2.3	0.0	46.1	1197.5	0.0	0.4	0.3	0.0	0.4	32.4	38.7	4.3	2.0	0.0	0.0
4.1	*4.6*	*0.0*	*62.4*	*1684.6*	*0.0*	*1.3*	*0.9*	*0.0*	*1.0*	*24.8*	*29.1*	*6.4*	*3.9*	*0.0*	*0.0*
0.1	2.6	0.0	78.7	782.0	0.0	0.2	0.0	0.0	0.3	38.8	83.8	3.5	5.2	1.2	0.7
0.3	*2.6*	*0.0*	*225.7*	*1321.4*	*0.0*	*0.4*	*0.0*	*0.0*	*0.7*	*36.2*	*96.1*	*6.4*	*6.3*	*3.8*	*1.3*
0.5	3.5	0.0	7.0	60.1	0.3	0.0	0.6	0.0	0.1	50.9	17.4	0.4	0.4	0.0	0.6
1.6	*10.0*	*0.0*	*15.0*	*117.6*	*0.9*	*0.0*	*1.3*	*0.0*	*0.3*	*60.7*	*18.7*	*0.7*	*0.7*	*0.0*	*1.1*
0.1	3.0	0.0	8.5	331.7	0.3	0.0	0.0	0.1	0.1	98.8	20.1	5.7	4.2	0.0	0.1
0.3	*7.2*	*0.0*	*21.8*	*629.9*	*0.9*	*0.0*	*0.0*	*0.3*	*0.3*	*212.5*	*16.0*	*11.3*	*5.7*	*0.0*	*0.3*
0.0	2.5	0.0	7.9	19.5	4.5	0.4	0.0	0.0	0.9	77.0	25.5	1.9	8.7	0.0	1.5
0.0	*4.2*	*0.0*	*12.4*	*42.8*	*14.2*	*1.3*	*0.0*	*0.0*	*2.8*	*66.4*	*27.4*	*2.4*	*10.8*	*0.0*	*3.2*
0.0	32.5	0.0	77.3	1345.2	10.8	3.7	0.0	0.0	0.0	50.8	22.7	6.5	9.5	0.0	2.7
0.0	*47.7*	*0.0*	*106.8*	*1452.2*	*16.8*	*5.4*	*0.0*	*0.0*	*0.0*	*34.3*	*22.2*	*4.2*	*17.0*	*0.0*	*3.4*
0.3	8.3	0.0	10.4	2155.0	0.0	0.6	0.0	0.0	0.0	130.6	70.1	2.0	35.2	0.0	1.5
0.9	*9.6*	*0.0*	*22.6*	*4252.7*	*0.0*	*1.3*	*0.0*	*0.0*	*0.0*	*149.5*	*84.5*	*2.3*	*43.9*	*0.0*	*2.9*

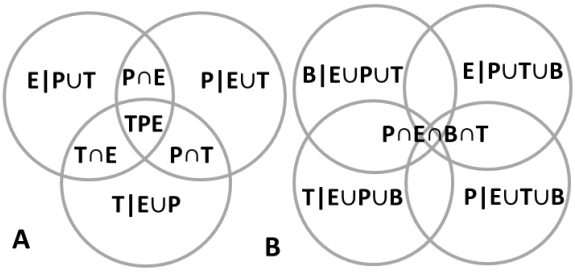

Figure S1. Visual representation of variance components estimated for total macrofauna species composition (A) and each macrofauna group on the level of order (B)

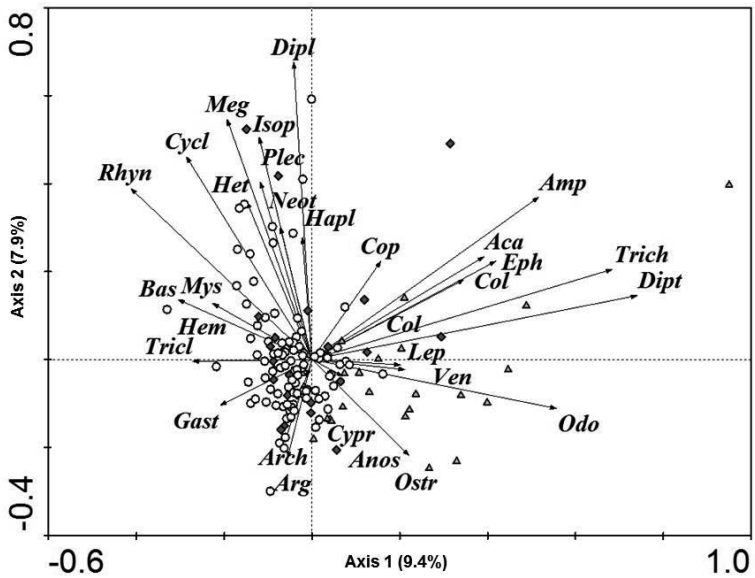

Figure S2. Principal component analysis of Hellinger-transformed macrofauna abundance on the level of Order (N=145). Abbreviations can be found in Figure 1.