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The effect of pesticides on the composition of aquatic macrofauna communities in field ditches

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Abstract

Ditches surrounding agricultural fields in the Netherlands are predominantly used for flood control, and they accommodate aquatic plant and animal species. Studies addressing the effects of pesticides in combination with abiotic and biotic factors on aquatic biota in ditches are scarce. The current study aimed to investigate the effects of pesticides along with environmental factors, presence of other biota and time on the community composition of aquatic macrofauna in ditches next to flower bulb fields and pastures. Macrofauna samples and environmental data were collected during two consecutive years. Ponds in a sandy dune area of a nature reserve 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 (water chemistry parameters and macrophyte coverage), time (study year and the number of the month), shared variance, and unexplained variance. The total explained variance in macrofauna community reached 22.6%. The largest proportion of explained variance was attributed to environmental factors (10.1%) followed by pesticides (5.4%) and time (4.8%). When each macrofauna group was analyzed separately, 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 contexts of aquatic ecosystems.

Zusammenfassung

Die Gräben, die in den Niederlanden landwirtschaftliche Flächen durchziehen, dienen hauptsächlich der Wasserstandskontrolle. Sie beherbergen aquatische Pflanzen- und Tierarten. Wir untersuchten den Einfluss von Pestiziden zusammen mit Umweltfaktoren, Vorhandensein anderer Taxa und Probetermin auf die Zusammensetzung der aquatischen Makrofauna in Gräben in der Nachbarschaft von Blumenzwiebelfeldern und Weiden. Proben wurden in zwei aufeinanderfolgenden Jahren genommen. Tümpel im Sanddünengebiet eines nahegelegenen Naturschutzreservats dienten als Kontrollen. Die Daten wurden mit der Varianzpartitionierung nach Redundanzanalyse analysiert. Die Gesamtvarianz der Zusammensetzung der Makrofaunagemeinschaft wurde untergliedert in die Varianzanteile, die durch Pestizide, Umweltfaktoren (hydrochemische Parameter und

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Makrophytenbestand) und Termin (Untersuchungsjahr und Monat) erklärt wurden, sowie gemeinsame Varianz und unerklärte Varianz. Die insgesamt erklärte Varianz der Makrofaunagemeinschaft erreichte 22,6%. Die größten erklärten Anteile entfielen auf Umweltfaktoren (10,1%), Pestizide (5,4%) und Termin (4,8%). Wenn die Makrofaunagruppen getrennt analysiert wurden, erklärten Umweltfaktoren und die Anwesenheit anderer Taxa in den meisten Fällen die größten Varianzanteile. Unsere Ergebnisse zeigen, dass Umweltfaktoren, biotische Interaktionen und zeitliche Variation die Süßwassermakrofauna erheblich neben den Pestiziden beeinflussen. Das Umweltmanagement sollte die vielfältigen Zusammenhänge von Stressoren in aquatischen Ökosystemen berücksichtigen.

Keywords: Abiotic factors; Biotic factors; Freshwater macrofauna; Pesticides; RDA

Introduction

Ditches are the typical aquatic ecosystems in the Netherlands and in addition to their direct function of water level control also have high aquatic biodiversity (Verdonschot, Keizer-Vlek, & Verdonschot 2012). Drainage ditches contain high numbers of aquatic plant and animal species, as well as semi-terrestrial species. Macrofauna in turn play an important role in the food chain and biochemical cycles in aquatic ecosystems. Thus, the presence of macrofauna in the sediments enhances the microbial nitrogen cycle by bioturbation. Bioturbation facilitates the transport of inorganic and organic nitrogen between sediment and water (Kristensen & Kostka 2005; Laverock, Gilbert, Tait, Osborn, & Widdicombe 2011). Thus, macrofauna take 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 aquatic ecosystems (Stief 2013).

To protect aquatic biodiversity and its ecosystem functions, it is important to understand the effects of chemicals on aquatic biota in the field. The reason for this is that in the real environment various abiotic and biotic factors influence the performance of aquatic organisms and affect the fate of pesticides in the aquatic environment. Several studies emphasized the importance of considering ecological parameters in ecotoxicological studies (Maund et al. 1997; Liess et al. 2003). A number of studies did include ecological factors in the assessment of pesticide effects on aquatic biota in the field. For instance, in the field study of Berenzen, Kumke, Schulz, and Schulz (2005) the effects of pesticides on macroinvertebrates were analyzed in combination with stream characteristics and water chemistry parameters. The study of Szöcs, Kefford, and Schäfer (2012) addressed the effects of pesticides in combination with salinity and other environmental factors (habitat and water chemistry parameters) on macroinvertebrate communities in streams. Martin, Bertaux, Le Ber, Maillard, and Imfeld (2011) investigated the responses of macroinvertebrate communities in stormwater wetlands to pesticide runoff taking into account physicochemical characteristics, hydromorphological features and vegetation coverage. Bollmohr et al. (2011) assessed the effects of pesticides along with physicochemical characteristics on meiobenthic communities in estuary. However, to our knowledge, the effects of pesticides on aquatic biota in combination with abiotic, biotic factors and time have not been previously studied in the field.

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

Sampling sites

During the year 2011, macrofauna and water chemistry samples were collected at 14 sites within the area: two sites in ponds within the nature reserve area, two sites in

ditches alongside pastures and ten sites in ditches alongside bulb fields within the same area as described in Ieromina, Peijnenburg, de Snoo, and Vijver (2014). Sampling was performed during the period April–November 2011 six times in order to account for seasonal fluctuations in water chemistry 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. In addition, four sites located in the area next to the polders were sampled four times in 2012. In total, 148 macrofauna and water chemistry samples were collected. Coordinates of the sampling sites are given in Appendix A: Table S1. The schematic map of the research area can be found in Fig. S1.

Pesticide and nutrient measurements

Concentrations of pesticides were measured by Omegam Laboratoria BV. Pesticides were measured according to the standard guidelines (analysis GC–MS and LC–MS/MS). Nutrients were measured according to the following guidelines: NEN 6663 for phosphate and NEN-EN-ISO 13395 for nitrate and nitrite. Pesticide samples were taken in the nature reserve (site D1) and a number of sites within the agricultural area (P1, P2, P3, F1, F2, F3, F5, F6, F8, and F10). Pesticide and nutrient levels were found to be below detection limits in all samples collected on the nature reserve site D1. Therefore, pesticide and nutrient concentrations were assumed to be below detection limits at other nature reserve sites D2, D3 and D4. Samples for water chemistry and pesticide analysis, as well as macrofauna samples were collected on the same day. If the concentration of a pesticide was below its limit of detection, half of the detection limit value was used in the data analysis (Clarke 1998). An overview of water chemistry parameters and pesticide concentrations at the sampling sites is given in Appendix A: 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). The percentage of water surface covered with floating macrophytes was recorded on each sampling day.

Macrofauna sampling and determination

Macrofauna samples were collected via dipping net with a 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, Goedhart, and Verdonschot (2011) and Vlek, Sporka, and Krno (2006), resulting in a multi-habitat sampling strategy.

Larger organisms (for instance, Gastropoda, Coleoptera) were identified in the field or photographed for further identification. 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 Appendix A: Table S4.

Statistical analysis

Principal component analysis (PCA) of macrofauna community composition

Principal component analysis (PCA) was performed on macrofauna abundance data at the level of order to identify variation patterns in macrofauna community composition and to visualize the groups of sampling sites containing similar macrofauna taxa. This analysis was done for macrofauna collected in all sampling locations ($N = 148$) and separately for macrofauna collected at sites where pesticides were measured ($N = 79$). Prior to analysis, all biological data was transformed using the Hellinger transformation (Legendre & Gallagher 2001).

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. Variance partitioning analysis was based on the data from sampling sites at which pesticide concentrations were measured ($N = 79$). 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 can be found in Table 1. The percentage of explained variance obtained by canonical analysis was measured as R^2, similar to the previous study of Peres-Neto, Legendre, Dray, and Borcard (2006).

The response variable dataset consisted of the total macrofauna species composition, and of the composition of individual macrofauna orders (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 and Fig. S2).

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

Response variables	Components of variance	Individual explanatory variables included in components of variance
Total species composition	Pesticides (P)	Concentrations of 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 year, number of month
Species composition of different macrofauna groups (Hemiptera, Diptera, Ephemeroptera, Trichoptera, Odonata, Coleoptera, Gasterosteiformes, Haplotaxida, Diplostraca, Basommatophora, Heterostropha, Veneroida, Neotaenioglossa, Rhynchobdellida)	Biota (B)[a]	Abundance of 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

[a] When each taxonomic group was analyzed separately, the additional Biota component was included in the analysis.

The variance in each macrofauna order (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 analyzed) (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 an RDA model affects the model outcome: the explained variance R^2 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). In order to account for the different numbers of explanatory variables in variance components, we performed a PCA on each set of explanatory variables. Sample scores of the first four principal components (PC) axes from each component were included in the RDA as explanatory variables. Results of the PCA on datasets containing pesticide, environmental factors, biota, and time datasets can be found in Appendix A: Tables S5 and S6. In addition, the correlation between PC scores was checked prior the analysis. The correlation coefficient between PC scores was below 0.5.

Missing values in the water chemistry dataset were calculated as average values of the parameter measured at the same date or sampling site (as recommended by Lepš & Šmilauer 2003). In addition, we performed a PCA on a dataset without missing values (including samples with all parameters measured) and a PCA on a dataset containing both measured and estimated values in order to test if PCA results differed between the two datasets. The results of PCA on datasets with and without estimated missing values were similar (Appendix A: Tables S5 and S6). Therefore, further analysis (RDA on PC 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 represented the sum of unconstrained and constrained eigenvalues. The total explained variance (P∪E∪T for total macrofauna composition and P∪E∪T∪B for macrofauna groups) represented the amount of variance explained by all components, or the sum of the constrained eigenvalues. The total explained variance was obtained by constructing an RDA in which all groups of explanatory variables (PC scores obtained from a PCA on pesticides, environmental factors, time and biota datasets) were included in the analysis as explanatory variables.

The proportion of variance explained by pesticides was estimated by constructing a partial RDA in which pesticide data (PC scores obtained from a PCA on pesticide dataset) were included in the analysis as explanatory variables, and PC scores obtained from a PCA on environmental, biota, and time datasets were included in the analysis as covariables (Table 2). A similar analysis was performed to determine the proportion of variance explained by environmental factors, time and biota. Proportion of variance in macrofauna community composition shared by different factors was estimated based on Eqs. (1) and (2) presented in Table 2.

In addition, Ezekiel's R^2 adjustment was applied to the estimated explained variance (Peres-Neto et al. 2006):

R^2 adjusted $= 1 - \frac{n-1}{n-p-1}(1 - R^2)$.

where n is the number of samples, p is the number of explanatory variables, and R^2 is the proportion of explained

Table 2. Summary of variance partitioning analysis.

Response variable	Parameter	Explanation	Calculation procedure
Total macrofauna community composition	Total variance	All variance	Assumed to be 100%
	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
	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 (Eq. (1))
	P[a]	All variance explained by pesticides	Pesticides included as explanatory variables, no covariables
	E[a]	All variance explained by environmental factors	Environmental factors included as explanatory variables, no covariables
	T[a]	All variance explained by time	Time included as explanatory variables, 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 variable, 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 (Eq. (2))

[a] Including shared variance with other factors.

variance. In the manuscript, we refer to R^2 adjusted. Multivariate analysis was performed in Canoco software version 4.5 (Lepš & Šmilauer 2003).

Results

Variance in macrofauna community composition

The order Diptera contained the highest number of species followed by the order Coleoptera (Appendix A: Table S4). The PCA showed that the first four PCs explained 61.6% of variance in macrofauna abundance for sites where pesticide concentrations were measured ($N = 79$) (Appendix A: Table S7). Diplostraca contributed mostly to PC1, Ephemeroptera to PC2 and Diptera to PC3. High abundances of the Diplostraca and Haplotaxida, as well as Basommatophora and Heterostropha were associated with sites located in the flower bulb area (Fig. 1). On the other hand, sites in the nature reserve contained the highest numbers of Ephemeroptera, Odonata, Diptera, Trichoptera as well as Veneroida. A similar pattern was obtained when a larger dataset including macrofauna from all sites sampled was analyzed ($N = 148$) (Appendix A: Fig. S3 and Table S8). Average abundances of macrofauna on the level of order and standard deviations are given in Appendix A: Table S9.

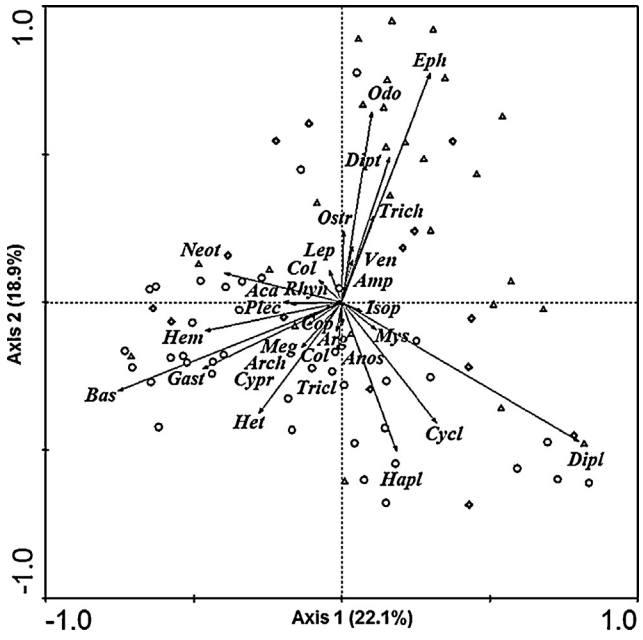

Fig. 1. Principal component analysis of Hellinger-transformed macrofauna abundance on the level of Order. Hem: Hemiptera; Dipt: Diptera; Eph: Ephemeroptera; Trich: Trichoptera; Plec: Plecoptera; Odo: Odonata; Col: Coleoptera; Lep: Lepidoptera; Meg: Megaloptera; Colm: Collembola; Gast: Gasterosteiformes; Cypr: Cypriniformes; Rhyn: Rhynchobdellida; Hapl: Haplotaxida; Tricl: Tricladida; Isop: Isopoda; Ostr: Ostracoda; Cycl: Cyclopoida; Dipl: Diplostraca; Cop: Copepoda; Amp: Amphipoda; Arg: Argyloida; Anos: Anostraca; Mys: Mysida; Bas: Basommatophora; Het: Heterostropha; Ven: Veneroida; Neot: Neotaenioglossa; Arch: Architaenioglossa; Aca: Acari; Tricl: Tricladida; Triangles: nature reserve sites; circles: ditches located in flower bulb fields; diamonds: ditches located in pastures. Shown are the sites sampled during 2011–2012 at which pesticide concentrations were measured ($N = 79$).

Variance partitioning of macrofauna community composition

When the total community composition was analyzed, the explained variance reached 22.6% (Table 3). The results of partial RDA showed that environmental factors contributed mostly to the explained variance (10.1%), followed by pesticide and time (5.4% and 4.8%, respectively). Shared variance between all factors was 1.6% (Table 3).

From all macrofauna groups analyzed, the percentage of total explained variance was the highest for Rhynchobdellida and Ephemeroptera (59.2% and 51.8% respectively). The variance in Diplostraca was least explained by RDA model (12.5%) (Table 4). All factors explained between 1% and 17% of 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 studies conducted in the Netherlands (Keizer-Vlek et al. 2011). The following orders showed the highest similarity in terms of the number of taxa with the study of Keizer-Vlek et al. (2011): Bivalvia, Trichoptera and Diptera (Appendix A: Table S4).

As seen in the PCA plots (Fig. 1, Appendix A: Fig. S2), higher densities of insect larvae Trichoptera, Odonata, Ephemeroptera and Diptera were associated with ponds of the nature reserve. Concentrations of dissolved oxygen generally were higher, and nutrient and pesticide levels were lower in the nature reserve area than in the agricultural area (Appendix A: Tables S2 and S3). Therefore, the good water quality of the nature reserve created favorable conditions for 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 the Hemiptera, Bassomatophora, Heterostropha, Diplostraca, Rhynchobdellida and Haplotaxida were larger in ditches inside the agricultural area. A similar result of large abundances of Gastropoda, Hirudinea and Oligochaeta at high pesticide levels was observed 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, chap. 6). In case of Oligochaeta that feed on organic matter, previous studies suggested a positive effect of nutrients on their abundances through positive effects on the biomass of bacteria and

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 variable	P∪E∪T	Residual variance	P|E∪T	E|P∪T	T|E∪P	P∩E∩T	P∩E	P∩T	T∩E	TPE
R^2	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*	*0.0*	*−0.3*

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

algae associated with the sediment, used by Oligochaeta as a food source (Armendáriza, Ocóna, & Rodrigues 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, Carlisle, Meador, and Potapova (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, Grönroos, Soininen, Virtanen, and Muotka (2012). Out of 22.6% of 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 the environmental factors in shaping community composition of aquatic biota. For instance, in the study of Larsen, Mancini, Pace, Scalici, and Tancioni (2012), environmental factors were found to be 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 the research area. For instance, the average phosphate and nitrate concentrations at the sampling sites varied 100-fold, while average DOC levels changed about 6-fold (Appendix A: Table S2). DOC and nutrients also relate to food availability for aquatic macrofauna and limit the performance of many aquatic species. Large variation in such 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 was two times lower than the contribution of environmental factors (5.4%). Studies quantifying the contribution of toxicants to the variance in community composition of aquatic biota are scarce. In the study of (De Zwart, Dyer, Posthuma, & Hawkins 2006), the toxicants explained 3% of the total variance in fish communities in 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 time to the total variance (4.8%) that can be explained by seasonal variation in macrofauna community composition. Shared variance between different factors 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 largely depends on environmental conditions (Maund et al. 1997). In addition, nutrients co-occur with pesticides due to their similar origin (pesticides and fertilizers are applied concurrently on the bulb fields).

The average percentage of total explained variance for all macrofauna groups was 30.0%. On average, biota and environmental factors explained the largest percentage of variance in different macrofauna groups, followed by pesticides, shared variance and time. These results can possibly be explained by the importance of biotic interactions and site-specific environmental conditions in structuring macrofauna community composition.

The RDA yielded a negative value for shared variance for Diptera, Trichoptera, and Veneroida (Table 4). Such a result for the shared variance means that the groups of variables separately explain the variance in the response variable dataset better than when combined (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 community composition of aquatic macrofauna. The results of our study indicate that the contribution of environmental factors and the presence of other biota to the variance in most of the macrofauna groups 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 50% higher contribution to the total explained variance than pesticides. Based on our results we can conclude that the responses of the 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 differences in responses of distinct macrofauna groups due to their varying ecological preferences.

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Appendix A. Supplementary data

Supplementary data Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2015.08.002.

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