Author: Ieromina, Oleksandra
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CHAPTER 4

VARIANCE IN TRAIT MODALITY DISTRIBUTION OF AQUATIC MACROFAUNA EXPLAINED BY PESTICIDES IN THE FIELD

O. Ieromina[†], C.J.M. Musters[†], P. M. Bodegom[†], W.J.G.M. Peijnenburg[†,‡], 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

Analyzing functional characteristics of species helps to understand the impacts of various stressors on aquatic communities in the field. This research aimed to study the effects of pesticides combined with other environmental factors (temperature, dissolved oxygen, dissolved organic carbon, coverage with floating macrophytes, phosphate, nitrite, nitrate), and time (seasonal and annual variation) on the trait modality distribution of aquatic macrofauna. To address the research aim, field work was performed in the flower bulb growing area of the Netherlands characterized by intensive agricultural activities. Field work included sampling of macrofauna in ditches next to flower bulb fields followed by taxonomic identification, measurements of physico-chemical parameters and pesticide concentrations. Each taxon was classified into the trait modalities of nine traits (feeding mode, respiration mode, locomotion type, diapause form, reproduction mode, life stage, voltinism, saprobity, maximum body size). Relationships between trait modality distribution per trait, pesticides, and environmental factors were analyzed with redundancy analysis (RDA). A variance partitioning based on the redundancy analysis (RDA) was applied to divide the total variance in trait modality distribution per trait into the variance explained by pesticides, environmental factors and time. On average, the largest proportion of variance in the trait composition was explained by environmental factors (11.2%) followed by time (7.2%) and pesticides (2.2%). To obtain a mechanistic understanding of community responses to pesticides, trait composition of aquatic communities should be analyzed explicitly, in combination with taxonomic composition.

Keywords: aquatic community, traits, chemical stress, environmental factors, pesticides

Introduction

Traditionally, the responses of biotic communities to human-induced disturbances have been evaluated based on the taxonomic approach, i.e. estimating the species diversity or the performance of selected indicator species (Mouillot et al., 2006). During the recent decades, the use of trait approaches, i.e. characterizing communities according to the functional characteristics, gained an increasing interest. The reason is that functional traits are supposed to provide an insight in the mechanisms underlying community responses to disturbances (Poff, 1997; Statzner et al., 2010). Information obtained using trait-based approaches can be extrapolated to a broader range of species, geographical zones and ecotypes (Baird et al., 2008; Dolédec et al., 2006; Charvet et al., 2000). These approaches have been successfully developed for a wide array of plant (for instance, Engelhardt 2006; Quétier et al., 2007; Suding et al., 2008) and animal communities, e.g. invertebrates (for instance, Poff et al., 2006; Charvet et al., 2000; Dolédec et al., 2006; Vieira et al., 2006; Magbauna et al., 2010; Menezes et al., 2010; Statzner et al., 2010; Ippolito et al., 2012).

The trait approach has been also adopted by ecotoxicologists. Liess & Von Der Ohe (2005) developed a trait indicator of community responses to pesticides. Ippolito et al. (2012) modelled the effects of pesticides on the trait composition of invertebrate communities. Rubach et al. (2010) linked traits of aquatic invertebrate species to pesticides. Yet, the applicability and the predictive potential of the trait approach in quantifying the effects of pesticides on aquatic communities in the multi-stressor field conditions require further understanding.

A number of key drivers may influence the performance of aquatic biota in water systems around agricultural areas. First, the use of pesticides in the agricultural fields results in the presence of pesticide mixtures in surface waters. Therefore, aquatic biota may be affected by mixtures of pesticides. Second, nutrients (phosphorus and nitrogen) are commonly applied in the fields to enhance yields and are often transported to surface waters in relatively large amounts along with pesticides (EPA, 2012; Tilman et al., 2002). Nutrients affected the responses of aquatic invertebrates to pesticides in the laboratory and semi-field conditions (Alexander et al., 2013; Ieromina et al., 2014a,b). Third, other physico-chemical parameters are highly variable in surface waters around agricultural fields. As an example, transportation of sediment particles from agricultural fields to surface waters has been shown to increase the levels of suspended/dissolved solids and dissolved organic carbon (DOC) in water (Ruark et al., 2009; Neung-Hwan et al., 2013). Various drivers concurrently affect the aquatic biota in agricultural areas. However, to which extent these drivers affect trait composition, i.e., the distribution of the modalities per trait within the community, remains poorly understood. As far as we know, no research has tried to distinguish the effects of pesticides on the trait modality distribution of aquatic communities from the effects of environmental factors and time.

This study therefore aimed 1) to analyze relationships between trait modality distributions, environmental factors and pesticides, and investigate the functional traits of aquatic communities in ditches adjacent to flower bulb fields and in watersheds of nature reserve 2) to quantify what proportion of the total variance in trait composition of aquatic macrofauna community can be explained by pesticides, other environmental factors and time. Traits likely to respond to chemical stress, such as traits related to the external exposure (feeding mode, life stage), internal sensitivity (respiration mode, maximum body size), population recovery (locomotion type, diapause form, voltinism, reproduction mode) (as classified by Rubach et al., 2011) and ecological tolerance (saprobity) were analyzed. We hypothesized that pesticides explain a larger proportion of variance in community trait composition than other environmental factors and time, because traits directly relate to the mechanisms of pesticide effects on communities and therefore are expected to be a sensitive indicator of pesticide pollution.

Materials and methods

Macrofauna sampling, measurements of environmental parameters and pesticide concentrations

Field work was performed in a freshwater ditch system located in the flower bulb growing region of the Netherlands. Samples were collected from a total of 16 sites in the research area repeatedly in the period April-November 2011 - 2012: 12 sites located in ditches next to flower bulb fields and pastures, and 4 sites located in watersheds of a nature reserve close to the flower bulb growing area. A detailed description of the research area, macrofauna sampling strategy, and taxonomic identification level for each group, procedures for measurements of physico-chemical water parameters and pesticide concentrations, as well as basic data on macrofauna community composition and abundances, water chemistry and pesticide concentrations can be found in O. Ieromina et al. (submitted). In brief, macrofauna samples were collected using a dipping net dragged over the total length of 5 m using multihabitat sampling strategy. The following water chemistry parameters were monitored: temperature (T, ^{o}C), dissolved oxygen (DO, mg/L), pH, conductivity (mS), dissolved organic carbon (DOC, mg/L). Coverage with floating macrophytes (Macr) (expressed in percentage) was estimated to account for habitat structure. Measurements of the concentrations of phosphate ($PO4^{-}$), nitrite ($NO2^{-}$), nitrate ($NO3^{-}$) and pesticides commonly applied in bulb fields (chlorprofam, pirimiphos-methyl, tolclophos-methyl, carbendazim, ethiofencarb, imidacloprid, isoproturon, imazalil, methiocarb, and prochloraz) were performed in the OMEGAM laboratory (Amsterdam, the Netherlands) using the standardized protocols.

Assigning trait modalities

First, each species was classified into the preselected trait modalities of nine traits: feeding mode, locomotion type, diapause form, voltinism, reproduction mode, life stage, respiration mode, body size and saprobity (Table 1). Trait data were found in the online database *www.freshwaterecology.info* (Schmidt-Kloiber & Hering, 2012 accessed in the years 2012 - 2014, last accessed 04.04.2014) or in literature. If a species was characterized by more than one modality of a trait, each of these modalities was assigned a coefficient ranging from 0 to 1, depending on how the given modality is represented in this species. For instance, the trait "feeding mode" included 7 modalities: deposit feeding, predating, grazing, shredding, filter feeding, gathering, and parasite types of feeding. If a species feeds 80% by grazing and 20% by predation, then the modality "grazing" was assigned a coefficient 0.8 and modality "predation" was assigned a coefficient 0.2. If a species was characterized by one modality of a trait, this modality was assigned a coefficient of 1, and the other modalities of this trait were assigned a coefficient of 0. If trait data for a given species was not found in literature, the information on the other species from the same genus or family was used to estimate the missing data. If a larger taxonomic unit was analyzed (macrofauna was identified to the genus, family or order levels), the trait data most characteristic of this taxonomic group was included in the analysis. As a result, a matrix containing trait modality coefficients for each species was obtained.

Second, the trait data was weighted by the biomass. For that, the data on the maximum body size for each species was found in literature. After that, each trait modality coefficient of the given species was multiplied by the maximum body size and by the abundance of this species.

Table 1. List of traits and trait modalities analyzed in this study

Trait category	Trait*	Trait modality	Abbreviation
Physiological	Feeding mode	deposit feeders	FDep
		predators	FPred
		grazers	FGraz
		shredders	FShred
		filter feeders	FFIlt
		gatherers and/or collectors	FGath
		parasites	FPar
	Respiration mode	gill respiration	RGill
		aerial respiration (hydrostatic vesicle)	RAir
		plastron	RPlas
		tegument respiration	RTeg

Table 1. List of traits and trait modalities analyzed in this study (*Continued*)

Trait category	Trait*	Trait modality	Abbreviation
Dispersal	Locomotion type	scatting	LScat
		diving	LDiv
		sprawling, walking	LWalk
		sessile	LSess
		burrowing	LBur
	Diapause form	egg and/or statoblast	ResEgg
		cocoons	ResCoc
		houses against desiccation	ResHous
		diapause and/or dormancy	ResDiap
		quiescence	ResQui
		none	ResNone
Life history	Reproduction mode	ovoviviparity	ROviv
		free isolated eggs	RFreeE
		fixed clutches	RFixCl
		free clutches	RFreeCl
		clutches in vegetation	RClVeg
	Life stage	pupa	Pupa
		larvae	Larv
		adult	Ad
	Voltinism	semivoltine	Sev
		bivoltine	Biv
		multivoltine	Mult
		univoltine	Uni
		trivoltine	Triv
		flexible	Flex
Ecological	Saprobity	xenosaprob	Xeno
		oligosaprob	Oligo
		beta-mesosaprob	Beta
		alpha-mesosaprob	Alpha
		polysaprob	Poly
Morphological	Maximum body size	0.05 cm - 1 cm	0.05-1
		1cm - 2 cm	1-2
		2 cm - 5 cm	2-5
		5 cm – 10 cm	5-10

*Traits and trait modalities were selected based on the literature data: Rubach et al., 2011; Magbauna et al., 2010; Statzner et al., 2010; Vieira et al., 2006; Ippolito et al., 2012; Charvet et al., 2000

Statistical analysis

The relationships between the trait modality distribution per trait, environmental factors, and pesticides were analyzed with redundancy analysis (RDA). Trait modalities per trait weighed by biomass were included in the analysis as response variables, while the concentrations of individual pesticides (chlorprofam, pirimiphos-methyl, tolclophos-methyl, carbendazim, ethiofencarb, imidacloprid, isoproturon, imazalil, methiocarb, and prochloraz) and environmental factors (temperature, DO, DOC, nitrate, nitrite, phosphate, macrophyte coverage) were explanatory variables. Time (the number of the month and the number of the year) was included in the analysis as covariable. The number of explanatory variables (17) was lower than the number of objects (79) fulfilling the requirement of RDA.

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According to Legendre & Birks (2012), before the ordination analysis, data need to be examined with regard to the symmetry of data distribution (normality) and the difference in measurement units between variables. Therefore, prior to RDA, the skewness (the symmetry of distribution), the kurtosis (the shape of the distribution), and Shapiro-Wilk test (normality of distribution) were calculated for each variable. These tests showed that the data were asymmetric and not normally distributed. To increase the normality, data were log (x+1) transformed. As a result, skewness and kurtosis values decreased. For many parameters, skewness reached the range between -2 and 2, which corresponds to the univariate normal distribution (George & Mallery, 2010). Log-transformation also removed the effect of measurement units. Results of RDA analysis were presented in triplots. Only explanatory variables having high correlation with the first two ordination axis (correlation coefficient above 0.2) were displayed in RDA triplots (similar to the study of Wesolek et al., 2010). Significance of the first RDA axis and the significance of the sum of all canonical axes per trait were tested by a Monte Carlo permutation test (based on 999 unrestricted permutations). Eigenvalues, F-ratios and p-values were derived. If the first ordination axis was not significant for a given trait ($p>0.1$), the RDA plot for this trait was not shown.

To quantify the percentage of variance in trait composition explained by pesticides, environmental factors and time, the variance partitioning based on pRDA was applied, following the method described in Borcard et al. (1992). The total variance was divided into five components: variance explained by pesticides, other environmental factors, time, shared variance between three components and unexplained (residual) variance. Variance partitioning was performed following the procedure described in Ieromina et al. (submitted). The variance components differed by the number of explanatory variables. According to Freedman (1983), the number of explanatory variables affects the explained variance (R^2). To account for the different number of explanatory variables in variance components, first a Principal Component Analysis (PCA) was performed on each explanatory variable dataset. After that, sample scores of the first four Principal Components (PC) were derived and included in the RDA as explanatory variables. In addition, Ezekiel adjustment was applied to the R^2 (according to Peres-Neto et al., 2006). In the manuscript, we refer to R^2 adjusted. Before the multivariate analysis, data were centered by species, and not centered by sample (Lepš & Šmilauer, 2003). Normality tests were performed in SPSS software (Version 21, IBM Corp. Released 2012). Multivariate analysis was performed in Canoco software v.4.5 (Lepš & Šmilauer).

Results

Linking trait modalities, pesticides and environmental factors

The relationships between the trait modalities, pesticides and environmental factors are shown in RDA triplots (Figure 1 and 2). Figure S1 (Supplemental Data) shows the relative contribution of traits modalities per trait for each sampling site. The first ordination axis for traits locomotion type and maximum body size was not significant, and RDA triplots for these traits are not shown.

High biomass of predators was associated with watersheds of the nature reserve (Figure S1). The biomass of animals breathing through a plastron was negatively correlated to the concentrations of phosphate and tolclophos-methyl. Gill respiration was characteristic of agricultural ditches (Figure S1). Biomass of macrofauna in their pupa and larvae life stage negatively correlated to phosphate, while the biomass of adults was positively correlated to nitrite and negatively to the dissolved oxygen (Figure 2). Large biomass of macrofauna in the larvae and pupa life stage was associated with watersheds of the nature reserve (Figure 2, S1). Semivoltine animals were typical for agricultural ditches (Figure S1). The biomass of animals reproducing by clutches in vegetation was positively correlated to isoproturon and was found in high amounts in agricultural ditches (Figure 2, S1). Biomass of animals reproducing by free clutches, ovoviviparity and fixed clutches was negatively correlated to tolclophos-methyl, chlorpropham and phosphate (Figure 2). The biomass of animals having diapause form was negatively correlated to phosphate (Figure 2). High biomass of these animals was associated with the nature reserve (Figure 2, S1). In contrast, the biomass of animals without a diapause form and a resistance form of egg or statoblast was positively correlated to macrophyte coverage and chlorprofam, respectively (Figure 2). Biomass of xenosaprobic species was positively correlated to the dissolved oxygen (Figure 2).

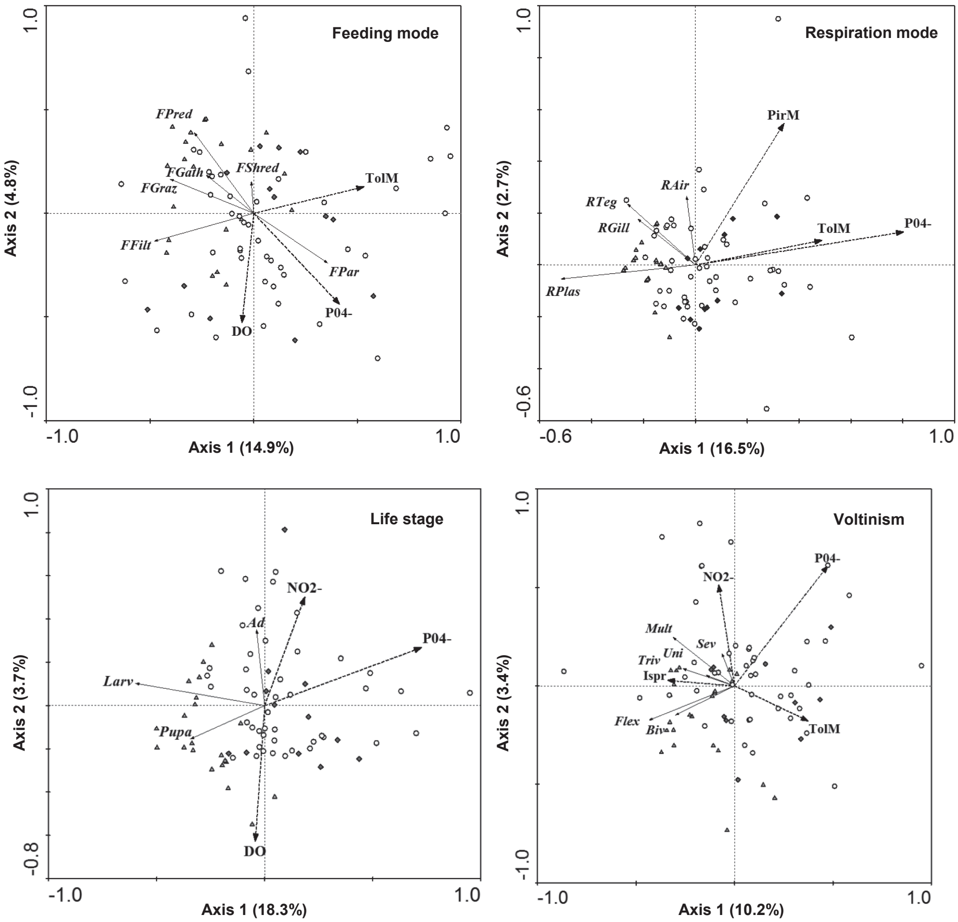

Figure 1. Redundancy analysis triplot of macrofauna trait distribution per trait in relation to pesticides and environmental factors (for traits feeding mode, respiration mode, life stage, voltinism). Abbreviations for trait modalities can be found in Table 1. Dashed lines represent pesticides and environmental factors, solid lines represent trait modalities. Chlor = chlorprofam, PirM = pirimiphos-methyl, TolcM = tolclophos-methyl, Imid = imidacloprid, Ispr = isoproturon. Triangular = sites sampling sites in watersheds of nature reserve, circles = sampling sites in ditches next to flower fields, diamonds = sampling sites in ditches next to pastures.

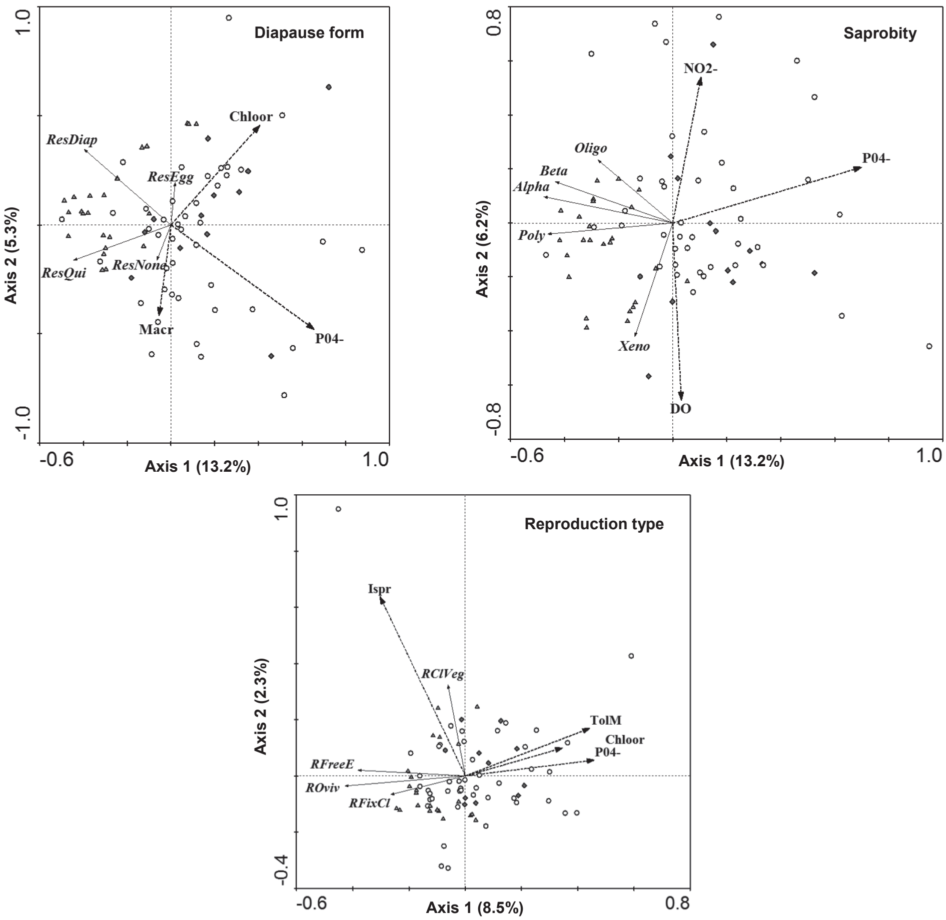

Figure 2. Redundancy analysis triplot of macrofauna trait distribution per trait in relation to pesticides and environmental factors (for traits diapause form, saprobity, reproduction type). Legend can be found in Figure 1.

Table 2. Summary of Monte Carlo test identifying the significance of the first canonical axis and the significance of all canonical axes in RDA, as presented in Figures 1 and 2

Trait		Test of significance of the first canonical axis	Test of significance of all canonical axes
Diapause form	Eigenvalue	0.132	0.222
	F-ratio	11.2	1.4
	p-value	0.011*	0.057**
Reproduction type	Eigenvalue	0.125	0.165
	F-ratio	11.4	1.1
	p-value	0.049*	0.371
Saprobity	Eigenvalue	0.138	0.218
	F-ratio	11.0	1.3
	p-value	0.057**	0.128
Respiration	Eigenvalue	0.165	0.211
	F-ratio	13.4	1.2
	p-value	0.056**	0.197
Feeding mode	Eigenvalue	0.149	0.224
	F-ratio	12.2	1.4
	p-value	0.025*	0.069**
Voltinism	Eigenvalue	0.102	0.187
	F-ratio	7.8	1.1
	p-value	0.063**	0.318
Aquatic life stage	Eigenvalue	0.183	0.239
	F-ratio	14.7	1.4
	p-value	0.064**	0.115
Locomotion type	Eigenvalue	0.126	0.193
	F-ratio	9.7	1.1
	p-value	0.298	0.363
Body size	Eigenvalue	0.363	0.201
	F-ratio	10.1	1.1
	p-value	0.416	0.384

*p<0.05
**p<0.1

Table 3. Components of variance estimated for macorfauna trait 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) and shared variance (P∩E∩T). Presented are the explained variance (R^2) and R^2 adjusted by Ezekiel's transformation (in italic)

Trait	PUEUT	Residual variance	P|EUT	E|PUT	T|PUE	P∩E∩T
Diapause form	28.0	72.0	2.3	10.9	7.8	7.0
	31.5	*68.5*	*2.5*	*12.2*	*8.7*	*7.8*
Reproduction type	25.1	74.9	1.0	5.1	14.4	4.6
	28.2	*71.8*	*1.0*	*5.6*	*16.1*	*5.1*
Saprobity	23.2	76.8	2.5	12.8	6.0	1.9
	26.1	*73.9*	*2.7*	*14.3*	*6.7*	*2.0*
Respiration	22.3	77.7	2.1	10.2	5.5	4.5
	25.1	*74.9*	*2.2*	*11.4*	*6.1*	*5.0*
Feeding mode	21.0	79.0	1.5	13.8	5.4	0.3
	23.6	*76.4*	*1.6*	*15.5*	*6.0*	*0.2*
Voltinism	19.3	80.7	2.3	8.0	6.1	2.9
	78.3	*2.5*	*8.9*	*6.8*	*3.1*	*21.7*
Aquatic life stage	19.2	80.8	1.7	12.4	5.9	-0.8
	21.6	*78.4*	*1.8*	*13.9*	*6.5*	*-1.0*
Locomotion type	18.1	81.9	1.6	7.7	5.1	3.7
	20.3	*79.7*	*1.7*	*8.6*	*5.6*	*4.1*
Body size	15.2	84.8	3.5	9.0	1.9	0.8
	17.1	*82.9*	*3.8*	*10.0*	*2.0*	*0.8*
Average R^2 adjusted	23.9	76.1	2.2	11.2	7.2	3.0

Variance partitioning of trait modality distribution

The average total explained variance in trait community composition was 23.9% with the minimum of 17.1% found for trait body size and the maximum of 28.0% found for trait diapause form (Table 2). On average, the largest proportion of variance was explained by environmental factors (11.2%, varying in the range 5.6% - 15.5%), time (7.2%, varying in the range 2.0% - 16.1%), followed by the shared variance between three components (3.0%, varying in the range 0.8% - 7.8%) and pesticides (2.2%, varying in the range 1.0% - 3.8%) (Table 2).

Discussion

The effects of pesticides and other environmental factors on the trait modality distribution

The trait modality distribution of aquatic macrofauna was influenced by pesticides and environmental factors (Figure 1 and 2). Remarkably, nutrients affected the distribution of all trait modalities. Such high importance of nutrients in structuring aquatic macrofauna can be possibly explained by the presence of nutrient gradients in surface waters, as a result of relatively high fertilizer application at the flower bulb fields (Centraal Bureau voor de Statistiek, 2015). Previous studies highlighted the importance of nutrient gradients in structuring aquatic biota in freshwater ecosystems. Verdonschot (1992) showed that nutrients along with the acidity and the extent of droughts determine the differences in macrofauna community structure in small ponds in the Netherlands. As found in the study of Scheffer et al. (2002), after the nutrient enrichment, the vegetation structure in a freshwater ecosystem (independently of its type) becomes dominated by phytoplankton. As a result, water turbidity increases, while dissolved oxygen concentration and the amount of light decrease. Subsequently, nutrients induce direct and indirect effects on aquatic biota.

High biomass of predators was found in watersheds of the nature reserve (Figure 1). Predators represent the upper level of the food chain and depend on organisms of lower trophic levels in terms of food source. The effects of stressors on organisms of lower trophic levels are likely to be negatively reflected in the performance of predators. The highly disturbed environment of agricultural ditches was not favorable for predators that were found in high amounts in clean waters of nature reserve.

The biomass of plastron–breathing animals was negatively correlated to nutrients and pesticides. This result suggests a high dependence of plastron-breathing invertebrates, exchanging oxygen and carbon dioxide in a thin layer of air around the body (Flynn & Bush, 2008), on the water quality. In contrast, the biomass of aerial-breathing insects using a hydrostatic vesicle for respiration was not strongly correlated to pesticides and environmental factors. Species using a hydrostatic vesicle obtain the air from an air bubble attached to the body (Database http://www.freshwaterecology.info/), and therefore do not fully depend on the chemical composition of the water for respiration. The biomass of animals with tegument respiration (through the body surface) was also not correlated to pesticides and environmental factors. The high biomass of animals with gill respiration was characteristic of agricultural ditches.

The biomass of pupa was positively correlated to dissolved oxygen and negatively to nutrients, suggesting that the clean waters of the nature reserve were suitable to accommodate macrofauna at this sensitive life stage. Macrofauna having a semivoltine reproduction cycle was associated with ditches of the agricultural area. In contrast, Díaz et al. (2008) reported that semivoltinism is a characteristic feature of an undisturbed environment. As a possible explanation, mollusks (semivoltine species) generally tolerant to organic pollution were found at high numbers in agricultural ditches. The reproduction by clutches in vegetation was associated with agricultural ditches, where high macrophyte abundance created favorable conditions for reproduction of species fixing egg clutches.

Variance partitioning

The variance partitioning results revealed that the environmental factors other than pesticides explained the largest proportion of variance for five out of nine traits. The environmental factors had the largest contribution (15.5%) to the variance in the trait feeding mode. This can be explained by the high dependence of food availability for macrofauna on environmental conditions. For the remaining four traits, time constituted the most important factor. Time accounted for the largest proportion of variance in reproduction type (16.1%). This can be explained by the seasonal succession of macrofauna exhibiting different reproduction types throughout the year.

In contrast to our hypothesis, pesticides explained the smallest proportion of variance in trait composition from all factors. A similar result was observed in the analysis of the taxonomic composition of macrofauna from the same research area (Ieromina et al., submitted). Trait modality distribution of several traits differed between agricultural ditches and watersheds of the nature reserve. This result suggests that macrofauna in agricultural ditches could possibly adapt to toxic stress. As found in our study, traits more typical to agricultural ditches (gill and aerial respiration, reproduction by clutches in vegetation, semivoltinism) possibly helped to ensure resilience of the macrofauna community to pesticides. In contrast, traits of the nature reserve (predation, xenosaprobity, presence of diapause form) were characteristic features of undisturbed environment.

The current study showed that the trait composition of aquatic communities next to agricultural fields is influenced by pesticides and other environmental factors. Understanding the responses of community composition to disturbances is important with regard to biodiversity conservation (Vandewalle et al., 2010). Species diversity and ecosystem resilience are closely related, and the resilience increases with the number of species within each functional group (Cleland, 2011; Ives & Carpenter, 2007; Naeem, 1998). As a general rule, communities containing high species diversity better withstand natural or anthropogenic disturbances than communities containing low species diversity, because communities with more species are likely to have a large variety of functional characteristics (trait modalities) that facilitates adaptation of such communities to changing conditions (Cleland, 2011). Like Fischer et al. (2006) concluded, conservation measures should aim at preserving species diversity within and between different functional groups.

Conclusions

Trait-based approaches are increasingly used to assess and monitor the health of ecosystems. As found in our study, the trait modality distribution differed between ditches next to flower bulb fields and nature reserve. Pesticides did not induce a larger effect on the community trait composition than environmental factors, in contrast to the initial hypothesis. However, our data indicates that the presence of an excess of nutrients and pesticides affects the distribution of certain macrofauna trait modalities (related to feeding, respiration, reproduction, ecological tolerance), hinting that macrofauna traits can potentially be used as a tool to monitor the ecological status of aquatic ecosystems in environmental assessment practices.

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Supporting information

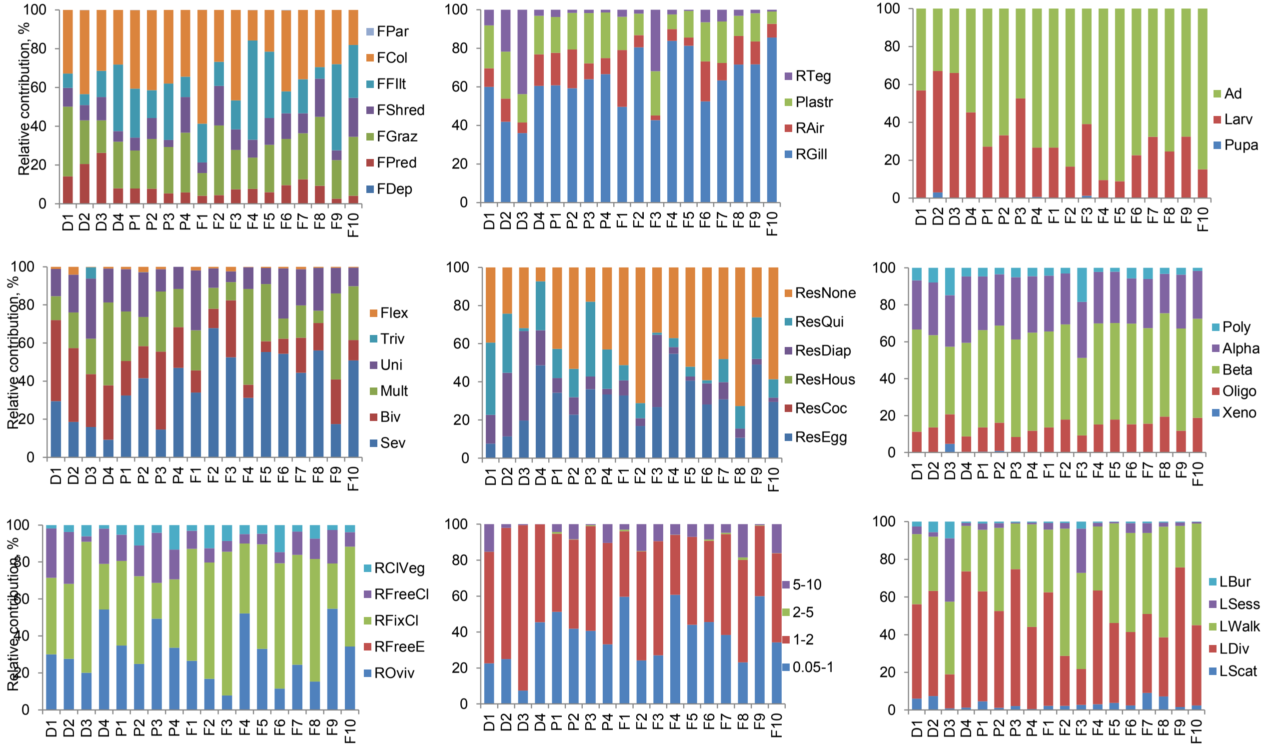

Figure S1. Relative contribution of trait modalities per trait for each study site. For legend, see Table 1. D = sites in watersheds of nature reserve, P = sites in ditches next to pastures, F = sites in ditches next to flower bulb fields